Kidney International, Vol. 27, 1985, pp. 99–352

Abstracts

American Society of Nephrology
Washington, D.C., USA
December 9–12, 1984

GENES IN THE MAJOR HISTOCOMPATABILITY COMPLEX REGULATE THE INTENSITY AND SPECIFICITY OF THE IMMUNE RESPONSE. Hugh O. McDevitt. Stanford University School of Medicine, Dept. Of Medical Microbiology, Stanford, California.

The Class II MHC antigens regulate the helper T cell (T_H) response. The T_H cell receptor sees both the self Class II MHC molecule and the foreign antigenic determinant on the antigen presenting macrophage.

To understand the molecular basis of this regulation we have undertaken study of the primary structure of the murine I-A and I-E Class II MHC molecules. The evidence from these structural studies indicates that these molecules have many structural and genetic similarities to the immunoglobulins. These molecules may have an "active site" which interacts with foreign antigens. It is the combination of this "active site" with a foreign antigenic determinant which is recognized by the TH cell receptor.

This knowledge can be utilized to regulate the immune response in vivo using monoclonal antibodies directed against Class II MHC molecules. The same monoclonal antibodies can be used to suppress autoimmune processes, such as experimental allergic encephalomyelitis and lupus-like nephritis in NZB/NZW F_1 mice.

ADVANCES IN THE ETIOLOGY AND PATHOGENESIS OF DIABETES. Arthur H. Rubenstein. Univ. of Chicago, Dept. of Medicine, Chicago, Illinois.

During the past decade significant progress has been made in our understanding of the etiology and pathogenesis of diabetes mellitus. It is now appreciated that diabetes mellitus comprises a heterogenous group of disorders with a common metabolic end-point of hyperglycemia. The two most frequent categories of diabetes are Insulin Dependent (IDDM; Type I) and Non-Insulin Dependent (NIDDM; Type II). IDDM is characterized by severe insulin deficiency, a tendency to develop ketoacidosis, circulating anti-islet cell antibodies and increased frequency of HLA-Dw3 and DW4. The beta cell destruction may be mediated by an autoimmune process and tiggered by an exogenous agent (e.g. virus, chemical).

NIDDM is more frequent in older individuals, many of whom are obese. The inheritance pattern is autosomal dominant with no specific HLA association. The defect is likely to reside in the insulin receptor and/or post-receptor pathway, altnough beta cell dysfunction may also play a role.

Both types of diabetes are associated with long-term complications involving the kidneys, eyes, nerves and cardiovascular system. Important issues regarding the relationship of these complications to the metabolic abnormalities are now being studied. Information concerning the impact of hypertension and hyperglycemia on kidney function and structure and the potential reversibility of diabetic renal lesions is becoming available and will be reviewed.

HEMATOLOGICAL PROBLEMS IN RENAL FAILURE. Robert M. Lindsay (Moderator), Guiseppe Remuzzi, and James W. Fisher. Univ. of Western Ont., Canada; Negri Institute, Bergamo, Italy and Tulane Univ. School of Medicine, New Orleans, USA.

The anemia of renal failure is due primarily to erythropoietin (Ep) deficiency. Ep present in sera of anemic renal failure patients appears to have immunologic but not biologic activity; inhibitors in uremic sera interfere with the latter but this may be improved by dialysis. Purified Ep, via recombinant DNA techniques, may become available to treat patients.

The anemia may be aggravated by blood losses secondary to thrombosis within extracorporeal circuits or by excessive bleeding because of a uremic bleeding tendency. The major cause of this bleeding tendency is a platelet defect. Uremic platelets produce less thromboxane A_2 whereas uremic vascular specimens produce more prostacyclin than controls. This bleeding tendency may be improved by increasing the hematocrit, by adequate dialysis, and by DDAVP (a synthetic derivative of antidiuretic hormone which causes release of factor VIII-Von Willebrand factor from storage sites).

To prevent thrombosis during dialysis heparin is used which, in turn, may aggravate the bleeding tendency. Heparin dosage cannot be judged on a patient body weight basis as complex relationships exist between heparin, platelets, and the blood-foreign surface interaction. Of importance are: 1) heparin may enhance platelet release, 2) platelets release a factor which neutralises heparin and 3) adequate dialysis may improve platelet function.

ANTICOAGULANT PROBLEMS IN DIALYSIS PATIENTS. Robert M. Lindsay. Renal Unit, Victoria Hospital and the Department of Medicine, the University of Western Ontario, London, Canada.

Patients on hemodialysis often experience either thrombosis in the extracorporeal circuit or excessive bleeding from cannulation sites in spite of standard doses of heparin. It is tempting to incriminate variability of heparin preparations, since commercially available forms are a variable polydisperse mixture of polysaccharides of markedly uneven anticoagulant activity. However, the clinical observations may also be explained by the finding of elevated heparin neutralizing activity (HNA) in the plasma of patients on maintenance hemodialysis. HNA appears to be related to the release of platelet factor 4 (PF4) as a result of blood-foreign surface interactions and the inadequate elimination of the PF4 molecule by hemodialysis therapy. Improvement of the uremic platelet defect by dialysis leads to an increased ability of platelets to liberate PF4 and, thus, improving the adequacy of dialysis may actually change dialysis heparin requirements because of increased plasma HNA. A protocol for dialysis anticoagulation, in which HNA is considered, minimizes the occurrence of clinical problems.

ERYTHROPOIETIN (Ep) IN THE PATHOGENESIS OF RENAL ANEMIA. James W. Fisher, Dept. Pharmacology, Tulane Univ. Sch. of Med., New Orleans, Louisiana

The anemia of renal failure is due primarily to Ep deficiency where there is insufficient amounts of Ep produced in response to the increased demands for new red blood cell formation created by renal disease. In severe renal failure inhibitors of the erythroid progenitor cell compartment and shortening of red cell life span are secondary factors adding further to the severity of the anemia. Evidence has been presented implicating polyamines such as spermine, parathyroid hormone, lipid-like substances of renal origin and other uremic toxins as inhibitors of the erythroid progenitor cell compartment. Guanidine derivatives have been mainly implicated in the reduction of red cell life span. Even though immunoreactive Ep has been demonstrated to be elevated in the anemia of renal failure it is apparently not sufficiently elevated to correct the anemia and is elevated to a much less extent for the degree of anemia than that seen in other types of anemia in patients with normal renal function. The Ep present in sera of patients with anemia of renal failure appears to have immunologic but not biologic activity. It is possible that the inhibitors in sera of renal failure patients interfere with the biologic but not the immunologic assay of erythropoietin. Improved dialysis procedures may also be helpful in correcting this anemia. Additional quantities of purified erythropoietin perhaps provided through recombinant DNA techniques, are needed to treat patients with anemia of renal failure.

METABOLIC ALKALOSIS: SYMPOSIUM INTRODUCTION. Jordan J. Cohen, Dept. of Med., Michael Reese Hospital & Univ. of Chicago, Chicago, Illinois.

Metabolic alkalosis of the common "chloride-dependent" variety is unique among the primary disturbances of acid-base equilibrium; once generated, this acid-base abnormality can be perpetuated by the normal kidney which can sustain the elevated plasma bicarbonate even after passage of the initiating process (e.g., diuretic administration, gastric aspiration). Metabolic acidosis, by contrast, is a self-limited disturbance; once the acidifying process abates, this acid-base abnormality is promptly repaired by normal renal mechanisms which augment net acid excretion appropriately, thereby returning plasma bicarbonate to normal. By way of further contrast, neither selective nor total dietary deprivation constrains the kidney in its ability to repair metabolic acidosis. Yet, in "chloride-dependent" metabolic alkalosis, the mere absence of dietary chloride forestalls renal repair by preventing the requisite reduction in net acid excretion.

This symposium will focus special attention on how chloride deficits interdict renal repair of metabolic alkalosis. New information will be reviewed on the precise renal mechanisms responsible for sustaining a high bicarbonate concentration; the relative roles of volume depletion, reduced GFR, augmented proximal reabsorption and collecting duct acidification will be examined.

ABNORMALITIES OF PLATELET AND COAGULATION FUNCTION IN RENAL FAILURE. Giuseppe Remuzzi. Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

Uremics suffer from a bleeding tendency whose laboratory hallmark is a prolongation of bleeding time (BT). Since BT is affected by platelet-vessel wall interaction we studied arachidonic acid metabolism at platelet and vascular level in uremics. We found that uremic platelets produced significantly less thromboxane A2 (a potent platelet aggregating agent) whereas uremic vascular specimens produced significantly higher prostacyclin (a potent antiaggregating agent) than controls. Moreover since BT may be influenced by packed cell volume (PCV) and uremics are often anemic, we studied the relation between the two parameters and found platelet-mediated hemorrhagic tendency in uremia may be succesfully managed by raising PCV values to above 30%. After the work of Janson et al showing that cryoprecipitate infusion transiently shortened the prolonged BT in uremics, we undertook a double-blind controlled study to evaluate the effect of DDAVP (a synthetic derivative of the antidiuretic hormone, which causes the release of FVIII-VWF from storage sites into plasma) on BT in uremics. DDAVP temporarily shortened BT in uremics. On this basis we studied the effect of giving sequentially washed red cell transfusions and /or DDAVP to 12 patients with uremia due to acute renal failure with the aim to improve hemostasis and allow a diagnostic renal biopsy. Our procedure resulted in normalization of BT in 10 patients, who underwent the bioptic procedure with no major bleeding complications.

ROLE OF CHLORIDE DEPLETION. John H. Galla. University of Alabama in Birmingham.

Evidence will be presented from studies of animal models of chloride-depletion alkalosis that chloride depletion, independently of volume depletion, contributes to maintenance of a high plasma bicarbonate, thus facilitating conservation of sodium and fluid volume until chloride can be supplied.

These changes are:

a) Stimulation of the renin-aldosterone system.
b) Reduction of glomerular filtration rate by tubuloglomerular feedback.
c) Alterations in collecting duct function.

Such changes promote maintenance of sodium balance and extracellular fluid volume despite hypochloremia and hyperbicarbonatemia. During correction of alkalosis with chloride but without volume expansion, bicarbonate is excreted, chloride conserved in the distal convolution and collecting duct, and glomerular filtration rate increased without changes in fractional fluid and chloride reabsorption in the proximal tubule and loop segment. It is emphasized that, in clinical examples, the effects of chloride depletion are adjuvant to any effects of volume depletion on the maintenance of metabolic alkalosis.

METABOLIC ALKALOSIS: ROLE OF GLOMERULAR FILTRATION RATE (GFR) AND PROXIMAL TUBULAR BICARBONATE REABSORPTION. Martin G. Cogan, Dept. of Med., Univ. of Calif., San Francisco, CA.

Chronic metabolic alkalosis can be maintained by either or both of two mechanisms. If GFR were to reciprocally fall as the plasma bicarbonate concentration rises, the filtered bicarbonate load would remain normal; a normal rate of tubular bicarbonate reabsorption would then suffice to prevent bicarbonaturia. Alternatively, if GFR remains normal, renal H^+ secretion must increase in proportion to the rise in plasma bicarbonate concentration to avoid bicarbonate excretion. In rats, the first mechanism is operative: as plasma bicarbonate rises, both chloride and potassium deficiencies cause GFR to be reciprocally reduced. Filtered bicarbonate load is unchanged from normal values. Absolute proximal bicarbonate reabsorption as well as bicarbonate delivery out of the proximal tubule (and thus net distal acidification) are normal and not increased. Cl and K supplementation induces bicarbonaturia and repairs the alkalosis by normalizing GFR, without changing the absolute rate of tubular bicarbonate transport. In man, however, both a reduction in GFR and a stimulation of tubular bicarbonate reabsorption can participate in maintaining the alkalotic state. For instance, when HCl is selectively removed from humans (by gastric aspiration) in order to cause a 27% increase in plasma bicarbonate concentration (from 25.3 $\pm$ 0.1 to 32.1 $\pm$ 0.3 mEq/l), there is a 10% reduction in GFR (from 93 $\pm$ 4 to 84 $\pm$ 4 ml/min) accompanied by a 17% increase in net renal H^+ secretion (from 2.7 $\pm$ 0.1 to 3.3 $\pm$ 0.2 mEq/min). In conclusion, metabolic alkalosis in rats is maintained principally by a reduction in GFR, while in humans, alkalosis can be maintained by changes in both GFR and net renal acidification.

THE ROLE OF THE CORTICAL AND MEDULLARY COLLECTING TUBULES IN METABOLIC ALKALOSIS. H.R. Jacobson. Univ. TX Hlth. Sci. Ctr., Dallas, TX.

If renal acid excretion is primarily responsive to systemic pH, then a role for the kidney in the generation or maintenance of metabolic alkalosis (MA) requires that certain factors override the influence of pH. These factors are felt to include increased mineralocorticoid activity, K depletion and volume (Cl) depletion.

The cortical collecting tubule (CCT) of both rabbits and rats can reabsorb as well as secrete HCO_3. In contrast, in both species the medullary collecting duct (MCD) only reabsorbs HCO_3 (secretes H^+) and at a rate several times that seen in CCT. Although evidence exists from in vitro studies of rabbit tubules that mineralocorticoid stimulates proton secretion in both CCT and MCD, if CCT from DOCA treated rabbits that develop MA are studied in vitro net HCO_3 secretion is observed. Similarly, in rabbit CCT and MCD studied in vitro acute increases in peritubular HCO_3 (i.e., in vitro MA) suppress acidification. These studies argue against a role for increased CCT and MCD acidification in the maintenance of MA. Acute reduction in peritubular K in MCD does not affect acidification and in CCT from alkalotic DOCA animals zero bath K^+ does not suppress HCO_3 secretion. Studies in CCT and MCD from chronically K-depleted animals are required to more completely address the role of K^+ depletion in collecting duct acidification. Finally, low luminal [Cl] (volume depletion) augments MCD acidification and may limit CCT HCO_3 secretion (i.e., serve to generate or maintain MA).

MORPHOLOGIC INSIGHTS INTO TRANSPORT MECHANISMS: AN OVERVIEW. C. Craig Tisher, Laboratory of Experimental Morphology, University of Florida College of Medicine, Gainesville, FL.

The past several years have brought an increased awareness that structural analysis of transport epithelia can expand our knowledge of their functional properties. This symposium presents three examples. Dr. Sherman Levine describes how light microscopy, transmission electron microscopy (TEM), freeze-fracture electron microscopy and scanning electron microscopy (SEM) have been employed to localize pathways of water flow and to estimate barrier permeabilities in the toad bladder and the collecting tubule (CT). Dr. Kirsten Madsen describes the use of SEM and TEM, the latter coupled with morphometric analysis, to define cellular events associated with hydrogen ion secretion in the parietal cell of the gastric mucosa, the carbonic anhydrase-rich cell of the turtle bladder, and the intercalated cell of the CT. Finally, Dr. Bruce Stanton presents morphologic data acquired with TEM and morphometric analysis describing the subcellular events associated with K reabsorption by intercalated cells and K secretion by principal cells in the CT. New and improved morphologic techniques such as immunocytochemistry employing immunoperoxidase and immunogold coupled with monoclonal antibody probes, intramembrane cytochemistry, photoaffinity labelling combined with autoradiography, freeze-fracture electron microscopic autoradiography, differential interference contrast microscopy, and computer-assisted image analysis hold great promise to advance our knowledge of transport mechanisms in various epithelia.

MORPHOLOGIC INSIGHTS INTO TRANSPORT MECHANISMS: WATER TRANSPORT. Sherman D. Levine, Dept of Medicine, Albert Einstein College of Medicine, Bronx NY

Although the rate of water transport across epithelial tissues is easily measured using a variety of techniques, progress in establishing structure-function relationships, localizing flow pathways, and estimating barrier permeabilities has largely been the result of morphologic approaches.

In the presence of transepithelial water flow down an osmotic gradient, transporting cells swell or shrink, while flow through intercellular spaces is suggested by an increase in the width of these spaces. Such changes are observable by light microscopic techniques in vivo, as well as in thin section of fixed specimens. Cell shape deformations suggest local alterations in cell compliance, while alterations in the size of cell organelles (e.g. mitochondria) have been used to estimate the local osmolality. In vasopressin-responsive tissues such as toad bladder and renal collecting tubule, freeze-fracture electron microscopy reveals luminal membrane particle clusters ("aggregates") whose presence correlates closely and specifically with the permeability of that membrane to water. These aggregates, which may indeed be the luminal water transport pathways, exist preformed within cell structures, visible with both freeze-fracture and scanning techniques. The aggregate-containing structures in turn move to and fuse with the luminal membrane after vasopressin stimulation, eventually delivering the aggregates to the luminal surface.

HYDROGEN ION TRANSPORT. Kirsten M. Madsen Division of Nephrology and Hypertension, University of Florida, Gainesville, Florida.

Stimulation or inhibition of hydrogen ion secretion is associated with characteristic ultrastructural changes in various acid secreting epithelial cells such as the parietal cell of the gastric mucosa, the carbonic anhydrase-rich cell of the turtle urinary bladder, and the intercalated cell of the mammalian collecting duct. These three cell types have several morphological characteristics in common. They are rich in mitochondria and possess numerous tubulovesicular membrane structures in the apical region of the cells. An electroneutral H^+-K^+-ATPase is responsible for acidification in the stomach, whereas hydrogen ion secretion in the turtle bladder and the mammalian collecting duct is an electrogenic process mediated by a proton translocating ATPase. In spite of differences in the mechanism of acidification, the ultrastructural changes associated with hydrogen ion secretion appear to be similar. Morphometric analyses of parietal cells, carbonic anhydrase-rich cells, and intercalated cells following stimulation of hydrogen ion secretion have demonstrated a significant increase in the surface area of the apical plasma membrane concomitant with a depletion of the tubulovesicular membrane structures in the apical region of the cells. These findings suggest that membrane, possibly containing a proton pump, is being transferred from the tubulovesicular membrane compartment to the apical plasma membrane in association with hydrogen ion secretion.

POTASSIUM TRANSPORT. Bruce Stanton, Dartmouth Medical School, Dept. of Physiology, Hanover, NH

Dietary K loading enhances K secretion and dietary restriction of K converts net secretion into reabsorption by renal collecting tubule. However, little is known about the cellular mechanisms of these transport processes. Morphological studies have provided important new information on the cellular mechanisms of K transport. K loading increases basolateral membrane area of principal cells. An increase in plasma aldosteorne and hyperkalemia both contribute to this effect. Because membrane area correlates with Na-K ATPase activity it is likely that membrane amplification reflects an increase in the number of ATPase pumps. The increase in membrane area is accompanied by an elevation in the number of vesicles in proximity to the basal membrane and in the number of fusion events of vesicles with the membrane suggesting that amplification occurs by recruitment of membrane from cytoplasmic vesicles. In contrast, K restriction stimulates amplification of the luminal membrane of intercalated cells and also stimulates K reabsorption. The active step in K reabsorption occurs across the luminal membrane. Amplification of luminal membrane is accompanied by a decrease in the number of apical cytoplasmic vesicles thereby suggesting that membrane, possibly containing the H pump, is recruited from these vesicles. In conclusion, morphological studies suggest that K is secreted by principal cells and is reabsorbed by intercalated cells. Each cell type increases K transport, in part, by recruiting membrane from cytoplasmic vesicles which may contain specific although different transport mechanisms.

NON-PARATHYROID HORMONE MEDIATED HYPERCALCEMIA. Gregory R. Mundy*, University of Texas Health Science Center, San Antonio, Texas.

Hypercalcemia has been noticed frequently since the routine measurement of serum calcium has become available. This has led to the realization that a) primary hyperparathyroidism is a much more common disease than previously realized, particularly in the elderly female population. Current estimates indicate the annual incidence is 250 new patients per million population. b) Hypercalcemia associated with malignancy is also much more common than previously thought. In the general population (hospitalized and non-hospitalized), these two causes of hypercalcemia comprise approximately 90% of all patients. Of the remaining causes, recent studies of abnormalities of vitamin D metabolism and action have revealed insights into the pathophysiology in sarcoidosis, possibly in some patients with the idiopathic hypercalcemia of infancy and occasional patients with T cell lymphomas. This symposium will review recent studies on the hypercalcemia of malignancy and abnormalities of vitamin D metabolism responsible for hypercalcemia. Hypercalcemia of malignancy will be considered in terms of patients with hematologic malignancies, (myeloma and T cell lymphomas), solid tumors associated with extensive osteolytic bone destruction and patients with solid tumors without metastases who have the syndrome of the humoral hypercalcemia of malignancy, where a humoral factor which has osteotropic and possibly renotropic effects is responsible. This latter syndrome has received considerable attention recently and a number of newly described factors have been implicated as potential mediator(s), simultaneously with the realization that prostaglandins and parathyroid hormone seem unlikely to be the cause in the majority of cases. During this symposium, several speakers studying this syndrome will describe recent studies.

GRANULOMATOUS DISEASES, LYMPHOMA AND VITAMIN D. John S. Adams*, Orthopaedic Hospital, Univ. of Southern California, Los Angeles, CA

Although elevated serum concentrations of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$-D) have been observed recently in some hypercalcemic patients with lymphoma, good evidence for the pathological extrarenal synthesis of an active vitamin D sterol exists only for sarcoidosis. Work from this laboratory, employing human pulmonary alveolar macrophages (PAMs) from patients with sarcoidosis as a model, has shown the macrophage to be the synthetic source of an active vitamin D_3 metabolite that is functionally and structurally identical to 1,25-$(OH)_2$-D_3. The cellular production of 1,25-$(OH)_2$-D_3 *in vitro* from 25-hydroxyvitamin D_3 (25-OH-D_3) is disease specific for sarcoidosis and greatest in PAMs from patients with diffuse infiltrative pulmonary disease, disordered calcium homeostasis, and high circulating levels of 1,25-$(OH)_2$-D *in vivo*. The high affinity of the PAM 1a-hydroxylation reaction for 25-OH-D_3 (53nM) and its apparent lack of an accompanying 25-OH-D_3-24-hydroxylase suggest that the PAM may be a more efficient producer of 1,25-$(OH)_2$-D_3 than its naturally occurring competitor, the renal 1a-hydroxylase. The PAM 1a-hydroxylation reaction is exquisitely sensitive to inhibition with dexamethasone *in vitro* which may explain the therapeutic efficacy of glucocorticoids in hypercalcemic patients with sarcoidosis.

HYPERCALCEMIA OF MALIGNANCY - ROLE OF TRANSFORMING GROWTH FACTORS (TGFs) AND OAF. Ellen Simpson*. Department of Medicine, Division of Endocrinology, University of Texas Health Science Center, San Antonio, Texas 78284

Patients with the hypercalcemia of malignancy can be classified arbitrarily into three clinical groups: a) hematologic malignancies, b) solid tumors with extensive bone metastases and c) solid tumors without metastases. In patients with the hematologic malignancies, it is likely that osteoclast activating factor(s) are responsible for activating osteoclasts, causing increased bone destruction and subsequent hypercalcemia. These factors are secreted by cultured myeloma cells and by HTLV-infected T cell lymphomas, which are almost universally associated with hypercalcemia.

In patients with solid tumors with metastases, the mechanism of hypercalcemia is due to local factors which increase osteoclastic bone resorption, or to direct effects of tumor cells on bone. In patients with the solid tumors without metastases or the humoral hypercalcemia of malignancy syndrome, the major candidates for the humoral factor responsible have been parathyroid hormone, the transforming growth factors, and parathyroid hormone PTH-like factors, which interact with some PTH receptors. It seems that PTH is rarely if ever responsible for the hypercalcemia of malignancy since serum immunoreactive PTH concentrations are suppressed in patients with this syndrome and these tumors are unable to synthesize PTH messenger RNA. In some tumors, we will present evidence that a family of tumor derived growth factors which are responsible for mesenchymal and tumor cell replication and maintenance of the neopolastic phenotype have the capacity to stimulate osteoclastic bone resorption and may be responsible for hypercalcemia.

ATRIAL NATRIURETIC FACTOR. AN OVERVIEW. Adolfo J. de Bold. Pathology Department, Queen's University and Hotel Dieu Hospital, Kingston, Ontario, Canada K7L 3H6.

The bulk of mammalian atrial cardiocytes are morphologically differentiated as both, contractile and secretory cells. As secretory cells, atrial cardiocytes display numerous storage granules referred to as specific atrial granules. These granules arise from the Golgi complex through a process of condensation and maturation which is common to cells producing secreted proteins. Atrial granules share morphological, tinctorial and histochemical properties with polypeptide hormone-storing granules. In addition, their number may change in experimental conditions known to upset water and electrolyte balance. After nearly 25 years of research on these granules it was found that their function is that of storing a polypeptide - atrial natriuretic factor - (ANF) which is a powerful natriuretic agent. ANF is synthetized by atrial cardiocytes as a precursor of approx. 150 amino acids. The C-terminus portion of this precursor contains the biologically active sequence. In rat atrial muscle the most abundant sequence is present in the form of a 28 amino acid peptide referred to as cardionatrin 1-28. The renal, cardiovascular and endocrine effects of ANF are compatible with the view that this peptide plays a major role in the maintenance of electrolyte balance and in blood pressure regulation.

HYPERCALCEMIA OF MALIGNANCY-ROLE OF PTH RECEPTORS. A. Broadus‡, K. Insogna‡, E. Weir‡, A. Vignery‡, W. Burtis‡, D. Goltzman* and A. Stewart‡* Yale Univ., New Haven, CT, and McGill Univ., Montreal, Que.

The biochemical marker for humoral hypercalcemia of malignancy (HHM) in vivo is an increase in nephrogenous cyclic AMP excretion, and this abnormality has been observed in some 75 percent of unselected patients with malignancy-associated hypercalcemia. Additional findings in patients with HHM include a marked increased in fractional calcium excretion, a profound reduction in plasma 1,25-$(OH)_2$D, normal or reduced serum iPTH, and intense osteoclastic bone resorption with near-complete uncoupling of bone turnover. Thus, the putative mediator is "PTH-like" in only a very limited sense.

Our strategy in vitro has been to establish detection systems based on the findings in vivo, these detection systems including PTH-sensitive adenylate cyclase assays in canine renal membranes and cultured rat osteosarcoma cells, a bone resorption assay, and the cytochemical bioassay for PTH. Activity in a combination of these assays has been regularly demonstrated in extracts of HHM tumors and conditioned medium from cultured tumor cells. This activity can be inibited by [Nle^8, Nle^{18}, Tyr^{34}]bPTH(3-34)NH_2 but not by preincubation with a variety of PTH antisera. Material from a human tumor and the rat Leydig cell tumor model has been purified 30,000-fold by conventional protein techniques. In both systems, the factor appears to be a basic protein of 28,000 daltons. Media from Xenopus oocytes microinjected with mRNA from several human and animal model tumors stimulate the cytochemical assay, and mRNA from the Leydig cell tumor has been enriched some 20-fold by preparative acrylamide gel electrophoresis.

THE SPASMOLYTIC AND NATRIURETIC PROPERTIES OF ATRIOPEPTINS. Philip Needleman,* Mark G. Currie,* David M. Geller,* Korekiyo Wakatani,* Takeshi Oshima,* Steven P. Adams,§* Tom Hintze,†* and Barbara R. Cole. Dept. of Pharmacology and Pediatrics, Washington Univ. Medical School, St. Louis, MO and †Dept. Physiol, N.Y. Medical Coll., Valhalla, NY.

Mammalian atrial extracts have been shown to contain bioactive peptides which exert natriuretic, diuretic, and smooth muscle relaxant effects. These rat atrial peptides include several low molecular weight (<5,000 Mr) atrial peptides (atriopeptins) which exhibit identical sequences over a central core region differing only in additions or deletions at the N- or C-termini. The atriopeptins are derived from a single high molecular weight precursor (atriopeptigen) which was characterized by microsequence analysis and the preparation of cDNA clones. The presence of phe-arg residues at the carboxy terminus of the atriopeptins (i.e. atriopeptin II and III) is essential for reducing vascular tone in: a) isolated blood vessel segments; b) ex-vivo Krebs perfused rat kidneys; c) in-situ blood autoperfused rat kidneys; and d) in kidneys in vivo in anesthetized or unanesthetized dogs. Atriopeptin III selectively reduces renal blood flow at doses that don't effect vascular resistance in other vascular beds in anesthetized rats or in trained unanesthetized dogs.

EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF) ON RENAL HEMODYNAMICS, BLOOD PRESSURE, RENIN AND ALDOSTERONE
S. Atlas,* M.J. Camargo, H. Kleinert,* J. Laragh, J. Lewicki,* T. Maack. Hypertension Ctr. and Dept. of Physiol., Cornell Univ. Med. Coll., New York NY.

We previously isolated and sequenced rat atrial peptides (auriculin A and B) that mimic the actions of the ANF found in atrial extracts. Synthetic ANF increases GFR, $U_{Na}V$ and renal vascular resistance in functioning isolated kidneys (IK) perfused without vasoconstrictors. ANF causes vasorelaxation in IK or aortic rings only if precontracted with hormonal or non-hormonal agents. In anesthetized and conscious dogs, constant ANF infusion decreases BP and induces a major, sustained increase in GFR and $U_{Na}V$. Renal blood flow (RBF) increases, but only transiently (1-2 min), and then falls to or below control levels, suggesting an additional direct or indirect renal vasoconstrictive effect in vivo. Current evidence indicates that the natriuresis is due wholly or in part to a combination of increased GFR and washout of the papillary interstitium which does not depend on a sustained increase in total RBF. In dogs, ANF decreases renin secretory rate and plasma renin activity (PRA), perhaps due to increased Na load to the macula densa. Plasma aldosterone (Aldo) also falls. While this may be due to lowered PRA, auriculin also decreases Aldo production by isolated adrenal cells. Furthermore, in renin-dependent Goldblatt hypertensive rats, ANF markedly reduces Aldo (as well as BP), independent of changes in PRA. In summary, ANF has a unique combination of functional properties, increasing GFR and $U_{Na}V$ without a sustained increase in total RBF, and decreasing BP, PRA and Aldo. These findings indicate the potential importance of ANF in extracellular fluid volume and BP homeostasis.

RAT MODEL OF AUTOIMMUNE DIABETES MELLITUS
George S. Eisenbarth, MD, Joslin Diabetes Center

Type I diabetes of both man and the BB rat results from immunologically destruction of pancreatic beta cells. Severe insulin deficiency and hyperglycemia develop. In the BB rat at least two "genes" contribute to the development of diabetes. One gene produces a severe circulating T cell immunodeficiency that is inherited as a simple autosomal recessive with 100% penetrance. In crosses of BB rats with normal inbred rats, lymphopenia is required for diabetes to develop. The thymus is histologically normal. An independently segregating gene in BB rats histocompatability region is also required for the development of diabetes. In crosses to normal rats the great majority of diabetic animals are homozygous for the BB's RTIu and the remaining animals are heterozygotes. The specific gene products of the histocompatibility gene complex and the manner in which lymphopenia predisposes to diabetes is unknown. Immunotherapies which further suppress immune function of the BB rat and therapies which partially correct the lymphopenia prevent diabetes. Diabetes can be transferred with conA-activated T cells and transplanted islets are rapidly destroyed in tolerant animals.

Type I diabetes of man appears to differ from the BB rat primarily in the severity of BB's T cell abnormality. Many of the immunologic abnormalities disappear after beta cell destruction is complete but segmental pancreatic transplantation from identical twins is blocked by reenactment of the beta cell destructive process, including recurrence of anti-islet antibodies. Attempts to "cure" recent onset diabetics or prevent Type I diabetes with immunotherapy are the subject of current research.

RENAL TUBULAR EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF).
Harald Sonnenberg. Dept. of Physiology, University of Toronto Canada.

The natriuretic action of atrial extracts and pure synthetic peptides is usually associated with increases in glomerular filtration rate and renal blood flow. It has, therefore, been suggested that alteration of renal hemodynamics is the major mechanism of the natriuresis. However, the renal response to ANF can not be mimicked by known vasodilators. Furthermore, in isolated perfused kidneys a decrease in fractional sodium reabsorption can be observed when vasoactive effects have dissipated. Micropuncture experiments in vivo show a small, but statistically significant reduction of net reabsorption in the loop of Henle, and an increased delivery to, and decreased reabsorption in, the medullary collecting duct system. It is concluded that renal hemodynamic mechanisms may contribute to the natriuretic response to ANF, but that a direct effect on distal nephron transport is an essential feature of the increased sodium excretion.

SPONTANEOUS SYSTEMIC LUPUS ERYTHEMATOSUS IN THE MOUSE.
Alfred D. Steinberg*, John D. Mountz*, Michael L. Miller*, Howard R. Smith*, Bonnie J. Steinberg* and J. Frederic Mushinski*, National Institutes of Health, Bethesda, Maryland.

Several inbred strains of mice and their crosses develop autoimmune diseases resembling human systemic lupus. The cellular, genetic, and molecular genetic bases for disease are being studied. In different strains, different cellular abnormalities are critical to disease expression. Single accelerator (lpr, gld, BXSB-Y) genes have been described. However, these often require important "background" genes for full expression of lupus, especially the renal disease. In NZB mice, at least six genes are required for illnes; there are two families of unlinked genes, one underlying stem cell defects and the other leading to anti-DNA and other autoantibodies. We have begun to study the molecular basis for disease. Several oncogenes were studied for abnormal expression. The myb oncogene, which codes for a protein which regulates the expression of other genes, is expressed 30 times higher in lpr/lpr and gld/gld mice than in normals. Myc expression is especially abnormal in B cells of NZB and BXSB mice. All of the mice have increased raf expression. The xid gene has been found to markedly retard or prevent autoimmunity in all of the mice studied. This single gene did not affect myb expression, but did dramatically reduce the expression of the other oncogenes. Therapy with cyclophosphamide has been found to prevent the renal disease of the various mice and to prolong survival. It also favorably influences abnormal gene expression.

THE HUMAN T CELL RECEPTOR. Ellis L. Reinherz,* Dana-Farber Cancer Institute, Boston, MA.

Recent studies using cloned antigen-specific T lymphocytes and monoclonal antibodies directed at their various surface glycoprotein components have led to identification of the human T cell antigen receptor as a surface complex comprised of a clonotypic 90KD Ti heterodimer and the monomorphic 20/25KD T3 molecules. Approximately 30,000-40,000 Ti and T3 molecules exist on the surface of human T lymphocytes. These glycoproteins are acquired and fully expressed during late thymic ontogeny, thus providing the structural basis for immunologic competence. The α and β subunits of Ti bear no precursor-product relationship to one another and are encoded by separate genes. The presence of unique peptides following proteolysis of different Ti molecules isolated by noncrossreactive anticlonotypic monoclonal antibodies supports the notion that variable regions exist within both the α and β subunits. Moreover, N-terminal amino acid sequencing of the Ti β subunit shows that it bears homology to the first V-region framework of immunoglobulin light chains and represents the product of a gene that rearranges specifically in T lymphocytes. The physiology of this receptor complex and its implications for the understanding of autoimmune processes and development of rational therapeutic strategies will be discussed.

ANOXIC INJURY TO THE RENAL TUBULE. J.M. Weinberg, H.D. Humes and D. Hunt*. VA Med. Cent. & Univ. of Michigan, Ann Arbor, MI.

Oxygen deprivation (OD) of isolated rabbit proximal tubules in suspension results in a rapid severe decrease in cellular ATP levels and disruption of monovalent cation homeostasis with loss of intracellular K^+. 60' of oxygenated incubation following OD allows for full recovery of these parameters when the duration of OD is brief but recovery is partial with longer durations of OD as a result of both complete loss of some tubule cells during OD and incomplete recovery of remaining intact tubule cells. The duration of OD required to produce widespread lethal cell injury is 15-30' of hypoxia, but when tubules are subjected to OD under ischemic conditions 60' of OD still produces mainly reversible injury. A fall in pH which occurs during ischemic but not during hypoxic incubation appears to be a major protective factor contributing to this difference. Although interpretation of the data may be complicated by the presence of free mitochondria, when OD produces widespread lethal tubule cell injury a subpopulation of tubules can be identified which do not appear to be lethally injured but which exhibit a rise in total intracellular Ca^{++} that is partly reversible with reoxygenation, suggesting that alterations of plasma membrane Ca^{++} permeability may indeed precede lethal ischemic tubule cell injury. Alterations of tubule cell viability and Ca^{++} homeostasis are accompanied by enhanced arachidonate release from prelabelled tubules. These data thus provide insights into major pathogenetic factors in injury produced by OD and serve as a basis for directly assessing how manipulation of these factors can alter the severity of such injury.

ROLE OF LIPID ALTERATIONS IN MEMBRANE DAMAGE. E. Matthys, J. Kreisberg, Y. Patel, D. Troyer, and M.A. Venkatachalam. UT HSC, San Antonio, Texas.

Cellular energy depletion caused by ischemia/anoxia may lead to imbalance between phospholipid synthesis and breakdown. This can lead to net loss of phospholipid, accumulation of lipid breakdown products and membrane damage. We have characterized these changes during ischemic renal injury. Our results show that ischemia is attended by rapid increases in the renal cortical levels of unesterified fatty acids, diglycerides and lysophospholipids. Accumulation of fatty acids and diglycerides was progressive with time, reaching levels greater than 10X control (at 60' of ischemia) for polyunsaturated fatty acids. These alterations were accompanied by decreases in the mass of phosphatidylcholine and phosphatidylinositol. With blood reflow after a period of ischemia that causes membrane damage, but not cell death, the lipid changes were largely reversed. However, following a duration of ischemia which causes progressive and lethal membrane damage, the lipid alterations persisted during blood reflow. Because fatty acids and lysophospholipids are potentially toxic, their effects on cultured renal cells were tested. At concentrations of 33 μM-165 μM in the incubation medium, both arachidonic acid and lysophosphatidylcholine were lethal. Thus, accumulation of toxic lipid breakdown products is a potential mediator of lethal cell injury. The role of ATP depletion in membrane damage was investigated in cultured LLC-PK_1 cells. The results show that ATP depletion, per se, is a proximate cause of membrane damage, and cell injury, but that reduction of ATP to near zero levels is necessary.

ANOXIC INJURY TO CULTURED RENAL EPITHELIUM CELLS. Patricia D. Wilson. Dept. Med., Univ. Colorado Med. Sch., Denver, CO.

Individually microdissected rabbit proximal tubules of S_1, S_2 and S_3 segments, medullary thick ascending limbs of Henle's loop (MAL) and cortical collecting tubules (CCT) were cultured for 7 days in hormonally defined media and subjected to anoxia by incubation for 45 min at 25°C in a humidified atmosphere at 95% nitrogen. Immediately after this anoxic insult, percentage of viable cells determined by nigrosine dye exclusion was S_1 90±1 = CCT 90±1 > S_2 86±1 > S_3 83±1 = MAL 79±3 ($p<.005$). Transmission electron microscopy showed cells with varying degrees of damage including nuclear chromatin condensation, endoplasmic reticulum dilatation, mitochondrial swelling, cytosolic thinning and plasma membrane breaks. Effects of "reflow" were tested by returning cells to normal media at 37° after the anoxic insult. Reflow for 5 hr in normal calcium-containing media resulted in the death of all cells from each segment. Reflow in media lacking calcium for 2 hr immediately after the anoxic period followed by return to normal calcium-containing media resulted in the survival of some cells for 48 hr: S_3 46±2%, MAL 39±7%, S_1 35±2%, S_2 30±2%, CCT 28±2%. Addition of verapamil (1 μM) to calcium-containing reflow media supported the survival of some cells for 24 hr: CCT 38±5%, S_3 24±6%. It is concluded that cells from different nephron segments have different intrinsic susceptibilities to anoxic death S_3 = MAL > S_2 > S_1 = CCT and the catastrophic effect on cell survival of reoxygenation can be attenuated by restricting calcium in the reflow environment.

ANOXIC INJURY TO THE THICK ASCENDING LIMB.
M. Brezis, S. Rosen, P. Shanley*, P. Silva and F.H. Epstein. Depts. of Med. and Path., Harvard Med. Sch. and Beth Israel Hosp., Boston, MA

Anoxic injury to the medullary thick ascending limb (mTAL) is consistently produced during isolated rat kidney perfusion and associated with its poor concentrating ability. A distinct injury pattern progressing rapidly from mitochondrial swelling to nuclear pyknosis and cytoplasmic disruption leads to cell death as early as 15 min of perfusion. Improved medullary oxygenation by including erythroeytes (or hemoglobin) in the perfusate preserved mTAL structure and function. Decreasing transport in the mTAL (furosemide, ouabain, non-filtering mode) prevents the damage while increasing active transport (polyenes) extends it, demonstrating strong dependency of the injury upon the rate of energy utilization within the cell.

Mitochondrial blockade (rotenone, antimycin, oligomycin) and inhibition of aerobic or anaerobic metabolism, associated with major declines in renal ATP content, do not reproduce the anoxic mTAL lesion, while they induce anoxic-like damage in the proximal tubule (PT). These and other observations suggest distinct modes of anoxic injury for different nephron segments. In the PT, cell injury correlates with ATP depletion. In the mTAL, damage appears strongly conditioned by the rate of energy demand imposed upon mitochondria facing O_2 deprivation.

Differences in functional characteristics along the nephron, such as water permeability, capacity for gel-sol transformation or rate of active transport may underlie these disparate mechanisms for anoxic cell injury.

Ca, P, PTH VITAMIN D AND BONE DISEASE

CONTROL OF HYPERPHOSPHATEMIA WITH ORAL CALCIUM CARBONATE. Jeffery F. Addison*, CPT MC and Charles J. Foulks, MAJ MC. Nephrology Service, Department of Medicine, Brooke Army Medical Center, Fort Sam Houston, Texas.

Aluminum deposition occurs in many organs in patients with ESRD on dialysis and has been closely associated with dialysis dementia, vitamin D resistant osteomalacia, and anemia. Though original reports indicated aluminum induced diseases were associated with elevated aluminum levels in inadequately treated dialysate water; marked elevations in tissue aluminum levels, aluminum toxicity in children, and aluminum associated osteomalacia have been reported in patients on oral aluminum phosphate binders. Since control of hyperphosphatemia in chronic renal failure is important to prevent hyperparathyroidism and continuous oral aluminum intake may be toxic, we converted stable hemodialysis patients from oral aluminum hydroxide to oral calcium carbonate for control of hyperphosphatemia. The results are shown in the table. No adverse effects were seen.

	Al(OH)3	CaCO3	
Ca	9.1±0.2	9.3±0.2	NS
PO4	6.1±0.3	5.4±0.2	NS

n=19

Calcium carbonate is as effective as Al(OH)3 for control of hyperphosphatemia and should be considered to prevent potential aluminum intoxication.

INFLUENCE OF 1,25, $(OH)_2D_3$ ON ALUMINUM ABSORPTION BY RAT DUODENUM. A.J. Adler*, G.M. Berlyne. Brooklyn V.A. Hosp., Brooklyn, NY 11209

Aluminum absorption by the duodenum was studied in vitamin D deficient (N=10) and 1,25, $(OH)_2$ D_3 treated (N=16) male Sprague Dawley rats (100-200g), using in vivo isolated gut segments. Perfusion solutions contained NaCl 150 mM; $CaCl_2$ 0.9 mM; glucose 3.5 mM; ^{3}H-polyethelene glycol 5μCi/L; and increasing concentrations of $AlCl_3$ (0.5 -10mM). Solutions were kept at 37°C and perfused at 0.4 ml/min for 30 min at each Al concentration. Sample were obtained at 10 min intervals.

Aluminum was determined by atomic absorption spectrometry and fluid absorption was assessed from ^{3}H-PEG levels determined by β-scintillation.

Aluminum uptake could be resolved into saturable and non-saturable components. Both groups demonstrated an identical linear non-saturable mechanism with an aluminum uptake of about 20% of the amount perfused per 100 mg dry intestinal weight/hr. Saturable absorption was curvilinear in nature and was significantly lower ($p<.05$) in the vitamin D deficient group (J_{max}=10.0± 1.1 μM/hr/100 mg DW), than in the vitamin D replete group (J_{max}=21.5± 4.0 μM/hr/100 mg DW). These results indicate that aluminum is absorbed in the duodenum by both a concentration dependent non-saturable mechanism and a vitamin D-dependent saturable mechanism.

EFFECTS OF ALUMINUM ON BOVINE PARATHYROID ADENYLATE CYCLASE (AC). E. Bellorin-Font, M. Weaver*, T.J. Stokes*, C. McConkey, Jr*, E. Slatopolsky, and K.J. Martin. Renal Division, Washington Univ. Med. School, St. Louis, MO and Centro Nacional de Dialisis, Caracas, Venezuela.

Recent studies have shown that the secretion of parathyroid hormone (PTH) is often impaired in association with aluminum (Al) accumulation in patients with renal failure. The mechanisms involved remain ill defined. Since AC plays a role in the regulation of PTH secretion, the present studies examine the effects of Al on parathyroid AC. In plasma membranes from normal bovine parathyroids, basal AC activity, in the presence of 0.2 mM ATP, 20 mM Mg^{++}, increased by 40% as Al was raised from 0 to 50 μM. Higher Al concentrations caused a progressive decrease in AC activity, reaching 30% of basal at 2 mM Al. Since AC activation is influenced by the interaction of multiple sites within the AC complex, the nature of the inhibition was explored by examining the interaction of Al with the substrate ATP and the allosteric activating metal ion Mg^{++}. In the presence of 20 mM Mg^{++}, increasing Al from 0 to 2 mM resulted in non-competitive inhibition with respect to ATP (decrease in Vmax from 3296 to 895 pMol cAMP/mg protein/15 min.; Km for ATP was unchanged). In contrast, at fixed ATP (0.2 mM), increasing Al from 0 to 2 mM resulted in mixed inhibition of AC with respect to Mg^{++}. These data suggest that the inhibition of parathyroid AC by Al occurs at the level of the allosteric metal activating site. These data provide a potential mechanism for the inhibition of PTH secretion by aluminum.

BONE EFFECTS OF STREPTOZOTOCIN (SZ) DIABETES

M. M. Beyer, T. A. Einhorn*, A.H. Burstein* and V. J. Devlin*. State Univ. of NY, Downstate Medical Center, Brooklyn, NY

Twenty-five % of new uremic pts. are diabetic (DM), of whom a significant subset develop pathological fractures at a greater rate than non-DM pts. Non-uremic DM pts. have radiologic evidence of diminished bone mass. Animal models of SZ induced DM have shown decreased serum $1,25(OH)_2D_3$, decreased duodenal absorption of Ca, diminished bone turnover, and increased levels of corticosterone. In an attempt to elucidate the contribution of long standing DM to the further loss of bone integrity with uremia, we examined the mechanical properties of intact control and SZ-DM rat femoral bone.

Thirty-one male Lewis rats (mean BW 253±9 gms) were assigned to 2 groups. Six acted as controls and 25 were made hyperglycemic (BG>400 mg/dl) with SZ. All animals received lab chow and H_20 ad. lib. At 3 mos the rats were weighed and killed. The R and L femora were potted in epoxy and "loaded to failure" in a rapid load torsion tester. Data were photographed from an oscilliscope and calculated by digital computer. Deformation, stiffness, torsional strength and energy absorption were determined. The tibiae were measured for changes in length.

When compared to age matched controls, SZ-DM rats had increased BG ($p<0.0005$) and decreased BW ($p<0.0001$). The bones exhibited reduced tibial length ($p<0.0001$), deformation ($p<0.005$), torsional strength ($p<0.0001$), and energy absorption ($p<0.0005$). Stiffness, although reduced, was NS. These findings are similar to those seen in osteoporotic bone. Attention to preservation of bone intergrity in the uremic diabetic will enhance rehabilitation.

NEPHRON SITES OF ENHANCED PHOSPHATE REABSORPTION DURING HYPOCAPNIA. T. J. Berndt* and F. G. Knox, Mayo Medical School, Rochester, MN

Micropuncture of the superficial late proximal (LP) and early distal (ED) tubule was performed to localize the nephron site(s) of enhanced phosphate reabsorption (PR) during hypocapnia in the presence and absence of parathyroid hormone (PTH). All rats were acutely thyroparathyroidectomized and mechanically hyperventilated. Blood P_{CO_2} was controlled by changing the composition of the inspired air. Two groups of rats were studied: 1) Normocapnia + PTH (NC + PTH, n=11), and 2) hypocapnia + PTH (HC + PTH, n=7). After a 2-hour equilibration period, clearance and micropuncture samples were collected. PTH (synthetic 1-34, 33 μ/kg + 1 μ/kg/min) was infused for 1 hour and samples were recollected.

		LP		ED		Urine	
		C	E	C	E	C	E
NC+PTH	FD_p%	25	36*	9	39*	2	37*
	±SE	±3	±4	±4	±7	±0.5	±5
HC+PTH	FD_p%	13§	34*	4	13*§	2	8§
	±SE	±4	±5	±2	±4	±1	±3

*$p<0.05$, paired t; §$p<0.05$, group t;
FD_p, fractional delivery of phosphate

Hypocapnia significantly increased PR along the superficial proximal tubule in the absence of PTH. PTH inhibited PR in the proximal tubule in both normocapnic and hypocapnic rats. However, enhanced phosphate reabsorption between the LP and ED tubule during hypocapnia blunted the phosphaturic effect of PTH.

EFFECTS OF MAGNESIUM DEPLETION ON SKELETAL MUSCLE CELLULAR BIOENERGETICS. N. Brautbar, K. Anderson,* M. Magott,* and S.G. Massry. Div. of Nephrology, Univ. So. Calif. Sch. Med., Los Angeles, CA.

Magnesium (Mg) depletion is associated with skeletal muscle weakness, fasciculations and histological evidence of mitochondrial swelling. We examined the effects of experimental Mg depletion (MgD) on muscle bioenergetics. 150 gram rats were raised on a magnesium deficient diet and muscle mitochondria and myofibrills were isolated. Serum Mg fell from 1.85±0.02 to 0.53±0.08 mg/d in 8 weeks MgD ($p<0.01$). Mitochondrial oxygen consumption fell from 227.0±10.7 to 197.0±9.9 mM O_2/mg prot/min ($p<0.01$) with no change in respiratory control rate and ADP:O ratios. Mitochondrial Mg ATPase activity was not different: 329.0±27.0 in controls vs 330.0±21.0 nmol P_i/mg protein/min in MgD. Mitochondrial creatine phosphokinase CPK activity was reduced significantly, 2.9±0.17 in controls vs 2.47±0.07 IU/mg protein/minute in MgD ($p<0.01$). Myofibrillar Ca ATPase and CPK activity were reduced significantly: 375.0±20.0 in controls vs 215.0±20.0 nmol P_i/mg protein/minute in MgD ($p<0.01$) and 1.97±0.1 in controls to 1.20±0.10 IU/mg protein/minute in LMg ($p<0.01$), respectively. Tissue Mg ash content was also reduced, 134.0±5.0 in controls vs 118.0±1.5 mg/mg dry weight in MgD ($p<0.05$). These data show that MgD is associated with: 1) impaired mitochondrial energy production; 2) reduced mitochondrial energy transport; 3) impaired energy utilization. The impaired cellular energy metabolism secondary to the reduced activity of the key enzymes regulating cellular bioenergetics may mediate muscle dysfunction in Mg depletion.

Na UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES (BBMV) AND Na-K ATPASE ACTIVITY ALONG THE NEPHRON IN HYPOPHOSPHATEMIC (HYP) MICE. Michèle G. Brunette, Ghazi El Mernissi* and Alain Doucet*. Maisonneuve Hospital, Montreal, Canada; Collège de France, Paris, France*.

To investigate the possible role of a sodium (Na) transport defect in the pathogenesis of phosphaturia in Hyp mice, we studied the Na uptake by BBMV, the interrelationship between phosphate (PO_4) and Na uptake, and the Na-K ATPase activity in microdissected segments of the nephron, in Hyp and normal (N) mice.

With 20-25-50-80-100 mM Na in the incubation medium, initial PO_4 uptake by BBM vesicles from Hyp mice was half that of normal. Increasing Na in the incubation medium similarly increased Vmax and decreased Km values of PO_4 uptake in the two series of animals and for a given concentration of Na in the medium, Km of the PO_4 uptake was the same in N and Hyp mice.

In contrast to the defect in PO_4 uptake, Hyp mice did not show any abnormality in Na transport. In both series of animals, Na uptake similarly increased with Na concentration in the medium, without reaching a maximal value. Addition of 5 mM PO_4 did not significantly enhance Na uptake.

The Na-K ATPase activity in microdissected nephrons was similar in Hyp and N mice in all the nephron segments except in the granular part of the distal tubule, where the enzyme activity was twice as active in Hyp as in N mice (3908 ± 576 vs 1529 ± 409 pmol $mm^{-1}h^{-1}$). No significant difference was found in the PCT.

In conclusion: in Hyp mice 1) The defect in PO_4 uptake by the BBMV is not due to an abnormal Na transport nor Na-PO_4 relationship 2) the Na-K ATPase activity in PCT is normal 3) For unexplained reasons, Na-K ATPase is enhanced in DCTg.

HIGH SPATIAL RESOLUTION VISUALIZATION OF SODIUM, POTASSIUM AND CALCIUM ON THE SURFACE OF NEONATAL MOUSE CALVARIAE: EFFECTS OF REDUCED MEDIUM PH. DA Bushinsky, G Crow*, S Deganello*, R Levi-Setti*, Y Wang*, FL Coe. Univ. of Chicago, Chicago, IL.

Surface elemental maps of cultured, lyophilized calvariae are obtained using a new high resolution Ga+ scanning ion microprobe by secondary ion mass spectrometry (SIMS). To determine the effect of mild metabolic acidosis on surface calcium we cultured 4 day old mouse calvariae for 3 hr in control (pH = 7.47) or acid (pH = 7.19) medium. With control there is no net calcium flux into the medium. This calvarial surface contains regions that are rich in sodium 23 (Na) and potassium 39 (K) atoms relative to calcium 40 (Ca); corrected relative ion yields (CRIY) of Na, K and Ca = 10, 4.1 and 0.5 respectively. Deliberate erosion of approximately 50 superficial atomic layers with the Ga+ beam increased Ca relative to Na and K (CRIY of Na, K and Ca = 10, 6.9 and 3.5 respectively). The gradual increase of Ca is visible on SIMS maps that resemble scanning electron microscopic images except they are specific for a given isotope. Analysis of the calvarial cross section revealed an abundance of Ca relative to Na and K (CRIY of Na, K and Ca = 10, 6.6 and 107 respectively). With acid treatment calvariae lost 89 nM/bone/3hr of Ca into the medium. Surface Ca of these calvariae is greater than controls relative to Na and K (CRIY of Na, K and Ca = 10, 3.1 and 4.0 respectively). Ga+ beam erosion enhanced surface Ca (CRIY of Na, K and Ca = 10, 9.4 and 11 respectively). Neonatal mouse calvariae have at least some of their surface covered by Na and K that overlies Ca. Brief exposure to acid medium seems to expose this calcium for release into the medium.

EFFECT OF RESPIRATORY ACIDOSIS ON BONE COMPOSITION J.M.Burnell,E.Teubner*,M.Bodvarsson*,V.J.Canzanello J.A.Kraut,C.A.Johns*, and N.E.Madias. Univ. of WA. Seattle, WA and Tufts-N.Engl. Med. Ctr.,Boston,MA.

Chronic metabolic acidosis is associated with decreased bone mineral content, CO_3/Ca and Na/Ca. To examine the effect of chronic respiratory acidosis on bone, we studied bone composition (iliac crest biopsies) in 10 dogs, on a normal controlled diet, before and after 10 wks of hypercapnia produced by exposure in an environmental chamber to 10% $FiCO_2$. Plasma changes were:

	pH	P_{CO_2} mmHg	HCO_3 mEq/L	$[Ca^{++}]$ mEq/L
Pre	7.38	36	21	1.32
Post	7.26	87	38.6	1.25
	p< .001	<.001	<.001	<.05

Serum iPTH, Ca, Mg, Na, & P were unchanged. Changes in iliac crest composition were:

	Density $\frac{g.bone}{cm^3 bone}$	%Min. $\frac{g.min}{g.bone}$	Porosity $\frac{cm^3 bone}{cm^3 core}$	CO_3/Ca mEq	Na/Ca mEq	Mg/Ca mEq
Pre	1.80	54.7	29.6	.129	.0305	.0268
Post	1.87	57.2	37.6	.153	.0276	.0239
	p< .005	<.02	<.005	<.001	<.001	<.001

While the bone carbonate change adds emphasis to the equilibrium between solution ions and hydroxyapatite,the increase in mineral content and trabecular bone mass cannot be explained without postulating stimulation of net mineralization and trabecular bone formation by bone cells under the influence of severe chronic hypercapnia. Thus the bone response to respiratory acidosis was opposite to that of metabolic acidosis except for the unexplained mineral Na loss.

CHRONIC RESPIRATORY ACIDOSIS AUGMENTS URINARY CALCIUM EXCRETION. V.J. Canzanello*, M. Bodvarsson*, J.A. Kraut, C.A. Johns*, E. Slatopolsky, and N.E. Madias. Tufts-N. Engl. Med. Ctr., Boston, MA, UCLA-VA Wadsworth Med. Ctr., Los Angeles, CA, and Washington Univ., St. Louis, MO.

Chronic metabolic acidosis is associated with hypercalciuria. By contrast, the effect of respiratory acidosis on urinary calcium excretion remains unclear. We, therefore, examined the effect of prolonged hypercapnia on calcium metabolism.

Chronic hypercapnia was induced in 10 dogs by exposure to a 10% F_ICO_2 in an environmental chamber for 10 weeks. Animals were fed a diet containing $CaCO_3$ (7.5 meq/Kg) and normal quantities of sodium and potassium.

During chronic respiratory acidosis mean blood pH ranged from 7.24 to 7.28 and mean $PaCO_2$ from 82 to 93 mm Hg. By day 2, urinary calcium excretion increased, reached a peak by week 4 and remained elevated thereafter (Control 1.8 mg/Kg/day, Day 2 3.1*, Day 3 3.9*, Week 1 2.6*, Week 3 2.9*, Week 4 4.2*, Week 6 4.8*, Week 10 4.5*, *p<0.05 vs. Control). Hypercalciuria occurred even though filtered load of calcium fell. Consequently, fractional excretion of calcium rose (Control 0.61%, Week 4 2.60%*, Week 10 2.61%*). Urinary excretions of sodium, phosphate and magnesium did not change, but significant bicarbonaturia occurred (Control 0.7 meq/Kg/day, Week 1 1.1*, Week 4 1.5*, Week 10 1.4*). Serum ionized calcium fell slightly but no changes in iPTH were noted.

These data suggest that respiratory acidosis, just like metabolic acidosis, augments urinary calcium excretion by depressing the tubular reabsorption of calcium.

INHIBITION OF PHOSPHATE (Pi) TRANSPORT BY PARATHYROID HORMONE (PTH) IN THE OPOSSUM KIDNEY (OK) CELL LINE. Joseph Caverzasio* and Jean-Philippe Bonjour, (intr. by V. Dennis), Univ. of Geneva, Div. of Pathophysiology, Dept of Med., Geneva (Switzerland)

In the present study, we have investigated the characteristics of Pi transport in confluent OK cells which have been shown to possess PTH receptors. A Na^+-dependent Pi transport (NaPiT) has been identified with Km = 93,2 ± 16,2 μM, Vmax = 4,14 ± 0,55 nmol/mg prot · 2 min^{-1}. Preincubation with PTH for 1 h. at $10^{-7}M$ inhibited NaPiT from 2,76 ± 0,11 to 1,08 ± 0,1 nmol/mg prot.·2 min^{-1}, $p < 0,001$. The inhibitory effect of bPTH was detectable at $10^{-9}M$ and was already expressed 5 min. after hormonal exposure at $10^{-7}M$. It was associated with a 40 fold increase in cAMP and was not suppressed by a 90 min. preincubation with cycloheximide at 70 μmol/l. It corresponded to a decrease in Vmax (from 4,14 ± 0,55 to 2,41 ± 2.24 nmol/mg prot · 2 min^{-1}) with no change in Km (0,093 ± 0,016 vs 0,094 ± 0,012 μM). bPTH at $10^{-7}M$ did not inhibit the uptake of methylglucopyranoside, a glucoside which is transported in OK cells also by a Na^+-dependent process. In conclusion OK cells possess a NaPiT system which is selectively inhibited by factors such as PTH and forskolin which increase cyclic AMP generation.

THE EFFECTS OF $1,25(OH)_2D_3$ ON PTH SECRETION BY MONOLAYER CULTURES OF BOVINE PARATHYROID (PT) CELLS. W Chan,* C McKay,* E Dye,* and E Slatopolsky, Department of Medicine, Washington University, St. Louis, MO.

Controversy exists regarding a direct effect of $1,25(OH)_2D_3$ on PTH secretion. We showed previously, in short term studies (4 hr), that $1,25(OH)_2D_3$ did not suppress PTH secretion by bovine PT cells in vitro. However, we recently found that I.V. $1,25(OH)_2D_3$ given thrice weekly to 20 patients on dialysis, caused a fall in serum i-PTH before detectable increases in serum Ca. Therefore, the suppressive effect of $1,25(OH)_2D_3$ on PTH secretion may only be demonstrable in $1,25(OH)_2D_3$ depleted tissue and/or after longer periods of exposure to $1,25(OH)_2D_3$. To investigate this hypothesis, primary monolayer cultures of bovine PT cells was established in 1:1 DMEM/HAMS F-12 media supplemented with 2% calf serum, insulin, hydrocortisone and transferrin but not $1,25(OH)_2D_3$. I Ca was maintained at 1.0 mM. Confluency of the cultured cells was usually achieved after 5 days. PTH secretion was measured before, 4 and 48 hours after adding $1,25(OH)_2D_3$, using both a mid-region/carboxy and an amino-terminal PTH antisera. $1,25(OH)_2D_3$ at concentration of 0.1 ng/ml had no effect on PTH secretion at 4 hours but suppressed PTH secretion by 33 ± 6% after 48 hours. This effect was not obviated by low calcium (0.5mM) in the media. High calcium (2.0 mM) suppressed PTH secretion by 45 ± 7% and this effect was not additive over that of $1,25(OH)_2D_3$. PTH secretion rate recovered fully 48 hours after removal of the influence of high Ca but not after the removal of $1,25(OH)_2D_3$. We conclude that $1,25(OH)_2D_3$ directly suppresses PTH secretion by monolayer culture of bovine PT cells.

ALUMINUM EXCRETION IN RATS RECEIVING VITAMIN D, DIHYDROTACHYSTEROL (DHT), 1,25-DIHYDROXYVITAMIN-D (1,25-D) AND ALUMINUM HYDROXIDE. James C M Chan, Mary Jacob, Sue Brown, John Savory, Michael R Wills. Med Coll Virg, Richmond, VA; Cal State Univ, Long Beach, CA and Univ Virginia, Charlottesville, VA.

In order to study the effects of vitamin D on aluminum excretion when different forms of vitamin D and phosphate-binders are used simultaneously for therapeutic purposes, 30 Sprague-Dawley weanling rats, weighing 44 to 62 gm, were randomly assigned to five groups: (A) control, (B) aluminum hydroxide, (C) DHT at 16 mcg/kg/day, (D) 1,25-D at 16 ng/kg/day and (E) vitamin D2 at 2,000 IU/kg/day. Aluminum hydroxide (60 mg/kg/day) in the feed was provided to all except the control group. The vitamin D or metabolites were fed by stomach tube daily for a period of 10 days. At the end of the study, mean ± SEM of serum aluminum (5.0±0.5 mcg/l) concentrations as determined by flameless atomic absorption spectrophotometry, did not reach significant differences between any of the groups. During the last three days of the study, 24-hour urine collections were made with the usual precautions against trace mineral contamination. The means ± SEM of aluminum excretion (mcg/100 gram body weight/day) were:

Group A	B	C	D	E
0.75	1.99	1.70	1.47	2.17
±0.18	±0.85	±0.51	±0.27	±0.65

Thus, urinary aluminum excretions doubled with treatment but were not significantly different between treated groups compared to control except between groups A and E, ($p < 0.05$). We conclude that at therapeutic doses of aluminum hydroxide and vitamin D or metabolites, hyperaluminemia was not observed, and urinary aluminum excretions were not significantly different between treated groups.

THE PATHOGENESIS OF HYPERCALCIURIA IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). P Chen*, C Hsu, and D. Smith*. Dept. of Medicine and Col. of Pharmacy, Univ. of Michigan, Ann Arbor, MI.

The etiology of hypercalciuria remains unknown in SHR. In order to differentiate absorptive versus renal hypercalciuria, serial measurements of 4 h urinary calcium excretion ($U_{Ca}V$) were made weekly under fasting and after gavage of $CaCl_2$ (50mg/100g) from age 8 to 14 wks in SHR (N=14) and normotensive Wistar Kyoto rats (WKY) (N=14). Fasting $U_{Ca}V$ were significantly greater in WKY than in SHR. However, after oral Ca loading $U_{Ca}V$ were greater in SHR after 13 wks of age (13wk:SHR, $U_{Ca}V$=954ug/mg creatinine; WKY, $U_{Ca}V$=541 ,$p < 0.01$. 14wk:SHR, $U_{Ca}V$=988; WKY, $U_{Ca}V$=534, $p < 0.01$). Ca disposition kinetic studies were performed at age 16 and 17 wks. No significant difference of i.v. Ca^{45} was observed between WKY (N=6) and SHR (N=6) in total plasma clearance, non-renal clearance, biologic half-life, and elimination rate constant from the central compartment. The renal clearance of oral Ca^{45} was, however, substantially greater in SHR (2.36 ± 0.27ml/h, N=8) than in WKY (1.18 ± 0.16, N=8, $p < 0.01$). Calculated fractional absorption of Ca of SHR was 17% greater than that of WKY. Hypercalciuria persisted after oral Ca loading in SHR despite chronic normalization of blood pressure. P content of the kidney, liver, muscle and bone as well as Ca content of the bone were not different between WKY and SHR. Urinary cyclic AMP excretions of SHR, but not WKY, were decreased after Ca loading when compared to the fasting values. Conclusions: 1) Hypercalciuria of SHR is of absorptive origin; 2) Cyclic-AMP excretion is decreased after $CaCl_2$ in SHR but not in WKY. 3) Hypercalciuria is not associated with phosphate depletion. 4) Normalization of hypertension does not abolish the hypercalciuria.

ROLE OF PHOSPHOLIPIDS AND FATTY ACIDS IN THE MODULATION OF THE 1,25-DIHYDROXYVITAMIN D_3 RECEPTOR. T.C. Chen, J. P. Mullen*, N.J. Meglin*, and J. B. Puschett, Renal-Electrolyte Division, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA.

In a previous study, we have documented that the specific binding of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) to its receptor obtained from rat kidney was inhibited differentially by phospho - lipids (PL) with differing head group. In the present study, the mechanism of PL inhibition between PL with defined fatty acid (FA) compositions and their respective capacities to inhibit the specific binding of [^{3}H]-$1,25(OH)_2D_3$ was examined. The results indicate that (1) 3-sn-phosphatidylcholine (PC) containing one or two unsaturated FA have higher inhibitory activity than those with two saturated FA, (2) the unsaturated FA themselves are potent inhibitors,whereas their methyl esters are essentially inactive, and (3) kinetic and Scatchard analysis reveal a pattern of noncompetitive inhibition and a decrease in total available hormone binding sites. These findings suggest that (a) PL or free FA may bind at a site different from the $1,25(OH)_2D_3$ binding site and therefore inhibit the hormone binding via a noncompetitive conformational change in the receptor molecule, (b) the PL-FA binding site on the receptor molecule may consist of a region lined with nonpolar amino acid side chains which recognize and interact with the hydrocarbon chain of FA and a positive charged residue which is responsible for an electrostative interaction with the carboxylate anion of FA or the phosphate anion of phospholipids, and (c) PL and FA may play an important role in the structure and function of the 1,25 $(OH)_2D_3$ receptor in rat kidney.

DISTINCT SITES OF ACTION OF CHLOROTHIAZIDE (CTZ) AND AMILORIDE (AM) IN RAT DISTAL CONVOLUTED TUBULE (DCT). Linda S. Costanzo. Medical College of Va., Richmond, Va.

Experiments were performed in rats to examine the distal site of action of thiazide diuretics and the additive hypocalciuric properties of CTZ and AM. In clearance experiments, the maximal natriuretic and hypocalciuric dose of CTZ was established (2mg/kg; 0.25 mg/kg.min). When AM was added, there was further augmentation of Ca reabsorption ($p < 0.025$), but no additional natriuresis. AM blunted CTZ-induced kaliuresis ($p < 0.001$).

Localization of the CTZ effect was studied in 8 early and 7 late DCT, microperfused in vivo with control and CTZ-containing solutions. The maximally effective luminal drug concentration, 5×10^{-4}M, decreased Na transport from 131±12 to 89±10 pmole/min.mm ($p < 0.001$) and increased Ca transport from 3.24±0.29 to 4.00±0.18 pmole/min.mm ($p < 0.01$) in early DCT; late DCT were, on average, unaffected by CTZ. It is suggested that thiazides interact with the distal convoluted tubule cell whose predominant location is the early DCT. In two additional long DCT, with early and late segments, the maximal CTZ concentration increased Ca transport and decreased Na transport in both tubules. Addition of 10^{-5}M AM caused additional increments in Ca reabsorption with no further decline in Na transport. We conclude: (1) CTZ and AM have distinct sites of action in the rat DCT, CTZ acting in the early and AM in the late portion, consistent with DCT heterogeneity. (2) Long DCT having early and late segments, have a hypocalciuric response to both diuretics. (3) A basis for additive hypocalciuric actions of CTZ and AM is different sites of action within the DCT.

MANAGEMENT OF POST-PARATHYROIDECTOMY (PTX) HYPOCALCEMIA IN HEMODIALYSIS (HD) PATIENTS: USEFULNESS OF 1,25 diOH VITAMIN D3 (CALCITRIOL). F. Clair*, L. Leenhardt*, D. Robert*, J. Zingraff*, C. Dubost* and T. Drüeke* (intr. by D.A. McCarron). Hôpitaux Necker and F. Widal, Paris, France.

Severe, prolonged hypocalcemia is observed in some, but not all, HD patients after (PTX) performed because of uncontrolled hyperparathyroidism. The aim of the present study was to investigate whether calcitriol and Ca supplementation in the immediate post-PTX period was of more help in the control of plasma Ca than Ca supplementation alone. Forteen HD patients were enrolled in a prospective, randomized, double blind and placebo controlled study. From the day after PTX seven patients received calcitriol and the remaining seven a placebo using incremental doses adjusted to the degree of hypocalcemia (up to 4 µg/day for calcitriol). Pre-PTX plasma Ca, P, alkaline phosphatases and iPTH were comparable in both patient groups, as was the lowest plasma Ca achieved in the post-PTX period. The mean decrement of post-PTX (days 3 through 9) as compared to pre-PTX plasma Ca was however less pronounced in calcitriol treated than in placebo treated patients (0.25±0.06 SEM vs 0.45±0.05 mM, $p<0.025$). Moreover, treatment with placebo was interrupted before day 14 because of persistent severe hypocalcemia in 4/7 patients whereas calcitriol treatment was continued in all 7 patients up to 14 days, i.e. the end-point of study. Finally, patients on calcitriol treatment needed less mean Ca supplements (days 1 through 9) than patients receiving placebo (37.4±3.2 vs 49.4±3.7 g, $p<0.02$).

In conclusion, oral calcitriol when given to dialysis patients during the immediate post-PTX period is associated with a lesser degree of hypocalcemia and reduced need for Ca supplements.

CORRELATION OF CYTOSOLIC pH AND PHOSPHATE TO NH_4Cl INDUCED PHOSPHATURIA IN VIVO. L.D. Cowgill and R.T. Bogusky. School of Vet. Med. and School of Med., Univ. of Calif. at Davis.

Metabolic acidosis promotes renal phosphate (P) excretion, but associative changes in cytosolic P metabolism and pH are incompletely understood. We studied renal P excretion, cytosolic P and ATP metabolism and cytosolic pH with sequential ^{31}P-NMR spectroscopy of the exposed mouse kidney in vivo. After one hour of control acquisitions, one group of mice (MA) were given 10-15 mmol/kg BW 2M NH_4Cl in 2 or 3 hourly doses by gastric gavage. A second group (C) was given a similar dose of 2M NH_4HCO_3. NH_4Cl decreased arterial pH from 7.33 ± 0.02 (SEM) to 6.92 ± 0.06 ($p<.001$) and plasma HCO_3^- from 17.9 ± .8 to 7.15 ± 1.5 ($p<.001$). In MA, cytosolic pH fell progressively from 7.38 ± .02 to 7.15 ± .03 ($p<.001$) by the 5th hour; in C pH fell slightly but insignificantly (7.36 ± .06 to 7.25 ± .02). Renal P excretion increased 1.9-fold by the 5th hour in MA, but was unchanged in C. ATP did not change from the control period during 5 hours of acquisition in either group. In MA, cytosolic P fell to 88.45 ± 3.21% of control ($p<.025$) in the first hour of NH_4Cl administration and declined further to 76.41 ± 3.21% ($p<.01$) by 5 hours. In C, cytosolic P was 103.15 ± 3.06% and 103.57 ± 13.7% of control in hours 1 and 5, respectively. We conclude NH_4Cl causes a prompt acidification of the cytosol which parallels the changes in arterial pH. Systemic acidosis promotes an NMR-detectable increase in renal P excretion. The decrement in cytoslic P and pH correlate with and may participate in the phosphaturic response.

CHANGES IN RENAL PHOSPHATE EXCRETION AND CYTOSOLIC METABOLISM IN RESPONSE TO ACUTE PHOSPHATE LOADING: ^{31}P-NMR STUDY IN VIVO. L.D. Cowgill, R.T. Bogusky, and K.M. Neu.* School of Vet. Med. and School of Med., Univ. of Calif. at Davis.

Dietary phosphate (P) modulates the phosphaturic response to an acute P load, but changes in cytosolic P metabolism which may accompany the response are unknown. We examined cellular metabolism in vivo in exposed kidneys of intact male mice using ^{31}P-NMR spectroscopy. Mice were fed a control diet (A) or a P-deficient diet for either 7-10 days (B) or 25-45 days (C) before the study. Following 1 hour of control acquisitions, neutral P was given orally at 1 mmol/gm BW/hr for 4-6 hours. During the loading period spectra were continuously acquired. Relative changes in pH and P metabolites were gathered from the serial spectra. Plasma P increased from 7.63 ± 0.94 (SEM) to 11.0 ± 2.0 in A; 5.9 ± 0.77 to 12.8 ± 0.99 in B, and 4.4 ± 0.7 to 20.13 ± 3.67 in C after 5 hours. P excretion rose 2-fold in A; 2.5-fold in B but was unchanged in C. Cytosolic pH was identical in each group before loading but fell from 7.351 ± .028 to 7.204 ± .021 ($p<.025$) in C after 5 hours. pH was unchanged in A and B. ATP content was unchanged in A and B but increased 17% in C ($p<.05$) with P loading. Concomitant with the phosphaturia, cytosolic P decreased 23% ($p<.05$) in A and 18% ($p<.05$) in B but was unchanged in C which lacked a phosphaturic response. We conclude the phosphaturia in response to acute P loading persists in intact mice with short-term P deprivation but is abolished in chronically deprived mice. Changes in P metabolism and pH accompany the altered responsiveness to chronic P deprivation. Decreases in cytosolic P may participate in the phosphaturic response to acute P loading.

HYPOCALCEMIA IN CRITICALLY ILL PATIENTS. Tusar K. Desai, Marilyn T. Haupt, Richard W. Carlson, Michael A. Geheb, (Intr. F. D. McDonald), Detroit Receiving Hospital, Wayne State University, Detroit, Michigan.

We evaluated the incidence of low ionized calcium (iCa) levels in critically ill patients admitted to the MICU. Serum iCa was measured in 88 of 108 consecutive admissions. iCa (1.10±.09 mMol/L; $\bar{x}$±SD) was decreased as compared to a group of normals (1.24±.03, n=23). In 62 (70%), iCa was 2 SD below the normal value (1.04±.09).

Hospital mortality was significantly greater (27/62, 44%) in the hypocalcemic group compared to the normocalcemic group (4/23, 17%)($p<.05$). The potential etiologies of hypocalcemia were evaluated.

Etiology	n	Etiology	n
(1) Hypomagnesemia	17	(5) Pancreatitis	2
(2) Renal Insufficiency	4	(6) Unexplained	32
(3) Hyperphosphatemia	3	(7) Normocalcemia	23
(4) Alkalosis	4	(8) Hypercalcemia	3

In Groups (6) and (7), serum PO_4, Mg, and creatinine were no different, however, there was a significant correlation between iCa and albumin ($r=+0.41$, $n=41$, $p<.01$). In 17 patients, the hemodynamic response to calcium infusion was evaluated. A significant vasopressor and positive inotropic response was seen.

Conclusion: 1) Hypocalcemia is common in the critically ill and portends a poor prognosis. 2) In almost half of the patients, the etiology is unclear, however, the association between iCa and albumin suggests that malnutrition may have a role. (3) Correction of the hypocalcemia leads to a positive inotropic and vasopressor response which may alter patient outcome.

THE INFLUENCE OF CYCLIC ADENOSINE - 3',5'-MONOPHOSPHATE (cAMP) ON AMINO ACID ACCUMULATION BY RAT RENAL BRUSH BORDER MEMBRANE VESSICLES (BBMV). Shermine Dabbagh, Russell W. Chesney, Naomi Gusowski, Univ. of Wisconsin, Dept. of Pediatrics, Madison, WI.

Aminoaciduria in vitamin D deficiency has been ascribed to the influence of PTH on the renal tubule. Rats placed on a low Ca, vitamin D-deficient diet for 4-6 weeks had a significant ($p<.01$) rise in urinary taurine 63.3 ± 36.8 µmoles/mg Cr (SD) vs 17.7 ± 3.9 (nl), in serum PTH (160.3 ± 24.1 µL Eq/ml vs 20.5 ± 12.1) and in urinary cAMP (70.1 ± 56.2 µmoles/gCr vs 30.3 ± 21.4). We explored the possibility that cAMP could influence the uptake of the amino acid, taurine, by BBMV prepared from normal rats, not chronically exposed to PTH or cAMP. Direct exposure BBMV with dibutyryl cAMP did not influence taurine uptake at 10^{-7} to 10^{-3} M in BBMV. Non-physiologic, hyperosmolar levels of cAMP (10^{-2} M) reduced uptake. Preincubation with cAMP for times between 0 and 60 min. and at 4° to 23° did not change uptake. Hypotonic lysis of BBMV, permitting entry of cAMP, followed by isotonic resealing of BBMV and stimulation with KF did not influence taurine uptake. Incubation in the presence of 10^{-5} M cAMP, 10^{-3} mM ATP, 10 mM KF resulted in an uptake of 30.5 ± 5.4 p moles/mg protein/60 sec vs. 29.07 ± 7.2 in lysed and resealed control BBMV. All combinations of cAMP, ATP, KF and lysis did not alter uptake. These data indicate that aminoaciduria in vitamin D is unlikely to be related to a PTH-induced increase in intracellular cAMP or in cAMP at the urinary surface since debutyryl cAMP does not influence the handling of the aminoacid taurine at the renal brush border surface.

PTH, cAMP AND SODIUM TRANSPORT IN RABBIT PROXIMAL TUBULES: ROLE OF TRANSMEMBRANE FLUX AND CYTOSOLIC CONCENTRATION OF CALCIUM. G.M. Dolson, M.K. Hise, E.J. Weinman. Dept. Medicine, University of Texas Medical School, Houston, Texas.

PTH and cAMP decrease sodium transport in the proximal tubule by inhibiting sodium-hydrogen exchange in the brush border membrane. To determine if an alteration in flux or cytosolic concentration of calcium is required for PTH or cAMP to express this effect, oxygen consumption was measured in an enriched suspension of proximal tubules and the oubain sensitive component of O_2 consumption taken as an index of sodium transport. PTH (1IU/ml) and cAMP 10^{-4}M) decreased O_2 consumption by 33.4 + 3.5% (n=10) and 29.2 ± 3.7 (n=12) respectively ($p<0.005$ versus control for both agents). Neither PTH or cAMP effected O_2 consumption in tubules pretreated with oubain or nystatin. In the presence of lanthanum (5 to 50µM), PTH and cAMP inhibited O_2 consumption by 22.5 ± 3.4% (n=7) and 27.6 ± 7.1 respectively. In the presence of verapamil (2 to 200µM), PTH and cAMP inhibited by 33.9 ± 5.5% and 35.4 ± 3.1.

Cytosolic calcium concentrations averaged 44.6 ± 4.9 nM (n=12) as determined by Quin2 fluorescence. PTH decreased cytosolic calcium concentrations from 53.9 ± 5.4 nM in control periods to 47.0 ± 4.9 (n=7, $p<0.01$). cAMP had no effect in the cytosolic concentration of calcium.

These studies demonstrate that in the rabbit proximal tubule, PTH and cAMP specifically inhibit sodium transport and that this inhibitory effect does not require an influx of calcium into the cell. Determination of cytosolic calcium concentrations by Quin2 fluorscence indicates that PTH decreases the cytosolic concentrations of calcium.

THE INTRACELLULAR MESSENGER OF ANGIOTENSIN II ACTION ON TRANSPORT IN RABBIT PROXIMAL TUBULES. J.H. Dominguez, A.B. Borle,* T. Brown* and K.W. Snowdowne.* Depts. of Med. & Physiol., School of Medicine, Univ. of Pittsburgh, Pittsburgh, PA.

These studies were conducted to determine if cAMP or cytosolic free calcium [Ca_i] were the intracellular messengers of the actions of angiotensin II (AII) on proximal tubular transport. We measured the effects of AII on fluid absorption (Jv) and lumen to bath phosphate transport (Jl-b PO_4) in the rabbit proximal convoluted (PCT) and straight (PST) tubules. We also studied the effects of AII on adenylate cyclase (AC) activity in the PST and on Ca_i in suspensions of rabbit proximal tubules (RPT). In the PCT (n=8), 10^{-10}M AII did not change Jv from control rates, 0.96±0.08 (S.E.) nl/mm/min, but 10^{-8}M and 10^{-6}M AII decreased Jv by 18% and 25% (P<0.05 for both) respectively. Control Jl-b PO_4, 6.02±1.33 pmol/mm/min, was not affected by AII. In the PST (n=11), 10^{-10}M AII did not alter Jv (control=0.39±0.03) but at 10^{-8}M and 10^{-6}M, AII decreased it by 21% and 23% respectively (P<0.02 for both). Control Jl-b PO_4, 2.14±0.25, was not affected by AII. In the PST, AII did not change the activity of AC. In suspensions of purified RPT loaded with aequorin by hypotonic shock treatment (Science 217:252, 1982) AII 10^{-8} and 10^{-6}M increased the Ca_i to 312±64 nM (n=11) and 460±58 (n=9) respectively. There was a decreased responsiveness of the cells to repeated administration of 10^{-6}M AII (-52±5%; n=4; P<0.05). We conclude that in RPT the effects of AII are mediated by Ca_i and not cAMP. The stimulation of this system by AII does not appear to change the transport rates of phosphate.

ACUTE ALUMINUM INTOXICATION IN THE RAT PRODUCES OSTEOMALACIA. A.J. Felsenfeld, M. Rodriquez*, and F. Llach. Dept. of Med., Univ of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

Chronic aluminum (AL) toxicity has been shown to produce osteomalacia in the rat. The present study assesses the effect of acute AL intoxication in rats with chronic renal failure. Ten mgs of AL were administered intraperitoneally for 2 consecutive days. Of 14 rats, 4 died within 4 days; the other 10 were sacrificed 25 to 27 days after AL exposure. Compared to control rats, levels of creatinine, calcium, phosphate, and parathyroid hormone were not different, but mean plasma (334±168 vs 43±3 µg/l; p<.005) and bone (104±53 vs 14±3 µg/g; p<.005) AL concentrations were increased in rats receiving AL. In the AL group, a direct correlation was observed between bone and plasma AL (r=.89, p<.001). In the 10 surviving rats in the AL group, bone formation as determined by double tetracycline labeling was absent in 5 and subnormal in 2. In rats receiving AL, relative osteoid volume was increased (21±18 vs 4±2%, p<.05), and an inverse correlation was present between osteoblastic osteoid and both plasma AL (r=-.90, p<.001) and bone AL (r=-.84, p<.001).

In summary, in 14 rats with chronic renal failure exposed to high dose AL for 2 consecutive days: 1) death occurred in 4 within 4 days; 2) a direct correlation was present between plasma and bone AL; 3) bone formation was decreased; and 4) an inverse correlation was present between osteoblastic osteoid and both plasma and bone AL. In conclusion: 1) an acute but heavy exposure to AL can produce osteomalacia; 2) an exchangeable bone-plasma AL pool appears to be present; and 3) AL may be toxic to the osteoblast.

LOCALIZATION OF ALUMINUM IN BONE DETERMINES THE PATHOLOGIC EFFECTS ASSOCIATED WITH ACCUMULATION OF ALUMINUM IN BONE IN HEMODIALYZED PATIENTS. MC Faugere*, K Abreo, HH Malluche, Univ. of KY, Div. of Nephrology, Bone & Min. Metab., Lexington, KY.

We reported bone aluminum accumulation (BAA) in 40-50% of chronically dialyzed patients (pts). BAA can be documented by measurement of aluminum content of bone (BAC) or by histochemical staining for aluminum (Al) at the mineralization front (sBA). We studied effects of BAA on quantitative histology in 55 hemodialyzed pts and compared predictive value of BAC vs. sBA for these effects. sBA was found in 47% and BAC was high in 55% of the pts. Pts. with sBA had increased volume of lamellar osteoid (16 ± 2.2 vs. 5.2 ± 1%), less volume of woven osteoid (5 ± 1 vs. 8 ± 1%), lower osteoblastic and osteoclastic indices (370 ± 72 vs. 1173 ± 173 and 89 ± 17 vs. 231 ± 27), less doubly labeled osteoid seams (5.8 ± 1.8 vs. 27 ± 4.3%), lower mineralization rate (0.5 ± 0.07 vs. 0.8 ± 0.07 um/d) and lower bone formation rate (10 ± 3.9 vs. 38 ± 7.8 mm^3/cm^3/year x 10^3). sBA correlated with volume and thickness of lamellar osteoid (r: 0.7) and with osteoblastic and osteoclastic parameters (r: -0.7 to -0.8), mineralization rate (r: -0.8) and bone formation rate (r: -0.7). In contrast, pts with high BAC had only an increase in volume of lamellar osteoid (15.5 ± 3.8 vs. 6.2 ± 1.5%), and BAC correlated only with volume of lamellar osteoid (r: 0.7).

These findings allow to conclude that (1) BAA is associated with abnormal mineralization and decreased turnover of bone, (2) localization of Al in bone is the major determinant for these abnormalities and (3) sBA is a more useful parameter than BAC for assessment of bone changes associated with BAA.

THE "TRADE-OFF" HYPOTHESIS REVISITED: N. Ferran*, G.M. Berlyne, A.J. Adler, Brooklyn VA Hospital Brooklyn, NY.

Progressive retention of inorganic phosphate (Pi) is thought to contribute to the development of secondary hyperparathyroidism in uremia. The mechanisms proposed to explain this effect constitute the "trade-off hypothesis". This states that small increases in serum Pi induce $CaHPO_4$ complex formation which decreases serum ionized calcium (Ca^{2+}) and thereby stimulates the parathyroid glands to increase PTH secretion.

To examine this hypothesis free from hormonal and physiological influences, we determined the effect of increasing Pi on Ca^{2+} in vitro.

Aqueous solutions consisting of 1mMCaCl2; ionic strength 0.15; pH 7.40±.01 and serum samples from 7 healthy human volunteers were studied. Ca^{2+} was measured by an Orion SS-220 ion selective electrode (C.V. in aqueous solutions 1.2%; C.V. in serum 2.5%). Total calcium was analyzed by atomic absorption spectrometry and Pi by standard autoanalyzed methods.

All samples were analyzed at pH 7.40±.01. The slope of the regression line for changes in Ca^{2+} (mM/L) associated with changes in Pi (mM/L) was -0.019±.001 for aqueous solutions and -0.018±.003 for serum. These were not significantly different. Based on these results serum Pi would have to increase by 1.2mM(3.7mg%) before serum Ca^{2+} was sufficiently depressed (0.1mg%; Blum et al, Endocrinol. 95:753 1974) to stimulate the parathyroid glands. We conclude that Pi retention does not induce secondary hyperparathyroidism by a direct physiochemical effect on ionized Ca in plasma.

CALCIUM (Ca) AND PHOSPHATE (Pi)BALANCE IN CHRONICALLY ALCOHOLIC RATS.E.J. Fillipone, G.M. Berlyne, A.J. Adler. Brooklyn VA Hosp. Brooklyn, NY 11209.

Chronic alcolholism is associated with derangements of Ca and Pi in both serum and muscle. Despite considerable study the mechanisms underlying these changes remain uncertain. To examine whether the abnormalities of Ca and Pi involve either renal or g.i mechanisms we performed metabolic balance studies on 3 groups of 10 male Sprague Dawley rats (100-150gm) each. Group A- 9% w/v ethanol in water as its sole source of fluid; group B- sucrose in water isocaloric to group A; group C- untreated water. All animals were fed Purina rat chow (.6% Ca, .6% Pi) ad libitum. At 20 weeks individual 72 h metabolic ballance studies were performed. Urine, serum and feces were collected and analyzed. Creatinine (Cr) and Pi were measured by autoanalyzer and Ca by atomic absorption spectrometry. Cr clearances were similar in all groups. Total Ca and Pi intake was significantly lower ($p<.02$) in group A compared to both B and C. But, net balance per 24 h was not different for either Pi or Ca. The % retention by A was greater for both Pi ($p<.05$) and Ca ($p<.02$) and was due to increased g.i. absorption. UV_{Pi} was greater ($p<.05$) in B (12.7±4.6 mg) than in C (9.3±3.0 mg) which was greater ($p<.02$) than A (5.4±1.9 mg) and correlated directly with the level of diuresis ($r=.72$; $p<.001$). UV_{Ca} was less ($p<.02$) in A (1.09±.63 mg) than B (1.70±.65 mg) but greater ($p<.01$) than C (.67±.16 mg) despite the larger diuresis in C than A. Conclusions: Net balance of Pi and Ca is unaltered in alcoholic rats. G.I adaptation to differences in intake is normal. Urinary excretion of Ca may be excessive in the alcoholic rat but is balanced by an increased g.i. uptake.

ABNORMALITY IN Ca^{++} UPTAKE IN UREMIC RAT BRAIN SYNAPTOSOMES.C.L. Fraser*, P. Sarnacki*, A.I. Arieff. Neph. Research, VA Med. Ctr. & U.C.S.F., San Francisco, CA

Ca^{++} in uremic brain is elevated - the functional significance is unclear. We evaluated Ca^{++} uptake in the brain of acutely uremic rats (BUN 250 mg/dl). Studies were carried out in synaptosomes obtained from rat brain cerebral cortex by homogenization and differential centrifugation. Synaptosomes are presynaptic nerve endings from brain which are metabolically active. Two mechanisms of Ca^{++} transport were evaluated using ^{45}Ca as a tracer.

We first evaluated the Na^{+}-Ca^{++} exchange mechanism in which vesicles were loaded with NaCl in the presence of 10 uM CaCl external media. Ca^{++} uptake in uremic synaptosomes was significantly greater than normal by 30% ($p<0.05$) over time. This increased Ca^{++} accummulation in uremic synaptosomes could be due to either increased Ca^{++} uptake or decreased Ca^{++} efflux. To evaluate Ca^{++} uptake we measured initial Ca^{++} uptake between 0-20 sec. Initial uptake was also found to be increased in uremic synaptosomes. Then to assess Ca^{+} efflux, Ca^{++} uptake via the ATP dependent system (1 mM ATP) was studied in inverted synaptosomes. In the uremic synaptosomes a significant decrease by 20% ($p<0.05$) in ATP dependent Ca^{++} uptake was also observed.

These studies show that: a) Ca^{++} accumulation via the Na^{+}-Ca^{++} exchanger is increased in uremia. b) Ca^{++} efflux from synaptosomes via the ATP dependent Ca^{++} transport mechanism is decreased in uremia. c) Thus the increased Ca^{++} accumulation in uremic synaptosomes is due to both increased Ca^{++} influx and decreased Ca^{++} efflux in uremia. The defects observed in uremia appear to be not easily reversible and may affect neurotransmission in the uremic state.

EFFECT OF HYPERPARATHYROIDISM IN ESRD ON RBC OSMOTIC FRAGILITY (OF). Charles J. Foulks, MAJ MC and Glenn M. Mills*, MAJ MC. Nephrology Service and Hematology/Oncology Service, Department of Medicine, Brooke Army Medical Center, Fort Sam Houston, Texas.

The anemia of CRD is multifactorial and is related to factors other than ineffective erythroporesis. The decreased RBC survival in uremic serum is postulated to be secondary to serum factors that are elevated in the uremic state. PTH is thought to be one of the responsible factors, perhaps working, through enhanced RBC osmotic fragility. PTH (N-terminal and intact) has been shown to increase RBC osmotic fragility in vitro. To examine the clinical significance of the effect of secondary hyperparathyroidism on RBC survival we tested the OF of 12 chronic HD patients. The PTH values (C-terminal, intact, midmolecule) were 9475±4127pg/ml, ionized CA 4.1 ± 0.1 mg/dl (nl 4.60-5.40), PO4 6.3 + 0.5 mg/dl, alkaline phosphatase 111 ± 23 u/ml (nl 30-125). The mean osmotic fragility was normal and no patients exhibited enhanced OF. We conclude that in patients without severe HPTH decrease that RBC OF in uremia is not enhanced and is not responsible for the decreased RBC survival.

METABOLIC ACIDOSIS ENHANCES $1,25(OH)_2D_3$ INDUCED INTESTINAL ABSORPTION OF CALCIUM AND PHOSPHORUS IN RATS. Uzi Gafter*, Samuel Edelstein*, Judith Hirsch*, and Joseph Levi. Dept. of Nephrology, Hasharon Hosp., Petah-Tivka, and Tel Aviv Univ. Med. School, Dept. of Biochem, Weizmann Institute of Science, Rehovot, Israel.

The effect of metabolic acidosis on the intestinal absorption of calcium (Ca) and phosphorus (P), plasma vitamin D metabolites and urinary excretion of Ca and P in adult rats treated with $1,25(OH)_2D_3$ were investigated. The rats in the experimental group (n=7) received 1.8% NH_4CL and a commercial chow (Ca 1%, P 0.7%) ad lib, their pair-fed controls (n=7) received NaCl 0.45% ad lib. After 3 days both groups were given subcutaneously $1,25(OH)_2D_3$ 7.5 ng/day for 6 days. Intestinal absorption of Ca and P was measured by everted gut sac uptake of ^{45}Ca and ^{32}P-phosphate. In the acidotic rats pH-7.14, HCO_3^- 12.9 mEq/L vs controls pH 7.4, HCO_3^- 20.7 mEq/L ($p<0.01$) duodenal, jejunal and ileal ^{45}Ca uptake was increased compared to controls 927±71, 990±73, and 719±73 vs 723±51, 626±92 and 471±70, (CPM/mg wet weight/0.5h), respectively ($p<0.05$). Jejunal and ileal ^{32}P-phosphate uptake was increased in the acidotic rats 995±75 and 706±60 vs 639±52 and 482±27, respectively ($p<0.01$). Plasma 1,25(OH)D, 25(OH)D and $24,25(OH)_2D$ were similar in both groups. $1,25(OH)_2D_3$ treatment induced a greater calciuria in acidotic rats, fractional excretion of Ca- 4.6±0.4 vs 3.3±0.5% ($p<0.05$). Duodenal ^{45}Ca uptake (n=12) from an acidic medium pH 7.0 vs 7.4 was increased 1209±122 vs 735±125 ($p<0.01$). The present study suggests that NH_4Cl induced metabolic acidosis enhances the effect of $1,25(OH)_2D_3$ on intestinal absorption of Ca and P in the rat.

BIOLOGICAL EFFECTS OF ALUMINUM ON THE ISOLATED PERFUSED BONE. T. Galceran,* J. Finch,* M. Bergfeld,* J. Coburn, S. Teitelbaum, K. Martin and E. Slatopolsky. Washington Univ. School of Medicine, St. Louis, MO., V. A. Hospital, Los Angeles, Ca.

Although it is well known that aluminum (Al) plays a role in the development of osteomalacia in patients with chronic renal failure, the mechanisms are not fully understood. Since the osteoblasts are the cells responsible for the formation of osteoid tissue, which is greatly affected in patients with aluminum-induced osteomalacia (Al-Ind.Ost.), it is possible that Al may interfere with the function of the osteoblast. To further characterize this potential mechanism, we performed studies in isolated perfused tibiae from normal and Al treated dogs. In this system when PTH is added to the perfusate, cAMP a major marker of osteoblastic activity is released into the perfusate. The dogs were divided in two groups: 1·) Control 2·) Aluminum 0.75 mg/kg I.V. 5 days a week for 3 months. Thereafter, the dogs were sacrified and the tibiae perfused in vitro. PTH (1-34) at the concentration of 3-4 ng/ml and IBMX (an inhibitor of phosphodiesterase) were added to the perfusate. Basal cAMP secretion was the same in both groups of dogs. However, after PTH was added to the perfusate, cAMP increased to a peak of 183 ± 34 pmol/min in the normal dogs vs 113 ±8 in Al treated dogs. Cumulative cAMP secretion over a 30 m period was 734 ± 147 in the normal dogs vs 455 ± 38.2 in the experimental animals. In summary; these studies indicate that Al impairs the release of cAMP by the isolated perfused bone suggesting an abnormal function of the osteoblast. Further studies are necessary to determine the relation between the abnormal function of the osteoblast and the development of osteomalacia.

SITE OF CALCIUM REJECTION IN MINERALOCORTICOID ESCAPE. M. Gehr, M. Goldberg. Department of Internal Medicine, Univ. of Cincinnati Medical Center, Cincinnati, Ohio.

Mineralocorticoid escape (ME) is characterized by restoration of sodium homeostasis and hypercalciuria. To examine the nephron site responsible for calcium rejection we prepared ME rats under strict metabolic balance conditions by injecting doca (15 mg/kg/day IM) in saline expanded animals. Saline control (SC) rats were prepared similarly except without doca. ME rats escaped sodium retention in 5 days and were hypercalciuric compared to the SC group: FE_{Ca} (SC 1.3±0.3; DE 2.8±0.6; $p < 0.05$). On day 7 micropuncture was performed to determine the fractional delivery of calcium along the nephron. Our results show that fractional delivery of calcium was not significantly different between SC and ME animals along the puncturable nephron segments. However, fractional excretion of calcium in the final urine was very significantly increased in ME rats.

	Late Proximal	Early Distal	Late Distal	Final Urine
SC	61.0±3.3	7.91±1.1	4.71±0.83	0.48±0.13
	n (9)	n (16)	n (10)	n (13)
DE	58.9±2.1	8.93±1.3	3.56±0.32	1.62±0.24†
	n (14)	n (7)	n (12)	n (12)

†$p < 0.001$

Fractional excretion of sodium was not significantly different: SC 1.21±0.22; DE 1.82±0.29. We conclude that as in other circumstances which dissociate sodium and calcium excretion, i.e. hyperparathyroidism and hypophosphatemia, the hypercalciuria of mineralocorticoid escape is probably the result of depressed calcium reabsorption in the collecting duct or the deeper nephrons.

CHLORTHALIDONE-INDUCED CA MALABSORPTION: EVIDENCE FOR A DIRECT MECHANISM. J. Garno*, B. Eby*, C. Langman, and K. Lau. Michael Reese Hosp. & Univ. of Chicago, Chicago, IL.

Previous studies demonstrated Ca malabsorption in response to chlorthalidone (CTD). The mechanism and the long-term effects are obscure. It has been hypothesized that the gut effect is a compensation to the hypocalciuric action. To test this hypothesis, we examined the role of PTH and vitamin D. In parathyroidectomized rats, despite comparable Ca intake, CTD decreased net Ca absorption (11 vs 29 mg/d§) [§, $p<0.05$ vs control (C)]. Although urine Ca was reduced (1.0 vs 1.7 mg/d§), Ca retention was markedly diminished by CTD (10±6 vs 28±5 mg/d§), arguing against a mere homeostatic adjustment to the renal effects. Weanling rats were vitamin D deprived for 6 weeks and then treated with CTD. Despite undetectable serum $25OHD_3$ and diminished serum $1,25(OH)_2D_3$ (82±8 vs 60±7 pg/ml, p=N.S.) Ca malabsorption, far exceeding renal Ca retention, was again produced, reducing Ca balance (Table).

	N	Oral Ca (mg/d)	Fecal Ca (mg/d)	Net Ca Absorbed (mg/d)	(%)	Urine Ca (mg/d)	Retained Ca (mg/d)	Serum 25OHD (ng/ml)
C	(7)	69.5	45.2	24.4	33	2.6	21.7	≤ 2
CTD	(7)	69.4	64.4§	4.9§	7§	0.7§	4.2§	≤ 2

In vitamin D-repleted rats, Ca malabsorption (10 vs 24 mg/d) also dominated over renal Ca retention (0.8 vs 2.3 mg/d) during chronic (8 weeks) CTD treatment, reducing Ca balance (from 21.5 to 9.3 mg/d).

These findings indicate the inhibition of Ca absorption by chlorthalidone is direct, mediated independent of PTH, 25OHD and serum $1,25(OH)_2D_3$ levels. Ca malabsorption is sustained, resulting in reduced Ca balance with chronic treatment.

EVIDENCE THAT THE PHOSPHATURIC EFFECT OF ACUTE HYPERCAPNIA (HC) IS NOT DUE TO THE LOW SYSTEMIC pH OF HC. J. Guntupalli, E. Bourke. Allegheny Singer Research Institute, Pittsburgh, Pennsylvania.

The consequences of HC and respiratory acidosis on phosphorus (Pi) excretion are not clearly delineated. Hence acute clearance experiments were performed in six groups of chronic PTX'ed rats after two days of dietary Pi deprivation. Group I: HC (10% CO_2 in inspired air) alone increased the fractional excretion (FE) of Pi (0.32 ± 0.11 to 7.56 ± 0.78%*). The GFR was stable. During HC the systemic pH decreased (7.36 ± 0.02 to 7.07 ± 0.02*) & PCO_2 increased (40 ± 3 to 88 ± 14*). The plasma (P) Pi increased during HC (9.56 ± 0.49 vs 12.88 ± 0.98 mg%*). Group II: Pi infusion to comparable PPi as in group I (13.06 ± 0.78 vs NS) during normocapnia, was not phosphaturic. Group III: Addition of PTH 3 $U.kg^{-1}.h^{-1}$ during HC further increased Pi excretion compared to HC alone, FEPi (22.15 ± 1.93 vs *). Group IV: PTH infusion during normocapnia was not phosphaturic. Group V: Addition of PTH to Pi infusion resulted in less phosphaturia compared to HC alone, FEPi (7.56 ± 0.78 vs 3.92 ± 0.31*). The PPi was comparable between groups. Group VI: Neutralization of the systemic acidemia of HC with isotonic $NaHCO_3$ resulted in Pi excretion similar to HC alone, FEPi (10.10 ± 1.37 vs 7.56 ± 0.78% NS). The increment (Δ) in the cAMP excretion vs the control phase in groups III and IV were similar (Δ 47 ± 8 vs 54 ± 8 pmols/ml GFR. * = $P < 0.001$. Conclusions: These studies suggest that 1) hypercapnia per se is phosphaturic independent of changes in GFR, plasma & diet Pi, and cAMP excretion. 2) restores the phosphaturic action of PTH in dietary Pi deprivation. 3) low systemic pH per se is not responsible for phosphaturia of HC.

LACK OF INFLUENCE OF VOLUME FLUX (J_v) ON PHOSPHATE (P) REABSORPTION IN THE ISOLATED PERFUSED PROXIMAL CONVOLUTED TUBULE (PCT). R.J. Hamburger, C. Soeldner*, and J.S. Kaufman, Renal Section, Boston VA Medical Center, Boston, MA

We examined the effect of changes in J_v, with or without changes in sodium flux, on P reabsorption (J_p) in the PCT of the rabbit. There was no difference in J_p in tubules from superficial or juxtamedullary regions, so the results have been combined. PCT were studies: 1) before and after 10^{-4} M ouabain (n=18), 2) with a bath of 260, 290, or 320 mOsm/kg and perfusate of 290 (n=11), 3) with change in perfusion rate from 15 to 5 nl/min (n=15). Results are summarized below (mean±SE).

	J_v (nl/min)	J_p (pM/mm min
Cont	0.69+.09	4.436+.681
Ouabain	-0.01+.05[a]	1.129+.369[a]
260 bath	-0.27+.10[b]	5.717+.635
290 bath	0.66+.09[b]	6.516+.655
320 bath	1.42+.12	6.970+.540
15 nl/min	1.27+.15[c]	4.683+.609[c]
5 nl/min	0.68+.10	2.587+.451

Significantly different ($p<0.5$) from a) control, b) 320, or c) 5 nl/min.

When J_v was altered without a change in sodium flux, by altering bath osmolality, no change in J_p was noted, even if net fluid secretion occurred. With addition of ouabain, J_v was zero and J_p decreased. When perfusion rate was decreased from 15 to 5 nl/min, J_v decreased by 47% and J_p decreased by 45%. These results are consistent with a relationship between sodium, but not fluid reabsorption, and P reabsorption. The absence of a relationship between J_v driven by osmatic gradients and J_p would suggest that the paracellular pathway is not a significant route of P flux.

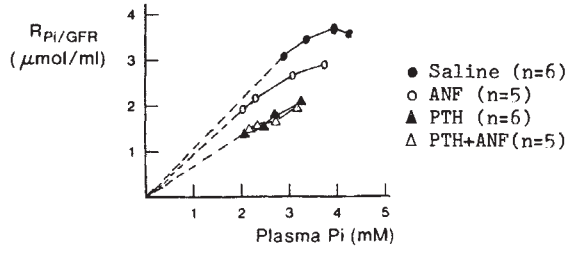
TUBULAR CAPACITY OF PHOSPHATE REABSORPTION IN SUPERFICIAL AND DEEP NEPHRONS. Aviad Haramati, Department of Physiology, Mayo Medical School, Rochester, MN

The present study was performed to determine the maximum capacity of phosphate (Pi) reabsorption (MAX R_{Pi}) in superficial and deep nephron proximal tubules, in vivo. Micropuncture experiments were conducted in acutely TPTX rats (n=20) fed a normal Pi diet (0.7%). Pi was infused at 0, 2, 4 or 6 µmol/min to increase filtered Pi load. Fractional Pi delivery (FD_{Pi}%) from superficial and deep nephron proximal tubules was measured at the superficial early distal tubule and deep nephron loop of Henle. With Pi infusions, plasma Pi increased from 3.03 to 7.01 mM and FE_{Pi} rose from 2 to 58%. FD_{Pi}% increased from both superficial (14±4 to 58±2, $p<.05$) and deep nephron proximal tubules (4±1 to 27±4, $p<.05$), but always remained lower from deep nephrons, reflecting more avid Pi reabsorption in deep nephrons. Indeed, the MAX R_{Pi}/SNGFR was significantly lower in the superficial than in the deep nephron proximal tubule (3.2 ± 0.4 vs 5.1 ± 0.1 pmol/nl, $p<.05$).

In seven Pi-infused rats, parathyroid hormone (PTH, 33 U/kg bolus; 1 U/kg·min) was added subsequent to the initial collections. During PTH the MAX R_{Pi}/SNGFR was significantly lower in deep than superficial proximal tubules (0.4 vs 1.6 pmol/nl, $p<.05$). Thus, a maximum capacity for Pi reabsorption was demonstrable in single nephron proximal tubules, in vivo, which was greater in deep than superficial nephrons in TPTX rats. Furthermore, PTH inhibited the capacity for Pi reabsorption to a greater extent in deep than superficial proximal tubules.

ATRIAL NATRIURETIC FACTOR (ANF) DECREASES TUBULAR CAPACITY FOR PHOSPHATE REABSPORPTION (TmPi/GFR). T. G. Hammond*, A. Haramati, F. G. Knox. Mayo Medical School, Rochester, Minnesota

Synthetic 26 amino acid ANF increases glomerular filtration rate and sodium and phosphate excretion. We tested the hypothesis that ANF reduces the tubular capacity for phosphate reabsorption. The effect of ANF(4.0 µg/kg·hr) was evaluated in TPTX rats given successive infusions of phosphate (0, 1, 2, and 3 µmol/min) to raise the filtered phosphate load, in the presence and absence of parathyroid hormone (PTH, 1 U/kg·min). The results are summarized in the figure.



The TmPi/GFR in the presence of ANF (2.7±0.3 µmol/ml) was significantly lower compared to saline controls (3.6±0.4 µmol/ml $p<0.05$). PTH further decreased TmPi/GFR (2.0±0.1 µmol/ml, $p<0.05$), which was not further reduced with PTH+ANF (2.1±0.2 µmol/ml). We conclude that ANF decreases the tubular capacity for phosphate reabsorption, and that this decrease is not additive to the effect of PTH.

REGULATION OF PROXIMAL TUBULAR (PT) CELL CYTOSOLIC CALCIUM ($Ca^{2+}i$) BY PARATHYROID HORMONE (PTH). K.A. Hruska, S. Mills,* and S. Westbrook.*, Renal Div., The Jewish Hospital of St. Louis, St. Louis, MO.

The effects of PTH on $Ca^{2+}i$ were analyzed in canine PT or mouse kidney cells grown in primary culture. PT segments were prepared from collagenase treated cortical slices by centrifugation in Percoll. PT cells were grown in defined serum free media to confluency at which time they were noted to form numerous hemicysts. PT cells removed from culture dishes by Na^+-EDTA and suspended in a Krebs-Hensleit buffer responded to b-PTH (1-84) 10^{-7}M with a 3.5 ± 0.7 (N=6) fold increase in cAMP production but did not respond to vasopressin or calcitonin. PT cells exhibited Na^+ dependent glucose uptake, PEPCK activity and minimal hexokinase activity. $Ca^{2+}i$ was 90 ± 26 nM, N=7 (PT cell) and 104 ± 10, N=5 (mouse kidney), as determined by the fluorescence of Quin-2. The addition of PTH caused an immediate (<15 sec), but small (2%) increase in $Ca^{2+}i$ that was maximal at 30 min (30%) and returned towards baseline with longer incubation times. $Ca^{2+}i$, uncorrected for a change in cytosolic pH, at 30 min was 117 ± 22 (canine) and 187 ± 30 (mouse) in the presence of PTH ($p<.05$, N=7). PT cells exposed to PTH exhibited a decrease in acridine orange fluorescence indicating cytosolic acidification which would tend to decrease the fluorescence of Quin-2. Thus, the increase in $Ca^{2+}i$ may have been greater than the uncorrected calculation. These studies demonstrate PTH stimulated changes in $Ca^{2+}i$ and, they suggest that Ca^{2+} may indeed represent a second messenger for the hormone.

EFFECT OF CHRONIC HYPERPARATHYROIDISM (HPTH) ON RENAL BICARBONATE ABSORPTION. P. Jaeger, W. Jones*, G. Segre* and J.P. Hayslett. Departments of Med., Yale Univ. Sch. of Med. and Univ. of Lausanne.

Although tubular absorption of HCO_3 is reduced after acute administration of parathyroid hormone (PTH), it has not been shown that chronic HPTH has a similar effect on renal HCO_3 handling. Studies were therefore performed to determine the effect of chronic HPTH on renal HCO_3 excretion in the absence of changes in P_{Ca} or P_D. TPTX rats were infused with I-34 bPTH via an Alzet minipump at 0.7 U/hr (normal replacement) and at 2.1 U/hr (experimental). The high rate tripled plasma PTH levels, as in primary HPTH, and caused a rise in P_{Ca}. To prevent hypercalcemia the experimental group was fed a low Ca-low P diet prior to TPTX and both groups were treated with Cl_2MDP and a low Ca diet along with PTH. Studies were performed on day 6, 12 hrs after HCO_3 was given by peritoneal dialysis to increase the excretory load of alkali.

	Plasma				Urine		
	Ca	P	HCO_3	pH	pH	FE_{HCO_3}	TmP/GFR
		mM					mmol/L
Cont. (n=6)	1.83 ±0.07	3.00 ±0.17	21.3 ±0.9	7.42 ±0.01	6.29 ±0.29	0.03 ±0.02	3.74 ±0.33
Exper. (n=6)	2.01 ±0.13	2.86 ±0.27	22.7 ±0.6	7.41 ±0.01	7.37* ±0.06	0.52* ±0.11	2.75** ±0.31

x ± SEM;*p<0.005;**p<0.05

FE_{HCO_3} was positively correlated with PTH (r=0.8, p<0.01) and TmP/GFR was negatively correlated (r=0.7, p<0.05). These data indicate that HPTH per se causes HCO_3 wasting as well as reduced P reabsorption. Since this model mimics primary HPTH in man, these results suggest that PTH acts to impair renal HCO_3 transport in clinical states of HPTH.

EVALUATION OF PARATHYROID(PT) AUTOGRAFT GROWTH AND FUNCTION IN HEMODIALYSIS PATIENTS G. Karsenty*, A. Petraglia*, D.J. Gambini*, J.F. Moreau*, A. Bourdeau*, J. Zingraff*, F. Bounérias*, C. Dubost* and T. Drüeke*(intr. by D.A. McCarron). Hôpitaux Necker, St. Cloud et F. Widal, Paris, France.

The aim of the present study was to evaluate the growth and function of PT tissue autografted into the forearm of chronic hemodialysis patients (pts) using several methods. In a first prospective study 7 pts had repeated determinations of plasma iPTH in both forearms, a radionuclide evaluation of autograft function using 201- thallium (Tl)-chloride as well as real time ultrasonography to appreciate the growth of implants. A significant gradient, i.e. < 2, of plasma iPTH concentration between both forearms developed in three out of the seven pts. In two of these, but in none of the other four, the radionuclide study disclosed an increased uptake of 201-Tl-Cl and ultrasonography detected a mass at the site of autograft implantation. In both, hyperplastic PT tissue was removed and it revealed no structural abnormality. One of them had a repeat radionuclide study one month after surgical graft removal. Increased tracer uptake was no longer demonstrable. In a second dynamic study the function of autografted PT tissue was evaluated using a zero Ca dialyzate and a Ca infusion procedure. It showed that an increase in plasma Ca was followed by a significant depression, and a decrease in plasma Ca by a significant augmentation of plasma iPTH. In conclusion, 1) hyperplasia of autotransplanted PT tissue in hemodialysis pts can be quantitatively assessed by radionuclide and ultrasonographic technics, and 2) the secretory activity of PT autografts appears to be adequately modulated by extracellular calcium.

TRIFLUOPERAZIN(T) ABOLISHES PARATHYROID HORMONE(PTH) EFFECT ON PHOSPHATE(P) UPTAKE BY ISOLATED RABBIT RENAL BRUSH BORDER MEMBRANE(BBM). Ok Jo* and Norimoto Yanagawa. Nephrol. Div., Sepulveda VAMC, UCLA Sch. of Med., Sepulveda, CA.

Our recent observation(Intl.Cong.Neph. 9:63A,1984) suggested the involvement of intracellular calcium mediators(ICM) in PTH action on P reabsorption in rabbit renal proximal tubules(PT). To further examine the role of ICM in PTH action on P uptake by BBM, the effect of T, an ICM inhibitor, was studied. Purified rabbit PT was prepared by sieving the cortex homogenate. BBM was isolated from PT by Ca precipitation method with modification. The effects of PTH(1 U/ml), 8-br-cAMP(Ø.1 mM) and T(Ø.1 mM) on P uptake by BBM were examined by adding these agents to PT incubation medium before the isolation of BBM. Incubation of PT with T alone did not affect P uptake by BBM(1172.Ø±132.2 v.s.1187.9±137.9 pmol/mg, n=3), but abolished the inhibitory effect of PTH or 8-br-cAMP on P uptake by BBM.

(n=5 each) (* $p < Ø.Ø2$)

(pmol/mg)	Control	PTH	Control	8-br-cAMP
-T	1126.2 ±89.76	985.2* ±82.2	1003.1 ±131.6	874.9* ±127.Ø
+T	1223.7 ±141.6	1250.6 ±150.2	1371.3 ± 35.8	1337.8 ± 57.2

In conclusion, these studies suggest the involvement of ICM in PTH or cAMP effects on P uptake by the BBM of rabbit renal PT.

EFFECT OF NICOTINAMIDE ANALOGS ON RENAL PHOSPHATE TRANSPORT. Stephen A. Kempson. Physiology Dept., Indiana University, Indianapolis, Indiana.

Nicotinamide functions both as an inhibitor of NAD hydrolysing enzymes and as a precursor for NAD synthesis. Treatment with nicotinamide increases renal NAD content, inhibits phosphate (Pi) uptake across the renal brush border membrane (BBM), and elevates urinary Pi excretion. The cellular action of nicotinamide was probed with 5-methylnicotinamide (5MN) and nicotinic acid (NA). While 5MN inhibits NAD hydrolysis it does not support NAD synthesis. NA neither inhibits NAD hydrolysis nor supports NAD synthesis in the kidney. A single i.p. injection of either 5MN or NA (4 mmol/kg body wt) did not alter renal cortical NAD content in low Pi diet rats. In the 5MN rats urinary excretion of creatinine (Cr) and Ca was not changed, but urinary Pi excretion was increased ($p < 0.05$) to 6.21 µmol/mg Cr compared to 0.17 in controls (n=12). Initial Na^+ gradient dependent uptake of Pi in BBM vesicles was 1504 ± 212 pmol/mg protein/15 sec in 5MN rats, and was decreased ($p < 0.01$) compared to 2487 ± 195 in controls. This was due to a decreased Vmax. Uptake of Na^+ and L-proline were not affected. In the NA rats, neither urinary Pi excretion nor BBM transport of Pi was significantly different from the controls.

In summary, data obtained with 5MN suggest that inhibition of NAD hydrolysis may be an important factor which contributes to the phosphaturic action of nicotinamide, independently of changes in NAD synthesis. The absence of changes in renal Pi transport in the rats treated with NA provides indirect support for this conclusion.

FURTHER EVIDENCE FOR EXTRA-RENAL SYNTHESIS OF 1,25-$(OH)_2$-VITAMIN D IN SARCOIDOSIS. A.B. Korkor, R.W. Gray,* and J. Lemann, Jr. Departments of Medicine and Biochemistry, Medical College of Wisconsin, Milwaukee.

We studied a 36 year old anephric black man. He had previously undergone bilateral nephrectomy, splenectomy and removal of a rejected cadaver kidney graft. He became hypercalcemic. The diagnosis of sarcoidosis was documented by liver biopsy and infiltrates on chest radiograph. He was studied while he ate a constant diet and underwent thrice-weekly hemodialysis before and during prednisone therapy. Values are means ± SD.

	Pre-Prednisone	During Prednisone
Serum Total Ca, mM	3.14 ± 0.25	2.44 ± 0.07
P, mM	1.32 ± 0.06	1.32 ± 0.05
Creatinine, µM	1430 ± 180	1250 ± 100
iPTH, µlEq/ml	68 ± 17	450 ± 50
1,25-$(OH)_2$-D, pM	110 ± 23	undetectable
25-OH-D, nM	40 ± 4	117 ± 26
Diet Ca, mmol/day	19.4	21.9
Fecal Ca, mmol/day	18.0	31.8
Net Intestinal Ca Absorption, mmol/day	1.4	-9.9

When plasma obtained before prednisone therapy was extracted and chromatographed, using two different solvent systems known to separate 1,25-$(OH)_2$-D from other dihydroxylated metabolites (JBC 258: 1152, 1983), only material comigrating with authentic 1,25-$(OH)_2$-D could be detected by radioreceptor assay. These data support the view that in patients with sarcoidosis, tissues other than the kidney or spleen can produce 1,25-$(OH)_2$-D.

HYPERCALCEMIA ASSOCIATED WITH SILICONE INDUCED GRANULOMAS. Gregory A. Kozeny,* Anthony L. Barbato,* Vinod K. Bansal, Leonard L. Vertuno, and Jessie E. Hano. Loyola University Medical Center, Maywood, Illinois.

Hypercalcemia occurs in a variety of chronic granulomatous diseases such as sarcoidosis, tuberculosis and berylliosis. We report a 33 year old white patient who became hypercalcemic after receiving bilateral silicone hip injections for cosmetic reasons. These sites, subsequently, developed persistent and massive (10x20 cm) areas of inflammation. Within two years of the injections she developed hypercalcemia, otosclerosis and renal insufficiency. Her chest x-ray, liver functions and serum angiotensive converting enzyme levels were normal. Evaluation revealed suppressed levels of parathyroid hormone and 25-OH vitamin D_3, but elevated serum calcium (14.7 mg/dl), calcitriol level (85 pg/ml) and urinary calcium excretion (507 mg/24 hr). A Galium ^{67} scan revealed uptake only in her hips at the site of the silicone injections. A biopsy of the area demonstrated fibrosis and granuloma formation. The serum calcium, urinary calcium excretion and calcitriol levels decreased to normal after a 5-day trial of prednisone (30 mg/day) and remained normal on a maintenance dose of prednisone (10 mg/day) only to increase again after the prednisone was discontinued. The data suggest extra-renal production of calcitriol or a chemically similar vitamin D metabolite by the granuloma reminiscent of the mechanism postulated for the hypercalcemia of sarcoidosis

FAMILIAL HYPOCALCIURIC HYPERCALCEMIA (FHH): MINERAL BALANCES AND BONE HISTOMORPHOMETRY ARE NORMAL. A.B. Korkor, A.B. Gustafson*, M.D. Fallon*, R.W. Gray* and J. Lemann. Jr. Departments of Medicine and Biochemistry, Medical College of Wisconsin, Milwaukee and Department of Surgical Pathology, University of Pennsylvania, Philadelphia.

We studied 3 affected members of a kindred with FHH, 2 males ages 14 and 23 and 1 female age 16 years. $U_{Ca}V$ averaged only 3.1 ± 1.4 SD mmol/day (estimated E/F Ca 1.0 ± 0.3%) despite a mean serum total Ca of 2.98 ± 0.03 mM. Mineral balances, serum vitamin D metabolites, iPTH and bone histology were observed while they ate constant normal diets providing 27 ± 9 mmol Ca/day. Serum 1,25-$(OH)_2$-D averaged 55 ± 20 pM and net intestinal Ca absorption averaged 4.7 ± 3.4 mmol/day. The individual estimates of net intestinal Ca absorption were appropriately correlated to individual serum 1,25-$(OH)_2$-D levels (r = 0.99). Ca balances averaged + 1.6 ± 4.1 mmol/day. Despite hypercalcemia, serum iPTH was not low, averaging 4.3 ± 2.9 µlEq/ml. Serum PO_4 averaged 1.21 ± 0.19 mM and fasting $TmPO_4$/GFR 1.13 ± 0.24 mM. Serum total Mg averaged 0.96 ± 0.04 mM and estimated E/F Mg was normal averaging 4.4 ± 1.2%. Bone histomorphometry demonstrated active bone remodelling: relative osteoid volume 2.2 ± 1.0% (normal adults < 3.5), osteoclasts/mm^2 0.20 ± 0.06 (normal adults < 0.19) and mineralization rate 0.69 ± 0.08 µm/day (normal adults > 0.60). Despite hypercalcemia among patients with FHH, the components of Ca balance, serum iPTH and 1,25-$(OH)_2$-D concentrations as well as bone histology are normal, thus indicating a new set-point for Ca transport in the parathyroids, as well as in the kidney.

1,25$(OH)_2D_3$ DIRECTLY STIMULATES PHOSPHATIDYLCHOLINE (PC) TRANSFER. B.R.C. Kurnik and K. Hruska. Renal Div., Jewish Hosp., St. Louis, MO.

Controversy exists as to whether the increase in duodenal and renal brush border membrane (BBM) PC content and stimulation of ion transport by 1,25$(OH)_2D_3$ is through a genomic mechanism or a direct membrane effect. To investigate direct membrane actions of 1,25$(OH)_2D_3$, dioleyl PC liposomes were prepared using self-quenching concentrations of fluorescent phospholipid derivatives, either NBD-PC or N-Rh-phosphatidylethanolamine (PE). Dilution of these fluorescent phospholipids through direct transfer or fusion with another membrane results in measurable increases in relative fluorescence (r.f.). When these liposomes were incubated with rat renal BBM vesicles, an immediate increase in r.f. of NBD-PC was detectable (.41 at 15 min.). Liposomes containing, 1,25$(OH)_2D_3$, 10^{-7}M, increased the initial rate and amount of NBD-PC r.f. in BBMV to .70 at 15 min. Peripheral fluorescence was visible when the BBMV were viewed with a fluorescent microscope. There was no detectable fluorescence of N-Rh-PE when incubated with BBMV in the presence or absence of 1,25$(OH)_2D_3$. To quantitate the amount of NBD-PC transferred, the BBMV were isolated, their lipids extracted, separated with thin-layer chromatography, and quantitated. 1,25$(OH)_2D_3$ stimulated an increase in transfer of NBD-PC and DOPC from 1.18±0.15 µg and 23.2 µg, respectively to 1.9±0.18 and 37.8 µg ($p<.05$, N=4). There was no significant transfer of Rh-PE. We conclude that facilitation of specific lipid transfer is a mechanism whereby 1,25$(OH)_2D_3$ and other hormones may directly modify membrane lipid composition and ion transport.

CHRONIC METABOLIC ACIDOSIS (CMA) INHIBITS cAMP-AND PHOSPHATE (Pi)-STIMULATED 1,25(OH)$_2$D$_3$ (1,25D) SYNTHESIS. CB Langman, MJ Favus*, FL Coe. Michael Reese Hosp. and University of Chicago, Chicago, IL.

Low Ca diet (LCD) and in vitro cAMP and low Pi stimulate 1,25D production by proximal tubules (PT). CMA inhibits the rise in serum 1,25D during LCD, but the effect on synthesis is unknown. To investigate in vitro synthesis of 1,25D during CMA, Sherman rats were fed LCD±1.5% NH_4Cl for 10d. to produce CMA (pH=7.34±.03, LCD vs. 7.23±.03, LCD+NH_4Cl, $p < .05$). PT in suspension were incubated for 20min. in Krebs-Henseleit bicarbonate (KHB) media (pH=7.4), or in KHB low in Ca (KHB-Ca), low in Pi (KHB-Pi), or low in both (KHB-CaPi), and then for 5min. at 37°C with 16.6uM 25(OH)D$_3$ to measure 1,25D synthesis. PT from rats fed LCD produced 10±1 pmol 1,25D/mg prot. (mean±SE) in KHB; 11±1 in KHB-Ca (p=NS vs. KHB); 20±2 in KHB-Pi; and 21±3 in KHB-CaPi ($p < .005$ vs. KHB for each). PT from rats fed LCD+NH_4Cl produced 5±3 pmol 1,25D/mg protein in KHB ($p < .001$ vs. LCD, KHB); 4±2 in KHB-Ca; 4±2 in KHB-Ca; 4±2 in KHB-Pi; 4±1 in KHB-CaPi (all p= NS vs. KHB). PT cAMP from rats fed LCD and LCD+NH_4Cl were similar (2260±330 vs. 1950±520 pmol/mg protein, p=NS), and were not altered by media Ca or Pi. Forskolin, 10^{-5}M, elevated cAMP 2.5x in PT from rats fed LCD±NH_4Cl, but did not alter 1,25D synthesis. Conclusions: normalization of medium pH did not restore diminished 1,25D synthesis in PT from CMA rats. CMA blocks cAMP-dependent 1,25D synthesis distal to cAMP generation, and also blocks cAMP-independent (low medium Pi) 1,25D synthesis. The site of inhibition of 1,25D synthesis in CMA is unknown, but may be distal to the plasma membrane.

JEJUNAL PHOSPHATE TRANSPORT: SOME UNUSUAL FEATURES. **D.B.N. Lee and M.W, Walling*. Sepulveda VAMC, UCLA Sch. of Med., Los Angeles.**

Brush border (BB) phosphate (P) influx is the initial event in active, transcellular absorption of this molecule across renal and intestinal epithelium. The rate of BB influx varies directly with extracellular (EC) [Na] in a manner consistent with a Na-cotransport model. We measured 32-P influx across BB in intact, rat jejunal epithelium mounted as a flat sheet in an Ussing system. Tritiated PEG (MW=900) was used for correction of adherent fluid. At pH 7.4 P influx increased from 4.9±0.3 to peak at 13.3±1.0 nmol/cm2/min as EC [Na] was increased from 0 to 100 mM by isosmotic choline substitution. However, with further increase in [Na] to 140 mM, P influx dropped to 6.9±0.7. This biphasic response to EC [Na] is quite different from reported P uptake studies in intestinal and renal BB membrane vesicles. In addition, up to 65% of total P influx remained intact at "0" EC [Na]. This "Na-independent" influx increased linearly with increases in EC [P]. We also examined the effect of changing [Na] on transepithelial, active P absorption and found a direct relationship between these two parameters for [Na] up to 140 mM. Conclusions: 1. Na-dependent P influx across BB of intact cells is different from that across isolated BB membrane vesicles and exhibits a biphasic pattern with influx inhibition at higher [Na]. 2. Up to 65% of BB P influx may be mediated through Na-independent carriers. 3. Changes in BB P influx is not always accompanied by parallel changes in transepithelial, active P absorption.

BLOCKADE OF LUMINAL CALCIUM ENTRY BLOCKS THE INHIBITORY EFFECT OF DIBUTYRL cAMP (DBcAMP) ON FLUID REABSORPTION (J_V) IN RABBIT PCT. **E. G. Laughry,* and J. W. McKeown. VAMC, and Univ. of Kentucky, Lexington, KY.**

Previous studies have demonstrated a role for luminal calcium in the inhibitory effect of PTH on fluid reabsorption in the rabbit proximal convoluted tubule. The present studies were designed to eliminate the possibility that this effect might be caused by a failure of cAMP generation in response to PTH resulting from changes in external calcium. Segments of rabbit PCT were perfused in vitro and exposed to bath addition of DBcAMP (10^{-4}M) both before (control) and after either 1) replacement of control perfusate with calcium-free perfusate or 2) addition of 50 µM lanthanum (L) to the perfusate. Under control conditions, DBcAMP resulted in a significant and reversible reduction in J_V. Replacement of control perfusate with calcium-free perfusate caused no change in J_V (1.67±0.28 vs. 1.65±0.24) and subsequent addition of DBcAMP to the bath caused no reduction in J_V (1.64±0.25 vs. 1.67±0.28) in contrast to the significant reduction seen with calcium containing control perfusate. L addition to the perfusate caused significant stimulation of J_V (2.22±0.21 vs. 1.95±0.31, $p<0.05$, n=6) and resulted in blockade of inhibition by subsequently added DBcAMP (2.17±0.24 vs. 2.22±0.22).

These data demonstrate that cAMP leads to a reduction in J_V by a mechanism which is dependent on changes in luminal membrane calcium handling. This suggests that the expression of PTH action on the renal tubule is dependent on steps distal to cAMP generation which induce changes in luminal membrane calcium handling.

EFFECT OF ALUMINUM ON DUODENAL CALCIUM ABSORPTION IN VITAMIN D DEFICIENT AND VITAMIN D REPLETE RATS. J. Lee*, G.M. Berlyne, A.J. Adler. Brooklyn VA Hospital, Brooklyn, NY.

The effect of aluminum (Al) on net calcium (Ca) absorption by rat duodenum was studied using an in vivo isolated intestinal segment technique. Groups consisted of vitamin D deficient (N=10) and 1,25(OH)$_2$D$_3$ treated (N=16) male Sprague-Dawley (100-200g) rats. Perfusion solutions contained NaCl 150 mM; $CaCl_2$ 0.9 mM; glucose 3.5 mM; ^{45}Ca µ2Ci/L; ^{3}H-polyethelene glycol (PEG) 5 Ci/L; and varying concentrations of $AlCl_3$ (0.5-5.0 mM). Solutions were maintained at 37°C and perfused at 0.4 ml/min for 30 min. Samples were collected at 10 min intervals. Al and Ca were determined by atomic absorption spectrometry and ^{45}Ca and ^{3}H-PEG by β-scintillation. Net Ca flux was determined from standard formulae. Ca absorption in vitamin D replete rats was 15.5±3.8 µM/hr/100 mg DW and fell to 10.3±3.9 µM/hr/100 mg DW with the addition of Al to the perfusate ($p<.02$) Ca absorption in vitamin D deficient rats was 9.6±3.2 µM/hr/100mg DW and fell to 8.1±2.9 µM/hr/100mg DW (P NS) with the addition of Al.

Conclusion: The presence of aluminum suppresses vitamin D dependent but not vitamin D independent Ca absorption in the rat duodenum.

HYDROCHLOROTHIAZIDE (HTZ) INHIBITS BONE RESORPTION IN MEN. J. Lemann, Jr., R.W. Gray*, W.J. Maierhofer and H.S. Cheung*. Departments of Medicine and Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin.

We evaluated the effects of HTZ on the components of Ca metabolism in relation to the PTH-vitamin D endocrine system and bone. We studied 6 healthy men adapted to constant diets providing only 5.1 ± 0.7 SD mmol Ca/day. 3/6 were also given calcitriol, 0.5 µg 6-hourly. Steady-state balances were compared during control and during HTZ, 25 mg 12-hourly. Directional changes during HTZ did not differ among subjects given or not given calcitriol. For all 6, control net intestinal Ca absorption, serum $1,25(OH)_2D$, serum iPTH and urine cAMP/creatinine averaged 0.5 ± 2.2 mmol/day, 162 ± 51 pM, 4.3 ± 2.2 µlEq/ml and 0.26 ± 0.05 µmol/mmol respectively; none changed during HTZ. HTZ, as expected, reduced $U_{Ca}V$ by - 1.4 ± 0.8 mmol/day; p < 0.01. During control, Ca balance, urine OHproline and fasting U_{Ca}/creatinine averaged - 3.7 ± 2.0 mmol/day, 0.39 ± 0.04 mmol/day and 0.26 ± 0.17 mmol/mmol, respectively. During HTZ, Ca balance became less negative by + 1.6 ± 1.0 mmol/day; p < 0.025 while urine OHproline and fasting U_{Ca}/creatinine decreased by - 0.13 ± 0.09 mmol/day; p < 0.025 and - 0.09 ± 0.05 mmol/mmol; p < 0.025, respectively. Thus HTZ reduced net bone resorption. HTZ also raised blood pH + 0.04 ± 0.02; p < 0.005 and serum HCO_3 + 2.7 ± 0.5 mEq/L; p < 0.001. Since HTZ did not change PTH, cAMP nor $1,25(OH)_2D$, inhibition of bone resorption may be mediated by 1) relative alkalosis, 2) a reduced skeletal sensitivity to PTH and/or $1,25(OH)_2$-D or, possibly, 3) by a direct skeletal effect of HTZ.

THE EFFECT OF ACUTE ACIDOSIS ON PHOSPHATE TRANSPORT BY THE RENAL PROXIMAL TUBULE BRUSH BORDER MEMBRANE. B.S. Levine, J.A. Kraut, P.W. Crooks, and D.R. Mishler.# VA Wadsworth Medical Center, UCLA School of Medicine, Los Angeles, CA.

Metabolic (M) and respiratory (R) acidosis (A) are associated with phosphaturia. The phosphaturia of MA is due, in part, to inhibition of Na-dependent phosphate (P) transport by the proximal tubule, an effect noted at 16 hrs. To examine if RA causes phosphaturia by a similar mechanism, we measured P and glucose (G) uptake by brush border membrane vesicles (BBMV) from TPTX rats with RA for 60 min. and from controls (C). RA was induced by ventilation with 10% CO_2; C were ventilated with room air. Arterial pH, PCO_2 (mmHg) and HCO_3 (meq/l) were 7.4±0.1, 39.2±7.3, 21.8±0.6, in C and 7.06±0.05, 97.8±17.0, 26.7±1.2 in RA. Serum P was higher in RA than C, 12.2±1.9 and 9.4±2.0 mg/dl, respectively (p<.01). Serum Ca in C was 5.9±0.5 and 6.0±0.5mg/dl in RA (p > .05). Na-dependent P uptake at 15 sec. incubation in RA was decreased by 15.7±4.9% compared to C (p<0.02, n=7). At 60 min. P uptake was not different. Na-dependent G uptake was similar at both 15 sec and 60 min. These data show that by 1 hr. of RA, Na-dependent P uptake is inhibited. To determine if MA of short duration also suppresses P uptake, MA was induced for 3 hrs. by I.V. NH_4Cl; C received NaCl. Despite severe acidemia (pH 7.4±0.02 in C, 7.1±0.04 in MA), P uptake was not different between MA and C. These data demonstrate that suppression of P uptake by BBM with RA occurs sooner than with MA. This could be due to a more rapid fall in intracellular pH in RA than MA, or to a rise in serum P observed with RA but not MA.

MECHANISMS OF CALCIUM MEDIATED CELLULAR DYSFUNCTION **M.Levi, B.A.Molitoris*, R.W.Schrier, and F.R.Simon*, VAMC, UTHSC and UCHSC, Dallas, TX, Denver, CO**

Increased cystosolic Ca impairs transport processes and may initiate cell injury, but the mechanisms are unclear. We studied the effects of in vivo hypercalcemia (HCa) on rat renal cortical brush border (BBM) and basolateral (BLM) membrane vesicles and mitochondria (Mito). Compared to normocalcemic (Con) rats, HCa did not alter Mito succinic dehydrogenase activity, succinate-, ADP-, or uncoupler-induced respiration, implying normal Mito function. In BLM HCa decreased Na,K-ATPase activity, 162± 3 in HCa vs 229± 9 µM /hr /mg in Con, p< .001, decreased total phospholipid content, 0.65 ±.04 in HCa vs. 0.74 ±.02 µM / mg in Con, p< .05, and decreased the total phospholipid to cholesterol molar ratio, 1.9 ±.1 in HCa vs 2.2 ±.1 in Con, p < .05. In BBM HCa decreased alkaline phosphatase activity, 568 ±44 in HCa vs 836 ±46 µM /hr / mg in Con, p < .001, decreased the total phospholipid content, 0.43 ± .02 in HCa vs 0.48 ±.02 µM /mg in Con, p <.05, and decreased total phospholipid to cholesterol molar ratio, 1.0 ± .05 in HCa vs 1.2 ±.04 in Con, p< .05. Thus, HCa causes significant alterations in BLM and BBM enzyme activity, phospholipid metabolism and possibly fluidity. These plasma membrane defects may impair transport processes and initiate cell injury.

ALUMINUM (AL) TOXICITY DECREASES PTH RESPONSIVENESS TO HYPOCALCEMIA IN THE RAT. F. Llach, M.Rodriguez*, A.J. Felsenfeld. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

Dialysis osteomalacia has been associated with both AL toxicity and a relative PTH deficiency. To test if AL toxicity is capable of inhibiting PTH secretion, hypocalcemia (↓Ca) was produced by a zero calcium (Ca) peritoneal dialysis (PD) in 3 groups of rats: I, control; II, received 5 mg of intraperitoneal (IP) AL daily for 3 days and then studied; and III, rats of Group II studied 30 days later. As shown below, the plasma Ca (mg/dl) fell with PD.

Group	0 (min)	30 (min)	120 (min)
I	9±.3	7.8±.3+	7.9±.2+
II	9.4±.3	8.3±.2+	7.9±.1+
III	9.6±.2	8.6±.3*	8.3±.3*

Mean±SE *p<.02; +p<.005 compared to baseline.

In all groups, the PTH levels [both amino (N) and carboxy (C)] significantly increased from baseline. As shown below, AL inhibited basal PTH levels and PTH responsiveness.

Group	N-PTH (0';ng/ml)	C-PTH (0';ng/ml)	N-PTH (30';ng/ml)	C-PTH (30';ng/ml)	N-PTH (120';ng/ml)	C-PTH (120';ng/ml)
I	.4±.1	.8±.1	1.2±.3	1.6±.1	1.2±.1	1.6±.1
II	.2±.1*	.9±.1	.4±.1*	1.3±.1	.5±.1*	1.2±.1*
III	.3±.2	.7±.1	.7±.2	1.5±.2	.5±.2	1.4±.2

Mean±SE *p<.05 compared to Group I at same time. In addition, ΔN-PTH and ΔC-PTH were reduced in Group II at 30 and 120 minutes (p<.05).

In summary, in this model: 1) zero Ca PD lowers plasma Ca; 2) PTH increases in response to ↓Ca; and 3) Groups II and III have decreased PTH responses to ↓Ca. In conclusion: 1) zero Ca PD provides an effective animal model to study the PTH response to ↓Ca; and 2) AL toxicity inhibits PTH secretion.

HYPOCALCEMIA MAY NOT BE THE CAUSE OF THE DEVELOPMENT OF SECONDARY HYPERPARATHYROIDISM S. Lopez,* T. Galceran,* W. Chan,* N. Rapp,* K. Martin and E. Slatopolsky. Washington University Medical School, St. Louis, MO.

It is currently accepted that hypocalcemia is the main factor responsible for the genesis of secondary hyperparathyroidism (S.H.) in chronic renal disease. Recently, evidence has been published from "in vitro" studies with parathyroid cells obtained from uremic patients, indicating that there is a shift in the set point for calcium. Thus, in uremia a higher concentration of ionized calcium (ICa) may be necessary to suppress the release of PTH. The present studies were performed in dogs to further clarify this new potential mechanism. To prevent the development of hypocalcemia six dogs were fed a high calcium diet (6.4 gm Ca/day); after obtaining control studies the GFR was reduced by approximately 70%. Catheters were implanted in the jugular vein, and blood samples were obtained every 4 hours for one week and then 3 times a week for a month. To measure biologically active PTH a new highly sensitive (1 pg/tube) RIA for amino-terminal PTH was developed in our laboratory. Initially, ICa was 4.79 ± 0.09 mg/dl and gradually increased after 1 week of renal failure to 4.98 ± 0.14 and after 1 month to 5.12 ± 0.10 mg/dl. Despite a moderate increase in ICa, i-PTH increased from 64 ± 7.7 to 118 ± 21 pg/ml. In summary: during one month of renal failure, the dogs at no time developed hypocalcemia; however, there was a 84% increase in i-PTH levels, suggesting that hypocalcemia, per se, may not be the only factor responsible for the genesis of S.H. Thus, an abnormal secretory mechanism in the parathyroid cells of uremic dogs may play an important role in the development of S.H.

EVIDENCE FOR INVOLVEMENT OF LUMINAL MEMBRANE PHOSPHOLIPIDS IN THE INHIBITORY EFFECT OF PARATHYROID HORMONE (PTH) ON TRANSPORT IN RABBIT PROXIMAL CONVOLUTED TUBULES. **J. W. McKeown, VAMC, Lexington, KY and Univ. of Kentucky, Lexington, KY.**

Exposure of rabbit renal cortical tubules to PTH results in an increase in the acidic phospholipid content of these cells, an effect which is inhibited by cycloheximide (CH) (Farese, et al, Ann. N.Y. Acad. Sci., 1981). The present studies were designed to investigate the possible role of luminal membrane phospholipids in the inhibitory effect of PTH on fluid (J_V) and phosphate (J_{PO_4}) transport in rabbit proximal convoluted tubules perfused in vitro. The effect of PTH on J_V and J_{PO_4} was measured under control conditions followed by two maneuvers designed to alter 1) calcium binding to luminal acidic phospholipids (Gentamicin (G), 200 μM to perfusate) or 2) the increase in acidic phospholipids in response to PTH (CH, 2 mM to bath). Under control conditions, PTH significantly and reversibly inhibited both J_V and J_{PO_4}. G added to the perfusate resulted in no change in J_V or J_{PO_4} and subsequent addition of PTH to the bath caused no inhibition of J_V (1.24± 0.14 vs. 1.24±0.17) or J_{PO_4} (1.24±0.38 vs. 1.12± 0.41) compared to the significant reduction seen with PTH under control conditions. Similarly, bath addition of CH blocked the inhibition by PTH of J_V (1.27±0.24 vs. 1.34±0.27) and J_{PO_4} (1.90±0.36 vs. 3.02±1.12).

These results demonstrate that changes in acidic phospholipids induced by PTH are importantly involved in the inhibitory effect of PTH on transport and suggest that these changes occur in the luminal membrane where the mechanism involved is sensitive to blockage by G.

SERUM PARATHYROID HORMONE LEVELS PREDICT PREFERENTIALLY OSTEOBLASTIC ACTIVITY IN PATIENTS WITH RENAL FAILURE. HH Malluche, R Reitz*, D Endres*, MC Faugere*. Univ. of KY, Div. Nephrology, Bone & Min. Metab., Lexington, KY; Nichols Inst., San Juan, Capistrano, CA; Endo. Metab. Ctr., Oakland, CA.

Parathyroid hormone levels (PTH) in blood have been shown to correlate with number of osteoclasts (OC) in bone. However, PTH receptors were found in osteoblasts (OB) but not in OC in vitro. Improved histologic techniques allow us to assess number and activity of OB and OC in vivo. We correlated parameters of cell number and cell activity with results of different PTH assays. Bone biopsies were obtained in 15 patients on chronic dialysis. Blood was drawn at time of biopsy for determination of PTH recognizing the C-terminal (C), mid-molecular (M) and N-terminal region (N). Coefficients of correlation are given below:

	C	M	N
Osteoblastic parameters			
Number of osteoblasts	0.84∞	0.63Ψ	0.90∞
Bone-osteoblast interface	0.87∞	0.61Ψ	0.87∞
Bone formation rate	-0.13	0.38	0.73∞
Osteon formation time	-0.57	-0.64Ψ	-0.66Ψ
Osteoclastic parameters			
Number of osteoclasts	0.54	0.35	0.71Ψ
Bone-osteoclast interface	0.38	0.17	0.54
Bone resorption rate	-0.11	0.25	0.72Ψ
Osteon resorption time	-0.21	-0.39	0.05

Ψ $p<0.01$ ∞ $p<0.001$

The data indicate that serum PTH predicts preferentially osteoblastic activity. This provides in vivo evidence that osteoblasts are the main target cells for PTH action on bone. Coupling between osteoblasts and osteoclasts might explain previous data on relationship between PTH and osteoclasts.

ABNORMAL BONE DENSITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT: DIFFERENTIAL EFFECT OF DIETARY NA^+ and CA^{2+}. J. Metz,*N. Karanja,*D. McCarron, Oregon Health Sciences University, Portland, OR.

The spontaneously hypertensive rat (SHR) exhibits multiple cellular and end-organ defects of Ca^{2+} metabolism. Dietary Ca^{2+} exerts a protective BP effect that is enhanced by Na^+ in the SHR and its control, the WKY. We assessed bone mineral content and density in the SHR and WKY on varying Ca^{2+} and Na^+ diets.

27 SHRs and 23 WKYs were assigned to 1 of 5 diets at 6 wks of age (WOA): Low Ca^{2+} (.1%)/low Na^+ (.25%); low Ca^{2+}/high Na^+ (1%); high Ca^{2+} (2%)/low Na^+; high Ca^{2+}/high Na^+ and control 1% Ca^{2+}/.45% Na^+. At 54 WOA, animals were sacrificed and femurs excised. Bone density was measured using direct photon absorpitonmetry. ANOVA and multiple range testing were used.

Mean (±SD) Bone Densities (g/cm^2) by Diet:

	LC/LN	LC/HN	HC/LN	HC/HN	Control
SHR	.205±.01	.185±.02	.232±.04	.202±.05	.242±.03
WKY	.206±.02	.199±.02	.260±.02	.263±.02	.269±.02

Independent of diet, bone density was reduced ($p<.0001$) in the SHR and modified ($p<.0001$) by diet, however, the SHR's response differed ($p<.03$) from the WKY. High Na^+ lowered bone density in the SHR from control ($p<0.05$) and was worsened by a low-Ca^{2+} intake, whereas high Ca^{2+} increased bone density in the WKY compared to low Ca^{2+} despite Na^+ intake. Low Ca^{2+}/high Na^+ resulted in the lowest bone density in both SHR and WKY ($p<.05$). Consistent with its other Ca^{2+} defects, the SHR has abnormal bone mineral metabolism. Its differential response to diet Ca^{2+}/Na^+ manipulation compared to the WKY may provide a relevant model of diet-related bone disease.

PHOSPHATE (P) AND CALCIUM METABOLISM IN CHRONIC RENAL FAILURE (CRF). R. Mitro,* T. Pitts, B. Piraino, G. Segre,* T. Chen, A. Greenberg and J. Puschett, Univ. of Pittsburgh, Pittsburgh, PA., and Mass. General Hospital, Boston, MA.

A popular thesis regarding the genesis of the secondary hyperparathyroidism of CRF holds that P retention is the initial event. Alternatively, deficiency of 1,25-dihydroxycholecalciferol (1,25D) may play a primary role. We measured serum P, ionized calcium (Ca^{++}), intact immunoreactive parathyroid hormone (IPTH), 1,25D, creatinine clearance/1.73 m^2 (CCR) and fractional P excretion (FEP) in normals and 41 patients with a wide range of CRF (CCR 5-91ml/min). 1,25D correlated directly with CCR ($r=0.71, P<0.001$) but did not fall below the normal range until advanced CRF occurred (~30 ml/min). IPTH and FEP were directly correlated ($r=0.53, P<0.001$) and each correlated inversely with CCR ($r=-0.46, P<0.01$; and $r=-0.75, P<0.001$, respectively). Both IPTH and FEP were elevated with early CRF. IPTH correlated directly with serum P ($r=0.53, P<0.01$), although serum P remained normal until late in CRF (~20-30 ml/min). IPTH did not correlate with 1,25D or Ca^{++}. 1,25D correlated inversely with serum P ($r=-0.54, P<0.001$) and directly with Ca^{++} ($r=0.41, P<0.01$) We conclude that 1,25D levels do not fall until late in the course of CRF, well after the rise in IPTH and FEP has occurred. Either P retention or loss of renal mass with consequent refractoriness to IPTH may account for the diminished production of 1,25D in response to elevated IPTH. This refractoriness could also account for the lack of correlation between Ca^{++} and IPTH. In addition, renal tubular resistance to the antiphosphaturic effects of 1,25D could contribute to the phosphaturia seen in early CRF.

NATURE OF THE RENAL PO_4 TRANSPORT ABNORMALITIES IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). T. Northrup* D. Zikos and K. Lau. Michael Reese Hospital and University of Chicago, Chicago, Il.

Compared to their normotensive Wistar Kyoto (WKy) control, 9 to 14 weeks old SHR reabsorbed PO_4 more avidly (KI 25:789). To evaluate the mechanism, balance and brush border membrane (BBM) vesicle studies were performed in 14 weeks old rats fed a normal P (0.60%) diet. Serum P (5.6 vs 5.1 mg/dl) and PO_4 metabolism (as means in mg/d) were similar (Table), arguing against PO_4 deficiency.

	(N)	Oral	Fecal	Absorbed	Urine	Retained
WKy	7	91±2	41±2	50±2	37±2	13±1
SHR	7	94±4	45±2	49±2	38±3	11±2

BBM vesicles data (in p moles/mg protein) are: (§, $p<0.05$ vs WKy)

	Na-specific Pi uptake			V max
WKy	554±78	195±40%	(of equilibrium)	980±72
SHR	983±86§	414±70%§		1,245±67§

There were no differences in Na-independent uptake, equilibrum uptake at 2 hours, uptake of D-glucose or apparent Km values (111 vs 108μM). Although hydralazine in prior studies normalized PO_4 excretion rates in SHR, hydralazine treatment of PTX WKy rats was also associated with an increase in fractional PO_4 delivery to late proximal tubule (57 vs 45%) and urine (10.3 vs 2.7%), suggesting a pressure-independent effect exerted by this agent and arguing against hypertension per se as the mediator of the transport abnormalities in SHR.

We conclude that the enhanced capacity for PO_4 reabsorption in young SHR is not due to PO_4 deficiency. Furthermore, this abnormality is expressed at the level of proximal tubule brush border membrane as an increase in PO_4 transport carrier. The role of hypertension remains to be defined.

THE EFFECT OF LITHIUM ON PARATHYROID HORMONE (PTH) SECRETION AND THE PHOSPHATIDYLINOSITOL CYCLE. J. J. Morrissey, Washington University School of Medicine, St. Louis,MO.

Lithium is known to increase the set point for calcium for the inhibition of PTH secretion. The molecular mechanism by which lithium affects PTH secretion was examined in collagenase-dispersed bovine parathyroid cells. The incorporation of [^{3}H]inositol into inositol phosphate, isolated by anion exchange chromatography, or into thin layer chromatographically isolated phosphatidylinositol as a function of medium calcium and lithium concentrations was determined. An increase in calcium concentration in the medium from 0.5 to 1.0 or 2.0 mM caused a significant decrease in the amount of measurable [^{3}H]inositol 1-phosphate and a significant increase in measurable [^{3}H]phosphatidylinositol along with an inhibition of PTH secretion. These alterations were evident within 2 minutes of the change in medium calcium concentration. At a medium calcium concentration of 1.0 mM, 10 mM lithium significantly stimulated PTH secretion and [^{3}H]inositol incorporation into inositol 1-phosphate and phosphatidylinositol. The stimulation of secretion and inositol 1-phosphate were to levels indistinguishable from those measured at 0.5 mM calcium. At either 0.5 or 2.0 mM calcium, 10mM lithium did not significantly affect secretion or labeled inositol incorporation into inositol 1-phosphate. These results suggest that calcium and lithium affect the activity of the phosphatidylinositol cycle of parathyroid cells. The activity of the cycle determines diglyceride levels and, in turn, protein kinase C-driven PTH secretion.

VOLUME EXPANSION (VE) - INDUCED PHOSPHATURIA IN THE RABBIT - PARTICIPATION OF THE PROXIMAL STRAIGHT TUBULE (PST). Thomas O. Pitts, Tracy Buckenheimer*, Thomas Brown*, Fred Schuler*, Jules B. Puschett. Univ. of Pittsburgh, Dept. of Med., Pittsburgh, PA.

Micropuncture data indicate that VE inhibits phosphate (P) reabsorption in the proximal convoluted tubule. It is not known if a similar effect occurs in the PST. In acute clearance studies, we examined the effects of 6% (body weight) VE on P excretion in intact New Zealand rabbits fed a 1.6% calcium, 0.45% phosphorous diet, intended to suppress parathyroid hormone secretion. In separate sets of similarly-fed animals, we used isolated tubular perfusion to examine the reabsorption of fluid (JV) and P (JP) in the PST during control conditions (Group I, n=8) and following VE (Group II, n=8). In both groups, perfusion was carried out for two sequential periods of 30-45 min. to determine the persistence of any observed transport alterations between the groups. Time control clearance studies (n=7) revealed no changes in any measured parameter. VE (n=6) increased the clearance of inulin: 10±1 to 18±3 ml/min ($P<.02$), and % excretion of P:2±1 to 7±2%, ($P<.01$), but did not alter blood pressure, acid-base status, or ionized calcium. Group I tubules revealed the following transport values: JV=0.31±.03 nl/mm·min and JP=2.62 ±.47 pmol/mm·min with no change over two collection periods. PST's of Group II rabbits showed: JV=0.18±.03 nl/mm·min ($P<.02$ vs Group I), and JP= 1.58±.10 pmol/mm·min ($P<.05$ vs Group I). These differences persisted throughout the two collection periods. We conclude that VE induces a phosphaturia in the rabbit, which results in part from an inhibition of P reabsorption in the PST.

VARIABILITY OF THE PHOSPHATURIC ACTION OF PARATHYROID HORMONE (PTH) IN THE RABBIT - EFFECT OF PTH PREPARATION. Jules B. Puschett, Thomas O. Pitts, and Tracy Buckenheimer*. University of Pittsburgh School of Medicine, Dept. of Med., Pittsburgh, PA.

Induction of phosphaturia in the rabbit by PTH infusion has been observed by some workers but is not a consistent finding. We compared the effects of equivalent (by weight) doses of highly purified synthetic bovine, 1-34 PTH from three manufacturers on the urinary excretion of phosphate (P) and calcium (CA) in acute clearance studies in the intact rabbit and on the reabsorption of P (JP) in the isolated perfused rabbit proximal straight tubule (PST). Fractional excretion (FE) of P (FEP) and Ca (FECa) are shown below during control (C) and PTH (0.3 mcg/kg priming, 0.15 mcg/min sustaining) infusions:

	FEP(%)		FECa(%)	
Preparation	C	PTH	C	PTH
Beckman(n=6)	5±2	9±1(P<.01)	19±3	11±2(P<.01)
Bachem(n=7)	4±1	8±1(P<.01)	15±2	6±1(P<.01)
Peninsula(n=5)	2±1	2±1(P>0.5)	12±3	4±1(P<.05)

Phosphaturia was uniformly associated with a decline in serum bicarbonate and carbon dioxide tension without pH change. Serum ionized Ca rose similarly in response to PTH in all studies. Beckman PTH (1 mcg/ml in bathing fluid) inhibited JP in the perfused PST: C:2.61±.54, PTH:1.82±.29 pmol/mm·min, P<.01, n=8. Peninsula PTH (1 mcg/ml) did not inhibit JP: C:3.56±.36, PTH:3.50±.33, P:NS, n=14. We conclude that the phosphaturic effect of PTH may be dissociated from its calcemic and anticalciuric actions due to variations in its commercial preparation. This may underlie, in part, the discrepancies in the literature regarding its phosphaturic action in the rabbit.

MODULATION OF THE BASOLATERAL PHOSPHATE TRANSPORT SYSTEM IN THE RENAL CELL LINE LLC-PK$_1$. **Carlos A. Rabito. Nuclear Medicine Division, Dept of Radiology, Massachusetts General Hospital, Boston, Massachusetts.**

Although the mechanisms involved in the inorganic phosphate (P_i) transport across the apical membrane during the P_i absorption by the proximal tubule are well characterized, the mechanisms involved in the exit step through the basolateral membrane are still imperfectly understood. Previous studies performed in the renal epithelial cell line LLC-PK$_1$ (Kidney Int 25:152, 1984) show the presence of two Na^+-dependent P_i transport systems localized in the apical and basolateral membrane of the epithelial cells. The apical system is similar to the system observed in the apical membrane of the renal proximal tubular cells. The basolateral system, is also Na^+-dependent and inhibited by As, but transport specifically monobasic P_i and is inhibited by bicarbonate. The present study analyzes the effect of different inhibitors, anion composition and pHs of the incubation media on the Na^+-dependent P_i transport by LLC-PK$_1$ monolayers under different growing conditions. The results show that not only bicarbonate and Glimidine but also $^-$OCN, acetate, formate and PAH are non-competitive inhibitors of the basolateral P_i transport system. The transport of Glimidine and PAH, however, are not inhibited by P_i suggesting an indirect mechanism in the inhibitory effect of these agents. The P_i influx across the basolateral system is markedly reduced in cells in exponential growth. No differences were observed, however, in the influx through the apical system. Inhibition of cell division or incubation of pH 7.0 do not increase the P_i influx. Full recovery occur, however, when the monolayers reach confluency. It is concluded that the apical P_i transport system under special circumstances could supply the P_i to cover the metabolic needs of the epithelial cells.

CHARACTERISTICS OF PHOSPHATE TRANSPORT ACROSS THE BASOLATERAL MEMBRANE OF THE PROXIMAL TUBULE. Gary A. Quamme, Department of Medicine, Health Sciences Center Hospital, University of British Columbia, Vancouver, B.C.

Studies were designed to characterize phosphate (Pi) transport across the basolateral membrane (BLM) of the rat proximal tubule. The effects of pH, Na and trans-stimulation by Pi were studied on the apparent kinetics of Pi uptake. All incubations were voltage (Em=0 mV) and pH (pH_i=pH_o) clamped and terminated at 6 sec. Two components of Pi uptake were observed; a sodium-dependent and a Pi trans stimulated component. The sodium-dependent Pi uptake demonstrated bell-shaped pH dependence, maximally at pH 7.0 and low uptake at pH 8.0 > pH 6.0 (pH_i = pH_o). At a fixed Pi (0.1 mM) J^{Pi} increased in sigmoid fashion as the external Na was increased 0 to 200 mM; the Km was 27 mM at pH 8.0 with an apparent Hill coefficient >1.0. Saturation kinetics of sodium-dependent Pi uptake at fixed Na (100 mM) was Jmax 2600 picomole.mg^{-1} protein .6 sec^{-1} and Km, 16 mM. Sodium-dependent Pi uptake under these conditions, was inhibited by external (5 mM) SITS, DIDS, and arsenate but not sulfate, bicarbonate, chloride or acetazolamide. Trans stimulated, sodium-independent Pi uptake was not affected by these anions and was maximally stimulated at a transmembrane Pi gradient of 2.5 mM. This data suggests that the BLM movement of Pi may be stimulated by the presence of sodium, trans stimulated by Pi and highly dependent on the pH. In addition, these results suggest that this transport is not shared by the other major anions.

INTERACTION OF BLOOD IONIZED CALCIUM (Ca++) AND SERUM 1,25(OH)$_2$D$_3$ (1,25D) DURING PTH INFUSION IN UNANESTHETIZED RATS. GS Riera*, DA Bushinsky, MJ Favus*, FL Coe. University of Chicago, Chicago IL.

In at least one example, metabolic acidosis, Ca++ down-regulates serum 1,25D levels. Because 24 hr PTH infusion raises [Ca++], and PTH itself can stimulate 1,25D production, the net effect of PTH infusion on 1,25D may be difficult to predict. A 24 hr infusion of bovine PTH 1-34 (10u/kg/hr) decreased serum 1,25D to 12 ± 3 pg/ml in 11 rats vs 35 ± 6 in 11 controls ($p < .005$). PTH infusion increased [Ca++] from 1.26 ± .02 mM to 1.37 ± .01 ($p < .001$). To determine if the increase in [Ca++] suppressed 1,25D, we infused rats with PTH (10u/kg/hr) plus varying doses of EGTA for 24 hr through venous catheters placed 12 days prior to the infusion. We drew blood from arterial catheters implanted simultaneously. Rats ate a normal calcium (1.2% calcium) diet and drank distilled water. During PTH + 0.67 um/min EGTA, [Ca++] was 1.20 ± .01 ($p < .03$ vs control or PTH alone) and 1,25D was 90 ± 33 ($p < .01$ vs PTH alone) in 7 rats. During PTH + 1.00 um/min EGTA, [Ca++] was 1.19 ± .03 ($p < .002$ vs PTH alone) and 1,25D was 148 ± 29 ($p < .001$ vs control or PTH alone) in 6 rats. During PTH + 1.33 um/min EGTA, [Ca++] was 1.14 ± .04 ($p < .03$ vs control or PTH alone) and 1,25D was 267 ± 46 ($p < .001$ vs control or PTH alone) in 7 rats. Serum phosphate and arterial blood pH were the same in all groups. Log 1,25D varied inversely with [Ca++] during all PTH infusions ($r = -.737$, $n = 31$, $p < .001$).

During 24 hr PTH infusion in the rat the effects of increasing blood [Ca++] to suppress 1,25D are more potent than the known stimulatory actions of PTH on 1,25D.

INTRAVENOUS (IV) ALUMINUM (AL) INCREASES TOTAL PLASMA CALCIUM (Ca), DECREASES IONIZED CALCIUM (iCa), AND SUPPRESSES THE PARATHYROID HORMONE (PTH) RESPONSE TO HYPOCALCEMIA (↓Ca) IN THE RAT. M. Rodriguez*, A. Felsenfeld, and F. Llach. Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

Aluminum toxicity is associated with a relative PTH deficiency. While assessing the effect of IV AL infusion on the PTH response to ↓Ca in the rat, it became apparent that IV AL produced hypercalcemia. Initially, 2 groups of rats were studied: Group I, a 2 hour Ca-free peritoneal dialysis (PD); and Group II, IV AL infusion (.2 mg/100g of body wt/hr) begun 20 minutes before Ca-free PD.

	0'		30'		120'	
	Ca (mg/dl)	PTH (ng/ml)	Ca (mg/dl)	PTH (ng/ml)	Ca (mg/dl)	PTH (ng/ml)
I	9±.3	.4±.1	7.8±.3	1.2±.3	7.9±.3	1.2±.2
II	10.8±.3	.4±.1	11.1±.5	.6±.1	11.2±.8	.6±.1

Mean±SE. In Group II, plasma iCa at 120' was less than normal (3±.2 vs 5±.1 mg/dl, $p < .01$).

To study the effect of AL IV on total and iCa, 2 additional groups received 2 hour IV AL infusions without Ca-free PD: Group III, .2 mg/100g/hr; and Group IV, .1 mg/100g/hr.

	0'		30'		120'	
mg/dl	Ca	iCa	Ca	iCa	Ca	iCa
III	9.7±.2	5.1±.1	10.5±.2*	4.4±.2*	12.7±.7*	4.1±.2*
IV	9.7±.1	4.9±.1	9.7±.2	4.6±.1*	10.1±.1*	4.6±.1*

Mean±SE; *$p < .05$ compared to baseline.

In summary, after IV AL, the PTH response to ↓Ca is decreased and IV AL increases total Ca with a simultaneous decrease in iCa. In conclusion, IV AL: 1) reduces PTH secretion during ↓Ca and 2) produces hypercalcemia which is likely due to increased binding of plasma Ca and a shift of Ca into the vascular space to restore iCa levels.

IDENTIFICATION OF ENLARGED PARATHYROID GLANDS BY RADIONUCLEOTIDE SUBTRACTION IMAGING IN CHRONIC HEMODIALYSIS PATIENTS. J.E. Rubin*, M.M. Maayan*, R. Johnson*, E.M. Volpert*, L. Bierman*, R.V. Sellitto*, G.M. Berlyne, Brooklyn Veterans Administration Medical Center, Brooklyn, NY.

Seventeen chronic hemodialysis patients underwent radionucleotide subtraction scanning to identify parathyroid gland enlargement using ^{99m}Technetium pertechnetate and 201 Thallium chloride (in a method similar to that of Young et al British Med J. 286, 1384-86, 1983). After scanning, the images were subtracted from each other by computer with the resulting image found to correlate anatomically with the location of the parathyroid glands. The patients were then divided into two groups; Group I which had very enlarged images suspected to be that of very large parathyroid glands and Group II: those which either failed to have parathyroid visualization or had very poor imaging of these glands. Eleven patients were in Group I while six patients were in Group II.

Group I patients were found to have statistically significantly higher values of N-terminal PTH ($p < .03$) and alkaline phosphatase ($p < .01$) levels (Mann-Whitney test) compared to those levels found in the patients in Group II. Good anatomical correlation was proven in one instance in a patient who underwent partial parathyroidectomy several years ago. Only the remaining parathyroid glands were visualized in this instance.

We conclude subtraction imaging is a safe and effective way to confirm the anatomic status and location of the parathyroid glands in patients with end stage renal disease. It is valuable at time of parathyroidectomy enabling rapid localization of the enlarged glands. In addition, the anatomical size of these glands can be easily verified and better follow-up achieved using this technique.

EFFECT OF SALMON CALCITONIN ON RENAL MAGNESIUM TRANSPORT IN THE MAGNESIUM-LOADED RAT. Denis R. Roy, Division of Nephrology, Royal Victoria Hospital, Montreal, Canada.

The mechanism whereby salmon calcitonin (SCT) lowers urinary magnesium excretion is still controversial. To determine the effect of SCT on renal magnesium transport, clearance and micropuncture experiments were performed in the following groups of thyroparathyroidectomized, mildly magnesium-loaded rats: a) Group 1 (N=8) water diuretic controls; b) Group 2 (N=8), similar to Group 1 but also receiving synthetic SCT (10mU/min); c) Group 3 (N=8) similar to Group 2 but receiving $CaCl_2$ to prevent the fall in plasma calcium (Pca 2.4 vs 1.8* vs 2.6 mM in Groups 1, 2 and 3 respectively). Plasma magnesium was similar in the three groups but SCT increased GFR significantly in Groups 2 and 3. SCT decreased urine flow rate (V) significantly from 52 ± 6 to 20 ± 3* and 26 ± 5* ul/min/gKW and fractional magnesium excretion (FE Mg) from 71 ± 4% to 45 ± 3%* and 54 ± 3%* in Groups 1, 2 and 3 respectively. A significant relationship was observed between FE Mg and V ($Y=42.9 + 0.42X$, $r=0.56$, $p < 0.01$). Within each group fractional magnesium delivery to the superficial end-accessible proximal tubule and juxtamedullary end-descending limb was similar. Our results suggest that: 1) in magnesium-loaded water diuretic rats, the fall in FE Mg following SCT is related in part to the fall in V; 2) the fall in FE Mg occurs even if plasma calcium is maintained at normal value; and 3) the effect of SCT is localized in part in the thick ascending limb. (* $p < 0.05$ vs Group 1).

THIAZIDES STIMULATE Ca ABSORPTION IN THE ISOLATED TURTLE BLADDER INDEPENDENT OF AN EFFECT ON Na TRANSPORT. S Sabatini, University of Illinois College of Medicine, Chicago, Illinois.

Thiazides (Tz) have long been noted clinically to decrease Ca excretion in a variety of hypercalciuric states. It is generally accepted that the primary mechanism of action is due to volume contraction and enhanced Ca absorption by the proximal tubule. This study was designed to examine the effect of Tz on Na transport, proton secretion, and bidirectional and unidirectional Ca^{45} fluxes in the isolated turtle bladder, an analogue of the mammalian collecting duct. When added to the mucosal solution, 1 mM Tz did not affect Na transport or proton secretion, but significantly increased mucosal to serosal Ca^{45} flux at 60 min (Control 118.9 ± 38.7; Tz 286.0 ± 64.9 pmol/mgm wet wt/60 min, n=16, $P<0.02$). In the presence of 1×10^{-4}M ouabain (serosa), Tz significantly enhanced mucosal to serosal Ca^{45} flux. The increment of Ca transport under these conditions was 83.4 ± 35.2 pmol/mgm wet wt/60 min, n=8, $P<0.05$. When bidirectional Ca^{45} fluxes were measured, Tz decreased the normally present net Ca^{45} secretory flux to zero by increasing the absorptive flux. These results show that Tz directly increase mucosal Ca transport in the isolated turtle bladder independent of an effect on Na transport. If these results can be extrapolated to the mammalian nephron, it is likely that the effect of Tz to decrease Ca excretion in hypercalciuric states is mediated in part by a direct effect on the distal nephron.

INTRACELLULAR CALCIUM AND ITS REGULATION IN PROXIMAL RENAL CELLS. Lakhi M. Sakhrani,* Bill Lambert,* Achour Laradi,* and Shaul G. Massry. Division of Nephrology, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California.

The ability to measure intracellular calcium and calcium transients is an important prerequisite for understanding cellular calcium homeostasis. Therefore, we have utilized the fluorescent probe Quin 2 to measure intracellular calcium and calcium transients in primary culture of proximal rabbit renal cells. The cells were loaded with the ester of Quin 2 and changes in cellular fluorescence (excitation 339 nm, emission 492 nm) followed during various maneuvers. Basal cytosolic calcium was 90±15 nM and increased by 4-5 fold to 420±10 nM in the presence of the calcium inophore A23187 (100 nM). The mitochondrial inhibitor rotoneone (50 uM) resulted in an increase in cytosolic calcium to 300±15 nM. Since renal cells have been reported to possess Na-Ca exchanger, we evaluated the importance of this exchanger in regulating renal cell cytosolic calcium. The addition of 1 mM ouabain resulted in no change in cystolic calcium. Furthermore, the substitution of extracellular sodium with choline gave similar levels of cytosolic calcium 80±16 nM and the addition of 10 mM Na to sodium-free solution resulted in no change in cytosolic calcium. We conclude: 1) renal cell calcium and calcium transients can be measured by Quin 2, 2) the basal cytosolic calcium in proximal renal cells is 90±15 nM, and 3) Na-Ca exchanger does not play a role in regulating cytosolic calcium in these cells.

Na^+ INDEPENDENT PHOSPHATE (Pi) TRANSPORT IN BASOLATERAL MEMBRANE VESICLES FROM DOG KIDNEY. S.J. Schwab,* and M.R. Hammerman. Washington Univ. School of Med., Dept. of Int. Med., St. Louis, MO.

We previously measured Na^+ independent ^{32}Pi uptake in canine basolateral membrane vesicles (BLMV) in order to characterize the mechanism by which reabsorbed Pi exits from the proximal tubular cell across the basolateral membrane (Clin.Res. 32:535A, 1984). Our data were consistent with the Na^+ independent exit of negatively charged Pi across this membrane down an electrical-chemical gradient. Measurements of ^{32}Pi uptake in BLMV did not clearly differentiate among binding, diffusion, and transport of ^{32}Pi. In order to isolate the mechanism for transport of ^{32}Pi in BLMV we examined the effects of membrane potential and of an inhibitor of anion exchange (DIDS) on counterflow of ^{32}Pi in BLMV measured in the absence of Na^+. Counterflow was documented by preloading BLMV with 2 mM Pi and demonstrating increased uptake of ^{32}Pi at early times of measurement. Counterflow of ^{32}Pi was inhibited 46% by the induction of a membrane potential with valinomycin (intravesicular negative) suggesting that the transport component of uptake in BLMV was accompanied by movement of negative charge. The addition of DIDS (50 uM) in SO_4^- preloaded BLMV inhibited counterflow of $^{35}SO_4^-$ by 50%. In contrast, the addition of DIDS had no significant effect on counterflow of ^{32}Pi in BLMV from the same preparation. Thus no evidence for anion exchange of Pi in BLMV was found. We conclude that carrier mediated, Na^+ independent Pi transport can be demonstrated in BLMV. This process may reflect the mechanism by which Pi absorbed across the luminal membrane exits from the proximal tubular cell.

RELIABLE MEASUREMENT OF URINE AND PLASMA OXALATE BY GC/MS. Jon I. Scheinman, Diane Gale,* Charles R. Roe,* and David S. Millington.* Duke Univ. Med. Ctr., Div. of Nephrology & Metabolism, Dept. of Pediatrics, Durham, North Carolina.

Difficulties in the measurement of plasma oxalate by all except in vivo isotope dilution methods include spontaneouus changes in the composition of the sample and the lack of appropriate internal standards. Stability of both plasma and urine oxalate is provided by acidification (pH < 1) and storage at -70°. The use of stable ^{13}C- oxalate as internal standard obviates problems of recovery. Analysis is by combined gas chromatography/mass spectrometry (GC/MS). One-step extraction with ethyl acetate and derivitization with TBDMS provides single, distinguishable and reproducible GC peaks defined by selected ion current profiles at M/Z 261 (unlabelled oxalic) and at M/Z 263 (^{13}C-oxalic). The ratio of signals enables rapid quantitation of oxalic acid with high accuracy in the linear standardization range of 0.9-45 mg/dl for urine and 0.018-1.8 mg/dl for plasma. Analytical reproducibility in plasma is ± 2.2%, for samples ± 11%. Normal plasma oxalate, 0.057 ± .007 mg/dl is similar to that measured by in vivo isotopic dilution, and that obtained by CGC without MS. Accuracy, reproducibility, lack of interference, independence of recovery, simplicity of sample preparation and speed of analysis (< 5 min/sample), makes the method cost effective for clinical applications.

Specific applications have included measurement of oxalate clearance in vivo and by dialysis and the assessment of therapy in patients with oxalosis prior to and after transplantation.

IMMUNOCYTOCHEMICAL LOCALIZATION OF CALELECTRIN IN RENAL AND NON-RENAL TISSUES. Karen Sherrill*, Dennis K. Stone, Thomas C. Sudhof*, and Fred Silva. Univ. Texas Hlth. Sci. Ctr. at Dallas, TX.

Calelectrins are a new class of Ca^{2+}-binding proteins, which are distinct from calmodulin and synexin. Their presence in numerous species and distinctive localization in organ homogenates suggest a role in Ca^{2+}-triggered cell membrane traffic and fusion events. Previous studies have not determined the exact cellular sites of calelectin within the organs studied.

Primary antiserum from New Zealand white rabbit against bovine liver calelectrin was affinity purified and was studied utilizing the peroxidase-antiperoxidase method on fixed, embedded tissues from Lewis rats. Control studies included lack of tissue staining with normal rabbit serum, peroxidase-conjugated secondary antiserum, or the primary antisera alone. Moreover, the affinity purified antibody did not bind to calmodulin.

The most intense cellular staining for calelectrin was found in the renal distal convoluted tubule and medullary collecting ducts, hepatic bile ducts, Paneth cells, pancreatic ducts and islets, intercalated discs of cardiac muscle, adrenal medulla, pulmonary bronchi, and leptomeninges. The distinctive immunocytochemical tissue localization of calelectrin corresponds to sites demonstrated in crude organ extracts and is strikingly different from the distribution of any known Ca^{2+}-binding proteins, thus suggesting a common function specialized to these cellular sites.

DIFFERENTIAL RESPONSE OF RENAL OSTEODYSTROPHY (RO) IN CAPD AND HEMODIALYSIS (HD) PATIENTS. N. Shusterman, M. Fallon*, F. Kaplan*, P. Audet*, G. Morrison, A. Wasserstein. Univ. of Penn. School of Medicine, Philadelphia, Pennsylvania.

To compare RO in ESRD we studied asymptomatic patients, 4 on CAPD and 6 on HD, receiving standardized treatment from the initiation of regular dialysis. CAPD and HD groups had similar age, sex, cause of ESRD, prior treatment [Al-containing phosphate binders (ALPB) only]; and did not differ in initial serum or bone parameters. Bone biopsy with tetracycline labeling was performed initially and at nine months. Serum PO_4 was maintained at $\leq$5.5 mg/dl with ALPB and total serum calcium (Ca_T) at 10-11 mg/dl with $CaCO_3$ 1.3 gm/d and varying doses of calcitriol. Doses of ALPB and calcitriol were similar in HD and CAPD. Results (mean±SD) including Ca_T (mg/dl), amino-terminal PTH (N-PTH-pg/dl), osteoclast count (OC-cells/mm^2), fibrosis (FIB-%), mineralization front (MF-%) and appositional rate (AR-μ/day) are shown. No biopsy showed bone Al-staining. Serum ionized calcium (Ca_i-mEq/L) was similar in HD and CAPD initially and rose to 2.77±0.13 and 2.50±0.13, respectively ($p<0.02$, HD vs CAPD).

CAPD	Ca_T	N-PTH	OC	FIB	MF	AR
PRE	9.5±0.7	107±29	2.1±1.9	2.9±3	15.5±19.8	0.3±0.6
POST	10.5±0.8*	73±69	1.6±1.6	2.1±2	50.3±21.9*	1.2±0.3*
HD						
PRE	9.4±0.5	103±78	1.4±1.3	4.6±8	32.0±32	0.5±0.8
POST	10.8±0.6†	40±31*	0.3±0.3*	0.1±.3	23.7±37	0.3±.05‖

*$p<0.05$, pre vs post, †$p<0.01$ pre vs post, ‖$p<0.05$, HD vs CAPD

PTH, OC and FIB were reduced in HD but not in CAPD. Conversely, MF and AR remained abnormally low in HD but improved in CAPD. We conclude that in comparably treated patients: 1) osteomalacia (OM) improved in CAPD but not in HD; 2) osteitis fibrosa (OF) improved in HD but had a variable course in CAPD; and 3) the differential response of OF, and possibly OM, may have been due to greater PTH suppression during treatment in HD, a consequence of greater increment in Ca_i. Other factors in CAPD may also account for these differences.

ROLE OF TRANSFUSION IRON (Fe) OVERLOAD IN METABOLIC BONE DISEASE IN MAINTENANCE DIALYSIS PATIENTS (MHD). J. Stivelman*; M. Lazarus, R. Hakim, T. Thornhill*, S. Doppelt*, A. Schiller*, and G. Schulman*; Brigham and Womens' and Mass. General Hospitals, Boston, Mass. We evaluated the relationship of Fe accumulation through blood transfusions (BTx) to development of bone disease in MHD patients (pts). Iliac crest biopsies (bx) from 11 MHD pts were obtained for bone pain, pathologic fractures, lower than expected serum PTH and occasional hypercalcemia. Ages:27-61, on MHD 57-188 months (m=112±14.3). All pts had at some time taken vitamin D analogues and $Al(OH)_3$ binders. Five pts had had subtotal parathyroidectomy; 5 were anephric. Mean [Ca++] 9.80±.18 mg/dl (range 8.66-10.4 mg/dl); [$PO_4^=$] 4.25±.30 mg/dl (2.58-5.40 mg/dl); PTH 534±176 uLEq/ml (130-1938 uLEq/ml); ferritin 12749±2587 mcg/l (6-28080 mcg/l); BTx rate 2.03±.38 u/mo (.6-5.1 u/mo); Fe burden 858.7±167 mg/kg body weight (126-2066 mg/kg). Histopathology of bx revealed both osteitis fibrosa cystic (OFC) and osteomalacia (OM) in 7, OFC alone in 2 and OM alone in 2. Eight bx were stained for aluminum (Al) and Fe. Al was in mineralization fronts (MF) in 7, and in cement lines (CL) in 2. Fe was found in 6 bx; in MF in 5 and CL in 3. Of the 5 bx with both OFC and OM, 4 were positive for Al and Fe, and one had Al alone. Of the 2 bx with OFC alone, 1 was positive for Al and Fe; one had Fe alone. Al only was present in the 1 bx with OM alone. The findings of heavy Fe exposure in pts with symptoms of metabolic bone disease, taken together with these bx findings, suggest Fe, as well as Al, may have pathogenic significance in OFC and OM in MHD pts.

VERAPAMIL BLOCKS THE ACUTE PTH-INDUCED DECREASE IN cAMP IN VASCULAR SMOOTH MUSCLE CELLS. R.C. Stanton, S.B. Plant,* D.A. McCarron, Oregon Health Sciences University, Portland, OR.

Parathyroid hormone (PTH) produces vasodilation in animals. The cellular mechanism of this vasodilation has not yet been determined. Experments with cultured bovine vascular smooth muscle cells (VSM) have shown that PTH induces an acute decrease in cAMP level that is maximal at 30 sec. Since PTH has been shown to act as a Ca++ ionophore, we assessed the effect of verapamil (V) on this acute decline in cAMP level.

Bovine VSM were grown in flasks using a subcultured cell technique. Each experiment used 4 flasks in which PTH (0.3 μg), PTH + V (0.1 mM) or V alone in a 1.5 mM Ca^{++} buffer was administered. Each flask was incubated for different times:

	cAMP pmol/mg Cell Protein (Mean±SEM)				
(±S.E.)	0 Min	0.5 Min	1 Min	3 Min	N
PTH	4.6±0.8	3.2±0.3	4.6±0.7	6.6±1.3	20
V	12.9±2.3	11.1±1.2	14.9±4.8	11.6±1.9	8
V+PTH	6.3±1.3	5.3±0.6	4.3±0.5	6.1±1.4	12

PTH caused a significant decrease in cAMP level at 30 sec ($p<.01$). After preincubating with V, incubation with PTH failed to decrease cAMP content. V alone, raised the baseline value ($p<.01$) but did not alter cAMP levels.

In conclusion, PTH causes an acute decrease in cAMP level in smooth muscle cells. The Ca^{2+} antagonist V, blocks this decrease. Thus, the PTH-induced decrease in VSM cAMP content may in part represent an action of PTH on Ca^{2+} channels.

RENAL MAGNESIUM WASTING FOLLOWING CISPLATIN THERAPY. R.A.L. Sutton, N.L.M. Wong, J.H. Dirks, C.M.L. Coppin*, and V. Mavichak*, Dept. of medicine, University of British Columbia, Vancouver, B.C.

We have studied 6 patients with persistent hypomagnesemia 2 to 6 years after the completion of cisplatin therapy for testicular cancer and 6 matched normal control subjects. The patients exhibited hypomagnesemia (1.3±0.1 vs 2.0±0.1 $p<0.01$) with a normal urinary Mg excretion (116±20 vs 114±12, NS), together with striking hypocalciuria (82±20 vs 206±20 mg/day respectively, $p<0.01$). Urinary electrolyte excretions were determined for 3 hours following a single i.v. dose of 40 mg furosemide. The patients showed a significantly smaller response than the controls in the first hour, with respect to urinary Na/Cr (1.0±0.1 vs 1.7±0.2 mEq/mg $p<0.05$), Mg/Cr (0.20±0.01 vs 0.31±0.03 mg/mg $p<0.05$) and Ca/Cr (0.48±0.03 vs 0.86±0.1 mg/mg, $p<0.05$) ratios, while in the subsequent 2 hours the patients showed a significantly exaggerated increase in these ratios, so that the net cumulative excretion of Mg after furosemide was greater in the patients (34 vs 27 mg Mg, $p<0.05$), and the hypocalciuria was abolished (83 vs 80 mg Ca, N.S.). These observations may be explained by a cisplatin induced defect in tubular furosemide secretion accounting for the blunted initial response to furosemide, together with a defect in the distal nephron resembling that caused by chronic thiazide administration. Such a distal lesion could account for magnesium wasting and enhanced calcium reabsorption, as well as the enhanced net response to furosemide.

MICROCYTIC ANEMIA AND ALUMINUM TOXICITY. R Swartz, J Dombrouski*, K Kluin*, M Burnatowska-Hledin*, G Mayor. Univ. of Michigan Med. Center, Ann Arbor MI, and Michigan State Univ., Lansing MI.

Aluminum (Al) accumulation in chronic dialysis is associated with bone and neurologic disease, but an easily recognized marker for Al toxicity has yet to emerge. Among 11 patients on dialysis from 3 to 13 yrs having musculoskeletal and/or neurologic symptoms with high serum Al levels, microcytic anemia without iron deficiency appears to be an important objective marker for Al intoxication in some cases. Desferrioxamine treatment in these 11 cases (500 to 1500 mg 3/wk, IV in 6 hemodialysis cases, IP in 5 peritoneal dialysis cases) was prospectively based on musculoskeletal signs (11/11), serum Al over 100 mcg/l (10/11), and microcytic anemia (7/11). Biochemical and x-ray findings were suggestive of coexistent hyperparathyroid bone disease in 4 cases, and abnormalities in EEG, speech and cognitive testing could not be distinguished from a group of patients without serum Al elevation except in 1 case of marked dialysis encephalopathy. Results of therapy included: (1) improved bone pain (9/11) in 2 to 6 wks, weakness (7/11) in 4 to 8 wks, and microcytic anemia (6/7) in 4 to 12 wks; (2) early worsening in the single case of marked encephalopathy when serum Al rose to 1380 mcg/l, but improvement with continued therapy; (3) a tendency to equal or improved phosphate control and decreased serum calcium despite switching from $Al(OH)_3$ to $CaCO_3$; and (4) a marked rise in alkaline phosphatase in 2 cases but no consistent change in parathyroid status. We conclude that Al toxicity comprises a complex clinical syndrome of musculoskeletal and neurologic disease with high serum Al levels in which reversible microcytic anemia may signal early diagnosis and responsiveness to appropriate treatment.

PATHOPHYSIOLOGY OF SPONTANEOUS HYPERCALCIURIA (SH) IN LABORATORY RATS: ROLE OF DERANGED $1,25(OH)_2D_3$ (1,25D) METABOLISM. D. Thomas*, C. Langman, B. Eby* K. Lau. Michael Reese Hosp. & Univ. of Chicago, IL

Recent data suggest a causal role of deranged 1,25D metabolism in the syndrome of idiopathic hypercalciuria (IH). To test this hypothesis, we evaluated if D availability and/or increased serum 1,25D are critical for the expression of hypercalciuria. Ca balance and D metabolites were studied in D deprived (-D) and D repleted (+D) ♂ progeny (p) born to the 7th generation normocalciuric (NC) and SH rats, a model analagous to IH (JCI 70: 835, 1982). Seven of 14 pSH and 2 of 21 pNC were found to have SH, defined as 2 S.D.>mean of control on a low Ca +D diet. Similar to man with IH, serum 1,25D was elevated in these 9 SH rats. However, their Ca excretion was also increased during D deprivation, when serum 1,25 was comparably reduced by substrate availability (§, $p<0.05$ vs NC).

	(+D)NC	(+D)SH	(-D)NC	(-D)SH
25OHD(ng/ml)	7.6±0.7	8.6±0.4	undetectable	
1,25D(pg/ml)	182±17	251±13§	102±6	106±6
urine Ca(mg/d)	0.6±0.03	1.2±0.1§	0.5±0.04	1.5±0.3§

Since urine cAMP was higher in SH rats (339 vs 259 nMoles/d), the data argue against a primary role of deranged 1,25D metabolism in the hypercalciuria. On -D diet, net Ca absorption (mean of 4 days) was uniformly negative (-0.8 vs -0.6 mg/d in NC). Thus, the hypercalciuria in SH (2±0.4§ vs 0.4±0.03 mg/d) was not secondary to increased absorption.

These findings indicate that spontaneous hypercalciuria is independent of absorption, serum 25 or 1,25D. The data best fit the hypothesis of a renal Ca leak, eliciting a compensatory increase in serum 1,25D if and when substrate is not limited.

EFFECT OF LONG-TERM DIETARY PHOSPHATE AND PROTEIN RESTRICTION (DPPR) ON VITAMIN D METABOLITES IN EARLY RENAL FAILURE(ERF). N.Tessitore,Bj.Lund*,L.Oldrizzi*,C.Rugiu*,G.Maschio* - Divisione di Nefrologia , Verona , Italy.

$1.25(OH)_2D_3$(1.25D)deficiency has been reported in adults with ERF and phosphate restriction has been shown to increase serum levels of 1.25D in healthy subjects.This study investigates the effect of long-term(30 ± 2 months) DPPR(P 700 mg,prote in 40 g/24 h)on vitamin D metabolites levels in 17 adults with ERF(Iothalamate Cl. 45 ± 4 ml/min). 11 patients(GROUP 1) had no change in renal function (GFR 45 ± 4 ml/min before(B) and 46 ± 6 after DPPR(A)); 6 patients(GROUP 2) had decreased renal function (GFR 44 ± 5 ml/min B and 13 ± 2 A).

	Controls (n=20)	GROUP 1(n=11) B	GROUP 1(n=11) A	GROUP 2(n=6) B	GROUP 2(n=6) A
s-P (mg/dl)	2.4-4.4	2.9±.2	2.7±.2	3.5±.3	3.6±.3
s-Ca (mg/dl)	8.8-10.3	9.4±.3	9.6±.1	9.0±.1	9.1±.1
i-PTH (mU/ml)	1.0-2.4	3.1±.4	3.2±.3	3.7±.5	6.2±.7*
25D (ng/ml)	12 - 42	19 ± 4	24 ± 3	27 ± 9	21 ± 5
1.25D (pg/ml)	18-46	9 ± 2	29 ± 7*	13 ± 5	15 ± 5
24.25D (ng/ml)	.7-2.2	.6 ±.1	1.7±.2*	1.0±.4	1.1±.4
Urine P (mg/24 h)	500-900	563±40	432±31*	494±20	320±22*
Muscle P (mM/100 g FFST)	27-31	-	27± 1	-	30± 2

* p <0.02 A vs.B

Our data show that long-term DPPR 1)halts deterioration of renal function in the majority of patients with ERF (65%) ; when associated with no further changes in GFR, 2)prevents secondary hyperparathyroidism and 3)increases serum levels of 1.25 D and 24.25D. This latter effect is not dependent on a decrease in serum phosphate but is possibly related to a mild reduction in intracellular phosphate.

REGULATION OF Ca^{2+}-ATPASE IN THE BASOLATERAL MEMBRANE OF RAT KIDNEY CORTEX. Yusuke Tsukamoto* and Wadi N. Suki. Baylor College of Medicine, Houston, Texas.

We have previously shown that high affinity (Km = 172 nM free Ca^{2+}) and low affinity (Km = 25 μM free Ca^{2+}) Ca^{2+}-ATPases exist in the basolateral membrane of rat kidney cortex. In the present study, we investigated the regulation of these Ca^{2+}-ATPases.

The effects of cations on these Ca^{2+}-ATPases were first investigated. In the absense or presence of various cations (0.1 mM: high affinity, 3 mM: low affinity), both high and low affinity enzyme activities were measured at 0.1 mM $CaCl_2$ (free Ca^{2+}:10^{-7}M) and 3 mM $CaCl_2$ (free Ca^{2+}: 0.365 mM), respectively. Al^{3+}, Ba^{2+}, and Sr^{2+}, did not affect either enzyme's activity. Mn^{2+}, Cu^{2+}, and Zn^{2+} stimulated high affinity enzyme activity significantly (control: 29 ± 2.3 vs. Mn^{2+}: 104 ± 15, $P < 0.01$, Cu^{2+}: 116 ± 26, $P < 0.05$, Zn^{2+}: 143 ± 13, $P < 0.001$), while the same cations were potent inhibitors of low affinity enzyme activity (control 644 ± 74 vs. Mn^{2+} and Zn^{2+}: 0, Cu^{2+}: 33 ± 7.8, $P < 0.01$; n = 3, mean ± S.E.). Mg^{2+} inhibited both enzyme activities significantly. (The activities are expressed as n mole $Pi.min^{-1}.mg^{-1}$ protein.)

Hormonal regulation of these enzymes was next investigated. TPTX rats (n = 3) showed a significantly lower ativity of high affinity Ca^{2+}-ATPase 8-9 days after operation as compared to sham-operated rats (93 ± 3.5 vs. 154 ± 16 $P < 0.05$). Since acute reduction of PTH induced by 24-hour dietary Pi deprivation had no effect on enzyme activity, it is suggested that decreased production of $1,25-(OH)_2D_3$, the result of long term PTH deficit, is instead responsible for the depressed enzyme activity.

SUSTAINED HYPOCALCEMIA, INHIBITION OF PTH SECRETION AND CORRECTION OF VIT D INDUCED HYPERCALCEMIA WITH CHRONIC ADMINISTRATION OF WR-2721 (WR). J. Weiss*, J. Wolf, E. Slatopolsky, M. Fallon*, M. Attie*, and S. Goldfarb, Univ. of PA and Wash. U., St. Louis, MO and Phila., PA

Single IV or intraperitoneal (IP) doses of WR acutely (4-6 hours) reduce serum Ca and PTH secretion in man and animals. We now test WR as a chronic inhibitor of PTH secretion and as a hypocalcemic (hypoCa) agent. WR, a sulfhydryl compound, was oxidized by heating in 0.1 N HCl to mimic gastric conditions. Oxidized IP WR decreased SCa from 10.8±.1 to 8.4±.3 mg/dl at 3 hr., while intact WR decreased SCa from 10.7±.1 to 8.3±.4 mg/dl. Oral WR (240 mg/kg) decreased SCa from 10.6±.1 to 9.0±.2 mg/dl ($p<.001$) at 6 hrs. Oral WR (120 mg/kg) given daily for 7 days, resulted in a similar hypoCa effect at 6 hrs. with recovery by 16 hours.

To test the effect of chronic WR, IP osmotic pumps infused WR at 15mg/kg/hr. SCa fell from 11.3±.1 to 6.0±.2 mg/dl by 96 hours. Serum PTH was reduced (38 µL-Eq/ml) compared to normocalcemic controls (54 µl-Eq/ml) despite profound hypoCa. Removal of the pumps led to recovery of SCa to 8.6 mg/dl 12 hours later. Histologically, femurs from rats infused with WR for 96 hours showed inhibition of osteoclastic activity. Rats made hypercalcemic by $1,25(OH)_2D$ received WR by IP pumps. After 7 days of WR, SCa was 9.2±1.2 while 13.6±0.1 mg/dl in controls ($p<.02$).

These studies demonstrate 1) the feasibility of sustained reduction of serum Ca and PTH secretion with chronic WR-2721 administration and 2) the possibility of chronic oral treatment with this unique hypocalcemic and PTH inhibitory agent.

INSULIN(I) ACTIONS ON IN VITRO ISOLATED PERFUSED RABBIT RENAL PROXIMAL TUBULES(PT). Norimoto Yanagawa. Nephrol. Div. Sepulveda VAMC, UCLA Sch. of Med., Sepulveda, CA.

In vivo studies have shown I to suppress fluid, while enhance phosphate(P), reabsorption by PT(J.Clin.Invest.58:83, 1976). However, the direct I effects on PT remains unclear. We have examined the effects of I on net fluid(Jv), and lumen to bath glucose(Jg) and P(Jp) transport rates across the in vitro isolated perfused rabbit proximal convoluted tubule (PCT) and proximal straight tubule(PST) where I receptors are present. Both perfusate and bath were identical artificial solution except for 6% albumin and higher calcium(Ca) concentrations in bath. At 3mM Ca bath(1mM Ca^{++}), I(1Ø-1ØØuU/ml) in the bath did not affect Jv, Jg or Jp in PCT or PST. Since Ca has been implicated to be involved in I actions, and we have reported the restorement of responsiveness to other hormone in rabbit PCT by raising the bath Ca(Kid.Intl.25:156A,1984), effects of I were studied in rabbit PCT with 5mM bath Ca(2mM Ca^{++}). Increase in bath Ca alone did not affect Jv, Jg or Jp, but allowed I(1ØuU/ml) to induce a reversible inhibition on Jv(1.1Ø±Ø.11 v.s. Ø.47±Ø.Ø7 nl/mm/min., n=15, $p<Ø.Ø1$), and Jg(68.39±3.7Ø v.s. 54.67±4.31 pmol/mm/min., n=5, $p<Ø.Ø2$), while Jp remained unchanged(6.96±1.24 v.s. 6.33±1.25 pmol/mm/min., n=1Ø, $p>Ø.6$). In conclusion, in a Ca dependent manner, I directly inhibited Jv and Jg, but not Jp, in rabbit PCT.

EFFECT OF FUROSEMIDE ON CISPLATIN-INDUCED HYPOMAGNESEMIC RATS. N.L.M. Wong, R.A.L. Sutton, V. Mavichak*, and J.H. Dirks, Dept. of Med., Univ. of British Columbia, Vancouver, B.C.

Recent studies on the magnesuric effect of furosemide in cisplatin induced hypomagnesemic patients suggested that there is a defect in proximal secretion of the drug, together with a defect in the distal tubule. In the present study we have examined the effect of furosemide (1mg/Kg b.w.) in an animal model with renal Mg wasting following cisplatin administration. Two-phase recollection micropuncture studies were performed on acutely thyroparathyroidectomized (TPTX) rats fed a low Mg diet and injected weekly with cisplatin (5 mg/kg I.P.) for 3 weeks (CIS), while control animals remained on a low Mg diet and were injected with normal saline solution (N). The cisplatin rats had a significantly lower GFR than normal rats (1.93 vs 2.41±0.17 ml/min respectively $p<0.05$). Significant hypomagnesemia was noted in cisplatin rats compared with control (0.44±0.02 vs 0.53±0.04 mM respectively $p<0.05$). Following furosemide, an exaggerated response was observed in FE Na (CIS 0.3±0.1 to 14.0±1.0% vs N 0.3±0.1 to 9.6±0.85%, $p<0.01$) and FE Mg (Cis 9±2 to 56±3% vs N 8±2 to 48±2%, $p<0.05$). Tubular fluid obtained from the superficial late proximal and early distal tubule of cisplatin and normal rats after i.v. furosemide were similar (Distal RF Na: Cis 16±3 to 84±8% vs N 21±5 to 76±10%; RF Mg Cis 7±1 to 66±5 vs N 5±1 to 64±8% respectively NS). These results are consistent with the presence of a defect in the juxta-medullary nephron, and/or a defect in the superficial distal tubule.

RENAL Ca CONSERVATION DURING DIETARY Ca DEPRIVATION: EVIDENCE FOR EXTRA-RENAL AND LOOP OF HENLE ADAPTATION. D. Zikos and K. Lau, Michael Reese Hospital & Univ. of Chicago, Chicago, IL.

Young rats respond to a low Ca diet by markedly reducing, though not completely eliminating Ca excretion. To define the role of hormonal and tubular adaptation and the mechanism for the suboptimal renal Ca conservation, we studied 150 gm SD rats fed a normal (N) (0.87%) or low (0.005%) Ca diet (LCD). Urine Ca fell within 2 days of LCD (0.73† vs 2.93 mg/d), reached a minimum by days 3 and 4 (0.37† vs 3.35 mg/d) and leveled off at days 5,6, and 7 (0.35† vs 3.8 mg/d) (†, $p<0.05$ vs NCD). Although PO_4 absorption was increased by LCD (net = 152† vs 126 mg/d), urine PO_4 was increased by a greater extent, diminishing PO_4 balance (70 vs 91 mg/day). Ca conservation was not affected by vitamin D deprivation since urine Ca was comparably low despite absent serum 25 OHD_3 and reduced 1,25 $di(OH)D_3$ (102 pg/ml). In acutely PTX rats, glomerular filtration rate was similar (2.7 vs 2.8 ml/min). Plasma ultrafilterable Ca was reduced by LCD (3.6† vs 4.7 mg/dl). Fractional delivery (FD) and excretion (E) data are:

	Late Proximal			Early Distal			Urine		
	TF/P In	FDNa (%)	FDCa (%)	TF/P In	FDNa (%)	FDCa (%)	$FEPO_4$ (%)	FENa (%)	FECa (%)
NCD	2.0	50	48	3.4	13	16	1.4	5.6	6.2
LCD	1.9	49	45	3.5	12	6†	1.6	7.3	1.4†

Conclusions: (1) The hypocalciuric effects of a low Ca diet are independent of PTH, 25 OHD_3 and $1,25di(OH)D_3$, but related to the reduced filtered load and increased Ca transport by the loop of Henle. (2) Despite increased absorption, P balance is reduced by the hyperphosphaturia, producing the suboptimal renal Ca conservation.

RELATIONSHIPS BETWEEN Ca AND Cl TRANSPORT IN FROG SKIN (FS). F.N. Ziyadeh*, E. Kelepouris and Z.S. Agus. Dept. of Med., Univ. of Penn., Phila., Pa.

The β-adrenergic/CAMP system stimulates net Ca secretion in FS. As FS contains subepidermal glands which secrete Cl with β-adrenergic stimulation, studies were designed to test the role of Cl in Ca transport. ^{45}Ca fluxes were measured in paired short-circuited FS pretreated with amiloride (A,10^{-4}M) to abolish Na absorption. Addition of 8-p-Cl-CAMP (CAMP,10^{-4}M) increased short-circuit current (SCC) from 1.4±0.6 to 7.4±0.9μA/cm^2 ($p<0.01$,n=5) reflecting net Cl secretion. Furosemide(F,10^{-3}M) (n=5) and Cl-substitution with NO_3 (n=5) abolished stimulation of SCC by CAMP. CAMP also stimulated net Ca secretion (from +0.7±0.3 to -1.6±0.4 nmol/cm^2/hr, $P<0.01$,n=7) which correlated significantly with A-resistant SCC (r=0.84) and was inhibited by F (-0.2±0.3, n=5, NS) or NO_3 (-0.2 ±0.4,n=4,NS).

To examine the role of Ca in Cl secretion,A23187 (10^{-5}M)was added instead of CAMP to A-treated FS. SCC increased from 0.2±0.1 to 3.5±0.4($p<0.01$,n=5) and the increase was prevented by F or NO_3. Increasing bath Ca concentration from 0.9 to 3.5 mM enhanced the SCC response to A23187 from 3.2±0.4 to 5.2±0.5 ($p<0.01$,n=5). Addition of CAMP to split-thickness FS, a gland-free Na-absorbing epithelial preparation, resulted in no change in net Ca transport (+0.5±0.2 vs +0.5±0.3 in control, n=6,NS) or A-resistant SCC.

Thus, CAMP-stimulated active Ca secretion in FS is Cl-dependent, and stimulation of Cl secretion may be a Ca-regulated process. These effects are localized to subepidermal glands which may be a useful model for the study of Ca transport in Cl-transporting epithelia.

AMELIORATION OF LACTIC ACIDOSIS BY SUPERIMPOSED RESPIRATORY (RA) OR INORGANIC METABOLIC ACIDOSIS (MA). S Abu-Romeh* and RL Tannen, Dept. of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Recent studies have demonstrated that metabolic acidosis suppresses ketoacid production in humans with fasting ketoacidosis. To determine whether inhibition of cellular acid production is a generalized defense mechanism, we examined the effect of acidosis on lactic acid production by hypoxic rats.

Anesthetized rats were mechanically ventilated using 8% O_2 for 3 hours. In some rats after 1 hour of hypoxia either RA (pCO_2 increase from 40 to 58 mmHg) or MA (intra-arterial infusion of 0.6 mEq of HCl) was superimposed on the hypoxia.

BP and p_aO_2 (38 mm Hg) were similar in the 3 groups. Arterial pH averaged 7.27 ± .02 in the controls, 7.24 ± .02 in the RA, and 7.23 ± .02 in the MA groups at the end of the 2nd hour. pCO_2 was comparable in the control and MA groups and elevated significantly in the RA rats. Plasma lactate, which averaged 0.8 mM during normoxia, is shown below for each hour of hypoxia.

	1 hr	2 hr	3 hr
Control (n=11)	2.4	4.2	7.0
RA (n=5)	1.9	2.8	1.7*
MA (n=5)	2.6	2.9	2.7*

* $p < .05$ in comparison with control

During 3 hours of hypoxia, lactic acid levels rose progressively in the control group. By contrast, the superimposition of RA or inorganic MA at the start of the 2nd hour completely inhibited a further rise in blood lactate and resulted in levels substantially and significantly lower than controls by the end of hour 3.

Thus, systemic acidosis appears to feedback in a protective fashion to inhibit net lactic acid production in rats with hypoxic induced lactic acidosis.

CLINICAL NEPHROLOGY

ASSESSMENT OF PROTEINURIA BY URINE PROTEIN CREATININE RATIOS (U P/Cr)IN NEPHROTIC CHILDREN Carolyn Abitbol,Jose Strauss*, Gaston Zilleruelo, Michael Freundlich. Univ. of Miami School of Med., Dept. of Pediatrics, Miami, Florida.

The feasibility of using random urine protein: creatinine ratios (U P/Cr) to predict daily protein excretion in nephrotic patients was tested by studying 119, 24 hour urine collections in 57 children with the nephrotic syndrome (age 19 mos-16 years). Six patients were steroid non-responsive and had focal segmental sclerosis by biopsy. All others were considered to have minimal change disease either by biopsy or Prednisone responsiveness. Urine protein was measured by the Coomassie blue binding technique (Bio-Rad Lab) and creatinine by the spectrophotometric Pierce Test Kit. Total protein excretion ranged from 2 mg to 32.1 g/m^2/day. U P/Cr ranged from .002 to 33.3 mg/mg in 24 hour urine samples. The correlation between daily protein excretion and the U P/Cr was highly significant ($n = 119$, $r = .76$, $p < .001$). The scattergram of all data suggested non-linearity when proteinuria exceeded nephrotic ranges ($\geq$ 1g/m^2/d). Moreover, when the linear regression equation ($y=.5x + .3$) was used to calculate U P/Cr for physiologic protein excretion ($<$ 0.1g/m^2/d),it was negative. Therefore, the data were analyzed separately. Values below nephrotic ranges were more linear ($n = 67$, $r = 0.87$), whereas a semilogarithmic regression was required to better define the nephrotic proteinuria ($n = 52$, $r = .71$). These data support the concept that U P/Cr can accurately assess physiologic or minimal proteinuria. However, non-linearity limits its use as an accurate assessment of proteinuria of greater magnitude.

PROLONGED ALTERNATE DAY PREDNISOLONE THERAPY IN ADULT NEPHROTIC SYNDROME WITH MESANGIOCAPILLARY GLOMERULONEPHRITIS. A Akinsola and J Thomas, University College Hospital, Ibadan, Nigeria.

Twenty-six adult Nigerian patients suffering from the nephrotic syndrome with normocomplementaemic mesangiocapillary glomerulonephritis were studied. 13 of these were treated with prednisolone at a dose of 1.5mg per kilogram body weight per day with maximum dose of 90mg per day initially for two weeks. Thereafter, a quarter of the daily dose was given on alternate days for a period varying from six to twelve months. The thirteen others were similarly longitudinally followed up, only on dietary and diuretic regime. Oedema disappeared completely in all the steroid group except one whilst it persisted in five of the controls. Proteinuria persisted in all the controls but remitted in five of the test patients. Renal function deteriorated with rising plasma creatinine in five of the controls in contrast to one of the test patients. Repeat biopsies in five steroid responders showed substantial histological changes.

MORBIDITY AND MORTALITY OF RESPIRATORY (RA) AND METABOLIC ALKALOSIS (MA) IN HOSPITALIZED PATIENTS (P). **L.Anderson*, J.Breyer*, and W.Henrich, DVAMC and SW Med Sch, Dallas, TX.**

The exact prognostic significance of alkalemia in hospitalized P is unsettled. Further, whether pure RA or a combination of RA+MA have different prognoses is also unclear. Accordingly, we prospectively followed to outcome 122 patients with pH ≥7.48 ($\bar{x}$ 7.56, range 7.48 to 7.84) and either pure RA or RA+MA as the cause of the alkalemia. The overall frequency of modest alkalemia (pH> 7.48) was 19% (661 of 3,550 blood gases). Results: *p<.01

	Pure RA (n=68)	RA+MA (n=54)
pH	7.52±06	7.62±0.4
Age	60±1.4	62±1.1
K^+ (mEQ/L)	3.95±.1	3.88±.1
Hospital Days	12.5±.9	19.3±2.2
Intubated (n)	18 (26.5%)	30 (56%)*
In ICU (n)	41 (60%)	50 (92.6%)*
Died (n)	13 (19.1%)	23 (43%)*

Associated with a poor prognosis were pH> 7.55 (for both pure RA and RA+MA), and admission to the surgical ICU. Of interest is that even modest alkalemia (pH 7.48 to 7.55) was associated with a high mortality: 16% in P with pure RA and 32% in P with RA+MA. We conclude that modest alkalemia is frequent in hospitalized P and denotes an extremely poor prognosis. In this regard, the combination of RA and MA impart a particularly grave outcome.

RENAL FAILURE IN ACUTE PANCREATITIS: IMMUNE COMPLEX DEPOSITS IN GLOMERULAR BASEMENT MEMBRANE. Spiro Arbes* and Sushil K. Mehandru, UMDNJ - Rutgers Medical School and Jersey Shore Med. Ctr., Neptune, New Jersey.

Acute renal failure is a frequent complication of pancreatitis. One group reports incidence as high as 78% in 23 cases studied with acute hemorrhagic pancreatitis. Various pathological changes in renal tissue have been described which include: acute tubular necrosis, focal interstitial inflamation, glomerular endothelial swelling, enlargement of mesangial cells, presence of IgG, fibrin and fibrinogen in the glomerular basement membrane. We studied a case of acute pancreatitis, without hypotension who developed acute renal failure and kidney biopsy showed focal interstitial inflamation, mesangial cell proliferation, glomerular capillary endothelial swelling and granular glomerular deposits of C3 and properidin in the subepithelial and intramembranous location. These renal changes suggest possible presence of immunological mechanism as an important factor in the pathogenesis of renal changes in acute pancreatitis. Patients renal functions were normalized in 3 weeks with improvement of pancreatitis.

TREATMENT OF HYPERLIPIDEMIA OF THE NEPHROTIC SYNDROME(NS): A CONTROLLED TRIAL. G.Appel, J.Gelfand*, C.Kunis, C.Blum*, Columbia U., N.Y., N.Y.

Dyslipoproteinemia is common in NS, with elevated low density lipoprotein cholesterol (LDL)(C) and decreased high density lipoprotein (HDL)C predisposing to atherosclerotic heart disease (ASHD). Drug therapy in nephrotic patients has not been well evaluated. We studied the effect of colestipol treatment in 7 adult pts with unremitting NS . The pts, 5M and 2F, had a mean plasma albumin of 2.29 gm/dl and 24 hour protein excretion of 10.7 gms. Control fasting plasma samples (2-4/pt) were obtained at least 3 weeks after dietary instruction for measurement of C, LDL C, HDL C and triglycerides(TG). The subjects were then treated with 15 to 25 gms/day of colestipol with multiple plasma specimens after 3 or more weeks of therapy. The results of therapy (mean +/-SD) are:

	C	LDL C	HDL C	TG
control	397(72)	298(73)	45(14)	261(112)
drug	317(99)	203(47)	46(20)	284(168)
% change	-20	-32	+2	+8
p(T test)	.07	<.05	NS	NS

We conclude that colestipol selectively lowers LDL C in nephrotic patients on a low C diet, which may decrease the long term risk for ASHD. Further studies should compare this therapy with other antihyperlipidemic drugs for safety and efficacy.

ASSOCIATION BETWEEN IDIOPATHIC HEMOLYTIC UREMIC SYNDROME (HUS) AND INFECTION BY VEROTOXIN PRODUCING E COLI (VTEC). GS Arbus, MA Karmali*, M Petric*, C Lim*, PC Fleming*, H Lior*. Hospital for Sick Children, Research Institute and University of Toronto, Department of Pediatrics, (Nephrology), Bacteriology, Virology, Toronto, Canada.

Forty (18 males, 22 females) pediatric cases of idiopathic HUS, ages 6 to 226 (mean 45.2; median 26.5) months were investigated for evidence of VTEC. Fecal VTEC belonging to five different O serogroups (O26, O111, O113, O145, O157) or neutralizable free fecal verotoxin (VT) or both were detected in 24 (60%) cases but in none of 40 matched controls. Ten of fifteen of the former developed a 4-fold or greater rise in VT-neutralizing antibody titer, as did a further 6 cases who were negative for both fecal VTEC and VT. Thus, a total of 30 (75%) cases of idiopathic HUS had evidence of VTEC by one or more criteria.

The detection of free fecal VT is the most important procedure in the diagnosis of this infection, since VTEC were never isolated in the absence of fecal VT while fecal VT was often present even when VTEC were undetectable. This may be explained in that the first stool was received after a mean of 10.6 (range 3-28) days after the onset of the acute diarrheal illness that preceded the development of HUS.

We conclude that a significant association exists between idiopathic HUS and infection by VTEC.

PERMANENT NEUROLOGICAL DISABILITY FROM HYPONATREMIA IN HEALTHY WOMEN UNDERGOING ELECTIVE SURGERY. A.I. Arieff. V.A. Med. Ctr. and U.C.S.F., Nephrology Research, San Francisco, CA.

Permanent brain damage from hyponatremia usually occurs in elderly subjects with serious medical illness. Nine healthy women aged 21-59 yrs with pre-op Na 138-141 mM underwent elective surgery (4 hysterectomy, 1 D&C, 1 cholecystectomy, 2 ENT procedures, 1 shoulder repair). No patient had serious medical illness, surgery was uneventful & all awoke from general anesthesia & ambulated. Fifty-72 hrs post-op, all patients had grand mal seizures with coma, & serum Na was 93-118 mM. Net fluid intake (all 5% D/W) averaged 5.2L/65hrs & urine Osm was 400-574 mOsm/kg. Therapy with 514 mM NaCl elevated serum Na to 128-135 mM within 48 hrs. Six patients awoke after correction of hyponatremia & were lucid, but 5 again lapsed into coma. Evaluation included lumbar puncture & CAT scan (9 patients), ventriculogram (3 patients), carotid & vertebral angiogram (6 patients) & open brain biopsy (2 patients). Only cerebral edema was found. Despite therapy with hypertonic NaCl, all patients had permanent neurological disability: 3 died, 5 are institutionalized & unable to care for themselves, 1 had permanent paralysis of a leg. 19 CAT scans, 2 brain biopsies and 2 autopsies revealed no evidence of central pontine myelinolysis (CPM).

These data show that: a) healthy women undergoing elective surgery in a setting where ADH is known to be elevated can rapidly develop severe symptomatic hyponatremia; b) there is often a transient response to therapy whereby patients become lucid but then revert into irreversible coma; c) invasive diagnostic procedures are common despite the presence of severe hyponatremia; d) resultant neurologic sequelae are permanent, unassociated with CPM or other obvious CNS lesion & are often unaffected by conventional therapy.

MALIGNANT PROTEINURIA (MP). A NEW LIFE-THREATENING SYNDROME AND OPTIONS FOR ITS MANAGEMENT. Avram MM, Gan A, Pahilan AN, and Iancu M. The Long Island College Hospital, Division of Nephrology, Brooklyn, New York

We describe herein a newly discovered, life-threatening syndrome, "malignant proteinuria" (MP) in 3 patients who presented with massive proteinuria of over 25mg/day in 24 hr urine collection. This resulted in hypoproteinemia, reduced oncotic pressure and massive edema, with life-threatening hypotension and shock in 2 of the 3 cases. Patients were severely azotemic, requiring dialytic therapy. Although the creatinine clearance was below 3ml/min, large protein amounts, paradoxically, were still lost through the non-clearing kidneys, probably due to a few, overactive remaining glomeruli. Ablation of remaining nephrons with metallic salts was followed by cessation of massive protein loss and restoration of blood pressure.

Other authors have recently described massive protein losses (over 25gm/24 hrs) in uremic and non-uremic patients such as post-transplantation and have used a variety of therapeutic approaches to reverse this life-threatening state (e.g. metallic salts, gelfoam embolization of renal arteries, stainless steel rings, uninephrectomy and binephrectomy--the latter in dialysis-maintained patients.

The lesion in most cases, including the post-transplant patient, was focal sclerosis, although a full range of minimal change to complete histological sclerosis of the nephron is encountered. We have described the MP syndrome, a new, life-threatening clinical entity and its management, in our hands, with medical nephrectomy using nephrotoxins such as metallic salts.

HEPATITIS B VIRUS VACCINE: A RANDOMIZED TRIAL OF A REDUCED DOSE REGIMEN IN HEMODIALYSIS PATIENTS George R. Aronoff*, Douglas R. Maxwell, Byron E. Batteiger*, and Naomi Fineberg*. Dept. of Med., Indiana Univ., Indianapolis, In.

To test the hypothesis that increasing the dose enhances response to hepatitis B virus vaccine in hemodialysis patients, we performed a randomized, double blind, controlled clinical trial. Twenty-four hemodialysis patients were randomly assigned to receive either three 20 mcg or three 40 mcg intramuscular injections over 6 months. Nineteen normal volunteers also received three 20 mcg doses of the vaccine. Anti-HBs was determined qualitatively and quantitatively. Statistical comparisons were made using chi square and two-way ANOVA. The qualitative development of Anti-HBs is shown below (seroconverted/total):

Time after first dose	Normal 20 mcg	Dialysis 20 mcg	Dialysis 40 mcg
1 month	10/19	4/11	3/13
2 months	15/19	4/11	4/13
6 months	18/19	5/11	6/13
8 months	19/19	8/11	7/13

Non-uremic subjects seroconverted more frequently than did either of the dialysis patient groups. Doubling the individual doses of the vaccine did not improve the response of the dialysis patients. We conclude that the response to the vaccine is not diminished when dialysis patients are given half the recommended dose of the vaccine and that the cost of vaccinating this high risk population could be reduced.

OVERCORRECTION RATHER THAN RAPID CORRECTION INDUCES CENTRAL PONTINE MYELINOLYSIS (CPM) IN PATIENTS WITH SEVERE HYPONATREMIA (SHN). J.C Ayus, R. Krothapalli, A.I. Arieff and J.P. Frommer, VAMC, Baylor Col. of Med., Houston, Tx. and U.C.S.F., San Francisco, Calif.

Previous studies in rats have shown that no pathologic lesions are found when SHN is rapidly corrected to mild hyponatremia, however, 100% of the animals corrected to normo or hypernatremic levels develop demyelinating lesions of the central nervous system (CNS). The present study examines the effects of rapid correction and overcorrection of SHN on the CNS. Five patients were divided into 2 groups: A) rapid correction of SHN to mild hyponatremia (N=3; of which 2 were alcoholics) and B) overcorrection of serum Na levels to hypernatremic levels (N=2; both alcoholics). Neurological examination, computerized tomography (CT) of the brain and pathologic examination of the brain (one case in group B) were used to assess the effects of treatment. Our results show that in group A, initial serum Na (iSNA) (mEq/l) was 113±4 and serum Na after 24h (cSNa) was 130±5. Neurologic examination and CT scan of the brain were normal in all 3 patients after correction. In group B, iSNa was 119±16 ($p=NS$ vs A), cSNa was 157±3 ($p<0.05$ vs A) and both patients died. CPM was found by CT scan in one patient and by pathologic examination of the brain in the other. Our results show that 1) rapid correction of SHN to mild hyponatremia does not induce neurologic lesions, even in alcoholics. 2) overcorrection to hypernatremia, however, results in demyelinating lesions of the CNS. We conclude that the neurologic lesions seen after correction of SHN are not the result of the rapidity of correction but rather of the magnitude of the osmolar change.

FUROSEMIDE (F) AS A TOOL TO EVALUATE CORTICAL COLLECTING DUCT (CCD) FUNCTION. DC Batlle, West Side VA and Univ of Illinois, Chicago IL.

In the CCD, but not in the medullary collecting duct, both H+ and K+ secretion are, in part, Na-dependent and thereby inhibitable by amiloride (A), an antagonist of Na transport. We reasoned that if the stimulatory effect of F on H+ and K+ secretion were to be prevented by A one could infer that this effect of F is secondary to enhancement of Na transport in the CCD. Accordingly, we gave F to normal subjects receiving A (20 mg). A prevented the fall in urine (U) pH and the increase in acid excretion observed when F was given alone. At comparable U flow and Na excretion, the increase in K excretion elicited by F was blunted by A. These findings indicate that the effect of F on H+ and K+ secretion requires intact CCD function and suggest that F can be used to segmentally evaluate collecting duct dysfunction in patients with distal tubular acidosis (DRTA). Accordingly, F was given to 6 patients with DRTA and to 9 with selective aldosterone deficiency (SAD). In the patients with SAD, F resulted in a further decrease in UpH (from 5.3±0.06 to 4.9±0.07, $p<0.005$) and an increase in acid and K excretion while plasma aldosterone did not change. Thus, Na-dependent CCD acidification is responsive to F even in the face of SAD. F had no effect on UpH in 4 DRTA patients while it elicited a fall in UpH (below 5.2) in the remaining 2. These data suggest that patients with DRTA unresponsive to F have CCD dysfunction, while those responsive to it have intact (or partially preserved) CCD function. Thus, a definition of the defective nephron segment may be possible in patients now classified under the vague term DRTA.

ACUTE RENAL FAILURE: IMPACT OF ETIOLOGY AND TREATMENT ON OUTCOME. R Bennett*, R Hodder*, S Gouge* J Moore. Walter Reed Army Med Ctr, Washington DC

Acute renal failure (ARF) continues to have an inordinately high mortality despite the almost routine use of aggressive dialysis, nutritional support, and antibiotics. To determine if there were clinical features in patients with ARF which correlated with outcome, we retrospectively examined the records of 87 patients with ARF who received hemodialysis. Variables recorded in all patients included medical and/or surgical conditions existent prior to ARF, presumed cause(s) of ARF, and details of treatment, including nutritional, respiratory, and antibiotic therapy. There were 3 possible outcomes: survival with normal renal function, survival with chronic renal failure, or death. At diagnosis of ARF 41% were non-oliguric, while 59% were oliguric. Levels of azotemia were not different between non-oliguric and oliguric patients. Azotemia and volume overload were the most common reasons for dialysis. The overall mortality rate was 64%; 23% survived with chronic renal failure, while 13% survived with normal function. When outcome was analyzed in context with all variables, sepsis, hypotension and oliguria were found to be the most important factors, as patients with these features exhibited mortalities of 90%, 85%, and 80% respectively. Little correlation was seen between treatment variables, including type and amount of nutrition, respiratory support, antibiotics, and outcome. These results underscore the impact of sepsis and hypotension on the severity of ARF, and suggest that aggressive therapy, including dialysis, has not been associated with a substantial decline in the mortality of ARF.

PRIMARY RENAL AMYLOIDOSIS (PRA) PRESENTING AS IDIOPATHIC NEPHROTIC SYNDROME (INS). A. Bellucci*, M. Susin and L. Mailloux, Dept of Medicine and Pathology, North Shore University Hospital, Manhasset, New York

Since 1981 we have biopsied 12 patients (pts) 50 yrs and older with normal renal function and clinical presentation suggestive of INS. Six pts had membranous, 4 had PRA, 1 had diffuse mesangial proliferation and 1 focal glomerulosclerosis. Of the 4 pts with PRA, 2 had IgG lambda in urine and serum and another developed serum IgG lambda spike one year after presentation. The hematocrit was normal and bone marrow and skeletal survey were not diagnostic of multiple myeloma. All the pts were treated with Alkeran and Prednisone. One pt developed ESRD within 24 months and expired 3 months after the initiation of hemodialysis. One pt has developed chronic renal insufficiency and disabling orthostatic hypotension 22 months after presentation. The other 2 pts are well after 12 and 14 months, respectively. We conclude that PRA is common and typically presents in pts above age 50 as INS with normal hematocrit, renal function and no evidence of multiple myeloma or systemic amyloidosis. Although the value of treatment is yet to be defined, the prognosis of untreated renal amyloidosis is so dismal that clinical trials with early chemotherapy are warranted. Our findings do not support the policy of "blind" steroid therapy (Kassirer, Kidney Int. 24:561, 1983) as the initial approach to pts 50 yrs and older with INS.

ROLE OF PLASMA VASOPRESSIN (Pavp) AND OF PLATELET FRACTION VASOPRESSIN (PlatAVP) IN THE ABNORMAL WATER EXCRETION OF PATIENTS (pts) WITH SEVERE CONGESTIVE HEART FAILURE (CHF). D. Bichet, C.Kortas*, B. Mettauer*, C. Manzini*, J.L. Rouleau*, Dept. of Medicine, Sacré-Coeur Hosp., Univ. de Montréal,Can

The mechanisms involved in the abnormal water excretion in pts with CHF are poorly understood. 25 pts with CHF (cardiac index (CI) 2.1±0.1 l/min/m^2 and pulmonary wedge pressure 27.5±1.5 torr) received two 15 ml/kg of body weight water loads:the first off all drugs on day 1, and the 2nd after 2 days of vasodilator therapy with either captopril or prazosin on day 3. Hemodynamic, renal and hormonal measurements were obtained for 5 hrs. Plat AVP was measured according to Preibiz (Hypertension I:129,1983). Basal Pavp was detectable (>.5, mean 3±.4pg/ml) in 17 out of 25 pts (group I) despite low effective plasma osmolality (Eosm,mOsm/kg H_2O) (262±3). The remaining 8 pts (group II) had suppressed Pavp (<.5pg/ml, undetectable) for their Eosm (268±2). CI (1.9 vs 2.6, $p<.005$) and the % of water excreted (31.4 vs 57.1, $p<.005$) were lower in group I but GFR were similar (55 vs 54 ml/min/$1.73m^2$). Plasma renin activity and aldosterone were higher in group I suggesting a decreased effective blood volume. In group I, vasodilators ↑ CI from 1.9 to 2.11 and the % of water excreted from 31 to 53% (both $p<.001$). Pavp↓ from 3 to 1.8 pg/ml ($p<.01$) PlatAVP from 8.6 to 5.1 pg/ml ($p<.005$) and minimum urinary osmolality (375 to 208 mOsm/kg H_2O $p<.001$). Changes in the renin-aldosterone system were unrelated to changes in H_2O excretion or to Pavp. Pavp and PlatAVP seem therefore to be independent major determinants of the abnormal H_2O excretion in many pts with CHF.

EOSINOPHILIA IN THE DIAGNOSIS OF ATHEROEMBOLIC RENAL DISEASE. A. Bidani, B.S. Kasinath, H.L. Corwin, M.M. Schwartz, E.J. Lewis, Dept. of Medicine, Rush Medical College, Chicago, IL.

In spite of frequent occurrence of atheroembolism to the kidneys, either spontaneously or following procedures such as angiography and aortic surgery, it is rarely diagnosed antemortem. We present data in four patients in whom atheroembolic renal disease was documented and in all of whom significant eosinophilia was noted, defined as eosinophil count greater than 350 mm^3. The results:

Patients	Age/Sex	BUN/S.Cr.		WBCX10^3/	%Eos.	Eos.Count
1	52 M	71	4.6	14.9	5	1188
2	69 M	66	4.4	8.9	6	540*
3	63 F	67	5.7	8.7	17	2000
4	67 F	24	1.5	9.0	6	540*

Eos=Eosinophils. *Estimated eosinophil count.

Renal histologic diagnosis of atheroembolism was obtained in 3 patients, and in the other, skin biopsy demonstrated cholesterol emboli. The renal diseases seen in our patients included acute renal failure (pt.3), chronic renal failure (pts.1&2) and uncontrolled renovascular hypertension (pt.4). Urine eosinophils were absent in 3 in whom urine was examined. Tissue eosinophilia was not present in the biopsy specimens. Eosinophilia could not be attributed to radiocontrast dye or drugs. On review of the literature we have noted eosinophilia in 20 out of 25 patients(80%) in whom total and differential WBC counts were reported, but this finding has not been adequately appreciated and emphasized.

We suggest that eosinophilia is commonly associated with atheroembolic renal disease and that it is a helpful clue in the clinical diagnosis of this disease.

IMMUNE SUPPRESSIVE THERAPY (ImST) IN CORTICOSTEROID RESISTANT MINIMAL CHANGE NEPHROPATHY (MCGN) WITH NEPHROTIC SYNDROME (NS). R. Cade, M. Privette, B. Thompson, and B. Croker. Depts. of Medicine and Pathology, University of Florida, Gainesville, FL.

We have used corticosteroid therapy (CST) in 31 adult patients with NS due to MCGN; 18 responded, 12 were resistant and one relapsed frequently. The 12 resistant and 1 who relapsed were then treated with ImST (12 Imuran, 1 Cytoxan) for 4 yrs. Age at advent of CST resistant NS ranged from 17 to 61 yrs, 9 were young adults. Selective proteinuria (Sp) was present in 6; non-selective (NSp) in 7. There were no other significant differences between the two groups. Before treatment creatinine clearance was 60±25 ml/min, plasma albumin 1.53±0.6 gm/dl, serum cholesterol 491±57 mg/dl and urine protein 14±5.7 gm/24 hrs. All patients responded to ImST, those with Sp more rapidly. After 1 month ImST 2 patients with Sp were improved, after 4 months 5 of 6 were in remission. Two of 7 patients with NSp were in remission after 4 months, 3 of 7 after 8 months. The remaining Sp and 4 NSp patients gradually improved and all were in remission after 24 months ImST. One patient with NSp stopped ImST after 30 months and relapsed but responded when ImST was restarted. During the post therapy observation period which ranges from 3 to 16 years there have been no relapses. Retrospective review of glomerular histology showed 2 patients with findings compatible with focal sclerosis.

USE OF CA^{++} ION ELECTRODE TO DETECT INHIBITION OF CALCIUM OXALATE MINERALIZATION (CaOxM) BY URINE FROM STONE FORMERS. Frank J. Bruns, Kai Jun Wu*, Sheldon Adler, and Shang J. Yao*. Depts. Med. and Surg., Montefiore Hospital and Univ. Pittsburgh Sch. Med., Pittsburgh, Pennsylvania

A calcium ion electrode can determine the rate of CaOxM by adding oxalate to a solution containing calcium and measuring the fall in ionized Ca. After an initial rapid fall there is a stable induction period (IP) for Ca^{++} before it falls again during the final mineralization. IP is prolonged by various substrates so this technique was used to study the effect of urine on CaOxM.

Urine from 7 normal fasting subjects (NL) increased IP in control (C) solutions from 7.7±0.5 to 12.13±1.0 sec. ($p < .001$). Urine from stone formers (SF), 9 CaOx and 2 combined CaOx-uric acid, prolonged IP from 7.7±0.6 to 9.0±0.6 sec. ($p < .01$ pair difference). Prolongation of IP by SF was 19.32±5.2%; a value significantly less than the 54.1±5.1% prolongation by NL ($p < .001$). As shown below neither urinary phosphate (P) nor citrate (Cit) accounts for the results ($\bar{X}$±SEM).

	N	IP	Ca/Cr	P/Cr	Cit/Cr
C	18	7.71±0.4			
NL	7	12.13±1.0*	.12±0.1	.38±0.1	2.1±0.5
SF	11	9.0±0.6*	.12±0.1	.49±0.1	1.7±0.3

*(NL vs SF = $p < .001$)

Summary: (1) The Ca ion electrode technique demonstrates reduced inhibition to CaOx crystalization in SF. (2) This reduced inhibition is not due to an alteration in urinary Ca, P or citrate. Conclusion: The Ca ion electrode technique can identify stone formers and may be useful for monitoring the effect of therapy on CaOxM.

THE CONSTANCY OF CREATININE EXCRETION IN HEALTHY, VERY OLD WOMEN. Bruce S. Chang, Helen L. Lang, and Calvin A. Lang,*, Dept. of Medicine and the Louisville Longitudinal Longevity Program, Dept. of Biochemistry, Univ. of Louisville Sch. of Med., Louisville, KY.

A senescence-related decline in creatinine excretion is well-accepted. However, to our knowledge there is no information on healthy, very old female subjects to serve as normal reference values. Thus in a double blind design we studied 29 women of 63-102 years age, who were ambulatory volunteers from our Longevity Program. They had been well-characterized previously by a battery of physical and biochemical assessments to be in excellent health and free of diabetes, cancer, anemias, and kidney diseases. Their overall serum creatinine values were normal 1.01 + 0.0371 mg/dl. All had high blood glutathione levels, our biochemical index of young functional age. For each age group the mg. creatinine excreted/kg. body weight/24 hours (mean + SEM (n)) were: 60-69 yr, 13.8 + 1.26 (7); 70-79 yr, 14.1 + 0.718 (14); 80-89 yr, 12.9 + 0.856 (6); and 90+ yr, 6.2 and 9.9 (2). From 60-89 years there was no statistically significant difference, but the two values after 90 yr were lower ($P<0.001$). These results indicate that creatinine excretion was constant in this healthy, very old female population, in contrast to the previously reported aging decline. These results further support the hypothesis that healthy individuals are functionally young, even though they are chronologically old.

RELATIONSHIP OF URINARY ALBUMIN TO GLOMERULAR STRUCTURE IN INSULIN DEPENDENT DIABETES MELLITUS (IDDM). Blanche M. Chavers, Eileen Ellis,* Michael W. Steffes,* S. Michael Mauer, Univ. of Minnesota, Minneapolis, Minnesota.

Renal biopsies were evaluated using electron microscopic morphometric techniques in 21 patients with longstanding IDDM but without clinical nephropathy. Creatinine clearances (CrCl) ranged from 95-182 ml/min/1.73m^2 in these patients (14 female) aged 9 to 53 years who had diabetes for 5 to 29 years (18.7 ± 6.2 yrs, $\bar{X}$ ± S.D.). Twenty-four hour urinary albumin excretion (UAE) was measured using a sensitive fluoroimmunoassay. The normal range is 0 to 20.5 mg/day. Microalbuminuria is defined as 21 to 203 mg/d. UAE in these IDDM patients varied from 2.8 to 203 mg/d (33.9 ± 44.8 mg/d). Twelve of the 21 patients had albumin levels in the range of microalbuminuria. Glomerular basement membrane thickenss (GBMT, 575.5 ± 149.8 nm), percent mesangial area (21.3 ± 6.8%) and/or surface density of the peripheral capillary wall (S/V, 0.106 ± 0.028) differed from normals in 20/21 patients. There was no significant correlation between UAE and GBMT (r = +0.39), percent mesangial area (r = -0.06), S/V (r = -0.09), duration of IDDM (r = +0.10) or CrCl (r = +0.34). In these IDDM patients without overt clinical nephropathy UAE did not reflect the underlying diabetic glomerular pathology. Thus, some patients with longstanding IDDM and minimal structural nephropathy have microalbuminuria while some with shorter duration of IDDM and more advanced renal lesions may have no microalbuminuria. The mechanism by which UAE predicts later clinical nephropathy is unclear and deserves detailed study.

THE CLINICAL USEFULNESS OF KIDNEY BIOPSIES IN THE DIAGNOSIS AND MANAGEMENT OF RENAL DISEASE AH Cohen, CC Nast,* SG Adler, JD Kopple Dept. of Pathology and Div. Nephrology, Harbor-UCLA Medical Center, Torrance, CA.

To assess the usefulness of renal biopsies (RB) in the diagnosis and management of non-transplant related disorders, we surveyed, for 4 consecutive months, nephrologists (56% board certified) whose patients' biopsies (n=83) were evaluated in our referral laboratory. RB were obtained from full-time academicians (25%) or private practitioners (75%). Nephrologist interview data with regard to clinical diagnosis, planned therapy, and an assessment of prognosis were analyzed in the various renal syndromes leading to RB. For nephrotic syndrome (n=32), there was an incorrect clinical diagnosis (ICD) in 75%, a change of therapy (TC) was effected in 50%, and a change in prognosis (PC) in 50%. For chronic renal insufficiency (12), ICD-50%, TC-17%, PC-8%. For asymptomatic urinary abnormalities (9), ICD-44%, TC-11%, PC-0%. For rapid onset renal failure (n=21), ICD-67%, TC-52%, PC-33%. For systemic disease involving kidneys (15), ICD-40%, TC-40%, PC-33%. In 28% of the patients, there were major errors of diagnosis (e.g. amyloid vs membranous nephropathy, acute interstitial nephritis vs crescentic glomerulonephritis), and the RB results led to dramatic changes in therapy or diagnostic procedures. There was no difference between academicians and private practitioners or between nephrologists who were or were not board certified. These data indicate that RB can markedly alter diagnosis, treatment and prognostication, and can increase the likelihood of rational management in patients with renal disease.

IS CLINICAL EVALUATION ADEQUATE FOR ASSESSMENT OF EXTRACELLULAR FLUID VOLUME (ECFV) STATUS IN HYPONATREMIA? H.M. Chung,* R.J. Anderson, R. Kluge,* and R.W. Schrier. Univ. of Colo. Hlth. Sci. Ctr., Dept. Med., Denver, Colorado.

Assessment of ECFV status is important in evaluation of the cause and in selection of appropriate therapy in hyponatremic disorders. However, the sensitivity and specificity of clinical assessment of ECFV status in hyponatremic states remains unknown. We therefore evaluated prospectively 58 nonedematous patients with serum sodium < 130 mEq/L. Patients were initially assessed by two experienced clinical nephrologists who performed a record review, history and physical examination. Subsequently, patients were judged to be either normovolemic (no response to saline infusion) or hypovolemic (saline infusion significantly corrected hyponatremia). Hypovolemic patients had significantly higher plasma renin activity (5.0±1.5 versus 2.5±05 ng/ml/3 hr, p<0.05) and norepinephrine (1,054±252 versus 519±55 pg/ml, p<0.05) concentrations than did normovolemic patients. Clinical assessment correctly identified only 47% of hypovolemic patients and 51% of normovolemic patients demonstrating limited sensitivity and specificity in identifying ECFV status in these hyponatremic patients. However, concentration of sodium in a spot urine sample clearly separated hypovolemic (mean U_{Na}=18.4±3.1 mEq/L) from normovolemic, hyponatremic patients (mean U_{Na}=72±3.7 mEq/L, p<.001). Moreover, a U_{Na}<30 mEq/L was highly sensitive and specific in identifying saline responsive hyponatremia. These results suggest that spot U_{Na} is a more precise method of determining ECFV status than clinical assessment in hyponatremic states.

PREDICTION OF OUTCOME IN ACUTE RENAL FAILURE (ARF). H.L. Corwin, M.J. Schreiber, R. Teplick*, L.S.T. Fang, J.V. Bonventre, C.H. Coggins. Departments of Medicine and Anesthesiology, Massachusetts General Hospital, Boston, Ma

A number of factors have been suggested as influencing prognosis in ARF, mostly on the basis of correlation with outcome rather than by multivariate analysis. In an attempt to predict outcome in ARF we have utilized logistic regression to analyze clinical data from 151 patients with ARF seen over a 14 month period. Recovery of renal function occurred in 60% of patients with 58% patient survival. The most common etiology was ischemic acute tubular necrosis (47%), followed by drug related (19%), contrast agents (15%), and pigment (9%). The following clinical variables were analyzed by logistic regression; age, sex, ARF etiology, initial and maximun BUN and creatinine, oliguria, duration of oliguria, dialysis, surgery, and complications. This analysis demonstrated sepsis, respiratory failure, and oliguria to be the major predictors of non-recovery of renal function. A score for each patient was derived from which sensitivity/specificity curves generated for prediction of outcomes in ARF. The sensitivity and specificity of the prediction is dependent on cutoff score chosen. In conclusion 1) using clinical variables a score predictive of outcome in ARF can be generated 2) sepsis, respiratory failure, and oliguria were the major predictors of non-recovery of renal function 3) age, sex, ARF etiology, dialysis, surgery, and other complications were less important in predicting outcome.

ATHEROEMBOLIC RENAL DISEASE CAUSES HYPOCOMPLEMENTEMIA. FG Cosio, RA Zager and HM Sharma*. Departments of Medicine and Pathology, Ohio State University, Columbus, Ohio.

The purpose of this report is to indicate that hypocomplementemia is a frequent finding in patients with atheroembolic renal disease. Seven consecutive patients with documented atheroemboli demonstrated depressed C_3 levels and 4 of the 7 depressed C_4 levels at or near the time of diagnosis. Serial complement levels in 3 patients demonstrated persistent hypocomplementemia in 1 and return of complement levels to normal in 2. In addition, 6 of 7 patients demonstrated thrombocytopenia and 5 of 7 eosinophilia. We postulated that atheromatous material could activate the complement system *in vivo*. To prove this postulate we developed an experimental model of atheroembolic disease in the rat. Intra-arterial injection of atheromatous material, extracted from human aortas, resulted in decreased C_3 levels 30 minutes after the injection in all 8 rats tested. C_3 levels returned to normal 20 hours after the injection in 4 of 5 rats. Renal histology in these animals revealed: One day post injection: Atheroemboli, focal fibrin deposition in glomeruli and vessels and microinfarcts. Three days post injection: Prominent perivascular infiltration with mononuclear cells and eosinophils. Six days post injection: Vascular intimal proliferation.

Conclusion: These findings document for the first time that atheroembolism causes hypocomplementemia in both man and experimental animals. Atheroemboli should be considered in the differential diagnosis of patients with renal failure and hypocomplementemia with or without thrombocytopenia and eosinophilia.

ADRENERGIC MODULATION OF POTASSIUM METABOLISM DURING EXERCISE. RA DeFronzo, DC Simonson*, P Castellino*. Yale University Sch Med, New Haven, CT.

Potassium (K) homeostasis was examined during 40 min of moderate intensity (40% VO_2 max) ergometer exercise in 6 normal (N) subjects and 7 insulin-dependent diabetics (IDD) receiving a basal insulin infusion (0.07 mU/kg/min). Each subject participated in 3 protocols: exercise alone (EX), EX + Propranolol (P), EX + Phentolamine (PH). P and PH were administered IV 30 min prior to EX and had no effect on basal plasma K(P_K). Following EX + P the increment in P_K was greater than with EX alone in both N (0.88±0.16 vs 0.38±0.07 meq/L, $P<0.01$) and IDD (0.74±0.09 vs 0.47±0.06, $P<0.01$). The rise in K during EX+PH was similar to EX alone in N (0.46±0.08) and IDD (0.35±0.10). To examine the effects of chronic beta adrenergic blockade on K tolerance 12 N and 10 IDD participated in 3 studies: EX, EX plus P (40 mg BID x 5 days), EX plus Metoprolol, (M)(50 mg BID x 5 days). Following P the rise in P_K was greater in both N (1.13±0.14 vs 0.70±0.10) and IDD (1.09±0.10 vs 0.64±0.05). M had no effect on the EX-induced rise in P_K in IDD but was associated with a slightly greater rise in N (0.97±0.05, $P<0.05$). Baseline U_KV remained constant in all studies. The deterioration in K tolerance after both acute and chronic beta blockade with P could not be explained by changes in acid base status or plasma insulin or cathecolamines. In summary, 1) in N both non-selective (P) and selective β_1 (M) blockade impair K tolerance; 2) in IDD non-selective (P) but not selective β_1 (M) blockade impairs K tolerance: 3) α-blockade had no effect on K tolerance; 4) the deleterious effect of β-blockade on K tolerance is exerted via extrarenal mechanism.

EFFECT OF DIPYRIDAMOLE (Dp) ON DIABETIC NEPHROPATHY (DN). Lidia G. de Bermúdez. Francisco J. Bermúdez.* Univ. of Costa Rica, Depts. of Medicine and Pharmacology. San Jose, Costa Rica.

Due to the known importance of altered platelet function in DN, we studied the effect of Dp on the natural history of this complication in 14 patients: 2 were males type I (ages 30 and 31) and 12 were type II: 7 males and 5 females whose average age was 69.3 ± SE 2.25. Two of the type II patients were eliminated from the study: 1 because of poor compliance and 1 because of an associated uric acid nephropathy. Ccr and proteinuria (P) before and after 3 to 24 months of treatment with Dp in a daily dose of 225 to 300 mg. were analyzed. Mean Ccr before treatment was 43.18 ± SE 10.06 ml./min. with a range of 5.9 to 89.0. We tried as much as possible to mantain constant 3 variables: control of BP, absence of UTI and an acceptable blood sugar. The effect of the treatment was a gradual and progressive increase of the Ccr in all the cases; the individual values were analyzed by a pair t test and the p value was <0.005. Ccr decreased while Dp was temporarily discontinued. Improved general condition sometimes raised Scr in spite of increased Ccr. The response of P to Dp was variable: as Ccr rose, it decreased in some patients and increased in those with the greater improvement in GFR. Dp treatment seems to improve renal function in DN in type I and Type II diabetics.

REVERSAL OF RENAL INSUFFICIENCY IN SARCOID NEPHRITIS AND SMALL KIDNEYS, WITH PREDNISONE THERAPY. Douglass T. Domoto, Diane Palmer* and Cheng C. Tsai*. St. Louis University, Department of Internal Medicine & Pathology, St. Louis, Missouri.

Two female Black patients, P.S. and M.E. had sarcoidosis and renal involvement as demonstrated by renal biopsy. The kidneys were small and were 6 cm. and 8 cm. in length when measured with ultrasonography. Creatinine clearance was reduced to 6 ml/minute and 10 ml/minute respectively with serum creatinines of 9.3 mg/dl. and 5.7 mg/dl. Prednisone therapy was begun at 60 mg/day.

Within two months after starting prednisone serum creatinine decreased to 7.0 mg/dl. and 4.3 mg/dl. In both patients, when steroids were discontinued or reduced below 30 mg/day, serum creatinine returned to or toward pretreatment levels. When prednisone was restarted or increased, serum creatinine again decreased. P.S. has remained off dialysis for over a year.

Conclusion: (1) Sarcoid nephritis with decreased glomerular filtration can be stabilized with prednisone. (2) Some improvement in GFR can be expected even if the kidneys are scarred and reduced in size. (3) However, continued high dose is necessary to maintain improvement in renal function.

EFFECT OF PROTEIN RESTRICTION IN CHRONIC RENAL INSUFFICIENCY: A PROSPECTIVE RANDOMIZED TRIAL. Ab J. Donker*, Johan B. Rosman*, Truus Ph. Piers-Becht*, Wim J. Sluiter* (Intr. by L.W. Statius van Eps). Univ. of Groningen, The Netherlands.

Restriction of protein intake is effective in preventing functional deterioration in rats with nephrotoxic serum nephritis or reduced renal mass. Yet, a prospective randomized trial on early protein restriction in man is still lacking. We assessed the effect of protein restriction in a group of 228 patients with various renal diseases and a creatinine clearance (Ccreat) of 60ml/min/1.73 m^2 or less, after stratification for sex, age and renal function (Ccreat 10-30 and 30-60 ml/min). Patients randomly allocated to the protein-restricted groups were advised to adhere to a diet with 0.4 and 0.6 g of protein/kg body weight, respectively. Log rank analysis revealed a lower rate of increase of serum creatinine in the protein-restricted groups during the follow-up of maximal 24 months. Patients (n=149) who could be followed for at least 18 months showed an overall reduction in urea excretion of 100 mmol/24h. Moreover, a reduction in proteinuria was observed . Regression analysis of the reciprocals of median serum creatinine values against time in these patients revealed that protein restriction diminished the median progression rate of renal insufficiency by a factor 3 and 5, respectively. Detailed analysis of the data showed that patients with glomerulonephritis had more benefit from protein restriction than patients with polycystic kidney disease or nephrosclerosis. The results of this prospective randomized study confirm that dietary protein restriction is an acceptable and effective regimen for delaying progression of functional renal deterioration in man.

INTERRELATIONS BETWEEN PARATHYROID HORMONE, 1,25 VITAMIN D_3 AND BONE GLA-PROTEIN IN PATIENTS WITH RENAL FAILURE. P Fanti*, A Smith, R Friedler, PA Price*, R Reitz*, HH Malluche, University of Kentucky, Division of Nephrology, Bone & Mineral Metabolism, Lexington, KY.

Parathyroid hormone (PTH) is known to regulate production of 1,25 VitaminD_3 (1,25) and 1,25 in turn was shown to stimulate production of bone GLA-protein (BGP) in vitro. Reduction of PTH by parathyroidectomy (PTX) is often needed for therapy of patients with uremic osteodystrophy. The present study was designed to evaluate the relationship between PTH, 1,25 and BGP in patients with renal failure. Five chronically dialyzed patients and one patient with a functioning renal transplant (creatinine clearance 50 ml/min) were studied. All patients underwent PTX because of severe hyperparathyroid bone disease. Serum levels of PTH, 1,25 and BGP were measured before PTX, once weekly during the first month after PTX and once monthly for the following 2 months. None of the patients was treated with any Vitamin D compounds. After PTX, there was a fall in PTH (23,830 $\pm$ 9793 to 1574 $\pm$ 828 pg/ml; $p<0.05$) associated with a decrease in 1,25 (20 $\pm$ 4 to 13 $\pm$ 3 pg/ml; $p<0.005$) and a decrease in BGP (1703 $\pm$ 987 to 1085 $\pm$ 588 ng/ml; $p<0.05$). The decline in the blood concentration of PTH correlated both with the decline in 1,25 ($p<0.02$) and the decrease in BGP ($p<0.002$).

The data indicate that 1) PTH controls production of 1,25 even in patients without kidney function; 2) PTH induces directly or indirectly production of BGP. These findings suggest that increased levels of PTH in patients with renal failure represent a compensatory mechanism to offset reduced renal generation of 1,25.

RENAL SIZE IN TYPE 2 DIABETES MELLITUS: A CLINICOPATHOLOGIC STUDY. Francis Dumler, Vijay Kumar, Raymond N Romanski*, Pedro Cortes and Nathan W. Levin. Henry Ford Hospital, Depts. of Medicine and Pathology.

It is known that renal size is increased in Type 1 diabetes mellitus but whether such change occurs in Type 2 diabetes is unclear. We have studied the autopsy reports of 80 consecutive patients with Type 2 diabetes (D) and compared their results to those of 68 non-diabetic age matched autopsied controls (C). Results are expressed as mean±SEM. No differences in absolute kidney weights (KWT) were found between C and D (295±12 vs 294±11 g respectively; p=ns) and no differences were found when KWT was factored by body weight, surface area or ponderal index. Patients were divided into three groups according to serum creatinine concentrations (SC), and D were further subdivided according to the presence or absence of diabetic glomerulosclerosis (DGS). Results were as follows:

	S. CREATININE (mg/dl)		
	< 2.1	2.1-7.9	> 7.9
C:	324±11 (53)	227±22 (9)	141±25 (6)
D: DGS +	360±26 (17)	269±18 (7)	165±23 (8)
DGS -	294±19 (29)	300±21 (18)	250 (1)

In D there was a significant difference ($p=.04$) in KWT between DGS+ and DBS- only in patients with mild renal function impairment. No differences were noted between C and DGS- patients. Conclusion: despite the apparent shorter duration of diabetes when compared to Type 1, patients with type 2 diabetes and DGS+ have a significant increase in KWT when compared to DGS- patients when renal function is relatively normal. As renal insufficiency progresses this difference is lost.

HYPERTENSION AND PROTEINURIA POST RENAL DONATION (PDx). N.L. Ferran*, B.G. Delano, M.M. Beyer, J. Uribarri, E.A. Friedman. Downstate Medical Center, Brooklyn, NY.

We evaluated 42 kidney donors for long term PDx changes in blood pressure (BP), protein excretion and renal function; 27 women, 15 men (31% blacks and 69% whites) with a mean age of 46+-13 yrs. and a mean time PDx of 8.4 yrs. The mean pre vs post BP (BP) for the group was 93+-6 vs 104+-16mm/Hg ($p<0.001$). We defined hypertension (HTN) as a BP >107 mm/Hg. PDx 40% became HTN; 41% were black and 59% white with a BP of 118+-11 mm/Hg for both groups. The incidence of HTN increased with time PDx (max. 123mm/Hg at >9 yrs PDx). All HTN subjects had low or normal renin levels. Proteinuria of >150 mg/24 hrs. was exhibited by 40% of donors; 58% were also HTN. Pre donation values were unavailable for 2/17 of them, but the remaining 15/17 had urinary protein <150 mg/24 hrs. The mean PDx protein excretion was 395mg/24hrs. (range 191-1180 mg). Mean age difference was N.S. in the proteinuric and non-proteinuric groups. Ccr were available for 22/42 subjects pre Dx, mean 104+-39 vs 82+-33 ml/min at time of study ($p<0.001$). The only significant difference was seen between donors with or without proteinuria ($p<0.05$).

Although the BP at >9 yrs. PDx was significantly increased compared to pre Dx values, the importance of this increase when compared with those matched for age and race in the general population remains to be determined. Proteinuria was found with a much greater frequency post vs pre Dx, although no effect on Ccr could be demonstrated. Whether the risks of parenchymal kidney disease will increase in this population remains to be seen.

HYPERTENSION IN BABIES FOLLOWING DISCHARGE FROM NEONATAL INTENSIVE CARE UNIT (ICU). Aaron L. Friedman, Univ. of Wisconsin Hospital, Department of Pediatrics, Madison, Wisconsin.

We followed 17 babies who were found to have elevated blood pressure (BP) at or after discharge from neonatal ICU (systolic BP > 115 mm Hg, repeated 3 times under quiet conditions); babies with hypertension during their stay were excluded. All 17 babies had nursery stays longer than 48 hr. Follow-up was 6 months to 3½ years. Discharges over the follow-up period of babies with stays longer than 48 hours totaled 800. Five babies had the following secondary diagnoses: 2 UPI obstruction, 1 coarctation of the aorta, 1 neuroblastoma and 1 renal artery thrombosis. Five babies had umbilical artery catheters (UAC) for more than 24 hours; three of these babies are included in the group with secondary diagnoses. Thus, 10 babies developed hypertension without obvious cause. Possible associations with development of hypertension, such as sex, age of gestation, type of feeding, ventilator use or antibiotic use, proved insignificant. Sixteen of 17 babies were treated with 1-2 mg/kg/day of propranolol and/or 10-20 mg/kg/day of chlorthiazide. All children older than 2 years were off medication and were normotensive.

We conclude: 1) follow-up of babies discharged from neonatal ICU should include careful BP measurements; 2) high BP may develop in babies who have not had prolonged use of a UAC; 3) even when no secondary cause can be elucidated, hypertension responds to medication; 4) further study is needed to determine if babies discharged from neonatal ICU are at high risk for elevated BP and to determine the natural history, prognosis and best form of treatment for these babies.

SAFETY AND COST EFFICACY OF A RESTRICTIVE POLICY FOR AMINOGLYCOSIDE ANTIBIOTIC (AA) USAGE. J.Gelfand*, H.C.Neu*, H.Morelli*, G.Appel. Columbia U., N.Y.,N.Y.

Despite differences in nephrotoxic potential among the AAs, cost containment may dictate a restrictive policy of hospital usage. In 1/83, guidelines for use of AAs were instituted at Presbyterian Hospital. Pts at high risk for renal failure were given the less nephrotoxic tobramycin(T) upon physician request: all others received the less expensive gentamicin(G). 99 courses of G and 74 courses of T in the subsequent 5 mos were reviewed. T pts were older($p<.01$), with higher initial creatinines($p<.01$) and with more associated serious illness ($p<.001$). Sex distribution, weight, total dose or duration of therapy were not significantly different. Nephrotoxicity (N) was present in 26% of T pts and 13% of G pts, but could be attributed solely to AAs in only 4 pts(3G,1T). N was associated positively with shock($p<.01$) and mortality($p<.01$) but clearly prolonged hospital stay in only 5 pts (2G,3T). Institution of the guidelines resulted in a change from 60% T and 40% G usage, to 30%T and 70% G usage. The savings were approximately $5000. per month, with no increase in the percentage of N with G over that expected from previous studies. We conclude:1) AAs contribute to N in a significant number of patients, although rarely as the only factor. 2) A balanced restrictive policy of AA usage can lead to decreased cost with a low incidence of N.

CYSTOPATHY COMPLICATING DIABETIC NEPHROPATHY **EA Friedman, G Laungani*, L Cohen*, KMH Butt Downstate Medical Center New York.**

We studied the prevalence of cystopathy in uremic diabetics referred for a kidney transplant. A total of 23 diabetics (16 type I, 7 type II) had air cystograms, and measurement of: bladder residual volume (V), maximal pressure (P), and capacity (C). Mean duration of diabetes was 21.4 yrs (type I 21.0 yrs, type II 22.3 yrs). The group contained 13 men and 10 women of mean age 40.7 yrs of whom 18 were on hemodialysis, 2 on peritoneal dialysis, and three on dietary treatment when studied. Cystopathy had not been previously recognized in any patient prior to starting uremia therapy.

Bladder function was graded from 1) (normal) to 4) (detrussor paralysis). There were 12 normal pts (52%) - grade 1: P >75 cm$H_2$0, and C 300-500ml. A minimal defect, was present in 3 pts - grade 2: P normal, and C >500 ml. Grade 3 cystopathy was noted in 1 pt (4%) - grade 3: C >500ml, and P >15 <25 cm $H_2$0. Detrussor paralysis, P = 0 was present in 5 (23%) pts - grade 4: all of whom had C >600ml (mean 676 ml). 2 pts (9%) had normal dynamics in an infected bladder. Repeat study 2 to 6 mos. post-transplant showed no change in 4 grade 4 pts. We infer that cystopathy is frequent (39%) in uremic diabetics - its detection permits distinction from allograft rejection in post-transplant azotemia.

DIABETIC (DGS) AND NON-DIABETIC NEPHROPATHY IN PATIENTS (Pts) WITH DIABETES MELLITUS (DM). J.L. Glickman*, B.C. Sturgill and W.K. Bolton, Univ. of Va. Sch. of Med., Charlottesville, Va.

Indications for renal biopsy in pts with DM are controversial; no large systematic studies have examined this question. We reviewed 112 biopsies of pts with the clinical diagnosis of DM. Of these pts, 100 (89%) had DM defined as a fasting blood glucose of ≥120 mg/dl on 3 or more occasions, episodes of ketoacidosis, abnormal GTT, and/or need for insulin therapy. 49/63 evaluable pts with DGS had retinopathy, while 14 (22%) did not. Hematuria was observed in 37/60 pts (62%). 20% of pts with DM had diseases other than or in addition to DGS - lipoid nephrosis, focal sclerosis, membranous nephropathy, sarcoidosis, acute crescentic glomerulonephritis (GN), SLE, Henoch-Schoenlein purpura, acute interstitial nephritis, membranoproliferative GN, Goodpasture's syndrome, post-infectious GN and PAN. Parameters indicative of non-DGS nephropathy were 1) decreased serum complement, 2) positive antinuclear antibody, 3) DM of ≤5 years duration, 4) red cell casts, 5) presence of another systemic disease with potential for renal involvement. The type of nephropathy (DGS or other) did not correlate with retinopathy, rate of change in renal function other than when very rapid (≤1 month), or degree of proteinuria. Appropriate therapy resulted in an improvement in situations in which the underlying non-diabetic nephropathy was amenable to therapy.

Thus, the present study shows that certain parameters are indicative of non-diabetic nephropathy in pts with documented DM and that some "classic" findings of DM may be absent in pts with DGS. Renal biopsy should be predicated on consideration of these new data.

THE DIAGNOSTIC VALUE OF GALLIUM-67 (Ga) SCANS IN VARIOUS NEPHROPATHIES. T.A. Golper, D.C. Houghton, H.D. Specht,*G.A. Porter. Oregon Health Sciences University, Portland, OR.

The Ga scan is purported to have utility in diagnosing and/or staging various nephropathies. Ga scans were performed prospectively in 22 patients (pts) such that the 48-hr imaging coincided with the renal biopsy. For quantitative evaluation clinical parameters (serum creatinine, quantitative proteinuria, urinary sediment activity and Ga uptake) and histological parameters (glomerular sclerosis, endothelial and extracapillary hypercellularity, interstitial fibrosis, edema and inflammation; tubular atrophy, casts, vacuolization and acute injury) were graded 0 (normal) through 4 (extremely abnormal). A positive correlation was observed between tubular and interstitial scores ($p<.01$) and between glomerular and interstitial scores ($p<.05$). Ga accumulation did not correlate with any clinical or histological parameter.

Abnormal Ga accumulation was observed in all 3 pts with crescentic RPGN, 1/3 with IgA disease, 2/2 with acute pyelonephritis, 1/2 with membranous GN, 1/1 with amyloidosis, 1/4 with acute interstitial nephritis, 1/1 with minimal change disease, 0/2 with proliferative lupus nephritis, 0/2 with arteriolar nephrosclerosis, 0/1 with focal glomerulosclerosis and 0/1 with membranoproliferative GN. Autoradiographs of renal tissue from pts with "positive" scans failed to demonstrate Ga localization.

We conclude that Ga renal scans are nondiscriminatory in sorting out various types of nephropathies and cannot predict the extent of glomerular, tubular or interstitial injury.

METABOLIC EFFECTS OF $NaHCO_3$ THERAPY OF HYPOXIC LACTIC ACIDOSIS (HLA) IN DOGS H. Graf*, W. Leach*, A.I. Arieff. University of California Medical School, VAMC, San Francisco, CA.

The standard of therapy of lactic acidosis has been intravenous $NaHCO_3$, but the mortality remains above 60%. Recent evidence suggests that $NaHCO_3$ might be harmful in the therapy of lactic acidosis. Therefore the metabolic effects of $NaHCO_3$ treatment were investigated in a dog model of HLA.

HLA was induced in 3 groups of dogs by ventilating animals with 8% oxygen, resulting in arterial pO_2 of less than 30 mmHg, pH less than 7.20, bicarbonate less than 12 mM, and lactate more than 5 mM. In this situation the bulk of lactate is produced by the gut (55%) and carcass (45%) in the presence of a greatly diminished capacity of the liver to extract lactate (4% vs control of 15%), probably due to a decreased liver intracellular pH (6.67 vs control of 6.99).

60 min of administration of $NaHCO_3$, NaCl or no treatment resulted in similar decreases of blood pH and bicarbonate in all 3 groups. The concommittant increase in blood lactate was significantly higher in the $NaHCO_3$ group (12.0 mM) vs NaCl (9.4 mM) or no treatment (9.0 mM). Gut lactate production was significantly higher in the $NaHCO_3$ than in the NaCl group. $NaHCO_3$ therapy resulted in a further significant decrease of liver pHi (6.53) and a significant increase in portal venous pCO2 (from 37 to 58 mmHg) both of which were not observed in the other 2 groups.

This data show that $NaHCO_3$ has harmful metabolic effects in the treatment of HLA as evidenced by: a) no increase in blood pH or bicarbonate; b) higher gut lactate production than with NaCl; c) decreasing hepatic pHi and d) higher blood lactates than with NaCl or no treatment. Thus, therapy of HLA with $NaHCO_3$ should be re-evaluated.

PROGNOSTIC VALUE OF THE INTERSTITIAL CHRONICITY INDEX IN IDIOPATHIC MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS (IMPGN). Janet U. Gorkin,* Richard B. Molina,* and Leonarda B. Sablay,* (intr. by Norman Bank). Montefiore Med. Ctr., Depts. of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, New York.

The prognosis of IMPGN in adults is often difficult to determine at the time of presentation. In order to evaluate renal biopsies more precisely and to assess their prognostic value in IMPGN, we studied the biopsies of 7 adult patients with IMPGN using a semiquantitative scoring method. Biopsies were reviewed by a nephrologist and nephropathologist who were "blinded" as to clinical data. Ten specific lesions were scored and categorized to reflect changes of activity and chronicity as well as glomerular and interstitial involvement. Scores were correlated with clinical course obtained retrospectively. Clinical course was expressed as change in serum creatinine over time. The average length of follow-up was 39.8 mos.,(range 4-114 mos). We found a strong correlation between the interstitial chronicity index, a measure of interstitial fibrosis and tubular atrophy, and progression of renal insufficiency ($R=0.85$, $p<0.025$), wheras there was no correlation between glomerular indices and clinical progression.

We conclude that the degree of tubular atrophy and interstitial fibrosis on initial biopsy is more predictive of eventual outcome in IMPGN than is the nature or degree of the glomerular involvement. Our observations extend the growing body of evidence that chronic interstitial changes are more predictive of outcome than glomerular lesions in certain forms of chronic glomerulonephritis.

LEAD EXCRETION AND PRE-ECLAMPSIA. Bruce A. Greenfield,* Robert J. Carpenter,* Paul Claman,* Gregory Matter,* and Garabed Eknoyan, Baylor College of Medicine, Houston, Texas.

Chronic lead toxicity has been implicated as a causative factor in tubulo-interstitial nephritis, gouty nephropathy and hypertensive renal disease. Because pre-eclampsia shares several features of chronic lead toxicity (hypertension, hyperuricemia, renal insufficiency) and a shared predilection for lower socio-economic groups, lead toxicity has also been implicated as a possible causative factor in the toxemia of pregnancy. In order to determine whether such a relationship exists, the urinary excretion of lead was determined after the intravenous infusion of 1 gram of calcium disodium edetate ($CaNa_2EDTA$), 48 hours post-partum, in 5 pre-eclamptic women (diastolic pressure >105 mmHg, 2+ proteinuria, no history of hypertension or nephropathy) and compared to that of 5 normotensive primi-paras. The use of $CaNa_2EDTA$ infusion to mobilize lead stores, and thus enhance urinary lead excretion, has been shown to be an accurate and safe tool for assessing excessive body lead content.

There was no difference between the 2 groups in creatinine clearance or in lead excretion (49.2±7 mcg/day in pre-eclamptics and 58.2±14 mcg/day in normals). These levels are well below the toxic level of 600 mcg/day.

The results of the present study indicate that: 1) lead toxicity is not a contributory factor in pre-eclampsia; 2) the low levels of lead excretion obtained in our subjects, relative to those reported for normal males from New Jersey (340±39 mcg/day), would suggest a geographical variation in the exposure to lead.

ALTERED IMMUNOGLOBULIN STATUS IN CONGENITAL NEPHROTIC SYNDROME (CNS) H. William Harris, Jr.*, Dale Umetsu*, Raif Geha* and William Harmon
Department of Pediatrics, Harvard Medical School Boston, MA

Infants with CNS suffer from severe failure to thrive, frequent infections and high mortality. Since lowered immunoglobulin levels might contribute to the morbidity of CNS, we studied the serum and urine immunoglobulin concentrations of a patient with CNS during an 11 month period following diagnosis at 6 weeks. Serum IgG levels were never higher than 25% and most were below 2% of normal infant values. Serum IgM levels rose to three times normal and IgA concentrations varied. Repeated measurements of the selective protein index (SPI) while the patient was hypogammaglobulinemic demonstrated that the proteinuria appeared highly selective (SPI < 0.06) under steady state conditions throughout the 11 months. Intravenous infusions of γ-globulin were performed to determine their effect on serum IgG levels. The infused IgG was initially contained within the intravascular space and produced expected increases in the serum IgG levels. Within 30 hours, however, 55% of the IgG was excreted in the urine, and by 80 hours the serum IgG had returned to pre-infusion levels. Additionally the renal clearance of IgG increased 20-fold immediately post-infusion rendering the proteinuria "non-selective". We conclude that children with CNS should be considered agammaglobulinemic throughout the first year of life and perhaps longer. Intravenous IgG infusions provide only hours of sufficient IgG replacement due to urinary losses and may be detrimental.

SERUM COPPER AND ZINC OF NON-UREMIC PATIENTS WITH VARIOUS RENAL DISEASES. Shinichi Hosokawa, Tetsuya Imai,* Toshiji Nishio,* Tadao Tomoyoshi,* Kenji Sawanishi.* National Utano Hospital, Shiga University, Kyoto University, Kyoto, Japan.

We examined the clinical significance of serum copper (Cu) and zinc (Zn) levels in patients various sorts of renal diseases. We measured serum Cu and serum Zn concentrations in 10 patients with chronic glommerulonephritis (CGN), 10 with diabetic nephropathy (DN), 10 with essential renal bleeding (ERB), 10 with acute pyelonephritis (APN) and 10 recovering from APN (R.APN). In all patients, serum protein values and protein analysis were normal. Serum Cu and Zn were measured by atomic absorption spectrophotometer (Hitachi, Japan). Serum Zn levels were significantly below normal (58 ± 10 μg/dl; normal values 100 ± 20 μg/dl) in all patients with CGN, DN, ERB and APN. However, serum Zn levels increased to normal values (96 ± 8 μg/dl) in 10 patients with R.APN. In CGN, DN, and APN patients, there was a significant correlation between serum Cu levels and BUN and between serum Cu and serum creatinine. The ratio of serum Zn to serum Zn plus serum Cu (Zn/(Cu+Zn) x100%) were 48 ± 4% in normal subjects, 39 ± 5% in CGN, 34 ± 6% in DN, 37 ± 5% in ERB, 34 ± 5% in APN and 43 ± 3% in R.APN patients. In ERB patients, red blood cell counts in urine were significantly correlated with serum Zn levels ($r=0.88$, $p<0.01$). These results show that serum Zn concentration can be used clinically as a diagnostic indicator of renal disease. The same is true of the ratios of serum Zn to serum Cu plus serum Zn (Zn/(Cu+Zn) x100%). High serum Cu levels correspond to more advanced stages of renal disease.

DIETARY AND SERUM LIPID LEVELS IN HUMANS: RESPONSE TO ORAL CA^{2+}. H. Henry,* N. Karanja,* C.D. Morris,* D.R. Illingworth,*D.A. McCarron. Oregon Health Sciences University, Portland, OR.

Cardiovascular risk factors cluster in individuals. A randomized trial of 1 g Ca^{2+} assessed relationships between BP status, dietary and plasma lipids in 62 hypertensives (HTN) and 29 normotensives (NL).

Reported dietary intakes from 3 24-hr recalls revealed no differences between HTN and NL in kcal, cholesterol (chol), saturated, unsaturated or total fat intakes. Plasma lipids in 27 NL and 40 HTN showed significant ($p<.05$) differences between NL and HTN females. HTN values were 28 ± 15, 138 ± 73 and 57 ± 15 mg/dl for VLDL, triglycerides and HDL respectively, and in NLs values were 18 ± 10, 92 ± 49 and 67 ± 17 mg/dl respectively. No changes were observed between HTN and NL males.

The group as a whole had no changes in plasma lipids as a result of Ca^{2+} supplementation. However, in those individuals above the 75th percentile (at baseline) for chol, LDL chol and triglycerides and below the 10th percentile for HDL chol, Ca^{2+} caused a drop in total chol from 250 ± 25 to 238 ± 20 mg/dl compared to placebo in NLs only. LDL chol dropped from 187 ± 17 to 176 ± 17 mg/dl. Subdividing this group further into males and females revealed a downward (non-significant) trend of total chol and LDL chol in both sexes. No changes in serum lipids were observed in HTNs.

We conclude that: 1) abnormal plasma lipids may be more prevalent in HTN females, and 2) 8 weeks of supplemental Ca^{2+} will lower BP and may favorably modify plasma lipids, but longer periods of supplementation may be required for HTNs with subnormal plasma lipids.

REVERSIBLE HYPORENINEMIC HYPOALDOSTERONISM IN S.L.E. R.M. Hurley, M.D.; G.A. Kozeny, M.D.; R. Fresco, M.D., Ph.D.; L.L. Vertuno, M.D.; and J.E. Hano, M.D. Loyola University Medical Center, Maywood, IL.

Spontaneous hyperkalemia has been noted in patients with long standing S.L.E. without advanced renal insufficiency. The patients previously described all had normal renin-aldosterone systems and the hyperkalemia was presumed secondary to a primary defect in renal tubular handling of potassium. We report a 10 year old girl who presented with hyperkalemic, hyperchloremic metabolic acidosis secondary to lupus nephritis but without significant renal insufficiency. Renal function studies revealed: a GFR of $68ml/min/1.73m^2$; a maximum FE_K of 6.8% (in the face of a serum K+ of 5.5 mEq/L); a normal sodium bicarbonate and sodium sulfate loading test; and markedly surpressed spontaneous and furosemide stimulated plasma renin & aldosterone levels. A renal biopsy demonstrated diffuse proliferative glomerulonephritis, mild intersititial nephritis, and necrotizing vasculitis involving the afferent and efferent arterioles and obscuring the juxtaglomerular apparatus/macula densa. Three months after treatment with prednisone (1mg/kg/day) the clinical symptoms abated and the electrolytes, urinalysis, and acid-base status normalized. Renin & aldosterone levels responded appropriately to furosemide stimulation. The patient demonstrates a reversible form of hyporeninemic-hypoaldosteronism associated with lupus nephritis.

EFFECT OF REDUCED DIETARY PROTEIN INTAKE ON ALBUMIN HOMEOSTASIS AND ALBUMINURIA IN MAN. F.N. Hutchison*, J. Gambertoglio, I. Jiminez, H. Jones, Jr.*, G.A. Kaysen, VA Medical Center, Martinez, CA, UC Davis, Davis, CA, U.C.S.F., San Francisco, CA.

High protein diets are prescribed for nephrotic patients to replace urinary protein loss, maximize albumin (alb) synthesis (syn) and optimize serum alb concentration (Salb). However, high protein feeding may accelerate the course of some renal diseases. To determine whether reduction in dietary protein adversely affects alb pool sizes or Salb in nephrotic patients alb homeostasis was measured in six subjects on sequentially high and low protein diets. Patients were placed on a diet containing 1.6 grams protein and 35 Kcal/Kg body weight for 14 days and then dietary protein was reduced to 0.8gm/Kg body weight with an isocaloric switch of carbohydrate for protein. Alb syn, catabolism (cat) and distribution were measured using ^{125}I alb, and urinary alb was measured for each 14 day period.

Urinary alb fell consistently in every patient median -2.11 g/1.73m^2/24h, -28% P <.05. Alb cat and syn fell in 5 patients, median -3.58g and -8.62 g/1.73 m^2/24h respectively. Salb was not significantly changed in any patient, median +.04g/dl. Total alb mass and extravascular alb mass each rose in 5 patients, median +11.5g and +7.45g/1.73 m^2, while plasma alb mass did not change. In summary, dietary protein restriction is followed by a fall in urinary alb loss in all, and a decrease in both alb syn and cat in most nephrotic subjects studied. There is no depletion of any alb pool. Protein restriction does not adversely affect alb homeostasis in nephrotic patients.

RELATIONSHIP OF CELLULAR COMPOSITION TO RESPONSE TO THERAPY (Rx) IN ACUTE CRESCENTIC RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (AC-RPGN). D. Innes*, B.C. Sturgill and W.K. Bolton, Univ. of Va. Sch. of Med., Charlottesville, Va.

AC-RPGN has a poor prognosis with most patients (pts) progressing to renal failure with conventional Rx. Certain pts will improve with pulse methylprednisolone. The present study evaluated the type of cellular infiltration in kidney biopsies of responding (R) and non-responding (NR) pts after pulse Rx using monoclonal antibodies. Frozen tissue sections were examined with an avidin-biotin method using the following monoclonal antibodies: T-cells (Leu-1 and Leu-4); T-suppressor (T_S Leu-2-A); T-helper (T_H Leu-3); natural killer (NK, Leu-11-B); B-cells (Leu-14); myelomonocyte (Leu-M 1) and monocyte-macrophage (MΦ, Leu-M 3). Slides were evaluated without knowledge of the clinical course or Rx of the pt. There were 13 evaluable pts - 5 anti-GBM, 2 vasculitis, 4 no-immunoglobulin-deposit and 2 granular type AC-RPGN. 9 pts improved with pulse Rx including 1 anti-GBM, while the other 4 anti-GBM pts were NR. Crescents were not different (63±11% for R, 82±16% for NR). T/B in NR was 5.8:1 compared to 5.1:1 for R. T_H/T_S in NR was 3.5:1 compared to 3.1:1 in R. Few NK cells were observed. MΦ were seen in and around crescents, in glomeruli, and in interstitial infiltrates. Discrete clusters of lymphocytes were seen in 7/9 R pts and 1/4 NR. Interstitial infiltration with T-cells and MΦ was mild in 7/9 R pts and severe in 3/4 NR pts.

These findings suggest that the degree of periglomerular and interstitial cellular reaction, rather than the type of cells involved, and the pattern of distribution of cells, are important determinants in response to therapy.

MYELINOLYSIS IN RABBITS FOLLOWING RAPID CORRECTION OF HYPONATREMIA. Barbara Illowsky* and Robert Laureno* (intr by Leslie Pierce) Dpts. of Medicine and Neurology, Washington Hospital Center and Dpt. of Neurology, George Washington Univ., Washington, D.C.

Pontine and extrapontine myelinolysis has been produced in dogs and rats by the rapid correction of hyponatremia. (Ann Neurol 13:232, 1983) Despite these results some authors still suggest that uncorrected hyponatremia can cause myelinolysis.

To resolve this issue thirty-five rabbits were made hyponatremic by the daily administration of IP D5W and IM Vasopressin. Serum sodium was measured daily in each animal. Group I animals were maintained with severe hyponatremia (serum $[Na^+]$=122 mEq/l). For the 8 animals surviving severe hyponatremia at least 7 days (mean 9 days), mean $[Na^+]$ was 108 mEq/L (range 90-122). Three animals appeared well; 5 were lethargic or diffusely weak. No myelinolytic lesions were present on autopsy. Group II animals were severely hyponatremic for 3 days with mean serum $[Na^+]$=107 . These were corrected with IP administration of 3% saline to a mean serum $[Na^+]$ of 131 mEq/L (range 123-136) at 24 hours after correction. Six of 7 rabbits surviving correction developed neurologic illness (seizures, monoplegia, quadriplegia); 3 had myelinolytic lesions in the thalamus; one also had a lesion in the central pons.

In summary, severe, protracted, uncorrected hyponatremia is insufficient to produce myelinolytic lesions in rabbits. However rapid correction of hyponatremia of shorter duration can cause central pontine and extrapontine myelinolysis.

LITHIUM-INDUCED NEPHROPATHY: A 3 YEAR PROSPECTIVE STUDY. D. Jorkasky, J. Amsterdam* and M. Cox. Departments of Medicine and Psychiatry, University of Pennsylvania School of Medicine, Phila., Pa.

Considerable controversy exists as to whether lithium (Li) maintenance therapy is associated with renal insufficiency. In 1980 we initiated a prospective study of renal function in manic-depressives beginning Li therapy, and who had no evidence of pre-existing renal disease. Sixty-five pts were entered, 37 remain active, and 12 have reached 3 year follow-up. Li was titrated to the lowest level consistent with control of symptoms; there have been no episodes of Li-intoxication.

Year	0(n=65)	1(n=39)	2(n=24)	3(n=12)
SLi	-	0.69±.40	0.68±.21	0.72±.27
SCr	0.92±.16	0.97±.20*	0.98±.19*	1.03±.24
CrCl	104±28	101±24	90±33	71±33
UP	65±64	125±36	114±21*	81±48
Uosm	903±111	840±126*	867±121*	764±105
UV	14.1±8.5	18.8±9.7*	14.5±6.8	16.1±9.3

SLi=serum Li(mEq/l), SCr=serum creatinine (mg/dl), CrCl=Cr clearance (ml/min), UP=urine protein (mg/d), Uosm=maximal urine osmolality (mOsm/Kg), UV=urine volume (dl/d); mean±SD, *=p<0.05, paired t-test; 3 year data not subjected to statistical analysis due to small sample size.

Thus, Li causes renal insufficiency. Considering the short duration of Li therapy, the relatively low SLi levels, and the avoidance of Li intoxication, this finding assumes even greater importance for the long-term treatment of manic depressives in the community at large.

RADIOLOGICAL AND BIOCHEMICAL PARAMETERS IN EVALUATION OF BONE DISEASE IN CHILDREN ON CAPD H. Kangarloo,* I.B. Salusky, J.W. Coburn, L. Paunier,* E. Slatopolsky & R.N. Fine. Dept Radiol, Peds, & Med, UCLA Sch Med, VA Wadsworth Med Ctr, Los Angeles, CA & Dept Med, Wash U Sch Med, St Louis, MO

The ability to predict the severity of renal osteodystrophy from serum (S) parameters has not been studied in children on dialysis. Therefore, we performed a prospective study in 17 children, ages 9.5±4.1 yrs (SD) and treated with CAPD for 22±7.4 mos. They were followed for 17±5.6 mos while calcitriol (1,25D) was given to maintain SCa above 10.5 mg/dl; S-Ca, P, alk p'tase (AP) and iPTH were measured monthly, and S-Al and X-rays of the left hand were obtained every 6 mos, using the same radiographic factors for each X-ray exposure. Semi-quantitative scores for sub-periosteal erosions (E) and growth-zone changes (GZ, rickets-like lesions) were developed and applied by one of us (HK) independent of clinical data. Total X-ray score (TX) was taken as the sum (0-18) of E (0-9) and GZ (0-9). Means of 3 values of SiPTH,AP,Ca & P at the time of X-ray were used for correlations(r) with E,GZ and TX (total n=51):

CORREL. COEFF between	TX	E	GZ
S-iPTH	0.62	0.59	0.30
S-AP	0.73	0.66	0.53

S-iPTH correlated inversely with SCa (r= -0.57) but not with SP, r=0.04. Bone biopsy in 2 atypical patients with high SAl showed Al-related osteomalacia. Utilizing bone X-ray as the index of bone disease in the growing skeleton, these data indicate that SiPTH and S-AP are reasonable predictors of erosions and growth zone changes but provide less indication of Al-related bone disease.

INCREASED SERUM POTASSIUM (K) DUE TO COMBINED CALCIUM CHANNEL AND BETA ADRENERGIC BLOCKADE. Stephen Kelleher*, David Gillum*, SUNY at Stony Brook, NY and UCHSC, Denver, Co.
(Introduced by George J. Kaloyanides)

Unexplained hyperkalemia was observed in a patient after initiation of nifedipine (N). A retrospective analysis of the chronic effect of N on K was then performed in all patients receiving N at the Denver VAH. Subjects with renal insufficiency, diabetes, obstructive uropathy, or receiving oral K, K sparing diuretics or NSAID were excluded. Chemistries before and during N were available in 46 patients. In the entire group K increased significantly ($p<.01$) from 4.2±0.5 to 4.5±0.2 meq/L. Eight subjects summarized in the table developed modest hyperkalemia (5.1-5.6 meq/L) in the absence of other electrolyte abnormalities.

	Na	K	CO_2	Cl	Glu	Creat.
Pre-N	139±4	4.2±.3	25±2	104±5	114±25	1.2±.2
DuringN	141±5	5.3±.2	26±4	105±6	105±25	1.2±.2

Dose of N (43±15 mg/d) in these 8 subjects was not different from the entire group (42±11 mg/d). However, in 6/8 N was added to a stable dose of propranolol (P). Serum K before and during therapy was then compared in 3 groups:N alone, P alone, and N added to a stable dose of P. Differences in doses between groups were not significant.

Group	n	dose N	dose P	K pre	K during	p value
N	22	40±6		4.2±0.5	4.3±0.5	NS
P	24		156±99	4.3±0.2	4.4±0.3	NS
N+P	24	44±15	204±111	4.2±0.5	4.7±0.5	$p< .01$

It is concluded that the addition of N to P induces a significant increment in serum K. The mechanism underlying this observation remains to be elucidated.

PROGNOSIS OF LUPUS NEPHRITIS. K. Kawala*, J. Bernstein, H. Shapiro, E.J. Lewis, M.M. Schwartz, Rush Medical College, Chicago and Wm. Beaumont Hosp., Detroit.

We report our experience with 128 SLE patients with active renal disease and renal biopsies analyzed according to ISKDC-WHO classification. 5 yr survival(surv) for segmental GN-category III (49 pts)=81.3%; DPGN-category IV (37)=50.4%; membranous GN-category V (30)=88.4%; for mesangial GN-category II(10)=40% and for all (128)=71%. Deaths were categorized as renal (RD)(5% at 5 yr) and nonrenal (NRD)(17% at 5 yr). ESRD was 7% at 5 yr. Overall caucasian(CA) surv at 5 yr = 74%, non-caucasian (n-CA) = 66%; for CA in category IV = 40% at 5 yr; n-CA category IV = 50% at 5 yr. Quantitative proteinuria did not correlate with outcome. Proteinuria >2G/d plus Cr >2 mg/dl strongly correlated with category IV. Early deaths in category IV clustered within 6 mos. Initial serum Cr(Cr_i) was an important risk factor particularly for RD/ESRD. Patients Cr_i ≤1.2 had 1 yr surv = 92%; 5 yr surv 80%: Cr_i 1.3-2.0 had 1 yr surv = 95%; 5 yr = 63%; Cr_i 2.1-4 had 1 yr surv = 78%; 5 yr = 40%: Cr_i ≥4.1 had 1 yr surv = 51%; 5 yr = 39%. However, of 30 patients who NRD/RD/ESRD, 13 had Cr_i ≤1.2. 35 patients increased Cr >40%. Of these 19 had Cr_i ≤1.2; 5 had Cr_i 1.3-2.0; 6 had Cr_i 2.1-4 and 5 had Cr_i ≥ 4.1. 11/35 patients with Cr increased >40% were III, 14/35 were IV and 7/35 were V. Our results emphasize the significance of DPGN(IV) and serum Cr_i as predictors of clinical outcome in SLE. DPGN(IV) had significantly poorer prognosis than segmental GN(III). ESRD and RD correlated with Cr_i, NR deaths did not. Decreasing GFR was observed irrespective of Cr_i and was noted in categories III, IV and V. Race was not a significant risk factor.

ANGIO ACCESS COMPARISON OF AUTOGENOUS FISTULA VS. PROSTHETIC GRAFTS. George Kherlakian*, Kenneth J. Newmark, Lionel R. King, James Arbough*, and L. Richard Roedersheimer*. Depts. of Medicine and Surgery, Good Samaritan Hospital, Cincinnati, Ohio.

Angio access remains a critical problem in chronic hemodialysis (CH). The autogenous AV fistula (AAVF) has been shown to have fewer complications and longer patency rates; however 25-30% of patients requiring CH do not have suitable vessels for creation of AAVF. In these patients use of a prosthetic material has been an acceptable alternative; and expanded polytetraflouroethylene (E-PTFE) has been shown to have the best long term patency, ease of insertion, lowest infection rate, and ease of thrombectomy. Over a period of66 mo. during which 200 patients entered our CH pool, we compared 100 primary AAVF insertions to 100 primary E-PTFE grafts. The groups were analyzed for incidents of early and late thrombosis, infections, pseudoaneurysms and venous hypertension with life table analysis and cumulative patency.The mean age of both groups was similar (50 yr. in the AAVF group vs. 53 yr. in the E-PTFE group). There were a greater no. of males in the AAVF group.Incidence of diabetes and hypertension was similar. There was a higher incidence of peripheral vascular disease, coronary artery disease, infections, venous hypertension in the E-PTFE group. Early thrombosis was significantly higher in the AAVF group. Life table method showed similar patency for 24mo. but after 36 mo. cumulative patencies in theE-PTFE group falls. AAVF is the preferred method of angio access in appropriately selected patients. E-PTFE graft insertion as a primary procedure in potentially difficult patients has proven to be an acceptable alternative.

DECEPTIVE PATTERNS OF PULMONARY EDEMA IN UREMIC PATIENTS. Jeffrey Kohen,* John Opsahl*, Carl Kjellstrand. Hennepin County Medical Center, Department of Medicine, Minneapolis, Minnesota.

Pulmonary edema is a common complication in patients with chronic renal failure. The etiology is multifactorial and includes: increased extracellular space, decreased oncotic pressure, and toxic effects of uremia on pulmonary capillaries. It is usually first seen in the hilar region of the lungs (butterfly or bat-wing edema), later spreading to the periphery. Chest x-ray is important in following these patients and important in making therapeutic choices.

We have encountered atypical pulmonary edema patterns in 3 patients. Two were originally mistaken for lobar pneumonia, in one the pattern simulated metastatic carcinoma or fungus balls. All patients had evidence of increased right sided hydrostatic pressure, 2 were severely uremic, but only one was hypoalbuminemic and one showed weight gain. Moist rales over the lungs were present diffusely in 2 patients. Based on the x-ray findings, antibiotics only were suggested as therapy in one patient. In one, a lung biopsy was advocated. However, all patients were immediately dialyzed and ultrafiltrated with fluid removal of 5-11% of body weight. Within 12-36 hours the chest x-rays were normal.

It is important to be aware of such deceptive radiographic patterns, otherwise, potentially disastrous delay in fluid removal may result.

EFFECT OF DOPAMINERGIC BLOCKADE ON PLASMA (P) ALDOSTERONE (A) LEVELS IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF). G. Krishna, N. Kopyt*, R. Hoeldtke*. Department of Medicine and Clinical Research Center, Temple Univ., School of Medicine, Philadelphia, PA.

Dopamine is an important regulator of A release. Dopaminergic blockade using metoclopramide (MCP) increases PA levels in healthy subjects. This is accompanied by an increase in plasma prolactin (PRL) levels while plasma renin activity (PRA) remains unchanged. To study the effect of dopaminergic blockade on PA levels in patients with CRF, and to investigate its applicability in the management of hypoaldosteronism associated with CRF 7 subjects with creatinine clearance values ranging from 13-62 ml/min were placed on a diet containing 80 meq of Na and 60 meq of potassium daily for 5 days. The plasma K at the end of this period was 4.7±0.2 mEq/L and creatinine clearance 32±6 ml/min. Four of these patients with CRF were hyperkalemic (>5 mEq/L) with inappropriately low PA (8±3 ng/dl) levels. 10 mg of MCP was administered as an IV bolus to each of these subjects with CRF. PA, PRA and PRL levels were measured pre study and at 15 minute intervals.

Time (min)	Pre-study	15'	30'	60'
PA (ng/dl)	19.8±5.5	19.8±5.5	21.4±5.9	18.6±4.7
PRA (ng/ml/hr)	1.1±0.3	0.9±0.4	1.1±0.4	1.2±0.5
PRL (ng/ml)	33±5	130±59	143±37*	190±68*

*p<0.05 compared to prestudy levels.

MCP administration failed to stimulate A release in patients with CRF with and without hypoaldosteronism. PRL levels rose while PRA and plasma concentrations of Na and K were unchanged from prestudy levels. Thus, doses of MCP which increase PA levels in normal subjects failed to do so in patients with CRF. The effect of multiple dose administration as well as the potential inhibitory role of uremia on PA response to dopaminergic blockade is currently under study.

EVALUATION OF ^{99m}Tc-DTPA NUCLEAR IMAGING TO QUANTITATIVELY DETERMINE THE GLOMERULAR FILTRATION RATE OF DOGS. Donald R. Krawiec, Robert R. Badertscher II,* A. Robert Twardock,* and Stanley I. Rubin.* Univ. of Illinois, Dept. of Vet. Clin. Med., Urbana, Illinois

The suitability of ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) as an agent to assess glomerular filtration rate (GFR) in dogs was studied. Glomerular filtration rates of 13 dogs before and after amphotericin B induced acute renal failure were calculated using data obtained from inulin and 24 hour endogenous creatinine clearances. These dogs were then injected intravenously with 1-2 mCi of ^{99m}Tc-DTPA. Percent total renal ^{99m}Tc-DTPA uptake was determined with background activity subtracted at 15-second intervals over a 6-minute period. Linear regression analyses (LRA) were performed to determine the correlation between percent ^{99m}Tc-DTPA uptake at several time intervals postinjection and GFR's calculated from creatinine and inulin clearance data. Percent dose ^{99m}Tc-DTPA corrected for kidney depth differences was also determined in a subgroup of dogs and LRA's against GFR's performed. Percent ^{99m}Tc-DTPA correlated better with inulin clearance (r=.87) than with endogenous creatinine clearance (r=.74). Both correlations improved only slightly with kidney depth correction. The 1-2 minute, or 1-3 minute intervals post ^{99m}Tc-DTPA administration appeared to be the best periods for analysis of data. It is concluded that ^{99m}Tc-DTPA can be used as a agent for the rapid, noninvasive and accurate determination of GFR in dogs.

EFFECT OF SIMPLE AND COMPLEX CARBOHYDRATE INTAKE ON THE NATRIURESIS OF CALORIE RESTRICTION. G. G. Krishna, C. Gross*, G. Hercz*, C. R. Kleeman, L. P. Dornfeld, M. H. Maxwell. UCLA School of Medicine, Department of Medicine, Los Angeles, CA.

Interruption of starvation with a low calorie simple carbohydrate diet markedly reduces the natriuresis. To determine whether initiation of a low calorie diet with carbohydrates prevents the copious natriuresis and to see if simple and complex carbohydrates have a varying influence on the sodium excretion, the following studies were performed. Three groups of obese subjects (>25% over ideal body weight) were placed on differing isocaloric 300 calorie diets for 5 days. Group I (n=5): 45g protein + 30g simple carbohydrates (glucose); Group II (n=4) simple carbohydrates (glucose); Group III complex carbohydrates (starch). All diets contained 45 meq Na, 40 meq K^+ per day plus vitamin and mineral supplements. Net negative Na balance (meq/5 days) of equal magnitude was noted in all 3 groups (Group I 190±61; Group II 244±120; Group III 140±94). The creatinine clearance values (ml/min/1.73m^2) fell from pre-study levels in Group II (87±7 to 67±9 p<0.05) and Group III (82±5 to 57±5 p<0.05), while there was no significant alteration in Group I (78±17 vs 67±9). The diastolic blood pressure (mmHg) fell from pre-study levels only in Group III 92±3 to 78±7 (p<0.05) while it was unchanged in Group I (81±9 vs 73±3) and Group II (82±7 vs 81±3).

These studies suggest that low calorie diets utilizing simple or complex carbohydrates do not alter the ensuing natriuretic response. However, the presence of protein in the diet attenuates the decline in GFR accompanying calorie restriction. The potential beneficial role of complex carbohydrates in reducing blood pressure during calorie restriction deserves further study.

HYPERCALCIURIA AND RISK FACTORS FOR CALCIUM NEPHROLITHIASIS (CN) DURING PREGNANCY (PG). C. Kristensen*, P.A. Abraham, M. Davis*, C.L. Smith, Hennepin County Medical Center, Mpls, MN.

Urinary calcium excretion increases in PG, but it is unclear if the risk for CN is enhanced. We studied potential risk factors for CN in normal PG subjects (n=13). Studies during the third trimester (PG3) and after PG or lactation (NPG) included diet history, serum Ca, ionized Ca, ultrafilterable Ca, parathyroid hormone (PTH), and 24 h urine for creatinine clearance (Ccr), volume (V), excretion of Ca (UcaV), oxalate (UoxV), phosphorus, sodium, uric acid, creatinine, citrate (UcitV), magnesium (UmgV), and urinary saturation studies including activity product ratios for brushite (APR_B) and calcium oxalate (APRox).

	Ccr (ml/min)	UcaV (mg/d)	UoxV (mg/d)	UcitV (Mmole/d)	UmgV (mg/d)
NPG	95	121	20	3.8	7.8
PG3	120*	259*	29*	6.1*	11.4*

Data are mean values; *$p < 0.05$, NPG vs PG3

From NPG to PG3, serum Ca decreased from 9.5 to 9.1 mg/dl ($p<0.05$), APR_B increased from 1.24 to 1.77 and APRox from 1.99 to 3.13, with no change in V. During PG3, hypercalciuria (UcaV 250>mg/d) occurred in 7/13 subjects compared to 1/13 NPG. This correlated with dietary Ca (p 0.05) which increased from 1275 mg/d (NPG) to 1820 mg/d (PG3) ($p< 0.01$). In addition, PTH decreased from 0.37 ng/ml (NPG) to 0.22 ng/ml (PG3) ($p < 0.05$). There was no change in other parameters.

Urinary supersaturation increases during PG as a result of higher UcaV and UoxV and should enhance the risk of CN. However, the increase in UcitV and UmgV (inhibitors of Ca stone formation) may prevent CN in normal PG.

RACIAL DIFFERENCES IN GENETIC EPIDEMIOLOGY OF ESRD. Douglas M. Landwehr, Richard J. Loveluck,* Walter E. Nance* and Joann A. Baughman.* Medical College of Virginia, Depts. of Human Genetics and Medicine, Richmond, VA, and Allegheny General Hospital, Dept. of Medicine, Pittsburgh, PA.

A population-based genetic and demographic study was carried out to elucidate risk factors responsible for the high incidence of ESRD in blacks(B). All 459 ESRD patients residing in central Virginia during an 8 month survey period were interviewed and their medical records examined. B represented a disproportionately large percentage of ESRD patients(62.3%) relative to their percentage of the population(28.8%), $p<.01$, and had significantly increased relative risks(RR) for ESRD associated with hypertension(HBP)(14:1), diabetes(D)(6:1), glomerulonephritis(GN)(2.5:1), and interstitial nephritis (IN)(2.3:1), all $p<.01$. Since the RR for B vs whites(W) of ESRD due to HBP and D is greater than the RR of B for HBP(2.4) and D(3.3), ESRD occurred as a complication of these diseases more frequently in B than W, $p<.01$. Although there was no correlation between age of onset and educational attainment in W, a significant negative correlation($r=-.36, p<.01$) was found in B, implying that education is associated with later onset of ESRD in B. Heritability estimates were obtained for etiologies of ESRD from analysis of reported frequencies of HBP, D, GN in parents of affected probands. Tetrachoric correlates showed that D in ESRD patients had a high degree of genetic determination in both B and W, as did HBP in W. In striking contrast, there was no evidence for a genetic component of HBP in B HBP ESRD patients, indicating that the large excess of ESRD due to HBP in B may be largely environmental in origin.

ABNORMALITIES OF MAGNESIUM (Mg) AND OTHER ELECTROLYTES AFTER REPEATED DOSES OF CIS-PLATINUM (CP): A PROSPECTIVE STUDY. Mildred Lam and David J. Adelstein,* Dept. of Medicine, Cleveland Metro. Gen. Hosp., Case Western Reserve U.; Cleveland, Ohio.

Hypomagnesemia and renal Mg wasting have been reported following CP therapy. To more completely characterize the effect of repeated doses of CP upon serum magnesium (s Mg) and renal Mg excretion, we prospectively studied 24 patients (pts) who received 59 doses of CP (1-4 doses/pt). CP was given in 2-hr IV infusions at least 3 wks apart, 50-75 mg/m^2 with saline/mannitol/furosemide diuresis. A 24-hr urine was obtained prior to each dose; serum chemistries and urinalyses were done before and after each dose. All pts developed hypomagnesemia (s Mg 0.3-1.6 mg/dL): s Mg decreased by 0.5±0.1 mg/dL (mean±SE) after 1 dose (n=20), by 0.6±0.1 mg/dL after 2 doses (n=18), 0.8±0.1 after 3 (n=9), and 0.8±0.1 after 4 (n=5)(all $p<0.01$ compared to baseline; $p<0.05$, 1 vs 2, 1 vs 3, 2 vs 4). Hypomagnesemia persisted for up to 6 mos. Fractional excretion of Mg was >5% in 17/17 pts despite hypomagnesemia. Renal tubular cells and macrophage-like cells were seen 2-4 days after CP in 19/19 pts. No pt had a significant rise in serum creatinine or evidence of other renal tubular defects. Transient decreases in serum potassium, calcium, and phosphate often occurred within 24 hrs after CP was given and were probably related to diuresis.

We conclude that CP causes significant and prolonged renal Mg wasting and hypomagnesemia which is dose-related. Evidence of renal tubular injury is frequently present on urinalysis after CP without clinically-detectable renal insufficiency. We propose that CP primarily affects renal tubular handling of Mg.

RESTING ENERGY EXPENDITURE IN UREMIC PATIENTS. Derrick Latos, M.D., F.A.C.P., Denise Strimel, R.D.,* William Boring, R.R.T., * Wheeling Clinic and Wheeling Dialysis Center, Wheeling, W.Va.

It remains unclear whether energy requirements for uremic patients are different than those for persons with normal renal function. Several aspects of uremia suggest that an abnormal metabolic state may be present, but available data are inconclusive. Resting energy expenditure (REE) was determined by indirect calorimetry 2-4 hours after a standardized meal in 5 nonobese, normal controls (N), 4 uremic nondialyzed patients (U), and 20 stable hemodialysis patients (D). Mean creatinine clearance in U was 5.8 ± 1.6 ml/min/1.73 m^2, and mean duration of dialysis therapy in D was 31 ± 27 months (range 3-91 months). REE was found to be 106 ± .05, 94 ± .11, 106 ± .14 Kcal/1.73 m^2/min in N, U, and D, respectively. REE for both N and D was higher than for U ($p<.05$). These results represent 103%, 94% and 107% of predicted basal energy expenditure for each of the groups, respectively. Fifty-seven percent of the U group were hypometabolic and 43% were normometabolic. Twenty-five percent of the D patients were hypometabolic, 45% were normometabolic, and 30% were hypermetabolic. Two patients in the U group were restudied after several months of hemodialysis. REE increased by 9-21%.

These data suggest that actual REE among uremic and hemodialysis patients is variable and often inconsistent with predicted energy expenditure. REE appears to increase after initiation of maintenance hemodialysis, which may be due to an improved metabolic state or to a catabolic effect associated with hemodialysis.

HYPO-OSMOLALITY IN PATIENTS WITH SPINAL CORD INJURY (SCI). D.J. Leehey, A.A. Picache*, G.L. Robertson*, J.T. Daugirdas, T.S. Ing. Hines-Loyola Medical Center, Hines, IL, and University of Chicago Hospitals, Chicago, IL.

Chronic hypo-osmolality (H) is common in SCI patients, but its etiology is unknown. We measured plasma osmolality (Posm) and arginine vasopressin (AVP) levels in 22 patients with SCI with essentially normal renal function (creatinine clearance 74-148 mL/min). H was present in 12 patients (Posm 264-278 mOsm/kg H_2O). Although urine osmolality (Uosm) ranged from 107-492 mOsm/kg H_2O, plasma AVP levels were uniformly low ($\leq$ 0.7 pg/mL). Urine output was significantly higher in patients with H than in the 10 patients without H (4652 ± 2183 ml/day vs. 2818 ± 1136, $p < 0.05$).

Water load tests were performed in 6 patients with H. Minimum urine osmolality (Umin) was normal (44-82 mOsm/kg H_2O). Maximal free water clearance (CH_2O) and percent load excreted were normal in 3 patients (mean values 9.38 ml/min and 118%, respectively). In the other 3 patients, maximal CH_2O, percent load excreted, and maximal osmolal clearance (Cosm) were all subnormal (mean values 3.98 ml/min, 58%, and 1.79 ml/min, respectively). In one patient with normal maximal CH_2O, after overnight dehydration, AVP was 1.9 pg/mL and urine was hypertonic despite a Posm of 276 mOsm/kg H_2O.

The results exclude the classic syndrome of inappropriate antidiuretic hormone secretion (SIADH) as the explanation for H in SCI patients. H may be related to habitually high fluid intake coupled with altered water excretion due to a mild resetting of the osmostat and/or to deficient solute excretion.

RHABDOMYOLYSIS AND ACUTE RENAL INSUFFICIENCY ASSOCIATED WITH DECOMPRESSION SICKNESS. James W. Loewenherz. Univ.of Miami, Dept.of Medicine and Anesthesiology, Miami, Fl.

A 39 y/o WM plastic surgeon in excellent prior health was SCUBA diving in 70 feet of seawater for 30 minutes. Upon surfacing he experienced rough seas and performed heroic effort to regain contact with the dive boat. He collapsed on the boat complaining of lethargy,back pain and calf pain. Dx of Decompression Sickness was made based on Hx & Px revealing bilat. lower extremity weakness and hyperreflexia. A Babinski reflex was on the left.Adm.Lab: CBC,W=12.3K,Hgb=12,Hct=42;CPK=10,840;U.A. 3+Prot., 3+Occ.Bld.,Micro: WBC=8-10/hpf,RBC=8-10/hpf,Epith. 2-3,casts=none; Scr=2.4;BUN=25;K^+=4.0;ECG=WNL,CXR=WNL. Hospital Course: The Pt. was re-compressed on a U.S.N. Tx.Table 6 with extensions. Pt's neuro.deficit was completely reversed. Renal insufficiency progressed with a rise in Scr to 3.0, U_O fell to 700cc/24hr. Ccr=35cc/min. on a 24hr clearance with a prot.=516mg/24hr. The CPK-MB fraction was elevated in all samples; and at the time of discharge the CPK=3,185. The Scr fell to 2.4. Discussion: This case illustrates the possibility that the rhabdomyolysis that this patient experienced was aggrivated if not mediated by his decompression sickness. It is understood that strenuous excersise following a SCUBA dive will frequently result in decompression sickness, due to the increased tendency for intravascular bubble formation. A common analogy is the soda bottle, where if it is not disturbed when opened, little bubbling occurs, however if it is shaken, vigorous bubbling occurs. It is proposed that this patient experienced a similar phenomenon where the intravascular bubble load in the circulation to the muscles caused a secondary ischemia and resultant rhabdomyolysis. It is concluded that rhabdomyolysis and renal failure are a possible concomitant to decompression sickness. This is the first such case reported in the literature.

CORRELATION OF PREDICTED GLOMERULAR FILTRATION RATE (PGFR) WITH MEASURED CREATININE CLEARANCE AND INULIN CLEARANCE. Arthur Lerner*, Antonio De-LosAngeles*, Steven Goldstein*, Mark Kramer, and Rasib Raja. Kraftsow Division of Nephrology, Albert Einstein Med. Ctr., Philadelphia, PA.

Since the original report by Cockcroft and Gault in 1974, the formula for PGFR

$$PGFR = \frac{(140-age)(wt.,\ kg.)}{72 \times Scr}$$

has been used for both bedside estimation of GFR and drug dosage in patients with renal dysfunction. The accuracy of this formula is questionable and there are no reports correlating this formula to Inulin clearance which represents true GFR. This study was undertaken to compare the PGFR to both measured creatinine clearance and Inulin clearance. Eighty patients (41 male, 39 female) age 14-81 yrs (mean 50) and weight 40-100 kg (mean 70) with relatively stable renal function were studied. The measured Inulin clearance was 80 ± 15 ml/min (mean ± I.S.D.), creatinine clearance was 89 ± 11 and PGFR was 97 ± 20. With linear regression analysis the correlations are as follows;

PGFR vs. Cr. clearance	(M)	r=0.32	P= < 0.05
	(F)	r=0.51	P= < 0.001
PGFR vs. Inulin clearance	(M)	r=0.20	P= > 0.05
	(F)	r=0.14	P= > 0.05
PGFR vs. Age	(M)	r=0.33	P=< 0.05
	(F)	r=0.47	P=< 0.01

We conclude that the formula for PGFR has no correlation with Inulin clearance and little correlation with measured creatinine clearance. Caution should be used when estimating drug dosage using this formula. Both measured and predicted creatinine clearance over-estimate GFR but the error is greater with PGFR.

PROVIDING DIALYTIC THERAPY FOR UREMIC PRISONERS. G. M. Lowder*, W. N. Friedman*, T.K.S. Rao, E. A. Friedman Downstate Medical Center, Brooklyn, New York.

Our municipal hospital cares for prisoners incarcerated on site and transported from outside prisons. During dialysis, current policy requires assignment of 2 guards per prisoner. Over the past 28 months, we dialyzed 13 uremic felons, all men, of mean age 34.3 yrs (range 26 to 61 yrs). Narcotics abuse was responsible for renal disease in 10 of 13 patients presenting as typical heroin associated nephropathy in 9 and as amyloid nephropathy in 1. Uremia was attributed to rhabdomyolysis and acute renal failure in 1 patient, and was undiagnosed in 2 prisoners. A total of 218 hemodialyses and 2 peritoneal dialyses (no suitable limb vessels) were performed in the prison ward for 5 patients, and in an unlocked ward for 8. Prisoners were handcuffed during dialysis. Mean predialysis creatinine and hematocrit, and weight gain were 17.4 mg/dl, 23.6%, and 5.5 lbs. Prisoner-related disturbances were graded from 0 to 4 (severly disruptive): only 2 patients scored >0 (both 1).

As controls, we compared 10 nonprisoner heroin users (8 men, 2 women, mean age 34.1 yrs) who received 180 hemodialyses. Predialysis creatinine (17.3 mg/dl), hematocrit (24.2%), and weight gain (4.9 lbs) were not different from prisoners. We conclude that outpatient dialysis of prisoners can be effected routinely.

ACQUIRED PSEUDOHYPERALDOSTERONISM (PHA) OCCURRING AS A COMPLICATION OF GLOMERULONEPHRITIS (GN). **F.Y.H. Lui,* L.M. Gaudiani,* A. Sebastian, and M. Schambelan. Dept. Med., San Francisco General Hospital, Univ. of Calif., San Francisco, CA**

Renal K^+ wasting (RKW) in the setting of hypomineralocorticoidism is rare, occurring only in PHA (Liddle's syndrome), a condition in which these findings has been attributed to a genetically determined primary increase in renal tubule Na^+ reabsorption. We studied a 46 year-old man in whom hypokalemia (1.4 mEq/L) and hypomagnesemia (1.0 mg/dl) developed two months after he presented with nephrotic syndrome ($U_{prot}V$ 35 Gm/d), azotemia (Cl_{creat} 18 ml/min), and biopsy-proven post-infectious GN. While in Na^+ balance on a constant metabolic diet (Na 120, K 79 mEq/d), hypokalemia (2.7±0.1 mEq/L) and hypomagnesemia (1.4±0.2 mg/dl) were associated with inappropriate urinary excretion of these ions (U_KV 57±4 mEq/d, $U_{Mg}V$ 50±2 mg/d). RKW persisted despite correction of hypomagnesemia (2.0±0.1 mg/dl) with Mg oxide. Hypertension (146/93 mmHg) and hyporeninemia (PRA 0.1 ng/ml/hr) were present. Primary hypermineralocorticoidism was excluded by the findings of undetectable levels of plasma and urine aldosterone, low to normal levels of other adrenal steroids and persistent RKW during treatment with spironolactone (300-600 mg/d). Concomitant spontaneous natriuresis and complete resolution of the PHA occurred during the next two weeks, consistent with a primary enhancement of renal tubule Na^+ reabsorption in the pathogenesis of the syndrome. Thus, RKW can occur as an acquired and reversible complication of GN, and PHA should be included in the spectrum of the recently identified low-renin subgroup of patients with the nephrotic syndrome.

EFFICACY AND SAFETY OF NETILMICIN VS TOBRAMYCIN IN END-STAGE RENAL DISEASE PATIENTS. G.R. Matzke, C.E. Halstenson*, P.A. Abraham, D. Anderson*, D.W. Johnson*, W.F. Keane. Hennepin County Medical Center, Minneapolis, MN.

Thirty end-stage renal disease (ESRD) pts (26 on chronic dialysis) with suspected and/or documented serious systemic gram-negative infections received either netilmicin (N) (n=14) or tobramycin (T) (n=16) in a randomized double-blind prospective study. The two groups were similar in age, sex, weight, concommitant antibiotics and duration of treatment [8.6±3.3 days (mean ±SD) and 6.7±4.4 days for N and T, respectively]. The peak and trough serum concentrations of N (7.1 ± 1.3 and 1.9 ± 0.4 mg/l) and T (7.5 ± 0.7 and 2.3 ± 0.8 mg/l) were not significantly different. Audiograms (AUDIO) (250 to 20,000 HZ) and brain stem evoked auditory responses (BEAR) were performed initially, every five days during and post therapy. Criteria for AUDIO and BEAR ototoxicity were at least two 15 db changes and at least a 0.25 Msec increase in wave V latency, respectively. Although complete clinical (85.7% N, 65.3% T) and bacteriologic cure (83.3% N, 50% T) were observed in more N pts than T pts these differences were not significant. AUDIO ototoxicity was seen in 2/14 (14.3%) N pts and 3/16 (18.8%) T pts. BEAR ototoxicity was seen in 3/14 (21.4%) N pts and 5/16 (31.3%) T pts. Despite the maintenance of N and T serum concentrations within recommended ranges our incidence of ototoxicity was markedly greater than that reported in non-ESRD patients [3% N, 10% T; J Antimicrob Chemother, 1984;13(suppl A):37-42].

CORONARY ARTERY BYPASS IN THE CHRONIC DIALYSIS PATIENT. William G. Marshall Jr.*, Nicholas P. Rossi*, Ronald L. Meng*, (intro. by Annette Fitz). Univ. of Iowa, Divisions of Thoracic and Cardiovascular Surgery and Nephrology, Iowa City, Iowa.

Cardiac disease continues to be a major cause of death in patients on chronic dialysis. The results of coronary artery bypass grafting (CABG) for significant coronary artery disease in chronic dialysis patients have been studied in a group of twelve patients (age 51-67 years), who underwent CABG between Jan. 1979 and Dec. 1983, and of which 11 were male.

Hospital mortality was 8% (1/12) and this patient died of ventricular arrhythmia. Two late deaths occurred; from peritonitis in a patient on chronic peritoneal dialysis and from metastatic renal cell carcinoma. There were two post-operative complications (morbidity 17%)--sternal dehiscence secondary to mediastinitis and an intra-operative cerebrovascular accident. Ten of the 11 hospital survivors had complete relief of angina, with the other patient more easily controlled on medication.

Combining this series with another series partially reported in the literature allows determination of actuarial survival in a group of 23 patients followed from 1-79 mos. (mean 33 mos.). Actuarial survival was 83% at one year, 71% at three years and 54% at five years; not significantly different compared to the natural history of these patients.

It appears that CABG can be performed on chronic dialysis patients with only slightly higher risk of significant mortality and morbidity than in the routine patient, and that CABG provides significant symptomatic relief of angina. However, no significant change in actuarial survival can be demonstrated.

EFFECTS OF HYPERTONIC RADIOCONTRAST MEDIUM (HRCM) ON RENAL P-AMINOHIPPURATE EXTRACTION (EPAH) OF HEALTHY, EUVOLEMIC DOGS. B.A. McKenna*, R.C. Pabico, R.W. Katzberg*, T. Morris*, H. Fischer*, R.B. Freeman. Depts. of Medicine and Radiology, Univ. of Rochester Medical Center, Rochester, N.Y.

Using the canine model (anesthetized, ventilated, adult mixed-breed dogs with the left renal vessels cannulated, left ureter catheterized; renal hemodynamic functions measured by both electromagnetic flow probe/A-V extraction of technetium 99m DTPA and the clearances of inulin and PAH), our recent studies showed a dramatic decline in glomerular filtration rate (GFR) and renal blood/plasma flow (RPF) within seconds of I.V. administration of HRCM; in hydrated animals the changes are transient, but in dehydrated dogs the depressed GFR and RPF persist (Int Cong of Nephrology, 1984 Program p 118). The effects of HRCM on the kidney are presumed to be due to the high osmotic load. But adverse effects on the proximal tubules continue to be of clinical concern. Hence, EPAH was measured in 7 hydrated mixed-breed dogs prior to and subsequently after I.V. HRCM, 2 ml/kg; two other sets of dogs underwent similar studies but receiving 0.9% NaCl and hypertonic Mannitol (HM) equiosmolal to the HRCM, respectively. Animals receiving 0.9% NaCl showed no change in GFR, ERPF, or EPAH. Dogs receiving HM or HRCM showed comparable changes in GFR and ERPF. EPAH was unchanged in dogs receiving HM, whereas dogs receiving HRCM showed decline in EPAH by 14-47% within 2-5 minutes after I.V. injection. The different effects of HM and HRCM on EPAH implies that large osmolal load is not the major factor; that HRCM may have toxic effects on the proximal tubule.

REVERSIBLE RENAL FAILURE AND MEMBRANOUS NEPHROPATHY ASSOCIATED WITH FENOPROFEN.
Sushil K. Mehandru and Spiro Arbes.* UMDNJ Rutgers Medical School and Jersey Shore Med. Ctr., Neptune, New Jersey.

Non-steriodal anti-inflamatory drugs have been reported to cause reversible acute renal failure and nephrotic syndrome. We studied a patient who developed renal failure while on Fenoprofen (Nalfon). A 75 year old male was admitted to the hospital on August 10, 1983 with progressive dyspnea, lower limb edema and elevated blood pressure. He has had long standing history of mild essential hypertension, hypertensive heart disease and osteoarthritis. He was taking Digoxin, Furosemide, Alpha-methyl dopa and was treated with Fenoprofen from February until August 1983. His BUN on admission was 54mg/dl, creatinine 4.6mg/dl and he had 12.8 gm of protein in 24 Hr urine specimen. Serum complement levels were normal. Blood eosinophil count was 10%. Percutaneous renal biopsy specimen revealed acute inflamatory interstitial infiltrate, granular glomerular deposits of IgG and C3, extensive fusion of the foot processes and subepithelial electron-dense deposits. Patients renal functions normalized upon discontinuation of Fenoprofen and three week dialysis therapy. We conclude that Fenoprofen may, in rare instances, cause tubulo-interstitial nephritis and membranous nephropathy usually reversed by discontinuation of the drug.

COMPARATIVE DIAGNOSTIC ACCURACY OF COMPUTERIZED TOMOGRAPHY (CT) AND GREY SCALE ULTRASONOGRAPHY (US) IN ACQUIRED RENAL CYSTIC DISEASE (ARCD).
N. Narasimhan,* M. Wolfson, T.A. Golper, M. Rahatzad,* W.M. Bennett. Oregon Health Sciences University and VA Medical Center, Portland, OR.

Atypical hyperplasia of tubular epithelium with cyst transformation occurs in conjunction with renal failure and hemodialysis. More importantly, an increased incidence of neoplasm with chronic renal failure and hemodialysis has been reported. Neoplasms may arise in close association with cysts or even within cysts, necessitating the early detection of cystic disease.

To ascertain the value of current diagnostic techniques, 81 patients (49 hemodialysis, 11 peritoneal dialysis, 21 nondialyzed) with chronic renal failure not due to polycystic kidney disease were evaluated with both CT scan and US. CT scan is reportedly 100% accurate in the detection of renal cysts. Therefore, comparing US with CT scan for detecting ARCD, the sensitivity was 69% and specificity was 98.5%.

		CT +	CT -	
US	+	9	1	False negative = 6%
	-	4	67	False positive = 10%

The resolution of either test was inadequate to detect solitary (1-3) cysts.

		CT +	CT -
US	+	5	14
	-	12	50

Conclusion: There is an unacceptable level of diagnostic inaccuracy for both CT and US in the detection of multiple and solitary renal cysts in chronic uremia. It appears that both a negative CT and US are necessary to comfortably exclude either ARCD or solitary cyst.

A SIMPLE AND ECONOMICAL METHOD FOR CONTINUOUS MONITORING OF DIETARY PROTEIN (P) INTAKE.
DF Middendorf, LA Hebert, RA Zager, J Hartman*, Dept of Medicine, Ohio State Univ, Columbus, OH.

P restriction is recommended to retard renal disease. P restriction is difficult. Incompliance is common. Traditional means to assess compliance are unsatisfactory: Diet history is inaccurate; analyses of 24 hr urine urea N (UUN) are cumbersome, costly and patients may be compliant with diet on days when urine is collected but "cheat" on other days. To circumvent these problems, we devised the following method to assess P intake: We found in studies on 15 subjects ingesting a wide range of P intakes that UUN/UCr ratio of the last voided (LV) urine (about 11 pm, 5 h after last meal) was the same as that of the previous 24 h urine ($r=0.88$, $p < 0.001$). Thus 24 h UUN = (UUN/UCr) x UCr24h where UCr24 = calculated 24 h urine creatinine excretion(Nephron 16:31,76). P intake over previous 24 h for patient in P balance = Eq (1) = UUN24h + 0.031 x body wt (kg) x 6.25, where 0.031 = (gm fecal N + gm urinary nonurea N)/kg body wt/day(Maroni ASN abstr 1983). Thus, to monitor P intake continuously (eg, 1 mo) each evening for 30 days, about 10 ml of the LV urine is saved (in preservative or frozen). After 30 days an aliquot of each LV urine is pooled. The UUN/UCr of the pool is the average UUN/UCr ratio for the previous 30 day. The average daily P intake for the 30 day period is then calculated using Eq (1). Conclusion: A single UUN/UCr analysis of pooled daily LV urine aliquots accurately, economically and conveniently assesses P intake over that time period.

A NON-ALUMINUM CONTAINING PHOSPHATE BINDER: POLYURONIC ACID.
H.G. Nebeker,* H.W. Schneider,* and J.W. Coburn. VA Wadsworth Medical Center, UCLA School of Medicine, Los Angeles, CA and Katharinenhospital and Fraunhofer Inst., Stuttgart, Germany.

Aluminum (Al) preparations given to reduce intestinal phosphorus (P) absorption in dialysis patients are implicated as a source of Al accumulation, which can cause osteomalacia. A new non-Al containing binder, polyuronic acid (PUA) complexed with calcium, which binds P effectively in vitro (Proc. EDTA 20:725, 1984), was evaluated in vivo. Twenty studies were done in 5 fasting normal dogs (23.3±0.9 Kg BW, mean ± SEM); they were fed a standard meal (KalKan (R)) containing 2.7 gm P plus supplemental potassium phosphate, 1.0 gm P. The meal was given alone (control) and then in separate studies with 6 g each of: PUA caps, $Al(OH)_3$ caps, or $CaCO_3$ tabs. Serum P (SP) was measured every 30 minutes and urinary P excretion (UP), hourly for 6 hours; intestinal P absorption was estimated from peak SP (SPmax (mg/dl)) and cumulative 6 hr UP (mg/6hr). Prior to the meal, fasting SP was 2.86±.71 mg/dl, with no difference between groups. No side effects were noted, and the following responses were observed:

	Control	PUA	$Al(OH)_3$	$CaCO_3$
SP_{max}	6.96±.30	5.28±.26	6.02±.36	5.82±.38
6hr UP	873±90	285±43	564±73	436±45

SP_{max} and 6hr UP were below control with each of the binders ($p<.05$). PUA delayed SP_{max} to 4.8±.6 hrs after the meal vs. 3.1±0.8 in the control ($p<.05$). Also, 6hr UP was lower after PUA than after either $Al(OH)_3$ or $CaCO_3$ ($p<.05$). Thus, PUA is at least as effective as $Al(OH)_3$ or $CaCO_3$ as an oral P binder but without the risk of Al loading after $Al(OH)_3$.

FACTORS DETERMINING THE RENAL RESPONSE TO WATER IMMERSION (WI) OF NON-EXCRETOR CIRRHOTIC PATIENTS. K. Nicholls,* M. Shapiro,* and R. Schrier. Dept. Med., Univ. Colorado Med. Sch., Denver, CO.

Non-excretor cirrhotic patients (pts), defined by <80% excretion of a standard water load (WL), display variable responses to WI. The hormonal and hemodynamic profiles of 15 such pts were analyzed relative to WL excretion during WI. In group 1 (n=7), WL excretion was <35%, whereas in group 2 (n=8) >50% of WL was excreted. Before WI group 1 as compared to group 2 pts had more impaired water excretion (13 vs 36%, p<.001), inulin clearance (C_{In}, 28 vs 62 ml/min/1.73m^2, p<.02) and PAH clearance (213 vs 351 ml/min, p<.02); higher plasma renin activity (PRA, 11.6 vs 4.9 ng/ml/min) and arginine vasopressin (AVP, 2.3 vs 1.0 pg/ml; lower serum Na concentrations (125 vs 130 mEq/L), all p<.05; more ascites (4+ tense vs 1+ to 3+ non-tense) and more diuretic resistance (weight loss on comparable Na intake and diuretic regime of 0.13 vs 0.54 kg/d, p<.05). During WI, only group 2 significantly increased C_{In} (62 to 80 ml/min/1.73m^2) and suppressed PRA (4.9 to 3.8 ng/ml/min), aldosterone (75 to 39 ng%), norepinephrine (976 to 603 pg/ml) and AVP (1.0 to 0.8 pg/ml), all <.05. During basal and WI conditions, cardiac output, wedge pressure and systemic vascular resistance were comparable in both groups. Serum albumin, hepatic enzyme and bilirubin concentrations were similar in both groups. In conclusion, non-excretor cirrhotic pts constitute a spectrum, with the most impaired water excretion associated with the highest, non-suppressible hormonal concentrations, worse renal function and hyponatremia, tense ascites, diuretic resistance and unresponsiveness to WI.

DESFERRIOXAMINE THERAPY IN PATIENTS WITH ALUMINUM-RELATED OSTEODYSTROPHY. SM Ott, HG Nebeker*, DL Andress*, DS Milliner, NA Maloney*, JW Coburn, DJ Sherrard. Univ. of Washington & VA Seattle, WA; Wadsworth VA & UCLA, Los Angeles, CA.

To see if chelation therapy could improve the bone disease associated with aluminum in patients (pts) receiving hemodialysis, we treated 27 such pts with desferrioxamine (DFO) for 4- 14 months (mean 8.3 ± 3.7). Prior to therapy each patient had a bone biopsy which documented aluminum accumulation on histochemical staining, and a DFO infusion test which showed increased plasma aluminum (mean±SE 202±27 to 535+50). Ten pts had repeat bone biopsies. Bone pain and/or muscle weakness improved in 25/27 pts; analgesic use fell in 17/22. Serum calcium fell by 1.7±.17 mg/dl at 3-4 months and later rose in all. Alkaline phosphatase rose by >25% in 19 cases. Peak serum aluminum fell by 21±6% at 3 mos and 34±11% at 6 mos. Bone histomorphometric measurements showed no changes in total bone area, osteoid area, or osteoid surface. There was a decrease in stainable surface aluminum (45±25 to 20±15%, mean±sd, p=.006) and an increase in bone formation rate as measured by double tetracycline labelling (63±111 to 244±204 μ2/mm2/day, p=.01). The increment in bone formation rate was larger in pts who had not had parathyroid surgery. The correlation between bone formation rate and bone aluminum was -0.64, p=.006. These data provide further evidence that aluminum loading is associated with inhibition of bone formation, and that low PTH levels play a major role. Our results suggest that DFO will be a valuable treatment for aluminum-related renal osteodystrophy.

HYPERKALEMIA IN ORGANIC ACIDOSIS, Man S. Oh, Jaime Uribarri, and Hugh J. Carroll. Downstate Med. Ctr., Dept. of Med. Brooklyn, New York

Although the acid infusion data on animals and the data from seizure-induced lactic acidosis (LA) suggest that hyperkalemia does not occur in organic acidosis, the clinical data on diabetic ketoacidosis (DKA) and lactic acidosis suggest otherwise. The purpose of the present investigation is to determine the frequency and mechanism of hyperkalemia in organic acidosis. Data from 108 patients with DKA and 8 patients with LA were analyzed. Hyperkalemia (serum potassium (SK) 5.6 meq/l) was seen in 49 patients (45%) with DKA and in 8 patients (44%) with LA. The correlation was good between SK and blood pH, and SK and BUN, but poor between SK and anion gap. 69% of patients with pH below 7.10 and 26% with pH above 7.10 had hyperkalemia, while 50% of patients with BUN ≥21 mg/dl and 33% with BUN≤20 mg/dl had hyperkalemia. In LA, no specific factor could be found that correlated well with SK, but the absence of correlation with pH may have been due to that acidosis was almost uniformly severe. In conclusion, severe organic acidosis usually leads to hyperkalemia, especially when accompanied by renal impairment. The absence of hyperkalemia with acute organic acid infusion or with seizure may be attributable to the relative midness and the short duration of acidosis.

THE ACUTE EFFECTS OF SHORT-TERM LOW PROTEIN DIET ON RENAL FUNCTIONS OF UNINEPHRECTOMIZED (UNI-NX) LIVING RELATED DONORS (LRD). R.C. Pabico, S. Sandroni*, B.A. McKenna*, R.B. Freeman. Univ. of Rochester Medical Center, Rochester, New York.

We are concerned that the glomeruli of the hyperperfused remaining kidney of LRD may undergo sclerosis and lead to loss of function, as seen in the animal "remnant kidney" model. Since low protein intake lowers glomerular filtration rate (GFR) and renal plasma flow (RPF) in subjects with normal renal function, we tested the effect of short-term low protein intake in the hyperperfused kidney of LRD. Seven LRD (mean age: 44 years; mean duration post-uni-nx: 7 years) were admitted to the CRC and placed on an isocaloric diet containing 0.6 gm/kg/day of protein for 5 days. GFR, ERPF, tubular maximum secretion of p-aminohippurate (TmPAH), urinary concentration/dilution, acidification were measured before and after low protein intake. Serum urea N and creatinine, and the renal clearances of urea N (Curea N) and creatinine (Ccreat) were also measured. Following 5 days of protein-restricted diet, GFR decreased by 13-21%*, whereas ERPF was virtually unchanged; TmPAH and TmPAH/GFR on the other hand, increased by 12-14%*. Urinary concentration and dilution were unchanged, but urinary acidification, particularly UNH4V, was impaired*. Serum urea N decreased by 25-69%* but the change in serum creatinine was not significant; both CureaN and Ccreat decreased by 25% and 16%, respectively*. Thus, short-term protein restriction decreased the elevated renal hemodynamic functions and enhanced proximal tubular transport of the Uni-nx LRD.

* p<0.05

METABOLIC ACIDOSIS IN MINERALOCORTICOID-RESISTANT RENAL HYPERKALEMIA WITHOUT SALT WASTING. A NEW TYPE OF PROXIMAL RENAL TUBULAR ACIDOSIS. M. Paillard, H. Nahum*, A. Prigent*, F. Leviel*, M. Bichara*, J.P. Gardin*, and J.M. Idatte*. Hôpitaux Louis Mourier et Lariboisière, Inserm U13, Paris, France.

The mechanism of the metabolic acidosis was investigated in a 22 year-old man with chronic renal hyperkalemia (5.2 to 6.8 mM), metabolic acidosis (plasma pH 7.27 to 7.34, HCO_3 17 to 22 mM), chronic hypertension with extracellular fluid volume (ECFV) expansion, low plasma renin activity (<0.10 ng/ml/h), high plasma aldosterone (32 to 100 ng/dl), normal GFR (134±2.5 ml/min.1.73m^2) during normal dietary intake of sodium. During hyperkalemic period, urine was highly acidic (pH 4.6 to 5.0), NH_4 excretion (30 mmol/24 h) was not supernormal as expected from a chronic acid load. During $NaHCO_3$ infusion, maximal tubular reabsorption of HCO_3 ($TmHCO_3$/1 GF) was subnormal (19 mmol/1 GF), fractional excretion of HCO_3 when plasma HCO_3 was normalized was 20%, and urine-blood PCO_2 during maximal urine alkalinization (pH>7.8) was normal (31 to 38 mmHg). When kalemia was returned to normal value (4.0 to 4.6 mM) either with cation exchange resin (persistent ECFV expansion) or diuretic agents (normal ECFV), metabolic acidosis disappeared, NH_4 excretion rose normally after short NH_4Cl oral test from 18 to 64 µmol/min, while urine pH remained acid (4.9 to 5.2), and the Tm HCO_3/1 GF normalized (24.8 mmol/1 GF). We suggest that metabolic acidosis is due to reduction of HCO_3 reabsorption and ammonia production in the proximal tubule, and not to impairment of distal hydrogen secretion. This new type of proximal renal tubular acidosis is probably dependent on chronic hyperkalemia.

PROGRESSIVE DETERIORATION OF RENAL FUNCTION IN NON-AZOTEMIC CIRRHOTIC PATIENTS WITH ASCITES: A PROSPECTIVE STUDY. M.A. Papadakis* & A.I. Arieff. V.A. Med. Ctr. & U.C.S.F., Dept of Medicine & Nephrology Research, San Francisco, CA.

Many physicians believe that "hepatorenal syndrome"(HRS) is an acute disorder occurring in hospitalized patients. However, long-term studies are limited on renal function in non-azotemic cirrhotic patients with ascites. We prospectively evaluated renal function in 13 patients with cirrhosis & ascites who had normal BUN & plasma creatinine (Cr), & no other systemic illness. Studies included liver function tests & clearances of inulin (CI), creatinine (CCr), PAH & water.

The initial mean GFR was 56 cc/min with Cr=1.1 mg/dl and BUN=13 mg/dl. Patients were divided into 2 groups based on initial GFR: normal (group I,n=5) 107cc/min; severe impairment (group II, n=8) 25cc/min. In group I patients, CI (107 ml/min) & CCr (124 ml/min) were similar, but in group II patients, CCr was 43 ml/min while CI was only 19 ml/min. Among group II patients, creatinine index was only 10 mg/kg/day (normal 20-28), largely accounting for the normal Cr with very low GFR. Four of 5 group I patients maintained normal GFR over 25 wks. However, among group II patients (4 studies/patient), 5 underwent progressive deterioration of GFR & 2 improved, 1 of whom had a LeVeen shunt. After 25 wks, 4 of 8 had died (hemorrhage, sepsis, encephalopathy). Cr rose above 1.5 mg/dl only when GFR fell below 19 ml/min.

Conclusions: In patients with cirrhosis, ascites & normal BUN & Cr: a) when GFR is normal, renal function is generally stable over 6 months; b) when GFR is below 50 ml/min, renal function usually progressively deteriorates, with a 57% 6 month mortality, often with no change in BUN & Cr until GFR is below 19 ml/min; c) HRS is not an acute disorder of hospitalized patients.

EFFECTS OF EXERCISE TRAINING DURING HEMODIALYSIS P.Painter,* M.Hill,* J.Nelson-Worel,* D.Thornbery,* W.Shelp, A.Harrington, A.Weinstein. Univ. of WI, Dept. of Med., Methodist Hospital, Madison, WI

Hemodialysis (HD) patients show typical cardiovascular training responses to exercise training (ET) when exercised on 'off' dialysis days. This study was done to determine if similar responses will result following ET during the dialysis treatment. Twelve HD patients ($\bar{x}$ age 44y) pedalled exercycles for 30 minutes during the second hour of HD for 12 weeks. Compliance to the exercise was 91%. Six HD patients served as controls. Maximal treadmill (TM) tests were done on all patients pre and post 12 weeks of ET. Heart rates and blood pressures (BP) were determined every minute and expired air was collected for determination of maximal oxygen consumption ($maxVO_2$). $MaxVO_2$ increased significantly (12.4%) in EX (18.9±1.4 to 21.6±1.8 ml/kg/min)(p<.01). Maximal TM work increased 30% in EX (499±68 to 688±79 kgm/min)(p<.01). No changes in $MaxVO_2$ or TM work were noted in C. Resting SBP decreased significantly in EX (166±9 to 149±8)(p<.03). Antihypertensive medications were discontinued in 3 of 6 nondiabetic EX patients. Two others decreased dosage or number of medications. No changes in BP or BP medications occurred in C. EX had slightly lower total cholesterol levels following ET (p<.03); however no changes in the HDL/total cholesterol ratio resulted. No changes in hematocrit or hemoglobin were observed in either group. ET during the HD treatment is technically feasible and safe for appropriately screened patients and will 1) improve exercise capacity, 2) improve BP in some patients, and may 3) enhance compliance to regular exercise in HD patients.

PAUCITY OF MINIMAL CHANGE (MC) LESION IN YOUNG CHILDREN WITH EARLY FREQUENTLY RELAPSING STEROID SENSITIVE (FRSS) NEPHROTIC SYNDROME (NS): Kishore Phadke,* Anthony Nicastri, Howard Trachtman,* Fred Carroll,* C. Chen* and Amir Tejani. Downstate Medical Center, Brooklyn, New York.

The morphological lesion in young children with early FRSSNS is presumed to be MC, however, there are no prospective studies to validate this assertion. From 1980-83, we performed a percutaneous renal biopsy on all children with the NS, ages 1-8 years,(yrs) as soon as they developed a FRSS course.

14 children (8 male, 6 female) qualified to enter the study. The mean age at time of biopsy was 4.9 yrs (2.7-8 yrs). An average of 4 relapses per patient was noted during the initial 13 months - the mean interval from the diagnosis of the NS to the renal biopsy.

Only 4/14 patients exhibited the MC lesion. In 71% (10/14) of patients, the renal histology was other than MC. 2 patients had diffuse mesangial hypercellularity. 6 had mesangial IgM nephropathy and 2 patients had focal segmental glomerulosclerosis. No clinical parameters discriminated between patients with MC and those with severe lesions. Of the 10 children with non-MC histology 1 patient requires dialysis, 4 have persistent proteinuria despite cyclophosphamide (CY) and 4 are in prolonged remission following CY therapy.

Our study concludes that as in the adult, a variety of morphological lesions are seen in young children with FRSSNS and that the occurrence of frequent relapses even in children as young as 3 yrs may herald the presence of a more ominous histological lesion.

PREVENTION OF URINARY TRACT INFECTIONS IN POST-MENAPAUSAL WOMEN. M. Privette, R. Cade, D. Mars, and R. Thompson. Dept. of Medicine, University of Florida Medical School, Gainesville, FL.

Twelve postmenapausal women were referred to us because of frequent episodes of urinary tract infection. Each had experienced at least six episodes of UTI in a two-year period and none had remained free of infection for longer than one month when antibiotics were discontinued. Age ranged from 51 to 76 years, 9 were sexually active. In each an atrophic vaginitis with bacterial overgrowth was found. The patients were then started on estrogen therapy to restore glycogen deposits and lactobacillus growth and treated with an appropriate antibiotic, and Betadine douche daily for one week. Patients under the age of 60 were given oral estrogen replacement while older patients were treated topically. During follow up which has ranged from 4 months to 7 years there have been only three episodes of UTI while frequency of infection averaged 5 per year before estrogen therapy was instituted. Four patients who took sulfonamide preparations either as diuretic or antimicrobial therapy had eosinophiluria which cleared when the sulfonamide was discontinued. GFR improved significantly in each of these women. We think atrophic vaginitis with bacterial overgrowth is a cause of frequent UTIs in postmenapausal women and that interstitial nephritis due to sulfonamides is a common occurrence.

T-CELL SUBSETS AND STATUS OF HEPATITIS-B SURFACE ANTIGEN(HBsAg) AND ANTIBODY(HBsAb) IN END-STAGE RENAL DISEASE(ESRD) PATIENTS(Ps). D.Revie*,S.Shen, J. Ordonez*, R. Welik*, L. Litkowski*, F. Dagher*, J. Sadler, P. Chretien*, Univ. of Maryland, Baltimore, MD.

We examine the influence of T-cell subsets on responses to either spontaneous HBsAg exposure or to Hepatitis-B vaccination(HBVa) in ESRD Ps. Screening tests for HBsAg and HBsAb were done by Abbott enzymatic assays on all Ps in our dialysis unit. T lymphocyte populations were studied by immunofluorsecence staining with monoclonal antibodies and FACS in the pre-dialysis(D) blood. HBsAb is positive(P) in 7 Ps(G1), and 4 Ps are chronic HBsAg carriers(G2). Ps with negative(N)HBsAg and HBsAb were then given 3 doses of 40ug HBVa at 0,1,6month. Their sera were tested for HBsAb about 3 months later. T-cell subsets were compared between G1 and G2. They were also compared between 24 HBsAb P Ps(G3) and 23 HBsAb N Ps(G4) after HBVa; G3 and G4 were selected for matching on age, time on D and underlying causes leading to ESRD.

Absolute lymphocyte(L),T,Helper(H),Suppressor (S) cell counts and H/S ratio are all significantly higher in G1 than G2. G3 has significantly higher L, T and H counts than G4; S count and H/S ratio are slightly higher but not significant. When comparison was made among 4 groups, G1 has the highest and G2 has the lowest L,T,H,S counts. H/S ratio is highest in G3 and lowest in G2. There is no significant difference on pre-D Cr and Albumin among all Ps, but BUN is higher in G2.

These findings suggest that L and T cell subsets counts are more important than H/S ratio, nutritional status and adequacy of D on HBsAb production in ESRD Ps.

EPIDEMIC AIDS ASSOCIATED NEPHROPATHY EXPANDS. T.K.S. Rao, A.D. Nicastri, S.J. Bennett* and E.A. Friedman Downstate Medical Center, Brooklyn, New York.

Between 1981 and 9/83, we treated 11 AIDS patients, all black, (8 men and 3 women, 5 IV drug addicts, 2 homosexual men and 3 Haitians) with newly recognized rapidly progressing nephropathy, typified in 10 by focal segmental glomerular sclerosis (FSGS). We now extend this finding to report an additional 17 patients with AIDS associated nephropathy seen between 9/83 and 8/84. All of these were black men ranging in age from 27 to 43 yrs (mean 32 yrs): 9 were IV drug addicts, 5 were Haitians, one was a homosexual, and in two (twins), no known risk factor was identified. In 14 patients, renal histology revealed FSGS, in one diffuse glomerular IgM deposition with mild mesangial increase was found. In 8 patients, the course of renal disease progressed to irreversible renal failure in 4 to 16 weeks resulting in death despite intensive hemodialysis. AIDS was recognized as the underlying illness only 3 to 6 months after starting maintenance hemodialysis in 4 patients. In retrospect, the course of renal disease in these four (progression to renal insufficiency within 8 to 20 weeks - all died) was characteristic for AIDS associated nephropathy.

Of interest are 3 patients who recovered sufficient renal function to no longer require dialysis and are currently alive with a serum creatinine of 1.5, 1.2, and 6 mg/dl. In this small "recovery" group, 2 of 3 remain nephrotic. An additional subset of 2 patients have not evinced deterioration over 3 months (mean creatinine <2 mg/dl). We conclude that AIDS associated nephropathy is characterized by FSGS and a rapid decline to irreversible renal failure with a high mortality.

DOES LATENT LEAD (Pb) INTOXICATION INCREASE RISK FOR DETERIORATION OF RENAL FUNCTION?
Eberhard Ritz*, Dagmar Behringer, Markus Stoeppler, Peter Craswell (intr. by R. Glassock), Dept. Int. Med., Heidelberg; Kernforschungszentr. Jülich,FRG; Royal Brisbane Hospital, Brisbane, Australia

In 19th century, chronic renal failure (RF) and hypertension were known consequences of clinically manifest chronic Pb intoxication. With virtual disappearance of heavy occupational Pb exposure such renal involvement is rarely seen today and existance of Pb-induced RF has even been questioned. Pb mobilisation with $CaNa_2EDTA$ after Emmerson (Austr. Ann.Med. 12,310,1973)permits assessment of body Pb burden. Using this test, Pb burden was measured in 21 controls, 19 non-gouty pat. with known renal disease and RF and 16 pat. with gout and RF. Under rigidly contamination free conditions, using Zeeman compensation as internal double beam background compensation and L'vov platform to reduce matrix effects, Pb was measured with atomic absorption (VC 5%). Method was validated with voltammetry.Control blood Pb was 72 ng/ml (36-187). The above method yielded lower postinfusion Pb excess than reported in literature (306 nmol/4 days/1.73m^2; 95th percentile 1.000). In gouty (G) pat. who developed G while being in RF, blood Pb (185) and postinfusion Pb excess (1827) were higher than in G pat. who had first G and than RF (103 and 936 respectively). Surprisingly, of 19 non-gouty RF pat. 7 had increased postinfusion Pb excess (> 95th percentile) and history of past occupational Pb exposure. High prevalence of asymptomatic increase of body Pb burden in non-selected RF pat. raises possibility that Pb contributes to progression of RF in pat. with renal disease.

TRENDS IN THE US END STAGE RENAL DISEASE (ESRD) POPULATION. SJ Rosansky and PW Eggers, VA Hospital, Columbia, SC, and the Health Care Financing Administration, Washington, DC.

Data from the ESRD Medical Information System (MIS) from 1973-79 and data from facility surveys and reports to Congress from 1979-82 were analyzed to describe trends in the US ESRD population. ESRD prevalence was determined by ESRD enrollment figures to the HCFA. Survival was determined by the actuarial life table method. Although overall incidence of ESRD increased from 15,993 in 1974 to 19,500 in 1982 (2.5% annual increase), the annual percent increase in prevalence has progressively declined from 41.8% (1974-75) to 9.2% (1981-82). From 1979-82: overall incidence rate increased from 77 to 85 per million; the home dialysis population (HDP) increased from 13.1% to 17.9% of the dialysis population (DP); home CAPD use increased from 7.8% to 55.6% of the HDP; there was a 28% increase in the number of ESRD transplant patients vs. an 82% increase in the ESRD incenter DP. Between 1974-79, although the average age of the ESRD DP increased from 50 to 55, and the diabetic ESRD DP increased from 11.9% to 18.7%, the overall one year survival of the DP remained stable (81%) partially due to improved one year percent survival for patients greater than 75 years of age (52% to 65%). It is concluded that although the treated US ESRD population continues to grow, its rate of growth is declining. The rate of growth of the overall HDP and transplant population lags the incenter DP. Stable patient survival in the face of an increasing age and increasing percentage of diabetic dialysis patients may reflect an improvement in treatment.

RESPONSE OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE (U_{NAG}) TO TWO OSMOTIC DIURETICS IN THE DOG. Janet R. Rowe, Ben H. Brouhard, Judith K. Dunn, and Lavenia LaGrone. Univ. of Texas Med.Br.,Depts. of Pediatrics & Preventive Med., Galveston,Texas.

Urinary NAG, a lysozomal hydrolase located in the proximal tubule, has been used as a marker for subtle renal injury and as a potential parameter of metabolic control in the diabetic. The mechanism of rise of U_{NAG} in the diabetic is not known. To differentiate between the osmotic diuresis itself and the hyperglycemia and glycosuria, 2 groups of dogs were studied. 25% mannitol was infused into the left renal artery to double left urine flow (Group I, n=5) and 25% glucose was infused to also double left urine flow (Group II, n=5). Urine from both ureters was collected for three 20-min control periods, then for 10 experimental periods; $U_{Na}V$ (uEq/min), U_KV, V (ml/min) and $U_{NAG}:U_{cr}$ (units) were compared. Control period data were not different for both groups; experimental periods showed the following:

	Group I		Group II	
	L	R	L	R
V	1.6±.3	1.0±.4*	1.1±.4*	40±.1*
$U_{Na}V$	101±29	84±30*	111±29	71±20*
U_KV	36±8	35±6	31±4	26±3*
$U_{NAG}:U_{cr}$	2.8±.2	2.4±.4	11.7±2.0	5.2±0.6*
Osmolality	307±146		382±33	

*L vs R $p < 0.05$, ($\bar{x}$±SEM)

We conclude that osmotic diuresis does not affect excretion of U_{NAG} but that hyperglycemia and/or glycosuria itself stimulates such enzymuria.

ABNORMAL RENAL FUNCTION IN ADULTS WITH CYANOTIC CONGENITAL HEART DISEASE (CCHD). E. A. Ross*, G. Danovitch, J. K. Perloff*, J. S. Child*, and M. Canobbio*, Divisions of Nephrology and Cardiology, UCLA School of Medicine, Los Angeles, CA.

Adult patients with CCHD have been shown to have abnormalities in glomerular function, including diminished GFR and proteinuria, with large glomeruli and segmental sclerosis. These patients are frequently hyperuricemic and may suffer from gout and tophaceous deposits of uric acid. We studied 8 adult patients with CCHD (hematocrit 62 ± 10%). Plasma creatinine was normal (0.9 ± 0.1mg./dl.) yet GFR was mildly reduced (92 ± 14 cc./min. by creatinine clearance and 81 ± 6 cc./min. by Indium^{111} DTPA). Three pts. had significant proteinuria and one was nephrotic. Plasma uric acid was high in all but one of the pts. (8.2 ± 2.1 mg./dl.), mean 24 hr. uric acid excretion was normal (564 ± 221 mg.), and fractional uric acid excretion was relatively low (6.4 ± 2.7%). The two pts. with the highest plasma uric acid (12.0 and 10.2 mg./dl.) had the lowest fractional excretion levels (2.8 and 3.6%). These two patients also had diminished capacity to excrete a water load (38 and 27%/4 hrs.) and to maximally concentrate urine (520 and 635 mOsm./kg. after thirst and AVP). Thus, adults with CCHD became hyperuricemic because of inadequate renal excretion of uric acid. The diminished GFR and proteinuria could be manifestations of their altered hemodynamics, but may relate to uric acid induced renal disease, with single nephron hyperfiltration and eventual segmental sclerosis.

DIAGNOSIS OF PROXIMAL TUBULAR (PT) INJURY BY URINARY ASSAY FOR PT ANTIGEN (Ag). R.H. Rubin*, R.E. Thompson*, D.J. Piper*, N.H. Bander*, L.J. Old*, L.H. Klotz*, W.P. Hansen* and N.E. Tolkoff-Rubin. Mass. General Hospital and Cambridge Research Lab, Boston, MA and Memorial Sloan-Kettering Cancer Center, New York, NY.

Utilizing murine monoclonal antibodies (mAb's) to Ags specific to particular nephron sites, we have developed urinary assays to detect the site and extent of nephron injury. The first assay utilizes 2 mAb's, URO-4 and URO-4a, which react to different epitopes of a 120,000 dalton glycoprotein, the adenosine deaminase binding protein, present on the brush border of PT cells. Urines from patients with defined renal disease were assayed for the presence of this PT Ag.

Condition	No.of Pts.	Assay Unit
1. Normal Controls	37	<0.1
2. Glomerular Disease	29	<0.2
	9	0.2-0.4
	2	0.4-0.6
3. Acute Tubular Necrosis	51	>1.0
	23	0.6-1.0
(>4 days post-injury)	5	0.4-0.6
4. Contrast Induced Renal Failure	5	>1.0
5. Aminoglycoside Toxicity	5	>1.0

In four patients who came to biopsy the assay was "correct" while the initial clinical assessment was incorrect.

We conclude that this assay can reliably demonstrate the presence of PT injury, distinguish between glomerular and tubular disease, and that the search for Ags specific for particular portions of the nephron in the urine, utilizing mAb's, can provide important clinical information.

RENAL FUNCTION 9-17 YEARS AFTER CHILDHOOD LEAD POISONING (LP). Henrietta Sachs*, Donald Moel. Northwestern Univ., Children's Mem.Hosp.,Dept.Peds. Chicago, Illinois.

There is great debate as to whether LP occurring during childhood results in chronic nephropathy later in life. Renal function was studied in a cohort of 74 study subjects (S) who had blood lead levels (PbB) >100 mcg/dl (range 100-471 mcg/dl; median 142 mcg/dl) between 1966-1972 (aged 1-6 years) and 21 age-matched sibling controls (C) who had PbB < 40 mcg/dl. S were continued under observation in the lead clinic until PbB remained < 50 mcg/dl on 2 successive visits. PbB measured in 1983 in S was significantly higher than C (14.5±4.5 vs 11.6±2.6 mcg/dl, mean ± 1 SD, p<.01). The 2 groups did not differ in development of hematuria (S vs C, 3 vs 10%) or leukocyturia (7 vs 5%). The frequency of elevated serum creatinine (11 vs 5%), depressed creatinine clearance (3 vs 0%), elevated protein excretion (3 vs 5%), low urinary osmolality (7 vs 9%), elevated serum β2-microglobulin (β2-M) (8 vs 5%), and elevated fractional excretion β2-M % (1 vs 5%) was similar in the 2 groups; an abnormal test is a value >2 SD above/below the mean of C values. Mean values of these renal tests were similar in S and C. Mean systolic blood pressure was significantly higher in S compared to C, 117±12 vs 109±10 torr, but C contained a preponderance of females and S had more overweight females. Mean diastolic pressure was similar in S and C (77±10 vs 76±6 torr). We conclude that in our population of adolescent subjects with LP (maximum PbB > 100 mcg/dl) 9-17 years earlier, there is little if any evidence of chronic nephropathy. Followup re-evaluation will be necessary to determine whether subjects are at risk of developing nephropathy 20 or more years after childhood LP.

EFFECT OF Na DICHLOROACETATE (DCA) IN HYPOXIC LACTIC ACIDOSIS IN RATS. J Scheid*, S Abu-Romeh* and RL Tannen. University of Michigan, Ann Arbor, Michigan.

Although DCA reduces blood lactate in Type B lactic acidosis in experimental animals, it is considered ineffective in the Type A disorder. However, recently this drug was reported to successfully treat lactic acidosis in humans, some of whom appeared to have Type A lactic acidosis. In order to resolve this discrepancy, we tested the effect of DCA in pure hypoxic lactic acidosis in rats.

Rats were mechanically ventilated with 7.5% O_2 for 1 hour and received either DCA, 300 mg/kg IV over the first 20 min., n = 10, or normal saline, n = 10. pO_2 and pCO_2 were comparable in the two groups, 38 and 42 mmHg respectively. The degree of lactic acidosis was attenuated significantly in the DCA studies. Blood lactate after 1 hour was 2.4 ± 0.6 mM in the DCA rats vs. 4.8 ± 0.8 mM in the controls, p< .05. This was accompanied by a higher pH (7.24 ± 0.03 vs. 7.18 ± 0.04) and higher plasma HCO_3 (18.8 ± 1.1 vs. 15.1 ± 1.5 mM) in the treated group. The DCA treated rats also maintained a higher mean BP (59.1 ± 4.8 vs 35.6 ± 5.9 mmHg) and the rates of urine flow (21.1 ± 1.8 vs. 4 ± 0.5 ul/min) and sodium excretion (2.6 ± 0.22 vs. 0.14± 0.04 uEq/min) were substantially greater.

These studies clearly demonstrate that DCA is an effective form of therapy for hypoxic Type A lactic acidosis.

ACUTE RENAL FAILURE IN SAUDI ARABIA. ANALYSIS OF 100 CASES. Riyad Said, Magdi Hussein*, & E. Veverbrandts. Al Hada Hospital, Taif, Saudi Arabia.

One hundred cases of acute renal failure (ARF) were seen at this hospital in the last 4 years. Sixty-nine were of renal origin, 21 pre-renal, and 10 post-renal. 60 were males and 40 females. 12 were below 15 years of age. From the 69 renal cases, 38 were acute tubular necrosis (septic shock in 15, hypovolemic shock in 6, aminoglycoside toxicity in 7, contrast nephropathy in 4, and rhabdomyolysis in 4), 16 cases of glomerular origin (acute post-streptoccocal GN in 7 rapid progressive GN in 3 systemic lupus in 2 and 1 case of each acute cortical necrosis, ARF with viral hepatitis, renal thrombosis with amyloidosis), 9 cases of acute interstial nephritis (4 idiopathic, 3 with non-steroidal drugs, and 1 case of each with methacilline, and thiazide)., and 6 of metabolic etiology (hypercalcemia in 4 and 2 with acute urate nephropathy. Acute post-streptoccocal GN was seen in 7 of the 12 pediatric cases. Oligo-anuria was seen in 59 cases, and non-oliguric ARF in 41 cases. 16 of the non-oliguric were in the ATN group, and 7 of these were secondary to aminoglycoside toxicity. 23 patients required dialysis and 20 of them were oliguric. Outcome of the hundred cases was: a) 48 alive with normal kidney function. b) 23 alive with mild renal insufficiency. c) 4 alive with irreversible ARF requiring chronic dialysis, and of these 3 has been successfully transplanted. d) 25 patients died; 7 in the pre-renal group, 1 in the post-renal and 17 in the renal group (12 of these were with ATN). 19 of the 25 deaths were oliguric. Septic shock, cardiac arryhthmias, are the commonest cause of death. We conclude that the spectrum of ARF in Saudis is similar to the West.

"PSEUDO-DISTAL RENAL TUBULAR (DRTA) ACIDOSIS" DUE TO LAXATIVE ABUSE: THE ROLE OF DECREASED DISTAL SODIUM DELIVERY. W. Schlueter*, A. von Riotte*, H. Rubenstein, N.A. Kurtzman, and D.C. Batlle. West Side VA Hosp, and Univ of Ill, Chicago, IL.

The metabolic acidosis (MA) of laxative abuse is felt to be the result of bicarbonate losses in the stool in the face of an appropriate increase in urinary acidification owing to the acidemic state. We studied a patient who had chronic MA (blood pH 7.28) associated with urinary (U) pH above 6.0 suggesting the diagnosis of DRTA. U Na was virtually absent (<1 mEq/L) and plasma aldosterone extremely high denoting enhanced avidity for distal Na reabsorption. In response to either oral furosemide or Na_2SO_4 infusion U pH fell below 5.0 in association with an increase in Na excretion. Urinary pH also fell when Na excretion was increased by $NaHCO_3$ infusion despite the fact that this maneuver resulted in alkalemia. After 2 weeks of saline infusion (3 L daily) and while the patient ingested her regular dose of laxatives, the MA was ameliorated (blood pH 7.36) and redeveloped after discontinuation of saline infusion (blood pH 7.31) The amelioration of MA occurred in association with lowering of U pH and plasma aldosterone and while U Na and acid excretion increased. This patient illustrates the importance of distal Na delivery for distal acidification which was suppressed in her despite acidemia and enhanced distal Na avidity and hyperaldosteronism. Moreover, the data demonstrate that the acidosis of protracted laxative abuse is, in part, due to inadequate distal Na delivery and therefore can be ameliorated with volume replacement.

REGIONAL CITRATE ANTICOAGULATION (RCA) FOR HEMODIALYSIS (HD): REPORT OF SIX HUNDRED TREATMENTS. Elliott N. Schwartz, Gretchen Turner*, Wanda Hunt*, Mary Brassfield*, Mervyn Sahud*, Peter H. Rowe, Robert Beallo, John C. Weaver, HD Services, Merritt-Peralta Med. Ctr., and Providence Hosp., Oakland, CA.

Heparin anticoagulation for HD has well-known risks especially bleeding. Regional citrate anticoagulation has been proposed for safer HD in high risk patients (Pinnick et al., N.E.J.M. 308: 258, 1983). We have developed a standard protocol for RCA. In 1½ years, over 600 RCA treatments in our inpatient and acute care units have been performed (12% of total per year). Indications for RCA included HD immediately preop and postop, major surgical procedure, GI bleeding, bleeding diathesis, invasive diagnostic procedure, pericarditis and heparin allergy. Treatments per patient varied from 1 to 24 (avg. 5.6). No routine lab test was helpful in determining adequate anticoagulation; serial calcium levels were helpful to prevent hypocalcemia; citrate levels were not clinically useful. Complications of RCA were rare. Fluid overload due to infusion volumes was common. HD was performed via standard accesses, including femoral and subclavian catheters. Twenty-seven HD with RCA were for plasma ultrafiltration only. The only absolute contraindication is a single needle HD procedure.

A PROSPECTIVE AUDIT OF RADIOCONTRAST (RC) NEPHROTOXICITY (N). Henry S. Shavelle and John Renner.* Memorial Hospital Med. Ctr., Long Beach, CA.

A prospective audit of all patients undergoing major angiography was performed at a large private medical center to determine the overall incidence of RCN. One hundred fifty five patients, mainly geriatric, were studied. N was defined according to criteria of Swartz et al, Am J Med,65:31-37,1978 and/or Eisenberg et al, Am J Med,68:43-46,1980. Hydration status prior to the study, type and amount of fluids given during the procedure, and amount of RC material used were uncontrolled and varied greatly. Amount of iodine (I) administered varied from 30 to 45 gm.

Overall incidence of N varied from 6.25% to 8.75% (depending on the criteria used), with the highest incidence for abdominal angiography, and is within the wide range of 0% to 12% of published experience at other institutions. Oliguria occurred in 1 patient. No relationship existed between % serum creatinine change and total amount of I administered. Six of 52 patients (11.5%) with preexisting renal dysfunction (serum Creat > 1.5 mg% or BUN > 20 mg%) developed N.

Compared to 0% N achieved by Eisenberg et al,AJR,136:859-861,1981, despite using greater amounts of I (35-66 gm.), the protective value of a concomitant forced saline diuresis employed by that group appears to be effective.

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (PKD) IN CHILDREN. Aileen Sedman,* Nancy Butler,* Patricia Gabow. Denver General Hosp. and Univ. of Colo. Health Sciences Center, Denver, CO, and Hosp. of University of Mich., Ann Arbor, MI.

Eighteen children in 13 PKD families had PKD before age 18. The mean age of the children was 9.6 ± 5.9 yrs. (range 0-18 yrs). Two children were diagnosed in utero. Nine children were symptomatic; 3 were hypertensive; 2 had decreased renal function; and 10 had abnormal urinalysis at diagnosis. Five children had hernias (2 bilateral inguinal, 2 umbilical, and 1 femoral) and 2 had congenital bony abnormalities. Fourteen children were seen more than once with a mean follow up of 6.4 ± 7.4 yrs. Three of 14 developed hepatic cysts. Only 2 of 14 developed ESRD; one received a transplant 3 years after diagnosis and one died of uremia 29 years after diagnosis. Ultrasonography in young children of PKD families may have suspicious rather than diagnostic findings of PKD, such as an inhomogenous pattern suggesting small cysts, only a few cysts, or unilateral cysts. There were 29 children from 22 families with such findings. Their mean age was 12.2 ± 3.2 yrs. at initial evaluation. Seven had PKD symptoms; none were hypertensive; 1 had an increased creatinine and 16 had an abnormal urinalysis. Five of 9 children seen subsequently developed definite PKD within 6.8 ± 3.4 yrs. at a mean age of 19.2 ± 5.9 yrs. Therefore: (1) children who manifest PKD frequently have other associated abnormalities, (2) PKD detected in childhood does not necessarily imply a bad short term prognosis, and (3) children in PKD families with suspicious ultrasonograms often develop PKD.

BUDD CHIARI AND NEPHROTIC SYNDROME DUE TO MASSIVE ACUTE FATTY LIVER. Stewart W. Shankel, M.D., Dept. of Medicine, Univ. of Nv., Reno, Nevada.

Associations between liver and renal disease are well known. Toxins often cause acute disease of both organs. Poor perfusion states, the classical hepatorenal syndrome and glomerulonephritis associated with cirrhosis of the liver are common. However, the nephrotic syndrome is rarely seen with cirrhosis of the liver. This is the first report of a case of nephrotic syndrome due to obstruction of the inferior vena cava from massive hepatic enlargement. A 24 year old man who drank 144 ozs. of beer daily for three years developed rapid enlargement of the abdomen, chronic diarrhea, edema, back and abdominal pain and SOB. He had no urinary complaints. BP was 170/60, P 140. He was in mild respiratory distress with generalized wheezes. He had marked jaundice, spider angiomas, and palmar erythema and massive edema below the ribs. Liver span was 24 cms. He was mentally confused with a fetor hepaticus. Lab tests showed BUN of 7, alb 2.4, bil 27.5 all in mg/dl. Urinary protein was 35.8 gms/d. An IVC venogram showed marked narrowing of the IVC in the intrahepatic portion. Over the next month, his liver function improved and the liver decreased in size. The ascites, edema and proteinuria cleared. Two months later, he developed bleeding esophageal varices, underwent shunt surgery and expired shortly thereafter. No narrowing of the vena cava was found at surgery or at autopsy. The liver weighed 3260 gms with Laennec's chirrhosis, alcoholic hepatitis and a clotted porta-caval shunt. The large kidneys showed mild tubular necrosis and no glomerular abnormalities.

MECHANISM OF IMPAIRED WATER EXCRETION IN NEPHROTIC SYNDROME (NS). M. Shapiro,* K. Nicholls,* and R. Schrier. Univ. Colorado Med. Sch., Denver, CO.

Hemodynamic and hormonal factors were studied in 9 patients (pts) with NS and evaluated relative to their capacity to excrete a 20 ml/kg water load (WL). In 5 non-excretor pts (37% of WL in 5 hr) and 4 excretors (105% of WL in 5 hr, p<.005, nl >80%), blood pressure (132/88 vs 119/79 mmHg), pulse (74 vs 77/min), cardiac index (3.7 vs 3.1 L/min/m^2), pulmonary wedge pressure (7.3 vs 7.3 mmHg), systemic vascular resistance (1538 vs 1254 dynes.sec.cm^{-5}) and plasma volume (42 vs 43 ml/kg, nl 40±4) were not different. Similarly, plasma renin activity (3.9 vs 3.9 ng/ml/hr), plasma aldosterone (8.5 vs 12.0 ng/dl) and arginine vasopressin (AVP, 2.7 vs 2.4 pg/ml) were not different in the 2 groups. AVP suppressed comparably in both groups (2.6 vs 0.9 pg/ml, p<.05) during WL. Inulin clearances (C_{In}) were, however, significantly different in the non-excretor and excretor groups (34 vs 77 ml/min/1.73 m^2, p<.02). Analysis by linear regression of these hemodynamic and humoral factors in all 9 pts demonstrated a significant correlation only between WL excretion and C_{In} (r=.68, p<.05). To enhance renal perfusion, central blood volume was expanded via head-out water immersion (HWI); this increased WL excretion (37 to 82%, p<.025) in the non-excretors. The change in %WL excretion during HWI correlated with the change in C_{In} during HWI (r=.70, p<.05). In summary, the results of the present study therefore indicate that a difference in GFR is the primary determinant of water excretion in NS, rather than any systemic hemodynamic or humoral factors.

MASSIVE ELECTROLYTE WASTING FOLLOWING CIS-PLATINUM: AMELIORATION DURING ACUTE RENAL FAILURE. Stephen C. Textor, David Goldberg. City of Hope National Medical Center. Duarte, CA

Although Cis-platinum (CP) commonly diminishes glomerular filtration rate (GFR) its tubular toxicity may produce life-threatening solute loss if GFR is preserved. We measured daily solute excretion for six weeks in a patient treated with CP (40 mg/Kg x 5 days i.v.) for metastatic seminoma. CP Rx produced a massive diuresis (5-7 l/daily) leading to hypovolemia and hypotension and characterized by wasting of Cl^-, Na^+, K^+, Ca^+, Mg^+, and $Po4^+$. Renal glycosuria and proteinuria (4.4 g/d) were also observed, but GFR was unaffected. Urinary chloride absorption was the most severely altered. Inappropriate solute loss persisted for 5 weeks. Tobramycin produced mild acute renal failure (ARF) and correction of serum values:

	CP Rx	ARF	LATE RECOVERY
GFR (ml/min)	76	31	76
FE Cl (%)	8.47	7.57	2.86
FE Na (%)	4.76	5.22	1.15
FE K (%)	71	45.8	14.4
U Prot (g/d)	4.4	0.7	3.3

Hence, ARF diminished the filtered load and total solute loss without changing the tubular abnormalities.

These studies underscore the potential for CP to selectively disrupt tubular reabsorptive function in man. Preservation of GFR under these circumstances may allow net solute wasting to reach massive levels. A decrement in GFR, and therefore filtered load, commonly encountered during aminoglycoside Rx may serve a protective role for electrolyte homeostasis in this setting.

HEPATITIS B ASSOCIATED MEMBRANOUS GLOMERULONEPHROPATHY (HBMGN) IN 11 AMERICAN CHILDREN-AN UNDERDIAGNOSED CLINICOPATHOLOGIC ENTITY. F.B. Stapleton, H.F. Krous*, & W.M. Murphy* for the Southwest Pediatric Nephrology Study Group, Memphis, TN.

The clinical and pathologic features of HBMGN in North American (NAm) children are unknown. We identified 11 pts with HBMGN--10 were diagnosed since 1979. All were male and 7 were black. The mean age at presentation was 5.3 yrs (range 2.2-8.4 yrs) compared to our pts with idiopathic MGN (10.6 yrs) or lupus MGN (13.4 yrs). Eight were evaluated initially for nephrotic syndrome (NS) and 3 for asymptomatic proteinuria (APr) or hematuria. No pt was icteric at presentation although 8/10 had elevated serum AST levels. Initial GFR was normal in all pts; BP was elevated in 3. Serum C3 and C4 concentrations were reduced in 11/11 and in 2/7 pts, respectively. Serum HB surface antigen was found in all pts, in 4 parents and in 1 sibling. Renal biopsies revealed stage II or III membranous glomerulonephropathy; IF studies revealed 3 or more glomerular immunoreactants in 10/11 biopsies; EM showed subepithelial and mesangial deposits in 9/9, intramembranous deposits in 8/9 and subendothelial deposits in 4/9 pts. Liver biopsies in 3 pts showed chronic persistent hepatitis in 2 and chronic active hepatitis in 1. Nine pts have been followed for a mean of 27 months (range 3-105 months). One child developed ESRD after 9 yrs, 3 have persistent NS, 3 have APr and 2 have normal urinalyses. We conclude: 1) HBMGN forms a distinct clinico-pathologic entity; 2) it occurs predominantly in young black males and is associated with decreased serum C3, and 3 or more glomerular immunoreactants; and 3) HBMGN in NAm children is more frequent than previously recognized.

ABNORMAL ANTIDIURETIC HORMONE (ADH) SECRETION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). H. Trachtman*, M. Rao*, E. Ginzler*, A. Tejani, L.Finberg* (Tech. Assist. L. Herod*, & J. Bellavia*) Depts. of Peds. & Med., SUNY Downstate, Brooklyn, N.Y.

Despite scattered case reports describing individuals with SLE and abnormal ADH secretion, no studies have assessed ADH secretion in a large group of SLE patients. We measured ADH levels in 31 stable patients (F:M,27:4) with SLE (GFR ≥80 ml/min) and correlated them with factors that regulate ADH release.

ADH samples were drawn after patients had been in the sitting position for 20 min, while blood for plasma renin activity (PRA) and aldosterone (A) was taken 60 min after oral administration of furosemide, 40 mg. A random urine osmolality and C_3 concentrations were measured concomitantly with the ADH determinations.

The patients were divided into two groups according to duration of SLE (I-less than; II-longer than 2 yr).

	GROUP I (N=13)	GROUP II(N=18)	p
Duration (mos) ($\bar{x}$±SD)	11.1±5.6	68.4±36.1	-
ADH (≤5.4uU/ml)	8.9±4.0	13.9±5.9	0.01
Uosm (mOsm/L)	445±207	697±269	0.02
PRA (≤4.2 ng/ml/hr)	5.1±3.9	5.6±3.2	NS
A (≤26.4 ng/ml)	10.9±12.0	10.4±6.6	NS
C_3 (83-177 mg/dl)	98.1±52.1	95.1±45.4	NS

No intra-group correlations were noted between ADH levels and Uosm, PRA, A or C_3.

These results demonstrate that patients with SLE develop progressively elevated plasma ADH levels which are unrelated to disturbances in volume status or impaired renal responsiveness to ADH. We propose that SLE of long duration is associated with subclinical cerebritis and primary hypersecretion of ADH. We cannot exclude altered hormone clearance or ADH-antibody complexes as causal in the elevated ADH concentrations.

ROLE OF SYSTEMIC HYPERTENSION IN DIABETIC NEPHROPATHY. W. Gordon Walker, Timothy Codd,* Robert P. Murphy,* Judy Hermann,* Lillian Rourke,* Loretta Young,* and Arnall Patz*. The Johns Hopkins Hospital, Baltimore, Maryland.

Sequential physical exam, renal function, renin-angiotensin-aldosterone status and diabetic control were evaluated semiannually in 133 patients with diabetes mellitus, representing more than 450 patient years of observation. Approximately half have had elevated systolic pressure throughout this period of follow-up (BP 152±3.7 mmHg); 67% of Type 2 diabetics were hypertensive and 73% of Type 1 diabetics were normotensive. Hypertensive diabetics had higher levels of plasma creatinine ($p<.02$); higher levels of B-2 microglobulin ($p<.001$); higher levels of prorenin ($p<.01$); and higher levels of angiotensin II ($p<.005$); higher levels of aldosterone ($p<.002$); and, as we previously reported, higher scores for most indices of retinopathy. Moreover, significant positive correlations were demonstrated between blood pressure and plasma renin activity ($r=+.20$; $p<.009$); plasma prorenin ($r=+.23$; $p<.04$); plasma angiotensin II ($r=+.21$; $p<.01$); plasma B-2 microglobulin ($r=+.29$; $p<.001$); plasma creatinine ($r=+.11$; $p<.009$). In addition, B-2 microglobulin correlates significantly with creatinine ($r=+.78$; $p<.001$), and angiotensin II ($r=+.35$; $p<.001$). These findings establish a clear association between hypertension, renal impairment and the renin angiotensin system in diabetes mellitus and are consistent with the hypothesis that hypertension may be a significant risk factor for the microvascular complications of diabetes.

IDIOPATHIC NEPHROTIC SYNDROME (NS) WITH GLOMERULAR COLLAPSE: ? A VARIANT OF "MALIGNANT FOCAL SEGMENTAL HYALINOSIS" (FSH). Mark A. Weiss and Renata Maryniak, Dept. of Pathology, University of Cincinnati Medical Center, Cincinnati, Ohio.

Six black patients (mean age 35 yrs.) presented with renal disease of acute onset (mean 2 wks.); 5 had NS with urine protein ranging from 11-22 gm./24 hrs. and serum Cr. 1.4-6 mg.% and 1 acute renal failure (serum Cr. 12 mg.%, 800 mg. protein/24 hrs.). There was no exposure to nephrotoxic drugs or intravenous narcotics.

Renal biopsies showed global and/or segmental glomerular collapse with cuffing by hyperplastic, reactive visceral epithelium having markedly swollen, vacuolated cytoplasm with hyaline droplets. Segmental sclerosis and hyalinosis were absent. EM documented diffuse, circumferential effacement of foot processes and focally prominent subepithelial formation of laminated new basement membrane material over collapsed loops. Immunofluorescence was non-contributory. All biopsies had patchy tubular necrosis with interstitial edema and mild inflammation.

The clinical course was characterized by persistent NS (steroid resistant in 3 patients) with rapid progression to chronic renal failure (mean 16 mos.) or irreversible acute renal failure. Repeat biopsy in 2 patients (at 8 and 17 mos.) showed diffuse (global and segmental) glomerular sclerosis with insudative deposits and marked tubular atrophy with persistent tubular damage. These cases may represent a variant of "malignant FSH", with features of glomerular ischemia and rapid progression to sclerosis possibly reflecting severe hypoperfusion induced by hemodynamic factors.

ACUTE HYPERGLYCEMIA (HG) DOES NOT INCREASE GLOMERULAR FILTRATION RATE (GFR) IN NON-DIABETIC NORMAL MAN. M. Walzyck,* J.P. Pulliam, W.M. Bennett, Oregon Hlth Sci. Univ., Portland, OR.

Early uncontrolled diabetes mellitus (DM) is characterized by an increased GFR which can be decreased by insulin therapy. Since hyperglycemia per se could cause the increased GFR, 8 normal volunteers received glucose infusions to achieve steady state blood sugar concentrations >500 mg/dl. Inulin (C_{In}), creatinine (C_{Cr}), and para-aminohippurate (PAH) clearances were determined during control, glucose and mannitol periods. Mannitol and glucose dosage was adjusted to achieve comparable osmolar clearances. Extracellular fluid volume was maintained so that body weight, serum electrolytes and hematocrit did not change during the study. Clearance data were analyzed using each patient as his own control.

Results $\bar{X}$ ±SD:	Baseline	Glucose	Mannitol
C_{Cr} (ml/min/1.73 m^2)	99±28	115±36	102±41
C_{In} "	87±28	87±26	81±19
C-PAH "	595±140	340±106*	549±144

*$p<.05$ vs baseline and mannitol. Plasma growth hormone was assayed in 7 patients and did not change predictably during glucose infusion.

Conclusions:

1. HG per se does not increase GFR (C_{In}) in non-diabetic normal man.
2. C_{Cr} does not increase with HG or high urine flow and thus can be used to measure the extent of GFR increase in uncontrolled diabetes when the subject is euvolemic.
3. It is possible that renal vasoconstriction induced by HG in normal man but not DM prevents hyperfiltration. Alternatively, HG could interfere with tubular secretion of PAH.

BLOOD PRESSURE AND ELECTROLYTE HOMEOSTASIS DURING FUROSEMIDE ADMINISTRATION: ROLES OF RENIN-ANGIOTENSIN-ALDOSTERONE (RAA) AND ALPHA-$_1$ ADRENORECEPTORS (A_1R). C. Wilcox, N. Guzman*, W. Mitch, R. Kelly, B. Maroni*. Harvard Med. Sch., Boston, MA

We showed that normal subjects given furosemide (F) and high Na^+ diet remain in precise Na^+ and K^+ balance despite the diuresis due to a compensatory decrease in electrolyte excretion for the ensuing 18 hrs. Even though plasma angiotensin II increased (+34.9±8.8 pg/ml; mean ± SE, $p<0.05$), RAA blockade with captopril (C) did not alter this compensation (K.I. 24:233, 1983). Since plasma norepinephrine (NE) also increased after F (+137±50 pm/ml, $p<0.05$) and since increased BP and Na^+ reabsorption during sympathetic nervous system activation in animals are mediated by A_1R receptors we gave prazosin (P), 2 mg/6h, to 6 normal subjects on a 270 mmol Na^+ and 80 mmol K^+ diet for 9 days. After 5 days of P alone, mean BP fell 6.9±1.8 mm Hg ($p<0.05$) and wt. increased 1.0±0.38 kg ($p<0.05$); however, each subject was in Na^+ and K^+ balance by day 5. F, 40 mg/d, was then given on days 6-9. Neither A_1R blockade alone with P on days 6-8 nor addition of RAA blockade with C (25 mg/6h; P+C) on day 9 changed the acute diuretic response or the ensuing compensatory fall in Na^+ and K^+ excretion. Na^+ and K^+ balances were precisely neutral on each diuretic day. Despite a diuresis of 1-2 liters, BP did not change acutely when F was given with P or C alone, but with P+C on day 9, BP fell 13±5 mm Hg ($p<0.05$) after F. Thus, when Na^+ intake is not restricted, electrolyte balance is maintained during administration of F despite RAA or A_1R blockade, but BP maintenance requires that one of these systems remains intact.

CONCOMITANT RENAL LESIONS IN DIABETIC PATIENTS WITH RENAL INSUFFICIENCY OR NEPHROTIC SYNDROME. B.M. Wong*, H.I. Al-Mufti* & A.I. Arieff. V.A. Med. Ctr. & U.C.S.F., Nephrology Research, San Francisco, CA

Renal involvement in patients with diabetes mellitus typically presents with nephrotic syndrome and usually leads to renal insufficiency. There is no known therapy for diabetic nephropathy, but other renal lesions may be present in diabetic patients which might be amenable to therapy. To evaluate the possible existence of other renal lesions in diabetic subjects with kidney dysfunction, we undertook a prospective study over 7 years to define the extent of concurrent renal diseases in selected patients who had diabetes mellitus & either nephrotic syndrome or renal insufficiency. Over 7 years, a total of 144 patients had renal biopsies performed for diagnostic purposes and among these, 12 patients had a diagnosis of diabetes mellitus. These 12 patients were biopsied for reasons other than diabetic nephropathy. The reasons for biopsy included microscopic hematuria, red cell casts, absence of diabetic retinopathy or a clinical course which was felt to be atypical of diabetic nephropathy. Six of 12 diabetic patients had in addition to diabetic nephropathy another renal lesion on biopsy. The renal lesions were: heroin associated focal sclerosis; light chain nephropathy; proliferative post-streptococcal glomerulonephritis; rapidly progressive glomerulonephritis; Ig A nephropathy; acute interstitial nephritis.

These data show that in selected diabetic patients who have nephrotic syndrome or renal insufficiency, there may be renal lesions other than diabetic nephropathy. These lesions may be amenable to therapy and may be present in up to 50% of a selected group of diabetic patients with renal dysfunction.

PROPRANOLOL-INDUCED HYPERKALEMIA IN NON-DIABETIC UREMIC PATIENTS. W.C.Yang*, T.P.Huang*, L.T.Ho*, H.M.Chung*, Y.L.Chan, and D.C. Batlle. Veterans General Hosp., Taiwan; and Univ of Ill, Chicago.

In normal subjects β -adrenergic blockade decreases K tolerance by interfering with K translocation into the cells. The occurrence of hyperkalemia with the use of β-blockers, however, has not been well documented. In 10 uremic patients receiving Propranolol (P) chronically, plasma K was higher than that of 10 age-matched uremics not receiving P (5.6±0.2 and 4.6±0.1 mEq/L $p<0.005$). Both groups were virtually anuric and had a comparable BUN, plasma HCO3, and erthrocyte Na-K ATPase. To examine whether extra-renal β adrenergic-mediated K disposal is intact (or enhanced) in uremics we infused epinephrine (E) and E + P to 10 uremics and 6 normal controls. (Table) (*$p<0.01$).

	Plasma K mEq/L	ΔK after E mEq/L	ΔK after E+P mEq/L
Uremic	4.9±0.20	-0.69±0.00	-0.04±0.07
Control	3.7±0.02*	-0.63±0.07	-0.02±0.05

E resulted in a comparable fall in plasma K (ΔK) in both groups, an effect which was totally abolished by the concomittant infusion of P. Neither plasma insulin nor plasma aldosterone changed 15 and 30 min after infusion of E or E+P, a time when E alone resulted in a fall in plasma K and an increase in heart rate in both groups. The data demonstrate that β-adrenergic-mediated control of K disposal is intact in non-diabetic uremics and that the use of P on a chronic basis results in hyperkalemia. Thus, β-adrenergic control of K disposal assumes a critical role in the defense against hyperkalemia in uremics.

SEVERE GLOMERULONEPHRITIS (GN) WITH LATE EVOLUTION TO TYPICAL WEGENER'S GRANULOMATOSIS (WG): REPORT OF THREE CASES AND REVIEW OF THE LITERATURE. Thasia Woodworth*, Alfredo Esparza and J. Gary Abuelo, Rhode Island Hospital, Depts. of Medicine and Pathology, Providence, Rhode Island.

Three patients (pts) who had presented with renal failure due to GN, 2-7 years later developed cough, hemoptysis, fever and multiple cavitary pulmonary nodules. "Blind" nasal biopsies were non-diagnostic. Lung biopsies showed WG. We analyzed 11 previously-reported pts along with our 3 pts. 11/14 were female. Mean age was 43 years (range 12 to 65 yrs). Initially azotemia (creatinine 1.7-12 mg/dl), proteinuria and hematuria (gross in 2) were each seen in 13/14 pts. 11/14 had fever, rash, and/or arthralgias; 6/14 initially had pulmonary hemorrhage, which cleared after immunosuppressive therapy in 4 and after bilateral nephrectomy in 2. 8/14 had crescentic GN by renal histology. 11/14 developed end-stage renal disease despite treatment with corticosteroids (4/14 also had cytotoxic agents). 12/14 pts were receiving dialysis, while the other 2 had creatinines of 2.6 and 4.4 mg% at time of late pulmonary involvement. The lapse between original GN and late pulmonary manifestations was 10-78 mos. 11/14 had vasculitis and granulomata on lung biopsy; the other 3 had necrotizing granulomata on nasal mucosal biopsy. 1/14 pts was treated with prednisone alone and 10/14 were treated with cyclophosphamide or azathioprine (3/10 also had steroids) with resolution of respiratory abnormalities. 1/14 pts became quiescent without treatment. Conclusions: WG may present with severe GN alone. Respiratory manifestations that are essential for diagnosis may not appear for years. Relapse of WG may occur, despite the "immunosuppression" of uremia.

MORPHOLOGICAL CHANGES OF RAT KIDNEYS AFTER HYPOKINESIA. Yan G. Zorbas* and Vasiliv G. Andreyev*. Danielopolu Institute of Physiology, Bucharest, Rumania.

Previous experimental studies under hypokinesia (HK) revealed an elevation in activity of juxtaglomerular system of the kidneys and increase in their weight. Against this background the objective of this investigation was to examine the state of kidneys of wistar rats after the exposure to 49 days of HK. They were divided into three groups: the 1st group(10-rats) subjected to pure HK, the 2d group (5-rats) submitted to combined HK and PE (physical exercise) and the 3d group (10-rats) placed under ordinary vivarium conditions and served as control. The rats were sacrificed within 4-12 hours and on the 25th day of readaptation period (RP), and morphological examinations were performed. A comparative analysis of the data obtained revealed that there was a statistically reliable and equally marked increase in kidney weight of rats subjected to HK, as compared to the control rats. No differences in the kidneys were demonstrated among the 1st and 2d groups of rats. It was concluded that further studies are essential in order to establish the mechanisms that led to the increase in kidney weight following the exposure to hypokinesia, while at the same time it was revealed that PE cannot be used to counteract effectively the development of morphological alterations in the kidneys under the influence of diminished muscular activity conditions.

HEMODIALYSIS

A PROSPECTIVE STUDY OF ERTHYROCYTOSIS IN HEMODIALYSIS (HD) PATIENTS. F.A. Adedeji*, A. R. Eiser, M. S. Neff, E. Diakoumakis*, R. F. Slifkin. Mt. Sinai Services, City Hospital at Elmhurst, Elmhurst, N.Y. and Mt. Sinai School of Medicine, New York, N.Y.

A group of HD patients with a hematocrit > 35% (Group I) were compared with a group of patients with hematocrits < 26% (Group II). Each patient underwent renal sonography. Erthyropoietin was measured by a hemagglutination inhibition assay. Clinical parameters and whether patients received androgens were noted.

	Group I (n=12)	Group II (n=11)
Multi-cysts	11	3
Single cysts	0	2
No cysts	1	6
Erthropoietin	12.3 mu/ml	22.41

The patients in Group I were significantly more likely to have multiple cysts (Ch square = 9.3 P < .01). Although 8 of 12 in Group I received parenteral androgens, of 4 of 11 in Group II likewise did. There was not statistically significant difference between the groups regarding androgens. (Chi square = 1.04 P > .10).

Erthropoietin assays failed to reveal significant differences between the two groups (P .10).

Preliminary data suggest a role for multiple cysts in dialysis - associated erthrocytosis. Failure to find a correlation with erthropoietin levels may reflect insensitivity and lack of specificity of the particular assay used.

DIALYSIS, ULTRAFILTRATION AND SHAM-DIALYSIS IN NORMAL SUBJECTS. Jonas Bergström, Anders Danielsson and Ulla Freyschuss (intr. by Lee Henderson) Dpts of Renal Medicine and Clinical Physiology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden.

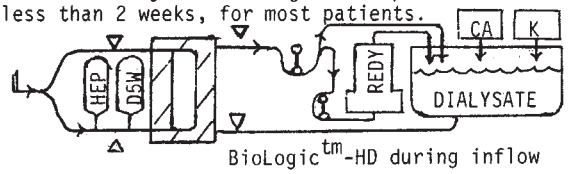
To obtain reference data we have studied cardiovascular response in healthy volunteers to isovolemic, isonatremic, acetate hemodialysis (IHD) (7 cases), isolated ultrafiltration (IUF) with (6 cases) and without (2 cases) preloading with Ringer (3% body weight) and sham-dialysis i.e. with no UF and no dialysate (8 cases); blood-membrane interaction was studied in the sham experiments, too. Cuprophane 1.2 m^2 dialyzers were used. Blood flow was 200 ml/min. Cardiac output (Q) (thermodilution) pulmonary (PAP) and brachial arterial pressure (BAP), lower limb vascular resistance (LVR) (occlusion pletysmography), blood gases, A-V O_2, acetate, catecolamines, white cells, complement factors (C3, C5, C3d) and acute phase reactants were determined. IHD induced an increase in heart rate (HR) associated with a fall in systemic vascular resistance (SVR) and LVR, but no change in BAP and PAP. IHF induced a fall in stroke volume and Q with an increase in SVR, but HR and BAP were unchanged. These responses to IHD and IUF were similar to those in uremic patients without cardiovascular complications. During sham-dialysis there was a transient leucopenia and a fall in arterial oxygen tension but no change in SVR or in PAP and pulmonary vascular resistance as has been observed in the sheep (Lindsay et al. 1983). The results indicate that in normal man the circulatory responses are related to the type of procedure (IHD, IUF, respectively) and not to blood-membrane interaction.

A SINGLE-ACCESS, SORBENT-BASED, FLOW-CONTROLLED SYSTEM FOR HOME HEMODIALYSIS: THE BIOLOGIC^{tm}-HD. Stephen R. Ash, David J. Carr*, Donald L. Blake*, and Thomas W. Schultz*. Ash Medical Systems, Inc., West Lafayette, Indiana.

In absolute numbers, there are fewer home hemodialysis patients today than in 1973. A major limitation is the prolonged training time (8-12 wks./pt.) The BioLogic^{tm}-HD machine is designed to provide single-access, sorbent-based hemodialysis with maximum simplicity, safety, mobility, and ease of patient training.

Unique features of the system include: 1) Use of the plate dialyzer membranes as a pump, avoiding a blood side pump. 2) Sensitive optical monitors for blood flow and particle content. 3) Control and optimization of blood flow and UFR by computer. 4) Machine controlled, variable dialysate regeneration rate. 5) Continuous ammonium monitoring of dialysate. 6) Pre-assembled dialysate and blood side components and solutions, completely disposable. 7) Portability on battery power for 30 minute periods during dialysis (wt. less than 40 lbs).

In vitro and in vivo tests have demonstrated blood flows and clearances approximating standard single access dialysis machines. Ion balance is similar to the Redy^{R} system used in the "bicarb" mode. Clinical testing will determine whether home hemodialysis training can be performed in less than 2 weeks, for most patients.



BioLogic^{tm}-HD during inflow

COMPARISON OF ACETATE (Ac) AND BICARBONATE (Bi) DIALYSATE IN AN OUT-PATIENT DIALYSIS UNIT. Joseph H. Brezin, Allan B. Schwartz, Joel L. Chinitz, Arthur R. Olshan, Larry E. Krevolin, Richard A. Friedman, Hahnemann University Hospital Division of Nephrology, Philadelphia, Penna.

105 patients using Ac entered a prospective, double blind, crossover study of Ac alternating with Bi over four 2 week periods. Dialysate concentrations (centrally mixed) in mEq/L:

	Na	K	Cl	Ac	Bi	Ca	Mg	Dext (mg/dl)
Ac	136	2.0	105	37.75		3.25	1.5	100
Bi	135	2.0	105		36	3.0	1.0	100

The mean maximum fall ($\overline{\Delta BP}$) in systolic BP (sBP) was 29.11 during 1172 Ac Rx's and 26.38 during 1136 Bi Rx's, (P=0.007):

	Initial BP	Low BP	End BP
Ac	145.21 ± 26.22	116.10 ± 30.65	134.79 ± 27.43
Bi	145.30 ± 25.00	118.92 ± 26.61	136.55 ± 25.62

Saline requirements were greater for Ac (205.56 cc/Rx) than Bi (161.66 cc/Rx). Nine patients had a $\overline{\Delta BP} > 50$ during Ac (mean = 56.17 mm Hg). On Bi, their $\overline{\Delta BP}$ was only 43.84, (P< 0.005); 7 of 9 patients improved and 2 of 9 were unchanged.

There was no difference in the amount of hypertonic saline, dextrose, or mannitol given during Ac or Bi to relieve symptoms. There were fewer episodes of shock (8 vs 19) and headache (10 vs 17) with Bi (N.S.). Vomiting was less frequent with Bi (6) than Ac (30), (P<0.005). Muscle cramps were more frequent during Bi (26.31%) than Ac (22.06%), (P<0.02).

We conclude that Bi offers only a modest benefit to most hemodialysis patients. Those who experience hemodynamic instability with Ac may benefit from switching to Bi.

EFFECT OF DESFERRIOXAMINE (DFO) AND ETHYLENEDIAMINE N, N'-bis (2-HYDROXYPHENYLACETIC ACID (EDBHPA) IN ALUMINUM (AL) LOADED RATS. M.A. Burnatowska-Hledin,* K. Schwartz,* J. Kovan,* G. Mayor. Michigan State University, Dept. of Medicine, East Lansing, MI.

The effects of DFO and theoretically more effective chelators on tissue Al burdens and Al toxic manifestations have not been critically evaluated. These experiments therefore evaluate the effects of DFO and EDBHPA on tissue Al stores and hematological toxicity. To induce anemia and increase tissue Al, Al was given for 2 weeks via intraperitoneal osmotic minipumps. All rats developed microcytic anemia and four groups (G) were studied for an additional 2 weeks: G-I was sacrificed on day (D) 0 (n=4); G-II was a time control (n=6); G-III was given DFO (n=6); and G-IV was given EDBPHA (n=5). DFO and EDBPHA were also administered by osmotic minipumps that delivered 7.8 and 2.9 mg/day respectively for 2 weeks. Rats were bled on D 0,7 and 14; 24 hour urines (U) were collected. Hct, Hb, MCV and plasma (P) Al on D 0 were not significantly different between G I-IV. The microcytic anemia was reversed by DFO only on D 7. U_{Al} in G-III and IV was significantly higher on D 1-7 when compared to either D 0 or G-II on D 1-7. Bone, kidney and liver Al concentrations in G-III were not significantly different from that in G-II, but were significantly higher in G-IV when compared to either G-II or III on D 14. P iron was significantly higher in G-IV when compared to either G-II or III on D 14. These results indicate that (1) treatment with DFO for 1 week leads to a reversal of Al induced anemia; (2) prolonged treatment with either chelator leads to a return of anemia; (3) selected tissue Al stores are not affected by DFO, and are further increased with EDBHPA.

THE ROLE OF THROMBOXANE IN PULMONARY HYPERTENSION (PHTN) INDUCED BY CUPROPHAN-ACTIVATED PLASMA (CAP). Alfred K. Cheung, Robert L. Baranowski, Div. of Nephrology, Univ. of Utah and VA Med. Ctr., Salt Lake City, Utah.

Acute PHTN occurs in patients undergoing hemodialysis with cuprophan membrane. This PHTN can also be seen in experimental animals in response to intravenous infusion of CAP. We have previously suggested that complement activation products are at least partially responsible for this hemodynamic alteration. In this study, we evaluated the role of another mediator, thromboxane A_2 (TX), in the pathogenesis of CAP-induced PHTN. Anesthetized swine (n=4) weighing 30-35 kg were studied. CAP was prepared by in vitro circulation of autologous plasma through a cuprophan dialyzer for 45 min. at 37°C. Acute bolus intravenous infusion of 5 ml of CAP produced marked PHTN with mean pulmonary artery pressure (PAP) increasing from 23.8±1.7 to 60.3±7.1 mmHg ($p<0.02$). After continuous infusion of OKY1581, a specific TX inhibitor, at 0.05 mg/kg BW/min, 5 ml of CAP failed to increase PAP significantly. A larger dose of CAP (20 ml), however, still increased PAP to 46.5±1.2 mmHg ($p<0.01$). An additional intravenous bolus (2.5mg) of OKY1581 abolished the PHTN response to 20 ml of CAP. Conclusion: (1) Besides complement activation products, thromboxane also mediates PHTN in response to acute intravenous CAP infusion. (2) Inhibition of this PHTN by specific TX inhibitor is dose-dependent. (3) The interaction between complement activation products and thromboxane in mediating this hemodynamic change warrants further studies.

A DETOXIFYING DEVICE WITH IMPROVED BIOCOMPATIBILITY AND CAPACITY: THE BIOLOGIC^{tm}-DT. D.J. Carr,* Stephen R. Ash, G.McC. Kaufman,* D.E. Blake,* and Aida A. Shihab-Eldeen.* Ash Medical Systems, Inc., W. Lafayette, IN.

Nonspecific sorbents such as charcoal, zeolite, and amberlite are effective in removal of drugs and liver failure toxins. However, they have limited capacity and marginal biocompatibility when used as large particles in hemoperfusion columns.

We have utilized sorbent suspensions in place of dialysate for the removal of uremic toxins in standard cellulosic plate dialyzers. 2.4 L of suspension containing 40 g/L powdered charcoal and 220 g/L Ca-Na loaded zeolite was circulated through the dialysate side of ExP 400 or Cobe PPD 1.6 dialyzers. Alterations in pressure of the sorbent suspension and one-way valves or clamps propelled blood and sorbent into and out of the appropriate dialyzer compartments. During in vivo tests on dogs, creatinine removal efficiencies were up to 50%. The same sorbent was used in custom-built reciprocating plate dialyzers. At blood flow rates of 119-190 ml/min clearance of a variety of drugs (phenobarbital, acetaminophen, digoxin, etc.) was similar to creatinine. These clearances were maintained in vitro for 12-24 hours. Biocompatibility was similar to that of standard cellulosic dialysis. A disposable cartridge including the plate dialyzer, sorbents, tubing, etc. will be attached to the BioLogic^{tm} machine, a computer-controlled, flow-monitored dialysis machine (Ash, et al, ASN, '84), for clinical tests. The BioLogic^{tm}-DT is expected to provide safe and effective therapy for serious drug overdose and liver failure.

SHORT EFFICIENT HEMODIALYSIS WITH REDUCED SYMPTOMS. A. Collins, P. Keshaviah*, R.Berkseth*, K. Ilstrup*, C. McMichael*, J. Ebben*. Hennepin County Medical Center, Minneapolis, Minnesota.

Reduction in treatment time is an attractive concept for dialysis centers and patients. However, there are concerns related to treatment adequacy, disequilibrium, acetate intolerance, increased symptoms, etc. We decided to study shortened treatment times without compromising solute and fluid removal in 12 stable patients on standard 4 hr. acetate hemodialysis (S_{Ac}). Therapy duration progressively decreased over a 4 mo. period till each patient reached the tolerance limit, manifested by increased complications. Treatment times being individualized, dialyzer configurations (single and dual dialyzers) and blood flow were adjusted to match the small and middle molecule clearances of S_{Ac}. Once shortened schedules (170 ± 7 min) were established, a 2 mo. surveillance period was initiated. Patients were then switched to bicarbonate therapy for 2 mo., everything else remaining constant. The data for S_{Ac}, rapid acetate (R_{Ac}) and rapid bicarbonate (R_{Bi}) therapy is shown below:

	S_{Ac} (N=191)	R_{Ac} (N=301)	R_{Bi} (N=301)
Hypotension (%)	17	21	12+
Vomiting (%)	8	10	1+*
Nausea (%)	16	20	5+*
Headache (%)	6	6	2+*
ΔWt (kg)	2.1	1.6*+	2.2

(*$p < .05$ relative to S_{Ac}, +$p < .05$ R_{Bi} vs R_{Ac})

Without sacrificing therapy adequacy or patient comfort and with stable serum chemistries we have successfully reduced treatment time by 33%.

EVALUATION OF HEPATITIS B VACCINE (HEPTAVAX-B IN HEMODIALYSIS PATIENTS. Eugene E. Cunningham, Mary T. Pasko*, William R. Bartholomew*, Thomas R. Beam, Jr.*, Daniel S. Camara* and Daniel Amsterdam*. SUNY at Buffalo, Depts. of Med. & Microbiol., Buffalo, New York.

A total of 111 hemodialysis (HD) patients were screened for Hepatitis B surface antibody (HBsAb) at the Erie County Medical Center and Veterans Administration Hospital in Buffalo, New York from January, 1983 to July, 1984. Thirty-one patients (28%) were found to have HBsAb present. This compares with the finding of HBsAb in 30 (7%) of 420 employees screened. A total of 42 patients received 3 doses (40mcg/dose) of the Hepatitis B vaccine (Heptavax-B). Of these, 18 responded to the vaccine by developing HBsAb. Of the responders, 6 had a progressive loss of antibody within 6 months following completion of vaccination. Two other patients subsequently became negative. Among the non-responders, 11 received a 4th dose of vaccine 6 months after the 3rd. One of these patients developed a transient antibody response, while the others failed to respond. To date, 276 employees have received the full course of vaccine (20 mcg/dose). Of these 21 (8%) failed to respond. Hepatitis B vaccine appears to be much less effective in HD patients than in employees (42% vs 92% conversion rate). Periodic surveillance is necessary to detect loss of antibody response in HD patients. The reason for the diminished response rate in HD patients is not known. The long term effectiveness of the vaccine in HD patients remains to be determined.

STREPTOKINASE TREATMENT FOR THROMBOSED AV FISTULAE (AVF)

Kevin M. Denny*, Robert A. Graor*, Emil P. Paganini, Barbara Risius*, Saturo Nakamoto, Victor Klimas, Jess R. Young*. Cleveland Clinic, Cleveland, OH.

AVF thrombosis usually results in loss of the access site and need for percutaneous support and reconstruction of a new fistula. Salvage of the thrombosed AVF may avoid these events. Attempts at reopening thrombosed fistulae were undertaken in 70 chronic hemodialysis patients. Low dose streptokinase (10,000 U/hr) was continuously infused directly into the thrombosed AVF.

57 patients presented within 4 days of thrombosis and successful lysis occurred in 87%. 23% of the remaining patients lysed when the clot was older than 4 days ($p < .001$).

Systemic effects of the infusion were noted in all patients. Serum fibrinogen levels fell by greater than 50% in all patients and thrombin times were prolonged to greater than 1 and 1-1/2 times control in 89% of patients. Serious complications were noted in 2 patients. Venous anastomosis stenosis was noted in 92% of patients and found to be correctable by surgical revision or transluminal angioplasty.

The study demonstrates the effectiveness of low dose streptokinase infusion for salvage of thrombosed AVF's.

VASORELAXANT EFFECTS OF PYRUVATE, LACTATE, ALANINE AND OTHER INTERMEDIARY METABOLITES.

J.T. Daugirdas, Z.M. Nawab*, D.J. Leehey, T.S. Ing. Hines-Loyola Medical Center, Hines, IL.

To help clarify the mechanism of acetate-induced vasorelaxation, the vasorelaxant potencies of similar compounds (lithium acetoacetate, sodium malonate, sodium propionate) and of unrelated intermediary metabolites (sodium pyruvate, sodium lactate, alanine) were compared to that of sodium acetate in a rat tail artery helical strip preparation. Vessel strips were first constricted using a submaximal dose of arginine vasopressin (3×10^{-9} M). Sixteen mM of the compound to be tested was then added, and relaxation was measured 5 minutes later. To control for increases in bath sodium level and osmolality, additional experiments were performed with addition of 16 mM NaCl.

Substantial vasorelaxant effects were demonstrable with sodium acetate (relaxation 65 ± 22 %), sodium propionate (80 ± 23) and sodium malonate (47 ± 22%). Lithium acetoacetate, however, had minimal vasorelaxant effect (5 ± 8.3%). Further studies in baths containing a constant level of lithium demonstrated that the lack of acetoacetate effect was not due to the lithium cation. Modest to absent vasorelaxant potencies were demonstrated by sodium lactate (19 ± 18%), sodium pyruvate (15 ± 21%) and alanine (-5 ± 19%). Relaxation with sodium chloride (controls) was 10 ± 19%)

The results suggest that the vasorelaxant effects of acetate are shared by certain chemically similar compounds. Sodium pyruvate, sodium lactate, and alanine evidenced minimal vasorelaxant effects. Pyruvate and lactate may be useful as bicarbonate-generating dialysate bases.

INCREASED STAINABLE BONE ALUMINUM (AL) DEPOSITION AFTER SUBTOTAL PARATHYROIDECTOMY (PTHx) IN DIALYSIS PATIENTS (PTS). M.C. de Vernejoul*, S. Marchais*, G. London*, J. Bielakoff*, and L. Miravet*. Hospital Lariboisiere, Paris, France (Intr. F. Llach).

An association between AL overload and dialysis osteomalacia has been reported. To test if AL may deposit in bone as the result of a decrease in parathyroid hormone (PTH), 10 dialysis pts were studied; they had bone biopsy prior to and 19±9 months after PTHx; age ranged from 24 to 70 years; duration of dialysis from 2 to 10 years. After PTHx serum Ca and P remained unchanged, alkaline phosphatase decreased from 2683±1441 to 1291±751 nkat/l ($p<0.001$), and intake of AL-containing binders was significantly decreased. Significant changes ($p<0.01$) in bone histomorphometric parameters after PTHx were: resorption surface from 6.6±2.6 to 1.5± 1.6%, osteoblastic surface (OBS) from 16±8 to 4± 5.7%, mean osteoid thickness from 11.8±3.4 to 8.8± 3.2 µm, double labeled surface from 18.7±10.5 to 5.3±8.8%, formation at tissue level from 0.333±0.25 to 0.098±0.09 $\mu^3/\mu^2/d$, AL stained surface (ALS) from 11.6±17.1 to 31.8±29%, and AL stained trabecular bone (TAL) from 0.69±0.79 to 1.20±0.95 mm/m^2. There was no significant relation between bone resorption or formation and PTH. Pre and post PTHx OBS and ALS were negatively correlated ($p<0.01$); so were ALS and TAL ($p<0.01$).

In summary, in dialysis pts after PTHx: a) bone formation decreased; b) stainable AL deposits were enhanced, and c) pre-PTHx AL deposits were a major factor in the post PTHx AL load.

In conclusion, in dialysis pts, PTHx may, by inducing low bone remodelling, facilitate bone AL deposits; however, it is not clear if such deposits play a role in observed bone changes.

STAPHYLOCOCCUS AUREUS CARRIAGE IN HEMODIALYSIS PATIENTS. Stephen F. DeWitt*, John P. Speck*, Milagros P. Reyes*, Franklin D. McDonald. Wayne State University School of Medicine, Detroit, MI

One hundred eleven chronic outpatient hemodialysis patients had serial nasal and skin cultures to identify carriers of Staphylococcus aureus. Forty two patients (37.8%) were identified as chronic carriers; 22% of the personnel (9/32) had a single positive nasal culture for S. aureus. In a prospective, double blind manner, carriers were randomized to receive rifampin 600 mg/day (22 patients) or placebo (16 patients) for 10 days. Follow up nasal and skin cultures at days 5 and 10 of therapy and 4,8,and 12 weeks post therapy revealed significant reduction ($P<.05$) in the number of culture positive patients in the rifampin treated group at each interval. Fifty percent of the rifampin treated group was still culture negative at 12 weeks. Two rifampin treated patients (9%) developed rifampin resistant S. aureus post therapy: their MICs pre-therapy were $\leq .015$ mcg/ml and post therapy >8 mcg/ml. Bacteriophage typing of all isolates showed phage types 95,96, 94/96, 29/52, and 29/52/80 to be the most common. Rifampin is effective in eliminating nasal and skin carriage of S. aureus in chronic hemodialysis patients; however, rifampin resistance may develop.

ERYTHROPOIETIN LEVELS IN ESRD BY ENZYME IMMUNOASSAY. Charles J. Diskin, K.L.O. Jones,* and E.C. Mora.* Auburn Univ., Auburn, Alabama.

Deficiency of erythropoietin production by the ESRD kidney is one of the putative reasons for the anemia of chronic renal failure. However, conflicting reports of erythropoietin levels and response to transfusion have led to confusion concerning the contribution of erythropoietin deficiency to the anemia. Much of the confusion arises from the inaccuracy and insensitivity of bioassays and hemagglutination inhibition assays. The radioimmune assays often are difficult to perform with complex and expensive equipment required to read the results. We therefore investigated a new, accurate and more easily performed double sandwich solid phase enzyme immunoassay (EIA) on serum erythropoietin levels in 7 transfusion dependent and 5 transfusion independent patients undergoing chronic maintenance hemodialysis. All patients with possible secondary causes of anemia were excluded. Erythropoietin levels were found to be elevated in all patients and were not statistically different in transfusion dependent ($\bar{x}$ 205 ± 77.1 SD) vs transfusion independent ($\bar{x}$ 160 ± 98.8 SD). Furthermore, there was no suppression of erythropoietin levels by transfusion ($\bar{x}$ 232 ± 76.6 SD). There was no increase in erythropoietin levels in patients given androgens. There was no correlation with the level of hematocrit. We conclude that deficiency of erythropoietin is not a major cause of anemia in ESRD and suppression of erythropoietin is not routinely accomplished by transfusion. Any increase in hematocrit effected by androgens is not mediated through erythropoietin. EIA is an accurate and inexpensive method of measuring erythropoietin.

DEVELOPING A WORKABLE CASE-MIX INDEX THAT RELATES TO COST: EXPERIENCE FOR PATIENTS WITH CHRONIC RENAL FAILURE. Louis H. Diamond, Philip J. Held, and Mary Jo Palumbo. Georgetown Nephrology Section, D.C. General Hospital, Georgetown School of Medicine; and The Urban Institute, Washington, D.C.

Are there differences in patient mix among the different types of dialysis settings? Do hospital units dialyze a more complex diagnostic group of patients than do the freestanding dialysis facilities? These are the questions analyzed in this paper.

Medicare claims forms data including diagnostic information from 1977-1981 were reviewed on 66,700 patients across the U.S. Changes in patient mix were examined over time and across dialysis unit type. Measures of patient mix include six diagnostic groups based on expected cost and risk, patient age, sex, race, and length of time since renal failure.

Findings reveal a dramatic increase in patient complexity over period. Aside from clear differences in racial mix divergences across dialysis unit types were slight. Greater variation was exhibited among the dialysis units within a given type of setting than was observed across dialysis unit type.

The quantitative impact of these patient groupings on reimbursement and costs will also be presented.

HEMODIALYSIS (HD) WITH EPOPROSTENOL (PGI_2):SAFETY AND EFFICACY OF BICARBONATE (B) VERSUS ACETATE (A) DIALYSATE. A. Dubrow†, N. Mittman, J. Crow†, A. Cato†, M. Lifschitz, W. Flamenbaum, Beth Israel Medical Center, New York, NY, Wellcome Research Labs, Research Triangle Park, NC, Univ. of Texas Health Science Center, San Antonio, TX

Seven patients underwent 2 randomized sequences of HD with A or B. The initial HD was with heparin (H); subsequent HD's used constant infusions of PGI_2 at 4 and 2 ng/kg/min. Efficacy was assessed using decrement in serum creatinine (Scr) or BUN concentrations. Safety was based on maximum change from baseline in diastolic blood pressure (DBP) and heart rate (HR). Results are presented as mean and range. Comparable changes were observed at both PGI_2 doses; only results using the higher dose are presented.

	A		B	
	H	PGI_2	H	PGI_2
BUN (mg/dl)	-31	-35	-35	-34
range	(9-46)	(22-56)	(28-48)	(17-53)
Scr (mg/dl)	-4.5	-6.1	-5.1	-4.8
range	(1.3-6.2)	(3.6-9.8)	(3.3-6.6)	(3.5-6.3)
DBP (mmHg)	-14	-18	-11	-18
range	(5-33)	(8-31)	(0-30)	(1-45)
HR (bpm)	+7	+13	+8	+5
range	(0-19)	(0-31)	(0-27)	(0-21)

No significant differences were observed in any of the parameters comparing A and B. No HD was stopped due to side effects with PGI_2 or H, using A or B. No side effect was clinically significant.

HD may be performed safely and effectively using PGI_2 and A or B. The cardiovascular instability in prior HD using PGI_2 and A may be related to a lower dialysate [Na+], since the present study used a dialysate [Na+] of 139-140 mEq/l.

CAPTOPRIL MONOTHERAPY IN HEMODIALYSIS PATIENTS. K. L. Duchin, R. M. Hakim, T. P. Parker, J. M. Lazarus, and L. Husni*. E. R. Squibb & Sons, Brigham and Women's Hospital and Dallas Kidney Disease Center, Princeton, New Jersey, Boston, Massachusetts, and Dallas, Texas.

This study was designed to determine the efficacy and safety of captopril in hypertensive patients maintained on chronic hemodialysis. Following a dose-titration period of 1-4 weeks, 21 patients received captopril at an average dose of 18.75 ± 3.3 mg t.i.d. of captopril (range: 6.25-50 mg) for 4.5 ± 0.3 weeks (range: 3-7 weeks). Predialysis sitting blood pressures were not significantly different during captopril (152 ± 4/87 ± 2 mmHg) compared to values (153 ± 4/89 ± 3 mmHg) obtained one month prior to captopril on other antihypertensive agents (1.4 ± 0.1 drugs/patient). Post-dialysis sitting blood pressures were similar before (136 ± 5/80 ± 2 mmHg) and during captopril administration (134 ± 4/79 ± 2 mmHg). The average interdialytic weight gain was identical before (2.9 ± 0.2 kg) and during (2.9 ± 0.2 kg) captopril therapy. Three patients had a mild decrease in leukocyte counts; however, the mean decrease in the whole population was 11%. The clearance of captopril by dialysis averaged 59.7 ± 5.4% of urea clearance and was 120 ± 10 ml/min. Minimum steady-state blood levels of unchanged captopril were proportional to the oral dose ($r=0.94$, $p<0.001$). During the study, transient minor adverse reactions included rash and taste disturbance in 2 patients. These data indicate that monotherapy with captopril provides effective blood pressure control in hemodialysis patients comparable to multiple-drug therapy.

ALUMINUM CAUSES NOT ONLY OSTEOMALACIA BUT ALSO OSTEOPENIA IN HEMODIALYZED PATIENTS. RM Friedler, MC Faugere*, HH Malluche, Univ. of KY, Div. of Nephrology, Bone & Min. Metab., Lexington, KY.

Aluminum (Al) induced osteomalacia has been extensively studied. The present study evaluates whether other bone changes ensue with bone aluminum accumulation (BAA). Bone biopsies and histochemical staining for aluminum (sBA) were done in 91 chronically dialyzed patients (pts). Fifty-two of these had sBA and 39 did not. Bone mass and parameters of bone formation and resorption were measured by histomorphometry. Bone mass was significantly lower in pts with sBA (19.3 ± 0.9 vs. 27.4 ± 1.6%). This was due to a decrease in osteoid-osteoblast interface (8.7 ± 1.7 vs. 31.7 ± 3.9%) associated with a disproportionately lesser decrease in bone-osteoclast interface (4.3 ± 0.6 vs. 9.3 ± 1.0%). Another 8 pts were studied who underwent two bone biopsies 11-16 months apart. All these pts had sBA at the first and/or second bone biopsy. On second bone biopsies there was a decrease in bone mass (mean difference: -19.4 ± 6.2%; $p<0.01$). Osteoid-osteoblast interface was also decreased (4.3 ± 1.1 vs. 9.8 ± 2.4%) associated with no change in bone-osteoclast interface (3.2 ± 0.7 vs. 3.9 ±0.7%). Another 7 patients were studied before and after 10 months of therapy with desferrioxamine for removal of Al from bone. Bone mass increased significantly in all these pts associated with reversal of sBA (22 ± 2.3 vs. 29 ± 3.2%; $p<0.01$).

These data show that Al does not only affect--directly or indirectly--the mineralizing function of osteoblasts, thereby inducing osteomalacia. It also depresses bone forming activity of osteoblasts resulting in osteopenia in hemodialyzed patients.

COMPARISON OF ACETATE (A) VS. BICARBONATE (B) HEMODIALYSIS (HD) IN ACETATE TOLERANT (T) OR INTOLERANT (I) PATIENTS. J. Fitzgibbons, G. Braden, M. Germain, Renal Service, Baystate Med. Ctr., Springfield, MA.

There is continuing controversy concerning the value of B HD. We carried out a double-blind crossover study on chronic HD patients who had been classified clinically as T or I based on previous HD treatments (rxs). With A HD, I patients developed symptoms (hypotension, headache, cramps, nausea, and vomiting) during 16 of 18 rxs, while with B HD symptoms occurred in only 5 of 17 rxs ($p < .01$). For T patients there was no difference in the frequency of symptoms between A HD and B HD. A Quality of Treatment Score (1-poorest, 10-best) was assigned to each rx by the patients.

	QUALITY OF TREATMENT SCORE	
	A HD	B HD
I	4.5 ± .8*	8.8 ± .4
T	9.6 ± .2	9.7 ± .2

*$P < .01$ compared with B HD and T patients on A HD.

With B HD the changes in Na, K, Cl, and HCO_3 occurring during the rx were not different for I vs. T patients. However, during A HD:

		Before	After
I	HCO_3 mEq/L	20.1 ± .7	16.9 ± .9*
T	HCO_3 mEq/L	19.6 ± 1.0	20.0 ± .7

*$P < .01$ compared with before HD.

CONCLUSION: 1) Some HD patients can be classified as acetate I on the basis of the frequency of symptoms and lower Quality of Treatment Scores during A HD. 2) I patients decrease their HCO_3 during A HD which may be due to impaired A metabolism.

MAXIMAL EXERCISE (ME) DURING HEMODIALYSIS (HD): PHYSIOLOGIC EFFECTS. M. Germain, E. Burke*, J. Fitzgibbons, G. Braden, Renal Service, Baystate Med. Ctr. and Springfield College, Springfield, MA.

Exercise training has been reported to have beneficial effects in HD patients. A ME test should be done prior to initiating an exercise program. This study investigates the feasibility and physiologic effects of ME during the HD treatment in 4 stable chronic HD patients. This is compared to ME on a non-HD day. Ventilation ($\dot{V}_E$), respiratory gases ($\dot{V}CO_2$, $\dot{V}O_2$), systolic BP (SBP), pulse (HR), electrolytes, ABG's, and blood lactate levels were measured. Maximal work capacity (MWC) was determined.

*$P < .05$	OFF HD	ON HD
SBP	214 ± 24	177 ± 21*
HR	148 ± 6	148 ± 13
Max. $\dot{V}O_2$ (ml/kg/min^{-1})	18 ± 3.0	14 ± 2.3
K+ (mEq/L)	5.9 ± .3	4.2 ± .6
Lactate (mmol/l)	9.7 ± 1.6	8.4 ± 1.6
pH	7.29 ± .04	7.37 ± .02*
PO_2 (mm/hg)	108 ± 3	117 ± 7
PCO_2 (mm/hg)	31 ± 2	28 ± 1*
HCO_3 (mEq/L)	11.9 ± 1.7	13.5 ± 1.0
MWC (kg/m/min^{-1})	662 ± 129	537 ± 121*

1) ME can be carried out during the HD treatment without adverse effects. 2) Since MWC is greater off HD, exercise prescriptions on HD are more appropriately based on a ME test during HD. 3) Hyperkalemia and acidemia occur during ME off HD. These potentially dangerous effects may be avoided by carrying out ME during HD. 4) Despite the lack of acidemia and hyperkalemia with ME during HD, MWC was greater off HD. Further studies are necessary to investigate the cause of decreased MWC during the HD treatment.

INTENSIVE DIALYSIS OF NO VALUE IN ACUTE RENAL FAILURE (ARF). D.M. Gillum* and J.D. Conger. Dept. of Med., Univ. of Colorado, Denver, CO.

Previous reports comparing different time periods and a single prospective study with a small patient population (Conger, J. Trauma, 1975) suggested that daily dialysis improved survival in ARF. Since these reports, intensive dialysis in ARF has become a common practice. Because of the deficiencies of previous studies, we questioned the validity of this practice. Thus the following prospective study was performed. During a 3-yr period, 34 medical-and-surgical-related ARF patients requiring dialysis (Scr < 8 mg/dl) were paired on the basis of specific underlying disorder or operation-injury. One patient of each paired was treated with sufficient dialysis to maintain predialysis Scr < 5 mg/dl (A). The other paired patient was dialyzed when Scr reached 9 mg/dl (B). Predialysis Scr were 5.3 ± 1.4 and 9.1 ± 1.3 mg/dl in A and B, respectively ($p<.001$). HCO_3, pH and Hct were lower and PO_4 higher in B. Duration of treatment, caloric and protein intake, serum antibiotic levels and complications were not different. Survival rates, as determined by X^2 analysis for paired proportions, were not different for A and B in either medical or surgical categories:

Outcome	Medical		Surgical		Total	
	A	B	A	B	A	B
	(8)	(8)	(9)	(9)	(17)	(17)
Lived	5	5	2	4	7	9
	63%	63%	22%	44%	41%	53%
Died	3	3	7	5	10	8
	37%	37%	78%	56%	59%	47%

Within the limits of this study it is concluded intensive dialysis is of no clinical benefit in ARF.

COMPLEMENT ACTIVATION IN FIRST-USE AND HYPERSENSITIVE HEMODIALYSIS PATIENTS. Raymond M. Hakim[1], Julian Breillatt[2],* Friedrich Port[3], Douglas Fearon[1],* and J. Michael Lazarus[1]. [1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]E.I. Dupont, Wilmington, DL; [3]Veterans Administration Hospital, Ann Arbor, MI.

We investigated complement activation during hemodialysis using new cuprophane membranes in six patients known to have recurrent first-use syndrome (FUS) and compared then to 10 other hemodialysis patients without clinical symptoms. FUS patients had maximum complement activation at 10 minutes following initiation of dialysis, with mean C3a desArg levels = 8533 ± 157 ng/ml, whereas the maximum complement activation in asymptomatic patients occurred at 15 minutes and was only 2907 ± 372 ng/ml ($P \leq 0.0001$). In-vitro complement activation with zymosan also showed a significant difference in C3a desArg levels between the two groups of patients. At a concentration of 3.8×10^{-5} gm/ml of zymosan, FUS patients had C3a desArg levels of 29.6 ± 1.4 μg/ml, whereas it was only 16.6 ± 2.3 μg/ml for asymptomatic patients ($P \leq 0.001$). Three other patients with anaphylactic response within minutes of initiation of dialysis had C3a desArg levels of 18,900 ng/ml, 7800 ng/ml, and 34,000 ng/ml in the efferent line. C3b INA, a regulatory protein of the alternative pathway, was significantly less in FUS patients than in asymptomatic patients (252 ± 37 vs. 351 ± 38, $P \leq 0.05$). No difference between the two groups of patients was seen in the mean levels of βlH, Factor B, or carboxypeptidase enzyme activity.

MEASUREMENT OF WHOLE BODY ULTRAFILTRATION (Kf) AND UREA MASS TRANSFER (KU) COEFFICIENTS: IMPLICATIONS FOR Na & U MODELING IN DIALYSIS. Frank Gotch, Fred Heineken*, Marcia Keen*, & Mary Evans*. Franklin Hospital, San Francisco, CA & Cobe Laboratories, Lakewood, CO.

Sizeable U & Na osmotic gradients may develop between intra and extracellular water (Vi,Ve) causing water shift to Vi and dialysis morbidity. Modeling optimal dialysate Na (CDNa) and/or use of mannitol (M) to prevent H2O shift requires values for Kf & KU. We measured Kf & KU in 10 dialysis patients. Dialyzer ultrafiltration rate and CDNa were adjusted to selectively remove either 1 L. of pure H2O or 150 mEq. of pure Na over 30 min. after which the dialyzer was in bypass for 30 min. equilibration (E) period. Blood U & Na (CBNa) were measured every 5 min. during H2O or Na removal and E. A 2 compartment model was used to calculate Vi,Ve,Kf & KU and total body water (VT) was determined from prior urea kinetics.

The results in 10 patients were (M+SE): VE/VT= .277±.017; Kf=.195±.004 ml/min/mmHg; KU=886± 129 ml/min. The KU agrees well with prior reports. Kf has not been previously reported to our knowledge and is surprisingly low, compared to 5 ml/min/mmHg reported for transcapillary ultrafiltration coefficient.

Use of these parameters to individualize CDNa or M will be presented. For the average patient dialysis, CDNa 6 mEq/L>CBNa during first hour followed by CDNa 1 mEq/L>CBNa remainder of dialysis results in constant Vi. Current recommended M doses appear to be far in excess of those required to achieve constant Vi and would appear to result in cell dehydration.

DESFERRIOXAMINE (DFx) CHALLENGE TEST FOR CHELATABLE IRON (Fe) BURDEN IN LONG-TERM DIALYSIS (HD) PATIENTS. R.M. Hakim, M. Fosberg*, G. Schulman*, J. Stivelman*, L. Wolfe*, W. Harmon &J.M.Lazarus. Brigham and Women's Hospital & Children's Hospital Medical Center, Boston, Massachusetts.

Chronic transfusion therapy for anemia in long-term HD patients results in pathologic Fe accumulation in viscera and may contribute to Fe deposition in the calcification fronts of bone. We have found that the amount of ferrioxamine (Fx) (the Fe-chelated form of DFx) formed 60 mins after 2 gm DFx infusion at the end of HD correlated with total body Fe burden as documented by transfusion history. In 14 chronically transfused, Fe-overloaded adult and pediatric HD patients, total Fe burden was 909±447 mg/Kg; serum ferritin 8776 ± 5304 ng/ml. Patients received 2 gm or 25 mg/Kg DFx over 15-20 min immediately post HD. Two distinct responses to a single dose were observed at one hour post infusion; one in which a substantial amount of Fx appeared in the serum (n=9) and a second in which little Fx appeared (n=5) ($p < .0005$). Serum response to DFx challenge correlated well with cumulative Fe burden ($r=.9295$), as documented by transfusion history and with dialysate Fe excretion ($r=.7995$), but was independent of DFx dose provided the dose exceeded 25 mg/Kg. Following second or third dose of DFx, DFx and Fx serum concentration reached a stable plateau. We conclude that the 60 min post-DFx Fx level identifies those HD patients with sufficient Fe overload to excrete Fe in response to DFx therapy.

ALTERATIONS IN THE CONTACT PHASE OF COAGULATION DURING HEMODIALYSIS. Raymond M. Hakim, Cynthia Perzanowski,* and Jocelyn Spragg.* Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

The residual capacity of plasma to activate kallikrein as a measure of activation of the contact phase of coagulation was studied prospectively in seven chronic hemodialysis patients. Patients were dialyzed for one month each on first-use hollow fiber membranes of three types: cuprophane (C), cellulose acetate (CA), and polymethylmethacrylate (PMMA). Blood samples were obtained pre-dialysis and simultaneously from the efferent and afferent blood at 15 and 90 minutes into dialysis. Samples were collected into citrate solution without glass contact, centrifuged and frozen at -70°C within 5 minutes. After cold activation in dextran sulfate, plasma samples were assayed in duplicate for cleavage of H-D-Pro-Phe-Arg-p-nitroanilide (S-2302). Spontaneous enzymatic activity was not observed. The mean residual capacity of plasma to activate kallikrein showed a significant decrease with CA and PMMA, but not with C membrane dialyzers 15 minutes after initiation, but was not significantly different from pre-dialysis at 90 minutes for any dialyzer. There was no statistically significant difference between efferent and afferent samples. These findings suggest that circulating components of the contact phase of coagulation are depleted early during hemodialysis with CA and PMMA, but not with C membranes. Subsequently, they may be released from the C membrane or from platelet sources. These findings are consistent with the relative inability of CA and PMMA membranes to activate complement and platelets as compared to C membranes.

PRESCRIBING DIALYSATE BICARBONATE CONCENTRATION (DBC) FOR HEMODIALYSIS PATIENTS **F. Heineken, M. Brady-Smith, J. Haynie, J. Van Stone. Cobe Labs., Lakewood, CO. Univ of Missouri, Columbia, MO.**

The following rearranged equation of Sargent and Gotch was used to determine DBC: $C_{Di}=(J+Q_F C_B)/(D(1-Q_F/Q_B))+C_B$ where C_{Di} is the prescribed inlet DBC, J is the acid generated between dialysis treatments, Q_F is the ultrafiltration rate, D is the HCO_3 dialysance, Q_B is the blood flow rate and C_B is a targeted serum HCO_3 concentration (SBC). Nine patients were studied over a 35 week period to verify this method of determining DBC. Urea kinetics were used to estimate the acid generated, urea clearance and hematocrit were used to estimate the HCO_3 dialysance and the targeted SBC was chosen to be 25 mEq/l. Prescribed DBC for the nine patients varied from 29 to 38 mEq/l with five patients having a prescribed DBC of 35 mEq/l. After a baseline period of five weeks, four patients (Group A) were switched from acetate to their prescribed DBC while five patients (Group B) who were already on HCO_3 dialysis at a DBC of 35 mEq/l were switched to their prescribed DBC. Patients were then followed for a study period of 30 weeks.

Results (Pre & Post Dialysis Blood Chemistries)

	HCO_3		pH		pCO_2		pO_2	
Group A	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Baseline	17	20	7.34	7.38	32	34	115	100
Study	20*	25*	7.35	7.44*	37*	37*	100*	97
Group B								
Baseline	21	25	7.38	7.45	35	36	92	88
Study	21	25	7.34*	7.42*	40*	39*	87	86

***P<.01 for Study versus Baseline**

Conclusions: The equation used to determine DBC results in more normal acid/base chemistries and shows that patient DBC needs are variable.

SYSTEMIC ABSORPTION OF ORAL VANCOMYCIN IN HEMODIALYSIS PATIENTS WITH ANTIBIOTIC ASSOCIATED COLITIS. C.E. Halstenson*, A.J. Collins, P.L. Olson*, G.R. Matzke. Hennepin County Medical Center, Minneapolis, MN.

The systemic absorption of orally administered vancomycin (V) was evaluated in four hemodialysis (HD) patients (pt) with documented antibiotic associated colitis (AAC) (i.e. positive stool culture for Clostridium difficile). The patient's age and weight ranged from 32 to 63 years [46±13 years (mean ± SD)] and 52.5 to 76.2 kg (68.1 ± 10.6 kg), respectively. V doses were 125 mg (1 pt), 250 mg (2 pt) and 500 mg (1 pt) every 6 hours. Multiple serum concentrations (SC) were obtained (range 4 to 19) during the 7 to 28 day course of V therapy. V SC were determined in duplicate by fluorescence polarization immunoassay. Systemic absorption of V was observed in three of the four patients. In these three patients V SC were rapidly attained and plateaued within 4 days. V SC were detectable as soon as 2 hours after the first dose and increased to a maximum of 2.6, 6.4 and 12.8 mg/l. An apparent linear relationship between dose and peak V SC achieved after two days of fixed dosing (r^2=0.84, p 0.05) was also observed. Oral V dosing with 500 mg every 6 hours is projected to produce V SC of 11-13 mg/l in HD pt with AAC. V SC in this range have not been associated with toxicity. Potential toxic SC have been observed (Int J Ped Neph 1983;4:1-4). Monitoring of V SC in HD pts receiving oral V is recommended.

CARDIAC RISKS IN PATIENTS WITH CHRONIC RENAL FAILURE. B. Höfling, E. Erdmann, R. Hässler, L. Castro, H. Hillebrand, H.J. Gurland. (intr. by G. Schreiner). Klinikum Grosshadern, Universität München, FRG.

Patients (pts) with end-stage renal failure (CRF) have a high cardiovascular mortality. A prospective differential survival-analysis with respect to coronary (CHD)- and valvular heart disease has not been demonstrated so far.
We performed cardiac catheterisation and coronary angiography in 77 pts with CRF and clinical symptoms for heart disease. The patients were followed up an average period of 1.6 years. In 49 pts we found coronary stenoses over 50%.
1.6 years after catheterisation 7 of these 49 pts had died (=14%), in 5 cases from myocardial infarction. In 17/77 pts hemodynamically significant valvular heart disease could be identified, which was not apparent before catheterisation in 9 cases. After 1.6 years 6 of those 17 pts had died (=35%) due to complications of their valvular disease. In 18/77 pts we could not demonstrate either CHD or valvular heart disease; only one patient had died after 1.6 years.
We conclude, that the mortality due to valvular heart disease was twice as high as that due to CHD. The frequency and severity of valvular heart disease is clinically often underestimated in these patients because of regular fluid withdrawal by dialysis.

ELEVATED BASILIC VEIN A-V FISTULA INSTEAD OF PTFE GRAFT. Arthur Humphries, William Hamilton,* Doug Rives,* Rao Kondur,* Gene Colburn,* Robert Nesbit,* and Charles Wray.* Depts. of Surg., Anesth., and Anat., Medical College of Georgia, Augusta, GA.

In 25 patients without suitable superficial veins we have created A-V fistulas in the upper arm elevating the basilic vein subcutaneously. The brachial vein was utilized if it was larger than the basilic. Mobilization of the vein from antecubital space to axilla was similar to that described by Dagher (J. Surg. Res. 20:373, 1976). The new feature of our operation is that the vein is not rerouted through a subcutaneous tunnel. Instead after the end of the vein is anastomosed to the side of the distal brachial artery, interrupted sutures are placed in Scarpa's Fascia under the vein to elevate it, and the skin is stapled immediately over it. Each incision healed quickly. The veins could be used in 2 to 3 weeks. No chronic arm swelling has occurred. One patient with nephrotic syndrome leaked lymph from the incision for 3 weeks. Entrapment of the large vein between thumb and forefinger permitted easy introduction of needles. The operation is simpler than Dagher's and results in a longer (approx. 13cm), straighter, more uniformly superficial segment of vein. The operation is simpler than use of PTFE and less likely to cause a steal syndrome. In patients requiring dialysis before the basilic vein is mature, a subclavian vein cannula is left inlying. Although a PTFE graft might be usable sooner, the lower risk of infection and expected better patency leads us to prefer the elevated basilic vein.

MORBIDITY AND MORTALITY IN A TWICE-WEEKLY CHRONIC MAINTENANCE HEMODIALYSIS PROGRAM, John Josselson, Matthew R. Weir, John H. Sadler, Univ. of Maryland Hosp., Div. of Nephrology, Baltimore, MD.

We retrospectively evaluated morbidity and mortality in our twice weekly(2W) chronic hemodialysis(HD) program from 1/1/78 through 12/31/82. 100 patients were in the program for at least 3 months after 1/1/78. Average time on HD was 28.1 months (range 1-79) at 1/1/78. 72 remained only on 2WHD; 8 moved to other locations; 20 changed modalities to CAPD or transplantation(TP) (8 CAPD; 16 transplants in 14 patients). The study covered 4,600 patient(PT) months for an average of 46.3 months/PT. Five year survival was 73%. For those patients changing modality survival was 85%, possibly reflecting their lower age at starting HD (33 vs 43.4 yrs overall). No relationship to disease category, sex or race was significant. Mortality data were complete.

Morbidity was reported as hospitalizations(HP), transfusions(TX) and access replacement(AR). Data were complete on more than 90% of patients. Patients undergoing CAPD or TP had more HP(8.2/5 yrs for 85 days/PT/ 5 yrs vs 3.5/5 yrs for 34 days/PT/ 5 yrs for the 2WHD. Of 72 patients only on 2WHD, data were 99% complete. 20% had no HP; 54% had no TX; 60% had no AR during the period studied. AR for that group averaged .9/PT/5 yrs. TX were 11.2 units/PT/5 yrs or 23.4 units each for 32 patients who received blood.

Our survival data for 2WHD are comparable to those reported for 3WHD. Comparable morbidity data are generally not reported, however we believe that our results indicate that dialysis frequency is not a critical factor in determining morbidity and mortality on HD.

ABSENCE OF TACHYPHYLAXIS TO ACETATE IN A VESSEL STRIP PREPARATION. T.S. Ing, J.T. Daugirdas, Z.M. Nawab*, D.J. Leehey, M.A. Klok*. Hines-Loyola Medical Center, Hines, IL.

Hypotension associated with intravenous acetate infusion tends to be maximal during the first few minutes, suggesting the possibility of tachyphylaxis. To assess tachyphylaxis, helical strips (n=27) cut from rat tail arteries, incubated in a bicarbonate-buffered bath (B) were sequentially constricted four times at 45-minute intervals using a submaximal dose (2×10^{-9} M) of arginine vasopressin. Strips were randomized into 3 groups. In group X, all constrictions (C) were performed with the bicarbonate-buffered bath (B). In group Y, C#1 was done in (B), but immediately before C#2, 16 mM of chloride in the bath were replaced by an equal amount of acetate (A). (A) remained in the bath thereafter. In group Z, (A) was used as in group Y, but was removed from the bath just prior to C#4. Results (expressed as hundredths of a gram tension) were:

	C#1	C#2	C#3	C#4
X (B1,B2,B3,B4)	74±24	76±25	74±26	72±29
Y (B1,A2,A3,A4)	73±13	46±23	47±23	45±23
Z (B1,A2,A3,B4)	69±12	51±20	53±15	70±17

With acetate in the bath, reduction in tension (C#2 vs C#1) was highly significant ($p < 0.01$). In group Y, there was no attenuation of the acetate effect during incubation times of 0 (C#2), 45 (C#3), and 90 (C#4) minutes. In group Z, removal of acetate from the bath just prior to C#4 resulted in full restoration of tension.

The findings suggest that (a) there is little tachyphylaxis to acetate over a 90-minute period, and (b) on removal of acetate, its vasorelaxant effect is quickly dissipated.

THE EFFECT OF PREDILUTION DURING CONTINUOUS ARTERIO-VENOUS HEMOFILTRATION (CAVH). Andre A. Kaplan* (intr. by P.R. Steinmetz). Univ. of Conn. Health Ctr., Division of Nephrology, Farmington,CT

The predilution mode (replacement fluid infused before the filter) has been shown to increase net urea clearance with machine-driven hemofiltration. To document the net effect of predilution during CAVH, an 8 hour crossover study was performed. Replacement fluid infusions were kept constant at 400 ml/hr and the mode of replacement (predilution or postdilution) was changed each hour: Mean postdilution output was 512±12 ml/hr (SEM) (n=4), mean predilution output was 600±20 ml/hr (n=4). Outputs were significantly different ($p<0.02$). The 17% increase in output was associated with only a 6% dilution in filtrate urea, giving a net increase in urea clearance.

To investigate the mechanism of this increase in urea clearance, 13 paired samples of prefilter plasma and filtrate were analyzed for % dilution of urea and creatinine. The mean urea dilution of 18±3% was significantly less than that of creatinine: 22±2% ($p<0.01$). Since intraerythrocytic urea equilibrates with plasma far more rapidly than creatinine (Katz and Hull,Nephron 12:171-177,1974) these data suggest that the predilution mode increases net urea clearance by causing shift of red cell urea into the plasma compartment, making it available for filtration. The increased dilution of creatinine may reflect its decreased permeability across red cell membranes.

In conclusion, predilution was found to increase net urea clearance during CAVH. This increase may reflect a shift of red cell urea into the plasma compartment.

HEMODIALYSIS UREA KINETICS IS NOT SINGLE POOL. P. Keshaviah*, K. Ilstrup*, W. Shapiro*, G. Hanson*. (intr. by A. Collins) Hennepin County Medical Center, Minneapolis, Minnesota.

Urea kinetics has become an established tool in hemodialysis. The underlying premise is that urea obeys single pool kinetics with a distribution volume similar to total body water (TBW). Urea kinetic calculations were performed on 12 hemodialysis patients based on urea removal by total dialysate collection. The urea distribution volume (V_u) was only 36% of body weight, significantly less than TBW. V_u was also calculated using the traditional, but less accurate, A-V clearance method, to yield V_u of 51% of body weight. In order to elucidate these discrepancies, we studied 3 acutely uremic dogs. ^{14}C urea and ^{3}H water were administered pre-dialysis to establish their volumes of distribution. The dogs were then dialyzed for 3 hours, clearances being scaled to body weight. ^{14}C urea clearances and the time course of urea removal were determined, along with total dialysate collection. The data is shown below expressed as % body weight:

Dog	TBW (^{3}H)	V_u(^{14}C)	V_u(Kinetic)
1	70	68	58
2	73	79	65
3	71	77	64
Mean ± SE	71 ± 0.9	75 ± 3.4	63 ± 2.2

V_u (^{14}C) urea is similar to that of TBW, but during dialysis, urea removal is from a ^{14}C volume that is 16% smaller. A significant ^{14}C urea rebound of 13% was noted post dialysis. The smaller V_u (Kinetic) and the post-dialysis rebound both indicate that urea obeys 2 pool rather than single pool kinetics.

HEMODIALYSIS CLEARANCE OF METRONIDAZOLE AND ITS METABOLITES by Alan Lau, Chih-Wen Chang* and Sandra Sabatini, Colleges of Pharmacy and Medicine, University of Illinois, Chicago, IL.

Metronidazole (M) is often used in septic patients with renal failure requiring hemodialysis (HD). We reported previously that M was removed by HD with a mean±SD clearance (Cl) of 107.0±16.3 ml/min for CDAK (Cordis Dow Corp.) dialyzers in 3 patients (12 sample pairs). However, Cl of the metabolites were not evaluated. We studied these parameters in 6 patients using TE (Terumo Corp.) dialyzers of comparable urea and creatinine Cl. Three patients received M (500mg IV q6h) for presumed sepsis. The other 3 volunteers received 1 dose of M (750 mg PO) 12 hours before and 500mg IV at the start of HD. Arterial and venous samples were obtained 1-4hr into HD to determine Cl. M and metabolite concentrations were assayed by HPLC. Hemodialysis Cl of M was 68.3±15.9 ml/min at blood flow rate of 200-250 ml/min, based on 21 sample pairs. There is a statistically significant difference between the Cl of TE and CDAK dialyzers ($p < 0.001$). Cl of hydroxy metabolite was 65.4±20.2ml/min; acidic metabolite concentrations were below detectable limit in all patients. These results indicate M and the hydroxy metabolite are dialyzable; however, Cl depends on the dialyzer type. Membrane properties and dialyzer design may affect the Cl. Compared with the total body Cl of M previously reported in chronic renal failure patients while not on HD (≈60ml/min), total Cl is increased by 100-175% during HD. An extra dose of M is thus needed to assure therapeutic concentrations in the seriously ill patient for each 3-6 hours of HD, depending on dialyzer type.

THE CARDIOVASCULAR (CV) AND METABOLIC EFFECTS OF MIXTURES OF ACETATE (AC) AND SUCCINATE (SUC). A POTENTIAL IMPROVEMENT IN DIALYSATE SOLUTIONS. Paul L. Kirkendol, Efrain Reisin, James E. Pearson and Francisco M. Gonzalez. Departs. of Pharmacology and Medicine, LSUMC, New Orleans, LA.

Succinate produces substantially fewer untoward CV changes than does AC; however, after SUC, HCO_3^- regenerates too slowly to be used alone. The present study determined the effects of AC/SUC at ratios of 25:75%, 50:50%, or 75:25%. For the CV studies each mixture was infused into 3 anesthetized dogs at doses of 0.125, 0.25, 0.5, and 1.0 mEq/kg/min for 10 min at each dose level. The effects on BP, HR, CO, dp/dt and TPR were determined after each infusion period. For the metabolic studies each mixture was infused at a rate of 0.25 mEq/kg/min for 1 hr in 7 acutely nephrectomized dogs. Blood pH, hct, Na^+, K^+, Cl^- and HCO_3^- were determined every 20 min. The effects on HCO_3^- regeneration showed that 25:75% AC/SUC is similar to 100% SUC, and the 50:50% mixture offers the advantages of rapid but less marked and more sustained HCO_3^- production (Δ16.3 mEq/L; $p < 0.05$). Corresponding pH changes in all mixtures followed HCO_3^- level with increases in Na^+ and decreases in CL^-, but no changes in K^+ levels or hct. BP, HR, and dp/dt were not significantly affected. Gradual CO increases (from 3.5 to 6L/min) and 50% decreases in TPR were noted. CV changes in all combinations closely resembled those for SUC alone. Thus, the addition of SUC in dogs seems to reduce the untoward CV changes seen with AC; consequently the ratio of AC/SUC should be based on metabolic considerations only. In summary we suggest that a 50:50% AC/SUC mixture may be the best combination for optimal metabolic and CV effects on dialysis.

THE EFFECTS OF DESFERRIOXAMINE (DFO) ON ALUMINUM- INDUCED OSTEOMALACIA IN THE RAT. J. Lewis-Finch*, M. Bergfeld*, K. Martin, S Teitelbaum, and E. Slatopolsky. Washington University Medical School, St. Louis, MO.

Studies in patients on dialysis have shown that aluminum (Al) accumulation in bone plays a major role in the development of osteomalacia. Aluminum-induced osteomalacia (Al-Ind.Ost.) also occurs in patients with CRF before dialysis. It has been suggested that DFO may be beneficial in treating Al-Ind. Ost. The present studies were performed in 4 groups of uremic rats to determine if DFO and/or discontinuation of Al administration have an effect on bone histomorphometry and blood chemistries. The groups were: 1) Uremic (U) control; 2) U + Al (.75 to 1.0 mg/kg I.P. 5 times per week for 12 weeks); 3) as 2) after 12 weeks Al was D/C and the rats received DFO (75 mg/kg I.P. 2 times per week for 9 weeks); 4) as 2) after Al was D/C a period of 9 weeks elapsed before the animals were sacrificed.

Group	Creat. mg/100 ml	BUN	Ca	PTH ul/Eq/ml	Serum Al ul/L	Bone Al ug/gm D.W.
1	1.34	48	9.8	120	19 ± 4	13 ± 2
2	1.13	42	9.5	55	513 ± 42	235 ± 55
3	1.31	51	9.7	109	132 ± 8	197 ± 33
4	1.13	41	9.5	94	129 ± 15	145 ± 15

High levels of Al in serum and bone and low levels of PTH were seen in rats receiving Al. Bone histology revealed Al in the mineralization front, abnormal tetracycline uptake and increased osteoid seams. DFO treatment or D/C of Al for 9 weeks reversed towards normal the above described lesions. In conclusion, these studies suggest that DFO and/or/D/C of Al in rats with approximately 30% of renal function greatly improve the Al-Ind.Ost.

THE INFLUENCE OF STERILIZING AGENTS ON THE BIO-COMPATIBILITY OF REUSED DIALYSERS. Robert M. Lindsay, J. Frank Walker,* William J. Sibbald,* and Adam L. Linton Univ. of Western Ontario, London, Canada.

There is evidence that reused dialysers are more biocompatibile than new ones. Degrees of neutropenia and complement activation, incidences of chest and back pain, and hospital admissions for dialysis related complications are reportedly less with reused dialysers. The question as to whether the type of sterilization used in reprocessing dialysers influences the improvement in biocompatibility has yet to be answered.

Studies in sheep have shown that blood exposed to dialysis membranes causes acute pulmonary hypertension (APH), neutropenia, and hypoxia. This animal model was used to examine the effect of sterilization of reused Cuprophan dialysers by 2% formaldehyde (F), 0.5% hypochlorite (H), 0.5% and 5% Warexin (tetradecyl-benzene sulphonate-hypochlorous acid) (W) on the pulmonary artery response.

Ten ml blood, previously in static contact with new Cuprophan dialysers, reinjected into sheep increased the mean pulmonary artery pressure (Δ MPAP) by 28 $\pm$ 11* mmHg. The use of F with the first reuse significantly reduced this APH (ΔMPAP 10 $\pm$ 4* mmHg); APH was totally abolished with subsequent reuses. Even the first reuse with W abolished APH. Such improvement was not obtained with H; Δ MPAP = 33 $\pm$ 7* mmHg after third reuse.

Thus, F and W improve biocompatibility, H does not.

* M $\pm$ SD (n=7)

DISSOCIATION OF HEMODIALYSIS LEUKOPENIA AND HYPOXEMIA FROM COMPLEMENT CHANGES DURING CITRATE ANTICOAGULATION. MacDougall, M., Diederich, D., Wiegmann, T.*, Kidney Urology Research Center, University of Kansas, Kansas City, KS and VAMC, Kansas City, MO.

We studied extent and time course of leukopenia and hypoxemia during hemodialysis (HD) in relation to changes of C3a and C5a complement components. A cellulose acetate membrane (CDAK-4000) was used with a bicarbonate bath and chronic anticoagulation (AC) was provided by either Heparin (H) or Citrate (C). C-AC complexes calcium during passage through the dialyzer. Arterial samples were collected at 0, 20, 60, 120, and 240 minutes. Initial and 20 minute values (mean) are given:

Var	PO_2	WBC	C3a	C5a	Ca2+
H-0	110	7,780	150	19.2	2.25
H-20	101*	5,470*	5209*	53.3*	2.20
C-0	94	7,510	230	23.1	2.11
C-20	83*	5,730*	1044*	27.0	1.86*

(* = $p<0.05$ non-paired T-test 0 versus 20 minutes)

C3a and C5a changes occurred in parallel during H-AC. Activation of C3a was diminished and the increase of C5a was abolished with C-AC. C5a increased at the end of the dialysis, but CBC and pO_2 were normal at that time. We conclude that C-AC depresses complement activation during dialysis. Furthermore, only the early component of complement activation appears to be required for the induction of HD-related hypoxemia and leukocytopenia.

HYPERKALEMIA CAUSES LOW THYROID UPTAKE OF THALLIUM IN PATIENTS ON CHRONIC HEMODIALYSIS. M.L. Maayan*, J.E. Rubin*, R. Johnson*, E.M. Volpert*, R.C. Sellitto*, L. Bierman*, G.M. Berlyne. Brooklyn VA Medical Center, Brooklyn, NY.

Seventeen chronic hemodialysis patients were injected intravenously with a mixture of 1 mCi each of ^{99m}Technetium pertechnetate and ^{201}Thallium chloride for parathyroid subtraction scanning. In 8 of these patients, the thyroid was not visualized, in contradistinction to a group of 5 patients with normal renal function suspected of having parathyroid adenomas who were injected with the same radionucleotides.

All had normal thyroid function studies; but there was a significantly higher serum potassium level in those patients whose thyroid glands were not imaged compared to those patients who had their thyroid visualized. The patients who had thyroid visualization had a mean serum potassium level (mEq/l $\pm$ S.D.) of 5.01 $\pm$ 0.7, while the eight patients who had no thallium uptake had a mean serum potassium level of 5.94 $\pm$ 0.5, ($p<.01$ by the Mann-Whitney test).

We conclude that this finding in some chronic hemodialysis patients is due to a higher than normal serum level of potassium which inhibits thallium uptake by thyroidal tissue. Other tissues, such as salivary glands and cardiac muscle, are able to pick up this tracer, so that the deficit appears to be specific for the thyroid.

TIME AVERAGE CONCENTRATION UREA TAC PREDICTS MORBIDITY IN STABLE HEMODIALYSIS PATIENTS. Mackenzie, T.A.*; Martino, J.A.; Ramsdell, R.; VAMC, Hampton, Va., Eastern Virginia Medical School, Norfolk, Va.

The TAC has been demonstrated to be a marker for adequacy of dialysis. To confirm the use of it as an index to predict expected morbidity, we correllated the absolute deviation from our target TAC 50mg% with the absolute number of hospital days (for any reason, in a group of stable dialysis patients - on ambulatory hemodialysis for greater than 6 months. TAC was averaged for three month intervals, and the corresponding total hospital days for the same interval was calculated). Patients were followed for a minimum of 6 months to a maximum of 14 months. The results indicate that there is a distinct correllation between deviation from target TAC, and total hospital days $r = 0.51$. Furthermore, for every increase in TAC of 5 mgm %, there will occur 1.8 days of hospitalization. The results also suggest, but are not conclusive, that the effects are cumulative; i.e., consecutive months of deviation from Target TAC is followed by consecutive months where a hospitalization occurs. The findings: (1) confirm the TAC is a marker of adequacy of treatment, and (2) is also a marker of cost effectiveness of a hemodialysis prescription.

CARBON FILTERS AS A SOURCE OF ALUMINUM (Al) IN WATER TREATMENT SYSTEMS. C MacLaren*, R Swartz. Univ. of Michigan Medical Center, Ann Arbor, MI.

The advent of routine water treatment during hemodialysis has resulted in virtual elimination of many contaminants from raw water used in preparing dialysate. However, the standard deionization (DI) and reverse osmosis (RO) procedures are ineffective in removing the chloramine which many communities add to sterilize the water supply, and adsorbent filters are required when chloramine is present. Our experience with some activated carbon filters used to remove chloramine from water already DI or RO treated has revealed the presence of significant Al in water destined for delivery to the dialyzer. In vitro irrigation of two different types of charcoal filters demonstrated from 160 to 1600 mcg/l of Al in post-filter water, with gradual washout of Al over 30 to 60 min and no Al in the water entering the filter. Although the total Al exposure from each filter is not extreme, frequent filter changes or failure to flush filters before dialysis use could increase the Al burden in patients at risk. Several maneuvers are possible: (1) placement of filters proximal to the DI or RO system to insure that Al is removed from water, although such filters are rapidly exhausted and require frequent replacement; (2) use of a sorbent dialysis system that does not require additional water treatment; or (3) use of charcoal filters that contain no Al, particularly those made from petroleum carbon sources rather than vegetable carbon sources. Although it is unclear whether Al originates in the carbon source itself or in the materials used in processing the carbon, or whether this Al contributes to clinical disease, filters free of Al are available and should be used.

SERUM CREATININE LEVEL (Cr) AT THE START OF CHRONIC HEMODIALYSIS (CH). John Malangone*, J.Gary Abuelo, John Pezzullo*, Kathleen Lund*, and Charlene McGloin*. Rhode Island Hospital, Dept. of Medicine, Providence, Rhode Island.

We analyzed the relation between Cr and laboratory and clinical data on 402 consecutive patients at the initiation of CH. The ages of the patients ranged from 6 to 90 yrs (mean 51.6); 202 were female, 200 were male; 351 were white, 50 were black and 1 was oriental. Mean Cr was 11.6 mg% with a S.D.=4.6 mg% and a range of 3.5 to 35 mg%. 4.7% of patients had Cr<6 and 5.6% had Cr≥20. Cr was correlated with a number of variables. Males had higher Cr's than did females (12.2 vs. 11.3 mg%, p=0.02). Diabetics, independent of sex, had lower Cr's than did non-diabetics (10.2 vs. 12.1 mg%, p=0.0004). Patients with ischemic heart disease, independent of sex, also had lower Cr's than did those without heart disease (10.0 vs. 12.0 mg%), p=.0015). Cr was inversely correlated with age (Cr=15.1-.066 age, p<.0001). After controlling for age, high levels of significance were maintained when Cr was compared to sex (p=0.009) and diabetes mellitus (p=0.005). Correction for age reduced the significance of the correlation with heart disease (p=0.083). Cr was inversely correlated with hematocrit (Cr=17.8-Hct x 0.24, p=.0001). Race, rate of progression of renal failure, and obesity were not significantly related to Cr.

These data support some common observations regarding the effect of sex, age and diabetes mellitus on Cr; interesting correlations between Cr and hematocrit and Cr and heart disease were also noted.

ANAPHYLATOXIN RELEASE AND LEUKOPENIA DURING COOL HEMODIALYSIS (HD). Maggiore Q, Enia G, Catalano C, Misefari V, Mundo A, Creazzo G, Zaccuri F. Centro Fisiologia Clinica, CNR, Reggio Calabria, Italy.

During cuprophan-HD the cellulosic membrane causes activation of the complement alternative pathway (CAP) with anaphylatoxin (C3a, C5a) release and leukopenia. Activation of CAP is known to be temperature (T) dependent. We investigated whether cooling of the extracorporeal blood could prevent anaphylatoxin release and leukopenia. C3a, C5a, and WBC count were determined in 7 pts both during standard HD and cool HD. Cool HD was accomplished by lowering pre-dialyser blood T by means of an especially designed circuit and by using dialysate at room T: blood T was 35.98 °C in pt arterial outlet, 28.4 in dialyser inlet, 22.4 in dialyser outlet, and 34.2 in pt venous inlet. Maximal changes in WBC count (arterial line), C3a, and C5a (venous line) occurred after 15' of treatment.

	Standard-HD		Cool-HD		
	Pre	15'	Pre	15'	
WBC x 10^3 mm^3	8.40	2.98	8.20	6.13	**
C3a µg/ml	0.20	3.60	0.25	1.58	*
C5a ng/ml	3.40	44.30	5.40	8.70	°

Standard vs cool HD at 15' Student's t test
** $p < 0.01$ * $p < 0.05$ ° $p < 0.02$

We conclude that cooling extracorporeal blood markedly attenuates anaphylatoxin release and intradialytic leukopenia.

FREE AND CONJUGATED PLASMA NOREPINEPHRINE IN HEMODIALYSIS PATIENTS. Francisco Monteon*, William J. Raum*, John Shaib*, and Joel Kopple. Divisions of Nephrology and Endocrinology, Harbor-UCLA Medical Center, Torrance, CA

Free norepinephrine (FNE) may be important for maintaining blood pressure during hemodialysis (HD). However, several studies report discrepant results concerning pre- and post-HD plasma FNE levels. We therefore reevaluated plasma levels and clearances during HD for FNE and conjugated NE (CNE), a major metabolite of FNE, using a radioimmunoassay (Raum, AJP 247:E4, 1984). Blood was obtained at the exact onset and finish of HD. Ten stable maintenance HD patients were studied during an uneventful HD. Age was 38±24 (SD) years; duration of HD therapy, 27±35 months; and blood pressure, 138±36/74±12 mm mercury. Pre-HD FNE and CNE were 278±228 and 5506±3114 pg/ml, respectively; CNE was increased (p 0.001) about 2-3 times normal. During HD, FNE decreased by 77±22% to markedly low levels, 53±39 pg/ml, while CNE decreased by 62±9% to 2034±900 pg/ml. Clearance of plasma FNE and CNE during mid-HD were 94±29 and 68±20 ml/min, respectively. There was no correlation between pre-HD or post-HD FNE or CNE and blood pressure or pulse at these times. Changes in FNE or CNE did not correlate with changes in blood pressure or pulse. These data suggest that in clinically stable HD patients, plasma FNE is normal pre-HD. Plasma CNE is increased, probably due to impaired renal excretion, and is cleared well by HD. FNE falls markedly during HD which may reflect its high clearance and possibly impaired release due to autonomic neuropathy. In unstable patients, FNE clearance may predispose to hypotension.

HEMODIALYSIS HEMODYNAMICS IN A NON-ANESTHETIZED ANIMAL MODEL: EFFECT OF ACETATE. Z.M. Nawab*, J.T. Daugirdas, I.L. Ing*. Hines-Loyola Medical Center, Hines, IL.

Acetate-buffered (40 mM), isonatric dialysate was used to perform diffusion dialysis (zero ultrafiltration rate) in conscious, non-uremic, splenectomized dogs. In control studies, the same animals were also dialyzed using bicarbonate-buffered dialysate. Dialysis was begun after 1 hour of extracorporeal circulation without weight removal.

After 90 minutes of dialysis, the following hemodynamic changes were observed:

	Acetate	HCO3	p <
Δ MAP (mm Hg)	-12 ± 9	-4 ± 6	.05
Δ CO (L/min)	+0.65 ± 0.33	-0.40 ± 0.63	NS
Δ TPR (d·s/cm^5)	-400 ± 264	+80 ± 272	.01
Δ Pulse (b/min)	+23 ± 17	-8 ± 11	.01
Δ PAP (mm Hg)	+4.0 ± 2.4	-0.7 ± 1.2	.01
Δ Weight (kg)	+0.02 ± 0.001	-0.03 ± 0.002	NS
Δ Hcrit (%)	-0.9 ± 0.03	-0.6 ± 0.03	NS

Also noted with acetate-buffered dialysis were dramatic acute (1-2 minutes) increases in mean pulmonary artery pressure (Δ PAP: 10 ± 3.1 mm Hg, $p < 0.001$) and cardiac output (Δ CO: 2.5 ± 1.3 L/min, $p < 0.001$), without associated changes in mean arterial pressure (MAP). These phenomena were not observed during bicarbonate-buffered dialysis (Δ PAP: 0.5 ± 1.0, NS; Δ CO: 0.3 ± 0.5, NS).

The results suggest that acetate-buffered hemodialysis causes adverse hemodynamic effects even in non-uremic animals with presumably intact cardiovascular systems.

PROTEIN-CALORIE MALNUTRITION IN CHRONIC HEMODIALYSIS PATIENTS. Stephen H.Norris, Mary Bess Kohrs*, and Neil A.Kurtzman. Univ of Illinois at Chicago, Department of Medicine, Chicago IL.

In order to assess dietary compliance and the state of nutrition in patients with end-stage renal disease, a survey of 10 randomly chosen stable hemodialysis (HD) pts compared recorded protein-calorie (P-C) intakes with their protein catabolic rates (PCR) as determined by urea kinetic modeling. The prescribed protein and caloric intakes were 1g/kg/day and 35 kcal/kg/day, respectively. The midweek predialysis serum urea nitrogen was 72±21 mg/dl, mean intra-dialytic weight gain was 1.5±1.3 kg, mean PCR was 0.85±0.29 g/kg/day. The post-dialysis wts of 3 pts were <90% of ideal body wt; in 1 pt triceps skin-fold thickness and in 3 mid-arm muscle circumferences were below the 5th percentile. In 6 pts serum albumins were <3.8 gm/dl, and in 6 serum transferrins were <200 mg/dl(normal 200-400 mg/dl). The prescribed P-C intakes exceeded both the PCR and the recorded intakes for 9/10 pts. Poor dietary compliance was thus documented in 9/10 pts. This study shows that dietary intake in these patients is less than that prescribed and that there is objective evidence of P-C malnutrition in almost all of these individuals. A long-term, controlled prospective study will be required to determine whether: 1) the prevalence of P-C malnutrition seen in this survey applies to the entire HD population; 2) what the possible sequellae of this degree of P-C malnutrition may be and; 3) what the cost/benefit ratio may be for "normalizing" these parameters by substantially amplifying the dietary and dialysis requirements of HD patients.

INTRAVENOUS CALCITRIOL FOR SEVERE SECONDARY (2°) HYPERPARATHYROIDISM IN DIALYSIS PATIENTS K C Norris,* J A Kraut, D L Andress,* A Koffler, D J Sherrard & J W Coburn, Wadsworth V A Med Ctr & UCLA Sch Med Los Angeles, CA & VA Med Ctr & Univ. Wash, Seattle, WA

Preliminary studies suggest that intravenous (IV) calcitriol (125D) suppresses serum (S) iPTH in dialysis patients with mild-to-moderate 2° hyperparathyroidism. To study the effectiveness and safety of IV 125D in patients with severe 2° hyperparathyroidism and normal SCa, we measured SiPTH, Ca, P, and alk P'tase (AP) frequently before and during IV 125D in 8 HD patients with iPTH of 1160±426 (SE) uLEq/ml, range 211-3523 uLEq/ml and SCa of 10.3±0.1mg/dl; 2 had recurrent disease after parathyroidectomy (PTX). Bone biopsies showed osteitis fibrosa in 7 and a mixed lesion in 1. They received 125D IV, 1 to 2.5 ug, 3 times/week for 8-64 weeks, mean 32 wks. The mean dose varied from 3.0-5.6 ug/wk. S-AP, and P before Rx were 205±53 IU/L and 5.0±0.2 mg/dl, respectively. Average SCa during Rx was 10.71±.14 (range, 10.1 to 11.3 mg/dl). During Rx, SiPTH levels fell by 44.4±6.2% and AP by 37±10%; indeed, SiPTH decreased by 30±6% in 7 pts during the 1st 2-18 wks of Rx as SCa was unchanged, suggesting a direct effect of 125D on the parathyroid glands. After 16-56 wks of Rx, repeat bone biopsies in 3 patients showed reduced fibrosis and bone formation, indicating less hyperparathyroidism. SCa exceeded 11.5 mg/dl 27 times during 64 patient-mos of Rx (20 episodes in 2 patients). Thus, IV 125D can be effective in suppressing SiPTH and reversing parathyroid bone disease in dialysis patients with severe 2° hyperparathyroidism; such Rx may provide an alternative to PTX.

A COMPARISON BETWEEN THE ADVERSE EFFECTS OF BICARBONATE AND ACETATE DIALYSATE IN CHRONIC RENAL PATIENTS. Oettinger, C., Alperin, L., Oliver, J., Emory University, Atlanta, Georgia

151 chronic hemodialysis patients participated in a 18 week single blind crossover study at Dialysis Clinic Incorporated. Atlanta, Georgia. The study was performed to evaluate clinical symptomatology in hemodialysis patients when bicarbonate was substituted for acetate as the dialysis base buffer.

Data collection consisted of documentation of clinical symptoms that occurred and medical interventions used throughout each treatment. Evaluation of each dialysis treatment (total of 8,183 treatments) consisted of subjective criteria (flushing, nausea, mental confusion, paresthesias, and shortness of breath) and objective criteria (vomiting, angina, cramps, hypotension, and the frequency of use of mannitol, hypertonic saline and nitroglycerine). The patients' symptoms were evaluated on a daily basis 12 weeks before and 6 weeks after switching from acetate to bicarbonate dialysate. The patients were unaware of the change in dialysate solutions. Statistical analysis of the data revealed a significant decrease in ($P<0.001$) incidence of vomiting, cramps, hypotension, nausea, flushing and the use of mannitol and hypertonic saline. Shortness of breath, angina, mental confusion and paresthesias were not statistically changed.

In our large dialysis population, the date reported support the conclusion that bicarbonate is the buffer of choice due to decreased incidence of side effects when compared to acetate.

A NEW PROCESS FOR THE LONG TERM STABILIZATION OF BICARBONATE HEMODIALYSATE (BD). C.W. Oettinger, J.C. Oliver, Emory University, Atlanta, Georgia

It is generally agreed that BD is preferable to Acetate dialysis (AD), but major limiting factors to its general usage are high cost and technical difficulties in maintaining its stability for prolonged periods. The procedure we have developed resolves both limiting factors, rendering BD feasible and practical for routine outpatient dialysis. The instability of BD is due to decomposition of HCO_3 into free CO_3 and CO_2 which is lost into the atmosphere resulting in a decline in HCO_3 and increased dialysate pH. This is prevented by maintaining a blanket of CO_2 at atmospheric pressure over the solution in an airtight storage tank. 1000 gallons of BD is freshly constituted in a mixing tank and transferred to an airtight storage tank where CO_2 is kept at atmospheric pressure by use of a two stage regulator connected to the CO_2 gas cylinder. Dialysate pH and HCO_3 concentrations were 7.33 + 0.11 and 34.3 + 2.2 respectively. HCO_3 concentration remained stable up to 30 days. The storage process is fully automated and requires only 1-2 hours of personnel time per batch. The process may be adapted to store 50-1000 gallons use of BD for use with single or multiple patient proportioning systems. Three dialysis units totaling 240 patients have been switched to BD without significant problems. Using this process, BD costs are similar to acetate. If BD is desired, this new process seems to be the best available method for the practical low cost utilization of BD.

DIETARY CALCIUM DEPRIVATION: A LEADING CAUSE OF SECONDARY HYPERPARATHYROIDISM IN PATIENTS WITH CHRONIC RENAL FAILURE UNDERGOING DIALYSIS. Mordecai M. Popovtzer, Ulrich F. Michael, Kathleen S. Johnson, Connie Ronstadt, Dianne Nelson, and David A. Ogden. Veterans Administration Medical Center and University of Arizona Health Sciences Center, Tucson, Arizona.

Dietary phosphate (P) restriction entails a substantial reduction in calcium (CA) intake in patients with end-stage renal disease. The present study was designed to evaluate the relationship between the intakes and serum (S) concentrations of CA, P and parathyroid hormone (PTH). In 39 patients treated with chronic dialysis the intakes of CA and P averaged 488±51 (x±SE) and 788±58 mg/24h respectively, and exhibited an intimate linear relationship $Y = 0.88X + 360$, $r=0.63$, $p<0.001$. There was no correlation between S-PTH and either SCA or SP concentrations. There was, however, an inverse exponential relationship between dietary CA intake and S-PTH, $Y = 126e^{-0.011\ x}$, $r = 0.51$, $p<0.001$. Likewise, when divided into low CA (<500): 269±22 mg/24h and modest CA (>500): 789±65mg/24h intake groups, S-PTH, (121±15 pg/ml) in the low CA intake group was almost twofold greater than that in the modest CA intake group (63±14 pg/ml), $p<0.01$.

The present data confirm a previously noted inadequacy of CA intake in patients with chronic renal failure undergoing dialysis and demonstrate a highly significant inverse relationship between CA intake and S-PTH. These findings suggest that dietary CA deprivation may play an important role in the evolution of secondary hyperparathyroidism in patients on chronic dialysis.

HIGH DOSE INTRAVENOUS DEFERIOXAMINE DOES NOT PRODUCE HEMODYNAMIC CHANGES IN THE DOG. J. Pederson, M. Rodriguez*, C. Haygood*, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VAMC, Okla. City, Okla.

Deferioxamine (DFO), a chelating agent, is used to treat dialysis patients with aluminum induced osteomalacia. Hypotension has been reported in dialysis patients during the intravenous administration of DFO. The reason for the development of hypotension is unclear. DFO may have a direct effect on the cardiovascular system or possibly produce hypocalcemia which may affect systemic vascular resistance or cardiac output.

To study the effects of DFO on hemodynamic parameters and plasma calcium (Ca), 5 dogs were infused with DFO (90 mg/kg) over a 2 hour period. Hemodynamic parameters determined with a Swan Ganz catheter included mean arterial blood pressure (BP), cardiac output (CO), heart rate (HR), and systemic vascular resistance (SVR), and are listed below:

Time	0'	30'	60'	120'
Ca (mg/dl)	9.5±.2	9.1±.3	9.2±.2	9.3±.2
BP (mmHg)	140± 5	140± 9	160± 4	156±12
CO (l/min)	3.5±.1	3.4±.2	4.0±.5	3.9±.2
HR (per min)	96± 8	101± 7	96± 6	94± 8
SVR (dynes $cm^{-1}sec^{-5}$)	3200±160	3440±240	3550±400	3200±240

Mean±SE

In summary, in dogs, DFO, in a dose comparable to that given to dialysis patients, does not produce any significant changes in plasma calcium, blood pressure or hemodynamic parameters. Thus, DFO in doses similar to those administered to patients, seems to be safe and does not induce hypotension in the dog.

SAFETY OF LONG-TERM MULTIPLE USE OF DIALYZERS. V. E. Pollak, K. Kant, M. Cathey,* S. Parnell,* G. Zasuwa,* and N. W. Levin. Univ. of Cincinnati, Cincinnati, Ohio and Henry Ford Hospital, Detroit, Michigan.

Multiple use of dialyzers is safe and effective in short-term studies and is associated with fewer symptoms. Long-term effects are unknown. We report outcomes from two dialysis units where multiple dialyzer use has been routine practice, in Cincinnati for 6.5 and in [Detroit] for 12 years (y). 259 [1059] patients, average age 49 [50] y were so treated. 38 [30]% were >60 y; 34 [24]% had diabetes mellitus. 160 [362] were dialyzed in multiple use settings for 1 y, 76 [153] for 3 y, and 24 [116] for 5 y. Average dialyzer use was 6.1 [5.0]. The 259 [1059] pts were followed a total of 535 [2209] pt y; 72 [430] died. Pts were admitted to hospital 1.6 [1.6*] times/y; average hospitalization was 14.2 [18.4*] days/y, a rate comparable to or lower than in the literature. Average survival on hemodialysis was 7.43 [5.13] y; the case fatality rate was 13.46 [19.46] deaths/treatment year. National and ESRD Network unadjusted case fatality rates are expressed: as deaths/calendar year divided by total patients who received 1 or more outpatient treatments (all modalities in all centers) in that calendar year. In 1980-1983 inclusive case fatality rate was: in the US 15.2%; Ohio Valley Renal Disease Network 16.2%; Michigan Renal Disease Network 16.3%. For Cincinnati patients it was 11.3% ($p < 0.0002$ vs OVRDN) for Detroit patients 15.7% [p NS vs MRDN]. We conclude that treatment by hemodialysis using dialyzers many times was not associated with increased morbidity or mortality.
[*Jan 1983-June 1984 only.]

SOLUTE CLEARANCE (C) AND ULTRAFILTRATION (UF) WITH VARYING DIALYSATE TEMPERATURE (T). Rasib M. Raja, Mark S. Kramer, Steven J. Goldstein,* Antonio De-LosAngeles* and Arthur M. Lerner*, Albert Einstein Medical Center, Kraftsow Division of Nephrology, Philadelphia, Pennsylvania.

Dialysate with low T has been reported to improve vascular stability during hemodialysis (HD). The effect of varying dialysate T on C and UF has not been reported. This study compares single midweek HD with dialysate T of 37.0°C ($T_{37.0}$), 35.5°C ($T_{35.5}$) and 34.0°C ($T_{34.0}$) each in 10 chronic HD pts. The dialyzer, Q_B, Q_D, TMP, T_D and dialysate composition was kept constant for each pt. Body wt, supine and standing BP were recorded before and after but BP every 30 mins during. Hct, platelets, WBC, BUN, Cr and Osm were determined at the start (S), 1 hr during and the end (E) of HD. The change in systolic BP (Δ BP), platelets (Δ P), C_u, C_{Cr} and UF/hr are:

	Δ BP (mmHg)	ΔPlatelets (%)	C_uE ml/min	C_{CrE} ml/min	UF ml/mmHg
$T_{37.0}$	20*	-6.1*	150*	108*	3.7*
$T_{35.5}$	8	-4.9	152	113	3.5
$T_{34.0}$	7	+2.7	161	120	3.2

$P < 0.05$ ($T_{37.0}$ vs $T_{34.0}$)

The HD decrease in BUN, Cr, Osm, C_uS and $C_{Cr}S$ were similar but increase in Hct was more marked in $T_{34.0}$ ($P < 0.05$). These data suggest that hypotension in HD may be less marked with lower dialysate T. Lower UF rate with cool dialysate may indicate decreased membrane permeability and may contribute to improved vascular stability. Small molecule solute C may not decrease during HD with cool dialysate due to less platelet adherence to the membrane at lower temperature.

HEMODIALYSIS (HD) IN CHRONIC RENAL FAILURE (CRF) DOES NOT PRODUCE CARNITINE (C) DEPLETION. Edmond S. Ricanati and Charles L. Hoppel,* Case Western Reserve University, Cleveland, Ohio.

Hypertriglyceridemia (HTG) is frequent in CRF patients (pts) on HD and has been attributed to C depletion during HD. C, a carrier of long-chain fatty acyl groups into the mitochondria for oxidation, plays a key role in triglyceride removal. The purpose of this study was to determine the clearance of C during HD. Mean weekly losses of total carnitine (TC), free carnitine (FC) and acylcarnitine (AC) fractions were measured in 5 CRF pts during routine HD (3x/wk). They were on a balanced isocaloric diet (proteins: 1.0 gm/kg/day). Simultaneous arterial (A) and venous (V) bloods were obtained at hourly intervals during HD and the concentration of C and its metabolites were measured by methods well established in this laboratory. The rate of loss of C and its metabolites during HD was calculated by (A-V) in μmoles/ml x plasma flow rate (ml/min) through the dialyzer maintained constant by a calibrated pump. Total losses per HD were x by 3 to estimate weekly losses. In 10 controls, 24 hr urinary losses of TC, FC and AC fractions were measured and then extrapolated to 1 week.

Mean losses/week (μmoles)

	TC	FC	AC
HD pts (5)	1891.3	637.2	1014.3
Controls (10)	1474.9	591.4	883.6
P	< 0.4	< 0.8	< 0.5

Muscle (4 pts) and liver (3 pts) tissue (obtained during elective abdominal surgery) were analyzed for TC, FC and AC and were found to be within the normal range. It is concluded that C depletion does not occur in pts with CRF on HD.

PRE-EXISTING HYPONATREMIA IS ASSOCIATED WITH A REDUCED TOTAL PERIPHERAL RESISTANCE (TPR) RESPONSE DURING ISOLATED ULTRAFILTRATION. **P.E. Razma*, J.T. Daugirdas, T.S. Ing, J.L. Izzo, Jr. Hines-Loyola Medical Center, Hines, IL.; University of Rochester Medical Center, Rochester, NY.**

Critically ill, fluid-overloaded patients requiring extracorporeal isolated ultrafiltration are often hyponatremic. To determine whether or not hyponatremia affects tolerance to ultrafiltration, isolated ultrafiltration (IU) was performed in ureter-ligated or nephrectomized, conscious dogs (n=13) previously rendered hyponatremic (HN: plasma Na 128 ± 3 mM) by use of a hyponatric dialysate. Ultrafiltration was continued (600 mL/hr) until mean arterial pressure (MAP) decreased to less than 80 mm Hg (end-point, EP). On a separate occasion, IU was performed in the same animals at a normal plasma sodium level (NN: plasma Na 148 ± 4 mM). Results were as follows:

	HN	NN	p<
EP MAP (mm Hg)	74 ± 4.2	72 ± 6.0	NS
EP CO (L/min)	1.94 ± 0.35	1.50 ± 0.29	.001
EP TPR (d·s/cm^5)	3120 ± 520	4000 ± 736	.01
EP Weight (kg)	24.5 ± 2.3	22.5 ± 1.4	.001
EP Blood vol (mL)	1795 ± 300	1667 ± 236	.05
ΔHct (%)	5.5 ± 1.6	8.3 ± 2.8	.001
ΔWeight (kg)	1.4 ± 0.5	1.9 ± 0.9	NS

The results suggest that hyponatremia per se is associated with a reduced TPR response during fluid removal and with an elevated post-ultrafiltration body weight.

THERE IS A SYNDROME ASSOCIATED WITH THE USE OF NEW DIALYZERS. M Robson,* V E Pollak, K S Kant, R Charoenpanich,* and M Cathey.* Department of Internal Medicine, Univ. of Cincinnati Medical Center, Cincinnati, Ohio.

It has been suggested that more symptoms occur during first than subsequent use of a dialyzer, and that there is a "first use syndrome." To examine these questions we analyzed computerized records of symptoms and events in 147 patients dialyzed on 26592 occasions over 28 months. All complications, considered together, were 1.3 times more frequent with the first (n=4933) than with subsequent (n=21659) dialyzer use. None was more frequent with the second or subsequent use. The following were significantly more frequent ($p < 0.0001$) with first use: hypotension, cramps, itching, chest pain, back pain, dyspnea, chills, clotting of dialyzer and lines, and dialyzer blood leaks. Chest pain and back pain were 2.8 and 6 times more frequent with first use. Their concurrence was not observed after the 4th use; and was 43 times more frequent with first use ($\chi^2_{[2]}=83.6$; $p < 0.0001$). Concurrent chest and back pain occurred more frequently than expected by chance ($\chi^2_{[3]}=72.4$; $p < 0.0001$) in patients with a high rate of total complications and a high ratio of total complications with first/subsequent dialyzer use. Thus, concurrent chest and back pain occurred with high rates of total complications during dialysis and with high rates during the first dialysis. These observations quantitate the advantages to patient well-being associated with reuse of dialyzers, and describe a syndrome associated with first use of the dialyzer.

ACUTE HEMODIALYSIS MORBIDITY. Stephen Sandroni, Betty Dillman*, Jan Currie*. Nephrology Division, University Hospital, Jacksonville, Florida.

The staffing and funding of dialysis units should be influenced by the amount of morbidity experienced by the unit's patients. The spectrum of morbidity of dialysis patients has not been well defined, although many individual problems are described. We reviewed 100 patients and identified 693 acute morbid events occurring during hemodialysis in a calendar year. These required the documented intervention of a physician during dialysis. Patients were 85% black; leading cause of ESRD was hypertension. 9 deaths occurred and there were 97 acute hospital admissions.

CLASSIFICATION OF MORBID EVENTS

CATEGORY	% OF TOTAL EVENTS
Acute complications of dialysis	33.5%
Severe psychological disturbance	26.3%
Medical/Surgical urgent admission	14.0%
Routine access inadequate/ unavailable	12.8%
Infection	11.5%
Acute inflammatory process	1.9%

Hypertension as the cause of renal failure was the single factor most clearly associated with morbid events. ($p < 0.001$). Age, sex, duration of dialysis, and presence of hypertension during the study period were not predictors of morbidity.

EFFECT OF BACKFILTRATION (BF) ON PERFORMANCE OF HEMODIALYZERS WITH HIGHLY PERMEABLE MEMBRANES (HPM). Matthias Schmidt, Wilhelm Schoeppe, Conrad A. Baldamus (intr. by Georg M. Eisenbach). Univ. Hospital, Nephrol. Dept., Frankfurt, FRG.

Only recently, occurence of BF, i.e. filtration from the dialysate into the blood compartment under standard hemodialysis (HD) working conditions, has been discussed as a possible explanation for the efficient clearance, especially for solutes of higher molecular weight, in dialyzers with HPM. In order to quantify BF we studied in-vitro and in-vivo the effect of a) oncotic pressure, b) pulsations of blood and dialysate pumps, and c) blood flow properties (hematocrit, protein content). A mathematical model was developed and evaluated which permits for a simulation of the local flow rates along the fibers of hemodialyzers with HPM. The Fresenius F 60 hemodiafilter (polysulfone membranes) with an in-vivo UFR of 40 ml/h/mmHg (Q-B = 200 ml/min; Q-D = 500 ml/min) was used. Total BF amounted to 30 to 40 ml/min in vitro and to approx. 20 ml/min in vivo. Abrupt pressure changes caused by the pulsations of the blood and the dialysate pump contributed approximately 40 to 50 % to this. They were observed for ultrafiltration rates as high as 800 ml/h. The periodical reversal (once every second) of convective flow may influences the protein layers on the membrane and might account for the stable performance. The total convective transport in dialysis with HPM could explain the improved vascular stability, described for other treatment regimes, based on convection (such as hemofiltration).

EFFECT OF DIFFERENT DIALYZERS ON PROTEINASES AND PROTEINASE INHIBITORS DURING HEMODIALYSIS. Roland M. Schaefer, August Heidland, and Walter H. Hörl (intr. by Joel D. Kopple). Department of Internal Medicine, Universities of Würzburg and Freiburg, FRG.

During hemodialysis (HD) activation of leukocytes occurs due to the contact with the dialysis membrane. Activated leukocytes release a variety of proteinases. Aim of the present study was to evaluate, the effect of different membranes (cuprophan, polymethylmethacrylate, polyacrylonitrile and polysulfone) on the release of granulocyte elastase (measured as an elastase-α_1-proteinase inhibitor complex: E-α_1PI) and its impact both on its main plasmatic inhibitor α_1-proteinase inhibitor (α_1PI) and on the proteolytic activity of plasma. Cuprophan (+420%) and polymethylmethacrylate (+620%) caused a higher maximal increase of plasma E-α_1PI compared with polyacrylonitrile (+280%) or polysulfone (+200%). The activity of α_1PI increased during HD using polyacrylonitrile (+36%) or polysulfone (+29%), whereas this increase was less with cuprophan (+6%) or polymethylmethacrylate (+8%). A reverse effect could be observed analyzing α_1PI concentrations. During HD with cuprophan (+33%) and polymethylmethacrylate (+23%) marked elevations occured, whereas α_1PI concentrations were only slightly enhanced with polyacrylonitrile (+12%) and polysulfone (+7%). Cuprophan membranes decreased the proteolytic activity of plasma by 35%, whereas polymethylmethacrylate, polyacrylonitrile and polysulfone caused a fall of more than 60%. From our data we may conclude: 1. The release of elastase depends strongly on the membrane used. 2. Membranes with a high elastase release induce marked elevations of α_1PI concentrations. Membranes with a low release of elastase induce high increases of α_1PI activities.

IRON OVERLOAD IN PATIENTS WITH CHRONIC RENAL FAILURE BEING TREATED BY HEMODIALYSIS. Ila Shah*, John Speck*, Latha Subramanian*, James Sedensky*, (intr. by Franklin D. McDonald). Wayne State Univ. School of Med., Dept. of Med. and Physiol., Detroit, Michigan.

Oral iron is routinely prescribed for patients with end stage chronic renal failure on regular hemodialysis. Many of these patients frequently receive red cell transfusions. Serum ferritin is a reliable indicator of reticuloendothelial iron stores. We evaluated iron stores in 143 patients with chronic renal failure on thrice-weekly hemodialysis. Fifty patients were receiving 1-2 transfusions per month on a regular basis. All the patients were receiving ferrous sulphate 300mg tid PO. The mean serum ferritin level was 569 ±524 ng/ml in transfused group with 10 patients having gross iron overload, and 119 ±45 ng/ml in the non-transfused group ($P < 0.001$). The mean duration on dialysis was the same for 2 groups. Only 13 of 143 patients had low ferritin levels. (Nl 20-230 ng/dl)

	Transfused Group N-50	Nontransfused Group N-93
S. Ferritin	569±524ng/dl	119±45ng/ml P<0.001

We conclude that, 1) routine oral iron therapy may not be necessary in patients on hemodialysis; 2) patients receiving PRBC transfusions should be treated prophylactically with iron chelating agents such as desferoxamine.

SORBENT RECYCLING OF ULTRAFILTRATE (SRUF) IN MAN. W.B. Shapiro, T.P. Schilb* and J.G. Porush. Division of Nephrology and Hypertension, Brookdale Hospital Medical Center, Brooklyn, N.Y.

In order to eliminate the need for substitution fluid during hemofiltration, we have developed a system for recycling ultrafiltrate utilizing a REDY sorbent cartridge to remove urea, creatinine and "uremic toxins" from the ultrafiltrate, which is then returned to the patient via a closed system. Eight patients with chronic ESRD previously treated with hemodialysis (HD) were studied. Four received 15 weeks of HD followed by 15 weeks of SRUF and 4 the reverse. Pre-treatment weights, standing mean arterial blood pressure (MAP), serum electrolytes, urea nitrogen, creatinine, uric acid, Ca and P were measured weekly. Symptoms during and between treatments were elicited by questionnaire and the frequency of symptomatic hypotension (requiring saline infusion) recorded. No untoward incidents occurred during either treatment modality. There were no statistically significant differences between patients treated with HD and SRUF in regard to weight urea nitrogen, Ca, P, Na and number of blood transfusions. Serum creatinine, uric acid, Mg and K were significantly lower pre-SRUF treatment ($p<0.01$). MAP was significantly higher pre-SRUF. Symptomatic hypotension occurred in 24.7% of HD vs 10.5% of SRUF treatments ($p<0.001$). Patients reported significantly more episodes of dizzyness and fatigue during and between HD ($p<0.05$) and significantly more nausea during SRUF ($p<0.05$). These data suggest that SRUF modification of hemofiltration is a safe, effective means of treating patients with end stage renal disease retaining many of the objective and subjective advantages of hemofiltration without the need for replacement fluid.

ANION EXCHANGE CHROMATOGRAPHY AND THE STUDY OF MIDDLE MOLECULAR CLEARANCE BY CELLULOSIC AND PAN DIALYZERS. Aida Shihab-Eldeen,* Stephen R. Ash, and Fred E. Regnier.* Ash Medical Systems, Inc., and Purdue University School of Biochemistry, West Lafayette, Indiana.

Direct anion exchange chromatography of uremic serum separates organic uremic substances into 10-20 peaks, many of which dialyze slowly in vitro. (Ash, et al, Kid Int 24:250-255, 1983). The method has recently been employed to study the efficiency of removal of such substances by cellulosic (Discap) and PAN (RP-1210) dialyzers. Five uremic patients participated in this study with a total of 28 dialyses. Arterial and venous serum samples were collected at the beginning and the end of each dialysis. The serum was injected onto a Synchropak AX300 anion exchange column and the area under the peaks was estimated by a mechanical integrator (Varian CDS111). The 20 or so peaks identified were common to all the uremic patients. The clearance of most of these peaks by the cellulosic dialyzers was 20-60 ml/min, indicating effective molecular weights of 1500-3000. Some clearances were lower at the end of dialysis. Clearances were somewhat lower with PAN and there was more variability. A generation of several peaks occurred during dialysis with the cellulosic dialyzers, fewer peaks were generated with PAN. The low and variable clearance of many uremic substances could be due to protein binding. Generation of peaks may be due to leaching, cellular release, or metabolic production during dialysis, and lower generation with PAN may be a measure of higher biocompatibility.

IMMEDIATE HYPERSENSITIVITY TO DIALYZER CONTENTS. Sherlock, J. and Olmer, J. Nassau County Medical Center, E. Meadow, N.Y., and SUNY Stony Brook, N.Y.

RAST for IgE, specific for a water wash of cuprophan hollow fiber (CuHf) dialyzers, ethylene oxide sterilized (Etox), were performed on 64 hemodialysis (HD) patients. Controls were 13 uremics before Hd. RAST ratio of controls was 0.88+/-0.021. Values > 1.17 (3 SD above control) were considered positive. 25/64 (39%) had positive RAST. Two patients post anaphylaxis (Ana) had RAST ratios of 11.68 and 9.77, and one with hives, 12.69. Symptoms per month (total, hypotensive, or allergic) did not correlate with RAST. 23/64 (36%) patients had eosinophile counts > 300/mm3, and 30/64 (47%) had increased total IgE. Neither correlated with RAST. No CuHf specific IgG (blocking antibody) was found with increased total IgE and normal RAST. Specific IgE to Etox was noted in 6/8 patients positive to CuHf. Two patients post Ana reacted to Etox but also to radiation sterilized CuHf. There was no difference in reaction to methanol washed vs. freon washed CuHf. Reactions to Etox sterilized cuprophan plate and cellulose ester Hf were ≤ 20% those to CuHf.

39% of Hd patients have IgE specific for CuHf contents. In some the allergen is Etox, in others, unknown. While not all patients with specific antibody to CuHf are symptomatic, some near fatal reactions occur. Other untested allergens may cause increased total IgE and eosinophilia.

DECREASED FIBRONECTIN(F) AND NEUTROPHIL(N) KILLING DURING HEMODIALYSIS(HD). Dale H. Sillix, J. Francis*, Wayne State University and Hutzel Hospital, Section of Nephrology, Detroit, MI.

Leukopenia, seen in the first hour of cellophane membrane HD, is due to pulmonic sequestration. This is thought to be secondary to C_5a activation of N but other mediators may be involved. Plasma F is a soluble opsonin antigenically related to surface adhesive F which may be involved in N adherance to endothelial cells. Plasma F was measured by an immunoturbidimetric assay. While F levels can be decreased by heparin, F was within normal limits at 2 hrs and 20 min levels were not correlated with amount of heparin used, drip vs bolus infusion, or clotting studies. Phagocytosis (Ph) and intracellular killing(K) of live Staphylococcus aureus(SA) by N during HD was measured by acridine orange staining of pre HD, 20 min, and 2 hr N which had been isolated by density centrifugation, washed, and studied using pooled human serum.

(n)	pre	20 min	2 hr	±SEM
F(11)	288±21	215±20[a]	284±20	
WBC(9)	9±1	4±1[a]	9±1	
%K(5)	88±4	77±5[b]	89±3	
#SA/N	16±1	13±1	13±1	

a: $p \leq 0.01$ vs pre & 2hr b: $p < 0.05$ vs pre

The N found circulating during the peak period of leukopenia have decreased K although Ph is unchanged. This functional difference suggests that they may be a distinct subpopulation. Changes in F parallel the changes in leukocyte counts and may be mediators of pulmonic sequestration of N during HD.

DETERMINANTS OF SERUM CREATINE KINASE ACTIVITY (CK) IN DIALYSIS PATIENTS (Pts). PC Singhal*, RH Barth, NS Ginsberg*, RI Lynn. Baumritter Kidney Center, Albert Einstein College of Medicine, Bronx NY.

Elevated CK has frequently been described in pts on chronic dialysis, but little is known about its cause and distribution. We measured CK in 105 pts on hemodialysis (HD) and CAPD and related it to simultaneous biochemical, nutritional and anthropometric data. None of the pts had evidence of hypothyroidism or muscle disease and none had received an IM injection within 3 days. In the entire group, CK was 130.3 ± 15.0 IU/L (mean ± SEM). CK correlated positively with serum LDH ($P < 0.001$) and with mid-arm muscle circumference (MAMC; $P = 0.05$), and negatively with age ($P < 0.01$). CK levels in various subgroups were as follows:

Group	n	CK (IU/L)	P
Men	56	166.0 ± 25.8	< 0.01
Women	49	82.4 ± 9.0	
White	59	92.6 ± 12.5	< 0.005
Black	46	158.8 ± 21.7	
HD	77	126.8 ± 17.0	NS
CAPD	28	142.7 ± 33.1	

Men had higher Cr ($P < 0.01$), Wt ($P < 0.01$) and MAMC ($P < 0.02$); there were no such differences between the black and white groups. Isoenzymes, determined in the 30 pts with CK > 130 IU/L, were all > 97% MM. Post-HD CK was measured in 10 pts and did not rise.

We conclude that CK is elevated in both HD and CAPD pts, particularly in men and in blacks; that elevated CK is not an effect of HD itself; that CK levels are related to muscle mass; and that CK declines with advancing age. The strong correlation with LDH suggests that production and/or clearance of CK and LDH are altered in dialysis pts by a similar mechanism.

INDICATIONS FOR AND THE RESULTS OF LONG-TERM IRON CHELATION IN DIALYSIS HEMOSIDEROSIS. Glen H. Stanbaugh, Diane Gillit*, and A. W. Holmes*. South Plains Dialysis Center and Texas Tech University Health Sciences Center, Lubbock, Texas.

An evaluation of 110 ESRD pts. at our dialysis center with quantitative ferritin screening & subsequent liver biopsy in 10 suspected of having non-A, non-B hepatitis, demonstrated markedly elevated serum ferritins, dense hepatic hemosiderosis with iron granulomas, rather than chronic viral hepatitis. Since DESFERAL is an effective iron chelator, 4 dialysis pts. with iron overload at our center have received DESFERAL for a total duration of 2-18 mos. After demonstrating distinct hepatic iron involvement rather than viral hepatitis, we felt it was justifiable to chelate those pts. with progressive liver dysfunction secondary to iron loading. Two pts., both treated with DESFERAL for up to 18 mos., demonstrated improvement in hepatic & myocardial function, along with a decline in serum ferritin. Two other pts. have not been on therapy long enough to show significant changes, & an additional cadaveric transplant pt. is presently undergoing phlebotomy for a similar condition. On the basis of these results, we feel that in selected individuals, iron removal with DESFERAL may reverse permament organ damage. Indications for therapy include progressive hepatic dysfunction, cardiomyopathy, neuromyopathy, or evidence of endocrine involvement. Not all pts. with a high ferritin or mild elevation in hepatic isoenzymes justify this long-term expensive therapy. We will present data summarizing results of our periodic ferritin screening in 110 dialysis pts., hepatic histology in 10, & results of long-term iron chelation in 2 pts.

CALCIUM CARBONATE IS AN EFFECTIVE PHOSPHOROUS-BINDER IN DIALYSIS PATIENTS. E. Slatopolsky, C. Weerts,* S. Lopez,* K. Norwood,* M. Zink,* D. Windus. Washington University School of Medicine, St. Louis, MO.

Hyperphosphatemia plays a key role in the development of renal osteodystrophy. Thus, control of serum phosphorus (Pi) is mandatory to prevent its deleterious effects on the parathyroid-vitamin D axis in uremia. In order to control Pi physicians usually prescribe phosphate-binders containing aluminum. Although these drugs are effective in preventing hyperphosphatemia, aluminum accumulation, particularly in the skeleton and parathyroid glands, may occur leading to the development of severe osteomalacia in some patients. We have shown recently in normal and uremic dogs, in short term studies (7 hrs) that Ca CO_3 is an effective agent to control hyperphosphatemia (Clin. Res. 32:452,1984). To evaluate the efficacy of Ca CO_3 in uremic patients on a more chronic basis studies were performed in 10 patients maintained on dialysis. Blood was obtained 3 times a week before dialysis. The study was divided in 3 periods: I Control, on aluminum phosphate-binders (one month) II No Al phosphate-binders (one month), III Ca CO_3 (2.5 to 15/gm for 2 months).

Period	I	II	III
	n= 119	n = 119	n = 229
PO_4	4.49 ± 0.10	6.95 ± 0.14	4.83 ± 0.12
	$p < 0.001$	$p < 0.001$	
Ca	9.66 ± 0.08	9.23 ± 0.06	10.0 ± 0.1

In summary, administration of Ca CO_3 to dialysis patients corrected the hyperphosphatemia and increased serum calcium levels suggesting that this drug may be an adequate substitute for traditional phosphate-binders in dialysis patients.

FOUR YEAR EXPERIENCE WITH ENDSTAGE RENAL DISEASE IN SAUDI ARABIA. Egils Veverbrants, Riyad Said and Magdi Hussein*. Al Hada Hospital, Taif, Saudi Arabia and Univ. of Rochester, Rochester, New York

Little is known about chronic renal disease in Saudi Arabia. Our dialysis unit accepted 120 patients for treatment in a 4 year period. 60 were males and 60 were females. Average age on entry was 40.9 years (range 6-70 years). Etiology was bilateral small kidneys in 49%, chronic glomerulonephritis 22%, diabetes 8%, obstruction 7%, interstitial nephritis 6% and single cases of cortical necrosis, lupus, amyloidosis and polycystic kidney disease.Only 2 definite cases of renal failure due to kidney stone disease were identified.

72% of patients had significant hypertension. Liver enzyme abnormalities were present in 42% and HBsAg was positive in 20%. 16% of patients needed therapy for active tuberculosis which was mostly extrapulmonary. 9% had diabetes. 39% had prolonged hypocalcemia and 33% had bone disease by X-ray. 10% had pericarditis and 4 patients needed surgery for tamponade. 25% had acetate intolerance. Blood transfusion requirement was high.

15 patients died (average age 51.I years). Most had multiple diseases. 44 patients were sent for transplantation and 27 have been transplanted so far. 18 had cadaveric, 6 live-related and 3 live-nonrelated transplants.

We conclude that most of chronic renal failure in Saudi Arabia is due to glomerulonephritis and untreated hypertension. There is a high incidence of tuberculosis, HBsAg positivity and liver function abnormalities. Nephrolithiasis is common but is not a frequent cause of renal failure.

INCIDENCE OF INAPPROPRIATE METABOLISM OF ACETATE (A) IN CHRONICALLY HEMODIALYSED PATIENTS-(CHDP). P. Vinay, M. Prud'homme, B. Vinet, G. Cournoyer, M. Leveille, Y. Piette, G. St-Louis, A. Gougoux, L. Lapierre. Notre-Dame Hospital and Univ. de Montreal, Canada.

The capacity to metabolize acetate was studied using a prospective study(60 CHDP) and a retrospective(211 CHDP) survey of the computerized records of each HD performed between 1974 and 1984. For each CHDP the intercept (I) of the regression line relating the change in plasma HCO_3 post-HD(ΔHCO_3) to the pre-HD concentration was used as an index of A metabolism. I indicates the plasma HCO_3 where the HCO_3 loss by HD is matched by HCO_3 generation from A metabolism. Under our HD conditions, a lack of HCO_3 increment post-HD together with a I <16 mM indicate inappropriate acetate metabolism. In 60 active CHDP this index identified 11 abnormal (10F;1M) and 49 normal CHDP. Whole blood acetate measurements (GC) showed that 44/49 (90%) of the normal CHDP had a normal I (mean = M=1.27 mM;F=2.98 mM). All the 11 CHDP with abnormal HCO_3 generation had an elevated I (mean : 5.74 mM, p <0.01) exceeding the normal 95% C.I. In the retrospective study 17/211 (<10%) in 6/11(55%) CHDP did not metabolize A optimally; women were affected more often than men(14/3); the anomaly occurred at the first HD, may fluctuate in severity with time and was not usually acquired with HD duration (up to 10 years of observation).

LIPO- AND APO-PROTEIN ABNORMALITIES IN CHRONIC HEMODIALYZED(CHD) PATIENTS WITH HYPERTRIGLYCERIDEMIA(HTG). Wakabayashi,Y.*, Shimada,H.*, Okubo,M.* & Marumo,F., Dept.Med., Kitasato Univ.Sch.Med., Sagamihara, Japan

Not all CHD patients showing HTG, our patients after a 2 year period of treatment with stable CHD, were divided into HTG(TG≥170mg%,n=10, age 45±12) and normo-TG(NTG,TG<170,n=9,42±10) groups and their lipo- and apo-protein composition was examined. Blood was collected following HD treatment preceded by a 14 hrs fast period. TG,T-chol.,VLDL and LDL were measured by the standard method. Serum apoproteins(Apo AI,B,CII &E) were measured by the single immunodiffusion method. Apo C subcomponents in VLDL were analyzed by SDS-PAGE. The conc. of TG,VLDL,T-chol. and LDL in the 2 groups (HTG vs NTG) were: 344±181mg% vs 127±31,p<0.001; 369±272mg% vs 76±46,p<0.002; 217±34mg% vs 154±17, p<0.001; and 727±67mg% vs 430±15,p<0.001, respectively. The ratio of AI/B, the atherogenic index, significantly decreased in HTG. In the apoprotein profile, although the serum conc. of Apo B,CII and E were elevated, the ratio of CII/VLDL and E/VLDL as well as TG/VLDL, significantly decreased in HTG compared with NTG. In NTG, CII/VLDL showed a strong inverse correlation with TG,r^2=0.7. Further Apo CII in VLDL significantly decreased, while CIII increased more in HTG than in the normal control. Accordingly, the ratio of CII/CIII decreased. These results indicate that the CHD patients with HTG were also suffering from deranged metabolism of cholesterol associated with the decreased ratio of AI/B. Secondly, they also indicate change in VLDL-apoprotein composition and function in HTG.

HIGH FLUX HEMODIAFILTRATION. B. von Albertini*, J. H. Miller, P. W. Gardner*, K. C. Norris*, C. E. Roberts*, and J. H. Shinaberger. Wadsworth VAMC and UCLA School of Medicine, Los Angeles, California.

Six chronic ESRD patients (wt 82.7±17.0 Kg, GFR < 1 ml/min) underwent 4 weeks of high flux hemodiafiltration (HDF, 3 x 2 hrs/week) and conventional hemodialysis (HD, 3 x 4 hrs/week). HDF was performed in a new mode: a pair of hollow fiber dialyzers (polysulfone 1.25 m^2, polymethylmethacrylate 2.1 m^2, cellulose acetate 1.8 m^2) were used in a serial or parallel configuration in the extracorporeal circuit to provide simultaneous high diffusive and convective solute removal. Sterile pyrogen-free bicarbonate dialysate was delivered in counter current mode by a volumetrically controlled automated system providing maximal ultrafiltration in one and simultaneous volume replacement by backfiltration of dialysate in the other device. In this configuration the entire surface area of both devices was available for diffusion. In HDF mean Q_B was 566±85, Q_D 1004±22 (Na 140, HCO_3 35), Q_F 112±38 ml/min and total Q_F/Rx 13.4 L. Control HD was performed with cuprophane 1.2 m^2 at Q_B 267±51, Q_D 500 (Na 140, Acetate 39 or HCO_3 35) ml/min. During the study the nutritional status was monitored by dietary surveys and urea nitrogen appearance rates and was unchanged. Results: Total mass removal of BUN, creatinine and phosphate, measured in dialysate, was similar in HD and HDF (n = 26).

pre Rx plasma n=48 (means ± SD)	BUN mg/dl	Creat mg/dl	Phos mg/dl	CO_2 mEq/L	Δ wt/Rx Kg
HDF (6 hrs/wk)	91±17	17.1±3	4.6±1.8	21.0±2*	2.7±1.2
HD (12 hrs/wk)	88±22	16.7±3	4.8±2.2	18.5±2	2.8±1.4

* p < .02

There were significantly fewer hypotensive episodes in HDF. Conclusions: 1) High solute and weight removal rates are well tolerated in HDF. 2) Compared to HD, HDF provides equivalent treatment in half the time.

DIGITOXIN LEVELS IN CHRONIC HEMODIALYSIS PATIENTS. J.A. Walker, R.A. Sherman, V.C. Walker,* G.B. Bialy,* R.P. Eisinger. UMDNJ-Rutgers Medical School, Dept. of Medicine, New Brunswick, New Jersey.

Spurious elevations in serum digoxin levels by radioimmunoassay (RIA) have been found in up to 63% of renal failure patients.

We investigated whether or not a similar problem exists with the RIA for digitoxin. Serum samples were obtained from healthy controls and patients receiving chronic hemodialysis; none was receiving any cardiac glycoside. RIA for digitoxin was performed on each sample utilizing two commercial kits according to the manufacturers' directions. All samples were assayed in duplicate; results from duplicate samples that differed by more than the assay's level of sensitivity (Kit A ≥1.5 ng/ml, Kit B ≥2 ng/ml) were excluded (Kit A: 3 patients, 0 controls; Kit B: 0 patients, 0 controls). The therapeutic range of digitoxin is 9-25 ng/ml. Results are summarized below:

Mean(±SD) Serum Digitoxin Levels (ng/ml)

Kit	n (Patients/Controls)	Patients	Controls
A	38/25	0.18±0.28	0.46±0.31
B	41/25	0.10±0.31	0.12±0.36

Therefore, it is unlikely that an endogenous digoxin-like substance in end-stage renal disease also interferes with the RIA for digitoxin. As measured by two different RIA kits, spurious elevation of serum digitoxin does not occur in chronic hemodialysis patients.

THE EFFECTS OF ULTRAFILTRATION (UF) OR DIALYSIS COMPOSITION ON DIALYSIS RELATED ACUTE PULMONARY HYPERTENSION (APH) AND HYPOXIA. J. Frank Walker,* Robert M. Lindsay, William J. Sibbald,* and Adam L. Linton. Univ. of Western Ontario, London, Canada.

Studies in sheep have shown that blood exposed to dialysis membranes causes APH, leukopenia and hypoxia. Sequential UF/dialysis or the use of bicarbonate dialysate (B) rather than acetate (A) in humans, has been reported to result in greater vascular stability and less hypoxia. We therefore examined the effect of isolated ultrafiltration, B or A dialysate on APH and PaO2 in 5 sheep with a Cuprophan HFAK.

Mean pulmonary artery pressure increased from 16 to 42 mmHg with UF, 16 to 39 mmHg with B and 17 to 38 with A over the first 10 minutes of dialysis ($p < 0.05$). The PaO2 decreased significantly from 96 to 81 mmHg with UF, 104 to 90 mmHg with B and 100 to 84 mmHg with A over the first 15 minutes of dialysis ($p < 0.01$). The use of UF or dialysate composition did not effect the degree of APH or PaO2.

These results suggest that the initial hypoxia of dialysis is associated with APH and is the direct result of a blood-foreign surface interaction. It is separate from the hypoxia found later in dialysis which may be caused by bicarbonate loss and, therefore, influenced by dialysate composition.

CENTRAL VENOUS CATHETERS FOR VASCULAR ACCESS IN CHILDREN: THE DEMISE OF THE SHUNT AND FISTULA? Alan R. Watson, Andre Bahoric,* and David Wesson*. Dept. of Pediatrics and Surgery, Univ. of Toronto and Hospital For Sick Children, Toronto, Canada.

Although CAPD is the dialysis treatment of choice for most children with CRF in our institution vascular access is still required for acute complications and at the time of transplantation (Tx). Evolving from our experience with central catheters for TPN we have developed a silastic (W-B-W) catheter which is placed at the SVC/right atrium junction under ECG control via a venotomy in the external or internal jugular vein. The catheter is available for immediate use and is filled with Heparin only at the end of dialysis or once weekly. It can easily be removed by simple traction. In 8 months the W-B-W catheter has been placed in 19 pts (age 1.8-19 yrs); weight 8.6-69 kg. Mean duration of catheter placement todate is 47 days (6-186) with average no. of dialysis treatments 17 (1-59). BUN clearances of > 2 mls/kg/min have been achieved with blood flows of 30-230 mls/min. Average recirculation in 7 pts was 15% (7-40%). Complications include 2 episodes of catheter related sepsis (both on immunosuppressants); intermittent blood flow requiring non-surgical repositioning (4 pts); accidental dislogement (2 pts); catheter breakage (successfully repaired) 2 pts. Other catheter uses have included post Tx CVP monitoring (6 pts); TPN (9 pts); antithymocyte globulin administration (2 pts) and multiple blood samples (13 pts).

We conclude that the W-B-W catheter offers pain free and relatively safe vascular access in children of all ages. With multiple possible uses and preservation of blood vessels it is the access of choice for acute and possibly chronic hemodialysis.

ELIMINATION OF A METALLO PROTEINASE DURING HEMODIALYSIS IN PATIENTS WITH ACUTE AND CHRONIC RENAL FAILURE. Christoph Wanner, Walter H. Hörl, Friedrich Thaiss, Peter Schollmeyer (intr. by Joel D. Kopple). Department of Internal Medicine, University of Freiburg, FRG.

The effect of different dialyzer membrane materials (cuprophan, polyacrylonitrile, polymethylmethacrylate, ethylene-vinyl alcohol copolymer, and cellulose hydrate) on the elimination of proteinases during hemodialysis was investigated in 26 patients with acute renal failure (ARF) and 40 patients undergoing regular hemodialysis treatment (RDT). Furthermore, the proteinase activity was characterized in vitro using azocasein and phosphorylase kinase as substrates in the absence and presence of different proteinase inhibitors. Proteinase activity of ultrafiltrates obtained from RDT patients was identical independent of the dialyzer used. However, proteinase activity of ultrafiltrates obtained from ARF patients was significantly enhanced using the dialyzer KF 101 (ethylene-vinyl alcohol copolymer). Digestion pattern of phosphorylase kinase revealed an identical type of proteinases in ultrafiltrates of ARF and RDT patients. The pH optimum of this proteinase was at alkaline pH. Proteinase activity could be inhibited in the presence of EDTA, whereas serine proteinase inhibitors were ineffective. Furthermore, the inactivated proteinase after Sephadex G-10 chromatography (in order to separate ultrafiltrate electrolytes and trace elements from protein) could be reactivated after the addition of Mg^{++} and/or Ca^{++}.

We conclude that a metallo proteinase can be eliminated during hemodialysis in ARF and RDT patients and that KF 101 is more effective in elimination of proteinase activity in ARF patients than other dialyzer membranes.

BI-WEEKLY VS. THRICE-WEEKLY HEMODIALYSIS: FIVE YEAR ANALYSIS OF MORBIDITY AND MORTALITY. Matthew R. Weir, John Josselson, John R. Bartholomew*, Gwendolyn Bolling*, Michael C. Yen, John H. Sadler Departments of Medicine, University of Maryland Hosp. and Maryland General Hosp., Baltimore, MD.

We reviewed the clinical data from 1/1/78 to 12/31/82 of two large Baltimore hemodialysis(HD) programs to assess morbidity and mortality of bi-weekly(2W) vs. thrice-weekly(3W) HD. One center exclusively dialyzed patients(194) using 2W whereas the other center (176 patients) used 3W. All patients maintained on chronic HD for a period of at least 3 months between 1/1/78 and 12/31/81 were included. Average(AVG) time on HD during the study for each patient was 34.5 months. Less than 10% were excluded for incomplete data. The two programs were well-matched for race, sex, and etiology of renal disease. AVG age was 51.7 years in 3W and 44.2 in 2W. AVG hours HD/week was similar: 11.2 in 3W and 12.6 in 2W. AVG monthly pre-dialysis BUN/Creat were similar: 83.5/15.3 in 3W and 86.1/16.2 in 2W. Mortality in the 3W program: 55 deaths/158 patients (34.8%) vs. 2W: 34 deaths/179 patients (19.0%). Morbidity in the 3W group: 1.8 AVG Hospitalizations/year/patient(H/YR/PT) and 18.7 AVG H-days/YR/PT compared with 2W: 0.8 AVG H/YR/PT and 7.2 AVG H-days/YR/PT. Access problems were similar with 3W having 1.0 access surgery(AS)/YR/PT and 2W having 0.8 AS/YR/PT. No patients had to withdraw from the 2W program for dialytic problems. We conclude that 2W HD is as efficacious as 3W HD and may be associated with less morbidity and mortality.

EFFECTS OF EXERCISE TRAINING (ET) ON PHYSICAL WORK CAPACITY OF HEMODIALYSIS (HD) PATIENTS (PTS): A CONTROLLED STUDY. GA Wolfe*, EI Feinstein, HJ Dwyer*, and SG Massry. Dept Phys Ther & Div Nephrol, USC Sch Med, Los Angeles, CA.

Exercise training is reported to increase physical work capacity and decrease serum triglycerides (TG) in HD PTS. To investigate further these effects we studied 21 HD Pts, 11 of whom underwent ET 3 times weekly for 8 weeks on a bicycle ergometer and 10 who did not (control). Symptom-limited graded exercise testing was done on a bicycle ergometer pre- and post-ET. Anaerobic threshold was determined by venous blood lactic acid changes. There was no difference between exercised and control groups in age, sex, hemoglobin, or duration of HD. Initially, exercised and control groups did not differ in peak oxygen consumption (VO_2) or anaerobic thershold but were 40 to 50% lower than in age-matched normal subjects ($p<0.01$). Control PTS showed no changes but exercised PTS fell into 2 groups: 6 who improved in day-to-day performance (Hi-Perf) and 5 who did not (Lo-Perf). There were more males in the Hi-Perf group ($p<0.05$). Hi-Perf had mean initial peak VO_2 of 1.55±0.67 L/min and anaerobic threshold of 0.96±0.19 L/min, compared to Lo-Perf, 0.93±0.24 L/min and 0.73±0.23 L/min ($p<0.05$). After ET Hi-Perf improved in peak VO_2 to 2.03±0.75 L/min and in anaerobic threshold to 1.56±0.49 L/min ($p<0.05$) while Lo-Perf did not (0.87±0.25 L/min and 0.54±0.20 L/min). Serum TG fell from 279±72 mg/dl to 244±102 mg/dl in Hi-Perf ($p<0.05$) but did not change in Lo-Perf. These data show that the effect of exercise training in HD PTS is not uniform and an improvement in physical work capacity and a decrease in serum TG levels occurs in about 50% of the patients.

UNTAPPED BLOOD FLOW RESERVE FOR DIALYSIS. D.T. Yamaguchi,* J.H. Shinaberger, B. Stabile,* and B. von Albertini. VA Wadsworth Medical Center, UCLA School of Medicine, Los Angeles, CA.

Efficiency of solute removal in dialysis depends greatly on the blood flow (Q_B) in the extracorporeal circuit. Q_B used in conventional dialysis rarely exceeds 300 ml/min. Actual Q_B in the vascular access was measured in 8 ESRD patients (4 PFTE grafts, 4 Cimino Brescia fistulae). All accesses were functioning for at least two years prior to the study. A non-invasive technique was used: vessel diameter and blood velocity were measured with a duplex ultrasonic sector scanner with pulsed Doppler capability along straight portions of the vessel. The diameter of the vessel was obtained by ultrasonic imaging and automatic caliper. Velocity profiles were obtained in the middle of each vessel by pulse-Doppler, Q_B was calculated for the product of the cross sectional area of the vessel and blood velocity. Each value is the average of measurements at 3 points (cm^3/min):

					$\bar{x}$ ±SEM
PFTE graft	575	1617	890	1200	1070±222
C-B fistula	2511	1731	1677	1800	1930±195*

*p<.05

Conclusions: 1) Mature fistulae provide higher Q_B than grafts 2) Blood flow is an underutilized resource to increase efficiency of solute removal in dialysis.

ACETATE INHIBITS KCl-STIMULATED CALCIUM INFLUX AND INCREASES CYCLIC AMP GENERATION IN RAT TAIL ARTERY STRIPS. M.C.M. Yang*, J.T. Daugirdas, P.K.T. Pang*. Texas Tech University Health Sciences Center, Lubbock, TX, and Hines-Loyola Medical Center, Hines, IL.

To study the mechanism of the vasorelaxant action of acetate in rat tail artery strips, the effects of acetate on calcium influx and cyclic AMP generation were explored. Calcium influx was assessed by determining the low affinity lanthanum-resistant pool of calcium. The effect of KCl-stimulation (60 mM) was first established. Then we measured the ability of D-600, a calcium-channel blocker, and of sodium acetate (16 mM) to inhibit KCl-stimulated calcium uptake.

In other experiments, generation of cyclic AMP was measured during incubation in the presence of theophylline. The control tissue bath used was a bicarbonate-buffered solution. In the acetate studies, 16 mM of chloride in the tissue bath were replaced by an equivalent amount of acetate.

KCl did stimulate calcium uptake in this model (control tissue/medium ratio: 0.37 ± 0.02; KCl: 0.73 ± 0.10, $p < 0.01$). KCl-stimulated uptake was inhibited by both 10^{-6}M D-600 (0.36 ± 0.02), and by acetate (0.48 ± 0.07).

Cyclic AMP generation was increased by acetate in both unstimulated vessel strips (10.7 ± 1.2 to 14.1 ± 1.3 pmole/g protein, $p < 0.01$) and in KCl-stimulated strips (10.7 ± 1.2 to 15.9 ± 1.9, $p < 0.01$).

The results suggest that acetate decreases KCl-stimulated calcium uptake and increases generation of cyclic AMP. Both actions are consistent with the vasorelaxant effects of acetate in this model.

ACETATE ANTAGONIZES VASOCONSTRICTION BY BOTH ALPHA-1 AND ALPHA-2 ADRENERGIC AGONISTS AND BY POTASSIUM CHLORIDE. T. Yang*, J.T. Daugirdas, D.J. Leehey, M. Lichter*, P.K.T. Pang, T.S. Ing. Hines-Loyola Medical Center, Hines, IL; Texas Tech Univ. Health Sciences Center, Lubbock, TX.

In an attempt to clarify mechanism of action, the effect of acetate on dose-response curves to the alpha adrenergic agonists, phenylephrine (alpha 1 & 2), methoxamine (alpha 1), and BHT-920 (alpha 2), was measured. To determine whether acetate antagonizes constriction dependent upon voltage-stimulated calcium entry, its effect on the dose-reponse curve to potassium chloride was determined.

16 mM acetate shifted all dose response curves to the right, BHT 920 > methoxamine; phenylephrine > KCl (all $p < 0.01$).

In other experiments, the calcium dependence of KCl-induced constriction (60 mM) in this model was demonstrated by recording a progressive increases in strip tension as bath calcium level was raised from 0 to 2.0 mM. Acetate shifted to the right the dose-response curve to calcium (in the presence of 60 mM KCl), but in a non-competitive fashion.

The results suggest that acetate does not selectively antagonize any one class of vasoconstrictor Although antagonism of constriction by KCl and BHT-920 might suggest inhibition of calcium entry, this mechanism of action is not consistent with: (a) failure of increased bath calcium to reverse inhibition of KCl-induced constriction, and (b) antagonism of vasoconstriction by methoxamine, an agent usually resistant to calcium entry blockers.

PSEUDOANGINA IN THE DIALYSIS PATIENT. Melvin Yudis, Robert A. Sirota and Harold D. Stein,* Dept. of Med., Abington Mem. Hosp., Abington, PA.

Coronary artery disease is a frequent complication in patients on chronic hemodialysis and is the major cause of death among these patients. We have recently seen a young female with ESRD on chronic hemodialysis develop unstable angina. Despite the strong likelihood of finding significant coronary artery disease, her coronary arteriogram was normal.

This patient is a 49 y.o. black female with chronic glomerulonephritis and hypertension as a cause of her ESRD. After 5 months on chronic hemodialysis, the patient developed a typical syndrome of angina pectoris. The pain initially responded to nitrates. Later unstable angina ensued which was refractory to high dose nitrates, beta blockers, and calcium channel blockers. Coronary visualization revealed the absence of any coronary artery disease or spasm.

This young female presented with typical angina pectoris initially responsive but later refractory to anti-anginal medication. Coronary arteriogram revealed normal coronary arteries. UGI revealed hiatus hernia, reflux, and an esophageal diverticulum. Her chest pain disappeared with the use of Carafate and Reglan.

Even though patients with ESRD on chronic hemodialysis commonly have coronary artery disease and angina as a cause for their chest pain, other etiologies must be considered and their diagnosis pursued. Gastrointestinal disease should rank high on this list. Proper diagnosis is important in relieving the patient of the psychological implications of coronary disease as well as in the appropriate treatment of the disease.

NON-INVASIVE ULTRASOUND-DUPLEX SCANNING (USDS) FOR THE DIAGNOSIS OF A-V VASCULAR ACCESS COMPLICATION IN THE HEMODIALYSIS POPULATION. Bernard S. Zoranski*, Harry G. Zegel*, Arthur R. Olshan, Allan B. Schwartz. St. Agnes Med. Ctr. and Hahnemann University Hospital, Depts. of Nephrology and Radiology, Philadelphia, Pennsylvania.

A non-invasive, rapid, reproducible sonographic technique was compared to invasive angiography to evaluate normal and abnormal AV grafts in hemodialysis patients.

Twelve (12) patients with clinically suspected AV graft abnormalities were studied: brachial artery AV graft, N = 9 (straight); femoral artery AV graft, N = 3 (looo). Results: arterial venous anastomosis stenosis - 1, mid graft stenosis - 1, venous anastomosis stenosis - 6, post venous anastomosis stenosis - 1. Perigraft collection - 3 (abscess - 1, seroma - 1, hematoma - 1).

Seventeen (17) clinically normal AV grafts were evaluated for control. Surprisingly, 2 grafts showed dampened doppler systolic amplitude flow abnormalities. Angiography confirmed early venous anastomosis stenosis which was then surgically corrected.

Summary: Ultrasound duplex scanning offers a safe, rapid, noninvasive technique for:

(a) diagnosis of AV graft structural and flow abnormalities suspected clinically; and (b) screening technique for all AV grafts to detect early unsuspected flow abnormalities and occult stenosis.

PERITONEAL DIALYSIS

PERITONITIS IN CHILDREN UNDERGOING INTERMITTENT PERITONEAL DIALYSIS(IPD) AND CONTINUOUS AMBULATORY PERITONEAL DIALYSIS(CAPD). H.Jorge Baluarte, Bruce Morgenstern,* Martin Polinsky,* Bruce Kaiser,* and Alan Gruskin. St. Christopher's Hosp. for Children Temple Univ. School of Medicine, Phila., PA.

Peritoneal dialysis (PD) was used to treat 66 children with end stage renal disease. Forty-four, ages 0.9-19.5yrs (mean 10.6)received IPD for periods ranging from 2-62mos (560 pt-mo) and 22, ages 0.8-17.8yrs (mean 10.9) CAPD from 2-42 mos(292 pt-mo). Peritonitis(P) was the major complication in both forms of dialysis but was twice as frequent in the CAPD group averaging 1 episode/5 pt-mo compared to 1 episode/9 pt-mo in the IPD group. Acturial analysis of the risk of having the 1st episode of P indicates that over 50% of the CAPD group had their initial episode by 3 mos and after 12 mos over 95% had at least 1 episode of P. In the IPD group 50% had their initial episode of P by 7 mos, and after 12 mos 75% developed P. Approximately 90% of the episodes were home acquired and 70% were successfully treated as outpatients. A relatively small group(30%)accounted for the majority(65%) of episodes of P. Gram positive organisms were responsible for 69% of P in the IPD and CAPD groups,respectively; gram negative organisms caused 6.5% of P in the IPD and 15% in the CAPD; fungal P caused 6.5% and 5% of episodes in the IPD and CAPD respectively, indicating no difference in etiology between the 2 modalities. P remains a significant problem in pediatric PD with a small group of pts responsible for the majority of episodes. Aside from new and innovative approaches to prevention of P a more strict selection criteria to enter the PD Program or an earlier change to an alternate modality would decrease the incidence of P.

RIFAMPIN INDUCED SUNSET DIALYSATE. R.L. Benz*, C.R. Schleifer, J. Raimondo, K. Read, H. Ziemek, Lankenau Hospital, Philadelphia, PA

Rifampin, a semisynthetic derivative of rifamycin-B, has been used clinically since 1966. Its major indications have included mycobacterium and resistant staphylococcal infections. Rifampin has been reported to cause an orange discoloration of multiple body fluids including urine, sweat, saliva, sputum and feces. Adequate CSF levels have been demonstrated but without record of discoloration.

We have used rifampin in 5 CAPD (Continous Ambulatory Peritoneal Dialysis) patients (4 for resistant staphylococcal peritonitis along with Vancomycin and 1 for presumed tuberculosis). In each case a discoloration of the peritoneal dialysate occurred which several patients noted to be sunset golden orange in description. Interestingly the discoloration began to fade after 24-48 hours despite persistent use of antibiotic. The reason for this clearing remains to be defined.

We conclude that patients on CAPD given rifampin should be alerted to expect dialysate discoloration. Further, patients should be instructed to expect the bags to clear up despite continued use of the antibiotic.

Such information will help avoid anxiety on the part of patient or staff that bleeding or infection is responsible for the change in dialysate appearance.

PERITONEAL CATHETER (PC) COMPLICATIONS AND SURVIVAL. M.H. Bierman, M. Hammeke, A. Kusek,* J. Kasperbauer,* R. Fitzgibbons, Jr.,* and J.D. Egan. Creighton Univ. Sch. Med., Omaha, Nebraska.

One hundred twenty-four end stage renal disease (ESRD) patients requiring 222 PC placements were retrospectively analyzed over 8 years. PC replacement was required for 107 failures; 91% of failures were due to malposition (38 episodes), obstruction (31), or peritonitis (28). Sixty-two per cent of failures occurred by the 8th week post-insertion, mainly due to malposition and obstruction. PC cumulative % survival by life table analysis was 46% at 52 wks and was not affected by sex, type of PC used (straight or curled), or use of x-ray during placement. The probability of replacement of the first PC was 25% at 4 wks and 25% from 4 wks to 52 wks.

Fifty-nine patients had diabetic ESRD. Total PC failures and failures due to peritonitis were less in diabetics. Diabetic PC cumulative % survival was greater than nondiabetic survival (55% vs. 39%, $p<.05$). Patients with multiple PC placements did not have an altered PC survival.

We conclude that PC malposition and obstruction account for early failures; the type of PC or use of x-ray during placement may not improve PC survival; PC failure does not alter future PC survival; PC survival is better in diabetics possibly due to less peritonitis.

PHENYTOIN DISPOSITION AND PROTEIN BINDING DURING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). T.J. Comstock*, D.A. Sica, T.B. Allison*, A.H. Harford, S.M. Lisby*. Medical College of Virginia, Depts. Pharmaceutics and Medicine, McGuire V.A. Hospital, Dept. Pathology, Richmond, Virginia

Phenytoin (P) is 90% bound to plasma proteins in normal serum and decreases to approximately 80% with uremia due to competitive binding from retained middle molecular weight peptides. In theory, CAPD will clear these larger molecules and may result in normalization of P binding. We determined the disposition and protein binding of P following a single IV dose of 5mg/kg in 6 pts. undergoing CAPD. Four exchanges were performed with dwell times of 6,4,4 and 8 h. Simultaneous blood and dialysate samples were obtained for determination of P in serum (total and free) and dialysate. Separation of free P was achieved with ultrafiltration at 37° C. Fractional binding (FB) of P was 0.78±0.05 (mean±SD) for 93 observations and is consistent with data from pts. with uremia as well as those undergoing hemodialysis. Calculated pharmacokinetic parameters based on fit to a two compartment model for free and total P were:

Parameter	Free P	Total P
Cl (total) l/h	13.04±4.5	3.19±.92
Cl (peritoneal) l/h	0.16±0.04	0.04±0.01
Vd l/kg	5.91±0.45	1.16±0.21
t 1/2 h	22.84±9.9	17.33±6.4

1.2% of the dose was removed by dialysis during the 24 hour study period. We conclude that 1) no consistent relationship exists between the end-of-dwell dialysate and serum concentrations. Removal of P by CAPD should not require dose adjustment, but the FB of 0.78 indicates caution be used in the interpretation of the total serum P.

SERUM IMMUNOREACTIVE ERYTHROPOIETIN (Ep) LEVELS IN PATIENTS ON CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). Manju Chandra, G. Clemons*, M. McVicar, P. Bluestone, and L. Mailloux. Dept. of Pediatrics and Medicine, North Shore Univ. Hospital, Manhasset, NY, & Cornell Univ. Medical College, and Lawrence Berkeley Lab, Univ. of California.

We examined the influence of CAPD on serum Ep levels to understand the mechanism for the improvement in hematocrit (Hct) observed in some uremic patients on CAPD. In 17 patients who were on CAPD, 73 determinations of serum Ep were made at monthly intervals over a 14 month period. Nine patients showed improvement in Hct by at least 10% over their pre-CAPD Hct values within 12 months of starting CAPD, while 8 patients failed to manifest such increase. Serum Ep levels were significantly higher (69.5 ± 5.5 mU/ml, Hct 35.5 ± 0.2%) in patients whose Hct improved as compared to those who did not show increase in Hct (Ep 35.1 ± 1.8 mU/ml, Hct 27.5 ± 1.2%, $p < 0.01$). Serum creatinine, SGPT and duration on CAPD were similar in both groups. Two patients showed higher serum Ep levels of 212 and 59.6 mU/ml while on CAPD as compared to their Ep values of 21.8 and 8 mU/ml respectively, obtained on a different mode of dialysis. A ten-fold or greater elevation in serum Ep over the normal control value of 18.5 ± 0.7 mU/ml was observed in 3 patients within 13 weeks of starting CAPD. We conclude that (1) the improvement in Hct observed in some patients on CAPD may be related to increased availability of Ep, and (2) CAPD facilitates increased Ep production. We hypothesize that the latter may be related to improved clearance of certain uremic toxins that may blunt the sensor mechanism for Ep production.

COMPARISON BETWEEN HEMODIALYSIS AND CAPD IN THE SAME PATIENT POPULATION. M.A. Datzman*, S.R. Acchiardo, A.P. Kraus*. Univ. of Tennessee Center for the Health Sciences, Memphis, Tennessee.

Clinical and biochemical parameters were measured in 18 patients (15 female and 3 male, ranging in age from 22 to 66 yrs) who had undergone both CAPD and hemodialysis (HD) for a minimum of 5 months on each mode of therapy. Twelve patients were changed from HD to CAPD due to access problems (2 patients) or patient preference (10 patients). Six patients initially on CAPD were transferred to HD secondary to patient noncompliance with the dialysis regimen and/or multiple episodes of peritonitis. The mean values for Hgb (10.0 vs 7.5 gm%), Hct (29.2 vs 21.7%), cholesterol (212 vs 182 mg/dl), and CO_2 (23.3 vs 20.2 mmol/l) were significantly higher while on CAPD. The total protein was significantly lower on CAPD although serum albumin (3.4 vs 3.7 gm/dl) was not significantly different. There was no significant difference in the urea nitrogen, creatinine, calcium, phosphorus, alkaline phosphatase, triglyceride or uric acid values. Six of the 10 patients requiring antihypertensive medication on HD were able to discontinue these on CAPD and 1 was able to decrease the amount of antihypertensives. Body weight tended to increase on CAPD but did not reach significance. The average number of hospital days/year was greater while on CAPD, (23.4 vs 8.0 days/yr) but in the 6 patients who were placed on HD due to noncompliance, there was not a significant decrease in their hospitalization requirement when changed to HD. Therefore, treatment with CAPD offers advantages by improvement in hypertension and anemia but is commonly complicated by lower total protein levels and more frequent hospitalization.

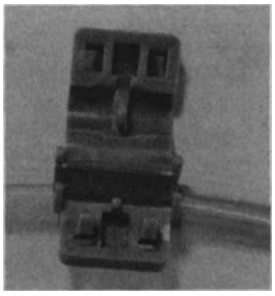
SAFE, SIMPLE INEXPENSIVE DISCONNECTING DEVICE FOR CCPD. Jose A. Diaz-Buxo, Donald A. Kay*, Kenneth L. Holt*. Metrolina Kidney Center, Charlotte, North Carolina.

A simple, disposable, plastic device was developed to allow termination of CCPD in one quick step without the need for aseptic control or disinfectants. The device is used in the morning to occlude the patient-cycler line distal to its connection with the PD catheter. The line is then cut with unsterile scissors. The procedure takes less than 1 minute and saves the cost of a daily sterile tray (approximately $1,500/yr).

Microbiological studies were done using 10 sets of sterilized 24" segments of PVC tubing clamped in the middle to form 2 chambers. One chamber was inoculated with a pure broth of the microorganism (S. aureus, E. coli, P. aeruginosa, S. marcescens, and control). Each organism was incubated for 14 days at 30-35°C. No growth was observed in any of the sterile chambers.

Pressure testing across the clamped segment was performed in 20 sets at 20 psi without a pressure drop. Ten sets were filled with an India ink solution and subjected to 20 psi without evident leaks.

Four patients used the device for 150 patient days. No episodes of peritonitis or malfunction were reported. Patient acceptance was excellent due to time savings and simplicity.



DECREASED FIBRONECTIN (FN) SECRETION BY PERITONEAL MACROPHAGES (PM) IS ASSOCIATED WITH A HIGHER INCIDENCE OF PERITONITIS AMONG CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD) PATIENTS, C.S. Goldstein, E.G. Neilson, R.A. Polin*, J.S. Gerdes*, S.D. Douglas*, Univ. of Penn., Phila., PA

We investigated the role of the opsonic glycoprotein FN in the host defense of the peritoneum in patients undergoing CAPD. FN promotes mononuclear phagocyte clearance of biologic particles and has a binding site for staphylococci. FN concentration in peritoneal dialysate from high infection rate CAPD patients (>1.80 episodes peritonitis per year) was significantly less than from low infection rate CAPD patients (<0.48 episodes peritonitis per year); 0.96 ± 0.10 vs. 1.35 ± 0.10 ug/mg total protein, $p<0.01$. Plasma FN in all CAPD patients was significantly less than in healthy normal volunteers (244.5 ± 39.0 vs. 328.8 ± 14.5 ug/ml, $p<0.001$), but there was no difference between plasma FN in low and high infection rate cohorts. PM from CAPD patients with high infection rate produced significantly less FN than PM from CAPD patients with low infection rate by days 10 and 14 in culture, $p<0.05$. Additionally, PM from CAPD patients with high infection rate produced less FN than PM from healthy volunteers on day 7 ($p<0.001$), day 10 ($p<0.01$) and day 14 ($p<0.01$) of culture. PM from CAPD patients with low infection rate produced less FN than healthy volunteers only on day 7 ($p<0.001$), but not by days 10 and 14 of culture. Thus, the decreased capacity of PM from high infection rate CAPD patients to secrete FN may be an important factor in the increased susceptibility to infection.

SELECTIVE INCORPORATION AND RELEASE OF EICOSANOID (EIC) PRECURSOR FATTY ACIDS (FA) BY PERITONEAL MACROPHAGES (PM) FROM PATIENTS ON CHRONIC AMBULATORY PERITONEAL DIALYSIS (CAPD). R Garrick*, JS Bomalaski*, CS Goldstein, EG Neilson, SD Douglas* RB Zurier*. Univ. of Penn., Phila., Pa.

PM participate in peritoneal host defense. Mononuclear phagocytes elaborate chemoattractants that interact with membrane phospholipids (PL). Leukocyte chemotactic responsiveness is related to synthesis of methylated phosphatidylcholine (PC) and EIC production. PM from CAPD have increased in vitro chemotaxis compared to PM from women undergoing tubal ligation (TL-PM). In order to further investigate this difference we studied the uptake and incorporation of FA into membrane-PL. Uptake by CAPD-PM of radiolabeled EIC precursor FA linoleic acid (LA), dihomogammalinolenic acid (DHLA) (precursor to PGE_1) and arachidonic acid (AA) (precursor to PGE_2) is less than uptake by TL-PM:

Precursor Uptake (% per 10 µg cell protein ±SEM)

PM	N	LA	DHLA	AA
TL	10	12.7±0.9	19.6±2.2	15.4±3.0
CAPD	7	8.0±1.5*	8.3±1.7*	9.4±1.5*

*p<0.01

CAPD-PM incorporated significantly less LA and AA into PC than TL-PM, but DHLA incorporation was similar for both groups:

FA Incorporation into PC (% total radiolabel)

PM	N	LA	DHLA	AA
TL	4	53.5±24.5	65.6±3.2	54.7±16.8
CAPD	5	8.9± 6.3	71.5±7.2	13.3± 3.7

Stimulation of CAPD-PM with calcium ionophore A23187 resulted in preferential hydrolysis of LA from phosphatidylethanolamine, the immediate methylation precursor of PC. EIC precursor incorporation into PC may be an important determinant of the chemotactic and functional response of PM in CAPD patients.

EFFECTS OF TIME, DIABETES AND HYPERTENSION ON PERITONEAL TRANSPORT. HANNELORE HAIN*, Ramesh Khanna, Harold Moore*, Karl Nolph. Univ. of MO., VA Hospital and Dalton Res. Ctr., Columbia, Mo.

In 27 patients treated with continuous ambulatory peritoneal dialysis (CAPD) peritoneal transport was assessed during the first week and at varying intervals thereafter. Initial and later measurements have been analyzed separately in patients with diabetes mellitus, patients with 5 - years of totally controlled hypertension prior to CAPD and, in non-diabetics with normal blood pressures. Transport measurements were determined during 2L long-dwell exchanges (200-300 min.) with 1.5% dextrose solutions. Measurements included ultrafiltration (UF), urea clearance (CUN), creatinine clearance (CCR), inulin clearance (CIN), dialysate protein (DPR), dialysate glucose (DG) & glucose absorption per exchange (GABS). The results overall are shown in Table 1.

	UF	CUN	CCR	CIN	DPR	DG	GABS
	ml	ml/mn	ml/mn	ml/mn	mg/dl	mg/dl	mg/ex
initial	238	8.3	5.8	2.1	79	648	16
Last	94	7.5	5.5	1.9	65	605	18
P	.025	01	.001	NS	NS	NS	NS

The mean time on CAPD between studies was 11±1.3 SEM months. The findings do not suggest any significant changes in peritoneal area or permeability. Decreases in UF explain changes in CUN & CCR & may relate more to mechanics or compliance than membrane alterations. These findings suggest no significant effects of time on CAPD, diabetes or a history of hypertension on peritoneal transport during the first year of CAPD.

CHRONIC USE OF DOCUSATE SODIUM (DSS) IN PERITONEAL DIALYSIS. Lawrence J. Hak, Ralph H. Raasch*, J. Charles Jennette. Schools of Pharmacy and Medicine, Univ. of North Carolina, Chapel Hill, NC.

Previous studies from our laboratory have shown an increase in the peritoneal dialysis clearance of Cr, urea, phosphate, and protein when DSS was added to a single dialysis exchange (Kidney Int 1981; 20: 563; DICP 1983; 17:440). This study examines the effect of multiple doses of DSS on clearance and toxicity. Five 2-3 kg male New Zealand rabbits with normal renal function were dialyzed once weekly for 4 weeks. Each dialysis consisted of 4 exchanges of control fluid (Dianeal 1.5% with 4 mEq K/L), a 5th exchange of dialysate containing 0.02% DSS, followed by 3 exchanges of control fluid. Blood samples were obtained from the ear vein at the beginning of the 4th dialysis exchange of each treatment. At the end of the 4th dialysis treatment, the animals were sacrificed and tissue was immediately obtained from brain, lung, liver, kidney, spleen, small bowel and abdominal wall for histologic examination. Results:

Wk	Curea ml/kg/min Pre-DSS	Post	Protein Loss mg/kg/exchange Pre-DSS	Post	Serum SGOT	Serum Alk P'tase
1	.12±.08	.52±.10	10±7	28±6	12±6	47±39
2	.37±.14	.56±.21	18±10	35±8	10±6	59±68
3	.27±.09	.46±.15	14±10	29±9	13±15	48±50
4	.29±.09	.52±.11	15±8	32±9	9±2	60±71

Histologic studies revealed no morphologic evidence of toxicity in any of the tissues examined. These data indicate that when DSS is added to a single exchange of peritoneal dialysate, a significant increase in clearance occurs; the increase in clearance continues to occur with subsequent doses; and no toxicity is evident with this dosage regimen.

EFFECTS OF INTRAPERITONEAL AND INTRAVENOUS DESFERRIOXAMINE ON ALUMINUM REMOVAL DURING CAPD G. Hercz,* I.B. Salusky, D.S. Milliner, H.G. Nebeker,* & J.W. Coburn. Med & Resch Services, VA Wadsworth Hosp & Depts Med & Peds, UCLA Sch Med, Los Angeles, CA

Desferrioxamine (DFO) has been used to treat Al-related bone disease in uremic patients, including those on CAPD. The effect of DFO on Al removal during CAPD is not well defined. Therefore, we carried out 13 studies of Al removal after DFO infusion in 10 CAPD patients with Al-related bone disease. After DFO, 40 mg/kg intravenously (IV), plasma (P) Al rose from 309±182 (SE) to 688±279 ug/L after 24 hours, and total dialysate (D) Al increased from 529±383 to 1585±668 ug/24 hr (n=9). After intraperitoneal (IP) DFO, 40 mg/kg in the overnight exchange (duration, 8-10 hrs), PAl rose from 262±103 to 800±293 ug/L at 24 hrs and DAl removal increased from 434±162 to 1383±357 ug/24 hr (n=4); 2 patients have received DFO by repeated IP dosing without ill effects. When measured before and both 24 and 48 hrs after DFO, DAl removal rates were 266±111, 1197±334, and 1268±313 ug/24 hrs, respectively (n=7); Al removal the 3rd day after DFO was 1333±531 ug (n=3). The D/P Al concentration ratios increased from 0.17±0.04 before DFO to 0.30±0.03 afterwards ($p<0.05$). We compared the effect of DFO on Al removal during hemodialysis (HD): DFO, 40 mg/kg IV, increased Al removal from 320±80 to 2940±680 ug during a 4 hr HD. Prior to DFO, Al removal was greater with CAPD than HD at comparable PAl levels. Thus, DFO administration, via both IV and IP routes, markedly augments Al removal during CAPD by increasing both PAl and its diffusable fraction. The effect of DFO on Al removal persists for several days after one dose.

SIGNIFICANT REDUCTION IN PERITONITIS USING A PATIENT ASSIST DEVICE. Alan E. Handt, St. Vincent Hospital and Health Care Center, Indianapolis, Indiana and Stephen R. Ash, Arnett Clinic, Lafayette, Indiana.

During the past nine months we have accumulated a total of forty patient months using the PAD 200, (Abbott Patient Assist Device) in our peritoneal dialysis program. During this forty month period we have had no episodes of peritonitis. This rate is statistically lower than that of our general CAPD population. The patients represented include those with sight impairment, strength impairment and learning disabilities. The longest period of time that we have had someone on the PAD 200 has been nine months and the shortest period of time three months. There are many contributing factors to peritonitis in the general CAPD population. These include demographic characteristics, patient training, tubing changes, exit site infections and in some cases underlying disease to mention a few. We believe that breaks in technique during the bag exchange procedure is still a significant source of contamination and peritonitis. The use of an exchange device greatly reduces the possibility of inadvertent contamination or a break in the exchange steps that can lead to contamination.

PANCREATIC ENZYMES IN CHRONIC RENAL FAILURE (CRF). D.M. Jensen*, V.L. Royse*, H.L. Corwin. Department of Medicine, Rush Medical College, Chicago, IL

The diagnosis of acute pancreatitis in the setting of renal failure is difficult as both conditions can cause an increase in serum amylase and amylase/creatinine clearance. We have used 2 new assays, isoamylase fractionation by gel electrophoresis and lipase concentration [L] by RIA (NuClipase, Nuclin Diagnostics) to assay pancreatic enzymes in pts with CRF. [L] was also compared to a standard lipase activity method (DuPonts' ACA). Evaluated were 29 stable hemodialysis pts (HD), 13 stable peritoneal dialysis pts (PD), 4 PD pts with peritonitis, 1 PD pt with documented acute pancreatitis. We found: 1. Amylase increased in 86% & 61% of HD and PD pts respectively. 2. P3 isoamylase activity, which is considered more specific for pancreatitis, was increased in 48% HD & 33% PD pts. 3. [L] was increased in 68% of CRF pts vs 42% for lipase activity. Nine pts (5PD & 4HD) had an increased [L], but normal activity. 4. Mean results were elevated in all assays and there was no significant differences between the HD and PD groups. 5. Amylase and [L] were found in the PD fluid of the pancreatitis pt, but not in the fluid of stable PD or PD peritonitis pts. We conclude: 1. total amylase, P3 isoamylase and lipase concentration were similarly elevated in HD and PD. 2. Detection of amylase and lipase in PD fluid may be an indication of pancreatitis.

THE EFFECT OF DIFFERENT DIALYSIS MODALITIES ON CALCIUM AND PHOSPHORUS METABOLISM AND RENAL OSTEODYSTROPHY IN CHILDREN. Bruce Kaiser,*Allen Root,*Barbara Wolfson,*Martin Polinsky,*Alan Gruskin, Jorge Baluarte. St. Christopher's Hospital for Children, Temple University School of Medicine, Phila., PA.

Abnormalities of Calcium(Ca) and Phosphorus (P) metabolism and the progression of renal osteodystrophy(ROD)are major problems for children(Ch)with renal failure. Ch starting either hemodialysis(HD, n=17),intermittent peritoneal dialysis(IPD,n=15) or continuous ambulatory dialysis(CAPD,n=10) and remaining on the same modality for 9-12 mos.were compared for serum Ca,P,Parathyroid Hormone(PTH), the oral aluminum hydroxide dose(Al-d)and the radiologic deterioration of ROD. All Ch received either dihydrotachysterol or calcitriol.

Onset	Ca(mg%)	P(mg%)	PTH(pg/ml)	Al-d(mg/kg/day)
HD	8.8±.3	6.6±.4	1078±275	134±20
IPD	8.9±.4	6.1±.4	1733±381*	96±17
CAPD	9.1±.3	6.7±.7	513±95*	105±27
9-12mos				
HD	9.6±.2	5.8±.3	1606±337	105±20
IPD	9.5±.1	6.6±.5	1786±478	106±13
CAPD	9.9±.2	5.9±.4	1127±431	78±22

$\bar{x}$SEM; Significantly different between group*p<.05

ROD progressed in 12 of 17 Ch on HD,6 of 15 on IPD and 6 of 10 on CAPD, and there was no statistical difference between modalities. Although some parameters of Ca and P metabolism improved,the changes were not significant,ROD progressed in the majority of Ch,and there was no advantage to any specific dialysis modality. Therefore, earlier medical therapy and early transplantation may be the best approach to normalize Ca and P,and improve ROD, since dialysis offers little benefit.

CARBAMOYLATION OF PLASMA PROTEINS IN CAPD AND HD. A.P. Kraus, Jr.,* M.S. Stephens,* and L.M. Kraus* (Intr. by F.E. Hatch). Univ of Tennessee Ctr. Health Sci., Depts. of Med. and Biochem., Memphis, Tennessee.

Effects of ESRD upon the post translational modification of blood protein was compared in CAPD and HD. Urea in solution is in isomeric equilibrium with cyanate which reacts under physiologic conditions to irreversibly modify available amino groups on proteins. This modification was reported in a comparative study of pts.on CAPD or HD where a significantly lower level of carbamoylated Hb was found in pts on CAPD (Fed Proc. 43: 1578,1984). Plasma from 17 HD pts and 13 CAPD pts was fractionated by non-denaturing polyacrylamide gel electrophoresis revealing differences in the separation of the albumin and gamma globulin indicating molecular microheterogeneity in these proteins. Normal whole blood was then incubated with ^{14}C cyanate and the plasma isolated and fractionated in the same system as the pt plasma resulting in a similar pattern of microheterogeneity. Radioactivity was found in the albumin fraction (37%) and in the gamma globulin fractions (19%) indicating carbamoylation of these proteins. When plasma electrophoresis from HD pts and CAPD pts was compared at the initiation of ESRD therapy similar abnormal patterns were found. However, when comparison is made after 10-12 months of therapy less carbamoylation is found in the CAPD pt. Carbamoylation has been shown to alter the biologic activity of Hb and insulin. The decrease in carbamoylated protein found in the CAPD population may play a role in some of the clinical differences between pts maintained on CAPD and those on HD.

THE VARIOUS SPECTRUM OF ULTRAFILTRATION FAILURE (UFF) AND ITS PATHOGENESIS AMONG CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD) PATIENTS (PTS.) D. Kim,* G.C. Madubuko,* F. Thome,* D.C. Cattran and S.S.A. Fenton*. Toronto General Hospital, Toronto, Canada.

Nine of our 126 CAPD population, unable to achieve their ideal weight despite frequent uses of 4.25% solutions and 9 controls (C) were studied. Following an overnight dwell, a complete drainage obtained and a flush test confirming a good catheter function, dwell times of 4, 2 and 1 hrs. were utilized in succession with 2 litre, 4.25% Dianeal^{R} solution. Aliquots of dialysate and blood samples were taken hourly. UFF was defined by ultrafiltrate volume (UFV) less than the mean of C-2SD. Group I and II had a true UFF whereas Gr.III had an excessive fluid and salt intake without UFF.

TABLE I ULTRAFILTRATE VOLUME (ml)

	1HR.	2HR.	4HR.	D/P(Cr)	D/P(UREA)
Gr.I(4pt)	307±149	282±88	177±83	.78	.96
Gr.II(1pt)	-	-	40	.59	.68
Gr.III(4pt)	523±221	432±117	640±59		
C(9pt.)	487±213	608±112	773±125	.74	.98

*D/P represents dialysate/plasma ratio at 4 hrs.

TABLE II OSMOLARITY IN DIALYSATE

	1HR.	2HR.	3HR.	4HR.
Gr.I(4pt.)	380±20	356±15	333±20	307±55
Gr.II(1pt)	450	435	420	409
Gr.III(4pt)	373±18	335±23	330±9	318±10
C(9pt.)	357±57	324±44	308±41	298±41

All pts. with UFF had been on CAPD for longer than 4 yrs. In conclusion, UFF can be categorized into 2 groups. 1)UFF with normal solute clearance despite an osmotic gradient comparble to C. 2)UFF with impaired solute clearance. This may be related to a loss of peritoneal membrane surface area.

ELEVATED RENIN ACTIVITY (PRA) ASSOCIATED WITH INCREASED CATECHOLAMINES IN CHRONIC AMBULATORY PERITONEAL DIALYSIS (CAPD). D. Narendra Kumar*, Paul M. Zabetakis, Mark H. Gardenswartz, Gilbert W. Gleim*, Meenakshi Agrawal*, Michael F. Michelis. Nephrology Section, Dept. of Med., Lenox Hill Hospital, New York, New York.

Based on our previous data suggesting fluid excess as a cause for hyporeninemia and hypoaldosteronemia in hemodialysis patients (HD) (J Lab Clin Med 96:734, 1980), the use of PRA and plasma aldosterone levels (PA) as an index of volume status appeared warranted. Since patients on CAPD are reportedly less hypervolemic than HD patients, 6 patients on CAPD were studied after 9.2±1.2 mo. Markedly elevated levels of PRA 10.2±2.9(nl 1-5 ng/ml/hr) and PA 47.4±16.2(nl 5-20 ng/dl) were observed.

Despite the increase in PRA and PA, no apparent decrease in plasma volume(PV) was observed in CAPD pts:PV 3620±358(nl 3083±200 ml).In 4 pts. data obtained post-HD and later on CAPD could not entirely explain the increases in PRA and PA. HD vs CAPD:PRA 0.9±0.3 vs 14.1±4.6(P<0.05),PA 3.4±0.3 vs 67.4±24.9(P<0.05),Glucose 95.3±6.7 vs 102.3±6.3(P<0.05),WT 76.9±8.3 vs 74.0±7.9 kg(NS), Hct 23.4±1.3vs 33.6±5.1%(NS). However, elevated catecholamine levels were seen in 5 CAPD pts.

(*P<0.01):	CAPD	HD	NL
NE,pg/ml	868.0±104.4*	400.5±104.5	358.4±41.5
Epi,pg/ml	386.3± 49.2*	7.1± 5.1	58.3±10.6

The data suggests that an elevation in PRA and PA can occur in CAPD patients despite the absence of volume contraction.Augmented sympathetic stimulation due to glucose loading may explain this. Unlike HD patients, changes in PRA and PA cannot be used in CAPD to reliably assess volume status.

IMPAIRMENT OF ULTRAFILTRATION WITH ACETATE BUFFERED PERITONEAL SOLUTIONS MADE IN FRANCE. M. Kwong*, G. Wu*, H. Rodela*, L. Brandes*, R. Ogilvie*, H. Husdan* and D.G. Oreopoulos. Toronto Western Hospital, Toronto, Canada.

Using the radioidinated albumin dilution technique we studied hourly ultrafiltration in normal rabbits (n=9-12) undergoing 5 hour dialysis with either a lactate solution (Dianeal Travenol) or two acetate ones (McGaw USA-Aguettant France). Hypertonic (4.25%) lactate solution produced a significantly ($p < 0.005$) higher UF (37 ± 19 ml) than the French acetate solution (9 ± 23 ml). The US acetate solution produced UF that was between that of the other two brands (22 ± 19 ml) not statistically different from either of them. The glucose absorbed during the first hour was 1782 mg for the French-acetate solution, significantly higher than the amount absorbed with the lactate (1404 mg). Again the USA acetate provided values between them (1584 mg). We did not observe any difference in the hourly dialysate osmolality and glucose concentration levels among the 3 brands. Our results indicate that acetate solutions made in France produce lower UF than lactate solutions made in Canada probably as a result of a vasodilation causing higher glucose absorption. The different behaviour between French and USA acetate solutions suggest that factors other than acetate may be responsible for this effect.

PERITONEAL ULTRAFILTRATION IS INCREASED BY AMPHOTERICIN B, NOT BY ITS SOLVENT. J.F. Maher, P. Hirszel,* E. Chakrabarti,* R. R. Bennett,* Dept. of Med., Uniformed Serv. Univ. Health Sci., Bethesda, MD

Intraperitoneal (ip) instillation of commercially prepared amphotericin B (Amp) was recently shown to selectively augment peritoneal osmotic ultrafiltration (UF) without affecting solute transport appreciably. To exclude the solvent, Na desoxycholate (des), accounting for the changes we conducted peritoneal dialyses in rabbits with and without ip des. With 1 mg/kg of des UF decreased from 0.33 to 0.21 ml/kg/min ($p<0.01$, n=4), but 10 mg/kg had no effect (0.31 vs 0.30, n=4). The low dose changed clearances (C) inconsistently. With 10 mg/kg of des, C_K rose from 1.02 to 1.46, C_{urea} from 0.71 to 1.25, C_{PO_4} from 0.30 to 0.78 and $C_{dextrose}$ from -0.38 to -0.65 (ea. ml/kg/min, & $p>0.01$) suggesting that loss of gradient induces decreased water flux. Des caused pain. As dissolving Amp in DMSO or alcohol raised dialysate osmolality such studies were abandoned. Powdered Amp (10-15 mg/kg) was added directly to dialysis fluid and instilled ip as a suspension. This did not affect ($p>0.1$, n=8) C_K (1.05 vs 1.21), C_{urea} (0.80 vs 0.93), C_{PO_4} (0.37 vs 0.31) or $C_{dextrose}$ (-0.31 vs -0.37) ea. ml/kg/min, but raised UF from 0.31 to 0.44 ml/kg/min ($p<0.02$). Amp, itself raises peritoneal UF. The solvent des causes peritoneal irritation and a nonspecific rise in C. Amp should be prepared for clinical ip use by dissolving it in a non irritant.

TSH RESPONSE TO TRH STIMULATION IN PATIENTS ON CAPD. H.B.Lee, C.H.Kim*, H.Na*, M.H.Yoo*, and S.D.Hwang*. Dept. of Intern. Med., Soon Chun Hyang Univ. Hosp., Seoul, Korea.

TSH response to TRH stimulation has been reported to be decreased in magnitude and delayed in time in both patients with chronic renal failure (CRF) not on dialysis and patients on chronic hemodialysis (HD). Similar data are not available in patients on CAPD. TRH (0.2mg) was administered iv. to 6 healthy adults (NC), 10 patients with CRF not on dialysis, and 10 patients on CAPD. Blood was drawn before and 30,60,90,120,180, and 240 min. after TRH and the samples were analyzed for TSH, T3, T4 and FT4. Baseline TSH level was normal (range: 1.4-5.4 μU/ml) in all 20 uremic patients but tended to be higher in CRF (2.11±0.80 μU/ml) and significantly higher in CAPD patients (3.12±1.08 μU/ml, $P<0.05$) than in NC (1.63±0.79 μU/ml). The ratio of maximal increment of TSH after TRH over baseline TSH (Δ max/B) was significantly lower in CAPD patients than in NC (1.94+ 0.61 vs. 4.41±2.73, $P<0.05$). Δ max/B for CRF was 2.44±1.27 and was not different from that of NC or CAPD patients. Peak TSH level was observed at 30 min. after TRH in all 6 NC, 7 CAPD and 5 CRF patients. Two of the 3 CAPD patients and 3 of the 5 CRF patients with delayed response achieved peak level at 60 min., 2 CRF patients at 90 min. and one CAPD patient at 120 min. In conclusion, patients on CAPD showed submaximal and delayed TSH response to TRH as was observed in patients with CRF not on dialysis and those on HD.

CAPD IMPROVES INTRACELLULAR AMINO ACID LEVELS AND PROTEIN SYNTHESIS, J. Metcoff, J. Pederson, F. Llach, Univ. Oklahoma Health Sc Ctr and Veterans Admin Hosp Oklahoma City, Ok.

ESRD patients stabilized by hemodialysis have intracellular (IC) deficits of essential amino acids (AA) and reduced protein synthesis (PSyn= ^{3}H-Leu incorporation), using the circulating granulocyte (LEUK) as a cell model (Kidney Intl 24:S-87-92, 1983). Neither IC levels of AA nor of PSyn, were significantly improved by 3 months of a standard AA infusion + the 3x/wk hemodialysis. Bergstrom et al (Per Dial. Bull, 1984) reported most plasma AA levels decreased, with ASP, ARG, GLU and CIT elevated, in 17 CAPD patients vs controls. In muscle, TYR and TAU were low, with LYS, ASP, GLU and CIT high. VAL was not reduced. PSyn was not measured. We studied 13 adult CAPD patients and 30 normal controls, concurrently. AA in plasma and cells (LEUK) were determined by HPLC with fluorometric detection; PSyn by (^{3}H-Leu incorp) and cell water by weight and ^{14}C inulin. Compared to controls, PSyn was low (2158 vs 2725 pmoles/hr/mg DNA, $p<.02$). Most plasma essential AA were low, while ASP, GLU, GLY and CIT were high. In cells, VAL was low and GLU high (all $p<.04$). The IC-AA concentrations differed significantly ($p<.05$) (usually higher) from their plasma concentrations. 7 of the CAPD patients had repeat studies after 3-4 months. Plasma AA levels were not significantly changed. However, PSyn was normalized, (3265 pmoles/hr/mg DNA) as were most IC-AA's except for LYS, GLU, GLY, ALA and ARG, which were increased. VAL was normal. Thus IC-AA levels and PSyn may be normalized by CAPD without apparent improvement in plasma AA levels, confirming the dissociation between the two compartment pools. CAPD for at least 6-8 months appears to improve IC-AA patterns and PSyn.

IS FAT ABSORBED FROM THE PERITONEUM? A. Mitwalli*, H. Rodela*, L. Brandes*, I. Wanless*, G. Wu*, R. Ogilvie*, H. Schilling* and D.G. Oreopoulos. Toronto Western Hospital, Toronto, Canada.

A 10% fat solution (Liposyn) was infused (50 ml/kg) into the peritoneal cavity of 4 normal rabbits where it was allowed to dwell for 5 hours. We studied changes in the intraperitoneal volume using the radioactive albumin dilution technique. After an initial ultrafiltration, that peaked at 1 hour, the solution started being absorbed and approximately 30% of the initial volume was absorbed at 5 hours. At the end of the infusion, dialysate fat concentration decreased to only 85% of the initial value but the absolute amount of fat absorbed was approximately 40% of the infused amount. Plasma total fat increased from 0.16 ± 0.03 to 1.35 ± 0.49 g/100 ml and plasma TG increased from 1.07 ± 0.65 to 14.10 ± 7.23 mM/L. We observed no histological changes in the liver of the rabbits. Our data indicate that fat emulsion is absorbed through the peritoneum slowly as the infused volume is absorbed. Under these conditions the solution cannot be used as a dialysis solution, but may be used in patients with normal kidney function who have no vascular access for total parenteral nutrition. A mixture of fat with glucose and amino acids may overcome this problem and allow ultrafiltration with simultaneous fat absorption.

CAPD IN PATIENTS (PTS.) WITH POLYCYSTIC KIDNEY DISEASE (PKD): A.R. Nissenson, D.E. Gentile, R.E. Soderblom, C. Brax and the Medical Review Board, NCC #4, Los Angeles, Ca.

It has been suggested that Pts. with PKD have more peritonitis and higher drop-out rates than others on CAPD. No large series of such patients has been published, however. As of 12/31/83, 775 Pts. had started on CAPD in NCC #4, 62 of whom (8%) had PKD. Total experience on CAPD for PKD Pts. was 914 mos., while mean time on CAPD was 15 mos. (range 0.5-44). 55% of Pts. were over 45 at initiation of CAPD while only 5% were ≤20 years old. Life table analyses of selected outcome variables revealed:

	6 mo	12 mo	18 mo	24 mo	30 mo
Pt. Survival	98	93	93	85	76
Technique Success	83	73	54	44	26
1st Episode of Perit.	33	42	55	58	62
1st Hospitalization	34	51	64	71	71
Catheter Replacement	3	7	12	15	19

PKD Pts. spent 18 days per year in hospital, 8 of those for peritonitis (44%) and 5 for other CAPD-related problems. Of those switching to hemodialysis, 45% did so because of peritonitis, exit site or tunnel infection, 21% because of patient or family choice, and 7% because of inadequate control of uremia.

Compared to other CAPD Pts., those with PKD have similar overall survival, peritonitis frequency, and technique success. The percent of Pts. remaining free of hospitalizations or catheter replacements was significantly better in PKD vs other CAPD Pts. More PKD than other Pts. switched to HD, however, because of patient or family choice. PKD Pts. do as well as others on CAPD and should not be excluded from this treatment option.

SCLEROSING PERITONITIS ON CAPD;THE ACETATE-LACTATE CONTROVERSY. L.H. Nielsen*, K.D. Nolph, R. Khanna and H. Moore*. Univ. of Mo. Dept. of Med. and Dalton Research Center, Columbia, Missouri.

Acetate has been implicated in sclerosing peritonitis and associated with rapid glucose absorption with low ultrafiltration rates in patients on peritoneal dialysis. Most patients are now dialysed with lactate solutions.

We designed a rat model to study the effect of commercially available buffers on the peritoneum during 3 months of CAPD. All rats, when spontaneously infected, were immediately treated with a standard protocol of cephalotin and tobramycin. Some results from this study are indicated below:

Study Group	Acetate	Lactate	P
# Rats	7	6	
# CAPD days	675	598	
# infections/rat	5.1	5.5	NS
# days pos. culture/infection	5.2	1.8	<.05
# days cloudy drainage/infect.	17.8	4.0	<.02
Dialysate protein mg/dl/@ 90 d.	526	224	<.02
Glucose absorption mg/dl/@90d.	1208	543	<.05
Ultrafiltration ml/min/@90 days	4.0	4.7	NS

On sacrifice 4 of 7 rats in the group dialysed with acetate were found to have sclerosing, encapsulating peritonitis. No rat in the lactate group showed any gross pathology. By light microscopy rats from the acetate group showed marked disruption of normal mesothelial architecture.

In summary, we have carried out peritoneal dialysis in a small animal for 90 plus consecutive days. Acetate was associated with protracted infections and more profound functional and morphological membrane alterations than was lactate.

FERROKINETICS IN PATIENTS ON CAPD: INFLUENCE OF CAPD ON THE ANEMIA OF UREMIA. H.S.Park*, S.W.Koh*, S.K.Hwang*, C.H.Lee*, and H.B.Lee. Dept. of Intern. Med., Soon Chun Hyang Univ. Hosp., Seoul, Korea.

Anemia improves in many ESRD patients (pts) while on CAPD and the reported mechanisms include higher serum erythropoietin level, increased red cell mass, and decreased plasma volume (PV). Ferrokinetic studies were carried out utilizing 59Fe-citrate in 5 healthy volunteers (NC), 6 pts. with iron deficiency anemia (IDA), 6 pts. with chronic renal failure (CRF) not on dialysis, 7 pts on CAPD in whom Hct was 30% or over (CAPD I) and 4 pts. on CAPD with Hct less than 30% (CAPD II) to evaluate the role of iron metabolism in the improvement of anemia in CAPD pts. PV was measured by use of 59Fe-citrate and red cell volume (RCV) was calculated. Hct was significantly lower in IDA (28.03±5.09) and 3 uremic groups when compared to NC (44.4±0.89) and was significantly higher in CAPD I (32.4±3.08) than in CRF (23.82±3.42) and CAPD II (24.88±1.84). IDA had significantly faster plasma iron disappearance rate (PID), higher red cell iron utilization rate (RCIU), red cell iron turnover rate (RCIT), and marrow transit time (MTT). There was no difference in PID, RCIU, RCIT and MTT between NC and 3 uremic groups. PV was significantly smaller in CAPD I (40.06±7.62ml/kg) when compared with UC (53.33±8.26 ml/kg) and CAPD II (57.71±6.36 ml/kg) but was not different from NC (39.72±4.21 ml/kg). RCV was larger in CAPD I than in UC and CAPD II but the difference did not reach significance. In conclusion, the improvement of anemia in CAPD is not related to improved iron metabolism but is, at least in part, due to decrease in PV.

PLASMA iPTH LEVELS AND INHIBITION OF IN VITRO ERYTHROPOIESIS BY UREMIC SERUM. A. Pierratos*, P. Toor*, D.G. Oreopoulos and A. Keating*. Toronto Western Hospital, Toronto, Canada.

Inhibition of erythoid colony (BFU-E, burst forming unit-erythoid; CFU-E, colony forming unit-erythoid) growth in vitro by uremic serum has been previously demonstrated. The in vitro effect of iPTH (immunoreactive PTH) on the inhibition of BFU-E however remains controversial. We studied the possible correlation between the plasma level of iPTH (normal range 3.1 - 25.1 pM/L) from patients on CAPD and the in vitro inhibition of BFU-E by their sera. We examined 12 patients (group A) with high levels (141±129; X±SD) and 14 patients (group B) with low levels (9.6±8.9) of iPTH. BFU-E were grown from the peripheral blood of a normal volunteer in the presence and absence of the serum of each patient. There were no differences in the etiology of renal failure, renal function, duration of dialysis, frequency of blood transfusions or hemoglobin levels between the two groups. We found no correlation between hemoglobin level and iPTH concentration. Mean BFU-E growth was 83±20.6% of the normal control for group A and 98.7±28.3% for group B. Thus we found no correlation between BFU-E growth and iPTH levels ($p>0.1$). These results suggest that iPTH is unlikely to contribute to the inhibition of erythropoiesis by uremic serum.

FACTORS INFLUENCING ACID BASE (AB) BALANCE IN ESRD PATIENTS ON IPD. J.M. Roscoe and R.M.A. Richardson Dept. of Medicine, Wellesley Hospital and Toronto General Hospital, Univ. of Toronto, Toronto, Ont.,

This study was designed to assess some of the factors influencing AB balance in ESRD patients on IPD. Two of these factors may be the level of dietary protein intake and net dialysis buffer gain. Eight evaluations of intake were obtained via carefully supervised diet histories in 5 stable IPD patients. Eight IPD balance studies were performed on the same patients using standard IPD technique, (20 hour biweekly treatment; 2 litre; ~ 35 minute exchange). Total dialysate volumes varied between 54 to 64 litres per treatment. Dialysate lactate concentration was 35mM.

Patients were acidemic with a mean predialysis pH of 7.27, and mean plasma HCO_3 of 17mM. Mean total protein ingestion was 70g/day (1.3g/kg) and correlated positively with plasma H^+ ($r=0.73$), and negatively with plasma HCO_3 ($r= -0.74$). Mean plasma HCO_3 rose to 21mM post dialysis; the increase being inversely related to predialysis plasma HCO_3 ($r= -0.52$). Dialysate HCO_3 rose to a mean of 6.4mM after 20min dwell. Dialysate L-lactate fell 3.4mM therefore total lactate (L and D) is estimated to have fallen 6.8mM. The decrease in L-lactate correlated positively with the increase in dialysate HCO_3 ($r=0.57$). HCO_3 loss exceeded lactate gain, with a net alkali balance of -53mM.

We conclude that these patients on IPD have a metabolic acidosis which is only partially corrected by dialysis. The acidosis is related to high protein intake and inadequate dialysis buffer gain. Thirty-five mM lactate in the dialysate is probably too low.

BICARBONATE, D-LACTATE AND L-LACTATE BALANCE IN INTERMITTENT PERITONEAL DIALYSIS (IPD). R.M.A. Richardson and J.M. Roscoe, Department of Medicine, Toronto General Hospital and Wellesley Hospital, University of Toronto.

This study was designed to assess acid-base balance and the role of D-lactate as a buffer in patients on IPD receiving standard dialysate containing 40 mM lactate (20mM L-lactate, 20 mM D-lactate). Blood samples and complete dialysate effluent were collected in 14 treatments in 7 patients. The dialysis protocol was 3 treatments /week, each consisting of 33L dialysate given in 17 exchanges over 10 hours.

Mean plasma HCO_3 rose from 22 mM predialysis to 25 mM postdialysis; the increase in plasma HCO_3 was inversely related to predialysis plasma HCO_3 ($r=-.90$). Dialysate HCO_3 rose from 0 to a mean of 6.1 mM during the dwell time; L-lactate and D-lactate fell a comparable amount, 5.9 and 5.8 mM respectively. Buffer gain during dialysis treatment (L-lactate 172±16 mmoles, D-lactate 161±20 mmoles) exceeded HCO_3 loss (215±17 mmoles), giving net alkali balance of +118±25 mmoles/ treatment, or 51 mmoles/day. Alkali balance was negatively correlated with pre-dialysis plasma HCO_3 and dialysate effluent volume and positively correlated with peritoneal clearance of lactate and HCO_3.

We conclude that patients on IPD have mild metabolic acidosis which is corrected during dialysis. D- and L-lactate contribute equally to alkali gain. Positive alkali balance of 51 mmol/d is similar to expected daily hydrogen ion load from diet. Large negative fluid balances in IPD may be deleterious to acid-base balance.

DIALYSATE PROTEIN LOSSES DURING PERITONEAL DIALYSIS. Jack Rubin, Gordon Deraps,* Cathy Adair,* & John Bower. Univ. of Mississippi Med., Ctr., Dept. of Med., Jackson, Mississippi.

We investigated the effects of peritonitis (P)-(+) dialysate (dial) culture, increased cell count, pain-upon dial proteins (prot) & uptake of cephalothin (K) and/or tobramycin (Tob) from the peritoneal cavity in 12 infected & 7 control (con) patients (pts). We delivered (AMP cycler) 16 L of dial in 480 min (set) - 40 min for 2 L to fill-dwell, 20 min drain. Antibiotic doses were 125-250 mg/L K &/or 5-10 mg/L Tob. We studied pts for up to 6 sets. Each parameter was reduced to 1 value/pt. Group means were compared by t test. Prot losses, measured by trichloroacetic precipitation (T), were more in the P group (5.8±.6 g vs 3.2±.6/ set; $P<.01$). The dial losses of IgG, IgM, IgA, C'3, & transferrin (nephlometry (N)) were more with P (0.5±0.1 vs 0.3±0.1/set; PNS) but the % (N/T) was the same (9±2% (PNS) suggesting that the major prot lost during P is albumin. K removal from dial was 25±6 (n=3) ml/min during con & 28±3 (n=8) with P. Serum K levels were 13±4 & 27±11 ug/ml during the 2nd & 3rd con sets (n=3) while P levels were 29±6 (n=8) & 30±6 ug/ml (n=10). Tob uptake was 11±2 ml/min (n=6) with con & 16±1 (n=11) with P ($P<.025$). Con Tob levels were 2±1 (n=5) & 3±1 ug/ml (n=6) during the 2nd & 3rd (n=6) sets while both P levels were 4=1 ug/ml (n=9). We suggest that K uptake was not increased during P because K was bound to dial prot.

CALCIUM CARBONATE AS A PHOSPHATE BINDER IN CHILDREN ON DIALSIS. I.B. Salusky, J.W. Coburn, J. Foley*, P. Nelson*, R.N. Fine. Dept. Peds. & Med., UCIA Sch. Med.; V.A. Wadsworth Med Ctr., Los Angeles, CA.

A substitue for Al-containing phosphate binders, a major source of increased body burden of Al in uremic children, is badly needed. We evaluated oral calcium carbonate ($CaCO_3$) as a phosphate binder and its effect on the evolution of plasma Al (P-Al) in children on dialysis. Eight CCPD patients, ages 4.6±3.7 (SD) yrs and body weight 11.8±4.5 Kg, were followed for 11.9 ±1.5 mos. All received calcitriol (1,25D), 0.25 to 0.75 ug/day. Serum (S) Ca, P and CO_2 were measured monthly and P-Al every 2-3 mos. PO_4 intake was 61±25 mg/kg/day (133±60% of RDA); the dose of $CaCO_3$ was 6.4±2.9 g/day (range, 2.4-12). S-Ca, P, and CO_2 were stable and P-Al fell*:

Time:	(0-3 mos)	(5-7 mos)	(Last 3 mos)	
SCa mg/dl	10.1±1.2	10.1±0.6	10.3±0.4	NS
S P mg/dl	4.6±0.9	5.5±1.0	5.6±0.9	NS
CO_2 mEq/l	24.0±3.4	21.0±2.1	23.0±1.9	NS
PAl*ug/L	133.0±48	96.0±48	48.0±20	<.02

*in 5 pts. previously receiving $Al(OH)_3$. In 3 infants given only $CaCO_3$, initial and final pAl were 31±19 and 28±3 ug/L (NS). Asymptomatic hypercalcemia (S-Ca>11.5mg/dl) occurred in 8 instances, with peak S-Ca, 13.2±1.2 mg/dl; S-Ca was normal when measured 5-21 days later after the 1,25D and $CaCO_3$ were reduced. Hyperphosphatemia and metabolic alkalosis did not appear. Thus, $CaCO_3$ can be used as a primary phosphate binder in young children with renal failure until other phosphate binders are available.

PLACEMENT OF CATHETER USING PACEMAKER-LIKE INTRODUCER WITH PEALAWAY SLEEVE. Stuart Updike,*Mark O'Brien, *William Petersen, and Stephen Zimmerman, Dept. of Medicine, Univ. of Wisc., Madison, WI.

Since March of 1983 we have used a pacemaker-type introducer, modified to our specifications by Cook, Inc., for placement of Tenckhoff catheters. Thirty six catheters were inserted and all functioned immediately without repositioning. To initiate the procedure a saline-filled 20 cc syringe was fitted onto a plastic-sleeved 19 gauge needle and directed through a one cm midline or paramedian incision. Puncture of the peritoneal cavity was carried out with simultaneous forceful infusion of saline followed immediately by withdrawal of the needle. A 20 gauge soft tipped guide wire was passed into the peritoneum through the plastic sleeve. An 11 gauge cutting needle was passed over the guide wire to enlarge the hole. A 16 French Cook dilator was then passed over the guide wire and promptly withdrawn leaving only the pealaway sleeve needed for easy placement of the Tenckhoff catheter. Only two patients (5.5%) developed exit site leaks which spontaneously resolved. Moderate bleeding occurred in one patient with a past history of candida peritonitis. Bowel puncture with the needle occurred in one patient, however, this was not associated with peritonitis and a PD catheter was successfully placed at a new location. Delayed dehiscence in one debilitated patient followed prolonged coughing. Drain failure occurred only once in a patient with a recent candida peritonitis. In summary this technique has been associated with infrequent complications, and can be performed at the bedside. It is easy to learn and was performed successfully by 11 different physicians over the past year.

NUTRITIONAL STATUS OF PATIENTS ON LONG-TERM CAPD. H. Schilling*, G. Wu*, J. Pettit*, J. Harrison*, K. McNeil*, Z. Siccion*, D.G. Oreopoulos. Toronto Western Hospital and University of Toronto, Toronto, Canada.

We analysed the changes in dietary protein and caloric intake, serum albumin, body weight (BW), total body potassium (TBK), and nitrogen (TBN) in 26 patients on CAPD for 2 years and in 13 of them on CAPD for 3 years. Protein intake remained stable at approximately 0.9 g/kg BW/day in women and 1.0 g/kg BW/day in men. Serum albumin remained stable at low levels (3.5 g/dl). TBK remained unchanged whereas TBN, after a decrease during the first year (to 85% of the predicted normal for women and 75% for men), remained stable thereafter. There was a significant inverse ($p< 0.05$) correlation between the losses in TBN and protein and caloric intake when the latter were expressed per kg of TBN. There was also a significant ($p< 0.001$) correlation between TBN loss and initial TBN, i.e. the higher the initial value, the greater the loss. Changes in TBN were more pronounced in men than in women. Our results indicate that 1) TBN and not TBK is a good index of nutrition in long-term CAPD. 2) On a daily protein intake of 0.9 g/kg BW for women and 1.0 g/kg BW for men most patients will lose body nitrogen and equilibrate at a lower level. A higher protein intake seems to prevent this loss.

DIALYSANCE OF ADRENAL STEROIDS DURING CAPD PG Zager, *CT Spalding, *HJ Frey, *MD Nevarez

We postulated that significant quantities of circulating steroids are removed during CAPD. To test this hypothesis we measured the dialysance of aldosterone(aldo), 18-hydroxycorticosterone(18OHB) and cortisol during 4h exchanges performed between 0800h and 1200h with 2L 1.5% Dianeal (n=5). Steroid levels were measured in plasma (P) (0800h, 1200h) and dialysate (D) (1200h).

	aldo(ng/dl)	18OHB(ng/dl)	cortisol(µg/dl)
P 0800h	43 ± 18	198 ± 93	23 ± 3
P 1200h	34 ± 12	135 ± 56	16 ± 2
D 1200h	13 ± 7	30 ± 14	2 ± 0.3

D aldo correlated with P aldo at 0800h ($r=.99$ $p<.001$) and 1200h ($r=.95$ $p<.01$). D 18OHB correlated with P 18OHB at 0800h ($r=.99$ $p<.001$) and 1200h ($r=.96$ $p<.01$). Correlations between D cortisol and P cortisol failed to attain significance. In separate experiments (n=6) the removal rates of aldo, 18OHB and cortisol were 2.0±1.4, 4.3±2.5 and 62.6±9.9µg/24h respectively.

To determine the effects of steroid-protein binding on dialysance, a series of equilibrium dialyses (n=6) were performed in vitro. In P most of the aldo (61±1%), 18OHB (64±1%) and cortisol (91±0.3%) was bound to P proteins. In contrast, in D most of the aldo (97±1%), 18OHB (96±1%) and cortisol (79±4%) was unbound. There were no significant differences between the concentrations of unbound aldo, 18OHB and cortisol in P and the concentrations of the respective steroids in D.

Conclusion: Unbound steroid readily equilibrates between P and D. Significant quantities of circulating steroids are removed during CAPD.

TENCKHOFF CATHETER DRAINAGE PROBLEMS ABOLISHED WITH LATERAL LOWER QUADRANT PLACEMENT.
Anthony R. Zappacosta, Susan T. Perras. The Bryn Mawr Hospital, Bryn Mawr, Pennsylvania.

Irreversible drainage problems of the Tenckhoff catheter have caused patient morbidity and expense. Omental entrapment of catheters migrated out of pelvis to subdiaphragmatic location is the main reason for this and occurs in 10% of midline placed catheters. Entrapment evidently does not occur in pelvic location. Several major design changes have been introduced to prevent migration, most of which require laparotomy. Although midline placement is traditional we place standard Tenckhoffs percutaneously in lateral lower quadrants. After 38 catheters with 224 patient months we had no occurance of drainage problems. Migration usually occurs within one week of placement but can occur anytime, therefore repeated X-rays would be needed to document pelvic location and this was not justified. Healing and hemostasis were good. Complication rate was comparable to percutaneous or surgical midline placement.

We conclude that lateral lower quadrant placement abolishes Tenckhoff drainage problems probably because this site prevents migration. The mechanism is obscure but this experience suggests there is no need to abandon the standard Tenckhoff catheter for designs that do not migrate such as the column-disc device, etc.

TENCKHOFF REMOVAL NOT NECESSARY FOR TREATMENT OF BACTERIAL PERITONITIS, EXCLUDING PSEUDOMONAS.
Anthony R. Zappacosta, Susan T. Perras. The Bryn Mawr Hospital Dialysis Program, Bryn Mawr, Penna.

Tenckhoff colonization or other foreign body effects of the catheter could prevent eradication of peritonitis organisms causing recurrent peritonitis or prolonging its course. This is documented for fungus but remains theoretical for bacterial peritonitis. Catheter removal for bacterial peritonitis continues to be viewed as helpful to shorten the course of and/or eradicate the infection. Excluding Pseudomonas peritonitis, catheter removal may not be necessary for eradication nor may the benefit of a shortened course of peritonitis outweigh the risk of removal, substitution of hemodialysis, and replacement of the catheter.

In 88 consecutive cases of bacterial peritonitis (10/80-8/84, excluding Pseudomonas) clinical recovery and no growth with negative serum antibiotic levels was achieved without catheter removal. Antibiotic course did not exceed 21 days. 59% of cases were hospitalized. Hospital stay was 1 to 38 days (median 12). Organisms were gram positive 81.8%, gram negative 18.2%.

Morbidity of Pseudomonas sepsis and peritonitis outweighs risk of catheter removal with substitution of hemodialysis, therefore all measures are taken to shorten the course of this infection.

We conclude that catheter removal in therapy for bacterial peritonitis (except Pseudomonas) should be last resort in CAPD, since risk benefit ratio favors pursuing antibiotics with catheter in place.

LOW INCIDENCE OF OBSTRUCTIVE CATHETER FAILURE WITH THE CURVED PERITONEAL DIALYSIS CATHETER (CDC).
Stephen Zimmerman, *Mark O'Brien, *Bill Petersen, and Stuart Updike. Univ. of Wisconsin, Madison, Wisc.

Since January of 1983 we have used a double-cuff CDC in our adult peritoneal dialysis patients. Through July, 1984 we have placed 56 CDC, for 347 patient months of experience (range 1-20 months) with a mean of 6.2 months per catheter. Thirty eight catheters were first time placements and 18 were replacement catheters. Nephrologists placed 35 catheters and surgeons 21. One year catheter survival for catheters with immediate function is 84%. Six percent (3) of the catheters were removed for complete drain failure. These were all repeat catheter placements; one following candida peritonitis, one following bowel perforation requiring colostomy, and peritonitis, and one in a patient with multiple hernia repairs and peritonitis. There were no drain failures in primary catheter placements. Catheter displacement has not been noted, and there have been no peri-catheter hernias. The incidence of exit site infection is .34/patient year. Using straight Tenckhoff catheters (STC) from August 1981-November 1982 we had 11% complete drain failures, usually associated with catheter displacement, in 63 primary placements. Although we have no controlled prospective data comparing the CDC and STC, our results suggest less early failure with CDC. For this reason the CDC remains our catheter of choice.

HYPERTENSION

A POSSIBLE MARKER FOR NATURETIC HORMONE IN HEMODIALYSIS PATIENTS. *
Suhail Ahmad, Margaret A. Kenny and B. H. Scribner. University of Washington, Seattle, Washington.

Some chronic hemodialysis (HD) patients, none of whom are receiving digitalis compounds, give a false positive test for digitalis in their plasma. No association with any clinical condition has been observed with a positive test for this digitalis like immunoreactive substance (DLIS). We explored the possibility that DLIS might serve as a marker for naturetic hormone, which also has digitalis like properties and may be a factor in the pathogenesis of hypertension in HD patients.

Plasma DLIS levels were determined in two subgroups of HD patients. Group A contained 12 patients who had hypertension requiring antihypertensive medications and were brittle in the sense that they became very hypertensive with increase in extra-cellular volume (salt overload) before dialysis. Group B consisted of 10 normotensive hemodialysis patients who remained normotensive pre-dialysis despite salt overload. The clinical profile of the patients in the 2 groups were similar except that there were more blacks in Group A. 10 of 12 patients in Group A had measurable DLIS levels (0.43+0.31 ug/1, $\bar{x}$+SD, normal=<0.1 ug/1), where as none of the patients in Group B had detectable levels of DLIS. DLIS was unstable decaying at a rate of 0.058 ug/1/day at -70°C. It was not dialysable. Dilution with healthy sera revealed a 200% recovery.

This difference between Group A and Group B with respect to the level of DLIS supports our hypothesis that DLIS might represent a marker for naturetic hormone which may be important in pathogenesis of hypertension in HD patients.

INTRAVENOUS MEDROXALOL FOR THE TREATMENT OF SEVERE ARTERIAL HYPERTENSION. Ali AlHaddad*, Donald G. Vidt. Cleveland Clinic Foundation, Cleveland, Ohio.

Medroxalol, a new alpha and beta adrenergic blocking drug, was administered intravenously to 6 patients with severe hypertension (essential-4, renovascular hypertension-1, renal parenchymal disease-1). A supine diastolic blood pressure (SDBP) of $\geq$ 120 mm Hg. was a condition for patient entry into the study.

Repeated intravenous injections of medroxalol (0.5 mg/kg.) were administered at 30 minute intervals until an SDBP of $\leq$ 100 Hg. was achieved or until a total of 4.0 mg/kg. was administered.

Five of 6 patients responded to IV medroxalol. Among the 5 responders, an average SDBP of 125 $\pm$ 4.73 mm Hg. pretreatment fell to 96 $\pm$ 4.24 mm Hg. following an average total dose of 118 mg. (range 54-240 mg.). The average total dose per kg. ranged from 1-2.5 mg/kg. Effects persisted for an average of 6 hours (range 4-8 hours) following the last dose of medroxalol. Therapy was not associated with significant clinical or biochemical adverse effects. One patient did experience brief orthostasis which required no specific therapy.

In the one non-responder, SDBP decreased to only 110 mm Hg. No adverse effects were observed and DBP was subsequently controlled with nitroprusside.

Medroxalol by repeated intravenous injection is a safe and effective treatment for severe arterial hypertension of varied etiologies.

RENAL CHEMORECEPTOR ACTIVATION DURING MODEST REDUCTIONS IN RENAL PERFUSION PRESSURE. Joan Barber and Nicholas Moss (intr. by Carl W. Gottschalk), Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

Afferent renal nerve activity (ARNA) was recorded in rats during graded reductions in renal perfusion pressure (aortic clamp). Afferent activity increased 25% $\pm$ 8.8 SEM in 12 multiunit preparations when renal perfusion pressure was reduced from 114 mmHg $\pm$ 1.9 SEM to 88mmHg $\pm$ 3.5 SEM. Further reductions in perfusion pressure resulted in a progressive increase in afferent activity with a peak response of 116% $\pm$ 30.5 SEM above control values at 40 mmHg $\pm$ 1.6 SEM. The receptors responsible for this excitation were identified as renal R2 chemoreceptors by recordings of unitary ARNA (8 units) during similar reductions in renal perfusion pressure. This activation of renal chemoreceptors during modest reductions in renal perfusion pressure offers new insight into the potential significance of these afferent nerves and their ability to signal alterations in renal function to the central nervous system.

ANTIHYPERTENSIVE THERAPY (RX) IN NEPHRITIC (GN) SPONTANEOUSLY HYPERTENSIVE RATS (SHR): EFFECTS ON NEPHRON DYNAMICS AND MORPHOLOGY. S. Azar, W. Keane and L. Raij, Univ. of Minnesota, Minneapolis, MN.

Hypertension and progressive loss of renal mass may hasten glomerular destruction in GN. Thus, we studied the effects of Rx (hydralazine, thiazide, rauwolfia-Kidney Int 12:332,1977) in 2 kidneys (K) and 1K SHR with ferritin-antiferritin GN. Rats with 2K had Rx for 5 weeks (2K Rx) and 1K rats for 15 weeks (1K Rx), control rats had no Rx (Rx0). All rats had measurements of blood pressure (AP), and glomerular hydrostatic pressure gradient (ΔP), mm Hg; SNGFR, nephron plasma flow (QA), nl/min; and morphologic injury scores in cortical (c) and deep (d) glomeruli (glom) of mesangial expansion, (ME=c+d), and sclerosis, GSL. Results: $p \leq .025$; *:2K vs 2K, 1K vs 1K; †2K vs 1K; n=7-8/group.

GROUPS	AP	ΔP	QA	NGFR	ME	GS-d
2K Rx0-	*177±3	*34±1	*118±11	*39±4	†113±16	0
2K Rx -	119±4	40±1	†197±13	†54±4	130±16	0
1K Rx0-	*175±4	*35±.9	*251±21	*58±4	161±12	*29±6
1K Rx -	117±2	40±.8	344±19	82±5	131±13	12±1

Two K rats: Rx0 had no cortical or deep GS; Rx reduced AP but elevated ΔP, flows and c ME:*77±4 vs 57±7 in 2KRx0. Nephrectomy in Rx0 induced GS in d glom and elevated QA and ME in c glom †(126±12 vs 57±7 in 2K Rx0) inspite of unchanged ΔP. In 1K, Rx elevated c ΔP and QA, did not improve ME, but reduced GS in d glom. Thus the effect of Rx in c and d glom injury differs according to renal mass. Due to further adaptation, the high c ME induced by nephrectomy is unchanged by Rx, while d gloms, perhaps maximally adapted, respond to reduced AP by decreasing GS. Thus, in nephritic SHR, Rx that reduce glom resistances may offset the full benefit of low systemic BP.

MECHANISM OF DECREASED VASCULAR RESPONSIVENESS TO ANGIOTENSIN II (AII) IN EARLY 2-KIDNEY, 1 CLIP HYPERTENSIVE RATS (RVH). R.G. Benedetti,* and S.L. Linas. Univ. of Colo. HSC, Denver, Colorado.

Compared to many forms of hypertension, vascular reactivity to AII is decreased in RVH. To determine the role of the AII receptor, we examined vascular responsiveness and I^{125} AII binding to mesenteric artery membrane fractions after 3 wks of RVH. Systolic pressure was 165±8 in RVH and 105±4 mm Hg in controls, $p<.001$. Plasma renin activity was 7.1±.5 in RVH and 2.2±.7 ng AI/hr, $p<.001$ in controls. The pressor response to exogenous AII was reduced by 40% in RVH. Since administration of a single dose of converting enzyme inhibitor (CEI) did not alter the response to exogenous AII, the decreased reactivity was not caused by receptor occupancy but to alterations in either AII binding to its receptor or to post-receptor events. I^{125} AII binding to mesenteric arteries was equal or greater by RVH than controls at all concentrations of AII. Scatchard analysis showed an increase in receptor number (Bmax): 109.2±7.7 in RVH; 84.5±6.9 fmoles/mg in controls, $p<.025$; while receptor affinity was unchanged: .93±.14 in RVH; 1.19±.18 nM in controls. To determine if the increase in circulating AII caused these binding abnormalities, rats were treated with CEI for 3 days. Chronic AII antagonism reversed the decrease in vascular responsivness as well as the increase in AII receptor number (Bmax 105±13 in RVH vs 83.3 ±13 fmoles/mg in RVH plus CEI, $p<.05$). Conclusions: 1) since AII binding is increased, a post-receptor event mediates the decreased vascular reactivity in RVH; 2) in the absence of Na depletion, AII receptor number in vascular smooth muscle varies directly with endogenous AII.

DIVERGENT EFFECTS OF BETA-1 RECEPTOR ANTAGONISM ON ACTIVE AND INACTIVE RENIN IN ESSENTIAL HYPERTENSION. Grace B. Bialy,* Michael C. Ruddy. Dept. of Med., UMDNJ-Rutgers Med. Sch., New Brunswick, NJ 08903

Plasma renin exists predominantly in an inactive form (IR) that may serve as a precursor of enzymatically active renin (AR). Beta adrenergic activity, a potent stimulus for renin release, may regulate plasma renin activity (PRA), in part by influencing the conversion of IR to AR. We investigated this hypothesis by studying the effects of the beta-1 selective antagonist atenolol (A) 50 mg/day on mean ± SEM PRA (AR), total renin (TR), IR and IR/TR in 10 patients (7 male, 3 female, mean age 42.9 ± 2.7) with essential hypertension. TR was determined by pre-incubation of plasma aliquots with 1.5 mg/ml trypsin, in the presence of 5 mM benzamadine, for 1 hour at -4°C prior to assay of renin activity. AR was defined as the rate of generation of angiotensin I in 37°C plasma at pH 5.7. IR was calculated as TR minus AR.

	PreTreatment	A 50 mg/day	P
Systolic BP mmHg	150.8 ± 6.9	132.4 ± 6.1	<.01
Diastolic BP mmHg	98.2 ± 3.7	87.8 ± 2.8	<.02
Heart Rate	78.6 ± 4.3	68.4 ± 3.1	<.01
AR(PRA) ng/ml/hr	3.28 ± 0.98	1.31 ± 0.31	<.05
TR ng/ml/hr	19.0 ± 3.1	19.2 ± 3.2	>.2
IR ng/ml/hr	15.7 ± 2.6	17.9 ± 3.1	>.2
% IR/TR	80.4 ± 5.0	92.3 ± 1.8	<.05

In essential hypertensives, A lowered PRA to less than 50% of pretreatment values, consistent with an important role of beta-1 adrenoreceptor-mediated sympathetic control of AR in the resting state. In addition, A increased IR/TR while TR remained unchanged. These findings suggest that beta-1 receptor blockade lowers PRA at least partly through inhibition of renal or extra-renal conversion of IR to AR.

NIFEDIPINE IN ACCELERATED HYPERTENSION: NONINVASIVE DOPPLER CARDIAC OUTPUT AND OUTCOME. Stuart Bursten*, Douglas Stewart*, Pamela Keeley*, Margaret Kenny*, Howard Steinberg & Robert Davidson, Univ. of Washington Hosp., Dept. of Med., Seattle, WA.

Nifedipine is safe & effective in acute hypertension; its advantages include increased cardiac output (CO). We have treated accelerated hypertension with nifedipine, using a non-invasive Doppler CO determination (UltraCOM) in some pts to monitor hemodynamics. Fifteen patients with mean BP $\geq$ 130, & without cardiac or neurologic problems, were treated with 10mg nifedipine administered as broken capsules p.o. BP was monitored q 10-15 min. with redosing of 10mg @ 30 min. if response was less than 15% decrement in mean BP. Patients were monitored for 4 hrs. At the beginning and end, standard lab. measurements were obtained; random serum levels were obtained at 0,1½,3,& 4 hrs. CO determinations were made in 4 of 15 pts @ 10-15 min. intervals. All pts responded to nifedipine with BP decrements varying from 10-42% from baseline within 15-30 min, with an avg. of 32%. All mean BP remained $\geq$ 90; no adverse symptoms other than slight headache. 4 pts required re-dosing, following which avg. decrement in BP was 18% from baseline. All renin levels remained constant. 4 pts with CO determinations had rises in CO, an avg. of 40%. Two with LVH by EKG had larger rises (60%) than those without LVH. Larger CO increase is predicted for diminished response to nifedipine. Pts with increased CO have all responded to addition of beta blockers. Nifedipine, an effective and rapid acute treatment for accelerated hypertension, increases cardiac output. Doppler CO is useful in determining and predicting efficacy of therapy.

ERYTHROCYTOSIS AND HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE (SHR) NORMOTENSIVE (WKY) AND DOMESTIC WISTAR RAT. John W. Boylan and Judith B. Van Liew. Veterans Administration Hospital, Newington, CT and Buffalo, NY.

In 1972 Sen et al (JCI 51:710) described erythrocytosis concomitant with the development of hypertension in a strain of SHR (SH). Neither domestic Wistars (DW) nor Sprague-Dawley strains exhibited increased RBC counts over the same period (from 130 to 300 gm BW). Since the RBCs of the SH strain had also smaller cell volumes (MCV) than controls (45.4 vs 54.2μ^3) and the surface area of RBCs is independent of volume, these findings could have important implications to widely reported differences in Na flux between RBCs of SHR vs normotensive Wistars (WKY) in units of mEQ/L cells. Accordingly,we measured RBCs(Coulter) and Hct in domestic Wistar, SHR and WKY rats from 7 to 15 wks. of age. These values,with derived MCVs,are given in the table.

Age (wks)	Wistar RBC	Wistar MCV	WKY RBC	WKY MCV	SHR RBC	SHR MCV
7	6.71* ± 0.17	54.0* ± 0.8	7.76 ± 0.15	47.8 ± 1.3	7.51 ± 0.06	47.9 ± 0.8
15	8.82* ± 0.12	47.1 ± 0.9	9.04 ± 0.19	47.9 ± 0.7	9.42 ± 0.12	46.4 ± 0.6

Values are mean ±SE. RBCs x 10^6
*Significantly different from SHR ($p < .05$).

Unlike Sen et al's study, all strains showed increased RBC counts with age. Counts are significantly higher in SHR than DW at every age, and MCVs significantly smaller except at week 15. Mean BP at week 15 were for DW 150, WKY 131 and SHR 180 mm Hg. We conclude that erythrocytosis is a normal event in these strains,that its relationship to hypertension is not established, and that RBC size is not a determinant of pressure and flux differences in SHR vs WKY.

HYPERTENSION (H) AND DECREASED GRAFT SURVIVAL IN LONG-TERM KIDNEY TRANSPLANT RECIPIENTS. J.S.Cheigh, J.Wang, P.Fine, R.R.Riggio, L.Tapia, A.L.Rubin and K.H.Stenzel. Rogosin Kidney Center, Cornell University Medical College, New York, N.Y.

To determine long-term effects of H on graft function and survival and the consequences of therapeutic intervention we studied the clinical course of 132 kidney transplant recipients whose grafts functioned for over 2 years (yrs). 77 were male and 55 female, age 5-63 yrs (33.4±11.1) at the time of transplantation (txpl) and followed for 30-120 months (68.2±32.4). 23 were normotensive (diastolic BP<90:Gr.1), 109 were H post-transplant. Among the H patients, 45 maintained their BP within normal ranges with antihypertensive agents (Gr.2), 63 were hypertensive despite treatment (Gr.3). Each group's graft survival rates (GSR) and serum creatinine at 2,5 and 8 yrs after txpl were:

	GRAFT SURVIVAL(%) 2Yrs.	5Yrs.	8Yrs.	SERUM CREATININE 2Yrs.	5Yrs.	8Yrs
Gr1	100	96	86	1.4±0.3	2.1±2.1	1.7±1.1
Gr2	100	70	24	1.9±0.8	2.0±1.1	1.5±0.6
Gr3	100	63	17	2.3±1.1	2.2±1.3	2.3±1.3

The GSR of Gr1 was significantly ($p<.005$) better than Gr2 or 3. However, there was no difference in GSR between Gr2 and 3. Creatinine level was lowest in Gr1 and highest in Gr3 ($p<.05$) at 2yrs but all 3 groups had stable and similar levels in subsequent yrs. This study indicates that H patients have a significantly lower GSR than normotensive patients. Lower GSR does not appear to be caused by H. Rather the lower GSR and H seem to be the consequence of low graft function associated with underlying pathology. Control of BP within normal ranges with therapy does not appear to improve graft survival to that of normotensive patients.

HEMODYNAMIC AND METABOLIC EFFECTS OF HYPOMAGNESEMIA IN THE SHR. Steven G. Chrysant, Louis Ganousis,* and Catherine Chrysant,* VA Med. Ctr., and Univ. of Kansas, Kansas City, Mo., and Kansas City, Kansas.

The hemodynamic and metabolic effects of dietary induced hypomagnesemia (HM) were studied in 2 groups of 2 month old male SHR. Both groups of rats were given distilled water to drink. However, group 1 (control, 12 SHR) were given regular diet, whereas group 2 (experimental, 12 SHR) were given magnesium free diet. The rats were observed for one month and metabolic and hemodynamic studies were done at the end of observation. Group 2 rats had significantly higher MAP, HR, TPR, renal vascular resistance (RVR), $U_{Na}V$, and serum Na^+; lower HCt, RBF, serum K^+ and serum Mg^{++} than group 1 rats. Group 2 rats had also increased calcium deposition with fibrosis and tissue necrosis in the heart and kidneys. There were no significant differences between the two groups with respect to weight, fluid intake, urine volume, serum calcium, BUN, CI, and GFR. We conclude: 1) Dietary induced HM aggravated the hypertension of SHR. 2) These effects of HM were mediated through an increase in vascular tone and systemic and renal vascular resistance. 3) The underlying mechanism for these changes was probably a combination of decreased tissue Mg^{++} and increased tissue Ca^{++}

ABNORMAL SODIUM HANDLING IN ESSENTIAL HYPERTENSION. Harriet P. Dustan, Cardiovascular Research and Training Center, University of Alabama in Birmingham, Alabama.

Inability to excrete sodium normally is thought to be a factor in the genesis and maintenance of some fraction of essential hypertention. To examine this possibility, studies were done during a 3 day control (C) period, then 4 days of salt deprivation (SD) (Na intake - 9 mEq Na/d) followed by 3 days of salt loading (SL) (Na intake - 3.88 mEq Na/kg/d) in 69 normotensive control subjects (NBP) and 23 essential hypertensive patients (EH) categorized by the arterial pressure (BP) response to SD : 10 responders (EH-R) with BP average for 4 days of SD <140/90 with SD and 13 nonresponders (EH-NR) with neglible BP change. Plasma aldosterone (PA) was measured at the end of each period and sodium retention (NaR) was calculated during SL as Na intake - urinary Na and was expressed as mEq/kg. Results appear in the Table as means ± std. deviation.

	PA-pg/ml			NaR-mEq/kg
	C	SD	SL	SL
NBP	106±66	532±310	78±60	6.0±1.6
EH-R	81±38	227±79*	80±28	6.4±1.0
EH-NR	125±66	332±143*	101±53	4.6±1.5**

*$p<.01$ vs NBP, ** $p<.01$ vs EH-R and NBP

Thus EH-R retained more sodium than EH-NR at the same level of PA. Because EH-R had normal NaR at abnormally low PA, the data suggest that kidneys of EH-R may be abnormally sensitive to aldosterone.

LONG-TERM ANTIHYPERTENSIVE EFFECTS OF CAPTOPRIL IN DIALYSIS PATIENTS. G. R. Dreslinski and A. C. Jenkins*, Squibb Institute for Medical Research, Princeton, New Jersey.

Efforts to control hypertension in dialysis patients by ultrafiltration are not uniformly successful. 330 patients enrolled in dialysis programs worldwide participated in a surveillance study of captopril in hypertension. Their entry blood pressure was 191/112 ± 30/13 (mean ± S.D.) while receiving an average of 2 antihypertensive drugs (range 0-6). The mean captopril dose and blood pressure response at various time points were as follows (mean ± S.D.; *=p<0.001 vs. entry):

	N	Blood Pressure(mmHg)	Dose(mg/day)
Month 1	293	161/94 ± 22/10*	108
Month 3	217	152/88 ± 21/10*	124
Month 6	167	152/88 ± 20/11*	113
Month 9	128	149/86 ± 19/10*	106
Month 12	92	150/85 ± 19/ 9*	96

Captopril was discontinued in fourteen patients due to adverse drug reactions, and an additional seven had therapy withdrawn due to treatment failure. Captopril prescribed in these doses was effective and safe in the treatment of hypertension in dialysis patients.

EVIDENCE FOR HEMODYNAMICALLY MEDIATED GLOMERULAR INJURY DESPITE ANTIHYPERTENSIVE THERAPY IN RATS WITH DESOXYCORTICOSTERONE-SALT (DOC-SALT) HYPERTENSION. Lance D. Dworkin, Helen D. Feiner, and Joseph Randazzo.* Depts. of Medicine & Pathology, New York Univ. Med. Ctr., New York, N.Y.

Elevated glomerular capillary pressure ($\overline{P}_{GC}$) and flow (Q_A) may cause glomerular injury in rats with DOC-SALT hypertension. To determine whether normalization of mean arterial pressure ($\overline{AP}$) might ameliorate glomerular capillary hypertension and injury in DOC-SALT rats, we performed micropuncture and morphologic studies 6 weeks after uninephrectomy (UNX) in groups of control (C) Munich-Wistar rats, UNX rats given DOC (10 mg/wk, s.c.) and 1% saline for drinking (D), and identical DOC-SALT rats ingesting hydrochlorthiazide, hydralazine and reserpine (DA). Micropuncture studies revealed:

GROUP	$\overline{AP}$	$\overline{P}_{GC}$	SNGFR	Q_A
	---mmHg----		---nl/min----	
C (n=9)	101±3	52±2	83±6	241±24
D (n=10)	156±3*	62±1*	88±7	279±30
DA (n=10)	96±1†	60±2*	77±8	245±26

Mean±S.E.M.; * P<0.05 v. C; † P<0.05 v. D

Protein excretion and histologic studies revealed that, along with hemodynamic abnormalities, D and DA rats had significant proteinuria (70±12 and 57±9 v. 15±2 mg/24h in C) and morphologic evidence of glomerular injury including mesangial expansion and focal segmental proliferation. We conclude: 1) Glomerular injury is associated with glomerular capillary hypertension in DOC-SALT rats. 2) Intrarenal hypertension may persist despite normalization of systemic blood pressure. 3) Treatment which successfully lowers mean arterial pressure may fail to protect DOC-SALT rats from hemodynamically mediated glomerular injury.

RENAL CHOLESTEROL EMBOLIZATION AND RENOVASCULAR HYPERTENSION. George Eisele*, Donald G. Vidt, Andrew C. Novick, Gordon M. Gephardt. Cleveland Clinic Foundation, Cleveland, Ohio.

Renal cholesterol embolization is often considered a cause of irreversible renal failure. Review of biopsy material at the Cleveland Clinic from 1978 to 1984 yielded 16 proven cases. Clinical manifestations of generalized atherosclerosis were common, including cerebrovascular events (11 patients), retinal emboli (5), peripheral emboli (6) and mesenteric infarction (1).

Evaluation of suspected renovascular hypertension (7 patients) or surgical revascularization (8) prompted 15 biopsies. One patient had new onset of hypertension (6 months); 15 had recent worsening of control of hypertension (mean duration 11.8 years). All 16 had angiographic evidence of severe abdominal aortic atherosclerosis; 13 had renal artery stenosis. In 12 patients, aortic surgery or femoral approach angiography caused worsening of azotemia (mean increase in serum creatinine of 5.7 mg/dl.). Seven patients had successful angioplasty or revascularization. Six had improved blood pressure control (mean decrease of 2 in number of medications postoperatively). Average serum creatinine decreased from 4.9 mg/dl. (preoperatively) to 2.4 mg/dl. (postoperatively). Six month follow up in 4 patients revealed an average creatinine of 2.3 mg/dl.

We conclude that cholesterol embolization is a complication of diagnosis and management of renovascular hypertension. The natural history is so variable that embolization does not constitute an absolute contraindication to surgical revascularization.

THYMOCYTE SODIUM CONTENT AND ^{22}NA EFFLUX IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RATS Terrence Forrester,* Eric Marks, and Harry Keiser,* NHLBI, NIH and USUHS, Bethesda, MD

The relationship between changes in systemic hemodynamics and alterations in thymocyte sodium content (TSC) and rate constants for ^{22}Na efflux were evaluated in SHRs and WKRs age matched at 4, 6,8,12 weeks. Na transport inhibitors, ouabain and furosemide, discriminated between active transport mechanisms. Thymocytes were Na loaded to evaluate the effect of intracellular concentration on transport activation rates. Measurements of cardiac output, arterial pressure, and total peripheral resistance (TPR) were obtained.

Age	Rats	TSC•	ktot†	kos	koi	TPR
4	SH	54.5*	11.72*	9.35	2.50*	1.7*
	WK	37.9	14.11	10.08	3.90	1.1
6	SH	53.6*	10.92*	8.41*	2.48	3.4*
	WK	18.8	12.79	10.66	2.13	1.6
8	SH	58.1*	9.73*	7.32	2.38*	5.0*
	WK	50.4	10.19	7.62	2.72	2.2
12	SH	44.0*	9.20*	7.33	1.95*	7.6*
	WK	27.7	10.42	7.04	2.78	2.3

†=hr^{-1}; •=mmol/kg; *= p<.05 vs WKR

TSC was always higher while total efflux (k_{tot}) was always lower in SHR. Lower k_{tot} was due mainly to the "passive" component (leak) of the ouabain insensitive (k_{oi}) efflux. The other component, furosemide sensitive efflux (co-transport), was not diminished. Ouabain sensitive efflux (k_{os}) (Na-K ATPase) also was not different between the two groups. Rate constants for k_{tot} and k_{os} fell with age in both groups. Activation of Na transport was identical in both groups. Higher TSC, normal Na-K ATPase and co-transport are present at the onset of increased TPR in SHRs.

CELL VOLUME REGULATION AND WATER CONTENT OF NORMOTENSIVE WISTAR-KYOTO (WKR) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR). P.U. Feig, M.A. D'Occhio,* and J.W. Boylan, Univ. of Conn. Health Center, Farmington and VA Hospital, Newington, CT.

To determine whether the abnormalities in membrane ion transport described in tissues of human and rat genetic hypertension affect cell volume regulation, we studied, by electronic cell sizing, thymocytes from WKR and SHR after sequential exposure to hypotonic and isotonic media. The anisotonic shock caused a greater immediate (at 1 min) response in WKR than in SHR both in swelling (to 140% of normal in WKR vs 130% in SHR, p<.001) and in shrinking (to 75% in WKR vs 81% in SHR, p<.02). Subsequently both WKR and SHR cells returned their volumes back towards normal: to 112% in WKR vs 107% in SHR 30 min after hypotonic shock at 20°C (p=0.065) and to 95% in WKR vs 97% in SHR 120 min after "hypertonic" shock at 37°C. Since the differences in immediate response to anisotonicity suggest that water content in WKR is larger than in SHR, a van't-Hoff plot was performed on cells of the two strains, at 4°C (to prevent volume regulation), with buffers spanning 0.5 to 1.75 x isotonicity. This yielded a lower y-intercept in WKR than in SHR.

Our studies show that while both WKR and SHR thymocytes can regulate their volume, the osmotically responsive water content is smaller in SHR than in WKR. This difference could be due to inequality in either cell solids or the fraction of osmotically inactive water. Our results indicate a need for caution in interpreting ion content data in thymocytes (and possibly other cells) of these strains of rats, when expressed as concentration per volume or weight of cells.

DIFFERENCES IN RENAL DIVALENT ION HANDLING CHARACTERIZE HUMAN KIDNEY DONORS WITH BP INCREASES AFTER UNINEPHRECTOMY (uninx). M.A. Friedlander and J.H. Lemke*, Departments of Medicine and Preventive Medicine, University of Iowa, Iowa City, IA.

To identify factors which contribute to BP elevations in the first 6 months (M) after uninx, we compared 10 donors who developed at least 1 postnx diastolic BP>90 (HYP) with 11 whose BP remained unchanged (NONHYP), using repeated measures ANOVA.

Time	n	BP (mmHg)	Creat Clear (ml/min)	U Ca (mg/TV)	U Pi (mg/TV)
pre NON	(11)	118/75	121±32	141±104	912±337
HYP	(10)	120/76	119±9	259±102	1123±256
1 M NON	(11)	116/76	81±18	127±111	853±274
HYP	(10)	131/83	86±18	220±101	1419±726
6 M NON	(9)	117/76	93±26	112±94	823±450
HYP	(8)	126/87	97±13	113±45	1141±186
p across BP		.002	NS	.06	.03
p across time		.001	.0001	.0001	.003

[All values: mean ± S.D.] The male/female ratios and ages were similar in both groups. HYP were heavier (84±18 kg) than NONHYP (67±11) (p<.01). But a relationship of weight or BP, with total urine Na or FeNa was not found. There was a marked decrease in tubular phosphate reabsorption (TRP) after uninx in both groups (p<.001), but total phosphate excretion (UPi) was higher in HYP at all time periods. At 6 M diastolic BP was correlated positively with UPi (.73, p<.001), and negatively with TRP (-.61, p<.01). Total calcium excretion (UCa) was greater in HYP, and a greater decrease in UCa was seen in HYP than NONHYP at 6 M (p=.02). Thus, both phosphaturia and a fall in calciuria are more pronounced in HYP. These data support a role for divalent ions in the pathophysiology of HTN associated with decreased renal mass.

DEVELOPMENT OF RENAL INNERVATION IN THE SHR. Vincent H. Gattone, Andrew P. Evan and J. Marc Overhage.* Departments of Anatomy, Pennsylvania State University, Hershey, PA., and Indiana University, Indianapolis, IN.

The renal nerves have been implicated in the pathogenesis and/or maintenance of hypertension in a number of aninal models including the spontaneously hypertensive rat (SHR). The present study examines the normal time course for the development of renal innervation in SHR, Wistar Kyoto (WKY) and Wistar (WR) rats. Newborn, 1, 2, 3 and 6 week-old rats are used. Renal innervation is followed by the SPG histofluorescence method for the demonstration of monoamines.

In newborn and 1 week-old WKY and WR, histofluorescent fibers are limited to the larger segmental-arcuate vessels with a few single fibers present in the perihilar cortex. The newborn SHR's innervation pattern is similar to that of the WKY and WR, however, in the 1 week-old SHR, cortical fibers are found not only in the perihilar cortex but also in the cortical region opposite the hilum. Cortical nerves are present in all cortical regions of all strains at 2 weeks of age. At 3 weeks of age there are qualitatively more cortical nerve fibers present than at 2 weeks with a similar pattern in all rat strains.

The present study indicates that the kidneys of the SHR establish cortical innervation earlier than do the normotensive rats. This earlier innervation could alter the early functional differentiation of tubular and/or vascular elements of the kidney, leading to the development of the hypertension or conditions which would be permissive for the elevation of blood pressure.

A COMPARISON OF TWO ISOTOPIC RENAL SCANNING TECHNIQUES IN THE ASSESSMENT OF NORMAL RENAL FUNCTION. M.L. Gross, W. Potvin*, J. Riccobono*, J.V. Nally, J. Windham*, Medical College of Ohio, Toledo, Ohio

Computer assisted 90 sec Tc-99m DTPA renal scans are utilized at our institution for the detection of renal artery stenosis. Preliminary results show excellent correlation with arteriography in patients with renal artery stenosis, in contrast to the conventional I-131 Hippuran renogram. The current study was designed to assess false positive responses for both the I-131 Hippuran and the 90 sec DTPA scanning techniques in normal subjects. Twenty subjects were studied who were normotensive and without evidence of kidney disease as assessed by urinalysis and serum creatinine. All renal scans were performed by usual clinical protocol without any special attention to hydration status. Ninety second Tc-99m DTPA and 30 minute I-131 Hippuran time activity curves were derived from regions of interest established for the aorta, kidneys and bladder. Mean values and deviations (typically 15-25%) were calculated for several Tc-99m curve parameters. These 90 sec studies were quite consistent with respect to curve shape, and allowed a standard template to be synthesized from the 40 normal kidney curves. In contrast, the shapes of the 30 min. Hippuran curves were inconsistent, with parameter deviations in the range of 30-40%. Only 9/20 Hippuran studies were judged to be unequivocally normal compared to 18/20 for the Tc-99m DTPA studies, based on conventional clinical criteria. These results suggest that the Tc-99m DTPA may be the renal scanning method of choice, and that the I-131 Hippuran scan as currently employed may have limited utility in renal scanning.

EFFECTS OF ENALAPRIL ON GROWTH, HEMATOCRIT % AND Rb^+ TRANSPORT IN SPONTANEOUSLY HYPERTENSIVE RATS FED A DIET CONTAINING 4% NaCl + 2.6% KCl. Anne B. Gould and Susan Goodman*. Hahnemann Univ., Dept. of Physiol. & Biophys., Philadelphia, PA.

Current evidence suggests a relationship between the renin system, erythropoiesis and hypertension. A common factor may be an imbalance in the phosphorylation potential ratio resulting from the demand for ATP exceeding the supply. To increase the demand for ATP, 40 day old male rats were given a moderately high salt diet for 5 to 6 months. Hematocrit values and rubidium transport were measured as indirect evidence of energy need and blood vessel adaptation respectively. Four groups of rats were treated as follows:

Group	Diet supplement	Medication
1. WKR	4% NaCl + 2.6% KCl	- - - - -
2. SHR	"	- - - - -
3. SHR	"	Enalapril
4. SHR	- - - - - - - -	Enalapril

A linear relationship between blood pressure and weight was observed in normotensive male rats between the ages of 6 and 19 weeks (Group 1), whereas in hypertensive rats the relationship was not linear, blood pressure increased after a gain in body weight. Although Enalapril (an angiotensin I converting enzyme inhibitor) was effective in keeping blood pressure within the normal range, rats fed the moderately high salt diet and given Enalapril had higher hematocrit values and lower body weights than rats not feed added salt. Rubidium uptake in abdominal aorta and caudal artery correlated positively to blood pressure; $r = 0.79$, $p<0.001$ and $r = 0.58$, $p< 0.005$. It is concluded that angiotensin II helps the growing rat adapt to the increased energy demand of a moderately high salt diet.

GAMMA ($\gamma 2$) MSH: A HYPERTENSOGENIC PEPTIDE DERIVED FROM PRO-OPIOCORTIN. K.A. Gruber, M.G. Callahan,* R.G. Kirby,* L.D. Wilkin,* L.D. Mitchell,* A. McCrae-Degueurce,* L.E. Ohman,* J.R. Lymangrover,* and A.K. Johnson.* U. of Iowa, Iowa City, IA 52242; INSERM U249, Montpellier France; Wake Forest University Med. Center, Winston-Salem, NC 27103.

We have shown that γMSH, a peptide derived from pro-opiocortin, has dose dependent natriuretic and pressor activity. We now report further studies. γMSH: 1) pressor effects (3-100 ug/kg increased MAP 15-100 mmHg) are attenuated by prazosin (α_1 blockade) or chlorisondamine (ganglionic blockade); 2) inhibited the baroreceptor reflex, even after β_1 blockade to prevent sympathetic chronotropic responses; 3) infusions (50 ng/kg/min) raised MAP 30-40 mgHg within 3-4 days; 4) produces no change in renal blood flow during its pressor response; 5) increased the firing rate of supraoptic magnocellular single units after systemic administration of 300 ng/kg. Circulating γMSH peptides are mainly secreted from the pars intermedia, which is regulated by the arcuate nucleus (AN). Using a retrograde tracer, we have demonstrated that AN receives efferents from the subfornical organ, organum vasculosum, and B 7&8 areas of the brainstem. Thus, γMSH peptides activate the central sympathetic nervous system while preserving renal blood flow (a logical action for a "natriuretic hormone:) and their secretion may be controlled by fore and hindbrain areas known to regulate hydromineral status.

METABOLIC EFFECTS OF INDACRINONE IN ESSENTIAL HYPERTENSION, Gordon P. Guthrie, Jr.,* E. Douglas Rees,* Theodore A. Kotchen. University of Kentucky College of Medicine, Lexington, KY 40536.

Most antihypertensive diuretics produce adverse metabolic side effects, including hyperuricemia and increased LDL cholesterol. Indacrinone (IN) is a new, long-acting loop diuretic containing natriuretic and uricosuric enantiomers. We treated 16 patients having mild to moderate essential hypertension with IN by a double-blind placebo (P) controlled protocol for 18 weeks and measured biweekly blood pressure (BP) and metabolic effects:

WEEK	2	4	6	8	12	16	18
DRUG	P	P	IN	IN	IN	IN	P
BP	138	138	132*	133*	133*	130*	135
	91	94	89	89	86	87	90
Potassium	3.9	4.0	3.6*	3.5*	3.8	3.9	4.3
Uric Acid	6.0	6.3	5.1*	5.5	5.4*	5.7	6.9
Glucose	102	106	105	106	104	107	105
Triglycerides	170	190	205	190	220	170	136
Cholesterol	222	231	243	228	234	220	213
HDL	44	43	44	43	46	43	42
LDL	145	150	163	151	149	148	141
VLDL	34	39	34	38	37	29	30

*($p<0.05$ vs P)

IN modestly reduced BP and serum potassium and uric acid, and produced no significant changes in serum total lipids, glucose, or lipoprotein concentrations. Thus, IN is a new antihypertensive diuretic that unlike some others is without several adverse metabolic side effects.

ROLE OF INCREASED RENAL ARTERIAL PRESSURE IN ESCAPE FROM ANTIDIURETIC EFFECTS OF VASOPRESSIN. J.E. Hall and Jean-Pierre Montani*. Dept. of Physiology, Univ. Miss. Med. Ctr., Jackson, MS.

This study examined the role of increased renal artery pressure (RAP) in mediating escape from the antidiuretic action of vasopressin (AVP) in conscious instrumented dogs. In normal dogs in which RAP was permitted to increase, chronic AVP infusion, at a rate (0.2 mU/kg/min) that was acutely subpressor, gradually increased mean arterial pressure (MAP) from 98±5 to 119±5 mmHg after 4 days while decreasing urine output (UO) and increasing urine osmolality (U_{osm}). However, after 4 days of AVP infusion, UO and U_{osm} returned to control while the hypertensive effects of AVP waned so that MAP averaged only 110±6 mmHg after 7 days of AVP. In contrast, when RAP was prevented from increasing in 4 dogs with an electronic servo-controlled aortic occluder, chronic AVP infusion caused sustained decreases in UO while U_{osm} was elevated from 575±51 to 1230-1690 mOsm/l throughout the 7 days of AVP infusion. In addition, the hypertensive effects of AVP did not wane when RAP was servo-controlled; instead, MAP continued to increase so that after 7 days of AVP infusion, MAP averaged 149±6 mmHg, compared to a control of 98±3 mmHg. These findings suggest that escape from the chronic antidiuretic action of AVP is mediated by increased RAP which causes diuresis thereby diminishing the hypertensive action of AVP. However, when pressure diuresis is prevented, AVP causes severe hypertension, suggesting that increased levels of AVP may be an important hypertensive mechanism when renal function is impaired and pressure diuresis is blunted.

CORRELATION BETWEEN DIFFERENT CATION TRANSPORT ABNORMALITIES OF ERYTHROCYTE AND PLASMA NOREPINEPHRINE LEVELS IN ADOLESCENTS WITH ESSENTIAL HYPERTENSION. Jean-Guy Mongeau, Univ. of Montreal, Ste-Justine Hospital, Dept. of Pediatrics, Section of Nephrology, Montreal, Canada.

The maximal rate of Na+ and K+ cotransport, the maximal rate of Na+ lithium countertransport and the rate constant of Na+ and K+ passive permeability of red blood cells were determined in 39 adolescents with labile essential hypertension, and 10 with stable essential hypertension. In labile hypertension, 21% were found to have a decreased Na+ cotransport, 17% an increased Na+ countertransport and 6% an increased Na+ leak. In stable hypertension, 10% had a decreased Na+ cotransport, 29% an increased Na+ countertransport and 25% an increased Na+ leak.

In 32 of these patients, plasma norepinephrine levels were also determined and the results were the following:

	n	Hyperadrenergic	Type of HT
Decreased cotransport	12	10/12	labile 11/12 stable 1/12
Increased countertransport	8	3/8	labile 3/8 stable 5/8
Na+ leak	12	5/12	labile 8/12 stable 4/12

Our results suggest that in labile hypertension, plasma norepinephrine is elevated and cotransport decreased whereas in stable hypertension, normal values of plasma norepinephrine are usually found with an increased countertransport.

RENAL VASCULAR HYPERTENSION IN PATIENTS AGE 60 & OVER, RESULTS OF OPERATION. M. Hanna,* A. Fitz. Department of Medicine, Veterans Administration Hospital & University of Iowa, Iowa City, Iowa.

It has been suggested that older patients should not be aggressively evaluated for renal vascular hypertension (RVHT) because of relatively poor long term prognosis related to vascular disease in other organ systems.

Twenty eight patients over age 60 (Group I) (age 64.2 ± 3.9 yrs.) operated on for arteriosclerotic RVHT during a 5 year period were compared to 32 patients under 60 (Group II) (52.1 ± 6.8 yrs.). Pre op blood pressure was the same in both groups, 195/96 ± 29/10 mm Hg in Group I and 171/100 ± 30/13 mm Hg in Group II, renal function measured by Serum Creatinine (Scr mg%) was similar in both groups 1.6 ± 0.6 and 1.6 ± 0.7 and duration of hypertension was similar. The average number of medications taken was the same, 3.3 ± 1.1 in each group, and the type of operative proceedures used were the same in both groups. There was one post operative death in each group.

Group I was followed for 37.1 ± 17.3 months compared to 35.3 ± 20.0 in Group II. Blood pressure was cured or improved in 24/27 (88%) in Group I. Scr remained stable in Group I, 1.6 ± 0.6 mg%, but increased slightly to 2.2 ± 2.1 in Group II.

This data obtained in patients age 60 to 73 who underwent a variety of operations for RVHT suggests that outcome in terms of survival, BP and renal function is as good in this group as in younger patients. Surgical intervention for hypertension should not be withheld on the basis of age if indicated by usual clinical criteria.

EFFECTS OF CAPTOPRIL ON RENAL HEMODYNAMICS AND HIPPURAN EXCRETION IN RENOVASCULAR HYPERTENSION (RVH). A.K. Hopla,* C.E. Kaufman, V. Ficken,* C. Ryals,* W. Allen,* J.C. Leonard.* Dept. of Med. and Radiol. Univ. of Oklahoma and VAMC, Oklahoma City, OK.

Captopril (Cp) may cause acute renal failure in patients (pts) with bilateral renal artery stenosis (RAS) but the hemodynamic effects of Cp in unilateral RAS are undefined. Eighteen hypertensive pts undergoing arteriography and Cp stimulated renal vein renins (SRVR) were divided into 3 groups Group I (6 pts) had >75% RAS, lateralizing SRVR, and a good response to surgery (5/5). Group II (5 pts) has RAS but nonlateralizing SRVR. Group III (7 pts) had no RAS. Single kidney glomerular filtration rate (SKGFR) and effective renal plasma flow (SKERPF) were measured by radionuclide methods before and 1 hour after 25 mg. of Cp. The SKGFR fell >10% in all kidneys with RAS in Group I (mean pre/post = 45.5/33.3cc/min; p<.01), but showed no consistent changes in the contralateral kidneys or in Groups II or III. Blood pressure fell similarly in the 3 groups. The table presents mean values expressed as a % change from baseline.

	Group I	Group II	Group III
Δ SKGFR%	-30.1±6**	23.4±12	17.8± 9
Δ SKERPF%	-32.5±11*	15.6±16	5.0 ± 6

*= p<.025; **= p<.01

The baseline hippuran excretion curve, abnormal in 3/11 stenotic kidneys (Group I + II) showed delayed excretion in 9/11 kidneys, including all of Group I, after Cp (p<.05).

In conclusion, Cp reduces both renal perfusion and GFR and delays hippuran excretion in kidneys with functionally significant RAS. It also impairs hippuran excretion in the presence of RAS which is otherwise of no apparent significance.

IDENTIFICATION OF THREE NAK-ATPASE INHIBITORS IN HUMAN PLASMA. R.A. Kelly,* D.S. O'Hara,* W.E. Mitch, T.W. Smith.* Harvard Med. Sch., Boston, MA.

Endogenous NaK-ATPase (NKA) inhibitors have generated intense interest because they might play an important role in the regulation of blood pressure and sodium homeostasis. Indeed, we have shown that NKA inhibitory activity is higher in desalted plasma from hypertensive patients than from normotensive controls ($12.5\pm1.8 \times 10^{-10}$M, n=12, vs $2.9\pm2.5 \times 10^{-10}$M, n=6, ouabain equivalents; mean ±SE; p<0.02). Using reverse-phase HPLC, we have identified three fractions (EI_1, EI_2 and EI_3) in desalted, deproteinized plasma from normal human subjects that inhibit NKA activity and displace ^{3}H-ouabain from the enzyme. They also crossreact with digoxin-specific polyclonal antibodies, implying a structural similarity with digitalis. In addition, we found one other fraction (IR_1) that shows crossreactivity with polyclonal and monoclonal digoxin-specific antibodies, but does not inhibit NKA. All fractions have a molecular weight <2,000 and are resistant to acid hydrolysis and protease digestion. To determine if binding of EI_1, EI_2 and EI_3 to NKA resembles that of digitalis, we examined whether increasing concentrations of KCl or the absence of ATP in the incubation media would lower the affinity of each fraction for the enzyme. Only the EI_3 peak shows a reduced affinity for NKA in the presence of KCl; unlike ouabain, none of the inhibitory fractions requires ATP for binding. Thus, desalted, deproteinized plasma contains at least three factors which may be physiologically important inhibitors of NKA, exhibiting some characteristics of the digitalis glycosides.

EFFECTS OF CA^{2+} AND NA^+ ON BLOOD PRESSURE, FOOD CONSUMPTION AND WEIGHT IN THE SPONTANEOUSLY HYPERTENSIVE RAT. N. Karanja,* J. Metz,* D. Lee,* T. Phanouvong,* D. McCarron. Oregon Health Sciences University, Portland, OR.

The spontaneously hypertensive rat (SHR) exhibits abnormal Ca^{2+} metabolism. Previous studies have shown a protective effect of Ca^{2+} and Na^+ on the expression of hypertension in the SHR. We assessed systolic blood pressure (BP), food consumption and weight (wt) in the SHR on varying Ca^{2+} and Na^+ diets.

75 SHRs were assigned to 1 of 5 diets at 6 wks of age (WOA): high Ca^{2+} (2%)/high Na^+ (1%); high Ca^{2+}/normal (N) Na^+ (.45%); control 1% Ca^{2+}/.45% Na^+; low Ca^{2+} (.1%)/NNa^+; low Ca^{2+}/low Na^+ (.25%). With 15 animals per diet, 5 were assigned to a pair fed, weight fed or ad libitum feeding regimen. Weight and BP were measured every 2 wks from 10 WOA. Food was administered daily. ANOVA and multiple range testing were used. Mean (±SD) BP (mmHg) by diet at 16 WOA:

HCa/HNa	HCa/NNa	NCa/NNa	LCa/NNa	LCa/LNa
197±15	179±21	202±22	204±23	224±18

No differences were noted in BP or wt between feeding methods. BPs in the HCa^{2+} diets were lower (p<.01) than in the LCa^{2+}/LNa^+ diet. The HCa^{2+}/HNa^+ group consumed the greatest amount of food, while the HCa^{2+}/NNa^+ consumed the least (p<.05). The high Ca^{2+}/NNa^+ had the lowest BP and body wt, despite receiving a smaller dose of Ca^{2+} and Na^+ overall. We conclude that: 1) HCa^{2+} diets result in lower BP, 2) Ca^{2+}'s BP effect may be maximized by a NNa^+ diet, and 3) These effects of Ca^{2+}/Na^+ are independent of feeding method.

ANTIHYTERTENSIVE DRUGS AND SODIUM RESTRICTION - ANALYSIS OF THEIR INTERACTION BASED ON THE RENAL FUNCTION CURVE. G.Kimura, F.Deguchi, S.Kojima, M.Yokouchi, T.Ashida, M.Kuramochi, K.Ito and M.Ikeda. Natl. Cardiovascular Ctr., Osaka, Japan.

The hypotensive effects of some drugs are augmented under sodium restriction, while those of others are not. The mechanisms of these interactions were theoretically analyzed based on the renal function(arterial pressure-natriuresis)curve.

Four-week studies were performed in 24 patients with essential hypertension who were given a regular sodium diet(12-15 g/day of NaCl) in the 1st & 3rd weeks and a sodium restricted diet(0-3 g/day) in the 2nd & 4th weeks. One of three antihypertensive drugs, 60 mg/day of nicardipine(Ca-antagonist), 120 mg/day of propranolol(ß-blocker) or 150 mg/day of captopril(converting enzyme inhibitor) was administered in the 3rd & 4th weeks. Urinary sodium excretion was plotted on the ordinate as a function of mean arterial pressure before and after administration of the antihypertensive drugs.

The hypotensive effect of nicardipine, being independent of the amount of sodium intake, was based on the leftward shift of the renal function curve, probably due to the decrease in renal vascular resistance. The effects of propranolol and captopril, being augmented under sodium restriction, were based on the combination of the left-ward shift and the decrease in the slope. The decrease in the slope might be due in part to the inhibition of the renin-angiotensin system. Diuretics, the effect of which is suppressed under sodium restriction, may increase the slope of the renal function curve and potentiate the hypotensive effects of propranolol and captopril.

CNS ADMINISTRATION OF CLONIDINE ON RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) AND SODIUM EXCRETION ($U_{Na}V$) IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR). J.P. Koepke & G.F. DiBona. Dept. Int. Med., Univ. Ia. Col. Med & VAMC, Iowa City, IA

The effects of intracerebroventricular (i.c.v.) injection of clonidine on basal and air stress evoked responses of RSNA (integrator resets/min), $U_{Na}V$ (μEq/min/100g BW), and MAP (mmHg) were examined in conscious SHR. Data are mean±SE; n=6; C=Control, R=Recovery; *p< 0.05 vs. C, + p < 0.05 R vs. C; 10 min periods

	Saline i.c.v. 2 μl				Clonidine i.c.v. 15 μg		
	C	Air	R		C	Air	R
RSNA	8.2 ±0.6	14.1* ±1.4	8.1 ±0.7	+	3.4 ±0.6	3.8 ±0.7	3.4 ±0.6
$U_{Na}V$	1.2 ±0.2	0.7* ±0.2	1.3 ±0.3	+	2.3 ±0.3	2.0 ±0.3	2.0 ±0.5
MAP	169 ±12	176* ±11	170 ±12	+	145 ±8	148 ±8	144 ±8

Air stress increased RSNA and MAP, and decreased $U_{Na}V$. In the same conscious SHR, i.c.v. clonidine (15 μg) lowered basal RSNA and MAP, and increased basal $U_{Na}V$. RSNA, $U_{Na}V$, and MAP responses to air stress were abolished by i.c.v. clonidine. The effect of clonidine to decrease basal RSNA and to inhibit the air stress induced increase in RSNA and decrease in $U_{Na}V$ were reversed by prior administration of rauwolscine (30 μg, i.c.v.), an α_2-adrenoceptor antagonist; rauwolscine did not affect the clonidine induced increase in basal $U_{Na}V$. A lower dose of clonidine (1 μg i.c.v.) abolished the RSNA and $U_{Na}V$ responses to air stress, but had no effect on basal values.

Conclusion: Central α_2-adrenoceptors mediate the RSNA, $U_{Na}V$ and MAP responses to air stress in conscious SHR.

DIETARY NaCL AS A DETERMINANT OF DISORDERED CALCIUM METABOLISM IN THE DAHL SALT-SENSITIVE RAT. **T.W. Kurtz and R.C. Morris. General Clinical Research Ctr., Univ. of California, San Francisco.**

In Dahl salt-sensitive (S) rats, but not in Dahl salt-resistant (R) rats, provision of supplemental dietary sodium chloride (NaCl) will induce hypertension. To investigate whether supplemental dietary NaCl induces an abnormality in calcium metabolism in S rats, we determined whether provision of supplemental dietary NaCl induces a greater increase in urinary excretion of calcium (UCaV) in S rats than in R rats, before hypertension develops. Ten S rats and Ten R rats (females) were individually housed in metabolic cages and pair-fed a low salt diet for two weeks. Over this two week period, the urinary excretion of calcium in the S rats was not significantly different from that in the R rats. However, within one day of supplementing the diet with NaCl, the urinary excretion of calcium in the S rats, 18 ± 4 μmoles/day became significantly greater than that in the R rats, 8 ± 1 μmoles/day ($p < .01$). Over a four week period in which supplemental dietary NaCl was provided, the urinary excretion of calcium in S rats, 92 ± 5 μmoles/week, was significantly greater than that in R rats, 58 ± 4 μmoles/week ($p < .001$). After 4 weeks of supplemental dietary NaCl, mean arterial pressure in the S rats, 133 ± 2 mm Hg, was significantly greater than that in the R rats, 121 ± 2 mm Hg ($p < .001$). These findings suggest that in Dahl salt-sensitive rats, an amount of dietary NaCl that induces hypertension, also induces a disorder in calcium metabolism that could contribute to the pathogenesis of salt-sensitive hypertension.

INHIBITION OF THE SODIUM PUMP IN HEALTHY SUBJECTS: HEMODYNAMIC CHANGES AND EFFECTS OF CALCIUM ENTRY BLOCKADE. H.J. Kramer, K. Glänzer *,T. Freitag *,J. Schönfeld *, M. Sorger *, and R. Düsing. Med. Poliklinik, Univ. of Bonn, W-Germany.

Since an endogenous inhibitor of Na-K-ATPase may play a pathogenetic role in various forms of hypertension, the present study was performed in 6 healthy volunteers to investigate the hemodynamic effects of ouabain (8.5 μg/kg b.wt. i.v.), a potent inhibitor of this enzyme. The role of intracellular calcium was studied indirectly by administration of nifedipine (10 mg orally). Within 2 h this dose of ouabain decreased RBC Na-K-ATPase from 12.5±2.0 during the control period (C) to 6.3±1.0 nmol P_i/mg protein x h ($p < 0.05$) and was accompanied by a rise in RBC ATP from 1.39±0.19 to 1.69±0.20 mmol/l cells and a decrease in K^+ concentration from 166.9±2.4 to 162.5±2.5 mmol/l cell water ($p < 0.05$). Peripheral vascular resistance index (C: 1914±257 dyn x sec x cm^{-5} x m^{-2}) rose by 24.3±7.8% ($p < 0.05$). Cardiac output (C: 3.85±0.42 l/min x m^2) and heart rate (C: 73.8±2.9 beats/min) decreased by 13.8±5.2% and 8.5±3.2%, resp. ($p < 0.05$), while stroke volume (C: 49.0±5.0 ml/m^2) and diastolic blood pressure (71.2±2.8 mm Hg) remained unaltered (-5.4±5.0% and 2.5±1.9%, resp.). Nifedipine had no effects on ouabain-induced changes in RBC Na-K-ATPase, ATP, and K^+, but completely prevented its hemodynamic effects.

The results support the concept that endogenous inhibition of Na-K-ATPase, e.g. following blood volume expansion and decreased baroreceptor reflex sensitivity, plays a pathogenetic role in volume-dependent human hypertension which depends on transmembrane calcium flux.

IS HYPERTENSION WORSE IN BLACKS? COMPARISON OF CREATININE LEVELS AT EQUAL BLOOD PRESSURES; EFFECT OF POTASSIUM. H. G. Langford, C. E. Ford*, Don Blaufox for the Hypertension Detection and Follow-up Program. Univ. of Mississippi Med. Ctr., Dept. of Med., Jackson, Univ. of Texas, School of Public Health, Houston, Albert Einstein College of Med., Bronx, New York.

Hypertension is often said to be worse in blacks; i.e., more organ damage is present with the same blood pressure. Blacks in the U.S. ingest and excrete significantly less potassium (K) than whites. This study compares the serum creatinine (cr.) of blacks and whites at equivalent blood pressures, and considers the possibility that K intake may be the cause of the differences found. The data is from the 7,000 Hypertension Detection and Follow-up Program participants who were not on antihypertensive therapy at baseline. Serum cr. was higher in black men than in white men, and in black women than in white women. This was true overall, and in each diastolic blood pressure strata (Stratum I 90-104 mmHg, Stratum II 105-114 mmHg, and Stratum III 115 mmHg and above). In Stratum I, whites with cr. 1.0-1.09 were at the 62nd percentile, compared to the 46.5 percentile for black men. For women, the comparable figures were 88.7 for whites and 82.9 for blacks. Serum K was lower for blacks than whites in the lowest blood pressure strata, but higher in the upper blood pressure strata. We attribute the lower K in blacks in the lowest strata to the lower K intake of blacks, and the higher K in the higher strata to increasing renal damage. The data is compatible with Tobian's experimental findings of increased renal damage at equal blood pressures produced by low K intake in Dahl rats.

EFFECT OF MILD POTASSIUM DEPLETION (KD) ON THE NATURAL HISTORY OF HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). S.L. Linas, R. Marzec-Calvert. Univ. of Colorado Health Sciences Center, Denver, Colorado.

Considerable controversy exists about the hemodynamic effect of K in hypertension. Whereas we have shown that mild K depletion prevents and reverses renovascular hypertension, others have suggested that K depletion leads to hypertension. Since mild KD often occurs in patients treated with diuretics, we determined the hemodynamic effect of mild KD in the SHR. SHR with systolic blood pressure (SBP) of <130 mm Hg were placed on a K replete (KR) (210 mEq/kg) or a mild KD (54 mEq/kg) diet. After 3 wks SBP reached 144 ± 2 in KR but only 123 ± 3 mm Hg in KD ($p<.001$). After 6 wks SBP was 157 ± 4 in KR and only 124 ± 3 mm Hg in KD ($p<.001$). Plasma K was reduced from 3.9 ± .1 in KR to 3.3 ± .1 mEq/L in KD ($p<.01$) and muscle K was reduced by 6% from 385 ± 7 in KR to 363 ± 8 mEq/kg in KD ($p<.01$). Although KD rats gained 75% as much weight at 3 wks, by 6 wks weight gain was comparable in KD and KR. To determine if mild KD could reverse established hypertension, SHR with SBP >150 mm Hg were continued on KR or changed to the KD diet. After 10 days SBP was 157 ± 3 in KR but only 123 ± 3 mm Hg in KD ($p<.001$). After 21 days SBP was 160 ± 3 in KR and 126 ± 2 mm Hg in KD ($p<.001$). The protective effect of KD was mediated by a 29% reduction in vascular resistance: .65 ± .05 in KR to .46 ± .02 U in KD ($p<.01$). In summary, mild potassium depletion prevents the development of hypertension and reverses established hypertension in the SHR. Conclusion: Mild potassium depletion may be a mechanism by which diuretics reduce blood pressure.

EFFECTS OF CALCIUM CHANNEL BLOCKADE OR DIETARY CALCIUM SUPPLEMENTATION ON BLOOD PRESSURE, SODIUM HOMEOSTASIS, RENIN SYSTEM, AND SYMPATHETIC NERVOUS SYSTEM. Friedrich C. Luft, George R. Aronoff, Rebecca S. Sloan*, Myron H. Weinberger. Indiana University, Indianapolis, Indiana.

To test the hypothesis that the antihypertensive effects of either the calcium channel blocker nitrendipine (N) or dietary calcium supplementation (Ca) are in part related to natriuresis, we gave 8 hypertensive and 8 normotensive subjects placebo for 8 days followed by either N 20 mg bid or Ca carbonate 500 mg bid for 8 days. Sodium intake was fixed for each subject. All urine was collected. Blood was drawn at the end of the placebo and drug periods for renin (PRA), aldosterone (ALDO), and norepinephrine (NOREPI). Data (mean + SD) are below:

	UNaV (mEq/d)	PRA (ng/ml/3hr)	ALDO (ng/dl)	NOREPI (pg/ml)
Placebo	127±30	6.5±1.5	11±2	360±35
N	144±27	14.5±2.5	12±1	468±59
p value	<0.01	<0.01	NS	<0.01
Placebo	144±41	8.6±3.6	10±4	368±58
Ca	150±33	8.6±4.7	10±3	373±62
p value	NS	NS	NS	NS

N lowered diastolic BP by 5 mm Hg in hypertensives and caused significant natriuresis, increased PRA and NOREPI. Ca did not lower BP, cause natriuresis, or effect the renin or sympathetic nervous systems. We conclude that the blood pressure lowering effects of N may in part be related to natriuresis, but that dietary Ca supplementation does not lower BP or effect Na excretion.

INHIBITION OF MYOCARDIAL SODIUM POTASSIUM PUMP ACTIVITY IN SEA WATER ADAPTED GOLDFISH. Jerome Lowenstein, NYU Medical Center, N.Y., N.Y.

The goldfish (Carassius auratus) a fresh water teleost, survives transfer to dilute sea water. Increased ingested sodium is offset by increased renal excretion; total body sodium is increased (Lahlou et al, Comp Biochem Physiol. 28:1427, 1969). We have examined the effects of salt loading during sea water adaptation in vascular and cardiac sodium-potassium pump activity (Na^+K^+ATPase).

Goldfish were maintained in fresh water (Na=9 mEq/L). transferred directly to 1/3 sea water (Na=137 mEq/L) and studied after 1-8 days. Fragments of aorta and ventricle were incubated in oxygenated Ringer's solution. Na^+K^+ATPase was estimated from the difference between ^{86}Rb uptake in the presence and absence of ouabain ($10^{-4}M$).

Myocardial Na^+K^+ATPase was inversely related to the duration of sea water adaptation ($r=.92$) and was significantly reduced after 3 days (sea water = 1.65±.37, fresh water = 2.84±.38 pmole ^{86}Rb/mg/min, $p<.03$). Aortic Na^+K^+ATPase was unchanged in sea water adapted fish. Plasma $[Na^+]$ increased from 149 mEq/L to 179 and 189 mEq/L after 1-3 and 4-8 days in sea water.

The finding that sodium loading results in inhibition of sodium-potassium pump activity in myocardial muscle but not in aortic smooth muscle suggests that pump inhibition is mediated by an endogenous circulating inhibitor acting on a specific receptor rather than by a nonspecific toxic effect of sea water adaptation.

PRESSOR RESPONSE TO ANGIOTENSIN II IN THE SHR: MODIFICATION BY DIETARY CALCIUM AND SODIUM. D. McCarron and L. Wegener,* Oregon Health Sciences University, Portland, Oregon.

Moderate chronic increases in dietary Ca^{++} and Na^+ have been shown to modify hypertension development in the SHR when initiated at an early age. We sought to determine the effects of later dietary modification on BP and pressor response to IV angiotensin II (AII) in the SHR and WKY.

At 10 wks, 24 SHRs and 24 WKYs were randomized to 3 diets for 12 wks: control - 0.45% Na^+/1% Ca^{++} by weight; 1% Na^+/2% Ca^{++}; or 0.25% Na^+/0.1% Ca^{++}. A similar number of animals received the diets beginning at 18 wks for only 4 wks. Mean intra-arterial pressure (MAP) was monitored during the first min and at 3-min intervals, while AII was given IV in 10,30 and 100 µg doses.

Both strains demonstrated a dose-response relationship to the three doses of AII ($p<.0000$). Baseline BP was lower ($p<.01$) in both the SHRs and WKYs on the $\uparrow Na^+/\uparrow Ca^{++}$ diet for 12 weeks than their counterparts on the other diets (180±4.0mmHg $\uparrow Na^+/\uparrow Ca^{++}$ SHR vs 192±6.7 $\uparrow Na^+/\uparrow Ca^{++}$ SHR; 127±2.2 $\uparrow Na^+/\uparrow Ca^{++}$ WKY vs 137±2.7 $\uparrow Na^+/\uparrow Ca^{++}$ WKY. The maximum BP reached with AII was also lower ($p<.05$) (224±6.5mmHg $\uparrow Na^+/\uparrow Ca^{++}$ SHR vs 235±4.9 $\uparrow Na^+/\uparrow Ca^{++}$ SHR; 181±4.1 $\uparrow Na^+/\uparrow Ca^{++}$ WKY vs 190±4.6 $\uparrow Na^+/\uparrow Ca^{++}$ WKY, at 100 µg AII). ΔMAP did not differ significantly between groups. No significant differences were found between diet groups in either strain receiving the diets for only 4 weeks.

Moderate increases in dietary Na^+ and Ca^{++} reduce both baseline BP and maximal BP under AII stimulation when initiated early and/or continued for more than four weeks in both SHRs and WKYs.

ETHANOL IMPAIRS HUMAN BARORECEPTOR FUNCTION. R.H. Merrill, A-R.A. Abdel-Rahman* and W.R. Wooles.* Depts. of Medicine and Pharmacology, East Carolina University School of Medicine, Greenville, NC U.S.A.

The association between ethanol intake and hypertension has been well established but the mechanism has not been defined. Recent studies from our laboratories have suggested that acute ethanol administration produced an alteration in baroreceptor sensitivity (BS) in experimental animals. The present study investigated the possibility in humans that BS alteration was involved in ethanol-induced hypertension. BS was evaluated in volunteers as the gain in baroreceptor function following intravenous bolus injections of phenylephrine (PE; 25-150 µg), which produced a 10-25 mmHg rise in mean arterial pressure (MAP), before and after the administration of graded doses of ethanol. BS was expressed as the ratio of change in heart period (ΔHP) to the change in MAP (ΔMAP). There was a dose-related decrease in BS with increasing doses of ethanol; a decrease to 55% of control values after low doses and to 20% of control after high doses. HP was decreased in a dose-dependent fashion whereas MAP was not altered suggesting that impairment of BS was not secondary to changes in MAP. The PE pressor response curves were shifted to the left after ethanol perhaps as a result of the partial loss of baroreceptor function. These data suggest that acute ethanol impairs baroreceptor function and this effect may be involved in ethanol-induced hypertension. Further studies are necessary to establish whether this effect is of central or peripheral origin.

DIETARY CHLORIDE AS A DETERMINANT OF DISORDERED CALCIUM METABOLISM IN DEOXYCORTICOSTERONE (DOC) HYPERTENSION **R.C. Morris and T.W. Kurtz. Gen. Clin. Res. Ctr., Univ. of CA, San Francisco**

In rats given DOC, the finding that dietary NaCl induces hypertension but an equimolar amount of $NaHCO_3$ does not, might result from differing effects of the two Na^+ salts on calcium (Ca) metabolism. Uninephrectomized rats were pair-fed equimolar amounts of NaCl or $NaHCO_3$ and given DOC (IM) for five weeks. Over the first two weeks, urinary excretion of calcium (UCaV) (µmoles/100 g body wt/week) in rats given NaCl, 345 ± 50, was significantly greater than that in rats given $NaHCO_3$, 150 ± 30, $p < .005$. After two weeks, mean arterial pressure (MAP) (mm Hg) in rats given NaCl, 124 ± 2, was significantly greater than that in rats given $NaHCO_3$, 112 ± 3, $p < .005$. At this point, the rats initially given NaCl were switched to $NaHCO_3$; rats initially given $NaHCO_3$ were switched to NaCl. Replacing NaCl with $NaHCO_3$ corrected both the hypercalciuria and hypertension initially induced by NaCl. Replacing $NaHCO_3$ with NaCl induced hypercalciuria and hypertension. In another study of the DOC model, we sought to determine if UCaV became greater in rats given NaCl than in those given $NaHCO_3$, before their blood pressures became different. On day 1, UCaV (µmoles/100 g body wt) in rats given NaCl, 21 ± 2, was significantly greater than that in rats given $NaHCO_3$, 5 ± 1, $p < .025$. At 4 days, UCaV was also different; MAP was not. These findings indicate that in the DOC model, dietary NaCl has an effect on calcium metabolism different from that of $NaHCO_3$. The findings suggest that a disorder in calcium metabolism might contribute to the pathogenesis of DOC hypertension.

CA^{2+} ATPASE ACTIVITY IN HUMAN HYPERTENSION. Cynthia Morris,*Frank Vincenzi,*David McCarron. Oregon Health Sciences University, Portland, OR and Washington Health Sciences Center, Seattle, WA.

Membrane Na^+/K^+/ATPase activity has been linked to hypertension (HTN). The physiologic relation among cellular Na^+, K^+, Mg^{2+} and Ca^{2+} transport and the recognized disturbances of Ca^{2+} metabolism in HTN prompted us to measure RBC membrane, Na^+/K^+, Mg^{2+} and Ca^{2+} ATPase (both basal and maximal) activities in normal (N=12) and HTN (N=25) humans. RBC ATPase was measured at baseline (no Rx), placebo and oral Ca^{2+} (1000 mg/day x 8 wks) phases in a randomized, double-blind treatment trial. Baseline membrane RBC ATPase activity (Nm Pi/cells 10^6/min) (mean±SEM) were:

	Na^+/K^+	Mg^{2+}	Ca^{2+}/Basal	Ca^{2+}/Maximal
NL	4.96±0.49	12.95±0.67	7.98±0.48	33.55±3.50
HTN	5.04±0.36	12.82±0.48	5.96±0.29*	34.04±0.94

* $p \leq .001$

Only baseline unstimulated Ca^{2+} ATPase was significantly ($p<.001$) lower in HTNs compared to the NLs. Treatment with Ca^{2+} was associated with a significant increase in Mg^{2+} ATPase ($p<.05$) and Na^+/K^+ ATPase ($p=.07$) activity. Basal and maximal Ca^{2+} ATPase were unchanged during Ca^{2+} Rx. A significant reduction in BP with oral Ca^{2+} Rx was associated with an increase ($p<.001$) in Na^+/K^+ ATPase activity of the HTN subjects.

We conclude that RBC Ca^{2+} ATPase activity is reduced in untreated HTNs and that successful Ca^{2+} intervention for BP is associated with enhancement of Na^+/K^+ ATPase activity of RBC plasma membranes, but does not correct the reduced basal Ca^{2+} ATPase.

^{99m}Tc-DTPA FLOW STUDIES WITH CONVERTING ENZYME INHABITION (CEI) IN UNILATERAL RENAL ARTERY STENOSIS (uRAS). J.V. Nally, H.S. Clarke*, G.P. Grecos*, M. Saunders*, M.L. Gross, J.P. Windham*, Medical College of Ohio, Toledo, OH

This study was designed to evaluate our computer assisted 90 second and 15 minute ^{99m}Tc-DTPA renal flow studies vs ^{131}I-Hippuran renography with and without CEI in uRAS. In group I (n=10), angiograms, PAH clearances (C_{PAH}), DTPA and Hippuran studies were performed in dogs before and after the creation of moderately severe uRAS. Group II animals (n=8) with mild stenosis underwent the same protocol, plus DTPA and Hippuran studies and split function C_{PAH} and C_{IN}, performed during CEI [Captopril (1.5 mg/kg bolus and 1.5 mg/min x 60 min) provided by E.R. Squibb]. Recovery DTPA and Hippuran studies (Rec) were obtained and repeated using nitroprusside (NP) to lower MAP.

RAS in group I reduced ipsilateral C_{PAH} by 58 ± 9% and increased MAP by 12% ($p<.005$). Both DTPA studies were diagnostic in all 10, while the Hippuran study was diagnostic in only 6 of 10.

Group II DTPA or Hippuran studies alone were less diagnostic (37% vs 50%). CEI resulted in a 40% decrease in ipsilateral C_{IN} with no change in contralateral C_{IN} while decreasing MAP by 20%. CEI produced a striking depression in the DTPA curves such that all were now diagnostic. These changes reversed during Rec and were not seen with NP.

Conclusion: 1) DTPA studies proved superior to Hippuran renography in our model of acute uRAS. 2) With mild RAS, CEI induced a significant decrease in ipsilateral GFR resulting in striking changes in the DTPA curves. 3) DTPA nuclear studies coupled with CEI offers great promise as a diagnostic tool for RAS.

STUDIES ON THE VASCULAR MECHANISM OF ACTION OF ATRIAL NATRIURETIC FACTOR. E.H. Ohlstein,* S.S. Sherman,* B.A. Berkowitz* (intr. by L. Kinter) Dept. of Pharmacology, Biological R&D Smith Kline & French Laboratories, Philadelphia, PA

Atrial natriuretic factor (ANF) is an endogenous peptide that is believed to be an important mediator of renal hemodynamics and natriuresis and may be of therapeutic utility in the treatment of hypertension. Using a synthetic biologically active 23 amino acid sequence (atriopeptin II, AP II) we have studied the molecular mechanism of AP II vasodilatation in the rabbit thoracic aorta. AP II elicited dose-dependent vascular relaxation with an ED_{50} of 1 nM. Cyclic GMP levels were increased 5 fold. The elevation in tissue levels of cyclic GMP preceded the onset of relaxation. Tissue cyclic AMP levels were not altered by AP II.

To investigate the relationship between AP II and cyclic GMP we manipulated tissue cyclic nucleotide levels with methylene blue (MB, an inhibitor of guanylate cyclase) and phosphodiesterase inhibitors. MB inhibited AP II-induced vasorelaxation (ED_{50}=8 nM) and inhibited the stimulation of cyclic GMP accumulation by 35%. MB had similar effects on nitroglycerin-stimulated responses. The phosphodiesterase inhibitors isobutylmethylxanthine and M&B 22,948 (specific for cyclic GMP) potentiated the AP II-induced vascular relaxation and tissue cyclic GMP levels. These data indicate that cyclic GMP is a second messenger for the vascular relaxation mediated by ANF. The interrelationship between ANF and cyclic GMP may be important in the control of renal hemodynamics and as a target site for the treatment of hypertension.

EFFECT OF GUANABENZ (G) ON PAPILLARY COLLECTING DUCT CHLORIDE (Cl) REABSORPTION. RW Osgood*, TA Fried and JH Stein. Univ of Tx Hlth Sci Cntr, San Antonio, Texas.

Guanabenz, an α_2 agonist, has been reported to lower blood pressure (BP) while maintaining glomerular filtration rate (GFR) constant and increasing water and solute excretion. The site of the increase in solute excretion is still unknown. Surface micropuncture experiments have suggested deep nephrons or the collecting duct (CD) as the site of this action. To evaluate the possible role of the CD, Munich Wistar rats were studied using the exposed papilla technique. G infusion lowered BP 18.2 mmHg (n=7) with no change in GFR and an increase in water and solute excretion. In the control group (n=5), 68% of the delivered Cl was reabsorbed as compared to only 25% after G infusion. This decrease in the reabsorptive rate may have been minimized due to the decrease in BP seen with G. To eliminate this variable, an additional group of experiments (n=4) was performed. In this group, BP was initially lowered by an aortic snare (AS) to approximately 95 mmHg and micropuncture samples were obtained. The AS was released and when BP returned to baseline levels, the BP was lowered in a second period by G infusion to a level comparable to that produced by the AS and CD samples were again collected. Fractional excretion of Cl averaged 5.5% of the filtered load during the initial period compared to 10.3% during G. During the AS period, CD Cl reabsorption was 40.5 $\pm$ 7% of the delivered load, and 12.8 $\pm$ 3.4% after G ($p < .05$).

These data suggest that G is a signficant inhibitor of CD Cl reabsorption and this inhibition may explain the increased solute excretion following G administration.

ANGIOTENSIN II INHIBITION OF RENAL NERVE ACTIVITY IN CONSCIOUS DOGS. J.L. Osborn and G.E. Huhnke*, Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.

Angiotensin II (AII) may increase arterial pressure (AP) in part by altering peripheral sympathetic outflow from the central nervous system. The goal of this study was to determine the effects of AII infusion (2.5 and 5.0 ng/kg/min) into the common carotid (i.c.) and vertebral (i.vert.) arteries and jugular vein (i.v.) on mean arterial pressure (MAP), renal blood flow and renal nerve activity (RNA) in conscious dogs. Dogs (n=6) were instrumented with chronic arterial and venous catheters, doppler flow transducers and bipolar renal nerve electrodes at least 24 hours prior to study. Mean data (+SEM) are shown (C=control, MAP=mmHg, RNA=μv/sec, *p <0.05):

		Jugular	Carotid	Vertebral
	C	101±6	100±6	109±5
MAP	2.5	109±4	97±2	115±5*
	5.0	114±2*	106±5	119±6*
	C	122±43	76±33	127±38
RNA	2.5	45±9*	52±18	84±36*
	5.0	21±6*	44±15*	67±22*

Renal, carotid and vertebral blood flows were unchanged by AII infusion. Nonpressor AII (2.5 ng/kg/min) infused i.v. and i.vert. decreased RNA. Slightly pressor AII infusion (5.0 ng/kg/min) i.v, i.c. and i.vert. decreased RNA further. Thus, circulating AII at nonpressor doses may decrease RNA by a peripheral rather than a central action. We conclude that circulating AII may importantly influence renal tubular function by inhibition of RNA in conscious dogs.

EFFECT OF SELECTIVE CHANGES IN SODIUM (Na) AND CHLORIDE (Cl) INTAKE ON BLOOD PRESSURE (BP) AND RENIN AFTER UNILATERAL NEPHRECTOMY (UNx) IN THE RAT. CE Ott, BJ Holtzclaw,* JA Downs,* CE Bea,* and TA Kotchen, Depts. of Physiology and Medicine, Univ. of Kentucky, Lexington, KY.

BP increases in rats ingesting a low Na diet following UNx but not in rats on a normal Na diet. Captopril treatment prevents this increase. We have shown that Cl feeding, without Na, decreases plasma renin activity (PRA) compared to animals on a low Na and low Cl diet. This study examined the effect of selective changes in Na and Cl intake on BP and renin following UNx. A total of 46 rats was fed one of 3 diets: a normal NaCl diet, a low NaCl diet or a low Na-normal Cl diet. One week after UNx, BP as measured by tail cuff, was higher ($p<0.01$) in the low NaCl group than the other 2 groups. At 2 and 3 weeks BP was higher ($p<0.05$) in both the low NaCl (128.6 $\pm$ 1.9 mmHg) and low Na-normal Cl (132.1 $\pm$ 3.2) groups than the normal NaCl (117.1 $\pm$ 2.3) group and were not different from each other. At 3 weeks, 24 animals were decapitated for PRA measurements. The other 22 were given converting enzyme inhibitor. PRA was increased ($p<0.001$) in the low NaCl group compared to the other groups, which were not different from each other. BP after captopril was lower ($p<.05$) in the low NaCl group (73.1 $\pm$ 3.6 mmHg) than the low Na-normal Cl group (92.0 $\pm$ 6.7) or the normal NaCl group (99.9 $\pm$ 2.8) which were not different from each other. Thus: 1. low Na intake increased blood pressure following UNx in the presence or absence of Cl; 2. increased BP in the low NaCl group is renin dependent; 3. part of increased BP with Cl feeding is renin independent.

EFFECT OF SALT LOADING AND SALT RESTRICTION ON HUMAN ERYTHROCYTE RUBIDIUM INFLUX. Gilbert J. Perry*, Clinton Joiner*, and Harriet P. Dustan, UAB, Birmingham, Alabama.

A circulating inhibitor of Na-K ATPase has been suggested as the mediator of vasoconstriction and hypertension with volume expansion. Since Na-K ATPase is also present in red blood cells (RBC) we investigated the possibility of a circulating inhibitor by measuring the effect of changing sodium (Na) balance on RBC ouabain sensitive rubidium (Rb) uptake (OSRb) in 8 normotensive and 10 hypertensive black volunteers. OSRb, plasma renin activity (PRA) and mean arterial pressure (MAP) were measured during a 3 day control period (C-150 mEq Na day), 3 days of Na loading (SL-3.88 mEq Na/kg/day) and 4 days of salt depletion (SD-9 mEq Na diet with furosemide, 1 mg/kg, on the first day). In normotensive controls, OSRb was unchanged by SL and SD (Table). In hypertensives it was increased by SL and normalized by SD. The rise in OSRb during SL correlated with the subsequent fall in MAP during SD ($r = .79$, $p<.007$). This increase was found only in the salt sensitive (SS) hypertensives (i.e. those with a fall in MAP >15 mmHg during SD), and in those with very low C PRA (< .03). In non-salt sensitive (NSS) hypertension, or in patients with C PRA >.03, OSRb did not change.

	N	OS Rb Influx (mM/L·RBC/h) C	SL		SD
Normotensives	8	1.71	1.75	p=NS	1.67
Hypertensives	10	1.50	1.66	p<.016	1.58
SS	6	1.42	1.67	p<.006	1.60
NSS	4	1.63	1.66	p=NS	1.54
PRA <0.03	6	1.47	1.75	p=<.0001	1.67
PRA <0.03	4	1.55	1.53	p=NS	1.46

Thus we found no evidence for a Na-K ATPase inhibitor during SL. However, these results suggest that changes in cellular ion transport may play a role in the salt-sensitivity of arterial pressure in patients with very low PRA.

ACUTE RENAL DYSFUNCTION IN 1-KIDNEY, 1 ARTERY STENOSIS HYPERTENSION (1-K,1C HT) DURING RENIN-ANGIOTENSIN BLOCKADE. D.W. Ploth, R. Ingram*, and K. Kleeman*. Nephrol. Res. Trng. Ctr., Univ. Ala. in Birmingham, and VA Med. Ctr., Birmingham, AL.

We studied the mechanism of acute renal failure reported in settings of solitary kidneys with renal artery stenosis following administration of converting enzyme inhibitor (CEI) in 1-K,1C HT rats. Renal hemodynamic, clearance and excretory function were assessed in two groups of 1-K,1C HT rats (3-4 wks post-clip;5-6 wks post-nephrectomy). Under pentobarbital anesthesia identically prepared animals of both groups (Group I: hydropenic conditions; Group II: 12-18 hrs following 2-4 mg of Furosemide ip) were studied during a control period and during infusion of CEI (Captopril, 1 mg/kg·hr). In response to Captopril, animals of group I (n=5) evidenced no change in BP (164±7 to 162±4 mmHg; $\bar{x}$±SE), GFR (1.52±.11 to 1.66±.16 ml/min), estimated RPF (4.6±.2 to 4.4±.4 ml/min), or renal vascular resistance (RVR, 20±2 to 24±5 units), although Na excretion ($U_{Na}V$, .33±.1 to .78±.3 µEq/min) tended to increase. Animals of group II (n=6) lost 8-20 gm weight after Furosemide and exhibited higher baseline levels of BP (191±6 to 173±11 mmHg; $p<.01$). GFR (1.08±.11 to 0.66±.12 ml/min; $p<.01$) and filtration fraction decreased (.32±.05 to .21±.04; $p<.05$) in response to CEI, while estimated RPF (4.1±.9 to 3.0±.3 ml/min) and $U_{Na}V$ (.27±.13 to .08±.04 µEq/min) tended to decrease and RVR (40±12 to 53±19 units) tended to increase. These observations indicate that BP and renal function of 1-K,1C HT in rats is exquisitely sensitive to CEI during conditions of apparent activation of the renin-angiotensin system due to prior volume depletion.

RENOMEDULLARY INTERSTITIAL CELLS (RIC) DETERMINE SALT-RESISTANCE AND SALT-SENSITIVITY TO HYPERTENSION. J.A. Pitcock*, P.S. Brown*, B. Brooks*, J.P. Rapp*, W. Rightsel*, E.E.Muirhead. Univ. of Tennessee Cen. for Health Sci., Memphis, TN.

Dahl sensitive (S) rats develop hypertension when on a high salt diet. Dahl resistant (R) rats resist hypertension under similar conditions. Dahl determined that the difference resides in the kidneys. The Belgrade Wistar rat (W) of Susic and Kentera (WB) resists partial nephrectomy-salt hypertension. This resistance is abolished by papillectomy. We have compared resistant and sensitive strains of rat with emphasis on RIC. The RIC were studied in situ and in tissue culture. In situ, the RIC from resistant (R and WB) animals were more numerous, larger and have more lipid granules when compared to S animals. These differences were maintained in tissue culture. Transplants of R RIC produced a greater antihypertensive effect than transplants of S RIC in recipient hypertensive animals. In addition the dark intercalated cell of the collecting duct appears to extend more distally down into the papillary collecting duct in the resistant strains than in the sensitive strains. Serum chemistry studies also showed some difference between the resistant strains and the sensitive strains, most notably in serum cholesterol and serum alkaline phosphatase.

	R vs. S		WB vs. W	
Cholesterol	61.1	43.2	52.7	32.3
Alkaline Phosphatase	766.2	297.7	466.7	349.0

All $p<0.05$

Conclusion: RIC of S and R differ markedly. These differences are involved in salt-resistance and salt-sensitivity.

INHIBITORS OF THROMBOXANE SYNTHESIS AMELIORATE THE DEVELOPMENT OF HYPERTENSION IN WISTAR RATS WITH SPONTANEOUS HYPERTENSION (SHR). M.Purkerson, K.Martin, J. Yates* and S.Klahr. Washington U. School of Medicine, St.Louis,MO.

We have shown recently that administration of inhibitors of thromboxane (TBX) synthesis ameliorate the progressive renal disease and hypertension that occur in rats with a remnant kidney (PNAS, in press). To determine whether the effect of inhibitors of TBX synthesis on blood pressure (BP) was due to improved renal function or mediated "directly" we examined the effects of administering an inhibitor of TBX synthesis (OKY 046) on BP and renal function in SHR rats a model in which the renal lesions are secondary to the high BP. Six SHR received OKY 046, 20 mg/Kg BW bid for 100 days whereas 6 SHR controls received vehicle. Results at the end of this period were:

SHR	Mean BP mmHg	C_{in} ml/Kg BW	Cpah	TBX Exc. pg/min
Control	154±2.7	11.0±0.3	34.4±2.0	189.5±38.5
Treated	109±8.7	14.1±1.1	52.5±2.9	55.4±11.8

Urinary excretion of TBX was greater in SHR than in WKY (normotensive)rats and in these rats OKY 046 had no effect on TBX excretion or blood pressure. The results indicate that inhibitors of TBX synthesis prevent the development of hypertension in SHR rats. These compounds may represent a new type of antihypertensive drug with mechanisms of action different from most antihypertensive drugs currently in clinical use.

RENAL PAPILLARY COLLECTING TUBULAR (RPCT) CELLS DIFFER IN PGE_2 SYNTHETIC CAPACITY IN PRE-HYPERTENSIVE DAHL SALT-SENSITIVE (S) AND SALT-RESISTANT (R) RATS. G.M. Reid* and M.J. Dunn. Case Western Reserve University and University Hospitals, Dept. of Medicine, Cleveland, Ohio.

The kidneys of R rats have a higher natriuretic capacity and their papillae contain more PGE_2 than those of S counterparts. We tested the hypothesis that RPCT cells from R and S animals may differ in their capacity to produce PGE_2, an autacoid which inhibits collecting duct NaCl transport. R and S rats were placed on a 0.4% NaCl diet, after weaning and studied at 5 wks of age. Direct, lightly anesthetized, intra-aortic blood pressures were measured (R, 86±2.1/71±1.7 mmHg, mean±SEM; S, 101±3.9/80±2.8). R rats excreted more urinary PGE_2 than S rats (R, 23.9±1.7 ng/24 hrs, n=22; S, 18.8±1.3 ng/24 hrs, n=22, p<.025). RPCT cells were cultured, in parallel, from 8 groups of 2-3 R and S 5 wk rats, in fully defined K-1 media for 72 hrs. Thirty min incubations were carried out in at least 6 replicate wells per experimental condition, in buffered media. PGE_2 was measured by RIA.

	PGE_2 (pg/μg protein/30 min) R	S	P†
Basal	3.3±.5	2.0±.4	.01
A23187 (2μM)	23.2±4.2	15.2±3.1	.01
Arachidonate (5μg/ml)	78.9±24.1	57±15.6	08

†Wilcoxon Signed Ranks Test

These results are consistent with decreased phospholipase and cyclo-oxygenase activity in RPCT of S rats. The underproduction of PGE_2 by RPCT cells from S rats may contribute to enhanced papillary collecting duct sodium reabsorption and may be an important factor in the differing natriuretic and antihypertensive capacities of R and S kidneys.

HIGH DIAGNOSTIC VALUE OF CAPTOPRIL CHALLENGE TEST (CCT) FOR UNILATERAL RENAL/RENOVASCULAR DISEASE IN HYPERTENSIVES. M.C. Ruddy, G.B. Bialy.* UMDNJ-Rutgers Med. Sch., New Brunswick, NJ

Forty-three hypertensive patients (pts), suspected of having unilateral renal/renovascular (RV) disease (severe, resistant or new-onset hypertension, or abdominal bruits), underwent radiologic investigation (angiography, renal DTPA scan, intravenous pyelography). All pts had a CCT, off drugs on an ad lib diet, as follows: plasma renin activity (PRA) was obtained immediately before (pre) and 90 minutes after (post) a single oral dose of 25-50 mg captopril. A positive CCT was defined as a fall in diastolic BP $\geq$ 10% from baseline and either a rise $\geq$ 6 ng/ml/hr in PRA (pre to post-captopril) or an absolute post-captopril PRA $\geq$ 20 ng/ml/hr.

Eighteen of the 43 patients (41.8%) had radiologic evidence of unilateral renal parenchymal (4 pts) or unilateral RV (14 pts) disease with 16/18 yielding positive CCT results (88.8% sensitivity). All pts with lateralizing renal vein renins had a positive CCT. Only 4 of the remaining 25 patients without unilateral lesions (including 1 of 6 with bilateral RV disease and 3 of 19 with normal angiograms) had positive CCTs (84% specificity). Out of the 43 study pts the CCT outcome correctly predicted the presence or absence of radiologically demonstrable unilateral disease in 37 pts (86% accuracy rate).

Our finding that the CCT was not a useful screening procedure for bilateral RV disease is consistent with the hypothetically lesser role of renin-angiotensin in this condition. However, in pts suspected of having a secondary form of hypertension, the CCT shows great promise as a screening test for unilateral disorders including renal parenchymal and and RV disease. The CCT may prove to be superior to any other currently available non-radiologic technique for detection of unilateral lesions.

SODIUM SENSITIVITY AND CALCIUM METABOLISM IN ESSENTIAL HYPERTENSION. Lawrence M. Resnick, John P. Nicholson, John H. Laragh. Cardiovascular Center, Cornell University Medical Center, NY, NY.

To investigate the clinical significance of the reciprocal blood pressure (BP) effects of oral calcium and 1,25 dihydroxyvitamin D (1,25D) (Resnick and Laragh, 1983), we measured BP, serum ionized calcium (Ca^{++}), and 1,25D in 10 essential hypertensive (EH) inpatients (IP) after 5 days of low (10 mEq/day) vs. high (200 mEq/day) sodium balance diets, as well as in 10 outpatients (OP) with EH after 3 weeks on both low (32±4 mEq/day $U_{Na}V$) and high (287±36 mEq/day $U_{Na}V$) sodium diets.

On high salt intake BP rose in OP (135±6/90±4 to 154±3/95±3 mmHg, p<0.01) accompanied by increases in 1,25D (54±6 to 76±10 pg/ml, p<0.005) while Ca^{++} fell (2.54±0.04 to 2.45±0.05 mEq/L, p<0.05). In IP, BP didn't change significantly (155±7/106±4 to 162±6/105±4 mmHg, p=NS), and neither did Ca^{++} (2.51±0.03 to 2.52±0.03 mEq/L, p=NS) or 1,25D (64±7 to 71±6 pg/ml, p=NS). However, for a given individual, both IP and OP demonstrated strong, positive relationships between the %ΔBP and %Δ1,25D (OP: r= 0.79, p<0.01; IP: r= 0.81, p<0.01). Additionally, the %ΔBP was also significantly and inversely linked to the ΔCa^{++} (OP: r=-0.87, p<0.01; IP: r=-0.81, p<0.01).

We conclude: 1) short or longer-term dietary salt loading in EH produces changes in BP among individual subjects in accordance with induced changes in both circulating levels of ionized Ca^{++} and 1,25D; 2) the greater the induced fall in serum Ca^{++} or the reciprocal rise in 1,25D, the greater the elevation in BP with salt loading. Altogether, these results suggest that alterations in calcium metabolism may mediate sodium sensitivity in EH.

EFFECTS OF CAPTOPRIL (CAP) AND NIFEDIPINE (NIF) ON EXCRETION OF A SALINE LOAD IN NORMOTENSIVES AND PATIENTS WITH ESSENTIAL HYPERTENSION. L.M. Ruilope,* J.L. Rodicio,* B. Miranda,* L.F. de Villa,* T.G. Hammond,* and J.C. Romero. 1° de Octubre, Madrid, Spain, and Mayo Medical School, Rochester, MN, U.S.A.

Patients with essential hypertension (HPT) excrete a saline load more rapidly than normotensives. Nifedipine increases sodium excretion acutely in normotensives (NOR). We tested the hypotheses that (i) captopril restores excretion of a saline load to normal in patients with HPT, and (ii) that nifedipine increases excretion of a saline load in NOR. Following basal measurements all patients had 2 liters of normal saline infused at 500 ml/hr and hourly urine collections. Sodium excretion in μeq/min is shown below (mean ± SE).

	Basal	2 hours	4 hours
NOR (n=8)	111±23	275±37'	263±51
NOR+CAP (n=8)	267±38	403±80	334±78
HPT (n=9)	108±33	373±53*	570±78*
HPT+CAP(n=9)	189±93	234±43'	341±56'
NOR (n=5)	177±71	263±40	220±54
NOR+NIF (n=5)	189± 6	463±76'	572±78'

*p<0.05 HPT compared to NOR by unpaired t-test
'p<0.05 pre compared to post CAP or NIF by paired t-test

PRA was suppressed with saline loading in all groups. Blood pressure fell with CAP in the HPT group (p<0.05) but remained greater than NOR (p< 0.01). We conclude that CAP restores excretion of a saline load to normal in HPT, and that NIF increases excretion of a saline load in NOR.

EVIDENCE AGAINST A PATHOGENETIC ROLE OF CA DEFICIENCY IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). D. Rydell*, U. Gafter*, I. Tropp*, M. Pesigan*, D. Zikos and K. Lau. Michael Reese Hosp. & Univ. of Chicago, IL.

Despite recent data suggesting an association between Ca deficiency and genetic hypertension, no causal relationship was established. Although massive oral Ca loading is associated with a reduced B.P., the effect is due to PO_4 deficiency (AJP April 1984). We tested the "Ca deficiency" hypothesis by evaluating other maneuvers that vary Ca balance: (1) Chronic hypercalcemia by IP $CaCl_2$ (Δ=2.0 to 3.5 mg/dl) failed to reduce B.P. as oral Ca loading. (2) Increased Ca balance (41 vs 31 mg/d) by high Mg diet (0.50 vs 0.15%) in 13 weeks old SHR also failed to reduce B.P. (180 vs 183 Torr). (3) Conversely, decreased Ca balance (16 vs 31 mg/d) by mild dietary Ca restriction (0.35% vs 0.87%) did not aggravate the increase in B.P. (Δ=13 vs 14 Torr) expected with growth. (4) To exclude the possibility of an offsetting effect by the increased PTH due to Ca restriction, parathyroidectomized SHR were randomized to a slightly high (1.35%) or low (0.35%) Ca diet. Despite large differences in Ca balance (79 vs 22 mg/d), B.P. was not altered for up to 5 weeks of treatment. (5) Ca retention was slightly increased, not decreased, in pre-hypertensive 3 weeks old SHR (data in mg/d, §, $p<0.05$ vs normotensive Wistar Kyoto (WKy) control).

	N	Oral	Fecal	Absorbed	Urine	Retained
WKy	9	75.5	35.3	40.2(53%)	3.2	37.0(49%)
SHR	10	73.9	29.8§	44.1(60%§)	1.7§	42.3(57%§)

Coupled with our data documenting Ca excess in adult SHR (AJP Nov 1984), these 5 lines of evidence offer no support for a causal role of Ca deficiency in the genetic hypertension of SHR.

DIET NA^+/CA^{2+} BP INTERACTION. J. Stanton,* C. Morris,*D. McCarron. Oregon Health Sciences University, Portland, Oregon.

A report from the Health and Nutrition Examination Survey I (HANES I) has associated both higher Ca^{2+} diets with lower BP across all subgroups of hypertensives compared to normotensives. We analyzed HANES I to assess whether a Na^+/Ca^{2+} interaction occur in humans.

HANES I measured BP and assessed nutrient intake in 20,749 persons. We identified 10,372 individuals with no history of hypertension or diet modification between ages 18 and 74. Cases were divided into quartiles of Na^+ intake within age, race and sex strata and grouped over low, mid-1, mid-2 and high quartiles of Na^+. By regression, Ca^{2+} intake by 25 mg intervals was inversely related to systolic BP (SBP), the proportion with SBP $\geq$160 mmHg, and with SBP in the upper 10th percentile. Similarly, the population was divided into Ca^{2+} quartiles, with analysis of SBP by 100 mg intervals of Na^+ intake.

Ca^{2+} is significantly linearly inversely related to SBP in each Na^+ quartile (low, r=0.42, p<.001; mid-1, r=0.36, p=.001; mid-2, r=0.17, p=.14; high, r=0.47, p<.001). However, the slopes and intercepts of each line were similar, and the lines coincident. The same relationships were found in the proportions $\geq$160 mmHg and in the 10th percentile. By Ca^{2+} quartiles, no significant relationship between Na^+ and SBP was observed in any quartile (low, r=0.06; mid-1, r=0.11; mid-2, r=0.07; high, r=0.13).

We conclude that dietary Ca^{2+} intake is inversely related to SBP uniformly across Na^+ intakes. Conversely, there is no significant relationship of Na^+ to BP across Ca^{2+} intakes.

NEPHROTIC PROTEINURIA IN ESSENTIAL HYPERTENSION. Kamaljit Sethi, Saiid M. Zonozi,* Louis H. Diamond, Robert Currerri and Sharda Sabnis and Tatiana T. Antonovych. Georgetown Nephrology Section, D.C. General Hospital, Georgetown Sch. of Med., and Nephropathology Section, Armed Forces Inst. of Pathology, Washington, D.C.

Essential hypertension (HBP) and benign nephrosclerosis are not associated with nephrotic range proteinuria (NP). The clinical characteristics of 16 patients presenting with essential hypertension and NP were studied and renal biopsy was performed. The diagnosis of benign nephrosclerosis was confirmed by the absence of glomerular pathology, and the presence of arteriolar hyaline sclerosis and tubular atrophy. NP was defined as 24 hr urine protein (UP) $\geq$ 3 G with normal GFR or normal GFR for age $\bar{X}$ UP. There were 13 males and 3 females, all black, between the ages of 25-68 yrs. The duration of HBP was 1-12 yrs in 14/16 and unknown in 2/16. Only 2/16 patients had serum creatinine (S_{creat}) < 2 mg%, while 14/16 had S_{creat} > 2 mg% or GFR < 50 ml/min. 4/16 had UP between 3-5 GM, and 12/16 > 10 GM, the highest being 80 Gm. 9/16 patients required treatment for ESRD within 1 yr of presentation. Three additional patients developed ESRD within 4 yrs. Of the remaining 4 patients, 3 had S_{creat} between 2.2-4.8 mg% at last follow-up at 3-5 yrs. There was no correlation between HBP control and ultimate outcome.

We conclude that NP 1) can occur in essential HBP & benign nephrosclerosis; 2) may be a bad prognostic factor in essential HBP; and 3) may not be related to HBP control.

EXAGGERATED GLOMERULAR CIRCULATORY RESPONSE TO VERAPAMIL BY ISOLATED SPONTANEOUSLY HYPERTENSIVE RAT KIDNEYS. T.H. Steele and L. Challoner-Hue*, University of Wisconsin, Madison, Wisconsin.

We investigated the possibility that abnormalities of cell calcium regulation may contribute to dysfunction of Kyoto spontaneously hypertensive rat (SHR) kidneys, compared to kidneys from Wistar-Kyoto controls (WKY). Kidneys were isolated and perfused at 120 and 160mmHg with a Krebs-Henseleit solution containing glucose, albumin, and amino acids. At 120mmHg, SHR glomerular filtration rate (GFR) was 0.24±.04 ml/min compared to WKY GFR of 0.70±.10 (P=.001). At 160mmHg, SHR GFR was 0.48±.05 ml/min compared to WKY GFR of 1.09±.05 (P<.001). At 120mmHg, addition of norepinephrine (NE) increased SHR and WKY renal vascular resistance (RVR) by 50%, and decreased SHR GFR by 27% and WKY GFR by 57%. At 160mmHg, NE elicited similar changes in SHR and WKY RVR and GFR. Addition of verapamil (VER), 5-10 µM, in the presence of NE returned RVR to 100-110% of control. Concomitantly, at 120mmHg, SHR GFR increased to 0.84±.23 ml/ min, a value 3.5 times control (P=.03). In contrast, WKY GFR in the presence of NE and VER was 0.97±.07 ml/min, unchanged from control (P=.07). At 160mmHg, NE and VER also failed to increase WKY GFR above control (P=.4), but increased SHR GFR by 52% over control (P=.03).

Isolated SHR kidneys exibited exaggerated GFR responses to VER but not to NE. Abnormal cell calcium regulation may underlie the markedly decreased GFR when SHR kidneys are perfused acutely at normotensive perfusion pressures.

K PREVENTS STROKE DEATH IN HYPERTENSIVE RATS WITHOUT LOWERING BP. L Tobian, J Lange‡ K Ulm‡ L Wold‡ J Iwai‡ University of Minnesota, Minneapolis, MN

24 SHRsp rats were fed normal chow containing 4% NaCl (0.75% K). 50 other SHRsp were fed this same diet with either KCl or K citrate added up to a 2.11% total K concentration. After 16 weeks, 20 out of 24 SHRsp with no added K had died, mainly from strokes(83% mortality, no uremic deaths). Only 1 of 50 rats on the high K diets had died(2% mortality), a 98% reduction in mortality($p<.00001$). BP averaged 233 vs 187 in these groups ($p<.00001$); thus K definitely lowered BP. However, the 11 SHRsp rats with the lowest BP on normal K & the 11 SHRsp rats with the highest BP on high K had the same average BP, 212mmHg. These 2 groups matched for BP had 64% mortality with normal K vs 9% mortality with high K, an 86% lower mortality ($p<.003$). Dahl S rats were fed similar diets with 8% NaCl. After 9 weeks, 18 out of 33 on normal K had died, mainly from strokes (55% mortality); while only 2 out of 45 rats on high K had died(4% mortality). Added K reduced mortality 93% in Dahl S rats($p<.00001$) & reduced average BP from 215 to 201($p<.005$). However, the 21 S rats with the lowest BP on normal K & the 39 S rats with the highest BP on high K had the same average BP, 205mmHg. In these 2 groups with matched BP, mortality was 38% on normal K vs 5% on high K, an 87% lower mortality($p<.001$). In both SHRsp & Dahl S rats, added K reduced BP. However, in matched BP groups among both SHRsp & Dahl S rats, added K strikingly reduced stroke mortality even when BP was comparable & not at all lower. After 28 weeks, 2 out of 24 SHRsp on K citrate were dead(8.3%) vs 8 out of 26 SHRsp on KCl(30.8%). Thus the death rate on K citrate at 28 weeks was 73% lower than on KCl ($p<.05$), even though BP's were comparable, 182 vs 191 (NS).

RENAL DENERVATION PREVENTS THE DEVELOPMENT OF ONE-KIDNEY LOW SODIUM HYPERTENSION. R.C. Vari*, R.H. Freeman, J.O. Davis, W.D. Sweet*. University of Missouri, Columbia, MO.

Contralateral renal denervation prevented the increase in systolic blood pressure (SBP) produced by uninephrectomy of sodium restricted rats. Comparison of SBP is shown over time for normal sodium sham denervated (NS-SHAM), low sodium sham denervated (LS-SHAM), and low sodium renal denervated (LS-DEN). * =$P<0.05$ from NS-SHAM + = $P<0.05$ between LS-DEN and LS-SHAM.

	Pre	Days after Nephrectomy			
Group	Neph	3	7	10	14
NS-SHAM (n=5)	109 ±4	126 ±2	127 ±2	130 ±2	132 ±3
LS-SHAM (n=9)	106 ±4	143*+ ±2	160*+ ±4	149*+ ±3	153*+ ±3
LS-DEN (n=15)	106 ±3	122 ±4	132 ±6	128 ±3	136 ±4

Renal denervation was confirmed by a 96% decrease in kidney norepinrephrine content. Fifteen days after nephrectomy plasma renin activity (PRA) was significantly elevated ($P<0.05$) in both LS groups compared to the control NS-SHAM group. However PRA in the LS-DEN group was decreased compared to the LS-SHAM group (9.5±1.0 vs. 14.3±1.5 ngAl/ml/hr respectively; $P<0.05$). No difference was observed in urinary Na^+, or fluid excretion between LS-SHAM and LS-DEN. These results suggest that intact renal nerves are required for the development of hypertension in this experimental model.

CHILDHOOD FAMILIAL PHEOCHROMOCYTOMA: CONFLICTING RESULTS OF DIAGNOSTIC IMAGING. Turner, M., Brennan, P., DeQuattro, V., Ansari, A., Lieberman, E., Childrens Hospital of Los Angeles, Depts. of Nephrology/Surgery, Dept. of Medicine/Nuclear Medicine, USC School of Medicine, Los Angeles, CA.

Childhood familial pheochromocytoma was investigated in 4 cases by abdominal CAT and 131 meta-iodobenzylguanidine (MIBG) scans and/or vena caval catecholamine (VCC) sampling. Results conflicted with surgical findings.

CASE	I	II	III	IV
Surgery	L.adrenal tumor; midline tumor	L.adrenal tumor	L.adrenal tumor	Tumor below L.renal artery
CAT	Positive; False -	Positive; False +	Positive	Positive
MIBG	Positive; False +	Positive; False +	Positive	Not Done
Vena Cava	Not Done	Positive; False +	Positive; False +	Positive

CAT scan identified all adrenal tumors but missed a midline tumor in Case 1. MIBG scan identified all adrenal tumors but suggested extra-adrenal tumors not confirmed at surgery in 2 of 3. VCC confirmed all left adrenal tumors but suggested additional tumors not verified at surgery in 2 of 3 patients. Two VCC samplings prior to our evaluation of Case IV suggested numerous tumors; repeat study did not. All cases are asymptomatic, have normal urinary catechols 3-36 mos after surgery. Due to suspicion of multiple tumors in familial pheochromocytoma, different diagnostic techniques were employed; false + results were more frequent with MIBG/VCC. More pediatric experience with MIBG/VCC in familial pheochromocytoma is needed.

PUTATIVE CENTRAL EFFECTS OF URAPIDYL AND PRAZOSIN IN RATS. R. Veelken, F. Luft, H. Becker, Th.Unger R.E. Lang, D. Ganten. Dept. Pharmacology, Univ. Heidelberg, FRG.

To test the hypothesis that urapidyl (URA), and prazosin (PRA), exert central effects, we gave these drugs, as well as clonidine (CLO), either iv or intracerebroventricularly (icv), to SHR in doses to lower blood pressure (BP) by 30 mmHg. BP, heart rate (HR), and splanchnic nerve activity (SNA), were measured directly in conscious, resting SHR. Peripheral alpha blockade of the drugs was tested with iv phenylephrine.

CLO, either iv or icv, failed to influence the effect of phenylephrine, while URA and PRA, either iv or icv, blocked the response. CLO iv and URA icv initially and transiently increased BP prior to decreasing BP. Data at stabilization (mean ± SD) are summarized below (n=6):

	dose(µg)	ΔBP(mmHg)	ΔHR(bpm)	ΔSNA(%)
CLO icv	5	-35± 5	-92±42	-51
CLO iv	5	-33± 7	-80±42	-45
PRA icv	2	-34±12	+47±26	+13
PRA iv	2	-28±17	+47±45	+29
URA icv	300	-38± 6	-76±39	+10
URA iv	300	-32± 5	+ 3± 6	+42

Only CLO decreased HR and SNA. URA and PRA increased SNA. Thus, the main action of both drugs is to block peripheral alpha-adrenoceptors. The depressant effect on HR by URA icv indicates a possible additional central action of URA. The dissociation of HR and SNA also demonstrates circumstances of selective efferent sympathetic inhibition.

EXAGGERATED DIURESIS AFTER WATER LOADING IN ESSENTIAL HYPERTENSION: ROLE OF VASOPRESSIN AND BLOOD PRESSURE. M.T. Velasquez, M.M. Skelton, *A.W. Cowley, Jr., Dept. Physiol. & Med., Med. Coll. Wis., Milwaukee, Wis. (INTRO: Richard J. Roman)

The present studies were designed to determine the response to water loading (20ml/kg;30-40 mins.) in normal (N;aged 35±3;N=10) and subjects with essential hypertension (H;aged 40±4;N=13). All subjects were placed on a fixed diet (100 mEq Na and 75 mEq K/day) for 3 days before the test. The changes from control of mean arterial pressure (MAP), urine excretion (V), plasma vasopressin (PAVP), and plasma osmolality (POsm) at 20,60,100 and 120 mins following water load are given below.

		CON	20"	60"	100"	120"
MAP(mmHg)	N	90±2	+6*	+3	+1	+2
	H	113±2	+11*	+10*	+6	+5
V(ml/min)	N	.6±.1	+11*	+8*	+10*	+9*
	H	1±.2	+27*	+10*	+7	+7
PAVP(pg/ml)	N	4.0±.7	-1	-1.5	-1.7	-
	H	2.4±.4	-1.2	-1.2	-.4	-
POsm(mOsm/kg)	N	281±1	-6*	-6*	-3	-
	H	280±1	-5*	-5*	-4	-

Plasma renin activity and heart rate remained unchanged in both N and H groups with water loading. The slopes of the regression relating PAVP and POsm did not differ between N and H (r=.50). We conclude 1) that the AVP osmotic sensitivity is preserved in hypertensive subjects; 2) that hypertensive subjects excrete the water load more rapidly than normal subjects; 3) that the exaggerated diuresis is not a result of greater suppression of AVP in hypertensive subjects; 4) that the increased arterial pressure in hypertensive subjects appears to account for the exaggerated diuresis observed.

DISASSOCIATION BETWEEN THE HYPOTENSIVE EFFECT OF THIAZIDES AND IONIZABLE PLASMA CALCIUM (ICa++). A. Von Riotte*, W.C. Yang*, S. Fontana*, T. Grosskopf* and D.C. Batlle, Univ of IL, Chicago.

Recent work suggests that ICa++ is low in some patients with essential hypertension (HTN) and that dietary Ca supplementation lowers blood pressure. It has also been reported that patients with low renin HTN may have a lower ICa++ than those with normal or high renin HTN. Thiazide diuretics decrease Ca excretion and thereby could increase ICa++. We reasoned that an increase in ICa++ could contribute to the hypotensive effect of these agents, and that the attendant rise in plasma renin activity could be correlated with the rise in ICa++. To test this hypothesis, we sequentially measured ICa++, Mg, and P after 1, 2, 4, and 8 weeks of thiazide treatment (50 mg daily) in 30 patients with essential HTN not receiving any other medications. Thiazides resulted in a marked fall in blood pressure (>10 mmHg) which was already apparent one week after its administration and was sustained thereafter. No significant change in ICa++, Mg, or P, however, was found at this time or throughout the 8 weeks of observation. Moreover, we found a lack of correlation between the change in blood pressure and the change in ICa++ (r = 0.07). This lack of correlation between ionizable Ca and the hypotensive effect of thiazides was observed despite the fact that thiazides resulted in a four-fold increase in plasma renin activity (from 1.0 to 4.0 mg/ml/ hr). Hence, our data do not support a role for ICa++ in the hypotensive effect of thiazides. Moreover, a marked fall in blood pressure without a change in ICa++ occurs despite the fact that plasma renin activity increases markedly during thiazide treatment.

SYNTHETIC ATRIAL NATRIURETIC FACTOR (ANF) REDUCES BLOOD PRESSURE AND ALDOSTERONE IN RENIN-DEPENDENT RENOVASCULAR HYPERTENSION.
Massimo Volpe,* Geoffrey Odell,*Hollis D.Kleinert, Maria J. Camargo, John A. Lewicki,* John H. Laragh, Thomas Maack, E. Darracott Vaughan, Jr. and Steven A. Atlas*. Cornell University Medical College, New York, NY.

We have shown that ANF antagonizes angiotensin II (AII)-induced contraction of the aorta and renal vasculature and AII-stimulated aldosterone production by isolated adrenal cells. To examine the significance of these effects in vivo, synthetic ANF ("auriculin A") was administered i.v. (2 µg/kg bolus followed by 0.3 µg/kg/min constant infusion) to instrumented, conscious, unrestrained 2-kidney 1-clip (2K1C) and 1-kidney 1-clip (1K1C) rats on normal sodium intake and their sham-operated controls. Measurements (mean±SE) of mean blood pressure (BP,mmHg) plasma renin activity (PRA,ng/ml/hr), and plasma aldosterone (PA, ng%) were made before and after 60 min infusion. In saralasin-responsive 2K1C rats (n=9), ANF reduced BP by 16±2% (from 190 ±12 to 158±11, p<0.001) and reduced PA by 49±13% (from 198±65 to 137±66, p<0.05),while PRA increased (64±17 to 88±21,p<0.05). In contrast, only minimal changes in BP occurred in saralasin non-responsive 2K1C (148±13 to 144±10, n=4), 1K1C (201±10 to 195± 11, n=5),and sham 2K (121±4 to 115±4,n=7) or 1K (115±3 to 112±2,n=7) rats. Control PRA and PA were not elevated in these groups and did not change significantly during ANF infusion. These data demonstrate that ANF has potent anti-hypertensive and aldosterone-suppressing effects in vivo, and they suggest that sensitivity to the vascular and adrenal effects of ANF may be enhanced when the activity of the renin-angiotensin system is increased.

BLOOD PRESSURE EFFECTS OF LONG-TERM VS SHORT-TERM INCREASED DIETARY CA^{++} and NA^{+} INTAKE IN THE SHR.
Lucinda Wegener,*Robert Shneidman,*David McCarron, Oregon Health Sciences University, Portland, OR.

Previously, moderate chronic increases in dietary Ca^{++} and Na^{+} have been shown to retard the development of hypertension in the SHR when initiated at an early age (6 weeks). Increased dietary Ca^{++} alone has been found to lower BP in the SHR when given for as short an interval as 2 weeks. We sought to determine the relative effects on blood pressure of diets with either increased or decreased Na^{+} and Ca^{++} contents introduced at different ages and continued over different durations.

24 SHRs and 24 WKYs were randomized to 3 diets at 10 wks (control: 0.45% Na^{+}/1% Ca^{++} by weight; 1% Na^{+}/2% Ca^{++}; and 0.25% Na^{+}/0.1% Ca^{++}). An additional 24 SHRs and 24 WKYs were randomized to the same diets at 18 wks. Serum and urines were collected at 2-4 wk intervals. Intra-arterial BP (MAP) was measured in all animals at 22 wks.

After 12 weeks on the diets, MAP was significantly lower in SHRs receiving the ↑ Na^{+}/↑ Ca^{++} diet [MAP mmHg ± SEM=158±3.4] versus the ↓ Na^{+}/↓ Ca^{++} diet [190±4.9] and control [183±2.6], (p<.0001). WKYs' MAP was unchanged. SHRs and WKYs receiving the diets for 4 weeks showed no significant changes in MAP. The BP response of the SHR was independent of growth and changes in serum and urine parameters.

We conclude that moderate increases in dietary Na^{+} and Ca^{++} content lowers BP in the SHR. This effect, though, requires introduction of the diet at a relatively early age, and/or continuation for a period longer than 4 weeks.

CAPTOPRIL TESTING TO SEPARATE PATIENTS WITH ESSENTIAL (EHBP) FROM UNILATERAL RENOVASCULAR (URVHBP) HYPERTENSION. James O. Wells and M. A. Boles*. Emory Univ. School of Med., Department of Medicine, Division of Nephrology, Atlanta, Ga.

We sought to determine if blood pressure response following a single dose of captopril would distinguish between patients with URVHBP and EHBP. There were 15 patients in each group. All were off medications for two or more days prior to testing. BP was measured in the sitting position in the same arm by the same observer every 20 min. for two hours following a 25 mg. oral dose of captopril. Patients did not eat within one hour of testing. All patients had diastolic BP of 90 or greater, a serum creatinine of < 2.0 mg/dl, and subsequently were studied with renal vein renin (RVR) determinations after furosemide, renal arteriograms and routine other tests.

URVHBP patients had unilateral stenosis on arteriogram, RVR ratios of $\geq$ 1.7 lateralizing to the side of the stenosis, and underwent either angioplasty, nephrectomy or bypass. All were followed at least 6 months and were classed as cured (4), improved (10), or failed (1), by Cooperative Study criteria. EHBP patients had normal arteriograms and RVR ratios of $\leq$ 1.2.

Observed parameters used for analysis included stimulated IVC renin (IVC) and percent change in mean (MAP) and diastolic (DBP) blood pressure.

Significant differences for the two groups were found in IVC renin ($p < .005$) and MAP ($p < .025$), but not in DBP ($p > .05$). By using discriminant analysis, patients could be correctly classified with the following accuracy: DBP = 53%, MAP = 70%, IVC renin = 83%, and DBP + MAP + IVC = 85%.

CALCIUM-INDUCED VASCULAR RESPONSES MAY BE DUE IN PART TO ALTERED MAGNESIUM. E. T. Zawada, Jr., J. A. Ter Wee,* D. E. McClung,* University of South Dakota & R. C. Johnson VAMC, Sioux Falls, SD.

These studies were undertaken to determine the role of magnesium in the systemic and renal hemodynamic consequences of hypercalcemia produced by dietary supplementation with calcium and vitamin D. Eight mongrel dogs were given two weeks of daily dietary supplements of 100 mg/kg calcium gluconate and 10,000 units/kg vitamin D. Eight control dogs were on a normal diet. Observations were made in the conscious state of cardiac output (CO), mean arterial blood pressure (MAP), total peripheral resistance (TPR), glomerular filtration rate (GFR), renal blood flow (RBF), ionized serum calcium (iCa), and serum magnesium (SMg). Systemic hemodynamics were measured by thermodilution and intraarterial pressure recording. Renal hemodynamics were measured by standard clearance techniques. iCa was measured with an ion-specific electrode. SMg was measured by the ACA method. iCa increased from $1.2 \pm .04$ to $1.6 \pm .05$ mMol/L, $p < .001$. There were no significant differences in CO, MAP, or TPR. TPR was higher in hypercalcemic dogs: 2849 ± 234 vs. 2572 ± 264 dyne-sec/cm^5. GFR was reduced from 73 ± 4 to 40 ± 6 cc/min, $p < .001$, and RBF was lower at 398 ± 8 to 230 ± 31 cc/min, $p < .001$, in hypercalcemic dogs. SMg was lower at $1 \pm .04$ vs. $2 \pm .04$ mg/dl, $p < .001$, in hypercalcemics due to enhanced magnesium excretion. We conclude that increased vascular resistance associated with hypercalcemia may be due in part to altered magnesium balance.

PREDICTIVE VALUE OF STANDARD AND VAUGHAN-LARAGH RENAL VEIN RENIN RATIOS IN UNILATERAL RENAL ARTERY STENOSIS. Daniel J. Wilson, M.D., Richard Benya, Vincient D'Souza, M.D. and George Plonk, M.D.. Departments of Medicine, Radiology and Surgery, Wake Forest Medical Center, Winston-Salem, NC.

We measured renal vein renins (RVR) in 36 patients with unilateral renal artery stenosis and suspected renovascular hypertension, in an effort to compare the predicitve value of standard RVR with Vaughan-Laragh RVR ratios. Unstimulated RVR were obtained 12-24 hours following the withdrawal of all anti-hypertensive medications, with the patient supine, and prior to arteriography. Renal arteriograms showed that 24 patients had atherosclerotic lesions and 12 patients had fibromuscular dysplasia. Nineteen patients underwent balloon angioplasty, while 17 patients had surgery. At 6-12 month follow-up (mean 10.1) 12 had cure of hypertension, 19 had improvement of hypertension, and 5 patients were treatment failures. Standard RVR ratios, comparing the affected to the non-affected kidney, and Vaughan-Laragh RVR ratios were calculated. Nine patients with standard RVR ratios < 1.5:1, and 14 patients with Vaughan-Laragh RVR ratios < 0.48 had cure or improvement of hypertension. Standard RVR ratios had a sensitivity of 70%, a specificity of 20%, and a positive predictive value of 85%, while Vaughan-Laragh RVR had a sensitivity of 50%, a specificity of 25%, and a positive predictive value of 82%. Vaughan-Laragh RVR ratios offer no advantage over standard RVR ratios in predicting the response to angioplasty or surgery for renovascular hypertension.

ABNORMAL RENAL CATECHOL PRODUCTION IN LONG-STANDING RENAL TRANSPLANT RECIPIENTS (RTR). M. Ziegler*, Lee Woodson*, R. Steiner*. University of California at San Diego Medical Center, San Diego, California. Intr. by D. Fanestil.

In 17 RTR 32.5±8.3 months post transplant (mean±SE) and 10 controls (C) measurements (pg/ml) were made of plasma (P) and urine (U) epinephrine (E), norepinephrine (NE) and dopamine (DA). C and RTR did not differ in P creatinine, (CR), PE, PNE or PDA. Fractional excretion (FE) of E and NE was lower in RTR, indicating reduced renal contribution to UE and UNE in RTR. C and RTR did not differ in FEDA or UDA/Cr (230±40 vs 370±70 mcg/gm, $p=.31$). In RTR there was no relation between time post transplant and FEE, FENE, or FEDA. As FENE was not different from FECR, a renal contribution to UNE in RTR was not shown. The high FEE and FEDA in RTR suggests intrarenal production of these catechols, but the lower UE and UNE are consistent with abnormal, reduced sympathetic reinnervation, not related to time post transplant. As suggested by animal studies, excretion of UDA in RTR may not depend on the presence of intrarenal sympathetic neurons. The respective reductions in UE and UNE in RTR indicate that at least half of the UE and UNE in C originated from renal sympathetic nerves.

	PE	PNE	UE/Cr	UNE/Cr	FEE	FENE
C	88±11 pg/ml	516±91 pg/ml	32±3 mcg/gm	126±13 mcg/gm	4.2±.5	3.4±.8
RTR	79±9	578±74	17±5	62±13	2.7±.7	1.5±.4
P	NS	NS	.001	.001	.021	.01

PDA-C 283±106 pg/ml FEDA-C 530±493
PDA-RTR 365±58 (N.S.) FEDA-RTR 67±37(N.S.)

IMMUNOLOGY/PATHOLOGY

MECHANISMS OF PROGRESSIVE GLOMERULAR SCLEROSIS IN THE RAT. Stephen Adler, Liliane Striker, Gary Striker, Diana Perkinson*, James Hibbert* and William Couser. Univ. of Wash., Seattle, WA.

To obtain a better understanding of the sequential development of sclerosis in immune glomerular disease we induced experimental membranous nephropathy in unilaterally nephrectomized rats by injection of sheep anti-rat FxlA(tubular brush border antigen) and evaluated the kidneys over a 9mo period. Serial renal biopsies were examined by light microscopy and IF for IgG, C3, membrane attack complex neoantigens(MAC), interstitial(type III) and basement membrane(type IV) collagen.

Urinary protein excretion increased from 208±19 mg/d(mean±SEM;n=13) at 2wks to 308±36mg/d(n=9) at 29wks. Mesangial hypertrophy, segmental sclerosis and thickening of Bowman's capsule were first seen at 8wks and progressed to diffuse involvement of glomeruli at 35wks without thickening of the capillary wall. Subepithelial deposits of sheep and rat IgG, C3 and MAC were initally present but gradually decreased with time. Beginning at 8wks coarse granular deposits of C3 and MAC were detected in thickened mesangial areas and sclerotic areas. Type IV collagen was observed in the widened mesangium, sclerotic areas and Bowman's capsule. Type III collagen accumulated in the interstitium around sclerotic glomeruli.

Progressive mesangial sclerosis developed following an initial immunologic injury to the capillary wall which did not appear to progress. The appearance of rat MAC in sclerotic areas raises the possibility that they are of pathogenetic importance. The absence of interstsitial collagen in sclerotic glomeruli suggests that the components of the lesion are produced solely by glomerular cells.

GRANULOCYTOPENIA OF HEMODIALYSIS IS ASSOCIATED WITH INCREASED SURFACE EXPRESSION OF A GRANULOCYTE ADHESION PROMOTING GLYCOPROTEIN (Mol) M. Amin Arnaout, Raymond M. Hakim, Nava Dana*,& Robert P. Todd,*III. Harvard Med. Sch., Boston, MA, Univ. Mich., Ann Arbor, MI.

Hemodialysis is often associated with a transient granulocytopenia presumably due to complement mediated leukoaggregation and microembolization. Quantitative kinetic studies of a granulocyte adhesion promoting surface glycoprotein (Mol) were undertaken to elucidate the mechanisms accounting for hemodialysis-induced granulocytopenia. In eight patients on maintenance-hemodialysis, a five fold increase in the mean cell surface expression of Mol (measured by quantitative immunofluorescense analysis) occurred within 15 minutes and persisted for 90 minutes after starting dialysis on a new cuprophane membrane. The peak increase in surface Mol coincided with the maximal drop in neutrophil count and with the peak rise in the plasma levels of complement C5a desArg and C3a desArg. During dialysis on a fifth reuse membrane, no complement activation, increase in Mol expression or a change in neutrophil count were seen. C5a desArg induced a comparable increase in Mol expression on normal neutrophils in vitro at concentrations similar to those detected in vivo. C5a-induced granulocyte aggregation (an index of increased cell adhesiveness) was specifically blocked by a mouse monoclonal antibody to Mol. These data suggest that the quantitative increase in expression of Mol on granulocytes in vivo is in part mediated by C5a and that it may provide a mechanism initiating leukoaggregation, sequestration of granulocytes and neutropenia during hemodialysis.

LONGITUDINAL STUDIES ON STREPTOCOCCUS MUTANS-INDUCED NEPHRITIS OF RABBITS. Albini, B., Glurich, I., Barua, M.W., Nisengard, R., Neiders, M., and Stinson, M.W. (introduced by G.A. Andres). Depts. of Microbiology, Periodontology, and Oral Pathology, SUNYAB, Buffalo, N.Y.

Repeated administration of disrupted Streptococcus mutans (MT 703; SM) i.v. into rabbits results in a severe glomerulonephritis characterized by IgG, C3, and SM antigen deposits, endothelial and epithelial cell proliferation, subendothelial and subepithelial electron dense deposits, and in over 60% rabbits, humps. Eluates from diseased kidneys show antibodies to four components of the microbe (65K, 50K, 35K, 24K) These components bind to normal kidney tissue and to kidney extracts in vitro. Six rabbits were studied chronologically for up to 38 weeks. Blood and urine were collected weekly, and open kidney biopsies were performed several times on the same animal. The rabbits showed two peaks of proteinuria (at wk 11.6 ± 3.7 and 34.0 ± 1.7). Hematuria was frequently observed during the observation period. High titers of antibodies to SM were detected by ELISA showing characteristically two drops (at wk 8.0± 2.6 and 28.5± 2.1). Circulating immune complexes (CIC) (Raji cell assay and PEG-precipitation) were present at various times in the circulation. Peaks of CIC often followed the drop of antibodies and coincided with periods of proteinuria. Analysis of biopsy material revealed capillary granulocytosis (>24 hr after first injection), endothelial proliferation (>4 wk), degenerative changes (>26 wk) and cresent formation (>30wk) Antibodies cross-reacting with kidney tissue showed low but steadily increasing titers in sera tested by ELISA. This animal model may be useful in understanding the pathogenesis of streptococcus-associated nephritis in man.

IMMUNOSUPPRESSIVE THERAPY OF LUPUS NEPHRITIS. H. Austin, J. Klippel*, N. leRiche*, J.L. Decker*, and J.E. Balow. National Institutes of Health, Bethesda, MD.

Treatment strategies for lupus nephritis have been compared in 107 patients entered into prospective trials from 1970 to 1981. Patients were randomized to oral prednisone (P) alone or P combined with oral azathioprine (A), oral cyclophosphamide (C), oral cyclophosphamide plus azathioprine (CA), or trimonthly intravenous cyclophosphamide (IVC). After a median followup of 75 months, patients treated with cyclophosphamide regimens tended to be at less risk of renal failure(ESRD) than those treated with P or A.

	P	A	C	CA	IVC
No. Pts.	28	19	18	22	20
Risk @ 75 mos.	.27	.22	.12	.13	.07

In a high risk subgroup identified by active glomerulonephritis and moderate to severe chronic histologic change, IVC afforded a significant advantage over P. ESRD developed in 0/15 IVC compared to 7/18 P ($p < 0.05$, Gehan). Tabulation of toxicity revealed that hemorrhagic cystitis occured only in patients treated with oral cyclophosphamide (6/40 C,CA vs. 0/67 IVC,P,A; $p = 0.0002$). Malignancies (bladder, thyroid and cervical carcinoma and polycythemia rubra vera) were noted only in patients treated with oral immunosuppressives. Herpes zoster infections were significantly increased in patients treated with cyclophosphamide (18/60 C, CA, IVC vs. 4/47, P,A; $p < 0.01$) as was premature ovarian failure (19/32 C, CA, IVC vs. 4/25 P,A; $p < 0.01$). Thus, treatment of lupus nephritis with IVC appears to reduce the the risk of ESRD with few serious complications.

INCREASED TRANSTUBULAR TRANSPORT MEDIATES PROXIMAL TUBULAR CYST FORMATION (PTCF) IN SERUM-FREE METANEPHRIC ORGAN CULTURE (SFMOC). E.D. Avner, W.E. Sweeney*, N.P. Piesco*, D. Ellis. Children's Hosp. of Pittsburgh, Pittsburgh, PA.

Recent data suggest that glucocorticoids induce Na-K ATPase activity and organic anion transport in developing proximal tubules. We have therefore studied Na-K ATPase activity, and the effects of ouabain and PAH in our SFMOC model of glucocorticoid-induced PTCF (Lab Invest 50:208,1984).

Renal Na-K ATPase activity was determined during 5 days of control (CON) and cystic (CY) nephrogenesis utilizing a kinetic enzymatic microassay. Further, CON & CY explants were studied 5 days following daily incubations of ouabain (0.2 mM x 120 min) or continuous treatment with PAH (1×10^{-5}M).

	Na-K ATPase (nmol/min/mg protein)				
Incubation Day	1	2	3	4	5
Control	2.3±.7	4.7±.2	5.3±.4	5.5±.2	6.2±.5
Cystic	3.3±.5	5.3±.3	6.1±.4	12.2±.6	9.8±.4
	NS	NS	NS	p<.001	p<.001

Changes in enzyme activity directly paralleled PTCF. Ouabain significantly decreased PTCF in glucocorticoid treated explants (cyst index: 2.8 ± .6 vs. 0.5 ± .4, p<.001). PAH treatment caused marked PTCF in CON explants (cyst index: 2.9 ± .8 vs. 0, p<.001) & augmented PTCF in CY explants (cyst index: 3.8 ± .3 vs. 2.6 ± .4, p<.01). The ultrastructural features of both glucocorticoid & PAH induced PTCF suggested ↑ paracellular solute transport.

We conclude that: 1) ↑ transtubular solute transport driven by ↑ ATPase activity mediates PTCF in SFMOC, 2) ↑ organic anion transport can induce PTCF in SFMOC independent of ATPase mediated transport processes.

CELL-MEDIATED AUTOIMMUNE TUBULO-INTERSTITIAL NEPHRITIS (TIN) IN THE LEWIS (LEW) RAT. Kym M. Bannister,* Thomas R. Ulich,* and Curtis B. Wilson. Scripps Clinic and Research Foundation, Dept. of Immunology, La Jolla, CA.

This study examined the pathogenic importance of both humoral [anti-tubular basement membrane (TBM) antibody] and cell mediated immunity in TIN in rats using an approach which isolates each component. LEW rats, which lack the immunofluorescently (IF) reactive TBM antigen (TBM Ag$^-$), were immunized with Brown Norway (BN) rat (TBM Ag$^+$) renal basement membrane (RBM) and adjuvants. A severe nodular TIN developed by day 10-14 characterized by granuloma-like mononuclear cell infiltrates comprised of cells staining with monoclonals OX-6 (anti-Ia), 90-100%, and OX-19 (anti-pan T cell), 60%, surrounding tubules. Day 14 direct IF and elution studies did not demonstrate TBM bound IgG despite high titers of circulating antibody reactive with TBM Ag$^+$ TBM (radioimmunoassay, indirect IF). In cell proliferation studies, stimulation with TBM Ag$^+$ but not TBM Ag$^-$ RBM demonstrated sensitized lymphocytes from draining nodes of immunized LEW rats. Adoptive transfer of lymphoid cells ($4\text{-}6 \times 10^8$) IV to naive LEW rats resulted in histologically identical TIN in 7 days. IV transfer of immune serum (absorbed with packed BN blood cells) to normal BN rats (TBM Ag$^+$) resulted in a non-nodular TIN within 3 days, associated with a time-dependent binding of IgG to cortical TBM (direct IF, elution studies and paired-label binding). This new LEW model of TIN appears to be cell mediated despite the presence of circulating anti-TBM alloantibody and has demonstrated pathogenic roles for both antibody and immune cells in TIN in the rat.

BINDING OF HUMAN FIBRONECTIN TO IMMUNE COMPLEXES. AP Bakaletz* and FG Cosio, Department of Medicine, Ohio State University, Columbus, Ohio.

Fibronectin (FN) is a large glycoprotein present in normal plasma. We have previously demonstrated that FN enhances the binding and phagocytosis of IgG coated erythrocytes by human monocytes and suggested that FN binds to immune complexes (IC) (J Lab Clin Med 103:613, 1984). In the present study we have examined the binding of FN to HSA(Ag)-rabbit IgG anti HSA(Ab) IC. ^{125}I FN was incubated with preformed precipitable IC under different conditions and the percent binding was calculated after separation of the precipitate by centrifugation. Binding occurred at both 4°C and 37°C and reached equilibrium after 60 minutes. Similar to the binding of FN to C1q, binding of FN to IC was enhanced by low ionic strength solutions. In addition, binding was inhibited by unlabelled FN and soluble IC formed in Ag excess but not by equivalent concentrations of Ag or Ab alone. Precipitable IC formed at different ratios of Ab to Ag bound FN and the binding increased with increasing Ab/Ag ratio. Thus IC containing 3ug of Ag and 20ug of Ab bound 25ng of FN while IC containing 3ug of Ag and 61ug of Ab bound 45ng of FN.

Conclusion: We have demonstrated for the first time that human FN binds to IC in the absence of C1q. Given the opsonic properties of FN and its capacity to bind C1q, binding of FN to IC could affect their removal from the circulation and their capacity to activate complement. Because FN is present in the subendothelial space of the glomerular capillaries it could bind IC formed or deposited in that space.

GLOMERULAR NEURAMINIDASE(Nase) ACTIVITY IN PUROMYCIN AMINONUCLEOSIDE(PAN) NEPHROTIC SYNDROME(NS) by William H. Baricos, Shirley Cortez*, Susana Dipp*, and Sudhir V. Shah, Depts. of Biochem. and Medicine, Tulane Medical School, New Orleans, LA.

The loss of the sialic acid-containing anionic coat of the the epithelial cell podocytes is well documented in both human and experimental NS. The mechanisms responsible for this loss are unknown. Release of glomerular Nase, an enzyme which cleaves sialic acid from sialoglycoproteins, may account for this loss of podocyte anionic charge. Utilizing a fluorometric assay we have measured Nase activity(nmoles/mg prot/hr) in normal rat glomeruli(5.6±0.4,n=18) and for comparison cortex(13.3±0.9,n=18) and liver(2.5±0.1,n=18). Measurement of rat WBC Nase(0.19+.02 nmoles /hr/ 10^6 cells) and studies utilizing an ^{125}I- blood marker demonstrated that less than 1.0% of glomerular Nase activity was due to residual WBC. Nase activity was measured in glomeruli and cortex isolated from PAN-treated (15mg/100 gm BWt,IP) and control rats sacrificed at the onset(day 4: 172±61 mg/24 hr) and peak(day 9: 901±63 mg/24 hr) of proteinuria. Loss of colloidal iron staining was observed in the PAN-treated group. Activity was:

	Day	Control	n	PAN
Glom	4	5.5±0.4	(10)	4.5±0.4
	9	5.1±0.4	(10)	4.7±0.4
Cortex	4	13.6±2.1	(10)	11.9±1.2
	9	11.1±0.6	(10)	11.7±1.4

In contrast to Nase we observed significant($p<.01$) decrease in the activity of glomerular lysosomal α-fucosidase.These results indicate that loss of podocyte anionic charge in PAN-induced NS is not associated with changes in the specific activity of glomerular Nase.

GLOMERULAR LOCALIZATION OF PLATELET CATIONIC PROTEINS FOLLOWING IMMUNE COMPLEX INDUCED PLATELET ACTIVATION. J.L. Barnes, G. Camussi,* C. Tetta,* and M. A. Venkatachalam. Univ. Tx. HSC, San Antonio, TX and Univ. Turin, Turin, Italy.

Synthetic polycations bind to glomerular polyanions(GPA) and increase permeability to macromolecules and immune complexes(IC) (J Exp Med 160: 286, 1984). Platelet factor 4(PF4) and other platelet cationic proteins also bind GPA and may play a role in IC deposition. Here we examined the potential of locally released cationic proteins to bind to GPA following IC induced platelet activation within the renal microvasculature. Rabbits immunized against bovine serum albumin(BSA) with > 1.8 mg of anti-BSA antibody/ml of serum were used. BSA (2 or 4 mg/ml in buffered saline) was infused into the left renal artery to deliver 8 or 16 mg of BSA over 20 mins. Thirty mins. later kidneys were removed and tissue processed for light and electron microscopy and immunofluorescence localization of PF4, platelet cationic proteins, IgG, BSA, and complement(C3). Glomerular capillaries contained large IC and platelet aggregates. Also, numerous deposits were observed within subepithelial and subendothelial aspects of the glomerular basement membrane(GBM). Immunofluorescence revealed prominent granular-linear localization of PF4, platelet cationic proteins, IgG, BSA and C3 within peripheral capillary walls of glomeruli. Glomeruli of contralateral kidneys did not show GBM localization of IC or platelet proteins. Thus, nascent formation of IC in capillaries was associated with platelet activation and deposition of cationic proteins in the GBM. This mechanism may mediate the increased permeability which leads to GBM deposition of IC.

A RAT MODEL OF OXALOSIS

M.J. Blumenfrucht*, C. Cheeks*, R.P. Wedeen, Veterans Administration Medical Center, East Orange, NJ and UMDNJ, New Jersey Medical School, Newark, NJ.

We have used a single infusion of sodium oxalate (Ox) to induce oxalosis in rat in order to examine the mechanism of CaOx crystal deposition. Section freeze-dry autoradiography was used to trace the tissue distribution of ^{14}C-Ox. Autoradiographs were prepared from tissues snap frozen 8 sec to 2 wks after injection of ^{14}C-Ox, 33 umol/100 gm. IV injection resulted in renal retention of up to 21% of the dose per gm kidney by 24 hours which fell to 0.5% after 1 wk. CaOx crystals could be seen within the lumens of proximal tubules within 1 min. The autoradiograph pattern from ^{14}C-Ox associated with tubular cells was indistinquishable from that obtained with ^{14}C-inulin. Microcrystals were found occasionally in glomeruli and heart muscle indicating the presence of diffuse oxalosis. Too small to be found in the light microscope, the microcrystals appear as point sources in the autoradiograph. Crystals reached a maximum length of 33 u after 24 hrs within tubular lumens but were not detected within cells during the first week. One week after IV injection crystals remained in tubular lumens but no inflammatory response was evident. Two weeks after injection, kidneys showed focal interstitial nephritis in association with CaOx crystals. Within regions of interstitial nephritis, crystals were present in proximal tubule and inflammatory cells. Although large crystals form within the tubular lumens, microcrystals seed glomerular and cardiac capillaries.

PHENOTYPIC IDENTIFICATION OF INTRAEPITHELIAL LYMPHOCYTES (IEL) IN ACUTE RENAL ALLOGRAFT REJECTION. W.E. Beschorner*, J.F. Burdick*, G.M. Williams*, and K. Solez. The Johns Hopkins Univ. School of Medicine, Dept. of Pathology & Surgery, Baltimore, Maryland.

In an earlier monoclonal antibody study we demonstrated that Leu 7+ IEL between tubular epithelial cells were common in transplant kidney specimens with early acute rejection (9 of 13), but not in transplants without rejection (0 of 24). Although having apparent diagnostic value, Leu 7 staining did not identify the effector cells since Leu 7 stains both some natural killer (NK) cells and a fraction of the cytotoxic/suppressor cells (OKT8+, Leu 2a+). We have stained and evaluated 5 transplant biopsies from patients with early acute rejection and Leu 7+ IEL using the avidin-biotin immunoperoxidase procedure on frozen material. Most of the IEL in each of the specimens were Leu 7+, OKT11+, OKT8+, Leu 2b+, B1-, Leu 3a-, and OKT4-. Leu 11b, which stains most NK cells, stained only rare IEL in two specimens, no IEL in one specimen, and was uninterpretable in two specimens due to epithelial staining. OKM1+ IEL were occasionally evident in 3 of 5 specimens and not evident in two specimens. This suggests that in these cases of acute rejection, most of the IEL are cytotoxic/suppressor T cells rather than NK cells or monocytes. Panning studies of peripheral blood lymphocytes demonstrate that OKT8+, Leu 7+ lymphocytes do not stain with Leu 15 (suppressor T lymphocytes, monocytes) providing evidence that the IEL are cytotoxic T lymphocytes rather than suppressor cells. These cytotoxic T cells may be important in producing tubular damage in acute rejection.

EFFECT OF PROTAMINE SULFATE (PS) ON ESTABLISHED GLOMERULAR IMMUNE DEPOSITS IN THE RABBIT. Wayne A. Border, Univ. of Utah Medical Center, Salt Lake City, Utah.

Injection of PS has been shown to prevent and possibly dissolve subepitheial deposits (SD) in experimental membranous nephropathy (MN) produced by cationic BSA administration (JCI 71:487, 1983). The present study was undertaken to better define the effect of PS on established deposits after termination of BSA injections. Subepithelial deposits (SD) were produced by injection of cationic BSA and mesangial deposits (MD) by anionic (native) BSA for 3 weeks; animals were divided into groups receiving: 50 mg IV PS or IV saline twice daily for 3 wks or direct renal artery perfusion of PS or saline for 120 min. Renal tissue was obtained at weekly or hourly intervals for immunofluorescence (IF) and electron microscopy (EM).

Before PS treatment began all animals had well developed SD (cationic BSA) or MD (anionic BSA) by IF and/or EM.. Compared to saline controls (n=9) IV PS animals (n=7) showed a rapid disappearance of deposits, restoration of podocyte architecture with only isolated SD remaining. In most animals the major effect of PS occurred in the first 7 d of treatment. PS (n=4) had a more modest effect versus saline alone (n=5) on MD; however, 2 animals showed disappearance of deposits. In none of the groups was there a clear effect of IV PS on proteinuria. Renal artery perfusion of PS showed no effect on SD (n=6) or MD (n=7).

These results show that PS is capable of both preventing and dissolving immune deposits in experimental MN.

"IN SITU" FORMATION OF IMMUNE COMPLEXES (IC) INDUCED BY ANTIBODY REACTIVE WITH PLASMA MEMBRANE ANTIGENS. RELEVANCE TO THE PATHOGENSIS OF HEYMANN GLOMERULONEPHRITIS (HG). Jan Brentjens*, Seiichi Matsuo*, Peter Caldwell* and Giuseppe Andres, State Univ. of NY at Buffalo, Buffalo, NY, and College of Phys. & Surg. Columbia Univ., New York, NY.

The hypothesis was tested that "in vivo" interaction of antibody with antigens expressed on the plasma membrane of cells adjacent to a basement membrane or to a basement membrane-like structure causes "in situ" formation of IC. Because angiotensin-converting enzyme (ACE) was found on the oolemma of mature rabbit oocytes, we studied the interaction of ovaries with goat antibody to rabbit ACE (GtARbACE). Rabbits were injected i.v. for 4 days with GtARbACE and the ovaries studied at weekly intervals for one month by morphological and immunocytochemical techniques. Interaction of divalent, but not monovalent, GtARbACE induced a redistribution ("patching") of ACE in the oolemma, followed by "shedding" of ACE IC into the perivitelline space, and by progressive centrifugal migration of IC in the zona pellucida. Similar ACE redistribution was observed in isolated ovaries perfused with GtARbACE IgG, but not with Fab. The results indicate that "in situ" formation of IC can be induced "in vivo" by mechanisms comparable to those occurring in well established "in vitro" systems (B lymphocytes, virus-infected cells) characterized by reaction of surface antigens (or receptors) with appropriate ligands. These studies might have relevance to the pathogensis of Heymann glomerulonephritis, a disease induced in rats by antibody reactive with antigen expressed on the plasma membrane of glomerular visceral epithelial cells (J. Exp. Med. 157:667, 1983).

EFFECTS OF CYCLOSPORIN (CY) ON THE EARLY AND LATE PHASES OF ACTIVE HEYMANN'S NEPHRITIS (AHN). Daniel C. Cattran, G. Moller*, Women's College Hospital, University of Toronto, Toronto, Ontario.

Effects of CY given 1) at initiation of AHN (Group 1), 2) after significant anti-FxlA antibody formation (Ab) (Group 2), 3) after significant proteinuria appeared (Group 3) were compared to control animals (Group 4). AHN was induced by a human FxlA fraction and all animals were followed 180 days. The immune pathophysiology was monitored by serial sampling for free Ab and circulating immune complexes (CIC). Function was determined by proteinuria, urea nitrogen and histology by serial biopsies examined by light, immunofluorescence and electron microscopy. CY was given S.C. and dose adjusted to keep 24 hour trough level 100-200 ug/mL. As previously described, CY completely suppressed both Ab and CIC during the 50 days of administration in Group 1. In Groups 2 and 3, both Ab and CIC were suppressed by a mean of 50% during the 50 days of CY but, both Ab and CIC rebounded in Groups 1-3 above control (Group 4) when CY stopped. Immuno pathology was not altered in Groups 2 and 3 compared to Group 4. Rats in Groups 2-4 developed or remained proteinuric. In contrast, Group 1 - despite free Ab and CIC elevation equal to Groups 2-4 post CY - remained morphologically and functionally normal up to 150 days after CY stopped.

These results suggest that CY must be given at antigen initiation time to alter AHN. CY does induce a permanent amelioration of the disease process as seen by normal function and morphology, despite increased levels of both free FxlA Ab and CIC after CY stopped.

ANTIBODY-INDUCED REDISTRIBUTION OF HEYMANN'S ANTIGEN (HA) ON THE SURFACE OF VISCERAL GLOMERULAR EPITHELIAL CELLS (GEC) IN LEWIS RATS. G. Camussi*, J.R. Brentjens*, D. Kerjaschki*, F. Malavasi*, B. Noble*, O.A. Roholt*, M.G. Farquhar and G. Andres. Depts. of Microbiol., Pathol. and Med. SUNY/AB, Buffalo, NY; Univ. of Torino, Italy; Section of Cell Biol., Yale Univ., New Haven, CN.

This study was performed to evaluate the potential of antibodies directed against the nephritogenic antigen of Heymann's nephritis to induce redistribution of that antigen on the plasm membrane of GEC. Antibodies used in the study included polyclonal antibodies to proximal tubule brush border vesicles, polyclonal antibodies to purified HA (gp330) and monoclonal antibodies to gp330. At 4°C, all 3 antibodies bound to the plasma membrane of GEC grown "in vitro". At 37°C, the polyclonal antibodies were detectable in clusters on the surface of GEC. Single and multiple patches and caps were observed. Prolonged incubation produced disappearance of HA from the cell surface. Antibody-induced redistribution of HA was energy dependent, required divalent antibodies and was related to the contractile activity of GEC. The monoclonal anti-gp330 antibodies were less efficient than the polyclonal antibodies in inducing antigen redistribution. Polyclonal IgG directed against brush border vesicles, injected into LEW rats, produced granular deposits "in vivo" in glomeruli. In contrast, Fab fragments of the same antibodies failed to produce a similar effect. The observations are consistent with the hypothesis that the formation of subepithelial immune deposits in Heymann's nephritis results from the shedding of immune complexes formed "in situ" on the plasma membrane of podocytes.

CLEARANCE OF IMMUNE COMPLEXES (IC): MODULATION BY POLYCLONAL B CELL ACTIVATION (PBA). Tito Cavallo, Kerry Graves*, Rodney Nunley*, and Norman A. Granholm*. Univ. of Texas Medical Branch, Pathology Department, Galveston, Texas

Clinical and pathological manifestations of lupus are commonly exacerbated by associated infections. We induced PBA in mice using bacterial lipopolysaccharide (LPS), 0.05 mg twice weekly for 5 weeks, and we studied the clearance of soluble preformed IC (BSA-anti-BSA) before (at 2.0 mo. of age) and after a course of LPS (at 3.5 mo. of age), and after withdrawal of LPS (at 5.0 mo. of age). At the dosage and schedule used, LPS causes PBA, as reflected by hypergammaglobulinemia, induction of autoantibodies, and circulating immune complexes (DNA-antiDNA, rheumatoid factor). The time (hours) required to clear 90% of IC administered (0.1 mg/g body weight) is shown below.

Group	2.0 mo.	3.5 mo.	5.0 mo.
Control	17.8 ± 1.2	16.1 ± 0.9	9.8 ± 0.5
LPS	---	22.0 ± 0.4	24.6 ± 0.6

Values are means ±SE; LPS vs. control $p < 0.001$

The data indicate that administration of LPS results in an impairment of clearance of IC from the circulation, and that this impairment not only persists, but also worsens with age, in spite of withdrawal of LPS. Exposure to bacterial antigens (that cause PBA) may exacerbate lupus due to increased formation of IC, impaired clearance of IC, or both.

EXPERIMENTAL AUTOIMMUNE GLOMERULONEPHRITIS (EAG) IN CHICKENS (Ch): INFLUENCE OF ANTIGEN IN A SYNGENEIC STRAIN. M. Chandra*, T. Tyson*, B. Sturgill and K. Bolton, U. of Va. Med. Sch., Charlottesville, Va.

We previously described the development of EAG in Ch immunized with bovine (Bo) glomeruli (GBM). The present study examined the role of human (Hu), Bo, turkey (Tu) and Ch GBM in an inbred strain of Ch. 120 syngeneic S.C. Ch 6 wks old were divided into 5 groups of 24 Ch. Ch in the first 4 groups were immunized with 10 mg dry wt/wk of Hu, Bo, Tu or Ch GBM in complete Freund's adjuvant (CFA). Controls in group 5 received only CFA. After the 5th immunization, 4 Ch from each group were sacrificed/wk. Pathological lesions (0-4+) of varying severity developed in all GBM recipient birds without correlation between IgG deposits and nephritis in individual Ch.

Parameter	Hu	Bo	Tu	Ch
IgG on GBM - %	92	50	55	33
- intensity	tr-4+	tr-3+	tr-2+	tr-1+
Proliferative GN - %	88	71	55	67
- intensity	1-4+	1-3+	1-4+	1-2+
Epithelial proliferation	+	+	+	-
Interstitial infiltrate	-	+	-	+

Both histopathological and immunofluorescence lesions were most severe in Ch with Hu and least in Ch with Ch GBM. Ch immunized with Hu and Bo GBM showed increased intensity of GBM deposits of IgG with time. There was no influence of Ch sex on the occurrence and severity of disease.

The present studies show 1) EAG can be produced in this syngeneic strain of Ch, 2) antigen source is an important factor with Hu > Bo > Tu > Ch in causing EAG, 3) no relationship between the occurrence and severity of histopathological lesions and deposits of IgG on GBM in the same Ch, and 4) no sexual predilection on precipitation of disease.

ISOLATION AND CHARACTERIZATION OF THE NEPHRITOGENIC TUBULAR ANTIGEN PRODUCING ANTI-TUBULAR BASEMENT MEMBRANE (αTBM) DISEASE. M. Clayman*, A. Martinzez-Hernandez*, L. Michaud*, R. Mann*, N.A. Kefalides, and E. Neilson, Univ. of Pennsylvania and Hahnemann University, Phila., PA

αTBM-mediated interstitial nephritis can be induced in susceptible rodents with a complex mixture of rabbit renal tubular antigens (SRTA) consisting of over 15 distinct TBM components. With a monoclonal antibody to a relevant TBM antigen [α3M-1-Ab: BN-TBM$^+$; LEW-TBM$^-$(LEW-TBM is generally accepted as being devoid of the relevant disease-producing antigen)], we utilized immunoaffinity chromatography to isolate a 48,000 M.W. glycoprotein (3M-1) from collagenase solubilized TBM. This moiety represented 0.05% of the starting material. Solid-phase radioimmunoassay with the reference α3M-1-Ab showed this protein to be markedly enriched for the 3M-1 epitope compared with SRTA. 50 μg of 3M-1 in adjuvant induced αTBM antibodies and interstitial nephritis (2.5±0.8 on a scale of 0-4) in Strain XIII guinea pigs. 50μg of SRTA also resulted in disease (2.2±0.4). SRTA depleted of 3M-1 did not produce interstitial lesions. Biochemical analysis of the 3M-1 protein revealed it to be a non-collagenous glycoprotein containing only glucose (6-8%). Immunofluorescent and immunoelectron microscopy localized 3M-1 to TBM and Bowman's capsule, but not to glomerular basement membrane. Immunoelectron microscopy showed 3M-1 to be associated with the interstitium and adjacent to or attached to the most lateral aspect of the TBM.

These results suggest that 3M-1 is the nephritogenic antigen producing αTBM disease.

TRANSFER OF EXPERIMENTAL AUTOIMMUNE GLOMERULONEPHRITIS (EAG) IN CHICKENS (Ch) BY SENSITIZED CELLS. M. Chandra*, T. Tyson*, B. Sturgill and K. Bolton, Univ. of Va. Sch. of Med., Charlottesville, Va.

We have shown that immunization of both normal (nl) and bursectomized (Bsx) non-inbred Ch with bovine (Bo) GBM in complete Freund's adjuvant (CFA) results in EAG. The present study examined the effect of transferred sensitized lymphocytes isolated from the kidneys (K) and spleens (S) of syngeneic Ch immunized with human (Hu), Bo, turkey (Tu) and Ch GBM. 42 Ch 6 wks of age were divided into 7 groups and given 1.3 x 10^6 to 3.1 x 10^8 syngeneic isolated lymphocytes. K biopsies were taken 2 wks later and Ch sacrificed 1½ mo after getting cells.

Group	n	Donor Immunization	Recipient Status	Cell Source	EAG 2 wk	EAG 6 wk
1	4	Hu	nl	K	0/1	
2	4	Bo	nl	K	2/2	
3	4	Tu	nl	K	1/2	
4	6	Ch	nl	K	2/2	5/6
5	9	Ch	5nl, 4Bsx	S	-	9/9
6	9	CFA	nl	S	-	0/9
7	6	CFA	nl	K	0/2	0/2

Proliferative glomerulonephritis was present in 5/7 two wk biopsies and 14/15 six wk biopsies in recipients of GBM cells, and absent in recipients of CFA control cells. Lesions were more severe 1) in Bsx Ch than normal, 2) with K than S cells, and 3) with time. No IgG deposits on GBM were present in any group.

These studies show that EAG in the Ch is transferable by sensitized cells in the absence of antibody to GBM, and provide further evidence for the role of cell mediated immunity in the pathogenesis of this model.

RENAL EXTRACELLULAR MATERIAL REACTING WITH A B CELL MARKER IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). D. Droz*, and E. Adafer* (intr. by G.S. Hill). Dept. of Nephrol., Hôpital Necker, Paris, France.

Indirect immunoperoxidase analysis using monoclonal antibodies (MoAb) was performed on 28 renal biopsies (RB) with various types of glomerulonephritis (GN). We used MoAb recognizing T and B cell markers (OKT3, OKT8, T4, B1), HLA-DR related antigen (I2) and monocytes (Leu M3). Eleven RB were obtained from patients with SLE, 9 of which had active lesions. We also studied 5 idiopathic membranoproliferative GN (MPGN), 2 acute postinfectious GN (AGN), 4 membranous GN (MGN), and 6 lipoid nephrosis. Various numbers of both T4 and T8 cells, were found in the interstitium in 23 cases; some B1+ cells were present in 7 cases. Leu M3+ cells were observed in the interstitium in 19 cases and in glomeruli in 13 cases (5 SLE, 4MPGN, 2AGN, 2MGN). In all cases HLA-DR antigen was detected within the glomeruli, both on endothelial and mesangial cells, on the vascular endothelium and on interstitial cells. HLA-DR was also expressed within tubular cells in 4 cases. Large amounts of extracellular material reacting with B1MoAB were found in the 9 active SLE RB. Such B1+ material was distributed with a granular pattern in mesangial areas, along glomerular capillary walls, and some tubular basement membranes. B1+ material co-distributed with IgG and C1q deposits. All non-SLE RB, even those containing IgG and C1q deposits, were negative for extracellular B1 binding except 2 MPGN in which faint labelling was detected in some glomerular areas. In conclusion, the presence of B1+ extracellular material appears to be a marker for active lupus nephritis and could be related to the B cell hyperactivity in SLE.

RELATIONSHIP OF GLOMERULAR STRUCTURE TO FUNCTION IN INSULIN DEPENDENT DIABETES MELLITUS (IDDM). E.N. Ellis,* M.W. Steffes,* and S.M. Mauer. Univ. of Minnesota, Minneapolis, Minnesota.

Glomerular mesangial expansion is an important determinant of renal dysfunction in IDDM. The mechanism proposed is the impingement of the expanded mesangium on contiguous structures, especially the glomerular capillary. For further investigation, renal biopsies and creatinine clearances (C_{Cr}) were obtained in 28 patients, aged 29±6 years with IDDM for 18±6 years. C_{Cr} ranged from 35 to 212 ml/min. 3 glomeruli from each patient were photographed by electron microscopy at X6000 and a photomontage was arranged to reconstruct the entire cross section of each glomerulus. Quantitative stereology was performed on each montage. Mean glomerular volume was determined for each patient using an estimation of area as determined on a digitizing tablet. Absolute values per glomerulus were determined for mesangial volume (Mes), capillary lumenal volume (CL), capillary length, capillary filtering surface (CS), and average capillary diameter (D). In addition, 2 million glomeruli were estimated per patient and corrected for the % sclerosed glomeruli. The total capillary filtering surface per patient (TCS) was then calculated.

C_{Cr} correlated inversely with Mes ($r=-.47$, $p<.01$), CL ($r=.38$, $p<.05$), and CD ($r=.45$, $p<.05$). A highly significant direct correlation was seen between C_{Cr} and CS ($r=.63$, $p<.001$) and between C_{Cr} and TCS ($r=.66$, $p<.001$). Thus, the TCS is the most important parameter in determining glomerular filtration rate at both the hyperfiltration range and when GFR is declining.

PHENOTYPE OF LEUCOCYTES IN THE HUMAN KIDNEY. Helen D. Feiner and Ralph M. Steinman*. NYU Medical Center & Rockefeller University, New York City, New York.

Frozen sections from 6 nephrectomy specimens were reacted with antileucocyte monoclonal antibodies (Mabs) followed by peroxidase-labeled secondary antibody, or an avidin-biotin-peroxidase complex. The leucocyte common antigen, identified by Mab 4B2, was localized to a few glomerular cells (mean 1.3-3.0 cells per glomerulus in 8 micron sections in the 6 cases, with sample sizes of 21-81 glomeruli), and to interstitial cells of cortex and medulla. Cell specific Mabs were applied to adjacent sections to classify the 4B2+ leucocytes. Neutrophil specific antigens were identified by Mabs Leu M1 and OKM1, and 3G8 an antibody directed against the Fc receptor for IgG. Neutrophils represented some of the white cells in glomeruli (mean .02-1.2 cells per glomerulus), but were rare in the interstitium. The monocyte restricted Mabs 3C10 and 63D3 stained round and stellate cortical and medullary interstitial cells, but only rare glomerular cells (mean 0.02-0.2 per glomerulus). T & B lymphocyte markers were found on few if any cells in glomeruli or uninflamed interstitium. OKIa and 9.3F10, Mabs directed at Ia related antigens, labeled most glomerular cells, all cortical peritubular capillaries, medullary vasa recta and endothelium of arteries, arterioles and venules. OKM5 stained a subpopulation of cortical capillaries and vasa recta , but not glomerular capillaries. This study demonstrates that in the human kidney mononuclear phagocytes are predominant interstitial leucocytes while glomerular leucocytes are quite heterogeneous and not yet fully characterized.

FUNCTIONAL ROLE OF TERMINAL COMPLEMENT COMPONENTS IN IMMUNE COMPLEX NEPHRITIS (ICN) IN MICE. Ronald J. Falk and J. Charles Jennette, Departments of Medicine and Pathology, University of North Carolina, Chapel Hill, NC 27514 USA.

The terminal complement (C) components C5-C9 have been localized by immunohistochemical studies in glomeruli (G) in human and experimental ICN. To determine the functional role of C5-C9 in ICN we used a congenic pair of mice which differ in the single gene coding for the presence of C5 (B10·D2-NSN) (NSN) or lack thereof (B10·D2-OSN) (OSN). A total hemolytic complement assay demonstrated that OSN mice serum had negligible hemolytic activity. Twenty NSN and 20 OSN mice were given intraperitoneal injections of 4 mg horse apoferritin (HAF) six days a week. In each experimental group, animals were sacrificed on days 10 (N=4), 28 (N=8), and 42 (N=8). Five additional NSN and 5 OSN were cage-controls. Prior to sacrifice, plasma was obtained from each animal to quantify HAF antibody production. Tissues were examined by light (LM), immunofluorescence (IF), and electron microscopy (EM). No histologic differences between OSN and NSN groups were apparent at day 10. By day 28, the G of NSN mice had 2-4+ diffuse deposition of IgG, IgM, C3 and HAF in mesangial loci, and in the most severely affected G, along capillary loops. By LM, NSN mice had diffuse proliferative glomerulonephritis with occasional crescents. At 48 days, the severity of these lesions was greater. In contrast at 28 and 48 days, OSN mice had minimal focal deposition of immune reactants without a LM morphologic difference from control mice. This study documents a critical role for C5 in the pathogenesis of the glomerular lesion of ICN.

NEPHRITOGENIC POTENTIAL OF ANTIBODIES (Ab) TO GLOMERULAR BASEMENT MEMBRANE (GBM) CONSTITUENTS. I.D. Feintzeig*; D.R. Abrahamson*; J.E. Dittmer*; D.J. Salant, Boston Univ. Med. Ctr., Boston, MA and Univ. Alabama, Birmingham, ALA.

The composition of GBM is similar to other BMs and includes Type IV collagen, laminin and proteoglycans. The murine EHS sarcoma produces abundant BM material from which these substances have been isolated. Abs against such proteins have produced linear GBM deposits but little glomerular injury. In these studies, sheep anti-EHS (Sh A-EHS, n=9) produced intense linear GBM deposits of Sh IgG but consistently failed to induce heterologous or autologous phase proteinuria (Mean urine protein <4mg/24h for up to 2 weeks). Similar results were obtained after the autologous phase was augmented by preimmunization with Sh IgG (n=4) or passive immunization with rat A-Sh IgG (n=3). GBM deposition of rat C3 in vivo was absent despite the proven ability of both Sh A-EHS and rat A-Sh IgG to fix C. In contrast, when kidneys containing Sh A-EHS were transplanted into naive recipients that were passively immunized with rat A-Sh IgG, significant proteinuria occurred (20-350mg/24h, n=3, on day 5). Immunofluorescence of the transplant showed rat C3 as well as Sh and rat IgG on the GBM, whereas rat C3 was absent from nonimmunized controls. We conclude that C fixation appears essential for injury when Abs react with certain GBM constituents and speculate that: a) a large "sink" of extrarenal Sh A-EHS normally buffers the kidneys from autologous phase injury; and b) the absence of C fixation by Sh A-EHS on the GBM might be due to a low density of the relevant epitope. This could explain the lack of injury in other studies of mono- and polyclonal Abs against known GBM constituents.

ISOLATION AND CLONING OF T LYMPHOCYTES FROM END STAGE RENAL DISEASE (ESRD) KIDNEYS. C. Flaa*, D. Roth, and J. Miller*. Univ. of Miami, Miami Fl.

In the mixed lymphocyte kidney culture (MLKC), we have demonstrated a lymphoproliferative response of T cell-enriched infiltrating lymphocytes (TK) isolated from the kidneys of patients with ESRD to collagenase extracted autologous renal cortical cells (KC). In the current study, further analysis of TK by T cell cloning techniques was performed. Clones were derived from lymphocytes growing from pieces of ESRD kidney tissue placed in IL-2 conditioned medium. Increasing numbers of lymphocytes were noted adjacent to the tissue after 7-21 days. Cloning of selected parent wells were performed by limiting dilution in the presence of IL-2, irradiated autologous lymphocytes and KC cells for antigen exposure. Phenotypic analysis of several clones by indirect immunofluorescence demonstrated a predominance of OKT4 positive clones with some expressing both T4 and T8 marker and all positive for DR antigen. This technique allows for the propagation of in vivo activated T cells to ESRD kidneys. Further phenotypic and functional studies may help evaluate in situ lymphocyte responses to damaged renal tissue.

ENVIRONMENTAL ANTIGENS (EAg) IN IgA NEPHROPATHY (IgAN). J.H. Galla, M.W. Russell*, D. Hammond*, M. Spotswood*, J. Mestecky*. University of Alabama in Birmingham.

To examine the role of EAg in IgAN, we studied serum and kidney biopsy tissue in 29 patients with IgAN, 8 patients with other renal diseases (CRF) and 22 normal subjects (CON). Radioimmunoassay with IgA1 and IgA2 monoclonal antibodies (Abs) to bovine (B), pig, and chicken γ-globulin (GG), casein, ovalbumin, β-lactoglobin, soy flour, wheat gliadin, phosphocholine, and surface protein I/II of **Strep. mutans** were used. In IgAN and CON, Abs to BGG and surface protein I/II **Strep. mutans** were much higher than other Abs but Abs levels for all EAg were not different between these groups. IgA2 Abs were only occasionally seen in either group. In selected sera, the polymeric/monomeric pattern also did not differ (Mann-Whitney U test; p-NS). Selected kidney biopsy tissue from all three groups were stained with monoclonal Abs to IgA1 and IgA2 and rabbit Abs to selected EAg. Normal kidney tissue was obtained from 4 nephrectomy specimens. All IgAN kidney tissue stained positive for IgA1 and none for IgA2. In IgAN, Abs to soy flour were seen in 9 of 14 biopsies, to milk whey in 16 of 19, and to I/II **Strep. mutans** in 0 of 5. In CRF, Abs to soy flour were seen in 2 of 7 and to milk whey in 1 of 4. No IgA or EAg fluorescence was observed in CON.

These data suggest that, although EAg may have an important pathogenetic role in IgAN, the occurrence, subclass or molecular form of serum IgA Abs to EAg does not cause deposition of these substances.

EFFECT OF CYCLOPHOSPHAMIDE PRETREATMENT ON DELAYED-TYPE HYPERSENSITIVITY (DTH) SKIN REACTION IN CHRONIC EXPERIMENTAL UREMIA. Raymonde F. Gagnon*, and David S. K. Lu*, (intr. by Bernard S. Kaplan). Division of Nephrology, Montreal General Hospital, McGill University, Montreal, Canada.

The reduction in immune responsiveness associated with chronic uremia has been attributed to increased suppressor cell activity. Since high dose cyclophosphamide can preferentially affect suppressor cells we assessed the effects of cyclophosphamide administration on the DTH response of chronically uremic mice. DTH testing, using oxazolone as the contact sensitizing agent, was performed in normal, sham-operated (right kidney electrocoagulation and left kidney mobilization) and uremic (right kidney electrocoagulation and left nephrectomy) C57BL/6 mice six weeks after the onset of uremia. We ascertained by the degree of myelosuppression that the level of active cyclophosphamide metabolites was similar in each of the drug-tested groups. Following pretreatment with a single sublethal cyclophosphamide dose (250mg/kg) enhancement of DTH response was observed in all mice over a 3 day period. This enhancement, however, was less marked in mice with renal failure when compared with controls.

Increase in DTH response (%)

	Normal	Sham	Renal failure
24 hr	29.3	54.8	15.6
48 hr	71.6	100.5	61.6
72 hr	86.2	65.9	54.7

These results suggest that suppressor cell activity is not increased in chronic uremia. Our study also shows that biotransformation of cyclophosphamide by the liver is maintained during renal failure.

INTERACTION OF IMMUNE-COMPLEXES WITH HEPARAN SULFATE ENRICHED ANIONIC SITES OF GLOMERULAR EXTRACELLULAR MATRICES. T.C.Glaser,* Y.S.Kanwar, G.R.Gallo and M.E.Lamm. Northwestern Univ., Chicago, IL.; New York Univ., New York, NY & Case Western Univ., Cleveland, OH. (Introduced by Frank A. Krumlovsky).

The binding characteristics of cationic and heterogeneous immune-complexes (ICs) with heparan sulfate enriched anioinc sites of the glomerular basement membrane (GBM) and mesangial matrix (MM) were studied. The rat kidneys were treated either with buffers alone or buffers containing heparitinase or chondroitinase-ABC (PNAS 76:1303-1307,1979) followed by perfusion of either cationic or heterogeneous ICs (JCI 67:1305-1313,1981). The tissues were processed for immunofluorescent and electron microscopy after fixation with glutaraldehde or tannic acid-glutaraldehde (TAG). The cationic ICs localized in the inner & outer layers of the GBM & MM. The distribution of ICs seemed to be similar to the anionic sites of the GBM & MM. TAG fixation remarkably enhanced the visualization of ICs. A linear pattern along the GBM & a granular pattern in the MM of ICs was observed by immunofluorescent microscopy. Heparitinase treatment resulted in the loss of binding of ICs both in the GBM & MM. Chondroitinase-ABC treatment did not cause any appreciable loss in binding of ICs. The heterogeneous ICs localized in the mesangium and their distribution was unaffected by the enzymatic treatments. These results indicate that the ICs containing cationic fractions bind to the heparan sulfate enriched anionic sites of the GBM & MM, while the heterogeneous fractions are non-specifically trapped in the mesangium of the renal glomerulus.

EICOSANOID PRODUCTION BY ISOLATED GLOMERULI IN NEPHROTOXIC SERUM NEPHRITIS (NSN). R.J. Glassock, P. Anderson*, R. Zipser*, Department of Medicine, Harbor-UCLA Medical Center, Torrance, California.

We have previously shown that the glomerular microcirculation undergoes an adaptive change in the course of NSN and that both enhanced angiotensin-II (A-II) action and augmented synthesis of vasodilatory prostaglandins (PG) are involved in this response. The present study was undertaken to further explore the interactions of A-II and PG in NSN in isolated glomeruli using enalapril (E) as an angiotensin converting enzyme inhibitor. An accelerated autologous model of NSN was induced in rats by I.V. administration of goat-anti-rat glomerular basement membrane antibody following immunization to goat IgG. PG synthesis was evaluated at 2, 6, 10, 14, and 18 days after immunization in control (C) and NSN rats by measuring PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 by radioimmunoassay in the supernate of isolated glomeruli incubated in medium containing 5 mg/ml of arachidonic acid. Synthesis of PGE_2, but not 6-keto $PGF_{1\alpha}$ or TxB_2, was increased in NSN compared to C rats ($p < .01$). Peak production was noted at 10 days following immunization. E at 3.0 mg/kg/d p.o. for 10 d, a dose sufficient to inhibit the hypertensive effect of angiotensin-I in-vivo, significantly reduced glomerular PGE_2 production in NSN rats. These results suggest that increased PGE_2 production in NSN in vivo may be the consequence of enhanced intraglomerular A-II action. The mechanism of enhanced A-II action in NSN remains unknown.

ABSENCE OF HEYMANN NEPHRITOGENIC GLYCOPROTEIN IN HUMAN KIDNEY. P.R. Goodyer*, M. Mills*, and B.S. Kaplan. Department of Nephrology, The Montreal Children's Hospital, Montreal, Quebec, Canada.

In passive Heymann nephritis, proteinuria is induced by injecting rats with antiserum to a specific nephritogenic glycoprotein (NGP) present in proximal tubular brush border membranes (BBM) and at the glomerular epithelial cell surface. We purified the NGP from rat kidney BBM as described by Kerjaschki and Farquhar (PNAS 79: 5557, 1982). On Coomassie blue-stained 5% SDS-PA gels this material had an apparent MW of 420K daltons; with storage at 4°C, a second fainter band (400K daltons) became apparent. Antisera to rat BBM and to purified NGP were then raised in rabbits and used to identify NGP in immunoblots of various membrane fractions of rat kidney transferred to nitrocellulose from 5% SDS-PA gels. A pair of immunoreactive bands (420 and 400 K daltons) were identified in lanes containing rat BBM or isolated rat glomeruli but not rat glomerular basement membrane. Since Heymann nephritis bears histopathologic resemblance to idiopathic epimembranous glomerulonephritis in humans, we also prepared BBM from fresh normal human kidney obtained at the time of nephrectomy for a well-demarcated renal tumor. On 5% SDS-PA gels, we did not observe Coomassie blue-staining bands in the 400-420 K dalton region, nor did we find high molecular weight immunoreactive bands when human BBM was immunoblotted against anti-rat BBM or anti-rat NGP. These data suggest that human kidney does not possess the Heymann nephritogenic antigen.

CELL-MEDIATED IMMUNITY IN CHRONICALLY UREMIC MICE. Jonathan Gold*. Raymonde F. Gagnon*, and William Gerstein*, (intr. by Bernard S. Kaplan). Department of Medicine, Montreal General Hospital, McGill University, Montreal, Canada.

Cell-mediated immunity was evaluated in C57BL/6 mice by delayed-type hypersensitivity (DTH) skin testing using oxazolone as the contact sensitizing agent. Mice were divided into 1) normal, 2) sham-operated (right kidney electrocoagulation and left kidney mobilization), and 3) chronically uremic (right kidney electrocoagulation and left nephrectomy) and tested six weeks after the onset of uremia. Skin sensitization with oxazolone (2.0mg on rump) was followed by challenge (0.1mg on right ear) four days later. The magnitude of the DTH response was determined by serial measurements of ear thickness following challenge. Results are expressed as specific increase in ear thickness in cm^{-4}.

	Normal(N)	Sham(S)	Renal failure
Number of mice	13	14	12
BUN (mg/dl)	22.3±1.5	21.5±2.4	105.0±8.0[a]
DTH response			
24 hr	7.1±1.1	11.9±0.7	5.9±0.9[b]
48 hr	12.6±1.0	12.9±0.7	9.3±1.3[c]
72 hr	13.4±1.8	10.6±1.2	7.7±1.6[d]

Values are means±SEM, a=p<0.001 vs N and S, b=p<0.001 vs S, c=p<0.02 vs S, and d=p<0.01 vs N.

We conclude that 1) the induction and maintenance of DTH responses are curtailed in chronic uremia, and 2) the mouse, using appropriate skin testing, appears to be a useful model for studying cell-mediated immunity in renal failure.

MURINE LUPUS NEPHRITIS: PATHOGENIC ROLE OF ANTI-DNA ANTIBODIES. Norman A. Granholm*, Kerry Graves*, and Tito Cavallo. Univ. Texas Medical Branch, Pathology Department, Galveston, TX.

The pathogenic role of antiDNA (αDNA) antibodies in the induction of lupus nephritis is controversial. We studied the αDNA activity of plasma (P) and renal eluate (E) of NZB/W mice at 5 mos. of age, when nephritis became apparent (group PT), and at 8 mos. of age, when renal failure developed (group UN). We performed similar studies in 3 groups of NZB/W mice, all at 8 mos. of age, whose nephritis had been arrested with a course of 12 weeks of methylprednisolone (MP), azathioprine (AZ), or cyclophosphamide (CY). We determined the P and E concentrations of IgG and αDNA antibodies by solid phase immunoassays. To compare αDNA activities, we adjusted the concentration of immunoglobulins to 1.0 μg/ml in all samples. The mean values of assays, and the relative concentration factor (αDNA E/αDNA P) of αDNA activity in the eluate are shown below.

Group	P IgG mg/ml	P αDNA cpm	E IgG μg/g tissue	αDNA E/P
MP	3.7	1997	17.6	0.6
AZ	4.7	1272	12.5	0.8
CY	5.0	782	9.6	1.4
PT	8.5	422	3.7	1.6
UN	10.5	307	31.1	2.5

The data indicate that a) the P αDNA activity reflected neither renal involvement nor renal deposits of elutable αDNA activities, b) although decreased by immunosuppression, the relative concentration of αDNA antibodies in kidneys was minimal, and c) immune complex systems other than, or in addition to, DNA-αDNA likely play a role in the pathogenesis of lupus nephritis.

CHANGES IN GLOMERULAR BASEMENT MEMBRANE (GBM) HEPARAN SULFATE (HS) IN EXPERIMENTAL MEMBRANOUS NEPHROPATHY (MN). G.C. Groggel, W.A. Border, P. Hoving,* A. Linker*. Univ. of Utah Med Center and VA Med Center, Salt Lake City, Utah.

The existence of a charge-selective barrier to macromolecular filtration in the glomerulus is well-established. The primary GBM polyanion contributing to this barrier is HS. We quantitated and characterized HS in glomeruli of normal (NG) and diseased (DG) rabbits by studying the in vivo incorporation of $^{35}SO_4$. MN was produced by 3 weeks of injection of cationic BSA (Border JCI 69:451, 1982), mean protein excretion 581 mg/24 h. 24 hours after injection of $^{35}SO_4$ glomeruli were isolated and studied.

NG and DG incorporated similar amounts of total $^{35}SO_4$ (232±65, N=8, vs 319±180, N=6, DPM/mg dry wt of glomeruli, p>0.05). The HS in DG was shifted to a fraction with reduced sulfation as shown on ion exchange chromatography. NG had 29.6±5.3% of HS in the 1.0M NaCl fraction while DG had 44.0±9.0%, p<0.01. The ratio of the percentage of HS in the 1.0M fraction to the 1.25M fraction was 0.71±.16 in NG (N=8) and 1.37±.42 in DG (N=6), p<0.01. A similar trend in uronic acid content was found. The ratio of uronic acid (µg/mg dry wt) in the 1.0M fraction to the 1.25M fraction was 0.91± 0.07 in NG (N=5) and 1.46±.19 in DG (N=6), p<0.001.

These results indicate that in MN polyanion synthesis is quantitatively normal but qualitatively abnormal with a shift toward species possessing less sulfate and lower negative charge. This change would theoretically reduce the charge density of GBM and increase permeability to anionic molecules.

CLEARANCE AND TISSUE UPTAKE OF IgA VS. IgG_1 IMMUNE COMPLEXES (IC) IN PRIMATES: EFFECTS OF ERYTHROCYTE (E) BINDING CAPACITY FOR IC. LA Hebert, FJ Waxman*, FG Cosio, DJ Birmingham*, WL Smead*, ME VanAman*. Dept of Medicine, Ohio State University, Columbus, Ohio.

Studies in nonhuman primates (JCI 71:236, 1983 and Oct 1984-IN PRESS) have shown that primates possess an unique mechanism for clearing IC from the circulation: IC bind to E-CR_1 and then IC are deposited in liver or spleen. This study examines if failure of IC to E binding changes clearance and tissue uptake of IC. Rationale: mouse monoclonal (mm) IgG_1-DNP-BSA IC (IgG_1-IC) are large and bind well to E but mm IgA DNP-BSA-IC (IgA-IC) are smaller and bind less well to E. In 3 baboons, IgA-IC and IgG_1-IC were infused simultaneously. In 2 baboons IgA-IC and IgG_1-IC were infused sequentially. We found that, compared to IgG_1-IC, IgA-IC were cleared from the circulation more quickly, despite smaller size. The more rapid clearance of IgA-IC correlated with its lower IC to E binding ($r=0.958$, $p < 0.02$). More IgA-IC were taken up at nonhepatic, nonsplenic sites, principally kidney and lung (mean + SE IgA/IgG: 2.9+0.4, 1.7+0.1, $p < 0.001$ for both). Less IgA-IC were taken up by liver and spleen (IgA/IgG 0.83 +.01, 0.81 +.01 $p < 0.001$ for both). The hypothesis which best explains the findings is: decreased IC to E binding accelerates clearance from the circulation because unbound IC are more susceptible to uptake throughout the circulation. Conclusion: IC to E binding is critical for safe removal of IC from the circulation. Failure of IC to E binding results in excessive IC uptake by kidney, especially glomeruli.

MECHANISM AND SIGNIFICANCE OF CHANGES IN RENAL MHC PRODUCT EXPRESSION IN MOUSE. Philip F. Halloran* and Peter Autenried, Dept. of Research, Mount Sinai Hospital, Toronto, Ontario

We have previously reported that renal Ia and H-2K products are increased up to 10x during strong systemic immune responses in kidney, the principal alterations being in the proximal tubule. Cyclosporine, a known inhibitor of release of mediators from lymphocytes, blocked the GVH-induced induction of Ia in vivo, raising the possibility that lymphocyte products may mediate the induction of Ia in renal tubules. To examine this possibility, supernatants of Con-A stimulated spleen cells were added to fresh (5 day) cultures of neonatal kidney cells and incubated for a further 4 to 8 days. The control cells expressed small amounts of H-2K and no Ia antigens. The cells incubated with ConA sup. had increased amounts of H-2K and Ia. Indirect evidence suggested that the mediator of this change was not interleukin 2, since certain preparations of IL2 failed to alter MHC product expression. The possibility that γ interferon mediates this change is being investigated. Finally, to determine whether alterations may be relevant to autoimmune diseases in vivo, absorption and immunofluorescence studies were performed to assess Ia expression in autoimmune MRL/l mice, compared to +/+ controls. The development of nephritis was associated with increased total renal Ia by absorption, part of which may be expressed in renal tubules as well as in infiltrating lymphocytes. Thus the changes in renal Ia and H-2K may be mediated by soluble products of lymphocytes, and may be relevant to spontaneous immunologic renal diseases.

THE EFFECTS OF ANTIGEN CHARGE ON MURINE HETEROLOGOUS PROTEIN-INDUCED GLOMERULONEPHRITIS (GN). J. Charles Jennette, J.E. Kylander*, S.D. Wall* and Ronald J. Falk, Dept. of Pathology and Medicine, Univ. of North Carolina, Chapel Hill, N.C.

A murine model was used to evaluate the pathologic and functional effects of antigen charge on horse apoferritin (HAF)-induced GN. BALB/c mice were given daily 4 mg. i.p. injections of cationic (C) or anionic(A) HAF. At 7, 10 and 14 days, groups of mice were sacrificed. Serum was obtained for creatinine, BUN and anti-HAF determinations; urine for albumin quantification; and kidney tissue for light, immunofluorescence and electron microscopy. At 7 days, mice given AHAF had only low intensity mesangial immunostaining for IgG, IgM, C3 and HAF; while those given CHAF had intense capillary IgG and HAF, low intensity capillary C3, and moderate intensity mesangial IgM. This pattern persisted at days 10 and 14 in mice given CHAF. In mice given AHAF, there was a progressive increase in mesangial IgG, C3 and HAF at days 10 and 14, and the appearance of relativiely low intensity capillary IgG, C3 and HAF. By light microscopy, mice given CHAF developed no discernable lesions; but mice given AHAF developed a mild GN by day 10 and a severe GN by day 14. Electron microscopy revealed predominately subendothelial dense deposits in CHAF mice; and extensive mesangial with scattered subepithelial and subendothelial deposits in AHAF mice. Neither AHAF nor CHAF mice developed renal failure. CHAF mice developed marked proteinuria by 7 days, but AHAF mice had minimal proteinuria. These data show that antigen charge effects both the pathologic and functional characteristics of immune complex-induced GN.

THE EFFECT OF WHEAT GERM AGGLUTININ ON RENAL FUNCTION IN THE ISOLATED PERFUSED KIDNEY. N.Jermanovich G.Closkey,*A.DeGuzman,* M.Kostianovsky,* Jefferson Medical College, Philadelphia, PA.

Wheat germ agglutinin(WGA) is a small molecular weight protein which binds to surface components of the glomerular epithelial cell membrane (J Cell Biol 98:1591,1984). The carbohydrate specificity of this lectin involves sialic acid and N-acetyl-glucosamine saccharide determinants. Epithelial cell retraction with membrane bleb formation has been described following glomerular exposure to WGA (Renal Physiol 3:330,1980). The relationship between lectin induced structural disturbances of the capillary wall and glomerular functional abnormalities is unknown. Therefore the effect of WGA infusion on renal function was investigated in the isolated perfused kidney using a modified technique of Ross et al.

Rat kidneys were perfused for 120 min. with oxygenated Krebs-Henseleit bicarbonate, glucose, and 5% bovine serum albumin. WGA (50ug/ml) was added at 50 min. As shown below, proteinuria developed in WGA perfused kidneys. Transmission electron-microscopy showed focal epithelial cell fusion with bleb formation.

PERFUSION	40min	50	60	80	100
Control (n=2)					
GFR (ml/min/gm)	.73	1.00	.80	.64	.37
Albumin (mg%)	24	15	30	24	36
Wheat Germ (n=5)					
GFR (ml/min/gm)	.87	.82	.61	.66	.88
Albumin (mg%)	19	23	27	70	322

Preliminary data suggest a possible role for membrane carbohydrates in the regulation of glomerular capillary wall permeability.

PATHOGENIC AUTOANTIBODY SPECIFICITIES IN HEYMANN NEPHRITIS (HN). Kouju Kamata*, Lynn G. Baird*, Mark E. Erikson*, A. Bernard Collins* and Robert T. McCluskey. Mass. Gen. Hosp. and Harvard Med. Sch., Dept. of Pathology, Boston, Massachusetts.

Specificities of serum autoantibodies (AA) produced after immunization of rats with FxlA and partially purified proximal renal tubular microvillar membrane preparations (MV, gp 330 enriched) were compared. MV was partially purified according to the method of Kerjaschki and Farquhar (PNAS 79: 5557,1982). Rats were immunized with 6 mg FxlA, MV, gp 330-depleted MV or saline emulsified in CFA and with pertussis vaccine. Serum AA were analyzed by solid phase RIA, immunoprecipitation and indirect immunofluorescence. In biopsies performed at 9 wks, small subepithelial glomerular deposits consistent with HN were demonstrated in MV rats; larger deposits were found in FxlA rats. From 10 wks following immunization, 22 of 26 FxlA rats had proteinuria (>20 mg/day); no rats in other groups were proteinuric by 24 wks. Serum AA titers of FxlA and MV groups, as detected on soluble MV, showed no difference during the first 8 wks. However, AA titers to soluble FxlA were significantly higher in FxlA immunized rats than in MV immunized rats ($p<0.001$). Proteinuria in individual rats at 10 wks showed a positive correlation with AA detected on FxlA ($r=0.526$, $p<0.005$) but not MV ($r=0.263$, $p=0.19$). Immunoprecipitations performed with radiolabeled MV indicated that all FxlA and MV rats produced AA recognizing gp 330. It is concluded that AA against gp 330 are sufficient to produce small glomerular deposits but that an additional AA specificity(s) is required for the development of large deposits and proteinuria characteristic of HN.

ASSEMBLY AND CATABOLISM OF DNA-ANTI-DNA COMPLEXES IN VIVO IN NORMAL AND AUTOIMMUNE MICE. F.S. Jones,* V.W. Dennis, D.S. Pisetsky,* and R.J. Kurlander.* Duke Univ. Med. Ctr., Durham, N.C.

Circulating immune complexes containing anti-DNA antibody bound to DNA antigen are considered important in the pathogenesis of lupus nephritis. To study the interaction of free DNA with anti-DNA in blood, the clearances of I-125 labeled monoclonal IgG2a anti-DNA antibody (60) and I-131 labeled myeloma IgG2A (C18.4) without anti-DNA activity were compared in normal B6D2 mice and in autoimmune NZB and MRL-lpr/lpr mice. The infusion of single or double-stranded DNA intraperitoneally caused a marked reduction in the levels of anti-DNA but not in the levels of myeloma IGg2A in blood. The cleared antibody was sequestered mainly in the liver and spleen. The amount of anti-DNA removed was proportional to the amount of DNA administered over a range from 1.88 ug to 30 ug per gram body weight. Pretreatment of normal mice with an anti-Fc receptor antibody to block Fc receptor function only slightly reduced anti-DNA clearance after DNA infusion indicating that anti-DNA was not being removed primarily by an Fc receptor-mediated process. In the absence of DNA, both antibodies disappeared from the blood at similar rates in all strains studied. Thus, DNA-anti-DNA complexes formed in vivo are cleared rapidly, primarily by an Fc receptor independent process, and the amount of DNA circulating intravascularly both in normal and autoimmune mice is insufficient to accelerate the clearance of anti-DNA. These findings suggest that intravascular DNA generation is not increased in the murine lupus-like syndromes.

LIPOPROTEINS ALTER BALANCE OF PLASMINOGEN ACTIVATOR (PA) AND INHIBITOR (IPA) PRODUCTION BY ENDOTHELIAL CELLS (EC). K. S. Kant, J. Sexton*, M. L. Kashyap*, P. Glas-Greenwalt*, V. E. Pollak.

EC possess receptors for low density lipoprotein (LDL) and EC growth and production of prostacyclin are inhibited by LDL in vitro. This effect is opposed by high density lipoprotein (HDL). Patients with renal disease commonly manifest alterations in lipoprotein levels and are known to have abnormal fibrinolysis. Hypertriglyceridemia decreases capacity/release of PA from EC in humans. The influence of lipoproteins on EC production of PA and IPA was studied using well characterized clones of bovine aortic EC. Different concentrations of LDL, HDL, and very low density lipoprotein (VLDL) were incubated with EC grown in serum free medium. Supernatant medium was sampled at 6, 24, and 72 hours. The samples were assayed for PA and an inhibitor of urokinase induced lysis (IPA) by a radiofibrinogen lysis assay. LDL and VLDL from 2 patients with uncorrected type 4 hyperlipemia depressed PA production significantly as compared to control medium and to the same fractions from 2 patients treated with gemfibrozil. IPA production was significantly increased in only one of the four - by the VLDL fraction of one of the untreated patients. We conclude that lipoproteins may be responsible for increased risk of microvascular thrombosis: a nonimmunologic means for progressive injury and sclerosis in glomerulonephritis.

EFFECTS OF β-D-XYLOSIDE ON THE GOLGI APPARATUS OF RENAL GLOMERULAR CELLS. Yashpal S. Kanwar, Dept.of Pathology, Northwestern University, Chicago, Ill.

The effect of P-nitrophenyl β-D-xylopyranoside on the glomerular cells was investigated. The cells were exposed to β-xyloside for 7 hr in an organ perfusion system and radiolabelled with [^{35}S]-sulfate. With the treatment of xyloside, numerous vesicles seemingly budding from Golgi saccules, distributed throughout the cytoplasm, were seen. The extent of vesiculation of Golgi saccules was much more in the visceral epithelium as compared to endothelial or mesangial cells. These vesicles were acid-phosphatase negative and contained osmium-impregnated deposits. The cells remained adherent to the extracellular matrices and did not lose their surface-associated sialoglycoproteins. Electron-microscopic autoradiographic studies revealed ∿10 times more sulfate incorporation into the cells with concomitant ∿3 fold reduction in incorporation into their matrices. Most of the intracellular radioactivity was associated with Golgi-derived vesicles. Biochemical studies indicated that xyloside treatment resulted in ∿5 fold reduction in sulfate incorporation into the proteoglycan fraction, synthesis of smaller-sized proteoglycan and ∿10 fold greater re-release of free glycosaminoglycan chains into the medium. These results indicate that xyloside or its metabolites, in this particular system, can cause drastic alterations in the Golgi-apparatus and selectively interferes in the synthesis of extracellular proteoglycans of the renal glomerulus.

DEMONSTRATION AND CHARACTERIZATION OF C3 RECEPTORS ON RAT GLOMERULAR CELLS IN CULTURE. B.S. Kasinath, M. Maaba*, E.J. Lewis, Department of Medicine, Rush Medical College, Chicago, IL.

Receptors for C3 (CR) are said to be present on the glomerular podocyte in humans. There is conflicting evidence regarding the presence of CR in kidneys of nonprimate species. Even when shown to be present on rat glomerular cells, the type of CR i.e. CR1 (for immune adherence), CR2 (for C3d), CR3 (for C3bi, non C3d) has not been elucidated. Our objective was to test for the presence of CR and to characterize them on cells grown from rat glomeruli (GC). Denuded glomeruli obtained from male Sprague-Dawley rats weighing 50 gm were planted in enriched culture media. By day 4 cells of epithelial morphology were seen growing out of the glomeruli and were cloned on day 6. CR was detected by rosette formation of sheep RBC coated with antibody and complement (EAC). Two types of assays for EAC rosettes were performed: using C5 deficient mouse serum as source of C and adding components of complement sequentially. EAC rosettes were detected by phase contrast microscopy. The results:

	Glomer. Cells(GC)	Cloned GC	Human Mononuc.	Human Kidney
Sheep RBC(E)	-	-	-	-
EAC 14	+	+	+	+
EAC 1243	+	+	+	+
EAC(C5 def)	+	+	ND*	ND
EAC(C5 def) + trypsin	+	+	ND	ND

*ND = Not Done

We conclude that CR1 and CR2 are present on epithelial-like cells grown from rat glomeruli. The functional significance of these CR is not known.

EARLY DMSO ADMINISTRATION AMELIORATES GLOMERULAR DISEASE IN NZB/WF$_1$ LUPUS MICE. B.S. Kaplan, L.S. Milner*, J.P. de Chadarevian*, P.R. Goodyer*, and J.S.C. Fong*. Departments of Nephrology and Pathology, The Montreal Children's Hospital, Montreal, Quebec, Canada.

Successful reduction of proteinuria in passive Heymann nephritis was found with DMSO treatment. DMSO was therefore given to NZB/WF$_1$ lupus mice to see if onset of proteinuria could be prevented in this model. Twenty mice were randomized into a saline, 0.1 ml/d, and a treatment group, DMSO 4 mg/gm/d. Therapy was commenced at age 10 weeks. Monthly and bimonthly 24 hour urine collections were performed. Significant differences in urine protein excretion between controls and treated groups were evident at 5 months (DMSO: 5.5 ± 0.46 mg/24 hrs; Controls: 7.35 ± 0.59 mg/24 hrs; $p < 0.05$) and at 6.5 months of age (DMSO: 6.75 ± 0.73 mg/24 hrs; Controls: 15 ± 2.15 mg/24 hrs, $p < 0.01$). By 7 and 7.5 months, the protein excretion was not significantly different (DMSO: 18 ± 8.3 mg/24 hrs; Controls: 41 ± 8.7 mg/24 hrs; $p > 0.05$); 7.5 months: DMSO: 20 ± 7.4 mg/24 hrs; Controls: 29 ± 5.7 mg/24 hrs; $p > 0.05$. At the 99% confidence limit, fewer DMSO treated mice had significant proteinuria compared to controls by 6.5 months of age ($p = 0.034$), and 7.5 months of age ($p = 0.014$). By 7.5 months of age, 5/6 treated mice had normal renal histology on light microscopy, while 6/8 untreated mice had focal proliferative glomerulonephritis, crescents and glomerular obsolescence ($p < 0.02$). These findings demonstrate a protective effect of DMSO on the progression of glomerular injury in this model.

INCREASED RENAL THROMBOXANE PRODUCTION IN MURINE LUPUS NEPHRITIS. Vicki E. Kelley, Brigham & Women's Hospital, Boston, MA.

To determine if there was a role for cyclooxygenase metabolites in lupus nephritis, intrarenal eicosanoid production was measured in autoimmune mice. Disease progression evaluated by monitoring proteinuria and renal pathology was related to kidney biosynthesis of thromboxane (TXB2), prostaglandin (PGE2) and prostacyclin (6 Keto PGF1α). These eicosanoids were analyzed in two autoimmune strains: MRL-lpr with the more rapidly progressive disease and NZBxW mice. MRL-++ mice served as controls.

	TXB2 pg/mg tissue					
	MRL-lpr		MRL-++		NZBxW	
Age(mo)	2	4	2	4	2	8-11
Cortex	60±10	247±56*	27±4	30±6	27±4	80±11*
Medulla	133±27	385±63*	73±7	105±21	87±8	192±23*

*p<.01 n=5-12 Means±SEM

Results showed: 1) renal synthesis of TXB2 increased in autoimmune MRL-lpr and NZBxW but not in aging control MRL-++ mice 2) there was no consistent elevation in the renal cortical or medullary levels of PGE2 or 6 Keto PGF1α 3) in both autoimmune strain, increased TXB2 was produced in the medulla, cortex and within glomeruli 4) enhanced production of TXB2 correlated with increased proteinuria ($r=.98$, $n=10$, $p<.01$) and with increased severity of renal pathology ($r=.8$, $n=9$, $p<.05$) 5) when renal disease was prevented by PGE2 therapy or dietary enrichment with fish oil, renal TXB2 did not rise. These data indicate that renal TXB2 increases in relation to the severity of lupus nephritis. Because of the potential deleterious effects of TXB2, enhanced production of this eicosanoid may be an important mediator of renal injury.

A TUBULAR ANTIGEN-SPECIFIC SUPPRESSOR T CELL (Ts) IN BN RATS MEDIATES ONTOLOGIC TOLERANCE TO AUTOLOGOUS TUBULAR ANTIGEN (TBM^+). C. Kelly*, W. Silvers*, and E. Neilson, U. of P., Phila., PA.

We have previously observed that BN rats (TBM^+) develop αTBM-Ab and tubulointerstitial nephritis (TIN) when immunized with rabbit-TBM, but only make small amounts of αTBM-Ab and do not develop TIN when immunized with BN-TBM. Using MHC identical/TBM^+ or TBM^- rats and delayed-type hypersensitivity (DTH) responses we examined the mechanism preventing a BN response to BN-TBM. To eliminate any alloreactivity, LEW.1N (TBM^-) and F.BN (TBM^+) rats were neonatally tolerized (T) to BN alloantigens (F.BN rats develop TIN in response to BN-TBM whereas F.BN(T) rats carrying BN skin grafts do not). The serum dilution of αTBM-Ab at 25% maximum binding for BN = 1:50, LEW.1N(T) = 1:700, and F.BN(T) = 1:950.

LEW.1N(T) rats (TBM^-) produce DTH reactions to BN-TBM, while BN and F.BN(T) (TBM^+) rats do not (LEW.1N(T) DTH to BN-TBM = 33.5 ± 0.5 vs. F.BN(T) = 7.5 ± 1.8 vs. BN = 3.8 ± 1.4 X 10^{-3} inches; $P<0.001$, whereas DTH to PPD was similar among the three groups). Immune cells from LEW.1N(T) donors transfer this T-cell dependent DTH to BN-TBM into LEW.1N recipients (αT+C' = 3.8 ± 1.3 vs. C' alone = 21.8 ± 1.1.), but not into LEW(4.3 ± 1.2). Admixture and co-transfer of immune LEW.1N(T) cells with either immune BN lymph node cells (LNC) or normal BN Thymus (THY) inhibited the DTH to BN-TBM (BN-LNC + LEW.1N(T): BN-TBM = 5.0 ± 0.5/PPD = 18.3 ± 1.7 vs. BN-THY + LEW.1N(T): BN-TBM = 7.0 ± 2.1/PPD = 21.5 ± 1.6 vs. Control BN + LEW.1N(T): BN-TBM = 20.8 ± 2.4/PPD = 20.2 ± 0.8; $P<0.001$). The Ts cell in BN-LNC was an $RT7.1^+$, $OX8^+$ T cell by phenotypic criteria.

These findings suggest that BN rats do not develop TIN after immunization with BN-TBM because the genetic association of BN $RT1^n$ with TBM^+ induces an $OX8^+$ Ts cell which inhibits effector T cell function to BN-TBM.

SURFACE ANIONIC SITES OF PERITUBULAR CAPILLARIES. Valsala Koshy, Pratap S. Avasthi. VAMC and Univ. of New Mexico, Albuquerque, New Mexico.

Peritubular capillary basement membranes have been shown to contain anionic sites. Since peritubular capillaries have diaphragmmed fenestrated endothelial cells, this study was designed to map the distribution of anionic sites at the luminal surface of the endothelial cells. Furthermore, the composition of the anionic sites was partially characterized by in vivo digestion with specific enzymes.

Native (pI 4·5) or cationized ferritin (CF, pI 8·4) were perfused through the cannulated renal artery in Sprague-Dawley rats following the removal of blood. Excess ferritins were removed by a further wash and kidneys were fixed by perfusion and examined by electron microscopy. Native ferritin did not bind to the peritubular capillary surface. CF bound both to the diaphragms and to the endothelial glycocalyx forming a continuous layer at the luminal surface.

Digestion of proteoglycans was carried out by the infusion of neuraminidase, heparitinase, hyaluronidase, chondroitin sulfate ABC. Alterations in CF binding pattern indicated that majority of the anionic sites in glycocalyx are from neuraminic acid. In contrast, the majority of anionic sites at fenestral diaphragms are from heparan sulfate proteoglycans. Papain digestion to assess whether glycolipids were also present showed that the endothelial glycocalyx has a few glycolipid containing anionic sites.

This anionic luminal surface may serve as the electrostatic barrier of the peritubular capillaries.

DEFECTIVE SIALYLATION OF PODOCALYXIN (PC) -- THE MAJOR GLOMERULAR SIALOPROTEIN -- IN PUROMYCIN AMINONUCLEOSIDE (PAN)-INDUCED NEPHROSIS. Dontscho Kerjaschki, Anthony Vernillo, and Marilyn G. Farquhar. Dept. of Cell Biology, Yale University School of Medicine, New Haven, CT., and Dept. of Pathology, University of Vienna, Austria.

PAN induces minimal change nephrosis, characterized by reduced glomerular sialic acid (SA), proteinuria, and loss of foot processes in rats. We have recently identified podocalyxin -- the major glomerular sialoprotein with an apparent MW of 140 kd which contains 4.5% SA and carries most of the glomerular SA (J. Cell Biol. 98:1591, 1984). In this study, rats were made proteinuric by daily injections of PAN for 10 days, and the PC in glomerular lysates was analyzed by SDS-PAGE. A decrease was observed in the mobility and staining intensity of PC from PAN-glomeruli after silver staining. When similarly prepared gels were transferred onto nitrocellulose and overlayed with rabbit anti-PC IgG or [^{125}I]WGA, a similar shift of the PC-band was observed in the PAN-lysates. PC of normal and PAN-rats was purified by preparative SDS-PAGE, and subjected to sugar analysis by gas liquid chromatography. SA was found to be reduced to 1.5%, Gal was slightly reduced, but Man and GlcNac were unchanged in PAN-PC. Fuc and GalNac were not detected in either normal or PAN-PC. When PC was localized on the plasmalemma of podocytes of normal and PAN-rats by immunoelectron microscopy (indirect immunogold procedure) similar numbers of gold particles were bound/μ membrane. These data indicate that in PAN: 1) the SA content of PC is reduced to 30% of that of normal PC; 2) other sugars are not affected; and 3) the density of PC on podocyte membranes is similar to controls.

THE FUNCTIONAL SIGNIFICANCE OF HUMAN LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN - 1. Alan M. Krensky, Donald Anderson,* Steven J. Burakoff,* and Timothy A. Springer.* Dept. of Pediatrics, Stanford University, Stanford, CA; Dept. of Medicine, Baylor University, Houston, TX, and Depts. of Pediatrics and Pathology, Harvard Medical School, Boston, MA.

Cytotoxic T lymphocytes (CTL) are important effectors in cell-mediated allograft rejection. The interaction of CTL and target cells is mediated by a number of cell surface molecules, including MHC gene products, the polymorphic T cell receptor, OKT3, OKT4, OKT8, and the lymphocyte function-associated antigens, LFA-1, LFA-2, and LFA-3 (Krensky, et. al., J. Immunol. 131: 611-616). Human LFA-1 is a 177, 95 kd heterodimer present on essentially all leukocytes and not on other cell types. Anti-LFA-1 monoclonal antibodies (mAb) inhibit cell-mediated cytolysis and proliferative responses in vitro. Recently, a group of immunodeficient patients with recurrent bacterial infections and deficiency (or absence) of the LFA-1 cell surface molecule have been identified, offering a unique opportunity to study the functional role of this molecule. We have studied three LFA-1 deficient individuals, family members, and unrelated controls. The LFA-1 deficient individuals show deficient CTL and NK cell-mediated cytolysis and T cell proliferative responses. LFA-1 expression correlates with lymphocyte functional competency, implying a fundamental role for this cell surface molecule. These results suggest that anti-LFA-1 mAb may inhibit T cell function in vivo and should be considered in trials of new agents of potential therapeutic utility in the treatment of allograft rejection.

THE SIGNIFICANCE OF RENAL HYALINE ARTERIOLOSCLEROSIS IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS). Hyun Soon Lee and Benjamin H. Spargo. Univ. of Chicago, Dept. of Pathol., Chicago, IL.

Sixty adult patients ($\geq$17 yrs.) with idiopathic FSGS were studied by light-, electron- and immunofluorescence microscopy in order to determine the clinicopathological significance of renal hyaline arteriolosclerosis (HA) in FSGS. HA was defined as definite hyaline material present entirely displacing the normal layers of wall and involving one or more afferent arterioles. Thirty-six biopsies (60%) exhibited a definite HA (HA [+]) in association with high levels of proteinuria (9.0 $\pm$ 6.0 gm/24 hr. vs 5.0 $\pm$ 3.6 gm/24 hr., $p<0.0024$) and serum creatinine (2.2 $\pm$ 2.0 mg% vs 1.3 $\pm$ 0.7 mg%, $p<0.0316$) and a high frequency of global sclerosis (13.9 $\pm$ 13.7% vs 5.0 $\pm$ 7.3%, $p<0.0014$) when compared to the remaining 24 biopsies without definite HA (HA [-]). By electron microscopy, the HA (+) group also exhibited a higher frequency of podocyte degeneration and necrosis than the HA (-) group ($p<0.05$). Fourteen (39%) of these HA (+) biopsies showed continuity of afferent arteriolosclerosis with hyalinosis of the adjacent glomerular axial segmental sclerosis. These findings suggest (1) that HA (+) patients show more severe disease than HA (-) patients in terms of proteinuria, renal function and incidence of global sclerosis, (2) that renal hyaline arteriolosclerosis is common in idiopathic FSGS and (3) that the glomerular change in the HA (+) group is related to the antecedent vascular lesion, while in the HA (-) group other factors may be operative.

MACROPHAGE SUPERNATANTS STIMULATE MOUSE MESANGIAL PROLIFERATION BY A PROSTAGLANDIN-E (PGE) DEPENDENT MECHANISM. E. P. MacCarthy, A. Hsu,* Y. M. Ooi, and B. S. Ooi. Dept. of Medicine, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

In-vivo nephritis is characterized by monocyte/macrophage infiltration and mesangial hypercellularity. To examine the mechanisms by which monocytes/macrophages cause mesangial cell proliferation, we have studied the effects of macrophage supernatants on mouse mesangial cell growth. Under conditions (2.5% FCS) in which mesangial cells were relatively quiescent the addition of macrophage supernatants produced a dose dependent increase in mesangial cell [^{3}H]-thymidine uptake. This stimulatory effect of macrophage supernatants could be partially abrogated by prior pretreatment of mesangial cells with indomethacin, suggesting a PGE dependent mechanism. Further proof that this was the case was provided by the demonstration that macrophage supernatants stimulated PGE production by mesangial cells and that exogenous PGE stimulated mesangial cell [^{3}H]-thymidine uptake. In conclusion, our studies provide evidence that macrophages stimulate mesangial cell proliferation by a PGE dependent mechanism and provide the rationale for therapeutic strategies which alter PGE production as a means of normalizing mesangial cell growth in nephritis.

RENAL IMMUNE INJURY: EFFECTS OF COMPLEMENT DEPLETION ON GLOMERULAR PROSTAGLANDIN (PG) AND THROMBOXANE (TX) SYNTHESIS AND RENAL HEMODYNAMICS. Elias A. Lianos, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI.

The effects of complement (C) depletion on glomerular PG (E_2, $F_{2\alpha}$, 6-keto-$PGF_{1\alpha}$) and TxB_2 synthesis were studied in the heterologous phase of rat nephrotoxic serum nephritis (NSN) and were correlated with changes in GFR (C_{IN}) and RPF (C_{PAH}). Male Sprague-Dawley rats (n = 4-6) received 4 intraperitoneal injections of purified cobra venom factor (CVF), 2 units/100 g body wt, prior to a single intravenous injection of anti-GBM immunoglobulin (nephrotoxic serum - NTS; 1.8 µg/100 g body wt), raised in rabbits. GFR and RPF were measured prior to and at 5 hrs following administration of NTS. Glomeruli were then isolated by differential sieving in Hank's balanced salt solution at 37°C for 45 min and media were assayed for PG's and TxB_2 (pg/mg glom. prot.). Control groups received a) NTS without prior decomplementation and b) CVF alone. Results were as follows:

	C-Replete		C-Depleted	
	PRE	POST-NTS*	PRE	POST-NTS*
GFR	0.73±0.05	0.43±0.04*	0.75±0.06	0.62±0.05
RPF	1.83±0.25	1.35±0.12	1.76±0.21	1.48±0.19
TxB_2		13.3±1.0		2.73±0.3†
PGE_2		1.6±0.2		6.41±0.1†
$PGF_{2\alpha}$		6.0±0.5		8.7±0.3†
6-keto-$PGF_{1\alpha}$		0.3±0.02		0.4±0.02

*$p < 0.05$ compared to PRE-NTS, † $p < 0.05$ compared to C-replete. In conclusion: In NSN complement depletion induces a redirection of glomerular PG-endoperoxides toward vasodilatory PG's, primarily E_2. This effect could mediate the amelioration of NTS-induced decrements in GFR and RPF.

MONOCLONAL ANTI-DNA ANTIBODIES(MαDNA) BIND DIRECTLY TO INTRINSIC GLOMERULAR ANTIGENS AND FORM IMMUNE DEPOSITS. MP Madaio, JA Carlson*, S Hodder*. Tufts-New Engl. Med. Ctr. Boston, MA.

Individual monoclonal lupus autoantibodies have been observed to react with diverse antigens that share common epitopes, including polynucleotides, phospholipids, cell membranes, cytoskeleton and bacteria. The present studies examine the capacity of MαDNA to bind directly to glomerular antigens and initiate immune deposit formation. MαDNA were derived from MRL-lpr/lpr mice, an inbred strain that spontaneously develops an accelerated form of SLE. Selected ^{125}I MαDNA bound to isolated normal glomeruli(G) significantly greater than controls (H130:15.7%,H241:11.6% vs control Igs:3-4%). This interaction was not affected by preincubation of G with DNAase, however, it was inhibited by preincuation of MαDNA with anti-idiotypic antibodies. In paired label studies, after i.v. injection of equal amounts of ^{125}I H241(IgG2a) and ^{131}I UPC10(control IgG2a)to normal mice, glomerular binding of H241 was observed, whereas UPC10 did not bind. In a similar in vivo study, ^{125}I H130(IgM) did not bind to G. The capacity of H241 to form immune deposits was further analyzed following i.p. injection of $2x10^6$ hybridoma cells to normal mice. After 2 weeks, diffuse granular deposits of IgG in the glomerular capillary wall and mesangium were observed by direct immunofluorescence. Normal mice injected with control hybridomas had only scant mesangial IgG deposits.

We conclude that certain anti-DNA autoantibodies can react directly with intrinsic glomerular antigens. Furthermore some of these MαDNA appear to bind directly to glomerular antigens in vivo initiating glomerular immune deposit formation.

ALTERED GLOMERULAR BASEMENT MEMBRANE (GBM) ANIONIC SITES IN THE MINIMAL CHANGE NEPHROTIC SYNDROME (MCNS) IN MAN. John D. Mahan,* Susan P. Sisson,* and Robert L. Vernier. Univ. of Mn., Dept. of Ped., Mpls., Mn.

A decrease in the number of stainable anionic sites in the lamina rara externa (LRE) occurs in aminonucleoside nephrosis in the rat and in the congenital nephrotic syndrome (GNS) in children. By routine EM, GBM appears normal in patients with MCNS, and similar alterations in anionic sites could explain the charge selective abnormalities in glomerular permeability seen in this disorder.

Using an *in vitro* method, kidney sections from 5 proteinuric steroid responsive MCNS patients and 5 normal individuals were examined after overnight incubation in polyethelenimime (M.W. 1200, pH 7.4). Random capillary loops were photographed (55,000 X) from 17 MCNS and 19 normal glomeruli and the distribution of stained sites calculated by grid-point method. Preliminary studies demonstrated normal LRE sites (22.6 ± 1.6/1000 nm GBM) but a less prominent lamina rara interna (LRI) network in MCNS. Quantitatively, anionic sites in MCNS occupied 14.1% and in normals 13.0% of GBM LRE volume (NS). LRI anionic sites in MCNS, however, were less common, 6.1%, than in normals, 9.45% ($p < 0.05$).

In contrast to CNS, LRE anionic sites in MCNS are normal. This study demonstrates a 35% decrease in LRI anionic sites in MCNS. These observations are consistent with the marked difference in the natural history and therapeutic responses seen in these two disorders. We propose that in MCNS the decrease in stainable LRI anionic sites may be critical to altered glomerular permeability and be a consequence of soluble factors accessible to this region from the circulation.

NEPHROTOXIC SERUM GLOMERULONEPHRITIS INDUCED IN THE RABBIT BY ANTI-ENDOTHELIAL ANTIBODIES. Seiichi Matsuo*, Peter Caldwell*, Jan Brentjens* and Giuseppe Andres. State University of New York at Buffalo, Buffalo, NY, and College of Physicians and Surgeons, Columbia University, New York, NY.

Previous experimental studies have shown that the basement membrane and the visceral epithelium of renal glomeruli can be damaged by heterologous antibodies. This study was designed to test the hypothesis that the glomerular endothelium (GE) can be the target of a similar pathogenetic mechanism. Goat anti-rabbit lung angiotensin-converting enzyme antibodies (GtARbACE), morphological, immunocytochemical and quantitative radioisotope techniques were used. Angiotensin-converting enzyme (ACE) was detectable by indirect immunofluorescence in the pulmonary but not the GE. GtARbACE IgG were perfused through isolated kidneys or injected into the abdominal aorta or intravenously in rabbits untreated or treated with captopril. The results show that: 1) GtARbACE IgG specifically bind to GE of perfused kidneys, and the binding is twofold increased in rabbits treated with captopril; 2) after intravascular injection, GtARbACE IgG bind to the palsma membrane of GE forming small "patches" that are not observed after Fab injection; 3) inflammatory changes occur during the heterologous phase, and after 8 days deposits of rabbit IgG and C3 are present in glomerular capillary wall; and 4) the lesions are most severe in rabbits treated with captopril. The results indicate that the GE can be the target of injury induced by a nephrotoxic serum, especially when an increased expression of plasma membrane ACE is induced by captopril.

L3T4$^+$ HELPER T CELLS FROM MICE WITH TUBULOINTERSTITIAL NEPHRITIS (TIN) INDUCE NEPHRITOGENIC AND DISEASE PRODUCING Lyt 2$^+$ EFFECTOR T CELLS IN VITRO. R. Mann*, M. Clayman*, and E. Neilson, Renal Section, Univ. of Penn., Phila., PA

We have previously reported that an L3T4$^+$, I-A restricted, antigen-specific helper T cell line could adoptively transfer TIN (αTBM disease). We now report that this cell line (RTA-2^S), or a soluble factor from its culture supernatant, when placed in culture for 5 days with renal tubular antigen (SRTA), macrophages, IL-2, and naive syngeneic spleen cells will induce an Lyt 2$^+$ cell from Lyt 1$^+$, 2$^+$ precursors. The induced Lyt 2$^+$ cell has effector function in delayed-type hypersensitivity (DTH) to SRTA (footpad swelling DTH to SRTA = 19.0 ± 2.0 X 10^{-3} inches vs. 4.3 ± 0.9 in controls vs. 3.3 ± 0.3 with soluble liver antigen) and causes TIN within 5 days when placed under the kidney capsule (histologic severity 2.0 ± 0.0 vs. 0.2 ± 0.1 in controls; $P < 0.001$). Removal of suppression by pretreatment of precursor spleen cells with αI-J^{S+} C' permits the induction of the Lyt 2$^+$ DTH effector cell as well as an L3T4$^+$ cell sharing the functional and phenotypic characteristics of effector cells in non-susceptible strains. These L3T4$^+$ cells are DTH-reactive (DTH to SRTA = 18.3 ± 0.3 X 10^{-3} inches vs. 3.8 ± 0.3 in controls), but incapable of intrarenal effector function (histologic severity 0.3 ± 0.3 vs. 0.2 ± 0.1 in controls).

Finally, both L3T4$^+$ and Lyt 2$^+$ cells can be harvested from the lesions of TIN. Only the Lyt 2$^+$ cell has effector function for DTH to SRTA. The L3T4$^+$ renal lymphocyte, placed in culture with naive spleen cells, will induce an Lyt 2$^+$ DTH effector cell (DTH to SRTA = 16.7 ± 0.3 X 10^{-3} inches vs. 3.7 ± 0.3 in controls). In summary precursor cells in disease susceptible mice are pluripotential, but T cell help and I-J$^+$ suppression induces a nephritogenic effector cell not found in non-susceptible mice.

INFLUENCE OF PGE_2 TREATMENT OF MURINE IMMUNE COMPLEX GLOMERULONEPHRITIS ON HUMORAL AND CELLULAR IMMUNE RESPONSE. KR McLeish, GT Stelzer,* and JH Wallace,* Depts. of Medicine and Microbiology and Immunology, University of Louisville, Louisville, KY.

To identify the mechanism by which prostaglandin E_2 (PGE_2) inhibits the development of apoferritin (HAF)-induced immune complex glomerulonephritis, serial determinations of glomerular histology, BUN, total immunoglobulin levels, anti-HAF IgG, peripheral blood T-cell subsets, and splenic suppressor cell activity were compared between mice receiving HAF and mice additionally treated with PGE_2. Mice receiving HAF alone developed marked glomerular hypercellularity and a significantly increased BUN at the third and forth weeks, while PGE_2 treated mice maintained normal histology and BUN levels. The inhibition of glomerular damage by PGE_2 was associated with a reduction in anti-HAF IgG. No alterations in total IgM or IgG subclasses occurred. Mice receiving HAF alone demonstrated serial reductions in both peripheral blood pan-T-cells and suppressor/cytotoxic T-cells identified phenotypically. Non-specific suppressor cell activity, measured by the ability of spleen cells from experimental mice to suppress either a mixed lymphocyte reaction (MLR) or MLR-induced polyclonal IgG synthesis, was not different between the two groups. These studies suggest that changes in specific antibody levels are not caused by an inhibition of antibody subclass production. Since no evidence for increased suppressor cell activity was found, down regulation of specific antibody may result from normal homeostatic mechanisms produced by the maintenance of normal peripheral blood T-cell subsets.

Fc RECEPTOR (Fc-R) AND C3b RECEPTOR (C3b-R) MEDIATED BINDING AND DEGRADATION OF IMMUNOGLOBULIN AGGREGATES BY U937 CELLS. R.L. Mehta,* H. Takahashi,* and D.W. Knutson. Univ. of Rochester Med. Ctr., Dept. of Med., Rochester, New York.

Mononuclear phagocytes are known to clear soluble immune complexes from the circulation by means of Fc-R and receptors for fragments of the third component of complement (C3b-R; C3bi-R). We studied Fc-R and C3b-R function using a human monocyte cell line, U937. C3b was incorporated into stable soluble heat aggregates of $IgM^{125}I$ (A-IgM) and $IgG^{125}I$ (A-IgG) using purified classical pathway components (up to 40 C3b molecules per aggregate). C3b incorporation was verified by the ability of aggregates to bind to human red cells, by co-sedimentation of ^{125}I and ^{131}I during ultracentrifugation, and by autoradiography of SDS-PAGE preparations. Cell uptake and degradation of A-IgG·C3b was up to two-fold greater than A-IgG. In contrast, A-IgM·C3b was bound but not degraded by U937 cells. (Uptake of A-IgM without C3b was negligible). C3b-R mediated binding was specifically inhibited both by a murine monoclonal antibody against the human C3b-R and by C3b oligomers generated by trypsin activation of C3. Low ionic strength caused marked increases in both Fc-R and C3b-R mediated binding but did not abrogate inhibition by C3b oligomers or anti C3b-R antibodies. We conclude that C3b receptor mediated binding enhances uptake and thus increases endocytosis and catabolism of immune complexes by U937 cells. However, C3b binding, even when multivalent, does not itself lead to endocytosis and degradation of ligands bearing C3b.

COLCHICINE REDUCES PROTEINURIA IN PASSIVE HEYMANN NEPHRITIS (PHN). L.S. Milner, D. Lotan, P.R. Goodyer, J.S.C. Fong and B.S. Kaplan, The Renal Lab.,Montreal Child. Hosp.,Montreal,P.Q., Canada. The effect of colchicine(C) treatment was studied in the heterologous and autologous phase of PHN rats. In each study the dose of C was 0.06mg I.P./rat/d x 14 d. Rats given C had reduced urine protein and albumin excretions compared to controls: protein, 94±49mg/d vs 239±44mg/d, $p < 0.05$;albumin, 54±30mg/d vs 200±38mg/d, $p < 0.05$. The urine albumin:creatinine ratio was 8±4 vs 29.8±6, $p < 0.02$. C treatment after PHN was well established did not reduce urine protein or albumin excretion: protein, 178±40mg/d vs 249±85mg/d, $p > 0.1$; albumin, 130±40 mg/d vs 249±85mg/d, $p > 0.5$. The effect of C was abolished by concomitant administration of indomethacin so that urine protein and albumin excretion were similar in experimental and control rats:144±43mg/d vs 249±86mg/d, $p > 0.05$; albumin, 108±35mg/d vs 130±43mg/d, $p > 0.05$. C treated rats had decreased GFRs compared to controls; 3.8±43ml/min vs 2.4 ±0.2ml/min, $p < 0.01$;rats given indomethacin and C had an even greater reduction in GFR; 1.26±0.3ml/min vs 3.8±0.4ml/min, $p < 0.001$. Serum cholesterol was reduced in C treated rats, 69±16mg/dl, compared with controls: 132±92mg/dl, $p < 0.05$;no significant reduction in cholesterol occurred in rats treated with C and indomethacin: 96±16mg/dl vs 132 ±92 mg/dl, $p > 0.05$.

Administration of C to rats with PHN prior to the onset of the autologous phase of the disease resulted in decreased proteinuria and albuminuria possibly as a result of endogenous stimulation of intrarenal prostaglandins.

ANTIBODIES TO HEPARAN SULFATE PROTEOGLYCANS (ANTI-HSPG) BIND TO THE LAMINA RARAE OF THE GBM AND INDUCE PROTEINURIA AND GBM THICKENING. Aaro Miettinen, Jennifer L. Stow and Marilyn Gist Farquhar. Dept. of Cell Biology, Yale University School of Medicine, New Haven, CT.

A rabbit antiserum was raised against purified glomerular proteoglycans which specifically recognizes basement membrane (BM) HSPG: it immunoprecipitates only HSPG and by indirect immunofluorescence (IF) or immunoperoxidase (IP) stains only BM's -- i.e., the GBM, TBM, pericapillary BM, and Bowman's capsule (Stow and Farquhar, "in press"). Anti-HSPG IgG (3-20 mg) was injected IV into rats, and the distribution of rabbit IgG was determined 15 min to 19 days thereafter by IF and IP. Rabbit IgG was detected in the mesangial matrix and in the GBM where it bound to both laminae rarae (LRI and LRE) at all time points. At 15 min it was more concentrated in the LRI. At 1-4 days it was equally concentrated in both laminae rarae, giving the GBM a tram-track appearance. By 15 days it was found predominantly in the LRE, and the GBM was irregularly thickened on its epithelial aspect. Other findings were binding of C3 and adherence of PMNs to the GBM up to 4 days, and a gradual increase in mesangial deposits up to 4 days, with a gradual decrease thereafter. Rats given immune IgG showed mildly increased albuminuria by radial immunodiffusion.

The results show that 1) heterologous anti-HSPG IgG binds to the laminae rarae of rat GBM in vivo -- a distribution which corresponds to that of the heparan sulfate-rich anionic sites detected previously with cationic probes, and 2) binding of anti-HSPG induces thickening of the GBM which implies an effect on the biosynthesis or degradation of the GBM.

CHARGE RESTRICTION OF MESANGIAL IgA IN PRIMARY IgA NEPHROPATHY. R.C. Monteiro,* L.H. Noël,* L. Halbwachs-Mecarelli,* J. Berger,* and P. Lesavre* (Intr. by G. Hill) INSERM U25, Hôpital Necker, Paris, France.

We previously demonstrated that approximately 70% of the eluted IgA's have a polymeric nature in IgA nephropathy (Contr.Nephrol.40:107,1984).In order to characterize the charge of mesangial IgA, we studied 5 percutaneous renal biopsy eluates from patients with IgA nephropathy. The acid eluates were obtained from 1248±598 glomerular sections (range 800-2300). After 3 washes, the sections were incubated for 4 h at 37°C in 200 µl of 0.02 M citrate buffer, pH 3.2, centrifuged and the eluates neutralized. Citric acid-treated eluates, purified serum IgA and NHS were analyzed by isoelectric focusing (IEF) on agarose. The agarose gel was cut into 0.5 x 1cm slices and incubated overnight at 4°C in neutral buffer, containing 1% ovalbumin and 0.05% Nonidet P40. Anodic and cathodic fractions were neutralized to pH 7.0. IgA content of the IEF fractions was measured by radioimmunoassay (RIA). The sensitivity of this IgA-RIA was 0.02ng. The isoelectric point (pI) of eluted IgA ranged from 4.5 to 5.6 contrasting with the broader and more neutral pI of purified serum IgA and NHS (4.5 to 6.8). For all eluates studied, a basic IgA peak was observed with pI between 7.8 and 8.0, but the main peak consistently corresponded to the acidic IgA peak. In conclusion, three conditions may favor mesangial IgA deposition in IgA nephropathy: 1) the multimeric nature of IgA; 2) immune complex formation as previously described and 3) as shown in the present study the anionic charge of IgA.

ANALYSIS OF A SUPPRESSOR T CELL (Ts) NETWORK THAT INHIBITS αTBM DISEASE PRODUCING TUBULOINTERSTITIAL NEPHRITIS (TIN) IN MICE. E. Neilson, R. Mann*, C. Kelly*, and M. Clayman*, U. of P., Phila., PA.

We have previously reported that SJL mice primed with tubular antigen (SRTA)-derivatized lymphocytes develop Ts-1 cells which can inhibit the development of TIN (αTBM disease) if transferred at the time of disease induction. This Ts-1 cell is $L3T4^+$, $I\text{-}J^+$, $RE\text{-}Id^+$ (idiotype), and antigen-binding. We now report that Ts-1 cells induce Lyt 2^+, $I\text{-}J^+$, and RE-Id-binding (anti-idiotypic) Ts-2 cells. Ts-2 cells will prevent the development of TIN when transferred 7 days after recipients have been immunized to produce αTBM disease (histologic severity; Ts-2 = 0.9 ± 0.4 vs. 3.2 ± 0.4 in Controls; $P < 0.001$). This protective effect was abrogated by the pretreatment of Ts-2 cells with αThy 1.2 or $\alpha I\text{-}J^s$. The fine specificity of these Ts-2 cells were further analyzed using delayed-type hypersensitivity (DTH) to SRTA in mice immunized to produce disease. Ts-2 cells are only effective after immunization (DTH to SRTA = 19.6 ± 1.9 X 10^{-3} inches on day 0 vs. 4.3 ± 1.2 on day 7; $P < 0.001$). The suppression is SRTA-specific (Ts-2 SRTA = 4.8 ± 1.4 vs. PPD = 18.0 ± 1.7 vs. Control SRTA = 17.0 ± 1.3; $P<0.001$). Ts-2 suppression is Igh-V restricted as BALB/c Ts-2 cells transfer suppression into BALB/c (V^a/C^a) = 5.8 ± 0.8, and BAB/14 (V^a/C^b) mice = 6.0± 1.4, but not into C.B-20(V^b/C^b) = 17.8 ± 1.0. Ts-2 suppression is also I-J restricted as SJL($I\text{-}J^s$) Ts-2 cells transfer suppression into B10.S($I\text{-}J^s$) = 5.5 ± 1.0 and B10.HTT($I\text{-}J^s$) mice = 5.8 ± 0.9, but not into B10.S(9R) ($I\text{-}J^k$) = 17.3 ± 1.1. Finally, Ts-2 suppression is only induced in donor mice primed with Ts-1 + SRTA = 7.5 ± 0.9 vs. Ts-1 + SLA = 21.3 ± 1.7 vs. Control + SRTA = 23.8 ± 2.1. In summary, we have observed that complementary interactions within an inducible Ts network can be therapeutically effective in the treatment of TIN.

STUDY OF COLLAGENOUS AND NONCOLLAGENOUS BASEMENT MEMBRANE COMPONENTS IN NON-AMYLOID LIGHT CHAIN DISEASE (LCD). L.H. Noël,* D. Droz,* and J. M. Froidart* (intr. by G.S. Hill), Dept of Nephrol., Hôpital Necker, Paris, France and Hôp. de Bavière, Liège, Belgium.

Renal and liver biopsies from 4 patients with kappa LCD were studied by indirect immunofluorescence (IF) with polyclonal antisera directed against Types I, III, IV, and V collagen, laminin and fibronectin. By light microscopy, all the 4 patients had renal nodular glomerulosclerosis and thickened tubular basement membranes (TBM); by IF kappa light chain deposits were present in glomeruli and along the TBM. In the glomerular nodules, type I collagen was present in 2 cases corresponding to the observation of characteristic striation of collagen fibers by electron microscopy; type V collagen was present once. Type III collagen and laminin were not detected in nodules. Conversely, fibronectin and type IV collagen were always demonstrated in mesangial nodules, along the glomerular basement membranes and TBM. In liver specimens, kappa light chain deposits were present along the sinusoids. In 3 cases, type I collagen was detectable in sinusoidal spaces; type III collagen was detectable once. Type V collagen was never present. Type IV collagen was always present along the sinusoids.

In renal and liver biopsies, type IV collagen and light chain deposits have exactly the same localization. That would suggest that the light chains deposited in the tissues have a peculiar affinity for type IV collagen.

APPLICATION OF MONOCLONAL ANTIBODIES TO THE STUDY ON THE MECHANISMS OF PUROMYCIN AMINONUCLEOSIDE (PAN) NEPHROSIS. Tadahiro Nishi*, Kazuo Nosaka*, Shoji Kuwata*, Hiroshi Kawasaki* and Hitoshi Endou* (Intr.by Dr.Yasuhiko Iino).Dept.of Medicine and Pharmacology, University of Tokyo,Tokyo, Japan

Two monoclonal antibodies(C3,D9),which have been made against rat glomerulus by the hybridoma technique in our laboratory, were applied to the study on mechanisms of PAN nephrosis. Both antibodies bind to the glomerular epitheliar cell surface on immunoelectron microscope. Nephrosis was induced in Sprague-Dawley rats (150-200g) by a single i.v.injection of PAN (150mg/kg). Massive proteinuria occured by day 5 and persisted for 2-3 weeks,then declined. The kidneys were removed by unilateral nephrectomy or sacrifice on day 3,7,14 and at week 5 and stained for C3 and D9 by immunofluorescence. Compared to the control, there was marked reduction of staining for C3 on day 3,7 and less reduction on day 14, whereas at week 5 the staining recovered to the normal level. Staining for D9 did not change at any stage of PAN rats. By enzyme linked immunosorbent assay,the antigens reactive to C3 and D9 in the urine and sera of PAN rats were measured sequentially. C3 reactive antigen increased in the urine of PAN rats on day 3 and 5 and returned to the control level after day 7, while D9 reactive antigen did not show any significant change. In conclusion,the reduction of a monoclonal antibody-defined podocyte antigen at the glomeruli and it's increase in excretion in urine were observed in PAN rats,indicating that detachment and loss in the urine of this antigen may be associated with the mechanism of proteinuria of PAN nephrosis.

ASSOCIATION OF LOCALIZATION OF POLY C9 WITH PROTEINURIA AND LOCAL LOSS OF ANIONIC SITES IN THE GLOMERULAR BASEMENT MEMBRANE (GBM) IN ACUTE SERUM SICKNESS NEPHRITIS. G. Parra,* Y. Takekoshi,* R.L. Vernier, Dept. of Pediatrics, Univ. of Minnesota, Minneapolis, Minnesota.

Acute immune complex disease was induced in New Zealand White (NZW) and C6 deficient (C6D) rabbits by a single intravenous injection of bovine serum albumin (BSA). Multiple biopsies from 6 NZW and 5 C6D rabbits were compared by immunofluorescence (IF) and immunoperoxidase microscopy (IEM) for localization of IgG, C3, Poly C9 and BSA. The quantitative distribution of anionic charge sites in the lamina rara externa (LRE) and interna (LRI) were evaluated by in vitro localization of polyethyleneimine (New Engl J Med 309:1001, 1983). By IF and IEM in NZW rabbits, IgG, C3 and BSA were distributed diffusely in both small subepithelial (SE) deposits and in large SE "humps". In contrast Poly C9 was present only in large SE "humps" found only in NZW rabbits. In 3 C6D rabbits IgG and C3 were present in small SE deposits; Poly C9 and BSA were negative. Anionic charge sites in baseline biopsies were identically distributed in the LRE of NZW and C6D rabbits: 26/1000 nm GBM length. Proteinuria occurred only in NZW rabbits with large SE "humps". Anionic sites were decreased (7/1000 nm GBM) only at the base of the humps and were normal in all other regions of the GBM even with proteinuria. These data suggest that formation of Poly C9, the membrane attack complex of complement, within SE "humps" results in local alteration of GBM permeability and proteinuria in this model.

EFFECT OF DR MATCHING ON THE CELLULAR IMMUNE RESPONSE OF RENAL TRANSPLANT RECIPIENTS. K.J. Pennline,* S. White,* M.C. Gelfand, and G.B. Helfrich.* Georgetown Univ. Med. Ctr., Dept. of Pathol., Dept. of Nephrol., and Dept. of Surg., Div. of Transplant., Washington, D.C.

Analysis at the time of transplantation indicated that the magnitude of the relative response (RR) in the mixed lymphocyte reaction was affected by the donor-recipient DR match. Transplant recipients with 2,1 and 0 DR matched donor kidneys exhibited RRs of 15%, 10-87% respectively. T-cell subset analysis on peripheral blood lymphocytes to assess T4 (helper/inducer): T8 (cytotoxic/suppressor) ratios were performed twice a week post transplant. The results indicated that mean T4/T8 ratios of 2 DR matched recipients remained within the normal range (1.70-2.25) post transplant. In contrast, successful transplantation in 1 and 0 DR matched recipients was always indicated by a drop in the mean T4/T8 to 1.35 and 1.10 respectively. Prior to and during rejection episodes, a significant rise in the ratio to 2.00 and above was apparent and appeared to reflect a T4 activation stage. Anti-rejection therapy initiated during this stage had a high reversal rate and resulted in a return of the T4/T8 ratio to 1.00. Rejection was not reversed in recipients entering therapy with low T4/T8 ratios (0.9-1.1) and appeared to be in a stage of irreversible rejection with high T8 numbers (cytotoxic T8). We are currently attempting to correlate these changes with gamma interferon production. These results suggest that DR matching influences the change in T4/T8 ratios and that T-cell monitoring may be significant in staging graft rejection and anti-rejection therapy.

EFFECT OF ANTIGENIC CHARGE ON IMMUNE COMPLEX LOCALIZATION AND EICOSANOID PRODUCTION BY RAT GLOMERULI. M.A. Rahman*, S.N. Emancipator and M.J. Dunn. Case Western Reserve Univ. and University Hospitals Dept. of Medicine and Pathology, Cleveland, Ohio.

We studied the effect of cationic antigen on glomerular arachidonic acid (AA) metabolites and immune complex (IC) localization in rat chronic IC glomerulonephritis. We hypothesized that different AA metabolites might play a role in the hemodynamic alterations and pathogenesis of the disease. Rats were divided into 3 groups; control, native bovine gamma globulin (NBGG) and cationic bovine gamma globulin (CBGG). The latter two received 10 mg I.V. of the respective antigen daily x 21 d. Five to 7 days after the last injection we measured inulin (GFR) and PAH (RPF) clearances. Glomerular synthesis of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2) were measured by RIA.

	GFR	RPF[1]	TxB_2[2]	PGE_2[2]
Control	2.15±.25	5.54±.56	.48±.74	1.04±.16
NBGG	2.46±.66	6.05±.91	1.04±.39	1.19±.37
CBGG	2.39±.25	8.37±.90[3]	3.12±.50[4]	2.23±.37[5]

[1]ml/min [2]ng/mg glom. dry wt. [3,4,5]$p<.05$, .001, .01

Only the CBGG rats developed proteinuria, 86.6±18 mg/24 h which correlated with TxB_2 synthesis, R= 0.67, p=0.01. Immunofluorescent microscopy showed mesangial deposits of IC in the NBGG group and capillary wall deposits in the CBGG group. Electron microscopy showed subepithelial deposits and fusion of foot processes in CBGG, but not cellular infiltrate. We conclude that cationic antigen induces a glomerular disease similar to membrane nephropathy with concomitant increases of PGE_2 and TxA_2. The increased RPF is probably due to increased PGE_2. Whether TxA_2 has a role in the pathogenesis is undetermined.

ALTERATIONS IN THE GENERALIZED SCHWARTZMAN REACTION (GSR) INDUCED BY CYCLOSPORINE (CY). Craig Porter*, G.H. Bock, B.A. Fivush*, S.P. Kapur*, C.R. Smith*, N.L.C. Luban*, K. Marinelli*. Depts of Nephrol., Pathol. and Lab. Med. Child. Hosp. Nat. Med. Ctr., Washington, D.C.

Hemolytic-uremic syndrome (HUS) has occurred in CY-treated recipients of bone marrow and renal allografts. In order to investigate the possible role of CY on the evolution of HUS-like renal disease, the GSR was studied in the term pregnant rat. Control and experimental rats received endotoxin, 1.1 mg/kg i.v. on day 20 of pregnancy. Experimental rats were pretreated with therapeutic CY doses on days 16 to 20 of pregnancy. A histological grading system, platelet counts and plasma fibrinogen levels were used to measure outcome.

The incidence of renal GSR in control vs. CY-treated animals was 80% and 20% respectively. Further, the degree of glomerular involvement in animals with GSR was significantly less ($p < .05$) in CY animals when compared to controls. However, mortality was 20% in control vs. 43% in CY animals. Decreased platelet counts correlated ($p < .01$) with mortality but not with CY treatment. Brain, liver and lung histology failed to explain these paradoxical findings.

Previous studies have demonstrated inhibition of in vitro prostacyclin production by CY. Our studies demonstrate that CY treatment attenuates rather than potentiates the renal lesion of the GSR. This suggests the possibility of heretofore undefined interactions of CY with prostaglandins, vascular endothelium or both.

OXYGEN RADICAL (O_2) INDUCED PROTEINURIA IN RATS FOLLOWING INTRAVASCULAR ACTIVATION OF COMPLEMENT. Ahmed Kehan*, Kent J. Johnson* and Roger C. Wiggins. Depts. of Internal Med. and Path., Univ. of Michigan, Ann Arbor, MI 48109.

The complement system was activated by infusion of 40 units of cobra venom factor (CVF) into the left renal artery of 250-300 gm male Sprague-Dawley rats. Urine collected over the first 24 hours after the infusion of CVF contained significantly more protein than saline injected controls (51.2 ± 5.8 mg/24 hr in CVF injected vs 9.0 ± 0.9 mg/24 hr in saline injected). This proteinuria occurred in a dose-dependent fashion and was also neutrophil-dependent.

Polyethylene glycol-coupled catalase (1000 units given i.v. 15 min before) CVF injection suppressed the proteinuria (23.7 ± 3.7 mg/24 hr in CVF + catalase vs 52.1 ± 1 mg/24 hr in CVF alone, P less than 0.05), suggesting that H_2O_2 and its metabolites are important mediators of proteinuria in this model. In contrast, superoxide dismutase (SOD) (40 mg injected i.v. before) did not prevent proteinuria (52.4 ± 15.9 mg/24 hr in SOD + CVF vs 54.7 ± 10.6 mg/24 hr in CVF alone) suggesting that superoxide anion was not the important O_2 metabolite involved. Similarly, Dimethyl sulfoxide[2](DMSO) (1 ml given 10 min before and 0.5 ml 15 min and 30 min after CVF) did not prevent proteinuria (58.9 ± 5.5 mg/24 hr in DMSO + CVF vs 54.7 ± 10.6 mg/24 hr in CVF alone) suggesting that the hydroxyl radical is not an important O_2 metabolite involved.

These studies suggest that H_2O_2 is capable of causing proteinuria following intravascular activation of complement.

IgA MOLECULAR FORM-NEPHRITOGENICITY CORRELATES: IMMUNE COMPLEX FORMATION AND GLOMERULAR DEPOSITION.
Abdalla Rifai*, Keith Millard* and Regina Verani. University of Texas Medical School, Houston, Texas.

Clearance kinetics and renal deposition of soluble IgA immune complexes (IgA-IC) were examined to determine the nephritogenic potential of IgA molecular form in mediating experimental IgA nephropathy. The immune complexes were prepared by mixing purified radiolabeled monomeric (mIgA) or polymeric (pIgA) IgA anti-dinitrophenyl (DNP) derived from MOPC 315 myeloma, with DNP-conjugated Ficoll. Clearance of IgA-IC from the circulation was curve-fitted by two exponential components. The first component was similar for both mIgA-IC and pIgA-IC. The second component was slightly more rapid for IgA-IC than for pIgA-IC. Immunofluorenscence studies, however, showed that only pIgA-IC deposited in the kidneys. Examination of renal tissues by electron microscopy revealed electron dense deposits in the gomeruli of mice that received pIgA-IC. Analysis of IgA-IC by gradient polyacrylamide gel electrophoresis indicated that mIgA formed only small-latticed complexes. The critical role of IgA-IC lattice-size in renal deposition was confirmed by demonstrating that large-latticed mIgA-IC, prepared by covalent cross-linking mIgA with a specific affinity-labeling antigen, deposited in the kidneys in a pattern similar to pIgA-IC. These findings suggest that only large-latticed IgA-IC are nephritogenic. Multivalent pIgA is nephritogenic because it forms predominantly large-latticed complexes. In contrast, monovalent mIgA forms only small-latticed complexes that persists in circulation without glomerular deposition.

IN VIVO LOCALIZATION OF MONOCLONAL (Mab) ANTI GP 330 ANTIBODIES : HETEROGENEITY OF THE ANTIGEN ANTIBODY SYSTEM.
By P. Ronco*, B. Baudoin*, M.Brunisholz*, P. Verroust*, INSERM U64 Hopital Tenon Paris, France. (Intr. by R. R. Robinson).

The factors controlling deposition of anti bodies in the glomerular subepithelial space are poorly defined. We have produced 3 Mab reactive with gp330 -a brush border (BB) antigen present on glomerular epithelial cells- and describe their in vitro and in vivo binding properties. Mab 12, Mab 28 and Mab 237, are IgG. They have similar isoelectric points, and are able to bind a 330 KD protein, as shown by immunoprecipitation analysis of radiolabelled (BB) and binding studies on affinity purified material. The binding affinities, estimated by adding increasing amounts of radiolabelled antibodies to solid phase BB are comparable for the 3 Mab . However, the epitopes identified are distinct, as demonstrated by cross inhibition studies : binding of a given labelled Mab to solid phase BB was only inhibited by the homologous antibody. In vivo binding was demonstrable 2 hours after intravenous injection of Mab 12 or Mab 28 and remained stable for 72 hours. Using paired label techniques, the amount of Mab 28 bound was significantly greater. No binding could be demonstrated using Mab 237. Since size, charge, and affinity of the 3 Mab are similar, we suggest that their differential in vivo behaviour is related to their fine specificity. This factor should be taken in account in studies attempting to demonstrate the role of physico chemical factors in glomerular localization of antibodies. This antigenic analysis may be also of value to understand membrane insertion, and function, of gp 330.

GLOMERULAR IgG SUBGROUP DISTRIBUTION IN MEMBRANOUS (MGN) AND PROLIFERATIVE (PGN) GLOMERULONEPHRITIS.
Michael T. Rissell*, Melvin M. Schwartz, and Edmund J. Lewis, Rush Medical College, Departments of Medicine and Pathology, Chicago, IL.

Immune complex mediated MGN and PGN may have comparable amounts of glomerular IgG but there is a paradoxical absence of glomerular inflammation and hypocomplementemia in MGN. These differences may result from the varying complement (C) fixing ability and the differential glomerular localization of the IgG subgroups. By indirect immunofluorescence microscopy using monoclonal subgroup specific antisera and the biotin-avidin system we semiquantitated the glomerular IgG subgroup distribution in 4 groups of patients: (1) systemic lupus erythematosus (SLE) MGN (n=7), (2) SLE PGN (n=11), (3) non-SLE MGN (n=10), and (4) non-SLE PGN (n=5). In all groups the total IgG deposition was similar. In SLE the C-fixing subgroups were greater in PGN (IgG2>IgG1>IgG3) while the non-C-fixing subgroup IgG4 was most prominent in 5 MGN biopsies. In non-SLE PGN IgG3 was dominant while IgG4 was virtually absent. The remaining subgroups were greater in MGN (IgG1>IgG4>IgG2) with IgG4 the most abundant subgroup in 4 biopsies. In light of the presence of C-fixing subgroups, the large relative increase of IgG4 in both SLE and non-SLE MGN of this subgroup may determine glomerular reactions. Increases of this non-C-fixing subgroup in immune aggregates could cause steric hindrance to the requisite multivalent binding and thus activation of C1q by the C-fixing subclasses. Thus, the increased glomerular deposition of IgG4 in patients with SLE and non-SLE MGN could account for the relative absence of hypocomplementemia and inflammation in these patients.

IMMUNOPATHOLOGICAL SIGNIFICANCE OF A GLOMERULAR EXPRESSED BRUSH BORDER RELATED ANTIGEN (BBRA).
P. Ronco*, V. Gay-Bellile*, A. Bernard* and P. Verroust* INSERM U 64, Hop. Tenon, Paris, and Laboratoire d'Immunologie, Villejuif. France (Introd. by C. Le Grimelec.)

We previously reported a monoclonal antibody (Mab) directed against a 90 KD protein present on rat brush border (BB) and glomerular epithelial cells which induces in vivo transient glomerular deposits. We have since observed identical kinetics with polyclonal antisera of the same specificity. This work shows that a similar system can be found in the rabbit and probably in man. Mab85, one of 11 Mab produced against rabbit BB, is specific for a protein of molecular weight(MW) slightly <90KD and binds in vitro to BB and glomeruli in a similar pattern. When injected intravenously in rabbits, it induces glomerular deposits, maximum 4 hours after I.V., which decrease to undetectable levels within 6 days. Mab99, a Mab raised against human BB binds a protein of identical MW and displays a similar binding pattern in vitro . It is specific for the same BB protein as J5 -a Mab to common acute lymphoblastic leukemia antigen (Calla)- and binds a significant percentage of acute leukemic cells. This observation prompted us to analyse rat lymphoid cells for BBRA. Using FACS, Mab8 as well as Mab anti gp330, bind antigens present on a significant proportion of peripheral lymphocytes and bone marrow cells. In conclusion:1. Binding kinetics described in the rat can be observed in other species.2.The antigen involved is related to surface antigens of lymphoid cells and shows analogy to Calla.3. This system may be relevant to short term humam membranous glomerulonephritis

SOLUBLE IMMUNE RESPONSE SUPPRESSOR(SIRS) IN URINES OF NEPHROTIC PATIENTS. H. William Schnaper* and Thomas M. Aune* (intro. by Barbara R. Cole), Jewish Hospital of St. Louis, Washington University School of Medicine, St. Louis, MO.

Patients with nephrotic syndrome (NS) frequently have suppressed immune responses of uncertain origin. Because enhanced suppressor cell activity has been reported in these patients, we evaluated urines from children with NS for presence of the lymphokine, SIRS, a product of concanavalin A- or interferon-activated suppressor T lymphocytes. SIRS suppresses in vitro antibody production as measured by the plaque-forming cell assay. Dialyzed urine from patients with minimal change NS in relapse similarly suppressed plaque formation by lymphocytes. Suppressive activity was identical to that of human SIRS by the following functional and physical criteria: 1) molecular weight estimated by gel filtration; 2) kinetics of suppression; 3) activation by H_2O_2; 4) inhibition of suppression by catalase, levamisole, or 2-mercaptoethanol; 5) elution pattern on HPLC; and 6) cross-reactivity with monoclonal anti-murine SIRS antibodies. SIRS excretion ceased after initiation of therapy but before clinical response. We tested 11 patients with minimal change disease, 2 nephrotic patients with acute glomerulonephritis and 2 with NS from membranoproliferative glomerulonephritis. All excreted SIRS, whereas patients with focal glomerulosclerosis or congenital NS, and those with proteinuria without NS (as well as normal controls) did not. Identification of SIRS excretion by certain nephrotic children represents the first association of this lymphokine with a disease state; its relationship to the immune suppression in NS remains to be determined.

FOCAL SEGMENTAL GLOMERULAR SCLEROSIS (FSG): THE EARLY LESION. Melvin M. Schwartz and Edmund J. Lewis. Rush Medical College, Departments of Pathology and Nephrology, Chicago, Illinois 60612

When FSG is found early in the course of the nephrotic syndrome (NS), it is a bad prognostic sign, but it is a non-specific glomerular response to injury which provides no insight into the the pathogenesis of the scarring. We studied renal biopsies from 60 patients with FSG by light, fluorescence and electron microscopy to determine whether a characteristic lesion could be identified to precede the segmental scar. The pathologic features were correlated with the interval between the onset of proteinuria and/or the NS and clinical and laboratory features present at the time of biopsy. Forty biopsies had only focal segmental glomerular scarring (Classic FSG). In 20 biopsies a significant and readily identifiable lesion of glomerular visceral epithelial cells was superimposed upon classic FSG. The epithelial lesion consisted of proliferation, cytoplasmic swelling and vacuolization, and cell necrosis (Active FSG). The other morphologic parameters were similar between the two groups. 19/40 with classic FSG and all the patients with active FSG had proteinuria $\geq$ 3.0g/d. Active FSG had a shorter interval between onset of proteinuria and biopsy than classic FSG (3.4 $\pm$ 3 vs. 34 $\pm$ 36 months, $p < .01$). The serum creatinine, diastolic blood pressure, and level of protein excretion were not different. These results suggest that 1) severe, segmental epithelial cell injury is the initial event in the pathogenesis of FSG, 2) the resulting epithelial cell reaction precedes the development of the classic segmental scar, and 3) the consistent finding of visceral epithelial cell pathology early in the course of FSG with the NS may be a clue to the pathogenesis of the classic sclerotic lesion.

THE MODULATION OF GLOMERULAR ANGIOTENSIN II (AII) RECEPTORS (R) DURING THE COURSE OF ACUTE GLOMERULONEPHRITIS (GN). George F. Schreiner and Thomas Moore*. Depts. of Pathology and Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts.

We report the down-modulation of rat glomerular AIIR in the heterologous phase of GN induced by rabbit anti-rat glomerular basement membrane antisera (aGBM). Our technique consists of enzymatically dissociating (collagenase/EDTA) freshly harvested glomeruli into a single cell suspension. Cells bearing AIIR are visualized by fluorescent, anti-AII antibody sandwich labeling of AII bound to the cells. AIIR+ cells typically comprise 25-30% of the cell suspensions. Receptors are quantitated by assaying binding of I^{125}-AII. We have observed a progressive, reversible decrease in glomerular AIIR after the administration of aGBM. There is a mean, maximum loss of 50% of AIIR 16 hrs. after the initiation of GN. A mean 25% decrease is apparent as early as 8 hrs. In a representative experiment, the AIIR K_D of 4.7×10^{-10}M in normal glomeruli was unchanged by GN. Immunofluorescent labeling shows no decrease in the number of AIIR+ cells, indicating the modulation of AIIR is occurring on a per cell basis. Prior decomplementation supplies partial protection against receptor loss, suggesting a contribution of complement activation to AIIR regulation. AIIR expression returns to baseline at 48 hrs. but progressively increases as the GN evolves into the autologous phase. We propose that glomerular AIIR modulation may underlie some of the filtration abnormalities observed in acute and chronic experimental GN.

PLATELET ACTIVATING FACTOR STIMULATES OXYGEN RADICAL RELEASE BY CULTURED RAT MESANGIAL CELLS. J.R. Sedor, and H.E. Abboud, Case Western Reserve University, Cleveland, OH.

Mesangial cells (MC) release oxygen radicals (OR) when exposed to immunologic stimuli and OR may mediate glomerular injury. Platelet activating factor (PAF), a mediator of inflammation, stimulates superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) release by inflammatory cells and may play a role in glomerular injury. We therefore examined the effect of PAF on OR release by MC. MC were incubated with PAF in the presence of 80 µM ferricytochrome C (FC). Control incubations included 10 µg/ml superoxide dismutase (SOD), a O_2^- scavenger. O_2^- release was quantified spectrophotometrically as the SOD-suppressible reduction of FC. PAF (1 µM) stimulated O_2^- release 10.4±2.2 after 5 min incubation (nmoles/mg protein, mean ± SEM, n=4). The effect of 1µM PAF on O_2^- release was seen as early as 1 min (8.11±1.1), and persisted for at least 10 min (9.67±0.99). PAF stimulated O_2^- release in a dose-dependent manner. After 5 min incubation, 0.1µM PAF stimulated O_2^- release 9.67±0.99, 1µM PAF 19.77±2.43, 10µM PAF 21.75±1.81 (nmoles/mg protein). Similar to its effect on O_2^-, PAF also stimulated H_2O_2 released by MC. H_2O_2 release was measured fluorometrically by the oxidation of scopoletin. In the absence of PAF, MC released 2.39±0.1 nmoles H_2O_2/mg protein after 30 min incubation. PAF (1µM) increased H_2O_2 release to 3.96±0.44 nmoles/mg protein. The H_2O_2 scavenger catalase (3000 U) inhibited PAF-induced H_2O_2 release by > 85%. These results show that PAF causes OR release by cultured MC and suggest that OR released from MC may mediate PAF-induced glomerular injury.

DENSE DEPOSIT DISEASE (DDD) IN CHILDREN: PATHOLOGIC PROGNOSTIC INDICATORS. F Silva, T Cavallo*, S Guggenheim*, and R Hogg, for the Southwest Pediatric Nephrology Study Group, Dallas, TX.

Clinical and pathologic features of DDD were examined in 16 children (mean age 9.3 yrs, range 5 to 15 yrs; 9 boys and 7 girls). Initial clinical features included hypertension and decreased GFR in 50% of pts, nephrotic syndrome in 69%, and gross hematuria in 73%. Serum C3 levels were low in 9 of 9 pts. All but 1 pt received steroid therapy. Of the 16 pts, 6 developed ↓GFR and 6 have normal GFR after 7-12 yrs; 4 have have been followed for less than 6 yrs. No clinical or laboratory feature at presentation was of prognostic value. Renal biopsies were studied by light (LM) and electron microscopy (EM) in all 16 pts and by IF in 12 pts. LM changes varied from mild glomerular mesangial hypercellularity to a marked membrano-proliferative pattern. EM showed variable DD alteration of the glomerular basement membrane in all cases and in the mesangium of 13/16 biopsies. IF showed C3 in 12/12, IgM in 9/12, IgG in 2/12 and IgA in 1/12 biopsies. Repeat biopsies showed little or no change in 5 pts who maintained normal renal function for a mean interval of 97 months but marked increase in sclerosis in 1 pt who deteriorated clinically. The following pathologic features correlated with poor outcome: excessive prominence of glomerular lobules ($p=0.01$), severe mesangial hypercellularity (0.02) and sclerosis (0.03), severe glomerular capillary loop obliteration (0.01), and mesangial DD alteration (0.02). We conclude that the clinical course of DDD in children is variable and that certain pathologic features may be helpful in predicting the clinical outcome.

ULTRASTRUCTURAL SIMILARITIES OF MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS TYPE II AND LIGHT CHAIN NEPHROPATHY. Eric Staros, Raymond Seiler and Sheila Moriber Katz, Hahnemann University, Philadelphia, PA.

The hallmark of membranoproliferative glomerulonephritis type II (MPGNII) is large intramembranous dense deposits, but, on occasion light chain nephropathy (LCN) demonstrates similar findings by electron microscopy (Randell et al, Amer J Med, 60:293, 1976). Both LCN and MPGNII can show mesangial widening and thick basement membranes by light microscopy, thereby making the distinction between these two entities difficult. In an attempt to evaluate morphologic similarities of these two diseases and to identify cases of LCN misdiagnosed as MPGNII a retrospecitive study was undertaken. Two cases initially found to have light and electron microscopic findings compatible with MPGNII were later reevaluated by immunofluorescence for light chain deposition. Both cases demonstrated 4+ immunofluorescence staining for kappa chains. One patient was subsequently diagnosed as having a kappa chain secreting plasma cell dyscrasia by immunoperoxidase studies of bone marrow sections, and the other patient was later found to have a monoclonal spike (kappa chain, 1.0mg/dl) by serum protein electrophoresis. These two cases illustrate the histopathological similarities between LCN and MPGNII, and demonstrate the need to perform immunofluorescence for light chains when deposits suggestive of MPGNII are seen by electron microscopy. Additionally, our study indicates that some cases previously diagnosed as MPGNII may, in fact, be LCN associated with plasma cell dyscrasia.

ISOLATION AND CHARACTERIZATION OF ANTIGENS IN NORMAL RAT SERUM WHICH CROSS REACT WITH THE NEPHRITOGENIC GP600-ANTIGEN OF HEYMANN NEPHRITIS. A. K. Singh* and S. P. Makker, Department of Pediatrics, University of Texas Health Science Center, San Antonio, Texas.

Previously we reported the isolation of gp600-the kidney antigen of Heymann nephritis. Following experiments using polyclonal antibodies to gp600 as a probe documented and characterized cross reactive antigens present in normal rat serum. 1) By competitive radioimmunoassay the level of the antigen in serum was determined to be 45.4 ± 12.1 ug/ml (N=17). 2) ^{125}I affinity purified anti-gp600 antibodies formed soluble immune complexes of MW 1.1×10^6 when mixed in vitro with normal rat serum. 3) Rat serum isoelectrofocussed in pH range 3.5 to 10, transblotted and reacted with anti-gp600 using indirect immunoperoxidase (IIP) showed staining of 3 bands in the pI region of 4.4 to 5.4. The serum antigens were isolated by immuno-affinity chromatography of normal rat serum on anti-gp600 column. SDS-PAGE of the eluted proteins showed a predominant band at 70K. Rabbit antiserum to these antigens reacted in a linear fashion to the GBM and interstitial capillary areas by IIF. Isolated perfusion of kidney with this antibody produced granular deposits in the glomerular capillary wall. The antibody showed reactivity to the 70K glycoprotein antigen in gp600 by IIP on nitrocellulose blots of SDS-PAGE of gp600. These results show that there is present in normal rat serum a predominant 70K antigen which cross reacts with 1) the 70K glycoprotein antigen of gp600 2) an antigen present in the glomerular and interstitial capillary wall.

TRANSFER OF SIGNIFICANT OR PROGRESSIVE ANTI-GBM NEPHRITIS (AGN) IN SHEEP BY PLASMA ANTI-GBM AUTOANTIBODIES (AAb) FROM NEPHRITIC SHEEP. Raymond W. Steblay and U. Rudofsky.* Center for Labs. and Research, N.Y. State Dept. of Health, Albany, N.Y.

Because of renewed interest in cell-mediated immunity in AGN, there is need to show that AAb alone can cause progressive AGN in the same species. Plasma was obtained from donor sheep made nephritic by immunization with human GBM and complete Freund's adjuvant (CFA). A plasma pool (1 to 10 donors in pool) was infused I.V. or I.P. in each of 24 recipients under different conditions. All 24 had linear staining for IgG (indicating transfer of AAb) and C3 by immunofluorescence (IF) along the GBM and TBM. Seven of 24 developed significant GN (severe or progressive). Of these 7, 6 were pretreated with CFA, and 2 of this 6 also received peripheral blood cells. The 7th recipient was unilaterally nephrectomized and infused once I.P. with 5 liters of pooled plasma. It developed kidney petechiae, progressive exudative, hemorrhagic and crescentic AGN and renal failure. The remaining 17 recipients got one infusion I.V. or I.P. of .29 to 2.5 liters. Three of the 17 had moderate AGN:petechiae, hematuria and proteinuria for 2 to 18 days. The other 14 had proteinuria for 1 to 13 days; some had mild transient exudative and proliferative glomerular changes. Eleven controls got 1.6 to 3.4 liters of plasma from CFA injected donors. They had no linear staining by IF for IgG or C3 along the GBM. Seven of the 11 had proteinuria for 2 to 7 days; some had transient exudative glomerular changes. Plasma from 3 of the 4 pools inducing progressive AGN had high titers of AAb (80 to 160 by indirect IF). Thus AAb appear to induce progressive AGN.

BINDING AND RELEASE OF C3 FRAGMENTS FROM SOLUBLE AGGREGATES OF IgG ^{125}I (A-IgG). H. Takahashi*, J.A. Sloand*, and D.W. Knutson. Univ. of Rochester, Dept. of Med., Rochester, N.Y.

Incorporation of C3 into soluble immune complexes (ICx) is known to affect the clearance of ICx from the circulation and probably affects the pathogenicity of ICx in other ways as well. However, the precise mechanisms for incorporation and release of C3 fragments from ICx remains unclear. We studied the incorporation of human C3 ^{131}I into A-IgG by purified classical pathway components (C1, C4 and C2) and the release of C3 ^{131}I fragments from A-IgG by the action of Factor I (C3b inactivator) with Factor H (βlH) or C3b receptors (CR1) on human RBC's. SDS-PAGE analysis (reducing conditions) of A-IgG·C3b revealed the expected alpha' chain and beta chain of C3b and the heavy and light chains of IgG; however, heavier bands of ^{131}I and ^{125}I activity were also observed. Direct counting of gel slices following SDS-PAGE of A-IgG·C3b treated with 1M hydroxylamine suggested the heavier bands contained both IgG and the alpha' chain of C3b. Treatment of A-IgG·C3b with Factor H 170 ug/ml and I 25 ug/ml and hydroxylamine demonstrated the conversion of C3b to iC3b whereas similar analysis following treatment with Factor I and CR1 showed the generation of A-IgG·C3d, A-IgG·C3d,g and free C3c fragments. A-IgG·iC3b, A-IgG·C3d,g and A-IgG·C3d showed negligible binding to CR1 on RBC's, whereas A-IgG·C3b bound up to 67.5%. We conclude: (1) C3b binds covalently to the IgG molecules in A-IgG (2) C3b bound to A-IgG are cleaved to iC3b, C3d,g, C3d and C3c in a manner similar to C3b bound to the surface of particulate ligands.

INTRAGLOMERULAR T CELL PHENOTYPE IN CYTOMEGALOVIRUS (CMV) - ASSOCIATED ALLOGRAFT GLOMERULOPATHY. T.V. Tuazon*, A.K. Bhan, E.E. Schneeberger*, A.B. Cosimi*, R.T. McCluskey, and R.B. Colvin. Mass. General Hospital, Dept. of Pathology, Boston, MA.

To provide insight into the pathogenesis of the allograft glomerulopathy associated with CMV, we have analyzed intraglomerular mononuclear cells from 10 biopsies with typical glomerulopathy. All patients (5) with adequate virology studies had evidence of CMV infection. Using the avidin-biotin immunoperoxidase technique, we found a predominance of OKT8+ over Leu-3a+ cells (12.9±3.0 vs. 4.1±1.1 cells per glomerular cross-section [GCS], respectively). The intraglomerular ratio of Leu-3a/OKT8 (0.26±0.04) was lower than the corresponding blood ratio (0.8±0.3; p=0.035). In contrast, renal allografts with acute rejection without glomerulopathy showed rare intraglomerular OKT8+ (2.0±0.9/GCS) and Leu-3a+ (1.4±0.7/GCS) cells. Leu-7+ cells were also present in the glomerulopathy (6.2±1.2/GCS). Intraglomerular mononuclear cells were positive for the activation markers Leu-10 (HLA-DC) and TAC (IL-2 receptor). No B cells were identified. Immunoelectron microscopy confirmed the presence of T8+ lymphocytes in glomerular capillary lumens and occasionally in the mesangium. Large lymphocytes and monocytes stained positively for HLA-DR. These studies suggest that the glomerulopathy is mediated by a subset of T cells, in that it is characterized by selective localization of T8+ lymphocytes in glomeruli and some of the intraglomerular mononuclear cells bear activation markers. The glomerulopathy appears to be a distinct form of allograft rejection related to CMV infection, in which glomeruli become the principal target.

DE NOVO MEMBRANOUS GLOMERULONEPHROPATHY IN RENAL ALLOGRAFTS: A REPORT OF EIGHT CASES. L. Truong*, J. Gelfand*, V. D'Agati*, J. Tomaszewski*, M. Hardy*, G. Appel and C. Pirani. Depts. of Path., Med. & Surg., Columbia U., NY, NY and U. of PA., Phila, PA.

Membranous glomerulonephropathy (MGN) is emerging as the most commonly recognized de novo glomerular disease (GN) in renal allografts. We describe 8 new cases (age 18-53 yrs; M/F, 6/2). When proteinuria was first detected 1-9 mos post-transplantation (PTx), SCr was normal in all pts. MGN was first diagnosed 6-33 mos PTx. Of the 4 pts who developed graft failure 8-31 mos PTx, all had hypertension (HT); 3 nephrotic syndrome (NS) and 3 renal insufficiency (RI) when MGN was first diagnosed. Four pts had stable proteinuria with functioning grafts 20-58 mos PTx ($\bar{x}$ SCr 2.2 mg/dl). There was no association of MGN with infections, drug therapy, systemic disease or HLA matching. Renal biopsies (Bxs) showed 3 patterns: Focal segmental (FS) MGN (2), MGN Stage I (1) and "atypical" MGN with distinct but unclassifiable features (5). Vascular and/or cellular rejection was present in all Bxs. Repeat Bx in 4 pts showed: no change (2), progression from FS to Stage I MGN (1) and from Stage I to Stage II-III MGN (1). One pt developed MGN in 2 successive allografts, the second associated with steroid-induced diabetic nephropathy. We conclude: 1) MGN is the most common de novo GN in renal allografts. 2) At time of diagnosis, NS, RI and HT portend poor prognosis. 3) De novo MGN is morphologically different from "idiopathic" MGN. 4) Glomerular rejection changes, present in all cases, may facilitate the development of MGN.

ULTRASTRUCTURAL STUDIES OF EXPERIMENTAL AUTOIMMUNE GLOMERULONEPHRITIS (EAG) IN CHICKENS (Ch). F.L. Tucker*, B.C. Sturgill and W.K. Bolton, Univ. of Va. Sch. of Med., Charlottesville, Va.

We have shown that immunization of Ch with bovine GBM produces EAG; animals lacking antibody to GBM develop nephritis comparable to those with antibody. The present studies examined the ultrastructural lesion associated with EAG. Normal and bursectomized (Bsx) Ch were immunized with complete Freund's adjuvant (CFA) or CFA-GBM. 8 CFA/Bsx controls, 5 GBM, 6 Bsx-GBM negative and 4 Bsx-GBM antibody positive birds were studied. Controls had a distinct trilaminar GBM, normal podocytes and mesangial (type I) cells. These are characterized by small, regularly shaped nuclei with coarsely clumped chromatin and scanty cytoplasm. A mild increase in type I cells was occasionally observed in controls. In contrast, proliferation observed in GBM immunized animals was characterized by a marked increase in type I cells, and occurrence of type II cells - those with an irregular, folded, lobulated nucleus, finely granular chromatin, abundant cytoplasm, and cytoplasmic dense granules or vacuoles. These cells had the appearance of macrophages and were observed in direct contact with small lymphocytoid (Lym) cells. The increase in type II cells and Lym was present in antibody negative as well as positive Ch, but never in controls.

The finding of cells with the appearance of macrophages and lymphocytes with normal mesangial cells suggests a cellular interaction in production of the proliferative lesion, irrespective of the presence or absence of antibody. This provides further support for the role of cell mediated immunity in the pathogenesis of this model of EAG in Ch.

ANTIBODIES TO GRANULOCYTE CYTOPLASM (ACPA) ARE A SPECIFIC MARKER FOR ACTIVE WEGENER'S GRANULOMATOSIS. F.J. van der Woude*, A. Wiik*, S. Lobatto*, L.A. van Es*, M. van der Giessen*, G.K. van der Hem*, T.H. The* (Intr. by G.A. Andres). Univ. of Groningen and Leiden, The Netherlands and Rigshospitalet, Copenhagen, Denmark.

Immune complex (IC) levels were prospectively investigated in 26 patients with Wegener's Granulomatosis (WG). IC were studied with the fluid phase Clq-binding assay, α-IgA-inhibition binding assay, G-PEG-test, PEG-precipitation test, Clq-ELISA-test and the indirect granulocyte phagocytosis test (IGPT). Positive results related to disease activity were obtained only in the IGPT. In this test normal granulocytes are incubated for one hour with patient's serum. Thereafter, cells with cytoplasmic inclusions of immunoglobulin are detected by fluorescent antibodies and scored. In patients with WG, IgG-containing inclusions with a specific morphology could be observed. No IgM or IgA were present. After only 5 min. incubation, fluorescence was more diffuse. This diffuse pattern was also observed when sera were incubated with ethanol-fixed granulocytes and monocytes. Fluorescent staining was observed with purified IgG. Positivity was also obtained with Fab_2 fragments. All patients with symptoms had significantly higher ACPA titers than patients without symptoms ($p<0.05$). Moreover, a rise in ACPA titer seemed to predict recurrence of disease activity. ACPA could not be demonstrated in sera of more than 10,000 blood donors, 35 SLE patients, 35 patients with seropositive rheumatoid arthritis, and 10 patients with idiopathic proliferative glomerulonephritis. ACPA seem to be a new, specific marker for active WG and could be of major importance in the pathogenesis of the disease.

TRANSFERRIN (Tf) RESTORES THE ABILITY OF HUMAN NEPHROTIC SERUM TO SUPPORT IN VITRO IGG SYNTHESIS BY NORMAL HUMAN LYMPHOCYTES. Barry L. Warshaw, Irene J. Check*, Christine Papadea*, Pamela Wagaman*, and L. Hymes. Emory Univ. Sch. of Med. Depts. of Pediatrics and Pathology, Atlanta, Ga.

To examine whether decreased serum IgG in children with the nephrotic syndrome is related to their decreased serum Tf, we studied the ability of nephrotic serum to support lymphocyte proliferation and IgG synthesis in vitro. Normal human lymphocytes were cultured with concanavalin A or pokeweed mitogen (PWM) for 3 or 6 days in RPMI-1640 supplemented with various amounts of normal or nephrotic serum or with 0 to 25 ng/ml of Tf. Tritiated thymidine uptake was proportional to Tf or serum concentration and, with <0.5% serum, to serum Tf. Tf facilitated IgG synthesis in a time and dose-dependent fashion. IgG assayed by ELISA after 3 days with PWM and 0 and 6 ng/ml Tf was 20 and 100 ng/ml, respectively, and after 6 days with 0, 6, and 25 ng/ml Tf was 200, 260, & 320 ng/ml, respectively. To determine whether hypotransferrinemic, nephrotic serum limits IgG synthesis, normal human lymphocytes were precultured for 6 days with PWM in 0.3% normal human serum (Tf=300 mg/dl), nephrotic serum (Tf=81 mg/dl), or nephrotic serum supplemented with purified Tf (25 ng/ml). The cells were washed free of human serum and then cultured in 1% fetal calf serum for 24 hrs. IgG synthesis was 40 ng/ml with normal human serum, 12 ng/ml with nephrotic serum, & 46 ng/ml with nephrotic serum plus Tf. Thus, hypotransferrinemia contributes to the inability of nephrotic sera to support normal IgG synthesis in vitro.

CHARACTERIZATION AND QUANTITATION OF THE LEUCOCYTE INTERSTITIAL INFILTRATE IN ACUTE IDIOPATHIC INTERSTITIAL NEPHRITIS (IN). R. Waldherr, K. Andrassy, R. Ritz (intr. by R.J. Glassock), Depts. Pathol. & Med., University of Heidelberg, Heidelberg, FRG.

Renal biopsies from 6 patients (4♂, 2♀; age 24-67 y)were examined by immunohistochemical methods (IF, three layer peroxidase or alkaline phosphatase technique). In addition to conventional IF (by which anti-TBM or IC mediated IN could be excluded), various monoclonal antibodies to leucocyte surface epitopes were used: LC (leucocyte common antigen), C3b receptor and HLA-Dr (monocytes, B cells), OKM1 (monocytes, null cells), B cells (HD 5,6, 12,28,37,39, Pan B), (natural) killer cells (Leu-7), T cells (OKT3, OKT11, Lyt 3), and T cell subsets (OKT4, OKT8, Leu-2a, Leu-3b). Positive cells were quantitated with an eye-piece graticule (at 400:1) and expressed as cells/$0.1mm^2$ ±SEM.

The majority of interstitial cells were (64-94%) were T cells (83.3±18.9). Monocytes/macrophages constituted 4.2-17.2%, polymorphs, B cells and plasma cells were only a minor proportion of the total cellular infiltrate. The T helper(inducer)/cytotoxic (suppressor), i.e. OKT4/OKT8 ratio in the kidney markedly differed from the normal blood ratio (1.9±0.7) since it was ≤1 in 5/6 cases.
The predominance of T cytotoxic/suppress. cells in the interstitial infiltrate underlines the pathogenic role of this T subset in the mediation of renal injury.

IMMUNE CELL ABNORMALITIES IN THAI CHILDREN WITH MINIMAL CHANGE GLOMERULONEPHRITIS. W.P. Wiesmann*, V. Vanapruks*, L. Srirasanasomboon*, P. Phisphumuithi*, S. Kongcharden* and H.K. Webster*. (Intr. by J.L. Atkins) Walter Reed Army Institute of Research, Wash, D.C. and Bangkok, Thailand and Phramongkutklao Hosp, Bangkok, Thailand.

Peripheral blood mononuclear cells (PBMC) from Thai children with nephrotic syndrome and biopsy proven minimal change glomerulonephritis (MCGN) were studied in vitro to evaluate the cellular immune system during active disease. Dramatic changes were observed in T lymphocyte subsets defined by monoclonal antibodies, and in the proliferative response to mitogens by patient PBMC and normal PBMC exposed to MCGN sera. MCGN patients showed an imbalance in the relative proportions of T lymphocyte subsets. While total T cells (OKT3) were unchanged, helper/inducer T cells (OKT4) were consistently decreased and suppressor/cytotoxic T cells (OKT8) were consistently elevated in MCGN resulting in depressed helper/suppressor T cell ratios (0.8 ± 0.1 SEM [MCGN] vs. 1.5 ± 0.1 [controls] $p < .001$). PBMC from MCGN patients had a suppressed response to phytohemagglutinin, conconavalin A and polkweed mitogen. MCGN sera also markedly suppressed the proliferative response of normal PBMC to all three mitogens suggesting the presence of a circulating inhibitory factor. These data demonstrate major defects in the cellular immune system of MCGN patients which may be confered by a serum factor uniquely associated with this disease.

PROCOAGULANT ACTIVITY IN URINE AND GLOMERULI OF RABBITS WITH NEPHROTOXIC NEPHRITIS. Roger C. Wiggins, Arthur Glatfelter*, and Jorge Brukman.* Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Glomerular fibrin formation is necessary for crescent development in nephrotoxic nephritis (NN) in the rabbit. To study the mechanisms responsible for fibrin formation a telescoped model of nephrotoxic nephritis using guinea pig (GP) anti rabbit GBM IgG in 41 rabbits preimmunized with GP IgG was used. Procoagulant activity (PCA) was measured by one stage clotting assay in urine and in sonicated isolated glomeruli as well as in glomerular culture supernatants from rabbits sacrificed at various times after induction of NN. PCA increased significantly in both glomerular sonicates and culture supernatants by days 3 and 4, reached a peak by days 5 and 6, and declined towards normal by days 9 and 10. The peak of PCA on days 5 and 6 corresponded with the first appearance of fibrin in Bowman's space as assessed by light, immunofluorescent and electron microscopy. Detectable PCA appeared in urine reaching a peak by day 8, and was preceded by increased protein excretion which peaked at 610 mg/24 hrs over days 4, 5, and 6. PCA in urine was mostly Hageman factor (HF)-dependent over the 4-13 day period studied, while PCA in glomeruli was variably HF, Factor VII, Factor IX, Factor X or prothrombin-dependent. These findings show that detectable procoagulant activity appears in glomeruli and in urine of rabbits with nephrotoxic nephritis, and that the time course corresponds with the appearance of fibrin in Bowman's space. The forms in which PCA appears in glomeruli and urine suggests a relationship between Hageman factor, thromboplastin, and vitamin K-dependent proteins in this model of renal injury.

C3 ACTIVATION IN IgA NEPHROPATHY. Robert J. Wyatt, Yoshiharu Kanayama,* Bruce A. Julian, Sandra Sugimoto,* John G. Curd*. Univ. Tenn. Ctr. Health Sci., Memphis, TN, Osaka City Univ. Med. School, Osaka, Japan, Univ. Alabama in Birmingham, Birmingham, AL, Scripps Clinic and Res. Fdn. LaJolla, CA.

Plasma C3 activation was determined for 26 IgA nephropathy patients (pts), 16 adult, 10 pediatric, (<18 yrs) using monoclonal antibody to the neoantigen (C3bi-Neo) present on the activated fragments C3bi, C3dg, C3d but absent on C3, C3b, C3c. Classical pathway activation was assessed by C4d/C4 ratio using rocket immunoelectrophoresis. Most plasmas were obtained at regular follow-up when pt had no intercurrent infection. Eight adult pts had serum creatinine concentration (SCr) <1.5 mg/dl, while 8 had chronic renal insufficiency (CRI) with SCr 1.9 to 8.2 mg/dl. None were on dialysis or immunosuppressive medications. Mean plasma C3bi-Neo concentration for 31 healthy adults was 15.6 μg/ml. [Normal range ($\bar{x}$+2SD) 8.6 to 22.6 μg/ml] and 16.0 μg/ml for pediatric pts. Adult pts had significantly elevated plasma C3bi-Neo concentrations ($\bar{x}$+1SD=34.2 ±10.3 μg/ml) as compared to normals ($P<0.01$). Two pediatric pts (20%) and 8 adult pts (50%) had significant elevation of plasma C3bi-Neo level. Plasma C3 concentration was normal for all pts. C4d/C4 was negative for 6/6 pediatric pts and 6/9 adult pts. C4 activation was absent in 4 pts with increased plasma C3bi-Neo levels. History of macroscopic hematuria, degree of proteinuria, presence of CRI were similar for adult pts with and without elevated C3bi-Neo levels. These studies indicate, for the first time, that C3 activation and to a lesser degree C4 activation occur in IgA nephropathy.

ABNORMAL CHARACTERISTICS OF HUMAN POLYCYSTIC KIDNEY (PKD) EPITHELIA GROWN IN VITRO. P. Wilson, R. Breckon,* R. Schrier, and P. Gabow. Dept. Med., Univ. Colorado Med. Sch., Denver, CO.

PKD cyst epithelia were grown in hormone supplemented media and compared with normal renal cells derived from proximal straight tubules (PST) and cortical collecting tubules (CCT). Segments of epithelial lining of superficial and deep PKD cysts (sPKD, dPKD) and individually microdissected PST and CCT were explanted onto collagen-coated plastic and grown in media containing dexamethasone (10^{-8} M), insulin (4 IU/ml) and fetal calf serum (3%). PKD epithelia started growing and were confluent sooner (20 days) than normal tubules (41 days). Light and transmission electron microscopy identified cells with polygonal and polarized morphology but PKD cultures also contained some elongate cells. All cells contained a kidney epithelial specific cytokeratin antigen demonstrated by immunocytochemistry. Adenylate cyclase was measured in PKD, PST and CCT cultures using ^{32}P-ATP as substrate and response to parathyroid hormone (PTH IU/ml), arginine vasopressin (AVP, 1 μM) and forskolin (F, 25 μM) compared. Activity in fmoles/30 min/μg prot was as follows:

	Basal	AVP	PTH	F
PST	54±17	16±4	826±212	5597±465
sPKD	37±9	9±3	43±15	168±14
p value	NS	NS	<.05	<.05
CCT	89±27	710±140	56±13	6520±600
dPKD	38±9	20±2	83±24	569±102
p value	NS	<.05	NS	<.05

We conclude that PKD epithelia show abnormally rapid growth, altered cell morphology and an impaired response to AVP, PTH and forskolin.

PATHOPHYSIOLOGY OF ACUTE RENAL FAILURE AND TOXIC NEPHROPATHY

1,25(OH)2D AND HYPERCALCEMIA (↑Ca) IN DIURETIC PHASE OF ACUTE RENAL FAILURE DUE TO RHABDOMYOLYSIS (ARF-RB). M. Akmal, A.W. Norman*, J. Bishop* and S.G. Massry. Div. Nephrol., USC Sch. Med., Los Angeles and Dept. Biochem., Riverside, CA.

↑Ca occurs in diuretic phase of ARF-RB. It is attributed to mobilization of deposited calcium in injured muscles. Increased levels of vitamin D metabolites may play a role since vitamin D is stored in muscle and could be released with muscle injury. To examine the role of vitamin D in the genesis of the ↑Ca, we studied 4 pts with acute renal failure without rhabdomyolysis (ARF) and 7 patients with ARF-RB. The results in diuretic phase were as follows:

	ARF	ARF-RB	(n=7)
		No ↑Ca	↑Ca
	(n=4)	(n=3)	(n=4)
Ca (mg/dl)	9.5±0.13	9.3±1.7	12.3±0.7
P (mg/dl)	4.0±0.10	4.3±0.6	5.9±1.0
PTH (uleg/ml)	8.0±1.10	12.6±1.7	undetected
25(OH)D (ng/ml)	18.3±0.90	17.3±1.7	17.5±0.8
1,25(OH)2D(pg/ml)	21.8±2.70	34.0±1.7	85.5±12.3

The data show that there is an appropriate response of PTH to ↑Ca in ARF-RB making the role of excess PTH unlikely. Blood levels of 25(OH)2D were significantly higher in ARF-RB with and without ↑Ca than ARF. The results are consistent with the notion that 1,25(OH)2D plays a major role in ↑Ca of ARF-RB. The increased production of 1,25(OH)2D could not be related to PTH or P levels. It is possible that 1,25(OH)2D production by the recovering kidney is not tightly controlled as in normal kidney and with availability of vitamin D from injured muscles, its blood levels are increased.

SODIUM BICARBONATE PRE-LOADING IS PROTECTIVE IN ISCHEMIC RENAL FAILURE.
J.L. Atkins, A.Y. Allen* and J. Roberson*. WRAIR, Washington, D.C.

Sodium bicarbonate loading is protective in some forms of toxic renal injury but it has not been examined in ischemic renal failure.

We have looked at the effect of bicarbonate loading on the initiation phase of ischemic renal failure. Sprague-Dawley rats were given sodium bicarbonate (.28M) in place of their drinking water for three days and then were subjected to bilateral renal artery occlusion for 50 minutes. After release of the clamp all animals were allowed normal food and drinking water. The increase in serum creatinine during the first 24 hours after release of the clamp was less in the sodium bicarbonate group than in controls (2.9 vs 4.0; p=.01). In order to cause bicarbonaturia without systemic alkalosis, animals on normal water intake were given Diamox. Animals given Diamox (30mg/kg) po tid for two days prior to clamp were also protected relative to controls (2.9 vs 4.2; p =.01). Urine pH at the time of clamping was higher in both treatment groups than in controls (8.3 vs 7.0). These results demonstrate that sodium bicarbonate loading is protective in ischemic renal failure and suggest that this protection is secondary to changes in tubular fluid pH or flow rates rather than systemic alkalosis or sodium loading.

THEOPHYLLINE ATTENUATES RADIOCONTRAST-INDUCED INTRARENAL VASOCONSTRICTION. G.L. Bakris* and J.C. Burnett, Jr., Mayo Medical School, Rochester, MN

Injection of hyperosmolar radiocontrast medium results in both a biphasic renal blood flow (RBF) response - transient vasodilation followed by prolonged vasoconstriction - and a decline in glomerular filtration rate (GFR) which persists in spite of return of renal blood flow to baseline. We hypothesized that injection of this hyperosmolar solution would result in release of intrarenal adenosine, which is known to cause both intrarenal vasoconstriction and a decrease in glomerular filtration rate. To test this hypothesis, we investigated the effects of theophylline, a competitive inhibitor of adenosine, in five nonvolume-expanded, anesthetized dogs during bolus intrarenal injection of the radiocontrast medium, Renografin, in the presence and absence of intrarenal infusion of theophylline at a dose of $5x10^{-6}$ mol/min.

	%ΔRBF (ml/min/gm kidney)	%ΔGFR (ml/min/gm kidney)
CONTROL	-16.4 ± 1.8	-34.1 ± 2.4
THEOPHYLLINE	- 7.5 ± 1.6*	-10.0 ± 4.0*

*p<.05

Arterial pressure was 126 ± 2 mmHg before and 125 ± 2 mmHg during theophylline infusion. We conclude that theophylline infusion results in attenuation of both radiocontrast medium-induced intrarenal vasoconstriction and decrements in glomerular filtration rate. These studies suggest a possible role for adenosine in the mediation of radiocontrast medium-induced decreases in renal hemodynamics.

SELECTIVE ANTAGONISM OF THROMBOXANE A_2 (TxA_2) AND SULFIDOPEPTIDE LEUKOTRIENES (LTs) AMELIORATES ENDOTOXIN-INDUCED RENAL ISCHEMIA. K.F. Badr*, V.E. Kelley and B.M. Brenner. Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Bolus IV administration of 100 μg/kg of E. Coli lipopolysaccharide endotoxin (LPS) to adult male Munich-Wistar rats (n=18) resulted in a progressive fall in RBF and GFR from 6.9 ± 0.2SE and 1.1 ± 0.05 ml/min to minimal values at 50 min of 3.8 ± 0.4 and 0.32 ± 0.08 (p <.05), respectively, without a fall in mean arterial pressure. At this time, renal cortical generation rates of PGE_2 (1075 ± 108 pg/mg tissue) and TxA_2 (106 ± 12 pg/mg) were significantly higher than those of sham treated control rats (n=10; PGE_2= 466 ± 107, TxA_2=35 ± 9 pg/mg) and morphologic examination revealed normal histology with notable absence of leukocytes and platelets. Pretreatment of a third group of 9 rats with the TxA_2 synthetase inhibitor UK-37248 (10 mg/kg) selectively abolished the LPS-induced rise in TxA_2, but not PGE_2, generation (TxA_2=35 ± 3, PGE_2 =837 ± 62 pg/mg), prevented the fall in RBF (6.3 ± 0.4 ml/min), and allowed for significant preservation of GFR (0.67 ± 0.08 ml/min). In addition, antagonism of endogenously produced LTs with the putative receptor antagonist FPL55712 (n=6, 500 μg/kg/min x 40 min) significantly ameliorated the LPS-induced fall in RBF (5.9 ± 0.5 ml/min), whereas GFR remained depressed (0.46 ± 0.13 ml/min). These observations point to a major role for intra-renally generated vasoconstrictor products of both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism in mediating the renal functional impairment of experimental endotoxemia.

MITOCHONDRIAL (M) RESPIRATORY ENZYME INTEGRITY DURING CONTINUOUS GENTAMICIN (G) TREATMENT: CORRELATIONS WITH RENAL FUNCTION. W.M. Bennett, D.C. Houghton, L.L. Widener, L. Mela-Riker, Oregon Health Sciences University, Portland, OR.

G decreases M bioenergetics prior to a fall in glomerular filtration rate (GFR) and overt tubular necrosis. To study chronic G effects on M ATP synthesis, respiratory enzyme concentrations, and calcium kinetics, M were isolated from renal cortex of male Fisher 344 rats given G 20 mg/kg bid for 3-21 days and controls. State 3 (substrate+ADP) and State 4 respiration were measured using multiple substrates. Cytochrome aa_3,b and c concentrations, M calcium (Ca) accumulation kinetics, inulin clearance (C_{IN}), serum creatinine (Cr), histology and renal G concentrations were also determined.

Results:

	Control (n=9)	Day 5 (n=6)	Day 10 (n=5)	Day 21 (n=5)
Cytochrome aa_3 nmol/mg	.33	.22*	.16*	.22*
State 3 (% control) (glutamate)	100	63*	33*	134*
Ca^{++} accum.(% control)	100	54*	30*	117
GFR ml/min/100 gr	.75	.38*	.03*	.43*
Cr mg/dl	.30	.57*	4.5*	.95*

*p<.01 vs control.

Other cytochromes are maximally decreased at 10 days. In recovery, enzyme activity increased relative to concentration. Renal G varied in parallel with tubular necrosis and regeneration.

Conclusion: M respiratory enzymes are primary target for G since concentrations and activity fall with injury. Recovery of M activity generally precedes increased GFR with continous G; heterogeneity of M dysfunction or dissociation of GFR from tubular events may be operative.

EFFECT OF CIS-DIAMMINEDICHLOROPLATINUM (CISPLATIN) ON ORGANIC CATION TRANSPORT IN CHICKEN AND HUMAN RENAL CORTICAL SLICES. J.A. Berg*, J.E. Bird*, J.E. Heil* and A.J. Quebbemann. Dept. of Pharmacology, Univ. of Minnesota, Mpls., MN 55455

Earlier work has demonstrated that the organic cation transport inhibitor, quinine, can block the nephrotoxic and lethal effects of cisplatin in the chicken. The present study was undertaken to examine the effects of cisplatin on organic cation transport in cortical slices from Highline hens and cortical slices from a human cadaver kidney which was found to be healthy but unsuitable for transplant. Slices were pre-incubated for 30 min in Cross-Taggart media under 100% O_2 with or without 3mM cisplatin. Uptake of the organic cation, ^{3}H-tetraethylammonium (TEA, 10 μM), was expressed as slice-to-medium concentration ratio (S/M). After 90 min incubation, the control S/M for TEA was 15.9±0.9 and 13.3±1.6 in chicken and human kidney slices, respectively. In the presence of cisplatin, the S/M of TEA decreased by 40% to 9.5±1.7 in chicken kidney slices, and by 44% to 7.5±0.2 in human kidney slices indicating a significant inhibition ($P<.01$) of organic cation uptake in both species. In contrast, cisplatin had no significant effect on the renal tubular uptake of p-aminohippuric acid (PAH, 10 μM). When chicken kidney slices were incubated for 60 min rather than 90 min, cisplatin again significantly ($P<.01$) inhibited TEA but not PAH uptake. The results suggest that cisplatin inhibits the renal tubular uptake of the organic cation TEA in chickens and humans at a concentration that has no significant effect on the uptake of PAH.

TUBULAR "UNLOADING" WITH FUROSEMIDE (FS) REVERSES THE $HgCl_2$-INDUCED DEPRESSION IN INTRARENAL pO_2: A POSSIBLE EXPLANATION FOR ITS PROTECTIVE EFFECT IN ACUTE RENAL FAILURE (ARF). R Brems,* RL Baranowski, C Westenfelder. University of Utah and VA Medical Centers, Salt Lake City, Utah.

FS, when administered early, reduces the severity of subsequent ARF. The exact mechanism for this protective effect is unknown. We showed previously that intracortical pO_2 (measured *in vivo* with a Pt-microelectrode) fell strikingly when $HgCl_2$ was administered. It is conceivable that the $HgCl_2$-induced tubular injury is further enhanced by this tissue hypoxia. The purose of this study was therefore to examine whether FS (2 mg/kg i.v.) given 60 min. after $HgCl_2$ (3 mg/kg i.m.) could be use to reduce oxygen consumption and thus prevent tissue hypoxia. In five adult rats *in vivo* pO_2 was monitored in cortex (0 to 3 mm depth) and red medulla (3 to 5 mm depth) prior to, 60 min. after $HgCl_2$ and again 30 to 60 min. after FS administration. GFR, RPF and Na reabsorption were measured in the punctured and contralateral kidneys. pO_2 in cortex and medulla fell by 30 to 50% after $HgCl_2$ ($p<0.01$). GFR, RPF and T_{Na} fell by 50%. Administration of FS caused a natriuresis and 40% rise ($p<0.01$) in cortical pO_2. Conclusion: These data show that FS can reestablish a normal intracortical pO_2 profile. This results probably from improved intrarenal blood flow and tubular "unloading." This effect of FS on intrarenal pO_2 might in part explain it's protective effect in early ARF.

PROTECTIVE EFFECT OF QUININE ON NEPHROTOXICITY INDUCED BY CIS-DIAMMINEDICHLOROPLATINUM (CP). J.E. Bird*, M.M. Walser*, and A.J. Quebbemann, University of Minnesota, Minneapolis, MN 55455.

Nephrotoxicity is a dose limiting side effect of CP, an important chemotherapeutic agent. A Sperber *in vivo* chicken preparation in which the renal bypass vessels were ligated to direct the flow from the saphenous vein (SV) into the peritubular network of the ipsilateral kidney, was utilized to examine the nephrotoxic effects of CP. The effects of CP (3 mg/kg) injected into the SV was studied in nine birds. Four birds died within four days of CP administration. In the remaining five birds, renal tubular excretory transports in control (C) and 4 days after CP treatment (CP) were (C: .59±.15; CP: .14±.24) for ^{3}H-tetraethylammonium (TEA) and (C: .55±.16; CP: .06±.26) for p-aminohippuric acid (PAH). All 5 birds had acute multifocal necrosis of the proximal tubules. Quinine, an organic cation transport inhibitor, (1.5 μmol/min) infused into the SV for 30 min before and 15 min after CP injection, prevented the lethality, the tubular necrosis, and the inhibition of renal tubular transports. In 4 quinine treated birds, renal tubular transports were (C: .67±.08; CP: .67±.04) for TEA and (C: .60±.03; CP: .59±.08) for PAH. Cyanine 863, another organic cation transport inhibitor, also protected against the nephrotoxic effects of CP.

INDOMETHACIN IMPROVES RENAL MEDULLARY FUNCTION WHILE PREDISPOSING TO ANOXIC INJURY: IMPLICATION FOR ANALGESIC TOXICITY. M. Brezis, S. Rosen, K. Spokes*, P. Silva and F.H. Epstein. Depts. of Med. and Path., Harvard Med. Sch. and Beth Israel Hosp., Boston, MA.

Since renal hypoperfusion predisposes to acute renal failure from non-steroidal anti-inflammatory agents (NSAI), and medullary ischemia might play a role in chronic analgesic nephropathy, a possible synergism between analgesics and medullary anoxia was evaluated in the isolated perfused rat kidney. In this preparation, indomethacin (5 mg/kg to the rat and 10^{-4}M in the perfusate) effectively suppresses prostaglandin production but does not improve concentrating ability (U/Posm 1.3+0.02).

Quantitation of the anoxic injury to the medullary thick ascending limb showed that indomethacin extended the severe lesion from 58+7% ($\bar{x}$+SE) of tubules in controls to 83+4% ($p<0.005$), in the deeper, most anoxic portion of the inner stripe. By contrast, when indomethacin was given to kidneys perfused with erythrocyte-enriched medium, concentrating ability was remarkably improved with a maximal Uosm of 755+50 mOsm and U/Posm 2.4+0.2 ($p<0.05$ vs no indomethacin) and morphological damage was no longer present.

Thus, in medullary hypoxia (as produced by cell free perfusion) prostaglandins may play a protective role by either vasodilatation or reduction in active transport. Indomethacin, by interfering with these processes, appears to predispose the medulla to anoxic damage, which might be of pathogenic significance in NSAI-induced acute renal failure and analgesic nephropathy.

HYPEREMIA FOLLOWING UNILATERAL ISCHEMIA IN THE RABBIT IS UNAFFECTED BY INDOMETHACIN (INDO) PRETREATMENT. Marc Browning. Wake Forest Univ. Med. Ctr., Dept. of Ped., Winston-Salem, N.C.

Reflow after 25 min. of unilateral renal ischemia in the volume expanded, anesthetized rabbit is characterized by reactive hyperemia and increased urine flow (UV, ml/min). These experiments examined the effect of INDO on postischemic hyperemia.

Rabbits were anesthetized with pentobarbital and infused with 0.9% NaCl at 10 ml/kg/hr. Renal blood flow (RBF) and mean arterial pressure (MAP) were monitored. INDO 8-10 mg/kg was given as a bolus 5-20 min. before left renal artery occlusion and, in some animals, continued in the sustaining infusion at 2 mg/kg/hr. Clearances of inulin (Cin) and PAH (Cpah) were measured from the postischemic (I) and contralateral nonischemic (N) kidney. After 40 min. of reflow the kidneys were removed to determine cortex-to-serum concentration ratios of inulin (C/Sin) and PAH (C/Spah).

Administration of INDO was followed by a 6% increase in MAP ($p < .05$) and a 16% decrease in RBF ($p < .05$). Additional results are tabulated:

	RBFt1	RBFt2	RBFt3	Cin	Cpah	C/Sin	C/Spah	UV
CTRLI	23.9	-	37.0+	1.6*	10.7	1.8	14.5	0.27*
CTRLN	24.3	-	26.1	3.4	17.1	1.8	7.8	0.05
INDOI	23.1	20.4	38.0+	3.2	21.8	2.1	13.2	0.39*
INDON	22.9	16.7	21.3	3.8	15.7	2.6	10.1	0.04

t1=preINDO, t2=postINDO, t3=postISCH;
*$p < .05$, I vs N; + $p < .05$, t3 vs t1

In conclusion INDO does not prevent the hyperemia or increased urine flow which follows renal artery occlusion in this model, despite clear effects on pre-ischemic MAP and RBF. In addition, INDO pretreatment appears to ameliorate the renal dysfunction seen 0-40 min. after reflow.

DOCUMENTATION OF POST-RECEPTOR DEFECTS IN INSULIN-MEDIATED MUSCLE METABOLISM IN ACUTE RENAL FAILURE (ARF). A. Clark,* R. May,* W. Mitch, Dept. Med., Brigham and Women's Hospital, Boston, MA.

To characterize the insulin (I) resistance of muscle in ARF, dose-response relationships between I and glucose uptake (GU) and glycogen synthesis (GS) were measured in incubated rat epitrochlearis muscles after 48 h of ARF or sham operation (SO). GU was measured as the release of ^3H_2O from D(2-^{3}H)glucose and GS was measured as incorporation of D(U-^{14}C)glucose into glycogen. ARF depressed I-stimulated GU; at 10 mU/ml I, (V_{max}), GU was 24% lower in ARF ($p<0.01$). Transport of D-2-deoxy(U-^{14}C)glucose was equally depressed. ARF also decreased I-stimulated GS; V_{max} was 54% lower in ARF ($p<0.01$). Since glycolysis was unaffected by ARF, the decrease in GS was not due to the lower rate of GU. To identify why GS was low, glycogen synthase and phosphorylase activities ±10 mU/ml I were measured in homogenized muscle (mean ±SE; *, $p<0.01$ vs SO).

	Insulin	Glycogen Synthase (Units/g protein)	(%I)	G-6-P (μMol/g mus)
ARF	-	1.8±0.1*	22±2*	298±25
	+	1.7±0.1*	25±1*	347±22
SO	-	2.3±0.1	28±2	267±18
	+	2.2±0.1	32±1	349±18

ARF decreased total and activated (%I) glycogen synthase activity. There was no increase in muscle glucose-6-phosphate (G-6-P) which might have modified enzyme activities in intact muscle. Glycogen phosphorylase activity was unchanged by ARF. Thus, in ARF: 1) GU in muscle is resistant to I; 2) I-stimulated GS is impaired; 3) reduced glycogen synthase activity, a post-receptor defect, causes the lower GS.

EFFECTS OF VERAPAMIL ON RENAL FUNCTION AND ADENINE NUCLEOTIDE METABOLISM AFTER WARM ISCHEMIA IN THE ISOLATED PERFUSED RAT KIDNEY. L Chan, J I Shapiro*, C Cheung*, A Itabashi*, R W Schrier. Dept. Med., Univ of Colorado Med. Sch.,Denver, CO.

Ischemia was induced in the isolated perfused rat kidney after 30 mins of perfusion by clamping the arterial line of the perfusion circuit. Perfusion was re-started after 40 mins of ischemia; after 60 mins of reflow the kidney was then freeze-clamped for adenine nucleotide level using enzymatic assay. Three groups of experiments were performed: Group I (n=6) no verapamil was added. In group II (n=6), verapamil 2.5 uM and in Group III (n=6), verapamil 5 uM, were added to the perfusionmedium. Before ischemia, inulin clearance(C_{In}) and urine flows rate (V) were similar in the 3 groups. The mean results of 60 mins of reflow are summarized below:

Verapamil		0	2.5 uM	5uM
C_{In}	ul/min/g	30.6±11	86.1±28	89.3±23#
V	ul/min/g	11.9± 7	39.2±13	27.9± 2*
T_{Na}	umol/min/g	3.6± 1	7.4± 2	8.2± 1#
ATP	umol/g dry	1.78±0.04	3.65±0.05*	3.83±0.04#
ADP	umol/g dry	1.66±0.12	3.18±0.29#	3.04±0.31#
AMP	umol/g dry	0.46±0.10	0.71±0.10	0.64±0.23
TAN	umol/g dry	3.89±0.45	7.45±0.60#	7.54±0.75#
Pi	umol/g dry	13.2±1.71	11.4±0.22	14.3±1.39

(Results are expressed as mean±sem; * $p < 0.05$; # $p<0.01$). GroupIII showed significantly higher C_{In}, net tubular sodium reabsorption(T_{Na}) and V than the control group I. In both verapamil groups (II, III), higher ATP, ADP and total adenine nucleotide (TAN) contents than the control group were found. Thus, calcium entry blocker, verapamil, exerts significant functional and metabolic protection against total warm ischemia in the rat kidney.

RENAL Na-K-ATPase IN POTASSIUM DICHROMATE-INDUCED ACUTE RENAL FAILURE IN THE RAT. E. Cooney* and S.K. Mujais. Univ of Illinois, Chicago, IL.

Toxic insults to the kidney may lead to a decrease in Na-K-ATPase with consequent disruption of cell volume regulation leading to cell death. In male Sprague-Dawley rats (250-300g), we examined the changes in renal Na-K-ATPase after the subcutaneous administration of K2Cr2O7 (KCr) (15 mg/kg). Sham animals received saline injections. Renal function and ATPase activity were determined at 24 and 48 hours. There was no difference in right kidney weight between the two groups at 24 (0.967±.03 vs 0.9±0.02g) or 48 hrs (0.935±0.02 vs 0.958±0.05g). Total cortical ATPase is shown below (in mmoles $Pi.hr^{-1}$). (*$p<0.05$)

	24 hr			48 hr		
	BUN (mg/dl)	ATPase Mg	ATPase Na-K	BUN	ATPase Mg	ATPase Na-K
Sham	14.5 ±1.0	2839 ±189	1173 ±65	13.5 ±1.3	2076 ±31	1647 ±137
KCr	23.6* ±2.9	2061* ±104	1286 ±111	77.0* ±34	1474* ±163	1031* ±49

When ATPase activity was normalized to protein content, an index to which intact and damaged cells likely contribute, there was no difference in enzyme activity between sham and KCr rats. This latter finding indicates a normal or possibly increased ATPase activity in intact cells, which may have been responsible for their resistance to the toxic insult. This suggests that the enhanced renal recovery from toxic insults induced by maneuvers such as Mg-ATP or thyroxine may be mediated by their effect on renal ATPase.

EFFECT OF VITAMIN D INDUCED HYPERCALCEMIA (HC) ON THE OUTCOME OF ISCHEMIC ACUTE RENAL FAILURE (ARF). K. Cooper*, G. Thulin*, H. Rasmussen*, and N.J. Siegel. Yale Univ. Sch. of Med., New Haven, CT.

Recent evidence suggests that Ca++ influx may be a primary mediator of cellular injury in ischemic ARF. To further define the role of cellular Ca++ overload in the pathogenesis of ARF, C_{in} was determined in normocalcemic (NC) and hypercalcemic (HC) Sprague-Dawley rats prior to and 24 hrs. after 45 min. of bilateral renal ischemia. HC was induced by the daily i.p. injection of 30, 60, or 120 pmol. of $1,25(OH)_2D_3$ for 4 days. Serum Ca++ was determined at the time of post-ischemic reperfusion. Prior to ischemic injury, HC control (sham-operated) rats had a C_{in} which did not differ from NC controls (958.1 ± 31.6 (SEM) vs. 972.6 ± 33.4 µl/min/100 gm N.S.)

24 hrs. after ischemic injury, those rats with moderate/severe HC(Ca++ > 11.0, mean 12.2 ± 0.2 mg/dl) had a significantly lower C_{in} than did NC controls (184.4 ± 29.2 vs. 353.4 ± 28.9, p<.001). Unexpectedly, rats with mild HC(Ca++ < 11.0, mean 10.6 ± 0.1) had a C_{in} (488.8 ± 35.7, p<.01) significantly greater than NC ischemic controls. In addition, serum Ca++ correlated inversely with post-ischemic C_{in} for both groups of vitamin D treated rats (r = 0.76, p<.001).

In summary, in vitamin D treated ischemic rats: 1) moderate/severe HC significantly augments renal injury, 2) mild HC, on the contrary, may be protective, and 3) serum Ca++ is inversely correlated with C_{in} determined 24 hrs. post-ischemia. These studies support a role for calcium as a mediator of cellular injury, however, Ca++ has multiple and complex effects on the outcome of ischemic ARF.

THE ROLE OF OXYGEN FREE RADICALS IN AMINONUCLEOSIDE NEPHROSIS. Jonathan R. Diamond, Morris J. Karnovsky* and Joseph V. Bonventre. Massachusetts General Hospital and Harvard Medical School, Boston, MA

The cellular processes responsible for the proteinuria induced by the aminonucleoside of puromycin (PA) remain inadequately defined. Hypoxanthine is both a metabolic breakdown product of PA as well as a substrate for xanthine oxidase, which catalyzes its enzymatic conversion to xanthine and uric acid yielding the superoxide anion in the process. We examined whether oxygen free radical production contributes to the development of proteinuria in this model. Seven groups of rats were studied with 5 animals per group. Proteinuria was quantitated 7 days after rats were treated with PA (5 mg/100g) intravenously over 5 min. PA-treated animals received either saline, dimethyl sulfoxide (DMSO, total dose =.08 or 4 gm/kg), catalase (Cat, 40 mg/kg), or superoxide dismutase (SOD, 15 mg/kg), over 30 min prior to and 30 min following PA administration. Another group received allopurinol (Allo, 100 mg/kg) over 4 hrs prior to PA. Results are tabulated below.

Treatment	24 hr Protein Excretion (mg)
Control	31.6 ± 6.9(SE)
PA	363.8 ± 84.2
PA + DMSO(.08gm/kg)	378.6 ± 67.4
PA + DMSO(4 gm/kg)	218.6 ± 73.8
PA + Cat	234.8 ± 61.3
PA + SOD	111.0 ± 41.7*
PA + ALLO	75.8 ± 11.3**

*p<.005,**p<.001 compared with PA group by ANOVA.

The protective effects of SOD and ALLO indicate that oxygen free radicals generated by xanthine oxidase are important mediators of PA-induced proteinuria.

PATHOGENESIS OF THYROXINE (T4) PROTECTION IN GENTAMICIN (G)-INDUCED ACUTE RENAL FAILURE. R. Cronin. VAMC and UTSW Med School, Dallas, Texas.

T4 given to rats prior to and during G protects against a rise in plasma creatinine. In this study, renal hemodynamics, renal excretion, and renal cortex homogenate Na-K ATPase activity were studied in normal rats given daily T4 (10 µg/100 g b.w. sub cut.) for 10 days prior to and during an 8 day course of G (30 mg/kg b.w. twice daily sub cut.). Results: In the control group G, creatinine clearance was significantly reduced, 3.0±0.3 to 1.5±0.2 ml/min, p<.001, but was unchanged in group GT4, 2.9±0.3 to 2.6±0.5 ml/min, NS. Urinary osmolality was reduced in groups G and GT4, but the decline was greater in G, 1970±88 to 927±79 mOsm/kg, p<.001, compared to GT4, 1889±153 to 1200±110 mOsm/kg, p<.05. Ten days of T4 prior to G had no significant effect on creatinine clearance, urine volume, solute excretion, urinary K+ excretion, urinary Ca++ excretion, but did increase slightly urinary Na+ excretion, 3.5±0.4 to 4.7±0.1 mEq/kg b.w./day, p<.05. At sacrifice, renal cortex Na+ (mEq/100 g fat free dry solids), an index of cell injury, in group G (33.0±0.1) and group GT4 (27.8±1.2) was higher than normal (18.9±0.4, p<.001), but also was significantly higher in G compared to GT4, p<.001. Renal cortex Na-K ATPase activity was reduced in group G, was normal in group GT, and correlated with plasma total T4, r=0.6, p<.001.

In summary, T4 protects against gentamicin-induced acute renal failure by a mechanism apparently independent of renal hemodynamics and renal excretory changes. Stimulation by T4 of membrane bound transporting enzymes such as Na-K ATPase may be important in protection.

EFFECT OF PARATHYROIDECTOMY (PTX) ON RENAL GENTAMICIN (G) ACCUMULATION, NEPHROTOXICITY (NTX) AND CALCIURIA. Clay Elliott, Debra Patchin,* David Jones. Med. & Path. Depts., SUNY-Upstate, Syracuse, NY.

PTH increases renal levels of G-binding acidic phospholipids. Since dietary Ca^{++} supplementation both suppresses PTH and attenuates G-NTX in rats, we compared G-NTX in PTX rats (PTH-) (normal Ca^{++} diet) to PTH stimulated rats (PTH+) (low Ca^{++} diet). Baseline serum Ca^{++} and U_{CAMP} were lower in PTH- (0.9±0.1 (SD) vs 1.3±0.1 mM/L and 10±1 vs 22±2 nM/mg Cr; p<0.001) but Scr was identical (0.3±0.02 mg/dl). G 20 mg/kg/d was administered for 3-21 d. Peak renal cortical G ([G]) and Scr were higher in PTH+: (Mean±SD; N=4-6; *=p<0.05).

Day	[G] (µg/100 mg cortex) PTH+	PTH-	Scr (mg/dl) PTH+	PTH-
3	40±13	18±7*	0.8±0.2	0.4±0.02*
7	54± 5	36±9*	0.6±0.03	0.4±0.1*
10	38±13	36±14	2.3±1.3	0.7±0.3*
14	31± 3	27±3	3.0±1.7	0.9±0.7*
21	42±10	40±11	1.3±0.3	0.7±0.2*

In addition, in PTH+ renal epithelial lysosomes were larger and more frequent and tubular necrosis was more extensive and severe compared to PTH-. G increased urinary calcium in PTH+ (2.0±0.7 (baseline) to 17±5 µm/mg Cr; p<0.025) but not in PTH-.

Conclusions: 1) PTX attenuates G-NTX in rats in spite of hypocalcemia compared to PTH stimulated normocalcemic controls, 2) PTX slows early G accumulation by the renal cortex, perhaps by decreasing G transport, 3) PTX dampens or prevents G-associated calciuria, suggesting that G alters PTH-mediated Ca^{++} handling.

PROXIMAL TUBULAR MORPHOLOGY AFTER SINGLE NEPHRON OBSTRUCTION IN THE RAT KIDNEY. Andrew P. Evan, Philip Blomgren,* Lisa C. Knopp,* and George A. Tanner. Indiana Univ. School of Medicine. Depts. Anatomy and Physiology & Biophysics. Indianapolis, IN

Previous work has shown that chronic obstruction of single proximal tubule lumens results in discrete functional changes; however, it was not known whether interruption of tubular fluid flow might alter the morphology of downstream tubular segments. We therefore injected a short column of solid paraffin wax into the lumen of single proximal tubules (PT) of superficial cortical nephrons. We observed these same nephrons one day, one week, or one month later by both light and electron microscopy. Control and obstructed nephrons were fixed by either whole kidney or individual tubular perfusion. At one day, the entire length of downstream PTs showed cell injury. The S_1-S_2 segments presented both reversible and irreversible injury, and S_3 showed primarily reversible cell damage. Some cells showed evidence of recovery. By one week, obstructed PTs were relined by less differentiated cells and possessed a collapsed lumen. Interstitial fibrosis was present. At one month, there was severe PT atrophy, indicated by a reduction in cell size and number. Extensive interstitial fibrosis surrounded the obstructed nephrons. In conclusion, prolonged obstruction of PTs leads to cell injury and tubular atrophy. These morphological changes may be due to ischemia and/or interruption of tubular fluid flow (disuse atrophy).

EXPERIMENTAL TOBRAMYCIN NEPHROTOXICITY IS REDUCED BY CONCOMITANT TICARCILLIN. D.C. Houghton, J. English,* G. Mayor, D.N. Gilbert,* W.M. Bennett. Oregon Health Sciences University, Portland, OR; Michigan State University, East Lansing, MI.

The antimicrobial activity of aminoglycosides is reduced by concomitant penicillin, although the combination is clinically synergistic. To examine the nephrotoxic potential of the combination, we treated male Fischer 344 rats with tobramycin (T) alone, tobramycin and ticarcillin (TT) injected simultaneously, or a pre-incubated tobramycin/ticarcillin mixture (TTi) (120 mg/kg/day of tobramycin and 250 mg/kg/day of ticarcillin). BUN, serum creatinine (SCr), inulin clearance (C_{In}), in vitro renal slice/medium uptake ratios of [1-^{14}C] tetraethylammonium (TEA) and para-aminohippurate (PAH) were measured at 14 days. Light microscopy was done without knowledge of experimental groups.

Gp(n=6)	BUN	SCr	C_{In}	TEA
T	61±13†§	1.1±0.5	0.2±0.02†§	3.1±0.6†§
TT	39±7†	0.6±0.1¶	0.4±0.06†¶	4.4±0.6†
TTi	36±6†	0.9±0.2	0.5±0.15†	4.8±1.5†
C	25±4	0.7±0.2	0.8±0.12	8.3±1.5

†p<.02 vs C; §p<.02 vs TT and TTi; ¶p<.02 vs TTi. PAH uptake was similarly depressed in T compared to TT and TTi. Only T developed severe proximal tubular necrosis correlating with reduced cortical drug concentrations. Conclusion: In vivo and in vitro interaction between tobramycin and ticarcillin modifies nephrotoxicity, possibly representing a feasible approach in clinical therapeutics.

VANADATE (V) POTENTIATES GENTAMICIN (G) NEPHROTOXICITY IN THE RAT. E. Fernández-Repollet and M. Martínez-Maldonado. School of Medicine, University of Puerto Rico and VA Center, San Juan, Puerto Rico.

Gentamicin and vanadate inhibit renal Na+K+ATPase activity in the rat. Both agents accumulate in the renal cortex and interact with the enzyme from the cytoplasmic side. V increases intracellular calcium, a mechanism by which G is nephrotoxic. To investigate the possibility of a synergistic effect of V on G-induced nephrotoxicity, male Wistar rats were injected with V (5mg/kg/BW/day; n=10) for 12 consecutive days; control rats received an equivalent volume of saline (n=10). On day 13, 5 V-treated rats and 5 control (C) rats also received G (100mg/kgBW/day) for 8 consecutive days. Body weight (BW;g), kidney weight (KW;g/100 gBW), urine flow rate ($\dot{V}$;ml/24hr/100gBW), and endogenous creatinine clearance (CrCl;ml/min/100gBW) were measured.

	BW	KW	$\dot{V}$	CrCl
C	255 ± 9	0.36 ± 0.01	5.6 ± 0.3	0.37 ± 0.01
G	250 ± 5	0.41 ± 0.01*	8.6 ± 1.1*	0.29 ± 0.01*
V	216 ± 9*	0.72 ± 0.16*	13.9 ± 1.1*	0.25 ± 0.02**
V+G	183 ± 7*	0.73 ± 0.04**	16.9 ± 3.3*	0.11 ± 0.02+

Values are means ± SEM. *p<0.05 (compared to control) + p<0.05 (G vs. V+G). Despite a lower CrCl, both G and V+G rats had a higher $U_{Ca}\dot{V}$ (G: 333%; V+G:373%) and $U_{Pi}V$ (G:75%; V+G 386%) than control rats indicating inhibition of Ca and Pi reabsorption. Morphological studies revealed severe tubular necrosis in the V+G group compared to other groups. The data indicate that chronic treatment with V worsen G nephrotoxicity in the rat. This may be related to alterations in the renal handling of Ca and /or P.

DIFFERENCES BETWEEN HYPOXIC AND ISCHEMIC INJURY TO ISOLATED TUBULES. D. Hunt*, M. White*, H.D. Humes and J.M. Weinberg. VA Medical Center and Univ. of Michigan, Ann Arbor, Michigan

Suspensions of rabbit proximal tubules subjected to 30' oxygen deprivation (OD) under ischemic (I) conditions appear to be substantially less susceptible to cell injury than when OD is produced under hypoxic (H) conditions based on gross structural integrity and maintenance of respiratory capacity (Clin. Res. in press). To more fully assess cellular metabolic features of this phenomenon we measured cell K+, Ca++, ATP and total adenine nucleotides (ADN) at the end of OD (N) and following 60' reoxygenation post OD (R) in tubules subjected to 30' H or I (Ns=7-8) as well as in time controls (TC). Values are means ± SE in nmol/mg protein. *p<.05 or better vs. TC, †p<.05 or better H vs. I.

	TC	HYPOXIA		ISCHEMIA	
		N	R	N	R
K+	335±7	60±6*†	206±23*†	115±3*	318±7
Ca++	18±2	27±3*†	24±2†	15±1	19±2
ATP	8.8±.3	.3±0*†	1.7±.2*†	.5±0*	7.1±4*
ADN	10.5±.4	2.6±.2*†	2.3±.2*†	5.1±.2*	8.5±.5*

These data indicate that the protection afforded by I includes major indices of cellular metabolic integrity. Available data indicate that one factor present during OD under I conditions but not during H which appears to have a major protective effect is a fall in pH from 7.43±.01 to 6.97±.10 (n=4, p<.01). Further delineation of the mechanisms involved in this protection should provide substantial insight into the processes responsible for tubule cell injury during ischemic acute renal failure.

RENAL TUBULAR CELL (RTC) INJURY AND ARACHIDONIC ACID (AA) METABOLISM: INVOLVEMENT OF $^{\cdot}OH$ RADICAL. WF Keane, BS van Asbeck*, G Gekker*, PK Peterson*, Hennepin County Med Ctr, U of Minn., Mpls., MN.

Hemodynamic and morphologic consequences of ATN have been extensively evaluated. However, the cellular mechanisms involved in RTC damage are unknown. Toxic O_2 species have been implicated as important mediators of cell injury. Since oxidative metabolism of AA generates toxic O_2 species, we reasoned that these could mediate injury to RTC. To test this hypothesis, freshly isolated rat cortical RTC were incubated at 37°C with 3 probes known to stimulate AA metabolism: H_2O_2 (0.9 mM), Ca^{++} ionophore A23187 (1 µg/ml) and lipid A (1 µg/ml). RTC death after 60 min incubation determined by trypan blue exclusion was 53.6±3.7% with H_2O_2, 40.9±1.0% with lipid A and 51.4±2.9% with A23187. Inhibition of RTC AA metabolism by methylprednisolone (10 µg/ml), indomethacin (1 µg/ml) and piriprost (1 µg/ml) significantly inhibited (34-70%; $p<0.001$) RTC death by all probes. Preincubation of RTC with superoxide dismutase, catalase, thiourea, mannitol or deferoxamine, agents which ameliorate oxidant injury, significantly inhibited (9-65%; $p<0.001$) RTC death and thus suggested $^{\cdot}OH$ was the toxic oxidant. Viable RTC incubated with any of the probes demonstrated marked chemiluminesence (CL), a measure of toxic O_2 species. Inhibition of CL was observed with all the above inhibitors or scavengers studied and correlated with improved RTC survival. Chelation of extracellular Ca^{++} with EGTA also inhibited RTC injury (>90%) and CL (>80%) induced by any probe. We conclude that Ca^{++} dependent generation of $^{\cdot}OH$ via AA metabolism is an important mechanism of RTC death.

RENAL SYMPATHETIC NERVES MEDIATE GLOMERULAR HYPOPERFUSION IN SYSTEMIC CIRCULATORY IMPAIRMENT. V. Kon, A. Yared* & I. Ichikawa. Child. Hosp., Boston, MA.

The observation that glomerular dynamics of normal animals are unaffected by renal denervation (DNx) suggests that renal sympathetic nerves (N) are contributing little to glomerular function in normal physiologic condition. To evaluate the potential significance of N in pathophysiologic circumstances, we measured the parameters of glomerular microcirculation before and after DNx in 3 groups of rats: congestive heart failure following myocardial infarction (MI, n=6), water deprivation for 48 hrs (WD, n=5) and normal controls (NC, n=4). Results include (Δ, change after DNx; †, $P<0.05$):

	SNGFR	Q_A	$\bar{P}_{GC}$	R_A	R_E	K_f
	---nl/min---		mmHg	$\times 10^{10}$ dyn·s·cm^{-5}		nl/(s·mmHg)
MI	23.1	69.4	54.0	3.1	2.9	0.046
Δ	+6.1†	+34.0†	-3.9	-0.4	-1.2†	+0.027
WD	34.6	99.0	59.1	2.1	1.9	0.044
Δ	+2.0	+38.0†	-7.8†	-0.5†	-0.8†	+0.017†
NC	53.8	205.8	48.6	1.5	0.9	>0.106
Δ	+2.3	+ 3.9	-1.1	-0.0	-0.1	–

Both MI and WD were characterized by a profoundly vasoconstricted glomerular microvasculature, as indicated by the depressed glomerular plasma flow (Q_A) and SNGFR, and high glomerular capillary pressure ($\bar{P}_{GC}$), afferent (R_A) and efferent (R_E) arteriolar resistances. In both MI and WD, removal of sympathetic influence by DNx led to a fall in R_A and R_E in all but one animal. In addition, ultrafiltration coefficient (K_f) rose after DNx. In MI, these changes were sufficient to elevate SNGFR. Thus, although renal nerves may have no vasoconstrictive effect in normal physiologic condition, they act as a primary mediator of glomerular hypoperfusion in prerenal circulatory impairment.

EFFECTS OF ACYCLOVIR ON CANINE RENAL FUNCTION. A. Kimes*, D. Holtzclaw*, K. McCullough*, D. Teller*, D. Spector, D. Dobyan and K. Kumor*. Div Renal Med., F. Scott Key Med Ctr, Johns Hopkins Sch Med, Baltimore, MD and U. Texas HSC, Houston, TX

Serum creatinine has been observed to rise in some patients treated with acyclovir (AC). To study the effect of AC on renal function, unanesthetized dogs were given 210 mg/kg/day AC by continuous iv infusion for 43 hrs (high dose-acute group) or 15 mg/kg every 8 hrs by 30 min iv infusions for 28 days (low dose-chronic group). These doses resulted in plasma AC concentrations comparable to high dose therapy in humans. Control dogs were sham treated with drug vehicle. Inulin clearance (GFR), maximum renal concentrating ability (Umax) after ADH or dehydration, and maximum PAH tubular secretion rate (Tm PAH) were performed before (baseline) and during treatment periods (weekly during chronic treatment).

Acute AC dogs had a 22% decrease in GFR from baseline after 43 hrs of AC treatment ($p<0.05$). Control and low-dose chronic dogs showed no change in GFR. Mean Umax declined 481 mOsm/kg after acute AC ($p<0.05$), and 442 mOsm/kg after chronic AC ($p<0.05$), and was unchanged in the control dogs. Tm PAH was unchanged by AC in both groups of animals. Administration of AC was associated with a decline in plasma K+ concentration (acute - 0.5 mEq/L, $p<0.05$; chronic - 0.3 mEq/L, $p<0.05$), but no change in plasma sodium. AC administration was not associated with changes in fractional excretion of potassium.

In conclusion modest but significant reductions in GFR, Umax and plasma K+ were associated with AC administration. A decline in Umax appears to be the earliest sign of AC induced renal dysfunction.

BASOLATERAL TRANSPORT MAINTAINING NORMAL CORTICAL CELL AMINO ACID CONCENTRATION IN ACUTE RENAL FAILURE. Kronfol, N.O., Duran, M.A., Spencer, P.*, Weise, M.* and Oken, D.E. Dept. Med., VA Hospital and Medical College of Virginia, Richmond, VA.

Since filtration is grossly reduced 24h p 1h. total renal ischemia, the delivery of amino acids (AA) to the brush border transport site must be very scant. Proximal tubule Na transport with which AA transport occurs is also greatly reduced and, if the tubules are "leaky," cell AA retention should be impaired. Using ^{14}C dansylation and thin layer chromatography, we have measured the concentrations of 17 AA in cortical cell water 3h or 24h after 1h unilateral total renal ischemia using the contralateral right kidney as control. Kidneys were frozen in situ and AA extracted in the presence of 4 protease inhibitors (EDTA, n-ethylmaleimide, e-aminocaproic acid and DFP). All AA were found at normal concentrations in ischemic kidney cell H_2O 3h after circulation was re-established ($p > 0.05$ or higher) and concentrations of all but Phe and Arg ($p < 0.01$) were normal 24h after ischemia. Cell:plasma (C:P) concentration ratios ranged up to 30:1 (Glu), and even Phe and Arg had C:P ratios of 6.3:1 and 2.9:1, respectively. With minimal delivery of AA to the brush border absorption site, we suggest that normal amino acid concentrations in cell water were maintained by uptake at the basolateral cell membrane, that cell energetics were adequate to maintain large C:P ratios despite severe injury, and that pathologic permeability to AA was not demonstrable.

INHIBITION OF PROSTAGLANDIN E_2 (PGE_2) RELEASE BY INDOMETHACIN (IND) DOES NOT DECREASE MUSCLE PROTEIN DEGRADATION IN ACUTELY UREMIC RATS. Stewart Laidlaw*, Robert Zipser*, Thomas Tasaki*, Sien Ha Wong Wu* and Joel Kopple. Harbor-UCLA Med Ctr, Torrance, CA.

Recent studies indicate that PGE_2 may stimulate release of muscle proteases. Since muscle protein wasting is increased in acutely uremic rats, we examined whether IND, an inhibitor of PGE_2 synthesis, could reduce protein degradation. Male rats underwent bilateral nephrectomy (n=3) or sham surgery (n=6). 30 hrs after surgery, the posterior hemicorpus was perfused for 2 hrs. In the acutely uremic rats as compared to controls, protein degradation was increased ($p<0.03$) and protein synthesis was slightly but not significantly reduced. Acutely uremic rats were then randomly assigned to one of 2 groups; during hemicorpus perfusion, one group was given IND (IND+, n=6), 14 μM in perfusate, and the other group was not (IND-, n=5). During the 30 hrs before perfusion, urea N appearance was similar in the 2 groups: 148±56 (SD) and 169±76 mg/30 hr, respectively. Net PGE_2 release into perfusate was lower in the IND+ rats (-6±4 vs 30±10 pg/g hemicorpus/hr; $p<0.001$). However, the IND+ and IND- rats did not differ in the rate of protein degradation (138±24 vs 151±25 nMol phenylalanine released/g/hr, respectively) or protein synthesis. Thus, although IND suppresses PGE_2 release from the hemicorpus of acutely uremic rats, it does not decrease the enhanced muscle protein degradation. These data suggest that either PGE_2 does not cause the increased muscle protein degradation in acutely uremic rats or that muscle protein degradation, once stimulated by PGE_2, is not reversible during short term inhibition of PGE_2 synthesis by IND.

TRANSMISSION ELECTRON MICROSCOPY (TEM) OF URINARY SEDIMENT (US) IN PATIENTS WITH AMINOGLYCOSIDE (A) THERAPY. Anil K. Mandal, George N. Mize,* and P. Allen Bowen, II. Med. Col. of Georgia, and Vet. Admin. Med. Ctr., Depts. of Med. and Pharm., Augusta, Georgia.

TEM of US showing renal tubule epithelial cells (RTEC) has been found to be valuable in the assessment of severity of acute renal failure (ARF). Myeloid bodies (MB) in the proximal tubule epithelial cells of renal tissue have been considered to be a specific marker of aminoglycoside nephrotoxicity (AN). Our study investigates: 1) whether TEM of US can distinguish AN from other causes of ARF, and 2) whether TEM findings correlate with the development of AN in patients (pts) receiving A. US from 22 A pts were studied. Controls were 9 ARF pts (5 post-transplant, 4 post-surgical) not receiving A, and 10 normals. ARF was diagnosed if serum creatinine rose greater than 0.5mg%. 19/22 (86.3%) of the pts received combination antibiotics with A (group A). Three pts received A alone (group B). There was no ARF in group B, two had negative TEM and one formed inadequate pellet for analysis. Two group A pts without ARF also formed no pellet for analysis. Twelve of 19 (63.1%) pts in group A had ARF and necrotic RTEC and MB were found in all 12. Five of 19 group A pts had no ARF. MB alone were found in 4 and TEM was negative in one. ARF non A pts showed necrotic RTEC without MB in all cases, and normal controls were all negative. We conclude: 1) TEM shows necrotic RTEC and MB in all pts with AN, thus TEM of US can distinguish AN from other causes of ARF and 2) Necrotic RTEC correlates better than MB with AN.

ACUTE RENAL FAILURE (ARF) PRODUCED BY NON-HYPOTENSIVE SEPSIS IN A LARGE ANIMAL MODEL. Adam L.Linton, J.Frank Walker*, Allan C.Cumming*, Robert M.Lindsay and Kim Solez. University of Western Ontario, London, Canada and Johns Hopkins University School of Medicine.

The pathogenesis of ARF in systemic sepsis remains unknown and most animal models of sepsis fail to reproduce the hemodynamic disturbances seen in man. We induced generalized sepsis in sheep (n=11) by cecal perforation and produced a high cardiac output(CO), low peripheral resistance (SVRI) state similar to that seen in sepsis in man. IV fluids were given to maintain pulmonary capillary wedge pressure (PCWP). Hemodynamic and renal functional measurements were recorded pre-sepsis (1) and at 24 hrs (2) and 48 hrs (3) post-sepsis:

	CO ($L/min/M^2$)	PCWP (mm Hg)	C.Cr. (ml/min)	FE_{Na} (%)	U.Osm. (mOsm/kg)	P.renin (ng/ml/hr)
1.	4.9	12	162	1.93	668	0.9
2.	5.3	11	114	0.45	634	2.5
3.	6.9	13	78	0.47	683	6.2

Tubular cell damage was evidenced by marked elevation in Fc lysozyme and by low molecular weight proteinuria. There were only minor tubular abnormalities on light microscopy but all showed hyperplasia of the juxtaglomerular apparatus. Electron microscopy showed no convincing tubular damage. All animals suffered a significant reduction in GFR. The urinary indices, the plasma renin level and the histology all suggested that the ARF was due to hypovolemia but all of the hemodynamic parameters (CO,BP,SVRI,PCWP) suggested that adequate volume and flow had been maintained. Apparently adequate fluid replacement does not maintain renal function in this model of generalized sepsis.

PROTECTION FROM ACUTE RENAL FAILURE(ARF)BY CALCIUM CHANNEL BLOCKERS(CCBs) IN HUMANS. N.L.Manohar, E. Retuta*,E.Jerome*,Maimonides Med Ctr,Brooklyn,N.Y.

CCBs are known to protect animals from experimental ARF. If such protection occurs in humans is unknown. In an uncontrolled preliminary study in 14 hospitalized pts who were on CCBs for cardiac reasons,a rise in S.Creat was seen after a noted renal insult.Data analysis:Mean age 69(range 58-86). Sex 8♂:6♀. Eight recovered (5♂:3♀),mean aged 69 (58-79),while six (3♂:3♀)aged 72(58-86)died in ARF. Pre-existing CRF due to HTN-Nephrosclerosis,AODM, membraneous GN,stones,& UTIs did not deter the recovery from ARF. Concomitant diuretic & aminoglycoside use negated any protection offered by CCBs. Acute reversal of CCB induced myocardial depression with I.V. $CaCl_2$ did not alter the protection of CCBs and such protection lasted for 7 days after withdrawal of CCBs. Concomitant β-blocker use had no adverse effect. Pre-existing cardiac,Pulmonary (COPD,ARDS) disease in the absence of sustained hypotension was not detrimental while presence of liver disease(cirrhosis,cholangitis)lead to poor out come. Pre-existing CRF or its severity had no effect on the CCB offered protection. Mean S.Creat prior to ARF was 4.0 in the recoverers while it was 2.2 in the non-recoverers. Only one of the recoverers needed transient dialysis while 5 of the non-recoverers needed dialysis. All 3 commercially available CCBs in USA(Verapmil,Nifedipine,Diltiazem) were effective when given on chronic maintenance basis even in small doses,while acute single doses by sublingual & I.V.routes offered little protection against ARF. We conclude that CCBs may minimize the severity & the duration of ARF in humans. Other variables which magnify or nullify such protection by CCBs in humans need to be established.

PROTECTION FROM ISCHAEMIC, ACUTE RENAL FAILURE - THE CONTRIBUTION OF VASCULAR DECONGESTION. June Mason*, J. Welsch*, J. Torhorst*, (intr. by L.C. Moore) Physiology Inst., Univ. Munich, FRG and Pathology Inst., Univ. Basel, Switzerland.

Rat kidneys display a vascular congestion with densely-packed erythrocytes after ischaemia, most conspicuous, but not limited to the outer medulla. The severity of this congestion has been found to reflect renal function after 45 min of ischaemia.

To determine the contribution of congestion to renal function, arterial haematocrit was lowered to 30% to impede its development. To determine if protection from renal failure is due to relief of congestion, a metabolic substrate (glucose), an ATP-precursor (adenosine) and a scavenger for free radicals (SOD) were employed as protective agents.

GFR was raised to a similar degree after each treatment. Congestion was completely absent after lowering haematocrit and was considerably reduced after treatment with glucose, adenosine or SOD.

GFR at 3 hr.	untreat.	↓Hct	glucose	aden.	SOD
µl/min/100 g	61	257	255	210	225
(mean ± SEM)	±10	±64	±46	±62	±32

It is concluded that early after ischaemia half the loss in GFR can be avoided if vascular congestion is prevented. Thus, one half of the fall in GFR can be attributed to vascular congestion and one half must be attributed to other mechanisms. Each of the protective procedures examined so far was able to restore GFR by about one half of the lost amount. This was achieved solely by relieving vascular congestion, not by other mechanisms.

ISCHEMIA INDUCES LOSS OF EPITHELIAL SURFACE MEMBRANE (SM) POLARITY AND ACCUMULATION OF PUTATIVE Ca^{2+} IONOPHORES. B.A. Molitoris,* R.W. Schrier, P. D. Wilson, and F. Simon.* UCMC, VAMC, Denver, CO.

To define the subcellular location of phospholipid (PL) degradation occuring during ischemia, rat renal brush border (BBM) and basolateral (BLM) membranes were isolated following 50 min of renal pedicle clamping. Ischemia decreased specific activity (SA) of BBM leucine aminopeptidase (LAP) to 67% of control ($p<.01$) but not enrichment (E)(15x). Neither SA nor E (10x) of BLM (Na^+,K^+)-ATPase was altered. Contamination of BBM and BLM by intracellular organelles was unchanged. Ischemic BBM did show an increase in (Na^+,K^+)-ATPase E (2.3 vs 4.3, $p<.01$). To determine if this was due to an isolation artifact or loss of the cell's ability to maintain SM polarity during ischemia, (Na^+,K^+)-ATPase localization was determined cytochemically. While (Na^+,K^+)-ATPase could only be detected in control BLM, specific deposits of reaction product were present in both BBM and BLM from ischemic kidneys, suggesting loss of SM polarity. Alterations in BBM cholesterol to PL ratio (0.80 vs 0.63, $p<.05$) and PL composition, i.e., sphingomyelin (38.5 vs 29.6%, $p<.01$) phosphatidylcholine (PC) (17.2 vs 29.7%, $p<.01$), occurred. These changes did not result from new PL synthesis, as the SA (^{32}P dpm/nmol Pi) of prelabeled individual PL was unaltered and suggest loss of SM polarity, as BBM and BLM differ in PL. Increases in lysoPC in BBM (1.0 vs 1.8%, $p<.05$) and BLM (1.3 vs 2.5%, $p<.05$) and BLM phosphatidic acid (0.4 vs 1.3%, $p<.05$), both reported to enhance Ca^{2+} influx, did occur. These data indicate ischemia impairs the cell's ability to maintain SM polarity and results in the accumulation of SM putative calcium ionophores.

UNILATERAL URETERIC OCCLUSION PROTECTS THE OBSTRUCTED KIDNEY FROM GENTAMICIN NEPHROTOXICITY. J.S. McNeil*, J. Roberson*, D. Hydlebeerg-Davis* and J.L. Atkins. WRAIR, Washington, D.C.

Most agents that protect the kidney from gentamicin induced renal failure have a mode of action that is not specific to the kidney. In search of a renal specific mechanism of protection we have examined the effect of unilateral ureteric occlusion on gentamicin nephrotoxicity.

Toxic doses of Gentamicin were given to Sprague-Dawley rats with an obstructed ureter. Gentamicin administration (20mg/Kg, ip, bid) was begun 3 days after occlusion of the left ureter. After seven days the ureteric obstruction was relieved and gentamicin therapy was stopped. In sham operated controls, serum creatinine continued to rise for 2 days after stopping the gentamicin therapy (0.4 to 4.2). In the animals which had had ureteric occlusion during the gentamicin administration, serum creatinine showed little increase during the two days following gentamicin therapy (0.3 to 0.7). It would appear that the obstructed kidney had not been as severely damaged by exposure to gentamicin. This model may be useful in the search for renal specific protective maneuvers in gentamicin nephrotoxicity. These results suggest that in some specific cases with both unilateral obstruction and gentamicin-induced renal failure, relief of the obstruction may have therapeutic value.

AN EVALUATION OF CALCIUM IN A MODEL OF POSTISCHEMIC ACUTE RENAL FAILURE. Mark L. Moody*, Sally B. Bates* and Peter M. Andrews. Georgetown University Medical Center, Washington, D.C.

There is evidence to suggest that calcium may play a major role in the etiology of postischemic acute renal failure (PIARF). In this investigation, we used in situ vascular perfusion to flush the left kidneys of Sprague-Dawley rats with (1) a protective isotonic phosphate buffered sucrose (50 mOsmol sucrose) solution (N11), or this solution containing (2) excess calcium (0.2 mg/ml calcium) (N14); (3) the calcium channel blocker, verapamil (0.125 mg/ml) (N9); or (4) the calcium chelator, EDTA (0.2%) (N11). Immediately after flushing the kidneys with one of the above four solutions, the renal pedicle was clamped to render the kidneys ischemic and hold the flushing solution in the kidneys. After one hour of normothermic ischemia, the pedicle clamp was removed and a contralateral nephrectomy of the right kidneys was performed. Following ischemia, only two of the rats with flushed kidneys (both EDTA treated) died. All the remaining rats in the four experimental groups recovered and exhibited normal serum creatinine levels within a week following ischemia. No statistically significant difference was evident in daily serum creatinine levels of the four experimental groups studied. It appears, therefore, that in this model system of PIARF, the presence or absence of excess calcium, a calcium channel blocker ,or a calcium chelator in a vascular flush during ischemia does not significantly affect recovery from ischemia.

A MODEL OF POST-TRAUMATIC RENAL INSUFFICIENCY: THE EFFECTS OF MYOGLOBIN AND HEMORRHAGE. J Moore, SF Gouge*, S Ingram*, JP Johnson. Walter Reed Army Med Ctr and Inst. of Research, Washington, DC.

While hemorrhagic hypotension is a common feature of post-traumatic acute renal failure (ARF) in man, ARF cannot be reliably produced in animals by this maneuver. We sought to determine whether ARF, similar to that seen after trauma, could be produced in conscious dogs using combinations of sublethal hemorrhage and myoglobin (Myo) infusion. All dogs were given a single dose of furosemide (2mg/kg) and 3 days of low salt diet and NH_4Cl drinking water to produce aciduria. Hemorrhage, to 50mm Hg mean arterial pressure for 2 hrs, was performed through femoral artery catheters. ARF was assessed by serial changes in serum creatinine 1-4 days after insult, and when present, was accompanied by increases and decreases in fractional sodium excretion and urinary osmolality, respectively. Low dose myo (0.5g/kg) produced mild, reversible ARF in half the animals, while high dose myo (1.0 g/kg) produced more severe, but still reversible ARF in all animals. Hemorrhage alone had no effect on renal function, while hemorrhage and low dose myo produced ARF similar to that seen in high dose myo alone. Hemorrhage plus high dose myo produced severe ARF, in which recovery did not occur. We conclude that hemorrhage to a degree insufficient to cause ARF alone can potentiate the nephrotoxic effects of myoglobin, and demonstrate that graded levels of ARF can be produced in unanesthetized animals by these methods. A model capable of producing ARF of varying severity may prove useful in the evaluation of prophylactic and attenuating maneuvers in post-traumatic acute renal failure.

ROLE OF THROMBOXANE A2 (TXA2) AND PROSTAGLANDIN I2 (PGI2) ON ALTERED GLOMERULAR FUNCTION IN GENTAMICIN TREATED RATS. Armando L. Negri,* Richard I. Levin* and Lance D. Dworkin. Dept. of Medicine, New York University Medical Center, New York, N.Y.

Indomethecin administration exacerbates the decline in glomerular filtration rate (GFR) observed in rats given gentamicin suggesting that vasodilator prostaglandins may oppose and offset the effects of vasoconstrictor hormones such as angiotensin II on GFR. To further examine the pattern of prostaglandin production in gentamicin nephrotoxicity we studied 2 groups of male Sprague-Dawley rats treated for 8 days with daily intraperitoneal injections of normal saline (C, n=12) or gentamicin sulfate (100 mg/kg/d; G, n=16). GFR was assessed by inulin clearance (C_{In}). Renal plasma flow (RPF) was calculated according to the Fick equation from plasma concentrations of inulin in femoral artery and renal vein. Production of TXB2 (metabolite of TXA2) and 6 keto $PGF1\alpha$ (metabolite of PGI2) by cortical slices, in vitro, during a 30 minute incubation was measured by radioimmunoassay. Results include:

GROUP	C_{In} (ml/min/kid/100g)	RPF	TXB2 (pg/mg wet wgt/30 min)	6ketoPGF1α
C	0.48±0.01	1.55±0.09	3.4±0.2	2.4±0.2
G	0.26±0.03*	1.05±0.15*	3.4±0.2	7.9±1.5*

Mean±S.E.M. *P<0.01 vs. C

We conclude that increased TXA2 production does not contribute to the fall in RPF and GFR observed in this model of acute renal failure, but that G induces a selective increase in PGI2 which may counteract the effects of vasoconstrictor substances and thereby preserve glomerular plasma flow and filtration rate.

PATHOPHYSIOLOGY OF IMPAIRED RECOVERY FROM ACUTE RENAL FAILURE (ARF) IN MAN. Mark Moran* and Bryan D. Myers. Dept. Medicine, Stanford University, Stanford, CA.

We attempted to define the role of transtubular backleak (TL) and tubular obstruction in 10 patients with postischemic ARF in whom no discernible recovery was evident after 19-53 days. Study after day 19 revealed inulin clearance=5±2 and urine flow 0.6±0.2 ml/min. The clearance of unrestricted filtration markers of graded size including DTPA (radius [r]=4Å), inulin (r=15.5Å) and 6 dextran fractions (r=17-23Å) were used to detect TL, while larger dextrans (r=24-40Å) were used to characterize tubule permeability to filtration markers. Whereas filtration marker clearance relative to inulin (θ) was 1.0 for DTPA, θ for smaller dextrans was progressively elevated above unity, with a peak value of 1.3±0.3 for the 23Å fraction. θ for larger dextrans, r=24-40Å, was significantly elevated above control values. A model of mass conservation revealed that 44% of filtered inulin and DTPA was lost through TL. TL of filtered dextran varied inversely with r, declining from 40% (17Å) to 0% (34Å). The blood-to-bladder residence time (τ) of an IV inulin bolus, computed from a 3-compartment model of inulin kinetics, was 34±9 vs. 9±1 min in normal controls. When the model was adjusted for TL and filtration marker recirculation, τ was nonetheless prolonged to 20±7 min, exceeding 9 min in 6/10 patients, and suggesting a reduced rate of tubule fluid flow. We conclude that failure to recover from ARF within 2 or more weeks is associated with (1) persistent TL of glomerular filtrate and (2) reduced velocity of tubular fluid flow, due possibly to intraluminal obstruction.

DO CALCIUM ANTAGONISTS IMPROVE THE COURSE OF POST-ISCHEMIC ACUTE RENAL FAILURE IN CONSCIOUS DOGS? H-H Neumayer, K Wagner, M Raffelt, v Achenbach, A Distler, Freie Universitat Berlin, and R Dusing, Universitat Bonn, FRG.

Calcium channel blocking agents may ameliorate acute renal failure in anesthetized animals in some experimental models (Kidney Int. 25, 519 (1984)).

We have investigated the effect of Diltiazem (D) in a postischemic model of ARF in conscious dogs. An electromagnetic flow probe and a pneumatic cuff were placed around the left renal artery in 19 uninephrectomized Beagle dogs on a high sodium diet. ARF was induced by inflating the cuff (180 min). Group A (n=6) received a continuous infusion of 0.9% NaCl (7 days), group B (n=6) received one of $5\mu g \cdot kg^{-1} \cdot min^{-1}$ D (7 days) immediately after deflating the cuff (posttreatment), whereas, in group C (n=7), the Diltiazem infusion was started 3 days prior to ischemia and then also continued for 7 days (pretreatment).

Before ischemia		Group	After ischemia 1st	3rd	7th day
RBF ($ml \cdot min^{-1}$)	131	A	120	84	128
		B	207*	117	95
		C	149	107	72
GFR ($ml \cdot min^{-1}$)	33	A	5	10	11
		B	8	10	16
		C	12*	15	17*

Median, *p<0.05, tested vs. control group A

Conclusion: In our model, Diltiazem improves the course of postischemic ARF only in the pretreatment group but not in the posttreatment group.

RENAL FUNCTION STUDIES DURING CISPLATIN TREATMENT AND CAPTOPRIL OR VERAPAMIL PROTECTION.
Joop J.G. Offerman*, Ab J.M. Donker*, Sijtze Meijer*, and Dirk Th. Sleyffer* (intr. by L.W. Statius van Eps). Univ. of Groningen, The Netherlands.

Patients (n=24) with histologically proven non-seminomatous testicular tumor were treated with Cis diammine dichloroplatinum (CDDP) according to the Einhorn regimen. Renal function studies (clearances of 125 I thalamate and 131 I hippurate) were performed before, during and after the first CDDP infusion on day one, before the second course of CDDP (=day 21) and finally, on the 10th day of the 4th course (=day 73).

The first 8 patients were treated with CDDP alone. On day 1, a persistent fall in effective renal plasma flow (ERPF) was found without a change in glomerular filtration rate (GFR). On day 21, also GFR was significantly reduced. On day 73, a further decrease in GFR and ERPF was noticed.

The next 8 patients were treated with CDDP and captopril (50 mg t.i.d.), and the last 8 patients with CDDP and verapamil (80 mg t.i.d.). Both captopril and verapamil were started one day before the first CDDP infusion and continued for the whole 73 days. In both groups of patients, the initial decrease in ERPF on day one now did not occur. However, on days 21 and 73, the decreases in GFR were not significantly less compared to that from the first 8 patients only receiving CDDP.

We conclude that the first renal function change during CDDP treatment is a fall in ERPF. This fall can be prevented by both captopril and verapamil. Despite this, these drugs do not protect against the CDDP-induced fall in GFR.

GLYCOLYSIS, ATP AND MEMBRANE INTEGRITY IN LLC-PK$_1$ CELLS.
Y. Patel,* J. I. Kreisberg and M. A. Venkatachalam. Univ Texas HSC, San Antonio, TX.

Cells were grown in the presence of ^{14}C-acetate to label lipids to steady state. Confluent cells were exposed 5 hrs to medium containing label with or without glucose(GLU), with or without an inhibitor of aerobic metabolism, antimycin(AM). Lipids were separated and quantified by counting for ^{14}C. Without AM,omission of GLU resulted in significant declines of phospholipids(PL), no major declines of ATP, and cells were viable. With AM, anaerobic glycolysis was the sole source of ATP; GLU restriction resulted in depletion of ATP and significant declines of PL. No loss of viability occurred as long as ATP was measurable. With zero GLU and with AM, ATP was reduced to zero, PL were severely depleted, and unesterified fatty acids(FA) accumulated. Cells were nonviable within 3 hours.

	NO ANTIMYCIN			1.5 μM ANTIMYCIN		
GLU	0	10	450	0	10	450
ATP	15.9 ±0.64	16.7 ±1.7	17.3 ±0.6	0.0	1.29 ±0.5	10.4 ±0.6
PC	14.6 ±0.6	N/D	21.2 ±0.2	11.1 ±0.9	13.3 ±0.5	20.9 ±0.6
PE	3.8 ±0.8	N/D	4.9 ±0.4	2.8 ±0.1	3.92 ±0.1	5.5 ±0.03
FA	3.6 ±0.3	N/D	2.4 ±0.06	21.3 ±1.3	4.8 ±0.1	4.0 ±0.1

GLU--mg/dl; ATP--nM/mg prot.; PC--phosphatidylcholine; PE--phosphatidylethanolamine. Lipids--DPM X 10^3/mg prot. N/D--not done.

Glycolysis provides substrates for lipid synthesis. Thus, depletion of glycolytic metabolites and ATP may both contribute to loss of membrane integrity.

FUCOSYLATION OF MICROVILLAR MEMBRANE (MVM) GLYCOPROTEIN AFTER RENAL ISCHEMIA.
Jean K. Paddock, Arthur R. Taddeo,* John Paddock,* and Leah M. Lowenstein. Thomas Jefferson University, Dept. of Biochemistry and Medicine, Philadelphia, Pa.

Fucose has been shown to be an integral part of the normal MVM and ischemia damages the MVM of the proximal tubule cell. However, at four hr of reflow, the MVM regenerates. We studied the effect of 25 min of ischemia, induced by left renal artery occlusion, on the fucosylation of the regenerating membrane. Injection of ^{3}H-fucose at zero reflow, followed by 15 min of reflow, resulted in a decrease in the incorporation of the label into TCA insoluble glycoprotein in homogenate, 63.0±9.0% and MVM, 67±5.2%, $p<0.05$, of the ischemic kidney. Labelling of the MVM 4 hr prior to ischemia showed no differences in ^{3}H-fucose specific activity (dpm/mg protein) of ischemic kidney vs contralateral or sham. However, the amount of ^{3}H-fucose (dpm/total fraction) was significantly reduced by 42.5±9.2%, $p<0.05$. When ^{3}H-fucose was injected at zero reflow, followed by 4 hr of reflow, incorporation of the label into the MVM of the ischemic kidney was lower in dpm/total fraction, 63.6±15%, $p<0.05$ and in dpm/mg protein, 73.7±6.9%, $p<0.05$, while MVM from the kidneys of rats prelabelled for 21 hr showed no significant differences.

These data suggest that 1) fucosylated glycoproteins are lost from the MVM in a moiety with other protein, 2) fucosylation of MVM is decreased during regeneration and 3) recycling of membrane glycoprotein may be important in re-establishing the MVM after ischemia.

RENAL FUNCTION IN CALVES WITH TOTAL ARTIFICIAL HEARTS (TAH).
R. Quinn,* D. Olson,* G. Pantelos,* D. L. Holmberg,* C. Kablitz, R. L. Baranowski, C. Westenfelder. Divisions of Artificial Organs and Nephrology, University of Utah and VA Medical Centers, Salt Lake City, Utah.

We reported previously that the first patient with a TAH developed recurrent episodes of acute renal failure and other abnormalities in renal function. These complications were felt to be the result of antibiotic nephrotoxicity and the TAH per se. In order to corroborate this impression, renal function was monitored in six healthy male Holstein calves, weighing 78.6±6.9 kg (mean±SE) at the time of TAH implantation. All animals received gentamicin postoperatively. Survival with the TAH was 243±9.7 days. Serum creatinine and BUN values were 1.0±0.1 and 14.2±2.0 mg/dl preoperatively. During the first post-implant week, the respective values were 1.2±0.3 and 12.5±3.5 and during the last week of life 1.0±0.1 and 18.2±3.5 mg/dl. There were no statistically significant differences between these variables. One animal developed non-oliguric acute renal failure after TAH implantation, probably caused by sepsis, hemoglobinemia and gentamicin toxicity. Acid-base and fluid and electrolyte balance was unchanged. Conclusion: Implantation of a TAH in growing calves causes neither short nor long-term deteriorations in renal function. It specifically appears that the TAH per se does not increase the renal susceptability to nephrotoxic insults.

EFFECT OF GENTAMICIN ON LIPID PEROXIDATION IN RAT RENAL CORTEX. L.Ramsammy,* K.Ling,* C. Josepovitz,* R. Levine* and G.J.Kaloyanides, Division of Nephrology, SUNY-Stony Brook and VAMC, Northport, NY.

Lipid peroxidation (LP) has been implicated in the pathogenesis of tubular cell injury caused by several nephrotoxins. In the present study we explored the possible role of LP in gentamicin (G) nephrotoxicity. Rats were injected with G, 100mg/kg per day, for 2 to 4 days and were sacrificed at 24 hr (a) or 48 hr (b) after the last injection. LP was assessed by the change in the ratio of double bonds (Δ) per mole fatty acid (FA), the decrease in arachidonic acid (AA-20:4), the formation of the LP product malonaldehyde (MA) and alterations in superoxide dismutase (SOD), catalase (CAT) and reduced (GSH) and oxidized (GSSG) glutathione in renal cortex. The results are summarized below:

	Δ/mol FA	AA(20:4) %	MA nmol/mg	CAT k/min	GSSG %
C	1.62+.01	27.6+.5	.67+.02	.22+.01	2.0+.1
Gx2	1.46+.04†	21.9+1.4†	-	-	-
Gx3	1.36+.07†	18.8+2.0†	.69+.04	.15+.01†	2.5+.2†
Gx4a	1.20+.02†	14.4+1.1†	.75+.04	.14+.01†	3.5+.2†
Gx4b	-	-	.93+.05†	.14+.01†	4.1+.4†

†=significantly different from control (C). The decrease in Δ/mol FA and AA after 2 doses of G indicates increased LP. After 2 doses CAT was depressed, % GSSG was increased but SOD was unchanged. The decrease in CAT suggests an inhibitory effect of G on this enzyme. This generates further LP exceeding the capacity of the GSH-GSSG antioxidant system and results in increased MA formation. We conclude that G induces LP in renal cortex by at least two mechanisms. Moreover, the early onset of these changes implicate LP as a proximal event in the injury cascade of G nephrotoxicity.

ANOXIA INCREASES CALCIUM (Ca) INFLUX IN RAT NEPHRON SEGMENTS. A. Schieppati,* V. Van Putten,* T. Burke, and R. Schrier. Dept. Med., Univ. Colorado Med. Sch., Denver, CO.

Increased renal cellular and mitochondrial Ca concentrations are seen during reflow following in vivo renal ischemia in the rat. The present study was undertaken to examine whether in vitro anoxia is associated with an increased rate of cellular Ca influx. A ^{45}Ca radiotracer method was used to examine Ca influx. Isolated proximal tubules were exposed to 95% O_2-5% CO_2 (Cont) or 100% N_2 (anoxia). Serial measurements following 30 min of anoxia showed a significant increase in Ca influx (nmol/mg prot):

Time (min)	1	5	10	20	30	60
Cont	3.29 ±.49	4.88 ±.54	5.33 ±.78	6.05 ±.71	6.65 ±.72	7.17 ±1.4
Anoxia	8.52 ±.58	11.2 ±.89	12.0 ±.56	13.0 ±.52	12.9 ±.52	15.2 ±1.4
p value	<.005	<.005	<.005	<.005	<.005	<.005

Kinetic analysis of the above Ca influx curves by non-linear least square method of Uchikawa and Borle demonstrated that anoxia increases the compartment size (nmol/mg prot) of both cell surface (Cont 5.63 vs anoxia 11.31) and intracellular compartment (Cont 8.53 vs anoxia 16.2). Ca influx rate (nmol/min/mg) was also increased for cell surface (Cont 2.06 vs anoxia 5.23) and for intracellular compartment (Cont 0.050 vs anoxia 0.081). The present studies therefore demonstrate in nephron segments that in vitro anoxia results in increased cellular Ca influx; both cell surface and intracellular compartment demonstrated increased Ca flux rate and compartment size.

NEPHROTOXICITY OF AMPHOTERICIN B IN THE DOG: COMPARISON OF RAPID INTRAVENOUS VERSUS SLOW INTRAVENOUS ADMINISTRATION. Stanley I. Rubin,* Donald R. Krawiec and Howard B. Gelberg.* Univ. of Illinois at Urbana-Champaign, Dept. of Vet. Clin. Med., Urbana, Illinois.

The nephrotoxicity of two methods of administration of amphotericin B (AB) were compared in 12 normal dogs. Six dogs received alternate day doses of 1 mg/kg of AB administered as a rapid infusion in 25 ml dextrose 5% in water over 5 minutes. Another 6 dogs received alternate day AB at a dose of 1 mg/kg by slow infusion in 1 liter. of dextrose 5% in water over 5 hours. Dogs in both groups received a total cumulative dose of 6 mg/kg. Both treatment groups experienced significant reductions in glomerular filtration rate (GFR) as measured by inulin and endogenous creatinine clearance. This reduction in GFR was most marked in the group receiving the AB as a rapid infusion. The fractional excretion of Na, K, P, and Ca increased as GFR decreased, with a significantly greater fractional excretion occurring in the rapid-infusion group. Increased 24-hour urinary excretion of calcium was noted in both treatment groups with a greater loss occurring in the rapidly infused dogs. There was no evidence of increase loss of urinary sodium and potassium and no alteration in acid-base balance on the basis of venous blood gases. The dogs which received AB as a rapid infusion had a significantly greater number of tubular lesions than the slow-infusion group. It is concluded that slow infusion of AB causes less renal functional impairment and less renal morphological damage than fast infusion.

GENTAMICIN-INDUCED ALTERATIONS IN PIG KIDNEY EPITHELIAL CELLS (LLC-PK_1) IN CULTURE. D.W. Schwertz,* O.F. Gonzalez, J.I. Kreisberg and M.A. Venkatachalam. Univ Texas HSC, San Antonio, Texas.

The effect of gentamicin (gent.) exposure was investigated in LLC-PK_1 cells in culture. Gent. (0.5-2.5 mM) was added to the media of cells which had been grown to confluency in the absence of antibiotics and antimycotics. Exposure to gent. (1-4 days) did not affect total cellular protein or DNA levels, total cell number, or the release of lactate dehydrogenase, γ-glutamyltranspeptidase, β-glucuronidase or N-acetyl β-D-glucosaminidase to the media. ATP levels in gent.-treated cells did not differ from control cells; however, media from the gent.-treated cells contained significantly lower lactic acid levels. Morphological examination by electron microscopy revealed gent.-elicited myeloid body formation. Further, total phospholipid (PL) level was markedly elevated in gent.-treated cells. Analysis of specific PL classes showed only phosphatidylcholine (PC) and phosphatidylinositol (PI) levels increased in a concentration and time-dependent manner (1mM gent., 4 days 133 ± 10 and 144 ± 12 percent of control, respectively). Raising the normal media Ca2+ concentration (1.36 mM) 1.5, 2.0 or 3.0-fold did not alter gent.-induced elevation in cellular PC and PI. However, total Ca2+ transported into the monolayer was inhibited in the presence of gent. Gent.-induced alterations in cultured LLC-PK_1 cell phospholipids are similar to those detected in the rat in vivo (Knauss et al, Am J Physiol, 1983). The results suggest the LLC-PK_1 cell provides a reproducible model for elucidating the mechanism of gent.-elicited alterations in renal epithelium.

TRANSPORT DEPENDENT CELL INJURY IN THE S3 SEGMENT OF THE PROXIMAL TUBULE. P. Shanley*, M. Brezis, K. Spokes*, P. Silva, F.H. Epstein and S. Rosen. Depts. of Pathology and Medicine, Harvard Med. Sch. and Beth Israel Hosp., Boston, MA.

Two distinct types of injury, cytoplasmic edema (E=cell swelling + luminal occlusion) and cell fragmentation (CF=mitochondrial swelling + membrane disruption), occur in the S3 segment of the proximal tubule in isolated hypoxic perfused rat kidneys (Krebs-albumin medium gassed without O_2). The % of S3 tubules with CF correlated with the GFR and urine output during the perfusion ($r>.9$) and approached 100% when the GFR was increased by high perfusion pressure. Conversely, the CF lesion was absent and the E lesion extensive when tubular transport was inhibited by perfusion with hyper-oncotic medium to prevent glomerular filtration or by addition of ouabain (10^{-2}M) to the perfusate. The protective effect of ouabain was overcome by very high perfusion pressure.

Polyene antibiotics increase membrane permeability and thus the work of active electrolyte transport. Perfusion with amphotericin ($3x10^{-3}$M) or nystatin (200 u/ml) in oxygenated medium also produced CF in S3. The lesion was prevented in the non-filtering kidney. Ouabain completely eliminated the CF due to nystatin and reduced that due to amphotericin from 50%±13 (SD) tubules involved to 15%±9 ($p<0.001$).

These results suggest that CF is a type of injury in S3 in which transport activity plays an important role. The E lesion seems fundamentally different and more akin to lesions described in ischemia where tubular flow is absent, active transport is diminished, and the morphology appears related to loss of cell volume regulation. Thus, depending on the rate of energy utilization in active electrolyte transport, two modes of anoxic damage are possible.

PATHOLOGICAL RENAL CHANGES WITH BIOLOGICALLY INACTIVATED GENTAMICIN. G. Stein,* N. Mummaw,* M. Burnatowska-Hledin,* S. Sleight,* M. Gurwith,* K. Champney,* G. Mayor. Michigan State University, Dept. of Medicine, East Lansing, Michigan.

Administration of active gentamicin may result in adverse changes in renal function and histology. To examine if biological inactivation of gentamicin with carbenicillin prior to administration produces the same changes, gentamicin and carbenicillin were given subcutaneously to 200g male Fischer 344 rats BID for 10 days. All rats received equal amounts of NaCl either in the form of carbenicillin disodium or as saline. Group I (n=3) received only saline. Group II (n=8) was given active gentamicin (100 mg/kg/d). Group III (n=8) received active gentamicin (100 mg/kg/d) and carbenicillin (5 g/kg/d). Group IV (n=8) was given gentamicin (100 mg/kg/d) that had been biologically inactivated by carbenicillin (5 g/kg/d) in vitro. BUN and creatinine were measured in serum on days 0,4,8, and 11. Rats were sacrificed on day 11 and kidney tissue was examined microscopically for pathological changes. Pathology was rated on a scale of 0-4 with 4 being the most severe change. Results of day 11 (means) are:

Group	BUN mg/dl	Creatinine mg/dl	Pathology
I	21.6	0.73	0
II	121.6*	4.60*	4
III	22.7	0.70	1
IV	21.2	0.61	3

*$p < 0.01$ vs Group I,II,IV or vs Day 0 (not shown)

Biological inactivation of gentamicin with carbenicillin did not influence serum urea nitrogen or creatinine but did produce pathological changes in the renal cortex.

MODEST HYPERCALCEMIA CAN MARKEDLY INCREASE THE NEPHROTOXICITY OF BENCE JONES PROTEIN. P Smolens and R Kreisberg*. Univ of Texas Hlth Sci Ctr, Dept of Med, San Antonio, Texas.

Hypercalcemia is frequently observed in patients with multiple myeloma and renal failure. Whether Bence Jones Protein (BJP) is directly nephrotoxic and how and whether the hypercalcemia might contribute to this putative nephrotoxicity is currently unclear. To examine this issue, we studied the effect of modest hypercalcemia on the GFR of rats exposed to a BJP which by itself had been found previously to be non-nephrotoxic (Kid Int 24: 192, 1983). S/D rats weighing 280-330 g were anesthetized and prepared for measurement of inulin clearance (C_{in}). The initial C_{in} was performed with the rats receiving Ringers solution at 4.7 ml/h. Following this, Group I rats (N=6) were given 100 mg of BJP and the Ringers infusion was continued for 2 hours. Group II rats (N=4) received no BJP but were given a $CaCl_2$ containing infusion instead of the Ringers solution. The mean Serum Ca (S_{Ca}) at 90-120 min was 12.8 ± 0.2 mg%. Group III rats (N=5) received both the 100 mg of BJP and the $CaCl_2$ containing infusate. At the end of the 2 hour infusion period, a second C_{in} was performed. Only small decrements in C_{in} were seen in the rats receiving either the BJP or $CaCl_2$ infusate alone (Group I = -9.2 ± 4.4%; Group II = -10.3 ± 5.8 %). In contrast, Group III rats had a marked decrement in C_{in} (-58.4 ± 3.81%, $p < 0.001$ vs. Group I or II) despite the fact that the rise in S_{Ca} was not different from that in Group II. These results indicate the presence of a unique interaction between hypercalcemia and the BJP which results in nephrotoxicity much greater than that which would be predicted from the effect of either agent given alone.

GENTAMICIN INDUCED GENERATION OF HYDROGEN PEROXIDE BY RENAL MITOCHONDRIA (MITO). **Patrick D. Walker, Clare Das*, Sudhir V. Shah. Tulane Medical School, Depts. Pathology and Medicine (Nephrol.).**

The effect of gentamicin on mito respiration has been postulated to be the initial event in gentamicin nephrotoxicity. Gentamicin's effect on the production of reactive oxygen species (ROS) by mito has not been previously examined. The aim of this study was to examine the effect of gentamicin on the production of hydrogen peroxide (measured spectrofluorometrically as the decrease in scopoletin fluorescence) in rat renal cortical mito isolated by differential centrifugation. Mito with succinate as substrate but without gentamicin produced approximately 0.04 nm/mg/min of hydrogen peroxide. Gentamicin produced a dose dependent increase in hydrogen peroxide as follows:

		Gentamicin (mM)				
		0.5	1	2	3	4
H_2O_2	mean	0.28	0.50	0.65	1.53	1.96
(nm/mg/min)	SEM±	0.01	0.04	0.04	0.16	0.16
	N	6	6	6	6	6

The gentamicin induced change in fluorescence was completely inhibited by catalase (but not by heat-inactivated catalase) indicating that the decrement in fluorescence was due to hydrogen peroxide. Hydrogen peroxide was not produced when gentamicin was added to incubation media in which mito or substrate were omitted or heat-inactivated mito were used. Thus our study demonstrates that gentamicin enhances the production of hydrogen peroxide by mitochondria. Gentamicin induced generation of ROS may play an important role in gentamicin nephrotoxicity.

NITROFURANS CAUSE CHRONIC INTERSTITIAL NEPHRITIS AND ARE ACTIVATED BY PROSTAGLANDIN H SYNTHASE: A PROPOSED MOLECULAR MECHANISM OF CHRONIC NEPHROTOXIC REACTIONS. Frederick Walters,* Bernard B. Davis, Sonny L. Johannsson,* Samuel M. Cohen,* and Terry V. Zenser.* VA Med. Ctr., GRECC, St. Louis Univ. Schl. Med., St. Louis, Missouri and Univ. of Nebraska Med. Ctr., Omaha, Nebraska.

Nephrotoxic reactions cause a sizable proportion of chronic renal failures. Delineation of molecular mechanisms of nephrotoxicity would allow development of treatment and prevention methodologies. 3-hydroxymethyl-1-[(3-(5-nitro-2-furyl)-allydidene)amino]-hydantoin (HMN) and formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide (FNT) are 5-nitrofurans which are reported to cause chronic nephrotoxicity when fed to rats as part of the diet. This was evaluated by feeding HMN as 0.2% of diet. After 9 weeks, 8/11 rats had histological evidence of renal papillary necrosis and at 11 weeks, 80% had chronic interstitial nephritis. Rats fed FNT developed adenomatous hyperplasia and severe cystic nephritis. HMN was metabolized by inner medullary microsomes in the presence of arachidonic acid (AA), 8.5 ± 0.3 nmol/ng protein/min. AA-dependent metabolism was not detected in the presence of indomethacin or aspirin. A similar metabolic profile was noted with FNT. Horseradish peroxidase also metabolized FNT and like PHS, catalyzed the formation of a thioether conjugate identified by NMR spectroscopy to be bound to the 5 position of the thiazole ring of FNT. These studies indicate the hydroperoxidase component of PHS oxidized these 5-nitrofurans to reactive intermediates. It is suggested this mechanism is involved in chronic nephrotoxic reactions.

EFFECTS OF ALLOPURINOL ON ISCHEMIC INJURY TO ISOLATED TUBULES. M. White*, D. Hunt*, H.D. Humes and J.M. Weinberg, VA Medical Center and Univ. of Michigan, Ann Arbor, Michigan.

The xanthine oxidase inhibitor, allopurinol (A), has been reported to be protective during ischemic acute renal failure as a result of effects to preserve the intracellular adenine nucleotide pool and to prevent free radical generation resulting from purine degradation. To better define the efficacy and mechanisms of action of A directly on renal tubule cells we studied the effects of 200 uM A during 60' of ischemia (I) and 60' of oxygenated recovery (R) in a well characterized suspension of isolated, predominantly proximal tubules from the rabbit. Uncoupled respiratory rates (RR), previously established to be a good general index of tubule integrity, are in natoms/min/mg protein. Adenine nucleotides are in nmol/mg protein. All values are means ±SE for N=6. *p<.05 or better vs. time control (TC).

	TC	I	R	I+A	R+A
RR	145±3	-	88±9*	-	94±7*
AMP	.2±0	2.2±.2*	.2±0	2.2±.2*	.2±0
ADP	1.2±.2	.7±0	.9±.1	.7±0	.8±.1
ATP	8.4±.4	.3±0*	4.5±.5*	.3±0*	4.6±.5*

Thus, A did not influence changes in cell integrity or intracellular adenine nucleotides occurring during and after 60' I. A was similarly ineffective when studied with 30' of ischemia, with 15' or 30' of hypoxia, or when used in combination with exogenous ATP. These observations do not support a role for allopurinol in altering determinants of ischemic acute renal failure at the tubule cell level and suggest that any efficacy in vivo is due to effects on other nephronal processes.

"BACKLEAKAGE" OF FUROSEMIDE (FS) PREVENTS ITS URINARY EXCRETION IN $HgCl_2$-INDUCED ACUTE RENAL FAILURE (ARF). C. Westenfelder and R. L. Baranowski. University of Utah and VA Medical Centers, Salt Lake City, Utah.

We reported previously that rats with glycerol-induced ARF showed no diuretic response to FS because of defective tubular secretion. It has been demonstrated further that animals with uranyl-nitrate or $HgCl_2$-induced ARF develop a proximal tubular lesion that permits backleakage of ultrafiltrate. It was therefore the purpose of the present study to examine whether back-diffusion of filtered FS occurs in $HgCl_2$-induced ARF. Two groups of adult Sprague-Dawley rats were studied. Both received 2 mg/kg BW FS i.v. The control group (C, n=6) received FS only, whereas the ARF group (n=6) was given FS 24 hrs after HgCl2 (3 mg/kg BW s.c.). Plasma FS levels in the C group were 9.5±1.5 (mean ± SE) and in the ARF group 17.5±1.5 ug/ml (p<0.01). Plasma binding of FS was 92.3±1.5% in C and 71.3±6.3% in ARF animals (p<0.01). FS caused a marked natriuresis in C animals only. GFR in ARF animals was 10% of that of C. The filtered load of FS in C and ARF animals was 1.5±0.2 and 1.5±0.1 ug/min respectively. Urinary excretion of FS in C rats was 9.8±1.5, in ARF rats 0.3±0.1 ug/min (p<.01). Negative secretion, i.e. backleakage occurred in ARF rats at 0.9±0.4 ug/min. These data demonstrate that transtubular back-diffusion prevents the urinary excretion of FS in this model of ARF.

ELECTRON SPECTROSCOPIC IMAGING (ESI) OF INTRACELLULAR ELEMENTS IN NORMAL KIDNEY AND IN ISCHEMIC ACUTE RENAL FAILURE (ARF). D.R. Wilson, E. Spitzer,* F.P. Ottensmeyer,* and G.T. Simon,* Dept. of Pathology, McMaster University, Depts. of Medicine and Medical Biophysics, University of Toronto, Canada.

Electron spectroscopic imaging (ESI) is a new technique which permits the mapping of elements at high mass and spatial resolution within subcellular organelles, allowing visualization of the intracellular distribution of Ca, P, and S. (Ottensmeyer, J. Ultrastruct. Res. 71:336, 1980, J. Cell Biol. 98:911, 1984.) Using a freeze-drying embedding technique to minimize translocation of diffusible elements, proximal tubular cells of rat kidney were studied. In normal cells Ca was mainly in the mitochondrial (mito) cristae, between the layers of mito membrane, and in the endoplasmic reticulum and plasma membrane, with very sparse distribution in the cytosol. Sulphur distribution was similar to Ca. Phosphorus was similarly distributed to Ca but was also seen in membranes without Ca. In 24 hour ischemic ARF produced by 45 minute clamping of renal artery, cytosolic Ca was increased and Ca was diffusely distributed in mito matrix. P was also more widely distributed and more extensively associated with Ca. Some S was associated with Ca in mito matrix. Previous studies using energy dispersive spectroscopy confirm the increase in cytosolic and mito Ca in ischemic ARF (IX Congress, p.336A). The results demonstrate the intracellular distribution of Ca, P, and S in normal proximal tubular cells and the marked changes which occur after ischemic renal injury.

GLOMERULAR DYNAMICS IN $HgCl_2$ ACUTE RENAL FAILURE: THE ROLE OF INCREASED GLOMERULAR CAPILLARY RESISTANCE. Wolfert, A.I., Laveri, L.A.,* Riley, K.M.,* Oken, K.R.,* and Oken, D.E. Dept. of Medicine, V.A. Hospital and Medical College of Virginia, Richmond, Virginia.

Glomerular dynamic abnormalities of Munich-Wistar rats were examined 16-26h p 9mg/kg $HgCl_2$ injection. Virtual absence of filtration was manifest by: 1) retention of low viscosity silicone or lissamine green (LG) injected at the glom.-tubular junction (GTJ) s movement over sev. min.; 2) inability to collect filtrate from either Bowman's space (B.S.) or the GTJ 3) nephron ^{14}C-inulin clearances. After i.v. L.G. injection, pale staining of a segment of B.S. was common, but coloration disappeared in 5-20 sec s ever entering the GTJ or the rest of Bowman's space. Bowman's space pressure (P_{bs}), 11.0±SEM 0.7mmHg, was not sig. elevated ($p>0.2$), and mean glom. cap. pressure (P_g) was reduced from 48 to 23.8±1.3mmHg ($p<0.001$). Individual gloms. showed an abnormally wide range of P_{bs} (up to 18mmHg) on multiple measurement. Net filt. pressure estimated from means for each glom. was essentially 0mmHg, but markedly positive and negative values were obtained in the same glom. due to intraglom. variability in P_g. Since P_g cannot fluctuate + and + axially along the capillary, intraglom. variation in P_g is presumed to reflect random sampling of a marked but orderly axial + P_g secondary to greatly + glom. capillary resistance. Such change is consistent c the segmental appearance of LG at the proximal end of the cap. with positive filtration pressure and reabsorption more distally with negative filtration pressure. Increased glomerular capillary resistance appears to play a key role in $HgCl_2$ acute renal failure.

PATHOPHYSIOLOGY OF CHRONIC RENAL FAILURE

THE INHIBITORY ROLE OF DIETARY PROTEIN RESTRICTION ON THE DEVELOPMENT AND EXPRESSION OF IMMUNE-MEDIATED αTBM DISEASE PRODUCING TUBULOINTERSTITIAL NEPHRITIS (TIN) IN RATS. D.Agus*, R.Mann*, D. Cohn*, M.Clayman*, C.Kelly*, and E.Neilson, Renal Section, Univ. of Penn., Phila., PA.

To test the role of dietary protein on the development of αTBM disease, pair-fed BN rats received low protein (3% Casein;LP) or normal protein (27% Casein;NP) normocaloric diets. After six weeks each group were immunized with renal tubular antigen in adjuvant to produce αTBM-Ab and TIN. The kidneys harvested from NP rats after 4 more weeks on the diet had worse TIN than the LP rats (histologic severity(out of 4.0); NP = 3.1±0.2 vs. LP = 1.1±0.3; $P<0.001$), and serum creatinines were concordantly different (NP = 1.34 vs. LP = 0.82; $P<0.05$). Titers of αTBM-Ab were similar in both groups, while T cell-mediated immunity (DTH footpad reactions) were non-specifically depressed in LP rats when compared to NP rats (NP: SRTA = 25.8±0.8 $X10^{-3}$ inches/PPD = 27.5±1.6 vs. LP:SRTA = 5.2 ± 0.6/PPD = 6.5±0.9; $P<0.001$). Admixture co-transfers of LP+NP cells failed to demonstrate active suppression as an explanation for the depressed DTH in LP rats (LP+NP cells: SRTA = 26.5±0.9/PPD = 28.2±1.5 vs. 2XNP: SRTA = 27.5±1.2/PPD = 27.5±0.9).

To test the therapeutic role of dietary protein restriction, rats were first immunized and fed NP diets for 4 weeks (histologic severity = 3.0±0.2; creatinine = 1.78), then divided into 2 groups, and followed for 6 more weeks either on LP or NP diets. LP rats, under these conditions, developed less disease than those fed NP diet (histologic severity; NP = 3.2±0.3 vs. LP = 1.4±0.2; $P<0.001$), and creatinines were concordantly different (NP = 1.92 vs. LP = 0.97; $P<0.05$). Again, the titers of αTBM-Ab in both LP and NP groups were similar.

These data suggest that LP diet prevents both the development and progression of TIN by the selective abrogration of effector T cell immunity.

RESPONSES OF THE NORMAL RAT KIDNEY TO SEQUENTIAL ISCHEMIC EVENTS. R. A. Zager, The Ohio State University, Department of Medicine, Columbus, OH.

Acute renal failure (ARF) often follows multiple bouts of renal ischemia. The goal of this study was to define how 1 ischemic event, insufficient to cause ARF, influences renal resistance to a second bout of ischemia. Rats underwent 15 min of bilateral renal artery occlusion (RAO) which induced extensive proximal tubular brush border (BB) loss, tubular cell swelling and severe ATP depletion (+86%) but no renal insufficiency. Either 30 min, 3-1/2 hr, or 24 hr later, they were subjected to 25 min of RAO. Functional (GFR, BUN) and histologic responses to this 2nd bout of RAO were compared (over 48 hr) to those seen in control rats subjected to a single 25-min ischemic event.

Rats with a 30-min hiatus between the 15 and 25 min bouts of RAO had significantly worse ARF than control rats subjected to only 25 min RAO. However, rats which were 3-1/2 hr or 24 hr post 15 min RAO had normal resistance to 25 min RAO. Recovery of normal resistance at 3-1/2 hr post 15 min RAO correlated with reversal of prior tubular cell swelling and with reappearance of the BB membrane but not with improvement in ATP concentrations (+25% at 30 min and 3-1/2 hr post 15 min RAO). Conclusion: Prior mild ischemic injury can lower renal resistance to a subsequent ischemic event. However, recovery of normal resistance occurs rapidly and correlates with restoration of cell volume regulation and with repair of the BB membrane. Resistance to ischemia can be normal despite persisting depressions in renal ATP content.

PROGRESSION OF CHRONIC RENAL FAILURE IN MAN AS INFLUENCED BY FREQUENCY AND QUALITY OF CLINICAL FOLLOW-UPS. Anders Alvestrand, Härje Bucht, Alberto Gutierrez and Jonas Bergström (intr. by Lee Henderson) Department of Renal Medicine, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden

Retrospective data suggest that protein restriction may be beneficial by slowing the rate of progression in CRF. We followed prospectively 25 CRF-patients without protein restriction with monthly clinical check-ups and determination of blood chemistries and creatinine clearance (CCR); after 12 months' randomization into 2 groups, 1 on low protein diet and 1 control group, takes place. Care is taken to reduce blood pressure (BP) to < 140/90, correct fluid overload, acidosis, hyperphosphatemia etc. Before entering the study most patients had been controlled at our out-patient clinic for ≥ 2 years but less rigidly and frequently. In 10 out of 17 patients followed for 6-12 months with monthly check-ups progression of CRF was retarded or halted; only 3 patients had an accelerated deterioration. In some patients retardation of progression was associated with improved BP-control; in others no specific factor could be singled out, but compliance to therapeutic measures may have been better with more frequent check-ups.

Conclusions: In prospective studies of the effect of nutrition on progression of CRF it is important that the frequency and quality of check-ups are the same over a period before as well as after the change in diet to allow the patient to be his/her own control. This principle should also be applied when comparing groups of patients or different regimens with each other.

STIMULATION OF NA-H ANTIPORT INDUCES HYPERTROPHY IN RENAL PROXIMAL TUBULAR (PT) CELLS. B Badie-Dezfooly, A Hamzeh* and LG Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

We have previously documented an increase in oubain-sensitive ^{86}Rb uptake in a primary culture of PT cells induced to hypertrophy (Clin Res 32:89A, 1984). To determine whether stimulation of Na-H antiport is the event which initiates the hypertrophy with secondary activation of the basolateral pump, we measured Na uptake and H efflux directly. Confluent, quiescent PT cell monolayers exposed to insulin (Ins) 10 µg/ml, PGE_1 1 µM and hypertonic NaCl (+20 mM NaCl) for 2 hrs. These stimuli lead to a 20-30% increase in protein/cell and cell size but no increase in thymidine incorporation at 24 or 48 hrs. Amiloride-sensitive ^{22}Na uptake and H efflux (pH-stat method) was measured in control and stimulated cells at 100 mM Na. Initial rates of both processes were equal, i.e., ± 100 pmoles/10^6 cells/sec. indicating 1:1 stoichiometry. Ins and PGE_1 but not NaCl increased amiloride-sensitive Na uptake and Na dependent H efflux by greater than 100% in Na depleted cells (the Na depletion step abolished the acute effect of the NaCl). PT cells exposed to growth stimuli for 24 hrs were hypertrophied and continued to demonstrate an increase in Na-H antiport even after the stimuli were withdrawn (Ins + 120%; PGE + 153%; NaCl + 40%).

Conclusions: An early effect of stimuli which lead to PT cell hypertrophy is stimulation of Na-H antiport. Once hypertrophied, the cell retains an augmented rate of Na-H exchange when the growth stimulus is removed.

DIABETIC NEPHROPATHY (DN): RESPONSE TO PROTEIN LOADING. J.P. Bosch*, S. Glabman, S. Lew*, A. Lauer*. Mount Sinai School of Medicine, Dept. of Medicine, New York, N.Y.

Early diabetic nephropathy (DN) is characterized by renal vasodilation, increased renal plasma flow (ERPF) and GFR. In normal man protein loading results in similar hemodynamic changes. To elucidate the effects of protein loading in DN, diabetic patients with various GFR's were studied. Disease free (n=5) and diabetic (n=11) subjects underwent the protein loading test. Following hydration, all subjects received an oral protein load (60-80 gms). Urine and blood samples were collected for clearance studies: A Baseline period for 1 hr preceding the load and a Test period for 120 min at 1 hour after the protein load. Inulin and PAH clearances were obtained using standard techniques. Results: Mean values, (FF = Filtration Fraction, TRR = Total Renal Resistance, $p<0.05$ = *).

	BASELINE				TEST			
	GFR ml/min	ERPF ml/min	FF %	TRR mmHg/ml/min	GFR ml/min	ERPF ml/min	FF %	TRR mmHg/ml/min
Control	123	656	19	0.080	150*	794*	19	0.065*
Diabetics								
Grp 1 n=3	181	777	23	0.062	148*	678*	22*	0.071*
Grp 2 n=4	123	638	19	0.090	112*	604*	19	0.094
Grp 3 n=4	71	367	19	0.192	62*	360*	17*	0.270*

In the control group protein loading resulted in a significant fall in TRR and a rise in ERPF and GFR. Grp 1 (early diabetes) was characterized by renal vasodilation with increased ERPF and GFR in the baseline period as compared to the control group. Protein loading failed to produce additional vasodilation in the diabetics. Conclusion: These studies demonstrated an abnormal response to protein loading in DN as compared to normals and patients with other renal diseases.

GLOMERULAR PRESSURE IN RATS WITH DIABETES AND HYPERTENSION. Norman Bank, Ruth Klose*, and Hagop S. Aynedjian*. Montefiore Hosp. and Med. Ctr. Dept. of Med., Bronx, N.Y.

It is well established that hypertension accelerates renal damage in patients with diabetes. In order to study the mechanism of this effect, micropuncture measurements of glomerular capillary pressure (PG) were made in SHR made diabetic by Streptozotocin injection. Stopped-flow pressure (SFP) and free-flow pressure (FFP) were measured 5-7 days after Streptozotocin, and PG and effective filtration pressure (EFP) were calculated. Control groups consisted of WKY, WKY-diabetics, SHR non-diabetics, and SHR-diabetics treated with antihypertentive drugs. Blood glucose averaged 403 and 445 mg% in 2 diabetics groups (pNS). Mean BP was 159 and 168 mm Hg in the SHR and SHR-diabetics (pNS), and was 112 mm Hg in the SHR-diabetics treated for hypertension ($p<0.01$). RPF was 1.70 ml/min/gkw in SHR and 3.37 in SHR-diabetics ($p<0.01$). GFR was 0.50 ml/min/gkw in SHR and 1.00 in SHR-diabetics ($p<0.01$). Comparable differences were found in WKY vs WKY-diabetics. PG was as follows: WKY 42.5; SHR 43.8 WKY-diabetics 48.3; SHR-diabetics 52.6 mm Hg. Thus, PG was significantly higher in WKY-diabetics vs WKY, and in SHR-diabetics vs SHR and WKY-diabetics. Treatment of hypertension lowered PG to 47.1 mm Hg. We conclude that hypertension causes a marked elevation of PG in diabetes. Correction of hypertension reduced PG to a level equal to that in WKY-diabetics, but not to normal. These findings provide an explanation for the damaging effect of hypertension in diabetes and the partial protection of antihypertensive treatment.

FUNCTIONAL AND MORPHOLOGIC CONSEQUENCES OF REDUCTION OF RENAL MASS IN DOGS. J.J. Bourgoignie, G. Gavellas,* E. Martinez,* and V. Pardo. Univ. Miami Sch. Med. and V.A.M.C., Miami, FL.

Renal mass reduction is associated with a progressive glomerulopathy in rat but an interstitial nephritis in rabbit. To determine which evolves in dog, renal function and morphology were evaluated in female remnant dogs fed 27% protein. Five dogs with fasting GFR below 9 ml/min. survived only 3 to 6 months. In contrast, GFR remained stable for 12 to 39 (mean 25.5) months in 8 of 10 dogs with fasting values of 10 to 24 ml/min. In the latter, filtration reserve, as estimated by comparing fasting GFR and peak GFR after ingestion of 1-2 g. protein/kg. plus 100 mEq sodium was as follows:

DOG (n)	Fasting GFR ml/min	Peak GFR ml/min	Δ ml/min	Δ %
Normal (5)	43.8	57.0	13.6	31.3
Remnant (7)	15.9	19.4	3.4	26.8
P	< .001	< .001	< .001	NS

Hypertension (BP 160/100 mm Hg) was the rule and a progressive proteinuria (0.2-3.7 g/d) commonly developed. Glomerular histology showed mesangial hyper plasia (MH) in 5 dogs, focal and segmental sclerosis (FSS) in 3 dogs, and was normal in one. There was no interstitial nephritis.

Thus, reduction of renal mass in dog, as in rat, leads to systemic hypertension, progressive proteinuria, glomerular MH and/or FSS. The presence of a filtration reserve in remnant dogs with modest CRI may help support GFR and delay a progressive fall in GFR.

THE POTASSIUM-ALDOSTERONE AXIS IN DOGS WITH CHRONIC RENAL INSUFFICIENCY (CRI). J.J. Bourgoignie, G. Gavellas,* V. Van Putten,* and T. Berl. Univ. Miami Sch. Med., Miami, FL and Univ. Colorado Med. Sch., Denver, CO.

To evaluate the potassium-aldosterone axis in CRI, the plasma concentration of aldosterone (PA) was measured before and after an oral potassium challenge in 9 dogs with intact kidneys (GFR 52 ± 3.1 ml/min) and in 9 dogs with one remnant kidney (GFR 16.1 ± 2.1 ml/min). Prior to KCl, serum potassium (SK), PA and urinary potassium excretion rates were similar in both groups. In the 5 hrs. following 50 mEq KCl, less of the potassium load was excreted by remnant (30.2%) than by normal (55.5%) dogs ($p < .01$); SK increased more in remnants than in normals. After KCl, PA increased in both groups, but the increments in remnant were twice the increases in normal dogs.

		Minutes after KCl				
	Control	30	90	150	210	270
		Normal dogs (n=9)				
SK mEq/L	4.7	5.0	5.6	5.8	5.4	5.0
PA ng/dl	8.6	13.6	20.3	22.0	19.1	14.0
		Remnant dogs (n=9)				
SK mEq/L	5.0	5.2	6.9*	7.0**	6.7	6.2
PA ng/dl	11.7	16.3	28.7	38.3**	41.7**	35.3*

*$p < .02$; **$p < .01$ compared to normal dogs.

The increase in PA per unit increment in SK was similar in both groups (14.5 vs 13.8 $ng.dl^{-1}/mEq.L^{-1}$). We conclude that the potassium aldosterone axis is normal in CRI. The hyperkalemia that follows a potassium load is not due to a failure to secrete aldosterone but to a decrease in potassium excretion in the setting of decreased renal mass.

LONGITUDINAL STUDY OF RENAL FUNCTION AND MORPHOLOGY IN UNI-NEPHRECTOMIZED RATS: K.M.H. Butt, A. Tejani, I. Lancman*, C. Chen*, and M. Beyer, Downstate Medical Center, Brooklyn, NY

To evaluate the long term effects of a 50% reduction in nephron population, we studied male Sprague-Dawley rats weight (BWT) 250 ± 20 gm. Animals were subjected to either a sham operation (S) or right nephrectomy (N). Animals were maintained on standard lab chow. Intra-arterial blood pressure (B.P), glomerular filteration rate (GFR) and renal plasma flow (RPF) were measured at 4, 8 and 12 months (Mo.). GFR and RPF were measured with labeled inulin and paraminohippurate. Animals were then sacrificed, left kidney (LK) weighed and processed for histology.

Results: (Mean ± S.D. * = $p < 0.5$)

Mo.		LK wt gm	B wt. gm	GFR ml/min	RPF ml/min	BP mmHg
4	N	2.6±.3*	505±31	1.5±0.4	3.1±0.9	160±05.6*
	S	2.0±.4	516±34	1.6±0.3	2.6±0.6	133±12.3
8	N	3.4±.5*	570±43	1.4±0.6	2.5±1.0	149±16.0
	S	2.1±.3	572±84	1.7±0.5	3.5±1.5	135±18.0
12	N	3.6±.5*	562±42*	0.9±0.7*	2.7±1.2	140±10.0
	S	2.6±.1	644±46	2.1±0.9	3.9±0.8	142±07.0

No morphologic changes were noted in 4 and 8 mo. S animals. Eight mo. N and 12 mo. S rats showed 0-2%, segmental glomerulosclerosis. Twelve mo. N rats showed Mean 13%, segmental glomerulosclerosis. Our study shows that in the male rat, a 50% reduction in renal mass leads to functional and morphological damage to the contralateral kidney over a one year period.

"NEPHRON LOSS ADAPTATIONS" (NLA) IN CHRONIC RENAL DISEASE (CRD). "Gain": The Concept, Its Quantification and Implications. N.S. Bricker, M.Shapiro, M. Levine, J.Wang and F.Gotch, Univ. of Calif. Los Angeles and San Francisco.

Gain is defined as "an increase in output over input." Gain Amplificationis the fundamental event in compensatory changes in excretion for all controlled solutes (c.s.) in all forms of CRD. It thus, is the basis of NLA and could provide a means of "decoding" the pathogenetic events responsible for NLA. Optimal gain (O.G.) implies maximal excretory compensation for the lost nephrons. It reduces to GFR_{CRD}/GFR Control. Actual Gain (A.G.) is the value obtained following administration of an identical challenge load of a c.s. in CRD vs. the Control state. A.G = UV/GFR(CRD) ÷ UV/GFR (Control). Gain Coefficient (G.C.)=AG/OG (%). Representative results: O.G. is identical for all c.s. A.G.,on the other hand,may vary widely for different c.s.; hence G.C. can range from"negative gain" to "supergain." For any c.s., G.C. is dynamic, an in-vivo phenomenon with no memory, often independent of the prior c.s. intake, inversely related to P_K, but linearly related to $\Delta ICF_K/\Delta ECF_K$(CRD/Control).

Gain is a measure of excretion; hence amplification depends upon precise modulation of diverse transport mechanisms. The differences in the values for G.C. for various c.s., and the nature, type and direction of the transport process(es) modulated, strongly support a network of solute-specific biologic control systems, operating through a common final pathway, the residual nephrons.

ROLE OF PARATHYROID HORMONE (PTH) AND PROSTAGLANDIN (PG) IN THE REDUCED VASCULAR RESPONSE TO NOREPINEPHRINE (NE) IN CHRONIC RENAL FAILURE (CRF). V.M. Campese, K. Iseki,* J. Collins,* and S.G. Massry. Div. Nephrol., USC Sch. Med., Los Angeles, CA.

Patients with CRF have secondary hyperparathyroidism and display reduced responsiveness to NE; this abnormality may underlie at least partly their autonomic nervous system dysfunction. We carried out studies in normal and CRF rats and in CRF patients to delineate the role of PTH in the genesis of this derangement. Both 1-84 and 1-34 PTH significantly ($p<0.01$) reduced the rise in mean arterial pressure (MAP) induced by NE infusion. This effect of the hormone was abolished by pretreatment with indomethacin; also, PTH induced significant rise in urinary 6 keto $PGF_{1\alpha}$ from 5.2±1.0 to 13.7±2.6 ng/mg creatinine. CRF rats also displayed reduced response to NE. This abnormality was prevented by indomethacin treatment. Parathyroidectomized-normocalcemic rats with CRF did not show reduced response to NE. Patients with CRF displayed a significant ($p<0.01$) inverse relationship between the rise in MAP during NE infusion and the blood levels of PTH. Treatment with indomethacin corrected the reduced response to NE in these patients. These data demonstrate 1) CRF in animals and humans is associated with reduced MAP response to NE, 2) PTH plays a major role in this abnormality, and 3) this action of PTH is most likely mediated through increased production of vasodilating prostaglandins. These results provide a therapeutic maneuver that could be useful in alleviating some of the manifestations of autonomic nervous system dysfunction in uremia and assign a new facet to the overall toxicity of PTH in CRF.

THE EFFECT OF INTRAVENOUS AMINO ACID INFUSION ON RENAL HEMODYNAMICS IN MAN. P Castellino*, B Coda* and RA DeFronzo. Yale University School of Medicine, New Haven, CT.

The effect of amino acid (AA) infusion on renal function was examined in 7 healthy young subjects, who participated in 3 experimental protocols: Study I-10% AA inf (Travasol) at a rate of 0.043 ml/kg/min for 3 hr and which increased plasma AA levels 2-4 fold; Study II-the same AA inf plus Somatostatin (SRIF) inf (8μg/min) with peripheral replacement of insulin (I), glucagon (G) and growth hormone (GH); Study III-the same SRIF inf with I, G, and GH replacement (no AA inf was given). GFR and RPF (ml/1.73 m^2S.A./min) were determined with inulin and PAH respectively.

STUDY	GFR		RPF	
	Basal	AA inf	Basal	AA inf
I	117±6	137±6(P<0.01)	633±58	720±52(P<0.02)
II	126±3	127±4(N.S.)	649±36	639±36(N.S.)
III	118±7	114±5(N.S.)	622±43	591±43(N.S.)

Plasma levels of I (13 to 27 μU/ml), G (124 to 275 pg/ml), and GH (1 to 10 μg/dl) rose during Study I and remained constant during Studies II and III. 5 subjects participated in an additional study; after a basal determination of GFR and RPF they consumed a low protein diet (0.6 gm of protein/kg/day) for 7 days at which time the same AA inf was administered.

	Pre Diet	Post Diet	AA inf Post Diet
GFR	105±7	96±4	116±6 (P<0.01)
RPF	615±29	517±26 (P<0.01)	594±16(P<0.01)

We conclude that 1) AA inf is associated with a modest increase in GFR and RPF; 2) SRIF blocks the AA-mediated increase in GFR and RPF, suggesting that the hyperfiltration is hormonally mediated; 3) dietary protein restriction causes a decline in RPF and GFR but does not alter the response to AA inf.

FURTHER CHARACTERIZATION OF OUABAIN-SENSITIVE AND INSENSITIVE Na TRANSPORT IN UREMIA. D.B. Corry, M.L. Tuck*, D.B.N. Lee, Olive View MC, Sepulveda VAMC, and UCLA Sch. of Med., Los Angeles, CA.

We have reported reduced red cell (RBC) Na efflux rates through the Na, K Cotransport system (CoT) and normal to high efflux through the Na, K pump (P) in patients on hemodialysis. We now report the effect of increasing RBC intracellular Na concentration (Na_i) on Na efflux rates through the CoT and P pathway in 14 dialysis patients (D) and 14 controls (C). RBC were Na-loaded by the nystatin method to attain 5 levels of Na_i with a range from 5 to 50 mM/L cells. Pre-loaded [Na_i] was similar in D and C (7.12± 0.5 vs 7.23± 0.4 mM/L cells) and the nystatin-induced rate of Na loading didn't differ in D and C. Mean efflux rates expressed in mM/L cells were:

Dialysis					
Na_i	5.1±.30	12.8±.5	22.5±.8	33.73±1.2	48.1±2.1
P	1.6±.11	4.0±29	5.2±41	5.5 ±41	6.3±.68
CoT	.074±.04	.096±.05	.210±.03	.264±.05	.267±.05
Control					
Na_i	4.5±.20	12.2±.4	21.3±.8	32.6±1.1	46.3±1.6
P	1.7±.13	4.9±25	5.9±.3	6.0±.3	6.7±.68
CoT	.327±.08	.412±07	.490±.01	.694±.09	.590±.09

Calculated kinetics of CoT revealed an identical Kt (13.5 mM/L cells) in both groups but markedly reduced Tmax in D vs C (0.380 vs 0.910 mM/L cells/hr). There was no significant difference however in Kt (11.5 mM/L cells) and Tmax (8.7 vs 9.5 mM/L cell/hr) for the RBC P in D and C. Thus, this model for examining Na transport extends our previous findings by showing normal affinity of Na_i for the CoT carrier but diminished functional capacity in D. The pump appears intact in D.

RELATIOSHIP BETWEEN GLYCOLYTIC ACTIVITY IN ERYTHROCYTES AND GLUCOSE INTOLERANCE IN UREMIA(U),HEMODIALYSIS(HD), AND CONTINUOUS AMBULATORY PERITONEAL DIALYSIS(CAPD). N.Contreras,* J.Cajías,* E.Bellorín Font, V.Sylva,* P.Amaír,* L.Gutierrez,* V.Paz-Martínez and J.R.Weisinger. Division of Nephrology, Hospital Universitario and Centro Nacional de Diálisis, Caracas, Venezuela.

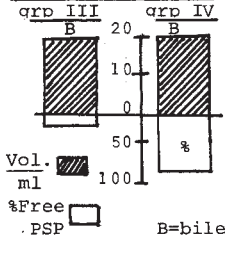
Insulin resistance in uremia involves a postbinding defect which might consist of an impairment in the intracellular capacity for glucose utilization. To explore this hypothesis, in vitro glycolytic activity by RBC and oral glucose tolerance was studied simultaneously in normal controls(C) and in U,HD, and CAPD patients. Washed RBC were incubated at 37 °C in Krebs medium containing 5mM glucose in the presence or absence of 1mM DB-cAMP. Lactate production was determined after 1 and 2 hours of incubation. Glucose intolerance and hyperinsulinemia was demonstrated in the uremic and dialysis patients, with areas under the glucose curves of 21.1±0.63, 25.8±1.56, 22.4±0.88 and 29.0±3.42 g min for the C,U,HD and CAPD groups, respectively. In the same groups, basal lactate production by RBC (2h) was 4.0±0.15, 6.6±0.74, 8.2±1.2 and 4.5±0.36μmol/mlRBC. DB-cAMP increased lactate production by 1.55±0.18, 1.97±0.36, 1.76±0.26 and 2.02±0.16 μmol/ml RBC.

Thus, no evidence of impairment of the glycolytic pathway was obtained when the uremic or dialysis groups were compared to controls. No Correlation was found between the degree of glucose intolerance and basal or stimulated glycolytic activity. If this model truly reflects glucose handling by peripheral cells, the postbinding defect in the response to insulin in uremia could be due to an alteration in the β subunit of the insulin receptor or to circulating factors.

BILIARY DIVERSION: AN ALTERNATIVE ROUTE FOR REMOVAL OF UREMIC TOXINS IN RATS. J.P. de la Rosa, S.Kerkar*, S.R.Dunn, M.L.Simenhoff. Division of Nephrology, Jefferson Medical College, Phila., PA.

The purpose of the present study is to investigate the liver as an alternative route for excretion of small molecular weight (SMW) compounds via the bile in chronic renal failure (CRF) Wistar rats. We used the SMW dye phenol red (PSP) M.W. 354 daltons as a marker for theoretical SMW toxin in control (n=4) and chronic (n=7) uremic rats. Rats were anesthetized and bile duct cannulated with PE50. Intravenous injection of PSP 0.3 mg/kg low dose, group I, (n=4) and 10 mg/kg high dose, group II, (n=3) were given in CRF. Bile samples were collected for 2 and 24 hours. For 24 hours, a biliary diversion (BD) via a bile catheter tunnelled subcutaneously and out over a swivel for bile collection was done. Results show that for a 2 hour bile collection, 25±3% was excreted in control, whereas for CRF group I 30.77±2.6% and group II 38.21±3% were excreted as free PSP of the total injected dose. A 24 hour bile collection in a subpopulation of normal rats with BD (group III) was compared with CRF with BD and renal pedicle tied (group IV). See graph. Note 88.9% excretion of free PSP in CRF.

Conclusion: Using PSP as a marker for SMW uremic toxin our data indicate that bile is an important alternative excretory route for excretion of SMW compounds. Absorption and recirculation via the enterohepatic route masks this effect.



CELL PROLIFERATION PRECEEDS CYST FORMATION IN A NEW MODEL OF RENAL CYSTIC DISEASE.

K.D. Gardner and A.P. Evan. Depts. of Medicine and Anatomy, Univ. of New Mexico, Albuquerque, NM, and Indiana University, Indianapolis, IN.

We have shown that bacterial contamination enhances cyst development in nordihydroguaiaretic acid (NDGA) - exposed germ-free rats. The kinetics of this rapid cyst development were explored by comparing mitotic activity with cyst growth. Germ-free rats were fed 2% sterilized NDGA for 14 days before being exposed to bacteria. Animals were killed 0, 1, 2, 3, 4, 7, 10 or 15 days after contamination. Two different types of control groups were also examined. Cell proliferation was measured with tritiated thymidine. Each kidney was given a pathology score from 0-4.

Animal Type	Days of Deconditioning	Path. Score	Labeling Index, % PT	CD
Germ-free	0	0	0.5	0.5
NDGA-fed	1	0	1.0	1.0
Contaminated	2	0	1.0	1.0
	3	0	3.0	5.0
	4	0	2.0	5.0
	7	2.5	15.0	23.0
	10	2.5	12.0	20.0
	15	3.25	12.0	24.0
Controls	15	0	.01	.01
	29	0	.01	.01

No evidence of cystic changes was seen until day 7. Mitotic activity was already elevated by day 1 in some proximal and collecting tubules. This activity increased the next 15 days concomitant with cyst growth and an accumulation of neutrophils. These data suggest that cyst growth seen in this model is the result, in part, of accelerated cell proliferation triggered by an environmental factor: bacterial contamination.

CHEMICAL BASIS OF IMPAIRED SMALL LIGAND BINDING IN UREMIC PLASMA: P. Gulyassy, P. Igarashi,* L. Stanfel,* E. Jarrard,* T. Depner. Univ. Calif. Davis, CA.

We have used impaired binding of small ligands to plasma proteins as a model in the search for uremic toxins. Our previous studies showed that uremic plasma could be normalized by adsorption at pH 3 with a resin and that a methanol extract of the resin added to normal plasma reproduced the uremic defect. Chromatographic isolation, NMR and mass spectroscopy revealed that a family of aromatic organic acids (OA) are the cause of the binding defect. Hippurate (HIP) has been identified as one of these OA's. Another possible component is indoxyl SO_4 (IND).

We have developed a reversed phase C-18 silica HPLC method at pH 3.0 to measure HIP and an ion-pairing (with TBA) C-18 silica HPLC method at pH 7.5 to measure IND in uremic plasma. In 14 non-dialyzed patients with far-advanced uremia (mean creatinine = 13.6 mg/dl) HIP was proportional to plasma creatinine but the mean value of 3.52 ± SD 4.13 mg/dl (196 ± 230 μmole/L) was far less than reported by others using a non-specific method. In 10 patients with far-advanced uremia (mean creatinine = 15.2) IND was 3.55 ± 1.44 mg/dl (167.4 ± 68.7 μmole/L). IND binds more tightly to normal plasma proteins than HIP. Therefore, IND may be the more important OA causing impaired binding in uremic plasma.

Studies are in progress to precisely define the relative importance of IND and HIP in the impaired binding by spiking normal plasma with HIP, IND or both at levels found in uremic plasma and measuring their effects on binding of a model ligand, ^{14}C-salicylate.

ANALYSIS OF PROGRESSION OF RENAL FAILURE

M.C. Gregory * and U.R. Shettigar.* (Introduced by W.A. Border)
Divs of Nephrology and Artificial Organs, University of Utah, Salt Lake City, Utah.

In most patients with progressive renal failure there is a linear relationship between the reciprocal of plasma creatinine (Cr) and time (t) once Cr is greater than about 0.2 mmol/L. There is a satisfactory explanation for neither the reason behind this relationship nor for its failure at lower values of Cr. Based on the assumption that the rate of loss of renal function is proportional to the degree of existing renal damage, mathematical analysis yields the general relation

$$\text{Exp}(Cr_o/Cr) = -kt + e.$$

For Cr > 0.2 mmol/L this simplifies to the known linear relation between 1/Cr and t.

The records of 31 patients with various progressive renal diseases beginning at a median Cr of 0.15 mmol/l and followed for a median duration of 96 months were examined. Regression of Exp(1/Cr) against t fitted the data better than 1/Cr or ln(Cr) in 27/31 cases. In 2 patients 1/Cr and in 2 cases ln(Cr) gave the best fit. Inspection of the plots showed that Exp(1/Cr) fitted the data well for Cr values < 0.2 mmol/L whereas the other expressions did not.

Hydrodynamically mediated renal injury could cause progression of this type, but other explanations such as retention of a toxic metabolite would yield the same theoretical relationship.

A DIET RICH IN LINOLEIC ACID IMPROVES RENAL FUNCTION AND DECREASES BLOOD PRESSURE IN RATS WITH A REMNANT KIDNEY. Michael Heifets*, Mabel Purkerson and Saulo Klahr, Washington University School of Medicine, St. Louis,MO.

Female rats with 1-3/4 nephrectomy were pair fed isocaloric diets, containing either 20% safflower oil (Group I,n=7) or 20% beef tallow (Group II, n=7), differing in polyunsaturated fatty acid (linoleic) content. Diets were otherwise identical and contained 24% casein. Five weeks after nephrectomy inulin (C_{in})and paraaminohippurate clearances(C_{PAH}), protein excretion and systolic blood pressure (SBP) were determined in awake rats. Cardiac index (CI), the ratio of heart weight to body weight, was also calculated. Mean values ± SEM are shown below:

	C_{in}	C_{PAH}	SBP	CI
	ml/min		mmHg	
Group I	0.85	2.47	158.7	410
	±0.23	±.581	±7.1	±19.8
Group II	0.37	1.23	205.9	506
	±0.07	±.204	±12.0	±26.9
P	<.05	<.05	<.05	<.05

Histology of the viable portion of the remnant kidney revealed glomerular sclerosis in both groups, but the percent of affected glomeruli was greater in Group II. Although proteinuria was greater in Group II than in Group I, the values were not significantly different. Thus, a diet rich in linoleic acid lowers blood pressure and improves renal function in rats with a remnant kidney. We conclude that in addition to protein, changes in dietary lipids may influence the progression of chronic renal disease.

EFFECTS OF GENETIC OBESITY ON RENAL STRUCTURE AND FUNCTION IN THE ZUCKER RAT (Z). BL Kasiske, MP Cleary*, MP O'Donnell, WF Keane, Dept. of Med., Hennepin Co. Med. Ctr., Univ. of Minn., Mpls., and Austin, MN.

Hyperphagia and obesity in Z are associated with spontaneous development of focal glomerulosclerosis (FGS). To investigate the pathophysiology of this glomerular injury, 24 h urine albumin ($U_{Alb}V$), awake tail cuff blood pressure (BP), and semiquantitative morphometric analysis of mesangial matrix (MM) and FGS were performed in lean (LN) and obese (OB) rats 14, 28, and 68 wks old. Inulin clearance (C_{In}) and filtration fraction (FF) were determined in LN and OB rats at 14 wks. Results (mean±SE; *p≥0.05 vs. LN):

	14wks		28 wks		68 wks	
	LN (n=10)	OB (n=10)	LN (n=10)	OB (n=10)	LN (n=7)	OB (n=6)
$U_{Alb}V$ (mg/24h)	0.5 ±0.1	5.3 1.5	1.9 0.5	112.9* 32.0	7.1 2.8	129.9* 33.7
MM	139 ±20	263* 18	184 13	307* 11	300 34	305 28
FGS	0	0	0	9* ±5	15 10	143* 38

At 14 wks, BP was 126±12 mmHg in LN and 140±10 in OB ($p<0.05$). BP elevations were also seen in OB rats at 28 and 68 wks. Prior to the development of FGS, C_{In} was similar in LN and OB, 2.0±0.4 and 2.2±0.6 ml/min respectively ($p>0.05$). FF was 0.34±0.06 and 0.32±0.08 in LN and OB ($p>0.05$). Thus progressive FGS developed spontaneously in OB Z. This was preceeded by increased BP, MM and $U_{Alb}V$, but increments in C_{In} or FF were not observed.

VASCULAR REACTIVITY OF THE KIDNEY: ASSESSMENT BY PROTEIN LOADING. A.Lauer*, S. Glabman, S, Lew* and J.P. Bosch*. Mount Sinai School of Medicine, Dept. of Medicine, New York, N.Y.

In normal man, after an acute oral protein load (PL) there is a rapid and transient increase in GFR. This change in GFR is associated with a fall in renal resistance and a rise in plasma flow (ERPF). To determine how various drugs may affect the renal vascular reactivity, disease free volunteers underwent a PL test. The PL test was done twice in each subject: CONTROL and EXPERIMENTAL. In the experimental PL test a drug (Indocin 50 mg or Minoxidil 10 mg) was administered prior to and during the test. PL test:All subjects received an acute oral protein load (60 - 80 gms). Following hydration, urine and blood samples were collected for creatinine, inulin and PAH clearances: 1 hr preceding the load (Base GFR) and a 120 min period 1 hr after the protein load (Test GFR).Results: mean values (ml/min), * = $p < 0.05$ by paired analysis.

		INDOCIN STUDIES			
SUBJECTS		CONTROL		EXPERIMENTAL	
		BASE	TEST	BASE	TEST
Creatinine Clearance	(n=4)	125	150*	116	117
Inulin Clearance	(n=2)	118	148*	115	120
PAH Clearance	"	630	832*	638	670
		MINOXIDIL STUDIES			
Inulin Clearance	(n=3)	125	146*	118	125*
PAH Clearance	"	698	767	753*	883*

Protein loading in normal subjects resulted in a significant increase in ERPF and GFR. Since filtration fraction remained unchanged it is likely that the vasodilatory effect of PL affected both the afferent and efferent arterioles. The response to PL appears to depend on the synthesis of prostaglandins. In Minoxidil treated subjects prior to PL, the increment in ERPF was not accompanied by a proportional rise in GFR. This suggests a predominant vasodilatory effect on the efferent arteriole that was unmasked further after PL. Conclusion: The PL test may permit the assessment of the effects of drugs on the vascular reactivity of the kidney.

EFFECT OF MILD CHRONIC RENAL FAILURE ON THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF THE PANCREAS. G.A. Kaysen, A.P.N. Majumdar*, M.A. Dubick*, G.A. Davis*, G. Mar* and M.C. Geokas*. Department of Medicine, VA Medical Center, Martinez, California, University of California, Davis, California.

Chronic renal failure (CRF) produces defects in synthesis of hepatic secretory proteins and may affect the synthesis of muscle protein. Although patients with CRF have evidence of pancreatic dysfunction, little is known about the direct effects of CRF on the exocrine pancreas. Mild CRF was produced in 7 female Sprague-Dawley rats by 7/8 nephrectomy, as evidenced by creatinine clearance of 0.91±.33 vs 2.39±1.03 cc/min in sham operated (S) controls ($P<.005$) BUN 40±4 vs 15±4 mg/dl ($P<.001$) Four weeks after operation, CRF rats had a significantly higher pancreas weight +67% ($P<.005$); increased pancreatic DNA +28% ($P<.025$), RNA +101% ($P<.001$) and protein +74% ($P<.001$) content and a marked rise in trypsin-like activity in pancreatic extract 51% ($P<.025$), despite no difference in total body weight. In contrast, protease inhibitor activity in the pancreas and serum of CRF rats was decreased by 50% and 23% respectively ($P<.025$) CRF produced no change in serum immunoreactive trypsinogen levels. The ability of the dispersed pancreatic acini isolated from CRF rats to incorporate ^3H leucine into protein in the absence and presence of 0.25 nm cholecystokinin (CCK) was lower than in acini from group S rats. CCK induced discharge of trypsinogen and chymotrypsinogen from dispersed pancreatic acini of CRF rats was similarly depressed. In summary, mild CRF produces cellular hypertrophy and hyperplasia in the exocrine pancreas, and marked alterations in both the synthesis and secretion of proteins.

DEMONSTRATION OF THE CARCINOGEN DIMETHYLNITROSAMINE (DMNA) GENERATION IN GUT BY ETHANOL INHIBITION OF ITS FIRST PASS METABOLISM BY LIVER IN CHRONIC RENAL FAILURE (CRF). P. Lele, S.R. Dunn*, M.L. Simenhoff, Jefferson Medical College, Philadelphia, PA.

N-nitroso compounds, including DMNA, are etiologically linked to carcinogenesis in many animals. DMNA is metabolized in the liver by P450 oxidase enzymes to the carcinogenic species. Ethanol, a P450 oxidase inhibitor, is known to prevent first pass metabolism of DMNA in the liver. This study aims at demonstrating intestinal DMNA generation utilizing the inhibitory action of ethanol. Six patients on maintenance hemodialysis, (CHD) and 2 controls (c) took 25 grams of 200 proof ethanol (diluted) orally. Blood was collected at 0, 5, 10, and 20 min. and assayed for DMNA. Table shows results in 6 CHD patients. Mean blood DMNA in ng/kg:

	0min.	5min.	10min.	20min.
CHD	255±57	437±37	408±31	350±26
C	0	0	0	0

Results show significant ($p<.01$) rise in DMNA at 5 and 10 min. and inhibition hepatic DMNA metabolism. Three CHD patients previously shown to produce DMNA via gut intubation were included in above series. Since blood levels do not reflect continuous intestinal DMNA generation, and obtaining intestinal samples is difficult, the above technique represents a simple method to demonstrate DMNA generation in the gut in ESRD. This is important because of the reported increased incidence of cancer in ESRD.

THE ROLE OF A RISING EXCRETION INDEX (E.I.) ON THE INDUCTION AND AMPLIFICATION OF GAIN IN NEPHRON LOSS ADAPTATIONS (NLA) IN CHRONIC RENAL DISEASE (CRD). M. Levine*, M.Shapiro,* N.Bricker, J.Wang,* R.Makoff,* and F.Gotch. Univ. of Calif. L.A. and San Francisco. E.I. is the ratio of the net daily acquisition rate of a controlled solute (c.s.) to GFR. Because the habitual dietary intake of solutes fails to decrease in CRD, E.I. rises progressively. The consistency of this event suggests that it could play a key role in the pathogenesis of NLA. This hypothesis was examined in dogs and rats, subjected to progressive reduction of renal mass and maintained either on a constant intake of a c.s. or on a regimen of proportional reduction of intake of the c.s. In the latter case, nephron loss occurs with no rise in E.I. E.I. may also be elevated by a stepwise increase in the intake of a c.s. with no loss of nephrons. The data were analyzed using the gain formulations described in an associated abstract. The results were different from predictions. Gain Coefficients were determined following identical challenge loads of each c.s. in matched animals with CRD and normal renal function. Representative values for Gain with rising values for E.I. were: Na, 130%, K 70%, PO_4 45% and Mg 38.5%. Comparable Gain values with constant E.I. values were: Na, 30%, K 55%, PO_4 64% and Mg 41%. When E.I. was increased progressively in the absence of nephron reduction, there was no evidence of amplification of the Gain Coefficient. Hence, the traditional view about the role of E.I. in the pathogenesis of NLA is not universally valid. Indeed, its validity is now in question about each of the c.s. studied with the possible exception of Na.

PHOSPHATE DEPLETION (PD) INDEPENDENT OF PROTEIN INTAKE ARRESTS PROGRESSION OF CHRONIC RENAL FAILURE (CRF). D Lumlertgul,* A Alfrey, T Burke, and R Schrier. Univ. Colorado Med. Sch.; and VA Hosp., Denver, CO.

In previous studies phosphate restriction was accompanied by protein restriction, thus any beneficial effect in preventing progression of chronic renal disease may have been due to protein rather than phosphate restriction. In the present study, two groups of rats with 5/6 nephrectomy (P-Nx) were pair fed so that protein, carbohydrate, caloric, phosphate, Ca, Na, and K intake was identical. PD was induced in the experimental (Exp) group by supplementing the normal diet with 15 g% dihydroxyaluminum aminoacetate while the control (Cont) group received the normal diet supplemented with the vehicle. Thirty days after P-Nx and prior to pair feeding animals in each group were matched individually by serum creatinine (SCr, 1.61 vs 1.63 mg/dl, NS) and body weight (BW, 273 vs 272 g, NS). After 12 wk of pair feeding, solute, urea, Na and K excretion and BW were not significantly different between the groups. Serum phosphorus was lower in the Exp than the Cont group (3.6 vs 12.9 mg/dl, $p<.001$). SCr (Cont 2.4 vs Exp 1.3 mg/dl, $p<.025$) and creatinine clearance (Cont 75 vs Exp 300 µl/min, $p<.001$) were also significantly different. Urine protein excretion was lower (Cont 375 vs Exp 75 mg/d, $p<.001$) with PD. Histologic examination revealed severe glomerular sclerosis, tubulointerstitial inflammation, tubular atrophy and luminal dilatation in the Cont but not the Exp group. PD alone, therefore, exerts a beneficial effect to prevent the progression of CRF in the remnant kidney model in rats.

PROTECTIVE EFFECT OF LOW SERUM TRIIODOTHYRONINE ON NITROGEN METABOLISM IN PATIENTS WITH CHRONIC RENAL FAILURE(CRF). VS Lim, MJ Flanigan and RM Freeman, Dept. Med., VA and Univ. Hosp., Iowa City, Iowa.

To test the hypothesis that low serum triiodothyronine (T_3) may minimize protein catabolism in CRF patients, we determined nitrogen balance(Nb) and urea generation rate (Gu, calculated from urea kinetics) in 7 CRF and 4 control subjects in the basal state and during periods when serum T_3 was elevated by L-T_3 (Cytomel, 0.78±0.05ug/Kg/day) and suppressed by Sodium Ipodate (1g/day) treatment. Changes in serum T_3 and N content in the food (Nin), dialysate(Nd), urine(Nu), feces(Nf) as well as Nb and Gu (Means ± SEM) are as follows:

CRF Patients	Basal	Cytomel	Ipodate
T_3 (ng/dl)	74±5	172±16‡	66±9*
Nin (g/day)	9.21±0.83	9.17±0.81	8.70±0.78
Nd (g/day)	7.43±0.55	8.60±0.43*	6.60±0.32
Nu (g/day)	0.22±0.12	0.26±0.15	0.27±0.13
Nf (g/day)	0.93±0.05	0.99±0.10	0.84±0.11
Nb (g/day)	0.58±0.34	-0.80±0.39‡	0.87±0.37
Gu(mg/min)	4.62±0.55	6.00±0.50‡	4.72±0.60
Controls			
T_3 (ng/dl)	111±6	214±12‡	74±8‡
Nin (g/day)	14.72±1.12	14.79±1.12	14.61±0.90
Nu (g/day)	13.18±0.70	12.92±0.53	12.88±0.73
Nf (g/day)	1.52±0.07	1.65±0.08	1.69±0.30
Nb (g/day)	0.02±0.51	0.22±0.67	0.05±0.60

*$p<.05$ and ‡ $p<.01$ by paired t test to basal values.

Furthermore, there was a significant negative correlation between serum T_3 and Nb in the CRF patients ($r=0.63, p<0.005$); changing serum T_3, however, had no effect on Nb in the controls. These data, thus, suggest that the low T_3 state may exert a beneficial effect on protein-nitrogen conservation in the CRF patients with low serum T3.

THE COURSE OF RENAL FAILURE IN PATIENTS WITH PRIMARY CHRONIC GLOMERULONEPHRITIS(CG). Giuseppe Maschio*, Lamberto Oldrizzi*, Carlo Rugiu*, Antonio Lupo* (Intr. by W.E.Mitch), Division of Nephrology, Verona, Italy.

The course of renal failure was evaluated in 2 groups of patients (p.) with CG. Group 1 had 35 p. with mean serum creatinine (SCr) of 1.87 mg/dl, kept for 6 to 132 months (mean 44) on a diet containing 0.6 g/Kg protein and 700 mg phosphorus (P). Group 2 had 11 p. with mean SCr of 2.29 mg/dl, retrospectively studied for 8 to 60 months (mean 24) on an estimated dietary intake of 1 g/Kg protein and 900 mg P. The mean SCr values rose to 3.31 mg/dl, with a slope in the regression analysis of 1/SCr against time of -0.0010, in group 1, and to 4.05 mg/dl, with a slope of -0.0190, in group 2 ($t = 3.35, p < 0.01$). The rate of progression was significantly affected by proteinuria and hypertension, and not by the various histologic and immunofluorescence patterns (mesangial proliferative, focal glomerular sclerosis, membranous or membranoproliferative).

Fifteen p. in group 1 (43%) and 3 p. in group 2 (27%) had no progression of renal failure; 20 p. in group 1 (57%) and 8 p. in group 2 (73%) had continued deterioration of renal function; 14% of p. in group 1 and 45% of those in group 2 required dialysis during follow-up. The differences between the 2 groups are statistically significant.

It is concluded that an early dietary protein and phosphorus restriction may drastically improve the course of renal failure in a large percentage of p. with immunologically-mediated primary CG.

ADRENERGIC DYSFUNCTION IN UREMIA. Leonard G. Meggs, Alvin I. Goodman. New York Med. Coll., Dept. of Med., Valhalla, New York.

Adrenergic dysfunction in uremia has been well described. Several lines of evidence suggest disorders of blood pressure regulation and myocardial response may occur secondary to adrenergic dysfunction; 1) in vitro myocardial subsensitivity has been demonstrated in uremia; 2) attenuated chronotropic responses during dialysis induced hypotension; 3) attenuated pressor response to norepinephrine. We have employed the partially nephrectomized uremic rat model to assess the effect of chronic uremia (4-6 weeks) of moderate severity (BUN 50, Cr 1.5) on myocardial beta receptor function, and vascular alpha $_1$ (α_1) binding properties.

Following the preparation of myocardial membrane vesicles, adenyl cyclase activity was measured and radioligand binding performed. Maximal isoproterenol (10^{-4}) stimulation was 58.5 vs 32.8 in nonuremic sham operated controls, ($p<.01$). Basal and fluoride simulated activity did not differ significantly (n=5). Beta receptor density was similar in the two groups (B_{max} 43.2 vs 48.7), however, the affinity of the beta receptor (K_D) was decreased in uremic rats (1.03 nm vs .281 nm $p<.02$) n=3.

Particulate fractions of rat mesenteric arteries were prepared and the binding properties of α_1 receptor compared by Scatchard Analysis (n=3). K_d 446 pm, B_{max} 63 fmol/mg in uremic rats vs K_D 280 pm B_{max} 72 fmol/mg in sham ($p<.05$).

Our preliminary data suggest chronic exposure to moderate levels of uremia results in altered binding properties and function of adrenergic receptors possibly related to end organ responses.

INFLUENCE OF AGE, SEX AND DIET ON THE GLOMERULAR RESPONSE TO ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODY (aGBM). H. Miyazawa*, D. Salant, A. Yared*, M. Purkerson, S. Klahr and I. Ichikawa. Harvard U., Boston U., MA and Washington U., St. Louis, MO.

It has been shown previously that the amount of glomerular aGBM deposition (G_{aGBM}, μg/g) is a linear function of serum concentration of aGBM (S_{aGBM}, μg/ml). To examine the influence of age, sex and dietary protein intake on the in vivo glomerular binding of aGBM, we compared G_{aGBM}:S_{aGBM} ratio (G/S) after 3-20 μg/g BW iv of ^{125}I-labelled aGBM between 1) 6 wk (Y, n=17) vs. 10 mo old rats (A, n=13), 2) male (M, n=13) vs. female adult rats (F, n=17) and 3) rats fed an isocaloric diet with 40% (H, n=11) vs. those with 6% protein (L, n=11) for 4 wks. Influence of the above factors on the severity of aGBM-induced glomerular functional damage was also evaluated by comparing percentage maximum reduction in GFR (ΔGFR) between these groups. Values for G/S averaged 0.068 (Y) vs. 0.098 (A)($P<0.001$), 0.085 (M) vs. 0.075 (F)($P>0.20$) and 0.087 (H) vs. 0.099 (L) ($P>0.10$), indicating that in vivo glomerular binding to aGBM increases with age while sex and dietary protein intake are without effect. Values for ΔGFR ranged from ∿5 to ∿90% in all animals studied. When animals with comparable glomerular aGBM binding were compared, ΔGFR was significantly greater in Y (48%) than A (32%) so that, even at a comparable S_{aGBM}, ΔGFR was uniformly greater in Y than A. In contrast, ΔGFR was comparable between M vs. F and H vs. L when comparison was made for animals with comparable aGBM binding or S_{aGBM}. We conclude that, of the three epidemiological factors examined, age, but not sex and protein intake, has a significant influence on a glomerular susceptibility to this form of immune insult.

CONTROL OF GLOMERULAR HYPERTENSION RETARDS PROGRESSION OF ESTABLISHED GLOMERULAR INJURY IN RATS WITH RENAL ABLATION. T.W. Meyer, S. Anderson,* H.G. Rennke, and B.M. Brenner. Brigham and Women's Hosp. & Harvard Med. Sch., Boston, MA.

Early treatment with the converting enzyme inhibitor (CEI) enalapril prevents development of glomerular capillary hypertension and limits glomerular injury following renal ablation in rats (Clin. Res. 32:564A, 1984). The current study examined whether CEI could lower glomerular capillary pressure ($\bar{P}_{GC}$) and modify disease progression in rats with established glomerulopathy. Munich-Wistar rats followed for 18 wks after 85% ablation of renal mass received no therapy (C), CEI throughout the 18 wks (CEI), or CEI beginning 8 wks after ablation when systemic hypertension and proteinuria were apparent (C/CEI). Results;(mean±SEM, † $p<.05$ vs C, § $p<.05$ vs CEI)

	$\bar{AP}$	$\bar{P}_{GC}$	SNGFR	Q_A	$U_{prot}V$
	----mmHg---		---nl/min----		mg/day
C (n=8)	147±9	70±3	119±7	441±37	120±13
CEI (n=9)	98±2†	52±2†	116±7	386±29	25±4†
C/CEI(n=7)	111±3†	52±2†	123±7	493±29	60±14†§

Both late and early institution of CEI reduced arterial pressure ($\bar{AP}$) and normalized $\bar{P}_{GC}$ without lowering the supranormal single nephron glomerular filtration rate (SNGFR) and plasma flow rate (Q_A) seen in remnant kidneys of C rats. Late institution of CEI treatment prevented further increase in protein excretion ($U_{prot}V$) and limited the prevalence of segmental glomerular lesions to 12±5% in C/CEI rats as compared with 32±6% in C rats and 1±1% in CEI rats. These studies show that progression of established glomerular injury can be limited by control of glomerular hypertension.

CONTROL OF RENAL HYPERTROPHY. THE ROLE OF A KIDNEY EPITHELIAL CELL GROWTH INHIBITOR. H Nasri, B Badie-Dezfooly*, RW Holley* & LG Fine. Department of Medicine, UCLA School of Medicine, Los Angeles, and The Salk Institute, San Diego,

Most growth factors stimulate cell proliferation (hyperplasia). When renal mass is reduced, the growth response is one of hypertrophy rather than hyperplasia. Why does the renal tubular cell of the hypertrophied kidney increase its protein content without multiplying? We examined the possibility that an inhibitor produced by the kidney epithelial cell can convert a mitogenic stimulus. Primary cultures of rabbit proximal tubular cells were grown to confluence and were arrested in G_0/G_1 by removal of growth factors for 48 hrs. A mitogenic stimulus, i.e., insulin (Ins), 10 μg/ml, plus hydrocortisone (HC) 5×10^{-9} M, was applied for 24 hr in the presence and absence of partially purified growth inhibitor derived from BSC_1(African green monkey kidney epithelial) cells (BSC-I).

	Protein content (pg/cell)	[^{3}H]thymidine (CPM/10^6 cells)
Control	200.8	2406
Control+BSC-I	207.8	2226
Ins+HC	236.3*	11546*
Ins+HC+BSC-I	237.3*	3997

(*$p<.05$)

Conclusion: BSC-I inhibited the stimulation of [^{3}H]thymidine incorporation but allowed the cells to hypertrophy. Endogenous growth inhibitors may thus modulate the pattern of growth in renal hypertrophy to determine whether hypertrophy predominates over hyperplasia.

EFFECT OF LOW PROTEIN DIET AFTER RENAL INJURY IS ESTABLISHED. K.A. Nath*, D. Kaufman*, and T.H. Hostetter, Univ. of Minnesota, Minneapolis, MN

Dietary protein restriction reduces renal injury in virtually all models of experimental renal disease. However, studies demonstrating this effect in remnant kidneys have uniformly imposed the protein restriction before injury is established, never after, and have often compared the restricted diet to one with a high, rather than standard, protein content. We examined whether protein restriction would still protect if instituted after renal injury is advanced. Sub totally nephrectomized rats were fed standard rat chow (~24% protein) for 3 months. Rats with total protein excretion rates exceeding 25 mg/day were paired for serum creatinine, divided into two groups and fed isocaloric diets with 20% (NlP) or 6% protein (LoP) for an additional 3 months. Systolic arterial pressure, body weight and protein excretion rates were indistinguishable between the groups at the time of initiating the two diets. Results after three months on the two diets (mean ± SEM, *p < .05).

	UprotV	UalbV	UIgGV	GFR	MAP
	- - - - -	mg/24h - - - -		ml/min	mmHg
NlP	36	15	2.3	.40	130
n=6	± 7	3	.5	.12	6
LoP	7*	2*	.5*	.68*	141
n=6	± 1	1	.1	.13	5

Total protein (UprotV) excretion rate as well as those of albumin (UalbV) and IgG (UIgGV) were all reduced, and GFR was preserved, on the low protein diet. Thus, glomerular filtration and permselectivity are beneficially influenced by a low protein diet even after renal injury is established.

THE EFFECT OF DISCONTINUATION OF DAILY ANTIGEN INJECTIONS ON CHRONIC SERUM SICKNESS GLOMERULONEPHRITIS. Bernice Noble*, Judith B. Van Liew and Jan R. Brentjens*. Pathology, Physiology and Microbiology Depts., SUNY/Buffalo and VA Medical Center, Buffalo, NY.

Previous studies have shown that chronic serum sickness glomerulonephritis progresses through three discrete stages, mild, moderate and severe. The diffuse, proliferative, necrotizing glomerulonephritis of severe chronic serum sickness has a fatal outcome. In this study daily injections of bovine serum albumin (BSA) were discontinued at the transition from the moderate to the severe stage of glomerulonephritis. Retrospectively, rats could be divided into two categories. Some, called non-survivors, died within two weeks of the cessation of antigen injections. Others, called survivors, remained alive for many months. At the time BSA injections were stopped, non-survivors were distinguishable from survivors by significantly decreased glomerular filtration rate and increased sodium retention. Two months later, immune deposits had become considerably less extensive than in the moderate stage of chronic serum sickness glomerulonephritis; C3 deposits were entirely undetectable. These changes were associated with a membranous transformation of the glomerular capillary wall. Partial recovery of glomerular function was evident, despite persistent proteinuria. In conclusion, rats with chronic serum sickness exhibiting abnormal retention of sodium, progress rapidly to end-stage renal disease, whereas those maintaining normal sodium balance may survive indefinitely despite persistent proteinuria. Maintenance of severe proteinuria does not appear to depend on an active immunological process.

NEPHROTOXIC SERUM NEPHRITIS (NSN) WITH HYPERTENSION: AMELIORATION BY ANTIHYPERTENSIVE THERAPY. J Neugarten, B Kaminetsky*, H Feiner, RG Schacht, DS Baldwin. Depts Med, Path, Peds. NYU and VA Med Centers, NY, NY.

Having developed a model of NSN in which hypertension is a regular feature, we examined the effects of antihypertensive therapy on the course of nephritis. NSN was induced in uninephrectomized male SD rats, all of which drank .9% saline ad lib. One half were randomly assigned to a treated group (Rx) which received diuretic, hydralazine and reserpine and became normotensive. At six weeks the following data were obtained:

	BP (mmHg)	Heart Wt (g)	$U_{prot}V$ (mg/24h)	S. Alb. (g/dl)
Rx(n=8)	103±3	1.11±.07	254±81	2.9±0.3
C (n=7)	148±5	1.53±.21	437±110	1.6±0.4
	p<.01	p<.001	p<.05	p<.01

Diffuse glomerular endo and extracapillary proliferation with arteriolar medial hypertrophy was commonly observed in untreated hypertensive nephritics. By contrast, with therapy, structural abnormalities were limited to focal segmental proliferation.

8-16 days after randomization hemodynamic measurements were obtained:

	BP (mmHg)	GFR (ml/min)	P_{GC}* (mmHg)	SNGFR (nl/m)	Q_A (nl/m)
Rx(n=8)	111±2	0.7±0.1	46±1	42±4	115±20
C (n=8)	169±5	1.0±0.2	55±1	56±5	160±20
	p<.001	p<.01	p<.001	p<.001	p<.01

*estimated from $P_{stop\text{-}flow}$

Our data demonstrate that treatment of hypertension ameliorates the clinical and histologic manifestations of NSN. This effect may be attributed to reduced glomerular hydraulic stress.

IMPAIRED ORGANIC ION TRANSPORT IN HEYMANN NEPHRITIS (HN) E.K. Park*, B. Noble, G. Andres and S.K. Hong. Depts. of Microbiology, Pathology and Physiology, SUNY at Buffalo, NY

The tissue slice technique was used to measure organic ion transport across the basolateral membrane (BLM) of proximal tubules (PT) in different stages of HN. Antibody-mediated injury to PT in stages 2 and 3 of HN leads to loss of brush border microvilli and basal invaginations. These histological changes were accompanied by severe decreases in slice to medium ratio (S/M) of p-aminohippurate (PAH) concentration (1.3 vs 2.7) and tetraethylammonium (TEA) concentration (6.9 vs 17.5). Other aspects of cell metabolism (Na-K-ATPase activity, O_2 consumption) remained normal. Study of the kinetics of organic ion transport in stage 3 revealed a reduction in the number of cation (TEA) carrier sites. In contrast, depression of organic anion (PAH) transport was associated with decreased substrate affinity of the anion carrier. Partial recovery of normal PT morphology in stage 4 of HN was accompanied by partial recovery of TEA, but not PAH, transport. Immunological injury appears to affect the BLM directly, producing impairment of organic anion and cation transport across the BLM. Reduced cation transport could result from decreased BLM surface area. However, qualitative changes detected in the PAH carrier lead us to postulate that autoantibodies in rats with HN react specifically with the anion carrier to alter its functional properties.

EFFECTS OF VALINE AND TYROSINE SUPPLEMENTS ON GROWTH OF RATS WITH RENAL FAILURE. David Powell*, Naum Spiegel*, Jean Harrah* and Malcolm Holliday, University of California at San Francisco, Dept. of Pediatrics, San Francisco, California.

It has been proposed that valine (VAL) and tyrosine (TYR) are limiting amino acids (aa) in patients with chronic renal failure (CRF). Supplementing low protein/aa diets with VAL and TYR may improve nitrogen retention and plasma and muscle fasting aa patterns. We put 110 gm rats into CRF (7/8 nephrectomy) and then evaluated growth and aa levels while feeding them either a standard 14% protein/aa diet (S diet) or one in which VAL and TYR supplements replaced some of the leucine and phenylalanine, respectively (VT diet). Sham operated rats served as controls (CON). At 3 and 9 weeks, serum Cr was 3X higher in CRF rats. Food and growth data after 9 weeks were as follows:

	food intake(gm)		Δ rat wt(gm)		Δ length(mm)	
Diet	CONrats	CRFrats	CON	CRF	CON	CRF
VT	1023±39	800±94*	227±16	148±39*	124±8	104±9*
S	1032±43	841±99*	237±26	157±42*	124±7	108±9*

* different from both CON groups, p<.05

Whereas CRF was associated with reduced intake and growth; the VT diets had no effect on any parameter measured; weight of soleus and EDL muscles at 9 weeks paralleled body wt. CRF rats as a group exhibited lower fasting serum TYR and lower 3 hour post feeding VAL and TYR vs CON rats (p<.05); higher serum VAL and TYR levels found in supplemented rats with CRF were not associated with improved growth or food intake. It is concluded that dietary TYR and VAL supplements offer no advantages for growth in young rats with CRF. Post feeding aa levels appear to be more sensitive than fasting levels.

ENHANCED NA-H ANTIPORT IN ISOLATED PROXIMAL TUBULAR CELLS 24 HRS FOLLOWING UNILATERAL NEPHRECTOMY. S Salehmoghaddam, EP Nord, W Trizna,and LG Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

In the ablative model of chronic renal failure, the increase in sngfr is matched by a parallel increment in proximal tubular salt and water reabsorption (Jv). Furthermore, the Jmax of the Na-H antiporter is increased approximately two-fold in brush border membrane vesicles. The present study was designed to investigate whether the adaptation documented at 3 weeks post ablation, could be observed at an earlier timepoint to explain previously documented early changes in Jv.

Intact proximal tubular cells from rabbit renal cortex (Kidney Int 25:311, 1984) were isolated from the removed kidney (control) and at 24 hr or 3 wks post-nephrectomy from the remainng kidney. Na influx (J_{Na}) was measured using ^{22}Na and H efflux (J_H) monitored by the pH-stat method. The initial rate of amiloride-dependent J_{Na} (10mM Na) and Na-dependent J_H (100mM Na) are reported. (*p<.05)

	Control	24 hrs	3 wks
J_{Na} (pmoles/10^6 cells/min)	125	313*	332*
J_H (nmoles/10^6 cells/min)	4.9	9.0*	12.8*

Conclusion: The major component of the increment in Na influx and H efflux from the proximal tubular cell, which is apparent 3 wks after unilateral nephrectomy, is manifest within 24 hr. Enhanced Na-H antiport is an early adaptive event.

ROLE OF SALT INDUCED HYPERTENSION AND DIETARY PROTEIN IN THE PROGRESSION OF GLOMERULAR DAMAGE IN GLOMERULONEPHRITIS. L. Raij, S. Azar, W.F. Keane, VAMC and University of Minnesota, Minneapolis, MN.

Immune mediated glomerulonephritis (GN) is the most common cause of renal failure. Development of glomerulosclerosis (Glom-Scl) is often a harbinger of progressive Glom destruction. We studied the effects of variations in dietary protein and NaCl content on the development of Glom-Scl in 4 groups of Dahl salt sensitive "S" rats with ferritin antiferritin immune complex GN (ferritin, 8 mg. ip for 8 wks.). Per cent protein/NaCl in chow in Groups I and II was 12/0.1 and 12/8 for 8 wks. followed by 9/0.1 and 9/8, respectively for another 8 wks. Groups III and IV received 40/0.1, 40/8, respectively for 16 wks. BP, Glom-Scl [(0-4+ x % Glom involved)] and proteinuria were evaluated after 16 wks. Results± SEM: † p < 0.05 vs Group I (n=6 to 8)

GRP	DIET	B.P.	U.PROT/24h	GLOM-SCL	B.W.
I	12-9/0.1	145±6	85±5	17±4	463±8
II	12-9/8.0	205±8†	123±8†	157±19†	405±7†
III	40/0.1	138±5†	136±10†	59±10†	459±5
IV	40/8.0	183±11†	271±7†	376±5†	455±10

Thus, hypertension (HPN) is the most important risk factor in progressive Glom-Scl in "S" rats with GN. Low protein diet does not prevent development of HPN and affords only partial protection againstHPN induced Glom-Scl (Group II). On the other hand, concomitant HPN and high dietary protein synergistically interact and strikingly increase Glom-Scl. Clinically, these studies suggest that in patients with GN, strict control of HPN and modification of dietary habits may slow the progression to renal failure.

BRANCHED CHAIN AMINO ACID (BCCA) METABOLISM IN CHRONIC RENAL FAILURE (CRF). M Schreiber, S. Kalhan*, A. McCullough*, S. Savin*. Cleveland Clinic Foundation and Case Western Reserve University at Cleveland Metropolitan General Hospital, Cleveland, Ohio.

Alterations in BCAA (leucine, isoleucine,valine) metabolism have previously been described in CRF. Oral ketoanalogues of BCAA have been employed in dietary therapy of chronic uremia. Therefore, we quantified the rate of leucine (LEU) turnover and LEU oxidation in 5 patients with CRF (mean Cr. 7.8 ± 2.7, mean BUN 87.4 ± 9.3) prior to the initiation of dialysis, and 2 patients currently on hemodialysis. Six normal subjects served as controls. (1-^{13}C)LEU tracer was used as a primed constant rate infusion for a period of 6 hours. ^{13}C enrichment of plasma LEU and of expired CO_2 was measured by mass spectrometry. The rate of CO_2 production and O_2 consumption was measured by respiratory calorimetry. Total body water measurements using $H_2(^{18}O)$ tracer were performed.

	Leucine*		Protein†	
	Turnover	Oxidation	Catabolism	Synthesis
Normals (6)	86.93 ±19.46#	12.42 ± 3.29	3.54 ±0.79	3.03 ±0.68
CRF (5)	79.35 ±16.99	7.58 ± 2.05	3.23 ±0.69	2.92 ±0.62

mean ± SD *umoles/kg.hr †gm/kg.day

In the 2 dialyzed patients the LEU turnover was 109.85 and 109.99 and LEU oxidation 11.5 and 14.7.

In CRF decreased nitrogen excretion results in decreased net nitrogen loss from LEU and decreased LEU oxidation. These studies provide a basis to examine the therapeutic effects of ketoanalogues and BCAA in CRF.

THE TWO-TIERED CONTROL SYSTEM FOR POTASSIUM. M.Shapiro* M.Levine* R.Makoff* G.Krishna, F. Gotch, I. Sargent, R. Korsak*,N.Wang, N.S.Bricker. Univ. of Calif., Los Angeles and San Francisco. Over 2-3 times ECF K content may be ingested in one meal. Were the volume of distribution of K limited to the ECF, death would follow rapidly despite maximum kaliuresis. Tier 1: Up to 90% of acute K loads can be transposed into the ICF (muscle and liver). Onset time is minimal, capacity is large and saturability is difficult to demonstrate (e.g., during a 60 minute K infusion = to 4.4% of ECF K/min in rats, 60-80% was sequestered continuously at a rate of up to 50 times the maximum rate of kaliuresis. Preliminary measurements of ^{86}Rb influx suggest that the augmented influx is due to stimulation of transport into the ICF rather than reduced efflux from the ICF. Net K flux does not correlate with P_K. Post infusion, net flux is from ICF to ECF and there is a linear correlation with U_KV. Tier 2: Using the newly developed techniques of gain analysis (accompanying abstract), NLA for K occurs with rare exception in all species with CRD. The % of maximal (or optimal) gain (Gain Coefficient or G.C.) changes over short intervals and is quite responsive to ongoing homeostatic needs. Following an acute K load, G.C. may change in a short time from negative to greater than 70%. The changes do not correlate directly with P_K or with many other testable variables. They do correlate linearly with $\frac{\Delta ICF_K}{\Delta ECF_K}$ (CRD/Control). Although Na-K ATPase presumably sets the upper limit of K secretion, the ongoing values for G.C. reflect changes in kinetics rather than the number of pumps.

TUBULAR SECRETORY VS. GLOMERULAR FILTRATION RATES OF CREATININE IN PRIMARY GLOMERULAR DISEASES. Ovadia Shemesh* and Bryan D. Myers. Dept. of Medicine, Stanford University, Stanford, CA.

We attempted to elucidate the tubular secretory (TS) component of the creatinine (creat) clearance in 173 patients with primary glomerular diseases (PGD). We compared simultaneous creat clearance to that of 3 filtration markers of graded size. The clearance ratio (θ) relative to inulin (radius [r]=16Å) was 1.02±0.14 for DTPA (r=4Å) and 0.98±0.13 for a narrow dextran fraction (r=19Å), with neither ratio differing from unity. In contrast, θ for creat (r=3Å) exceeded 1.0 and increased in a hyperbolic fashion as inulin clearance (GFR) declined. When GFR (ml/min)>80, θ creat = 1.2±0.4, GFR 40-80, θ creat = 1.6±0.4 and GFR<40, θ creat = 1.9±0.7. Corresponding values for plasma (P) creat were 0.9±0.3, 1.3±0.5 and 2.9±1.7 mg/dl. In deteriorating PGD (N=28), GFR declined from 61±12 to 32±6 ml/min while θ creat increased 1.6±0.1 to 2.3±0.3. With remitting PGD (N=26), GFR increased from 49±5 to 65±8, while θ creat fell, 1.8±0.1 to 1.5±0.1 (all changes $p<0.005$). IV infusion of 300 mg Cimetidine, a blocker of TS of creat, lowered θ creat acutely from 1.7±0.4 to 1.2±0.2 (N=12, $p<0.001$) without altering GFR. We conclude (1) that when r<19Å true filtration markers, including inulin, are unrestricted in PGD, (2) that the TS rate approaches the GFR of creat as the latter declines, thereby (3) maintaining P creat in a normal range until GFR<40 ml/min. We propose that in PGD attempts to evaluate GFR from creat clearance or predict it from P creat lead to gross overestimates. Similarly, GFR in PGD cannot be reliably monitored with creat because of an opposite change in tubular secretion of creat.

CATABOLISM OF LDL IS ALTERED IN EXPERIMENTAL CHRONIC RENAL FAILURE. R. Jean Shapiro*, Dept. of Medicine, Univ. of British Columbia, Vancouver, B.C. and R. Elam*, J. Witztum*: (intr. by John H. Dirks), Dept. of Medicine, Univ. of California, San Diego, La Jolla, CA.

Chronic renal failure is associated with abnormalities of lipoprotein (LP) metabolism. There is triglyceride enrichment in VLDL and LDL classes, whereas HDL cholesterol is low. To determine whether differences in LP particles, or differences in host clearance mechanisms might account for the altered LP metabolism of uremia, a two- way crossover study was done. Male Hartley guinea pigs (GP) were made uremic by unilateral nephrectomy and ligation of upper and lower poles of the contralateral kidney. BUN and creatinine rose approximately four-fold. ^{125}I-LDL isolated from donor uremic GP, and ^{131}I-LDL from donor control animals were injected simultaneously into recipient groups of control (C) and uremic GP (U), and the plasma disappearance followed. Fractional catabolic rates (FCR pools/hr) for the two sets of animals were as follows:

	CONTROL LDL	UREMIC LDL	
C(N=4)	0.0892±.002	0.0839±.0048	p<.075*
U(N=4)	0.0779±.0094	0.0708±.0091	p<.002*
	p<.057**	p<.043**	

* CONTROL LDL vs UREMIC LDL paired t test
** C vs U GP's, unpaired t test

The slower FCR of uremic LDL in both U and C GP's suggests an alteration in the LP particle itself. In addition, FCR of either uremic or control LDL was slower in the U compared to C GP, reflecting altered LDL clearance mechanisms in the uremic GP.

SUPERFICIAL NEPHRON SEGMENTAL HANDLING OF K AND NA IN ACUTE GLOMERULONEPHRITIS (AGN). E. Simon,* D. Martin,* D. Trigg,* J. Buerkert. Renal Div., Jewish Hosp., Washington Univ., St. Louis, MO.

Nephrotoxic serum nephritis was induced in 13 rats to examine the role of all superficial segments in K and Na homeostasis in AGN. About 22 d after initiation of AGN and in 14 paired controls (C), end (E) and beginning (B) distal (DT) and end proximal (EPT) tubule fluid was sampled from the same nephron. During AGN GFR fell from 1.4±0.1 to 0.9±0.1 ml/min ($p<.001$). Fractional excretion (FE) of Na was reduced from 0.23±0.05 in C to 0.10±0.02% ($p<0.05$). In contrast, due to an increase in FE(K) (from 12±1 in C to 18±2%, $p<0.02$), absolute K excretion was similar in the two groups. At the EPT, fractional delivery (FD) of H_2O and Na were not affected by AGN. However, due to a decrease in sodium reabsorption in the loop segment (LS) of AGN rats (from 87±1 to 82±2% of the delivered load, $p<0.02$), Na delivery to the BDT was similar and did not change to the EDT. Though K delivery to the BDT was not different in the two groups (14.1±2.3 in C vs 12.3±1.3 peq/min in AGN), delivery to the EDT doubled after AGN (to 27.5±4.9 peq/min, $p<0.025$) but remained unchanged in C (15.3±2.3 peq/min).

In conclusion, during AGN: 1) GT balance is maintained in the PT. 2) Due to a significant reduction in Na reabsorption by the LS, Na delivery to the EDT was similar to C. Therefore, sites distal to the EDT reabsorb Na more avidly. 3) The DT contributes significantly to K balance by enhancing secretion of K.

LOW PROTEIN BUT NOT LOW PHOSPHATE DIET INHIBITS PROTEINURIA IN AGING RATS. David Spector, Gary Hill, Nancy DiCiani*, Diana Teller*, Bertram Sacktor*, Div. Renal Med., F. Scott Key Med. Ctr., Johns Hopkins U. Sch. Med., GRC,NIA,NIH,Balto,MD.

We previously showed that proteinuria precedes the development of glomerulosclerosis and reduced GFR in aging rats (Abst.16thASN:107A,1983) To examine whether high dietary protein (pro) or phosphate (Pi) induced the renal dysfunction, we placed groups of 11 to 14 six mo. old male Wistar rats on four (pair-fed) diets: normal (20% pro, 0.5%Pi), low pro (10%pro, 0.5%Pi), low Pi (20%pro, 0.1%Pi), low pro-Pi(10%pro,0.1%Pi). After 6 mos, (age 12 mos) renal function and histologic tests were performed.

Rats on normal and low Pi diets had greater 24 hr. proteinuria ($\bar{x}$=61±17mg,63±18mg) than rats on low pro and low pro-Pi (22±5mg,9±1mg) and 6 mos rats (15±4mg)($p<0.05$). Semi-quantitative histology (scale:0-3) indicated that focal glomerulosclerosis was minimal in 6 mos rats($\bar{x}$= 0.25±.11) but developed in normal (0.77±0.23) and low Pi (0.79±0.12) rats ($p<0.05$). Glomerulosclerosis did not progress in rats on low pro (0.27±.16) or low pro-Pi(0.21±0.9) diets. Mean GFR (inulin clear.) was similar for all rat groups except the low pro-Pi rats which had significantly lowered GFR-perhaps due to gross and/or microscopic calculi noted in 13/14 animals.

Thus, in the aging rat, 6 mo dietary protein restriction, but not Pi restriction, protects against the development of proteinuria and glomerulosclerosis. Urinary calculi form in animals fed low protein-low Pi diet.Long term dietary effects (12 and 18 mo on diet) are being evaluated.

THE EFFECTS OF UNILATERAL NEPHRECTOMY ON GLOMERULAR SOLUTE TRANSPORT IN THE HUMAN. R.J. Weiss,* J.K. Leypoldt,* R.P. Frigon,* D.C. Otsuka,* L.W. Henderson. VAMC, San Diego, Calif.

Inulin clearance (C_{IN}) is a reference standard against which other measurements of glomerular filtration rate (GFR) have been qualified. Endogenous creatinine clearance (C_{CR}) in normal man approximates C_{IN}; fractional excretion of CR (C_{CR}/C_{IN}) approaches unity. Work in human kidney donors showed that C_{CR}/C_{IN} significantly exceeded unity; impairment of inulin filtration was postulated (Kidney Int 1979; 16:179-86).

We assessed C_{CR}/C_{IN}, C_{IN} with respect to ^{125}I-iothalamate (IO) (C_{IN}/C_{IO}), and clearance of polydisperse dextran (D) with respect to IO in 6 human kidney donors and 7 normal subjects. D concentrations were determined by high performance liquid chromatography. Data are presented as mean ± SEM.

	Normals	Donors	P
C_{CR}/C_{IO}	1.21 ± 0.10	1.20 ± 0.06	NS
C_{IO}/C_{IN}	1.05 ± 0.07	1.02 ± 0.08	NS
C_{CR}/C_{IN}	1.31 ± 0.16	1.21 ± 0.09	NS

We noted 51% functional hypertrophy in donors and no significant difference in fractional D clearance between the groups as would be expected if inulin were retained at higher single-kidney GFRs. C_D/C_{IO} curves were similar across the spectrum of intermediate-sized solutes. We suggest that inulin is not retained by the glomerulus at higher GFRs, and that tubular secretion of creatinine is responsible for higher C_{CR}/C_{IN} and C_{CR}/C_{IO}. We conclude that inulin serves as an adequate marker of GFR in kidney donors and that the degree of hyperfiltration found in this population does not result in significant impairment to glomerular transport of intermediate-sized molecules.

DIMINISHED LIPID STORES MAY MODULATE PROTEIN TURNOVER IN STRESSED UREMIC RATS. Steven J. Wassner and Jeanne B. Li*, Penna State U. Coll. of Med., Hershey Pa.

During starvation depletion of lipid stores leads to accelerated protein catabolism. We studied chronically uremic rats (U) to learn whether the increased muscle protein catabolism seen in U was associated with alterations of body lipid content. Protein synthesis (S) and net protein degradation (D) were measured using phenylalanine (Phe) incorportation and release from muscle during hemicorpus perfusion studies. In fed animals, muscle protein turnover was no different than normal. Significant differences were present after only 24h of fasting. By 48h S was 37 vs 28 nmol/h/g muscle and D 140 vs 88 nmol/h/g HC (both p[.05) in U compared to ad-lib fed controls (ALC). In pair-fed controls (PFC), D increased but S was not significantly different. U had lower body lipid content than ALC (29.6 vs 54 mg lipid/g carcass) and with fasting, body lipid stores were depleted sooner. After 48 h of starvation lipid levels dropped to 14.7 (U) vs 22.5 (ALC) mg lipid/g carcass respectively. Epididymal fat pad weights correlated with total body lipid content. After fasting, fat pad weight was 0.14% of body weight in U and 0.34 or 0.36 % in ALC or PFC (both p[.02). Percent weight loss was related to fat pad weight in U but not controls and rates of protein degradation were inversely related to lipid levels in both groups. Estimates of lipolysis during fasting in U suggest that lipolysis is not impaired but that lower initial concentrations of body lipids are more rapidly exhausted leading to enhanced net protein catabolism under conditons of metabolic stress.

UREMIC SERUM IS SUPERSATURATED WITH RESPECT TO CALCIUM OXALATE MONOHYDRATE. E.M. Worcester*, Y.N. Nakagawa* and F.L. Coe. Univ. of Chicago, Chicago, Illinois.

Oxalate is an end product of metabolism; serum levels rise in uremia because urine is its only route of excretion. Calcium oxalate (CaOx) crystals occur in the tissues of some uremic patients which suggests that their serum must have been supersaturated with respect to CaOx. The degree and consistency of supersaturation (SS) are impossible to know by direct measurement since ionic oxalate can not be measured. We have used a seeded crystal growth system to estimate the degree of SS in serum ultrafiltrates (UF). Ionic Ca (Ca++) and total oxalate (Ox) were measured in 6 uremic (mean predialysis creatinine 15 ± 1 mg/dl) and 7 normal subjects. Serum ultrafiltrate (pH adjusted to 7.4) was seeded with 1 mg/dl preformed CaOx monohydrate crystal, and then incubated (37 C) for 48 hours with stirring; Ca++ and Ox were remeasured. Preincubation (pre) [Ca++] [Ox] concentration products (all x10-9 M2, ±SEM) were 86 ± 9 (uremic) and 7 ± 2 (normal); postincubation (post) products were 51 ± 5 (uremic; p<.05 pre vs post), and 11 ± 3 (normal, p=NS pre vs post). Saline-Tris buffer (pH 7.4) was incubated similarly; concentration products were 64 ± 15 (pre) and 25 ± 9 (post)(p<.005). In another 11 patients, post dialysis [Ca++] was 36 ± 0.5, (p<.001, vs predialysis). The solubility product of calcium oxalate is 23 x 10-9 M2, corrected for activity but not for ionic oxalate. Our study suggests that oxalate retention in uremia leads to SS which creates a driving force for crystal deposition; SS may be present even after dialysis.

LOWERING OF ARTERIAL PRESSURE (MAP) LIMITS GLOMERULAR HYPERTENSION AND ALBUMINURIA IN EXPERIMENTAL DIABETES. R. Zatz,* T.W. Meyer, B.R. Dunn,* S. Anderson,* R.L. DeGraphenried,* J.L. Noddin,* A.W. Nunn,* J.L. Troy,* and B.M. Brenner. Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA.

Lowering of MAP with enalapril has been shown to limit both glomerular transcapillary hydraulic pressure gradient ($\overline{\Delta P}$) and glomerular sclerosis in rats with renal ablation. To determine whether similar lowering of MAP also prevents glomerular injury in experimental diabetes, rats were studied 4-6 weeks after streptozotocin injection (60mg/kg). Blood glucose (BG) was kept between 200 and 400 mg/dl by daily injections of ultralente insulin. Rats received either no additional treatment (Group DM), or enalapril, 15 mg/l in the drinking water (Group DMX). Age-matched normal control rats (Group C) were also studied. (Results: mean ±1SE, †p<.05 vs. C §p<.05 vs DM)

	BG	MAP	KW	SNGFR	Q_A	$\overline{\Delta P}$
	mg/dl	mmHg	g	---nl/min--		mmHg
C (n=7)	87±3	118±3	1.1±0.1	46±4	154±18	39±1
DM (n=8)	350±11†	115±4	1.5±0.1†	82±6†	269±26†	52±2†
DMX(n=8)	346±14†	98±2†§	1.3±0.1†§	72±7†	227±14†	37±1†

DM rats had marked elevations in kidney weight (KW), single nephron GFR (SNGFR), glomerular plasma flow (Q_A), and $\overline{\Delta P}$, as compared to C. Despite similarly increased BG levels, DMX showed a normalization of $\overline{\Delta P}$, as well as lesser elevations in SNGFR and Q_A, mostly as a result of lower MAP. Additional rats were kept for 6-8 months under similar conditions. Albuminuria (mg/24h) was: C=3±1, DM=32±8 and DMX=4±1 (p< 0.05 DM vs C and DMX vs DM). We conclude that intraglomerular hypertension is a readily reversible factor in the initiation of diabetic glomerular disease.

UNOCCUPIED RENAL ESTRADIOL RECEPTOR CONCENTRATION DIFFERS IN MALE & FEMALE RATS. SG Adler, PS Anderson,* CL Bowlus,* KL Rood,* AH Cohen, JD Kopple, and RJ Glassock. Dept. of Medicine and Pathology, Harbor-UCLA Medical Center, Torrance, CA.

Although renal estradiol receptors have been identified by a number of investigators, little is known about the effect of sex or diurnal variation on receptor characteristics. Since this could affect experimental results, we studied the concentration of free estradiol receptors (i.e. receptors unoccupied by hormone), and the dissociation constant (KD) in male (n=9) and female (n=8) Sprague Dawley rats. Unbound receptor concentration was higher in males than females (20.2 ± 4.3 SEM vs. 5.39 ± 1.6 fmol/mg cytosol protein; p<.02). There was no difference in KD (18.9 ± 5.8 vs. 7.6 ± 1.7 X 10^{-10} M) between the two sexes.

Peak and trough serum corticosterone levels varied predictably in all rats (18.1 ± 2.0 vs. 5.2 ± 1.1 µg/dl). Renal estrogen receptor concentration at peak (n=9) and trough (n=8) periods (15.6 ± 4.9 vs. 10.5 ± 8.9 fmol/mg cytosol protein) did not differ, nor was there a significant difference in the KD (10.9 ± 5.6 vs. 16.6 ± 3.6 X 10^{-10} M).

Thus, while the KD of renal estradiol receptor is similar in both sexes, unbound receptor concentration is higher in male than female Sprague Dawley rats. Therefore, any effect estrogens may have on the rat kidney may be expressed differently in males vs. females. Unbound estradiol receptors do not vary diurnally with serum corticosterone levels.

PROSTAGLANDINS, KININS AND OTHER RENAL HORMONES

SEROTONIN STIMULATES PGE_2 PRODUCTION IN CULTURED RAT MESANGIAL CELLS. Hanna E. Abboud and Thomas C. Knauss. VA Medical Center, Case Western Reserve University, Cleveland, Ohio.

Platelet activation and deposition are prominent components of many forms of glomerular injury. Serotonin (5-HT) is a potent vasoactive amine that may be released from platelets or synthesized locally in the kidney. Cyclo-oxygenase inhibitors have been shown to modulate the renal hemodynamic actions of 5-HT. We studied the effect of 5-HT on PGE_2 production in cultured rat mesangial cells (MC). 5-HT (10^{-4}M) stimulated PGE_2 production in a time dependent manner. After 0.5 min. ΔPGE_2 (mean ± SEM; pg/µg protein) was 2.5±2.1; at 2 min 9.1±3.0; at 5 min 11.7±5.0; at 10 min 16.9±6.7; at 20 min 23.2± 9.1; and at 30 min 33.2±10.4 (n=4 cultures). 5-HT stimulated PGE_2 production in a dose dependent manner. After 5 min incubation, basal PGE_2 levels (pg/µg protein) were 5.11± 1.0; 10^{-6}M 5-HT 8.3± 1.6; 10^{-5}M 10.44±3.3; 10^{-4}M 11.30±2.1; 10^{-3}M 13.25±2.7 (n=6 cultures). The vasoactive compounds dopamine and adenosine did not stimulate PGE_2 production in the same MC. Ketanserine, a specific 5-HT receptor antagonist, abolished the stimulatory effect of 5-HT. After 5 min incubations, basal PGE_2 levels (pg/µg protein) were 7.1±2.1; 5-HT (10^{-4}M) 14.9± 3.3; ketanserine (10^{-4}M) 7.2±4.1; 5-HT (10^{-4}M) + ketanserine (10^{-4}M) 5.7±1.3 (n=7 cultures). Similar inhibition was observed with the 5-HT antagonist cinanserine and methiothepin but not the histamine antagonists metiamide and diphenhydramine or the β -adrenergic antagonist propranolol.

These studies show that 5-HT stimulates PGE_2 production in MC via specific receptors. PGE_2 may modulate the effects of 5-HT on MC functions.

PERTUSSIS TOXIN REVERSES PROSTAGLANDIN E_2 INHIBITION OF ARGININE VASOPRESSIN (AVP) AND FORSKOLIN IN RABBIT COLLECTING TUBULAR EPITHELIUM. R.J. Anderson, P.D. Wilson,* M.A. Dillingham,* R. Breckon,* U. Schwertschlag, and J. Adolfo Garcia-Sainz*. Univ. Colo. Hlth. Sci. Ctr., Den., CO. and Univ. Nacional Autonoma de Mexico.

Prostaglandin E_2 (PGE_2) inhibits the hydro-osmotic response to AVP but not to cAMP in rabbit cortical collecting tubule (CCT) suggesting that PGE_2 inhibits AVP-stimulated cAMP formation. In initial studies we found that 10^{-6}M PGE_2 completely inhibited the effect of AVP (10^{-9} through 10^{-6}M) to stimulate adenylate cyclase in primary cell cultures of rabbit CCT (p<.001). An inhibitory guanine nucleotide regulatory protein (Ni) which is a target site of hormone action and which prevents cAMP formation has been identified in several cyclic nucleotide systems. We therefore examined the effect of pertussis toxin (an agent which inactivates Ni) on PGE_2 inhibition of AVP-stimulated adenylate cyclase. Incubation of cultured rabbit CCT in pertussis toxin (10 ng/ml) completely reversed PGE_2 inhibition of AVP-stimulated (10^{-9} through 10^{-6}M) adenylate cyclase. To confirm these results, we examined the effect of 10^{-6}M PGE_2 on forskolin-stimulated hydraulic conductivity (Lp) in isolated rabbit CCT perfused in vitro (25°C; 100 mOsm lumen-bath gradient). Forskolin alone increased Lp to 71±16x10^{-7} cm/atm·s. PGE_2 (10^{-6}M) pretreatment inhibited by 80% the Lp response to forskolin (n=6, p<.02). This inhibition was no longer present in rabbits treated with pertussis toxin (n=5). Together, these results suggest that PGE_2 inhibits AVP in the rabbit CCT by acting, at least in part, at a site distal to the AVP receptor. Our results are compatible with an effect of PGE_2 mediated via Ni.

THE GLOMERULAR ANGIOTENSIN II (AII) RECEPTOR: EVIDENCE FOR COUPLING TO A NUCLEOTIDE REGULATORY UNIT. B.J. Ballermann, R.J. Nadler,* and B.M. Brenner. Brigham and Women's Hosp., Boston, MA.

Guanine nucleotides reduce, and $MgCl_2$ enhances the affinity of many receptors for their agonists by interacting with nucleotide regulatory units stimulatory (N_S) or inhibitory (N_I) to adenylate cyclase. We determined the effects of $MgCl_2$, GTP and EDTA on rat glomerular (G) AII receptor affinity by studying AII dissociation rates and AII equilibrium binding parameters. We also determined the influence of AII on histamine-stimulated cAMP accumulation in G. In the presence of 10 mM $MgCl_2$ the equilibrium dissociation constant and AII receptor density (R_o) averaged 0.79±.08SE nM and 1.73±.08 pmol/mg glomerular protein, respectively, compared to 1.95±.13 nM and 0.90±.14 pmol/mg in the absence of divalent cations (5mM EDTA) (n=3). In studies of AII dissociation with infinite dilution 97±2% and 83±3% of AII remained bound at 3 and 60 min, respectively, with 5mM $MgCl_2$, compared to 76±4% and 52±4% without added $MgCl_2$ (n=4). GTP (100μM) enhanced AII dissociation ($p<.001$) after addition of excess unlabelled AII, with 39±3% and 1±1% of AII remaining bound at 3 and 60 min, compared to 89±3 and 62±3% without GTP (n=5). AII dissociation rates were similarly enhanced by GTP in crude glomerular membranes (n=2). AII reduced histamine-stimulated cAMP accumulation by 39±10% and 51±3% at 10^{-7} and 10^{-6}M AII, respectively. The effects of GTP and $MgCl_2$ on AII receptor affinity and inhibition of cAMP accumulation by AII suggest that the AII receptor in G couples to N_i. The doubling of R_o with $MgCl_2$ raises the possibility that this AII receptor may bind two AII molecules when coupled to N_i.

CONTROL OF PROSTAGLANDIN (PGE_2) SYNTHESIS BY RAT INNER MEDULLARY COLLECTING TUBULE (RIMCT) CELLS: ROLE OF cAMP. T. Berl,* and I. Teitelbaum. Dept. Med. Univ. Colorado Med. Sch., Denver, CO

In inner medullary slices cyclic nucleotides do not inhibit PG synthesis (J Pharm Exp Ther 219: 442, 1981) whereas in MDCK cells they do (Am J Physiol 244:C369, 1983). We have explored the ability of the cAMP analogue, 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (ClPheS-cAMP, 10^{-4}M) to modulate PGE_2 synthesis in RIMCT cells.

	PGE_2 Synthesis (pg/μg prot/hr)		
	Basal (n=6)	Ionophore ($5x10^{-5}$M,n=4)	Bradykinin (10^{-6}M,n=3)
Control	20.21±1.10	135.58±13.63	49.36±5.32
ClPheS-cAMP	10.46±0.87	85.71±9.84	20.98±1.48
p value	<.001	<.05	<.01

This effect to suppress PGE_2 production is not seen at 10^{-6}M ClPheS-cAMP. As AVP does not stimulate PGE_2 production by RIMCT cells (Am J Physiol 241:F94, 1981), we explored whether this failure to stimulate PGE_2 synthesis might be due to the concomitant AVP-stimulated increase in cAMP. $5x10^{-5}$M adenosine (Ad) inhibits 10^{-7}M AVP-stimulated cAMP production by 56% (417±23 to 181±8 fm/μg prot, $p<.001$). Yet, PGE_2 production in the presence of AVP+Ad, 21.37± 4.89 pg/μg prot/20 min, is not significantly different than that seen in the presence of AVP alone, 26.17± 5.43, (n=5). We conclude that 1) a cAMP analogue at a dose that increases hydraulic conductivity suppresses basal and stimulated PGE_2 synthesis thereby providing a feedback control of PGE_2 synthesis and 2) despite a significant reduction in cAMP, AVP fails to stimulate PGE_2 synthesis in RIMCT cells.

DEGRADATION OF ANGIOTENSIN II (AII) BY ISOLATED GLOMERULI. R.L. Baranowski and C. Westenfelder. Division of Nephrology, University of Utah and VA Medical Centers, Salt Lake City, Utah.

Receptors for AII have been demonstrated by us and others in isolated glomeruli. Virtually all receptor studies add large quantities of a scavenger peptide (ACTH 1-24) to the incubation medium to suppress degradation. It is possible that under physiologic conditions large quantities of scavenger peptides may not be present. Therefore, we studied glomerular AII binding without the addition of ACTH using physiologic concentrations of AII. Glomeruli were isolated from rat kidneys using a passive sieving technique. Binding was determined after incubation with 18 fmoles of AII at 37°C. All incubation solutions were extracted and subjected to reverse phase high performance chromatography to determine the extent of AII breakdown. Binding of AII under these conditions was never more than 10% and did not increase with time. Chromatography revealed that only 6% AII remained intact after 2 min. When a high concentration of unlabeled AII was added to the incubation mixture the remaining intact AII increased to 25%. In addition, different metabolites appeared in the chromatogram indicating a different pathway for breakdown. We conclude that glomeruli have a large capacity for AII degradation which may alter availability of physiologic concentrations of AII for binding, unless naturally occurring polypeptides can assume the role of scavenger peptides in vivo.

BRADYKININ STIMULATION OF RENAL OXIDATIVE METABOLISM: POSSIBLE ROLE OF ARACHIDONIC ACID. Peter C. Brazy and Paul E. Klotman, Duke Univ. and Durham VA Medical Centers, Durham, NC.

Bradykinin (BK) stimulates oxidative metabolism in cortical tubules from rabbit kidney (Clin. Res. 32:451A). BK also stimulates release of arachidonic acid (AA) from renal plasma membranes. We evaluated the possibility that the effect of BK on oxygen consumption was related to changes in AA availability. We compared the effects of BK and AA on oxygen consumption rates (QO_2) measured with a Clark-type electrode in suspensions of cortical tubules from rabbit kidney. Tubules were prepared by partial digestion with collagenase and separation on a Ficoll density gradient. Addition of BK (10 nM) to suspensions of cortical tubules increased QO_2 by 0.79±0.09 nmol/mg protein·min. Addition of AA (1 μM) increased QO_2 by a similar amount (0.68±0.10 nmol/mg protein·min). If BK was added after AA, there was no further increase in QO_2. Pretreatment of tubules with mepacrine (10 μM) to inhibit release of AA from membrane phospholipids blocked the BK effect on QO_2 but did not prevent stimulation of QO_2 by exogenous AA. Mitochondrial inhibitors, rotenone and antimycin A, blocked the effect of both BK and AA on QO_2. Indomethacin, a cyclooxygenase inhibitor, did not prevent BK or AA from stimulating QO_2 (+0.52±0.12 and +0.83±0.15 nmol/mg protein·min, respectively). These data indicate that both BK and AA stimulate mitochondrial respiration. The BK effect on QO_2 appears to depend on phospholipase activity and can be reproduced by addition of exogenous AA. These results are consistent with the hypothesis that BK activates the enzymatic release of AA from plasma membranes and thereby stimulates QO_2.

ALTERATIONS IN ANGIOTENSIN II (AII) RECEPTORS DURING PREGNANCY. Gail Brown* and Rocco C. Venuto. SUNY at Buffalo, Dept. of Med., Buffalo, New York.

During pregnancy glomerular filtration rate and renal blood flow (RBF) increase while systemic blood pressure and the pressor response to exogenous AII fall. Pregnancy induced changes in AII receptors could contribute to these changes. AII binding kinetics were determined using ^{125}I-AII in vascular (glomeruli, mesenteric vessels) and non-vascular (adrenal) tissues from 21-28 day pregnant (preg) rabbits. Comparisons were made using tissues from non-preg rabbits. Glomerular preparations were 90 ± 3% pure (n=16). Binding in all tissues was saturable and dissociable. The number of receptors (N), shown below, and binding affinities were obtained by Scatchard analyses of data.

		N (fmol/mg protein)		
	(n)	Non-Preg	Preg	P
Glomeruli	(7)	521 ± 98	325 ± 57	<.02
Mesenteric vessels	(4)	304 ± 21	112 ± 23	<.005
Adrenal	(8)	2552 ± 390	2447 ± 365	NS

Binding affinity differed only in adrenal plasma membranes. K_d was 0.9 ± .1 nM (non-preg) and 1.0 ± .1 nM (preg) ($P < .02$). The effect of the prostaglandin inhibitor, meclofenamate (M) on glomerular receptors was studied in preg rabbits. Following 3 days administration of M, the number of glomerular receptors increased (63 ± 14%; $P < .05$). We conclude that AII receptors are down regulated during pregnancy in glomeruli and mesenteric vessels. This could affect RBF and vascular responses. The increment of receptors following M suggests endogenous prostaglandins may contribute to receptor down regulation.

LEUKOTRIENE B_4 SYNTHESIS IN NORMAL RAT GLOMERULI V. Cattell*, J. Smith*, H.T. Cook*, S. Moncada*+ and J.A. Salmon*+, Dept. of Pathology, St. Mary's Hospital Medical School, London and +Dept. of Prostaglandin Research, Wellcome Research Labs., Beckenham, Kent, England.

The synthesis of the lipoxygenase metabolite of arachidonic acid (AA), leukotriene B_4 (LTB_4) by normal rat glomeruli has been studied. Isolated glomeruli from perfused rat kidneys were incubated in Krebs' buffer for 15 min at 37°C. The concentrations of LTB_4 and cyclo-oxygenase metabolites of AA in cell-free supernatants were determined by direct, specific radioimmunoassays (RIA). Mean basal synthesis were (n=11): thromboxane (TX) B_2 0.70 ± 0.08 > 6-keto-prostaglandin $F_{1\alpha}$ 0.50 ± 0.07 > PGE_2 0.43 ± 0.04 > LTB_4 0.11 ± 0.01 ng/mg glomerular protein. The dual inhibitor of cyclo- and lipoxygenase, BW755C (50 µg/ml), reduced the synthesis of immunoreactive LTB_4 (i-LTB_4) by a mean 44% whereas the cyclo-oxygenase metabolites were suppressed by more than 70%. To confirm LTB_4-synthesis, pooled supernatants from 30 rat kidney incubations were extracted using ODS silica cartridges and subjected to reverse-phase high pressure liquid chromatography (RP-HPLC). Immunoreactive LTB_4 in HPLC fractions was determined by RIA. A peak of i-LTB_4 was eluted at the exact retention time of authentic LTB_4 thus confirming the presence of LTB_4. However, i-LTB_4 was also detected at other retention times indicating that the i-LTB_4 measured by direct RIA includes a contribution from other unknown compounds as well as LTB_4 itself. The physiological role of LTB_4 in the normal kidney is unknown.

ROLE OF INTRACELLULAR CALCIUM IN RENIN SECRETION (RSR) BY RENAL NERVE STIMULATION (RNS). P. Cadnapaphornchai, D. Kellner*, and F.D. McDonald, Wayne State University School of Medicine, Department of Medicine, Detroit, Michigan.

RNS increases both RSR and efflux of Ca^{++}. We evaluated the effects of altering cellular Ca^{++} on RNS mediated renin release in 3 groups of anesthetized dogs. Each dog received RNS (0.5 Hz) twice as follows: control, RNS, recovery; vehicle, RNS and recovery. Group 1 (n=7) served as a time control. Group 2 (n=5) received Ca ionophore A23187 (Io) (0.6 ug/Kg-min) and Group 3 verapamil (V) 5 ug/Kg-min during second RNS. In Group 1, RSR increased from 95 ± 22 to 223 ± 73 ($p < .05$) and from 13 ± 5 to 108 ± 20 ($p < .005$) ng AI/hr-min giving the ratio (RNS/control) of 2.2 ± .3 and 7.7 ± 2.0 ($p < .02$) for the first and second RNS respectively. In Group 2 RSR increased from 211 ± 85 to 402 ± 118 ($p < .02$) and from 88 ± 11 to 157 ± 39 (ns) ng AI/hr-min giving the ratio of 2.3 ± 0.2 and 1.71 ± 0.4 (ns) for the first and second RNS. In both groups systemic and renal hemodynamics and UNaV did not change. In Group 3 RSR increased from 63 ± 17 to 109 ± 14 ($p < .004$) ng AI/hr-min during the first RNS. Prior to second RNS, V increased RSR from 42 ± 12 to 273 ± 71 ($p = .03$) ng AI/hr-min. With RNS, RSR did not increase further. UNaV increased significantly with V. The ratio for RSR was 2.2 ± 0.4 and 1.02 ± .2 (ns) for the first and second RNS respectively. These data suggest that, increased intracellular Ca^{++} by Io, suppresses RNS mediated RSR. V which decreases intracellular Ca^{++} does not enhance RNS mediated RSR. We conclude that alteration of intracellular Ca^{++} plays an important role in renal nerve mediated renin release.

STIMULATION OF NA-H ANTIPORT BY ANGIOTENSIN II IN THE PROXIMAL TUBULAR CELL IS MEDIATED BY PGE_2. A Chaudhari*, B Badie-Dezfooly*, BM Homadani*, and LG Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

Angiotensin II (Angio II), at low concentrations, has been reported to increase fluid reabsorption by the proximal tubule (PT). The specific Na transport process involved and the intracelluar signal for this process have not been determined. We examined the effect of Angio II on Na-H antiport and PGE_2 production in a confluent, primary culture of PT cells in which growth factors were removed 48 hr prior to study. 2 hr exposure to Angio II (10^{-9} M) caused uptake of 100 mM Na to increase from 36.8 ± 0.7 to 49.5 ± 5.6 nmoles/10^6 cells/min ($p<.01$). The increase was completely inhibited by 10^{-4} M amiloride. Angio II also increased PGE_2 production from 193 to 1335 pg/10^6 cells/2 hr. Exogenous PGE_1 increased amiloride-sensitive Na uptake by 195%; we thus wished to determine whether the effect of Angio II was mediated by intracellular PGE_2. Indomethacin (200 µM) inhibited PGE_2 production by 40% and completely inhibited the Angio II dependent increase in Na uptake (34.4 ± 2.8).

Conclusion: Angio II stimulates Na-H antiport in the PT cell. This effect is mediated by intracellular PGE_2. PGE_2 may be an important mediator of the hormonal regulation of this transport process.

STIMULATION OF IN VITRO VASOPRESSIN SECRETION FROM THE RAT NEURAL LOBE BY PROSTAGLANDIN E_2 (PGE_2), PROSTAGLANDIN D_2 (PGD_2) AND ARACHIDONIC ACID (AA). Gil Clifton and John Wallin. Tulane Univ. Sch. of Medicine and Veterans Administration Hospital, New Orleans, LA.

In vitro release of vasopressin from the neurohypophysis can be stimulated by a variety of conditions including electrical shock, increase in extracellular potassium concentration and exposure to cold. The critical role of calcium in regulation of vasopressin secretion is well recognized, however, the function of other second messangers such as prostaglandins and cyclic nucleotides in the secretion mechanism has not been clearly resolved. Previous investigations using both in vivo and in vitro models have shown PGE_2 stimulatory to vasopressin release. In the present studies we show that PGE_2 (1 and 100 nM), PGD_2 (1 and 100 nM) and AA (100 µM) increase both basal and potassium stimulated vasopressin secretion in amounts 1-2 fold greater than control.

Addition	Concentration	Per Cent Increase	
		6mMK+	60mMK+
PGE_2	1 nM	153*	172*
PGE_2	100 nM	98*	55**
PGD_2	1 nM	172	0
PGD_2	100 nM	160**	20
AA	100 µM	148*	23***

* $p<0.001$ ** $p<0.005$ *** $p<0.025$

These results not only suggest that prostaglandins other than PGE_2 may be important mediators of vasopressin secretion, but the effect of AA implies that prostaglandin synthesis occurs in the neurohypophysis where these second messangers act proximally to stimulate vasopressin release.

PERTUSSIS TOXIN BLUNTS PGE_2 INHIBITION OF ADH-STIMULATED Ve IN MOUSE mTALH. R. Michael Culpepper. Univ. Texas Med. School, Houston, Houston, Texas.

ADH stimulates the transport-dependent, transepithelial voltage (Ve) in mouse medullary thick ascending limbs (mTALH) by activating adenylate cyclase (AC) and increasing cAMP production. PGE_2 inhibits ADH stimulation of Ve but not that of cAMP, indicating inhibition of ADH generation of cAMP. Forskolin (FSK), a non-hormonal activator of AC, reverses PGE_2 inhibition of the ADH-dependent Ve. Pertussis toxin binds to an inhibitory, GTP-binding subunit of AC and prevents specific inhibition of AC by agents in a number of tissues. This study examined the effectiveness of PT in blocking PGE_2 inhibition of the transport Ve in mouse mTALH. The results of experiments on unpaired tubules are given below. Identical lots of ADH, PGE_2 and PT were used in all experiments. ADH was present at 10µU/ml in all experiments; PGE_2, at 10^{-6} M; and PT, at 1µg/ml, incubated for at least 40 min. Data are Ve in mV.

		ADH	ADH + PGE_2	Diff	p	n
PT	-	10.8±0.5	4.7 ± 0.9	6.1±0.6	<0.002	4
PT	+	9.0±0.7	8.1 ± 0.5	0.9±0.2	=0.04	4

The PGE-induced decrement in the ADH-dependent Ve in tubules not exposed to PT (6.1 ± 0.6 mV) was significantly greater than the small change (0.9±0.2 mV) seen in tubules incubated with PT ($p<.001$). PT at 100ng/ml and PT incubations of 20-30 min did not significantly alter PGE_2 inhibition of Ve. These data are consistent with a PGE_2 interaction with an inhibitory (Ni) subunit of AC which blocks ADH stimulation of AC, and Ve, in mTALH. PT, as in other tissue, appears to inactivate the Ni subunit and block PGE_2 inhibition of the ADH-dependent Ve.

EVIDENCE FOR RENAL TUBULAR ADENOSINE RECEPTORS. Richard Coulson* and Steven J. Scheinman* (intr. by M.E. Trimble). Depts. Pharmacology and Medicine, VA and Upstate Medical Centers, Syracuse, NY.

Tubular effects of the adenosine analog PIA (N6-(R-phenylisopropyl)adenosine) were studied in isolated rat kidneys perfused under conditions that enhance PTH-responsiveness (Scheinman & Coulson, AJP 246:F907). When PIA (0.5 mM) was used it was added to the medium at zero-time. Bovine 1-34 PTH was added as a bolus (40 pmol) after a 20 min equilibration and a 20 min control period. Three 20 min urine samples were then collected. Data ± SEM:

	n	Urinary cAMP (pmol/ml GFR)			
		Control	PTH (1 nM)		
		period 1	period 2	period 3	period 4
-PIA	10	12 ± 3	150 ±40	130 ±30	46 ±15
+PIA	10	11 ± 4	22 ± 5	21 ± 4	19 ± 4
p (Student t):			< 0.005	< 0.005	< 0.1

The decrease in PTH-stimulated UcAMP by PIA was not associated with an increase in kidney or perfusate cAMP, suggesting that PIA was not inhibiting cAMP efflux from cell to lumen. PIA did not alter renal ATP or GTP levels. In the presence of PTH, PIA did not affect fractional phosphate or calcium excretions, vascular resistance or GFR. PIA did increase the fractional excretion of sodium: 3.2 ± 0.4% with PTH alone and 5.6 ± 0.1% with PTH + PIA ($p < 0.05$). Failure of PIA to inhibit the PTH-induced phosphaturia may be due to PIA-induced natriuresis. Similar data were obtained at different levels of PIA (0.1 mM) and PTH (120 pmol bolus + 3 pmol/min infusion).

The data would support the hypothesis that PIA inhibits PTH-stimulated renal tubular adenylate cyclase activity by interaction with Ri-type receptors.

CORTICAL INTERSTITIAL CELL INTERACTIONS INDUCE SENSITIVITY OF HYDRONEPHROTIC KIDNEY (HNK) TO BRADYKININ (BK). **Bernard B. Davis, David Thomasson,* and Terry V. Zenser* VA Med. Ctr., GRECC, and St. Louis Univ. Schl. Med., Depts. of Int. Med. and Biochemistry, St. Louis, Missouri.**

Unilateral ureteral ligation induces both rabbit renal cortical prostaglandin H synthase and sensitivity to BK, effects attributable, at least in part, to the proliferation of macrophages (M) and fibroblasts (F) in the cortical interstitial area. The role of each cell type, M and F, in these effects was evaluated using mixed and purified cell cultures. Explants from unilaterally HNK, contralateral (CLK), and normal (NK) kidneys were incubated for 2 days, removed, and cells which migrated from explants allowed to grow for 11 days. BK increased PGE_2 production in HNK from 1.4 ± 0.2 ng/plate to 15.2 ± 2.1 ($p < 0.01$) but had no effect on CLK, 2.1 ± 0.4, or on NK, 1.9 ± 0.3. Neither pure F nor pure M from HNK responded to BK by increasing PGE_2 production, 0.9 ± 0.1 and 1.7 ± 0.9, respectively. However, the addition of M from HNK to HNK F restored BK responsiveness, 10.3 ± 1.4. M from CLK had no effect on HNK F. M from HNK when added to mixed cultures of either CLK or NK induced BK responsiveness, 12.1 ± 2.3 and 14.9 ± 3.1, respectively. These data indicate a unique interaction between M and F in HNK which induces the capacity of HNK cortex to respond to BK by increasing PGE_2 production. This capacity can be transferred to interstitial cells from nondamaged kidneys by M, derived by HNK cortical interstitium. Because M do not respond to BK it is suggested that the M from HNK uncover responsiveness to BK in F.

LOW DOSE ANGIOTENSIN II (AII) INFUSION AFFECTS IN VITRO CONTRACTILE RESPONSES AND PGE_2 SYNTHESIS OF ISOLATED RAT GLOMERULI. Janice G. Douglas and Carson White. Case Western Reserve University, Department of Medicine, Cleveland, Ohio.

AII binds to high affinity receptors of glomeruli and induces dose-dependent effects on glomerular surface area (GSA). We have previously demonstrated that low dose AII infusion in rats increases glomerular AII receptor density and affinity. The present studies were designed to determine the extent to which such receptor changes influence glomerular responses to AII. GSA was determined using a digitizing screen attached to a microscope. Control rats had a GSA of 11335±619 as compared to 9797±725μ^2(n=6) after AII pretreatment. The AII concentration that induced a maximum decrease in GSA was 100-fold lower in AII infused group (.08±.02pM, n=4) as compared to 7.8±2.3pM (n=4) for controls. The maximum contractile response was also significantly greater (11.5±1.5% as compared to 6.7±1.4% for controls). Because PGE_2 modulates GSA, we were interested in the extent to which PGE_2 might contribute to the enhanced contractile response. Basal and arachidonic acid stimulated PGE_2 was decreased in pretreated glomeruli (0.39±.006 & 1.14±.08 ng/mg protein) as compared to controls (0.92±0.2 & 2.6±0.01). In conclusion, AII pretreatment decreased GSA of isolated glomeruli and enhanced the sensitivity and magnitude of the in vitro contractile response to AII. This effect is in part mediated by a change in basal PG production and may also be caused by enhanced AII binding. This is the first evidence that altered AII binding to isolated glomeruli is linked to altered glomerular function.

THE EFFECTS OF LEUKOTRIENE C_4 (LTC_4) ON RAT GLOMERULAR MESANGIAL CELLS IN CULTURE. M.J. Dunn and M. Simonson*. Case Western Reserve University and University Hosp., Dept. of Medicine, Cleveland, OH

Isolated rat glomeruli have specific LTC_4 receptors which may mediate reductions of glomerular filtration rate. Since mesangial contraction could be a factor regulating glomerular responses to LTC_4, we investigated the effects of LTC_4 on the morphology of cultured rat mesangial cells.

Using image analysis microscopy, we measured the cross-sectional area (CSA) of individual mesangial cells. Changes in CSA during 30 min experimental periods were calculated as a percentage of each cell's basal CSA. In cells that responded, LTC_4 reduced CSA at 5 min and maximum contraction occurred between 10-20 min. With angiotensin II or a high K^+, the time course was similar to LTC_4; however, both the maximum decrease of CSA and the percentage of cells responding were greater. Since LTC_4 exerts some biological actions through stimulation of thromboxane A_2 (TxA_2) we evaluated the possible role of vasoconstrictor TxA_2 on LTC_4-mediated reductions of CSA. EP-092, a TxA_2 receptor antagonist, did not block the actions of LTC_4 on mesangial cells.

	% cells responding	CSA Decrease*
LTC_4, 1μM	33	23.1 ± 3.6
LTC_4, 1nM	31	16.2 ± 2.2
LTC_4, 1μM+EP092, 10μM	33	24.0 ± 6.0
Angiotensin II, 1μM	41	28.0 ± 4.4
High K^+ (30 mM)	67	30.5 ± 5.5

*Data are mean ± SE, % decrease

We conclude that LTC_4 contracts rat glomerular mesangial cells in culture, and that LTC_4 may have important intraglomerular actions to reduce filtration surface area.

CHRONIC CORTICOSTEROID EFFECTS ON SHORT-CIRCUIT CURRENT (I_{SC}) AND MORPHOLOGY OF CULTURED A6 (TOAD KIDNEY) EPITHELIA. R.L. Duncan*, T.M. Harris* and C.O. Watlington. Med. Coll. Va.-VCU, Richmond, Va.

Corticosterone (CS) induces greater I_{SC} increase than aldosterone (A) in A6 epithelia at 24h (abstracts these proceedings). This study examines chronic effects of A, CS and dexamethasone (Dex) at maximal effective concentration ($3.2x10^{-6}$M) on I_{SC} and morphology (electron microscopy) in cells placed in serum free medium 14-21d after seeding. CS stimulated I_{SC} was reduced 50% at 3d compared to 1d and remained so at 5 and 7d. In a shorter study I_{SC} (μAmp/5cm^2, mean ± SD;N=5) comparing 1d to 2d was:

	Control	A	CS	Dex
Day 1	4.0±.9	36.2±2.1	55.7±7.1	53.3±4.5
Day 2	3.9±.9	33.9±3.0	25.6±3.2	34.6±1.4
P	NS	NS	<.001	<.001

Control epithelia at 5d showed predominant multilayering (3-5 strata) of flattened cells with large intercellular spaces. Epithelia exposed to CS for 5d differed by showing primarily low columnar cells in monolayer, extensive lateral interdigitation, basal plasmalemma infolding, dense subapical bodies and mitochondrial changes. Cells exposed to CS and Dex for 2d were indistinguishable from those exposed 5d to CS. Although less multilayered than control, 2d A treated cells differed from CS and Dex series. They did not exhibit the extensive and regular low columna appearance, had widened intercellular spaces, less lateral interdigitation and less basal infolding. Enhanced I_{SC}, its down regulation and morphologic differences produced by CS and Dex compared to A may be mediated by receptors other than those shown previously to be co-occupied by the 3 steroids.

BIOSYNTHESIS OF PGI_2 IN SPONTANEOUSLY HYPERTENSIVE RATS: INCREASED OR DECREASED? Pierre Falardeau and André Martineau, Clinical Research Institute of Montreal, CANADA.

Recent experiments in our laboratory point to the existence of an impaired capacity for spontaneously hypertensive rats (SHR) to synthesize PGI_2 *in vivo*, contrary to the situation prevailing *in vitro*. Our conclusion that normal Wistar-Kyoto rats (WKY) can produce more PGI_2 than SHR *in vivo* relied on the quantification of urinary 2,3-dinor-6-oxo-$PGF_{1\alpha}$ (PGI_2-M), a major metabolite of PGI_2.

Since the urinary levels of PGI_2-M are determined by the balance between the amount of PGI_2 synthesized and the extent of its further metabolic oxidation, it became imperative to determine whether or not the metabolic fate of PGI_2 is identical in WKY and SHR. Therefore, we monitored the urinary excretion of PGI_2-M during the intravenous infusion of either PGI_2 or 6-oxo-$PGF_{1\alpha}$ in conscious, unrestrained rats aged 12-15 weeks, in doses ranging from 300 to 1000 ng. The proportion of substrate excreted in urine as PGI_2-M did not vary over this dose range and was found to be similar in SHR (17.1 ± 7.2%) and in WKY (16.7 ± 2.8%).

These results validate the use of urinary levels of PGI_2-M as an index of the *in vivo* production of PGI_2 in both normal and hypertensive rats and confirm the relevance of the conclusions drawn from our previous *in vivo* experiments. In addition, they incite to a certain caution about extrapolating directly to *in vivo* situations the conclusions derived from *in vitro* experiments.

THROMBOXANE: AN EARLY INDICATOR OF KIDNEY ALLOGRAFT REJECTION. M.L. Foegh,* M.R. Alijani,* G.E. Schreiner, P.W. Ramwell* and G.B. Helfrich* (Intr. by W.P. Argy). Georgetown Univ. Med. Ctr., Dept. of Medicine, Div. of Nephrol., Dept. of Surg., Div. of Transplant., and Dept. of Physiol. and Biophys., Washington, D.C.

Thromboxane A_2 (TXA_2) is derived from activated platelets and inflammatory cells which invade the rejecting allograft. TXA_2 is a powerful pathogenic mediator which rapidly hydrolyses to thromboxane B_2 (TXB_2). Urinary immunoreactive TXB_2 (i-TXB_2) is reported elevated prior to clinical diagnosis of renal allograft rejection in 12 patients. This phenomenon has been studied in a further 50 patients who exhibited 62 rejection episodes. The day of rejection is defined as the day of the clinical diagnosis of rejection and initiation of treatment. Urinary i-TXB_2 was compared with serum creatinine and B_2-microglobulin (B_2-MG) as indicators of kidney allograft rejection. Two days prior to rejection, an increase in urine i-TXB_2 occurred in 50% of the rejection episodes, whereas serum B_2-MG was increased in 35% and serum creatinine was increased in all but 5% of the episodes. On the day prior to rejection elevated urine i-TXB_2, serum B_2-MG and serum creatinine values were seen in 63%, 50% and 15%, respectively, of the rejection episodes. The incidence of false negative values was the same for all three parameters. The incidence of false positive values for both urine i-TXB_2 and se um B_2-MG was double that for serum creatinine. We conclude that urine i-TXB_2 is an earlier indicator that both serum B_2-MG and serum creatinine of kidney allograft rejection. A statistical evaluation will be presented.

THE ANTI-RENIN/ANTI-ALDOSTERONE ACTIONS OF SYNTHETIC ATRIAL NATRIURETIC FACTOR IN CONSCIOUS DOGS WITH CAVAL CONSTRICTION AND ASCITES. R.H. Freeman, J.O. Davis, R.C. Vari*, W.D. Sweet*. University of Missouri, Columbia, MO.

Atrial natriuretic factor (ANF) is synthesized in mammalian atria, but its physiological function has yet to be defined. Constriction of the thoracic inferior vena cava to decrease venous return and atrial filling, markedly elevates plasma renin activity (PRA) and plasma aldosterone concentration (PAC) and produces chronic sodium retention and ascites in the dog. In this study, we infused synthetic ANF (BBRC 117:859, 1983) into conscious dogs with caval constriction and ascites at doses of 57 and 114 pM/kg/min for 30 min each. Both doses of ANF decreased PRA from elevated control levels of 24±6 and 24±5 ng/ml/hr to 16±4* and 14±4* ng/ml/hr, respectively (*P<0.05); PAC decreased from 72±26 and 63±26 ng/dl to 29±10* and 23±8* ng/dl, respectively (*P<0.05). The infusion of ANF at these doses into conscious, normal dogs did not suppress basal levels of PRA or PAC, but produced a natriuresis. Arterial pressure and the clearance of PAH did not change during the infusion of ANF in these dogs with caval constriction, but creatinine clearance increased from 78±9 and 79±9 ml/min to 95±10* and 92±10* ml/min, respectively (*P<0.05). Infusion of ANF also produced significant diuresis and kaliuresis in this low output model of chronic sodium retention, but urinary excretion of sodium remained low. These results are consistent with the concept that ANF might function to tonically inhibit the renin-angiotensin-aldosterone system and to maintain glomerular filtration.

CAUSAL ROLE OF THROMBOXANE IN ORGAN ALLOGRAFT REJECTION. M.L. Foegh,* B.S. Khirabi,* G.F. Schreiner, and P.W. Ramwell* (Intr. by W.P. Argy). Georgetown Univ. Med. Ctr., Dept. of Medicine, Div. of Nephrol., and Dept. of Physiol. and Biophys., Washington, D.C.

Increased excretion of urinary immunoreactive thromboxane B_2 (TXB_2), which is the stable hydrolysis product of thromboxane A_2 (TXA_2), is associated with kidney allograft rejection in patients and experimental cardiac allograft rejection in rats. The source of thromboxane is most likely platelets and inflammatory cells accumulating in the rejecting allograft. A causal role for this indicator is postulated on the basis of the powerful pathogenic properties of TXA_2. This hypothesis was tested. A thromboxane synthase inhibitor (OKY-1581, ONO, Japan) as well as a thromboxane antagonist (L640035, Merck-Frosst, Canada) were administered to rats receiving a cardiac allograft. The rats were divided into four groups: Group A (n=6) received saline; group B (n=9) received azathioprine; group C (n=11) received azathioprine and OKY-1581; group D received azathioprine and L640035. Group A rejected the allograft on day 8.5 ± 0.4; group B rejected the cardiac allograft on day 9.2 ± 0.8 (n.s.), group C on day 11.5 ± 1.2 ($p< 0.05$), and group D on day 14.2 ± 1.0 ($p< 0.002$). We conclude that the increase in allograft survival with the thromboxane receptor antagonist and the thromboxane inhibitor is highly suggestive of a causal role for TXA_2 in allograft rejection; thus thromboxane may be an indicator with a causal role.

PGE_2 IS AN IMPORTANT INTRACELLULAR MODULATOR OF NA-H ANTIPORT IN THE RENAL PROXIMAL TUBULAR (PT) CELL. D Goldfarb*, EP Nord, A Chaudhari*, S Vaystub*, A Hafezi*, H Rahimizadeh* and LG Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

Prostaglandins (PG) have been shown to be important intracellular modulators of transport events in the renal tubule, but no role for PG in the PT has been identified. We thus studied PGE_2 production and the effect of PGE_2 on Na-H antiport.

Intact isolated PT cells prepared from rabbit kidney (Kidney Int 25:311, 1984) were used to measure PGE_2 production, Na influx (J_{Na}) and H efflux (J_H). PGE_2 biosynthesis from arachadonic acid (AA) was determined by radioimmunoassay, J_{Na} was measured using ^{22}Na, and J_H monitored by the pH-stat method. PGE_2 production increased from 82 to 275 pg/mg/hr with addition of 15 µM AA and 1 mM glutathione (GSH). This increment was inhibited approximately 50% by addition of either 200 µM indomethicin or 200 µM meclofenamate or 2 mM aspirin to the incubation medium. Since PGE_2 appears to be a major AA metabolite in PT cells the effect of PGE_2 on Na-H antiport was examined. In the presence of PGE_2 (10^{-6} M) the initial rate of amiloride-sensitive J_{Na} (1 mM Na) increased by 65%. J_{Na} (control) 7 ± 0.5 vs 11 ± 1 (PGE_2) pmoles/10^6 cells/15 secs. Na-dependent (100 mM Na) J_H increased 60%: J_H (control) 2.9 vs 4.6 (PGE_2) nmoles/10^6 cells/15 secs.

Conclusions: 1. PT cells produce PGE_2 as a major AA metabolite. 2. PGE_2 enhances Na-H antiport in the proximal tubule suggesting an important modulator role for this compound.

EXTRA-RENAL ROLE FOR ALDOSTERONE IN ADAPTATION TO HIGH POTASSIUM (K) INTAKE IN THE RAT. M. Goldstein,* B.S. Dixon,* S. Anderson,* and R.J. Anderson. Univ. Co. Hlth. Sci. Ctr., Den., CO.

The mechanisms underlying the early phase of adaptation to high K intake in the rat remain unknown. We therefore examined the effect of high K intake (1,400 μEq/gm food) in conscious rats in metabolic balance cages. An increase in urine K excretion occurred with the first feeding period and reached a stable plateau after 3 days. The increase in K excretion was associated with polydipsia, polyuria and natriuresis. Prevention of polyuria (water restriction), rendering the urine Na-free (5 days of Na depletion) and maintenance of constant blood aldosterone (adrenalectomy with minipump replacement of physiologic concentrations of corticosterone and aldosterone) did not affect the kaliuretic response to high K intake. Combined water restriction and Na depletion resulted in a significant 17% decrease in K excretion. When adrenalectomized rats replaced with physiologic concentrations of corticosterone and aldosterone were Na depleted and fed high K a kaliuretic response comparable to controls was observed. Despite comparable kaliuresis, these rats had either high mortality or high plasma K (9.1±0.3 versus 4.7±0.3 mEq/L in controls). In the absence of aldosterone, rats ate virtually no K and replacement of aldosterone restored K appetite. These results demonstrate that the kaliuretic response to high K intake occurs independent of single factors such as polyuria, Na in the urine and increase blood aldosterone. Aldosterone however appears to exert critical extrarenal effects (K appetite and plasma K concentration) in adaptation to high K intake.

EXTRARENAL RENIN DURING ONTOGENY IN THE MALE MOUSE Julie R. Ingelfinger, Richard E. Pratt*, Kristin E. Ellison* and Victor J. Dzau*, Harvard Medical School, Boston MA

Certain strains of mice (e.g. CD-1) contain 2 renin genes (Ren-1 and Ren-2). Ren-1 is predominantly expressed in kidney, whereas Ren-2 is expressed in the submandibular gland (SMG). SMG renin is under androgenic influence, but it is unclear if other extrarenal renins are under similar control. To that end, we examined tissue renin expression in the male mouse at various ages from 1 to 40 days. Renal renin concentration remained relatively constant (approximately $3x10^4$ ng AI/h/mg protein). In contrast, SMG, testes and adrenals all showed low renin levels from birth to puberty and dramatic increases thereafter, coinciding with androgen increase.

Renin Activity (ng AI/h/mg Protein ± SEM)

Organ	Pre-puberty (D22)	Post-puberty (D38)	Sig
Kidney	$(3.0\pm1.2)x10^4$	$(2.7\pm0.36)x10^4$	NS
SMG	$(1.0\pm0.12)x10^5$	$(2.0\pm1.0)x10^6$	p<.001
Testis	24±0.25	60±0.37	p<.001
Adrenal	32.6±1.26	412±155.0	p<.01

Our data demonstrate that renal renin is unchanged during development of the male mouse. In contrast, SMG renin expression, under androgen influence, increases post-puberty. Since other extrarenal tissue renin levels parallel that of SMG during ontogeny, we hypothesize that these renins may also be under androgen regulation and are probably products of Ren-2 gene expression. Thus, it appears that in the mouse, the local tissue renin-angiotensin system is controlled by mechanism distinct from those in kidney.

MECHANISM OF RENAL ACTION OF PURE ATRIAL NATRIURETIC FACTOR (ANF). C.-L. Huang,* J. Lewicki*, and M.G. Cogan. CVRI, Depts. of Med. and Physiol., Univ. of Calif., San Francisco, and Calif. Biotechnology, Inc., Palo Alto, Calif.

Controversy exists as to whether the increased solute excretion induced by crude extracts of ANF is mediated hemodynamically or by direct tubular transport inhibition. 7 rats were studied during a control euvolemic period (C) and following 12 μg/kg then 0.1 μg/kg/min pure rat 24 amino acid ANF. ANF increased $U_{Na}V$ and $U_{Cl}V$ by 10- to 25- fold, with a kaliuresis but little bicarbonaturia. GFR increased from 1.17 ± 0.04 to 1.52 ± 0.07 ml/min (p<0.005). ANF increased SNGFR from 34 ± 1 to 44 ± 2 nl/min (p<0.001) measured at the end-proximal site (EP) and from 26 ± 1 to 37 ± 2 nl/min (p<0.005) in the early distal (ED) sites. Significantly (*) increased absolute and slightly decreased fractional solute reabsorption were appropriate for increased flow.

	Absolute Reabs.			Fractional Reabs.		
	HCO_3^- (pmol/min)	Cl^- (peq/min)	H_2O (nl/min)	HCO_3^-	Cl	H_2O
EP: C	820 ± 28	1605 ± 102	17 ± 1	.90 ± .01	.41 ± .02	.51 ± .02
ANF	976* ± 35	1786 ± 182	20* ± 1	.82* ± .02	.35* ± .03	.46* ± .02
ED: C	683 ± 35	2873 ± 35	22 ± 1	.98 ± .01	.96 ± .01	.84 ± .03
ANF	947* ± 60	3836* ± 234	29* ± 2	.96* ± .01	.91* ± .01	.78* ± .03

To exclude the possibility that ANF increases GFR only in anesthetized animals, 8 further unanesthetized rats were studied. GFR increased 45%, from 2.04 ± 0.17 to 2.97 ± 0.27 ml/min (p<0.005), without a change in renal plasma flow (C_{PAH}): 5.4 ± 0.5 to 5.6 ± 0.4 ml/min. In conclusion, ANF significantly increases GFR, which appears to account for the increased solute excretion.

A METABOLITE OF CORTICOSTERONE IS ANTINATURETIC BUT NOT KALURETIC IN RAT. JP Johnson, JL Atkins, JS McNeil* AND CO Watlington. WRAIR, Washington, DC and Medical College of Va, Richmond, Va.

Cultured epithelial cells metabolize corticosterone (Corti) to the 6β-OH derivative (6B-Corti), a metabolite which increases short circuit current in these cells (Abstracts, these proceedings). We sought to determine whether 6B-Corti has any action in mammalian kidney. Preliminary studies in intact rats showed significant antinaturesis. We then studied adrenalectomized (AX) rats. Rats were AXed and maintained on saline drinking water. Adequacy of AX was assessed by measurement of plasma Corti. Rats were kept in metabolic cages for three day control urine collections. Rats then received, vehicle (control), aldosterone (Aldo, 15ug/100g), Corti (15ug/100g), 6B-Corti or a combination of the above, by IP injection along with an acute load of 0.45% NaCl, 0.25% KCl (3ml/100g). Initial 0-2 hr urine samples and subsequent 2-5 hr samples were analyzed separately for Na+, K+ and creatinine (Cr) concentrations. Aldo produced a significant antinaturesis and kaluresis in both periods. Corti produced a slight decrease in Na+ excretion and an increase in Cr excretion, neither of which was significant. Corti produced significant kaluresis in the first two hours. 6B-Corti produced a significant antinaturesis in both periods but was not additive to Aldo or Corti. 6B-Corti did not result in kaluresis and did not effect Cr excretion. These results demonstrate that a metabolite of corticosterone produces an antinaturesis but not a kaluresis suggesting that it acts at a different site or through different receptors than Aldo or Corti in mammalian kidney.

PROSTACYCLIN (PGI_2) AND PROSTAGLANDIN E_2 (PGE_2) BIOSYNTHESIS BY ISOLATED RENAL MICROVESSELS (MV). M.A. Kirschenbaum, A. Chaudhari*, D. Aframian*, J. Cherry*, J.J. Kownacki*, and H. Pae*. Div. of Neph., UCLA Dept. of Medicine, Los Angeles, CA.

Blood vessels can produce prostanoids (PG) which may have direct and indirect effects on their function (modulating effects of vasoactive factors, i.e., angiotensin II, norepinephrine, ADH). These properties may be important to the kidney since both glomerular and tubular function may be influenced by changes in perfusion. Studies have been designed to evaluate directly the ability of isolated MV (interlobular arteries and afferent arterioles) to produce PG. A method has been developed to obtain large numbers of renal MV with high purity. A homogenate of renal cortex from rabbit kidneys previously perfused with magnetized iron particles is purified by graded sieving and MV are separated from nonvascular tissue with a magnet yielding a preparation showing a >50X enrichment of PGI_2 production/mg prot when compared to the crude homogenate. The yield of MV is 3-5 mg prot/kidney. MV are capable of synthesizing PGI_2 and PGE_2 (as determined by RIA) in a ratio approx. 4-5:1 both in the absence and presence of arachidonic acid (AA). The addition of AA leads to a dose-dependent increase in both PGI_2 and PGE_2 synthesis. Indomethacin and meclofenamate (both 100 μM) reduced MV PG synthesis by 70%. In conclusion, a method has been described in which isolated renal MV can be easily prepared in large quantity and high purity. They are capable of producing both PGI_2 and PGE_2 in both the presence and absence of exogenous AA. This production can be blocked by cyclooxygenase inhibitors.

THE EFFECT OF AN INTRACELLULAR CALCIUM ANTAGONIST UPON MESANGIAL CELL PROSTAGLANDIN PRODUCTION. T.C. Knauss. Case Western Reserve University and Cleveland VAMC, Cleveland, OH.

The trimethoxybenzoate derivitive TMB-8 has received wide use as an intracellular calcium antagonist. In the present studies the effects of this drug upon rat mesangial cell PGE_2 production and phospholipid (PL) metabolism are assessed.

	VEHICLE	TMB-8 10μM	TMB-8 100μM
Basal ng PGE_2/ml	1.2±0.1	1.0±0.4	0.6±0.1
Angiotensin II(AII)	1.9±0.1	1.2±0.1	0.6±0.1
Arachidonic Acid(AA)	8.8±1.5	9.0±2.5	4.7±1.8

TMB-8 10μM blocked AII stimulated PGE_2 production without affecting basal synthesis. This effect is proximal to AA liberation as PGE_2 production in the presence of AA 5μg/ml was unaffected. In contrast 100μM TMB-8 decreased basal levels by 50% and AA stimulated production by 47%.

PL turnover was measured by ^{32}P or ^{14}C-choline labeling experiments. Data are expressed as the mean fractional isotope incorporation into phosphatidylcholine(PC) phosphatidic acid (PA) or phosphatidylinositol (PI) compared to matched controls.

	TMB-8 10μM	TMB-8 100μM	TMB-8 1mM
PC: ^{14}C:	0.79±.05	0.35±.05	0.18±.05
PC: ^{32}P:	0.90±.09	0.40±.06	-
PA: ^{32}P:	1.08±.10	1.70±.10	-
PI ^{32}P:	1.12±.10	2.14±.10	-

This dose dependent re-direction of PL synthesis away from PC and towards PA and PI is most compatible with an inhibition of the enzyme phosphatidate phosphohydrolase. We postulate that increased levels of negatively charged PL may bind intracellular calcium. At 10μM TMB-8 this may stabilize a hormone sensitive pool. Larger doses inhibit PGE_2 synthesis by less specific mechanisms.

EFFECT OF FUROSEMIDE (F) ON SUPERFICIAL NEPHRON CHLORIDE (CL) TRANSPORT DURING PROSTAGLANDIN SYNTHESIS INHIBITION (PGI). Kent A. Kirchner, Dept. of Med., Univ. of MS Med. Ctr., Jackson, MS.

PGI antagonizes the chloruretic action of F. To identify the site of this effect, cortical micropuncture was performed in F (4 mg/kg/hr) treated rats (n=10/group) during meclofenamate (M) or indomethacin (I) infusion. Control (C) rats received the PGI vehicle. PGI reduced fractional Cl excretion compared to C values (I: 6.58±0.93% vs C: 10.6±1.0, P<.01; or M: 5.7±0.7% vs C: 10.6±1.0, P<.001). Mean arterial pressure (MAP), C_{In}, RBF, late proximal tubule (PT) and distal tubule (DT) SNGFR were not different between PGI and C rats. Cl delivery out of superficial cortical segments is as follows:

	Late PT		Early DT		Late DT	
	Abs (pEq/min)	Fract (%)	Abs (pEq/min)	Fract (%)	Abs (pEq/min)	Fract (%)
C	1253	48	1428	38	553	15
	±91	±3	±210	±2	±60	±1
I	1493	43	906*	22††	353†	9**
	±78	±3	± 83	±3	±27	±1
M	1293	40	780**	21††	345†	9**
	±68	±2	± 83	±2	±46	±1

*P<.025 compared to C; **P<.02; †P .01; ††P<.001. Loop segment chloride uptake was less in C than either I (Abs: 138±144 pEq/min vs 587±113, P<.02; and Fract: 9±2% vs 18±2%, P<.05) or M (Abs: 514±65, P<.02; and Fract: 18±2%, P<.05) rats. DT Cl uptake (both fract and abs), however, was greater (P <.02) in C than either PGI group. We conclude that attenuation of F's effect by PGI is not dependent on alterations in MAP, C_{In}, RBF, SNGFR, PT or DT Cl handling but may, at least in part, result from a PGI induced change in loop segment Cl handling.

CELL-TO-SUBSTRATE ADHESION IN MESANGIAL (MS) CELLS IS AFFECTED BY HORMONES WHICH AFFECT CONTRACTILE BEHAVIOR. J.I. Kreisberg and M.A. Venkatachalam. Univ Texas Health Science Center, San Antonio, TX.

MS cells grown on plastic have bundles of stress fibers which terminate on substrate attachment plaques. Tight cell-to-substrate adhesion may be responsible for the inability of MS cells to contract after treatment with vasoactive agonists. We examined cell adhesion by a trypsin assay as well as by interference-reflexion (IR) microscopy after treatment with agents that raised intracellular cAMP (e.g., db-cAMP and isoproterenol plus MIX [ISO])and vasoactive agents (e.g., vasopressin [V] and PGE_2). For the trypsin assay, data are expressed as % cells removed from the plastic. Both ISO and db-cAMP decreased mesangial cell adhesion (Detached cells: control = 12.3 ± 3.11%; ISO = 48.3 ± 7.4%; db-cAMP = 29.3 ± 3.7%). Also, the cells became arborized, stress fibers were fragmented, and the IR image was altered. That is, the areas of adhesion between the cell and substrate disappeared and were replaced by areas of greater separation. Addition of PGE_2 or V to ISO treated cells or to the incubation medium containing cAMP elevating agents reversed or prevented the above changes, respectively. PGE_2 or V alone increased MS cell adhesion (Detached cells: control = 11 ± 2.4%; V = 5.4 ± 0.4%; PGE_2 = 6.8 ± 0.5%). The areas of adhesion as seen by IR microscopy were also increased. Thus, the lack of isotonic contraction after exposure of cultured MS cells as well as smooth muscle cells to contractile agonists may be due to the development of tight adhesions to the substrate.

RADIOIMMUNE DETERMINATION AND CHARACTERIZATION OF ATRIAL NATRIURETIC PEPTIDE IN PLASMA. R.E. Lang, F. Luft, D. Ganten, H. Thoelken, Th. Unger. Dept. of Pharmacology, Univ. of Heidelberg, FRG.

The mammalian cardiac atria contain biologically active peptides, termed atrial natriuretic peptides (ANP), which are capable of producing natriuresis, diuresis, and vasorelaxation. Although ANP presumably acts as a hormone, the available assays were not as yet sufficiently sensitive to demonstrate its presence in blood. Using ^{125}J-labelled α-rANP (5-28) as tracer, and antibodies raised against synthetic rat α-ANP (5-27) (Kangawa et al., BBRC 121:585-591, 1984), we developed a sensitive radioimmunoassay which recognizes α-rANP (5-25) as well as (5-27) and (5-28). The final dilution was 1:80.000; 50% intercept 59 pg α-rANP (5-28). The total content of α-rANP (5-28)like immunoreactive material in both rat atria was 3,73 ± 0,19 µg α-rANP (5-28) ($\bar{x}$ + SEM), n=12). Separation of extracts from rat atria by high pressure liquid chromatography (HPLC) revealed a number of immunoreactive peaks with the highest eluting at the position of β-rANP. Acute volume load (iv administration of 20 ml saline /kg body weight) increased the α-rANP immunoreactivity in plasma of male rats from 157 ± 17 to 992 ± 332 pg/ml ($p<0.05$) within 1 min. On HPLC, plasma extracts showed a single peak of immunoreactive material with a similar retention time as α-rANP (5-28).

This demonstration of circulating ANP suggests an endocrine function for cardiac atria with respect to regulation of extracellular fluid.

LACK OF RENAL RENIN RESPONSE TO ACUTE ALTERATION OF SERUM POTASSIUM. A. Leahy*, N. Bondar, P. Cadnapaphornchai, F.D. McDonald. Wayne State University School of Medicine, Department of Medicine, Detroit, Michigan.

The effect of acute alteration of K^+ on renin release was evaluated in the isolated perfused rat kidney perfused with a recirculating pass. The perfusate contained Krebs-Henseleit solution with 5 mM/L of K^+, albumin and glucose. The perfusion pressure was maintained constant at 110 mmHg. After stabilization and two five-minute control clearance periods, the perfusate was switched to a new perfusate containing K^+:5.0 (Group 1), 0.1 (Group 2) or 7.5 mM/L (Group 3) for an additional 25 min of perfusion. The first and last switched clearance periods were compared and expressed as mean ± SE. In all three groups GFR and % FE Na^+ did not change significantly. In Group 1 (n=5) % FE K^+ increased from 56 ± 10 to 84 ± 6 ($p < .05$). Renal venous effluent renin (R) increased from 9.1 ± 1.8 to 26.8 ± 4.5 ng AI/hr-min (ratio 3.14 ± .28). In Group 2, (n=7), % FE K^+ decreased from 88 ± 12 to 44 ± 4 ($p < .005$). R increased from 20.5 ± 6.8 to 45.3 ± 13.4 ng AI/hr-min (ratio 2.3 ± .28). In Group 3 (n=6), % FE K^+ increased from 51 ± 9 to 75 ± 9 (ns). R increased from 8.3 ± 1.9 to 27.7 ± 9.6 ng AI/hr-min (ratio 3.05 ± .41). The FE K^+ was significantly lower in Group 2 than Group 1 and 3. The changes in R were not significantly different among the three groups. We conclude that acute alteration of K^+ concentration does not influence renal renin release.

KININ-GENERATING ACTIVITY IS PRESENT IN THE MADIN DARBY CANINE KIDNEY (MDCK) CELL LINE W. Lawton & J. Quinn,* V.A. MED. CTR. & UNIV. HOSP., Dept. of Internal Medicine, Iowa City, Iowa.

The renal kallikrein-kinin system (KKS) is a putative regulator of Na^+ and H_2O transport and other vasoactive systems but the regulation and precise role of KKS is poorly understood. Studies of KKS were conducted in a distal tubular epithelial cell line, MDCK cells. Confluent cells were incubated in Eagles MEM without FBS or gentamicin for 20 hrs. Incubant was removed from cells and exogenous kininogen (KGN) added and further incubated. KGN was removed by QAE Sephadex and generated kinins (BK) measured by BK RIA. Antibody to BK was generously provided by Drs. Pisano and pierce, NIH. BK increased with increasing concentrations of KGN and with time.

INCUBATION TIME WITH KGN	1hr.	3hrs.	6hrs.	20hrs.
ng BK/3x10^7 cells	2.0	1.5	4.1	5.3

The kinin generating activity was inhibited by aprotinin. Cell incubant also had kininase activity not inhibited by 10^{-3}M EDTA. BK recoveries were: 1hr=81%, 3hrs=63%, 6hrs=34%, 20hrs=16%.

MDCK cells were sonicated, treated with Triton-X (1%) and contained an antigen which cross reacted with our antibody to purified dog urinary kallikrein (DUK). Antigen estimate=8.8ng DUK/3x10^7 cells (DUK RIA). The pH optimum for kinin-generating activity of DUK is 8.5. When KGN was added to intact cells, BK was found, however kininase activity was present, not inhibited by captopril or EDTA.

Incubant from MDCK cells form BK and the cells contain a kallikrein-like enzyme. MDCK cells should be productive for studies of the regulation and function of the KKS.

PROSTAGLANDIN E_2 (PGE_2) INHIBITS CHLORIDE (Cl) TRANSPORT IN MEDULLARY THICK ASCENDING LIMB (MTAL) CELLS, Stephanie Lear, Patricio Silva, Harvard Medical School and Beth Israel Hospital, Department of Medicine, Boston, Massachusetts.

The inhibitory effect of PGE_2 on Cl transport in isolated rabbit TAL tubules has not been consistently observed. We reinvestigated the effect of PGE_2 on transcellular transport of Cl using MTAL cells isolated from rabbit outer medulla. After harvest, the cells were suspended in indomethacin (10^{-4}M) to inhibit endogenous prostaglandin production. Transport dependent oxygen consumption (QO_2), as a marker of transcellular Cl transport, was measured using a polarographic oxygen electrode at 37 C. PGE_2 produced a dose-dependent inhibition of QO_2. The inhibition at $3x10^{-4}$M was 33% (control 4.56+0.44 MO_2/mg/hr, and 3.04+0.23 after PGE_2, $p<0.0005$). PGE_2 had no effect on QO_2 after either ouabain (10^{-4}M) or bumetanide(10^{-3}M). Ouabain after PGE_2 decreased QO_2 further to 50% of control. Bumetanide after PGE_2 reduced QO_2 to 55% of control. PGE_2 had no effect on QO_2 in the absence of sodium or Cl.

These results indicate that PGE_2 inhibits transcellular Cl transport in isolated rabbit MTAL cells. The data are consistent with a modulating effect of PGE_2 on Cl transport in the TAL.

INHIBITION BY HALOPERIDOL OF THE NATRIURESIS INDUCED BY ATRIAL NATRIURETIC FACTOR (ATRIN). Marcos Marin-Grez*, Gisela Schubert*, Josephine P. Briggs, and Jürgen Schnermann*. University of Munich, Department of Physiology, Munich, West Germany.

To study the possibility of an interrelationship between the natriuretic effects of the two renal vasodilators, dopamine and atrin, we examined the influence of the dopamine receptor antagonist haloperidol on atrin-induced natriuresis. Experiments were done in anesthetized Sprague-Dawley rats. Rat atrial extracts were purified by chromatography on Bio-Gel P-60. Intravenous injection of the pooled fractions containing the low molecular weight natriuretic activity at three dose levels increased Na excretion by 58±7.5%, 90±14%, and 125.7±35%; after giving 50 µg haloperidol changes in Na excretion induced by the same doses of atrin were completely inhibited (-10.6±15.9%, -11.0±21.6%, and 11.7±7.4%). Haloperidol also blunted the natriuretic effect of dopamine, but did not interfere with the action of furosemide.

In another series of experiments the effect of haloperidol on saline diuresis (2.5% BW at 22.5 ml/hr followed by 9 ml/hr) was studied. Mean Na excretion during saline loading fell by 2.8±0.66 (17%) and 3.6±1.64 µM/min (23%) and mean urine flow by 14.5±2.8 (23%) and 24.4±5 µl/min (35%) when haloperidol in doses of 50 or 100 µg was administered. Kidney GFR was not significantly altered (1.32±0.02 vs. 1.29±0.05 ml/min) while SNGFR fell slightly from 39.8±1.67 to 34.9±1.42 nl/min.

We conclude that the natriuretic effects of dopamine and atrin appear to be interrelated. The mechanism of this interrelationship may involve release of dopamine by atrin or an interaction at the receptor level.

RENAL PROSTAGLANDIN E_2 SYNTHESIS (PG SYN) AND DEGRADATION (PG DEG) IN THE DEVELOPING RAT. Donald Moel, Richard Cohn, and John Penning*. Northwestern Univ., Children's Mem.Hosp., Dept.Peds, Chicago, Illinois.

Postnatal development of GFR and RBF is associated with a fall in renal vascular resistance that may be mediated by vasoactive substances. We examined differences in the regulation of one such substance, PGE_2. The present studies examined renal cortical (C) and medullary (M) PG syn and PG deg in rats aged 20 d (30.7g), 31 d (101g), and 120 d (413 g). PGE_2 syn from ^{14}C-arachidonic acid was determined in C and M microsomes by thin layer chromatography (TLC). PGE_2 degradation was determined by following the disappearance of ^{3}H-PGE_2 substrate in cytosolic fractions of C and M by TLC. Mean values (±1SE) for PG syn (%arachidonate conversion), PG deg (%PGE_2 disappearance), and GFR (ml/min/gmKw) are shown below: *=p<0.05, 20 d vs 31 d; †=p<0.05, 31 d vs 120 d; Ψ=p<0.05, 120 d vs 20 d; n= number of animals.

age	n	GFR	PG syn		PG deg	
			Cortex	Medulla	Cortex	Medulla
20	5	.61±.05*	5.0±.2*	4.7±.2*	41.7±0.9*	27.4±1.3*
31	6	.85±.03	2.5±.2†	8.8±.7†	21.5±4.4†	12.2±4.7
120	6	.90±.04Ψ	1.1±.1Ψ	12.3±.4Ψ	5.5±2.6Ψ	11.0±3.4Ψ

PG syn in C microsomes is highest in 20 d rats and decreases with age; in contrast M PG syn is lowest in 20 d rats and increases with age. Both C and M PG deg are highest in 20 d rats and decrease with age. Despite demonstrating significant age-dependent differences in C and M PG syn, 11 days of aspirin (300 mg/kg/day) to 20 d rats (n=8) blocked PGE2 syn in C and M by 60 and 75% but GFR was similar to 31 d rats (n=5) (.78±.04 vs .85±.03), suggesting that observed age-dependent difference in renal PG syn is not a major determinant of development of GFR.

EFFECT OF PROSTAGLANDIN INHIBITION (PGI) ON NON-LOOP DIURETICS. C. Martin,* J.D. Bower, R. Mueller,* S. Brandon,* and K.A. Kirchner. Univ. of Mississippi Med. Ctr., Jackson, Mississippi.

PGI antagonizes the effect of loop diuretics. Whether PGI antagonizes diuretics acting at other nephron sites is unknown. To evaluate the effect of PGI on diuretic action, acetazolamide (Acet) (20 mg/kg/hr), hydrochlorothiazide (Hctz) (5 mg/kg/hr), or amiloride (Amil) (3 mg/kg/hr) were given to rats (n=7/group) receiving continuous infusions of indomethacin (I), meclofenamate (M), or the vehicle for PGI administration (C). Mean arterial pressure and percent change in plasma volume were not different between C and PGI rats for any diuretic group. Inulin clearance and fractional sodium excretion were as follows:

	Acet		Hctz		Amil	
	C_{In} (ml/min/gKW)	FeNa (%)	C_{In} (ml/min/gKW)	FeNa (%)	C_{In} (ml/min/gKW)	FeNa (%)
C	0.83 ±.07	5.40 ±.48	1.29 ±.07	1.30 ±.36	1.47 ±.13	1.39 ±.23
I	0.81 ±.06	2.51* ±.59	1.36 ±.09	0.13* ±.04	1.51 ±.10	0.49* ±.09
M	0.83 ±.05	1.75* ±.38	1.41 ±.07	0.21* ±.08	0.98** ±.09	0.22* ±.08

*P<.001 compared to C in same group; ** P<.005.

Renal blood flow (by inulin extraction) in Hctz rats was not different between C and PGI groups (5.5±1.5 ml/min vs 4.8±.55, C vs I; and 5.5±1.5 ml/min vs 4.3±.37, C vs M). We conclude (1) PGI antagonizes the effects of proximal, early distal, and late distal tubule diuretics; and (2) the mechanism of antagonism is unknown but is not restricted to a single class of diuretics or diuretics acting at a single nephron site.

β-CATECHOLAMINE-SENSITIVE cAMP SYSTEM IN PROXIMAL TUBULES OF DOG KIDNEY. N. Murayama,* B.T. Ruggles,* J.L. Werness,* S. Gapstur* and T.P. Dousa. Nephrol. Res. Unit, Mayo Clinic, Rochester, MN.

To address the question whether adrenergic neurotransmitters act directly on proximal tubules via β-receptor-linked adenylate cyclase (AdC), we studied the responsiveness of AdC in segments of cortical tubules microdissected from canine kidneys to isoproterenol (ISO), norephinephrine (NE), β-blockers, PTH and [8-Arg]-vasopressin (AVP). The AdC activity both in the proximal convoluted tubule (PCT) and in proximal straight tubule (PST) was stimulated by PTH, but not by AVP. Addition of ISO or NE caused marked stimulation of AdC in PST (Δ + 806%), but had no effect in PCT. AdC in the late distal convoluted tubule was stimulated by AVP and by ISO, but to a lesser degree than in PST (Δ + 242%). Stimulatory action of ISO on AdC in PST was blocked by propranolol (PRO) and butoxamine, but not by phentolamine or metoprolol. In the intact segments of proximal tubules, the accumulation of cAMP (fmol/mm) was increased by ISO (10^{-6}M) and by PTH (10 U/ml) in a similar way as the AdC:

	Basal	ISO	ISO + PRO	PTH
PCT:	1.9 ± 0.4	4.8 ± 1.1	----	20.3 ± 3.4†
PST:	3.1 ± 0.5	24.0 ± 5.0†	5.2 ± 2.0	34.0 ± 3.6†

†Significantly higher than basal (t-test).

Our studies show that proximal tubules of canine nephron, namely PST, contain β-adrenergic receptors coupled to AdC, of β_2 subtype. Therefore, the present findings indicate that the direct β-effects of catecholamines in proximal tubules are mediated by cAMP.

DECREASED PRESSOR REACTIVITY TO ANGIOTENSIN II IN CIRRHOTIC RATS. EVIDENCE FOR A POST-RECEPTOR DEFECT IN ANGIOTENSIN ACTION. B.M. Murray* and M.S. Paller, Univ. of Minnesota, Minneapolis, MN.

Resistance to the pressor effects of angiotensin II (AII) in cirrhosis has been recognized for 20 years, but the mechanisms involved remain unclear. In a model of cirrhosis in the rat produced by inhalation of CCl_4 for 6 wks, the pressor response to 5 doses of AII was significantly lower in conscious cirrhotic animals compared to controls. On the other hand, cirrhotic animals had normal pressor responses to norepinephrine, suggesting that a generalized defect in vascular reactivity did not exist. No alteration of baroreceptor function was found in cirrhotics to account for the decreased pressor response to AII. PRA was increased in cirrhotics (9.5 vs. 3.4 ng/ml/hr, $p < .01$) but urinary PGE and 6-k-$PGF_{1\alpha}$ were no different from controls. Pretreatment with either captopril to reduce circulating AII or meclofenamate to inhibit prostaglandin synthesis failed to normalize the response to AII. Studies of AII binding by mesenteric artery smooth muscle particles showed that in cirrhotic animals, receptor affinity for AII, K_d, was decreased (cirrhosis 1.11 ± 0.09, control 0.94 ± 0.13 nM, $p < .02$) whereas receptor number was increased (315 ± 42 vs. 277 ± 43 fmol/mg, $p < .01$). However, total binding of AII by vascular receptors from cirrhotics was no different than in controls since the decrease in affinity negated the increase in receptor number. Although similar receptor abnormalities have been found in potassium deficiency, plasma and muscle K were normal in cirrhotic rats. We conclude that the decreased pressor response to AII in cirrhosis is the result of a post-receptor defect in angiotensin action.

PERTUSSIS TOXIN (PT) POTENTIATES ANESTHESIA-INDUCED RENIN SECRETION (RS). J.Pedraza-Chaverri*, M.C. Alatorre*, M.E.Ibarra*, J.A.García-Sáinz*, y J.C. Peña. Instituto Nacional de la Nutrición Salvador Zubirán & UNAM, México, D.F., México.

Anesthetics seem to increase RS by adrenergic mediated mechanisms. The pharmacological effects of anesthetics was studied preparing the Wistar rat with propranolol (PR) to inhibit and PT to magnify the effect of these agents on RS. Wistar rats were injected with PT (50 µg/100 g) 3 days before anesthesia or with PR (1 mg/kg) 30 minutes before anesthesia.

Animals were anesthetized with: pharmacological doses of: Ether (E), Pentobarbital (P), Inactin (I), Ketamine (K), Urethane (U) or Chloralose (CH); 15 minutes later the rats were decapited and blood collected. Plasma renin concentration was determined by radioimmunoasay and expressed in ng ml^{-1} $hour^{-1}$. All values are the mean ± SE in a minimumm of 5 animals. The basal values were: 58±6 in the control group (C) and 86±7 in the PT groups. The table summarizes the results.

	E	P	I	K	U	CH
C	250±49	116±8	140±34	206±23	528±57	250±28
PR	106±14	146±25	186±20	96±14	124±22	94±27
PT	830±31	826±32	433±72	598±82	1438±269	1079±64

All anesthetics increased RS in control and PT groups. PR diminished RS 95-90% in groups with E, K, U and C, and remain stable in groups P and I. It is concluded that PT magnifies and PR minimizes the stimulation of RS produced by anesthetics. These data indicates that the adrenergic system is involved in the RS induced by anesthetics.

EFFECT OF RENAL ISCHEMIA ON PRODUCTION OF ERYTHROPOIETIN AS MEASURED BY RADIOIMMUNOASSAY (RIA) AND BIOASSAY (BA). John Paddock,* Teresa Morone,* Jaime Caro,* Allan Erslev,* Leah M. Lowenstein, and Jean K. Paddock. Thomas Jefferson University, Dept. of Biochemistry and Medicine and Cardeza Institute, Philadelphia, PA.

Erythropoietin (EPO) production has been shown to be increased in response to an hypoxic stimulus. We studied the effect of renal ischemia, produced by 45 min of left renal artery occlusion, on the ability of the kidney to respond to hypoxia. After the kidneys were made ischemic they were allowed to reflow for 5 hrs during which time they were exposed to 4 hrs of hypoxia (.4 atm). Plasma and kidney EPO levels were measured by RIA and BA on the same sample. This time period of ischemia has been shown to spare the gloumeruli morphologically but damages the tubular cells as indicated by decreased microvillar enzymes specific activities, alkaline phosphatase, leucine amino-peptidase and gamma glutamyl transpeptidase. EPO levels measured by RIA and BA were found to be similar in plasma but in all kidneys, both ischemic and shams, the levels measured by RIA were much higher than those measured by BA ($P<0.025$). The EPO content of the ischemic kidneys were consistently lower by both BA and RIA $P<0.025$.

EPO CONTENTS mU/ml or gm ± S.E.M.

	EXPERIMENTAL		SHAM	
	BA	RIA	BA	RIA
Plasma	1066±306	1162±380	1148±374	1294±578
Left Kidney	259± 42	476± 71	730± 69	1589±440
Right Kidney	497± 48	1968± 63	550±194	1483±516

These studies suggest 1) that kidney tissue contains an immunoreactive but biologically inactive EPO "precursor" and 2) tubular cells may play an important role in the production of this precursor and/or its conversion to biologically active EPO.

EFFECT OF RENAL α_2-ADRENOCEPTOR (α_2-A) STIMULATION DEPENDS ON THE HORMONE-SPECIFIC ADENYLATE CYCLASE (AC) SYSTEM ACTIVATION. William A. Pettinger,* Satoshi Umemura,* Elsa Yang* and Donald D. Smyth*. (intr. by Juha Kokko). Univ. of Texas Health Science Center, Dallas, Texas.

A number of hormones mediate their effects through activation of AC in the kidney. If α_2-adrenoceptors act through inhibition of AC, then the effect of stimulation of these receptors may depend on the predominating hormone-AC mediated effect. We therefore studied two substances, furosemide (F) and vasopressin (AVP). Both activate renal AC but have disparate effects on Na and H_2O excretion in the isolated perfused rat kidney (Krebs Henseleit solution; 6.5g% albumin or 1g% albumin + 3.5g% ficoll; prazosin 30nM; propranolol 100nM; 36°C). F (30µM) increased Na and H_2O excretion ($p<.05$) in association with an increase in cAMP excretion ($p<.05$). At present it is not clear whether the rise in cAMP is a causal or an epiphenomenon. α_2-A stimulation with l-epinephrine 28nM (E) blocked these increases in cAMP as well as Na and H_2O excretion ($p<.05$). AVP (10µU/ml), which is known to act by stimulating AC, decreased Na and H_2O excretion ($p<.05$). In this situation α_2-A activation with E or AC inhibition with adenosine P-site activation blocked this effect of AVP and increased Na and H_2O excretion ($p<.05$). Yohimbine, an α_2-A antagonist, (300nM) blocked all effects of E ($p<.05$); thus, α_2-A mediate E effects. These results show that α_2-A activation may increase or decrease Na excretion, depending on the predominating function-specific AC-mediated effect.

A NATRIURETIC FACTOR THAT STIMULATES Na TRANSPORT IN ISOLATED TUBULES. David H. Petzel*, Henry H. Hagedorn*, and Klaus W. Beyenbach. Section Physiology, Cornell University, Ithaca, N.Y.

To this date rat atrial natriuretic factors have failed to affect electrolyte and fluid transport in isolated perfused renal tubules. In contrast, we have found that an extract of mosquito heads stimulates fluid secretion in isolated Malpighian tubules (the functional unit of the mosquito kidney) in parallel with marked effects on the transepithelial voltage and resistance (J.Comp. Physiol. 149,511,1983 and 154,301,1984). We have now subjected the extract to HPLC chromatography and have recovered a fraction that selectively stimulates active Na transport in vitro. Isolated Malpighian tubules spontaneously secrete fluid at rates averaging 0.65 nl/min. Secreted fluid consists primarily of Na, 76±3 mM; K, 114±3 mM; and Cl, 181±3 mM (n=44). The active HPLC fraction promptly increases fluid secretion to 2.3 nl/min (250%, $p<0.001$). $[Na]_{sf}$ in secreted fluid increases to 148±7*mM, $[K]_{sf}$ decreases to 31±6*mM, and $[Cl]_{sf}$ remains unchanged ($n=12$, $*p<0.001$). Hence the HPLC fraction significantly ($p<0.001$) increases Na and Cl secretion but not K secretion. In isolated perfused Malpighian tubules the HPLC fraction hyperpolarized the transepithelial voltage from 52 to 66 mV (lumen-positive, $n=7$, $p<0.05$) These effects on voltage and NaCl secretion are mimicked by cAMP (J.Comp.Physiol. 154,301,1984). Hence we appear to have isolated a mosquito natriuretic factor (MNF) that may stimulate diuresis via cAMP. MNF is probably a peptide. Preliminary molecular weight estimates (Bio-Gel, P-4) have indicated a weight of approximately 1900 daltons.

EFFECT OF LEUKOTRIENES (LT) D4 and B4 ON URINARY SODIUM EXCRETION. G.E. Plante*, R.L. Hébert*, P. Maheux*, C. Lamoureux*, and P. Sirois* (intr. by T. Nawar). Dept. Physiol., Univ. Sherbrooke, P.Q., Canada.

Lipoxygenases which are responsible for the production of LTs from arachidonic acid, exist in renal tissues. The present study examines the effect of LTD4 and LTB4 on the net renal transport of sodium by infusing small doses (100 ng/min) of these hormones into the left renal artery of anesthetized hydropenic dogs, to minimize systemic effects. The right kidney was used as control.

LTD4 reduces urinary sodium ($\dot{U}_{Na}V$) from 138±15 to 103±15 uEq/min, but after cessation of infusion, $\dot{U}_{Na}V$ rebounds to 198±24, 166±28, 147±20 and 155±20. No change is observed in the control kidney. GFR and RPF remain stable throughout experiments in both kidneys. Saralasin blockade abolishes the renal response to LTD4 and also the rebound effect. In contrast, indomethacin fails to alter the reduction of $\dot{U}_{Na}V$ induced by LTD4. LTB4 does not influence $\dot{U}_{Na}V$ but the combined infusion of LTB4 and PGE2, a known natriuretic substance, results in a marked elevation of $\dot{U}_{Na}V$, from 112±11 to 225±18 during PGE2 alone, to 368±26, 317±30 and 342±52 under PGE2 and LTB4.

The absence of significant changes in GFR and RPF suggests that LTD4 as well as the combined infusion of LTB4 and PGE2, exert direct effects on sodium transport. In addition, it appears that angiotensin II is required for the expression of LTD4 action, whereas the production of other arachidonic acid metabolites is not critical. Furthermore, this model indicates that systemic physical and/or humoral events are not needed for the expression of LT on renal function.

INDUCTION OF AMILORIDE (AM) SENSITIVE NA TRANSPORT IN RAT COLON: AN EFFECT OF MINERALO- AND GLUCOCORTICOID STIMULATION. G. Piskorska* and R.D. Perrone. Tufts-N. Engl. Med. Ctr., Boston, MA.

Aldosterone(aldo) or dexamethasone(dex) have been previously demonstrated to induce AM inhibitable short-circuit current(I_{SC}) and mucosa to serosa Na flux($J_{Na}M\text{-}S$) in rat distal colon. Although aldo acts via mineralocorticoid receptors (MR), it is unclear whether dex acts via cross-occupancy of MR or via specific glucocorticoid receptors(GR). To address this question, we administered dex or RU26988 (a highly specific GR agonist) to control or spironolactone(SP) treated rats (1 gm/kg) and measured the in vitro effect of AM on $J_{Na}M\text{-}S$ and I_{SC} in distal colon 5 hr after injection. Results are mean±SE in μEq/hr.cm² and indicate the Δ after AM. Steroid dose is in μg/100 gm. * $p\leq0.05$ compared to SP at same dose.

	+SP		-SP	
	ΔI_{SC}	$\Delta J_{Na}M\text{-}S$	ΔI_{SC}	$\Delta J_{Na}M\text{-}S$
DEX(10)	-1.1±0.3	-2.0±0.8	-1.0±0.5	-1.3±0.3
DEX(50)	-1.2±0.2	-1.4±0.6	-2.6±0.5*	-3.6±0.6*
DEX(600)	-2.1±0.3	-2.1±0.5	-3.3±0.6	-2.4±0.7
RU(600)	-1.1±0.2	-2.0±0.8	-1.2±0.3	-1.6±0.5

Since vehicle and SP rats responded similarly to AM, these were combined (ΔI_{SC}= -0.1±0.1, $\Delta J_{Na}M\text{-}S$= -0.6±0.6). The lowest dose of dex increased AM inhibition to the same degree as RU26988 and there was no effect of SP. By contrast, larger doses of dex further increased AM inhibition and were blocked by SP at the 50 μg dose of dex but not at 600. We conclude that the effect of low dose dex or high dose RU26988 represents a relatively pure glucocorticoid effect. Larger doses of dex result in cross-occupancy of MR as evidenced by SP inhibition. Dex stimulates AM sensitive I_{SC} and $J_{Na}M\text{-}S$ via occupancy of both MR and GR.

DE NOVO FORMATION OF ANGIOTENSIN II (AII) IN THE KIDNEY DURING STIMULATED RENIN SECRETION IN ANESTHETIZED DOGS. L. Rosivall, A.J. Narkates, S. Oparil, and L.G. Navar. Univ. of Alabama in Birmingham, Birmingham, Alabama.

Previous studies were unable to quantify net intrarenal formation of AII due to the high intrarenal AII degradation rate (DR). This study was designed to allow estimation of AII-DR in each animal and determination of intrarenal AII formation under conditions of normal and enhanced renin secretion rate (RSR) elicited by renal arterial constriction (RAC). In 7 anesthetized dogs, plasma renin activity, angiotensin I (AI) and AII were measured in arterial (A) and renal venous (RV) blood by radioimmunoassay in 4 periods: control, RAC, converting enzyme inhibition (MK422) and MK422 plus systemic arterial infusion of AII. AII-DR was determined from the A-RV AII concentration difference during MK422 + AII infusion. During MK422 infusion both the arterial and venous AII concentrations were <1 pg/ml. AII infusion (0.12±0.02 μg/ml) yielded an arterial concentration of 127±24 pg/ml with a venous concentration of 13±4 pg/ml; average DR was 91.5±2.2%. During RAC, RSR (8.9±4.1 ng AI/min•g•h) increased by 162±63%. Arterial AII (66.3±16.5 pg/ml) and venous AII (25.3±6.6 pg/ml) increased by 155±92 and 107±25%, respectively. Net intrarenal AII formation increased from 31.7±7.1 to 54.0±12.1 pg/min•g after RAC. This increase could not be explained by changes in arterial AI (not altered) or by an increased conversion rate (shown previously to not be changed during RAC). These findings demonstrate de novo AII formation within the kidney occurring as a consequence of increased intrarenal AI generation due to enhanced RSR.

MECHANISM OF POTASSIUM DEPLETION (KD) HYPERRENINEMIA: STUDIES IN THE ISOLATED RAT KIDNEY (IPK). S.G. Rostand, J. Work, and R.G. Luke. Univ. of Alabama in Birmingham, Nephrology Research and Training Center, Birmingham, Alabama.

We studied the effects of KD on renin release (RR) in IPK. KD in 5 rats elevated RR when compared to 7 controls (3.4 ± SE 0.7 ng/A1/min/ml perfusate vs. 1.7 ± 0.2, $p<0.05$). Perfusate flow (42 ± 1.1 ml/min vs. 40 ± 2.2) GFR (0.39 ± 0.04 ml/min vs. 0.31 ± 0.04) and $U_{Na}V$ (0.90 ± 0.03 mEq/min vs. 0.91 ± 0.03) did not differ from control. Our prior studies, using control IPK, show that reduction of perfusate chloride by nitrate substitution progressively and significantly elevates RR by a macula densa signal; however, RR by KD IPK was not further increased by perfusate chloride reduction to 7 mEq/L (2.1 ± 0.7 ngA1/min/ml, $p > 0.1$ vs. KD with normal perfusate chloride). Inhibition of KD-stimulated RR by papaverine has been used as evidence for vascular mediation of the KD effect on RR. However, we found that papaverine (0.4 mM) completely blocked RR stimulated by furosemide, 10^{-4}M, (a macula densa signal) without affecting perfusate flow or furosemide's natriuretic effect (RR=0.85 ± 0.15 ng/A1/min/ml for control + furosemide vs. RR=0.01 ± 0.01, for papaverine + furosemide, n = 5 both groups). Therefore, since (1) papaverine can inhibit RR by other than a vascular mechanism, (2) since KD can stimulate RR without affecting perfusate flow, and (3) a macula densa signal such as reduced perfusate chloride failed to stimulate RR in IPK from KD rats, we conclude that the normally tonic suppressive macula densa signal is defective in KD.

INTERACTIONS OF VASOPRESSIN, cAMP, PROSTAGLANDINS AND PHOSPHOLIPIDS IN TOAD URINARY BLADDER Joseph A. Satriano* and Detlef Schlondorff, Albert Einstein Col. of Med. Bronx, NY

Prostaglandin (PG) inhibits the hydroosmotic effect of vasopressin (VP). While VP's hydroosmotic effect is mediated by cAMP, its stimulation of PG synthesis is not. We therefore reexamined the interaction of VP, cAMP, and prostaglandins in toad bladder epithelial cells. VP slightly, but reproducibly stimulated PGE_2 synthesis in cells isolated using EDTA or collagenase. Increasing cAMP either by 8 bromo cAMP or by phosphodiesterase inhibition with isobutylmethylxanthine (MIX) significantly inhibited both basal and VP-stimulated PGE_2 synthesis. This inhibition was overcome by addition of arachidonic acid suggesting that cAMP blocked phospholipase. VP also enhanced [^{32}P] labeling of phosphatidylinositol and phosphatidic acid in epithelial cells. This effect was prevented by phosphodiesterase inhibition. These results support the view that cAMP inhibits PG formation via inhibition of phospholipase. We conclude that VP, similar to its effect in the liver, may also increase phosphatidylinositol turn-over in toad bladder. This cAMP-independent action of VP may initiate PG synthesis and provide a link among VP, cAMP and calcium, possibly via a calcium-phospholipid dependent protein kinase C. A dual feed-back is proposed, whereby VP stimulates PG synthesis in a cAMP-independent manner and also inhibits PG synthesis in a cAMP-dependent manner.

ENDOGENOUS BRADYKININ (BK) INHIBITS ANTIDIURETIC HORMONE (ADH)-INDUCED FLUID ABSORPTION (Jv) IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT). Diane Rouse*, Claudia Soroka, Frank Williamson, and Wadi N. Suki. Baylor College of Medicine, Houston, Texas.

This study was designed to determine the role of endogenous BK, identified in CCT cells, in the regulation of the hydroosmotic effect of ADH. Rabbit CCT segments were perfused in vitro at 25°C with a 125 mOsm/kg perfusate and a 250 mOsm/kg bath. After a 2-3 hour equilibration time, Jv, in 6 CCT's, was 0.1 ± 0.1 nl/mm.min. The addition of ADH to the bath (10^{-2} uU/ml), raised Jv to 1.5 ± 0.2 nl/mm.min. When captopril (CP), which inhibits BK degradation, was added to the bath (10^{-4}M), Jv fell to 0.2 ± 0.1 nl/mm.min. To study the mechanism of this inhibition, adenylate cyclase was stimulated directly by the addition of forskolin (F, 10^{-5}M) to the bath. In 7 CCT's, F enhanced Jv from 0.1 ± 0.1 nl/mm.min to 1.6 ± 0.3 nl/mm.min. Addition of CP to the bath, decreased Jv to 0.8 ± 0.2 nl/mm.min. However, in 5 CCT's, CP failed to inhibit the hydroosmotic effect of cAMP added to the bath (10^{-4}M). Jv was -0.1 ± 0.1 nl/mm.min, in control, 1.8 ± 0.2 nl/mm.min, after cAMP and 1.8 ± 0.2 nl/mm.min after CP. To test the possible role of prostaglandins (PG) in mediating the action of BK, 5 CCT's were exposed to 5×10^{-6}M indomethacin in the bath. When ADH (10 uU/ml) was added to the bath, Jv rose from 0.0 ± 0.1 to 1.5 ± 0.2 nl/mm.min; addition of CP (10^{-4}M) failed to inhibit Jv (1.6 ± 0.1 nl/mm.min).

We conclude that endogenous BK inhibits the hydroosmotic effect of ADH in CCT. This effect is exerted either by direct inhibition of adenylate cyclase, or by activation of an inhibitory regulatory subunit. PG appear to mediate this action of BK.

THE EFFECTS OF FISH OIL EICOSAPENTAENOIC ACID (EPA) ON PGE SYNTHESIS IN MESANGIAL CELLS AND IN THE 5/6 NEPHRECTOMIZED (NX) RAT. L. Scharschmidt*, L. McGarry*, and P. Berger* (Intro. by R.M. Hays) Albert Einstein Col. of Med., Bronx, NY

Arachidonic acid (AA) is the precursor for synthesis of "2-series" prostaglandins (PG). EPA is a precursor of "3-series" PGs, whose actions differ from those of the "2-series". The effect of manipulating PG precursors was assessed in vitro in cultured mesangial cells and in vivo on the course of renoprival nephropathy.

Mesangial cells were incubated with 3μM radiolabelled AA or EPA and lipids analyzed by TLC. Uptake into and distribution among cell phospholipids were similar for AA and EPA, however the rate of AA conversion to "2-series" PG's was 2.5 x that for EPA conversion to "3-series" PG's. Thus AA is the preferred substrate for cyclooxygenase in mesangial cells.

The effect of EPA substitution was also assessed in pair-fed female rats which underwent 5/6 NX 1 month prior to dietary manipulation. Diets differed only in the composition of fats. Groups of 8 rats were fed a diet high in either beef tallow (control) or menhaden oil (rich in EPA). 15 weeks later, 1 of 8 control and 6 of 8 EPA rats had died. The 6 rats fed EPA which died had significant decreases in both creatinine clearance and urinary PGE_2, while these remained stable in the remaining 2 experimental rats and controls. These data show that substitution of EPA for AA interferes with PGE_2 production. The chronic decrease in renal PGE_2 production in the experimental rats may have contributed to their decline in renal function and accelerated death rate.

CHARACTERIZATION OF THE COLONIC ALDOSTERONE RECEPTOR. G. Schulman*, A. Miller-Diener*, G. Litwack* and C. Bastl. Temple Univ. Health Sci. Center, Phila., PA

The rat colon is an aldosterone (ALDO) target site where the ALDO receptor (AR) can be studied in isolated epithelium. The receptor has not been well characterized in colon due to binding of ALDO to the glucocorticoid receptor (GR). Using excess RU26988, a very specific glucocorticoid, we blocked binding of ^{3}H-ALDO to the GR which represented 50% of specific ALDO binding in the absence of RU26988. Specific ALDO binding is present along the entire colon but is highest in the most distal segment followed by the most proximal segment. Late proximal and early distal have only 30% of maximal binding. The Kd is 3.6±0.5nM. The Bmax of distal colon is 8×10^{-14}M/mg protein. Competitive binding assay demonstrates glucocorticoids to be poor competitors but at 4°C progesterone>DOCA>corticosterone=ALDO. Warming cytosol to 30°C completely reverses this order suggesting a temperature dependent modulation of the ligand binding site. Anion exchange column chromatography results in a single peak of bound radioactivity eluting at high salt which implies a net negative charge. This elution profile is distinct from serum corticosterone binding globulin but identical to that of the unactivated GR. This suggests a similarity of structure of the two receptors in the unactivated state. With activation, the GR elutes with low salt suggesting a conformational change. Under similar conditions the elution profile of the AR remains unchanged. The AR in kidney cortex behaves similarly.

Thus, the AR in rat colon has a specificity and distribution distinguishing it from the GR. Inability to separate unactivated from "activated" cytosolic AR distinguishes it from the GR and suggests that conformational changes do not occur in AR's cytoplasmic phase.

DOPAMINERGIC STIMULATION OF cAMP ACCUMULATION IN CULTURED RAT MESANGIAL CELLS. Pamela Shultz, John Sedor and Hanna Abboud. VA Med. Center, Case Western Reserve University, Cleveland, OH.

Dopamine (DA) has profound effects on renal hemodynamics and dopaminergic neurons are present at the vascular pole of the glomerulus. We studied the effect of DA on cAMP accumulation in rat cultured mesangial (MC) and epithelial (EC) cells. In the presence of 0.5 mM isobutyl methyl xanthine, a phosphodiesterase inhibitor, DA stimulated cAMP accumulation in a time- and dose-dependent manner. After 0.5 min incubation, 10^{-5}M DA elicited +Δ273%, at 2 min + Δ498%, at 5 min + Δ 856%, at 10 min + Δ919%. After 5 min incubation, basal cAMP levels (pmoles/mg protein; mean ± SEM) were 85±17, 10^{-7} M DA 94±7, 10^{-6}M 239±51, 10^{-5}M 1131± 251, 10^{-4}M 2035±598 (n=5). The DA antagonists haloperidol (hal) and trifluperazine (trif), but not the β adrenergic antagonist propranolol (prop), abolished the stimulatory effect of DA. Basal cAMP levels (pmoles/mg protein) were 58±6; DA (10^{-5}M) 1137±17; DA (10^{-5}M) + hal (10^{-5}M) 111±14; DA (10^{-5}M) + trif. (10^{-5}M) 91±31; DA (10^{-5}M) + prop. (10^{-5}M) 1067±95 (n=6-9). In contrast to its effect in MC, 10^{-5}M DA did not influence cAMP accumulation in EC, while forskolin, a direct activator of adenylate cyclase, markedly stimulated cAMP accumulation in the same EC cultures. Basal cAMP levels (pmoles/mg protein) were 41±4, DA (10^{-5}M) 53±3, forskolin (10^{-5}M) 1029±213 (n=4-6).

These results show that MC possess specific DA receptors that are different from previously characterized β adrenergic receptors. DA, acting via cAMP, may influence MC functions.

BRADYKININ (BK) INCREASES RELEASE OF FREE INOSITOL POLYPHOSPHATES IN RENAL PAPILLARY COLLECTING TUBULE (RPCT) CELLS. James A. Shayman* and Aubrey R. Morrison, Departments of Medicine and Pharmacology, Washington University Medical School, St. Louis, Missouri.

RPCT cells were harvested from New Zealand white rabbits via mechanical dissociation, collagenase digestion and hypotonic lysis. Cells were grown subsequently as primary cultures in fully defined media. Cell morphology by EM revealed sparse mitochondria and tight junctions typical for these cells; arginine vasopressin (AVP) (10^{-7}M), induced time dependent cAMP formation; and BK (10^{-7} M) induced $iPGE_2$ formation. Cells were labelled with ^{3}H inositol and extracted by the Bligh-Dyer technique with base hydrolysis of lipid soluble components and reextracted into an aqueous phase. Both free inositol phosphates and lipid-soluble inositides were separated by anion-exchange chromatography. BK (10^{-7}M) induced 228 and 215 percent rise in inositol tri and diphosphate respectively (n=4; $p < .05$) as compared to time matched controls. This was maximal by 15 seconds and essentially reversible by 5 minutes. Phosphatidyl inositol 4 monophosphate and 4,5 bisphosphate demonstrated initial increased followed by decreased labelling suggesting ongoing formation and consumption of polyphosphatidyl inositides. Cells preincubated with LiCl (10 mM), a known inhibitor of inositol-1-phosphatase, demonstrated a marked increase in inositol-1-phosphate when stimulated with BK. We conclude that RPCT cells when stimulated by BK, a known agonist of PGE_2 production, release free polyphoinositols.

ATRIOPEPTINS: MECHANISM OF CHLORIDE STIMULATION IN THE SHARK RECTAL GLAND (RG). **P.Silva, R.Solomon, L.Cantley*, and F.H.Epstein. Brown Univ. and Harvard Univ., Depts. of Medicine, Providence, RI and Boston, MA and the Mount Desert Island Biological Laboratory, Salisbury Cove, ME.**

Atriopeptins (A) stimulate active chloride transport in the rectal gland both *in vivo* and *in vitro*. In dispersed cell preparations and rectal gland slices, vasoactive intestinal peptide (VIP) but not A stimulates oxygen consumption. Since VIP resides in neurons within the RG itself, we investigated the possibility that A acts to stimulate chloride secretion by releasing VIP.

Perfusion of the *in vitro* gland with a concentration of calcium and magnesium which inhibits neurotransmitter release completely inhibited the effects of A but not VIP on chloride secretion. Procaine also inhibited the effects of A but not those of VIP.

In vivo, intraarterial infusion of shark Ringer's (30ml/kg) produced a prompt fourfold increase in chloride secretion from and blood flow to the rectal gland. Similar secretory and hemodynamic changes were seen in denervated, transplanted glands. However, infusion of tetrodotoxin (10-6M) into the transplanted gland completely prevented the stimulation of chloride secretion following volume expansion. Volume expansion-induced vasodilation was unaffected.

These studies suggest that atriopeptins may have direct vascular effects but in addition produce effects on chloride transport mediated by release of a neurotransmitter such as VIP.

THE INTRARENAL LOCALIZATION OF ANGIOTENSINOGEN mRNA BY RNA-DNA DOT BLOT HYBRIDIZATION. EI Simpson* and TA Fried. The University of Texas Health Science Center, Department of Medicine, San Antonio, Texas.

It is known that angiotensinogen is synthesized and stored in the liver. The kidney has also been proposed as a possible site of its synthesis and/or storage. The reports evaluating intrarenal angiotensinogen have, however, not all agreed. Because of the importance of the angiotensin system in the regulation of renal hemodynamics, glomerular filtration and fluid reabsorption and because of the advantage that local synthesis would offer in the control of this regulation, we undertook an investigation of possible intrarenal synthesis. This study utilized the technique of RNA-DNA dot blot hybridization. A radiolabeled synthetic oligonucleotide DNA primer complementary to the coding region of angiotensinogen mRNA was used to perform dot blots on polydenylated RNA prepared from normal rat kidney, kidney medulla, kidney cortex, liver, muscle and spleen. The results reveal the presence of mRNA specific for angiotensinogen in the kidney. Indeed this mRNA appears to be confined mainly to the renal medulla and not cortex. There also appears to be intrarenal regulation as there was less hybridization to equal amounts of mRNA prepared from kidneys harvested 24 hrs following uninephrectomy when compared with that from normal kidneys. As expected the liver revealed significant hybridization and both muscle and spleen, no hybridization. We conclude that both liver and kidney can synthesize angiotensinogen, and we suggest that the kidney may, in part, regulate its own angiotensinogen synthesis.

ATRIOPEPTIN (A) STIMULATES SHARK RECTAL GLAND SECRETION. **R.Solomon, P.Silva, F.H.Epstein. Brown Univ. and Harvard Univ., Depts. of Medicine, Providence, RI and Boston, MA and the Mount Desert Island Biological Laboratory, Salisbury Cove, ME**

In the isolated shark rectal gland (RG) perfused at constant pressure, a bolus of A(1ug) produces an increase in salt secretion which occurs within minutes and is sustained for 30 min. Stimulation ofsecretion occurs without a change in vascular resistance. Extracts of shark atria(EA) and ventricle(EV) but not skeletal muscle, brain or kidney are also capable of stimulating rectal gland function in vitro without a change in hemodynamics.

In vivo, A (10ug/kg) produces a threefold increase in secretion accompanied by a fall in systemic blood pressure and a parallel increase in blood flow through the rectal gland.

(N)	CHLORIDE SECRETION (uEq/h/gww)	
IN VITRO	CONTROL	EXPERIMENTAL
A (12)	96±16	428±71*
EA (11)	126±23	594±120*
EV (9)	119±19	558±102*
IN VIVO		
A (7)	540±216	1624±341*

*= P<.01

We conclude that A is a physiologic regulator of rectal gland function both in vivo and in vitro. Stimulation of secretion occurs in the absence of a primary vascular effect although vascular effects are seen in vivo. Crude homogenates of cardiac tissue also contain a substance capable of stimulating rectal gland function.

RENAL TUBULAR POSTSYNAPTIC α_1- AND EXTRASYNAPTIC α_2-ADRENOCEPTORS IN THE RAT. Donald D. Smyth,* Satoshi Umemura,* Elsa Yang* and William A. Pettinger*. (intr. by Juha Kokko). Univ. of Texas Health Science Center, Dallas, Texas.

We tested the hypothesis that, as in vascular tissue, α_1-adrenoceptors (A) are postsynaptic and α_2-A are extrasynaptic using two models. One was α_1-A stimulation of tubular sodium reabsorption and the second was α_2-A inhibition of vasopressin (AVP) retention of Na and H_2O in the isolated non-recirculating perfused rat kidney. We confirmed the α_1-A nature of Na retention using subpressor threshold (10V, 1ms, .85±.14Hz) renal nerve stimulation (RNS) and α_1-A blockade with prazosin (P) 30nM or α_2-A blockade with yohimbine (Y) 300nM (see table). * µEq/min

	C	RNS	RNS+Y	RNS+P
V(µl/min)	87.4±4.1	57.9±3.9	59.2±7.5	82.5±3.3
$U_{Na}V$ *	4.50±.42	1.71±.23	1.96±.37	3.98±.34
$\%FE_{Na}$	4.58±.41	1.91±.27	2.75±.40	4.69±.79

In the second model, epinephrine (E) reversed ($p<.05$) the cAMP mediated Na and H_2O retention induced by AVP. This E effect was blocked by yohimbine confirming α_2-mediation of this effect.

	Control	AVP	AVP+E	AVP+E+Y
$U_{Na}V$ (µEq/min)	4.7±.6	1.9±.4	5.0±.6	2.9±.6
V(µl/min)	88±5	61±5	87±8	59±6
$\%FE_{Na}$	4.9±.4	2.1±.5	4.4±.5	3.2±.6

We attempted to activate this α_2-mediated effect by renal nerve stimulation as above (10V, 1ms, .65±.1Hz) and with higher levels (2.80±.32Hz). Even with higher rates of RNS, α_2-A could not be activated. Thus, α_1- but not α_2-A are activated by nerve stimulation. These results suggest postsynaptic location of renal α_1-A and extrasynaptic location of α_2-A as occurs in arteries.

SPECIFIC ACTIVATION OF PLATELET THROMBOXANE (TX) SYNTHETASE BY HUMAN GLOMERULI IN VITRO. J. Sraer, M. Bens, J.D. Sraer, R. Ardaillou. INSERM U.64, Hôpital Tenon, Paris, France.

Platelet activation plays a major role in many glomerular diseases, particularly thrombotic microangiopathy and Goodpasture's syndrome. This prompted us to investigate whether or not human glomeruli (G) interacted with human platelets (P) on arachidonic acid (AA) metabolism during in vitro coincubation. Using radiometric HPLC, we found that P and G coincubated with a tracer dose of $|^3H|$AA produced a greater amount of TXB_2 (17,231 ± 6,392 cpm/tube) than the sum of the synthesis by P (5,308 ± 646) and G (321 ± 159) incubated separately (n = 3). The degree of activation was even greater when TXB_2 was measured by RIA (P : 1.16 ± 0.30 ; G : 0.1 ± 0.015 and P + G : 26.6 ± 7.1 ng/ml) n = 6. The additional TXB_2 synthesis was from platelet origin since pretreatment of P by aspirin (1 mM) completely abolished TXB_2 production whereas similar pretreatment of G did not affect TXB_2 synthesis. Increase in TXB_2 production was not associated with stimulation of 12-HETE synthesis and was suppressed by 5 µM OKY 046. This suggests that TX synthetase was specifically activated. The factor stimulating P was released by G in the incubation medium. It was thermostable and acted within 2 min. Activation critically depended on both glomerular protein content and P/G protein ratio. These data demonstrate that G release a factor stimulating TX synthesis by P. This local increased TX production may in turn enhance glomerular resistance and promote intracapillary coagulation.

GLOMERULAR PROSTAGLANDINS MODULATE GFR IN RATS WITH REDUCED RENAL MASS ON HIGH PROTEIN INTAKE: Rolf A.K. Stahl, Sabine Kudelka and P. Schollmeyer. Dept. of Medicine, Univ. of Freiburg, F.R.G. Following reduction of renal mass (80-85%) rats were placed on either a low protein (6%) LP or high protein (45%) HP diet for up to 2 and 5 weeks (wks). Total kidney weights, urine volumes and 24 h urinary protein excretion were greater in rats on HP 2 and 5 wks after ablation of renal mass. GFR (µl/min/100 gr bw) fell from the second to the fifth week in both animal groups, however, was greater in rats on HP (2 wks:LP 354±46, HP 486±79; 5 wks:LP 274±29, HP 365±37). Glomerular in vitro prostaglandin E_2 (PGE_2) and thromboxane B2 (TxB_2) production (pg/mg/min) in rats on HP were greater compared to LP.

	2 weeks		5 weeks	
	PGE_2	TxB_2	PGE_2	TxB_2
HP	461±43	172±48	688±89	207±43
LP	323±40	103±18	512±29	157±45

The in vivo application of the cyclooxygenase inhibitor indomethacin reduced glomerular PGE_2 formation in both animal groups by about 50%. GFR in rats on HP fell significantly, whereas it did not change in animals on LP intake. The data suggest that the elevated PGE_2 formation in rats on HP intake modulates GFR in these animals.

STIMULATION OF PROSTAGLANDIN E2 AND THROMBOXANE B2 PRODUCTION IN CULTURED RAT MESANGIAL CELLS BY PLATELET ACTIVATING FACTOR: INHIBITION BY A SPECIFIC RECEPTOR ANTAGONIST. J.E. Stork, T.Y. Shen, and M.J. Dunn. Case Western Reserve Univ. and Univ. Hosp., Dept. of Medicine, Cleveland, OH, and Merck Sharp and Dohme, Rahway, N.J.

1-alkyl-2-acetyl-sn-glycero-3-phosphocholine or platelet activating factor(PAF) is a powerful mediator of inflammation. PAF, originally described in basophils, can be produced by kidney, and may be important in glomerular immune injury. Interactions of PAF and the glomerular mesangial cell could effect both glomerular hemodynamics and permeability. We have examined the effect of PAF and a specific PAF receptor antagonist on mesangial cell eicosanoid production.

PAF significantly stimulated both prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) synthesis in a dose dependent manner in primary cultures of rat glomerular mesangial cells. Stimulation was first seen at 10^{-9}M PAF, was maximum at 10^{-7} M and varied from 3-8 fold for PGE_2, and 5-80 fold for TXB_2. Kadsurenone, a substance isolated from the Chinese medicinal herb heifenteng, is a specific, competitive inhibitor of PAF receptor binding. The effect of kadsurenone was examined with a constant concentration of 10^{-7}M PAF. At equimolar concentrations there was no consistent inhibition of PAF-stimulated PGE_2 or TXB_2 production. Significant inhibition (~60%) was seen at 10^{-6}M, and maximum inhibition (~90%) was seen at 10^{-5}M. Kadsurenone had no agonist activity at these concentrations. We conclude that PAF is a potent stimulator of both PGE_2 and TXA_2 production in the glomerular mesangium, which may contribute to the glomerular actions of PAF. The mesangial effects of PAF can be inhibited by a specific PAF receptor antagonist, which could prove of benefit in further investigations of the role of PAF in glomerular injury.

INCREASED THROMBOXANE B_2FORMATION BY GLOMERULI FROM RAT KIDNEYS AFTER INDUCTION OF IN SITU IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN). R.A.K.Stahl, F.Thaiss*,S.Kudelka*,P.Schollmeyer*. Dept.of Nephrol., Freiburg,F.R.G.

In vitro glomerular prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2) formation (pg/mg protein/min) was evaluated 2,24,48 h and 7 d after inducing an in situ ICGN in the left kidneys of male Wistar rats. The right kidneys served as controls (C). A PP 10 polyethylene catheter was advanced from left carotic artery to the abdominal aorta. Right kidney and aorta were clamped and the left kidney was selectively perfused with cationized human IgG. Total ischemia averaged 30 s. Antibody was infused after circulation was reconstituted. 2 h after induction of ICGN TxB_2formation in glomeruli of left kidneys was significantly greater than in right kidneys, with no differences at 24,48 h and 7 d. Glomerular PGE_2production of ICGN-glomeruli was only slightly greater at 2 h than in C. At 24 and 48 h PGE_2formation was greater in C than in ICGN. 7 d following experiments no significant differences were present.

	TxB_2		PGE_2	
	ICGN	C	ICGN	C
2 h	448 ± 116	238 ± 29	372 ± 34	295 ± 30
24 h	78 ± 7	75 ± 11	92 ± 4	147 ± 12
48 h	32 ± 3	47 ± 13	174 ± 25	344 ± 87
7 d	122 ± 28	166 ± 26	303 ± 108	318 ± 85

2 h after inducing nephritis GFR in ICGN kidneys was 164 + 38 µl/min/100 g bw and 295 + 82 in C. In the first and second hour after induction of ICGN proteinuria was not different between both kidneys. The data demonstrate increased TxB_2formation 2 h following induction of in situ ICGN in the rat. This might have pathogenic implications.

TISSUE CULTURE OF ISOLATED RENAL TUBULES OF RABBIT DISTAL SEGMENTS. K Takeda,* S Torikai,* M Araki,* T Saito,*, Y Asano,* and M Imai.* (Intr. by RW Schrier). Jichi Med.Sch., Tochigi, Japan; and Natl. Cardiovascular Ctr., Osaka, Japan.

Cortical thick ascending limbs (CAL), distal convoluted tubules (DCT), connecting tubules (CNT) and cortical collecting tubules (CCT) were dissected from collagenase-treated rabbit kidney and cultured in hormone-supplemented low serum medium on thin collagen membranes. The collagen membranes were supported by a special device that limited the exposed surface area to a disk 1.0 mm in diameter. The cells usually proliferated to form an epithelial monolayer covering the collagen membrane within 2-6 days. Morphological studies of cultured cells derived from the CAL and CCT indicated that the cells maintained their original characteristics. The cultured collecting tubule cells revealed the presence of only one cell type that resembles the principal cell of the CCT. The hormone-dependent increment of cAMP generation (fmoles/mm^2/10 min) in cultured DCT, CNT and CCT cells were as follows:

	DCT	CNT	CCT
AVP(10^{-6}M)	-2.2±1.7(3)	26.6±11.5(5)	35.8±8.0**(11)
ECT(100ng/ml)	28.8±3.5*(4)	0.6±7.2(3)	
PTH(10U/ml)		2.6±1.8(5)	-2.0±3.9(3)
ISO(10^{-6}M)		40.0±9.3**(5)	3.1±2.9(3)
FORS(10^{-4}M)		211.3(1)	210.7±70.2(3)

*p<.001, **p<.02, mean ± SE

These data indicate that cultured rabbit distal nephron segments retain the cAMP response to certain hormones. This technique may allow us to select a single type of cell from a nephron segment composed of multiple cell types.

DEVELOPMENT OF TOLERANCE TO THE NATRIURETIC EFFECT OF ATRIAL NATRIURETIC PEPTIDE (ANP). C.J. Taylor*, H.H. Saneii*, and J.H. Ludens. The Upjohn Co., Kalamazoo, MI.

Vascular and renal effects of ANPs have been characterized, largely, as single doses in anesthetized animals. In the present study, synthetic ANP (atriopeptin II, Science 223:67, 1984) was infused for 5 hrs in conscious rats (160-180 g). Cannulas for mean arterial pressure (MAP) and infusion were implanted in carotid and jugular vessels, respectively, under Metofane anesthesia 2 hrs prior to study. ANP (administered as 6 pulses/hr) was given in a .9% saline load of 2 ml/hr and urine was collected at 0-1, 1-2, 2-3 and 3-5 hrs. Dose dependent increases in Na excretion of 1.6, 2.3 and 2.8 times the control were apparent with 12, 36 and 108 µg/rat/hr, respectively, during the first hr. During all subsequent collection periods, however, Na excretion ranged from 1.9 to 0.6 times the control with no dose dependent relationship. A similar profile was obtained when the saline load was reduced to 0.8 ml/hr. In all cases, tolerance or compensation developed to the natriuretic effect of ANP at some point during the 5-hr infusion and the greater the initial response the sooner tolerance developed. In addition, tolerance or compensation occurred at a rather modest level of negative Na balance since total Na excreted in similar animals during a 5-hr furosemide infusion was substantially greater. While MAP fell 5-20 mmHg with ANP, this cannot account for loss of the natriuretic response in that MAP after an initial drop did not change appreciably over the remainder of the study.

PROSTAGLANDIN ALTERATIONS IN PRE-HYPERTENSIVE DAHL S RATS. Y Uehara*, L Tobian, J Iwai*, University of Minnesota, Minneapolis, MN & Brookhaven Lab, N.Y.

19 Dahl S rats & 21 R rats were fed a .3% low Na Cl diet for 11 weeks(BP's, 140 & 125 respectively). Kidneys of these rats were quickly excised & frozen in N_2. Prostaglandins were measured in frozen renal cortex(ng/gm dry wt): I) 6-keto-PGF1α(from prostacyclin), R rats, 91 vs S rats, 64(-30%,p<.02); II) PGE2, R rats, 151 vs S rats, 114(-25%,p<.05); III) PGD2, R rats, 69 vs S rats, 47(-32%,p<.03); IV)TXB2 (from thromboxane A2) R rats, 6.6 vs S rats, 10.0 (+52%,p<.02). In frozen outer medulla in these same kidneys(inner + outer stripes), prostaglandin levels (ng/gm dry wt) were: I) 6-keto-PGF1α (from prostacyclin), R rats, 75 vs S rats, 52(-31%, p<.03); II) PGE2, R rats, 110 vs S rats, 70 (-36%, p<.03); III) PGD2, R rats, 208 vs S rats, 118 (-43%, p<.03); IV) TXB2 (from thromboxane A2) R rats, 2.47 vs S rats, 3.64 (+47%, p<.04). Prostacyclin, PGE2, & PGD2 are vasodilators & had significantly lower renal cortical & outer medullary concentrations in the borderline hypertensive S rats compared to normotensive R rats. Conversely, thromboxane is a vasoconstrictor which had significantly higher renal cortical & outer medullary concentrations in borderline hypertensive S rats compared to normotensive R rats. Thus in borderline hypertensive S rats, both renal cortex & outer medulla have a prostaglandin pattern which favors vasoconstriction in cortical vessels & in descending vasa recta. This could partially account for the increased renal vascular resistance & low papillary plasma flows which are integral components of Dahl hypertension. The low PGE2 in S medullas would enhance Na reabsorption in collecting tubules & ascending limbs, thereby encouraging Na retention and hypertension.

ISOLATION OF EPOXYEICOSATRIENOIC ACIDS FROM HUMAN URINE. **R. Toto, S. Manna*, J. Tannenbaum, J. Capdevila*, A. Siddhanta*, and J.R. Falck*, Univ. TX Hlth. Sci. Ctr., Southwestern Med. Sch., Dallas, TX.**

Enzymatic epoxygenation of arachidonic acid in both kidney and liver results in the formation of four regioisomeric epoxyeicosatrienoic acids (EET). EET are potent selective in vitro stimulators of peptide hormone release and the 5,6 isomer has recently been shown to inhibit sodium and potassium transport in the isolated perfused rabbit cortical collecting duct (Jacobson, ASN 16:193A, 1983). The purpose of the present study was to isolate EET from human urine of normal volunteers. Accordingly, pooled urine collected over an 8-h period from 17 normal humans was extracted with ethyl acetate and purified by reverse and normal phase high performance liquid chromatography (HPLC). After methylation and repurification by HPLC the extract was subjected to gas chromatography (GC)/mass spectrometry (MS). Urinary EET was identified by the following criteria: 1) the GC retention time of the biological sample was identical to the chemically synthesized, purified 14,15 EET standard (S); 2) MS monitoring of the GC column eluent revealed coelution of EET-derived peaks of the major ions: 285, 303, 317, 335, 345 and 363 which are common to the S; 3) MS of the extract demonstrated that the relative abundance of these ions was similar to S. These results represent the first demonstration of EET in the urine of any species. Taken together with the in vitro studies our findings raise the possibility that the EET play a role in renal physiology.

α_2-ADRENOCEPTOR STIMULATION AND CELLULAR CAMP LEVELS IN ISOLATED SINGLE NEPHRON SEGMENTS IN THE RAT. Satoshi Umemura,* Donald D. Smyth,* and William A. Pettinger*. (intr. by Juha Kokko). Univ. of Texas Health Science Center, Dallas, TX

A functional role for the numerically predominant renal α_2-adrenoceptors, which in other tissues inhibit adenylate cyclase, remains undefined. We therefore examined the effect of α_2-adrenoceptor stimulation with l-epinephrine (E) on cell cAMP content in the isolated proximal convoluted tubule (PCT), cortical thick ascending limb of Henle (cTAL) and the cortical collecting tubule (CCT) of the rat kidney. Nephron segments were incubated in the presence of 1-methyl-3-isobutylxanthine (phosphodiesterase inhibitor) with propranolol and parathyroid hormone (1-34, PTH) (for PCT and cTAL) or arginine vasopressin (AVP) (for CCT and cTAL) and varying concentrations of E at 37°C for 2 min. E ($5x10^{-7}$~$5-10^{-6}$M) suppressed (p<.05) cellular cAMP stimulation by PTH by 35% in PCT. E also inhibited the increases in cellular cAMP following AVP in CCT by 45% (p<.01). This suppression by E in PCT and CCT was inhibited by $5x10^{-6}$M yohimbine or $5x10^{-7}$M phentolamine but not by $5x10^{-6}$M prazosin. However, E did not suppress the PTH- or AVP-stimulated increase in cellular cAMP in cTAL. These studies show that there are α_2-adrenoceptors in the rat nephron. Activation of these α_2-adrenoceptors can inhibit cAMP formation stimulated by PTH in PCT and AVP in CCT. Pathophysiologic roles of these receptors are yet to be described.

TECHNIQUES TO FACILITATE THE EXPRESSION OF ISOTONIC CONTRACTION IN CULTURED MESANGIAL CELLS. M.A. Venkatachalam and J.I. Kreisberg. University of Texas Health Science Center, San Antonio, Texas.

Under conditions of prolonged culture mesangial cells lose their ability to contract in the usual manner (i.e., isotonically). One explanation for this may be that contraction is prevented by tight cell adhesion to an immobile substrate. Two models in which substrate adhesiveness was expected to be diminished were used to test this hypothesis. In one, cells were seeded onto collagen coated dishes and used within 40 mins. of plating. In the other, cells were plated onto dishes coated with poly 2-hydroxyethyl methacrylate (poly-HEMA) and used, depending on thickness of the poly HEMA substrate, up to periods of 1 week. When challenged with either PGE_2 ($2X\ 10^{-6}$ and $2X\ 10^{-9}$ M), arginine vasopressin (10^{-6} M - 10^{-9} M) (AVP) or the calcium ionophore A23187 (5 µg/ml) cells plated onto such substrates contracted. Contraction took place within 5-15' at 37°C. The contraction seen with AVP was dependent on extracellular Ca++ while that observed with PGE_2 was not. Cells returned to their normal configuration between 2 and 3 hours in the continued presence of agonists. With poly HEMA, the length of time required to reach relaxation depended on the thickness of the substrate (between 30 mins. to 3 hours). Cells similarly plated on plastic did not show isotonic contraction. Thus, cell-substrate interaction may play a role in the expression of isotonic contraction by cultured mesangial cells. This may apply also to other contractile cells in culture such as smooth muscle. This technique should facilitate the study of contraction in cultured contractile cell types.

EFFECT OF PLATELET ACTIVATING FACTOR (PAF) ON RENAL PROSTAGLANDIN (PG) RELEASE IN UNILATERAL URETER OBSTRUCTION (UUO). Steven M. Weisman*, Diane Felsen*, and E. Darracott Vaughan, Jr. Cornell Univ. Med. Ctr., Depts. of Surgery (Urology) and Pharmacology. New York, New York.

PAF is a lipid with potent platelet-stimulating and hypotensive properties, which has been shown to stimulate PG release from a few cell types. It is produced by a number of inflammatory cells and the renal medulla. We, therefore, studied the effect of bolus injections of PAF on the isolated perfused rabbit kidney subjected to aseptic ureter ligation for 72 hrs. Intrarenal resistance (RVR), as reflected by changes in perfusion pressure was measured. The release of PGs by both the hydronephrotic (HNK) and contralateral kidney (CLK) was quantified by RIA. Intrarenal administration of PAF causes a dose dependent stimulation of the release of large amounts of PGE_2, Thromboxane B_2 (TxB_2) and the PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$ from the HNK and the CLK. The release from the HNK is 50X greater than from the CLK. RVR increased significantly following PAF injection in the HNK, but fell slightly in the CLK. Product identity was confirmed by perfusing the kidney with a selective TxA_2 synthesis inhibitor (OKY-046, 0.1ug/ml) which inhibited the PAF-stimulated release of TxB_2 by 87% without affecting PGE_2 and 6-keto-$PGF_{1\alpha}$ release. Indomethacin (2ug/ml) inhibited the release of all PGs while simultaneously inhibiting the increase in RVR. These data show that PAF is a potent stimulus for renal PG release and suggest a possible role of PAF in mediating some of the hemodynamic changes which accompany UUO.

A CORTICOSTERONE METABOLITE PRODUCED BY A6 (TOAD KIDNEY) CELLS IN CULTURE: IDENTIFICATION AND EFFECTS ON NA^+ TRANSPORT. C.O. Watlington, W.M. Grogan*, M.L. Fidelman*, D.E. Newton*, and R.L. Duncan*. Med. Coll. Virginia-VCU, Depts. Med., Physiol., and Biochem., Richmond, VA.

Aldosterone (A) and corticosterone (CS) stimulate active Na^+ transport (short-circuit current, I_{sc}) in A6 epithelia. Previous studies with [^{3}H]-CS (10^{-8}-10^{-6}M) showed saturable nuclear binding of polar CS metabolites produced by these cells. Since these metabolites may be agonists cells were incubated in [^{3}H]-CS (10^{-8}-10^{-4}M) for 24h to obtain sufficient quantities of the metabolite(s) for identification. Approximately 25-35% of the radiolabel, recovered in ethyl acetate extracts of medium, chromatographed on reverse phase HPLC as a single peak more polar than CS. This derivative cochromatographed with 6β-OH-CS on HPLC and normal phase high performance TLC. Mass spectroscopy of 6β-OH-CS and the unknown yielded 10 identical molecular ions including the molecular ion with a mass to charge ratio of 362 corresponding to the molecular weight of 6β-OH-CS. 6β-OH-CS stimulated I_{sc} in A6 epithelia with a time course typical of a steroid and an EC_{50} of 10^{-6}M. The I_{sc} was equivalent to net Na^+ flux. At maximum effective concentrations of corticosteroids, 6β-OH-CS plus A induced greater I_{sc} stimulation than A alone. Also, CS produced twice the I_{sc} increase produced by A. Thus, 6β-OH-CS may contribute to the enhanced CS stimulation of I_{sc} compared to aldosterone alone. This effect may be mediated by receptors other than those previously shown to be co-occupied by A and CS and could have relevance in vivo at high glucocorticoid concentrations, e.g. in stress.

ANGIOTENSIN II (AII) METABOLISM BY ISOLATED GLOMERULI: EVIDENCE FOR NON-RECEPTOR MEDIATED AII DEGRADATION. Barry M. Wilkes, Peter Mento*, George Kiesel. Dep't. of Med., North Shore Univ. Hosp., Manhasset, N.Y.

Isolated glomeruli have been shown to convert angiotensin I to AII and to degrade AII to inactive fragments. This study was undertaken to determine whether AII degradation in isolated glomeruli occurs by a receptor-mediated mechanism. Glomeruli were isolated from male Sprague-Dawley rats and suspended in media containing: 2.65nM ^{125}I-AII, 5mM DTT, 0.1M PMSF, 100µM Bacitracin, 1.2mM Ca^{++}, 20mM Tris and 30µg glomerular protein. The competitive AII antagonist, SAR^1-ILE^5-AII (SAR), was added to half the experimental tubes (10^{-5}M). At the appropriate times, specific AII binding was determined, and integrity of ^{125}I-AII was measured by thin layer chromatography (butanol) (4): acetic acid (1): water (5). Results:

Time	Bound AII(% max) -SAR/+SAR	Intact AII (% recovered) -SAR/+SAR
0'	0/0	94.2/92.6
5'	58.3/0*	89.7/92.8
15'	85.5/7.7*	82.6/85.0
30'	100.0/19.4*	59.2/68.9
45'	89.5/1.0*	59.9/62.8

* -SAR vs +SAR, $p < .001$

SAR significantly decreased AII binding, but did not affect glomerular degradation of AII. We conclude that isolated glomeruli contain potent AII-degrading enzymes. Most, if not all, of the degradation is independent of hormone binding.

IN VIVO EVIDENCE AGAINST FUNCTIONAL SIGNIFICANCE OF INTRARENAL PGI_2-ANGIOTENSIN II (AII) INTERACTION IN THE RAT. T. Yoshioka*, A. Yared*, H. Miyazawa* and I. Ichikawa. Children's Hospital, Boston, Mass.

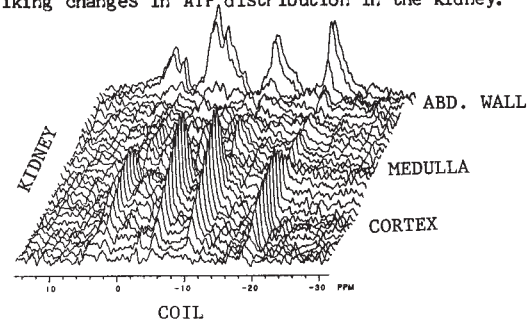
In many animal species, PGI_2 given in vivo fails to induce renal vasodilation. This has been interpreted to reflect species difference in the PGI_2-sensitive AII system, which dampens the vasodilator action of PGI_2. To test this hypothesis, we examined in rats treated with saralasin (s) both renal and systemic influence of PGI_2 (3.6 μg/kg/hr,i.ao.) by measuring systemic blood pressure (AP), cardiac output (CO), renal blood flow (RBF), total peripheral (TVR) and renal (RVR) vascular resistances. The effect of acetylcholine (ACh, 0.35 mg/kg/hr) and nitroprusside (NPr, 0.40 mg/kg/hr) was examined for comparison. Results (mean; †P<0.05) include:

	AP	CO	RBF	TVR	RVR	RVR/TVR
	mmHg	---ml/min---		mmHg/(ml/min)		
CONTROL	109	100.9	10.5	1.11	11.2	10.4
PGI_2(n=6)Δ	- 8†	+27.3†	-0.3	-0.38†	-0.1	+5.6†
ACh (n=7)Δ	-13†	+11.7†	+2.7†	-0.38†	-4.2†	-1.1
NPr (n=5)Δ	-16†	+10.3†	-0.6	-0.25†	-1.2†	+1.4

While PGI_2 and ACh caused comparable reduction in TVR, a marked difference was noted in their renal effect: whereas RBF rose and RVR fell markedly with ACh, RBF and RVR were hardly affected by PGI_2 so that RVR:TVR ratio rose markedly during PGI_2. This contrasted to the near-constancy of RVR/TVR during ACh and NPr. In a separate group of s-untreated rats (n=5), PGI_2 caused changes in these indices which were indistinguishable from those seen with s-treatment. The data indicate that the absence of renal dilation by PGI_2 in vivo is not due, as previously postulated, to highly efficient offsetting influence of intrarenal AII release, but rather to its uniquely weak direct renal dilatory action.

NONINVASIVE ANALYSIS OF TISSUE HETEROGENEITY: NUCLEAR MAGNETIC RESONANCE STUDY OF IN VIVO RAT KIDNEY. Ronald T. Bogusky, Michael Garwood,* Galo Acosta,* Larry Cowgill, Gerald Matson* and Thomas Schleich.* Univ. of Calif. at Davis and Santa Cruz, CA.

Techniques were developed to noninvasively measure tissue sodium, ATP and inorganic phosphate levels in different regions of rat kidney. A 350g rat was anesthetized and its left kidney exposed through a flank incision. The kidney was supported by copper shielding that also separated the exposed kidney from the abdominal wall. The exposed kidney was then covered with Saran Wrap and placed beneath a two-turn surface coil in an NMR probe. The probe containing the rat and the exposed kidney were then placed in the center of a wide-bore 4.7 Tesla magnet with a Nicolet spectrometer. Application of graded pulses and phase-cycling techniques yield resonances at varying depths to produce a metabolite map of the kidney. The method can distinguish metabolic changes that occur in the renal cortex from those in the renal medulla with a resolution of 1-2 mm. In normal kidney the cortex has the highest content of ATP and the medulla the least. Sodium is concentrated to the highest levels in the outer medulla. Kidneys from rats made acidotic with NH_4Cl or hypokalemic with DOCA injections reveal striking changes in ATP distribution in the kidney.



RENAL METABOLISM

GLUTAMATE DEHYDROGENASE (GDH) DOES NOT REGULATE AMMONIA FORMATION DURING ACUTE METABOLIC ACIDOSIS IN VITRO. Ronald T. Bogusky and Richard L. Dietrich,* Dept. of Med., Univ. of California, Davis, CA.

The role of GDH in renal NH_3 formation during acute acidosis was tested in isolated perfused rat kidneys by using amino[^{15}N]glutamine as substrate. Urine and perfusate were analyzed by mass spectrometry to measure the rate of ^{15}N enrichment into free NH_3. Rate of NH_3 formation from 1 mM amino [^{15}N]glutamine in perfused kidneys from methionine sulfoximine treated rats was 0.66 μmol/g kidney/min. Of this rate 0.25 μmol/g kidney/min is from the amino group of glutamine. When 1N HCl was added to the perfusate to lower the perfusate pH to 7.0 NH_3 formation from glutamine increased from 0.66 to 1.10 μmol/g kidney/min. The rate of NH_3 formation from the labeled amino group of glutamine, however, did not increase but remained the same as controls at 0.27 μmol/g kidney/min. The most likely source of NH_3 following addition of HCl to the perfusate is the unlabeled amido-nitrogen of glutamine. Analysis of kidney tissue metabolites showed no correlation of α-ketoglutarate levels with ammonia production from glutamine. The results of this study show (1) GDH is not rate-limiting for NH_3 formation from glutamine during acute in vitro acidosis; (2) GDH is probably not near equilibrium in kidney; (3) metabolism of α-ketoglutarate is not rate-limiting for NH_3 formation from glutamine and (4) glutamine deamidation following enhanced glutamine transport probably regulates NH_3 formation during acute in vitro acidosis in rat kidney.

CITRATE REABSORPTION IN THE PROXIMAL TUBULE. S. Brennan*, S. Klahr, L. Hamm. Department of Medicine, Washington University School of Medicine, St. Louis, MO

Citrate is an important substrate for renal metabolism. Its uptake has been well characterized in renal brush border membrane vesicles; however, citrate reabsorption (Jcit) in the intact renal epithelium has not been well studied. Our aim was to characterize Jcit in the proximal tubule. Isolated proximal convoluted (PCT) and proximal straight tubules (PST) from normal rabbits were perfused and bathed with artificial solutions. Bath contained 6 gm% albumin. Collected fluid citrate measured by microassay was unaffected by tubular production or bath content of citrate. Therefore, other studies used ^{14}C-citrate to measure lumen-to-bath Jcit; perfusate and bath contained 3 mM and 0 mM citrate, respectively. Ion exchange chromatography was used to show that citrate was the predominant ^{14}C species in both perfusate and collected fluid. Jcit in PCT was 2.9 ± 1.0 $pmol \cdot mm^{-1} \cdot min^{-1}$ (Range=0.32-7.90) and was inhibited 87% by ouabain. In comparison, glucose reabsorption in similar PCT was 24.4 ± 5.6 $pmol \cdot mm^{-1} \cdot min^{-1}$. In contrast to PCT, Jcit in PST was minimal (0.2 ± 0.1 $pmol \cdot mm^{-1} \cdot min^{-1}$). In summary, the magnitude of citrate reabsorption is small compared to glucose reabsorption. Citrate reabsorption is an active, ouabain inhibitable process primarily localized to the PCT.

THE EFFECTS OF SHORT-TERM ANOXIA ON ADENYLATE LEVELS AND K-UPTAKE IN A SUSPENSION OF THICK ASCENDING LIMB TUBULES. Mary E. Chamberlin* and L.J. Mandel. Duke University, Dept. of Physiology, Durham, North Carolina

A suspension of medullary thick ascending limb tubules was prepared from rabbit kidney and incubated for 10 minutes at 37°C in a saline containing glucose as the sole metabolic substrate. In the presence of O_2 the ATP and ADP levels (in nmoles/mg protein) were 6.9 ± 0.8 and 1.1 ± 0.1, respectively. The addition of nystatin, a drug which enhances the Na-K-ATPase activity by elevating intracellular sodium, caused a significant drop in ATP levels (5.0 ± 0.9) and rise in ADP levels (1.8 ± 0.2). The addition of ouabain had no effect on the adenylate levels. When the tubules were incubated in an anoxic saline the ATP levels dropped to 4.9 ± 0.9 and ADP levels rose to 2.1 ± 0.3. The addition of nystatin caused slight, but no significant changes in ATP and ADP. The addition of ouabain produced no change in ATP levels but did significantly decrease ADP levels to 1.1 ± 0.1. The rate of active K uptake was measured in the presence and absence of O_2 by monitoring the disappearance of extracellular K when K was added to a suspension of K-depleted tubules. In the presence of O_2 the K uptake (in nmoles K/min·mg protein) was 450.0 ± 75.0, while in anoxic conditions the K uptake was only 48.5 ± 20.0. These results indicate that under anoxic conditions, 71% of the ATP is maintained, yet active K-uptake is severely depressed.

EFFECT OF ADRENAL STEROIDS ON RENAL AMMONIAGENESIS D.J. Colao*, R.M. Serra, and A.C. Schoolwerth. The Pennsylvania State University, Hershey, PA

Although a role for adrenal steroids in renal ammonia metabolism has long been recognized, the specific nature of this effect has not been elucidated. Recent studies in adrenalectomized (ADX) rats suggested that mineralocorticoid repletion promoted urinary acidification whereas glucocorticosteroids resulted in enhanced ammonium excretion (Kidney Int. 21:546, 1982). However, definitive evidence for a role of adrenal steroids in renal ammonia production is lacking. To provide further insight into the effect of adrenal steroids on ammoniagenesis, studies were performed in perfused kidneys and mitochondria isolated from acidotic ADX and sham rats. Kidneys were perfused with 2 mM glutamine and 5 mM glucose at pH 7.4. Although GFR, urine flow, and sodium reabsorption were similar in ADX and sham kidneys, total ammonia production and glutamine uptake were more than twofold greater in sham than ADX kidneys. Ammonium excretion was 50% greater in sham than ADX kidneys. However, there was no significant difference in ammonia production by isolated mitochondria from sham and ADX kidneys. Moreover, there was no difference in mitochondrial ammoniagenesis in ADX rats treated with dexamethasone or aldosterone even though perfused kidneys from these animals produced significantly more ammonia than did ADX kidneys. These studies provide support for a role of adrenal steroids in ammonia production and excretion, both of which are depressed in acidotic ADX animals. Furthermore, the data suggest that the effect of adrenal steroids on ammoniagenesis is not exerted on mitochondrial ammoniagenic pathways.

INDEPENDENCE OF CITRATE REABSORPTION ($\dot{T}cit$) AND UTILIZATION ($\dot{Q}cit$) FROM TISSUE [CITRATE]. Julius J. Cohen, N.A. Anaizi*, A.J. Black* and S.W. Wertheim.* Univ. of Rochester, Roch. NY.

During alkalosis in vivo, renal tissue [citrate] ($[cit]_t$) increases and simultaneously, both $\dot{T}cit$ and $\dot{Q}cit$ decrease. Decreased $\dot{Q}cit$ has been interpreted to cause the increased $[cit]_t$ which in turn, decreases $\dot{T}cit$. Renal citrate handling and $[cit]_t$ could be regulated by other mechanisms in vivo, since alkalosis changes [substrate] and [hormone] in arterial blood. Therefore we perfused the isolated rat kidney (n=80) at pH 7.2, 7.4 or 7.6; pH was changed by varying $[HCO_3^-]$. Citrate 0-2 mM was the only substrate available in a KRB perfusate containing 6% substrate-free albumin. At pH 7.6 $\dot{Q}cit$ and $\dot{T}cit$ were significantly reduced below the values at pH 7.2 or 7.4, but $[cit]_t/[cit]_p$ was the same at all pH values. Thus, as occurs in vivo, both $\dot{T}cit$ and $\dot{Q}cit$ are lowered by a high ECF-pH, but in contrast, $[cit]_t/[cit]_p$ was not increased at high ECF pH when citrate was the only substrate present. In 11 additional perfusions, done in the presence of glucose + lactate + malate, but without added citrate, $[cit]_t$ = 0.6 μmol g^{-1} at pH 7.6 and 0.3 μmol g^{-1} at pH 7.2 ($p<0.01$). No citrate was detectable in the perfusate and citrate excretion was negligible. Thus $[cit]_t$ increases in alkalosis in the absence of citrate in the ECF. We conclude that increased $[cit]_t$ in alkalosis: a) is not the result of decreased citrate utilization taken up from ECF but is due to increased synthesis from other substrates; b) is not required to reduce citrate reabsorption. The decreases in both $\dot{T}cit$ and $\dot{Q}cit$ are independent of $[cit]_t$.

LLC-PK_1 CULTURED RENAL TUBULE CELLS: DIRECT EXPOSURE TO OXYGEN PERMITS A DIRECT AND ADAPTIVE EFFECT OF pH ON AMMONIAGENESIS. LA Cole*, J Scheid*, and RL Tannen. Univ. of Michigan, Dept. of Internal Medicine, Ann Arbor, MI.

With the objective of finding a cell culture system for investigating proximal tubule ammonia metabolism, LLC-PK_1 cells (pig kidney tubules, predominantly proximal) were examined. Under normal culture conditions (cells under 2 mm serum-containing media in flasks or dishes in a 5% CO_2 incubator) neither a direct effect of pH on NH^+_4 production nor adaptation in response to prolonged exposure to an acid pH was found. When LLC-PK_1 cells were cultured in 75 cm^2 flasks on a Bellco rocker (1.2 cycles/min) so that all cells 50% of the time were exposed to the flask atmosphere (5% CO_2 incubator), strikingly different results occurred. Aerobically cultured LLC-PK_1 cells produced NH^+_4 in a glutamine-concentration dependent manner (1.6 nmol $NH^+_4 \cdot 24\ hr^{-1} \cdot$ mg cell protein$^{-1} \cdot$ mM media glutamine^{-1}). When 70% confluent cells were cultured 3 hr at pH 7.05, 7.40 or 7.60, 0.97 ± 0.04, 0.72 ± 0.03 and 0.49 ± 0.02 nmol NH^+_4/mg protein respectively were produced. Appropriate controls indicated that this represented a direct effect of pH on ammoniagenesis. When cells were cultured 3 days to 70% confluency in pH 7.05, 7.40 or 7.60 media, then transferred to pH 7.40 media, after 16 hours 7.84 ± 0.49, 7.10 ± 0.32 and 6.28 ± 0.31 nmol NH^+_4/mg protein respectively were produced, demonstrating adaptation of NH_4 production to pH ($P < 0.04$).

These studies demonstrate: 1) a striking difference in the metabolism of cells cultured in an aerobic vs. normal (i.e. under sera) manner; 2) pH-dependence and pH-adaptation of ammoniagenesis in cultured LLC-PK_1 cells. This should provide a useful tool to delineate the pathways and mechanisms of NH_4 regulation.

THE EFFECTS OF AMINO-ACID (AA) INFUSION ON GFR, OXYGEN AND SUBSTRATE UPTAKE AND Na^+ REABSORPTION IN THE DOG. A. Fine, University of Manitoba, St. Boniface General Hospital, Winnipeg, Canada.

Changes in Na^+ reabsorption (R) are usually correlated with changes in oxygen uptake. However virtually all previous data are with reductions in Na^+R. Increased GFR following protein loading has been demonstrated in man and the dog. We infused AA (Travesol 10%) into normal and chronic metabolic acidotic dogs to determine the effects on Na^+R and oxygen/substrate uptakes by the kidney.

In normal dogs GFR rose from 51.1 ± 5.8 to 99.4 ± 9.42 ml/min. ($p < 0.001$). $\dot{F}Na^+$ rose from 7.96 ± 0.87 to 15.95 ± 1.52 mEq/min/100 g ($p < 0.001$). $\dot{T}Na^+$ rose from 7.61 ± 0.84 to 15.6 ± 1.26 mEq/min/100 g ($p < 0.001$). In spite of this marked elevation in Na^+ reabsorption oxygen consumption was unchanged at 259 ± 29 vs. 248 ± 27 umoles/min/100 g. Renal blood flow did not change. In chronic acidotic dogs similar results were obtained; the uptakes of glutamine and lactate, (the major renal energy substrates) and renal NH_3 production were unchanged following AA infusion.

These results demonstrate that 1. AA infusion in dogs gives rise to a marked increase in GFR but without RBF change. 2. Very marked increases in Na^+ reabsorption occur without expenditure of metabolic energy or alteration in substrate uptake. This novel and unexpected finding suggests that peritubular-physical factors may predominate to augment Na^+ reabsorption in this situation of increased GFR following a protein load, which itself may well represent the commonest single pertuburation of GFR in man and carnivorous animals.

MALEATE-INDUCED STIMULATION OF RENAL GLUTAMINE METABOLISM IN THE DOG. A. Gougoux, and P. Vinay, Notre-Dame Hospital and Depts. of Medicine and Physiology, University of Montreal, Montreal, Canada.

Studies were performed in dogs with normal acid-base equilibrium to evaluate the effects of maleate on renal metabolism. The i.v. administration of maleate (50 mg/kg) increased markedly fractional excretion of bicarbonate (to 32%), sodium, potassium and phosphate, and the urinary excretion of glutamine, glutamate, alphaketoglutarate, lactate, pyruvate, alanine and citrate. Glutamine utilization per 100 ml GFR increased from a control value of 48 to 92 µmoles 120 minutes after maleate administration whereas total ammonia production per 100 ml GFR increased threefold from 60 to 181 µmoles, most of this ammonia being diverted into the renal vein. The renal production of alphaketoglutarate rose from 0 to 78 µmoles per 100 ml GFR, a value almost equal to the renal utilization of glutamine; this finding indicates a metabolic block at the alphaketoglutarate dehydrogenase step. The maleate-induced stimulation of renal glutamine metabolism occurred despite a fourfold rise in renal cortical concentration of alphaketoglutarate. Maleate reduced renal alanine production but did not change lactate utilization. These findings suggest that: 1) the mitochondrial entry of glutamine is not regulated only by cytoplasmic alphaketoglutarate; 2) the deamination of glutamate into alphaketoglutarate is accelerated by maleate, probably through an impaired mitochondrial NADH production; 3) the resulting decrement in intramitochondrial glutamate concentration deinhibits the phosphate-dependent glutaminase.

LOCALIZATION OF RECEPTORS FOR INSULIN-LIKE GROWTH FACTORS (IGFs) IN CANINE RENAL PROXIMAL TUBULAR CELLS. J.R. Gavin, III* and M.R. Hammerman. Washington Univ. Sch. of Med., St. Louis, MO.

Insulin and its related growth factor MSA/IGF II have been shown to regulate acute metabolic events in suspensions of renal proximal tubular segments (PTS) from dog kidney. We have shown that insulin effects are likely mediated through receptors which are asymmetrically distributed on the plasma membrane of this cell, being localized predominantly in the basolateral membrane. The present study was performed to determine if the acute effects of insulin and MSA/IGF were mediated through unique receptors and to determine the distribution of such binding sites. We examined PTS, isolated basolateral (BLM) and brush border membranes (BBM) for the presence of IGF receptors using highly-purified tracers and radioligand assay techniques. Specific binding of ^{125}I-IGF II, but not ^{125}I-IGF I was found in PTS. Whereas insulin binding to BLM was 20-fold greater than to BBM, IGF II binding was only 15-25% greater. BLM and BBM bound very little ^{125}I-IGF I. Binding of ^{125}I-IGF I was inhibited by IGF II, but not by IGF I, and was not affected by insulin-receptor antibody, suggesting that ^{125}I-IGF I bound to an IGF II receptor. ^{125}I-IGF II was covalently crosslinked with disuccinimidyl suberate to a protein with M_r 250,000 in both BLM and BBM. No detectable crosslinking of ^{125}I-IGF I was found in either membrane, consistent with binding studies. We conclude that receptors for IGF II, but not IGF I are present in renal proximal tubular cells. These receptors are localized to both basolateral and brush border membranes and may have functional roles in regulation of acute metabolic events in kidney.

LACK OF IMPORTANCE OF LACTATE METABOLISM IN THE REGULATION OF THE MAXIMUM RATE OF AMMONIUM PRODUCTION BY THE DOG KIDNEY. M.L. Halperin, M.B. Goldstein, A. Gougoux and P. Vinay. Univ. of Montreal and Toronto, Canada.

Cells remain in ATP balance by adjusting their rate of ATP synthesis to equal that of ATP utilization. In the kidney cortex of the dog with chronic metabolic acidosis, close to half the ATP is produced from glutamine and half from lactate; the vast bulk of this ATP is used to pump sodium. Our purpose was to evaluate whether a reduced rate of renal lactate extraction would lead to an increased rate of glutamine metabolism. Acidotic dogs were divided into two groups based on their steady-state lactate extractions - i.e. the low lactate group (n = 6) had a blood lactate of less than 0.5 mM whereas the other group had a mean blood lactate of 1.1 mM (n = 6). Extractions were expressed at a constant rate of ATP turnover.* When substrate oxidations were evaluated in the kidneys of both groups of dogs, the total ATP turnover was unchanged, close to 3500* as judged from renal oxygen consumption. However, the ATP derived from lactate oxidation fell from 1400* in the higher lactate group to 400* in the lower lactate group; ATP derived from glutamine extraction was equal in both groups of dogs (close to 1700*) and this amount is equivalent to the majority of ATP turnover in the proximal convoluted tubule. These data suggest that lactate is not a principal fuel in the proximal tubule; by inference, lactate is the principal fuel for the distal nephron. When lactate is not utilized in distal segments, fuels other than glutamine (fat) appear to supply their ATP.

*µmoles/100 ml GFR.

SUBCELLULAR LOCALIZATION OF INSULIN IN RAT RENAL CORTEX. J. Herrman*, T. Glaser*, E. Vasquez*, B. Marck * and R. Rabkin. Stanford University and VA Medical Center, Palo Alto, CA.

Renal protein degradation occurs in lysosomes, but insulin degrading activity is present in both lysosomal and non-lososomal structures. With EM autoradiography, we were unable to find significant localization of ^{125}I-insulin over lysosomes suggesting an alternate pathway for insulin degradation. (Proc. Int. Cong. Neph., 370A, 1984). To confirm these findings and to exclude the possibility that rapid hydrolysis may have obscured the identification of lysosomal insulin localization by autoradiography, we examined the localization of ^{125}I-insulin in renal cortical subcellular fractions. Fractions were prepared from isolated kidneys perfused with ^{125}I-insulin for 10, 25 or 60 minutes in the presence or absence of 25-100 μm chloroquine, an inhibitor of lysosomal degradation. Separation of fractions was achieved by centrifugation in a linear sucrose gradient. Distribution of insulin was bimodal with peak densities of 1.096± .002 and 1.179±.002 g/ml which were unaltered by the presence of chloroquine. By contrast, lysosomal enzyme activity (n-acetyl-β-glucosomidase) peaked at a density of 1.206±.009 g/ml a value which differs significantly from that of insulin ($p < 0.05$).

These findings indicate that lysosomes are not a major site of insulin localization, supporting the hypothesis of a major non-lysosomal pathway for insulin degradation. This suggests that the classic route of protein metabolism may not apply to all proteins.

STOICHIOMETRIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION, NA REABSORPTION, AND ATP SYNTHESIS OF THE KIDNEY IN VIVO. A.P. Koretsky, A. Xuan, and M.W. Weiner. VA Med. Ctr., and Univ. Calif. San Francisco, CA 94121.

Previous investigators have suggested that the kidney reabsorbs Na with a Na/O_2 ratio greater than the theoretical maximum value of 18, predicted from a ATPO/O=3 and a Na/ATP=3. To investigate this problem, the rate of ATP synthesis of the kidney in vivo was measured by ^{31}P NMR. The measurements were performed using chronically implanted wire radiofrequency coils. ATP synthesis was quantitated using the saturation transfer technique. The fractional change of the Pi peak and T_1 apparant of Pi were measured while saturating gamma ATP. A chronic indwelling renal vein catheter measured renal inulin and O_2 extraction; thus renal blood flow, O_2 consumption, and Na reabsorption could be calculated. The fractional change of Pi, produced by saturating gamma ATP was 13%. The T_1 apparant of Pi was 0.91 $sec.^{-1}$ The k for the rate of ATP synthesis was 0.14 sec^{-1}. The O_2 consumption was 9 umol/min and Na reabsorption was 140 umole/min. The rate of ATP synthesis was 18 umoles/min and the calculated ATP/O was 1. This is considerably less than the predicted ATP/O of 3. The Na/ATP was 8. However the rate of ATP synthesis may be spuriously underestimated because of NMR-invisible Pi. In addition, the T_1 of Pi may be overestimated because of the presence of non-exchanging metabolites which overlap with the Pi peak. Nevertheless, these experiments demonstrate the feasibility of measuring the efficiency of oxidative phosphorylation and the utilization of ATP for Na transport in the kidney in vivo.

ROLE OF SPECIFIC LIPIDS IN RENAL MEMBRANE ORDER. M.K. Hise, and E.J. Weinman. University of Texas Medical School, Houston, Texas.

To determine the role of fatty acyl chain length and degree of unsaturation in membrane order (fluidity), liposomes of rat renal brush border membranes (BBM) were digested with sphingomylinase and reconstituted to specific phosphatidylcholine (PC) to sphingomyelin (Sg) ratios with PC's containing known fatty acids. Fluorescence anisotropy (r) of DPH was used to reflect acyl chain mobility. As compared to controls (r= .205 ± .001 at 37°C), addition of PC with unsaturated bonds significantly decreased (r).

	PC/Sg Ratio		
Fatty Acid	1.1	1.6	2.4
Oleic (18:1)	.197±.002	.193±.002	.186±.002
Linoleic (18:2)	.194±.003	.189±.003	.186±.002
Arachidonic (20:4)	.195±.001	.187±.002	.179±.003
	$p<0.02$	$p<0.003$	$p<0.0003$

The number of unsaturated bonds (1 to 4) had no independent effect. Reconstitution with PC's with saturated fatty acids of varying chain lengths (14 to 24) did not effect (r). Reconstitution with natural PC's of mixed fatty acyl chain composition, yielded (r) values comparable to those of PC's containing unsaturated fatty acids.

These studies demonstrate that in the complex lipid mixture of BBM: (1) over a small range of concentrations, the presence of unsaturated fatty acids exerts a powerful influence on order while the number of unsaturated bonds and the chain length of the fatty acids have little effect, and (2) the PC/Sg ratio is a determinant of order by virtue of the types of fatty acids contained in natural PC's.

ACCELERATED MUSCLE VALINE OXIDATION IN ACUTE RENAL FAILURE (ARF). B.J. Maroni,* G. Karapanos* and W.E. Mitch. Harvard Med. Sch., Boston, MA.

ARF causes insulin resistance and protein hypercatabolism in skeletal muscle. Since muscle is the major site of branched-chain amino acid oxidation, we studied the effect of ARF and insulin on metabolism of valine (V) and leucine (L) in rat epitrochlearis muscle. Muscles from ARF and sham-operated (SO) rats were incubated with physiologic concentrations of V or L and the release of $^{14}CO_2$ from L(1-^{14}C)valine or L(1-^{14}C) leucine was measured, i.e., oxidation. Transamination (TRNS) was calculated as oxidation plus $^{14}CO_2$ released after decarboxylation of the ketoacid with H_2O_2. At 100 μU/ml insulin, rates of V and L oxidation were linear for 2 h. Insulin dose-response curves (0-10 mU/ml) indicated that V oxidation was significantly higher in muscles of ARF compared to SO rats, but varying insulin had no consistent effect on V oxidation. Despite a persistent trend, increased L oxidation in ARF muscle was significant only at 10 mU/ml insulin, yet L oxidation rates increased in both groups at ≥100 μU/ml insulin. In ARF, the higher oxidation rate of V was not due to increased TRNS since rates were similar in both groups. Total uptake of V or L (the sum of TRNS, accumulation within muscle and incorporation into protein) at 100 μU/ml insulin was unaffected by ARF. Thus, V oxidation in muscles is increased in ARF and must be due to enhanced branched-chain dehydrogenase (BCD) activity. An independent effect of ARF on L oxidation may be obscured because the ketoacid of L stimulates BCD. Insulin is not a primary factor regulating muscle oxidation of V.

THE EFFECTS OF METABOLIC ACIDOSIS (MA) ON INSULIN-MEDIATED PROTEIN TURNOVER IN MUSCLE. R.C. May,* G. Karapanos,* and W.E. Mitch. Harvard Medical School, Boston, MA.

In MA, muscle glutamine (GLN) release contributes to the increased renal ammoniagenesis. Since muscle GLN arises from amino acids, we investigated whether MA affects muscle protein turnover. In the perfused hindquarter, lowering the media pH from 7.40 to 7.15 increased GLN release 10% but did not change PD measured as tyrosine (Tyr) release; a lower media pH also did not increase PD in incubated muscle. To study chronic MA, two groups of rats were gavage-fed 12 g/d of a 14% protein diet ±4 mMol/100g/d NH_4Cl and protein synthesis (PS) and PD were measured in their incubated epitrochlearis muscles. After 5 days, arterial pH was lower (7.12 MA vs 7.33; $p<0.01$) and growth was stunted (-2 g MA vs +8 g; $p<0.05$). PS (phenylalanine, Phe, incorporation into protein) was unaffected by MA in the absence of insulin (44.1±2.6 MA vs 44.1±2.3 nMol Phe/g/h; mean ±SE) or with 10 mU/ml insulin (60.1±1.8 MA vs 65.6±4.6 nMol Phe/g/h). In contrast, PD was significantly higher at all (0-10 mU/ml) levels of insulin. To control for the effects of NH_4, group III rats were gavage-fed an equimolar amount of NH_4acetate, leading to an arterial pH of 7.36 and a 43% lower nitrogen excretion (88 vs 126 mgN/100g/d MA; $p<0.01$). Their muscle PD at 0.1 mU/ml insulin was 41% lower than that of MA (100±12 vs 141±13 nMol Tyr/g/h, MA; $p<0.05$). Thus, in muscle: 1) acute changes in pH did not change protein turnover; 2) chronic MA stimulated PD without changing PS; 3) the increased PD persisted despite insulin; 4) this effect caused negative nitrogen balance and was not due to NH_4.

RENAL PROTEIN CHANGES IN HYDRONEPHROSIS. John B. Nanninga, Lyda Oliver*, Julia Sensibar*, and Chung Lee*. Northwestern University Med. Ctr., Dept. of Urology, Chicago, Illinois.

Changes in the tissue proteins in the hydronephrotic kidney reflect the pathologic process caused by renal obstruction. This study was performed by comparing normal and hydronephrotic rat kidneys and normal regions from human kidneys with hydronephrotic human kidneys. The rat kidneys were studied after 1 and 2 weeks obstruction. The renal tissue was analyzed by homogenizing and centrifuging it at 100,000 x g for 1 hour. The super natant containing 200 µg of protein was applied on a LAEMMLI gel for electrophoresis. Following this the gels were stained for proteins with Coomassie Blue. The results in the hydronephrotic rat kidney demonstrated an increase in the protein band in the 60-70 thousand (K) range. There were no changes in the contralateral non-obstructed kidney or the sham operated kidneys. In the human kidneys the length of obstruction varied. However, all kidneys functioned on x-ray (IVP) or isotope study. The protein analysis demonstrated changes in the 60-70 K region as well as more clearly defined bands in the 30 K range. These changes would seem to be related to the recognized changes in hydronephrosis such as weight gain, macrophage infiltration and decreasing blood flow and anoxia. There did not appear to be any change in the contralateral kidney as demonstrated in this study.

Na-K-ATPase ALONG THE RAT NEPHRON FOLLOWING UNINEPHRECTOMY. SK Mujais, S Kauffman*, and NA Kurtzman, Univ of Illinois, Chicago IL.

Uninephrectomy (UNx) leads to an increase in Na-K-ATPase in kidney homogenates, but the segmental location and mechanism of this increase are unknown. We evaluated this issue in male Sprague-Dawley rats weighing 140-160 g subjected to right UNx. Compensatory hypertrophy was evident at 2 wk by greater left kidney weight (1.2±0.03 vs 0.96±0.03 g; $p<0.001$) and GFR (1.13±0.1 vs 0.75±0.07 ml/min, $p<0.05$) in UNx than in sham rats. Daily excretion of Na (1269±164 vs 1272±231 µEq/d) and K (1674±207 vs 1619±259 µEq/d) was identical in sham and UNx rats, respectively. Na-K-ATPase in proximal convoluted (PCT), medullary (MAL) and cortical (CAL) thick ascending limb, cortical (CCT) and medullary (MCT) collecting tubules (MCT) was: (pmoles.mm.hr.-1) (*$p<0.05$).

	PCT	MAL	CAL	CCT	MCT
SHAM	2776	4507	4738	1984	1278
	±317	±402	±615	±276	±190
UNx	2707	4617	4257	2788*	1129
	±262	±333	±553	±174	±138

The segmental specificity of the increase in Na-K-ATPase suggests that this increase is not a non-specific effect of compensatory hypertrophy but the consequence of functional adaptation to transport requirements. The selective increase in Na-K-ATPase in the CCT, a major site of K adaptation in the rat, suggests that this increase is in response to a greater secretory K load. The lack of change in Na-K-ATPase in segments with greater capacity for Na reabsorption suggests that adaptation to larger filtered Na load is mediated by mechanisms other than increases in tubular cell Na-K-ATPase, possibly by increases in tubule length.

PYRIMIDINE NUCLEOTIDE (PN) METABOLISM IN CULTURED RAT MESANGIAL CELLS AND IN WHOLE GLOMERULI INCUBATED IN VITRO. T.W. Ott*, F. Dumler, P. Cortes, D. Paielli,* K.S.S. Sastry,* and N.W. Levin. Henry Ford Hospital, Detroit, MI.

PN-sugars are important precursors of glomerular basement membrane (GBM) synthesis. We have studied PN metabolism using confluent rat mesangial cells (MC) in RPMI media and in isolated glomeruli (G) incubated in vitro with Krebs-Ringer bicarbonate buffer containing 10% fetal calf serum and 20 mM glucose. The cellular content of RNA, uridine triphosphate (UTP), UDP-glucuronic acid (UDPGA), UDP-glucose (UDPG), and UDP-N-acetyl-glucosamine (UDPAG), and the incorporation rate of ^{3}H-orotate into RNA and UTP were measured after a 2 hour labeling period. PN were measured by high pressure liquid chromatography. Results are expressed per mg DNA as mean±SEM. UTP content in MC was 40 times greater than in G (325±47(6) vs 8.2±0.3(8) nmol respectively; $P<.0001$). UDP-sugar pools (nmol) were 40 to 100 times higher ($P<.0001$) in MC than in G (MC: UDPGA 38.1±6.2; UDPAG 173±19; UDPG 119±13; G: UDPGA .951±.02; UDPAG 1.64±0.03; UDPG 1.87±0.03). RNA content was 5.9 times greater in MC than in G (333±28 vs 56±1 µg respectively; $P<.0001$). When using 10 µM orotate as precursor, the incorporation of ^{3}H-orotate into UTP and RNA was greater in MC than in G (4158 and 466 pmol/min into UTP and RNA for MC vs 7.0 and 4.3 pmol/min for G). PN metabolism is more active in MC than in G as a result of cellular hypertrophy and/or exclusion of other cell types in tissue culture. The increased metabolic activity of PN in MC facilitates the study of factors regulating GBM synthesis.

BICARBONATE INHIBITION OF KIDNEY CORTEX SUCCINATE DEHYDROGENASE: INFLUENCE ON GLUTAMATE METABOLISM. R.C. Scaduto* and A.C. Schoolwerth. The Pennsylvania State University, Hershey, Pennsylvania

Recent studies have demonstrated a pH-independent effect of bicarbonate on the metabolism of glutamine by kidney cortex tubules. To evaluate the mechanism of this effect, studies were performed with isolated kidney cortex mitochondria. Mitochondria were incubated with glutamine and glutamate in the presence or absence of 5% CO_2/28 mM HCO_3^- buffer at 28°C. Bicarbonate was found to inhibit glutamate oxalacetate transaminase (GOT) flux and to stimulate glutamate dehydrogenase flux. Since the addition of malonate, a specific inhibitor of succinate dehydrogenase (SDH), duplicated these results, the effect of bicarbonate on succinate metabolism was investigated in rotenone-inhibited mitochondria. With 1 mM succinate as substrate, malate production was suppressed 51% by 0.1 mM malonate and 57% by bicarbonate. To access further the specificity of the bicarbonate effect, the activity of SDH in isolated inner mitochondrial membranes was determined. Bicarbonate ion, in the physiological range, was found to be a potent competitive inhibitor of SDH. Inhibition of SDH lowers GOT flux due to a decreased availability of malate. This, in turn, stimulates glutamate deamination because of the inhibition of GOT-derived α-ketoglutarate. Thus, these data demonstrate that bicarbonate ion exerts an effect on glutamate metabolism by kidney cortex mitochondria that is mediated through inhibition of SDH activity. They also suggest that bicarbonate ion, independent of hydrogen ion, has a physiological role in the control of kidney glutamate metabolism.

ROLE OF RENAL METABOLISM IN GENESIS OF CHEMICALLY INDUCED TRANSITIONAL CELL CARCINOMA. **Leslie A. Spry, Terry V. Zenser,* and Bernard B. Davis. VA Med. Ctr., GRECC, and St. Louis Univ. Schl. of Med., Depts. of Int. Med. and Biochem., St. Louis, Missouri.**

N-[4-(5-nitro-2-furyl)-2-thiazole] (FANFT), a 5-nitrofuran, after deformylation to ANFT is a potent transitional cell carcinogen. Renal excretion and metabolism of FANFT and ANFT were evaluated using the isolated rat kidney perfused for 1 hr with modified Krebs-Henseleit and amino acid mixture. Analyses were by simultaneous electrochemical and UV detection after HPLC separation. Mean GFR and Fe Na were 0.65 ± 0.5 ml/min and 7.7 ± 0.5%, respectively. With FANFT, Fe FANFT was 2.7 ± 0.5% and with ANFT, Fe ANFT was 6.3 ± 0.8%. However with FANFT, the Fe ANFT was 183 ± 45%. The half-life of FANFT in perfusate was 41 ± 3 min and of ANFT was 147 ± 21 min. Of the FANFT which disappeared from perfusate, 57 ± 10% was recovered as ANFT in perfusate or urine. Neither aspirin nor probenecid had an effect on metabolism or excretion. Renal deformylase activity was demonstrated in broken cell preparations with a Km of 6 μM. Over 90% of deformylase activity was in the 100,000 x g supernatant fraction, was stimulated 3-fold by 0.25 M NaCl, and was not inhibited by 1 mM EDTA, 0.1 mM PCMB, 0.1 mM paraoxan, or 0.3 mM aspirin. These data document a unique coupling of deformylation to urinary excretion of FANFT/ANFT. Deformylation increases the rate of excretion of the putative proximate carcinogen, ANFT. It is suggested that this metabolic/excretory coupling is important in the mechanism by which rats fed FANFT develop urinary tract cancer.

UTILIZATION OF ENDOGENOUS AND EXOGENOUS FATTY ACIDS BY THE PROXIMAL TUBULE. S.P. Soltoff and L.J. Mandel, Physiol. Dept., Duke Univ., Durham, N.C.

To examine the contributions of various substrates to the maintenance of active transport in the proximal tubule, the ouabain sensitive rate of O_2 consumption (QO_2) and the adenine nucleotide content (by HPLC) were measured in a suspension of rabbit proximal tubules. In some experiments the ionophore nystatin (Nys) was used to stimulate maximal Na pump activity. Endogenous substrate support of QO_2 was tested in a substrate-free (0-Sub) medium. In this medium, QO_2 was inhibited by 70% in the presence of 10^{-5} M TDGA or 0.6 mM bromooctanoate (Bromo), which inhibit mainly long-chain fatty acid oxidation. Nys stimulated the 0-Sub QO_2 by 80% in the absence but not the presence of either inhibitor. Thus, the proximal tubule uses mainly fatty acids as endogenous substrates to supply energy for the Na pump during basal as well as pump-stimulated conditions. For 0-Sub tubules, the combined presence of TDGA plus Bromo decreased the QO_2 by more than 80%, decreased ATP content by 65%, and caused a large increase in the AMP and hypoxanthine content. In the presence of 5 mM glucose, 4 mM lactate, and 1 mM alanine, TDGA or Bromo significantly inhibited control and Nys-stimulated respiration. However, 1 mM butyrate prevented the inhibition of QO_2 by TDGA. Therefore, endogenous fatty acids continue to be oxidized unless exogenous fatty acids are supplied to the proximal tubules.

INTRACELLULAR RESPIRATORY DYSFUNCTION AND CELL INJURY IN SHORT-TERM ANOXIA OF RABBIT RENAL PROXIMAL TUBULES. T. Takano, S.P. Soltoff, S. Murdaugh, and L.J. Mandel. Dept. of Physiology, Duke Univ. Med. Ctr., Durham, N.C.

The effects of short-term anoxia were studied by using a proximal tubule suspension in order to avoid the hemodynamic consequences of clamp-induced ischemia. The suspension was subjected to anoxia for 10-40 min and the effects on a number of cellular transport and respiratory parameters were monitored. Cellular respiration was measured upon addition of nystatin (Nys) to maximally stimulate Na pump activity. Mitochondrial respiration was measured in the tubules by addition of digitonin and ADP to obtain the State 3 respiratory rate. Results show that 10 and 20 min of anoxia partially inhibited Nys-stimulated and mitochondrial respiration, and partially decreased ATP and K^+ contents, but all these effects were largely reversible after 20 min of reoxygenation. After 40 min of anoxia and 20 min of reoxygenation, all these variables remained irreversibly inhibited: Nys-stimulated respiration by 54%, mitochondrial respiration by 50%, K^+ content by 42%, ATP content by 75% and ADP content by 55%. In addition, hypoxanthine levels increased 15 to 25-fold, and lactate dehydrogenase release was 40% after 40 min of anoxia. Addition of extracellular Mg-ATP significantly ameliorated these changes. We conclude: 1) Short term anoxia elicits multiple cellular dysfunctions at the plasma and mitochondrial membranes; 2) Irreversibility of cellular function may be associated with loss of cellular adenine nucleotides.

CHARACTERIZATION OF RENAL BRUSH BORDER(BB) AMINOPEPTIDASE BY A MONOCLONAL ANTIBODY(Mab). M. Tauc* P. Poujeol*, P. Verroust*, P. Ronco*, E. Brianti* F. Chatelet*, and A. Vandewalle*, INSERM U246 CEN Saclay, INSERM U64 and Dept. Histopathologie Hop. Tenon, Paris, France.(Intr.by T. Anagnastopoulos). Eleven previously reported Mab against rabbit renal cortical cells were screened for antibody activity against constitutive enzymes of the BB. For this purpose solubilized brush border membrane vesicles (BBMV)were incubated with Mab previously adsorbed on plastic tubes. One antibody, Mab 5, was able to bind leucine aminopeptidase (LAP) activity, and immunoprecipitated a protein with an apparent MW of 115 KD from radiolabeled BBMV. Using Mab5 coupled to Sepharose 4B(S4B-Mab5), it was possible to deplete 95% of BBMV LAP activity. This depletion was specific since no LAP activity was removed when BBMV were incubated with immunoadsorbents coupled to 2 other antiBB Mab. When the immunoadsorbents were eluted with alkaline buffer LAP activity was only recovered in the eluates of S4B-Mab5 with a specific activity over 500 times higher than in the control eluates. In additional experiments, when radiolabeled BBMV was added to the soluble BBMV incubated with S4B-Mab5, the eluted protein migrated with an apparent MW of 115 KD. By indirect immunofluorescence with Mab5, LAP was localized on the renal and intestinal BB. Immuno-ultrastructural analysis on renal tissue indicated that the enzyme is localized over the entire surface of the microvilli. In conclusion, Mab5 allowed the initial characterization and ultrastructural localization of LAP and may be useful to study membrane turnover of proximal tubular cells.

DESIGN OF A PERIFUSION CHAMBER ALLOWING NMR STUDIES OF IMMOBILE RENAL TUBULES FOR PROLONGED PERIODS. Patrick Vinay, Yvan Boulanger, Tan Phan Viet, Binaye Soowamber and Lise Lessard. Dept. of Med., Physiol. and Biomed. Engineering, Univ. of Montreal, Canada.

NMR spectroscopy allows the non-invasive study of living tissue. Due to its low sensitivity, this technique requires the accumulation of signals for minutes or hours according to the nuclei of interest. It is thus necessary to study the tissue during optimal steady-state conditions. We have designed a perifusion chamber allowing the observation of renal tubules for 10 consecutive hours or more with no loss of biochemical activity - Dog renal tubules (1.5g) are prepared by collagenase dispersion and introduced in a tuft of hollow-fiber dialysis membrane (200 µm i.d.; cuprophane) obtained from a conventional capillary dialyser. The fibers are sealed with paraffin, and introduced in a glass chamber (20-ml) where the heated (37°C) and gassed (95% O_2-5% CO_2) perifusate containing metabolic substrates is circulated at 250ml/hr. The substrates (lactate + alanine + glutamine (2mM)) are kept constant by continuous infusion in the reservoir. Using this system, we have observed a constant and maximal glucose production by the tubules for 10 hours (glucose vs time: $r^2 = 0.99$; glucose = 0.72 µmol g wet wt^{-1} min^{-1}) while obtaining ^{31}P-NMR spectra. This procedure 1) removes the necessity for stirring (and destroying) the cells 2) allows rapid changes of experimental conditions without interrupting NMR settings 3) allows the continuous monitoring of the perifusate outside the magnet 4) improves the quality of the NMR specta obtained.

ISOLATION OF DISTAL CELL POPULATIONS FROM RABBIT KIDNEY BY IMMUNOADSORBENT CHROMATOGRAPHY. A. Vandewalle*, F. Cluzeaud*, P. Ronco*, P.Verroust* and P. Poujeol*, INSERM U 246, C.E.N. Saclay and INSERM U 64, Hôpital Tenon, Paris, France. (Intr.by R. R Robinson)

This study describes a method for distal cell separation based on the sequestration of proximal tubular cells by Sepharose 6MB covalently linked to 3 monoclonal antibodies (Mab) directed against renal brush border and glomerular cells. Isolated cortical cells were prepared by mechanical dissociation of rabbit kidney in the presence of EDTA(0.25mM).In 7 experiments,157 to 360x10^6cells were submitted to 2 sequential chromatography steps. 92.6% of the applied cells were first retained on 4 ml of S6-Mab by incubation for 60 min at room temperature.The unbound cells were submitted in a second step, to free flow passage over 1 ml of S6-Mab: 29.3% of the cells, representing 2% of the starting material, were recovered. 98% of the cells in the final suspension excluded eosin. The efficiency in proximal cell depletion was attested by 74.5% and 84% decreases in leucine aminopeptidase and alkaline phosphatase activities. The basal oxygen consumption was high : 32.1 ± 4.6 (n=5) as compared to 13.9 ± 1.7 (n=6) nmolesO_2.min^{-1}.mg $protein^{-1}$ for the initial cell suspension. There was an increase in Na^+-K^+ ATPase (x1.65) , succinyldehydrogenase (x1.5) and hexokinase (x3.4) activities,indicating the distal origin of these cells. The efficiency and reproducibility of this method for distal cell preparation should be useful for biochemical studies and for production of Mab against distal cell subpopulations.

AMINO ACID (AA) UPTAKE (U) THROUGH THE BASOLATERAL MEMBRANE OF THE TOAD BLADDER: DEPENDENCE UPON HORMONES. B.J. Wilk* and C.P. Carvounis, Div. Nephrology, SUNY, Stony Brook and Upstate Medical Center, Syracuse, NY.

We previously showed that AA influence vasopressin (V) and cAMP stimulated water flow. These effects of AA follow their entry in V sensitive epithelia and depend upon AA-U. AA-U in the toad bladder occurs exclusively through the basolateral membrane. We showed distinct systems for acid, basic and neutral AA. AA-U follow Michaelis-Menten kinetics and specific Km and Vmax have been identified, i.e. for lysine a Km of 1.7 mM and a Vmax of 912 pM · min^{-1}· mg^{-1} for glutamine a Km 0.6 mM and a Vmax 223, alanine Km 0.7 mm, V max 156 and so forth. AA-U depends upon various hormones. Insulin (I) increased early (5') uptake of lysine and histidine and decreased that of glutamine, but had no effect on steady-state U. Kinetic analysis showed that I decreased Km of lysine and histidine and increased that of glutamine while it had no effect on Vmax. cAMP increased steady-state U of histidine, lysine, glutamate. Kinetic analysis demonstrated that cAMP increased Vmax in all these cases. V was found to also affect U of selected AA. V decreased early U of lysine and glutamate due to increase in Km. In conclusion a) AA-U in the toad bladder occurs exclusively through the basolateral membrane; b) U occurs through mechanisms having specific characteristics (Km, Vmax); c) U appears to depend upon peptide hormones and cAMP; d) the discrepancies between effect of I, V, and cAMP suggest that I and V influence AA-U by altering directly the U mechanisms rather than through cAMP generation.

FASTING INCREASES CITRATE UPTAKE BY BRUSH BORDER BUT NOT BASOLATERAL MEMBRANES OF RAT KIDNEY. David W. Windus. Washington Univ. School of Med., Dept. of Internal Medicine, St. Louis, Missouri.

Fasting in rats decreases urinary citrate excretion and increases peritubular uptake of plasma citrate by the kidney. To determine if these findings result from an adaptation in citrate transport across the membrane of the renal tubular cell, Na-gradient dependent ^{14}C-citrate uptake was examined in brush border (BBMV) and basolateral (BLMV) membrane vesicles prepared from the kidneys of fed and fasting rats. After 72 hours of fasting, the blood pH fell from 7.44 ± .02 to 7.33 ± .03 and the bicarbonate from 23.3 ±1.6 to 16.6 ± 1.3 mEq/L. The initial rate (10 s) of 100 uM Na-dependent citrate uptake was significantly increased in BBMV isolated from the kidneys of fasting rats (491 ± 24.9 pmol/mg prot) compared to fed rats (346 ± 23.9 pmol/mg prot). To analyze the role of the acidosis of fasting a separate group of fasting rats was given 100 mM $NaHCO_3$ to drink during the 72 hour fasting period. The initial rate of 100uM Na-dependent citrate uptake in BBMV from kidneys of fasting rats given $NaHCO_3$ (477 ± 28.9 pmol/mg prot) was not significantly different from fasting rats given only tap water. There was no significant difference in the initial rate of Na-dependent citrate uptake in BLMV isolated from the kidneys of fasting compared to fed rats. An adaptation occurs in the brush border membrane of the renal tubular cell of fasting rats, unrelated to acidosis, that results in increased reabsorption of citrate.

RENAL PHYSIOLOGY—ACID BASE

MEASURMENT OF RENAL PH BY ^{31}P NMR: EFFECTS OF ACIDOSIS AND POTASSIUM DEPLETION. W.R. Adam*, A.P. Koretsky* and M.W. Weiner. VA Med Ctr., San Francisco and Univ. of Calif. at Berkeley and San Francisco.

Metabolic acidosis and potassium depletion both stimulate renal ammoniagenesis. It has been suggested that intracellular acidosis is a common signal which increases ammonia production. To examine this problem, the pH of the rat kidney in vivo was non-invasively measured using ^{31}P NMR. Renal pH in control rats was 7.39 ±0.04 (n=8), (arterial blood pH 7.45 ± 0.04). Acute metabolic acidosis lowered renal pH, and there was a direct relationship between arterial blood pH and renal pH (renal pH=0.43 x blood pH +4.05, r=0.96, n=7). Chronic acidosis (arterial blood pH=7.26 ± 0.20) was associated with renal pH of 7.30 ± 0.24 (n=9), significantly lower than control. Potassium depletion (K free diet 14-28 days, arterial blood pH 7.44 ± 0.05) lowered renal pH to 7.17 ±0.018, n=12. This value was significantly lower than controls and chronic metabolic acidosis. There was a direct relationship between renal pH and cardiac K^+ (as a measure of potassium status) (renal pH = 0.0183 x cardiac K^+ + 5.81, r=0.87, n=16). Rapid repletion with KCl (1 mmole) significantly increased renal pH from 7.14 ± 0.03 to 7.31 ± 0.01, n=5 over 40 minutes. The finding that acute metabolic acidosis lowers renal pH, suggests that intrarenal acidification may be the initial signal which stimulates ammoniagenesis. However, the different results with chronic acidosis and potassium depletion clearly demonstrate the absence of a direct relationship between renal pH and the maintenance of increased ammoniagenesis. Therefore it is concluded that factors other than intracellular pH are responsible for the chronic adaptive increase of renal ammonia production.

DIFFERENTIAL EFFECT OF TRIIODOTHYRONINE (T_3) ON BRUSH BORDER MEMBRANES (BBM) IN SUPERFICIAL AND JUXTAMEDULLARY RENAL CORTEX. A.N.K. Yusufi* and T.P. Dousa. Nephrol. Res. Unit, Mayo Clinic and Foundation, Rochester, MN.

We observed that administration of T_3 to TPTX rats increases the Na^+-dependent uptake of phosphate (Pi) and the Na^+-H^+ antiport in BBM, but the uptake of Na^+, and Na^+-dependent uptake of L-proline, D-glucose and citrate is not changed. We further studied whether the action of T_3 differs in BBM vesicles (BBMV) prepared from superficial cortex (BBMV-SC) and those prepared from juxtamedullary cortex (BBMV-JM). TPTX rats received two i.p. injections of either 2.6 µmoles T_3/kg b.wt., or the vehicle. The Na^+-dependent Pi uptake (pmol/30s/mg prot.) increased only in BBMV-JM, the rate of amiloride-sensitive Na^+-H^+ exchange (pmol/15s/mg prot.) increased only in BBMV-SC; the $^{22}Na^+$ uptake (nmol/30s/mg prot.) was not changed.

		Pi-uptake	$Na^+ \leftrightarrow H^+$	$^{22}Na^+$ uptake
BBMV-SC:	Cont.	2436 ± 119	167 ± 19	53 ± 2
	+ T_3	2571 ± 33	240 ± 7†	52 ± 3
BBMV-JM:	Cont.	933 ± 41	58 ± 12	32 ± 1
	+ T_3	1465 ± 78†	54 ± 5	33 ± 4

†Significantly (t-test) different from controls.

T_3 had no effect on uptake of L-proline either in BBMV-SC or BBMV-JM. We conclude that T_3 stimulates selectively Na^+-dependent uptake of Pi only in BBM of juxtamedullary nephrons, and in contrast, the Na^+-H^+ antiport only in BBM of superficial nephrons. We suggest that T_3 may regulate separately the acidification and Pi reabsorption, respectively, in different populations and/or different segments of proximal tubules.

DIFFERENTIAL TIME COURSE OF SITS EFFECTS IN THE RABBIT SUPERFICIAL PROXIMAL STRAIGHT TUBULE. Bruce A. Biagi, * and Barbara V. Brown. The Ohio State University, Dept. of Physiology, Columbus, OH.

Conventional microelectrodes were used to measure the basolateral membrane potential (Vbl) in superficial proximal straight tubules perfused in vitro. The time course of the effects of 0.1mM SITS (4-acetamido-4'- isothiocyanostilbene-2,2'disulfonate) added to the bathing solution on Vbl, and on the transient depolarization of Vbl in response to lowering HCO3 from 22 to 6.6mM at constant pH (L-HCO3), and on the depolarizations resulting from total sodium replacement with N-methyl-d-glucamine (0-Na), and increasing bath potassium from 5 to 16.7mM (HK) were examined. The control and steady state responses 15 min. after SITS were:

	Control	SITS
Vbl (mV)	-42±2.1(13)	-79±1.4(13)
L-HCO3 (ΔmV)	21±2.5(5)	0±0.0(4)
0-Na (ΔmV)	35±0.7(4)	8±0.8(4)
HK (ΔmV)	3±0.9(4)	26±1.3(4)

The times required to reach 50% of the observed responses were 4.0±0.60(13), 0.36± 0.045(4), 6.1±1.11(4), and 4.8±1.68 (4) min., respectively. The time for inhibition of the L-HCO3 transient was significantly different ($P<.05$) from that observed for the other responses. It is concluded that SITS blockage of the HCO3 exit pathway(s) is a primary event and that changes in Vbl and the Vbl responses to 0-Na and HK may represent secondary events associated with changes in intracellular ion actvities.

EFFECT OF PTH ON THE RENAL HANDLING OF HYDROGEN IONS IN TPTx SPRAGUE DAWLEY RATS. M. Bichara*, O. Mercier*, M. Paillard, A. Prigent*, and F. Leviel*. Laboratoire de Physiologie Rénale, Colombes, Hôpital Louis Mourier and Inserm U 13, Paris.France.

The effect of PTH on the renal handling of hydrogen ions is not established, since either decrease, increase or no change in urinary net acid excretion has been reported. First, we studied the distal hydrogen secretion rate (DHSR) by observing urinary minus blood PCO_2 over urinary HCO_3 ratio taken as DHSR index (mmHg/mM) in maximally alkaline urine (pH>7.8) after 0.9 M $NaHCO_3$ infusion. In 6 rats (G1), infusion of bPTH (2.3 u/hr.100 g Bwt) decreased urinary pH (7.9 vs 8.0, p<0.02), enhanced DHSR index (0.27±0.02 vs 0.21±0.02, p<0.01) and urinary phosphate (782±48 vs 309±42 nmol/min, p<0.01). In 6 rats with constant urinary phosphate (937±80 vs 1110±99,NS) obtained by infusion of phosphate before PTH administration (G2), PTH infusion did not change DHSR index (0.17±0.02 vs 0.21±0.02,NS) or urinary pH. In 6 rats with constant PTH activity obtained by PTH infusion throughout the experiment (G3), the infusion of neutral phosphate (2.5 µmol/min), decreased urinary pH (7.8 vs 7.9, p<0.01), enhanced DHSR index (0.31±0.02 vs 0.18±0.01, p<0.01) and urinary phosphate (2068± 57 vs 874±70, p<0.01). Second, we studied the proximal hydrogen ion secretion rate by performing end proximal micropunctures in another series of 6 TPTx rats, during plasma repletion (PR)(39 tubules), then during PTH infusion (Recollections) ; tCO_2 was measured by microcalorimetry. Results (means±SEM) are shown in the table.

	SNGFR	End Prox $[tCO_2]$	Absolute end-Prox delivery of tCO_2	Fractional reabsorpt. of tCO_2
	nl/min	mmol/l	pmol/min	
PR	36.7±2.2	7.5±0.6	154±20	0.86±0.01
PR+PTH	39.3±1.7	10.7±0.6	273±24	0.75±0.02
p	NS	<0.01	<0.01	<0.01

We conclude that 1) PTH per se inhibits proximal reabsorption of HCO_3; 2) PTH enhances distal hydrogen secretion rate, via the augmentation of urinary phosphate; 3) the final effect of the presence of PTH on urinary net acid excretion depends on the balance between the magnitude of the two opposite effects.

VIP IS A FIRST MESSENGER FOR cAMP-DEPENDENT, ELECTROGENIC LUMINAL ALKALINIZATION IN TURTLE BLADDERS. William A. Brodsky and John H. Durham* Mt. Sinai Sch.Med., Dept. of Physiology, N.Y.,N.Y.

We have found in urinary bladders from post-prandial turtles, a cAMP-dependent, electrogenic luminal alkalinization process as well as a cAMP-independent acidification process. Acidification is suppressed while alkalinization is enhanced in bladders from alkalotic turtles; and vice-versa, in bladders from acidotic turtles. Initiation of luminal alkalinization, determined by pH stat (J_{alk}) and short circuiting current (Isc) occurs after serosal addition of IBMX or cAMP (Satake et al, 1983); or after luminal addition of A-23187 Ehrenspeck, 1984). Suspecting that a G.I. hormone could be the first messenger, it was found (in 20 alkalotic bladders) that nanomolar levels of VIP in the serosal fluid reproducibly activate a luminal alkalinization process; and that this effect is maximal in the presence of sub-effective levels of IBMX. With no luminal HCO_3 (pH statting), VIP increased J_{alk} from 40 to 56 µa and the concomitant Isc, from 13 to 40 µa, on the average. With identical (HCO_3 + CO_2)-containing, NaCl-free Ringer on both surfaces, VIP increases Isc from 11 to 52 µa, on the average. VIP fails to induce any further increase in the cAMP-stimulated luminal alkalinization. Data from microelectrode impalements (Durham and Nagel, 1984) show that these effects do not occur in the granular cells of this tissue. These effects suggest that VIP is an up-regulator of alkali secretion and could be the first messenger which triggers the post-prandial alkaline urinary tide.

PERITUBULAR BICARBONATE CONCENTRATION BUT NOT PCO_2 REGULATES ACIDIFICATION IN THE CORTICAL COLLECTING TUBULE (CCT). **M.D. Breyer*, J.P. Kokko, and H.R. Jacobson, Univ. TX Hlth. Sci. Ctr., Southwestern Med. Sch., Dallas, TX.**

Bicarbonate (HCO_3) transport in the isolated perfused CCT responds to chronic in vivo changes in systemic acid-base balance. The acute response of HCO_3 transport in CCT to changes in ambient [HCO_3] or PCO_2 is unknown. In CCTs from normal rabbits perfused in vitro with 25 mM HCO_3-containing solution, we measured net HCO_3 flux by microcalorimetry under 4 circumstances: 1) elevation in peritubular [HCO_3] from 25 to 50 mM (metabolic alkalosis, pH 7.6); 2) depression in peritubular [HCO_3] from 25 to 5 mM (metabolic acidosis, pH 7.0); 3) elevation in ambient PCO_2 from 40 mmHg to 80 mmHg (respiratory acidosis, pH 7.05); 4) reduction in ambient PCO_2 from 40 mmHg to 14 mmHg (pH 7.8) or 8 mmHg (pH 8.2).

HCO_3 fluxes in pmol/mm/min are listed.

	Control	Exp	p
Met Acid	2.8±1.3	5.1±1.2	<0.02
Met Alk	-0.7±2.4	-4.5±1.4	<0.05
Resp Acid	1.4±0.7	2.2±1.5	>0.40
Resp Alk (PCO_2 14)	3.1±1.2	3.3±1.3	>0.50
Resp Alk (PCO_2 8)	3.9±0.7	0.3±0.2	<0.03

We conclude: 1) acute changes in ambient PCO_2 within the physiologic range do not affect HCO_3 transport in CCT; 2) peritubular [HCO_3] regulates acidification in CCT by increasing net HCO_3 absorption (during low peritubular HCO_3 and pH) or net HCO_3 secretion (during high peritubular HCO_3 and pH). Whether this regulation is due primarily to changes in a H^+ secretory or a HCO_3 secretory process is being examined.

SURFACE NEPHRON (SN) HANDLING OF ACID DURING ACUTE RESPIRATORY ACIDOSIS (ARA). J. Buerkert, D. Martin,* and D. Trigg.* Renal Div., Jewish Hosp. and Wash. Univ., St. Louis, MO.

How ARA affects acid handling by SN was assessed in 15 Munich-Wistar rats. Before (period 1) and after (period 2) ventilation with 8% CO_2, in situ pH and the tubule fluid content (TF) of bicarbonate (HCO_3-) and (NH_4^+) were measured at the end of the proximal (EPT) and along the distal tubule (DT) of the same nephron. ARA decreased urine pH and increased NH_4^+ excretion from 0.6±0.1 to 1.1±0.1 µeq/min/g kidney wt (KW). During ARA in situ pH fell from 6.89±0.02 to 6.81±0.03 (p<.05) and TF[NH_4^+] increased from 3.3±0.3 to 4.3±0.3 mM (p<.01) at the EPT. ARA increased (p<.025) EPT delivery of NH_4^+ from 43±6 to 58±6 peq/min/g KW. Proximal HCO_3- reabsorption increased from 73±3% to 80±2% of that filtered. Distal delivery of NH_4^+ increased from 9±1 to 22±4 peq/min/g KW (p<0.001) but DT in situ pH and bicarbonate reabsorption were unchanged by ARA. In both periods DT NH_4^+ delivery was less than to the EPT (p<.001). In period 1 loss in the loop segment (LS) was 39±7 peq/min/g KW and was not different in period 2 (36±5 peq/min/g KW). Thus, 79±4% of the NH_4^+ delivered to the loop was lost before, in contrast to only 59±4% after the induction of ARA (p<.001).

We conclude: ARA enhances acid formation in the PT but decreases NH_4^+ loss from the LS. This latter finding may be due to a decreased pH in the LS of SN as has been reported in that of deep nephrons (KI 25: 272, 1984).

BICARBONATE REABSORPTION IN THE RAT DISTAL TUBULE. EFFECT OF LOW POTASSIUM DIET. G. Capasso*, V. Guckian*, G. Malnic*, R. Kinne and G. Giebisch. Dept. Physiol., Yale Univ., New Haven, CT.

There is controversy about the quantitative importance of H^+ secretion in the distal tubule (DT) of the rat. Measurements of luminal pH have shown significant acidification, while measurement of total CO_2 reabsorption by microcalorimetry in microperfused DT in vivo have not detected bicarbonate (HCO_3^-) reabsorption. In the present study we investigated this issue by measuring the fractional HCO_3^- reabsorption in free flow conditions using standard micropuncture techniques. The $[HCO_3^-]$ was estimated by measuring total CO_2 with microcalorimetry. Two groups of rats were studied: Group I, control (n=11, blood pH 7.42±0.04, plasma K 4.8±0.2 mEq/l and HCO_3^- 27.1±1.1 mM, GFR 1.13±0.30 ml/min/kidney, fractional HCO_3^- excretion 0.985±0.320); and Group II, rats fed a low K diet for 3 weeks (n=6, blood pH 7.52±0.03, plasma K 2.5±0.2 and HCO_3^- 36±3 mM, GFR 1.00±0.22 ml/min/kidney, fractional HCO_3^- excretion 0.405±0.200). In Group I the fractional HCO_3^- delivery (TF/P) HCO_3^-/Inulin x 100 (mean±SD) to the early DT (8.9±3.3%) was significantly higher ($p<0.01$) than to the late DT (4.2±2.8%). In Group II the (TF/P) HCO_3^-/Inulin x 100 was 4.8±1.9% to the early DT and 1.2±0.6% to the late DT ($p<0.001$). These values were significantly lower ($p<0.01$) than the corresponding values in Group I. The data indicate that cortical distal segments reabsorb HCO_3^-. Furthermore, they demonstrate that chronic depletion of K maintains HCO_3^- reabsorption of the rat distal tubule, even in the presence of steeper $[HCO_3^-]$ gradients.

PHYSIOLOGIC REGULATION OF PROXIMAL $NaHCO_3$ AND NaCl REABSORPTION BY RENAL NERVE ACTIVITY. Martin G. Cogan. CVRI and Dept. of Med., Univ. of Calif., San Francisco, CA.

While renal nerve activity is known to alter proximal reabsorption, it is unclear whether absolute proximal reabsorption (APR) of $NaHCO_3$ or of NaCl or both are affected. 10 Sprague-Dawley rats were studied using free-flow micropuncture techniques during euvolemia and following acute denervation. APR of HCO_3^- fell following denervation (933 ± 40 to 817 ± 30 pmol/min, $p<0.005$) as did APR of Cl^- (1643 ± 116 to 1341 ± 129 peq/min, $p<0.025$), accounting for decreased APR of water (18.8 ± 0.9 to 15.7 ± 0.9 nl/min, $p<0.001$). Such parallel changes in APR of HCO_3^- and Cl are unique compared to previously studied determinants: peritubular protein (selectively changes APR of NaCl) and peritubular bicarbonate concentration (selectively changes APR of $NaHCO_3$). Denervation also caused significant, ≥2-fold increases in urinary Na, K, HCO_3^-, Cl, and H_2O excretion. GFR and SNGFR were stable. To further assess the physiological significance of changes in proximal transport induced by alteration in renal nerve activity, 6 other rats were subjected to acute unilateral nephrectomy (AUN). AUN induces a contralateral natriuresis (reno-renal reflex), known to be mediated by inhibition of efferent renal nerve activity. AUN caused significant changes in proximal transport in the same pattern as had denervation: reduction in APR of HCO_3^- (975 ± 45 to 889 ± 48 pmol/min), of Cl (1471 ± 122 to 1234 ± 112 peq/min), and of H_2O (18.4 ± 0.9 to 15.8 ± 1.0 nl/min), associated with the expected increase in urinary solute excretion. SNGFR and GFR were stable. In conclusion, a depression in renal nerve activity inhibits proximal reabsorption of both $NaHCO_3$ and NaCl, which may explain, at least in part, the observed whole kidney response.

OPTICAL MEASUREMENTS OF INTRACELLULAR pH IN SINGLE PK_1 CELLS: EVIDENCE FOR Cl-HCO_3 EXCHANGE. **J. Richard Chaillet*, Kurt Amsler*, and Walter F. Boron. Depts. of Physiology and Human Genetics, Yale Univ., School of Medicine, New Haven, CT.**

PK_1 cells, an epithelial line derived from pig kidney, were loaded with the pH-sensitive dye 4',5'-dimethyl-5(and -6)-carboxyfluorescein. A single cell attached to a cover slip was illuminated by a 10-μm diameter spot of white light. The transmitted light was projected on a diffraction grating and the resulting spectrum focused on a photodiode array. We calculated intracellular pH (pH_i) from absorbance spectra of intracellular dye, obtained at intervals of ~3 sec. The experiments were performed on both rapidly growing and quiescent cells. The results were qualitatively the same for both groups of cells. When cells were incubated in CO_2/HCO_3^- Ringer at pH 7.4, replacing all extracellular Cl^- with glucuronate caused pH_i, initially ~7.0, to reversibly increase by ~ 0.3. These pH_i changes induced by altering $[Cl^-]_o$ were greatly attenuated by 50 μM DIDS or by HCO_3^--free (2 mM PIPES) solutions. Lowering pH_o from 7.4 to 6.7 (achieved by reducing $[HCO_3^-]_o$ from 25 to 5 mM at constant pCO_2) caused pH_i to reversibly decrease by ~0.2. The magnitude of these pH_i changes induced by altering pH_o was greatly reduced by DIDS, by HCO_3^--free solutions, and by Cl-free solutions. These results are consistent with the existence of a Cl-HCO_3 exchanger in PK_1 cells. Because the pH_i changes induced by altering either $[Cl^-]_o$ or $[HCO_3^-]_o$ were not substantially affected by removal of Na^+, it is unlikely that the transport system is Na linked.

IMPORTANCE OF THE KIDNEY IN THE CORRECTION OF CHLORIDE DEPLETION ALKALOSIS (CDA) BY CHLORIDE. D. M. Craig*, J. H. Galla, D. N. Bonduris*, R. G. Luke (intr. by D. Tharpe). Nephrology Research and Training Center, University of Alabama in Birmingham.

CDA produced by peritoneal dialysis (PD) against 0.15 M $NaHCO_3$ can be corrected by infusion of isotonic fluid with Cl^- 80 meq/L without plasma volume expansion (JCI 73:96, 1984). To determine the extent to which this correction is dependent upon renal as distinct from extrarenal mechanisms, Gps 2, 2A and 3 were functionally nephrectomized (BNX) by renal pedicle ligatures prior to PD. After PD, rats were infused with isotonic solutions for 3 h; anions: Gp1 (Sham) and 2 - Cl 70, acetate 40, HCO_3 40; Gp2A - Cl 140, HCO_3 10; Gp3 - PO_4 70, acetate 40, HCO_3 40 meq/L at 0.5 ml/h/100 g BW in Gp1. The infusion rate was halved in 2,2A and 3 to compensate for anuria; infusate Cl was doubled in Gp2A to match the Cl input in Gp1. Plasma (P) Cl (81±1) and P_{HCO_3} (39±1 meq/L) after PD did not differ among the groups.

Gp	Hct (%)	P Protein (mg/dl)	Δ P_{Cl} (meq/L)	Δ P_{HCO_3} (meq/L)	P_{K^+} (meq)
1	2.5±1.8	5.3±0.1	6.1±1.9§	-6.0±1.5§	3.1±0.1
2	1.9±0.8	5.3±0.2	0.4±1.2	-2.0±0.6§	4.8±0.2
2A	-1.2±0.4§	4.8±0.1	0.6±0.5	0.8±0.8	4.0±0.2
3	2.9±1.0§	5.3±0.1	-0.9±0.9	-1.7±0.5§	4.8±0.2
p†	<0.01	NS	<0.01	<0.01	<0.01

§ $p<0.05$ within group paired t; † ANOVA

The reciprocal changes in plasma Cl^- and HCO_3^- over 3 hrs occurred only in Gp1; the small decreases in plasma HCO_3^- in Gp2 and 3 are attributed to BNX. We conclude that the acute correction of CDA by Cl in non-volume expanded rats occurs by renal mechanisms.

AN ELECTROGENIC PROTON-TRANSLOCATING ATPase (H^+-ATPase) IN HUMAN INNER MEDULLARY MEMBRANE VESICLES. F.D. Díaz Díaz,* E.F. LaBelle,* D.C. Eaton,* and T.D. DuBose, Jr. The University of Texas Medical Branch, Galveston, Texas.

An electrogenic H^+-ATPase was described recently in vesicles from bovine renal medulla (JCI 73:1706). The purpose of the present study was to determine if a similar H^+-ATPase is present in human kidney. Inner medullary membrane vesicles were prepared by step ultracentrifugation from human cadaver kidneys unsuitable for transplantation. Intravesicular acidification was assessed by ATP-generated acridine orange fluorescence quenching (excitation 490 nm and emission 530 nm). H^+ ion accumulation was rapidly reversed by addition of the protonophore 1799 (2.5×10^{-5}M). However, proton transport was not affected by orthovanadate (5×10^{-4}M), ouabain (5×10^{-5}M), or low Na^+-media (3 mM). Similarily, amiloride (5×10^{-4}M), oligomycin (5 µg/ml), and K^+-free media did not affect H^+ accumulation. Moreover, H^+ transport was inhibited by N-ethylmaleimide (10^{-3}M). Substrate specificity of the pump was demonstrated for ATP, not GTP. These characteristics are similar to those described for bovine renal medulla and turtle urinary bladder but not ($Na^+ + K^+$) ATPase, mitochondrial F_0-F_1 ATPase, lysosomal H^+-ATPase or gastric ($K^+ + H^+$)ATPase. In addition, an increase in the rate and extent of H^+ accumulation after addition of valinomycin (3×10^{-6}M) in the presence of KCl suggests that the pump is electrogenic. Under control conditions H^+ transport was balanced by the parallel conductance of chloride since substitution of chloride by gluconate or methanesulfonate decreased H^+ accumulation. In summary, these findings demonstrate for the first time, that H^+-ATPase activity is present in human renal medulla and may play an important role in urinary acidification.

CO_2 STIMULATION OF H+ SECRETION BY TURTLE BLADDER (TB): ROLE OF EXOCYTOSIS, INTRACELLULAR pH (IpH) AND CALCIUM. G. Dytko* and J.A.L. Arruda, UAMS and VAMC, Univ. Ark., Little Rock.

CO_2 stimulates H+ secretion in the TB either by inducing exocytosis or by decreasing IpH. We measured exocytosis and monitored IpH continuously with the pH probe 6-carboxyfluorescein in the turtle bladder during stimulation of TB with 1% or 5% CO_2. Exocytosis was measured by the mucosal release of fluorescein dextran incorporated into TB. One percent CO_2 resulted in a significant increase in H+ current at 3 min of 6.0±0.5 µA and this was associated with an increase in exocytosis and a decrease in IpH. The decrease in IpH and the increase in exocytosis preceded the increase in H+ current and there was an excellent correlation between the increase in exocytosis and the increase in the H+ secretion. The initial increase in H+ secretion and exocytosis by 5% CO_2 was of the same magnitude as that observed with 1% CO_2 despite the fact that the decrease in IpH was greater (0.32 vs 0.13). This suggests that an initial decrease in IpH is associated with the increase in H+ secretion and exocytosis but the magnitude of decrease in IpH does not influence the early increase in H+ secretion. Lanthanum (an agent that displaces membrane bound Ca) and trifluoperazine (an inhibitor of calmodulin) inhibited exocytosis and partially inhibited the increase in H+ secretion induced by CO_2. These agents failed to inhibit the decrease in IpH suggesting that this decrease can stimulate the H+ pumps already inserted into the apical membrane. Thus the stimulation of H+ secretion by CO_2 is the result of an interaction of IpH, exocytosis and cell Ca.

ACETAZOLAMIDE (AZ) INHIBITS EXOCYTOSIS IN THE TURTLE URINARY BLADDER (TUB). T.E. Dixon, C. Clausen*, and D. Coachman. Dept. of Med. Northport VAMC, & Dept. Physiol. SUNY Stony Brook, N.Y.

Proton transport (JH) in TUB can be regulated by exocytotic fusion (EX) of proton pump containing vesicles (V) with the apical membrane, or by endocytotic (EN) removal of these V, causing changes in the surface area. Using impedance analysis to follow apical capacitance, (CA), as a marker of surface area, we find in 5 bladders bathed in CO_2 free solution that 50µm AZ causes CA to decline .24±.07 uF/cm2 from its baseline value of 3.16±.51 ($p<.05$). To determine whether AZ affects EX we incubated bladders with fluorescent dextran (FD), which is taken up by V, and then measured the rate of FD release into the apical medium. In 7 hemibladders stimulation of JH by changing to a CO_2/HCO_3 solution was associated with a 48±18% increase in the fluorescent signal, whereas pairmates pretreated with .5m AZ, had blunted stimulation of JH and the increase in fluorescent signal was only 13 ±7%. Using the uptake of FD as a marker of EN in 6 bladders bathed in CO_2 free solution, no difference between controls and hemibladders treated with .5 mM AZ was found during the first 15 minutes of AZ treatment (1.8±.25 vs 1.79±.17 nl/mg protein/min., despite a 64% decline in JH in the AZ group. These studies show that one mechanism by which AZ can effect JH is by inhibition of EX.

INTERACTION BETWEEN AMMONIUM AND BICARBONATE FLUX. G.M. Feldman, R. Cohen* R. Stephenson* VAMC, Dept. of Med., Univ. of Pa. Sch. of Med., Phila., Pa.

Renal total ammonia (TNH_3) transport occurs at nephron sites that transport H^+ and/or HCO_3^-. Since TNH_3 can exist in two forms, nonionic NH_3 and ionic NH_4+, the flux of TNH_3 ($JTNH_3$) may be influenced by epithelial acid-base transport. We examined these interactions in a tissue which normally secretes HCO_3^- and absorbs TNH_3, the rat distal colon perfused in vivo. $JTNH_3$ varied directly with luminal [NH_3], and the ratio of permeabilities (NH_3/NH_4+) was >50:1. To acidify luminal fluid, CO_2 entry into the lumen was induced by buffering perfusate with HEPES rather than HCO_3^-/CO_2. At perfusate pH 7.6, luminal acidification reduced $JTNH_3$ from 17.3 ±0.9 to 8.8±1.0 µmole/min/g, $p<0.001$. To alkalinize luminal fluid, HCO_3^- secretion was permitted by using perfusate containing Cl^- rather than $SO_4^=$. At perfusate pH 6.1, luminal alkalinization increased $JTNH_3$ from 3.6±1.0 to 9.2±0.5 µmole/min/g, $p<0.001$. The limiting influence of HCO_3^- secretion (luminal alkalinization) on $JTNH_3$ was demonstrated by the reduction of $JTNH_3/[TNH_3]$ from 0.58±0.07 to 0.28±0.04 cm^3/min/g ($p<0.005$) as perfusate [TNH_3] was increased from 5 to 60mM. The presence of luminal TNH_3 altered HCO_3^- flux in an absorptive direction: the increment in HCO_3^- absorption was equal to $JTNH_3$. CONCLUSIONS: The observations that the colon is more permeable to NH_3 than NH_4+ and that luminal TNH_3 causes HCO_3^- absorption at a rate equal to $JTNH_3$ are best described by coupled nonionic diffusion of NH_3 and CO_2. Furthermore, $JTNH_3$ is strongly influenced by epithelial acid-base flux, and ongoing epithelial acid-base flux can lead to nonlinear $JTNH_3$ kinetics.

THE ENHANCED NA-H ANTIPORT ACTIVITY OF THE RENAL BRUSH BORDER MEMBRANE (BBM) INDUCED BY REDUCTION OF RENAL MASS IS DUE TO AN INCREASED TURNOVER OF INDIVIDUAL ANTIPORTERS. C Frelin*, LG Fine, P Vigne* and M Lazdunski*, Centre de Biochimie, Universite de Nice, France, and Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

We have previously reported that the increase in bbm Na-H antiport activity which follows subtotal nephrectomy is a Vmax effect (Abst Am Soc Nephrol 16:108A, 1983). This could be due to insertion of additional antiporters into the bbm or to an increase rate of turnover of a fixed number of antiporters. To distinguish between the two possibilities, studies were performed on bbm vesicles of normal rabbits and 15-20 day uninephrectomized (UniNx) rabbits. In UniNx the initial rate of amiloride-sensitive uptake of ^{22}Na (pH_{in}=5.5 to 7.0; pH_{out}=8.5) was increased 1.5 to 2 fold compared with normals. Bbm were solubilized and the specific binding of [^{3}H]-ethylpropylamiloride (EPA) to the Na-H antiporter was assayed. EPA binding is closely associated with the Na-H antiporter as evidenced by the comparable order of potency of amiloride analogs in competitions experiments. In normal kidneys Scatchard analysis revealed a single family of binding sites with a Kd of 30 nM and a maximum binding capacity of 1.5 pmoles/mg protein. An identical Scatchard plot was obtained on UniNx kidneys. Alkaline phosphatase activity was also comparable in the two preparations, indicating comparable solubilization characteristics.

Conclusion: Since no increase in EPA binding was detected, uninephrectomy presumably increases the Vmax of the Na-H antiporter by increasing the turnover of the carrier.

EFFECTS OF AMILORIDE (AM) ON HCO_3 REABSORPTION BY THE RAT KIDNEY. J.P. Frommer, and D.E. Wesson*, (intr. by J. C. Ayus), V.A. Med. Ctr., Baylor Coll. of Med., Houston, Tx.

Micropuncture studies have shown that AM inhibits distal acidification when distal HCO_3 delivery is increased by the administration of acetazolamide. The present experiments examine the effects of AM on HCO_3 reabsorption along the nephron of the intact animal. Cortical and papillary micropuncture was used to obtain tubular fluid samples from the superficial late proximal (LP) and early distal (ED) tubule and the base (B) and tip (T) of the papillary collecting duct of 18 Munich-Wistar rats. Total CO_2 (tCO_2) was determined using microcalorimetry and fractional excretion of HCO_3 ($FEHCO_3$) from the urine of the contralateral kidney. Two groups of animals were studied: A) Controls (N=5); and B) AM (2.5 mg/kg bw/hour i.v.) (N=13). Results expressed as fractional delivery of tCO_2 to the micropuncture sites (%) are depicted in the table (mean ± SEM):

	LP	ED	B	T
Control	18.8±3.9	5.0±1.0		
AM	17.9±1.9	8.9±1.2*	0.7±0.2	0.2±0.1†

* = P<005 vs control; † = p<0.05 B vs T

$FEHCO_3$ Control was 0 and AM was 0.6±0.1% (p<0.05)

Our results show that: 1) AM inhibits acidification between the LP and ED micropuncture sites, 2) there is significant tCO_2 reabsorption by the the papillary collecting duct of AM-treated rats, and 3) AM significantly increases $FEHCO_3$. We conclude that the systemic administration of AM at these doses inhibits acidification in the loop of Henle in addition to its well known action in the cortical collecting duct and 2) active tCO_2 reabsorption by the papillary collecting duct still occurs in the presence of amiloride.

BICARBONATE SECRETION BY THE DISTAL NEPHRON OF THE RAT KIDNEY DURING CHRONIC METABOLIC ALKALOSIS (CMA). J.P. Frommer and D.E. Wesson*, V.A. Med. Ctr., Baylor Coll. of Medicine, Houston, Tx.

Studies in the isolated collecting duct of the rat and rabbit have shown that these tubular segments have the capacity to secrete HCO_3. Previous studies from our laboratory failed to demonstrate significant distal HCO_3 secretion during acute metabolic alkalosis (AMA) in the rat. The present studies examine the possibility of HCO_3 secretion by the distal nephron in 14 Munich-Wistar rats using cortical and papillary micropuncture during CMA. Tubular fluid samples were obtained from the superficial late distal tubule (LD). Fractional excretion of HCO_3 ($FEHCO_3$) was determined from the urine of the intact contralateral kidney. Total CO_2 (tCO_2) was determined using microcalorimetry. The rats were prefed 77 mM Na HCO_3 in the drinking water for 1-2 days prior to the experiment and then loaded acutely with hypertonic HCO_3. All rats were administered 20 mg of i.v. bromethylamine hydrobromide (BEA) 18-24 hr prior to the experiment in order to abolish juxtamedullary nephron contribution, since previous studies have established a significant role for juxtamedullary nephrons in the disposal of acute HCO_3 loads. Our results show that the fractional delivery of tCO_2(%) to LD was 30.1 ± 2.5 and $FEHCO_3$ was 39.1 ± 2.5% (p< 0.02 vs LD). Thus, there is net addition of HCO_3 between LD and the final urine. Previous experiments during AMA (in which the rats had not been prefed $NaHCO_3$)revealed that every rat reabsorbed HCO_3 between LD and urine after BEA. We conclude that the net addition of HCO_3 observed in the present studies after abolishing juxtamedullary nephron contribution with BEA, is due to HCO_3 secretion by the distal nephron at a site between LD and the final urine.

MEDULLARY BICARBONATE GRADIENTS DURING ACUTE METABOLIC ALKALOSIS (AMA) IN THE RAT. J.P. Frommer and D.E. Wesson*, (intr. by J. C. Ayus), V.A.Medical Center, Baylor College of Medicine, Houston, Tx.

Previous studies from our laboratory have demonstrated that, in the rat, juxtamedullary nephrons (JN) deliver a higher fraction of the filtered load of HCO_3 to the final urine than superficial nephrons (SN) during AMA. The present micropuncture studies were performed in order to examine possible driving forces that could account for these findings. AMA was induced by hypertonic HCO_3 loading in 12 Munich-Wistar rats. Standard papillary micropuncture was used to obtain tubular fluid samples from the bend of the loop of Henle (LH). Plasma from adjacent vasa recta (VR) was obtained in a paired fashion. Total CO_2 (tCO_2) was determined by microcalorimetry. VR tCO_2 was corrected for plasma protein and Donnan equilibrium. Results are depicted in the table (mean ± SEM):

	LH	VR corrected	VR uncorrected
tCO_2 (mM)	36.7±3.8	43.1±4.3*	39.4±4.0

* = p< 0.05 vs LH

Our results suggest that during AMA there is a tCO_2 gradient between the interstitium and tubular fluid of LH favoring net entry of bicarbonate into the tubular lumen. Since JN are known to have long thin limbs of loops of Henle, the presence of a bicarbonate secretory gradient could account for a significant net bicarbonate entry into these nephrons. This may be a mechanism by which JN deliver a higher fraction of the filtered load to the final urine than superficial nephrons during AMA in the rat.

UNIDIRECTIONAL BICARBONATE FLUXES IN CORTICAL COLLECTING DUCTS (CCD) FROM DEOXYCORTICOSTERONE (DOC)-TREATED RABBITS. J. Garcia-Austt,* D.W. Good,* M.B. Burg, and M.A. Knepper. NHLBI, NIH, Bethesda, MD.

Previously, we showed that chronic DOC administration to rabbits stimulates their CCD, when perfused in vitro, to secrete bicarbonate at high rates. Oral NH_4Cl loading of the rabbits (50 mEq/L in drinking water) or removal of luminal chloride during perfusion in vitro completely inhibited the net bicarbonate secretion. Net bicarbonate transport in CCD is the algebraic sum of two processes, namely bicarbonate secretion and proton secretion (bicarbonate absorption). To determine which process changed to inhibit net bicarbonate secretion, unidirectional bicarbonate fluxes were measured. Net bicarbonate transport (pmol/min/mm) was measured with 25 mEq/L bicarbonate in bath and perfusate, and the unidirectional fluxes by removing bicarbonate isohydrically from either the bath (L-to-B or absorptive flux) or perfusate (B-to-L or secretory flux).

	Net	B-to-L	L-to-B
DOC	-20.4	-30.6	+3.6
DOC + NH_4Cl loading	+1.6*	-4.6*	+6.1*

*P<0.05 vs. DOC alone, unpaired t-test.
+ = absorption; - = secretion

In CCD from rabbits treated with DOC (but not NH_4Cl), replacement of luminal chloride by sulfate inhibited the secretory flux (from -30.6 to -11.4) with no significant change in the absorptive flux (+3.6 to +3.0). Thus, acid loading of the animals or chloride removal from the lumen greatly inhibited direct bicarbonate secretion, but had little or no effect on proton secretion.

AMMONIA AND BICARBONATE TRANSPORT BY RAT CORTICAL COLLECTING DUCTS PERFUSED IN VITRO. D.W. Good,* M.B. Burg, and M.A. Knepper. NHLBI, NIH, Bethesda, MD.

Little direct information is available about acid-base transport in the rat cortical collecting duct (CCD) because it is inaccessible to micropuncture. Consequently, we perfused CCD from deoxycorticosterone(DOC)-treated rats in vitro and measured total CO_2 and total ammonia transport. Perfusate and bath were identical. With 25 mM bicarbonate and no ammonia in the solutions, there was net bicarbonate secretion (6.4 ± 1.4 pmol/min/mm). Acid loading of the DOC-treated rats (40 mM NH_4Cl drinking water) abolished the bicarbonate secretion. With 25 mM bicarbonate and 4 mM total ammonia in perfusate and bath, CCD from rats treated with DOC (but not acid loaded) absorbed total CO_2 (5.1 ± 2.4) and secreted total ammonia (3.2 ± 0.5). The collected total ammonia concentration (5.4 mM) exceeded the value predicted from the bath and collected bicarbonate concentrations based on the diffusion trapping model. Luminal carbonic anhydrase reversibly decreased the collected total ammonia concentration to the predicted value.

Conclusions: 1) CCD from DOC-treated rats can secrete bicarbonate at substantial rates. 2) CCD bicarbonate secretion may be an important response to metabolic alkalosis. 3) Addition of 4 mM NH_4Cl to perfusate and bath prevents bicarbonate secretion, possibly by altering cell pH. 4) CCD from DOC-treated rats secrete ammonia by luminal acidification and nonionic diffusion. 5) The ammonia secretion is in part dependent on an acid pH disequilibrium in the lumen.

ISOLATION OF SOLUBILIZED RENAL H+ATPASE BY HIGH PERFORMANCE ION EXCHANGE CHROMATOGRAPHY. Stephen Gluck and Jerry Caldwell*. Univ. of Chicago Depts. of Medicine, and Pharmacol. and Physiol., Chicago, IL.

Bovine renal medulla membranes (RMM) contain a proton pump which is resistant to oligomycin (OLI), but sensitive to N-ethyl maleimide (NEM), disulfonic stilbenes (SITS), and Zn^{++}. We report the solubilization and initial chromatographic isolation of this enzyme.

RMM were solubilized with buffer containing the zwitterionic detergent CHAPS and centrifuged at 150,000 x g. The clear supernatant had NEM-sensitive ATPase activity (NSA), and mixing the supernatant with sonicated soybean azolectin followed by detergent removal by gel filtration yielded ATP-induced proton transport, measured by the uptake of acridine orange, that was resistant to OLI and inhibited by NEM. Supernatant was loaded onto an HPLC anion exchange column, eluted with a KCl gradient in buffer containing CHAPS, and fractions were assayed for NSA. Four main peaks of NSA were found with up to 90% NSA recovery. Peak 1 was broad and NSA in it was inhibited by Zn^{++} and SITS. Peaks 2 and 3 were sharper and not SITS sensitive. Peak 4 was unstable.

The results show that NSA of renal membranes is likely derived from at least 4 different proteins. Of these, the NSA in peak 1 may represent the H^+ ATPase.

CL-HCO_3 EXCHANGE IN RABBIT RENAL CORTICAL BASOLATERAL MEMBRANE VESICLES (BLMV). Steven M. Grassl*, Lawrence P. Karniski*, and Peter S. Aronson. Yale Sch. Med., Depts. Med. & Physiol., New Haven, CT.

Pathways for Cl transport were evaluated in BLMV isolated from rabbit renal cortex by Percoll gradient centrifugation. Uptake of 4.2 mM ^{36}Cl was assayed by filtration. Inward gradients of K or Na did not stimulate Cl uptake, arguing against K-Cl or Na-Cl cotransport. An inside-positive, valinomycin-induced K diffusion potential stimulated Cl uptake by 3.1 nmoles/mg/min. This conductive component of Cl influx was inhibited 30% by 1 mM DIDS. An inside-alkaline pH gradient (pH 7.5 in, 6.0 out) in the absence of CO_2 stimulated Cl uptake by 5.2 nmoles/mg/min, suggesting modest Cl-OH exchange. The same pH gradient with a CO_2-HCO_3 buffer system stimulated Cl uptake by 30 nmoles/mg/min and induced a 4x overshoot over equilibrium Cl uptake, indicating appreciable Cl-HCO_3 exchange. Stimulation of Cl uptake by an outward HCO_3 gradient was unaffected by the addition of valinomycin with $K_i=K_o$ to shunt any anion diffusion potentials. Cl-HCO_3 exchange was inhibited 50% and 95% by 0.01 and 0.10 mM DIDS, respectively. Cl-HCO_3 exchange was unaffected by the presence of Na. In contrast to these findings in BLMV, there was no evidence for Cl-OH-(HCO_3) exchange in luminal membrane vesicles. These data indicate that renal BLMV contain a DIDS-sensitive transport system that can mediate Cl-HCO_3 exchange. The higher flux rate of Cl-HCO_3 exchange compared to Cl-OH exchange may explain the importance of converting intracellular OH to HCO_3 in order to facilitate base exit from the proximal tubular cell.

SERUM ELECTROLYTE PATTERNS IN END-STAGE RENAL DISEASE. A. Greenberg, R. Wallia*, B. Piraino and J.B. Puschett. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Metabolic acidosis (MA) typically accompanies the development of end-stage renal disease (ESRD). Although some patients (pts) with renal failure from tubulointerstitial disease may have MA with hyperchloremia (HC) and normal (nl) anion gap (Δ), most patients with ESRD are thought to develop the high Δ variety. A review of the electrolytes of 70 consecutive patients beginning dialysis over a 2 year period (1981-83) disclosed the following patterns (mean ± SEM):

Group	% of pts	tCO_2 mEq/L	Cl mEq/L	Δ mEq/L
MA, HC, nl Δ	30	17±3	114±4	13±3
MA, HC, high Δ	16	14±5	110±3	19±2
MA, nl Cl & Δ	14	19±2	102±3	15±1
MA, high Δ	20	15±3	101±5	21±3
no abnormalities	20	25±3	97±4	13±3

Overall, only 36% of patients had a high Δ. Four of the 21 patients with MA, HC and no Δ had ESRD due to glomerulonephritis and/or nephrosclerosis. Furthermore, the distribution of observed causes of ESRD was similar among the 5 groups listed above. In all groups, serum potassium was normal. We conclude that: 1) Several different electrolyte patterns, including normal, may be seen in ESRD; 2) MA with HC and no Δ may occur with glomerular as well as tubulointerstitial disease; and 3) In ESRD, MA with HC is as or more common than MA with high Δ.

H^+/OH^- PERMEABILITY OF PROXIMAL TUBULE BRUSH BORDER VESICLES. Harlan E. Ives. Dept. of Medicine, UCSF, San Francisco, CA 94143.

Proximal tubule brush border vesicles from the rabbit kidney contain a H^+ permeability in parallel with a Na^+/H^+ antiporter. However, the magnitude of this permeability has never been directly determined. The net H^+/OH^- permeability of brush border membranes was ascertained by measuring the rate of collapse of preformed pH gradients with acridine orange in voltage clamped membrane vesicles made either by Mg^{++} aggregation or by sucrose density gradient. Intravesicular buffer capacity was determined by titration with KOH and by titration of measured Na^+/H^+ rates with exogenous buffers. These two independent methods both yielded a buffer capacity of 125-135 mM/pH unit at pH 6.0 and 20°C. Using this buffer capacity, the net H^+/OH^- permeability was found to be 5×10^{-3} cm/sec. Further studies were aimed at characterizing the H^+ permeation pathway. Activation energy of the pathway was 4.9 kcal/mole compared to 11.4 kcal/mole for the Na^+/H^+ antiporter. The pathway was not inhibited by 100 μM amiloride or 2 mM DCCD but was inhibited by 0.2 mM pCMBS. The H^+/OH^- permeability was not increased in vesicles from acidotic rabbits (plasma $[HCO_3^-]$ = 10 mM) in which Na^+/H^+ antiporter activity was increased 2.5 fold. These findings are consistent with the idea that H^+ do not diffuse across the Na^+/H^+ antiporter in the absence of Na^+ but rather may permeate through another ion transport channel or the brush border water channel. The magnitude of the H^+/OH^- permeability of proximal tubule brush border membranes is high compared to other biological membranes. However, assuming that the in vivo H^+ permeability is similar to that determined in vitro, it would not result in a significant backleak of H^+ in relation to net H^+ secretion in the proximal tubule.

PHYSIOLOGIC SIGNIFICANCE OF NEGATIVE URINE (U) - BLOOD (B) pCO_2 GRADIENTS. C Gutterman*, DC Batlle and NA Kurtzman. Univ of Ill, Chicago IL.

The U pCO_2 was consistently lower than blood in animals with either acute or chronic hypercapnia (H) (55±6.9 and 85±0.7 mm Hg, $p<0.001$) and (51±6.2 and 80±0.7 mm Hg respectively, $p<0.001$). When bicarbonate was infused to rats with acute H the U pCO_2 rose to levels significantly greater than that of blood. Carbonic anhydrase (CA) infusion to bicarbonate-loaded animals is known to lower U pCO_2 to a value equal to that of B. This is the result of rapid dehydration of carbonic acid, allowing equilibration across the distal nephron. When bicarbonate was administered with CA the U pCO_2 in H rats was 77±2.2 and B was 86±0.9, $p<0.005$, while normocapnic rats had identical U and B pCO_2. The secretion of H+ into a U free of bicarbonate but rich in non-bicarbonate buffer will regenerate bicarbonate and consume CO_2 in the process. These data suggest H+ secretion consumes CO_2 and lowers U pCO_2 below that of B when the predominant U buffer is not bicarbonate and raises U pCO_2 when bicarbonate is the main buffer. To test this hypothesis, we gave amiloride, an agent which inhibits distal acidification, to rats with chronic H. These animals had a U pCO_2 not significantly different from that of B both before and after bicarbonate administration. Thus, an agent which inhibits distal acidification inhibits the ability to lower U pCO_2 in a bicarbonate-free U and likewise inhibits the ability raise U pCO_2 in a bicarbonate-rich U. These data suggest that H+ secretion both raises and lower U pCO_2 dependent upon the buffer composition of the U and that the final U pCO_2 is the sum of these opposing forces.

PROSTAGLANDINS (PGs) INHIBIT AMMONIAGENESIS (AG) VIA AN INTRACELLULAR EFFECT. E.R. Jones; R. Shay; T.R. Beck; and R.G. Narins. Temple Univ. Health Sci. Ctr., Phila., PA.

To define whether PGs inhibit AG by a direct effect on intracellular metabolism or via a primary action on the cell membrane, we compared the inhibitory effect of added PGs on intact rat cortical slices with that on cortical homogenates. The sensitivity of AG to inhibition by intracellular PGs was compared with that caused by adding either PGE_2 or $F_2\alpha$ to the incubation media. Intracellular PGs synthesis was stimulated with calcium ionophore (CI) (2-20 μmol/ml KHB). Tissue was incubated in Krebs-Henseleit buffer (KHB) with 2mM glutamine at pH 7.4 with or without the PGs synthesis inhibitor meclofenamate (M) for 1 hr. The KHB was assayed for NH_3, PGE_2 and $F_2\alpha$.

AG in slices and homogenates was stimulated by M. The addition of PGE_2 (0.1-10,000ng/ml) or $PGF_2\alpha$ (0.1-500ng/ml) did not alter M-stimulated slice AG except at the highest levels of PGE_2 (10,000 ng/ml). However, M-stimulated AG in homogenates was suppressed by markedly lower levels (30ng/ml) of PGE_2 and $F_2\alpha$. Although added PGE_2 or $F_2\alpha$ had no effect on unstimulated slice AG, increasing intracellular PGs synthesis with CI, resulting in PGE_2 media levels of only 1ng/ml, decreased AG by 30%. In addition, M-stimulated slice AG was normalized when PG synthesis was returned to basal values with CI.

In summary, the greater sensitivity to PGs inhibition of homogenate AG compared with that of slices, plus the enhanced inhibitory effect of intracellular PGs compared with extracellular PGs, suggest that the suppression of AG by PGs is an intracellular event. Moreover, the inhibition of AG is specific for PGE_2 and $F_2\alpha$ and not due to a nonspecific affect of M or CI.

THE CHEMICAL BASIS FOR THE REDUCTION OF INSULIN BINDING DURING METABOLIC ACIDOSIS. R.L. Jungas*, S. Cheema-Dhadli*, K. Desai*, C. Yip* and M.L. Halperin. Univ. of Toronto, Toronto, Ontario, and Univ. of Connecticut, Framington, Ct.

There is a resistance to insulin action during metabolic acidosis; with regard to mechanisms, there is a striking dependence of insulin binding to its plasma membrane receptor with pH - optimal binding was observed at pH 7.5 to 7.8 with a sharp reduction on either side of this range. The purpose of this study was to determine the chemical groups responsible for this pH dependent binding of insulin over the pathophysiological pH range. We observed a linear relationship of the equilibrium binding constant with pH - since the slope was close to unity up to pH 7.6 on a logarithmic plot, one dissociable group in the region of pH 7.6 appears to be deprotonated for optimal binding of insulin. Furthermore, a single binding isotherm could describe all the results with variable insulin concentrations and pH. Since the enthalphy change during dissociation was very small, the group responsible for pH sensitive binding seems to be a very weakly acidic carboxyl group on the insulin receptor. Four positively charged groups on insulin could be attracted to this carboxyl. Insulin analogues which cannot bear a positive charge at 3 of these sites (A or B N-terminus, lysine B_{29}) retained their pH sensitivity - thus the best candidate for this function was the arginine on the B chain. We conclude that the receptor contains the pH sensitive group required for binding and suggest that the cationic group of arginine at B_{22} is important for this interaction. This property could be important in the recycling of the insulin receptor.

BICARBONATE REABSORPTION IN ISOLATED PERFUSED COLLECTING TUBULES FROM OBSTRUCTED RABBIT KIDNEYS. ME Laski, C Sabo*, VE Morgan*, and NA Kurtzman. West Side VA and Univ of Illinois, Chicago IL.

Urinary tract obstruction (UTO) may cause distal renal tubular acidosis with or without hyperkalemia. This acidosis may be the result of impaired proton secretion in the cortical (CCT) and/or the medullary (MCT) collecting tubule. Since active Na transport and a lumen-negative potential difference are present only in CCT, decreased K excretion and hyperkalemia should develop only from CCT and not MCT injury. Anatomically, it is likely that the acidification defect in UTO occurs first in MCT and later in CCT. To examine the pattern of this disorder in UTO we simultaneously studied total CO_2 flux (JTCO2) in isolated perfused MCTs and CCTs obtained from both the normal and the obstructed kidney of rabbits one and two days after unilateral ureteral obstruction.

At one day, no defect of JTCO2 was seen in either MCT or CCT, though urine bladder pH was lower than the pH in the matched pelvic urine of the obstructed side. At two days JTCO2 was lower in the MCT from obstructed kidneys as compared to tubules from the normal kidney (25.5±10.93 vs. 9.95±8.21 pMol/mm/min $p<.05$). No difference was noted in the CCT. Urine pH was again lower in the bladder than in the obstructed pelvis.

We conclude: 1) The in vivo acidification defect in the dog with unilateral UTO previously reported by our lab at one day and suggested by the high urine pH of the obstructed kidney of the rabbit is not seen in vitro. 2) The earliest demonstrable defect in JTCO2 in this model of UTO occurs in the MCT.

EFFECT OF ORAL ACID INGESTION AND AMILORIDE ON TOTAL CO_2 (tCO_2) ABSORPTION IN THE RAT SUPERFICIAL DISTAL TUBULE (SDT). RT Kunau and KA Walker*. Dept of Med, Univ of Texas Hlth Sci Ctr, San Antonio, Texas.

Previous studies in the rabbit cortical collecting tubule and rat SDT have shown that oral acid loading, sufficient to induce acidemia prior to study, stimulated H^+ secretion resulting in absorption of tCO_2 in these segments when they were perfused with solutions isohydric to plasma. The present studies assessed the influence of oral acid ingestion, sufficient to lower urinary pH but not cause acidemia, and the effect of amiloride on SDT tCO_2 absorption. SDTs were microperfused in vivo at 10 nl/min with an isohydric solution ($NaHCO_3$ 28 mM). tCO_2 was measured by microcalorimetry. The absorptive rate of tCO_2, $JtCO_2$, is expressed as pmol·mm^{-1}·min^{-1}. In rats ingesting a commercial (Teklad) diet, urine pH immediately prior to study was 6.81 ± .16, plasma tCO_2 29.3 ± .7 mM, and $JtCO_2$ 12.8 ± 5.5. In a second group of rats, after eating 18 gms of a diet with 32% casein one night before study, the values were 5.78 ± .12, 28.0 ± .6 and 38.8 ± 5.4, respectively. Amiloride (10^{-4}M) added to the perfusate in casein fed rats reduced $JtCO_2$ to 22.5 ± .1. Plasma tCO_2 and urine pH were 27.3 ± .3 and 5.5 ± .03, respectively, in this third group.

These data suggest that acidification in the SDT can be stimulated by oral acid loads which do not induce a systemic acidemia. The rate of acidification can be influenced by amiloride, presumably by causing a relative hyperpolarization of the luminal membrane.

RENAL CONCENTRATING DEFECT ASSOCIATED WITH GASTRIC ALKALOSIS IN THE RAT. D.Z. Levine, L.N. Peterson, D. Sztorc*, and J. Kucharczyk*. Depts. of Pediatrics, Physiology, Medicine, University of Ottawa, Ottawa, Ontario, Canada.

To determine how chloride depletion alkalosis may alter renal concentrating ability, we developed a chronic model of metabolic alkalosis in rats fed a chloride-free diet (without $NaHCO_3$ replacement) and subjected to drainage of acid through indwelling gastric fistulae. Alkalotic animals displayed persistent hyponatremia, reduced plasma osmolality (P_{osm}), polydipsia (2x control) and a renal concentrating defect. When animals were pair-watered to prevent polydipsia, P_{osm} did not increase and urine concentrating ability was not improved. Despite reduced P_{osm} (281±4.0 vs 304±1.7, $p<.001$) in alkalotic rats, ADH by kit radioimmunoassay suggested adequate hormone levels. When ADH was administered to pair-watered dehydrated alkalotic rats (500 mU b.i.d.) there was a significant increase in maximal U_{osm}, although not to the same values of sham-operated controls.

	MAXIMUM U_{osm} (mosm/Kg H_2O)	
	Sham Rats	Alkalotic Rats
Ad-Lib H_2O	2754±145.1	1853±107.8*
Pair-Watered	2851±130.0	1967±131.7*
Pair-Watered+ADH	2615±75.6	2342±57.8*

*$p<0.05$ or less compared to sham.

These results indicate that the concentrating defect in chronic metabolic alkalosis may be attributed to a primary derangement in the renal concentrating process and to an impairment in either ADH release or to the renal response to the hormone.

INHIBITORY EFFECTS OF LITHIUM, AMILORIDE AND QUINIDINE ON SODIUM UPTAKE IN BRUSH BORDER MEMBRANE VESICLES (BBMV). Andrew G. Lowe*, Herbert Y. Lin*, Victoria J. Yee* and David Warnock. VA Medical Center and CVRI, Univ. California, San Francisco, San Francisco, Ca.

Quinidine (Quin) is a mixed inhibitor of the Na/H antiporter. The mode of inhibition by amiloride (Amil) and lithium (Li) are controversial, perhaps due to differences in isotopic versus fluorescence techniques. The present studies examined the combined effects of inhibitors on initial rates of sodium (22Na) uptake. BBMV were prepared from rabbit renal cortex by Mg^{++} aggregation, and loaded with 100 mM MES/KOH at pH 6.0. 22Na uptake was linear between 0, 0.5 and 1 sec. The uptake buffer contained 90 mM Na Gluconate, 3 μCi 22Na, and 100 mM HEPES/KOH at pH 7.5.

Per Cent Inhibition of 22Na Uptake

	Lithium	
Amiloride	0 mM	1 mM
0 uM	control	11.6±2.0#@
25 uM	26.9±1.7#	26.3±1.8#
Quinidine		
0 uM	control	7.2±3.3#@
250 uM	35.6±5.4#	31.1±3.0#

(# $p < 0.05$ versus control; @ $p < 0.05$ versus 0 mM Li)

The control 22Na uptake was 16.4±3.7 nmol/mg/sec (n=7 preps). The zero time value (determined at 4°C with 10 mM amiloride) was 2.0±0.2 nmol/mg protein.

Conclusions: 1) Li, Amil and Quin each inhibited 22Na uptake. 2) The inhibitory effect of 1 mM Li was not additive to that of Amil or Quin, suggesting similar modes of inhibition. 3) These results are consistent with effects at the external "modifier" site which we previously described with the acridine orange technique.

DCCD-SENSITIVE ATPase ACTIVITY IN PROXIMAL AND DISTAL SEGMENTS OF THE RABBIT NEPHRON. D. Marver. Univ. Texas Hlth. Sci. Ctr., Southwestern Medical School, Depts. Int. Med./Biochem., Dallas, Tx.

Corticosteroids influence acid excretion, and indirect evidence in turtle bladder suggests that aldosterone, in particular, stimulates this process by increasing the number of luminal membrane proton pumps. Stimulation may be due to an enhanced synthesis of enzyme or due to recruitment of latent pumps. Accumulated data suggests that luminal membrane H^+ ATPase is inhibited by DCCD, and insensitive to ouabain and the mitochondrial H^+ ATPase inhibitor, oligomycin. This study, therefore, examines the level of DCCD-sensitive enzyme in segments from normal (N) and adrenalectomized (Adx) rabbits, as an aid to identifying steroid-sensitive enzyme along the nephron. Freeze-dried proximal convoluted tubules (PCT) and cortical and medullary collecting ducts (CCT, MCT) were assayed for activity (37°C) using the method of enzymatic recycling. Assays included: 100 mM Tris, 53 mM NaCl, 5 mM KCl, 0.1 mM EDTA, 3mM MgATP, 1 mM ouabain, and 12.5 μg/ml oligomycin ± 500 μM DCCD. Activities (mol/kg dry wt/hr) are shown below.

	PCT	CCT	MCT
DCCD-sens.			
N	1.44 ± 0.12	3.14 ± 0.28	3.46 ± 0.25
Adx	0.71 ± 0.19*	0.60 ± 0.14*	0.55 ± 0.12*
DCCD-insens.			
N	0.80 ± 0.22	1.00 ± 0.30	1.14 ± 0.28
Adx	1.05 ± 0.21	0.90 ± 0.26	0.79 ± 0.24

* $p < 0.05$, Adx vs N

Thus corticosteroid-dependent changes in DCCD-sensitive ATPase activity are apparent along the rabbit nephron: MCT,CCT> PCT.

PLASMA POTASSIUM [P_k] AND ACIDOSIS RELATED TO VOLUME EXPANSION [VE] IN SELECTIVE HYPORENIN/HYPOALDOSTERONISM (SAD). Martino, J.A.; Barbour, G.L. VAMC Hampton, and Eastern Virginia Medical School, Norfolk, Va.

The syndrome of selective deficient renin/aldosterone is characterized by increased P_k. The associated acidosis is characterized by normal urine acidification. We studied such patients, urine ph≤5.0 with 2gm sodium intake, (VI), ad lib sodium (VE), and with flourinef (VEF). All patients demonstrated depressed renin/aldo levels at VI, and poor response to diuretic stimulation. All were in sodium balance. All patients had hyperchloremia, which did not change.

Results	Mean	P_k	$PHCO_3$	ΔKg
VI	meq/L	5.70	19.5	-
VE		6.65	13.0	+2.3
VEF		5.55	13.0	+2.2

Notable observations are; P_k was lower during VI with acidemia; P_k increased as acidemia worsened during VE; when flourinef was added; P_k decreased with no change in acidemia. The findings are consistant with: (1) Increased distal delivery of filtrate due to VE may cause a bicarbonate "leak", and/or impair Na/K exchange. (2) Two separate mechanisms for Na/K exchange exist, one related to VE, and distal delivery of Na and other responsive to aldosterone.

pH-DEPENDENCY OF AMILORIDE-SENSITIVE SODIUM HYDROGEN ANTIPORTER IN LLC-PK_1. A. Moran and N. Moran (intr. by J. S. Handler). Physiology Dept., AFRRI and NINCDS, NIH, Bethesda, MD.

LLC-PK_1 is an established cell line which manifests many properties of the proximal tubule. Our previous work indicates that there is a large Na flux into LLC-PK_1 epithelia via an amiloride-sensitive Na-H antiporter. Such an exchange has been postulated to be one of the major acidification mechanisms in the proximal tubule. The present report examines the pH dependency of this amiloride-sensitive Na uptake. Epithelia were grown in cluster-12 wells. Cells were preincubated for 2 hr in low Na (0.1 mM) Ringer containing 128 mM N-methyl glucamine, 0.5 mM $CaCl_2$, 4 mM KCl, 1 mM $MgCl_2$, 1 mM ouabain, and 20 mM Tris-Hepes, pH 7.4. Initial uptake was measured at room temperature for 1 min in the presence of 1 mM NaCl or in various sodium concentrations in Na-dependence experiments. Sodium influx as a function of [Na] exhibited saturation kinetics with an apparent K_{Na} of 25 mM. Lowering the extracellular pH inhibits sodium influx. Protons appear to act via a single inhibitory site with an apparent K_H of 66 nM (equivalent to pH of 7.2). Decreasing external pH from 7.5 to 6.4 resulted in an increase in the apparent K_m for sodium without a significant change in the V_{max} of the system. Protons also modulate the inhibition of sodium uptake by amiloride. The same pH change (as above) causes an increase of the K_i for amiloride from 30 to 68 μM. The data indicate that one of the major sodium entry pathways through the apical membrane in LLC-PK_1 epithelia is via Na-H exchange similar to the Na-H exchange of the proximal tubule. The LLC-PK_1 cell line may thus provide a controlled and convenient system to study the regulation of this pathway.

AMMONIA PRODUCTION BY ISOLATED PERFUSED MOUSE PROXIMAL TUBULES. EFFECT OF METABOLIC ACIDOSIS. G. T. Nagami, C. M. Sonu*, and K. Kurokawa. Nephrol Div, VA West Los Angeles Med Center, and Dept Med, UCLA Sch Med, Los Angeles, CA.

Ammonia production (AP) by the proximal tubule plays a key role in the defense against metabolic acidosis (MA). To study the regulation of AP by the proximal tubule in response to MA, we examined the rates of total AP by isolated perfused proximal segments dissected from control and NH_4Cl-induced acidotic mice (blood HCO_3^- = 16.8±0.8 mM (±SE)). Isolated segments of mouse pars recta were cannulated and perfused with Krebs-Ringer bicarbonate buffer (KRB). The perfused segments were incubated under oil at 37°C in KRB containing 0.5 mM L-glutamine and sodium acetate equilibrated with 95% O_2:5% CO_2, pH 7.4. Bath and luminal fluid samples were collected at timed intervals and ammonia was measured by a bioluminescence reaction. The rates of AP by unperfused tubules from control and MA mice were 5.9±0.3 pmol/mm/min and 22.6 ±2.9, respectively ($p<0.001$). Perfusion of the lumen resulted in increased rates of total AP in both control and MA groups (21.0±0.8 pmol/mm/min for control group vs. 43.3±7.0 for the MA group; $p<0.01$). In both control and MA tubules, the luminal ammonia output (LAO) accounted for greater than 90% of the increment in total AP observed with perfusion. These observations suggest that total AP by unperfused mouse proximal tubules is enhanced by in vivo MA, that total AP is enhanced further by perfusion of the tubule lumen, and that the LAO contributes a major portion of the increase in total AP observed with perfusion in MA and control proximal tubules.

DISPARATE EFFECTS OF RACEMIC (DL) AND LEVO (L) LACTIC ACID (LA) ON PLASMA PHOSPHORUS [P]. James R. Oster, Carlos A. Vaamonde, and Helen Alpert*. VAMC & Dept. Med., Univ. Miami, Miami, FL.

The mechanism(s) for the hyperphosphatemia associated with lactic acidosis is unknown. Experimental lactate-induced hyperphosphatemia seems to require acidemia because we have shown that prevention of acidemia with $NaHCO_3$ obivates increases in [P] (Canad J Physiol Pharmacol, in press). Because the rate of lactate metabolism (by utilizing NAD or other mechanisms) might modulate transcellular movement of P, we assessed the plasma [P] response to 3 hr infusions of DL-LA vs. L-LA. Since the dog metabolizes only the L moiety of DL-LA (thereby consuming H^+), we expected that more L-LA (group 3) would be needed to produce as much acidemia as obtained with DL-LA. Group 1 (n=6) mongrel dogs received 12 mEq/kg DL-LA; group 2 (n=6) 12 mEq/kg L-LA, and group 3 (n=4) 16 to 19 mEq/kg L-LA. After 3 hr:

	pH	$[HCO_3]$ (mEq/L)	Δ[P]* (mg/dl)	Δ[P]* (%)
Group 1	7.08±0.03	8±1	1.9±0.4	51±12
Group 2	7.29±0.02†	18±1†	0.9±0.3	17± 6
Group 3	7.04±0.03	11±1	0.9±0.5	19±10

X ± SE; *Δ=hr 3 - baseline; †$p<0.05$ from group 1.

Both infusion rates of L-LA increased [P] less (p=NS) than DL-LA, indicating the importance of factors other than pH. Also, since presumably more lactate was metabolized in group 3 than in group 2 (but the Δ[P] was the same in both), the findings do not support the hypothesis that the rate of lactate utilization is a major modulator of plasma [P] in acute lactic acidosis.

INTRACELLULAR pH REGULATION IN RABBIT PROXIMAL STRAIGHT TUBULES: BASOLATERAL HCO_3^- TRANSPORT. **Nazih L. Nakhoul* and Walter F. Boron. Dept. of Physiol., Yale Univ. Sch. of Med., New Haven, CT.**

We studied the basolateral transport of HCO_3^- and its contribution to intracellular pH (pH_i) regulation in isolated perfused proximal straight tubules (PST) of the rabbit. pH_i was calculated from absorbance spectra (sampled ~1/sec) of the pH-sensitive dye 4',5'-dimethyl-5(and -6)-carboxyfluorescein. The average initial pH_i of 10 PST's incubated in pH-7.4 CO_2/HCO_3^- Ringer at 38° was 7.23 ± 0.04. In one series of experiments, the basolateral (b) pH was lowered from 7.4 to 6.7 by reducing $[HCO_3^-]_b$ from 25 to 5 mM at constant pCO_2. This reduction of pH_b and $[HCO_3^-]_b$ caused pH_i to rapidly fall by 0.61 ± 0.04 (n=13 tubules). pH_i rapidly recovered when pH_b and $[HCO_3^-]_b$ were returned to normal. When pH_b was lowered from 7.4 to 6.7 in 2 mM PIPES Ringer, the pH_i decrease was reduced by 70.1 ± 7.9% (n=3). The pH_i decrease induced by lowering pH_b and $[HCO_3^-]_b$ was inhibited by 37.6 ± 4.0% (n=7) by replacing all luminal and basolateral Cl^- with glucuronate. The inhibition of the pH_i change was not substantially increased by removing Na^+ in addition to Cl^-. These results indicate that the majority of basolateral HCO_3^- transport in the PST is not coupled to either Cl^- or Na^+, and could be mediated by a simple HCO_3^- conductance. However, the small Cl^- dependent component could be due to Cl-HCO_3 exchange. In support of this hypothesis is the observation that removing Cl^- from bath and lumen caused pH_i to transiently increase by ~0.23 ± 0.04 (n=6), whereas returning the Cl^- had the opposite effect.

MODIFICATION OF URINARY BICARBONATE SECRETION BY CYCLIC AMP AND 9-ANTHROIC ACID. J. Palmisano*, D. L. Stetson*, R. Beauwens*, P. Mitchell* and P. R. Steinmetz. Univ. of Conn. Health Center, Farmington, CT.

To arrive at a double membrane model for the cellular organization of HCO_3^- secretion (J_{HCO_3}) by the turtle bladder we examined the effects of 8-bromo-cAMP (see Satake et al AJP 244:C259, 1983), 3-isobutyl-1-methylxanthine (IBMX) and the Cl^- channel blocker 9-anthroic acid (9-AA) on the electroneutral and electrogenic components of J_{HCO_3}. J_{HCO_3} was measured by pH stat titration in the mucosal (M) compartment after Na^+ transport and H^+ secretion were abolished by ouabain and a ΔpH, respectively. Serosal (S) addition of cAMP in the presence of S HCO_3^- increased J_{HCO_3} from 1.32±0.6 μmoles/hr to 2.24±0.27 and caused a short circuit current (Isc) equivalent to 1.82 μmoles/hr (n=5) of anion secretion; the neutral component was reduced, consistent with reduced net Cl^- absorption. IBMX (50 μM) similarly increased J_{HCO_3} and Isc (n=6), while acetazolamide inhibited both components. 9-AA (100 μM to M) inhibited the IBMX-induced electrogenic J_{HCO_3} by 50% in the presence and absence of ambient Cl^-. In summary: Control J_{HCO_3} involves electroneutral exchange for Cl^-. The double membrane model consists of a primary H^+ pump at the S membrane driving J_{HCO_3} and a Cl-HCO_3 exchanger at the M membrane with variable parallel anion conductance. cAMP activates the parallel anion conductance resulting in electrogenic HCO_3^- secretion. This pathway is inhibited by 9-AA. These transport elements may be contained in a subpopulation of carbonic anhydrase-rich cells (Stetson and Steinmetz, ASN, 1984).

EFFECT OF CHANGE OF LUMINAL pH, P_{CO2} and $[HCO_3]$ ON INTRACELLULAR pH (pH_i) IN THE PROXIMAL TUBULE OF NECTURUS. G. Planelles*, J. Teulon*, A. Edelman*, S.R. Thomas*& T. Anagnostopoulos. INSERM U.192, Hôpital Necker, Paris, France.

pH_i was measured by double barreled selective microelectrodes (liquid ion exchanger and voltage sensing barrels) during high flow rate luminal microperfusions with double barreled micropipettes. Three pairs of perfusates were used. A : 5 % CO_2, pH 6.9 vs 1.1 % CO_2, pH 7.5 (constant $[HCO_3]$ 8 mM). B : $[HCO_3]$ 27 mM, pH 7.4 vs $[HCO_3]$ 8 mM, pH 6.9 (constant CO_2 5 %). C: 5 % CO_2, $[HCO_3]$ 27 mM vs 1.1 % CO_2, $[HCO_3]$ 6 mM (constant pH 7.4). Results. A: increasing lumen CO_2 from 1.1 % to 5 % while lowering pH from 7.5 to 6.9 (at constant $[HCO_3]$) resulted in a reversible fall of pH_i by 0.28 ± 0.02 pH units. B : lowering $[HCO_3]$ from 27 to 8 mM and pH from 7.4 to 6.9 (at constant P_{CO2}) decreased pH_i by 0.18 ± 0.03 pH units. C : increasing CO_2 from 1.1 % to 5 % and $[HCO_3]$ from 6 to 27 mM (at constant pH) brought about 0.12 ± 0.03 cell acidification. pH_i was always more acid than lumen pH. The transmembrane ΔpH and H^+ electrochemical potential gradient ($V-E_H$) were little affected by changes of luminal fluid composition. Acetazolamide 10^{-4} M considerably reduced pH_i changes in series A and B, and totally suppressed them in series C. Conclusions : 1°) Changes of luminal pH, at constant P_{CO2} or at constant $[HCO_3]$ produced homologous changes of pH_i. 2°) When lumen P_{CO2} and $[HCO_3]$ have opposite effects on pH_i, those of P_{CO2} prevail. 3°) The relative constancy of ΔpH and $V-E_H$ transmembrane differences during luminal fluid disturbances is consistent with some control of transmembrane H^+ distribution. 4°) Carbonic anhydrase plays a dominant role in this control.

CHARACTERIZATION OF THE ACIDIFICATION DEFECT IN OBSTRUCTIVE UROPATHY. C. Ribeiro* and W.N. Suki. Baylor Col. of Med., Houston, Texas.

A defect in urine acidification has been described in obstructive uropathy. Since the collecting tubule from the inner stripe of the outer medulla ($OMCT_i$) is the major site for distal acidification, isolated $OMCT_i$ nephron segments from control rabbits and from rabbits after 24-hours of unilateral or bilateral ureteral obstruction (UUO or BUO) were studied. Tubules were perfused (4 nl/min) and bathed with an artificial solution resembling rabbit serum ultrafiltrate. Bicarbonate absorption (J_{HCO_3}) was measured from the difference between total CO_2 (tCO_2) in perfused and collected fluid samples. Apparent fluid absorption (Jv) in UUO was significantly negative and J_{HCO_3} reduced, as compared to control (C). In BUO, Jv was also significantly negative, however, J_{HCO_3} was not significantly different from C. To determine whether the acidification disorder was due to a gradient or capacity defect, the difference between perfused and collected bicarbonate (ΔHCO_3) was measured at perfusion rates of 1 nl/min in some tubules. No difference was found in ΔHCO_3 among $OMCT_i$ from C, UUO, or BUO rabbits.

	n	Jv nl/mm.min	J_{HCO_3} pmol/mm.min	n	ΔHCO_3 mmol
C	10	-0.03±0.03	11.61±1.21	5	8.98±0.54
UUO	15	-0.48±0.12*	7.59±1.09+	5	9.95±1.76
BUO	18	-0.29±0.07*	7.96±2.75	7	8.93±2.19

*P <0.05 vs. 0 and C, + P < 0.05 vs. C

We conclude that H^+ secretion is impaired in the $OMCT_i$ during UUO due to a capacity defect. The defect was not observed in BUO probably because of the high degree of morphological and functional heterogeneity observed in tubules from these animals.

MODULATION OF Na/H ANTIPORTER ACTIVITY BY PARATHYROID HORMONE IN A CULTURED RENAL CELL LINE: A RAPID ONSET cAMP MEDIATED EFFECT. A.S. Pollock, R.T. Miller*, G.J. Strewler* and D.G. Warnock, VA Med. Center, CVRI, Univ. of Calif., San Francisco, CA.

The continuous opossum kidney cell line (OK) retains the proximal tubule characteristics of a Na/H antiporter, Na coupled hexose transport and PTH-responsive adenylate cyclase. Steady state intracellular pH (pHi) of OK cells is lowered by 1 picomolar (pM) PTH, presumably due to inhibition of the Na/H antiporter. The purposes of the present studies were to define the time course and cAMP dependence, and the specificity of this inhibitory effect on the Na/H antiporter.

Amiloride-sensitive 22Na uptake was used to measure Na/H antiporter activity. OK cell monolayers were depleted of Na in HEPES buffered medium at pH 7.42 in the absence of bicarbonate/CO2. PTH, 0.1 pM to 100 nM, 0.1 mM 8BrcAMP or 1μM forskolin were added 10 min to 4 hrs before 22Na uptake. Two minute 22Na uptake was measured at 37°C in HEPES buffered medium containing 10 mM Na.

Without hormones, amiloride-sensitive Na uptake averaged 16.6±2.1 nmol/2 min/mg protein. 25 nM PTH decreased 22Na uptake by 64%. 0.1 mM 8BrcAMP or 1 μM forskolin, an adenylate cyclase activator, produced similar inhibition. The inhibitory effect was fully developed after 10 min exposure to PTH. Concentrations of PTH as low 10 pM caused measurable depression of amiloride-sensitive Na uptake. Amiloride-insensitive Na uptake was unaffected by these agents.

Conclusions: 1) The proximal-like OK cell line has an amiloride-sensitive Na/H antiporter whose activity is decreased by picomolar levels of PTH. 2) This effect is reproduced by cAMP analogues and forskolin, indicating that the response to PTH is mediated by cAMP. 3) Inhibition of the Na/H antiporter is probably the mechanism of PTH-induced lowering of pHi in this cell line. 4) The rapid onset and sensitivity of this hormone response suggests that PTH is a physiologic regulator of Na/H antiporter activity.

VOLTAGE REGULATION OF CARBONIC ANHYDRASE INDEPENDENT ACIDIFICATION IN TURTLE BLADDER. S.Sabatini and N.A. Kurtzman, Univ of Illinois College of Medicine, Chicago, IL.

In vitro, carbonic anhydrase inhibition (CAI) virtually completely inhibits acidification in most membranes. In vivo, however, approximately 50% of filtered HCO_3 is reabsorbed by the kidney at sites other than the superficial proximal tubule after CAI. While the sites and mechanisms of carbonic anhydrase independent acidification are not completely understood, micropuncture studies from this laboratory have shown that substantial reabsorption occurs in the distal nephron which is partially inhibited by amiloride. Proposed mechanisms for this carbonic anhydrase independent acidification include favorable electrical gradients (abolished by amiloride) or passive events (i.e., favorable concentration gradients). To examine the effect of the former, we studied acidification (J_H) in the turtle bladder after CAI, 10^{-4} M acetazolamide, over a range of voltage clamped conditions. In the presence of 1%CO_2 and normal turtle Ringers' solution, voltages ranged from 0 to 75 mV. J_H, when PD was 0, was 1.24 ± 0.14 μmol/hr. This effect was linear and is described by the equation as follows: Y = 0.012 X + 1.129, correlation coefficient = 0.42, n=47, P < 0.01. This increase is identical to that seen with control bladders. These results show that favorable electrical gradients contribute significantly to carbonic anhydrase independent acidification. Thus, carbonic anhydrase independent HCO_3 reabsorption by the kidney may, in part, be mediated by voltage gradients established by electrogenic sodium reabsorption in the cortical collecting tubule.

ISOLATION AND CHARACTERIZATION OF A H+ ATPASE FROM RABBIT AND HUMAN KIDNEY MEDULLA.

A. Sallman, H. Lubansky*, L. Barker*, Z. Talor and J.A.L. Arruda, VAMC and Univ. Ark., Little Rock.

Distal acidification is thought to be mediated by a H+ ATPase. We isolated a plasma membrane (PM) fraction containing a H+ ATPase from rabbit and human kidney medulla by using differential and gradient centrifugation. In the presence of ouabain to inhibit Na K ATPase and in the absence of Ca to inhibit Ca ATPase and in the presence of oligomycin to inhibit mitochondrial ATPase, the PM fraction of both rabbit and human medulla contained ATPase activity. This ATPase was inhibited 60-70% by dicyclohexylcarbodiimide (DCCD). The PM fraction containing this ATPase was enriched 7 to 10-fold in alkaline phosphatase and maltase, considered to be markers of the luminal membrane. Thus, the DCCD sensitive ATPase seems to be present in the luminal membrane of the medulla and also present in the luminal membrane of the human cortex. In the presence of oligomycin and the absence of Na, ATP addition to the human PM fraction led to quenching of acridine orange. This quenching could be dissipated by protonophores indicating the generation of a H+ gradient. Acidification was not affected by replacement of Na by K but was critically dependent on the presence of chloride. In the presence of K and valinomycin to create a negative intravesicular voltage, the quenching of acridine orange was enhanced, indicating that the H+ ATPase is electrogenic. Thus, the rabbit and human kidney medulla contain an oligomycin resistant, DCCD sensitive ATPase. In addition, the human medullary H+ ATPase is capable of acidification and is electrogenic.

TWO FUNCTIONALLY DISTINCT TYPES OF MITOCHONDRIA-RICH (MR) CELLS IN CORTICAL COLLECTING TUBULE (CCT) AS DETERMINED BY CHANGES IN CELL pH (pH_i) IN INDIVIDUALLY IDENTIFIED CELLS.

G.J. Schwartz and Q. Al-Awqati. Albert Einstein Coll. of Med., Bronx, N.Y. and Columbia Univ. N.Y., N.Y.

MR cells probably secrete both H^+ and HCO_3^-. We recently found that only 1% of the MR cells of the CCT (which usually secretes HCO_3^-) but $\gg$ 50% of MR cells in the medullary collecting tubule (MCT), which secretes H^+, can endocytose luminal macromolecules (E+). In isolated perfused tubules, we measured HCO_3^- transport by picapnotherm (in pmol/mm.min) and pH_i in identified MR cells using excitation ratio fluorometry of 6-carboxyfluorescein. Solutions simulated rabbit plasma ultrafiltrate; bath pH at 38° C was 7.32 ± 0.02; pCO_2 was maintained at 48 ± 3 mm Hg to permit alkaline and acid changes in pH_i. Replacement of bath Cl^- by gluconate stimulated HCO_3^- secretion (J_b) in CCT (from 3.3 ± 1.6 to 12.0 ± 2.3, n=13), but inhibited H^+ secretion (J_H) in MCT (from 9.1 ± 1.6 to 2.1 ± 1.6, n=5). We added rhodamine-BSA luminally to identify endocytosing (E+) and non-endocytosing (E-) cells and measured pH_i of single MR cells. Removal of bath Cl^- increased pH_i in E+ cells in both MCT (from 7.1 ± 0.1 to 7.3 ± 0.1, n=6) and CCT (from 7.2 ± 0.1 to 7.4 ± 0.1, n=6) but reduced pH_i in E-cells of CCT (from 7.4 ± 0.1 to 7.0 ± 0.1, n=6) (n= no. of tubules, $p < 0.05$ for all results).

Since MCT secretes H^+ and most of its MR cells are E+, these cells must be responsible for H^+ secretion in MCT and CCT. Removal of bath Cl^- should then inhibit J_H and increase pH_i due to reduced basolateral Cl^-:HCO_3^- exchange. Stimulation of HCO_3^- secretion and reduction in pH_i in E- cells by removal of bath Cl^- suggests that these MR cells of the CCT secrete HCO_3^- by a luminal Cl^-:HCO_3^- exchanger.

CYCLIC-AMP-STIMULATED BICARBONATE SECRETION (BiS) IN RABBIT CORTICAL COLLECTING TUBULES (CCT).

V.L. Schuster, Department of Medicine, University of Iowa, Iowa City, IA.

Although BiS has been described in CCT, little is known about this process. The present studies examined the hormonal factors regulating BiS in CCT and their mechanism(s). CCT's from HCO_3-loaded rabbits were perfused and bathed with 25 mM HCO_3 Ringers. BiS (pmol/mm/min) was measured by microcalorimetry. In time-controls, BiS fell (Δ=-3.84±1.09, p<.02). In contrast, 8-bromo-cAMP (0.1 mM) stimulated BiS (Δ=+4.53±1.29, p<.025). Whereas isoproterenol (ISO) (10^{-6} M) also stimulated BiS (Δ=+1.89±0.89, p<.005 compared to time controls), ADH (200 μU/ml) did not (Δ=-4.68±1.33). ISO and ADH each decreased the lumen negative voltage, but had opposite effects on BiS. ISO-induced BiS is thus unlikely due to an effect on passive HCO_3 distribution. Likewise, electrogenic BiS due to ISO is unlikely. To determine if the stimulation of BiS by cAMP is due to inhibition of H+ secretion, bath DIDS (0.1 mM) was added to control CCT. Since no increase in baseline BiS was seen, minimal H+ secretion was present, making this cAMP mechanism unlikely. The cAMP effect on BiS was reversibly eliminated by 0 Cl perfusate, but not by luminal DIDS. Bath containing 10^{-3} M amiloride and 5 mM Na did not inhibit BiS due to cAMP. Conclusions: 1) cAMP stimulates BiS in CCT, probably via Cl-HCO_3 exchange; 2) Basolateral Na-H exchange is not the driving force for cAMP-stimulated BiS. Since both ADH and ISO are known to stimulate CCT adenylate cyclase, the present results suggest separate cell cAMP pools or cellular heterogeneity in cAMP response.

INTRACELLULAR ALKALINIZATION INDUCED BY DEPOLARIZATION IN AMBYSTOMA PROXIMAL TUBULES.

Arthur W. Siebens* and Walter F. Boron. Dept. of Physiol., Yale Univ. School of Medicine, New Haven, CT.

We examined the effect of changes in membrane potential on intracellular pH (pH_i) in isolated, perfused proximal tubules from the tiger salamander, Ambystoma tigrinum. Basolateral membrane potential (V_{bl}) and pH_i were measured with conventional and pH-sensitive, liquid-membrane microelectrodes, respectively. The solutions were nominally HCO_3^--free. Increasing bath (b) $[K^+]$ from 2.5 to 50 mM caused an immediate depolarization of 40 ± 3 mV (initial value: 60 ± 2 mV, n=15 tubules) and a pH_i increase of 0.24 ± 0.03, developing over ~4 min. These changes were fully reversible. Increasing luminal $[K^+]$ resulted in a slow, continuing depolarization; after 4 min, V_{bl} depolarized by 14 ± 3 mV while pH_i increased by only 0.03 ± 0.04 (N.S., n=4). When 1 mM $[Ba^{++}]_b$, rather than 50 mM $[K^+]_b$, was used to depolarize the cell (by 33 ± 1 mV), pH_i increased by 0.17 ± 0.01 (n=3). Thus, depolarization per se appears to induce cell alkalinization. Replacement of all extracellular Na^+ with N-methyl-D-glucamine decreased by 93 ± 16% the magnitude of the alkalinization induced by raising $[K^+]_b$ to 50 mM (n=5). Two Na-dependent ion-transport systems are known to affect pH_i in these cells, Na-H exchange and electrogenic Na/HCO_3^- cotransport (J. Gen. Physiol. 81:29-52, 53-94). Because Na/HCO_3^- cotransport does not occur under nominally HCO_3^--free conditions, the depolarization-induced alkalinization is most likely caused by stimulation of the Na-H exchanger.

BICARBONATE SECRETION BY RABBIT CORTICAL COLLECTING DUCT: ROLE OF CHLORIDE/BICARBONATE EXCHANGE. Robert Star*, Mark Knepper, and Maurice Burg. NHLBI, NIH, Bethesda, MD.

Cortical collecting ducts (CCD) from rabbits treated with deoxycorticosterone (DOC) secrete bicarbonate at high rates. To investigate the mechanism of bicarbonate secretion, we measured bicarbonate and chloride transport (pmole/mm/min) in CCD from rabbits treated with DOC for 9-24 days. Chloride or bicarbonate was removed from the perfusate and/or bath and replaced with gluconate. Removal of chloride from both perfusate and bath inhibited bicarbonate secretion (-9 ± 1 to 0.5 ±1 [±SE]) without a change in transepithelial voltage. Removal of chloride only from the bath increased bicarbonate secretion, while removal of chloride only from the perfusate inhibited secretion. In contrast to the effect of removing chloride, removal of sodium from both the perfusate and bath (replacement with N-methyl-d-glucamine) altered transepithelial voltage (-26 ± 7 to 15 ± 5 mv), and did not change bicarbonate secretion (-13 ± 4 to -16 ± 3). The rate of bicarbonate secretion equaled the rate of chloride absorption (13 ±3) in tubules bathed with 0.5 mM ouabain to inhibit any cation dependent chloride transport. Under those conditions removal of bicarbonate from both the perfusate and bath inhibited chloride absorption. Removal of bicarbonate only from the bath inhibited chloride absorption, while removal of bicarbonate from the lumen stimulated chloride absorption. We conclude that the CCD from DOC loaded rabbits secrete bicarbonate by a sodium-independent, chloride-dependent mechanism which involves electroneutral chloride/bicarbonate exchange.

PARTIAL RESOLUTION AND RECONSTITUTION OF THE CLATHRIN-COATED VESICLE PROTON PUMP. Dennis .K. Stone, Univ. TX Hlth. Sci. Ctr., Dallas, TX.

Clathrin-coated vesicles (CCV), which contain an N-ethylmaleimide (NEM)-sensitive proton ATPase, shuttle this pump to the luminal surface of medullary collecting duct. The CCV proton pump, functionally identical to the renal medulla vesicle pump, was examined to define the final effector of distal nephron acidification.

The ouabain and azide resistant, NEM-sensitive ATPase of bovine brain CCV was solubilized with 0.8% nonylglucopyranoside and was reconstituted into asolectin liposomes by the detergent-dilution method; NEM-sensitive proton pumping was demonstrated by both ATP-generated acridine orange quenching and $^{32}P_i$-ATP exchange. The NEM-sensitive ATPase was purified 200-fold by cation exchange, anion exchange and hydroxylapatite chromatography; the partially purified ATPase had a specific activity of 13 μmoles $P_i \cdot mg\ protein^{-1} \cdot min^{-1}$ and was fully sensitive to NEM.

The NEM-sensitive ATPase became resistant to dicyclohexylcarbodiimide (DCCD) with purification; 50% of the NEM-sensitive ATPase of intact CCV was inhibited by 1.4 nmoles DCCD/15 μg CCV protein, whereas a 10-fold higher ratio was needed to inhibit the partially purified ATPase by 50%. Reconstitution of NEM-sensitive proton pumping with the partially purified ATPase required ATPase-depleted CCV membranes or liposomes plus a chromatography side fraction; ATPase alone could not be reconstituted into liposomes.

It is concluded that the CCV proton pump is composed of at least two major subunits: one responsible for ATP hydrolysis and a second serving as a transmembranous proton channel.

A SUBPOPULATION OF CARBONIC ANHYDRASE-RICH (CA) CELLS IN TURTLE BLADDER: POSSIBLE ROLE IN URINARY HCO_3^- SECRETION. David L. Stetson* and Philip R. Steinmetz. Univ. of Conn. Health Center, Farmington, CT.

Physiologic studies (Palmisano et al., ASN, 1984) suggest that urinary HCO_3^- secretion (J_{HCO_3}) is driven by a H^+ pump at the basolateral cell (BL) membrane and that HCO_3^- is transported across the apical (A) membrane by either a Cl-HCO_3 exchanger and/or a conductive anion channel. Since the CA cells are known to be involved with acid-base transport we examined the ultrastructural characteristics of 96 CA cells from 8 tissues exposed to 0-5% CO_2. The CA cells were classified operationally on the basis of their luminal surface projections as microplicated (MP) cells, microvillated (MV) cells, and cells with both microvilli and microplicae (MPV cells). Of the 96 CA cells, 69 were of the MP or MPV type and 27 were pure MV cells. By freeze fracture the MP and MPV cells had a characteristic distribution of rod-shaped intramembrane particles (RSP). The A membrane and many cytoplasmic membrane vesicles contained a dense population of RSP's, whereas the BL membrane contained no RSP's. In contrast, the MV cells revealed a reversed distribution of RSP's. RSP's were abundant primarily in BL membranes and absent or very sparse in the A membrane and vesicular membranes. Thin sections revealed studs coating the cytoplasmic surfaces of the membranes in the same distribution as RSP's. Stimulation of H^+ secretion by CO_2 changes MPV cells into MP cells, but has little effect on MV cells. We suggest that H^+ secretion occurs in MP and MPV cells and that the MV cells with RSP's in BL membrane are responsible for urinary HCO_3^- secretion.

CHRONIC HYPERCAPNIA ENHANCES THE V-MAX OF NA-H ANTIPORTER. Z. Talor, J. Shuffield*, G. Dytko*, J.A.L. Arruda. VAMC and Univ. Ark., Little Rock.

Chronic hypercapnia is associated with increased proximal HCO_3 reabsorption. Proximal HCO_3 reabsorption is mediated by a Na-H antiporter. We hypothesized that chronic hypercapnia would be associated either with increased Vmax or with decreased K_m of the Na-H antiporter. To test this hypothesis we made rabbits hypercapnic for 48 hours by exposure to 10% CO_2. Chronic hypercapnic rabbits had significantly higher pCO_2 than controls (71±5 vs 37±1 mmHg). Plasma HCO_3 was higher (30±2 vs 24±2 mEq/l) and urine pH was lower (5.94±0.2 vs 8.12±0.1) indicating renal adaptation to chronic hypercapnia. In both control and hypercapnic animals, cortical luminal (L) membranes were enriched 14-fold in alkaline phosphatase over the homogenate. The kinetic activity of the Na-H antiporter was measured by the dissipation of the quenching of acridine orange by addition of different Na concentrations. Eadie-Hofstee analysis of these data showed a linear relationship, with the slope representing the K_m and the intercept, the Vmax. Chronic hypercapnic rabbits had significantly higher Vmax of the Na-H antiporter of L membranes than controls (593±81 vs 252±40 arbitrary fluorescence units, $p < 0.01$). However, the K_m was not different (12 vs 14 mM). The uptake of ^{3}H-glucose by L membranes was not different between control and hypercapnic rabbits. Thus, chronic hypercapnia induces a selective and specific increase in the Vmax of Na-H antiporter and this may mediate the adaptation to chronic hypercapnia.

EFFECT OF CHRONIC RESPIRATORY ACIDOSIS (CRA) ON THE INTRACELLULAR pH OF THE PROXIMAL TUBULE AS REFLECTED BY CHANGES IN pH SENSITIVE METABOLITES. B Trivedi*, L Cole*, and RL Tannen. Univ. of Michigan, Ann Arbor, Michigan.

In contrast to chronic metabolic acidosis, CRA does not result in an adaptation in either renal ammonia or glucose production. To examine the possibility that this might be explained by a difference in proximal tubule intracellular pH, the response of two pH sensitive metabolites, α KG and citrate, was analyzed.

Metabolic acidosis of 3 days' duration, induced by drinking 1.5% NH_4Cl, significantly reduced urinary citrate excretion (237 to 22 umols/day), and renal cortical citrate (1.13 to 0.88 umols/g) and αKG (.97 to .48 umols/g) concentrations in comparison with pair fed normal rats. CRA, produced by 3 days in a 10% CO_2 environment, lowered systemic pH similar to metabolic acidosis. However, urinary citrate excretion (201 umol/day) and renal cortical citrate (1.18 umol/g) and α KG concentrations (1.07 umol/g) were unaffected by CRA in comparison with pair fed controls. Both metabolic and respiratory acidosis of 1 day's duration decreased urinary citrate excretion as well as renal cortical citrate and α KG concentration.

These data suggest that proximal tubular intracellular pH falls with short term acidosis of either metabolic or respiratory etiology. However, intracellular pH appears to return to normal when respiratory acidosis persists, while it remains low with metabolic acidosis. If the filtered HCO_3 load modulates intracellular pH by influencing the rate of proximal tubule H^+ secretion, this divergent pH response may be explained by the persistence of a low plasma HCO_3 with metabolic acidosis, and by the progressive increase in plasma HCO_3 in response to respiratory acidosis.

CO_2 CAUSES EXOCYTOSIS BY INCREASING CALCIUM IN TURTLE BLADDER EPITHELIAL CELLS. Janet van Adelsberg* and Q. Al-Awqati, Columbia Univ., New York, N.Y. CO_2 stimulates H^+ secretion by causing exocytosis of vesicles whose membranes contain H^+ pump. We recently found that exocytosis and H^+ secretion are blocked by MAPTAM, an agent that buffers intracellular calcium (Ca_i). To measure the effect of CO_2 on Ca_i directly, turtle bladder epithelial cells were isolated and loaded simultaneously with esters of the fluorescent Ca^{+2} indicator Quin2 and of the pH indicator 6-carboxyfluorescein (6-CF), which are hydrolyzed to their active forms in the cell. Cell pH (pH_i) was measured fluorometrically using the change in 490/460 excitation ratio of 6-CF. Ca_i was measured comparing the fluorescence of intracellular Quin2 with its fluoresence after cell lysis at external calcium concentrations of 1mM and 0mM.

Ca_i was found to be 101±15nM (n=9). CO_2 transiently increased Ca_i to 228±99nM (n=4). This effect was probably caused by a decrease in pH_i, since 10mM butyrate, which decreased pH_i by 0.32±0.09 units (n=3), also increased Ca_i to 210±43nM. In addition, both 10mM NH_4Cl and 0.5mM acetazolamide increased pH_i by 0.80±0.10 units and decreased Ca_i to 67±12nM (n=4). (All changes $p<0.05$.) Low pH (<5.00) quenches the fluoresence of Quin2; pH_i was never in this range.

These results suggest that pH_i affects Ca_i. Since CO_2 is known to decrease pH_i, these results also suggest that CO_2 causes exocytosis by reducing pH_i which in turn increases intracellular calcium.

EFFECT OF PRIOR POTASSIUM DEPLETION (KD) ON NH_4Cl-INDUCED ACUTE CHANGES IN PLASMA [K]. Carlos A. Vaamonde, James R. Oster, Helen C. Alpert*, and Genaro R. Rodriguez*. VAMC & Dept. Med., Univ. Miami, Miami, FL.

It is unclear if KD prevents acidosis-induced increases in plasma [K]. If it does not, this might enhance urinary K loss. We produced KD in seven female mongrel dogs in 12 days with desoxycorticosterone acetate (DOCA), NaCl added to the drinking water, and a K-free diet. Four control dogs (C) received regular chow, identical NaCl supplements, and sham injections of DOCA. The achievement of KD was shown by differences between KD and C in both baseline plasma [K] and muscle [K] (C: 30±1, DK: 21 ± 1 mEq/100 g, $p<0.001$). Plasma [K] in the 3 hr following NH_4Cl (5 mEq/kg IV in 0.45% NaCl) were:

	baseline (B) (mEq/L)	max (mEq/L)	max-B (mEq/L)	max-B (%)
C	3.5±0.1 (3.3-3.7)	5.3±0.5 (4.3-6.6)	1.8±0.4 (1.0-3.0)	51±12 (30-84)
p	<0.001	<0.025	NS	NS
KD	1.9±0.1 (1.7-2.2)	2.5±0.1 (2.1-3.0)	0.6±0.1 (0.3-1.1)	31±5 (14-54)

X ± SEM, ranges in parentheses. NS=p>0.05

Despite virtually identical baseline values, minimal pH and [HCO_3] after NH_4Cl were somewhat lower in C (C: 7.13±0.03, 11.7±1.3 mEq/L; KD: 7.17±0.03 [NS], 13.4±0.7 [NS]). Plasma [K] increased both in C ($p<0.025$) and KD ($p<0.005$), but less (p = NS) in KD. Thus, KD reduces but does not prevent acidosis-related increments in plasma [K]. Whether KD attenuates the degree of metabolic acidosis induced by NH_4Cl requires further study.

CARBONIC ANHYDRASE-INDEPENDENT HCO_3 REABSORPTION IN THE RAT: EFFECT OF ETHOXZOLAMIDE (EZ). D.E. Wesson* and J.P. Frommer, V.A Med. Ctr., Baylor Col. of Med., Houston, Tx.

The present studies examined the effects of the liposoluble carbonic anhydrase inhibitor EZ on HCO_3 reabsorption by the rat kidney and were compared to the effects of acetazolamide (AZ). Cortical and papillary micropuncture was used in 39 Munich-Wistar rats. Tubular fluid was sampled from the late proximal (LP); and early distal tubule (ED), base (B) and tip (T) of the papillary collecting duct. Total CO_2 (tCO_2) was determined by microcalorimetry. Three groups were studied: 1) EZ (10 mg/kg BW/hour i.v.) (N=22); 2) AZ-50 (50 mg/kg BW/ hr i.v.) (N=11); and 3) AZ-20(20 mg/kg BW/hr i.v.) (N=6). Fractional deliveries of tCO_2 ($FDtCO_2$%) (mean±SEM) to the micropuncture sites are depicted in the table:

	LP	ED	B	T
EZ	85.4±8.0	52.4±5.2	31.7±4.6	24.6±3.9*
AZ-50	84.2±8.2	59.4±5.1	--	--
AZ-20	79.6±10.1	52.1±10.1	--	--

*=p<0.01 B vs T

Fractional excretion of HCO_3, and $FDtCO_2$ at LP and ED were the same for the 3 groups of animals. There is significant tCO_2 reabsorption between B and T in the rats treated with EZ. We conclude that 1) the more liposoluble carbonic anhydrase inhibitor EZ does not have any additional inhibitory effects on carbonic anhydrase-independent HCO_3 reabsorption in the kidney of the rat as compared to AZ; 2) administration of AZ in the above described doses most likely totally inhibits carbonic anhydrase in the kidney of the rat. These findings confirm our previous studies that there is significant carbonic anhydrase-independent HCO_3 reabsorption by the papillary collecting duct.

TRANSPORT OF BICARBONATE BY THE AMPHIBIAN NEPHRON. C.B. Yucha* and L.C. Stoner. Upstate Medical Center, Syracuse, NY.

Unlike the mammal, the primary site of HCO_3^- reabsorption in the amphibian is thought to be the distal nephron. To determine the site of urinary acidification, tubules from four different segments of the salamander nephron (Ambystoma maculatum) were perfused (8-9 nl/min) in vitro. Both perfusate and bath solutions contained 25 mM bicarbonate. Bicarbonate transport rates were calculated from microcalorimetric measurements of total CO_2 content in the perfusate and collected tubular fluid. Bicarbonate was not transported by the proximal tubule (10.3±5.4 pmol·mm^{-1}·min^{-1}, p:ns, n=4), diluting segment (7.8±6.2 pmol·mm^{-1}·min^{-1}, p:ns, n=6), or the mid-distal tubule (-16.9±11.2 pmol·mm^{-1}·min^{-1}, p:ns, n=7). Because the late distal (LDT) and initial collecting tubules (CT) appeared structurally similar and were difficult to differentiate, data from these late distal nephron segments were pooled. Bicarbonate reabsorption was observed in these segments (34.6±11.7 pmol·mm^{-1}·min^{-1}, p<.01, n=11). When the carbonic anhydrase inhibitor ethoxzolamide (10^{-4} M) was added to the perfusate and the bath in 3 LDT and 4 CT, bicarbonate reabsorption was significantly reduced from 26.0±7.5 to 3.9±8.2 pmol·mm^{-1}·min^{-1} (p<.05). Ethoxzolamide had no significant effect on transepithelial voltage (-0.1±0.6 mv vs 0.5±0.4 mv, p:ns, n=7). From these data it is concluded that bicarbonate is reabsorbed by the late distal and collecting tubules in the amphibian by an electroneutral mechanism that is dependent largely on carbonic anhydrase.

ATP-DEPENDENT INTRACELLULAR pH (pHi) REGULATION IN RABBIT MEDULLARY COLLECTING DUCT (MCD) CELLS.

ML Zeidel*, P Silva, and JL Seifter*, Brigham and Women's and Beth Israel Hospitals, Boston, Mass.

Using the flourescent intracytoplasmic pH dye, 6-carboxyflourescein (CF), we examined pHi regulation in suspensions of single MCD cells from rabbit outer medulla. Cells were separated by enzyme digestion, purified by Ficoll gradient centrifugation, and loaded with CF by incubation with CF-diacetate. pHi was determined using the flourescence excitation ratio, 492/450nm. at emission 530 nm. and standardized with the K/H ionophore, nigericin. Resting pHi was 7.19±.05 (S.E.) in nonbicarbonate medium, pH 7.30. Exposure of cells to acetate solutions at constant extracellular pH resulted in a rapid decrease in pHi followed by a gradual (3-5 min.) linear recovery to baseline pHi. Acid-loaded control cells recovered pHi at a rate of 2.72±.28x10^{-3} pH units/sec. This rate was unaffected by 0.5mM amiloride or by removal of extracellular Na^+, suggesting that Na/H exchange is not present in these cells. KCN, 2mM, reduced cellular ATP levels by 95% in the absence of glucose, but by only 40% in its presence. Acid-loaded cells exposed to KCN in the absence of glucose exhibited a pHi recovery rate 49±5% of that observed in cells poisoned in the presence of glucose. ATP dependence of pHi recovery was also observed in cells acid-loaded by pretreatment in NH_4Cl followed by dilution into ammonium-free medium. Neither the resting pHi nor the buffer capacity were altered in ATP-depleted cells. Ouabain, lmM, did not alter pHi recovery. These results suggest the presence of an H-ATPase in MCD cells which may play a role in pHi regulation and urinary acidification.

RENAL PHYSIOLOGY—HEMODYNAMICS

RENAL HEMODYNAMIC RESPONSE TO CONTRAST MEDIA: EVIDENCE FOR MEDIATION BY INTRARENAL ADENOSINE. L.J. Arend*, C.I. Thompson*, and W.S. Spielman. Michigan State University, East Lansing, MI.

The intrarenal injection of contrast media (CM) results in a transient fall in renal blood flow (RBF) and a decrease in glomerular filtration rate (GFR). These effects are enhanced by dietary sodium restriction and attenuated by sodium-loading. A similar sodium-dependent response in RBF and GFR occurs with the intrarenal injection of adenosine. In view of these similarities, we sought to determine if the renal hemodynamic response to CM is mediated by endogenous adenosine. The intrarenal injection of CM (meglumine/Na diatrizoate-76%) in 6 sodium-depleted, anesthetized dogs resulted in a 22 ± 3% decrease in RBF and a 36 ± 9% decrease in GFR. During the intrarenal infusion of dipyridamole (DP, 24 ug/kg/min), a potentiator of adenosine through its action to inhibit the cellular uptake of nucleosides, the hemodynamic response to CM was significantly greater (p <.05); RBF fell 31 ± 3% and GFR fell 44 ± 7%. This enhanced response to CM during DP was reversed by the intrarenal infusion of the adenosine receptor antagonist, theopylline (5 umol/min); RBF was decreased 17 ± 2% and GFR decreased 22 ± 4% (p <.05). The urinary excretion of endogenous adenosine increased following the injection of CM (472 ± 79 vs 874 ± 255 nmol/min). In summary, the CM-induced fall in RBF and GFR was augmented by dipyridamole and attenuated by theophylline, and the administration of CM resulted in an increase in the excretion of endogenous adenosine. These results suggest that endogenous adenosine mediates the renal hemodynamic response to CM.

POSTPRANDIAL RENAL HEMODYNAMICS IN HUMANS. P.S. Avasthi, E.R. Greene*, W.F. Voyles*, D.G. Fisher*. VAMC and Lovelace Foundation, Univ. of New Mexico, Albuquerque, NM.

Postprandial increase in renal blood flow (RBF) has been demonstrated in healthy humans. However, the temporal relationship of postprandial changes in RBF and cardiac output have not been investigated.

Using a noninvasive echo Doppler method to measure RBF (J. Ultrasound Med. 3:208, 1984), we determined phasic blood flow in the right renal artery (RRBF). Cardiac outputs (CO) were concurrently measured (AHJ 107:339, 1984). Both measurements were made before and at 30, 60, 90, 120, 180 and 240 min following a meal in 4 healthy adults (2F) after 18 hours of fasting. The meal consisted of 150g protein, 30g fat and 30g carbohydrate in a 500 ml volume. Results are given as mean ±SD.

	Basal	30'	60'	90'	120'	180'	240'
RRBF (ml/min)	559 ±86	517 ±46	555 ±62	604* ±54	643* ±52	692* ±61	687* ±55
CO (L/min)	5.84 ±0.56	7.04* ±1.26	8.19* ±1.08	8.02* ±0.92	7.56* ±1.13	7.85* ±1.02	7.52* ±1.63

*indicates p value <.05 versus basal value.

Although CO increased significantly within 30 min, RBF remained constant during the first 60 min postprandial. RBF increased significantly between 90 and 120 min and remained elevated up to 240 min. These noninvasively obtained data in humans confirm that RBF increases postprandially and provide the temporal profile of postprandial changes in RBF. Furthermore, these results suggest that the increases in RBF are temporally dissociated from the postprandial increases in CO.

DOSE DEPENDENT MOVEMENT OF CATIONIC MOLECULES ACROSS THE GLOMERULAR WALL. Sally B. Bates* and Peter M. Andrews. Georgetown University Medical Center, Washington, D.C.

Different concentrations of the polycation polyethyleneimine (PEI) were either injected intravenously or administered by vascular perfusion through the kidneys. At fixed time intervals after administration of PEI, the kidneys were fixed and the distribution of PEI in the glomerular wall evaluated by electron microscopy. At the lower concentrations, PEI bound only to the glomerular endothelial glycocalyx and preferentially to the villous-like projections on this endothelium. At higher concentrations, PEI also bound to discrete anionic sites in the lamina rara interna (LRI). At higher concentrations, PEI was also seen to move deeper into the GBM and bind to discrete anionic sites in the lamina rara externa (LRE). Although anionic sites in the LRI and LRE appeared nearly saturated with PEI, this cationic molecule was rarely seen to cross filtration slits and pass into the urinary space. At higher concentrations, however, PEI appeared to move freely across the filtration slits and bind extensively to the glomerular epithelial glycocalyx. When high concentrations of PEI were vascularly perfused through the kidneys, PEI binding to the epithelial glycocalyx caused adherence of adjacent podocyte processes and the narrowing of filtration slits. Also, in these latter samples, PEI was diffusely distributed throughout the GBM, discrete anionic sites in the LRE were no longer apparent ,and a dense band of PEI was localized under foot processes. The above correlation between glomerular permeability and polycation concentration may result from a neutralization of anionic sites in the glomerular wall and /or consequent changes in the GBM gel-like structure.

EVIDENCE OF A DEFECTIVE TUBULO-GLOMERULAR FEEDBACK (TGF) CONTROL IN YOUNG RATS OF THE MILAN HYPERTENSIVE STRAIN (MHS). U.Boberg, G.Bianchi, A.E.G. Persson (Intr. by F Wright). Depts Physiology & Urology, University of Uppsala, Sweden.

It is earlier documented that MHS rats have a different GFR, salt and water regulation than age-matched Milan normotensive strain (MNS) rats. Therefore we investigated the urine salt excretion, GFR,interstitial subcapsular hydrostatic (P_{sc}) and oncotic (π_{int}) pressures in one series of experiments and the TGF in another series of young animals. MHS rats 4-5 weeks old (MHS_p) had no hypertension (93 mm Hg) but 5-7 weeks old they developed hypertension (MHS_d) (109 , control 92 mm Hg).

The TGF was examined by proximal tubular stop-flow pressure (P_{sf}), the maximal drop in P_{sf} (ΔP_{sf}) that could be released and the tubular flow rate that released a half maximal response, the turning point (TP). GFR was similar in all groups during hydropenia (HP) but during (5 % B.W.) volume expansion (VE) in MNS animals there was an increase with 40-50 % while in all MHS no increase was observed. Urine flow and sodium excretion rates were higher in MHS than in MNS during both HP and VE. The P_{sc} was significantly higher in the MHS_p,2.9 mm Hg, than in all other groups (control 0.9 mm Hg) and in MHS_p the TGF response was absent in all situations. In the MHS_d , TGF sensitivity was significantly higher than in the MNS controls with a TP of 14 nl/min (control 21 nl/min) and during VE this high sensitivity persisted in contrast to a decreased sensitivity in the control.

Thus, in MHS_p the lack of GFR increase during VE might be due to absent TGF that in turn was caused by the high P_{sc}. In MHS_d the absent GFR increase during VE could be caused by the high sensitivity of the TGF. Thus, altered TGF response seems important for altered GFR and electrolyte handling during development of arterial hypertension in the MHS rats.

TUBULO-GLOMERULAR FEEDBACK ACTIVITY (TGF) IN 12 DAY PREGNANT (12P) RATS. C. Baylis* and R.C. Blantz, Dept. of Med., UCSD and VAMC, La Jolla, CA.

GFR and nephron filtration rate (SNGFR) are increased and plasma volume is expanded (VE) at 12P. VE is known to suppress TGF which might cause increased SNGFR. Using microperfusion techniques, we examined TGF in 12P and virgin (V) rats by monitoring proximal SNGFR changes while late proximal tubules were perfused at 0, 10, 20 and 40 nl/min with artificial fluid. Also, proximal-distal SNGFR differences (P-DΔ) were measured to determine actual late proximal flow rate (V_F) and the corresponding (distal) SNGFR. In V rats % reductions in SNGFR from the maximum (0 nl/min perfusion rate) values were: 10 nl/min, -1±3% (NS); 20 nl/min, -15±5% ($p<0.01$) and 40 nl/min, -23±4% ($p<0.001$). In P rats, % SNGFR reductions were: 10 nl/min, -4±3% (NS), 20 nl/min, -29±4% ($p<0.001$) and 40 nl/min, -32±4% ($p<0.001$). SNGFR in rats in which P-DΔ and perfusion responses were conducted were as follows: *=$p<0.01$

Perfusion (nl/min)	0	10 (nl/min)	20	40	P-DΔ
V	26±2	28±3	22±2*	18±2*	6±1*
12P	34±2	34±3	23±3*	20±2*	5±1*

The true V_F in V rats was 13±1 nl/min. At this V_F, TGF appeared maximally activated, as assessed by the large % P-DΔ observed. In P rats V_F was 17±2 nl/min which was near the mid-point of the maximum TGF response. TGF responses in P rats are intact in spite of VE, and all absolute values for SNGFR and actual V_F are shifted upward and rightward in proportion. This shift in the TGF curve in P could be due to primary effects on SNGFR or tubular reabsorption to which the TGF system adapts appropriately.

LACK OF EFFECT OF DOPAMINE-4-SULFATE ON RENAL VASCULATURE. T.Bradley*, P.Hjemdahl*, L.I.Goldberg*, G.F.DiBona, B. Osikowska*, P.Sever*. Pharm., Karol. Inst., Stockholm, Sweden; Clin. Pharm., Univ. Chicago, Chicago, IL; Int. Med., Univ. Iowa, Iowa City, IA; Clin. Pharm., Univ. London, London, U.K.

Dopamine-4-sulfate (DA-4-S) is found in high concentrations in canine and human plasma. We studied the effect of DA-4-S on renal vascular α-adrenoceptors and DA receptors in 4 anesthetized dogs. Two methods were used for synthesis of DA-4-S (Biochem. J. 135:109, 1973; Biochem. Pharmacol. 31: 2279, 1982). TLC and NMR, respectively, were used to verify the synthesis product. After each experiment the amount of free DA and DA-4-S in the DA-4-S solution was determined by HPLC. In the DA-4-S solution from 2 experiments there were detectable amounts of DA (0.75 and 0.91%, respectively). The renal blood flow (RBF) response to the amount of DA in each injection of the DA-4-S solution was interpolated from each dog's individual dose response curve for DA and subtracted from the response to the DA-4-S solution. Phenylephrine (Phe), 49 or 98 nmoles; DA, 0.08 nmoles/kg to maximal renal vasodilation at 100-340 nmoles/kg; and DA-4-S, 0.08-309 nmoles/kg, were given as bolus doses in 0.2 ml normal saline into the renal artery. DA caused dose-dependent renal vasodilation with a maximal increase in RBF of 32±5%, whereas, DA-4-S had no effect on RBF. The injections of Phe, DA, and DA-4-S were repeated after a phenoxybenzamine (POB) infusion into the renal artery. The vasoconstrictor response of Phe was then abolished,the vasodilation caused by DA was larger than before POB (57±12% increase in RBF), and DA-4-S had no effect on RBF. Thus, DA-4-S has no agonist activity on α-adrenoceptors or post-junctional vascular DA receptors.

DISSOCIATION BETWEEN STOP FLOW PRESSURE (P_{SF}) AND FILTRATION RATE RESPONSE TO CHANGING LOOP OF HENLE FLOW RATE (V_{LP}). Josephine P. Briggs, and Jürgen Schnermann*. University of Munich, Department of Physiology, Munich, West Germany.

Both SNGFR (or early proximal flow rate, V_{EP}) and P_{SF} have been used as indexes of glomerular function in studies of the tubuloglomerular feedback mechanism, but comparison in the same nephron has not been made. The effect of varying V_{LP} on V_{EP} and P_{SF} was assessed in Sprague-Dawley rats by paired measurements of both variables in 8 nephrons at 6 rates of loop perfusion (0,10,15,20,30,40 nl/min). With maximum flows, V_{EP} fell 14.0±1.5 nl/min and P_{SF} fell 11.4±1.4 mm Hg from the non-perfused value. The percentage of the total response in each flow interval was:

(nl/min)	0-10	10-15	15-20	20-30	30-40
V_{EP}	25±10	52±12	20± 4	4±10	-2± 4
P_{SF}	6± 3	29± 9	48± 9	19± 7	-3± 3

In the subnormal flow range (<15) P_{SF} was much less sensitive to changing loop flow than was V_{EP}. Both V_{EP} and P_{SF} could be described by a sigmoidal function of V_{LP}, using a computerized method to obtain curve parameter estimates for each nephron. The flow rate at which the response was half-maximum differed significantly (13.0±1.2 nl/min for V_{EP} and 16.7±0.8 nl/min for P_{SF}, $p<0.02$).

We conclude that in the subnormal flow range P_{SF} (and probably glomerular capillary pressure) is less sensitive to changes in loop flow than is filtration rate. Previous studies have shown that in the supranormal flow range increasing V_{LP} constricts the afferent arteriole. The present finding suggests that in the subnormal flow range the response is a balanced constriction of the afferent and efferent arterioles.

ALTERATIONS IN RENAL VASOACTIVE HORMONAL SYSTEMS IN DOGS WITH CHRONIC LOW CARDIAC OUTPUT (CO). S.Y. Chou, D.G. Blackstock*, P.F. Faubert and J.G. Porush. Division of Nephrology and Hypertension, Brookdale Hospital Medical Center, Brooklyn, N.Y.

In chronic low CO states associated with Na retention, the renal vasoactive hormonal systems have not been characterized. In the present studies, systemic and renal hemodynamics and renal output of norepinephrine (NE), renin and PGE_2 were determined in anesthetized normal and chronic caval dogs (constriction of the thoracic inferior vena cava, edema and ascites). NE was measured by the radioenzymatic method and PGE_2 by radioimmunoassay after extraction. Results (Mean±SE):

				NE		PRA		PGE_2	
	MAP	CO	RBF	A	RV	A	RV	A	RV
	mmHg	L/min	ml/min	pg/ml		ng/ml/min		pg/ml	
Normal	140	3.2	207	101	147*	2.5	3.1*	19	30*
(n=7)	±4	±.4	±15	±12	±25	±.4	±.6	±2	±3
Caval	135	1.9	201	387	637*	13.6	21.6*	24	74*
(n=7)	±5	±.2	±13	±43	±58	±2.1	±3.5	±3	±8
P <	NS	.02	NS	.01	.01	.01	.01	NS	.01

MAP=mean arterial pressure; RBF=renal blood flow; PRA=plasma renin activity; A=arterial plasma; RV=renal venous plasma; *P<.01 vs A.

Renal output of NE, renin and PGE_2 was 5-fold, 12-fold and 4-fold greater, respectively, in caval than normal dogs. GFR was similar in the 2 groups with Na excretion markedly reduced in caval dogs. These data demonstrate that in chronic caval dogs with low CO and avid Na retention, vasoconstrictor systems (adrenergic and renin-angiotensin) are activated, contributing to maintenance of arterial blood pressure and are opposed by an augmented renal vasodilator system (PGE_2), which may be responsible for maintaining normal renal hemodynamics.

HEMODYNAMIC EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITOR (CEI) IN EXPERIMENTAL CHRONIC PARTIAL URETERAL OBSTRUCTION (CPUO). Robert L. Chevalier and Anthony V. Broccoli, Univ. of Virginia, Dept. of Pediatrics, Charlottesville, Virginia.

Unilateral CPUO results in significant vasoconstriction of the ipsilateral kidney (Pediatr Res 18:359A,1984). To evaluate the role of endogenous angiotensin II in this vasoconstriction, guinea pigs subjected to constriction of the left ureter during the first 2 days of life were given enalapril, a CEI, 6 mg/day for 7-12 days prior to study (N=11). These were compared to a control group of littermates receiving no drug (N=11). Renal blood flow (RBF,ml/min), renal vascular resistance (RVR, mmHg/ml.min) and number of perfused glomeruli (NPG, thousands) were measured at 21-26 days of age (mean ± SE, ns = P>0.05):

	Left (obstructed) Kidney			Right (intact) Kidney		
	RBF	RVR	NPG	RBF	RVR	NPG
Enalapril	4.5	14.1	79.5	9.8	6.3	83.7
±SE	0.6	1.6	2.4	1.1	0.7	2.1
Control	2.8	24.4	64.2	8.9	7.9	78.8
±SE	0.2	2.0	5.1	0.8	0.8	2.6
P<	0.03	0.01	0.03	ns	ns	ns

While the intact kidney was unaffected by enalapril administration, RVR was reduced and NPG increased in the obstructed kidney. This resulted in a 60% increase in RBF of the obstructed left kidney. We conclude that angiotensin II plays a significant role in reduction of RBF during CPUO, and acts in part by diminishing the proportion of perfused glomeruli.

PERSISTENT PROTEINURIA FOLLOWING PRESSURE REGULATION IN THE PROTAMINE SULFATE PERFUSED RAT KIDNEY. G. Closkey* N. Jermanovich, A. DeGuzman*. Jefferson Medical College, Philadelphia, PA.

Protamine Sulfate (PS) causes epithelial cell fusion, proteinuria and vasoconstriction in the isolated perfused rat kidney (IPK). The contribution of hemodynamic factors to vascular permeability in this model is unclear. The effect of vasodilation on protein excretion in PS perfused kidney was evaluated using either isoproterenal(10 ngm/ml), dopamine(1.2 ugm/ml) or histamine(100 ugm/ml). Kidneys were perfused for 120 min under standard conditions with KHB, 5%BSA and 5%RBCs. Post control(50min) vasodilators were given 5 min before PS(500 ugm/ml) infusion. Vasoconstriction was not altered by the above regimen. However histamine in combination with manual pressure reduction maintains adequate GFR although proteinuria persists.

PROTAMINE (N=4)	40min	50min	70min	110min
RBF (ml/min)	15.7	12.2	9.7	10.5
BP (mm Hg)	101	133.5	159.5	163.7
GFR (ml/min/gm)	.83	.26	.17	.22
PROTEIN (mg%)	11.6	25.5	289.0	653.1†
HISTAMINE (N=3)				
RBF (ml/min)	16.5	23.6	27	28.3
BP (mm Hg)	103	96	72	80
GFR (ml/min/gm)	1.17	1.07	1.04	.97
PROTEIN (mg%)	17.0	18.8	14.6	13.0
HISTAMINE + PS (N=5)				
RBF (ml/min)	20	21	10	9
BP (mm Hg)	97	92	107	108
GFR (ml/min/gm)	.70	.66	.24	.35
PROTEIN (mg%)	11.3	20.4	269.5	950.8†

†not statistically significant

Hypertension does not appear to be a significant factor in PS induced capillary permeability.

ATRIOPEPTIN-INDUCED NATRIURESIS DEPENDS ON RENAL VASODILATION. B.R. Cole, M.A. Kuhnline‡ T.Oshima‡ K. Wakitani‡ M.G. Currie‡ and P. Needleman‡ Washington U.Sch. of Med., Depts. of Peds. and Pharmacol., St. Louis, MO.

Atrial peptides are natriuretic and relax precontracted smooth muscle strips. Synthetic Atriopeptin I(AP I), a 21 amino acid peptide which selectively relaxes intestinal smooth muscle, is natriuretic when injected into intact, anesthetized rats but has no effect in dogs. To study possible mechanisms of action, we evaluated AP I-induced vasodilation and natriuresis in the 2 species. During i.v. infusions of 0.5 to 5 μg/kg/min AP I, renal blood flow (RBF), measured by microspheres in rat and renal artery flow meter in dog, glomerular filtration rate (GFR), blood pressure (BP) and Na+ excretion ($U_{Na}V$) were measured. Data expresses mean per cent of control ± S.E.M. *p<.05

Time of infusion (min)	B.P.	RBF	GFR	$U_{Na}V$
		RAT (N=5)		
5	86±5*	113±4*	94±6	201± 21
30	76±6*	120±2*	136±10*	1942±471*
		DOG (N=3)		
5	99±1	99±2	103± 6	103± 7
30	99±2	103±3	110±18	137± 19

AP I produces increased RBF and natriuresis in the rat, but in the dog, there is no change in RBF and $U_{Na}V$ does not increase. We believe that the renal vasodilation caused by atriopeptins is a major determinant of the increased Na excretion.

SYSTEMIC AND RENAL VASCULAR RESPONSE TO VASOCONSTRICTORS DURING PREGNANCY: SERIAL STUDIES IN CONSCIOUS RATS. Kirk P. Conrad* and Mary C. Colpoys* (intr. by H. Valtin). Dept. of Physiol., Dartmouth Medical School, Hanover, NH.

We examined the pressor response to angiotensin II (AII) and norepinephrine (NE) before and after indomethacin (I; 7mg/kg; N=5 rats) and meclofenamate (M; 6mg/kg; N=4). We also assessed the response of renal hemodynamics to AII ($5ng \cdot kg^{-1} \cdot min^{-1}$ i.v.) before and after I (N=5). Beginning 7-10 days after implantation of catheters, each rat was studied before mating, and during pregnancy.

V:vehicle	ΔMAP(mmHg) Virgin V	Virgin I	Day 20 V	Day 20 I	Virgin V	Virgin M	Day 20 V	Day 20 M
AII 3.1 ng/kg	-	-	-	-	8	9	5	7
6.3	-	-	-	-	12	12	8	8
12.5	22	25	13†	12	27	26	13†	12
25	36	36	21†	24	39	38	17†	19
50	45	44	29†	31	-	-	-	-
NE 50 ng/kg	-	-	-	-	13	11	5†	6
100	21	24	9†	11	18	18	7†	10
200	30	33	18†	17	27	30	15†	14
400	40	42	21†	23	-	-	-	-

P<0.05 virgin vs. pregnant†	Virgin AII+V	Virgin AII+I	Day 19 AII+V	Day 21 AII+I
MAP	+20	+21	+12	+10†
GFR %Δ	-15	-15	-2†	+7†
ERPF	-34	-36	-16†	-8†
RVR(MAP-5/ERBF)	+89	+110	+37†	+21†

Both the systemic and renal vasculature are refractory to exogenous vasoconstrictors in the rat during late pregnancy. Cyclooxygenase inhibitors fail to reverse this apparent refractoriness, which suggests that it is not mediated by vasodilating prostaglandins.

EFFECT OF INDOMETHACIN (I) ON RENAL AND SINGLE NEPHRON (SN) HEMODYNAMICS IN 12 DAY PREGNANT (P) AND VIRGIN (V) FEMALE AND MALE (M) MUNICH-WISTAR RATS. R.C. Collins* and C. Baylis* (intr. by R.C. Blantz). Dept. Med., Univ. of Calif., San Diego, CA.

Pregnancy is associated with elevations in GFR, renal plasma flow rate (RPF) and prostaglandin (PG) production. These studies were performed to investigate the effect of acute PG synthesis inhibition (with I; 2 mg/kg bw/h) in euvolemic P, V and M rats on GFR, RPF, renal vascular resistance (RVR), SNGFR and glomerular plasma flow rate (Q_A).

		GFR	RPF	RVR	SNGFR	Q_A
		ml/min		mmHg/(ml/min)	nl/min	
V	C	0.81±0.03	2.2±0.1*	27.1±2*	25±1*	77.8±5*
	I	0.93±0.04*	2.7±0.1*	22.1±1*	29±2*	97.5±8*
P	C	0.95±0.04*	2.7±0.2*	23.6±2*	30±2*	104.8±11
	I	1.13±0.04*	3.3±0.2*	18.0±2*	36±2*	122.0±13
M	C	1.32±0.07	3.8±0.4	14.9±2	44±5	224±36
	I	1.27±0.10	3.4±0.4	14.4±1	43±7	186±42

(Mean±SE; all n=5; p<.05 by paired t-test, C vs I).

Surprisingly, PG inhibition led to significant *increases* in GFR, RPF, SNGFR and Q_A due to reductions in RVR in both P and V rats. In the M, no change in the measured variables occurred in response to I. Therefore, vasodilatory PGs do not appear to be responsible for increased GFR in midgestation, since the higher GFR seen in P vs V rats in control (C) was sustained (although reset at a higher level) during I (p<0.05). These studies did reveal a sex-related difference in the response to I; female rats whether P or V showed changes in renal hemodynamics characteristic of an I-induced renal vasodilatation, presumably by attenuation of a vasoconstrictor stimulus since RVR was significantly higher in P and V compared to M during C (p<0.005).

INFLUENCE OF ALBUMIN CONCENTRATION ON THE ULTRAFILTRATION BARRIER IN THE ISOLATED PERFUSED DOG GLOMERULUS. TA Fried, RN McCoy‡ RW Osgood* and JH Stein. Univ of Texas Hlth Sci Ctr, Dept of Med, San Antonio, Texas.

We have previously reported (ASN 1981) that when the perfusate albumin concentration of the in vitro isolated perfused dog glomerulus is decreased from 3.6 gm% to 0.1 gm% there is a small though significant increase in the ultrafiltration coefficient (Kf), 2.79 to 3.74 nl/min-mmHg ($p < .05$). This is supportive of the in vitro findings of Mason et al from mesenteric capillaries (Microvas Res 13: 185), but is in contrast to the in vivo findings of others that suggest a direct relationship between plasma protein concentration and Kf. We hypothesized that the discordance between the in vivo and in vitro studies may be explained by secondary systemic effects encountered in vivo. To further investigate the in vitro phenomena we studied the effect of decreasing the albumin concentration even further to levels < 0.005 gm%.

In these studies (n=4) Kf increased even further to 31.6 ± 12.8 (mean ± SE), $p < .05$. Three of these experiments included a second period (0.1gm%) to insure that the baseline values of this set of experiments were comparable to our earlier ones. In these three experiments Kf increased 12, 18 and 39 fold over baseline values.

We conclude that in vitro, at very low albumin concentrations, there is a marked increase in Kf as seen in other capillary beds and in support of our earlier findings. Further, when extremely low albumin concentrations are reached, an important role of albumin in the ultrafiltration barrier is demonstrable.

EFFECT OF INTERMITTENT FEEDING ON RENAL FUNCTION IN CONSCIOUS RATS. J.J. Gehrig, Jr.,* T.W. Meyer, R.L. Jamison,* C. Baylis, J.L. Troy,* B.M. Brenner, and R.L. Jamison. Dept. Medicine, Stanford Univ., Stanford, CA and Brigham and Women's Hospital, Boston, MA.

Excess protein intake has been shown to have adverse effects on renal function in anesthetized rats but the influence of diet in conscious rats is not known. We performed 56 clearance (Cl) studies in 20 unanesthetized male Sprague Dawley rats at mean age 30 wks. Group (Grp) 1 rats (N=10) were fed standard chow only on alternate days for 25 wks and were studied after a fed day (Grp 1A) and a fasted day (Grp 1B) while Grp 2 (N=10) were fed ad lib during the same period. Results (mean) for body wt (BW, g), mean arterial pressure (MAP, mm Hg), GFR and effective RBF (Cl inulin and Cl PAH/(1-Hct), ml/min), Na excretion ($U_{Na}V$, μM/min) and protein excretion ($U_{Pr}V$, μg/min) were:

Grp	BW	MAP	GFR	ERBF	$U_{Na}V$	$U_{Pr}V$
1A	396†§	119	4.25†§	22.2†§	3.5†	35
1B	383§	118§	3.47§	18.6§	1.3§	20
2	545	125	5.56	29.7	4.9	27

The results indicate that food intake substantially increases GFR and ERBF both acutely (Grp 1A vs 1B) and chronically (Grp 2 vs 1) in awake rats. This is associated with moderately increased Na excretion and no reduction in plasma protein conc or Hct, suggesting the hemodynamic effects were not mediated through volume expansion. † $p<0.03$, Grp 1A vs 1B; § $p<0.05$, Grp 1A or 1B vs 2.

SULINDAC (S) ACCENTUATES RENAL ISCHEMIA DURING HEMORRHAGE (H). **W.L.Henrich, D.C.Brater, and W.B.Campbell*, Dallas VAMC and SW Med Sch, Dallas, TX.**

The effect of S on renal function is controversial. In order to assess the impact of S on renal hemodynamics during acute circulatory stress, we measured GFR (ml/min), and RBF (ml/min), in anesthetized dogs before and after an acute 20% reduction in mean arterial pressure by H. Four groups of dogs (all n=5) were studied: Control (C), no drug; Indomethacin (I), 10 mg/kg IV before H; Benoxaprofen (B), 75 mg kg IV before H; and S, given as the sulfide .4mg/kg IV then .03 mg/kg min as an infusion. This dose of S sulfide resulted in plasma levels (3.6 μg/ml) comparable to therapeutic levels in humans and did not appear in the urine. Results: * <C dogs, $p<.01$

	C		I		B		S	
	PreH	H	PreH	H	PreH	H	PreH	H
GFR	56	30	38	15*	43	19*	39	16*
RBF	380	237	250	46*	156	45*	223	57*

Each drug prevented an increase in renal venous 6-keto $PGF_{1\alpha}$ (the stable metabolite of PGI_2) during H. In summary, each drug caused similar sharp decrements in renal hemodynamics (especially RBF) during H. We conclude that therapeutic plasma levels of S sulfide are capable of enhancing renal ischemia during ciculatory stress. This ischemic response appears related to PG synthesis inhibition.

CARDIOVASCULAR AND RENAL EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF) IN THE SPONTANEOUSLY HYPERTENSIVE (SHR) RAT. M. Gellai,* L.B. Kinter and R. Beeuwkes, Department of Pharmacology, Smith Kline & French Laboratories, Philadelphia, PA

Changes in blood pressure and renal hemodynamics were assessed in SHR and control (WKY) rats undergoing ANF-stimulated natriuresis.

Low dose (LD) (1 μg/kg + 2 μg/kg/hr) and high dose (HD) (10 μg/kg + 20 μg/kg/hr) of synthetic ANF, atriopeptin II (APII) were given iv for 90 minutes to conscious, trained SHR (n=5) and WKY (n=5) rats surgically prepared for renal clearance studies one week prior to the experiments. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by the clearance of polyfructosan (Inutest) and PAH, respectively. Blood pressure (MAP) decreased ($p<0.05$) in SHR rats with the LD and HD (192±5 to 173±5 and 182±4 to 146±5 mm Hg) and in WKY rats with the HD of APII (122±2 to 98±3 mm Hg). The changes in renal functions, expressed as % of control, were:

	LD		HD	
	WKY	SHR	WKY	SHR
GFR	99±8	101±5	88±10	100±7
ERPF	83±8	90±5	59±7*	69±3*
$U_{Na}V$	153±19*	189±32*	328±24*	338±22*

(* $p<0.05$)

Thus, APII is a potent systemic, but not a renal vasodilator in conscious SHR and WKY rats. Urine flow and the excretion of Na^+ and Cl^- increased, despite no change, or decreases in GFR, ERPF, and MAP.

We conclude that changes in GFR and/or ERPF can not account for ANF-stimulated natriuresis in Okamoto strain rats.

VARIATION IN EFFERENT ARTERIOLAR PLASMA PROTEIN CONCENTRATION REFLECTS VARIABILITY IN SAMPLING TECHNIQUE RATHER THAN TRUE INTERGLOMERULAR HETEROGENEITY. M. Hughes* & I. Ichikawa. Child. Hosp., Boston, MA.

Based on a wide variability seen in protein concentration (C) of postglomerular plasma collected by micropuncture from surface efferent arterioles (EA), it has been postulated that rat glomeruli are highly heterogenous in function. We tested if such a variability represents true interglomerular heterogeneity or technical inconstancy in collecting or assaying EA blood samples. The variance in C_{EA} (g/dl) of samples from randomly chosen EAs ($s^2_{inter\text{-}G}$) was compared to samples collected and recollected from the same EAs ($s^2_{intra\text{-}G}$) in 20 Munich-Wistar rats. During EA blood collection, oil was maintained with gentle suction downstream to the collection pipette to avoid disturbance of natural glomerular blood outflow. $s^2_{inter\text{-}G}$ averaged 0.083, a value not different from mean $s^2_{intra\text{-}G}$ (0.079), indicating that the degree of true interglomerular heterogeneity is too small to detect with this micropuncture technique. When 10 additional samples were collected using uncontrolled suction pressure, $s^2_{inter\text{-}G}$ averaged 1.13, a value markedly greater than that obtained with controlled pressure, yet comparable to some recently reported values. In 5 nephrons with surface glomeruli and EAs, glomerular capillary pressure (P_{GC}) was monitored during EA blood collection. Applying widely varied suction pressure, P_{GC} changed as much as 18 mmHg, indicating that improper suction pressure can alter glomerular pressures and flows, hence filtration fraction and C_{EA}. Thus, unlike renal tubules, EAs appear not to have a sensitive Starling resistor-like property, which may, in turn, account for the wide variability in C_{EA} reported previously.

METOCLOPRAMIDE (MCP) DECREASES RENAL PLASMA FLOW (ERPF) IN MAN. R. Israel*, B. Austin*, R. Meyer*, A. Bellucci*, and R. Mossey, Dept of Med., North Shore University Hospital, Manhasset, New York

High dose MCP is standard treatment for chemotherapy-induced vomiting. Although renal function abnormalities have not been reported, the antidopaminergic effect of MCP could cause a fall in ERPF. We tested this hypothesis in 8 patients (5 males, 3 females; age range 46 to 70 yrs., s. creatinine 1.0 ± 0.2 mg/dl) receiving MCP (0.7-2.0 mg/kg, I.v. b.w.) prior to chemotherapy. After at least 2 hrs. normal saline hydration (100 cc/hr.), ERPF was measured with I-131 Hippuran by single injection technique (Slekalns et al, J. Ped. 38:749, 1973). Pre MCP ERPF was not different from age matched control (test group, n = 8, 418 ± 168 vs. control, n = 7, 514 ± 72 cc/min; p: N.S.), but fell significantly to 368 ± 146 cc/min following MCP (2 tailed t test p <0.025). Two of the patients received MCP 0.7 and 1.2 mg/kg, I.v., b.w. respectively and had <3% change in ERPF. The other 6 patients were given MCP 2 mg/kg, I.v., b.w. and had a mean decrease in ERPF of 14.5% (range 6.0 to 26%). Blood pressure, body weight and s. creatinine did not change. We conclude that despite hydration, I.v. MCP (2 mg/kg, b.w.) decreases ERPF in man. Our study suggests that dopaminergic stimuli may play an important role in maintaining ERPF even during isotonic volume expansion. Alternatively, the fall in ERPF could be the result of the direct effect of the drug. Although further studies are necessary to evaluate the clinical significance of these findings, high dose MCP should be used with caution especially when administered together with other nephrotoxins.

GFR FAILS TO INCREASE FOLLOWING PROTEIN INGESTION IN GROWTH HORMONE DEFICIENT ADULTS. Kenneth S. Kleinman* and Richard J. Glassock. Department of Medicine, Harbor-UCLA Medical Center. Torrance, California.

Growth hormone (GH) levels have been reported to be elevated in patients with poorly controlled type I diabetes mellitus in association with enhanced GFR. These abnormalities often return to normal with improved glycemic control. In short term studies, an elevation in GFR has been observed in normal human adults fed large quantities of protein, but this change is blunted in patients with renal disease. The mechanism by which protein causes augmentation in GFR is currently unknown. In order to study a possible role of GH in the response of GFR to a protein meal we studied four chronically GH deficient adults and age matched controls, free of renal disease, before and after ingestion of a protein meal as red cooked meat. GFR, measured by inulin clearance and corrected for body surface area, was 18.5% lower during basal conditions (89.1± 6.0 ml/min) in the GH deficient population when compared to controls (109.7±9.1 ml/min) but this difference did not achieve statistical significance (p> 0.1, <0.2). For a three hour postprandial period, mean GFR increased by 25.3% in the control population (136.8±10.7 ml/min) (p<0.02) while mean GFR failed to increase in the GH deficient patients (86.8±10.9 ml/min) (p>0.25). Male control subjects, not pretreated with diethylstilbestrol, had no change in GH levels (p>0.2). However, the one female control was found to increase GH levels ten times preprandial levels. These studies suggest that GH itself or a growth-hormone dependent factor may be mediator in the augmentation of GFR observed following a large protein load in human adults.

RELIABILITY OF CLASSICAL CLEARANCE TECHNIQUES. RW Katzberg*, RC Pabico, TW Morris*, BA McKenna*, HW Fischer*, Depts. of Radiology and Medicine, Univ. of Roch. Med. Ctr., Rochester, NY 14642

A widely accepted measure of whole kidney GFR is the plasma clearance of inulin: UV/P. Theoretical considerations and indirect experimental evidence have justified wide acceptance. A less widely employed method to assess GFR uses the plasma extraction radio of inulin (arterial (A) minus renal venous concentration (V)/arterial concentration (A): A-V/A) multiplied by renal plasma flow (RPF). The purpose of this study was to directly compare these determinations in steady state and non-steady state conditions. Animal preparation (8 anesthetized dogs) consisted of cannulation of the left renal vein and aorta at the level of the left renal artery; catheterization of the left ureter; measurement of RPF by electromagnetic probe; and a priming dose of 10% inulin with infusion to provide a continuous plasma level of 15 mg/dl. Steady state conditions (no treatment; i.v. 0.9% saline; n=17) and non-steady state conditions (2 cc/kg 25% mannitol or 2 cc/kg 76% meglumine/sodium diatrizoate; n=25) were assessed. The anticipated decrease in GFR with osmotic diuresis was observed using the A-V technique; whereas, an increase in GFR was noted using the UV/P technique. There was no correlation between these two methods (r=0.01; n=42, p>0.05). Our results suggest greater reliability with the A-V extraction technique for GFR determinations.

REVERSAL OF PROSTAGLANDIN-INDUCED VASOCONSTRICTION BY DILTIAZEM: STUDIES IN ISOLATED SMOOTH MUSCLE AND PERFUSED RAT KIDNEY. Rodger Loutzenhiser*, Charles Horton*, Phillip Sonke*, and Murray Epstein. Nephrol. Sect., V.A. Med. Ctr. & Univ. of Miami Sch. Med., Miami, FL 33125

U44069, a PGH_2 analogue and potent vasoconstrictor, mimics many of the actions of thromboxane A_2. The effects of U44069 and the inhibitory actions of diltiazem were assessed in both the isolated perfused rat kidney (IPRK) and isolated vascular smooth muscle (VSM). We anticipated that combined studies with these two models would delineate the mechanisms mediating the renal vascular effects of these agents. U44069 induced contractions in isolated rings of rat aortae and renal arteries, with maximal tension (1.9 + 0.1 g) attained at 10^{-6} M. The contractions were associated with a stimulation of ^{45}Ca uptake by VSM (from 255 + 3 µmol/Kg to 314 + 4 µmol/Kg). Both tension development and ^{45}Ca uptake stimulated by U44069 were inhibited by diltiazem in a dose dependent manner (EC_{50} 369 ± 20 nM and 321 + 41 nM for tension and ^{45}Ca uptake). In the IPRK, 10^{-6} M U44069 caused a 82 ± 3 % and 80 + 4 % decrease in GFR and filtration fraction, while reducing RPF by only 13 + 8%; suggesting pre-glomerular vasoconstriction. Diltiazem administration completely reversed the decrease in GFR and RPF. The EC_{50} for the effects of diltiazem on GFR and RPF were 378 ± 11 nM and 571 + 204 nM. Diltiazem, thus, reversed the U44069-induced decrease in GFR at a dose similar to that inhibiting both tension development and ^{45}Ca uptake by VSM. Thus, our findings suggest that diltiazem acts by blocking Ca uptake of pre-glomerular vessels.

RELEASE OF PROSTAGLANDIN E_2 (PGE_2) BY A SINGLE ISOLATED PERFUSED GLOMERULUS. RN McCoy*, TA Fried, RW Osgood* and JH Stein. Univ of Texas Hlth Sci Cntr, San Antonio, Texas.

Prostaglandins play an important role in the control of renal hemodynamics. Numerous studies have reported prostaglandin production by glomeruli and glomerular cells in culture. The factors controlling production have not been fully evaluated. The possibility that intravascular perfusion and/or the resultant capillary hydrostatic pressure alter PGE_2 release has not been addressed. Using the isolated perfused glomerulus technique, we perfused 6 individual midcortical glomeruli with intact capsules through their afferent arterioles with a modified Krebs Ringer solution; 6 comparably dissected and manipulated glomeruli served as nonperfused controls. Glomerli were perfused or incubated for 2-4 hrs at 37° C, the perfusate and bath collected, and analyzed for PGE_2 using a radioimmunoassay capable of detecting 0.25 pg PGE_2 per sample.

Perfused dog glomeruli (n=6) released 0.65 $\pm$ 0.04 pg PGE_2/hr whereas nonperfused glomeruli (n=6) released .44 $\pm$ .04 pg PGE_2/hr ($p < .02$). Identical experiments in rabbits were qualitatively similar though PGE_2 release by the rabbit glomerulus appeared greater: perfused (n=2) released 4.77 $\pm$ 1.06 pg PGE_2/hr, whereas nonperfused (n=5) released 0.86 $\pm$ 0.21.

We conclude: 1) PGE_2 release by a single glomerulus is measurable; 2) intravascular perfusion increases this release; 3) there appears to be a species difference in glomerular PGE_2 release between dog and rabbit.

EFFECTS OF INTRARENAL ADMINISTRATION OF DOPAMINE (DA) ON RENAL BLOOD FLOW (RBF) IN CONSCIOUS FETAL AND ADULT SHEEP: RELATION TO IN VITRO DA RECEPTOR FINDINGS. Kenneth T. Nakamura,* Robin A. Felder,* Pedro A. Jose and Jean E. Robillard. Univ of Iowa, Dept Peds, Iowa City, IA, and Georgetown Univ Med Ctr, Dept Peds, Washington, D.C.

Effects of intrarenal boluses of DA (0.125 to 16 ug/kg of body wt.) on RBF were studied in chronically catheterized fetal (F) (129-137 days; term 145 days) (n=8) and adult (A) (n=6) sheep. Changes in RBF were continuously monitored by doppler flowmeter. Effects of DA alone and during α and β adrenergic blockade were studied. Blood pressure and heart rate were unchanged during DA infusion. DA doses were corrected for RBF and expressed in ug/kg/ml RBF. DA alone in F produced no change in RBF at doses <0.08 ug/kg/ml RBF; doses >0.08 ug/kg/ml RBF decreased flow in a dose response relation (r=0.84). Decreased RBF in A was seen only at >0.014 ug/kg/ml RBF. Following adrenergic blockade, DA produced vasodilation in F and A. The smallest dose producing vasodilation in F was 0.08 ug/kg/ml RBF compared to A at 0.004 ug/kg/ml RBF. In vitro DA-receptor characterization was done using the opposite kidney removed at surgery. Similar DA receptor affinity between F (Kd 66.3±10.4 nM) and A (Kd 70.5±6.4 nM) as well as DA receptor density (1.08±0.13 and 0.90±0.22 pM/mg protein respectively) were found. These results suggest that DA has less vasodilatory effect in F than A during adrenergic blockade; however this effect is not secondary to a difference in DA receptor affinity or density, but possibly to a post-receptor phenomenon.

PROSTAGLANDIN (PG) DEPENDENCE OF RENAL BLOOD FLOW (RBF) DURING CHRONIC CONVERTING ENZYME INHIBITION (CEI) WITH MK421 IN THE RAT. P. Mento* and Barry Wilkes, Div. of Nephrology & Hypertension, North Shore University Hospital, Manhasset, N.Y. 11030

Several reports have described increased PG production as a direct consequence of CEI. The following protocol was devised to study the contribution of PGs to the cardiorenal effects of MK-421. Male Sprague-Dawley rats were maintained for 21 days on either moderate sodium diet (0.26 mEq/24h), low sodium diet (0.04 mEq/24h) or low sodium diet plus MK421 in the drinking water (300 mg/L). Half the rats were given an acute infusion of indomethacin (Indo), 2 mg/kg/hr, immediately prior to study. Results ($\bar{X}\pm SE$, $n \geq 7$):

	Mod Na^+	Low Na^+	Low Na^++MK421
MAP (mmHg)			
-Indo	130±3	135±5	66±4†,§
+Indo	129±5	121±5	65±7†,§
CO. (ml/min/100g b.w.)			
-Indo	31±2	38±4	43±2§
+Indo	31±3	41±4	35±4
RBF (ml/min/100g b.w.)			
-Indo	3.1±0.1	3.3±0.3	3.2±0.3*,†,§
+Indo	3.3±0.2	3.8±0.4	1.9±0.3*,†,§

* $p < 0.05$ compared to same group without Indo
† $p < 0.01$ compared to same group without MK421
§ $p < 0.05$ compared to moderate Na^+ group

Acute PG inhibition did not affect MAP or CO in any of the groups, but reduced RBF by 41% in MK-421-treated rats. We conclude that PGs are important for the maintenance of RBF, but not for lowering MAP during chronic CEI.

OBESITY IN YOUNG ZUCKER RATS IS NOT ASSOCIATED WITH GLOMERULAR HYPERFILTRATION. MP O'Donnell, BL Kasiske, MP Cleary*, and WF Keane, Dept. of Medicine, Hennepin County Medical Ctr., Univ of Minn., Mpls., and Austin, MN.

Obese male Zucker rats (OZ) developed spontaneous microalbuminuria and mesangial expansion by 3 months of age (ASN, 1984). At 6 months, focal glomerulosclerosis (FGS) was evident and progressive nephron destruction occurred over the ensuing 12 months. To determine whether specific alterations in glomerular hemodynamics might play a role in the pathogenesis of these lesions, we performed micropuncture studies in OZ and lean male littermates (LZ) 9-13 weeks of age.

Results (mean±SEM; *$p<0.01$; †$p=0.05$):

Group	BW (gm)	KW	SNGFR (nl/min)	SNPF	$\Delta\bar{P}$ (mmHg)	K_f (nl/sec.mmHg)
OZ	313*	0.93†	34.4	96.3	41.1	0.027
(n=8)	±17	0.06	2.3	4.9	2.1	0.002
LZ	237	0.78	29.3	82.6	41.2	0.024
(n=8)	±12	0.05	2.1	4.9	1.3	0.003

Despite significantly greater BW and KW in OZ, single nephron function and the determinants of glomerular ultrafiltration were similar in both groups. Morphologically, expansion of mesangial matrix was evident in OZ but no glomeruli exhibited FGS. Urinary albumin excretion was 2.0 vs 0.5 mg/24h ($p<0.01$) in OZ compared to LZ. Thus, hemodynamic factors do not appear to significantly contribute to the spontaneous development of microalbuminuria, mesangial expansion or FGS in OZ.

EFFECT OF BETA ADRENERGIC STIMULATION WITH ISOPROTERENOL (I) UPON GLOMERULAR HEMODYNAMICS. J. C. Pelayo*, B.J. Tucker*, and R.C. Blantz. Dept. of Med., UCSD and VAMC, La Jolla, CA and Dept. of Ped. Harbor-UCLA Med. Cen., Torrance, CA.

Previous studies from this laboratory demonstrated that intravenous infusion of 50 ng/kg BW/min of I resulted in a significant reduction of 40% in the glomerular ultrafiltration coefficient (LpA) and increases in the glomerular capillary hydrostatic pressure gradient (ΔP) of 5 mmHg while nephron filtration rate (SNGFR) and plasma flow (SNPF) remained unchanged. These changes in LpA and ΔP with I were prevented by infusion of converting enzyme inhibitor or saralasin, suggesting that angiotensin II (AII) mediated these beta agonist effects. However, it was possible that AII stimulation was not a direct renal beta or I effect but secondary to systemic effects on blood pressure or cardiac output, resulting in AII release due to neural or baroreceptor mechanisms. We examined this issue by infusing vehicle into the renal artery in a control period (C) and I into the renal artery (8 ng/kg BW/min) in the experimental period. This dose was ∿16% of the systemic dose and resulted in no change in pulse rate or blood pressure. Results: *p<0.05

	SNGFR nl/min	SNPF nl/min	ΔP (mmHg)	LpA (nl/sec/mmHg)
C	50±3	216±20	37±2	0.11±.02
I	47±3	181±16	36±1	0.06±.01*

Both systemic and intrarenal I infusion decrease LpA but intrarenal infusion does not increase ΔP. These results suggest that renal beta adrenergic stimulation results in a decrease in LpA independent of systemic effects and does so via the local action of AII.

HYPONATREMIA, PLASMA VOLUME EXPANSION AND PRE-RENAL AZOTEMIA DURING CHRONIC CONVERTING ENZYME INHIBITION (CEI) IN THE SODIUM RESTRICTED RAT. Judy Rabin*, Barry M. Wilkes, Peter Mento*, Div. of Nephrology/Hypertension, North Shore University Hospital, Manhasset, NY 11030.

Several clinical reports have linked decreased renal function with CEI. The following protocol was designed to study the effects of chronic CEI on renal hemodynamics. Male Sprague-Dawley rats were maintained for 21 days on either moderate sodium diet (0.26 mEq/24h), low sodium diet (0.04 mEq/24h) or low sodium diet plus MK421 in the drinking water (300 mg/L). Studies were performed on day 21. Results ($\bar{X} \pm SE$):

	MOD Na^+	Low Na^+ (21 days) -MK421	+MK421
MAP (mmHg)	130 ± 3	135 ± 5	66 ± 4*,†
RBF (mL/min/100g)	3.1 ± 0.1	3.3 ± 0.3	3.2 ± 0.3
Cortical RBF (mL/min/100g)	2.9 ± .2	3.1 ± 0.3	3.0 ± 0.2
Pl Vol (mL/100g)	4.23±0.18	3.61 ± 0.12	4.86 ± 0.13*
S_{Na^+} (mEq/L)	142 ± 1	145 ± 1	128 ± 4*,†
BUN (mg/dL)	14.3 ±0.6	24.0 ± 1.4	62.5 ± 17.4§
S_{creat} (mg/dL)	0.41 ±0.03	0.58 ± 0.17	0.77 ± 0.12†

*p < 0.01 compared to same group without MK421
†p < 0.05 compared to control
§p < 0.05 compared to same group without MK421

Despite normal RBF and excretory function (S_{creat}) MK421-treated rats had elevated BUN and plasma volume. Since renal hemodynamics and plasma volume were not reduced, pre-renal azotemia occurred at the level of the nephron. Retention of water causing severe hyponatremia suggests an additional abnormality in renal dilution.

TUBULO-GLOMERULAR FEEDBACK (TGF) IN ADULT HYPERTENSIVE RATS OF THE MILAN STRAIN (MHS). A E G Persson, G Bianchi, U Boberg (intr. by F Wright). Depts Physiol and Urology, University of Uppsala, Sweden

In MHS rats the disease can be transplanted with the kidney to a Milan normotensive strain (MNS). GFR, salt and volume regulation differ between MHS and MNS. Since TGF is important for this regulation we measured TGF activity during hydropenia (HP) and during (5 % B.W.) saline volume expansion (VE) and during two hours of ureteral occlusion (UO). The TGF sensitivity can be influenced by interstitial pressure conditions. In a first series ,GFR, urine sodium excretion rate, subcapsular interstitial hydrostatic pressure and interstitial oncotic pressure were measured . In a second series, proximal tubular stop-flow pressure (P_{sf}) was measured upstream to a wax block when late proximal segments were perfused with a Ringer solution from 0 - 40 nl/min. The maximal drop in P_{sf} (ΔP_{sf}), and the tubular flow rate at which 50% of this response was achieved, the turning point (TP) were determined. The result showed that GFR was similar in MHS and MNS during HP, but during VE, GFR was less in MHS. Interstitial pressures were similar in both MHS and MNS during HP and VE. ΔP_{sf} was slightly increased in the MHS during HP (8,5 versus 5.2 mm Hg) while P_{sf} and Tp was similar.However, during VE, TGF sensitivity in MHS was significantly higher than in MNS with a TP of 25 nl/min (MNS 38 nl/min) and a ΔP_{sf} of 5.6 mm Hg (MNS 2.5 mm Hg). During UO TGF sensitivity was reduced to a low level in MNS and other control rats but unaltered in MHS.

Thus, TGF sensitivity was reset to a low level in MNS rats during VE and UO but MHS animals were unable to reset TGF sensitivity in a normal way. This finding indicate an important influence of the TGF on the development and maintenance of the hypertensive disease.

ROLE OF INCREASED VASA RECTA PRESSURE AND FLOW IN THE PRESSURE DIURESIS RESPONSE. Richard J. Roman, Medical College of Wisconsin, Milwaukee, WI.

To determine whether the pressure diuresis response may be triggered by changes in the inner medullary circulation, the relationships between vasa recta pressure, papillary blood flow and renal perfusion pressure (RPP) were characterized. Experiments were performed using uninephrectomized, 250-400g Sprague Dawley rats whose left kidney was specially prepared for exposure of the renal papilla. RPP was varied between 90 and 160 mmHg using aortic clamps and ligatures on the mesenteric and coeliac arteries. Vasa recta pressures were measured from at least 8 capillaries at each level of RPP using a servonull pressure system. Blood flow in the papilla tip was measured using a laser-doppler flowmeter (Perimed). The flowmeter was calibrated in 28 rats by relating the flow signal to measurements of papillary blood flow obtained using ^{51}Cr-labeled RBC. Vasa recta pressure and blood flow were linearly related to RPP over the entire range of pressures studied. Vasa recta pressure increased significantly from 6.3±0.8 to 16.6±1.0 mmHg (N=11 rats) as RPP was increased from 96±3 to 166±4 mmHg in steps of 25 mmHg. Vasa recta flow increased by 68%, from 25±3to 42±4 ml/min·100g papilla wt (N=15 rats), as RPP was varied over this range. These results suggest that the pressure diuresis phenomenon may be a consequence of weak autoregulation of inner medullary blood flow. Increases in vasa recta pressure could possibly affect tubular reabsorption in medullary nephron segments by altering physical forces in a manner similar to the mechanism influencing proximal tubular function.

EFFECT OF CAPTOPRIL ON RENAL HEMODYNAMICS IN RATS DEVELOPING SPONTANEOUS HYPERTENSION.
M. Audrey Rudd,* Richard S. Grippo,* and William J. Arendshorst. Univ. of North Carolina, Sch. of Med., Depts. of Med. & Physiol., Chapel Hill, NC.

We have previously shown that the kidneys of 6-wk-old spontaneously hypertensive rats (SHR) are vasoconstricted with a reduced renal plasma flow (RPF), glomerular filtration rate (GFR) and filtration coefficient relative to age-matched Wistar-Kyoto control rats (WKY). To determine the potential contribution of the renin-angiotensin system to the vasoconstriction and reduced GFR, we conducted clearance experiments on anesthetized, euvolemic, 6-wk-old SHR (n=9) and WKY (n=11). After a control period, we infused captopril (SQ 14,225, 4 mg/kg prime, 0.2 mg/min infusion) to produce angiotensin I-converting enzyme inhibition (CEI). Before CEI, moderately hypertensive SHR had lower GFR and RPF. CEI produced renal vasodilation in SHR; RPF increased while arterial pressure (AP) decreased. Despite the increase in RPF, GFR was unchanged. In WKY, RPF, GFR and AP were unchanged by CEI.

	WKY		SHR	
	Control	CEI	Control	CEI
GFR, ml/(min.gKW)	1.2	1.2	0.9#	0.9
RPF, ml/(min.gKW)	4.2	4.3	2.8#	3.3*
AP, mmHg	93	94	113#	98*

#, P < 0.02, SHR vs. WKY in control period.
*, P < 0.05, CEI vs. control period within group.

Our observations suggest that the renin-angiotensin system mediates some of the enhanced renal vasoconstriction in young SHR developing hypertension, but that other factors are primarily responsible for the reduced GFR.

DIETARY PROTEIN: EFFECT ON TUBULOGLOMERULAR FEEDBACK SIGNAL AND SENSING MECHANISM.
FD Seney, Jr*, AEG Persson*, GV Desir*, FS Wright. Yale Univ and VA Med Ctr, New Haven, CT.

We have previously shown that feeding rats a high protein diet reduces activity of the tubuloglomerular feedback (TGF) system. The present experiments examined whether protein intake affects either the signal initiating TGF or the function of the TGF sensing mechanism. Adult male rats fed diets containing either 6% or 40% casein for ~8 days were prepared for micropuncture. In one group (11 rats) free flow samples of early distal tubule (EDT) fluid were collected and Na and Cl concentrations (mM) were measured:

	[Na]	[Cl]	
Low protein (LP)	53 ± 2	48 ± 2	* = P <0.001
High protein (HP)	38 ± 2*	25 ± 2*	(LP vs HP)

The same nephrons were then perfused from the late proximal tubule (LPT) at 15, 20, 30 and 40 nl/min with a Ringer's solution and all fluid reaching the EDT was collected. Absorption of Na and Cl between LPT and EDT was 25% greater for the HP rats. Na and Cl concentrations in fluid reaching the EDT were 30% lower for HP rats.

In a second group (8 rats) Henle's loop was perfused backwards from the EDT with 15, 30 and 50 mM NaCl. TGF responses (change in stop flow pressure) increased with increasing NaCl concentration but did not differ between diet groups.

Thus we found no evidence that protein intake influences the function of the TGF sensing mechanism. We did find that absorption of Na and Cl between the LPT and EDT is more avid in rats fed a protein rich diet. We conclude that GFR is increased after protein feeding because the signal eliciting TGF responses is diminished.

EFFECTS OF ANGIOTENSIN II ON GLOMERULAR FUNCTION IN VITRO.
Virginia J. Savin. Univ. of Kansas Med. Center, Department of Medicine, Kansas City, Kansas.

Infusion of Angiotensin II (AII) during micropuncture studies decreases ultrafiltration coefficient (K_f) and AII causes contraction of isolated glomeruli or cultured mesangial cells. To test the hypothesis that mesangial contraction in response to AII decreases K_f, we studied K_f of isolated rat glomerli after incubation for 20 min. at 37°C in control medium (C) or medium containing AII (5.2×10^{-9} to 10^{-6} M). Glomeruli were isolated from adult Munich-Wistar rats and filtration was induced by creating an oncotic gradient across the capillary wall. Glomerular volume, filtration rate and K_f were estimated from frame-by-frame analysis of video-recordings. This technique provides an estimate of glomerular hydraulic permeability independent of perfusion. Average glomerular volume prior to filtration was 8% lower after AII incubation than after C incubation while K_f was not different (4.2±1.0 and 4.7±1.2 nl/min·mmHg, respectively). To further define the response to AII, we performed paired studies of filtration using individual glomeruli. Glomerular volume decreased by 7.4±0.8% during AII incubation. K_f was not significantly altered, averaging 6.5±1.3 and 4.7±1.2 nl/min·mmHg before and after exposure to AII. In summary, incubation with AII decreases glomerular volume but does not alter Kf. We suggest that mesangial contraction does not diminish total capillary area or capillary hydraulic conductivity in vitro. Diminished K_f in vivo may reflect an altered pattern of intraglomerular perfusion rather than a change in the hydraulic permeability of the glomerular capillary.

EVIDENCE THAT NON-CYCLOOXYGENASE DERIVED ARACHIDONIC ACID (AA) METABOLITES ALTER RENAL HEMODYNAMICS AND SODIUM REABSORPTION IN INTACT DOGS.
J. Tannenbaum, A. Womack*, and R. Toto, Univ. TX Hlth. Sci. Ctr., Southwestern Medical School, Dallas, TX.

The kidney enzymatically converts AA to several identifiable non-cyclooxygenase metabolites (NCO). The purpose of the present studies was to investigate the effects of NCO on renal hemodynamics and solute excretion in intact dogs. AA was continuously infused at a rate of 30 µg/kg/min into the left renal artery of 8 anesthetized dogs undergoing a water diuresis. AA metabolism was sequentially inhibited by intravenous indomethacin (I) and intrarenal eicosatetraynoic acid (ETYA). Renal plasma flow (RPF), glomerular filtration rate (GFR), filtration fraction (FF), and urinary sodium excretion (Na) were monitored during four periods of study:

	RPF (ml/min)	GFR ml/min	FF	%ΔNa
Control	160±6	41.0±2.7	.25	
AA	200±8*	38.2±2.8	.18	+124±34*
I	151±17*	36.8±3.6	.24	+51.4±30*
ETYA	130±16	23.4±4.5*	.16†	-42.8±6*

*p<0.05, †p<0.01 compared to previous period. Values are mean ±SEM.

I infusion reduced RPF while GFR remained constant and FF increased. After ETYA RPF was unaltered while GFR fell significantly. Urinary sodium excretion increased after I but decreased after ETYA. Urinary prostaglandin E_2 excretion decreased after I but showed no further change after ETYA. These data suggest that NCO support GFR during inhibition of RPF and inhibit sodium reabsorption in the distal nephron.

THE EFFECT OF AMINO ACIDS AND/OR LOW-DOSE DOPAMINE ON RENAL FUNCTION.

Piet M. ter Wee*, Wim J. Sluiter*, Ab J. Donker* (Intr. by L.W. Statius van Eps).Univ. of Groningen, Dept. of Nephrology, Groningen, The Netherlands.

Both intravenous infusion of amino acids and a low dose dopamine (1.5-2.0 µg/kg/min) increase glomerular filtration rate (GFR), provided that renal function is not severely impaired. Since infusion of amino acids is accompanied by a stable filtration fraction (FF) while infusion of dopamine leads to a fall in this variable, we assessed the effect of amino acids and/or dopamine intravenously on renal function in healthy volunteers and patients with varying degrees of renal insufficiency during a simultaneous measurement of the clearances of 125 I-iothalamate and 131 I-hippurate. The highest values for GFR were obtained with the combined infusion of amino acids and dopamine. However, in patients with moderate (GFR 90-30 ml/min) to severe (GFR < 30 ml/min) renal insufficiency, hardly or no reserve in filtration capacity was present. Dopamine infusion increased effective renal plasma flow much more than infusion of amino acids. The highest values were obtained with the combination. We conclude that amino acids and dopamine increase filtration by different mechanisms as expressed by the FF. The combination leads to the highest values for the GFR without an influence on systemic blood pressure or heart rate. The results support the idea that if in renal disease GFR is markedly impaired, no significant reserve is present anymore.

DISTRIBUTION OF MICROSPHERES OF DIFFERENT DIAMETER IN THE CAT KIDNEY. J. Torretti*, P.Randall*, F.M.Fischer* and J.Koerner*. Depts. of Pharmacology, Anesthesiology, Radiology and Physiology, SUNY Upstate Medical Center, Syracuse, N.Y.

We measured intra-renal distribution of radiolabeled microspheres 15±3 and/or 9±1 µ diameter, given by intraventricular injection to 20 cats. Reference blood was collected simultaneously from brachial and femoral arteries (all cats), from subcapsular vein (9 cats), and from subcapsular and deep renal veins (4 cats).

Fractional distribution of right kidney mass sectioned freehand was: outer cortex (OC) 29.8±.7%; inner cortex (IC) 28.7±.7%; medulla (M) 32±1.2%; poles 9.2±.4%. Intrarenal distribution of 15 and 9µ spheres given simultaneously to 6 cats was uneven. OC/IC was 2.2±.3 for 15µ spheres and 1.3±.1 for 9µ spheres. 5.4±.9% of 15µ spheres vs 8.8±1.4% of 9µ spheres ($p<.01$) trapped in the kidney were counted in the medulla. The ratio of M/IC counts was also higher for 9µ spheres. 96±2% of 15µ and 97±1% of 9µ spheres were entrapped in the area drained by subcapsular veins. Simultaneous collections from subcapsular and deep renal veins in cats injected only with 9µ spheres showed similar entrapment by both areas of venous drainage.

We conclude that in the cat kidney (1) 15µ diameter spheres are streamed to the outer cortex. (2) 9 µ diameter spheres may be used to measure flow to areas of subcapsular and deep venous drainage when all the tissue is counted (3) Counting only samples of deep cortex will underestimate its flow on account of spilling past juxta medullary glomeruli, with subsequent entrapment in the vasa recta.

FUNCTION OF ISOLATED PERFUSED KIDNEYS FROM HYPOTHYROID RATS. R. Tomford, C. Vacca*, D. Beckwith* and P.W. Hall, Department of Medicine, Cleveland Metro. General Hosp., Case Western Reserve Univ., Cleveland, Oh.

Hypothyroidism (HT) significantly affects renal function in humans and experimental animals, including decreased GFR, RBF, and Na reabsorption. Thyroidectomy (Tx) prevents progression of chronic renal failure in the nephrotoxic serum nephritis model. To determine whether the changes in renal function produced by HT are a result of systemic or direct renal effects, kidneys from Tx rats with intact parathyroids were perfused *in vitro* (IPK) 2 weeks post Tx with 7% BSA Krebs-Henseleit buffer at constant pressures for 60 min. The GFR of Tx rats (n=6) was > the control rats (n=5) (0.96 ± 0.16 vs. 0.71 ± 0.18ml/min/gKW; $p < 0.05$)*. The FENa was not statistically different between the groups, but the HT kidneys had higher urine flow rates and developed significantly more proteinuria (0.333 ± 0.140 vs. 0.166 ± 0.056 mg/min; $p < 0.05$)*. The etiology of the increased proteinuria remains to be determined. The similar FENa for both groups suggests that Na/K ATPase is not the limiting factor in Na reabsorption by the IPK from HT rats. In contrast to HT rats *in vivo*, the GFR of IPKs from HT rats was increased suggesting that the HT induced alterations in renal hemodynamic are mediated predominantly through extrarenal signals.

* Mean ± S.D.

HEMODYNAMIC DIFFERENCES BETWEEN LEFT AND RIGHT RAT KIDNEYS SHOWN BY TUBULAR ANTIBODY DEPOSITION. HJ Ward, T Ishidate* & JR Hoyer, Harbor-UCLA Med.Ctr., Torrance, CA & Children's Hosp., Philadelphia, PA.

Hemodynamic and antibody(Ab) binding properties of left(L) and right(R) kidneys(K) of normal rats were previously assumed to be quantitatively comparable. However, we now show quantitative differences in L & R kidney tubular Ab deposition of IgG Ab to Tamm-Horsfall protein(TH). Marked differences (LK>>RK) were seen in all 8 groups of male Sprague-Dawley(SD) rats removed and counted 8 hours to 14 days after a single IV injection of 500µg of I^{125}-rabbit IgG ab to TH with I^{131}-normal IgG as a pair label. K of SD, Munich-Wistar(MW) rats given anti-TH, & rats given I^{125}-anti-GBM were studied at 24 hours. Rats kept in various positions under Inactin anesthesia for 8 hours were also studied for renal anti-TH binding.

GROUP	N	µg Ab LK	µg RK	LK/RK	p
SD male	13	10.9±3.6	5.0±1.1	2.2	<0.001
SD female	4	8.9±2.3	6.0±0.6	1.5	<0.05
MW	6	6.5±0.7	3.2±0.3	2.0	<0.001
Anti-GBM	6	17.0±1.1	17.6±1.4	1.0	NS
Anesthesia					
Prone	4	4.5±1.4	4.7±1.5	1.0	NS
Supine	7	5.6±1.3	5.3±1.1	1.1	NS
L Flank up	8	5.5±1.3	5.0±1.0	1.1	NS
Conscious	6	12.0±5.2	5.9±1.4	2.0	<0.02

Renal homogenates incubated *in vitro* showed equal uptake of I^{125}anti-TH by LK and RK. These data show the greater uptake of Ab to tubular antigen (but not GBM) by the LK of rats is abolished by anesthesia and indicate that the intrarenal hemodynamics of the LK and RK are significantly different in conscious rats.

CATIONIC DEAE-^{3}H-DEXTRAN - A PROBE OF GLOMERULAR AND POSTGLOMERULAR CAPILLARY FUNCTION IN VIVO. C. Whiteside and M. Silverman, Department of Medicine, University of Toronto, Toronto, Ontario.

Interaction of cationic DEAE-^{3}H-dextran (-^3H_d) (pI∿9) with glomerular (G) and postglomerular (PG) capillaries was examined using the multiple indicator dilution method in anaesthetized dogs. A .3cc pulse of ^{125}I albumin (plasma reference), ^{14}C-inulin (G ref) and DEAE-^3H_d was injected into the left renal artery, followed by left renal venous and left and right urine outflow sampling. Urine recoveries of the indicators measured unidirectional glomerular extraction (E_G). The urine recovery ratio (E_{Gd}/E_{Gi}) of DEAE-^3H_d 22 Å to 32 Å Stokes-Einstein radius, relative to ^{14}C-inulin was .49±.02 to .07±.02 (mean ± SD). Total urinary plus renal vein recoveries of DEAE-^3H_d was less than 100% indicating net renal uptake. Excess unlabelled DEAE-dextran added to the injection solution increased E_{Gd}/E_{Gi} to 1.00 for the 22Å probe and to .35 for the DEAE-^3H_d 32A. Unlabelled DEAE-dextran (pI∿9) injected into the renal artery after the tracer pulse caused a delayed release of bound DEAE-^3H_d into the urine (10mg/100gm kidney released 50% of DEAE-^3H_d; 100mg/100gm kidney released 100% DEAE-^3H_d). Intrarenal infusion of protamine sulfate (30mgm) before the tracer pulse increased E_{Gd}/E_{Gi} by 20%. The results demonstrate saturable, reversible binding of DEAE-^3H_d persumably to fixed G anionic sites. Unlabelled DEAE-dextran or protamine sulfate infusion also decreased the renal vein mean transit times of DEAE-^3H_d by 25%±9% indicating reversible binding in the PG microcirculation. Hence, cationic DEAE-^3H_d can be used to analyze the effective anionic charge in the G and PG capillaries.

DOSE DEPENDENT ACTIONS OF SODIUM-ORTHOVANADATE (V) AND EFFECT OF POTASSIUM DURING ISOLATED KIDNEY PERFUSION IN RATS (IPK). Wiegmann, T.*, MacDougall, M, Kidney Urology Research Center, University of Kansas, Kansas City, KS and VAMC, Kansas City, MO.

Intrarenal administration of V leads to dose dependent increases in vascular resistance in dogs. The same response was found during IPK of rats. The effect is magnified by K addition. Diluent or vanadate (5 and 20 µM) was added to perfusate after equilibration and control periods. Perfusate K was increased from 5 to 10 mM after 3 V-periods in additional animals. Major effects were seen in perfusion pressure and sodium excretion. Mean results during period III and VI are compared to preceeding control periods C:

Var	µM V	K µM	C	III	VI
Press	0	5	108	108	107
	5	5	109	114*	115*
	20	5	109	131*	130*
Fx Na	0	0	9.5	11.8	13.0
	5	0	11.3	14.7*	16.8*
	20	0	14.2	19.9*	24.8*
Press	0	10	109	110	110
	5	10	110	117*	122*
	20	10	110	131*	134*
Fx Na	0	10	9.8	11.1	15.9*
	5	10	11.9	18.2*	30.4*
	20	10	12.1	24.2*	33.7*

(* = p<0.05 paired T-test)

Pressure and sodium excretion rose promptly with V addition and remained elevated at a stable level after III. Urine flow increased similarly. Further increases were obtained after K addition. We conclude that K augments V effect on renal resistance, fluid and sodium transport, possibly by inhibition of Na-K-ATPase.

IN VIVO ASSESSMENT OF RAT RENAL VASCULAR α-ADRENOCEPTORS. DW Wolff,* FA Gesek,* & JW Strandhoy. Wake Forest Univ. Med. Ctr., Dept. of Physiol. and Pharmacol., Winston-Salem, NC.

Rat renal cortex contains a ratio of 3 α2: α1-adrenoceptors; the α2 receptors are largely postsynaptic. We have developed a method for examining the effect of selective α-adrenergic agonists on RBF in anesthetized Wistar rats. The right suprarenal artery was cannulated for intrarenal arterial infusion and an HPLC valve was used for random and reproducible 6 µl bolus injections. Decreases in RBF were recorded with an ultrasonic probe and flowmeter, and expressed as a percentage of the control value.

Agonists	n	r	slope	ED(-20% RBF) ±SEM (µg/kg)
Norepinephrine	2	-.843	-90.3	.042±.016
Cirazoline (α1)	5	-.998	-116	.013±.003
Phenylephrine (α1)	10	-.720	-102	.323±.188
Guanabenz (α2)	10	-.970	-14.6	47.2±37.2
B-HT 920 (α2)	4	-.991	-8.61	72.8±19.6
UK-14304 (α2)	6	-.887	-7.30	165±122

α1-Agonists caused minimal systemic effects. α2-Agonists were neither potent nor efficacious in the renal vascular bed, but vasoconstricted systemically. Norepinephrine and the α1 agonists were competitively antagonized by prazosin whereas there was slight if any antagonism of α2 responses. Furosemide or renal vasodilation did not modify α2 vasoconstriction suggesting that release of vasodilators or tubuloglomerular feedback could not explain the weak vasoconstriction. We conclude that α1 receptors mediate renal sympathetic vasoconstriction and that renal vascular α2 receptors are either relatively sparse or silent.

DEPRESSION OF RENAL HEMODYNAMIC AND TUBULAR FUNCTIONS DURING PICHINDE VIRUS INFECTION IN STRAIN 13 GUINEA PIGS. **S.R. Yaffe*, C.T. Liu, E.C. Staley*, P.B. Jahrling*, and C.J. Peters*. US Army Med.Res. Inst.Infect.Dis. Ft. Detrick, MD.**

Pichinde virus causes death in guinea pigs (GP) 14 days postinoculation (PID 14). Negative balances of water and electrolytes during Pichinde infection have been previously observed (Physiologist 26:A-59, 1983). This investigation was to study renal responses to Pichinde virus infection in strain 13 GP. Jugular vein, common carotid artery and urinary bladder of each GP were cannulated under anesthesia one day before experiments (day 0,PID 7&14). The GP were allowed to recover. Solutions containing inulin, PAH, and glucose or mannitol were infused iv to all GP. Blood pressure was unchanged by PID 7, but GFR, TmG and TmPAH decreased 24%, 56% and 77%, respectively from control values. Renal reabsorption for Na^+, Cl^-, and HCO_3 decreased to 88%, 89% and 93%, respectively, from a baseline average of 98%. The excretory rate of K^+ increased from 3.9 to 4.5 µEq/min. ERPF and TRBF decreased by 50%, while total renal resistance increased by 40% as compared to controls. More drastic changes of renal functions were observed by PID 14. Since fluorescence-labled viral antigen was only found in renal tubular cells, the data suggest that reduced GFR of infected GP may be due to a decreased TRBF and a simultaneously increased renal resistance. The severely depressed renal tubular secretory and reabsorptive functions may be induced by the virus directly or acted upon through mediators. Thus, renal hemodynamic and tubular dysfunction during progression of Pichinde virus infection may contribute to critical disturbances of body fluids and electrolyte metabolism.

EFFECT OF ARGININE VASOPRESSIN (AVP) ON VASA RECTA BLOOD FLOW. B. Zimmerhackl,* C.R. Robertson,* and R.L. Jamison. Depts. of Med. and Chem. Eng., Stanford University, Stanford, CA.

The role of AVP in the regulation of medullary blood flow is uncertain. The effect of AVP on vasa recta (VR) blood flow (Q_{VR}) was studied in the exposed renal papilla of 4 groups of chronically water diuretic rats using fluorescence videomicroscopy. The protocol consisted of 3 periods: control (period 1), experimental (2), and recovery (3). In period 2 Group I rats (AVP, N rats=7) received AVP, 75 pg/min/100 g BW; Group II (Time, N=7) received dilute saline; Group III (AVP + inhib., N = 5) received AVP plus the vascular AVP antagonist $d(CH_2)_5$Tyr(Me)AVP, 200 ng/min 100 g BW; and Group IV (inhib., N=6) received the antagonist alone. Group III rats concentrated their urine as well as Group I (U_{osm} = 747 ± 83 mOsm/kg H_2O vs. 673 ± 68) while urine remained dilute in Groups II and IV. GFR did not change significantly in any group. Q_{VR} (nl/min, mean ± SE) for DVR was:

Period	I(AVP)	II(Time)	III(AVP+inhib.)	IV(inhib.)
1 $\bar{x}$	11.7	13.2	10.6	12.9
SE	1.5	2.4	1.6	2.8
2 $\bar{x}$	10.4*†	14.2	10.7	15.7
SE	1.6	2.4	1.8	3.5
3 $\bar{x}$	13.0	15.7	13.2	15.3
SE	2.4	2.6	2.0	3.1

*P<0.05 compared to period 1; †P<0.05 compared to Group II.

The AVR values were lower as expected but changed in the same way. The results establish that AVP in physiological doses reduces Q_{VR} and suggest the possibility that AVP may have a direct vasoconstrictive as well as an indirect effect.

RENAL PHYSIOLOGY—Na, K AND Cl

ACTIVE TRANSCELLULAR CHLORIDE ABSORPTION IN THE RAT PROXIMAL CONVOLUTED TUBULE (PCT). R.J. Alpern and K.J. Howlin*, Dept. Med., Univ. of Calif., San Fran., CA.

In order to examine the mechanism of Cl absorption (J_{Cl}), rat PCT were microperfused in vivo with solutions of varying [Cl] in the presence and absence of cyanide (CN). Volume flux was measured using [^{3}H]-inulin, J_{Cl} microtitrimetrically, and transepithelial potential difference using low resistance microelectrodes.

J_{Cl} was a linear function of the transepithelial electrochemical gradient, yielding an apparent chloride permeability of 20.6 ± 0.6 x 10^{-5} cm/sec (r=0.99). In the absence of an electrochemical driving force, J_{Cl} was 131 ± 6 peq/mm•min, thus demonstrating active, transcellular chloride absorption. In order to confirm this, the effect of the metabolic inhibitor, CN, was examined. In tubules perfused with a high luminal [Cl] which absorbed Cl, CN addition inhibited net Cl absorption by 147 peq/mm•min. This effect could be due to inhibition of active Cl absorption or could be due to a decrease in passive Cl absorption secondary to cell swelling. In tubules perfused with a low luminal [Cl] which secreted Cl, active and passive fluxes were in opposite directions. In this setting, CN addition enhanced net Cl secretion by 167 peq/mm•min, thus demonstrating inhibition of active chloride absorption by CN. The effect of CN on J_{Cl} was not attributable to changes in the electrochemical driving force. When J_{Cl} was examined as a function of the electrochemical driving force, CN did not affect the slope (Cl permeability), but reduced the Y-intercept (active transport rate) to zero. We therefore conclude that: 1) J_{Cl} involves two parallel components: a passive paracellular flux with a permeability of 20.6 x 10^{-5} cm/sec and an active transcellular Cl flux of 130-160 peq/mm•min. 2) Cyanide inhibits J_{Cl} by inhibiting active transcellular Cl absorption.

^{23}Na-NMR COMBINED WITH A K^+-ELECTRODE TO MEASURE RAPID Na^+ AND K^+ FLUXES IN RABBIT PROXIMAL TUBULES. M.J. Avison*, S.R. Gullans*, T. Ogino*, R. G. Shulman*, and G. Giebisch. Yale Univ. Depts. Physiol.& Molec. Biophys. Biochem., New Haven, CT.

With the use of NMR it is now possible to measure rapidly intracellular Na^+ in mammalian kidney tubules. Rabbit renal proximal tubules, prepared by collagenase digestion, were suspended in a modified Ringer solution containing the shift reagent, dysprosium tripolyphosphate. The shift reagent enabled us to resolve intra- and extracellular Na^+ using a Brüker WH360 wide bore NMR spectrometer with a 20 mm probe tuned to 95.26 MHz. Simultaneously, a K^+-selective electrode was used to measure extracellular [K^+] without introducing r.f. interference. Initial experiments demonstrated that the presence of the shift reagent did not adversely affect spontaneous or ouabain-sensitive respiration. Furthermore, intracellular Na^+ changed in a predictable manner following ouabain inhibition of the sodium pump or addition of nystatin. In another series of experiments, K^+-depleted tubules were incubated in a K-free Ringer at 25°C and ^{23}Na-NMR spectra were collected every 5 sec. After 40 sec, during which Na^+_i and K^+_o remained constant, 4 mM K^+ was added to the suspension. This addition of K^+, which maximally stimulates the Na^+, K^+-ATPase, caused an initial rapid efflux of Na^+_i (corresponding to +0.52 mM min^{-1} Na^+_o) and an influx of K^+_o (-0.36 mM min^{-1}). We conclude that a combination of ^{23}Na NMR with a K^+-selective electrode allows simultaneous and rapid measurements of net Na^+ and K^+ fluxes in suspensions of rabbit proximal tubules.

ELECTROLYTE FLUX IN THE RAT RENAL PELVIS. Joanne M. Bargman,* and Rex L. Jamison. Division of Nephrology, Stanford University, Stanford, CA.

We recently discovered the urine-to-plasma (U/P) osmolality (osm) ratio is lower in the renal pelvis and fornix (Fnx) than at the end of the collecting ducts (CD) because of transepithelial addition of water. The purpose of this study was to compare urine electrolyte concentration at the two sites and to account for any differences. Microcatheters were inserted through the ureter into the Fnx in 12 antidiuretic rats. Samples of urine from Fnx and from the papillary tip (Tip) were analyzed for osm, inulin, and for electrolyte composition using the electron probe. The mean ± SEM osm of Tip urine was 1486 ± 102 mOsm/kg H_2O versus 705 ± 33 mOsm/kg H_2O in Fnx ($p<0.001$). Tip (U/P) inulin was 377 ± 50 vs. 170 ± 26 in Fnx ($p<0.001$), confirming our previous observations. (U/P) ratios for electrolytes were (mean ± SEM):

Electrolyte	Tip	Fornix	P
Na	0.3 ± .04	0.5 ± .10	<0.02
K	26 ± 5.3	14 ± 2.4	<0.01
Cl	1.4 ± .15	1.0 ± .09	<0.005
Ca	1.2 ± .13	1.0 ± .10	=0.05
P	20 ± 6.9	7.6 ± 2.0	<0.05
Mg	25 ± 3.8	13 ± 1.7	<0.005

The lower Fnx osm and electrolyte values are consistent with transepithelial addition of water. In striking contrast, the higher Fnx (U/P) Na indicates net Na addition. We suggest a Na concentration gradient is generated between urine refluxing into the pelvis and medullary interstitium, favoring Na diffusion from medulla to pelvic urine.

PERITUBULAR PROTEIN MODULATES DIRECTLY NEUTRAL ACTIVE NaCl ABSORPTION IN THE RABBIT PROXIMAL CONVOLUTED TUBULE (PCT). Michel Baum* and Christine A. Berry, CVRI, Dept. of Med. and Physiol., Univ. of Calif., San Francisco, CA.

We have previously proposed that peritubular oncotic pressure influences net volume absorption from PCT by a specific effect on active NaCl absorption (JCI 71:263, 1983). In the present study we directly tested this hypothesis. PCT were perfused with a high chloride, low bicarbonate solution simulating late proximal tubular fluid and were bathed in a symmetrical high chloride solution containing 6 g/dl albumin in the control and recovery periods. In this setting all NaCl transport is active, for there are no anion gradients responsible for passive transport. We examined the effect of decreases in peritubular protein to zero g/dl and of increases to 10 g/dl. In our first protocol protein removal decreased volume absorption (Jv) 44% from 0.39 $\pm$ 0.07 to 0.22 $\pm$ 0.08 nl/mm•min. In our second protocol protein addition increased Jv 42% from 0.26 $\pm$ 0.04 to 0.37 $\pm$ 0.04 nl/mm•min. The transepithelial potential difference was zero in all periods, demonstrating that active NaCl transport was electroneutral and transcellular. In the final protocol tubules were bathed in a high chloride, low bicarbonate solution containing either 0, 6, or 10 g/dl albumin at 20°C to confirm that there was no effect of peritubular protein in the absence of active transport. Jv was not different form zero in all periods, demonstrating that changes in peritubular protein were not affecting a passive process.

In conclusion these data demonstrate that changes in peritubular protein concentration (1) can both inhibit and stimulate directly neutral active NaCl transport in the rabbit PCT and (2) can modulate Jv within the physiologic range of peritubular plasma protein, 6-10 g/dl.

DIRECT VISUALIZATION OF THE ISOLATED AND PERFUSED MACULA DENSA. P. Darwin Bell, Kevin Kirk*, Maria Ribadeneira*, and Delon Barfuss. Nephrol. Res. & Training Ctr. and Dept. of Physiol. & Biophysics, Univ. of Ala. in Birmingham, Birmingham, AL.

The macula densa may serve an important role in the control of glomerular function. Until recently, however, direct study of these cells in a living preparation has not been possible. We report the isolation and perfusion of the tubular segment containing the macula densa and visualization by differential-interference contrast (DIC) microscopy of the macula densa during changes in luminal fluid osmolality. Individual thick ascending limbs ranging from 300 to 525 μm in length with glomeruli attached were dissected free from rabbit kidneys. Tubules were bathed with an albumin containing artificial medium and initially perfused with iso-osmotic Ringer's solution. Following dilution of perfusate (290 mOsm to 70 mOsm) by removal of NaCl, the lateral intercellular spaces within the macula densa were observed to increase markedly and there was a small, but significant increase in cell height of the macula densa plaque from 11.2±0.3 μm to 12.5±0.3 μm (n=4). These structural responses were prevented by maintaining perfusate osmolality at 290 mOsm with mannitol. Also the morphologic responses obtained with dilution of the perfusate were reversible and specific for the macula densa, ie there were no structural changes in the remaining thick ascending limb. The increase in intercellular space width in the macula densa may indicate transepithelial water flow and thus suggests that the macula densa is a small water-permeable plaque within the otherwise water-impermeable thick ascending limb.

STUDIES WITH A MONOCLONAL ANTIBODY TO SODIUM POTASSIUM ATPase. Daniel Biemesderfer*, Michael Caplan*, Bliss Forbush* and Michael Kashgarian. Yale Univeristy, Dept. of Pathology, Physiology and Cell Biology, New Haven, CT

A monoclonal antibody to the α-subunit of the Na+-K+-ATPase was developed. Partially solubilized membranes from the outer medulla of dog kidney were used as antigen. A monoclonal antibody (C62.4) was selected with ELISA and an assay for inhibition of Na+-K+-ATPase activity. Immunoprecipitation of membranes of MDCK cells biosynthetically labeled with ^{35}S methionine with C62.4 identified a single 96 KD protein. MDCK cells labeled with the tritiated N-Azido 85 NZOYL derivative of ouabain which binds specifically to the α-subunit of Na+-K+-ATPase, again precipitated a 95 KD protein with C62.4. In both cases, preincubation with Jorgensen purified Na+-K+-ATPase blocked precipitation of the labeled polypeptide. C62.4 inhibited ^{3}H ouabain binding by >70% in the presence of Na, Mg and ATP but did not inhibit ouabain binding in the presence of Mg and Pi. When Na+-K+-ATPase was complexed with the antibody Na+-K+-ATPase, Na+-ATPase and K+-ATPase activity were found to be 25%, 60% and 100% of maximal. C62.4 cross reacted with rat kidney Na+-K+-ATPase. Rat kidneys fixed with periodate-lysine-paraformaldehyde were immunolabeled. Colloidal Au and peroxidase markers were localized solely to the cytoplasmic domain of basolateral plasma membranes. The most intense labeling was in the thick ascending limb, the distal convoluted tubule and the principal cells of the collecting tubule. Intercalated cells and the thin descending limb of Henle had minimal labeling.

CHLORIDE (Cl) TRANSPORT BY PROXIMAL TUBULE (PT): EFFECT OF BICARBONATE ABSORPTION. K.Bomsztyk and M.B. Calalb*, University of Washington, Seattle,WA.

In the early PT increased Cl concentration is attributed to preferential absorption of $NaHCO_3$ with water. We postulated that increased Cl may also reflect HCO_3 dependent Cl secretion. To test this, in vivo paired perfusions were done in surface PT of the rat kidney. Cl was measured by microtitration, tCO_2 by microcalorimetry,Na by atomic absorption, V_{TE} and luminal pH(pH_L), with double barrel microelectrodes. Control, HCO_3 solution (I) was similar to early PT fluid. In test solution(II) all HCO_3 was replaced with SO_4;(III), 1 mg/ml of dextran bound carbonic anhydrase inhibitor was added to (I);(IV),1 mM amiloride was added to (I). All solutions contained sufficient mannitol to reduce water flux (J_V) to zero. Avg. values: net ion fluxes (J_i), pmol/min(+,absor;-,secr);total concentrations in collected fluid,$[i]_L$,mM;transepithelial voltage (V_{TE}), mV, (lumen +) and pH_L were:

Soln.	$[tCO_2]_L$	J_{tCO_2}	$[Cl]_L$	J_{Cl}	$[Na]_L$	pH_L	V_{TE}
I	17.7	187	125	-39	130	7.15	0.6
vs. II	2.9*	-14*	118*	58*	129	6.71*	0.5
I	15.2	231	126	-46	133	7.36	0.8
vs.III	26.1*	38*	122*	49*	141*	7.24*	0.6
I	15.7	233	124	-5	128	7.25	1.3
vs. IV	20.0*	177*	123*	48	132*	7.33*	1.1

*p<.025 test vs. control. Compared to (I): with all test soln. $[Cl]_L$ and J_{tCO_2} were reduced, $[tCO_2]_L$ was lower,(II),or higher,(III),(IV);pH_L was lower, (II)(III) or higher(IV);$[Na]_L$ was the same,(II) or higher,(III),(IV);V_{TE} was the same for all. When J_V=0,HCO_3 absorption drives Cl into the lumen independent of luminal HCO_3,Na,pH or V_{TE}. This effect may, in part,account for increased Cl in early PT.

ASYMMETRIC RESPONSE TO DIETARY SODIUM RESTRICTION AND RELOADING. **J. Brensilver, F. Daniels, G. Lefavour, C. Hoy, M. Ponte,* and S. Cortell. St. Luke's - Roosevelt Hospital and Columbia Univ. New York, NY.**

According to the kinetic model of sodium excretion of Strauss et al (Arch. Int. Med. 102:527 1958), elimination of sodium from the diet results in an exponential decline in urinary excretion, with a 24 hour half-life in man and a cumulative loss approximating the previous daily intake. Thus man maintains excess sodium in the body equivalent to about one day's intake.

In twenty, 14 month old Fisher rats, removal of sodium from a diet providing about 400 microEq/day resulted in the elimination of sodium from the urine within two days. A cumulative loss of about one-quarter of the previous day's intake, 94±11 microEq, was sustained, which suggests a half-life of about six hours. Strauss's kinetic model predicts that restoration of dietary sodium would result in retention of one-quarter of the new daily intake. However, when sodium was replaced in the diet to provide 256±16 (n=5), 456±25 (n=10), or 956±64 (n=5) microEq/day, the rats retained 83±2, 74±4, and 65±2 %, respectively, of the sodium ingested on the first day. These results indicate an asymmetry in the urinary response to dietary sodium restriction and reloading which is not consistent with Strauss's kinetic model.

MECHANISMS OF RITODRINE (R)- AND TERBUTALINE (T)-INDUCED HYPOKALEMIA (HK) AND PULMONARY EDEMA (PE). G. Braden, P. Von Oeyen*, M. Smith*, D. Gingras*, M. Germain, J. Fitzgibbons, Renal & Ob-Gyn Services, Baystate Med. Ctr., Springfield, MA.

R & T are the two beta-adrenergic (BA) drugs commonly used to inhibit preterm labor. Both may produce severe HK and PE, but the mechanisms for these effects have not been completely elucidated. To determine the extrarenal and renal effects of R & T, blood and urine samples were obtained and Holter monitoring was performed for 2 hrs. before and 4 hrs. during either I.V. T (10 µg/min) or R (100 µg/min) in 4 women in preterm labor. Results are expressed as the mean of 4 studies at each time: *P < 0.05 vs. time 0.

Blood Test	0"	15"	60"	120"	240"
K+ (mEq/L)	4.18	3.67*	2.70*	2.60*	2.50*
HCO_3 (mEq/L)	19.9	18.6	18.0*	17.7*	16.7*
pH (venous)	7.35	7.36	7.36	7.35	7.35
Lactate (mmol/L)	1.2	0.9	2.2*	2.7*	4.7*
Glucose (mg%)	65	72	90*	135*	125*
Insulin (µU/ml)	7.4	12.8	27.7*	29.1*	32.5*
Renin (ng/ml/hr)	9.1	8.7	13.8*	17.3*	24.0*
Aldo (ng/ml)	116	87	110	230*	152*

Renal studies before and during R & T showed: the GFR fell from 125 to 107 ml/min, urine V fell from 4.3 to 1.0 ml/min, $U_{Na}V$ fell from 238 to 71 µEq/min, & U_KV fell from 81 to 25 µEq/min. One woman developed supraventricular tachycardia.

We conclude: 1) Early HK may be due to BA-mediated cellular K+ uptake; 2) BA-induced changes in glucose, insulin and aldo may play a role in R & T induced HK; 3) Lactic acidosis is induced by R & T; 4) Enhanced U_KV does not contribute to R & T-induced HK; 5) Na+ & H_2O retention and arrhythmias may play a role in R & T-induced PE.

RAT RENAL PAPILLA: COMPARISON OF TWO TECHNIQUES FOR X-RAY ANALYSIS. R.E.Bulger, A.J.Saubermann*, V.L.Scheid*, and D.C.Dobyan. Depts. of Anes.& Path., Univ. of Texas Health Science Ctr.,Houston, Texas.

Differences in elemental and H20 content in cells of rat papilla have been reported by us and by Beck et al (K.I.25:397-403,1984). To address this problem, we analyzed paired left and right renal papilla (N=6) by microanalysis using both techniques. Our method was used on undipped (U) papilla while our method and that of Beck et al were simultaneously applied to the contraleteral papilla dipped (D) in an external albumin (ALB) standard. Elemental concentrations in mmol/kg wet wt. for collecting duct (CD), papillary epithelial (PE), and interstitial (IC) cells and interstitium (I) for U and D papilla are shown below (Values=X±SEM; *=P < 0.01):

	Na		Cl		K		S		H_2O(%)	
	D	U	D	U	D	U	D	U	D	U
CD	124	227*	154	269*	125	154*	29	34*	75	69*
	±12	±16	±13	±17	± 4	± 7	±1	±1	±1	±1
PE	151	254*	186	278*	141	132	39	40	70	68
	±11	±31	±11	±32	± 6	±12	±2	±4	±1	±2
I	518	654*	517	652*	41	38	9	2*	82	83
	±15	±31	±15	±32	± 3	± 2	±1	±1	±1	±1
IC	391	362	429	401	132	137	22	23	66	69
	±23	±28	±24	±31	± 6	± 8	±2	±2	±1	±2

The results show two major problems with the use of an external ALB standard:1) ALB dipping changes elemental and H_2O content in CD and PE cells and in I; 2) H_2O and elemental content in the ALB change in a direction consistent with Na and Cl movement from tissue to ALB and H_2O from ALB to tissue. Hence, calculations based on the assumption that ALB remains unchanged are erroneous. Such errors are sufficient to account for the differences between our results and those of Beck and coworkers.

EFFECTS OF SYNTHETIC ATRIAL NATRIURETIC FACTOR ON RENAL FUNCTION AND RENIN RELEASE. J. C. Burnett, Jr., J. P. Granger*, and T. J. Opgenorth*, Mayo Medical School, Rochester, MN

Studies were performed in anesthetized dogs (n=5) to determine the effects of synthetic atrial natriuretic factor (ANF) on renal function and renin release. This synthetic factor is composed of 26 amino acids from residues 8 to 33 of ANF. Intrarenal infusion of ANF (0.3 $\mu g/kg\text{-}min^{-1}$) resulted in a unique transient increase in renal blood flow (126±8 to 148±11 ml/min, $p<.05$) with a duration of 3.1±0.4 min, followed by a slight decrease in renal blood flow (126±8 to 117±8 ml/min, $p<.05$), and an increase in glomerular filtration rate (23.1±3.5 to 30.7±1.9 ml/min, $p<.05$), thus increasing filtration fraction (0.19±0.04 to 0.27±0.03, $p<.05$). These hemodynamic alterations were associated with increases in fractional sodium excretion (0.6±0.2 to 5.8±0.8%, $p<.05$), fractional potassium excretion (30.8±9.4 to 56.3±7.3%, $p<.05$), fractional lithium excretion (32.2±7.1 to 60.3±5.7%, $p<.05$), and fractional phosphate excretion (8.7±3.5 to 41.6±11.7%, $p<.05$). Intrarenal infusion of synthetic ANF markedly suppressed renin secretion rate (295.5±84.6 to 17.2±10.6 ng/min, $p<.05$) despite a slight reduction in arterial pressure (123±9 to 118±9 mmHg, $p<.05$). Our studies demonstrate that synthetic ANF results in a marked natriuretic response which is in part mediated by an increase in glomerular filtration rate. The increase in fractional lithium and phosphate excretion suggests that this factor may also have an action on proximal tubule reabsorption. Further, these studies demonstrate that synthetic ANF markedly inhibits renin secretion.

CELL SWELLING INCREASES INTRACELLULAR CALCIUM, A REQUIREMENT FOR THE INCREASE IN K^+ PERMEABILITY WHICH UNDERLIES VOLUME REGULATION IN TOAD BLADDER H.Chase and S.Wong*, Columbia University, N.Y. NY

Epithelial cells exposed to a reduced extracellular osmolality first swell and then shrink to their original volume following an increase in basolateral K^+ permeability (P_K) and loss of KCl. Calcium (Ca) may play a role because lowering extracellular Ca prevents volume regulation (VR).

We examined the role of intracellular Ca (Ca_i) by measuring Ca_i and VR in a suspension of Na-transporting cells obtained by soaking toad bladders in EGTA. Cells were loaded with quin 2, resuspended in Ringer's and fluorescence recorded at 340/485nm. When cells were swelled ~ 10% by adding hypotonic buffer, Ca_i rose from 160±12 to 193±12nM (n=5). Reducing extracellular [Na] isosmotically did not cause Ca_i to increase. The rise in Ca_i after swelling was due to an increase in Ca influx: Ca did not increase when cells were swelled in EGTA (124±12 vs. 122±6nM, n=4).

We measured cell volume (V) with a Coulter counter to see if the rise in Ca_i was required for VR. Cells swelled in EGTA failed to return to their original V, whereas control cells, incubated in 1mM Ca, did. VR was retarded by high extracellular [K] and completely inhibited by the K^+ channel blockers phencyclidine, tetraethylammonium, $DiSC_3 5$ and quinidine.

These studies provide direct evidence that Ca_i plays a fundamental role in VR: cell shrinking, following swelling, is due to a Ca-stimulated loss of K^+, probably via a channel. If cell swelling due to an increase in solute entry also increases Ca_i, then the rise in Ca_i may increase P_K, coupling it to the rate of Na transport.

DEVELOPMENT OF THE Na^+-H EXCHANGE SYSTEM IN LLC PK_1 MONOLAYERS. **Horacio F. Cantiello* and Carlos A. Rabito Nuclear Medicine. Department of Radiology. Massachusetts General Hospital, Boston, Massachusetts.**

The apical membrane of the proximal tubular cells has a Na^+-H^+ exchange system that mediates the active efflux of H^+. This efflux is coupled to the influx of Na^+ moving down its electrochemical gradient. Although the properties of the Na^+-H^+ exchange system have been well characterized by studies performed in renal brush border membrane vesicles, data pertaining to the modulation and development of this system are very limited at present. A significant limitation has been the immutable tissue organization of the renal proximal tubule. Confluent monolayers of LLC-PK_1 cells have two amiloride inhibitable Na^+ transport systems. These systems have been characterized as a Na^+-H^+ exchange and as a conductive anion dependent Ca^{++} inhibitable Na^+ pathway (Kidney Int 25:296,1984). Removal of the cells by trypsin-EDTA treatment of confluent monolayers inhibit the Na^+-H^+ exchange without affecting the conductive pathway. A progressive increase of the Na^+-H^+ exchange was observed, however, after plating the cells at saturation density. Steady-state was obtained 24 hr after plating. Incubation in presence of cycloheximide but not actinomycin D inhibit the development of the Na^+-H^+ exchange. Cells in exponential growth do not have the Na^+-H^+ exchange system. Inhibition of the cell division alone or incubation at pH 7.0 do not have any significant effect on the system. Inhibition of the cell division at low pH, however, produce a significant increase in the Na^+-H^+ exchange system. From these results we conclude that the synthesis of the Na^+-H^+ exchange system is regulated at the translational level. In addition, the pH of the incubation medium and the growing conditions of the cells have an important role in signaling the sythesis of the Na^+-H^+ exchange system.

GRADED VOLUME REPLETION MODIFIES THE NATRIURETIC AND THE KALIURETIC RESPONSES OF THE REMAINING KIDNEY (RK) TO ACUTE UNILATERAL NEPHRECTOMY (AUN) IN THE RAT. C.C. Chaves* and M.H. Humphreys, Div. of Nephrology, San Francisco General Hospital and University of California, San Francisco, CA.

Previous work from this laboratory has shown that AUN in euvolemic rats increases sodium ($U_{Na}V$) and potassium (U_KV) excretions, while in the hypovolemic rat, AUN results only in increased U_KV. In order to examine the effects of different degrees of volume repletion on the response of the RK to AUN, rats were infused 2 hours prior to AUN either with 0, 1.0, 1.5, or 2.0% body weight (bw) of a 3 gm/dl bovine serum albumin in normal saline (BSA-NS) solution, the 0 group receiving only NS at 2.4 ml/h. After infusion and 1 h. recovery, control urine (C) was collected for 1 h, AUN was then performed and experimental urine (E) collected for 2 h. Data are means ±SE. Paired "t" test before and after AUN was performed: $\alpha<.05$; $\alpha\alpha<.001$. Analysis of variance between groups (Barlett's test): $\phi<.05$; $\phi\phi<.001$.

BSA-NS (%bw)	$U_{Na}V$, nEq/min C	$U_{Na}V$, nEq/min E	U_KV, nEq/min C	U_KV, nEq/min E
0 (n=6)	150±50	358±83$^{\alpha}$	463±46	1300±93$^{\alpha\alpha}$
1.0(n=5)	805±184	925±196	1049±125	1442±147$^{\alpha}$
1.5(n=5)	1028±212	1939±369$^{\alpha}$	954±112	1899±796$^{\alpha\alpha}$
2.0(n=6)	1571±113	4099±894$^{\alpha}$ $^{\phi\phi}$	1454±205 $^{\phi}$	2382±2035$^{\alpha\alpha}$ $^{\phi}$

These experiments indicate the following: 1) Graded volume repletion progressively increased the magnitude of $u_{Na}V$ and U_KV after AUN in all groups; 2) the increments in U_KV are greater than in UN_aV, after AUN for each group. These results suggests that the effect of volume expansion with BSA-NS are additive to those of AUN alone.

COUPLING OF TUBULAR Mg TRANSPORT TO NaCl TRANSPORT ? William H. Cliff*, Douglas B. Sawyer*, Maureen M. Wilhelm*, and Klaus W. Beyenbach. Sect. Physiology, Cornell University, Ithaca, N.Y.

Transepithelial Mg transport is conveniently studied in isolated flounder proximal tubules because Mg is secreted from the bath (1 mM Mg) into the small compartment of the tubule lumen where [Mg] up to 62 mM can be measured (electron probe, WDS). The kinetics of transepithelial Mg secretion was studied for bath [Mg] ranging from 0.1 to 20 mM. To detect measurable changes of luminal [Mg], the lumen was perfused at low rates (0.3 - 0.9 nl/min). Mg secretion exhibited saturation kinetics with half saturation at a bath [Mg] of 0.24 mM, indicating saturation of transport at the plasma [Mg] of 1 mM. Transport maximum lies near 2 pmoles/min-mm tubule length. Since the studies were done in the absence of bath SO_4, SO_4 is not essential for Mg transport. Most notably, in the absence of perfusion, when tubules secrete fluid spontaneously, secreted fluid is a solution consisting primarily of Mg (35.1±5.6 mM), Na (105.5±9.8 mM), and Cl (181.0±6.1 mM), n=12. There are no major anion, cation, or osmotic deficits. As [Mg] in secreted fluid rises, [Na] falls, ($[Na]_{sf} = -1.7\ [Mg]_{sf} + 165.9$ mM; r=0.84), with Cl always providing electroneutrality. Mg and fluid secretion are inhibited when bath Na or Cl are removed (Beyenbach, Nature, 299,54,82). Dependence of Mg transport on bath Na and Cl, and the presence of Na and Cl as principal ions in secreted fluid when Mg is secreted, suggests that Mg transport is (directly or indirectly) coupled to NaCl transport. In this context it is of interest that mammalian tALH, the major site of Mg reabsorption, also reabsorbs NaCl, and that inhibitors of NaCl transport also inhibit Mg transport.

CHARACTERIZATION OF ALDOSTERONE-INDUCED PROTEINS WITH LECTIN- AND ANTIBODY-AFFINITY CHROMATOGRAPHY. M. Cox, B. Blazer-Yost*, & J. Wade*. Depts. Med. VAMC & Univ. Pa. Sch. Med., Phila., Pa., & Dept. Physiol., Univ. Md. Sch. Med., Balt. Md.

We have identified several proteins in toad urinary bladders (TUBs) whose synthesis may be related to aldosterone (aldo)-induced Na+ transport (aldo-induced proteins, AIPs). They occur in both membrane (mem) and cytosolic (cyto) fractions, and exhibit polymorphism (variation in molecular weight, MW, 65-70kDa) and microheterogeneity (variation in isoelectric point, pI, 5.5-6.0). We examined the relationship between the mem and cyto AIPs using concanavallin A (conA)- and antibody-affinity chromatography. AIPs were identified with 2D-polyacrylamide gel electrophoresis and autoradiography. ConA bound both mem and cyto AIPs with high selectivity, a 10-20 fold purification being achieved. Although all the AIPs bound, the lower MW AIPs had greater affinity than the higher MW AIPs. Several polyclonal antibody preparations (raised against TUB proteins) also exhibited considerable selectivity, and provided 10-20 fold further purification of both mem and cyto AIPs.

Thus, aldo induces a group of glycoproteins with very similar characteristics (MW, pI, conA affinity, antigenic determinants) in mem and cyto fractions of TUBs. The polymorphism of these proteins is due, at least in part, to differences in glycosylation. Since glycoprotein binding to conA is inversely proportional to the degree of substitution of the terminal mannose residues of the oligosaccharide side chains, the higher MW AIPs appear to contain more complex, substituted mannosyl residues than the lower MW AIPs.

VARIATIONS WITH AGE AND STATE OF HYDRATION IN MEDULLARY CONCENTRATIONS OF SOLUTES AND WATER IN THE RAT KIDNEY. Percy J. Colon, III,* Roberta M. O'Dell-Smith,* and Clelmer K. Bartell.* Univ. of New Orleans, Dept. of Biol. Sciences, New Orleans, LA.

High osmotic concentration of solutes in the adult rat kidney medulla has been well established by many investigators. The question arises as to which solutes contribute the most and how long it takes them to accumulate in the kidney after birth. This study was undertaken in an attempt to answer that question. A series of Sprague-Dawley rats were divided into 3 groups: neonates-5 days post partum, juveniles-21 days post partum and adults-75 days or older. The groups were subdivided into those receiving water ad libitum and those deprived of water. The kidneys were excised from the rats and the central portion containing the inner and outer medulla isolated. Wet and dry weights of tissue were determined and analyzed for total osmolality, sodium and potassium, chloride, urea and amino acids using aliquots of the same tissue for each analysis. Results indicate that there are variations in the percentages of solutes that make up the total osmolality particularly in the neonate and the juvenile with regard to relative proportions of urea and sodium. These results will be discussed in terms of the countercurrent mechanisms for renal concentration.

SYNTHETIC ATRIAL NATRIURETIC FACTOR (ANF) INHIBITS Na-COUPLED SOLUTE REABSORPTION IN PROXIMAL TUBULES. T. P. Dousa, T. G. Hammond*, A. N. K. Yusufi*, and F. G. Knox, Depts. of Physiology and Medicine, Mayo Medical School, Rochester, MN

The intrarenal location and the cellular mechanism by which ANF elicits natriuresis is not yet clarified. We explored whether ANF influences proximal tubular transport of solutes. TPTX rats were infused with synthetic ANF, or controls (CON) with vehicle, and their kidneys were removed for preparation of brush border membrane vesicles (BBMV) and transport studies. Infusion of ANF resulted in an increase of fractional excretion (FE) of Na^+, Pi, and HCO_3, but not of K^+, Ca^{2+}, or Mg^{2+}. The Na^+ gradient-dependent uptake of ^{32}Pi and Na^+-H^+ exchange (pmoles 15 sec/mg protein) were markedly inhibited in BBMV from ANF-infused rats. Uptake of $^{22}Na^+$ and Na^+-dependent uptake of L-proline by BBMV were not changed.

	FE_{Na^+}	FE_{Pi}	FE_{HCO_3}	^{32}Pi uptake	Na^+-H^+ exchange
CON:	1.1±0.3%	0.8±0.4%	5.8±2.1%	1862±69	295±36
ANF:	3.7±0.7%†	5.0±2.0%†	9.9±1.9%†	1378±143†	173±28†

†different from controls (P<0.05, t-test)

The change (Δ) in response to ANF in Δ FE_{Na} was correlated both with Δ FE_{Pi} (r=0.9; P<0.01) and Δ FE_{HCO_3} (r=0.89; P<0.01), but not with FE of any other solutes. Therefore, infusion of ANF decreases reabsorption of Pi and of Na^+ reabsorption coupled to HCO_3 in proximal tubules, and also inhibits luminal brush border membrane transport systems. We conclude that ANF inhibits reabsorption of Na^+, Pi and HCO_3 in proximal tubules of rat nephron.

INCREASED BINDING OF FUROSEMIDE BY TAMM HORSFALL GLYCOPROTEIN OF DIABETICS - RELATION TO ABNORMAL TUBULAR Na HANDLING?

Jan Dulawa*, Joachim Greven, Max Notohamibrodjo and Eberhard Ritz (intr. by R. Glassock). Dept. Internal Medicine, University of Heidelberg and Dept. Pharmacology, Technische Hochschule Aachen, FRG

The affinity of Tamm Horsfall glycoprotein (THP) for furosemide (F) and diuretically active congeners (in press) correspond well with affinity of F to Henle loop thick ascending limb Na, K, Cl_2 Co transport in vitro (Greger, 1983). We have previously demonstrated abnormal composition and colloid stability of THP in diabetics (DM) (Kidney Int. 25, 237,1984). Therefore, we examined whether chemically abnormal THP in DM also shows impaired ^{14}C-F-binding. THP was isolated from 8 non-proteinuric type I diabetics, 8 matched controls and 4 Bartter pat. THP was pure by immunediffusion and SDS. Binding of ^{14}C-F (spec. act. 8 Ci/mol) was determined using Amicon ultrafiltration system at 0°C. Control THP showed Na and Cl dependent saturable F binding; binding isotherm suggested pos. cooperativity (Hill coeff. 1.53). Half max. F-binding by THP occured at 1.2 mmol Na/l in CO and 0.47 in DM. Max. binding in a NaCl system was 2.5±0.3 nmol F/mg THP in CO, 5.1±0.5 in DM ($p < 0.025$) and 1.0±0.3 in Bartter.

The data shows that in two clinical states with opposite changes of tubular NaCl transport, binding of furosemide by Tamm Horsfall glycoprotein changes in the same direction as avidity of tubular NaCl transport.

KCl COTRANSPORT IN RABBIT RENAL CORTICAL BASOLATERAL MEMBRANE VESICLES. J. Eveloff*, J. Calamia*, and D.G. Warnock. Nephrology Sec., VA Medical Center, and Dept. of Physiology, CVRI, Univ. of California, San Francisco, CA.

KCl cotransport is thought to be a major route for K and Cl exit from epithelial cells. Our experiments examined KCl cotransport in the basolateral membrane of the proximal tubule. Basolateral membrane vesicles were prepared from rabbit renal cortex by differential and sucrose density gradient centrifugation at pH = 7.0 (JBC 258:13513, 1983). Rubidium (86Rb) and chloride (36Cl) uptake were measured by the rapid filtration technique at 25°C. The uptake buffer contained 1 mM K Anion, 80 mM N-MethylGlucamine (NMG) Anion, 100 mM sucrose, 50 mM Hepes/Tris at pH 7.0. Valinomycin (Val) was 7 μM when present.

	86Rb uptake (pmol/mg/10sec)	
Anion	- Val	+ Val
Chloride	786.4±201.8	812.4±138.6
Nitrate	426.5±50.4	2054.7±180.1#
Gluconate	128.2±36.6	231.9±64.7

(Mean ± SEM, n = 5, #P < 0.05 compared to control)

In addition, 36Cl uptake (15mM) was stimulated more by an inwardly directed 80 mM K gradient (3.59 ±1.55 nmol/mg/10 sec) than with a 80 mM NMG gradient (0.92± 0.01 nmol/mg/10 sec), and there was no effect of Val on 36Cl uptake in the presence of the K gradient.

Conclusions: (1) The K conductance of the basolateral membranes was low in these experiments since 86Rb uptake was increased with K Nitrate plus Val. (2) The Cl conductance was low since K gradients plus Val had no effect on Cl uptake. (3) The low conductances and the stimulation of 86Rb uptake by chloride compared to nitrate suggest electrically neutral KCl cotransport in rabbit cortical basolateral membrane vesicles.

CALCIUM ENTRY MODULATION AS A DETERMINANT OF SODIUM EXCRETION BY THE ISOLATED PERFUSED RAT KIDNEY. Murray Epstein, Rodger Loutzehniser*, Charles Horton*, and Phillip Sonke*. Nephrology Sec., V.A. Med. Ctr. & U. of Miami, Miami, FL.

Recent observations implicate cytosolic Ca^{+2} as a regulator of renal Na transport. Since calcium entry blockers (CEB) modulate cytosolic Ca^{+2}, we examined their effects on Na excretion using the isolated perfused rat kidney (IPRK). The effects of manganese (Mn, 10^{-2} M), an inorganic CEB exhibiting little tissue selectivity, and Ca^{+2} removal by chelation with 5mM EGTA (EG), were compared to those of three organic CEB's [10^{-7} M nisoldipine (N); 10^{-6} M nitrendipine (NT) and 10^{-5} M diltiazem (D)]. At these doses, D, N and NT each produced identical reversal of norepinephrine-induced vasoconstriction. When administered in the absence of norepinephrine, however, these equivalent vasoactive dosages produced different effects on UNaV (μEq/min) and FENa (%). (Mean ± SE; † $p < 0.05$)

	BASAL UNaV	DELTA UNaV	DELTA FENa
D	4.8±0.8	0.7±0.8	0.2±0.3
N	9.0±0.9	1.4±1.3	1.8±1.1
NT	5.2±0.7	3.5±1.9†	4.1±2.2†
Mn	7.3±2.1	4.5±1.2†	7.2±1.7†
EG	9.7±1.4	16.7±2.8†	14.6±1.4†

Thus, Ca entry blockade by NT, Mn or EG was natriuretic rather than antinatriuretic in the IPRK. The striking differences in the effects of D, N and NT on FENa indicate that organic CEB's differ in their relative potency for evoking natriuresis and vasodilation. An interaction of Mn and NT with a different class of Ca^{+2} channels, on the epithelial plasmalemma, may mediate the natriuretic effects of these agents.

SIMULATION OF VOLUME AND SOLUTE FLUX ACROSS A FIBRE MATRIX TIGHT JUNCTION (TJ) IN PROXIMAL CONVOLUTED TUBULES (PCT). William D. Fraser* and Andrew D. Baines, Dept. of Clinical Biochemistry, Univ. of Toronto.

Assuming that the TJ is a protein fibrous mesh Curry (The Physiol,23:90) developed equations to describe volume and solute flow across endothelial membranes. We have applied these equations to the PCT. Values for the length (l)(1.6×10^{12}cm/cm^3TJ) and radius (r)(3.5×10^{-8}cm)(which determine the void volume (ϵ)), were estimated from known ultrastructure of the TJ and proteins. Values for the TJ fractional area (A)(1.0×10^{-4}) and thickness (ΔX)(1.0×10^{-4}) were obtained from the literature. The equations predict values of the hydraulic permeability (Lp), solute permeabilities (Ps), and reflection coefficients (σ), that are similar to experimentally determined values.

Lp(calc) 1.8×10^{-7} (exp) 1.7×10^{-7}cm·s^{-1}(cm H_2O)$^{-1}$

	Ps(calc)	Ps(exp)	σ(calc)	σ(exp)
Mannitol	7.4×10^{-6}	8.7×10^{-6}cm·s^{-1}	.63	.69
Sucrose	5.7×10^{-6}	4.3×10^{-6}cm·s^{-1}	.74	.82
Inulin	6.5×10^{-7}	--	.96	--

To simulate protein fibre contraction r was increased by 29% with constant ϵ. This produced a 7% increase in P_{suc} and a 70% increase in Lp. When ϵ was decreased from .994 to .90 with constant r, Lp decreased dramatically from 1.8×10^{-7} to 4.9×10^{-10}cm·s^{-1}(cm H_2O)$^{-1}$ with only a 25% decrease in P_{suc}. Thus in a fibrous TJ:1. Lp is very sensitive to r and ϵ. 2. Experimental values of Ps cannot be used to predict Lp without detailed knowledge of the TJ ultrastructure. 3. Depending on the structure, the TJ could account for the Lp and Ps for nonelectrolytes, such as sucrose and mannitol, in the PCT.

EFFECT OF DIETARY CHLORIDE ON CL UPTAKE IN THE COLLECTING DUCT (CD). J.H. Galla, D.N. Bonduris*, R.G. Luke (intr. by C.W. Old). Nephrology Research and Training Center, University of Alabama in Birmingham.

To explore further the mechanism of the effect of prior (7-10 days) dietary Cl intake on CD Cl uptake (AJP 239:F552, 1980), we compared late distal (LD) Cl delivery to and ^{36}Cl efflux from the Cl in the following groups after injection of ^{36}Cl and ^{3}H into LD tubule: I - drinking 0.15M NaCl, IIA - regular diet, IIB - regular diet + DOC 2 mg IM (at 24 and 2 h before study), IIIA - low NaCl diet; 0.15 M $NaHCO_3$ at 5% body weight/hr was infused to increase tubule fluid flow rate. Because P_{Cl} was significantly lower in group IIIA, group IIIB was infused with 0.15 M Na^+ with Cl 55 and HCO_3 85 meq/L. The results were:

Group	n	P_{Cl} (meq/L)	TF_{Cl} (meq/L)	V_{TF} (nl/min)	Cl delivery (peq/min)	^{36}Cl recovery (%)
I	6	84±2	33±3	9±2	308±67	92±4
IIA	7	83±2	23±4	9±1	223±53	82±2§
IIB	6	83±2	19±3§	9±1	165±36	82±2§
IIIA	7	75±2§	24±4	6±1†	118±13§	66±6§†
IIIB	8	84±1	20±3§	10±1	193±37	50±3§†

§ $p<0.05$ compared to I; † $p<0.05$ compared to IIA.

There was no significant correlation between Cl delivery to the CD and ^{36}Cl recovery within any of the groups or for all animals studied ($r=0.313$; d.f. 29).

We conclude that progressive restriction of dietary Cl enhances Cl uptake in the CD unrelated to changes in P_{Cl}, Cl delivery from the superficial LD tubules or increases in mineralocorticoid.

EFFECTS OF ALDOSTERONE, ADH, AND TRYPSIN ON ^{3}H-PHENAMIL BINDING TO TOAD BLADDER. J.L. Garvin, S.A. Simon, L.J. Mandel & E.J. Cragoe*. Dept. of Physiology, Duke Univ. Med. Ctr., Durham, N.C. & Merck, Sharp & Dohme Res. Lab., West Point, Pa.

We previously reported that phenamil irreversibly inhibits short circuit current (Isc) of toad urinary bladder by specifically binding to mucosal membrane sodium channels.[1] This irreversible binding makes phenamil a useful tool to measure channel density of the mucosal membrane. ^{3}H-Phenamil binding experiments were performed using either intact bladders, to correlate binding with inhibition of Isc, or isolated epithelial cells. The relationship between ^{3}H-phenamil concentration and binding saturated at 0.05 µM. Therefore, concentrations slightly greater than 0.05 µM were used in all experiments.

Specific binding corresponded to a channel density of 422/µm^2, and was not correlated with the inhibition of Isc. In paired experiments, specific binding to ADH-treated tissues was not different from that of controls. Similarly, specific binding of aldosterone treated tissue did not differ from paired controls. However, pretreatment of bladders with trypsin reduced specific binding by 80%. From these data, we conclude: 1) The ratio of open/closed channels is small, about 1/60; 2) ^{3}H-phenamil binds to all forms of the channel, whether open, closed, or inactivated within the mucosal membrane or inside the cell; 3) A new model of the mucosal sodium channel may be formulated which includes several new states.

[1]Garvin, J.L. et al. Fed. Proc. 42:1282, 1983.

MODULATION OF NA-K-ATPASE IN CORTICAL COLLECTING DUCT (CCD) BY DIETARY K^+ IN ABSENCE OF ALDOSTERONE (A). Lal C. Garg and Neelam Narang* Univ. of Florida College of Medicine, Gainesville, Fla.

Na-K-ATPase activity in CCD has been shown to be influenced by the dietary intake of K^+. This has been attributed to a change in plasma A which also influences Na-K-ATPase activity in CCD. To investigate whether or not the dietary K^+ can modulate Na-K-ATPase in CCD independent of A, we determined Na-K-ATPase activity in CCD of adrenalectomized (adx) rabbits given 4 different diets for 1 week before experimentation. All diets were similar in composition except their K^+ contents which were 100 meq/kg (group I), 300 meq/kg (group II), 500 meq/kg (group III) and 700 mq/kg (group IV). Na-K-ATPase activity in CCD of all four groups is given below as mean ± SEM of 4-6 animals in pmol·min^{-1} mm^{-1}.

Group I	Group II	Group III	Group IV
9.5±1.2	16.0±3.7	23.8*±0.9	32.5**±3.1

*$P<0.01$ vs group I; **$P<0.01$ vs groups I and II.

Na-K-ATPase activity in CCD of groups III and IV was significantly greater than group I. In addition, there was a significant ($P<0.02$) linear relationship between Na-K-ATPase activity in CCD and K^+ excretion in adx animals from all 4 groups. Our data indicate that the dietary K^+ can influence Na-K-ATPase activity in CCD independent of A.

EFFECT OF SYNTHETIC ATRIAL NATRIURETIC FACTOR IN NEWBORN PUPPIES. M. Gharagozloo*, J. C. Burnett, Jr., and A. Haramati, Dept. of Physiol., Mayo Medical School, Rochester, MN

Synthetic atrial natriuretic factor (ANF), 26 amino acid peptide, has been shown to increase glomerular filtration rate (GFR), urine flow (V), and fractional excretion (FE) of sodium (Na) in adult dogs. Since the neonatal dog does not respond well to some natriuretic stimuli (e.g., volume expansion), we tested whether ANF would elicit a natriuresis in young, immature puppies. Clearance experiments were performed in 6 puppies (27-37 days of age). ANF (0.3 µg/kg·min) was infused into the left renal artery via a small 30 gauge needle. Results: († = $p<.05$ vs control).

	V ml/min	FE_{Na} %	FE_K %	FE_{Pi} %	GFR ml/min/gkw	BP mmHg
Control	.07 ±.01	1.3 ±.3	32 ±1	19 ±4	0.45 ±.12	86 ±6
ANF	0.55† ±.15	11.1† ±3.2	67† ±7	31† ±7	0.52 ±.10	75† ±4

ANF significantly increased V and FE of Na, of potassium (K), and phosphate (Pi). Mean arterial blood pressure (BP) decreased significantly, but there were no significant changes in GFR. Parallel clearances from the right kidney showed no changes in renal function between the periods. We conclude that 4-5 week old puppies respond to ANF with increases in water and electrolyte excretion in the absence of significant changes in GFR.

ELECTRICAL HETEROGENEITY OF AMPHIUMA PROXIMAL TUBULES. G. Giebisch, H. Oberleithner, F. Lang. Yale University, Dept. of Physiology, New Haven,CT

In Amphiuma, early (EPT) and late (LPT) proximal tubules can be discriminated by differences in color, i.e. EPT are dark brown, whereas LPT are white. Electrophysiological techniques (cable analysis and step changes of extracellular ion concentration) show striking differences between EPT and LPT. The following table summarizes our observations:

	Rte	Rm	VDR	Rs	ΔPDk	ΔPDb	ΔPDg
EPT	27±7	29±3	1±0.2	33±8	23±2	21±2	11±2
LPT	4±1	64±5	1±0.4	4±1	6±1	7±1	3±2

Rte, Rm and Rs: Resistance across the epithelium, both cell membranes in parallel, and the shunt pathway (kohm cm). VDR: Cell membranes resistance ratio. ΔPDk: depolarisation (mV) of the peritubular cell membrane to step changes of potassium from 2.5 to 12.5 mmol/l. ΔPDb: hyperpolarisation of the peritubular cell membrane following replacement of Hepes by bicarbonate. ΔPDg: depolarisation of the luminal cell membrane by luminal application of 4 mmol/l glucose. All except VDR are significantly different , EPT vs LPT.

The most striking observation is the electrical tightness of the paracellular pathway in EPT, and differences in basolateral ion conductances. Transport of glucose is more pronounced in EPT than in LPT. The basolateral cell membrane is dominated by a bicarbonate conductance in EPT, and a potassium conductance in LPT. Transport of substrates such as glucose is more pronounced in EPT than in LPT. This implies differences in transport across the two nephron segments.

Ba^{++} SENSITIVE, Ca^{++} ACTIVATED K^+CHANNELS IN CULTURED RABBIT MEDULLARY THICK ASCENDING LIMB CELLS (MTAL) AND CULTURED CHICK KIDNEY CELLS (CK). S.E. Guggino*, B.A. Suarez-Isla*, W.B. Guggino, N. Green* and B. Sacktor*. LMA, LNS, NIA and Dept. of Physiology, Johns Hopkins University, Baltimore, MD and LKEM, NHLBI, Bethesda, MD.

A Ba^{++} sensitive K^+ conductance is present in the luminal membrane of the thick ascending limb and cortical collecting tubule. Patch clamp experiments were performed on cultured kidney cells to study the conductive properties of the apical cell membrane. We have identified in cell attached patches a Ca^{++} activated K^+ channel, in passage 24 of rabbit MTAL cells (GRB-MAL2) which demonstrated a specific differentiated property of MTAL, the Tamm-Horsfall protein, in passage 12. The channel has a single channel conductance of 133 ± 24 pS and is blocked by Ba^{++}. Both the mean open time and fractional open time of spontaneous fluctuations increased with depolarization. We conclude that a K^+ channel is a component of Ba^{++} sensitive K^+ conductance in the MTAL. We have also identified a Ca^{++} activated K^+ channel in the apical cell membrane of a population of CK cells. The single channel conductance is 96 ± 17 pS and shows a voltage dependent Ba^{++} block. Perfusion of either forskolin or ADH but not Ringers, from a pipet near the surface of the patched CK cell caused a rapid and reversible 17 fold increase in the fraction of the time spent in the open state from 0.17 ± 0.04% to 3.0 ± 0.9% (at -40mV applied voltage) and an increase in mean open time. The stimulation of a Ca^{++} activated K^+ channel may be a mechanism by which K^+ secretion is enhanced in some nephron segments through c-AMP mediated pathways.

ELECTRICAL PROPERTIES OF COLLECTING DUCT PRINCIPAL CELLS IN TISSUE CULTURE. Peter Gross*,Will Minuth*,Wilhelm Kriz*,Eberhard Frömter*(intr.by Robert J.Anderson).Univ. of Heidelberg,Dept.of Medicine and Anatomy, Univ.of Frankfurt,Dept.of Physiology,FRG.

We have recently succeeded at isolating and growing in tissue culture a collecting duct epithelium derived from kidney cortex of newborn rabbits.Morphological,ontogenetic,biochemical and immunologic properties were typical of collecting duct while electron microscopy revealed a composition of principal cells only.This monolayer was now characterized by measuring its potential difference(PD)and its transepithelial resistance(R)in a microperfusion chamber using Ringer's solution.Thus,epithelia cultured for 4-8 days under standard conditions(n=8)had a PD of -7.75±6.4 mV,while R was 2.1±.86 $k\Omega cm^2$.Comparably cultured epithelia,preincubated in 10^{-6}M aldosterone had a significantly higher PD of -32.3±15 mV(n=18;p<.05),while R remained at 2.87± 1.2 $k\Omega cm^2$(NS).10^{-6}M amiloride reduced this PD to -3.9±2 mV and increased R slightly. In contrast to collecting duct in vivo $5x10^{-3}$M Ba^{++} neither changed PD nor R significantly. - It is concluded that principal cells in tissue culture develop electrical properties characteristic of active sodium absorption,which can be stimulated by aldosterone.The experiments with barium suggest that apical K^+-permeability may be preferentially associated with dark cells or may be suppressed during culture. (Data reported as means ± SD).

SODIUM DELIVERY FROM SUPERFICIAL AND DEEP PROXIMAL TUBULES IN RESPONSE TO CHANGES IN RENAL PERFUSION PRESSURE. J. A. Haas*, J. P. Granger*, and F. G. Knox, Dept. of Physiology, Mayo Clinic and Foundation, Rochester MN

Previous studies have demonstrated that superficial proximal sodium delivery does not change in response to alterations in renal perfusion pressure (RPP). However, preliminary studies measuring whole kidney proximal sodium reabsorption utilizing the lithium clearance technique demonstrated that proximal sodium delivery is changed during alterations in RPP. In the present study, micropuncture was performed to determine if the changes in whole kidney proximal sodium delivery are due to changes in superficial and/or deep proximal sodium delivery. A comparison was made of fractional sodium delivery (FD_{Na}) to the superficial late proximal tubule (Superficial), the descending limb of Henle's loop of juxtamedullary nephrons (Deep) and the papillary tip (Urine) in response to acute changes in RPP by means of suprarenal aortic constriction in the rat (n = 8).

	Renal Perfusion Pressure (mmHg)		
	117±5	141±5	
	Proximal Tubule (FD_{Na}%)		P
Superficial	56±6	57±4	NS
Deep	31±2	47±7	.02
Urine	3±1	6±1	.01

These studies demonstrate that FD_{Na} from deep, but not superficial, proximal tubules is altered during changes in RPP. We conclude that proximal tubules of deep nephrons are more sensitive to changes in RPP than superficial nephrons.

ATYPICAL ACTION OF ALDOSTERONE ON TARGET TISSUES. J. Halevy*, H.J. Binder*, E.L. Boulpaep and J.P. Hayslett. Yale Univ. Sch. of Med., New Haven, CT.

The effect of aldosterone (Aldo) to increase Na and K transport in rat distal colon (D) and other target tissues involves a rise in apical membrane Na conductance (G_A), transepithelial PD (V_T) and I_{SC}. Since Aldo also stimulates Na and K transport in rat proximal colon (P) in the absence of these typical changes (KI 25:265,1984), studies were performed in controls (C) and rats on a Na-free diet for 7 days (E) to analyze the effect of Aldo on the electrical properties of the adjacent colonic segments in vitro. Cell impalements permitted estimates of apical (V_A), and basolateral (V_{BL}) voltages, and $\alpha \cong (R_A/R_T)$. Values are mean ± SE; *p<0.05.

		V_T	V_A	V_{BL}	I_{SC}	
			mV		μA·cm^{-2}	
Distal	C	-7±0.8	31±18	-38±1	31±5	0.87±0.02
	E	-20±4*	21±4*	-42±1*	179±49*	0.75±0.05*
Prox	C	-2.6±0.5	31±2	-33±2	40±6	0.70±0.04
	E	-5.6±1.1	32±3	-38±2	79±14*	0.65±0.04

In contrast to D where aldosterone causes significant changes in cell membrane voltages, a 6 fold rise in I_{SC} and a fall in α, voltage and conductive changes were absent in P, and I_{SC} increased only 2 fold. Moreover, in E animals Na channel (amiloride) and K channel (TEA) blockers increased α significantly in D only. Conclusion: Aldo stimulates Na and K transport in target tissues by different mechanisms. In rat D Aldo increases G_A for Na and electrogenic Na absorption. In P, in contrast, Aldo primarily stimulates electroneutral Na transport and has no effect on G_A for Na or K.

AMILORIDE ANALOGS INHIBIT Na-GLUCOSE AND ALANINE COTRANSPORT IN RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV). R.C. Harris,* R.A. Lufburrow III,* E.J. Cragoe, Jr.,* and J.L. Seifter* (intr. by B.M. Brenner). Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA and Merck, Sharp and Dohme Research Lab., West Point, PA.

The pyrazine diuretic amiloride (Am) is a known inhibitor of Na/H exchange, Na/Ca exchange, Na channels and Na-K ATPase. We examined the effects of Am, 5-ethyl-isopropyl Am (5-EIA) and Benzamil (B) on initial rates of Na coupled D-glucose (glu) and L-alanine (ala) cotransport in rabbit renal BBMV and tested the effects of the analogs on Na-H exchange and Na-K ATPase. The percent inhibitions (±SE) at inhibitor concentration [I] and at the indicated [Na] were as follows:

	[Na]mM	[I]mM	Am	5-EIA	B
^{3}H glu	50	1.0	17±5	84±4	65±2
^{3}H ala	50	1.0	51±7	65±2	61±5
Na/H	1	0.1	76±5	88±4	35±7
Na-K-ATPase	100	1.0	24±6	89±1	79±6

Am gave dose dependent inhibition of glu and ala uptake. Decreasing external [Na] increased the inhibition of ^{3}H-glu uptake by 1mM Am; inhibition was independent of glucose concentration (0.1μM-1.0mM). Maximum ^{3}H-phlorizin binding (0.1μM) was unchanged by 2mM Am (16.6±2.2 vs 17.4±2.3pmoles/mg protein), whereas K_{Na} increased from 7.9±0.6 to 13.4±2.2mM (p<.05), further indicating competitive inhibition by Am of Na in Na-glu co-transport. These results extend the spectrum of Am inhibition to include Na-glu and Na-ala co-transport in renal BBMV. The sensitivity of Na-glu, Na-ala cotransport, Na/H exchange and Na-K ATPase to inhibition by Am analogs implies a conformational similarity among Na sites of these processes.

BASOLATERAL ANION CHANNELS IN RABBIT URINARY BLADDER EPITHELIUM. John W. Hanrahan*, William P. Alles*, and Simon A. Lewis*. (intro. by Peter S. Aronson) Yale Univ. Sch. Med., New Haven, CT

There is a large Cl conductance in the basolateral membrane of urinary bladder epithelium and the cortical thick ascending limb, but little is known regarding the properties of these pathways. Two groups of rabbit urinary bladders were mounted in Ussing-type chambers: The first group was equilibrated in NaCl Ringers, the second was exposed on the luminal side to 50 μM gramicidin and high-K Ringers to reduce apical membrane resistance. Diffusion currents were measured immediately after replacing serosal Cl with other anions. In both groups, NO_3, SCN, and Br replacement caused small increases in transepithelial current whereas gluconate and acetate caused large decreases. Gluconate substitution on the luminal side of bladders in group 1 had no effect, indicating that little of the anion current flowed through the paracellular shunt, which has very low conductance in this tissue. These data suggest that the basolateral membrane is relatively non-selective between Cl, NO_3, Br, and SCN, but has much lower permeability to acetate and gluconate. The patch clamp technique was used to record single channel currents through the basolateral membrane of dissociated bladder cells. We observed anion-selective channels that were open 91% of the time, had a conductance of 64.0 ± 4.7 pS at -50 mV, and showed the permeability sequence 1.0 Cl ≃ Br ≃ I ≃ NO_3 ≃ SCN > 0.52 F > 0.49 acetate > 0.10 gluconate. We conclude that this anion channel may be responsible for basolateral Cl conductance in the urinary bladder and perhaps other epithelia.

VASO-ACTIVE INTESTINAL PEPTIDE (VIP) EFFECT ON RENAL FUNCTION. R.L. Hébert*, A. Fournier*, S. St-Pierre*, and G.E. Plante* (intr. by T. Nawar). Dept. of Physiology, Univ. Sherbrooke, Sherbrooke, Canada.

VIP influences the intestinal transport of sodium and water. This study examines the effect of VIP on sodium excretion ($U_{Na}V$) and renal dynamics using clearance techniques in anesthetized volume expanded dogs (EXP). VIP was either infused at a rate of 250 pM/kg/hr or given in bolus of 100, 150 and 200 pM in the femoral vein. In addition, the entire peptide and its fragments were administered in the left renal artery.

Continuous infusion of VIP reduced by 100% the normal response to EXP: $U_{Na}V$ only rose from 107±20 to 203±58 uEq/min (control: 98±15 to 558±47). When VIP infusion ceased, $U_{Na}V$ rose to 371±82 and 489±69 but decreased again to 401±63 and 325±60 upon reinfusion of VIP. GFR remained stable. Given in bolus of increasing doses, VIP reduced $U_{Na}V$ from 702±91 to 431±73, from 469±73 to 325±46, and from 404±98 to 290±70. GFR remained stable, but a small decrement in RPF occurred. A dose of 100 pM in the renal artery reduced $U_{Na}V$ from 465±79 to 273±58. Saralasin blockade failed to alter the renal response to VIP. Only the 15-28 VIP fragment reduced $U_{Na}V$ from 422±72 to 358±64. Two other fragments, 1-14 and 2-28 VIP, had no effect on $U_{Na}V$.

In conclusion, the continuous infusion or single bolus of VIP are both associated with a reduction of $U_{Na}V$. This phenomenon appears to result from a direct action of VIP on sodium transport, since the small effect on renal hemodynamics could not be correlated with the observed changes of $U_{Na}V$. Finally, fragment 15-28 of the VIP molecule seems to be responsible for this effect.

PERITUBULAR PHYSICAL FACTORS ARE ESSENTIAL FOR SUPPRESSION OF PROXIMAL FLUID REABSORPTION DURING IN VIVO INHIBITION OF CARBONIC ANHYDRASE (CAI). I. Ichikawa. Children's Hospital, Boston, Mass.

CAI suppresses transepithelial fluid absorption in isolated proximal tubules. Using in vivo micropuncture technique, the physical forces and reabsorption coefficient (K_r) which determine peritubular capillary uptake of proximal tubule reabsorbate were measured before (CONT) and during CAI with Benzolamide (2 mg/kg/hr, iv). Results [n=7 rats; mean; P<0.05 vs. CONT (†) and vs. CAI (§)] include:

	AR	FR	$\overline{\Delta\Pi}$	$\overline{\Delta P}$	$\overline{P}_r$	K_r
	nl/min		mmHg	mmHg	mmHg	nl/s/mmHg
CONT	26.7	0.51	23.9	10.7	13.2	0.037
CAI	17.7†	0.36†	24.6	15.2†	9.5†	0.033
CAI + ↑π	25.6§	0.48§	27.3†§	14.3†§	13.0§	0.036

CAI reduced both absolute (AR) and fractional (FR) proximal tubule reabsorption. This was accompanied by a fall in net peritubular capillary reabsorptive pressure ($\overline{P}_r$), a result of elevated transcapillary hydraulic ($\overline{\Delta P}$) and constant oncotic pressure difference ($\overline{\Delta\Pi}$). The rise in $\overline{\Delta P}$, in turn, was due primarily to increased intracapillary hydraulic pressure. The potential causal link between the fall in $\overline{P}_r$ and AR was evaluated by normalizing $\overline{P}_r$, without affecting SNGFR or serum HCO_3^- concentration, by infusing hyperoncotic, high hematocrit blood (↑π). Despite persistent CAI, elevation in $\overline{\Delta\Pi}$ and normalization of $\overline{P}_r$ brought AR to pre-CAI level. Continued CAI alone, with unchanged $\overline{P}_r$, did not raise AR or FR (n=4 rats). The results indicate that, in vivo, CAI, either directly or indirectly depresses peritubular capillary uptake forces, a feature essential for its inhibitory influence on proximal fluid reabsorption to be effectively expressed.

INCREASED FLOW RATE FAILS TO INDUCE ADAPTATION IN ISOLATED PERFUSED SUPERFICIAL PROXIMAL STRAIGHT TUBULES (SFPST). J.R. Johnston,* S.C. Hebert, and B.M. Brenner. Brigham and Women's Hosp. and Harvard Medical School, Boston, MA.

In the rabbit reduction of renal mass induces glomerular hyperfiltration and increased intrinsic volume reabsorptive capacity (J_v, [nL/(mm·min)]) of perfused proximal tubules isolated from these animals. To evaluate whether an acute increase in luminal flow rate (V_i, nL/min) in vitro could reproduce this in vivo adaptive increase in J_v, we perfused SFPST from normal rabbits and compared J_v measured before and after a 60 min period of increased V_i. All tubules were bathed in rabbit serum and perfused with an ultrafiltrate of the bath. Two ranges of luminal flow rate were examined: a high flow range where control V_i=10, experimental V_i=30 for 60 min, and recovery V_i=10; and a low flow range where V_i values for the respective periods were 3, 10 and 3 nl/min. The J_v values ([nl/(mm·min)] ±SEM) were:

	Control	Experimental	Recovery
High flow (n=7)	.55±.05	.55±.11	.52±.05
Low flow (n=8)	.49±.04	.54±.04**	.50±.05

(** p<.025 compared to control and p<.005 compared to recovery)

In the high flow range J_v did not increase with elevating V_i, and no adaptive increase in J_v occurred upon return to control V_i. In the low flow range, despite a small apparent increase in J_v at the higher V_i, J_v fell to the control value upon return to the lower flow rate. We conclude that a 60 min period of increased V_i over high or low flow ranges provides an insufficient in vitro stimulus to cause adaptive increases in the intrinsic rate of volume absorption in SFPST.

INTRARENAL EFFECT OF ATRIOPEPTIN III IN THE ISOLATED PERFUSED RAT KIDNEY. A. Itabashi,* J. Shapiro,* C. Cheung,* and L. Chan. Dept. Med. Univ. Colorado Med. Sch., Denver, CO

The effects of the synthetic atrial peptide atriopeptin III (Geller et al, BBRC 120:333, 1984) were examined in the isolated perfused rat kidney. Atriopeptin III, 1 µg, was added to the perfusate (100 ml of 6.7% albumin Krebs-Hensleit bicarbonate buffer) in a recirculating system. As compared to control kidneys, the peptide increased Na excretion ($U_{Na}V$, 1724±719 to 7698±1897 mEq/min/g kidney, p<.05), urine flow (UV, 28±9 to 111±13 µl/min/g kidney, p<.05), fractional excretion of Na (1.6±0.5% to 6.1±1.6%, p<.05) and clearance inulin (C_{In}, 660±84 to 871±68 µl/min/g kidney, p<.05). Fractional distal delivery ($C_{Na}+C_{H_2O}/C_{In}$) was increased (6.7±0.8 to 10.6±1.3, p<.05) as solute-free water clearance (C_{H_2O}) increased (25.7±3.1 to 40.1±5.3 µl/min/g kidney, p<.05). In summary, these results demonstrate that the atrial peptide, atriopeptin III, exerts both a hemodynamic and natriuretic effect in the isolated perfused kidney. The tubular effect of atriopeptide appears to occur in the proximal nephron.

DOPAMINE RECEPTORS MODULATE SODIUM EXCRETION IN THE DENERVATED KIDNEY. Pedro A. Jose, Robin A. Felder*, Robert R. Galloway*, and Gilbert M. Eisner. Georgetown University Medical Center, Washington, D.C.

We have previously reported(AJP 245:F247, 1983) that dopamine(D) modulates sodium excretion by the innervated kidney. To examine a role of D on sodium excretion in the denervated (DNX) kidney, the effects of the nonselective D antagonist cis-flupenthixol(CF)(given intravenously 5 ug/kg/min) to saline loaded Wistar Kyoto rats after acute unilateral left DNX were compared with a placebo group(P) and another group that received the D_1 antagonist SCH 23390(1 ug/kg/min). Adequacy of DNX was functionally assessed by a natriuresis in the left DNX kidney and antinatriuresis in the innervated right kidney. The results (left kidney) are tabulated:

	P (n=5)		CF (n=7)		SCH (n=6)	
	GFR[a]	FENa[b]	GFR	FENa	GFR	FENa
BASAL (I)	774 +143	1.55 +.37	542 +50	2.13 +.28	598 +52	2.01 +.27
DNX (II)	529	2.95[c]	496	3.56[c]	598	3.68[c]
DNX+P,CF or SCH (III)	533 +63	3.23[e] +.44	392[d] +56	1.95[df] +.27	497 +37	2.82[dg] +.25

a=ul/min/gm kidney
b=%fractional sodium excretion
c=p<.05 IvsII paired-t test
d=p<.05 IIvsIII paired-t test
e=p<.05 IvsIII paired-t test
f=p<.05 P vs CF, g=p<.05 CF vs SCH ANOVA

Mean arterial pressure was similar among groups.

These studies demonstrate that D receptors play a role in the natriuresis of acute renal denervation. This effect is in part due to renal D_1 receptors since the D_1 antagonist SCH 23390 was effective in attenuating the denervation natriuresis. The role of D receptors in other sites (i.e, adrenal D_2) or renal D_2 receptors remains to be explored.

MECHANISM FOR NA-COUPLED CL TRANSPORT IN DOG RENAL MICROVILLUS MEMBRANE VESICLES (MMV). Lawrence P. Karniski* and Peter S. Aronson. Yale Sch. Med., Depts. Med. & Physiol., New Haven, CT.

We have screened dog renal MMV for mechanisms of ion-coupled Cl transport. There was no evidence for Na-Cl cotransport or Cl-OH(HCO_3) exchange. ^{36}Cl uptake 3-4x in the presence of valinomycin and $K_i=K_o$, indicating Cl-Cl exchange. Cl-Cl exchange was inhibited 60, 30 and 20% by oxalate (10 mM), sulfate (20 mM) and formate (10 mM), respectively, suggesting that these anions might share the exchanger. Indeed, an outward oxalate gradient stimulated Cl uptake 2x, and an outward Cl gradient stimulated oxalate uptake 6x, demonstrating Cl-oxalate exchange. Cl-oxalate exchange was inhibited 90% by 0.1 mM DIDS. Because of the low specific activity of ^{36}Cl, ^{82}Br was used as a tracer for Cl in subsequent studies. ^{82}Br uptake was stimulated by an outward gradient of Cl, confirming Br-Cl exchange. An outward oxalate gradient in the physiologic range (1.0 mM in, 0.08 mM out) stimulated uptake of ^{82}Br 1.7x and induced its transient uphill accumulation. For exchange of luminal Cl with oxalate or other anions to account for Cl absorption, there must be recycling of these anions back into the cell. We confirmed the presence of Na-SO_4 cotransport. In addition, an inward Na gradient caused uphill oxalate accumulation, indicating Na-oxalate cotransport. Finally, an inward Na gradient stimulated ^{82}Br uptake 2x in the presence but not in the absence of oxalate, suggesting that Na-anion cotransport in parallel with anion-Cl exchange is a mechanism for Na-coupled Cl absorption in the mammalian proximal tubule.

INTRARENAL INFUSION OF SARALASIN IN DOGS WITH CHRONIC THORACIC INFERIOR VENA CAVA (TIVC) CONSTRICTION. J. Keiser,* A. Chakravarthy,* T. Opgenorth,* and J.C. Romero. Mayo Clinic, Rochester, MN.

TIVC constriction in dogs promotes profound sodium retention with development of ascites and edema. Systemic infusion of renin-angiotensin system (RAS) blockers in this model of low output failure results in variable natriuretic and hemodynamic responses. The present study was undertaken to assess the effects of intrarenal blockade of the RAS on renal hemodynamics and electrolyte excretion with an intrarenal dose of saralasin (S) (0.07μg/kg/min) devoid of systemic effects. Dogs (n=4) were chronically prepared with indwelling catheters and a balloon cuff around the TIVC. After 4-6 days of constriction the acute experiment was performed in anesthetized animals. Ascites fluid was present in the abdomen of all the dogs and daily Na excretion ranged from 2-20% of intake. Kidneys were exposed via a flank incision and fitted with flow probes, renal artery and vein needles and ureteral catheters. Unilateral clearances were performed before, during and after intrarenal infusion of S. During S infusion Na excretion rose from 1.04±.27 to 1.95±.50 μEq/min. After discontinuing S infusion Na excretion returned to control levels (1.09±.30 μEq/min). Urine volume doubled during the treatment period and fell towards but did not reach control levels during the recovery period. There were no significant changes in GFR or RBF during S infusion. In conclusion, we have demonstrated that blockade of intrarenal angiotensin II promotes Na excretion independent of changes in renal or systemic hemodynamics in this model of low output failure.

EFFECT OF PROSTAGLANDIN (P) INHIBITION ON POTASSIUM (K) HOMEOSTASIS. J.S. Kaufman, D.H. Chase,* and R.J. Hamburger, Renal Section, Boston VA Med. Ctr. Boston, MA.

Indomethacin (I) has been noted to cause clinical hyperkalemia. It has been suggested that this finding may be due to an effect of PG inhibition on aldosterone secretion or impairment of extrarenal K disposition. We examined the effect of acute I administration in rats on the disposition of an i.v. K load. After a control period, I, 2 mg/kg, or the vehicle was given i.v. over 2 min. Thirty min were allowed to elapse, then an infusion of KCl, 0.5M, was begun. Ninety min later two 30 min clearance periods were performed. In separate groups similar studies were performed after acute adrenalectomy (Adx). In a third group bilateral ureteral ligation (BUL) was performed and blood samples obtained every 30 min for 150 min after initiation of the K infusion. Results (mean±SE are shown below.

	GFR (ml/min)	P_K (mM)	FE_K
Sham (n=19)	3.2±0.3	5.95±.25	58.8±5.8
Indo (n=20)	3.3±0.3	6.94±.32[a]	37.9±4.1[a]
Adx-Sham (n=8)	2.4±0.2	7.30±.14	37.5±3.4
Adx-Indo (n=8)	3.0±2.4	8.52±.55[b]	15.8±3.9[b]

Significantly different ($p<0.05$) from a) sham or b) adx. No differences in plasma K(P_K) were seen in the two groups of BUL rats.

These results indicate that acute I administration inhibits renal K excretion in response to a K load. This effect appears to be independent of adrenal hormones, since the defect was still apparent after adx. No effect was noted after BUL, suggesting that the I effect was renal not extrarenal. The results are consistent with a role of PGs in renal K excretion.

HORMONAL REGULATION OF MEMBRANE-BOUND ADENYLATE CYCLASE IN THE RECTAL GLAND OF SQUALUS ACANTHIAS. G Kelly*, DR Gifford*, F Wang* and JN Forrest, Jr. Department of Medicine, Yale Univ., New Haven, CT.

Although the shark salt gland has been used extensively as a model of cAMP dependent NaCl secretion, the regulation of adenylate cyclase (AC) activity in this tissue has not been examined previously. We determined the effects of agents known to modify NaCl secretion in perfused glands on AC activity in an enriched basolateral membrane preparation. Basal AC was 7.4±0.3 pmoles/min/mg protein at 30°C (n=82, 13 exps). Vasoactive intestinal peptide (VIP), adenosine receptor agonists and forskolin (F) each stimulated AC activity in a dose-dependent manner. Activity was unchanged by atrial natriuretic factor (ANF) or carbachol. VIP increased AC from 7.8±0.3 to 47.9±1.2 at 1μM(EC_{50}, 0.4μM). N-ethyl carboxamide (NECA) (EC_{50}10μM) was a more potent agonist than phenylisopropyl adenosine (PIA) and the response to NECA was inhibited in a dose-dependent manner by adenosine receptor antagonists: theophylline, 8-p-theophylline and IBMX. Somatostatin (SRIF) (1-100μM) inhibited both basal AC and the AC response to VIP, NECA and F. We conclude that (1) VIP and adenosine stimulate NaCl secretion through activation of membrane-bound AC; (2) The potency order of adenosine agonists in stimulating AC (NECA >PIA) and the inhibition by methylxanthines parallel the effects of these agents in the perfused gland and are consistent with an Ra adenosine receptor; (3) The effect of SRIF to inhibit basal, hormone stimulated and F-stimulated AC suggests that SRIF regulates NaCl secretion at the level of AC, probably through an inhibitory nucleotide protein; and (4) The effects of ANF and carbachol to stimulate NaCl secretion appear to be independent of AC.

PHOTOINCORPORATION OF ^{3}H BROMOBENZAMIL INTO MAMMALIAN KIDNEY CORTEX MEMBRANES. T.R. Kleyman*, M. Markell*, E.Cragoe* and Q.Al-Awqati. Columbia University NY,NY and Merck,Sharp &Dohme,West Point PA

Amiloride inhibits the Na channel of tight epithelia with high affinity and also blocks the Na:H exchanger but at much lower affinity.

We measured the binding of an amiloride analog ^{3}H benzamil by equilibrium dialysis. We found that rat kidney cortex microsomal membranes contained a single class of high affinity binding sites with a Kd of 3 nM. By contrast, rat kidney brush border membrane vesicles which showed Na:H exchange had low affinity ^{3}H benzamil binding sites with a Kd of 20 uM. Beef kidney and human kidney cortical microsomes also contained the high affinity binding sites.

We incorporated the photoactive analog, ^{3}H bromobenzamil(20nM)+ luM unlabelled benzamil into bovine, rat, and human kidney cortical microsomes using 360 nm light and studied the proteins by SDS-PAGE. Two peaks of specific incorporation were consistently found in all tissues with appearent MWs of 45 and 70 kDa, 50 and 80 kDa, and 60 and 95 kDa, respectively.

These proteins may be integral components of the sodium channel.

TROPHIC EFFECTS OF EFFERENT RENAL NERVE ACTIVITY (ERNA) ON RENORENAL REFLEX RESPONSES TO MECHANORECEPTOR (MR) STIMULATION. U.C.Kopp, L.A. Smith* & G.F. DiBona. Dept. Int. Med., Univ. Ia. Coll. Med. & VA Med. Ctr., Iowa City, IA

T_6 spinal cord section abolishes the increase in ipsi afferent RNA (ARNA), the decrease in contra ERNA and the increase in contra V and $U_{Na}V$ produced by renal MR stimulation by increased ureteral pressure (↑UP) in the rat. These results suggest that ERNA has a trophic influence on the ipsi ARNA response to renal MR stimulation. To test this hypothesis ERNA was decreased by saline volume expansion (VE, 10%, n=5) and hexamethonium (Hex, n=8), 15 mg/kg, i.v.

Before VE, ↑UP 32 mmHg increased ipsi ARNA 31±5 counts/s from 8±1 counts/s ($p < 0.05$), contra V and $U_{Na}V$ 41±6 and 55±9% ($p < 0.05$), respectively. VE did not affect MAP but increased contra V from 4.6±1.1 to 48±12 µl/min/g and $U_{Na}V$ from 0.6±0.2 to 9.7±2.4 µmol/min/g. ↑UP after VE did not affect ipsi ARNA, -1±2 counts/s, contra V, 6±16%, or contra $U_{Na}V$, 5±15%. Similarly Hex blocked the responses to ↑UP; before Hex ipsi ARNA increased 47±6 counts/s ($p < 0.01$) and after Hex -1±1 counts/s, contra V increased 34±5% ($p < 0.05$, n=4) before and 5±9% after Hex, and contra $U_{Na}V$ increased 34±5% ($p < 0.05$, n=4) before and -5±9% after Hex. Hex decreased MAP from 139±4 to 109±5 mm Hg. Restoring MAP to control (146±5 mmHg) by i.v. norepinephrine did not restore the responses to ↑UP.

Conclusion: ERNA has a trophic effect on the ipsi ARNA response and the associated contra renorenal reflex responses to renal MR stimulation.

ELECTROPHYSIOLOGIC HETEROGENEITY OF THE RABBIT COLLECTING DUCT. Bruce M. Koeppen, University of Connecticut Health Center, Farmington, CT.

Collecting duct segments were isolated from the cortex (CCD), and outer (OMCDo) and inner (OMCDi) stripes of the outer medulla, perfused in vitro, and studied with intracellular microelectrodes. In isotonic Ringer the values of transepithelial voltage (Vt), resistance (Rt), basolateral membrane voltage (Vb), and fractional resistance of the apical membrane (fRa) were: (mean ± SE)

	Vt (mV)	Vb (mV)	Rt (kΩcm)	fRa
CCD	-16.2	-85.2	14.7	0.40
n=34	±2.0	±3.4	±1.4	±.03
OMCDo	-2.1	-55.6	22.1	0.86
n=8	±4.2	±3.7	±3.6	±.04
OMCDi	+16.5	-29.2	56.6	0.99
n=23	±1.9	±2.1	±6.4	±.01

Na^+ and K^+ conductances of the apical membrane were assessed with luminal amiloride and $BaCl_2$, respectively. Amiloride increased fRa from 0.35 to 0.49 in CCD, from 0.79 to 0.98 in OMCDo, and had no effect in OMCDi. $BaCl_2$ increased fRa from 0.40 to 0.77 in CCD, but had no effect in OMCDo or OMCDi. Thus, apical membrane conductances exist for Na^+ and K^+ in CCD, and for Na^+ in OMCDo. Bath ion substitutions were used to assess basolateral membrane conductances. The major ion conductances of this membrane were; K^+ and Cl^- in CCD, K^+ in OMCDo, and Cl^- in OMCDi. This electrophysiologic heterogeneity is consistent with the known transport properties of each segment; Na^+ reabsorption in CCD and OMCDo, K^+ secretion in CCD, and H^+ secretion in OMCDi.

K NMR OF KIDNEY AND MUSCLE OF RATS IN VIVO. A.P. Koretsky*, W.R. Adam* and M.W. Weiner, Repatriation Gen. Hosp., Univ. of Melbourne, Australia, VA Med Ctr., Lawrence Berkeley Lab, Univ. of Calif., Berkeley and Univ. of Calif., San Francisco.

The aim of these experiments was to develop ^{39}K NMR as a non-invasive measure of tissue potassium, to facilitate (patho)physiological studies. Experiments were performed in an 8 cm. horizontal bore 5.7 magnet using coils tuned to 11.05 MHz. After shimming on protons, spectra were obtained with a signal to noise ratio of 20-30 with 5 minutes of acquisition time. The spectra were compared with an external standard of K. The coefficient of variation (n=10) was 15%. In vitro, ^{39}K NMR detected only 48 ± 1.7% of muscle and 45 ± 3.5% of kidney potassium. Therefore about 50% of potassium is NMR invisible due to either binding or quadrupolar interactions of the ^{39}K nuclei. ^{39}K spectra were readily obtained in vivo from kidney and thigh muscle. Acute infusion of KCl (1 mmole) increased the ^{39}K NMR signal by 86 ±21%. However, muscle K, measured by biopsy, extraction, and flame photometry increased by only 15 ±%. Dietary potassium depletion decreased the ^{39}K NMR signal by 50%. In contrast, muscle K, measured by extraction, decreased by only 20%. The results demonstrate the feasibility of measuring tissue K by ^{39}K NMR in vivo and variation of the ^{39}K signal by physiological maneuvers. The greater change observed by ^{39}K NMR, compared to changes of absolute tissue K, suggests that NMR is a more sensitive, but may be a less specific measure of tissue K. The ability to obtain rapid repetitive non-invasive measurements of tissue potassium by ^{39}K NMR make it a valuable technique to investigate the composition of intracellular fluids in vivo.

EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF) IN RAT PREGNANCY. C. Kristensen*, Y. Nakagawa*, F. Coe, M. Lindheimer. Departments of Medicine, Obstetrics & Gynecology & Pathology, University of Chicago, Chicago, Illinois.

Renal hemodynamics and intravascular volume increase markedly in rodent pregnancy, yet acute sodium loads are excreted similarly in gravid and virgin controls. This study was designed to identify and compare ANF activity in pregnant and virgin rat hearts and to test renal responsiveness to ANF during gestation. Acid extracts of atria from gravid (n=36) and virgin (n=39) rats were assayed in awake virgin animals (n=6). The natriuretic effects were similar, despite increments of ≈70% in the pregnant rats' vascular volumes, changes which would increase activity in nongravid animals. We then purified a natriuretic peptide identical by HPLC to synthetic 23 amino acid atriopeptin II (APII) and tested the renal responsiveness of awake gravid and virgin rats. With equimolar injections of ANF or APII, gravid rats demonstrated a blunted natriuretic response ($p < .03$). However, when dose was based on estimated extracellular volume, virgin (n=6) and pregnant (n=6) animals demonstrated similar, highly significant dose response curves (r's >0.8). When hydropenic rats were assayed, both gravid and virgin animals required ≈10 fold more ANF to initiate a diuresis, and dose response curves were again similar. Conclusion: The hypervolemia of pregnancy does not increase atrial ANF content, suggesting resetting of the system. The natriuretic response to ANF is similar in gravidas and virgins, a surprising observation, as the renal vasculature of the former is already vasodilated.

DIRECT EFFECTS OF INSULIN ON TRANSPORT PROPERTIES OF PROXIMAL RABBIT RENAL CELLS. Achour Laradi,* Lakhi M. Sakhrani,* and Shaul G. Massry. Division of Nephrology, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California.

Insulin affects a variety of renal transport processes. It is not known whether these actions are due to a direct effect on the renal tubule or to secondary events induced by the hormone. We utilized proximal rabbit renal cells in suspension to investigate probable direct action of insulin and to delineate the mechanism of such an action. Insulin (200 μU/ml) stimulated the initial rate of uptake of phosphate by 60±10% and of alpha methyl glucoside by 40±8% by these cells. The hormone also produced a 64±12% stimulation of ouabain sensitive ^{86}Rb uptake, a measure of Na-K-ATPase activity. In addition, ^{22}Na uptake was increased by 80±12% in the presence of insulin. To delineate if the stimulation of Na-K-ATPase was a direct effect of insulin or secondary to the stimulated sodium uptake, we measured the effect of insulin on ouabain sensitive ^{86}Rb uptake in the presence of 1 mM amiloride. The latter partly reduced the response of the ^{86}Rb uptake to insulin. These studies show 1) insulin directly stimulates phosphate, alpha methyl glucose and sodium uptake by these cells, 2) insulin directly stimulates Na-K-ATPase in proximal renal cells, and 3) this effect on Na-K-ATPase may partly underlie the stimulation of the uptake of phosphate alpha methyl glucoside and sodium.

NMR MEASURED INTRACELLULAR Na^+ IN PROXIMAL TUBULES OF RAT KIDNEY. Adarsh M. Kumar, Raj K. Gupta, and Adrian Spitzer. Albert Einstein Coll. of Med., Depts. of Pediatr., and Physiol. and Biophys., Bronx, N.Y., 10461.

Na^+ sensitive glass electrodes and Na^+ selective resins are currently used for the measurement of intracellular Na^+ activity. These methods disturb the integrity of cells, and some may have a relatively low degree of specificity. Consequently, the reported values for intracellular Na^+ activity in proximal tubule cells vary widely. In Necturus kidney, at room temperature, it was found to be 12.9 ± 0.6 when measured with Na^+ selective glass microelectrodes (Lorenzen et al) and 29.7 ± 1.2 when measured with ion selective resin microelectrodes (Kimura and Spring). Using NMR and a paramagnetic shift reagent, dysprossium tripolyphosphate Dy $(PPPi)_2$, we measured intracellular Na^+ in suspensions of rat proximal tubules prepared by collagenase digestion. The tubules were washed in Ringer's solution and purified on a Ficoll gradient. Final suspension was incubated for 15 min at 25°C in an oxygenated solution containing Dy $(PPPi)_2$. Intracellular NMR-observable Na^+ concentration was 37.4 ± 1.9 mM at 25°C. Raising the temperature to 37°C decreased the intracellular concentration to 16.5 ± 0.7 mM. Addition of 0.1 mM ouabain, a Na^+ pump inhibitor, increased Na^+ concentration to 29.0 ± 3.2 mM, while the channel former nystatin raised it to 81.0 ± 2.4 mM. Thus, intracellular Na^+ can be measured in renal cells by NMR spectroscopy. The results are consistent with those obtained with ion selective electrodes and suggests that NMR measures free cytosolic Na^+. The direction and magnitude of the changes observed under various experimental conditions followed the expected pattern indicating that the biological properties of the cells were preserved.

PRIMARY CULTURE OF RAT RENAL PROXIMAL TUBULAR CELLS RETAIN DIFFERENTIATED TRANSPORT FUNCTIONS. C. Lechene, R. Harris*, and S. Larsson*, Harvard Medical School, Boston, MA.

Proximal cells were isolated from adult Sprague Dawley rats by collagenase perfusion, grown in DME+10% FBS, and studied 72 hours after plating on non-permeable supports. Ionic content of individual cells, rate coefficients of ionic fluxes, Na-K coupling ratio, and volume changes were measured by electron probe analysis with the method of Abraham et al. (Am. J. Physiol, in press).

The cells, although non confluent, exhibited α methyl glucoside accumulation, 90% inhibitable by 100 μM phlorizin. Na/K coupling ratio of Na, K-ATPase, measured during the first 5 minutes of recovery after incubation in K-free DME, was 1.30±0.11SE(N=11), N.S. from 1.5. Cells grown in normal 5mM external K had an intracellular K=136±2 mM (N=6) and a K/Na ratio = 19±5 (N=6). In the presence of 1mM ouabain they exchanged K for Na with a T1/2 = 26±2 min. At 90 minutes, cellular content of Na+K+Cl had increased 14%, suggesting cell swelling. Simultaneous administration of 1mM amiloride strikingly slowed down Na entry and the T1/2 increased to 190±21 min. At 90 minutes, Na+K+Cl content decreased 22%, suggesting cell shrinkage. Amiloride inhibition was equivalent in cells serum deprived for 24 hours, and was dose dependent with a K_i of 30-50 μM.

The results show that proximal tubular cells, in short primary culture, retain differentiated functions even when grown on non-permeable supports, or in the absence of serum. Cells accumulate α methyl glucoside. Amiloride dramatically inhibits Na influx. The K_i is compatible with inhibition of a Na-H exchanger. Na, K-ATPase coupling ratio suggests a value of 3Na for 2K.

MODULATION OF RENAL MEMBRANE PERMEABILITY BY COPPER. M.S. Lipkowitz and R.G. Abramson. Mount Sinai School of Med., Dept. of Med., N.Y., N.Y.

To evaluate the conductive properties of rat renal cortical cell membranes, brush border (BB) and basolateral (BL) membrane vesicles (MV) were obtained simultaneously using free-flow electrophoresis (FFE). Permeability to KCl was assessed from the [KCl] achieved inside the vesicles after 3 hrs of incubation in 100 mM KCl. The potential sensitive fluorescent probe DiS-C3-(5) was used to determine $[KCl]_{in}$. Despite the prolonged incubation, equilibrium was not attained: BBMV contained only 24.4±3.4 and paired BLMV only 12.1±2.4 mM KCl (n=22). Paired vesicles were also prepared with 20 uM $CuCl_2$ (n=8). $[KCl]_{in}$ reached only 5.6±1.0 and 3.0±0.2 mM in BB and BL copper exposed vesicles. In vesicles not exposed to copper, membrane copper content (measured by atomic absorption) correlated closely with $[KCl]_{in}$: the higher the copper the lower the $[KCl]_{in}$; BLMV contained significantly more copper than BBMV. $[KCl]_{in}$ was not affected by either the addition of 2 mM $MgCl_2$ or the intrinsic membrane magnesium content. Relative conductances calculated from the constant field equation indicated that K^+ conductance was approximately 3 times Cl^- conductance under all experimental conditions.

These findings indicate that: 1) vesicles prepared by FFE have extremely low KCl permeability; 2) Cl^- conductance limits transmembrane KCl movement; and 3) membrane KCl permeability is inversely related to membrane copper. It is suggested that trace amounts of this divalent ion may modulate the ionic permeabilities of renal cell membranes in vivo.

ROLE OF ADRENAL FUNCTIONS IN ELECTROLYTE IMBALANCE OF PICHINDE VIRUS-INFECTED GUINEA PIGS. C.T. Liu, R.P. Sanders*, P.B. Jahrling*, and C.J. Peters*. US Army Med.Res. Inst.of Infect. Dis. Ft. Detrick, MD.

Negative balances of electrolytes and water were among signs observed in Pichinde virus-infected strain 13 guinea pigs (GP)(Physiologist 26: A-59,1983). Since viral antigen was found in the adrenal gland without major histological changes, this study searched for possible hormonal mechanisms of hyponatremia, hypokalemia and hyposmolality. Strain 13 GP (n=16) were inoculated with 10^4 PFU Pichinde virus. On day 7 and 14 post-inoculation(PID 7&14) both infected and control GP (n=8) were anesthetized, and blood samples were taken for radioimmunoassay of aldosterone and cortisol. Results of plasma hormone values and wts. of adrenal glands are summarized as follows:

GROUP	ALDOSTERONE (ng/dl)	CORTISOL (µg/dl)	ADRENAL WT (mg/100g BW)
Control	8.6±1.3	46.3±5.1	36.6±1.7
Infected		*P<0.01	
PID 7	41.4±2.1*	46.3±3.5	43.6±2.8*
PID 14	105.5±3.2*	90.3±11.3*	71.0±6.5*

These findings suggest that hyperaldosteronism was responsible for negative renal balance of K^+. The higher values of plasma cortisol postinoculation imply that the infected GP were under stress without adrenal insufficiency. A stressful situation with possible concurrent release of ADH as evidenced by high urine osmolality (1500 mOsm) might have accounted for hyponatremia. We suggest that hypertrophy of the adrenal glands may be associated with Pichinde virus-induced electrolyte imbalance and stress. The release of aldosterone from the adrenal cortex may play a key role in altering renal transport of electrolytes in the infected GP.

EFFECTS OF Ca ON TRANSPORT PROCESS IN RAT RENAL BRUSH BORDER MEMBRANES. Jiann-Trzuo Lin, Ling-Yu Chiu, Erich Heinz and Erich E. Windhager, Cornell University Medical College, New York, N.Y.

The possible role of Ca on transepithelial transport was evaluated in studies of sodium-D-glucose cotransport and Na/H exchange in renal brush border membranes. Vesicles were isolated by Mg-precipitation. Using Quin-2 (free acid), intravesicular and membrane-bound Ca together was determined to be 400 to 500 nM (65 to 80 pmol/mg protein). When the freshly prepared membranes were pre-loaded with 0.2 mM Ca, the initial D-glucose uptake, measured in a 150 mM NaSCN and 0.1 mM glucose inward-gradient, was reduced by about 40%. The degree of inhibition became progressively significant only at $[Na]_o > 50$ mM suggesting that Ca might act on the membrane potential. Valinomycin-($[K_i] > [K_o]$) stimulated D-glucose uptake by brush border vesicles was indeed abolished by pre-loading with 0.2 mM Ca. On the other hand, if D-glucose exchange across the membrane was determined at zero chemical and electrical potentials (tracer exchange), no Ca-inactivation was observed suggesting that Ca may not directly affect the sodium-D-glucose cotransport system. Determining the Na-uptake (1 mM Na in the medium) under a pH gradient ($pH_i < pH_o$), at least 30% of the amiloride-sensitive Na/H-antiport was inhibited by Ca. The results suggest that Ca may control the sodium flux across brush border membrane vesicles partly via its effect on the membrane voltage, and possibly by direct inhibition of Na/H antiport.

EFFECT OF GRAMICIDIN OR REDUCTION IN LUMINAL (Na) ON CYTOSOLIC $Ca^{2+}(a^i_{Ca})$ AND $Na^+(a^i_{Na})$ ION ACTIVITIES IN ISOLATED PERFUSED NECTURUS PROXIMAL TUBULES. M. Lorenzen, C.O. Lee* and E.E. Windhager, Medizinische Hochschule Hannover, FRG and Cornell Univ. Med. College, N.Y., N.Y., USA.

Cytosolic Ca^{2+} is thought to play a regulatory role in the negative feedback mechanism that links a^i_{Na} with the rate of Na entry across the apical cell membrane (AJP 236:F505, 1979). To test whether a^i_{Ca} changes in parallel with a^i_{Na}, as predicted by this hypothesis, a^i_{Ca} and a^i_{Na} were measured with ion-selective microelectrodes during maneuvers thought to decrease (low luminal Na concentration) or increase (gramicidin) the rate of apical Na entry. When (Na) in the luminal perfusate was reduced from 100 to 10 mM by choline substitution a^i_{Na} fell from 12.7 ± 1.3 to 7.8 ± 1.4 mM ($n=5; p<0.003$); a^i_{Ca} decreased from 65.7 ± 4.8 to 50.3 ± 3.7 nanoM($n=5; p<0.001$) while luminal membrane voltage (cell negative) increased from -44.4 ± 0.6 to -56.6 ± 2.9 mV ($n=5; p<0.03$). Addition of 5×10^{-6}M gramicidin A to control perfusate increased a^i_{Na} from 10.1 ± 1.2 to 41.8 ± 5 mM ($n=5; p<0.002$); a^i_{Ca} increased from 86.4 ± 20.5 to 403.2 ± 70.7 nanoM ($n=5; p<0.01$) while the luminal membrane was depolarized from -33.0 ± 6.1 to -6.8 ± 1.7 mV ($n=5; p<0.02$). Results are consistent with the view that increased apical Na entry first raises a^i_{Na} and secondarily augments a^i_{Ca}, presumably by diminishing basolateral Na-Ca exchange. Reduced luminal Na entry has opposite effects. The data are in agreement with the feedback hypothesis quoted and with the operation of a Na-Ca exchange process in the basolateral cell membrane.

PROSTAGLANDIN (Pg) INHIBITS ADH-STIMULATED LOOP CHLORIDE ABSORPTION IN THE RAT IN VIVO. Robert G. Luke, Beverly B. Booker,* and John H. Galla. University of Alabama in Birmingham, Nephrology Research and Training Center, Birmingham, Alabama.

To examine the influence of Pg on chloride absorption in the TAL, microperfusion of superficial nephron loop segments was performed from latest proximal to earliest distal tubule at 20 nl/min (in vivo perfusion rates were measured with ^{3}H inulin) in Sprague-Dawley (SD), Brattleboro (DI) rats, and DI rats treated with ADH for 1-3 days with 1 unit daily, a dose known to increase renal excretion of PgE_2. After a control period indomethacin (IN, 5 mg/kg bolus then 6 mg/kg/hour) was infused in all 3 groups (n = 5-7). Separate time controls (n = 5 or 6) were studied for the 3 groups and no significant changes were seen. Initial (CON) values (± SE) and the changes associated with IN (Δ = IN-CON, ± SE) infusion in paired studies in the 3 groups were:

	Fluid Reab. %		Cl Reab. %		ED Cl (meq/L)	
GROUP	CON	Δ	CON	Δ	CON	Δ
SD	30±4	4±2	56±8	7±2*	80±9	-9±3*
DI	22±7	2±4	50±6	1±4	83±5	-4±2
DI + ADH	44±4	6±4	75±2	10±3*	59±3	-19±3*

*P, at least, < 0.05 ED = Early Distal

Plasma Cl remained normal throughout. Thus IN does not alter in vivo absorption of Cl in the loop in the absence of ADH; this suggests that Pg inhibits the ADH-stimulated component of net chloride absorption in the TAL.

INTERRELATIONSHIPS AMONG QUINIDINE, AMILORIDE AND LITHIUM AS INHIBITORS OF THE RENAL NA-H EXCHANGER. Rex L. Mahnensmith* and Peter S. Aronson. Yale Sch. Med., Depts. Med. & Physiol., New Haven, CT.

The kinetic mechanism of inhibition of Na-H exchange by amiloride and Li is controversial. We re-examined these interactions, using quinidine as a kinetic probe of Na-H exchange activity. In renal microvillus membrane vesicles with pH_i 6.8, pH_o 7.4, quinidine is a mixed-typed inhibitor of ^{22}Na influx, as 200 uM Quin increased K_{Na} (12 to 18 mM) and decreased V_{max} (470 to 260 nmol/min/mg). The plot of 1/V vs [Quin] was curvilinear, with Hill coefficient 1.4, indicating 2 or more inhibitory sites. In contrast, both amiloride and Li are competitive inhibitors of ^{22}Na influx, as K_{Na} was increased from 12 to 35 mM by 20 uM Amil, and from 11 to 23 mM by 2 mM Li, with no changes in V_{max}. Plots of 1/V vs [Amil] and 1/V vs [Li] were linear, indicating single inhibitory sites. Addition of 2 mM Li increased the intercept with no change in slope of the 1/V vs [Amil] plot, indicating that Li and amiloride are mutually exclusive inhibitors. Addition of 200 uM Quin increased both the intercept and the slope of plots of 1/V vs [Li] and 1/V vs [Amil], indicating that binding of quinidine is only partially exclusive of Li and amiloride. We conclude: (1) amiloride and Li interact at a single site where they compete with each other and with Na; (2) quinidine interacts at this site and at an additional site that is not shared by Na, Li or amiloride.

EFFECTS OF SYNTHETIC ATRIAL NATRIURETIC FACTOR (AURICULIN) ON KIDNEY FUNCTION AND THE RENIN-ALDOSTERONE SYSTEM IN THE DOG. T. Maack, D.N. Marion,* M.J.F. Camargo,* H.D. Kleinert,* J.H. Laragh,* E.D. Vaughan, Jr.,* and S.A. Atlas.* Cornell University Medical College, Depts. of Physiol., Surg., and Med., New York, N.Y.

To characterize the functional properties of auriculin, we studied its effects on renal function, blood pressure (MBP), plasma renin activity (PRA), renin secretory rate (RSR) and plasma aldosterone (PA) in intact dogs. Auriculin was administered i.v. as a prime (1 μg/kg BW), and constant infusion (0.1 $\mu g.min^{-1}$/kg BW, for 1 hr.), to 5 anesthetized, and 2 conscious, dogs. Auriculin decreased MBP from 134±5 to 122±4*mmHg (*p<0.05, paired t test), and increased GFR (25.5±2.7 to 32.3±4.1*$ml.min^{-1}$/kidney (k)), diuresis (0.21±0.03 to 1.06±0.14*$ml.min^{-1}$/k), natriuresis (38±0.6 to 187±35* $\mu Eq.min^{-1}$/k) and kaliuresis (14.8±1.6 to 35.7±6.3*$\mu Eq.min^{-1}$/k). These effects were sustained throughout auriculin infusion, and entirely reversible. RBF increased only transiently (1-2 min) and then returned to, or below, control levels. Urine osmolality decreased by 40%, while free water clearance remained unchanged. Auriculin reversibly decreased PRA (11.6±2.3 to 7.1±1.5*$ng.ml^{-1}$/hr.), RSR (895±313 to 262±94*$ng.hr.^{-1}$/min) and PA (8.4±1.6 to 5.0±0.7*ng/100 ml). Results demonstrate that auriculin has a unique combination of functional properties, increasing GFR and natriuresis without a sustained increase in total RBF, and lowering blood pressure, plasma renin and aldosterone. These properties suggest a potential role for endogenous atrial natriuretic factor in the regulation of extracellular volume and, possibly, blood pressure.

SINGLE "DELAYED RECTIFIER" POTASSIUM CHANNELS IN NEUROBLASTOMA CELLS: IDENTIFICATION BY VOLTAGE DEPENDENT FEATURES. Stanley Misler,* and Lee Falke* (intr. by B.D. Nidus). Renal Division, Jewish Hospital of St. Louis, St. Louis, MO.

Neurons, like epithelial cells, possess a variety of plasma membrane K^+ conductances (g_K). In neurons the voltage dependence of each g_K serves as a major identifying signature. One g_K, the delayed rectifier (g_{K-DR}), is fully activated within several msec after onset of a constant membrane depolarization (V_c) and "inactivates" over secs. At a given time during a V_c, g_K is larger, the larger the V_c. In differentiated clonal neuroblastoma cells (N1E115 treated with DMSO), we have tentatively identified the single channel conductance underlying g_{K-DR} by correlating the voltage dependence of one of three classes of single K^+ channel events with g_{K-DR}. In "cell attached" membrane patches gigasealed to pipettes filled with 5 mM K^+ Ringers, steps of V_c from rest give rise to surges of outward current which are initially maximal and then decay over sec. but are composed throughout of constant amplitude rectangular steps. The conductance (γ) of this channel is 22 pS for $10<V_c<120$ mV, while its zero current potential, E_{rev}, is 10-20 mV negative to V_{rest}; V_{rest} ~-40 → -60 mV. With 110 mM K^+ Ringer pipettes, E_{rev} is shifted positively by 40-60 mV and γ increases to 32 pS. For $10<V_c<60$ mV, the average number of channels open at a given time increases with V_c due to increases in both the frequency of channel openings (f) and the open channel duration (τ_o). The time dependent inactivation of g_{K-DR}, seen at a given V_c, is correlated with a decrease in f rather than τ_o.

COMPARISON OF DISTAL CONVOLUTED TUBULE (DCT) WITH CORTICAL COLLECTING TUBULE (CCT) VIA ELECTROPHYSIOLOGIC METHODS--EVIDENCE FOR DISTAL NEPHRON HETEROGENEITY. DA Molony,* HR Jacobson, Univ. TX Hlth. Sci. Ctr., Houston, TX and Dallas, TX.

The DCT is functionally distinct from the CCT. Utilizing in vitro microperfusion and cell puncture with 1 M KCl-filled microelectrodes, we compared the effects of 50 mM bath K on transepithelial (V_T) and basolateral (V_{BL}) voltages (mV) in individual cells of the isolated rabbit DCT and CCT. DCT was identified by the criteria of Imai (KI 15:346, 1979). Control electrophysiologic measurements were performed in tubules perfused and bathed in Ringer's bicarbonate. Measurements were repeated after a log increase in bath K concentration (5 mM to 50 mM). The table lists our results.

	Control V_T	ΔV_T*	Control V_{BL}	ΔV_{BL}*
DCT	-1.3	13.7	-73.3	44.3
CCT	-14.7	32.4	-88.1	29.6
	p<.05	p<.02	NS	p<.05

We conclude: 1) the V_T but not the V_{BL} of the DCT differs from that of the CCT; 2) the relative basolateral membrane K conductance as measured by the change in V_{BL} with 50 mM bath K is greater in the DCT than in the CCT; 3) the apical membrane depolarizes in the DCT but not in the CCT with 50 mM bath K; 4) the known low rates of K secretion in the true DCT as opposed to the high K secretion in CCT may owe in part to the higher basolateral membrane K conductance in DCT.

DISSOCIATION OF HEMODYNAMIC FROM NATRIURETIC EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF). Robert D. Murray*, Oscar A. Carretero, A. Guillermo Scicli*and Tadashi Inagami* Hypertension Research Division, Henry Ford Hospital, Detroit, MI and Vanderbilt U., Nashville, TN

Recent studies suggest that ANF increases glomerular filtration rate (GFR), but the extent to which increased filtered sodium load influences ANF-induced natriuresis (UNaV) is not clear. We used a single-pass isolated rat kidney perfused at constant pressure to study the relationship between GFR and ANF-induced UNaV. Following a control clearance period, synthetic ANF was infused at either 31 (n=6) or 61 (n=6) ng/ml perfusate and two clearances were performed; infusions were then stopped and a recovery clearance was performed. GFR and UNaV as percents of the control group (NaCl-infused;n=6) values are presented below. GFR increased (p<0.005) from the

		Infusion Period 1		Infusion Period 2	Recovery Period
GFR	31 ng/ml	127%	<N.S.>	130%	101%
	61 ng/ml	145%	<N.S.>	145%	105%
UNaV	31 ng/ml	349%	<.001>	395%	189%
	61 ng/ml	476%	<.001>	515%	222%

control period to the first infusion period, then remained constant. UNaV continually increased during both infusion periods. GFR returned to control values in the recovery period, but UNaV remained elevated (p<.05). Continually increasing UNaV with stable GFR's during the infusion period, and elevated UNaV with normal GFR's during the recovery period, indicate that changes in GFR alone do not explain ANF-induced natriuresis.

EFFECTS OF ACUTE POTASSIUM LOADING ON HENLE'S LOOP ELECTROLYTE TRANSPORT: A MICROPERFUSION STUDY IN VIVO. Roland Müller-Suur* and Rex L. Jamison. Dept. of Clinical Physiology, Uppsala, Sweden and Dept. of Medicine, Stanford University, Stanford, CA.

Previous studies from our lab support Stokes' hypothesis that K recycling exerts a regulatory influence on reabsorption of K and NaCl by the medullary thick ascending limb of Henle's loop. To study this further, superficial loops of 8 rats fed a normal diet were studied before (C) and after intravenous KCl infusion (KCl). They were microperfused at the late proximal tubule downstream from an immobile wax block with end-proximal Ringer's solution containing ^{14}C-inulin at 15 nl/min (L) and 41 nl/min (H). Fluid at the beginning distal tubule was collected and analyzed for inulin and electrolytes. After KCl loading, excretion of H_2O, Na and K increased, as did distal tubule concentrations of Na, K, Cl (P<0.01). Fractional reabsorption (FR) was (*P<0.05, **P<0.001):

	FR_{H_2O}		FR_{Na}		FR_{Cl}		FR_K	
	L	H	L	H	L	H	L	H
C	31	17	66	30	68	28	64	31
KCl	35	19	59	21*	60*	18*	31**	-6**

FR of Ca and Mg did not change. In 13 of 20 tubules after KCl, more K was recovered from the distal site than was perfused into the loop at H flow, indicating net K addition. The results suggest a selective effect of acute KCl loading on Henle's loop: net K reabsorption was profoundly inhibited and NaCl reabsorption was also reduced.

ALPHA$_2$-ADRENERGIC AGONIST ENHANCES NA-H ANTIPORT IN ISOLATED PROXIMAL TUBULAR (PT) CELLS. EP Nord, KE Meier*, D Goldfarb*, A Hafezi*, S Vaystub*, PA Insel*, and LG Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA and Department of Medicine UCSD, La Jolla, CA.

Catecholamines have been shown to augment salt and water flux in renal proximal tubules. The specificity of the adrenergic receptor and transport pathway involved have not been defined. In this study, a purified preparation of PT cells, isolated from rabbit kidney (Kidney Int 25:311, 1984) was used to identify the specificity of the adrenergic receptor and test the effect of a catecholamine on a major Na transport pathway, the Na-H antiporter.

Catecholamine receptors were evaluated by radioligand binding of [^{3}H]prazosin, [^{3}H]rauwolscine and [^{3}H]CGP 12177 (selective α_1, α_2 and β-adrenergic ligands respectively) to intact PT cells. Binding with [^{3}H]rauwolscine was avid, but minimal with both [^{3}H]prazosin and [^{3}H]CGP 12177. Thus the effect of an α_2-adrenergic agonist, clonidine, on Na/H exchange was tested in intact cells. Na influx was measured using ^{22}Na, and H efflux monitored using the pH-stat method. Under defined experimental conditions, 10^{-5} M clonidine enhanced the initial rate of amiloride-sensitive Na influx (1 mM Na) 2-fold: J_{Na} (control) 5 ± 0.8 vs 11.5 ± 1 (clonidine) pmoles/10^6 cells/15 secs. Na-dependent H efflux (100 mM Na) was similarly enhanced: J_H (control) 1.36 vs 2.59 (clonidine) nmoles/10^6 cells/15 secs. Conclusions: 1. Alpha$_2$ adrenergic receptors are the predominant subtype of catecholamine receptor present on rabbit PT cells. 2. Alpha$_2$-adrenergic agonists stimulate Na-H antiport in isolated PT cells.

ROLE OF ANGIOTENSIN II (AII) IN ESCAPE FROM ALDOSTERONE INDUCED SODIUM RETENTION. TJ Opgenorth,* JP Granger,* A. Chakravarthy,* FG Knox, JC Romero. Dept. of Physiol., Mayo Clinic, Rochester, MN.

This study was designed to test the hypothesis that escape from the Na retaining effects of mineralocorticoids requires the suppression of intrarenal AII. Five dogs were uninephrectomized and instrumented for chronic intrarenal infusion, pressure measurement, and daily fluid and electrolyte balances. Aldosterone (ALDO) infusion at 10 ng/kg/min i.r.a. decreased UnaV from 90.6±5.8 to 31.3±6.5 mEq/day the 1st day. On the 5th day of ALDO, UnaV was back to control (84.7±9.0 mEq/day). During ALDO, MAP increased (89.1±1.3 to 101.2±1.4 mmHg), Ccreat increased (37.9±3.4 to 44.4±3.0 ml/min), and Cpah did not change. After 8 days ALDO was terminated and the dogs were allowed to come back into Na balance. Then in the same 5 dogs AII was infused intrarenally at 1.5 ng/kg/min. AII produced a transient Na retention, increased MAP to 101.2±2.3 mmHg, decreased Ccreat to 25.2±2.7 ml/min and decreased Cpah from 84.8±10.0 to 43.8±2.9 ml/min. While the AII infusion was maintained, ALDO was again infused decreasing UnaV from 93.0±6.4 to 32.2±4.9 mEq/day on the 1st day. Again, on the 5th day of ALDO, UnaV had returned to pre-ALDO levels (86.9±12.4 mEq/day). MAP increased further to 118.7±2.5 mmHg, Ccreat increased to 38.7±5.0 ml/min, and Cpah did not change. After 8 days of ALDO the AII infusion was terminated producing a marked natriuresis even though the ALDO infusion was maintained. In conclusion, these data suggest that intrarenal infusion of AII does not alter the time course of "escape". However, the level of intrarenal AII may effect the level of arterial pressure necessary to achieve Na balance.

REGULATION OF RAT KIDNEY Na/K-ATPase ACTIVITY BY CHRONIC OUABAIN TREATMENT. Barbara M. Rayson, Dept. of Physiol., Cornell Univ. Med. Coll., New York, N.Y.

The possibility that a chronic increase in intracellular Na^+ levels might raise the number of Na/K-ATPase sites in kidney tubular cell plasma membranes was tested by superfusion of a suspension of rat outer medullary tubules in medium containing ouabain. The tubular suspension was prepared and superfused as previously described (Rayson & Edelman, Am.J.Physiol.,243, F463,1982). Na/K-ATPase activity, measured under Vmax conditions, was raised, after superfusion in medium containing 10^{-4}M ouabain, for 18 hrs., at 37°C. This measurement provides an estimate of the number of Na/K-ATPase sites, in the absence of non-competitive activators/inhibitors. The control level observed was 35.2±5.4(n=6, SE) µM Pi/hr/mg prot. That determined after ouabain treatment was 61.1±13.9(n=6, SE,$p<0.05$(pd t tst)). No significant difference was observed at earlier time intervals. Some specificity in the response was indicated by the absence of any change in MgATPase activity, under the same conditions. The control level observed was 14.4±2.2(n=6,SE) µM Pi/hr/mg prot. That observed after ouabain treatment was 17.6±6.2 (n=6,SE,$p>0.5$(pd t tst)).

These results suggest the existence of a homeostatic mechanism in kidney tubular cells, whereby chronic increases in intracellular Na^+ levels raise the apparent number of Na/K-ATPase transport sites.

CIRCADIAN K EXCRETION AND ADAPTATION TO A HIGH K DIET IN RATS. L. Rabinowitz, C.J. Wydner*, H. Yamauchi. School of Medicine, Univ. of California, Davis, CA.

Although most authorities assert renal adaptation to a high K diet in rats requires many days, we have recently shown that it occurred within 24h (AJP:Renal Oct. 1984). To more precisely identify the rapidity of adaptation we studied undisturbed male rats that were kept in metabolism cages and given a liquid diet (Nutrament) ad lib. Urine was collected in sequential 90 min periods. The diet on days 1-3 had $[K^+]$ 39 mEq/L and days 4-6 173 mEq/L. Light was from 07.00h to 19.00h and food was given each day at 10.00h. On all days there was a marked diurnal rhythm for K, Na, and water excretion with peak excretion shortly after the beginning of the dark phase. Peak K excretion rates were 142 and 586 µEq/h with basal and elevated K diets. These maxima were 7.1 and 4.4 times the respective minima. Providing the high K diet led to a transition within 6h to a cycle of K excretion with elevated maxima and minima but with no change in phase. This transition was during the light interval when food intake is low in rats. No change in Na or water excretion occurred. These results show there must be highly sensitive receptor and rapidly responding effector mechanisms involved in recognizing and adapting to changes in dietary K intake in rats. Excretory K adaptation is essentially immediate and is superimposed on normal daily excretory rhythms.

NULL POINT ESTIMATE OF PROXIMAL TUBULE INTERSPACE NaCl CONCENTRATION. Henry Sackin and Eric Roth*. Department of Physiology, Cornell University Medical College, New York, New York.

The role of the paracellular interspace in solute coupled water transport was investigated in isolated salamander (Ambystoma) proximal tubules, perfused and bathed with identical solutions. Interspace NaCl was determined by a null point method, in which tubules were subjected to an abrupt and sustained decrease in temperature simultaneous with a change in bath NaCl. Rapidly decreasing bath temperature from 23°C to 1°C in 400 msec initially depolarized the transepithelial potential (Vte) from -3.4±.4 mV to -1.1±.2 mV (n=12). Subsequent to this initial depolarization there was either a slow depolarization or a slow hyperpolarization, depending on the concentration of NaCl in the low temperature bath. Since, at the tight junctions of these tubules, the Cl transference number exceeded the Na transference number by .57 ±.03 (n=12), a slow depolarization of the lumen negative Vte indicates diffusion of NaCl from interspace to bath, whereas a slow hyperpolarization indicates diffusion of NaCl from bath to interspace. The absence of a slow change in Vte after cooling indicates a match between the interspace NaCl concentration and the test concentration of NaCl in the bath. The results of this null point method in 12 proximal tubules indicate that at 23°C the interspace NaCl concentration is about 3 to 4 mM above the NaCl concentration in either the lumen or bath, demonstrating that the interspaces of the salamander proximal tubule can function as a local hyperosmotic compartment which facilitates fluid transport between solutions of identical composition.

MINERALOCORTICOID REGULATION OF CELL MEMBRANE Na AND K PATHWAYS IN RABBIT CORTICAL COLLECTING DUCT (CCD). Steven C. Sansom* and Roger G. O'Neil,* (Intr. by R.M. Culpepper). Univ. of Texas Medical School, Houston, Texas

Microelectrode techniques were applied to the perfused CCD (37 C) to quantitate the influence of deoxycorticosterone acetate (DOCA, 2 mg/kg/day, i.m.) on the Na and K pathways using equivalent circuit modelling. After 1 day of treatment (acute), the apical membrane amiloride sensitive Na conductance (G^a_{Na}) and current (Na entry) increased from .7 $mScm^{-2}$ and 78 μAcm^{-2} to 1.6 $mScm^{-2}$ and 185 μAcm^{-2}, respectively, and were maintained with chronic treatment. The apical membrane K current (K secretion) also increased after 1 day (from -39 to -98 μAcm^{-2}) due to an increase in the driving force. However, the apical membrane Ba^{2+}-sensitive K conductance did not increase until 4 days or more (chronic) of treatment (from 4.0 to 10.3 $mScm^{-2}$), thereby maintaining a high K current. The basolateral membrane K conductance also increased after 4 days or more of treatment, which was paralleled by a hyperpolarization of the basolateral membrane from -75 to -99 mV, resulting in a net driving force for passive K uptake across the basolateral membrane. Further, this hyperpolarization was shown to arise from an increase in the active Na pump current (ouabain and amiloride inhibitable) due to a greater elevation in Na efflux over K influx via the pump, resulting in an increase in the Na:K pump coupling ratio from near 1.5:1 to 3:1 after chronic treatment. It is concluded that the acute effect of mineralocorticoids is to increase G^a_{Na} with delayed increases in the membrane K conductances and Na pump current.

PROXIMAL TUBULE (PT) CELLS: SIMULTANEOUS COMPARISON OF TECHNIQUES FOR X-RAY ANALYSIS. A.J.Saubermann*, V.L.Scheid*, D.C. Dobyan, and R.E.Bulger. Depts. of Anes. & Path. & Lab. Med., UTHSC, Houston, Texas.

Differences in elemental and water content have been reported in kidney cells using two different methods for x-ray microanalysis. Our method uses frozen hydrated sections cut at -53°C while Beck et al (K.I.17:756-63,1980) use freeze-dried tissue sections dipped in an albumin-electrolyte solution and cut at -80°C. To determine if the two techniques used in these reports could account for the differences measured, we analyzed the H_2O content and the elemental concentrations in rat PT cells using both methods simultaneously on the same cells. The H_2O content in % wet wt. and the elemental concentration in mmol/kg wet wt. of PT cells are shown in the Table below (Values are means ± SEM):

	Saubermann Method		Beck Method
Cut Temp.	-53°C	-80°C	-53°C
H_2O	71.1 ± 0.6	71.3 ± 0.8	67.4
Na	51.6 ± 1.6	44.3 ± 2.7	45.5 ± 1.5
K	132.2 ± 2.6	136.9 ± 5.0	116 ±11
Cl	51.7 ± 1.6	45.5 ± 2.3	44.3 ± 2.4
P	190.8 ± 3.8	192.7 ± 5.7	-----

No significant differences were observed between the two methods. Elemental concentrations of Na and Cl were equal to each other in this study but were higher than that previously reported by Beck and coworkers. No differences in elemental or H_2O content of PT cells cryosectioned with albumin at -53°C and those cells cryosectioned at -80°C were seen. The differing values reported by the two groups for elemental analysis of rat kidney cannot be ascribed to either cryosectioning at warmer temperatures used by our group or to the analytical algorithms used by either group.

EVIDENCE FOR MATURITY OF THE CHEMICAL GRADIENT DRIVING K^+ SECRETION IN THE CORTICAL COLLECTING TUBULE (CCT) OF THE NEWBORN (NB) RABBIT. Lisa M. Satlin* and George J. Schwartz, Dept. of Peds., Albert Einstein College of Medicine, Bronx, N.Y.

The serum K^+ concentration in the NB is higher than in the adult, possibly reflecting a diminished K^+ secretory potential in distal segments of the nephron. We chose to study whether a reduced intracellular K^+ concentration in the NB CCT limits K^+ secretion by a decreased cell-to-lumen K^+ gradient. Approximately 5-10 CCTs from NB and adult rabbits were isolated, measured for length and outer diameter, extracted with 0.1M acid, and analyzed for K^+ content by helium glow photometry. Acridine orange was used to count cells; tubules were weighed on a quartz fiber. (n= number of animals; mean ± SE)

		CCT K^+ content			
Age	n	(pmol/mm)	(pmol/cell)	(pmol/ng)	(mEq/L)
NB	7	56.4 ± 4.8	0.105 ± 0.011	.627 ± .069	95.6 ± 9.8
Adult	6	90.4 ± 5.3	0.164 ± 0.012	.675 ± .052	100.4 ± 6.3
p		< 0.001	<0.005	0.60	0.70

K^+ content in the CCT was unchanged when corrected for the 50% increase in tubular volume and dry weight during development; however, thigh muscle cellular K^+ rose during the same period (85.7 ± 1.9 to 132.5 ± 4.0 mEq/L tissue water, $p<0.001$). Although CCT K^+/mm and K^+/cell increase with age, without a change in cells/mm, the accretion of K^+ can account for only about 10% of the observed tubular weight gain; addition of protein and other solids contributes significantly to CCT growth.

We conclude that K^+ in the neonatal CCT has already reached mature levels, a finding not paralleled in muscle, a non-secreting tissue. Assuming no change in the K^+ activity coefficient during development, we find no increase in the chemical gradient favoring K^+ secretion and speculate that changes in cellular K^+ do not mediate maturation of K^+ secretion by the CCT.

MECHANISM OF FLUID SECRETION BY PROXIMAL TUBULES IN THE GLOMERULAR KIDNEY OF THE SHARK. Douglas B. Sawyer*, William H. Cliff*, Maureen M. Wilhelm*, Robert O. Frömter* and Klaus W. Beyenbach, Section Physiology, Cornell University, Ithaca, N.Y.

We have recently found electrophysiological evidence for active Cl secretion in shark proximal tubules (Beyenbach and Frömter, Am.J.Physiol., in press). We now present evidence for net fluid secretion that is driven by Cl transport. Isolated shark proximal tubules (PT) were crimped closed at one end, and secreted fluid flowing from the open end was collected (Beyenbach, Nature, 299,54,82). Secreted fluid and peritubular bathing medium were analyzed by X-Ray wavelength dispersive spectroscopy and nanoliter osmometry. When bathed in shark Ringer PT spontaneously secreted fluid at rates averaging 21.2 ± 1.9 nl/min-mm (19 tubules). Secreted fluid contained, in mM

	Na	Cl	K	Mg	Ca	mOsm
Secreted Fluid	291	272	4.8	6.2*	2.6	1106*
Bathing Ringer	280	285	4.0	3.0	2.5	1023

(* sig. different from bath, $p<0.05$). db-cAMP (1mM) in the bath significantly increased fluid secretion by more than 60% in 7 of 11 tubules. Presumably, the other 4 tubules were spontaneously stimulated and refractory to cAMP. However, furosemide (10^{-4}M, bath) significantly inhibited fluid secretion, 49.6 ± 10.7%, n=6) in every tubule. Fluid sectrion completely stopped with the mixture DNP, azide, and cyanide (5,5,1 mM), linking fluid secretion to cellular metabolism. These results indicate that net fluid secretion in PT is driven in part by a cAMP-stimulated and furosemide-sensitive Cl transport system. Our present finding of fluid secretion in the shark (class elasmobranch) and in the flounder (class teleost) indicates that fluid secretion in vertebrate glomerular tubules, that is physiological, is not an isolated observation.

VASOPRESSIN STIMULATES RUBIDIUM SECRETION IN THE ISOLATED RAT CORTICAL COLLECTING TUBULE. James A. Schafer and Susan L. Troutman*. Nephrol. Research and Training Center, University of Alabama in Birmingham, Birmingham, AL.

In order to examine the effects of arginine vasopressin (ADH) on K+ transport in the rat cortical collecting tubule (CCT), we measured unidirectional $^{86}Rb^+$ fluxes. Tubules were dissected from Sprague-Dawley rats maintained on a normal ad libitum diet, and were perfused and bathed with isotonic media at 38°C. Under control conditions, the $^{86}Rb^+$ flux (pmol/min·mm) from bath to lumen (J-bl) was 4.2±0.8 (SEM), and the lumen to bath flux (J-lb) was 2.0±0.3 (measured in separate experiments). By nonpaired comparison there was a net secretory flux of 2.2 ($p<.01$). Addition of 100μU/ml ADH to the bathing solution gave a reversible increase in J-bl to 7.5±1.4 ($p<.01$), a smaller decrease in J-lb to 1.4±0.2 ($p<.05$), and a significant increase in the net secretory flux to 6.1 ($p<.01$). Addition of 2mM Ba++ to the perfusate, with ADH still in the bath, rapidly reduced J-bl to 3.5±0.7 and J-lb to 1.1±0.2, with a net secretory flux of 2.4 that was still significantly different from zero ($p<.01$) but not from control. J-bl measured with 10μM amiloride in the perfusate was 2.8±0.5 in the absence of ADH, and rose significantly to 3.3±0.6 ($p<.01$) after ADH addition. Luminal Ba++ decreased this flux to 1.5±0.4, suggesting that ADH stimulates Rb+ secretion by enhancing apical Rb+ permeability directly. We conclude that ADH increases Rb+ (and thus probably K+) secretion in the rat CCT, and that the cellular component of K+ (Rb+) secretion depends on Ba++-sensitive exit across the apical membrane.

PARADOXICAL K^+ DEPLETION: A RENAL MECHANISM FOR EXTRARENAL K^+ ADAPTATION

A. Spital and R. Sterns. Rochester General Hospital, University of Rochester School of Medicine, Rochester, N.Y.

An acute K^+ load given after nephrectomy (Nx) causes a smaller increase in plasma K^+ in rats previously fed a 10% KCl diet (HK) than in rats fed a regular diet (C). In studies demonstrationg this "extrarenal K^+ adaptation" (EKA), rats were fasted prior to acute K^+ loading (Alexander & Levinsky, J.C.I. 47:740,1968). Previous work has shown that during fasting, high urinary K^+ excretion persists in HK rats leading to paradoxical K^+ depletion (Clin Res 29:476A,1981). To see if K^+ depletion contributes to EKA, rats were fed a C or HK diet for 7 to 14 days, fasted for 5, 16, or 40 hours, then Nx and K^+ loaded (2.5 mEq/kg). During fasting, urinary K^+ losses in HK were twice those in C. In the table, plasma K^+ before (K_o) and 2 hrs. after K^+ loading (K_f) and the increase in plasma K^+ after K^+ loading (ΔK) are shown in mEq/L as means ± SEM (*=p<.01,**=p<.001, †=p>.10, HK vs C).

	5-hour fast		16-hour fast		40-hour fast	
	HK	C	HK	C	HK	C
K_o	4.08 †	3.87	3.16 **	3.87	2.57 **	3.58
	±.08	±.09	±.11	±.10	±.13	±.08
K_f	6.38 †	6.69	5.25 *	6.22	3.64 **	5.63
	±.24	±.29	±.25	±.18	±.13	±.11
ΔK	2.31 †	2.81	2.09 †	2.35	1.07 **	2.05
	±.18	±.28	±.19	±.10	±.09	±.11

In HK, ΔK and muscle K^+ (not shown) were significantly lower than C only after prolonged fasting. We conclude that EKA can only be demonstrated after a critical period of fasting has allowed sufficient K^+ depletion to develop in HK rats. Renal K^+ wasting resulting in paradoxical K^+ depletion is an important mechanism in EKA.

INCIDENCE AND ETIOLOGY OF HYPERKALEMIA IN HOSPITALIZED PATIENTS. R. Shaker, M. Kleinfeld, and S. Borra, Dept. of Med., Kingsbrook J. Med. Ctr., Bklyn, New York.

A prospective study was designed to ascertain the incidence of hyperkalemia (serum K >5.5mEq/L). As reported by our laboratory, it was 3.9% of a total of 11,830 test over 102 days.

Forty two patients with persistent hyperkalemia were selected on a randomized basis. The group's mean age was 66.3 years with a range of 24 to 94 years. A battery of laboratory tests, including serum electrolytes, glucose, lipids, arterial pH, peripheral blood count, creatinine clearance, urine Na, K, and pH, serum aldosterone and renin before and after stimulation were obtained in each patient.

On the basis of the clinical and laboratory data, the cause of hyperkalemia were determined to be multifactorial. The predominant causes were hyporeninemic hypoaldosteronism, 13 patients (30.9%) and chronic renal failure requiring dialysis, 13 patients (30.9%) with iatrogenic causes as the next most frequent etiology, 8 patients (19.0%). Acute renal failure accounted for 5 patients (11.9%) and hyperkalemia due to increased tonicity for 3 patients (7.1%).

When the group was separated into those 60 years or less (15 patients) and those older than 60 (27 patients) hyporeninemic hypoaldosteronism and iatrogenic induced hyperkalemia were predominant in those above 60 years with iatrogenic causes present exclusively in those above 60 years. Hyperkalemia due to chronic renal failure requiring dialysis was significantly higher in the group of patients 60 years or less.

CALCIUM CHANNEL BLOCKERS (CCB) ENHANCE EXTRARENAL POTASSIUM (K) DISPOSAL. Allen Sugarman and Thomas Kahn, VA Med. Ctr., Bronx, N.Y. and Mount Sinai School of Medicine, New York.

The effect of CCB on the extrarenal disposition of an acute potassium load was examined in acutely nephrectomized rats infused with KCl 0.75mEq/kg/hr for 60 minutes alone or in combination with either verapamil (V) 150μg i.v. bolus or nifedipine (N) 50μg/kg i.v. bolus. ΔP_K with either V or N was reduced from control (C). Since both V and N caused a transient drop in arterial pressure, studies were repeated in acutely adrenalectomized animals (ADX) to evaluate whether the changes in P_K were dependent on adrenal hormones or peripheral sympathetic activity. ΔP_K with ADX was higher than C. ADX rats subjected to chemical sympathectomy with 6 hydroxydopamine had a similar ΔP_K to ADX alone. ADX with either V or N produced a lower ΔP_K than ADX alone.

Group	n	P_{K_o}±S.D.	ΔP_K±S.D.
C	8	4.4±0.3	1.2±0.2
V	7	4.4±0.2	0.7±0.3*
N	6	4.1±0.2	0.7±0.2*
ADX	8	4.7±0.5	2.3±0.5*
V+ADX	8	4.7±0.4	1.7±0.5**
N+ADX	7	4.8±0.7	1.8±0.4**

*$p < 0.02$ vs.C; **$p < 0.02$ vs. ADX

Conclusions: 1)During K infusion ΔP_K is less in the presence of CCB. 2)The alteration in K transport produced by CCB does not appear to be dependent on adrenal function or peripheral sympathetic activity. Impaired calcium entry into cells may alter K transport in the intact animal.

HORMONAL REGULATION OF CHLORIDE (Cl) TRANSPORT IN THE CORTICAL COLLECTING TUBULE (CCT). K. Tago, V.L. Schuster, J.B. Stokes, Dept. of Med., Univ. of Iowa, Iowa City, IA.

Previous studies have shown that a major part of Cl flux in CCT occurs by electrically silent Cl-Cl exchange, and that net Cl absorption is enhanced by isoproterenol (ISO). We studied hormonal regulation of Cl transport in the rabbit CCT using lumen-to-bath ^{36}Cl flux, expressed as the efflux rate coefficient (K_{Cl}, mm/sec). The perfusate contained amiloride (50 μM) to render the transepithelial voltage near zero. Bath and perfusate were HCO_3-free solutions (HEPES, pH 7.5).

K_{Cl} spontaneously decreased in time control experiments. 8-Bromo-cAMP (0.1 mM) increased K_{Cl} (ΔK_{Cl}=+29.9±8.7, p<0.05) in the presence of bath Cl but not in its absence. Similarly ISO (10^{-6} M) increased K_{Cl} (+18.7±5.5, p<0.05) in the presence of bath Cl. The ISO stimulation was prevented by pretreatment with either propranolol (10^{-6} M) or PGE_2 (10^{-6} M) in the bath; it was also inhibited by subsequent addition of PGE_2 to the bath. In contrast, vasopressin (ADH, 200 μU/ml) added to the bath did not increase K_{Cl}. These experiments demonstrate that cAMP stimulates cellular Cl efflux without altering the paracellular component. Since under the present experimental conditions net Cl absorption is low, the majority of the stimulation represents Cl-Cl exchange. The ISO effect is modulated by β-adrenergic receptors and is inhibited by PGE_2. These results, together with the failure of ADH to stimulate K_{Cl}, suggest separate pools for cAMP mediated events and/or cell heterogeneity in CCT cAMP regulated functions.

EFFECTS OF VASOPRESSIN AND BRADYKININ ON SODIUM AND POTASSIUM TRANSPORT IN RAT CORTICAL COLLECTING DUCTS IN VITRO. K. Tomita, J.J. Pisano,* and M.A. Knepper. NHLBI, NIH, Bethesda, MD.

To determine whether vasopressin and bradykinin directly affect sodium or potassium transport, cortical collecting ducts from deoxycorticosterone treated rats were perfused in vitro. Net fluxes of sodium (J_{Na}, pmol/min/mm) and potassium (J_K, pmol/min/mm), fluid transport (J_v, nl/min/mm), and transepithelial potential difference (P.D., mV) were measured. Perfusate and bath solutions were identical (Na, 146 mEq/L; K, 4 mEq/L). Arginine vasopressin (AVP, 10^{-10} M in the bath) had the following effects:

	J_{Na}	J_K	J_v	P.D.
Control	12.5	-5.1	-0.01	-10.7
AVP	58.0*	-16.6*	0.26*	-26.6*
Recovery	27.7	-7.3	0.09	-13.6

*P < 0.05, AVP vs. control and recovery (n = 6). Bradykinin (BK, 10^{-9} M in the bath) in the presence of AVP had the following effects:

	J_{Na}	J_K	J_v	P.D.
AVP(initial)	51.0	-12.0	0.24	-24.5
AVP+BK	31.2*	-9.6	0.18*	-22.1
AVP(final)	45.3	-6.2	0.23	-21.6

*P< 0.05, AVP+BK vs. AVP alone (n = 6). Added to the lumen, bradykinin had no effect.

We conclude: 1) Vasopressin directly stimulates net potassium secretion and net sodium absorption. 2) Bradykinin reversibly inhibits net sodium absorption without affecting the potential difference, consistent with an effect of bradykinin on electroneutral sodium transport.

ADAPTATION OF K^+ TRANSPORT BY THE COLON TO DIETARY K^+ RESTRICTION. RL Tannen, R Marino*, and D Dawson*, University of Michigan, Ann Arbor, MI.

We have shown recently that ingestion of a low K diet for 3 days activates an intrinsic renal adaptation to conserve K^+, which is independent of other known K^+ regulatory factors. The present studies were carried out to determine whether the colon, another K^+ transporting epithelium, responds in a similar fashion.

Unidirectional K^+ fluxes across pieces of rat colon mounted in Ussing chambers and bathed in normal Ringer's were estimated under short circuited conditions using ^{86}Rb. K^+ transport was determined in the proximal and distal segments of the terminal 1/2 of the colon from rats fed for 3 days a control (.15 mmol/g), K^+ free, or high K (1.13 mmol/g) powdered diet of otherwise identical electrolyte content.

The low K^+ diet decreased net K^+ secretion by the proximal segment from 0.45 to -0.02 $uEq/cm^2/hr$ ($p < .01$), by decreasing the serosal to mucosal flux, with no change in mucosal to serosal flux. Conductance and short circuit current were similar in the control and low K^+ colons. Net K^+ secretion by the distal segment averaged .17 $uEq/cm^2/hr$ and was decreased, but not significantly to .01 $uEq/cm^2/hr$ by the low K^+ diet. By contrast, in comparison with controls the high K^+ diet had no significant effect on K^+ secretion by the proximal segment (.48 $uEq/cm^2/hr$), but increased net secretion by the distal segment substantially (.77 $uEq/cm^2/hr$) due entirely to an increase in the serosal to mucosal flux.

Thus a low K^+ diet modifies the intrinsic K^+ transport properties of the rat colon, analogous to its adaptive effects on K^+ handling by the kidney. Furthermore different portions of the colon serve as the primary sites for altered K^+ transport in response to a low and high K^+ intake.

THE FLOW-DEPENDENT ENHANCEMENT OF FLUID REABSORPTION (Jv) IN THE PROXIMAL TUBULE IS MEDIATED BY MECHANICAL STIMULATION OF PGE SYNTHESIS. W Trizna, B Badie-Dezfooly*, and LG Fine, Division of Nephrology, UCLA School of Medicine, Los Angeles, CA.

We have previously shown that proximal straight tubule (PT), exposed to high luminal flow rates, have increased Jv even after flow has been returned to normal levels (Abst Am Soc Nephr 16:181A, 1983). Amiloride prevents this effect, suggesting that it is mediated by an increase in Na-H antiport. Since mechanical stimulation (e.g., distention, compression) has been shown to stimulate PGE release in a variety of cell systems, we examined the possibility that tubule distention during high flow could mediate the stimulation of Jv by enhancing PGE production. Exposure of PT to high flow (30 nl/min) in the presence of 225 μM Indomethacin blocked the effect of flow on Jv (control$_\Delta$ + .29 ± .06; Indomethacin$_\Delta$ + .03 ± .09 nl/mm/min). In contrast, 50 μM arachidonic acid (aa) + 1 mM GSH increased Jv by 0.19 ± 0.08 nl/mm/min at low flow (10 nl/min). Since we have previously shown that PT synthesize PGE, studies were performed on PT cells in primary culture to determine whether acute exposure PGE_1 stimulates Na-H antiport. Amiloridesensitive uptake of 100 mM was 10.7 ± 5.8 in control and 31.6 ± 7.9 nmoles/10^6 cells/3 min with PGE_1. A similar increase in Na-dependent H efflux occurred. Conclusion: Stimulation of Jv by high flow is blocked by a PG inhibitor. ↑PG synthesis mimics the effect of high flow. Since PGE stimulates Na-H antiport in PT cells the effect of high flow is mediated by mechanical stimulation of PGE synthesis.

K & Cl PERMEABILITIES IN CELLS OF RABBIT EARLY DISTAL CONVOLUTED TUBULE. Heino Velázquez* and Rainer Greger*. Max-Planck-Institute für Biophysik in Frankfurt, FRG. (introduced by Fred S. Wright).

In vitro microperfusion and electrophysiological methods were used to study cells of the rabbit early distal convoluted tubule (D), identified by dissecting the thick ascending limb (TAL), glomerulus and DCT, and excising D within 600 μm distal to the transition from TAL to DCT. Luminal and peritubular sides were bathed with the same solution. With Soln I, (similar to ultrafiltrate of plasma) transepithelial voltage (PD_{te}, mV) was -2.5 ± 0.8 SE and resistance (R_{te}, ohm-cm^2) 56 ± 10. With Soln II, a simplified solution containing (in mM) 150 Na, 150 Cl, 4 K, 2 PO_4, 1.3 Ca and 5 glucose, PD_{te} increased to -5.6 ± 0.8, R_{te} decreased to 40 ± 5.8 and basolateral membrane voltage (PD_{bl}), cells impaled from bath side, was -73 ± 11.4 mV, n = 21. Adding magnesium to Soln II (1mM, bath) lowered PD_{te} by 4 ± 0.6, n = 9, and raised PD_{bl} by 3.4 ± 0.96, n=10. Ouabain (0.01 mM, bath) reversibly lowered PD_{te} by 4.1 mV and PD_{bl} by 53 mV. Amiloride (1mM, bath) lowered PD_{bl} by 24 ± 3.8 mV, n=8. Barium (3mM, bath) lowered PD_{bl} by 50 ± 3.9 mV, n = 6. PD_{bl} response to changes in bath [K] was Nernstian and was inhibited by Ba. Diphenylaminecarboxylate, a Cl channel blocker, (0.1mM, lumen) raised PD_{bl} by 5.5 ± 2.0 mV, n = 6. Chlorothiazide (0.1mM, lumen) raised PD_{bl} by 11 ± 4.6 mV, n = 6. We conclude that in D cells: 1) a basolateral Na-K ATPase is ouabain sensitive, 2) the basolateral conductance is highly selective for K, 3) a luminal conductance to Cl, decreased by bath Mg generates the lumen negative PD_{te}, and 4) a chlorothiazide sensitive pump or carrier transports Cl across the luminal membrane.

STRUCTURAL AND FUNCTIONAL COMPARISON OF MESONEPHRIC AND METANEPHRIC PROXIMAL TUBULES OF RABBIT. Larry W. Welling, Klaus Tiedemann*, Pat Basto*, and Dan J. Welling. Laboratory Service, VA Medical Center, Kansas City, MO, Anatomisches Institut I, Univ. of Heidelberg, Fed. Rep. Germany, and Depts. of Pathology and Physiology, Univ. of Kansas Medical Center, Kansas City, KS.

Electron microscopy and in vitro microperfusion were performed on mesonephric proximal tubules from 17-18 day fetuses. In 18 distally occluded tubules volume transport measured by ^3H_2O tracer method was 0.91 ± 0.08 nl/min·mm at 37°C, was reduced $59 \pm 4\%$ by cooling to 25°C, and was independent of transmural hydrostatic pressure of 1 to 10 cm H_2O. For comparison, metanephric S_2 segments are reported to transport 0.33 ± 0.02 nl/min·mm under the same conditions. Because mesonephric tubule OD ≈ 107 μm and S_2 OD ≈ 39 μm, however, both segments transport 2.7 to 2.8 nl/min·mm^2 outer tubule surface area. Computer-assisted morphometric analysis of 6 mesonephric and 8 metanephric S_2 segments gave lateral cell membrane surface densities of 0.93 ± 0.05 and 2.77 ± 0.16 μm^2/μm^3, respectively, to yield total lateral areas of $12.2 \pm 1.6 \times 10^6$ and $13.2 \pm 1.0 \times 10^6$ μm^2/mm^2. Thus, there is exact correspondence between lateral membrane area and transport ability in the mesonephric and metanephric S_2 segments. Further analysis also shows the cells of both segments to increase in cell circumference from apex to base in a manner which, in a previous study of S_2, was consistent with absorption driven both by active small ion transport at lateral membranes and an added effect of peritubular protein diffusing retrograde into the channels.

EFFECTS OF ADRENALECTOMY (ADX) AND GLUCOCORTICOID REPLACEMENT ON LOOP OF HENLE Cl^- TRANSPORT AND RENIN RELEASE. William J. Welch*, Cobern Ott, and Theodore Kotchen. Univ. of Kentucky, Departments of Medicine and Physiology, Lexington, Kentucky.

We have suggested that inhibition of renin release by NaCl is related to increased absorptive Cl^- transport in the thick ascending limb of the loop of Henle. Renin release is increased in the ADX rat and is not suppressed by NaCl. The purpose of this micropuncture study is to evaluate the relationship between renin release and loop function in ADX. Loop function and plasma renin concentration (PRC) were measured in three groups of rats (n=7/gp) before and after acute NaCl infusion: ADX 7 days before study, sham operated rats, ADX treated with dexamethasone (DEX). ADX and DEX drank 0.9% NaCl. Unrelated to arterial pressure, plasma volume, or GFR, before NaCl, loop Cl^- reabsorption in ADX (836 pEq/min + 172 SE) was lower ($p<.05$) than sham (1646 + 353) and DEX (1377 + 318). After NaCl infusion, Cl^- delivery to the loop increased in all groups ($p<.01$); loop Cl^- reabsorption increased ($p<.05$) in sham (2688 + 271) and DEX (2885 + 364), but not ADX (1151 + 114). Early distal tubular [Cl^-] was higher in ADX than in the other 2 groups ($p<.01$). Baseline PRC was increased ($p<0.01$) in ADX and did not decrease after acute NaCl (350 U/ml + 108 to 400 + 99). Baseline PRC decreased ($p<.01$) after NaCl in both sham (56 + 6 to 20 + 2) and DEX (107 + 32 to 52 + 24). Thus inhibition of renin release by NaCl is related to increased Cl^- transport in the loop; increased renin release in ADX is associated with decreased loop Cl^- transport. DEX normalized both the loop defect and baseline PRC, and restored renin responsiveness to NaCl.

EFFECT OF DIETARY NA AND K CONTENT ON THE ^{22}NA RATE EFFLUX COEFFICIENT OF RABBIT OUTER MEDULLARY COLLECTING TUBULES. C.S. Wingo. Division of Nephrology, University of Florida and VA Medical Center, Gainesville, FL.

Previous studies from this laboratory have shown that dietary K restriction increases the ^{42}K rate efflux coefficient (K_K) in medullary collecting tubules dissected from the inner stripe of the outer medulla (MCT_{is}). The present studies examined whether or not similar alterations in the Na efflux coefficient (K_{Na}) occur during dietary K restriction and whether inhibitors of active Na or H transport affect K_{Na} during K restriction. Rabbits were maintained on either a NaCl diet containing 254 meq/kg of Na and 139 meq/kg of K or a KCl diet containing 11.6 meq/kg of Na and 399 meq/kg of K for three to seven days. Tubules were perfused in vitro in symmetrical Ringer's bicarbonate solution at 37°C. Volume reabsorption was negligible. K_{Na} was 87.3 ± 16.1 nm/sec for animals maintained on the KCl diet and was less ($.1 > p > .05$), 53.0 ± 9.5 nm/sec, for those rabbits on the NaCl diet. Acetazolamide (2×10^{-4}M) had little effect on K_{Na}, but ouabain (2×10^{-4}M) reduced K_{Na} by approximately 26%. Based on these and previous observations, the ratio of K_K/K_{Na} increased from 0.94 for rabbits on the KCl diet to 4.15 for rabbits on the NaCl diet, rather than changing in parallel. This latter value is substantially different from the limiting mobilities of these ions in aqueous solutions. The effect of dietary K on the apparent K and Na permeabilities suggests that these ions traverse the MCT_{is} by separate selective pathways.

ARE ALDOSTERONE AND CORTICOSTERONE NECESSARY FOR THE NORMAL DAILY CYCLES IN K AND Na EXCRETION IN RATS? H. Yamauchi, L. Rabinowitz, C.J. Wydner*. School of Medicine, Univ. of Calif., Davis, CA.

Male Sprague-Dawley rats (200-250g) were kept individually in metabolism cages in a 12 h-light 12 h-dark environment, given 65 ml/day of a liquid diet ([K] 60 meq/l), and given ad lib access to water and 0.9% NaCl. Treatment groups (4 rats each) were: normal, adrenalectomized (ADX) + no steroid, ADX + aldosterone, ADX + dexamethasone, ADX + aldo and dex, and sham operated. Steroids were given at a constant rate by implanted miniosmotic pumps at rates of 2.3 µg/day for aldo and 3.2 µg/day for dex. Urine was collected in serial 90-min periods over 3.5 days beginning 7 days after adrenalectomy. In all rats in all groups there was a highly reproducible daily cycle in both K and Na excretion, with little or no difference between groups in phase (time of peak or trough). The major difference between groups was that ADX + dex treated rats had significantly greater Na intake and Na excretion than all other groups. Terminal blood samples showed all ADX + no steroid treated rats had plasma corticosterone levels of 1.5-8.7 µg/dl but most other rats had essentially no measureable levels, a finding suggesting substantial activity of accessory adrenal cortical tissue or adrenal regeneration in ADX rats when ACTH or renin were not suppressed. The results show that cyclic changes in secretion of aldosterone or of a glucocorticoid are not necessary for expression of normal circadian rhythms in K or Na excretion in rats.

MORPHOLOGICAL ORGANIZATION OF THE MALPIGHIAN TUBULE: THE ENDOPLASMIC RETICULUM (ER) AS AN EPITHELIAL TRANSCELLULAR ROUTE. M. Bergeron, F. Berthelet, M. Beaudry-Lonergan, H. Linares *and G. Whittembury. Dept. of Physiology, Université de Montréal, Montréal and Dept. of Biophysics, IVIC, Caracas, Vénézuéla.

Isosmotic fluid absorption carried out by many mammalian epithelia appears to be similar to the isosmotic secretion of insect epithelia such as the Malpighian tubules, which are responsible for urine formation and osmoregulation. We have studied by electron microscopy (80 kV) the 3-D characteristics of organelles in the Malpighian tubules of *Rhodnius prolixus* using thick sections (0,3-0,5 µm) and uranyl and lead impregnation.

The ER presents a different organization in the upper (distal) and lower (proximal) segments. In the upper secretory segment, the ER forms a network made of chains of vesicles having irregular shapes (ca. 0,06 µm in diameter) while in the lower absorptive segment, the ER is arranged in parallel stacks or forms whorls in the central region of the cytoplasm. In both segments, the ER network extends throughout the cytoplasm from the basolateral infoldings to the apex between the many mitochondria present in these areas. A unique feature of these cells, revealed by thick sections is the presence, in each microvillus, of either a mitochondrion or an ER chain in continuity with the network. The ER does not seem to have any specific association with mitochondria or other organelles.

As in the mammalian nephron, this ER organization is most likely related to specific segmental functions and adds support to its potential role as a transcellular epithelial route.

RENAL PHYSIOLOGY—WATER VASOPRESSIN AND SOLUTES

NATURAL HISTORY OF UNTREATED SEVERE HYPONATREMIA (SHN) IN THE RAT. J. Carlos Ayus and R. Krothapalli, V.A. Medical Center, Baylor College of Medicine, Houston, Tx.

SHN in humans carries a high morbidity and mortality. The present study examines the natural history of untreated SHN in 40 Sprague Dawley rats. SHN was induced by administration of vasopressin tannate in oil and 5% dextrose in water over 3 days. After induction of SHN the rats were allowed a normal diet. Thirteen rats (32.5%) died during induction of SHN. Of the rest: group I (N=6) died within 24 hours of induction. Group II (N=5) died between 24 hours and 10 days of induction. Group III (N=8) died between 24 hours and one month after induction. Group IV (N=8) survived. A total of 32 rats (80%) died. Serum Na (mEq/l) measured initially (iSNa), after induction of SHN (aSNa) and 24 hours post induction (pSNa) is depicted:

Group	iSNa	aSNa	pSNa
I	144.7±1.7	103.3±3.7*	---
II	142.2±2.6	99.8±2.3*	110.1±2.4†
III	146.7±2.1	114.3±0.8	144.3±2.6
IV	145.2±1.7	109.6±1.6	137.6±2.3†§

*p< 0.01 vs III; § p=0.07 vs III; † p<0.05 vs iSNa

Our results show that: 1) untreated SHN has a very high mortality (80%); 2) rats with lower aSNa, and who remained SHN (groups I and II) died early; 3) overcorrection of SHN to baseline levels in group III (pSNa = iSNa) resulted in late mortality; 4) correction of SHN to SNa levels significantly lower from baseline in group IV (pSNa < iSNa) resulted in survival of all animals. This study suggests that early death (groups I and II) is the result of SHN itself while late mortality (group III) may be secondary to neurologic lesions caused by SHN and overcorrection.

VASOPRESSIN (VP) AND ANGIOTENSIN II (AII) INCREASE CYTOSOLIC FREE Ca^{2+} [Ca^{2+}_f] LEVELS IN GLOMERULAR MESANGIAL CELLS. Joseph V. Bonventre, Joseph Y. Cheung, Karl Skorecki, Jeffrey I. Kreisberg, and Dennis A. Ausiello, Harvard Medical School, Boston, MA and U. Texas H.S.C. San Antonio, TX.

Changes in mesangial cell Ca^{2+} homeostasis have been implicated in this cell's response to VP and AII. [Ca^{2+}_f] under basal conditions or after hormone stimulation, however, has not been measured in this cell. Using quin 2, an intracellular fluorescent indicator, we have monitored [Ca^{2+}_f] before and for 5 min after addition of VP, AII, isoproterenol, and PGE_2 and compared the hormone response to that in glomerular epithelial cells. Furthermore we have compared the [Ca^{2+}_f] response to activation of adenylate cyclase and cAMP production in both cell types. In mesangial cells only VP (.1-100nM) and AII produced changes in [Ca^{2+}_f] (from 108±24 to 851±362nM with 100 nM VP, n=6, p<.05; from 96±9 to 261±96nM with 1 µM AII, n=4, p<.05) within seconds after the introduction of the hormone. No effects of these agents on [Ca^{2+}_f] were found in epithelial cells. cAMP production and membrane adenylate cyclase activity are unaffected by VP (1 µM), AII or PGE_2 (1 µg/ml) in both mesangial and epithelial cells. By contrast isoproterenol (1 µM) increased cAMP production in both cell types (from 28±2 to 414±16 pmol/mg protein, n=3, p<.001 in mesangial cells; from 14±2 to 266±33, n=3, p<.001 in epithelial cells) during 40 min of incubation.

In conclusion VP and AII acutely elevate [Ca^{2+}_f] in mesangial cells. These changes in [Ca^{2+}_f] likely are related to the contraction and PGE_2 production stimulated by these agents. Stimulation of cAMP production by isoproterenol is not associated with acute changes in [Ca^{2+}_f].

REGULATION OF CELLULAR pH IN THE TOAD URINARY BLADDER. Andrew Brem and Maryann Pacholski* Brown University, Providence, Rhode Island.

While serosal bath pH (pH_S) clearly can affect cellular pH (pH_i), the pH_i in unstimulated toad hemibladders is measurably more alkaline than the bathing medium at a pH_S below 7.7 (Nature 206:511, 1965). This epithelia appears to regulate its pH_i relative to changes in the serosal bath (AJP 13:F 712, 1983). To examine the mechanism pH_i control, cellular $[H^+]$ was estimated using the DMO method under a variety of experimental conditions known to alter ADH induced water flow and/or pH_i. Isolated cells or intact hemibladders from Dominican toads were bathed in a 2.4 mM HCO_3^- amphibian Ringer's bubbled with 1% CO_2-99% O_2 containing ^{14}C DMO, ^3H inulin as a marker of extracellular space (ECS), and 10 mM Hepes at pH 7.1. Bilateral radioactive labeling was used for 30 min. Tissue water was determined by drying to constant weight. $[H^+]_i$ in isolated cells was $6.18 \pm 0.22 \times 10^{-8}$M ($pH_i$ 7.21); a value similar to intact hemibladders (n=8). The pH_i was dependent on serosal Na. With a 20 mM Na-choline Ringer's, the $[H^+]_i$ was $6.41 \pm 0.17 \times 10^{-8}$ (pH 7.19) in contrast to the $[H^+]_i$ in control hemibladders of $5.10 \pm 0.24 \times 10^{-8}$M (pH 7.29)(n=5; $p<0.01$). The anion transport inhibitor, DIDS (2.5×10^{-4}M) in the serosal bath did not appear to affect the $[H^+]_i$, $5.97 \pm 0.36 \times 10^{-8}$M (pH 7.22) vs. control $[H^+]_i$ $5.98 \pm 0.34 \times 10^{-8}$M (pH 7.22)(n=6). The diuretic, furosemide (6×10^{-5}M) in the serosal bath decreased $[H^+]_i$; $4.71 \pm 0.22 \times 10^{-8}$M (pH 7.33) when compared to control $[H^+]_i$ $5.39 \times 0.27 \times 10^{-8}$M (pH 7.27)(n=9; $p<0.05$). ECS was unaffected by the various experimental maneuvers. These data suggest that regulation of pH_i is dependent on serosal Na and may be influenced by coupled ion transport.

RELATIVE CONTRIBUTIONS OF LUMINAL MEMBRANE (L) AND CYTOSOL (C) TO VASOPRESSIN STIMULATED WATER FLOW (Jv). C.P. Carvounis, H.P. Thippeswamy,* and G. Carvounis,* Div. Nephrology, SUNY, Stony Brook and Upstate Medical Center, Syracuse, N.Y.

We have reported that between 22° and 37°C the activation energy (E_A) for vasopressin-Jv in toad bladders varies inversely to water flow (range, 4 to 11 Kcal/mol). We explained the variability by considering that as L permeability increases progressively approaching and/or exceeding that of C, the physical properties of transepithelial Jv depend more on constraints imposed by C. Using appropriate equations we found that L-E_A remained stable about 11 Kcal/mol irrespective of overall E_A and Jv, consistent with two barriers in series. In the present studies we show that serosal hypotonicity (SH=1/2N), established following vasopressin stimulation and formaldehyde fixation of L, resulted in increased permeability by almost 2-fold (0.49 ± 0.1 vs 0.28 ± 0.05 $\mu l \cdot min^{-1} \cdot mOsm^{-1}$, n=4, $p<0.05$). If C and L permeabilities are about equal, SH increases C permeability by almost an order of magnitude, thus making L permeability (resistance) the only significant limiting factor to Jv. This was further confirmed by showing in paired hemibladders that in vasopressin stimulated and L fixed bladders, SH resulted in much higher E_A compared to control (11.7 ± 1.2 vs 5.2 ± 1.6, n=4, $p<0.05$), similar to L-E_A. In conclusion, SH eliminates C-resistance to water and permits examination of physical properties of L independent of C. This may be a useful maneuver to identify whether agents influencing Vasopressin-Jv do so via alterations of Jv at the L or C level.

EFFECTS OF BENZYL ALCOHOL AND OCTANOL ON THE ACTIVITY OF ENZYMES AND TRANSPORT SYSTEMS IN RAT KIDNEY BBM. Brigitte Carrière* and Christian Le Grimellec. Univ. of Montreal, GRTM, Montreal, Quebec.

Benzyl alcohol (BA) is a "fluidizing" agent which has been used in artificial and biological membranes to investigate relationships between membrane physical state and function. The present experiments were performed to examine the effects of BA and octanol (OC) on rat kidney brush border membranes (BBM) as regards to: 1) their physical state, estimated by fluorescence depolarization of diphenyl-hexatriene (DPH); 2) the activity of alkaline phosphatase and γ-glutamyltranspeptidase; and 3) the Na-dependent glucose transport system. At 50 mM, BA and OC decrease the anisotropy from 0.2334 ± 0.0009 to 0.205 ± 0.002 ($p<0.001$) and 0.192 ± 0.001 ($p<0.001$) respectively with DPH at 37°C. The anisotropy changes are dose-dependent but not necessarily linear. At concentration up to 45 mM, only OC decreases K_m of γ-glutamyltranspeptidase from 841 to 685 μM at 37°C and from 1907 to 477 μM at 20°C with γ-glutamyl p-nitroanilide as substrate. OC also decreases V_{max} but at 20°C only from a control value of 32.5 to 25.1 μM p-nitroaniline/mg prot/min. On the other hand, neither BA nor OC have any effect on alkaline phosphatase K_m and V_{max}. Both BA and OC, in concentration up to 40 mM, act on Na-dependent glucose transport system by decreasing the initial uptake (6 sec) of glucose at 24 mM from 12.8 to 8.2 ($p<0.005$) and from 22.0 to 12.5 nmoles glucose/mg prot ($p<0.005$) respectively. It is concluded that when the same degree of fluidity is achieved, OC has a much greater effect than BA on rat kidney BBM. These results also indicate that these compounds do not only act on the BBM as fluidity modifiers but also directly as alcohols.

THE ROLE OF EXTERNAL CHLORIDE IN RENAL BRUSH BORDER MEMBRANE VESICLE (BBMV) AMINO ACID UPTAKE. Russell W. Chesney, Shermine Dabbagh and Naomi Gusowski, Univ. of Wisconsin, Med. Ctr., Dept. of Pediatrics, Madison, WI.

Taurine secretion by the flounder kidney is dependent on external Cl^- at the basal lateral membrane. Although taurine uptake by renal BBMV in mammalian species is Na^+-dependent (Rozen et al., Biochem J 180:245, 1979), little is known about the role of Cl^- on the accumulations of this β-amino acid. Of all anions tested; Cl, NO_3, SO_4, SCN, and tartarate, a typical overshoot was noted only in the presence of an NaCl gradient. Preincubation with choline Cl resulted in reduced uptake after imposition of an external NaCl (100mM) gradient. Since choline is poorly permeant, BBMV were preloaded with a series of Li^+ salts prior to incubation in 100 mM NaCl. LiCl preincubation reduced taurine uptake by 40-60%, $p<.001$ whereas preincubation in $LiNO_3$ or LiSCN did not inhibit and in $LiSO_4$ actually stimulated uptake at 15, 60 and 360 sec.; at equilibrium, (45 min) this Cl^- effect disappears. Nigericin in the presence of external KCl stimulated uptake by 30%, $p<.001$, but reduced uptake when KCl was inside, $p<.01$. These findings are consistent with the hypothesis that external Cl^- is required to fully demonstrate the Na^+-dependent uptake of the amino acid taurine and indicates that the anonic species present may govern amino acid transport.

DIFFERENCES IN N^1-METHYLNICOTINAMIDE (NMN) TRANSPORT FROM TETRAETHYLAMMONIUM (TEA) TRANSPORT BY ISOLATED PERFUSED SNAKE PROXIMAL RENAL TUBULES. William H. Dantzler and Olga H. Brokl* Dept. of Physiol., Col. of Med., Univ. of Arizona, Tucson, Arizona.

Our previous work on TEA with these tubules suggested transport into cells against electrochemical gradient at both luminal and peritubular membranes but net transepithelial transport in bath-to-lumen direction. Although NMN was considered to be transported by same mechanism as TEA, it inhibited TEA transport only in high concentrations. Therefore, we compared NMN transport by these isolated perfused tubules to TEA transport. Unidirectional lumen-to-bath ($J^{l\to b}_{NMN}$) and bath-to-lumen ($J^{b\to l}_{NMN}$) fluxes saturated, but mean $J^{l\to b}_{NMN}$ exceeded mean $J^{b\to l}_{NMN}$ at each concentration studied. K_m was slightly lower (64 vs 88μM) and V_{max} was slightly higher (500 vs 490 fmol min^{-1} mm^{-1}) for $J^{l\to b}_{NMN}$ than for $J^{b\to l}_{NMN}$. Net transport was from lumen to bath in each tubule studied and rate was identical to difference between unidirectional fluxes. Cell/bath or cell/lumen NMN concentration ratios during unidirectional or net flux measurements averaged 3-4, far below expected ratio (about 16) for passive distribution at electrochemical equilibrium. At steady-state, this ratio was even lower (about 2). TEA (even 20mM vs 3μM NMN) did not inhibit NMN transport. Mepiperphenidol, an organic cation closer in structure to NMN than TEA, in bath (1mM vs 10μM NMN) inhibited $J^{b\to l}_{NMN}$ by 75% but lead to cell/bath ratio (about 1.4) significantly lower than control steady-state value. Data suggest that NMN, in contrast to TEA, enters cells down electrochemical gradient, probably by carrier-mediated process, at both luminal and peritubular membranes and is transported out of cells against electrochemical gradient at both membranes.

FLOW RATE DEPENDENCE OF NET VOLUME FLUX (Jv) AND HYDRAULIC CONDUCTIVITY (Lp) RESPONSE TO ARGININE VASOPRESSIN (AVP) IN RABBIT CORTICAL COLLECTING TUBULE (CCT). **B.S. Dixon,* and R.J. Anderson. Univ. Colo. Hlth. Sci. Ctr., Denver, CO.**

In vivo studies suggest that high urine flow impairs the hydro-osmotic effect of AVP. However, the effect of tubular fluid flow rate on water transport response to AVP in CCT has not been previously investigated. We therefore studied rabbit CCT perfused in vitro at 25°C with a 100 mOsm lumen-bath gradient. After stable maximum stimulation with 200 μU/ml AVP, each tubule (n=8) served as its own control and was studied at both low (7+3 nl/min) and high (17+4nl/min) flow rates. The order of study was randomized. Increasing flow rate was associated with a 65% increase in Jv (0.81+0.11 to 1.34+0.14 nl/mm/min, $p<0.005$). Although Lp is not felt to be flow rate dependent, increasing flow rate was also associated with a 47% increase in Lp ($-9.9+1.6\times10^{-6}$ to $-14.6+1.8\times10^{-6}$ cm/atm·sec, $p<0.005$). To delineate the mechanism of increasing flow rate on Jv and Lp responses to AVP, collected fluid osmolalities were calculated and found to be lower at high tubular flow rates (196+4 high and 209+5 mOsm/kg H_2O low, $p<0.02$). A significant 27% increase in tubular diameter was also noted at high rates of flow. These results demonstrate that both Jv and Lp responses to AVP are directly related to tubular flow rate and have practical implications in interpretation of studies examining AVP response in perfused CCT. The direct relationship between flow rate and Jv and Lp may be due to greater surface area and maintenance of an osmotic gradient that favors water movement out of CCT in the presence of AVP.

PURINERGIC REGULATION OF HYDRAULIC CONDUCTIVITY (Lp) IN RABBIT CORTICAL COLLECTING TUBULE (CCT). **M.A. Dillingham, C. Burke,* and R.J. Anderson. Univ. of Colo. Hlth. Sci. Ctr., Denver, CO.**

Cell surface adenosine receptors which either stimulate (Rs) or inhibit (Ri) adenylate cyclase have been identified in several cell types. The presence and physiologic significance of adenosine receptors in mammalian CCT remain unknown. We therefore examined the effect of adenosine (10^{-7} through 10^{-4}M in bathing fluid) on Lp of rabbit CCT perfused in vitro at 25°C with 100 mOsm lumen to bath gradient. At 10^{-4}M, adenosine resulted in a significant sustained increase in Lp in each of 6 tubules. Maximum Lp with 10^{-4}M adenosine was 58% of that obtained with maximal concentrations (10^{-6}M) of arginine vasopressin (adenosine 63+16; AVP 108+7 ctm/s·atm 10^{-7}, $p<.02$.) To determine if adenosine affected the water flux response to AVP, AVP was given to tubules bathed in either 10^{-4}M adenosine or adenosine carrier solution. Maximum Lp following AVP was the same in the presence (n=6) and the absence (n=10) of adenosine (108+7 and 105+17 ctm/sec·atm 10^{-7}, respectively, NS). An intracellular purinergic receptor which inhibits adenylate cyclase (so-called "P site") has also been descibed in several cells. Since 2'5' dideoxyadenosine (DDA) is an agonist for the P site, we determined the effect of DDA on basal and AVP-stimulated Lp (n=9). DDA did not affect basal Lp but resulted in a significant 37% reduction in Lp response to 10^{-6}M AVP (AVP 107+7, AVP+DDA 67+15, ctm/sec·atm, 10^{-7}, $p<.02$). These observations demonstrate that adenosine and DDA are capable of modulating basal and AVP-stimulated Lp in the mammalian CCT. Our results are compatible with functional Rs and P site receptors which regulate rabbit CCT adenylate cyclase activation.

COMPARATIVE RATES OF URINARY PAH AND URATE EXCRETION IN ANESTHETIZED RABBITS: RELATION TO ISOLATED PERFUSED TUBULES. V.S. Donoso* and J.J. Grantham, Univ. of Kansas Med. Ctr., Dept. of Medicine, Kansas City, Ks.

Para-aminohippurate (PAH) and urate (UA) transport have been studied extensively in isolated rabbit proximal tubules in vitro. However, the relative rates of secretion of these anions in vivo have not been rigorously determined in rabbits. We used i.m. acepromazine, ketamine, and xylazine to achieve stable anesthesia. Animals were initially volume expanded to 2% BW with an isotonic salt solution. Plasma levels of NaPAH or LiUA were sequentially increased by intravenous infusion. Clearances of PAH, UA and inulin were determined every 10 minutes. Clearance values were corrected for plasma protein binding. Excretion rates of both PAH and UA increased with increasing plasma concentration. At low plasma concentrations (20-100 μM), 4/5 rabbits reabsorbed urate while secretion was observed in all rabbits with P_{UA} concentrations in excess of 100 μM. In contrast, PAH was extensively secreted at all plasma levels. Probenecid maximally supressed FE_{PAH} from 8.0 to 2.5 and reduced FE_{UA} from 1.8 to 0.3, confirming significant urate reabsorption. At plasma concentrations that stimulate anion secretion to a half-maximal rate in vitro, net urinary PAH secretion (10.0 μmoles/min) was 12.8 fold greater than UA secretion (0.78 μmoles/min). The difference in net secretion rates between PAH and UA agrees favorably with the 7.5 fold difference in maximal secretion rates in isolated perfused proximal tubules. We conclude that absolute and relative tubule secretion rates for PAH and UA in vivo are maintained in isolated perfused tubules in vitro.

EFFECTS OF VERAPAMIL ON THE CHANGES IN URINARY SODIUM PRODUCED BY HYPAQUE. Cristobal G. Duarte and Christopher W. Old. Walter Reed Army Inst. Res., Dept. of Nephrology, Washington, D.C.

This study was done to determine whether a calcium channel blocking agent can alter the changes in urinary sodium caused by a radiocontrast agent. The glomerular filtration rate was measured by the clearance of inulin (C In) and the fractional excretion of sodium (FE Na) was calculated in 2 groups of 8 rats each. In the first group (RC) studies were done during a saline diuresis (control), during a 1 hour iv infusion of diatrizoate meglumine (Hypaque 76), 0.01 ml/min/kg, and the following 2 hr recovery. In the second group (RC V), Hypaque administration was followed by a 1 hour iv infusion of Verapamil, 50 µg/min/kg.

	FE Na %				
	Control	Hypaque	p^a	Recovery	p^a
RC	0.54 ±0.28	1.1±0.16	<0.0025	0.37±0.03	<.05
	Hypaque	Verapamil	p^b	Recovery	p^b
RC V	1.1±0.25	1.34±0.22	NS	1.06±0.12	NS
p^c	<0.005	NS		<0.0005	

[a]Significant difference from control (RC experiments) or [b]Hypaque (RC V experiments). [c]Significant difference between RC and RC V experiments.

The increase in FE Na during Hypaque infusion is related to the osmotic diuresis and an increased filtered load (due to a rise in C In). The fall in FE Na during recovery in RC experiments could have been the result of the fall in vascular perfusion known to follow infusion of a radiocontrast. The latter effect was prevented by Verapamil as demonstrated in RC V experiments in which the natriuresis persisted throughout the recovery period.

OXYTOCIN-PROSTAGLANDIN INTERACTIONS IN DEHYDRATED BRATTLEBORO (DI) RATS. Brian R. Edwards, Dept. of Physiology, Dartmouth Medical School, Hanover, NH.

Although lacking vasopressin, DI rats can synthesize and release oxytocin (OT). In previous studies (Am.J.Physiol. 247: F453-F465, 1984) we demonstrated that 3 h of dehydration (3hD) of DI rats were associated with a 6.5-fold rise in plasma OT, marked increases in fractional sodium excretion (FE_{Na}) and urine osmolality (Uosm), but with no change (or a slight rise) in glomerular filtration rate (GFR) despite a weight loss of 7.6%. Administration of a combined antidiuretic-pressor antagonist after 3hD abolished the natriuresis, largely reversed the rise in Uosm, and induced a 20% fall in GFR, thereby indicating the OT-dependency of these changes. The present experiments were designed to determine whether the effects of OT on FE_{Na} and GFR were mediated via increased synthesis of renal prostaglandins (PG). Sequential clearance measurements were made in 10 chronically catheterized conscious DI rats in the normally hydrated control state, during 3hD, and for 90 min after injecting 4-6 mg/kg indomethacin (INDO). Mean control, 3hD, and INDO values were, respectively: for FE_{Na}, 0.18 ± 0.03, 1.53 ± 0.09, 0.86 ± 0.05 %; and for Uosm, 108 ± 8, 352 ± 14, 745 ± 57 mOsm/kg H_2O. GFR (as a % of control) rose to 108 ± 2 during 3hD, fell to 81 ± 7 after INDO, but returned to 108 ± 7 in the last 30 min. INDO had no effects in 4 hydrated DI rats. We conclude that PG synthesis is stimulated during 3hD and that PGs: (1) reduce the antidiuretic potential of OT; (2) partially account for the natriuresis; and (3) influence the maintenance of GFR during the volume contraction, although additional factors are involved.

DIABETES INSIPIDUS (DI) DUE TO CIRCULATING VASOPRESSINASE. J.A. Durr,* J.G. Hoggard,* R.W. Schrier, and J.M. Hunt. Univ. Colorado Med. Sch., Denver, CO, and Univ. Texas Med. Sch., San Antonio, TX.

A 21 yr old woman with preeclampsia developed postpartum polyuria (>25 L/day). Plasma Na 161 mEq/L, plasma osmolality 340 mOsm/kg H_2O, urine osmolality (Uosm) 94 mOsm/kg H_2O and undetectable plasma arginine vasopressin (AVP) levels were most compatible with central DI. However, IV aqueous vasopressin in progressive doses of 5, 10, 15 and 30 U q2h failed to alter urine volume. In contrast, dDAVP (24 µg) reduced urine volume from 800 to 50 ml/h and increased Uosm to 831 mOsm/kg H_2O. Radioimmunoassay for AVP 30 min after 30 U of IV AVP measured 225 pg/ml of plasma. However, serial dilutions comparing standard AVP and the patient's plasma extract showed markedly different characteristics suggesting that this was not intact hormone but rather possible multiple immunoreactive degradation products of AVP. An extract of this plasma demonstrated no antidiuretic activity by rat bioassay. No endogenous AVP binding antibodies could be demonstrated. Vasopressinase activity was measured in the patient's plasma and found to be 10x higher 12 days postpartum as compared to a 3rd trimester plasma. Moreover, administration of the patient's plasma to a rat resulted in a marked diuresis (16 to 83 µl/min). At 6 wk postpartum circulating vasopressinase activity was undetectable and the polyuric state had resolved. We, therefore, propose that a DI state can be caused by excess vasopressinase activity and treated by the peptidase resistant dDAVP.

LONG TERM EFFECTS OF VASOPRESSIN ON RENAL FUNCTION, URINARY PROSTAGLANDIN EXCRETION, AND ARTERIAL PRESSURE. J. P. Granger*, T. J. Opgenorth*, A. Chakravarthy*, F. G. Knox, and J. C. Romero, Mayo Medical School, Rochester, MN.

Renal prostaglandins have been shown to be an important modulator of the renal actions of vasopressin under acute conditions. The purpose of this study was to determine the long term effects of arginine vasopressin (AVP) on renal function, urinary prostaglandin excretion, and mean arterial pressure (MAP). AVP was continuously infused at a rate of 150 µU/kg/min for 14 days in seven chronically instrumented dogs (sodium intake = 160 mEq/day). AVP decreased urine volume (UV, 1244±123 to 751±79 ml/day) and increased urine osmolality (U_{osm} 627±63 to 1278±91 mOsm/kg) during the first 3 days of infusion. Escape from the water retaining actions of AVP occurred on day 4, whereas UV and U_{osm} returned to and remained at control levels for the remainder of the infusion period. Urinary excretion of prostaglandin E_2 decreased from 251± 44 to 140±23 ng/day on day 1 of AVP infusion and then returned toward control by days 7 and 14. AVP increased MAP from 85±3 to 99±4 mmHg by day 5, but MAP gradually waned toward control levels. AVP significantly increased glomerular filtration rate (GFR, 73±6 to 93±9) while having no effect on urinary sodium or potassium excretion. We conclude from these studies that (1) renal prostaglandins do not appear to play a major role in escape from the water retaining actions of AVP and causing the gradual waning of MAP during chronic AVP infusions, and (2) increases in MAP and GFR may be important factors in maintaining escape from AVP.

ADH-DEPENDENT HYPERTONIC CELL VOLUME REGULATION IN MOUSE MEDULLARY THICK ASCENDING LIMBS (mTALH). Steven C. Hebert. Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Previous studies, using a computer assisted optical technique coupled with conventional electrical methods, demonstrated that the cells of the *in vitro* microperfused mouse mTALH volume regulated in hypertonic peritubular media by a HCO_3^- - dependent salt entry mechanism located in basolateral cell membranes. The present paired studies evaluated the role of ADH in the volume regulatory increase (VRI) response to a 50 mOsm elevation in bath osmolality produced with mannitol. All studies were performed using 25 mM HCO_3^- - containing solutions at pH 7.40. The rate of tubule cell volume increase after initial osmotic shrinkage, V_T (nL/min cm), was negligible in the absence of ADH, 0.000 ± 0.012, but 0.105 ± 0.020 in the presence of hormone (n = 4; ΔV_T +0.105 ± 0.032, $p < 0.05$). This effect of ADH on the VRI response could be mimicked by 10^{-3}M dibutyryl cyclic AMP (db c-AMP) in the bath: V_T with or without db c-AMP were 0.098 ± 0.021 and 0.013 ± 0.006, respectively (n = 3; $p < 0.05$). In these studies ADH or db c-AMP increased the transepithelial voltage from 5.0 ± 1.0 to 8.6 ± 1.1 mV and net NaCl absorption from 3190 ± 350 to 7000 ± 990 pM/sec cm^2; however, abolishing net salt absorption with perfusate 10^{-4}M furosemide had no effect on the ADH - dependent V_T (0.167 ± 0.032; n = 9). These results indicate that ADH (via c-AMP) regulates the ability of mouse mTALH cells to restore their volume in hypertonic media and that this regulatory function is independent of the hormone-mediated increase in net NaCl absorption.

POTASSIUM DEFICIENCY (KD) DECREASES VASOPRESSIN (AVP) STIMULATION OF ADENYLATE CYCLASE (AC) IN ISOLATED RABBIT CORTICAL COLLECTING TUBULES (CCT). S.D. Holland*, K.H. Raymond*, T.D. McKinney and M.S. Katz*, Univ. of Tx. Hlth. Sci. Ctr., Dept. of Medicine, San Antonio, TX.

KD induces a urinary concentrating defect in rabbits and a decreased hydroosmotic response to AVP in isolated perfused CCT (Raymond et al., Clin Res 32: 455A). This defect may result from diminished AVP stimulation of AC in CCT. AC activity, expressed as pmol cAMP/mm tubule/30 min (p/mm/30), was measured in isolated CCT from KD and control rabbits by the method of Morel et al. (Meth Pharmacol 4B, 1978). CCT from KD rabbits showed decreased AC response to AVP.

[AVP] in M	10^{-10}	10^{-9}	10^{-8}	10^{-7}
$\frac{(AC_{control} - AC_{KD})}{AC_{control}} \times 100$	43*	26* (*$p<0.05$)	20	33*
$N_{control}$ (N=no. of expts)	6	13	8	8
N_{KD}	4	6	6	7

Maximal AVP stimulation of AC occurred at 10^{-7}M and half-maximal at 6×10^{-9}M in both KD and control CCT. In control and KD CCT maximal AVP stimulation of AC was 0.66± 0.06 (SEM) p/mm/30 and 0.44±0.07 p/mm/30, respectively. Unstimulated AC activities in control (0.05 ±0.01 p/mm/30) and KD CCT (0.04±0.01 p/mm/30) did not differ. Our results suggest that decreased AVP stimulation of AC in KD CCT contributes to the urinary concentrating defect in KD rabbits. In preliminary experiments stimulation of AC by 5'-guanylyl imidodiphosphate and NaF, which act at the nucleotide regulatory protein of AC, is similar in KD and control CCT. Thus, decreased AVP stimulation of AC in KD rabbits may be due to alteration of the AVP receptor or its functional interaction with AC.

GLUCOCORTICOIDS DO NOT DIRECTLY AFFECT WATER DIURESIS David J. Hirsch*, Dalhousie University, Dept. of Med. and Physiol., Halifax, N.S., Canada. Intro by Dr. Allan D. Cohen.

It is known that steroid deficiency is associated with a defect in free water excretion, but the mechanism is not established. Hemodynamic effects of adrenalectomy (adx) including reduced GFR impair water excretion while reduced blood pressure (BP) elevates plasma antidiuretic hormone (ADH) levels. Using an ADH-free animal model in which hemodynamic variables were controlled, a direct effect of glucocorticoids on water diuresis was sought, as proposed by Stolte et al (Pflugers, 299:99, '68). ADH-free Brattleboro rats were studied under anesthesia after a 3% body weight IV water load. Group I rats were intact, while Group II and III were adx. Group III was supplemented with corticosterone to yield constant low physiologic plasma levels of 5 µg/dl, and all adx rats were studied at least 10 days post-adx. All groups received a similar volume load at surgery, and GFR was determined by inulin clearance, while urine osmolality (Uosm) and free water clearance (CH_2O) were assessed.

	GFR/gm renal wt.	Uosm	CH_2O
I	0.63±0.08 ml/min-gm	170 ±12 mosm/kg	0.09±0.01 ml/min
II	0.44±0.06	279 ±74	0.05± 0.03
III	0.65± 0.11	277 ±74	0.04± 0.03

Mean BP was similar in all groups. GFR, Uosm and CH_2O were statistically similar in all groups by ANOVA methods (n=5 each group). These data suggest that physiologic levels of natural glucocorticoids have no effect on free water excretion if ADH levels and hemodynamic variables are controlled.

MOLECULAR BASIS OF DIABETOGENIC EFFECTS OF DIURETICS. D.B. Jacobs*, B.K. Mookerjee and C.Y. Jung*. VA Medical Center and SUNY, Buffalo, New York

In this report, we explore further our observation that Furosemide (F) inhibits 3-O-methyl glucose (3-O-MG) equilibrium flux in adipocytes in presence or absence of insulin (ASN proceedings 1983). We report here the molecular basis of this inhibition. Isotopic equilibrium exchange flux of 3-O-MG was measured in epididymal adipocytes obtained from male Sprague-Dawley rats (150-200 gm) in presence and absence of diuretic drugs. Cytochalasin-B (CB) is known to be a specific inhibitor of the function of the glucose carrier molecule (Cushman, S. J.Biol.Chem. 255:4788, 1980). D-glucose displaceable CB binding was used to assess function of glucose-carrier proteins. Furosemide, Hydrochlorothiazide and Piretanide inhibit 3-O-MG flux in saturable, non-competitive manner with comparable potencies. Bumetanide is much less potent. After 48 hrs incubation with 10^{-4}M of F at 37°C, 50% inactivation of 3-O-MG transport occurred. Furosemide inhibits the specific glucose displaceable portion of CB-binding of plasma membrane and microsomal fractions of adipocytes in saturable, non-competitive manner. The inhibition develops slowly with incubation, with the first order rate constant of 0.12/hour at 4°C. Apparent Ki of this inhibition was 3.5 and 0.7($\times 10^{-3}$M) after 2 and 18 hours incubation respectively. The effect is identical in basal or insulin stimulated cells. In conclusion, direct inactivation of glucose-carrier molecules of plasma membrane and microsomal pools in peripheral tissue cells may operate in the genesis of diuretic induced glucose intolerance.

EFFECTS OF MALEIC ACID (MA) ON VASOPRESSIN STIMULATED WATER FLOW. S.K. Kad and C.P. Carvounis, SUNY, Stony Brook and Upstate Medical Center, Syracuse, N.Y.

Addition of MA to the serosal bath of the toad urinary bladder produces a dual effect: at low concentrations (from 0.5 to 1 mM) it decreases vasopressin stimulated water flow while at high concentrations (2.5 to 10 mM) it increases it. Similar effects were also seen following cAMP stimulation, suggesting actions at steps subsequent to cAMP generation. Both effects were readily reversible, and there was no evidence of loss of epithelial integrity, since C^{14} sucrose permeability and electrical resistance did not differ from control. The mechanisms for the phenomena are very different. Low concentrations appear to work through inhibition of oxidative metabolism. Addition of pyruvate increases vasopressin stimulated water flow in (1 mM) MA-treated bladder (26.1 ± 5.1 vs 18.9 ± 4.4, n=8, $p < 0.01$), but not in control (36.9 ± 3.8 vs 36.0 ± 3.2 µl/min, n=4, N.S.). Also, 1 mM MA was ineffective in the presence of pyruvate (30.1 ± 4.6 vs 33.1 ± 3.1, n=8, N.S.). The enhancing effect of high-dose MA (10 mM) on vasopressin stimulated water flow cannot be explained by an action on oxidation, since the latter is not rate-limiting for vasopressin stimulated water flow. It appears that the action is Ca^{++} dependent in that it disappears with low serosal Ca^{++}. (Serosal Ca^{++} 1 mM: 21.3 ± 5.0 vs 13.8 ± 4.5, n=5, $p < 0.02$; serosal Ca^{++} 0.2 mM: 22.4 ± 7.0 vs 21.2 ± 6.3, n=4, N.S.). We hypothesize that chelation of cell Ca^{++} has some role in this effect of MA.

CELLULAR ACTION OF ARGININE VASOPRESSIN (AVP) IN THE ISOLATED RENAL TUBULES OF HYPOTHYROID (HT) RATS. J.K. Kim, S.N. Summer,* A.E. Erickson,* and R.W. Schrier. Dept. Med., Univ. Colorado Med. Sch., Denver, CO.

HT has been demonstrated to be associated with an impaired renal concentrating capacity and specific morphological changes in the thick ascending limbs. The present study was undertaken to evaluate the adenylate cyclase (AC) response to AVP in the isolated renal tubules from control (Cont) and HT rats. HT was induced by feeding aminotriazole to the rats for 4 wk. Urinary volume was higher in HT rats (Cont 13.5±0.9, HT 17.7±0.9 ml/24 hr, $p<.005$) and Uosm was lower in HT rats (Cont 1707±48, HT 1229±35 mOsm/kg H_2O, $p<.001$). Plasma AVP level was significantly higher in HT rats (Cont 1.9±0.6, HT 4.1±0.6 pg/ml, $p<.05$), thus documenting AVP resistance. The AC response to AVP (10^{-6}M) was significantly lower ($p<.05$) in the medullary thick ascending limb (MAL) in HT (14.3±2.4 to 41.7±5.8 fm/30 min/mm, $p<.001$) than in MAL in Cont (14.4±2.8 to 110.1± 24.9 fm/30 min/mm, $p<.001$). In contrast, the AC response to AVP was not significantly different in cortical collecting tubules of Cont (47.4±6.9 to 205.9±61.7 fm/30 min/mm, $p<.001$) and HT (66.6±6.9 to 168.8±58.5 fm/30 min/mm, $p<.001$); in medullary collecting tubules of Cont (54.1±4.1 to 187.94± 34.43 fm/30 min/mm, $p<.001$) and HT (53.9±8.6 to 129.3±15.4 fm/30 min/mm, $p<.005$); and in papillary collecting tubules of Cont (57.8±7.6 to 481.5±44.8 fm/30 min/mm, $p<.001$) and HT (53.3±8.0 to 493.5± 125.7 fm/30 min/mm, $p<.005$). These results therefore suggest that the impaired cellular action of AVP in the MAL is involved in the impaired concentrating capacity in HT rats.

ORGANIC ANION TRANSPORT IN RAT RENAL BASOLATERAL MEMBRANE VESICLES: URATE-ANION EXCHANGE; Na^+-PAH COTRANSPORT. Andrew M. Kahn* and Edward J. Weinman. Univ. of Texas Med. School, Houston, Texas.

These studies were performed to determine the mechanisms for urate transport in basolateral membrane vesicles from rat kidney, and to examine the relationship between the transport of urate and that of PAH in this membrane. The 10 sec. uptake of [^{14}C]urate was inhibited 39 and 49% by 2.4 mM probenecid and DIDS, respectively, and was trans-stimulated and cis-inhibited by unlabeled urate. The uptake of urate at early time points was stimulated by outwardly directed gradients for Cl^- or OH^-, and these effects were not the consequence of a more electropositive intravesicular space.

As opposed to rat renal brush border vesicles, urate uptake in basolateral vesicles was not cis-inhibited by PAH nor trans-stimulated by PAH or L-lactate. Whereas urate uptake in basolateral vesicles was not affected by an inwardly directed Na^+ gradient, PAH uptake was stimulated 86%. Na^+ gradient-stimulated [^{3}H]PAH uptake was cis-inhibited by unlabeled PAH, but not by urate.

These studies indicate: 1) urate transport in rat renal basolateral membrane vesicles is mediated via an anion exchanger with affinity for urate, Cl^- and OH^-; 2) unlike the brush border urate anion exchanger, the basolateral system does not have affinity for PAH or L-lactate; 3) a Na^+ cotransport process for PAH but not for urate is present in rat renal basolateral membrane vesicles.

CELL VOLUME MAINTENANCE IN THE ISOLATED PERFUSED PROXIMAL STRAIGHT TUBULE. Kevin L. Kirk*, James A. Schafer, and Donald R. DiBona*. Nephrology Center, Univ. of Ala. in B'ham, Birmingham, AL.

Volume-regulatory responses of the isolated, perfused rabbit proximal straight tubule were examined by differential interference-contrast microscopy and computer-assisted morphometry. Following an increase in the osmolality (290 to 390mOsm.) of the bath and perfusate, cell volume (V_c) decreased within 1 min to a steady state value predicted for a perfect osmometer (75±2% [SEM] of control). In contrast, following dilution (290 to 190 mOsm.) of the bath and perfusate, V_c increased to a maximum within 30 sec and then declined to a steady state value equal to the initial V_c (103±2% of control). In most tubules (11 of 16) the change in V_c followed a biphasic time course: 1) After the initial swelling, V_c returned to the control value within 3.0±0.3 min but then swelled again, often to the same extent as in the first swelling. 2) This reswelling was followed by reshrinking to the initial V_c, which lasted an additional 10-12 min. Qualitatively-similar results were observed following cell swelling using iso-osmotic, urea-containing (100mM) perfusate and bath, indicating that the signal for the volume regulatory decrease was not a reduction in intracellular osmolality per se. Since nonperfused lumen-collapsed tubules exhibit incomplete, monotonic recovery of volume following exposure to dilute media (Dellasega and Grantham, Amer. J. Physiol. 224:1288, 1973), our results imply that transport processes at the luminal membrane may exert an important modifying influence on cell volume maintenance.

EFFECT OF PHLORIZIN ON THE FLUORESCENCE OF RENAL BRUSH BORDER MEMBRANES. Barry B. Kirschbaum, Medical College of Virginia, Dept. of Medicine, Richmond, Virginia.

Phlorizin is capable of binding to cell membranes and competitively inhibiting Na+-dependent transport of D-glucose. In a recent study, phlorizin was demonstrated to quench specifically the fluorescence output of the complex formed by intestinal brush border membrane and 8-anilino-1-naphthalene sulfonic acid (ANS) (J. Biochem. 93:1167-1173, 1983). This effect was appropriately dependent on the presence of Na^+ and reversible upon addition of D-glucose. Utilizing rat kidney brush border membranes isolated by a $MgCl_2$ precipitation technique, we evaluated the effect of phlorizin on the fluorescence properties of the renal membrane-ANS complex. With excitation and emission wavelengths of 340 and 490 nm, quenching by phlorizin was observed. The quenching occurred with isotonic saline or mannitol buffers and was therefore not dependent on the presence of Na^+. Quenching was not reversed by addition of D-glucose. The absorption spectrum of phlorizin showed a peak extinction coefficient of 19.9×10^3 at 325 nm and coefficients of 12.9×10^3 and 0 at 340 and 380 nm respectively. When the fluorescence measurements were repeated using a wavelength of 380 nm to excite the membrane-ANS complex, phlorizin no longer quenched emission intensity. We conclude that the reported quenching by phlorizin is not a reflection of a membrane configurational change attributable to the binding of phlorizin to its membrane receptor. Rather, phlorizin competes with ANS for light absorption at 340 nm.

RENAL CLEARANCE OF DEXTRAN: A NEW, IMPROVED METHODOLOGY. John K. Leypoldt,* Ronald P. Frigon,* Karl W. DeVore* and Lee W. Henderson. VA Med. Ctr., Dept. of Med., San Diego, Calif.

Dextrans (D) are useful molecular probes of the size-selective properties of the glomerular capillary wall. We have developed new methods using high performance gel permeation chromatography for separating neutral D. After quantitative isolation of D from plasma and urine, molecular weight (MW) distributions (5000-60,000) were determined on a TSK-3000SW column. The chromatograph was interfaced with a microcomputer that automated data acquisition and facilitated computations. The speed (40 min./chromatogram) and ease of data manipulation allow a detailed examination of the accuracy of D clearance (Cl) studies.

A putative requirement for precise Cl experiments is a steady state plasma concentration (PC) of the test solute. We were unable to achieve this condition in preliminary Cl experiments with polydisperse D. We thus examined the effect of rapidly changing PC of D by 3 different protocols designed to provide various degrees of change in D PC. These protocols were examined on 7 unanesthetized dogs: (B) bolus D injection only; (S) bolus D injection plus a sustaining infusion; and (UR) urine reinfusion. Although creatinine ($p < .01$) and PAH ($p < .05$) Cl were significantly reduced during UR versus S, no significant changes were noted in fractional D Cl at any MW with the 3 protocols. The fractional D Cl was also similar to those reported previously by others. We conclude that (1) these chromatographic methods provide an efficient alternative to conventional techniques and (2) large (up to 5 fold) changes in PC of D do not significantly affect fractional D Cl measurements.

PHYSIOLOGICAL AND PHYSICO-CHEMICAL MODIFICATIONS OF THE AGING RAT KIDNEY. D. Lajeunesse*, M.C. Giocondi & C. Le Grimellec. Dept. of Biochemistry, Univ. of Montreal, Montreal, Quebec, Canada.

Kidney aging is associated with decreasing normal physiological properties in human and in laboratory animals, but the nature of these modifications are not fully understood. Hegner recently proposed that aging resulted from progressive alterations of membrane physico-chemical properties (Mech. Aging and Dev. 14 (1980), 101). We studied these points with Fisher 344 male rats aged 4, 10, 15 and 29 months old. Their body weight increased up to 15 months of age and then showed a slight decrease while the kidney cortex weight increased steadily with age. The (^{3}H)-methoxy-inuline clearance declined from 2.18 ± 0.19 ml/min for the 4 month old rats to 1.26 ± 0.09 ml/min for the 29 month old group. The Tm_g was reduced from 2.24 mg glucose.ml^{-1}.g $cortex^{-1}$ for the 4 month old group to 1.1 for the 29 month old group. This reduction in Tm_g is accompanied by a gradual decrease in the uptake of (^{3}H)-D-glucose by isolated brush border membrane vesicles (BBM) prepared with the same four age groups. The BBM preparations also showed reduced activities of alkaline phosphatase (30%) and γ-glutamyltranspeptidase (16%) with age. Chemical analysis of the BBM revealed that their phospholipid content did not change significantly with age while cholesterol increased from 453 nmole/mg protein for the 10 month old group to 564 for the 29 month old group. These results thus show that reduced physiological parameters in Fisher 344 rats are accompanied by modifications of the BBM D-glucose transport and by reduced enzymes specific activities. The aging kidney also shows compositional alterations of BBM lipids.

EFFECTS OF PROSTAGLANDINS(PGs) ON THE VASOPRESSIN(Vp)-INDUCED OSMOTIC WATER FLOW IN THE TOAD BLADDER: ROLE OF INHIBITORY EFFECT OF PGE_2. Fumiaki Marumo, Dept. Med., Kitasato Univ.Sch.Med., Sagamihara, Japan

PGE_2 inhibits Vp-action on the water flow, while Vp stimulates PG production. In the present study, the role of the inhibitory effects of PGE_2 and stimulatory effects of TXB_2 along with influence of calmodulin on this action are discussed. The urinary bladder of the toad, Bufo bufo japonicus was used.

Both 10^{-7}M PGH_2 and 10^{-8}M PGE_2 inhibited 10mU/ml Vp-induced water flow. However, it was inhibited by $PGF_{2\alpha}$ at 10^{-6}M, but neither PGI_2 nor PGD_2 had any effect at 10^{-5}M. 3 PGs had no physiological effect. PGE_2 significantly inhibited both 10mU/ml Vp- and 5mM cAMP-induced water flow. It inhibited the Vp-action in dose-dependent manner, but not the cAMP action. Indomethacin stimulated both Vp- and cAMP-induced water flow. The half maximum activation dose was approx. 5×10^{-10}M for Vp, and 5×10^{-8}M for cAMP. These results indicate that PGE_2 physiologically inhibits pre-cAMP sites such as adenylate cyclase, but pharmacologically inhibits post-cAMP sites. 10^{-6}M TXB_2, after preincubation with indomethacin inhibited Vp-induced water flow, but not that induced by cAMP. Our data show that PGs physiologically modulate Vp-action at pre-cAMP sites. W-7, a specific inhibitor of calmodulin, was found to inhibit both Vp- and cAMP-induced water flow in a dose-dependent manner. The ID_{50} for Vp and cAMP were 6×10^{-5}M and 2×10^{-4}M, respectively. 5×10^{-5}M W-7, which has no effect on cAMP action, inhibited Vp-induced water flow additively with 10^{-5}M verapamil. Thus, carmodulin may possibly activate the adenylate cyclase.

PHORBOL MYRISTATE ACETATE INDUCES EXOCYTOSIS, ENDOCYTOSIS AND HYDROOSMOSIS IN THE TOAD URINARY BLADDER. Sandra K. Masur, Daneen Rivero*, Victor Sapirstein*, Mt. Sinai Sch. of Med., Dept. of Physiol. & Biophys., New York, New York

The induction of the hydroosmotic response in the toad urinary bladder associated with membrane addition is mediated by exocytosis at the affected luminal membrane and is reversed by endocytic retrieval at that surface. The permeability, exocytosis and endocytosis are initiated by ADH-receptor interaction on the basolateral membrane. In other hormone responsive systems, phorbol ester (PMA), a tumor promoter, has been implicated in the regulation of various transport processes through the activation of protein kinase C leading to the phosphorylation of cytoskeletal proteins and localized release of intracellular Ca^{2+}. We now report that addition of 10^{-6}M PMA to the mucosa induces an hydro osmotic response which is gradual and which reaches a maximum within 40-90 min equal to 1/3 the maximal ADH response. Morphologically PMA causes rapid exocytosis of the granules, endocytosis of a mucosal marker into tubules and multivesicular bodies and elongation of apical microvilli. Controls treated with 0.1% DMSO or serosal PMA lack the hydroosmotic, exocytic, endocytic and cytoskeletal changes. ADH plus mucosal PMA enhances the initial hydroosmotic response and exocytosis stimulated by ADH but lowers the maximal ADH hydroosmotic response and prevents the ADH-induced increase in transepithelial PD. Addition of mucosal PMA during ADH washout prevents the return to the basal permeability. Thus, apical events mediating the ADH hydroosmotic responsecan be initiated without ADH by an agent which promotes alteration of the apical cytoskeleton and initiates exo- and endocytosis.

ORGANIC BASE TRANSPORT BY PROXIMAL TUBULE BRUSH BORDER MEMBRANE VESICLES (BBMV)). T.D. McKinney and M. Kunneman* Audie Murphy V.A. Hospital and Univ. TX Health Sci Ctr, San Antonio TX.

We have previously characterized the active secretion of the organic base procainamide (P) in isolated perfused rabbit proximal tubules. To evaluate transport events in the apical cell membrane ^{3}H-P transport was studied in BBMV prepared from rabbit renal cortex by a calcium precipitation method. Leucineaminopeptidase activity was enriched 10 fold in BBMV compared to the inital homogenate, whereas enrichment for markers of lysozomes, mitochondria, endoplasmic reticulum, cytosol, and basolateral membranes was less than 1. Equilibrium uptake of P was inversely related to media osmolality (r=.99). A proton gradient from vesicle interior (pH=6) to exterior (pH=7.5) was associated with higher initial rates and a transient overshoot of P uptake above equilibrium both of which were reduced by the proton ionophore FCCP. In the absence of a proton gradient FCCP was without effect. A proton gradient from vesicle exterior (pH=7.5) to interior (pH=8.2) reduced P uptake. Initial rates of pH-stimulated P uptake over the range of .05-1.0 mM P showed saturation with an apparent Km of 5.11×10^{-4} M and Vmax of 1.42×10^{-9} mol/mg protein. pH-stimulated P uptake was inhibited by the organic bases quinidine and amiloride. Finally, pre-loading vesicles with 10 mM procainamide enhanced uptake of ^{3}H-P. These studies demonstrate mediated transport of P across rabbit renal cortical BBMV. Transport is enhanced by an oppositely directed proton gradient and inhibited by a similarly directed gradient of protons or other organic bases. The data are consistent with an organic base proton exchanger.

MORPHOGENETIC CLONAL GROWTH OF CELL LINE MDCK: FORMATION OF POLARIZED EPITHELIAL CYSTS WITH ULTRASTRUCTURAL FEATURES OF DISTAL NEPHRON. James A. McAteer*, Ellen E. Vance*, Kenneth D. Gardner and Andrew P. Evan. Indiana Univ. Sch. of Med., Dept. of Anatomy, Indianapolis, Indiana, and Univ. of New Mexico, Dept. of Medicine, Albuquerque, New Mexico.

Cell line MDCK (Madin-Darby Canine Kidney) is a recognized model for studies of transepithelial transport and has recently found application for the study of cell interactions with the extracellular matrix. MDCK monolayers atop collagen substrate will reorganize to form epithelium-lined lumena when overlaid with hydrated collagen gel (HCG) (Hall, PNAS 79:4672, 1982). We have observed similar organized cell behavior, but, under clonal growth conditions. When dissociated MDCK cells were seeded into HCG ($2-5 \times 10^5$ cells/ml), cells exhibited "morphogenetic" clonal growth. Single cells, by successive division, formed solitary cysts lined by a simple, polarized epithelium. MDCK-cysts increased in size with time in culture (>500 um dia. by 10-14 days). This type of "morphogenetic" clonal growth did not occur in conventional monolayer culture, regardless of substrate (plastic, glass, collagen). The ultrastructure of MDCK cysts was similar to cells of distal nephron. Most cells were cuboidal, with numerous stubby apical microvilli, and a primary cilium. Lateral and basal surfaces were simple, with few villi or infoldings. Occluding junctions were typical of distal nephron. Desmosomes occurred intermittently along the intercellular interface. In conclusion, the morphogenetic clonal growth of MDCK cells reported in this study offers some unique advantages for study of structure and function of cells of the distal nephron. (1). Each cyst is derived from a single cell. (2). MDCK-cyst epithelium possesses a true basal and luminal surface.

VOLTAMMETRIC MICROELECTRODE TECHNIQUE TO STUDY PROXIMAL TUBULE REABSORPTION. **Leon C. Moore, Chris Clausen*, Aija Birzgalis*, Edward Bowden*. SUNY Stony Brook, NY, and North Carolina State University, Raleigh, NC.**

We have developed a voltammetric microelectrode which permits rapid, repeated measurement of fractional proximal tubule reabsorption in vivo using ferrocyanide ion (FC) as an extracellular volume marker. Also, [FC] can be measured in nanoliter samples of plasma and proximal tubular fluid in vitro. FC is easily oxidized at the surface of a polarized, inert electrode, thereby generating a current that is proportional to [FC]. Electrodes are constructed by heat sealing a 5 um carbon fiber into the tip of a 9 um micropipet. The tip is double beveled, siliconized, and electrochemically treated to reduce protein adsorption. The electrical circuit is completed by attaching a fine copper wire to the carbon fiber and with a Ag:AgCl reference electrode. The carbon-fiber electrode is polarized for 100 ms to 400 mV with a custom-designed voltage clamp. An integral nanoammeter and sample/hold circuits record the oxidation current. To evaluate the method, rat proximal tubular fluid and plasma samples were split and analyzed in vitro for FC and tritiated inulin. The results showed excellent agreement between the paired inulin and FC TF/P ratios. In vivo measurements of FC in proximal tubule fluid show that the electrodes are stable, responsive, and give consistent readings upon repuncture. These results demonstrate the feasibility of using voltammetric microelectrodes to monitor proximal volume reabsorption continuously in a freely-flowing single nephron.

CHOLERA TOXIN (CT), FORSKOLIN (FSK) AND PGE_2 INTERACTIONS IN ISOLATED PERFUSED RABBIT CORTICAL COLLECTING TUBULES (CCT). S.P. Nadler,* S.C. Hebert, and B.M. Brenner. Brigham and Women's Hosp., Harvard Medical School, Boston, MA.

To investigate the mechanism of PGE_2 inhibition of the ADH-stimulated increase in hydraulic conductivity (Lp) in CCT, we studied the interactions of CT, FSK, and PGE_2 in isolated perfused CCT. At 25°C, CT (1nM) had no effect on Lp. At 37°C, following a 45 min lag phase, CT caused an increase in Lp from 16±2SE to 107±12x10^{-7} cm/s/atm (n=10). Whereas at 37°C ADH- and FSK-stimulated Lp declined with time, CT-stimulated Lp achieved stable plateau values. In paired studies with PGE_2, tubules were exposed sequentially to effector alone, effector plus PGE_2 (0.1μM), and then effector alone. In all experiments the bath contained 5μM indomethacin. (** p<.01, p<.05 vs 1st period)

			Lp (x10^{-7}cm/s/atm)		
Effector	T°C	n	-PGE_2	+PGE_2	-PGE_2
ADH 10μU/ml	25	4	94±13	52±8**	78±15
FSK 10μM	25	6	107±4	117±8	96±9
FSK 1μM	25	4	58±6	74±4	52±2
CT 1nM	37	6	113±17	87±17**	100±22

PGE_2 reversibly inhibited the Lp stimulated by ADH and CT but not the Lp stimulated to the same degree with FSK. PGE_2, alone or in the presence of 1μM bath FSK, caused a small increase in Lp. These studies suggest that the inhibitory action of PGE_2 occurs at a functional site linking the nucleotide regulatory protein with the catalytic unit of adenylate cyclase. The stable CT-stimulated Lp at 37° indicates that this pre-cAMP stimulation of Lp can offset the post-cAMP event(s) known to be responsible for the temporal decline in ADH-stimulated Lp.

EFFECT OF AGE ON LYSOZYME ABSORPTION AND PROTEINASE ACTIVITY IN RAT PROXIMAL TUBULES (PT). C.J. Olbricht, J.K. Cannon* and C.C. Tisher, University of Florida, Gainesville, Florida.

We demonstrated previously that lysozyme-induced proteinuria stimulates the activity of the intralysosomal proteinases, cathepsin B and L (CBL), in rat PT. In the present studies we examined the effect of age on endocytosis and basal and stimulated proteinase activity in rat PT. CBL activity was determined with a fluorometric microassay in single dissected S1, S2, and S3 segments of PT from 4 groups of female rats: nonproteinuric controls weighing 90-125 g (Group A) or 140-165 g (Group C) and rats of the same weight injected i.p. with lysozyme (Groups B and D) to induce proteinuria. PT of lysozyme-injected rats were also examined histologically for evidence of endocytosis. CBL activities represent the mean ± SD in pmol/mm tubule/min. The asterisk denotes a p value <0.01 against respective control values.

Group	Animals	S1	S2	S3
A	4	26.5±3.7	13.0±7.5	2.6±0.9
B	3	21.1±6.2	13.0±5.7	1.7±0.7
C	6	13.1±1.3	11.8±2.2	2.2±0.6
D	6	20.2±2.2*	13.6±2.1	3.2±1.4

CBL activity was highest in S1 segments of young control rats (A) and did not increase in any segment with proteinuria (B). In contrast, in adult controls (C) basal CBL activity was lower in S1 segments, but increased significantly with proteinuria (D). Histologically, lysosomes were increased markedly after lysozyme injection in adult rats only. We conclude that lysozymuria has no effect on endocytosis or CBL activity in young rats, whereas in adult rats it increases endocytosis which stimulates CBL activity selectively in the S1 segment.

PAH TRANSPORT IN ISOLATED PROXIMAL TUBULAR CELLS: COMPARATIVE STUDIES OF INFLUX AND EFFLUX PROCESSES. C.N. Nagineni,* D.B.N. Lee and N. Yanagawa. Sepulveda VAMC, UCLA Sch. of Med., Los Angeles, CA.

Initial PAH influx (Ix) and efflux (Ex) rates were measured in isolated rabbit proximal tubular cells (Biochem J, Vol 223, in press) at 37°C using tritiated PAH. Both fluxes were reduced by 50% at 25°C and abolished at 4°C. Probenecid (2mM) as well as metabolic inhibitors (Antimycin 50 uM, Retenone 50 uM) caused 80-90% reduction in both Ix and Ex. Ix was decreased by replacement of extracellular (EC) NaCl with KCl, ChCl or mannitol from 82±12 to 39±2, 36±3 or 20±2 pmol/10^6 cells (n=4) respectively. Kinetics of this Na^+ dependent Ix exhibited increase in Tmax without changes in Kt as EC [Na] was increased from 0 to 140 mM. Ex was not affected by EC [Na]. Increasing EC pH from 6.8 to 7.4 doubled Ix but only caused a 10% increase in Ex. Further increase in pH to 8.0 caused no further increase in Ix but an additional small increase in Ex. Our data suggest that both PAH Ix and Ex are mediated through carrier- and energy-dependent processes and are sensitive to inhibition by probenecid. However, Ix is Na^+ dependent and exhibits greater sensitivity to pH changes, while Ex is Na^+ independent and is less sensitive to pH changes. The kinetics of Na^+ dependent Ix is not characteristic of Na^+ cotransport model. Since the viability of the cells is unchanged during incubations at 37°C and initial rates of Ix and Ex could be studied conveniently as early as one or two min., isolated proximal tubular cells offer an ideal model to study the cellular mechanisms of PAH transport in this nephron segment.

TIME COURSE OF ALBUMIN ABSORPTION, ACCUMULATION, AND METABOLISM IN ISOLATED PERFUSED RABBIT PROXIMAL CONVOLUTED TUBULES (PCT). C.H. Park*: (intr. by C.C. Tisher), University of Florida, Gainesville.

In PCT filtered proteins are reabsorbed by endocytosis and gain entrance to the lysosomal compartment where they undergo hydrolysis. Hydrolysis products exit across the basolateral membrane. To examine the time course of absorption, accumulation and hydrolysis of protein, isolated PCT were perfused with ^{3}H-labeled albumin (^{3}H-Alb) at a physiological concentration of 36.4 μg/ml. Absorption was measured by the difference in perfused and collected ^{3}H-Alb. Hydrolysis was measured by appearance of acid (TCA) soluble ^{3}H in the bath. Accumulation was determined by measurement of ^{3}H in PCT that were extracted in nitric acid or was calculated as the difference between the ^{3}H that was absorbed and that which appeared in the bath. Tubule absorption of ^{3}H-Alb was constant at 99.9± 6.7 pg·min^{-1}·mm^{-1} for at least 70 min. Hydrolysis increased for about 40 min and remained constant at 71±6% of the uptake during the next 30 min. TCA soluble ^{3}H first appeared in the bath at 6-7 min after the start of perfusion with ^{3}H-Alb. Tubules accumulated protein for up to 100 min in a non-linear fashion, while the rate of accumulation decreased with time. The efflux of ^{3}H correlated linearly with that accumulated in the tubule. The results demonstrate that protein accumulation and hydrolysis in PCT are nonlinear functions of time, while absorption remains constant. Furthermore, hydrolytic activity appears to be dependent on the protein load delivered to the lysosomes. The transcellular processing of protein in PCT appears to be complete as early as 6-7 min after the initial absorption at the apical membrane.

EFFECT OF 8-p-(CPT)-cAMP (cAMP), FORSKOLIN (F) AND ISOBUTYL METHYL XANTHINE (IBMX) ON THE HYDROOSMOTIC RESPONSE OF CORTICAL COLLECTING TUBULES (CCTS) FROM POTASSIUM DEPLETED (KD) RABBITS (R). KH Raymond* and TD McKinney. Univ of Texas HSC/VA Hospital, San Antonio, Texas.

The present experiments study the cellular events involved in the decreased vasopressin (VP) responsiveness previously shown in CCTS from KD R perfused in vitro. CCTS were perfused at 25°C with a 200 mOsm/kg H_2O gradient from bath to lumen. The hydroosmotic response to cAMP (10^{-6}-10^{-3}M) was similar in CCTS from 5 KD and 4 control (C) R. F (a stimulator of the catalytic unit of adenylate cyclase) was added to the bath and net water flux, Jv, and the hydraulic conductivity coefficient, Lp, were measured in CCTS from 5 KD and 4 C R. There was no significant difference in Lp as shown below:

Forskolin [M]	Lp ($cm.s^{-1}.atm^{-1}.10^{-7}$) C	KD
0	2.28 ± 5.10	19.14 ± 7.35
10^{-6}	22.01 ± 5.01	45.18 ± 10.47
10^{-5}	40.04 ± 7.58	58.72 ± 10.62
10^{-4}	134.79 ± 18.29	159.72 ± 23.52

Finally, when IBMX (10^{-4}M) was added with VP (200 μU/ml), Lp and Jv increased significantly in CCTS from 4 KD and 4 C R. However, the hydroosmotic response in KD tubules was still significantly decreased ($p < .025$).

In conclusion, these studies indicate that the decreased hydroosmotic response of CCTS from KD R to VP primarily involves a step at or proximal to activation of adenylate cyclase. In addition, increased phosphodiesterase activity appears to make no contribution to this decreased response.

CONTRIBUTION OF CELL MEMBRANES AND DIFFUSION BARRIERS TO DIFFUSIVE WATER FLOW IN RAT PROXIMAL CONVOLUTED TUBULES (PCT). P. Preisig and C.A. Berry, Depts. of Physiol. Nsg. and Physiol., U.C.S.F., S.F., CA.

To evaluate the pathway and mechanism of ^3H_2O diffusion, rat PCT were microperfused in vivo at 25 nl/min with a solution containing raffinose to reduce volume flux to zero. The permeabilities of ^3H_2O ($P_{^3H_2O}$) and butanol (P_{BUT}) were determined. P_{BUT} was used as a minimum estimate of non-lipid epithelial diffusion barriers in series with cell membranes. The membrane permeability (P_{mem}) was calculated from $P_{^3H_2O}$, P_{BUT}, and their respective free solution mobilities. In control volume contracted rats P_{mem}, P_{BUT}, and $P_{^3H_2O}$ were 5.90±0.47, 2.21±0.14, and 2.77±0.18 x 10^{-3} cm/s, respectively. Epithelial diffusion barriers contributed 52.3±1.5% of the series resistance to ^3H_2O diffusion. Volume expansion, a condition that increases paracellular conductances in vivo, did not change any of these values, suggesting that the pathway of ^3H_2O diffusion was transcellular. 1 mM luminal pCMBS, a RBC inhibitor of ^3H_2O diffusion via aqueous channels, decreased P_{mem} 25% without affecting P_{BUT}, consistent with water channels in cell membranes. These data suggest: 1) ^3H_2O diffusion is transcellular, 25% pCMBS sensitive, and has a minimum membrane permeability of 5.90 x10^{-3} cm/s. 2) Non-lipid epithelial diffusion barriers contribute at least 52% of the series resistance to ^3H_2O diffusion.

PROSTAGLANDIN-E_2 DOES NOT INHIBIT VASOPRESSIN ACTION IN THE ISOLATED PERFUSED RAT CORTICAL COLLECTING TUBULE. Max C. Reif and James A. Schafer, Nephrology Research and Training Center, Univ. of Alabama in Birmingham, Birmingham, AL.

We have shown that arginine vasopressin (ADH) produces stable increases in osmotic water permeability (P_f), lumen-to-bath Na^+ flux (J-1b), and lumen-negative transepithelial voltage ($\Delta\psi$) in rat cortical collecting tubules (CCT), and that the J-1b and $\Delta\psi$ responses are enhanced by prior deoxycorticosterone acetate (DOCA) administration (Abstr. IX Internat. Congr. Nephrol., p.430A, 1984). The present experiments examined whether prostaglandin E_2 (PGE_2) could inhibit or reverse ADH effects as reported for toad bladder and rabbit CCT. Pathogen-free Sprague-Dawley rats were given DOCA (20 mg/kg/day i.m.) for 4-7 days. CCT from these rats were perfused at 38°C with a hypotonic (236 mOsm) medium containing 25μCi/ml $^{22}Na^+$, and bathed in isotonic (320 mOsm) medium. Control P_f was 27.8±12.6 μm/sec (SEM, n=5), which rose to 888±196 ($p<.05$) after adding 100 μU/ml ADH to the bath, and was unchanged at 839±217 by further addition of 10 μM PGE_2 to the bath. Control $\Delta\psi$ was -6.8±1.6 mV (n=7), -17.5±2.8 with ADH, and not significantly different at -20.0±2.5 after further addition of PGE_2. J-1b rose from 111±18 pmol/(min·mm) to 179±23 ($p<.025$) with ADH, and remained unchanged at 177±16 after addition of PGE_2. Adding PGE_2 after the control period caused no change in P_f, J-1b or $\Delta\psi$, and did not affect the subsequent response to ADH. We conclude that PGE_2 does not alter ADH actions in the isolated rat CCT, and that important species differences in local actions of prostaglandins may exist.

DEXAMETHASONE ACCELERATES COORDINATED DIFFERENTIATION OF EPITHELIA FORMED BY KIDNEY CELLS (A6) IN CULTURE. A.S. Preston*, J. Muller*, and J.S. Handler, NHLBI, and FDA, Bethesda, Md.

A6 cells form a differentiated monolayer epithelium seven days after they are seeded at confluent density on filters. In such mature epithelia, dexamethasone and aldosterone stimulate sodium transport (Isc). Vasopressin stimulates adenylate cyclase activity fivefold, tenfold in epithelia incubated with dexamethasone or aldosterone. We find that the addition of dexamethasone to the culture medium after cells are seeded results in dramatic acceleration of epithelial differentiation. One day after seeding, cultures incubated with dexamethasone have PD = 5mV, resistance = 3kohm-cm2, and an adenylate cyclase response to vasopressin. On day two, PD = 39mV, Isc = 11uA/cm^2, resistance = 3.4kohm-cm^2, and adenylate cyclase is stimulated fivefold by vasopressin, characteristics of mature epithelia. In contrast, controls have no PD, negligible resistance, and no adenylate cyclase response to vasopressin. Morphologic differences are also striking on day two. Control cultures show little differentiation, consisting of flattened, elongated, non-polarized cells in a multilayer arrangement. Dexamethasone-treated cultures consist of a monolayer of cuboidal cells with typical epithelial polarization and attachments. Thus, in addition to the previously described effects of dexamethasone and aldosterone on sodium transport and the adenylate cyclase response to vasopressin, dexamethasone has a pleiotropic effect on differentiation.

ALPHA(α)$_2$-ADRENERGIC INHIBITION OF FLUID ABSORPTION (Jv) IN RABBIT SUPERFICIAL PROXIMAL CONVOLUTED TUBULES (PCT). D. Rouse* and W.N. Suki. Baylor Col. of Med., Houston, Tx.

In a previous study on rabbit superficial (SF) PCT segments, perfused in vitro, Jv was enhanced by beta (β)-adrenergic stimulation and inhibited following α-adrenergic stimulation. The present study was designed to characterize the α-adrenergic modulation of Jv in isolated, perfused rabbit SFPCT's. Tubules were perfused and bathed in an artificial solution resembling rabbit serum ultrafiltrate. ^{3}H-methoxy inulin was added to the perfusate as a volume marker, and 0.35 g/dl protein was added to the bath. With the addition of 10^{-4}M clonidine (CL), an α_2-adrenergic agonist, to the bath in 7 PCT segments, Jv fell from 0.8±0.0 nl/mm.min, control (C), to 0.6±0.01 ($P < 0.05$). Potential difference (PD) increased from -1.6±0.2 mV, C, to -2.3±0.4 ($P < 0.05$) after CL. In another 5 PCT segments, the addition of 10^{-6}M methoxamine (MX), an α_1-adrenergic agonist, to the bath, did not significantly alter Jv (0.8 ±0.01 nl/mm.min, C, and 0.8±0.1, MX), or PD (-1.3±0.5 mV, C, and -1.7±0.7 MX). When isoproterenol (I), a β-adrenergic agonist, was added to the bath of 4 PCT, Jv rose significantly from C, 0.6±0.1 nl/mm.min to 0.8± 0.1. CL addition significantly reduced Jv, to 0.5±0.2 nl/mm.min. PD was not significantly different among C, I or CL periods.

We conclude that fluid absorption in the superficial PCT is modulated by both α- and β-adrenergic receptors, and that an α_2-adrenergic receptor inhibits baseline and β-adrenergic-enhanced Jv in this segment. This effect may be responsible in part for the natriuresis which follows the administration of α_2-adrenergic agonists.

TECHNIQUES FOR MEASURING INSENSIBLE WATER LOSS AND ITS SIGNIFICANCE FOR WATER BALANCE STUDIES IN PICHINDE VIRUS INFECTED GUINEA PIGS. R.P. Sanders*, M.J. Griffin*, and C.T. Liu, US Army Med.Res.Inst. Infect.Dis. Ft. Detrick, MD.

Pichinde virus infection in strain 13 guinea pigs (GP) causes a 25-28% reduction of body weight and a negative water balance in 14 days. Since water intake and urine output were extremely low at a late stage, other possible routes for water loss were considered. This study established techniques for measuring insensible water loss (IWL) and examined its significance in the distribution of total body water output (TBWO) in control and infected GP. For control GP, IWL (ml/day) was determined as daily H_2O intake + H_2O content of food intake-(body weight gain . K*) - (H_2O content of collected urine & feces). *For infected GP, H_2O content of the lost body weight was added; K=0.702 for control and 0.773 for infected GP, determined by dessication. Results show that IWL slightly decreased in the infected GP despite fever. However, when IWL were expressed as a percentage of total water intake (TWI) or TBWO, these values increased significantly:

Group	IWL ml/100g BW	H_2O Balance ml/100g BW	IWL/TWI %	IWL/TBWO %
Control[a]	10.0±1.3	2.4±0.2	46±3	53±5
Infect.[b]	mean ± SE,	*P<0.05	[a]n=5	[b]n=9
Day 12	6.1±1.3	-1.9±0.9*	78±6*	64±8*
Day 13	8.1±1.4	-1.9±0.4*	106±13*	78±2*

These data indicate that IWL accounts for 53% of TBWO in control GP. In the infected GP, IWL becomes the main route of water dissipation from the body (78%), while urine output contributes 22% of TBWO. In conclusion, insensible water loss is important for studying water balance in infected animals.

ACTION OF [8-ARG]-VASOPRESSIN (AVP) AND FORSKOLIN (FN) ON ADENYLATE CYCLASE (AdC) IN DISTAL TUBULES FROM HUMAN AND CANINE KIDNEY. B.T. Ruggles,* N. Murayama,* J.L. Werness,* M. Bentley,* S. Gapstur,* and T.P. Dousa. Nephrol. Res. Unit, Mayo Clinic, Rochester, MN.

Morphology of the canine nephron resembles more closely the human nephron than other frequently used experimental animal species. Therefore, we determined responsiveness of AdC to AVP in cortical ascending limb (CAL) and medullary thick ascending limb (MAL) of Henle's loop, as well as in medullary (MCT) and cortical (CCT) collecting tubules microdissected from these two types of kidneys. In segments of human nephron, both AVP (10^{-6}M) and FN (10^{-5}M) stimulated the AdC in CCT and in MCT; FN potentiated the stimulation of AdC by AVP in MCT and CCT. However, in CAL and in MAL only FN but not AVP elicted stimulation of AdC. Addition of FN did not uncover the stimulatory response of CAL and MAL to AVP. Tubules dissected from the canine kidney responded to AVP and FN in a similar way as human tubules. AdC in the MAL and CAL was insensitive to AVP, while AVP readily stimulated AdC in MCT and in CCT. The cAMP accumulation in intact tubules from canine kidney was markedly increased by AVP in MCT and also slightly increased in MAL. Therefore, in CAL and MAL from both human and canine nephron the AdC-cAMP system is virtually insensitive to stimulation by AVP, while collecting tubules, MCT and CCT, responded to AVP in a manner similar to other mammalian species. We conclude that with respect to responsiveness of the cAMP-generating system to AVP, the distal nephron of dog kidney is analogous to human nephron and thus a suitable model for studies of AVP actions.

SALICYLATE REABSORPTION BY THE PROXIMAL TUBULE OF RABBIT KIDNEY. Laurent Schild* and Françoise Roch-Ramel*, (intr.by L.C. Stoner). Univ. of Lausanne, Dept. of Pharmacology, Switzerland.

Micropuncture data demonstrated that salicylate (S) reabsorption from proximal tubules is not only due to simple diffusion (Roch-Ramel et al.,J.Pharmacol.Exp.Ther. 207:737-747,1978). To investigate the mechanisms involved in S reabsorption(J_S), S_2 segments were perfused with ^{14}C-S (0.5 mM). Varying the pH of bath and of perfusate, J_S was measured (fmoles.min^{-1}.mm^{-1}). When perfusate pH was decreased from 7.4 (HEPES 20 mM) to 6.6 (MES 20 mM), without change in bath pH, no increase in J_S was observed. In contrast a decrease of 0.5 pH unit of perfusate and bath, by increasing pCO_2 from 40 to 135 mmHg, increased J_S from 128±17 to 433±34 (n=10).

Apparently factors other than perfusate pH play a role in S reabsorption, i.e. basolateral pH or pCO_2. To investigate the role of basolateral pH, pCO_2 was kept constant (40 mmHg) and bath pH was decreased from 7.4 to 7.0 (HEPES/HCl buffer): J_S increased from 172+42 to 365+38.

In the absence of CO_2, the same decrease in bath pH did not stimulate J_S. Ethoxyzolamide (0.1 mM in bath) suppressed J_S. PAH (1 mM in lumen) decreased J_S by 33%. Removal of Cl^- from lumen stimulated J_S by 88%.

These data support the existence of a facilitated transport mechanism for S reabsorption and an OH^- (or HCO_3^-)/ S^- exchange mechanism sensitive to Cl^- and PAH^-.

PROTEIN KINASE C INHIBITS VASOPRESSIN (VP)-STIMULATED WATER FLOW IN TOAD URINARY BLADDER. Detlef Schlondorff and Sherman D. Levine. Albert Einstein Coll of Med. Bronx, NY

VP-stimulated transport in toad bladder is mediated by cyclic AMP (cAMP), while its effects in hepatocytes are mediated by calcium, phosphoinositide phospholipids, and a diglyceride-stimulatable, calcium-phospholipid-dependent protein kinase (kinase C). Based on our recent observation that VP also stimulates phosphoinositide turnover in toad bladder, we examined the role of kinase C as a modulator of VP-stimulated water flow. Both phorbol myristate acetate (PMA) (which can substitute for diglyceride as an activator of kinase C) and RHC-80267 (a glyceride lipase inhibitor which should increase diglyceride levels) inhibited VP-stimulated water flow by 30-50%, but not water flow stimulated by cAMP, consistent with inhibition of cAMP production. Inhibition persisted when prostaglandin synthesis was blocked by naproxen. Furthermore, water flow was not inhibited by the inactive diglyceride substitute phorbol didecanoate, supporting the specificity of the PMA inhibition. Toad bladder epithelial cell supernatant demonstrated calcium-dependent protein kinase activity which co-eluted from DEAE columns with kinase C, and was stimulated by phospholipid, diglyceride and PMA. Our data suggest that kinase C modulates VP-stimulated water flow in toad bladder by inhibiting cAMP generation, and support the hypothesis that VP-stimulated membrane turnover antagonizes the effects of VP via changes in diglyceride, phospholipid, and kinase C activity.

SOLUBILIZATION AND PARTIAL PURIFICATION OF THE NA-DEPENDENT GLUCOSE CARRIER FROM DOG KIDNEY BRUSH BORDER MEMBRANE VESICLES. M. Silverman, Univ. of Toronto, Toronto, Ontario.

This report describes solubilization and partial purification of the Na-dependent phlorizin (Phl) receptor in renal proximal tubule brush border membranes (BBM). BBM prepared by Mg precipitation (Am. J. Physiol. 242:F711, 1982) are subject to (i) 0.1% deoxycholate (DOC) for 1 hr 4°C; (ii) 50,000 g pellet is exposed to 0.5% DOC for 60 min 4°C and then 30 min 37°C; (iii) 100,000 g supernatant is subjected to chromatofocussing (Pharmacia PBE 94) and exclusion gel chromatography on S-200. At each step protein is incubated at 37°C for 2.5 min in 0.01 μM ^{3}H-Phl with 140 mM NaCl or KCl. The reaction is stopped by placing the sample on ice and then immediately on a 0.7 x 20 cm G75 column. Elution is carried out at 4°C with either NaCl or KCl buffer. Bound ^{3}H-Phl appears in the void volume; free ^{3}H-Phl in the column volume. Na specific binding is temperature dependent, unaffected by DOC detergent but abolished by proteolytic agents. No specific binding is observed with buffer alone, solubilized basolateral membranes or dog plasma protein. Specific binding is inhibited by 10 μM Phl and 10 mM D-glucose but not 10 mM L-glucose. Chromatofocussing of the 100,000 g DOC extract exhibits 3 protein peaks eluting at pH 8-8.5, 7-7.2 and 6.2-6.4 respectively. Only peak 2 exhibits specific Na-Phl binding. Gel filtration with S200 yields Na-Phl binding in the column volume. SDS PAGE suggests that specific ^{3}H-Phl binding is attributable to a 40-65K protein enriched some 10-15 times relative to the starting BBM.

THE EFFECT OF A POTENT Na:Ca EXCHANGE INHIBITOR ON THE CALCIUM PERMEABILITY OF A MONOLAYER OF CULTURED PROXIMAL TUBULAR (PT) CELLS. J.E. Scoble,* S.L. Westbrook,* C.J. Cragoe,* R. Duncan,* C.O. Watlington,* K.A. Hruska. Jewish Hosp., St. Louis, MO; Merck Institute for Therapeutic Res., West Point, PA; Medical College of Virginia, Richmond, VA.

We have shown parathyroid hormone (PTH) stimulated Na:Ca exchange in canine basolateral membrane vesicles (BLMV). Benzamil, when applied to BLMV, inhibited Na:Ca exchange with an IC_{50} of 175 μM. In canine PT cells prepared from cortical slices by Percoll density gradients and grown on collagen treated Nucleopore filters in serum free defined media, PTH increased cAMP production 3.23 ± 0.77 fold, N=6. Vasopressin and calcitonin were nonstimulatory. The cells exhibited Na^+ dependent glucose uptake and PEPCK but no hexokinase activity. After 14 days of culture, the monolayers formed a transepithelial potential (Ψ) of -0.5 ± 0.1 mV, apical side negative. Monolayers incubated in a modified Krebs Ringer solution exhibited undirectional Ca^{2+} fluxes of 0.092 ± .001 μmol/cm^2/hr apical → basal vs 0.083 ± 0.006 basal → apical. Benzamil (1 mM) added to basolateral PT cell surfaces abolished the Ψ within one hour and increased unidirectional Ca^{2+} fluxes to 0.348 μmol/cm^2/hr which were not different from the fluxes in collagen coated filters alone. Thus, an inhibitor of Na:Ca exchange in the renal proximal tubular cell produces loss of monolayer integrity demonstrating a potential role of Na:Ca exchange activity in cell adherence and cellular calcium metabolism.

BASOLATERAL CELL MEMBRANE WATER PERMEABILITY (Lp) IN PERFUSED RABBIT CORTICAL COLLECTING DUCTS (CCD). K. Strange* and K.R. Spring, N.I.H., N.H.L.B.I., Bethesda, MD 20205

We report direct measurements of basolateral membrane Lp in isolated perfused CCD. Short segments of CCD are perfused at 37°C on the stage of an inverted microscope equipped with Nomarski optics and a T.V. camera. Intercalated (IC) and principal (PC) cells are readily distinguished and their borders easily seen. Cell membrane Lp is determined from initial rates of cell shrinkage following a 100 mOsm increase of bath osmolality. A laminar flow bath chamber allows rapid bath exchanges ($t_{1/2}$ = 55 mSec) without tubule movement during fluid switching. Volume changes of the cells are complete within 600 mS after switching solutions. Initial and final cell volumes are determined by recording enface optical sections through an IC or PC. Because cell volume changes too rapidly to record the transient by this method, the rate of shrinkage is determined from the rate of change of the cross-sectional area of an IC or PC measured at the midlevel of the lateral wall of the tubule. The final percent change of cell volume and cross-sectional area are in excellent agreement. Using these data estimates of basolateral membrane Lp, uncorrected for unstirred layer effects, are: PC = 7.98 ± 0.65 (x 10^{-4} cm . sec^{-1} Osm^{-1}), n = 6; IC = 5.46 ± 0.87 (x 10^{-4} cm . sec^{-1} Osm^{-1}), n = 6. (Kevin Strange is supported by an NKF Fellowship.)

EFFECT OF CALCIUM ION ON VASOPRESSIN (AVP)-DEPENDENT cAMP CONCENTRATION IN MOUSE MEDULLARY THICK ASCENDING LIMB OF HENLE (MAL) AND COLLECTING TUBULES (MCT). K. Takaichi*, S. Uchida* and K. Kurokawa. Div Nephrol, Dept Med, Univ Tokyo Sch Med, Tokyo, and Wadsworth VA Med Ctr and UCLA Sch Med, Los Angeles, CA.

AVP regulates transport function of MAL and MCT by adenylate cyclase activation and hypercalcemia is known to inhibit AVP action in the kidney. To further clarify the effect of Ca^{++} on AVP-sensitive cAMP production, we examined cell cAMP content in MAL and MCT dissected from outer medulla. Tubules were preincubated at 37°C in Eagle MEM medium with 1.2 mM MIX and with 0, 1.0, or 5.0 mM Ca^{++} for 6 min and then with AVP for 2 min. Results in 11 mice of cAMP content (fmol/mm/2 min) measured by RIA are summarized in the Table.

Ca^{++}	0	1.0	5.0 mM
MAL	15.6±1.5	14.6±1.1	5.7±0.6
MCT	76.7±5.4	80.0±6.7	72.5±4.9

Thus, an increase in Ca^{++} from 1 to 5 mM suppressed AVP-dependent cAMP accumulation only in MAL but not in MCT. Neither verapamil nor diltiazem prevented a decrease in cAMP by 5 mM Ca in MAL. Ca-ionophore, A-23187, did not suppress cAMP accumulation in the MAL in the presence of 1 mM Ca^{++}. These results show that Ca^{++} inhibits AVP-dependent cAMP acccumulation only in MAL and not in MCT. In addition, the lack of effects of Ca-channel blockers and a Ca-ionophore suggests that high ambient Ca^{++} per se may inhibit AVP-sensitive cAMP formation in MAL. Our present data indicate that inhibition of AVP action by hypercalcemia may be at the medullary thick ascending limb of Henle and not at the collecting tubule.

TETRAETHYLAMMONIUM UPTAKE BY RABBIT S_2 PROXIMAL TUBULES. Joan B. Tarloff* and Paul H. Brand, Medical College of Ohio, Dept. of Physiology, Toledo, Ohio.

Tetraethylammonium (TEA) undergoes net secretion by the renal proximal tubule. Using isolated perfused and nonperfused rabbit proximal tubules, we are investigating the mechanism whereby TEA is accumulated within the tubular cells. In all experiments, the bath concentration of TEA was 25 x 10^{-6} M. Ringer solutions contained glucose, alanine and Na acetate. In perfused tubules, the unidirectional bath-to-lumen flux of TEA was similar when perfusate and bath were either HCO_3 -Ringer or phosphate-Ringer. However, in nonperfused tubules, TEA uptake was dependent on the presence of HCO_3 and CO_2 in the bath. In HCO_3-Ringer after 60 min incubation, the cell/bath ratio for TEA was 127.1 + 9.42 (n=6). The intracellular TEA concentration exceeded by 10-fold that predicted by the Nernst potential for passive accumulation of a cation across the basolateral membrane. With HCO_3-free bath and 100% O_2, the TEA cell/bath ratio was 31.73 + 8.46 (n=6). Omission of other anions (e.g. sulfate, phosphate, acetate) did not depress TEA uptake.

In the presence of HCO_3/CO_2, TEA uptake was reduced by maneuvers that inhibit the Na^+,K^+-ATPase and depolarize the basolateral membrane, e.g. 10^{-4} M ouabain, 0mM K^+ and 2mM Ba^{2+}. These results suggest that: (1) TEA uptake is dependent on the negativity of the cell interior and the action of Na^+,K^+-ATPase; and (2) HCO_3, CO_2 or intracellular pH are determinants of TEA accumulation at the basolateral membrane.

ANION EXCHANGER IS PRESENT IN BOTH LUMINAL (L) AND BASOLATERAL (BL) RENAL MEMBRANES. Z. Talor, R.M. Gold, A. McMoran*, J.A.L. Arruda. VAMC and Univ. Ark., Little Rock.

We have previously shown specific binding of the anion exchanger inhibitor ^{3}H-DIDS to both L and BL cortical membranes of beef kidney. To assess the role of this putative anion exchanger on transport we measured $^{35}SO_4$ uptake by highly purified L and BL membranes. Sulfate uptake was measured in presence of an outward directed chloride gradient, or OH gradient or HCO_3 gradient. To eliminate sulfate uptake by pH or voltage the experiments were performed in presence of protonophore and valynomicin. In both L and BL membranes sulfate uptake was significantly greater in presence of a Cl gradient, OH gradient or HCO_3 gradient than in the absence of these gradients. The sulfate taken in could be released by lysis of the vesicles indicating true uptake and not binding of sulfate. There was an early overshoot of anion dependent sulfate uptake of 5 to 10-fold magnitude as compared to the equilibrium uptake at 60 minutes. The anion dependent sulfate uptake was completely inhibited by either DIDS or furosemide in both L and BL membranes. Furthermore, an inward directed Na gradient stimulated sulfate uptake in L but not in BL membranes. The Na dependent sulfate uptake in L membranes was also inhibited by DIDS and furosemide. This finding suggests that Na and anions stimulate sulfate uptake by interacting with the same transport system. Thus, in addition to the well known Na dependent sulfate uptake in L membranes, there exists an anion exchanger in both BL and L membranes capable of sulfate transport.

CONTROL OF VP-STIMULATED cAMP SYNTHESIS IN CULTURED RAT INNER MEDULLARY COLLECTING TUBULE (RIMCT) CELLS: ROLES OF EXTRACELLULAR (Ca_E) AND INTRACELLULAR (Ca_I) CALCIUM AND PROSTAGLANDINS (PGE_2). Isaac Teitelbaum,* and Tomas. Berl. Dept. Med., Univ. Colorado Med. Sch., Denver, CO.

The effects of Ca_E and Ca_I on cAMP production by RIMCT cells were studied by obtaining dose-response curves to VP at various concentrations of Ca_E in the absence and presence of the Ca ionophore A23187 (I, 5x10^{-5}M). 10^{-7}M VP-stimulated cAMP generation (fm/µg prot/7.2 min) was (n=6):

Ca_E mM	0.0007	1.0	4.0
VP	500.98±80.75	315.96±63.10	274.93±54.61
VP+I	380.49±86.30	104.55±23.39	70.70±24.68
p=	NS	<.02	<.01

Ca_E exerts a tonic inhibitory influence on VP-stimulated cAMP production but further increases in Ca_E only minimally decrease cAMP accumulation. I does not significantly decrease cAMP in the absence of Ca_E; in the presence of Ca_E, I markedly impairs cAMP production. These changes in cAMP are associated with reciprocal changes in PGE_2 synthesis. The role of PGE_2 was explored by the use of 5 µM meclofenamic acid (M), a dose that inhibits I-stimulated PGE_2 production >95%.

Ca_E mM(n=4)	0.0007	1.0	4.0
VP	470.28±116.1	264.74±62.07	235.17±61.72
VP+M	411.46±77.49	243.90±41.36	218.11±33.39
VP+I	379.16±130.27	140.26±33.59	87.92±41.32
VP+I+M	314.54±99.77	121.94±39.79	100.26±43.91

The effects of Ca_E and Ca_I seen in the PG-intact state are not altered by PG inhibition. We conclude that both Ca_E by providing a tonic inhibition and Ca_I by potently inhibiting cAMP production can affect the hydroosmotic response to VP. These effects occur by a PG independent mechanism.

TARGET ANALYSIS STUDIES OF RENAL BRUSH BORDER WATER AND UREA TRANSPORT. A.S. Verkman, J.A. Dix, J.L. Seifter, K.L. Skorecki, C.Y. Jung, and D.A. Ausiello (intr by F.C. Rector). University of California, San Francisco; Harvard; S.U.N.Y. Buffalo and S.U.N.Y. Binghamton.

Radiation inactivation was used to determine the nature and mol. wt. (MW) of water and urea transport pathways in brush border membrane vesicles (BBMV) isolated from rabbit renal cortex. BBMV were frozen to -50° C, irradiated with 1.5 MeV electrons, thawed, and assayed for transport or enzyme activity. The freezing process had no effect on enzyme or transport kinetics. BBMV alkaline phosphatase activity gave linear ln(activity) vs dose plots with MW = 76 kDa, similar to reported values (JBC 257:10794). Water and solute transport were measured using stopped-flow light scattering. The rates of acetamide and osmotic water transport did not depend on radiation dose (0-7 Mrad), suggesting that transport of these substances does not require a protein carrier. In contrast, urea and thiourea transport gave linear ln(activity) vs dose curves with MW = 135 - 150 kDa. 400 mM urea inhibited thiourea flux by ~50% at 0 and 4.7 Mrad, showing that radiation does not affect inhibitor binding to surviving transporters. These studies demonstrate the use of target analysis to explore transport mechanisms and suggest that BBMV urea transport is facilitated by a protein carrier.

PROXIMAL TUBULES RESPOND TO DROP IN OSMOLALITY OF MEDIUM WITH REDUCTION IN RATE OF SOLUTE TRANSPORT

James C. Williams Jr.* and James A. Schafer. Nephrology Research and Training Center, Univ. of Alabama in Birmingham, Birmingham AL.

We have previously reported that proximal straight tubules (PST) perfused under oil respond to dilution of a simplified perfusate (no bicarbonate or amino acids) with increases in both the rate of volume absorption (Jv) and the rate of glucose absorption, with no apparent change in the rate of transport of total solute (Fed. Proc. 43:892). We have repeated these experiments using more complete (ultrafiltrate-like) perfusates in PST and in convoluted tubules (PCT), and determined the rate of total solute transport by measuring absorbate osmolality. In 7 PST, reduction of perfusate osmolality from 290 to 200 mOsm/kg by reducing only the NaCl concentration caused no change in Jv from the control value of 0.49±0.08 nl/min·mm (mean ± SEM; paired change in Jv was -0.01±0.11), but the rate of transport of total solute was reduced from 143±23 to 97±25 pico-osmoles/min · mm ($p<0.025$, paired t-test). Similarly, in 6 PCT, the control Jv of 1.25±0.18 nl/min·mm was unaffected by the maneuver (a paired change of +0.05±0.06), but the transport of total solute was reduced from 393±65 to 282±51 pico-osmoles/min·mm ($p<0.001$). It may be that a reduction in the rate of apical solute entry is necessary to permit a net loss of intracellular solute for counteracting cell swelling. With the simplified perfusates used previously, the lower rate of apical entry of solute may allow transepithelial transport to continue at the same rate following dilution without compromising regulation of cell volume.

CONTROL OF PROXIMAL TUBULE PAH SECRETION: INHIBITION BY PERITUBULAR SERUM PROTEINS IN VITRO IS DEPENDENT ON LUMENAL AND PERITUBULAR ORGANIC SUBSTRATES. D.E. Webb*, R.M. Edwards, and J.J. Grantham. Univ. of Kansas Med. Ctr., Dept. of Med. and Physiol., Kansas City, Ks.

Para-amino hippurate (PAH) secretion from bath to lumen was measured in isolated perfused segments of rabbit S_2 proximal tubule in vitro in order to study the mechanism of inhibition of PAH transport caused by rabbit serum proteins. Tubules were perfused and bathed in medium containing D-glucose as the only carbon substrate, and different concentrations of dialyzed rabbit serum proteins (RSP) and bovine serum albumin (BSA) were added to the peritubular bath. PAH transport was corrected for binding of PAH to RSP and BSA. BSA did not inhibit PAH secretion. By contrast, PAH inhibition by RSP was dependent on the presence of citrate, lactate and alanine in either the perfusate or the peritubular bath. In the presence of citrate, lactate and alanine, RSP (5.7 gm/dl) reversibly inhibited PAH transport by 42.6%. Maximal inhibition was observed with 1 gm/dl RSP in the bath; thus, PAH transport is highly sensitive to the protein inhibitors. With ultrafiltrate of serum as perfusate, RSP inhibited PAH transport 21.6% and in the absence of all organic substrates save D-glucose, RSP inhibited PAH transport by only 16.8%. Citrate, lactate and alanine did not directly inhibit the basolateral PAH transporter; rather, these substrates promoted the inhibitory effect of RSP. These studies provide strong support for the view that plasma proteins, by virtue of their effect to inhibit basolateral PAH transport, are potential modulators of renal organic anion excretion.

CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO VASOPRESSIN (VP). J. Work, J.F. Kearney*, and S.J. Ram*. Nephrology Research and Training Center and Dept. of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama.

Monoclonal antibodies to VP were developed by immunization of BALB/c mice with 8-arginine VP covalently coupled to bovine thyroglobulin. Nine clones from lymph node fusion with myeloma Ag8.653 produced antibodies that recognized VP. Inactivation of antidiuretic activity of VP was evaluated by infusion of three of the monclonal antibodies (2 μk, 18 μg/24 hr; 1 γ2bk, 52 μg/24 hr) into Sprague-Dawley rats (n=5) using osmotic minipumps. Urine volume before infusion was 3.6±0.4 ml/24 hr and increased to 19±5 ml/24 hr ($p<0.05$). Urine osmolality was 2086±108 mOsm/kg before and 1079±136 after infusion of monoclonal antibody ($p<0.01$). Inactivation of VP pressor activity was determined in anesthetized Sprague-Dawley rats infused acutely with 1 μg/kg/hr VP and then either monoclonal antibody to VP or monoclonal antidextran, as a control. The blood pressure increased from 106±2 mm Hg to 146±4 after VP infusion and then fell to 103±2 after infusion of monoclonal antibody to VP but remained unchanged with infusion of the control antidextran monoclonal antibody. These results demonstrate that chronic infusion of monoclonal anti-VP results in a partial diabetes insipidus and that pressor activity of VP was eliminated by infusion of the monoclonal antibody. We conclude that monoclonal antibodies to VP may be useful in defining both VP modulated transport processes and VP mediated pressor responses.

ROLE FOR VASOPRESSIN (AVP) IN RATS WITH CONGESTIVE HEART FAILURE (CHF). A. Yared*, V. Kon, B.M. Brenner and I. Ichikawa. Harvard Med. Sch., Boston, MA.

We studied rats with CHF ∿4 wks following myocardial infarction, using selective antagonists of the pressor [anti-P, $d(CH_2)_5$Tyr(Me)AVP] or hydroosmotic [anti-H, $d(CH_2)_5$D-Tyr(Et)VAVP] actions of AVP. Six anesthetized CHF rats had low systemic arterial ($\overline{AP}$, 100±2SE mmHg) and high left ventricular filling pressures (16±1 mmHg). Anti-P (10 µg, iv), given in the presence of saralasin, further reduced $\overline{AP}$ in each CHF rat (by 17±3 mmHg) but not in 6 normal controls (NC). In other 8 awake CHF rats, the renal excretory response to an acute oral water load (12% BW) was compared to that of 12 NCs in the absence ($\overline{s}$) and presence ($\overline{c}$) of anti-H. Values of volume excreted (V), U_{osm}, osmolar (C_{osm}) and free water (C_{H_2O}) clearances during the 2nd hr (2°), and cumulative V (ΣV) at the end of 2° (Mean; †P<0.05 vs. NC) are:

		V(2°)	ΣV(2°)	U_{osm}(2°)	C_{osm}(2°)	C_{H_2O}(2°)
		--% of load--		mOsm/L	-ml/min/kg BW--	
$\overline{s}$ anti-H	CHF	18.5†	19.9†	138	0.17	0.19†
	NC	24.7	26.7	111	0.18	0.30
$\overline{c}$ anti-H	CHF	27.9	37.4	95	0.16	0.40
	NC	27.0	37.8	88	0.15	0.39

CHF rats without anti-H demonstrated enhanced water reabsorption during 2°, as evidenced by normal C_{osm} but depressed C_{H_2O}. Without water supplement, V (17.8† in CHF vs. 12.1 in NC) and C_{H_2O} (0.23† vs. 0.15) became higher and U_{osm} lower than NC during the subsequent 2 hrs so that ΣV reached normal level (67.9 vs. 70.0). This delay in water excretion in CHF was abolished when water loading was repeated with anti-H (15 µg, iv). The data demonstrate significant enhancement of vasopressor and antidituretic action of AVP in CHF.

VIROLOGIC AND IMMUNOLOGIC STUDIES IN RECOMBINANT INTERFERON- α (rIFN) TREATED RENAL TRANSPLANT RECIPIENTS (RTR). J.D. Bell, L.L. Bohannon, B.R. Mehta, K.M. Ramsey, and R.B. Pollard. University of Texas Medical Branch, Galveston, Texas.

A Phase I trial utilizing rIFN was conducted on 10 cadaveric RTR who had received thoracic duct drainage. Administered dose of rIFN was either 3 or 6 million units IM 3x/week for 8 weeks. Evaluations of T-cell subpopulations and natural killer (NK) cell activity were performed and monitoring the incidence of herpes group virus infections and allograft rejection was accomplished. Mean values for immunologic studies prior to and 2 weeks after the start of rIFN therapy were as follows:

	T3(%)	T4(%)	T8(%)	NK(%50:1)	NK(LU)
Pre	53.4	24.4	27.4	53.1	155.3
2 Wks	31.9	10.1	21.1	39.1	44.3

While 4/10 RTR experienced viruria (3 CMV, 1 HSV), none had symptomatic viral infections. Those with positive viral cultures exhibited a greater reduction in NK activity (-56%) compared to those with negative viral cultures (-14%). In addition, the decrements in the T3 and T8 subsets were greater in those RTR with positive viral cultures. Only 2 episodes of early (1 and 2 months) allograft rejection were noted and graft survival rate (50%) at one year was similar to comparably treated historical controls.

In conclusion: 1) rIFN treatment results in the retention of substantial NK activity despite potent immunosuppression in the early post-transplant period, 2) ultimate allograft survival was unaffected by rIFN treatment, and 3) the lack of symptomatic viral infections among rIFN-treated RTR encourages further trials with this agent.

TRANSPLANTATION

IMPROVING EURO-COLLINS FLUSHING SOLUTION'S ABILITY TO PROTECT KIDNEYS FROM ISCHEMIA. Peter M. Andrews and Sally B. Bates*. Georgetown University Medical Center, Washington, D.C.

In this investigation, we describe a modification of Euro-Collins flushing solution which enables this solution to be effective in preventing normothermic as well as hypothermic postischemic acute renal failure (PIARF). The left kidneys of Sprague-Dawely rats were briefly flushed in situ by vascular perfusion with Euro-Collins solution and the renal pedicle clamped to render the kidney ischemic and hold the flushing solution in the kidney. Following one hour of in situ normothermic ischemia, the pedicle clamp was removed and a contralateral nephrectomy of the right kidney performed. In two other groups of rats the same experimental protocal was followed using Euro-Collins solutions in which the dextrose in this solution was replaced with a similar osmolal contribution of either sucrose (64 g/L) or mannitol (35 g/L). Rats with kidneys flushed with the standard Euro-Collins solution containing dextrose (N24) exhibited significantly higher postischemic daily serum creatinine levels, a greater degree of tubular necrosis, and a higher mortality (75% versus 31%) than unflushed ischemic controls (N 22). Rats with kidneys flushed with Euro-Collins containing either sucrose (N 25) or mannitol (N 22) in place of dextrose all survived, exhibited only focal tubular damage as observed by electron microscopy, and most returned to normal serum creatinine levels within 72 hours following ischemia. These findings, together with our other recent reports that sucrose based flushing solutions provide improved protection from cold ischemia, strongly argue for the substitution of sucrose (or other similar protective impermeant agents) for dextrose in flushing solutions such as Euro-Collins.

EVALUATION OF PRESENSITIZATION BY FLOW CYTOMETRY IN CADAVERIC KIDNEY RECIPIENTS. J.P. Bernhardt, C. Stabile, B.W. Colombe, K. Huebner, D. Wood, V. Lim, W. Amend, F. Vincenti, J. Melzer, N. Feduska, O. Salvatierra, M.R. Garovoy. University of California San Francisco.

The flow cytometry (Fluorescent activated cell sorter) crossmatch- (FACS) which is up to 250 times more sensitive than current cytotoxicity tests, was used retrospectively to investigate presensitization in 79 cadaver kidney recipients transplanted in 1983. Overall, the graft survival at 6 months was 73% with a negative FACS T crossmatch, and only 55% with a positive FACS T crossmatch. Among the presensitized recipients (PRA ≥10%) a positive FACS T crossmatch was detrimental with 46% graft survival at 6 months vs. 85% for FACS T negative patients. Among the patients with B warm cytotoxic antibodies we could identify by FACS, at least 2 types of antibodies in these sera- one binding to T cells, and one binding to the B cells only. Table 1 shows the graft survival at 6 months:

		Graft Survival	(n)
B warm antibodies neg.	FACS T pos.	43%	(7)
	FACS T neg.	69%	(29)
B warm antibodies pos.	FACS T pos.	62%	(13)
	FACS T neg.	81%	(16)

Therefore, among panel reactive individuals (PRA ≥ 10%) FACS crossmatching appears to detect an adverse state of presensitization, while distinguishing those patients with B warm cytotoxic antibodies who have an enhanced rate of graft survival.

TREATMENT OF STEROID AND ATG RESISTANT REJECTION WITH CYCLOSPORINE. M.J.Bia, K. Gaudio, A. Kliger, D. Smith, W. Flye* Yale Sch of Med., New Haven, CT

Little data is available concerning the value of cyclosporine in the treatment of acute rejection episodes. We evaluated the efficacy of cyclosporine in this setting in 5 patients in whom acute rejection had been unresponsive to treatment with both pulse steroids and Upjohn anti-thymocyte globulin (ATG). All patients (ages 9-54 y.o.) were recipients of their first cadaveric transplant and all were originally maintained on azothiaprine and steroids. Acute rejection occurred within the first month and was confirmed by renal biopsy in all patients. Rejection was first treated with pulse steroid therapy (1.5-2.5g solumedrol over 4 days) and, when renal function failed to improve, ATG (15 mg/km/d for 21 days). One patient did not receive ATG because of leukopenia. Because of continued deterioration in renal function, the patients were then switched from maintenance azothiaprine to cyclosporine (at doses that maintained trough serum levels between 50-150 ng/ml). One patient received an additional short course of monoclonal antibodies (against OKT3 cells). Serum creatinine values ranged 2.3-5.9 mg/dl at the time of the drug change and patients have now been followed for 2-10 mos. (mean 7 mos.). Although creatinine continued to rise in 1 patient (now back on dialysis), it failed to rise further and started to decrease in the remaining 4 patients over 1-4 wks. (down to 0.8-2.2 mg/dl, where values are now stable). Regarding infectious complications, 2 patients experienced mild symptomatic CMV disease, one of whom also suffered an episode of Listeria sepsis. We conclude that a change from maintenance azothiaprine to cyclosporine may be a useful mode of therapy in patients experiencing steroid and ATG resistant rejection.

INDUCTION OF IMMUNE ALTERATIONS AND SUCCESSFUL RENAL TRANSPLANTATION WITH A SIMPLIFIED METHOD OF DONOR-SPECIFIC BLOOD TRANSFUSION (DST). J.S.Cheigh, M.Suthanthiran, R.R.Riggio, W.Stubenbord, M.Kaplan, M.Fotino, M.Evelyn, N.Schechter, K.H.Stenzel, and A.L.Rubin. Rogosin Kidney Center, Cornell University Medical College, New York, N.Y.

We developed a simple method of DST protocol for prospective kidney transplant recipients (R) and examined its immunologic effects and clinical efficacy. Prospective kidney donors (D) gave 400ml of blood which was stored at bank. 39R were transfused with 100ml of whole blood (5 from HLA-identical, 32 one-haplotype mismatched and 2 two-haplotype mismatched D at 1,8 and 15 days of its storage. R were screened for lymphocytotoxic antibodies weekly during and for 3 weeks following DST. In addition, they were monitored with D-specific and non-specific MLC pre and post-DST. Post-DST only 3 (7.7%), all from one-haplotype mismatched D developed D-specific antibodies; 2 with T and B warm and 1 with B warm only. Following DST, D-specific MLC only was significantly suppressed without any accelerated (secondary type) response in early MLC. 24 patients (4 from identical, 18 one-haplotype and 2 from two-haplotype mismatched) subsequently underwent a kidney transplantation from the blood D. All of these have functioning grafts except one for 3 to 36 months with a graft survival rate of 96% at one year. In conclusion, 1)100ml of stored, whole blood DST, three times at weekly intervals is simple, less sensitizing and effective approach to enhance graft survival from living related D, 2)DST produces D-specific cellular adaptive responses that might be conducive to successful graft outcome.

PROGNOSTIC FACTORS FOR RENAL FUNCTIONAL OUTCOME IN PATIENTS (Pt) WITH LIVER CIRRHOSIS (CI) AFTER LIVER TRANSPLANTATION (LTX).

R. Brunkhorst*, A. Armbrecht*, Ch. Brölsch*, H. Liebau*, K.M. Koch*, R. Pichlmayr*, K.Kühn*. Hannover Medical School, Dept. Nephrol. and Dept. Abd. Surg., Hannover, W.-Germany

In 1972 Iwatsuki et al described in 3 cirrhotic Pt with hepatorenal syndrome a significant improvement of kidney function after LTX. We examined in 15 Pt with CI prior and on day 1-2, 8-10, and 28-30 after LTX creatinine clearance (C_{Cr}), serum sodium (S_{Na}), urinary sodium concentration (U_{Na}) and plasma renin activity (PRA). Cyclosporin A was given 6 hours prior and daily after LTX. Pt received no diuretic therapy.

According to PRA prior and post LTX and to C_{Cr} post LTX, 2 groups (gr I and gr II) of Pt could be distinguished (means ± SEM):

	gr	prior LTX	day 1-2	day 8-10
PRA	I	2.7 ± 1.9	1.4 ± 1.3	1.6 ± 1.7
PRA	II	16.0 ± 8.2	16.9 ± 13.7	16.0 ± 23.2
C_{Cr}	I	136.5 ± 48.2	57.9 ± 46.7	116.8 ± 55.7
C_{Cr}	II	94.2 ± 15.8	17.7 ± 21.2	37.2 ± 27.6

In gr II S_{Na} prior and U_{Na} post LTX were significantly lower than in gr I. In gr I all 9 Pt, in gr II only 1 of 6 Pt survived after 28 days.

Pt with high PRA levels developed a significant decrease in C_{Cr} after LTX. High PRA levels correlated with low preoperative S_{Na} and low U_{Na} after LTX.

Conclusion: Low S_{Na} and high PRA before LTX seem to indicate an unfavourable outcome of renal function after LTX in CI patients.

PROLIFERATIVE GLOMERULONEPHRITIS SECONDARY TO ATG (ATGAM^R) ADMINISTRATION. Yong Chi*, Eugene E. Cunningham, Jan Brentjens* and Rocco C. Venuto. SUNY at Buffalo, Dept. of Med., Buffalo, New York.

Antithymocyte globulin (ATG) is a gammaglobulin preparation obtained from various animals immunized with human thymus lymphocytes. It is used most commonly for the treatment of acute kidney allograft rejection. Heterologous ATG is a foreign protein capable of producing such side effects as fever, arthralgias, urticaria, anaphylaxis and a serum sickness reaction. Purified ATG raised in horses is available commercially (ATGAM^R). To our knowledge acute proliferative glomerulonephritis (PGN) has not been reported with this preparation (ATGAM^R). A kidney transplant patient developed fever, arthralgia, and a nephritic syndrome near the end of a course of ATGAM^R. A kidney biopsy revealed acute proliferative and exudative glomerulonephritis. Granular deposits of C3 and human and horse IgG were observed in the periphery of capillary tufts on immunofluorescence microscopy. Mesangial and subendothelial deposits of dense material were observed by electron microscopy. The acute renal failure in this patient resolved after cessation of ATG. Twelve other patients who were biopsied after or while receiving ATGAM^R demonstrated a picture compatible only with continued or resolving allograft rejection. A PGN was not seen. While some patients demonstrated weak linear staining for horse IgG, the granular pattern seen in the patient with PGN was not observed. A kidney biopsy is important in differentiating the development of a PGN secondary to ATG from allograft rejection.

NATIVE NEPHRECTOMY IMPROVES FUNCTION AND DECREASES REJECTION IN RAT RENAL ISOGRAFTS. T.M. Coffman,* W.E. Yarger, P.C. Brazy and P.E. Klotman, Duke Univ. and Durham VA Medical Centers, Durham, NC.

The presence of native kidneys may influence renal function and cellular rejection in renal transplants. We performed isogeneic kidney transplants in litter mates of Lewis rats with bilateral native nephrectomy (TxBNN) or sham native nephrectomy (TxSNN). Eight days after transplantation, RBF, C_{In}, and C_{PAH} were measured in the engrafted kidneys. The isografts were examined histologically for evidence of cellular infiltration. Function of transplanted kidneys was compared to that of two-kidney controls and 8-day uninephrectomized Lewis rats (UNx).

	C_{In}	C_{PAH}	RBF
	(ml/min/kg/kidney)		
TxSNN	1.17 ± 0.35	3.79 ± 1.27	17.83 ± 0.85
TxBNN	4.20 ∓ 1.56	13.71 ∓ 6.07	34.98 ∓ 4.31
Control	5.52 ∓ 0.19	14.89 ∓ 0.56	34.80 ∓ 2.99
UNx	7.44 ∓ 0.46	21.91 ∓ 1.03	47.27 ∓ 2.76

Renal function in TxSNN was significantly reduced when compared with TxBNN. Moreover, kidneys from TxSNN rats had marked perivascular and interstitial round cell infiltration. TxBNN isografts demonstrated minimal evidence of cellular rejection and renal function was not different from controls. However, isografts from TxBNN rats did not increase renal function to the level of UNx rats. Thus, the presence of native kidneys had a significant detrimental effect on renal isograft function. Bilateral native nephrectomy improved renal function and decreased histologic evidence of cellular rejection.

DOPAMINE (D) FUROSEMIDE (F) INFUSION FOR PREVENTION OF POST TRANSPLANT OLIGURIC RENAL FAILURE (TORF). Antonio DeLosAngeles*, Ashley Baquero*, Aaron Bannett*,and Rasib Raja. Kraftsow Div. of Neph. and Section of Transplant Surgery, Albert Einstein Med. Ctr., Philadelphia, Pennsylvania.

Several studies have used infusion of D and F for the treatment and/or prevention of acute oliguric renal failure. This study evaluates D and F infusion for prevention of TORF in cadaveric renal transplant recipients (CTR). Twenty consecutive CTR who received infusion of D(3 µg/kg/min(and F 200 mg IV q 6 hr) for 24-48 hrs (A) were compared to 20 consecutive CTR who did not receive the infusion (B). Renal preservation, surgical techniques and immunosuppression were similar in both groups. Serial BUN, Cr, electrolytes, C_{Cr} were determined. EKG monitoring was done for 24-48 hrs. Pts with progressive azotemia requiring hemodialysis (HD) were included to have TORF. Clinical characteristics were similar in both groups. Eleven pts in A and 7 in B had TORF and 61 post-transplant HD were needed in A and 36 in B. C_{Cr} was similar in pts while receiving infusion and 6-12 hrs after. Urine output with TORF was 1145 ± 525 ml/24 hrs and 1010 ± 544 and without TORF 4321 ± 936 and 2436 ± 322 in A and B respectively ($P < 0.05$ for pts without TORF). BUN was 56 ± 6 mg/dl and 64 ± 5.9; serum Cr 10.2 ± 1.4 and 9.6 ± 1.4; serum K 4.5 ± 0.3 and 4.7 ± 0.4 in A and B respectively after 24 hrs. Mean serum K was 3.6 in A, 4.5 in B ($P < 0.05$) in pts without TORF after 24-48 hrs. Two pts had cardiac arrhythmias in A but none in B. These data suggest that D and F infusion may not decrease the incidence of post transplant renal failure and may aggravate cardiac arrhythmia, fluid and electrolyte inbalance in pts without TORF.

CAPTOPRIL THERAPY OR NATIVE KIDNEY NEPHRECTOMY RESULTS IN MARKED INCREASES IN RENAL PLASMA FLOW RATES (ERPF) IN HYPERTENSIVE TRANSPLANT PATIENTS (HTXP). Curtis JJ, Luke RG, Jones P, Whelchel JD, Diethelm AG. University of Alabama Medical Center, Birmingham, AL.

The presence of the retained native kidneys (NK) is one cause of persistent post-transplantation hypertension. The renin-angiotensin system may be involved in its pathogenesis. We have reported that captopril decreases ERPF markedly if the cause of post-transplantation hypertension is functional renal artery stenosis (N Engl J Med 308:377,1983). We measured the change in ERPF in 8 NK caused HTXP's, all of whom became normotensive on follow up after NK nephrectomy (NX). ERPF measured one week before and 4.5±3 months (±SD) after NX increased (182±57 to 301±75 ml/min; $p<0.01$) despite the marked contemporaneous fall in M.A.P. (125±8 to 100±9 mm Hg; $p<0.01$). In 5 of these patients pre-nephrectomy administration of captopril (25 mg t.i.d. for 2-3 days) led to a similar fall in blood pressure (126±7 to 99±8 mm Hg; $p<0.05$) and increase in ERPF (196±47 to 350±102 ml/min; $p<0.06$). Serum creatinine was not significantly altered despite the increases in ERPF by either captopril or NK nephrectomy.

We conclude that: (1) The native kidneys can cause both hypertension and decreased allograft plasma flow via a mechanism that is reversed by captopril or by NX. (2) In HTXP's with well maintained renal function and late persistent hypertension, captopril may permit differentiation between functional renal artery stenosis (fall in ERPF) and native kidney dependent hypertension (increase in ERPF). The long term effects of NK dependent allograft vasoconstriction are unknown.

TRANSPLANT (TX) OUTCOMES IN PERITONEALLY DIALYZED (PD) PATIENTS. Peggy Devney, William J.C. Amend, Jr., Flavio Vincenti, Oscar Salvatierra, Nicholas Feduska, and Juliet Melzer. Univ. of California, San Francisco Transplant Service.

Increasingly, patients on PD are being TX'ed. We evaluated the TX outcomes of patients on PD from 9/79-3/84. 43/683 pts. (7%) were on PD. Despite initial clearance, 2 did not receive TX's due to preop peritonitis. Preop dialysis employed conventional PD techniques. Postop, dialytic techniques were variable (depending on operative findings). The tunnel and exit sites were inspected daily postop without routine irrigation of unused catheters. 3/48 pts developed postop clinical peritonitis with resolution following standard therapy. 5 pts developed tunnel infections. Overall TX outcomes were comparable between hemo and PD.

TX TYPE	GRAFT SURVIVAL HEMO (#Pts)	1 Yr% Survival	PD (#Pts)	1 Yr% Survival
CADAVER NON-DIABETIC	(346)	57%	(27)	54%
CADAVER DIABETIC	(38)	37%	(10)	30%
RELATED NON-DIABETIC	(200)	93%	(9)	89%
RELATED DIABETIC	(51)	83%	(2)	100%

There were no differences in pt. survival between the groups. Of pts. returning to dialysis, 12/14 returned to PD. In summary, with appropriate management, TX can effectively be provided to those patients receiving PD.

ASEPTIC NECROSIS IN RENAL TRANSPLANT RECIPIENTS. D Farge, PS Parfrey, JA Hanley, RD Guttmann, Royal Victoria Hospital, Montreal and Memorial University, Newfoundland.

Before 1971 the incidence of aseptic necrosis (AN) in renal transplant (T) recipients was 29% and after 1971 it was 4%. To investigate the reasons for this decreased incidence we studied all 26 T with AN and 42 controls matched for year of transplantation, age and sex.

Development of AN was not related to duration of dialysis before transplant, severity of uremia at time started dialysis, adequacy of dialysis before T, transplant dysfunction at time AN diagnosed, serum albumen when started dialysis, at T or at diagnosis, hemoglobin, platelet levels, or presence of hyperparathyroidism on x-ray at time AN diagnosed. SGOT was not different but in AN 50% (5/10) had parenchymal iron on liver biopsy 1 year after transplant compared to 7% (12/13) of those without AN ($x2 = 4.5$ $p < .05$). Total steroid dose 1 month after T was lower in AN compared to non AN (2.47 ± 0.3 vs 3.6 ± 0.3 gm) and was similar after 4 months (6.72 ± 0.55 vs 7.14 ± 0.6 gms) as were total number of I.V. doses of solumedrol or solucortef. There was a tendency to longer duration of high oral steroid doses in AN: duration of oral prednisone > 40 mg/day was 44.3 ± 8.6 days in AN compared to 34.2 ± 3.8 in controls, and 23% of AN had previous T compared to 2% of controls.

In conclusion the incidence of aseptic necrosis following transplantation has decreased recently for reasons that are unclear but probably multifactorial, and may include decreased use of prolonged high oral steroids and a decreased prevalence of iron overload.

EFFECT OF LIPOSOME ENCAPSULATION ON THE TOXICITY OF CYCLOSPORIN A. S. M. Gibson*, T. Shimamura, G. Strauss*, and J. K. Maesaka. UMDNJ, Rutgers Med. School, Pathol. Dept., Piscataway, Rutgers Univ., Chem. Dept., New Brunswick, VAMC, E. Orange and UMDNJ, N.J. Med. School, Newark, New Jersey.

The major side effect of Cyclosporin A (CsA), a powerful immunosuppressive drug, is its hepatorenal toxicity. We have attempted to reduce this toxicity by incorporating CsA in the lipid bilayer membrane of large multilamellar liposomes made from egg phosphatidyl choline (PC). Rats were injected i.v. daily for 5 days with either: a) Ringers; b) drug-free liposomes (90 mg PC/kg); c) CsA (30 mg/kg); or d) liposomes containing CsA at levels corresponding to b) and c). On day 6, inulin and PAH clearances were measured. Kidneys and livers were studied by light microscopy. Bilirubin levels were found to be elevated by administration of free CsA (1.910 mg%) compared to 0.244 and 0.275 mg% for Ringers and drug-free liposomes respectfully. Liposome entrapped CsA, in contrast, elevated the bilirubin levels only slightly (0.362 mg%), despite a dosage level 4-10 times higher than suggested therapeutic doses. Protection of renal function by entrapped CsA was not demonstrated, as assessed by elevated BUN levels and reduced GFRs in rats receiving either form of CsA. Administration of free or entrapped CsA caused no overt structural changes in hepatocytes or renal cellular elements, but did significantly reduce spleen weight compared to controls. It is concluded that incorporation of CsA in liposomes reduces the hepatotoxicity of the drug following i.v. injection. However, a similiar reduction of nephrotoxicity was not apparent at the high drug level used.

MAGNETIC RESONANCE IMAGING (MRI) FOLLOWING RENAL TRANSPLANTATION. N.J. Feduska, H. Hricak, F. Terrier, and F. Vincenti. Transplant Service, Univ. of California, San Francisco.

MRI in 27 renal transplant (Tx) pts early post-Tx evaluated ATN, acute rejection (AR), and cyclosporine toxicity (CyT). 5 patients had MRI at 72 hrs and at the time of Tx biopsy (bx). 22 pts had MRI only at the time of bx. Bx confirmed diagnoses included: ATN 3, AR 9, and CyT 5; 10 pts had normal histology. MRI was performed with a 0.35 Tesla unit Spin-echo technique with variable settings (TR=0.5 sec & 2 sec, TE=28 & 56 msec) and inversion recovery technique (TR=1800 msec, TI=420 msec, TE=28 & 56 msec) were used. Images were analyzed for Tx size, corticomedullary (cm) differentiation, visualization of hilar adipose tissue, perirenal fluid collection, and anatomy of the pelvocalyceal system. T1 & T2 relaxation values, spin density and intensity were measured. Ultrasound and renal scan studies also provided diagnostic data. ATN showed mild renal enlargement with good cm differentiation and normal hilar adipose tissue. T1 and T2 values for the cortex were normal. In the medulla, T2 values were prolonged and spin density was decreased. With AR, there were variable degrees of Tx swelling while cm differentiation was obscured with marked decrease in the renal cortex intensity. Spin densities and T1 values in the cortex and medulla were significantly prolonged and T2 changes were inconsistent. With CyT there was no Tx swelling, T1 of the cortex was unremarkable, and T2 was decreased. No T1 or T2 relaxation or spin density changes within the medulla were detected. Conclusion: The dramatic difference in the appearance of AR vs CyT illustrates that MRI has great potential for the early diagnostic evaluation of renal Txs.

PREDICTING THE COURSE OF CHRONIC RENAL FAILURE(CRF) IN RENAL TRANSPLANT RECIPIENTS. Susan K. Glocheski* and Douglas M. Landwehr. Depts. of Medicine, Medical College of Virginia, Richmond, VA, and Allegheny General Hospital, Pittsburgh, PA.

To determine if models used to predict rate of progression of CRF in nontransplant patients is applicable to allograft recipients, we analyzed the course of patients developing CRF following cadaveric transplantation. Patients transplanted between 1976-83 were studied if graft function persisted for >4mos., if S_{cr} rose by >2mg/dl to a level >4.5 mg/dl over a period of no less than 3mos. duration. In 22 patients meeting these criteria, S_{cr} rose from 2.4 ± 0.3 SEM mg/dl to 7.3 ± 0.4mg/dl over 13 ± 0.1 mos. Regression analyses in 14/22 patients showed single linear slopes vs time for: $1/S_{cr}$(r: .67-.98, all $p<.001$) and log S_{cr}(r: .73-.98, all $p<.001$); 8/22 had variable slopes. Rate of progression was not correlated with primary renal disease, HLA matching, immunosuppressive therapy, episodes of acute rejection, or level of proteinuria. Despite constant rate of change in $1/S_{cr}$ and log S_{cr} in most patients, rate of decline in creatinine clearance accelerated with time since creatinine production, as manifested by urinary creatinine excretion, fell. Thus, although $1/S_{cr}$ and log S_{cr} transformations may be used to predict rate of change of S_{cr} in a majority of transplant patients with CRF, they underestimate rate of loss of renal function. Similarity in the pattern of change in $1/S_{cr}$ and log S_{cr} in transplant and nontransplant patients with CRF suggests they may have common underlying mechanisms responsible for progression of CRF.

ORTHOCLONE OKT*3 TREATMENT OF ACUTE RENAL ALLOGRAFT REJECTION. Gideon Goldstein, Huei C. Tsai, Linda A. Barnes and Margaret M. Sheahan. (intro. by Jeffrey Friedman). Ortho Pharmaceutical Corp., Raritan, New Jersey

Acute renal allograft rejection was treated with ORTHOCLONE OKT*3 (5 mg IV daily for 10-14 days, with concomitant lowering of other immunosuppressives) in protocols involving 233 patients treated with ORTHOCLONE OKT*3 and 76 control patients. From these and other supportive studies, ORTHOCLONE OKT*3 is a highly effective therapy for the reversal of acute renal rejection (94 percent reversal), including cases resistant to all available conventional therapy (59 percent reversal). ORTHOCLONE OKT*3 is a murine monoclonal antibody that reacts with an important human T cell antigen since it blocks T cell functions in vitro. Early symptoms related to the first injection of ORTHOCLONE OKT*3 were for the most part tolerable with concomitant symptomatic therapy. Pulmonary edema occurred after the initial rejection in five (5) cases, each of which was shown to have pre-existing fluid overload. A careful assessment of fluid balance and weight gain, with reduction to dry weight if necessary has prevented the occurrence of pulmonary edema in 91 consecutive patients.

COMPARATIVE PATHOLOGICAL REVIEW OF 72 RENAL TRANSPLANT NEPHRECTOMIES OF PATIENTS TREATED WITH CYCLOSPORINE OR AZATHIOPRINE. Linda Green*, Regina Verani, Linda Schoenberg*, and Barry Kahan*. Univ. of Texas Health Science Ctr., Dept. of Pathology and Surgery, Houston, Texas.

The objective of this study was to review the pathological findings of renal transplant nephrectomies (TN) and to compare the results on Cyclosporine (CsA) and Azathioprine (Aza) treated kidneys. From 1978 to 4/1984, 145 renal transplant pts have been treated with Aza and 260 with CsA at the UTMSH. During this period 72 TN (41 Aza and 31 CsA) have been performed. The graft survival for the Aza treated pts was < 1 mo in 10 cases (24%) and 1 to 48 mo (mean 10 mo) in 31 cases. In the CsA treated kidneys the survival was < 1 mo in 15 cases (48%) and 1 to 7 mo (mean 2 mo) in 16 cases. A systematic semiquantitative histologic evaluation on a scale of 0 to 3+ was performed. We were unable to document a higher degree of interstitial fibrosis related to CsA therapy. Causes of nonfunctioning graft were: acute humoral rejection (16% CsA and 44% Aza), chronic rejection (26% CsA and 36% Aza), hyperacute rejection (3% CsA and 2.5% Aza), thrombosis of hylar vessels without signs of acute humoral rejection (26% CsA and 10% Aza), perirenal hematoma (13% CsA and 2.5% Aza), perirenal inflammation (16% CsA and 5% Aza). We concluded that TN were done earlier for the CsA as compared to the Aza treated kidneys. Thrombosis of hylar vessels and perirenal hematoma and inflammation were more frequent complications in CsA treated kidneys. However, rejection was the most frequent cause of TN in Aza treated kidneys (82.5%) as compared with CsA treated kidneys (45%).

IMPACT OF LIVING RELATED DONOR TRANSPLANTATION ON THE QUALITY OF LIFE. S Gouge*, J Moore, JP Johnson, B Bremer* and CR McCauley*. Walter Reed Army Med Ctr, Washington DC and Bryn Mawr College, Bryn Mawr, PA.

Objective and subjective measures were used to assess quality of life of 52 patients who received living related transplants (LR tx) and 66 related donors (D) and potential donors (PD). Both D and PD were similar on all measures, including present health and subjective well-being, and were equivalent to national norms for subjective quality of life. No impact of relative's tx failure was found for either D or PD respondents. Compared to D and PD, both successful and failed tx patients showed diminished employment, more discontinued activities, and less satisfaction with health. However, successful LR tx patients showed generally normal subjective well-being, while failed LR tx patients showed increased morbidity and diminished subjective well-being on 5 different measures. These results confirm and extend results of a previous study (K.I. 22:286-291, 1982) using the same measures, that found normal and near-normal quality of life for successful cadaveric tx patients and never-transplanted dialysis patients, respectively, but reduced quality of life for failed cadaveric tx patients. We conclude: (1) kidney donation has no effect on perceived health, activity, or subjective well-being, and (2) successful tx is associated with generally normal quality of life, but unsuccessful tx is associated with substantial impairment in the quality of life.

IMMUNOHISTOLOGICAL ANALYSIS OF SEQUENTIAL RENAL BIOPSIES FROM PATIENTS WITH ACUTE REJECTION (Rj). W.W. Hancock* and R.C. Atkins, Nephrology, Prince Henry's Hospital, Melbourne, Australia.

Cell infiltrates in 36 biopsies from 14 patients with acute Rj were analyzed using a 4-layer peroxidase technique and a panel of monoclonal antibodies (McAb): all leukocytes (PHM1), T cells (OKT3,9.6), T subsets (OKT4,OKT8), activated T cells (TAC), macrophages (FMC32, OKM1, Mac-120), activated macrophages (A1-3), B cells (B1,PHM14), NK and K cells (HNK1), and granulocytes (FMC10). Counts of labeled interstitial cells were expressed as a % of $PHM1^+$ cells:

McAb	Biopsy(Days Post Onset of Rj) 2-3	10-12	>30	p(t-test)
9.6	54.7%	55.7%	57.0%	
OKT4	15.4	20.6	15.7	
OKT8	36.4	37.1	41.7	
Ratio 4:8	0.47	0.57	0.44	
TAC	1.0	14.6**	2.2**	$p < .01$
FMC	41.6	41.9	36.8	
A1-3	4.7	27.6*	22.9	$p < .05$
HNK1	17.5	3.8**	6.8	$p < .01$
B1	6.0	2.6	2.0	
FMC10	2.5	2.2	6.6	

These studies show that the proportions of intragraft T cells, T subsets, macrophages and B cells are not significantly altered during Rj. However, the onset of Rj is accompanied by an NK cell influx, which resolves, followed by significant numbers of both activated T cells and macrophages around day 10. These dynamic events, possibly indicating intragraft cell activation, may be of clinical and therapeutic importance.

POST RENAL TRANSPLANT POLYCYTHEMIA. R.M. Hansen*, H.M. Kauffman*, W.F. Piering, W.J. Maierhofer, M.B. Adams*, and W. Fried*. Depts. Med. and Surg., Medical College of Wisconsin, Milwaukee, Wi and Dept. Med. Rush Presbyterian Medical School, Chicago, Il.

463 renal transplant patients (pts.) had graft function for at least six mo. Sixty-one (13%) developed polycythemia (PCT) (Hct. >52% men, >47% women). In 43, the native kidneys were intact. Fifteen of 20 pts. had either an absolute increase in red cell mass and/or plasma volume contraction. Of 43 pts. with native kidneys intact, avg. time to onset of PCT was 12.3 months (range 2-42). Polycythemia in 2 and 6 pts. was temporally related to rejection and hepatic dysfunction, respectively. Serum erythropoietin (ERP) levels were elevated in 9 of 11 pts.; elevated ERP production by native kidneys was proven in 2 pts. Polycythemia in these 2 pts. resolved after native kidney nephrectomy. Of 18 pts. with native kidneys previously removed, avg. time to onset of PCT was 30 mo. (range 4-53). Polycythemia in 3 and 5 pts. was temporally related to rejection and hepatic dysfunction, respectively.

Twenty-two pts. required an avg. of 5 phlebotomies (range 1-15) to maintain Hct.<55%. In spite of this, 4 pts. (7%) developed life threating thromboembolic phenomena while the Hct. was >50%: myocardial infarction (2), pulmonary emboli (1), mesenteric vein thrombosis (1). Elevated renal vein ERP levels in pts. with post transplant PCT are a probable indication for native kidney nephrectomy.

CY A RESCUE: SALVAGE OF RENAL TRANSPLANTS UNDERGOING ACUTE REJECTION UNRESPONSIVE TO CONVENTIONAL THERAPY. J.H. Helderman, A. Sagalowsky*, I. Dawidson*, W. Emerson*, A. Hull, P. Vergne-Marini, R. Dickerman*, D. Nesser, R. Velez, and K. Brinker, Univ. TX Hlth. Sci. Ctr. at Dallas, Southwestern Med. Sch., and Methodist Hosp. Dallas, TX.

Failure to reverse the first acute allograft rejection with the initial course of therapy augurs poorly for transplant success. One-third of such rejections escape reversal by prednisolone therapy and 10-15% escape ATG therapy when conventional immunosuppression is used as background therapy to forestall rejection. To salvage these kidneys we have tested the efficacy of cyclosporin A (Cy A) used as rescue therapy. Sixteen of 20 recipients who failed bolus prednisolone followed by ATG and were switched to daily prednisolone and Cy A (14 mg/kg PO) responded to the rescue attempt. These patients had a mean creatinine of 1.8 mg/dl, 1-9 months later. The four nonresponders had substantial humoral rejection on biopsy. Five additional patients were switched to Cy A because of ATG complications (4 serum sickness, 1 thrombocytopenia) or were ATG dependent (2). All experienced maintenance of excellent allograft survival. We conclude: 1) Many renal allografts hitherto lost to steroid and/or ATG- resistant rejections may be rescued by switching to Cy A; 2) conventional therapy fast humoral rejections may be resistant to Cy A rescue attempts; 3) Cy A may also have an important role in the treatment of rejections when ATG must be discontinued because of important drug toxicity.

LOW DOSE MAINTENANCE CYCLOSPORIN A (CsA) THERAPY AFTER RENAL TRANSPLANTATION. E.C. Haygood,* S.Dosa, A.M. Thompson, and S. Karmi.* Div. of Nephrol., George Washington Univ. Med. Ctr., Washington, D.C.

CsA nephrotoxicity in renal allograft recipients appears to be dose related and in most cases reversible. The effective CsA maintenance dose is reported to be 5-10 mg/kg/day. However, the minimum dose required to maintain good graft function is yet to be established.

Fifteen patients (mean age 38.4 yrs, range 28-60 yrs) with cadaveric renal allografts were treated with CsA and Medrol. CsA was given orally starting at 13-16 mg/kg/day. At 4 wks post-transplant, the mean CsA dose (mg/kg/day) was 8.8 $\pm$ 2.7; at 8 wks 6.2 $\pm$ 1.62; at 12 wks 4.6 $\pm$ 1.13 and at 16 wks 3.65 $\pm$ 1.16. Medrol was tapered from the initial mean dose (mg/day) of 144.7 $\pm$ 48.2 to 25.7 $\pm$ 10.4 at 4 wks, 13.2 $\pm$ 4.8 at 8 wks and 8.3 $\pm$ 1.62 at 16 wks post-transplant. Trough whole blood CsA levels were measured by HPLC. The mean follow up period post-transplant was 19.4 wks (range 4-35 wks). At present 14/15 patients studied have functioning grafts. The mean serum creatinine (mg/dl) at 4,8, 12 and 16 wks was: 1.94 $\pm$ 1.13, 1.80 $\pm$ .73, 1.65 $\pm$ .59 and 1.45 $\pm$ .36 respectively. The mean CsA levels (ng/ml) at 4,8,12 and 16 wks were: 221 $\pm$ 34; 214 $\pm$ 26; 229 $\pm$ 49 and 206 $\pm$ 27.8. The changes in the CsA blood levels from 4-16 wks were not significant ($p<0.01$).

Our data show that in renal allograft recipients the maintenance dose of CsA used in combination with steroids could be much lower than previously reported. The long term effect of low dose CsA therapy on the incidence of nephrotoxicity and allograft survival requires further studies.

COLD STORAGE (CS) VERSUS MACHINE PERFUSION (MP) FOR PRESERVATION OF CADAVER KIDNEYS FROM THE SAME DONOR. G.B. Helfrich,* J.A. Cutler,* D.J. Kelley,* C.J. Del Valle,* D.N. Morres,* B.W. Pechan,* C.B. Currier Jr., M.R. Alijani,* and J.A. Light. Georgetown Univ. Med. Ctr. and Washington Hosp. Ctr.; Divs. of Transplant., Depts. of Surgery, Washington, D.C.

Cadaver kidneys have been transplanted on CS for up to 48 hours, and preserved by MP and transplanted up to 72 hours with speculation as to which method is superior. We compared preservation methods on paired kidneys presumed to have the same function.

One kidney was preserved via CS, the other with MP. The quality of preservation for the CS and MP kidneys was evaluated by comparing the incidence of acute tubular necrosis (ATN) post-transplant.

CS kidneys were flushed and preserved with Euro-Collins solution. MP was performed with the Waters MOX-100 machine with Plasma Protein Fraction perfusate.

Of the 58 kidneys in this report, 24 were transplanted locally, and the remainder shared with 19 different transplant institutions. The mean preservation time of the CS kidneys was 29 hours 41 minutes. MP kidneys had a 32 hours 30 minutes mean preservation time. ATN was observed in 63% of the CS kidneys with the recipients requiring a mean of 2.66 dialyses. 17% of the MP kidneys required a mean of 2.3 dialyses.

Results of this study with 22 recipient institutions indicate that MP is superior to CS in reducing the rate of ATN in cadaver kidneys from the same donor ($P<.01$). CS is reported to be an acceptable preservation method. Graft survival at 1 year was not altered by method of preservation. This report of cadaver kidneys removed enbloc and separately preserved favors MP over CS as a method of cadaver kidney preservation when analyzed with respect to early graft function.

CONTROL OF HYPERTENSION IN RENAL TRANSPLANT RECIPIENTS WITH CAPTOPRIL. A. C. Jenkins* and G. R. Dreslinski, Squibb Institute for Medical Research, Princeton, New Jersey.

The initiation and maintenance of hypertension in renal transplant recipients may be related to fibrotic vascular lesions, with renin-angiotensin-mediated ischemia playing a fundamental role. 110 renal transplant recipients with hypertension were given captopril while continuing immunosuppressive therapy. At entry, their blood pressure was 170/108 ± 26/12, and serum creatinine 2.8 ± 0.6 mg/dl (mean ± S.D.). The changes at various time points were as follows (*=p<0.001 vs. entry):

	N	Blood Pressure (mmHg)	S. creatinine (mg/dl)
Month 1	81	147/92 ± 19/10*	2.8 ± 0.6
Month 3	68	141/88 ± 19/11*	2.7 ± 0.5
Month 6	56	142/88 ± 20/ 9*	3.0 ± 0.6
Month 9	48	138/77 ± 17/ 8*	2.6 ± 0.6
Month 12	24	145/93 ± 17/ 9*	2.3 ± 0.5

Renal function remained stable despite blood pressure control with a mean daily captopril dose of 122 mg. Nine patients had therapy discontinued due to adverse drug effects, and three were withdrawn as treatment failures. In this subgroup of hypertensive patients, perhaps due to the mechanism of the hypertension, captopril was shown to be safe and highly effective.

IN VITRO AND IN VIVO INTERACTION OF SULFAMETHOXAZOLE (SMX) WITH CYCLOSPORIN A (CSA) MEASUREMENT BY HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC) P.L. Kimmel, T.M. Philips,* N.C. Kramer, and A.M. Thompson. George Washington Med. Ctr., Renal Division, Dept. of Medicine, Washington, D.C.

Sulfamethoxazole/trimethoprim (TMP) combinations have been reported to potentiate CSA nephrotoxicity. Intravenous sulphadimidine/TMP administration reportedly results in reduction in CSA whole blood (WB) concentrations, measured by radioimmunoassay. We have observed elevated CSA levels measured by HPLC in renal transplant patients with stable hepatic function treated with SMX/TMP. CSA levels in our laboratory are measured by HPLC in 0.5 ml samples of deproteinized WB developed in 25% H_2O/75% acetylnitrile isocratically on a C-18 column (Altex) as a peak at 15.56 min (PK). In an attempt to elucidate a possible interaction of SMX/TMP in the CSA assay 1 mg SMX/TMP (Bactrim, Roche) was dissolved in 1 ml of normal human WB and run under the same conditions described. An interfering peak identical to the CSA peak was detected at 15.56 min. 1 mg SMX alone produced the same peak. TMP, furosemide, cimetidine and ranitidine produced no HPLC peak in the measured range.

In addition four volunteers with normal renal function taking neither SMX nor CSA had WB CSA determination by HPLC before and 3 h after a single oral dose of 800 mg SMX. PK was unmeasureable in the pre samples, but 3 h post SMX PK was comparable to a CSA level of 128 ± 43 ng/ml.

We conclude that SMX produces in vitro and in vivo interactions with CSA measurements by HPLC. Artifactual elevations of CSA determinations by HPLC must be considered in patients taking oral SMX.

VARIABILITY OF COURSE IN DE NOVO MEMBRANOUS NEPHROPATHY(DNMN). Matthew R. Kaplan, Lewis Reisman*, and Wallace W. McCrory. Cornell Univ. Med. College, Div. of Pediatric Nephrology, New York, New York.

DNMN has been well described in renal transplant recipients. Long term followup data in patients with DNMN is scanty and its natural history is unclear. Data from 3 recipients in the pediatric age group who developed heavy proteinuria and were documented by biopsy to have DNMN are presented below. Original causes of renal failure were hemolytic uremic syndrome(#1), dense deposit disease (#2) and tubulointerstitial nephritis(#3).

Patient#		1	2	3
Age(yrs)/Sex		8/M	19/F	18/M
Transplant Type		C*	LR*	C
Followup(mos)	From Transplant	24	91	31
	From DNMN	17	29	15
Pre-Biopsy:	CCr(cc/min/1.73m^2)	89	50.5	51.4
	Urine protein(mg/day)	4360	1725	3530
	Serum albumin(mg/dl)	2.7	4.0	4.2
Most Recent:	CCr	95	53.6	39.8
	Urine protein	527	570	15,900
	Serum albumin	3.6	4.7	3.1

*Cadaver **Living Related

These data demonstrate decreased protein excretion and stable GFR during prolonged followup in patients #1 and #2 who are assymptomatic. Patient #3 has become nephrotic with increased proteinuria and decreased GFR despite a course of high dose, alternate day steroid therapy. The cause of the marked variability in the course of DNMN is unclear. Possible explanations include differing antigenic components of the responsible immune complexes and their relationship to concurrent rejection, time post-transplant or original disease.

LYMPHOCYTE FUNCTIONS IN RENAL TRANSPLANTS ON CYCLOSPORINE(CY) vs AZATHIOPRINE(AZ). T. Kovithavongs, M.O'Brien*,J.French*,and J.B.Dossetor. University of Alberta, Edmonton, Alberta, Canada.

CY is a potent immunosuppressant with proven efficacy in clinical organ transplantation and has a margin of superiority over AZ of 10-20 % in prolonging renal allograft survival in various clinical trials. As a participating centre in the Canadian CY Trial, we have studied lymphocyte functions in these two groups of patients following transplantation in 3 areas: 1) the ability to generate donor specific cytotoxic T cells(CTL) in vivo as detected in the lymphocyte mediated cytotoxicity test; 2) the ability to produce interleukin 2(IL-2) following PHA stimulation in vitro and assayed with an IL-2 dependent mouse T cell line MTL2.8.2; and 3) the activity of natural killer cells(NK) in long term surviving patients using K562 target. The last aspect is of particular interest as these cells may have a role in immune surveillance against tomor. We found no difference in CTL generated in vivo with or without clinical evidence of rejection in the first 90 days of transplantation between these two patient groups. IL-2 production capacity fell to negligible levels in the first 4-6 weeks of CY administration in patients without rejection or infection; thereafter it was more variable and high levels of IL-2 could be produced in patients during quiescence and infection free. In contrast to AZ which depressed NK activity to low levels(<20%), CY only marginally effected NK (79%) in long transplant survivors(p<0.001). Thus, Immunological functions are relatively intact after the first several weeks of CY despite continued well being of the renal allograft, a finding in favour of CY as a drug for organ transplantation.

CYCLOSPORINE AND TEGRETOL--ANOTHER DRUG INTERACTION. P. Lele*, P. Peterson*, S. Yang*, B. Jarrell*, J.F. Burke, Jr., Divisions of Nephrology and Renal Transplantation, Thomas Jefferson University Hospital, Philadelphia, PA

Cyclosporine (CyA) has dramatically changed the outcome of organ transplantation by virtue of its highly specific immuno-suppressive effects. Increasing use of CyA has uncovered drug interactions with phenytoin, ketoconazole, and cimetidine due to modulation of the liver P450 oxidase enzyme system. We present an interaction not previously described.

A 55 year old white male received a cadaver kidney and at four months post-transplant had a serum creatinine of 2.2 mg/dl and a therapeutic serum CyA level of 346 ng/ml (RIA). For severe eye pain, he was started on Tegretol (carbamazepine), 200 mg TID, with a therapeutic level of 9.4 mcg/ml. Within three days, the CyA level had fallen to 64 ng/ml and a week later was down to 37 ng/ml. Tegretol was stopped and the CyA level rose to between 100 and 200 ng/ml until Tegretol was restarted at 100 mg TID. CyA dosage then had to be increased to keep serum levels therapeutic.

Tegretol is a hepatic P450 oxidase enzyme inductor, the system responsible for CyA metabolism. The dramatic decreases in CyA levels clearly demonstrate increased CyA degradation. The patient was not taking any other drugs known to affect this enzyme system. As usage of CyA increases, more interactions are sure to occur, and knowledge of metabolic effects is essential to avoid clinical problems.

THE EFFECT OF HYPOTENSION, RENAL INSUFFICIENCY, AND DIABETES INSIPIDUS IN CADAVER DONORS ON THE INCIDENCE OF POST-TRANSPLANT ACUTE RENAL FAILURE. Robert E. Lordon, and Kenneth E. Watson*. Univ. of Louisville, Louisville, Kentucky.

Although post-transplant acute renal failure (PTARF) does not affect graft survival, it does increase patient morbidity. We studied the association of clinical parameters in cadaver donors with PTARF using non-pulsatile cold storage. PTARF occurred in 46% of the 56 patients who received kidneys from 33 donors. Donor hypotension with systolic BP <80 mgHg (88%) or <60 mmHg (52%) did not increase the incidence of PTARF (48% and 45% respectively). Dopamine Rx used in 79% and renal insufficiency (serum creatinine 1.6-4.4 mg/dl) occurring in 52% of donors also had no effect on PTARF. Cold ischemia time (CIT) was significantly longer ($p<.01$) in kidneys with PTARF (mean 32 hrs) compared to those with immediate function (mean 24 hrs). Diabetes insipidus (DI) was diagnosed by a serum Na >156 meq/L and a urine Sp. gr. <1.006 in 55% of donors. Treatment of DI with vasopressin (VP) increase the incidence of PTARF an association independent from CIT as shown below.

DONORS	NO. KIDNEYS	PTARF	CIT
DI Rx with VP	13	77%*	29 hrs
DI no VP	17	29%	24 hrs
No DI	26	42%	29 hrs

*Significantly different from other 2 groups

Hypotension, dopamine therapy and mild renal insufficiency in cadaver donors do not alter the incidence of PTARF. DI, a common complication, is associated with an increase in PTARF when treated with VP but not when treated with fluids alone.

MECHANISM OF ACTION OF MLR GENERATED HUMAN SUPPRESSOR T LYMPHOCYTES (Ts). R. Loertscher*, J. Williams*, H.M. Shapiro*, C.B. Carpenter, T.B. Strom. Dept. of Medicine, Beth Israel and Brigham&Women's Hospitals Harvard Medical School, Boston, MA.

RNA synthesis and the expression of certain proteins e.g. IL-2 receptor (IL-2R) and 4F2 protein are premitotic indicators of T cell activation(TCA) Monoclonal antibodies 49.9 (anti IL-2R) and 4F2 and specific stains for RNA and DNA coupled with flow cytometric analysis were used to assess the stage at which human Ts interrupt the TCA pathway. Fresh PBL (R) and heat inactivated (44.5°/1hr) EBV transformed Laz 509 (B cell line) stimulators (S) were interacted in 1° bulk MLRs. After 10 days of culture the Ts containing R cell blast fraction (M) was isolated on a Percoll density gradient, irradiated with 1200r and placed into fresh bulk MLRs combining R+Sx. M:R ratios were 14, 1:2 and 1:1. RNA content, IL-2R and 4F2 protein were simultaneously analyzed with DNA content in individual cells. R alone and R+Sx served as negative and positive controls (C) respectively. Ts rich M cells reduced RNA synthesis from 52.2% (+C) to 14.4% (1:1), DNA synthesis from 13.8% to 6.3%, and activation antigen (49.9, 4F2) positive cells from 52.5% to 19.2% and from 61.6% to 28.8%, respectively. We conclude that Ts generated against heat treated S interfere with RNA synthesis in G_{1A} before progression to G_{1B} thus preventing the consequent steps, e.g. synthesis of new proteins and DNA in the TCA cascade. These data are in contrast to recent work in the mouse demonstrating an interference of Ts generated through other means in TCA later than IL-2R expression.

DONOR FACTORS ASSOCIATED WITH DELAYED GRAFT FUNCTION (DGF) IN CADAVER DONOR RENAL TRANSPLANTATION (CDRT). Bruce A. Lucas, Univ. of KY Med. Ctr., William K. Vaughn, Vanderbilt Univ., Nashville, TN., Everett X. Spees, Francis Scott Key Hospital, Baltimore, MD., Fred Sanfilippo, VA Hosp., Durham, N.C.

Delayed renal allograft function has become an issue of increasing interest since the advent of cyclosporine. Previous reports from the South-Eastern Organ Procurement Foundation (SEOPF) Prospective Study have documented less DGF with machine preservation (30%) than with ice storage (42%) or combined modalities (37%) and compromised graft survival in CDRT for kidneys with DGF. This report documents donor factors associated with DGF from the SEOPF Prospective Study. From 1977 to 1982, data are available on 3811 kidneys retrieved and transplanted within SEOPF. Significant donor factors and DGF include:

Age ≤ 30 vs >30 years. $p=0.003$

Length of hospitalization ≤ 48 vs >48 hours. $p=0.001$

Last hour urine output ≤ 300 vs > 300 ml/hr. $p=0.0005$

Terminal serum creatinine (Scr) ≤ 2 vs >2 mg/dl. $p=0.003$

Average systolic BP ≤ 90 vs >90mmHg. $p=0.01$

Heartbeating vs off ventilator. $p=0.000004$

Proteinuria and multiple arteries are significant factors only with ice storage.

ZINC METABOLISM IN CYCLOSPORIN-PREDNISONE VS. AZATHIOPRINE-PREDNISONE TREATED RENAL TRANSPLANT RECIPIENTS. S.K. Mahajan, J. Abraham*, L. Bennett*, S. Migdal, D. Abu-Hamdan and F.D. McDonald. V.A. Medical Center, Allen Park and Hutzel Hospital, Detroit, Michigan.

We have recently reported that abnormalities of Zinc(Zn) metabolism of uremia persist following renal transplantation(RT) in Azathioprine-Prednisone (AP) treated patients and may be related to increased Urinary Zn(UZn) losses. High dose steroids known to increase UZn, may account for post RT hyperzincuria in AP treated patients. Since cyclosporin-Prednisone(CP) treated patients receive lower steroid dosage, we compared plasma Zn levels and UZn excretion in CP treated (N=8) and AP treated (N=8) patients following RT. Data (Mean ± S.D.) were obtained up to 6 months post RT in both groups.

	CP (N=8)	p	AP (N=8)
Plasma Zn (ug/dl)	88±13	N.S.	80±10
Urinary Zn (ug/24°)	486±210	<.01	1470±520

All patients had significantly lower plasma Zn levels up to 6 months post RT as compared to those in normal controls (112±10 ug/dl). However, hyperzincuria was present only in AP-treated patients. UZn excretion in CP-treated patients were similar to those in normal controls (420±160ug/24°). The 2 groups of patients were similar in all aspects except the steroid dosage. The mean steroid dosage in AP (44±12mg/day) was significantly higher than that (18±2mg/day) in CP recipients. The results of this study suggest that steroids may, in part, account for high UZn losses. Persistence of low plasma Zn post RT may, in addition, be related to factors other than renal losses of Zn.

HLA-DR TISSUE TYPING IN 324 CADAVERIC RENAL TRANSPLANTS. R Migliori, D Fryd, H Noreen, N Ascher, D Sutherland, W Payne, R Simmons, J Najarian. Dept. of Surgery, University of Minnesota.

324 cadaveric renal transplants (1979-84) were reviewed to determine patients who might benefit by HLA DR matching. Of 237 first cadaveric transplants, no significant differences in graft function or patient survival occurred as a result of mismatching at the DR Locus. Even after controlling for diabetes, immunosuppressive regimen, associated HLA A & B match, or sex differences could be observed between well-matched or mismatched grafts.

2 YEAR CADAVERIC GRAFT FUNCTION

Number of HLA-DR Mismatches	All first transplants	1st transplants younger than 45	All multiple transplants
0	81%	89%	67%
1	78%	82%	75%
2	74%	71%	55%
P value (2vs0 mismatch)	0.44	0.06	0.09

A nearly significant difference in graft function was noted in the group of first cadaveric transplants when the recipient was younger than 45 years. Graft function was 18% greater at 2 years in the absence of mismatching as compared to transplants with complete 2 Ag mismatches in this group of young patients. Near significant differences in both function and survival also was seen in the 87 patients who received cadaveric grafts following previous renal transplantation.

DONOR-SPECIFIC CELL MEDIATED LYMPHOLYSIS RESPONSIVENESS AND SUPPRESSOR CELL STUDIES IN RENAL ALLOGRAFT RECIPIENTS. M. Mathew,* T. Kovithavongs & J.B. Dossetor. University of Alberta Hospital, Edmonton, Alberta, Canada.

The immunological responsiveness of a panel of 30 renal allograft recipients (R), was studied against cells of the specific donor (D). 15 had rejected their transplants (Tx) within 1 year while the other 15 had good function at the same period. Serial samples were obtained from all the patients, pre Tx (R0), post Tx 0-30 days (R1), 31-90 days (R2) & 91 days-1 year (R3). Two donor-specific immunological systems were used. In the first, the cell mediated lympholysis (CML) inducibility of pre and post Tx R cells to stimulation by D or healthy controls (Y) were compared (R0Dx, R1Dx etc). In the second, the suppressive capacity of irradiated post Tx R lymphocytes, was assayed (R0Dx+R1x etc). These two assays were run simultaneously on all the samples in individual patients. In the 15 patients with rejected allografts, the mean CML specific lysis of R0Dx was 47%, post Tx R1Dx remained elevated at 37%, & R2Dx at 41%. Control values, R0Yx, R1Yx & R2Yx were 55, 52 & 50% respectively. Suppressor cell studies showed no significant difference between patients and controls, i.e. no donor specific suppression. In the other 15 patients with good function, R0Dx was 40%, all the post Tx samples dropped to 20% or less, ($p<0.005$), with no drops in the controls. Suppressor cell studies in this group showed a small but statistically significant suppression, ($p<0.02$). These results suggest that the CML inducibility post Tx may be useful as a predictive index of ultimate transplant outcome.

GLOMERULAR THROMBI IN RENAL ALLOGRAFTS ASSOCIATED WITH CYCLOSPORIN THERAPY. Guy Neild, Rowena Reuben, Barry Hartley, J. Stewart Cameron, Guy's Hosp. Dept. of Nephrol. and Path,, London SE1. UK. (intr. by R.J. Glassock)

We have reviewed 110 renal biopsy specimens from 47 renal allograft recipients, who were all receiving prednisolone and cyclosporin. Glomerular capillary thrombi or afferent arteriolar thrombosis were observed in 13 specimens from 10 recipients. Biopsies were performed either routinely one and four weeks after transplantation or during periods of renal dysfunction. None of the patients whose biopsy material contained glomerular thrombi were considered, in retrospect, to have been under going rejection at the time of biopsy. In every case, trough whole blood cyclosporin levels were high with a mean of 1490 µg/l (range 755-2000). Thrombi consisted of finely granular material partially obstructing glomerular capillaries. By light microscopy, the staining characteristics of the thrombi were compatible with platelet-fibrin aggregates and this was confirmed by immuno-peroxidase examination using anti-sera against fibrin, factor XIIIa and platelets. Large thrombi were associated with mononuclear macrophages. Such thrombi were never previously seen in biopsy material from recipients treated with prednisolone and azathioprine, except very rarely associated with acute vascular rejection. In none of these patients was there haematological evidence of a haemolytic uraemic syndrome as has been reported in bone marrow recipients treated with cyclosporin. The findings are further evidence that cyclosporin may initiate or accentuate vascular damage, by, we believe, an effect on platelet-endothelial interactions.

TUBULAR FUNCTION AFTER LIVE DONOR TRANSPLANTATION. D. J. Norman, D. Hatch,* and J. M. Barry. Oregon Health Sciences University, Portland, Oregon.

It is assumed that the minimal ischemia time to which live donor kidneys are subject causes reversable tubular damage. Therefore, to avoid volume depletion from a urinary concentrating defect, urine output is matched by intravenous input after transplantation. We questioned the wisdom of this practice. Therefore, a randomized prospective study was conducted using 18 patients and 2 IV replacement protocols. Nine patients were given the previous hours' urine output plus 30 cc each hour (high replacement) and another nine patients were given 125 cc per hour regardless of output (low replacement). The study was conducted for 48 hours following transplantation. Several urine and serum measurements were made including Na, K, PO4, uric acid, protein, creatinine, glucose, osmolality, amino acids, and lysozyme. Urine output was less than input in both groups. The high replacement group has a significant positive sodium balance. The low replacement group could concentrate their urine and reabsorb sodium normally and did not become volume depleted. The groups had identical clinical outcomes, but the conventional high replacement group required significantly more IV replacement.

In summary, a clinically significant urinary concentrating defect was not present after live donor transplantation. Massive IV fluid replacement is unnecessary.

CHRONIC NON B HEPATITIS IN RENAL TRANSPLANT RECIPIENTS. PS Parfrey, D Farge, R Dandavino, RDC Forbes, RD Guttmann. Royal Victoria and Maisonnueve-Rosemont Hospitals, Montreal and Memorial University, Newfoundland.

To determine the outcome of chronic hepatitis in ESRD we studied all 358 renal transplant recipients (T) and 295 hemodialysis (D) patients treated for > 1 year since 1970. The incidence of chronic hepatitis (elevated SGOT for > 1 year) was 15% (N=54) in T and 3.4% (N=10) in D. 48% (26) of T and 50% (5) of D were HBsAg positive (HB).

In T the clinical outcome was significantly better in non B compared to HB: 11% died, none from liver disease and 35% remitted after a mean follow-up from start of liver disease of 77.3 $\pm$ 8.2 months. In HB 54% (14) died, 9 from liver disease, and none remitted after a follow-up of 90.2 $\pm$ 8.9 months. Adverse prognostic factors (age, duration of diabetes and heart disease) present before ESRD treatment began were similar in both groups as was duration of follow-up. 14% (2/14) of non B progressed to chronic active hepatitis compared to 71% (15/21) of HB but follow-up to last biopsy was longer in HB. However, histological stability in those with serial biopsies occurred in 66% (4/6) of non B and only 18% (13/16) of HB with a similar duration of follow-up. No D died from liver disease.

We conclude that chronic hepatitis occurs more frequently in T than D, and that chronic non B hepatitis has a more benign clinical and histological outcome than chronic HB hepatitis in renal transplant recipients.

DECREASED RENAL BLOOD FLOW AFTER CYCLOSPORINE INFUSION. M.S. Paller, B.M. Murray* and T.F. Ferris, Univ. of Minnesota, Minneapolis, MN.

Cyclosporine (CSA) is now widely used in transplantation, but its use is complicated by a high incidence of renal dysfunction. We studied the effect of acute infusions of CSA in doses of 20 mg/kg (CSA 20) and 10 mg/kg (CSA 10) on systemic and renal hemodynamics in conscious rats using radiolabelled microspheres. CSA 20 caused a decrease in renal blood flow (RBF) (3.4 vs. 6.1 ml/min/g, $p < .001$) and an increase in renal vascular resistance (36.9 vs. 19.9 mmHg/ml/min/g, $p < .05$), whereas CSA 10 caused no significant decrease in RBF (5.3 vs. 6.5 ml/min/g). Neither dose of CSA caused a change in cardiac output, although mean arterial pressure was lower after CSA 20 (112 vs. 122 mm Hg, $p < .05$). Both doses increased PRA (con: 5.6 ng/ml/hr, CSA 10: 11.4, CSA 20: 24.7) and urinary 6-keto $PGF_{1\alpha}$ excretion (con: 14 ng/6 hrs, CSA 10: 22.7, CSA 20: 25). Animals treated with meclofenamate prior to CSA 10 had lower RBF than similarly treated controls (4.3 vs. 7.0 ml/min/g, $p<.05$). Pretreatment of animals with captopril did not prevent the fall in RBF by CSA 20 (4.7 vs. 7.1 ml/min/g, $p < .05$) indicating that the CSA-induced decrease in RBF was not directly mediated by increased angiotensin II. In summary, high-dose CSA infusion (CSA 20) in the rat caused renal vasoconstriction accompanied by increases in PRA and prostacyclin production. Although lower doses of CSA (CSA 10) alone did not decrease RBF, RBF did fall after prostaglandin synthesis was inhibited. Renal dysfunction secondary to CSA may be mediated, in part, by decreased RBF, and particularly in patients with limited renal protaglandin production.

PROGNOSTIC VALUE OF PERIPHERAL BLOOD AND RENAL BIOPSY EOSINOPHILIA IN ACUTE RENAL ALLOGRAFT REJECTION. J.N.Posner*, M.R.Weir, M.Hall-Craggs*, S.Shen, S.V.Alongi*, F.J. Dagher*. Transplantation Service, Univ. of Maryland Hospital, Baltimore, MD.

The significance of eosinophilia (E) in the peripheral blood(PB) and allograft biopsy specimens(AB) of renal transplant(TX) recipients undergoing rejection(R) has not been reported. We reviewed the clinical course of 132 consecutive cadaveric and living-related donor TX treated with azathioprine and prednisone over a 6 year period. 150 acute R occurred. PB E($\geq$4% in the peripheral smear)was noted within 3 days of R in 60 episodes of which 25 were irreversible(42%). 90 R occurred without E of which 24 were irreversible(27%). These results barely miss statistical significance but show a strong trend(Fisher's Exact(1 tail)p=.04,(2 tail)p=.08, Chi Square p=.055. Of 124 renal AB revealing acute R, E($\geq$2% E of inflammatory cells)was noted in 8; 6 of these patients lost their kidney. Of 116 AB without E, 43(37%)had irreversible R. These results again barely miss statistical significance. (Fisher's Exact (1 tail)p=.04, (2 tail)p=.4, Chi Square p=.03(Yate's p=.08). All 4 patients with both PB E and AB E lost their kidneys. No other causes for E were identified. We conclude that during acute R, E of either the PB, or AB, or both, is suggestive of a more aggressive form of R with a lower allograft survival rate.

FACTORS CONTRIBUTING TO THE IMPROVED GRAFT SURVIVAL RESULTS IN RECIPIENTS OF CADAVER KIDNEY TRANSPLANTS. K.V. Rao, A. Umen*, R.C. Andersen*. Hennepin County Med. Ctr and Univ. of Minnesota Med. School, Minneapolis, MN.

Using the Cox regression model, we analyzed the "independent effect" of the following risk variables on graft survival in 400 consecutive cadaver transplants (TXs) performed at our center between 1964 and 1983. Recipient's age, sex, race, diabetic status, dialysis duration, previous TXs, blood transfusion, splenectomy, cytotoxic antibodies, HLA mismatches, co-existing medical problems, cold ischemia time, post TX ATN, prophylactic ALG and time periods (period A transplantation before June 1975 and period B, after June 1975). Only conventional immunosuppressive drugs (steroids, Imuran and ALG) were used in both periods. Of the 15 variables screened, only 5 exerted an independent effect ($p < 0.05$). Longer duration of dialysis (relative risk, RR = 1.15/yr) older age (RR = 1.02/yr) and diabetes mellitus (RR = 1.97) had a negative effect. Prophylactic ALG (RR = 0.42) and transplantation in period B (RR = 0.38) had a positive effect. The graft survival rate at one and five years had improved from 52% and 31% respectively in period A to 78% and 64% respectively in period B, after adjusting for the effects of the other 4 significant variables i.e., dialysis duration, age, diabetes and ALG (A vs B, $p = 0.000003$). Perhaps the comprehensive patient care and consistency in the treatment protocols adapted at our center since July 1975 may have played an important role.

RENAL INSUFFICIENCY IN LONG-TERM ALLOGRAFTS: IS INSIDIOUS REJECTION THE ONLY CAUSE? R. R. Riggio, J. S. Cheigh, M. Suthanthiran, R. Haschemeyer,* L. Tapia, W. T. Stubenbord,* and K. H. Stenzel. Rogosin Kidney Center, New York, NY.

The Graft Survival Rate (GSR) for various donor-source allografts at 1 and 10 years, respectively are: Living Related Donors (LRD); two haplotype (2H) matches, 84±0.4% and 62±0.7%; one haplotype (1H) matches, 56±0.3% and 31±0.4%, and cadaver renal transplants (CRT), 42±0.2% and 24±0.2%. The 10 year GSR for those recipients who retained their grafts for at least 1 year: 72±0.8% (2H); 53±0.6% (1H); and 57±0.4% (CRT). Thus graft attrition rates can be seen as a continuum even after the first post-operative year wherein most losses are usually observed. In order to address the question - can the rejection process alone account for these late graft losses or, are other additional factors involved, we examined serum creatinine values (Cr) in long-term survivors (≥10 years) from the time of their discharge from the hospital (D/C) to the present. We postulated that if rejection alone accounted for long-term loss of grafts, then Cr values (mg/dl ± S.D.) at a given point in time (the 10 year value) should be significantly higher in the at-risk group of survivors (CRT>1H>2H). The Cr values observed, however, were nearly identical despite different levels observed at their time of D/C: 1) CRT at D/C, 1.4±0.6%; at 10 years 1.6±0.5%, 2) 1H at D/C, 1.1±0.3%; at 10 years 1.7±0.8%, 3) 2H at D/C, 1.2±0.3%; at 10 years 1.8±0.8%. The findings that renal insufficiency developes at a steady and equal rate in all recipients irrespective of the donor source suggests a role for additional factors (hyperfiltration?, hypertension?, etc.) in the late attrition rate of renal grafts.

LONG TERM FOLLOWUP OF CHILDREN TREATED WITH CONTINUOUS EXCHANGE PLASMAPHERESIS(CEP) FOR ALLOGRAFT REJECTION. Lewis Reisman*, Matthew R. Kaplan, and Wallace W. McCrory. Cornell Univ. Med. College, Div. of Pediatric Nephrology, New York, New York.

Early acute renal allograft rejection with histological evidence of humoral damage is often unresponsive to conventional high dose steroid therapy. CEP remains a controversial mode of therapy although theoretically useful for the removal of circulating antibodies. Followup data on 6 children aged 12-19 years, all recipients of cadaver renal allografts who underwent CEP is presented. The subjects had either first or subsequent episodes of rejection in the first month post transplantation unresponsive to I.V. methylprednisolone therapy. All had percutaneous allograft biopsies which demonstrated primarily humoral rejection. All 6 were treated with a course of high dose steroids (methylprednisolone 10 mg/kg/day x 3 days) together with CEP (4-5 single volume exchanges in a 4-6 day period). All patients responded with decreased serum creatinines to less than 3.0 mg% 1 week after cessation of CEP. Two patients had subsequent rejections unresponsive to steroids and CEP requiring nephrectomies at 50 days and 6 months. Four patients continue to have good long term allograft function (mean serum creatinine 1.9±0.4 mg%, range 1.2-3.2 mg%) at 20-42 months post transplantation. These data suggest that CEP provides a useful adjunctive therapy in selected patients undergoing acute humoral rejection in the first month after transplantation.

NATURE OF CYCLOSPORINE-INDUCED TRANSPLANT TOLERANCE: UNIQUE SUPPRESSOR T CELLS DO NOT EXIST. D.R.Salomon*, L.C.Uhteg*, L.L.Rocher*, J.W.Kupiec-Weglinski*, J.L.Araujo*, M.Rubin*, N.L.Tilney* and C.B.Carpenter . Brigham and Women's Hosp., Boston, MA.

Cyclosporine (CyA) treatment of cardiac allografted rats results in long term graft survival. The mechanism by which tolerance is induced and maintained is unknown. We have studied the relationships between thymic(THY), splenic(SPL), lymph node(LN) and peripheral blood lymphocytes(PBL) as a function of time and immune compartment in CyA-treated tolerant(TOL), untreated acutely rejecting (AR), and naive control animals. Two recipient/allodonor combinations were studied-LEWIS/LBN F1 and WF/LEWIS. PERCOLL continuous density gradients were used for cell separations. Suppressor(Ts) and helper activity(Th) was assessed in 3-party MLR's, and NK activity against Cr-labeled YAC targets. Ts were nylon wool adherent, FcR positive, antigen nonspecific, and stained with anti-T MoAb. Density gradient separations revealed two populations of SPL Ts, high and low density, the latter clearly expanded in TOL and AR compared to controls. Kinetic studies revealed that identical waves of potent PBL and SPL NK activity occur during the first 2 wks following transplantation in both TOL and AR thus excluding early NK as a marker of tolerance. A striking pattern of potent Ts in THY, SPL and LN also occurs in both TOL and AR indicating that the compartmental generation of Ts is not unique to the TOL. In contrast, at 2 wks AR develop a second wave of SPL, LN and THY Th, while TOL still possess potent Ts. Thus a relative lack of Th rather than the presence of a unique or antigen specific Ts marks the tolerant state.

RELATIVE RISK OF FACTORS ASSOCIATED WITH GRAFT AND PATIENT OUTCOME IN RENAL TRANSPLANTATION. Fred Sanfilippo,* William K. Vaughn,* and Everett K. Spees.* S.E.O.P.F., Richmond, Virginia. (Introduced by Vincent W. Dennis)

Data collected prospectively from 1977 through 1982 with subsequent follow-up to 1984 on 3811 of all 3874 cadaveric renal transplants performed by the 42 member institutions of the South-Eastern Organ Procurement Foundation (SEOPF) were analyzed to identify factors associated with graft and patient outcome. Using multivariate analysis (Cox regression), 23 variables were examined for their association with overall graft survival, irreversible rejection, and patient death on an actuarial basis. Factors found to have neither significant nor suggested associations with any outcome included recipient: sex, history of pregnancy, blood group, length of time on dialysis, or most recent PRA; donor/organ: race, preservation method, preservation time or source; or the number of transplants performed by center per year. In decreasing order of relative risk (RR), factors having a significant association with irreversible graft rejection were: loss of 2 or more prior grafts, HLA-A,-B match=0, lack of pretransplant transfusion, highest PRA>60%, delayed graft function, no splenectomy, diabetes, no use of ALS, and non-white recipient. Patient death was significantly associated with splenectomy (RR=1.86, $P < 0.004$), diabetes (RR=1.79, $P < 0.003$), and use of ALS (RR=1.67, $P < 0.001$). The year of transplant was associated with graft and patient outcome, and recipient age was associated with patient survival. Center effects were quantitated by entering each center as a separate covariate in the analysis. These results provide the basis for determining the potential risk of graft rejection or patient death in patients being considered for renal transplantation.

HLA-D MATCHING AND THE OUTCOME OF LIVING-RELATED KIDNEY TRANSPLANTATION (LRKT). S. Shen, R. Welik,* J. Schwartz,* J. Haimes,* D. Evans,* F. Dagher,* Nephrology and Transplant Service, Univ. of Maryland Hosp.; Tissue Typing Lab and Transplant Service, Johns Hopkins Hosp.; Transplant Service, F. Scott Key Med. Ctr., Baltimore, Maryland.

From Oct. 1974 to Nov. 1983 mixed lymphocyte culture (MLC) was done in 139 LRKT without donor specific transfusion (DST) among three transplant centers in Baltimore. To determine the predictive value of HLA-D matching by MLC, we analyze the stimulation index (SI) and the graft (G) outcome on each of these transplant patients (T).

The mean SI among 48 T who did not have any acute rejection (AR) is 6.59 ± 6.83, which is significantly ($p<0.01$, t test) higher than 4.83 ± 5.23 among 91 T who had at least one AR. Neither of them are significantly different from mean SI of 5.28 ± 4.61 among 29 T who lost their G due to AR. Fifteen T lost their G from chronic rejection (CR); their mean SI of 9.08 ± 9.69 is significantly higher than that of T with AR ($p<0.02$) and also higher than those who lost G due to AR ($p<0.05$), but it is not significantly different from T who had no AR. We further analyze the SI with cut-off points at 2, 3, 5, 10 in relation to the incidence of AR, G loss due to AR or CR, and one year G survival by two tails Fisher's exact and X^2 with Yates tests. None of them are found to be significantly related to the G outcome.

We conclude that higher SI is related to G loss from CR, otherwise it is not significantly predictive on the G survival or AR. We do not find any SI cut-off point that is critical for selecting potential donors, or, for recommending DST.

THE PROTECTIVE EFFECT OF VERAPAMIL ON RENAL FUNCTION AFTER COLD ISCHEMIA IN THE ISOLATED PERFUSED RAT KIDNEY. J.I. Shapiro,* C. Cheung,* A. Itabashi,* L. Chan, and R.W. Schrier. Dept. Med. Univ. Colorado Med. Sch., Denver, CO

The isolated perfused rat kidney was used to investigate the effect of verapamil, a calcium channel blocker, on renal function following cold ischemia. Three groups of kidneys were studied: Group 1 was a control group in which the kidney was flushed in situ with Collins C2 solution (K 107, Mg 60, Na 9.3, HPO_4 93, SO_4 60, Cl 14, HCO_3 9.3 mEq/L and glucose 2.5%) at 0°C, removed and stored at 0°C for 8 hr and then perfused with albumin (6.7%) Krebs-Henseleit saline supplemented with 20 amino acids and glucose (5 mM) under constant pressure (100 mmHg at cannula tip) at 37°C for 1 hr. In Group 2, verapamil was added to the flush-solution at a concentration of 2.5 μM. In Group 3, verapamil (2.5 μM) was added to both the flush and perfusion solutions. Results are summarized:

	C_{In} (μl/min/g)	T_{Na} (μmol/min/g)	V (μl/min/g)
Group 1(6)	24±9	2.7±1.1	8±2
Group 2(7)	106±21+	10.7±3.6*	39±8+
Group 3(6)	106±33*	9.3±2.8*	32±10*

(mean ± SEM; *p<.05; +p<.01)

Both verapamil groups 2 and 3 showed significantly higher inulin clearance (C_{In}) net tubular Na reabsorption (T_{Na}), and urine flow rate (V) than the control group 1. Verapamil appears to exert a protective effect on renal function in this model of cold ischemia. Better protection from ischemic injury might be afforded by inclusion of a calcium membrane blocker such as verapamil in a flushing solution for kidney preservation in transplantation.

THE EFFECT OF RENAL TRANSPLANTATION IN INFANCY ON PSYCHOMOTOR DEVELOPMENT. SKS So,* PN Chang,* SM Mauer, RL Simmons, JS Najarian, TE Nevins. Univ. of Minnesota, Depts. of Surgery & Pediatrics, Minneapolis, Minnesota.

Chronic renal insufficiency in infancy can result in progressive encephalopathy which may not be reversed by dialysis or by transplantation (tx) in later life. The effect of tx in infancy on psychomotor development (PMD) has never been reported. We assessed the PMD of 9 infants transplanted between Jan. 1978 and July 1983. Followup ranged from 1-6.5 yrs. The mean age at tx was 7.5 months (6-11 mos.) and the mean weight was 6 kg (5.0-7.7 kg). Six infants were dialysed for a mean of 129 days (68-210 days), 3 were never dialysed. Standard immunosuppression was used. Eight children are alive with functioning grafts (1,1.7,2,2.2,2.6,2.7,3.4,6.5 yr after tx). Serial head circumferences (OFC) and the Bayley Scales of Infant Development were assessed pre- and post-tx. At the time of tx, 5 infants had experienced seizures; 6 were microcephalic; and of 8 infants tested, 6 had abnormal motor or mental functions. After tx, all were seizure-free and off all anticonvulsants. Eight showed dramatic catch-up growth in OFC and 1 had normal growth velocity. PMD was normal or significantly improved in all 6 infants assessed after tx. The 1st infant, now 6.5 yr post-tx, has a normal IQ of 98. Furthermore, normal graft function was associated with accelerated or normal statural growth. This preliminary report suggests that earlier tx might optimize the potential for PMD in uremic infants.

EFFECT OF SINGLE PREDNISOLONE DOSE ON LYMPHOCYTES OF LONG TERM SUCCESSFUL DST PATIENTS. C. Stabile, M. Gumbert, J. Gambertoglio, W. Amend, F. Vincenti J. Melzer, N. Feduska, O. Salvatierra, M.R.Garovoy University of California, San Francisco.

Sensitivity of long term renal transplant recipients to the immunosuppressive action of prednisolone remains a controversial matter. In order to evaluate the pharmocokinetics and immunologic effects of a small dose of prednisolone in chronic immunosuppressed renal recipients we studied 10 DST recipients (5M,5F), age 32.5±4.3 years, 13.2± 2.9 mo. post transplant with serum creatinine at 1.35±0.08 mg%. All patients were receiving prednisone (16.7±2.3 mg/day) and azathioprine. After 24 hours without medication, 0.25mg/kg weight of IV prednisolone was administered to each patient. Total lymph count, Leu4 (Pan T), Leu3 (Helper) and Leu2 (cytotoxic/suppressor) cells from peripheral blood, as well as serum prednisolone levels were determined for the next 10 hours. The mean results were:

	count/mm^3	% Decrease					
Time (hour)	0	1	2	4	6	8	10
Total Lymph	1932	0%	34%	50%	50%	45%	2%
Leu 4	1455	0%	39%	63%	53%	56%	18%
Leu 3	922	4%	52%	72%	70%	66%	19%
Leu 2	681	0%	16%	37%	36%	38%	1%
Pred (ng/ml)	38	432	325	248	166	102	55

These results show that a small dose of IV prednisolone has an effective role in decreasing the peripheral blood lymphocyte count in stable transplant recipients. The helper-inducer subset is particularly susceptible and recovers more slowly from the immunosuppressive effects of prednisolone than the cytotoxic-suppressor cell. Moreover, the immunologic effect persists despite minimal drug levels detectable in the serum.

DETERMINANTS OF PHOSPHATE (P) WASTING IN RENAL TRANSPLANT RECIPIENTS (RTR). R. Steiner*, M. Ziegler*, N. Halasz*, L. Deftos*. University of California at San Diego Medical Center, San Diego, California. Intr. by D. Fanestil.

Renal P wasting was evaluated in 22 stable RTR, 14 on P replacement (PR) with serum creatinine (SC) 1.35±0.48mg% (mean±SD). Of 22 RTR, urine cyclic amp was normal in 20, alkaline phosphatase was normal in 21, and 16 had normal N-terminal PTH (NPTH). NPTH correlated well with mid-molecule PTH (MPTH, $p<.001$) and C-terminal PTH (CPTH, $p<.001$), respectively, yet values for MPTH & CPTH were uniformly elevated. Each of the 3 PTH assays correlated ($p<.05$) inversely with serum P and directly with PR and fractional excretion (FE) of P. SC correlated with MPTH (p=.004) and CPTH (p=.030), but not NPTH (p=.274), serum P (p=0.34), or FEP (p=.264). 1-25 dihydroxycholecalciferol levels were normal or elevated and unrelated to other study variables. Serum calcium (CA) correlated inversely with both FEP (p=.045) and prednisone (steroid) dosage (DS, p=.034). DS correlated directly with NPTH, MPTH (p=.01) and CPTH (p=.031).

	P	FEP	PR	CA	DS
Mean± SD	3±0.7 mg%	.45±.41	2.1±2.1 gm/d	9.7±0.6 mg.%	0.27±.14 mg/kg/d
p(vs NPTH)	.036	.003	.017	.063	.043

Contrary to many reports, P wasting in RTR can be shown to be influenced by PTH, as well as CA and DS, even in RTR who do not appear to be hyperparathyroid.

HYPERCHLOREMIC METABOLIC ACIDOSIS WITH HIGH SERUM POTASSIUM: CYCLOSPORINE A (CyA) ASSOCIATED SIDE EFFECT IN PATIENTS AFTER KIDNEY TRANSPLANTATION? RAK Stahl, L Kanz, B Maier, P Schollmeyer, Dept. of Medicine, Univ. of Freiburg, FRG.

In 4 out of 13 patients treated with CyA and prednisolone following renal transplantation hyperchloremic metabolic acidosis (MA) with high serum potassium were observed. Serum levels (mEq/l) of potassium (5.13±0.1), chloride (116±1.0) and bicarbonate (17,5±1) were significantly different from those patients on CyA who did not have MA (K^+:4.42±0.1; Cl^-:110±0.6; HCO_3^-:25±1.0). Urine pH in all 4 patients was <5.5. CyA serum levels were higher (740±132 ng/ml) compared to controls (493±60). Glomerular filtration rate in patients with MA was lower compared to controls. MA was no uremic acidosis since the anion gap was 9.4±0.8 mEq/l. Histologic evaluation of renal tissue from 2 patients revealed CyA associated vasculopathy. In these patients plasma-renin-activity and serum aldosterone levels were in the subnormal range. Reduction of CyA levels in patients with MA (390±10) increased GFR from 34±10 to 51±10 ml/min, however, MA improved only moderately (K^+:4.8±0.2, Cl^-:112±1.2, HCO_3^-:20.4±2.2) We conclude that CyA induces tubular dysfunction in some patients after renal transplantation, which results in hyperchloremic metabolic acidosis.

GRAFT TOLERANCE PRODUCED AN ANTI-INTERLEUKIN-2 RECEPTOR MONOCLONAL ANTIBODY. Terry B. Strom, Armelle Ythier,* Vicki E. Kelley, Glen Gaulton,* and Robert L. Kirkman.* Harvard Med. Sch., Beth Israel & Brigham & Women's Hosp., Depts. of Med., Surg. & Path., Boston, MA

As all recently activated, dividing, but not resting or memory, T-cells express interleukin-2 (IL-2) receptors, this "activation antigen" offers an attractive target for therapy in transplantation. A panel of monoclonal antibodies (MAb) were made in rats in response to immunization with murine cytotoxic T lymphocytes (CTL). The MAb was tested for its capacity to bind to ConA blast and resting T cells by flow cytometry. The MAb binds to activated, but not resting, T cells. MAb M7/20 totally prevented IL-2 directed DNA synthesis of an IL-2 dependent CTL line. The purified MAb competitively inhibited binding of radiolabel IL-2 to the CTL line with a Kd of 1.2×10^{-9}M. M7/20 precipitated an N-glycosylated 58,000 dalton glycoprotein from activated T cells. These data indicate that M7/20 binds to murine IL-2 receptors at or near the IL-2 binding epitope. Administration of 5 µg i.p./day x10 of M7/20 to BIO.AKM mouse recipients of H-2 incompatible C57B1/6 heterotopic heart grafts caused indefinite survival of 4 of 5 test grafts, the other graft was rejected on day 18 while control, untreated recipients rejected grafts on days 7,7,8,8 and 15. The dramatic effects of the anti-IL-2 receptor MAb suggest that all alloreactive clones have been destroyed.

ACTIVATION OF ALLOIMMUNE MEMORY CELLS BY RECOMBINANT INTERLEUKIN 2 (rIL-2): A MODEL FOR ELICITATION OF SPECIFIC ALLOIMMUNITY BY IMMUNOLOGICALLY NON-SPECIFIC SIGNALS.

M. Suthanthiran and K.H. Stenzel, Rogosin Institute The New York Hospital-Cornell Medical Center, New York, New York.

We investigated the ability of rIL-2 to activate antigen-primed memory cells generated in primary long-term MLCs and examined the effects of cyclosporine (CSA) or methylprednisolone (MP) on rIL-2 or alloantigen associated activation of memory cells. While rIL-2 failed to activate unprimed cells, exposure of memory cells to rIL-2 resulted in significant proliferation and generation of specific secondary cytotoxic T cells(CTL). ^{3}H-thymidine incorporation icreased from 2298±663 CPM/culture (MEAN±SE,N=4) to 68983±9514 CPM/culture($p < .01$) when memory cells were incubated with 100units/ml of rIL-2. Percent specific chromium release (SCR) increased from 4±2% to 46±4% ($p < .005$) when memory cells activated with rIL-2 were effector cells and specific allogeneic cells were target cells. The cytolytic activity exhibited by CTL was specific for the original priming stimulus. Percent SCR was 0.2±0.2% and 2.5±1.5% (p=NS) when memory cells incubated with syngeneic cells or rIL-2 were effector cells, respectively and syngeneic cells were target cells. CSA (250ng/ml) or MP (500ng/ml) mediated minimal inhibition (20%) of rIL-2 associated activation, and marked inhibition (50-90%) of alloantigen associated activation of memory cells. Our findings, besides indicating a pathway by which immunologically non-specific signals can elicit specific alloimmunity, indicate a signal dependent inhibitory capacity for currently used immunosuppressants.

THE SPECTRUM OF HUMORAL REJECTION: CLINICO-PATHOLOGIC CORRELATION: Luis Tapia, Janet Morodian*, Annie Strup*, Jhoong Cheigh, Robert Riggio, and Kurt Stenzel. Rogosin Kidney Center, New York Hosp. Cornell University Medical College, New York, N.Y.

One hundred and four transplant renal biopsies, in 94 patients, done during an episode of Acute Rejection were evaluated. Glomerular, interstitial and vascular lesions, were rated according to their severity, from 0 to 10. Thus, 30 represent the maximal degree of severity of the three lesions. Seventy four (79%) of the biopsied patients lost their graft, despite solumedrol "pulse" with or without plasmapheresis treatment. Twenty patients (21%). 10 living related and 10 cadaver donors, responded to treatment and had a functioning graft up to the end of this study. The overall degree of severity was greater in the group that lost their graft (16.3± 6.7) Vs the group that had a functioning graft at the end of the study (13.8 ± 3.9) (P= <.02). Although the severity index in the interstitum was significantly higher than the glomerular and vascular, there was no statistical difference between the two groups. On the other hand the glomerular and vascular lesions were significantly greater in the group that lost their graft.

	Glomerular	Interstitial	Vascular
Functioning	2.7 ± 1.9	7.8 ± 2.0	3.7 ± 2.5
Lost graft	4.7 ± 2.9	7.6 ± 2.5	5.0 ± 3.2
("t" test) P=	<.005	N.S.	<.005

Clinical manifestation (ΔCr., fever, tender graft, etc...) or response to treatment can not distinguish between cellular and humoral rejection. Thus the vascular lesions detailed by renal biopsy are the best predictors of the severity of humoral rejection.

FATE OF TRANSPLANTED CADAVERIC KIDNEYS (K) WITH MULPTIPLE VESSELS. M.B.Tallent, M.D., TC Krueger, M.D., C.M.Ynares, M.D., H.E.Warner, B.S., H.K.Johnson, M.D., R.M. MacDonell, M.D., R.E.Richie, M.D., G. Niblack, Ph.D., W. Green, Ph.D. , Nashville, Tn.

Many centers are reluctant to accept shared K with multiple vessels. Between Dec.1978 & April 1984, 586 K were transplanted by our center. Fifty-five of these were cadaver K with multiple vessels. Forty-three had 2 arteries & 12 had 3 arteries. Forty of these K had all vessels on a single cuff of donor aorta. Arterial diameters ranged from 1-7 mm. Eleven K were preserved with pulsatile perfusion. There were 2 lost to hyperacute rejection, 1 lost to bleeding from a disrupted anastomosis of a 1 mm artery,& 1 lost to an ischemic ureter that developed 2 mo. post-op. The ATN rate was 44% compared to 50% for the series overall. Renal artery stenosis developed in 5% (3/55) compared to 2.6% in the total series. All 3 K were machine preserved without a cuff of donor aorta. At a follw-up time of 4 mo. to 51/2 yr, 62% are functioning. The presence of multiple vessels on a shared donor K is not a contraindication to transplant. Our results are comparable to single artery K.

CYCLOSPORINE (CY) AS THE SOLE MAINTENANCE IMMUNOSUPPRESSIVE IN CHILDREN WITH RENAL TRANSPLANTS. Amir Tejani, Khalid Butt, Howard Trachtman*, Kishore Phadke*, Orlando Adamson*. DMC, S.U.N.Y. Brooklyn, N. Y., Depts. of Peds. and Surgery.

Transplanted children with a good functioning graft continue to show growth inhibition even with low dose prednisone (P). We have utilized the steroid sparing effect of CY by attempting to discontinue P after induction of graft tolerance in 23 children. Fifteen (Group A) started with CY and 0.5 mg/kg P at the time of transplant. Eight children with stable renal function (Group B) were switched to CY from Imuran. In both groups, P was tapered with complete withdrawal in 16-20 weeks. Results: (1) In 5 children P could not be reduced below 0.2-0.4 mg/kg without raising the serum creatinine (SCR) unacceptably. (2) In 10 children P is still being reduced and ranges from 0.1 mg/kg to 0.3 mg/kg. (3) In 8 children (4 cadaveric, 4 parent) P has been withdrawn completely for 1-11 months. Their mean SCR is 1.2 mg/dl (0.7-2.3 mg/dl), the higher values being noted in Group B. Serum cortisol level measured in these patients after ACTH stimulation showed a 2 1/2 fold rise from base line (normal 2 fold). Growth hormone levels measured in 4 pre-pubertal children off P, with methylodopa stimulation showed a peak level of 27.5 ng/ml from 2.5 ng/ml (normal >7 ng/ml). Our study shows that (1) withdrawal of P is possible with cadaveric as well as live related grafts. (2) Withdrawal is tolerated better in patients started on CY then in those who are switched to CY later and (3) Patients whose P has been discontinued have enough endogenous cortisol to prevent Addisonian crisis, and have adequate growth hormone levels.

DIAGNOSIS OF TRANSPLANT REJECTION AND CYCLOSPORIN TOXICITY BY URINARY ASSAY FOR PROXIMAL TUBULAR (PT) ANTIGEN (Ag). N.E.Tolkoff-Rubin, A.B. Cosimi*, F.L. Delmonico*, P.S. Russell*, R.E. Thompson*, D.J. Piper*, N.H. Bander*, L.J. Old*, L.H. Klotz*, W.P. Hansen* and R.H. Rubin*. Mass. General Hospital and Cambridge Research Lab., Boston, MA and Memorial Sloan-Kettering Cancer Center, New York, NY.

Two murine monoclonal antibodies (URO-4 and URO-4a) which detect different epitopes of a PT glycoprotein, the adenosine deaminase binding protein, have been formatted into a sandwich enzyme immunoassay for detection of the PT Ag in urine. Serial urines from 34 renal transplant patients during the first 6 months post-transplant were analyzed to determine the correlation of this test with clinical rejection and cyclosporin (CyA) toxicity.

In 29/29 acute rejection episodes the PT Ag was elevated, beginning 1-7 days prior to treatment of rejection. Eighteen patients were treated for rejection with courses of OKT3 (13) or anti-thymocyte globulin (5): 0/6 whose PT Ag level fell to normal during therapy had re-rejection; 10/12 whose PT Ag level remained elevated during therapy had re-rejection within 7 days of therapy completion. Of 15 patients treated with CyA, 7 had no rejection or drug toxicity; all 7 had normal PT Ag levels. The remaining 8 had CyA nephrotoxicity as characterized by increased blood levels, elevated serum creatinines, and fall in the serum creatinine with decrease in CyA dose. All 8 had elevated PT Ag levels in association with toxicity that fell to normal levels with change in dosage.

We conclude that serial performance of this assay on urines from transplant patients provides information useful for the diagnosis and prevention of rejection and cyclosporin toxicity.

TECHNETIUM-99M TIN COLLOID - ITS VALUE IN THE DIAGNOSIS OF RENAL TRANSPLANT REJECTION AND ITS POTENTIAL FOR DIFFERENTIATING CYCLOSPORIN NEPHROTOXICITY. *E.C.R. Wijeyesinghe*, T. Hawkins*, P. Keavey*, R. Wilkinson*. Depts. of Nephrology and Nuclear Medicine, Freeman Hospital, Newcastle upon Tyne, U.K. (introduced by C. Whiteside)*

A rising creatinine post transplant commonly suggests rejection although in patients on Cyclosporin it could also be due to nephrotoxicity.

There have been previous reports that the accumulation of Technetium sulphur colloid is of value in the diagnosis of renal transplant rejection.

We evaluated the accumulation of intravenously administered Technetium Tin Colloid (Tc-99mTC) in the context of a rising creatinine occurring more than 1 month after grafting in 14 patients. 3 patients with normal creatinines who were at similar intervals post transplant to 3 in the former group were also studied.

A quantitative method was used which related the accumulated activity at 12-16 min. post injection (X) to the initial activity in the graft over 0-4 min. (Y). An index range of 64-172% was obtained in those patients in the test group with adequately perfused kidneys in whom rejection was diagnosed on the basis of clinical and/or biopsy evidence (7/8). The 3 'normals' and 4 patients whose rising creatinine was due to causes other than rejection had an index ranging from 5-45%. The latter included 1 patient on Cyclosporin who suffered a clinically proven rejection episode at a later date at which time his index became elevated.

This study suggests that Tc-99mTC may help not only in diagnosing graft rejection but also in differentiating Cyclosporin nephrotoxicity from rejection in the event of a rise in creatinine.

AUTOPSY FINDINGS IN RENAL TRANSPLANTATION: A PATHOLOGICAL STUDY OF PATIENTS ON CYCLOSPORINE AND ON AZATHIOPRINE. Elaine Wagner* and Regina Verani, Dept. of Pathology, The Univ. of Texas Medical School, Houston, Texas.

The objective of this study was to compare the autopsy findings in renal transplant patients (pts) treated with cyclosporine (CsA) and Azathioprine (Aza). From 1978 to 4/1984, 145 renal transplant pts have been treated with Aza and 260 with CsA at the UTMSH. During this period 29 autopsies (10 CsA and 19 Aza) were performed. The time of death post-transplant was 1 to 4 mo (mean 2 mo) for the CsA and 1 to 46 mo (mean 10 mo) for the Aza treated pts. Pneumonia was observed in 22/23 cases with infection. Bacterial infection (BI) was present in 26/29 cases (17/19 on Aza and 9/10 on CsA). Fungal infection (FI) was present in 10/29 cases (9/19 on Aza and 1/10 on CsA). CMV inclusions were observed in 5/19 cases on Aza. One pt on Aza had a Strongyloidiasis. Infection was the cause of death in 17/19 Aza and 6/10 CsA pts. Other causes of death were cardiovascular (2 CsA pts), intracerebral hemorrhage (1 Aza and 1 CsA pt), GI bleeding (1 Aza pt) and pelvic hemorrhage (1 CsA pt). The two pts on CsA with a cardiac death had heart weight of 800 gm. Acute humoral rejection was seen in 1/10 CsA and 2/19 Aza chronic rejection in 1/10 CsA and in 10/19 Aza, perinephric hematoma in 2 CsA and perinephric abscess in 1 Csa. We concluded that pts on CsA died earlier in the post-transplant period than the pts on Aza therapy. FI was more frequent in Aza pts (47%) than on CsA pts (10%), however the incidence of BI was similar in both groups. Infection as the cause of death was more common in Aza treated pts (89%) than on CsA pts (60%).

LONG TERM EFFECTS OF KIDNEY DONATION: A SIBLING STUDY. S. Williams* and D. Jorkasky. Dept. of Med. Univ. of Penn. Sch. of Med., Phila., Pa.

Hypertension is reported as a sequala of renal donation. To study familial factors which may account for this result, we evaluated blood pressures (BP) and renal function in 38 donors who underwent nephrectomy more than 10 years ago (mean: 13 years, range 10-17 years), 16 of whom were compared to a group of their healthy, unaffected siblings. All donors were also compared to age, sex, race matched controls (D=diastolic; mean±1SD):

	n	Mean D BP	Mean BP	n	Mean D BP	Mean BP
donors	16	87±10	103±11	38	86±10	102±10
siblings	16	81±14	96±13	-	-	-
controls	-	-	-	38	80±10	97±11
p		<0.05	<0.01		<0.05	<0.005

Seventy-five percent of the potential number of donors and 59% of the available number of siblings were studied. The age and sex distributions of subjects in each group were comparable. The prevalence of diastolic BP≥90 mm Hg was the same in each group (Chi Square). Current donor creatinine clearances were 80% of available predonation values and of current sibling creatinine clearances. Urinary protein excretion (mg/24 hrs; mean±1SD) was significantly higher in male donors (186±152; p<0.001) and in female donors (80±77; p<0.05) than in both male and female siblings (36±31).

We conclude that 13 years after uninephrectomy kidney donors have higher blood pressures and more proteinuria than either sibling or population controls. The clinical significance of these findings is unknown, but renal function as measured by creatinine clearance is unaffected.

CARCINOEMBRYONIC (CEA) ANTIGEN IN HEMODIALYZED AND IN SUCCESSFULLY TRANSPLANTED (Tx) INDIVIDUALS.
N. Zerefos, E Koulentianos, J Alexopoulos, A Sahin, H Stathakis, A Agrafiotis and G. Daikos, University of Athens Medical School, 1st Department of Propedeutic Medicine, Laikon Hospital, Athens, Greece.

CEA levels were measured by radioimmunassay in plasma from 42 patients on regular chronic hemodialysis (C.H.D.) and 22 Tx individuals (Creatinine clearance >60 ml/min, 6-48 months folloroing renal transplantation (R.T.). They were compaired to 90 trealthy controls (H.C.). Smokers as well as pts with cirrhosis, diverticulitis, pancreatitis and emphysema were excluded from this study. CEA levels were elevated in 73% of CHD pts, in 3% of the healthy controls, but normal in all successfully Tx individuals.

The mean CEA levels among CHD pts, Tx individuals and H.C. were:
10.80 ng/ml (S.D. 7.45), 3.02 (S.D. 4.02) and 3.21 ng/ml (S.D. 1.07) respectively. Paired t-test evaluation showed a significant difference between CHD pts and HC ($t=9.31$, $p<0.001$) as well as between CHD pats and Tx individuals $t=4.34$, $p<0.001$. There was no statistically significant difference between Tx and HC.

We conclude that successful R.T. normalizes the high levels of CEA observed in CHD pts, thus suggesting a significant renal contribution in the metabolism of the above antigen.

Clinical Nephrology (continued)

EVALUATION OF ASYMPTOMATIC RENAL VEIN THROMBOSIS IN PATIENTS WITH IDIOPATHIC NEPHROTIC SYNDROME.
Francisco Velasquez-Forero, Nestor Garcia-Prugue. Centro Hospitalario 20 De Noviembre, ISSSTE, Dept. of Pathology & Nephrology, Mexico, D.F.

In the recent past, scientists have observed that patients with nephrotic syndrome have a concomitant hypercoagulability state. With this in mind, we conducted, in otherwise asymptomatic patients, a prospective study in order to evaluate the incidence of asymptomatic renal vein thrombosis (ARVT) in these patients. We used inferior venocavograms in 19 adult patients with Idiopathic Nephrotic Syndrome. These patients were negative by P.E., laboratory studies and kidney biopsies. From the cavograms, we found asymptomatic renal vein thrombosis present in eight of the nineteen patients corresponding to 42 percent incidence. In two of these patients, the thrombosis was bilateral, while in 6 patients it was unilateral. Membranoproliferative glomerulomephritis was the most frequent cause of Idiopathic Nephrotic Syndrome, and Membranous glomerulonephritis was most commonly associated with renal vein thrombosis. We conclude that asymptomatic renal vein thrombosis with its concomitant hypercoagulability state is a much more frequent complication of nephrotic syndrome than has previously been reported.

Immunology and Pathology—Basic (continued)

LOW PROTEIN DIET PREVENTS AND HIGH PROTEIN DIET EXACERBATES EXPERIMENTAL NEPHROSIS.
Giuseppe Remuzzi, Carla Zoja, Andrea Remuzzi, Magda Rossini, Cristina Battaglia, Tullio Berteni. Mario Negri Institute for Pharmacological Research, Via Gavazzeni 11, Bergamo, Italy.

Adriamycin (ADR) induces in rats a nephrotic syndrome (NS) with persistent proteinuria which develops 13-15 days after a single i.v. injection (5 mg/kg). Electron microscopy (EM) shows alterations of glomerular visceral epithelial cell with foot processes fusions. The disease resembles minimal change nephropathy in humans. We studied the effect of dietary manipulation on proteinuria, inulin and p-aminohippuric acid clearances and glomerular morphology in ADR nephrosis. Group 1 rats (n 10) received ADR and were fed a standard diet. Group 2 (n 10) were fed a low protein diet starting 7 days before ADR. Group 3 (n 5) were fed a low protein diet starting the day after ADR. Group 4 (n 10) were fed a high protein diet starting 7 days before ADR. Group 5 (n 5) served as control. Proteinuria in rats on low protein diet was lower than in rats on standard diet ($p<.001$), whereas rats on high protein had more proteinuria than those on standard diet ($p<.001$). GFR and RPF were both higher in group 4 ($p<.05$) and ($p<.001$) in respect to group 1 and 5. An increase in RPF ($p<.01$) but not in GFR was also observed in group 2.

days after ADR	PROTEIN EXCRETION* 14th	21st	28th	GFR** 28th	RPF** 28th
Group 1	199±61	398 ±112	607±136	0.76±0.24	2.14 ±0.37
Group 2	6± 2	6 ± 2	8± 3	0.95±0.21	3.82 ±0.75
Group 3	5± 3	6 ± 4	7± 4	---	---
Group 4	378±92	838 ± 177	1122 ±195	1.24±0.32	4.33 ± 0.92
Group 5	6± 4	8 ± 4	8 ± 5	0.74±0.12	2.42± 0.55

*(mg/day); **(ml/min/100 g).

EM showed the usual epithelial cell abnormalities in group 1, more severe damage in group 4, no alterations in group 2, 3 and 5. We conclude that low protein diet has favorable effect on the evolution of NS.