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Abstracts

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PARATHYROID HORMONE (PTH) RESPONSE TO HYPOCALCEMIA (CA↓) IN DIALYSIS OSTEOMALACIA (OM). D. Andress*, A. Felsenfeld, A. Voigts*, and F. Llach. Dept of Med., Univ. of Okla. Health Sci. Ctr., and VA Med. Ctr., Oklahoma City, Okla.

Dialysis patients (pts) with OM generally have low PTH levels and aluminum (AL) toxicity. To study PTH response, 8 dialysis pts with OM and 7 pts with osteitis fibrosa (OF) had a zero calcium (Ca) dialysis for 150 min or until Ca was below 7 mg/dl. Blood samples were obtained at baseline and every 30 min. Osteoblastic osteoid, active resorption, osteoclasts/mm^2, and endosteal fibrosis were greater in OF pts ($p<.005$). The Ca↓ was greater in OM pts at all intervals. The peak amino (N) PTH response was greater in OF ($0.86\pm.38$ vs $0.39\pm.33$ ng/ml±SD, $p<.025$). NPTH failed to increase in 4 OM pts while all OF pts increased NPTH by >0.2 ng/ml. Carboxy (C) PTH failed to increase in 4 OM pts. At the nadir of plasma Ca, NPTH was less than peak response in 6 OM and 6 OF pts. Similarly, CPTH was less than peak response in 4 OM and 6 OF pts. In OM pts, the peak ΔCPTH correlated with bone resorption ($p<.001$) and osteoclasts/mm^2 ($p<.05$); and the surface density of trabecular bone AL directly correlated with relative osteoid volume ($p<.005$) and inversely with the peak NPTH response ($p<.025$).

In summary, 1) the Ca↓ is greater in OM: 2) the peak NPTH response is less in OM; 3) the peak CPTH and NPTH response often occurs prior to the nadir of plasma Ca; and 4) in OM, the peak ΔCPTH correlated with bone resorption and osteoclasts/mm^2 and the peak NPTH inversely correlated with trabecular bone AL. In conclusion: 1) OM pts fail to appropriately increase PTH secretion with Ca↓; 2) OM pts may be unable to mobilize Ca from bone: and 3) the AL burden may diminish PTH secretion.

MECHANISM OF DOPAMINE (DA) INDUCED PHOSPHATURIA IN PERFUSED KIDNEYS FROM PHOSPHATE (Pi) DEPLETED RATS. A. D. Baines, R. Drangova*, H. James* and P. Ho* Univ. of Toronto, Dept. of Clinical Biochem. Toronto, Ont. Canada.

Pharmacological doses of DA are phosphaturic in men and dogs. To obtain evidence of a physiological role for DA in Pi excretion we examined (1) DA production by Pi-depleted (3-6 days 0.07% Pi diet) and Pi-replete rat kidneys and (2) the effects of exogenous DA on Pi excretion. Isolated perfused replete kidneys produced 3±1 ng/min of DA in the period from 20 to 50 min. At the same time depleted kidneys produced 0.5±0.1 ng/min ($p<0.001$). DA (6×10^{-8} M) added to the perfusates at 50 min produced phosphaturia, ($p<0.01$) natriuresis and increased free water formation ($p<0.02$) in replete kidneys but only phosphaturia in depleted kidneys ($p<0.01$). DOPA (10^{-7} M) was also phosphaturic in depleted kidneys. Haloperidol 10^{-4} M, inhibited the phosphaturic action of DA and DOPA. PTH (0.3 U/ml) was phosphaturic in replete but not in depleted kidneys & increased cAMP excretion in both. DA did not increase cAMP excretion. We conclude that phosphate depletion is associated with reduced renal DA production from endogenous renal sources; addition of exogenous DA, or a DA precursor, to these kidneys inhibits phosphate but not sodium reabsorption by acting on an adenyl cyclase independent dopamine receptor distinct from PTH receptors.

IN VITRO MODULATION OF RENAL 25-HYDROXYVITAMIN D_3 METABOLISM BY $1,25\text{-}(OH)_2\text{-}D_3$ AND CALCIUM. H.J. Armbrecht,* N. Wongsurawat,* T.V. Zenser,* and B.B. Davis. Geriatric Ctr., St. Louis VA Med. Ctr., St. Louis, Missouri

It has been shown that $1,25\text{-}(OH)_2\text{-}D_3$ and dietary Ca modulate renal metabolism of $25\text{-}OH\text{-}D_3$ to $1,25\text{-}(OH)_2\text{-}D_3$ and $24,25\text{-}(OH)_2\text{-}D_3$ in the rat. However, it is not known if $1,25\text{-}(OH)_2\text{-}D_3$ and Ca act directly on the kidney to modulate $25\text{-}OH\text{-}D_3$ metabolism or indirectly through other mechanisms such as the modulation of parathyroid hormone secretion. Therefore, we have used isolated renal cortical slices from the rat to study the effect of $1,25\text{-}(OH)_2\text{-}D_3$ and Ca in vitro on renal $25\text{-}OH\text{-}D_3$ metabolism. Incubation of renal slices from rats fed a vitamin D-deficient, low Ca diet with 50 nM $1,25\text{-}(OH)_2\text{-}D_3$ for 3 hr resulted in a 40% decrease in $1,25\text{-}(OH)_2\text{-}D_3$ production and a 120% increase in $24,25\text{-}(OH)_2\text{-}D_3$ production. Increasing media Ca concentration from 0.5 to 1.25 mM resulted in a 44% decrease in $1,25\text{-}(OH)_2\text{-}D_3$ production but no change in $24,25\text{-}(OH)_2\text{-}D_3$ production. The inhibitory effect of $1,25\text{-}(OH)_2\text{-}D_3$ was blocked by cycloheximide, but the inhibitory effect of Ca was not blocked by cycloheximide. Renal $1,25\text{-}(OH)_2\text{-}D_3$ production was inhibited to a greater extent by incubation with $1,25\text{-}(OH)_2\text{-}D_3$ and Ca together than by incubation with $1,25\text{-}(OH)_2\text{-}D_3$ and Ca separately. These studies indicate that $1,25\text{-}(OH)_2\text{-}D_3$ and Ca act directly on the kidney to modulate renal $25\text{-}OH\text{-}D_3$ metabolism. They also suggest that the mechanism of modulation is different for each agent.

RENAL PHOSPHORUS (P) WASTING IN THE ELDERLY BY PARATHYROID HORMONE (PTH) INDEPENDENT MECHANISM: RENAL BRUSH BORDER MEMBRANE (BBM) P TRANSPORT. N. Beck and D.B.N. Lee. Long Beach VA Hospital, Univ of California, Irvine & UC Los Angeles, CA.

In the elderly, PTH is elevated, and P as well as Ca metabolism is altered (e.g. senile osteoporosis). Since kidney is a major organ involved in the regulation of P homeostasis, renal P metabolism was investigated in senescent Fischer 344 rats: 12 vs 28 month old rats (MOR).

In pair-fed animals with intact PTH glands, urinary P excretion was increased in 28 MOR: 19.5±1.9 vs 35.0±2.5 μmol/day, $p<0.01$; suggesting renal P wasting in the elderly. In TPTXed rats, the fractional excretion of P remained higher in 28 MOR: FEp, 0.57±0.04 vs 19.38±3.05%, $p<0.01$; suggesting that the renal P wasting was independent of PTH. Urinary cyclic AMP excretion in response to 5 U of PTH was decreased in 28 MOR: 722±108 vs 363±39 pmol/min, $p<0.01$; suggesting that the renal P wasting in the elderly is not at or before the step of cyclic AMP generation.

The biochemical mechanism of the renal P wasting was further investigated at a step distal to cyclic AMP generation. P uptake by renal BBM vesicles was significantly decreased in 28 MOR: 1341±119 vs 713±81 p mol P-uptake/mg protein/2 min, $p<0.01$; while alkaline phosphatase activity in BBM vesicles was not different: 28.1±4.0 vs 24.4±3.4 μmol PNP/mg protein/30 min, $p>0.05$.

These results suggest that the renal P wasting in the elderly is by PTH-independent mechanism, and it is at least in part at the step of renal brush border membrane P transport.

CHARACTERIZATION OF A PHOSPHATE BINDING PROTEIN IN RAT RENAL BRUSH BORDER MEMBRANES. Richard Béliveau* and Michèle G. Brunette, Research Center Maisonneuve-Rosemont Hospital, University of Montreal, Mtl, Quebec, Canada.

The luminal membrane of the proximal convoluted tubule is critical for phosphate ion reabsorption. The transport kinetics and bioenergetics are rather well understood phenomena, but the nature of the molecular events implied remains obscure. In search of the molecular nature of the phosphate carrier, we studied the binding of inorganic phosphate on isolated brush border membrane (BBM) from rat kidney cortex. Incubation of rat renal brush border membranes with ^{32}P orthophosphate reveals a single radioactive band when membrane proteins are separated by SDS polyacrylamide gel electrophoresis. The same protein can also be specifically labelled by γ-^{32}P ATP if the temperature is lowered to 0°C or magnesium ions are deleted from the medium. The pH profile shows a maximum incorporation at pH 6.5, but half of this incorporation is still evident under alkaline conditions. Lineweaver-Burk plots revealed two different binding Km's, one at 50 uM obtained with low Pi concentration and one at 280 uM obtained with high Pi concentration. The bound phosphate is completely exchangeable with phosphate in the medium. Phosphate binding and phosphate release are completed within 5 seconds. Bromotetramisole, a specific inhibitor of alkaline phosphatase, blocks Pi binding, but does not release the phosphate already bound. A 6.5 to 9.4 pH transition results in the release of half of the bound phosphate, while bromotetramisole fails to affect this release. According to our results, it is suggested that this phosphate binding protein is alkaline phosphatase.

SITE OF INHIBITION OF PHOSPHATE REABSORPTION BY CALCITONIN. T. J. Berndt* and F. G. Knox, Dept. of Physiology, Mayo Clinic and Foundation, Rochester, Minnesota

Calcitonin is phosphaturic in the rat. Since calcitonin-sensitive adenylate cyclase is limited to the distal portion of the rat nephron, we evaluated whether calcitonin-induced inhibition of phosphate reabsorption occurs exclusively along the distal tubule, consistent with the site of stimulation of adenylate cyclase. Clearance and free-flow micropuncture samples were collected from thyroparathyroidectomized rats fed a normal phosphate diet (0.7%). Following control collections, salmon calcitonin (9.9 U/kg & 0.3 U/kg/min) was infused for 1 hr and recollection micropuncture was performed (n = 6).

	FRACTIONAL DELIVERY OF PHOSPHATE %			
	LP	ED	LD	Urine
Control	23±4	8±3	3±1	2±1
Calcitonin	63±5†	43±5†	29±6†	26±4†

†significant from control; LP = late proximal; ED = early distal; LD = late distal

Calcitonin markedly increased delivery of phosphate to the late proximal tubule. There was continued phosphate reabsorption in the pars recta and distal nephron. We conclude that calcitonin inhibits phosphate reabsorption in the proximal rather than distal tubule.

SILICON CONCENTRATIONS IN THE BRAIN MITOCHONDRIA OR NORMAL AND UREMIC RATS. Berlyne GM, Adler AJ, Caruso C.*Brooklyn Veterans Administration Medical Center, Brooklyn, NY 11209, USA.

Silicon was measured in pure mitochondria separated from the whole brain of Sprague Dawley male rats using a flameless furnace atomic-absorption technique. The rats were divided into two groups; (1)normal rats and (2)chronically uremic rats which had undergone 5/6 nephrectomy four weeks earlier and had an elevated BUN. Mitochondria were separated by differential centrifugation using density gradients and purity confirmed by enzyme studies and by electron microscopy. Results were expressed as µg/mg of protein.

Mitochondrial Silicon Content (µg/mg of protein)

Group:	Normal	Chronic Uremic
No.	10	8
Mean	6.59	9.16*
SD	±1.25	±2.00

* $2p < .001$

There was a significant increase in the silicon content of mitochondria in uremia ($2p < .001$); on a molar basis there was more silicon than calcium in mitochondria. It is likely that silicon in the mitochondria is related to the presence of calcium in a similar fashion to the high Si/Ca ratio found in growing regions of chick periosteal bone.

CONCLUSION

Silicon plasma levels are elevated in uremia, so that it is likely that intracellular silicon and intramitochondrial silicon elevations are a function of raised ECF silicon concentrations in chronic renal failure.

CALCIUM ABSORPTION BY PROXIMAL TUBULE STIMULATED BY REDUCING LUMINAL SODIUM CONCENTRATION. Karol Bomsztyk,* and Fred S. Wright. Yale Univ. School of Med. and VA Med. Ctr., New Haven, CT.

To determine whether proximal absorption of Ca and Na are necessarily linked, we perfused surface tubules of anesthetized rats in vivo at 20 nl/min with four solutions: I, contained (in mM) 147 Na, 4 K, 1.5 Ca, 143 Cl, 10 HCO_3 and mannitol to make net fluid absorption zero; in II and III, Na was partially replaced by TMA; in IV, by Li. Measuring total Ca conc. ([Ca], mM) by atomic absorption, Ca activity by liquid ion exchanger microelectrodes, and transepithelial voltage (V_{TE}, mV) by 3M KCl microelectrodes, permitted calculation of net Ca absorption (J_{Ca}, pmol/min·mm), free Ca ion conc. ($[Ca^{2+}]$, mM), and transepithelial electrochemical potential difference ($\Delta\tilde{c}_{Ca}$, mM).

Soln.	Na/Cation	$[Ca]_{CF}$	$[Ca^{2+}]_L$	J_{Ca}	V_{TE}	$\Delta\tilde{c}_{Ca}$
I.	147/ -	1.21	1.10	2.9	1.6	.064
II.	110/ 37 TMA	1.02	0.97	4.6	4.0	.202
III.	74/ 73 TMA	0.87	0.82	7.2	7.4	.390
IV.	10/137 Li	1.06	0.99	4.8	1.6	-.001

CF=collected fluid, L=lumen. Blood $[Ca^{2+}]$ 1.23 mM. Reducing Na decreased Na absorption. With TMA, V_{TE} increased favoring the increase in J_{Ca}. The slope of the relation between J_{Ca} and $\Delta\tilde{c}_{Ca}$ is 22 x 10^{-7} cm^2/s, similar to apparent P_{Ca} values under different circumstances. With Li, J_{Ca} increased even though V_{TE} did not change and $\Delta\tilde{c}_{Ca}$ decreased to zero. Thus, proximal Ca and Na absorption can change in opposite directions. J_{Ca} has both passive and active components. TMA replacement revealed a voltage dependent diffusive flux. Because Li replacement stimulated J_{Ca} while reducing luminal Na, the active Ca flux could be mediated by Na-Ca exchange across the basolateral membrane.

MECHANISMS BY WHICH LIMITED PHOSPHATE AVAILABILITY IMPAIRS PROXIMAL RENAL FUNCTION. P.C. Brazy, S.R. Gullans,* and S.P. Soltoff.* Duke University and Durham VA Medical Centers, Durham, NC.

Removal of intraluminal inorganic phosphate (Pi) inhibits solute transport and oxygen consumption rates (QO_2) in rabbit proximal convoluted tubules. The present study quantified the Pi-dependence of oxidative phosphorylation and tests whether the addition of selected metabolic substrates can circumvent the effects of Pi limitation. The Pi-dependence of oxidative phosphorylation was examined by measuring ADP-stimulated QO_2 in tubules made permeable with digitonin. With excess ADP, QO_2 is a saturating function of ambient Pi with an apparent Km of 0.7mM Pi. Thus, oxidative phosphorylation is limited at an ambient Pi which may occur under physiologic conditions. Because Pi also serves as a counter anion for mitochondrial uptake of substrates such as dicarboxylic acids, we examined the possibility that additions of specific substrates could overcome the effects of limited Pi. Reductions in uncoupled QO_2 were used to indicate impaired substrate delivery. With standard media, the removal of Pi reduced uncoupled QO_2 by 30%. Additions of malate, citrate or succinate at 1mM preserved uncoupled QO_2 despite limited Pi. Butyrate or valerate did not protect. Moreover, butylmalonate, an inhibitor of Pi:dicarboxylate exchange, reduced both coupled and uncoupled QO_2 in Pi-containing media, but caused no further reduction in QO_2 in Pi-free media. We conclude that Pi limitation in proximal renal tubules inhibits energy metabolism by reducing the availability of Krebs cycle intermediates that enter via the mitochondrial dicarboxylate anion exchange mechanism.

MECHANISM OF PHYSIOLOGICALLY INCREASED INTESTINAL CALCIUM (Ca) ABSORPTION AND SERUM 1,25 DIHYDROXY-VITAMIN D_3 (1,25D) IN MALE (M) RATS. DA Bushinsky, MJ Favus*, BA Rosenfeld* and FL Coe, Michael Reese Hospital and Univ. of Chicago, Chicago, Il.

Because they grow faster than females (F), M rats must accumulate larger Ca stores from dietary sources. The augmented Ca absorption could be due to elevated 1,25D or an intrinsic gender-related difference in Ca absorption. To study this, equal weight adult Sherman rats were offered 13 gm/day of 0.6% Ca, 0.65% phosphorus (P) diet for 11 days. During days 7-11 inclusive, M displayed relative Ca hyperabsorption and increased serum 1,25D levels. Cumulative Ca balance (values mean±SEM) was:

	n	Intake	Urine	Fecal	% Abs	1,25D
M	8	390±0	3.6±0.3	208±10	47±2	264±34
F	8	378±4*	10.5±1.0*	249±8*	34±2*	112±17*

Intake, urine and fecal Ca in mg/5 day; % Abs=(intake-fecal)/intake X 100; 1,25D in pg/ml; *p<.02, M vs F.

^{45}Ca uptake by proximal duodenal gut sacs (nM/mg protein/30 min) was higher in M (28±2 vs 21±1; p<.025) and directly correlated with 1,25D (r=.796; p<.001), as was cumulative Ca retention (r=.772; p<.001). Elevated 1,25D was not due to low serum Ca or P (10.34±.09 vs 10.56±.08 mg/dl, 8.47±.09 vs 8.16±.18 mg/dl for Ca and P, M vs F, respectively; p NS for both). Urine cAMP (nM/mg creat) was higher in M than F (19.2±.7 vs 15.9±.4; p<.001) and urine Ca in M was less than F. Serum 1,25D correlated positively with urine cAMP/creat (r=.635; p<.01) and inversely with urine Ca (r=-.744; p<.001).

Relative Ca hyperabsorption in M seems due to increased 1,25D and occurs in proximal duodenum, at least. Increased 1,25D levels with increased urine cAMP and reduced urine Ca suggest increased parathyroid hormone secretion.

BRUSH BORDER MEMBRANE FROM HUMAN KIDNEY. PROTEIN COMPOSITION AND PHOSPHATE UPTAKE CAPACITY. Michèle G. Brunette, Richard Béliveau*, Maisonneuve Hospital and Univ. of Montreal, Montreal, Canada.

Brush border membrane (BBM) vesicles have been prepared from fresh samples of normal kidney cortex obtained from 3 patients undergoing nephrectomy. Protein composition, phosphate (Pi) transport capacity and alkaline phosphatase activity were investigated. The protein pattern was determined by SDS polyacrylamide gel electrophoresis and the Na gradient-dependent-Pi uptake was studied according to the filtration technique. For comparison, similar studies were performed in rats, mice and rabbits. Aside a few variations, human BBM present a protein pattern similar to that of the animal species. 22 proteins are shared by man and these animals. Incubation with inorganic ^{32}P reveals a Pi binding protein whose molecular weight is 78000. This Pi binding protein is also common to the animal species but its binding capacity is lower in man (7.36 pmoles/mg) than in the other species (36.8, 29.8, 9.9 pmoles/mg). The Na gradient dependent Pi uptake was studied at 20°C. Lineweaver-Burk plot reveals a Km at 89 ± 3.3 µM (compared to 92 ± 5.5,58 ± 1.2, 76 ± 4.6 in rats, mice and rabbits), and a Vmax at 0.97 ± 0.05 nmol/mg/20 sec. (compared to 5.2 ± 0.2, 1.5 ± 0.2, 1.3 ± 0.1 nmol/mg in the animal species), indicating a comparable apparent affinity, but a smaller phosphate uptake capacity in man. Alkaline phosphatase activity in homogenate and BBM is also low in human kidney compared to the other species (rat>mouse>rabbit>man). It seems therefore that when comparing these four species, a parallelism exists between the phosphate binding protein, the alkaline phosphatase activity and the Na gradient dependent Pi uptake by the vesicles.

NET OXALATE SECRETION BY RAT SMALL INTESTINE. KA Calhoun,* SC Kathpalia, MJ Favus* and FL Coe. Michael Reese Hospital and Univ. of Chicago, Chicago, Il.

Overabsorption of dietary oxalate (Ox) by colon is a primary cause of hyperoxaluria and calcium oxalate nephrolithiasis in patients with extensive small bowel resection or inflammatory small bowel disease. Little is known about absorption or secretion of Ox by small intestine in normal or pathologic conditions. We measured the two unidirectional Ox fluxes in vitro under short-circuited conditions across jejunum and ileum from 200 g male Sherman rats fed normal chow. Tissues were bathed in phosphate buffered Krebs Ringer solution (pH 7.4) containing 13.5 µM Ox and .125 mM Ca. Absorptive fluxes from mucosa to serosa (Jms) and secretory fluxes from serosa to mucosa (Jsm) were measured with ^{14}C-Ox.

Segment	n	Jms	Jsm	Jnet	Isc	G_t
		pmol·cm^{-2}.hr^{-1}			µA·cm^{-2}	mΩ·cm^{-2}
Jejunum	8	113±15	242±15*	-129±41†	34±4	26±4
Ileum	8	151±14	322±28*	-171±24†	32±2	23±1

Values are mean±SEM; Jnet=Jms-Jsm; Isc=short-circuit current; G_t=conductance. *Jsm>Jms (p<.03); †, differed from no net flux (p<.02).

Net $Ox^=$ secretion (Jsm>Jms) occurred across both regions of small bowel in the absence of electrochemical gradients, consistent with an active transport mechanism.

Net Ox secretion may occur normally in the small intestine. In patients with extensive small bowel resection or inflammatory small bowel disease, loss of normal Ox secretion could contribute to the hyperoxaluria.

EFFECT OF PHOSPHATE DEPLETION (PD) ON BLOOD PRESSURE. V.M. Campese, Y. Saglikes*, N. Brautbar, R. Barndt* and S.G. Massry. Div. Nephrol., USC Sch. Med., Los Angeles, CA.

PD affects adversely myocardial function, but its influence on mean arterial pressure (MAP) is not well elucidated. We examined the effect of PD on MAP, hormones involved in regulation of MAP and the vascular response to pressor agonists. Both MAP and cardiac index were lower ($p<0.01$) in PD than normal rats (NR) (112±2 vs 122±2 mmHg, and 95±11 vs 140±7 ml/min/kg, respectively). Systemic vascular resistance (SVR) was higher (1.2±0.12 vs 0.9±0.04 mmHg/ml/min/kg, $p<0.05$) in PD than in NR. Plasma norepinephrine (NE) in the resting state (44±5.5 vs 27±3.4 ng/dl) and during the stress of immobilization was higher ($p<0.01$) in PD rats. Basal PRA was also higher ($p<0.05$) in PD and increased similarly in the two groups of rats after isoproterenol. Bolus injections of NE (10, 30, 100, 300 ng) or angiotensin II (3, 10, 30, 100 ng) produced smaller rise in MAP in PD than in NR. When NE was infused to achieve a rise in MAP of 300 mmHg, the dose required in PD (1.3±0.34 µg/min) was higher than in NR (0.3±0.07 µg/min). Pretreatment with indomethacin did not affect the response of MAP to NE. The content of inorganic phosphorus, ATP and AMP was lower ($p<0.05$) in the mesenteric vessels of PD than NR. The data show that PD leads to a decrease in MAP and vascular response to pressor agonists and with appropriate compensatory rise in NE, and PRA but with inadequate increase in SVR. The results indicate that the reduced vascular response to pressor agonists is a major mechanism underlying the decrease in MAP in PD and it is probably due to reduced energy availability in blood vessels.

EFFECT OF AMILORIDE (AM) ON CA TRANSPORT BY THE DISTAL CONVOLUTED TUBULE (DCT): IN VIVO MICROPERFUSION STUDY. Linda S. Costanzo, Michael P. Conrad,* and Linda S. Beahm*. Med. Coll. of Va., Richmond, Va.

Clearance experiments were performed in rats to examine the effect of AM on Ca reabsorption. 5% mannitol in saline was infused to produce high excretion rates. AM infusion (0.018mg/kg•min) caused no effect on GFR, plasma electrolytes or fractional Na excretion. Fractional Ca excretion fell from 0.120 ± 0.033 (control) to 0.074 ± 0.019 (AM), $p < 0.05$). The ratio of Ca to Na clearance fell from 0.69 to 0.46, $p < 0.01$. AM reduced fractional K excretion from 0.507 ± 0.064 to 0.093 ± 0.031, $p < 0.001$.

To localize the site of the hypocalciuric action of AM, rat DCT were microperfused at 6.3 nl/min with a solution simulating early distal fluid and containing C-14 inulin. DCT were perfused with control solution or one containing 10^{-5} M amiloride. Ca and Na concentrations of perfused and collected fluids were measured by flameless atomic absorption spectrophotometry. Ca transport increased from 3.77 ± 0.64 (control) to 5.53 ± 0.36 pmol/min • mm (AM), $p< 0.05$. AM inhibited Na and fluid reabsorption. In a separate experiment one DCT received both control and AM solutions. AM increased Ca reabsorption from 3.47 to 6.69 pmol/min • mm and decreased Na and water reabsorption from 120 to 95 pmol/min • mm and 1.35 to 0.99 nl/min • mm, respectively. We conclude: (1) The hypocalciuric action of AM is the result of increased Ca transport in the DCT. (2) AM acts from the luminal side of the distal epithelium. (3) Effects of AM on Ca transport are opposite to those on Na and H_2O.

PARATHYROID HORMONE (PTH) INCREASES ENZYMATIC PHOSPHOLIPID METHYLATION IN KIDNEY CORTICAL MEMBRANE. Tai C. Chen and Jules B. Puschett. Renal-Electrolyte Division, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Recent studies have shown that stepwise methylations of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) by methyltransferase I (MT-I) and methyltransferase II (MT-II) play an important role in the transduction of receptor-mediated signals through membranes in a variety of cellular systems. Furthermore, membrane phospholipid alterations have been implicated in the regulation of phosphate transport by renal cortical tissue in response to a number of agents, including PTH. Therefore, we investigated the possibility that methyltransferases are important in the physiological actions of PTH on the kidney. The MT-I and MT-II activities were determined in renal cortical membrane preparations obtained either from thyroparathyroidectomized (TPTX) rats or TPTX rats given PTH (15µg). The assays were performed by measuring incorporation of the methyl group from S-adenosyl-L-[methyl-^{3}H] methionine (SAM) into membrane phospholipids. The MT-I and MT-II activities in TPTX rats were 12 and 480 pmoles of SAM/mg protein, respectively. PTH stimulated MT-I activity by 50 ± 20% over TPTX levels, but had little or no effect on MT-II. In vitro incubation of membrane preparations or cortical slices with PTH failed to enhance either MT-I or MT-II activity. We conclude that: 1) MT-I and MT-II are present in renal cortical membranes; 2) the first methylation step in converting PE to PC is stimulated by PTH. The data suggest that one mechanism of PTH effect on the kidney may be an increase in PC synthesis.

PGE_2 HAS PARATHYROID HORMONE (PTH)-LIKE EFFECTS IN THE PROXIMAL SEGMENT OF THE RABBIT NEPHRON. J.H. Dominguez, T.O. Pitts*, and J.B. Puschett. Renal-Electrolyte Division, University of Pittsburgh School of Medicine, Pittsburgh, PA.

PGE_2 administered in vivo blocks the phosphaturia induced by PTH (Endocrinology, 109:2267, 1981). This suggests that PGE_2 might desensitize the renal tubule to the effects of PTH. For this to occur, PGE_2 should act at the same tissue level as PTH. Since PTH inhibits phosphate transport in the proximal straight segment (PSS) of the rabbit nephron, we have examined the effects of PGE_2 on fluid (Jv, nl/mm/min) and lumen to bath phosphate fluxes ($Jl\text{-}bPO_4$, pMol/mm/min) in this segment. Rabbit PSS were perfused and bathed in vitro at 37°C with and in chemically defined media. We examined two groups of tubules during two sequential periods (I and II). We collected 3-5 samples/period. Group I, n=8, was studied during two control periods. Perfusion rates (nl/min) were: 13.36 ± 0.54 and 13.07 ± 0.42, respectively ($P >.20$). Jv I and II were: 0.46 ± 0.05 and 0.42 ± 0.06 ($P >.05$). $Jl\text{-}bPO_4$ I and II were: 3.18 ± 0.42 and 3.06 ± 0.41 ($P >.20$). Group 2, n=10, was first studied during control conditions, followed by addition of PGE_2, 10^{-5}M, to the bath. The perfusion rates were: 12.15 ± 0.40 and 12.13 ± 0.41 for periods I and II, respectively ($P >.50$). Jv I and II were: 0.52 ± 0.06 and 0.35 ± 0.06, $P <0.01$. $Jl\text{-}bPO_4$ I and II were: 3.29 ± 0.54 and 2.90 ± 0.46, $P <0.02$. These results demonstrate that PGE_2 produces a PTH-like effect in the PSS of the rabbit nephron. Accordingly, failure of PTH to act in vivo in dogs pretreated with PGE_2 could represent tubular desensitization to PTH by PGE_2.

DISTURBANCES OF LIPID METABOLISM IN PATIENTS (pts) WITH SECONDARY (RENAL) OR PRIMARY HYPERPARATHYROIDISM (HPTH). I Drüeke*, B Lacour*, V Jorgetti*, M Thévenin*, A Poirier*, C Dubost* (Intr. by D.A.McCarron) Dépt. de Néphrol. et de Biochimie A, Hôp. Necker, and Serv. de Chir., Hôp. F.Widal, Paris, France.

The occurrence of anomalies of lipid metabolism in human HPTH remains controversial. Therefore, we decided to study serum lipids and lipoproteins in 30 uremic pts with 2°HPTH and 65 pts with 1°HPTH before and 6-8 days after parathyroidectomy (PTx). Results in pts with 2°HPTH (means± SEM) are shown in the table (TG=triglycerides; chol=cholesterol; PL=phospholipids; R1=apoA/apoB; R2=HDL-chol/(LDL+VLDL)-chol; R3=HDL-PL/(LDL+VLDL)-PL.

	before	after PTx	P
TG(mM)	2.22±0.21	1.46±0.08	< 0.001
chol(mM)	5.02±0.26	4.19±0.18	< 0.001
PL(mM)	2.92±0.19	2.54±0.20	< 0.001
R1	2.10±0.15	2.10±0.11	NS
R2	0.27±0.03	0.32±0.03	< 0.01
R3	0.68±0.05	0.76±0.05	< 0.05

In 1°HPTH pts as a whole a similar sigificant decrease was observed for TG, chol, and PL. In contrast to 2°HPTH pts, R1, R2, and R3 were decreased. However, only 25% of 1°HPTH pts had lipid anomalies that all were improved by PTx.

In conclusion, lipid anomalies of HPTH pts were improved by PTx. Howevever, the changes observed in 2°HPTH pts were different from that in 1°HPTH pts.

PARATHYROID HORMONE (PTH) STIMULATES PHOSPHOLIPID (PL) PHOSPHORYLATION AND TURNOVER IN BASOLATERAL MEMBRANES (BLMs) OF RENAL TUBULAR CELLS IN VITRO. Pedro Esbrit* and Keith Hruska. Jewish Hospital, St. Louis, Missouri.

Synthesis and turnover of PLs in the phosphatidylinositol cycle are stimulated by PTH in isolated renal tubular segments. These PLs stimulate calcium binding and translocation in renal tubular brush border cell membranes. The present studies utilized BLMs prepared by centrifugation of renal cortical homogenates in Percoll. BLMs were incubated with 1 mM [γ^{32}Pi] ATP, 1 μM Ca^{2+} and +/- b-PTH 1-84 (10^{-6}M). PTH produced a rapid stimulation of PL phosphorylation which was detectable by 15 sec of incubation. The PL phosphorylation products were phosphatidic acid (PA), phosphatidylinositol 4'-phosphate (DPI), and phosphatidylinositol 4'5'-diphosphate (TPI). After 5 min of incubation with PTH, Pi incorporation into these PLs was 5.2, 61.9, and 12.2 pmol/mg protein respectively, while it was 4.1, 56.2, and 10.2 in control BLMs ($p<.05$). After 5 min of incubation, dephosphorylation became predominant indicating turnover of the phosphorylated PLs. PL phosphorylation was not stimulated by incubation with 3'5' c-AMP (10^{-6} or 10^{-3}M). The absence of Ca^{2+} did not alter the effects of PTH on PL phosphorylation, but dephosphorylation was impaired by either zero calcium or chloropromazine addition. These data indicate a direct action of PTH on PL phosphorylation in the BLM independent of c-AMP and CA^{2+}. Ca^{2+} stimulates degradation of the phosphorylated products. These effects of PTH may be a mechanism for modulation of Ca^{2+} binding and translocation in the BLM directly associated with hormonal binding to the receptor.

POLYDYPSIA AND POLYURIA IN THE CHRONICALLY PHOSPHATE-DEPLETED RAT. W.B. Duffy,* R. Krothapalli* and H.O. Senekjian. VA Medical Center and Baylor College of Medicine, Houston, Texas.

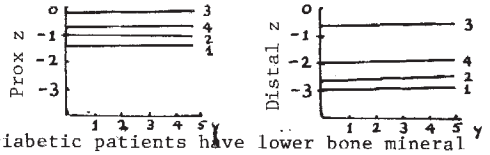
The effect of chronic phosphate depletion (CPD) on water metabolism was studied in three groups of rats: group 1 was maintained on a normal control diet (0.44% phosphate) and had free access to water; group 2 animals were fed a low phosphate diet (0.01%) and had ad lib water intake while group 3 animals were fed the same low phosphate diet, but had their water intake matched to that of group 1. Water intake, urine output, and urine osmolality were determined daily. Maximum urine concentrating ability was measured weekly. The daily rate of urinary osmolar excretion was similar in all three groups of animals. Daily water intake, urine output and urinary osmolality of groups 1 and 3 were not significantly different from each other throughout the study. By the 4th day of study, however, group 2 animals had a significantly greater water intake and urine output as well as a lower urinary osmolality as compared to either group 1 or 3. On day 15, maximum urinary osmolality (following 24hr water deprivation) of group 1 rats was 1905.2±40.9 mOsm/kg water. Maximum urinary osmolality of group 2 animals averaged 1743.8± 53.5 mOsm/kg water ($p < 0.025$ compared to group 1), while group 3 animals averaged 2035.1±68.6 mOsm/kg water (p=NS vs group 1, $p < 0.005$ vs group 2). We conclude that CPD results in an increase in water intake and a subsequent polyuria. Urinary concentrating ability is well preserved, as seen in group 3 animals. CPD animals provided an ad lib fluid intake have a submaximal urinary concentrating ability, probably on the basis of medullary washout.

BONE MINERAL CONTENT (BMC) IN DIABETIC HEMODIALYSIS PATIENTS. R. Estes,* G. Willie,* C. Cruz, G. Zasuwa,* N.W. Levin, A.M. Parfitt,* Detroit, Michigan.

Measurement of bone mineral content reproducibly permits evaluation of one aspect of renal osteodystrophy. In 4 Type I diabetics (DI), 12 Type II diabetics (DII), and as non-diabetic controls, 6 polycystic (PC) and 15 hypertensive (HTV) patients, BMC was measured by photon absorptiometry at the initiation of dialysis ± 6 months and serially thereafter. Results are given as standard deviations from age, sex and race adjusted means (z scores). Mean results follow.

	DI(1)	DII(2)	PC(3)	HTV(4)
Prox z	-1.73C	-1.04B	-.32A	-.74AB
Distal z	-3.01B	-2.65B	-.91A	-1.90AB

Means which do not share letters differ at the $p<.05$ level (Duncan's test). The changes in z score over time are shown below.



Diabetic patients have lower bone mineral content than either polycystic patients or hypertensives. In all four diagnosis groups dialysis patients maintain their bone mineral content on par with age, sex, and race adjusted controls. The major portion of BMC loss antedates the initiation of dialysis, suggesting the need for early therapeutic intervention.

DOES BONE ALUMINUM INTOXICATION (BAI) CAUSE RESISTANCE TO VITAMIN D THERAPY (VDT)? M.C. Faugere*, H.H. Malluche. Univ of Kentucky, Div of Nephrol, Bone & Mineral Metabolism, Lexington, Kentucky.

Data on the therapeutic effectiveness of Vit D in low turnover osteomalacia (LTOM) are conflicting. Aluminum (Al) has been shown to be one of the causes of LTOM. The study evaluates the response of 20 non-dialyzed (D-) and 27 dialyzed (D+) patients (pts) to VDT by comparing quantitative bone histology before and after 9-36 months of VDT using various Vit D metabolites. $1,25(OH)_2$ Vit D was given to 19 pts, 25(OH) Vit D to 11 pts, 5,6-trans-25(OH) Vit D to 12 pts and Vit D_3 to 5 pts. Doses of all Vit D compounds were adjusted to achieve normal intestinal absorption of Ca. Response to VDT was correlated with the extent of trabecular bone surface that had a positive reaction to a histologic stain specific for Al (Al+). Eighteen pts were Al+; 16 of these were D+ and 2 were D- (creatinine clearance 66 and 25 ml/min). Bone histology before VDT revealed low turnover osteomalacia (LTOM) in 6 out of 18 pts with Al+ and 1 out of 29 without Al intoxication (Al-). Mixed uremic bone disease (MUBD) was found in 50 pts. Twelve of these had Al+. Degree of hyperparathyroid component of MUBD was lower in Al+ than in Al- (osteoclastic index 11.5±5.2 vs 27±4; fibrosis 11.7±9.7 vs 50±17, $p<0.05$). LTOM did not improve after VDT despite the presence or absence of Al. MUBD improved in 90% of Al- pts and only 62% of Al+ pts.

In summary: (1) BAI is not confined to dialysis pts with LTOM. (2) BAI ameliorates hyperparathyroid component of MUBD. (3) BAI reduces but does not abolish response to Vit D in MUBD. (4) LTOM is unresponsive to VDT independent of BAI.

EFFECT OF ASYMPTOMATIC PRIMARY HYPERPARATHYROIDISM (PTHD) ON RENAL CONCENTRATION AND ACIDIFICATION
C. J. Foulks, L. F. Wright. Nephrology Service, Brooke Army Medical Center, Ft Sam Houston, TX.

Seven patients with PTHD have been studied while on a 60gm protein, 3gm Na^+, 400mg Ca^{++} diet. The diagnosis was made by demonstrating elevated PTH levels with hypercalcemia, absence of tumor, vit D intake, or milk-alkali syndrome. The mean age was 57.5 yrs (31-76); mean duration of disease was 8.3 yrs (2-16); mean creatinine clearance was 93ml/min (72-110). All were normotensive. All had a normal chest x-ray and bone survey. Tests performed included overnight water deprivation, NH_4Cl loading, and $NaHCO_3$ infusion to determine $TmHCO_3$. Results are shown in the table.

$S_{Ca^{++}}$ (mEq/L)	S_{Cl^-} (mEq/L)	S_{CO_2} (mEq/L)	$TmHCO_3^-$ (mEq/L GFR)	Max UpH	Max Uosm (mOsm/kg)
11.1	104	27	26.6	4.55	782
10.6-11.7	103-108	23-28	23-30	4.40-4.73	578-1002

The max Uosm was decreased and there was a slight decrease in the $TmHCO_3^-$. None of the patients had nocturia or a systemic hyperchloremic acidosis despite these findings. We conclude that the mild reduction in $TmHCO_3^-$ is attributable to the effect of PTH on proximal bicarbonate handling, since it bears no relation to $S_{Ca^{++}}$, Ccr or the duration of the disease. This decrease in $TmHCO_3^-$ does not appear to be clinically important as there is no impairment of urinary acidification.

EFFECT OF CIMETIDINE ON SECONDARY HYPERPARATHYROIDISM (HPT)
Fein, P.A., Soncuya, L.F.*, Iancu, M., Avram, M.M. The Long Island College Hospital, Department of Medicine, Division of Nephrology, Brooklyn, N.Y.

Numerous previous reports in the literature have considered excess parathyroid hormone (PTH) to have deleterious effects on multi-organ systems in uremia. Other reports have proposed cimetidine usage to counteract the effects of PTH excess. However, there are conflicting reports as to the effectiveness of cimetidine on dialysis patients in this setting.

We compared 7 chronic hemodialysis patients with HPT who received 300 mg. cimetidine twice a day with 7 other dialysis patients with HPT over a 5 month period. Intact PTH was measured by Upjohn using the Hawker method at the beginning, after 2 months and after 5 months (N=163-347). In the cimetidine group PTH was initially 775±277. After 2 months PTH was 670±326 and after 5 months PTH dropped to 618±304. The average difference was -157±247 which did not reach statistical significance. ($P<.10$). However, 5 patients (71%), reported clinical improvement in bone pain after 2 months. In the group which did not receive cimetidine, the intact PTH was 499±301, at 2 months it was 360±107 and at 5 months 367±78 with an average change of -132±359. Moreover, in these patients there was no clinical improvement in bone pain.

We conclude that, while there is no statistical significance in measured excess PTH when patients are treated with cimetidine, in a definite subset of uremia patients on dialysis, bone pain symptoms are improved.

31P-NMR SATURATION TRANSFER IN THE FUNCTIONING KIDNEY: REGULATION OF SODIUM TRANSPORT. D. Freeman, S. Bartlett, G. Radda and B. Ross. Nuffield Dept. of Medicine and Dept. of Biochemistry, Univ. of Oxford, Oxford, U.K.

ATP from oxidative phosphorylation, in which 3 ATP/oxygen atom may arise, is hydrolysed by Na-K-ATPase, where 3 Na/ATP are transported. Alternatively, sodium may be pumped against a gradient by free energy from ATP hydrolysis. ΔG_{ATP} is dependent on the cytoplasmic phosphorylation potential. We used quantitative ^{31}P nmr to follow these events in the perfused rat kidney. Values for [ATP] by nmr were similar to those from enzymatic assay. Most ATP is thus presumably cytoplasmic. Pi was 0.6mM or only 27% of that determined chemically. ADP was 25% of the enzymatic value. Saturation transfer occurred between the γ atom of ATP and Pi, confirming that Pi is in chemical exchange with cytoplasmic ATP. Oxidative phosphorylation rate was 16 µmole/min/g kidney and O_2 consumption was 3.3 µmole O_2/min/g kidney. The P:O ratio is thus 2.4±0.3. Allowing 30% of ATP production for Na transport, we conclude that Na transported by ATP was 4 times that attributable to the accepted mechanism of Na-K-ATPase. On the other hand the phosphorylation potential by nmr was $10,230M^{-1}$. The free energy yield of hydrolysing ATP at this phosphorylation potential is 59.7KJ/ mole. At this rate, 30% of the ATP could yield the total energy required to transport sodium between the cell (Na_i 22mM) and the perfusate (Na_e 145mM). We suggest that nmr measures a pool of Pi and other phosphates that is directly involved in energy metabolism. Free energy of ATP hydrolysis rather than a strict stoichiometry between ATP and Na-K exchange may determine the rate of sodium pumping.

ALTERED MINERAL HOMEOSTASIS IN CHILDREN WITH NEPHROTIC SYNDROME (NS) AND NORMAL RENAL FUNCTION (NRF) M Freundlich, AI Jacob, JM Canterburry*, G Zilleruelo, H Gorman*, JJ Bourgoignie, J Strauss. University of Miami; Depts. of Pediatrics and Medicine, Miami, Florida.

Mineral metabolism may be altered in adults with NS and NRF; data in children are scanty. We studied 16 patients ($\bar{x}$ age 10.5 yr) with NS and NRF; 6 were on steroids at testing. All had proteinuria (Uprot>1gm/day) and/or hypoalbuminemia (Alb < 3.0gm/dl) and NRF (GFR > 90ml/min/1.73m^2 or s. creatinine < 1.1mg/dl). Serum parathormone(iPTH, C-terminal), ionized calcium(Ca++), 25OHD and 1,25 $(OH)_2D$ were measured by specific methods. Results were as follows:

	Alb (gm/dl)	Uprot (gm/day)	Ca++ (mEq/L)	iPTH (uLEq/ml)	25OHD (ng/ml)
NS $\bar{x}$	2.3	2.3	2.11	100	7.6
(range)	(1.2-3.5)	(0.28-8.5)	(1.44-2.37)	(10-270)	(3-15)
Normal	$\geq$3.5	$\leq$0.20	2.36-2.64	10-80	14-43

Four/9 patients had increased cortical/trabecular ratios on bone densitometry, compatible with hyperparathyroidism or osteopenia. All patients had low 25OHD; 1,25$(OH)_2D$ varied. Ca++ was decreased in 15/16; nevertheless, 8/16 had normal iPTH. Conclusions: 1)hypocalcemia, low 25OHD and high iPTH may contribute to the genesis of bone disease in NS children with NRF, 2)hypocalcemia failed to induce abnormal elevation of iPTH in some children while in others abnormally elevated iPTH failed to correct hypocalcemia, 3)low Ca++ accompanied low 25OHD but a variable 1,25$(OH)_2D$ independently of steroid therapy. Thus, bone disease exists and mineral homeostasis is altered in NS children with NRF.

INDUCTION OF OSTEOMALACIA IN DOGS FOLLOWING SHORT-TERM INJECTIONS OF ALUMINUM. W.G. Goodman, D.A. Henry,* R.A. Nudelman, A.C. Alfrey, & J.W. Coburn. Med & Resch Svc, Sepulveda, Wadsworth, & Denver VA Med Ctrs & Dept Med, UCLA Sch Med, Los Angeles, CA

There is a strong association between the presence of Al in bone and the occurence of osteomalacia (OM) in dialysis patients; however, it has been suggested that Al may passively bind to osteoid and not be a pathogenic factor. To evaluate whether Al is pathogenic in OM, quantitative bone histomorphometry was performed in 6 dogs given intravenous AlCl3, providing 1 mg/kg/day, for 3-5 weeks; iliac crest bone biopsies were done before (Bx1) and after (Bx2) Al loading. Percent osteoid ($\bar{x}\pm$sd) (2.8±0.8 vs 7.0±4.3, $p<0.05$) and osteoid width (5.7±0.6μ vs 8.0±1.2μ, $p<0.01$) increased from Bx1 to Bx2, whereas forming surface did not change significantly (24.8±3.3 vs 37.2±21.9%). Bone Al was markedly increased in Bx2 (105±26 vs 1.3±1.6 μg/kg in Bx1, $p<0.001$) and was evident by histochemical stain at Bx2 only. The severity of OM (% osteoid) correlated with bone Al as measured by histochemical technique ($r=0.87$, $p<0.01$). Bone formation could not be assessed in Bx2 due to minimal uptake of tetracycline by bone; the latter finding is indicative of impaired mineralization. Serum calcium increased in 5 of 6 dogs in the absence of histologic evidence of increased bone resorption, providing evidence for reduced capacity of the skeleton to buffer hypercalcemia. These data clearly indicate that short-term parenteral administration of Al can produce OM in dogs; the severity of OM appears to be related to the extent of trabecular bone Al deposition.

BICARBONATE INDUCED SUPPRESSION OF DUODENAL ABSORPTION OF CALCIUM (Ca) AND PHOSPHORUS (P) INDEPENDENT OF 1.25$(OH)_2D_3$. U. Gafter, S. Edelstein, J. Levi (intr. by C.R. Kleeman), Nephrol. Unit, Hasharon Hospital, Petah-Tikva and Tel Aviv Univ. Med. School, and Dept. of Biochem, Weizman Inst. of Sci., Rechovot, Israel.

To study the effect of metabolic alkalosis on intestinal absorption of Ca and P, male rats were fed comercial diet (Ca 1%, P 0.7%). A bicarbonate fed group (n=12) drank 2.5% $NaHCO_3$ while pair-fed controls (n=12) drank distilled water. 9 days later they were sacrified and the gut was removed for measurement of ^{45}Ca and ^{32}P uptake by everted gut sac technique. HCO_3 and pH increased in $NaHCO_3$ fed rats compared to controls, 23.1±0.6 vs 19.8±0.9 mEq/l ($p<0.01$) and 7.52±0.02 vs 7.44±0.02 ($p<0.05$) respectively. PCO_2, Ca and P did not differ in both groups. Duodenal uptake of ^{45}Ca and ^{32}P by $NaHCO_3$ fed rats was less, compared to control rats 789±87 vs 1143±143 ($p<0.02$) and 400±39 vs 649±56 CPM/mg wet weight/0.5h ($p<0.01$). Ca and P uptake by Jejunum, ileum and colon did not differ in both groups. In a similar experiment Vitamin D metabolites levels were measured in pooled plasma from pairs of rats in $NaHCO_3$ fed group (n=7) and control group (n=7). Both 25(OH)D and 1.25$(OH)_2D$ were increased in $NaHCO_3$ fed rats compared to controls 18.5±0.4 vs 15.9±1.1ng/ml ($p<0.02$) and 958.2±150 vs 278.8±34.3 pg/ml ($p<0.001$) respectively. 24,-25$(OH)_2D$ was similar in both groups.

In summary, bicarbonate feeding increased 1.25-$(OH)_2D$ and 25(OH)D plasma levels in rats. It also decreased duodenal absorption of Ca and P independently of the effect on Vitamin D.

PTH INCREASES ADP-RIBOSYLATION OF CANINE RENAL BRUSH BORDER MEMBRANE PROTEINS. Marc R. Hammerman, Dunell E. Cohn,* Juan Tamayo,* and Kevin J. Martin. Washington Univ. School of Med., Dept. of Internal Med., St. Louis, Missouri.

We recently demonstrated mono-ADP-ribosylation of a protein band (M_r 62,000) on autoradiograms of SDS-polyacrylamide gels of isolated canine renal brush border membrane vesicles (BBMV) associated with decreased Na^+-dependent Pi transport in BBMV (JBC in press). The present studies were performed to ascertain whether PTH-induced phosphaturia may be effected through ADP-ribosylation of the renal brush border membrane and if so to study the effect of duration of renal PTH exposure on Pi transport across the luminal membrane and on ADP-ribosylation of membrane proteins. To this end we examined the effects of 2 and 24 hour exposure of isolated perfused canine kidneys to PTH, on Pi transport in BBMV, and on ^{32}Pi-ADP-ribosylation of BBMV. Initial rates of Na^+-dependent Pi uptake were decreased 25 ± 3% and 28 ± 3% respectively in BBMV prepared from isolated kidneys perfused +PTH for 2 or 24 hours compared to Pi uptake in BBMV from kidneys perfused -PTH. [^{32}Pi]-ADP-ribose incorporation in a protein band (M_r 62,000) was demonstrable in gels of BBMV from kidneys perfused ± PTH for 2 or 24 hours. ^{32}Pi-ADP ribosylation of the band was significantly decreased (52 ± 4%) in BBMV from kidneys perfused +PTH for 24 but not 2 hours, reflecting increased ADP-ribosylation in intact kidneys perfused with PTH for 24 hours. We conclude that PTH may induce phosphaturia via ADP-ribosylation of the renal brush border membrane. ADP-ribosylation may mediate phosphaturic effects resulting from long term PTH elevations.

PHOSPHATE TRANSPORT OF SUPERFICIAL AND DEEP NEPHRONS IN RESPONSE TO PARATHYROID HORMONE. Aviad Haramati, John A. Haas* and Franklyn G. Knox, Dept. of Physiology, Mayo Clinic and Foundation, Rochester, Minnesota

The importance of parathyroid hormone (PTH) in the regulation of renal phosphate (P) excretion (E) is widely recognized, but the effect of PTH on intrarenal P handling between nephron populations is unknown. In this study we evaluated the response of superficial and deep nephron proximal tubules to PTH in TPTX rats fed a normal P diet (0.7%). Since P reabsorption is not detectable in the ascending loop of Henle, fractional P delivery (FDp%) to the early distal tubule and loop of Henle reflect delivery from superficial and deep nephron proximal tubules, respectively. Recollection micropuncture experiments were performed in 10 acutely TPTX rats before and after the infusion of PTH (30 U/kg bolus; 1 U/kg·min). Following PTH, FEp% increased from 3.3% to 26.2%.

	FDp%		
	Superficial	Deep	P
Control	15.7 ±3.8	5.7 ±1.1	<.05
PTH	29.7 ±5.2	28.0 ±2.1	NS

During control, FDp% was less from deep nephrons indicating enhanced P reabsorption by deep compared to superficial proximal tubules. During PTH, FDp% increased in both groups, but the relatively greater P reabsorption in deep nephrons was abolished. We conclude that PTH inhibits phosphate transport to a greater extent in deep than superficial nephrons.

IMPAIRED PHOTOPRODUCTION OF VITAMIN D (D) LEADS TO D DEFICIENCY IN UREMIA. A. I. Jacob, A.L. Sallman Z. Santiz*, and B. Hollis*. Univ. of Miami, Florida, and Case Western Reserve, Cleveland, Ohio.

The initial step in D metabolism is the photoconversion of 7-dehydrocholesterol (7-DHC) to pre-D in the epidermis and requires ultraviolet light (UV(B)). We measured plasma D, 25D and 1,25D in 14 normal white (NW), 9 normal black (NB), 9 uremic white (UW) and 16 uremic black (UB) subjects.

	D (ng/ml)	25D (ng/ml)	1,25D (pg/ml)
NW	4.00±1.00	31.3±3.0	34.5±4.7
NB	.96± .06[a]	17.7±1.5[c]	41.6±5.6
UW	6.68±2.55	43.8± 9.7	--------
UB	.15± .06[b]	24.9±3.5[d]	--------

a= p<.05 vs NW c=p<.01 vs NW
b= p<.005 vs NB+UB d=p<.05 vs UW

D was not detectable in 11 of 16 UB, including 2 treated with 1,25D. In all uremics, plasma 25D correlated with D (r=.79, p<.001). We exposed 5 NW and 5 UW subjects to equal doses of UV(B). The maximal increase in circulating D averaged 21.3±2.8 ng/ml in NW and 6.3±1.9 ng/ml in UW (p<.01) 7-DHC content was similar in epidermis from site matched skin of fresh cadavers and UW pts., 131±23 and 125±14 ng/ml skin, respectively.

Four uremics and 4 normals were given 2000 U of D orally for 3 days. The increase in plasma D was 4.75±.78 and 1.93±.84 in normals and uremics, respectively (p<.05).

We conclude that 1)NB have less circulating D and 25D than NW, 2)uremia inhibits photoproduction of D, 3)the combination of black skin and uremia leads to marked D deficiency in UB subjects, 4) the concentration of circulating 25D in uremia is dependent on plasma D.

PARATHYROID HORMONE (PTH) INHIBITS VOLTAGE DEPENDENT CALCIUM (Ca^{2+}) UPTAKE IN BRUSH BORDER MEMBRANE VESICLES (BBMV) OF CANINE PROXIMAL TUBULE CELLS. K. Hruska, S. Khalifa,* and S. Mills,*. Jewish Hospital, St. Louis, Missouri.

Specific phospholipid phosphorylation stimulated by PTH increases Ca^{2+} binding and translocation in BBMV. In the present studies, Ca^{2+} uptake was stimulated when transmembrane potentials, intravesicular negative, were generated by exposure of BBMV to extravesicular gradients of highly permeant anions or exposure of KCL loaded BBMV to valinomycin. Tetraphenylphosphonium (TPP) was used to monitor the intravesicular negative transmembrane potential. The addition of extravesicular 120 mM NaSCN, increased both TPP and 25 μM Ca^{2+} uptake in BBMV, 152 and 390 pmol Ca^{2+}/mg protein at 10 secs and 1 min of incubation, compared to addition of 120 mM NaCl, 89 and 316 pmol/mg protein, p<.02 NaSCN >NaCl. The Ca^{2+} uptake stimulated by negative transmembrane potential was released by hypotonic lysis of the BBMV demonstrating that voltage dependent Ca^{2+} uptake occurred through a mechanism besides binding of Ca^{2+} to the BBMV. PTH (2.5 μg/kg) administered to dogs prior to nephrectomy and preparation of BBMV completely inhibited the voltage dependent stimulation of Ca^{2+} uptake. PTH had no effect on the generation of the transmembrane potential. These data demonstrate a voltage dependent Ca^{2+} uptake mechanism in renal tubular BBMV which is inhibited by PTH. PTH by stimulating phospholipid phosphorylation and Ca^{2+} binding may stabilize the BBM and decrease the activity of the voltage dependent Ca^{2+} uptake mechanism.

LOW CALCIUM (Ca) DIET AND PARATHYROID HORMONE (PTH) ENHANCES RENAL COMPENSATORY HYPERTROPHY (RCH). J.Jobin, J.-P. Bonjour, J.Caverzasio, and C. Taylor, (intr. by V. Dennis). Univ. of Bern, Department of Pathophysiology, Bern, Switzerland.

The effects of variations in Ca and PTH status on RCH after unilateral nephrectomy (U-NX) have been studied in rats. U-NX rats were pair fed diets with high (1.1%, HCaD) or low (0.1%, LCaD) Ca content. After U-NX the wet weight (w.w.) of the remaining kidney was (mg ± SE): at 3 days HCaD: 722 ± 40, LCaD: 810 ± 35; at 8 days HCaD: 1175 ± 30, LCaD: 1405 ± 34 (p <0.001); at 15 days HCaD: 1387 ± 44, LCaD: 1928 ± 104 (p <0.001). The higher w.w. of the remaining kidney under LCaD was accompanied by a proportional and highly significant increase in dry weight, total protein and DNA content. LCaD did not display such effects in sham-operated rats with intact renal mass. In U-NX rats fed HCaD chronic administration of PTH mimicked the effect of LCaD. In conclusion, LCaD can enhance markedly RCH. The data suggest that among the Ca-regulating hormones, PTH could be involved in the stimulating action of Ca restriction on renal compensatory hypertrophy.

EARLY EFFECTS OF VITAMIN D METABOLITES ON PHOSPHATE (Pi) FLUXES IN ISOLATED RAT ENTEROCYTES. G. Karsenty*, B. Lacour*, A. Ulmann*, E. Pierandrei*, and T. Drüeke* (Intr. by D.A. McCarron). INSERM U 90, Hôp. Necker, Paris, France.

The purpose of the present study was to investigate possible early, direct effects of vit. D metabolites on Pi movement in isolated enterocytes of normal rats. Jejunal enterocytes were isolated and incubated in working medium (Lacour et al, BBA 648 : 151, 1981). After centrifugation they were reincubated during 20 min at 37°C or 4°C in the presence of either vit.D$_3$ metabolites or ethanol (0.5%) vehicle. ^{33}Pi uptake velocity (V_{Pi}) was then measured during the first 8 min. In this model, V_{Pi} was a Na^+- and temperature-dependent phenomenon. V_{Pi} was enhanced by increasing medium Pi conc. from .1 to 8.0 mM (.05±.01 SEM to 1.37±.17 nmol Pi/mg.min). When medium Pi conc. was 3.0 mM, 1,25(OH)2D3 (1 pM) and 25 OHD3 (1 nM) significantly enhanced V_{Pi} vs control within 20 min (1.3±.16 vs .79±.09 and 1.39±.18 vs 1.09±.1 nmol Pi/mg.min). The effect of 1,25(OH)2D3 was Na^+-dependent. The hormone failed to stimulate V_{Pi} in the presence of trans-vaccinic acid (MTVA, 0.12 mM) known to change membrane lipid composition. In contrast to Pi influx, Pi efflux rate constant remained unchanged in the presence of 1,25(OH)2D3 when compared to its vehicle.

In conclusion, 1,25(OH)2D3 added in vitro enhanced enterocyte Pi uptake velocity within 20 min possibly by changing membrane fluidity.

NAD$^+$-DEPENDENT ADP-RIBOSYLTRANSFERASE IN RENAL BRUSH BORDER MEMBRANES. S.A. Kempson and N.P. Curthoys*, University of Pittsburgh School of Medicine, Pittsburgh, PA

Oxidised nicotinamide adenine dinucleotide (NAD$^+$) in cytosol may interact with the renal brush border membrane (BBM) and inhibit Na-dependent BBM phosphate transport. The mechanism of interaction between NAD$^+$ and BBM was investigated in the present study. Incubation of rat renal BBM with [adenine-^{3}H]-NAD$^+$ led to acid-stable binding of [^{3}H] to BBM. Binding was time-dependent and reached 10.3 ± 1.0 pmol/mg protein at 30 min. In contrast there was no binding of [^{14}C] when [carbonyl-^{14}C]-NAD$^+$ was used. Acid-stable binding of [^{32}P] from [adenylate-^{32}P]-NAD$^+$ was 10.8 ± 0.5 pmol/mg BBM protein/30 min. Equimolar incorporation of [^{3}H] and [^{32}P] into BBM is consistent with the presence in BBM of an ADP-ribosyltransferase which transfers ADP-ribose from NAD$^+$ to BBM. ADP-ribosylation increased as the NAD$^+$ level increased in the range 0.02-0.20 mM and the Km was 44 µM. After sucrose gradient centrifugation of BBM the ADP-ribosyltransferase was recovered at the same isopycnic density as known BBM enzymes indicating that it is an intrinsic component of BBM and not a contaminant. The activity of the ADP-ribosyltransferase in BBM was unaltered in the presence of thymidine, nicotinamide or Mg^{2+} at levels which markedly affect the activity of nuclear ADP-ribosyltransferase. These findings indicate that cytosolic NAD$^+$ may be used by a unique ADP-ribosyltransferase in BBM for covalent modification of BBM proteins, and this may be a mechanism for regulating the BBM phosphate transport system.

EFFECTS OF RICINOLEATE (RIC) ON RAT COLON PERMSELECTIVITY: MECHANISM FOR OXALATE (OX) HYPERABSORPTION. SC Kathpalia, MJ Favus,* FL Coe, Michael Reese Hospital and Univ. of Chicago, Chicago, Il.

Colonic hyperabsorption of dietary Ox is the primary cause of hyperoxaluria in patients with inflammatory bowel disease or small bowel resection. Fatty acid (FA) malabsorption may lead to increased colon Ox permeability but the exact mechanism is unclear. To study the effect of Ric (a long-chain hydroxy FA) on colon permselectivity, we measured steady-state unidirectional fluxes of radiolabeled polar nonelectrolytes under short-circuited conditions. The molecular weights ranged from 76-594 with Stokes radii (Sr; in Å) of 2.2-6.0 for thiourea (Thio), Ox, erythritol (Eryth), mannitol (Mann) and raffinose (Raff). Apparent permeability (flux/medium concentration) of the absorptive (mucosa to serosa, Pms) and secretory (serosa to mucosa, Psm) fluxes was measured in the absence and presence of mucosal 1 mM Ric, across segments of rat ascending colon.

	Ox	Thio	Eryth	Mann	Raff
Control	11±1	27±3	10±1	12±1	7±1
+ Ric	23±1*	40±2*	20±3*	22±.5*	11±1*

Values are mean±SEM for Pms; *p<.05. For each molecule, the Psm was comparable to the Pms value.

Transport of molecules with Sr above 3.0 was retarded compared to molecules with Sr below 3.0. Ox Pms and Psm were retarded to a greater extent than predicted by size alone, reflecting the cation selectivity of the colon paracellular channels. Ric increases Pms and Psm for all molecules without changing the membrane size or charge selectivity. Ric effects are consistent either with an increased number of aqueous channels or with greater access to existing channels.

DECREASED BASAL 1,25-(OH)$_2$-VITAMIN D$_3$ (1,25 D) PRODUCTION BY CULTURED CORTICAL KIDNEY CELLS FROM X-LINKED HYPOPHOSPHATEMIC (HYP) MICE. A.Korkor, J. Kuchibhotla,* R.W. Gray,* and R. Meyer, Jr.* Departments of Medicine and Biochemistry, Medical College of Wisconsin and School of Dentistry, Marquette University, Milwaukee, WI.

Serum 1,25 D levels have been shown to be normal in HYP despite very low serum P levels. Furthermore, serum 1,25 D does not increase in response to dietary P deprivation in those animals. We have now measured 1,25 D production by cultured kidney cells from 4 week old HYP and normal (NL) littermates. The kidneys were removed, the cortex minced and digested in a collagenase the cells cultured in a serum-free medium containing transferrin, insulin and PGE$_1$. The cells reached confluence in 8 days when 1,25 D production was measured. 5 ml of fresh culture medium containing 5 µM 25-OH-D$_3$ was added to each flask and incubations continued for 1 to 8 hours. The flasks were then frozen and the cells + medium extracted with $CHCl_3$: methanol. The extracts were purified by LH-20 and HPLC and assayed for 1,25 D by receptor assay. Results are expressed as pmol 1,25 D/mg protein ± SD (n=3-5 for each time).

Hours	1	2	4	6	8
NL Cells	1.8±0.2	3.0±0.7	4.9±2.0	4.4±1.3	5.4±1.4
HYP Cells	1.1±0.2	2.3±1.2	2.8±1.0	3.1±0.9	4.1±1.8
P	<0.05	NS	<0.02	<0.05	<0.05

There was no difference in cell growth between NL and HYP. The lower 1,25 D production by HYP cells, presumably reflecting lower 1αOHase activity, suggests that decreased basal renal 1,25 D production may account for the inappropriately normal serum 1,25 D levels and the lack of its rise during P deprivation in HYP mice.

EFFECT OF CHRONIC METABOLIC ACIDOSIS ON VITAMIN D METABOLISM IN MAN. J.A. Kraut, E. Gordon, R.L. Horst*, J.C. Ransom, E. Slatopolsky, J.W. Coburn & K. Kurokawa. Depts. Med., VA Wadsworth Med. Ctr., UCLA & Wash. U. Sch. Med., Los Angeles, CA & St. Louis, MO & Natl. Animal Disease Ctr., Ames, IO.

Bone disease may occur in disorders associated with chronic metabolic acidosis (MA). This has been attributed, in part, to reduced production of $1,25(OH)_2D_3$. Although MA in the vitamin D deficient animal has been associated with reduced conversion of radiolabeled $25(OH)D_3$ to $1,25(OH)_2D_3$, studies in D replete man revealed no effect of MA on vitamin D metabolism. To examine this issue further, we measured serum iPTH, $25(OH)_2D$, $1,25(OH)_2D$ and $24,25(OH)_2D$ levels in 6 healthy subjects before and after 9 days of MA induced by the ingestion of ammonium chloride, 2 mEq/kg per day. In four subjects, we also measured changes in serum levels of $1,25(OH)_2D$ in response to a 10 hr infusion of parathyroid extract (PTE), 1 u/kg/hr both during control (C) and MA. Plasma HCO_3, pH and Ca were lower in MA (7.32±.01, 18.0±.4 mEq/L, and 9.2±.1 mg/dl, respectively) than C (7.42±.01, 28.0±.7 mEq/L, and 9.5±.1 mg/dl). Serum P, Mg, and iPTH did not differ. Serum levels of 25(OH)D, $1,25(OH)_2D$, and $24,25(OH)_2D$ in C (29±7 ng/ml, 13.6±1.3 pg/ml, 2.7±1.2 ng/ml, respectively) and MA (28±7 ng/ml, 14.3±0.9 pg/ml, 2.4±1.1 ng/ml) were not different. Moreover, serum $1,25(OH)_2D$ levels in each subject rose to a similar degree during infusion of PTE in C and MA. These data provide strong evidence that MA does not have a substantial impact on the plasma levels of $1,25(OH)_2D$ or generation of $1,25(OH)_2D_3$ in vitamin D-replete man and suggest that other mechanisms may account for bone disease in MA.

RENAL RESPONSE TO DIETARY PO_4 DEPRIVATION AND PARATHYROID HORMONE (PTH) IN SPONTANEOUS HYPERCALCIURIC (SH) RATS. K. Lau, T. Romani*, C. Rogers*, and B. Eby*. Div. of Neph., Dept. of Medicine, Univ. of Mich., Ann Arbor, Mich.

Previous work by us indicates that rats with SH are characterized by high urine cyclic AMP, corrected by chronic chlorothiazide. To examine the role of PTH resistance and disturbances in PO_4 balance, SH (N=25) and normocalcuric (NC) (N=11) rats were fed a 0.03% Ca and 0.60% PO_4 diet for 7 days. Urine Ca (μg/mg of creatinine), higher in SH rats (106 vs. 80, $p < .05$), increased by greater magnitude (Δ = 313, vs 110) with low PO_4 diet (≤ 0.03%) potentiating the hypercalciuria (419 vs 189). This exaggerated response to PO_4 deprivation, a maneuver known to diminish distal Ca reabsorption, supports our earlier findings of intrinsic proximal defects in SH rats. On day 4 of PO_4 deprivation, urine PO_4 was higher in SH rats (14.9 vs 11.3 mg) despite a lower plasma PO_4 (5.9 vs. 6.6 mg%). High PO_4 diet (1.2%) produced a greater fall in urine Ca (Δ = 66.6 vs 44.4, $p < .05$), attenuating but not abolishing the hypercalciuria (40 vs 31, $p < .005$). PTH (100 u/Kg, I.P.) elicited similar hypocalciuric (Δ urine Ca = 242 vs 268 μ g/5h) and phosphaturic (Δ urine PO_4 = 0.68 vs 0.58 mg/5h) response. We conclude (1) SH rats respond appropriately to chlorothiazide, PO_4 and PTH, primarily distally-acting hypocalciuric agents. (2) PO_4 deprivation exaggerates the hypercalciuria, consistent with our micropuncture findings of a proximal defect. (3) A subtle renal PO_4 leak is present, further emphasizing the pathophysiologic similarities between idiopathic hypercalciuria in man and SH in laboratory rats.

NUCLEOTIDES COMPETITIVELY INHIBIT NA-DEPENDENT PHOSPHORUS (P) UPTAKE BY BRUSH BORDER MEMBRANE (BBM) OF RABBIT PROXIMAL TUBULES. Ronald P. Lang*, Norimoto Yanagawa, Edward Nord*, and Leon G. Fine. Divisions of Nephrology, Center for the Health Sciences, and Sepulveda VA Medical Center, UCLA School of Medicine, Los Angeles, California.

It has been proposed that cellular NAD may be a modulator of tubular P absorption since it inhibits Na-dependent P uptake in BBM vesicles. Perturbations such as acidosis, + PTH and P deprivation alter the V_{max} for P uptake by BBM. If NAD is relevant to these situations, it should act by decreasing V_{max}. The present study examined 1) whether nucleotides other than NAD exert similar effects on Na-dependent P uptake in BBM; 2) whether the effect is seen in intact proximal tubules; 3) whether these nucleotides alter the K_m or the V_{max} of this process. 0.3 mM NAD, ADP-ribose, ADP, ATP and GDP (but not nicotinamide) all decreased lumen-to-bath P flux in proximal tubules at concentrations above 10^{-6} M. In BBM, all of these nucleotides exerted an effect on K_m which was increased from 0.2 mM to approximately 0.4 mM. V_{max} (30 nmol/µg/min) was not affected. The effect was also seen when alkaline phosphatase was maximally inhibited with theophylline. No evidence was found to indicate that BBM converts NAD to ADP-ribose. The fact that nucleotides lacking ribose exerted similar effects suggests that ADP-ribosylation is not involved in this process. Summary: A variety of adenine and guanine nucleotides inhibit P uptake by BBM and intact proximal tubules by acting as competitive inhibitors. It is unlikely that these molecules mediate physiological adaptations in P transport in which V_{max} is changed.

DIFFERENT SODIUM REQUIREMENTS FOR $1,25(OH)_2D_3$-STIMULATED Ca AND PO_4 TRANSPORT: FURTHER EVIDENCE FOR A "TWO-CELL" THEORY OF INTESTINAL TRANSPORT. D.B.N. Lee, M.W. Walling,* & J.W. Coburn. Research & Medical Services, Sepulveda & Wadsworth VA Med Ctrs & UCLA Sch Med, Los Angeles, CA

Studies from our laboratory have provided evidence for separate $1,25(OH)_2D_3$(1,25D)-stimulated Ca and PO_4(P) pumps (or cells) in the intestine. We studied the need for Na in these 1,25D-stimulated transport processes. Ca transport was examined in the colon, which only transports Ca in response to 1,25D, and P transport was studied in the jejunum, which mainly absorbs P after 1,25D. Transmural fluxes of Ca and P were measured in Ussing chambers using isotopic tracers. The cellular influx of ^{32}P across brush border was corrected for extracellular trapping using ^{3}H-polyethylene glycol (MW, 900). Substitution of Na by choline in the bathing media caused a marked reduction in short-circuit current (Isc, 38±7(SE) to 4±2 uA/cm^2, $p < 0.01$) but had no effect on 1,25D-stimulated Ca absorption (Jnet, 14±4 vs 12±3 nmole/cm^2/hr). On the other hand, graded reduction in bath Na levels led to proportional decreases in 1,25D-responsive P absorption; Isc and P Jnet fell from 142±6 to ~5±2 and from 102±9 to 19±6, respectively as bath Na was reduced from 144 to 25mM. Measurement of cellular P influx revealed a Na-dependent, saturable process and a Na-independent, non-saturable process. Only Na-dependent P entry was stimulated by 1,25D. These different Na requirements for 1,25D-stimulated Ca versus P transport provide further evidence for the existence of distinct Ca and P pumps ("two-cell" theory) for 1,25D stimulated transport in the gut.

STEROID-SENSITIVE HYPERCALCEMIA ASSOCIATED WITH COMMON VARIABLE IMMUNODEFICIENCY:? PSEUDOHYPERVITAMINOSIS D. J.M. Lemire*, L. Paunier*, S.C. Jordan*, J.W. Coburn, E. Slatopolsky, R.L. Horst*, D.J. Sherrard & R.N. Fine. UCLA Sch. Med., V.A. Wadsworth Med, Ctr., Wash. Univ. Sch. Med., Natl. Animal Dis. Ctr. & Seattle V.A. Hosp. L.A., Cal.

A 15 year old boy,with soft tissue calcification of the arm, had growth retardation,band keratopathy and splenomegaly. Serum (S) Ca was 11.2-12.8 mg/dl (ionized Ca, 5.76). S-Mg, P, albumin, alk. p-tase, iPTH (carboxy terminal) were normal (N); S-25(OH)D, 23 ng/ml (N, 10-55)and $1,25(OH)_2$ D3 25pg/ml (N,20-76). GFR (creat clearance) was 19.9 ml/min/1.73M^2 and urinary Ca, 358 mg/day (10.3 mg/Kg/d). X-rays showed increased bone density and nephrocalcinosis. There was panhypogammaglobulinemia and immunologic studies showed common variable immunodeficiency syndrome (CVI). Angiotensin converting enzyme was normal. Ca balance, conducted on 366 mg dietary Ca/day, showed persistent hypercalciuria and hypercalcemia. With prednisone (2 mg/Kg/day), S-Ca fell to 8.4 mg/dl, $1,25(OH)_2D_3$ to 7.4 pg/ml, iPTH tripled and GFR rose by 36%; on stopping prednisone, S-Ca rose to 12.5 mg/dl and $1,25(OH)_2D_3$ to 33 pg/ml. Bone biopsy revealed slightly increased osteoid but no increased resorption surface. The persistent hypercalciuria with high bone density suggests increased intestinal Ca absorption. The prompt response of S-Ca to steroids is similar to that noted in sarcoidosis;however, the normal $S-1,25(OH)_2$ D_3 levels suggest increased sensitivity to normal amounts of circulating vitamin D sterols. The relationship between this Ca disorder and CVI syndrome is uncertain at present.

EFFECT OF FILTERED LOAD AND PARATHYROID HORMONE ON PHOSPHATE UPTAKE BY RENAL BRUSH BORDER MEMBRANES. B.S. Levine, J.W. Coburn, and K. Kurokawa. Resch & Medical Services, VA Wadsworth Med Ctr & Dept Med, UCLA Sch Med, Los Angeles, California.

It is uncertain whether changes in filtered (F) phosphate (Pi) due to altered GFR affect its tubular transport. In the present study, uninephrectomy (NX) was used to increase both GFR/nephron and FPi and thereby assess the effect of increased FPi on proximal tubular transport. Transport was assessed using brush border membrane vesicles (BBMV) prepared from the renal cortex of sham-operated (C) or NX rats weighing 200-250g. BBMV, suspended in 320mM sorbitol, pH 7.5, were incubated in 100 mM NaCl, 120mM sorbitol and 0.1 mM ^{32}Pi, pH 7.5. Two days after NX or C, serum (S) creatinine was 0.71±.03mg/dl in C and 0.77±.02 in NX; SPi was 8.6±.2mg/dl in C and 8.6±.5 in NX. Pi uptake by BBMV was 1083±270 pmol/mg protein at 15sec incubation and 2117±434 at 1.5min in C and 1413±352 and 2963±623, respectively, in NX, ($p < 0.02$, n=5); at equilibrium (60 min incubation), Pi uptake was not different in C and NX. Na-dependent glucose uptake at 1.5 and 60 min was similar in both groups. In chronic (1 week) parathyroidectomized (PTX) rats, Pi uptake by BBMV 2 days after NX was 2841±598 in C and 3522±649 in NX ($P < .01$,n=5). In PTX rats studied 7 days after NX, Pi uptake at 1.5 min incubation was 2528±529 in C and 2757±457 in NX ($p > .05$). Thus, Pi uptake by BBMV is enhanced within 2 days after NX in both intact and PTX rats. These studies suggest that increments in FPi that occur via augmented GFR can enhance Pi transport by BBMV. This effect occurs within 2 days and in the absence of PTH; the lack of difference 7 days after NX may be due to compensatory hypertrophy.

EFFECT OF CHRONIC HYPERCALCEMIA (HCa) ON RENAL CORTICAL MITOCHONDRIAL CALCIUM (MCa) AND MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION. M. Levi, P.E. Arnold*, T.J. Burke, T. Berl, and R.W. Schrier. Univ. Co. Hlth. Sci. Ctr., Denver, Colorado.

In vitro studies have shown that MCa (nmoles Ca/mg) accumulation and state 3 respiration (S3, ADP stimulated oxidative phosphorylation, natoms O_2/mg/min) compete for the proton gradient across the mitochondrial inner membrane. Thus, the energy delivered by electron transport can be used to facilitate either MCa accumulation or ATP formation, but not both simultaneously. The present experiments were therefore performed to examine the effect of HCa (SCa 12.9 mg/dl) on MCa and S3 in the rat renal cortical mitochondrial tissue. MCa was significantly greater in HCa than normocalcemic (C) rats (41 vs 16, $p<.01$). This effect on MCa was associated with diminished S3 in HCa (110 vs 135 in C, $p<.05$). HCa resulted in moderate impairment of RBF (6.4 vs 7.2 ml/min/gkw in C, $p<.01$). Since impaired tissue perfusion may result in impaired S3 with subsequent MCa accumulation, the possibility that these alterations in MCa and S3 may be a direct consequence of the renal hypoperfusion was examined. MCa and S3 were therefore measured in HCa after chronic angiotensin II (AII) inhibition, which resulted in complete normalization of RBF (7.4 vs 7.2 in C). In these AII inhibited HCa rats, in spite of normal renal perfusion, MCa was still significantly increased (38 vs 16 in C, $p<.05$) and S3 was significantly impaired (112 vs 135 in C, $p<.05$). Our studies therefore suggest that HCa in the rat, even in the presence of normal renal perfusion, causes MCa overload which may result in impairment of S3.

THE ROLE OF THE KIDNEY AND THE SKELETON IN THE METABOLISM OF BIOLOGICALLY ACTIVE AND INACTIVE BOVINE PTH 1-34. J. Lewis*, K. Martin, K. Hruska, and E. Slatopolsky. Washington University Medical School, St. Louis, MO

We have shown in dogs that the metabolic clearance rate of biologically active PTH (1-84) is greater than that of oxidized (inactive) PTH (1-84). Moreover, the uptake of PTH (1-34) by the isolated perfused canine bone was greater than that of inactive PTH (1-34). The present studies were performed to further characterize the disappearance rates of PTH (1-34) in the rat. Syn-b-PTH (1-34) or oxidized syn-b-PTH (1-34), 25 µg/kg/b.w., were injected into three groups of rats: 1) normal, 2) bilateral ureteral ligation (BUL), and 3) bilateral nephrectomy (BNX). Since BUL does not decrease renal plasma flow in the first 2 hr, the results in this group reflect renal handling of PTH in the absence of GFR with maintenance of PTH delivery to the peritubular membrane receptors. Blood was collected at 2,20,40 and 60 minutes after injection of PTH. PTH (1-34) was measured by RIA. The results shown are the 20 minute values expressed as a percent of the 2 minute value.

	CONTROL	BUL	BNX
b-PTH (1-34)	17.7 ± 2.4	20.9 ± 0.9	28.4 ± 4.3
Oxidized b-PTH (1-34)	20.1 ± 1.0	39.0 ± 2.5	47.0 ± 1.3

The results demonstrate that the disappearance of oxidized PTH is slower in the BUL group reflecting a role of the kidney (presumably at the peritubular membrane) in the removal of PTH. In the BNX group, the difference in the results with active vs inactive PTH (1-34) suggests a major role of bone in the uptake of active PTH (1-34).

THE EFFECT OF ORAL ALUMINUM HYDROXIDE ON SERUM 1,25-$(OH)_2$-VITAMIN D LEVELS IN HEALTHY MEN. W.J. Maiefhofer, * R.W. Gray, * and J. Lemann, Jr., Departments of Medicine and Biochemistry, Medical College of Wisconsin, Milwaukee, WI

Dietary PO_4-deprivation (-P) in animals rapidly increases renal synthesis and plasma levels of 1,25-$(OH)_2$-D_3. However, we previously reported that serum 1,25-$(OH)_2$-D levels did not change when men ate a liquid diet providing only 3 mmol PO_4/day, a result that is likely due to a subsequently recognized effect of liquid diets to lower serum 1,25-$(OH)_2$-D levels. We have now measured fasting serum 1,25-$(OH)_2$-D levels in 5 men eating whole food diets: a) during control (C) when diet PO_4 = 54 ± 4 SD mmol/day and b) during 18 days (-P) when diet PO_4 = 31 ± 6 mmol/day and the subjects were given 115 to 165 mmol $Al(OH)_3$ baked into cookies and given in divided doses. Serum PO_4 averaged 1.34 ± 0.16 mM during C and was identical during -P, while urinary PO_4 fell from 30 ± 2 to 4 ± 2 mmol/day ($p<0.001$). Serum iPTH averaged 8.2 ± 2.6 and 7.7 ± 3.4 µlEq/ml during C and -P, respectively (NS). Despite the undetectable changes in serum PO_4 and iPTH levels, serum 1,25-$(OH)_2$-D levels rose from 85 ± 22 pM during C to 107 ± 29 pM during -P ($p<0.05$). Serum Ca averaged 2.42 ± 0.12 mM during C and also did not change during -P, while urinary Ca rose from 3.7 ± 1.2 to 6.0 ± 2.3 mmol/day ($p<0.025$). We conclude that phosphate deprivation in men can increase serum 1,25-$(OH)_2$-D levels by a mechanism which appears to be independent of fasting serum PO_4, Ca and iPTH concentrations.

EFFECT OF INTACT PARATHYROID HORMONE (PTH) AND ITS AMINOTERMINAL FRAGMENT ON HUMAN POLYMORPHONUCLEAR LEUKOCYTES: IMPLICATIONS IN UREMIA. S.G. Massry, C.C. Doherty*, P. Kimball*, D. Moyer* and N. Brautbar. Div. Nephrol., USC Sch. Med., Los Angeles, CA.

Derangement in leukocyte function occurs in patients with primary hyperparathyroidism (HPTism) and in those with uremia which is a state of secondary HPTism, suggesting that PTH affects leukocyte function. We examined the interaction between PTH and random motility (RM) of human polymorphonuclear leukocytes (PMNL) utilizing a modified Boyden chamber technique. Intact (1-84) PTH produced marked and significant stimulation of RM from 22.8±1.3 (SE) to a peak of 41.1±2.5 µ/60 min with 50 U of PTH/ml. There was a dose-response relationship between PTH concentration and the increase in RM. This effect of 1-84 PTH was independent of calcium concentration in media, was not mimicked by calcium ionophore and was not blocked by verapamil. PTH did not stimulate cyclic AMP production by PMNL. Quinidine also produced significant ($p<0.01$) increase in RM which was not additive to the effect of PTH. Prolonged exposure to PTH (16-20 hr) was associated with marked reduction in RM. RM of PMNL from uremic patients was significantly reduced and was not stimulated by PTH. The 1-34 PTH and the 53-84 PTH did not enhance RM. Calcitonin, glucagon, insulin or ADH did not affect RM. The data indicate 1) PMNL are target organs for the intact but not the 1-34 PTH, 2) PTH effect is specific for the hormone, 3) effect of PTH is not mediated by calcium influx or stimulation of cAMP, and 4) prolonged exposure of PMNL to PTH may adversely affect RM. The secondary HPTism of uremia may be responsible for altered PMNL function.

SERUM LEVELS OF BONE GLA-PROTEIN (BGP) PREDICT UNDERLYING BONE HISTOLOGY IN DIALYZED PATIENTS. H.H. Malluche, M.C. Faugere*, P. Fanti*, P.A. Price*. Univ of Kentucky, Div Nephrol, Bone & Min Metab, Lexington KY & Univ California, San Diego.

Recently a radioimmunoassay (RIA) became available for BGP, the most abundant non-collagenous bone protein. The present study correlates quantitative bone histology with results of serum BGP measured by RIA in serum of 21 dialyzed patients (pts). Blood samples were obtained at time of biopsy. Bone histology showed low turnover osteomalacia (LTOM) in 5 pts, mixed uremic osteodystrophy (MUO) in 11 pts and predominant hyperparathyroid bone disease (HPT) in 5 pts. Values for BGP were above normal in LTOM (71.4±28.1 vs 6.78±0.20 ng/ml, $p<0.01$), clearly elevated in MUO (257±76.5 ng/ml) and extremely elevated in HPT (874±210 ng/ml). Differences in BGP between each histologic group were significant ($p<0.01$).

There was a significant correlation between BGP and the following histologic parameters: surface of osteoid ($r=0.83$), bone osteoblast interface ($r=0.66$), osteoblastic index ($r=0.73$), surface and volume of fibrosis ($r=0.77$ and 0.86) and mineral apposition rate ($r=0.69$). There were no significant differences in serum levels of 1,25$(OH)_2$Vit D, alkaline phosphatase, parathyroid hormone (PTH), serum Ca and P between the 3 histologic groups.

The data indicate that: (1) BGP is a good marker of osteoblastic activity. (2) BGP is a useful diagnostic tool for uremic bone disease and, thus, (3) the need for diagnostic bone histology in uremia may decrease. (4) Using BGP levels LTOM can be clearly differentiated from HPT. This is not always possible using serum PTH and 1,25$(OH)_2$ Vit D measurements.

THE EFFECT OF ALUMINUM (Al) LOADING ON SPECIFIC TISSUE CALCIUM (Ca) AND MAGNESIUM (Mg) CONCENTRATIONS IN NORMAL RATS. GH Mayor, MA Burnatowska-Hledin*, Dept. of Medicine, Michigan State University, E. Lansing, Michigan.

Considerable evidence implicates Al in the etiology of dialysis dementia but the mechanism of Al toxicity is not known. Since increased brain Ca concentrations have also been associated with disturbances in the central and peripheral nervous system, this study examines the effects of Al loading on Ca and Mg concentrations in brain and other tissues. Normal rats fed a normal diet were given daily intraperitoneal injections of either acidified saline (controls, n=5) or 2.7 mg of $AlCl_3$ (n=5) for 10 days. Tissue Al, Ca, and Mg concentrations were determined by plasma emission spectrophotometry and total serum Ca (S_{Ca}) by atomic absorption. Al loaded rats had significantly higher Al concentrations than controls in bone (70.6±19.0 vs. 7.4±2.2 ppm, $p<.001$); liver (379 ±109.8 vs 1.2±0.3 ppm, $p<.001$); spleen (332.6± 141.7 vs 5.5±5.1 ppm, $p<.001$); heart (9.3±1.0 vs 4.5±1.6 ppm, $p<.05$) and brain (4.6±0.6 vs 3.0± 0.5 ppm, $p<.05$) and significantly higher Ca concentrations than controls in liver (373.4±50.4 vs 117.1±18.5 ppm, $p<.01$); spleen (265.9±33.0 vs 117.4±8.5 ppm, $p<.001$), and brain (4329.3±2900.9 vs 255.2±56.8 $p<.05$), but not heart (124.9±0.3 vs 129.6±5.8 ppm, ns). Following Al loading, brain Ca concentrations varied over a wide range and were significantly correlated to brain Al concentrations ($r=0.971$). Al loading had no significant effect on Mg concentration or on S_{Ca}. These results indicate that Al affects specific tissue Ca but not Mg concentrations, which ultimately may be involved in the mechanism of Al intoxication.

EFFECTS OF DIHYDROTACHYSTEROL (DHT), 25-OHD_3 (25D) AND 1.25$(OH)_2D_3$ (1,25D) ON GROWTH VELOCITY (GV) IN CHILDREN WITH CHRONIC RENAL FAILURE (CRF). Alice T. Mazur,* Margaret Vaughan-Norton,* and Michael E. Norman. Children's Hosp. of Phila., Phila. PA.

Nine children, ages 2.8 to 14.9 yrs (mean 6.5 ±0.7 yrs) with CRF and renal osteodystrophy have completed one year of a comparative trial of the effects of DHT (0.1-1.0mg/d), 25D (1-2ug/kg/d), and 1,25D (15-50ng/kg/d) on GV, calcium metabolism, and bone histology. Sex, age, bone biopsy, and GFR were used to allocate each patient into three treatment groups. One patient in each group received DHT, one 25D, and one 1,25D. GV was defined by standard deviation scores using Tanner growth charts.

GV (mean±SEM) was -1.1±0.4 pre-treatment and increased to -0.4±0.4 for the entire patient population. Individual group GV scores were: DHT: -1.6 ±1.1 to 0.3±0.4 ($p<.05$); 25D:-1.0±0.6 to -0.8±0.8; and 1,25D:-0.5±0.6 to -0.5±0.9. GV correlated with mean increase in plasma 1,25D (46.9±4.8 to 50.7±8.1 $r=.6$, $p<.01$) for all nine patients, but not in any of the individual treatment groups. Serum calcium did not increase (9.6±0.3 to 9.6±0.1). Urine calcium did increase after treatment in all pts. and correlated with drug dosage only in the 1,25D group. Serum bicarbonate and phosphorus levels remained normal. There was no significant increase in weight for height index (WHI) despite dietary counceling. WHI did not correlate with GV. Serum creatinine and GFR did not change after treatment.

In conclusion, DHT, but not 25D or 1,25D, improved GV in the initial yr of therapy; the increase in plasma 1,25D could reflect an increase in plasma 25OH-DHT as measured by HPLC.

EFFECTS OF CALCIUM IONOPHORE A23187 ON FLUID AND PHOSPHATE REABSORPTION IN RABBIT PROXIMAL CONVOLUTED TUBULES (PCT). J. Wade McKeown. VA Med. Ctr.and Univ. of Michigan, Ann Arbor, Michigan.

Previous work has demonstrated an increase in both fluid and phosphate transport in PCT when either lanthanum or verapamil are added to the lumenal perfusate (Clin. Res. 30:458A, 1982). This effect could be due either to a decrease in movement of calcium (Ca) across the lumenal membrane or a decrease in Ca binding to the lumenal membrane. Studies were performed to assess the effect of increased lumenal membrane Ca entry by the addition of Ca ionophore A23187 (2 µg/ml) to the perfusate of the isolated PCT [perfused and bathed in artificial fluids designed to simulate ultrafiltrate and rabbit serum].

Effects of Lumenal A23187

J_V			J_{PO4}		
Control	A23187	Recontrol	Control	A23187	Recontrol
1.099	1.313	0.962	4.56	5.06	5.28
±0.14	±0.10	±0.24	±0.72	±0.81	±1.03
$P<0.001$		$P<0.05$	NS		NS
N=11		N=9	N=11		N=10

Addition of A23187 to the bathing solution in six tubules resulted in no change in either J_V or J_{PO4}.

Conclusions: 1) Increased lumenal entry of Ca results in an increase in J_V and not J_{PO4}. 2) This effect is reversible on removal of A23187 from the perfusate. 3) Addition of A23187 to the bath had no effect on J_V or J_{PO4}.

Combined with previous work, these findings suggest the existence of two Ca pools, one membrane bound and one cytosolic which both importantly affect transport processes in PCT.

IMPAIRED NEPHROGENOUS cAMP RESPONSE IN THE SPONTANEOUSLY HYPERTENSIVE RAT. David A. McCarron, Division of Nephrology, Oregon Health Sciences University, Portland, Oregon.

The spontaneously hypertensive rat (SHR) has lower serum ionized Ca^{2+} and higher parathyroid hormone (PTH) concentrations throughout its lifetime than its normotensive control, the Wistar-Kyoto rat (WKY). The mature SHR develops hypercalciuria and nephrolithiasis. We characterized further the SHR's mineral metabolism during the developmental phase of its hypertension. At 4 weeks of age (WOA), 6 SHRs and 6 WKYs (males) were begun on a standard diet (1% Ca^{2+} by weight). Between 11 and 17 WOA, repeated timed urines for creatinine (Cr), cAMP and electrolytes and serums for Mg, PO_4 and Cr were obtained.

BP rose ($p<.001$) in the SHR but not the WKY. Mean (±SEM) serum Mg [1.54±.04 (mg/dl) SHR vs 1.41±.04 WKY] was higher ($p<.05$), and serum PO_4 lower [5.78±0.68 mg/dl, SHR vs 6.82±0.50, WKY] ($p=.06$) in the SHR. Cr [0.47±.02 (mg/dl) SHR vs 0.48±.02 WKY] and urinary electrolyte (Na,Ca,PO_4 and Mg) excretion were similar. Mean nephrogenous (N) cAMP (nM/mg Cr) was lower ($p<.02$) in the SHR (10.3±.9, SHR vs 15.2±1.7, WKY).

In conclusion, the SHR has multiple abnormalities of mineral metabolism that include alterations in serum Mg and PO_4 as well as impaired NcAMP generation in the face of low serum Ca^{2+} and elevated PTH levels. This impaired renal NcAMP response may reflect, in part, the SHR's known abnormalities in membrane Ca^{2+} and may contribute to the development of hypercalciuria and nephrolithiasis in the adult SHR. The reduction in serum PO_4 appears to be independent of PTH's effects on PO_4 reabsorption.

MOBILIZATION OF CALCIUM BY METABOLIC ACIDOSIS: EVIDENCE FOR ENHANCED CELL MEDIATED BONE RESORPTION. D.R. Mishler*, J.A. Kraut, & K. Kurokawa, Med. & Resch. Svc., Wadsworth VA Med. Ctr. and UCLA Sch. Med., Los Angeles, CA.

Metabolic acidosis (MA) is associated with increased urinary Ca excretion which is thought to arise as a result of increased mobilization of Ca from bone. The mechanism responsible for this mobilization of Ca from bone is, however, unclear. To study the mechanism, we examined whether the administration of colchicine or calcitonin, agents which block cell-mediated bone resorption, could modify the mobilization of Ca from bone that was induced by MA. The release of Ca from bone was assessed by the increments in serum Ca in thyroparathyroidectomized (TPTX) rats studied after MA for 16 hrs. TPTX rats were gavaged with 1.8% NH_4Cl; 16 hrs later, serum Ca was measured and colchicine, 0.2 mg/100 g body wt. i.p., or calcitonin, 0.6 mU/g body wt. s.c., was injected. Controls received similar volumes of saline, i.p. or s.c. The rats were again gavaged with NH_4Cl, and serum Ca was measured after either 2 hrs (calcitonin) or 4 hrs (colchicine). From a baseline of 7.2±0.2 mg/dl, serum Ca rose by 1.2 to 1.5 mg/dl after the acid feeding. The administration of colchicine or calcitonin caused a fall in serum Ca of -1.1±.2 mg/dl ($p<.01$) and -0.7±1.2 mg/dl ($p<.05$), respectively, whereas the saline had no effect. These data show that inhibition of bone cell activity abolishes or attenuates the calcemic response to MA and thus suggest that the mobilization of Ca from bone by MA is due, in part, to stimulation of cell-mediated bone resorption.

REPETITIVE FIRING OF MOTOR NERVE TERMINALS IN LOW EXTRACELLULAR Ca and Mg. Stanley Misler*(intr. by R.E. Longnecker) Rockefeller Univ., New York, NY

Hypocalcemia and/or hypomagnesemia result in neuromuscular hyperexcitability manifested as twitching, hyperreflexia, or tetany. Since short bursts of action potentials are a frequent EMG correlate of these symptoms, repetitive firing was investigated at the neuromuscular junction (NMJ). At the isolated frog NMJ, reducing $(Ca + Mg)_o$ from 2.0 to 0.4 mM consistently results in doublet muscle endplate potentials (epp) following a nerve impulse, strongly suggesting that the nerve terminal is firing twice within 10 msec in response to a single shock. Under similar ionic conditions, direct stimulation of a muscle or a sheathed nerve trunk did not result in repetitive firing of these tissues suggesting that, in vitro, nerve terminal is the site of maximal excitability. Short periods of nerve trunk stimulation at 20 Hz resulted in triplet or quadruplet epp's. When $(Ca + Mg)_o$ = 0.55mM, replacing 50mM NaCl with 95mM sucrose converted repetitive firing from a rare to a consistent occurrence. Repetitive firing could be reversed by: a) replacing the sucrose with 50mM chloride salts of impermeant univalent cations such as glucosamine or arginine; b) increasing $(Ca)_o$ or $(Mg)_o$ by 0.2mM; or c) adding 0.05mM Mn or Co. This suggests that repetitive firing is influenced by a negatively charged external site, which is electrostatically screened by Ca, Mg or Na, but may bind trace metals. These cation effects are at least qualitatively predicted from data on cation modulation of Na channel gating in nerve, which is often attributed to cationic screening or binding of gate-associated surface charge.

HUMAN RENAL CANCER CELLS PRODUCE HYPERCALCEMIA AND A NOVEL PROTEIN RECOGNIZED BY PARATHYROID HORMONE RECEPTORS. Robert A. Nissenson*, Richard D. Williams*, and Gordon J. Strewler* (intr. by Allen I. Arieff). Depts. Medicine and Surgery VAMC and University of California, San Francisco, Calif.

The purpose of this study was to develop a model system for human malignancy-associated hypercalcemia (MAH) and to determine whether a PTH-like factor is produced. To accomplish this, we injected dysthymic ("nude") mice subcutaneously with cultured cells derived from a renal cell adenocarcinoma (RCC) from a patient with MAH. Animals developed tumors with one week, and died within 4-8 weeks of innoculation. Mean serum calcium rose from 9.1 ± 0.4 (S.D.) mg/dl to 15.0 ± 3.0 mg/dl 20 days after innoculation ($p<0.001$). Serum calcium normalized within two days after total tumor resection. Production of a PTH-like factor by these RCC cells was tested in a renal adenylate cyclase (AC) assay previous shown to be specific for PTH. Spent culture medium from the RCC cells markedly stimulated AC; activation curves were parallel to those produced by PTH. Activation by the RCC factor was inhibited by the competitive PTH antagonist [^{8}Nle,^{18}Nle,^{34}Tyr]bPTH(3-34)amide, and was abolished by preincubation with trypsin. Gel filtration chromatography demonstrated that the factor was not coeluted with PTH(1-84), but rather was eluted between albumin (MW 67,000) and PTH(1-84) (MW 9,500). Furthermore, the factor was not recognized by 4 region-specific PTH antisera. Thus these RCC cells produce both hypercalcemia in nude mice and a protein factor which interacts with renal PTH receptors, but is distinct from native PTH. This factor may be representative of the etiologic agents producing hypercalcemia in many patients with MAH.

CHARACTERIZATION OF CALCIUM-ATPase IN PARATHYROID CELL MEMBRANES. J. Morrissey and S. Klahr. Washington Univ. Med. School, St. Louis, MO

Changes in cytosolic Ca^{++} are believed to be a major determinant in parathyroid hormone secretion. The concentration of cytosolic Ca^{++} is maintained by extrusion from cells and uptake by organelles. Extrusion mechanisms for Ca^{++} at the cell membrane include a Na/Ca exchange and a Ca^{++}-ATPase. These systems have not been characterized in parathyroid cells. We recently described a Na/Ca exchange mechanism (Endocrinology 111: 225, 1982) in parathyroid cells. We, therefore, sought to characterize a Ca^{++}-ATPase in membrane fractions from canine parathyroid glands. ATPase activities were measured by cleavage of [γ-^{32}P]ATP in the presence of 10 mM azide in order to inhibit mitochondrial ATPases. The membranes had an active Mg^{++}-ATPase (4.8 ± 0.4 nmole ATP/mg prot/10 min) and a ouabain inhibitable Na^{+}/K^{+}-ATPase (1.5 ± 0.3 nmole ATP/mg prot/10 min). Assays for Ca^{++}-ATPase were performed with azide and 1 mM ouabain to inhibit Na^{+}/K^{+}-ATPase activity. Without calmodulin the membranes had an active Ca^{++}-ATPase (1.3 ± 0.6 nmole/ATP/mg prot/10 min) with a Km for Ca^{++} of 4 μM. With calmodulin the Ca^{++}-ATPase activity increased to 3.6 ± 0.3 nmole ATP/mg prot/10 min with a Km of 0.3 μM for Ca^{++}. The Km for calmodulin was about 0.5 μM. The Ca^{++}-ATPase activities were stimulated (25%) by 2-5 mM K^{+} and were not inhibited by 10 mM theophylline, suggesting respectively that the ATPase resides in the plasma membrane and does not represent alkaline phosphatase activity. Thus, parathyroid cells can extrude Ca^{++} by a Na/Ca exchange mechanism or by a Ca^{++}-ATPase system.

VITAMIN D THERAPY IN ALUMINUM-RELATED OSTEOMALACIA. SM Ott*, RR Recker , JW Coburn, DJ Sherrard, VAMC & Univ. of Washington, Seattle, WA.

In a previous study of 47 patients (pts) with renal osteodystrophy, 14 pts with osteomalacia (increased osteoid area without marrow fibrosis) did not improve after 1,25 $(OH)_2D$ therapy. All had high bone aluminum (Al), whereas responders had low Al. Since 25 (OH)D may have a different action than 1,25 $(OH)_2D$, we examined bone Al in 34 pts who were given 25 (OH)D for 17 weeks. Bone biopsies after treatment revealed 8 pts with osteomalacia; Al levels were heavy in 5, mild in 1, and low in 2 (.80 ± .64 mm/mm^2, mean ± SD). In the other 26 pts only 1 had heavy Al, 7 mild, and 18 low levels (.10 ± .21, $p < .01$ by chi-square).

Next we treated 23 pts with 24,25 $(OH)_2D$. All had osteomalacia refractory to 1,25 $(OH)_2D$ and high bone Al. Clinical improvement was seen in 69% within 4 months of therapy. The 8 clinical responders who had repeat biopsies had definite histologic improvement, with decrease in osteoid area from 37 ± 22% to 10 ± 4, $p=.01$. Bone Al, however, was unchanged: 1.28 ± .47 to 1.14 ± .71. The histologic pattern of Al deposition demonstrated increased amount in cement lines. 24,25$(OH)_2D$ was not helpful in other types of osteodystrophy. We conclude that high bone Al is associated with a form of osteomalacia which is refractory to therapy with either 1,25$(OH)_2D$ or 25 (OH)D, but which often responds to 24,25$(OH)_2D$. How 24,25 $(OH)_2D$ mineralizes osteoid despite persistant Al is a problem demanding further study.

25(OH)D3 BLOCKS PTH-INDUCED DEPRESSION OF NET FLUID ABSORPTION BY THE RABBIT PARS RECTA. R.A. Peraino, Baylor College of Medicine, Department of Medicine, Houston, Texas.

Parathyroid hormone depresses the net rate of fluid absorption in vitro in the isolated rabbit pars recta. The mechanism of the hormone effect is due to inhibition of bicarbonate - dependent fluid absorption. In studies examining the effects of vitamin D metabolites on phosphate transport by the pars recta, I observed that the effect of PTH to depress fluid absorption was blocked in the presence of a physiological concentration of 25(OH)D3. Segments of pars recta, isolated from New Zealand white female rabbits were bathed in an artificial solution simulating plasma water with 6 g/dl bovine serum albumin. The segments were perfused with an ultrafiltrate of the bath containing ^{3}H-inulin as the volume maker. In Group I, after control collections, synthetic (1-34) PTH was added to the bath in a concentration of 0.2 U/ml and samples obtained. In Group II, 25(OH)D3 was added to the bath prior to filtration in a concentration of 100 nM. After collections were obtained, PTH was then added to the bath with 25(OH)D3 and samples obtained. The results were:

	control	PTH	25(OH)D3	25(OH)D3+PTH
Jv	0.38	0.24	0.27	0.25
nl/min/mm	±0.03	±0.05	±0.02	±0.03
p	< .005		NS	
n	8		13	

Perfusion rates did not vary in either group while the potential difference fell in both groups.

In summary, 25(OH)D3 blocks PTH induced depression of Jv in the rabbit pars recta. Given that PTH works via inhibition of HCO3 absorption, then 25(OH)D3 may be a modulator of PTH action, and renal acidification/ bicarbonate absorption.

$24,25(OH)_2D_3$ ENHANCES THE CALCEMIC EFFECT OF $1,25(OH)_2D_3$: EVIDENCE FOR HYPOCALCIURIC ACTION OF $24,25(OH)_2D_3$. Mordecai M. Popovtzer,* Tony Hayek,* and Hanna Wald * (intr. by Stanley Cortell). Hadassah-Hebrew Univ. Hosp., Nephrology Services, Jerusalem , Israel.

Whereas $1,25(OH)_2D_3$ has been shown to augment serum (S) and urinary calcium (UcaV), the response to $24,25(OH)_2D_3$ has been studied in less detail. The present study examined the effects of $24,25-(OH)_2D_3$ alone and with $1,25(OH)_2D_3$ on Sca and on UcaV. Three groups (g) of rats received for 5 days the following metabolites of vit D_3 : g.1 $24,25-(OH)_2D_3$, g.2 $1,25(OH)_2D_3$, and g.3 $24,25(OH)_2D_3$ with $1,25(OH)_2D_3$. Sca and UcaV were recorded daily . In g.1 neither Sca nor UcaV changed. In g.2 Sca rose from 5.78 ± 0.07 (x ± SE) to 6.39 ± 0.09 ($p<.01$) after 24 h , and subsequently averaged 6.41 ± 0.15 mEq/L, UcaV rose from 50 ± 8 to 135 ± 35 after 24 h ($p<0.01$) and averaged 561 ± 41 µEq/24 h thereafter. In g.3 the rise in Sca from 5.78 ± 0.07 to 6.33 ± 0.11 ($p<0.01$) after 24 h did not differ from that in g.2, however the rise in UcaV from 50 ± 8 to 99 ± 16 was less than that in g.2 ($p<0.01$). In the subsequent 4 days the Sca that averaged 6.82 ± 0.12 was higher than that in g.2 ($p<0.01$),while UcaV that averaged 549 ± 43 did not differ from that in g.2. These results show that $24,25(OH)_2D_3$ alone does not alter the Sca or the UcaV, however when it is given with $1,25(OH)_2D_3$ 1) it elicits a decrease in UcaV after 24 h, and 2) during the course of the subsequent days it produces an increase in the Sca without causing a commensurate rise in UcaV. These observations suggest that $24,25-(OH)_2D_3$ potentiates the calcemic action of $1,25-(OH)_2D_3$ and that this response , at least partly is due to increased tubular reabsorption of ca.

CORRELATION BETWEEN BONE HISTOLOGY (BH) AND CT SCAN OF LUMBAR VERTEBRA (LV) IN RENAL OSTEODYSTROPHY (RO). B. Piraino*, D. Herbert*, A. Greenberg, R. Rault, J. Dominguez, M. Sorkin, and J. Puschett. University of Pittsburgh School of Medicine, Pittsburgh, PA.

To determine whether a dialysis patient with RO has osteomalacia (OM) or osteitis fibrosa (OF), a bone biopsy (BB) is necessary. Neither PTH levels nor skeletal survey reliably predict BH. In this study the CT scan-determined bone mineral content (BMC) of the LV was compared to BB, in an attempt to find a noninvasive predictor of BH. A CT scan of the first LV was obtained with each patient lying over a phantom containing four known K_2HPO_4 solutions. BMC was determined by reference to the calibration curve obtained from these standard solutions. Bone biopsies were obtained after tetracycline labelling. Based on fluorescence and the Goldner stain, the BB were divided into two groups: OF alone; or OM with or without OF (OM ± OF). The following results (means ± SE) were obtained:

	OM ± OF	OF
Number of patients	5	7
Age (years)	51.8 ± 5.4	49.9 ± 4.0
BMC* (mg/cm^3)	77.2 ± 17.8	153.1 ± 17.7

*$p < .05$

Thus, mean bone mineral content is higher in patients with OF than in patients who have a component of OM. CT scan-determined LV bone mineral content shows promise as a means of differentiating OF from OM. Studies of other factors that affect bone density, including race, sex, and concomitant osteoporosis, will be needed to better define the role of this non-invasive technique.

EFFECT OF RESTRICTION AND SUPPLEMENTATION OF DIETARY PHOSPHATE ON PLASMA $1,25(OH)_2D$ AND SERUM iPTH In CHILDREN WITH MODERATE RENAL INSUFFICIENCY (MRI) A.A. Portale*, B.E. Booth*, B.P. Halloran*, and R.C. Morris Jr., GCRC, Depts. of Pediatrics and Medicine, Univ. of California, San Francisco, CA.

In children with MRI in whom dietary intake of phosphate (Pi) is normal but in whom remaining nephrons process greatly increased amounts of Pi, a Pi-mediated suppression of the activity of renal 1α-hydroxylase might reduce plasma levels of $1,25-(OH)_2D$ and thereby contribute to the hyperparathyroidism of these patients. To test this hypothesis, we measured circulating levels of $1,25(OH)_2D$ and iPTH in 8 children with MRI (mean GFR 46±4 ml/min/1.73M^2) in whom daily dietary intake of Pi was first normal (1.2g), then either restricted (0.4g, n=7) or supplemented (2.4g, n=6) for 5 days. With normal Pi intake, a) the mean (±SE) plasma level of $1,25(OH)_2D$ was lower than that in age-matched controls (n=17) (28±2 vs 36±2 pg/ml, $p<0.02$); b) serum levels of iPTH were increased (C-iPTH, 59±9 vs 17±3 µleq/ml; N-iPTH, 198±14 vs 119±8 pg/ml; $p<0.001$); c) throughout the day, serum levels of Pi were not higher than those in 5 normal children.

Dietary Pi	$1,25-(OH)_2D$	C-iPTH	N-iPTH	FEPi
	% change from control (normal Pi diet)			
Restricted	+60±11	-25±6	-25±3	-68±3
Supplemented	-32±6	+131±39	+45±17	+102±23

all changes significant at $p<0.005$

Plasma levels of 25-OHD remained normal and unchanged. The changes induced in $1,25(OH)_2D$ varied inversely with those in iPTH ($r=0.88$, $p<0.001$). These data demonstrate that in children with MRI, dietary intake of Pi is a major determinant of the blood levels of both $1,25(OH)_2D$ and iPTH. The data provide support for the hypothesis stated.

INHIBITION OF RENAL BRUSH BORDER PHOSPHATE TRANSPORT AND STIMULATION OF RENAL GLUCONEOGENESIS BY CYCLIC AMP. J B Puschett, S A Kempson, and J C Kowalski*, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Gluconeogenesis (GNG) in the proximal tubule may play a role in intracellular regulation of phosphate transport across the luminal brush border membrane (BBM). The possible relationship between GNG and BBM transport was examined by testing whether administration in vivo of phosphaturic doses of 8-bromo-cyclic AMP (8BcAMP) or parathyroid hormone (PTH) cause changes in both GNG and BBM phosphate transport. Thyroparathyroidectomized dogs were anesthetized, equilibrated and control urine collections obtained prior to removing the left kidney. Subsequent intravenous infusion of 8BcAMP at 50 mg/hr for 2 hrs increased fractional excretion of phosphate from 4 ± 1 (controls) to 29 ± 4% ($p < 0.001$) without changing GFR. In BBM vesicles isolated from the renal cortex the initial Na-dependent transport of phosphate was decreased from 747 ± 135 (controls) to 564 ± 126 pmol/mg/15 sec after 8BcAMP ($p < 0.025$), but Na-independent phosphate uptake and Na-dependent L-proline uptake were not changed significantly. Renal GNG was assessed in the same animals by incubating renal cortical slices with 10 α-ketoglutarate. The rate of glucose production was increased from 2.5 ± 0.3 (controls) to 5.3 ± 0.5 μmol/g tissue/hr after infusion of 8BcAMP ($p < 0.001$). PTH in vivo, like 8BcAMP, inhibited BBM phosphate transport and stimulated renal GNG. We conclude that the inhibitory action of cyclic AMP and PTH on BBM phosphate transport is accompanied by stimulation of GNG. This suggests a possible role for GNG in the regulation of BBM transport of phosphate.

THE EFFECT OF DIETARY SODIUM LOADING ON DIVALENT IONS IN HYPERTENSION. L.M. Resnick*, D.B. Case, T.G. Pickering*, J.H. Laragh. Hypertension Center, New York Hospital-Cornell University Medical Center, New York, N.Y.

As both magnesium (Mg^{++}) and calcium (Ca^{++}) may contribute to blood pressure homeostasis, we studied the effects of different sodium intakes on blood pressure (BP) and the metabolism of these ions in hypertension. We measured urine aldosterone and electrolyte excretion, blood Mg^{++}, ionized and total Ca^{++}, plasma renin activity (PRA), and parathyroid hormone (PTH) levels in 6 untreated patients on both low (U_{Na}=48±11 mEq/day) and high (U_{Na}=312±42 mEq/day) sodium diets. Sodium loading caused a) a significant rise in systolic BP (9.1±3.9%, 145±7/96±2 to 157±6/100±2 mmHg, $p<0.05$), b) a consistent (6/6) fall in serum ionized Ca^{++} (2.16±0.04 to 2.03±0.05 mEq/L, $p<0.005$), with c) a reciprocal rise in serum Mg^{++} (1.83±0.05 to 1.97±0.06 mEq/L, $p<0.05$). Urinary Ca^{++} excretion rose (154±32 to 218±31 mg/day, $p<0.02$). There were no significant changes in intact or C-terminal PTH levels.

The baseline systolic blood pressure observed in all studies was directly related to ionized calcium (low sodium group, $r=0.76$, $p<0.05$; high sodium, $r=0.88$, $p<0.025$). Moreover, the increment in systolic blood pressure with sodium loading was an inverse function of the induced decrement in ionized calcium ($r=-0.75$, $p<0.05$).

We conclude: (1) systolic blood pressure is a function of serum ionized calcium, (2) sodium loading as it raises pressure causes significant reciprocal changes in serum ionized calcium and magnesium levels, (3) changes in calcium and magnesium levels may mediate the pressor effects of dietary sodium administration.

INFLUENCE OF LUMINAL pH ON THE ADAPTATION OF PROXIMAL PHOSPHATE TRANSPORT TO CHANGES IN DIETARY PHOSPHATE. Gary A. Quamme, Susan J. Whiting* and Norman L. Wong, Dept. of Medicine, Health Sciences Centre, U.B.C., Vancouver.

Studies were performed in rats to determine the effects of luminal pH on the tubular adaptation to low phosphate (Pi) diets. Early proximal convoluted tubules were perfused in vivo with equilibrated Ringer buffered with HEPES, pH 7.65 or MES, pH 6.5 at 5% CO_2. Unidirectional lumen-to-plasma flux was greater at luminal pH 7.65 than 6.5 in rats on a normal Pi diet, urinary Pi excretion, FE 5%. The apparent Jmax was 34.5±7.8 pmol.min^{-1}.mm^{-1} and Km 1.7±0.2 mM at pH 7.65 and at pH 6.5 was 15.6±0.9 and 1.0±0.1 mM, respectively. In dietary restriction (FE 0.01%), the apparent values were pH 7.65: Jmax 22.7±4.6 and Km 0.7±0.2*; pH 6.5: Jmax 32.6±5.3* and Km 1.2±0.1. Uptake studies were performed on isolated brush border membrane (BBM) vesicles. BBM vesicles were prepared from rats on normal (ND) or Pi restricted diets (LD) and Pi uptake was determined with external vesicle pH of 7.5 or 6.5 and internal pH of 7.5. The Vmax of ND with pH 7.5 and 6.5 was 1.0±0.2 and 0.6±0.2 nmol.mg^{-1} protein .20 s^{-1}, and the Km was 0.28 ±0.01 and 0.08±0.04 mM, respectively. The 6.5: 7.5 ratio for Vmax increased from ND to LD, 0.6 to 1.2, and the Km from 0.3 to 2.1, respectively. The in vivo and in vitro data indicate that Pi absorption is normally greater from alkaline pH due to direct affects on the BBM and adaptation to LD results in relatively greater absorption from luminal acidic pH values.

BLOOD IONIZED CALCIUM (Ca++) AND NOT PROTON CONCENTRATION (H+) MODULATES SERUM 1,25$(OH)_2$D3 (1,25D) DURING CHRONIC METABOLIC ACIDOSIS (CMA) IN THE RAT. GS Riera*, DA Bushinsky, MJ Favus*, FL Coe. Michael Reese Hospital and Univ. of Chicago, Chicago, Il.

Previously we have shown that CMA due to NH_4Cl administration (1.5% in drinking water) prevents the rise in 1,25D during low calcium diet (.002% Ca, LCD; 1,25D, 27±8 vs 204±24 pg/ml; $p<.001$ vs LCD without acid). Suppression was not due to reduced parathyroid hormone concentration, elevated serum phosphorus ($PO_4^{=}$) or total calcium concentration. However, Ca++ and H+ were inversely correlated with 1,25D ($r=-.84$ and $r=-.67$, respectively; both $p<.01$).

To determine whether Ca++ or H+ modulated 1,25D, we infused rats with 1.33 uM/min EGTA (a calcium chelator) alone (EGTA), with 1.33 uM/min $CaCl_2$ (EGTA + 1.33 Ca) or with 2.2 uM/min $CaCl_2$ (EGTA + 2.2 Ca) to vary Ca++ without altering H+. Rats ate LCD and had 1.5% NH_4Cl in their drinking water during the 12-day experiment. For the last 24 hr, rats were infused continuously through venous catheters, before blood was drawn through arterial catheters, both placed prior to the experiment.

	EGTA	EGTA + 1.33 Ca	EGTA + 2.2 Ca
n	7	7	9
1,25D (pg/ml)	997±179	428±114†	59±21†¶
Ca++ (mM/L)	1.29±.01	1.37±.02†	1.51±.03†¶
H+ (nEq/L)	48±1	48±3	50±3
$PO_4^{=}$ (mg/dl)	8.5±.4	8.4±.3	9.1±.6

Results are mean±SEM. †, vs EGTA, $p<.02$; ¶, vs EGTA + 1.33 Ca, $p<.02$.

There was no difference in H+ or $PO_4^{=}$ between any group. 1,25D was inversely correlated with Ca++ ($r=-.57$; $p<.01$). Ca++, not H+, is a prime regulator of 1,25D during NH_4Cl acidosis.

HYPOCALCEMIA AND INHIBITION OF PTH RELEASE AFTER WR2721 (WR) - A RADIO AND CHEMOTHERAPY PROTECTIVE AGENT. L. Riley*,D. Glover*,B. Spar*, Z. Agus, E. Slatopolsky, J. Glick* and S. Goldfarb. Wash. U., St. Louis, MO., and U. of PA., Phila., PA.

WR, S-2-(3-aminopropylaminoethyl) phosphorothioic acid, an organic thiophosphate compound, in animals selectively protects normal tissues against the toxicity of radiation and alkylating agent chemotherapy (AAC). In Phase I Clinical Trials of WR and AAC, one patient (pt) developed symptoms of hypocalcemia (HC). Subsequently, serial Ca, PO_4, and Mg were obtained pre and post WR (740mg/m^2 IV) in 16 pts (18 treatments). All 16 pts developed HC [Ca: 9.33±.62 pre and 7.62±.70mg/dl post WR (p<.001)]. Ca nadir occurred within 2-5 hours and remained at 7.88± 0.67 mg/dl for at least 24 hrs post WR. In 4 pts re-studied, Ca was unchanged with AAC without WR. Ionized Ca paralleled serum Ca changes in 12 pts studied. No in vitro interaction between WR and the serum Ca assay was found. No changes in serum PO_4 or pH were noted, but Mg fell [1.91±.49 to 1.48±.21mg/dl 12 pts (p<.001)]. In 9 pts studied, despite a fall in Ca from 9.08±.24 to 7.68±.26 mg/dl (p<.01), UV_{Ca} rose from 7.62±1.16 to 21.36±2.80μEq/min (p<.01). There was no change in UV_{PO4} or GFR. Despite severe HC, serum PTH fell in all 8 pts studied (4.75 to 1.6pL-Eq/ml; undetectable in 5 pts). 1mM WR (~10X plasma levels) did not interfere with the PTH assay.

In conclusion, WR caused HC which in part may be related to direct inhibition of PTH release. The rapidity of the fall in Ca also suggests altered skeletal and soft tissue uptake. As UV_{Ca} rose despite the lower filtered load, renal calcium loss may also contribute to HC.

PARATHYROID HORMONE (PTH) - A NATURALLY OCCURRING Ca IONOPHORE. S Sabatini, J T McCreary*, and N A Kurtzman, Univ of Illinois, Chicago IL.

We studied the effect of PTH on water and Ca transport in isolated toad bladder sacs and toad bladder epithelial cells. Serosal addition of PTH (0.1 mgm/ml) stimulated baseline water flow (7.04± 0.64 vs 9.0±0.8 μl/cm^2/hr, n=24, p<0.05). In contrast, vasopressin (20 mU/ml) (AVP) and cyclic AMP (10 mM) -stimulated water flow following PTH was markedly inhibited (AVP 104.8±6.5 vs AVP+ PTH 77.2±7.4 μl/cm^2/hr, n=24, p<0.02) (cAMP 103.2 ±17.2 vs PTH 58.8±13.7 μl/cm^2/hr, n=14, p<0.05). Concentrations as low as 1 ngm/ml significantly inhibited AVP-stimulated water flow. Pretreatment of the toad bladders with 10^{-6}M indomethacin had no effect on baseline or hormone-stimulated water transport but totally abolished the effect of PTH on water transport. PTH significantly stimulated Ca^{45} uptake by isolated bladder epithelial cells at 5, 10, and 15 min. As little as 3 ng/ml (but not 0.3) of PTH caused a rapid uptake of Ca by bladder cells as measured by dual wavelength spectrophotometry. AVP had no effect on Ca uptake. These results show that the effect of PTH on water transport is independent of the generation of cyclic AMP, and is not merely a reflection of competition of PTH and AVP for receptor sites on the serosal membranes (PTH blocks cAMP-stimulated water flow). These data are identical to those seen following the exposure of this membrane to the Ca ionophore A23187. We suggest that PTH is a naturally occurring calcium ionophore. These data may provide one explanation for the mechanism of the concentrating defect seen in patients with hyperparathyroidism and hypercalcemia.

THE INFLUENCE OF CALCIUM (Ca) ON PHOSPHATE (PO_4) TRANSPORT IN PROXIMAL CONVOLUTED (PCT) AND STRAIGHT (PST) TUBULE SEGMENTS OF THE RABBIT NEPHRON, PERFUSED IN VITRO. Diane Rouse* and Wadi N. Suki, Baylor College of Medicine, Department of Medicine, Houston, Texas.

Hypercalcemia has been shown to increase, decrease or have no effect on PO_4 reabsorption in the mammalian kidney. The purpose of this study was to determine if increasing ionized Ca in the bath and perfusate had an effect on lumen-to-bath PO_4 flux (J^{lb}_{PO4}) in proximal tubule segments. Tubules were obtained from rabbits maintained on a normal PO_4 diet and perfused and bathed with artificial solutions (mimicking plasma ultrafiltrate) of identical composition except for the addition of $^{32}PO_4$ to the perfusate and 0.3 g/dl fetal calf serum to the bath. Superficial (SF) or juxtamedullary (JM) segments were verified by their voltage response to an imposed chloride gradient. The order in which the tubules were exposed to normal (2.5 mEq/l) or high (5.0 mEq/l) ionized Ca was randomized. In five SFPST and five JMPST segments, high ionized Ca had no effect on J^{lb}_{PO4}, transepithelial potential difference (PD),or water absorption (Jv). Identical studies in five SFPCT segments showed an enhancement of J^{lb}_{PO4} from 3.1 ± 1.3 to 7.1 ± 0.9 pmol/mm.min (P< 0.025) with high Ca, and no change in PD or Jv. In five JMPCT segments, J^{lb}_{PO4} also rose with high Ca from 3.5 ± 1.5 to 6.1 ± 0.7 pmol/mm.min (P<0.01). There was no change in PD; however, there was a small but significant decline in Jv from 0.56 ± 0.11 to 0.46 ± 0.11 nl/mm.min (P< 0.02) with high Ca.

These studies show that high ionized calcium concentrations enhance PO_4 absorption in the PCT apparently by a direct effect on the transport mechanism. In contrast, no such effect was seen in PST segments.

PHOSPHATURIA IN THE ISOLATED PERFUSED RAT KIDNEY (IPRK): DEPENDENCE ON PERFUSATE INORGANIC PHOSPHORUS (Pi) CONCENTRATION. Steven J. Scheinman*, Richard Coulson* and R. H. Bowman* (intr. by M. E. Trimble). Depts. of Med. and Pharmacol., SUNY-Upstate and V.A. Med. Ctr., Syracuse, NY

We studied the effect of various perfusate concentrations of Pi on the fractional excretion of Pi (FePi) in the IPRK. Kidneys from normal male Sprague-Dawley rats were perfused for 2 or 3 hours with Krebs bicarbonate medium containing glucose, amino acids and 6 g% albumin. Cyclic AMP (cAMP) was measured by RIA. In the absence of PTH, FePi was steady at .018 ± .004 from 60 to 120 minutes of perfusion at 1.2 mM Pi. From 2.0 to 4.0 mM Pi, FePi rose in a linear fashion from .030 ± .005 to .157 ± .022 (r = .761, p<.005). Synthetic 1-34 bovine PTH (bPTH), beginning at a dose of $2x10^{-10}$M (=.01 U/ml) raised FePi significantly over control At $2x10^{-9}$M, bPTH caused comparable degrees of phosphaturia and urinary cAMP (UcAMP) excretion whether given by continuous infusion or by multiple boluses. At that dose of bPTH, UcAMP rose after an injection at time 0 but declined by 30 minutes and did not rise again despite injections at 30, 60 and 90 minutes. Phosphaturia was maintained despite return of UcAMP to near-control levels.

Conclusions: (1) in the IPRK, despite early development of resistance to the cAMP response to bPTH, the stimulation of phosphaturia persists, suggesting possible down-regulation of the PTH receptor-adenylate cyclase complex; (2) in this system, the optimal range for study of phosphaturic influences is 2.0-2.5 mM Pi, while higher Pi levels should be more useful for the evaluation of potentially antiphosphaturic factors.

LONG TERM PROSPECTIVE STUDY OF RENAL OSTEODYSTROPHY. E. Schwartz, R. Reitz,* P. Rich,*P. Rowe, R. Beallo, J. Weaver, S. Manolagas,* B.D. Catherwood,* and L. Deftos.* Providence Hosp., Oakland, and Univ. of Calif., San Diego, California.

The population of an outpatient dialysis center including 140 patients on HD and 20 patients on CAPD have had biochemical and radiologic evaluation. Mean PTH concentrations were elevated in specific assays for N terminal(3245±1541 pg/ml), Midmolecule(20802±2944 pg/ml), and C terminal(3339±450 pg/ml) fragments, and were significantly decreased in patients on 1,25 OH_2D or DHT treatment(N=1381±312 pg/ml, M=13512±3103 pg/ml, and C=2306±462 pg/ml, $p<0.001$). Measurement of 1,25 OH_2D revealed low values in both untreated and D treated patients(15.1±1.2 pg/ml vs.18.5 ±1.6 pg/ml, $p<0.001$). Following 6 hours of HD, 1,25 OH_2D levels increased in both untreated and treated patients (19.3±2.2 and 25.3±2.4 pg/ml, $p<0.01$). Mean levels of GLA protein (71.6, n=<7 ng/ml) and calcitonin (308, n=<75 pg/ml) were significantly elevated. Bone density measurements by CT scan revealed a wide spectrum varying from 25% below to 30% above normal.

Conclusions: 1) 1,25 OH_2D levels are increased and PTH levels are decreased in D treated vs. untreated patients; 2) 1,25 OH_2D levels are low and PTH levels high in D treated patients. 3)PTH-M is a useful indicator of biologic activity in HD patients(M vs. N cc=0.76); 4) the reason for increases in 1,25 OH_2D following HD are unclear; and 5) the wide spectrum of radiologic and biochemical abnormalities in HD and CAPD patients indicates the need for closer evaluation to optimize therapy.

RENAL OSTEODYSTROPHY: ETIOLOGIC FACTORS. DJ Sherrard, SM Ott*, DL Andress*, NA Maloney*, VAMC & Univ. of Washington, Seattle, WA

Using quantitative histology and tetracycline labeling we have identified 5 forms of renal osteodystrophy. In 197 patients bone biopsies were divided into groups on the basis of bone formation rate (BFR), osteoid area (OA) and marrow fibrosis (Fib) as shown in the top half of the table. All biopsies had increased osteoid surface. We then obtained bone aluminum (Al) values, C-terminal PTH, and calcium-phosphate product (CaXP) as shown:

	Aplastic	Malacic	Mild	Fibrotic	Mixed
N	27	45	28	47	46
BFR	low	low	N1-hi	hi	low-hi
OA	N1	hi	N1	N1	hi
Fib	0	0	0-+	++	++
CaXP	50±2	50±3	55±2	53±3	42±3*
PTH	213±31+	182±24+	464±110	670±71	775±180
Al	.74±.08*	1.33±.11*	.30±.08	.13±.05*	.29±.08

*$p < .02$ vs all other groups (mean ± SEM)
+$p < .02$ aplastic & malacic vs other groups

These data suggest that the mild lesion (which is associated with only moderate PTH excess) progresses in response to unchecked PTH to fibrotic if CaXP is high or mixed if CaXP is low. Low BFR's are associated with low PTH as well as high Al. With Al excess the aplastic or malacic lesions are seen, the latter with heavier exposure to Al. Low PTH may also be caused by Al and may participate with Al in causing a low BFR. Future studies of renal osteodystrophy are needed to clarify the prevention and treatment of this Al and PTH mediated bone disease.

PHOSPHATURIA IN THE RAT FOLLOWING SALIVARIADENECTOMY. Stewart W. Shankel.* Dept. of Med., University of Nevada, Reno, NV 89520.

The salivary glands elaborate 2 hormones that affect the integrity of the teeth. PH-Aβ enhances movement of fluid through the teeth. Since the parotid ducts have many similarities to the renal tubule and the parotid hormones effect transport of calcium and possibly other electrolytes in the teeth, we decided to look at the effect of salivariadenectomy on renal function. 9 mature female Sprague-Dawly rats were studied prior to salivariadenectomy, 1, 3 and 7 days after salivariadenectomy. Serum Ca, Pi and ^{14}C inulin clearances were measured every 20 mins for 4 hrs and the means of the 12 periods were calculated. The values are shown below ± SEM.

	Serum Ca	Serum Pi	TRP	GFR
before	10.16±.13	3.42±.22	95.00±1.24	2.47
post 1d	10.16±.08	3.51±.16	80.38±2.02	2.44
post 3d	10.47±.08	3.48±.16	81.42±2.14	2.27
post 7d	10.29±.17	3.13±.09	85.39±1.66	2.11

One Sham operated animal showed no change in TRP in all 4 phases of the study. From the above data, it is clear that salivariadenectomy markedly reduces the tubular reabsorption of Pi. $P < .01$ inspite of a constant or decreased load. It is unlikely that this effect is due to PTH since it was present in all 9 animals studied; there was no change in serum Ca, except in day 3, no change in serum Pi, except in day 7, and no change in TRP in the Sham operated rat. It is tempting to postulate that the salivary glands secrete a hormone capable of controlling TRP.

25-HYDROXYVITAMIN-D_3-24R-AND 1α-HYDROXYLASES: REGULATION OF ACTIVITY ALONG THE RAT NEPHRON AND IN ISOLATED RENAL CELLS. Justin Silver*, Walter Czaczkes* and Deborah Elstein* (Intr. by L. Feld) Nephrology Services, Hadassah Hospital, Jerusalem, Israel.

The kidney is central to the cascade of vitamin D activation; however, until recently it has not been possible to study rat renal 25(OH)D_3 metabolism in vitro because of a serum inhibitor. We have studied the regulation of 25(OH)D_3 metabolism by isolated cells of one kidney and microdissected single nephron segments from the contralateral kidney.

Preparations from rats fed a normal diet produced only 24,25(OH)D_3, by isolated cells (7.9 pmol/1.25.10^6cells/min), proximal conducted tubules (PCT) (1.19 pmol/mm/min), and proximal straight tubules (PST) (1.5 pmol/mm/min). Distal tubules (DT) did not metabolize 25(OH)D_3. Rats pretreated with 1,25(OH)$_2D_3$ produced more 24,25(OH)$_2D_3$ by PST (2.49 pmol/mm/min), with no change in metabolism by isolated cells, PCT and DT.

Preparations from vitamin D deficient rats produced both 24,25(OH)$_2D_3$ and 1,25(OH)$_2D_3$: 7.2 and 4.6 pmol/min respectively by cells, 0.9 and 0.3 pmol/mm/min by PCT and 1.2 and 0.4 by PST.

These results demonstrate that rat PCT and PST have 24-hydroxylase activity, and that in vitamin D deficiency rat renal cells, PCT and PST all have both 24-hydroxylase and 1-hydroxylase activity. They confirm that 1,25(OH)$_2D_3$ pretreatment induces the 24-hydroxylase activity in the PST but not in the PCT.

ADHERENCE OF CALCIUM OXALATE (CAOX) CRYSTALS TO RAT UROEPITHELIUM. Charles L. Smith, and Jerold Napier.* Regional Kidney Disease Program at Hennepin County Medical Center, Minneapolis, Minnesota.

The adherence of CAOX crystals to rat bladder uroepithelium was studied in anesthetized female rats after bladder catheterization and ureteral ligation. Group 1 bladders were irrigated with 0.9% NaCl (C) or 0.1N HCl acid (A). Group 2 bladders were acid treated and then irrigated with heparin sulfate (HS), ^{14}pentosanpolysulfate (PS), or rat urine (RU). ^{C}-CAOX crystals were introduced into the bladder and nonadherent crystals washed out with 0.9% NaCl. Bladders were excised, digested and the radioactivity counted. Crystal adherence is expressed as percent radioactivity remaining after saline wash ($\bar{X}$ ± SEM).

	C(N=5)	A(N=14)	PS(N=12)	HS(N=4)	RU(N=4)
%	0.6±0.1	14.8±2.7	5.5±1.6	1.3±0.6	18.5±1.9

(CvsA:$p<0.02$; CvsPS:NS; CvsHS:NS; CvsRU:$p<0.001$) In a third group, C and A bladders were studied with a colloidal iron stain. A mucin layer which stained iron positive was found in C but not A bladders. Further studies showed iron positive material lining the distal and collecting tubules, the surface of the papillary tip, pelvis and the ureter. Conclusions: An iron positive mucin layer is present on the rat uroepithelium from distal tubule to the bladder. The mucin layer in the bladder has antiadherence properties for CaOx crystals. The mucin layer is removed by acid treatment and this treatment is associated with increased crystal adherence. Antiadherence is restored by HS or PS but not by RU.

THE EFFECT OF PROSTAGLANDIN METABOLISM ALTERATION ON INTESTINAL CALCIUM ABSORPTION. M.K. Song*, M.A. Wong* and D.B.N. Lee, Research and Medical Services, VA Med. Ctr., Sepulveda, California and UCLA-San Fernando Valley Medical Program, UCLA School of Medicine, Los Angeles, California.

Calcium absorption (CaAB) in female S-D rats was estimated by the plasma contents of ^{45}Ca 1 hr. after its intraduodenal (ID) administration. In one group of rats (8 wks or older) CaAB was increased by the administration of subcutaneous 1, 25-dihydroxyvitamin D_3 (1,25D: 270 ng/D x 4) or ID indomethacin (IND: 1 mg 24 and 4 hs) before study. Simultaneous administration of 1 mg of PGE_2 with ^{45}Ca caused a reduction in CaAB. These data suggest that 1,25D and PGE_2 have opposite effects on CaAB, and the stimulatory effect of IND may be attributed to the suppression of PGE_2 or other related metabolites. In a younger group of rats (4-6 wks old), 1,25D did not stimulate CaAB, and PGE_2 and IND had no significant influence on this parameter. Arachidonic acid (1 mg ID 24 and 4 hrs before study), however, caused a clear increase in CaAB. Thus, changes in PG metabolism may alter Ca transport, but the metabolites and mechanisms involved are probably different from those in the 1,25D-responsive and older rats. We also identified three mucosal cytosolic Ca-binding ligands (MW=85,000; 10,000 and 800) by passing ^{45}Ca labeled cytosol through Sephadex G-75 column. The major Ca-binding activity was not found in the two large proteins, but was located in the MW 800 fraction which appeared to represent the Ca-bound PG family members ($Ca(PG)_2$). We conclude that PGs modulate CaAB in normal rats and that this is achieved through the participation of different metabolites in different physiological settings.

RENAL CA HANDLING BY SPONTANEOUS HYPERTENSIVE RAT (SHR): EVIDENCE AGAINST A RENAL CA LEAK. J. Spirnak*, B. Eby*, & K Lau. Neph. Div. Med. Dept. U. of Mich. Ann Arbor, Mich.

Hypercalcuria has been found by some previous metabolic studies in SHR. As plasma (P) ionized Ca was reportedly reduced and parathyroid hormone increased, a renal Ca leak has been postulated. To directly test this thesis, clearance studies were done on 23 week old fasted ♀ SHR and normotensive Wistar Kyoto (WKY) rats. Glomerular filtration rate was similar (1.7 vs 1.8 ml/min). Both P_{Ca} and ultrafiltrate (UF) Ca were reduced in SHR (5.6 vs 6.6 mg%). At comparable clearance (C) of Na (97 vs 95 µl/min), C_{Ca} was not different (117 vs 133 µl/min). Two hours after acute parathyroidectomy, P_{UFCa} remained lower (4.8 vs 5.4 mg%), but again, at similar C_{Na}, C_{Ca} was almost identical (138 vs 130 µl/min). The acute response to PTH (100 units/Kg) was also not different (ΔC_{Ca} 23 vs 27 µl/min). Similar plasma and clearance results were found for 15 week old rats. To resolve the discrepancy between our clearance and prior metabolic data, the role of diet Ca and H_2O intake was specifically examined. Between 14 and 24 weeks of age, urine Ca (mg/d), as means of ≥2 collections per week was consistently lower in SHR over a wide range of diet Ca (<0.03% to 2.6%). However, H_2O intake was also lower (22 vs 32 ml). When H_2O was restricted in WKY rats, Ca excretion was similar at all ages. Only one of 30 urine exhibited a slight hypercalciuria (1.4 vs 1.1 mg/d in SHR), but they also ate more food (13 vs 10.7 gm/d). We conclude: SHR are characterized by (1) fasting hypocalcemia, (2) normal tubular reabsorption of Ca in both the presence or absence of PTH, and (3) apparent differences in 24h urine Ca largely stem from hypodipsia in SHR and differences in food ingestion.

PTH-INDUCED cAMP RESPONSE OF VASCULAR SMOOTH MUSCLE CELLS: A CALMODULIN DEPENDENT ACTION. R. Stanton*, S.B. Plant*and D.A. McCarron. Division of Nephrology, Oregon Health Sciences University, Portland, Oregon.

Bovine (b) PTH (1-84), plus synthetic human (h) and bPTH (1-34) analogs possess specific, vasodilator actions when administered to both normo- and hypertensive animals. This effect is log-dose dependent, maximal at 60 sec. and reduces mean arterial pressure by as much as 55%. We assessed the cAMP response of cultered bovine vascular smooth muscle (VSM) cells to incubation with 0.3 µg of bPTH (1-34) alone and following pre-incubation with either 1 mM theophylline (THE) or 10 µM trifluoperazine (TFP). Isoproterenol (ISP) served as a positive control. VSM cAMP (p mole/mg cell protein; mean±SEM) results:

Incubation	Time	0 Min	0.5 Min	1 Min	3 Min
bPTH(1-34)	N=12	4.1±0.4	3.3±0.2	1.9±0.1	3.0±0.4
THE +bPTH	N=12	12.2±3.0	3.5±0.6	5.6±0.8	1.2±4.1
TFP +bPTH	N=8	4.1±1.0	4.1±1.0	2.4±0.4	3.3±0.9
ISP	N=8	3.4±0.4	6.3±1.4	5.7±1.0	6.6±1.7

bPTH (1-34) induces 54% reduction ($p<.005$) in VSM cAMP that is maximal at 1 min. THE-induced phosphodisterase inhibition increased basal cAMP 3-fold ($p<.05$) but failed to block ($p<.025$) the cAMP response to bPTH (1-34). TFP inhibition of calmodulin, prevented the PTH-induced suppression of cAMP, though it did not modify basal cAMP. Isp stimulated ($p<.01$) VSM cAMP content.

In contrast to the bone and kidney, in VSM bPTH (1-34) suppresses cAMP. The time course and magnitude of cAMP suppression parallels, PTH's hypotensive effect. This cAMP response requires the Ca^{2+}-calmodulin interaction. It is not modified by phosphodiesterase inhibition.

PARATHYROID HORMONE (PTH) RESPONSE TO HYPOCALCEMIA (Ca↓) IN DIALYSIS PATIENTS (Pts) WITH OSTEITIS FIBROSA (OF). A. Voigts*, A. Felsenfeld, P. Wilson*, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

To assess PTH response and its correlation with bone histology, 18 dialysis pts with OF underwent a zero calcium (Ca) dialysis. Sampling was obtained at baseline and every 30 minutes (min). The plasma Ca declined most rapidly during the first 30 min. Both amino (N) and carboxy (C) terminal PTH attained peak levels by 30 min and remained elevated throughout dialysis. Despite basal hypercalcemia (Ca↓) in 3 pts, C and N-PTH increased in each at 30 min in the absence of Ca↓.

	Ca (mg/dl)	N-PTH (ng/ml)	C-PTH (ng/ml)
0 min	9.38±.6	.53±.4	1.50±.5
30 min	8.45±.7**	1.27±.8**	2.14±.7*
120 min	7.75±.7**	1.20±.8**	2.21±.8**

X±SD p<.01*; p<.005**

Osteoclasts/mm^2 (OC/mm^2), % active resorption (RES) and % endosteal fibrosis (EOF) were correlated with maximum (max) ΔN-PTH and ΔC-PTH. While max ΔN-PTH did not correlate with any parameter, max ΔC-PTH correlated with OC/mm^2, % RES, and % EOF ($p<.005$).

In summary, in dialysis pts with OF: 1) Ca↓ produces a rapid N and C-PTH response; 2) the N and C-PTH response occurs despite Ca↓; 3) after 30 min, N and C-PTH did not increase despite progressive Ca↓ and 4) max ΔC-PTH correlated with bone histologic parameters. In conclusion: 1) a minimal decline in plasma Ca produces a max N and C-PTH response; 2) an altered PTH set point may be present; and 3) C-PTH response correlates with bone histology.

SEVERE HYPOPHOSPHATEMIA (SH) DURING HYPERALIMENTATION (HA) IN PATIENTS WITH RENAL FAILURE (RF) AND HYPERPHOSPHATEMIA (H). Stephen J. Sweet, Glen Schwartzberg*, and James Corsones*. Baystate Medical Center, Springfield, MA.

During a 42 month period we encountered 10 pts. (5 on chronic hemodialysis and 5 with acute RF) who developed SH (serum phosphorus (P_S) less than 1.1 mg/dl) during HA. All pts. were hospitalized for an acute illness and were malnourished either on admission or became so during their prolonged confinement (mean 28 days). The mean age was 71 years and 9/10 were female. Prior to HA the P_S ranged from 3.8 to 9.9 mg/dl (mean 6.9 mg/dl) while receiving phosphate (P) binding antacids (5/10) and dialysis (9/10). During HA the mean P_S fell to 1.0 mg/dl by day 3 and SH (range 0.3 to 1.1 mg/dl, mean 0.7 mg/dl) was present in all by day 4. With SH, all pts. became symptomatic: Altered mental status (4), muscle weakness (4), unexplained anemia (2) and abnormal liver function tests (5). There was a significant correlation between the total cumulative or the previous 24 hr. caloric load administered by HA and the change in or lowest P_S, respectively. The pts. received 0.04 to 0.44 (average 0.20) m mole of P/kg body wt/6 hrs. with a normalization of P_S by 24 hrs. The calculated apparent space of distribution of P was 0.22 to 6.4 (average 2.36), a value somewhat greater than reported for pts. with normal P_S (0.69) or SH but without RF (1.68). Despite normalization of P_S, 9/10 pts. died. In conclusion, pts. with RF and H: 1) Are not immune from developing SH during HA, particularly when malnourished; 2) may be severely P depleted as suggested by the increased P space; and 3) require careful monitoring during HA.

RENAL MAGNESIUM TRANSPORT IN ACID-BASE DISTURBANCES. Norman L.M. Wong, Gary A. Quamme and John H. Dirks, Department of Medicine, Univ. of British Columbia, Vancouver, B.C., Canada.

The renal transport of divalent cations are greatly affected by changes in acid-base status of the animal. Clearance studies have suggested that alkalosis enhances and acidosis reduces the reabsorption of these ions by the renal tubules. Micropuncture and clearance experiments were performed on thyroparathyroidectomized (TPTX) dogs to assess the role of plasma and luminal bicarbonate concentration on magnesium transport by the various nephron segments. Three groups of dogs were studied, Group I: normal dogs (n=29) with P HCO_3 21 mM; Group II: acidotic dogs (n=27) produced by feeding NH_4Cl (10 gm/day) for 3 days, P HCO_3 12 mM; Group III: acute alkalosis (n=37) induced by intravenous infusion of $NaHCO_3$, P HCO_3 31 mM. The overall GFR and UF Mg were similar in all three groups. The mean FE Na was 9% in group III compared to 4.2% and 4.8% in group I and II respectively. FE Mg as a function of FE Na demonstrated a reduction of Mg reabsorption in acidosis and an enhancement in alkalosis. The fractional reabsorption to the late proximal tubule was 19%, 21% and 21% in group I, II and III respectively. In the distal tubule, the fractional Mg reabsorbed was 63% in normal animals and 52% in acidotic dogs. Fractional Mg reabsorption was dependent on bicarbonate delivery. These results suggest that acidosis inhibits and alkalosis enhances Mg transport reabsorption in the loop of Henle.

LACK OF REGULATORY ROLE OF CYTOSOLIC REDOX POTENTIAL ON PHOSPHATE TRANSPORT ACROSS RABBIT PROXIMAL TUBULES. Norimoto Yanagawa, Glenn Nagami*, Ok Joe*, Kiyoshi Kurokawa, and David B.N. Lee. Nephrol Div, Sepulveda VAMC and Wadsworth VAMC, UCLA Sch Med, Los Angeles, CA.

Based on the observation that nicotinamide adenine dinucleotide (NAD) added in vitro directly inhibits phosphate uptake by renal brush border membranes, it was hypothesized that cytosolic redox potential (NAD/NADH) and/or NAD content may regulate phosphate transport across the proximal tubule (J Clin Invest 67: 1347, 1981). Since cytosolic NAD/NADH is proportional to the ratio of cellular concentrations of pyruvate/lactate, we examined the effect of using either pyruvate or lactate as the substrate in the bathing medium on the lumen-to-bath phosphate flux (Jp) in isolated perfused rabbit proximal straight tubules. When the substrate was changed from 5mM pyruvate to 5mM lactate, Jp did not change (from 1.01±0.32 to 1.20±0.20 pmol/mm/min). When the substrate was changed in the reverse order, i.e., from lactate to pyruvate, Jp again did not change (from 2.27±0.47 to 2.44±0.46). We further examined the effect of change in redox potential induced by methylene blue (MB), a proton acceptor which converts NADH to NAD. When MB (0.1 mM) was added to the bath which contains 5 mM lactate as the substrate, no decrease in Jp was observed; 1.47±0.33 and 2.05±0.51 in the absence and presence of MB, respectively. These data suggest that changes in the NAD/NADH ratio or NAD content in the cell cytosol do not play a significant role in the regulation of Jp in the proximal tubule.

ENZYMATIC REMOVAL OF ALKALINE PHOSPHATASE (AP) FROM RENAL BRUSH BORDER MEMBRANE (BBM): EFFECT ON Na^+-DEPENDENT TRANSPORT OF PHOSPHATE (Pi). A. N. K. Yusufi,* M. G. Low,* S. T. Turner,* and T. P. Dousa, Mayo Clinic, Rochester, MN and Okla. Med. Res. Fdn., Oklahoma City, OK.

Previous studies suggested that AP may be related directly or indirectly to Na^+-dependent Pi transport across renal luminal BBM. We examined the effect of deletion of AP from the BBM structure on its transport properties. Sheets of BBM isolated from rabbit kidney cortex were incubated with and without purified phosphatidylinositol-specific phospholipase C (PI-PLC). BBM vesicles (BBMV) were then prepared from BBM sheets and used for transport studies. This PI-PLC treatment resulted in almost complete (> 90%) removal of AP from BBM, but leucineaminopeptidase, gamma glutamyltransferase or maltase activities were not different from control BBM with intact AP. Enzymatic removal of AP did not change the capacity of BBMV for Na^+-dependent uptake of ^{32}Pi, [^{3}H]-D-glucose and of [^{3}H]-L-proline. Also, removal of AP did not abolish the increased rate of Pi uptake by BBMV prepared from rabbits fed low Pi diet; in contrast, Na^+-dependent BBMV uptake was further increased (Δ%94±17; $P<0.005$) after AP removal. However, deletion of AP did not enhance the Pi transport rate in BBMV prepared from rabbits fed normal Pi or high Pi diet. The results provide direct evidence that AP is not an essential component Na^+-gradient dependent renal BBM transport system for Pi, D-glucose, or L-proline. Results also suggest that increased AP activity of BBM in response to low Pi diet may be a secondary adaptation due to enhanced Pi transport by BBM.

CHARACTERIZATION OF ALKALINE PHOSPHATASE IN CANINE RENAL CORTICAL BRUSH BORDER MEMBRANES AS PHOSPHOPROTEIN PHOSPHATASE. J.E. Yourist*, M. Ashby*, G. Gavellas*, J.J. Bourgoignie. University of Miami School of Medicine, Miami, FL.

Several mechanisms have been proposed to explain the cellular basis of PTH inhibition of phosphate transport and phosphaturia. These include a PTH induced increase in intracellular $NAD^+/NADH$ ratio and endogenous phosphorylation of 96000 and 62000 dalton proteins in brush border membranes. NAD^+ also inhibits alkaline phosphatase of renal brush border membranes. The function of alkaline phosphatases is unknown but since they are non-specific phosphomonoesterases, they can catalyze the hydrolysis of phosphate from protein. Therefore, we tested the possibility that alkaline phosphatase from renal brush border contains phosphoprotein phosphatase activity.

In purified canine renal brush border membranes, NAD^+ inhibited both alkaline and phosphohistone phosphatase in a dose dependent manner. For both, the inhibition was characterized as non-competitive. Also, histones inhibited alkaline phosphatase and, conversely, p-nitrophenyl phosphate inhibited phosphohistone phosphatase in a competitive manner. Moreover, both enzyme activities, eluted from Sephacryl 200, coincided at about 80000 daltons.

These results support the hypothesis that alkaline phosphatase and phosphoprotein phosphatase in renal brush border represent the same enzyme. An increase in $NAD^+/NADH$ ratio under the influence of PTH would inhibit dephosphorylation of phosphoproteins, thereby inhibiting phosphate transport and resulting in phosphaturia.

AGE RELATED NEPHROCALCINOSIS IN THE RAT. Rudolfs K. Zalups and Peter Haase. (intr. by J. C. Romero) University of Western Ontario, Dept. of Anatomy, London, Ontario, Canada

It has been suggested that age is an important factor that influences the development of nephrocalcinosis in rats. We therefore tested the hypothesis that weanling rats develop a morphologically different type of nephrocalcinosis than do adult rats. To induce nephrocalcinosis, a group of female weanling (45-50g) and adult (210-225g) Sprague-Dawley rats were given single daily injections (IP) of 0.5 M neutral sodium phosphate (15.5 mg Pi/100g bw/24h) for 10 days. Following the experimental period, cross-sectional slices were taken from the kidneys of the animals and were fixed in 80% ethanol for 48 h. After fixation the tissues were dehydrated, cleared and embedded in paraffin. Sections (5-10 μm) were stained with alizarin red S and von Kossa methods for calcium and phosphate, respectively. The weanling rats developed a type of nephrocalcinosis that was characterized mainly by large intraluminal calciferous deposits situated in the terminal segments of proximal tubules at the junction of the outer and inner stripes of the outer medulla, and in collecting ducts and ascending thick limbs of Henle in the inner stripe of the outer medulla. In contrast, the young adult rats developed a type of nephrocalcinosis that was characterized mainly by small granular calciferous deposits in the basement membranes of proximal tubules in the mid- and outer cortex. We conclude that injections of phosphate induce intraluminal nephrocalcinosis in weanling rats, while they induce basement membrane nephrocalcinosis in adult rats.

Clinical Nephrology

VARIATIONS WITHIN 24 HOURS OF URINARY CALCIUM OXALATE (CX) AND BRUSHITE (B) SATURATION: STONE FORMERS (S) AND NONSTONE FORMERS (N). P.A. Abraham* and C.L. Smith, Hennepin County Medical Ctr., Dept. of Med., Mpls., MN.

The mechanism of CX stone formation is not understood. Urine collections for 24 h show supersaturation of CX in N and S and thus do not explain urolithiasis. However, CX saturation could vary within a 24 h period to distinguish N from S. Consecutive 4 h urine specimens were collected for 24 h from N and metabolically active S to determine: pH; activity product ratio (APR) and formation product ratio (FPR) for CX and B; concentration product ratio for uric acid and monosodium urate; excretion and concentration of Ca, oxalate, phosphate, uric acid, pyrophosphate, citrate and Mg. Data presented are mean ± SEM,(*P<.05).

APR(CX)	6A	10A	2P	6P	10P	2A
N(7)	2.1±.2	1.7±.2	1.9±.2	2.9±.7	2.1±.4	2.4±.6
S(5)	2.2±.2	3.8±1.7	2.2±.2	3.8±1.1	4.2±1.2	3.5±.9
APR(B)						
N(7)	.7±.3	.4±.2	.4±.2	.3±.1	1.1±.3	1.3±.6
S(5)	1.3±.2	1.8±.5*	1.7±.5*	2.9±.4*	3.7±1*	2.0±.3

Of the factors measured, the higher APR(B) in S is explained in part by intermittently higher pH (6P, P<.01) and higher Ca concentration (10A, 2P; P<.02). FPR (CX) was not different for N and S. In determination of FPR (B) 16% of N did not precipitate and 22% of S spontaneously precipitated, suggesting a difference in FPR(B).

Our data shows that saturation for B not CX was higher in S than N. Of 30 CX containing stones from our clinic, 20 (66%) had centers of pure hydroxyapatite, a possible transitional phase of B. Saturation for B may be important for CX stone formation.

REDUCTION IN PREDNISOLONE METABOLISM DURING UREMIA. William Amend, John Gambertoglio, Jytte Birnbaum,* Patricia Lizak,* and Nicholas Holford*. Univ. of Calif., San Francisco, CA

Prednisone or prednisolone (Po) is commonly used in renal disorders such as SLE, membranous glomerulonephritis, kidney transplantation in which there can be wide variations in renal function. The principle mode of Po's elimination is metabolism. However, to test the hypothesis that there may be differences in Po metabolism in uremia, we studied Po clearance in 12 stable renal transplant pts with a wide range of renal function. All pts had normal serum albumin.

A dose of 0.2 mg/kg of Po was administered i.v. Po levels were measured using HPLC and unbound Po concentrations were obtained using equilibrium dialysis.

	Creatinine Clearance (ml/min)	Total Po Clearance (ml/min)	Unbound Po Clearance (ml/min)
Group I (n=6)	83 ± 9	98 ± 10	489 ± 22
Group II (n=6)	23 ± 13	73 ± 11	310 ± 71

There was a significant correlation between total ($p < .05$) or unbound ($p < .01$) clearance and creatinine clearance. Unbound Po clearance may be predicted according to the relationship: Po clearance (ml/min) = 2.5 x creatinine clearance(ml/min)+ 266 ml/min, $r^2 = 0.46$.

Conclusion: Po clearance is reduced in patients with decreased renal function which may be a reflection of diminished hepatic or renal metabolism of the drug.

COMPARATIVE EVALUATION OF DOUBLE FILTRATION PLASMAPHERESIS (DFPP) TO STANDARD CENTRIFUGATION PLASMAPHERESIS (CFPP) IN SLE PATIENTS. Tetsuzo Agishi,* Hidehiro Amemiya,* Nobuhiro Sugino, and Kazuo Ota.* Kidney Center, Tokyo Women's Medical College, Tokyo, Japan.

Therapuetic plasmapheresis was performed either by DFPP or CFPP in 7 SLE patients. DFPP is a technic to concentrate and selectively remove macromolecular components of the plasma utilizing the difference of pore size of two membrane filters in order to conserve an amount of the replacement fluid. For DFPP, 4 to 8 procedures were repeated every other day replacing with 0.6 liters of 0.5% albumin in lactated Ringer's solution. For CFPP, 3 to 5 procedures were done replacing 2 to 3 liters with the same substitution fluid. In terms of improvements in the clinical signs and the laboratory data, there were no significant differences between both modalities, while the amount of albumin supplemented per series in DFPP was almost half compared to that in CFPP (DFPP 198g vs CFPP 356g). Conclusion from these observations is drawn that DFPP is advantageous in the reduction of human blood product for substitution with satisfactory effectiveness of plasmapheresis therapy.

POLYMORPHIC ULTRASTRUCTURAL RENAL FINDINGS IN PLASMA CELL DYSCRASIA. Tatiana T. Antonovych and Sharda G. Sabnis. Division of Nephropathology, Armed Forces Institute of Pathology, Registry of Nephropathology and Veterans Administration Special Reference Lab., Washington, D.C.

Approximately 17 per cent of plasma cell dyscrasias and 21 to 25 per cent cases of overt myeloma are characterized by exclusive production of monoclonal light chains, either kappa or lambda. Excessive light chain production may lead not only to the classical renal lesion of myeloma kidney, amyloidosis but at times to granular or crystalloid deposits in multiple organs with the result of multisystem disease. Light, electron, and immunofluorescence microscopy changes of 16 patients illustrating the differences in morphologic appearances of light chain diseases will be demonstrated.

LONG TERM FOLLOW-UP OF LUPUS NEPHRITIS (LN) CLASSIFIED BY WHO HISTOLOGIC CRITERIA. G.B.Appel, D.Estes‡ D.Cohen‡ J.Meltzer, C.L.Pirani, Depts. of Med. and Path., Columbia Univ., New York, N.Y.

Non-uniform histologic criteria, failure to classify pure mesangial disease separately, and short periods of observation have obscured the natural history of LN. In 1978 we published the renal histopathology, using the WHO classification, and the clinical course of 56 patients (pts) with LN followed for a mean of 3.7 yrs. We now report an additional 6 yr. follow-up. Of 37 pts. living at the end of the first study, additional information was available on 33 with 26 currently followed ($\bar{x}$ 10 yrs. from biopsy).

Class IIA (N=5) pts. have had a benign renal course ($\bar{x}$ follow-up 11.5 yrs). One Class IIB pt (N=7) transformed to Class IV, developed the nephrotic syndrome and renal failure; another pt died with stable renal function. The other 5 have done well ($\bar{x}$ 11.7 yrs). One of 9 Class III pts progressed to Class IV and renal failure; another on dialysis died. The remaining 7 have stable renal function ($\bar{x}$ 8.4 yrs). Of 6 Class IV pts, 1 is stable after Txp; 1 died; 2 have worse renal function; and 2 are stable ($\bar{x}$ 12.2 yrs). 3 of 6 Class V pts have developed renal dysfunction late in their course ($\bar{x}$ 7.7 yrs).

Survival of LN pts has improved in recent years. Of 38 pts biopsied before 1973, 20 are dead (53%) and two in renal failure ($\bar{x}$ follow 7.8 yrs). Of 18 pts biopsied since 1973 only 2 have died (11%) and 3 are in renal failure ($\bar{x}$ follow 6.4 yrs). The number of pts in each class was similar in both groups. Thus, the survival is improved for each class of LN, and renal biopsy provides important prognostic information.

CADAVERIC RENAL TRANSPLANTS IN CHILDREN UNDER 6 YEARS OF AGE. GS Arbus, BE Hardy‡ JW Balfe, CP Rance, BM Churchill‡ BT Steele, R Baumal, RN Curtis*. The Hospital for Sick Children, and University of Toronto, Toronto, Canada.

We reviewed our experience with cadaveric renal transplantation in children <6 yr of age, to determine whether the procedure was of value in the younger patient. From March, 1973 to June 1982, 42 cadaveric renal transplants were performed in 31 children (24 male, 7 female) aged <6 yr. Thirteen of the children, two 3 months of age, weighed <10 kg when transplanted. Primary renal diseases were glomerulonephritis (14), dysplasia/hypoplasia (7), reflux/obstructive nephropathy (7), other (3). Before transplantation, 10 patients had never been dialyzed; only 8 patients had bilateral nephrectomies. Twenty patients now have functioning grafts (following a mean time of 40 mo); in 16, the original graft is still functional. Four patients are back on dialysis and 7 have died. Psychosocial and physical development was retarded in the pre-transplant period, but dramatic catch-up occurred in the first year after successful transplantation. Causes of graft failure were primary non-function of donor kidney (5) including 4 donors < 1 yr, recurrence of hemolytic uremic syndrome (4 times in one patient), rejection (4), sepsis (2), other (2). Our data indicates that cadaveric renal transplantation is feasible in children <6 yr of age. However we find that kidneys from donors <1 yr are unlikely to function and vessels of large kidneys placed in small children may thrombose, especially when the native kidneys are left in situ.

HYPERLIPIDEMIA IN ADULTS WITH THE NEPHROTIC SYNDROME (NS). GB Appel, C Kunis, C Blum,* Dept. of Med., Columbia University, New York, NY.

Although hyperlipidemia is a well recognized feature of the N.S., conflicting data exist regarding the nature and significance of these lipid abnormalities. This may stem from reports including diabetics, patients with renal insufficiency, and patients on steroid treatment, as well as reports of children with nil disease who might not be at long-term risk from atherogenic complications. Little data is also available concerning high density lipoprotein (HDL) cholesterol (chol) and its relation to total and low density lipoprotein (LDL) chol despite increased awareness of its significance in terms of atherogenesis. To clarify the patterns of lipid elevations in nephrotic adults we studied 22 serial samples on 11 patients. No patient had diabetes mellitus, a serum creatinine greater than 2.0 mg/dl, known liver disease, or was receiving corticosteroids. Diagnoses included membranous 2, focal sclerosis 4, luetic 1, drug related 1, IgA disease 1, Amyloid 2. Mean values for the 22 samples as shown below:

Cholesterol (Chol)				Triglycerides (TG)			
Total	HDL	LDL	VLDL	Total	HDL	LDL	VLDL
359	41	275	39	276	28	109	138

Of 22 samples 19 had elevated total chol (>95 percentile for age and sex), 17 had elevated LDL chol, and only 8 elevated VLDL chol. No patient had an elevated HDL chol. HDL chol was low (<10 percentile) in 11 of 22 specimens. TG were elevated in 14 samples. Those patients with unremitting NS with major increases in total and LDL chol and low values for HDL chol may be at increased risk for accelerated atherosclerosis. These patients may benefit from therapy with lipid lowering drugs.

EVALUATION OF A NONINVASIVE ECHO-DOPPLER METHOD FOR THE DIAGNOSIS OF RENAL ARTERY STENOSIS. Pratap S. Avasthi, Ernest R. Greene,* and Wyatt F. Voyles.* University of New Mexico and Lovelace Medical Foundation. Albuquerque, New Mexico.

Beat-to-beat blood flow variables in normal human renal arteries have been noninvasively characterized using a two-dimensional, real-time sector imager combined with a pulsed Doppler velocimeter (Kidney Int 20:523, 1981). This method is termed duplex scanning (DS). In this study, altered flow characteristics were utilized to diagnose renal artery stenosis. Twenty-two of 28 (79%) consecutive patients (44 arteries) were studied, both by biplane renal arteriography and blindly by noninvasive DS. Twelve vessels in 6 patients (21%) had technically inadequate DS exams. Any of the following four predetermined alterations in flow variables was deemed diagnostic for renal artery stenosis: a) peak velocity (V) $\geq$100 cm/sec in a localized area of the imaged renal artery, b) widening of pulsed Doppler audio-spectra generated by a fast Fourier transform indicating disturbed blood flow, c) absence of the normally high diastolic flow pattern, d) absence of flow in an imaged vessel. The sensitivity and accuracy of the noninvasive DS method was evaluated using the results of arteriography. The DS method was 90% sensitive and 75% specific in detecting hemodynamically significant ($\geq$50% diameter reduction) renal artery stenosis. Positive predictive and negative predictive indices were 75% and 90%, respectively. These data suggest that the DS method can be used in the noninvasive diagnosis of renal artery stenosis and may provide a sensitive screening method in selecting patients for subsequent angiography.

SAFETY OF RAPID CORRECTION OF SEVERE HYPONATREMIA (SHN) IN SPRAGUE-DAWLEY RATS. J.C. Ayus, R. Krothapalli*, E. Pace* and H.O. Senekjian. Ben Taub General Hospital, VA Medical Center and Baylor College of Medicine, Houston, Tx.

We recently demonstrated the safety and benefit of the rapid correction of SHN in patients (Am. J. Med. 72:43). Others, however, (Science 211:1068) have shown that the rapid correction of serum sodium (^{S}Na) from 106.3 to 151.8 mEq/L in rats results in Central Pontine Myelinolysis (CPM). The present studies were performed to examine the effect of rapid correction (without over correction) of SHN. SHN was induced by the daily injections of pitressin in oil and 2.5% dextrose in water. On day 4, hypertonic saline was given in a dose calculated to raise ^{S}Na to 130 mEq/L. Initial ^{S}Na averaged 146.4±6.8 mEq/L. Fourteen rats (28%) died during the induction of SHN. Of the remaining 36 rats, 15 (42%) (group 1) died shortly after correction of SHN, while 21 rats (58%) (group 2) were alive two months following correction of SHN. Both groups 1 and 2 had similar degrees of SHN with ^{S}Na of 107.7± 7.5 and 112.1±7.7 mEq/L respectively. On day 5, ^{S}Na was 137.1±9.9 and 132.2±7.7 mEq/L in groups 1 and 2 respectively (p=NS). Two months following correction of SHN, brains of group 2 rats were examined. ^{S}Na at that time was 149.2±3.2 mEq/L. CPM was not present in any of the rats. In a third group of rats, SHN was induced and no correction was attempted except for water restriction. The immediate mortality rate was 100%. We conclude that 1) SHN is associated with a high mortality rate and 2) The rapid correction (without over correction) of SHN decreases the mortality without the development of CPM.

THE OUTCOME OF HYPONATREMIA IN A GENERAL HOSPITAL POPULATION. Dana Baran,* and Tom A. Hutchinson* (intr. by Dr. J.F. Seely). Royal Victoria Hospital, Division of Nephrology, Department of Medicine, Montreal, Canada.

In order to determine how often hyponatremia causes CNS symptoms, we studied a group of 67 consecutive patients on the wards of a general hospital whose serum sodium levels fell to 128 mEq/l or below during their hospitalization.

Thirty-six patients had no CNS abnormalities. An observer blind to the actual sodium levels examined the records of the remaining 31 patients to assess the likelihood that the hyponatremia caused the CNS symptoms observed. A modification of a diagnostic algorithm for assessing causality in adverse drug reactions was used to standardize the judgements made (Kramer et al, J.A.M.A. 242: 623-632, 1979). Twenty-two of the 31 patients had CNS symptoms that were unlikely to be related to their hyponatremia and that could be attributed to other factors (eg. hypoxemia, hepatic encephalopathy, etc.). Only 9 patients appeared to have CNS abnormalities related to a low serum sodium. These tended to be patients whose lowest sodium was below 120 mEq/l and in whom the sodium level fell rapidly (i.e. $\geq$ 5 mEq/l in $\leq$ 24 hrs.).

We conclude that hyponatremia of 128 mEq/l or less causes CNS symptoms in a minority of patients. When these symptoms are present, they are usually related to other factors. These findings should be kept in mind when considering potentially harmful therapy (e.g. hypertonic saline) for this relatively common disorder.

STUDY OF LUPUS NEPHRITIS WITH GALLIUM SCANS. Asad A. Bakir*, Vincent Lopez-Majano*, and George Dunea. Cook County Hospital, Chicago, Illinois.

To better assess the activity of nephritis in SLE patients, Gallium^{67}citrate scans (G.S.) were done on 34 patients(33 female,1 male). Scans were considered positive if the kidneys were still seen after 48 hours. Hematuria, 24-hr proteinuria over 500mg, declining renal function, and active glomerulonephritis on biopsy were considered signs of active renal disease (ARD). A positive G.S. was seen in 21 patients and a negative G.S. in 13 (see table).

Gallium Scan	Positive		Negative	
	Range	Mean	Range	Mean
Age	15-65	30.6	24-54	32.1
ANA(dilutions)	40-10240	2736	0-10240	2004
Anti-DNA(dilutions)	0-80	11.4	0-10	1.6
C3 mg/dl	42-122	78.4	30-145	87.9
C4 mg/dl	12-74	26.1	17-42	25
Urine protein(gm)	0.08-9.2	3.6	0.12-1.7	0.62
BUN mg/dl	8-59	21.8	8-73	19.7
Creat. mg/dl	0.7-3.5	1.28	0.8-3.6	1.53
Biopsy	19 Patients		7 Patients	
DPN	11 (58%)		1 (14%)	
SPN	4 (21%)		2 (28%)	
MN	4 (21%)		1 (14%)	
MMN	0		3 (44%)	

DPN denotes diffuse, SPN,segmental proliferative, MN,membranous, and MMN,mild mesangial nephritis. ARD was seen in 22 patients,19 of whom (86.4%) had positive G.S. Renal disease was inactive in 12 patients,10 of whom (83.3%) had negative G.S. Only anti-DNA,urine protein,and DPN correlated positively with positive G.S.and ARD. We conclude that G.S. is very useful in evaluating ARD in SLE.

INTERSTITIAL NEPHRITIS (IN) AS HIGH RISK FACTOR FOR ACUTE RENAL FAILURE (ARF) AFTER USE OF RADIOGRAPHIC CONTRAST MEDIA (RCM) IN DIABETICS (D) AND NON DIABETICS (ND) . Lidia de Bermudez and Francisco Bermudez*. Hospitales Calderon Guardia y San Juan de Dios, Universidad de Costa Rica, San Jose, Costa Rica.

We want to emphasize IN as a common predisposing factor in the development of ARF due to RCM. One hundred and two D and 82 ND patients from the literature plus 14 D of our own were analized looking for common risk factors. Nine of our 14 patients had acute urinary tract infection in the 2 weeks prior to excretory urography (EU) , 10 were hypertensive , 7 had history of congestive heart failure and all of them had diabetic retinopathy. Six presented persistent nephrogram during EU and instead of considering this an early sign of ARF, prompted a new EU that worsened it. Of 184 cases from the literature, 78 had well known causes of IN (tuberculosis, lithiasis, gout, nephrocalcinosis, etc.). If we add hypertension and diabetes as causes of IN, the number of patients with IN who developed ARF after RCM rises from 70.6% to 88.9%.

The pathologic characteristics of IN worsen the hemodynamic, rheologic and obstructive changes described in RCM's ARR

Diabetics with acute urinary tract infection should not have EU done.

EFFECT OF DIET ON GLOMERULAR FILTRATION RATE (GFR): FUNCTIONAL RESERVE (FR) OF THE NORMAL KIDNEY. J.P. Bosch, A. Saccagi, A. Lauer, M. Belledonne, S. Glabman. Mount Sinai School of Medicine, New York, New York.

To study the effect of altering diet on GFR, experiments were performed in normal volunteers. GFR was measured by creatinine or in ulin clearance over 24 hours in the chronic studies and hourly in the acute. In four normal subjects, on a regular protein diet (70 gms), GFR averaged 113 ± 7 ml/min. After seven days, on a low protein diet (20 gms) GFR fell significantly to 100 ± 5 ml/min. In a group of vegetarians without known kidney disease matched for age and body surface area with the above group, GFR averaged 66 ± 20 ml/min ($p < .05$). To ellucidate the effect of sudden changes in diet on GFR acute studies were also done in four normal volunteers. After a fasting control period a protein load was ingested (50-70 gms) and GFR measured. In the control period GFR averaged 92 ± 10 ml/min and increased significatnly after the protein load to 149 ± 22 ml/min. Conclusions: these studies suggest that dietary intake may be an important determinant of GFR. This capacity to vary GFR with changes in protein load may reflect the functional reserve of the normal kidney. The loss of this functional reserve may preceed the absolute fall in GFR in renal failure.

CLINICAL AND LABORATORY FINDINGS IN 53 PATIENTS WITH TYPE I RTA (RTA-I). R. J. Caruana and V. M. Buckalew, Jr., Bowman Gray School of Medicine, Winston-Salem, North Carolina.

We reviewed the records of 53 patients with RTA-I seen at our institution over the last 15 years. Fourteen of 15 pediatric cases occurred in infants (mean age 6.8 mos.). All infants had complete RTA-I (cRTA-I) and presented with vomiting, diarrhea or failure to thrive. Two had bone disease, but none had kidney stones (KS), nephrocalcinosis (NC), renal failure or a family history (FH) of RTA-I. The other pediatric case was an 8 year old with incomplete RTA-I (iRTA-I) and bilateral staghorn calculi (SC). Twenty-nine of 38 adults (76%) presented with KS (19 females, 10 males). Twenty-two of 29 (76%) had cRTA-I and 7 of 29 (24%) had iRTA-I. Mean age at first KS was 29.5 yrs. and mean age at diagnosis of RTA-I was 37.3 yrs. Twenty-five of 29 had formed more than 5 KS. Twenty had NC and 9 had SC. Twenty-three had had at least one surgical procedure for KS and 23 had had urinary tract infections. Five had hypercalciuria, 11 had hyperuricosuria and 12 had hypokalemia. Five had a FH of KS; 2 had a FH of RTA-I and 5 had rheumatologic disease (RD). Mean creatinine clearance (C_{Cr}) in this group was 59.5 ml/min. The mean age of the 9 adults (7 females and 2 males) without KS was 41.5 yrs. Six had cRTA-I and 3 had iRTA-I. Mean C_{Cr} was 63 ml/min. Four had RD. Two had FH RTA-I and 2 had malignancy. At our institution RTA-I is usually diagnosed in children before serious renal complications occur. In contrast, adults with RTA-I have usually experienced severe morbidity from KS, NC, SC, infection and mild renal failure by the time their RTA-I is discovered.

RENAL, SPLENIC AND COELIAC ARTERIAL LESIONS IN FIBROUS DYSPLASIA OF THE BONES. Claude R. Caron, C. Léger* and J.M. Pépin*. Univ. of Sherbrooke, Dept. of Med., CHUS, Canada.

Various endocrinopathies have been described in association with bone dysplasia, such as precocious puberty, hyperthyroidism, hyperparathyroidism, Cushing's disease and osteomalacia.

This report describes a new anomaly in association with bone dysplasia, namely multiple arterial aneurysms.

A fifty-year old male consults for hemiparesis of sudden onset. A few days later, he develops severe lumbar pain and presents proteinuria and microscopic hematuria. The physical examination reveals "café-au-lait" spots marked deformities of his limbs which show, on radiological examination, typical dysplasic changes: osteosclerosis, increased trabeculation and thinning of the cortex with lacunar zones.

Associated hypocalcemia, hypophosphoremia, increased alkaline phosphatase and decreased tubular phosphate reabsorption suggest osteomalacia.

Arteriographic studies show aneurysms up to 2.5cm diameter in the coeliac artery, both renal arteries and a markedly tortuous splenic artery.

In this condition, there exists abnormal proliferation of the fibrous tissue of bones and there could well exist a similar disorder in the arterial wall.

HYPERCALCEMIA IN CHILDREN DURING 1,25-VITAMIN-D_3 TREATMENT. JCM Chan, RB Young* and P Mamunes,* Medical College of Virginia, Richmond, Virginia

Forty-two children received 1,25-vitamin-D_3 for treatment of renal osteodystrophy (n=29), sex-linked dominant hypophosphatemic rickets (n=9), hypoparathyroidism (n=2) and pseudohypoparathyroidism (n=2). Serum calcium, phosphorus and creatinine concentrations were measured monthly for a total of 1079.5 patient months. Patients with renal osteodystrophy manifested hypercalcemia (>11 mg/dl) once in every 13 months of treatment on the average. Of three children with hypophosphatemic rickets who experienced hypercalcemia, two proved to have tertiary hyperparathyroidism. Among children with hypoparathyroidism and pseudohypoparathyroidism, three episodes of hypercalcemia were observed during 124.5 patient-months, an incidence of one hypercalcemic episode per 39 treatment months. The incidence of hypercalcemia is correlated to parathyroid activity.

Before and after initiation of treatment with 1,25-vitamin-D_3 the mean calcium x phosphorus solubility products were 42.9 and 47.2, respectively, values within the normal range. Renal function, as represented by reciprocals of serum creatinine determined retrospectively and prospectively for a mean of 22 months before and 26 months, respectively, after 1,25-dihydroxy-vitamin-D_3 treatment, underwent no significant changes, except in seven children with chronic renal insufficiency, six of whose rates of renal function deterioration worsened on the treatment. Hypercalcemia as the cause of such deterioration can be implicated in only three of these children.

EFFECTIVENESS OF THIAZIDES IN PREVENTING RECURRENT CALCIUM UROLITHIASIS: THE QUALITY OF THE EVIDENCE. David N. Churchill.* Faculty of Medicine, Memorial University, St. John's, Nfld., Canada.

Nine clinical trials of thiazide effectiveness in calcium urolithiasis prophylaxis have defined interventions and clinical outcomes. Seven concluded that thiazides are effective. Three of 7 (Yendt 1970, Coe 1974, Yendt 1978) have a one group pre-test post-test design which fails to protect against historical (co-intervention) and statistical regression threats to internal validity. Four of 7 (Coe 1977, Backman 1979, Ljunghall 1981, Pak 1981) have a one group, with a non-equivalent comparison group, design (both with pre-test and post-test) which fails to protect against selection and experimental mortality threats to internal validity. The remaining 2 trials (Scholz 1981, Brocks 1981) are randomized controlled trials which find no statistically significant difference between thiazide and placebo but lack statistical power to conclude that there is no difference. No conclusion, based on these 9 clinical trials, can be made regarding the effectiveness of thiazides for urolithiasis prophylaxis.

ACUTE RENAL FAILURE DUE TO ACUTE TUBULAR NECROSIS IN LUPUS NEPHRITIS. A.H. Cohen, H. Wang*, W.A. Border, and R.J. Glassock. Departments of Medicine and Pathology, Harbor-UCLA Medical Center, Torrance, CA.

Patients with active systemic lupus erythematosus (SLE) who develop acute renal failure (ARF) are often considered to have severe diffuse proliferative glomerulonephritis (DPGN) on clinical grounds and a renal biopsy may not be performed. DPGN is usually treated aggressively with immunosuppressive drugs and plasma exchange. Our experience suggests that this clinical approach may not always be appropriate.

During a five-year period we identified 6 patients with SLE who presented with ARF. Our standard approach is to recommend renal biopsy before treatment but, in these patients, because of a presumptive clinical diagnosis of DPGN, treatment was started empirically. Five patients received pulse methylprednisolone and/or cyclophosphamide and/or plasma exchange before other causes of ARF were considered. A subsequent renal biopsy showed that all 6 patients suffered from mesangial lupus nephritis and acute tubular necrosis (ATN) but not DPGN. The initiating factor(s) of ATN were drugs in 4 patients (2 ibuprofen, 2 naprosyn) and volume depletion and hemolysis in 1 patient each. After recognition of the correct diagnosis, immunosuppressive measures were discontinued and with general medical therapy alone five patients returned to baseline levels of renal function within two months; one patient died of opportunistic infection probably related to aggressive therapy.

We conclude that careful consideration of all causes of ARF in patients with SLE, as in other patients, must be done prior to initiating treatment. A renal biopsy may be indicated to distinguish ATN from DPGN in order to avoid unnecessary aggressive therapy.

NEPHROSTOLITHOTOMY: A NON-SURGICAL APPROACH TO RENAL AND URETERAL CALCULI. Ralph V. Clayman,* V. Surya,* Wilfrido Castaneda-Zuniga,* Kurt Amplatz,* and Paul H. Lange.* Departments of Urologic Surgery and Radiology, University of Minnesota, and Urology Section and Radiology Service, Veterans Administration Medical Center, Minneapolis, Minnesota.

In the treatment of urolithiasis, approximately 10,000 surgeries are performed annually in the United States. The development of percutaneous nephrostomy tubes has provided the physician with a conduit for approaching urinary tract calculi. Between 1977 and 1982, 92 patients have undergone percutaneous stone manipulation (nephrostolithotomy, or NTL). All cases required only local anesthesia. Stones varied in diameter from 4 to 50 mm, and were removed from all areas of the calyces, renal pelvis and ureter. Techniques included rigid and flexible nephroscopy, fluoroscopy, and electrohydraulic stone disintegration. There were no mortalities. Morbidity was 2%, primarily due to hematuria requiring transfusion. Successful extraction occurred in 87% of patients. Following NTL, there has been no significant change in blood urea nitrogen or creatinine, although the hemoglobin has routinely fallen 1-2 gms. The average hospital stay for NTL is 6 days. Most patients were able to return to work within 1-4 days following discharge.

Percutaneous nephrostolithotomy, while a relatively new technique, seems to be both a safe and time/cost effective approach to the majority of urinary tract calculi.

HEROIN ASSOCIATED NEPHROPATHY: HOW WIDESPREAD? Eugene E. Cunningham, Maria A. Zielezny*, and Rocco C. Venuto. SUNY At Buffalo, Depts. of Medicine and Social & Preventive Medicine, Buffalo, New York.

Heroin associated nephropathy (HAN) is a form of renal disease seen in parenteral abusers of heroin or cocaine that manifests itself with proteinuria or nephrotic syndrome. Among 18-45 year olds in the Buffalo area it was found to be second only to diabetic nephropathy as a cause of end stage renal disease (ESRD). Between 10%-13% of all 18-45 year old patients treated for chronic renal failure in this area have HAN. Among blacks the percentage of patients with ESRD secondary to HAN was found to be 48%. The importance of this problem nationwide is not clear. To determine the extent of HAN we sent out questionnaires to 130 dialysis units or nephrology sections in 24 metropolitan areas. Twenty-two of 31 respondents from 13 metropolitan areas identified 94 patients with HAN. All patients were in the 18-45 year age group. Two-thirds of these patients had ESRD. Eighty-nine patients (95%) were black. Seventy-four patients (79%) were black males, 15 patients (16%) were black females and 5 patients (5%) were white. Within the 18-45 year age group HAN was identified as the cause of ESRD in 31.5% of black males, 8% of black females but only 1% of white males reported by the 22 respondents. HAN was found to be the cause of ESRD in 10% of all respondents' patients. Conclusions: HAN appears to be an important cause of chronic renal disease in many areas of the country. Young black males appear to be especially prone to develop this serious kidney disease.

HORMONAL RESPONSES TO VOLUME EXPANSION (VE) INDUCED BY SALINE INFUSION DURING DAY (D) AND DURING NIGHT (N). G Danovitch, G Krishna, J Sowers,* E Mendoza.* Divisions of Endocrinology and Nephrology, UCLA School of Medicine and VA Medical Center, Los Angeles, CA.

We have previously reported that the natriuretic response to VE is attenuated at night. To examine the role of hormonal factors mediating these variations, 10 salt-replete normal subjects received an infusion of 0.9% NaCl at 500 ml/hr for four hours during D (0900-1300) and N (0000-0400). Each study was preceded and followed by a control hour. In four subjects we measured levels of plasma renin activity (PRA), Aldosterone (PA), Norepinephrine (NE), Epinephrine (E) and Dopamine (DA) at hourly intervals. Prestudy, 2nd and 4th hour values are tabulated (Mean±SE).

		Pre	2 Hr	4 Hr
UNaV	D	169±23	265±50	416±60
uEq/min	N	143±30	134±19	181±25
PRA	D	3.5±1.0	2.1±0.6	1.3±0.4
ng/ml/hr	N	4.2±1.0	3.2±0.9	2.3±0.8
PA	D	108±11	73±9	75±6
pg/ml	N	83±6	54±7	72±7
NE	D	328±56	280±48	261±47
pg/ml	N	407±76	233±36	273±70

Levels of DA, E and GFR were stable. Hence, despite similar alterations in the measured hormonal variables in response to VE during D and N, there was marked nocturnal attenuation of the natriuretic response. We conclude that suppression of PRA, PA and NE is indicative of recognition of an expanded central volume. However, nocturnal attenuation of natriuretic response to VE occurs independent of the changes in PA and catecholamines.

PREDICTION OF THE RESULTS OF INTENSIVE DIALYSIS (ID) IN UREMIC PERICARDITIS (UP) BY DISCRIMINANT ANALYSIS (DA). N.L. DePace*, J.S. Schwartz*, G.S. Mintz,* P.F. Nestico,* M. Kotler,* and C. Swartz. Univ. of Pennsylvania and Hahnemann Univ., Philadelphia, Pennsylvania.

To identify predictors of the success or failure of daily ID in UP, we examined initial clinical, laboratory and echocardiographic (echo) data on 97 patients (pts) using univariate and multivariate techniques. Sixty-seven pts responded to ID, 30 did not (22 required surgery and 8 died). By univariate analysis, the following 9 factors correlated with ID failure ($p<0.05$); temperature >102, pulmonary rales, admission blood pressure less than 100 mmHg, jugular venous distension, chronic peritoneal dialysis treatment, WBC >15,000, WBC shift, large effusion, and both anterior and posterior effusion by echo. Whether the pt was already on maintenance hemodialysis or not was not a useful predictor of outcome. By DA, a 7 variable function was constructed which could classify pts into three categories: (I) high, (II) intermediate, and (III) low probability of response to ID. Seventy-seven of the 97 pt values feel into categories I or III. The positive predictive values of category I was .98 and of category III was .100. The outcome in 10 consecutive pts admitted with UP was correctly predicted. We conclude: DA of pts with UP allows improved selection of pts with above 90% likelihood to respond to daily ID and rapid identification of pts unlikely to respond to ID requiring surgical intervention.

ADULT POLYCYSTIC KIDNEY DISEASE (APCKD): A REVIEW OF 53 PATIENTS. V. Delaney*, D.P. Segel, D.S. Fraley, F. Bruns, M. Licina* and S. Adler. Univ. Pittsburgh, Montefiore Hosp., Pittsburgh, PA.

53 symptomatic patients with APCKD were studied retrospectively, mean follow of 12 years (range 10 months - 33 years). Diagnosis was confirmed by either x-ray, ultrasound, laparotomy, or autopsy. Commonest presenting clinical findings were: hypertension (21%), flank pain (30), symptomatic urinary tract infection (UTI) (19%), hematuria (19%) and palpable masses (15%). Four patients had creatinine> 2.5 mg/dl at diagnosis and 9 patients (17%) progressed to end stage renal disease. Of 19 patients followed 10 years in only 2 (10%) did creatinine rise to> 2.5 mg/dl. Renal function change was linear to the reciprocal of plasma creatinine versus time. Easily controlled hypertension developed in 64% attended by minimal retinopathy. UTI's were common (53%), often recurrent (52%), precipitated by instrumentation in 6/14 patients (43%) leading to death in 2 (33%). Renal stones occurred in 18/53 (34%). Hematuria (66%) bore no relationship to decline in renal function. Proteinuria although common (68%) never exceeded 300 mg/dl. Of 55 pregnancies 9 (16%) were complicated. Hepatic cysts (44%), pancreatic cysts (9%), ovarian cysts (38%) were of no clinical significance. We conclude: 1. Renal functional deterioration is linear, less than previously reported, and bears no relationship to hematuria. 2. Hypertension is common but easily treated and causes little end organ damage. 3. Renal stones are frequent. 4. Urinary tract instrumentation often induces infection with considerable morbidity and mortality and must be avoided.

THE DIURNAL RENAL EXCRETION OF CYSTINE AND ITS SIGNIFICANCE FOR TREATMENT WITH CHELATING AGENTS IN PATIENTS WITH CYSTINURIA. Joost T.M.de Wolf*, Willem P. Geus*, Abraham van den Ende*, Petra Hoenderdos-Blom* and L.W.Statius van Eps. Depts. of Internal Medicine and Clin.Chem.Lab., Sloter-vaart Hosp., Amsterdam, The Netherlands.

Urinary free cystine excretion in 3 young patients with classical cystinuria treated with D-penicillamine (D.P.A.) and α-mercaptopropionylglycine (M.P.G.) was studied collecting the urine every four hours during periods of 2 weeks to 3 months. Also the relation between fluid balance and cystine excretion was investigated by subjecting the patients to a strict fluid regime (intake every 4 hours 500 ml water).

Free cystine in urine was quantitated by a modification by Backer of the procedure according to Haux and Natelson (Clin.Chem.Vol.20, 1974, p. 871).

The highest levels of cystine excretion in the untreated patients were observed in the period of 4 - 8 hr. a.m. During this period the urinary concentration of cystine exceeded amply 300 mg/l, being the crucial level for precipitation. Adjusting the dosage and time of administration of D.P.A. and later M.P.G. to this diurnal variation in excretion, it was possible to keep the concentration of urinary cystine below the critical 300 mg/l. With this treatment scheme it was possible to obtain good results with a dosage considerably lower (1250 mg/day for D.P.A. and 1500 mg/day for M.P.G.) than advised and used in clinical practice (2000 - 4000 mg/day for D.P.A.). Since this regime no new stone formation has occured in all patients studied.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS (MPGN): A PROSPECTIVE CLINICAL TRIAL OF PLATELET INHIBITOR THERAPY. J. V. Donadio, Jr., C. F. Anderson, J. C. Mitchell, K. E. Holley, D. M. Ilstrup,* and V. Fuster.* Mayo Clinic & Foundation, Division of Nephrology, Rochester, Minnesota.

In 1978, we reported to ASN the effects of platelet inhibitor drugs in patients with MPGN in a double-masked, placebo-designed trial (Kidney Int. 14:649, 1978). Herein, we present further results of treatment in 40 children and adults with idiopathic Type 1 MPGN. Patients entered the study between 4/75 and 11/80 and were assigned by balanced randomization to treatment for 1-year with either dipyridamole 225 mg/d and aspirin 975 mg/d (Group 1) or matched placebo tablets (Group 2). Treatment was interrupted in 4 patients because of complications, 3 bleeding in Group 1 and 1 acute interstitial nephritis in Group 2.

In Group 1, average iothalamate clearance (C_{iot}) decreased 1.3 ml/min/1.73 m^2/yr (NS) and serum creatinine (cr) increased 0.18 mg/dl/yr (NS). In Group 2, renal function declined significantly; average C_{iot} decreased 19.3 ml/min/1.73 m^2/yr ($P<.01$) and cr rose 1.1 mg/dl/yr ($P<.01$). The difference between treatment groups was significant for C_{iot} ($P<.02$) but not for cr or 1/cr using linear regression analysis of slopes of lab. values over time. ^{51}Cr-labeled platelet survival in vivo improved significantly in Group 1 ($P<.01$) but not in Group 2. No differences were observed between treatment groups in levels of hematuria, proteinuria or serum C3. We conclude that dipyridamole and aspirin treatment for 1 year stabilizes glomerular filtration rate in patients with Type 1 MPGN perhaps by reducing platelet consumption which may have a role in pathogenesis.

RENAL INVOLVEMENT IN TYPE II DIABETES MELLITUS (DMII): A CLINICOPATHOLOGIC STUDY. Francis Dumler, Vijay Kumar*, Pedro Cortes and Nathan W. Levin. Dept. of Med., Henry Ford Hospital, Detroit, Michigan.

We have studied black (BD) and white (WD) autopsied patients with DMII and black (BC) and white (WC) nondiabetics matched for age, sex, race and weight as controls . In all groups,the prevalences of renal insufficiency (RI), end stage renal disease (ESRD), hypertension (HT), cardiovascular disease (CVD), diabetic glomerulosclerosis (DGS) and of severe renal (RA), aortic (AA) and coronary (CA) arteriosclerosis were studied. Results (as %) were as follows:

	RI	ESRD	HT	CVD	DGS	RA	AA	CA
BC(n=40)	29	12	55	70	-	40	72	55
BD(n=54)	54	30	80	81	41	74	72	59
WC(n=41)	20	10	54	73	-	50	78	70
WD(n=55)	29	7	71	84	36	55	76	73

There were no differences between BD and WD in age (66±1 vs 70±1 yrs), duration of diabetes (14±1 vs 13±1 yrs) and insulin use (44 vs 54%). No differences were noted between WC and WD nor between BC and WC. BD had a higher prevalence of RI ($p<.01$), ESRD ($p<.05$), HT ($p<.01$) and RA ($p<.001$) than BC, with no differences in CVD, AA and CA. BD had a greater frequency of RI ($p<.01$), ESRD ($p<.003$), and RA ($p<.03$) than WD while no differences were noted for HT, CVD, DGS, AA and CA. The severity of HT, assessed by left ventricular thickness measurements was also similar between BD and WD (1.43±0.04 vs 1.34±0.08 cm). In summary: BD have significantly higher prevalences of RI and ESRD than WD. This can be accounted for only in part by a greater prevalence of HT in BD.

HYPONATREMIA UNTREATED CAN CAUSE CENTRAL PONTINE MYELINOLYSIS (CPM). G.D. Dubois,* W. Leach,* and A.I. Arieff, Nephrology Research, V.A. Med. Ctr. and U.C.S.F., San Francisco, CA.

CPM is a demyelinating lesion of the brain white matter (usually in the pons) which is usually fatal and of unknown etiology. Recently, it has been suggested that treatment of hyponatremia with hypertonic (3%) NaCl can cause CPM. We investigated this hypothesis in 55 rabbits in whom hyponatremia (serum Na = 116 mM vs control of 145 mM) was induced over 7 days with daily nasogastric 10% glucose/water (5% body wt/day) and vasopressin (1 U/kg/day). As serum Na fell to 119 mM, there was hind limb weakness in (60%) and at Na = 115 mM for 3.6 days, hind limb paralysis occurred in 45% of cases. Nystagmus was present in 7% of animals while 27% also developed front limb paralysis. 92% of animals with Na below 110 mM developed hind limb paralysis within 2.1 days. Hyponatremia (mean serum Na = 117 mM) untreated for 5 days resulted in a 55% mortality. Brain water in rabbits with limb paralysis was normal (423 ± 11 gm/kg dry wt vs control = 426 ± 13) while brain content of Na and K were reduced by 26% and 17% vs control ($p < .01$). In rabbits with limb paralysis, animals were sacrificed and brains were fixed, sectioned and stained with luxol fast blue. Central pontine myelinolysis (CPM) was present in 20% of those with hyponatremia for over 1 week. No animal was treated with hypertonic NaCl. In animals with hyponatremia for less than 7 days, CPM was not observed. Conclusions: 1) serum Na < 117 mM for 5 days can cause a progressive syndrome of limb weakness, paralysis, nystagmus and mortality > 50%; 2) in rabbits with chronic hyponatremia, brain water content is normal; 3) hyponatremia, in the absence of therapy, can lead to CPM.

CAPTOPRIL-INDUCED FUNCTIONAL RENAL INSUFFICIENCY IN PATIENTS WITH BILATERAL RENAL-ARTERY STENOSES OR RENAL-ARTERY STENOSIS IN A "SOLITARY" KIDNEY. V.J. Dzau*, D.E. Hricik*, P.J. Browning*, R.I. Kopelman*, W.E. Goorno*, N.E. Madias, Brigham and Women's Hosp. New England Med. Ctr., Boston, MA

Acute renal failure (ARF) occasionally complicates captopril (C) therapy but the types of patients (pts) at risk have not been defined. We observed 12 pts who developed ARF while receiving C, for severe hypertension associated with arteriographic-documented bilateral renal artery stenosis (RAS) (n=7) or RAS in a solitary kidney (n=5). All were receiving diuretic therapy. The mean systolic blood pressure fell from 189+19 to 171+7mmHg (4 had no response), serum creatinine (cr.) and urea nitrogen (BUN) increased from 1.8+0.3 and 41+6.5 to 5.3+0.6 and 101+15mg/dl respectively. None had proteinuria or abnormal urine sediment. Time from C to peak renal impairment varied 4 days to 2 months. In all cases, renal function improved within 1 week of stopping C. ARF probably resulted from functional disturbance of autoregulation of glomerular filtration consequent to blockade of renin angiotensin system or activation of prostaglandins in the presence of markedly reduce renal perfusion pressure. Further support for this hypothesis is derived from a) the observation that other nonspecific vasodilators (e.g. minoxidil), did not induce ARF in these pts and b) one pt who developed recurrence of ARF when rechallenged with another converting enzyme inhibitor (MK421). Sodium depletion and preexisting renal insufficiency appear to be predisposing factors. We conclude that C must be used with caution in pts with bilateral RAS or RAS in a "solitary" kidney.

RENAL ALLOGRAFT FAILURE DUE TO RECURRENT DENSE INTRAMEMBRANOUS DEPOSIT DISEASE (DIDD). A. Eddy,* R. Sibley and Y. Kim, Depts. of Pediatrics, Lab. Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota.

The 28 published case reports of histologic recurrence (rec.) of DIDD in renal allografts conclude that rec. is almost universal but rarely results in graft failure. We retrospectively studied the clinical course and the renal histology of 6 patients (pt) with DIDD who received 10 renal transplants (tx) since 1961. All had characteristic histologic findings of DIDD in their native kidneys (7 biopsies, 5 nephrectomies) by light, immunofluorescent and electron microscopy.

Two pts have normal renal function 3½ and 14 yrs after the first tx; and one pt 5 yrs after a third tx. None of these pts had post-tx biopsies. Histological evaluation of 10 biopsies and 6 nephrectomies of the remaining 7 grafts showed evidence of rec. in all. Graft failure could be attributed to the rec. in 5 tx in 4 pts (4 nephrectomized, 1 with chronic renal failure). They all had marked mesangial proliferation with crescents, but with absent or minimal acute interstitial and vascular changes. In the remaining 2 grafts histological evidence of rec. was too mild to account for graft loss; one showed mainly acute tubular necrosis and the other chronic rejection. Clinically, all pts with graft failure due to rec. were male and nephrotic. In 2 pts, pre-tx clinical and histological features were those of rapidly progressive glomerulonephritis (RPGN). In conclusion, rec. of DIDD in renal allografts is not so benign as previously reported, especially in pts with RPGN.

URINARY ERYTHROCYTE MORPHOLOGY IN MICROSCOPIC HEMATURIA. Kenneth F. Fairley*, Douglas F.Birch*, (intr. by Priscilla Kincaid-Smith) Department of Nephrology, The Royal Melbourne Hospital, Melbourne, Australia.

Asymptomatic microscopic hematuria is a common diagnostic problem for which urologic investigation is frequently unable to determine the cause. Glomerular bleeding is distinguishable from other causes of hematuria on the basis of urinary RBC morphology under phase-contrast microscopy. The value of this test for clinical practice was assessed by examining urine samples from 117 consecutive patients referred for investigation of microscopic hematuria. Dysmorphic (morphologically variable) RBCs suggestive of glomerular bleeding were present in 86/87 patients later shown to have glomerulonephritis (sensitivity of test = 99%; specificity = 93%). In contrast 30/30 patients with non-glomerular lesions had isomorphic (morphologically uniform) RBCs in the urine (sensitivity = 100%; specificity = 90%). 10 patients yielded a mixed morphologic pattern suggestive of dual pathology which was confirmed in 4 patients. 95% of 376 control subjects gave < 8000 RBCs/ml in the urine and in each case cells were dysmorphic, suggesting that RBCs enter the urine of healthy individuals via the glomerulus. Electron microscopy demonstrated the range of dysmorphic changes characteristic of glomerular bleeding. Phase-contrast microscopy of the urine sediment provides a reliable guide to the appropriate direction of investigation in patients with microscopic hematuria.

RAPIDLY PROGRESSIVE IgA NEPHROPATHY (RPIGAN). Alfredo R. Esparza, J. Gary Abuelo, Richard A. Matarese, Raymond G. Endreny, Jaime S. Carvalho, and Salvatore R. Allegra*. Providence, Rhode Island.

We report 5 cases of RPIGAN. All are males, ages 16 to 60 years. Respiratory infections preceded the onset in 2 cases. One case each presented with uremia, nephrotic syndrome, and gross hematuria; 2 cases with microhematuria and proteinuria on routine urinalysis. One of these latter patients had ignored recurrent episodes of gross hematuria. All had hypertension, azotemia (creatinine 1.6 - 9.4 mg/dl), proteinuria (>6 gm/24 hr. in 4 cases), hypoalbuminemia (>3 gm/dl), and hematuria (gross in 2 cases). All experienced progressive renal failure ending in dialysis (3 cases) or death from unrelated causes (2 cases). Prednisone, 60 mg/day for one month with 2 1-gm doses of i.v. methylprednisolone in 1 case and alone in another case, had no effect. A crescentic glomerulonephritis was present in all biopsies (crescents in 31-80% of glomeruli-mean 50%). The size and stage of the crescents was variable. Numerous glomeruli had focal or diffuse sclerosis. In all cases there was a (3+) deposition of IgA. IgG and sometimes IgM were noted in 3 cases in lower intensity. C3 was strongly positive in 3 cases. On electron microscopy, dense granular mesangial deposits were noted in all cases and occasionally capillary subepithelial deposits were also observed.

This form of IgA nephropathy is not uncommon. Rapid loss of renal function unlikely to respond to steroids is the usual clinical course.

THERAPEUTIC PARATHYROID AUTOTRANSPLANTATION IN RENAL OSTEODYSTROPHY (RO). Newell Falkinburg, Bruce D. Baird,* Robert E. Berry,* Carl H. Bivens,* Michael H. Koch,* Susan N. VandenBerg.* Roanoke Memorial Hospital, Dept. of Med. & Surg., Roanoke, Virginia.

Significant numbers of patients on hemodialysis today are experiencing the sequelae and complications of long term dialysis, namely RO, from secondary hyperparathyroidism (HP). This is usually a result of dietary noncompliance as well as medical noncompliance. Our approach in treatment of RO and pruritis secondary to renal failure is total parathyroidectomy with autotransplantation (TPA). To date we have performed 16 radical parathyroidectomies with autotransplantation of a portion of the most normal appearing gland into the brachioradialis muscle. Twelve of the transplants have been for secondary HP, 4 for primary HP with diffuse hyperplasia. This report deals only with the secondary HP patients. These patients have shown dramatic clinical improvement with loss of bone pain and pruritis as well as radiographic improvement of hand x-rays. Pathological examination of all parathyroid glands removed has revealed hyperplasia. Graft function has been documented by comparing serum parathyroid hormone levels of the grafted and nongrafted forearm. At present, our longest follow-up is 31 months and we have experienced minimal morbidity and no mortality related to the procedure. We have also found gland localization with preop CT scan to be of no value. Real-time ultrasonography has been of benefit in parathyroid gland localization. We conclude that TPA can be performed with minimal morbidity and limited hospital stay in the patient with RO. An ongoing study of patients will be presented.

PARENTERAL NUTRITION (PN) WITH INCREASED NITROGEN (N) INTAKE IN THE TREATMENT OF ACUTE RENAL FAILURE (ARF). Eben I. Feinstein, Joel D. Kopple, Howard Silberman* and Shaul G. Massry. Div. Nephrol., USC Sch. Med. and Div. Nephrol., Harbor-UCLA Med. Ctr. Los Angeles, CA.

In a previous study of PN in patients (pts) with ARF, we found no reduction in negative N balance or improvement in survival using either 21 g/day of essential (EAA) or 42 g/day of essential and non-essential amino acids (ENAA), compared to PN with glucose alone. To assess whether larger amounts of ENAA improved catabolism and survival, 12 pts with ARF were randomly assigned to PN with EAA 21 g/day (Group I) or ENAA (Group II). In Group II, N intake from ENAA was adjusted to exceed the urea N appearance (UNA) by 2.0 g/day up to a maximum of 15 g/day. 8 pts had ARF due to trauma. 3 pts in I and one pt in II had nonoliguric ARF. Mean age was 44 ± 18.4 SD years in I (n=6), and 47 ± 19.3 in II (n=6). Duration of PN was 14.5 ± 9.6 days and did not differ in the two groups. Mean N intake was 2.3 ± 3 g/day (I) and 11.7 ± 1.9 g/day (II), $p<0.001$, calorie intake was 2540 ± 259 K cal/day (I) and 2459 ± 335 (II). Mean UNA was significantly greater in II, 14.3 ± 4.7 g N/day, as compared to I, 7.3 ± 2.8, $p<0.01$. N intake minus UNA, an estimate of protein balance, was slightly but not significantly less negative in II, -2.1 ± 4.0 g/day vs -5.1 ± 2.7. Recovery of renal function occurred in 3 pts in I and one in II. 2 pts in I and no pt in II survived. These data indicate that with PN containing 11.7 g/day of ENAA N, there was greater UNA and no improvement in N balance or survival as compared to 2.3 g/day of EAA N. Whether a different pattern of ENAA will be beneficial needs to be examined.

k-IgA NEPHROPATHY AN UNUSUAL MANIFESTATION OF A PLASMA CELL DYSCRASIA. Jose A. Ganel, Charles N. Gamble. Depts. of Medicine & Pathology, Kaiser Foundation Hospital & Sutter Memorial Hospital, Sacramento, California.

We describe a patient with nephrotic syndrome and renal biopsy showing diffuse deposition of a material reactive with anti-IgA and anti-kappa light chain anti-serums. Small amounts of a k-IgA M component were noted in the serum and urine prior to treatment with melphalan and prednisone. Several bone marrow studies did not reveal multiple myeloma. Renal functional deterioration was observed, but seemed to stabilize after chemotherapy resulted in virtual disappearance of the M component from serum and urine. The renal findings initially of obscure interpretation were thought to represent a secondary manifestation of an underlying plasma cell dyscrasia.

PROTEIN MALNUTRITION IN A DIALYSIS POPULATION: TRANSFERRIN BLOOD LEVEL AS AN INDICATOR. Ronald Fischman and Maher Azer, Los Alamitos Hemodialysis Center, Los Alamitos California.

Transferrin Blood Levels were monitored for a period of 6 months in a dialysis population consisting of 124 patients. Fifty-eight patients (46%) maintained a normal transferrin level above 200 mg% suggestive of an appropriate protein nutritional state; thirty patients (24%), maintained a level between 180-200 mg% suggestive of a state of mild protein depletion. Twelve patients (10%) maintained a level between 160-180 mg% suggesting a moderate state of protein malnutrition, while twenty-four patients (20%) had a level below 160 mg% suggestive of a state of severe protein malnutrition.

It is apparent that protein malnutrition in a dialysis population can exceed 50%. Special attention is needed for early diagnosis, prevention and correction.

PLASMA VOLUME (PV) MEASUREMENTS IN PATIENTS WITH NEPHROTIC SYNDROME (NS): A METHODOLOGICAL STUDY ON THE LABELED ALBUMIN METHOD. Anton B. Geers, Hendrik A. Koomans, Peter Boer, and Evert J. Dorhout Mees. Intr. by Ben B. Johnson, Dept. of Nephrology and Hypertension, University Hospital, Utrecht, The Netherlands.

According to the prevailing concept, edema formation in NS patients is attributed to the effect of intravascular hypovolemia on renal salt excretion. Yet, as previously reported, we found normal or increased PV and blood volumes (BV) in NS patients using the radioiodinated serum albumin (RISA)-method and hematocrit (Ht). This discrepancy might be explained by overestimation of PV with RISA in NS patients, because of an altered albumin distribution volume or increased transcapillary escape rate (TER) of the injected albumin. Assuming that an increased albumin distribution volume would be accompanied by a decreased F-cell ratio (whole body Ht/peripheral venous Ht), ^{51}Cr erythrocyte volume and PV were measured simultaneously. F-cell ratio was 0.842±0.06 in 24 patients with NS from various causes, vs 0.863±0.03 in 12 healthy controls. In a second group of 19 NS patients TER was 9.5±2.5% during the first hour after injection, an increase of approximately 4% vs normal TER. These findings indicate slight overestimation of PV with RISA not exceeding 3%, or only 1.5 ml/kg lean body mass. In a total of 55 NS patients we found a BV of 94.1±15.9 ml/kg lean body mass vs 88.5±9.5 in 48 normals. In only one patient BV was clearly reduced. As apparant from the above considerations this difference cannot be attributed to overestimation of BV in NS patients. We conclude that overestimation of PV with the RISA method is minimal in the NS. Normal or increased blood volumes are the rule rather than the exception in this condition.

INVESTIGATION OF CYSTINE CALCULI BY X-RAY DIFFRACTION AND SCANNING ELECTRONMICROSCOPY. Willem P. Geus*, John W.Geus*, Joost T.M. de Wolf* and L.W. Statius van Eps. Dept.Med., Slotervaart Hosp., Amsterdam and Dept.Inorg.Chem., Univ., Utrecht, The Netherlands.

Calculi of three patients suffering from classical cystinuria have been investigated to assess the mechanism of crystallization in vivo; dissolution has been studied too. The crystal structure has been established by X-ray diffraction. Accurate measurement shows that the diffraction pattern of the calculi almost completely coincides with that of pure L-cystine crystallized in vitro. Much information has been obtained by investigation of the calculi in the scanning electronmicroscope.

Whereas L-cystine crystallizes in vitro as hexagonal platelets (diameter 40 to 200 µm, thickness 5 to 20 µm), crystallites grow more three-dimensionally in vivo. The calculi appear to consist of symmetrical crystallites (200 to more than 400 µm) intimately grown together with a small amount of platelets analogous to those observed with L-cystine crystallized in vitro. The platelets are liable to desintegration to small spherical particles. Desintegration is observed to proceed preferentially at the external surfaces of the calculi.

Exposure of cystine calculi in vivo to α-mercaptopropionylglycine results in attack of the crystallites at special sites (presumably defects). In vitro cystine calculi immersed in solutions of trometamol and trometamol with D-penicillamine display a much more severe attack. The rate of dissolution shows decomposed material in the outer shell to dissolve less rapidly. Electronmicrographs indicate a non-specific attack of the crystallites.

MASSIVE G.I. HEMORRHAGE SECONDARY TO RECTAL ULCERS IN PATIENTS WITH CHRONIC RENAL FAILURE. Marvin Goldberg, Duane G. Wombolt. Norfolk, Virginia.

Massive lower G.I. bleeding from rectal ulcers is an unusual medical problem. The syndrome has never been described as a complication of chronic renal failure. We will discuss five patients with chronic renal failure who presented with a sudden onset of life-threatening hemorrhage secondary to solitary rectal ulcers. Four of the five patients were being treated with dialysis.

All of the patients had massive gastrointestinal hemorrhage with a passage of large amounts of bright red blood per rectum. Hypotension was present in all and none of the patients had been dialyzed within 24 hours of the bleeding and thus were not anticoagulated with heparin. All patients required transfusions. Two patients were considered for colectomy to stop the bleeding prior to the proper diagnosis. Two of the patients had mesentric arteriograms to help localize the source of bleeding. Three of the five patients had to be taken to the operating room for suture ligation to stop their bleeding. Biopsy of 1 ulcer revealed nonspecific inflammatory cells. It is important to consider the diagnosis of bleeding rectal ulcers in any patient with chronic renal failure who develops a sudden massive life-threatening lower gastrointestinal hemorrhage.

USE OF RANDOM "SPOT" URINE SAMPLE TO QUANTITATE PROTEINURIA. Jay Ginsberg,* Richard Matarese, Bruce Chang, and Serafino Garella. Rhode Island Hospital and Brown University, Providence, Rhode Island.

Quantitation of proteinuria has diagnostic and prognostic importance, and is used to assess therapy. Timed urine collections -- usually 24 hrs. -- are cumbersome to obtain and are often inaccurate. We measured 24-hr. protein and creatinine excretion rates in 36 patients with a wide range of proteinuria (0.03 - 22 gms) and of renal function (PCr 0.7-7.2 mg/dl), and compared them to the urine protein:creatinine ratio $[(P/Cr)_u]$ in a single "spot" sample collected the next day. Correlation was highly significant ($r = 0.96$); all patients with > 3.5 gms of proteinuria had a $(P/Cr)_u$ of >3.5; patients with <0.2 gms of proteinuria had a $(P/Cr)_u$ of < 0.1. Three patients with intermediate degrees of proteinuria had a $(P/Cr)_u$ of > 3.5: in each, it was shown that the timed urine collection was incomplete, verifying the greater accuracy of $(P/Cr)_u$. Comparison of $(P/Cr)_u$ values in samples obtained during standing and recumbency showed a clear orthostatic component.

The determination of $(P/Cr)_u$ in a single "spot" urine sample obtained after standing is a simple, rapid, accurate means of assessing protein excretion rates. Discrepancies between $(P/Cr)_u$ and 24-hr. protein excretion rates are due to inaccuracies in the timed sample. Variations in $(P/Cr)_u$ can be used to evaluate the importance of the orthostatic component of proteinuria. This method should therefore replace the collection of 24-hr. urine samples in the clinical quantitation of proteinuria.

LONGTERM FOLLOWUP OF RENAL FUNCTION IN KIDNEY TRANSPLANT DONORS. R.C. Goldszer,* R.M. Hakim, and B.M. Brenner. Brigham and Women's Hos., Boston, MA

Renal ablation in animals leads to progressive azotemia, proteinuria and glomerular sclerosis. We therefore examined renal function and blood pressure in 25 kidney donors (13 men, 12 women; mean age 60 yrs) 10 to 28 years post-nephrectomy (Nx). We compared current serum creat (Scr), creat clearance (Ccr) and proteinuria (UprotV) to pre-donation values; comparisons were also made with age and sex-matched non-Nx controls.

	Diast BP (≥90mmHg)	Scr (mg/dl)	Ccr current / Ccr pre-Nx	UprotV (mg/day)
Controls (n=25)	4/25	1.01±0.04SE	---	<50
Donors(n=19) 10-14yr p-Nx	7/19	1.29±0.06	0.75±0.05	130±19
Donors(n=6) ≥15 yr p-Nx	3/6	1.48±0.20	0.69±0.09	366±127

UprotV increased in all but 6 donors, exceeding 200 mg/day in 8 and reaching 500 and 900 mg/day in two. Scr exceeded 1.4 mg/dl in 6 donors (≥1.7 in 4). The ratio of Ccr current/Ccr at time of donation averaged 0.7 in both donor groups. In 6 donors (5 of whom had ↑ BP or ↑ UprotV) this ratio was ≤0.6, implying a reduced hyperfiltration response. These findings suggest the possibility of a slowly progressive, albeit relatively mild, syndrome of hypertension, proteinuria and declining Ccr in some kidney donors. Serial, longterm evaluation of all patients undergoing uninephrectomy appears warranted.

HEMOLYTIC UREMIC SYNDROME (HUS) AND DEAFNESS INDUCED BY POTASSIUM BROMATE ($KBrO_3$). D. Gradus*, M. Rhoads* & S.C. Jordan*. (Introduced by Isidro Salusky). UCLA Cntr. Health Sciences, Div. Ped. Neph. Los Angeles, California

A widely used home permanent hair neutralizer containing $KBrO_3$ is known to cause acute renal failure and deafness. A case of $KBrO_3$ poisoning is presented which mimicked the clinical and laboratory presentation of HUS. Diagnosis of deafness was therefore delayed until clinical manifestations occurred. A 17 mo. old black female ingested an unknown amount of home permanent cold wave neutralizer containing $KBrO_3$. She subsequently became stuporous with hypotension, vomiting, diarrhea and abdominal cramps. Lab data revealed: Hct=27%; HCO3=8 mEq/L; cl=122 mEq/L; Bromide=1.2 mg/dl (non-toxic level). The pt. developed progressive hemolysis (Hct=15%) and renal failure requiring peritoneal dialysis. HUS was considered because of the clinical presentation and the non-toxic bromide level. Further investigation of the ingested neutralizer revealed that the toxic agent was bromate rather than bromide, with different toxic manifestations of ARF and deafness. This, combined with clinical evidence of behavioral regression and loss of speech led to audiographic diagnosis of sensory deafness. $KBrO_3$ is stable in the body and is only minimally reduced to bromide which explains the non-toxic bromide level. Although reports of ARF and deafness with $KBrO_3$ exist, an HUS-like picture has not been reported. HUS is the most common cause of ARF in infancy; this case is presented to expand the differential diagnosis to include HUS-like syndrome induced by $KBrO_3$ and to emphasize the importance of early diagnosis and treatment of deafness.

OSMOTIC DIURESIS AFTER RADIO CONTRAST AGENTS (RCA). **F.N.Harris,Jr.* and R.J.Glassock, Dept. of Medicine, Torrance, CA.**
Although it is known that RCA increase urine volume and osmolar clearance (Cosm, ml/min), a prior systematic study of the extent of fluid and electrolyte loss after RCA has not been performed. Consequently questions as to the composition and quantity of fluid needed to replace these losses remain unanswered. To examine this problem 16 hospitalized patients were studied with blood and 2-hour urine collections for osmolality, creatinine, urea, Na, K, and Cl before (B) and three consecutive periods (I,II,III) after RCA. Fractional excretion of Na (FENa), creatinine clearance (CrCl,ml/min), Cosm, and urine non-chloride anion (NCA, mEq) were calculated from this data. The patients who underwent intravenous pyelogram and CT scan were the low dose group (LD); the patients who underwent angiography were the high dose group (HD). All patients were euvolemic and received .5 normal saline (.5NS) at 100 ml/hr for at least 12 hours before RCA and for the urine losses after RCA. Results are expressed as mean ± SEM. The dose (mOsm/kg) of Na and methylglucamine diatrizoate given LD was 1.8±.3 (1420 mOsm/L) and HD was 4.8±.5 (1690 mOsm/L). The 6 hour urine total of Na (mEq), K (mEq), and urine volume (ml) was 50±17, 19±3, and 838±158 respectively with LD, and 95±17, 41±7, and 2119±141 respectively with HD.

	LD (n=7)			HD (n=9)		
	B	I	III	B	I	III
CrCl	105±15	102±14	100±16	129±10	124±21	120±18
FENa	.7±.1	1.4±.3*	.8±.2	.3±.1	2.2±.7*	1.3±.4*
Cosm	1.6±.4	3.4±.7*	3.6±.9*	2.1±.3	5.8±.6	5.1±1
NCA	4.7±3	13±2*	11±4*	3±.7	17±3*	8±3*
Uosm	349±39		325±41	403±29		304±35*
Plasma K	4.6±.02		4.2±.1*	3.9±.1		3.8±.1*

*p<.05 vs. B

The data indicate that the osmotic diuresis after RCA is similar to mannitol with urine osmolality approaching isotonicity, a urine Na concentration approximately 50 mEq/L, and urine K concentration < 20 mEq/L. Fluid loss after HD can be massive but can be adequately replaced with .5NS without the addition of K.

MESANGIAL IGM NEPHROPATHY IN CHILDREN. K. Gurumurthy, Amir Tejani, and Anthony D. Nicastri.* Depts. of Peds. and Pathol. DMC, SUNY, Brooklyn, NY.

The clinical manifestations, course and prognosis of mesangial IgM nephropathy were studied in 25 patients. Glomeruli were normal by light microscopy or had mild mesangial hypercellularity. All patients showed diffuse mesangial deposition of IgM. 21 patients were nephrotic (N) at onset while 4 had hematuria and proteinuria. All N patients were treated with prednisone. 6 were resistant and of 15 responders, 9 became steroid dependent.(SD) 13 patients required cyclophosphamide.(C) Only 6 had complete remission. These results were compared to the outcome of 26 patients with minimal change nephrotic syndrome and negative immunoflorescence, who were biopsied because of being frequent relapsers and have been regularly followed.

	M.C.N.S.	IgM	p
Age	41 mo. (11-96)	63 mo. (6-140)	< .01
SD	14%	60%	< .01
C	35%	61%	< .05
Remission on C	100%	46%	< .01

On rebiopsy 2 of the IgM nephropathy patients have converted into focal sclerosis and are in chronic renal failure. Our study shows that children with IgM nephropathy are older at onset, and respond only partially to therapy. The prescence of such deposits is a marker for a more severe disease with a less favorable prognosis.

BLOOD ZINC PROTOPORPHYRIN LEVELS (ZPP) AS AN INDICATOR OF LEAD TOXICITY IN CHRONIC RENAL FAILURE. Stephen M. Hessl*, Asad A. Bakir*, Douglas Hoffman*, and George Dunea. Cook County Hospital, Chicago, Illinois.

ZPP is a useful test in the detection of lead toxicity (LT) in patients with normal renal function, but its utility in chronic renal failure (CRF) has not been well described. We measured blood lead levels (BPb) by atomic absorption spectrophotometry and ZPP levels by hematoflourometery in four groups of patients. History of lead exposure (EXP) was obtained by a detailed questionnaire. Group I consisted of 2 patients with CRF on chronic hemodialysis (CHD) who had elevated BPb and very significant EXP. Group II consisted of 35 patients on CHD with no EXP or elevated BPb. Their mean hematocrit was 26% (S.D. 4.3). Group III consisted of 14 patients with mild to moderate CRF not on CHD who had no EXP or elevated BPb. Their mean hematocrit was 30% (S.D. 4.4) and creatinine was 7.0 mg/dl (S.D. 2.5). Group IV consisted of 30 normal controls.

	N	Mean ZPP	(S.D.)
Group I	2	431 µg/dl	(78)
Group II	35	114 µg/dl	(51)
Group III	14	48 µg/dl	(28)
Group IV	30	5.4 µg/dl	(4.3)

p <0.001 (analysis of variance)

These data suggest the following: 1) CRF increases ZPP. 2)ZPP levels over 165 µg/dl in CHD and over 76 µg/dl in CRF should raise the suspicion of lead toxicity. 3) LT is probably not a common cause of CRF. 4)ZPP is a useful test in the evaluation of LT in patients whose impaired renal function precludes the EDTA provocative test usually done for the diagnosis of LT.

XAD-4 HEMOPERFUSION (HP) FOR DIGOXIN POISONING IN RENAL FAILURE (RF). WE Hoy, TP Gibson, VK Jain, AJ Rivero, RM Bayer, DF Montondo, RB Freeman. Univ. of Rochester, Dept. of Medicine, Rochester, N.Y.

Digoxin (dig) poisoning can be protracted in RF because of reduced dig clearance. We treated 3 patients with Cr.Cl. <5ml/min and refractory dig levels and manifestations of digitoxicity with XAD-4 HP. Hourly pre&post cartridge and red blood cell(RBC) dig levels were measured and dig extraction fractions, clearances and plasma $T_{\frac{1}{2}}$ calculated.

	Dig level ng/ml		Extraction Fraction		Clearance ml/min
	Plasma	RBCs	Plasma	RBCs	
Pt 1	S/E*	S/E	S/E	S/E	
5 hr HP	4.3/2.2	ND	.94/.73	ND†	218
6 hr HP	3.0/1.3	ND	.90/.82	ND	293
Pt 2					
$5\frac{1}{4}$ hr HP	5.8/3.6	5.4/4.0	.95/.70	.82/.73	192
Pt 3					
$6\frac{1}{4}$ hr HP	3.6/1.6	4.3/2.2	.92/.84	.8/.59	277

Digitoxic manifestations improved during each HP. Dig extraction from plasma and RBCs was efficient and well maintained. Total dig clearances greatly exceeded normal renal dig clearances and increased with increasing flow rates. Mean plasma $T_{\frac{1}{2}}$ dig was 64.5hr pre&post HP and 5.9hr during HP. Dig levels rebounded to intermediate levels 4 hrs. after HP, and mandated repeat HP in one pt. Dig recovery from used resin (176-300 micrograms) equalled or exceeded calculated dig removal.

With low toxic/therapeutic ratio, removal of modest amounts of dig should reverse toxicity. XAD-4 HP can provide dig clearance 2-3x normal renal dig clearance, abbreviate toxicity, and should be considered for other digitoxic RF patients.

*S/E - start/end of HP †ND - not done

ANTICYSTINURIC EFFECT OF GLUTAMINE AND OF LOW SODIUM DIET IN CYSTINURIA. Philippe Jaeger* and Luc Portman*. (intr. by G. Giebisch) Dept. of Medicine, Univ. of Lausanne, Switzerland.

The effects of glutamine (Gln) and of dietary sodium intake on cystine, ornithine, lysine and arginine excretion were studied in a 56 year old male recurrent stone former with cystinuria. L-Gln was given orally (2.1 g/d in 3 divided doses) for 18 days on 2 different occasions. Several 24-h. urine collections were obtained before and during treatment, and urine was analyzed for amino acids, Na and creatinine. After 7 days on Gln, cystine excretion started decreasing rapidly from 2.30±0.05 (n=10) to 1.15±0.08 (n=9) mmol/g creat. ($p<0.0005$), and so did ornithine, from 2.57±0.14 (n=10) to 1.36±0.23 (n=9) mmol/g creat. ($p<0.001$) (mean ± SEM). In contrast, lysine and arginine excretion rates which also were elevated, did not change after Gln.

Manipulation of the Na-intake of the patient over a wide range (20 to 350 mmol/day) demonstrated that urinary excretion of both cystine and ornithine are positively ($p<0.01$) correlated with that of Na. On a low Na diet, urinary cystine concentration decreased to the solubility level of this amino acid, and on Gln, it was below this limit. No correlations between urinary sodium excretion and that of lysine and arginine were found. It was also observed that the correlation between sodium excretion and that of cystine and ornithine was abolished by Gln. These results suggest that both Gln and a low sodium intake, the latter possibly via changes in extracellular fluid volume, are anticystinuric.

PROTEINURIA IN TREATED ESSENTIAL HYPERTENISON. Chia M. Huang, Antonio Quintanilla and Francesco Del Greco, Section Nephrology/Hypertension, V.A. Lakeside Medical Center and Northwestern Univ. Med. School, Chicago, Illinois.

Incidence and magnitude of proteinuria (Upr. g/24 hr) in the course of antihypertensive therapy are not well documented in patients with essential hypertension (EH). To clarify this issue, 248 benign EH men on step care therapy for at least one year were studied. Upr. endogenous creatinine clearance (Ccr, ml/min), systolic and diastolic blood pressure (SBP, DBP) were determined repeatedly. Upr greater than 0.2 gm was considered significant. On this basis, patients were divided into two groups. Results (mean ± SE): Upr was 0.85±0.3 in 64 patients. (Group I, and 0.13±0.04 in 184 patients. Group II, ($p<0.001$). Ccr was lower in Group I than in II, 80.8±3.2 v/s 90.7±1.8 ($p<0.05$). DBP was<90 mm Hg in both groups, 88.5±0.1 v/s 86.8±0.7, while SBP was higher in group I than II, 148.2±1.4 v/s 135±0.8 ($p<0.05$). Group I patients had been hypertensive longer than group II patients, 13.9±1.2 v/s 8.6±0.4 years ($p<0.001$). Kidney biopsy in 7 patients with Upr greater than 2.5 gm of group I revealed: IgA nephropathy 1, focal glomerulosclerosis 1, severe nephrosclerosis 5. Four of these patients progressed to renal failure within 2 years. Conclusions: (1) 25% incidence of significant Upr in treated EH is greater than reported previously, (2) persistent proteinuria despite decreased DBP<90 suggests progression of underlying renal lesion, (3) longer duration of hypertension prior to therapy and persistent higher SBP increase incidence and the amount of proteinuria.

ACUTE GLOMERULONEPHRITIS (AGN) CAUSING ACUTE RENAL FAILURE (ARF) IN ANGIOIMMUNOBLASTIC LYMPHADENOPATHY WITH DISPROTEINURIA (AILD). P Jenkins, M Schrager,* J Lodish,* R Hall.* Mount Sinai Medical Center, Milwaukee, WI.

An 84 y/o man developed ARF 3 mos after a diagnosis of AILD was made. He originally had fever, weight loss, generalized lymphadenopathy, hepatosplenomegaly, a creatinine of 0.8 mg%, and was treated with a short course of Prednisone. Three mos later he presented with generalized edema, lymphadenopathy, and splenomegaly. Creatinine was 4.6 and BUN 100 mg%. Ccr was 3.5 ml/min with 641 mg of protein/day. Urinalysis showed 4+ protein, 150+ RBC's and 10-15 WBC's with 5% eosinophils. U_{Na^+} was 11 mEq/L. Percutaneous renal biopsy disclosed diffuse proliferative glomerulonephritis and occasional intra-glomerular eosinophils. There were deposits of anti-IgA and anti-C3 peripherally and segmentally, without IgG, C4, or C1q. Electron microscopy showed massive sub-endothelial deposits. C3 was low (26 mg%) with a normal C4 and C1q. Rajii cell assay was high (183 AHG Eq/ml). Negative were cryoglobulins, ANA, RA factor. Increased were IgE (>2000 mg/dl) and IgA (359 mg/dl). Oliguria developed. The patient was treated with dialysis and steroids but progressively worsened and died on the 29th hospital day with refractory hypotension.

This represents the first comprehensively studied AILD patient with AGN. The findings suggest an immune complex etiology but with alternate pathway complement activation. IgA as found is uncommon. Eosinophils in glomeruli and urine may be unique for this unusual complication of AILD, and with elevated IgE suggests a possible allergic basis.

NOCTURNAL HYPOSTHENURIA AND POLYURIA-AN UNUSUAL MANIFESTATION OF PSYCHOGENIC POLYDIPSIA. Kirit K. Joshi, Southeast Al. Medical Center, Dothan, Alabama

Polyuria, hyposthenuria, hyponatremia & water intoxication have all been reported in pts. with Psychogenic Polydipsia. Nocturnal Polyuria is very unusual in a compulsive water drinker. Inability to concentrate urine at night(Uosm 119±16.9) & nocturnal polyuria in a pt. with Psychogenic Polydipsia form the basis of this case report. Sosm & Uosm are expressed in Mosm/Kg.. Pt. is a 51yr. old W/M who gave H/o of increased nocturia and polyuria (3000-3600 cc's at night)of 5yr. duration. Pts. renal function was normal & had no evidence of diabetes. Pt. gave H/o of ingesting 1½-2gals. of fluid a day, more in evening hrs..Pt's baseline Sosm was low (278±2.8).Serum Na was normal(138-140mEq). Uosm at daytime was 668.5±170 & at night only 119±16.9. After fluid restriction of 36hrs., Sosm was 278.6±6.42 & Uosm 819.33 ±102.5 but at night pt. was still excreting relatively dilute urine(Uosm 415.5± 19.09). After infusion of hypertonic (3%) saline Uosm was 607. After administration of 5 units of Aqueos Vasopressin percent change in Uosm was only 2.9. In conclusion, a pt. with Psychogenic Polydipsia can present with Noctrual Polyuria. Probable mechanisms in this pt: Maximal supression of Vasopressin at night by recumbency, hypervolemia & hypotonicity of plasma due to excessive fluid ingestion at end of the day.

NON-OLIGURIC PRERENAL AZOTEMIA. D.E. Kamm, A. Spital, and R. Sterns. Rochester General Hospital, Univ Roch Sch Med, Rochester, New York.

Prerenal azotemia is usually characterized by a high urine osmolality and U/P creatinine, and a low fractional Na+ excretion and urine output; these findings reflect the presence of a relatively normal, but poorly perfused kidney. This report describes 6 patients who presented with a picture typical of prerenal azotemia, except for the absence of oliguria: 4/6 had CHF and 2/6 volume depletion. G.I. bleeding, steroid therapy and fever were each present in 3/6. All 6 had received diuretics prior to, but not during, our studies. Exogenous protein intake varied from nil to 60 g/d. The mean BUN and S. creatinine were 91 (42-174) and 2.6 (1.6-4.9) mg/dl, and the BUN/S. creatinine 35 (23-44). The creatinine clearance was 43 ± 7 ml/min and urea clearance 19 ± 2. Urine studies revealed low [Na+] (1-13 mEq/L), [Cl-] (1-9 mEq/L) and fractional Na+ excretion (0.01-0.42%), and high osmolality (505-871 mOsm/kg) and [K+] (30-72 mEq/L). Urine volume ranged from 1200-2900 ml/d. Urine urea-N excretion was elevated, 24.9 ± 1.6 (15-36) gm/d and urea accounted for 72-82% of the urine solute. Conclusions: Prerenal azotemia should be considered in the differential diagnosis of non-oliguric renal failure. In catabolic patients, increased urea production and excretion provide sufficient urinary solute to prevent oliguria even when the urine is concentrated and sodium free.

SPONTANEOUS RECOVERY OF NEPHROTIC SYNDROME ASSOCIATED WITH TOLMETIN. S.J.Joshi and K.Stern*. Veterans Administration Medical Center,Department of Medicine, Saint Louis, Mo.

Renal disease associated with non-steroidal anti-inflamatory agents usually takes the form of interstitial renal disease, and Nephrotic Syndrome (NS) is rare. We have seen two patients who developed NS during tolmetin therapy. Both recovered after withdrawal of the drug.

Case 1: 71 year old black male was admitted with NS.Past history of laryngectomy for carcinoma of larynx.Also has arthritis for which he recieved Tolmetin. No gold or penicillamine. Serum Cr. was increased from 1.6 to 2.4 and he had an eosinophilia of 4%. Open renal biopsy showed sclerosis of many glomeruli and interstitial inflammation. Immunofluorescence (If.) showed immunoglobulin(Ig) localization in the tubules.Proteinuria decreased spontaneously over the next three months with improvement of renal function to baseline, after withdrawal of Tolmetin.

Case 2: 71 year old white male with psoriatic arthritis treated with gold and tolmetin. Gold was discontinued for skin rash. In Sept. 80 developed 3+ proteinuria. In Jan 81 had a 3.4 gm. protein in a 24 hour urine sample. Renal function was unchanged. Renal biopsy showed few sclerotic glomeruli, two normal glomeruli and one glomerulus with mild sclerosis. There was patchy interstitial change. If was negative. Proteinuria spontaneously reduced over the next year after withdrawal of tolmetin.

We conclude that interstitial nephritis and sclerosis may be an important part of renal disease associated with tolmetin, and the disease is reversible upon withdrawal of the drug.

REDUCED EXTRAVASCULAR T4 BINDING IN CHRONIC RENAL FAILURE (CRF) AND CRITICAL NONTHYROIDAL ILLNESS WITH NORMAL RENAL FUNCTION (CI) . E.M. Kaptein,* E. Chang,* E.I. Feinstein, J.T. Nicoloff,* and S.G. Massry. Div. Nephrology & Endocrinology, USC School of Medicine, Los Angeles, CA.

Patients with CRF and CI have reduced serum binding of T4 to TBG. In healthy euthyroid subjects such a defect accelerates the rate of T4 exit from serum (Kii). In contrast, in CI and CRF the Kii is reduced, presumably due to impaired extravascular binding of T4. To determine the extravascular site(s) of this binding abnormality, serum tracer T4 kinetic studies were compared in 7 CI and 7 CRF patients, 8 normals, 1 healthy euthyroid subject with increased serum TBG and 1 with absent TBG using a 3 pool model consisting of rapidly (liver and kidney) and slowly (muscle and skin) equilibrating tissues and serum. The pool sizes are shown below as the mean ± SE,* P<0.01.

POOLS(ug/m2)	RAPID	SERUM	SLOW
NORMAL	146 ± 16	118 ± 9	219 ± 24
HIGH TBG	130	363	582
ABSENT TBG	128	18	70
CI	31 ± 9*	39 ± 5*	58 ± 10*
CRF	109 ± 14*	132 ± 15	199 ± 12

The results indicate: 1) serum and slow pool sizes parallel serum T4 binding to TBG while rapid pool size does not, 2) in CI all 3 pools are reduced, and 3) in CRF only the rapid pool is reduced. Thus 1) rapid pool size is independent of serum T4 binding to TBG, 2) in CRF and CI the reduced rapid pool size must be due to impaired tissue binding, and 3) the decreased rapid tissue binding is responsible for the reduced rate of T4 exit from serum in both CI and CRF patients.

EFFECTS OF INCREASED URINE FLOW AND ALDOSTERONE ON K BALANCE DURING FUROSEMIDE ADMINISTRATION TO MAN. R. Kelly,* C.S. Wilcox, W.E. Mitch, P. Souney,* T. Meyer,* K. Skorecki,* and C. Rayment.* Harvard Medical School and Brigham and Women's Hospital, Boston, Mass.

Micropuncture studies have shown that Aldosterone (Aldo) and distal Na and fluid delivery can regulate K excretion (U_KV). We studied whether K depletion with diuretics was related to changes in these variables. K balance (B_K), $U_{Na}V$, urine flow (V) and plasma Aldo were measured in 6 normal subjects given 40 mg/d furosemide (F) for 3 days while ingesting a high-salt (270 mmol/d, HS) or a low-salt (20 mmol/d, LS) diet. V and $U_{Na}V$ were always higher during HS than LS both acutely following F and between doses of F ($p<.01$). Nevertheless, during LS, the acute increase in U_KV with F was greater than during HS ($p<.05$) and U_KV remained above basal values in the period between diuretic doses; this resulted in negative B_K during LS (-72±7 mmol/3d; mean ±SEM; $p<.001$). In contrast, during HS, B_K was neutral (-8±12 mmol/3d, ns) because U_KV fell below basal values between drug doses. Aldo accounted for negative B_K with LS since the rise in Aldo after F was always greater during LS than HS ($p<.01$) and B_K correlated with changes in Aldo ($r=-.70$, $p<.001$). To determine whether changes in V affected B_K, V was increased during HS to the level occurring with F by water loading. At this V, U_KV rose but only to 32% of that with F ($p<.001$). In conclusion, U_KV with F is strongly dependent on changes in Aldo but only weakly on V or $U_{Na}V$. The augmented Aldo response to F explains why F induced negative B_K only when dietary salt was restricted.

CATECHOLAMINE AND DOPAMINE RESPONSES TO CENTRAL VOLUME EXPANSION (CVE): POTENTIAL ROLE IN MEDIATING NATRIURESIS. G.G. Krishna, G. Danovitch, & J. Sowers*. Divisions of Nephrology & Endocrinology, UCLA Sch. of Med. and VA Medical Center, Los Angeles, CA.

A natriuretic role for dopamine (DA) and antinatriuretic role for norepinephrine (NE) has been suggested. To study the plasma levels of these hormones and their potential role in mediating natriuresis in response to CVE 4 salt replete healthy adults were subjected both to 4 hours of thermoneutral head-out water immersion (WI) and 2L 0.9% NaCl infusion (SI) over four hours on two separate occasions. Both these maneuvers resulted in significant natriuresis, peak values being noted during the fourth hour of the study period. The creatinine clearance remained stable. During WI studies plasma NE levels fell steadily from a pre-study value of 453±74 pg/ml to a nadir of 254±71 pg/ml ($p<0.05$) during the fourth hour. During SI, NE levels fell steadily from a pre-study value of 328±56 pg/ml to a nadir of 261±47 pg/ml ($p<0.05$) during the fourth hour. Both during WI and SI studies plasma DA levels rose and peak values were observed during the fourth hour. The urinary sodium excretion ($U_{Na}V$) correlated directly with the plasma DA levels and inversely with the plasma NE levels. To study the relationship of $U_{Na}V$ to the relative concentrations of DA vs. NE, we plotted $U_{Na}V$ against DA/NE ratio. A strong direct relationship ($r>0.90$) was noted. These data suggest that 1) plasma NE levels suppress and DA levels rise in response to CVE, 2) DA/NE ratio provides a measure of central volume changes, and 3) relative concentrations of these hormones may have a role in mediating natriuresis in response to CVE.

USE OF UREA KINETICS (UK) IN THE NUTRITIONAL CARE OF THE ACUTELY ILL PATIENT. John M. Kosanovich*, Francis Dumler, Mathilda Horst*, Chris Quandt*, John A. Sargent*, and Nathan W. Levin. Henry Ford Hospital, Depts. of Med., Surg., & Pharm., Detroit, MI; Quantitative Medical Systems, Emeryville, California

UK techniques were used in 12 acutely ill patients to study the effect of varying caloric (CAL) and protein (PR) intakes on protein catabolic rate (PCR) during parenteral hyperalimentation (PH). In Group I (GI, n=6),the caloric/nitrogen ratio was maintained at 125 and in Group II (GII, n=6) at 170 Kcal/gm N2. PR intake was gradually increased from 1.0± 0.1 to 1.5 ± 0.1 gm protein/kg** as CAL intake was increased from 20±2 to 30±3 Kcal/kg in GI and to 41±3 Kcal/kg in GII. As CAL and PR intake increased, PCR rose from 1.10±0.20 to 1.46±0.22 gm protein/kg in GI whereas in GII it fell from 1.23±0.14 to 1.13±0.14 gm protein/kg. To determine the effects on PCR of an increasing CAL intake at a fixed PR intake (1.3±0.2 gm protein/kg), in 7 patients CAL were increased from 27±4 to 35±4 Kcal/kg. PCR fell from 1.21±0.10 to 0.94±0.12 gm protein/kg ($p<.01$) as CAL intake increased; however, when CAL and PR intake were simultaneously increased, a rise in PCR occurred in 3 out of 7 patients. In summary, UK facilitates the rational use of PH in the nutritional support of acutely ill patients. The use of high caloric/nitrogen ratios and a stepwise increase in caloric intake while monitoring PCR prior to further increases in protein intake may offer a greater protein sparing effect than arbitrary CAL and PR changes in PH therapy.

** All results as mean ± SEM.

LOW MOLECULAR WEIGHT PROTEINURIA IS A POOR PREDICTOR OF AMINOGLYCOSIDE NEPHROTOXICITY. Kenneth Kuznetsky,* Ted E. Feldman,* Marion Macsai,* and Jimmy L. Roberts. Rush Medical College, Chicago, IL.

Acute renal failure (ARF) is a major complication of aminoglycoside antibiotic (AA) therapy. We have studied 19 patients receiving amikacin, gentamicin or tobramycin for bacterial sepsis in an effort to verify whether indices of low molecular weight proteinuria (LMWP) are predictive of the development of ARF. Patients receiving penicillins, cephalosporins and erythromycin were studied as an additional control group. Serum (s) and urine (u) creatinine (Cr), beta-2-microglobulin (β_2M), lysozyme (LYSO), and pre- & post-dose AA concentrations were obtained daily from the beginning of therapy through an average of 4 days post-therapy. Fractional excretion (%FE) for β_2M and LYSO was also calculated. Five patients (26%) receiving AA developed a $\geq$ 30% rise in Cr_S which was not attributable to other known causes of ARF. At the start of therapy 74% and 47% of patients demonstrated elevated β_2M_U ($\bar{X}$: 1.30 mg/dl) and $LYSO_U$ ($\bar{X}$: 0.33 mg/dl), respectively. Comparison of AA treated patients developing ARF with AA treated patients and controls who failed to develop ARF demonstrated that increases in β_2M_U (3.89 *vs* 3.41 mg/dl), $LYSO_U$ (2.05 *vs* 2.77 mg/dl), %FE β_2M (54.5 *vs* 33.5%) and %FE LYSO (18.7 *vs* 6.7%) were not significantly different. These studies suggest that abnormal LMWP is frequently present in septic patients prior to therapy with AA and may result from the effects of sepsis on the kidney. We conclude that LMWP is a poor predictor of AA nephrotoxicity in patients who are at greatest risk of developing this complication.

INFLUENCE OF ALUMINUM-CONTAINING ANTACIDS ON PLASMA ALUMINUM. Mildred Lam, Edmond S. Ricanati, Allen C. Alfrey, Jack W. Coburn. Cleveland Metropolitan General Hospital, Dept. of Medicine, and Case Western Reserve University; Cleveland, O.

Nine patients on chronic hemodialysis (HD) were studied after stopping their usual doses of aluminum-containing antacids (AA). All were on HD using dialysate made with deionized water. Plasma aluminum (Al) levels were determined by atomic absorption spectrophotometry. Mean baseline plasma Al while patients were taking AA was 54.3 ± 11.2 μg/L (mean ± SEM). The baseline value correlated with length of time patients had been on chronic HD, $r = 0.76$, $p < 0.01$. Mean plasma Al was 44.0 ± 9.3 μg/L after 2 weeks off AA, and 34.0 ± 6.9 after 3 weeks ($p < 0.01$). Plasma Al decreased in 8 of the 9 patients and did not change in 1 patient. Five patients were then studied at least 2 weeks after being placed back on AA. In 4 of 5, plasma Al increased to a value greater than baseline; mean change from baseline was 55 ± 26% ($p < 0.01$).

Oral Al loading tests were done in 5 patients. No significant change in plasma Al was seen over 4 hours following a standard 60-ml oral dose of aluminum hydroxide-containing antacid (2 gm Al).

We conclude that 1) AA are a significant source of Al for patients on HD; and 2) absorption of Al occurs either too slowly or in too small an amount to be reflected by plasma Al within 4 hours following a single oral dose of AA.

SHORT-TERM PROGNOSIS IN SEVERE LUPUS NEPHRITIS. Report of the Lupus Nephritis Collaborative Study Group. Rush Medical College, Chicago, Ill. 60612

Data were collected from 12 participating centers regarding the outcome of patients with severe lupus nephritis (DPGN) treated with various pharmacologic regimens during a calendar year following biopsy. Followup was obtained on 44 patients who had a mean serum Cr 2.4±2.6 mg/dl, and urinary protein 3.9±3.3Gm/day. Microscopic hematuria was reported in 70% and low serum C3 in 77%. The initial serum Cr was <1.2 in 14, 1.3-4.0 in 23 and >4.1 in 7. During 1 year, 7 died, 4 went into ESRD and 12 increased the serum Cr >40% above the initial value (ΔCr>40%). Of the 36 survivors, the serum Cr remained unchanged (<0.2>) in 12, decreased in 14 and increased in 11. 13 patients experienced a decreased Cr ≥ 0.5 while 10 experienced an increased Cr ≥ 0.5. Mean followup Cr for the survivors (excluding ESRD) was 1.6±0.9 mg/dl. Of 33 patients with initial Cr <2, 5 died, 2 ESRD and 10 ΔCr>40%. Among those with initial Cr <1.2 there were 2 deaths, 1 ESRD, 5 ΔCr>40%. Of 11 patients with an initial Cr >2.1, 2 died, 2 ESRD and 2 ΔCr>40%. The crude mortality/ESRD rate for those with initial Cr ≤ 1.2 was 21.5%, those with Cr 1.3-2.0 was 21% and Cr >2.0 was 36%. We conclude: (1) that the renal biopsy finding of DPGN in SLE continues to identify a poor prognostic group, irrespective of the serum Cr at the time of biopsy, (2) crude 1 year mortality/ESRD rate was 25%, (3) the highest mortality/ESRD rate was among those with initial Cr >2, (4) mean renal function was stable in those patients who did not die or enter ESRD, (5) the great variation in short term prognosis requires that therapeutic trials be controlled.

PROXIMAL RENAL TUBULAR DYSFUNCTION IN SEVERE BURNS. J. Lindquist*, C. Drueck*, B. Elson*, D. Hurwich*, D. Roxe, and N. Simon. Evanston Hosp. and Northwestern U. Med. School, Evanston, Illinois.

Increased clearance of amylase, tubular proteinuria, and hypouricemia have been reported in severe thermal injury. We investigated proximal tubular function in 11 patients with 40% or greater total body surface burns. Creatinine clearance (C_{Cr}), fractional excretion of sodium (FENa), urate (FEUr), and amylase (FEAm), renal threshold phosphate concentration ($TmPO_4/GFR$), clearances of beta-2 microglobulin (C_{B2M}) and lysozyme (C_{Ly}), 24-hour urine glucose, qualitative amino acid, and protein excretion were determined on paired specimens during the 4th-8th hospital days. C_{Cr} and FENa were normal in all but 1 patient.

	FEU_r	FEAm	$TmPO_4/GFR$	C_{B2M}	C_{Ly}
# abnorm. / # studied	8/11	9/11	4/11	10/10	9/10

Twenty-four hour urine glucose excretion exceeded 1 gm. in 5 patients, aminoaciduria was noted in 8, and proteinuria, predominantly globulinuria, was consistently present. Metabolic acidosis and hypokalemia occurred only rarely.

Abnormalities of tubular function were more marked in 5 patients with the greatest extent of third degree burns who died 17 to 73 days after admission. All patients were treated with topical antiseptics or sulfa drugs, while 5 received parenteral antibiotics during the study period. The cause of proximal tubular dysfunction is not clear and may be related to an adaptive response to severe injury, a toxic metabolite produced in burns, or to drugs used in burn management.

ZINC METABOLISM IN NEPHROTIC SYNDROME. S.Mahajan, J. Speck*, G. Varghese*, D. Abu-Hamdan, S.Migdal, W. Briggs, A. Prasad*, and F. McDonald. VA Medical Center, Allen Park MI.

It is not clear whether low plasma zinc (Zn) reported in nephrotic syndrome (NS) is indicative of Zn deficiency or reflects a decrease in Zn binding proteins. To determine the Zn status in NS, Zn concentration in plasma, hair, WBC, and urine was determined in 20 patients with NS. Taste acuity by Henkin's method and 3 day dietary recall for protein and Zn intake were also done in these patients. Data are Mean±S.D.,† $p<.025$.

	Controls (N=20)	Nephrotics (N=20)
Plasma Zn (μg/dl)	112±14	84±16†
Hair Zn (μg/gm)	192±6	144±28†
WBC Zn ($\mu g/10^{10}$ cell)	110±19	76±22†
Urine Zn (μg/24 hr)	380±120	1460±380†

Patients with NS had subnormal Zn concentration in plasma, hair and WBC while urine Zn was higher than controls. Mean daily Zn and protein intake in NS patients were subnormal (10±3μg and 68±21 gm respectively). Similarly taste detection and recognition threshold for sodium chloride (salty), sucrose (sweet), hydrochloric acid (sour) and urea (bitter) were significantly higher in patients with NS than those in normal controls. The results of this study suggest that Zn deficiency is present in NS and may be related to increased urinary Zn losses as well as decreased Zn intake. Hypogeusia may be a complicating feature of Zn deficiency in NS.

SLEEP APNEA IN DIALYSIS PATIENTS WITH DIABETES. Sumanta Mitra, Chauncey Maher, Jr., Pradeep Mehta, Randy Kienstra and Lanie Eagleton*. SIU School of Medicine, Springfield, Illinois, Division of Nephrology and Pulmonary Medicine, Department of Medicine.

Sleep apnea has been associated with diabetes and may exacerbate during hemodialysis. The aim of the current study is to determine the prevalence of sleep apnea in dialysis patients with diabetes and to assess the relationship of the apnea to neuropathies and abnormalities of chemoreceptor function. Sleep apnea was assessed by polysomnography during nocturnal sleep. Neuropathies were assessed by nerve condution using EMGs and by cardiac responses to posturing and the valsalva maneuver using EKGs. Ventilatory responses to progressive hypoxemia and hypercapnia were used to assess chemoreceptor function. Six of eight patients studied had sleep apnea. All of these patients had both autonomic and peripheral neuropathies. The patients with sleep apnea did not demonstrate quantitative difference in respect to the cardiac responses and the nerve conductions when compared to those without apnea. One patient with sleep apnea failed to increase his ventilation in response to hypoxemia. The other patients had chemoreceptor functions which were within normal limits. We suggest that sleep apnea is common in diabetics receiving hemodialysis. In this small sample, the sleep apnea does not correlate with the presence or the severity of the neuropathies or abnormalities in chemoreceptor function.

PREDICTION AND SIGNIFICANCE OF HEMATOMAS POST PERCUTANEOUS RENAL BIOPSY. Morris CR, Mittelsteadt CA,* Jennette JC,* Colindres RE, Mattern WD. Depts. of Med., Radiol., Path., Peds., Univ. of North Carolina, Chapel Hill, N. C. 27514.

Previous studies have documented a high frequency of hematomas (H) following percutaneous renal biopsy (RB). Neither factors which predispose to H nor the significance of H have been determined. Abdominal computerized tomography (ACT) was performed in 22 adults and 16 children 24 hours following ultrasound (US) guided RB. Twelve perirenal and one retroperitoneal H were detected (34%). The clinical data, biopsy morphology and US images of the patients with (Grp I) and without (Grp II) H were reviewed by observers unaware of the ACT results. Hypertension, severe renal insufficiency and nephrotic syndrome were of comparable frequency in both groups, and H could not be correlated with the presence of medullary tissue or transection of medium or large arteries. Neither arteriosclerosis nor the type of glomerular or tubulointerstitial injury related to H formation. H did not develop when US indicated a lower pole capsule to collecting system parenchymal thickness (PT) of >2 cm, but H occurred in 50% when PT was ≤2 cm. The incidence, tachycardia, pain and gross hematuria were low in both groups. Changes in hct failed to identify patients with H. ACT or US in 7 patients documented complete resolution of H without asymmetry in kidney size.

H is common when PT is ≤2 cm, and modification of biopsy technique should be considered. Biopsy morphology does not predict H. H cannot be detected clinically, but routine post biopsy ACT is unnecessary since uncomplicated resolution follows.

PERCUTANEOUS LARGE BORE-SIZE NEPHROSTOMY FOR RELIABLE URINARY DIVERSION AND ENDOSCOPIC MANIPULATION OF RENAL REVIS. Kanichi Mitsuno,* Tetsuzo Agishi,* Michiko Takahashi,* Shohei Fuchinoue,* Kazuo Ota,* and Ryuko Umezu* (introduced by Nobuhiro Sugino). Kidney Center, Tokyo Women's Med. College, Tokyo, Japan.

A safe technique of creation of a percutaneous nephrostomy has been established under real-time ultrasonographic guidance. Through so-created nephrostomies, urinary diversion was reliably made and endoscopic manipulation was safely performed. A percutaneous nephrostomy cannula system which has multistep cannula type dilators and allows easy insertion of as large bore size as 16 F balloon catheter immediately after creation of the nephrostomy was newly devised. More than 50 nephrostomies have been made with this new device without serious complications except a hemorrhage episode in a special patient with uremic bleeding tendency.

Via a largermost dilator cannula, a flexible endoscope, a bile duct fiber scope, was inserted and endoscopic manipulation including inspectory examination, removal of stones, installation of a pigtail ureteral stent catheter, and sampling of biopsy specimen were safely conducted.

DIETARY CALCIUM AND HUMAN HYPERTENSION: A U.S. SURVEY. C. Morris, J. Stanton, D, McCarron. Division of Nephrology, Oregon Health Sciences University, Portland, OR.

A recent report of a pilot study noted a 22% reduction in daily calcium (Ca) intake in adults with hypertension (HTN). The possible importance of this observation is suggested by the protective effect of Ca in animal models of HTN. The HANES I survey, done by the National Center for Health Statistics, gathered BP, health assessment and nutrition data in 20,749 Americans, ages 1-75. Between ages 12 and 75, 12,056 individuals were identified who denied a history of HTN or awareness of previous BP. The subjects were stratified into 3 BP [normal (NL); borderline (BL), 140-160/90-95; HTN, >160/95], 4 age [12-19, 20-34, 35-54, 55-75] and 2 racial groups.

Of 17 the nutrients assessed, reduced Ca intake was the only consistent variation between NL and HTN, particularly in subgroups with the highest prevalence of HTN. Across the 35-74 group, HTN consumed 18% less calcium than did NL and BL (697 mg vs 573 mg, $p<.001$). Depending upon the subgroups, HTN's Ca intake was 10-65% less than their NL controls. Ca consumption corrected for total calories (mg Ca/1000 Kcal) was:

	20-34 yrs		35-54 yrs		55-74 yrs	
	N	$\bar{x}\pm SD$	N	$\bar{x}\pm SD$	N	$\bar{x}\pm SD$
NL	3336	390±243	2166	381±203	1160	421±219
BL	466	355±191*	466	374±242	991	418±207
HTN	28	350±210*	159	310±171*	455	386±208*

(*$p<.001$, compared to NL)

Decreased calcium intake is a consistent nutritional difference between NL and HTN humans. These findings may have implications for the pathogenesis and/or therapy of HTN in the U.S.

RENAL LESIONS IN SICKLE CELL NEPHROPATHY. Anthony D. Nicastri,* Amir Tejani, Thomas Manis,* Dilip Sen, and Eli Friedman.* SUNY, DMC, Depts. of Peds., Medicine and Pathology, Brooklyn, NY

Over a 14 year period, 16 sickle cell disease (SS) patients ranging in age from 6-37 yrs. with either persistent proteinuria or the nephrotic syndrome underwent a renal biopsy.

	Mean Age	No. of Cases
Focal Sclerosis (FS)	15 yrs. (6-18)	8
Mesangial Proliferation (Mes+)	10 yrs. (7-12)	5
Membranoproliferative (MPGN)	37 yrs.	1
Membranous (MGN)	38 yrs.	1
Acute glomerulonephritis (AGN)	24 yrs.	1

Patients with FS presented with the nephrotic syndrome more often (6/8) than those with Mes+ (1/5) p <.05. Of 8 patients with FS, 1 has died of renal failure, 1 has been transplanted, 1 is on dialysis, 2 continue to be nephrotic following prednisone and cyclophosamide, 2 are proteinuric and 1 is lost to follow-up. Patients with MPGN and MGN are on dialysis. Mes+ patients are moderately proteinuric.

A selective review of literature revealed 46 SS patients with nephropathy of whom 48% had MPGN, 17% Mes+, 10% AGN, 10% MGN and 13% FS.

Our study with the largest number of children from a single center shows that the predominant lesion in SS nephropathy in childhood is FS. Children with SS have supernormal glomerular filtration rates suggesting that hyperfiltration, over a period of time, may be the cause of focal segmental sclerosis.

ALDOSTERONE AMELIORABLE HYPERKALEMIA INDUCED BY ANGIOTENSIN CONVERTING ENZYME INHIBITION. **W. Peters,* M. Schambelan, A. Sebastian, and E. Biglieri.* U.C.S.F., San Francisco, CA**

Captopril (CAP), an angiotensin converting enzyme inhibitor, is a potential hyperkalemia-producing agent owing to its ability to reduce plasma angiotensin II, thereby causing hypoaldosteronism. However, hyperkalemia is not a recognized complication of CAP-induced hypoaldosteronism, perhaps because, as in Addison's disease, hypoaldosteronism causes hyperkalemia only when associated with a potentiating factor such as glucocorticoid deficiency, dietary K excess or Na restriction, or renal disease. We administered CAP (450 mg/day for 11 days) to a 60 year old man with mild chronic renal insufficiency due to obstructive uropathy (GFR 74 ml/min) and to three normal subjects. The patient was normokalemic and normotensive and had low normal plasma renin activity (PRA) and urinary aldosterone excretion ($U_{Aldo}V$). CAP treatment reduced both urine aldosterone ($U_{Aldo}V$ 6.5±0.3 → 2.6±0.3 µg/24h p<0.001) and urine K (cumulative ΔU_KV -64 mEq), and increased plasma K ($[K]_p$ 4.3±0.1 → 5.4±0.1 mEq/L p<0.025). Normal subjects had no significant change in $[K]_p$ or $U_{Aldo}V$, and cumulative ΔU_KV was +9 mEq. The PRA response of the patient was blunted (+4 vs. +28 ng/ml/hr). In the patient, infusion of d-aldosterone (106 µg/day for 5 days), superimposed on CAP treatment, increased U_KV and normalized [K]p. Thus, captopril can unmask an impaired renin secretory response mechanism, resulting in hypoaldosteronism, renal K retention and hyperkalemia.

PROXIMAL AND DISTAL NEPHRON FUNCTIONS ARE ABNORMAL IN PATIENTS WITH IDIOPATHIC NEPHROLITHIASIS (IN). Rufino C. Pabico and Barbara A. McKenna.* Univ. of Rochester Medical Center, Rochester, New York.

Patients with IN may have hypercalciuria and 'phosphate leak' suggesting tubular dysfunction, but published reports are conflicting on the issue of tubular abnormalities in IN. To resolve this clinical problem, 16 patients with IN with normal glomerular filtration rate (GFR) underwent the following studies: measurement of renal hemodynamic functions (GFR; effective renal plasma flow-ERPF), tubular maximum reabsorption of glucose (TM_G) and secretion of p-aminohippurate (TM_{PAH}), phosphate reabsorption (TM_{P_i}/GFR), urine concentration after 14 hours of fluid deprivation (U/S osm), and short-term acidification and U-B pCO_2. The results are:

	IN (n=16)	Normal (n=17)	p
GFR (ml/min/1.73m^2)	108 ± 12	110 ± 9	N.S.
ERPF "	569 ± 91	550 ± 35	N.S.
TM_G (mg/min/1.73m^2)	208 ± 58	375 ± 50	<0.01
TM_{PAH} "	59 ± 12	89 ± 4	<0.01
U/S osm	2.1 ± .3	3.2 ± .4	<0.01

Five hypophosphatemic patients (serum Pi <2.5 mg/dl) have low TM_{P_i}/GFR (1.9 mg/dl vs 3.5 in normal, P <0.05). Both urinary NH_4+ and titratable acid excretion for 6 hours following NH_4Cl load were distinctly lower than normal. U-B pCO_2 following HCO_3^- loading ranged from -4 to 22 mm Hg, much lower than normal (≥ 30). Thus, proximal and distal tubular functions are abnormal in IN. Both hypercalciuric and normocalciuric subjects have similar defects. The role of the tubular defects in the causation or perpetuation of IN have yet to be defined.

MODIFIED CRITERIA (MC) FOR THE DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) IN THE PRESENCE OF DIFFUSE PROLIFERATIVE NEPHRITIS. Marc A. Pohl, Randall Krakauer,* Gordon Gephardt, Lawrence Hunsicker, Jimmy L. Roberts, Melvin M. Schwartz, Edmund J. Lewis, and The Lupus Nephritis Collaborative Study Group (LNCSG), Cleveland Clinic, Cleveland, Ohio, VA Hospital, Iowa City, Iowa, and Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois.

Traditionally, the diagnosis of SLE depends upon the presence of 4 of the 14 original ARA criteria (OC) for SLE. However, some patients with known nephritis are highly suspect of having SLE but fail to meet the OC. Three centers in the LNCSG reviewed 38 patients with biopsy-proven diffuse proliferative nephritis (DPN), believed due to SLE. Of these 38 patients with DPN, only 26 (68%) met OC. The OC were modified (MC) as follows: (1) antinuclear antibody (ANA) for LE prep; (2) proteinuria requirement 1 gm/24 hours; (3) persistently low C_4 for at least 2 weeks; (4) antibodies to native DNA or Sm antigen; (5) LE band test. At renal biopsy, 37 of these 38 patients (97%) met at least 4 of the MC and all 38 did so within 6 months of biopsy. There were no differences between negative and positive error in diagnosing SLE from the OC or MC among the 3 referral centers. For 27 patients from 2 centers, at biopsy 19 (70%) met at least 4 OC; all 27 patients had more than 4 MC, and 96% had at least 6 MC.

The OC for SLE have not dealt with SLE as a renal disease and appear inadequate for patients with DPN. Substitution of ANA for LE prep and the addition of anti-DNA and low C_4 may be useful modifications.

POSTSTREPTOCOCCAL GLOMERULONEPHRITIS IN TRINIDAD
Elizabeth Potter, David Earle, and Theo Poon-King*
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We have been studying poststreptococcal acute glomerulonephritis (PSAGN) in Trinidad since 1965 when an epidemic involving about 2000 patients was in progress. Now we are completing these studies and should like to report on the over-all picture. Four large and 2 small epidemics have occurred from each of which a predominant streptococcal strain has been isolated: the first two were so close together they were considered a single two phased epidemic. However, different strains were predominant in each phase, M55 and M49. The third also had two peaks with M60 and M57 isolated respectively. However, M60 has been found during endemic periods while the other epidemic strains disappeared completely when the epidemics waned. After 6 years, M55 reappeared during an outbreak of scabies and caused an even more explosive epidemic. Six years thereafter, first M49 and then M55 reappeared and caused an increase in PSAGN but not of the scale of the earlier epidemics. Reasons for the decrease in both endemic and epidemic PSAGN will be considered. During these studies, observations have been made on the relation of acute rheumatic fever, diphtheria in the skin lesions, and scabies to PSAGN as well as the effects of different treatments on streptococcal skin infections. Finally, 760 patients ill in 1964-68 were chosen for follow-up every five years. These follow-up studies indicate a good prognosis for the many patients with PSAGN in Trinidad, only 3 classical patients (one now dead) having abnormalities on every follow-up and 8 more developing proteinuria since 1976.

REVERSAL OF RENAL FAILURE IN LUPUS NEPHROPATHY AFTER SEVERAL MONTHS OF HEMODIALYSIS.
F. Quismorio*, E.I. Feinstein, M. Sugarman*, A. Kamdar, B. Barbour, E. Dubois and S.G. Massry. Div. Nephrol. and Rheumatol., USC Sch. Med., Los Angeles, CA.

Renal insufficiency in systemic lupus erythematosus (SLE) has been associated with a universally poor prognosis. Nine patients with serum creatinine over 3.9 mg/dl had improved renal function with eight surviving 2.5-3.5 years. Of six patients requiring dialysis for 1-12 months, three improved without recent treatment with corticosteroids or other immunosuppressive drugs. Two patients were receiving outpatient maintenance hemodialysis when renal function improved and hemodialysis was discontinued. Three patients were treated with steroids while on hemodialysis. One of these three underwent renal transplantation, graft rejection, and removal of the transplanted kidney several months prior to improvement in her natural kidney's function. The remaining three patients improved concurrently with steroid therapy, one also received nitrogen mustard. This study emphasized the natural variability of lupus nephritis, including amelioration of chronic renal failure while on supportive dialysis with or without steroids or alkylating agents. The necessity of dialysis does not preclude subsequent improvement, and an elevated serum creatinine of 3.9 mg/dl or greater does not mean an uniformly unfavorable prognosis in this disease.

1-DEAMINO-8-D-ARGININE VASOPRESSIN (DDAVP) INCREASES FACTOR VIII/VON WILLEBRAND FACTOR AND SHORTENS THE BLEEDING TIME IN UREMIA.
F. Pusineri, M.Massazza, G. Mecca, P. M. Mannucci, R. Lombardi, C. Valsecchi and G. Remuzzi (intr. by J.S. Stoff). Dept. of Nephrol., Ospedali Riuniti, Bergamo, Italy and Haemophil. and Thromb. Centre, Univ. of Milan, Milan, Italy.

1-deamino-8-D-arginine vasopressin (DDAVP) or placebo was given in a randomized double-blind cross-over trial to 12 patients with chronic uremia, hemorrhagic tendency and prolonged bleeding time (BT). DDAVP infusion significantly ($p<0.001$) shortened BT in all patients (basal levels: 15'->30' 1hr after DDAVP: 4'-18', 1hr after placebo 14'->30'), the effect lasting for at least 4 hours in the majority of cases. No significant changes in platelet count, platelet cAMP levels, platelet retention on glass beads, plasma fibronectin, serum Tx B_2 and residual prothrombin accompanied DDAVP infusion. A significant ($p<0.001$) increase of all the components of factor VIII molecule paralleled the effect on BT. The study of multimeric structure of factor VIII-von Willebrand factor showed that DDAVP induces the release in plasma of larger multimers than were present before DDAVP. 6 additional patients with acute renal failure and prolonged BT (mean value 17') were infused with DDAVP before undergoing renal biopsy. A significant ($p<0.001$ shortening of BT was observed and biopsies were performed without hemorrhagic complications.

FUROSEMIDE (F) STIMULATES URINARY ACIDIFICATION IN PATIENTS WITH DISTAL RENAL TUBULAR ACIDOSIS (dRTA).
S.P. Rastogi, C.Crawford*, R.Wheeler*, W.Flanigan, J.A.L. Arruda. UAMS and VA Hospitals, Little Rock, AR.

(F) stimulates distal acidification in normal subjects probably by increasing distal Na delivery and transport and thus creating a favorable electric gradient for H+ secretion. (F) should stimulate H+ secretion in pts with dRTA provided the H+ pump is intact and the distal nephron is capable of transporting Na. We examined the effect of (F) (1mg/kg body weight) on urinary acidification in 5 normal subjects, 7 normokalemic dRTA pts and in two pts with hyperkalemic dRTA, pretreated with mineralocorticoid to enhance Na transport. In the control subjects (F) decreased urine pH (6.19 ± 0.13 to 5.16 ± 0.23 $p< 0.001$) and increased net acid excretion (12.9 ± 5.7 to 38.7 ± 8.4 μEq/min $p< 0.01$). (F) administration to pts with normokalemic dRTA decreased urine pH (6.03 ± 0.28 to 5.09 ± 0.12 $p<0.001$) and increased acid excretion (17.0 ± 12.5 to 55.1 ± 16.7 μEq/min $p<0.01$), to values not different from controls. In pts with hyperkalemic dRTA, (F) failed to decrease urine pH and to increase net acid excretion. The pts that responded to (F) had a rate dependent or gradient RTA and thus increased Na delivery created a favorable electric gradient for H+ secretion. The hyperkalemic pts had a voltage dependent defect and hence increased distal Na delivery failed to create the favorable electric gradient for H+ secretion. These data demonstrate that (F) can be used to characterize the mechanism responsible for dRTA and may be helpful in the treatment of selected pts with dRTA.

COMPARISON OF DIFFERENT THERAPEUTIC REGIMEN. IN ONE PATIENT WITH FOCAL SCLEROSIS(FS) AND NEPHROTIC SYNDROME(NS). Aura Maria Rocha*, Luiz Carlos Ribeiro* Maria das Neves Maia* and José Roberto C. da Rocha. Hospital da Beneficência Portuguesa, Rio de Janeiro, Brasil

We studied a 15 y.o girl with severe NS and FS over a period of three years, using different therapeutic regimens. During the first year treatment consisted of oral prednisone (1.0 mg/kg). She developed a full-blow Cushing and remained with severe proteinuria and long-term admissions for anasarca. Chlorambucil was then added, in a maximal dose of 0,2 mg/kg and low dose prednisone maintained. Proteinuria persisted and she remained in anasarca after five months of treatment. In a last effort, methylprednisolone pulsetherapy (MPPT) was utilized, using an intermittent one-day-one dose schedule (±10 mg/kg/dose) combined with low-dose prednisone maintenance. Dramatic reversal of the clinical picture of NS occurred and proteinuria reached the lowest levels in years and the patient was able to resume full activities.

We conclude that MPPT used intermittently can help control clinical picture of NS in FS.

URINE ACIDIFICATION AFTER SPIRONOLACTONE AND AMILORIDE: J.M.Roscoe, G.Virani* and C.C.Williams, Wellesley Hospital, Univ.of Tor.,Toronto,Ontario

Spironolactone (S) and Amiloride (A) have been associated with hyperchloremic metabolic acidosis (HCMA) possibly as a result of decreased ability to acidify the urine. Urine acidification was studied in 10 normal subjects before and after 10-14 days treatment with S(400mg od) and in 8 normal subjects before and after A(5mg od) for 3-12 days. Acidification and bicarbonate load tests were done before and after. Results were compared between pairs. Urine Na excretion and serum K increased significantly after S and serum K and Cl increased significantly after A indicating drug effect. Serum Na, Cl and weight fell significantly after S only. No evidence of HCMA was noted in either group. Acidification results are as follows:

Parameter	Pre S	Post S	Pre A	Post A
Bl pH	7.31±0.3	7.29±0.04	7.32±.03	7.30±0.03
Ser.K	3.9 ±0.3	4.2 ±0.3*	4.1 ±0.2	4.5 ±0.3**
Min UpH	4.87±.25	5.08±.24**	4.84±.18	4.91±.14
TAueq/mg Cr	22.4 ±5.9	22.6±11.4	18.6± 7.1	14.7 ± 5.6
NH_4^+ "	55.7 ±28.8	42.7±30.7	49.5±28.4	37.4 ±20.7
NAE "	78.0 ±28.5	65.4±34.2	68.1±30.1	52.1 +25.9*
U-BpCO_2	31.0 ±10.0	43.0±26.0	68.0±23.0	45.0 ±13.0

The minimum UpH achieved after S is significantly higher than pre S but remains below the accepted normal of 5.30. The minimum UpH after A does not change. Net acid excretion falls significantly after A but does not change after S. We conclude that significant changes in urine acidification occur after both S and A. The minor changes in UpH after S are of unknown clinical significance, but the fall in NAE with A could result in acidosis.

DIURETIC EFFECT OF PULSETHERAPY (PT) IN NEPHROTIC SYNDROME (NS). José Roberto C. Rocha, Maria das Neves de P. Maia*, Luiz Carlos Ribeiro*, and Aura Maria Rocha* Hospital da Beneficência Portuguesa , Rio de Janeiro, Brasil

Patient with severe nephrotic syndrome may show extreme resistance to diuresis especially if they suffer from anasarca.

We treated three patients with NS with methylprednisolone pulsetherapy (MPPT) and observed an important diuretic effect. Two patient had NS due to Minimal Lesion (ML) and one had Focal Sclerosis. All three patients initiated large diuresis after MPPT one to three days after the last day of infusion, in spite of persistent proteinuria One patient with ML and severe azotemia, lost 25 kgs in two weeks, without the use of diuretics or albumin, with complete correction of azotemia.

No side-effects were observed and this diuretic effect may last for several days with a single course of treatment MPPT may be very effective in some patients with NS, inducing diuresis and correcting the accompanying azotemia, even withouth the need of diuretic and in the presence of persistent proteinuria.

THROMBOTIC MICROANGIOPATHY AND RENAL FAILURE ASSOCIATED WITH ANTINEOPLASTIC CHEMOTHERAPY. B D ROSE, A M Jackson,* L G Graff,* J B Jacobs,* J H Schwartz,* G M Strauss,* J P S Yang,* M R Rudnick, C P Bastl, R G Narins, and I B Elfenbein,* Depts. of Medicine and Pathology, St. Vincent Hospital, Worcester, Massachusetts and Temple Univ. Health Science Center. Philadelphia, Pennsylvania.

Four patients with cancer (2 testicular, 2 squamous cell of the head and neck) developed thrombotic microangiopathy characterized by renal insufficiency, microangiopathic hemolytic anemia, and in 3 cases, thrombocytopenia, after treatment with similar antineoplastic chemotherapeutic regimens: cis-platinum, bleomycin, and a vinca alkaloid. Studies included complete blood counts, peripheral blood smears, coagulation parameters, urinalysis, and renal biopsy or post-mortem examination. One patient had thrombotic thrombocytopenic purpura, 2 the hemolytic-uremic syndrome, and 1 an apparent forme fruste of one of these disorders. Histologic examination revealed evidence of intravascular coagulation primarily affecting small arteries and arterioles. Since 3 of the patients were free of tumor at the onset of this syndrome and no other cause was apparent, the thrombotic microangiopathy may have been induced by the chemotherapy. Two patients with acute renal failure died whereas 2 with a subacute course survived with the recovery of relatively normal renal function. Diagnosis may be delayed or missed if renal tissue or the peripheral blood smear is not examined since the renal failure may be ascribed to cis-platinum nephrotoxicity and the anemia and thrombocytopenia to drug-induced bone marrow suppression.

EARLY DIAGNOSIS OF ADULT POLYCYSTIC KIDNEY DISEASE (PCKD): CLINICAL FEATURES AND COMPARISON OF RADIOLOGIC METHODS OF DIAGNOSIS. R. P. Saylor,* H. Cable,* L. F. Wright, Brooke Army Medical Center, Fort Sam Houston, TX.

Fifteen children (ages 7-22) of patients with PCKD were investigated by gray scale ultrasonography (US), enhanced and unenhanced computed axial tomography (CT), and intravenous nephrotomography (NT). Renal function was normal in all subjects (Ccr 67 to 132 ml/min uncorrected) and all were normotensive. Subacute or acute flank pain episodes were reported by 4 subjects all of whom demonstrated PCKD by US. One had a single episode of gross hematuria and also had a positive US. Five subjects had culture-proven urinary tract infections; all had positive US examinations. For the entire group, 9 had positive US examinations, 8 had positive CT studies, and 5 had positive NT examinations. No subjects were found to have normal US and an abnormal CT or NT study, but one patient with a normal CT and four with a normal NT study had positive US studies. The sensitivity of the US examination cannot be calculated precisely in that we are not sure the negative studies are "true negatives" in four subjects due to their youth. This study does show that US is about twice as sensitive as the more traditional NT study, and is less hazardous and less expensive. We therefore consider US the diagnostic method of choice; CT scanning does not appear to add to the diagnostic yield, although it is nearly as sensitive. Our study also shows that clinical findings are often present in children with PCKD at a younger age than is generally appreciated.

UREMIC POLYNEUROPATHY: A MANIFESTATION OF THE MALNUTRITIONAL CATABOLIC STATE OF UREMIA. RESPONSE TO DAILY DIALYSIS AND HYPERALIMENTATION. Lewis Schainuck and Maher Azer. Los Alamitos Hemodialysis Center. Los Alamitos, California.

We are presenting a 64 year old uremic non diabetic female who was recently placed on hemodialysis. Acute Motor paralysis with inability to ambulate became manifest on two occasions. Clinical examination,nerve conduction studies (NCS) and electromyography suggested polyneuropathy. A regimen of daily dialysis and hyperalimentation was initiated with clinical improvement, Rt. Peroneal nerve conduction velocity (RPNCV) improved from 47 meters per second to 54 (N-44-56) and distal latency from 8.8 meters per second to 6.2 (N-Less than 6). Left Peroneal nerve conduction velocity (LPNCV) improved from 32 to 44 and distal latency from 7.2 to 3.2. Relapse occurred when alternate day dialysis was instituted, hyperalimentation discontinued and weight loss became manifest. The same regimen was instituted with excellent response; (RPNCV) improving from 35 to 44 and (LPNCV) from 38 to 50. Right Peroneal Nerve Distal latencies improved from 8.9 to 6.9, and left side from 7.6 to 6.1. The catabolic state of uremia induces polyneuropathy, the creation of an anabolic state, allows the polyneuropathy to revert prior to the occurrence of permanent neurological deficit.

IS CHRONIC PLUMBISM IMPORTANT IN PATHOGENESIS OF RENAL DISEASE IN GOUT? R. P. Saylor,* L. F. Wright, Brooke Army Med Ctr, Ft Sam Houston, TX

Nine persons with documented symptomatic gout, hypertension and moderate renal insufficiency (Ccr 20 to 85 ml/min) or proteinuria (mean 900 mg/day)were studied for possible lead nephropathy. Seven of 9 gave a history of previous lead exposure; 5 from illicit alcohol and 2 from industrial sources. Subjects were tested for lead intoxication by administration of 2 gm CaEDTA in divided doses with collection of all urine for 72 hours. Two mobilized 687 and 707 mcg Pb respectively and were considered to have lead nephropathy and saturnine gout secondary to ingestion of illicit alcohol. The other 7 had normal responses, (mean 257 mcg/72 hours). Thus, in contrast to others (N Engl J Med 1981; 304: 520), we were unable to demonstrate that lead was important in the development of renal disease in the majority of our patients with gout, despite a positive history of Pb exposure. Other factors such as hypertension are probably more important in the usual patient. However, the 2 positive responses were obtained in subjects whose history of "moonshine" ingestion dated to 30-40 years prior to study. Thus, a high index of suspicion for lead intoxication is still necessary in patients with symptomatic gout and unexplained renal disease.

RENAL β_2-MICROGLOBULIN (β_2-m.) AND N-ACETYL-GLUCOSAMINIDASE (NAG) EXCRETION IN SJÖGREN'S SYNDROME (SS) AND RHEUMATOID ARTHRITIS (RA). G.H.C.Schardijn*, L.W.Statius van Eps, R.M. van Soesbergen*,A.van den Ende* and W.J.Nooyen*. Depts. of Int.Med., Rheumatology, Clin.Chem., Slotervaart Hospital and Dept.of Clin.Chem., Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands.

SS may be associated with renal tubular acidosis (RTA). As the renal excretion of β_2-m. and NAG is elevated in tubular dysfunction, we studied the excretion of β_2-m. and NAG in 40 patients with SS and 20 patients with RA. In the RA-group, all patients had a normal β_2-m. excretion ($\leq$ 370 µgram/24 h), while 11 patients showed an elevated NAG excretion (normal $\leq$ 120 nmol/h/mg creatinine). In the SS-group 10 patients had an elevated β_2-m. excretion (range 507 - 6564 µgram/day); all these patients had an abnormal acidification and concentration test. Only 8 patients of this group had an elevated NAG excretion (range 166 - 1278 nmol/h/mg creatinine); moreover, just 4 patients had a significantly decreased maximum tubular reabsorption of phosphate ($TmPO_4/GFR$, range 0,51-0,79; normal 0,81-1,45.

From our results we can conclude, that proximal tubular involvement may occur more frequently in SS than assumed and that the urinary excretion of β_2-m. is a sensitive method to detect it.

TREATMENT OF MINIMAL CHANGE NEPHROTIC SYNDROME WITH LOW DOSE HYDROCORTISONE. Morris J. Schoeneman. Mount Sinai School of Med., Beth Israel Med. Ctr., Dept. of Ped., New York, N.Y.

Minimal change disease (MC) is the major cause of nephrotic syndrome in children and a frequent-relapsing course is present in 40%. Standard therapy with prednisone and cytotoxic agents can lead to serious toxicity. We treated 4 such children solely with low dose hydrocortisone (HC), 7.5 or 15 mg daily, 3 for 6 months and 1 for 13 months. ACTH stimulation testing was done before, during, and after completion of the HC trial. We compared the number of relapses during the previous 12 months, while on prednisone, with those while on HC. We also compared the mean monthly dosage of prednisone in the 12 months prior to HC with the mean monthly dose of HC, expressed as its prednisone equivalent. Three children had no relapses while on HC, compared to 3 relapses in the previous year. The mean monthly dose of prednisone equivalent was 8-18% of that required during the previous year. One child had 7 relapses during 13 months on HC, compared to 5 relapses in the previous year on prednisone, but was controlled with HC, requiring only 14% of the average monthly prednisone equivalent used during the previous 12 months. Adrenal response to ACTH testing was normal in all subjects. Steroid toxicity disappeared and no complications of HC appeared. We conclude that relapses in some children with MC can be prevented or treated with prolonged low dose HC. Children with MC and steroid toxicity should be given a trial of HC before initiating treatment with more toxic drugs.

EFFECTS OF FUROSEMIDE (F) AND BUMETANIDE (B) ON AUDITORY AND VESTIBULAR FUNCTION IN NORMAL HUMAN VOLUNTEERS. Marcia R. Silver, Margareta B. Møller*, F. Owen Black*, Conrad Wall, III*, and Jules B. Puschett. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Data obtained in animals suggest that B is a less ototoxic drug, but is otherwise similar to F in its actions. Furthermore, controlled comparisons of the drugs in humans have not been reported. We gave single doses of F and B intravenously to normal human subjects in a double-blind crossover study to investigate differences in ototoxicity between the two drugs. High frequency audiometry (up to 16,000 Hz) was used to measure auditory function. Rotation and fixed force platform posturography screening tests were used to assess vestibulo-ocular and vestibulo-spinal function. Subjects were 21-30 years old, had normal liver and kidney function, and had normal baseline auditory and vestibular test results. Nine subjects received low dose of drugs (1 mg of B and 40 mg of F) and 10 received high doses (2 mg of B and 80 mg of F). No significant changes in auditory function were seen. In four subjects, substantially decreased vestibulo-ocular responses were seen at one hour post-drug dose, followed by recovery back to baseline. Two of these subjects had received low dose F, and two had received high dose F. No subject receiving B had such a marked change. Patients with less than perfect baseline hearing and vestibular function and with impaired renal function are likely to experience more ototoxicity with these drugs than normal volunteers. The results of this study suggest that further investigation of ototoxicity of F and B in patients will be rewarding.

IMPAIRED GRANULOCYTE PHAGOCYTOSIS AND BACTEROCIDAL ACTIVITY IN NEPHROTIC SYNDROME. D Sillix,* J Francis,* S Mahajan, W Briggs. Nephrology Division Wayne State Univ. Sch. of Med., Detroit, MI.

Nephrotic syndrome (NS) is associated with a susceptibility to bacterial infection. Although much is known regarding defective opsonization in NS, very little is known about granulocyte (PMN) function in NS. PMN functions, including phagocytosis (Ph) and intracellular bacterial killing (IBK) of a pathogenic strain of Staphylococcus aureus (Staph) were studied in 10 nonuremic, (serum creatinine < 4.9 mg/dl), adults ages 20-81 yrs, with NS (24° urine protein 3-16 g), and in 10 age matched controls (C). Ph, expressed as % Staph ingested, was measured using a differential centrifugation technique and ^{3}H-labelled, heat-killed Staph opsonized with normal or NS serum. IBK, expressed as % intracellular Staph killed over 60 min., was measured using plating techniques after eliminating live extracellular Staph with lysostaphin. Results ($\bar{x} \pm$ SEM) of Ph and IBK using Staph opsonized with normal serum:

	Ph (%)			IBK (%)
	5 min	10 min	20 min	
NS	5 ± 1*	8 ± 1†	10 ± 1†	49 ± 6§
C	9 ± 2	14 ± 1	19 ± 1	70 ± 3

* < 0.05, † < 0.01, § < 0.001

Ph (20 min) by normal PMN was significantly less using Staph opsonized with NS serum than with Staph opsonized with normal serum (18.2 ± 2.5 vs. 27.0 ± 3.0, $p < 0.001$). In conclusion, impaired granulocyte Ph and IBK, as well as defective opsonization, contribute to the vulnerability to invasive bacterial infection in NS.

URINARY DIAGNOSTIC INDICES IN RENAL FAILURE ASSOCIATED WITH CARBON TETRACHLORIDE (CCl_4) INTOXICATION. R.A. Sinicrope, J.R. Little, J.A. Gordon and A.C. Schoolwerth. The Pennsylvania State University, Hershey, Pennsylvania.

Renal failure associated with CCl_4 exposure has been attributed to acute tubular necrosis (ATN). However, the evidence for direct nephrotoxicity of CCl_4, based largely on postmortem histology, is not compelling. CCl_4 intoxication often results in a clinical syndrome of nausea, vomiting, abdominal pain, diarrhea, anorexia and, frequently, altered consciousness, all of which could lead to volume depletion and prerenal azotemia. A prerenal etiology to the renal failure seen following CCl_4 exposure has not been previously considered. Moreover, most reports of CCl_4-associated renal failure appeared prior to recognition of the utility of the FE_{Na} and renal failure index (RFI) tests as aids in diagnosis.

We recently evaluated three patients exposed to CCl_4. In each the presenting symptoms included nausea, vomiting, poor intake and oligoanuria of 24-36 hours duration. Physical examination supported volume depletion. Liver function tests indicated severe hepatocellular disease. Urinalyses revealed proteinuria, pyuria and granular casts. In each case, the FE_{Na} was < 0.3% and RFI < 0.6, strongly indicative of prerenal azotemia. Each patient responded to aggressive volume repletion with prompt improvement in renal function.

Our experience suggests a prerenal etiology to the acute renal failure seen following CCl_4 exposure. Furthermore, since volume replacement alone corrected the abnormality, it is suggested that the evidence for direct nephrotoxicity of CCl_4 be reevaluated and extended.

FUNCTIONAL HEPATO-RENAL FAILURE AND ASCITES: CURED USING AN EXTERNAL MECHANICAL AUTO-INFUSION DEVICE: Burton H. Smith*, Maher Azer and Gary Nemhauser*. Los Alamitos General Hospital, Los Alamitos California.

We are presenting a case of Hepato-Renal Failure associated with massive ascites in which functional renal failure was corrected and ascites controlled. The use of a Mechanical external pump, allowed continuous auto infusion to occur through a Le-Veen Shunt that became dysfunctional. The rate of auto infusion was 600 cc/hour, suggestive that ascitic fluid accumulation was of such a magnitude that conventional diuretic regimens and Le-Veen non-assisted shunt were non-effective. The highest level of creatinine reached prior to initiation of manual auto infusion was 6.1 mg%; in association with a decrease urine output to 543 cc/24hr. and urinary sodium of 5 meq/L. Ascites occurence and reoccurence was massive with respiratory embarrassment. Manual auto infusion on two occasions corrected the ascites and the functional renal failure, decreasing the serum creatinine to 1 mg% and urinary output to 900 cc/24hr. External mechanical auto infusion through the Le-Veen Shunt, maintained the patient relatively free from ascites and stabilized renal function at a creatinine level of 1 mg%.

INCIDENCE AND PREVALENCE OF END STAGE RENAL DISEASE IN THE EASTERN UNITED STATES: 1973 1979, T Sugimoto* and SJ Rosansky, Depart of Prev. Medicine, School of Medicine USC, and VA Hospital, Columbia, SC.

Annual incidence (AI) and annual prevalence (AP) rates of end stage renal disease (ESRD) per population at risk for 88,968 patients by age, sex, race, and primary etiology (glomeronephritis, hypertensive nephropathy, diabetic nephropathy, and polycystic kidney disease) leading to ESRD were computed for twenty eastern states for the period 1973 to 1979, utilizing data from the U.S. ESRD Medical Information System.

AI rates for white males and females have stabilized at 60 and 40 per million population since 1977. AI rates for blacks, hypertensive and diabetic nephropathy patients and patients over 65 years of age continue to increase. Higher AI rates in males were noted for both races during the entire study period: black males had higher AI rates of all primary etiologies than black females (except for diabetic nephropathy); while white males had higher AI rates for glomeronephritis than white females. Blacks had twice the ESRD AI rate than whites primarily due to 7 and 2.5 higher AI rates of hypertensive and diabetic nephropathy respectively. AP rates between 1977 and 1979 showed higher rates of increase in those states with a larger proportion of blacks.

It is projected that stabilization of AP rates will occur several years after stabilization of AI rates, ("delayed accumulative effect") assuming no change in survival. The most significant single intervention to reduce incidence and prevalence of ESRD indicated by this study is control of hypertension especially in blacks.

^{67}GALLIUMSCINTIPHOTOGRAPHY (^{67}Ga), RENAL β_2-MICROGLOBULIN (β_2-m.) EXCRETION AND ANTIBODY COATED BACTERIA (ACB) IN UPPER AND LOWER URINARY TRACT INFECTIONS (UTI).
L.W.Statius van Eps, G.H.C.Schardijn*, C.Hoefnagels*, W.Pauw* and W.J.Nooyen*. Depts. of Int. Med. and Microbiology, Slotervaart Hospital, Depts. of Clin.Chem. and Nuclear Med., Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands.

We have described a method to diagnose upper or lower UTI by estimating urinary β_2-m. excretion. The present study compares the results of renal β_2-m. excretion, ^{67}Ga scintiphotography of infected kidneys and the ACB-test in 19 patients with upper and 14 patients with lower UTI. All patients with acute pyelonephritis showed a grossly elevated β_2-m. excretion and a significant ^{67}Ga uptake in one or both kidneys. A positive immunofluorescence with the ACB-test was only obtained in 9 cases with upper UTI. In the cystitis group, the β_2-m. excretion was always normal; ^{67}Ga uptake was negative in all the kidneys, while the ACB-test was positive in 3 cases. Prostatitis was accompanied by an increased ^{67}Ga accumulation in the prostate. Follow-up studies - in patients with pyelonephritis - revealed that recovery was accompanied by a normalization of β_2-m. excretion and ^{67}Ga uptake.

Urinary β_2-m. excretion and/or ^{67}Ga scintiscanning are reliable and useful additions for the localization of UTI.

TREATMENT OF PARAQUAT POISONING. Sutton, J.*, Israelit, A., Hartnett, M., Bennett, W. Providence Med. Ctr., Good Samaritan Med. Ctr., Univ. of Oregon Health Sciences Ctr., Portland, Or.

Three men accidentally ingested 30% paraquat (P). 20 hrs. after ingestion bentonite and gut lavage (1 pt.) were started along with charcoal hemoperfusion (HP) and forced diuresis (2 pts.). To minimize pulmonary toxicity corticosteroids, vitamin E and low F_IO_2 were employed. Each pt. developed pulmonary, renal and liver complications and died pulmonary deaths 5, 10 and 22 days post ingestion.

From 20 to 40 hrs. after ingestion each pt. received a total of 15 hrs. of HP and hemodialysis (HD) (not simultaneously), P levels dropped from 1.36 to 0.14, 2.1 to 0.7 (at 32 hrs.) and 0.8 to 0.07ug/ml. It was necessary to give platelet transfusions to treat thrombocytopenia due to prolonged HP. Extraction with HP was 94% at zero hrs. and still 90% after 4 hrs. with the same cartridge. Extractions with HD was 80%. Between the end of HP at 40 hrs. and start of next HP at 50 hrs. levels rebounded up into toxic range 0.14 to 0.43 and 0.07 to 0.20ug/ml in the 2 pts. measured. From 20 to 50 hrs. each pt. gained 2-3 kg. From 50 to 74 hrs. P levels again went up in each case possibly due to less aggressive treatment over this interval than 20 to 50 hrs. (41% of urine output, 10% of stool output, 35% of HP time).

Although combined treatment lowered P levels, it was suboptimal in that: (1) a marked rebound in P levels occurred after first HP, (2) attempts to increase urine and stool resulted in early weight gain contributing to pulmonary edema.

RENAL CALCULI AND ACETAZOLAMIDE (ACZ) THERAPY. R.A.L. Sutton, J.Dewar*,V.R.Walker*,and S.M. Drance* Departments of Medicine and Ophthalmology, University of British Columbia, Vancouver, B.C., Canada.

Renal calculi occurred in 28 of a total of approximately 350 patients during treatment of glaucoma with ACZ (0.25-1.0 gm/day). The interval between the start of ACZ and the first stone episode ranged from 3 months to 8 years. The ACZ was discontinued when renal calculi occurred. We have studied 9 of these 28 patients 3-16 years after ACZ withdrawal. The protocol included 24-hr urine collections and a standard 1 gm oral calcium load test. The data are compared with those from a local age- and sex-matched normal control group with no history of glaucoma or stone disease. No significant differences were observed in mean fasting serum levels of Ca, P, Mg, Na, and K, or in mean creatinine clearances. Urinary uric acid and oxalate were within the normal range but 3/9 patients had a 24-hr urinary Ca in excess of 250 mg. In response to the oral Ca load, both fasting urinary Ca/creatinine ratio (mg/mg) and increments in this ratio following the Ca load were greater in the patients than in the normal subjects (0.110 ± 0.015 vs 0.049 ± 0.005, $p<0.01$ and 0.145 ± 0.018 vs 0.079 ± 0.013, $p<0.01$ respectively). Fasting hypercalciuria was present in 4/9 patients. Two of these subjects in addition to one other also demonstrated Ca hyperabsorption. These observations suggest that many patients who form renal calculi during ACZ therapy may have a pre-existing abnormality of urinary Ca excretion. The screening of glaucoma patients for hypercalciuria before starting chronic ACZ treatment would permit the exclusion of many of those at risk for stoneformation and thus greatly reduce the incidence of this complication of treatment.

CLINICAL ASSESSMENT OF URINARY ACIDIFICATION USING A SHORT TEST WITH ORAL FUROSEMIDE (F). J Tarka*, NA Kurtzman, and DC Batlle. Univ of Ill, Chicago.

Distal acidification (DA) can be evaluated using maneuvers that do not require the presence of spontaneous or induced acidemia such as Na_2SO_4 infusion or HCO_3 administration (using urine $[U]_{pCO_2}$ as an index of DA). We examined the effect of F on DA in an attempt to develop a simpler test to evaluate DA independent of acidemia. F was given at a dose of 80 mg and DA evaluated at hrly intervals, 1 hr before and 4 hrs after F. The 6 normal subjects studied exhibited an initial rise or increase in U pH in association with a maximal rise in U flow, sodium, potassium, and chloride excretion. This was followed by a marked fall in U pH (from 5.9 ± 0.16 to 4.8 ± 0.04) by the 3rd hour following F. At this point, U flow and Na excretion declined; a significant correlation between minimal U pH and the decline of Na excretion was then observed, suggesting that F increased DA by increasing distal Na delivery and thus stimulating collecting duct Na reabsorption and, in turn, H+ secretion. The minimal U pH and the rate of acid excretion elicited by F was compared to those elicited by NH_4Cl or NH_2SO_4 administration. As shown below, F resulted in a similar U pH and acid excretion compared to either NH_4Cl or NH_2SO_4.

Test	Urine pH	TA^4 (μEq/min)	UNH_4V
F (n=6)	4.8±0.04	30±7.3	22±4.6
NH_4Cl (n=6)	4.9±0.06	24±1.5	20±2.0
Na_2SO_4 (n=3)	4.9±0.20	35±1.4	23±3.2

Conclusions: 1. F can effectively and simply be used to evaluate DA, obviating the need for inducing acidosis or IV infusion; and, 2. F may be a useful tool in the diagnosis and characterization of defects in DA.

EXPERIENCE WITH 200 ANGIOGRAPHICALLY CONTROLLED PERCUTANEOUS RENAL BIOPSIES (ACPRB). C. Swartz and Audrey Wilson*. Depts. of Med. and Rad., Hahnemann Univ., Philadelphia, PA.

Since 1978, we have routinely performed percutaneous renal biopsy under angiographic control. Retrospective review of the most recent 100 patients led to the following conclusions: (1) ACPRB provides the most accurate localization of kidneys and adequate tissue specimens were obtained in all patients actually biopsied; (2) sixteen percent of angiograms revealed no cortical tissue or a kidney smaller than 7 cm and no biopsy was attempted; (3) no patient required surgical intervention or intra-arterial epinephrine vasoconstriction after we learned that biopsy cuts must be within 2 cm of the kidney edge; (4) average Hgb decrease was 0.5 gm% and creatinine increase 0.2 mgm%; (5) the initial 100 patients confirmed the value of the technique in recognizing severe hemorrhage and intra-arterial vasoconstrictive intervention; (6) biopsy training of renal fellows is greatly enhanced; (7) local hematomas occurred in 4% of patients and all responded to conservative management; (8) the increased professional time and risk of femoral vascular complications must be balanced against the accuracy of needle placement and early detection and intervention in post biopsy hemorrhage. A cost benefit analysis has led us to continue ACPRB as the procedure of choice.

FAMILIAL FOCAL SEGMENTAL GLOMERULOSCLEROSIS. Amir Tejani, Anthony D. Nicastri,* K. Gurumurthy, Irving Dunn,* and Philip Calderone.* State University of New York, Downstate Medical Center, Depts. of Peds and Pathol., and Brooklyn Jewish Hospital, New York.

We have observed the nephrotic syndrome in more than 1 child in 3 unrelated hispanic families. Biopsy (B) showed either focal sclerosis (FS) or IgM nephropathy (IN) evolving into FS. Tissue typing revealed the presence of HLA DRW8 in 6 out of 8 patients.

Family	Sex	Age	1st B	2nd B	HLA DR	Outcome
1 A	M	12	IN	FS	5 W8	Renal failure
B	F	7	IN	-	5 W8	proteinuria
2 A	F	22	FS	-	5 6	Dialysis
B	M	20	IN	FS	5 6	Renal failure
3 A	F	15	FS	-	2 W8	Renal failure
B	F	22	IN	-	4 W8	proteinuria
C	M	21	IN	-	4 W8	proteinuria
Cousin	M	20	FS	-	4 W8	Renal failure

Two siblings in family 1, and 2 siblings in family 3 are normal and do not inherit DRW8.

HLA DRW8 is a rare antigen present in 7% of whites and 14% blacks. Its frequency in hispanics is not known. It was present in 0% of 34 normal hispanic people typed in our laboratory, in contrast to its frequency of 75% in our patients, $p < .0001$.

Our study suggests that there may be a genetic predilection towards developing focal segmental glomerulosclerosis.

CLINICAL USEFULNESS OF COMBINED DIURETIC THERAPY IN CONGESTIVE HEART FAILURE(CHF) AND EVIDENCE OF DRUG INTERACTION OF DIURETICS. Norihiko Terao, Yasushi Asano*, and Saichi Hosoda*. Jichi Med. School, Dept. of Cardiology, Tochigi, Japan.

Combined administration of diuretics has never been precisely evaluated as modality of therapy for severe CHF. In order to assess natriuretic effect in relation to urinary excretion of diuretics, we prospectively did strict balance study (Na 100mEq, K 75mEq, and fluid 1300ml per day) in a pt. with CHF resistent to loop diuretics. During control period, administraion of Furosemide(F) 200mg PO or IV twice a day resulted in only 54-82mEq Na excretion per day. In contrast, during experiment period, the addition of Hydrochlorthiazide(H) 75mg and 25mg to the same dose of PO(F) led to striking natriuresis of 320 mEq per day. However, in comparable condition, pretreatment with Spironolactone(S) 150mg previous-day and 50mg 2 hours prior to F+H administration produced less Na excretion(241 mEq/day)than F+H alone. Hourly urinary anlysis revealed delayed natriuresis with low peak in F+H+S study comparing with F+H one. This alteration of Na excretion was found to be associated with similarly delayed and less urinary excretion of F and H. Careful use of this treatment improved him remarkably, allowing to undergo curative surgery. It is concluded that combined diuretic therapy is safe and essential part of treatment for selected cases with CHF resistent to even large dose of loop diuretics. Drug interaction by addition of S to F+H was demonstrated, which probably occurs at organic acid transport system in the proximal nephron.

HEMATURIA IN CHILDREN: INDICATIONS FOR BIOPSY. Howard Trachtman*, Robert Weiss,* Ira Greifer, Children's Kidney Center of the Hospital of the Albert Einstein College of Medicine, Bronx, N.Y.

Previous reviews of hematuria in children have included patients with proteinuria or other renal functional abnormalities. We report the results of renal biopsies performed in patients age 3-19 years, referred for evaluation of persistent isolated hematuria during the period, 1972-1981 (N=77). Specimens were examined by light and electron microscopy and immunofluorescence techniques. The overall incidence of abnormal renal histology was 53%. The majority (39/41) of abnormal biopsies could be classified into four histological categories: 1) IgA nephropathy (n=9); 2) Alport's disease (n=9); 3) thinning of the glomerular basement membrane (n=14); 4) vascular C_3 staining (n=7). The children were divided into three subgroups 1) isolated microscopic hematuria (IMH), n=42; 2) IMH plus a family history of hematuria, n=15; and 3) IMH plus at least one episode of gross hematuria, n=20. An increased likelihood of obtaining an abnormal renal biopsy was demonstrated in groups two and three (X^2, $p<0.007$). Sex, age at onset and duration of hematuria had no predictive value for abnormal histology. The risk factors identified in this study are predictive of the yield to be expected from renal biopsy for isolated hematuria in the pediatric patient.

ACUTE RENAL FAILURE DURING CONVERTING ENZYME INHIBITION IN PATIENTS WITH RENAL ARTERY STENOSIS. Stephen C. Textor, Augustine Biscardi,* Emmanuel L. Bravo, Robert C. Tarazi,* and Fetnat M. Fouad.* Cleveland Clinic Foundation, Research Division, Cleveland, Ohio.

Inhibition of angiotensin converting enzyme lowers blood pressure and peripheral resistance, often improving regional perfusion. However, acute renal failure (ARF) may occur requiring cessation of therapy. We studied 7 patients with ARF (rise in creatinine > 2.0 mg/dl) during captopril administration. All patients had either bilateral atherosclerotic renal artery stenosis (RAS) (5) or stenosis to a solitary kidney (2) and were receiving diuretics. Mean duration of CEI before ARF: 8 days (range 1-21); age: 60 years (range 52-69). Pre-Rx PRA = 23±5 ng/ml/hr.

	Pre	CEI	Post
Creatinine (mg/dl)	2.4±.4	5.6±.8	2.0±.2
MAP (mmHg)	121±12	100±9*	112±11
Potassium (mEq/l)	3.8±.3	5.0±.4*	4.0±.3

MAP=mean arterial pressure; Mean±SEM; *p < .01

Transient oliguria (80%), Low FE_{Na} (<1%) in (50%) and normal urinary sediments were common. Two patients had repeat episodes during re-challenge. Although MAP fell in most cases, two developed oliguric ARF without a systemic blood pressure change.

These observations demonstrate that pre-existing vascular stenosis to the total renal mass is associated with impaired GFR and sodium excretion during CEI producing oliguric ARF. Advanced age, underlying renal impairment and widespread atherosclerosis are associated features. Unexplained ARF during CEI in this setting suggests bilateral renal artery stenosis.

USE OF COLCHICINE (C) IN A UREMIC PATIENT SECONDARY TO DIABETIC NEPHROPATHY.

A. Treviño M.D.*, C.S. Avilés M.D., Nephrology Department, Especialities Hospital, IMSS, México, D.F.

The use of (C) has been extended to diseases such as cirrhosis, scleroderma, familial Mediterranean fever and amyloidosis, where collagen synthesis increase occurs. (C) inhibits transport of collagen precursors and stimulates collagenase production.

It is possible that in diabetic glomerulosclerosis, (C) might have a beneficial effect. Literature reports state that survival, from the beginning of azotemia to death or dialysis is not more x 2.4 years; in our cases survival was 10.3 months.

Juvenile diabetic patient, 34 years old, on insulin therapy since 12. Referred to our hospital in 1974 because of proteinuria; GF 35 ml/min; ischemic heart disease in 75. In 76 neuropathy, piodermitis, otitis; in 77, diabetic cataracts and amaurosis Nephrotic syndrome and recurrent urinary infections with deterioration of renal function. In May 81 necrosis and infection of the 2nd 3rd and 4th toes of the right foot; July 81 supracondyloid amputation of the right lower limb and. Normotensive 130/80, 24hr. urine ± 2,000cc; Glucose always had been upper normal values. Started on (C) october 79, 1mgr. daily; proteinuria persists, (GF) decreased from 34 to to 6ml/min at present, creatinine 4mg. and urea 169mg. Our observations allow us to conclude that in this patient (C) has been useful to preserve renal function for 3 years.

REMISSION OF THE NEFROTIC SYNDROME(NS) AND RENAL VEIN THROMBOSIS(RVT) TREATED WITH THROMBECTOMY(T) AND ANTICOAGULANTS(A). F. Monteón; S. Gutiérrez; A. Treviño*; M.A. Schettino; E. Exaire*; A. Pérez; R. Mancilla; R. García Torres. Especialities Hospital, C.M.R. México,DF

The association of TVR due to membranous neprophaty (MN) is frequent and the usual treatment A is not always effective with patients ending up in chronic renal failure(CRF) or death. T has been utilized only exceptionally and remission is rare. 22 NS cases with MN and RVT of which 16 were idiopathic MN and 4 MN and Systemic Lupus Erythematosus (SLE) one MN due to gold salts and one with amyloidosis. 14 men and 8 women, ages from 25 to 75 years (40.2), follow up 3 to 61 months (28.6). 3 cases were drop outs and the amyloidosis. case was excluded. 14 cases treated with A and 4 with T were compared. The first and last creatinine clearances in the A treated group were 65.1±33 and 91.6±48.3ml/min respectively and proteinuria 9.12±6.15 and 3.64±3.25grs/24 hs. for the first and last readings respectively. (P <0.01).

In the T group, first and last levels of creatinine clearance were 61±23 and 91±25ml/min respectively and proteinuria 8.2±4.9 to 0.6±1.3grs/24hs for the first and last values respectively. (P <0.05). TvsA:"t"=4.8 (P <0.01).

2 A treated cases and 3 of the 4 T treated cases showed complete remission. We discuss here that MN without well defined characteris tics that can be complicated with extense RVT where the treatment of choice should be T with great possibilities of remission.

HYPOCITRATURIA IN RENAL STONE FORMERS. Jaime Uribarri and Man S. Oh, Downstate Medical Center, Brooklyn, N.Y.

Citrate is a well known inhibitor of urinary crystallization and therefore, urinary citrate excretion may be relevant to renal stone formation. In fact, some studies in the past have documented low values of urinary citrate in renal stone disease. The purpose of this study was to determine the frequency and mechanisms of this hypocitraturia. We have measured urinary excretion of citrate in 100 consecutive recurrent renal stone formers and found that 20 of them had values below the lower limit of normal (100 mg daily). None of these patients had urinary tract infection at the time of the urine collection and the creatinine clearance was over 80 ml/min in all of them. In 5 of these patients we have done further tests to elucidate the mechanism of their hypocitraturia. 1. Intestinal absorption of citrate was evaluated by measurement of plasma citrate levels following a fixed oral dose of citrate; the plasma levels did not differ significantly from control in all 5 patients. 2. Renal tubular reabsorption of citrate was determined in the fasting state (U/P citrate x P/U creatinine x 100). The fractional reabsorption was markedly enhanced (over 95%) in all 5 patients despite normal serum K and serum CO_2 levels (hypokalemia and metabolic acidosis are known to enhance tubular reabsorption of citrate). Conclusions: 1. Hypocitraturia appears to be a rather common abnormality in renal stone formers and probably an important factor in the genesis of nephrolithiasis. 2. This hypocitraturia is due to enhanced renal reabsorption of citrate; the exact mechanism awaits further investigation.

EFFECT OF CHRONIC FUROSEMIDE (F) ADMINISTRATION ON URINARY CALCIUM EXCRETION (U_{Ca}) AND TOTAL BODY CALCIUM (TBC) IN THE RAT. S.N. Tuma, A. LeBlanc*, G. Eknoyan, W.N. Suki. Baylor Coll. Med., Houston, Texas 77030.

The long-term effect of F on calcium (Ca) homeostasis is not well defined. The available data suggests a deleterious effect on the skeleton. To further investigate the effect of F on calcium balance, 16 Sprague-Dawley rats maintained on the same diet were divided into two groups. One group (F) received 40 mg F daily for 8 weeks in drinking water while control group (C) received tap water. 24-hour urine collection for Ca, Mg, Na, K and PO_4 were obtained weekly. TBC was measured monthly by neutron activation. The 24-hour excess loss of Na, K, Mg and PO_4 induced by F was quantitated and supplemented daily. Furosemide administration resulted in an increase in U_{Ca} (9.52±1.3 vs 5.45±.7 mg/day, p<.01) and U_{Na} (2323±201 vs 1505±115 μEq/day, p<.001). U_{Mg} and U_K were also higher in F during the first 5 weeks, but were not different from C thereafter. There was no difference between the two groups in TBC, which showed an equal increase of 7.0±1.4 mg/day in the F group and 6.4±1.4 mg/day in the C group. This accounts for a monthly increment of approximately 7% of TBC in both groups, representing normal growth. Thus, furosemide for 8 weeks in growing rats resulted in an increase in urinary calcium but no change in total body calcium. It is possible that enhanced absorption from the gut compensated for urinary loss of Ca. It is unlikely, then, that chronic F results in a detrimental effect on TBC.

PRESERVATION OF NORMAL GLOMERULAR FILTRATION RATE AFTER 7.7 YEARS OF CONTINUED LITHIUM THERAPY. Carlos A. Vaamonde, Nestor Millan*, Gaston Magrinat*, Guido O. Perez and James R. Oster. Depts.of Medicine and Psychiatry, Miami VA Medical Center and University of Miami, Miami, Florida.

From cross-sectional studies it has been postulated that chronic treatment of manic depressive disorders with lithium (Li) results in focal chronic interstitial nephritis and moderate decreases in glomerular filtration rate (GFR). The few available longitudinal studies have followed patients for only 1 to 2 years of Li therapy. Severe impairment of renal concentrating ability and episodes of acute Li intoxication have been proposed as determinants of the GFR impairment.

Creatinine clearance (Ccr) was measured using multiple short-duration collections shortly after beginning (baseline) and after an average of 7.7 ± 0.3 (SE) yr (5.9-8.6 yr) of sustained Li therapy (900-1200 mg/day Li carbonate) in 7 patients with manic depressive disorders. These patients had received Li from 1 to 20 weeks prior to the first measurement of Ccr. Plasma Li levels were monitored monthly. The mean age at baseline was 41 ± 4 yr (26-55). Baseline Ccr was 101 ± 8 ml/min/1.73 m^2 and 7.7 years later it was 104 ± 4. Plasma Li levels were 0.79 ± 0.1 and 0.59 ± 0.04 mM/L, respectively (non significant). No patient suffered recognized acute episodes of acute Li intoxication or severe dehydration.

In summary, these observations document the absence of deterioration of glomerular filtration rate in seven patients treated chronically with Li for an average of nearly 8 years.

FAMILIAL RENAL HYPOURICEMIA: NORMAL URATE TRANSPORT INTO ERYTHROCYTES. Vinay, P., Gattereau, A., Moulin, B., Gougoux, A. Departments of Medicine and Physiology, University of Montreal and Hôtel-Dieu Hospital, Montreal (Quebec), Canada.

The transport of 2-^{14}C-urate into erythrocytes of four patients with familial renal hypouricemia (P urate = 1.5 ± 0.5 mg/dl) was compared to that measured in 6 normal subjects (P urate = 5.4 ± 0.2 mg/dl). The urate/creatinine clearance ratio of the patients was 0.77, 0.65, 0.21 and 0.20 as compared to 0.12 ± 0.01 for the controls, and was minimally affected by pyrazinamide administration (3gm p. os.). The rate of urate uptake by erythrocytes was identical and hypoxanthine (1 mM) had the same competitive effect on this transport in both groups of patients. Thus the permeability of erythrocytes to urate is not affected in the familial renal hypouricemic syndrome. The transport of urate by red cells of Dalmatian and mongrel dogs was also studied. In contrast to the data of Harvey and Christensen (Science, 145: 826-1964) no difference was found between the two breeds of dog. This challenges the accepted view that the Dalmatian dog presents a generalized defect of urate transport across cell membranes.

CIMETIDINE (C) NEPHROPATHY: A PROBABLE DISORDER OF CELL MEDIATED IMMUNITY (CMI). Alan Watson*, Milton Dalbow*, Irene Stachura, Jorge Fragola*, Mario Rubin*, Rose Marie Watson*, and Edmund Bourke, Depts. of Medicine and Pathology, Allegheny General Hospital, Pittsburgh, PA.

The role of CMI in the pathogenesis of acute interstitial nephritis (AIN) remains speculative. To examine this further we subtyped the lymphocyte populations of peripheral blood and biopsy material from a patient who developed AIN with associated acute renal failure and polymyositis following C therapy. Cell mediated sensitivity to the drug was initially demonstrated by significantly increased lymphoblastogenesis and leukocyte migration inhibitory factor production following addition of C to the patient's lymphocytes when compared to controls. Using monoclonal antibody techniques the prominent mononuclear infiltrates in biopsies from both kidney and muscle contained 75% and 76% cytotoxic/suppressor T cells respectively. This was associated with a marked increase in cytotoxic/suppressor T cells in the peripheral blood with a reversal of the normal helper/inducer: cytotoxic/suppressor cell ratio. Thirty-eight percent of circulating T cells possessed DR (Ia-like) antigens, further indicating immune activation. Following steroid therapy the helper/inducer: cytotoxic/suppressor cell ratio in the peripheral blood normalized and the DR-antigen positive T lymphocytes fell to 2%. These effects coincided with a complete clinical remission of the acute renal failure and polymyositis. The findings indicate a prominent role for CMI in the pathogenesis of this drug induced disorder.

NEPHROTIC RANGE PROTEINURIA IN PATIENTS WITH IgA NEPHRITIS. John F. Walker,* Philip Landy,* and Allan Katz. Toronto Western Hospital, Depts. of Medicine and Pathology, Toronto, Canada.

Nephrotic range proteinuria is uncommon in patients with IgA nephritis. For this reason we compared the clinical and pathologic features in 63 non-nephrotic patients with those in 8 patients who had nephrotic range proteinuria at the time of biopsy. Both the mean age and the mean duration of the disease at the time of diagnosis were not significantly different in the 2 groups of cases. Significant associations were found between nephrotic range proteinuria and the following: the extent of glomerular hyalinization, the severity of interstitial fibrosis and tubular atrophy and the degree of foot process effacement and mesangial matrix increase. A significant association was also noted with the presence of renal failure on follow-up. Seventy per-cent of patients who presented with nephrotic range proteinuria had elevated levels of serum creatinine on follow-up compared with 20% of non-nephrotic cases. The results of our study suggest that patients with nephrotic range proteinuria have a more severe type of IgA nephritis from the outset and that a marked degree of proteinuria is not merely a consequence of the duration of disease.

TRENDS IN INCIDENCE AND PREVALENCE OF END-STAGE RENAL DISEASE (ESRD). John M. Weller, Shu-Chen Wu*, C. Wm. Ferguson*, and Victor M. Hawthorne.* Univ. of Michigan, Depts. of Internal Medicine, Biostatistics and Epidemiology, and the Michigan Kidney Registry, Ann Arbor, Mich.

Analysis of data on ESRD patients in Michigan from 1974 through 1981 shows an increase in ESRD incidence rates from 4.79/100,000 population to 8.90. Males predominate over females (7.76 to 5.55). Older age groups have higher incidence rates. The incidence rate for black ESRD patients is 20.75, while it is 4.78 for white patients. Black patients, compared to white, had relative risks of 3.8 for diabetes mellitus, 10.9 for hypertension and 1.7 for glomerulonephritis as perceived causes of ESRD. The incidence trend points to an increase in older age and diabetic patients; however, the incidence rates for glomerulonephritis and hypertension as causes of ESRD in the black population decreased in 1981.

Prevalence rates of ESRD increased from 11.59/100,000 population in 1974 to 31.77 in 1981 with males predominating; the prevalence rate being 36.85 for males and 26.90 for females. The prevalence rate for black ESRD patients in 1981 was 78.76 and for whites was 24.87. Rates for older patients increased faster. Prevalence rates for blacks in 1981 for glomerulonephritis, hypertension and diabetes mellitus as causes of ESRD were twice, more than 10 times and 4 times greater than the respective rates in whites.

Center hemodialysis is the most common treatment modality; the percent of all ESRD patients on home hemodialysis has steadily decreased from 1974 through 1981. Use of CAPD has risen rapidly from 1979 through 1981.

EVOLUTION OF PROGRESSIVE DISEASE IN IGA NEPHROPATHY. Robert J. Wyatt, Bruce A. Julian,* Eugenie C. Scott,* Frank J. Block,* Nancy H. Holland and Hartmut H. Malluche. University of Kentucky, College of Medicine, Lexington, Kentucky.

IgA nephropathy was found on renal biopsies obtained since 1973 in 63 patients (pts). Forty-seven pts were male and 16 female. Onset was defined by appearance of macroscopic hematuria (MH) in 38, abnormal urinalysis in 16 and hypertension (HT) in 9. Mean age ± SEM of onset was 23.5 ± 1.7 yrs. At last follow-up 46 pts had normal renal function. Chronic renal failure (CRF), defined by serum creatinine >2.0 mg%, was present in 17. Dialysis was initiated in 9 pts after a mean interval of 9.0 yrs from onset. CRF pts were older at onset than normal function pts ($p < 0.05$). However, onset with MH at 10, 13 and 18 yrs was noted in 3 CRF pts. HT was presenting sign in 35% of CRF pts. CRF pts were more likely to present with HT ($\chi^2 = 8.39$, $p < 0.01$). Pts with normal function were more likely to present with MH ($\chi^2 = 4.24$, $p < 0.05$). Anti-HT medication other than a diuretic (D) was used by 83% of CRF pts prior to dialysis. In normal function pts 67% did not require anti-HT medication, 17% required D only and 15% required medication other than D. Kidney survival predicted by life-table method was 76% at 10 yrs and 50% at 22 yrs from onset.

Thus, (1) our data differ from data of many U.S. studies which show a generally favorable prognosis but agree with data from European and Australian studies which document progressive course to CRF in a significant percentage of IgA nephropathy pts. Also, (2) childhood onset is not always associated with a benign outcome.

IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN) IN CHARCOT-MARIE-TOOTH (CMT) DISEASE. Melvin Yudis and Robert A. Sirota, Dept. of Medicine, Abington Memorial Hospital, Abington, Pa.

CMT Disease (peroneal muscular atrophy) has rarely been associated with an acute nephritic syndrome. We present a case of chronic ICGN in a patient with CMT Disease.

This 38 y.o. male presented with hematuria and proteinuria (8 gms/24 hrs) in 1975. Renal biopsy revealed ICGN characterized by the presence of dense deposits in the subepithelial, intramembranous subendothelial and mesangial positions. HBsAg was positive but liver function studies were normal. He was treated with a course of steroids with remission of the proteinuria. In 1976, upper G.I. series revealed Crohn's Disease. Laparotomy revealed ileovesical fistulas which were repaired. In 1982, he had a repeat course of steroids because of increasing muscular weakness. The steroids improved his neurologic picture.

Acute glomerulonephritis has been reported in association with CMT Disease. Our case concerns a man with chronic ICGN and nephrotic syndrome whose disease responded partially to steroid therapy. Other factors which can cause renal disease were present in this case including HBsAg and Crohn's Disease with urinary tract infection and ileovesical fistula. However, we do not feel his renal biopsy findings or clinical course can be explained on the basis of the positive HBsAg or the bacteriuria. This case of ICGN associated with CMT Disease may indicate a significant association of these two entities. Treatment with steroids may be efficacious when this association occurs both from the nephropathy and the neuromuscular picture.

Dialysis

PREVALENCE AND ETIOLOGY OF ALUMINUM INTOXICATION IN NON-DIALYZED (D-) AND DIALYZED (D+) PATIENTS WITH RENAL FAILURE. K. Abreo*, M.C. Faugere*, A. Smith, R. Yokel*, B.A. Julian*, R. Kluge*, R.M. Friedler, H.H. Malluche. Univ of Kentucky, Div of Neph, Bone & Min Metab, Lexington, KY.

Prevalence of bone aluminum intoxication (BAI) was correlated with serum aluminum (Al), Al in hair and drinking water, $Al(OH)_3$ intake and quantitative bone histology in 100 D- and 87 D+ uremic patients (pts) from various geographic areas. BAI was established using a specific histologic stain for Al. BAI was present in 5% of D- pts and 44% of D+ pts. BAI varied geographically from 23 to 88% in D+ pts. Bone histology in D+ pts showed low turnover osteomalacia (LTOM) in 42% and mixed uremic osteodystrophy (MUO) in 58%. All D- pts had MUO. BAI correlated with Al in city water ($r = 0.99$) irrespective of the use of reverse osmosis for dialysis H_2O treatment. Pts with BAI had higher total $Al(OH)_3$ intake (8.8±1.84 vs 1.8±0.53 kg, $p<0.01$) due to both longer time of renal failure with $Al(OH)_3$ therapy (87±14.1 vs 41±5.3 months, $p< 0.01$) and higher daily dose of $Al(OH)_3$ (4.8±1.0 vs 2.0±0.7 g/day, $p<0.02$). Serum Al levels were higher in BAI (137±32.3 vs 49±12.3 µg/L, $p<0.02$). Al in hair did not correlate with Al deposits in bone.

The data indicate that: (1) BAI is not confined to dialysis pts with LTOM, it is also found in 5% of non-dialyzed pts. (2) Increased Al in drinking water is a risk factor for BAI in D+ pts but not in D- pts. (3) Reverse osmosis of dialysate water is not sufficient to prevent BAI. (4) Cumulative $Al(OH)_3$ intake is a major etiologic factor of BAI in D+ pts and D- pts. (5) Serum Al is higher in BAI but does not correlate with degree of BAI. (6) Hair Al is not predictive of BAI.

POTASSIUM AND PHOSPHATE REMOVAL WITH BICARBONATE HEMODIALYSIS. Ronald Albright*, Barry Kram, Robert P. White*. St. Luke's Kidney Center, Bethlehem, PA.

Whether the decrease in serum potassium (K) and phosphate (PO4) seen in bicarbonate hemodialysis (BHD) is primarily the result of K and PO4 removal or to intracellular shifts is unclear. We therefore studied K and PO4 removal as well as pH changes in four patients on BHD versus acetate hemodialysis (AHD). Each patient had two dialysis sessions on AHD followed by two sessions on BHD one week later. K, bicarbonate (HCO_3), pH and PO4 were measured pre and immediately post dialysis. The total dialysate was collected and the amount of K and PO4 removed measured. All patients were dialyzed against a standard bath with a similar sodium concentration and a K concentration of 2 meq/L with a parallel plate dialyzer. On BHD pre-dialysis K, PO4, HCO_3 and pH were 4.4±1.5 meq/L, 5.9±1.7 mg/dl, 18.7±2.3 meq/L and 7.41±.48 respectively with post-dialysis values 3.7±.46 meq/L, 3.9±.7 mg/dl, 26.1±1.5 meq/L and 7.49±.31. On AHD pre-dialysis K, PO4, HCO_3 and pH were 5.0±1.2 meq/L, 5.8±1.6 mg/dl, 20±3.4 meq/L and 7.41±.29 respectively with post-dialysis values 3.6±.3 meq/L, 3.3±.4 mg/dl, 23.3±2.7 meq/L, 7.48±.31. Significant results are listed below.

	K Removal (meq)	PO4 Removed (mg)	pH Δ
BHD	74.6±20.4	557.9±289.2	0.7
AHD	71.1±31.1	416.2±310.7	0.8

We conclude that the change in serum K and PO4 with BHD is secondary to actual K and PO4 removal as opposed to intracellular shifts and that BHD can be performed for its reported beneficial effects (as compared to AHD) with comparable and satisfactory removal of K and PO4.

THE LIFECATH™ PERITONEAL IMPLANT: STUDY OF 46 CONSECUTIVE PATIENTS. Stephen R. Ash, Hemodialysis Lab., A.A. Potter Engr. Ctr., Purdue Univ., West Lafayette, Indiana.

The LifeCath™ is a peritoneal access device with a 1 3/4" diameter, 3/16" high, disc-shaped intraperitoneal portion. The disc is held in position on the anterior abdominal wall by the preperitoneal Dacron cuff. The large peripheral entry port of the disc yields a peritoneal fluid velocity during inflow and outflow 1/100 that of the Tenckhoff catheter.

Function of 46 LifeCath™ catheters was studied from February 1980 to July 1982, in multiple centers (250 patient-months). Two-thirds of the patients were "high risk" for catheter failure, with numerous previous surgeries and Tenckhoff catheters. All but 7 catheters worked well initially, with 4 catheter failures being due to surgical placement problems (intraperitoneal cuff location or pericatheter leaks). Only 1 catheter failure occurred in a "low risk" patient, and this was related to intraperitoneal cuff location. Late complications have been rare; cuff infections = 0, exit cuff erosions = 0, late outflow failures = 0, and infections which failed to clear (after 1 course of antibiotics) = 3. Least squares analysis of outflow curves for both Tenckhoff and LifeCath™ catheters indicated that deceleration during outflow, occurred earlier with the Tenckhoff (6 to 8 minutes) than with the LifeCath™ (12 to 18 minutes). Most patients drained 2 liters in 9 to 11 minutes with the LifeCath™ catheter.

The LifeCath™ is a suitable alternative catheter, especially for patients at high risk of peritoneal access failure.

ANALYSIS OF MORBIDITY OF DIALYSIS HYPERALIMENTATION. Maher Azer, Los Alamitos Hemodialysis Center, Los Alamitos, California.

The complications of dialysis hyperalimentation (DHA) were assessed in thirty-eight patients receiving a total of 1325 dialysis treatments. The solution used consisted of 1L. containing 25% glucose and 3.5% aminocin plus electrolyte additives tailored to the patients needs. The indications for the use of (DHA) included acute renal failure, malnutritional state, enteric fistula, perioperative in association with major surgery, septicemia, ascites, pericardial effusion, acute peripheral neuropathy and acute encephalopathy. The patient population was classified into two groups: Group I: Received (DHA) only; Group II: Received (DHA) plus continuous central hyperalimentation. The incidence of septicemia was (5.5%) and occuring only in Group II. The incidence of post dialysis hypoglycemia; blood glucose level below 80 mg%; was (2%) and occuring equally in Group I (1.8%) and Group II (2.16%). Post dialysis hyperglycemia above 300 mg%, was common (55.4%) and occuring equally in both groups. Hyperglycemia could be controlled with the use of intravenous regular insulin given at the start of (DHA) and or by the addition of regular insulin to the infused hyperalimentation.

UREMIC NON-DIABETIC HYPOGLYCEMIA. A LIFE-THREATENING SYNDROME ON THE INCREASE. Avram MM, Pahilan A*, Wolf RE*, Fein PA, Iancu M, Gan A. The Long Island College Hospital, Department of Medicine, Division of Nephrology, Brooklyn, New York

We diagnosed spontaneous hypoglycemia in 8 non-diabetic uremic patients receiving maintenance dialysis (5 men, 3 women) ranging in age from 25 to 71 years. Serum glucose was less than 25 mg/dl in all 8 and, surprisingly, less than 5 ml/dl in 2 patients. Interestingly, 6 of 8 patients were on propanolol and 1 on disopyramide. Both drugs are known to interfere with gluconeogenisis.

7 of 8 patients had hypoalbuminemia (2.5 mg/dl or less) and all had endogenous creatinine clearances of 5 ml/min or less. 7 of 8 patients were on hemodialysis and 1 on peritoneal dialysis. In 1 patient the syndrome occurred during a bout of pneumoccal bronchopneumonia and 6 of 8 patients refused to follow adequate oral diets. It was possible in all but 1 of the group to correct the hypoglycemia via prompt administration of IV hypertonic glucose.

While this newly recognized syndrome does not have an easily explainable pathogenesis, hepatic and nutritional causes and infection are contributing factors. Additionally, the presence in 6 of 8 patients of the beta blocker propanolol, a widely used medication for uremic patients, suggests a need for thorough reevaluation of propanolol-uremia-hypoglycemia interaction.

DOPPLER ULTRASONIC DETECTION OF PARTICULATE RELEASE DURING HEMODIALYSIS. S. Badylak,* S. Ash, D. Carr* Hemodialysis Lab., Purdue Univ., West Lafayette, IN.

Doppler ultrasound is a non-invasive technique for monitoring speed or quantity of moving objects, while ignoring stationary objects. A Doppler system was investigated to determine its sensitivity for detection of particulate release in blood during hemodialysis. Seven hemodialysis procedures were done in dogs and six in humans using a sorbent suspension reciprocating dialyzer (SSRD). Two additional trials were done in humans using a cellulose acetate hollow fiber dialyzer (HFD). Particulate release was monitored with a Parks 9.3 MHz continuous directional Doppler flowmeter which generated an output signal that was simultaneously recorded by a Visicorder Qscillograph, and electronically integrated for the recording of mean reflected power. We found a large increase (approx. 2-fold) in reflected Doppler signal in outflowing blood compared to inflowing blood from the SSRD and HFD in both dogs and humans. Numerous additional studies to characterize the nature of the particulate matter showed platelet aggregates in the dogs, and fibrinogen-fibrin degradation products in humans to be present in the outflowing blood. In vitro studies suggested microscopic air bubbles (<25μ diameter) may have contributed to the particulate matter detected during hemodialysis by this sensitive monitoring system.

QUALITY OF PATIENT LIFE (QOL) ON CAPD. M. Barnicle,* M. Simmons,* D. Hofman,* D. O'Neill,* M. Bierman, M. Hammeke. Creighton University, Omaha, Nebraska.

CAPD is a new dialysis treatment effective for management of ESRD. Many questions however remain about broad usage of CAPD, particularly the quality of patient life and patient acceptance. We reviewed all active CAPD patients at our institution (33% of all dialysis) using interviews and the Sickness Impact Profile (SIP) to assess the QOL of CAPD patients. Patients (N=28) were on CAPD for a mean of 1.4 years. They had 0.48 episodes of peritonitis/year and were hospitalized 13.2 days/year. SIP scores showed a high degree of disability especially in diabetic patients. Despite disability all patients could be managed at home with 71% being totally independent in this setting. Family members provided high levels of care for 15%. Pre-morbid attitudes were expressed by 7%, however, 82% felt that they contributed to the welfare of their families. Five (18%) earned an income. Most (85%) expressed satisfaction with their present life. Greater than 90% of the patients reported adequate opportunities to socialize, however only 50% of the patients accepted these opportunities frequently because of their CAPD schedule. Only 7% of the patients felt the dialysis treatment a burden.

We conclude that despite high level disability CAPD patients find satisfaction with their life and contribute to their families socially and economically.

RABBIT MODEL OF CHRONIC AMBULATORY PERITONEAL DIALYSIS (CAPD) AND PERITONITIS. Thomas Beam,* Thomas Raab,* and Victor Sacchi* (Intr. by C. Bentzel), Buffalo VA Medical Center and SUNY at Buffalo, Buffalo, New York.

An animal model of CAPD was developed in New Zealand white rabbits to assess host defenses against infection in the peritoneal space. A Tenckoff catheter was placed under general anesthesia; the exit site was the nape of the neck. The catheter remained patent for 3 to 5 days using Dianeal (1.5%). Addition of heparin (10,000 U per L) preserved function for 10 days longer. Two days after catheter placement, E. coli were injected. Serum possessed neither opsonic nor bactericidal activity against this strain. Iron increased virulence.

Group	Inoculum	Iron	Nephrectomy	Survival	Cult.
I	10^7	yes	no	>7 days	+/-
II	10^7	yes	Sham	5 days	+
III	10^7	no	partial	3-5 days	+
IV	10^2	no	yes	1-2 days	+

Group I animals had local absess formation along and remote from the catheter; Group II had similar findings; Group III were variably uremic; Group IV sustained a fulminant infection despite a 10^5 reduction in inoculum. Half of Group IV animals were bacteremic. We have found that local defenses fail in nephrectomized CAPD animals leading to sepsis and death. This model will allow detailed analysis of defense factors and possible favorable manipulation.

COMPARISON OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD) AND HEMODIALYSIS IN CHILDREN. Michel Baum*, David Powell*, Kathy McHenry,* and Donald Potter. Univ. of Calif., San Francisco, Dept. of Pediatrics, San Francisco, California.

The clinical and biochemical effects of CAPD in 20 children and of center hemodialysis in 16 children were compared over a 2½ year period. The CAPD patients had a higher serum bicarbonate (25 vs 20 mEq/L), a higher intake of calories (79 vs 64% of RDA), a higher intake of protein (167 vs 124% of RDA), a higher hematocrit (21.9 vs 19.6%) with a lower transfusion requirement (0.16 vs 0.73 transfusions/month), and a lower systolic blood pressure (110 vs 129 mmHg) than the hemodialysis patients. Serum cholesterol was higher in the CAPD patients (250 vs 179 mg/dl). Serum sodium, potassium, chloride, urea nitrogen, creatinine, glucose, albumin, triglycerides, calcium, alkaline phosphatase, phosphorus, parathyroid hormone, the incidence of renal osteodystrophy, and the growth rate were not significantly different between the two groups. The CAPD patients had more complications than the hemodialysis patients, but the incidence and duration of hospitalization were similar. There were four treatment failures with CAPD and one with hemodialysis. There was one death in each group. The cost of CAPD and hemodialysis, excluding medications and hospitalization, was $19,600 and $54,300 per patient-year respectively. Patients treated with both forms of dialysis prefered CAPD. We conclude that CAPD is a satisfactory alternative to hemodialysis in children.

MACROPHAGE ACTIVATION BY PARTICLES RELEASED FROM DIALYSIS TUBING. J.Bommer*, D.Gemsa*, R.Waldherr*, E.Ritz* (intr.by E.Friedman). Dept.Int.Med., Immunology and Pathology, Heidelberg (FRG)

Multiorgan abnormalities in dialysis patients (hepatosplenomegaly, granulomatous non-A,non-B-hepatitis, pancytopenia from hypersplenism) have recently been ascribed to storage by macrophages (MØ) of silicone (Si) particles released from tubing in the pump segment. We examined (1) whether the syndrome can be reproduced experimentally with Si as well as with PVC and polyurethane (PU), potential substitutes for Si, and (2) whether foreign material storage alters MØ function. 150g rats; daily iv solvent (CO) or 1-6 x 10^8 (Si, PVC or PU) particles for 3 weeks. Light microscopy, TEM and electron probe of lung, liver, spleen. Peritoneal MØ incubated 4h or 24 h; stimulated with zymosan or LPS; PGE_2 and TXB_2 with RIA. MØ with foreign material inclusion and granulomas were found in the lung of Si, PVC or PU animals. In liver and spleen the material was stored in lysosomes of MØ. As compared to CO, unstimulated PGE_2 was increased in MØ with Si) PVC)PU (CO 4.27±0.5 ng/ml; Si 51.9±13.2). There was a parallel increase of TXB_2. Zymosan increased PGE_2 in incubation medium after 4 h and decreased PGE_2 after 24 h for MØ of Si, PVC and PU animals but increased PGE_2 both after 4 h and 24 h in controls. Conclusion: Foreign material deposition in RES of dialysis patients can be reproduced with particles of silicone, PVC, and polyurethane. Foreign material deposition in macrophages is paralleled by marked abnormalities of arachidonic acid metabolism. PVC and PU are not safe substitutes for silicone.

AMBULATORY CHRONIC HEMODIALYSIS IN THE ELDERLY. Sonia Borra, Lynne Smith*, Henry Lipner, and Morris Kleinfeld*. So. Bklyn Nephrology Ctr., Brooklyn, New York.

The results of ambulatory hemodialysis in patients under 60 years were compared to those 60 and older. Eighty-seven patients, 47 males and 40 females, received hemodialysis from July 27th, 1981 to July 30th, 1982. The age range varied between 22 and 82 years, with 43 patients (49%) less than 60 years and 45 patients (51%) 60 and older. Patients less than 60 were dialyzed for a total of 296 months, (mean 6.8 months) whereas, those 60 and older were dialyzed for 313 months (mean of 6.9 months). Diabetics accounted for 9% of those less than 60 and 11% of the group 60 and older. Medical admissions for those under 60 totaled 163 days. Medical admissions for those 60 and older accounted for 225 days. The group less than 60 had surgical admissions totaling 20 days and those 60 and older accounted for 78 days. There were 4 deaths in the group less than 60 and 2 in those 60 and older. Five of the 6 deaths were attributed to cardiovascular deaths with 5 of those having hypertensive heart disease. Peritonitis was cause of death in 6th patient. The study indicates that elderly patients can be effectively dialyzed in ambulatory setting. Hypertension is a major contributing factor to mortality; the hypertension being more significant than age.

CHRONIC RENAL FAILURE (CRF) IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). Jhoong S. Cheigh, Kurt H. Stenzel, Albert L. Rubin, Jacqueline Chami, and John Sullivan. The Rogosin Kidney Center, The New York Hospital-Cornell Medical Center, New York, NY.

There is little information on the clinical spectrum of patients with SLE who develop CRF and require maintenance dialysis therapy. We studied the course of 36 patients with SLE who developed CRF and required dialysis for > 3 mos. between 1972 and 1981. There were 29 females and 7 males, ages ranged from 15-65 yrs ($M\pm SD=27\pm10$) when they began dialysis and they were followed for 4-168 (36 ± 39) mos. Clinical renal disease was present 3-173 (66 ± 40) mos. prior to dialysis. At the time dialysis was initiated, 12 patients (33%) had clinically active SLE. Renal disease progressed in the majority of patients in the absence of active SLE. Following dialysis, 8 patients died, 2 from cardiovascular and 6 from infectious disease after 4-27 (16 ± 10) mos. Actuarial survival rates at 1,2 and 5 yrs following dialysis were 91.1%, 78.8% and 68.9%. Twenty four patients survived on dialysis for >1 year and only 3 of these patients had episodes of clinically active SLE in subsequent years. Serologic abnormalities were, however, present in most patients. Twelve of these patients (50%) were maintained on prednisone 2.5-15mg/day. We conclude that: 1.Lupus nephritis often progresses to CRF in the absence of clinically active SLE; 2.Most SLE patients with CRF and treated with dialysis remain clinically inactive despite little or no prednisone therapy, and 3. Survival of SLE patients on dialysis is comparable to that of the general CRF population. Our study suggests that prognosis could be further improved by giving less immunosuppressive therapy to them.

COMPARISON OF ZIRCONIUM PHOSPHATE AND ZEOLITE AS SORBENT IN A RECIPROCATING DIALYZER. D.J. Carr,* S.R. Ash, and D.E. Blake.* Hemodialysis Lab, Purdue Univ., W. Lafayette, IN

Sorbent suspensions of activated charcoal, particulate-bound urease and either Na-loaded zirconium phosphate (ZrP) or Ca-Na loaded zeolite F (ZF) were tested in reciprocating dialyzers. Dialyzer design was similar to the sorbent suspension reciprocating dialyzer (SSRD) described in 1980. A 46L batch of buffered osmotically balanced salt solution containing 80 mg% urea nitrogen and 20 mg% creatinine was dialyzed at a treatment rate of 200 ml/min. In vitro dialysis using the ZrP containing sorbent resulted in slightly better urea and creatinine clearance than with ZF sorbent. However, ammonia return to the treated "artificial blood" was unacceptably high when dialyzed against ZrP (9.5 to 16.0 mM) as compared to ZF dialysis (3.2 to 7.1 mM). Similar results were found in vivo when dogs were dialyzed with the same sorbents. Also, while pH balance with ZrP was acceptable in vivo, high Na return and Ca++ removal were life threatening. However, Ca-Na loaded ZF has been shown to yield appropriate electrolyte balance for therapy of uremia. Therefore, zeolite sorbents are superior to ZrP for use in the SSRD.

ANAPHYLATOXIN FORMATION DURING CUPROPHANE DIALYSER RE-USE. **A. Cheung,*D. Chenoweth*, D.Ward, L. Henderson. VA Medical Center and University of California, San Diego.**

Leukopenia during exposure to cellulosic hemodialysis membrane is considered to result from alternative pathway complement activation and anaphylatoxin (C_{3a}, C_{5a}) release by the membrane. Leukoagglutination and pulmonary sequestration is thought to result from C_{5a} ($\mp$ des arg) interaction with polymorphonuclear leukocytes. Re-use of cellulosic dialyser membranes that have been formalin treated result in lesser degrees of hemodialysis leukopenia. The three previous studies quantitating complement activation with formalin-treated reused cellulosic dialysers employed indirect assays (CH-50 in 2 and PMN agglutination in 1) and their results are conflicting. The present study employs a sensitive radioimmunoassay for both C_{3a} and C_{5a} antigens in the venous (return) line of bracketing first use and 5 serial reuse treatments in 11 patients. Prior studies indicate maximum leukopenia and anaphylatoxin generation after 15-20 minutes of treatment.

x ± 1 SEM		New	1-5 Re-uses Min -- Max
Relative WBC (%)	Pre HD	100	100
	15 Min	31 ± 6	72 ± 10 -78 ± 9
Plasma C_{3a} (ng/ml)	Pre HD	452 ± 73	221± 55 -398± 69
	15 Min	5540±926	558±142-1166±410
Plasma C_{5a} (ng/ml)	Pre HD	62 ± 15	32 ± 9 -69 ± 21
	15 ml	200 ± 45	35 ± 45 -78 ± 21

All differences in new vs re-use values at 15 min. are statistically significant ($p<.05$). We conclude that formalin re-use of cellulosic dialysers markedly attenuates their anaphylatoxin producing capability.

THE EFFECTS OF THREE DIALYZER MEMBRANES ON BLOOD GASES (ABG) AND WHITE BLOOD CELL COUNT (WBC). S. Chopra, T. Chung, S. Vicks,* R. Rubin, W. Flamenbaum and R. Hamburger, Renal Sections, Lemuel Shattuck and Boston VA Medical Center, Boston, MA.

The etiology of the hypoxemia during hemodialysis has been ascribed to pulmonary leukostasis, the Bohr effect, increased O_2 consumption, and hypoventilation. Of these factors, pulmonary leukostasis and hypoventilation in response to CO_2 loss across the dialyzer membrane are best supported by the available data. We studied the effects of three different dialyzer membranes AN-69, RP 610; Cuprophane, HPF 300; and cellulose, CDK 4000 on ABG and WBC. The patients (n=16) were dialyzed 8 weeks with the RP 610 and then randomly assigned to either HPF 300 or CDK 4000 dialysis for an additional 8 weeks.

Comparisons were made between predialysis values and values obtained at 15 minutes. No significant change in PCO_2, pH, or bicarbonate occurred. Each dialyzer was associated with a similar fall in PO_2 (mmHg): RP 610, 89 to 83; HPF 300, 88 to 80; CDK 4000, 91 to 84. However, WBC decreased by 53±8% (SE) with HPF 300 and 31±3% with CDK 4000; both greater than 4±3% decrease with the RP 610. This dissociation of dialysis leukopenia from hypoxemia suggests that pulmonary leukostasis was not the primary cause of the observed hypoxemia. Since there was no change in pH, the Bohr effect cannot account for the hypoxemia. These data, therefore, are most consistent with hypoventilation or increased O_2 consumption as the cause of the observed decrease in PO_2 during hemodialysis.

IMPROVEMENT IN RENAL FUNCTION AND DISCONTINUATION OF CHRONIC HEMODIALYSIS (CHD) FOR END STAGE RENAL DISEASE (ESRD). Tai J. Chung, Shreekant Chopra, Steven L. Vicks* and Robert J. Hamburger, Renal Sections, Lemuel Shattuck Hospital and Boston VA Medical Center, Boston, Mass.

Because of improvement in renal function (RF), CHD was discontinued in 10 (4 males, 6 females) out of 270 patients (3.7%) in our ESRD program during the last 16 years. Age range was 35-72 years (mean 59). The etiologies of ESRD in this group were: Chronic obstructive uropathy 3, Rapidly progressing glomerulonephritis 2, Focal segmental glomerulosclerosis 1, Wegener's granulomatosis 1, Aminoglycoside nephrotoxicity 1, Analgesic nephropathy 1 and non-specific chronic interstitial nephritis 1. Diagnosis was made in accordance with standard clinical and laboratory criteria after excluding reversible causes of acute or acute-on-chronic renal failure.

This improvement in RF occured with 46-673 days (mean 182) after institution of CHD. Predialysis serum creatinine (S.Cr.) dropped to 6.6± 3.2 mg/dl at the time of discontinuation of CHD.

Duration of CHD was as follows: 4 less than 3 months, 2 between 3-6 months and 4 over 6 months.

Five patients restarted CHD within 28-468 days (mean 137). Of the remaining 5 patients, 2 died of non renal causes within 1-4 years, while 3 are still off CHD.

Duration of CHD before improvement of RF, subsequent reinstitution of CHD and ultimate prognosis were unrelated to the pre- CHD serum creatinine level.

The data demonstrates that spontaneous, delayed recovery of RF in ESRD patients may occur and may be long lasting in some instances.

EFFECT OF DIALYZER MEMBRANE AND DESIGN ON β THROMBOGLOBULIN (BTG) DURING HEMODIALYSIS (HD). T. Chung, S. Chopra, R. Rubin, W. Flamenbaum and R. Hamburger, Renal Sections, Lemuel Shattuck Hospital and Boston VA Medical Center, Boston, MA.

Platelet abnormalities resulting from activation during HD have been postulated as having a potential role in HD associated hemorrhagic complications and atherosclerosis. Dialyzer membrane composition and/or design may modulate platelet activation. To evaluate the role of these factors we determined platelet count and BTG prior to; 15 min. into and immediately post HD using a parallel flow (AN-69) and 2 capillary (cellulose, Ce, CDK 4000; Cuprophane, Cu, HPF 300) dialyzers. No significant decreases in platelet count were observed.

	BTG (± SD) levels (ng/ml)		
	AN-69	Ce	Cu
Pre	155±36	155±57	172±44
15 min	163±49	166±54[a]	202±28[a,b]
Post	122±35[a]	154±56[b]	188±34[b]

Significantly different ($p < .05$) from pre (a) or AN-69 (b). (AN-69 N=15, Ce N=8; Cu N=7).

Since patients were treated for 8 wks. with each dialyzer the effect of time was examined. While pre-HD values did not change with AN-69, there was a significant rise in BTG with both Cu (149 to 182) and Ce (137 to 178) comparing wk 1 and 8 of HD.

These studies demonstrate differences in platelet activation, without a change in platelet count, that are due to membrane composition rather than design. In HD patients in whom platelet abnormalities may be a problem dialyzer selection based on membrane type maybe important.

USE OF CALCITRIOL IN PROPHYLAXIS OF BONE DISEASE IN DIALYSIS PATIENTS: A PROSPECTIVE, DOUBLE BLIND STUDY. J.W. Coburn, N.C. DiDomenico,* G.F. Bryce,* L.W. Bassett,* S.A. Shupien,* E. Wong, R.B. Miller, C.M. Bennett, R.H. Gold,* J.P. Mallon,* O.N. Miller * & P.C. Chang.* Depts Med, VA Wadsworth Med Ctr, UCLA Sch Med, & Participating Hospitals, Los Angeles, CA, & Roche Research Center, Nutley, NJ.

To examine whether calcitriol (1,25) could prevent bone disease, we gave either 1,25 (A) or placebo (P) to 98 asymptomatic dialysis patients with normal bone X-rays and good biochemical control (x serum (S) P, 4.7 & Ca, 9.7mg/dl). All received Al gels & $CaCO_3$ (1g Ca/day); treatment (T) was increased, 1→4 caps/day (1cap=.25μg/d) and adjusted if hypercalcemia (↑SCa) or S(Ca X P)>70 developed. Followup over 30 mos of T included S-Ca, P, (2X/mo), alk p'tase & iPTH (1X/mo), bone X-rays (q 6mo) and bone mineral content (BMC) (q 4mo). With T SCa rose from 9.4±.6(SD) →10.2±.5mg/dl in A and 9.5±.6→9.7±.6mg/dl in P. In A and P, SiPTH responses were variable: SiPTH fell in 60 & 27% of A & P, respectively, group I; was unchanged in 30 and 34%, II; or rose in 11 and 39%, III; 1,25-T changed the distribution in groups, $p<.01$. Both pre-T SiPTH and SiPTH changes during T were predictive of new resorptive lesions on X-ray (OF) and a fall in BMC; thus, 20% of those with pre-T SiPTH >130μlEq/ml developed OF, compared to none of 36% with SiPTH≤ 130. In groups I, II, & III, respectively 0, 15 and 35% developed OF and BMC fell in 7, 22 and 65%. A side effect of 1,25 was asymptomatic ↑SCa without worsened soft-tissue calcifications. Thus, 1,25 can prevent 2° hyperparathyroidism and lessen bone mineral loss, particularly in "higher risk" patients with SiPTH levels >130-μlEq/ml, the upper 2/3 of this dialysis population.

TRACE ELEMENTS IN PATIENTS ON CAPD. Bo G. Danielson, N. Grefberg, P. Nilsson, L. Weiss.

Renal Division, Dept of Internal Medicine, University Hospital, Uppsala, Sweden.

Uremic patients on dialysis may be at risk of accumulation or depletion of various trace element due to transport across the peritoneal or hemodialysis membrane.

We have therefore studied trace element concentrations in blood of CAPD patients and compared these concentrations to those of normal controls. Since January 1979 57 patients have been trained on CAPD. Thirty of these patients, who have been on CAPD in average 12 months and at least three months, have been studied on one or more occasions for blood concentrations of trace elements. The results have been compared to those of 50 normal people of different age groups. The element studied have been aluminium, copper, tin, zinc, nickel, selenium, chromium, manganese, mercury, arsenic and cadmium.

The CAPD patients showed significantly higher blood levels than for the controls for selenium, cadmium and aluminium. Significantly lower concentrations of manganese, tin, zinc and nickel were obtained, while no significant differences for copper, chromium, mercury or arsenic between CAPD patients and normals could be found.

The results indicate that CAPD patients may have disturbed metabolism of trace elements, where both accumulation and depletion may occur. Since the CAPD patient is exposed to a large volume of dialysis fluid the risk of accumulation or depletion of trace elements has to be recognized.

ABDOMINAL HERNIAS IN PATIENTS UNDERGOING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). G.E. Digenis, R. Khanna, A. Pierratos, R. Mathews, S. Clayton, and D.G. Oreopoulos. Toronto Western Hospital, Toronto, Ontario. Canada.

The development of hernias through a weak area of the abdominal wall is a serious complication in patients undergoing continuous ambulatory peritoneal dialysis (CAPD).

Among 192 patients (101 men 50±17 years old and 91 women 50±14 years old) treated with CAPD (4 x 2 litre exchanges per day) through a peritoneal catheter implanted by an infraumbilical midline incision, 22 patients (6 men and 16 women) developed 31 hernias (10 in men and 21 in women). The mean age of those who developed hernias was significantly higher than the whole group. (68±8 years for men and 55±7 years for women). Diabetics and patients with polycystic kidneys did not have a higher incidence of hernias than other renal disease groups. Almost half of the cases (15/31) were incisional hernias at the site of the catheter insertion. Most of the hernias developed during the first two years since the initiation of CAPD. Twenty-five of the cases presented as a painless swelling while in six there was some intestinal obstruction. All but six of the hernias were surgically repaired and all but two of these patients returned to CAPD after a temporary period of intermittent peritoneal dialysis or hemodialysis. The patients who preferred to delay the repair, continued on CAPD by using a special supportive corset. In conclusion, abdominal hernias are a frequent complication of CAPD (11.5% of the patients) occuring mainly in women and older men. After surgical repair the patients can continue on CAPD.

MYOCARDIAL PERFORMANCE (MP) AND CHANGES IN STARLING FORCES ACROSS PULMONARY CAPILLARY VESSELS DURING HEMODIALYSIS (HD). O. Llan de Rosos*, M. Rodriguez*, J. Pederson, L. Rankin, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

Abnormal MP, high pulmonary wedge pressure (PCW) and low colloid osmotic pressure (COP) are factors leading to cardiovascular complications in HD patients. These factors were evaluated in 5 pts during 3 hrs of HD. Hemodynamic parameters were determined by invasive techniques. Myocardial performance was assessed by determining the Sarnoff Curve which establishes the relation of Left Ventricular Stroke Work Index (LVSWI) and PCW. The Starling Forces across the pulmonary capillary wall were assessed by the difference between COP and PCW (COP-PCW). The mean ± SE value for these data is shown:

Minutes(min)	0	60	120	180
PCW	19.0±2	9.8±1**	5.6±.7**	4.2±1**
LVSWI	99.0±14	72.0±11**	51.0±10**	42.0±3**
LVSWI/PCW	5.2±.6	7.6±.7*	9.4±2*	10.0±2*
COP-PCW	1.4±.5	14.0±2**	20.0±.6**	24.0±2**

p<.05*; p<.01**

In summary, during HD: a) though there is a decrease in LVSWI and PCW, the expected value of LVSWI for the PCW, observed at 0 and 180 min is greater than in normals (82±12 and 12±9); b) there is a progressive increase in COP-PCW which is most pronounced in the first hour. In conclusion, during HD: 1) there is marked increase in myocardial function and the Sarnoff Curve is established at a higher set point than normal, and 2) changes in the Starling Forces occur, favoring movement of fluid into the pulmonary vessels.

HEPATITIS B IN THE DIALYSIS CENTER: PREVALENCE, SURVEILLANCE AND PROPHYLAXIS IN 1982. Mark A. Dillingham* and Robert J. Anderson. Univ. of Colo. Hlth. Sci. Ctr., Denver, Colorado.

Although hepatitis B (HB) is a recognized problem in the chronic dialysis center, there is little current information regarding prevalence, surveillance and prophylaxis. We first determined that the majority (67%) of dialysis centers in ESRD Network 5 are free of HBS_{Ag}(+) patients and the point prevalence rate of HBS_{Ag} in this population of 1,007 patients is 2.6%. Next, we obtained information from 90 dialysis centers selected randomly from each ESRD Network. These centers provide care for 8,104 patients. Most centers (57%) are free of HBS_{Ag}(+) patients and the point prevalence of patients (+) for HBS_{Ag} in this population is 2.3%. Surveillance testing (tests/year) with Communicable Disease Center-CDC-recommendations revealed:

	HBS_{Ag}		HBAB		Other HB		LFT	
	Pts.	Staff	Pts.	Staff	Pts.	Staff	Pts.	Staff
Centers	11	8	4	3	3	1	31	10
CDC	12	4-6	4	4-6	0	0	24	0

Regarding prophylaxis, HBS_{Ag}(+) patients are sequestered by machine, room and dialysis center staff in 88, 82 and 32% of centers, respectively. HBS_{Ag} vaccine is either in use or planned for use in 15 of the 90 centers (16.7%). The frequency of vaccine use is slightly higher in HBS_{Ag}(+) (26%) than (-) units (10%). Ten of 26 (38%) of ESRD Networks have established guidelines for surveillance and prophylaxis of HB. We conclude that the point prevalence of HB is less than reported previously. Although current practices of surveillance appear adequate, improvements in selected aspects of prophylaxis may further decrease HB as a problem in the chronic dialysis center.

HYPERCALCEMIA (HCA) IN CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD) PATIENTS (PTS). M. Dratwa+, F. Collart+, Ch. Tielemans+ (Intr. by C.C. Tisher). Hôpital Universitaire Brugmann, Brussels, Belgium.

Due to a negative peritoneal balance and a poor dietary intake, oral calcium (Ca) supplements and vitamin D (Vit. D) therapy have been advocated in CAPD pts but, as shown in the present study, surprisingly often lead to HCa (serum Ca≥10.5 mg/dl). After 8.6±1.6 months of CAPD without Ca supplementation (time 0), oral Ca carbonate (3 gm/day) was prescribed to 16 pts using standard Dianeal (Ca : 7 mg/dl); Vit. D (calcifediol 25 µg/day) was added to this regimen 7.6±0.8 months later. Over a 6 to 20 months follow-up period, HCa occured in 8/16 pts 5.1±0.9 months after beginning Vit. D. HCa (12.1±0.4 mg/dl) was accompanied by a significant decrease in serum alkaline phosphatases from 251±21 before starting Vit. D to 202±21 I.U./l ($p<0.05$) and quickly subsided after stopping Ca and Vit. D. Comparing HCa pts (group I) to the pts remaining free of this complication (group II) revealed that, at time 0, serum phosphorus, albumin, alkaline phosphatases, elevated i PTH and low Vit. D metabolites levels were not different but that Fogelman score (N: 0 to 4) of 99 m Tc-pyrophosphate bone scintigrams was significantly lower in group I (4.6±0.9 versus 8.4±1.1; $p<0.02$). Furthermore, following 6 months of Ca supplementation, the only observed change was a rise in serum Ca from 8.9±0.2 to 9.8±0.3 mg/dl ($p<0.05$) in group I which was not the case in group II (8.8±0.2 and 8.9±0.2 mg/dl; N.S.). These findings suggest that Vit. D should not be given together with oral Ca supplements to those CAPD pts showing a rise in serum Ca with the latter therapy and a low Fogelman score possibly indicating a poor osteoblastic activity.

REPRODUCTIVE FUNCTION IN CHRONIC RENAL FAILURE IN ADOLESCENT FEMALES. Leonard G. Feld, Ruth Freeman Marjorie Werner*, Benjamin Thysen*, Ira Greifer, and Robert Weiss*. Albert Einstein College of Med. Depts. of Pediatrics and Obs/Gyn.,Bronx, New York.

Patients who have chronic renal failure are known to have abnormalities of their reproductive function. We have studied the frequency of these disorders and changes in this system in 8 girls 14 to 19 years of age, who have been maintained on hemodialysis from 1 to 9 yrs. FSH, LH, prolactin, Estradiol (E2) and progesterone were measured every 2 weeks for a minimum of 4 months. Quantitation of these hormones was based on radioimmunoassay procedures. In addition exogenous LRH(50µg) was administered with blood samples drawn for FSH and LH over a two hour period. Over the 4 month period only 3 girls demonstrated post-ovulatory rise in progesterone. LH was elevated (32.6±15.2sd mIU/ml) compared to controls (11.6±9.3sd mIU/ml), FSH and prolactin levels were not significantly different from controls. Plasma E2 levels ranged from less than 10 to 297 pg/ml. All girls had multiple follicular levels below 50pg/ml. After LRH injection, LH levels rose from 130-530% of baseline values with elevations sustained above baseline after 120 minutes. The normal gonadotropin response to LRH suggests normal pituitary sensitivity. The LH and normal FSH corresponding to low E2 levels implies a defective pituitary-ovarian feedback loop or a decreased ovarian steroidogenesis response to normal gonadotropins. The elevated LH may be due to inactive hormone that would normally be excreted by the kidney.

REGIONAL CITRATE ANTICOAGULATION FOR HEMODIALYSIS OF PATIENTS WITH ACTIVE BLEEDING. K. Duncan*,R. Seaton*, R. Pinnick*, T. Wiegmann*, D. Diederich* (Intr. by J. Grantham). Univ. Kansas Med. Ctr., Kansas City, KS; V.A. Hospital, Kansas City, Mo.

Hemodialysis (HD) of patients at high risk for bleeding is complicated by hemorrhage in about 25% of patients. We reported on the use of sodium citrate (C) as a regional anticoagulant for HD previously (Kidney Int. 21:175, 1982). Coagulation within the assembly was prevented by an infusion of C; C was removed by the dialyzer to reverse the anticoagulation (A). We now report our experience with 78 HD using C anticoagulation in 16 patients in whom systemic anticoagulation was contraindicated. Seven patients had bled persistently following major operations or trauma; 9 patients were at high risk for bleeding because of multiple problems. Mean laboratory values ± 1 SD obtained during the initial 1-3 HD in each subject are listed.

	N	Initial	Postdialysis
APTT (Sec)	37	33.7±6.6	31.9±4.5
Platelets % change	30		-0.6±21
Serum creat. (mg/dl)	35	8.3±1.9	4.9±1.3
Serum Na (mM)	36	141±8.	144±5.
Serum Ca (mg/dl)	32	8.0±0.9	9.3±1.
Serum HCO_3 (mM)	36	22±5.	27±4.

Activated PTT and clotting time invariably decreased during HD with C, confirming the absence of systemic anticoagulation during HD with C. No clotting or systemic side effects occurred. In conclusion, a larger experience demonstrates the advantages of regional citrate anticoagulation for HD of patients at high risk for bleeding.

PERITONITIS (P) IN CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (C.A.P.D.) PATIENTS (PTS.) S. Fenton, S. Flax*, D. Cattran, W. McCready, C. Gutman* & W.T.W. Clarke. Toronto, General Hospital² Toronto, Canada.

Between March 1978 and December 1981,227 episodes of P occurred in 150 pts. on C.A.P.D. The pts. mean age was 46, range 18-76. Patients exposure was over 2,400 mths. giving an overall incidence of P of 1/10.6 pts. mths. Despite "improvements" in technique such as the Travenol connector the incidence of P in new pts. didn't changeover the years i.e. 1978-61%, 1979-53%, 1980-54%, 1981-65%. However the incidence of P/yr. increased after the first P. episode, i.e. probability of first P-58%/yr. versus second P-67%/yr. $p=0.05$. The patients were assessed as compliant or non-compliant by both physicians and nursing staff. This was the only significant factor seen in the recurrent P group whose first P incidence rate was 1/7 pt. mths. Those patients with ≥3 P's was even greater with an incidence of P every 4.7 mths. Death from P was seen in 5/227 episodes (2.2%). There was no particular initiating organism and the pts. were not in the non-compliant group. Three of 5 had multiple abdominal abscess at autopsy despite catheter removal and surgical drainage.

These results indicate that new and innovative approaches to P prevention must occur before P rate will fall. Also since P rate increases after the first episode, alternate treatment modalities should be considered in this group after their first infection.

EARLY DETECTION OF PERITONITIS IN PATIENTS ON CHRONIC INTERMITTENT PERITONEAL DIALYSIS. Jorge A. Fragola*, Harold Bregman, Dept. of Medicine, Allegheny General Hospital, Pittsburgh, PA.

Peritonitis is a major source of morbidity in peritoneal dialysis. One of the earliest responses to peritoneal infection is an increase in dialysate white cells which will precede fever or abdominal pain. Routine dialysate cell counts are impractical as a screening method in the peritoneal dialysis population. We have shown (Abstr. ASN, 1981) the specificity and sensitivity of a leukocyte esterase-sensitive test strip in detecting peritoneal fluid leukocytosis and now extend these studies to the early recognition of peritonitis.

Eight hundred-sixty peritoneal dialysate specimens were sampled prospectively over 3 months from 42 patients receiving C.I.P.D. Test strip positive specimens (blue color in 10 minutes or less) were cultured by a blood culture bottle technique and a cell count was obtained. Eleven test strip positive specimens from 11 patients yielded 8 positive cultures and 3 had no growth; all 11 specimens had white cell counts greater than 60/mm^3 (range 60-2940/mm^3). Definite cloudiness was noted in only 2 specimens. Three specimens appeared hazy and 6 were judged by experienced observers to be clear. No other patients in the study developed peritonitis making the test strip highly sensitive (1.0) and specific (.99) in the detection of subclinical peritonitis. All patients were treated as outpatients with appropriate antibiotics avoiding the need for hospitalization.

We conclude that the leukocyte esterase-sensitive test strip is useful in screening patients for peritoneal fluid leukocytosis and in the early diagnosis of peritonitis during C.I.P.D.

ACUTE RENAL FAILURE(ARF) COMPLICATING AORTIC ANEURYSM(AA) SURGERY. Charles C. Gornick, Carl M. Kjellstrand. Dept. of Med., Hennepin Co. Medical Center, Minneapolis, MN.

We studied 47 patients who developed ARF after AA surgery, 1968-1980. Ten of 45 pre-, intra-, and post-operative factors studied were related to survival, ultimate renal function, or need for chronic hemodialysis(CHD).

Only 10/47 patients survived. 69% of deaths were due to infections, 52% in the abdomen. Major intraoperative complications, WBC> 15,000/mm^3 and fever(> 100°F) after three weeks, pulmonary infiltrates and the need for respirator were associated with death. Previous medical history, mean age, type of operation, and later cardiac or GI complications were not associated with death. No patient over age 70 years, or with coma, or not alert at 3 weeks, or on respirator > 2 weeks, or with positive blood culture, survived. Postoperative blood chemistries were not different between survivors and those who died. Patients treated before 1975 survived as often as patients treated later. Ten patients survived to discharge, three on CHD. All CHD patients had preoperative hypertension($P < 0.05$). No one recovered renal function if> five weeks of dialysis. Of ten survivors to discharge, six died in 6 mo. - 6 yrs., four of CV deaths, two of cancer. Four patients survive 2-10 yrs., one on CHD(>2 yrs.).

ARF after AA has a 79% death rate, a 6% CHD rate and a 15% renal recovery rate. We feel that dialysis can be discontinued if the patient remains on respirator, is comatose, or has a pos. blood culture after two weeks or probably after three weeks, if the patient is>70 yrs., has a white count over 15,000 and a temp. over 100°F.

AMELIORATION OF HEMODIALYSIS (HD) INDUCED FALL IN PaO_2 WITH EXERCISE (EX). M.J.Germain, G.L.Braden, E.J. Burke*, J.P.Fitzgibbons, Baystate Med. Ctr., Renal Section, Springfield, MA.

It is well documented that PaO_2 falls within 15 min. after initiation of HD with an acetate bath. The phenomena has been explained by either: 1) an increase in alveolar arterial gradient ($AaDO_2$) secondary to pulmonary sequestration of leukocytes; 2) decreased alveolar ventilation ($\dot{V}_A$) secondary to CO_2 dialysance or acetate metabolism; resulting in a decreased carbon dioxide production ($\dot{V}CO_2$). To test the role of the latter mechanism in producing a fall in PaO_2, we reasoned that a stimulus to increase $\dot{V}_A$ may reverse the fall in PaO_2. Thus, EX studies utilizing a bicycle ergotomer during HD were performed in 6 patients. ABG's and pulmonary gas volumes ($\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$) were measured. $\dot{V}_A$ and $AaDO_2$ were calculated.

*P <.05 $\bar{x}$ ± SEM	Pre-HD Rest	ON HD Rest	ON HD EX
PaO_2 (mm/Hg)	102 ± 4	92 ± 4*	102 ± 3*
$PaCO_2$ (mm/Hg)	36 ± 2	37 ± 1	36 ± 1
$\dot{V}_A$ (ml/min)	5.2 ± 1.0	4.2 ± 0.2	10.5 ± 1.4*
$\dot{V}CO_2$ (ml/min)	200 ± 30	170 ± 10	440 ± 60*
$\dot{V}O_2$ (ml/min)	220 ± 40	250 ± 10	520 ± 60*
$AaDO_2$ (mm/Hg)	8 ± 7	5 ± 5	2 ± 2

The linear correlation between $\dot{V}_A$ and $\dot{V}CO_2$ was .997 ($P < .001$). We conclude: 1) EX during HD reverses the fall in PaO_2 induced by HD; 2) the changes in PaO_2 are paralleled by changes in $\dot{V}_A$ and $\dot{V}CO_2$; 3) the changes in PaO_2 are not predicted by changes in the $AaDO_2$; 4) the close correlation between $\dot{V}_A$ and $\dot{V}CO_2$ in this study suggests that $\dot{V}CO_2$ may be a controlling factor for ventilation under these conditions.

COMPARISON OF INTRAVENOUS (IV) AND INTRAPERITONEAL (IP) ADMINISTRATION OF CEFTIZOXIME IN PATIENTS UNDERGOING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). M.L. GROSS, J.V. Nally, J.T. Higgins, E.H. Freimer* and P. Somani*. Medical College of Ohio, Department of Medicine, Toledo, Ohio.

This study was carried out to compare the pharmacokinetics, especially the rate and extent of absorption and elimination, of ceftizoxime (C) after IV or IP administration in patients undergoing CAPD. C is a new beta-lactamase stable cephalosporin. All patients were clinically stable on CAPD without active peritonitis. Each subject was given 500 mg first by IV bolus and then 1 month later by IP instillation. Serum and dialysate samples were collected simultaneously at 0, 0.25,0.5,1,1.5,2,3,4, and 6 hrs during 1 exchange with 2.0 liters of 1.5% Dianeal. In 8 patients, following IV injection of C, peritoneal fluid drug levels increased rapidly, starting at 15 min, and an equilibrium was reached in 2-4 hrs. However, only 15 ± 5% of the injected dose was eliminated in 6 hrs through peritoneal clearance. The serum elimination $t_{1/2}$ ranged from 5.1 to 20.8 hrs, which is much longer than 1.4 hrs reported for normal subjects. Following IP instillation, the serum drug levels increased rapidly in the first 2 hrs and 86 ± 5% of C was absorbed through the peritoneal membrane into the serum during the 6 hr dwell time.

It is concluded that the peritoneal membrane permits a rapid exchange of C from peritoneal to blood compartment and vice versa. These data suggest that IP instillation of C provides adequate serum concentrations and may provide optimal therapy for peritonitis or systemic infections in CAPD patients.

PROTEIN BALANCE MORE DEPENDENT ON PROTEIN INTAKE THAN ON ENERGY INTAKE IN HEMODIALYSED CHILDREN. W.E. Grupe, N.S. Spinozzi* and W.E. Harmon, Dept. of Pediatrics, Harvard Medical School, Boston, MA.

Previous data have projected that neutral nitrogen (N) balance in children on hemodialysis occurs at oral energy intakes of 10kcal/cm of height and protein intake of 0.3Gm/cm. Since advancing protein intake (Prot-I) could increase urea generation, be counterproductive to N-balance and promote inefficient N-utilization, the influence of Prot-I on protein balance (Pro-Bal) and protein catabolic rate (PCR) was measured over a broad range of energy and protein intakes during 43 balance periods in 15 diet adapted, chronically hemodialysed children, ages 0.8-18 years. There was a linear relationship between Pro-Bal and Prot-I ($R=0.66$; $p<0.005$) at both submaintenance and excessive intakes. Likewise, a linear relationship between PCR and Prot-I was present in both positive ($R=0.66$; $p<0.005$) and negative ($R=0.72$; $p<0.005$) Pro-Bal with the PCR, and hence urea appearance, uniformly lower for any given Prot-I during positive balance. Although there was a strong correlation between energy and protein intakes ($R=0.76$; $p<0.005$), multiple regression analysis indicated that Prot-I was more predictive ($p<0.01$) of Pro-Bal than energy intake ($p>0.20$).

Thus, under the conditions of this study, advancing Prot-I was correlated with improved Pro-Bal and increased protein utilization without an increase in either urea generation or dialysis requirements. These data suggest that restriction of Prot-I in hemodialysed children can be counterproductive and imply that relative energy excess might contribute to inefficient N-utilization.

FEASIBILITY OF A STERILE SPLICE FOR CONNECTION IN CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). Robert W. Hamilton, Patricia L. Adams, Betty Disher, Genevieve Dillingham and Cindy Crater. Bowman Gray School of Medicine of Wake Forest Uni. Dept. of Med., Winston-Salem, N.C.

Peritonitis due to bacterial contamination during the spiking step of the CAPD bag exchange procedure limits the acceptance of CAPD. DuPont has developed a sterile connection device (SCD), which has the potential to reduce the peritonitis rate. This SCD automatically makes a sterile connection between an air-filled extension tube of the dialysate bag and the solution transfer set in which an air bubble has been trapped, thus eliminating the need to spike. After DuPont demonstrated the sterility of the connection in vitro, we studied two prototypes (P_1 & P_2) of the SCD to determine the feasibility of its use in CAPD. We asked 2 questions: 1)Is trapping an air bubble in tubing feasible in the exchange procedure? 2)Are there toxic reactions to the splicing procedure? Nine patients learned to perform CAPD bag exchange using the SCD. A total of 241 exchanges were performed with 2 prototypes. Failure of splicing occurred in 5/80 exchanges with the P_1, and in 1/161 exchanges with P_2. Failures were primarily a result of improper technique. Each patient learned to use the device within one week and demonstrated the feasibility of the bubble trapping procedure. Patients found the procedure more convenient than the spike technique because it eliminated the need to scrub and mask. No significant time-savings was encountered. Transient changes in SGOT were noted in 3 of the patients; however, these appear to be related to intercurrent illness rather than device usage. We conclude the SCD technique is feasible for use in CAPD.

NON-ENZYMATICALLY GLUCOSYLATED SERUM PROTEINS IN PATIENTS WITH END STAGE RENAL DISEASE (ESRD). R.J.Haley*, D.M.Ward* (Intro. by J.H.Licht), University of California, San Diego, CA

Non-enzymatic glucosylation of extracellular proteins is potentially harmful (implicated as an etiologic factor in diabetic lesions). We measured the degree of glucosylation of whole serum proteins (GP) in patients with ESRD, using a modification of the Flückiger-Winterhalter method. This assay is unaffected by carbamylated protein, which contributes to the increase in fast-migrating hemoglobin (HbA1) seen in ESRD. Patient groups were: CONT= normal controls, N-HD= non-diabetic hemodialysis (HD) patients using a dialysate glucose (DG) = 0 mg%, non-diabetic patients on continuous ambulatory peritoneal dialysis (CAPD), and diabetics on HD using DG=200 mg%. The levels of GP are recorded as nmole of glucose/mg protein (mean ± SEM). Serum glucose (Glu, mg/dL) and HbA1 (% of total Hb) are also shown:

	n	GP	p vs 1	Glu	HbA1
1) CONT	14	1.61 .05			7.9
2) N-HD	20	1.91 .04	<.001	100	9.9
3) CAPD	13	1.96 .08	<.01	109	11.1
4) D-HD	5	2.04 .13	<.01	198	13.1

We also studied these parameters serially in two cohorts of non-diabetic patients changed from HD (DG=0 mg%) to HD (DG=100 mg%) (n=20) or to CAPD (n=6). There was no significant rise in GP levels following either change (although one CAPD patient became diabetic).

We conclude that there is a major increase in non-enzymatic glucosylation of serum proteins in non-diabetic patients with ESRD, and this is not affected by the mode of dialysis. The glucose load of CAPD therapy does not appear to cause any further increase of GP in non-diabetic subjects when compared to HD.

ANTIBIOTIC PROPHYLAXIS FOR STAPH. AUREUS COLONIZATION/INFECTION IN HEMODIALYSIS PATIENTS: A 3 YEAR PROSPECTIVE STUDY. J.E. Hanchett, V.L. Yu, A. Goetz,* M. Wagner,* and P.B. Smith,* VAMC and University of Pittsburgh, Pgh, PA 15240

80 hemodialysis patients were monitored for staph colonization and infection in an ongoing prospective study initiated in 1979. Nares cultures are taken every 3 months. 66% showed nasal carriage 1-12 times in 3 years. Of the 11 patients phage typed and followed for 1-2 years the type remained identical for 10. Staph carriage did not correlate with age, blood chemistries, years on hemodialysis or diabetes. Carriers were randomized to short term prophylactic regimens. Vancomycin (1-2 doses of 1 gm IV in 2 weeks) was ineffective. Topical bacitracin for 7 days eradicated nares carriage only short term; 73% re-colonized 1 week post prophylaxis. Rifampin 600 mgm bid for 5 days po eradicated nares carriage in 100% patients; 22 and 55% re-colonized (usually with the same phage type) at 1 and 3 months post prophylaxis respectively. Rifampin-resistant staph has not been seen even in patients who received rifampin on multiple occasions. 27 staph infections, including 6 bacteremias, occurred in 18 patients over 3 years. All 6 isolates from infected sites that were phage typed were identical to those carried in the nares. Three bacteremic patients were not staph carriers. Although at this point in the study, efficacy of intermittent (every 3 months) rifampin in prevention of staph infections is not statistically demonstrable, it may be useful in interrupting staph outbreaks involving hemodialysis patients.

CARDIAC BETA-RECEPTOR RESPONSE IS SELECTIVELY REDUCED IN ACUTE UREMIA. Michael Hausen*, Johannes F.E. Mann*, and Eberhard Ritz* (intr. by E. Friedman), Dept. of Medicine, University of Heidelberg, W-Germany.

Beta receptor mediated changes of heart rate in acute uremia and the specificity of the changes were investigated. Rats were bilaterally nephrectomized (NX) or sham-operated (SO) (n=6-9 per group) 36 hrs prior to experiments. For continuous recording of heart rate (HR) and blood pressure (BP) and i.v. injections, catheters were implanted in the femoral vessels. The renin-angiotensin- and the autonomous nervous systems were blocked with MK 421, pentolinium and atropine. (I) HR-responses to increasing doses of isoproterenol were blunted in NX-rats (maximum ΔHR: 132±12 vs 194±9 beats/min, $p < .01$. Baseline HR was reduced in NX rats (332±12 vs 375±12 beats/min, $p < .05$). The BP decreasing effect was not different between groups (32 vs 30 mm Hg). (II) In normokalemic (low dietary K^+ intake), and (III) in normokalemic normoacidemic ($NaHCO_3$ i.v.) NX rats (serum-K^+: 4.5±.3 mM, pH 7.41±.02), the maximal HR response to isoproterenol was also reduced ($p < .01$) with no change in the BP response. (IV) Increasing doses of dibutyryl-cAMP and glucagon iv yielded no differences in ΔHR (32±2 vs 44±9, and 68±14 vs 73±13 beats/min respectively) or BP between NX and SO. (V) In radioligand binding studies, ^{3}H-dihydroalprenolol binding was reduced in plasma membrane of NX rat hearts.

Our results suggest that the chronotropic response to isoproterenol is reduced in acute uremia. This phenomenon appears to be specific for the cardiac beta receptor and may be mediated - at least in part - by changes at the receptor level.

BICARBONATE (Bi) VS. ACETATE (Ac) HEMODIALYSIS (HD): DOUBLE-BLIND, CROSSOVER STUDY OF SIDE EFFECTS. W. Henrich, T. Woodard, * B. Meyer, * T. Chappell, * L. Rubin, * (Intr. by R. Cronin).

While Bi HD has been shown to improve symptomatic tolerance to HD in short-term studies using low osmolality dialysate, the superiority of Bi HD over Ac HD is not established when a higher osmolality dialysate is used. Thus, we compared Bi to Ac HD over 6 weeks each in 10 stable HD patients using a double-blind crossover design and a dialysate sodium concentration of 140 MEQ/L. The dialyzer, delivery system, and dialysate constituents were identical except for the substitution of Bi or Ac; blood work was obtained pre and post maneuver at the beginning of the week. Symptoms (Sx) were defined as nausea, vomiting, diaphoresis, chest pain, and systolic blood pressure (BP) <90 mmHg; therapeutic interventions (Rx) were either saline or mannitol. Results:

	Bi	Ac	p value
Pre HD WT (Kg)	70.6	70	NS
Interdialytic WT Gain (Kg)	2.06	2.15	NS
Upright mean pre BP (mmHg)	99.4	99.5	NS
Upright mean post BP (mmHg)	90.6	90.0	NS
Pre HD pH (units)	7.39	7.35	<.01
Pre HD Bi	19.1	15.1	<.001
Sx (per pt/6 wks)	2.0	2.5	NS
Rx Interventions (per pt/6 wks)	1.5	3.1	<.02

The results show that similar weights and BP's were observed with both HD procedures. The frequency of Sx was also comparable, although the Sx associated with Bi HD required Rx less often. Thus, the use of Bi HD is only of marginal benefit in further reducing adverse symptoms on HD if a higher osmolality dialysate is used.

IMPORTANCE OF CHANGES IN IONIZED CALCIUM (CA) AND BICARBONATE (Bi) ON LEFT VENTRICULAR CONTRACTILITY (LVC) DURING HEMODIALYSIS (HD). W.L. Henrich, J.V. Nixon,* U.TX. S.W. Med. Sch. and DVAMC, Dallas, TX

Although we have previously shown that HD induces a marked improvement in LVC (measured as increased velocity of circumferential fiber shortening, VCF) independently of changes in preload, the precise mechanisms responsible for this improvement are unknown. Thus, we systematically investigated the potential importance of changes in CA and Bi on LVC during 3 different isovolemic dialysis maneuvers in 4 stable HD patients. The same dialyzer was used in each 3 hour maneuver; 2D echocardiography was done pre and post each of the maneuvers. Dialysate calcium and bicarbonate were adjusted as follows: #1) CA 2.2 mg/dl, Bi 24 MEQ/L: #2) CA 7.6 mg/dl, Bi 24 MEQ/L, #3) CA 2.2 mg/dl, Bi 32 MEQ/L. Results:

		WT (Kg)	CA (mg/dl)	Bi (MEQ/L)	CR$^+$ (mg/dl)	ESV^{++} (ml)	VCF (circ/sec)
(1)	pre	79.4	4	18.5	20.5	69	.84
	post	79.4	3.7	18.0	13.4*	68	.84
(2)	pre	78.5	4.24	19.3	20	77	.62
	post	78.5	5.7*	20	12.9*	61*	.77*
(3)	pre	78.3	4.36	19.2	19.6	76	.70
	post	78.3	4.2	23.8*	12.7*	69	.73

+ creatinine; ++ end systolic volume; *$p < .05$

Changes in serum sodium, potassium, and magnesium were comparable in each maneuver. The results clearly implicate the increase in CA as an important factor in the improvement in LVC observed during HD. The results further suggest that the increase in Bi and HD itself may be dissociated from this improvement in LVC.

SIGNIFICANCE OF SERUM ALUMINUM IN DIALYSIS PATIENTS. Hood, S.A.,* Hodsman, A.B., Cordy, P.E. and Leung, F.Y.* Division of Nephrology, University of Western Ontario, London, Ontario, Canada.

The biological significance of serum aluminum (sAl) levels in dialysis patients (pts) has not been determined. In 152 unselected pts dialyzed against Al-free dialysate in a regional centre, mean sAl was 74 ± 5 (SE) µg/L; the distribution was skewed by pts with lower sAl (median 56 µg/L), but 16 pts (10.5%) had sAl levels classified as "high" (range 160-340 µg/L). Intra-individual variation in sAl was only 12% (determined in 3 pts over 12 wks). There were no significant correlations between sAl and, duration of dialysis therapy, prescribed dose of $Al(OH)_3$ gels, or seCa and alk. phos. Preliminary attempts were made to assess body Al burdens by histochemical staining of bone biopsies, presence of dementia, and the increase (Δ) in sAl two hrs after a test dose (6 gm IV) of desferrioxamine (DFO) [see table].

sAl	+ve bone biopsies	Dementia no. pts.	Δ sAl after DFO µg/L [n]
"High"	3/5	1/ 16	139 ± 64 (SE) [6]
"Average"	1/6	1/136	121 ± 75 [6]

Thus sAl levels alone do not detect the presence or absence of significant tissue Al accumulation. However, DFO infusion given to 6 pts on CAPD increased removal of chelated Al from an average of 325 µg/d by 300%; daily Al removal was significantly related to ambient sAl ($r = 0.91$) both before and after DFO therapy. This confirms that DFO mobilizes tissue Al stores, resulting in substantially increased Al clearance because of increased sAl levels.

BLOOD EOSINOPHILIA IN MAINTENANCE PERITONEAL DIALYSIS PATIENTS. H.M. Humayun*, T.S. Ing, S. Nawab*, J.T. Daugirdas, V.C. Gandhi. Veterans Administration Hospital, Hines, Illinois, USA.

The incidence of blood eosinophilia has been reported to be increased in hemodialysis patients. To define the incidence of an elevated blood eosinophil count (>500/cu.mm.) in maintenance peritoneal dialysis (MPD) patients, we reviewed routine blood differential counts performed during 555 months in 37 patients. For each patient, 17.8 ± 10.5 (SD) blood counts were available, over 15 ± 13 months of MPD.

In 18 patients, blood eosinophil (BE) counts were always less than 500/cu.mm. In the remaining 19 patients, BE counts exceeded 500 in 2 to 25% of the samples analyzed (mean: 11% ± 5.4). No patient had a persistently elevated BE count. On those occasions in which BE count was elevated, the elevation was mild (% count: 8.4% ± 3.2; total count: 751 ± 209 eosinophils/cu.mm.). Total white blood cell count (WBC) tended to be higher (9,700 ± 3,420) on those occasions when BE counts were increased ($p < 0.05$), compared to WBC counts in the same patients when BE counts were normal (8,316 ± 1,924).

Peritoneal fluid total non-erythrocyte counts and peritoneal fluid eosinophil counts were often elevated at the same time that eosinophilia was found in the blood.

The results suggest that sporadic elevations of the BE count occur in patients receiving maintenance peritoneal dialysis. Although MPD patients with peritoneal fluid eosinophilia do not commonly manifest elevated BE counts, peritoneal fluid eosinophilia is often present when high BE counts are found.

PERITONEAL DIALYSIS USING BICARBONATE-CONTAINING DIALYSATE PRODUCED BY AN ON-LINE METHOD. T.S. Ing, V.C. Gandhi, J.T. Daugirdas, J. Hunt*, R.W. Reid*, F.K. Merkel*, S. Gibson*. Hines VA Medical Center, Hines, Illinois, USA.

Sterile peritoneal dialysis (PD) solutions containing bicarbonate, calcium, magnesium, and glucose are difficult to prepare and store, yet may be useful in the management of certain acidotic states. We devised a method of preparing "on-line" bicarbonate containing PD solutions: With the aid of a roller pump, a sterile "acid solution" and a sterile "base solution" were mixed in equal proportion just prior to instillation into the peritoneal cavity. The "acid solution" contained glucose, hydrochloric acid, and chlorides of sodium, potassium, calcium, and magnesium. The "base solution" consisted of sodium chloride and sodium bicarbonate. Final dialysate had the following composition: sodium 133 mEq/L, calcium 3.7 mEq/L, magnesium 1.3 mEq/L, bicarbonate 41.8 mEq/L. Calcium and magnesium remain in solution when dialysate is prepared in this fashion.

Nine maintenance PD patients underwent a single 10-hour PD treatment using bicarbonate-buffered dialysate prepared in the above manner. Pre- and post-dialysis blood samples revealed:

	pH units	HCO3 mEq/L	Ca mEq/L	Mg mEq/L	UN mg/dL	Creat mg/dL
Pre	7.34	19.0	4.1	2.4	81	12.6
	±0.05	± 2.5	±0.4	±0.4	± 3	± 4.0
Post	7.43	24.6	4.7	2.3	52	9.5
	±0.04	± 0.9	±0.2	±0.6	± 4	± 4.1
p <	0.01	0.001	0.02	0.05	0.001	0.001

The results suggest that azotemia and uremic acidosis can both be adequately corrected by this method of bicarbonate-buffered peritoneal dialysis.

A CHANGE TO CHRONIC AMBULATORY PERITONEAL DIALYSIS AND STORT-TERM SURVIVAL — A MATCHED COHORT STUDY. T.A. Hutchinson,* C.H. Cole and M. Kaye. Royal Victoria and Montreal General Hospitals, Montreal, Canada.

Since starting a Chronic Ambulatory Peritoneal Dialysis (CAPD) program involves a considerable learning period for the staff involved, we wished to determine whether the initial patients treated are placed at increased risk. We therefore compared 1-year survival in the first 38 patients who began CAPD at 2 hospitals, with a matched group of prognostically similar patients who began treatment at the same institutions before CAPD became available.

Thirty-five of the 38 CAPD patients were successfully matched with other dialysis patients for age, diabetes, and heart failure - 3 variables that we had previously shown to be of major prognostic importance in end-stage renal disease (Ann. Intern. Med. 96: 417-423, 1982). The 35 matched CAPD patients were similar to their 35 controls in mean age (CAPD 60.2 years vs. non-CAPD 59.4 years) frequency of diabetes (9/35 in both groups) and frequency of heart failure (20/35 in both groups). The 1-year survival was 27/35 in CAPD patients and 28/35 in controls. This similarity in survival between CAPD patients and controls was consistent throughout the study period; when we divided patients into two approximately equal halves based on the order they started CAPD treatment we found that the first 17 patients treated by CAPD (1-year surv. = 14/17 in CAPD vs. 14/17 in controls) had similar results to patients treated later on (1-year surv. = 13/18 in CAPD vs. 14/18 in controls).

Although other studies will be needed to evaluate other risks and benefits of CAPD therapy, our results do not suggest that a change to CAPD has a detrimental effect on short-term patient survival.

CHLORAMINE-INDUCED HEMOLYTIC ANEMIA DESPITE CARBON FILTRATION (CF). M.P. Jacobson,* J.M. Schmidtlein,* J. Otrakji, T.H. Lee, D.E. Wells,* and A.R. Mir. Kansas City Dialysis and Transplant Center, Kansas City, Missouri.

An "epidemic" of hemolytic anemia occurred at a free-standing outpatient hemodialysis center during the period of 12/13/81 to 1/16/82. Twenty-seven of 63 regular dialysis patients (pts) required 70 units of blood during those 5 weeks. Although all dialysis water (DW) was treated by CF and reverse osmosis, a concentration of 0.5 mg/L of chloramines (NH_2Cl) was found in the product water. A retrospective review of the records of 45 pts who continually dialyzed at this unit from 11/1/81 to 3/13/82 showed: 1) significant decrease in Hct in 91% of pts, 2) 7-fold increase in transfusion requirements, 3) abnormally low serum haptoglobin levels in 46% of pts and 4) increase in LDH and bilirubin. All of these abnormalities normalized or improved promptly after removing NH_2Cl totally from the DW supply. We found routine laboratory testing of DW for NH_2Cl to be unsatisfactory. Over 30% of measurable NH_2Cl dissipates from water samples within 48 hours. The diethyl-P-phenylene diamine (DPD) method for detecting NH_2Cl is a simple and accurate test at low-level concentrations of NH_2Cl. We conclude that low-level NH_2Cl contamination may be an unsuspected problem in outpatient hemodialysis centers and that on-site testing for NH_2Cl using the DPD method should be performed routinely.

FUNGAL PERITONITIS: AN INCREASING CONCERN IN CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD).
Richard J. Johnson,* Paul G. Ramsey,* Nancy Gallagher* and Suhail Ahmad. Univ. of Washington, Dept. of Med., Seattle, Washington.

Peritonitis is a major problem in peritoneal dialysis (PD), especially for patients on CAPD. Fungal peritonitis (FP) is less common than bacterial, but more difficult to cure and often require discontinuation of PD. Out of 200 intermittent PD patients from the Northwest Kidney Center over an 11 year period there were 8 cases of FP. In contrast 9 of 50 CAPD patients over a 4 year period developed FP. Typical findings included pain (100%), fever(90%), cloudy fluid (95%) and poor dialysate flow (48%). The most common organisms were Candida, but Torulopsis and Rhodotorula were also seen. Although peritoneal cultures often became negative with treatment only 3 patients could continue on PD, the rest failed due to complications or recurrences of infection. Of 15 patients treated with systemic and/or intraperitoneal (IP) amphotericin (Am) only 1 patient could continue on PD. Five of 15 patients had their catheters replaced and 2 received 5-flucytosine in addition to Am. Recently, 2 patients were treated with oral ketoconazole, IP miconazole and catheter replacement. Both were cured and remain on PD 6 months after treatment. These data suggest: 1. there is an increased risk of fungal peritonitis for CAPD patients; 2. Am with or without 5-flucytosine was associated with complications and had a high failure rate for maintaining PD; 3. miconazole, ketoconazole and catheter replacement may be effective therapy for fungal peritonitis.

MICROCYTIC ANEMIA SECONDARY TO INTRAPERITONEAL (IP) ALUMINUM (Al) IN THE RAT.
L Kaiser*, MA Burnatowska-Hledin*, KA Schwartz*, GH Mayor, Dept. of Med., Michigan State Univ., E. Lansing, MI.

Dialysis patients exposed to high dialysate Al develop a microcytic anemia which is reversed by deionization (DI) of the dialysate. Although partial correction of the anemia after DI implicates Al, it does not prove a causal relationship. Consequently, these experiments were initiated to determine if Al causes anemia and if the anemia is enhanced by parathyroid hormone (PTH) or uremia (U). Six groups of 5 rats were studied: Control (C); PTH; U; Al; Al+PTH; and Al+U. Al treated rats received 1 mg of Al IP daily, a dose known to achieve bone levels comparable to dialysis patients. PTH was given as 5.5 IU subcutaneously daily. U rats were 1 5/6 nephrectomized; all others sham operated. Blood was obtained weekly (0.5 ml) and analyzed by Coulter Counter. No significant difference in HCT or MCV was noted prior to treatment. At 6 wks HCT of C and PTH groups was greater than all other groups ($p \leq 0.01$). HCT of Al, Al+PTH and Al+U was similar. HCT of Al+U was significantly less than U ($p \leq 0.01$). MCV of C and PTH groups was greater than Al and significantly greater than Al+PTH and Al+U ($p \leq 0.01$). No fecal occult blood was detected.

	C	PTH	U	Al	Al-PTH	Al+U
HCT%	44.8	43.5	33.6	30.9	27.9	26.8
MCVμ^3	58.6	57.8	58.7	53.2	50	49.2

We conclude: (1) anemia is a toxic manifestation of Al exposure (2) the anemia is microcytic (3) anemia of Al, Al+PTH, and Al+U rats is not significantly different (4) HCT of U+Al rats is significantly less than U rats indicating that the anemia of uremia can be exacerbated by Al.

AMYLOIDOSIS, A COMPLICATION IN LONG TERM RENAL FAILURE PATIENTS
H.G.Kachel*, P.Altmeyer*, O. Runne*, K.W. Kuehn*, K.M.Koch*, C.A.Baldamus*, Dept.Nephrol.,Dermatol. Univ.Frankfurt/m.,and Medical School Hannover,FRG

Carpal tunnel syndrome (CTS) occurs frequently in maintenance hemodialysis patients (HD). On the other hand CTS is known as a typical complication of amyloidosis. To further study, whether amyloidosis is a late complication in long term renal failure patients, skin and excised carpal tunnel tissue was histologically examined (Congored, birefringency, (EM) for amyloid (A) deposits.

236 chronic HD patients were retrospectively screened for CTS. Occurence of CTS was correlated with time on RDT. In a second study skin was randomly biopsied in 4 patient groups: I: Chronic renal failure on conservative treatment (S_{creat}.5-15mg/dl for > 2y). II:HD for2-3y, III: HD for8-13y, and IV: HD for 8-13 y plus CTS.

CTS was found in 17 patients (8%). Incidence increased with duration of RDT:0/97<5y HD; 4/95 5-9y HD; 13/44 >9 y HD. CTS-site was independent of vascular acces site. Positive A-staining in skin was found in 4/7 in I, in 15/22 in II, in 11/15 in III and in 9/9 in IV. Although Congored and birefringency indicated A the typical B-sheeted fibrils were not found in EM (0/6).

Summary: A-like deposits at predilection sites for amyloidosis are found in high incidence in long term renal failure patients. Frequency increases with duration of renal failure. A-like deposits seems to cause CTS in RDT patients. Site of CTS is independent of vascular access site. Until the A-like material has not been identified as A by immunological and biochemical methods, the described complication in long term RDT patients is only suggestive to result from amyloidosis.

DRUG CLEARANCES OF THE SORBENT SUSPENSION RECIPROCATING DIALYZER (SSRD), A DIALYZER WITH MINIMAL SORBENT SATURATION.
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The extent to which hemodialysis affects the total body clearance of a drug varies with the characteristics of the dialyzer and the distribution of drugs in the body. The clearances of 4 drugs* were demonstrated in non-uremic dogs using the SSRD, a parallel plate dialyzer with a reciprocating blood flow and a sorbent suspension (powdered charcoal and zeolite) which is freely mobile between dialysis membranes. D, GN, and TB were administered intravenously to dogs with 7/8 or 3/4 nephrectomies. PX was administered orally, to dogs with full renal function. 3-5 dialyzer procedures were tested for each drug. Drug clearance of the dialyzer was expressed as % of creatinine clearance. Results are charted below:

	DST	+60	+120	+180	+240	Minutes
GN	93	115	41	93	147	
D	65	41	40	30	56	
TB	151	117	37	21	150	
PX	15	18	80	-30	14	

DST = dialysis starting times
+x = x as the number of minutes after DST

Persistent drug clearance is consistent with the expected mixing and interchange of sorbent granules next to the membrane surface. This delays or avoids surface saturation of the sorbent.

*[gentamicin (GN), tobramycin (TB), propoxyphene (PX), digoxin (D)].

ALBUMIN HOMEOSTASIS DURING CAPD. George Kaysen, Patricia Schoenfeld, Division of Nephrology, San Francisco General Hospital, San Francisco, CA.

Since its introduction, continuous ambulatory peritoneal dialysis (CAPD) has been widely accepted as a modality for the treatment of end-stage renal disease. Loss of protein during this procedure comparable to that which occurs in severe nephrosis has been documented. The homeostatic mechanisms triggered in response to albumin loss in patients undergoing CAPD have not been reported.

Albumin and protein removal rates were studied in 16 patients undergoing (CAPD). In seven patients simultaneous studies of albumin distribution and turnover were performed. Total albumin loss was 5.77±2.85 g/1.76m^2/24 hr; total protein removed was 9.94±4.38 g/1.76m^2/24 hr. Although these values were within the range for severe nephrosis, serum albumin concentration remained nearly normal, 3.64±0.54 g/dl. Plasma albumin mass (PAM) 122±30.4 g/1.76m^2, and total albumin mass, (TAM) 259±56.1 g/1.76m^2, did not differ from those of the control group: PAM 111±11 g/1.76m^2, TAM 280±30 g/1.76m^2. Compared with controls, patients had reduced albumin catabolism, 10.45±1.8 vs 14.2± 1.00 g/1.76m^2/day ($p<0.005$). Within the patient group albumin synthesis increased with increased albumin loss. Serum albumin concentration correlated negatively with albumin losses. Serum Albumin Conc. = 4.46-.144xalbumin loss r=.770p<.001. The CAPD patients maintained albumin homeostasis through decreased albumin catabolism and increased synthesis. All major albumin pools were maintained despite massive albumin loss.

24-HOUR CONTINUOUS BLOOD PRESSURE MONITORING IN HYPERTENSIVE PATIENTS ON HEMODIALYSIS. G Lang, S Sabatini, A Von Riotte*, NA Kurtzman, and DC Batlle, Univ of Illinois, Chicago IL.

Most patients with end-stage renal failure have hypertension (H) that is ameliorated by fluid removal during dialysis. The decision to use antihypertensive agents in such patients is often difficult because the pattern of blood pressure following dialysis is unknown. Our study attempts to characterize this pattern by non-invasive blood pressure recording (Del Mar Avionics Pressurescan) at 15-minute intervals on an outpatient basis. Ten stable patients with pre-dialysis hypertension in whom blood pressure was documented to fall markedly in 3 successive dialysis treatments prior to the 24-hour blood pressure monitoring were studied. The patients did not receive any antihypertensive agents for at least 2 weeks prior to study. Blood pressure readings were analyzed during dialysis and during the 24 hours immediately following dialysis (immediately following dialysis, during sleep, and during the patients' daily routines). Prior to dialysis, mean diastolic blood pressure was 100.6±3.7 mm Hg; during dialysis, it fell to 91.0±2.6 mm Hg ($p<0.05$). In the post-dialysis period, the mean diastolic blood pressure was 87.5±2.7 mm Hg and fell further to 76.4±3.5 mm Hg ($p<.05$) during sleep. It increased to 85.0±2.4 mm Hg ($p<.05$) during the daily routine. We conclude that patients with volume-dependent hypertension respond to dialysis with a sustained reduction in diastolic blood pressure to normotensive levels. Thus, the use of antihypertensive agents in the 24 hours following dialysis might not be necessary. Continuous 24-hour blood pressure monitoring is useful in identifying such patients.

THE CARDIOVASCULAR EFFECTS OF MIXTURES OF SODIUM SUCCINATE AND ACETATE INFUSED INTO DOGS: POSSIBLE NEW DIALYSATE PREPARATIONS. Paul L. Kirkendol, Carlos J. Devia*, James E. Pearson, & Francisco M. Gonzalez. LSU Med. Ctr., Dept. of Pharmacology & Medicine, New Orleans, Louisiana.

We have reported that succinate (SUC) produces less cardiovascular (CV) effects than acetate (AC), but SUC generates HCO_3^- too slowly to be used alone in dialysis. Mixtures of AC & SUC were previously infused to determine their metabolic effects. The present study was performed to determine the CV effects of these mixtures. Mixtures containing 25-75, 50-50 or 75-25% AC/SUC. Each mixture was infused into anesthetized dogs at doses of 0.125, 0.25, 0.5 and 1.0 mEq/kg/min for 10 min at each dose level. (Doses calculated as mEq of sodium). At the end of each 10 min infusion period, the effects on blood pressure (BP), heart rate (HR), stroke volume (SV), cardiac output (CO), dP/dt, total peripheral resistance (TRP), femoral blood flow (FBF) and vascular resistance (FVR) were determined. It was found that none of the 3 solutions had any effect on BP, HR or dP/dt. All 3 solutions produced dose-related increases in CO from 3.5 up to 6 L/min. SV and FBF were also increased with each of the solutions. Likewise, all three solutions induced dose-related decreases in TPR and FVR. In all cases, the changes seen were similar whether the solution was 25-75, 50-50 or 75-25% AC/SUC. Their responses closely resemble those previously reported for SUC alone. Because the addition of SUC seems to reduce the CV changes seen with AC, these data suggest that the ratio of AC/SUC selected for future studies should be based on ionic and metabolic considerations.

HEMODIALYSIS CLEARANCE OF METRONIDAZOLE. Alan Lau* and Sandra Sabatini, University of Illinois, Chicago, Illinois.

Metronidazole is a nitroimidazole derivative which is used in the treatment of giardiasis and amebiasis. Recently the drug has been shown to be very effective in the treatment of anaerobic bacterial infections. Based on its broader pharmacological spectrum we anticipate increased usage in clinical medicine. Previous studies have not examined the hemodialysis clearance and extraction ratio (ER) for this agent in patients with renal failure undergoing hemodialysis. We studied these parameters in 3 patients during 4 hemodialysis treatments following both oral (250 mg TID) and IV (500 mg Q6H) administration of the drug. Paired arterial and venous samples were taken from 1 to 4.5 hr during hemodialysis. Metronidazole concentrations were determined by high pressure liquid chromatography. Standard formulas for calculating hemodialysis clearance and ER were used. At similar blood flow rates (200 ml/min) but varying transmembrane pressures (80-500 mm Hg), the hemodialysis clearance of metronidazole was 107.0± 4.7 ml/min (range, 80.4-130.5). ER was 0.65±0.02 (range, 0.57-0.85). These findings indicate that the drug is highly dialysable. This probably relates to its small molecular size and low protein binding (10-20%). The hemodialysis clearance is higher than the total body clearance previously reported for patients with chronic renal failure in the interdialysis period. This twofold increase in total drug clearance during dialysis may result in suboptimal serum drug concentrations. From these results we suggest that in addition to the usual maintenance dose of metronidazole, an additional dose be given per 3 hours of hemodialysis.

THE CASE AGAINST THE "TEMPORARY" PERITONEAL DIALYSIS CATHETER. D.J. Leehey, J.T. Daugirdas, T.S. Ing, W.P. Geis, J.L. Giacchino*, V.C. Gandhi. Hines-Loyola Medical Center, Hines, Illinois, USA.

Serious complications (e.g. bowel perforation, severe hemorrhage) related to the use of "temporary", stylet-guided peritoneal catheters have been described. We reviewed our experience with such "temporary" catheters from 1974 to 1976. During 250 catheter insertions, 4 episodes of perforation of intra-abdominal structures occurred (3 bowel; 1 aorta + inferior vena cava + ectopic kidney). All patients required emergency surgery. Since 1976, we have used "temporary" catheters rarely, relying on the Tenckhoff catheter for emergency as well as elective dialysis. From 1973 to 1981, 412 Tenckhoff catheters were surgically placed under direct vision using local anesthesia. One patient was re-explored shortly after insertion because of poor drainage, and later suffered a bowel perforation. No other serious complications occurred. Early replacement of a Tenckhoff catheter (within 5 days of insertion) was necessary in only 16 instances (4.0%). Reasons were: poor drainage (8), clotted catheter due to minor bleeding (3), omental wrap (2), catheter removal by patient (3).

Our results suggest that surgical placement of a Tenckhoff catheter as opposed to blind insertion of a temporary catheter results in fewer serious complications. A functioning Tenckhoff catheter can be achieved in a high percentage of cases, obviating the need for repeated "temporary" catheter insertions if dialysis is prolonged. We consider surgical placement of a permanent catheter to be the procedure of choice in most instances when peritoneal dialysis is required.

COLLOID OSMOTIC PRESSURE (COP) FROM THE ARTERIAL (A) AND VENOUS (V) PORTS OF A CAPILLARY DIALYZER DURING REGULAR DIALYSIS (RD) AS COMPARED WITH ISOLATED ULTRAFILTRATION (IU). F. Llach, M. Rodriguez*, J. Pederson, and C. Williams*, Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

Hypovolemia is considered the factor leading to dialysis-induced hypotension. The decrease in plasma osmolarity (OSM) during RD may delay the shift of fluid from the interstitial into the vascular space. COP is the main force recruiting fluid into the vascular space. The present study evaluates the effect of OSM on the ability of the plasma protein to exert COP. RD was done in 19 patients (pts) and IU in 11 pts. Plasma samples were obtained from the A and V ports of the dialyzer at 0,15,30, and 120 minutes (min). COP, total protein (TP) and OSM were determined. For each determination during RD and IU, there was a similar relationship between COP and TP. The mean + SD value of COP during RD and IU at 7 and 8 g/dl protein concentration from the A and V ports of the dialyzer was:

		RD		IU	
TP		7 g/dl	8 g/dl	7 g/dl	8 g/dl
COP	A	34±2	40±3	32±2	38±3
Cm H_2O	V	33±3	41±3	32±2	39±3

During RD, A OSM at 120 min (291±7 mOSM/L) was significantly greater than the V OSM (273±8 mOSM/L). During IU, no significant change was noted. In summary, there was a similar increase in COP during both procedures from A and V despite changes in OSM during RD. In conclusion: a) the ability to recruit fluid (COP) from the interstitial space of the V port plasma is similar during RD and IU; b) this occurs despite a decrease in OSM during RD.

THE PHYSIOLOGICAL BASIS FOR THE ROUTINE USE OF HIGHER Na BATH DIALYSIS IN DIABETICS. H.I.Lipner, B.M.Louis, P.Gorfien*, A.Lock*, C.Sreenivasan*, and N.L.Manohar, Maimonides Med. Ctr., Div. of Nephrology, Brooklyn, New York.

The known high complication rate of diabetics (DM) on hemodialysis (HD) & their higher intracellular (RBC) Na levels may be interrelated. To test this hypothesis, the HD bath Na level was raised above 132mEq/L (range 148-156) for a period of 13-18 mos. in 4 DM pts., all with stormy course on 132mEq/L Na bath HD in past. The infections, access failures & transfusion rates fell drastically on higher Na bath HD. However, the intracellular (RBC) Na levels stayed high at (7.39mEq/L of RBC) as compared to that of non DM pts. on similar HD (RBC Na 5.09). The resulting higher intracellular osmolality & hydration may be matched & corrected with higher Na bath HD. The hitherto gloomy outlook of DM pts. on HD may improve with the use of higher Na HD. The insulin lack may be responsible for the higher intracellular Na of DM pts. on HD.

Patient # & Problem	COURSE ON HD: Low Na Bath (months)	High Na Bath (months)
1. Severe Retinopathy	Can't read newsprint (3)	Drives auto with telescopic lenses (13)
2. Cerebro-vasc.dis.	C.V.A in wheelchair (24)	Walks with help (15)
3. Cerebro-vasc. & Cor. disease	C.V.A. & M.I. (25)	Symptom Free (18)
4. Peripheral vasc.Dis.	B.K. amputee wheelchair (10)	Walks with help (17)

EFFECT OF POLYACRYLONITRILE AND CUPROPHAN DIALYSIS MEMBRANES ON WHITE BLOOD CELL AND COMPLEMENT COMPONENT ACTIVATION. J. Richard Ludgin,* George B. Naff,* Philip Spagnuolo,* and Jay B. Wish. Case Western Reserve Univ. Sch. Med., Cleveland, Ohio.

Ten patients were dialyzed with the polyacrylonitrile (PAN) or cuprophan (CUP) membrane for 9 weeks, then with the other membrane for 9 weeks to assess the differential effect of these membranes on WBC and complement (C) activation. Venous blood samples were taken pre-and postdialysis and 15 min into dialysis and assessed for granulocyte count and Fc receptors (by RBC rosetting assay). Arterial blood samples were taken pre- and postdialysis and 10 min into dialysis and analyzed for CH50, C1, C3, C8 and factor B. WBC counts and granulocyte Fc receptors/100 cells fell significantly ($p<0.02$) to their nadir at 15 min with CUP but remained stable with PAN. Changes in C activity were similar in patients dialyzed with either CUP or PAN. CH50 fell significantly ($p<0.001$) to its nadir at 10 min with both membranes. C1, C3 and C8 activities showed no change at 10 min ($p>0.05$) with either membrane. Factor B fell significantly ($p<0.005$) at 10 min then increased ($p<0.02$) to baseline postdialysis with PAN. Factor B fell ($p<0.2$) at 10 min then increased ($p<0.01$) to baseline postdialysis with CUP. We conclude that: (1) The mechanism of C activation is via the alternative pathway. (2) Since both membranes activate C but only CUP induces leukopenia, C activation alone does not play a major causative role in dialysis leukopenia. (3) The selective depletion of Fc receptor-bearing granulocytes during CUP dialysis indicates that these receptors become unavailable for binding antibody-coated RBCs and perhaps other immune complexes.

EFFECTS OF A MINERALOCORTICOID ON EXTRARENAL POTASSIUM (K+) HOMEOSTASIS IN ANURIC PATIENTS (PTS)
N. Lyman, S. Mulgaonkar*, R. Walcer*, R. Viscuso, M.G. Jacobs, Saint Barnabas Medical Center, Livingston, New Jersey.

Fludrocortisone (F), a potent mineralocorticoid, has been used to treat symptomatic hypotension (SH) in pts with normal renal function. Five anuric dialysis dependent pts were treated with F for SH. Supine systolic blood pressures (BPmmHg) were increased by F from 106.7 ± 13.6 to 123.7 ± 22.6 ($\bar{x}$ ± SD; $P < .001$) during a 3 to 9 month trial. During this period, a fall in serum K+ was noted: pre F 4.7 ± 0.9 to 4.0 ± 0.8** on F (**$p < .01$). No significant changes occurred in serum Na, Cl, HCO_3, glucose, BUN or creatinine. The cause of a reduced K+ was studied in one CAPD pt on a 2.5g/day K+ diet using 4 exchanges of Dianeal^R solution per day. Results were ($\bar{x}$ ± S.D.):

	Control (5 Days)	0.3mg BID F (5 Days)
Serum K+mEq/L	4.9 ± 0.3	3.9 ± 0.1**
Dialysate K+		
Loss mEq/day	51.2	41.5
pH	7.45	7.44
Serum Aldo pg/ml	460	99
B/P	112.8 ± 7.7	123.5 ± 9.8

No significant changes in Na, Cl, HCO_3, glucose, BUN, weight, or pulse were noted. Conclusions:

1. F may be used to effect an important rise in supine BP in pts with SH.
2. F may also be used cautiously to treat intractable hyperkalemia in non-hypertensive pts on maintenance dialysis.
3. The F-induced reduction of serum K+ is not due to increased dialysate losses, but may be due to enhanced bowel excretion of K+, increased cellular uptake of K+, or both.

COMPARISON OF PLATELET ACTIVATION BY POLYACRYLONITRILE AND CUPROPHAN DIALYSIS MEMBRANES.
Kandice K. Marchant,* James M. Anderson,* Michael C. Smith, and Jay B. Wish. Case Western Reserve Univ. Sch. Med., Cleveland, Ohio.

A group of ten patients was dialyzed with the polyacrylonitrile (PAN) or cuprophan (CUP) membrane for nine weeks, then with the other membrane for an equal period of time to assess the differential effect of these membranes upon platelet activation. Post-dialyzer blood samples were taken pre- and 2 min post-heparin infusion and at 15, 30, 60, 120, and 180 min into dialysis. Platelet factor 4 (PF4) levels increased significantly ($p<0.05$) from predialysis level 2 min after the bolus of heparin was given to patients with either membrane. PF4 levels progressively fell during the course of dialysis, to a similar degree with both membranes, but remained significantly elevated over baseline at 180 min. Mean β-thromboglobulin (βTG) levels were elevated in the patients prior to dialysis and did not change significantly with heparin administration. However at 15 min the mean βTG level for patients dialyzed with PAN was significantly lower ($p<0.05$) than for patients dialyzed with CUP. Both membranes produced a similar and significant ($p<0.05$) decrease in ADP-induced platelet aggregation at 180 min, and a similar and significant ($p<0.025$) decrease in platelet thromboxane B_2 production at 60 min which persisted throughout the remainder of dialysis. Intradialytic platelet counts were unchanged with both membranes. These results suggest that the PAN and CUP membranes induce similar degrees of platelet activation, and that differences in βTG levels are due to increased clearance or adsorption of that protein by PAN.

HEMODIALYSIS WITH COOLED DIALYSATE (HCD): EFFECT ON BLOOD PRESSURE STABILITY AND SERUM CATECHOLAMINES.
B.H.Mahida*, G.Zasuwa*, G.Fleig* and N. W. Levin. Dept. of Med., Henry Ford Hospital, Detroit, MI.

To extend Maggiore's observation that HCD may maintain vascular stability during hemodialysis 15 patients were each subjected to 3 sequential dialytic treatments with dialysate at both 37°C (W) and 34°C (C). Mean arterial pressures (MAP) which were similar pre-dialysis in C and W, were measured at 30 min. intervals subsequently. The duration of dialysis was similar in both groups Results were as follows (mean±SEM).

	MAP (mmHg)	PULSE /min.	WT.CHANGE (kg)	TEMP.°C (post)
W	92.1±5.0	92±0.8	2.8±0.1	37.0±0.04
C	97.5±5.0	93±1.0	2.8±0.1	36.5±0.10
p	< .001	NS	NS	< .0001

Symptomatic hypotensive episodes were less frequent during C than W (3.6 vs 17.6% respectively, $p<.001$). To determine the role of the sympathetic nervous system in the maintenance of MAP during C, epinephrine (E) and norepinephrine (NE) blood levels were measured in 7 patients prior to beginning and 30 minutes before concluding dialysis. Changes in NE (C125±67vs W115±84pg/ml) and E (C1.00±.79, vs W 14.3±70.9) were not significantly different. Conclusion: HCD results in better maintenance of MAP and decreased incidence of hypotensive episodes. The favorable effects of HCD are not accompanied by consistent changes in serum catecholamine concentration during dialysis. The mechanisms responsible require identification.

EFFECTS OF TRACE ELEMENTS ON DEVELOPEMENT OF RENAL OSTEODYSTROPHY(ROD) AND ON DERANGED METABOLISM OF OTHER TISSUES CAUSED BY THEIR DEPOSITION.
F.Marumo, M.Nakamura*, Y.Tsukamoto*, & S.Iwanami* Dept.Med. & Radiol.,Kitasato Univ.Sch.Med.,Sagamihara,Japan

This study was designed to investigate the effects of ROD, and on deranged metabolism of other tissues caused by their deposition. Trace element contents in tissues were measured by nondistructive neutron activation analysis, and that in plasma by flameless atomic absorption spectrophotometry and tube-excited X-ray fluoresence analysis.

Ca contents in bone decrease in both undialyzed(CRF) and dialyzed(HD) pts(n=22) compared to the control(C,n=11,$p<.05$). The correlation of Ca-Al contents in bone of C,CRF & HD are $Y=1.56X^{0.92}$(r=.96,n=14),$Y=22.2X^{0.44}$(r=.64,n=22) and $Y=4.01X^{0.7}$(r=.55,n=22), respectively. This correlation indicates both CRF & HD pts have more Al and less Ca in bone compared to C. The similar result is obtained in the correlation of Ca-Mg contents in bone. Al content in aorta of CRF+HD(30±11.2μg/g,n=8) is higher than C(13.9±4.9,n=8,$p<.01$). Mg in aorta(.62±.24 mg/g) is also higher than C(.39±.16,$p<.05$). Ca content is skin of CRF(.56±.14mg/g,n=6) is higher than C(.39±.14,n=7,$p<.05$). In hair(n=10-14), Al(157±52 μg/g) and Ca(642±132μg/g) contents of CRF are higher than C($p<.01$), while Zn(32.5±9.9μg/g) is lower. Al(78.6±7.1),Ca(1020±192),Mn(946±282pg/g) and V(661±57pg/g) of HD are higher than C($p<.01$). In fingernail, As,Mn and Cu contents of CRF are higher than C($p<.05$). Al content in RBC of both CRF & HD are higher than C($p<.01$).

In conclusion, Al and Mg may affect the development of ROD. Deposition of several elements in the tissues may be related to atherosclerosis or metastatic calcification.

BLOOD PRESSURE RESPONSE TO CHANGES IN SERUM CALCIUM DURING HEMODIALYSIS. J. Maynard*, C. Cruz, J. Meister*, G. Zasuwa*, D. Valade*, N.W. Levin, M. Kleerekoper* Dept. of Int. Med., Henry Ford Hosp., Detroit, MI.

Recent clinical and experimental evidence suggests a relationship between serum calcium concentration ([Ca]) and blood pressure. This was studied prospectively in 7 maintenance hemodialysis patients. Each was dialyzed 3 times at each of 2 levels (5.5 mg/dl and 7.5 mg/dl) of dialysate calcium concentration after a 15 min period of isolated ultrafiltration. A stepwise multiple regression analysis relating changes in systolic blood pressure and diastolic blood pressure to changes in [Ca], osmolality and weight revealed Δ[Ca] to be the best single predictor of systolic blood pressure following ultrafiltration ($r=.52$, $p<.002$) and dialysis ($r=.43$, $p<.02$); Δweight and Δosmolality did not add significantly to the systolic blood pressure models. ΔWeight was the best single predictor of diastolic blood pressure following dialysis ($r=.44$, $p<.02$); Δ[Ca] and Δosmolality added a small but significant degree of predictive power to this model ($r=.52$, $p<.05$). Thus, diastolic blood pressure fell in those patients with significant volume losses despite increases in [Ca]. However, the data support the hypothesis of a positive correlation between [Ca] and the maintenance of systolic blood pressure during dialysis; this could be due to a calcium induced inotropic effect leading to increased systolic blood pressure.

ASSESSMENT OF COPPER (Cu) REMOVAL BY HEMODIALYSIS WITH PENICILLAMINE (P). J.A. Mitas II, R. McNeal, W.V. Roman*, C.A. Mosley, Jr.*, J.W. Koett*. University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma and Naval Regional Medical Center, San Diego, California.

A patient treated with P for Wilson's disease developed renal failure requiring hemodialysis. Cu determinations were made by flame photometry on arterial (A) and venous (V) blood, dialysate in (Di) and out (Do) and reported as mcg/dl.

Time (min)	A	V	Di	Do
0	670	560	3	-
30	698	700	3	5
120	660	692	3	4
240	730	740	4	3

Additional studies were performed to assess the rise in Cu concentration and the removal of Cu across a Travenol 12.11 membrane before and after addition of 25 mg P to whole blood after 60 min. with dialysis (#1) or ultrafiltration (UF) alone (#2) and mass balance studies (#3).

	#1			#2				#3			
Time (min)	Hct (%)	V	Do	A	V	Cu in UF	Vol. (cc)	A	V	Di	Do
0	42	163	14	54	86	0	0	97	6		
30		110	10	123	137	4		114	113	6	7
60	58	274	4	72	83	9	675	96	100	7	7
				--Add P--							
90	68	292	2	77	85	8	0	84	80	5	5
120				87	93			93	85	7	5
150				75	102						
180	60	230	0	63	69	16	1000				

Conclusions: (1) Some removal of Cu is possible with hemodialysis. (2) The increased venous Cu reflects fluid loss not extraction of significant Cu from the membrane. (3) P enhances Cu dialysance.

CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD) IN DIABETES. Gonzalo Mejia*, Stephen W Zimmerman and David P Simpson. Nephrology Section, University of Wisconsin, Madison, Wisconsin.

ESRD in 22 diabetics was treated with CAPD. None had received prior dialysis or transplant. Mean age was 44.3yr, 10 were females and 12 males. Mean duration of diabetes was 21.5yr. Neuropathy was initially present in 21, varying degrees of retinopathy in 19 and hypertension in 15 (Mean duration of the latter: 7.2yr). Six had had myocardial infarctions (MI). At 1 and 2 years, actuarial survival rate was 78%. There were 2 CAPD failures that were switched to hemodialysis, 5 received transplants and 3 died. All deaths occurred in patients with prior MI and 2 were due to recurrent MI. Of the 22, 17 were followed for more than 2 months and their data are analyzed. This group represents a total of 175.5 patient-months (pt-mo). Overall hospitalization was 2 days/pt-mo, 36% related to initiation of CAPD, 22% to peritonitis and 42% to other complications. There was 1 episode of peritonitis per 8 pt-mo with a total of 22 occurring in 13 patients. While on CAPD, 2/3 were able to completely discontinue antihypertensive drugs. No further clinical progression of neuropathy or retinopathy was observed. The level of patient activity remained stable. Compared to pre-CAPD levels there was significant improvement at 6 months in: Hct (25.3±.9 to 30.1±1.7, $p<.005$), Ca++ (8.3±.3 to 9.2±.1, $p<.01$), PO4 (7.8±.7 to 4.8±.4, $p<.0005$) and albumin (3.1±.1 to 3.5±.1, $p<.02$). HgbAlc did not significantly decline at 6 months. This study confirms that CAPD is a safe therapeutic modality for diabetic patients in ESRD. It may be the treatment of choice for those without living related kidney donors.

CAPD PROVIDES "ADEQUATE" DIALYSIS. A.R. Nissenson, D. Wolcott*, W.S. Brown*, and J.T. Marsh*. Division of Nephrology and Department of Psychiatry, UCLA School of Medicine, Los Angeles, CA.

Twelve CAPD patients (PTs) were studied using a battery of electrophysiological (EP) and behavioral (B) measures to assess the "adequacy" of this form of therapy. At testing, mean Cr and SUN levels were 16.8 and 67.5, respectively. Mean time on CAPD was 49 weeks. EP tests included brainstem auditory potentials (BAEPs), checkerboard inversion visual evoked potentials (VEPs) and event-related potentials (ERPs). The first two tests index the speed of conduction in sensory pathways. The remaining test assesses cortical information processing. B measures included the Span of Apprehension Test (SPT) and the Continuous Performance Test (CPT). SPT indexes the efficiency of visual information processing and the CPT indexes sustained selective attention. CPT and SPT results in PTs were no different from those obtained from a group of normals (N). BAEP latencies were comparable to N. VEP latencies for large and small checks were also N and shorter than those values reported for hemodialysed (HD) uremics. Latencies of ERP components were all shorter than the mean values of 49 control subjects suggesting normal cortical processing time. No significant correlations were found between any EP or B measures and either SUN or Cr. Significant correlations were found between ERP latencies and CPT and SPT. These data suggest that in PTs treated with CAPD, in spite of the low small solute removal when compared to HD, normal CNS and cognitive functions are maintained. Therefore, CAPD does provide "adequate" dialysis.

GLUCOSE IN THE DIALYSATE DOES NOT REDUCE THE FREE AMINO ACID LOSS DURING DIALYSIS. Keiji Ono*, Yoshihiro Waki*, & Takeshi Sasaki* (intr. by Dr. F.M. Gonzalez). Ono Geka Clinic, Morishita Pharm. Co. & Ehime Univ., Fukuoka, Osaka, Ehime, Japan.

In order to clarify the effect of dialysate glucose on free amino acid loss during routine 5 hr hemodialysis(HD), 10 stable non-fasting HD patients were dialyzed twice using a dialysate (Na 142, K 2.5, Ca 3.5, Cl 101, Mg 1.5, Acetate 36 mEqL) with or without glucose. Total free amino acid in the dialysate and plasma free amino acids were measured using a liquid chromatograph amino acid analyzer. It was possible to quantitate 10 essential and 9 non-essential amino acids. There was no significant difference in the dialysate total amino acid levels as shown below.

Dialysate glucose	No. of patients	Total amino acid in dialysate (mean ± SE)
500 mg/dl	10	6676 ± 482 mg
none	10	6527 ± 174 mg

The concentration in plasma of free amino acid fell considerably during dialysis but there was no significant difference between the two types of dialysate used. Plasma glucose increased up to 160 mg/dl with the glucose containing dialysate and decreased to 70 mg/dl with glucose free dialysate. It is concluded that the addition of glucose to the dialysate does not reduce the free amino acid loss during routine HD of non-fasting patients. This result does not accord with a previous report that adding glucose to the dialysate decreases the loss of free and bound amino acid during dialysis. (ASAIO XIX:309,1973)

ONE YEAR (YR) EVOLUTION OF RENAL OSTEODYSTROPHY (ROD) IN BONE BIOPSIES (BX) FROM UNMODIFIED CAPD PATIENTS (pts). J. Pederson, A. Felsenfeld, A. Voigts*, F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr., Okla. City, Okla.

To assess the affect of CAPD on ROD, basal (1) & follow-up (2) data (1 yr) from 4 male CAPD pts are compared as group means & mean paired differences (D) with range values (W) per Lord.

	1	2	D	W	p<0.1
Osteoblastic					
Osteoid (OB)%	1.9	8.6	+6.8	13.2	(-)
Resorption					
Active (AR)%	3.2	7.2	+3.7	8.7	(-)
Total (TR)%	4.9	10.2	+5.5	8.2	(+)
Bone Volume (BV)%	14.0	19.3	+5.7	7.7	(+)
Osteoclasts					
per mm^2 (OCC)	0.9	2.8	+1.9	3.4	(+)
Fibrosis					
Volume (FV)%	0.7	3.0	+2.3	4.8	(-)
Serum PO_4 (P) mg/dl	7.4	4.9	-2.4	3.5	(+)
Alk P'Tase (AP) U	81.0	139.0	+49.6	90.4	(+)
Hematocrit (H)%	23.0	28.6	+5.6	7.9	(+)

Initial iliac BX showed osteitis fibrosa (OF) & osteomalacia (OM) in 2 pts each. OB, AR, OCC & FV increased in the 2 OF pts. OM was unchanged in 1 pt but changed to severe OF in another. Aluminum in the initial BX of the 2 OM pts disappeared in one. No uniform changes were noted in weight, BUN, serum creatinine, calcium, albumin or parathyroid hormone (PTH). In summary: 2 pts had progressive bone resorption without changes in PTH while OM changed to severe OF in another & OM was unchanged in a fourth pt. In conclusion: overall bone metabolism appears increased with CAPD but a specific effect remains to be demonstrated.

PARATHYROID HORMONE (PTH) IN PATIENTS WITH DIABETES MELLITUS AND END-STAGE RENAL DISEASE (ESRD) ON CHRONIC HEMODIALYSIS (CHD). R.C. Pabico, A.J. Rivero,* B.A. McKenna,* R.B. Freeman. Univ. of Rochester Medical Center, Rochester, New York.

We have observed recently that juvenile-onset diabetics with ESRD tend to have lower serum PTH than non-diabetics with ESRD. We have extended this observation by comparing the serum PTH, measured by the C-terminal assay of diabetics with ESRD on CHD with the non-diabetic ESRD patients on CHD. Predialysis serum total calcium (Tca), ionized calcium (Ca++), magnesium (Mg++), and inorganic phosphate (Pi) were determined in both groups. They were comparable in age, duration on CHD, and predialysis chemical variables. Both groups were on 18-hour dialysis/week using plate dialyzer, against a bath containing 3.75 mEq/L of Ca++ and 2.0 mEq/L of Mg++. The results are as follows:

Serum	Diabetics (N=15)	Non-Diabetics (N=17)	P
PTH(ng/ml)	5.4±3.4	24.9±11.8	<.001
Tcalcium (mg/dl)	9.5±1.5	9.8± 2.1	N.S.
Ca++(mEq/L)	2.4±0.2	2.5± 0.6	N.S.
Mg++(mEq/L)	2.3±0.3	2.5± 0.4	N.S.
Pi(mg/dL)	4.2±1.1	4.5± 0.8	N.S.

Serum PTH is significantly lower in diabetics compared with non-diabetics. This finding could not be attributed to the effects of either Ca++ or Mg++ on PTH secretion since these cations were virtually identical in both groups. Adult-onset diabetics with ESRD on CHD (n=7) have values similar to non-diabetics. The possible roles of catecholamines and other determinants of PTH secretion in these patients have yet to be defined to explain the findings above.

SEVERE REACTIONS TO CUPROPHAN CAPILLARY DIALYZERS. S. Popli, T.S. Ing, J.T. Daugirdas, A.O. Kheirbek, R.M. Vilbar, G.W. Viol, and V.C. Gandhi. Veterans Administration Hospital, Hines, Illinois, USA.

Four patients developed severe reactions during their first exposure to a Cuprophan capillary dialyzer. On each occasion, symptoms developed within minutes of initiating dialysis, and included a burning sensation in the chest, a sensation of heat throughout the body, and hypotension. Dialysis was immediately discontinued in all instances. The first patient sustained a fatal cardiopulmonary arrest several minutes after onset of symptoms. The second and third patients became severely dyspneic, had marked bronchial spasm, and suffered respiratory arrest. They recovered after intensive supportive therapy. The fourth patient also recovered.

The cause of the reactions was ultimately not determined. However, prior to these episodes, it was our practice to rinse only the blood compartment of the dialyzer, using 1 L of 0.9% saline. Since occurrence of the reactions, we also recommended rinsing the dialysate compartment using 5 L of dialysate. After the new rinsing procedures were instituted, we encountered 2 additional, similar adverse reactions to Cuprophan capillary dialyzers. In both instances, inadequate rinsing of the dialyzer by a self-care patient was reported. In these last 2 episodes, it was not the patients' first exposure to a Cuprophan capillary dialyzer.

We suspect that substances leached from the dialyzer might have caused the syndrome described. The severity of such reactions may be lessened if care is taken to thoroughly rinse this type of dialyzer prior to use.

INCREASING DIALYSATE SODIUM WITH Na BICARBONATE ADDITION-LONG TERM USE. Rasib M. Raja, Mark S. Kramer, Kevin G. Barber,* and Shwu-Miin Chen*. Renal Section, Dept. of Medicine, Albert Einstein Medical Center, Philadelphia, PA.

Increasing dialysate Na (D_{Na}) has been reported to reduce intradialytic morbidity. D_{Na} has been increased by addition of Na chloride to the dialysate in batch systems. Our preliminary studies showed that increasing D_{Na} to 143 mEq/L with addition of Na bicarbonate (Na HCO_3) resulted in better improvement in dialysis morbidity and metabolic acidosis than Na chloride. This study compares hemodialysis (HD) for 6 months each with standard acetate dialysate (A) and Na HCO_3 added dialysate (B) in 6 chronic HD pts with frequent intradialytic hypotension (Hp). Na HCO_3 (75 gm) was added to the mixed dialysate. Adjustment of pH was not needed and D_{Ca} did not change. Dry wt, dialyzer, Q_B, Q_D were constant. Pre and post-HD wt and BP before, after and during hourly HD was recorded. Blood chemistries were done monthly. The results (mean) are:

	Wt (Kg)	BP mmHg Pre	Post	Hp (%)	pH (Units) Pre	Post	Ca (mEq/L)	Na
A	67.3	163	118	48	7.35	7.43	4.2	137
B	67.4	160	136*	16*	7.39*	7.49*	4.3	140

* $p < 0.01$

Muscle cramps were 21% and 9 ($p < 0.01$) while 25% mannitol used for Hp was 14 ml/HD and 6 ($p < 0.01$) with A and B respectively. These data suggest that long term use of D_{Na} 143 mEq/L with addition of Na HCO_3 to standard acetate bath is safe, results in lower HD morbidity and better correction of acidosis. Interdialytic wt gain and pre-HD blood pressure may not increase significantly with long term addition of Na HCO_3.

INCREASED PLASMA VITAMIN K1 CONC. (P-K1) IN HEMODIALYZED PATIENTS (HP) WITH VASCULAR AND SOFT TISSUE CALCIFICATIONS (calc). D. Robert*, V. Jorgetti*, B. Lacour*, M. Leclerc*, T. Drüeke* (Intr. by T. Nawar). Dépt de Néphrol., Hôp. Necker, Paris, and Inst. Pasteur, Lyon, France.

The mechanisms involved in the greater frequency of calc in some HP are not well understood. The demonstration of vit K-dependent proteins in calc have led us to examine vit K1 metabolism and its possible relation to calc in HP. Measurements of P-K1 were done in 16 volunteers (NV), 16 HP without x-ray calc, and 22 HP with x-ray calc. In 10/16 HP without and in 18/22 with calc osteitis fibrosa was present on x-ray exam. Mean P-K1 was 18.1±1.5 µg/L in NV, 27.3±9.4 µg/L in HP without calc and 40.8±8.5 µg/L in HP with calc. A significant difference in P-K1 was found between NV and HP with calc ($p<0.05$). Moreover, χ^2 test for P-K1 vs calc in HP showed a significant difference ($\chi^2=6.34$, $p<0.05$). Plasma Ca, Pi and alk. P.tases were not significantly different in both HP groups.

In conclusion, extra-osseous calcifications in hemodialyzed patients could be favored by an increase in circulating vit K1.

SEQUENTIAL VARIATIONS IN DIALYSATE Na (D_{Na}). Rasib M. Raja, Mark S. Kramer, Kevin G. Barber,* and Shwu-Miin Chen*. Renal Section, Department of Medicine, Albert Einstein Medical Center, Philadelphia, PA.

Hemodialysis (HD) morbidity may be reduced by increasing D_{Na}. Sequential use of hyper- and hypotonic dialysate has been reported to be better than fixed D_{Na}. Proportioning dialysis machines have been designed where D_{Na} can be varied during HD. This study compares fixed D_{Na} to hyper- hypotonic and hypo- hypertonic dialysate in 10 stable chronic HD pts. Each pt was dialysed for 2 wks with fixed D_{Na} of 135 mEq/L (A) and 140 (B) and then D_{Na} was sequentially varied each hr from 145 to 135 (C) and from 135 to 145 (D). Q_B, Q_D, TMP, dialyzer and HD time were kept constant. Body wt, supine and standing BP were recorded before and after HD and BP every 30 mins during. Serum electrolytes, Osm, BUN, Cr, proteins and Hct were measured before, after and hrly during HD. The wt gain (Kg), hypotension (Hp%), muscle cramps (Mc%), difference in pre- and post-HD (Δ) BP (mm Hg), Osm (Osm/L) and serum Na (mEq/L) are (mean):

	Wt Gain	HP	Mc	Δ BP	Δ Osm	Δ Na
A	2.3	46*	5	28*	24*	-3
B	2.4	20	2	16	15	-1
C	2.2	22	7	20	20	-3
D	2.6	32	0**	9**	13	+2

*$p < 0.01$ (A vs B, C and D)**$p < 0.01$ (D vs A and C)

These data suggest: 1) Intradialytic morbidity may be similar with fixed median D_{Na} and hyper-hypotonic dialysate but the latter may be preferred in pts with excessive wt gain. 2) Hypo-hypertonic dialysate may be used in pts having frequent Mc without hypotensive episodes.

EFFECTS OF REGULAR DIALYSIS (RD) AND ISOLATED ULTRAFILTRATION (IU) ON COLLOID OSMOTIC PRESSURE (COP) AND PLASMA REFILLING RATE (PRR). M. Rodriguez*, J. Pederson, F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

The effect of RD and IU on hypovolemia is dependent on the PRR which is thought greater during RD than IU. Plasma volume (PV) can be monitored with COP. A lesser PRR should be reflected in an increase in COP. This hypothesis was tested in 5 patients undergoing both RD (2 hrs) and IU (2 hrs) at different sessions. Predialysis weight gain (3.1±1 kg) and fluid removed (2.5±.4 L) was the same with each procedure. The percent changes (% Δ) ±SE in COP, calculated PV, mean arterial pressure (MAP), and plasma osmolarity (OSM) were:

		30 Min	60 Min	120 Min
COP	RD	1.0±1.2	5.1±2.9	13.1±3.3
	IU	4.5±2.1	8.5±1.5	18.1±4.3
PV	RD	1.9±3.0	4.2±1.2	7.5±2.5
	IU	2.7±0.5	6.1±1.2	11.0±2.3
MAP	RD	-3.7±3.0	-5.0±2.3	-15.0±7.1
	IU	-6.0±1.5	-8.0±4.5	-5.2±2.7
OSM	RD	-2.1±.2	-3.9±.9	-6.0±1.7
	IU	1.3±.4	-0.3±.7	1.2±1.0*

*$p < .01$

During both RD and IU, progressive, parallel and similar changes occurred in all parameters except for a decrement in OSM observed only during RD. Though there was a greater decrement in MAP with RD than with IU, the difference was not significant. In conclusion, since there was not a difference in the magnitude of change in PV and COP during RD and IU, a similar PRR may be present during both procedures provided that the same amount of fluid is removed.

OCCUPATIONAL THERAPY CRAFTS PROGRAM FOR HEMODIALYSIS PATIENTS. SJ Rosansky, J Tennant*, and P Rosenzweig*, Depart of Rehabilitative Medicine and Depart of Medicine, VA Hospital, Columbia, SC.

Two of the major problems with the end stage renal disease program are the rising costs and lack of patient rehabilitation. An occupational therapy (OT) crafts program could help resolve both of these problems. This study describes, gives a cost analysis, and explores the potential profit from the sale of the projects made in an OT crafts program used for ten chronic incenter hemodialysis patients.

Patient and staff questionaires were utilized to analyze the results of the program. Patient symptoms and compliance in the period prior to and after the OT program was initiated were analyzed by chart review. A students T test was used to compare the results of the average potassium, phosphorus, weight gain, and symptomatic episodes pre and post initiation of the OT crafts program, but no statistically significant differences were found. After initiation of the crafts program, patients spent increased wakeful hours and had improved self-esteem. All patients expressed willingness to participate in a "money making" OT crafts program. Staff felt that the OT program: improved patient and staff morale and did not interfere with patient treatment. Cost analysis revealed that the average cost per patient visit was $5.54. The average potential profit per patient per year was estimated at $642.24 to $930.24 depending on the project made.

Thus, an OT crafts program could help rehabilitate dialysis patients and could be used as a form of patient "pay back" for this life sustaining therapy.

PROTAMINE SULFATE (PS) AUGMENTS PERITONEAL CLEARANCES IN A RABBIT MODEL OF CAPD AND ENHANCES ANTIMICROBIAL ACTIVITY IN VITRO. V.A. Sacchi,* C.J. Bentzel, B.K. Mookerjee and T.B. Beam,* Buffalo VA Medical Center and SUNY at Buffalo, Buffalo, NY.

An animal model of CAPD was developed in New Zealand white rabbits. An indwelling Tenckoff catheter was inserted into the peritoneum and silastic catheters were placed into the femoral artery and vein.

1. Peritoneal clearances were measured sequentially using a constant infusion of inulin and urea. 100 ml of isotonic Ringers lactate (RL) was rapidly infused into the peritoneal cavity and 15 minute clearance periods were measured. PS, a cationic protein, pI≈11.0, was added to the dialysate at a concentration of 100 µg/ml. PS increased the clearance of inulin and urea acutely by 73% and 28%, respectively. This effect was sustained when rabbits were restudied following two 12 hr exchanges with RL containing PS. Addition of heparin (10 U/ml) did not lead to acute reversal of the PS-induced increment in permeability to inulin and urea but partial reversibility was observed after 24 hrs of dialysis with RL.
2. Inclusion of protamine (50 µg/ml) into an in vitro assay medium significantly enhanced phagocytosis of zymosan and consequent superoxide production (measured by cytochrome C reduction) by granulocytes.
3. The in vitro antibacterial activity of PS was tested in Mueller Hinton broth. MIC_s (µg/ml) were: E coli (12.5) S. aureus (6.25) and Pseudomonas (12.5). Heparin inhibited the antimicrobial effect.

We conclude that PS enhances dialysis efficiency of small and intermediate molecules and has the potential for reducing infections in CAPD.

PERITONEAL DIALYSIS IN THE DOG WITH A POLYMER SOLUTION. Rubin J, Jones Q, Planch A, Bower J. University of Mississippi Medical Center, Jackson, MS.

We compared the dialysate(dial) effluent volumes (VD) and protein lost into dial(prot) for peritoneal dialysis solution containing a glucose polymer (P) as the osmotic agent(3%P,6%P) to commercially available dial(C) containing glucose(1.5%G,4.25%G) in anephric 20 Kg dogs undergoing chronic peritoneal dialysis with 2L dial exchanges.Dial remained within the peritoneal cavity except during refreshment performed at 0800, 1200, and 1600 hrs. Twelve dogs underwent C solution for 7 ±2.5 days(SD)(range 1-10 days).Nine dogs also received P solution for 5 ± 2.5 days(range 1-7 days).No dog had peritonitis. Dial was chosen to maintain mean BP at 100 mm Hg. One mean value/dog was derived for C and P solutions for dial exchanges of 240 min(infusion to completion of dial drainage) and 960 min.

240 min exchange	1.5%G	3%P	4.25%G	6%P
n	11	4	9	6
VD(ml)	+1913	2119	2283	2431
Prot (g)	1.4	1.1	1.5	1.3
% absorbed from dial	75	50	79++	47
960 min exchange				
n	11	4	8	6
VD(ml)	*1339	2006	1730	2067
Prot (g)	3.0	2.0	3.2	2.3
% absorbed from dial	91	84	97	61**

+P<.05 1.5%g vs 4.25%G,6%P ++P<.05 4.25%G vs 6%P
*P<.05 1.5%G vs 3%P,6%P **P<.05 6%P vs 1.5%G,4.25%G

Protein lost into dial(an index of cellular irritation) was similar with P and C solutions.A solution with P as the osmotically active agent is effective in maintaining the intraperitoneal volume of dial when residence of dial within the peritoneal cavity is prolonged.

GROWTH AND NUTRITIONAL STATUS (NS) IN CHILDREN RECEIVING CAPD. Isidro B. Salusky, Richard N. Fine, Pauline Nelson*, Joel D. Kopple. UCLA Dept.Ped.and Harbor Med. Ctr., Dept. Med. L.A., CA.

Although CAPD is a rapidly growing therapy for uremic children, little is known about growth and NS during CAPD. We examined these factors in 21 children undergoing CAPD for 5 to 23mos(mean 12± 5.3SD). Age at onset of CAPD was 9.34±4.34yrs. Patients were strongly encouraged to ingest a prescribed high energy, high protein diet.Mean intake of protein and energy (diet+dialysate) during the study were 86% and 75% of the prescribed diet. Height(Ht), weight(Wt), midarm circumference(MAC), midarm muscle circumference(MAMC) and triceps skin fold thickness(TSF) were low for chronological age (Table).

	Ht[a]	Wt[a]	MAC[a]	MAMC[a]	TSF[a]	Albumin[b]
Initial	-3.1	-1.3	-1.5	-1.2	-1.0	3.60
Final	-3.1	-1.4	-0.9	-0.6	-0.8	3.65

[a]expressed as Z-score, the number of SD from mean of normal controls, [b]g/dl.

Each value in table is significantly below normal; p<.01-p<.001. Weight,MAC,MAMC and TSF were generally reduced in proportion to height and were close to normal for height/age.Only Z-scores for MAC(p<.001) and MAMC(p<.01) increased during CAPD. Serum total protein, albumin and transferrin were below and did not change with CAPD,neither did the pattern of plasma amino acids.These findings suggest that in children on CAPD,most anthropometric values increase normally but there is no catch-up growth, except for MAC and MAMC.Several serum proteins are slightly reduced and do not change with CAPD.Studies are needed to assess whether greater energy or nitrogen intake will improve growth and NS with CAPD.

EFFECT OF A CALCIUM INFUSION ON THE VASCULAR STABILITY DURING HEMODIALYSIS. K. Schaefer[x], D.von Herrath[x], M. Hüfler[x] (intr. by S.G. Massry), Med. Abt. II, St. Joseph-Krankenhaus I, Bäumerplan 24, 1000 Berlin 42, Germany.

Patients on chronic hemodialysis (HD) experiences hypotensive episodes more often than patients treated by hemofiltration (HF). As the precise pathomechanism responsible for the increased vascular stability (VS) during HF is not yet clear, it seemed feasible to test the hypothesis that the higher venous serum calcium during HF might be a contributing factor (HF:5.20 mEq/l ± 0.43; HD: 4.74 mEq/l ± 0.42, $2\alpha \ll 0.05$). Eight patients previously treated by conventional acetate HD received a calcium infusion during HD (15 mg/kg/bw) on several occasions. The venous serum calcium was thereby increased to 6.45 mEq/l ± 0.55. A careful observation of various circulatory parameters revealed a reduction of hypotensives episodes by 28 %, an increase, however, of the systolic blood pressure, as in healthy subjects, was not observed at any time.

Conclusions: 1) An increase of the venous serum calcium from 4.74 mEq/l to 6.45 mEq/l does not raise the systolic blood pressure during HD. 2) In spite of a slight reduction of hypotensive episodes during the application of a calcium infusion, the incidence of hypotensive episodes is still less frequent during HF. 3) Therefore it appears unlikely that the higher venous calcium during HF is a very important factor with regard to the improved VS of this method.

CHANGES IN PLATELET NUMBERS (PN), β-THROMBOGLOBULIN (BTG) AND FACTOR VIII ANTIGEN (F VIII) DURING HEMODIALYSIS (H.D.). G.W. Schmitt, R. Hamburger, J. Moake,* and S. Vicks,* Hematology and Renal Section, Boston VA Medical Center, Boston, MA.

Changes in PN, BTG, and % F VIII were measured in three patients who were selected because they had a 10% or greater decrement in platelet numbers during hemodialysis with the Gambro dialyzer (G). Each patient was hemodialyzed for periods of three weeks using the G, R-P 610 (R), and Disscap (D) dialyzers. Heparinization was identical for all patients and for all dialyzers. PN, F VIII, and BTG were measured on the day of the last dialysis of each period after 0, 15, and 45 minutes of dialysis.

The mean peak decrement in platelet numbers was > 11% for the D and G, and 7% for the R dialyzers. The BTG fell or did not change during the R dialysis. The peak rise in BTG was 22 and 26% with D and G dialyzers respectively. At 15 minutes the F VIII rise was 13, 21, and 110% for the R, D, and G dialyzers respectively.

Dialyzers have a variable effect on platelet numbers and platelet stimulation. In addition, H.D. is associated with an increase in F VIII. The magnitude of this increase appears to be a function of the dialyzer being used. Further studies are being conducted to evaluate the relative roles of dialyzer geometry and membrane composition on hemodialysis induced changes in F VIII.

SIMULTANEOUS HEMODIALYSIS AND PLASMAPHERESIS: TEN YEARS' EXPERIENCE. E. Scheiner, L. Reich*, M. Isaacs, P. Vanamee*, C.D. Flombaum*, S. Van Strien* and S.C. Gulati*. Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Eleven patients on hemodialysis for acute renal failure (ARF) with indications for massive plasmapheresis were treated with a simultaneous procedure. Blood at 60 ml/min. was diverted from the venous line of the dialyzer (D) into a continuous flow centrifuge (C) (IBM 2997 blood cell separator). Plasma was removed and replaced with normal human serum albumin, which was returned with the patient's cells to another more proximal venous site. One to two plasma volumes were exchanged. Heparin was infused into the arterial limb of D and anticoagulated both systems. Regional heparinization was used in bleeding patients. The joint procedure was carried out 21 times.

Seven patients had hepatic and ARF after major hepatic or pancreatic surgery. Encephalopathy improved temporarily, and one recovered renal and hepatic function. Three patients with cancer developed ARF, microangiopathic hemolytic anemia and thrombocytopenia after Mitomycin-C therapy. Anemia and thrombocytopenia, but not renal function, improved markedly in two, one of whom was maintained for two years on chronic dialysis. Hemoptysis improved in one patient with probable Goodpasture's syndrome.

The procedure was safe and without complications. This approach, used early, might be useful in thrombotic thrombocytopenic purpura, immune-complex mediated renal failure, or the hemolytic-uremic syndrome. The ability of C to separate cells might be utilized in renal transplantation rejection with ARF.

THE PHOSPHATE BINDING EFFECT OF SUCRALFATE. R.A. Sherman, E.R. Hwang*, J.A. Walker, and R.P. Eisinger. UMDNJ-Rutgers Medical School, Piscataway, New Jersey.

Sucralfate is a minimally absorbed anti-ulcer medication. Its effect on serum phosphate was studied in 5 stable hemodialysis patients with their informed consent. Conventional phosphate binders were discontinued for 4 days and sucralfate (1 gm four times daily) was then given for 7-14 days; subsequently there was another 4-day control period without phosphate binders. Dietary phosphate intake was maintained at 1 gm daily. Serum phosphate was measured at the end of each control period and during the study period.

	Pre-Sucralfate	Sucralfate	Post-Sucralfate
Serum PO_4 (mg/dl)	9.8	6.4	9.1
S.D.	±2.2	±1.1	±2.0

In each case serum phosphate declined during sucralfate administration and rose subsequently.

Sucralfate is a complex of sulfated sucrose and aluminum hydroxide. The aluminum content of 1 gm of sucralfate is equivalent to that of one capsule of aluminum carbonate (as Basaljel). Preliminary in-vitro evidence indicates that the phosphate binding effect of sucralfate is disproportionate to its aluminum content when compared with aluminum hydroxide (Amphojel tablets) and aluminum carbonate (Basaljel capsules). These data suggest a possible role for sucralfate as a phosphate binder in dialysis patients.

COMPARISON OF 4 DIALYSATE FORMULAE FOR PATIENT WELL BEING. A. Shimizu, D. Sackett,* W. Taylor,* G. Lennox,* J. Martin,* P. Hoda,* H. McNeaney.* McMaster University, Hamilton, Ontario.

In view of many studies which claim either bicarbonate or high Na dialysaet leads to less symptoms than standard low Na acetate dialysate, we studied 4 dialysate combinations delivered to bedside consoles by a central delivery system to determine which combination resulted in least and most symptoms during & after dialysis among 37 hospital patients. The follwoing dialysate combinations were used: 1. 142 mM/L Na, 40 mM/L acetate 2. 142 mM/L Na, 35 mM/L bicarb 3. 135 mM/L Na, 40 mM/L acetate 4. 135 mM/L Na, 35 mM/L bicarb. K was 2 mM/L in all dialysates and chloride 101.6 mM/L or 135 Na & 108.6 mM/L for 142 mM/L Na. The following symptoms were monitored at each dialysis through a questionnaire: cramps, headaches, vomiting, blahs all during & after dialysis at 3 degrees of severity.

Design features included blinding of physicians, nurses, technicians, research assistant who insured compliances to answering questions and the patients. Each dialysate formula was delivered for 2 weeks on 18 occasions thus the study took 36 weeks. Analysis of results were obtained form symptoms during the second week.

The percentage of sessions during which patients had moderate to severe symptoms are indicated in this table.

	↑Na Acet	↑Na Bic	↓Na Acet	↓Na Bic
Cramps	2.0	1.96	3.2	3.6
Blahs	10.5	7.1	13.2	8.9
Headaches	8.7	5.3	8.5	7.8
Vomiting	0.74	1.2	0.7	0.76
All symptoms	18.0	11.9	20.4	16.4

Thus, high Na bicarb solution lead to least symptoms and low Na acetates lead to most symptoms.

PROCAINAMIDE UTILIZATION IN CHRONIC AMBULATORY PERITONEAL DIALYSIS. Domenic A. Sica,* Antonia Harford,* and Ralph Small,* (introduced by Edward Zawada), Department of Medicine, Medical College of Virginia, Richmond, Virginia.

Antiarrhythmic therapy in end-stage renal disease(ESRD) is not uncommon. Of the agents available, quinidine and procainamide(P) would commonly be considered for use. We have recently had the opportunity to study a quinidine intolerant individual undergoing chronic ambulatory peritoneal dialysis(CAPD) placed on P as an alternative agent.

A total of 3125mg of P was administered orally over 54hrs. Serum P and n-acetylprocainamide (NAPA) values were obtained at various time intervals following the completion of drug administration and were as follows:

TIME (hrs)	PROCAINAMIDE (mg/L)	NAPA (mg/L)	COMMENT
8	6.2	12	
12 (predialysis)	4.5	20.8*	4 hr exchange
(postdialysis)	3.7	21.8*	
(dialysate)	2.6	12.2*	
24	1.2	20.4	
80	1.12	14.8	
128	.77	9.2	

The V_D of P was 1.5L/kg. $T_{\frac{1}{2}}$ of P was 40hrs and that of NAPA was 64hrs. The dialysance of both P and NAPA amounted to less than 2% of body burden. We conclude that 1)the $T_{\frac{1}{2}}$ of P and NAPA are markedly prolonged in ESRD, 2)the dialysance of both substances via the peritoneal route is limited, 3) dosing guidelines in peritoneal dialysis patients may require a daily dose only, and at an appropriately diminished strength, 4)this data suggests that the use of P is contraindicated as initial therapy for arrhythmias in ESRD treated by CAPD.

SIGNIFICANCE OF ERYTHROCYTE SEDIMENTATION RATE (ESR) DETERMINATION IN CHRONIC HEMODIALYSIS (HD) PATIENTS. N. Shusterman*,P. Kimmel*, M. Clayman*, G. Morrison. Univ. of Pa Sch of Med, Phila., PA.

Reported data on ESR in chronic renal failure (CRF) have indicated it may be elevated. This study was undertaken to determine the distribution of ESRs in stable HD patients. Thirty-six patients (22 male,14 female;mean age 47±14) on HD 44±39 months without rheumatic, granulomatous, malignant or recent infectious disease had pre-HD ESRs determined by the modified Westergren method. Mean ESR (mm/hr±SD) was 60±33 (males:57±31;females:63±36). Of significance, 20% had a normal ESR (male:0-20;female:0-30) and 22% had an ESR>90. The ESR correlated negatively($p<0.01$)with hematocrit and positively ($p<0.05$) with globulin level. There was no correlation of ESR with age, sex, race, duration of HD or cause of CRF.

In 11 patients who had 2 ESRs determined two months apart the mean ESR did not change (50±24 vs. 53±28) but individual changes ranged from -48% to +171% of the first value. Similarly, in 14 patients the mean post-HD ESR was unchanged from pre-HD values(pre:49±27; post:52±33) though individual post-HD values varied from -90% to +142% of the pre-HD ESR. Weight change, heparin dose or fluids administered during dialysis did not correlate with the post-HD change in ESR.

In summary, pre-HD ESR is elevated in the majority of stable HD patients. Marked day-to-day intrapatient variability occurs and HD alters ESRs unpredictably. Anemia may be a factor elevating the ESR. The significance of an abnormal ESR in HD patients is still uncertain, may be related to CRF per se, and may not be a useful diagnostic tool.

SPOUSE ACCEPTANCE AND ATTITUDES TOWARDS CAPD PATIENTS. M. Simmons,* M. Barnicle,* D. O'Neill,* D. Hofman,* M. Hammeke, M. Bierman. Creighton University, Omaha, Ne.

Sixteen spouses of active CAPD patients were interviewed for their attitudes toward CAPD, and completed the Sickness Impact Profile (SIP) to assess the impact of CAPD on their lifestyle. Thirty-one percent of the spouses were responsible for doing the dialysis and high levels of care of the CAPD patients. Most spouses (80%) reported satisfaction in their marriage relationships and 88% reported their relationship to be stable or improved since initiating dialysis. Only 5% felt that the family structure would be improved without CAPD. Spouses frequently reported that CAPD detracted from sexuality (38%) and 50% of the spouses reported a decrease in sexual activities since initiating CAPD. Socialization opportunities were frequently available but accepted only 50% of the time. Spouse satisfaction with life was less than patient satisfaction with life 56% vs. 80%. No divorces or separations were experienced in this population with 16.9 patient dialysis years.

We conclude CAPD has a significant impact upon the spouse of the patient. Marriage relationships are maintained and improved in many cases; however, many spouses do not find satisfaction in their lifes and their socialization outside the family is frequently limited. Sexual desirability of the dialysis patient is adversely affected.

MARKED SUPPRESSION OF SECONDARY HYPERPARATHYROIDISM (S.H.) BY INTRAVENOUS $1,25(OH)_2D_3$ IN UREMIC PATIENTS. E. Slatopolsky, C. Weerts*, J. Thielan*, K. Martin, and H. Harter. Washington University School of Medicine, St. Louis, MO

Controversy exists as to whether $1,25(OH)_2D_3$ has a direct effect on PTH secretion. We have shown that $1,25(OH)_2D_3$ does not suppress PTH release by isolated bovine parathyroid cells. The present studies were conducted in 20 dialysis patients. After three weeks of control studies, $1,25(OH)_2D_3$ was given I.V. three times a week after each dialysis for 2 months. The drug was then stopped and post-treatment blood samples were obtained for an additional 3 weeks. The dose of $1,25(OH)_2D_3$ was 0.5 µg and was gradually increased to a maximum of 4.0 µg per dialysis. From previous studies in our laboratory which correlated the levels of i-PTH with bone biopsies, we divided our patients in 3 groups characterized by mild (I), moderate (II), and severe (III) S.H. The levels of PTH in µLEq/ml were as follows:

Group	Control	$1,25(OH)_2D_3$	Post-treatment
I (n=9)	61	14	39
II (n=7)	150	42	113
III (n=4)	902	407	872

i-PTH decreased in all 20 patients with a mean fall of 71% during the treatment period and returned to high levels after $1,25(OH)_2D_3$ was discontinued. Although there was a significant correlation ($p<0.01$) between the levels of ionized calcium and PTH during the administration of $1,25(OH)_2D_3$, the fall in i-PTH appeared to preceed the changes in serum Ca^{++}, raising the possibility of an effect of $1,25(OH)_2D_3$ on PTH secretion independent of serum calcium.

SUBCLAVIAN VEIN(SV) STENOSIS AS A COMPLICATION OF SUBCLAVIAN CATHETERIZATION FOR HEMODIALYSIS(HD). Bruce S. Spinowitz, Marilyn Galler, Ronald A. Golden, Joel H. Rascoff, Lawrence Schecter*, Chaim Charytan. Booth Memorial Med. Ctr., Dept. of Med. and Radiology, Flushing, New York.

In a previous series of 50 subclavian catheter implantations(CI) for temporary HD we observed 3 episodes of ipsilateral arm swelling secondary to SV stenosis. We, therefore, undertook a prospective study to evaluate the incidence of this complication using routine peripheral venograms following SV CI.

Thirteen catheters were implanted which remained in place for a period of 13-41 days. Eleven were placed on the right side, 2 on the left. Peripheral venograms were performed on a routine basis 2-6 weeks following CI. Seven of the 13 studies were performed within 2 weeks of implantation.

Peripheral venography revealed total occlusion of the SV in one case, significant narrowing of the SV as it entered the superior vena cava in 5 cases, and no abnormalities in the remaining 7 cases. None of these stensosis or occlusions were clinically evident. Two different types of catheters were used; teflon or polyurethane. Two of the 4 polyurethane catheters and 4 of the 9 teflon catheters resulted in SV stenosis.

We conclude that SV catheterization for temporary HD results in a significant incidence of SV stenosis or occlusion. While the use of the technique obviates the need for repetitive transfemoral catheterization for temporary vascular access, alternate methods of interdialytic anticoagulation and/or the use of different types of catheter material must be investigated so that these complications may be minimized.

LISTEROSIS IN HEMODIALYSIS (HD) PATIENTS (P): ASSOCIATION WITH IRON OVERLOAD, AND SUCCESSFUL TREATMENT WITH VANCOMYCIN. James Sondheimer*, Robert Mossey, Barry Wilkes, Asif Rahman*, North Shore University Hospital, Div. of Nephrology, Manhasset, N.Y.

Listerosis is a rare infection, with a predilection for immunocompromised hosts. Although seen in transplant P, it has rarely been described in the HD population. Over the past year, we have seen three HD P with listeria monocytogenes bacteremia. The first P, a 54 year old (yr) black female with malignant hypertension, had been on HD for 5 years. The second P was a 36 yr white female with chronic glomerulonephritis on HD for 13 years. The third P is a 27 yr white female with focal segmental glomerulosclerosis on HD for 9 years. All three presented with malaise and fevers to 39°C mimicking a viral syndrome. There were no focal symptoms or signs, including meningismus or evidence of AV fistula infection or of endocarditis. Lumbar punctures on the first two were normal. The patients were cultured and treated with gentamicin (G), 2 mg/kg, and vancomycin (V), 1 gm with prompt defervescence and clearing of bacteremia. V was continued for 4-6 weeks at 750 mg/wk. All three had strong evidence for hemosiderosis: ferritin levels in excess of 10,000 ng/ml, transfusion history of over 200 units, and in vivo iron measurements (by nuclear resonant scattering) of 19 and 9 mg iron/gm of liver in patients 2 and 3, respectively. We conclude: 1. there exists an increased incidence of listerosis in the HD population with an apparent predilection for those P with hemosiderosis, 2. these P can be successfully treated with a loading dose of G and a 4-6 week course of V.

NON-A NON-B HEPATITIS(NANB) IN A LARGE HEMODIALYSIS(HD) POPULATION. Bruce S. Spinowitz, P. Sankarapandian,* George Martin,* and Chaim Charytan. Booth Memorial Medical Ctr., Dept. of Medicine, Flushing, New York.

Despite the decreasing frequency of hepatitis B in HD units, the incidence of acute elevations of transaminases(ET) has not shown a parallel decline. We noted 63 episodes of ET in 45 patients from a total population of 263 HD patients during a 33-month period. In 43 of these episodes, the patients had received a transfusion of packed RBC's within a 5-mo. period prior to the ET. The incidence of ET/yr. ranged from 4.5-11.6%, but the risk per unit transfusion each year was stable (2.5%). During this entire interval no staff member developed ET.

Only 6 patients developed symptoms referable to the ET and none became icteric. 33 episodes of ET persisted for 30-90 days, 13 for 91-180 and 16 have had ET for >180 days. These patients are currently being evaluated with liver biopsies. None of these patients sero-converted to HB_SAg or HB_SAb state. In 38 patients tested for HAAAb no acute phase antibody(IgM) was detected. Although we have not definitively excluded unlikely causes of hepatitis such as CMV or EBV infections, the most likely cause of ET in our HD population is transfusion-related NANB.

The natural history of this entity is still under investigation. There is a paucity of data to guide the dialysis staff in the management and containment of transfusion-related NANB. In view of the significantincidence noted above and the frequent protracted course, more data must be obtained in prospective studies in the severity of the disease and its epidemiology.

CAPD EXPERIENCE AT A UNIVERSITY CENTER. R Swartz, D Stone‡ C Dickinson‡ J Dombrouski‡ P Lees‡ University of Michigan Med Cntr, Ann Arbor, Michigan.

Over the past 3 years our referral center has trained 62 patients for CAPD, gaining 126 patient-months (P-M) experience in diabetics, 63 P-M experience in children, and 627 P-M experience in total. Median longevity of CAPD treatment in this mixed population is over 18 months, with drop-out most often due to peritonitis, psychosocial issues or death unrelated to CAPD itself. Clinical status of patients is noteworthy for improved anemia and transfusion requirement, stable or improved blood pressure control, activity status comparable to that for other treatment modalities, and excellent control of BUN, K, phosphorus and albumin with liberal dietary prescriptions.

Peritonitis remains the major medical complication, with 83 episodes in 627 P-M or 1.6/patient-year. Peritonitis has been characterized as gram positive in 65%, gram negative in 24%, mixed in 5%, culture-negative in 5% and eosinophilic in 1%. Two fungal infections occurred only after prolonged antibiotic therapy of bacterial peritonitis. Treatment of peritonitis without catheter removal was successful in 90% of episodes, although elective catheter removal was performed in 4 (5%) of episodes. Six (8%) episodes could not be cured without catheter removal, including 2 fungus superinfections and 3 Pseudomonas infections. Higher risk of peritonitis appears to occur in the first 2 months of CAPD, in patients with diabetes or systemic disease, in children, and in the presence of leukopenia. No higher risk was apparent in the presence of subcutaneous catheter infections or dialysate leaks, or in elderly patients.

HIGH VOLUME, LOW FREQUENCY CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD). Z. J. Twardowski,* B. F. Prowant,* and K. D. Nolph, Univ. of Mo. Hlth. Sciences Ctr., Div. of Nephrology, Columbia, MO.

Intra-abdominal pressure (IAP) and forced vital capacity (FVC) were measured in 18 stable CAPD patients maintained on 2 L exchanges, in the supine, sitting, and upright positions after infusing dialysis solutions in 0.5 L increments up to 4 L as tolerated. Thereafter, 5 patients did not increase to 3 L volumes (I), 4 used 3 L volumes occasionally (II), and 9 chose 3 L volumes for routine dialysis (III). IAP was similar in all groups and dependent on intraperitoneal volume (IPV). The mean IAP increased 2.0, 2.7, and 2.8 cm H_2O/L of IPV in the supine, upright, and sitting positions respectively. The patients of the I group had dramatic deterioration (up to 42%) of the FVC in the supine position with IPV above 2 L. The patients with the greatest deterioration of pulmonary functions could not continue the measurements above 3 L of IPV. Two of these patients were switched to 1 L overnight exchanges. Even in patients who tolerated up to 4 L, FVC decreased significantly in the supine and sitting positions, with IPV greater than 3 or 4 L respectively. In the upright position, the values did not decrease significantly below those with the empty abdomen up to 4.6 L of IPV. Each liter of IPV increased abdominal girth by 2.1 cm.

Exchange volume and exchange frequency should be individualized. In our studies, 50% of patients could increase daily dialysate volume (from 8 to 9 L) while decreasing daily exchanges (from 4 to 3). The advantages of fewer exchanges include decreased risk of peritonitis and reduced cost.

EOSINOPHILIA (EO) AND SUPPRESSIVE T CELL FUNCTION IN HEMODIALYSIS (HD) PATIENTS (PTS). *C. Tielemans*, F. Collart*, L. Schandene*, E. Dupont*, M. Dratwa*, J. Wybran*. (Intr. by C.C. Tisher). Brugmann and Erasme University Hospitals, Brussels, Belgium.*

Many HD pts display EO (≥ 400 eosinophils/μl). As T lymphocytes have been implicated in the control of eosinophilopoiesis, we studied blood T cells subsets with monoclonal antibodies identifying total (T3), helper (T4) and suppressor cytotoxic (T8) T cells in HD pts with (group A) or without (group B) EO. Con A-induced suppressor activity was assessed on the proliferative response of autologous monocytes to Con A (I Con A) or allogeneic lymphocytes (I MLC).

Percentages of T3, T8 and T4/T8 ratio were not different between the two groups, in contrast to T4 (46.2±2.9 in A v. 39.8±1.4% in B, p<.05). Other results (mean ± SEM) were:*

	Normal	A (n=12)	B (n=23)	p*
EO	< 400/μl	864±135	210±21	<.001
months on HD		67.8±9.8	26.0±8.7	<.01
I Con A	25-65%	10.4±3.6	40.5±4.9	<.001
I MLC	25-75%	3.8±2.2	32.5±8.1	<.02

* *Student's t test for unpaired data (A v. B)*

EO was correlated with T4 (p<.05), I Con A (p<.01), I MLC (p<.05) and time on HD (p<.01), while I Con A and I MLC were correlated with time on HD (p<.05 and p<.01, respectively).

Thus HD pts with EO are characterized by deficient suppressive function, a higher percentage of T4 and a longer duration on HD. This study suggests that T suppressor function deteriorates with time on HD and could be implicated in the observed EO.

ACUTE CHANGES IN PERITONEAL MORPHOLOGY & TRANSPORT PROPERTIES WITH INFECTIOUS PERITONITIS VS. MESOTHELIAL DRYING. C.Verger,* A.Luger,* H.Moore*, K. Nolph, U. of Missouri Hlth Sci. Ctr, Dalton Res. Ctr, and V.A. Hospital, Columbia, MO.

Capillary wall, interstitial, and mesothelial resistances to passive solute movements during peritoneal dialysis are unknown. Mesothelium may be so permeable as to be a minimal resistance. Peritoneal transport of water, urea, glucose, and protein and peritoneal ultra-structure post sacrifice were assessed in 34 rats with superficial or extensive peritoneal injury: control (n=3) morphology only; group 1 (n=8) single 2 hr, 22 mL exchanges (ex); group 2 (n=8) 2 hr ex before and 10 hr after mesothelial exposure to warm dry air for 10 min; group (n=15), three 3-4 hr ex daily until spontaneous peritonitis, transport studies with 2 hr ex and morphology thereafter. Results: *(p<0.001 from normal (group 1)) (mean values)

	Drainage (ml/ex)	Glucose Absorption (mg/ex)	Dialysate Protein (mg/dl)	Urea Clearances (ml/min)
1.Normal	33	245	117	0.14
2.Drying	21*	661*	327*	0.15
3.Peritonitis	21*	625*	408*	0.15

Transport changes suggest increased permeability. Both peritonitis and drying caused loss of mesothelial villi and widening of mesothelial intercellular gaps. Only peritonitis caused obvious peritoneal interstitial inflammation & vasodilation. Summary: Mesothelial injuries with or without deeper peritoneal changes were associated with similar transport changes. Mesothelium may contribute to peritoneal resistances.

STUDY OF THYROID FUNCTION IN PATIENTS ON CAPD. F. Walker, G. From, R. Khanna, G.E. Digenis, and D.G. Oreopoulos. Dept. of Medicine, Toronto Western Hospital, Toronto, Canada.

A variety of abnormalities of thyroid function have been described in patients with chronic renal failure and those undergoing hemodialysis. In contrast the long-term effect(s) of CAPD on thyroid function is unknown. The clinical records of 104 (54 men, 50 women) patients were examined retrospectively. Thyroid function tests (TSH, T_3 resin uptake and total T_4 were available prior to commencing CAPD and at 3 month intervals during treatment, the duration of which ranged from 6 to 54 (average 19) months. During the last year of study free thyroxine (Free T_4) measurements were also done whereas total T_3 was measured only once, during treatment in most patients.

Prior to CAPD 14 patients were treated with L-thyroxine and an additional 13 patients had elevated TSH but normal all other indices. T_3RU was increased in 11 patients and normal in the remainder. Total T_4 and free T_4 were normal.

During CAPD mean TSH increased significantly ($p=.01$) and an additional 7 patients, whose T_4 and Free T_4 were also decreased, had to be started on treatment. In patients in whom T_3RU was increased prior to CAPD this returned to normal during CAPD. Total T_4 F.T.I. remained normal. Total T_3 was low in 60% of the euthyroid patients in whom it was measured.

In conclusion we observed a high incidence (14%) of hypothyroidism among patients starting CAPD. During CAPD, TSH increases significantly and an additional 7% of the patients developed hypothyroidism requiring treatment. The pathogenesis for this elevated TSH is not known.

A SHEEP MODEL TO STUDY CARDIO-PULMONARY EFFECTS OF BLOOD-DIALYZER INTERACTIONS. J.F. Walker*,R.M. Lindsay, W. Sibbald*,S. Peters*,& A.L. Linton. The University of Western Ontario, London, Canada.

Acute chest pain, hypotension, & dyspnea occasionally occur during hemodialysis; cardio-pulmonary arrest has been reported. This syndrome may be related to the hypoxemia & neutropenia that uniformly occur with cuprophan hemodialysis. The cardio-pulmonary response to blood-dialyzer membrane interactions was studied in Suffolk sheep to see if comparable phenomena occur.

Using an ex-vivo circuit blood was exposed to different dialysis membranes (cuprophan, regenerated cellulose, cellulose acetate and polyacrylonitrate (PAN)-in sheet or hollow fibre form) for varying periods of time (10,20,30, mins.). Increasing doses (5,10,15,25 ml) of this exposed blood were reinjected into animals and the effects on mean pulmonary artery pressure (PAP), cardiac output (CO), peripheral neutrophil count and PAO2 were followed.

With cuprophan hollow fibres 5 mls of blood exposed for 10 mins produced no response whereas 25 ml produced a fourfold rise in PAP, a 50% reduction in CO, a marked neutropenia & subsequent hypoxia. With increasing duration of exposure these effects were magnified. Sheet cuprophan gave less effect than fibre. With cellulose acetate and PAN very little effect was seen; regenerated cellulose gave a maximum response with minimal doses & exposure times.

This response was both reproducible in the same sheep and in different animals. The model, thus, produces comparable phenomena to clinical hemodialyses even to the occurrence of a catastrophic reaction & may be used to study the mechanisms involved.

UNEXPLAINED RED BLOOD CELL MICROCYTOSIS IN CHRONIC HEMODIALYSIS. J.A. Walker, E.R. Hwang*, R.A. Sherman, R.P. Eisinger, M.J. Nissenblatt*. Department of Medicine, UMDNJ-Rutgers Medical School, Piscataway, New Jersey.

Anemia associated with end-stage renal disease (ESRD) typically is normocytic. When red blood cell microcytosis (RBCM) occurs, etiologies include iron deficiency, sideroblastic anemia, chronic infection, and aluminum (Al) toxicity.

We investigated 8 of our ESRD patients with microcytic anemia (mean corpuscular volume (MCV < 80 femtoliters (fl)) whose mean MCV was 74.4 fl (range 60-79). All received chronic hemodialysis (CHD) 12 hours weekly using dialysate water treated with cation exchange, charcoal filtration, and reverse osmosis. All had adequate bone marrow iron stores without ringed sideroblasts, normal serum ferritin levels, and normal serum Al levels (20.6 ± 6.8 µg/dL). No chronic infection or hypophosphatemia was present. Prior to initiating CHD, the mean MCV of these patients was 88.4 fl (range 80-95). Over 31 ± 23 months of CHD, the mean decrement in MCV was 14 ± 5.7 fl, but there was no relationship between duration of CHD and the magnitude of decline in MCV. There was no difference between these patients with RBCM and those without in the causes of ESRD, duration of CHD, dialyzing equipment, or degree of anemia. Dialysate water levels of 18 trace elements were within ASAIO limitations.

The incidence of unexplained RBCM at our unit was greater than that at two nearby CHD facilities (28% vs. 9% and 9%).

These data demonstrate that some ESRD patients develop RBCM not due to presently recognized causes, and suggest that a local toxin may be responsible.

FACTORS AFFECTING POTASSIUM (K) REMOVAL DURING HEMODIALYSIS. Richard A. Ward, Terry E. Williams* and Ronald L. Wathen. Univ. Louisville Sch. Med., Louisville, Kentucky.

Acid-base changes and glucose loading affect the intra-body distribution of K. We studied the effects of these phenomena on K removal during dialysis. Seven patients were dialyzed for one week against each of four dialysate formulations; acetate (A), acetate plus glucose (AG), bicarbonate (B), and bicarbonate plus glucose (BG). Acetate and bicarbonate concentrations were 35 mmol/l, glucose, when used, was 11.1 mmol/l, and all dialysates contained 2 mmol/l K. Net removal of K and glucose was obtained by integrating serial dialysate mass flux versus time data for the 4.5 hours of dialysis. The contributions of intra- and extracellular compartments to total mass removed were assessed by measuring total body water, assuming 42% to be intracellular, and taking pre- and postdialysis serum levels as representative of extracellular concentrations at these times. Changes were assessed in pre- and postdialysis pH. While the pre- and postdialysis concentrations of K were unaffected by the dialysate used, the total amount removed varied. Significantly more K was removed with A (84.8±6.0 mmol) and B (78.9±7.2 mmol) than with AG (67.3±5.3 mmol) and BG (60.1 ±6.4mmol). Although pH changes were greater with B and BG than with A and AG, they had no significant effect on K removal. Depending on the dialysate, 53-70% of K removed derived from intracellular stores. These results indicate that changes in serum K are a poor guide to dialytic removal. Maintenance of proper K homeostasis is dependant on selecting appropriate dialysate levels of K and glucose.

USEFULNESS OF HEMODIALYSIS THERAPY IN CYANIDE (CN) INTOXICATION. DE Wesson*, RJ Foley*, NA Kurtzman, and S Sabatini. Univ of Illinois, Chicago IL.

A dramatic response was noted in a patient at our hospital who received hemodialysis therapy (HD) for severe acidosis secondary to an unknown toxin, subsequently identified as CN. While the HD clearance of many toxins is well known (eg, thiocyanate, 178 ml/min), there is no available information concerning the HD clearance and extraction ratio of CN. We studied the effect of HD in 3 dogs receiving a constant infusion of IV CN (0.1 mg/kg/min) with and without thiosulfate infusion (12 mg/kg/hr) at a constant blood flow (145±5 ml/min) and varying transmembrane pressures (range 175-300 mm Hg). The HD clearance during constant CN infusion was 38.7±4.3 ml/min. The extraction ratio was 0.43±0.05. HD alone increased the total lethal dose from 2.4 mg/kg to 4.0 mg/kg. The continuous infusion of thiosulfate, in addition to HD, caused a further increase in the total lethal dose to 6 mg/kg. The lethal dose reported with thiosulfate infusion alone is 3.4 mg/kg. This study represents the first demonstration of the effectiveness of HD in cases of cyanide toxicity. The added effectiveness of thiosulfate administration is likely the result of increased conversion of CN to thiocyanate which is 5x more dialyzable and is less toxic. Based on these observations, we recommend that HD be considered as a primary form of therapy in suspected cases of CN intoxication. The dramatic response in our patient, coupled with the demonstration of a significant HD clearance of CN, suggests that hemodialysis played an important role in his recovery.

EFFECT OF VITAMIN B_6 DEFICIENCY ON PLASMA AMINO ACIDS IN CHRONICALLY UREMIC (U) and SHAM (S) RATS. M. Wolfson, M.R. Jones,* R. Flugel-Link* J.D. Kopple. Portland VAMC and Oregon Health Sciences Univ., Portland, Ore. and Harbor-UCLA Med. Center, Torrance, Cal.

Vitamin B_6 [e.g. pyridoxine (Pyr)] is a cofactor for many biochemical reactions involving amino acids (AA). Pyr deficiency is not uncommon in U patients who do not receive Pyr supplements, and it is reported that supplemental Pyr increases plasma AA in U patients. Since many plasma AA are increased in non-U Pyr deficient rats, we evaluated the relationship between Pyr deficiency and plasma AA in chronically U and S rats. Male albino U and S rats were randomly assigned Pyr deficient(-) and Pyr replete(+) diets (22 mg Pyr/kg diet). There were 6-7 rats in each of the 4 groups. All diets were given for 20 days; three groups were pair-fed to U rats fed the Pyr- diet. EGOT index increased in the U and S rats fed the Pyr- diet (4.8±1.3 SD vs 2.9±.6, $p<.001$) indicating Pyr deficiency. Plasma creatinine was increased in both groups of U rats vs S rats(.63±.26 mg/dl vs .34±.06, $p<.001$). In U vs S Pyr- rats most individual plasma AA were similar. In U and S Pyr- rats vs U and S Pyr+ rats there was a significant increase in each essential AA, except lysine and phenylalanine, and in tyrosine, ornithine and total AA. There were fewer differences in plasma AA between all U rats vs all S rats. Thus, the altered plasma AA levels in U rats do not appear to be due to Pyr deficiency. From these data, Pyr deficiency in U would be expected to raise not lower plasma AA, presumably due to impaired AA metabolism.

BLOOD VISCOSITY (V), SYSTEMIC VASCULAR RESISTANCE (SVR) AND BLOOD PRESSURE (BP) CHANGES DURING HEMODIALYSIS (HD). C. Williams*, M. Rodriguez*, O. Llan de Rosos*, J. Pederson, L. Rankin, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

During HD, a decrease in BP occurs due to both a decrease in plasma volume and a lack of appropriate increase in SVR. Viscosity is an important factor in SVR. This study evaluates the role of V on changes in SVR during HD-induced hypotension. Five patients (pts) underwent HD during 3 hrs and 3.4±.4 L of fluid were removed. Hemodynamic parameters evaluated by invasive technique were: Systemic, arterial and filling pressures, heart rate and cardiac index (CI). A rotational viscometer measured V at 450 shear rate. Mean changes (±SE) in mean arterial pressure (MAP), CI, SVR, and V during HD were:

Time (Min)	0'	60'	120'	180'
MAP	132 ± 5	125 ± 7	116* ±10	101** ±7.5
CI	4.4 ±.4	3.6* ±.3	3.0** ±.2	3.0** ±.4
SVR	2361 ±268	2695 ±157	3022** ±376	2726 ±464
V	3.49 ±.23	3.81 ±.29	4.08 ±.32	4.32** ±.30

*p<.04; **p<.01

In summary, there was a progressive decrease in BP and CI as well as an increase in V which was most pronounced at 180', and an increase in SVR most pronounced at 120'. In conclusion, in HD-induced hypotension, despite an increase in V, there is an insufficient response in SVR for the decrease in CI. This suggests relaxation of vascular tone.

UPRIGHT POSTURE AND EXERCISE LOWER PERITONEAL TRANSPORT RATES. S Zonozi‡ JF Winchester, N Kloberdanz‡ H Preuss, S Fox‡ C Crocker‡ K Sanders‡ W Barnard‡ L Fox‡ Georgetown U, Wash DC, and JF Maher, Uniformed Services U, Bethesda, MD.

Continuous ambulatory peritoneal dialysis (CAPD) involves erect posture and exercise, but their effect on transport kinetics has not been studied. To test effects on water and solute transport, 12 CAPD patients had timed hourly dialysate exchanges when randomly supine(S), upright(U), and while U exercising(X). Treadmill X was graded to 30% of capacity. For each period time(t) dialysate volume(V) ultrafiltration rate(UF), dialysate/plasma concentration(D/P) and clearance(C) of urea(Ur), creatinine(Cr) and phosphate(PO_4) and dialysate(in-out)/in $((D-D_t)/D)$, and C glucose(G) were measured. When S, mean C were Ur 18.1, Cr 13.5, PO_4 7.9 and G 10.4 ml/min, C tended to decrease when U or X but not by paired t test. Blood pressure did not change. V and t varied in study periods ($P<0.05,<0.02$), with incomplete collections without any UF influence. So data were analyzed as D/P with t adjusted

Mean D/P	S	U	X	p+	p++
Ur	0.44	0.33	0.35	0.01	0.01
Cr	0.33	0.28	0.29	0.01	0.01
PO_4	0.20	0.17	0.16	0.18	0.11
$(D-D_t)/D$	0.26	0.19	0.20	0.07	0.01

+ Comparing S and U: ++ Comparing S and X.

Since X did not lower transport more than U, X was raised to 50% of capacity in 5 patients, but neither D/P nor C decreased further. The data suggest that studies when S do not indicate transport when U or X. Lower transport when U or X may reflect lower splanchnic blood volume (peripheral pooling)or reduced dialysate surface contact.

Hypertension

CALCIUM BALANCE AND PTH-INDUCED VASODILATION IN THE SPONTANEOUSLY HYPERTENSIVE RAT. S. Anderson*, J. Grady*, D. Ellison, S. Hann*and D. McCarron. Division of Nephrology, Oregon Health Sciences University, Portland, Oregon.

Human PTH (1-34) produces log-dose ($p<.001$) dependent hypotension in the SHR. We assessed the influence of alterations in Ca^{2+} balance on the SHR's vasodepressor response to hPTH (1-34). Male SHRs raised on a 1% Ca diet, were switched at 16 weeks to either a 4% or a 0% Ca^{2+} diet. Timed urines and blood samples were obtained. After at least two weeks on the diets, they received graded IV infusions of hPTH (1-34) at doses between 1 and 100 µg/kg.

On the 4% diet serum total (T) Ca ($p<.001$) and $U_{Ca}V$ ($p<.001$) rose, serum Ca^{2+} was unchanged and uCAMP fell ($p<.001$). The 0% SHR's TCa was unchanged, while serum Ca^{2+} increased, ($p<.05$). $U_{Ca}V$ declined ($p<.001$) and cAMP increased. At baseline, mean arterial pressure (MAP) was higher ($p<.005$) in the 0% SHR (198±5) compared to 4% SHR (180±8 mmHg). In both groups, hPTH (1-34) produced log-dose dependent hypotension ($p<.001$), maximal at 1 min. Compared to 4% diet SHR's, the 0% SHR's log-dose curve was shifted to the right, i.e. reduced sensitivity. In addition, the 4% SHR demonstrated an enhanced ($p<.01$) maximal response to the hPTH (1-34) and a prolongation ($p<.01$) of the hypotensive action.

In conclusion, the PTH-induced vasodepressive response of the SHR is, in part, dependent upon the state of Ca^{2+} balance. The enhancement by the 4% diet and depression by 0% presumably reflects alterations in membrane and/or intracellular Ca^{2+} as it can be dissociated from changes in extracellular ionized Ca^{2+}.

THE ROLE OF THE RENIN ANGIOTENSIN SYSTEM IN COARCTATION HYPERTENSION. Michael D. Bailie, Norberto Gonzalez,* and Vicki Donoso.* Univ. of Kansas Medical Center, Depts. of Pediatrics and Physiology, Kansas City, Kansas.

The role of the renin-angiotensin system (RAS) in coarctation hypertension (CoA) remains unclear. We studied blockade of the RAS with Captopril (C) delivered by intraperitoneal minipump on CoA in the rat. Four groups of animals were studied: Group I received C for 2 days prior to CoA. II was a control for I (CoA without C). III underwent CoA then received C beginning 12 days later. IV was the control for III. Plasma renin activity (PRA), angiotensin I (AI) and angiotensin II were measured before, during and after C and CoA. At the end of the experiment, animals were lightly anesthetized and carotid (CBP) and femoral (FBP) arterial blood pressure determined. In Group I PRA increased 10-fold and AI 2-fold after C. In Group III PRA increased 5-fold and AI 2-fold after C. CoA alone did not cause an increase in PRA or AI. In Group I, CBP rose to 151±6 mmHg; significantly less than in II, III and IV (185±5, 175±6, 175±9 mmHg). The difference between CBP and FBP was also less in I (33±3 mmHg) than in II, III and IV (48±9, 48±7, 39±5 mmHg). In Group I and III blockade by C was demonstrated by reduced response to i.v. AI. Thus, blockade of the RAS prior to the onset of CoA reduces the maximal elevation of BP (final CBP in Group I less than II, III and IV). We conclude that the RAS is important in the generation phase of CoA but not in the maintenance of the elevated BP. We note that the effect of C is seen (I vs III) although PRA is not elevated when C is not used (II and IV). The effect of C on bradykinin may also play a role in the hypertension of CoA.

VASCULAR REACTIVITY TO ANGIOTENSIN II (AII) IN CANINE NEONATALLY-INDUCED COARCTATION HYPERTENSION (NICH). S.P. Bagby, G.M. Baur,* Portland VA Med Ctr and OR Health Sci Univ, Portland, OR.

Inbred dogs with NICH exhibit a small (4-5%) volume excess and plasma renin activity (PRA) similar to littermates, thus an abnormal renin: volume relationship. To determine if the pressor impact of this apparently small defect is augmented by increased vascular reactivity to AII, we determined dose-response curves in 6 coarcted and 5 littermate control dogs at two years post-aortic-banding during ad lib diet (NS) and after low sodium diet for 4 days with furosemide (5 mg/kg IV b.i.d.) on days 1 and 2 (LS/Lasix®). Using indwelling catheters to monitor mean pressure (MAP) in proximal (brachial) and distal (abdominal aortic) sites, dose-response curves were based on 4-6 AII doses, each dose given twice. Slope (mmHg/log dose) and threshold (ng/kg/min) were derived from linear regression analysis. Results for brachial responses (mean ± S.D.) are:

	NS		LS/Lasix®	
	Coarcted	Control	Coarcted	Control
Slope	55±11	43±12	45±11	47±12
Threshold	8.2±1.2	5.6±4.4	12.8±5.7	14.5±5.4

Distal responses were identical. Coarcted dogs did not differ from littermate controls. Threshold values for all dogs were significantly higher during LS/Lasix® ($p<.02$) and correlated positively with PRA ($r = .71$, $p<.001$). Results suggest that increased vascular reactivity to AII is not characteristic of NICH; if secondary structural vascular changes occur, the expected slope increase is offset by other factors.

COMPARATIVE EFFECTS OF ENALAPRIL (MK-421) VS HYDROCHLOROTHIAZIDE ON RENAL FUNCTION. John H. Bauer, Louise B. Jones*, Dept. Medicine, Univ. Missouri Health Sciences Ctr., Columbia, Missouri.

A randomized, double-blind, study was performed in 27 patients with primary hypertension to evaluate the effects of hydrochlorothiazide (HCTZ; 25-50 mg bid) vs. Enalapril (MK-421; an angiotensin II converting enzyme inhibitor; 10-20 mg bid) on blood pressure, electrolytes, plasma volume (PV by ^{125}IHSA), extracellular fluid (ECF by $Na_2{}^{35}SO_4$), glomerular filtration rate (GFR by inulin clearance), effective renal plasma flow (ERPF by para-aminohippurate clearance), free water clearance (C_{H_2O}), and fractional excretion of sodium (FE_{Na}) and potassium (FE_K). Patients were studied following 4 wks placebo and 8 wks active drug therapy. Mean results are presented below:

	HCTZ (N=13)		MK-421 (N=14)	
	placebo	8 wk	placebo	8 wk
syst (mm Hg)	156	135¶	152	130¶
diast (mm Hg)	103	89¶	106	86¶
PV (L)	3.32	3.19†	3.84	3.81
ECF (L)	17.04	15.97†	18.91	18.77
GFR (ml/min/1.73 m^2)	83	84	79	76
ERPF (ml/min/1.73m^2)	324	324	332	360
C_{H_2O} (ml/min/1.73 m^2)	7.2	4.9†	6.2	7.8†
FE_{Na} (%)	1.8	2.8†	2.6	2.3
FE_K (%)	16.0	20.7†	28.3	25.1†
P_K (mEq/L)	4.1	3.3¶	3.9	4.2§

†$p<0.05$, §$p<0.01$, ¶$p<0.005$ compared to placebo

HCTZ and MK-421 were equally effective in lowering blood pressure. Patients on HCTZ developed volume contraction, hypokalemia, impaired C_{H_2O}, and increased FE_{Na} and FE_K. In contrast, patients on MK-421 experienced no change in volume, an increase in plasma potassium (P_K) associated with a decrease in FE_K, and an enhanced C_{H_2O}. MK-421 is an effective first-step antihypertensive agent without deleterious renal effects.

SYMPATHOLYTIC EFFECT OF A NON-CNS PENETRATING BETA BLOCKER(BB) IN HYPERTENSION IS UNMASKED BY PARASYMPATHETIC INHIBITION(CARDIAC VAGAL INHIBITION,CVI). KN Bernstein*,AP Barg*,J Cervenka*,DT O'Connor. VA Med.Ctr. and Univ. of California, San Diego, CA.

BB antihypertensive effects may involve changes in sympathetic(SNS)function, usually thought to be central(brain)action of BB. We studied effects of nadolol(N), a non-CNS penetrating BB, on physiologic indices of SNS function in 9 hypertensive men after 1 month placebo and after 5-7 weeks of once-daily N monotherapy. After each period, we assessed mean arterial pressure(MAP,in mmHg),heart rate(HR, in bpm),baroreflex sensitivity(BRS,ΔRR/ΔMAP,in msec/mmHg)by phenylephrine(PEB)and amyl nitrite (ANI)stimuli,CVI(by atropine,in msec)cold pressor test(CPT,ΔMAP in mmHg)pre and post CVI,and phentolamine αblockade(Phentol,ΔMAP in mmHg). Results as mean ± SEM,with paired t-test. Decrement in CPT post CVI did not correlate with MAP lowering by N (r=0.37,p= NS). We conclude:1) N achieved lowered MAP with an associated decline in CPT response, a physiological index of efferent SNS vasomotor function. 2) N achieved this sympatholytic effect only after CVI, suggesting that the effect is masked by intact parasympathetic nervous function. 3) N achieved the sympatholytic effect despite non-CNS penetration, suggesting a peripheral, efferent SNS site of action. 4) The sympatholytic effect did not correlate with N's MAP lowering action.

	Placebo	Nadolol	p
MAP	124±3	100±2	<0.005
HR	78±4	66±3	<0.005
BRS-ANI	7.9±0.8	6.1±0.6	<0.03
BRS-PEB	15.7±4.1	20.2±6.9	NS
CVI	381±48	476±37	NS
CPTpreCVI	18±3	14±4	NS
CPTpostCVI	18±3	9±4	<0.05
Phentol	21±2.8	26±4.1	NS

MECHANISM FOR THE SALUTARY EFFECTS OF HIGH DIET CA ON BLOOD PRESSURE IN SPONTANEOUS HYPERTENSIVE RATS (SHR). S. Chen*, B. Eby*, and K. Lau. Neph. Div., Dept. of Med., Univ. of Mich., Ann Arbor, MI.

Earlier work suggests that high Ca diet lowers blood pressure (B.P.) in SHR. To examine the role of parathyroid hormone (PTH) and volume depletion due to hypercalcemia-induced diuresis studies were done on SHR and normotensive Wistar Kyoto (WKY) rats fed a Na supplemented (1.1%) diet to prevent volume contraction. At 8 weeks of age, B.P. was measured daily by tailcuff method. When stable, they were fed either a high (4.3%) or low (0.23%) Ca diet. Low Ca diet was associated with the expected rise in B.P. in intact SHR (158 to 184 torrs, $p < .01$), whereas high Ca diet increased plasma Ca (13.5 vs 11.1 mg%) but reduced B.P. (165 vs 184 torrs) despite comparable weight gain (20 vs 24 gm). Similarly, in stable chronic parathyroidectomized (PTX) SHR rats, high Ca diet increased plasma Ca (10.7 vs 7.5 mg%), but kept B.P. constant (162 to 162 torrs), vs the increase (153 to 168 torrs) with low Ca diet. Although on low Ca diet, B.P. was higher in intact than PTX SHR (184 vs 168, $p < .03$), their weight gain was also greater (25 vs 10 gm). Thus, as weight gain was similar (20 vs 21 gm) on high Ca diet, B.P. was similar, regardless of the presence or absence of PTH (165 vs 162 torrs). In WKY rats, at comparable weights, B.P. (122 vs 139 torrs, $p < .05$) was also reduced by high Ca diet as plasma Ca was raised (12.7 vs 11.1 mg%). Conclusions: (1) The antihypertensive effect of high Ca diet appears to depend on a relative increase in plasma Ca. (2) It is independent of (a) baseline B.P., (b) changes in body weight, and (c) the presence of PTH. (3) An effect of PTH per se on B.P. cannot be demonstrated.

EVIDENCE FOR PRIMARY CELLULAR VS HUMORAL CAUSE FOR THE SODIUM TRANSPORT ABNORMALITY OF RED BLOOD CELLS (RBC) OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR). J. W. Boylan, P.P. Mitchell*, M.A. D'Occhio*, and P.U. Feig. Dept. of Medicine, U. of Conn. Health Center, Farmington, CT and VA Med. Center, Newington, CT.

It has been suggested that the abnormalities of cellular sodium transport observed in essential hypertension are due to humoral factors. Abnormalities in sodium transport have also been found in cells of SHR compared to normotensive Wistar-Kyoto (WKY) rats. We previously reported higher unidirectional ^{22}Na efflux in RBC's of SHR vs WKY. To assess the role of plasma factors, we studied 5 pairs of SHR and WKY in which this flux difference was demonstrated. In this study, k was determined in RBC's after 5 hours of incubation in plasma at 37° C, at 25% Hct., according to the following protocol: A- WKY cells in WKY plasma; B- WKY cells in SHR plasma; C- SHR cells in WKY plasma and D- SHR cells in SHR plasma. Results of the rate constant k are given in hr^{-1}, as means±SEM:

A	B	C	D
1.42±.05	1.42±.11	1.69±.11	1.61±.08

Both A and B were significantly different from both C and D but there were no differences between A and B or between C and D (paired Student t test). The same qualitative results were also found if k was measured in presence of ouabain (10^{-4}-10^{-3}M).

We conclude that the difference in RBC sodium transport between SHR and WKY cannot be induced by short-term cross-incubation of the cells and is therefore more likely due to primary cellular than to humoral factors.

EFFECTS OF ACUTE RENAL DENERVATION ON KIDNEY FUNCTION IN DOCA HYPERTENSIVE SWINE. Charles D. Ciccone* and Edward J. Zambraski, Rutgers Univ., Physiology, New Brunswick, New Jersey.

In long term (4-5 month) DOCA treated hypertensive Yucatan miniature swine (YMS) renal vascular resistance (RVR) is elevated. Glomerular filtration rates (GFR), renal blood flow (RBF) and sodium excretion are normal despite renal perfusion pressure being 40-60 mmHg higher than normal. The purpose of this study was to determine the role of the renal nerves in controlling renal function in DOCA hypertensive YMS. Ten YMS were treated with DOCA (100 mg/kg) for 4-5 months. Mean arterial pressure (MAP) (conscious) increased to 164±4 mmHg (120 mmHg-control). Under phentobarbital anesthesia renal hemodynamics and electrolyte excretion were assessed before and after acute left kidney surgical denervation. (RBF (ml/min/gm)-electromagnetic flowprobe, GFR (ml/min)-inulin clearance; U-VOL-urine volume (ml/min); UnaV and UkV-sodium and potassium excretion (μEq/min)) MAP was lowered to 131±4 mmHg by anesthesia. All data are from the left kidney (†P<0.05 vs control).

	MAP	RVR	RBF	GFR
CONTROL	131±4	47.5±6.1	3.1±3	44±7
DENERVATION	†137±4	†37.0±3.9	†4.0±.4	†55±5

	U-VOL	UnaV	UkV
CONTROL	.46±.09	44.8±11.3	28.0±6.3
DENERVATION	†.84±.20	†81.7±24.9	36.0±6.3

In the intact right kidneys no increase in RBF, GFR, diuresis or natriuresis occurred. These data demonstrate that the renal nerves are important in controlling renal hemodynamics and sodium excretion in DOCA hypertensive Yucatan miniature swine.

CURE OF "ESSENTIAL HYPERTENSION" (EH) BY SUCCESSFUL RENAL TRANSPLANTATION (TX) IN BLACKS. J. Curtis, H. Dustan, M. Kashgarian, A. Diethelm, J. Whelchel, P. Jones and R. Luke. Univ. of Alabama, Birmingham, Alabama and Yale Univ., New Haven, Conn.

The effect of Tx on the EH associated with ESRD due to nephrosclerosis (NS) was studied in 6 black patients (EHTX). EH was confirmed by history and gross, microscopic, and ultramicroscopic exam of both native kidneys which showed no primary renal disease and severe arteriolonephrosclerosis. Results 4.7±1 years after Tx were compared to 6 age- and sex-matched black control patients (CG).

	Age(yrs)	MAP(mmHg)	Ccr(ml/min)	
EHTX	40 ± 2	92 ± 1.7	94 ± 8*	*P < 0.05
CG	40 ± 0.5	90 ± 1.0	113 ± 9*	

Before Tx, highest MAP of patients averaged 168 ± 8 mmHg and all had LVH and retinopathy. After Tx, BP was normal (92±1.7) without LVH or retinopathy. All patients and CG were admitted to the CRC for studies on normal, low (9 mEq/d) and high (4 mEq/kg/d) sodium intake of plasma volume (PV), renin activity (PRA), aldosterone (PAC) and catecholamines (PNE and PE). EHTX patients responded just as did the CG, for example:

		norm. Na	low Na	high Na
PRA	EHTX	0.3±.2	2.9±.7	0.1±.09
ng/ml	CG	0.4±.2	2.7±1.3	0.1±.04
PAC	EHTX	82±36	461±84	61±17
pg/ml	CG	84±27	433±75	85±17
PV	EHTX	1.5±.06	1.3±.06	1.7±.09
L/M^2	CG	1.4±.08	1.3±.06	1.7±.1

We conclude: (1) successful Tx can result in normal BP in blacks with EH and NS; (2) after Tx, such patients respond normally to decreases and increases of sodium intake.

HYPERTENSION, A LATE CONSEQUENCE OF KIDNEY DONATION. B.G. Delano, I.L. Lazar*, E.A. Friedman. Downstate Medical Center, Brooklyn, N.Y.

Donation of a kidney for transplantation induces no long term risk to the donor according to actuarial studies of the ensuing 6 yrs. Recently, however, Brenner hypothesized, based on studies of rat models of renal injury or reduced renal mass, that glomerular hyperfiltration may over the long term result in structural injury to glomeruli.

To evaluate the effect of a 50% reduction in nephrons in man, we examined 15 patients who had 1 kidney removed from 9 to 62 years previously. Thirteen patients donated a kidney for transplantation 9 to 19 years prior to examination. The mean age of this group was 49yrs (range 31 to 73yrs). One 62 yr old man had a unilateral multicystic kidney removed shortly after birth. Nine of the 15 patients (60%) were hypertensive (BP > 150/100mmHg) a finding not present at the time of kidney donation. Listed in the table are the blood pressure (BP), creatinine clearance (Ccr), and 24 hr urine protein excretion of the hypertensive group.

	PATIENT	AGE	BPmmHg	CCr	PROTEIN
1	W.M.	59	180/120	68 ml/min	582 mg
2	L.K.	62	160/100	90 ml/min	341 mg
3	G.M.	47	190/110	90 ml/min	15 mg
4	E.M.	54	150/100	83 ml/min	226 mg
5	D.S.	49	160/100	130 ml/min	203 mg
6	J.M.*	37	154/96	64 ml/min	230 mg
7	L.L.	73	170/100	108 ml/min	78 mg
8	W.D.	44	180/110	57 ml/min	598 mg
9	A.S.*	53	150/104	104 ml/min	321 mg

* On Antihypertensive therapy

The discovered prevalence of hypertension exceeds that of age, sex, and cadaver matched controls. We conclude that renal donation may predispose to hypertension and a larger series of donors should be studied.

RENIN ACTIVITY AND HEMODYNAMIC RESPONSE TO ISOLATED ULTRAFILTRATION IN CONSCIOUS, UREMIC DOGS. J.T. Daugirdas, T.S. Ing, R.R. Al-Kudsi, J.E. Hano. Hines-Loyola Medical Center, Hines, Illinois, USA.

Controlled volume depletion was effected by isolated ultrafiltration in 20 normal (N) and 20 uremic (U), splenectomized dogs, weighing 20-30 kg. Of the uremic dogs, 15 had undergone bilateral ureteral ligation (BUL) and 5 had bilateral nephrectomy (BN). Uremic animals were studied 1-2 weeks after the above surgery, having been supported by daily hemodialysis. Removal of plasma water, at a rate of 600-800 ml/hr, was continued until mean arterial pressure (MAP) decreased to less than 80 mm Hg. Hemodynamic indices and level of plasma renin activity were monitored.

Initial thermodilution cardiac indices were similar in the normal and uremic dogs (N: 4.65 ± 0.5, U: 4.35 ± 0.6 $L/min/M^2$, p=NS), but MAP (N: 110 ± 9.6, U: 135 ± 14.3 mm Hg, $p < 0.001$) and total peripheral resistance index (TPRI; N: 1924 ± 390, U: 2340 ± 420 $dyne \cdot sec/cm^5/M^2$, $p < 0.01$) were higher in uremic dogs. By the end of volume depletion, at end-point MAP less than 80 mm Hg, TPRI had increased to similar levels in both N and U dogs (N: 3307 ± 740, U: 3412 ± 760, NS). End-point blood volumes were also comparable. Heart rate, however, was increased to a greater extent in the uremic animals (N: 110 ± 35, U: 144 ± 30 beats/min, $p < 0.05$). The response of BN dogs was similar to that of BUL animals.

End-point plasma renin activity was 17 ± 14 ng AI/ml/hr in N, vs 2.8 ± 2.3 in U ($p < 0.01$).

Our results suggest that in dialyzed, uremic dogs, response to fluid removal resembles that in normal dogs (changes in heart rate excepted), despite diminished plasma renin activity.

VASOPRESSIN MAINTAINS ARTERIAL PRESSURE IN ADRENALECTOMY. Fernando Elijovich,* Madeleine Kirchberger,* Catherine R. Barry,* and Lawrence R. Krakoff,* (intr. by Robert Safirstein). The Mount Sinai School of Medicine, C.U.N.Y. New York, New York.

Changes in mean arterial pressure (MAP) produced by an antagonist of the vascular action of vasopressin (dPMeTyrAVP) followed by captopril (CAP) were studied in adrenalectomized (AX) and control (C) rats, which were either unanesthetized (U) or anesthetized with pentobarbital (P).

Animals were maintained in metabolic cages for 72 hrs before study, on 1% saline-2.5% dextrose.

AX had significant hyponatremia, hyperkalemia and lower serum osmolality as compared to C. Urinary K excretion was significantly lower in AX than in C. AX rats were in Na balance after 72 hrs while C showed a mild Na retention. Osmolar clearance and tubular water reabsorption were not different between AX and C. dPMeTyrAVP (50 µg/kg iv) significantly reduced MAP in PAX (17 ± 4 mmHg) and UAX (9 ± 1 mmHg) while it did not modify MAP in UC (0 ± 2 mmHg). In PC, dPMeTyrAVP reduced MAP by 7 ± 1 mmHg ($p<0.01$), an effect significantly smaller that that in PAX. CAP (1 mg/kg iv) given after dPMeTyrAVP significantly reduced MAP in PAX (50 ± 8 mmHg) and UAX (17 ± 3 mmHg) while it did not modify MAP in UC (0 ± 1 mmHg). In PC, CAP reduced MAP by 32 ± 3 mmHg ($p<0.01$).

These data demonstrate that: a) endogenous vasopressin participates in arterial pressure regulation in adrenalectomy and b) pentobarbital anesthesia augments the blood pressure dependency of adrenalectomized and control rats upon both vasopressin and the renin-angiotensin system.

RENAL AND CARDIOVASCULAR RESPONSE TO Ca^{2+} INFUSION AT VARYING Na^+ INTAKES IN HYPERTENSIVE HUMANS. D.H. Ellison and D.A. McCarron. Div. of Nephrology, Ore. Hlth Sci. Univ., Portland, OR.

Altered renal and vascular responses to Ca^{2+} have been reported in human hypertension (HTN). We measured BP; serum ionized Ca^{2+}, Na^+, K^+; urinary Ca, Na, K, cAMP; inulin (C_{IN}) and PAH (C_{PAH}) clearances before, during and following a 3-hour Ca^{2+} infusion (15 mg/kg) in 4 matched normal (NL) and 4 HTN subjects in balance on 3 Na^+ intakes (10 mEq/d, 150 mEq/d, 500 mEq/d).

HTN's serum Ca^{2+} (2.02±.01 mEq/l) was lower ($p<.01$) than NL's (2.12±.01 mEq/l) and their uCAMP higher ($p<.01$) on all Na^+ intakes. At baseline, C_{IN} (92±14 ml/min NL; 130±22 HTN), C_{PAH}, fractional excretion (FE) Na and FE_{Ca} (0.35±.1 NL; 0.32±.2 HTN) were similar for NL and HTN. Ca^{2+} infusion, increased serum Ca^{2+} (0.39 mEq/l HTN; 0.33 mEq/l NL) similarly. GFR and RBF were not changed. With the Ca^{2+} infusion, FE_{Na} rose ($p=.07$) in both groups. While FE_{Ca} increased for both NL and HTN, at all 3 Na^+ intakes the NL's FE_{Ca} increased more ($p<.05$). During the first 30 min of Ca^{2+} infusion, BP of HTN increased (147±2/91±1 → 162±2/97±2: $p<.05$). HTN's BP then returned to baseline ($p<.05$) inspite of progressive hypercalcemia. NL's BP were unchanged (112±1/71±1).

In conclusion, HTN demonstrate abnormalities of Ca^{2+} metabolism at baseline and altered renal and cardiovascular responses during acute hypercalcemia. The HTNs' initial pressor response can be dissociated from absolute changes in serum Ca^{2+} and Na^+ balance. HTNs' reduced FE_{Ca} during hypercalcemia is independent of Na^+ balance and not related to differences in GFR and RBF.

DO SMALL CHANGES IN SODIUM ACCOUNT FOR THE ATTENUATION OF THE BLOOD PRESSURE RESPONSE TO CAPTOPRIL? Roger K. Ferguson, Heschi H. Rotmensch,* Peter H. Vlasses* and Brian N. Swanson*, Jefferson Medical College, Philadelphia, Pennsylvania.

We and others have found that patients with marked ($\geq$10%) reductions in blood pressure after an initial dose of captopril (CAP) frequently manifest attenuation of this response with repeated dosing in the absence of changes in body weight or plasma volume. Because small, subtle changes in sodium (Na^+) might account for this attenuation in responsiveness to CAP, we studied the effect of i.v. Na^+ (75 mEq) on the acute response to CAP (25 mg) in 8 hypertensive patients who had previously manifested $\geq$10% decrease in seated diastolic blood pressure (SDBP) after CAP. After 2 weeks off all drugs and on their usual Na^+ diet, each patient received in random order one week apart each of the following treatments: (1) 150 ml D5W + CAP, (2) 150 ml 3% saline + placebo and (3) 150 ml 3% saline + CAP. Lying and sitting blood pressure were measured (DINAMAP®) before and at regular intervals for 6 hours after drug administration. Venous blood for the measurement of plasma renin activity (PRA), plasma aldosterone concentration (PAC) and angiotensin converting enzyme (ACE) activity was collected before and after each infusion and at 1.5 and 6 hours after placebo or CAP. Urinary Na^+ excretion 24 hours before each treatment was comparable. Compared to Na^+ + placebo, both D5W + CAP and Na^+ + CAP produced a significant ($p < 0.05$) decrease in SDBP. CAP, with both D5W and Na^+, caused an increase in mean PRA and significant ($p < 0.05$) decreases in mean PAC and ACE. Thus, small amounts of i.v. Na^+ had little influence on the acute response and does not account for the attenuation.

MEMBRANE PROTEIN CONTENT, SURFACE AREA AND SODIUM FLUX IN RED BLOOD CELLS (RBC) OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Peter U. Feig, Peter P. Mitchell,* and John W. Boylan. Dept. of Medicine, U. of Connecticut Health Center, Farmington, CT and VA Medical Center, Newington, CT.

Several cellular sodium transport abnormalities have been found in human essential hypertension and in SHR. We have previously shown that the rate constant of unidirectional ^{22}Na efflux (k) is higher in RBC's of SHR than of normotensive Wistar-Kyoto (WKY) rats (Clin. Res. 335A, 1982). Since an increase in transporting surface per cell volume could be the cause of the increased k, we determined in RBC's of 5 pairs of WKY and SHR the mean corpuscular volume (MCV), and in RBC ghosts the integral membrane protein (IP) content and surface area per ml RBC. Surface area was determined by phospholipid (PL) content and IP by treatment of the ghosts at pH=11.3 and ultracentrifugation. Results are given as means ± SEM, p by paired Student t test:

	k (hr^{-1})	MCV (μ^3)	PL ($\mu M\ P_i$)	IP (mg)
WKY	1.266±.057	52.9±1.2	4.92±.25	3.93±.12
SHR	1.481±.113	48.1±1.3	5.37±.24	4.41±.13
P	0.027	0.034	0.014	0.003

MCV was smaller in SHR, with an increase in PL and IP per cell volume. PL and IP per cell were identical in WKY and SHR. The k correlated with IP per cell volume (r=0.76, p=0.011). When k is factored for IP per ml cell, the flux differences between WKY and SHR disappear (0.33 vs 0.34, p=0.25).

We conclude that the increased rate constant for sodium efflux in RBC's of SHR is due to a decreased cell volume, with maintainance of cell surface area such that membrane surface area and integral membrane protein per volume of cells become increased.

GREATER RENAL VASOCONSTRICTION IN BLACK PATIENTS WITH ESSENTIAL HYPERTENSION. Edward D. Frohlich, Franz H. Messerli, Francis G. Dunn*, Wille Oigman*, and Hector O. Ventura*. Ochsner Medical Institutions, New Orleans, Louisiana.

Epidemiologic and clinical data suggest more severe hypertension in black patients with essential hypertenion (EH). However, systemic hemodynamic studies indicated that for any level of mean arterial pressure (MAP) total peripheral resistance (TPR), the hemodynamic hallmark of EH, was similar in age,sex, and MAP-matched black and white patients. This was confirmed in this study of 60 EH patients, 30 black and 30 white (15 men and 15 women in each racial group), using intra-arterial pressure and indocyanine green dye (IGD; cardiac output measurements). Splanchnic hemodynamics (IGD) were likewise similar. However, covariance analysis (to eliminate factors of age, sex, body surface area) revealed that renal blood flow (RBF I^{131}-p-aminoiodothalamate) was reduced ($p < 0.003$) and renal vascular resistance (RVR) was increased ($p < 0.002$) in blacks. In contrast with splanchnic hemodynamics, a highly significant relationship was demonstrated between the level of MAP ($r=.481$; $p < 0.0004$) and TPR ($r=.329$; $p < 0.0037$)and the degree of renal vasoconstriction in both racial groups. Moreover,the higher the MAP the lesser was RBF in the blacks ($r=.322$;$p < 0.047$) and the greater was RVR ($r=.592$;$p < 0.0004$), a significant difference between the two racial groups ($p < 0.05$). These findings lend credence to those reports suggesting that at any level of MAP hypertensive renal vascular disease and morbidity are more severe in black patients even though systemic and splanchnic hemodynamics may be no different from matched white patients with EH.

IDENTIFICATION OF A HEPTAPEPTIDE WITH DIGITALIS AND NATRIURETIC HORMONE LIKE PROPERTIES. K.A. Gruber, J.F. Hennessy*, V.M. Buckalew, Jr., J.R. Lymangrover*. Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Using immunochemical and fluorescence techniques, we have ascertained that the peptide ACTH/αMSH$^{4-10}$ - βLPH$^{47-53}$ has chemical properties similar to the putative natriuretic hormone. Since hormones containing this amino acid sequence have some natriuretic activity, we decided to test this peptide in appropriate assays. ACTH$^{4-10}$ inhibits (-40%) human red blood cell ouabain sensitive ^{86}Rb uptake at 10^{-7}M. Bolus injections of 4 X 10^{-9}M/kg in rats increase sodium excretion and urine volume 50% 90 minutes after injection, and 8 X 10^{-8}M/kg IV is pressor (20% increase in MAP). Although the peptide doses used are relatively large, we have determined that infusions of ACTH$^{4-10}$ result in a significantly greater response to a cumulative dose 10-100 fold less than bolus injections. This suggests that enzymatic degradation and/or renal excretion are limiting the peptide's activity. These data indicate that ACTH$^{4-10}$ is a natriuretic-diuretic and pressor peptide with digitalis-like properties. The release of this peptide or a physiologic (and perhaps chemical) analog could play a role in the pathophysiology of salt-induced and/or Cushing's Disease hypertension. ACTH,αMSH, and βLPH occur in many areas of the brain providing numerous sites for the synthesis of such a peptide. This peptide may be a member of a family of Na, K ATPase regulators.

ESCAPE FROM THE CHRONIC SODIUM RETAINING ACTIONS OF ANGIOTENSIN II:ROLE OF INCREASED RENAL ARTERIAL PRESSURE. John E. Hall and Joey P. Granger*. Dept. of Physiology, Univ. Miss. Med. Ctr., Jackson, MS.

This study was designed to examine the role of increased renal artery pressure (RAP) in allowing the kidneys to escape from the chronic Na retaining effects of angiotensin II (AII). In control dogs, where RAP was allowed to increase during AII infusion(5 ng/kg/min),urinary Na excretion ($U_{Na}\dot{V}$) decreased from 78.7±5.6 to 20.4±5.9 mEq/day on the first day, but returned to control on the second day of AII infusion and there was no net accumulation of Na and no significant change in Na-iothalamate space when AII infusion was continued for 8 days. Mean systemic arterial pressure (MAP) rose from 100±3 to 132±2 mmHg after 3 days,and remained at that level for the next 5 days of AII infusion. In dogs where RAP was prevented from rising with a servo-controlled aortic occluder, $U_{Na}\dot{V}$ decreased from a control level of 74.3±5.1 to 29.5±3.4 mEq/day on the first day and remained at 58% of the control level even after 5 days of AII infusion; cumulative Na balance increased by 187±33 mEq and Na-iothalamate space rose 1158±244 ml when RAP was servo-controlled during AII infusion. MAP did not plateau when RAP was servo-controlled during AII infusion, but continued to rise and after 6 days averaged 157±3 mmHg. In 3 of the 8 dogs where RAP was servo-controlled during AII infusion, Na and water retention became so severe that MAP increased to 165-180 mmHg and pulmonary edema developed within 4-6 days. These data indicate that a rise in RAP is essential in allowing the kidneys to escape from the chronic Na retaining actions of AII and to achieve Na balance and a stable level of MAP without severe volume expansion.

URINARY NOREPINEPHRINE METABOLITES IN SALT-SENSITIVE HYPERTENSION. H.-G. Güllner, J. Oliver, J.R. Gill, Jr., and I.J. Kopin. N.I.H., Bethesda, Md.

The increase in blood pressure with NaCl loading in "salt-sensitive" hypertension (SH) has been observed to be associated with an increase in plasma norepinephrine suggesting that increased SNS activity mediates the increase in BP. Recent evidence suggests that the sum of urinary metabolites of norepinephrine, MHPG and VMA, reflects overall NE synthesis, whereas normetanephrine (NM) is an index of sympathetic activity. The present study was designed to examine the effects of sodium intakes, normal (109 mEq/d), low (9 mEq/d) and high (249 mEq/d) on the urinary excretion of MHPG, VMA and NM in 6 patients with SH and 6 patients with "non-salt-sensitive" hypertension (NSH). The patients were fed each diet for 7 days. Daily 24-hour urines were collected for measurement of MHPG, VMA and NM by GC-MS. Results, analyzed by ANOVA, are shown in the table:

	MHPG (mg/d)		VMA (mg/d)		NM (mg/d)	
Na^+	SH	NSH	SH	NSH	SH	NSH
109	14.4 ±1.6	12.8 ±1.7	18.7 ±2.7	18.8 ±0.8	1.4 ±0.2	1.3 ±0.2
9	15.0 ±1.6	14.7 ±2.4	20.1 ±2.4	20.3 ±1.4	1.6 ±0.2	1.8 ±0.3
249	13.9 ±1.4	13.5 ±1.9	19.2 ±2.1	19.4 1.1	1.5 ±0.2	1.4 ±0.2

Neither low nor high sodium diets altered significantly the urinary excretion of normetanephrine, its deaminated metabolites, or the ratio among the metabolites in SH or NSH. The excretion of metabolites in SH was not different from that in NSH.

DIGITAL RENAL ANGIOGRAPHY AS A SCREENING TEST FOR RENOVASCULAR HYPERTENSION. Timothy Howland, Richard Chiang, Patricia Randall, John Cucinotta, Gunnar H. Anderson, Jr., and David H.P. Streeten (Introduced by Theodore Schroeder). Department of Medicine, SUNY, Upstate Medical Center, Syracuse, New York.

The value of digital renal angiography as a screening procedure for renovascular hypertension was studied. 69 consecutive patients referred for evaluation of hypertension were studied with digital renal angiograms, response to saralasin and a stimulated plasma renin activity (PRA). 5 were uninterpretable and 5 were equivocal. Of the digital studies 44 were normal. Only 2 of these patients had evidence for renovascular hypertension with a significant fall in blood pressure with saralasin or a high PRA. In addition, 6/8 renal vein studies had ratios <1.5.

15 of the 69 digital studies were abnormal. In 4 there was an associated blood pressure fall with saralasin. 3/6 of these patients had a renal vein renin ratios >1.5.

Five patients with abnormal digital studies had abdominal aortograms, four of which also showed significant renal artery stenosis.

In conclusion, the correlation between normal saralasin and stimulated plasma renin studies in patients with normal digital angiograms (42 of 44 patients) demonstrates the effectiveness of digital angiography as a screening procedure for excluding renovascular hypertension. An abnormal digital renal angiogram, on the other hand, suggests but is not diagnostic of renovascular hypertension.

ACUTE PLASMA RENIN RESPONSE TO ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITION BY CAPTOPRIL: A SCREENING TEST FOR RENAL HYPERTENSION. B. Jackson, B.P. McGrath*, P.G. Matthews* and C.I. Johnston*, Monash Department of Medicine, Prince Henry's Hospital, Melbourne, Victoria, Australia.

The acute response to a single dose of the orally active ACE inhibitor captopril was compared in patients with essential hypertension (EH), accelerated hypertension (AH) and renal hypertension (RH). In a preliminary study of 10 patients with EH, 6 with AH and 8 with RH it was observed that the degree of hypotensive response to 25mg captopril did not differentiate RH from AH and EH. Basal plasma renin concentration (PRC, ng/AI/ml/hr) also failed to distinguish RH from AH and EH. Sixty minutes following captopril the peak rise of PRC was 28±7% in EH, 72±50% in AH and 153±39% in RH. Using an absolute rise in PRC of >2.5 as a cut off RH was separable from EH and all but one of AH. Subsequently we have prospectively studied all patients undergoing renal arteriogram for hypertension. In a total of 29 with RH (based on angiogram and renal vein PRC measurements) the PRC response to captopril was "positive" in 26. Of 24 patients with a normal arteriogram 3 were "positive". In RH the rise in PRC was not adequately explained by either fall in blood pressure or plasma angiotensin II.

Following successful surgical repair the rise in PRC following captopril returned to the EH range in 4 cases studied, and remained "positive" in the one case who on re-angiogram had a re-stenosed renal artery.

The PRC rise following a single dose of captopril is a useful non-invasive screening test for renal hypertension.

THIAZIDE PRODUCED URIC ACID ELEVATIONS DO NOT CAUSE RENAL DAMAGE. H. G. Langford, D. Curb,* D. Blaufox, N. Borhani,* and A. Chakrin,* for the Hypertension Detection and Follow-up Program (HDFP). Univ. of Texas School of Public Health, Univ. of Mississippi, Einstein, Univ. of California, Davis, and Northwestern Univ.

Uric acid levels, frequently elevated in hypertension, are further elevated by antihypertensive therapy. A major worry in the past has been that these elevated levels of uric acid will cause further renal damage. This abstract reports changes in serum uric acid and creatinine (Cr) in chlorthalidone-treated hypertensive patients (pts), noting influence of initial Cr on initial uric acid and change in uric acid, as well as the effect of uric acid lowering agents on change in Cr following chlorthalidone. This report is based on 2,201 pts. Pts were divided into quartiles by baseline uric acid. Uric acid increased pari passu with Cr. The lowest quartile's Cr was 0.9 ± .2 mgm/dl, with uric acid 4.15 ± .64. The upper quartile's baseline Cr was 1.12 ± .24, with uric acid 7.42 ± .68. After chlorthalidone therapy, the Δ uric acid was 1.06 mgm% in the lowest quartile, and 0.80 mgm% in the highest quartile. The corresponding Δ Cr were 0.15 mgm/dl and 0.12 mgm/dl. Pts placed on uric acid lowering agents dropped uric acid from 7.5 mgm% to 7.3, but Cr increased from 1.36 mgm% to 1.74 (26%). We conclude that uric acid changes with Cr, but does not appear to injure the kidney.

COMBINED α AND β BLOCKER THERAPY IS NO BETTER THAN α BLOCKER ALONE IN DIURETIC TREATED HYPERTENSIVES. M.S. Kochar, J.H. Kalbfleisch,* S.S. Blumenthal, and W.J. Maierhofer. Wood VAMC and Med. Coll. of Wisconsin, Milwaukee, Wisconsin.

We conducted a single blind study to compare the antihypertensive efficacy of prazosin with and without propranolol in patients with essential hypertension whose diastolic blood pressure (BP) had remained ≥ 95 torr despite therapy with 50 mg daily hydrochlorothiazide and 240 mg daily propranolol. After monitoring the BP for 4 weeks, the 14 patients were divided randomly into 2 groups. In the first group, propranolol was reduced to 120 mg/day and prazosin added, starting with an initial dose of 1 mg b.i.d.; in the second group, propranolol was tapered and discontinued while adding prazosin. The data were analyzed using paired t test to compare BP at the end of 4, 8 and 12 weeks of therapy to the initial BP. After 4 weeks of therapy with prazosin (mean daily dose 3.5 mg), in the prazosin-added group the sitting BP (mean ± 1 SD) was reduced from 158±18/102±5 torr to 140±22/88±8 torr ($p<.01$). After 4 weeks of therapy with prazosin (mean daily dose 4.3 mg), in the prazosin-substituted group the sitting BP (mean ± 1 SD) was reduced from 151±14/102±5 torr to 141±19/88±7 torr ($p<.01$). During the next 8 weeks the BP remained essentially the same as at week 4 in both groups. There was no significant difference in the reduction of BP in the two groups when compared using an unpaired t test. Heart rate rose significantly in both groups ($p<.01$) but more so in the patients who were taken off propranolol. We conclude that, wherever possible, prazosin should be substituted for propranolol rather than added to a diuretic and β blocker.

ETHNIC DIFFERENCE IN RBC Na^+-K^+ ATPase Norman Lasker, Susan Grossman*, Abraham Aviv, Roni Rosenfeld*, Donna L. Kropp* and Stuart Baskin, Univ. of Med.+Dent.of N.J., Dept. of Med., Ped., Physiol., Newark, N.J.

Hypertension is more common and hypertensive sequelae more severe in American blacks compared to non-blacks. Although hypertension is most prevalent among young black males, the sex differential for blacks is less than for non-blacks. RBC sodium concentration is higher in normotensive and hypertensive blacks and higher in black women than in black men. (Love and Burch, J.Lab. and Clin.Med. 41:258-267, 1953 and Munro-Faure, Hill and Anderson, Nature 231:357-458, 1971). We measured Na^+-K^+ ATPase activity in RBC's of 24 blacks and 29 non-black doctors, nurses, students and technicians ranging in age from 20 to 46 years. They were all normotensive and non-pregnant with no history of thyroid disease, renal failure or hemolytic disorders. They were not taking diuretics, birth control pills, thyroid or digitalis. Serum creatinine and electrolytes were normal.

Results:

	Black		Non-Black	
	Male	Female	Male	Female
Number	9	15	16	13
Mean age	39	34	30	30
Mean Q.I.*	3.49	3.40**	3.21	3.01
Na^+-K^+ATPase	.088	0.062***	0.097	0.099

*Q.I.=Quetelet index=Weight/Height^2 x 100
Black vs. non-Black female, **$p<.01$, ***$p<.001$
Black women had a lower RBC-Na^+-K^+ ATPase activity than all other groups. There was no correlation between Na^+-K^+ ATPase activity and Q.I., age, diet, weight, and family history of hypertension. These findings should add to our understanding of the ethnic distribution of this disorder.

PRESSOR EFFECT OF PROPRANOLOL IN ANEPHRIC MAN. Bruce R. Leslie, Thomas G. Pickering,* and John H. Laragh. New York Hospital-Cornell Medical Center, New York, New York.

The role of the renin system in the antihypertensive effect of propranolol (PRO) remains controversial, in part because acute administration of PRO does not lower blood pressure (BP) despite prompt reduction of plasma renin activity (PRA). To test the importance of the renal renin system in the response to PRO, we studied the acute hemodynamic effect of PRO in 4 anephric subjects, in whom this system was absent. Subjects received either placebo (PCB) or 40 mg PRO orally on separate occasions. Supine BP was measured automatically. Plasma norepinephrine (NE) and serum PRO concentrations were measured before and 2 hours after each dose. The changes in BP at 2 hours compared to pre-dose were:

	ΔBP (systolic/diastolic)mm Hg	
Subject	PCB	PRO
1	+3/+1	+17/+13
2	+16/+13	+37/+15
3	+10/+9	+23/+10
4	+15/+13	+16/+14

NE increased following PRO by 17-104 pg/ml in subjects 1-3, but fell by 134 pg/ml in subject 4. Serum PRO ranged from 24-107 ng/ml.

In the absence of the renal renin system, PRO has an acute pressor effect associated with increased plasma NE. In intact man, this effect may be offset by the depressor effect of a lowered PRA, resulting in little initial change in BP. The renal renin system is thus important in the acute hemodynamic response to PRO.

THE MECHANISM OF THE ANTIHYPERTENSIVE EFFECT OF HEPARIN. Anil K. Mandal, Dinko Susic,* and Dusan Kentera.* Institute for Medical Research, Belgrade, Yugoslavia and V.A. Medical Center and Medical College of Georgia, Augusta, Georgia.

Chronic heparin (H) treatment lowers blood pressure (BP) in spontaneously hypertensive rats (SHR) (Mandal, Microvasc. Res. 16:373, 1978; Wilson, Am. J. Pathol. 102:62, 1981). The purpose of this study is to delineate the mechanism of the antihypertensive effect of H. Thirty Wistar rats with one-kidney, one-clip hypertension (1KGH) and 20 SHR were used. One-half of SHR and 1KGH rats received H (200 units/day/rat), the other half 0.2 ml. of saline s.c. After 4 weeks, mean arterial pressure (MAP), cardiac output (CO) (dye dilution), total peripheral resistance (TPR), hematocrit (Hct), heart rate (HR), and left ventricular weight/body weight ratio (LV/bw) were measured. Significant decreases in MAP, TPR, Hct, and LV/bw, and significant increases in CO were found in H treated rats with no difference in HR between the groups. In a group of H treated SHR in which Hct was kept at control level by repeated transfusions, H-induced decrease in BP was abolished, while in a group of SHR in which Hct was decreased to a level similar to that in H treated rats by repeated blood letting, BP was found to be decreased. The results indicate that H-induced decrease in Hct, with consequent decrease in blood viscosity, may be responsible for the decrease in TPR and BP in H treated rats and further supports the idea (Letcher, Am. J. Med. 70:1195, 1981) that increased blood viscosity in hypertensive subjects may participate in the maintenance of hypertension.

SODIUM-LITHIUM COUNTERTRANSPORT AND PASSIVE CATION PERMEABILITY ABNORMALITIES IN ERYTHROCYTES OF PREGNANT WOMEN. A.Logan,R.Bear, A.Steingart,* A. Chatzilias,* N.Zielinski,* and P.Flanagan,*Dept. of Med., University of Toronto, Toronto,ON.

The maximum rate of RBC sodium-lithium countertransport (SLC) and passive permeability for lithium were assessed in three groups of women:(1) pregnant hypertensives (PH),(2) pregnant normotensives (PN) and (3) nonpregnant normotensives (NPN). SLC, an ouabain insensitive membrane pathway for sodium transport,was found to be significantly increased in 32 PN and 19 PH, the mean being 0.35 + 0.02 (mean + SEM) and 0.51 + 0.04 mmol/L of RBC/hr respectively compared with 0.26 + 0.02 in 17 NPN ($P<0.001$). The increase in SLC was positively correlated with gestational age in PN ($r=0.55$, $P<0.002$) but not in the PH where a significant inverse relationship was observed ($r=-0.63$, $P<0.004$). SLC was significantly higher in the PH even when only those after the 28th week were compared (0.47+0.03 vs. 0.39 + 0.02,$P<0.05$).While no difference in RBC sodium concentration was found in the three groups of women, the RBC lithium concentration was significantly increased in PH being 6.8 + 0.2 mmol/L of RBC compared with that of 6.3+0.2 in PN and 5.9 + 0.2 in NPN ($P<0.05$). The passive permeability constant for lithium of 0.033 + 0.007 $(hr)^{-1}$ in PH and of 0.028 + 0.002 in PN was significantly higher than that of 0.021 + 0.001 in NPN ($P<0.05$). These results provide further evidence that defects in membrane cation transport are present in pregnant women and may contribute to the pathogenesis of hypertensive disorders of pregnancy. In addition, early detection of these abnormalities may prove to be a useful way to identify high-risk pregnancies.

EFFECTS OF PARATHYROIDECTOMY (PTX) ON BLOOD PRESSURE (BP), BODY FLUID-, AND ELECTROLYTE HOMEOSTASIS IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE (SHR) RATS. Johannes F.E. Mann*, Michaela Becker*, and Eberhard Ritz* (intr. by F. Llach). Dept of Medicine, University of Heidelberg, W-Germany.

The effect of parathormone (PTH) on BP development in SHR was evaluated.Four groups of rats were studied: sham-operated SHR(SHR-SO),SHR-PTX,normotensive Wistar-Kyoto(WKR)-SO, and WKR-PTX (n: 24 per group).PTX was done in 4 week old rats, and PTX-groups received high dietary Ca++ one week after PTX to maintain normocalcemia. Mean arterial pressure (MAP) (chronic arterial catheter) in the conscious rats was lower in PTX groups at 2 months ($p<.01$) (SHR-PTX: 153; SHR-SO:186; WKR-PTX:103; WKR-SO:121mmHg) and lower in SHR-PTX than in SHR-SO at 3 months following PTX (<.01) (SHR-PTX: 164; SHR-SO: 205 mmHg).Plasma Ca dropped in all PTX rats and was normal upon feeding high dietary Ca++. No differences between PTX- and SO-rats were found for plasma angiotensin II (radioimmunoassay) and-norepinephrine (radioenz. assay), but norepinephrine was higher in SHR than in WKR ($p<.01$). Plasma volume was expanded in PTX-rats and lower in SHR than in WKR ($p<.01$), (SHR-PTX: 4.14; SHR-SO: 3.77; WKR-PTX: 4.55; WKR-SO: 4.11ml/100g). Similar changes were found for total body sodium (SHR-PTX: 5.6; SHR-SO: 5.1; WKR-PTX: 5.4; WKR-SO: 4.8 mmol/100 g). Lanthan-resistant aorta-Ca++ content was reduced in PTX rats by 20-30% and aorta-cAMP (RIA) by 15-25%. The results suggest that endogenous PTH exerts a permissive effect from BP development in SHR, probably by enhancing intracellular Ca++ in vascular smooth muscle with subsequent increase in vasoconstrictor tone.

RENAL CATECHOLAMINES IN ACUTE II KIDNEY I CLIP HYPERTENSION. E.S. Marks, D.S. Goldstein,* H.R. Keiser,* Dept. of Med., Uniformed Services Univ.; NHLBI, NIH, Bethesda, MD.

Acute effects of renal artery constriction and ipsilateral denervation on renal catecholamines were evaluated in 5 anesthetized foxhounds. A screw clip reduced renal blood flow 50% for 20 min. After 40 min recovery, ipsilateral denervation and reclipping were performed. Samples were obtained every 20 min simultaneously from the aorta, renal veins and ureters. Norepinephrine (NE),epinephrine (E), and dopamine (DA) levels and clearances of inulin (C_I) and PAH (C_{PAH}) were measured. Results: Clipping raised continuously monitored mean arterial pressure 15 torr before and 10 torr after denervation. Baseline renal venous NE exceeded arterial NE ($\bar{x}$ 114%), with a net renal release (C_{PAH} x arterial-venous NE difference). Clipping decreased NE release and the arterial-venous difference on both clipped and unclipped sides with arterial NE essentially unchanged. Clip removal restored venous NE bilaterally toward baseline. Denervation reduced NE release 45% and 61% on the clipped and unclipped sides respectively. Urine NE fell bilaterally following denervation. Reclipping after denervation reduced arterial NE. Renal venous E was lower than arterial E by 53%. Arterial, venous, and urine E levels declined over time. Plasma DA distributed randomly while urinary DA fell progressively. Neither arterial nor venous NE correlated with C_{PAH} or C_I. Conclusions: 1) Clipping or denervating one renal artery reduces renal NE release bilaterally. 2) The combination of renal artery clipping and denervation lowers arterial NE.

EFFECTS OF ORAL INDAPAMIDE ON RENAL FUNCTION AND PLASMA VOLUME IN HYPERTENSIVE PATIENTS. U.F.Michael, S.K.Mukherjee, L.A.Meeks*, G.P. Lewis*, R.P.Paone*, R.R. Szabadi* and D.J.Blasucci* Medical and Research Services, VAMC, Tucson, AZ; Clin. Pharmacol. Depts. Lemuel Shattuck Hosp., Boston, MA and Revlon Health Care Group, Tuckahoe, NY.

Indapamide (I) is a new indoline derivative diuretic-antihypertensive drug related to the benzothiadiazine diuretics. In a double blind, placebo (P) controlled 12 wk trial, the effects of 2.5mg/24 h oral I on the clearances of inulin (C_{IN}), p-amino hippurate (C_{PAH}) and water (CH_2O) as well as on RISA plasma volume (PV) in 30 patients with mild to moderate hypertension were compared to those of P in 13 patients with similar blood pressure (BP) elevations. After baseline renal function studies, these were repeated after 4 and 12 wks on oral I or P. Standing systolic and diastolic BP dropped by 14.2 ($p<0.0001$) and 6.6 mm Hg ($p<0.001$) respectively in the I group, while decreasing non-significantly by 5.8 and 4.2 mm Hg respectively in the P group. PV, C_{IN} and C_{PAH} did not change significantly in either group. CH_2O following a 20 ml/kg water load decreased from 7.3±0.5 (SEM) to 4.5±0.4 ml/min/1.73 m^2 in the I group. ($p<0.0001$) while not changing in the P group.

It is concluded that (1) 2.5 mg oral Indapamide is effective in lowering standing systolic and diastolic BP without a significant decline in PV. (2) Indapamide has its diuretic effect in the diluting segment of the nephron. (3) No significant decrease of glomerular filtration rate or effective renal plasma flow is seen at an oral dose of 2.5 mg Indapamide

REINNERVATION OF THE KIDNEY AFTER NERVE CRUSH. Nicholas G. Moss* and W. Wallace Harrington*(intr. by C.W. Gottschalk). Univ. of North Carolina, Dept. of Physiol., Chapel Hill, North Carolina.

The return of afferent activity in reinnervating rat kidneys was studied up to 8 wks after crush denervation of renal nerve bundles. Acute nerve crush abolished all afferent nerve activity for at least 2 hrs but within 3 days normal basal levels of afferent activity had reappeared. Functional reinnervation was assessed from the responses of renal chemoreceptors (multi-units) to acute renal ischemia or to perfusion of 100 mEq/l KCl into the renal pelvis:

Time after crush	Bundles tested	Bundles responding to: Ischemia	KCl
Sham op. (5)	8	8 (100%)	8 (100%)
3 days (6)	9	0 (0%)	0 (0%)
1 wk (5)	8	8 (100%)	1 (13%)
2 wks (5)	5	5 (100%)	1 (20%)
4 wks (5)	5	5 (100%)	3 (60%)
6 wks (5)	5	5 (100%)	4 (80%)
8 wks (4)	5	5 (100%)	5 (100%)

Number of rats in parentheses

Three days after nerve crush unresponsive basal activity originated from the point of injury in the nerve bundles, as it was not blocked by re-crush of the nerve between this region and the kidney. The recovery of response to ischemia at 1 wk indicates re-entry of afferent nerves into the kidney at this time. The slower recovery to intrapelvic KCl suggests that further axonal growth is necessary before the nerve endings are in a position to respond to this stimulus. Thus, when interpreting the functional consequences of chronic renal denervation the early appearance of afferent nerve activity must be considered.

A COMPARISON OF 90 SECOND DTPA RENAL SCANS AND 30 MINUTE I-131 HIPPURAN RENOGRAMS IN THE ASSESSMENT OF RENAL ARTERY STENOSIS. J.V. Nally, M.L. Gross, W.J. Potvin*, H.S. Clarke, Jr.*, J.T. Higgins, and J.P. Windham*. Medical College of Ohio, Departments of Radiology and Medicine, Toledo, Ohio.

Computer assisted 90 second Tc-99m DTPA renal flow scans have been developed to assess early renal blood flow changes. This study was carried out to compare the 90 second DTPA scan to the standard 30 minute I-131 Hippuran scan in the evaluation of patients with known renal artery stenosis. Six patients with angiographically documented renal artery stenosis were studied using both methods. The time activity curves for both studies were derived from regions of interest selected from the computer acquired dynamic images. The following parameters were used to assess renal blood flow: differential maximum activity, minimum/maximum activity ratio, and peak width.

The results of the study demonstrate that the 90 second DTPA scan accurately predicts (6/6) and correlates with angiography, while the 30 minute I-131 Hippuran scan was clearly predictive in only 2/6. The differential maximum activity and peak width were good discriminatory factors when compared with a template synthesized from curves obtained from normal subjects.

In conclusion, the 90 second Tc-99m DTPA renal blood flow scan with the aforementioned parameters correlates extremely well with angiography and may be the non-invasive procedure of choice in patients suspected of having renal artery stenosis.

ANGIOTENSIN I CONVERTING ENZYME ACTIVITY IN HYPERTENSION. A.P. Niarchos, L. Resnick*, J.H. Laragh, Hypertension Center, New York Hospital-Cornell University Medical Center, New York, N.Y.

The activity of the angiotensin I converting enzyme (ACE) was measured in 70 untreated hypertensive and in 13 normotensive (NT) subjects, and during acute and chronic therapy with captopril (C). ACE was elevated (> mean ±2SD of NT subjects) in 25% (12 of 55 patients) with essential hypertension (EH) and in 18% (2 of 11) with renovascular hypertension (RVH), but ACE was normal in primary aldosteronism (N=4). In EH ACE was related ($r=0.34$, $p<0.01$, N=55) to the mean arterial pressure (MAP), to the plasma renin activity (PRA) ($r=0.31$, $p<0.05$) and to the patient's age ($r=-0.34$, $p<0.01$). During acute C therapy (N=18) ACE was decreased by C from 24±1.7 to 13.9±1.1 units/ml ($p<0.005$), but the decrease in MAP by C from 131±4 mmHg ($p<0.005$) was not related either to the pretreatment ACE ($r=0.15$) or to the % change in ACE ($r=0.42$) but to the pretreatment PRA ($r=0.75$, $p<0.001$). During chronic C therapy ACE was not related to the concurrent MAP but to the total daily dose of C ($r=-0.55$, $p<0.001$, N=35 measurements). These results indicate that although ACE is elevated in a proportion of patients with EH and RVH, PRA rather than ACE activity is a better predictor of the acute hypotensive effect of C. However, during chronic C administration measurement of ACE activity confirms compliance to C therapy.

ENKEPHALINS(enk)IN HUMAN PHEOCHROMOCYTOMA(pheo): SUBCELLULAR STORAGE IN AND RELEASE FROM CATECHOLAMINE STORAGE VESICLES(CSV), AND GENERATION FROM PRECURSORS BY CONTROLLED PROTEOLYSIS. DT O'Connor, R Frigon*. Dept. of Med.,VA Medical Center and University of California, San Diego, CA.

Enk are endogenous opioid pentapeptides which may be involved in blood pressure control. Because enk and enk precursors have been detected in bovine adrenal medullary CSV, we searched for enk and enk precursors in CSV from 6 human pheos.

Methods: CSV were prepared by sucrose gradient centrifugation and lysed hypotonically. Leucine-enk(by RIA)and catecholamines(cat)were measured in CSV and cell cytosol. CSV proteins were also trypsin digested(2h,37°C)to liberate enk from precursors. Results: Each tumor contained immunoreactive enk(57.6±32.2µg)which paralleled the assay standard. Total enk correlated with tumor cat($r=0.97$)and tumor weight($r=0.99$). Within the cell, both enk(55±17%)and cat(54±9%)were localized to CSV. In vitro CSV lysis released 81% of enk and 94% of cat. Proteolysis increased immunoreactive CSV enk by 59±23%

We conclude: 1)pheos contain immunoreactive enk in µg quantities localized in the cell along with cat to CSV. 2)enk are released into the soluble phase during in vitro lysis, and are thus examples of chromogranins(soluble,released CSV proteins). 3)Increased immunoreactive enk after proteolysis suggests higher molecular weight enkephalin precursors in CSV. 4)enk may be involved in the manifestations of pheo.

THE RELATIONSHIP OF SALT INTAKE AND THE RENIN-ANGIOTENSIN SYSTEM TO THE FIRST DOSE PRAZOSIN RESPONSE. J.P. Nicholson,* L.M. Resnick,* J.H. Laragh. Hypertension Center, New York Hospital-Cornell University Medical Center, New York, N.Y.

It has been reported that the first dose orthostatic response to prazosin is more often seen in patients who are volume or salt depleted or concomitantly on beta blockers. We studied the relationship between sodium intake, plasma renin activity (PRA) and the acute blood pressure (BP) response to oral administration of 1 mg prazosin in 15 (10 male, 5 female) patients with essential hypertension (Avg. BP=150/100±5/2) off therapy for 3 wks. BP was automatically monitored in the seated position for 2 hrs. after dosage, and then in the standing position for 1/2 hr. PRA was measured at T=0 and T=150 min. 10 of 15 patients experienced acute falls in BP associated with nausea and dizziness. This was markedly accentuated upon standing. The induced orthostatic depressor response was inversely correlated with both the baseline PRA ($P<0.005$) and the increment in PRA at T=150 ($P<0.0125$). There were no significant differences in 24 hr urinary sodium excretion between responders and non-responders (UNa=118±17, UNa=180±53 mEq/day). Thus, the orthostatic depressor response to first dose prazosin is more frequent than reported, (10/15, 66%), and its occurrence is inversely related to the baseline PRA. This unique responsiveness of low renin essential hypertension to prazosin may reflect their possible underlying increased alpha tone and/or their attendant blunted renin response. Whatever the case, the hypotensive response to prazosin is not a function of salt intake.

ROLE OF PRESSOR HORMONES IN THE MAINTENANCE OF PERIPHERAL VASCULAR RESISTANCE AND MEAN ARTERIAL PRESSURE IN THE CONSCIOUS RAT. M. Paller and S. Linas, University of Minnesota, Minneapolis, MN and University of Colorado, Denver, CO.

Three hormonal systems regulate mean arterial pressure (MAP): angiotensin II (AII), the alpha-adrenergic system (AAS) and arginine vasopressin (AVP). In this study we determined the ability of each hormone to support MAP when the other two systems were inhibited. Following converting enzyme inhibitor (CEI) plus the AAS antagonist phenoxybenzamine (POB), MAP decreased from 107.4 to 67.7 mmHg ($p<.001$) as a result of a 45% decrease in peripheral vascular resistance (PVR). Despite hypotension, plasma AVP levels were not increased and an AVP pressor antagonist (AVP-A) did not result in a further decrease in MAP. POB, rather than CEI, prevented AVP release since following hemorrhage, another nonosmotic stimulus for AVP release, plasma levels reached 51 pg/ml with CEI but only 4.7 pg/ml with POB. Thus, the hypotension after POB plus CEI was the result of inhibition of all three systems. To study the pressor effect of AVP alone, AVP was infused in POB plus CEI treated rats. AVP increased MAP (from 67.7 to 91.5 mmHg, $p<.005$) and plasma AVP (to 13.8 pg/ml). Since POB inhibited both the AVP and the AAS, the role of AII alone was determined in POB treated rats. In the presence of an intact AII system, MAP was 96.8 mmHg. In order to study the AAS, MAP was determined in CEI plus AVP-A treated rats. With an intact AAS, MAP was 101.2 mmHg. We conclude that PVR and MAP are profoundly decreased in the absence of all three pressor systems. In contrast, MAP is well maintained as long as a single pressor system remains intact. In addition, an intact AAS is critical for nonosmotic AVP release.

A COMPARISON OF DIURETIC REGIMENS IN HYPERTENSION (HBP). B.Pugh* J.H.Licht and R.J.Haley*, Nav.Reg.Med.Cen., Oakland, CA

Because of efficacy and low cost hydrochlorothiazide (HZ) is the most widely used diuretic in HBP. Due to side effects, including hypokalemia (serum K<3.5 mEq/L.=LoK) many patients must change to other diuretic regimens. We reviewed the records of 5094 hypertensives to find the most cost-effective alternative regimen to HZ. Patients were studied if they received HZ 50 mg/day (HZ 50) and were changed to or from one of the following: furosemide 40mg/day (F), triamterene 50 mg + HZ 25 mg 2/day (T+HZ), spironolactone 25 mg + HZ 25 mg 2/day (S+HZ), chlorthalidone 25 mg/day (C), or HZ 50 + 37 mEq KCl/day (HZ+K). Other medications were constant in both periods, which were consecutive and a minimum of 3 months long. The figures in the table represent mean differences from values during the HZ 50 period:

Drug	n	SBP	DBP	K	Glu	Cost/yr	
F	56	0	-1	0.4*	-6°	$ 49	°=p<.05
T+HZ	69	-2	-1	0.2*	3	$ 96	†=p<.01
S+HZ	15	-4	-3	0.4°	-2	$132	*=p<.001
HZ+K	42	-2	-1	0.2†	-1	$142	compared
C	17	-3	-1	0	0	$ 53	to HZ 50

Cost (source=1981 Redbook) for HZ=$37. To substantiate this apparent K-sparing by F we defined the mean serum K:prevalence of LoK and (n) in patients treated with these regimens only: No diuretics 4.5:1.7% (801); HZ 50 4.1:11.0% (500); F 4.4:3.8% (284); T+HZ 4.2:5.3% (357); HZ+K 4.0:11.6% (163); C 4.1:8.1% (37).

Each alternate regimen controlled HBP as well as HZ 50 and, with the exception of C, raised K. Because F increased K as well as other agents, while lowering Glu, and controlling HBP, F is the most cost-effective potassium sparing alternative to HZ 50 in HBP.

INVERSE CORRELATION BETWEEN MEAN ARTERIAL BLOOD PRESSURE AND SERUM ANGIOTENSIN CONVERTING ENZYME LEVELS IN MAINTENANCE HEMODIALYSIS PATIENTS. T.K.S. Rao, E. Silverstein*, J. Brunswick*, J. Friedland*. Downstate Medical Center, Brooklyn, New York.

Serum angiotensin converting enzyme (SACE) was measured fluorimetrically in 48 patients with various types of chronic renal disease (CRD), 52 patients on maintenance hemodialysis (MH) and 58 healthy adult controls. The CRD group included patients with a measured C_{cr} of 126 to 4.8ml/min. The results were as follows:

Group		SACE (n. mol/min/ml)
Control	n = 58	32.8 ± 9.8
CRD	n = 48	45.6 ± 16.7 (P .001)
MH	n = 52	43.2 ± 13.8 (P .001)

The increased SACE levels in CRD group did not correlate with the nature of renal disease, or the degree of renal insufficiency but there was an insignificant positive correlation with the 24 hr. urinary protein excreation (r = 0.254, n = 22). Following hemodialysis, the SACE levels increased significantly to 50.6 ± 18.0) as compared to 44.2 ± 15.3 before dialysis (P<.001), which was secondary to hemoconcentration. There was a significant (P< .05) inverse correlation between SACE levels and mean arterial blood pressure in MH patients during both pre and post dialysis.

The reasons for the increased SACE levels in CRD are unknown. The inverse correlation between mean arterial pressure and SACE in MH patients might suggest a negative feed back mechanism in the control of blood pressure.

EFFECT OF SALT INTAKE ON NA^+,K^+-ATPase ACTIVITY AND CATION TRANSPORT IN THE RED CELL. M. Rahman,* M.I. Primera,* T.P. Gibson, and A.P. Quintanilla. Department of Medicine, VA Lakeside Medical Center and Northwestern University Medical School, Chicago, Illinois.

It has been suggested that volume expansion induces formation of a factor inhibitory of Na,K-ATPase and cation transport in the renal tubule. Similar changes may occur in the red cell. To test this hypothesis we placed 9 healthy volunteers on either a low-salt (20 mEq/d) or a high-salt (200 or more mEq/d) diet for 4 days. The 24-hour urinary Na excretion ($U_{Na}V$), body weight, orthostatic BP, plasma renin activity (PRA) and plasma aldosterone (PA) were consistent with good adherence to diets. In the high- and low-salt diets, mean $U_{Na}V$ was 231 and 23 mEq/d (p<0.001), standing systolic BP 112.3 and 101 mm Hg (p<0.001), PRA 1.29 and 5.0 ng/ml/hr (p<0.001) and PA 116 and 302 pg/ml (p<0.001), respectively. The ouabain-sensitive Na,K-ATPase (ATPase) in the red cell was significantly higher in the low than in the high-salt diet (143 and 87 n moles of P_i/mg/hr respectively, p <0.02), whereas ouabain-resistant ATPase was not different. There was a correlation between ATPase and net Na efflux (R=0.83) and K influx (R=0.76) in the low-salt, but not in the high-salt diet. In conclusion, in healthy humans on a low-salt diet, ATPase activity in the red cell was enhanced, and correlated with net Na efflux and K influx, suggesting that ATPase activity is enhanced by volume contraction. When ATPase was inhibited by high-salt intake, other transport mechanisms may play a relatively greater role obscuring the relationship between ATPase and Na,K transport.

RENAL AND SYSTEMIC HEMODYNAMICS IN HYPERTENSIVE OBESE AND LEAN MATCHED PATIENTS. Efrain Reisin,* Hector O. Ventura,* Franz H. Messerli, Francis G. Dunn,* Wille Oigman,* Gerald R. Dreslinski,* Edward D. Frohlich. Ochsner Medical Institutions and Louisiana State University School of Medicine, New Orleans, LA

Accelerated hypertension with proteinuria and renal necrotizing arteriolitis has been found to be less frequent in obese than in nonobese patients. Ten obese (OH) were matched with lean (LH) hypertensive subjects with regard to race, sex, age (±5 yrs), mean arterial pressure (±10 mmHg, MAP). All the patients had a normal creatinine clearance. Cardiac output (CO, indocyanine green), renal blood flow (RBF, ^{131}I-PAH) and total blood volume (TBV ^{125}I-albumin) were compared in both groups. Results were:

	LH	OH
Weight (kg)	61±3	93±4**
Height (cm)	164±3	168±2
MAP(mmHg)	111±4	110±4
CO(L/min)	5.1±.3	6.1±.3*
TPR (units)	22±2	19±1**
RBF(ml/min)	876±52	1128±85**
RVR (units)	13±1	10±1**
TBV (ml)	3913±213	5171±380**

*p<0.05; **p<0.01. TPR=total peripheral resistance; RVR=renal vascular resistance.

It is concluded that despite a similar level of mean arterial pressure, the high output state and volume expansion in the obese hypertensive patients increase the renal perfusion and maintain a lower total peripheral and renovascular resistance than in the lean matched subjects. Thus, an increase in body weight may predispose to cardiac complications but may exert a protective effect on renal hemodynamics and mitigate the risk of renal damage.

SODIUM-CALCIUM EXCHANGE AND THE REGULATION OF VASCULAR SMOOTH MUSCLE TONE. Jack Rubenstein, Steve Bourla, Yuan Shih, Andre-Jacques Neusy,* and Jerome Lowenstein,* N.Y.U. Medical Center, Dept. of Medicine, Renal Section, New York, New York.

Transmembrane sodium gradient is postulated to be the driving force for sodium-calcium exchange, and critical in the regulation of vascular smooth muscle tone (Blaustein). We have examined the responses of rabbit aortic strips bathed in buffer in which the sodium concentration is reduced to 25-30 mEq/L by substitution with choline chloride. Strips preloaded with 2gm developed a prompt increase in tension averaging 0.9±.08gm and, unexpectedly, a late further increase in tension averaging 1.8±.09gm at 125±8 minutes.

Neither the early nor the late contractile responses appear to be mediated by catecholamine release since phentolamine (10^{-5}M), sufficient to significantly shift the dose-response to norepinephrine, failed to inhibit either response. Calcium-dependence of both the early and late contractile responses was established by the finding that reduced bath calcium concentration ($1X10^{-5}$M) or lanthanum (0.2-2mM) completely inhibited both responses. At intermediate bath calcium concentrations ($2\text{-}8X10^{-5}$M) or lower lanthanum concentration (.05-.1mM) only the early contractile response was blocked. These findings suggest different calcium-dependent mechanisms mediate the early and late contractile responses to reduced transmembrane sodium gradient.

MECHANISM OF THE HYPOTENSIVE ACTION OF THE INTACT AND AMINOTERMINAL FRAGMENT OF PARATHYROID HORMONE (PTH). Y. Saglikes*, V.M. Campese and S.G. Massry. Div. Nephrol., USC Sch. Med., Los Angeles, CA.

PTH is known to exert hypotensive action. This is probably not due to calcium movement into the cells of the vascular muscle, since calcium channel antagonists, such as nifedipine or verapamil produce vasodilation. We examined the effect of the intact PTH 1-84 and that of its NH_2-terminal 1-34 fragment on mean arterial pressure (MAP) and heart rate (HR) and on the vascular response to infused norepinephrine (NE) or angiotensin II (A II). Infusion of PTH 1-84 (30 U/h) did not alter MAP or HR. On the contrary, infusion of PTH 1-34 (30 U/h) lead to a decrease in MAP from 124±1.4 to 103±4.1 mmHg ($p<0.01$) and to a rise in HR from 359±30 to 437±13 beats/min ($p<0.02$). Bolus injections of 30 U of both PTH 1-84 and PTH 1-34 produced a significant ($p<0.01$) decrease in MAP and a rise in HR. However, the hypotensive response to 1-34 PTH (-28±4.6 mmHg) was more marked ($p<0.01$) than to 1-84 PTH (-9±1.8 mmHg). Both PTH 1-84 and PTH 1-34 inhibited the pressor effects as well as the reduction in HR produced by bolus injections of 10, 30, 100 and 300 ng of NE or 3, 10, 30, 100 ng of A II. Pretreatment with indomethacin (5 mg/kg) totally abolished the inhibitory effect of PTH 1-84 and PTH 1-34 on MAP and HR response to NE and A II. The data show that 1) the hypotensive action of PTH probably resides in its NH_2-terminal, 2) the hormone antagonizes the effect of NE and A II on MAP and HR, and 3) this antagonistic effect is probably not due to calcium movement into the vascular contractile cells but by activation of prostaglandin synthesis.

REVERSAL OF RENAL FAILURE FOLLOWING PERCUTANEOUS TRANSLUMINAL RENAL ANGIOPLASTY IN BILATERAL ATHEROSCLEROTIC RENOVASCULAR HYPERTENSION. R. Patterson Russell, Saadoon Kadir*, Mamdouh O. Darwish, and Daniel G. Sapir. The Johns Hopkins Med. Inst., Dept. of Med. & Radiol., Baltimore, Maryland.

Two caucasian males, 50 and 66 years of age, presented with longstanding hypertension (≥15 yrs), documented decrease in size and function of one kidney and moderate renal insufficiency (serum creatinine (SC) 3.8 & 5.8 mg%). One patient with chronic congestive heart failure became anuric following a prolonged episode of chest pain and required intermittent peritoneal dialysis (PD) for 8 days. The other patient with a history of a stroke was admitted for evaluation of refractory hypertension. The SC rose from 3.8 to 18.2 mg% 6 days following a renal arteriogram and intermittent PD was required for 15 days. In both patients serial renal scans documented a deterioration in function to the "less affected" kidneys and renal angiography documented occlusion of the renal arteries to those kidneys. A balloon catheter was successfully passed through the occluded arteries and both were dilated. In each case the blood pressure gradient was obliterated, there was an immediate diuresis and PD was terminated. The improved level of renal function (SC 1.9 and 4.4 mg%) has been maintained for 6 months and all antihypertensive medications have been discontinued. These cases demonstrate the value of percutaneous transluminal renal angioplasty in high-risk patients with acute renal failure resulting from occlusion of the main renal artery.

AMILORIDE CORRECTS THE KALIURESIS IN LIDDLE'S SYNDROME. Martin T. Starkman. McGuire Clinic and Dept. of Medicine, Va. Comm.Univ., Richmond, Va.

In 1963, Liddle et al (Trans.Am.Phys. 79, 199) described a kindred with severe hypertension associated with renal potassium wasting and hypokalemia. These patients had many of the features of Conn's syndrome. However aldosterone levels were not elevated. These patients were thought to have mineralocorticoid-type hypertension that may have been due to a yet undescribed kaliuretic hormone. These patients differed from those described by Welt and associates (Ibid p 221) in that patients with Liddle's syndrome were normomagnesemic.

A 64 year old white male presented with a 3 yr. history of severe hypertension (250/140) associated with hypokalemia (2.5). The hypokalemia persisted (2.2-2.6) despite discontinuation of diuretics. Serum magnesium was normal. Urinary K was greater than 60 mEq/liter when pt. was hypokalemic Renal angio was normal; creatinine 1.3; eight plasma renin determinations were non-detectable. U & P aldosterone were nl. Twenty-four hr. urine for 17 OH and pregnanediol was normal. Spironolactone had little effect on K excretion. The patient was subsequently placed on Amiloride 5 mg po qam. His serum potassium gradually normalized and has remained normal without supplementation. The hypertension is now controlled with Minoxidil 40 mg qd, Lasix 40 qd, Amiloride 5 mg qd and Atenolol 250 mg qam.

It appears as though the site of renal potassium wasting in Liddle's syndrome is located distal to the aldosterone responsive portion of the nephron. Amiloride is an effective agent in this rare syndrome.

PLASMA FACTORS IN DAHL S & R RATS WHICH AFFECT Na EFFLUX. L Tobian, B Norman, MA Johnson*, B Derauf*, J Iwai*, University of Minn. & Brookhaven Lab, NY.

Washed, Na^{22}loaded lymphocytes from Sprague-Dawley rats were incubated in Dahl plasma & Na^{22}efflux rate was measured(10rats/group). Lymphocytes in plasma from Dahl R rats(.3%low NaCl diet)had a slower rate of Na efflux than those in plasma from Dahl S rats(.3%NaCl diet)(81.6%Na^{22}remaining in lymphocytes in R plasma vs 72.9%remaining in lymphocytes in S plasma, $p<.01$). There was no decrease of Na efflux in plasma from S & R rats on a 4%high NaCl diet, hence no evidence for increase of "natriuretic" factors in response to a high NaCl intake. Similar incubations were made in plasma containing .1 mM added ouabain to inhibit Na-K ATPase. On .3%NaCl diets, Na efflux in S plasma(ouabain)was now slower than in R plasma(ouabain)(92%Na^{22}remaining with S plasma vs 80%with R plasma, $p<.001$). After 4 weeks on 4%NaCl diet, efflux in R plasma(ouabain)decreased, 89%remaining vs 80%remaining($p<.01$); while efflux in S plasma(ouabain)increased, 83%remaining vs 92%remaining($p<.01$). Thus R rats on 4%NaCl generate humoral agents which retard Na efflux in ouabainized lymphocytes, while S rats do the opposite. These differences were abolished with ouabain plus furosemide, which together inhibit Na-K co-transport as well as Na-K ATPase. Summarizing, R plasma induces slower Na efflux than S plasma. If this effect occurred in renal tubules, S plasma would retard natriuresis much more than R plasma & thereby lead to NaCl hypertension. Moreover, a 4%high NaCl diet brings out a different "natriuretic" hormone in R rats only, one which inhibits Na efflux in ouabainized lymphocytes. This factor could facilitate natriuresis & thereby account partially for resistance to NaCl hypertension in R rats.

COMPARATIVE COST OF ANTIHYPERTENSIVE DRUGS. Frederick S. Wilson* and Barry R. Walker. Wyeth Laboratories, Dept. of Clinical Research & Development, Philadelphia, Pennsylvania.

Diuretics (D) are used as initial antihypertensive therapy partly because they are inexpensive. Unlike α-agonists (A) or β-blockers (B), D frequently induce metabolic abnormalities requiring lab monitoring and supplemental rx: e.g., 20-40% of patients taking D require 30 mEq K/day. In this group, D are less cost effective than either A or B, i.e.

Inderal® 120 mg/day/yr.	$153.00
Catapres® 0.2 mg/day/yr.	153.00
Hydrochlorothiazide 100 mg/day/yr.	15.00
Hydrodiuril® 100 mg/day/yr.	30.00
KCl 30 mEq/day/yr.	90-180.00
SMA 12 2/yr.	20.00
Lipid profile 2/yr.	60.00

Other costs may be incurred because of hyperuricemia, elevated blood glucose, etc. Also, D induced metabolic abnormalities more frequently develop in blacks and the elderly (those least able to bear the additional expense). Detailed comparative cost analyses will be presented. In summary, D. is less expensive than A or B only in those patients who do not develop metabolic abnormalities requiring laboratory follow-up or supplemental drug rx. Comparative rx costs must reflect not only initial drug costs but the total cost impact of any given rx modality. In the interests of both cost and avoidance of potential risk factors, patients who develop metabolic abnormalities on D should be considered for sole rx with A or B.

The Effect of Pb^{++}, Hg^{++}, Cd^{++} and Ca^{++} ON THE VASCULAR SMOOTH MUSCLE CELL (VSMC) Na-K-ATPase. Aijiro Tokushige*, Hirohiko Higashino*, John D. Bogden*, Bernard M. Searle*, John W. Bauman, Jr., and Abraham Aviv. N.J. Medical School, Div. of Pediatric Nephrology, Newark, New Jersey.

The electrogenic Na-pump plays an important role in the contractile state of the VSMC. Recently we have developed techniques that enabled us to directly measure the kinetics and specific activity of the Na-K-ATPase (the enzymatic correlate of the Na-pump) in in-vitro preparations of VSMC's derived from the rat carotid artery (Aviv, Higashino et al, Am. J. Physiol.-Cell Physiol., in press). In the present experiments we examined the effect of trace metals and calcium on the Na-K-ATPase in VSMC's. The specific activity of the enzyme in the VSMC's was 2.3 ± 0.08 µMol P_i/mg cell protein/hr. (mean ± SEM) or 1.0 ± 0.03 µMol $P_i/10^6$ cells/hr. Pb^{++}, Hg^{++} and Cd^{++} exerted a potent inhibition on the activity of the Na-K-ATPase in the in-vitro preparations of the VSMC's. The I_{50} values for the respective ions were reached at concentrations of 10^{-5}, 10^{-6} and 10^{-5}M. Calcium also inhibited the enzyme. The I_{50} for Ca^{++} was reached at a concentration of 10^{-3} M, which is within the physiological range of extracellular ionized calcium. It is theorized that since inhibition of the Na-pump in vascular tissue may lead to an increase in peripheral resistance, a mechanism by which the aforementioned trace metals may produce toxicity in-vivo is via inhibition of the VSMC Na-K-ATPase. Furthermore, since the I_{50} for Ca^{++} is at its physiological extracellular concentrations, it is possible that calcium may participate in blood pressure regulation by its effect on the VSMC Na-pump.

PLASMA VOLUME IN TREATED HYPERTENSIVE PATIENTS. Daniel J. Wilson, Doris Wise,* and Robert Cowan.* Bowman Gray School of Medicine, Department of Medicine, Section of Nephrology and Hypertension, Winston-Salem, North Carolina.

Plasma volume may be increased or decreased in various primary and secondary forms of hypertension(HBP). However, few studies have attempted to correlate plasma volume with a patients response to antihypertensive therapy.

Plasma volume was measured in 41 male and 41 female treated hypertensive patients with ^{125}I human serum albumin. All patients had uncontrolled (HBP) with supine right arm blood pressures $\geq$ 140/90 mmHg. Fifty five patients had essential HBP, 20 renovascular HBP, 7 renal parenchymal HBP, and one patient had primary aldosteronism.

Plasma volume (mean + SD) was 18.5 $\pm$ 3.9 cc/cm in males and 14.9 $\pm$ 2.5 cc/cm in females. Twenty four patients, 13 male and 11 female, had resistant HBP; defined as a diastolic blood pressure $\geq$ 100 mmHg while on maximum tolerated doses of 3 or more antihypertensive medications, administered in a stepped care fashion. Plasma volume was 21.2 $\pm$ 5.2 cc/cm in males with resistant HBP, and 17.3 $\pm$ 2.5 cc/cm in non-resistant hypertensive males ($p \leq .001$), while plasma volume was 16.4 $\pm$ 2.3 cc/cm in females with resistant HBP and 14.1 $\pm$ 2.4 cc/cm in non-resistant hypertensive females ($p \leq .023$).

Thus, plasma volume was consistantly greater in males as compared to females. Patients with resistant or drug refractory hypertension have greater plasma volumes than non-resistant hypertensives.

Hypervolemia may be an important factor in resistant hypertension.

Immunology & Pathology — Basic

MULTIVALENT ANTIGENS ARE ESSENTIAL FOR IN SITU SUBEPITHELIAL GLOMERULAR IMMUNE DEPOSIT FORMATION. Lawrence Agodoa, V.J. Gauthier* and Mart Mannik*, Dept. Med., Univ. of Washington, Seattle, Wa. Cationic antigens bind to the glomerular basement membrane, and subsequent injection of specific antibodies leads to in situ formation of immune complexes in the subepithelial area. This study was designed to determine if multivalent antigens, capable of forming immune precipitates, are essential for formation of subepithelial deposits. For this purpose precipitating (multivalent=NAP_{high}·HSA)and nonprecipitating (oligovalent = NAP_{low}·HSA) cationized nitroazidophenyl human serum albumins were used. Rat renal arteries were injected with cationized antigens: NAP_{low}·HSA (600µg) or NAP_{high}·HSA (400µg). Separate groups of rats received injections of cationized antigens followed in 1 hr by intravenous 1 mg rabbit antibodies to NAP (anti-NAP), or 1 mg of antibodies to HSA (anti-HSA). The cationized antigens alone were present in the GBM within 1 min and 1 hr of injection, but disappeared by 24 hrs when stained for HSA. After intravenous injection of 1 mg anti-NAP to rats given NAP_{high}·HSA, both HSA and rabbit IgG were present in the GBM from 1 through 96 hrs. Electron microscopy revealed predominantly subepithelial deposits. 1 mg anti-NAP 1 hr after NAP_{low}·HSA caused no deposits. On the other hand, when anti-HSA was given 1 hr after NAP_{low}·HSA the antibody was detected through 96 hrs, and subepithelial deposits were present by EM, demonstrating that the antigen was bound to the GBM initially.
These studies indicate that the formation and persistence of subepithelial deposits depends on multivalent antigens capable of forming immune precipitates with antibody.

REACTIVE OXYGEN PRODUCTION ASSOCIATED WITH STIMULATION OF 12-LIPOXYGENASE DURING PHAGOCYTOSIS BY RAT GLOMERULAR MESANGIAL CELLS. Raymond Ardaillou*, Laurent Baud*, Jacqueline Hagege*, and Josée Sraer* (intr. by Michael Dunn). INSERM 64, HOPITAL TENON, PARIS, FRANCE.

To investigate the phagocytic capability of glomerular mesangial cells and the events associated to phagocytosis, rat cultured mesangial cells were incubated in the presence of opsonized zymosan (STZ). Mesangial cells were identified on the basis of morphological (presence of microfilaments and pattern of staining by an antimyosin antiserum) and physiological (contractile activity in response to AII) characteristics. No contamination by esterase-positive cells was observed. Electron microscopy studies revealed that the phagocytic process started after 5 min incubation, was maximum at 30 min and affected 20-30 % of the cells. Superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) generation by mesangial cells exposed to STZ increased with time and STZ concentration. Pretreatment by Cytochalasin markedly inhibited reactive oxygen production. Cells incubated with zymosan particles treated with heated serum produced undetectable amounts of O_2^- and 6 times less H_2O_2 than cells exposed to STZ. ^{14}C arachidonic acid conversion into PGs and HETEs by mesangial cells was measured by radiometric HPLC. Incubation with STZ did not modify the synthesis of PGE_2 and $PGF_{2\alpha}$ whereas it stimulated that of 12-HETE. This study demonstrates that a high percentage of rat cultured mesangial cells phagocytize opsonized particles. The phagocytic process results in an oxidative burst with stimulation of the 12-lipoxygenase pathway and without change in the cyclooxygenase activity. The latter result appears as specific of this type of cell.

STREPTOCOCCUS-ASSOCIATED NEPHRITIS IN RABBITS. Boris Albini, Russell J. Nisengard, and Murray W. Stinson, Dept. Microbiology, Sch. Med. and Dept. Periodontics and Endodontics, Sch. Dent., SUNYAB, Buffalo, N.Y.

New Zealand white rabbits were injected intravenously three times per week with disrupted Streptococcus mutans strain MT703 or K1R. Within 6-8 weeks, the rabbits developed proteinuria with subsequent weight loss and lethargy. Many of the rabbits died 4 to 6 months after first injection. The rabbits' sera contained antibodies to S. mutans and to rabbit heart and kidney tissues. Their sera had anti-S. mutans antibodies and some had reactivity with rabbit heart and kidney tissues. During acute disease, kidneys were pale, enlarged, and showed numerous red spots. Immunofluorescence tests showed IgG, IgM, and complement deposits in granular patterns in the GBM and the mesangium. Staining for fibrinogen was focal and segmental. Light microscopy showed marked lobulation of the glomeruli with endothelial and mesangial cell proliferation and infiltration with polymorphonuclear granulocytes. Later in the disease, many glomeruli showed areas of necrosis and sclerosis. Adhesions and crescents were seen frequently. Focally, the interstitium was expanded and was densely infiltrated by PMNs and plasma cells. S. mutans antigens could be demonstrated in the early lesions by immunofluorescence and in elution experiments. Tissue specific antibodies were also present in the eluates, but in lower titers. This severe nephritis should provide a useful model for the study of the pathogenesis of poststreptococcal nephritis in man.

IDENTIFICATION OF THE HUMAN COMPLEMENT C3bi RECEPTOR (CRIII) WITH MONOCLONAL ANTIBODIES. M.A. Arnaout, N. Dana*, J. Melamed*, A. van Agthoven* and R.A. Todd* Harvard Medical School and Children's Hospital Medical Center, Dept. of Medicine Boston, MA.

Receptors for the third component of complement (C3) are present on a wide variety of human cells. These receptors are important in various forms of proliferative glomerulonephritis and in clearance of circulating immune complexes. Receptors for three different fragments of C3 have been described but their relative functional importance in vivo is not clear. We developed two mouse monoclonal antibodies to the C3bi receptor (CRIII). These antibodies immunoprecipitate a previously described antigen (MO1) which consists of two noncavalently linked glycosylated polypeptide chains of 150 K and 94 K daltons. These antibodies specifically inhibited binding of C3bi coated sheep erythrocytes to neutrophils or monocytes in a dose dependent manner. There was no inhibition of C3b, C3d or Fc receptor functions by these antibodies. There were 70,000 and 140,000 C3bi receptors/cell on neutrophils and monocytes respectively. The availability of these reagents will allow a more quantitative analysis of the role of this receptor in leukocyte dependent immunopathologic disorders.

CYSTIC METANEPHRIC DEVELOPMENT (CMD) IN SERUM-FREE ORGAN CULTURE (SFOC). E.D. Avner, W.E. Sweeney*, N.P. Piesco*, and D. Ellis. Univ. Pgh. Schools Medicine & Dental Medicine, Pgh., PA.

The roles of tubular obstruction, tubular cell death & regeneration, $\downarrow[K^+]$, & tubular supporting wall abnormalities in the genesis of CMD are controversial. To study the pathogenesis of CMD we have developed a SFOC model in which hydrocortisone (HC) produces cystic tubular changes.

Whole metanephros was explanted from SW albino mice at 13 ± 0.4 days gestation. Controls (C) were grown in Dulbecco's MEM & Ham's F-12 with 10 nM selenium, insulin (5 µg/ml), PGE_1 (25 ng/ml), T_3 (3.2 pg/ml) & transferrin (5 µg/ml). The addition of HC (50 µg/ml) to growth media produced the cystic model (CY). $[K^+]$ of both C & CY media was constant (3.9 mEq/L). All tissue was incubated in humidified 5% CO_2/mixed air at 36.5°C. Tissue was harvested after 1-7 days growth & studied by light & electron microscopy.

Both C & CY underwent advanced organotypic tubulogenesis & unique epithelial glomerulogenesis without vascularization. CY showed marked CMD in proximal tubules. Cyst walls had normal microvilli & intracellular organelles without necrosis or proliferation, while tubular basement membranes (TBM) showed attenuation in areas where irregular, thinned supporting mesenchyme was retracted. All cystic changes could be prevented by adding 10% bovine serum to the CY growth medium.

We conclude: 1) CMD can be produced in SFOC by HC in the absence of perfusion, urine flow or obstruction, or changes in $[K^+]$; 2) CMD in this model is caused by abnormalities induced in TBM & supporting mesenchyme; 3) CMD in SFOC can be blocked by the addition of a serum factor.

GLOMERULAR SIEVING OF ANIONIC AND NEUTRAL BOVINE ALBUMINS (BSA) IN PROTEINURIA DUE TO HEXADIMETHRINE (HDM) OR ADRIAMYCIN (ADRIA). JA Bertolatus and LG Hunsicker. Dept. of Medicine, VA Medical Center and U. of Iowa, Iowa City, IA.

Studies with nonprotein markers in proteinuria have demonstrated alterations in both the charge and size selective properties of the glomerular filter. To determine the basis for albuminuria we measured the simultaneous fractional clearances (FC) of ^{131}I-labeled native BSA (aBSA, pI=4.9) and ^{125}I-labeled cationized BSA (nBSA, pI=7.5) in control rats infused with saline (N=4), and in rats made acutely proteinuric by HDM (20µg/m for 45 m, N=5) or chronically proteinuric by Adria (2 wks after 7.5 mg/kg IV, N=5). Protein excretion rates were 13±4, 303±186 and 136±87µg/m in the three groups. The left kidney was rendered non-filtering (inulin extraction=0) by mannitol diuresis and ureteral ligation. Right kidney marker filtration was measured as TCA insoluble marker in urine plus right kidney homogenate, corrected for interstitial marker in the non-filtering left kidney. GFR was measured by inulin clearance. (Data: mean±STD).

GROUP	C_{in}/100 g	FC(aBSA)	FC(nBSA)
Saline	.34 ± .05	.0002 ± .0001	.031 ± .015
HDM	.47 ± .13	.0116 ± .0060*	.042 ± .022
Adria	.30 ± .13	.0187 ± .0125*	.022 ± .017

* $p < .0001$ control vs experimental; others NS

There was little change in FC of nBSA, but FC of aBSA increased 48 and 78 fold in HDM and Adria rats, rising almost to the level for nBSA. These results indicate that in proteinuria due to HDM or Adria, increased filtration of albumin (MW=69,000) is due mainly to loss of charge dependent permselectivity, probably from neutralization (HDM) or loss (Adria) of glomerular anions.

SIZE AND CHARGE SELECTIVE PERMEABILITY DEFECTS INDUCED IN GLOMERULAR BASEMENT MEMBRANE BY A POLYCATION. J.L. Barnes, R.A. Radnik,* E.P. Gilchrist,* and M.A. Venkatachalam. Univ. of Tx. Health Science Center, San Antonio, Texas.

Polyethyleneimine, M.W. 1800(PEI) was given to rats (15 µg/g body wt, i.v.) followed 15 min. later by 0.1 mg/g body wt native ferritin (NF-pI =4.5-4.8) or one of three cationic ferritins (CF-pI=7.5-8.2;8.0-8.7 or 8.7-9.0). After 15 min. kidneys were fixed for electron microscopy. Controls(C) received vehicle without PEI followed by the appropriate ferritin. PEI-induced permeability change was measured as the ratio (PEI/C) of counted ferritin particles within the glomerular basement membrane (GBM) in the corresponding PEI and control groups. The effects of PEI on inulin clearance (C_{IN}) and renal blood flow(C_{PAH}) were measured. Ferritin permeation of GBM increased with rising pI in control rats. The effects of PEI, as measured by the permeability index for each tracer were: NF=107;CF(7.5-8.2)=3.3;CF(8.0-8.7)=1.7;and CF(8.7-9.0)=0.9. After PEI, C_{IN} and C_{PAH} decreased by half. Because filtration fraction remained constant,but ferritin particles in the GBM increased, PEI effects on permeation cannot be attributed to hemodynamic factors. If its effect on GBM permeability were to be mediated exclusively by neutralization of the charge barrier, PEI/C should be increased for NF, but decreased for all CF. The results show a marked effect of PEI on the charge barrier (PEI/C >100 for NF). But, PEI also enhanced GBM permeation by CF (7.5-8.2) and CF(8.0-8.7). These paradoxical results can be explained if polycations (including CF) not only neutralize anionic sites, but distort GBM gel structure, thereby altering porosity.

SHARING OF ANTIGENIC DETERMINANTS BY RAT Fx1A, AND ANTIGEN(S) LOCATED IN NORMAL GLOMERULI AND IN IMMUNE DEPOSITS OF HEYMANN NEPHRITIS: STUDIES WITH MONOCLONAL ANTIBODIES. Atul K. Bhan, Kristina Krok, Diane Duane, Eveline E. Schneeberger, A. Bernard Collins, Robert T. McCluskey, Dept. of Path., Mass. Gen. Hosp., Boston, MA.

Monoclonal antibodies against rat Fx1A were made by somatic cell hybridization of spleen cells of BALB/c mice, immunized with rat Fx1A in complete Freund's adjuvant, with NS1-myeloma cells. Two clones (AG3, 14C1) were selected, and ascites fluid obtained after i.p. injection of cloned hybrid cells was used as the source of monoclonal antibodies. Although both monoclonal antibodies were reactive with brush borders of all proximal tubules in frozen tissue sections of normal Lewis kidneys, granular staining of glomeruli was seen only with 14C1 antibody. In addition, 14C1 antibody, but not AG3 antibody, stained immune deposits in glomeruli of rats with Heymann nephritis. Intravenous injections of antibodies in normal Lewis rats or Lewis rats with Heymann nephritis, resulted in deposition of 14C1 antibody, but not AG3 antibody, in glomeruli in a granular pattern along capillary walls, as observed at intervals of 1 to 8 days. The results indicate that the monoclonal antibody 14C1, but not AG3, recognizes antigenic determinants shared by rat Fx1A and by antigen(s) present in normal glomeruli and in immune deposits of Heymann nephritis. Therefore, it appears that 14C1 antibody reacts with an antigen responsible for Heymann nephritis. Furthermore, since the antigen is present in normal glomeruli, it seems likely that the immune deposits form in situ.

GENETICALLY DETERMINED SUSCEPTIBILITY TO STEROID-INDUCED POLYCYSTIC KIDNEY DISEASE (PKD) IN INBRED MICE. Stan R. Blecher* and John F.S. Crocker. Dalhousie Univ., Dept. of Anatomy & Pediatrics, Halifax, Nova Scotia.

A form of PKD which resembles human infantile PKD can be induced in mice by steroid injections at birth. Because human PKD is genetically determined in part, we examined the total variance (V_t) in two parent inbred mouse strains and their F_1 and F_2 crosses in order to determine how much of the variance is environmental (V_e) and how much genetic (V_g). Hydrocortisone acetate was given on day 1 and, on day 5, cyst formation was histologically evaluated, using a score of 0 to 4+. We crossed a sensitive strain (C3H),($\bar{x}$=0.94; n=220), and a resistant one (DBA), ($\bar{x}$=0.31; n=72). The DBA · C3H F_1 cross had no cysts ($\bar{x}$=0; V_t=0) although the sample size was small (n=12). The F_2 cross showed some cyst formation ($\bar{x}$=0.69; V_t= 0.61; n=111). The V_g (difference between V_t of F_1 and F_2) is 0.61 (or virtually 100%). On the other hand, the reciprocal C3H · DBA F_1 cross had a cyst frequency intermediate between the parent strains ($\bar{x}$=0.79; V_t=0.41; n=21) and the F_2 cross somewhat less ($\bar{x}$=0.56; V_t=0.53; n=78), yielding a V_g of 0.53 (21.4 $\pm$ 0.3%). These results, though preliminary, indicate a significant genetic susceptibility to cyst induction by steroids, and reveal an intriguing but as yet unexplained difference between reciprocal crosses. We propose on the basis of these data, that the etiology of infantile PKD involves an endogenous abnormality of steroid metabolism superimposed on a genetically determined susceptibility.

NEPHROTOXICITY OF LYSOZYME (LZM): EVIDENCE FOR THE INTERACTION OF CATIONIC PROTEINS (CP) WITH POLY-ANIONIC (PA) TUBULAR (TE) GLYCOCALYX (Glx). Praveen N. Chander, Mark J. Todt*, and Steven C. Mohos*. New York Medical College, Valhalla, NY.

Most nephrotoxins are cationic at physiologic pH. LZM (pI,11; MW, 13,900) was injected IV in varying doses into rats to study the interaction of CP with PA TE Glx. To prevent a drop in BP, rats were preinjected with antihistamine. Urines were collected 24 hrs before and 6 hrs after LZM inj. Mean% changes from pre to post LZM inj. values follow:

Group**	Ccr	V.[1]	FeNa[1]	FeK[1]	FeGl[1]	Uosm[1]
Saline	-6.4	+34	+35	+9.1	+6.9	-39
25 mg	-14	+17	-1.1	-31	+350	-41
50 mg	-53*	+79	+240*	+120	+2500	-60*
100 mg	-59*	+130*	+270*	+96*	+2500*	-64*
200 mg	-78*	+130*	+1000*	+300*	+6700*	-72*

1=(Ccr, V=creatinine clearance, urine flow in ml/min/100g body weight; FeNa, FeK, FeGl=% fractional excretion of sodium, potassium and glucose; Uosm= urine osmolality in mOsm/kg.) *$p < 0.02$;**N=4. Morphologically, a dose related response was seen ranging from minimal subcellular damage to clumping, partial loss of proximal TE (PTE) brush borders (BB) (S_1 & S_2> S_3), and focal necrosis of TE cells, the latter involving thick ascending limb cells (TAL) most significantly. Colloidal iron stain (pH 1.9), revealed reduced intensity and degree of PTE BB staining at high dosages. In conclusion LZM, a CP of low molecular weight, induces a dose related, nonoliguric acute renal failure (ARF) with renal concentration defect. We hypothesize that renal functional and morphologic changes are due to interaction of cationic protein with polyanionic tubular glycocalyx. Significant early damage to TAL appears to be unique to this model of ARF.

EFFECT OF DOPAMINE AGONISTS ON SPONTANEOUS FOCAL GLOMERULAR SCLEROSIS (FGS) IN AGING RATS. W.K. Bolton and B.C. Sturgill, University of Virginia School of Medicine, Charlottesville, Virginia.

Spontaneous FGS with proteinuria develops in rats as a function of aging. We studied the effect of two dopamine agonists, bromocriptine (Br) and CU-32-085 (CU) on the clinical and pathologic course of this process in Fischer F-344 rats. These agents specifically inhibit prolactin secretion from the anterior pituitary and have little other effect. Sixty-seven 3 mo old male rats were uninephrectomized to accelerate development of FGS. Rats were divided into 3 groups - control (C), CU, and Br animals. The test rats continuously received the two drugs in food. Serial kidney biopsies at 3 mo intervals to age 15 mo revealed that both CU and Br treatment ameliorated progression of FGS with lesser effects on cast formation (on a 0-4+ scale, glomerular score CU-1.34; Br-1.44; C-2.78, $p<.001$). Abnormal total urinary protein developed in all rats, but was less than C for both agonists after 8 wks and significantly less for CU, $p<.05$ to $p<.001$. Serum creatinines were similar in all groups. Body wts were comparable throughout the study, kidney sizes at sacrifice were not different, and pituitary wts were similar. Prolactin levels were significantly depressed in agonist recipient rats (CU-3.9; Br-19.2; C-24.7 ng/ml).

These studies show that the dopamine agonists, CU and Br, significantly ameliorate progression of FGS in rats. This effect presumably occurs by specific inhibition of prolactin secretion, and further implicates prolactin as an important etiologic factor in FGS in rats.

A MECHANISM OF GLOMERULAR IMMUNE COMPLEX LOCALIZATION. William F. Clark, Gerald J.M. Tevaarwerk,* and Bruce D. Reid.* Department of Medicine, University of Western Ontario, London, Ontario, Canada.

Large lattice DNA-anti-DNA immune complexes are thought to be rapidly deposited in the glomeruli of patients with severe SLE nephritis. A mechanism for this localization has not been revealed. We have been studying the interaction of DNA-anti-DNA immune complexes and platelets at physiologic concentrations in human plasma. Platelet aggregation and release is mediated via the platelet Fc-receptors by large lattice immune complexes formed by high affinity anti-DNA antibodies. Whereas small lattice DNA-anti-DNA immune complexes produced no detectable aggregation or release. The formation of large lattice immune complexes behaves the laws of mass action and the platelet release reaction is concentration-dependent. Theoretically antigen, antibody and platelet concentration would exponentially magnify the large lattice immune complex, platelet interaction. We studied *in vitro* the effect of concentration of high affinity anti-DNA antibodies, DNA antigen and human platelets in plasma that would be expected with physiologic glomerular ultrafiltration and pathologic hyperfiltration. We noted that negligible platelet release would occur in the systemic circulation at the time of marked platelet release in the glomeruli. The large lattice immune complex, platelet interaction could provide the permeability factors that facilitate large lattice immune complex deposition. *In vivo* (pigs) we noted that intra-renal large lattice immune complex formation was associated with subendothelial-mesangial immune complex deposition and renal vein thrombocytopenia.

THE ROLE OF THE HUMAN MONOCYTE(MN) Fc AND C3b RECEPTORS(FcR,C3bR) IN BINDING AND CATABOLISM OF SOLUBLE AGGREGATED IgG (AIgG). FG Cosio, Ohio St Univ, Dept of Medicine, Columbus, Ohio.

It is believed that FcR of cells of the mononuclear-phagocytic system(MPS) play the principal role in the clearance of immune complexes(IC) from the circulation. The role of the C3bR in this process has not been clarified. MN were preincubated with AIgG at 37°C, washed and incubated with ^{125}I IC (HSA-anti-HSA) at 4°C. Preincubation with AIgG caused inhibition of subsequent binding of IC by FcR to a degree directly related to the concentration of AIgG and the time of incubation. By contrast, in the presence of human serum(HS), preincubation with AIgG, up to 100 ug/ml, did not cause significant inhibition of FcR binding and AIgG, 1000 ug/ml, caused significantly less inhibition than in MN preincubated without HS (18±12% vs 79± 3%, p<.001). The FcR protective effect of HS was lost in a C3 deficient HS and in heat decomplemented HS. In a second series of experiments, MN were incubated with ^{125}I AIgG at 37°C. HS significantly enhanced the endocytosis and catabolism of AIgG and the effect was complement dependent. After AIgG was bound to the MN surface, removal of HS from the incubation media did not alter the rate of catabolism. In conclusion, MN are able to bind soluble AIgG via both FcR and C3bR however binding to the former occurs only if C3 is not present during the incubation. Binding to C3bR is followed by catabolism of AIgG at a faster rate than after binding to FcR. These results suggest that the C3bR plays a primary role in the in vivo clearance of large, complement-fixing IC. Complement depletion will interfere with the uptake of IC by the MPS thus facilitating deposition in tissues.

GLOMERULAR ^{35}S-GLYCOSAMINOGLYCAN (GAG) METABOLISM IN AMINONUCLEOSIDE-INDUCED (PAN) NEPHROTIC SYNDROME IN RATS. Peter J. Dehnel,* David J. Klein,* Theodore R. Oegema,* Alfred F. Michael, and David M. Brown. Univ. of Minnesota, Depts. of Pediat., Lab. Med. and Pathol., Orthopedic Surg., and Biochem., Minneapolis, Minnesota.

The synthesis of GAG by glomeruli of PAN-nephrotic syndrome was studied by $^{35}SO_4$ labelling in vitro. Glomeruli were isolated 5 or 7 d. after injection of PAN and incubated 12, 24 or 48 h. with $^{35}SO_4$ after which GAG synthesis was studied by methods described previously (Diabetes 41:418, 1982). At all times, increased ^{35}S-GAG was present in nephrotic whole glomeruli and incubation media, whereas no differences from controls were found in the ^{35}S-GAG of nephrotic GBM. A smaller percentage of total glomerular ^{35}S-GAG was found in the nephrotic GBM. Similar results were obtained using GBM derived from sonicated or H_2O-detergent-DNAse glomeruli. Nephrotic and control glomeruli incubations had similar distribution of ^{35}S-GAG between whole glomeruli and incubation media at all times. The proportions of heparan sulfate and chondroitin sulfate-dermatan sulfate in the glomerular fractions and incubation media from nephrotics and controls were similar. CsCl density gradient and Sephadex G-50 chromatographic distribution of nephrotic and normal ^{35}S-proteoglycan were similar. If one speculates that nephrotic glomeruli have altered proteoglycan or GAG localization on filtration surfaces, these metabolic studies indicate that an abnormality is likely to be due to selective qualitative or matrix organization characteristics.

ACUTE TOXICITY OF MERCURY UPON CULTURED RENAL TUBULAR CELLS. Francis E. Cuppage, Abbas M. Behbehani,* John P. Devine,* and Stephen D. Tarver.* Univ. of Kansas Med. Ctr., Dept of Pathology, Kansas City, Kansas.

Heavy metal toxicity is known to adversely affect the function of renal tubules in vivo. We have used an in vitro system of cultured kidney tubular cells to evaluate heavy metal injury exemplified by $HgCl_2$. Canine renal tubular epithelial cells were harvested and grown in primary culture using a serum free defined medium. Transcellular transport of electrolytes was measured by the formation of domes in the monolayer of cells cultured upon an impermeable base in plastic culture dishes. Once dome formation was evident in the monolayers varying concentrations of $HgCl_2$ were then added to the media. Inhibition of transport was evidenced by alterations in dome maintenance as evaluated by light microscopy and both scanning and transmission electron microscopy. Control monolayers demonstrated dome formation in the absence of $HgCl_2$. With the addition of 2.0 µg Hg/ml to the medium, partial dome collapse occurred within 1 hr. and total collapse by 4 hrs. 10 µg Hg/ml resulted in total dome collapse by 1 hr and monolayer disruption occurred by 4 hrs. Associated ultrastructural evidence of cell injury included mitochondrial swelling and membrane disruptions. Thus, we have been able to grow primary canine renal tubular epithelia as functional monolayers and have developed an in vitro system for evaluation of toxicity to heavy metals using this culture system. The techniques should provide a model system for further studies in renal toxicity and altered transepithelial transport.

CHINESE TRADITIONAL HERBS AMELIORATE FIBRIN DEPOSITION IN CATIONIZED-BOVINE SERUM ALBUMIN (C-BSA) NEPHRITIS (GN). X. H. Du,* Y. K. Zhang,* W. Z. Zhou,* S. H. Wang,* (intr. by V.E. Pollak), Dept. of Medicine, First Teaching Hospital, Beijing Medical College, Beijing, China.

Extracts of Chinese traditional herbs, Salvia miltiorrhazae (SM) and Ligustrazini hydrochloride (LH), increase fibrinolytic activity, improve the microcirculation, and promote tissue repair. We studied the effects of these drugs in an experimental GN with glomerular fibrin deposition induced in 22 Chinese white 2-2.5 kg rabbits by daily injection of 10 mg C-BSA. The 12 controls received only C-BSA for 42 days; 10 experimental animals were injected daily IV, from 3 days before C-BSA, with 1 ml each of the SM and of the LH--containing respectively 1 g and 20 mg of crude extract. Proteinuria occurred on week 3, and increased thereafter. By 6 weeks the proteinuria was slightly greater in control (m = 235 mg/24 h) than in treated (m = 176 mg/24 h) animals. All animals developed endocapillary proliferative GN, proved by renal biopsy. At sacrifice after 6 weeks, the control glomeruli (m = 125 µ) were larger than those in the treated group (m = 107 µ; p <0.01). Microthrombosis (PTAH hematoxylin) occurred in 40% of control glomeruli; in none of the treated. Fibrin related antigen was found in glomeruli of 11/12 controls, in 0/10 of the treated group. These Chinese herbs prevented fibrin deposition and thrombosis formation in glomerular capillaries in this model of experimental GN, and ameliorated the inflammatory process in the glomeruli.

INFLUENCE OF URETERAL OBSTRUCTION ON IMMUNE COMPLEX MEDIATED TUBULOINTERSTITIAL NEPHRITIS. Anders L. Fasth*,John R. Hoyer,Marcel W. Seiler, Dept. of Pediatrics,Harbor-UCLA Medical Center, Torrance,CA and Dept. of Pathology,VA Hospital, West Roxbury,MA.

Immunofluorescence staining for Tamm-Horsfall Protein (TH),a distal tubular surface membrane protein,shows a diffuse intense cellular staining of the ascending thick limb of Henle's loop (ALH) and a similar less intense pattern in distal convoluted tubules (DCT) in normal mouse kidneys. Extratubular aggregates of TH form in mouse kidneys after unilateral ureteral obstruction (UUO) for 24 hours.These TH aggregates are seen in the interstitium,within Bowman's space of glomeruli,and in large aggregates at the base of some DCT.The effect of UUO on immune complex nephritis was studied in 26 Swiss-Webster mice immunized with rat TH (KI 17:284,1980) and 18 controls,1-3 weeks after temporary UUO.Mean IgG anti-TH antibody titers measured by ELISA in immunized mice were more than 10^4 times greater than baseline in control mice.TH-immunized mice formed granular immune deposits of IgG and TH at the base of ALH and DCT cells.UUO did not cause a further rise in anti-TH titers in immunized mice and only minimal increase in IgG deposits, primarily with large TH deposits at base of some DCT.TH within Bowman's space of glomeruli persisted for 3 weeks after UUO,but IgG deposits were not seen in this site.These studies suggest that the availability of tubular antigen is not the limiting factor for immune tubular injury in this model.

MONOCLONAL MARKERS TO HUMAN GLOMERULAR CELLS - IDENTIFICATION OF GLOMERULAR CELLS IN CULTURE. Eric F. Glasgow*, Wayne W. Hancock*, Norbert Kraft* and Robert C. Atkins. Department of Nephrology, Prince Henry's Hospital, Melbourne, Australia.

Cell markers for glomerular cells were produced by the hybridoma technique and these monoclonal antibodies were used to identify the individual glomerular cell types in kidney sections and in outgrowths from isolated glomeruli in culture. Human glomeruli were used for immunisation. Monoclonal antibodies were produced, and cloned, to glomerular epithelial cells (PHM 5, PHM6), mesangial cells (PHM 9, PHM 12) and endothelial cells (PHM 2, PHM 14). Glomerular cell specificity was established by light microscopy using a sensitive 4-layer PAP immunoperoxidase technique on paraffin and resin embedded kidney sections. Immunoperoxidase labelling of 7-21 day old glomerular outgrowths, using these antibodies as specific cell markers, allowed definition of two intrinsic glomerular cell types within glomerular outgrowths. Large, stellate Type I cells were stained by the epithelial markers PHM 5 and PHM 6 but not by the mesangial (PHM 9, PHM 12) or endothelial (PHM 2, PHM 14) markers. Fusiform Type II cells were stained with the mesangial markers (PHM 9, PHM 12) alone. No endothelial cells were detected. Thus these results confirm our previous contention that Type I cells in glomerular culture are epithelial in origin, and Type II cells are mesangial cells. Thus these monoclonal antibodies can be used to identify cells within glomeruli in tissue sections and also in glomerular outgrowths in vitro. They provide the basis for a fresh approach for studies of glomerular pathophysiology.

EFFECT OF ANTI-TUBULE BASEMENT MEMBRANE ANTIBODY (Anti-TBM-Ab) ON GLUCOSE UPTAKE BY RAT RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV). Aaron L. Friedman, Sue Langan*, and Keith McCrae.* Univ. of Wisconsin Hospitals, Dept. of Pediatrics, Madison, Wisconsin, and Duke University Medical Center, Dept. of Pediatrics, Durham, North Carolina.

Anti-TBM-Ab has been described in various forms of glomerular and interstitial nephritides. The functional significance of anti-TBM-Ab remains unclear. Renal cortical tissue extract from Sprague-Dawley rats, plus Freund's adjuvant and pertussis vaccine, were injected into Lewis-Brown Norway rats (modification from Sugisaki et al). At 12-14 days post-injection, animals were sacrificed and found to have substantial deposition of IgG on TBM with minimal glomerular deposition. Endogenous creatinine clearance (ml/min $\pm$ SEM) was not significantly different in immunized vs. control rats (1.24 $\pm$ .087 vs. 1.42 $\pm$ .090). No glucose was found in the urine of immunized or control rats as measured by autoanalyzer. Glucose transport by BBMV was assessed using 60μM glucose in a time course from 15 sec to 45 min. In controls, an eightfold to tenfold "overshoot" (Na^+-dependent uptake) in glucose uptake was found at 15-30 sec of incubation (peak of 100.1 $\pm$ 1.6 pmoles glucose/mg protein; steady state at 45 min of 11.6 $\pm$ 2.0). Immunized rats showed only a 4.5-fold "overshoot" at 30 sec (peak of 58.5 $\pm$ 18; steady state at 45 min of 11.2 $\pm$ 1.6). Anti-TBM-Ab causes derangement in luminal brush border function and may play an important role in overall renal dysfunction in immune-mediated kidney disease.

TERMINAL COMPLEMENT (C) PATHWAY IN PROTEINURIA OF CHRONIC SERUM SICKNESS. G.C. Groggel*, S. Adler*, H. G. Rennke, W.G. Couser and D.J. Salant, Boston Univ. Med. Ctr. and Harvard Medical School, Boston, MA.

In rat membranous nephropathy (MN) proteinuria is complement-dependent but neutrophil independent (Salant et al JCI 66:1339,1980). To examine the possible role of the terminal complement components acting through the membrane attack complex (MAC) in mediating glomerular immune injury, we studied the genesis of proteinuria in another MN-like lesion. Serum sickness was induced in preimmunized controls and C6 deficient rabbits (C6D) with cationized BSA (cBSA) (pI 8.9-9.2) (Border et al JCI 69:451,1982). C6 hemolytic activity in C6D was 0.01% of controls. After 1 wk of daily cBSA (25 mg) both groups had identical granular capillary wall deposits of rabbit IgG, C3 and BSA by IF, small, exclusively subepithelial, electron dense deposits and no cellular infiltrate. Proteinuria was present in 71% of controls (range 1-3,010 mg/24h) and none of the C6D group (range 2-12 mg/24h). Serum antibody levels as measured by ABC-33 were greater in controls (6.5-99.0 μg/ml) than in C6D (2.0-46.9 μg/ml) although there was considerable overlap. However, specific glomerular-bound rabbit IgG, as measured by an in vitro assay utilizing glomeruli isolated from individual rabbits and anti-rabbit IgG ^{125}I, was similar in both groups. After 2 wks, coincident with a prominent influx of mononuclear cells and neutrophils, proteinuria developed in C6D.

These results indicate that C6 is important for the development of the early, non-inflammatory component of proteinuria in this model of serum sickness and suggest a possible role for the MAC in the mediation of complement-dependent immunologic glomerular injury.

CELLULAR COMPOSITION OF CRESCENTS IN HUMAN RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (RPGN).
Wayne W. Hancock* and Robert C. Atkins, Prince Henry's Hospital, Department of Nephrology, Melbourne, Australia.

The relative contribution of epithelial cells and macrophages to crescent formation remains controversial. This is largely due to difficulty in macrophage identification within crescents since their morphology, enzyme content and surface receptors vary throughout maturation. We therefore compared the antigenic profile of macrophages, crescent cells and evolving granulomata. A panel of antimacrophage monoclonal antibodies (PHM2, PHM3, OKM1, FMC32, FMC34); a muramidase antiserum to macrophages and polymorphs; plus monoclonal markers for polymorphs (FMC10) and glomerular epithelial cells (PHM5) were used; the latter also stained proliferating epithelial cells. Cryostat and paraffin sections of renal biopsies from 11 patients with RPGN (cellular crescents n=8; sclerosed n=3), were stained by a 4-layer PAP immunoperoxidase technique. Labelled cells were expressed as a mean percentage (range) of crescent cells. Cellular crescents comprised macrophages 27% (0-62), plus polymorphs 16% (2-58), and epithelial cells 8% (2-19), whereas sclerosed crescents contained macrophages 3% (1-16), polymorphs 5% (2-10) and epithelial cells 10% (4-19). In general OKM1, PHM2 and PHM3 labelled only tuft macrophages while FMC32 and FMC34 stained both tuft and crescent macrophages including multinucleate giant cells. Only FMC32 consistently labelled cells of granulomata. These finding demonstrate that macrophage cell surface antigens are lost during maturation. Macrophages were the major cell type identified within cellular crescents.

DIFFERENTIAL EFFECTS OF STEROIDS ON LEUKOCYTE MEDIATED GLOMERULONEPHRITIS (GN) IN THE RABBIT.
S. Holdsworth* and R. Bellomo* (intr. by R. Atkins), Department of Medicine, Prince Henry's Hospital, Melbourne, Victoria 3004, Australia.

The effects of methylprednisolone (2mg/kg/12hr) on the development of injury in two models of experimental GN (one mediated by neutrophils, the other by macrophages) was compared. The neutrophil associated lesion (initiated by heterologous anti-GBM antibody) was characterized by an exudative GN with heavy neutrophil accumulation (mean 6.7 neutrophils/glomerular cross section (N/GCS)±2.6SD) minor macrophage infiltration (6.2 macrophages/glomerulus (M/G)±3.3) and heavy proteinuria (2162 mg/24hr±643). Steroid treated rabbits developed a marked monocytopenia, mild neutrophilia and abrogation of glomerular macrophage accumulation (0.3 M/G±0.4). However, neutrophil accumulation (7.4 N/GCS±3.1), histological appearances and proteinuria (1969 mg/hr±622) were unaffected. The macrophage associated model of GN was a passively induced model of the autologous phase of anti-GBM antibody induced GN. The glomerular lesion was a diffuse endocapillary proliferative GN with heavy macrophage infiltration (56 M/G±12), insignificant neutrophil accumulation (0.6 N/GCS±0.05) and proteinuria (401 mg/24hr±103). Steroid treated rabbits developed neutrophilia and monocytopenia associated with abrogation of glomerular macrophage accumulation (1.6 M/G±0.4). This was associated with prevention of the development of GN and proteinuria (18 mg/24hr±6.2). Thus, steroids abrogate macrophage but not neutrophil mediated GN and their effectiveness in proliferative leukocyte mediated GN depends on the nature of the infiltrating cells.

COMPLEMENT (C) DEPLETION ACCELERATES THE CLEARANCE OF IMMUNE COMPLEXES (IC) FROM THE CIRCULATION OF PRIMATES.
LA Hebert, FJ Waxman†, DJ Birmingham†, JB Cornacoff†, MA VanAman†, WL Smead†, EH Kraut†, Ohio St Univ, Depts of Med, Radiol and Surg, Columbus, OH.

It is commonly held that C plays no role in the clearance of IC from the circulation. However, this is based on studies in non-primates whose erythrocytes (E) lack C3b receptors (C3bR). Primate E bear many C3bR and play a major role in the removal of large C-fixing IC from the circulation: circulating IC bind to E via C3bR until IC-bearing E traverse liver or spleen where IC are deposited. E then exits liver to repeat the cycle (Clin Res 350 Apr 82). Since IC binding to primate E is C dependent, C depletion in primates might affect clearance of IC from the circulation. This hypothesis was tested. Baboons and IC were prepared as reported above. C depletion was done with cobra venom (CV) or heparin (H). CV exp: (N=2) 3.2 u/ml was given iv 24 h earlier. IC were then given iv and multiple blood samples taken for 30 min. Fresh plasma (170 to 230 ml) was then given iv to reverse C depletion and the experiment repeated. H exp: (N=1 baboon + 1 rhesus) the primates were studied as above first untreated and then after H 90 u/ml. Compared with normals, C depleted animals showed decreased % IC bound to E (61±9% vs 14±4%), correspondingly increased % IC free in plasma and greatly accelerated peak IC clearance from blood (7±2%/min vs 44±2%/min p<.01). This increased IC clearance could not be accounted for by increased hepatic or splenic uptake. Conclusion: In primates C depletion may be harmful since clearance of IC by E is impaired and non-hepatic uptake is increased. This may lead to increased IC uptake by vulnerable organs such as kidney.

HEXADIMETHRINE (HDM) INDUCED PROTEINURIA: LAG BETWEEN GLOMERULAR HDM BINDING AND PROTEINURIA.
LG Hunsicker, SJ Shaffer*, JA Bertolatus. Depts. of Medicine, VA Med Ctr and U of Iowa, Iowa City, IA.

Infusion of the polycation HDM induces reversible proteinuria in rats associated with binding of HDM to anionic sites of the glomerular basement membrane (GBM). To determine whether proteinuria correlates temporally with glomerular binding of ^{3}H-HDM female rats, 200 g, were infused with saline alone (CTL), ^{3}H-HDM 20 µg/m for 25 m (before proteinuria)(A), or the same dose followed by 2.5 µg/m until onset of proteinuria ($\bar{x}$ = 38m)(B). Others were made proteinuric then permitted to recover for 1 (C) or 2(D) h on saline. Urine was collected for 5 m prior to sacrifice for measurement of urinary protein. Glomeruli were isolated for measurement of bound ^{3}H-HDM and terminal plasmas were analyzed for ^{3}H-HDM.
(Data expressed as $\bar{x}$ ± STD):

Gp	N	UV Protein (µg/m)	glom HDM (µg/mg)	plasma HDM (µg/ml)
CTL	5	16 ± 8	0.00 ± .001	0.00 ± .001
A	5	10 ± 10	1.16 ± .22	1.75 ± .78
B	5	65 ± 38	1.03 ± .69	1.36 ± .61
C	5	244 ± 331	1.49 ± .18	0.50 ± .08
D	4	656 ± 975	1.03 ± .55	0.43 ± .13

Though plasma HDM drops after stopping HDM glomerular HDM does not change measurably in this period. But proteinuria, absent in group A, is present in groups B, C and D after a lag. Thus proteinuria seems not to result solely from GBM charge neutralization by HDM. These results suggest that HDM binding induces a delayed, reversible structural change in the GBM which is the proximate cause of proteinuria.

HETEROGENEITY OF GLOMERULAR PERMEABILITY IN IMMUNE COMPLEX NEPHRITIS. Takeo Ishidate*, John R. Hoyer, Marcel W. Seiler, Dept. of Pediatrics, Harbor-UCLA Medical Ctr., Torrance, CA and Dept. of Pathology, VA Hospital, W. Roxbury, MA.

Luminal rabbit IgG deposits (LIgD) form in the ascending thick limb of Henle's loop (ALH) in proteinuric rats injected with rabbit antisera to the distal tubular antigen, Tamm-Horsfall protein (TH). In rats with heterologous immune complex nephropathy (HICN) injected with anti-TH, the distance along the ALH that LIgD extended correlated closely with magnitude of proteinuria. LIgD were confined to the early ALH in less proteinuric rats and extended further in more proteinuric rats. The distance along the ALH that LIgD extend thus gives a measure of glomerular permeability changes in individual nephrons. HICN rats, 10 days after injection of sheep anti-FxlA, and autologous immune complex nephropathy (AICN) rats, 6 months after FxlA injection were studied after injecting anti-TH. The % of ALH with LIgD in 2 medullary zones and the cortex during the first day were determined in 7-10 month old HICN and AICN rats with mean albuminuria of 238 mg and 256 mg/day respectively. Nearly all ALHs in the inner medullary zone had LIgD in both models. Less than 5% of cortical ALHs in HICN had LIgD, while in AICN, 50% had LIgD and some extended to the macula densa region. LIgD were not formed in proteinuric rats given normal rabbit serum. These studies are in agreement with micropuncture studies (JCI 69:185, 1982) showing greater heterogeneity in glomerular ultrafiltration and its determinants in AICN than HICN and provide a new way to evaluate glomerular permeability in many individual nephrons/kidney.

ANGIOTENSIN II (AII) MODULATES MESANGIAL (MES) AFFERENT AND EFFERENT LIMB. WF Keane and L Raij, Univ of Minn., Dept. of Med., Mpls., Mn.

The factors that control MES uptake (afferent limb) and processing (efferent limb) of macromolecules are largely unknown. These studies clearly demonstrate a striking effect of AII on MES afferent and efferent limb. Rats were continuously infused with 25 ng/min/kg B.W. of AII for 8 h. At 2 h blood pressure (BP), inulin clearance and renal blood flow (RBF) were measured and filtration fraction (FF) calculated. AII did not change BP, but decreased RBF by 18% and FF proportionately increased. When AII and saralasin (SA), 300 ng/min/kg B.W., were simultaneously infused the FF changes were abolished. Twenty minutes after the infusions were started, radiolabelled aggregated human IgG (AG) 30 mg/100g BW was given iv to 15 saline control (C), 15 SA, 15 AII and 15 AII + SA rats, and sacrificed in groups of 5 at 2, 4, 8 h after AG injection. MES AG was measured in preparations of glomeruli from individual rats and compared to simultaneous blood >7S AG levels. Results: MES AG μg/mg glomeruli mean ± SEM (*$p < 0.05$ compared to C):

Time	C	SA	AII	AII+SA
2h	3.5±0.2	3.8±0.3	26.1±2.4*	8.9±1.4*
4h	2.9±0.2	3.1±0.2	25.5±1.9*	9.0±1.2*
8h	0.7±0.1	0.9±0.1	20.5±2.1*	8.7±0.9*

Blood AG>7S was similar in C, SA, AII, AII + SA rats at all time intervals and decreased by 95% between 2 and 8 h. As seen in the table, MES AG decreased by 80% between 2 and 8 h in C and SA, but not in AII or AII + SA indicating AII induced impairment of MES efferent limb. In addition, AII induced marked increases in MES afferent limb which only in part correlated with changes in FF.

REVERSIBLE CELLULAR AND TBM CHANGES IN DPT INDUCED CYSTIC DISEASE IN RATS. Yashpal S. Kanwar, and Frank A. Carone, Northwestern University Medical School, Dept. of Pathology, Chicago, Illinois.

A micropuncture study in 2-amino-4,5-diphenyl thiazole (DPT) treated rats suggested that the cystic disease is due to a defect in tubular basement membrane (TBM) (Kid. Int'l. 5:411,1974). In order to delineate the changes which lead to cystic disease, rats were fed on DPT for 2-8 wks and sacrificed at sequential intervals. To study reversibility of the disease, rats with cystic lesions were fed a normal diet for additional 4-8 wks. The kidneys were perfused fixed with either aldehyde fixative alone or with ruthenium red (RR) in the same fixative. The tissues were processed for LM and EM examination. The LM sections were stained with alcian blue (Ab). The LM revealed that the cystic change begins in corticomedullary junction at 2 wks, becomes progressively severe and regresses completely after 8 wks on a normal diet. TBMs did not stain with Ab in cystic kidneys but they did stain with recovery phase. No obstructive lesions were observed. Em revealed marked thickening and layering of TBM into multiple lamellae with loss and disorganization of RR stainable granules. Early cellular changes consisted of prominent Golgi complexes and increase in the smooth endoplasmic reticulum. In late stages there were extensive elongations of rough endoplasmic reticulum and a marked increase in the polyribosomes and lysosomes. These cellular and extracellular changes regressed during the recovery phase of the disease. The findings support the concept that DPT induced cystic disease is due to disordered synthesis and organization of TBM matrix.

DIET RICH IN EICOSAPENTENOIC ACID (EPA) DECREASES RENAL PROSTAGLANDIN E (PGE) LEVEL AND PREVENTS RENAL DISEASE IN MRL-lpr MICE. Vicki E. Kelley, Brigham and Women's Hosp., Dept. of Med., Boston, MA.

Autoimmune MRL-lpr mice develop a unique, predictable lupus disease with 1) massive T cell lymphoproliferation (lpr) 2) increased expression of peritoneal macrophage (PM) Ia surface antigen and 3) rapidly fatal nephritis. Since PGE's are potent modulators of the immune system, we designed experiments to investigate whether altering dietary fatty acid precursors of PGE changed disease expression in these animals. For this purpose, groups of male MRL-lpr mice were fed a similar diet supplemented with either 20% safflower oil (SO) or menhaden fish oil (MO). SO is a rich source of the PG precursor arachadonic acid, while MO contains EPA, a relatively poor substrate for PG synthesis. Animals were maintained on these diets between 2-5 mo of age then sacrificed and features of disease evaluated.

	lpr Lymph Node	PM	Glomerular Path. Light	Fluoresc.†	PGE Renal Med.
DIET	Cells x10^6	%Ia	Microscopy		pg/ml/mg
SO	902±80	50±2	3.0±0.3	2.0±0.5	1886±183
MO	593±120§	14±3*	0.4±0.2*	0§	1050±171*

§$p < .05$ *$p < .01$ mean±SEM †peripheral loop

These results show that a MO diet rich in EPA 1) prevents morphologic development of glomerulonephritis 2) decreases lpr and PM Ia surface expression 3) reduces renal medullary PGE as measured by radioimmunoassay. In conclusion, dietary reduction of renal PGE synthesis in MRL-lpr mice is associated with suppression of features of autoimmune disease and arrests the progression of lupus nephritis.

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE HEYMANN NEPHRITIS ANTIGEN (gp330) IN GLOMERULAR EPITHELIAL CELLS OF NORMAL LEWIS RATS. **Dontscho Kerjaschki and Marilyn G. Farquhar*, Section of Cell Biology, Yale University School of Medicine, New Haven, CT.**
We have recently purified the pathogenic antigen of HN from tubular brush border fractions and have identified it as a membrane glycoprotein, M_r 330,000 (PNAS, 1982). Both affinity purified rabbit and monoclonal anti-gp330 antibodies were prepared. We have localized gp330 in glomeruli by immunoprecipitation, immunofluorescence (IF), and immunoperoxidase (IPO). Isolated kidneys were labeled by perfusion with [^{35}S]methionine; purified glomerular fractions were prepared and extracted (with 0.2% Triton X-100 in 25 mM Tris-HCl, pH 7.2) and immunoprecipitated with anti-gp330. As seen by SDS-PAGE-fluorography, gp330 was specifically immunoprecipitated. Unfixed cryostat sections were incubated for indirect IF with monoclonal and polyclonal IgG; a fine granular staining was seen throughout the glomerulus, confirming the findings of others. Kidneys were aldehyde-fixed and cryostat sections incubated for indirect IPO. Gp330 was detected only in the epithelial cell where it was localized to: the ER, occasional Golgi elements, multivesicular bodies, and in coated pits located all along the cell membrane on the cell bodies, and the sides and base of the foot processes. Reaction was detected in the latter location only after predigestion with neuraminidase. When rats were given (IV) rabbit anti-gp330 IgG, and their kidneys stained for direct IPO 3 days later, rabbit IgG was localized beneath the slit diaphragms and in coated pits at the base of the foot processes. We conclude: 1) gp330 is an epithelial, rather than a GBM antigen; 2) it is synthesized by glomerular epithelial cells and becomes concentrated in coated pits at the epithelial cell surface; 3) coated pits located at the base of the foot processes are most likely the sites where the antigen and antibody meet.

THE EFFECT OF C1 ON SERUM-ENHANCED BINDING AND CATABOLISM OF SOLUBLE AGGREGATES OF IgG (A-IgG) BY MURINE PERITONEAL MACROPHAGES. D.W. Knutson. M.J. Petruzzi*, S.I. Rosenfeld*, Department of Medicine, University of Rochester, Rochester, New York.

Heat labile factors in mouse, guinea pig and rabbit sera enhance the binding and catabolism at 37°C of soluble immune complexes by homologous and heterologous macrophages, probably via C3b and C3b receptors. However, we find that human sera fail to enhance the uptake of A-IgG by autologous or heterologous mononuclear phagocytes. We investigated the possibility that this failure of human sera to enhance was due to the action of C1. Both human (Hu) and guinea pig (GP) C1^{131}I bound to and increased the S-rate of murine A-IgG (mw=10^7). In cell experiments, Hu and GP C1 (150 µg/ml) abrogated the 2-5 fold enhancement of A-IgG uptake seen with mouse serum and reduced total uptake of A-IgG to less than no-serum controls, suggesting that both C3b and Fc mediated binding were inhibited by C1. In the absence of serum, GP C1 inhibited cell uptake of A-IgG at both 4°C and 37°C. However, inhibition by Hu C1 was minimal at 4°C and Hu C1 may even have enhanced uptake at 37°C, a finding disparate from observations made in the presence of mouse serum. Up to 20% of both Hu and GP C1^{131}I were themselves bound and degraded by macrophages, and this was little affected by the presence of A-IgG. In conclusion, Hu and GP C1 can block C3b and Fc receptor mediated uptake of A-IgG in the presence of mouse serum. GP C1 in contrast to Hu C1 inhibits Fc mediated uptake in the absence of serum. These results suggest differences in the reactions of model immune complexes with C1 that may help explain certain phlogistic and pathogenic differences among immune complexes in patients.

TOLERANCE TO AUTOLOGOUS IMMUNE COMPLEX NEPHRITIS: EVIDENCE FOR SUPPRESSION OF 'B' CELL RESPONSE. Kanwal K. Kher. Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin.

The mechanism of tolerance to autologous immune complex nephritis (AICN) was studied in adult female Lewis rats by pretreatment with incomplete Freund's adjuvant (IFA) and the proximal renal tubular antigen (FxIA). Fifteen days after antigen pretreatment, each rat received a challenging dose of 10 mg FxIA emulsified with complete Freund's adjuvant (CFA), in order to induce AICN. Control animals received only FxIA and CFA. Five days after the antigen challenge, the spleen of each rat was removed aseptically under light ether anesthesia and a cell monolayer prepared. Solublized FxIA was coated on freshly obtained sheep red blood cells (SRBC), using chromic chloride to enhance the uptake of the antigen on SRBC. In vitro IgM antibody production by the spleen cells of tolerant and control animals was assayed using a modified hemolytic plaque assay, antigen coated SRBC served as indicators of hemolysis.

Splenic lymphocytes of the pretreated animals demonstrated significantly less (1181.8±226.8) number of hemolytic plaques (per 10^6 spleen cells) when compared to the controls (2648.75±1375.3). The data suggests that the mechanism of tolerance to AICN by pretreatment with FxIA and IFA is mediated via suppression of specific B cell activity. Although the involvement of a suppressor cell was not directly demonstrated, induction of such an underlying process for modification of antibody response and, therefore, tolerance to AICN can be postulated.

INFLUENCE OF ANTIGEN (Ag) CHARGE ON IMMUNE COMPLEX (IC) BINDING TO CULTURED GLOMERULAR EPITHELIAL CELLS. K.J. Lavelle - Indiana Univ. Sch. of Med., Indianapolis, Ind.

The electrical charge of the Ag or antibody (Ab) is a determinant of glomerular IC localization in vivo. We have previously demonstrated IC interaction with rabbit glomerular epithelial monolayer cultures and decided to investigate the effect of Ag charge on IC-cell interaction using radiolabeled rat albumin (RA) with rabbit Ab. RA was chemically modified to cationic (CAT), PI 8.0-9.0, or anionic (AN) PI 3.0-4.0, fractions. CAT IC, AN IC or IC with unmodified (UNM) RA, PI 5.0-6.0, were incubated with cells for 48 hrs. CAT IC binding to cells was sevenfold greater ($P<0.001$) than UNM or AN IC. The difference between UNM and AN IC binding was not significant. Addition of unlabeled CAT IC (5x excess) increased the cell binding of CAT and UNM IC 400 and 700% respectively ($P<0.01$), possibly by interaction of complexed cell bound Ag or Ab with complexed Ag or Ab in the medium. Unlabeled UNM IC (5x excess) reduced UNM IC binding by 40%. Unlabeled free CAT RA (10x excess reduced CAT IC binding 80%, perhaps by occupying the anionic receptors on the cell. Free CAT RA (10x excess) increased the binding of UNM IC fourfold ($P<0.01$), suggesting IC adherence to cell bound Ag or CAT RA may be incorporated into the unbound IC facilitating union with anionic cell receptors. The results suggest glomerular epithelial cells possess anionic sites which interact with cationic proteins in IC. Glomerular IC localization via electrostatic attachment noted in vivo may in part be due to IC binding to anionic receptors on epithelial cells.

EFFECTS OF GLOMERULAR THROMBOXANE (TxA_2) SYNTHESIS ON ACUTE HEMODYNAMIC CHANGES IN NEPHROTOXIC SERUM NEPHRITIS (NTN). E.A. Lianos* and M.J. Dunn. Dept. of Med., Case Western Reserve Univ. and Univ. Hosp., Cleveland, OH.

Glomerular immune injury following administration of anti-glomerular basement membrane (anti-GBM) serum in the rat, is characterized by acute reductions in whole kidney and single nephron GFR. We assessed the changes in GFR, renal plasma flow (RPF) and in vitro glomerular TxA_2 synthesis following a single IV injection of anti-GBM serum. TxB_2, the stable product of TxA_2, was measured by radioimmunoassay after incubation of isolated glomeruli with arachidonic acid, 5µg/ml, in Earle's minimum essential medium. GFR and RPF were monitored by the clearances of inulin and PAH respectively. There was a progressive decrement in GFR from 0.66±0.04 to a nadir of 0.25±0.04 ml/min/100g wt ($p<0.01$) at 2 hrs. accompanied by decrements in filtration fraction from 0.36±0.03 to 0.13±0.03 at 3 hrs., ($p<0.05$). These changes coincided with a 5-fold increase in glomerular TxB_2 at 2 hrs. and a peak of 11-fold at 3 hrs. compared to sham injected controls. Infusions of the thromboxane synthetase inhibitor, OKY-1581, at 1500 µg/hr completely inhibited glomerular TxB_2 synthesis and preserved GFR and filtration fraction at 1, 2 and 3 hrs. following injection of anti-GBM serum. GFR and filtration fraction were inversely correlated with glomerular TxB_2 synthesis ($n=22$, $r=-0.67$, $p<0.01$; $n=22$, $r=-0.57$, $p<0.05$ respectively).

In conclusion, TxA_2 is an important nonimmune mediator of glomerular hemodynamic events in NTN, possibly due to effects on glomerular vasculature and mesangial cell contractility, thereby reducing filtration surface area.

DMSO REDUCES PROTEINURIA IN RATS WITH MEMBRANOUS NEPHROPATHY. Danny Lotan,* and Bernard S. Kaplan. The Montreal Children's Hospital, Department of Nephrology, Montreal, Quebec, Canada.

Studies were designed to evaluate the effect of DMSO on protein excretion in rats with membranous nephropathy. Proteinuria and epimembranous deposits were produced by 2 spaced i.v. injections of rabbit anti-rat brush border membrane serum. A day later, 20 rats were randomly assigned to control and treatment groups. Treated rats were given DMSO 4 mg/g i.p. b.i.d. 5 d/week for 30 d. Controls received saline. The results were (protein excretion mg/24 hours):

Day	1	7	14	21	35	42	49	60
Control	1.4	3.7	25	19	17	21	20	24
DMSO	1.4	1.5*	2.3*	2.9*	4.4*	6.7*	6.2*	6.8*

Treated rats secreted significantly less protein (*$p < 0.01$, Wilcoxan Rank Sum Test) during and after DMSO. DMSO treatment was started in a second group of rats two months after onset of proteinuria. Rats with comparable protein excretion were paired prior to starting treatment. The same dosage schedule was used as before and continued for 3 weeks. Results were (mg/24 hours):

Week	0	2	3	6
Control (n=6)	45.6	141.9	94.8	120.5
DMSO (n=6)	51.3	85.4	47.6*	114.0

A significant (*$p < 0.05$) reduction in proteinuria occurred after 3 weeks of treatment. Cessation of DMSO led to increasing proteinuria. DMSO therefore reduces protein excretion in rats with membranous nephropathy. This beneficial effect was found when DMSO was given ab initio as well as in rats treated after well-established proteinuria.

GLOMERULAR ARACHIDONATE LIPOXYGENATION IN NEPHROTOXIC SERUM NEPHRITIS (NTN). E. Lianos* and M.J. Dunn, Dept. of Med., Case Western Reserve Univ. and Univ. Hospital, Cleveland, OH.

Glomerular immune injury induced by the administration of anti-glomerular basement membrane (anti-GBM) serum in the rat results in enhanced glomerular cyclo-oxygenation of arachidonic acid (AA) to prostaglandins and thromboxane. To evaluate the lipoxygenase pathway in this disease model, glomeruli, isolated at various time points following a single IV administration of anti-GBM serum, were incubated with ^{3}H-AA and Ca^{++} ionophore A23187 (3µM) in Earle's minimum essential medium at 37°C for 45 min. Media were acidified, extracted and the ^{3}H-monohydroxylated eicosatetraenoic products (HETEs) were identified by high performance liquid chromatography. Glomerular lipoxygenation was expressed as percent conversion of ^{3}H-AA to ^{3}H-12,5,8 and 9-HETE. Comparisons were made between glomeruli isolated from NTN and simultaneous control rats. After induction of NTN, conversion of ^{3}H-AA to 12-HETE was enhanced to 9.2±1.5% at 3-5 hrs., 19.5±3.5% at 24 hrs. and 8.5±2.1% by day 14 (autologous phase). ^{3}H-AA conversion by control glomeruli to 12-HETE ranged from 1.4 to 2.7%. AA lipoxygenation to 8- and 9-HETE was enhanced to a lesser extent, primarily on day 1 (heterologous phase) (5.2±0.9%) compared to 1.2±0.25%, $p<0.01$).

We conclude that in NTN there is enhanced glomerular AA conversion primarily via the 12-lipoxygenase pathway to 12-HETE, a potent chemotactic and pro-inflammatory fatty acid.

IMMUNITY AND IMMUNOSUPPRESSION IN EXPERIMENTAL NEPHRITIS. Robin P. Lowry, Clark Forbes*. Renal Immunol. Lab., Dept. of Med., Royal Victoria Hosp., McGill University, Montreal, Canada.

The role of immunologic and non-immunologic factors in progression of primary glomerular disease remains uncertain. We have studied host immunity in the pathogenesis of the Accelerated Autologous Form of Nephrotoxic Serum Nephritis (AA-NTSN) and identified histopathologic features predicating against successful immunosuppressive intervention with fractionated high dose (3400 rad) Total Lymphoid Irradiation (TLI).AA-NTSN was induced in LEW rats by i.p. injection of sheep IgG (SGG, day -5) followed by i.v. administration of a subnephritogenic dose of sheep anti-rat GBM antiserum (0.05 cc, day 0). Rats immunized with SGG as for induction of AA-NTSN develop significant cellular immunity characterized by antigen specific T cell proliferation, generation of Leukocyte Procoagulant Activity (PCA) and Leukocyte Inhibiting Factor (LIF) production on in-vitro culture with SGG. While renal histology and 24 hour urine albumin excretion was virtually normal at long term follow up of the majority of rats with established AA-NTSN subjected to TLI, a subset with more advanced endo- and extracapillary proliferation and fibrin deposition on initiation of therapy retained marked proliferative changes and significant albuminuria as noted in untreated controls. This model provides a useful system to ascertain whether failure of immunosuppressive intervention may be related to qualitative or quantitative differences in host immunity (i.e. to the planted antigen) or whether non-immunologic factors dictate progression of renal disease.

K PROTECTS AGAINST RENAL TUBULE LESIONS IN NaCl-FED DAHL S RATS. D MacNeill*, L Toblan, MA Johnson*, M Ganguli*, J Iwai*, Univ of Minn. & Brookhaven Lab.

Patchy tubular dilation is one of the most prominent early renal lesions in NaCl hypertension of Dahl S rats. Extra dietary K appears to partially protect against such lesions. S rats(20 per group) were given 6 different Purina diets for 24 weeks: 4%NaCl(BP171); 4%NaCl-3.8%K citrate(BP174); 4%NaCl-2.6%KCl(BP173); .3%NaCl(BP158); .3%NaCl-3.8%K citrate(BP160); .3%NaCl-2.6%KCl(BP157). Tubular lesions per unit area of kidney were graded in a rigorously blind microscopic study of 2 entire sections for each kidney, providing a "tubular dilation index"(TDI)(100, severest;0,normal). In renal cortex,TDI averaged 41,20,22,24,15,18 respectively in the 6 groups. Thus both dietary K citrate & KCl halved the number of lesions on 4%NaCl($p<.001$) and brought them down to the level seen in S rats on a low .3%NaCl diet. Even among S rats on .3%NaCl, adding K citrate or KCl reduced lesions -38%($p<.005$) & -25%($p<.05$)respectively. In renal outer medulla, TDI was 79,54,58,53,48,53 respectively. Again adding K citrate & KCl reduced lesions on 4%NaCl by -32% & -27%respectively($p<.001$)& brought them down almost to the level of S rats on .3%NaCl. In renal papilla, TDI was 49,28,28,26,22,22 respectively. Again adding either K citrate or KCl reduced lesions on 4%NaCl by -43%($p<.001$), almost down to the .3%NaCl level. The strong protective effect of K citrate & KCl can't be ascribed to decreased BP. A glomerular lesion index averaged 29,24,23,24,22,24 respectively. Again,adding K citrate & KCl reduced lesions 17% & 21% respectively in S rats on 4%NaCl ($p<.01$). The natural diet of all primitive mammals, including man, is very high in K. Such primitive diets may retard these renal lesions.

RAT PROXIMAL TUBULE BRUSH BORDER ANTIGENS CHARACTERIZED BY MONOCLONAL ANTIBODIES. Donna L. Mendrick* and Helmut G. Rennke. Brigham & Women's Hospital, Boston, Massachusetts.

Rat kidney antigens (Ag) were characterized ultrastructurally and biochemically with the use of hybridoma-derived monoclonal antibodies (MAbs). Five of such MAbs react with the proximal tubule (PT) brush border (BB). Two of these (K17/7 and K8/8) also cross-react with intracellular components of the glomerular capillary wall (GCW). K8/8 exhibits binding to the z bands in striated muscle cells suggesting it is directed against Ags associated with contractile elements. K17/7 recognizes a glomerular and PT Ag that has a MW of 104 k as revealed on proteins electrophoretically transferred to nitrocellulose paper from SDS-polyacrylamide gels. Both these MAbs are of the IgM class and are reactive across species lines. Three other MAbs (K9/9, K8/6, and K6/4) react with intestinal as well as renal BB and are species specific. K9/9 is a IgG_1 antibody which also recognizes Ags present on endothelial cells, the pericanalicular cytoplasm of hepatocytes, and in the GCW (which it binds to upon in vivo administration). In a renal membrane BB preparation, the Ags have apparent MWs of 108 k and 64 k. K8/6 is an IgM antibody which reacts with Ags of MWs 78 and 75 k of the BB membrane. K6/4 is an IgG_1 antibody that in the kidney binds exclusively to the PT BB. The tissue distribution exhibited by these 3 MAbs resembles that of alkaline phosphatase suggesting that they are directed against an Ag closely associated with this enzyme but in the case of K9/9 and K8/6, clearly different on the basis of MW of the Ags recognized. Such MAbs may prove useful in studies of the function of BB associated antigens.

DECREASE IN ANIONIC CHARGE SITES (CS) IN THE LAMINA RARA EXTERNA (LRE) IN AMINONUCLEOSIDE NEPHROSIS. J. Mahan,* S. Sisson,* and R.L. Vernier, University of Minnesota, Dept. of Pediatrics, Minneapolis, Minnesota.

Fixed anionic sites in the GBM participate in the charge selective function of this filter. Using the cationic stain polyethyleneimine (PEI; MW 1200), the distribution of electron dense CS in the LRE in random electron micrographs was quantitated during the course of puromycin aminonucleoside (PAN) nephrosis in the rat.

20 mg/kg of PEI, pH 7.4, was injected by tail vein and the animals were sacrificed 5 minutes later. In controls PEI labelled CS were demonstrated in regular latice-like arrays in the LRE. 4 hours after PAN, prior to recognizable changes in foot process morphology, the number of CS was reduced. The decreased numbers of CS were focally distributed and persistent through 7 days. Similar quantitative data were obtained by in vitro methods in which 30 μm slices of kidney from control and PAN rats were incubated in 1% PEI.

	Time After Pan (Hours)				
Control	4	24	48	120	168
21.63* ±1.99	16.47 ±0.79	18.68 ±1.04	16.56 ±0.28	15.96 ±0.76	14.02 ±1.40

*LRE CS (per 1000 nm GBM)

The decrease in the number of CS prior to the reported appearance of albuminuria and the persistent decrease throughout the study period suggests that loss of anionic CS in the GBM may be a major mechanism for proteinuria in this and other proteinuric states.

SPONTANEOUS INTERSTITIAL NEPHRITIS IN KdKd MICE: A NEW MODEL OF AUTOIMMUNE RENAL DISEASE. E.G. Neilson, R. Korngold,* M. Clayman,* and E. McCafferty,* U of Pa. & Wistar Inst., Phila., PA.

Kd mice ($H\text{-}2^K$) were bred out of CBA/Ca stock over ten years ago. The mice are normal at birth but after 10 weeks develop interstitial nephritis (IN) leading to endstage kidneys. In general this mouse has been assumed to have a developmental kidney lesion. We now report Kd mice actually exhibit a primary IN on an autoimmune basis. To demonstrate this 13/14 Kd→CBA bone marrow chimeras developed significant disease at 6 months (avg. lesion severity=2.5±0.3). In contrast 11/11 Kd→B10.BR ($H\text{-}2^K$) chimeras were completely normal, suggesting that only selected strains express the relevant interstitial antigen. Kd mice, T cell depleted by thymectomy and T cell antisera, showed a reduction in the severity of lesions at 3.5 months (1.4±0.2 vs 3.4±0.3 in cont.;$p<0.001$). Although Kd→CBA chimeras get disease, disease cannot be transferred into CBA mice with mature lymphoid cells from nephritic Kd mice. This finding suggests the development of disease may be related to loss of a dominant suppressor cell. Lesions in Kd mice were primarily interstitial with progressive cellular infiltrates, tubular dropout, and dilitation. Fluorescent staining revealed non-specific linear staining of involved tubules for IgG, IgA, IgM, C3, and albumin. Nephritic serum and a renal eluate from nephritic kidneys failed to stain normal Kd or CBA kidneys.

In summary, Kd mice spontaneously develop autoimmune cell-mediated IN. This model has many implications for the further understanding of the development of interstitial renal lesions.

QUANTITATIVE INDICES OF AMINONUCLEOSIDE-INDUCED NEPHROTIC SYNDROME (AMNS). T.E. Nevins, J. Basgen* and T. Gaston,* Dept. of Pediatrics, University of Minnesota Medical School, Minneapolis, MN.

Significant morphologic alterations and albuminuria occur within hours of aminonucleoside administration in rats. A sensitive immunodiffusion assay quantitated albumin excretion (A) in 12 hour collections of urine. Average glomerular epithelial foot process widths (FP) were estimated by morphometric analysis of random electron micrographs. These studies were performed in 56 rats following intraperitoneal administration of either saline or aminonucleoside. Within 24 hrs average FP were increased and significant albuminuria was detected by 36 hrs.

	Hours After Aminonucleoside					
Control	12	18	24	36	48	60
FP(nm) 218	219	232	245*	262*	251*	326*
A (mg).136	.117	.114	.182	.338*	.357*	.611*

*indicates values that differ significantly ($p<.05$) from saline injected controls.

AMNS has been extensively studied to gain insights into the pathophysiology of nephrotic syndrome. Sensitive, quantitative techniques permit investigation of earlier events in the sequence leading to nephrotic syndrome. These data document changes in epithelial morphology preceeding detectable increases in albuminuria. In AMNS, epithelial alterations are important in the initiation and propagation of albuminuria.

MODULATION OF MESANGIAL CELL FIBRONECTIN BIOSYNTHESIS BY MACROPHAGE SUPERNATANTS. Y. M. Ooi, M. A. Weiss, and B. S. Ooi, Univ. of Cincinnati Medical Center, Cincinnati, Ohio.

Fibronectin has been visualized in the mesangium by immunofluorescent methods. Fibronectin is a cellular protein which serves a number of important physiological functions including cell differentiation, morphology and architecture. Pertubations of fibronectin synthesis would be expected to have consequences on glomerular cell architecture. To investigate the mechanisms by which macrophages produce glomerular damage in nephritis, in-vitro cultures of mouse mesangial cells were obtained. De novo biosynthesis of fibronectin by mesangial cells was demonstrated by labeling the cells with ^{35}S-methionine, and subjecting cellular lysates and supernatants to immunoprecipitation with rabbit anti-mouse fibronectin and analyzing the precipitates by SDS-PAGE. The 240,000 dalton subunit of fibronectin was seen in the gels. Quantitative immunoprecipitation was also done on extracellular material obtained from mesangial cells cultured with macrophage supernatant. The results are shown below.

Concentration of macrophage supernatant %	^{35}S-fibronectin (c.p.m. x 10^{-4})
0	13.5
25	1.67
50	1.05
75	0

The data show that macrophage supernatants markedly depress the production of fibronectin by cultured mesangial cells, and provide a mechanism by which macrophages may profoundly alter mesangial cell function in immune nephritis.

ENHANCED SUSCEPTIBILITY TO HEYMANN NEPHRITIS (HN) IN RATS FAILING TO DEVELOP DISEASE AFTER PRIMARY IMMUNIZATION. B. Noble*, J.B. Van Liew, G.A. Andres and J.B. Brentjens*. School of Medicine, SUNY/AB and VA Medical Center, Buffalo, NY.

Although most LEW rats (Charles River) develop the nephrotic syndrome of HN 8 weeks after immunization with FxlA, protein excretion of some remains normal. We have compared those normal nonproteinuric (N_{pr}) rats with those that developed HN in order to identify factors influencing susceptibility to immunologically mediated kidney disease. In the primary response, anti-brush border (BB) titers and IgG deposition in glomeruli were similar in both groups. However, complement (C) was detected only in rats with HN. Although overall kidney function was normal in N_{pr} rats, primary immunization with FxlA resulted in a substantial alteration in the fractional composition of urinary protein by 30 weeks. Reimmunization with FxlA at 30 weeks stimulated identical anamnestic antibody responses in both groups. Following reimmunization 60% of N_{pr} rats developed severe HN with an unusually rapid (1 week) onset. In the remaining N_{pr} rats normal kidney function was maintained. Once again, the presence of C in glomerular immune deposits distinguished rats with proteinuria from others. It is concluded that anti-BB titers, measured by immunofluorescence tests, are not an index of the pathogenicity of an immune response to FxlA. Subpopulations of BB antibodies, or antibodies with other specificities, may be important. Immunological memory, leading to rapid expression of disease upon reexposure to antigen, can be established after primary immunization in the absence of clinical symptoms. Alterations in urine protein composition may reflect subclinical immunopathology. Variability of response suggests heterogeneity of the breeding stock.

RENAL ONTOGENIC STAGES IDENTIFIED BY MONOCLONAL ANTIBODIES (McAb). J.L. Platt,* T.W. LeBien* and A.F. Michael, Departments of Pediatrics, Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN.

The origin and characteristics of primitive metanephric cells destined to form the epithelium of the glomerulus and tubules are poorly understood. Compelling evidence for the presence of differentiation antigens in these cells that are also present in certain lymphoid cells was discerned by immunohistochemical studies using McAb. Primitive epithelial cells in human fetal kidney reacted with BA-1 (which identifies most peripheral B cells). With metanephric development and appearance of immunoreactive glomerular basement membrane BA-1 reactivity was lost and BA-3/J5 (leukemia associated antigen) appeared on visceral glomerular and proximal tubular epithelium. This was immediately followed by development of C3b receptors on visceral glomerular epithelium (indicated by C3b coated E. coli). Other McAb reactive with lymphoid cells also reacted with renal epithelial cells: BA-2 (leukemia associated antigen) with the primitive S-form of the glomerulus and ureteral bud and anti-B1 with ureteral bud and distal tubules. McAb which identify T cells and thymocytes (OKT3, TA-1, OKT4, OKT8, OKT6, OKT10) did not bind to renal epithelium. Lymphohemopoietic cells and renal epithelium share some differentiation antigens the acquisition and loss of which can portray metanephrogenesis.

CHARACTERIZATION OF A RAT GLOMERULAR ANTIGEN UTILIZING HYBRIDOMA-DERIVED ANTIBODIES. Helmut G. Rennke, Donna L. Mendrick*, Irmgard Montfort*, Brigham & Women's Hosp., Boston, Massachusetts.

Monoclonal antibodies (MAs) have been produced to study the antigenic composition of the glomerular capillary wall and the role played by such determinants in immune-mediated injury. Out of more than 60 stable positive clones, 3 MAs, of different IgG subclasses, recognize a single antigen present on endothelial and glomerular epithelial cell surfaces and the GBM proper as revealed by immunoelectron microscopy. The antigen was isolated by 1) immunoprecipitation from ^{125}I-labelled, detergent (NP-40, Triton X-100, CHAPS, Brij-35)-solubilized glomeruli and 2) electrophoretically transferred glomerular proteins onto nitrocellulose paper from SDS-polyacrylamide gels followed by exposure with MAs and ^{125}I-labelled sheep F(ab')$_2$ anti-mouse Ig or ^{125}I-labelled Staph. protein A. The antigens recognized are two polypeptides with apparent MWs of 129 and 117 k daltons and present roughly in a 2:1 proportion. The antigen is sensitive to enzymatic digestion by pepsin at pH 3.2 but resists the action of bacterial collagenases when tested on 1) tissue sections, 2) Brij-35-solubilized proteins followed by immunoprecipitation and 3) on nitrocellulose-transblotted proteins. One of these MAs appears to recognize a separate antigenic site since its binding to the transblotted antigen is additive after preexposure to either of the other two MAs. Hybridoma-derived antibodies provided a powerful tool for the immunological, biochemical and ultrastructural characterization of this previously undescribed constituent of the glomerular capillary wall.

PHAGOCYTOSIS OF E. COLI BY RENAL TUBULAR EPITHELIA OF RATS. Tetsuo Shimamura* and John K. Maesaka*, UMDNJ-Rutgers Med. Sch., Piscataway, NJ, VAMC, East Orange, NJ, and UMDNJ-NJ Medical Sch., Newark, NJ.

The phagocytic activity of renal tubular epithelia has not been well characterized. Studies were, therefore, performed to investigate the process of phagocytosis by proximal tubular epithelia. Isolated proximal tubular brush border membranes were smeared on a glass slide, dried and incubated in a solution containing E. Coli or control rat erythrocytes. The slide was then rinsed with three changes of phosphate buffered saline (pH 7.6), stained and examined by light microscopy. E. Coli were noted to adhere to brush border membranes whereas adherence was not detected with rat erythrocytes. Live and dead E. Coli were then microinjected into early proximal tubular sites of rats with or without prior ureteral ligation and examined by electron microscopy 1/2, 2, 4 and 6 hours after completion of microinjection. Live and dead E. Coli appeared initially to adhere to brush border membranes, which invaginated adjacent to the bacteria, and were localized within the cytoplasm as early as 2 hours after microinjection in both obstructed and nonobstructed kidneys. Since dead E. Coli localized within the cytoplasm of the epithelial cells, the bacteria must have entered the cytoplasm by phagocytosis. In conclusion, E. Coli appears initially to adhere to brush border membranes with eventual phagocytosis by the tubular epithelia. There appears to be some functional similarity between proximal tubular epithelial and reticuloendothelial cells.

INTERACTION OF MUTANT lpr (LYMPHOPROLIFERATION) GENE WITH STRAIN BACKGROUND INFLUENCES AUTOIMMUNITY AND RENAL DISEASE. John B. Roths*, Shozo Izui*, Vicki E. Kelley, Jackson Lab, Bar Harbor, ME, Hopital Cantonal, Geneva, Switz., Brigham and Women's Hospital, Boston, MA.

MRL-lpr mice have an lpr gene responsible for an early onset autoimmunity. These mice have massive T cell (Lyt 1$^+$) hyperplasia, circulating autoantibodies and rapidly fatal nephritis (50% mortality - 6 mo). Congenic mice lacking this gene do not have lymphoid hyperplasia but develop a late onset autoimmunity and die with nephritis (50% mortality - 17 mo). To study the relationship of the lpr gene in the development of autoimmunity and lupus nephritis, this gene was transferred to several nonautoimmune mouse strains and the new strains designated: C3H-lpr, B6-lpr, AKR-lpr. Effects of the lpr gene were evaluated by measuring lpr, circulating ss DNA antibodies and retroviral gp 70 immune complexes (IC), and the development of renal disease. Groups of C3H-lpr, B6-lpr and AKR-lpr female mice were analyzed at 2 and 4-6 mo of age and compared with the MRL-lpr strain. Massive lpr occurred in all strains (C3H-lpr>MRL-lpr>AKR-lpr) by 4-6 mo of age. However, only the MRL-lpr mice developed IC glomerulonephritis (evaluated by light, fluorescence and electron microscopy) and proteinuria. Although these other strains had circulating ss DNA antibodies, the levels were 4 times less than in MRL-lpr mice. Also, the MRL-lpr mice were the only strain with high levels of circulating gp 70 IC. In conclusion, these studies indicate that interaction of the lpr gene with strain background greatly influences the expression of autoimmunity and renal disease.

ROLE OF ANGIOTENSIN II - INDUCED RENAL FUNCTIONAL CHANGES IN MESANGIAL DEPOSITION OF FERRITIN IN RATS. H.D. Stein*, W.Feddergreen*, M.Kashgarian, R.B. Sterzel. Depts Med & Path, VA Med Center-Yale Univ School of Med, New Haven, CT.

The relationship between acute angiotensin II (AII)-induced functional changes of the kidney and mesangial localization of macromolecules was studied in rats using native horse spleen ferritin(Fe) as an exogenous tracer. A single iv injection of Fe (45mg/100g BWt) in all rats was followed in 7 by an 80 min infusion of AII (50ng/100g·min), while 6 controls received saline. Plasma Fe concentrations, C_{PAH} and C_{Inulin} were not different between the groups. AII-treated rats showed significant increases over controls of mean systolic arterial pressure (AP 135±7vs.105±7 mmHg), change of filtration fraction (ΔFF 0.26±0.04vs. 0.15±0.03), urinary excretion of albumin (E_{Alb} 13.7±5.4vs.1.5±0.3 µg/min) and of Fe (E_{Fe} 1.1±0.4 vs.0.6±0.1 µg/min). Semiquantitative evaluation of renal tissue by immunofluorescence and electron microscopy revealed that AII-treated rats had markedly enhanced mesangial localization of Fe when compared to controls, with little or no Fe in the peripheral loop in either group. The degree of mesangial Fe deposition correlated most strongly with E_{Alb} (r=0.84) and E_{Fe} (r=0.67). There were weaker yet significant correlations of glomerular Fe with AP and ΔFF. The results show that AII acutely augments the mesangial accumulation of Fe in association with increases of AP, FF and glomerular permeability to proteins. The findings suggest that related hemodynamic alterations, such as induced by AII, affect the passage of macromolecules across the glomerular capillary wall and their entry and trapping in the mesangium.

GLOMERULAR PERMEABILITY IN EXPERIMENTAL MEMBRANOUS NEPHROPATHY. J. A. Stevenson* and W. A. Border, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA.

Alterations in glomerular permeability as a function of molecular size and charge were studied in groups of 5 control (C) and 4 experimental (E) rabbits. Fractional clearances (C/GFR) were measured over the range of molecular radii from 15 to 65 Å for ^{3}H-labeled neutral dextran (ND) and anionic dextran sulfate (DS) radiolabeled with ^{35}S. After 3 weeks of daily injections of cationic bovine serum albumin, E glomeruli showed granular IgG and C3 in a diffuse epimembranous location. Clearance studies were performed between 48 and 72 hours following the last injection. Mean albuminuria in E was 298 ± 147 mg/24 hours, and inulin clearance revealed a reduction in single kidney GFR of 61% (C=4.6 ± 0.2 ml/min, E=1.8 ± 0.3 ml/min). C/GFR for ND were increased in E for molecular radius >47 Å with a four-fold increase over C at 62 Å (table below):

ND	15 Å	32 Å	54 Å	62 Å
C	.98±10%	.19±15%	.0028±9%	.0012±19%
E	.81±4%	.13±10%	.0077±22%*	.0046±14%*

(Values are C/GFR as mean ± SEM, *p < 0.05)

In C, C/GFR was less for DS than for ND by a factor of 4 to 90, over the range studied. DS sieving curves for E were unchanged from C over the range 15 to 24Å, but showed significantly increased C/GFR for molecules greater than 30 Å radius (table below):

DS	15 Å	23 Å	32 Å	62 Å
C	.147±14%	.049±11%	.0082±11%	.00036±25%
E	.145±7%	.050±9%	.012±6%*	.00098±20%*

The results show that proteinuria in this model of membranous nephropathy is due to a defect of size selectivity. In E, C/GFR for anionic DS < 24 Å radius is unchanged from C, indicating a functionally intact charge barrier.

HEMOLYTIC UREMIC SYNDROME: PERSPECTIVES FOR THE DEVELOPMENT OF AN ANIMAL MODEL. Regina Verani, Keith Hoots*, Deborah Bergman*, Ann Ince* and Eileen D. Brewer. University of Texas Medical School at Houston, Depts. of Pathology & Pediatrics, Houston, Texas.

The hemolytic uremic syndrome (HUS) is characterized by acute renal failure, microangiopathic hemolytic anemia (MHA), thrombocytopenia, glomerular thrombosis (GT) and in some cases cortical necrosis (CN). A suitable animal model for laboratory investigation of this disorder is lacking. The objective of this study was to develop such a model. In preliminary studies thrombin (T), 100-200U/100g BW, was administered via femoral vein catheter over 2 or 4 hrs. to rats. Extensive GT was observed in all kidneys; 24 hrs. later CN was present in many (12/26 rats). The microscopic findings were not different from those characteristically seen in HUS. Within 2 hrs. of administering T at a dose of 140U/100g BW, urine output decreased significantly or ceased. To investigate whether MHA and decreased platelets also occurred after infusion of T, 140U/100g BW, 6 rats were studied and compared to 6 controls receiving only saline. In T-treated rats, platelet counts were significantly reduced (226,000 ± 78,000 vs. 575,000 ± 163,000 in controls, $p<0.001$), and blood smears showed characteristic schistocytes and helmet cells not present in controls. GT was present in kidneys of T-treated rats, but not in controls. These results provide evidence that MHA and GT, which are hallmarks of HUS, can occur together after administration of T to rats. The T-treated rat may prove to be an appropriate laboratory model for HUS.

THE INHIBITORY EFFECT OF HUMAN RHEUMATOID FACTOR (RF) AND RABBIT RHEUMATOID FACTOR-LIKE SUBSTANCE (RFS) ON EXPERIMENTAL IMMUNE COMPLEX GLOMERULONEPHRITIS. Andres J. Valdes, Eugene Rodriguez,* and Frederick G. Germuth. Div. Immunopathology, St. John's Mercy Med.Ctr., St. Louis, Missouri.

A) It has been found that randomly bred New Zealand rabbits have a high serum level of a substance which reacts positively in a latex test for human rheumatoid factor (RFS). In the acute BSA-rabbit system(i.e. following a single injection of a large dose of BSA) at the time of BSA clearance, such rabbits are known to develop diffuse monocytic GLN accompanied by scarce glomerular deposits. RFS disappears together with immune disappearance of BSA and simultaneously cryoglobulinemia develops. In contrast, an inbred strain of New Zealand rabbits (JAX-III) was found to have no detectable RFS and all such animals developed a diffuse GLN characterized by the presence of numerous loop deposits, an influx of PMN's, and the absence of cryoglobulinemia. These observations suggest that RFS may serve to protect the glomerulus from the deposition of immune complexes, and that results obtained in classic studies of this system need to be re-evaluated.

B) When purified human RF was injected into mice developing passive immune complex GLN, the glomerular localization of immune complexes was inhibited. This was associated with the development of cryoglobulinemia.

Together these two sets of results raise the possibility of treating selected cases of early human GLN with human monoclonal RF in an attempt to prevent further progression of the lesions. Should cryoglobulins develop in large amounts, they could be removed by cryogelation.

CATIONIC, EXTRACELLULAR STREPTOCOCCAL ANTIGENS IN HUMAN IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN). A. Vogt*, S. Batsford*, B. Rodriguez-Iturbe* and R. Garcia* (Intr. by R. Sterzel). Inst. of Immunology, D-7800 Freiburg, FRG, Dept. of Nephrology, Univ. Hospital, Maracaibo, Venezuela.

Experimentally cationic proteins can bind to the GBM (planted antigen) and induce GN,via in situ IC formation. We posed the questions: 1) Do nephritogenic streptococci excrete cationic products and 2) can these be detected in the glomeruli of patients with acute post-streptococcal GN (ASPGN)? Nephritogenic strains of streptococci were cultured in protein free medium and found to excrete anionic neutral and cationic products. Up to 16 distinct fractions could be separated on the basis of their isoelectric points by the recently introduced technique of chromatofocussing (Pharmacia).Specific antisera were raised to each of 5 fractions with isoelectric points in the pH range 5.0 to 8.3 and to 9 fractions in the pH range 8.5 to 11.0. Frozen sections of renal biopsies from 18 cases of ASPGN were tested by immunofluorescence for the presence of antigen. Positive results were obtained only with antisera raised to fractions whose pI exceeded pH 8.5. Eight of 18 cases studied revealed cationic streptococcal antigens in a mesangial/capillary pattern. Positive results were more frequent in early biopsies and were associated with deposits of IgG and IgM. Prior absorption of antiserum with the appropriate antigen abolished staining. Ig positive biopsies from other types of glomerular disease were negative, confirming the specificity of the staining. Patients' sera contained antibody to the cationic antigens studied. This data suggests a role for extracellular cationic streptococcal antigens in ASPGN via in situ formation of glomerular immune deposits.

CHARACTERIZATION OF KIDNEY LESIONS (KL) DUE TO Ia DIFFERENCES IN ACUTE GRAFT VERSUS HOST DISEASE (GVHD). A. Wadgymar*, P. Halloran, S. Farkas*, S. Poucell* & R. Baumal. Dept. of Méd. & Path., Mount Sinai Hospital & Hospital for Sick Children, Toronto, Canada.

Ia antigens are known to induce GVHD and graft rejection. To study the role of renal Ia in the mechanisms of immunological renal diseases, we assessed the kidney lesions of GVHD. GVHD was induced in bm12 mice by lethal irradiation and reconstitution with parental bone marrow cells from B6 mice, which differ only at the I region, due to a point mutation. Normal, irradiated and syngeneic controls of both strains were also studied. GVHD was assessed by the Simonsen Assay.

By light microscopy, mice with GVHD developed KL by day 12 after reconstitution, characterized by mononuclear infiltrates around interstitial blood vessels and mild vasculitis. Immunofluorescence demonstrated Ia in peritubular capillaries (PTC) of normal kidneys. However in GVHD, the cytoplasm of tubular cells also became strongly positive, probably due to uptake of Ia released from damaged host lymphocytes. Survivors of acute GVHD showed deposition of IgG and IgM in glomerular capillaries, suggesting immune complex (IC) formation. Since the only difference between host and donor is in Ia, our findings suggest that the infiltrating cells might be responding to host Ia in PTC (cell mediated injury), and that donor anti-Ia antibodies combine with circulating Ia to form IC that fix to glomerular capillaries. This model may be useful to study the role of renal Ia in immunological renal diseases.

PROTEOLYSIS OF ^{125}I-RABBIT HAGEMAN FACTOR DURING ANTI GBM NEPHRITIS IN THE RABBIT. Roger C. Wiggins and Charles G. Cochrane. Dept. Int. Med., Univ. of Michigan Med. Sch., Ann Arbor, and Research Institute of Scripps Clinic, La Jolla.

Rabbit Hageman factor (HF) was purified from plasma, radiolabelled and used as a probe to determine whether proteolysis of HF occurred during acute glomerulonephritis (GN). Guinea pig (GP) anti rabbit GBM IgG in rabbits preimmunized (4 days) with non-immune GP IgG was employed to produce proteinuria starting 1 day after anti GBM injection and reaching a peak of 800 mg/24 hrs by day 5. In this model of GN circulating antigenic HF, prekallikrein and high MW kininogen levels did not change during proteinuria although C3 fell to 50% of normal. The rate of disappearance of plasma ^{125}I-HF increased during proteinuria, but the ratio of ^{125}I-HF:^{131}I-albumin did not change, indicating that no selective loss of ^{125}I-HF from plasma had occurred. Urine ^{125}I-HF was analyzed by first isolating the HF by ion exchange chromatography and then examining proteolytic fragmentation of ^{125}I-HF by reduced SDS-PAGE. Urine ^{125}I-HF analyzed by the above method showed that on the day of onset of proteinuria proteolysis of ^{125}I-HF into fragments of 50,000 and 30,000 M_r occurred (the fragments associated with HF activation), but on subsequent days little or no fragmentation of HF occurred. These results are compatible with the concept that in acute nephritis in the rabbit proteolysis of HF occurs at the time of initial glomerular insult, but that continuous HF activation during leakage of protein through the damaged glomerulus does not occur.

INFLUENCE OF ANTIGEN CHARGE ON ISOELECTRIC POINT AND BIOLOGIC PROPERTIES OF PREFORMED IMMUNE COMPLEXES. H. Wang* and W.A. Border, Dept. of Medicine, Harbor-UCLA Medical Center, Torrance, California.

Cationic antigens have been shown to be nephritogenic but it is unclear whether glomerular deposits form by deposition of preformed cationic immune complexes (IC) or in situ by free (dissociated) antigen and antibody. To study this question we prepared IC composed of cationic (pI 9.5) or anionic (native, pI 4.5) bovine serum albumin (BSA) and electrofocused sheep anti-BSA IgG (pI 5-7.0) formed in 30X antigen excess.

Direct measurement of IC pI was accomplished by chromatofocusing after isolation of IC (MW 3.5-7.0 X10^6) from a linear sucrose density gradient. Chromatofocusing peaks were confirmed to be IC by dual labelling of antigen and antibody, gel filtration chromatography and dissociation of IC into BSA and IgG by SDS-PAG electrophoresis. IC formed with cationic or anionic BSA were both anionic with pIs of 4.83±0.22 and 4.68±0.14 respectively (P=NS).

Similar IC prepared with cationic or anionic BSA were injected IV into groups of 6 rabbits each; blood was obtained at 10 min and blood and renal tissue at 1 h. Both antigens appeared as free BSA at 10 min, and increased in amount at 1 h. Cationic BSA IC produced diffuse glomerular capillary wall staining; whereas, anionic BSA IC resulted in granular mesangial deposits. When covalently cross-linked cationic BSA IC were injected (n=8), free BSA levels were diminished and capillary wall staining was abolished.

We conclude that antigen (BSA) charge does not predictably influence IC charge and induction of glomerular wall deposits by a cationic antigen is dependent on the presence of free antigen and not on deposition of preformed cationic IC.

ADOPTIVE TRANSFER OF MURINE INTERSTITIAL NEPHRITIS. B. Zakheim,* E. McCafferty,* and E.G. Neilson, U. of Pa., Phila., PA.

We have previously shown that interstitial nephritis (IN) is produced in SJL mice by injection of tubular antigen (TBM) in adjuvant. We now report disease is also produced by transfer of T cells or anti-TBM antibody (αTBM-Ab). 5-10 x 10^6 immune spleen (SP) or lymph node (LN) cells were injected IV into normal recipients. IN was noted 6 weeks after transfer; lesion severity graded at 4.0±0.0 vs. 0.0 in controls. To ascertain the cell population mediating disease unfractionated SP/LN cells and SP/LN cells depleted of T cells (TD) by treatment with T cell antisera were injected. After 4 weeks unfractionated cells caused lesions graded at 3.0±0.7. vs. TD cells graded at 0.8±0.4 (P<0.04). Serum and kidney sections in animals with IN were negative for αTBM-Ab. The nephritic infiltrate was typed and found to be T= 37.9%±6.1, NK=22.1%±6.1, MØ=26.4%±6.9, B=13.3%± 8.8, Lyl/Ly2 ratio = 0.76±.13. To determine whether IN is produced by transfer of αTBM-Ab, normal recipients were injected with nephritic mouse serum (titer 1:80). IN was first noted at 6 weeks: 3/4 recipients had lesions graded at 3.0±1.0; at 12 weeks 10/14 showed lesions graded 2.1±0.5. Controls were all normal. Infiltrating cells were found to be T=18.1%±1.1, NK=58.6%±3.8, MØ=16.0%±2.2, B=7.0%±4.8, Lyl/Ly2 ratio=.47±.05. Serum from recipients was negative for αTBM-Ab, but kidney sections were positively stained.

In summary, IN was produced in normal mice by injection of T lymphocytes or serum obtained from nephritic mice. Lesions were similar by light microscopy, but differences in sub-populations of infiltrating cells were noted.

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ANALYSIS OF GLOMERULAR LEUCOCYTE INFILTRATION IN HUMAN GLOMERULONEPHRITIS BY MONOCLONAL ANTIBODIES. Robert C. Atkins, David H. Hooke* and Wayne W. Hancock* Prince Henry's Hospital, Department of Nephrology, Melbourne, Australia.

The role of cell mediated immune mechanisms in human glomerulonephritis (GN) is unknown although recent studies have described altered ratios of circulating leucocyte subsets in some GN. We have therefore investigated the participation of leucocyte subsets and monocytes within glomeruli of 61 patients with GN. Monoclonal markers to specific leucocyte cell surface antigens were used:- leucocyte common antigen (PHM 1), monocytes (FMC 32, PHM 2), polymorphs (FMC 10), T cells (OKT 3) and T cell subsets (OKT 4, OKT 8) and B cells (29.110). Cells were identified using a 4-layer PAP technique applied to PLP-fixed frozen sections and were quantitated (mean ±S.E.M.) per glomerular cross-sections. In 21 patients with various forms of non-proliferative GN few leucocytes were detected; total 2.1±0.8, monocytes 0.8±0.3, polymorphs 0.5±0.3 and T cells 0.2±0.1. In contrast, in some forms of proliferative GN there was a marked leucocyte accumulation. In post-infectious GN (5 patients) total leucocytes were 19±1.6, monocytes 8.4±1.6, polymorphs 20±6.4 and T cells 0. Within glomerular tufts of 9 patients with RPGN total leucocytes were 19.7, monocytes 7.3±2.4, polymorphs 9.3±5.6 and T cells 0.5±0.3. Monocytes and polymorphs were infrequently present in normal or non-proliferative glomeruli but were greatly increased in post-infectious, crescentic and lupus GN. However, T cells appeared not to be directly involved in any form of human GN.

INVESTIGATION OF PREDICTIVE FEATURES IN LUPUS NEPHRITIS BY LIFE-TABLE AND DISCRIMINANT ANALYSIS. H. Austin , K. Joyce*, J. Balow, T. Antonovych, M. Kullick*, J. Klippel*. NIADDK, NIH, Bethesda, MD and Armed Forces Institute of Pathology, Washington, DC.

The predictive value of laboratory tests and renal histology was analyzed in 82 patients upon entry into prospective therapeutic trials of lupus nephritis (mean follow-up 62 months). Eleven have progressed to renal failure while 71 have not after at least 2 years of observation. Pathologic features emerged as the strongest predictors of renal failure. Individuals with diffuse proliferative or membranoproliferative glomerulonephritis were at modestly, but significantly, increased risk of end stage renal disease (ESRD) compared to patients with other classes of lupus nephritis. Semi-quantitative scoring of active and chronic irreversible histologic features further refined the prognostic information obtained from renal morphology as evidenced by computer derived life tables analyzed by both Breslow and Mantel-Cox tests using ESRD as the measure of outcome. Furthermore, histologic scores specified by Activity (AI) and Chronicity (CI) Indices, were identified as the most powerful predictive variables when entered into a stepwise discriminant analysis program along with laboratory parameters obtained at study entry and types of drug therapy. The computer program generated a classification function based on AI and CI that successfully predicted renal functional outcome in 80% of cases. Thus, certain heretofore underutilized features of the renal biopsy can contribute to the definition of the prognosis of patients with lupus nephritis.

ADRIAMYCIN INDUCED CHRONIC PROTEINURIA: A NEW MODEL OF GLOMERULAR FOCAL SCLEROSIS. T. Bertani, G. Rocchi, G. Mecca, G. Sacchi G. Remuzzi (intr. by C.L. Pirani). Div. of Nephrol., Ospedali Riuniti, Bergamo, Italy and Dept. of Pathol. II, Spedali Civili, Brescia, Italy.

Adriamycin given to 32 rats induced nephrotic sindrome (NS), proteinuria beginning 14 days after a single i.v. injection (5 mg/Kg/body weight). The full expression of NS developed after 28 days, proteinuria ranging between 161 and 454 mg/day. At 28 days no detectable abnormalities were seen by light microscopy (L.M.), but marked "fusion" of foot processes was observed by electron microscopy (E.M.) in all glomeruli examined (16 rats). After six months proteinuria was markedly increased (600 to 1100 mg/day). Focal glomerular sclerosis (F.S.) associated with severe tubular and interstitial changes was present in 50% of the bioptic specimens. At that time 50% of glomeruli showed by E.M. foot processes "fusion". After nine months proteinuria ranged between 800 and 1200 mg /day. By L.M., besides a variable degree of glomerular F.S., the most relevant findings were represented by tubular and interstitial changes. By E.M. foot processes "fusion" was detectable in 40% of glomeruli. This model is representative of the evolution from a "minimal change" type lesion to glomerular F.S. and suggests that tubular and interstitial changes might be an important factor in the development of F.S.

FOCAL SCLEROSIS (FGS) OF HYPERTROPHIED GLOMERULI IN SOLITARY FUNCTIONING KIDNEYS OF MAN. DB Bhathena, BA Julian, RG McMorrow & RW Baehler. Dept. of Path., Wayne State Univ. School of Med., Detroit MI and Central Baptist & St. Joseph Hosp.s, Lexington KY.

This biopsy study investigates glomerular size in relation to focal (segmental) glomerulosclerosis in three patients with solitary functioning kidneys. Control glomerular (Bowman's capsular) diameter (GD) (113.94±17.06μ,n=18) was calculated from published data on normals by Kincaid-Smith (1975). The biopsies of each of two patients with unilateral renal agenesis, proteinuria >2.5gm/24hr and renal failure showed FGS and focal interstitial scarring. Their mean GDs were 272.80±50.67μ(n=23) and 245.08±37.22μ (n=15) respectively. Excluding inflammatory glomerular diseases, these values are comparable to those reported for oligomeganephronie, a congenital condition with reduced nephron numbers estimated at 20% of the normal total body complement. The third patient had biopsy of a fully compensated unilateral functioning kidney prior to removal of the contralateral non-functional, calcified (tuberculous) kidney. Post-nephrectomy, serum creatinine remained unchanged at 0.7mg% with residual urinary protein excretion at 419mg/24hr. Mean GD of the functioning kidney was modestly increased to 192.87 ±27.60μ(n=58) and the biopsy did not show FGS or interstitial scarring. The greater degree of glomerular hypertrophy in our 2 patients with unilateral renal agenesis suggests that its induction, like that in oligomeganephronie, is likely associated with a reduction of the total body nephron population by >50%. The observations also suggest that in the setting of reduced nephron numbers, destruction of glomeruli via FGS occurs when their induced hypertrophy approaches or has reached its limits.

TUBULAR BASEMENT MEMBRANE CHANGES IN POLYCYSTIC RENAL DISEASE. Ralph Butkowski,* Frank Carone, Jared Grantham and Billy Hudson*. Univ. of Kansas Med. Ctr. and Northwestern Univ. Med. School.

Polycystic renal disease was induced in rats by feeding 2-amino-4,5-diphenylthiazole. Cortical and medullary tubular basement membrane (TBM) and glomerular basement membranes (GBM) were purified and analyzed for possible structural changes which may be a factor in development of the tubular dilations and cysts. Changes in the TBM were seen as differences in the relative intensities of several bands by sodium dodecylsulfate polyacrylamide gel electrophoresis. Without reduction of disulfide bonds, a component of M_r = 380,000 was observed to be increased by nearly 2-fold and a component of M_r = 55,000 was decreased by about 25% of control values. Upon reduction of disulfide bonds, additional polypeptides of M_r = 245,000 and 145,000 are increased about 2-fold. With reduction the M_r = 380,000 component dissociates, while the one of M_r = 55,000 remained in lower than normal concentrations, as it was before reduction. Other polypeptides from the TBM appear to change very little or not at all and the GBM is unaltered as analyzed by this technique. The changes take place most rapidly from 4 to 8 weeks of drug administration and remain constant between 8 and 16 weeks. If feeding of the drug is discontinued the TBM returns to normal. The components which were found in altered concentrations are readily soluble under non-reducing conditions, indicating that they are probably non-collagenous. These results indicate that TBM from animals with 2-amino-4,5-diphenylthiazole induced polycystic renal disease is abnormal and may be a factor contributing to the formation of cysts.

IgA NEPHROPATHY: STUDIES OF THE NATURAL HISTORY AND HISTOPATHOLOGY. Byron P. Croker, Deborah V. Dawson and Fred Sanfilippo (intr. by Dr. Robert A. Gutman). Departments of Pathology and Microbiology and Immunology, Duke University and Durham VA Medical Centers, Durham, N.C.

We attempted to identify features in 81 patients with IgA nephropathy (IgAN) which might provide additional insight into the natural history of the disease. Proliferative changes in glomeruli were semiquantitated on an eight point ordinal scale for statistical analysis. Global glomerulosclerosis was quantitated on a continuous scale. The age of onset of IgAN was defined as the age of documented hematuria or proteinuria. The median age at onset of clinical disease was 20 yrs. The median age at biopsy was 27 yrs. Glomerular hypercellularity showed weak correlation with interstitial fibrosis, global sclerosis, proteinuria or serum creatinine ($r < 0.4$). In contrast, global glomerulosclerosis showed a high correlation with serum creatinine and proteinuria ($r=0.5$, $p < .0001$). There was no association between serum creatinine and duration of clinical disease or subtotal glomerular lesions. We had 2-4 year follow-up in 28 patients. Of 15 patients with normal creatinine at biopsy, 3 developed mild azotemia. Nine patients had elevated creatinine but were not in chronic renal failure (CRF), of these 8 patients showed progressive azotemia with three developing CRF. Four patients had CRF at the time of diagnosis. In conclusion, IgAN is a chronic renal disease which begins in childhood. Generally patients with normal creatinine maintain a normal creatinine for 2 to 4 years whereas patients with an elevated creatinine at biopsy have progressive azotemia associated with proteinuria.

TWO VARIANTS OF IGM NEPHROPATHY? D.Cattran, P.Rance C.Cardella, R.Charron, R.Bear, J.Roscoe, E.Cole, S.Ritchie and the Toronto Glomerulonephritis Group (TGNR), Toronto, Ontario, Canada.

In the 2000 cases in the TGNR, there were 32 with mesangial IgM deposits; by light microscopy (LM) 19 had focal glomerulosclerosis(FGS), (Gp1) and 13 had nil lesion disease (MCD), (Gp2). These Gps were compared to 59 cases of FGS (Gp3) with no IgM (P value using Chi squared among Gps).

At Presentation	Gp1	Gp2	Gp3	P
Number patients	19	13	59	-
Sex M/F	10/9	8/5	31/28	NS
Age	14	9	23	NS
Hematuria - Micro %	28	0	25	0.03
- Macro %	25	0	2	0.02
Hypertension %	19	23	25	NS
GFR < 80%	25	42	55	NS
Proteinuria > 2.5gm/day	60	66	77	NS
Steroid Response %				
Responsive	11	55	13	0.05
Frequent Relapse	0	33	0	0.03
Non-Responsive	35	11	27	0.06
No Treatment	53	0	59	-

Change/year in creatinine clearance in mls/min with total follow-up in mos. in brackets was Gp1, -6.5 (48); Gp2, +78(50); Gp3, -9(51). Actuarial kidney survival at 5 years was Gp1; 72%; 2; 94% and 3;70%. General linear model analysis showed groups 1 & 3 identical with group 2 significantly different $P < 0.003$.

We believe this explains the contrast found by other authors re: significance of mesangial IgM deposits. When IgM present without LM changes the prognosis and treatment equals MCD and with FGS changes follow that disease prognosis. This suggests IgM is of no independant significance.

ELEVATED FIBRINOLYSIS INHIBITORS IN LUPUS NEPHRITIS (LN): RELATION TO FIBRIN CLEARING EFFECT OF ANCROD. A. K. Dosekun,* P. Glas-Greenwalt,* S. K. Kant, M. A. Weiss, C. Allen,* V. E. Pollak. Univ. of Cincinnati Med. Ctr., Cincinnati, Ohio.

In 6 patients with LN and glomerular fibrin deposition, we previously reported a deficiency of prostacyclin stimulating factor (PSF), and resolution of glomerular thrombosis (GT) with normalization of PSF after defibrination with ancrod (Kidney Int. 21:210, 212, 1982). We now report evidence of other abnormalities of endothelial cell function in LN, and their relation to the response to defibrination. A highly standardized fibrin plate method was used to assay fibrinolytic parameters in plasmas of 14 patients with LN and GT before and during ancrod treatment. Vascular plasminogen activator was trace/absent in 12/14 plasmas; an inhibitor of plasminogen activator was found in 10/14. Both abnormalities corrected within 48 h of defibrination. A potent inhibitor of the in-vitro action of plasmin on fibrin (PI) was found in only 3 of 14. Serial histology showed resolution or improvement of GT (mean score 1.85 to 0.7) in 9/11 patients with no PI. In 3 patients with PI, PI persisted during defibrination and GT failed to resolve in 2 (mean score 2.5 to 2.2). These findings strongly suggest an important role for the fibrinolytic system in the genesis of GT, and that a high PI blocks the therapeutic effect of defibrination. To test this hypothesis further 1 patient with PI was given a second course of ancrod and 2 plasmaphereses were done. PI normalized immediately in the plasma; a third biopsy showed resolution of fibrin.

RENAL DEPOSITION OF POLY C9: NEOANTIGEN OF THE MEMBRANE ATTACK COMPLEX (MAC). R.J. Falk,* Y. Kim, C.H. Tsai,* J.I. Scheinman, H. Gewurz,* A. Dalmasso,* and A.F. Michael, Depts. of Pediatrics, Lab. Medicine and Pathology, Univ. of Minnesota Medical School, Minneapolis, MN.

A monoclonal antibody (Poly C9-MA) has been developed and characterized which reacts specifically with a neoantigen on the polymerized C9 (poly C9) portion of the membrane attack complex (MAC) of complement. Poly C9-MA does not recognize native C9 nor any other constituent of the MAC. By immunofluorescence, poly C9 was found minimally in normal adult kidney along vessel walls and in juxtaglomerular regions, but was absent from fetal kidney. In 16 patients with various glomerulonephritides poly C9 was present in a pattern similar to that observed for C3. In 17 patients with non-nephritic renal disease (diabetes mellitus, hypertension, obstructive uropathy, congenital dysplasia, amyloidosis) poly C9 was deposited extensively in the mesangium, Bowman's capsule, tubular basement membranes, vessel walls, and especially in areas of sclerosis. There was disjunction of C3 and poly C9 deposition in non-nephritic diseases. These studies document the deposition of MAC, specifically poly C9, in nephritic and non-nephritic renal disease. The marked deposition of poly C9 in areas of sclerosis suggests that complement activation may play a role in the development of renal insufficiency.

EFFECTS OF EXERCISE ON RENAL HISTOLOGY IN RATS. Nancy E. Gary, Edward J. Zambraski, Ann Bretschneider,* and Willard A. Burns*. UMDNJ-Rutgers Medical School, Piscataway, N.J., V.A. Med. Ctr., Lyons, N.J. and Rutgers Univ., New Brunswick, N.J.

Transient proteinuria and decreases in renal blood flow have been noted in healthy animals and man following strenuous exercise (E). The purpose of this experiment was to study renal histology following E of a level, duration and intensity which has previously been associated with a decrease in glomerular anionic character. Eight adult Sprague Dawley rats were trained to run on a motor driven treadmill. After 3 days rest, 5 rats were exercised to exhaustion (running 180 minutes at 1 km/hour). Two rats (4,5) were kept for 24 hrs. for clearance studies (Ccr) and then sacrificed. Three rats (6,7,8) were sacrificed immediately post E. Control (C) rats (1,2,3) were sacrificed after 180 min. on a motionless treadmill. Results:

Rat	C or E	24 hr. urine ml	Serum Creatinine mg/dl	Ccr ml/min
1	C	16	0.7	1.1
2	C	18	0.5	1.6
3	C	11	0.7	1.3
4	E	7	0.8	0.6
5	E	26	1.2	0.3

Light microscopy of exercised rat kidneys (4-8) showed considerable vacuolization of renal tubular epithelial cells. Transmission electron microscopy showed minimal increase in glomerular mesangial cells and matrix without electron dense deposits when compared to control rats (1,2,3). In conclusion, these data suggest that strenuous exercise may cause non-specific glomerular and tubular histologic abnormalities in rats.

T LYMPHOCYTE SUBPOPULATIONS AND LYMPHOCYTE FUNCTION IN MINIMAL CHANGE NEPHROPATHY (MCN) DURING LONG TERM REMISSION. J. Feehally*, I.B. Houston*, R.Gokal*, NP. Mallick* (intr. by S. Dosa). Manchester Royal Infirmary, Manchester, England.

In order to assess the long term effect of cyclophosphamide (CY) therapy on immune function in MCN, 45 children were studied during prolonged remission: 21 children (mean age 12.1 yrs) who had received only corticosteroids; and 24 children (mean age 10.9 yrs) who, in addition to steroids, had received CY 2.5 mg/kg/day for 8 weeks. Mean period since CY was 5.1 yrs. Peripheral blood lymphocytes were assessed by: 1. Enumeration of T cell subpopulations using monoclonal antibodies to total T cells (OKT3), helper T cells (OKT4) and suppressor T cells (OKT8). 2. Lymphocyte response in culture to phytohaemagglutinin (PHA) and concanavalin A (Con A). 3. Co-culture assays in which suppressor cells were induced by culture with Con A and their suppressive effect on previously unstimulated autologous cells measured in a second co-culture with Con A. There were no significant differences between the two patient groups and controls in relative and absolute numbers of OKT3+, OKT4+ and OKT8+ cells; OKT4/OKT8 ratio; response to PHA and Con A, response in suppressor cell assays.

Children with MCN in remission have normal lymphocyte numbers and function and the use of CY in conventional dosage does not appear to confer any prolonged cellular abnormality which might prejudice the use of this drug.

ASSOCIATION OF WILMS TUMOR (WT) AND SCLEROSING GLOMERULONEPHRITIS (GN). D.C. Houghton, D. Ridgeway and Y. Talwalker. Oregon Health Sciences University (OHSU), Portland, Oregon.

Among 22 WT nephrectomy specimens, 3 had histologic evidence of GN at the time of nephrectomy. One patient, a 33 mo. old girl had bilateral WT 24 mo. after developing GN that progressed to renal failure. The others, boys, 3 and 16 mo., were hypertensive, and one, the latter, had nephrotic range proteinuria before nephrectomy. All had focal, segmental mesengial hypercellularity and glomerular sclerosis (GS) with hyalin deposition and crescent formation. EM showed variable membranous and mesangial GS with local 'hyalin' deposits; IF (case 1 only) showed focal, segmental deposits of IgM and C3.

Other than being younger (mean age: 17 vs 34 mo.), there were no clinical differences found between these 3 and the other WT patients. No consistent differences in WT size, site or histology were noted. Patients 1 and 2 are alive 12 and 36 mo. after nephrectomy without evidence of tumor; the latter is no longer hypertensive. Patient #3 died of septicemia 11 days after nephrectomy.

Other reports of the coexistance of sclerosing GN & WT provide evidence that the association is not happenstance. The pathogenesis of the GN is speculative; all three cases resemble the focal segmental GS and hyalinosis seen in other settings. There is little histological evidence to implicate intrarenal reflux or obstruction or immune complex diseases. The lesion may develop, as other forms of GS are thought to do, in response to glomerular hyperfiltration after progressive loss of functional tissue.

FALSE NEGATIVE ANTI-DNA ANTIBODY ACTIVITY IN AN INFANT WITH CONGENITAL NEPHROTIC SYNDROME AND SLE: DEMONSTRATION OF ANTI-DNA ANTIBODIES IN ISOLATED CIRCULATING IMMUNE COMPLEXES (CICs). Stanley C. Jordan*, Jacques Lemire*, Wayne Border, Rebecca Sakai*, Robert Ettenger and Richard N. Fine. Div. Ped. Neph., Dept. Peds., UCLA Sch. Med. and Div. Neph., Dept. Med., UCLA-HGH, Los Angeles, Calif.

A 2½ month old previously healthy female infant presented with nephrotic syndrome, progressive renal failure, anemia and thrombocytopenia. Renal biopsy revealed a proliferative glomerulonephritis with both glomerular and extra-glomerular deposits of IgG, IgM, C_3 and C1q, by direct immunofluorescence techniques (IF). Skin biopsy revealed positive IF for IgG and C_3. Despite strong clinical and IF criteria for SLE, ANA and anti-DNA antibody studies were negative. CIC levels were positive in the Raji-RIA, C1q-SPA and the $F(ab)_2$ anti-C_3 assays for CICs. Immunochemical analysis of CICs isolated from Raji cell eluates demonstrated anti-DNA antibody activity when dissociated with 0.5 M citric acid buffer pH 2.5 (CAB). Two sera negative for anti-DNA antibodies (4% DNA binding) increased to 87% and 85% DNA binding respectively after sera were preincubated with (CAB) to dissociate the CICs. Analysis of kidney biopsy eluates by immunochemical technique confirmed the presence of DNA and anti-DNA antibodies. These studies confirm that SLE may be a cause of congenital nephrotic syndrome and that "false negative" serologies may be secondary to binding of available antibody by excess antigen. Analysis of CICs may prove useful in confirming the diagnosis of SLE in seronegative patients.

A CLINICAL & IMMUNOLOGIC STUDY OF A CHILD WITH ACUTE POSTSTREPTOCOCCAL GLOMERULONEPHRITIS AND RHEUMATIC FEVER. H.A.Majeed, K.Lange*and D.Sharda. The Dept. of Ped., Kuwait Univ., Kuwait and Lenox Hill Hosp., New York Med. Coll., New York, N.Y.

The coincidence of acute poststreptococcal glomerulonephritis (AGN) and acute rheumatic fever (ARF) in the same patient is extremely rare and the occurrence of AGN *preceding* ARF has not been described. A 9 year-old girl was admitted with typical signs and symptoms of AGN including markedly elevated antibodies to streptococcal exoenzymes and the newly described antibody to the streptococcal *cytoplasmic* antigen, endostreptosin (ESS). ESS-Abs characteristically rise significantly in AGN above the normal maximal titer of 1:16 and remain elevated for long periods of time. C3 *fell* significantly to low levels. Five weeks after onset of AGN she came down with a typical first attack of ARF with carditis and joint swelling, high ESR but *high* C3 and a steep rise of the ESR which had already normalized after the AGN, further rise of ASLO but fall in the previously abnormally elevated ESS-Ab. It appears that the streptococcal antigen probably responsible for AGN is different from the antigen in ARF as judged by the behavior of the corresponding antibodies in these two poststreptococcal sequelae.

Streptococcal Abs, C3, BUN and ESR during the Clinical Course of AGN and ARF

Day	Disease	ASO	AH	ESS-Ab	C3	BUN	ESR	Urine
1	AGN	833	256	256	28	58	90	+++
14	"	833	256	128	ND	20	ND	+
28	"	ND	ND	ND	38	18	ND	tr.
36	ARF	1250	1024	64	70	29	150	±
55	"	625	512	64	52	14	64	tr.
242	"	333	256	64	ND	8	34	-
340	"	125	256	64	ND	ND	ND	-

A COMPARATIVE STUDY OF IDIOPATHIC AND SLE-ASSOSIATED MEMBRANOUS GLOMERULONEPHROPATHY IN 52 CHILDREN. Henry F. Krous* and James E. Wenzl, Southwest Pediatric Nephrology Study Group.

This retrospective multicenter study evaluated renal biopsy findings in 38 children (ages 1 9/12 to 17 yrs) with idiopathic membranous glomerulonephropathy (IMGN) compared to 14 children with SLE-associated membranous glomerulonephropathy (SMGN). Four patients with hepatitis B-associated MGN were studied but excluded from the comparative analysis. Forty-two variables were semiquantatively analyzed by light microscopy (LM) (n52), immunofluorescence (IF) (n39) and/or electron microscopy (EM) (n45). Compared to the IMGN group, the SMGN patients were predominantly female (80% vs 44%), older (13 5/12 yrs vs 10 3/12 yrs), ANA positive (100% vs 6%) and had lower serum C3 concentrations (50% vs 7%). Although glomerular subendothelial and mesangial deposits were significantly more common by EM in SMGN than IMGN (69% vs 14% and 92% vs 45%, respectively), mesangial hypercellularity and sclerosis were not significantly different by LM and were present in more than two-thirds of both groups. Although IgA was more frequently identified in SMGN than IMGN (35% vs 11%), there was no difference in the frequency of other immunoreactants (IgG in 100%, IgM in 50% of each group). The presence of three or more immunoreactants per biopsy was not different between groups. Glomerular obsolescence, tubulointerstitial disease, vasculopathy and the distribution of MGN pathologic stages (Gluck, et al, Ann Intern Med, 1973) were similar in SMGN and IMGN. We conclude that renal biopsy morphologic and IF findings are similar in these 2 groups except for the increased incidence of glomerular subendothelial and mesangial deposits by EM in the children with SMGN.

SELECTIVE BINDING OF IgG4 AND OTHER NEGATIVELY CHARGED PROTEINS IN NORMAL AND DIABETIC KIDNEYS. T. Melvin,* Y. Kim, A.F. Michael, Univ. of Minn., Dept. of Pediatrics, Minneapolis, Minnesota.

The linear deposition of albumin and IgG along glomerular basement membrane (GBM) in normal and diabetic kidneys is unexplained. Evidence that this binding is charge related is deduced from immunohistochemical studies with antibody probes directed against proteins of varying isoelectric points (pI). Kidney tissues from patients with diabetes mellitus (DM) (5 with end-stage renal disease and 5 with normal renal function), 5 normal human kidneys (NHK) and 3 kidneys with anti-GBM disease were examined by immunofluorescence techniques for the presence of the serum proteins indicated.

GBM BINDING IN NHK AND DM

Protein	pI	Binding
α-1-acid glycoprotein	2.7	+
Amyloid P	3.9-4.8	+
Albumin	4.9	+
IgG4	5.8-6.0	+
IgG1	7.0-9.5	-
IgG2	7.0-8.5	-
IgG3	8-9	-
Properdin	>10	-

Linear staining of GBM was demonstrated in normal and diabetic kidney for those proteins with a pI of 6.0 or lower. That this is not a consequence of serum concentration is indicated by the selective presence of IgG4 (≈4% of total serum IgG) and absence of IgG1, IgG2 and IgG3. In contrast, in anti-GBM nephritis linear GBM staining was present with IgG1, IgG2, IgG3 and IgG4. These studies suggest that there are positive charge sites in human GBM which bind anionic proteins in vivo.

THERAPY OF ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODY (A-GBM-Ab) DISEASE: A PROSPECTIVE STUDY. J Moore, JP Johnson, L Bohan, H Austin, J Balow, T Antonovych, C Wilson. Walter Reed Army Med Ctr, AFIP, Wash, DC, Nat Nav Med Ctr, NIH, Bethesda, MD, & Scripps Res Fnd, La Jolla, CA.

We compared therapy (Rx) with immunosuppresion (I) alone to I plus plasma exchange (PE) in 17 patients (PT) with A-GBM-Ab disease. PT were randomly assigned to Rx with I (prednisone +cytoxan, N=9) or I + PE (4L PE q 3 days, N=8). Rx was continued until A-GBM-Ab was < 5% binding or PT had remained stable on dialysis > 1 mo. Clinical characteristics (age, s. creatinine, HCT, pulmonary Sx) and duration of Rx were similar in both groups. Rate of disappearance of A-GBM-Ab (expressed as % binding by RIA) was linear over time of treatment and more rapid in PT receiving PE + I then I alone ($p < .005$). At termination of Rx, 6 of 8 PT receiving PE + I were off dialysis, 2 with moderate renal insufficiency. 3 of 9 PT receiving I alone were off dialysis and 1 died. Lung hemorrhage and infectious episodes were similar in both groups. No PT on dialysis at start Rx recovered function. During longterm followup, 2 PT (I + PE) died 2^{o} complications of ESRD and 1 PT (I + PE) progressed to dialysis 7 mo post-Rx. Discriminant analysis was performed employing semi-quantitative scoring of pathologic features. Among all variables cellular crescent score emerged as the most powerful predictor of outcome. Our results suggest that PE results in a more rapid disappearance of A-GBM-Ab and an improved outcome for PT with this disorder. PT with severe crescentic disease fared poorly and PT with mild crescentic involvement did well whichever therapy was used.

RENAL VEIN THROMBOSIS PLASMA BLOCKS FIBRINOLYSIS IN-VITRO: IMPORTANCE OF A PLASMIN INHIBITOR. P. A. Pajel,* P. Glas-Greenwalt,* A. K. Dosekun,* K. S. Kant, and V. E. Pollak. University of Cincinnati Medical Center, Cincinnati, Ohio.

Renal vein thrombosis (RVT) causes both acute renal failure and nephrotic syndrome. It occurs with membranous glomerulopathy, lupus nephritis and amyloid. The underlying cause of the thrombosis is unknown; coagulation studies have yielded little pathogenetic information. Using the fibrin plate technique, on plasmas from 73 healthy controls and 11 patients with RVT, we measured vascular plasminogen activator (VPA), an inhibitor of urokinase action on plasminogen (IPA) and an inhibitor of plasmin action on fibrin (PI). In normals, VPA (geometric mean ± 2 SD) was 7.67 (3.0, 19.9) activator units; VPA was unmeasurable in 3/11 RVT plasmas. The normal IPA was 732 (615, 872) inhibitor units; values in RVT plasmas, 1509 (883, 2578) inhibitor units, were elevated ($p < 0.01$). The normal PI was 812 (715, 909) inhibitor units whereas PI was elevated above the normal range in all 10 RVT patients. The data were compared with those from another condition with thrombosis--lupus nephritis (LN) with glomerular thrombi (GT).

	VPA↓	IPA↑	PI↑
RVT	3/11	10/11	10/10
LN-GT	12/14	10/14	3/14
p	<0.02	NS	<0.001

These observations suggest that fibrinolytic enzyme abnormalities play an important role in the pathogenesis of RVT; and that a plasmin inhibitor may predispose to the development of RVT.

EVIDENCE FOR A MONOCYTE PHAGOCYTIC DEFECT IN A SUBPOPULATION OF PATIENTS WITH NEPHRITIS. B. S. Ooi and Y. M. Ooi, University of Cincinnati Medical Center, Cincinnati, Ohio.

Experimental studies have demonstrated the critical role of the mononuclear phagocytic system in the removal of immune complexes from the circulation. It may be postulated that a phagocytic defect in the system may impair such mechanisms of removal, favoring the deposition of complexes in the kidney. To examine this possibility in human nephritis, an in-vitro assay for studying monocyte phagocytic function was performed on normal subjects, 10 patients with varying forms of mesangial proliferative glomerulonephritis, 8 patients with membranous nephropathy (MN) and 8 patients with chronic renal failure (CRF). Monocyte monolayers obtained by plating mononuclear cells onto cover slips were overlaid with sheep erythrocytes coated with IgG antibody (EAIgG SRBC) or with latex particles. The number of monocytes phagocytizing the particles were enumerated, performing triplicate assays. The percentage of monocytes (±SD) showing EAIgG SRBC phagocytosis in normal subjects was 89% ± 5%. In patients with renal disease, 4/10 patients with mesangial proliferative glomerulonephritis, and 1/8 patients with MN displayed a phagocytic defect. Monocytes from 2 of the patients with mesangial proliferative glomerulonephritis also had a defect in phagocytizing latex beads. All 8 patients with CRF showed normal monocyte phagocytic ability. The results of these studies identify a subpopulation of patients with nephritis with a monocyte phagocytic ability which may have pathogenetic significance in the development of nephritis.

SIGNIFICANCE OF TUBULO-INTERSTITIAL CHANGES IN LUPUS NEPHRITIS. Moon-Hyang Park,* Gerald B. Appel and Conrad L. Pirani. Columbia Univ., Dept. of Path. & Med., New York, New York.

The renal biopsies from 103 patients with SLE and clinical evidence of renal involvement were studied by light-, electron- and immunofluorescence-microscopy (LM, EM and IF). The presence and amount of immune type electrondense deposits in the tubular basement membranes (TBMD) and in the interstitium, the degree and type of interstitial inflammation, and the class (WHO Classification), severity and activity of glomerular lesions were assessed by semiquantitative methods. The findings in tubules, interstitium and glomeruli were compared with each other and with serologic and renal functional data. TBMD were detected in 33% of the biopsies by IF and in 23% by EM. IgG and C_3 were most commonly present in the deposits. Presence and amount of TBMD were positively correlated with the more severe and active type of glomerular lesions (Classes III and IV) but were not correlated with the degree of interstitial inflammation, since in many cases, interstitial inflammation was present in the absence of TBMD and in a few instances there was no interstitial inflammation in the presence of TBMD. A high Farr and low tot. serum complement correlated with presence of TBMD but did not with the degree of interstitial inflammation. A higher degree of interstitial inflammation was associated with more severe renal insufficiency (serum creat.), elevated diastolic BP and worse longterm prognosis.

CHARGES OF ISOLATED CIRCULATING IMMUNE COMPLEXES (CIC) IN PATIENTS WITH GLOMERULAR DISEASES. T.M. Phillips*, S. Dosa, A.M. Thompson, C. Short*, N.P. Mallick*, Divisions of Nephrol., G.W. Univ. Med. Ctr., Washington D.C. and Univ. of Manchester, U.K.

Experimental evidence suggests that interaction between cationic immune materials (antibodies or antigens) with glomerular polyanionic sites may enhance their glomerular deposition. However, there is little information available on the charge characteristics of CIC found in patients with immune complex glomerulonephritis (GN). We have developed a flatbed isoelectric focusing technique to determine the charges (PI) of isolated, intact CIC from the sera of patients with biopsy proven glomerular diseases. Confirmation of the isoelectric points for each CIC was obtained by chromatofocusing. CIC was considered neutral at PI 7.0, anionic in the PI range of 6.0-6.3 and cationic in the PI range of 7.1-8.5.

In this preliminary study, CIC were detected by C_1q, conglutinin and Raji cell assays. The individual CIC were isolated on PEG and sucrose density gradients. CIC were detected by one or more assays in 9/13 patients without demonstrable glomerular immune complex deposition (G1), in 6/8 patients with membranous GN (G2) and in 12/12 patients with mesangial proliferative GN (G3). The median PI in G3 was higher at 7.6 (range 7.0-8.1) than in G1: 7.3 (6.7-7.6) or in G2: 7.0 (6.4-7.3). However, due to the wide PI range found in all three groups, further investigations on a larger patient population are required to determine the significance of these observations.

PROGNOSTIC FACTORS DETERMINING THE COURSE OF MEMBRANOUS(M) GLOMERULONEPHRITIS(GN) OF SYSTEMIC LUPUS ERYTHEMATOSUS(SLE). Melvin M. Schwartz, Jimmy L. Roberts, Karen Kawala*, Carol Humes*, and Edmund J. Lewis. Depts. of Pathology and Medicine, Rush Medical College, Chicago, Il

We studied clinical and pathologic characteristics of 21 patients with SLE MGN to determine prognostic factors. Biopsies were classified according to WHO criteria. The distribution of subepithelial(EPI) and subendothelial(ENDO) deposits was quantified. Three cases had no glomerular proliferation(5A), 6 mild mesangial changes(5B), 10 segmental GN(5C), and 2 diffuse proliferative GN (5D). 5-year survival was 90%. Two deaths occurred in 5C. A serologic difference among MGN patients was that those with severe proliferation (5C/5D) had elevated cryoglobulins while those with mild changes(5A/5B) did not. Two of 3 patients whose creatinine increased died. Most of the biopsies had extensive EPI, but 3 patients who were clinically and serologically similar to the others had sparse, segmental EPI. All the biopsies had some ENDO, but in four the deposits were extensive. Two of these patients died. The 2 5D patients had extensive ENDO. Two patients developed more "active" lesions. One with massive ENDO died. The other without ENDO responded to therapy and has maintained stable renal function. We conclude 1) the presence of infrequent ENDO does not change the generally good prognosis of the patient with MGN, but extensive ENDO identify a group with a poor prognosis. 2) SLE MGN patients with sparse EPI seem clinically and serologically similar to those with generalized deposits. 3) The WHO histologic classification appears useful in SLE MGN as it identifies patients with poor prognostic features.

ALTERED PROSTAGLANDIN-MEDIATED ADHERENT CELL SUPPRESSOR ACTIVITY (ACSS) ASSOCIATED WITH IMPAIRED LYMPHOCYTE CYCLIC AMP PRODUCTION IN CHRONIC RENAL FAILURE (CRF). MC Ruddy, A Novogrodsky,* AL Rubin, KH Stenzel. UMD-Rutgers Med. Sch., New Brunswick, N.J. and NYH-Cornell Med. Ctr., N.Y., N.Y.

We have previously shown that impairment of the prostaglandin-mediated (monocyte-macrophage) ACSS in CRF is associated with decreased sensitivity of uremic lymphocytes to the suppressive effect of prostaglandin E_1 (PGE_1) on soybean agglutinin(SBA) and peanut agglutinin (PNA)-induced lymphocyte proliferation.

Reduced uremic lymphocyte sensitivity to PGE_1 may be attributable to an altered cellular response to cyclic AMP. We investigated the suppressive effects of several cyclic AMP analogues and 3-isobutyl methyl xanthine (MIX) on SBA and PNA-induced blastogenesis. Nine stable maintenance hemodialysis patients were compared with paired controls. Each of these agents (8 bromo cyclic AMP, 10^{-4}M; MIX, 20µg/ml) were found to inhibit mitogenic responses of the uremic cells to the same extent as in normals. Cyclic AMP production by non-adherent cells from patients and controls in response to PGE_1 and MIX was also determined. Significant differences between uremic and control cellular cyclic AMP generation rates were not present in the unstimulated state or when either PGE_1 or MIX were added alone. However, PGE_1, (1µg/ml) together with MIX (20µg/ml) resulted in a markedly greater production of cyclic AMP in the controls (420 pmol/10^7 cells ($P<.05$).

We conclude that PGE_1-stimulated production of cyclic AMP by uremic lymphocytes is defective, which may account for decreased activity of the ACSS in CRF.

ENDOSTREPTOSIN ANTIBODY DETERMINATIONS IN THE DIFFERENTIAL DIAGNOSIS OF GLOMERULONEPHRITIS. Gene Seligson*, Kurt Lange*, H.A. Majeed and D. Chug. Lenox Hill Hosp., New York Med. Coll., New York, N.Y. and The Dept. of Ped., Kuwait Univ., Kuwait.

Endostreptosin (ESS) is an immunologically well defined cytoplasmic antigen of all group A streptococci. ESS is probably the pathogenetic antigen of poststreptococcal glomerulonephritis (ASGN). ESS can be demonstrated on the glomerular basement membrane of patients with ASGN during the first 7-10 days of symptoms. This antigen deposition is followed by in situ coverage with specific antibody.

Antibodies to ESS (ESS-Ab) were determined by microcomplement fixation in 880 patients. The majority of tests were performed as double blind examinations. The results were as follows:

Patients		No.	% with titers>16	Mean Arith.	Mean Log2
Norm. infants	(<4 mos.)	6	29	21.3	3.80
" "	(>4 mos.)	10	0	1.8	0.50
Norm. children	(U.S.A.)	151	19	13.0	2.67
" "	(Mid-East)	153	28	11.9	2.56
Norm. adults	(U.S.A.)	186	7	6.0	2.10
ASGN	(U.S.A.)	90	87	43.0	4.51
"	(Mid-East)	40	85	61.4	5.50
Strep. infec.	(U.S.A.)	56	38	14.0	4.12
" "	(Mid-East)	188	37	23.3	4.10

ESS-Ab titers in patients with streptococcal infections without renal involvement were only transiently elevated in contrast to the persistent elevations in ASGN. ESS-Abs in patients with other types of glomerulonephritides were in range of normals. ESS is not related to known streptococcal exoenzymes. ESS-Ab titers did not parallel antibody titers of exoenzymes. They were most informative and diagnostic for ASGN and its immunologic basis and course.

MONOCLONAL ANTIBODY-DEFINED T CELL SUBSETS IN IDIOPATHIC MEMBRANOUS GLOMERULONEPHRITIS (MGN) C.D.Short*, P.Brenchley*, B.Coupes*, P.Dyer*, J.Feehally*, R.Pumphrey*, N.P.Mallick*. (intr. by S.Dosa).

In Caucasians MGN occurs more frequently in the subjects who are (A1-B8)-DR3. Seeking further clarification of immunological abnormalities in MGN we have used monoclonal antibodies to human T cells (OKT3, OKT4, OKT8) to quantitate the T cell subpopulations in 22 patients with histologically proven MGN and 26 normal controls.

MGN patients had a normal percentage of OKT3+ cells (71.5 ± 9.8) compared to the controls (71.6 ± 7.7) and a normal percentage of OKT4+ cells (48.0 ± 8.3 and 45.1 ± 11.2 respectively). However, the MGN patients exhibited a significant reduction in the percentage of OKT8+ cells (24.1 ± 7.5) compared to controls (30.2 ± 8.4). There was no significant reduction in the absolute number of OKT8+ cells when compared to controls.

MGN patients with a low percentage of OKT8+ cells (< control mean value - 1 S.D.) were no different from the other patients when compared by age, tissue type, degree of renal impairment or proteinuric state, but had a significantly lower absolute number of OKT8+ cells and a higher serum IgG concentration than the other patients.

Patients who were DR3+ had a higher percentage of OKT8+ cells (25.1 ± 8.6) compared to patients who were DR3- (21.9 ± 4.1).

The reduction in percentage OKT8+ cells in a subgroup of MGN, not identifiable immunogenetically is further evidence that a spectrum of immunological abnormality may occur in this disorder.

THE "UNIQUE" LESION OF FENOPROFEN NEPHROPATHY - AN OVERSIMPLIFICATION? K. Solez, W.E. Beschorner*, W.L. Bender*, M. Hall-Craggs, and A. Whelton, Johns Hopkins Hospital, Baltimore, Maryland.

It has been suggested that the nonsteroidal anti-inflammatory drug (NSAID) fenoprofen (Nalfon) produces a unique renal lesion characterized by a combination of minimal change disease and an interstitial nephritis with an infiltrate made up entirely of T-lymphocytes (Am.J.Med.72:81,1982). We compared biopsies from 4 cases of nephrotic syndrome and interstitial nephritis in patients receiving NSAID's (Nalfon-2, Zomax-1, Motrin-1) with 4 cases of minimal change disease with interstitial nephritis in patients not receiving NSAID's. Immunofluorescence studies demonstrated B lymphocytes staining for IgM in the inflammatory infiltrate in all cases in both groups. B cells constituted 2-26% of the infiltrating cells in the NSAID cases and 2-19% in the other 4 cases. Characterization of the infiltrating T-cells using monoclonal antisera showed that the ratio of OKT4+ cells to OKT8+ (helper cells/suppressor-cytotoxic cells) ranged from .6 to .9 in the NSAID cases and .5 to 1.4 in the others. Most of the OKT8+ cells in the NSAID cases reacted with the Cyt 1 antibody developed by one of us (W.E.B.) which is specific for cytotoxic T-cells. Three of the 4 NSAID cases showed occasional giant collecting duct cells, not seen in any of the 4 other cases. Otherwise, there were no histologic differences between cases with and without a history of NSAID treatment, or between the Nalfon cases and the Zomax and Motrin cases. The Nalfon lesion is not unique. Cell mediated immunity appears to be an important factor in this type of lesion. However, the inflammatory infiltrate contains B as well as T cells.

INTERSTITIAL CELL INFILTRATE (ISC) IN CYCLOSPORIN A (CyA) NEPHROPATHY IDENTIFIED IN TISSUE SECTIONS USING MONOCLONAL ANTIBODIES (Ab). R. Sibley, J.L. Platt,* R. Ferguson,* and A.F. Michael. Departments of Laboratory Medicine and Pathology, Pediatrics and Surgery, Univ. of Minnesota Medical School, Minneapolis, MN.

Nephrotoxicity due to CyA is a frequent cause of decreased renal graft function and is associated with renal interstitial inflammation. The ISC in 13 renal tissues from patients with CyA toxicity and in 7 renal tissues from patients with rejection were studied using monoclonal Ab to identify lymphoid cells, and ethidium bromide stain for total interstitial cells by indirect immunofluorescence on frozen tissue sections. The degree of infiltration in CyA toxicity was less than that in rejection tissues, however, total T cells (OKT3, TA-1) and total B cells (BA-1) were similar. CyA toxicity tissues contained a greater proportion of T helper cells (OKT4) and fewer monocytes (OKM1) than did rejection. The ratio of OKT8 reactive to OKT4 reactive ISC was less in CyA toxicity (.95 + .59) than in rejection (4.3 ± 1.5) ($p < .001$). Two tissues from patients on CyA who experienced rejection had ISC infiltrates characteristic of rejection (OKT8/OKT4 = 4.9 and 6.7). OKT8/OKT4 ratios in peripheral blood were broadly overlapping and not significantly different. CyA toxicity and rejection can be distinguished by analysis of ISC.

MONONUCLEAR CELL SUBSETS IN HUMAN IDIOPATHIC CRESCENTIC GLOMERULONEPHRITIS (ICGN) STUDIED WITH MONOCLONAL ANTIBODIES. Irene Stachura, Lusheng, Si*, and T L Whiteside*, Dept. Lab. Med., Allegheny Gen. Hosp., and Div. Clin. Immunopathol., Univ. of Pittsburgh School of Med., Pittsburgh, Pa.

Mononuclear inflammatory cells (MIC) in renal biopsies from 14 patients with ICGN were studied by avidin-biotin-immunoperoxidase technique utilizing monoclonal antibodies (Ab) to cell surface antigens: T 11 (total T), T4 (inducer/helper T), T8 (suppressor/cytotoxic T),B1 (B cells),M1 (monocytes) and Leu 7 (natural killer, NK cells). Six patients had glomerular immune complex deposits, three had anti-GBM disease and five had ICGN without immune deposits. Biopsies from three cases of minimal change disease served as controls. The distribution and numbers of MIC were determined in serial cryostat sections stained with monoclonal Ab. Results were expressed as mean cell number ±SD; in each biopsy five high power fields were counted. T lymphocytes (394 ± 150) constituted ~80% of renal MIC. Monocytes, B cells and NK cells accounted for the other 20%. T cells formed focal interstitial collections or surrounded renal glomeruli. They were occasionally observed in glomerular tufts and crescents. Monocytes were the most common MIC in glomeruli. Subtyping of T cells showed predominance of T4 cells (242 ± 102) over T8 cells (168 ± 88). There was no difference in the number and distribution of MIC in tissues from patients with various forms of ICGN. Control tissues contained few MIC, most of which were T lymphocytes (27 ± 20). These results indicate that T lymphocytes may be involved in the pathogenesis of all forms of human ICGN.

MEDIATOR SYSTEMS IN IN SITU IMMUNE COMPLEX GLOMERULONEPHRITIS (ICGN). F.Thaiss*, S.Batsford*, M.Mihatsch*, D.Bitter-Suermann*, A.Vogt(Intr.by R. Sterzel). Zentrum für Hygiene der Universität Freiburg, Institut für Medizinische Mikrobiologie der Universität Mainz, FRG, Institut für Pathologie der Universität Basel, Schweiz.

Mediators of inflammation, such as complement(C), polymorphonuclear leukocytes(PMN) and macrophages(M) are of varying importance in the different models of nephritis. C but not PMN is important in the passive Heymann model(Salant et al.,J.Clin.Invest., 66,1339,1980). In passive Heymann nephritis immune complexes are formed in situ, which may be a special situation. We studied the pathogenetic role of C, PMN and M in another model of in situ ICGN employing a cationic antigen(Batsford et al.,Clin.Neph., 15,211,1980). In brief the left kidneys of Wistar rats were perfused with 40ug of cationized HuIgG followed by an i.v.injection of rabbit anti-HuIgG one hour later. The following groups were studied:1) No additional treatment 2) C3 depleted(Cobra Venom Factor) 3) PMN depleted(anti PMN antiserum) 4) M depleted (anti M antiserum). The amount of Ag. deposited in the left kidney was the same in all groups examined, as revealed by studies with radiolabeled, cationized HuIgG. Proteinuria reached 100mg/24h in group 1 on day two, rising to 160mg/24h on day five, but did not exceed 20mg/24h until day 6 after C depletion, 40mg/24h until day 5 after PMN depletion and 40mg/24h until day 5 after M depletion; these differences were statistically significant. This shows that C,PMN and M are capable of increasing glomerular permeability and moreover all 3 mediators are required for full expression of the lesion. A cellular(PMN,M)component is involved in the in situ ICGN model described here, in contrast to the situation in passive Heymann nephritis.

SEGMENTAL NECROTIZING GLOMERULONEPHRITIS (SNGN): PATHOLOGICAL FEATURES AND CLINICAL SIGNIFICANCE. Mark A. Weiss and John D. Crissman*, Depts. of Pathology, Univ. of Cincinnati, Cincinnati, Ohio and Harper-Grace Hospital, Detroit, Michigan.

Forty-eight patients with SNGN were studied to evaluate pathogenesis, prognosis, and significance of associated intra-renal vasculitis (IRV). Immunofluorescence (FM) suggested immune-complex (IC) injury in 40% of bx's; however, both IgG and C3 were absent in 38%. EM demonstrated ill-defined dense deposits in only 18% of bx's. By contrast, glomerular thrombosis was identified by LM in 73% of bx's, fibrin was present by FM in 69% of bx's, and EM demonstrated intracapillary platelet activation/fibrin in 30% of bx's. Crescents, although present in 43 bx's, involved >60% of glomeruli ("RPGN") in only 14 bx's (30%). Chronic renal failure, however, ensued in 65% of patients, despite aggressive therapy with steroids with or without alkylating agents or anticoagulants. To examine the significance of IRV with SNGN, patients were consolidated into clinicopathological groups:

SNGN	No. Pts. (48)	Wegener's Granulomatosis 13 pts. (27%)		Systemic Vasculitis 15 pts. (31%)		? Primary 20 pts.(42%)
		Documented	Suspected	Documented	Suspected	
IRV absent	33	9	3	1	4	16
IRV present	15	0	1	7	3	4

In summary, SNGN is primarily related to coagulation rather than IC injury, has a poor prognosis independent of % of crescents and mode of therapy, and may occur as a primary form unrelated to Wegener's Granulomatosis or systemic vasculitis. IRV is a useful predictor of systemic vasculitis, but may be limited to the kidney, and is uncommon in Wegener's Granulomatosis.

CORRELATION BETWEEN HYPOTRANSFERRINEMIA AND HYPOGAMMAGLOBULINEMIA IN CHILDHOOD NEPHROTIC SYNDROME. B.L. Warshaw, L.C. Hymes,* and I.J. Check.* Depts. of Pediatrics and Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia.

Transferrin has recently been shown to regulate lymphocyte proliferation. Since children with nephrotic syndrome have large urinary transferrin losses, we investigated whether low levels of serum transferrin in these children could contribute to depression of their IgG. In 15 children with nephrotic syndrome (11 in relapse, 4 in remission) and 5 with other renal disorders, we analyzed proteins in parallel serum and urine samples by electrophoresis on high resolution agarose gels and measured serum levels of transferrin by rate nephelometry. We found decreased levels of serum transferrin in 10 of 11 patients with nephrotic syndrome in relapse (mean ± s.e.m. = 142 ± 17.5, range 56-259 mg/dl), and normal levels in all other patients (277 ± 13.7, range 217-317 mg/dl, $p < .001$). In the serum, transferrin levels correlated with total protein ($r = .87$, $p < .001$), albumin ($r = .91$, $p < .001$), and gamma globulin ($r = .78$, $p < .001$). Nine of the 10 patients with low serum transferrin levels, but only 2 of the 10 with normal levels had decreased serum gamma globulin (<550 mg/dl, chi square = 9.89, $p < .002$). Electrophoretic analyses showed minimal urinary losses of gamma globulin. These data raise the possibility that the unexplained decrease in IgG levels in children with nephrotic syndrome is related to the effects of hypotransferrinemia on lymphocyte function.

NATURAL KILLING (NK) AND ANTIBODY DEPENDENT CELL MEDIATED CYTOTOXICITY (ADCC) IN UREMICS. Hugh H. Williams, T. J. Yoo, Univ. of Tennessee, Div. of Nephrology and Immunology. Memphis, Tennessee.

Cell mediated immunity is suppressed in uremics. The activity of the natural killer cell, which probably also mediates ADCC, was tested in normals and uremics, for NK activity against K562, a human myeloid leukemia cell line. Four hour chromium 51 release assays were used. Washed lymphocyte rich cell populations from normals (Nc) and uremics (Uc) were suspended in standard media (SM), in plasma from normals (NP), in uremic plasma pre dialysis (UP pre) and in uremic plasma post dialysis (UP post). Similar NK and ADCC activities of 50-60% killing were seen in 23 Nc and 23 Uc with SM as the diluent. Suspension of Nc or Uc in NP, instead of SM, reduced NK activity by less than 10% ($p > .05$). UP pre and UP post, inhibited NK activity for Nc and Uc, as compared to SM ($p < .001$).

Cells/Medium	No. Tested	BUN in Medium	Percent inhibition of NK
Nc + NP	17	14	-3
Nc + UP pre	15	62	37
Nc + UP post	16	27	37
Uc + NP	10	13	8
Uc + UP pre	57	73	29
Uc + UP post	60	31	24

Nc were more sensitive than UC to this inhibition ($p < .001$). There was no significant difference in NK inhibition between UP pre and UP post ($p > .1$). This suggests that in the in vivo uremic state, NK activity is probably inhibited. The inhibitor(s) was (were) not dialyzable and could contribute to decreased immune surveillance in patients with end stage kidney disease.

Pathophysiology of Acute Renal Failure

PENICILLIN INDUCED HEMORRHAGE-A COMMON COMPLICATION OF ACUTE RENAL FAILURE

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Bleeding complications (GI and wound bleeding, epistaxis) are common in renal failure. The role of penicillin (P) or P derivatives in the genesis of bleeding episodes in ARF was analyzed. Among 60 consecutive ARF pat., clinically important bleeding was observed in 19. Trough P (deriv.) levels (5-50 µg/ml) were found by measurement with standard bact. tests in 11 (=60%). More severe bleeding (nasal > gastric > colonic and wound bleeding) was seen in P treated ARF pat. To further delineate the mechanism of P induced hemostasis defects, thrombocyte function was analyzed in 10 pat. (normal GFR) with 2x20 Mio P/d (trough levels 28.8, range 1-60µg/ml) and pat. with renal failure given P in recommended dose adjusted for GFR. Consistent prolongation of bleeding time (Ivy) > 30 min, elevation of platelet basal and PGE_1 (10^{-6}M) stimulated cAMP (basal 114±21 pmol/10^9 pl.; day 7 167± 38) and reduced platelet aggregation with ADP (ADP day 0 40-13.6% MA; day 7 20.7-7.3), epinephrin and collagen were observed. At such P concentrations, no impairment of plasmatic coagulation was observed.

Conclusion: In uremic patients, P in recommended doses at trough levels ~ 20 µg/ml causes clinically relevant impairment of platelet function. Uncontrolled retrospective analysis suggests a major role of P in bleeding of ARF patients.

CALCIUM MEDIATES THROMBOXANE (TX) SYNTHESIS IN THE UNILATERAL URETERAL OBSTRUCTION (UUO) MODEL IN RATS.

J.D. Baker,* P.E. Klotman, J. Boatman,* and W.E. Yarger. Dept. of Med., Duke Univ., Durham, NC

Peptide-stimulated prostanoid synthesis contributes to renal vasoconstriction following the release of 24 h of UUO in the rat. We explored the role of Ca antagonists in vivo and in the ex vivo perfused kidney. With a flowprobe on the left renal artery (LRA), we measured the hemodynamic response after 24 h of UUO to the LRA infusion of either the Ca antagonist diltiazem (D) 100 µg/min alone, bradykinin (BK) 1 µg/kg/min alone, or the combination of D+BK. BK decreased RBF 38%, D increased RBF 10%, and the addition of D to BK restored RBF to its baseline value.

In order to evaluate Ca's effect on TXA_2 synthesis, we perfused post-UUO kidneys ex vivo with 37°C Krebs Henseleit (2.5 mM Ca) at 10 ml/min. We measured TXB_2 in the venous effluent by RIA before and 4 h after q 30 min stimulation with 1 µg boluses of BK. In other groups, we infused the Ca antagonist verapamil (V) 150 µg/min or the Ca-calmodulin (CMD) inhibitors trifluoperazine (TFP) .12 mg/min or 1 M urea.

Ex vivo kidney	TXB_2 pg/min	
	Control	4 Hours
BK	134	597
BK + V	107	199
BK + TFP	118	212
BK + 1 M Urea	195	133

BK stimulated TXB_2 production in the UUO. V, TFP, and urea inhibited this peptide-stimulated thromboxane synthesis. We conclude that Ca channel blockers and inhibitors of the CMD complex improve RBF after UUO by blocking peptide-stimulated TXA_2 production.

PREVENTING POSTISCHEMIC ACUTE RENAL FAILURE

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Recently we reported that flushing and storing kidneys in certain phosphate buffered sucrose solutions (PBSucrose) will protect the morphological integrity of cold stored kidneys significantly better than other clinically popular cold storage solutions (Andrews and Coffey, Lab. Invest. 46:100-120, 1982). In this investigation, we evaluated the ability of our solution to maintain the function and morphological integrity of kidneys which have been subjected to 1 hour of normothermic ischemia. Our PBSucrose solution is isotonic and consists of 1.45g/L NaH2PO4, 4.94g/L Na2HPO4, and 68.3g/L sucrose. This solution was compared with the clinically popular Collins preservation solution. The left kidneys of pentobarbital sodium anesthetized Sprague-Dawley rats (225 gms.) were flushed in situ with either our PBSucrose or Collins preservation solutions. Flushing was accomplished by retrograde perfusion through a needle inserted into the abdominal aorta just below the level of the renal arteries. Immediately after the kidneys blanched, the left renal vessels were clamped. After one hour of ischemia, the renal vessel clamp was removed and a contralateral nephrectomy was performed. Control rats were subjected to 1 hour of ischemia plus contralateral nephrectomies, or unilateral nephrectomies without ischemia. After 48 hours of recovery, serum creatinine levels (mg/dL) were as follows (Mean±SD): Control Nonischemic(N5) 0.92±0.25; Control Ischemic(N6) 7.77±0.72; PBSucrose Flushed(N10) 2.37± 1.69; Collins Flushed(N6) 8.81±1.39. Histological examination revealed minor damage to the PBSucrose kidneys while the Collins flushed kidneys and nonflushed ischemic kidneys revealed luminal casts and extensive damage to the thick segments of Henle's loop. These results indicate that our PBSucrose solution can effectively reduce the ARF associated with ischemia.

ENHANCEMENT OF GENTAMICIN (G) NEPHROTOXICITY BY DIETARY SODIUM DEPRIVATION.

W.M. Bennett, D.C. Houghton, W.C. Elliott, D.N. Gilbert.* Oregon Health Sciences University, Portland, Oregon.

Dietary deficiencies of Na, K, Mg and Ca have all been reported to increase the risk of G nephrotoxicity in rats. With all other nutrients held constant, groups of male Fischer rats were placed on 2 weeks of either .1%, .35% or 1% sodium diet prior to 14 days of G, 40 mg/kg administered b.i.d. Prior to G, serum concentrations and urinary excretion of Ca and K did not differ between groups. Results of renal functional data revealed:

	.1% Na		.35% Na		1% Na	
RxDay	BUN mg/dl	Cr mg/dl	BUN mg/dl	Cr mg/dl	BUN mg/dl	Cr mg/dl
3	44	.75	39	.7	30	.6
7	201	8.1	120	5.6	34	1.3
10	435*	11.9	201	7.0	177	6.5
14	387*	11.5	282†	7.5	160	3.9

*3 animals died of uremia, †4 animals died of uremia. n=6 at all time points.

Statistically, .1% Na and .35% Na had worse renal failure than 1% animals at all time points beyond 3 days ($p<.01$). Cortical G peaked earlier (3 days) but no higher in .1% animals. Histologic damage by light microscopy and renal slice uptake of PAH paralleled functional deterioration in degree and severity.

Conclusion: Dietary sodium intake is an important risk factor in experimental G nephrotoxicity. Controls for Na and other dietary cations are essential for interpretation of studies using G as a model for acute renal failure.

REVERSIBLE POLYCATION-INDUCED PROTEINURIA. C.R. Bridges,* H.G. Rennke, W.M. Deen, J.L. Troy,* and B.M. Brenner, Depts. of Medicine and Pathology, Harvard Medical School, Boston, MA, and Dept. of Chemical Engineering, M.I.T., Cambridge, MA.

Female Munich-Wistar rats weighing ~175g received 126-420 μg/kg/min of hexadimethrine (HDM) i.v. for up to 30 min, until the onset of 2+ proteinuria (PEAK). Twelve rats (HH) received a 300 IU heparin bolus immediately after the PEAK period, while six rats (H) did not.

GROUP	PERIOD	UV/GFR(μg/ml) Albumin	IgG	GFR (ml/min)	RPF	$\Theta_{54Å}$
H	INITIAL	3	<2	0.47	1.57	----
H	PEAK	1021*	94	0.35	0.89*	----
H	RECOVERY	292*	35*	0.14*	0.55*	----
HH	INITIAL	5	<2	0.47	1.40	0.005
HH	PEAK	996*	195*	0.30*	0.81*	0.018*
HH	RECOVERY	18+	<2	0.46+	1.43+	0.005

* $p<0.05$ vs. INITIAL period of same group, paired
+ $p<0.05$ vs. same period in GROUP H, unpaired

Morphologic studies during the PEAK period in both groups showed that extensive intraglomerular microthrombosis, obliteration of foot processes, and disruption of filtration slit diaphragms was associated with a reduction in GFR and RPF and with non-selective proteinuria. One hour after the PEAK period (RECOVERY) the GFR and proteinuria returned to normal in group HH but not in H. Recovery in HH rats was associated with deposition of HDM-heparin complexes in the basement membrane. In HH rats neutral dextran fractional clearances (Θ) for radii >38Å were elevated above INITIAL (n=4) during the PEAK period (n=6) ($p<0.05$), but not during RECOVERY (n=3). The changes in Θ with HDM can be attributed to a reversible increase in glomerular pore dimensions.

ABSENCE OF GLOMERULAR ENDOTHELIAL PORE SIZE DIFFERENCES IN ACUTE RENAL FAILURE (ARF) IN RATS INDUCED BY MERCURIC CHLORIDE ($HgCl_2$) OR GENTAMICIN (G). R.E.Bulger, D.C.Dobyan, and G.Eknoyan. Dept. of Path., Univ. of Texas Health Science Center and Renal Section, Baylor Coll.Med., Houston, Texas.

Decreases in glomerular capillary endothelial pore size and density have been reported in different models of ARF. Using extensive morphometric analysis we quantitated the endothelial luminal surface characteristics in rats subjected to either $HgCl_2$ (2mg/kgBW) or G (40mg/kgBW BID for 8-9 days) induced ARF. GFR in μl/min/gm Kid.W was 548± 64 at 6 hr $HgCl_2$, 134±64 at 24hr $HgCl_2$ and 102±40 μl/min after G. The % of endothelial surface occupied by pores or ridges and unpored areas was:

	Pores	Ridges	No Pores
Control	53.6 ± 2.7	31.2 ± 1.5	15.5 ± 4.0
6hr $HgCl_2$	50.9 ± 1.9	29.8 ± 1.9	19.3 ± 2.0
24hr $HgCl_2$	53.9 ± 5.7	30.6 ± 3.1	15.6 ± 4.3
Gentamicin	56.7 ± 2.4	26.5 ± 1.5	16.9 ± 2.3

The pore area (nm^2), pore density ($\#/\mu m^2$) and total pore area/total area measured (PA/TA) in % were:

	Pored Area	Pore Density	PA/TA
Control	1494 ± 75	80.7 ± 4.1	12.8 ± 0.8
6hr $HgCl_2$	1326 ± 48	84.9 ± 2.4	11.2 ± 0.7
24hr $HgCl_2$	1559 ± 130	72.8 ± 2.7	10.9 ± 0.8
Gentamicin	1340 ± 101	87.3 ± 2.6	10.9 ± 0.7

No significant differences were present between the controls and the experimental groups in any of the parameters measured. A larger variation in pore size, however, was seen after G. In contrast to other studies using less rigorous methods, we conclude that when thorough quantitation is employed, no significant changes in glomerular endothelial pore characteristics are noted in either model of ARF.

EFFECT OF SALT AND FUROSEMIDE(F) ON GENTAMICIN(G)-INDUCED ARF IN THE RAT. Felix P.Brunner,* and Gilbert Thiel,* (intr.by F.C.Rector,jr.), Depart. Int.Med., University of Basel, Switzerland.

In contrast to other types of ARF, G toxicity appeared not to be reduced by salt loading (Bennett et al, Proc.Soc.Exp.Biol.Med.151:736,1976) and was aggravated morphologically by F(De Rougemont et al, Nephron 29:176,1981). Hence we studied these effects in a model where a single injection of G induces ARF provided the rats receive no food and drink 5% glucose containing 2 to 10 mEq/l NaCl. 50 female Wistar rats kept in metabolic cages on NaCl 10 mEq/l 5% glucose were injected s.c. with G 200 mg/kg. 24 h later, they were divided into 5 groups, implanted with osmotic micropumps (empty or loaded with F) and changed to NaCl 30 or 100 mEq/l 5% glucose for drinking. Serum creat.and weight loss 96 h after G as well as the number of rats that died within 4 to 7 days after G are shown below.

	gr.1)	gr.2)	gr.3)	gr.4)	gr.5)
NaCl mEq/l	10	30	100	100	100
F mg/kg/d	0	0	0	12-20	25-40
Scr mg%±SD	1.3±0.9	1.0±0.3	0.6±0.3	1.8±1.1	2.9±1.7
%w.loss±SD	11±6	10±3	5±4	14±4	16±6
died (x/10)	2	0	0	3	7

Thus NaCl loading, even when started 24 h after G intoxication, afforded protection against ARF. Drinking NaCl 100 mEq/l together with F was not protective or aggravated G-induced ARF. F could act by ECV depletion. However, since weight loss was similar in gr.2) compared to gr.4) but mean Scr higher and 3 rats dying in gr.4), we conclude that F may directly potentiate toxicity of G at the tubular cellular level.

HYPERCALCEMIA (HCa) AND ACUTE RENAL FAILURE (ARF): PROTECTION WITH ANGIOTENSIN II (AII) INHIBITION. T.J. Burke, M. Levi, P.E. Arnold*, T. Berl, and R. W. Schrier. Univ. Co. Hlth. Sci. Ctr., Den., Co.

Prostaglandin (PG) inhibition in HCa (Sca 12.9 mg/dl) rats resulted in ARF as RBF decreased from 6.4 to 4.1 ml/min/gkw, GFR decreased from 0.5 to 0.2 ml/min/gkw and FENa increased from 1.0 to 2.3%, all $p<.05$. The impairment in renal hemodynamics and tubular function was accompanied by significant alteration in mitochondrial respiration (MR, nmoles O_2/mg/min). State 3 respiration (S3, ADP stimulated oxidative phosphorylation) decreased from 110 to 26, state 4 respiration (S4, substrate induced respiration) decreased from 37 to 16, acceptor control ratio (ACR, S3/S4) decreased from 2.9 to 1.4 and uncoupled respiration (FCCP) decreased from 129 to 34. Mitochondrial calcium (MCa, nmoles Ca/mg) increased from 41 to 108, all $p<.05$. In these PG inhibited HCa rats prior or concomitant AII inhibition, using either an AII antagonist or a converting enzyme inhibitor protected against ARF and resulted in maintenance of all values at HCa levels, including RBF (6.0 vs 6.4), GFR (0.5 vs 0.5), FENa (1.2 vs 1.0), S3 (100 vs 110), S4 (37 vs 37), ACR (2.6 vs 2.9), FCCP (123 vs 129) and MCa (46 vs 41). Inhibition of PG synthesis in the HCa state may lead to ARF. The consequent renal hemodynamic, tubular and mitochondrial impairments are prevented by AII inhibition. The results therefore suggest that impairment of renal hemodynamics, tubular function and MR in this variety of ARF involves activation or enhancement of the renin-angiotensin system. The effect of AII on the renal vasculature may be enhanced in HCa in the absence of PG, thereby predisposing to disturbances of tubular function and MR.

STUDIES OF ISCHAEMIC HUMAN KIDNEYS BY 31_p NUCLEAR MAGNETIC RESONANCE (NMR). L. Chan.*(intr. by R.W. Schrier). Oxford University, CMR Laboratory, Biochem. Dept., Nuffield Dept. of Medicine & Surg.

Biochemical aspects of acute renal failure(ARF) in various rat models have been studied by ^{31}P NMR in vivo (Chan et al 1982). Validation of hypothesis derived from animal studies is important because of the differences in the pathological process of human post-ischaemic ARF. With the development of a wide-bore (20 cm) magnet, it is now possible to examine whole human kidney in vitro.

22 kidneys,removed from urological patients and perfused as if they were to be transplanted, were first examined in order to determine how these parameters obtained with NMR varied with increasing degrees of ischaemic damage. In contrast to earlier results with kidneys of small animals(rats & rabbits) in which ATP signal disappears rapidly after the onset of ischaemia, the human kidney retains some ATP for up to 12 hours in cold. The pH falls as anticipated reaching a value of 6.5 after 24 hours. Studies have also been made of human kidneys that were to be transplanted. Early graft function and the need for dialysis in the first week after transplantation were chosen as criteria of degree of ischaemic damage. The NMR result(in 40 kidneys) is in accordance with the finding in nontransplanted kidneys and may provide new impetus to the study of organ preservation

Whole body magnets for ^{31}P NMR study are expected to become available soon. Extensions of study to human kidneys in vivo (eg transplant kidneys) may provide a unique approach to the study and diagnosis of ARF in man.

Chan et al (1982):In Acute Renal Failure.p 35-41, ed H.E. Eliahou. London:John Libbey.

MITOCHONDRIAL CALCIUM TRANSPORT BY NORMAL RENAL CORTICAL MITOCHONDRIA DURING THEIR ISOLATION. Mary Clark,* Joel M. Weinberg,and H. David Humes. VA Med. Ctr. and Univ. of Mich., Ann Arbor, MI.

Renal cortical mitochondria (RCM) like those from other tissues avidly transport available calcium into their matrices. This process may be an important component of intracellular Ca regulation and the Ca transporting capacity and content of isolated RCM may provide valuable insights into cellular Ca homeostasis in vivo. However, RCM may gain or lose Ca during their preparation. To evaluate the extent to which such pertubations occur, RCM from normal rats were isolated from kidney cortex in homogenizing medium containing the usual levels of Ca (0.15-0.25 mM) and graded excesses of added Ca (up to 4 mM). Four different homogenizing mediums were tested: plain 275 mM sucrose (S), sucrose-1 mM EGTA (S-E), sucrose-20 μM ruthenium red(S-RR) and sucrose-1 mM EGTA-20 μM RR (S-E-RR). When isolated in S, normal RCM avidly took up Ca during preparation at all ambient Ca levels. In S-E,Ca uptake only occurred when medium Ca exceeded 0.6 mM. Results of isolation in S-RR and S-E-RR demonstrated that RR completely blocked Ca uptake but not a relatively small element of superficial Ca binding. These data establish that, during their isolation, in spite of low temperatures and limited exogenous substrate,large amounts of mitochondrial Ca uptake may occur. However, by comparing appropriately chosen isolation conditions Ca uptake and superficial binding during isolation can be readily distinguished from the endogenous Ca pool. Furthermore, Ca loss from undamaged RCM during isolation in S-E is unlikely given the favorable conditions for Ca uptake demonstrated in this study.

ABNORMAL MUSCLE PROTEIN TURNOVER AND GLUCOSE UPTAKE IN ACUTE RENAL FAILURE (ARF). A.S. Clark* and W.E. Mitch. Harvard Medical School and Brigham and Women's Hospital, Boston, Mass.

ARF in rats increases amino acid release from muscle and induces insulin (I) resistance. To study if net protein turnover and abnormal glucose metabolism were linked, we measured net tyrosine release (T, nmol/g/h), glucose uptake (GU) and release of the products of its anaerobic metabolism, lactate (LR) and alanine (AR), from perfused hindquarters of ARF and sham-operated (SO) rats. Basal T rose 40% after 24h and 98% after 48h of ARF. Responses to I at 48h (Table) also were present at 24h. *, $p<.02$; †, $p<.01$ compared to SO.

		T	GU	LR	AR	LR/GU
		(nmol/h/g)		(μmol/g/h)		Ratio
SO	-I	55±3	6.4±.5	4.6±.4	.74±.06	.66±.04
	+I	20±4	13.7±.7	5.3±.5	.14±.04	.41±.06
ARF	-I	109±12†	6.8±.4	6.3±.3†	.85±.05	.95±.03†
	+I	84±8†	10.9±.7*	7.5±.6†	.56±.06†	.75±.07†

Both increased T and impaired glucose utilization were less responsive to I in ARF. Protein synthesis (PS) and degradation (PD) were measured in epitrochlearis muscles to determine which causes the increase in T. After 24h, the increase in T was due to increased PD ($p<.001$) and the effect of I on PD was blunted (-10%, ARF vs -23%, SO; $p<.02$); PS was unchanged. At 48h, PS also was abnormal; it fell 25% even with I ($p<.01$). T was correlated with inefficient glucose utilization estimated as the LR/GU ratio ($r=+0.78$; $p<.001$). Thus, there are 4 defects in muscle metabolism in ARF: after 24h, there is resistance to I-stimulated GU, and increased LR and PD. After 48h, PS becomes depressed also. Inefficient glucose utilization may contribute to the high T occurring in ARF.

STUDY OF ISOLATED NEPHRON SEGMENTS IN A RABBIT MODEL OF DIABETES MELLITUS. K. Davidson* and M.J. Hanley. Univ. of Tex. Hlth. Sci. Ctr. at San Antonio, Texas

Insulin deficiency has been found to affect renal hemodynamics and to alter the response of glomerular capillaries to hormonal and pressor agents. To determine the effect of insulin deficiency on tubular function we have examined nephron segments from rabbits two weeks after they have been made hyperglycemic (> 400mg%) and glycosuric by the administration of alloxan (125 mg/kilo). Segments of proximal straight tubule (PST) from alloxan (A) rabbits perfused and bathed with standard artificial ultrafiltrate (SAU) showed normal fluid reabsorption (J_V) (J_V = 0.40 + 0.06 N=4) and a 60% inhibition of J_V with the addition of dopamine to the bath. In PST's from A rabbits perfused and bathed with a high glucose artificial ultrafiltrate (HGAU) (glucose concentration = 450 mg%), J_V was reduced (J_V = 0.17 + 0.05) and no significant dopamine inhibition of J_V was observed (J_V = 0.21 + 0.05 N.S., N=6). To assess whether this effect was due to the high glucose medium PST's from normal rabbits were perfused and bathed with HGAU. Control J_V was reduced (J_V = 0.08 + 0.02) and no significant dopamine inhibition of J_V was observed (J_V = 0.11 + 0.02 (N=4), NS). When HGAU perfusate and bath was replaced with SAU perfusate and bath in these tubules J_V was normalized (J_V = 0.38 + 0.03). From these results we conclude that 1) Insulin deficiency of short duration does not alter intrinsic PST transport or its response to dopamine 2) The hyperglycemia which results from insulin deficiency is capable of reducing PST fluid reabsorption and abolishing the response to dopamine 3) The polyuria of uncontrolled diabetes mellitus may be due in part to intrinsic alterations in proximal nephron function induced by hyperglycemia.

FUNCTIONAL AND MORPHOLOGICAL CORRELATES OF PROTECTION FROM GENTAMICIN (G) NEPHROTOXICITY BY ORAL CLONIDINE IN THE RAT. G. Eknoyan, H.O.Senekjian, R.E.Bulger and D.C. Dobyan. Baylor Coll. Med. and Univ. of Texas Med. School, Houston, Texas.

Clonidine (C) reduces the severity of ischemic and mercuric chloride induced acute renal failure. To determine the effect of oral C on the nephrotoxicity of G (40mg/kg BID for 8-9 days) metabolic cage studies were performed on two groups of 6 Sprague-Dawley rats each. For 5 days before G and throughout the study, one group (W) drank tap water while the other (C) had clonidine (5 mg/L) added to the drinking water. Compared to W, the C rats had a lower serum creatinine (2.3±.8 vs 5.0±.6 mg/dl, p<.05), higher creatinine clearance (347±103 vs 102±39 µl/min, p<.05) and lower fractional excretion of sodium (2.0±.7% vs 5.5±1.1%, p<.05). Urine osmolality fell progressively in both groups, but the fall was greater in the W group and by day 4 post-G the difference between C and W urine osmolality was significant. Varying degrees of cell injury were present in the proximal convoluted tubules (PC) and proximal pars recta (PR) of both groups, but C treated rats had a lesser degree of damage. In the outer cortex, the most severely injured region, C rats had fewer necrotic cells in the PC (55±8% vs 79±3%, p<.025) and in the PR (5±2% vs 43±10%, p<.02). In the inner cortex, necrosis was also less in C rats in PC (29±2% vs 60±7%, p<.01) and in PR (0.6±.3% vs 13±6%, p<.01). In the outer stripe of the medulla, the least affected region, C rats had fewer necrotic cells (2±.7% vs 6±1%, p<.05). Thus, clonidine provides partial functional and morphological protection from G nephrotoxicity.

ATTENUATION OF TUBULAR INJURY: IMPORTANCE OF THE REFLOW PERIOD FOLLOWING TEMPORARY RENAL ISCHEMIA. E. Fernandez-Repollet,* W.F. Finn, D. Goldfarb,* A. Iaina,* and H.E. Eliahou,* Univ. of N. Carolina Chapel Hill, N.C. and Chaim Sheba Medical Center, Tel-Hashomer, Israel.

Following unilateral renal artery occlusion (RAO) reflow of blood to the postischemic kidney is more complete and the secondary period of vasoconstriction does not occur if the contralateral kidney (RK) has been removed. To determine if reflow influences the severity of the injury, studies were performed at Tel-Hashomer (TH) and Chapel Hill (CH) 24 hrs after 60 min RAO in rats with (Gp I) or without (Gp II) the RK in place.

At TH, creatinine clearance (C_{Cr}), urine flow rate (V) and fractional sodium excretion (FE_{Na}) were measured. At CH, glomerular capillary hydrostatic pressure (P_{GCe}) was estimated and total (R_T) and preglomerular vascular resistances (R_{PG}) were calculated. Results expressed per 100 g BW are ± SEM. *denotes p <0.05 Gp I vs Gp II.

	Control	Group I	Group II
C_{Cr}, µl/min	354±46	96±42*	311±53
V, µl/min	4.9±1.7	14.7±5.7*	27.5±4.2
FE_{Na}, %	0.8±0.3	21.5±2.0*	13.2±4.1
P_{GCe}, mm Hg	51.8±1.3	28.0±1.8*	41.8±1.6
R_T, 10^5dyn·s/cm^5	35.2±2.6	64.5±6.1*	36.0±2.6
R_{PG}, 10^5dyn·s/cm^5	20.1±1.8	49.3±5.0*	24.2±0.2

In addition, light and electron micrographs demonstrated marked attenuation of the tubular epithelial cell damage in the Gp II animals.

Thus, substantive differences exist in the response to ischemia which depend upon the presence or absence of the contralateral kidney. The degree of reflow is an important factor in the ultimate amount of anatomical and functional injury.

STREPTOZOTOCIN-INDUCED DIABETES (SID) MODIFIES BUT DOES NOT PREVENT GENTAMICIN (G) NEPHROTOXICITY IN MALE RATS USING INULIN CLEARANCE (CIN) TO MEASURE GLOMERULAR FILTRATION RATE (GFR). W.C. Elliott, D.C. Houghton, D.N. Gilbert*, J. Hunter-Baines*, W.M. Bennett. Oregon Health Sciences University, Portland, Oregon.

SID reportedly confers complete protection from experimental G nephrotoxicity (KI 21:600, 1982). Because female rats are less susceptibile to G and to exclude artifactual increases of C_{Cr} due to high urine flow in SID, male Fischer rats with SID or isosorbide (I) induced osmotic diuresis received G 40 mg/kg in divided doses for 7 to 21 days. C_{In} and C_{Cr} (ml/min/100 g), cortical G concentration [G] in µg/gm and histology were assessed. Results: n= or >6 at each point.

	Baseline		7		14		17		21	
	SID	I	SID	I	SID	I	SID	I	SID	I
C_{Cr}	.53	.52	.53	.47	.53§	.26*†	.93§	.50*	.55§	.42
C_{In}	.69	.86	.47	.66	.19†	.11*†	.19†	.37*†	.18†	.48*
[G]	-	-	297	806*	584	333*	664	587	656	498

* p<.05 vs SID;† p<.01 vs baseline;§ p<.01 vs C_{In}.

Proximal tubular necrosis occurred in both groups but was worse in I with maximum damage on day 14. Untreated SID animals had proximal tubular vacuolar changes not evident in untreated I. In SID the marked increase in urine flow correlated directly with C_{Cr} (r=.56,p<.001). Osmolar clearances in SID and I were similar.

Conclusions: 1) SID in male rats modifies but does not prevent G nephrotoxicity if C_{In} is used to measure GFR, 2) In SID overestimation of GFR by C_{Cr} is most apparent at low GFR.

STUDIES ON THE PROTECTIVE EFFECT OF CHRONIC UNINEPHRECTOMY IN RENAL ISCHEMIA IN THE RAT. Terrance Fried*, Akira Hishida*, and J. H. Stein. Univ. of Tex. Hlth. Sci. Ctr. San Antonio, TX.

It has been suggested that prior removal of the contralateral kidney attenuates functional impairment after renal ischemia in the rat. In order to investigate this possibility initial studies were performed in two week sham nephrectomized (SHAM) and two week uninephrectomized (UNI) rats 3 and 48 hours after 40 minutes of left renal artery clamping (RAC). There was no significant difference at 3 hours but by 48 hours the inulin clearance (C_{IN}) was 11 ± 6 % (mean ± SE) of preclamped values in SHAM rats and 58 ± 15 % in UNI rats p< 0.01 vs. SHAM. This protective effect may have been related to either 1) some consequence of renal hypertrophy per se or 2) the different environments present in UNI versus SHAM animals. In order to differentiate these two possibilities three further groups were studied: I. Bilateral renal artery clamp (BRAC): both renal arteries were clamped for 40 minutes (N=6) II. Acute uninephrectomy (AUN): Right nephrectomy performed at the time of RAC (N=5). III. Ureterovenostomy (UV): Right ureterovenostomy created at the time of RAC (N=7). Despite the absence of hypertrophy, the C_{IN} in all three groups at 48 hours was significantly greater than in SHAM rats and equivalent to values found in UNI rats, (BRAC: 37 ± 4, AUN: 56 ± 9, UV: 49 ± 5% of initial values, p< 0.005 vs. SHAM). Each of these latter models was also associated with the development of azotemia. These studies indicate that UNI enhances the recovery of C_{IN} 48 hours after RAC. Further, this enhanced recovery appears to be the consequence of some environmental alteration which occurs when all excretory renal tissue is made ischemic.

EXPERIMENTAL STUDIES OF NEPHROTOXICITY BY ADMINISTERING LOCALLY CIS-DIAMMINEDICHLOROPLATINUM(CIS-DDP) TO NEPHRECTOMIZED RATS. Shinichi Hosokawa, Seigo Kohira,* and Tadao Tomoyoshi*. Shiga Medical Science, Department of Urology, Shiga, Japan

The nephrotoxicity of cis-DDP were studied in rats performed left nephrectomy to examine the effectivness to unilateral kidney treating by cis-DDP. We have examined the nephrotoxicity by administering cis-DDP, 1.5mg/kirogram, every two days for five weeks, to the peritoneal cavity of five male nephrectomized Wistar rats with 220gram body weight in average. Body weights were measured everyday and blood urea nitrogen(BUN), serum creatinine, serum potassium(K), serum sodium(Na) and hematocrit(Hct) were examined at the end of the 5th week. After that all rats were examined by autopsy, and size and weight of both kidneys were measured. In the nephrectomized control group, body weight was 342±10(gm) and right kidney weight was 1.89±0.05g and right renal size was 2.0X1.1X1.1 cm and right kidney weight/body weight X 100 was 0.72±0.04. In experimental group, these values were 181±11g, 2.42±0.36g, 2.4X1.3X1.2cm, 1.34. There were significant differences between control group and experimental group. The values of BUN, serum creatinine, Hct, Na, K were 21.0±1.2mg/dl, 0.61±0.02mg/dl, 48±1.2%, 140±2mEq/L, 4.2±0.3mEq/L in control group and 186±70mg/dl, 3.8±0.05mg/dl, 24±1.6%, 152±4mEq/L, 6.9±0.5mEq/L in experimental group. There in statistical differences between control and experimental group. Histological examination showed edema and necrosis of the renal tubules in experimental rats. We concluded that acute renal failure and compensatory renal hypertrophy were caused by cis-DDP administering locally to nephrectomized rats.

RENAL CYCLIC NUCLEOTIDES, BETA-ADRENERGIC RECEPTORS (BRec) AND THE AMELIORATION OF GENTAMICIN (G) ACUTE RENAL FAILURE (ARF) BY VERAPAMIL (V) IN RATS A. Iaina,* K. Thurau,* I. Serban,* S. Kapuler,* S. Gavendo,* H.E. Eliahou,* Sheba Med. Ctr. Dept. of Nephrology, Israel. Introduced by WF Finn.

ARF induced by i.p. G, 120mg/kg/day for 8 days resulted in serum urea (S_u) 233±43mg% and creatinine (Scr) 3.7±0.4mg%.(Normals were 39±1.6 and 0.97±0.05, $p<0.005$). Treatment with V given in drinking water (10mg/dl) as follows: (GV-2): V started 2 days before G. (GV): V started with G. (GV+6): V started on the 6th day of G. V continued to the end. Only the GV-2 rats developed milder ARF (S_u=119±9, Scr=2±0.14, $p<0.005$; their GFR= 239±85 ul/min compared to 80±5 in untreated G.ARF)

	BASAL & STIMUL. CYCLASE ACTIVITY			cAMP**
Groups	Basal*	NaF#	Isoprot.#	
CONTROL	17±2.5	416±43	126±4	686±87
G.ARF	21±2.8	399±62	138±11	459±40
GV-2	23±3.4	312±24	125±4	490±93
GV	15±2.5	464±71	118±10	515±52
GV+6	17±2	516±76	126±3	506-61

*pMol/m/mg membr. prot.**pMol/g cortex.#%of basal.

Analysis of variance showed no differences between groups. cAMP in all the G-treated rats (pooled) was (494±30),i.e. lower ($p<0.02$) than in controls, (686±87). BRec density (fMol/mg prot.) & K_D(nM) were respectively in controls:124 & 12; G.ARF:116 & 11; GV-2:76 & 9; GV:161 & 6; GV+6:129+6. Conclusions: Tubular plasma cell membranes BRec do not seem torespond to this therapeutic maneuvre. The alleviation of G.ARF in rats by V is neither through the renal sympathetic system activity nor through renal cyclic necleotides.

IS CALCIUM A COMPETITIVE INHIBITOR OF THE GENTAMICIN (G) RENAL MEMBRANE RECEPTOR INTERACTION WHICH AMELIORATES G NEPHROTOXICITY? H. David Humes, M. Sastrasinh,* and Joel M. Weinberg. VA Med. Ctr. and Univ. of Michigan, Ann Arbor, Michigan.

Dietary $CaCO_3$ loading has been reported to reduce experimental G nephrotoxicity(Kid.Int.21:216, 1982). To evaluate the mechanism for this effect, the ability of Ca to compete with G for membrane binding sites and the effects of oral $CaCO_3$ loading on BUN and renal cortical homogenate (RCH) Ca levels and mitochondrial State 3 respiration, biochemical parameters of renal tubular cell injury, were assessed. Ca was a competitive inhibitor of G binding to isolated renal brush border membranes with a K_i of 12 mM. For in vitro toxicity studies, rats received sc G in a single daily 100 mg/kg dose. Compared to simultaneously studied groups receiving G and a normal diet (ND+G), dietary supplementation with 4% $CaCO_3$ (Ca+G) ameliorated G nephrotoxicity. Data expressed as means±SE. n=5-8,*$p<.01$,†$p<.001$.

No. of G Doses	BUN (mg/dl)		State 3 (natom O/ min/mg prot)		RCH Ca (nmole/ mg prot)	
	ND+G	Ca+G	ND+G	Ca+G	ND+G	Ca+G
8	120±20	25±5*	136±14	224±20*	80±43	25±3
10	213±15	25±2†	111±7	190±5†	145±39	18±2*

This protective effect of Ca was observed in rats on both normal and Na deficient diets. However, dietary supplementation with NaCl or $NaHCO_3$ alone was also protective. Thus, $CaCO_3$ loading ameliorates G induced acute renal failure and renal tubular cell injury. Although these data suggest that Ca has a critical influence on the manner in which G interacts with renal membranes so as to ameliorate G nephrotoxicity, the protection afforded by $CaCO_3$ loading may be multifactorial in origin.

EFFECT OF d-PROPRANOLOL ON POST-ISCHEMIC ACUTE RENAL FAILURE. Masaaki Ishigami,* Nick Stowe, Philip A. Khairallah and Ray W. Gifford, Jr. Cleveland Clinic Foundation, Cleveland, Ohio 44106

The effectiveness of d-propranolol in preventing the reduction in glomerular filtration following a period of warm renal ischemia was studied in a rat model in which the left renal artery was occluded for 45 min and renal function measured in the immediate two hour post-ischemic period. Renal function of the kidneys subjected to ischemia was significantly enhanced by the prior administration of racemic propranolol (16 μg/kg/min), d-propranolol (8 μg/kg/min) and an analog of propranolol, pranolium chloride (8 μg/kg/min), but not by l-propranolol (8 μg/kg/min). In the untreated control group, glomerular filtration rate following ischemia returned to only 13% of its control value whereas with racemic propranolol it returned to 29%. The d-isomer of propranolol and pranolium chloride maintained glomerular filtration rate at 25% and 27% of its initial value respectively. This contrasted with the 10% recovery observed with l-propranolol. The salutary effects of racemic propranolol, d-propranolol and pranolium chloride was achieved without an increase in total renal blood flow. Since d-propranolol and pranolium chloride lack major beta-adrenergic receptor blocking actions, the beneficial effect observed with these drugs was mediated through properties unrelated to beta-adrenergic receptor blockade. The membrane stabilizing properties of d-propranolol and pranolium chloride may be an important factor in the salutary effect observed with these drugs in lessening the severity of post-ischemic acute renal failure.

EFFECT OF ISCHEMIA ON PROXIMAL TUBULE FLUID AND GLUCOSE REABSORPTION. Paul A. Johnston, Helmut Rennke, N.G. Levinsky, Boston Univ. Med. Ctr., University Hospital and Harvard Med. School, Boston Mass.

The effect of 35 minutes of ischemia on proximal tubule fluid (FR) and glucose reabsorption (GR) was assessed in rats using in vivo microperfusion. Measurements were made during the first hour (ER) and 2 and 4 hours (LR) after release of a renal artery clamp. Light and EM evaluation revealed 60-75% loss of proximal convoluted tubule brush border in ER, and essentially complete restoration in LR. No other tubule abnormalities were evident. Total renal blood flow was not significantly different from control during both ER and LR. During ER, FR was reduced to 29.8±5.2% of control ($p<0.001$). GR, at a normal load of 150 $pM.min^{-1}$ was reduced to 73.9±5.5% of control ($p<0.01$). During LR, FR remained depressed at 54.3±8.1% of control ($p<0.005$). GR was not different in ischemic and control tubules at glucose loads below 400$pM.min^{-1}$. However, at loads of 400-1,000 $pM.min^{-1}$, GR was significantly reduced by 23% ($p<0.001$) in ischemic tubules. Passive glucose flux, determined in the presence of 10^{-4}M phloridzin, was unaffected by ischemia.

We conclude that proximal tubule fluid (sodium) and, to a lesser extent, glucose reabsorption are decreased during the first hour after ischemia. This correlates with a significant loss of proximal tubule brush border. However, these transport defects persist even after the brush border has regenerated and proximal tubule cell morphology appears normal. Therefore, tubule structure and transport function may recover at different rates after ischemia.

CAPTOPRIL STIMULATES THROMBOXANE PRODUCTION AND EXACERBATES NEPHROTOXIC ACUTE RENAL FAILURE. P.E. Klotman, J. Boatman,* J.D. Baker,* and W.E. Yarger. Dept. of Med., Duke Univ., Durham, NC.

We previously demonstrated that captopril (C) exacerbates the toxicity of gentamicin (G) in K+ depleted rats. Indomethacin (In) blocked this effect. In order to explore the mechanism of C's toxicity and the protective effect of In, we examined 4 groups of K+ depleted rats. 24 h prior to study, all groups received G 100 mg/kg s.c. Groups 3 and 4 also received C 10 mg/kg s.c. and Groups 1 and 2 received C vehicle. Using an electromagnetic flowprobe placed on the left renal artery (LRA), we examined hemodynamic responses to the LRA infusion of C 10 mg/kg over 30 min, bradykinin (BK) 1 µg/kg/min, the kallikrein antagonist aprotinin (AP) 1000 KIU/kg/min, and the thromboxane synthetase inhibitor imidazole (IM) 5 µM/kg/min. Each animal served as its own control, receiving a 30 min LRA infusion of 0.9% NaCl to establish baseline RBF.

GROUP	TREATMENT	INFUSION	% CHANGE RBF
1	G	C	-50% ± 15%
2	G	BK	-58% ± 7%
3	G+C	AP	+35% ± 9%
4	G+C	IM	+73% ± 12%

Mean arterial blood pressure did not change more than 15mmHg in any group. C administration significantly reduced RBF. The administration of BK mimicked the changes observed with C. 24 h after G+C administration to K+ depleted rats, the LRA infusion of AP or IM significantly improved RBF. We conclude that the deleterious effect of C in this model is due to the inhibition of kininase II. The resulting increase in BK stimulates TXA_2 which produces a fall in RBF.

STOICHIOMETRY OF NETILMICIN BINDING TO PHOSPHATIDYLINOSITOL OF APICAL AND BASOLATERAL MEMBRANES. C. Josepovitz*, R. Levine*, T. Farruggella*, B. Lane and G.J. Kaloyanides, Departments of Medicine and Pathology, SUNY, Stony Brook, NY and VAMC, Northport, New York.

It has been postulated that the first step in the transport and accumulation of aminoglycosides (AG) by renal proximal tubular cells involves binding of AG to phosphatidylinositol (PI), the putative apical membrane (AM) receptor (R). The stoichiometry of AG/receptor binding has not been determined. To examine this question we measured the binding of ^{3}H-netilmicin (N) to isolated AM and BLM vesicles and determined the phospholipid (PL) composition of the membranes. AM and BLM vesicles from rat renal cortex were prepared by ultracentrifugation and percoll gradient techniques which yielded vesicles with purification factors of 14 and 12, respectively. Scatchard analysis of ^{3}H-N binding to AM and BLM revealed both membranes possess a single class of R for N with similar affinity constants ($Ka=0.013\pm0.003 \mu M^{-1}$ and 0.012 ± 0.002 μM^{-1}, N=5, respectively), but that the maximum binding of drug (B max) by BLM was higher than that by AM (48±5 vs 28±2 nmoles/mg membrane protein, $P<0.01$). Total PL of AM and BLM measured 623±30 and 910±20 nmoles PL-phosphorus/mg membrane protein ($P<0.01$) and PI measured 27±2 and 44±1 nmoles/mg membrane protein ($P<0.01$), respectively. The ratio Bmax/PI was 1.04 for AM and 1.09 for BLM. If PI is the membrane receptor for AG, these data indicate that the stoichiometry of AG/receptor binding is 1. The greater binding of N to BLM implicates a major role for BLM in the pathogenesis of AG nephrotoxicity.

SUBCELLULAR DISTRIBUTION OF THE ALTERATIONS IN RENAL CORTEX ACIDIC PHOSPHOLIPID (AP) CONTENT INDUCED BY GENTAMICIN (G). Thomas C. Knauss,* Joel M. Weinberg, and H. David Humes. VA Med. Ctr. and Univ. of Michigan, Ann Arbor, MI, and VA Med. Ctr., Cleveland, OH.

G preferentially binds to pure AP, binds to the AP of isolated renal brush border membranes, induces the development of increased levels of renal cortex AP after in vivo treatment and inhibits the in vitro interactions of phospholipases with AP. To ascertain whether the in vivo effects of G on renal tubular cell phospholipases and AP are limited to effects within lysosomes, the most prominent site of intracellular accumulation of G identified to date, or are present at additional subcellular sites, alterations in total phospholipid levels and in content of phosphatidylinositol (PhI) (µg lipid P_i/mg protein±SE), the AP most increased after G treatment, were determined in isolated mitochondria (M), brush border membrane vesicles (BBM) and a light membrane fraction consisting of endoplasmic reticulum and light lysosomes (ER + LYS) from control rats (C) and after 4 daily 100 mg/kg doses of G. Ns = 4-6.

	Total Phospholipid		PhI	
	C	G	C	G
ER+LYS	19.30±2.05	22.52±0.59	1.42±.17	2.03±.17*
BBM	19.60±1.13	19.70±0.58	.45±.03	.77±.03**
M	12.15±0.51	12.57±0.48	.28±.02	.46±.04**

*p <.05 **p <.001 vs control

These data suggest that the changes in renal cortical phospholipid content produced early during G treatment are not limited to lysosomes and suggest that G is available at multiple sites within the cell for interaction with subcellular components.

EVIDENCE THAT GENTAMICIN (G) IS NOT EXCLUSIVELY LOCALIZED TO LYSOSOMES (LYS). Carol Kreger,* Joel M. Weinberg, and H. David Humes. VA Medical Center and Univ. of Michigan, Ann Arbor, Michigan.

Although available data suggest that G in renal tubular cells preferentially localizes within LYS, this issue has not been critically assessed during protocols associated with G toxicity. We fractionated renal cortical homogenates (H) into cytosolic proteins (CP), mitochondria (M), endoplasmic reticulum + light LYS (ER + LYS), plasma membranes (PM) and heavy LYS (HL) and measured G content (μg/mg protein±SE) by RIA in kidneys from rats treated with G (100 mg/kg daily x 4) (in vivo) and H from control rat kidneys to which we added G during homogenization (in vitro) to levels similar to those found in vivo. Ns=3-5.

FRACTION	G Level-in vivo	G Level-in vitro
H	8.5±0.3	10.2±0.1
CP	2.0±0.1	5.2±0.2
ER + LYS	18.0±3.0	30.0±2.4
PM	4.4±0.3	6.2±0.7
M	4.1±0.5	5.2±0.2
HL	7.4±1.9	12.4±3.4

Except for HL which was greatly reduced in purity after in vivo G, all fractions showed comparable levels of purity in both groups. Relative to homogenates, G was enriched only in the ER + LYS fraction but degree of enrichment did not differ between in vivo and in vitro addition of G suggesting that the enrichment seen after in vivo treatment in the ER + LYS fraction cannot be unequivocally attributed only to high levels of G in light LYS but also reflects the availability of large numbers of acidic phospholipid binding sites for G on ER, binding sites which may play a significant role in the intracellular effects of G.

A POSSIBLE MECHANISM OF ACETAMINOPHEN-INDUCED RENAL CORTICAL NECROSIS. J.F. Newton, C-H. Kuo, D. Hoefle and J.B. Hook. Mich. St. Univ., Dept. Pharmacol./Toxicol., Ctr. for Environ. Toxicol., East Lansing, MI.

Acetaminophen (APAP) is an antipyretic analgesic, which in human overdose situations has produced hepatic centrilobular and renal cortical necrosis. In addition, APAP has been implicated as a causative agent in the chronic renal disease, analgesic nephropathy. Recently, we have demonstrated that p-aminophenol (PAP) is a urinary metabolite of APAP. PAP formation has also been demonstrated in isolated rat kidneys perfused with APAP. PAP is a potent, selective nephrotoxicant that produces a lesion histologically identical to that produced by APAP. Therefore, the mechanism of APAP-induced renal cortical necrosis may be related to the deacetylation of APAP to PAP. Bis(p-nitrophenyl)phosphate (BNPP) is an inhibitor of deacetylation. Therefore, experiments were designed to determine the effect of BNPP on the renal cortical necrosis produced in Fisher 344 rats by APAP and PAP. Pretreatment with BNPP reduced the rise in blood urea nitrogen (38±21 vs. 181±16 mg%) and the increase in kidney weight (2.187±0.090 vs. 2.578±0.047 g) after a nephrotoxic dose of acetaminophen. However, BNPP had no effect on blood urea nitrogen (200±1 vs. 190±8 mg%) or kidney weight (2.157±0.025 vs. 2.471±0.055 g) after a nephrotoxic dose of PAP. These studies and others indicate that APAP metabolism to PAP is a requisite step in APAP-induced renal cortical necrosis.

BRAIN ENERGY METABOLISM IN ACUTE RENAL FAILURE: EFFECTS OF ANESTHESIA. C.A. Mahoney,* P. Sarnacki,* A.I. Arieff, Nephrology Research, V.A. Med. Ctr., and U.C.S.F., San Francisco, CA.

Acute renal failure (ARF) is accompanied by profound depression of the central nervous system (CNS) but the cause is unclear. Previous studies of brain energy metabolism have been equivocal. Dogs with acute renal failure have changes in brain similar to those observed in patients. Studies were carried out in 4 groups of dogs: control; ARF; ARF without general anesthesia; control without anesthesia. General anesthesia was induced with IV pentobarbitol while analgesia was induced by acute volatile anesthesia (nitrous oxide), intubation, local analgesia (bupivicaine), sucinyl choline, and withdrawal of nitrous oxide. Studies were made in brain of ATP, ADP, AMP, energy charge (EC) creatine phosphate (CP), lactate (L), pyruvate (P) and glucose. Samples were obtained by a freeze suction device and snap frozen in liquid nitrogen.

In dogs with ARF and general anesthesia, levels in brain of ATP (1.8 mM), ADP (0.52 mM), AMP (0.13 mM), CP (2.6 mM), L/P (32) and EC (0.82) were not significantly different from control values in anesthetized dogs. In dogs with ARF but without anesthesia, values in brain for ATP (1.8 mM), ADP (0.29 mM), AMP (0.06 mM), CP (2.4 mM), L/P (16.5) and EC (0.91) were different from anesthetized dogs, but were not different from unanesthetized controls.

Conclusions: Although barbiturate anesthesia depresses the energy charge, there was no evidence that this obscured a difference between control and ARF. Thus, in brain of dogs with ARF, the resting energy state is normal and this effect is not due to anesthesia.

RAISED GLOMERULOCAPILLARY RESISTANCE IN $HgCl_2$ ACUTE RENAL FAILURE. Donald E. Oken and Leigh A. Laveri, Departments of Medicine, Medical College of Virginia and Veterans Hospital, Richmond, Virginia.

Munich-Wistar rats were studied by micropuncture 18-24h $\bar{p}$ 12mg/Kg BW $HgCl_2$. With pipets placed at the glomerulotubular junction (G-TJ), a volume sufficient for SNGFR measurement was obtained in only 1 of 44 attempts. Given i.v., lissamine green (LG) entered a segment of Bowman's space (BS) in 46 of 50 surface glomeruli, became progressively pale and disappeared in 12-44 sec ($\bar{x}$ 22±SE2.4sec). LG never entered the G-TJ. Early prox. tubule pressure (P_t), where measurable, was 8.6±SE0.3mmHg (N=22) vs control 11.8±0.4mmHg, $p<0.001$. LG stained saline injected directly into BS at ∿11mmHg pressure entered the tubule but remained static while coloration in BS disappeared rapidly. With ongoing filtration documented but not measurable, failure of filtrate to enter the G-TJ despite low P_t and rapid disappearance of dye entering BS, we propose that small vols. of filtrate were formed early in the capillary and quantitatively reabs. more distally. Network modelling shows that ↑ glom. cap. resistance (R_{cap}) to e.g. 1.9×10^{10} dyne sec cm^{-5} (i.e. = R_E) raises early glom. cap. pressure (P_{gA}) to 58mmHg (vs 43mmHg) c linear ↓ in P_g to 35mmHg (P_{gE}); SNGFR is ↓ 27%. If R_A now autoregulates to return P_{gA} to 43mmHg, P_{gE} ↓ to 24mmHg. Filtration is brisk in the prox. seg. of the cap. but stops and is reversed in the distal 40% with increasingly neg. net filtration pressure. Net SNGFR is ↓ 90%. No circumstance except ↑ R_{cap} can provide negative filtration but solitary ↑ R_{cap} that yields ↓↓ SNGFR also ↓ GBF 70-80%. The combination of ↑ R_{cap} and autoreg. of P_{gA} via Δ R_A fit well with our results.

MODIFICATION OF EXPERIMENTAL GENTAMICIN (G) NEPHROTOXICITY BY INCREASED DIETARY CALCIUM (Ca). M. Quarum*, D.A. McCarron, D.C. Houghton, D.N. Gilbert*, W.M. Bennett. Oregon Health Sciences University, Portland. Portland, Oregon.

Ca displaces G from its anionic phospholipid receptor *in vitro*. 4% calcium diets can delay the onset of G nephrotoxicity in rats maintained on low sodium intakes (KI 21:216, 1982). The present study examined .5% (normal) and 4% (high) calcium diets 14 days prior to and during G 40 mg/kg/day for 3 to 14 days in male Fischer rats. Diets were matched for sodium (.45%), potassium and magnesium. Prior to G, both groups had similar serum Ca and urinary sodium excretions. High Ca animals had marked hypercalciuria. Inulin clearance (C_{IN}) in ml/min/100 g, serum creatinine (Cr) in mg/dl and renal cortex concentration of [G] in µg/g are shown: N=6 at each time point.

	Baseline		3		7		14	
	.5%	4%	.5%	4%	.5%	4%	.5%	4%
C_{IN}	.77	.72	.76	.83	.30*	.78	.14	.38
Cr	.5	.5	.6	.6	1.3*	.8	8.9*	1.6
[G]	-	-	643	552	988*	707	578	449

*p</01 vs 4% calcium diet animals

.5% Ca diet animals who died demonstrated profound ionized hypocalcemia. 4% calcium animals had no mortality. Proximal tubular necrosis was earlier and more severe in .5% Ca animals.

Conclusions: Increased dietary Ca can 1) modify the severity and time course of experimental G nephrotoxicity, and 2) reduce renal cortex [G] accumulation. Calcium supplementation could be studied as a possible preventive or therapeutic modality in clinical G nephrotoxicity.

THE DEGREE OF GENTAMICIN (G)-INDUCED ACUTE RENAL FAILURE IN VARIOUS STATES OF Na BALANCE IS DUE TO STRUCTURAL RATHER THAN FUNCTIONAL RENAL ABNORMALITIES. M. Sastrasinh,* J.M. Weinberg, and H.D. Humes. VA Med. Ctr.& Univ. of Mich., Ann Arbor, MI.

The state of Na balance is a critical determinant of G nephrotoxicity, so that Na-depletion potentiates and Na-expansion ameliorates G nephrotoxicity.This phenomenon has been thought to be due to the effect of Na balance on functional determinants of glomerular filtration rate (GFR) rather than degree of structural damage of renal tubular cells. To test the influence of Na balance on tubular cell injury in G nephrotoxicity, biochemical indices of renal cell injury, including mitochondrial State 3 respiration (S3, natom O/min/mg prot) and renal cortical homogenate calcium content (RHC,nmole Ca/mg prot), were quantitated and compared to renal excretory function as measured by BUN (mg%). Rats were placed on normal (N), Na-deficient (ND), 5% NaCl (NC), and 5% $NaHCO_3$ (NH) diets, were given daily s.c. G (100 mg/kg) and were evaluated after either 6 doses (N6 and ND) or 8 doses (N8, NC, NH) of G. Data expressed as means±SE; n=6-14 for each group; *p<.05, †p<.01, compared to N6 or N8.

	N6	ND	N8	NC	NH
BUN	36±6	107±8†	120±20	51±5†	54±15*
S3	176±15	98±9†	136±14	162±6*	223±17†
RHC	21±4	71±11*	80±40	28±2*	27±5*

Na deprivation potentiated and Na supplementation ameliorated G nephrotoxicity. The degree of renal failure(BUN)directly correlated to the decline in S3(r=.66)and the rise in RHC(r=.60). The mechanism by which Na balance influences G nephrotoxicity is, therefore, due not to its influence on functional determinants of GFR but to its modification of the degree of renal tubular cell injury.

EFFECTS OF MINIMAL HYPOTENSION (H) ON THE COURSE OF ACUTE RENAL FAILURE (ARF) IN RATS WITH INNERVATED (N) AND DENERVATED (D) KIDNEYS. J.B. Robinette,* and J.D. Conger. Univ. of Colorado Health Sciences Ctr., Denver, Colorado.

A previous report from our laboratory showed that D restored renal blood flow (RBF) autoregulation lost in N kidneys during the maintenance phase of norepinephrine (NE)-ARF. It was hypothesized that H during ARF to the lower limit of autoregulation could adversely affect the course in N kidneys but not D kidneys because of recurrent ischemia in the former. Four groups of 8 SD rats were studied: 1, NE-ARF, N kidneys, given H; 2, NE-ARF, D kidneys prior to H; 3, control, NE-ARF, N kidneys, no H; 4, control, no NE-ARF, N kidneys, given H. Sham D was performed in all N kidneys. NE was infused on day 1, H was carried out on day 7 by phlebotomy to mean BP 90 mmHg for 4 hr; thereafter blood was returned. BUN (above), and S_{Cr} (below) for Groups 1 and 2 and BUN for Groups 3 and 4 were:

Day	0	2	6	9	14
Gr 1	22±3	58±13	35±4	67±11	30±2
	.4±.1	1.6±.4	.7±.2	1.7±.4	.8±.1
Gr 2	23±2	21±20	38±9	33±7	25±4
	.5±.1	1.6±.1	.8±.2	.7±.1	.5±.1
Gr 3	23±2	61±15	44±11	38±9	30±4
Gr 4	23±4	23±2	23±5	22±1	22±1

Compared to Group 2, the BUN and S_{Cr} in Group 1 on day 9 had risen after H (p<.001) and remained higher on day 14 (p<.001). It is concluded that transient decreases in BP only to the lower limit of autoregulation can adversely affect recovery from ARF because of loss of RBF autoregulation in NE-ARF rats.

^{31}P NUCLEAR MAGNETIC RESONANCE STUDIES OF RENAL ISCHEMIA. Norman J. Siegel, M.J. Avison,* H. Reilly,* and R.G. Shulman*. Depts of Peds and Molecular Biophy. and Biochem. Yale University Sch of Medicine, New Haven, Connecticut.

Alterations in renal ATP concentration during and after an ischemic insult have not been clearly delineated. To allow an *in vivo* determination of changes in renal ATP, we utilized nuclear magnetic resonance (NMR) to study rat kidneys. The left kidney was placed in a specially designed cup which contained a radiofrequency coil. The animals were then put in a TMR-32 spectrometer with 20 cm. bore operating at 80.2 MHz for protons and 32.5 MHz for ^{31}P.

Under control conditions: Distinct peaks corresponding to α, β and γ phosphate of ATP, sugar phosphate and inorganic phosphate (Pi) were observed in 7 min with good signal to noise ratio. Animals and ^{31}P spectrum were stable for 3-4 hrs.

During renal artery occlusion: The β-ATP peak (the only peak which is unique to ATP) declined appreciably within 10 min and had fallen to less than 10% of baseline values after 45 min. Concomitantly, Pi peak intensity increased and tissue pH fell significantly (at least 0.4 pH units).

After release of the renal artery: The β-ATP peak returned to low levels within 30 min and remained considerably below baseline values for 2-3 hrs. Pi levels fell progressively over the same period and tissue pH increased.

These data indicate that: 1) Changes in high energy phosphate and cellular pH can be determined continuously *in vivo* utilizing ^{31}P NMR, 2) Tissue ATP and pH fall rapidly during renal ischemia and 3) Recovery of ATP levels is incomplete as long as 3 hrs after an ischemic renal insult.

THE ADENINE NUCLEOTIDE PROFILE AS AN INDICATOR OF RENAL FUNCTION IN THE NOREPINEPHRINE (NE) MODEL OF ACUTE RENAL FAILURE. T. Sinsteden*, T. O'Neil*, M. Lifschitz and J. H. Stein. Univ. of Tx. Hlth. Sci. Ctr. San Antonio, Tx.

Bradykinin (Br), Furosemide (Fr), and Mannitol (M) when given before NE attenuate the degree of renal functional impairment induced by 40 mins of intrarenal NE (1μgm/kg/min). In addition to evaluating the renal functional protection offered by these agents on an anatomical basis (such as related to obstruction), this study investigates a possible biochemical basis to explain this protection. The adenine nucleotides were estimated by HPLC expressed in nmols/gm wet tissue, summed as TAN, and charge ratio (CR%) calculated. In addition to the above established protective agents phenoxybenzamine (P) was used to evaluate a direct toxic effect of NE on renal tissue. The inulin clearance at three hours post infusion (expressed as % of control) was: NE 5%, Br 37%, Fr 57%, M 51%, and P 98%. Renal blood flow fell to unrecordable levels during the NE (except P). Control levels of CR and ATP content fell precipitously in all groups (except P) during NE infusion. No significant change was observed in any parameters with P. Recovery of ATP and TAN was significantly ($p < .01$)* greater in the protected groups. CR recovered rapidly in the protected groups and recovered to 66% with NE alone.

CONT	30' INFUSION				180' POST INFUSION			
	NE	Br	Fr	M	NE	Br	Fr	M
CR-79	16	21	34	28	66	74	74	78
ATP1175	97	222	299	250	388	849*	861*	756*
TAN1768	1163	1671	1343	1417	776	1393*	1548*	1181*

The marked recovery of ATP and TAN at 180 min post infusion in the protected groups vs NE alone, suggest that these results reflect a major biochemical basis for the recovery in renal function in this model.

THE PROTECTION AFFORDED TO DIABETIC RATS AGAINST GENTAMICIN-INDUCED ACUTE RENAL FAILURE (G-ARF) IS NOT MEDIATED BY PROSTAGLANDINS (PG). C.A. Vaamonde, J. Pisegna*, C.M. Vaamonde*, B. Owens*, H. Alpert*, and V. Pardo. VA Med.Ctr. & Dept.Med., Univ. of Miami, Miami, Florida.

To assess the role of PG in the protection against G-ARF observed in untreated diabetic rats, streptozotocin-treated rats (DM) and matched controls (C) received gentamicin after PG synthetase inhibition with indomethacin (IN). Three DM and C groups were studied: (a) IN(2mg/Kg/d for 12 d, (b) gentamicin (G) (60 mg/Kg/d for 9 d,) and (c) IN+G (n=8 in each). Ccr, lysozymuria (Ly), renal cortical G content (rG) and histology (tubular necrosis score, TNS) were evaluated.

		Baseline Ccr ml/min	Ccr day 9 Δ %	rG μg/g dry wt	TNS (0-4)
DM	IN	2.3 ± .1	↑ 20 ± 12	-----	0
	G	2.3 ± .1	↓ 11 ± 6	1829 ± 183	0
	IN+G	2.2 ± .1	↓ 7 ± 4	1492 ± 167	0
C	IN	1.4 ± .1*	↑ 28 ± 10	-----	0
	G	1.6 ± .1*	↓ 86 ± 4*†	2536 ± 114*3.7±.2*	
	IN+G	1.2 ± .1*	↓ 76 ± 6*†	2515 ± 164*3.7±.2*	

X±SE * $p < .01$ vs DM groups; † $p < .01$ vs Baseline.

IN alone produced no changes in Ccr in either group. In DM, Ccr was preserved during G and G+IN, no differences were observed in rG, there was no Ly and TNS was 0. In controls, G and IN+G induced marked ↓ Ccr, severe Ly, higher rG, and tubular necrosis, with no differences between the two groups. We conclude that in C, IN does not influence G-ARF. If PG inhibition was equal in DM and C, the data suggest that the protection afforded to untreated DM rats against G-ARF is not mediated by renal prostaglandins.

RENAL EFFECT OF RADIOGRAPHIC CONTRAST MEDIA IN OLD DEHYDRATED DIABETIC RATS. Carlos A. Vaamonde, Barbara Owens*, Helen Alpert* and Victoriano Pardo V.A.Med.Ctr. & Dept. Medicine, Univ. of Miami, Miami, Florida.

We have shown that radiographic contrast media (CM) does not produce acute renal failure (ARF) in young (6 mos) non-dehydrated rats. Since dehydration (Dehy) and old age appear to be risk factors for CM-ARF, renal function was studied in 19-mos DM female Sprague-Dawley rats (DM,n=8) and age- and sex-matched control rats (C,n=9) after CM. DM was induced at age 2 mos with iv streptozotocin, 65 mg/Kg. Animals were Dehy for 24-hr before iv (5 ml/Kg) methylglucamine diatrizoate (Renografin 76%). Prior to Dehy Ccr was similar in DM(2.9 ± .2 [SE] ml/min) and C(2.8 ± .2) rats. Dehy resulted in lower urine flow and higher Uosm in both groups, and Ccr decreased by 40 ± 5% ($p<.001$) in DM and by 14 ± 5% in C (NS). Changes in Ccr were:

	Dehy (ml/min)	Contrast Media (% Δ from Dehy) Day 1	Day 2	Day 3
DM	1.7 ± .2	↑ 43 ± 19#	↑ 36 ± 15#	↑ 36 ± 14#
p	< .025	< .05	< .05	NS
C	2.4 ± .1	↓ 6 ± 5	↓ 5 ± 9	↑ 9 ± 7

#p < 0.05 from Dehy. NS = non significant.

Dehy reduced Ccr less in C rats than in DM (which rapidly recovered). Neither group had histologic evidence of tubular abnormalities. We conclude: (a) CM does not cause ARF in Dehy DM or C rats; (b) age does not influence the renal effects of CM in DM or C animals; and (c) factors other than age and dehydration are necessary for the appearance of CM-ARF in the DM rat with normal glomerular filtration rate.

POTASSIUM HANDLING IN CISPLATIN (CP)-INDUCED ACUTE RENAL FAILURE (ARF) IN RATS. S. Vicks,* J. Kaufman, S. Chopra, and R.J. Hamburger. Boston VA Medical Center, Renal Section, Boston, MA.

We examined the renal response to a potassium load 96 hr. after CP, 10 mg/kg i.p. Clearance studies were performed during 0.9% saline infusion at 1.2 ml/hr (C) and for the last 60 min. of the infusion of 0.5M KCl at 1.2 ml/hr for 150 min. (E). Results (±SE) were as follows:

	Control		CP	
	C	E	C	E
V (μl/min)	5.9±0.7	26.0±3.4[a]	7.5±1.9	30.4±7.2[a]
U/P_{In}	565±116	130±19[a]	36±13[b]	15±4[b]
C_{In} (ml/min)	2.7±0.1	2.9±0.1	0.3±0.1[b]	0.5±0.1[ab]
FE_{Na} (%)	0.1±0.1	0.6±0.2[a]	2.4±0.9[b]	4.5±1.0[ab]
FE_K (%)	27±4	79±8[a]	62±4[b]	119±11[ab]
P_K (mM/L)	4.2±0.2	5.4±0.2[a]	4.3±0.2	8.0±0.5[ab]
U_K (mM/L)	523±58	498±42	93±34[b]	121±24[ab]
U_KV (mM/min)	2.9±0.3	11.6±1.3[a]	0.8±0.3[b]	4.2±1.3[ab]

Significantly different ($p < .01$) from C (a) or control rats (b).

As we have previously demonstrated, CP induces ARF with well-maintained urine flow rates (V), increased FE_{Na}, and decreased urinary concentrating ability, suggested by the low U/P_{In}. After a potassium load, CP rats increased their FE_K to 119% providing evidence for an intact distal secretory mechanism. However, although the FE_K of the CP rats was greater than control, they excreted only 55% of the K load in 150 min. compared to 98% in controls and plasma K almost doubled. Since V was similar in both CP and control rats, this factor cannot account for the blunted kaliuresis. Whether it indicates distal tubule dysfunction or other factors cannot be determined from the present data.

IN VIVO ORIGIN OF MITOCHONDRIAL CALCIUM OVERLOAD DURING NEPHROTOXIC ACUTE RENAL FAILURE (NARF). Joel M. Weinberg and H. David Humes. VA Medical Center and Univ. of Michigan, Ann Arbor, Michigan.

Tissue and mitochondrial Ca overload occur during NARF at the time of advanced renal tubular cell injury and may be very useful in quantitatively assessing the extent of such injury (Kid. Int. 21:226, 1982). However, since mitochondria (M) may take up Ca during their isolation, the extent to which Ca overload in isolated M reflects their in vivo state as opposed to uptake or superficial binding during isolation is uncertain. To clarify this issue, M were obtained from kidneys with a broad range of tissue Ca (2.5-25 x nl) generated by studying water and saline drinking rats 12 and 24 hours after 5 mg/kg $HgCl_2$. M Ca was assessed after isolation in 275 mM sucrose-1 mM EGTA, sucrose-20 μM ruthenium red and sucrose-1 mM EGTA-20 μM-ruthenium red and was compared to Ca of M from control rats isolated with a comparable range of ambient Ca levels achieved by adding Ca directly to the isolating medium. Under every isolation condition, irrespective of the degree of tissue Ca overload, M isolated after $HgCl_2$ had substantially higher Ca levels than control M similarly isolated in the presence of similar ambient Ca levels. Furthermore, the Ca levels of M isolated from severely injured kidneys exceeded by 2-3 fold the maximum levels achievable by Ca loading during isolation. Similar results have been obtained with advanced gentamicin nephrotoxicity. These observations clearly demonstrate the major extent to which M Ca loading occurs in vivo during advanced NARF and establishes the usefulness of isolated M in assessing this process.

SEQUENTIAL CHANGES IN MITOCHONDRIAL FUNCTION IN ISCHEMIC ACUTE RENAL FAILURE (ARF) IN THE RAT. D.R. Wilson, P. Arnold*, T. Burke, R.W. Schrier. Dept. Med., UCHSC, Denver, Col.

Changes in mitochondrial (Mito) respiration and calcium occur in ischemic ARF induced by norepinephrine in the dog. Our aim was to determine the sequence of changes in Mito function in ischemic ARF induced by 45 min. of bilateral renal pedicle clamping in the rat. Results for state 3 (ADP), state 4 (succinate), acceptor control ratio (ACR, state 3/4) and uncoupled (FCCP) Mito respiration, mito Ca^{2+} and serum creatinine are shown.

		45'	Reperfusion		
	Sham	Clamp	1 h	4 h	24 h
n=	22	10	10	8	12
S creat	0.22	0.53†	0.68†	0.89†	1.93†
State 3	141	53†	118 †	103 †	60†
State 4	31	25	33	29	28
ACR	4.54	1.96†	3.64†	3.59†	2.01†
FCCP	165	78†	150 †	135 †	74†
Ca^{2+}	23	32*	44 †	46 †	101 †

P vs. sham* <0.05,† <0.01

Mito resp (state 3, ACR, FCCP) was severely depressed after 45' clamp, improved at 1 and 4 h reperfusion, but was markedly impaired with established ARF at 24 h. Mito Ca^{2+} increased progressively during clamp and reflow, reaching high levels at 24 h. Increased mito Ca^{2+} during reperfusion (1,4,24 h) correlated with decreased state 3 Mito resp and rising serum creat($p<0.001$). The results support the hypothesis that an early and progressive increase in mitochondrial, and, most likely, cytosolic Ca^{2+} is important in the pathogenesis of ischemic ARF.

INTRARENAL OXYGEN (O_2) PROFILES DURING THE INITIATION PHASE OF $HgCl_2$-INDUCED ACUTE RENAL FAILURE (ARF): C Westenfelder, and R L Baranowski, Dept of Medicine, University of Illinois and West Side VA Hospitals, Chicago, IL.

All models of ARF are characterized by proximal tubular dysfunction/damage. This proximal tubular defect is thought to be important in the pathogenesis of filtration failure. Because this nephron segment is almost exclusively dependent on an aerobic pattern of metabolism, O_2 supply during the initiation phase of ARF is critical. Therefore, in the present study intrarenal pO_2 and renal function were measured in Sprague-Dawley rats, 60 min before and 30 and 60 min after the administration of $HgCl_2$ (3mg/kg s.c., n=5). Intrarenal pO_2 was measured with an O_2-sensitive plantinum microelectrode which was inserted into the left kidney at a point opposite the renal hilus. Local pO_2 was measured at 0.2mm steps, advancing the pipette centripetally from the surface. GFR and renal plasma flow (RPF) before $HgCl_2$ administration were not different in control (right) and left (punctured) kidney. Both variables fell by 50% within 60 min of $HgCl_2$ injection (no difference between right and left). pO_2 (mean ± SE) before $HgCl_2$, at a depth of 0.2mm from the surface was 112 ± 17, at 1mm, 47 ± 8, at 2mm, 44 ± 6 and at 3mm, 40 ± 5 mmHg. 30 min after $HgCl_2$ administration, pO_2 at a depth of 0.2mm, was 106 ± 14 (NS), at 1mm, 24 ± 6 ($p<0.01$), at 2mm, 25 ± 7 ($p<0.01$) at 3mm, 18 ± 6 ($p<0.01$). 60 min after $HgCl_2$ pO_2 had returned to control levels. Conclusion: These data suggest that during the initial phase of $HgCl_2$-induced ARF cortical pO_2 falls, thereby aggravating proximal tubular injury and thus contributing to the severity of ARF.

IMPAIRED URINARY ACIDIFICATION FOLLOWING ACUTE RENAL ISCHEMIA IN THE DOG. J. Winaver*, D. Agmon*, R. Harari*, O.S. Better* (Intr. by R.T. Kunau, Jr.). Rambam Hospital and Technion School of Medicine, Haifa, Israel.

Metabolic acidosis is a common sequel to acute renal failure. The present study was undertaken to evaluate tubular secretory capacity for H^+ ions following acute unilateral one hour ischemia (E) in the dog. Acute clearance studies were performed 60 minutes following release of clamp. The contralateral kidney served as control (C).

	* min U_{pH}	** U-B pCO_2 mmHg	*** U-B pCO_2 mmHg
C	5.5 ± 0.3	34.7 ± 6.5	40.1 ± 14.8
E	6.5 ± 0.5	13.4 ± 4.6	-1.9 ± 3.4
P	<0.01	<0.02	<0.02

During infusion of: *0.1M Na_2SO_4, **Neutral phosphate, ***0.15M $NaHCO_3$. Results expressed as mean ± SEM

Thus, U-B pCO_2 gradient generation was impaired during both bicarbonate and phosphate infusion. In addition, ischemia resulted in an inability to normally decrease UpH during Na_2SO_4 infusion. These results are compatible with a primary secretory defect in H^+ ion transport. It is concluded that: (a) acute renal ischemia is associated with a distal tubular defect in hydrogen ion secretion in the dog; (b) this defect appears to be of a secretory type; (c) and similar tubular dysfunction may contribute to acidosis following acute renal failure in man.

DETERMINANTS OF DIMINISHED GLOMERULAR FILTRATION RATE (GFR) IN EARLY CISPLATIN (Pt) INDUCED RENAL FAILURE. J. Winston,* M. Daye, R. Safirstein. Mount Sinai School of Medicine, New York, NY.

Renal hemodynamics were studied in rats 48-72h after Pt (5mg/kg) when the first detectable fall in GFR occurs. At this time rats do not show defects in superficial proximal or distal tubule Na and H_2O transport during free flow micropuncture. Whole kidney GFR, single nephron (SN) GFR, renal blood flow (RBF), systemic pressure, stop-flow pressure (P_{SF}), intratubular pressure (P_T) and plasma protein concentration (C_A) were measured in euvolemic (1.25% body weight plasma infusion) control and Pt animals. In 11 Pt rats, GFR varied between normal and 30% of normal (mean .76±.11 vs 1.3±.05 ml/min $p<.005$) but individual rats showed consistent GFR. SNGFR was reduced from 0 to 50% of normal (mean 28.4±1.6 vs 40.4±1.7 nl/min $p<.001$) Mean P_{SF} was reduced from 40.6 to 31.8 mmHg, $p<.005$. P_{SF} was significantly correlated with SNGFR ($r=.74$, $p<.01$). C_A was unchanged. The reduction in P_{SF} did not entirely account for the reduced SNGFR. In Pt treated rats, mean P_T and mean RBF (by PAH extraction) were reduced from 12.3 to 10.3 mmHg ($p<.001$) and 8.4 to 4.3 ml/min ($p<.01$), respectively. Renal vasculature resistance increased 1.9 fold above normal ($p<.05$). In a separate group of animals RBF was measured by radioactive microspheres under hydropenic and euvolemic conditions in 3 day Pt and control animals. RBF was reduced 37%, and 51%, respectively, $p<.01$.

The earliest fall in GFR is in part due to renal vasoconstriction, reduced renal perfusion and reduced glomerular hydrostatic pressure. These changes occur without evidence of tubule obstruction.

Pathophysiology of Chronic Renal Failure

MITOCHONDRIAL CALCIUM LEVELS IN CHRONICALLY UREMIC RATS. A. J. Adler, G. M. Berlyne, Brooklyn Veterans Hospital, Brooklyn, NY.

Whole brain (whole brain) and mitochondrial (MT) calcium levels were determined in normal and chronically uremic Sprague Dawley rats. Uremia was induced by 5/6 nephrectomy 4-6 weeks prior to the study. Groups were divided into normal (BUN 16.6±4.1 mg/dl), mild uremia (BUN 39.2±6.8 mg/dl) and severe uremia (BUN 65.6±15.8 mg/dl). Mitochondria were isolated by gradient centrifugation and purity assessed by LDH and cytochrome C oxidase activities. Calcium was determined by flameless atomic absorption spectrophotemetry. Data are shown as mean ± S.D. (ug Calcium /mg Protein).

	WB Ca(N)	MT Ca(N)
Normal	.62±.11(45)	.35±.11(26)
Mild Uremia	.63±.09(21)	.31±.07(21)
Severe Uremia	.69±.09(24)*	.31±.07(17)

* $2p < .01$

Whole brain calcium is increased 11% in severely uremic rats ($2p < .01$) and correlated directly with Ca x PO4 ($p < .05$). Mitochondrial calcium was unchanged in uremia.

CONCLUSIONS: Severe uremia is associated with increased brain calcium probably secondary to interstitial deposition of calcium phosphate, whereas intracellular calcium as represented by mitochondrial levels are not elevated.

ORGANIC ANIONS (OAs) EXACERBATE EXPERIMENTAL ACUTE RENAL FAILURE (ARF). R.A. Zager, H.M. Sharma,* Ohio State University Hospital, Columbus, Ohio.

OA plasma concentrations and OA loads per nephron are increased in patients with renal insufficiency (e.g. hippurates) and during therapy with OA drugs (e.g. the penicillins). The purpose of this study was to determine whether these perturbations alter renal susceptibility to acute ischemic or nephrotoxic insults. Anesthetized Sprague Dawley rats were infused with a control infusate or control infusate plus an OA (hippurate, paraamino hippurate, cephalothin, 0.125-1.0 mg/min). This rate of infusion produced serum cephalothin concentrations within a therapeutic range for man. After a 40-min control period acute renal injury was induced (bilateral renal pedicle cross clamping x 25 min; or $HgCl_2$ 12 mg/kg IV). GFR was measured before and after these insults (0-2 h post ischemia; 0-4 h post $HgCl_2$).

OAs had no effect on GFR prior to renal injury. However, each OA exacerbated the loss of GFR post ischemia (OAs ↓89 ± 2%; no OA ↓51 ± 4%) and post $HgCl_2$ (OAs ↓84 ± 4%; no OA ↓40 ± 4%)($\bar{X}$ ± SEM; $p < .001$). This effect was not OA dose dependent. Discontinuing OA infusion immediately post renal injury resulted in low terminal OA serum levels (< 4 mg/dl) but GFR failed to rise. By light microscopy, OA treated ARF rats had greater proximal tubular injury (vacuolization, dilatation) than did non-OA infused ARF rats. OA infusion into normal (non-ARF) rats and equimolar Na_2SO_4 infusion into ARF rats had no effect on GFR or renal histology. Conclusion: OA infusions exacerbate functional and histologic parameters of experimental ARF. These findings suggest that OAs modulate renal susceptibility to ischemic and nephrotoxic injury.

ROLE OF UREMIA, INCREASED BRAIN CALCIUM (Ca) AND PARATHYROID (PTH) HORMONE ON CHANGES IN ENCEPHALOGRAM (EEG) IN CHRONIC RENAL FAILURE. M. Akmal, D.A. Goldstein, S. Multani* and S.G. Massry. Div. Nephrol., USC Sch. Med., Los Angeles, CA.

Acute uremia is associated with increased Ca in brain and changes in EEG and both derangements are related to excess PTH. Also changes in EEG in patients with chronic renal failure correlated directly with the blood PTH and fall in PTH was followed by improvement in EEG. We examined whether chronic uremia per se has an effect on brain Ca or EEG. Uremia was produced by 5/6 nephrectomy and maintained for 6-12 (10+2) months in 7 thyroparathyroidectomized (TPTX) dogs and 8 animals with intact parathyroid glands (control). EEG was recorded prior to sacrifice and Ca content of gray and white matter was measured. There were no differences in the serum Ca and phosphorus or creatinine clearance (TPTX 16±3, control 14±4 ml/min). Serum PTH was undetectable in TPTX and elevated in control dogs. Ca in gray matter was elevated in both groups but significantly higher in control (TPTX 637±46, control 1324±47 mg/kg/dry weight), but high in white matter only in control dogs (TPTX 221±8, control 604±10 mg/kg/dry weight). The % waves of <7 Hz in EEG were 4.8±1.1 and 4.6±1.1 prior to uremia and remained unchanged in TPTX dogs (4.5±0.9) but increased significantly in control animals (19.9±2.0). Data show that chronic uremia per se is associated with marked rise in Ca in gray matter and the increment is higher in the presence of PTH. Disturbance in EEG in state of chronic uremia requires the presence of excess PTH and is prevented if hyperparathyroidism is not allowed to develop.

SELECTIVE JM NEPHRON HYPERFILTRATION IN SHR RATS WITH REDUCED RENAL MASS. Norman Bank, Lloyd Alterman*, and Hagop S. Aynedjian*. Montefiore Hospital & Med. Ctr., Dept. of Med., Bronx, N.Y.

Studies were carried out to determine the consequences of reduction in renal mass on single nephron hemodynamics and proteinuria in the spontaneously hypertensive rat (SHR). Four groups were studied: 2 kidney SHR and WKY controls; uninephrectomized (UNX) SHR and WKY. UNX was performed at age 8-10 wks. Blood pressure and protein excretion were measured periodically, and micropuncture experiments carried out at age 32-40 wks. Systolic BP was as follows: SHR=170±4; SHR+UNX = 183±5; WKY=116±4; WKY+UNX=111±4 mm Hg. Protein excretion increased markedly only in the SHR+UNX rats. In cortical nephrons, SNGFR was lower in SHR vs WKY and in SHR+UNX vs WKY+UNX. SNPF was lower in SHR+UNX than in WKY+UNX. Glomerular pressure, calculated from stopped-flow measurements, were closely comparable in all groups, without any rise after UNX. Measurements of JM SNGFR were made by collections from loops of Henle in the papilla of SHR+UNX and WKY+UNX rats. Results were: SHR+UNX=88±4.7; WKY+UNX=57±6 nl/min ($p<0.005$). We conclude that cortical glomeruli are not exposed to increased P_G in the SHR following UNX, and that SNPF increases less than in controls. In contrast, P_G and/or flow appear to increase disproportionately in JM glomeruli of SHR+UNX rats. These findings suggest that JM nephrons in SHR are predisposed to injury as renal mass is reduced, due perhaps to inadequate autoregulation. They also provide an explanation for the previous observation that proteinuria in SHR emanates primarily from deep nephrons (Feld et al Kid. Internat. 12:332-343, 1977).

ROLE OF GLUCOCORTICOIDS AND MINERALOCORTICOIDS IN POTASSIUM (K) ADAPTATION AFTER DECREASED GFR. M Bia R. DeFronzo, K Tyler. Yale Med Sch, New Haven, Ct.

We have previously shown that K adaptation after a reduction in GFR does not occur in the absence of adrenal hormones (Clin Res 30:443A'82). To determine the importance of gluco and mineralocorticoid replacement in this process, 4 groups of rats were given an acute K load (0.11 mEq/100g over 40 min IV): Gp I- 5 rats with 3/4 nephrectomy (NPX); Gp II - 8 rats adrenalectomized before NPX (NPX-ADX); Gp III - 15 NPX-ADX maintained on dexamethasone, 1 µg/100g/d S.Q.; Gp IV - 7 NPX-ADX maintained on aldosterone (Aldo) 0.5 µg/100g/d S.Q. Hormones were given by continuous infusion. Rats were studied 2 wks after NPX and all NPX-ADX were maintained on saline. GFR was reduced to .5-.7 ml/min/100g in all groups and was slightly higher in Dex treated animals. Results:

Group	P_K(mEq/L)	ΔP_K	Base FE_K(%)	Peak FE_K(%)
I	3.8±1	1.1±.1	46±10	95±11
II	5.2±.3*	1.7±.2*	21±4*	34±4*
III	3.9±.1†	1.6±.1*	14±2*	35±2*
IV	3.5±.1†	1.6±.2*	23±6	57±3*†

FE_K = fractional K excretion. * = $p < .05$ vs Gp I. † = $p < .05$ vs Gp II.

Baseline hyperkalemia, present in NPX-ADX, was corrected with hormone replacement. After KCl, ΔP_K rose higher in all NPX-ADX even with hormone replacement. FE_K was lower in all NPX-ADX groups vs NPX alone. Following KCl, peak FE_K was improved with Aldo replacement ($p<.001$ vs Gp II) but was still less ($p<.01$) vs NPX alone. Conclusion: K adaptation following a decrease in GFR is impaired in ADX. Chronic, physiologic replacement with either Dex or Aldo does not return FE_K to the values observed in NPX with intact adrenal glands.

ADRIAMYCIN INDUCED CHRONIC PROTEINURIA: A NEW MODEL OF GLOMERULAR FOCAL SCLEROSIS. T. Bertani, G. Rocchi, G. Mecca, G. Sacchi, G. Remuzzi (intr. by C.L. Pirani), Div. of Nephrol., Ospedali Riuniti, Bergamo, Italy and Dept. of Pathol. II, Spedali Cavili, Brescia, Italy.

Adriamycin given i.v. (5mg/kg/body weight) to 32 rats induced a nephrotic syndrome (NS), proteinuria beginning 14 days after a single injection. The full expression of NS developed 28 days later, proteinuria ranging between 161 and 454 mg/day. At 21 days no detectable abnormalities were seen by light microscopy (L.M.), but marked fusion of "foot processes" was observed in all glomeruli examined (16 rats). After six months proteinuria was markedly increased (range 600 to 1100 mg/day). Glomerular focal sclerosis (F.S.) associated with severe tubular and interstitial changes was present in 50% of the bioptic specimens. At that moment 50% of the glomeruli showed by electron microscopy (E.M.) "foot processes" fusion. After nine months proteinuria ranged between 800 and 1200 mg/day. By L.M., besides a variable degree of glomerular F.S., the most relevant findings were represented by tubular and interstitial changes. At E.M. "foot processes" fusion was detectable in 30% of glomeruli. This model could be relevant to understand the evolution from a "minimal change type" lesion to glomerular F.S. Moreover our data suggest that tubular and interstitial changes should be regarded as a crucial event for the development of F.S.

DOGS WITH A REMNANT KIDNEY DO NOT DEVELOP PROGRESSIVE RENAL FAILURE. J.J. Bourgoignie, G. Gavellas and V. Pardo. University of Miami, Miami, FL.

Clinically, chronic renal disease progresses to end-stage failure. Evidence accrued in rats suggest that "adaptive" increments in SNGFR may represent a potentially adverse response to severe reduction in functioning renal mass and contribute to the progressive destruction of remaining glomeruli. This hypothesis, however, must consider the following facts in remnant dogs.

GFR (endogenous or exogenous creatinine) was measured serially in six remnant dogs (partial infarction of one kidney and contralateral nephrectomy). Mean GFR was 12.4 (range 7.6-20)ml/min. at 3 months and 14.1 (range 8.4-20.5)ml/min. at 18 months. The animal with the lowest GFR initially had the lowest GFR terminally. Four animals were followed for 27 and two for 36 months. In none was there evidence of progressive decrease in GFR. Total proteinuria averaged 66 (range 36-113) in remnants (N=4) and 55.0 (range 24-116)mg/24hr in normals (N=9). Focal mesangial changes were present in remnant glomeruli by light and electron microscopy. As in the rat, SNGFR increases by more than 50% in remnant dogs (Dirks and Wong Proc. Int.Congr.Nephrol., Athens 1981, p.255). Thus, while the adaptive increases of SNGFR that occur in the dog remnant kidney may be attended by focal mesangial sclerosis, they do not result in endothelial cell damage, proteinuria, or progressive destruction of remaining glomeruli. The hypothesis that hyperfiltration is the final common pathway of progressive nephron destruction requires careful scrutiny of disease models other than the remnant rat.

EFFECT OF PTH ON SKELETAL MUSCLE: ROLE OF PTH IN UREMIC MYOPATHY. N. Brautbar, R. Baczynski*, S. El-Belbessi* and S.G. Massry. Div. Nephrol., Dept. Med., USC Sch. Med., Los Angeles, CA.

Uremia as well as states of excess PTH and normal renal function are associated with myopathy. It is possible that the secondary hyperparathyroidism of uremia affects skeletal muscle metabolism and contribute to myopathy. High energy phosphate were measured in muscle biopsies, oxidative phosphorylation in isolated mitochondria and energy utilization in myofibrills from rats injected with 200 U PTH/day for 4 days. Skeletal muscle ATP and creatine phosphate were reduced in PTH rats: 37.6±2.2 vs 20.1±0.85 ($p<0.01$) and 86.8±8.9 vs 46.5±6.5 μmoles/g protein ($p<0.01$), respectively. Mitochondrial respiratory control ratio and oxygen consumption were reduced 8.0±0.22 vs 4.9±0.25 ($p<0.05$) and 112.5±6.1 vs 70.0±6.3 ($p<0.01$) nmole O_2/mg protein/min, respectively. Mitochondrial energy transport was impaired as shown by a reduced ability to increase QO_2 at state 4 (ATP in excess) in response to creatine: 1.35±0.12 in control vs 1.0±0.11 in PTH rats ($p<0.01$). Mitochondrial and myofibrillar creatine phosphokinase were reduced in PTH rats: 2.3±0.31 vs 1.52±0.06 ($p<0.01$) and 0.464±0.032 vs 0.273±0.034 ($p<0.01$), IU/mg protein, respectively. Myofibrillar energy utilization was also impaired since the activity of Ca-Mg ATPase was reduced: 170.1±15.0 vs 103.0±19.6 μmoles P/mg protein/min ($p<0.01$). These data show that excess PTH impairs 1) mitochondrial energy production and transport, and 2) myofibrillar energy utilization. The data provide a molecular cellular mechanism for the skeletal myopathy seen in uremia and other conditions of excess PTH.

EXERCISE ENHANCES MUSCLE SENSITIVITY TO INSULIN IN UREMIC RATS. T. Davis*, I. Karl*, E. Tegtmeyer*, A. Goldberg*, and H. Harter. Washington University School of Medicine, St. Louis, MO.

Exercise training has been shown to enhance tissue sensitivity to insulin in various disease states. To evaluate the effects of exercise and insulin on muscle protein catabolism in uremia, 3/4 nephrectomized (U) and sham-operated control (C) female rats were exercised (E) (swam 2h/d, 5d/wk x 4 wk) or remained sedentary (S). The epitrochlearis muscles were removed 24 hours after the last exercise bout and incubated with and without .01U/ml of insulin. Baseline muscle citrate synthase activity was reduced in muscles from SU rats (.3 ± .1 moles/Kg/hr) compared to control values (.6 ± .1 moles/Kg/hr) ($p<.005$). Exercise increased these values two fold in both EU and EC rats. Baseline tissue glycogen levels were also increased by 40% in muscles from EU and EC rats compared to sedentary values ($p<.02$). Phenylalanine (PHE), tyrosine (TYR), and alanine (ALA) release from muscles of SU rats were increased 40% above SC values ($p<.05$). Exercise reduced PHE and TYR release muscles of EU rats to control values, but no change in ALA release occurred.

Insulin reduced PHE, TYR, and ALA release from muscles of EC and EU rats by 20% at 1 hr. and 50% at 3 hrs ($p<.05$). Release of these amino acids from muscles of SC and SU rats was unaffected at 1 hr. and decreased by 20% at 3 hours. Glucose uptake was increased 20-30% in EC and EU muscles compared to SC and SU values. These data suggest that exercise reduces muscle protein catabolic rates in uremia. Exercise also increases muscle sensitivity to insulin.

METABOLIC ACIDOSIS AND PARATHYROIDECTOMY INCREASE NA^+-H^+ EXCHANGE IN ISOLATED RENAL BRUSH BORDER MEMBRANE VESICLES. Dunell E. Cohn,* Saulo Klahr, and Marc R. Hammerman. Washington Univ. School of Med., Dept. of Internal Med., St. Louis, Missouri.

We have recently identified an amiloride inhibitable Na^+-H^+ exchanger in canine renal brush border membrane vesicles (BBMV). The activity of this exchanger was found to be enhanced in BBMV prepared from the remnant kidneys of dogs with chronic renal failure (CRF) (Clin.Res.29:743A,1981) and it was suggested that this increased activity might contribute to the increased H^+ excretion per nephron that occurs in the remnant kidney model of CRF. In order to determine whether the increased need to excrete H^+ and/or increased levels of circulating parathyroid hormone (PTH) which accompany the development of CRF mediate the increased activity of the Na^+-H^+ exchanger, Na^+ uptake under conditions of a H^+ gradient (intravesicular pH < extravesicular pH) was compared in BBMV isolated from the kidneys of normal dogs, dogs with NH_4Cl induced metabolic acidosis and thyroparathyroidectomized dogs. The initial rates of Na^+ uptake in these three groups were 1.78 ± .07, 2.40 ± .14 and 2.63 ± .28 nmols Na^+/mg protein/20 sec, respectively. In both experimental groups, Na^+ uptake was significantly different from the control group ($p < .05$, Student's t test) and the increased Na^+ uptake was inhibited by amiloride, indicating that increased Na^+ uptake was a result of enhanced Na^+-H^+ exchange. These observations suggest that the adaptation in Na^+-H^+ exchange in CRF may result from the need to excrete more H^+ per nephron and that this adaptation occurs in spite of the increased levels of circulating PTH that accompany this condition.

EVIDENCE FOR TUBULAR SECRETION OF COMPOUNDS RETAINED IN UREMIA. Thomas A. Depner and Peter Igarashi*. Dept. of Int. Med., Univ. of California, Davis, California.

Decreased binding of small ligands to serum albumin in uremia is due to poorly dialyzable inhibitors extractable from whole uremic serum at low pH. Several properties of the extract (I_x) suggest that the major components are organic acids, shown to inhibit PAH transport in rat kidney slices. This study examines the mode of excretion of the major detectable components of this extract by the in vitro perfused rat kidney. I_x was isolated from uremic pleural fluid and added to chemically defined perfusate which contained PAH and inulin but no albumin. Samples of perfusate and urine were collected at timed intervals before and after addition of I_x. Major components were separated by high pressure liquid chromatography and detected at 280 nM. Fractional excretions by the kidney (FE) and HPLC retention time (Tr) for PAH and 5 major peaks are shown at 15 min after addition of I_x:

	PAH	A	B	C	D	E
Tr	5.0	7.0	13.5	21.0	22.2	23.3
FE	2.5	5.4	7.9	7.5	8.2	0.9

Several other minor peaks were also detected. Peak B is tentatively identified as hippurate. The high fractional excretion observed for most of these isolates indicates a major secretory mechanism most likely at the proximal nephron in this preparation. It is concluded that in addition to glomerular filtration demonstrated for middle molecules, tubular secretion plays a major role in the excretion of other retained substances that may account for protein binding inhibition in uremia.

DIMINISHED BARORECEPTOR FUNCTION AND VASCULAR RESPONSIVENESS IN CHRONIC RENAL FAILURE IN THE INTACT AWAKE RAT. D DiPette*, A Watson*, K Simpson*, (intr. by E Bourke). Allegheny General Hospital, Dept. of Medicine, Pittsburgh, Pennsylvania.

To determine whether baroreceptor dysfunction contributes to hypertension seen in chronic renal failure (CRF), we examined the heart rate (HR) response to blood pressure (BP) elevation induced by graded i.v. infusion with both norepinephrine (NE) and phenylephrine (PE) in rats five days post subtotal nephrectomy (SN) (NE n=7, PE n=8) and controls (C) (NE n=8, PE n=8). BUN was elevated in all SN rats (SN:88±9; C:18±1 mg/dl±SEM $p<.001$) as well as baseline mean BP (SN:130±2; C:120±2 mmHg $p<.001$). A significant inverse correlation was found between the increase in BP induced by NE or PE and the decrease in HR within both SN and C groups. The ratio of change in HR to change in BP was decreased in SN animals with both NE and PE respectively (NE, SN 2±.2 vs C 3±.2 $p<.01$; PE, SN 2.6±.2 vs C 4.3±.4 $p<.001$).

The change in mean BP in response to NE and angiotensin II (A) given via i.v. bolus in SN and C rats is shown below.

Dose NE (ng/kg)	Mean BP (mmHg) SN n=6	C n=7	Dose A (ng/kg)	Mean BP (mmHg) SN n=6	C n=5
100	11±2##	22±2	50	18±2	23±3
400	27±4##	44±3	200	35±4	44±3
800	37±5##	53±3	400	39±4#	49±2
	SN vs. C	#p<.01		##p<.001	

The decrease in BP response in the SN rats to both NE and A was correlated to the increase in BUN (NE $r=0.8$ $p<.05$; PE 0.9 $p<.05$). In contrast to either NE or A no response to i.v. vasopressin was seen between the SN and C rats.

Taken together SN rats with established chronic uremia exhibit 1) decreased baroreceptor function which may contribute to their hypertension and 2) decreased sensitivity to NE and A but not to AVP.

IN VIVO ALUMINUM ABSORPTION BY THE DUODENUM IN CHRONICALLY UREMIC RATS. E.J. Filippone*, A.J. Adler, G.M. Berlyne, Brooklyn, New York.

The toxicity of large oral doses of aluminum used for phosphate binding in chronic uremia has been previously suggested. In addition, intestinal absorption has been demonstrated by a rise in blood aluminum levels and urinary excretion following an oral aluminum load. However, the permeability of the intestinal barrier to aluminum has not yet been determined.

We studied the duodenal absorption of aluminum in normal and chronically uremic Sprague Dawley rats (BUN 76±2lmg/dl) by means of an in vivo perfusion method. Animals were rendered uremic by a two-stage 5/6 nephrectomy method and allowed to stabilize for 4 weeks. The duodenum was cannulated at the pyloric sphincter and at the ligament of Treitz. The duodenal loop ranged in length from 7-10cm and was continuously perfused at 0.3ml/min for 1 hour with an aluminum containing solution warmed to 37•C.

The solution contained $AlCl_3$-5μM/L, NaCl-140mM/L, KCl-4mM/L, $CaCl_2$-1mM/L; and glucose-23mM/L. Perfusate and duodenal effluent were collected at 10 minute intervals and analyzed for aluminum by flameless atomic absorption spectrophotometry. At the conclusion of the infusion, the loop of duodenum was removed and dried to constant weight.

Following a 10 minute equilibration period at steady state, the mean aluminum absorption, corrected for H_2O fluxes, was 0.51±0.14 μM/min/gm dry weight in normal rats and 0.54±0.14 μM/min/gm dry weight in uremic rats.

The results indicate that there is significant aluminum absorption by the duodenum and that the rate is not affected by chronic uremia.

ERYTHROPOIETIN (Ep) WORKS NORMALLY IN UREMIC ANEMIC SHEEP (UAS). J. Eschbach, J. Mladenovic*, J. Garcia*, J. Kaup*, G. Kaylor*, and J. Adamson.* Dept. of Medicine, University of Washington, Seattle, WA, and the Lawrence Berkeley Laboratory, University of California.

The effect of Ep on the anemia of uremia was studied in UAS made chronically uremic by subtotal nephrectomy compared to controls (C). Ep levels and $T_{½}$ were measured by RIA to exclude an effect of reduced Ep clearance on results. Baseline values were:

	N	Cr, mg/dl	Hct, %	Ep, mU/ml	Ep $T_{½}$, hr
UAS	5	6.5±3.4	19.4±2.2	46.5±16.9	9.8±3.1
C	9	1.0±1.1	32.3±2.1	36.0±8.3	10.8±2.7

Infusing Ep-rich sheep plasma, 11.5±1.2 U/kg/day for 10 days, caused identical increases in reticulocytes and plasma iron turnover and shortening of marrow transit time in both groups. All UAS corrected their anemia with a similar dose of Ep daily for 15-40 days. Increase in red cell mass (RCM) correlated with total dose of Ep infused ($r=0.84$); hct 19.4±2.2 → 31.8±1.4; RCM 8.0±4.2 ml/kg → 14.9±3.7 ml/kg. Two of the 5 UAS needed dialysis 2-3 times a week. Their response to Ep was similar to non-dialyzed UAS. In vitro, normal or uremic marrow responded equivalently to graded doses of Ep in the presence of normal or uremic sheep serum. Thus, in uremia: Ep clearance is normal; the effect of Ep on erythropoiesis is normal in vivo and in vitro; and Ep corrects the anemia. There is no evidence for inhibitors of Ep in UAS. These results predict that Ep should be an effective therapy for the anemia of chronic renal failure in humans.

THE NATURE OF THE ADAPTIVE RESPONSE OF THE PROXIMAL TUBULAR (PT) BRUSH BORDER MEMBRANE (BBM) IN THE REMNANT KIDNEY. Leon G. Fine, Edward P. Nord*, and Timothy Bradley*. Division of Nephrology, UCLA School of Medicine and Department of Developmental and Cell Biology, Univ of Calif, Irvine, CA.

In the remnant kidney (RK) model of chronic renal failure, SNGFR is increased 2-3 fold and absolute proximal reabsorption by the PT increases in parallel. To examine whether adaptation occurs at the level of the BBM, we studied the kinetics of two fundamentally different Na transport systems, i.e., a symporter (Na-succinate cotransport) and an antiporter (Na-H exchange) as well as Na permeability (P_{Na}) in normal and RK BBM. Normal and RK BBM were identical with respect to EM appearance and enzyme markers:

	K_t (mM)		J_{max}(nmol/mg/min)	
	Normal (N)	RK	Normal (N)	RK
Na-Succinate	0.65	0.71	51	49
Na-H (pH_i = pH_o)	37	42	50	45

P_{Na} was about 0.4 μl/mg·min in both normal and RK BBM. Histomorphometric analysis of PT fixed during perfusion revealed that BBM surface area/outer tubular diameter ratio was the same in N and RK tubules. Since outer diameter is increased by 25% in RK PT, and cell number/unit length is unchanged, there is an absolute increase in BBM surface area/cell. Conclusion: Hypertrophy leads to an increase in BBM per cell in the RK. Since the functional properties of the BBM are unchanged, enhanced Na uptake per cell is dependent on the extent of hypertrophy.

FILTRATION DYNAMICS AND BARRIER DYSFUNCTION IN GLOMERULONEPHRITIS. S. Friedman,* C.R. Bridges,* W.M. Deen, B.M. Brenner, and B.D. Myers. Depts. of Med., Stanford Univ., Stanford, CA and Harvard Univ., Boston, MA and Dept. of Chem. Engineering, M.I.T., Cambridge, MA.

Colloid volume expansion (CVE) magnifies the proteinuria associated with glomerulonephritis (GN). To elucidate this phenomenon we performed differential, fractional macromolecule clearances (θ) prior to and following infusion of hyperoncotic solutions in 15 subjects with GN. Plasma volume (+33%), oncotic pressure (+16%) and renal plasma flow (+16%) were all significantly higher during CVE than euvolemia. The glomerular filtration rate (GFR), by contrast, remained constant, 39 vs. 36 ml/min/1.73m^2, respectively. With CVE, urinary excretion of albumin and immunoglobulinG (IgG) increased from 6±1 to 13±4 and .5±.2 to .9±.2 mg/min, respectively. The corresponding fractional clearances (θ) increased 40% ($p<.005$) for albumin and 27% ($p<.025$) for IgG. CVE had no effect on θ for smaller, neutral dextrans (radii 20-36Å), but selectively increased θ for larger dextrans (radii 38-56Å) by between 20 and 31% ($p<.05$-$.005$). We subjected θ for dextrans (radii = 20-56Å) to a model of solute transport through a bimodal pore size distribution. Pore radius in the lower mode ($r_1 \simeq 53$Å) and upper mode ($r_2 \simeq 110$Å) in euvolemia was unaffected by CVE. In contrast, the fraction of total GFR permeating the large pores of radius r_2 increased from .04 in euvolemia to .06 during CVE. We conclude that CVE-induced elevation of glomerular pressures and flows increases the area-fraction of the nephritic membrane occupied by large, protein-permeable pores.

VERAPAMIL IMPROVES DEFECTIVE 1,25-DIHYDROXYCHOLECALCIFEROL SYNTHESIS AND DUODENAL CALCIUM ABSORPTION IN EXPERIMENTAL UREMIA. **M. Goligorsky, C. Chaimovitz, S. Shany and J. Rapoport. Soroka Med. Ctr., Beer-Sheba, Israel. (intr. by T. Berl)**

Accumulation of calcium (Ca) in kidneys has been observed in uremic rats. We examined whether this Ca accumulation contributes to the impaired 1,25-dihydroxycholecalciferol [1,25(OH)$_2$D$_3$] synthesis in uremia. Three groups of rats were studied: 1) subtotally nephrectomized (SNX) rats 3 weeks after surgery; 2) SNX rats treated with verapamil (Ver) intramuscularly 0.1 mg/kg twice a day for 3 weeks; 3) control rats with intact kidneys (C). The following studies were performed: Ca content of kidney remnants (CaR); in vivo fractional duodenal ^{45}Ca absorption (FDACa); and plasma concentration of 1,25(OH)$_2$D$_3$. Degree of azotemia was similar in SNX and SNX+Ver. Results were as follows.

	CaR mcg/g dry weight	FDACa %	1,25(OH)$_2$D$_3$ pg/ml
C	142+36	72+7	32+5
SNX	251+52*	23+5*	29+8
SNX+Ver	158+76**	53+9**	50+9**

*** $p<.05$, SNX vs C; ** $p<.05$, SNX+Ver vs SNX. Conclusions: 1) The elevated renal Ca content occurring in SNX rats is prevented by Ver; 2) Ver administration leads to elevation of the 1,25(OH)$_2$D$_3$ to supranormal levels and to consequent enhancement of FDACa; 3) The data support the hypothesis that renal Ca accumulation plays a critical pathogenetic role in the impaired synthesis of 1,25(OH)$_2$D$_3$ in chronic uremia.**

EXERCISE TRAINING RAISES HIGH DENSITY LIPOPROTEIN CHOLESTEROL, LOWERS PLASMA TRIGLYCERIDE AND NORMALIZES LIPOPROTEIN LIPASE ACTIVITY IN HEMODIALYSIS PATIENTS. A.P. Goldberg,*J. Hagberg‡ R. Florman,* T. Kuusi, * E. Nikkila, * H. Harter, Washington University School of Medicine, St. Louis, Mo. and University of Helsinki,Finland.

Hemodialysis patients (n=12) with high triglyceride (TG) (277±245mg/dl,x̄±SD) and low plasma high density lipoprotein (HDL) cholesterol (CHOL) (31±9) levels, exercise trained for 12±4mo. Their maximal aerobic capacity ($\dot{V}O_2$max), the best index of physical fitness, was low (21±7,ml O_2/Kg/min) compared to normal (38±4, $p<.01$). Exercise training increased $\dot{V}O_2$max by 21% (25±9, $p<.01$) and HDL-CHOL by 22% (36±11, $p<.01$), and lowered TG by 26% (177±98, $p<.05$). Total and low density lipoprotein CHOL did not change. Sedentary hemodialysis patients (n=8) with low $\dot{V}O_2$max (20±7), TG (174±76) and low HDL-CHOL (30±6) were followed for 8±4mo. There were no changes in their $\dot{V}O_2$max, TG or HDL-CHOL levels.

Hemodialysis patients had low postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities in the untrained state. Exercise training increased LPL by 30% (7.8±2.8 to 10.6±4.3, µmole FFA/ml/hr, $p<.01$) and lowered HL by 17% (13.3±10.2 to 10.4±7.0, $p<.01$). HL and LPL activity did not change in sedentary hemodialysis patients.

Thus, exercise training improves lipid profiles in hemodialysis patients by normalizing LPL activity. This suggests that a sedentary lifestyle contributes to the lipid abnormalities in hemodialysis patients.

RELATIONSHIP BETWEEN THE INTRACELLULAR Na LEVEL AND THE HEMODIALYSIS BATH Na LEVEL. P. Gorfien,* H.I.Lipner, B.M.Louis, C.Sreenivasan,*A.Lock,*and N.L.Manohar, Maimonides Med. Ctr., Div. of Nephrology, Brooklyn, New York.

The relationship between the hemodialysis (HD) bath Na level and the patient's intracellular (RBC) Na levels were tested in a group of 14 HD patients (3 diabetics, 10 stable nondiabetics and one ill nondiabetic). The diabetics consistently had higher RBC Na at 7.39(mEq/L of RBC), compared to 5.09 in the stable nondiabetics. One ill nondiabetic with complications due to noncompliance with HD regimen, has a high RBC Na of 8.28 and he is currently recuperating on a higher Na bath HD. The nondiabetics who were sick in the past and have been stabilized on higher Na (148-156mEq/L) bath HD are currently having lower RBC Na levels comparable to that of 42 healthy nonuremic controls (5.19mEq/L of RBC).

The hemodynamic stability of higher Na bath dialysis could lead to better clearance of the uremic toxin(s) that block the cell Na-K ATP'ase pump, and thereby lower the RBC Na towards the normal over a period of several months which lags behind the clinical improvement. This lowering of RBC Na has so far not been achieved in the diabetics at the end of 12 to 18 months of higher Na bath HD, despite marked improvement in clinical status and well being.

Frequent monitoring of the intracellular Na levels and reciprocal adjustments of the dialysis bath Na seems to be indicated to maximize the correction of cellular hypernatremia and cellular overhydration, in all groups of chronic HD patients.

INFLUENCE OF STRICT CONTROL OF DIABETES ON INTRARENAL HEMODYNAMICS. T.H. Hostetter, T.W. Meyer*, H.G. Rennke, and B.M. Brenner, Departments of Medicine & Pathology, Harvard Medical School and University of Minnesota, Boston and Minneapolis.

Glomerular capillary hypertension and hyperfiltration occur in moderately but not in severely hyperglycemic diabetic rats (KI 19:410). We further examined the relation of diabetic treatment to glomerular dynamics in three groups of unilaterally nephrectomized (NPX) rats. Control rats (CONT) had NPX only. Moderately hyperglycemic rats (MOD) received streptozotocin (S) (60 mg/kg) after NPX and then NPH insulin (2.5U q.d.). Strictly controlled rats (STRICT) also received (S) after NPX and then heat treated ultralente insulin (2.2-4.4 U q.d.). All rats were studied 2 to 3 weeks after NPX and S. Results: mean ± sem, * $p < .05$ vs. CONT., + $p < .05$ vs. MOD.

	Glucose mg/dl	SNGFR ---nl/min--	Q_A	BP --mmHg--	$\overline{\Delta P}$
CONT.	131	61	189	114	38
n=10	± 7	3	12	2	1
MOD.	380*	74*	261	116	52*
n=10	39	3	32	3	2
STRICT	60*+	60+	218	117	40+
n=14	7	4	17	3	1

MOD rats had single nephron hyperfiltration with increased net ultrafiltration pressure due to increases in the mean glomerular transcapillary hydraulic pressure gradient ($\overline{\Delta P}$) and glomerular plasma flow (Q_A). STRICT rats had values of SNGFR and its determinants not different from CONT. Others have prevented glomerular pathology with strict glucose control; the present data suggest this protection may be due to prevention of diabetic intrarenal hypertension and hyperperfusion.

ALTERATIONS OF NA-K PUMP IN ERYTHROCYTES FROM PATIENTS WITH CHRONIC RENAL FAILURE (CRF): D. Kaji, J.T. Cheng*, and Thomas Kahn. Bronx V.A. Med. Ctr. and Mt. Sinai School of Medicine, New York, N.Y.

Some patients with CRF are reported to have elevation of intracellular sodium. We have shown that RBC [^{3}H] ouabain binding (o/b) is decreased significantly in CRF patients with high red cell sodium as compared to normals (275±51 v/s 455±59, $p < .005$). Reduced [^{3}H] o/b in CRF patients may be consequent to 1)ouabain-like retained uremic toxin which binds tightly to Na-K pump sites and competitively inhibits radioligand ouabain binding 2)a factor in uremic plasma which alters pump conformation to render the sites inaccessible to [^{3}H] ouabain 3)decreased synthesis of pump sites during erythropoeisis.

In order to evaluate whether a retained uremic toxin decreases [^{3}H] o/b, normal RBCs were incubated in plasma for 24 hr at 37C as follows:

Normal erythrocytes in:	o/b after incubation:
(a) Own plasma (10)	414±35
(b) Heterologous normal plasma (33)	387±52
(c) Plasma CRF-low [^{3}H] o/b (47)	390±62

Plasma from CRF patients with reduced o/b (c) did not lower o/b in normal RBCs more than plasma, from normal subjects (b). Thus, lower o/b in RBCs from these patients does not appear to be due to competitive inhibitor in plasma of these patients.

In separate studies, the affinity of Na-K pump for [^{3}H] ouabain was found to be similar in RBCs from CRF patients with low o/b as in normals, suggesting that measured reduction in o/b is not consequent to a conformational change in Na-K pump.

The decrease in [^{3}H] o/b in CRF patients is probably consequent to a decreased synthesis of Na-K pump sites during erythropoeisis.

BLUNTED RESPONSIVENESS OF THE REMNANT KIDNEY TO CHANGES IN PHYSICAL FACTORS. K.H. Hwang*, M. Ashby*, G. Bourgoignie*, and J.J. Bourgoignie. University of Miami School of Medicine, Miami, FL.

We have recently described an inability of the remnant kidney to rapidly excrete Na or K loads in-vivo. To evaluate whether these functional limitations are intrinsic to the kidney, we compared normal (N) and remnant (R) rat kidneys during in-vitro perfusion. At 110mm Hg and 6.5 g% albumin, we observed:

	CIn ml/min	RPF ml/min	UV µl/min	UNaV µeq/min	UKV µeq/min	UPV µg/min
N	1.48	46	48	3.5	1.5	92
R	0.15*	27*	25	2.7	0.5	86

		Resistance mmHg/ml	U/PIn	FENa •%	FEK %	FEP %
+p<0.05	N	2.4	48	1.6	19	2
*p<0.01	R	4.0*	6.3	37+	118*	36*

Increasing perfusion pressure progressively from 70 to 190mm Hg increased markedly the differences in all parameters, including UNaV and UKV; only UPV remained constant in both groups. GFR autoregulation prevailed at lower perfusion pressure in N than in R. When perfusate albumin was decreased from 8 to 6.5 g% at constant perfusion pressure and flow, GFR, UV, UNaV and UKV increased 4 to 8 -fold in N without changes in R. Thus, 1) The increased fractional water and solute excretion characteristic of chronic renal insufficiency in-vivo prevails in-vitro in an isolated perfusion setting, away from neurogenic and hormonal influences; 2) R demonstrates a blunted diuresis, natriuresis and kaliuresis when hydrostatic pressure increases or oncotic pressure decreases. These observations define the functional abnormalities within the remnant kidney.

REDUCED FECAL ALUMINUM (Al) EXCRETION IN RATS FOLLOWING PARATHYROID HORMONE (PTH) OR BILE DUCT LIGATION. AM Klein*, MA Burnatowska-Hledin*, J Kovan*, GH Mayor, Dept. of Med., Michigan State Univ., E. Lansing, MI.

Rats fed Al and treated with PTH achieve total body Al burdens exceeding 3mg before, and 0.5mg 5 days after PTH withdrawal. Since the route of Al egress after PTH withdrawal is unknown, this study compares urinary and fecal Al excretion. Rats were fed a control diet (Group I, n=5), or 0.1% Al supplemented diet and given 2.75 IU PTH twice daily for 7 days (Groups II-IV, all n=5). On day 8, all rats were placed on a normal diet to allow fecal clearance of exogenous Al. Subsequent fecal Al appearance was taken as a measure of biliary Al excretion. To evaluate endogenous Al egress Group II continued receiving PTH, Groups I, III and IV received vehicle. Group IV was bile duct ligated and groups I-III were sham operated. Urine and feces collected on days 7,9,10 and were analyzed for Al by plasma emission spectrophotometry. Serum calcium and creatinine were normal in all rats. Urinary Al excretion was less than 1% of total Al excretion in all rats regardless of treatment. Mean fecal Al excretion (mg) on day 7 was 0.96±0.15 in Group I and 10.45±2.17 in Groups II to IV: 0.96± 0.16, 1.96±0.25, 4.17±2.1 and 0.92±0.48 on day 9, and 1.69±0.16, 0.94±0.18, 1.71±0.16, and 0.95±0.19 on day 10 in groups I to IV, respectively. The significant difference in fecal Al excretion between Groups II and III on days 9 and 10 accounts for previously observed decrease in body burdens after PTH withdrawal. The significant difference in Al excretion between groups IV and II on day 9 indicates that bilary excretion is responsible for a large fraction of Al eliminated from the body.

ALTERED CONTROL OF EXTRACELLULAR FLUID VOLUME (ECFV) IN CHRONIC RENAL FAILURE (CRF) AS STUDIED BY RAPID SALINE INFUSION. Hendrik A. Koomans, Anton B. Geers, Peter Boer, Jan C. Roos and Evert J. Dorhout Mees, intr. by John E. Kiley, Dept. of Nephrology and Hypertension, University Hospital, Utrecht, The Netherlands.

From animal experiments Floyer* postulated that the intact kidney has some positive influence on interstitial space compliance. A decrease of interstitial compliance in CRF may limit interstitial fluid expansion. In search of such an abnormality a standardized rapid infusion test was applied: 25 ml/kg body weight 0.9% saline was infused in 30 min in 9 normals and 11 patients with end stage CRF (supine, no antihypertensives, dry weight). The increments of plasma volume (PV, ^{131}I-albumin distribution volume) and blood volume (BV, from PV and hematocrit, Ht) were monitored during 2 hours using changes in Ht and ^{131}I dilution, whereas changes of ECFV (^{82}Br distribution volume) were calculated from control ECFV, infused volume and natriuresis. Immediately after the infusion ECFV had increased 10.7% in both groups, but the increases of PV and BV were larger in CRF patients (ΔPV 17.9± 4.0 vs. 11.9±3.2%, $p<0.05$ and ΔBV 13.2±3.5 vs. 7.8±1.4, $p<0.01$). At 90 min postinfusion BV/ECFV ratio was significantly decreased from control in the normal subjects, but slightly increased in the CRF patients. It is concluded that in severe renal failure the control of ECFV-distribution is changed, leading to a preferential distribution of rapidly infused saline in the intravascular compartment.

*Floyer, M.A. Clinical Nephrology, 4:152-156, 1975.

INTESTINAL GENERATION OF THE PRECARCINOGEN NITROSO-DIMETHYLAMINE (NDMA) IN UREMIA. P.S.Lele*, S.R.Dunn*, and M.L.Simenhoff, Division of Nephrology, Dept. of Medicine, Jefferson Medical College, Philadelphia, Pa.

We have previously demonstrated in advanced chronic renal failure (CRF), generation of intestinal dimethylamine and bacterial overgrowth. (Lancet II:818-821,1976). The latter is important in choline degradation to methylamines and in nitrosation in the small bowel. We now present additional data on in vivo generation of NDMA, a powerful precarcinogen formed when amines react with nitrite (NO_2^-). We also investigated the role of anti-oxidants such as L-ascorbic acid (AA) on the generation of NDMA since there is increasing evidence that AA reduces in vitro nitrosation in gastro-intestinal model systems, by competitive binding for NO_2^-. Eleven CRF patients and 9 controls had gastro-duodenal intubation. Three patients then ingested 6 gms AA daily for 14 days and were re-intubated. Mean blood AA levels were 8 mg% (N=0.2-0.5mg%). Blood and duodenal aspirates were analyzed for NDMA. The results of bacterial cultures confirmed small intestinal bacterial overgrowth of both aerobic and anaerobic organisms.

Mean NDMA levels (ng/kg ± S.E.M.) in control and CRF patients for blood was 223 ± 77 and 241 ± 62 (not statistically significant) and for duodenal aspirate: 70 ± 20 and 292 ± 35 ($p < .002$) respectively. Following AA administration in 3 patients mean NDMA in duodenum fell from 286 ± 50 to 210 ± 60 ng/kg. We conclude that there is increased generation of NDMA in uremia. A role for anti-oxidants in preventing nitrosation remains to be defined.

PHOSPHATE (P) RESTRICTION OVERRIDES THE DECREASE OF Tm_{PO_4}/GFR IN UREMIA, INDEPENDENT OF PTH. E. Kraus*, N. DiCianni*, G. Briefel, L. Cheng*, B. Sacktor*, D. Spector. Div. Renal Med., Balto. City Hosp. and GRC, NIH, Balto., MD.

To identify factors regulating renal P excretion in uremia, five groups of pair-fed male Sprague-Dawley rats underwent parathyroidectomy (PTX) or sham PTX, followed by 1 2/3 nephrectomy (NX) or sham NX, followed by normal (0.7%) or low (0.1%) P diet for three weeks. Plasma Ca (mM) and P (mM), GFR (CIn/Kg, ml/min.), and fractional excretion of P (FEP,%) were determined. Maximal tubular reabsorption of P (Tm_{PO_4}/GFR, μM/ml) was calculated after six 20 minute periods of phosphate infusion.

Group:	I	II	III	IV	V
Diet:	Normal	Normal	Normal	Low	Low
Kidneys:	Intact	NX	NX	NX	NX
Parathy:	PTX	Intact	PTX	Intact	PTX
Rats,(n)	6	7	7	11	7
GFR	7.5±.4	3.1±.2	2.4±.3	2.8±.3	2.9±.2
Ca	1.6±.2	2.2±.1	1.6±.1	2.4±.1	2.6±.1
P	4.1±.3	2.3±.2	4.0±.3	2.4±.2	2.3±.2
FEP	9±2	46±7	37±5	5±1	5±1
Tm_{PO_4}/GFR	5.0±.5	1.6±.2	3.0±.4	4.7±.4	6.9±.2

In uremia phosphate reabsorption (Tm_{PO_4}/GFR) is inhibited by both parathyroid hormone (II vs III, $p<.02$) and a non PTH factor (III vs I, $p<.01$). These factors are additive (I vs II + III). A low phosphate diet enhances phosphate reabsorption in uremia in the presence (IV vs II, $p<.001$) or absence (V vs III, $p<.001$) of parathyroid hormone. Thus, low P diet overrides the decrement in phosphate reabsorption found in uremia.

INCREASE NEUROMINIDASE ACTIVITY (NMA) IN UREMIA, ITS ROLE IN ANEMIA OF CHRONIC RENAL FAILURE (CRF). J. Levi, H. Levinsky, U. Gafter, D. Allaluf, (intr. by C.R. Kleeman), Depts. of Nephrology and Biochem. Hasharon Hospital, Petah-Tikva, and Tel Aviv Univ. Med. School, Israel.

Anemia in CRF is attributed to increased destruction and defective production of RBC's. Aging and shortened life span of RBC's is partially related to decreased amount of sialic acid (SA) in their membranes. A study was carried out on 22 ESRD patients to determine 1)SA content in erythrocytic membranes and 2) NMA in sera. Blood bank donors served as controls. SA was determined by the method of Schouer. Patients with hemoglobin of 6.6±0.3g/dl had 59.0±3 nmol/10^9 RBC's SA compared to 59.6±2.5 in controls (n=6) with hemoglobin in 13.7±0.2g/dl. The NMA of uremic sera was found by incubation with blood group matched RBC's from blood bank donors and determination of SA in the RBC's after the incubation. Furthermore, sera was incubated with fetuin (Sigma) a commercial substrate for NMA and the amount of SA was determined. Sera from blood bank donors incubated with the respective RBC's and fetuin served as controls. Erythrocytic SA after incubation with uremic sera (n=13) was 47.8±2.2 much less than after incubation with control sera (n=5) 61.6±2.1 nmol/10^9 RBC's ($p<0.001$). Also, uremic sera (n=15) released more SA from fetuin 4.26±0.47 nmol compared to control sera (n=13) 2.29±0.37 nmol ($p<0.0025$).

In conclusion: Increase NMA in the uremic serum suppress erythropoietic activity in uremia and play a role in the anemia of CRF. The similar SA values could be explained by the relatively younger RBC population in uremia.

LIVER BUT NOT PITUITARY HYPOTHYROIDISM IN A UREMIC RAT MODEL. V.S. Lim, C. Passo*, Y. Murata*, E. Ferrari* and S. Refetoff*. Dept. of Med., Univ. of Iowa Col. of Med., Iowa City, IA, and Thyroid Study Unit, Univ. of Chicago Pritzker Sch. of Med., Chicago, IL.

To assess thyroid status in uremia, we measured the following in a partially nephrectomized rat model (Nx): In the serum (S), T_3 (ng/dl), TSH (ng/ml) and urea nitrogen (UN, mg/dl). In the liver (L), nuclear T_3 (nucl T_3, pg/mg DNA), T_3 binding capacity (C_{max}, pg T_3/mg DNA) and affinity (Ka,x10^9M-1), and mitochondrial α-glycerophosphate dehydrogenase (GPD, ΔOD/min/mg protein) activity. In the pituitary (P), T_3 content (pg/mg pit). Comparisons were made with control (C), thyroidectomized (Tx) and rats pair-fed with Nx rats (PF) as follows:

Group (n)	C (17)	Nx (12)	Tx (13)	PF (10)
Body wt(g)	368±8	231±13*	233±8*	316±13*
S,UN	23±1	147±13*	28±2	21±1
S,T_3	42.9±3.3	34.6±3.9	8.2±1.4*	43.3±2.4
S,TSH	617±29	409±52*	2731±259*	556±94
L,Nucl T_3	104±14	52±6*	16±4*	82±9
L,C_{max}	121±11	74±7*	91±10*	121±9
L,K_a	4.30±0.52	5.28±0.38	3.62±0.36	4.65±0.32
L,GPD	1.62±0.13	1.06±0.07*	0.81±0.10*	1.64±0.12
P,T_3	7.0±1.5	13.0±3.6	3.1±0.9*	7.8±1.1

Values are presented as mean±SEM; *p<.05.

The reduction in nucl T_3, C_{max} and αGPD in the Nx rats suggests hypothyroidism in the liver. The pituitary, however, remained euthyroid as its T3 content was normal. Thus, it appears that hypothyroidism in the uremic rat occurs selectively in the peripheral tissue only. Thyroid dysfunction in uremia is not caused by decreased food intake as PF rats did not manifest any of the abnormalities.

PROTEIN RESTRICTION AND RENAL FUNCTION IN THE UREMIC RAT. Michael A. Madden, Stephen W. Zimmerman, Dept. of Medicine, Univ. of Wis., Madison, Wisconsin

Fourteen rats subjected to 5/6 renal ablation (uninephrectomy and partial infarction) were randomized by residual renal function to 8% protein (PR), or 24% protein (NPR) diets, which were otherwise identical. Survival at 22 wks was greater in PR (45% vs 10%). In rats surviving at least 10 wks Ccr (ml/min) at 3 wks (PR:0.16/NPR:0.26), and 10 wks (PR:0.37/NPR:0.55) was greater in NPR rats (P<.05), then fell linearly in NPR (0.29), and stabalized in PR (0.41) at 22 wks. The rate of decline was less in PR after 10 wks. Proteinuria reached 200mg/d, and albumin excretion 102 mg/d in NPR, vs 72 mg/d and 42 mg/d in PR. Histology showed segmental sclerosis in 46.8% (PR) and 71.8% (NPR) of glomeruli (P<.05), tubular dilation and cast formation, and arteriolar sclerosis (NPR>PR). Peak blood pressure by tail cuff, unanesthetized, (NPR vs PR) was not different (192 vs 189.5) nor was heart weight (1.32 g. vs 1.46). PR rats gained more weight (372 vs 295 gm., P<0.5). Serum calcium (NPR vs PR) (5.08 vs 5.66 mEq/L) serum phosphate (12.7 vs 9.9 mg/dl), and tubular phosphate reabsorption (.71 vs .58) were not significantly different.

PR was associated with increased survival and amelioration of proteinuria, but Ccr was less than NPR at 3 and 10 wks. After 10 wks Ccr fell at a greater rate in NPR. There was no difference in dietary phosphate intake. These data suggest that 8% PR is beneficial and compatible with adequate nutrition as judged by serum albumin and weight gain, but preservation of Ccr is late, possibly related to a blunting of early "hyperfiltration injury."

SERUM RIA-SOMATOMEDIN-C (Sm-C) IS DEPRESSED IN CHILDREN AND ADOLESCENTS WITH END STAGE RENAL DISEASE. Robert E. Lynch, Richard W. Furlanetto,* Luther B. Travis, Robert J. Cunningham and S. Michael Mauer. Cardinal Glennon Hos., St. Louis, Children's Hos., Philadelphia, Univ. Texas Medical Branch, Galveston, Univ. Minn. Hos., Minneapolis.

Studies of Somatomedin activity in growing patients with chronic renal failure have produced conflicting conclusions, related in part to variations in methodologies and Somatomedin fractions assayed.

We report here serum levels of Sm-C determined in children and adolescents with renal disease and compared to levels determined in age and sex matched controls. Patients with ESRD included 25 males, mean age 7.8 years (0.75-15), and 21 females, mean 9.8 years (0.92-17). Twenty five control males averaged 8.0 years (0.75-15), and 21 control females averaged 10.2 years (0.92-17.9). Mean Sm-C for ESRD, age 0-5=0.17 units, C=0.25; 6-10=0.20, C=0.84; 11-17=0.95, Control=1.65. Comparison of matched pairs by Wilcoxan signed ranks test confirmed a highly statistically significant depression of Sm-C in ESRD patients, Z=5.25, $p < 0.001$. The decrease was present in both sexes. Cross sectional data from this study suggests that a pubertal rise in Sm-C does occur in ESRD patients, but that the rise is both delayed and blunted compared to normals.

Nine additional patients age 9.2-17.9 years with well-functioning transplants did not differ significantly from age and sex matched controls for Sm-C level.

It is concluded that chronic renal failure leads to depression of Sm-C levels through an as yet unknown mechanism.

RELATIVE ROLES OF ERYTHROPOIETIN (Ep) AND INHIBITORS OF ERYTHROPOIESIS IN THE ANEMIA OF CHRONIC RENAL FAILURE (CRF). R.J. McGonigle*, J.D. Wallin, R.K. Shadduck*, W.M.O'Neill Jr., J.W. Fisher. Dept. Pharm. & Int. Med. (Neph), Tulane Univ. Sch. Med., New Orleans, LA and Dept. Int. Med. (Hematol.), Univ. Pittsburgh, PA.

Sixty predialysis CRF patients, 30 anemic patients with normal renal function and 40 normal subjects had the following studies performed: serum creatinine (Cr); hematocrit (Hct); Ep by radioimmunoassay (RIA); erythroid (CFU-E) and granulocytic (CFU-GM) colony formation using fetal mouse liver and human bone marrow cultures, respectively; and parathyroid hormone (PTH) using a mid-terminal RIA. In uremic patients the following correlations were found: a) Hct correlated (inversely) with Cr (p<.001); b) both Hct (inversely) and Cr (directly) correlated with % CFU-E inhibition (p<.001; p<.001,respect.);c) both Hct (inversely) (p<.01), Cr (directly) (p<.001) and % CFU-E inhibition (directly) (p<.01) correlated with PTH; d) Ep failed to correlate with Hct, Cr or % CFU-E inhibition. Mean Ep levels 33.6 mu/ml (range 11-406) were elevated above normal values 14.9 mu/ml (range 9-37). In anemic patients with normal renal function: a) Hct correlated(inversely) with CFU-E stimulation (p<.001); b) Ep correlated (inversely) with Hct (p<.001) and(directly)with CFU-E stimulation (p<.01). CFU-GM formation was significantly increased in uremic patients compared to normal subjects. We conclude that uremic toxin inhibitors of CFU-E, but not CFU-GM, which may possibly be polyamines or PTH, in addition to a relative Ep deficiency are primarily responsible for the anemia of CRF.

EFFECTS OF AMINO ACID INFUSIONS (AAI) ON CELL METABOLISM IN HEMODIALYZED UREMICS. Jack Metcoff, Seshachalam Dutta, Gayle Burns, Paul Costiloe, James Pederson, Billy Matter and Owen Rennert. Univ. of Oklahoma Health Sci., Ctr., Depts. Pediat., Biochem., Med., & Biostat., VA Hosp., Midwest Dialysis Ctr., Oklahoma City, OK.

Eighteen uremics received intravenous AAI (10% Aminosyn, Abbott) 3x/wk for 3 mo. based on the hypothesis that correcting cellular amino acid imbalance would improve reduced protein synthesis (Psyn = ^{3}H-leucine incorporation), energy level (energy charge = Ech = ATP + ½ ADP/ATP + ADP + AMP) and activity of the rate limiting glycolytic enzyme pyruvate kinase (PK) which were reduced in these patients vs 32 control normal subjects. The circulating leukocyte (88 ± 5% granulocytes) was used as a cell model. They were a subset of 42 uremics stabilized by maintenance hemodialysis and had reduced **cellular** levels of threonine, isoleucine, methionine and ornithine, with increased levels of aspartate, glycine, arginine, tyrosine and phenylalanine. Previously, multiple regression best subset analysis showed that a combination of the cell levels of aspartate, valine, isoleucine, ornithine, lysine and tryptophan could "explain" 40% of the variance in protein synthesis in these uremics. Similarly, a combination of the levels of aspartate, glutamic acid, glycine, ornithine and arginine were "predictive" of the level of energy charge. After 3 mo. of AAI, levels of the predictive intracellular amino acids did not improve, but deviated further from baseline values. Nor were Psyn, Ech and PK significantly above baseline, but Psyn was not significantly ($p > .05$) < controls. Failure to detect significant improvement in the cell bioactivities is attributed to uncorrected imbalance in the intracellular amino acid pool.

K HOMEOSTASIS IN CHRONIC INTERSTITIAL NEPHRITIS (CIN). Guido O. Perez, Rene Pelleya,* James R. Oster, Carlos A. Vaamonde, and David C. Kem.* VA Med. Ctrs. & Univ. of Miami, Miami, FL & Univ. of Oklahoma Health Sci. Ctr., Oklahoma City, OK.

We studied renal K handling and plasma K and aldosterone (PA) responses to acute oral K loading in 11 pts with CIN (Ccr 36±5 ml/min [SE]) and 13 controls (C) (Ccr 123±5). After 4 days of a 10 mEq Na, 50 mEq K diet, the pts received 0.25-0.50 mEq KCl/Kg. Blood and urine specimens were then collected for 4 hrs. Prior to KCl there were no significant differences between the groups in P_K or U_KV, but the $P_{[HCO_3]}$ was lower in CIN (22±1 vs 27±1 mMol/L; $p<.001$); five pts had suppressed PA (HA) and 5 had normal PA (NA).

	ΔP_K (mEq/L)	U_KVmax (µEq/min)	%Exc*	%ICF+	ΔPA (%)
C (13)	0.8±0.1	214±21	54±5	18±5	40±16
p	NS	<0.001	<0.01	<0.01	NS
CIN (11)	1.0±0.1	84±19	13±4	42±6	127±62

* % load excreted and + % retained in ICF at 4 hr.

Following KCl, maximal U_KV was lower, less of the load was excreted, and more was retained in the ICF of CIN than C. P_K was higher 3 hr post KCl and ΔP_K/dose given was higher in CIN($p<0.05$). Both the % change in PA and its ratio to ΔP_K tended to be higher in CIN. The 5 HA pts tended to have greater ΔP_K (NA: 0.8±0.1; HA: 1.2±0.3 mEq/L), ΔPA and lesser U_KVmax (NA: 125±23; NA: 51±11 µEq/min), % Exc (NA: 21±7; HA: 7±2) and % ICF. We conclude that patients with CIN and moderate renal insufficiency have impaired ability to excrete an acute oral K load even when their PA is normal or supranormal. Nevertheless, effective intracellular translocation of K obviated the development of hyperkalemia.

PRESERVATION OF RENAL STRUCTURE AND FUNCTION BY LONG TERM PROTEIN RESTRICTION IN RATS WITH REDUCED NEPHRON MASS. T.W. Meyer,* T.H. Hostetter, H.G. Rennke, J.L. Noddin,* and B.M. Brenner. Brigham and Women's Hosp., Harv. Med. Sch., Boston, MA.

We studied the effects of chronic dietary protein restriction on changes in renal function and glomerular morphology induced by reduction of renal mass. Rats with intact kidneys (C), a single kidney (Nx), or a single kidney of which 1/3 was infarcted (NxI) were kept for 8 months on either a 40% casein (HP) or 6% casein (LP) diet. BUN, left kidney inulin clearance (Cin), protein excretion (UprotV) and the percentage of glomeruli showing global and focal sclerosis (%glom. scler.) were then determined. Results: *$p<.05$ vs comparable LP group, + $p<.05$ vs C group on same diet (n=9 or 10 for each group).

	-Control-		---Nx---		---NxI---	
	LP	HP	LP	HP	LP	HP
BUN mg/dl ±1SE	10 ±2	30*	10 ±2	36*	9 ±2	74*+
Cin ml/min	.70 .09	1.30*	1.12+ .11	1.95*+ .13	.78 .10	1.22* .22
UprotV mg/day	5 1	15* 3	4 1	38*+ 9	16+ 3	57*+ 9
% glom. scler.	.5 .2	1.8* .4	5.2+ 1.6	11.3*+ 2.3	17.3+ 4.0	37.2*+ 6.6

With both diets graded loss of renal mass led to increases in UprotV and %glom. scler. Within each group LP was associated with lower BUN and Cin and with lesser UprotV and %glom. scler. than HP. Reduction in glomerular filtration rate accompanying protein restriction may thus limit progressive glomerular damage following loss of renal mass.

EFFECTS OF INSULIN ON HEMICORPUS PREPARATIONS FROM NORMAL AND UREMIC RATS. David Powell* and Malcolm Holliday. Dept. of Pediatrics, Univ. of California, San Francisco CA.

Uremia is associated with a decrease in insulin mediated glucose disposal, decreased insulin extraction by peripheral tissues and increased release of alanine (ALA) and glutamine (GLN) from incubated muscle. Muscle protein synthesis in the fasting state is also depressed. We examined glucose uptake, insulin extraction, amino acid exchange and muscle protein synthesis in a perfused rat hemicorpus preparation from uremic (U) and sham (S) operated animals. In each preparation one leg was perfused without and the other with insulin (225 uU/ml). Perfusate was Krebs-Henseleit buffer containing albumin (3 gm%), steer blood (Hct 33%), a.a. at plasma concentrations and glucose clamped at 200 mg%.

The U group showed no decrease in glucose uptake relative to shams either with or without insulin. Similarly, protein synthesis data revealed comparable basal values and increases with insulin in both U and S preparations. Insulin enhanced the uptake of branched chain amino acids to a greater extent in U (87%) vs. S (3%) preparations. ALA or GLN release into perfusate was unaffected by U or the presence of insulin. Insulin extraction was comparable in the two groups. We infer from these data that preparation washout fundamentally alters the uremic state; in particular the normal glucose uptake may be due to removal of circulating inhibitory factors.

ROLE OF HYPERTENSION (HPN) AND MESANGIAL (MES) INJURY IN PROGRESSIVE GLOMERULAR (GLOM) DAMAGE. L.Raij,S.Azar and W.F.Keane,U. of Minn.,Mpls.,MN.

HPN frequently accompanies chronic glomerulonephritis (GN). MES injury is common in GN and MES sclerosis (SC) often harbingers progressive Glom destruction. We hypothesized that HPN may influence the course and/or progression of MES-SC particularly when "protective" pre-Glom vasoconstriction is absent thus resulting in "Glom-HPN". To test this hypothesis experimentally, we induced MES ferritin-antiferritin immune complex (FIC) disease (ferritin 8 mg/100 g b.w., IP for 6 weeks) in a) 6 hypertensive SHR rats, b) 6 hypertensive Dahl salt sensitive (S) rats fed 8% NaCl chow, and c) 6 normotensive Dahl S rats fed 0.3% NaCl chow. Age-matched control (C) rats received IP saline. These strains were used because it has been shown that "protective" pre-Glom vasoconstriction is present in SHR but absent in Dahl S rats (Clin Sci 56:203,1979). Severity of MES-SC was evaluated by light microscopy (0-4+) and correlated with intensity of immunofluoresence microscopy (IF), 3 months after completion of immunization.

Group		BP	IF(IG/C3)	MES-SC
Dahl 0.3%	C	142±3	0/0	0-1+
	FIC	151±2	2+/2+	1-2+
SHR	C	170±2	0/0	0
	FIC	175±3	2+/2+	0-1+
Dahl 8.0%	C	175±4	0/0	1-2+
	FIC	183±5	2+/2+	3-4+

Thus, when "protective" pre-Glom vasoconstriction is absent (as in Dahl rats) MES injury and HPN act synergistically to induce progressive Glom damage. Clinically, the different rates of progression in human GN associated with HPN may be in part dependent on similar mechanisms.

ROLE OF 1,25 DIHYDROXY-VITAMIN D_3 ($1,25D_3$) IN PATIENTS (Pts) WITH EARLY RENAL FAILURE (ERF). L. Wilson*, M. Lam*, A. Felsenfeld, and F. Llach. Dept. of Med., Univ. of Okla. Health Sci. Ctr. and VA Med. Ctr., Okla. City, Okla.

Low levels of $1,25D_3$ may contribute to the skeletal resistance to parathyroid hormone and phosphate (P) retention in pts with ERF. This study evaluates these factors. EDTA infusion and oral P load (1 gm) were performed in 11 pts with ERF (C_{Cr} 40-90 ml/min) and 5 controls before (B) and after (A) 6 weeks of $1,25D_3$ (.25 mcg BID). Significant changes ($p<.01$) B and A $1,25D_3$ were: 1) Baseline: In ERF, serum calcium (S_{Ca}) increased from 9.0±.1 to 9.4±.2 mg/dl, Sp 2.6±.1 to 3.4±.1 mg/dl and 24 hr urinary Ca, 71±16 to 179±42. No changes were noted in controls.

2) EDTA Infusion: In ERF:

Time (Hrs)	0	2	8	12	24
S_{Ca}, B	9.1±.2	6.6±.2	7.7±.2	7.9±.2	8.2±.2
S_{Ca}, A	9.4±.2	7.0±.2	8.2±.2	8.4±.2	8.9±.2

No changes were observed in controls.

3) Oral P Load: In ERF:

Time (Hrs)		0	2	4	6
Sp	B	2.9±.2	3.6±.2	3.5±.1	3.5±.1
	A	3.3±.2	4.1±.2	3.7±.1	3.6±.1
Tmp/GFR	B	2.4±.2	2.3±.3	2.5±.2	2.2±.2
	A	2.9±.1	3.0±.2	2.5±.2	2.5±.09

No changes were observed in controls and their values were similar to those of ERF after $1,25D_3$.

In summary: In ERF treated with 1,25: a) Baseline S_{Ca},P and 24 hr U_{Ca} significantly increased; b) Correction of the EDTA-induced Ca↓ and recovery occurred: c)the renal handling of P normalized; d) no significant changes after $1,25D_3$ were observed in controls. In conclusion, these data provide support for the concept of $1,25D_3$ deficiency in ERF.

THE MAGNIFIED NATRIURESIS PER NEPHRON IN CRD: ON THE ROLE OF CHRONIC ECF VOLUME EXPANSION AND OF A PRE-EXISTING STATE OF Na ADAPTATION. Michael S.Shapiro*, Estela Mendoza*, Margalit Grunberger*, Neal S. Bricker, Prog. in Kidney Dis.,UCLA Sch. of Med.,Los Angeles,CA.

Na adaptation in CRD involves progressive increase in $U_{Na}V$ per nephron as nephron destruction proceeds assuming constant intake. A pivotal feature of the adaptation is that following a fixed Na+ load ΔFE_{Na} varies inversely with GFR. The present studies examined the relationship of a series of potential determinants of adaptation to the magnified natriuresis. 3 groups of Sprague-Dawley rats (150-250g)(N=58)were studied. Each received a specific Na diet: 1)Normal rats(NR): 3mEq/day; 2)Adapted uremic rats(AR):3mEq/day; 3)Unadapted uremic rats(UR):approximately 0.3 mEq/day (Na intake was reduced in proportion to GFR). Animals were in balance at the time of study. Measurement of ECF volume (ECFV) by inulin space revealed no difference between groups:AR(N=6)=19.4±2.0 (SD)% BW;UR(N=5)= 18.3± 1.9;NR(N=7)=19.6±2.7. 40 unanesthetized rats were volume expanded with isotonic NaCl equal to 5 or 10% of ECFV and then were studied for one hour. 10% expansion resulted in the following: 1)$\Delta U_{Na}V$: AR(N=8)=1.07±.56 (SD), UR(N=5)=0.32±.23,NR(N=7)= 0.82±.70 ($p<.05$=AR:UR); 2)ΔFE_{Na}%:AR=1.68±.84, UR= 0.77±.29, NR=0.26±.23 ($p<.05$=AR:VR,UR:NR); 3)% load cleared:AR=11.1±6.0, UR=3.1 ± 2.2, NR=8.1±6.9 ($p<.05$=AR:UR). Similar relationships were found with 5% expansion. These data indicate that: 1)Adaptation occurred independently of chronic ECFV expansion; 2) Nephron loss without prior adaptation in UR was associated with minimal to no magnification of FE_{Na} following acute volume expansion in contrast to striking magnification in AF, 3)Blunted excretion of Na load did not contribute to magnified FE_{Na} in AR. A sensitive Na control system in CRD preserves balance and ECFV by level of adaptation.

RENAL VESICLE GLUTAMINE TRANSPORT IN HEALTH AND CHRONIC RENAL FAILURE. Dave Windus,* Saulo Klahr, and Marc R. Hammerman. Washington Univ. School of Med., Dept. of Internal Med., St. Louis, Missouri.

To determine whether the increased rate of ammonia production per nephron which occurs in chronic renal failure (CRF) might result from an increase in L-glutamine transport across the brush border and/or basal-lateral membranes of the renal cortical cell and a consequent increased availability of this ammoniagenic amino acid, L-glutamine transport in isolated brush border (BBMV) and basal-lateral membrane vesicles (BLMV) from kidneys of normal dogs was characterized and compared to transport in vesicles from remnant kidneys of dogs with CRF. Na^+-dependent electrogenic transport of L-glutamine was demonstrated in BBMV which exhibited saturability over the concentration range of 25 uM to 500 uM L-glutamine (Km 230 uM, Vmax 128 pmol glutamine/mg protein/15s). Na^+-dependent L-glutamine transport was measured in BLMV which demonstrated saturability over the concentration range of 25 uM to 2 mM L-glutamine (Km 383 uM, Vmax 333 pmol glutamine/mg protein/15s). Initial rates of L-glutamine transport were not increased in either BBMV or BLMV obtained from remnant kidneys of dogs with CRF when compared to transports measured in vesicles from kidneys of normal dogs. Our studies provide evidence that transport of L-glutamine occurs across both luminal and contralateral membranes of the intact renal tubular cell. We conclude that an adaptation resulting in increased uptake of L-glutamine across the brush border or basal-lateral membranes of the tubular cell does not underlie the increased rate of ammonia production per nephron which occurs in CRF.

Renal Metabolism

ALTERATIONS IN GAMMA GLUTAMYL TRANSPEPTIDASE (GGTP) WITH CHRONIC EXPERIMENTAL UREMIA. Carolyn Abitbol, Jila Sharif* Dept of Ped, SUNY/Stony Brook, New York

GGTP is a major membrane enzyme involved in amino acid transport. Nutritional and metabolic consequences of chronic uremia may therefore involve abnormalities of this enzyme system. Specific tissue activity of GGTP was studied in chronic experimental uremia and chronic undernutrition. Male Wistar rats were rendered uremic(U) by 7/8 nephrectomy and compared to pair-fed (PC) and ad libitum fed (AC) sham-operated controls. After 10 days of controlled feedings, the animals were sacrificed and the leukocytes, intestinal mucosa, kidney and liver tissue analyzed for GGTP activity.

GGTP Activity (nM/min/mg Protein)

Group(N)	Leukocyte	Intestine	Kidney	Liver
AC(10)	2.3±0.5	3.1±1.4	100±26	0.66±.3
PC(10)		4.3±1.1	138.5±53	0.81±3
U (11)	8.7±6.4*	4.5±1.7	193.8±80*	0.91±2*

*Significantly different from Cp<.05

Intestinal mucosa GGTP was unchanged by uremia or undernutrition. However, kidney, liver, and leukocyte GGTP was significantly elevated in U as compared to C animals. Values in all tissues from PC did not differ from U or AC indicating a negligible influence of chronic undernutrition. In conclusion, GGTP is altered by the chronic uremic state which may contribute to alterations in plasma amino acid patterns.

MECHANISMS OF OXALATE (OX) ACCUMULATION IN RAT RENAL CORTICAL SLICES. Vecihi Batuman, Oya Levendoglu-Tugal* and Richard P. Wedeen, V.A. Medical Center, East Orange, N.J. and the UMDNJ, New Jersey Medical School, Newark, N.J.

Selective accumulation of ^{14}C-OX in proximal tubules in section freeze-dry autoradiographs from renal cortical slices incubated in vitro suggested concentrative transport of this actively secreted organic acid. ^{14}C-OX uptake and the effect of inhibitors was, therefore, studied in rat renal cortical slices incubated for 180 min at 25° C in Krebs-Ringer bicarbonate buffer. A slice to medium concentration ratio (S/M) of 2.7±0.13 SEM (n=39) was obtained with 0.001 mM oxalate in the incubation medium. 75% of the OX uptake occured within 15 min followed by slow accumulation for the next 165 min. Incubations conducted at 0° C resulted in a total inhibition of renal cortical slice uptake. Iodoacetemide, dinitrophenol and probenecid caused statistically significant reductions in the S/M ratio. However, addition of 3mM KCN or replacing O_2 with N_2 did not reduce slice accumulation of OX except in Ca^{++}-free medium. The S/M of 2.8±17 obtained with Ca^{++} plus KCN was reduced to 2.0±0.14 in Ca^{++}-free medium (n=28, p<.001). Similarly, the S/M of 3.0±0.39 in Ca^{++}-containing media with N_2 was reduced to 1.9±0.15 when Ca^{++} was removed (n=15, p<.001). OX uptake was not reduced by Ca^{++}-free media in the absence of inhibitors. These data indicate that at least two mechanisms participate in the accumulation of OX against its concentration gradient in proximal tubules; one appears to share the organic acid transport system while the other may involve Ca^{++}-OX precipitation.

PHOSPHATE LOADING ATTENUATES THE LYSOZYMURIA AND LYSOSOMAL ENZYMURIA INDUCED BY MALEIC ACID IN THE DOG. H. Al-Bander*, D. Mock*, T. Paukert*, M.H. Humphreys, and R.C. Morris, Jr., GCRC, Dept. of Medicine, Univ. of California, San Francisco, CA.

In the dog type 2 renal tubular acidosis/Fanconi syndrome (RTA-2/FS) induced by maleic acid (MA) is strikingly attenuated by prior phosphate loading. In the dog, we investigated whether prior phosphate loading attenuated the MA-induced increase in the urinary excretion of lysozyme, a low molecular weight protein normally virtually completely reabsorbed in the proximal tubule by a phagolysosomal endocytotic process, and N-Acetylglucosaminidase (NAG), a "marker" lysosomal enzyme of a size too large to be filtered by the glomerulus, but normally exocytosed into the tubular lumen at low rates. Paired studies were carried out in 5 conscious trained dogs to which MA (20-25mg/kg) was administered with and without prior administration of neutral sodium phosphate which raised the serum concentration of phosphate to 10.5± 2mg%. With administration of MA, and after the occurence of (RTA-2/FS) the urinary excretion of lysozyme and NAG increased and decreased in parallel and to the same extent. Prior phosphate loading strikingly attenuated the increment in both enzymes.

MA	TIME (min)	60	100	140	180
		% of maximal increase			
ONLY	NAG	13±5	56±17	95±4	47±7
	Lysozyme	12±7	56±5	87±4	44±10
WITH PHOS	NAG	4±1	7±2	14±7	10±3
	Lysozyme	6±2	12±4	14±4	9±7

These findings provide evidence that MA induces a dysfunction in endocytosis/exocytosis of the renal tubule that is mediated by a phosphate dependent metabolic abnormality.

SEGMENTAL DISTRIBUTION OF DNA POLYMERASE ACTIVITY IN COMPENSATORY RENAL GROWTH. Michel Bergeron and Tina Hoang*. Dept. of Physiology, University of Montreal, Montréal, Québec.

Compensatory renal growth (CRG) is not uniform in all nephron segments. Most biochemical stuies of CRG have previously been carried out on the whole kidney. The activity of DNA polymerase, an enzyme thought to be a good index of cell proliferation, has been measured in tubule fragments separated with collagenase and Percoll gradient centrifugation (1). In control rats weighing 150-160 g, the enzymic activity of glomeruli and distal fragments (F1-F2), proximal fragments (F4) was moderate and yielded values similar to the whole kidney extract (WK) when expressed per mg of DNA (2000 μμM dAMP); but different values are obtained if expressed per mg of protein (180, 200, 115 μμM dAMP). In uninephrectomized rats, DNA polymerase activity/mg DNA in F_4 measured at 24, 48, 72 and 96 h postnephrectomy increased to 100%, 200%, 350% and 450% of control respectively. Values from F1-F2 were not modified at 24 h but were increased by 90% after 48 h and plateaued at 72 h; WK had slightly higher activity than F1-F2 but were 2-3 times lower than F4. Our studies indicate that the DNA polymerase activity is most active in the proximal nephron whereas the hypertrophic response appears to occur more widely. They further illustrate that studying the whole organ in the case of CRG may provide misleading informations due to the uneven responses of different nephron segments in CRG.

(1) Vinay et al. Am J Physiol. 241, F403, 1981.

CELL DEATH MEDIATED BY TRANSPORT ACTIVITY IN PERFUSED KIDNEY. Meir Brezis,* Seymour Rosen, Patricio Silva, and Franklin H. Epstein. Harvard Medical School and Beth Israel Hospital, Departments of Medicine and Pathology, Boston, Massachustts.

During isolated perfusion of the rat kidney, a specific and consistent structural lesion, localized to the thick ascending limb of Henle's loop (T.A.L.), progresses from mitochondrial swelling at 15 min to complete cellular disruption by 90 min. At the same time, glomerular filtration rate (GFR) and fractional sodium reabsorption (TR_{Na}) fall and concentrating ability is severely impaired. The addition of amino acids to the perfusate attenuates the anatomical lesion and improves kidney function and stability.

In order to evaluate the possibility that the T.A.L. damage might result from transport activity, the histology of kidneys was evaluated under conditions in which T.A.L. cell transport activity was varied. The fraction of T.A.L. tubules involved with severe damage (0.44 ± 0.02, in kidneys perfused with glucose) was dramatically reduced by furosemide (0.07 ± 0.04, $p < 0.001$) and was eliminated in the presence of ouabain or in non-filtering kidneys. Protection of the T.A.L. cells was not seen with acetozolamide, which increased the amount of damage. The initial lesion, mitochondrial high amplitude swelling, was completely prevented by inhibitors of mitochondrial respiration, rotenone or antimycin. These results suggest that the T.A.L. lesion seen during isolated kidney perfusion is related to transport work activity of the T.A.L.

GLOMERULAR HYPERTROPHY IN EARLY EXPERIMENTAL DIABETES. P. Cortes, F. Dumler, and N.W. Levin. Dept. of Medicine, Henry Ford Hospital, Detroit, Michigan.

Renal hypertrophy is a characteristic change of short-term insulin-deficient diabetes. The occurrence of glomerular hypertrophy during the early stages of the disease may be pathogenetically related to the development of diabetic glomerulosclerosis in long-term diabetes. We have estimated the degree of glomerular hypertrophy in experimental diabetes of mild severity in rats by measuring RNA accretion and orotate incorporation into RNA in isolated glomeruli incubated in vitro for 3 h in a 20 mM glucose 10 M orotate media. After the injection of 30 mg/kg streptozotocin the results in diabetic (D) and saline injected controls (C) were:

	Plasma glucose (mg/dl)	RNA/DNA	pmol orotate in RNA/mg DNA
24h			
C	145±2.6(10)	20.6±1.6(4)	60.6±1.8(4)
D	176±15(10)	25.1±1.4(4)	140.8(2)
48h			
C	140±2.6(10)	20.5±2.0(4)	36.5±1.2(4)
D	278±65(10)	28.1±2.0(4)	196.1(2)
72h			
C	137±2.8(10)	20.7±1.4(4)	38.1±1.2(4)
D	328±28(10)	22.8±3.1(4)	297.4(2)

Results represent Mean ± SEM. Insulin administration prevented the changes found at 48h. It is concluded that the onset of glomerular hypertrophy occurs rapidly after the induction of mild diabetes even if plasma glucose concentration is only minimally increased.

PREPARATION OF A TUBULE SUSPENSION FROM RABBIT KIDNEY ENRICHED IN THICK ASCENDING LIMBS. Mary E. Chamberlin*, L.J. Mandel, and A. LeFurgey. Duke Univ., Dept. of Physiol.,Durham, North Carolina

A suspension of tubules enriched in thick ascending limbs (TAL) was prepared by a series of steps involving enzymatic tissue digestion, low speed centrifugation, filtration and density gradient centrifugation. Yields of 5-15 mg protein/rabbit were obtained which are sufficient for studies on the relationship between active transport and metabolism in this segment. Measurement of calcitonin-activated adenyl cyclase, a marker for the TAL, indicates that density gradient centrifugation results in a 3.5-fold purification of TAL. Light and electron microscopy indicate that the suspension consists of intact segments of TAL with open lumens. The major contaminant of the preparation is the S_3 segment of the proximal tubule. The oxygen consumption (QO_2) of the tubules is 26 nmol/min mg protein using glucose as the sole substrate. This rate is minimally stimulated by 1 mM succinate indicating intact TAL membranes. QO_2 is inhibited up to 50% by 10^{-3} M furosemide, whereas 10^{-4} M ouabain inhibits it by 50 to 60%. Nystatin, which enhances Na,K ATPase activity by increasing cellular Na, stimulates QO_2 by 50%. The nystatin-stimulated QO_2 is independent of furosemide, indicating no inhibition of the Na,K ATPase by furosemide. Oxidation of glucose alone can support the nystatin-stimulated QO_2 in contrast to what is found in the proximal tubule. Addition of other substrates in the presence of glucose enhanced the nystatin-stimulated QO_2 in the following order: fatty acids > acetate > lactate.

IDENTIFICATION AND PHOSPHORYLATION OF ACTIN-BINDING PROTEIN IN THE TOAD BLADDER AND TOAD EGG. Howard L. Corwin, John H. Hartwig*, and Dennis A. Ausiello. Medical Services, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

The hydro-osmotic effect of vasopressin may be mediated by cAMP induced alterations in the cell cytoskeleton. High molecular weight actin-binding proteins (ABP) interact with actin filaments to form a gel network. We have demonstrated a 270K peptide in toad bladder extracts which co-migrate with macrophage ABP in SDS-PAGE and promotes actin gel formation. In order to obtain large quantities of purified toad ABP we used the toad egg. Sequential chromatography yielded a 270K protein which accounted for all of the isotropic actin cross-linking activity in toad egg extracts. 79% of the ABP present in the starting extract was recovered during purification. Antibodies prepared against this toad egg protein demonstrated immunologic cross reactivity with toad bladder ABP and macrophage ABP. Toad egg ABP has the same Stoke's radius and cross links actin filaments into a gel with the same efficiency as the macrophage protein. It is also indistinguishable from the macrophage protein in the electron microscope. This is the first demonstration of an ABP identical to mammalian ABP in a non-mammalian cell. Purified toad egg ABP when reacted with P^{32}ATP and catalytic subunit of cAMP dependent protein kinase demonstrated P^{32} incorporation into gel slices corresponding to the migration of toad egg ABP on SDS-PAGE. It is possible that cAMP dependent phosphorylation of ABP is involved in the action of vasopressin.

INSULIN RESISTANCE IN UREMIA IS SPECIFIC FOR GLUCOSE METABOLISM. R DeFronzo, D Smith, A Alvestrand. Yale Univ, New Haven & Karolinska Inst, Stockholm.

Insulin resistance (IR) in uremia is a nearly universal finding. We have examined whether this IR extends to other aspects of insulin action, specifically potassium (K) and amino acid (AA) metabolism. 12 uremic (U)(Scr=11±1 mg%) & 24 controls (C) were studied. I clamps (+100 uU/ml) were performed with hepatic & femoral vein catheterization. Total body glucose (G) uptake was reduced by 44% in U vs C (3.7±0.3 vs 6.7±0.3 mg/kg·min, $p<0.001$), due to impaired peripheral glucose utilization (leg G uptake = 21±1 vs 48±8 umol/kg leg wt per min, $p<0.001$). Splanchnic G uptake (0.4±0.1 vs 0.5 ±0.1 mg/kg·min) was similar in U and C. Despite this marked IR, K homeostasis was not altered. The decline in P_K was similar in U (0.98±0.10 meq/L) and C (0.95±0.05). Splanchnic (67±10 vs 67±6 ueq/min) and leg (22±1 vs 24±2 ueq/min) K uptake were also similar. Most plasma AA conc were lower (by~ 30%, $p<0.01$) in U vs C. Following I, all AA conc declined by 10-50% ($p<0.05$). The greatest decline occurred for the branched chain AA. Both the percent (val=30%; leu & isoleu=50%, $p<0.001$) and absolute declines were similar in U and C. In the basal state all AA were released from muscle with a reciprocal uptake by the splanchnic area. Basal splanchnic alanine uptake was greater in U vs C ($p<0.01$). Hyperinsulinemia decreased release of all AA from leg to a similar extent in U & C. Following I, splanchnic alanine uptake declined but remained greater in U vs C ($p<0.01$). Splanchnic uptake of all other AA was unaffected by insulin.

CONCLUSIONS: In uremic man (1) I-mediated G metabolism is impaired, (2) I-mediated K and AA uptake is not altered, (3) Splanchnic alanine uptake is enhanced, suggesting increased gluconeogenesis.

HOMOGENEOUS PREPARATIONS OF CULTURED TUBULAR SEGMENTS FROM RABBIT RENAL CORTEX AND MEDULLA. G. Denis*; M.-F. Arthus* and M. Bergeron. Dept. of Physiology, University of Montreal, Montreal, Quebec.

A collagenase (0.2%) digested renal cortical suspension has been separated into four bands on a Percoll density gradient according to the method of Vinay et al. Am. J. Physiol. 241, F403, 1981. The F_1 band contained mainly glomeruli, the F_2 and F_3 bands, respectively distal tubules and short fragments of proximal tubules, while the F_4 band contained almost exclusively proximal tubules. All fractions were plated in a 50:50 mixture of Dulbecco's modified Eagle's and Ham's F_{12} media supplemented with 10% fetal calf serum; areas of homogeneous fragments were isolated with O rings. Confluent primary cultures and reseeding were obtained from all fractions. The growth rate was somewhat slower than under non Percoll conditions for the first 2 or 3 days with the F_4 fraction being the slowest. This protocol was also applied to the outer and inner medullary tissue. The composition of the resulting bands are now being characterized. This present study offers a model which delivers a large number of tubules from defined origin thus allowing the cultivation of many homogeneous tubules and a high yield of epithelial cells.

EFFECTS OF A23187 ON RABBIT RENAL CORTICAL TUBULE BIOENERGETIC FUNCTION. Bruno E. Delahaye,* H. David Humes and Joel M. Weinberg. VA Med. Ctr. and Univ. of Michigan, Ann Arbor, Michigan.

The divalent cation ionophore A23187 (A) has been used to assess the effects of elevated intracellular Ca^{++} on transport functions of renal tubules. However, the well established potential for A to promote cell injury by several mechanisms has not been directly assessed in this model. In order to evaluate this issue we isolated rabbit cortical tubules by in vivo collagenase perfusion. After resuspension in Krebs-Henseleit bicarbonate with 0.6% dextran we tested the effect of brief (15 min) exposure to 9 μM A on the bioenergetic capacity of these tubules. Relative to paired control tubules (Ns=4-6) A decreased mean basal respiration (natom oxygen/mg protein/min±SEM) with glucose, lactate, and alanine (GLA) as substrates (35.1±1.4 to 29.9±1.2 $p<0.02$), decreased respiration with GLA + valerate (45.2±2.2 to 38.4± 2.4 $p<0.05$) decreased respiration stimulated by nystatin (66.9±5.8 to 42.4±5.5 $p<0.05$) and decreased fully uncoupled respiration in the presence of carbonyl cyanide chlorophenyl hydrazone (117.4±3.6 to 58.0±1.4 $p<0.001$). The percentages of respiration inhibited by oligomycin (67.1 vs. 68.3) and ouabain (43.4 vs. 45.5) were similar in control and A treated tubules. Dose response studies established a threshold value for A effects of 0.35-0.70 μM. These data show that even brief exposure to A has deleterious effects on multiple tubular bioenergetic functions which confound interpretation of any observed inhibitory effect of A on tubular function and transport.

EFFECT OF NICOTINAMIDE (NiAm) ADMINISTRATION ON NAD CONTENT IN PROXIMAL TUBULES. T. P. Dousa, A. N. K. Yusufi,* E. Kusano,* and J. L. Braun-Werness* (Intr. by D. M. Wilson). Mayo Clinic, Rochester, Minnesota.

In previous studies (J. Clin. Invest. 67:1347, 1981) we found that injection of NiAm leads to phosphaturia, increase in renal cortical NAD, and inhibition of phosphate (Pi) transport across brush border membrane. To localize this NiAm effect, we determined NAD content in proximal convoluted tubules (PCT), proximal straight tubules (PST), and for comparison in cortical ascending limb of Henle's loops (CAL) and in glomeruli (GL). Thyroparathyroidectomized rats fed normal (0.7%) phosphate diet were injected either with 1 g of NiAm/kg i.p. or with saline (controls). After 9 hours, nephron segments were microdissected and NAD was determined by bacterial luciferase micromethod. NAD content is expressed as fmoles per mm of tubule length or per glomerulus. Values are mean ± SE (n = 17-27, from 4 experiments).

	PCT	PST	CAL	GL
Controls	851± 95	414±43	556±72	104±17
NiAm-treated	3208±349*	754±88*	793±96	235±33*
Δ%	+227%	+82%	+43%	+116%

* significantly higher than controls (t-test)

NAD content was significantly higher ($P<.05$) in PCT compared to other examined nephron segments, both in controls and after NiAm administration. Both relative (Δ%) and net increase of NAD content after NiAm injection was much higher in PCT than in PST. These results indicate that PCT is a nephron segment particularly rich in NAD. Results also show that PCT rather than PST is a major site of NAD accumulation in response to NiAm administration.

AMMONIA TRANSPORT BY THE TURTLE BLADDER. G. Dytko*, R.Wheeler*, L.Withers*, J.A.L.Arruda. UAMS and VA Hospitals, Little Rock, AR.

We studied ammonia transport by the turtle bladder by adding NH_4Cl to serosal or mucosal solution. By varying the NH_4Cl concentration and or pH it was possible to vary NH_4 and maintain NH_3 concentration constant or vice versa. At pH 6.4 (NH_4+/NH_3 ratio is 1000/1) the transport of ammonia into the mucosa was electrogenic. The increase in current could be totally accounted for by ammonia transport suggesting that NH_4+ is the transported species. NH_3 transported was quantified by measuring ammonia transport when NH_3 was increased and NH_4 concentration was maintained constant. NH_4+ transport was preferential from the serosa to the mucosa whereas NH_3 transport was of the same magnitude in both directions. The apparent permeability to NH_3 was 12 times greater than that of NH_4+ (3.6 vs 0.2 cm/s x 10^{-5} $p < 0.001$). The relative permeability (compared to Na and calculated from the Goldman equation) of the mucosa to NH_4+ was similar to that of K (0.19 ± 0.04 0.13 ± 0.05). The permeability of serosa to NH_4+ (compared to K) was greater than that of Na. NH_3 transport increased linearly as function of NH_3 concentration. NH_4+ transport achieved a maximum at 7.5 mM NH_4Cl concentration. ^{14}C-methylammonium (an analog of NH_4+) transport also exhibited saturation kinetics. NH_4+ was a competitive inhibitor of methylammonium transport suggesting that these substances share a common transport system. These data suggest the turtle bladder is capable of transporting ammonia as NH_4+ and as NH_3 and these species have different permeabilities and transport characteristics.

IMPAIRED RENAL MEVALONATE (MVA) METABOLISM IN NEPHROSIS: FURTHER EVIDENCE FOR DIRECT KIDNEY INVOLVEMENT IN HYPERLIPIDEMIA. T.A. Golper, K.R. Feingold*, M.H. Wiley*, M.D. Siperstein*, Portland and San Francisco VA Medical Centers.

MVA is an essential intermediate in cholesterogenesis because the rate limiting enzyme in its synthesis is the site of primary feedback regulation. Kidneys metabolize MVA to sterols or CO_2 (non-sterols) while the liver metabolizes MVA predominantly to cholesterol (Chol). The number five carbon of MVA is converted to CO_2 only through the non-sterol pathway.

5-^{14}C-MVA was injected into 9 puromycin nephrotic (N) and 7 control (C) female S-D rats. Their expired CO_2 was collected for 6 hours prior to sacrifice. N expired 3/4 as much $^{14}CO_2$ as did C ($p<.001$) and N livers synthesized twice as much Chol from MVA ($p<.001$). Kidney Chol synthesis from MVA was the same.

Liver and kidney slices from separate N and C were incubated in media containing 1-^{14}C-Acetate or 5-^{14}C-MVA. In liver slices from N and C, acetate and MVA conversion to Chol or CO_2 was not different. Per gram of kidney, $^{14}CO_2$ production in N was 57% of that in controls ($p<.001$) and Chol synthesis was increased 227% ($p<.005$).

Impaired renal metabolism of MVA can contribute to nephrotic hyperlipidemia by, 1) not diverting MVA to non-sterols, allowing more to be presented to the liver where it is synthesized to cholesterol, and 2) when renal circulation is intact, renal cholesterogenesis from MVA appears normal, but slice studies demonstrate enhancement.

PROLINE UPTAKE BY HUMAN RENAL BRUSH BORDER MEMBRANE VESICLES. J.W.Foreman, P.D.McNamara*, and S. Segal. The Children's Hospital of Phila., Dept. Peds., Univ. of Penna., Phila., PA.

Abnormalities in proline transport occur in several disorders such as iminoglycinuria and the Fanconi syndrome. The presumed abnormality in iminoglycinuria is a defect in transport across the renal brush border membrane. Proline transport by brush border membranes has been characterized in rodents, but not in humans.

Proline transport was studied in isolated brush border vesicles prepared from surgical specimens of normal human renal cortex. Purity of this preparation was demonstrated by a 7-10 fold increase in the specific activity of alkaline phosphatase over the initial homogenate. Uptake of 0.06 mM proline under an inwardly directed Na^+ gradient was characterized by an "overshoot" similar to that observed with rat brush border vesicles. An overshoot was not observed when Na^+ was allowed to equilibrate across the membrane or when a choline Cl gradient replaced the NaCl gradient. An Eadie-Hofstee plot of the concentration dependence of uptake over the concentration range of 0.02-4.5 mM in 5 different individuals revealed a two-limbed curve consistent with multiple transport systems. The kinetic parameters of these transport systems were Km_1 - 0.076 ± .008 mM, $Vmax_1$ - 0.328 ± .075 nmoles/mg protein per 15 sec and Km_2 - 0.724 ± .159, $Vmax_2$ - 0.966 ± .148. 1 mM hydroxyproline inhibited 0.02 mM proline uptake by 59% after 1 min and 1 mM glycine inhibited it by 29%. These data indicate that 2 transport systems for proline exist on the luminal membrane of the human and a defect in one could explain iminoglycinuria.

INTERACTIONS BETWEEN GLUCONEOGENESIS AND SODIUM TRANSPORT IN THE PROXIMAL TUBULE. **S.R. Gullans*, P.C. Brazy, V.W. Dennis, and L.J. Mandel. Depts. of Physiology and Medicine, Duke University Med. Ctr., Durham, N.C. (Intr. by G. Giebisch)**

Gluconeogenesis (GNG) has been proposed to compete with sodium transport for cellular energy. We have examined this relation in the rabbit proximal tubule under several substrate conditions. Gluconeogenesis, oxygen consumption, and ATP content were measured in suspensions of cortical tubules and fluid transport (J_v) was measured in isolated perfused tubules. In the presence of lactate and alanine GNG was 43.8±2.8 nmol/mg prot.·hr (n=25). Decreases in cellular ATP content caused by rotenone (10^{-7}M) or by stimulation of Na,K-ATPase activity with nystatin abolished GNG. However, inhibition of Na,K-ATPase activity by ouabain (10^{-5}M), which causes an increase in cellular ATP content, also reduced GNG by 54±1%. Additional studies showed that fatty acids such as butyrate and valerate could stimulate GNG by 136% to 151% yet not alter ATP content or J_v. Furthermore, these fatty acids affected the response of GNG to rotenone, nystatin, and ouabain independent of changes in ATP. J_v was also unaffected by 3-mercaptopicolinate which reduced GNG by 74±2%. We conclude that under substrate-sufficient conditions GNG and sodium transport are not inversely related and the proximal tubule can meet the energetic demands of both processes. Furthermore, although ATP is involved in the regulation of both GNG and sodium transport, there are additional factors which determine the interrelationship of these processes and which can be affected by fatty acid metabolism.

PHOSPHATIDYLCHOLINE SYNTHESIS DURING COMPENSATORY RENAL GROWTH IN RATS. Michael K. Hise*, Charles M. Mansbach*, and Robert H. Harris. Dept. of Medicine, Duke University, Durham, NC

Phosphatidylcholine (PC) is the principle renal phospholipid and a major constituent of renal membranes. Since increased PC synthesis is the earliest biochemical event described in a remaining kidney after unilateral nephrectomy (UNx), we sought to identify steps at which de novo PC synthesis was altered. At 24 h after UNx (n=14) or sham nephrectomy (SN, n=14), pool sizes of PC synthetic intermediates and the activities of the three PC synthetic enzymes were measured in the cortex of the contralateral kidney in adult male rats.

In the UNx rats, cortical PC content was 3.5 ± 0.1 μmoles/mg DNA compared with 2.8 ± 0.1 for SN ($p<0.02$). However, the pool sizes of the intermediates choline, phosphocholine, and CDP-choline were not significantly changed. The Vmax of choline kinase, the first enzyme of the pathway, was 2.0 ± 0.2 nmoles/mg protein/min compared with 1.2 ± 0.1 for SN ($p<0.01$); likewise, the Vmax of the third enzyme, phosphocholinetransferase, was increased in UNx (2.2 ± 0.2 nmoles/mg/min vs. 1.4 ± 0.1 for SN, $p<0.01$). Vmax of the second enzyme, citidyltransferase, was unchanged. For each enzyme, the Km was unchanged, and the substrate concentration for each was well above its KM. Thus, UNx stimulates PC synthesis rate, while increasing the first and last enzymes of the pathway. The finding that only the final product, PC, was increased by UNx suggests that phosphocholinetransferase is the regulatory step in the pathway.

INOSITOL UPTAKE AND RENAL FUNCTION IN THE ISOLATED PERFUSED RAT KIDNEY. John R. Little and Kathleen A. Pasko. Renal and Electrolyte Division, M. S. Hershey Medical Center of Pennsylvania State University, Hershey, PA.

Function of the isolated perfused rat kidney is improved by addition of glucose to the perfusate. As glucose is a precursor of inositol in kidney tissue, this effect could be due to inositol. To test this hypothesis the isolated rat kidney was perfused at 120 mm Hg with an apparatus which recirculated perfusate at 37°. The perfusate was Krebs-Ringer bicarbonate solution containing 6% bovine serum albumin (BSA), inulin as a measure of GFR and inositol. The BSA was purified by ion exchange chromatography to remove contaminating metabolites. In each experiment 20 min was allowed for equilibration followed by three 20 min observation periods. Inositol in the perfusate and urine was measured by gas-liquid chromatography of the butaneboronic acid derivatives using mannitol as an internal standard. Data are expressed per g wet weight of the normal left kidney:values are means of number of observation periods ± SE.

	Control (N=18)	Inositol (N=9)
Perfusate inositol conc.(mM)	-	1.12±0.06
Net uptake of inositol ($\mu mole \cdot g^{-1} \cdot min^{-1}$)	-	0.25±0.06
Perfusate flow rate ($ml \cdot g^{-1} \cdot min^{-1}$)	20.4±0.6	19.7±0.7
GFR($\mu l \cdot g^{-1} \cdot min^{-1}$)	328±18	309±31
Fractional Na^+ reabsorpt.(%)	65.4±1.6	68.8±0.9
Fractional K^+ reabsorpt.(%)	54.0±2.8	49.0±2.3

Inositol uptake was not accompanied by significant functional changes thus the effect of glucose is not due to it being metabolized to inositol.

GLYCOSAMINOGLYCAN (GAG) METABOLISM IN AMINONUCLEOSIDE NEPHROTIC SYNDROME (AMNS). Mark T. Houser, Dept. of Pediatrics, UTHSCD, Dallas, TX.

GAGs are now recognized to be an important component of the glomerular basement membrane (GBM) and to have a role in regulating macromolecular transport. With this consideration, we decided to investigate GAG metabolism in the AMNS.

Sprague-Dawley male rats in the experimental (E) group were given a single dose of aminonucleoside of puromycin; controls (C) were injected with saline. By day 9, protein excretion had increased dramatically in E (5.3 to 134.9 mg/day; $p<0.001$) but was unchanged in C. GBM GAGs were isolated following in-vivo pulse labelling with ^{35}S on day 10. Anion exchange chromatography was used to separate GAG components into low, medium and high salt fractions (HSF) followed by the biochemical analysis of uronic acid (UA) and sulfaminohexose (SAH) residues. In C, the total UA content was 175 ng/mg GBM and SAH content was 77.4 nmoles glucosamine/mg GBM. In E, total content was 186 and 58.5, respectively. HSF UA content decreased 4 fold in E although total UA content was similar in both groups. SAH distribution in the salt fractions was identical in both groups, although actual content was less for all fractions in E. ^{35}S incorporation into GBM GAG was augmented in E with a greater than 10 fold increase in specific activity in the medium and HSF. These findings suggest that the rate of GAG synthesis is increased in E and that while total GAG content is normal, distribution is shifted to lower charged density components. Furthermore, a decrease in N-sulfation of heparan sulfate chains is likely. These results may have significance relevance to the pathogenesis of AMNS.

ALKALINIZATION OF LYSOSOMAL pH INHIBITS RENAL HYDROLYSIS OF ABSORBED PROTEIN. T. Maack, E. Eich* and M.J.F. Camargo* Dept. of Physiol., Cornell Univ. Med. Coll., New York, N.Y.

Filtered proteins are absorbed by renal tubular cells and hydrolysed within lysosomes (L) to amino acids which are then returned to the circulation. To further study determinants of the hydrolytic process we tested whether the acid pH of L is essential for the normal metabolism of absorbed proteins. The probe protein was cytochrome c(CYTc), labeled with $^{14}CH_3$ by reductive methylation of the ε-amino groups of lysine. The rate of hydrolysis of $^{14}CH_3$-CYTc was determined by measuring the efflux rate of $^{14}CH_3$-lysine to the perfusate of isolated rat kidneys (IK) pre-loaded in vivo with $^{14}CH_3$-CYTc. The acid pH of L was raised by adding to the perfusate the lysosomotropic weak bases chloroquine (CQ-0.1mM) or NH_4Cl (10mM). In controls (n=4), 68±6% SE of $^{14}CH_3$-CYTc initially present in the IK was released to the perfusate as $^{14}CH_3$-lysine at the end of 40 min of perfusion. This value decreased markedly ($p<0.001$) to 29±2% (n=7) with NH_4Cl and to 18±4% (n=5) with CQ. This effect was partially reversible upon removal of NH_4Cl or CQ from the perfusate. Chromatographic analysis of kidney tissue and perfusate showed that CQ and NH_4Cl inhibited the hydrolysis of $^{14}CH_3$-CYTc within the cells and not the efflux rate of formed $^{14}CH_3$-lysine from the cells. Results show that the acid milieu of lysosomes is essential for the normal disposal of absorbed CYTc and suggest the hypothesis that intrinsic defects in lysosomal acidification may lead to deposition of protein absorption droplets within renal tubular cells.

INTRARENAL FORMATION OF SEROTONIN FROM ITS AMINO ACID PRECURSOR 5-HYDROXYTRYPTOPHAN (5-HTP). George McKendall*, Charles T. Stier, Jr., and Harold D. Itskovitz. Departments of Pharmacol. and Med., New York Medical College, Valhalla, New York.

The kidney is a rich source of the enzyme aromatic amino acid decarboxylase. We examined the ability of isolated kidneys to form 5-hydroxytryptamine (serotonin) by decarboxylation of its amino acid precursor 5-HTP. Kidneys from 6 male Sprague-Dawley rats were perfused at 37°C and 80 mmHg with modified Krebs-Henseleit solution gassed with 95% O_2-5% CO_2. 5-HTP was infused at rates of 1.5 and 15 µg/min. 5-HTP and serotonin were determined in the urine (U) and renal venous effluent (E) using high-performance liquid chromatography. Our results are shown in the table below which compares serotonin and 5-HTP excretion and renal vascular resistance (RVR) during control periods and with both doses of 5-HTP.

	Control U/E	1.5 µg/min U/E	15 µg/min U/E
Serotonin(ng/min)	0/0	29/218	34/591
5-HTP (ng/min)	1/0	5/565	34/2998
RVR (mmHg/ml/min)	5.1±0.7	5.5±2.0	30.2±25.1

Mean values ± SD.

Our data demonstrate direct intrarenal formation of serotonin accompanied by increases in RVR at the 15 µg/min dose. The changes in RVR could be reversed by administration of the decarboxylase inhibitor carbidopa or the serotonin receptor antagonist Ketanserin. These results are consistent with the possibility that serotonin can be formed within kidneys to play a role in the regulation of normal kidney function and/or the pathogenesis of various types of renal disease.

THE PENTOSE PATHWAY AND RENIN RELEASE (RR) FROM ISOLATED PERFUSED RAT KIDNEY. S.G. Rostand and J. Work, NRTC, Univ. of Alabama in Birmingham, Birmingham, AL.

Sodium depletion produces parallel increases in renal renin content (RRC) and macula densa and JG cell G6PD activity while sodium loading has the opposite effect. To study this association further, we perfused nonfiltering kidneys from Sprague-Dawley rats with KRB buffer containing 5mM glucose, and 14gm% BSA in the presence and absence of 6-aminonicotinamide (0.25), an inhibitor of G6PD, the rate limiting step of the pentose pathway. Perfusate renin activity was measured during four 15 min periods after which kidneys were frozen in liquid N2 and analyzed for RRC. Five kidneys perfused without 6-AN had RR of 646±(SE)185ngAI/gm/min. In 4 kidneys perfused in the presence of 6-AN, RR was depressed to 56±12.8, p<.001. The RRC of 4 control kidneys was 56±3.3ngAI/mg/hr while in 4 kidneys perfused with 6-AN, RRC was lower, 35.3±2.9 (p<.01). When 5mM lactate was used instead of glucose as the energy source in 7 kidneys, RR was 25.7±1.9 which was lower than RR in kidneys perfused with 5mM glucose (p<.001). Unlike the glucose studies, 6-AN failed to suppress RR in 4 kidneys perfused with lactate (25.8±3.7). The RRC of 3 kidneys perfused with lactate was 40.2±6.4 and was lower than glucose controls. We conclude that glucose but not lactate supports maximal RR and that G6PD inhibition by 6-AN depresses RR and RRC in the presence of glucose. 6-AN had no effect on RR in the presence of lactate. We suggest that the pentose pathway may play a role in renin production and release.

UTILIZATION AND RENAL HANDLING OF D-LACTATE IN MAN. Man S. Oh, Jaime Uribarri, Denise Alveranga*, Ira Lazar*, Nadine Bazilinski*, and Hugh J. Carroll. Downstate Medical Center, Department of Medicine, Brooklyn, New York.

The wide clinical use of dl-lactate as an alkalinizing agent and the recent discovery of d-lactic acidosis in man have sparked interest in d-lactate metabolism. The purpose of the present investigation was to study utilization and renal handling of d-lactate in man. Eleven healthy volunteers received infusion of 1/6 molar sodium dl-lactate (50% d- and 50% l-lactate). In 6 subjects the solution was infused at 12-15 ml/kg body weight/hour (low rate) and in 5 at 21-24 ml/kg/hr (high rate). The infusion was continued for 150 min., and the blood and urine samples were collected at 20 min. intervals from the start of infusion to 50 min. after the end of infusion. Plasma d-lactate concentration leveled off at 1.6 to 3.0 meq/l at low rate and at 4.5 to 6.0 meq/l at high rate. The maximal rate of d-lactate metabolism was 0.99 meq/kg/hr ± 0.12 at low rate and 1.52 meq/kg/hr ± 0.05 at high rate. Urinary excretion of d-lactate was substantial at the plasma concentration below 1.5 meq/l, whereas virtually no l-lactate excretion occured at concentrations below 2.5 meq/l. T_m for d-lactate appeared to be 0.15 to 0.17 meq/100 ml GFR at low rate, but it varied greatly at high rate, depending on the rate of l-lactate reabsorption. Reabsorption of d-lactate virtually ceased with increasing l-lactate excretion in the urine. Conclusions: in contrast to ruminants man utilizes d-lactate quite efficiently. Tubular reabsorption of d-lactate is much less efficient than l-lactate, and may be interfered with by l-lactate reabsorption.

IN VITRO AND IN VIVO SEX DIFFERENCES IN RENAL UDF-GLUCURONYL TRANSFERASE IN THE FISCHER 344 RAT. *Glenn F. Rush, John F. Newton and Jerry B. Hook. Mich. St. Univ., Dept. Pharmacol./Toxicol., Ctr. for Environ. Toxicol., East Lansing, MI.

Glucuronidation is an important reaction in the detoxification and elimination of xenobiotics from the body. While in vitro xenobiotic metabolism is generally very low in extrahepatic organs, the kidney contains UDP-glucuronyl transferase (UDPGT) activity equal to the liver suggesting that the kidney may make a significant contribution to total body glucuronidation. In vitro, male renal microsomal UDPGT activity was 4.15±0.64 nM/min/mg protein while female UDPGT activity was 11.61±1.58 nM/min/mg protein. Kinetic analysis revealed a greater Vmax in females (15.03±1.01 vs. 5.05±0.81 nM/min/mg protein). The Km was not different between males and females (0.13±0.01 vs. 0.14±0.02 mM, respectively). In vitro sex differences persisted after total release of enzyme latency with 0.05% deoxycholate. Sex differences in renal glucuronidation were also quantified in vivo using a modification of the isotope dilution method. During steady state infusion of p-nitrophenol (PNP), excretion of PNP-glucuronide (PNPG) from all sources (renal and extra renal) was greater in females than in males (916.9±26.0 vs. 715.6±65.9 nM/min/kg b.wt.). Renal glucuronidation of PNP could totally account for this sex difference as 22% of the total PNPG excreted in females was of nephrogenic origin as opposed to 11% in males. These results demonstrate: 1) in vitro sex differences in renal UDPGT activity correlates qualitatively with in vivo observations, and 2) renal glucuronidation makes a significant contribution to total body detoxification and elimination of certain xenobiotics.

EFFECTS OF VARIOUS K+ SALTS ON URINE ACID BASE PARAMETERS AND CITRATE CLEARANCE IN RECURRENT URIC ACID STONE FORMERS. K. Sakhaee, S. Corona, C. Skurla, A. Womack, C.Y.C. Pak, and H.R. Jacobson. UTHSCD, Dallas TX.

We have observed the complication of Ca++ stone formation in patients with recurrent uric acid stones treated with $NaHCO_3$ and have recently demonstrated that alkali therapy with K citrate prevents this complication. The present studies determined whether the effects of K citrate on urine acid-base parameters and citrate clearance are due to the administered K+, the administered citrate per se, or the alkalinizing effect of citrate. Four recurrent uric acid stone formers were studied during 4 phases: a control phase with no therapy and three randomly timed treatment phases, each of 3 wks duration with treatment consisting of 80 meq/day of KCl or $KHCO_3$ or K citrate. At the end of each 3 wk period patients were admitted to the GCRC, fed a constant low acid ash metabolic diet and daily urine and blood analyzed for the following (* $p<0.05$ compared to control).

	Control	KCl	$KHCO_3$	Kcit.
serum citrate,mg/L	23.4	21.6*	24.6*	26.5*
urine citrate,mg/24hr	561	779*	1026*	1138*
cit clearance,ml/min	16.3	25.1	26.7*	28.1*
urine pH	5.46	5.34	6.81	7.00

Urine, NH_4, titratable acid (TA) and HCO_3 excretion were not affected by KCl treatment while $KHCO_3$ and K citrate equally suppressed NH_4 and TA and increased HCO_3 excretion. We conclude that K citrate therapy in these patients produces changes in urine acid-base parameters/citrate clearance via 2 effects: an effect of K administration and the alkalinizing effect of citrate.

GLUTAMATE TRANSPORT IN RAT KIDNEY MITOCHONDRIA. A.C. Schoolwerth, K.F. LaNoue,* and W.J. Hoover.* The Pennsylvania State University, Hershey, PA.

The predominant pathway for oxidation of external glutamate by kidney mitochondria is transamination. In contrast, glutamate generated inside the mitochondrial matrix space from glutamine is deaminated to a greater extent and contributes to renal ammonia formation, particularly at acid pH and in metabolic acidosis. These findings suggest that transport of external glutamate may limit its deamination. The present studies were designed to characterize the glutamate hydroxyl transporter in rat kidney mitochondria. [U-^{14}C]glutamate transport was measured in rotenone-inhibited energized mitochondria at pH 7.0 and 28°C. Glutamate efflux was observed to be first order with respect to matrix glutamate with a rate constant of 0.457^{-1}. Uptake kinetic studies indicated that the Km of external glutamate was 1.4 mM and the Vmax 3.2 nmol/mg·min. These kinetic values were not different at pH 6.6 or in mitochondria from acidotic rats. Parallel metabolic studies were performed in mitochondria incubated in the absence of rotenone but in the presence of malonate to inhibit glutamate transamination. The deamination of 1 and 10 mM glutamate was identical to the simultaneously measured rates of glutamate transport. No glutamate was detectable within the matrix space under the conditions of these metabolic experiments.

These studies provide quantitative data for the first time characterizing glutamate hydroxyl transport in rat kidney mitochondria. The data indicate that the transporter is quite slow and rate limiting for the oxidative deamination of external glutamate in kidney mitochondria.

PRIMARY CULTURE OF PROXIMAL TUBULAR EPITHELIAL CELLS IN SERUM-FREE MEDIUM. CONTRIBUTION OF APICAL GLUCOSE (G) TRANSPORT TO OVERALL GLUCOSE METABOLISM. Lakhi Sakhrani*, Walter Trizna*, Mary Taub*, and Leon G. Fine. Division of Nephrology, UCLA School of Medicine, Los Angeles, CA, and Department of Biochemistry, State University of New York, Buffalo, NY.

Studies on isolated non-perfused proximal tubules (PT) indicate that these segments do not metabolize glucose. Since it is possible that there is restricted access of G to the lumen in isolated tubules, we examined the issue using primary cultures of rabbit PT in which the entire cell membrane is exposed to G. PT cells (98% purity) obtained from rabbit renal cortices were maintained in hormone-supplemented serum-free medium. Cultures at 4-6 days showed: 1) PTH-, but not ADH-sensitive adenyl cyclase, 2) dome-formation, and 3) morphological polarity by electron microscopy. Kinetics of α methyl glucose uptake (not metabolized; apical uptake only) showed K_m = 0.9 mM and V_{max} = 0.8 nmols/mg/min. Uptake was inhibited 95% by 0.5 mM phlorizin or Na-free medium, 70% by ouabain, and 57% by galactose, but not by other G analogues. Cells generated ^{14}C-CO_2 from ^{14}C-glucose in the medium at a rate of 2.3 nmols/mg/30 min. This was not inhibited by phlorizin. Metabolism of exogenous glucose was predominantly via the hexose-monophosphate pathway. Summary: PT cells in primary culture show Na-dependent G uptake which is 10-fold greater than that seen in LLC-PK_1 cells. These cells do metabolize G to CO_2. The contribution of luminal G uptake to overall metabolism of exogenous G is trivial.

ENZYMATICALLY INDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS) BY ISOLATED RAT GLOMERULI (GL) Sudhir V. Shah, and Deborrah Noble.* Tulane Med. Center, Dept. of Med. and VAMC, New Orleans, LA

ROS affect a variety of biological processes potentially important in GL diseases. We have previously demonstrated the ability of isolated rat glomeruli to produce ROS (J.Lab.Clin.Med.98:46,'81) To elucidate mechanism that may be relevent in renal pathophysiology, we examined whether production of ROS could be triggered enzymatically. GL and tubules were prepared from perfused rat renal cortices; production of ROS was quantified by measuring luminol amplified chemiluminescence (CL). No response was observed with thrombin (1,10units/ml) or purified collagenase (100,200,400units/ml). In contrast, chymotrypsin and trypsin caused a dose dependent (.01-2%) increase in CL from GL with maximum response as follows: Resting $16\pm2\times10^3$ cpm(n=12); Chymotrypsin $325\pm75\times10^3$cpm(n=6); trypsin $217\pm34\times10^3$cpm/mg protein (n=6). Tubules had only a minor response. Soyabean trypsin inhibitor caused a marked (>90%) inhibition of glomerular CL response indicating an enzymatic rather than non-specific effect of trypsin. The CL response was by GL rather than by "contaminating" leukocytes since a similar marked response (n=3) was observed in GL isolated from cytoxan treated leukopenic (WBC $<1000/mm^3$) rats. The involvement of superoxide and hydroxyl radicals in the CL response was demonstrated by using scavengers of ROS. Neutral proteases from infiltrating leukocytes and/or renal tissue have been shown to be released in GL diseases; our results which show the production of ROS in response to neutral proteases suggest a potential mechanism for the production of ROS in GL diseases.

SIMULTANEOUS QUANTIFICATION OF EXCRETORY METABOLISM OF 1-NAPHTHOL (N) AND EXCRETION OF ITS CIRCULATING METABOLITES BY TWO ORGANS IN THE RAT IN VIVO. L. M. Tremaine* and A. J. Quebbemann, Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN.

Excretory metabolism is defined as the process of precursor uptake by an organ, intracellular metabolism, and direct excretion of the metabolite(s), without entering the general circulation. The Specific Activity Difference Ratio (SADR) technique (Drug Metab. Disp. 9:402, 1981) permits simultaneous and separate quantification of the excretion of circulating metabolites and the excretory metabolism by any organ. A continuous infusion of ^{14}C-N, 1-naphthyl glucuronide (NG) and 1-naphthyl sulfate (NS), each at 1.0 μmol/min/kg, into anesthetized male Sprague-Dawley rats (n=3) resulted in an 86% recovery of the ^{14}C-label in urine (75%) and bile (11%). Greater than 97% of the excreted ^{14}C-label was identified as NG (69%) or NS (28%). 99% of the recovered NS appeared in urine. Of the NS formed in vivo and excreted in urine, 15% was derived from renal excretory metabolism of N. 81% of the recovered NG appeared in urine and 19% in bile. 22% of the NG formed in vivo and excreted in urine was derived from renal excretory metabolism of N. In contrast, all of the biliary NG was derived from the hepatic extraction of circulating NG. Therefore, at this infusion rate, virtually none of the biliary NG is derived from the intestinal-hepatic excretory metabolism of N, whereas the kidney couples metabolism of N with direct excretion as nephrogenic NS and NG. This demonstrates the usefulness of the SADR technique in simultaneously separating and quantitating excretory metabolism and the excretion of circulating metabolites in several organs in vivo.

VASOPRESSIN INDUCES GROWTH OF RENAL EPITHELIAL CELLS. Margaret M. Walsh-Reitz,* and F. Gary Toback. Department of Medicine, The University of Chicago, Chicago, Illinois.

In a previous study exogenous Na^+ was shown to stimulate the proliferation of renal epithelial cells from the monkey kidney line BSC-1. We have now examined the role of arginine vasopressin (V) as a growth-promoting polypeptide because this hormone is known to increase Na^+ permeability in epithelia. The effect of V on cell growth was studied in quiescent, high-density cultures to simulate the low proliferative capacity of kidney cells *in vivo*. The addition of V (100 pg/ml) stimulated [^{3}H]thymidine incorporation into DNA by 38%. The growth rate of confluent cultures was increased up to 33% by the addition of V (10 - 125 pg/ml), with a sharp optimum at 75 pg/ml. In contrast, the capacity of V to stimulate the growth of a fibroblast line was optimal at 20 ng/ml. To determine if V acts at the plasma membrane to exert its mitogenic effect, nutrient transport and ligand binding were assessed. Uptake of the nonmetabolizable amino acid, α-aminoisobutyric acid (AIB) was 49% greater in cells preincubated with V for 30 min. A similar increment in AIB uptake was obtained by raising the medium Na^+ concentration by 25 mM. V also increased the binding of [^{125}I] epidermal growth factor by 30%. The effects of V did not appear to be a consequence of altered cAMP metabolism because the addition of 8-bromoadenosine cAMP (10^{-11} to 10^{-3} M) did not stimulate DNA synthesis.

These findings suggest that V can promote kidney cell growth and appears to act at the level of the plasma membrane. Its effect could be mediated, at least in part, by alterations in cell Na^+ flux.

SUBSTRATE METABOLISM AND AMMONIAGENESIS DURING ACIDOSIS. L.Vertuno, D.Gaydos*, S.Bagnasco*, H. Preuss, Georgetown Univ., Dept. Med., Wash., D.C.

Oxidation of substrates by incubating rat renal slices decreases glutamine ammoniagenesis. Lactate has been shown to be less depressive when renal slices were obtained from acidotic rather than normal rats (Met. 27:1629,1978). We followed the effects of 10 other substrates on slice ammoniagenesis of normal and acidotic rats to discern patterns of inhibition. In addition to lactate, β hydroxybutyrate, acetate, pyruvate and acetoacetate showed relatively less depression to ammoniagenesis of acidotic slices. Citrate, succinate, fumarate, octanoate, and α ketoglutarate displayed as much depression in acidotic as normal slices. Glycerol had little effect under any circumstance. Thus, only substrates outside the TCA cycle (with exception of octanoate) had less depressive effects on ammoniagenesis during acidosis. To study this further, we followed glutamine and lactate metabolism in acidotic and normal slices. We found that the ability to resist depression of ammoniagenesis by lactate is present after 4 h of acidosis, peaks by 28 h, and remains constant through 76 h. Gluconeogenesis from lactate increases significantly, but $^{14}CO_2$ release and QO_2 decrease in acidotic slices. This suggests less oxidative decarboxylation of lactate via the TCA cycle in acidosis. Less oxidation of substrates entering the cycle would provide more NAD^+ to stimulate ammoniagenesis in the same manner as inhibitors (AJP 220:54,1971). Both studies suggest that a block in renal metabolism at or prior to citrate formation occurs during acidosis and may be important in augmented ammoniagenesis.

ALTERATION OF TRANSPORT PROPERTIES IN ISOLATED RENAL BRUSH BORDER MEMBRANES OF DIABETIC RATS. N.L.M. Wong, S.J. Whiting, and G.A. Quamme, Dept. of Medicine, Health Sciences Centre, Univ. of B.C., Vancouver, B.C.

Experiments were designed to determine the short-term (4-30 day) and long-term (11 mo.) changes in renal function associated with diabetes mellitus. Glucose titration studies were performed on age-matched control (C) and streptozotocin-induced diabetic (SD) Wistar rats using renal cortex brush border membrane (BBM) vesicle preparations on at least 3 separate days per experiment. The initial uptake (20 sec) of glucose by BBM vesicles was greater in 30 day SD rats compared to controls (55±5 and 91±4 pmol.mg^{-1}, respectively). The TmG of the kidney was also higher (1.5-2.0 fold) in SD rats compared to C. Similar BBM glucose uptake results were observed in the spontaneous diabetic BB Wistar rat, 4 days following insulin withdrawal. Glucose transport in long-term diabetes was significantly decreased (SD: 66±6 and C: 101±13 pmol.mg^{-1}.20 sec^{-1}) with a Na gradient and under Na-equilibrated conditions (SD: 19±1 and C: 39±4 pmol.mg^{-1}sec^{-1}). Chronic renal failure (7/8 nephrectomized, BUN: C,18 vs E, 51 mg%) rats exhibited a similar decrease in Na-gradient glucose uptake but no change in Na-equilibrated glucose transport. These data indicate that renal glucose transport is increased in short-term diabetes, due to alteration in BBM by the acute absence of insulin and/or hyperglycemia. In contrast glucose transport is decreased in long-term diabetes due to renal failure.

Renal Physiology — Acid Base

SIMULTANEOUS MEASUREMENT OF RENAL TUBULAR CELL pH BY DMO AND ^{31}P-NMR. Sheldon Adler, Eric Shoubridge* and George K. Radda*. Oxford Univ., Dept. of Biochemistry and Montefiore Hospital, Pittsburgh, PA.

Renal tubular cells were prepared from 1.5 kg white rabbits using collagenase separation. Tissue was incubated for 35-50 minutes in a specially designed NMR probe tube, stirred and bubbled continuously with 5% CO_2, 95% O_2 at 37°. The NMR spectrometer operates at 73.8 MHz for the ^{31}P nucleus. ^{14}C-DMO and ^{3}H-PEG were added at the start of the experiment. Viable tubules were defined by trypan blue exclusion before incubation and ATP and a small inorganic phosphorus peak in the NMR spectra. External pH (pH_E) was varied by altering bicarbonate in the K-R medium. Values obtained were:

N	pH_E	pH_{DMO}	pH_{NMR}	pH_{MITO}
(1)	7.20	7.34	7.27	7.55
(3)	7.00-7.08	7.18	7.04	7.60
(1)	6.85	7.08	6.90	7.62
(3)	6.68-6.75	6.98	6.70	7.82

In all experiments pH_{DMO} exceeded pH_{NMR}. Mitochondrial pH (pH_{MITO}) was calculated by assuming NMR pH to be a measure of cytoplasmic pH, DMO pH the sum of mitochondrial plus cytoplasmic pH, and a mitochondrial volume of 25%. These experiments indicate: 1. Both DMO and ^{31}P-NMR are valid measurements of tubular cell pH. 2. pH_{DMO} always exceeds pH_{NMR} reflecting DMO penetration into relatively alkaline mitochondria. 3. The transmitochondrial pH gradient increases as external bicarbonate and pH fall. We conclude that an elevated transmitochondrial pH gradient may be involved in the altered renal metabolism of metabolic acidosis.

EFFECT OF VOLUME EXPANSION (VE) ON LATE PROXIMAL TUBULAR BICARBONATE ABSORPTION ($JHCO_3$) IN THE IN VIVO MICROPERFUSED RAT PROXIMAL CONVOLUTED TUBULE (PCT). Robert J. Alpern and Floyd C. Rector, Jr., CVRI, Dept. of Med. and Physiol., Univ. of Calif., San Francisco, CA.

Previous studies from this laboratory demonstrated that in PCT perfused with an ultrafiltrate-like solution, VE had no effect on net $JHCO_3$ or active proton secretion, yet increased bicarbonate permeability ($PHCO_3$) by 50%. The failure to observe an effect of the increased $PHCO_3$ on net $JHCO_3$ was attributed to the small transepithelial bicarbonate gradients. It can be predicted that volume expansion will have its greatest effect on $JHCO_3$ when luminal [HCO_3] is low and gradients for HCO_3 backleak are large. To test this hypothesis, tubules were perfused with a late proximal tubule-like solution (5 mM HCO_3) in hydropenic (HYD) and VE rats. The rates of volume absorption (Jv) and $JHCO_3$ were measured using ^{3}H-inulin and microcalorimetry. As in previous studies, $PHCO_3$ measured in HYD and VE were used to divide net $JHCO_3$ into two components: passive HCO_3 diffusion (JPASS) and active proton secretion (JPROT). Results are mean ± SEM.

	HYD	VE	p
n	10	10	
$JHCO_3$ (pmol/mm•min)	19.0 ± 3.0	7.0 ± 3.0	<.01
JPASS (pmol/mm•min)	-33.0 ± 1.0	-45.0 ± 2.0	<.001
JPROT (pmol/mm•min)	52.0 ± 3.0	52.0 ± 4.0	NS
Collected [HCO_3] (mM)	4.9 ± 0.5	6.1 ± 0.4	< .1
Jv (nl/mm•min)	2.59 ± 0.11	1.93 ± 0.11	<.001

We conclude: 1) VE inhibits $JHCO_3$ in the late proximal tubule. 2) This effect can be explained totally by its effect on $PHCO_3$ and does not involve an altered rate of proton secretion. 3) The physiologic importance of this effect remains to be defined as it is small compared to the total rate of $JHCO_3$ in the proximal tubule.

EFFECTS OF CHRONIC CHANGES IN $PaCO_2$ ON COLLECTING DUCT H^+ SECRETION (CDH^+S) IN THE INTACT DOG. H.J. Adrogue and N.E. Madias. Baylor College of Medicine and Tufts-New England Medical Center, Houston, TX and Boston, MA.

Proximal acidification decreases in acute or chronic hypocapnia and increases in acute or chronic hypercapnia. By contrast, CDH^+S has been shown to decrease in both acute hypocapnia (JCI 58:77, 1976) and acute hypercapnia (Clin. Res. 29:454A, 1981). In the present study, we investigated the effect of chronic changes in $PaCO_2$ on CDH^+S. The urine-blood PCO_2 difference (U-BPCO_2) in alkaline urine was used as an index of CDH^+S; bicarbonaturia was induced by a sodium bicarbonate infusion (5 mmol/kg). Unanesthetized animals were examined under conditions of normocapnia ($PaCO_2$ 34.6 ± .9 mmHg, n=4), chronic hypercapnia ($PaCO_2$ 70.4 ± 2.8 mmHg, n=9) and chronic hypocapnia ($PaCO_2$ 21.3 ± .5 mmHg, n=8). Chronic hypercapnia and hypocapnia were produced in an environmental chamber. Bicarbonate excretion (uEq/kg/min) increased significantly as the chronic level of $PaCO_2$ increased (y=0.16 x + 4.85; r:0.781). Urine PCO_2 was a significant function of both $PaCO_2$ (y=1.14 x + 42.2; r:0.932), and [HCO_3^-] u (y=0.34 x + 11.3; r:0.798). Since the U-B PCO_2 was also a significant function of [HCO_3^-] u (y=0.117 x + 20.2; r:0.709), the evaluation of CDH^+S was performed by the ratio U-B PCO_2/[HCO_3^-]u. A significant negative correlation was found between the U-B PCO_2/[HCO_3^-]u and both $PaCO_2$ (y=0.257 - .0011x; r: -0.507) and [HCO_3^-]p (y=0.274 -.003x; r:-0.519).

The data suggest that CDH^+S in the intact dog diminishes as a function of the chronic level of $PaCO_2$. This finding provides a plausible explanation for the greater bicarbonaturia observed in hypercapnic as compared to hypocapnic animals following an identical alkali load.

PROSTAGLANDIN E_2 INHIBITS H^+ SECRETION IN THE TURTLE URINARY BLADDER. K. Ascer*, I. Loewenstein*, B. Mutz*, M. Schwartzman* and P. Lief. Montefiore Medical Center, Bronx, N.Y.

We unexpectedly found that furosemide but not chlorothiazide inhibited H^+ secretion in the turtle urinary bladder. Since furosemide stimulates tissue prostaglandin (PGE_2) production [and chlorothiazide does not] we speculated that PGE_2 might mediate the furosemide-related decrease in H^+ secretion. Initial studies, labeling the turtle bladder arachachadonic acid (AA) tissue pool demonstrated a spontaneous 30% conversion of AA to PGE_2. The conversion could be significantly prevented (>70%) by pretreatment with indomethacin. In short-circuited bladders, exogenous serosal PGE_2 [10μM] decreased H^+ secretion from 1.06±0.10 to 0.54±0.10 μM/h [n=6, p<0.025]. Inhibition of H^+ secretion by PGE_2 was dose related over a concentration range from 0.1-100 μM. Mucosal furosemide [6.25 x 10^{-4}M] also reduced H^+ secretion from 1.63±0.38 to 0.87±0.28 μM/h [n=5, p<0.01]. Indomethacin pretreatment completely blocked the inhibitory effect of furosemide but indomethacin alone had no effect on H^+ secretion. Neither PGE_2, furosemide nor indomethacin had any effect on Na^+ transport as estimated from short-circuit current. Our results suggest that both exogenous and endogenous (furosemide-stimulated) PGE_2 decrease H^+ secretion, perhaps by increasing intracellular C-AMP, a known inhibitor of H^+ secretion in the turtle bladder [KI 16:103, 1979]. We propose that prostaglandins may play an important role in the regulation of H^+ secretion.

DISTAL ACIDIFICATION (DA) AND URINARY pCO_2 DURING CHRONIC HYPERCAPNIA (CH). D Batlle, R Foley*, M Downer*, and N A Kurtzman. Univ of Ill, Chicago IL

Recent studies suggest that DA is unchanged or decreased during acute hypercapnia (H). That H decreases DA was inferred from the finding that urine-blood (U-B) pCO_2 during HCO_3 loading was lower than during normocapnia (N). We examined acidification in rats with CH induced by 3 days' exposure to 10% CO_2. This resulted in a marked compensatory rise in plasma HCO_3 (pH 7.32±0.02, pCO_2 77±2.0 mmHg, HCO_3 38±1.7 mEq/l), indicating that overall acidification was enhanced. Identical to that of rats with acute H, U-B pCO_2 was lower than in normocapnic rats following both HCO_3 and PO_4 loading. PO_4 infusion elicited a greater increase in titratable acid excretion in rats with CH than that of controls, suggesting that the low U-B pCO_2 of rats with CH might not portray decreased DA. To further examine this issue, we calculated the rise in urinary (U) pCO_2 from its baseline (i.e., preinfusion U pCO_2) value elicited by either HCO_3 or PO_4 infusions (ΔpCO_2). The baseline U pCO_2 of rats with CH was not significantly higher than that of controls (56±3.8 and 48±6.7 mm Hg). Both groups achieved an identical ΔpCO_2 after HCO_3 or PO_4 infusions. Thus, the low U-B pCO_2 of rats with CH is the result of their higher blood pCO_2 rather than failure to increase U pCO_2 in response to maneuvers that stimulate DA. We conclude that: 1. a chronic rise in blood pCO_2 does not lead to an equal rise in baseline U pCO_2; the reason U pCO_2 fails to rise during chronic H is unknown; 2. DA is probably enhanced during CH, and 3. U-B pCO_2 be replaced by ΔpCO_2 to examine DA when blood pCO_2 and U pCO_2 are widely different prior to HCO_3 or PO_4 infusion.

ROLE OF METABOLIC CO_2 PRODUCTION ($\dot{M}CO_2$) AND DIFFUSIVE GAS TRANSFER IN GENERATION OF ELEVATED RENAL CORTICAL PCO_2. A. Bidani, M.S. Lucci, E. Crandall, and T.D. DuBose, Jr. Univ. of Texas Med. Br., Galveston, TX and Univ. of Calif., Los Angeles, CA.

We have previously proposed that addition of HCO_3^- to stellate vessel (SV) plasma in disequilibrium with H^+ (pH_{Dq}) could explain the observation of elevated renal cortical PCO_2. Recent whole kidney perfusion studies by others have supported this hypothesis (Clin. Res. 30:106A). We examined the role of disequilibrium in SV by i.v. infusion of carbonic anhydrase (C.A.) (20 mg/hr) in 6 rats while monitoring SV PCO_2. Although a decrease in SV PCO_2 (61.6 ± 1.6 to 55.6 ± 1.1 mmHg, $p < 0.001$) was observed after C.A. the value remained higher than systemic $PaCO_2$. Therefore, pH_{Dq} in vivo cannot fully explain these findings. To examine additional mechanisms we have developed a model based on compartmental analysis utilizing mass balance calculations. To demonstrate agreement between experimentally measured and model values (pH, PCO_2, tCO_2) it was necessary to incorporate diffusive gas transfer of CO_2 ($\dot{F}CO_2$) as described for O_2 and heat. The model was extended to include calculations of the magnitude of FCO_2 (mM/L RBF) as $\dot{M}CO_2$ was varied from 0 to 1.1 mM/L RBF to maintain SV PCO_2 at 65 mmHg and pH at 7.28. At a $\dot{M}CO_2$ of 0, 0.5, and 1.1 the calculated FCO_2 was 2.2, 1.7, and 1.1 respectively, and described a linear relationship as expected from mass balance considerations. The efficiency of CO_2 gas transfer ranged from 75 to 85%. A small degree of pH_{Dq} would reduce the requirement for $\dot{M}CO_2$ and $\dot{F}CO_2$. In conclusion, metabolic CO_2 production coupled with a moderately efficient vascular gas exchange pathway in the cortex can adequately explain the findings.

TISSUE O_2 DELIVERY IS STABLE DESPITE RAPID CORRECTION OF CHRONIC METABOLIC ACIDOSIS (C MA). Benjamin, J.; N. Kopyt; E.R. Jones; R.G. Narins. Temple University Hospital. Phila. PA

Rapid in vivo correction of C MA transiently increases Hemoglobin-O_2 binding, as studied in vitro, suggesting that alkali therapy may be harmful. To determine if in vivo tissue hypoxia results from $NaHCO_3$ therapy of C MA, we studied exercise (Ex) tolerance and blood lactate (LA) as indices of effective O_2 delivery. Arterial studies were done in unexercised (UEx) and Ex normals (N-Ex;N-UEx) and in C MA rats (C MA-UEx;C MA-Ex). C MA was corrected prior to Ex by gavage feeding with $NaHCO_3$ (CMA+HCO_3-Ex); other groups received NaCl. Ex tolerance was studied utilizing a treadmill (end point:10 min or exhaustion).

PARA-METERS	N-UEx(5)*	N-Ex(5)	C MA UEX(5)	C MA EX(9)	CMA+HCO_3 UEx(7)
pH	7.44** ±.01	7.38 ±.01	7.30 δ ±.02	7.25 δ ±.02	7.47@ ±.01
HCO_3mM	22.1 ±.8	20.2 ±.1	16.0 δ ±1.0	11.6 δ ±1.0	23.4@ ±1.0
LA mM	1.2 ±.2	3.4 δ ±.9	1.2 ±.3	4.5 δ ±.7	3.2 δ ±.4
Ex(min)	-	10	-	6.3 ψ ±.7	5.4 ψ ±1.0

*() no. of rats; **mean ±SEM; p values: δ<.05 vs N-UEX; @ <.05 vs other C MA groups; ψ <.05 vs N-Ex.

Ex tolerance, lessened by CMA, was not worsened by $NAHCO_3$ nor was the increment in LA changed. Thus, 2 key parameters of tissue oxygenation show that rapid correction of C MA does not compromise O_2 delivery.

ANION SELECTIVITY ALTERS RENAL TUBULE CELL Na^+-H^+ EXCHANGE. S. Blumenthal*, R. Ware* and J. Kleinman, Department of Medicine, Wood VA Medical Center and the Medical College of Wisconsin, Milwaukee, WI.

Suspending Na^+ and ATP-depleted renal proximal tubules in Na^+-containing media produces transitory alkalinization of the cell interior, probably due largely to luminal Na^+-H^+ exchange (Clin. Res. 29:775a, 1981). To investigate the role of the accompanying anion in Na^+ gradient-induced alkalinization, suspensions of cortical renal tubules were Na^+ and ATP-depleted by incubation with rotenone in Na^+-deficient media. After centrifugation, the cells were resuspended in media containing 140mM Na^+ with anions of differing permeabilities. The magnitude of the initial alkalinization (estimated from the distribution of ^{14}C-DMO) correlated with the sequence, $SO_4^=$>isethionate >Cl^->NO_3^->SCN^-, an order of selectivity described for a number of membranes. SITS or the presence of CO_2/HCO_3^- only reduced the difference in cell alkalinization between the Na_2SO_4 and NaSCN gradients by 10-20%. In other experiments, cells were depleted of Na^+ and ATP in media containing 40mM K^+. These high-K^+ cells were then resuspended in K^+-free media containing 140mM NaCl with and without valinomycin. Valinomycin significantly inhibited the initial alkalinization (0.12±0.02 pH units vs. 0.17±0.01, P<0.01).

The results demonstrate that anionic permeability influences the magnitude of Na^+-H^+ exchange in renal tubule cells, probably through alteration of cell membrane potential.

EFFECT OF CARBONIC ANHYDRASE INHIBITORS ON BASOLATERAL HCO_3^- TRANSPORT IN SALAMANDER PROXIMAL TUBULES. Walter F. Boron and P. Fong*, Dept. of Physiol., Yale Univ. Sch. Med., New Haven, CT

Basolateral HCO_3^- transport in the proximal tubule (PT) of *Ambystoma tigrinum* appears to involve the movement of one Na^+, two HCO_3^- (or their equivalent) and negative charge, all in the same direction. Reducing basolateral (bl) $[HCO_3^-]_{bl}$ or $[Na^+]_{bl}$ lowers intracellular pH (pH_i) and Na^+ activity (a_i^{Na}), and transiently depolarizes the basolateral membrane potential (V_{bl}). Returning $[HCO_3^-]_{bl}$ or $[Na^+]_{bl}$ to normal raises pH_i and a_i^{Na}, and transiently hyperpolarizes V_{bl}. About half of this pH_i recovery is due to Na-H exchange, and half to Na/HCO_3 transport. In the present experiments, we examined the effects of acetazolamide (ACZ) and benzolamide (BNZ) on the Na/HCO_3 transporter of the isolated perfused PT, using pH- and voltage-sensing microelectrodes. Basolateral ACZ (10^{-4} M) reduced the rate constant of pH_i recovery from 1.74 to 0.88 min^{-1} ($n = 5$; $P = .0005$) when $[HCO_3^-]_{bl}$ was returned to normal, and from 1.86 to 1.01 ($n = 5$; $P = .007$) when $[Na^+]_{bl}$ was returned to normal. The transient hyperpolarization was reduced 52±7% (control: 13 mV) in the $[HCO_3^-]_{bl}$, and 15±4% (control: 32 mV) in the $[Na^+]_{bl}$ experiments. These last observations suggest ACZ may directly affect the Na/HCO_3 transporter, in addition to intracellular carbonic anhydrase. ACZ inhibition is about half as great from the bl as from the luminal solution. 50% inhibition by bl ACZ occurred between 10^{-7} and 10^{-8} M. BNZ has similar effects, but is more potent than ACZ.

PASSIVE AND ACTIVE COMPONENTS OF PROXIMAL TUBULAR BICARBONATE TRANSPORT. Yun Lai Chan, Gerhard Malnic and Gerhard Giebisch. University of Illinois, University of Sao Paulo and Yale University.

The effect of oncotic pressure changes on fluid (Jv) and net bicarbonate transport ($JHCO_3^-$), and the transepithelial bicarbonate permeability ($PHCO_3^-$) were measured by an improved luminal and capillary microperfusion method, allowing paired experiments on the same tubule. Rat proximal tubules were pump perfused and Jv and $[HCO_3^-]$ measured with ^{14}C inulin and a pH-glass electrode. Raising peritubular protein (0-8-15gm% bovine serum albumin) stimulated Jv and HCO_3^--reabsorption. The response to oncotic pressure changes was asymmetrical since luminal protein had no significant effects. Whereas solvent drag effects on HCO_3^- must be minimal peritubular protein stimulates translocation of bicarbonate from interspaces into peritubular capillaries.

$PHCO_3^-$ was measured from HCO_3^- net flux along a lumen to capillary directed electrochemical potential gradient. In these experiments active HCO_3^- transport and Jv were minimized by 10^{-4} M acetazolamide and luminal raffinose. $PHCO_3^-$ was 1.7 x 10^{-5}cm/s, and unaffected by increasing luminal flow rate from 10 to 45 nl/min. Since bicarbonate backflux is only a small fraction of physiological rates of JHCO3, net transport alterations at varying $[HCO_3^-]$ in the lumen must be due to changes in active HCO_3^- (H^+) transport. Thus, active H^+-ion secretion across the proximal tubule is gradient-dependent.

ACID HANDLING BY DEEP (DN) AND SURFACE NEPHRONS (SN) OF THE REMNANT KIDNEY (RK). J. Buerkert, D Martin,* and D Trigg,*. Renal Div., Jewish Hospital and Wash. Univ., St. Louis, Missouri.

The technique of micropuncture was used to assess acid handling in 11 rats 12 days after a 2/3 reduction in renal mass. The RK weight was 269±18 mg(SE) compared to a total renal mass of 867±21 mg in 15 control rats (CK). Arterial pH and bicarbonate content were reduced in the RK group. In situ pH measured near the end of the proximal tubule (EPT) of SN was 6.76±0.07, lower ($p<.005$) than the mean of 7.14±0.08 obtained near the bend of Henle's loop (BHL). Both values were less than those measured in the CK (6.92±0.03 at EPT and 7.34±0.05 at BHL, $p<.05$). The buffered acid content of fluid obtained at these sites was increased. At EPT titratable acid [(TA)V] delivery was 31±5 peq/min, threefold the value obtained for CK (7.1±1.2 peq/min, <.001). Similarly (TA)V to the BHL was greater (6.9±2.5 vs 1.6±0.5 peq/min in the CK, $p<.005$). After remnant formation delivery of ammonium [(NH_4^+)V] to the EPT increased from 18±2 to 66±6 peq/min, ($p<.001$). (NH_4)V to the BHL was 70±13 vs 46±7 peq/min, in CK ($p<.05$). Bicarbonate reabsorption [$T(HCO_3)$] in the proximal segments of SN and DN was increased. $T(HCO_3)$ at EPT was 338±52 vs 215±15 peq/min in the CK ($p<.025$). Near the BHL $T(HCO_3)$ was 552±63 for the RK vs 390±36 peq/min in the CK ($p<.05$). The data indicate that hydrogen secretion in the proximal segments of DN and SN is enhanced when nephron mass is reduced, and support the concept that acid retention in the RK group is due to alterations in the distal mechanisms for urine acidification.

RELATIVE EFFECTS OF SYSTEMIC pH, PCO_2 AND HCO_3 CONCENTRATION ON ILEAL ION TRANSPORT. **Alan N. Charney and Lloyd P. Haskell. VA Medical Center, NYU School of Medicine, New York, New York.**

We previously reported the effects of systemic acid-base balance on intestinal electrolyte transport (Am.J.Physiol.239:G427,1980; 242:G486,1982). To determine the relative effects of systemic pH, PCO_2 and HCO_3 concentration, states of acute metabolic acidosis (pH=7.14,HCO_3=14.7mM) and alkalosis (pH=7.52,HCO_3=35.5mM) were created in rats by gavage feeding $(NH_4)_2SO_4$ and $NaHCO_3$, respectively. During in situ perfusion of the ileum of mechanically ventilated rats, ion transport was measured before and after respiratory compensation of the systemic pH. Acute respiratory acidosis (pH=7.19, PCO_2=67.9 mmHg) and alkalosis (pH=7.60,PCO_2=23.8 mmHg) also were studied. When animals in all groups were considered, net sodium absorption ranged from 3.4 to 10.0 μeq/cm/hr and correlated with blood pH (-0.97). Net bicarbonate secretion ranged from -0.9 to -5.8 μeq/cm/hr and correlated with the plasma bicarbonate concentration (r=0.91) independently of blood pH and PCO_2. Net chloride absorption ranged from 4.1 to 12.4 μeq/cm/hr and correlated with blood PCO_2 (r=0.92). Chloride absorption only was altered when systemic pH and bicarbonate concentration changed in opposite directions. Alterations in luminal pH and PCO_2 did not affect ion transport. These results suggest that systemic pH affects a sodium chloride absorptive process and that the plasma bicarbonate concentration affects a chloride absorptive-bicarbonate secretory process in the rat ileum.

IMPACT OF ACUTE RESPIRATORY ALKALOSIS (ARA) ON PROXIMAL BICARBONATE REABSORPTION. M.G. Cogan, Dept. of Med., Univ. of Calif., San Francisco, CA.

Clearance studies have demonstrated a reduction in renal bicarbonate reabsorptive capacity by acute respiratory alkalosis (ARA). The quantitative role of the proximal nephron in the reduced reabsorptive capacity in ARA has not been previously examined in vivo. Free-flow proximal and whole kidney total CO_2 (tCO_2) reabsorption was therefore measured using microcalorimetry in hypocapnic and normocapnic rats. A respirator controlled ventilation. All rats received 1½-2% body weight plasma.

During ARA, a 48% drop in pCO_2 reduced absolute proximal bicarbonate reabsorption by 24%. Fractional bicarbonate reabsorption also fell in ARA despite the slightly lower filtered bicarbonate load, indicating specific inhibition of proximal acidification.

	ARA (n=8)	Control (n=6)	p <
pCO_2 (mm Hg)	22.8 ± 1.0	44.0 ± 1.4	0.001
Filtered [tCO_2] (mM)	23.9 ± 0.7	28.0 ± 0.4	0.001
SNGFR (nl/min)	47.1 ± 2.0	47.0 ± 2.7	NS
Absolute tCO_2 Reabsorption (pmol/min)	773 ± 36	1021 ± 49	0.005
Fractional tCO_2 Reabsorption	0.69 ± 0.02	0.78 ± 0.02	0.025

Despite similar distal tCO_2 deliveries (356 ± 42 vs. 296 ± 35 pmol/min), ARA induced significant bicarbonaturia (449 ± 107 vs. 18 ± 4 nmol/min, $p < 0.005$) compared to controls.

Conclusions: 1) ARA decreased absolute proximal acidification, which may be at least partially responsible for the diminished whole kidney bicarbonate reabsorptive capacity. 2) ARA also inhibited acidification by nephron segments other than the superficial proximal convoluted tubule since bicarbonaturia occurred in the setting of normal distal tCO_2 delivery.

COMPARISON OF DEEP LOOP OF HENLE (LOH) AND VASA RECTA (VR) TOTAL CO_2 CONCENTRATION [tCO_2]. T.D. DuBose, Jr. and M.S. Lucci. Univ. of Texas Medical Branch, Galveston, Texas.

We have previously demonstrated that alkalinization of LOH fluid occurs as the result of water abstraction in the descending limb (dLOH). A similar mechanism results in the generation of outwardly directed concentration gradients (ΔC) for passive Na^+ and Cl^- transport in the thin ascending limb (aLOH). This study was designed to determine if a ΔC exists for HCO_3 between LOH and VR before and after carbonic anydrase inhibition. [tCO_2] was determined by microcalorimetry and inulin from ^{3}H-methoxy-inulin activity in VR plasma and aLOH or dLOH using papillary micropuncture. Contamination of VR was ruled out using VR to collecting duct inulin ratios. The groups of rats investigated were: 1) controls (C)(n=10), 2) acetazolamide 50 mg/kg/hr (A)(n=12), and 3) A plus mannitol 12.5% (A+M)(n=11). The results are displayed as [tCO_2] mM ± SEM corrected in VR for H_2O and Donnan effect:

	LOH	VR	ΔC
C	20.5 ± 1.5	22.4 ± 1.1	NS
A	57.4 ± 1.6	39.3 ± 2.5	< 0.001
A+M	37.5 ± 1.5	36.3 ± 1.3	NS

The [tCO_2] in control LOH and VR was less than reported for Na^+ and Cl^- and differed further since a ΔC was not observed. An outwardly directed ΔC (18.3 ±3.4 mM) was present after A but abolished during A + M. It is concluded that passive transport of HCO_3 in the aLOH is unlikely and does not contribute to "loop" HCO_3 reabsorption. Significant passive transport could occur after A and may contribute to the "loop" component of carbonic anhydrase-independent bicarbonate reabsorption.

ALTERATIONS IN MEMBRANE AREA ASSOCIATED WITH H^+ TRANSPORT IN TURTLE BLADDER. Troy E. Dixon, and Chris Clausen*. SUNY Dept. of Medicine and Northport VA, and SUNY Dept. of Physiology and Biophysics, Stony Brook, L.I., New York.

Recent reports suggest that alterations of the apical membrane area, caused by fusion of cytoplasmic vesicles, is involved in regulation of H^+ transport in turtle urinary bladder. We have performed impedance studies in the tissue to measure the different membrane conductances and capacitances. Capacitance has been shown to be proportional to exposed membrane area. Our results from 9 bladders pretreated with ouabain and amiloride show that the apical-to-basolateral resistance ratio is 20 ± 2.2 and the capacitance ratio is 0.46 ± 0.04, both of which are comparable to data based on microelectrode studies and micrographs. The apical capacitance (Ca) in these bladders was 3.7 ± 0.67 $\mu F/cm^2$. Both apical and basolateral capacitance slowly and continuously decreased with time in each bladder, but only the rate of decrease of Ca appeared to be related to transport rate. Inhibition of H^+ transport with 0.5 mM SITS or 50 μM acetazolamide significantly increased the rate of decline in Ca from 0.35 ± 0.11 $\mu F/cm^2/h$ to 0.73 ± 0.11 $\mu F/cm^2/h$ (n=4), and from 0.68 ± 0.25 to 1.04 ± 0.36 (n=5), respectively. Removal of AZ and addition of 10 mM HCO_3^- and 5% CO_2 in the AZ-treated bladders increased H secretion and decreased the rate of decline in Ca to 0.35 ± 0.15. These studies demonstrate that changes in the rate of H^+ transport are associated with changes in the apical surface area in the turtle urinary bladder.

DISTAL TUBULAR CARBONIC-ANHYDRASE INDEPENDENT (CAI) BICARBONATE (HCO_3) REABSORPTION IN THE RAT: EFFECT OF AMILORIDE (AM). J P Frommer, M E Laski, M A Dudek*, D Wesson* and N A Kurtzman. Univ of Illinois, Chicago, IL

The sites of CAI HCO_3 reabsorption were localized in the Munich-Wistar rat using standard cortical and papillary micropuncture techniques and microcalorimetry. 17 rats received acetazolamide (AZ) (20 mg/kg/hr) and 14 rats received AM (2.5 mg/kg/hr) and AZ. Fractional distal delivery (FDD) of HCO_3 was determined by puncturing the early distal (ED) tubule of superficial (S) nephrons, and bend of the loop of Henle (LH) of juxtamedullary (JM) nephrons. The fractional excretion (FE) of HCO_3 was determined from urine of the intact contralateral kidney. The table depicts FDD of HCO_3 (%) to the different puncture sites. Results are expressed as mean ± SEM.

	FDD (S)	FDD (JM)	FE HCO3
AZ	39.6±2.0	78.1±8.4#	36.3±2.5#+
AZ & AM	34.4±2.8	64.6±6.0#	43.8±2.4#+

(#=p<0.05 vs preceeding value; +=p<0.05 AZ vs AZ & AM). There were no differences in TF/P inulin at the late proximal and ED and LH puncture sites between both groups. Our results show: 1) addition of AM to AZ treated rats does not affect proximal water or HCO_3 reabsorption; 2) fractional proximal CAI HCO_3 reabsorption is significantly higher in S than JM nephrons; and 3) AM induces net addition of HCO_3 between the superficial early distal tubule and the final urine. We conclude: 1) the administration of AM to these animals increases FE HCO_3 by inhibiting distal CAI HCO_3 reabsorption and 2) a significant proportion of CAI HCO_3 reabsorption must be voltage dependent since it can be inhibited by AM.

SEGMENTAL NEPHRON CHLORIDE HANDLING DURING RECOVERY FROM CL-DEPLETION ALKALOSIS (CDA). J. H. Galla, D. N. Bonduris*, R. G. Luke. Nephrology Research and Training Center, University of Alabama in Birmingham.

Acute CDA produced by peritoneal dialysis can be corrected by provision of Cl and without volume expansion. During this recovery, we have shown that Cl reabsorption is enhanced in collecting duct segment (CDS) (Abst, ASN 1981). To assess the contribution of other sites in the nephron to recovery, we studied by micropuncture techniques CDA rats dialyzed vs 0.15M $NaHCO_3$ and infused with isometric solutions containing 80 Cl and 40 HCO_3 meq/L at 0.5 ml/100 gm BW/h. Control rats (CON) were dialyzed vs Ringers HCO_3. All rats were studied 3 hrs later when serum Cl had increased (79±1 to 90±1 meq/L) in CDA.

SNGFR determined from distal nephron sites were lower in CDA (27.9±2.3 vs 37.9±2.6 nl/min; $p<0.02$). Late proximal (1.18±0.02 CDA & CON) and early distal (0.30±0.03 CDA; 0.25±0.04 CON) TF/UF Cl ratios did not differ. Reabsorptions of fluid (53±3 CDA; 51±2% CON) and Cl (38±4 CDA; 39±3% CON) in the proximal convoluted tubule did not differ. Fluid (65±3 CDA; 68±4% CON) and Cl (93±1 CDA; 92±1% CON) reabsorptions in loop segment also did not differ. Cl deliveries (4.8±0.7 CON; 4.5±0.9% CDA) to distal convoluted tubule (DCT) did not differ but out of DCT (4.8±1.0 CON; 2.9±0.3% CDA; $p=0.05$) did differ.

These data suggest that conservation of Cl in recovery from CDA is mediated in part by decreased filtered Cl and by alterations in Cl reabsorption within DCT as well as the CDS but not in more proximal segments.

AMMONIA PRODUCTION BY INDIVIDUAL SEGMENTS OF RAT NEPHRON. D.W. Good* and M.B. Burg. National Heart, Lung, and Blood Institute, Bethesda, Md. 20205

Sites of NH_3 production along the nephron previously had been assessed only indirectly by enzyme assay. Therefore, we measured directly NH_3 production by nephron segments of normal rats and rats given 0.28 M NH_4Cl to drink for 3-6 days. Tubules were microdissected and incubated in 2.5 µl fluid droplets under oil with and without 2 mM glutamine. NH_3 concentration in the droplets was measured at ten minute intervals using a new microfluorometric technique. The time-dependent increase in concentration was used to calculate production rates in pmol/min.mm tubule. Segments studied were proximal convoluted (S-1), proximal straight (S-2 and S-3), and distal convoluted tubule (DCT); cortical (CCT), outer and inner medullary collecting duct; cortical and medullary thick ascending limb; and thin descending limb (TDL). All segments produced NH_3 from glutamine. In normal rats total production was highest (>5 pmol/min.mm) in S-1, S-3 and DCT and lowest (<2) in CCT and TDL. Metabolic acidosis increased production by 120% in S-2 and 60% in S-1 without significant effect in any other segment. Proximal tubule segments from rats drinking 0.28 M $NaHCO_3$ for 4-8 days were also examined. This treatment decreased production by S-1 but had no effect on S-2 or S-3. We conclude: (1) all segments tested produced NH_3 from glutamine when incubated *in vitro* and (2) acid-base changes altered NH_3 production only in specific segments of proximal tubule.

CHARACTERISTICS OF A H^+ATPase FROM MAMMALIAN PAPILLA. S.Gluck, S.Kelly*, and Q.Al-Awqati. Depts. of Medicine and Physiol., Columbia Coll. P&S, New York, NY.

Urinary acidification by the collecting duct results from H^+ secretion. We report preliminary characteristics of a H^+ATPase mediating this renal process.

Bovine papillae were homogenized and a microsomal fraction was obtained by differential centrifugation which contained electrogenic ATP-dependent H^+ transport (measured by ATP-induced uptake of the weak base acridine orange which was reversed by protonophores) that was resistant to oligomycin (O), rutamycin, and efrapeptin, but inhibited by DCCD and N-ethyl maleimide (NEM), and had O-resistant DCCD and NEM-sensitive (NS-ATPase) ATPase activity. NS-ATPase, used as a H^+ATPase marker, was stimulated by protonophores, and was more specific than DCCD. Microsomes were separated on sucrose gradients; peak NS-ATPase was at density 1.11, separate from Na-K ATPase, and mito and lysosomal contaminants. The lysosomal fraction also had substantial NS-ATPase. Properties of the H^+ATPase in the microsomal peak NS-ATPase fraction were studied. H^+ transport was inhibited by the other sulfhydryl reagents PCMBS and NBD-Cl, but not by vanadate. PCMBS and NBD-Cl sensitive ATPase activity were the same as NS-ATPase, and all were inhibited by DCCD. The pH optimum of NS-ATPase was 7.0.

An electrogenic H^+ATPase is identified which is in a fraction distinct from mitochondria, lysosomes, and basolateral membrane, and likely represents a luminal H^+ pump. It appears to be a sulfhydryl enzyme which is not phosphorylated, and thus, like the turtle bladder H^+ATPase, resembles the pump of chromaffin granules and lysosomes.

EFFECT OF HCl-INDUCED ACIDOSIS ON NET ACIDIFICATION RATE IN THE PROXIMAL CONVOLUTED TUBULE (PCT). J.I. Hart*, O. Herrera*, R.M. Wong-Garcia* and R.T. Kunau. Univ. of Tx. Hlth. Sci. Ctr. San Antonio, Texas

Mineral acid induced metabolic acidosis is known to enhance acidification in the distal nephron. Study of the effect of metabolic acidosis on proximal tubular acidification by micropuncture techniques is hampered, in part, by the associated reduction in bicarbonate delivery. As the quantity of bicarbonate reabsorbed is used as an estimate of net acidification rate, this reduction makes comparison with control states difficult. In the present experiments the intratubular bicarbonate (TCO_2) concentration in the PCT was kept above 20mM by using in-vivo microperfusion techniques and monitoring the pH at the collection site with a micro pH electrode. The rate of tubular perfusion was adjusted to keep the pH at the collection site at 7.25. The pH electrode was then removed and the perfusate quantitatively collected to determine fluid (nl) and TCO_2 (pm) absorbed/mm·min^{-1}, i.e., J_V and J_{CO_2}, respectively. TCO_2 concentration was measured by microcalorimetry. Rats were made acidotic by adding 20 mM HCl/Kg BW to their diet daily for three days. Control (C) rats ate the same diet without HCl. Plasma TCO_2 and pH were 29 ± .3mM and 7.41 ± .003 in C rats, 21 ± .6mM and 7.25 ± .03 in acidotic rats, $p<.001$ and $p<.005$, respectively. J_V and J_{CO_2} were 3.05 ± .4 and 204 ± 11 in C rats and 4.48 ± .3 and 562 ± 71 in acidotic rats, $p<.02$ and $p<.005$, respectively. The ratio J_{CO_2}/J_V rose from 69 ± 5 in C rats to 125 ± 12 in acidotic rats, $p<.005$. If H^+ secretion in the PCT is mediated primarily by Na: H antiport, these findings suggest that HCl induced acidosis either increases the coupling ratio of Na to H or the number of antiport sites per unit tubular length.

IN VIVO MEASUREMENT OF pCO_2 IN THE RABBIT KIDNEY AND LIVER. R.J. Hogg, L. Pucacco*, J.P. Kokko and N.W. Carter. UTHSCD, Dallas, Texas.

It has been previously demonstrated that the pCO_2 of renal cortex ($KpCO_2$) is higher than systemic arterial pCO_2 ($ApCO_2$) in animals that normally excrete an acid urine (rat and dog). The purpose of the present studies was to examine potential mechanisms of the high $KpCO_2$ by 1) measuring $KpCO_2$ in the rabbit (an animal that normally forms an alkaline urine) and 2) determining pCO_2 in the liver (an organ without significant H secretory or HCO_3 reabsorption capacity). New Zealand white rabbits were micropunctured using pentobarbitol anesthesia and pCO_2 sensitive microelectrodes. Three series of pCO_2 measurements were made: a) left kidney surface proximal tubules (n=84); b) right renal cortex (n=31) and c) liver surface punctures (n=25). The latter two studies used a common mineral oil bath previously equilibrated with CO_2. Systemic arterial and renal vein pCO_2 were also measured. The results show:

	pCO_2	P
Arterial (A)	36.8±0.7	A vs RV=NS
Renal vein(RV)	39.2±1.9	
Renal cortex	58.3±0.8	Kidney vs A/RV=p<0.005
Liver surface	66.8±3.0	Liver vs kidney=p<0.05

There was no difference in the pCO_2 of left vs right kidney (57.2±1.2 vs 58.3±0.8). We conclude that 1) (K-A) pCO_2 values are elevated in animals that normally excrete urine of widely differing pH, and 2) the liver has a tissue pCO_2 that is significantly higher than that in the kidney. This suggests that metabolic CO_2 production may be an important component of the high CO_2 in body organs, including the kidney.

NH_3 METABOLISM IN RENAL DISEASE: ROLE OF SOLUTE EXCRETION AND GLUTAMINE (GA). Jones, E.R.; T. Katz; R.G. Narins. Temple University Hospital, Phila. PA

Factors regulating NH_3 production (AP) in discrete kidney diseases are poorly understood. Using clearance methods we studied, AP/GFR and the extraction of GA(EGA) in control (C) and experimental rats 1 week after iv Bromoethylamine HBr (BEA) - a specific medullary oxidant or normal NaCl. Data from basal and Acute Metabolic acidosis (AMA) are shown:

PARAMETERS	C (6)	BEA (8)	C+AMA(6)	BEA+AMA(7)
GFR δ	1.03±.11	.71±.06*	.87±.11	.57±.09 *
UNH_3V**	70±9	58±11	217±.23	156±.3
AP**	122±15	71±14*	312±31	192±38 *
EGA**	89±19	-110±40*	208±22	-56±69 *
FE NH_3(%)η	60±5	86±3 *	72±5	81±8

results are mean ± SEM; ( ) = n; δ ml/min/0.1Kg; **nmole/min/.1kg/mlGFR: η fractional excretion; *p<.05, BEA vs control;

BEA rats with histologically normal cortices, had a fall in GFR and AP, and GA addition to blood rather than extraction. FE NH_3 from non-GA precursors maintained UNH_3V equal to C. The FENa(%) and v(ml/min) rose in BEA vs C (2.1±.3 vs .95±.29; and .11±0.1 vs .078± .01; p<.05). AMA increased AP less in BEA but continued high $FENH_3$ kept UNH_3V close to C. Despite a 2.7 fold rise in AP EG was negative. Conclusions: 1. solute diuresis raises $FENH_3$ and maintains acid excretion in mild renal failure; 2. a N source other than GA is used for AP; 3. Failure of AP/GFR to rise above C as seen in other models of renal failure, suggests a key role for deep nephrons in the adaptation of AP.

THE Na^+/H^+ ANTIPORTER OF THE RABBIT PROXIMAL TUBULE IS CONFINED TO THE LUMINAL MEMBRANE. H.E. Ives*, V.J. Yee*, and D.G. Warnock, Dept. of Med., Univ. of Calif., San Francisco, S.F., CA.

It is believed that a Na^+/H^+ antiporter is responsible for proton secretion in the rabbit renal proximal tubule. For the Na^+/H^+ antiporter to effect net proton secretion, it must be located primarily on the luminal, and not basolateral membrane. In contrast, the Na^+/H^+ exchanger is found on both sides of the cell in the salamander proximal tubule. To study the location of the Na^+/H^+ antiporter in rabbit proximal tubule, we have separated luminal and basolateral membranes and measured Na^+/H^+ exchange in each using the acridine orange technique. Homogenates of renal cortex were fractionated with 38 ml linear 35-48% sucrose gradients. 3 ml fractions were collected and assayed for sucrose density (ρ), Na^+/H^+ antiporter rate, basolateral membrane marker (Na/K ATPase), and brush border marker (maltase). Enzyme activities are expressed relative to homogenate activity (RSA). The two fractions with maximal marker enzyme activity are:

ρ	Na/H Antiporter Arbitrary Units	Maltase RSA	Na/K ATPase RSA
1.150	0.0	0.0	29.7
1.170	13.7	14.3	1.4

Membrane vesicles capable of maintaining a pH gradient were found throughout the sucrose gradient. pH gradient collapse by K^+ and nigericin could be detected in all fractions, but pH gradient collapse by Na^+ could only be detected in the brush border fractions, suggesting the absence of a Na^+/H^+ antiporter in basolateral membranes. Thus, in distinction to the salamander proximal tubule, Na^+/H^+ antiporter of rabbit proximal tubule appears to be confined to the brush border membrane. This distribution could account for the vectorial transport of protons in the rabbit proximal tubule.

PROSTAGLANDINS (PG) MODULATE TOTAL AMMONIA PRODUCTION (AP). Kapoor, S.; T. Beck; R.G. Narins; E.R. Jones. Temple University Hospital, Phila. PA.

PG influence many vital renal functions but their effect on AP and the effect of acidosis on in vivo PG synthesis is unknown. Thus, in vivo rat AP was studied with clearance methods in: 1. controls (C); 2. mild acute metabolic acidosis (mAMA): 2mEq/kg of HCL in 40 min. Meclofenamate (M) 5mg/kg ip, was given to other C and acidotic rats.

PARAMETERS	C (7)	C + M(10)	mAMA (8)	mAMA+M (8)
pH	7.39±.01	7.39±.01	7.28±.02	7.31±.01
HCO_3mM	23.1±1.0	21.6±.4	17.8±.04	19.8±.8
AP*	87±12	216±27 δ	188±21	271±23ψ
UPG(pg/min)	175±24	<20 δ	771±129	70±12 δ

*nmole/min/0.1Kg/mlGFR; p values for M vs non-M δ<.01;<ψ.05

We show that M increases AP by 2.5 fold in C, demonstrating that PG inhibit AP. mAMA stimulates AP but we also show that it increases PG synthesis. The further increase in AP when M is added to mAMA shows that the net increase in AP in mAMA is the algebraic sum of positive direct effects of acidosis on AP and negative indirect effects from PG stimulated by mAMA. Thus mAMA, like ADH, stimulates renal PG synthesis which lessons the renal response (AP, H_2O reabsorption) to the primary stimulus.

FUROSEMIDE INDUCED METABOLIC ALKALOSIS IN RAT: HOMEOSTATIC ROLE OF CITRATE METABOLISM.
A.M. Kaufman*, C. Brod-Miller*, T. Kahn. Bronx VA Med. Ctr. and Mt. Sinai School of Med., N.Y., N.Y.

The metabolism of citrate results in the generation of bicarbonate. Therefore the excretion of citrate is considered equivalent to bicarbonate loss in terms of acid-base balance. Studies were performed to determine whether metabolic alkalosis produced by furosemide (F) results in an increase in citrate excretion and thereby ameliorates the alterations in P_{HCO_3}.

Seventeen rats fed a low Cl diet to which 4000 uEq Na_2SO_4 per day was added were studied during 1)baseline (7 days), 2)furosemide 6 ug/kg bid (3 days) and 3)post-F (4 days). Urine was collected daily and blood at the end of each period.

	Net Acid Excretion uEq/24 hr	P_{HCO_3} mEq/l	UcitV uEq/24 hr
Baseline	1214	23.0	59
Furosemide	1606	31.4	221
Post-F	1789	28.9	453

Net acid excretion (NAE), P_{HCO_3} and citrate excretion increased during F administration. During the post-F period NAE remained significantly higher than baseline NAE, citrate excretion was increased, but P_{HCO_3} did not rise. Similar results were obtained in studies performed on 8 rats fed 4000 uEq of Na neutral phosphate.

The findings that in the post-F period NAE remained higher than baseline NAE despite a high P_{HCO_3} was not consequent to Na depletion since the Na balance became positive but may have been due to sustained Cl and K depletion. Regardless of the explanation for high NAE in the post-F period an increase in citrate excretion appears to ameliorate the rise in P_{HCO_3} that would otherwise occur.

EFFECT OF CYCLIC AMP ON HYDROGEN ION SECRETION AND SODIUM TRANSPORT IN THE TURTLE BLADDER.
N A Kurtzman and S Sabatini. Univ of Ill, Chicago IL.

A previous study from another laboratory (Kidney Int 16:103,1979) demonstrated that serosal addition of 10 mM cyclic AMP inhibited hydrogen ion secretion in the isolated turtle bladder perfused with zero CO_2. The current studies were initiated to examine the mechanism responsible for the inhibition of hydrogen ion secretion induced by cyclic AMP. We examined the effect of cyclic AMP on hydrogen ion secretion in the isolated turtle bladder using both the pH stat technique and reverse short-circuit current. Sodium transport was measured using the short-circuit current. Studies were carried out at 0% CO_2 (low transport rates) and 1% CO_2 (high transport rates) at pH 7.4. 10 mM cyclic AMP was added either to the serosal or mucosal solution, and hydrogen ion secretion was measured from 0 to 120 minutes. In the presence of 1% CO_2, 10 mM cyclic AMP had no effect on hydrogen ion secretion. 1 mM dibutyryl cyclic AMP added either to the mucosa or serosa, likewise, had no effect on hydrogen ion secretion (high or low transport rates). This was true whether the agent was added to the mucosal or serosal solutions. Sodium transport, measured following the addition of 10 mM cyclic AMP to the serosal or mucosal solution at 0% CO_2 and 1% CO_2, was unchanged from control. These results indicate that cyclic AMP has no effect on sodium or hydrogen ion secretion in the isolated turtle bladder when studied at two different rates of acidification. We can demonstrate no role for this nucleotide in the modulation of acid excretion or sodium transport by this membrane.

STIMULATION OF DISTAL NEPHRON H^+ SECRETION BY K^+ DEPLETION.
C. Kornandakieti* and R. L. Tannen. Univ. of Michigan, Ann Arbor, MI.

The impact of in K^+ homeostasis on H^+ transport by the distal nephron is unclear. In previous studies utilizing the isolated perfused rat kidney, neither K+ depletion nor loading in vivo had any influence on the maximal urine to perfusate pH gradient, suggesting that the force of the proton pump was unaltered (KI 20:36, 1981). To examine whether K+ homeostasis might influence the intrinsic H+ secretory capacity of the distal nephron, kidneys from rats fed a K^+-free (n=10) or high K^+ diet (0.9 mmol/g, n=12) for 7 days were perfused in vitro at pH 6.8. Creatinine was added to the perfusate in stepwise fashion to provide sufficient urinary buffer excretion to determine the maximal distal nephron H+ secretory capacity (Abstr ASN 14:113A, 1981). Perfused kidneys from K^+-deficient rats had a lower urine pH (5.58 vs 5.76, $p < .01$) and excreted more titratable acid (6.11 vs 4.47 μmol/ml GFR, $p < .02$) than the K^+ loaded group. Titratable acid to pH 6.0, an index of distal nephron H^+ secretion, was also higher (3.81 vs 2.06 μmol/ml GFR, $p < .01$) in the K^+-depleted group. Following addition of amiloride (10^{-5}M) to the perfusate, urine pH remained lower (5.72 vs 5.96, $p < .001$) and T.A. pH 6.0 higher (2.31 vs 0.51 μmol/ml GFR, $p < .001$) in the K^+-depleted group. Thus K^+ depletion increases the intrinsic distal nephron H+ secretory capacity by a mechanism independent of distal nephron Na^+ transport.

Additional comparisons with kidneys from control, chronic metabolic acidotic and alkalotic rats suggest that the predominant effect on acidifiation is stimulation by K^+ depletion rather than suppression by a high K^+ intake. Therefore, the high urine pH which accompanies K^+ depletion in vivo results from increased renal ammonia production.

EFFECT OF ACUTE UREMIA ON ACID BASE BALANCE: RELATION TO UREA FORMATION.
Thayne Larson* and T.C. Welbourne. LSUMC, Dept. of Physiol. & Biophys., Shreveport, Louisiana.

The effect of eliminating renal excretory function on systemic acid base balance was studied in control, C, and metabolically acidotic, MA, rats; C and MA were pair fed $NH_4HCO_3^-$ and NH_4Cl^- over a 3 day period prior to study. The animals were anesthesized and their femoral arteries cannulated followed by bilateral ureteral ligation, BUL; serial blood samples were drawn before and at 3 hr after BUL. BUL of C rats resulted in a striking fall in plasma $[HCO_3^-]$, 27.9±1.2 to 20.8±1.4mM, rise in blood $[H^+]$, 39.7±4.4 to 49.0±3.6nM and reduction in pCO_2, 46±3 to 33±4mmHg; plasma $[Cl^-]$ rose from 90.8±2.4 to 97.4±1.8mM as did plasma $[K^+]$, 4.8±0.2 to 6.1±0.5mM, while plasma $[Na^+]$ remained unchanged, 147±4 vs 149±5mM. In contrast MA rats did not respond with a fall in the already reduced plasma $[HCO_3^-]$, 21.1±0.8 vs 20.0±1.3mM, nor changes in either $[H^+]$, 44.6±3.1nM vs 46.0±3.4nM or plasma $[Cl^-]$ 94.8±2.4 vs 96.5±2.1mM while plasma $[K^+]$ rose from 3.8±0.6 to 5.6±0.8mM. Hepatic urea formation potentially consumes 2 moles of HCO_3^- per mole of urea synthesized while urea production is greater in C vs MA, 3.1±0.3 vs 2.0±4μmole $min^{-1}100g^{-1}$, prior to BUL. However, BUL results in a 30 percent increase in urea formation by MA rats as compared to controls suggesting that metabolic HCO_3^- consumption is not the mechanism behind the fall in plasma $[HCO_3^-]$. Alternately the metabolic acidosis induced in C rats following BUL may be explained by either a paradoxically greater endogenous H^+ production in C than in MA rats or a shift of H^+Cl^- from intracellular or transcellular to the extracellular compartment.

EVIDENCE FOR VOLTAGE DEPENDENT ACIDIFICATION IN THE CORTICAL COLLECTING TUBULE (CCT) OF THE RABBIT. M E Laski, V E Morgan*, and N A Kurtzman, Univ. of Ill and West Side VA Hospital, Chicago, IL

Ouabain (O), and Li decrease acidification in open circuited bladders by eliminating the electrical gradient favoring acidification. The effect of O and Li on acidification by collecting tubules obtained from starved female New Zealand White rabbits was studied using the techniques of isolated nephron perfusion and microcalorimetric analysis of total CO2. Total CO2 flux (JTCO2) is reported in picomoles/mm/min, potential difference (PD) in millivolts. Solutions were always symmetric, with 25 mM HCO3, and were bubbled with 6.7% CO2 93.3% O2. After control (C) PD and JTCO2 were measured, O (10^{-8}, 10^{-10}M) was added to the bath, or 40mM LiCl replaced 40mM NaCl in bath and perfusate.

n=12	PD*	JTCO2**	n=11	PD**	JTCO2***
C	-16±4.0	6.03±4.46	C	-11.6±4.46	10.80±1.76
O	-2.2±2.0	1.48±1.63	LI	0.4±1.44	4.18±2.79

(*p<0.001, ** p<0.005, ***p<0.025)

In medullary collecting tubules (n=6) control PD was +9.6±5.6 and JTCO2 21.7±1.2. After O, PD in this segment was +10.5±5.6 and JTCO2 was 21.96±1.90 both NS.

We conclude: A) O and Li inhibit +JTCO2 and PD in rabbit CCT's and, B) O alters neither PD nor JTCO2 in medullary collecting tubules. The data CCT's is thus consistent with the data previously obtained in bladders, and also with the presence of a major effect of voltage on acidification in the CCT. The medullary tubule data agree with those of others and suggest that acidification in this segment is independent of sodium transport.

K^+ DEPLETION (KD) POTENTIATES THE METABOLIC ALKALOSIS (MA) OF Cl^- DEFICIENCY (CD). J.H. Licht and K. McVicker*, Naval Regional Med Cntr, Oakland, CA

In dogs chronic KD, produced by K^+ restricted diet, does not cause MA, but does potentiate the MA producing effects of mineralocorticoid hormone (MCH). The effects of KD on the MA of CD are unknown. We studied the effect of KD on MA of CD, produced by substituting $NaHCO_3$ for dietary NaCl in adrenalectomized dogs on fixed hormone replacement (n=5). In the first study dogs were fed a K^+ deficient diet for 25 days. Plasma K^+ (pK) fell from 4.4±0.1 to 2.8±0.1 mEq/L (deficit 5 mEq/kg). Plasma H^+, pCO_2 and HCO_3 did not change. When CD (3.4 mEq/kg) was superimposed over 12 days, $pHCO_3$ rose from 20.9±0.6 to 28.9±1.6 mEq/L ($p<0.01$). Effects of CD alone were examined by substituting $KHCO_3$ for $NaHCO_3$. While pK returned to baseline over 8 days, urine Cl^- fell from 3.7±0.7 to 2.3±0.3 mEq/day ($p<0.05$) and $pHCO_3$ fell to 23.6±1.0 mEq/L ($p<0.01$), a value still significantly higher than $pHCO_3$ during subsequent K^+ and Cl^- repletion (19.8±0.8; $p<0.01$). In a second study the order was reversed: KD was superimposed on CD. First, CD caused $pHCO_3$ to rise from 20.7±0.6 to 24.5±0.8 mEq/L ($p<0.001$). With superimposition of KD for 7 days $pHCO_3$ rose to 28.3±1.0 mEq/L ($p<0.001$), while pK fell from 4.4±0.1 to 3.0±0.1 mEq/L ($p<0.001$). Again Cl^- excretion was slightly greater during combined CD-KD than during CD alone. Weight did not change, but $pHCO_3$ was higher for any pCO_2 during combined KD-CD than during CD alone ($p<0.01$). We conclude that 1) chronic KD decreases renal Cl^- conservation during CD in MCH replete dogs and, 2) KD potentiates the alkalosis producing effect of CD, the result of enhanced renal bicarbonate reabsorption.

DISTAL CONVOLUTED TUBULE (DCT) TOTAL CO_2 REABSORPTION (J_{tCO_2}): AN IN VIVO MICROPERFUSION STUDY OF THE EFFECTS OF LOAD AND PERFUSATE COMPOSITION. David Z. Levine and Hans-Ulrich Gutsche*. Departments of Medicine and Physiology, University of Ottawa, Ottawa, Ontario, Canada.

Distal nephron HCO_3 reabsorption is thought to be influenced by cation exchange processes whereby H^+ secretion can be modulated by Na and K movements. To evaluate this at the DCT site, 2-loop surface DCT of normal and NH_4Cl rats were microperfused at 10 and 20 nl/min with (in mM): 25 HCO_3, 95 Na, 65 Cl, 0 K (Sol 1); 25 HCO_3, 95 Na, 65 Cl, 7.5 K_2SO_4 (Sol 2); 25 HCO_3, 33 Na, 0 Cl, 7.5 K_2SO_4, 102 urea (Sol 3). In each sample, H_2O movements were measured by H^3 inulin, J_{tCO_2} by microcalorimetry and reabsorption of Na and K (J_{Na}, J_K) by graphite furnace atomic absorption spectrophotometry. Normal rats perfused with Sol 1 and Sol 2 showed minimal J_{tCO_2}, which was significantly lower ($p<0.05$) than in NH_4Cl rats (below), and was not increased by perfusate changes. Data for NH_4Cl rats perfused at 20 nl/min (9-12 rats, 21-23 samples in each group):

	J_{tCO_2} pmol/mm.min	J_{Na} pmol/mm.min	J_K pmol/mm.min
Sol 1	98±17	283±77	-42±6
Sol 2	43±11	59±34	7±10
Sol 3	81±18	-184±73	19±21

F-testing showed no differences in J_{tCO_2}, at 10 or 20 nl/min perfusion. Thus, reversal of J_{Na} and J_K does not alter DCT J_{tCO_2} in metabolic acidosis. Accordingly, neither load nor these perfusate changes appear to augment minimal J_{tCO_2} in normal rats or suppress J_{tCO_2} in NH_4Cl acidosis.

STABILITY OF FILTERED BICARBONATE LOAD DURING RECOVERY FROM METABOLIC ALKALOSIS. F-Y Liu* and M.G. Cogan, Dept. of Med., Univ. of Calif., San Francisco, CA.

We recently reported that as the plasma [HCO_3^-] rose in chronic metabolic alkalosis, glomerular filtration rate (GFR) fell reciprocally so that filtered bicarbonate load remained normal. The present studies examined whether the mirror image of these changes occurs during recovery from metabolic alkalosis: whether GFR rises toward normal as plasma [HCO_3^-] falls. Twenty awake rats were studied in vivo during two clearance periods: Period (A), metabolic alkalosis induced with Na_2SO_4 added to an electrolyte-deficient diet for a week; and Period (R), repletion with Cl and/or K for a week. For alkalotic time controls, Na_2SO_4 was continued. All rats received daily DOCA. * represents $p < 0.001$.

Treatment	Plasma [HCO_3^-] (mM)		GFR (ml/min)		Filtered HCO_3^- (μeq/min)	
	A	R	A	R	A	R
Na_2SO_4/Na_2SO_4	33.6 ± 0.8	40.6* ± 0.6	2.1 ± 0.1	1.7* ± 0.1	72 ± 1	70 ± 1
Na_2SO_4/K_2SO_4	33.0 ± 0.7	29.9* ± 0.1	2.2 ± 0.1	2.4* ± 0.1	73 ± 1	72 ± 1
Na_2SO_4/NaCl	35.0 ± 0.6	30.8* ± 0.1	2.0 ± 0.1	2.3* ± 0.1	70 ± 1	71 ± 1
Na_2SO_4/KCl	31.3 ± 0.6	26.2* ± 0.4	2.2 ± 0.1	2.7* ± 0.1	71 ± 1	71 ± 1

The values following KCl were indistinguishable from normal. In summary, during partial repair (K_2SO_4 or NaCl) or full repair (KCl) of metabolic alkalosis, the fall in plasma [HCO_3^-] was accompanied by a reciprocal increase in GFR. Filtered bicarbonate load did not change. Thus, the rate of renal H^+ secretion needed to reabsorb the filtered bicarbonate remained unchanged from normal during the repair as well as the induction of metabolic alkalosis.

EFFECTS OF A DEXTRAN-BOUND AND UNBOUND CARBONIC ANHYDRASE INHIBITOR ON PROXIMAL TUBULE ACIDIFICATION. M. Lucci, J. Tinker, I. Weiner, and T. DuBose, Jr. Univ. Texas Med. Br., Galveston, TX and Upstate Med. Ctr., Syracuse, N.Y.

Previous experiments by our laboratory have examined the role of luminal carbonic anhydrase (CA) using a dextran-bound CA inhibitor, and suggested that luminal CA was required for the major portion of proximal bicarbonate reabsorption. Since dissociation of the unbound inhibitor (STZ) from the dextran molecule could not be excluded totally, the results were also compatible with inhibition of intracellular CA by STZ. Therefore, the present experiments were designed to compare the effects of dextran-bound STZ (DBI) and unbound STZ on total CO_2 reabsorptive rate ($JtCO_2$) and intraluminal pH. Rat superficial proximal convoluted tubules were perfused in vivo at 20 nl/min with artificial ultrafiltrate solution, and $JtCO_2$ was measured with ^{14}C inulin and micocalorimetry. Glass pH microelectrodes were used to determine pH in the first two loops closest to the perfusion pipette. STZ reduced $JtCO_2$ to 8 ±17 pmol/mm/min, not different from the effects of DBI. The intraluminal pH with STZ was 7.24 ± 0.04 (n=19), a value equal to the calculated equilibrium pH for a cortical PCO_2 of 60 mmHg and perfusate bicarbonate concentration of 25 mEq/L. In contrast, the pH measured with DBI was 6.80 ± 0.02 (n=22). Because both inhibitors decrease $JtCO_2$ similarly but only DBI resulted in luminal acidification, it is concluded that STZ remains bound to DBI in vivo. Unbound STZ permeates the cell and limits hydrogen ion secretion by inhibition of intracellular CA, while DBI inhibits only luminal CA and allows the generation of an acid disequilibrium pH, the result of continued hydrogen ion secretion.

UNIFORM VENTILATORY RESPONSE TO CHRONIC METABOLIC ACIDOSIS AND ALKALOSIS IN THE DOG. N.E. Madias, and H.J. Adrogué. Tufts-New Engl. Med. Ctr., and Baylor College of Med., Boston, MA and Houston, TX.

Previous studies have characterized the secondary changes in $[HCO_3^-]p$ resulting from chronic primary alterations in $PaCO_2$. By contrast, systematic data are not available with regard to the appropriate $PaCO_2$ response to chronic primary alterations in $[HCO_3^-]p$. To this purpose, dogs with graded severity of chronic metabolic acidosis (prolonged HCl-feeding;n=75) or metabolic alkalosis (three models:diuretics, gastric drainage and DOCA-alone or in conjunction with oral $NaHCO_3^-$;n=116) were examined. The ventilatory response was quantitated as the slope of the linear regression obtained by pooling the individual changes in $PaCO_2$ as a function of the prevailing changes in $[HCO_3^-]p$. The results demonstrate the existence of a significant and highly predictable ventilatory response, its magnitude in metabolic acidosis (y=0.86x+0.96) being indistinguishable from that in metabolic alkalosis (y=0.79x-0.78); the combined equation for all studies was, $\Delta PaCO_2$=0.74 $\Delta[HCO_3^-]p$-0.06, r=0.97. No significant differences were detected among the ventilatory relationships of the three models of alkalosis studied (diuretics, y=0.84x-1.41;gastric, y=0.61x+1.03; DOCA, y=0.79x+0.13. We conclude that the magnitude of the ventilatory response is uniform over a wide spectrum of chronic metabolic acid-base disorders extending from severe metabolic acidosis to severe metabolic alkalosis; on average, $PaCO_2$ is expected to change by 0.74 mmHg for a 1 mEq/L change in $[HCO_3^-]p$ of metabolic origin. In addition, our data indicate that the ventilatory response to chronic metabolic alkalosis is independent of the particular mode of generation.

EVIDENCE FOR CO_2 PRODUCTION IN THE EARLY PROXIMAL TUBULE OF THE MUNICH-WISTAR RAT. D.A. Maddox and F.J. Gennari. Univ. of Vermont, Burlington, VT.

We have found PCO_2 in the early proximal tubule (EP) to be greater than in either adjacent peritubular capillaries (PC) or the late proximal tubule (LP) (AJP 242:F78, 1982; Kid Int 21:237, 1982). If the high EP PCO_2 is related to CO_2 produced in the process of HCO_3 reabsorption, then PCO_2 should increase between Bowman's space (BS) and EP. To test this thesis, PCO_2 was measured in 6 Munich-Wistar rats. Arterial pH averaged 7.46 ± .02 and $PaCO_2$ was 39.2 ± 1.1 mmHg. The PCO_2 gradients (mmHg) between EP, BS, LP, PC, and systemic arteries (Art) were:

	EP-BS	EP-LP	EP-PC	EP-Art	Pc-Art
PCO_2 = (mmHg)	5.6 ±.7	5.1 ±.8	3.3 ±.8	22.8 ±1.7	20.3 ±1.7
p value =	<.005	<.005	<.025	<.001	<.001

The results again show significant EP-LP and EP-PC gradients. In addition, an increase in PCO_2 occurred between BS and EP which was largely dissipated by the LP. This increase in PCO_2 is consistent with a high CO_2 production rate in EP.

Values for EP-Art and PC-Art were double those we found previously in Sprague-Dawley rats and are in accord with higher values found by DuBose et al. (JCI 62:338, 1978). Because these rats were studied using a protocol identical to DuBose et al., we also measured renal blood flow (RBF) and proximal HCO_3 and Na reabsorption to determine the basis for the higher PCO_2. RBF was lower (p<.01) while proximal HCO_3 and Na reabsorption were comparable to our previous studies. Thus, the difference in cortical PCO_2 may be due, in part, to a decreased rate of CO_2 removal in the face of comparable CO_2 production.

EVIDENCE FOR HYDROGEN ION (H^+) SECRETION IN RAT OUTER MEDULLARY COLLECTING DUCT (OMCD). K.M.Madsen and C.C. Tisher, University of Florida, Gainesville

Studies in isolated perfused tubules have provided evidence for acidification by the OMCD. Recent studies in turtle bladder suggest that H^+ secretion in response to elevated CO_2 is regulated by exocytotic insertion of H^+ pumps into the luminal membrane of mitochondria-rich cells. Since intercalated cells (IC) in the OMCD resemble the mitochondria-rich cells, we examined the IC during acute respiratory acidosis (RA) for morphologic evidence compatible with the insertion of H^+ pumps into the luminal membrane. Rats were studied during normal acid-base conditions (n=5) and after 4 hr of RA (n=5). After collection of physiologic data the kidneys were fixed by in vivo perfusion. The surface density (S_V) in mm^2/mm^3 (±SD) of the apical and basolateral plasma membrane of IC was determined by electron microscopic morphometry. The blood pCO_2 was 35.8 mmHg in control and 92.5 mmHg in RA animals. Blood pH was 7.37 and 7.00, respectively.

	Outer Stripe		Inner Stripe	
S_V	apical	basolateral	apical	basolateral
Control	566 ± 89	1916 ± 162	383 ± 41	1574 ± 269
RA	850 ±167	1986 ± 288	565 ±105	1449 ± 206

There was a significant increase in the S_V of the apical membrane during RA (P=0.02). In addition, a decrease in the tubulovesicular membrane compartment was observed in the apical cell cytoplasm during RA. No changes were found in the S_V of the basolateral membrane. These findings are consistent with the working hypothesis that H^+ secretion in the OMCD of the rat is mediated by insertion of H^+ pumps into the luminal membrane of the IC.

INSULIN (I) RELEASE IN HYPEROSMOLAR STATES: L Nascimento, and J Shah*, VA West Side and Univ of Illinois Hospitals, Chicago IL.

We previously demonstrated that in nephrectomized rats the infusion of 3% saline (HS) or 18% mannitol (HM) is associated with severe metabolic acidosis. Alterations in membrane permeability and significant transcellular shifts seems to play a major role on the generation of this acidosis. Both groups were rendered hypertonic by infusion of similar volumes (15-20 mOsm/kg) of the corresponding solutions and the pCO_2 kept in normal range by mechanical ventilation. We evaluated immunosuppressive I release in these hypertonic states and its effects on the acid-base and electrolyte homeostasis. During HM infusion, serum Osm increased from 305 to 356 mOsm/kg H_2O ($p<0.02$) and blood pH decreased from 7.40 to 7.25 ($p<0.02$). In the HS group, 308 to 362 mOsm/kg H_2O ($p<0.02$) while blood pH changed from 7.36 to 7.25 ($p<0.01$). Changes in serum electrolytes were: Na 147 vs 169 mEq/l, Cl 107 vs 140 mEq/l and K 3.3 vs 3.3 mEq/l for HS and Na 142 vs 106 mEq/l, Cl 101 vs 74 mEq/l and K 3.7 vs 4.2 mEq/l for HM. These changes were highly significant ($p<0.001$) while glucose levels, serum Osm and blood pH were similar in both groups. I levels varied as follows: HM 19, 16 and 23 μU/ml at baseline 120 and 180 minutes respectively; HS 31, 94 and 76 μU/ml ($p<0.05$) a highly significant difference from baseline and HM group. These results may explain the higher K levels in HM group. The mechanisms involving the I response under these experimental conditions are not clear; however, serum sodium may be a significant factor. Further studies are in order to evaluate the role of I on the acid-base and electrolyte homeostasis in this model.

EVIDENCE FOR INTRACELLULAR pH REGULATION IN HEPATOCYTES. Allan S. Pollock* and Robert Park. V.A. Medical Center-Univ. of California, Div. of Nephrology, San Francisco, CA.

The stability of liver intracellular pH (pHi) has been previously noted in the intact dog (Park et al, Amer.J.Physiol. 236: F240, 1979). To further explore this phenomenon, we studied pHi regulation in one day old primary monolayer cultures of rat hepatocytes. Cultures were incubated overnite at 37°C in complete 1990R incubation medium prior to substitution with experimental media, and pHi was measured by the DMO method.

Under nonphysiologic conditions (1990R -HCO_3 + 40 mM HEPES in room air) with extracellular pH (pHe) ranging from 6.8 to 7.8, the measured pHi varied from 6.9 to 7.5 in a linear fashion so that pHi = 0.446(pHe) + 3.933 ($r = 0.8$). In contrast, physiologic conditions (1990R + HCO_3 2-40 mM + pCO_2 10-40 mmHg) of pHe ranging from 7.0 to 8.2 resulted in a plateau of pHi values between 6.8 and 7.0. Below pHe = 7.0, pHi values appeared to follow a slope similar to that observed in HEPES buffered media. Values for pHi were similar at given pHe values regardless of the respective values for HCO_3 and pCO_2, and the absence of medium glucose had no effect under either set of conditions.

We conclude that hepatocytes in monolayer cultures demonstrate evidence of pHi regulation in HEPES buffered incubation media. Under physiologic conditions, pHi regulation appears to be nearly complete for a pHe range which exceeds 1.0, with incomplete regulation at extreme degrees of acid pHe.

PROXIMAL REABSORPTION OF HCO3, Cl and WATER DURING ISOHYDRIC EXTRACELLULAR FLUID VOLUME EXPANSION (VE) IN SPRAGUE DAWLEY RATS. M. Paillard, M. Bichara* B. Corman*and C. de Rouffignac *(intr. by T. Anagnostopoulos). Hôpital L. Mourier, INSERM, and C.E.A., Paris, France.

It was recently reported in Munich Wistar rats that colloid free VE per se has no effect on proximal reabsorption of bicarbonate (HCO3), but specifically inhibits chloride (Cl) and water (H2O) proximal reabsorption, because of the decrease in peritubular protein concentration (Cogan et al, J. Clin. Invest 64:1168-1180, 1979). We examined the proximal HCO3, Cl, and H2O reabsorption during colloid free and colloid containing VE in plasma repleted Sprague Dawley rats, when GFR remained constant. Free flow micropunctures were performed 1) in 7 rats (Gr.1) while plasma repleted and then while expanded with colloid free (HCO3 Ringer, 10% body weight) solution ; 2) in 7 rats (Gr.2) while expanded first with colloid containing (albumine 70 g/l Ringer, 5% body weight) solution, then with colloid free solution. End proximal concentrations of total CO2 (tCO2) and Cl were determined by microcalorimetry and method of Ramsay respectively.

		Absolute reabsorption		
Gr.1	SNGFR	tCO2	Cl	H2O
	nl/min	pmol/min	pmol/min	nl/min
Plasma Repletion	45±2	1088±56	2006±163	23.1±1.3
Ringer VE	46±2	764±43	1202±118	15.4±1.0
	NS	$p<0.05$	$p<0.02$	$p<0.02$

(mean values ±SEM are factored by kidney weight)

During colloid containing VE (Gr.2) mean values for fractional proximal reabsorption remained lesser than in plasma repleted state, but were higher than in colloid free VE, for tCO2 (0.70±0.01 vs 0.67±0.02, $p<0.01$), Cl (0.34±0.02 vs 0.28±0.02, $p<0.05$) and H2O (0.44±0.01 vs 0.40±0.01, $p< 0.02$). Conclusions: 1) colloid free VE induces parallel impairment in proximal reabsorption of HCO3, Cl, and H2O in Sprague Dawley rats. 2) This effect is attenuated when the decrease in peritubular protein concentration is prevented. 3) VE per se and peritubular protein concentration probably alter the passive movements of solutes and H2O through the paracellular pathway.

ROLE OF ENDOCYTOSIS IN H^+ SECRETION (J_H) REGULATION BY TURTLE BLADDER. W.Reeves*, S.Gluck, and Q. Al-Awqati. Depts. of Medicine and Physiol., Columbia Coll. P&S, New York, NY.

J_H increases with CO_2 due to exocytotic insertion of H^+ ATPase into the luminal membrane (PNAS 79:4327,'82). We tested whether endocytosis could decrease J_H by removal of H^+ pumps.

Bladders bathed in 5% CO_2 Ringer and having a steady state J_H were found to have a rate of endocytosis of 1.4 nl/mg cell prot/min,as measured by uptake of fluorescent dextran (FD), which was linear up to 30 min. Microscopy showed uptake of FD into endocytic vesicles (which previously were shown to have H^+ pumps- PNAS, ibid.) and was restricted to mitochondrial rich cells (MRC). In paired hemibladders, removal of CO_2 decreased J_H by 80% over 30 min, and increased endocytosis by 57% to 2.2 nl/mg/min over the same period ($p/.05$).

As an independent test for this mechanism, the luminal surface of bladders was exposed to the lectins Con A and wheat germ agglutinin (WGA) which are known to induce endocytosis in other cell types. When fluorescently labeled, these lectins were seen bound to and internalized by MRC. Both lectins abolished J_H within 15 min. In paired hemibladders, WGA increased the rate of FD endocytosis by 100% (from 0.5 to 1.0 nl/mg/min; $p/.005$), and microscopy showed that FD uptake was again limited to MRC.

We conclude that MRC have a baseline rate of endocytosis during constant J_H which probably represents membrane recycling and shuttling of pumps between an intracellular pool and the cell surface. Removal of CO_2 or treatment with lectins can produce a decrease in J_H by enhanced endocytosis and net removal of luminal pumps.

EFFECT OF ACUTE HYPERCALCEMIA (HC) ON RENAL HCO_3 HANDLING IN THE RAT. R.M.A. Richardson, Dept. of Medicine, University of Toronto, Toronto, Ontario.

The association of HC with metabolic alkalosis has been attributed to a direct tubular effect of calcium to increase HCO_3 reabsorption, to inhibition of PTH, to a reduction in GFR due to HC itself or to reduced ECF volume. In this study, acute HC was induced in rats which had been volume expanded with saline-bicarbonate (6% BW). Whole kidney and proximal tubular HCO_3 handling (measured by microcalorimetry) were compared to control rats (C) which had not received calcium. Calcium infusion significantly increased serum calcium compared to C (12.1 mg/dl vs 8.6 mg/dl, $P < .01$). Plasma pH, HCO_3, total protein and hematocrit values were not different. At the same plasma HCO_3 level (34.5 mM), HC rats excreted less HCO_3-absolute excretion 1.5 vs 3.1 μEq/min/100 g BW ($P < .01$), and $F.E._{HCO_3}$ 5.3 vs 8.8% ($P < .01$). HC reduced whole kidney GFR (.86 vs 1.05 ml/min/100 g BW) and filtered HCO_3 load (28.4 vs 33.1 μEq/min/100 g). SNGFR of superficial nephrons was not reduced (34.3 vs 34.9 nl/min) nor was single nephron filtered HCO_3 load (1201 vs 1203 pEq/min). Superficial nephron proximal tubular HCO_3 and fluid reabsorption was similar in both groups.

It is concluded that acute HC in volume expanded alkalotic rats reduces urine HCO_3 excretion independent of ECF volume. A reduction in GFR and filtered HCO_3 load in deep nephrons may explain this effect. This study shows no evidence that HC increases proximal tubular bicarbonate reabsorption.

BICARBONATE TRANSPORT IN JUXTAMEDULLARY PROXIMAL CONVOLUTED TUBULES(JMPCT)-EFFECTS OF FLOW RATE AND LUMINAL ORGANICS. V. Schuster*, J.P. Kokko, and H.R. Jacobson. UTHSCD, Dallas, TX.

Both HCO_3 reabsorption ($JHCO_3$) and proximal glomerulo-tubular balance are felt to depend, in part, on the filtered load of HCO_3 as well as tubular fluid flow rate. We have recently shown that isohydric elevation of extracellular $[HCO_3^-]$ increases both $JHCO_3$ and net volume reabsorption (J_V) in in vitro perfused JMPCT's. The present studies examine the effects of perfusion rate on $JHCO_3$ and J_V in rabbit JMPCT's perfused in vitro. Because luminal organic solutes may prevent full expression of flow dependence of $JHCO_3$ via "competition" for the available electrochemical Na gradient, tubules were perfused with a solution free of transportable organic solutes. J_V, nl/mm min and $JHCO_3$, pmoles/mm min at perfusion rates of 5-8, 14-18, and 20-25 nl/min respectively were 0.7, 96.9; 0.94, 168.6; 0.94, 164.0. Mean luminal voltage was -0.4 mV and unaffected by flow rate. At all perfusion rates $JHCO_3$ was sufficiently high to account for all J_V as isotonic $NaHCO_3$ reabsorption. At perfusion rates >10 nl/min $JHCO_3$ in these tubules was significantly higher (166 vs 74) than $JHCO_3$ in similar tubules perfused with a solution containing organic solutes. These results suggest:1) Both $JHCO_3$ and J_V are flow dependent in JMPCT's with apparent saturation at flow rates >10 nl/min. 2)$JHCO_3$ is significantly greater when organic solutes are absent from the lumen suggesting that organic solute coupled Na entry competes with Na^+-H^+ exchange. 3) In the absence of luminal organic solutes and a luminal negative voltage J_V is accounted for by $JNaHCO_3$ without requiring JNaCl.

MECHANISM OF HCO_3^- EXIT ACROSS THE BASOLATERAL MEMBRANE OF RABBIT PROXIMAL CONVOLUTED TUBULES (PCT). S. Sasaki* and C.A. Berry. Univ. of Calif., San Francisco, CA

Acidification in the rabbit PCT is effected by Na-H exchange at the luminal membrane and HCO_3^- movement across the basolateral membrane. The mechanism of HCO_3^- exit, however, is not known. HCO_3^- could exit either conductively down its electrochemical gradient or neutrally in exchange for Cl. To examine whether HCO_3^- exit is conductive or neutral, we perfused PCT in vitro with ultrafiltrate-like solutions and measured net volume absorption (J_V) with inulin and net total CO_2 absorption ($JNTCO_2$) with microcalorimetry.

Conductive HCO_3^- exit was tested by measuring the effect of 2mM bath Ba^{++} which partially depolarizes the basolateral membrane. Bath Ba^{++} reduced $JNTCO_2$ 41% from 96.4 ± 13.4 to 56.4 ± 7.9 pmol/mm min (n = 9) and J_V 31% from 0.95 ± 0.11 to 0.65 ± 0.13 nl/mm min (n = 9). The Ba^{++} effect was specific for active H^+ transport. When acidification was first inhibited by 10^{-5} M acetazolamide in the perfusate and bath, bath Ba^{++} did not inhibit the residual $JNTCO_2$ (17.1 ± 6.2 v. 12.1 ± 5.2 pmol/mm min) or J_V (0.62 ± 0.07 v. 0.61 ± 0.05 nl/mm min (n = 6)). The hypothesis that HCO_3^- exit is neutral, in exchange for Cl, was tested by measuring the effect of Cl replacement with gluconate. Removal of perfusate and bath Cl reduced $JNTCO_2$ 30% from 69.2 ± 4.8 to 48.0 ± 5.1 pmol/mm min, but did not alter J_V (0.74 ± 0.03 v. 0.70 ± 0.07 nl/mm min (n = 6)).

In summary: (1) Bath Ba^{++} inhibits $JNTCO_2$ 41%. (2) Bath Ba^{++} inhibition is specific for the acetazolamide-sensitive component of proximal absorption. (3) Replacement of perfusate and bath Cl with gluconate inhibits $JNTCO_2$ 30%. From these data we conclude that HCO_3^- exits in part conductively down its electrochemical gradient and in part neutrally in exchange for bath Cl.

Na-K-ATPase ACTIVITY IN DEVELOPING RABBIT EARLY JUXTAMEDULLARY PROXIMAL CONVOLUTED TUBULE(EJMPCT). George J. Schwartz. Albert Einstein Coll. of Med. Dept. of Pediatrics, Bronx, N.Y.

HCO_3 transport ($JHCO_3$) in EJMPCT isolated from 2 week old rabbits is 1/3 that in EJMPCT from adults (ASN 1981). There is little change during the next 2 weeks, followed by a rapid increase during weeks 5-6. Because the developmental pattern for glucose absorption (Jglu) is similar, and both Jglu and $JHCO_3$ are driven by lumen-to-cell Na^+ flux, the role of Na-K-ATPase is considered to be critical. We developed a kinetic micro-assay for Na-K-ATPase (which couples ATP hydrolysis to NADH oxidation). Total and ouabain-insensitive ATPase are measured on the same 0.3-0.7 mm segment. Three to 9 EJMPCT were obtained after collagenase treatment of the kidney and 4-6 rabbits were studied at each week.

WEEKS	$JHCO_3$*	Na-K-ATPase*	$JHCO_3$/Na-K-ATPase
2	24±2	62±8	0.39
3	26±7	69±10	0.38
4	46±7	66±10	0.70
5	58±5	71±9	0.82
6	79±16	76±3	1.04
7	--	107±7	--
Adult	89±18	124±15	0.72

*mean ± 1 SE, pmol/min.mm

The increase in Na-K-ATPase tends to follow rather than precede that for $JHCO_3$; this is clear from the ratio of $JHCO_3$ to Na-K-ATPase activity. Thus, it is likely that the change in activity of Na-K-ATPase is secondary to the maturation of $JHCO_3$. $JHCO_3$ in the maturing EJMPCT may rise in parallel with the delivery of Na^+ to the cell(from increasing SNGFR) or may reflect increasing numbers of Na^+-H^+ exchangers in the luminal membrane.

INTRACELLULAR pH OF GRANULAR (G) AND MITOCHONDRIA RICH (MR) CELLS OF TURTLE BLADDER. John H.Schwartz and Maureen Tripolone*, Thorndike Mem.Lab.,Renal Section, Boston City Hospital, Dept. of Medicine, Boston University Med. School, Boston, Ma.

It has been proposed that H^+ transport (JH) by the turtle urinary bladder is primarily a function of MR cells but not G cells. As a consequence of JH, alkali is generated in the cell. Therefore, cells transporting H^+ should be more alkaline than cells not transporting H^+. To evaluate the hypothesis that MR cells are more alkaline than G cells we determined the intracellular pH of these isolated cells and the effect of inhibitors and stimulators of JH on cell pH. Bladder cells were separated into subpopulations of G and MR cells by Ficoll density gradient centrifugation. Cell pH was determined by DMO distribution. In a HCO_3^- free Ringer solution,the pH of MR cells was 7.30. This value was significantly greater ($p < 0.05$, $n = 8$) than both G cell pH (7.02) and media pH (6.98). With the addition of either acetazolamide ($5X10^{-5}M$) or SITS ($1X10^{-4}M$) MR cell pH increased to 7.38 and 7.39 respectively ($p < 0.05$, $n = 7$). These agents did not alter G cell pH. Isohydric addition of 2% CO_2 to the media reduced G cell pH by a greater extent than it did MR cell pH (6.71 vs 7.25, $p < 0.05$, $n = 6$). These studies document that: (a) MR cells are more alkaline than G cells or the incubation media; (b) agents that impair the disposition of alkali, generated as a consequence of JH, increase MR but not G cell pH and (c) CO_2 addition, which stimulates JH, resulted in a smaller reduction in the pH of MR cells than of G cells. These observations are consistent with the proposal that MR cells are primarily responsible for JH by turtle bladder.

ISOPROTERENOL AND cAMP STIMULATION OF H^+ SECRETION IN TOAD BLADDER. B. Spar*, E. Kelepouris*, R. Garrick*, M.M. Civan, Z.S. Agus. U. of Pa. School of Medicine, Philadelphia, Pa.

Preliminary studies have shown that cAMP stimulates and acetazolamide (Acz) inhibits reverse short circuit current (RSCC) in the amiloride (Am) treated Mexican toad bladder. To test the ionic basis of this effect and the influence of hormonal manipulation on RSCC, the following studies were performed:

To determine whether RSCC represented H^+ secretion or electrogenic Cl^- absorption, mucosal Cl^- was omitted. Hemibladders bathed in mucosal NO_3^- (Cl^- free), pretreated with Am ($2.0X10^{-4}M$), showed a stimulation of RSCC by $10^{-4}M$ 8-p-chloro cAMP (mean±S.E.Δ = -4.4±2.0μA, n=13, p<.05). Subsequent addition of Acz ($10^{-3}M$) eliminated RSCC and produced a rise above baseline (Δ= +7.6±2.3μA).

Vasopressin (VP)(100 mU/ml) and PTH (250mU/ml) did not stimulate RSCC after 30 minutes in Am treated bladders bathed in standard Ringers. Isoproterenol (ISO) (10^{-5}-$10^{-4}M$), however, produced a significant RSCC (Δ= -1.4±0.3μA, n=10, p<.001). In post Am bladders Acz produced a slight rise in SCC and prevented further stimulation of RSCC by both cAMP and ISO. Pretreatment with MIX, a phosphodiesterase inhibitor, accentuated the ISO effect compared to paired untreated hemibladders (Δ= -3.4 vs. -1.0μA, n=4, p<0.02).

We conclude that cAMP stimulates H^+ secretion in toad bladder, unlike its effect in turtle bladder. Similar stimulation by ISO, its potentiation by MIX, and the absence of effects with other adenylate cyclase stimulators suggest a catecholamine sensitive cAMP dependent process.

EFFECT OF ACUTE METABOLIC ACIDOSIS (AMA) ON AMMONIUM (NH_4^+) PRODUCTION BY THE PROXIMAL TUBULE (PT). E Simon,* D Martin,* and J Buerkert. Renal Div., Jewish Hosp. and Wash. Univ., St. Louis, MO.

In order to assess the role of the PT in NH_4^+ production during AMA, micropuncture studies were performed in two groups of rats. After tubule fluid collections were obtained near the end of the PT, Group I was given 0.2N HCl at 18 μl/min/100 g body wt. while Group II was given only saline. One hour later, samples were obtained from the previous sites of collection. During AMA, urine pH fell and total acid excretion doubled due to an increase in NH_4^+ excretion from 0.61±0.07 to 1.15±0.06 meq/min/g K.W. (p<.01). Acid excretion did not change in period 2 of the time controls. Tubule fluid NH_4^+ rose from 2.2±0.2 to 4.4±0.6 meq/L during acid infusion (p<.001) and its delivery [(NH_4^+)V] to the end of the PT nearly doubled (72±7 vs 36±3 peq/min/g K.W. in period 1, p<.01). In controls (NH_4^+)V changed only slightly. The increase in delivery during AMA was due to enhanced ammonia (NH_3) entry into the PT, since the filtered load was not affected (4.8±0.4 vs 4.3±0.3 peq/min/g K.W. in period 1). In situ pH at this site averaged 6.98±0.05 in period 1 and did not change after acid infusion (6.91±0.05). These data indicate that in AMA entry of NH_3 into the PT increases in near proportion to the increase in urinary NH_4^+ excretion. This occurs independent of a change in luminal pH. Further, proximal tubular entry of NH_3 is enhanced within an hour of the administration of an acute acid load.

CHLORIDE DEPENDENCE OF MEDULLARY COLLECTING DUCT ACIDIFICATION. D.K. Stone*, D.W. Seldin, J.P. Kokko and H.R. Jacobson. UTHSCD Dallas, Texas.

Recent in vitro microperfusion studies have demonstrated that the rabbit medullary collecting duct (MCD) possesses a high acidification capacity. We have shown that this acidification process proceeds by a sodium independent mechanism that can be stimulated by mineralocorticoids. These observations raise two major questions: what maintains net electroneutrality during proton secretion and what (if any) is the anionic dependence of the acidification process. The present studies were designed to address these issues. Rabbit MCD segments were harvested from inner stripe of outer medulla and perfused in vitro with measurement of bicarbonate reabsorptive rate ($JHCO_3$) by microcalorimetry and chloride flux (JCl) by electrometric techniques (Both expressed as pmol·mm^{-1}·min^{-1}). The role of Cl in the bath and lumen was then examined by replacing Cl by gluconate. $JHCO_3$ fell reversibly from 9.88 to -0.19 ($p<0.05$) with removal of Cl from the bath and increased from 10.68 to 15.01 ($p<0.05$) with removal of Cl from the perfusate. Addition of 5×10^{-4} M SITS to normal bath prompted an 80% reduction of $JHCO_3$. Luminal SITS had no effect. In a second series of experiments, $JHCO_3$ and JCl were measured simultaneously in normal MCD. In this series hydrogen ion secretion ($JHCO_3$) and Cl secretion occurred at near equal rates (9.37 vs 10.05 pmol·mm^{-1}·min^{-1}, respectively). We conclude that acidification in medullary collecting duct occurs by means of an HCl secretory process and that base exit across the basolateral membrane is effected by means of a Cl-dependent exchange mechanism.

ENHANCED URINARY AMMONIUM (NH_4) EXCRETION WITH L-DOPA. S. Striegel*, M. Gross, J. Higgins and J. Nally. Medical College of Ohio, Toledo, OH.

Studies by Zieve et al. (Gut 20:28, 1979) demonstrated that ammonium chloride induced coma in the rat model could be prevented by the administration of L-DOPA. The effect correlated with a decrease in blood and brain ammonia levels and an increase in urinary NH_4 excretion. We undertook clearance studies in the L-DOPA treated dog to examine the parameters known to affect NH_4 excretion. Mongrel dogs (n=7) were anesthesized and mechanically ventilated. A gentle water diuresis was established and urine was collected under oil via a Foley catheter. Two 20 min. control periods were followed by administration of L-DOPA (250 mg. IV over 10 min) with five 20 min. experimental periods.

The administration of L-DOPA did not alter serum pH (7.39 ± .02 vs 7.38 ± .02) nor decrease urinary pH (6.51 ± .24 vs 6.69 ± .19, NS). L-DOPA resulted in a significant increase in urine volume (3.49 ± .90 vs 6.68 ± 1.19 ml/min, $p < .05$). $U_{[NH_4]}$ was similar (5.65 ± 1.61 vs 5.44 + .96 mM/L) which resulted in a doubling in $U_{NH_4}V$ (16.3 ± 3.1 vs 33.6 ± 6.8μeq/min, $p < .05$). Urine Na^+ and K^+ excretion tended to increase (54 ± 10.7 vs 179 ± 75μeq/min, $p \geq .10$ and 34.4 ± 8.2 vs 52.2 ± 13.7 μeq/min, $p \geq .10$), but not significantly. We conclude: 1) L-DOPA enhances $U_{NH_4}V$, 2) the effect is proportional to the diuresis (and mild natriuresis) attributed to the known renal vasodilatory effects of L-DOPA, 3) the effect is not related to Δ serum pH or urinary acidification. Further studies employing a canine model of cirrhosis are warranted to examine if L-DOPA may ameliorate the hyperammonemia of liver failure.

ADAPTATION TO METABOLIC ACIDOSIS: ROLE OF INTRA CELLULAR pH AND CELL TRANSFORMATION. **R.Wheeler*, G.Dytko*, J.A.L.Arruda. UAMS and VA Hospitals, Little Rock, AR.**

The effect of in vivo metabolic acidosis on in vitro urinary acidification was examined in the turtle bladder. The turtles were made acidotic by the administration of NH_4Cl (20 mmol/day for 1-3 days). This maneuver resulted in a significant decrease in blood pH (7.04± 0.003 vs. 7.35 ± 0.003, $p< 0.001$) and urine pH (4.92 ± 0.18 vs 6.42 ± 0.18, $p< 0.001$) in the acidotic turtles as compared to controls. H+ secretion was significantly greater in bladders from acidotic than bladders from control turtles (57.0 ± 5.7 vs. 36.0 ± 6.4 μA, $p < 0.025$) despite identical mucosal and serosal pHs. This increase could be mediated either by a change in intracellular pH or by an alteration in the number of cells responsible for H+ secretion. Overall intracellular pH of isolated turtle bladder cells (measured by 6-carboxyfluorescein diacetate) was not different between control and acidotic bladders (6.77 ± 0.23 vs. 6.85 ± 0.14. The site of acid secretion was studied by fluorescence microscopy utilizing acridine orange. The cells thought to be responsible for H+ secretion appear red whereas the cells not involved in acidification appear green. Control bladders show approximately 2 cells with red fluorescence. In bladders from acidotic turtles there appears to be a striking increase in the area of red fluorescence. These data demonstrate that bladders from acidotic turtles show an adaptive increase in H+ secretion in vitro. This adaptive increase seems to be mediated by an increase in the number of cells thought to be responsible for H+ secretion.

CIS AND TRANS EFFECTS OF Li^+ ON THE Na^+/H^+ ANTIPORTER. D.G. Warnock, V.J. Yee*, and H.E. Ives*. Dept. of Medicine, Univ. of Calif., San Francisco, San Francisco, CA.

Li^+ inhibits the renal brush border Na^+/H^+ antiporter by several mechanisms. When Li^+ is present on the same (cis) side as Na^+ both competitive (Am.J.Phys. 241:C220, 1981) and non-competitive inhibition (Fed. Proc. 41:5793, 1982) have been observed. In addition, when Li^+ is on the opposite (trans) side to Na^+ there is inhibition of Na^+ efflux. We examined the concentration dependence of the cis and trans effects of Li^+ on the Na^+/H^+ antiporter. Rabbit renal brush border membrane vesicles were prepared at pH 6.0 using standard techniques, and were internally loaded with 0 to 30 mM Li^+ gluconate. Vesicles were then diluted into pH 7.5 buffer containing 90 mM Na^+ alone or 90 mM Na^+ and 1 mM Li^+; Na^+-dependent collapse of the outwardly directed proton gradient was measured with the fluorescent dye acridine orange. Rates of collapse are expressed as fluorescent units/sec/mg protein. % Cis Effect is inhibition due to 1 mM external Li^+ compared to 0 mM external Li^+.

Internal Li^+	External Li^+		% Cis Effect
	0 mM	1.0 mM	
0 mM	6.25	4.55	27%
1 mM	5.70	4.15	27%
5 mM	4.60	3.45	25%
10 mM	3.80	3.15	17%
30 mM	2.65	1.65	38%

1 mM external Li^+ caused ↶27% inhibition of the antiporter, greater than predicted by simple competition (Fed. Proc. 41:5793, 1982). This was true regardless of the degree of trans inhibition due to internal Li^+. Conclusion: The non-competitive cis inhibition by Li^+ is independent of trans inhibition and suggests the existence of a modifier site on the Na^+/H^+ antiporter.

ELECTROGENIC PROTON TRANSLOCATION BY AN H+-ATPase IN TURTLE BLADDER EPITHELIAL CELL PLASMA MEMBRANE VESICLES. H.J. Worman*, S.J. Youmans* and W.A. Brodsky. Mt. Sinai Sch. of Med., N.Y.; and Pritzker Sch. of Med., Univ. of Chicago, Chicago.

A proton-translocating ATPase in isolated plasma membrane vesicles from turtle bladder epithelial cells (Biophys. J. 37, 338a, 1982; Kidney Int. 21, 234, 1982) is shown by the ATP-dependent formation of transmembrane pH gradients (ΔpH), which are detected by quenching of acridine orange (AO) fluorescence of vesicles suspended in a (KCl + $MgSO_4$ + tris Hepes) medium at pH, 7.3. Replacement of KCl with K gluconate or sucrose reduced or abolished these responses. In sucrose media, an ATP-dependent transmembrane electrical potential (ΔΨ) was detected by oxonol-V fluorescence changes. Oligomycin (5 ugm/mg prot.) or DCCD (10 uM) inhibited over two thirds of mitochondrial ATPase activity but had no effect on plasma membrane ATPase activity. Higher levels of DCCD (500 uM) inhibited half the plasma membrane ATPase while oligomycin at over 1000 ug/mg prot. had no detectable effect. Correspondingly, levels of DCCD in excess of 100 uM were also needed to inhibit the ATP-dependent H+- translocation in these vesicles. Using free-flow electrophoresis, we find a tenfold enrichment in this ATPase which had been separated from the ouabain-sensitive, (Na + K) ATPase. In conclusion, the well-established ouabain-resistant ATPase (Am. J. Physiol. 215; 249, 1968) is further shown to be responsible for electrogenic H+= translocation in plasma vesicles from turtle bladder epithelial cells.

PROTON PERMEABILITY OF RENAL BRUSH BORDER MEMBRANES. Ernest M. Wright*, Richard E. Schell*, and Sally Krasne* (Intr. by Leon Fine). Department of Physiology, University of California, Los Angeles, California.

An important parameter in acid-base physiology of the renal proximal tubule is the proton permeability (P_H) of the brush border membrane. We have set out to estimate P_H of brush border vesicles from bi-ionic diffusion potentials and the constant field equation. Vesicles were prepared from rabbit kidney cortex by the Ca^{++} precipitation procedure and diffusion potentials were measured using the fluorescent dye 3,3' dipropylthiadicarbocyanine iodide (diS-C_3-(5)). Vesicles were incubated in salts of choline and gluconate buffered to pHs between 5.5 and 8.5 with 50mM Tris/HEPES or Tris/MES. Diffusion potentials were generated across the membrane by replacing choline and gluconate in the extravesicular solution with permeable ions or by changing the pH of the external buffer. The voltage response of DiS-C_3-(5) was calibrated with K-valinomycin and Na-ETH 1097 diffusion potentials. H diffusion potentials were essentially Nernstian in the absence of permeable ions, but were less than Nernst in the presence of permeable ions such as K. The P_H/P_K ratio was estimated from the magnitude of the H/K bi-ionic potentials, and in all experiments ranged between 1×10^7 and 1×10^8. We estimate from this P ratio that the proton conductance of the the renal brush border(G_H) membrane is in the same range as G_{Na} and G_K. Thus pH gradients between the proximal tubule epithelium and the glomerular filtrate will dissipate by H diffusion across the brush border membrane.

MOBILIZATION OF FEEDBACK RECEPTOR CYTOSOLIC CALCIUM DURING TRANSMISSION OF TUBULOGLOMERULAR FEEDBACK SIGNALS. P. Darwin Bell and Mary Reddington*. Dept. of Physiology and NRTC, Univ. of Ala. Med. Ctr., Birmingham, Alabama.

Recently we have proposed that feedback receptor cytosolic calcium (Ca^{++}) may be an intermediary step in the transmission of feedback signals. The purpose of these studies was to determine the source for the alterations in receptor cytosolic Ca^{++} during feedback responses. Stop flow pressure (SFP) feedback responses were evaluated in rats during retrograde microperfusion from an early distal tubule at 15 nl/min. During perfusion with an isotonic Ringer's solution (IRS) containing 4 mEq/L of Ca^{++}, SFP decreased by 13 ± 1.0 mmHg (n=21). Addition of 10^{-3} M verapamil, or decreasing perfusate Ca^{++} to 1 mEq/L or to zero Ca^{++} and 5 mM EGTA did not alter the magnitude of SFP feedback responses. In other studies, 8-(N, N-diethylamino)-octyl-3, 4, 5-trimethoxybenzoate (TMB-8), a putative inhibitor of intracellular Ca^{++} release, was added to IRS and perfused retrograde for 5 mins. During perfusion with IRS alone, SFP decreased by 11 ± 0.9 mmHg (n=14). With 100 μM TMB-8, feedback responses were reduced by 58 ± 7% (n=21) and were further inhibited by 80 ± 5% (n=10) and 70 ± 6% (n=16) at 300 and 500 μM TMB-8 respectively. Addition of the Ca^{++} ionophore A23187 (5μM) to the 500 μM TMB-8 solution restored feedback responsiveness (11 ± 0.9 mmHg; n=12). Thus, these results support a role for cytosolic Ca^{++} in the transmission of feedback signals. Furthermore, alterations in receptor cytosolic Ca^{++} during feedback responses may involve mobilization of Ca^{++} from bound or sequestered stores.

Renal Physiology — Hemodynamics

EFFECTS OF INFRARENAL INTRAAORTIC BALLOON PUMPING ON RENAL HEMODYNAMICS. Tetsuzo Agishi,* Satoshi Teraoka,* Tsutomu Sanaka,* Kazuo Ota* (introduced by Nobuhiro Sugino). Kidney Center, Tokyo Women's Med. Coll., Tokyo, Japan.

In some of heart failure patients, especially after major heart operations, prerenal renal failure may develop due to low cardiac output although sufficient to sustain patients' lives. Intraaortic balloon pumping is usually applied positioning a pumping balloon at the level of the descending aorta and is known to increase the coronary blood flow in synchronous collaboration with the natural cadiac pumping. Infrarenal intraaortic balloon pumping in which a pumping balloon was positioned between the renal tributaries and the iliac bifurcation was experimentally performed to investigate hemodynamic effects on renal circulation in healthy mongrels.

Pumping was performed in a very similar manner to usual intraaortic balloon pumping as triggered by R-wave on EKG. An amplitude of pulse pressure and pulse flow of the renal blood flow markedly increased during the pumping while the average pressure and flow rate did not show noticeable change. Neither blood pressure or blood flow rate in the ascending aorta and carotic artery were affected during the pumping. These observations may suggest that the infrarenal intraaortic balloon pumping be effective to selectively improve the renal perfusion in specific types of renal failure.

EFFECTS OF NORMAL AND RIGID ERYTHROCYTES ON THE ISOLATED PERFUSED RAT KIDNEY. A.Besarab, A.DeGugman, S.Hunter*, and A.Baseman*. Department of Medicine, Thomas Jefferson University, Philadelphia, Pa.

We determined the effects of hematocrit (Hct.), size, and deformability of erythrocytes on functions of the isolated rat kidney perfused at constant pressure for 2 hrs. with Krebs medium containing colloid (π 23mm.Hg.). Rat erythrocytes produced no effects on GFR, renal perfusate flow (RPF) or filtrating fraction (FF) at Hct. <2%. As Hct. was increased to 20%, GFR and RPF decreased but FF increased from 3 to 9%. Normalized protein excretion ($Up_r \times P/U_{In}$) decreased stepwise at a Hct. between 1 & 2% without further decreases at higher Hct. Fractional Na excretion (F_xNa) decreased with higher Hct. Human and rat erythrocytes produced identical effects. Gassing with carbon monixide - 0_2 did not alter the effects of human or rat erythrocytes. Small sheep erythrocytes required higher Hcts. to produce equivalent effects on hemodynamics.

Rigid erythrocytes produced by short heat treatment increased GFR and FF while decreasing RPF at Hct. <5%. F_xNa and protein excretion initially decreased at Hct. of .5 to 2% but then increased at higher Hct. Very rigid erythrocytes produced by gluteraldehyde fixation produced anuria at Hct. >2%. At Hct. of 0.5% GFR was decreased by 90% and associated with natriuresis and increased proteinuria. At lower Hcts., functional parameters returned toward those found with normal cells.

We conclude that erythrocytes have important effects on renal function dependent on their particulate properties. These effects are in turn affected by erythrocyte size and deformability.

VOLUME REGULATION IN MAN DURING NECK-OUT IMMERSION IN A MEDIUM WITH HIGH SPECIFIC GRAVITY (DEAD SEA WATER). O.S. Better*, N. Ish-Shalom* (Intr. by Robert T. Kunau, Jr.) Rambam Hospital and Technion School of Medicine, Haifa, Israel.

The effect of immersion for 4 hours on arterial blood pressure and the rate of urinary sodium excretion was studied in the same 5 subjects in (a) fresh water and (b) Dead Sea water (sp. gr. = 1.19) at 34°C. and the results were compared.

At 100 minutes of immersion mean systolic and diastolic blood pressure decreased ($P < 0.01$) in subjects immersed in fresh water and increased ($P < 0.05$) in those immersed in Dead Sea water.

While immersion in fresh water led to hypotension it was associated with an increased natriuresis. In contrast, the hypertensive response to immersion in Dead Sea water was not associated with an increased natriuresis.

It is concluded that under the unique conditions of this experiment urinary excretion of sodium becomes independent of systemic arterial blood pressure and is presumably governed by neurohumoral influences originating in the baroreceptors of the low pressure system.

SYSTEMIC PLASMA RENIN ACTIVITY (PRA) DOES NOT CHANGE SIGNIFICANTLY DURING RENAL AUTOREGULATION IN TRAINED, CHRONICALLY-CATHETERIZED, CONSCIOUS RATS. K.P. Conrad*, T. Brinck-Johnsen*, M. Gellai*, H. Valtin. Depts. Physiology and Pathology, Dartmouth Medical School, Hanover, NH.

Acute surgery and anesthesia perturb the renin-angiotensin system (RAS). In order to examine RAS in the undisturbed state and to assess a possible role of RAS in autoregulation, we used chronically-catheterized, conscious rats. Long-Evans male rats were prepared with an aortic constricting cuff to decrease or increase RPP, aortic catheters to measure mean aortic pressure (MAP) and RPP, and a bladder cannula. At least 5 days were allowed for recovery. Each rat served as its own control and only one altered RPP was studied in a rat on any given day. Absolute control values (n=29): RPP=112±2mmHg; GFR=896±31 µl/min·100gmBW; ERPF=3396±132 µl/min·100gmBW; PRA=2.07±.23ng/ml·h. All tabulated mean values are expressed as fraction of control (*$p<0.05$).

	Renal Perfusion Pressure			
	.65*	.76*	.85*	1.22*
# Observs./Rats	5/5	6/5	8/5	10/5
MAP	1.32*	1.09	1.03	1.22*
GFR (C_{In})	.52*	.92	1.06	1.00
ERPF (C_{PAH})	.51*	.84*	1.14	.94
PRA	5.06*	1.12	1.21	1.20

Conclusion: PRA does not change significantly within the autoregulatory range studied (ca. 90 to 137 mmHg), suggesting that systemic RAS does not mediate changes in vascular resistance during renal autoregulation. This study does not, however, exclude a role for intrarenal RAS in autoregulation.

DISTRIBUTION OF RENAL BLOOD FLOW (RBF) IN THE YOUNG RAT: EFFECTS OF UNINEPHRECTOMY (UN) AND MICROSPHERE SIZE. Robert L. Chevalier and Anthony V. Broccoli,* University of Virginia, Department of Pediatrics, Charlottesville, Virginia.

We have previously shown that autoregulation of RBF in the young rat takes place over renal perfusion pressure (RPP) of 70-100 mmHg, and is impaired by prior UN (ASN 116A, 1981). To determine whether distribution of RBF is altered by UN and decreased RPP, 22 rats were subjected to UN or sham operation during the first 5 days of life. At 30-40 days of age, RPP was adjusted to 100, 70, or 40 mmHg and radioactive microspheres, 9±1 and 15±1 µ in diameter were injected into the aortic root. The distribution of spheres was determined for the outer and inner halves of the renal cortex, and the percent in the outer cortex was as follows:

RPP (mmHg)	100		70		40	
Spheres (µ)	9	15	9	15	9	15
Sham (%)	78.7* ±0.9	88.1 ±1.3	81.2 ±0.8	81.2† ±0.2	79.3 ±2.2	82.9 ±4.9
UN (%)	78.3* ±1.0	87.5 ±0.8	80.8 ±2.7	83.0 ±2.0	80.0 ±2.0	80.1† ±0.2

Mean ± SE. *$p<0.05$ 9µ vs 15µ. †$p<0.05$ vs 15µ @ RPP 100 mmHg.

There was no redistribution of 9µ spheres when RPP was lowered below the autoregulatory range in either sham or UN animals. However, outer cortical distribution of 15µ spheres exceeded that of 9µ spheres within but not below the autoregulatory range. It is concluded that distribution of RBF is unaffected by reduction in RPP and that 15µ spheres overestimate outer cortical RBF at normal RPP in the young rat.

HEMODYNAMIC RESPONSES TO ADENOSINE AND ADENOSINE ANALOG IN THE ISOLATED PERFUSED RAT KIDNEY(IPRK). Richard Coulson*(intr. by M.E. Trimble). Dept. Pharmacol., VA & Upstate Med. Ctrs., Syracuse, NY.

Adenosine (Ado) is vasoconstrictive in the kidney in vivo but is vasodilatory in most other vascular beds. Previously, hemodynamic responses to Ado have not been demonstrable in the IPRK. I have shown both vasoconstriction and vasodilation in response to Ado and Ado-analog (N6-L-2-phenylisopropyl-, PIA) when norepinephrine(NE) or renin substrate tetradecapeptide (TDP) were added in appropriate amounts to the IPRK. Kidneys from Na-deficient Sprague-Dawley rats were recirculated for 1 hr with 50 or 100 ml Krebs-HCO3 medium containing 6g albumin (Fr.V) per dl. Vascular resistance (VR) = pressure/flow (mm Hg/ml per min). VR in control perfusions ranged from 2-4. This was increased by TDP-infusion (0.2-0.7 µg/min) to a more physiological range of 10-20. PIA or Ado (0.1 0.01 mM; as boluses in the reservoir) + TDP-infusion produced a profound, sustained (≥30 min) dilation. 3-isobutyl-1-methylxanthine (0.1 mM) abolished TDP-constriction and subsequent PIA-dilation. NE-infusion (0.2 µg/min) raised VR to 4-10 range. PIA (0.1 mM as a reservoir bolus) + NE-infusion produced a short (≤4 min) constriction and then a sustained (≥25 min) dilation. At higher NE-infusion rates (0.6 µg/min) or at lower PIA doses (50 nmol, intraarterial) only vasoconstriction was observed. It is concluded:(i) the renal vasoconstrictive effect of Ado augments NE-maintained vascular tone, (ii) the renal vasodilatory effect of Ado antagonizes angiotensin II-maintained vascular tone.

ENDOGENOUS DOPAMINE ACTIVITY IN CHRONIC HEART FAILURE. Andrew B. Covit*, John H. Laragh, Robert J. Cody*. Cornell Medical College, New York, New York.

Endogenous dopamine (D) is felt to result in renal vasodilatation and natriuresis; ischemic vasoconstriction and blunted natriuresis may be stimuli for enhanced dopaminergic activity. Therefore, plasma D levels (pg/ml; nl<90) were measured in 23 patients (pts) with the low-flow state of chronic heart failure (CHF). All pts had diminished cardiac index (1.51±.06 L/min/M^2) and elevated pulmonary wedge pressure (26±4 mm Hg). Renal ischemia was estimated by plasma renin activity (PRA, ng/ml/hr), an index of angiotensin-mediated vasoconstriction. D ranged from 1 to 1460 (mean 201±74 pg/ml). Pts on maintenance digoxin and diuretic treatment were divided into normal (nl) PRA (<5, n=8) and high PRA (>5, n=15) yielding a D of 40± 12 vs. 285±108 respectively (p<.01). D did not corelate with baseline hemodynamics, and revealed no response pattern on tilt. To evaluate Na+ influence on D, 8 pts were placed on 10 mEq and 100 mEq Na+ balance diets, off diuretics. Pts were divided into nl and high PRA on 10 mEq Na+ and the response of D to Na+ repletion was:

	PRA	10 mEq	100 mEq	%Δ
Group I (m=4)	2.9±.7	29±4	75±17	+159
Group II (m=4)	12.5±1.4	101±37	19±10	- 81

In conclusion, increased D activity is present in pts with PRA-mediated CHF. With Na+ repletion, nl PRA pts had a nl increase in D suggesting nl tonic inhibition of aldosterone secretion, whereas high PRA pts had paradoxical decrease in D, due to improved Na+ delivery to the distal nephron. This may explain the natriuresis of D administration in low-flow states.

THE ROLE OF ANGIOTENSIN II (AII) IN THE REGULATION OF PAPILLARY PLASMA FLOW (PPF) AND NA EXCRETION. P.F. Faubert, S.Y. Chou, J.G. Porush and E.M. Epstein*. Division of Nephrology, Brookdale Hospital Medical Center, Brooklyn, N.Y.

This study was undertaken to delineate the role of a physiological dose of AII in PPF (measured by the albumin accumulation technique) and Na excretion. Following AII (0.5 ng/kg/min) infusion into the left (L) kidney, Na excretion decreased ipsilaterally from 26±11 to 15±8 μeq/min (p<0.05) in 5 hydropenic anesthetized dogs, with no change in the right (R) kidney. After AII, PPF was 8.6±2.9 in the L and 27.9±1.8 ml/min/100g in the R kidney (p<0.01). Following IV saline loading superimposed on L intrarenal AII infusion in 6 dogs, Na excretion increased by 29±14 in the L and 65±20 μeq/min in the R kidney (p<0.01) with PPF 17.4±2.6 in the L and 40.8±3.4 ml/min/100g in the R kidney (p<0.005). The increase in PPF after saline loading was significant only in the R kidney (p<0.05). In 5 dogs when AII blockade was produced by saralasin (2 μg/kg/min IV) prior to saline loading and unilateral AII infusion, Na excretion and PPF were similar in the 2 kidneys. In all groups GFR and renal blood flow (RBF) were similar in the 2 kidneys before and after AII. These data demonstrate that AII reduces PPF and Na excretion without changing GFR or RBF. In addition, AII prevents the increase in PPF and blunts the natriuretic response to saline loading, both of which effects are abolished by AII blockade. These findings support our previous proposal that Na retention and blunted natriuretic response to saline loading in salt retaining states (e.g., chronic caval dogs) may be due, in part, to increased endogenous AII and its effects on medullary blood flow.

RESPONSE OF ISOLATED RENAL MICROVESSELS TO INTRALUMINAL PRESSURE, NOREPINEPHRINE (NE) AND ANGIOTENSIN II (AII). R.M. Edwards (Intr. by J.J. Grantham). Dept. of Medicine, Univ. of Kansas Med. Ctr. Kansas City, Kansas.

Interlobular arteries and superficial afferent and efferent arterioles were isolated from rabbit kidney and the effects of intraluminal pressure, NE and AII on lumen diameter were examined. A single microvessel was dissected and one end was cannulated and perfused. The other end of the vessel was occluded and lumen diameter was measured at fixed intraluminal pressures. In most experiments lumen diameters of interlobular arteries and afferent arterioles decreased by 5 to 10% with step increases in intraluminal pressure over the range of 80 to 180 mmHg. This increase in vascular tone with pressure was inhibited by papaverine. In contrast, lumen diameters of efferent arterioles continued to increase as pressure was elevated. In all three vessels NE (10^{-9} to 10^{-5}M) caused a dose-dependent decrease in lumen diameter. With 10^{-6}M NE lumen diameter decreased from 39.8 ± 4.6 to 14.3 ± 2.7 μm (7) in interlobular, 18.9 ± 1.5 to 3.9 ± 0.8 (8) in afferent and 12.2 ± 0.9 to 5.3 ± 0.9 (6) in efferent vessels. However, only the efferent arteriole responded to AII. Lumen diameter decreased by 10% with 10^{-12}M AII while the maximum response (11.8 ± 1.2 to 4.9 ± 1.7 μm (6) occurred with 10^{-8}M. The response to AII was blocked by saralasin. Conclusions: 1) Preglomerular vessels can increase their resistance in response to increased pressure in the absence of an intact macula densa. 2) NE constricts both pre and postglomerular vessels. 3) AII has a direct effect only on the efferent arteriole.

EFFECT OF ALPHA ADRENOCEPTOR BLOCKADE ON RENAL HEMODYNAMICS DURING MATURATION. R.D. Fildes*, P.L. Calcagno, G.M. Eisner & P.A. Jose. Georgetown Univ. Med. Ctr, Dept. of Peds & Physiol. Wash D.C.

An enhanced sensitivity to catecholamines might explain the increased renal vascular resistance (RVR) and decreased renal blood flow (RBF) observed in young puppies (Am J Physiol 225:796, 1974). To further characterize the role of alpha adrenergic activity on RVR and RBF, we studied the effect of the intrarenal infusion of phentolamine, an alpha blocker, during hydropenia in puppies. (Group I-15.67±0.67 days; Group II-42.17±1.81 days). Mean arterial blood pressure (MAP-mm Hg), RBF (ml/min/gm), RVR (units), glomerular filtration rate (GFR-ml/min/gm), and fractional sodium excretion (%FENa) were monitored. The results M±SEM are:

Dose[a]	MAP	RBF	RVR	GFR	% FENa
Group I					
C[b]	56±2	1.38±0.04	40.9±0.8	0.19±0.01	0.29±0.04
0.1	55±2	1.39±0.04	39.5±1.5	0.20±0.01	0.31±0.04
0.5	57±2	1.55±0.04*	36.0±0.4*	0.22±0.01*	0.39±0.03*
1.0	53±2	1.64±0.04*	32.5±0.7*	0.23±0.01*	0.40±0.03*
5.0	47±2*	1.44±0.03*	32.4±1.1*	0.21±0.01*	0.33±0.03*
Group II					
C	85±1	2.45±0.06	35±1.3	0.51±0.03	0.38±0.02
0.1	84±1	2.47±0.04	34±0.6	0.53±0.02	0.38±0.01
0.5	82±1	2.49±0.06	33±0.8	0.55±0.01	0.60±0.04*
1.0	80±1	2.62±0.05*	31±0.6*	0.59±0.02	0.76±0.05*
5.0	72±1*	2.41±0.08	30±1.1*	0.53±0.02	0.62±0.04*

a=ug/kg/body weight/min b-control; * = p<0.05, paired t test, compared to C; n=6/group.

This study demonstrates increased alpha adrenergic activity in the newborn kidney and provides the physiological expression of the increased alpha receptors reported from this laboratory in newborn puppies.

AFFERENT AND EFFERENT ARTERIOLES OF THE RABBIT
Vincent H. Gattone II, Friedrich C. Luft and Andrew P. Evan, Indiana University School of Medicine, Indianapolis, Indiana.

To identify the cellular components of the renal microvascular wall that determine a vessel's capability to modify renal blood flow, we exposed the arteriolar walls by our digestion-microdissection technique and examined samples by scanning electron microscopy. Most smooth muscle cells(SMC) of the afferent arteriole(AA) have a fusiform shape. These SMCs wrap around the vessel twice in both a clockwise and counter-clockwise direction. In the distal portion of the AA, near the glomerulus, the SMC is modified into a multipolar cell which contains renin granules. The shape of the SMCs of the efferent arteriole(EA) change over the length of the vessel. Proximally,for 50-100um from the glomerulus, the efferent's SMCs spiral around the vessel. These cells have irregular outlines and slight gaps between cells. More distally, multipolar pericytes are seen, possessing irregular processes which surround the vessel without appreciable orientation. There is a gradual rarefaction of the pericytes along the EA, as it approaches the peritubular capillaries or vasa rectae.

In conclusion: 1) the afferent arterioles possess typical smooth muscle cells along most of their length, 2)the proximal efferent arteriole possesses smooth muscle cells with sphincteric-like orientation and thereby would have potential for modifying glomerular and renal hemodynamics, and 3) the distal efferent arteriole possesses pericytes with a limited capability to modulate renal blood flow.

CHRONIC SALT LOADING: EVIDENCE FOR RESETTING OF TUBULOGLOMERULAR FEEDBACK (TGF) BY A HUMORAL FACTOR IN THE TUBULAR FLUID (TF). D.A. Haberle* and J.M. Davis# (introduced by G. Giebisch). *Yale Univ. Med. School, Dept. of Physiol., New Haven, CT. and #Univ. of Melbourne, Dept. of Physiol., Melbourne, Victoria, Australia.

Experiments were conducted in chronically volume expanded and control rats to determine whether resetting of TGF is caused by changes of the intrinsic sensivity characteristics of the juxtaglomerular apparatus or by changes of TF composition. The volume expansion was achieved by feeding the rats a high salt diet.Its effects were confirmed by measuring plasma volume which increased from 4.7 ± 0.75 (S.D.) to 6.18 ± 0.55 ml/100 g B.W. Rats from both groups were prepared in parallel and late proximal TF was collected by means of a microsuction/perfusion pump. TGF-response was assessed in each group from the change in early proximal flow rate (EPF) during loop of Henle perfusions at rates of 0, 10 and 40 nl/min with homologous, heterologous and artificial TF (Ringer's solution). EPF measured at loop perfusion rates of 10 and 40 nl/min were expressed as % of EPF at zero loop perfusion rate. In high salt rats loop perfusion with homologous TF elicited no significant TGF-response. However perfusion at 10 and 40 nl/min decreased EPF by 29 and 48% resp. (artificial TF) and by 19 and 38% (TF from control rats). In control rats perfusion with TF from high salt rats produced no significant TGF-response. However perfusion with homologous or artificial TF decreased EPF as in high salt rats. It is concluded that an inhibitory principle appears in TF of high salt rats which causes resetting of TGF sensivity.

INDOMETHACIN ALTERS THE SITE OF NOREPINEPHRINE'S ACTION ON ISOLATED PERFUSED RAT KIDNEY. G.W. Gleim*, G. Kao-Lo*, E. Ingenito*, D.L. Maude, Dept. of Physiol., N.Y. Med. Coll., Valhalla, N.Y.

Indomethacin-treated kidneys (I) have a lower GFR at any perfusion pressure (P) than control kidneys (C):

P (mmHg)		100-120	120-140	140-150
GFR	C	.65±.05	.75±.09	.83±.10
(ml/m/gm)	I	.19±.07*	.38±.06*	.49±.10*

*P (C v.I) < .05, N=6

Since renal vascular resistance (RVR) is higher in I (7.13±54 v. 5.18±.37 mmHg/ml/m/gm, P<.05) these data suggest that inhibition of prostaglandin (PG) synthesis leads to afferent arteriolar (AA) constriction.

To investigate further the effects of PG's on renal vascular activity we studied the effect of norepinephrine (NE) (1 µgm bolus in the arterial cannula) on kidneys perfused at constant flow (20±2.5 ml/m/gm) with a recirculating 5% albumin-containing bicarbonate-saline solution: The NE-induced increase in GFR and filtration fraction (FF) was attenuated in I (ΔGFR=.33±04 v. 66±.12; ΔFF=1.58±.23 v 3.33±.56%, p<.05, N=7). I and C kidneys had similar increases in overall renal vascular resistance (ΔRVR=1.84±.32 v. 2.08±.40 p>.1). NE, which raises GFR and FF in C by causing efferent arteriolar constriction, is an AA constrictor in I. Our findings indicate that PG's influence the resistance of the glomerular vasculature: Their inhibition by indomethacin is associated with AA constriction and with a shift of NE's dominant site of action from the efferent to the afferent arteriole.

ENDOGENOUS RENAL PROSTAGLANDINS ATTENUATE REFLEX RENAL VASOCONSTRICTOR RESPONSE TO SOMATIC RECEPTOR STIMULATION. H. Holdaas, U.C. Kopp & G.F. DiBona. Dept. Int. Med., Univ. Ia. Col. Med. & V.A. Med. Ctr., Iowa City, IA

Somatic receptor stimulation with capsaicin (Ca) or electrical stimulation of somatic afferent nerves (sciatic, SNS) produces reflex increases in heart rate, arterial pressure and vascular resistance. We assessed the ability of alterations in endogenous renal prostaglandins (increased by ureteral occlusion, UO; decreased by indomethacin, I) to modify the reflex renal vasoconstriction. Antegrade femoral arterial injection of Ca, 0.3 mg, in 7 vagotomized sinoaortic denervated anesthetized dogs decreased RBF by 35±9%; UO attenuated the decrease in RBF (-15±5%) the UO effect was reversible (-27±5%). UO reduced the renal vasoconstrictor response by ca. 50% (p< 0.01). In 10 vagotomized anesthetized dogs with bilateral carotid occlusion, graded (0.5-16.0 Hz) afferent SNS (20-40V, 1 ms) was examined during control (C), UO and recovery (R). Data are mean±SE% change in RBF at each frequency.

	0.5	1	2	4	8	16
C	-3±2	-7±2	-13±2	-17±2	-24±3	-28±4
UO	+2±1	-1±1	-5±1	-10±2	-12±2	-12±2
R	-1±1	-4±1	-10±2	-18±4	-23±4	-25±4

UO inhibited the renal vasoconstrictor response to afferent SNS by ca. 50% (p< 0.01) at all frequencies. In 7 similarly prepared dogs, I (2 mg/kg i.v.) restored the attenuated renal vasoconstriction to control despite continued UO.

Conclusion: The reflex renal vasoconstrictor response to somatic receptor stimulation is attenuated by enhanced endogenous renal prostaglandin synthesis.

VASOPRESSIN DECREASES PAPILLARY PLASMA FLOW, GFR, AND ERPF THROUGH DIFFERENT RECEPTOR SUBTYPES. W. Holt*, G. Sosnowski*, and V. Wiebelhaus., Pharmacology Dept., Smith Kline & French Laboratories, Philadelphia, Pa.

Renal vasopressin receptors can be separated into vascular (pressor) and tubular (antidiuretic) subtypes. We report characterization of the receptors mediating some of the renal responses to arginine vasopressin (AVP) in water diuretic anesthetized rats (N=66). AVP (52 pg/-kg/min) depressed papillary plasma flow (PPF, from 60.5±2.4 to 43.7±1.8 ml/min/ 100 gm papilla), inulin clearance (GFR, from 1.23±0.08 to 0.78±0.08 ml/min/ 100 gm rat), PAH clearance (ERPF, from 2.32±0.17 to 1.51±0.15 ml/min/100 gm rat), urine flow (V, from 27.5±4.5 to 6.7±1.4 µl/min/100 gm rat), and increased urine osmolality (U_{osm}, from 153±23 to 483±74 mOsm/kg H_2O); blood pressure (MAP) was unchanged. The nonpressor, antidiuretic agonist, dDAVP (1-deamino,8-DAVP;10 pg/kg/min) gave results quantitatively similar to AVP, except that PPF was at the control value. Similar results followed the concurrent infusion of AVP and a vascular receptor antagonist [$d(CH_2)_5$ Tyr(Me)- AVP, 16 µg/kg/min]. However, the concurrent infusion of AVP and a vascular-antidiuretic dual receptor antagonist [$d(CH_2)_5$ · Tyr(Et)VAVP, 16 µg/kg/-min] resulted in values for PPF, GFR, ERPF, V, U_{osm}, and MAP not different from those observed before drug infusion or in saline-infused controls.

We conclude that a vascular-type receptor mediates AVP-induced depression of PPF. Surprisingly, a tubular-type receptor appears to mediate AVP-induced depression of GFR and ERPF.

GLOMERULAR RESPONSE TO SEVERE CONGESTIVE HEART FAILURE IN THE RAT. I. Ichikawa, J.M. Pfeffer*, M.A. Pfeffer*, T.H. Hostetter, E. Braunwald*, and B.M. Brenner. Harvard Medical School, Boston, MA.

To define the glomerular response to severe heart failure, single nephron GFR (SNGFR) and its determinants were measured ∿3 weeks after ligation of the left coronary artery in male Munich-Wistar rats. Results from euvolemic rats with scarring of >40% of the left ventricular circumference (large MI, n=6) were compared with those from sham-operated controls (C, n=6). C and MI rats were also studied following indomethacin (I, 3.5 mg/kg BW/hr, iv).

	$\bar{P}_{GC}$	Q_A	SNGFR	SNFF	R_E	K_f
	mmHg	---nl/min--			¶	nl/(s·mmHg)
C	48±1	144±7	36±2	.25±.01	1.1±.1	.102±.011
MI	60±1†	81±4†	28±1†	.35±.01†	2.4±.1†	.031±.002†
MI+I	57±1	60±2†	20±1†	.34±.01	3.5±.3†	.020±.001†

(mean±1SE; † $p<0.05$ vs. preceding group; ¶ x 10^{10} dynes·s·cm^{-5})

Large MI rats had higher left ventricular end-diastolic pressures (29±1†vs. 6±1 mmHg) and lower UNaV (0.3±0.1† vs. 2.2±0.2 µEq/min). In MI rats an increase in efferent arteriolar resistance (R_E) caused glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) and single nephron filtration fraction (SNFF) to rise markedly so that SNGFR fell only slightly despite low values for glomerular plasma flow rate (Q_A) and ultrafiltration coefficient (K_f). Indomethacin led to a further rise in R_E and fall in Q_A and SNGFR in MI but not in control rats. Thus, prostaglandins appear to attenuate the action of a renal vasoconstrictor in MI rats. This delicate interplay between vasoconstrictor and vasodilator elements preserves GFR in heart failure despite marked renal hypoperfusion.

THE EFFECT OF ACUTE DECREASE IN RENAL PERFUSION PRESSURE (RPP) ON PAPILLARY PLASMA FLOW (PPF) AND ITS RELATION TO SODIUM EXCRETION (UNaV) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C. Hsu, K. Lau, and R. Caldwell*, Univ. of Michigan, Ann Arbor, MI.

The decreased PPF may be responsible for the decreased UNaV following reduction of RPF was studied in unanesthetized 13 week old female SHR. After 3% body wt. of isotonic saline expansin, followed by a constant infusion of .05 ml/min/100g saline solution, caridac output (CO, ml/min/100g) renal blood flow (RBF, ml/min/100g), GFR (ml/min/100g), mean arterial pressure (MAP, mmHg), PPF (ml/min/100g papilla), UNaV (uEq/min), and urinary osmolality (Uosm, mosm/kgH_2O) were measured in SHR-AC with decreased RPP by aortic constriction (AC), sham operated SHR (SHR-Sham), and Wistar Kyoto rats (WKY-Sham). The results are presented in the table (Mean ± SEM).

Gr.	CO	RBF	PPF	GFR	MAP	RPP	UNaV	Uosm
1 WKY-Sham	45	4.77	57.0	.73	119	119	3.4	701
(N=7)	±3	±.67	±4.2	±.04	±2.8	±2.8	±1.1	±102
2 SHR-Sham	37	3.77	43.8	.73	149	149	7.0	540
(N=8)	±3	±.42	±3.8	±.06	±3.0	±3.0	±1.5	±55
3 SHR-AC	30	3.19	43.6	.64	162	126	3.1	808
(N=9)	±1	±.37	±4.4	±.02	±3.2	±2.2	±.2	±25

The PPF of both groups of SHR was significantly lower than that of WKY. MAP was increased and CO was decreased when AC was applied to the SHR. Despite a significant decrease in UNaV and an increase in Uosm of SHR-AC when RPP was reduced to 126 mmHg, the PPF, RBF, and GFR of SHR-AC were not different from those of SHR-Sham, respectively. We conclude that enhanced tubular sodium reabsorption is primarily responsible for the decreased UNaV in SHR following acute reduction of RPP.

DIRECT DETERMINATION OF RENAL PAPILLARY BLOOD FLOW. R.L. Jamison, C. Holliger,* K.V. Lemley,* S.L. Schmitt,* F.C. Thomas,* and C.R. Robertson.* Depts. of Med. and Chem. Eng., Stanford Univ., Stanford, California.

To determine inflow and outflow of blood to the renal papilla, 18 rats were anesthetized and the left renal papilla exposed by ureteral excision. Fluorescence videomicroscopy and fluorescein-labeled gammaglobulin as a plasma marker allowed simultaneous determination of erythrocyte velocity (V_{rbc}) and capillary diameter (D) in descending (DVR) and ascending (AVR) vasa recta (N=97). The Fahraeus factor (F) relating V_{rbc} to blood velocity was determined in vitro; F = 1.4±0.06 (SE) (N=25); this enabled calculation of blood flow (Q_{vr}) in each vas rectum. Results:

	V_{rbc} (mm s^{-1})		D (µm)		Q_{vr} (nl min^{-1})	
	DVR	AVR	DVR	AVR	DVR	AVR
$\bar{x}$	1.04	0.38*	15.6	20.0*	9.2	4.8*
SE	0.10	0.03	0.3	0.4	1.0	0.3

(*different from corresponding DVR value, $P < 0.001$)

The total number of AVR and DVR at the base of the exposed papilla was determined in separate experiments. In a montage of electron micrographs of three papillas, VR were identified and counted. Mean AVR/DVR ratio was 3.96; total DVR=634; total AVR=2512. Total blood outflow (Q_{vr} X total AVR) in all AVR was 12.1 µl min^{-1}, twice as great as total blood inflow in all DVR, 5.8 µl min^{-1}. The difference, 6.3 µl min^{-1}, represents an estimate of water uptake by the microcirculation of the exposed renal papilla.

DIRECT EFFECTS OF CALCIUM INFLUX BLOCKERS ON RENAL FUNCTION, HEMODYNAMICS, AND RESPONSE TO ANGIOTENSIN II. Armando Lindner and Anthony Bell*. VA Medical Center and University of Washington, Seattle, Washington.

The purposes of this study were the evaluation of the direct effects of Verapamil (VP) or Nifedipine (NF) during unilateral renal artery infusion and the role of calcium in the hemodynamic response to angiotensin II. Dogs were anesthetized with pentobarbital (30 mg/kg/IV). Measurements included systemic blood pressure (BP); separate renal function, i.e.: GFR (C_{IN}), urine flow rate (V), electrolyte, and solute excretion rates; and RBF (electromagnetic flowmeters). Control measurements (C1, C2) were obtained for 2 hours prior to and following intrarenal artery infusion of either VP (5 µg/kg/min) or NF (0.32 µg/kg/min) for 90-120 min. Systemic and renal vascular effects of Angiotensin II (AII, IV bolus) were measured in each period. Group means (± SEM, n=6) for the VP-infused (I) and noninfused (N) kidneys were as follows:

	RBF (ml/min)		V (ml/min)	
	N	I	N	I
C1	134±12	136±30	.11±.02	.11±.01
VP	132±16	151±33 (p<.01)	.12±.03	1.5±.33 (p<.01)
C2	135±18	139±33	.13±.03	.37±.1
	C_{IN} (ml/min)		EFF Na^+ (%)	
C1	22±2	20±2	.23±.05	.29±.1
VP	22±2	35±3 (p<.05)	.21±.1	2.9 ±1 (p<.05)
C2	23±2	23±2	.34±.1	1.1 ±.3 (p<.05)

VP or NF infusion equally induced a diuresis and a rise in RBF. Unlike other vasodilators, these drugs markedly increase GFR and fractional excretion of Na^+. Further, they abolish the renal vascular response to angiotensin II. These findings suggest that VP or NF inhibit proximal tubular Na^+ transport and possibly mesangial cell contraction resulting in a stable K_F.

ADRIAMYCIN (A) NEPHROTIC SYNDROME: GLOMERULAR HEMODYNAMICS AND PERMSELECTIVITY. LD Michels, M Davidman and WF Keane, Dept. Med., Univ. of Minn., Hennepin County Medical Center. Mpls., MN.

The determinants of glomerular ultrafiltration and filtration barrier permselectivity were evaluated in Sprague-Dawley rats 4 weeks after i.v. A (7.5 mg/kg b.w.) and compared to age-matched control (C) rats. Proteinuria in A rats (223±43 mg/24 h) was significantly greater than in C rats (20±4 mg/24 h). Glomerular histology revealed fusion of epithelial cell podocytes and marked reductions in glomerular polyanion (colloidal iron stain). Results (mean±SE; * $p<0.05$):

Group	SNGFR	P_{UF}	K_f
	nl/min/gKw	mmHg	nl/min/mmHg/gKw
C (n=9)	35±3	17±2	2.5±0.4
A (n=9)	28±2*	23±3*	1.4±0.2*

The reduction in superficial nephron filtration rate (SNGFR) was principally a result of the 44% decrease in the ultrafiltration coefficient (K_f). This was partially offset by a 6 mmHg increase in mean ultrafiltration pressure (P_{uf}). Neutral dextran clearances revealed an 8% increase in filtration barrier mean pore size in A rats, but a 50% reduction in the pore surface/length ratio (S'/l). Hence, A induces a marked alteration in K_f, associated with a reduction in S'/l (fewer pores?). Anionic dextran sulfate (DS) clearances were not significantly different from C. Thus, proteinuria may be related to an increase in pore size. Since reduced gomerular polyanion in A rats did not significantly alter anionic DS clearance, changes in the charge-related barrier of the glomerular filtration surface may not correlate with loss of the glomerular polyanion.

CALCIUM ENTRY BLOCKER NISOLDIPINE PREFERENTIALLY AUGMENTS GLOMERULAR FILTRATION RATE (GFR) IN THE VASOCONSTRICTED ISOLATED PERFUSED RAT KIDNEY. Rodger Loutzenhiser,* Charles Horton,* and Murray Epstein. Depts. Med. and Pharmacol., Univ. of Miami School of Med. & VA Med. Center, Miami, FL.

We have previously demonstrated that the organic calcium entry blocker diltiazem preferentially increases GFR of the norepinephrine (NE)-vasoconstricted rat kidney. Nisoldipine (NS) differs markedly in molecular structure and physical properties from diltiazem, but shares diltiazem's ability to block NE-induced calcium entry into vascular smooth muscle. In the present study, we evaluated the effects of nisoldipine upon the NE-induced alterations in renal hemodynamics of the isolated perfused rat kidney. Under conditions of constant (100 mm Hg) renal arterial pressure, the infusion of NE ($3X10^{-7}$ M) caused a sustained reduction in renal perfusate flow (RPF) from a control of 37±2 (SE) ml/min/g to 19±4 ml/min/g and a sustained reduction in GFR from a control of 0.8±0.1 ml/min/g to 0.2±0.1 ml/min/g (n=7). In a second group of kidneys (n=7), NE infusion caused similar initial decreases in RPF and GFR:

	Pre-NE	NE	NE+NS
RPF (ml/min/g)	36±2	20±1*	25±2*
GFR (ml/min/g)	0.9±0.1	0.2±0.1*	0.7±0.1

Means ± SE *p<0.05 compared to Pre-NE

The administration of (10^{-7} M) nisoldipine completely reversed the NE-induced reduction in GFR, while increasing RPF moderately. These results suggest that the NE-induced ↓ in GFR is mediated by a stimulation of Ca entry and raise the possibility that NS might augment GFR in various disorders characterized by renal vasoconstriction.

A MECHANISM TO AUTOMATICALLY PROVIDE CONCOMITANT CHANGES IN AFFERENT AND EFFERENT ARTERIOLAR RESISTANCE. Donald E. Oken, Dept. of Medicine, Medical College of Virginia, Richmond, Virginia.

Pre- (R_A) and postglomerular (R_E) resistance are found experimentally to change concordantly and almost equally after a variety of maneuvers. The means of communication between the two resistances is unexplained. Solitary change in R_E causes significant change in glomerular capillary pressure (P_g), however, and, since P_g cannot be higher than end afferent arteriolar pressure (P_{art}), change in P_g must be transmitted back to the afferent arteriole. Current theory holds that ΔP_{art} induced by MAP will cause R_A to change proportionally (autoregulation). If R_A responds to ΔP_{art} induced by MAP, it should be equally sensitive when ΔP_{art} is induced by adjusting R_E. Constriction or relaxation of R_E would thus cause a concordant change in R_A entirely reflexly, returning P_g and P_{art} to basal values. Using a network thermodynamic model, we have found that SNGFR in the rat is minimally increased by large solitary ↑ in R_E but falls precipitously with ↓ R_E. Such a prediction is not in keeping with experimental results. Setting our model to automatically adjust R_A to maintain P_{art} and P_g constant despite ΔR_E, SNGFR now changes symetrically about control with both ↑ and ↓ in R_E. Results consistent with the literature are obtained. We propose that the intrinsic tone of R_A (and thus the value of P_g) is set at a given basal value by intrinsic and neurohumoral mechanisms. With ΔR_E, an ↑ or ↓ in P_g changes P_{art} and causes autoregulatory change in R_A to restore P_g to its basal value, thus explaining the concordant changes in R_A and R_E found in micropuncture studies.

DIFFERENTIAL EFFECTS OF METHOXAMINE (MTX) AND ISOPROTERENOL (ISO) ON GLOMERULAR HEMODYNAMICS. J.C. Pelayo,* B.J. Tucker,* and R.C. Blantz. Univ. of Calif., San Diego, and VA Med. Ctr., San Diego, CA.

To evaluate the specific contributions of alpha-(α) and beta-(β) components of the adrenergic nervous system to the regulation of glomerular ultrafiltration (sngfr), we measured sngfr and its determinants in plasma volume expanded Munich-Wistar rats in 1) control (C,n=8), 2) during MTX, an α_1-adrenergic agonist, (15-30 µg/kg/min,n=12), and 3) ISO, a β_{1-2} agonist (0.05 µg/kg/min,n=7) infusion. In addition, 4) we also examined the effects of concomitant infusion of sar^1,ala^8-AII (S) (5 µg/kg/min,n=6) or MK421, an angiotensin converting enzyme inhibitor (MK) (17 µg/kg/min,n=6) during ISO infusion, and 5) C rats were also given MK. Results of mean arterial pressure (MAP), glomerular hydrostatic pressure gradient (ΔP), nephron plasma flow (rpf), and glomerular ultrafiltration coefficient (LpA) are provided (±SEM)-(§=p<0.05 vs. C; †=p<0.05, ISO vs. ISO+MK+S, ϕ=minimum estimate).

	MAP (mmHg)	ΔP	sngfr (nl/min)	rpf	LpA (nl/s/mmHg)
C	120±4	39±1	56±3	204±18	0.08±0.01
MTX	143±5§	38±1	53±2	206±19	≥0.12±0.01ϕ§
ISO	117±5	44±1§	51±4	233±25	0.05±0.006§
ISO+MK+S	111±3	38±1†	53±3	256±24	0.09±0.02†
C+MK	111±3	40±1	56±2	198±16	0.09±0.01

MTX increased LpA above C. ISO decreased LpA below C, but sngfr was maintained due to the increase in ΔP. Both S and MK eliminated the effects of ISO on LpA and ΔP. Conclusions: 1) MTX and ISO exert significant and opposing influences on LpA and 2) ISO induced changes in LpA may be mediated by AII, but MTX may exert an independent effect in increasing LpA.

EFFECT OF INDOMETHACIN ON RENAL FUNCTION IN NORMOTENSIVE MALES. Garry Reams*, John H. Bauer, Rona E. Pasternak*. Dept. Medicine, University of Missouri Health Sciences Ctr., Columbia, MO.

Prostaglandin inhibition (PGI) with indomethacin (INDO) has been reported to decrease or have no effect on renal blood flow, glomerular filtration rate, fractional excretion of sodium (FE_{Na}) and/or free water clearance (C_{H_2O}).

Nine normal subjects underwent studies of inulin (Cin), para-aminohippurate (Cpah), sodium (C_{Na}) and osmolar (Cosm) clearances (ml/min/1.73 m^2), C_{H_2O}, FE_{Na} (%), urine osmolality (Uosm; mOsm/kg) and urine flow rate ($\dot{V}$; ml/min) following 2 wks of either 2-4 gm or 8-10 gm sodium (Na) diet. Renal studies were repeated 2 wks after INDO (50 mg tid). Mean results are indicated below:

	low Na (N=6)		high Na (N=3)	
	pre-INDO	post-INDO	pre-INDO	post-INDO
Cin	98	100	108	99
Cpah	498	520	510	440
C_{Na}	0.68	1.09§	2.07	3.44
FE_{Na}	0.69	1.06§	1.92	3.52
Uosm	53	85¶	74	82
Cosm	2.26	2.92†	3.25	4.79
$\dot{V}$	13.53	11.15§	14.11	18.36
C_{H_2O}	9.61	6.85¶	9.14	11.69

p†p<0.06, §p<0.025, ¶p<0.015 compared to pre-INDO

There were no changes in mean arterial pressure or weight in either group, and there were no differences in Cin or Cpah. C_{Na} and FE_{Na} increased in all subjects following INDO. C_{H_2O} and $\dot{V}$ decreased in the low Na group, but increased in all subjects on high Na diet. We conclude that INDO does not alter renal hemodynamics in normal man consuming a physiological range of Na. PGI is natriuretic in both sodium states. It is diuretic in the Na replete, but antidiuretic in the Na-deplete state.

AUTOREGULATION OF RENAL HEMODYNAMICS BY CONTRALATERAL KIDNEYS OF GOLDBLATT HYPERTENSIVE RATS DURING INHIBITION OF CONVERTING ENZYME ACTIVITY. David W. Ploth, L. Morrill*, L. G. Navar. NRTC, U.A.B. and V.A. Med. Ctrs., Birmingham, AL.

These studies were designed to assess the influences of systemically delivered Angiotensin II on the impaired autoregulatory behavior of renal blood flow (RBF) and filtration rate (GFR) of the contralateral kidney of 2 kidney, 1-clip Goldblatt hypertensive rats (GHR). During pentobarbital anesthesia, microhemodynamic and clearance techniques were used to examine GHR 3-4 weeks after clipping (0.2 mm) one renal artery. Measurements for the contralateral kidney at spontaneous blood pressure (BP) and at reduced BP's achieved by aortic constriction during an initial control period were repeated in the same animals during a second period of infusion of the converting enzyme inhibitor Teprotide (CEI;3mg/kg·hr). In studies where the pressure-flow relationship could be studied, BP decreased from 186±4 ($\bar{X}$+SEM) to 174±4 mmHg during CEI; urine flow rate, GFR, and sodium excretion all increased (p's <.05). RBF for the contralateral kidneys at spontaneous BP increased from 5.6±.6 to 7.2±.7 ml/min. During control conditions, RBF for the contralateral kidney changed nearly linearly in response to altered BP. The ΔRBF/40 mmHg change of BP (AI) was .52±.07. The pressure-flow relationship for these kidneys was improved during conditions of CEI (AI=.25±.06; p <.025). These results support the concept that systemically delivered angiotensin contributes to the altered hemodynamic behavior observed for the contralateral kidney and may attenuate its ability to moderate hypertensive blood pressures.

RENAL VASCULAR RESPONSE TO ANGIOTENSIN II (AII) IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES MELLITUS (DM). H. John Reineck and Jeffrey I. Kreisberg. Univ. of Texas Health Science Center, San Antonio, TX.

Previous studies have demonstrated that glomerular mesangial cells grown in cell culture contract when AII is added to the media. In the absence of insulin, however, these cells fail to demonstrate this normal contractile response. Studies were therefore designed to determine if this mesangial cell resistance to AII influences the in-vivo renal vascular response to the hormone. DM was induced in Sprague Dawley rats by the intravenous injection of streptozotocin, 60 mg/kg (N=8). Control rats (C) received an injection of the streptozotocin diluent (N=6). Ten to 14 days later the changes in renal blood flow (RBF) and renal vascular resistance (RVR) were determined in response to intrarenal AII administered at doses of 0.5, 1.0 and 2.0 ng/min. At the lowest dose, RBF decreased by 16% in C and 11% in DM and RVR increased by 17 and 10% respectively (NS). At higher doses, however, the response to AII was significantly decreased in rats with DM. At 1.0 ng/min RBF fell by 37% in C and 19% in DM and RVR changed by 57% in C and 20% in DM. In response to the highest dose, RBF fell by 42 and 23% in C and DM, respectively, and corresponding values for RVR increased by 72 and 27%. To determine if endogenous AII activity might account for these findings, saralasin was administered systemically to both C and DM rats. No difference was noted in either the blood pressure or RBF response to this agent.

We conclude that animals with early DM are hyporesponsive to the renal vascular effects of AII and that endogenous levels of AII cannot account for this finding.

GLOMERULAR ULTRAFILTRATION COEFFICIENT IN HUMAN RENAL DISEASE. Virginia J. Savin,* Walter P. Richardson, and Robert V. Pinnick,* Univ. of Ks. Med. Ctr., Kansas City, Kansas

We have studied the ultrafiltration coefficient (K_f) of glomeruli isolated from 20 renal biopsies obtained during evaluation of hematuria, proteinuria, nephrotic syndrome and acute and chronic renal failure. Glomeruli were isolated from a fragment of the biopsy by microdissection. The remainder was processed for pathologic study. Filtration was induced by applying an oncotic gradient across the capillary wall. Glomeruli without capsular fragments or crescents were selected; globally sclerotic glomeruli were excluded. K_f was calculated from volume changes observed during filtration (Kidney Int. 20:188, 1981). We have previously shown that K_f of glomeruli of normal human kidneys increases with increasing glomerular size (Clin. Res. 29:745A, 1981) and estimated average capillary hydraulic conductivity (Lp), 3.8 $\pm$ 0.4 nl/min·mm Hg·cm^2 (mean $\pm$ SD). Lp fell within the normal range for 11 patients with acute GN, membranous nephropathy, transplant rejection and toxic nephropathy. Lp of 7 patients was more than 2 SD below normal. These patients all had renal insufficiency; diagnoses were ATN, focal sclerosing, IgA and hypertensive nephropathies, and chronic GN. Lp of 2 patients was more than 2 SD above normal. Diagnoses were focal lupus GN and diabetes mellitus. We conclude that in vitro filtration may be used to examine functional characteristics of human glomeruli from routine renal biopsies. Further studies will be required to determine the role of altered K_f in human renal disease.

THE INFLUENCE OF VENOUS PRESSURE (Pv) ELEVATION ON TRANSCAPILLARY FLUID (Jv) AND PROTEIN MOVEMENTS (Js) IN HUMAN FOREARMS. James J. Szwed, J.J. Friedman, and P.A. Kesler. Indiana University, Med. Center, Depts. of Medicine and Physiology, Indianapolis, Ind.

Pv elevation produces an accentuation of normal transcapillary fluid and protein net flux from the intravascular to the extravascular space. We previously studied a mass-balance approach for estimating transcapillary fluid and protein movement in feline gut. (Friedman, Szwed, and Johns, Am J Physiol 242, H227-232, 1982) We have now applied this approach to the human forearm. Normal adult males and females were employed. The Whitney strain-gauge was used to measure changes in tissue volume. Jv and Js were measured during 30, 40 and 50 mmHg Pv elevation. A catheter was placed in an antecubital vein to obtain blood samples for hematocrit and protein Cv. The calculations were: Jv (total transcapillary fluid movement), Jo (fluid movement due primarily to Starling's forces), Jp (fluid movement due to Js). Since Js/Cv = Jp, then Js = Cv x Jp. Mean Jv in males (N=20) was 0.10 $\pm$ .02, 0.15 $\pm$ .02, and 0.24 $\pm$ 0.5 (S.E.) ml/min · 100 ml at 30, 40, and 50 mmHg Pv elevation. Js in males was 6.6 $\pm$ 2.1, 5.9 $\pm$ 1.8, and 6.9 $\pm$ 3.3 (S.E.) mg/min · 100 ml at 30, 40 and 50 mmHg Pv elevation. While Jv in females (N=15) was similar to males, statistically significant differences in Js in females were found: 1.0 $\pm$ 1.4, 3.9 $\pm$ 1.5, 11.7 $\pm$ 1.6 (S.E.) mg/min · 100 ml at 30, 40, and 50 mmHg Pv elevation. The results suggest an enhanced Js in females but the mechanism of this apparent increased capillary membrane permeability is not apparent.

INVOLVEMENT OF α-1 AND α-2 ADRENOCEPTORS IN RENAL BLOOD FLOW ALTERATIONS IN DOGS. J.W. Strandhoy, B.P.C.S Rao,* and D.W. Wolff.* Wake Forest Univ., Bowman Gray Sch. Med., Winston-Salem, NC

Clonidine(C) and guanabenz(G) are both α2 agonists used as antihypertensives. Whereas C has been reported to cause antinatriuresis proportional to renal vasoconstriction(VC) with acute infusion,G increases Na and water excretion with little change in RBF. We hypothesized that C-mediated VC involves α1 receptors. RBF in dogs was measured electromagnetically. Boluses of phenylephrine (P, α1 agonist; .5-5nmol/kg), C(.4-11) and G(3-103) were injected into the renal artery via a perfusion cannula. Responses were expressed as ΔRBF. Then prazosin or yohimbine were administered i.a. and dose-response curves repeated. Changes in ED50s and slopes were calculated.

	P	C	G
ΔED50 after prazosin / ΔED50 after yohimbine	52.9	4.7	0.62

G was a 10x weaker vasoconstrictor than C but G was still effective when α1 receptors were blocked. Ten fold differences were found between P,C and G for dependence upon α1 receptors. Denervation did not significantly shift the curves. Thus, postsynaptic α2 receptors can contribute to renal VC but the receptors are either less numerous on the vasculature or less efficiently coupled to contractile elements. Verapamil (i.a.) shifted the curve for P but obliterated responses to C and G. This suggests a greater dependence for α2 agonists on Ca movement through voltage dependent channels.

A NEW IN VITRO METHOD FOR THE MEASUREMENT OF GLOMERULAR CONTRACTILITY: C Westenfelder, and R L Baranowski*, University of Illinois and West Side VA Hospitals, Chicago, IL.

Isolated glomeruli (G) contract upon exposure to vasoactive compounds. It is postulated that this contraction causes a decrease in capillary surface area available for filtration, which translates into a fall in ultrafiltration coefficient (Kf or LpA). Changes in glomerular diameter measured in vitro might therefore be a simple anatomical correlate of the physiologic alterations in ultrafiltration dynamics assessed by glomerular puncture in vivo. In order to test this hypothesis, we isolated G from Sprague-Dawley rats utilizing a differential sieving technique. A nephelometer was used to quantitate changes in the intensity of light scattered by a stirred suspension of approximately 1,000 to 2,000 G's. Because the intensity of scattered light is directly proportional to the number of particles in the light path (number of G remains constant) and their surface area, changes in glomerular diameter can be continuously monitored. All nephelometric determinations on G correlated very closely with simultaneous morphometric measurements. Applying this method, we observed that isolated G contract in response to angiotensin II, norepinephrine, vasopressin and cAMP. This confirms morphometric results from other investigators and supports the notion that the contractile response of isolated G parallels in vivo changes in ultrafiltration dynamics. In conclusion, this method has proven to be much less cumbersome than morphometric techniques, it is highly sensitive and utilizes a large sample of G, which can be monitored continuously.

POSTGLOMERULAR CAPILLARY PERMEABILITY DETERMINATION IN DOGS. C. Whiteside* and M. Silverman, Univ. of Toronto, Toronto, Ontario.

In previous in vivo investigations of postglomerular (PT) capillary permselectivity it has been difficult to separate diffusive from convective solute flux. This study focusses exclusively on the diffusive PG flux of a series of neutral solutes including ^{14}C-inulin (I) and ^{3}H-dextran (D) (6-12K daltons) using the pulse injection multiple indicator dilution technique (MIDT) in anaesthetized mongrel dogs. A bolus of indicators including ^{125}I-albumin (plasma reference), creatinine (interstitial ref.) ^{14}C-I and a homogeneous ^{3}H-D fraction was injected into the left renal artery (LRA) during mannitol diuresis. L renal vein (RV) and urine outflow were rapidly sampled. Total renal blood flow (RBF) was determined with an electromagnetic probe. Glomerular extractions E_G (urine recoveries) were identical for ^{14}C-I and ^{3}H-D's indicating no solute flux limitation. The PG extraction (E_{PG}) was calculated from the RV upslope ratio: E_{PG} = 1-(RV indicator/^{125}I-albumin). Initial PG plasma flow was calculated: F=RBF(1-Htc)(1-E_{G_I}). Reduction (2-3x) in F significantly increased E_{PG} of ^{14}C-I and ^{3}H-D confirming PG capillary diffusion limitation. E_{PG} remained constant during LRA ouabain infusion indicating negligible convective backflux contamination of the RV upslope ratio. Permeability·surface area products were calculated PS= -Fln(1-E_{PG}) and ranged from 4.86 ± .89 to .97 ± .25 SD for ^{14}C-I to ^{3}H-D (12K) ml·sec^{-1}·100gm^{-1}. Using these values of PS an effective pore radius r = 47.5 ± .5 Å was calculated implying diffusion limitation via a small pore system.

REGULATION OF VASOPRESSIN (AVP) SECRETION IN THE PREGNANT RAT: ROLE OF ISOSMOTIC VOLUME DEPLETION. W.Barron*, B.Stamoutsos*,M.D.Lindheimer, Depts.of Ob/Gyn/Med/Path.,Univ.of Chicago,Chicago,Illinois.

Plasma osmolality (P_{osm}) decreases ≈10mOsm/kg during rat pregnancy (P). We have demonstrated that the osmotic thresholds for both AVP release and thirst decrease during P in the rat. (J Clin Invest 68:337,1981). Although blood volume (BV) increases in P,some suggest "effective" BV is decreased and the latter state may be responsible for changes in osmoregulation. Thus, we studied effects of isosmotic BV depletion on AVP release in 20 day gravid Sprague-Dawley animals and age-matched virgin (V) controls. BV was measured (Evans-Blue and ^{51}Cr-RBC) and in parallel studies 1-20% isotonic BV depletion was produced by i.p. injection of polyethylene glycol in saline of varying tonicity. Plasma AVP (P_{AVP}) response to hypertonicity was also studied in volume depleted animals. Basal P_{osm} was reduced ($p<.001$) in P (287±3mOsm/kg) compared to that in V (297±3mOsm/kg) while BV was greater ($p<.001$) in P (25±2ml) than in V (17±2ml). Nevertheless,P_{AVP} of V and P groups rose in a similar exponential manner in response to isotonic dehydration, P_{AVP} becoming signigicantly elevated only when BV depletion was > 6% [V: $P_{AVP}=1.80e^{0.14\Delta BV}$,r=0.9;P:$P_{AVP}=1.87e^{0.12\Delta BV}$ r=0.9]. 6-12% BV depletion accelerated the rise in P_{AVP} per osmole increment in plasma tonicity by ≈2-3X in both P and V. Conclusions: The AVP secretory response to percent isotonic BV depletion is similar in P and V rats. In fact,gravid animals must lose more absolute volume to evoke a similar rise in P_{AVP}. The hypothesis that decreased "effective" volume in pregnancy is responsible for the change in osmoregulation seems untenable.

Renal Physiology -- Water & Solutes

FUNCTIONAL CONSEQUENCES OF ADH-INDUCED THICK ASCENDING LIMB (TAL) HYPERTROPHY IN BRATTLEBORO RATS WITH DIABETES INSIPIDUS (DI). Lise Bankir*, M.M. Trinh*, N. Bouby*, M.Douté*,(intr. by B.M. Brenner). INSERM U90, Hôp. Necker, Paris, France.

We have previously shown that 1) rats with DI lack the normal nephron heterogeneity(Heter) with regard to filtration rate (SNGFR) and size; 2) medullary TAL (MAL) in DI rats are underdevelopped; 3) six wks ADH treatment (T) restores the nephron Heter and induces a marked hypertrophy of MAL epithelium (AJP 1981 240:F372 and Ann NY Acad Sci in press). We now examined the effect of abrupt ADH arrest (AT) after 6 wks T (dDAVP 200 ng/d) on urine osmolality (mOsm/kg H_2O, see Table) and SNGFR (ferrocyanide technique).

n Days	-2	AT ↓	+1	+2	+3	+7	+14	+42
5 DI	142		134	134	130	138	139	130
5 ATDI	2900*		176**	93**	72**	101**	106*	110

signif. diff. from DI (*) or from previous day (°)

Rats after AT became more diuretic than control DI. At day +3 SNGFR (n = 4) did not change in superficial (S) but decreased by 15% indeep (JM) nephrons. However SNGFR Heter regressed but did not disappear with loss of concentrating ability: S/JM SNGFR ratio was .88±.07 versus .71 ±.02 in 7 TDI and 1.12 ±.04 in 4 DI. Thus SNGFR Heter in TDI and in normal rats neither depends on a direct vascular effect of ADH nor on medullary hypertonicity. High SNGFR in JM could depend on a low macula densa signal, present only when MAL is well developped since JM have no cortical TAL. This and the exaggerated urine dilution after AT are possibly due to stimulation of hypertrophied MAL by the other hormones acting on this segment.

A CORTICAL AND PAPILLARY MICROPUNCTURE EVALUATION OF THE RENAL CONCENTRATING DEFECT OF POTASSIUM DEPLETION (KD). M. Bevan*, R. Hogg and J. Kokko. Univ. of Texas Hlth Sci Cntr, Dallas, Texas.

The purpose of these studies was to examine renal mechanisms which might contribute to the concentrating defect associated with KD. Rats were pair fed a K deficient (KD) or K supplemented (KS) diet. The KD diet was developed to produce a rat model of KD that was able to withstand micropuncture procedures. KD rats (n=19, initial weight 88.3 gm) gained less weight, had lower serum K (3.00±0.08 vs 4.17±0.017 mEq/L) and had deficient urinary concentrating capacities (U_{max} 1381±58 vs 1921±54 mOsm/L) when compared to KS rats (n=15, initial weight 86.1 gm). Both KD and KS rats withstood surgery well and showed no differences in GFR, proximal or distal transit times. Measurement of fluid osmolality of adjacent level papillary collecting ducts (PCD), loops of Henle (LOH), and vasa recta did not demonstrate osmotic disequilibrium between these structures either in KD or KS rats. However, linear regression analysis indicated a progressive rise of PCD fluid osmolality from base to tip in KS rats, but not in KD rats. Obliteration of the osmolality rise suggests decreased urea recycling into the papilla. To evaluate NaCl transport in the LOH, fractional and absolute Cl delivery to superficial early distal tubules was measured in KD and KS rats; no differences were found (12.2±0.62 vs 12.1±0.55%; 0.42±0.03 vs 0.39±0.8 mEq/min). Our studies suggest that the renal concentrating defect of KD is not the result of decreased Cl transport by the LOH or the failure of osmotic equilibration across the PCD but may result from failure of papillary accumulation of urea.

ACTIVE TRANSPORT OF Mg AND SO_4 BY ISOLATED PROXIMAL TUBULES OF THE WINTER FLOUNDER. K.W. Beyenbach. Cornell Univ., Ithaca, N.Y.

The winter flounder has a glomerular kidney and significant GFR's. However, isolated proximal tubules secrete fluid spontaneously without perfusion of the lumen (Beyenbach, Nature 1982). Since water flow across epithelia is linked to solute flow, the transepithelial electrochemical potential differences ($\Delta\mu$) were determined for the dominant solutes secreted into the lumen: Na, Cl, Mg, and SO_4. One end of the tubule was crimped closed and the other end opened into a collection pipet where secreted fluid was collected for electron probe analysis (WDS, JEOL 733). When bathed in flounder Ringer, secreted fluid/bath ratios were for Na 0.99±0.05 (22) SE, Cl 1.10±0.04 (22), Mg 25.7±10.9 (22)* and SO_4 14.5±5.2 (16)*. These ratios are remarkably similar to U/P ratios measured in the aglomerular teleost Lophius (Forster, 1975) and support the idea that proximal tubules of glomerular marine teleosts have the capacity to form urine without glomerular filtration. Transepithelial voltage (V_T) was measured with respect to ground in the bath in tubules perfused and bathed with Ringer according to the method of Burg et al. (1966); transepithelial resistance (R_T) was measured using cable analysis. V_T was -2.2±0.2 mV (93) and R_T was 3,373±268 Ωcm tubule length (20). On the assumption that values of V_T in perfused tubules are similar to those in spontaneously secreting tubules, $\Delta\mu$ was less than 5 mV favoring tubular secretion of Na and opposing secretion of Cl. In contrast, $\Delta\mu$ was 40 mV for Mg and 37 mV for SO_4 opposing secretion. These results indicate active transport of Mg and SO_4 from bath to lumen in isolated flounder proximal tubules and offer a useful model for the renal tubular transport of these divalent ions. * $p<0.001$.

THE RELATIONSHIP OF NATRIURETIC FACTOR (NF) TO THE NATRIURESIS OF CENTRAL VOLUME EXPANSION (CVE) DURING DAY (D) VS. NIGHT (N). N.S. Bricker and G.G. Krishna. UCLA Program in Kidney Diseases and Div. of Nephrology, Los Angeles, CA.

We have previously shown that the natriuretic response to CVE is markedly attenuated at N in comparison to D, despite similar changes in plasma levels of aldosterone, norepinephrine and dopamine. To study the relationship between NF and these responses 8 salt replete healthy adults underwent thermoneutral head-out water immersion (WI) during D (0900-1300) and during N (0000-0400). The data are tabulated below (Mean±SE).

	$\Delta U_{Na}V$ (μEq/min)	ΔFE_{Na}	C_{Cr} (ml/min)
DWI	99±9	0.69±0.05	126±1
NWI	40±19	0.23±0.09	126±2
p	<0.05	<0.01	NS

The gel filtration fraction of the urine from these studies was investigated for natriuretic activity using the standard bioassay in rats with reduced nephron mass. The rat bioassay data are tabulated below (Mean±SE).

	$\Delta U_{Na}V$ (μEq/min)	p	ΔFE_{Na}	p
DWI	1.99±0.43	NS	2.54±0.58	NS
NWI	3.00±0.97		3.77±1.26	

These data show that despite elevated NF in the urine, during D as well as N, natriuretic response to WI was markedly attenuated at N. The data suggest either a relative nephron insensitivity at night to potential hormonal mediators of natriuresis or that the changes in hormonal levels might simply indicate recognition of CVE and might not serve to mediate natriuresis.

ACTIONS OF CHLORPROPAMIDE (CPMD) ON CYCLIC AMP (cAMP) METABOLISM IN MEDULLARY TUBULES. J. L. Braun-Werness,* E. Kusano,* and T. P. Dousa (Intr. by C. G. Strong). Mayo Clinc, Rochester, MN.

The mechanism by which CPMD potentiates the antidiuretic effect of vasopressin (VP) is unknown. We examined the effect of CPMD on cAMP metabolism in medullary collecting tubules (MCT) and in medullary thick ascending limb of Henle's loop (MAL). Brattleboro rats with diabetes insipidus were treated with CPMD (20 mg/100 kg b.wt./day for 7 d) then the tubules were microdissected and analyzed. MCT from CPMD-treated rats did not differ from controls in activities of adenylate cyclase (AC), basal or stimulated by VP or by NaF, cAMP-phosphodiesterase (cAMP-PDIE) or in accumulation of cAMP. Also, MAL from CPMD treated rats did not differ in activities of cAMP-PDIE, basal and NaF-stimulated AC. In the presence of VP accumulation of cAMP (fmol/mm; mean ± SE) from CPMD-treated MAL (15.9 ± 1.9) was significantly ($P < 0.02$) higher than in controls (8.3 ± 1.6). AC activity assayed in the presence of VP, in 800 mOsm medium, was also higher (Δ+81%) in MAL from CPMD-treated rats. The direct addition of CPMD to tubules incubated in vitro increased (≅+60%) cAMP accumulation in MAL, but not in MCT. The results indicate that CPMD does not enhance the antidiuretic effect of VP directly, by potentiating its cAMP-mediated response in the collecting tubules. Increased cAMP response to VP in MAL may relate to enhanced NaCl reabsorption in MAL and to enhanced papillary hypertonicity found in response to CPMD administration.

RENAL CLEARANCE OF ALUMINUM (Al) IN NORMAL RATS M Burnatowska-Hledin*, T Doyle*, GH Mayor, Dept. of Med., Michigan State Univ., E. Lansing, MI.

Increased tissue Al has been associated with dementia, osteomalacia and anemia in individuals with chronic renal failure and on dialysis. However, it is not clear if renal failure is a prerequisite for increased tissue Al burdens. Consequently, this study examines renal Al handling employing standard clearance techniques. Flameless atomic absorption spectrophotometry was used for Al determination. The concentration of ultrafilterable plasma Al (UF_{Al}) estimated with Amicon filter cones (CF-50) was 9.6.±5.6% (n=5) in plasma drawn from the rats at the end of the experiment and 6.2.±3.5% after in vitro standard addition of Al (n=7). Neither infusion of acidified saline nor Al (26 μg/ml) at 3.4 ml/hr had any significant effect on GFR, urine flow rate (V), total plasma calcium or calcium clearance. Three hr Al infusion led to a significant increase in plasma Al (P_{Al}) (mean±SD) from 20.6±20.9 to 392.3 ±175.4 ng/ml (n=5, $p<0.01$), and urinary Al excretion ($U_{Al}V$) rose from 1.7±1.2 before to 92.1±41.2 after 2 hrs and 104.8±58.7 ng/min after 3 hrs of Al infusion (n=5, $p<0.05$). In controls $U_{Al}V$ was 1.4±.4, 5.2±6.2 and 4.9±5.9 ng/min. Fractional Al excretion was 68% before, 90.8% and 113.1% after 2 and 3 hours of Al infusion and 94% and 89% after saline infusion. The acutely administered Al appears to accumulate in the liver (1.95 vs. 4.72 μg/g in control vs. Al loaded animal). This study indicates that the kidney is not a major route of Al excretion as only a small fraction of Al is filtered at the glomerulus. Very little Al is reabsorbed, and reabsorbed Al is independant of P_{Al}. A significant fraction of administered Al is retained in the body.

Ca DEPENDANCE OF HEMODYNAMIC AND NATRIURETIC EFFECTS OF ATRIAL EXTRACT (AE) IN THE ISOLATED PERFUSED RAT KIDNEY (IK) M.J.F. Camargo*, H.D. Kleinert*, J.E. Sealey, J.H. Laragh and T. Maack, Dept. of Physiol. and Cardiovasc. Ctr. Cornell Univ. Med. Coll., New York, N.Y.

We recently reported(Kleinert et al.APS Fall Meeting,1982)that AE induces significant increases in GFR,filtration fraction(FF),renal resistance (RR)and absolute(UV)and fractional(FE)excretion of Na,K and H_2O in the IK. To test the Ca dependance of these effects we compared responses to AE in IK perfused with normal (NP)or very low(LP)perfusate [Ca] (NP_{Ca}=2.0mM,LP_{Ca}=0.2mM). After 2 control periods,100 µl of 1/10(w/v)homogenate of rat atria were added to 60ml of perfusate. Three experimental periods followed. Results below(mean±SE) are given as % change from control periods(C=100%)

	GFR	RR	FF	$U_{Na}V$	FE_{Na}
NP_{Ca} (n=9)	201±24%*	133±8%*	245±40%*	1259±289%*	641±129%*
LP_{Ca} (n=7)	101±20%§	103±2%§	110±22%§	199±19%*§	199±25%*§

*p<0.001,vs C(paired t);§p<0.001vsNP_{Ca}(unpaired t)

As shown,low Ca abolished the effect of AE on GFR RR and FF and markedly blunted the natriuretic response to AE. Low Ca per se decreased RR but had minimal or no effect on the other parameters. Low Ca also significantly blunted(p 0.001)the AE induced increased in FE_{H_2O}(NP_{Ca}=675±121%,LP_{Ca}= 182±5%),and U_KV(NP_{Ca}=1009±320%,LP_{Ca}=157±51%). AE by preferential efferent vasoconstriction and perhaps redistribution of flow increases GFR and FF and by direct tubular effect(s) inhibits Na reabsorption in the IK. Results show that the renal hemodynamic and natriuretic effects of AE are dependant on the availability of extracellular Ca.

LUMINAL AND PERITUBULAR SOLUTES COMPOSITION AND INTRACELLULAR POTENTIAL OF PROXIMAL CONVOLUTED TUBULE. Jean Cardinal, Raynald Laprade*, and Jean-Yves Lapointe*. Centre de Recherches, Hôpital Maisonneuve-Rosemont and Université de Montréal, Montréal, Québec.

Basolateral membrane potential (Ψ_{BL}) was measured in rabbit proximal convoluted tubule (PCT) perfused in vitro under control conditions and luminal and peritubular substitutions of Na, K, and Cl. Perfusate and bath solutions were continuously exchanged at a fast rate and tubule perfusion rate was higher than 100 nL min^{-1}. In control solution without proteins, Ψ_{BL} was 54 ± 3.1 mV, n = 37. Luminal substitution of K by Na had no effect. In contrast, a 75% luminal substitution of NaCl by mannitol, of Na by choline, and a 95% substitution of Cl by SO_4 produced a rapid hyperpolarization (13.5, 10.0, and 6.6 mV, respectively) followed by a return close to the control values in 5-8 min. Returning to control solution produced, in these three situations, a rapid depolarization (16.2, 11.4, and 5.8 mV) followed by a return to the control value. Curves very similar to those obtained with these three luminal substitutions were observed when stopping and starting perfusion of PCT. A 95% peritubular substitution of Cl by SO_4 had no effect while a complete substitution of K by Na, a 75% substitution of NaCl by mannitol, and Na by choline produced a sustained but reversible depolarization of 37.5, 19.6, and 10.2 mV, respectively. These results, with those of simultaneous transepithelial potential measurements, would suggest the presence of a rheogenic component of Ψ_{BL} which depends strongly on peritubular Na and K and possibly on luminal Na and Cl.

DECREASED LUMINAL MEMBRANE PERMEABILITY IN PROXIMAL TUBULES OF HYPOTHYROID RATS. EVIDENCE FROM MICROPUNCTURE STUDIES WITH AMPHOTERICIN B. G. Capasso*, R. Kinne, N.G. De Santo* and C. Giordano. Albert Einstein College of Medicine,Bronx,N.Y., and 1° Faculty of Medicine,University of Naples,Italy.

In the proximal tubules of hypothyroid rats the isotonic fluid reabsorption (J_v) is deeply impaired as indicated by a large increase of the half time of reabsorption (tl/2).This phenomenon has been explained by a reduction of the number of the sodium pumps.However,our recent experiments (Pfluegers Arch,in press) have demonstrated no correlation between the rise of J_v after short term administration of 10 µg/kg b.w. of tri-iodothyronine (T_3) and Na-K-ATPase acitivity.We therefore hypothesized that T_3 increases the permeability of the luminal membrane.To test this hypothesis Amphotericin B,known to increase membrane permeability,was applied to the lumen of late proximal tubules of hypothyroid rats,age matched normal rats and rats treated with T_3. tl/2 was measured using the shrinking droplet technique.Each tubule investigated served as it's own control.It was found that Amphotericin B did not affect tl/2 in age matched normal rats.However,when it was applied in hypothyroid rats a significant (p<0.005) tl/2 reduction of 13% was detected.Furthermore,in hypothyroid rats treated with physiological doses of T_3 (10 µg/kg b.w. for 3 days) Amphotericin B failed to decrease tl/2. These data support the hypothesis that luminal membrane permeability in the proximal tubule of hypothyroid rats is decreased.Moreover,they suggest that the early effect of physiological doses of T_3 is an increase in the permeability of luminal membrane rather than an increase in the number of sodium pumps.

α-ADRENERGIC AGONIST INHIBITS VASOPRESSIN (AVP) INDUCED cAMP ACCUMULATION IN THE COLLECTING TUBULE. D. Chabardes*, M. Montegut*, M. Imbert-Teboul*, and F. Morel* (intr. by M. Burg). L. Physiol. Cell., College de France, 75231 Paris, France.

cAMP was measured by radioimmunoassay in single isolated tubule segments, microdissected from the cortical (CCT) or medullary (MCT) collecting tubule of rat kidney. Experiments were performed in 4 µl, duration 4 min., in the presence of a phosphodiesterase inhibitor (IBMX, 10^{-3} M). Basal cAMP content was below the sensitivity of our assay (< 2.5 fmol). Addition of 10^{-6} M AVP produced no detectable cAMP in incubation medium but a maximal intratubular cAMP accumulation: MCT = 129.7 ± 6.3 (SEM) fmol/mm., N = 16 exp., CCT: 113 ± 5, N = 2, values very close to AVP stimulated Adenylate Cyclase activity. In the MCT, AVP 2 x 10^{-11}, 10^{-10} and 10^{-9} M induced 16% (N = 3), 63% (N = 8) and 95% (N = 2) respectively of the maximal cAMP increment. Addition of an α-adrenergic agonist (10^{-5} M Norepinephrine + 10^{-5} M propranonol: NE) decreased the accumulation of cAMP: MCT, AVP 10^{-10} M: 88.8 ± 14.1, AVP + NE: 10.1 ± 1.8, N = 5; CCT, AVP 10^{-10} M: 54.2 ± 3.5, AVP + NE: 8.8 ± 2.1, N = 3. Similar results were observed for all AVP concentrations used and with NE as low as 10^{-7} M. Inhibition by α-adrenergic agonist was reversed by 10^{-5} M phentolamine (PH) or yohimbine (YO): MCT, AVP 10^{-10} M: 94.1 ± 24.6, AVP + NE 10^{-5} M: 10.8 ± 3.2, AVP + NE + PH: 66.5 ± 17.6, AVP + NE + YO: 53.9 ± 7.8; N = 3; prazozin had no effect. No α-adrenergic agonist effect was observed on cAMP accumulation induced by glucagon in the CCT and MCT or by calcitonin in the CCT. These data suggest a specific effect of α-adrenergic agonist on cAMP accumulation stimulated by ADH in the collecting tubule.

SODIUM DOES NOT REDUCE THE SODIUM PERMEABILITY OF LUMINAL MEMBRANE VESICLES FROM TOAD BLADDER. H.Chase* and Q.Al-Awqati,Columbia U. New York,N.Y.

Sodium entry across the luminal membrane (LM) into the cell is rate-limiting for transepithelial Na transport and is thus the likely site of regulation by hormones and intracellular events. We have shown previously in whole bladders that maneuvers which increase cell Na reduce the Na permeability (P_{Na}) of the LM. Na could reduce P_{Na} either by a direct effect on the LM, or indirectly via a second messenger such as calcium. In this study we have directly measured P_{Na} in LM vesicles (LMV) and shown that Na has little effect on P_{Na}.

LMV were prepared by differential and sucrose density gradient centrifugation. ^{22}Na efflux from LMV, measured in a flow-quench apparatus, was fast ($T1/2 \simeq 150$ msec) and amiloride sensitive(K_I=39 nM). Initial Na spaces (Na_o) at 1 and 110 mM [Na] (at constant ionic strength) were 7.7±0.5 and 4.3±0.3 µl/mg respectively. The differing Na_o suggested that there was binding of ^{22}Na at low [Na].Because binding reduces the vesicular efflux rate coefficient (K), the measure of P_{Na}, we determined the extent of binding by measuring the osmotic behavior of Na_o and comparing it to the 14C-glucose space.We found that at 1mM [Na] 31% of Na_o was due to binding while there was no binding at 110mM.

K was calculated at 1 and 110 mM [Na] and was -2.8±0.8 and -3.9±0.4 sec^{-1} respectively. The K at 1 mM [Na], corrected for the effect of binding, was -4.5±0.4,not significantly different from that at 110 [Na].

These results suggest that sodium does not have an important effect on the P_{Na} of the LM. The effects on P_{Na} of changes in cell Na are probably due to the direct actions of cell calcium.

PROXIMAL TUBULAR COLLECTION PRESSURE AFFECTS NEPHRON FILTRATION RATE (NFR) IN UNINEPHRECTOMIZED SPRAGUE DAWLEY (SD) RATS. J.D. Conger, J.B. Robinette,* and S.A. Falk.* Univ. of Colorado Health Sciences Ctr., Denver, Colorado.

The effect of proximal tubular collection pressure on the measurement of NFR was examined in intact (Group 1) and 12 to 20 day uninephrectomized (Group 2) Sprague-Dawley rats by micropuncture studies of the left kidney. Collection and recollection were performed in last proximal tubular loops at the existing pressures and reduced pressures, respectively, as determined by a pipette placed two to three tubular diameters upstream from the collection pipette. There was no difference in NFR in intact rats (31.4±5.4 vs 34.2±6.1 nl/min, $p>.10$) between existing and lower pressure collections despite a significant mean difference in pressures of 8±2 mmHg. In uninephrectomized rats, differences in collection pressures between existing and lower pressure collections were similar to those in Group 1, but NFR varied inversely with pressure (42.1±6.5 nl/min at existing pressure vs 51.0±7.6 nl/min at lower pressure, ($p<.0005$). Tubular fluid collection rates also varied with collection pressure ($p<.0005$) while TF/P_{In} remained constant. It is concluded that reducing collection pressure below existing tubular pressure in late proximal loops modifies NFR in uninephrectomized Sprague-Dawley rats but does not affect measurement of NFR in animals with intact kidneys. It is presumed that collection pressure altered Bowman's space pressure in uninephrectomized rats, but not in rats with intact kidneys.

THE BLUNTING OF THE RENAL ADAPTATION TO ALTERED DIETARY SULFUR AMINO ACID INTAKE BY FASTING OCCURS AT THE BRUSH BORDER SURFACE. Russell W. Chesney, Aaron L. Friedman, Patti W. Albright,* and Naomi Gusowski.* University of Wisconsin, Department of Pediatrics, Madison, Wisconsin.

We have previously shown that there is renal adaptation to alterations in dietary sulfur amino acid intake. With a low-methionine, low-taurine diet, urinary and plasma taurine (T) concentrations fall, and the accumulation of T by collagenase-isolated tubules and brush border vesicles (BBV) is enhanced by 200%. On a T and methionine-supplemented diet (3%, w/w), urinary and plasma T values are increased, and accumulation by tubules and BBV is diminished. We evaluated the effect of fasting on adaptation to dietary intake change. Rats were given a high-taurine diet (HTD), low-taurine diet (LTD) or a normal diet (NTD) from age 56 to 70 days of life. Half of the rats in each group were fasted for 72 hr and lost 13±1% of body weight. Plasma T values fell in all three fasted groups, and urinary T excretion rose in LTD and fell in HTD-fasted animals. Renal cortex T fell in all fasted groups, indicative of T release. The in vivo tissue distribution ratio remained unchanged in fasted LTD and NTD animals, but rose into the normal range in fasted HTD animals. The initial rate of uptake (5 min) by isolated tubules was reduced in LTD-fasted and increased in HTD-fasted cortex. The Km of uptake was unaffected, but the Vmax was changed after fasting. Fasting also reverses the adaptive response in isolated BBV; hence, this membrane is involved in the blunting of adaptation. The signal for this adaptation and blunting remains uncertain, but may involve changes in tissue taurine content.

EVIDENCE FOR MOVEMENT OF WATER WITHOUT SOLUTES THROUGH KIDNEY PROXIMAL TUBULE. Bruno Corman* and Antonio di Stéphano*. dept. Biol. C.E.N. Saclay. 91191 Gif-sur-Yvette, France.

Convoluted proximal tubules from rabbit kidney were perfused in vitro with a control solution containing 50 mM/l of mannitol or raffinose in the bath. The resulting net water flux due to the present transepithelial osmotic gradient was measured when active solute transport was inhibited either by an external temperature of 26 °C of by serosal ouabain.

This measured water flux (Jv^{exp}) was compared to the theoretical net water flux (Jv^{th}) calculated with the assumption of movement of pure water through proximal tubule ;

$$\text{that is : } Jv^{th} = Vo\ (1 - osmO/osml)$$

with Vo the perfusion rate, osmO and osml the osmolalities of the delivered and collected fluid respectively. Results are (nl/min) :

Bath temperature Addition of	38 °C mannitol + ouabain (n = 9)	26 °C mannitol (n = 12)	26 °C raffinose (n = 6)
Jv^{exp}	1.65 ± 0.19	1.28 ± 0.05	1.23 ± 0.11
Jv^{th}	1.62 ± 0.16	1.28 ± 0.05	1.18 ± 0.12

It is concluded that an imposed osmotic gradient through kidney proximal tubule induces a movement of pure water without significant amount of solutes.

SITE OF PGE_2 INHIBITION OF ADH-MEDIATED NaCl TRANSPORT IN MOUSE MEDULLARY THICK ASCENDING LIMB(mTAL). R.M. Culpepper and T.E. Andreoli. Univ. of Texas Med. School, Dept. of Int. Med., Houston, Texas.

In perfused mouse mTAL, transepithelial voltage (V_e) is an accurate index of net NaCl transport (J^{net}_{NaCl}). ADH significantly enhances while PGE_2 inhibits both J^{net}_{NaCl} and V_e at a pre-cAMP step. We used pharmacologic probes to identify the cellular locus at which PGE_2 inhibits V_e. 10^{-6}M isoproterenol(ISO) increased V_e in mTAL from 4.7±0.8 to 10.8±1.0 mV. 10^{-5}M propranolol blocked ISO stimulation of V_e but did not affect ADH stimulation in paired observations on the same tubules. The Ka for ISO stimulation of V_e was 4.9×10^{-9}M but rose to 24×10^{-9}M in the presence of 10^{-6}M PGE_2. 10^{-6}M glucagon(GLU) also stimulated V_e in mTAL (2.8±0.6 to 9.8±1.1 mV) and was also inhibited by PGE_2. Forskolin(FSK), a diterpenenoid, activates adenyl cyclase(AC) in intact cells, acting directly on the catalytic subunit of AC. In mTAL, FSK stimulated V_e with a Ka of 1.4×10^{-7}M. Unlike with ISO and GLU, 10^{-6}M PGE_2 had no effect on the Ka for FSK stimulation of V_e. Furthermore, 10^{-5}M FSK reversed PGE_2 inhibition of V_e in ADH-stimulated tubules, causing V_e to rise from 5.8±0.6 to 10.4±0.6 mV. All these agonists failed to increase V_e in the mouse cortical TAL, in which V_e is unresponsive to cAMP. Since ADH, ISO and GLU each affect V_e via a receptor-mediated increase in cAMP, and are all inhibited by PGE_2, the PGE_2 interaction with ADH is unlikely receptor-specific. The failure of PGE_2 to inhibit FSK-stimulated V_e suggests no effect by PGE_2 on the catalytic subunit of AC. We conclude that PGE_2 inhibits a site, presumably the guanine nucleotide subunit of AC, that links the membrane receptors to the catalytic subunit of AC.

EFFECTS OF CALCITONIN (CT) ON THE RENAL CONCENTRATING MECHANISM C. de Rouffignac* and J.M. Elalouf* (intr. by D. Z. Levine). Département de Biologie, CEN Saclay, France.

The effects of CT were investigated in DI Brattleboro rats in the absence of the peptidic hormones (hormone deprived, HD) which might produce the same physiological effects as ADH on the thick ascending limb (glucagon, PTH and CT) and on the cortical collecting duct (CT) (Morel et al. -Kidney Int. in press). Recent data showed that ADH administered to HD rats strongly stimulates electrolytes (Na, Cl, Mg, Ca, K) reabsorption by the thick ascending limb. Clearance and papillary micropuncture experiments were performed on 10 HD and 15 HD infused salmon CT (SCT, Miacalcic, Ciba Geigy, 2.5 mU MRC/min/100g.) rats. Circulating level of hormones was reduced by acute TPTX and somatostatin administration. In HD rats the cortico-medullary concentration gradient was almost abolished (at the tip of juxtamedullary nephrons F/Pin = 2.28 ± .03; F/Posm = 1.19 ± .05). Administration of CT significantly restored the gradient to a level usually found in water diuretic rats (F/Pin = 3.17 ± 0.8 S.E.; F/Posm = 1.85 ± .14). In parallel both the absolute and fractional excretion (FE%) of water fell significantly in spite of a concomitant increase of the glomerular filtration rate (FE% : HD = 8.5 ± 0.6, SCT = 2.5 ± 0.3, $p < 0.001$) whereas the osmotic pressure rose (U/Posm : HD = 0.66 ± .06; SCT = 1.03 ± .10, $p < 0.02$). It is concluded that: 1) In the HD rats administration of CT consistently enhances the corticomedullary concentration gradient; 2) the effects of hormonal deprivation and CT administration on the urinary concentrating mechanism are compatible with a direct effect of CT on the electrolytes reabsorption by the thick ascending limb and/or on the water permeability of the cortical collecting ducts.

EFFECTS OF LOW CALCIUM AND LANTHANUM ON P-AMINOHIPPURATE (PAH) TRANSPORT BY ISOLATED, PERFUSED SNAKE RENAL TUBULES. William H. Dantzler and Olga H. Brokl.* Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona.

Net secretion of PAH (J_{PAH}) by isolated, perfused snake (Thamnophis spp.) renal tubules occurs by transport into cells at peritubular membrane against electrochemical gradient followed by mediated transport into lumen down electrochemical gradient. Since early data on flounder tubules suggested that transport across luminal membrane might be dependent on calcium, we explored the calcium requirement for PAH transport in these isolated, perfused snake tubules. Removal of calcium from bath led to disruption of cells without clear depression of J_{PAH}. However, removal of calcium from lumen with calcium (0.18, 0.54, or 1.8 mmol/l) present in bath reversibly depressed J_{PAH} by about 60% without altering net fluid absorption (J_V), intracellular PAH concentration ($[PAH]_{cell}$), or apparent permeability of luminal membrane to PAH (P_L). Therefore, removal of calcium from lumen depresses J_{PAH} without evidence of effect on luminal transport step. To explore calcium requirement more fully, we used lanthanum in presence of calcium (1.8 mmol/l) to block calcium binding and entry into cells. Lanthanum (2 mmol/l) in bath alone or in bath and perfusate reversibly depressed J_{PAH}, permeabilities of luminal (P_L) and peritubular (P_P) membranes, and, apparently, initial rate of PAH entry into cells, without altering $[PAH]_{cell}$ or J_V. Lanthanum (2 mmol/l) in perfusate alone reversibly depressed J_{PAH} and P_L without affecting $[PAH]_{cell}$ or J_V. In conclusion, calcium is required for PAH secretion, and lanthanum-inhibitable calcium entry into cells may be important for normal P_L, P_P, and initial PAH uptake at peritubular membrane.

RENAL NERVE ACTIVITY IN SALINE VOLUME EXPANSION: STUDIES IN CONSCIOUS RATS ON VARYING SODIUM DIET. G.F. DiBona and L.L. Sawin*. Dept. Int. Med., Univ. Ia. Col. Med. & V.A. Med. Ctr., Iowa City, IA.

Renal nerve activity (RNA) and function were measured before, during and after isotonic saline volume expansion (VE, 4 ml/kg/min x 30 min) in conscious rats eating high (HNa, N=8), normal (NNa, N=8) or low (LNa, N=7) sodium diets. Arterial pressure was constant throughout while heart rate uniformly decreased during VE. VE increased GFR similarly, 33-43%, in all groups. Peak increases in absolute and fractional excretion of Na and H_2O were greater in LNa than NNa = HNa. Control right atrial pressure (RAP): HNa>NNa>LNa; control RNA: LNa>NNa>HNa. The relationship of RNA on RAP during VE was inverse and similar in all groups. The relationship of $U_{Na}V$ on RNA during VE was inverse; a reduction in dietary sodium intake (HNa to LNa) shifted the relationship to the left so that a greater increase in $U_{Na}V$ per change in RNA occurred in LNa than in NNa than in HNa.

Conclusion: During HNa, RAP is increased and RNA is decreased so that further increases in RAP during acute VE result in reductions in RNA which do not contribute to a greater peak natriuresis than NNa. During chronic LNa, RAP is decreased and RNA is increased so that increases in RAP during acute VE result in reductions in RNA which contribute to a greater peak natriuresis than in NNa or HNa. These results indicate that the contribution of decreased RNA to the natriuresis of VE is greatest during LNa when other contributing variables (renal hemodynamics, GFR, physical factors, hormones) are less favorably set and least during HNa when those variables are more favorably set.

RENAL CHLORIDE TRANSPORT IN THE ADRENALECTOMIZED RAT (AX). S.L. Dumbauld, J.H. Galla, R.G. Luke. Nephrology Research and Training Center, University of Alabama in Birmingham.

We have shown that the conscious rat can conserve Cl normally in response to selective dietary deprivation of Cl in contrast to the response to Na restriction (JCI 54:1329, 1974). To examine the nephron sites at which this occurs, micropuncture studies were performed in AX (n=5) and sham-operated (S) (n=6) rats drinking NaCl for 10-14 days, then infused acutely with 0.15 M $NaHCO_3$ in a volume equal to 5% BW/h. BP (85±7 vs 106±7 mmHg), GFR (650±25 vs 938±111 µl/min) and SNGFR (21.7±3.4 vs 40.5±5.2 nl/min) were lower in AX rats; P_{Cl} (83±1 vs 84± meq/L) and FE_{Cl} (3.2±0.5 vs 2.2±0.6%) were not different. Nephron Cl reabsorption was 36 vs 23% (p=0.05) to the late proximal tubule and 94 vs 93% (p=NS) to the early distal tubule in AX and S respectively. Segmental loop reabsorption of delivered Cl load was not different (p=NS) (91 vs 91%), nor were early distal TF/P_{Cl} (0.19±0.02 vs 0.19±0.03) and urinary Cl (24±5 vs 27±4 meq/L) in AX and S respectively. To confirm the inability of AX rats to conserve Na, lysine HCl 0.075 M in a volume equal to 5% BW/h was infused. FE_{Na} was higher ($p<0.05$) in AX rats (1.7±0.5 vs 0.3±0.1%), but FE_{Cl} was not different (6.3±1.1 vs 4.6±0.7%; p=NS). Plasma aldosterone was lower in AX rats (2.5±1.0 vs 25.0±7.9 ng/dl; $p<0.05$).

We conclude that Cl can be conserved acutely in the absence of adrenal hormones. Enhanced Cl reabsorption in the proximal tubule may contribute importantly to such conservation.

OXYTOCIN (OT) SECRETION BY CONSCIOUS BRATTLEBORO HOMOZYGOUS (DI) RATS DURING DEHYDRATION. B.R. Edwards and F.T. LaRochelle*. Dept. of Physiology, Dartmouth Medical School, Hanover, N.H.

The rise in urine osmolality (Uosm)(to ∿700 mOsm/kg H_2O) that is seen after 24 hr dehydration of DI rats might be explained by the concurrent 73% fall in GFR (AJP 237:F100,1979). However, recent experiments have shown that this explanation cannot be invoked during the early hours of dehydration. Clearance measurements in 7 chronically-catheterized conscious DI rats were made in the control state (where fluid balance was maintained with 5% dextrose iv) and during the succeeding 3 hr after the D5W infusion was stopped. Over the 3 hr, the rats lost 7% body wt*, urine flow fell from 69 ± 5 to 28 ± 2 µl/min·100g*, Uosm rose from 95 ± 8 to 334 ± 16 mOsm/kg*, but GFR and PAH clearance demonstrated non-significant increases of 4% and 5%, respectively. Fractional excretion (FE) of urea remained unchanged at ∿66% while, unexpectedly, FE_{Na} increased from 0.2 ± 0.1 to 1.3 ± 0.1 %* (* $p<0.001$). Plasma OT (by RIA) increased from 5.0 ± 0.4 to 42.7 ± 6.6 pg/ml at 3 hr, but fell again (N=3) to 25.5 ± 7.3 pg/ml at 24hr. We have previously shown that OT infusion in non-dehydrated DI rats (resulting in P_{OT} of ∿46 pg/ml) induces a natriuresis and increases GFR and PAH clearance (Horm.Reg.Sodium Excr.,B.Lichardus et al(eds),1980 Elsevier/North Holland,p.121). Although still circumstantial, the evidence suggests that the release of OT during the early hours of dehydration may explain the paradoxical natriuresis and contribute to the maintenance of GFR and PAH clearance. Whether an antidiuretic effect of OT also contributes to the increased concentrating ability during dehydration remains to be determined.

RENAL HANDLING OF K IN SPONTANEOUS HYPERTENSIVE RATS. EVIDENCE FOR INTRINSIC TUBULAR DEFECTS. B. Eby*, J. Spirnak*, and K. Lau. Neph. Div., Dept. of Medicine, Univ. of Mich., Ann Arbor, MI.

Previous work suggests abnormal tubular Na handling in spontaneous hypertensive rats (SHR). Increased plasma K has been reported by some but not all studies. To examine renal K excretion and its role in the pathogenesis of the hyperkalemia, clearance and metabolic experiments were performed in 15- to 18-week-old SHR and normotensive Wistar Kyoto (WKY) rats. At similar glomerular filtration rate (GFR) (1.8 vs 1.6 ml/min) and clearance (C) of Na (86 vs 99 µl/min), K excretion was significantly (<0.05) reduced (1.46 vs 2.27 µEq/min). To evaluate the role of the reportedly reduced levels of aldosterone in SHR, rats were adrenalectomized (ADX) and maintained on regular rat chow and half normal saline water. Five days after ADX, plasma K was increased, but higher in SHR (5.5 vs 4.8 mEq/L, $p < .05$). The kaliuretic effects of deoxycorticosterone (15 mg/Kg I.M.) were similar (ΔU_K = 33.8 vs 29.6 µEq/h). Clearance studies performed during hydropenia and ≥2 weeks after ADX revealed tubular K retention, since SHR had reduced C_K (233 vs 347 µl/min, $p < .05$) and FE_K (16.2 vs 26.8, $p < .005$) despite higher plasma K (3.8 vs 3.3 mEq/L, $p < .01$), comparable GFR (1.5 vs 1.3 ml/min) and C_{Na} (26 vs 23 µl/min). We conclude (1) Spontaneous hypertensive rats are characterized by reduced rates of K excretion and increased plasma K. (2) Kaliuretic response to mineralocorticoid is, however, normal. (3) The renal K retention is independent of adrenal steroids and the tubular handling of Na.

INFLUENCE OF ADH ON POTASSIUM HANDLING BY THE DISTAL NEPHRON IN BRATTLEBORO RATS. Michael J. Field*, B.A. Stanton and G.H. Giebisch. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT

We have studied the relationship between movements of water, Na and K in the distal nephron during the conversion of water diuresis to antidiuresis by ADH. Simultaneous clearance and free-flow micropuncture experiments were performed in 10 male homozygous Brattleboro (D.I.) rats during water diuresis (0.45% NaCl infusion) and after arginine vasopressin (6mU/hr). Final urine flow rate fell markedly after ADH, associated with a rise in urine/plasma ratios for inulin (12.0±0.5 to 78.4±9.2) and osmolality (0.40±0.03 to 2.03± 0.15, means ± SEM, $p<.001$ in both cases). Final fractional excretion of Na (FE_{Na}) was not altered overall, but FE_K increased consistently and significantly after ADH from (7.3±1.4)% to (13.1± 2.3)%, $p<.02$. In late but not early distal tubular segments, ADH caused a rise in TF/P inulin (5.70±0.26 to 9.18±0.74, $p<.001$) and a fall in flow rate (6.2±0.6 to 3.2±0.5 nl/min, $p<.01$), while fractional delivery of Na and K was not significantly altered at either site. Thus, K secretion between late distal tubule and final urine was increased after ADH, despite lowered luminal flow rates. These changes were associated with significant increases in intraluminal Na concentration in late distal tubule (24±3 to 39±5 mM, $p<.01$) and final urine (21±4 to 143±9 mM, $p<.001$) during antidiuresis. We conclude that in this model ADH promotes K secretion beyond the distal tubule indirectly, by allowing intraluminal Na concentrations to rise above the low levels (known to be limiting for K secretion) which develop during water diuresis.

PERFUSION OF SINGLE ISOLATED TUBULES OF THE SHARK RECTAL GLAND: RESPONSE TO HORMONES AND ELECTRICAL CHARACTERISTICS. J.N. Forrest Jr., F. Wang and K.W. Beyenbach. Yale U. New Haven, Ct., Cornell U. N.Y.

The shark rectal gland has been used extensively as a model of hormone-mediated electrogenic Cl^- transport, but isolated tubules of the gland have not been perfused *in vitro*. To distinguish between vascular and tubular effects and to study directly the mechanism of NaCl transport, we isolated and perfused single tubules by the method of Burg. In segments (0.3-1.3 mm length) perfused and bathed with identical shark Ringer's, a basal lumen neg. transepithelial voltage (V_t) was consistently observed (-1.8±0.4mV, n=26). Addition of 8-cpt cAMP (100 μM) to the bath increased V_t to -8.3±1.6, n=7, $p<0.01$, paired t). Both vasoactive intestinal peptide (VIP) and adenosine (Ado) cause vasodilation and increase NaCl secretion in the whole gland. In perfused tubules VIP (1μM) increased V_t to -9.0±2.5 (n=8) and Ado increased V_t to-5.0±0.8, n=9 (both $p<0.02$ compared to basal V_t). The Ado response was reversed by theophylline, a specific inhibitor of Ado receptors. Furosemide (1mM in bath) reversibly inhibited the response to VIP and Ado ($p<0.01$). Replacement of bath Na with choline hyperpolarized the tubule due to a Na diffusion potential (ΔV_t-28 mV) whereas replacement of bath Cl^- with isethionate caused no change. Transepithelial resistances (12Ω $\cdot cm^2$) indicated high epithelial conductances. The results demonstrate that: (1) tubules are perfusable and have receptors for VIP and Ado; (2) secretagogues hyperpolarize the tubule consistent with electrogenic Cl^- transport; and (3) the cation selective permeability of the paracellular pathway is consistent with paracellular movement of Na during hormone stimulated Cl^- transport in this epithelium.

RENAL RESPONSE OF K DEPLETED (KD) RATS TO EXTRACELLULAR FLUID VOLUME EXPANSION (VE), DEOXYCORTICOSTERONE ACETATE (DOAC), FUROSEMIDE AND CHLOROTHIAZIDE (CTZ). B. Gelzayd*, B. Eby*, and K. Lau. Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Previous studies suggest that renal adaptation to KD is independent of aldosterone and plasma K. To evaluate the mechanism, clearance studies were done in KD (<0.01% K diet) and K repleted (0.47% K diet) rats. In KD rats, K clearance (C) rose from 35 (hydropenia) to, respectively, 212 and 323 μl/min with VE (5% and 10% B.W.). However, at all levels of C_{Na}, C_K was lower than K repleted rats (372 to 785 and 832 μl/min). In hydropenic KD rats, furosemide (F) (1.5 mg/Kg B.W. I.V.), but not CTZ (40 mg/Kg B.W.), increased C_K (46 to 153 μl/min) and fractional excretion of K (1.9 to 8.1%) despite lower increments in C_{Na} (34 vs. 63 μl/min), and despite reduced glomerular filtration rate (2.7 to 2.0 ml/min). Similar effects were seen in volume expanded KD rats after F (ΔC_K = 236 μl/min) but not after CTZ or DOCA (15 mg/Kg B.W. I.M.) despite the expected ΔC_{Na} (+95 and -47 μl/min, respectively). This effect of furosemide was almost identical to that in K repleted rats (ΔC_K = 258 μl/min). We conclude: (1) KD rats are resistant to the kaliuretic effects of distally acting agents (CTZ and DOCA), but sensitive to more proximal inhibitors (VE and F); (2) Furosemide is equally kaliuretic regardless of diet K, volume status and natriuresis; (3) These data imply that the renal adaptation to KD is primarily distal, but limited in capacity, hence unable to reclaim excessive K loads rejected by inhibited earlier nephron segments.

NA-K-ATPASE ACTIVITY IN THIN LIMBS OF RAT NEPHRON. Lal C. Garg and C. Craig Tisher. Univ. of Florida College of Medicine, Gainesville, Florida.

Potassium secretion has been demonstrated in the descending limb of the loop of Henle in juxtamedullary (JM) nephrons; however, it is unknown whether the process is active or passive. Although Na-K-ATPase is involved in active secretion of K^+, this enzyme has not been demonstrated in the thin descending limb (TDL). To examine the possible role of the TDL in the active secretion of K^+, we measured Na-K-ATPase activity in freshly dissected thin limbs from female Sprague-Dawley rats. The previously described four types of thin limbs were studied including outer medullary Type I and II segments from short- and long-looped nephrons, respectively, and inner medullary Type III and IV segments corresponding to descending and ascending limbs of JM nephrons. The findings were related to the morphology of these segments as determined by electron microscopy. Enzyme values are expressed as mean ± SEM of five animals in pmole/min/mm of tubule.

Type I	Type II	Type III	Type IV
5±3	30±5	4±3	3±2

Significant levels of Na-K-ATPase activity were found only in Type II segments. This segment was also the most complex in structure possessing cells with extensive basolateral cellular interdigitations, luminal microvilli and mitochondria. The presence of Na-K-ATPase activity in cells that resemble those known to be involved in solute transport suggests the possibility that Type II segments of long looped JM nephrons located in the outer medulla may be involved in active K^+ secretion.

CRITICAL ROLE OF CALCIUM IN THE REGULATION OF INTRACELLULAR VOLUME OF ISOLATED PROXIMAL S_2 RENAL TUBULES IN HYPOTONIC MEDIUM. T. Neufeld*, D. Terreros*, J. Grantham. Univ. of Kansas Med. Ctr., K.C., KS. 66103.

When isolated non-perfused proximal rabbit tubules are suddenly bathed in hypotonic medium in vitro the cells rapidly absorb water from the bath by osmosis and swell. This osmometric swelling phase is followed by a volume regulatory decrease (VRD) in cell volume accompanied by the loss of intracellular K, Na, Cl, and H_2O. The present study examined the role of calcium in triggering the VRD. When control non-perfused S_2 segments were bathed in hypotonic medium (290 reduced to 190 mOsm/Kg, by removing NaCl) containing 2.0 mM Ca^{2+} they rapidly swelled to a maximum value (+ 124.6 ± 5.5%). After 5 minutes in hypotonic medium, cell swelling had decreased to 107.9 ± 2.4%. In control tubules the fraction of the osmometric gain in cell volume in hypotonic medium lost within 5 min (% VRD) was 71.0 ± 8.4%. Removal of extracellular Ca^{2+} (zero Ca^{2+} bath plus 1 mM EGTA) virtually stopped VRD in hypotonic medium (% VRD 6.3 ± 1.3 at 5 min). Verapamil (10^{-5}M), a Ca^{2+} channel blocker, also inhibited VRD despite normal Ca^{2+} levels in the bath (% VRD zero at 5 min). Addition of A21387 (10^{-6} M), a Ca^{2+} ionophore, in the presence of verapamil and external Ca^{2+} restored VRD to 63.9 ± 12.1%. Barium (1 mM), a K^+ channel blocker in basolateral membranes strikingly retarded VRD. We conclude that the accelerated K^+ loss, instrumental in the regulation of intracellular volume of S_2 segments in hypotonic medium, is critically dependent on the level of intracellular Ca^{2+}.

HYPERTONIC FLUID REABSORPTION BY THE RAT RENAL PROXIMAL TUBULE. R.Green,* and G.Giebisch, Depts. of Physiology, University of Manchester,Manchester, England, and Yale University, New Haven, CT.

For many years it has been assumed that fluid is reabsorbed isotonically from the proximal convoluted tubule. Recent evidence suggests,however, that at least in some cases, this may not be so (Bishop,Green and Thomas, J.Physiol. 288:331-351, 1979; Barfuss and Schafer, Am.J.Physiol. 241:F597-F604, 1981), but measurement of the effective osmotic driving forces has been complicated because different solutes have different reflection coefficients (σ).

Male Sprague-Dawley rats (180-220 g) were prepared for micropuncture and individual proximal tubules were perfused with 154 mM NaCl at 45 nl/min; their surrounding capillaries were simultaneously perfused with the same solution. Fluid reabsorption was measured using [^{3}H] inulin and the osmolality and chloride concentrations of perfusates and collected tubular perfusate were determined.

In 19 tubules (8 rats) the rate of fluid reabsorption was 0.88 ± 0.14 nl/min/mm. There were no significant differences between the tubular and capillary perfusates with respect to either osmolality or chloride, but the collected tubular fluid had a lower osmolality (Δ Osm = −3.8 ± 0.8 mOsm/kg water) and a lower chloride concentration (Δ chloride = −1.8 ± 0.7 mmole/L) than the perfusate.

We conclude that when there is a single solute (NaCl), and therefore no effect of different σ, the proximal tubule reabsorbs a hypertonic fluid and the fluid in the tubule becomes hypotonic.

RELATIONSHIP BETWEEN CELL VOLUME AND ION TRANSPORT IN THE DILUTING SEGMENT OF AMPHIUMA KIDNEY. W.B. Guggino, H. Oberleithner* and G. Giebisch, Depts. of Physiol., The Johns Hopkins Univ., Baltimore, MD, Univ. of Innsbruck, Innsbruck, Austria, Yale Univ., New Haven, CT.

Video-optical techniques in combination with conventional microelectrodes were used to estimate simultaneously cell volume and basolateral cell membrane potential (V_{bl}) in isolated perfused diluting segments of Amphiuma kidney. Since we observed that alterations in cell size occurred primarily as changes in cell height, this parameter was used to evaluate changes in cell volume. Decreasing the osmolality of the basolateral solution by 23% results within 2 min in a 77±9% increase in cell height while increasing the osmolality decreases cell height by 31±6%. In contrast, similar osmolality changes of the luminal solution do not significantly affect cell height. Luminal perfusion with furosemide (5×10^{-5}M), or K-free or Cl-free solutions, maneuvers known to inhibit apical cotransport of Na/K/Cl do not result in detectable changes of cell height. Ouabain-induced inhibition of the basolateral Na/K pump within 10 min increases cell height by 97±16% and decreases V_{bl} by 49±12%. However, in the presence of either luminal furosemide or K-free, or Cl^--free solutions ouabain-induced changes of cell height and of V_{bl} are not observed. Cell swelling by a hypoosmotic bathing solution was also reduced 44±11% by luminal application of furosemide. We conclude that 1) the water permeability of the apical cell membrane is low, 2) swelling due to ouabain requires an intact apical Na/K/Cl cotransport system, and 3) this cotransport system must be the major luminal pathway for Na, Cl and K entry.

PARADOXICAL EFFECTS OF PYRAZINOATE (PZA) ON URATE TRANSPORT IN DOG RENAL BRUSH BORDER MEMBRANE VESICLES (BBMV). Sandra E. Guggino* and Peter S. Aronson, Depts. of Medicine and Physiology, Yale Sch. Med., New Haven, CT.

PZA can suppress or enhance urate excretion in a dose dependent manner, effects described as paradoxical. We examined the effect of PZA on urate transport in dog renal BBMV. PZA shares the anion exchange pathway for urate as evidenced by inhibition of pH gradient (pH 6.5^o, 7.5^i) stimulated urate uptake by external PZA (I_{50} 0.15mM), and stimulation of uphill urate accumulation (3-X overshoot) by an outward PZA gradient ($10mM^i$, $0.2\ mM^o$) in the presence of valinomycin and nigericin and $K^o=K^i$. In addition, PZA shares the Na-monocarboxylic acid cotransport system not shared by urate, as an inward Na gradient caused uphill accumulation (2-X overshoot) of PZA, and external PZA inhibited Na stimulated lactate uptake (I_{50} 1.8mM). We then tested whether the outward PZA gradient generated by an inward Na gradient would drive urate uptake. Indeed, 0.1mM external PZA induced a 1.6-fold urate overshoot above equilibrium in the presence of an inward Na gradient, but not in the presence of an inward K gradient. However, in the presence of the same inward Na gradient, 5mM external PZA inhibited urate uptake by 60%, the dominant effect at that PZA dose being cis inhibition of the urate exchanger. In summary, we find that the dose-dependent paradoxical effects of PZA can be explained on the basis of its interacting with the luminal membrane urate exchanger. Suppression of urate excretion by low dose PZA may reflect stimulation of urate reabsorption and not necessarily inhibition of urate secretion.

INFECTION CAUSES ENHANCED SODIUM EXCRETION DURING UNILATERAL SUSTAINED PARTIAL URETERAL OBSTRUCTION (USPUO) IN THE DOG. F.D. Gutmann. Department of Medicine, University of Wisconsin, Mount Sinai Medical Center, Milwaukee, Wisconsin.

This laboratory previously reported that the anticipated decrease in fractional excretion of sodium (FE_{Na}), observed in the experimental kidney (EK) within 1 hr of inducing USPUO in conscious dogs, was reversed by 24 hrs, and remained similar to that of the contralateral control kidney (CK) until the obstruction was released 3 days later. To determine the effect of infection in this model, 7 conscious split-bladder dogs were studied that were spontaneously infected prior to obstruction (as determined from EK ureteral urine). As in previous studies, 1 ureter was partially constricted via an externally inflatable cuff, distal to an ureteral catheter which permitted monitoring of EK ureteral pressure (UP). Mean FE_{Na}, GFR and maximal urine osmolality (U_{max}) were:

	Baseline		1 hr USPUO		3 days USPUO	
	EK	CK	EK	CK	EK	CK
FE_{Na}(%)	.62	.44	.13	.49	1.53	.59
GFR(ml/min)	37	44	29	49	23	42
U_{max}(mosm/kg)	1111	1592			519	1478
UP(mm Hg)	9		27		27	

Although the FE_{Na} invariably decreased in EK compared to CK after 1 hr of USPUO, the FE_{Na} was greater in EK compared to CK (1.53 vs 0.59, $p<.05$) after 3 days of USPUO. The decrement in GFR and abnormality in U_{max} tended to be greater in the infected EK as compared to previously reported non-infected EK. These 2 factors plus others associated with EK infection may explain enhanced FE_{Na} in EK after 3 days of USPUO in the dog.

ION FLUXES AND FUROSEMIDE BINDING IN RECTAL GLAND PLASMA MEMBRANE VESICLES;EVIDENCE FOR THE PRESENCE OF A Na,K,Cl COTRANSPORT SYSTEM. J.Hannafin,* E.Kinne-Saffran,* D.Friedman,* and R.Kinne. Albert Einstein College of Medicine, Bronx, N.Y. and MDIBL, Salsbury Cove, Maine.

The rectal gland of Squalus acanthias actively transports Cl in a manner analogous to the thick ascending limb of Henle's loop by means of a Bumetanide sensitive, Na,Cl cotransport system. To investigate the role of K in this system a basal-lateral membrane fraction was isolated from rectal glands and a rapid filtration technique was used to characterize ion transport and diuretic binding. KCl gradient dependent ^{22}Na uptake decreased 53% when the K concentration was decreased from 164 to 5mM. A similar degree of inhibition was seen with $4x10^{-4}M$ Bumetanide. The 15 second ^{86}Rb uptake (at 0.4mM)-Rb used as a K substitute-was 236 pmol/mg with a 164mM NaCl gradient, but only 69pmol/mg in $NaNO_3$ or LiCl media. This Na,Cl dependent Rb flux was also observed in tracer exchange experiments; it was abolished with $4x10^{-4}M$ Bumetanide or 10mM K.

Scatchard analysis of [^3H]N-methylfurosemide (NMF) binding to the membrane in 200mM NaCl/10mM KCl revealed the presence of low and high affinity binding sites. High affinity sites had a $K_D=4x10^{-6}M$ with n= 104 pmol/mg protein. At $0.8x10^{-6}M$, NMF binding decreased 51% when Na free or low K media were used. $10^{-3}M$ Bumetanide or 600mM Cl also reduced NMF binding by 53%. These results support the hypothesis that the Na,Cl cotransport system described previously is in fact a Na,K,Cl cotransport system whose interactions with Cl and the "loop diuretics" are affected both by Na and K.

ROLE OF ALDOSTERONE IN RENAL POTASSIUM ADAPTATION David Hirsch* and John P. Hayslett. Dept. of Med., Yale School of Medicine, New Haven, CT.

The action of aldosterone (Aldo) is characterized by an increase in PD (lumen negative), as well as an increase in the transport of Na^+ and K^+. Since chronic K^+ loading induces similar effects and an increase in Aldo production, the role of hyperaldosteronism in the mechanism of K^+ adaptation due to K^+ loading is unclear. To determine the role of Aldo in K^+ adaptation, late distal tubule PD was used as a marker for Aldo action in rats with hyperaldosteronism (Na^+ free diet), and adrenalectomized (Adx) rats with physiologic plasma levels of Aldo (5 ng/dl) and corticosterone (4 μg/dl) on a normal or high K^+ (10 X normal, 7 days) intake.

Group	Distal tubule PD, mV	
1.Intact-control	-33.8 ± 2.9	(18)
2.Intact-Na+ free	-35.6 ± 2.6	(12)
3.Intact-High K^+	-41.9 ± 2.6	(16)*
4.Adx - control	-40.3 ± 2.7	(13)
5.Adx - High K^+	-46.6 ± 1.4	(20)†

*p< 0.05 vs Group 1 (n)
†p< 0.05 vs Group 4

As in the Na+ deprived group, chronic administration of high dose Aldo (plasma levels >200 ng/dl) to intact rats, for 16 hrs or 6 days, had no effect on PD.

These data 1) show that the effect of chronic K+ loading on distal tubule is independent of Aldo and 2) fail to show an effect of Aldo on distal tubule.

UREA PERMEABILITY OF TOAD URINARY BLADDER IN HYPEROSMOTIC MEDIA. Marcos A. Hardy. University of Miami, Department of Medicine, Miami, Florida.

The environment of the medullary collecting tubule of the mammalian nephron is hyperosmotic compared to plasma. The toad bladder is used as a model of the collecting tubule. Therefore, the effect of hypertonicity (H,.22 M mannitol added to isotonic Ringer [R,.23 osM]) on the ^{14}C-urea permeability (Pu) of toad bladder was studied.

Agent	mM	Pu(nm/s) R	Pu(nm/s) H	n	P
-	-	10±2	10±3	7	NS
ADH	7.10^{-5}	311±24	128±16	8	<.001
8Br-cAMP	1	171±32	115±21	6	<.001
IBMX	1	336±28	112±25	4	<.001

While H does not alter basal Pu, it inhibits stimulation of Pu by either ADH, cAMP or the phosphodiesterase inhibitor IBMX. This suggests that the inhibition operates at a post-cAMP step. Thus, the effects of H on the cAMP content (in pmoles/mg prot.) of the epithelial cells was assayed. Hypertonicity did not modify basal cAMP (R: 32±4, H: 24±4; n=5, p>.1), ADH increased it (R: 48±6, R + ADH: 537±35; n=9, p<.001) and H did not alter the effect of ADH on nucleotide accumulation (R + ADH: 545±55, H + ADH: 480±72; n=8, p>.2). In conclusion, H inhibits the effects of ADH on Pu at a site beyond the synthesis of cAMP, i.e. on the distal events that trigger the increase in Pu at the luminal membrane. Hypertonicity of the medullary interstitium may have there a negative feed-back effect on the ADH-induced recycling of urea; thereby, interstitial hypertonicity could be self-limiting.

ALCOHOL-INDUCED WATER DIURESIS IS NOT PROSTAGLANDIN DEPENDENT. M.Johnson,* E.T. Zawada, Jr., McGuire VA and Med. Coll. of Va., Richmond, Va.

Previous studies have demonstrated an effect of alcohol on arachidonic acid metabolism. These studies were undertaken to determine if the water diuresis of alcohol is due to enhanced prostaglandin E_2 production, a known antidiuretic hormone antagonist. 6 rabbits weighing between 2.5 and 3.2 kg were studied in standard metabolic cages during 4 periods: control (C), indomethacin administration (I), alcohol administration (A), and (A+I). 10 ml of 100% alcohol was added to their water in periods 3 and 4, and 5mg/kg indomethacin was given during 2 and 4. We recorded urine output ($\dot{v}$), urine osmolality (Uosm), sodium excretion (UNav), potassium excretion (UKv), and prostaglandin excretion ($UPGE_2v$). The results as summarized below:

	$\dot{v}$ (cc/d)	Uosm (mosm)	UNav (mEq/d)	UKv (mEq/d	$UPGE_2v$ (ng/d)	# of Days
C	79±7	1514±70	7±1	33.9±1.8	897±71	4
I	84±11	1451±79	5±<1	30.9±2.2	105±22	6
A	177±13	765±67	8±1	28.8±1.6	125±13	6
A+I	165±11	814±68	8±<1	31.5±1.3	191±30	7

There were no differences in water intake or weights between periods. In these studies urine flow rate significantly increased during A which was not prevented by indomethacin. In addition, prostaglandin E_2 production by the kidneys did not increase during A. Conclusion: the water diuresis produced by acute alcohol administration is not mediated by enhanced renal PGE_2 production.

DISTURBED RENAL SODIUM HOMEOSTASIS DURING ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITION IN THE RAT.

C.I. Johnston* (intr. by B. Jackson), Monash University, Department of Medicine, Prince Henry's Hospital, Melbourne, Victoria, Australia.

The response to ACE inhibition during constant sodium intake and salt equilibration during changed sodium intake was examined in the rat. Captopril (50mg/kg/day) or Enalapril (1mg/kg/day) administered in drinking water to rats after seven days on a high salt (1% NaCl drinking solution), normal (NS) or low salt (LS) diet did not alter their urinary sodium excretion over the ensuing seven days. When ACE inhibitor was given to rats equilibrated on NS, and simultaneously they were placed on LS, they took longer to achieve sodium balance. 24 hr urinary sodium (μmol) fell in non-treated rats from 530±188 to 39±8 by the fourth day. ACE inhibitor treated rats excreted 113±18 μmol sodium (Captopril group) and 232±30 μmol (Enalapril group) on the fourth day, and did not achieve sodium balance till the seventh day. Plasma angiotensin II (AII, pg/ml) in treated rats was then depressed by 40% (Captopril group) and 64% (Enalapril group) compared to salt depletion alone. Despite suppression of AII by Enalapril 24 hr urinary aldosterone (fmol) rose in a similar manner in both groups (non-treated rats from 4.5±0.8 to 9.4±3.1, and treated rats from 4.8±0.9 to 8.8±0.6).

ACE inhibition appears to disturb renal sodium conservation independent of aldosterone during salt restriction.

URATE AND PAH TRANSPORT IN BASOLATERAL MEMBRANE VESICLES FROM RAT KIDNEY. Andrew M. Kahn and Edward J. Weinman. Univ. of Texas Med. School, Dept. of Internal Medicine, Houston, Texas.

To examine the mechanisms of transport of urate and PAH across the basolateral border of the cell and to determine if there was interaction between these two organic anions, basolateral membrane vesicles were prepared by a Percoll density gradient method. In control conditions, the uptake of of 53uM (^{14}C) urate and 2.4uM (^{3}H) PAH at 10 sec. of incubation were 20.4 and 0.67 pmol/min/mg protein respectively. Probenecid (2.4mM) inhibited the uptake of urate and PAH by 39%. DIDS (2.4mM) inhibited the uptake of urate by 49% and PAH by 73%. The addition of unlabeled urate (1.4mM) to the incubation solution inhibited the uptake of (^{14}C) urate by 35% and PAH by 10%. The addition of unlabeled PAH (4.8mM) inhibited the uptake of (^{3}H) PAH by 39% and that of urate by 15%. However, when vesicles were preloaded with unlabeled urate (1.5mM), the uptake of urate was stimulated by 82% but that of PAH was not. When vesicles were preloaded with unlabeled PAH (4.8 mM), the uptake of (^{3}H) PAH was stimulated by 65% while the uptake of urate was not.

These studies demonstrate that in basolateral membrane vesicles of the rat, the uptake of urate and PAH is saturable, capable of exchange diffusion and inhibitable by probenecid and DIDS. Neither organic acid trans-stimulated the uptake of the other although a small degree of cis-inhibition was demonstrated. This suggests that urate and PAH are probably transported by separate carriers with only a small degree of cross affinity.

PROSTAGLANDIN SYNTHESIS, INTRAMEMBRANOUS PARTICLE AGGREGATES, AND WATER PERMEABILITY IN TOAD URINARY BLADDER. William A. Kachadorian. National Institutes of Health, Bethesda, MD

The effects of naproxen-mediated inhibition of prostaglandin synthesis on toad bladder intra(luminal)membrane particle aggregates (presumed sites for transmembrane water flow) and total tissue osmotic water permeability (P_f) were examined in bladders prepared as sacs. In the absence of ADH, P_f was enhanced appreciably by naproxen treatment (16.9 ± 2.7 vs $3.4 \pm 0.5 \times 10^{-4}$cm/sec) and aggregates became disproportionately numerous ($29.4 \pm 11.1 \times 10^{6}/cm^2$) (n=5 pairs). In separate bladders (n=5) treated with submaximal ADH (0.125mU/ml), P_f was 2-fold greater than with naproxen treatment alone ($35.3 \pm 9.8 \times 10^{-4}$cm/sec), but aggregates were 3-fold less ($9.8 \pm 5.5 \times 10^{6}/cm^2$). In bladders paired to these, naproxen treatment potentiated the action of ADH so that P_f became about half-maximal ($128.3 \pm 11.5 \times 10^{-4}$cm/sec) and aggregates increased, again disproportionately, to a near maximal level ($98.2 \pm 17.6 \times 10^{6}/cm^2$). The results indicate that prostaglandin synthesis modulates aggregate frequency and P_f both prior to and during ADH stimulation. If the apparent water permeability of aggregates is constant, the data are consistent with the view that luminal membrane water permeability can be enhanced without a comparable increase in total tissue water permeability. This implies the presence of a barrier to ADH-stimulated water flow, in series with the luminal membrane, whose water permeability is altered directly by ADH.

BETA BLOCKADE AND K SECRETION IN THE ISOLATED PERFUSED RAT KIDNEY. LEE D. KATZ, William Jones*, and Ralph DeFronzo. Yale Univ Sch. of Med. New Haven,Ct

Previous studies from our laboratory employing the isolated perfused rat kidney have demonstrated that epinephrine has a direct inhibitory effect on K secretion. This antikaluretic effect was believed to be mediated via the β adrenergic receptor system as propranolol was shown to stimulate K excretion. To further delineate the adrenergic receptor mechanism involved in renal tubular K excretion, following a 30 min control period of perfusion with Krebs-Henseleit buffer, either isoproterenol(6ng/ml, a β^1 and β^2agonist), ITP(200ng/ml, a β^1agonist), butoxamine (1,10,100μg/ml, a β^2 antagonist), or metoprolol(50, 200ng/ml, a β^1 antagonist), was added to the perfusate to achieve the desired concentration and an additional 30 min exp period followed. Perfusate K was similar in all groups. The changes in K excretion were unrelated to changes in GFR, $U_{Na}V$, or flow.

	$U_K V$ (μeq/min)		C_K (ml/min)		FE_K %		GFR (ml/min)	
	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60
CON	.35	.38	.11	.12	25.8	31.1	.44	.43
ISO	.49	.27*	.10	.06*	30.9	16.3**	.38	.35
ITP	.51	.39*	.12	.08*	32.8	18.5**	.40	.39
BUT	.41	.47	.08	.09	22.5	27.1	.37	.37
MET	.56	.68*	.11	.16*	31.0	48.0**	.42	.43

*p<0.025 **p<0.01

When either ISO or ITP were used, a decrease in K excretion was observed. MET in either low or high concentrations stimulated K excretion. CONCLUSION : Renal K handling can be stimulated or inhibited by manipulation of the β^1adrenergic receptor system in the renal tubule of the isolated perfused rat kidney. Agents working via the β^2 receptor had no effect on renal K excretion.

POTASSIUM (K^+) FLUX COEFFICIENT IN THE PROXIMAL CONVOLUTED TUBULE (PCT). James S. Kaufman and Robert J. Hamburger, Boston VA Medical Center, Renal Section, Boston, MA.

K^+ flux coefficients were estimated in the isolated PCT of the rabbit by determining the effect of various bath K^+ concentrations on net lumen-to-bath K^+ flux (J_K). Perfusate K^+ concentration was 4mM and bath K^+ concentration was either 2, 4 or 6 mM. Studies were performed in the presence of volume flux (J_v). K^+ concentrations of the perfusate (P) and collected fluid (CF) were determined by electron probe microanalysis. Results (± SE) are as follows; J_v is in nl mm^{-1} min^{-1}, CF/P_K is the CF to P potassium concentration ratio and J_K is in pM $mm^{-1}min^{-1}$:

Bath K^+	J_v	CF/P_K	J_K
2 mM	1.05±0.16	1.00±0.04	4.00±2.02
4 mM	1.01±0.15	1.11±0.04[a]	-0.94±1.27[a]
6 mM	0.93±0.18	1.15±0.04[a,b]	-6.71±1.36[a,b]

Significantly different ($p < 0.05$) from value with 2mM (a) or 4mM (b) bath.

The K flux coefficient was estimated from the slope of the linear regression equation relating J_K to bath K^+. This value was 2.60±0.65 pM mm^{-1} $min^{-1}mM^{-1}$ or 0.55±0.14 μm s^{-1}. This flux coefficient is relatively high, about 50% of that of sodium (Kokko et al, JCI 50:69, 1971) and would be consistent with a predominant role for passive transport in potassium handling in the PCT.

SEQUENCE OF MORPHOLOGIC EVENTS IN ADH-STIMULATED WATER FLOW ACROSS RABBIT CORTICAL COLLECTING TUBULE. Kevin L. Kirk,* Donald R. DiBona* and James A. Schafer, Nephrol. Res. & Training Ctr., Univ. of Alabama in B'ham, Birmingham, Al.

The temporal sequence of morphologic events during ADH-induced hydroosmosis across the rabbit cortical collecting tubule was determined in longitudinal studies of individual isolated and perfused segments using differential interference-contrast microscopy. Computer-assisted morphometric analysis was employed to monitor total epithelial volume (V_T) and the rate of intercellular space dilation. In the absence of an osmotic gradient (perfusate and bath: 290 mOsm) the addition of 250μU/ml ADH to the bath resulted in no apparent structural changes. Switching to a hypotonic perfusate (130 mOsm) in the presence of ADH resulted in dilation of the intercellular spaces and prominent bulging of the cells into the lumen. V_T increased by 29± 6% (mean ± SEM) within 15-20 minutes, reaching a half-maximum at between 2 and 5 minutes. Cytoplasmic vacuolation, which occured to varying degrees, was not apparent until 15-20 minutes after the change in perfusate. Returning to the isotonic perfusate reversed this sequence of events and returned V_T to control values. Concerning the time course of these structural events two observations are pertinent to models of transtubular osmosis: 1) Following imposition of an osmotic gradient intercellular space dilation paralleled or slightly preceded the increase in cell volume. 2) Cytoplasmic vacuolation, which was often quite prominent, occured only in the latter stages of cell volume expansion.

VASOPRESSIN ANTAGONIST-ASSOCIATED AQUARESIS IN PRIMATES. L. B. Kinter, F. Stassen*, E. Horner*, V. Wiebelhaus and N. Yim*. Dept. of Pharmacology, Smith Kline & French Labs., Philadelphia, PA

The vasopressin (VP) analog $d(CH_2)_5$D-PheVAVP (SK&F 101071) is the most potent member of a new class of diuretic agents yet reported (Manning et al., J. Med. Chem. 25: 414, 1982). We have characterized the renal activity of this peptide in the rat and squirrel monkey. In conscious hydropenic animals SK&F 101071 (i.p.) increased urine flow (V; from <1.0 to >20 ml/kg/4 hr) and decreased urine osmolality (Uosm; from >1000 to <100 mOsm) in a dose-dependent fashion. Doses (μg/kg) to increase V to 10 ml/kg/4 hr (ED_{10}) and decrease Uosm to 300 mOsm (ED_{300}) were defined. Negligible increases in urinary solute excretion were observed.

SK&F 101071	ED_{10}	ED_{300}	Ki
Rat (9)	23	24	5.3 x 10-9 M
Monkey (6)	880	580	9.1 x 10-9 M

To investigate the molecular mechanism of SK&F 101071-stimulated urine flow we measured inhibition of VP-stimulated adenylate cyclase in rat and monkey renal medullary membranes. SK&F 101071 was a potent, competitive antagonist in both tissues (Ki). In addition, no peptide-associated agonist activity was demonstrable in either system.

We conclude that SK&F 101071 is a potent diuretic in conscious rats and monkeys and that the mechanism is antagonism of VP-stimulated cAMP formation. We propose that the specific water losing activity of SK&F 101071 and other vasopressin antagonists be termed "aquaretic" activity, as distinguished from the saluretic activity of conventional diuretics.

ENHANCED OSMOTIC EFFECT OF MANNITOL ON NEONATAL PROXIMAL TUBULE. L.I. Kleinman and T.A. Disney*, University of Cincinnati College of Medicine, Cincinnati, Ohio

In order to elucidate maturational characteristics of proximal tubule Na reabsorption, tubular response to mannitol loading was studied in 6 neonatal dogs aged 1-19 days and in 6 adult dogs. Proximal tubule function was determined using distal nephron blockade technique with ethacrynic acid and amiloride. Mannitol was infused IV at progressively increasing dosages to increase proximal tubular load. With increasing mannitol loads proximal tubular rejection of Na increased linearly. The proximal tubule osmotic effect of mannitol was quantitated from the slope of the plot, mannitol excretion vs. sodium excretion ($\Delta ENa/\Delta EM$) during distal nephron blockade. Newborn dogs had higher $\Delta ENa/\Delta EM$ (1.60±.25) than adults (.70±.20). Newborns also had higher $\Delta EK/\Delta EM$, $\Delta ECl/\Delta EM$ and $\Delta EH2O/\Delta EM$ than adults. This enhanced osmotic effect of mannitol declined exponentially with age. Prior to infusion of mannitol, proximal tubule U_{Na}/P_{Na} ratios were unity for both newborns and adults. In adults U_{Na}/P_{Na} declined linearly as osmotic load increased; in newborns the ratio remained close to unity. Proximal tubule U_{Osm}/P_{Osm} remained at unity for all animals. These results indicate that there is enhanced osmotic effect of mannitol in the neonatal kidney due to inability of the immature proximal tubule to generate a transtubular Na concentration gradient.

UREA TRANSPORT IN ISOLATED, PERFUSED THICK ASCENDING LIMBS (TAL) AND COLLECTING DUCTS (CD) FROM RATS. Mark A. Knepper, NHLBI, NIH, Bethesda, MD 20205

The permeability properties of many segments of the rat nephron have not been studied directly. Urea permeabilities were determined in isolated, perfused cortical and medullary TAL and cortical CD from pathogen-free Sprague-Dawley rats (75-125 g). TAL from the inner stripe of the outer medulla were directly identified as being from either short- or long-looped nephrons. Permeabilities were calculated from urea fluxes measured with 5mM urea either in the bath or perfusate and no urea on the opposite side of the epithelium. Urea concentrations were measured in bath, perfusate and collected fluid using microcolorimetry. The magnitudes of the bath-to-lumen and lumen-to-bath fluxes were not significantly different in any segment. Permeability coefficients (cm/sec x 10^{-5} $\pm$ S.E. [number of tubules]) in different portions of the TAL were: cortical, 1.5$\pm$0.3 [11]; outer stripe, 1.4$\pm$0.3 [9](P>0.6 vs. cortical); inner stripe,short loop, 0.9$\pm$0.2 [6] (P<0.05 vs. cortical); and inner stripe,long loop, 0.6$\pm$0.2 [8] (P<0.025 vs. cortical). Arginine vasopressin (100 μU/ml) in the bath did not affect the urea permeability of cortical CD: control, 0.4$\pm$0.1 x10^{-5} cm/sec; vasopressin, 0.5$\pm$0.1 (n=5). Conclusions: 1) Urea permeabilities of medullary TAL from short and long loops of Henle do not differ substantially. 2) Urea permeabilities of TAL from the cortex and outer stripe are greater than those from the inner stripe. Substantial passive urea absorption is predicted in vivo. 3) As in the rabbit, the cortical CD of the rat has a low urea permeability with and without vasopressin.

EVIDENCE FOR PLASMA FLOW DEPENDENCE OF PROXIMAL FLUID REABSORPTION. Valentina Kon*, Mary L. Hughes*, Iekuni Ichikawa. Harvard Med. School, Boston, MA.

Proximal fluid reabsorption (PR) is currently believed to be independent of peritubular capillary plasma flow rate (Q_C) since variation in Q_C is theoretically predicted to have little effect on mean peritubular capillary plasma oncotic pressure ($\bar{\pi}_C$). To examine the relationship between Q_C and PR, micropuncture measurements were performed in 12 rats with low Q_C during hydropenia (HP) and high Q_C during ~4% BW plasma volume expansion (VE). Results (mean$\pm$SE; † p<0.05) include:

	PR	Q_C	$\bar{\pi}_C$	$\bar{P}_C$	$\bar{P}_r$	Kr
	---nl/min---		------mmHg------			nl/(s·mmHg)
HP	11±3	52±10	28±1	13±0	10±1	0.023±0.005
VE	16±1†	162±17†	29±1	15±1†	9±1	0.032±0.005†

Thus, the rise in Q_C during VE was associated with a significant increase in PR, but no change in $\bar{\pi}_C$ due to a concurrent fall in filtration fraction. Mean peritubular capillary hydraulic pressure ($\bar{P}_C$) increased slightly, as did interstitial hydraulic pressure, thus mean net reabsorptive pressure ($\bar{P}_r$) remained unaffected. By contrast, the peritubular capillary reabsorption coefficient (Kr) increased substantially, accounting entirely for the rise in PR. This increase in Kr was accompanied by a profound fall in peritubular capillary resistance to axial flow (4.8±0.7†vs. 2.3±0.4 x 10^9 dynes·s·cm^{-5}) and suggests that the rise in Q_C led to an enlargement of capillary diameter and surface area available for reabsorption. The results indicate that the plasma flow dependence characterizing the GFR under various conditions also prevails in the process of proximal fluid reabsorption, thereby providing an important mechanism for preservation of glomerulotubular balance.

MICROELECTRODE STUDY OF RABBIT CORTICAL COLLECTING TUBULES (CCT): EFFECT OF MINERALOCORTICOID. B. Koeppen and G. Giebisch. Univ. of Connecticut, Department of Medicine, Farmington, CT., and Yale Univ., Department of Physiology, New Haven, CT.

The apical cell membrane of the CCT contains conductive pathways for Na^+ and K^+, and the basolateral cell membrane is conductive to K^+ (Kidney Inter. 21:280, 1982). Using intracellular microelectrodes, the effects of chronic mineralocorticoid treatment (DOCA 5 mg/d x 10-14 d) on the membrane electrical properties of the isolated perfused CCT were examined. Control tubules had a transepithelial voltage (V_T) of -12.4±1.5 mV, a basolateral membrane voltage (V_{bl}) of -84.0±2.5 mV, and the apical to basolateral membrane resistance ratio (R_a/R_{bl}) was 0.83±.11. With DOCA treatment, V_T and V_{bl} hyperpolarized to -44.8±4.4 and -105.8±3.1 mV respectively, and R_a/R_{bl} decreased slightly to 0.62±.10. Increasing the peritubular $|K^+|$ from 5-50 mV caused a 28.6±3.8 mV depolarization of V_{bl} in control tubules, while with DOCA treatment V_{bl} depolarized by 49.4±4.4 mV. Although the apical cell membrane conductance was increased by DOCA treatment, the response to luminal K^+ substitution was not appreciably altered, suggesting that the fractional K^+ conductance of this membrane was unchanged.

These results are consistent with a DOCA induced increase in the apical cell membrane Na^+ and K^+ conductances, and an increase in the basolateral cell membrane K^+ conductance. Also, with DOCA treatment, K^+ may enter the cell across the basolateral cell membrane both passively ($V_{bl}>E_K$), and via the Na^+-pump. Together these changes could account for the observed stimulation of net Na^+ and K^+ transport by chronic DOCA treatment.

RENORENAL REFLEXES: NATRIURETIC RESPONSES TO MECHANO-(MR) AND CHEMORECEPTOR (CR) STIMULATION. U.C. Kopp, M.D. Thames & G.F. DiBona. Dept. Int. Med., Univ. Ia. Col. Med. & V.A. Med. Ctr. Iowa City, Ia.

In anesthetized rats (N=11, paired), increased ureteral pressure (↑UP 28 mm Hg, MR) and retrograde isobaric ureteropelvic perfusion of hypertonic NaCl (0.9 M NaCl, 25 μl/min, UP 0 mm Hg, CR) each reversibly increase contralateral V and $U_{Na}V$ ($p<0.02$) without changing MAP, RBF or GFR. To separate MR from CR stimulation, contra responses to ↑UP with 0.1 M NaCl and ↑UP with rat urine were compared (N=6, paired); at the same ↑UP, increases in contra V and $U_{Na}V$ were comparable, indicating isolated MR stimulation. To assess neural pathways, effect of ipsi (N=6) and contra (N=8) renal denervation (RDNX) on contra responses to CR stimulation was measured. Prior to either ipsi or contra RDNX, CR stimulation increased contra V by 58±15% ($p<0.01$) and $U_{Na}V$ by 48±20% ($p<0.01$). Following either ipsi or contra RDNX, the increases in contra V and $U_{Na}V$ were abolished.

Conclusion: Renal MR and CR stimulation each produce a distinct receptor-specific contralateral renorenal reflex diuresis and natriuresis. Renal CR stimulation produces a contralateral renorenal reflex diuresis and natriuresis dependent on intact ipsi and contralateral renal innervation and may be related to decreased contralateral efferent renal nerve traffic.

EFFECT OF CATECHOLAMINES ON WATER ABSORPTION (Jv) IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT) R. Krothapalli,* W.B. Duffy*, W.N. Suki, and H.O. Senekjian. VA Medical Center and Baylor College of Medicine, Houston, Tx

The effect of alpha and beta adrenergic agonists on Jv in the CCT was studied by isolated tubule microperfusion. Bath and perfusate osmolalities were 290 and 125 mOsm/kg water, respectively. Antidiuretic hormone (ADH), isoproterenol (ISO), L-phenylephrine (PE), and phentolamine (PH) were added to the bath only. Experiments were performed at 25° C. In the presence of ADH, 100 μU/ml, Jv was 1.04 ± 0.1 nl/min/mm. Addition of ISO (10^{-6} M and 10^{-5}M) did not significantly affect Jv. ISO (10^{-4}M) resulted in irreversible cellular damage. ADH (2.5 μU/ml) resulted in a Jv of 0.6 ± 0.1 nl/min/mm; Jv did not change with the addition of ISO (10^{-6} or 10^{-5} M). ISO alone, in the absence of ADH, did not change Jv from zero at bath temperature of 25 and 37°C. In studies where PE was added, Jv in the presence of ADH (100 μU/ml) averaged 1.14 ±0.12 nl/min/mm. The addition of PE (10^{-6} and 10^{-5}M) decreased Jv to 0.83 ± 0.3 ($p < 0.001$) and 0.53 ± 0.05 ($p < 0.001$) nl/min/mm, respectively. In another group of tubules, Jv in the presence of ADH (100 μU/ml) and PE (10^{-5}M) was 0.32 ± 0.03 nl/min/mm. The addition of PH (10^{-6} or 10^{-5}M) resulted in a significant increase in Jv to 0.82 ±0.17 and 0.89 ± 0.06 nl/min/mm respectively. We conclude that 1) Beta-adrenergic agonists do not modulate ADH-mediated water absorption in the CCT. 2) By contrast, alpha adrenergic agonists inhibit ADH-mediated water absorption, an effect that can be blocked by specific alpha adrenergic antagonists.

ADENOSINE STIMULATES C-AMP ACCUMULATION AND SODIUM TRANSPORT IN EPITHELIA FORMED BY A6 (TOAD KIDNEY) CELLS IN CULTURE. M.A. Lang,*, J.N. Forrest, A.S. Preston* and J.S. Handler, NIH/NHLBI, Bethesda, MD, Boston Univ. Sch. of Med., Boston, MA and Yale Univ. Sch. of Med., New Haven, CN.

Adenosine (ADO) has been shown to alter cAMP accumulation and cAMP dependent events in a number of cells and tissues. The A6 line of *Xenopus laevis* kidney cells form an epithelium with high transepithelial resistance (10k Ωcm^2) when grown on millipore filters. Transepithelial potentials range between 25-50 mV. Na^+ transport is equivalent to short circuit current (I_{sc}). The response to ADO satisfies four criteria for external ribose specific stimulatory receptors: (a) Both ADO and 2-chloroadenosine (2Cl-ADO), the non-metabolizable analog, stimulate I_{sc} in a concentration dependent manner above 10^{-8}M; stimulation is 5-10 fold at 10^{-5}M. (b) The relative (to basal) increase in I_{sc} with 10^{-6}M 2Cl-ADO or ADO (2.20 ±0.16, SE) is inhibited by 10^{-4}M theophylline (1.16 ±0.03), an antagonist of ribose specific ADO receptors, to levels seen with theophylline alone (1.23 ±0.05). (c) 2Cl-ADO stimulates cAMP accumulation in the epithelium. (d) The relative potency of ribose specific analogs is similar to reports for activation of adenylate cyclase in other tisues, i.e., adenosine 5'-ethylcarboximide shows 50% greater relative stimulation of I_{sc} than either 2Cl-ADO or N^6-(phenylisopropyl)-adenosine at both 10^{-6}M ($p<0.025$) and 10^{-5}M ($p<0.01$); the latter two analogs are of equal potency. 10^{-5}M amiloride, an inhibitor of conductive Na^+ channels, inhibits both basal and 2Cl-ADO stimulated I_{sc} by >90%. The results indicate stimulatory receptors for adenosine in this kidney derived epithelium.

EFFECTS OF CHLORPROPAMIDE (CPMD) ON THE URINARY CONCENTRATING SYSTEM. E. Kusano,* J. L. Braun-Werness,* and T. P. Dousa (Intr. D. N. Wochos). Mayo Clinic, Rochester, Minnesota.

The mechanism whereby CPMD enhances the antidiuretic response to vasopressin (VP) is not yet known. Treatment with CPMD did not potentiate VP-dependent cAMP metabolism in collecting tubules. Therefore, we examined the CPMD effect on cortico-medullary gradient of solutes in kidneys of rats with hypothalamic diabetes insipidus (Brattleboro homozygotes). Rats were treated (s.c.) for 7 days with 20 mg CPMD/100 gr b. wt. and compared with controls. CPMD-treated rats did not differ from controls in urine flow, urine osmolality (Uosm) or in Na^+, K^+ and total solute excretion. In contrast, cortico-medullary gradient of total solutes and of Na^+ increased in CPMD-treated rats.

	Solutes (mOsmol/kg H_2O)		Na^+ (mEq/kg H_2O)	
	Controls	CPMD	Controls	CPMD
Cortex:	297±15	293±18	61±1	66± 6
Medulla:	295±10	408±15*	70±3	107± 7*
Papilla:	383±17	595±48*	114±9	187±25*
Urine (U_{osm}):	183±19	170± 7		

* significantly different from controls (P<0.025).

Rate of ^{14}C-lactate oxidation, putatively linked to NaCl transport, in medullary thick ascending limbs of Henle's loop (MAL) dissected from CPMD-treated rats was markedly increased (Δ%+113±33; P<0.05) compared to controls. These results indicate that CPMD enhances the antidiuretic effect of VP by increasing collecting tubule lumen-to-interstitium osmotic gradient, which is a prerequisite for the antidiuretic response. It is suggested that increased osmolality is due to the enhanced rate of NaCl reabsorption in MAL.

TRANSEPITHELIAL AND RATIO OF LUMINAL TO BASOLATERAL ELECTRICAL RESISTANCE OF RABBIT PROXIMAL CONVOLUTED TUBULE PERFUSED IN VITRO. Jean-Yves Lapointe*, Raynald Laprade*, and Jean Cardinal. Centre de Recherches, Hôpital Maisonneuve-Rosemont, and Université de Montréal, Montreal, Quebec.

Determination of the transepithelial electrical resistance (R_T) is of prime importance to the understanding of potential differences and electrolytes transport in a leaky epithelium such as the proximal convoluted tubule (PCT) since R_T reflects the magnitude of the paracellular solute fluxes. A new technique using a double-barrelled perfusion pipette has been developped in order to measure R_T in isolated rabbit PCT. Using the cable theory, the technique has been tested successfully: the transepithelial resistance did not change with tubule length and the measured core resistance of the lumen (R_C) varied normally with the lumen diameter and the perfusate resistivity. In control solutions, PCT's have a linear I-V relationship from -300 nA to +300 nA and a mean R_T of 1050 ± 400 Ω cm (n = 28) in agreement with the only yet published value of Lutz et al. (1973). Bath proteins and important variations in transtubular hydrostatic pressure had no significant effect on R_T, while R_T was not systematically related to the sodium to chloride permeability ratio (P_{Na}/P_{Cl}) (n = 16). Normal perfusate solution minus 50 mM NaCl increases R_T only from the luminal side of the tubule. Finally, the ratio of luminal to basolateral cell membrane resistance (R_L/R_{BL}) was determined with intracellular microelectrodes and was found to vary between 2 and 6 in control solutions containing proteins.

THE LENGTH OF THE SINGLE-FILE WATER CHANNEL IN TOAD URINARY BLADDER. Sherman D. Levine, Monica Jacoby*, and Alan Finkelstein*. Albert Einstein Coll of Medicine, Bronx New York.

Vasopressin (VP) and cyclic AMP (cAMP) increase water flow across the toad urinary bladder by inserting water transporting structures into the granular cell's luminal membrane (LM). We determined the ratio of osmotic water permeability (Pf) to diffusional water permeability (Pd) in the LM of the stimulated bladder, since Pf/Pd equals the number of water molecules in a single-file channel. To determine the true Pd of the luminal membrane (LM) itself, it is necessary to correct for the resistance to diffusion offered by series barriers (SB) in the bathing media and the tissue itself. We did this as follows: 1) Pf and Pd were measured simultaneously in well stirred flow chambers after stimulation by low levels of 8Br-cAMP, which stimulates water and not urea transport, thus opening water channels only. 2) SB in each tissue was measured by either stimulating the tissue maximally with VP and assuming the SB to be totally rate limiting for diffusion or by exposing it to amphotericin B, and calculating the SB required to correct the Pf/Pd of the amphotericin channel to its known value of 3. The SB's calcuated by these two methods were identical, and equivalent to an unstirred layer of 470±31μ (n=19) and 468±21μ (n=12) respectively. 3) Only tissues in which the SB made up less than half the total resistance to diffusion were considered. The LM Pf/Pd for the low dose 8Br-cAMP treated tissues was 11.4±1.5, suggesting that each water transport channel contains 11-12 water molecules in single file.

FUNCTIONAL RESPONSE OF THE CONTRALATERAL KIDNEY (CK) TO ACUTE UNILATERAL NEPHRECTOMY (AUN): REQUIREMENT FOR INTACT PITUITARY FUNCTION. S-Y.Lin*, Y-N Chou*, E. Wiedemann*, and M.H. Humphreys, Divisions of Nephrology and Endocrinology, San Francisco General Hospital, San Francisco, CA.

Endogenous opioids participate in the increase in sodium (UNaV) and potassium (U_KV) excretion by the CK after AUN. To determine if pituitary or adrenal opioids mediate this response, we carried out AUN in normal rats, hypophysectomized rats, (hypox), normal rats pretreated with a dose of dexamethasone (dex) known to suppress release of endorphin from the pituitary (2 mg/kg IP 3d), and rats with bilateral adrenalectomy (adr'ex). The last three groups also received a constant infusion of dex (100 ug/kg/hr) during the experiment.

	UNaV, neq/min		U_KV, neq/min	
	Control	p AUN	Control	p AUN
Normal(N=12)	953± 531	2386± 941*	1342±421	2519±706*
Hypox(N=8)	1477± 734	1041± 558	1143±582	1325±798
Dex (N=7)	2066±1093	1399±1245	1511±610	1281±478
Adrex(N=7)	1587± 957	4030±2035**	1423±550	3048±473*

*p<.001 vs. control; **p< .05 vs control

In normal rats, circulating plasma endorphins, measured by radioimmunoassay, did not increase after AUN.

These results demonstrate that the increased cation excretion by the CK after AUN is dependent on a intact pituitary gland, and that suppression of pituitary hormone release by pretreatment with dex also blocks the excretory response of the CK. The role of the pituitary in the response to AUN does not involve increased release of endorphins into the circulation but rather some other neuroendocrine function influenced by dex pretreatment.

RADIATION INACTIVATION OF BRUSH BORDER MEMBRANE TRANSPORT FUNCTIONS. J.T. Lin*, Chan Y. Jung*+ and R. Kinne. Albert Einstein College of Medicine, Bronx, N.Y. +Veterans Administration Medical Center and State University of New York, Buffalo, N.Y.

In order to investigate the target size of sodium cotransport systems, brush border membrane vesicles were isolated from calf kidney cortex and irradiated at -60°C with a van de Graff generator producing high voltage electrons. Sodium dependent and sodium independent D-glucose, and L-alanine and transport were investigated in tracer exchange experiments after various levels of irradiation. Irradiation with 1.25 Mrad decreased sodium-D-glucose cotransport by about 70%, sodium-L-alanine cotransport was completely abolished. At the same time sodium independent uptake was not affected. In contrast high affinity binding of phlorizin-determined at 0.6×10^{-6} M by a rapid filtration method - decreased only slightly with irradiation energies up to 2.9 Mrad. Preliminary studies indicate that in this energy range irradiation changes mainly the affinity of the phlorizin binding site but not the number of sites. These results suggest that the target size of the system mediating sodium-D-glucose cotransport is larger than that of the phlorizin binding site. They could be explained by the assumption that the sodium-D-glucose cotransport system contains at least two subunits, one responsible for D-glucose (or phlorizin) binding and another involved in sodium binding and translocation. A similar composition with an even higher number of sodium binding subunits might be postulated for the L-alanine cotransport systems.

EFFECT OF POTASSIUM DEPLETION (KD) ON LOOP FUNCTION. Robert G. Luke, Beverly B. Booker, * John H. Galla. University of Alabama in Birmingham, Nephrology Research and Training Center, Birmingham, Alabama.

We have suggested that impaired chloride reabsorption in the loop segment (LS) may contribute to a KD-induced concentrating defect and, via the macula densa signal, hypereninemia (KI 21:14, 1982). To determine the effect of KD on LS function microperfusion was performed at 22 nl/min with an artificial late proximal tubule perfusate (Cl 130 meq/L) from that site to the early distal tubule (ED) in control and KD rats. KD was produced by a low K diet and administration of DOC 2 mg on days 2 and 3; studies were performed at 6-9 days and during infusion of 0.08 M $NaHCO_3$ and 0.07 M NaCl. Controls (CON_1) ingested the same diet with the addition of neutral K phosphate and also received DOC. An additional control group (CON_2) was studied with aortic constriction to approximate the reduced renal perfusion pressure in KD. Muscle K was 36 ± 3 vs 24 ± 1 in KD, p<0.001; body weights were not different (CON 233 ± 4, n = 8; KD 240 ± 11 n = 11).

	Fluid (%) Absorption	Cl (%) Absorption	ED [Cl] (meq/L)	BP (mm/Hg)
CON_1	45 ± 3	76 ± 2	55 ± 4	122 ± 5
CON_2	40 ± 3	81 ± 5	41 ± 11	98 ± 4*
KD	42 ± 3	66 ± 3*	70 ± 5*	102 ± 4*

*p <0.05 vs CON_1

Plasma Cl was not different among groups.

We conclude that, unrelated to load or renal perfusion pressure, KD reduces reabsorptive Cl transport in the LS which may explain hypereninemia and contribute to the concentrating defect by reducing medullary interstitial [NaCl].

EVALUATION OF THE ROLE OF Na:Ca EXCHANGE IN THE CONTROL OF CYTOSOLIC FREE Ca IN PROXIMAL RENAL TUBULES. Lazaro J. Mandel and Elizabeth Murphy. Physiology Department, Duke University Medical Center, Durham, North Carolina 27710.

Cytosolic free Ca was measured in a suspension of proximal rabbit kidney tubules using the null point method (Murphy and Mandel, AJP:242,C124,1982). To measure the null point, tubules were normally bathed in a nominally Ca-free saline*. Addition of 10^{-4}M ouabain in this medium did not change the cytosolic free calcium concentration ($0.22\pm0.05\mu M$, control vs. $0.19\pm0.05\mu M$, ouabain, n=7). Ouabain (10^{-4}M) added to cells in Ca-containing (1mM) saline caused no change in total cellular Ca content in 20 min (95±5% of control) nor did it alter the cytosolic free Ca concentration ($0.18\pm0.11\mu M$, n=4). Other studies have used Na replacements to investigate Na:Ca exchange. We replaced 100mM NaCl by either 100mM KCl, choline-Cl, or tetramethylammonium-Cl (TMA). The null points under these conditions were, respectively, $0.36\pm0.06\mu M$ (control), $0.52\pm0.05\mu M$($P>0.05$), $0.72\pm0.08\mu M$($P=0.01$), and $0.94\pm0.10\mu M$($P<0.005$). All of these maneuvers would be expected to inhibit Na:Ca exchange. However, only the choline and TMA replacements raised cytosolic free Ca. Preliminary experiments suggest that choline and TMA increase the cytosolic free Ca by enhancing the rate of Ca efflux from the mitochondria without affecting the Ca influx. These results suggest that Na:Ca exchange does not play an important role in the regulation of cytosolic free Ca concentration in these tubules.

*Saline contained:(mM):NaCl(105), $NaHCO_3$(25), NaH_2PO_4(4), KCl(5), $MgSO_4$(1), D-glucose(5), lactate(4), alanine(1), butyrate(1), Dextran(.6%), HEPES(10), and Arzenazo III (40μM).

REGULATION OF LUMINAL MEMBRANE STRUCTURE BY OSMOTIC WATER FLOW DURING ADH STIMULATION AND WASHOUT IN TOAD BLADDER. J.Muller* and W.A. Kachadorian. Office of Biologics-FDA, and NIH, Bethesda, MD.

Intramembranous particle aggregates (presumed sites for water flow) which appear in the luminal membrane consequent to ADH treatment are derived from cytoplasmic membrane structures which fuse with the luminal membrane. Bladder stimulation in the absence of an osmotic gradient leads to ~2X as many aggregates and ~3X as many fusion sites as ADH treatment in the presence of a gradient (175 mOsm). We now report that ADH-related osmotic water flow itself can dramatically influence the occurrence of luminal membrane aggregates and fusion sites in the same bladder during uninterrupted stimulation as well as during hormone washout. Bladders (n=6) treated with ADH for 1 hr without a gradient and then for 1 hr with a gradient had ~1/3 as many aggregates and fusion sites as paired bladders treated for 2 hr without a gradient. Conversely, bladders treated with ADH for 1 hr with a gradient and then for 1 hr without a gradient had ~2X as many aggregates and fusion sites as bladders treated for 2 hr with a gradient. In other paired bladders (n=6) that were first stimulated with ADH in the absence of a gradient, aggregates and fusion sites persisted at near maximum levels for 15 min of washout in the absence of a gradient, whereas washout in the presence of a gradient for the same time reduced aggregate and fusion site frequencies almost to base-line levels. The degree of cell hydration and resulting changes in the concentration of cytoplasmic intermediates may underlie the regulation of luminal membrane water permeability.

EFFECTS OF SITS IN LUMEN AND BATH ON URATE TRANSPORT BY ISOLATED, PERFUSED SNAKE RENAL TUBULES. Suniti K. Mukherjee and William H. Dantzler. Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona.

Net secretion of urate (J_{Urate}) by isolated, perfused snake (Thamnophis spp.) proximal renal tubules occurs by transport into cells at peritubular membrane against electrochemical gradient followed by movement down electrochemical gradient into tubule lumen. Previous data provided no evidence for any form of mediated urate transport from cells to lumen. However, since effects of 4-acetamido-4'-isothiocyano-2,2'-disulfonic stilbene (SITS) in lumen and bath on p-aminohippurate (PAH) transport supported concept of separate mediated transport steps at luminal and peritubular membranes, we examined effects of SITS in lumen and bath on urate transport. SITS (10^{-4} mol/l) in lumen had no effect on J_{Urate} or net fluid absorption (J_V). These data do not support concept of mediated transport step for urate from cells to lumen and are compatible with simple passive diffusion. However, SITS (10^{-4} mol/l) in bath inhibited J_{Urate} by about 60% without affecting J_V. At time of maximum depression of J_{Urate} with SITS in bath, intracellular urate concentration ($[Urate]_{cell}$) was significantly ($p<0.001$) increased from control and apparent permeability of luminal membrane to urate (P_L) was significantly ($p<0.001$) decreased from control. Effects of SITS were completely reversible when it was removed from bath, suggesting that they result from electrostatic binding, not covalent binding, of SITS to membrane. These data suggest that SITS in bath may inhibit J_{Urate} by reducing efflux of urate from the tubule cells.

TRANSEPITHELIAL VOLTAGE IN THE REPTILIAN-AND MAMMALIAN-TYPES NEPHRONS FROM JAPANESE QUAIL. H. Nishimura, M. Imai,* and M. Ogawa.* Univ. Tennessee Ctr. Hlth. Sci., Dept. Physiol., Memphis, TN, and Jichi Med. Sch., Tochigi, Japan.

Bird kidneys consist of short superficial reptilian-type (RT) nephrons which lack a loop of Henle, and long mammalian-type (MT) nephrons that possess medullary loop structure. Isolated segments of renal tubules from the Japanese quail, Coturnix coturnix, were perfused in vitro to characterize the transepithelial voltage (Vt). The thick ascending limb (TAL) of the MT nephron, showed Vt positive in the lumen (+9.4±0.7 mV, n=32). VT decreased with increases in hydrostatic perfusion pressure. Furosemide added to the lumen (10^{-5}M), and Na cyanide and ouabain added to the bath (10^{-3}M) reversibly reduced Vt. Removal of Cl (replaced by cyclamate) or Na (replaced by choline) in the perfusate and the bath decreased Vt (mV) of the TAL from +10.3±3.0 to −0.3±0.3 n=5, $P<0.05$, and from +7.4±1.5 to +1.2±0.2 n=8, $P<0.01$, respectively. The distal tubule of the RT nephron showed two types of Vt: a lumen-negative Vt (−2.7±1.0 mV, n=5) in the middle to late segment, and a lumen-positive Vt (+4.9±2.0 mV, n=5) in the early segment, where the parent glomerulus attaches firmly. Both Vts were reversibly reduced by ouabain and sodium cyanide added to the bath. These results suggest that in quails: 1) The TAL of the MT nephron resembles that of the mammalian kidney with both Na and Cl required for generation of luminal positivity. 2) The distal tubule of the RT nephron appears heterogenous in which the early segment may have evolved as a proto-type of the TAL.

THE QUANTITATIVE CONTRIBUTIONS OF THE NA^+/H^+ ANTIPORTER AND NA^+ DIFFUSIVE PATHWAY TO TOTAL NA^+ FLUX ACROSS THE LUMINAL MEMBRANE OF THE PROXIMAL TUBULE IN THE ABSENCE OF ORGANIC SOLUTES. Edward P. Nord*, Ernest M. Wright*, and Leon G. Fine. Division of Nephrology and Department of Physiology, UCLA School of Medicine, Los Angeles, California.

In the absence of organic solutes, Na^+ transfer across the luminal membrane of the rabbit renal proximal tubule has been reported to be via an electroneutral Na^+/H^+ antiporter. Studies using a pH-sensitive dye probe suggest the presence of a Na^+ diffusive pathway that parallels the antiporter. Limitations in experimental methods have precluded quantitation of the role of each pathway. The present experiments examine the kinetics of Na^+ transport over a Na^+ concentration range of 1.0-200 mM at different pH conditions. Brush border membrane vesicles were prepared from rabbit renal cortices by the Ca^+ precipitation method, and ^{22}Na transport assayed by a rapid filtration technique. Kinetics of Na^+ transport were:

pH (in)	pH (out)	K_t (mM)	J_{max} (nmol/mg·min)	Perm Coef (µl/mg·min)
7.5	7.5	37	50	0.40
6.0	6.0	34	57	0.41
6.0	7.5	6	53	0.46

We conclude that, in the absence of organic solutes, Na^+ uptake by the luminal membrane is via two pathways - a Na^+/H^+ antiporter, and a Na^+ diffusive pathway. An opposing H^+ ion gradient enhances the affinity of the antiporter for Na^+ (lowers the K_t) by 6-fold, whereas the maximal rate of uptake (J_{max}) is unaffected. At physiological Na^+ concentrations, diffusion accounts for about 50% of total Na^+ transfer.

AMILORIDE UPTAKE INTO RABBIT RENAL EPITHELIAL CELLS: A FLUORESCENT STUDY. Roger G. O'Neil* and William P. Dubinsky.* Univ. of Texas, Med. Sch., Dept. of Physiol. & Cell Biol., Houston, TX. (intr. by P.A. Friedman).

Uptake of amiloride (a fluorescent molecule) into renal epithelial cells was evaluated using microscopic epifluorescent techniques (excitation, 330-380 nm; emission barrier, 420 nm). Selected tubule segments were isolated and bathed at room temp. in a NaCl Ringer, pH 7.4. From photomicroscopic observation of non-perfused tubules amiloride uptake into cells could be detected readily above the low-level autofluorescence in the proximal convoluted tubule, proximal straight tubule (PST), cortical thick ascending limb, and cortical collecting tubule (CCT) when bathed in a solution containing from 0.1 to 1.0 mM amiloride. Similar results were obtained when PST and CCT were perfused with an identical amiloride containing solution. Furthermore, when the relative fluorescence of the perfused tubules was quantified via a photometer connected to the camera port of the microscope, amiloride uptake into the PST and CCT was shown to increase in a concentration-dependent manner (0.01 to 1.0 mM amiloride in the perfusate), but only if the tubule cells were viable as evidenced by exclusion of the stain trypan blue. It is concluded that renal cells can take up amiloride and, hence, intracellular affects of this drug, such as the recently identified inhibition of carbonic anhydrase (Dubinsky and O'Neil, unpublished), must be evaluated.

EFFECTS OF GENTAMICIN (G) ON VASOPRESSIN (V) STIMULATED WATER FLOW (WF). Maria Noya*, Christos P. Carvounis, Georgia Carvounis* and Mark Darrow*. Div. Nephrology, SUNY-Stony Brook, New York.

The effects of G on VWF in the toad bladder were examined. Appropriate care was taken to maintain bath pH at 7.4 since G is known to alter pH. 1mM G on the serosal side moderately increased VWF (V= 2mU/ml) compared to paired control hemibladder (21.9±2.9 vs 17.6±3.3, n=6, $p<0.05$). A similar effect was noted on cAMP stimulated WF (6.7±0.8 vs 4.9±0.7, n=5, $p<0.01$). G mucosally produced a variable response on VWF. In experiments where control hemibladders responded significantly to 2mU/ml V (WF>20µl/min) G decreased VWF (28.0±3.0 vs 34.0±2.3, n=5, $p<0.05$). In experiments where the effect of V was less pronounced (WF<20µl/min) G significantly increased VWF (27.5±2.5 vs 14.3± 1.2, n=13, $p<0.001$). In both sets of experiments the effects were reversible, in that following wash out and restimulation with V, both hemibladders responded equally (18.8±5.2 vs 18.2±4.2 and 15.1±2.9 vs 16.1±2.9, respectively. Since WF in response to V occurs through both small and large pores (C.Carvounis et al., J. Membrane Biol., 1979) one could explain the variability observed if G has a dual effect simultaneously increasing small pores and inhibiting large pores. As urea permeates only through the large pores one would expect that the reflection coefficient (σ) of urea during V stimulation would increase in the presence of G. Indeed we found that σ_{urea} is substantially increased in the presence of G (1.0±0.1 vs 0.35±0.2, G vs control, n=8, $p<0.001$), a finding consistent with our hypothesis.

EFFECT OF PROTAMINE SULFATE (PS) ON THE CELLULAR PATHWAY IN NECTURUS GALLBLADDER. C.E. Palant* and C.J. Bentzel, Dept. of Medicine, SUNY/Buffalo and VA Medical Center, Buffalo, NY.

Cationic proteins such as PS (pI>11) increases depth of the tight junctional intramembranous meshwork of strands, while increasing transepithelial resistance and spontaneous potential difference in Necturus gallbladder. We now explore the effects of PS on cell membrane ionic permeability. Gallbladders were mounted in an Ussing-type chamber, bathed in Amphibian Ringers (K^+=6.0 mM) and transmucosal membrane potential difference (Vmc) and voltage divider ratio (R_m/R_s) were determined with 3M KCl intracellular microelectrodes. PS (20 to 50 µg/ml) dissolved in the mucosal bath rapidly hyperpolarized Vmc from -52± 1.0 to -62±1.0 mV. R_m/R_s increased from 0.76±0.08 to 1.55±0.18 ($p<0.01$, n=4). In 5 tissues bathed on both sides with high K^+ Ringers (K^+=48 mM) PS hyperpolarized Vmc from -24±8.6 to -32±9.8 mV ($p<0.001$). Even in high K^+ media, PS increased R_m/R_s suggesting that membrane permeability to K^+ decreased. Cl^- substitution with Na gluconate Ringers (in both baths) abolished the effects of PS on Vmc but not on R_m/R_s. Cell membrane effects were reversed when PS was neutralized with heparin.

Conclusions: PS acts both on non-junctional and junctional domains of the apical membrane. The increase in R_m/R_s suggests that PS decreases membrane permeability to cations. The Cl^- dependence of the PS effect on Vmc can be explained by increased permeability to anions. Thus, the coupled anion-cation transport entry step across the mucosal membrane may have been disassociated by PS.

TUBULAR ABSORPTION OF ALBUMIN (ALB) IN THE ISOLATED PERFUSED PROXIMAL CONVOLUTED TUBULE (PCT) OF THE RABBIT: INFLUENCE OF CHARGE. C.H. Park* and T. Maack, Cornell Univ. Med. Coll., Dept. of Physiol., New York, N.Y.

Molecular determinants of tubular absorption of proteins were further studied by comparing tubular uptake rates of native anionic $ALB^{\ominus}$ and derivatized cationic $ALB^{\oplus}$. ALB was cationized by amination of carboxyl groups to a pI of 8.4. $ALB^{\ominus}$ and $ALB^{\oplus}$ were labeled by reductive methylation with ^3H and tubular absorption rates (J_{ALB}) were determined in PCTs as previously described (Park & Maack, ASN meeting, 1981). Perfusate (P) concentrations of $ALB^{\ominus}$ or $ALB^{\oplus}$ varied between 0.1 and 1.0 mg/ml and perfusion rate was 11.9 ± 0.2 nl/min (n=29). At P_{ALB} =0.1 mg/ml, $J_{ALB^{\oplus}}$ was 0.25 ± 0.04 ng/min.mm tubule (n=7), a value 5 fold greater ($p<0.001$) than the corresponding $J_{ALB^{\ominus}}$ of 0.05 ± 0.005 ng/min.mm (n=6). At P_{ALB} of 0.1 mg/ml fluid reabsorption (J_v) of PCTs perfused with $ALB^{\oplus}$ and $ALB^{\ominus}$ were not significantly different (1.15 ± 0.2 vs 1.02 ± 0.12 nl/min.mm, respectively). At higher P_{ALB}, $ALB^{\oplus}$ but not $ALB^{\ominus}$ markedly inhibited J_v to 0.52 ± 0.08 ($P_{ALB^{\oplus}}$=0.4 mg/ml, n=7) and 0.38 ± 0.09 nl/min.mm ($P_{ALB^{\oplus}}$=1.0 mg/ml, n=6). At the P_{ALB} of 1.0 mg/ml $J_{ALB^{\oplus}}$ was smaller than the corresponding $J_{ALB^{\ominus}}$ (0.64 ± 0.12 ng/min.mm, n=6 vs 1.63 ± 0.32 ng/min.mm, n=3, respectively $p<0.01$). Therefore, high tubular fluid concentrations of polycationic $ALB^{\oplus}$ but not native polyanionic $ALB^{\ominus}$ cause marked impairment of proximal tubular function. At loads nearer the physiological range, cationization of ALB greatly enhances its tubular absorption rate, probably by a more efficient binding of the protein to anionic sites at the luminal membrane.

ALTERED RENAL BRUSH BORDER MEMBRANE (BBM) ALANINE (ALA) TRANSPORT IN EXPERIMENTAL DIABETES. Ralph Rabkin and Paula Hirayama.* Dept. Med., Stanford Univ. and VA Medical Center, Palo Alto, CA.

Diabetes is associated with altered amino acid metabolism including increased intestinal amino acid transport. As renal and intestinal BBM have several functional similarities, we examined the effect of diabetes on renal BBM ALA transport. Diabetes was produced with streptozotocin and 18 days later, BBM were isolated from normal rats, untreated diabetic and insulin treated diabetic rats. Under initial 100mM Na gradient conditions, maximum ALA uptake at peak of overshoot, was significantly higher in BBM of untreated diabetics as compared to normals (264±4 vs. 203±7 pm/mg protein). Insulin treatment of diabetics abolished this difference, thus excluding a direct drug effect. Equilibrium uptake values did not differ between groups. Initial rates of ALA transport were then measured with the membrane potential clamped by pre-equilibration with KCL and valinomycin (Wright et al, J. Biol. Chem. 257:1773, 1982). In the presence of a 100mM Na gradient, the initial rate was significantly reduced in the untreated diabetics as compared to normals (21±5 vs. 46±8 pm/mg protein/2 sec.), suggesting a decrease in the ALA transporter. Conclusion: Diabetes is associated with altered BBM ALA transport; in the presence of a Na gradient, maximum uptake is increased although there appears to be a decrease in the ALA transporter. The increase in maximum uptake may be explained by an ability of the diabetic BBM to maintain a higher driving force for active ALA transport, a phenomenon which could potentially be due to altered Na permeability.

AN IN VIVO STUDY OF THICK ASCENDING LIMB (TAL) TRANSPORT IN POTASSIUM DEPLETED (K-DEP) RATS. L.N. Peterson*, H.-U. Gutsche*, and D.Z. Levine. Departments of Pediatrics, Medicine and Physiology, University of Ottawa, Ottawa General Hospital, Ottawa, Ontario, Canada.

In K-depletion there is impairment of urine concentrating and diluting ability which depends on normal function of the TAL. TAL transport was assessed in vivo in control rats (n=7), K-Dep: 14 day diet (n=7), and K-Rep: K-Dep given 2.5 mEq KCl by gavage (n=3). Loops were perfused with 110 mM NaCl and 80 mM mannitol. Using a micro stop-flow technique, 3-15 conductivity probe measurements in 48 nephrons were made at 10, 30, 45 and 60 sec on fluid emerging from the TAL. The conductivity at each stop-flow interval was divided by the initial conductivity at 0 sec to obtain the fraction of perfusate concentration remaining (F).

	Minimum (60 sec) Values	
	F60	Corresponding [Na]
Control	.19±.025	18.3±2.73
K-Dep	.37±.031*	36.5±2.97*
K-Rep	.10±.025*	9.2±1.71*

*$p<.05$ compared to control

Thus, conductivity of the tubular fluid was strikingly elevated at 60 sec (and at other time intervals) in K-Dep rats and the minimum value, F60 correlated significantly ($r=.91$, $p<.001$, n=17) with plasma [K]. Further, after acute administration of KCl, conductivity was within the range of normal. We conclude that K-Dep causes an impairment in TAL transport which correlates with the severity of the deficit and is reversed when K stores are repleted.

CATION FLUXES AND GROWTH REGULATION IN AN EPITHELIAL CELL LINE(MDCK). Vivian M. Reznik‡ Stanley A. Mendoza, Univ. of California, San Diego, School of Medicine, La Jolla, California.

We have developed a technique to reversibly arrest the growth of an epithelial canine kidney line (MDCK). Over a period of 3 days, serum is sequentially removed from the media of subconfluent MDCK cells. They are demonstrably quiescent by autoradiography after an additional 24 hours in serum free media. Addition of fresh serum produces DNA synthesis after an 18 hour lag period. These cells then grow to confluency and retain their transport capacities as seen by the formation of "domes".

This system allows for measurement of monovalent ion fluxes and its relationship to growth regulation. Addition of fresh serum to quiescent MDCK cells produces an increase in ouabain sensitive ^{86}Rb uptake, a measure of Na-K pump activity. This stimulation is mediated by increased uptake of Na into the cells as demonstrated by measurements of intracellular Na-K in the presence and absence of ouabain. Serum stimulated DNA synthesis is blocked by the addition of ouabain in doses that inhibit the Na-K pump. It appears that serum stimulates growth by increasing the amount of intracellular Na available to the Na-K pump. In other cell culture models, vasopressin has been shown to be mitogenic. Four to 6 hours after the addition of vasopressin to quiescent MDCK cells, there is an increase in ouabain sensitive ^{86}Rb uptake. There is no such effect on ouabain insentitive ^{86}Rb uptake. As in other cells systems, monovalent ions appear to mediate early events in growth regulation of epithelial cells. Vasopressin may be mitogenic for epithelial cells.

SUCCINATE AND PROTEIN REQUIREMENT FOR SODIUM REABSORPTION BY RAT PAPILLARY COLLECTING DUCTS. Richard J. Roman and Claude Lechene. Med. Col. of Wis. Milwaukee, WI and Harvard Med. Sch., Boston, MA.

Several permeability properties of the collecting duct have been measured, *in vitro*, using the excised rat papilla, but it had not been possible to demonstrate sodium reabsorption in this preparation. We examined the experimental conditions which are necessary for collecting ducts to reabsorb sodium in the excised papilla. Excised rat papillae were incubated at 37°C in a Ringer-like solution containing glucose and 0.3% albumin. Collecting ducts (n=31) were perfused at 10-30 nl/min in the presence of a 100mM bath to lumen NaCl concentration gradient. Under these conditions, the collected to perfused fluid sodium concentration ratio averaged 1.8±0.2, indicating a bath to lumen sodium flux into the collecting duct. Despite this abnormal sodium influx, these tubules exhibited water reabsorption which was enhanced by ADH (200 μU/ml) and inhibited by PGE_2(10^{-5}M). Addition of sodium succinate (10mM) to the bath, that has been shown to increase papillary oxygen consumption, not only eliminated the bath to lumen sodium influx but produced reabsorption of sodium against a concentration gradient. Collected to perfused fluid sodium concentration ratio (C/P) in 22 tubules averaged .86±.06 ($p<.05$ from 1). Removal of albumin from the bath containing succinate again produced a bath to lumen sodium influx, the (C/P) Na ratio of 17 tubules averaged 1.3±.05. These results indicate that collecting ducts of excised papillae reabsorb sodium if supplied with succinate and protein, and that this preparation can be suitable to study papillary collecting duct function *in vitro*.

CL TRANSPORT IN RABBIT RENAL MICROVILLUS MEMBRANE VESICLES (MMV): EVIDENCE AGAINST CL-OH EXCHANGE. Julian Seifter*, Roy Knickelbein*, and Peter S. Aronson. Yale Sch. of Med., Depts. of Med. and Physiol., New Haven, CT.

The mechanism of Cl^- transport was studied in MMV isolated from the rabbit renal cortex. Inwardly directed K^+ gradients in the presence of the K^+ ionophore valinomycin (Val) enhanced 10mM ^{36}Cl uptake 2.5x, confirming a Cl^- conductive pathway (Warnock and Yee, JCI 67, 103, 1981). An inwardly directed H^+ gradient (pH_i 7.5, pH_o 6.0) stimulated 10mM Cl^- uptake 1.5x compared to pH_i = pH_o = 6.0. However, this H^+ gradient stimulation of Cl^- uptake appeared secondary to the H^+ diffusion potential rather than to Cl^--OH^- exchange, as it was abolished by Val and $K^+_i = K^+_o$. The same inwardly directed H^+ gradient inhibited 1mM ^{22}Na uptake 80% compared to $pH_i = pH_o = 6.0$, an effect unaltered by Val and K^+ indicating that Val had not dissipated the pH gradient. Additional evidence against Cl^- transport via anion exchange was the lack of significant inhibition of Cl^- uptake by 1mM DIDS, furosemide or probenecid; the failure of an inwardly directed Cl^- gradient to generate an inside-acid pH gradient as monitored by quenching of acridine orange fluorescence; and the lack of acceleration of ^{36}Cl efflux by external Cl^- as compared to gluconate when vesicles were short-circuited with the H^+ ionophore FCCP and $pH_o = pH_i$ (i.e., no Cl-Cl exchange). Finally, Cl^- influx was the same in the presence of inwardly directed gradients of Na, K, Cs, Li and Rb, arguing against Na-Cl cotransport.

These data confirm a conductive mode of Cl^- transport but fail to demonstrate a significant Cl-OH exchange pathway in rabbit renal MMV.

ORIGIN OF THE POSITIVE TRANSEPITHELIAL VOLTAGE IN THE SALAMANDER RENAL DILUTING SEGMENT. Henry Sackin and Emile L. Boulpaep. Depts. of Physiol., Yale Univ. Sch. of Med., New Haven, CT and Cornell Univ. Med. College, New York, N.Y.

The positive transepithelial voltage (V_3) of both the amphibian diluting segment and the mammalian thick ascending limb could result either from the difference in ionic diffusion potentials across luminal and basolateral membranes or directly from a diffusion potential across the tight junction. The relative contribution of these two voltage sources depends critically on the ratio of basolateral to paracellular resistance (R_1/R_3). The larger the value of R_1/R_3, the greater the fraction of the tight junction diffusion potential which contributes to the value of V_3. Earlier studies in the amphibian diluting segment have indicated that luminal application of 10^{-5}M furosemide blocks net ion transport and abolishes V_3. In six salamander diluting segments, inhibited by furosemide, addition of 2mM $BaCl_2$ to the lumen depolarized the basolateral potential (V_1) by 27.7± 3mV but produced a change in V_3 of only -0.5±0.6mV. Since transport has been inhibited by furosemide, luminal $BaCl_2$ is unlikely to affect the electrical properties of either the basolateral membrane or the paracellular space. The voltage changes after $BaCl_2$ should reflect only the effect of Ba on the properties of the luminal membrane. In this case, the ratio R_1/R_3 is simply equal to the negative ratio of the Ba induced voltage deflections $\Delta V_1/\Delta V_3$. The average R_1/R_3 value of 57±9 (n=6) requires that a significant fraction of the observed positive V_3 in the diluting segment arises from a diffusion potential across the tight junction.

EFFECTS OF AMBIENT IONIC ENVIRONMENT ON Na^+/GLUCOSE COTRANSPORT IN RABBIT RENAL BRUSH BORDER MEMBRANE VESICLES. David Shiuan* and Stephen Weinstein Division of Nephrology, SUNY, Stony Brook, N.Y.

The effects of changes in non-sodium ambient ionic environment on the Na^+- gradient dependent glucose transport in the brush border membrane vesicles were studied by varying the medium ionic strength symmetrically across the membrane. It was found that high concentrations of monovalent (Li^+, K^+, Cs^+, TMA^+) and divalent(Mg^{2+},Ca^{2+}) cation salts inhibit the Na^+-gradient dependent glucose uptake without affecting the Na^+-independent passive diffusion of D-glucose. The mode of the inhibition kinetics is most consistent with a competitive or mixed type inhibition. Inhibition of the Na^+-gradient dependent glucose efflux by high electrolyte concentration was also observed, indicating that changes in any existing Donnan potential can not explain the inhibitory effect of nonsodium salts on this process. The possible involvement of residual transmembrane potential was also examined and eliminated. The inhibition may occur when the negative charge on the membrane is screened by these cations resulting in a decrease of surface potential and sodium concentration near the membrane surface as predicted by Gouy-Chapman theory. A concomitant reduction of the negative membrane surface potential by these maneuvers was noted using the fluorescent probe TNS (sodium-2-p-toluidylnaphthalene-6-sulfonate). We conclude that the mechanism of inhibition by these salts is primarily due directly or indirectly to the modification of membrane surface potential. This inhibitory process may be important in sodium and glucose reabsorption in vivo.

AMILORIDE DIRECTLY INHIBITS THE Na,K-ATPase OF THE RABBIT PROXIMAL TUBULE. S.P.Soltoff* and L.J. Mandel. Duke Univ. Dept. of Physiology and Machu Picchu Research Foundation, Durham, NC

Amiloride (A), a K^+-sparing diuretic, inhibits Na entry across the apical membrane of tight epithelia at low (uM) concs. At higher conc. (mM) it inhibits Na-H exchange in proximal membrane vesicles. We now report that A directly inhibits the Na pump activity of intact tubules in suspension and the Na,K-ATPase enzyme activity of proximal mem. fragments. Na pump activity was determined by measuring the ouabain-sensitive oxygen consumption (QO_2). All expts. were performed at 37 C. We observed that A inhibited by about 50% the steady-state ouabain-sensitive QO_2 of intact tubules exposed to 15-150 mM extracellular Na. The following results indicate that A directly inhibited the Na pump activity and not the Na entry: (1)Cellular Na increasedand K decreased after A; (2)A inhibited the ouabain-sensitive nystatin-stimulated QO_2 by about 25% (Nys. is an ionophore which equilibrates the intra- and extracellular Na, thus removing Na entry as a rate-limiting barrier to transport). Since these results suggested that A directly inhibited the Na,K-ATPase of the intact tubules in suspension, we measured Na,K-ATPase enzyme activity in lysed mem. fragment of our prox. tubule preparation. A inhibited this activity in a dose-dependent manner. At 30mM Na/120mM K and 140mM Na/10mM K, the inhibition by 0.5 mM A was 18±2% and 11±1%, respectively, and the inhibition by 1 mM A was 30± 3% and 20±2%,respectively(n=3). Conclusion: Under conditions prevailing in the in-vivo kidney, A at high concentrations may inhibit net Na transport in the proximal tubule by a direct effect on the Na,K-ATPase.

ROLE OF THE ADRENERGIC NERVOUS SYSTEM IN HYPERKALEMIA. R.H. Sterns, J.L. Izzo, Jr., D. Mahany*, and K. Turgeon*, Rochester General Hosp., Univ. of Rochester, Rochester, N.Y.

Beta adrenergic blockade markedly impairs the cellular uptake of acute K+ loads, but in normokalemic subjects, the effect on basal plasma [K+] is minimal. To assess the effect of β- blockade in hyperkalemia, awake, anephric dogs were studied. Immediately after nephrectomy, dogs were given 1 mEq/kg of either KHCO3 (n=4) or NaHCO3 (n=5). The next day, plasma [K+] was stable, ranging from 4.7 to 5.3 mEq/L in KHCO3 dogs and 3.6 to 4.7 mEq/L in NaHCO3 dogs. After a 1-hr control period, propranalol was infused (0.3 mg/kg loading, 0.005 mg/kg/min sustaining). In each KHCO3 dog, plasma [K+] increased markedly (2.6 ± 0.4 mEq/L; mean ± SEM), peaking in 30 to 70 min. and then (in 3 dogs) decreased back towards baseline and peaked again over the course of 80 to 160 min. This large oscillation in plasma [K+] was strikingly correlated with parallel changes in plasma glucose. In the NaHCO3 dogs, the early rise in plasma [K+] was more variable, ranging from 0.2 to 3.4 mEq/L (1.4 ± 0.6 mEq/L). In the 9 dogs, the magnitude of the initial propranalol-induced increase in plasma [K+] was positively correlated with basal plasma [K+] ($r= .85$, $p<.01$).

Conclusion: The adrenergic nervous system plays a critical role in the maintenance of basal plasma [K+] in hyperkalemia. Beta-adrenergic blockade under these conditions can cause large acute elevations in plasma [K+] which appear to be counteracted by a mechanism involved in glucose homeostasis.

ROLE OF PHYSIOLOGICAL LEVELS OF ALDOSTERONE IN REGULATION OF DISTAL TUBULE MORPHOLOGY AND POTASSIUM TRANSPORT. B. Stanton, A. Janzen*, T. Klein-Robbenhar*, J. Wade*, G. Giebisch, and R. DeFronzo. Yale Univ. School of Med., Depts. of Physiol. and Med., New Haven, CT

Stimulation of K secretion by rat distal tubule after K adaptation is accompanied by an increase in plasma aldosterone and basolateral membrane surface density (S_v) of principal cells. This suggests that K secretion by principal cells is regulated by aldosterone and dependent on membrane area. To evaluate the influence of normal levels of aldosterone on K transport and cell structure adrenalectomized (ADX) rats without hormone replacement were compared to animals receiving aldosterone replacement continuously by minipump at 0.5μg/100gm/day for 10 days. During clearance experiments animals received a K load 0.42 mEq/h. ADX sharply depressed fractional K excretion (FE_K) from 58±5% in controls to 20±1% ($p<0.001$). Basolateral membrane S_v decreased from 3.57±0.13 to 2.53±0.22 ($p<0.01$). Aldosterone restored plasma aldosterone to normal levels (7 ng/dl) and increased S_v to 3.49±0.35, a value similar to control. FE_K increased sharply compared with ADX 43±4% vs 20±1% ($p<0.01$). Thus normal aldosterone levels play an important role in maintaining principal cell morphology and enhance the ability of the kidney to increase potassium excretion in response to an acute K load. This effect of aldosterone is mediated via binding to Type I (mineralocorticoid) receptors (∿30% occupancy) and not by binding to Type II (glucocorticoid) receptors since aldosterone occupancy of Type II receptors is less than 1%.

MINERALOCORTICOID STIMULATION OF K+ TRANSPORT BY THE CORTICAL COLLECTING TUBULE (CCT): EVIDENCE FOR INCREASED CELL K+ PERMEABILITY. John B. Stokes, University of Iowa, Department of Internal Medicine, Iowa City, IA.

These experiments were designed to examine whether mineralocorticoid enhancement of K^+ secretion includes (in addition to increasing Na-K pump activity) changes in cell K^+ permeability of the CCT. All experiments were conducted on rabbit CCT perfused in vitro. Three main observations emerged. First, the amiloride-induced change in transepithelial voltage (V_T), an index of the magnitude of Na absorption and the "mineralocorticoid state" of the tubule, was correlated with the ΔV_T induced by raising lumen [K^+] to 50 mM ($r=0.79$, $n=35$). This increase in the K^+ diffusion voltage indicates an increasing transference number for K^+ with increasing mineralocorticoid effect. Second, simultaneously determined ^{22}Na and ^{42}K lumen-to-bath fluxes before and after amiloride were closely correlated. DOCA pretreatment increased the amiloride-sensitive Na absorption (J_{Na}) from 24.8±4.2 to 72.9±9.7 peq/mm·min and the post-amiloride K^+ rate coefficient (K_K) from 201±30 to 621±95 nm/s. When all tubules were considered together, the J_{Na} and K_K showed a linear relationship ($r=0.92$) with an intercept near zero. Third, aldosterone (0.2 μM) in the bath increased K_K (amiloride present) from 178±16 to 240±43 nm/s after 150 min ($p<0.05$) while time controls showed a modest decline in K_K. The results indicate that mineralocorticoid hormones increase a passive, conductive, cellular pathway for K^+ in the CCT. Mineralocorticoid stimulation of K^+ secretion probably involves increased luminal membrane K^+ permeability.

EFFECT OF ACUTE K REPLENISHMENT ON NA, CL AND K REABSORPTION BY HENLE'S LOOP. C.R. Sufit* and R.L. Jamison. Div. of Nephrology, Stanford Univ., Stanford, California.

Stokes suggested that a K gradient across the medullary thick ascending limb, due to K recycling, inhibits Na and Cl reabsorption and alters K transport to accelerate urinary K excretion. To test this hypothesis, rats were fed a K-free diet for 3 days, prepared for re-collection micropuncture of the end-accessible proximal tubule (end-PT) and beginning of accessible distal tubule (early-DT) and divided into 2 groups. Both Control (N=7) and KCl (N=7) groups received NaCl infusions in periods I and II; the KCl group also received KCl in period II. There were no significant differences in fractional delivery of Na or Cl to end-PT or early-DT, or in "loop" reabsorption [(end PT - early DT)/(end-PT)] of Na or Cl between groups. Fractional K delivery (% of filtered K) was: (P, I v. II: *<.01, **<.001)

	End PT		Early DT		Urine	
Group	I	II	I	II	I	II
Cont $\bar{x}$	53	66**	12	17*	5.0	7.0
SE	2.7	3.0	0.8	1.2	1.7	1.3
KCl $\bar{x}$	50	62*	11	25*	2.6	44.6**
SE	4.7	3.5	1.0	2.7	0.8	4.9
P, C v. KCl	NS	NS	NS	<.01	NS	<.001

Loop reabsorption of K was significantly depressed from 75 to 58% (P<0.02) in the KCl group but not in controls (77 to 73%). The results suggest that after acute K loading, K delivery to the early DT is increased due to decreased net loop reabsorption and, contrary to current concepts, contributes substantially to urinary K excretion.

EVIDENCE OF TRANSPORT IN ISOLATED PERFUSED MESONEPHRIC TUBULES OF RABBIT. Daniel A. Terreros, Klaus Tiedemann*, and Larry W. Welling. Departments of Pathology and Medicine, University of Kansas Medical Center, Kansas City, Kansas, Anatomisches Institut I, Universitat Heidelberg, Federal Republic of Germany, and Laboratory Service, Veterans Administration Medical Center, Kansas City, Missouri.

Rabbit fetuses (New Zealand white) of 17-18 days gestational age and average weight 1.3 gm were obtained by caesarean section. Mesonephric tubules were dissected and perfused at 37°C by the technique of Burg et al. The basolateral transmembrane potential difference (PD) in 354 cells was measured by conventional microelectrode techniques. PD was -43 ± 1 mV (SEM) in 106 cells of 23 proximal tubules, -54 ± 1 mV in 66 cells of 17 distal tubules, and -55 ± 1 mV in 86 cells of 20 collecting ducts. The cellular PD in all segments was depolarized by 10^{-4} M ouabain and increases in extracellular potassium concentration. The PD of proximal tubule cells also was depolarized by the addition of glucose or amino acids to the perfusion fluid. In separate studies of 15 distally occluded proximal segments from 11 fetuses volume transport was 2.7 ± 0.3 $nl \cdot min^{-1} \cdot mm^{-2}$. These results support the idea that the mesonephros of the rabbit may be a functional organ.

EFFECT OF INDOMETHACIN (INDO) ON PAPILLARY SOLUTE CONTENT (PSC) IN THE POTASSIUM DEFICIENT (KD) RAT. Y. Takamitsu* and R.T. Kunau. Univ. of Tx. Hlth. Sci. Ctr. San Antonio, Tx.

A prominent aspect of the concentrating defect in the KD rat is a reduction in PSC. INDO increases PSC in normal rats, presumably by abolishing an inhibitory effect of PGE_2 on NaCl transport in the thick ascending limb (TALH). The present studies examined the effect of INDO on PSC in the KD rat. Control (C) and KD rats were fed equal quantities of appropriate diet and water for 21 days. The rats received either INDO, 5 mg/kg, i.p., or diluent (D) for three days. The papillary Na content (mM/kg H_2O) of the 4 groups was: C-D, 181 ± 11 SEM vs. C-INDO, 271 ± 12, p<.001; KD-D, 135 ± 4 vs. KD-INDO, 129 ± 6, pNS. Papillary urea content paralleled Na content. Thus, INDO increased papillary Na and urea content in C but not in KD rats. An effect of INDO to increase PSC may be obscured if NaCl delivery to the TALH were less or INDO enhanced papillary "washout" in KD rats. Previous studies have shown equal NaCl delivery rates to the loop of superficial nephrons in C and KD rats. "Washout" was indirectly examined by measuring papillary plasma flow (PPF). INDO increased PPF in KD rats from 13 ± 2 to 23 ± 2 ml/min/100 gm papilla, p<.01. INDO had no effect on PPF in C rats, being 51 ± 3, C-INDO, and 49 ± 3, C-D. The rise in PPF seen in KD-INDO rats presumably would not be sufficient to negate an increase in TALH solute transport as PPF in KD-INDO rats was still lower than in C rats. The results suggest that the TALH transport mechanism in KD rat may be unresponsive to INDO. If prostaglandin related effects in the TALH are necessary for appropriate solute accumulation in the papillary interstitium, defective operation of this system may contribute to the concentrating defect seen in the KD state.

A SCANNING ELECTRON MICROSCOPE STUDY OF THE ACTION OF ADH ON THE TOAD BLADDER LUMINAL MEMBRANE. S. Tilles‡ J. Sasaki‡ L. Meiteles‡ N. Franki‡ and R. Hays, Albert Einstein Coll. of Med., New York.

Freeze-fracture electron microscopy has shown that ADH induces fusion of cytoplasmic tubules with the luminal cell membrane and delivery of particle aggregates to the membrane. Information about fusion is limited, since freeze-fracture images of fusing tubules are rare. We have used high-resolution scanning electron microscopy (SEM) to study fusion and its relation to the cytoskeleton. Luminal membranes were removed from epithelial cells by Dounce homogenization, and allowed to settle, cytoplasmic surface up, on polylysine-coated coverslips. Membranes were viewed by SEM at 10,000 to 30,000X. In both control and ADH-treated preparations tubules were seen in contact with the membranes. Fine filaments appeared to anchor some tubules to the membrane. In epithelial cells which were rapidly frozen and split transverely, a thick mat of cytoskeleton, granules and tubules was seen underlying the luminal membrane. The free (luminal) ends of the tubules protruded from the mat, and, in ADH-treated preparations, were fused with the luminal membrane. The opposite ends of the tubules remained anchored in the mat. Tubules were concentrated by gentle sonication of luminal membranes, and sedimentation in sucrose. Tubular structure was more complex than previously reported; they had a spherical head piece, a narrow neck, and a straight or segmented body.

SEM is a powerful technique for studying epithelial transport. Following ADH, fusing tubules emerge from a thick cytoskeletal matrix underlying the luminal membrane. Tubule structure is complex, and a system of filaments may aid tubule fusion.

RENAL TRANSPORT OF ADENOSINE. M.E. Trimble and R. Coulson.* Depts. of Physiology and Pharmacology, Upstate and VA Med. Ctrs., Syracuse, N.Y.

Transport of adenosine into or out of cells may be important in determining its concentration at external receptor sites. We studied transport of adenosine in the isolated perfused rat kidney (IPRK) and in luminal (L) and antiluminal (AL) membrane vesicles isolated from rat renal cortex. In IPRK, exogenous adenosine in perfusate at 0.1 or 1.0 mM was rapidly metabolized and the clearance (C) ratio, C_{aden}/C_{inulin}, was 1.0. Conversely, PIA (N6-(L-2-phenylisopropyl)-adenosine) is not metabolized and C_{PIA}/C_{inulin} = 0.12 ± 0.05 (mean ± SD, n=4), indicating reabsorption. Considerable adenosine deaminase activity is retained by vesicles (L activity = 3x AL = 1/3 whole homogenate). Therefore, 30 μM EHNA (erythro-9-(2-hydroxy-3-nonyl)adenine) was added in all experiments to inhibit metabolism. HPLC analysis of incubation medium showed no metabolism of [^{3}H]adenosine after 10 min in the presence of EHNA. Initial uptake of adenosine showed saturation only in L vesicles as medium concentration was varied from 0.1-200 μM. K_m was 30-40 μM (n=5 preps). V_{max} varied from 50-260 pmoles·min^{-1}·mg^{-1}. Uptake of 1-10 μM adenosine was inhibited by 20 μM guanosine and 10 μM dipyridamole (n=3). When L vesicles were preloaded with [^{3}H]adenosine, countertransport was observed as efflux of [^{3}H]adenosine was stimulated by 5-10 mM extravesicular adenosine. Although the direction of in vivo transport cannot be stated with certainty, experiments demonstrating saturation, competition and countertransport, indicate that mediated transport of adenosine occurs across the L membrane.

THE EFFECT OF SITS, DIDS AND FUROSEMIDE ON URATE ABSORPTION IN THE RAT PROXIMAL TUBULE. Edward J. Weinman, Steven C. Bennett,* and Andrew M. Kahn. Univ. of Texas Medical School, Dept. of Internal Medicine, Houston, Texas.

To examine the effect of anion exchange inhibitors on the absorption of urate, proximal convoluted tubules of the rat were microperfused between oil blocks at a rate of 17.5 nl/min with a solution containing varying concentrations of (2-^{14}C) urate. In control animals, Jv averaged 2.31 ± 0.04 nl/min/mm. Urate absorption increased with increasing initial concentrations of urate. The relationship between the flux of urate and the mean intraluminal concentration of urate yielded the following kinetic parameters: Km 0.14mM, Vmax 0.25 pmol/min/mm and passive permeability coefficient 0.8 pmol/min/mm/mM. The addition of SITS (10^{-5}M) resulted in a lower Jv of 1.66 ± 0.09 nl/min/mm ($P<0.05$). Urate absorption was significantly decreased at any given intraluminal concentration of urate. The flux of urate in the presence of SITS closely approximated the passive permeability coefficient determined in control animals. The addition of DIDS (10^{-5}M) or Furosemide (10^{-3}M) resulted in significant lower Jv's of 1.33 ± 0.03 and 0.81 ± 0.03 nl/min/mm respectively. Both drugs also significantly inhibited urate absorption and the residual flux was that estimated to occur by passive permeation.

These findings indicate that SITS, DIDS and Furosemide significantly inhibit the absorption of water and urate in the rat proximal tubule and suggest that one component of the active transcellular absorption of urate in this nephron segment involves an anion exchange mechanism operating at the luminal border of the cell.

RENAL DISTAL TUBULE PATHWAYS FOR SODIUM, CHLORIDE AND POTASSIUM TRANSPORT ASSESSED BY DIURETICS. Heino Velázquez* and Fred S. Wright. Yale Univ. School of Med. and Va Med. Ctr., New Haven, CT.

To characterize distal tubule ion transport mechanisms, we tested effects of amiloride (AML), furosemide (FUR), and chlorothiazide (CTZ) separately and together. Surface distal tubules of anesthetized Sprague-Dawley rats were perfused in vivo at 16 nl/min. A control solution (I) contained (in mM) 75 Na, 68 Cl, 4.5 PO_4, and urea to make net fluid absorption zero. Maximally effective concentrations of diuretics were added to 6 test solutions: 0.1 AML in II, 0.1 FUR in III, 1.0 CTZ in IV, 0.1 AML + 0.1 FUR in V, 0.1 FUR + 1.0 CTZ in VI, and 0.1 AML + FUR + 1.0 CTZ in VII. Solutions were tested in pairs in the same tubule. Na and K were measured in perfused and collected fluids by atomic absorption, Cl by microtitration. Average net ion fluxes (J_i, pmol/min, + absorption, - secretion) were:

Soln	I	II	III	IV	V	VI	VII
J_{Na}	391	262	275	103	212	132	31
J_{Cl}	274	237	134	18	107	30	-42
J_K	-126	-68	-134	-124	-97	-124	-105

J_{Na} was decreased by AML, FUR, and CTZ. J_{Cl} was decreased by FUR and CTZ, but not by AML. J_K was decreased by AML, but not by FUR or CTZ. AML inhibition of J_{Na} added to inhibition by either FUR or CTZ. FUR inhibition of J_{Na} added to inhibition by AML but not by CTZ. CTZ inhibition of J_{Na} added to inhibition by either AML or FUR. We conclude that at least two distinct pathways for Na transport are present: one, for Na without Cl, affects K transport, and is inhibited by AML; the other, for Na and Cl, does not affect K transport, and is inhibited more completely by CTZ than by FUR.

EFFECTS OF AMILORIDE AND SITS ON NaCl PERMEABILITY IN ISOLATED PERFUSED RABBIT PROXIMAL TUBULE. S.W. Weinstein and Allen Williams*, Div. Nephrology, Dept. Medicine, SUNY, Stony Brook, New York.

High concentrations of amiloride, a sodium transport inhibitor, and of SITS, an anion transport inhibitor, have been shown to reduce proximal tubular fluid reabsorption. The mechanism for their action has not been determined. One possibility is that they alter passive permeability to NaCl, as has been shown for amiloride in the gall bladder. To study this, ionic diffusion potentials, capable of assessing low resistance and thus paracellular pathway passive ionic permeability were measured in the S-2 segment of the superficial cortical straight proximal tubule. NaCl dilution potentials, 100mM NaCl replaced isosmotically with raffinose, and biionic diffusion potentials were measured. (Na^+ vs the impermeant cation tetramethylammonium (TMA) and Cl^- vs the impermeant anion isothionate). The results show that amiloride, $5x10^{-3}$M, increased the 100mM NaCl gradient diffusion potential 1.12± 0.09mV and decreased the biionic diffusion potential NaCl vs TMACl, 1.00±0.07mV indicating that amiloride specifically reduces low resistance pathway sodium permeability. SITS 10^{-3}M, decreased the 100mM gradient NaCl potential 1.01±.14mV and decreased the sodium isothionate vs NaCl biionic potential 2.30±0.35mV, indicating that SITS specifically inhibits chloride permeability through the same pathway. These data provide evidence that separate and specific reductions in proximal tubular paracellular pathway permeability to sodium and to chloride can be achieved. Such changes may explain the reported reduction in fluid reabsorption produced by these inhibitors.

VERY RAPID VIDEO MEASUREMENTS INDICATE LOW NaCl REFLECTION COEFFICIENT ACROSS BASOLATERAL CELL MEMBRANES OF RABBIT S_1 AND S_2 PROXIMAL TUBULES. Dan J. Welling, Larry W. Welling, Toni Ochs*, and Gregg Bliss*. Departments of Pathology and Physiology, University of Kansas Medical Center, Kansas City, Kansas, and Laboratory Service, VA Medical Center, Kansas City, Missouri.

The reflection coefficient σ for a solute across a cell membrane can be defined in the context $J_{V_O}/\Delta C = \sigma L_pA$ where J_{V_O} is the initial transmembrane water flux induced by a change ΔC in the bathing solute concentration and L_pA is the membrane hydraulic conductivity. The σ for a permeant solute thus can be derived by comparing the L_pA measured using the permeant solute to the L_pA measured using a known impermeant solute ($\sigma = 1$). We have used a video camera and recorder to measure in 1/60 sec increments the initial rate of trans-basolateral cell membrane water flux and tubule swelling that occurs in isolated, non-perfused S_1 and S_2 segments within 0.1 sec after acute reductions in the bathing concentrations of the impermeant solute raffinose or of NaCl. Using raffinose, L_pA per unit membrane area was found and previously reported by us to be quite large ($P_f \simeq 250\ \mu m \cdot sec^{-1}$) and not statistically different in the two segments. Using NaCl, the apparent L_pA per unit membrane area now is found to be considerably smaller ($P_f \simeq 123\ \mu m \cdot sec^{-1}$) but again not statistically different in the two segments. From the ratio of the mean raffinose (43 tubules) and NaCl (39 tubules) hydraulic conductivities, the apparent reflection coefficient for NaCl across basolateral membrane in S_1 and S_2 rabbit proximal tubule segments is 0.48 ± 0.01 and thus surprisingly low.

RENAL GLUCOSE TRANSPORT IN PREGNANT RATS. S.F.Wen, and N.R. McSherry.* Dept. of Medicine, University of Wisconsin, Madison, Wisconsin.

In order to evaluate the mechanism of renal glycosuria in pregnancy, we performed clearance and micropuncture studies in pregnant (PG) and age-matched non-pregnant (NP) rats. In 6 PG and 8 NP rats, daily urine collections were made during gestation days 11-20 and 1-3 days post partum. In PG, plasma glucose at 4.12 ± .14 mM was significantly lower than that in NP (6.87 ± .45 mM), while 24 hr urinary glucose excretion (U_GV) (44 ± 3 vs 27 ± μmol), urine flow (V) (17 ± 2 vs 10 ± 1 ml), $U_{Na}V$ (1.1 ± .06 vs 0.9 ± .06 mEq) and creatinine clearance (1.4 ± .08 vs 1.0 ± .03 ml/min) were higher. Replacing drinking water with saline or 5% glucose increased V and U_GV and equalized $U_{Na}V$ for the two groups but the difference in U_GV between the two groups (51 ± 2 vs 40 ± 2 μmol/24 hr) persisted. The higher GFR and U_GV and lower plasma glucose in PG reverted to normal within 2 days post partum. Micropuncture of the late proximal tubule in 9 PG (vs 7 NP) rats showed higher single nephron GFR (34 ± 1 vs 29 ± 1 nl/min), similar tubule fluid-to-plasma (TF/P) inulin ratios (2.09 ± .08 vs 2.16 ± .13), higher TF/P glucose (0.26 ± .01 vs 0.22 ± .02) and lower fractional proximal glucose reabsorption (87 ± .6 vs 90 ± .6%, $P < 0.05$). The higher GFR in PG was offset by lower plasma glucose so that the filtered load of glucose remained lower. Conclusions: (1) Lower plasma glucose in PG cannot be explained by renal glucose leak. (2) Reduction in absolute and fractional glucose reabsorption in proximal tubule despite the maintenance of glomerulotubular balance for sodium accounts for the increased U_GV in PG. (3) High U_GV in PG is not related to high $U_{Na}V$ or GFR.

EFFECT OF BARIUM ON CELL VOLUME REGULATION IN HYPOTONIC MEDIUM. Paul Welling*, Michael A. Linshaw, Larry Sullivan, Univ. of Ks. Med. Ctr., Department of Pediatrics and Physiology, Kansas City, Kansas.

The acute stress of immersion in hypotonic medium causes renal proximal straight tubules (PST) to swell quickly to a peak then shrink over 3-5 min as they regulate their volume towards control. The regulatory phase occurs presumably as K^+ and water are passively extruded from the cells. Ba reduces K^+ conductance of the basolateral membrane of epithelial cells and may be a useful tool for studying cell volume regulation. We mounted 30 rabbit PST with collapsed lumens between 2 micropipets, incubated them in 10^{-3}M BaCl (20 min) and/or 10^{-4}M ouabain (1 min) and observed their response to sudden immersion in dilute medium. All tubules swelled to a similar peak in 1 min (@ 55% above baseline). Control tubules then reduced their volume to a steady state level 17% above baseline in 4½ min. The recovery phase of tubules previously exposed to Ba was significantly prolonged, reaching a volume of 22% above baseline in 13½ min. Tubules exposed to ouabain for 1 min swelled quickly upon addition of hypotonic medium, then shrank slightly and subsequently gradually swelled to 108% above baseline in 20 min. Tubules exposed to Ba and then ouabain for 1 min swelled 57% within 1 min of immersion in hypotonic medium and then very slowly swelled to 72% above baseline at 20 min. Thus, Ba slowed the volume recovery phase in control tubules presumably by reducing K^+ efflux. Surprisingly Ba also reduced the slow swelling phase caused by ouabain. It is possible that Ba interferes with the passive entry of NaCl.

AMILORIDE INHIBITION OF K SECRETION IN THE CORTICAL COLLECTING TUBULE(CCT) IS INFLUENCED BY MINERALOCORTICOID STATUS. C.S. Wingo. Division of Nephrology, University of Florida, Gainesville, Florida.

We demonstrated recently that CCT K secretion that is ouabain inhibitable in adrenalectomized (ADX) rabbits is nearly the same as that in normal rabbits. The present studies examined the sensitivity of CCT K secretion to luminal 10^{-5}M amiloride (Am) and to 10^{-4}M ouabain (O)(in bath), sequentially. Tubules (N=13) from female New Zealand white rabbits, ADX either 7 days before experimentation, or treated with 5mg/day of DOCA for 3-14 days, were perfused and bathed at 37°C with an artificial ultrafiltrate. J_V was negligible. Mean perfusate [K] was 5.2 mEq/L. Below are the mean values ± SEM for collected [K] in mEq/L, transepithelial voltage (V_T) in mV and K flux in pmoles $mm^{-1}\ min^{-1}$.

Condition	Effluent[K]	V_T	K Flux
ADX:CONTROL	8.71±.72	+5.2±1.5	-3.62±.37
ADX:A	6.60±.56+	+3.4±0.8	-1.52±.21+#
ADX:A+O	6.43±.37+	+1.7±0.4+	-1.37±.29+#
DOCA:CONTROL	16.7± 3.9	-20.1±6.3	-28.60±9.4
DOCA:A	5.4 ±.32+	+8.0±2.1+	+0.35±.49+
DOCA:A+O	5.3 ±.45+	+6.5±2.0+	+0.34±.53+

+Significantly different from respective control, $P<.01$; #Significantly different from zero, $P<.01$.

Am inhibited K secretion by 58% in ADX rabbits, but V_T was unaltered. No further effect on K secretion was produced by O, but V_T was decreased. Am completely inhibited K secretion in DOCA-treated animals and V_T became lumen positive. Conclusion: 1)A substantial amount of K secretion is inhibited by Am independent of the rabbits' mineralocorticoid status; 2)These data and additional electrophysiologic data suggest that Am affects K secretion in ADX rabbits by an electroneutral mechanism.

RUBIDIUM TRANSPORT IN RAT MEDULLARY THICK ASCENDING LIMB. Jack Work and James A. Schafer, Nephrology Research & Training Center, University of Alabama in Birmingham, Birmingham, Alabama.

In order to examine the K^+ transport properties of the rat medullary thick ascending limb, unidirectional lumen-to-bath $^{86}Rb^+$ fluxes (J^{lb}, pmol min^{-1} mm^{-1}) were measured as an index of K^+ permeability. Limb segments from barrier-maintained male Sprague-Dawley rats were bathed and perfused (9-13 nl/min) with phosphate-buffered solutions at 38° C. Under control conditions in 18 tubules transepithelial voltage (V_e) was +2.8 ± 0.4 mV (lumen positive), the net Cl^- flux measured titrimetrically was 187.8 ± 67.5 peq min^{-1} in the absorptive direction, and J^{lb} for Rb^+ was 25.00 ± 1.88 at an average luminal concentration of 4.6 mM. This flux was used to calculate an apparent Rb^+ permeability coefficient of 5.4 ± 0.4 pmol min^{-1} mm^{-1} mM^{-1}, or about 1.5 μm/sec. Removing Rb^+ from the bathing solution or adding 0.1 mM ouabain to it reduced J^{lb}-Rb^+ to 17.7 ± 3.0 and 15.36 ± 3.4, respectively. V_e was not significantly different from zero after either maneuver. In contrast, the addition of luminal furosemide (0.01 mM) had no significant effect on J^{lb}-Rb^+ but reduced V_e to zero. These results suggest that Rb^+ (and presumably K^+) transport in the medullary thick ascending limb of the rat is similar to that reported previously for the rabbit, that is, a major fraction of the Rb^+ flux is transcellular and the high permeability could account for rapid passive reabsorption of K^+ down its concentration gradient.

SUGAR UPTAKE INTO A PRIMARY CELL CULTURE OF DOG KIDNEY PROXIMAL TUBULAR CELLS. C. Yau*, L. Rao*, D. Fraser* and M. Silverman, Univ. of Toronto, Toronto, Ontario.

A suspension of renal proximal tubular (PT) cells was prepared from canine kidney cortex as previously described (Ann. N.Y. Acad. Sci. 372: 468, 1981). Cells were plated on petri dishes and grown in α minimal essential medium with 15% fetal calf serum supplementation. By day seven, confluent monolayers were obtained showing: 1) typical transporting epithelial morphology and multiple dome formation; 2) strong binding with specific rabbit-anti-dog brush border membrane antibody; 3) histochemically demonstrable alkaline phosphatase; 4) metabolism of $25(OH)D_3$ to $24,25(OH)_2D_3$; 5) PTH stimulatable c-AMP synthesis. Sugar uptake of ^{14}C-α-methyl-D-glucose (αMG) (1mM) and ^{14}C-3-O-methyl-D-glucose (3OMG) (1mM) was measured relative to ^3H-L-glucose (1mM) at 37°C (pH 7.4, 140 mM Na). Individual plates of cells were first washed free of growth medium and incubated at 22°C for 2 hrs. with a balanced salt solution prior to transport assay. Timed uptake studies revealed that αMG entry into cells was slow, progressively increasing even after 2 hrs, and inhibited by 10 μM phlorizin. 3OMG uptake was rapid, reaching ~ 50% of maximal value by 30 sec, was 100% blocked by 10 μM cytochalasin B, 70% by 10 μM phloretin but not inhibited by phlorizin. These findings suggest that the primary culture system derived from a purified canine PT cell suspension has retained functions typical of in vivo PT cells and may prove useful in studying regulation of PT transport systems.

EFFECT ON TUBULO-GLOMERULAR FEEDBACK OF HUMAN URINE OBTAINED DURING SALT DEPLETION AND SALT LOADING. Wunderlich P.,* Hermle M.,* Thiel G.,* Brunner F.P.* (intr. by F.C.Rector,jr.). Depart.Int.Med., University of Basel, Switzerland.

A possible role of tubulo-glomerular feedback in altering GFR in response to salt depletion and salt loading was studied by microperfusing human urine in situ through the loop of Henle of surface convolutions in the anesthetized rat. Salt depletion and maximal antidiuresis was induced in a healthy individual by feeding a Na free diet (< 10 mEq/d Na) for 5 days and thirsting for the last 24 h. Ccreat fell from 140 ml/min. to 100 ml/min. and Na excretion to 2.8 mEq on day 5. Aliquots of the refrigerated urine collected on day 5 (osmolality 1017, Na 7, Cl 40 mEq/l) perfused at 5, 20, and 40 nl/min. decreased stop flow pressure (SFP) by 13% ($p < 0.005$), 24% ($p < 0.001$) and 32% ($p < 0.001$) respectively. Urine obtained from the same individual after 9 days of salt loading (300 to 500 mEq/d supplemental NaCl with Ccreat rising to 163 ml/min. and Na excretion of 390 mEq on day 9; urinary osmolality 961, Na 229 and Cl 250 mEq/l) failed to lower SFP at any perfusion rate. Control perfusions with Ringer's at 40 nl/min. decreased SFP by 27% ($p < 0.001$). It is concluded that tubulo-glomerular feedback was activated by urine from a salt depleted subject, while urine obtained during massive salt loading completely blocked feedback response. The presence or absence of humoral factors in the urine could thus be responsible for or contribute to changes in GFR during salt depletion and salt loading.

Renin, Prostaglandins and Other Hormones

SODIUM CHLORIDE INCREASES PROSTAGLANDIN E_2 (PGE) BINDING TO RAT RENAL MEDULLARY RECEPTORS. Nancy L. Allison, Thomas R. Beck, Temple Univ. Phila.PA.

PGE increases water excretion by antagonizing the action of ADH on medullary NaCl transport and water permeability. We studied ^3H-PGE binding to a 35,000xg particulate fraction prepared from rat renal medulla. Binding achieved equilibrium by 60 min at 37° (k_1=.099min^{-1}). Specific binding accounted for 89±2% of total binding. Scatchard analysis revealed a single high affinity binding site with an dissociation constant (Kd) of 1.93±.26 nM and a receptor density of 284±36 fmols/mg protein(n=6). NaCl, in a concentration dependent fashion, markedly enhanced PGE-receptor binding. Maximal binding was observed at a NaCl concentration of 375 mM, which represented a 90% increase compared to NaCl-free conditions. Equiosmolar concentrations of urea and sucrose had no effect on binding. Experiments performed over a range of low NaCl concentrations (15-90mM) demonstrated that the effect of high NaCl was to increase receptor affinity (Kd=3.83+.42nM in low NaCl vs. 1.93+.26nM in high NaCl) without changing receptor number.

We conclude that over a range of physiologic medullary concentrations, NaCl regulates renal medullary PGE-receptor binding. ADH, by increasing NaCl transport and medullary NaCl concentration, would lead to enhanced PGE-receptor affinity. The interdependence of ADH, medullary NaCl concentration, and PGE-receptor binding represents an important mechanism by which PGE can exert its known negative feedback inhibitory role on ADH action.

PROSTACYCLIN (PGI_2) IS THE MAJOR METABOLITE OF ARACHIDONIC ACID IN CANINE BLOOD VESSELS AND RENAL CORTEX. Billy S. Arant, Jr, Dept. of Pediatrics, UTHSC, Dallas, TX.

Previous studies have suggested PGE_2 and $PGF_{2\alpha}$ to be the primary prostaglandins (PG) synthesized by the kidney. In contrast to the rat, PGI_2 has been found to be the major PG synthesized by human glomeruli. The purpose of this study was to characterize PG synthesis in canine blood vessels and renal cortex and to identify any species variation. Vascular tissues and renal cortex (1.0 gm) obtained from 3 mongrel dogs were finely minced and incubated in Krebs-Ringer solution with ^{14}C-arachidonate for 60 minutes (pH 7.4,37°C,95%O_2:5%CO_2). The supernatant was acidified, extracted in ethyl acetate: cyclohexane, dried under N_2 and reconstituted in 0.02M H_3PO_4. PG were separated by reverse-phase HPLC (CH_3CN). Radiochromatographic peaks were identified by comparison with known compounds. Results are expressed as mean values of [cpm for each PG metabolite/cpm total PG synthesized] x100. (PGI_2=6ketoPGF$_{1\alpha}$ +6,15diketoPGF$_{1\alpha}$.)

	PGI_2^{α}	TxB_2	$PGF_{2\alpha}$	PGE_2
Aorta	64.1	11.0	7.7	17.2
Pulmonary artery	78.1	0.0	6.8	15.1
Carotid artery	52.1	23.8	11.6	12.5
Femoral artery	64.0	9.8	13.6	12.6
Renal artery	45.0	25.0	15.9	14.1
Renal cortex	50.0	25.2	8.9	15.9
Lung	53.4	8.4	4.6	33.6
Jugular vein	50.8	16.6	7.5	25.1
Femoral vein	59.4	5.0	6.7	28.9

These findings suggest that in the mongrel dog, vascular and renal cortical synthesis of PG is predominantly PGI_2 and similar to the human.

DISSOCIATION OF THE RENAL HEMODYNAMIC AND RENIN RELEASE EFFECTS OF ADENOSINE BY VERAPAMIL. Lois J. Arend* and William S. Spielman. Department of Physiology, Michigan State University, East Lansing, Michigan.

Adenosine produces a vasoconstriction in the kidney and results in a decrease in glomerular filtration rate (GFR). Intrarenal adenosine also produces a marked inhibition of renin release. In the present study, adenosine was infused (.226 μmol/min) prior to and during the infusion of verapamil (10 μg/kg/min) to investigate the role of calcium influx in the renal actions of adenosine. In 6 anesthetized (sodium pentobarbital) dogs, verapamil infusion abolished the decrease in GFR produced by adenosine. However, the suppression of renin release seen with adenosine infusion was not affected. Results (mean ± sem; *= $p< 0.05$):

				V		
	con	ado	rec	con	ado	rec
GFR	24±2	*10±2	19±2	22±1	25±3	23±3
RR	309±53	*71±26	252±55	1151±229	*181±159	881±211
MAP	108±7	106±8	107±10	96±6	89±6	88±8

(V = verapamil; con = control; ado = adenosine; rec = recovery; MAP = mean arterial pressure, mmHg; RR = renin release, ng AI/min; GFR, ml/min)

We conclude that calcium influx has an important role in the renal hemodynamic response to adenosine, but that the inhibition of renin release produced by adenosine is not dependent on either the hemodynamic response or an influx of calcium.

ENDOGENOUS KALLIKREIN-KININ SYSTEM (KKS) MODULATES VASOPRESSIN (V) ACTION IN THE RAT. L.A. Arbeit, Div. Nephrology, SUNY Stony Brook, New York.

In an in vitro model of the distal nephron, the toad urinary bladder, inhibition of the KKS increases V stimulated water flow. The effects of KKS inhibition was studied in a rat subjected to water diuresis with .45% saline (S). After basal (B) collections, the KKS inhibitor aprotinin (A) was infused in the experimental group (n=5) and S was infused in a control group (n=7). After 30 minutes a 200μU bolus of V was infused. There was no significant difference between B collections in the A or S treated groups. Following V stimulation, A treated animals were significantly more responsive to the hormone than S animals:

	Flow	U_{osm}	C_{osm}	T_{CH_2O}/C_{osm}	GFR
S	111±19	337±50	120±16	.052±.11	2.5±.2
	NS	p<0.05	NS	p<0.05	NS
A	90±27	548±86	147±38	.53 ±.06	2.6±.2

In S and A treated animals kallikrein (K) was measured by an activity assay (generation of kinins) and an immunologic assay which measures total K(active and inactive). A ratio of active to total(A/T) K, which represents the activity per weight of K was calculated. K was measured in the S and A rats.

	CONTROL(S)		APROTININ	
	Basal	Vaso	Basal	Vaso
Active	10.1±2.6	10.5±2.1	7.1±2.2	0
Total	41.5±4.6	24.7±5.5*	20.6±0.4	13.4±3.2*
A/T	.3± .07	.58±0.2*	.23±.07	0* *p<.05

The increase in the A/T ratio in the S rats post V suggests that V activates K. A completely inhibited K activity. In conclusion: 1. inhibition of the KKS enhances V action and 2. V activates the KKS. Thus KKS is a feedback regulator of V.

ALTERED GLOMERULAR ANGIOTENSIN II (AII) RECEPTOR MODULATION IN UNTREATED DIABETIC RATS. B.J. Ballermann,* V.J. Dzau,* K.L. Skorecki, and B.M. Brenner. Brigham and Women's Hosp., Boston, MA.

Decreased in vitro vascular reactivity to AII has been demonstrated in aortas from diabetic rats. We explored the AII receptor density (R_o) and dissociation constant (K_D) in isolated glomeruli from rats 3-4 weeks after streptozotocin (60 mg/kg i.v.). Three groups of animals were studied: untreated diabetics (D), diabetic rats treated with NPH or heat-treated ultralente insulin 2.5-3.5 U sc/day (D+I), and non-diabetic controls (C).

	R_o (fmoles/mg glm protein)	K_D (nM)
C (n=15)	1172±52 (SE)	.463±.027
D (n=13)	987±57*	.457±.043
D+I(n=21)	1078±57	.423±.031

* C vs D $p<.05$

No differences in K_D values were observed among groups. Since R_o was decreased significantly in D vs C we measured plasma renin concentrations (PRC) to assess the role of the renin-AII axis in this decline in R_o. An inverse relationship between PRC and R_o was obtained in C and D+I whereas R_o was reduced in D despite low values for PRC. Thus, modulation of glomerular receptor density in C and D+I appears to be dependent on the degree of stimulation of the renin-AII axis. In D, however, despite suppression of PRC the expected upregulation of R_o does not occur. This defect in AII receptor modulation may account for the observed decrease in vascular reactivity in untreated diabetes.

INABILITY OF RENAL ANGIOTENSIN CONVERTING ENZYME (RCE) TO DEGRADE BRADYKININ. RL Baranowski, BL Currie,* and C Westenfelder, Dept. of Medicine, University of Illinois, Chicago, Illinois.

We have previously demonstrated the presence of an angiotensin converting enzyme (ACE) in a crude deoxycholate extract of rat kidney cortical microsomes. We have also shown differences in kinetic properties and sensitivity to inhibitors between the detergent form of RCE and lung CE with angiotensin I as substrate. The present study was performed to examine whether RCE degrades bradykinin, another known natural substrate of ACE. ACE was extracted from the microsomal membrane of dog kidney cortex of lung with detergent (Triton X-100) and the extract was subsequently purified on columns of Sephadex G-200 and DEAE Sephadex. Both enzyme preparations were purified approximately 100 fold. The catalytic generation of the dipeptide phe-arg from bradykinin by ACE was established using a high performance liquid chromatographic method. The relevant peptides were further identified by mass spectrometry. The lung enzyme generated the carboxyterminal dipeptide phe-arg from bradykinin and was inhibited by CE inhibitors captopril or SQ 20881. The renal enzyme, however, did not catalyze cleavage of the pro-phe bond resulting in the dipeptide phe-arg. We conclude therefore, that the detergent form of RCE which generates angiotensin II and does not degrade bradykinin differs from lung CE. These differences may be related to membrane lipid modification of renal converting enzyme activity.

GLUCOCORTICOID RECEPTORS (GR): THE CATION TRANSPORT RECEPTOR IS DISTINCT FROM THE METABOLIC RECEPTOR. C.P. Bastl, C.A. Barnett and G. Litwack. Temple Univ. Health Sc. Center, Phila.,PA.

Glucocorticoids alter sodium and potassium transport and Na-K-ATPase activity in both kidney and colon. This study provides evidence that a distinct GR mediates this effect.

The GR in epithelial cell cytosol of rat proximal colon demonstrated the following affinities by competitive binding assay: triamcinalone acetonide (TA)>corticosterone=DOCA>progesterone>>cortexolone>aldosterone> spironolactone. Scatchard analysis revealed a single high affinity binding site with a dissociation constant of 10nM, similar to that of GRs in kidney and liver. The unactivated form of the [^{3}H] TA-GR was elutable by 0.4M KCL from DEAE-Sephadex anion exchange columns, a characteristic of the unactivated GR in other tissues. However, the heat-activated (25^{0}C) GR complex eluted in the prewash. This elution profile identifies proximal colon GR as IB, a form previously thought to be the major GR only in kidney cortex. In liver and thymus the activated GR complex, II, elutes at 0.2 M KCL. The activated colonic GR binds to DNA, and its formation is inhibited by molybdate but not by protease inhibitors suggesting it is a true receptor and not a proteolytic product.

The described features of the colonic-renal GR distinguish it from GRs in other tissues with low rates of Na^+-K^+transport. This finding suggests that G mediated electrolyte transport is effected via binding to a transport GR, IB, which is distinct from the GR, II, which regulates metabolic functions of glucocorticoids.

THE CATALYTIC PROPERTIES OF THE AMPHIPHATIC AND HYDROPHILIC FORM OF RENAL ANGIOTENSIN CONVERTING ENZYME. RL Baranowski, C Westenfelder, and NA Kurtzman, Univ of Ill and West Side VA Hospitals, Chicago IL.

Detergent extracts of membrane bound enzymes are generally difficult to purify. Thus, alternative methods have been utilized which include autolysis or limited proteolytic enzyme digestion of the membrane to effect solubilization. These procedures, however, do not guarantee the extraction of an intact enzyme. A hydrophobic portion which interacts with the lipid environment of the membrane is usually absent from the solubilized enzyme. The loss of this portion and/or its association with the membrane may adversely alter catalytic activity. This hypothesis was examined using renal angiotensin converting enzyme as a model system. The enzyme was extracted from the microsomal membrane with detergent (Triton X-100) or Trypsin with subsequent purification on columns of Sephadex G-200 and DEAE Sephadex. Characterization of the catalytic activity of these preparations was established by development of a high performance liquid chromatographic method to identify enzymatic peptide fragments. The detergent form was not chloride dependent or inhibited by Captopril or Teprotide. The Trypsin form, however, was chloride dependent and inhibited by converting enzyme inhibitors. These data demonstrate clear differences in catalytic activity between the different forms of the enzyme. If the amphiphatic form resembles the native enzyme, these data suggest that membrane lipids may modify renal angiotensin converting enzyme activity in-vivo.

REGULATION BY GUANYL NUCLEOTIDES OF RAT RENAL CORTICAL PROSTAGLANDIN E (PGE) RECEPTORS. Thomas R. Beck. Temple Univ., Phila., PA.

Although PGE influences many renal physiologic events, renal PGE receptor binding has received little attention. We studied ^{3}H-PGE_2 binding in a 35,000g particulate fraction of rat renal cortex. Specific binding was rapid (second-order rate constant = .0308 $min^{-1}10^9M^{-1}$) and achieved equilibrium in 60 minutes at 37^{0}C. Scatchard analysis revealed a single specific high affinity receptor site with a dissociation constant (Kd) of 1.96 + .41 nM and a receptor density of 70+10 fmols/mg protein (n=6). Competition experiments demonstrated that binding was specific for PGs of the E series. Other PGs (F,A,B) were 75 to 250 fold less potent at displacing ^{3}H-PGE_2. Studies of the dissociation kinetics revealed a rapidly dissociable component of binding (rate constant = .139 min^{-1}) and a slowly dissociable component (.012min^{-1}). Increasing concentrations of GppNp (25-250uM), a non-hydrolyzable analog of GTP, reduced total binding up to 65%. Additionally, GppNp (100uM) increased the percentage of ^{3}H-PGE bound in the rapidly dissociable form from 24% to 48% of the total bound.

We conclude that rat renal cortex posesses a single, specific PGE receptor which exists in two states, rapidly and slowly dissociable. Furthermore, guanyl nucleotides regulate the transition between these two states, specifically by increasing the rapidly dissociable form of the PGE-receptor complex.

BLOOD KININ SECRETION IN RESPONSE TO REDUCED RENAL PERFUSION IN THE DOG. WH Beierwaltes, ML Arora*, OA Carretero and AG Scicli*. Henry Ford Hospital, Detroit, MI.

Kinins may act within the kidney to maintain renal blood flow (RBF) when renal circulation is compromised. We have measured renal venous kinin secretion rate (KSR=[venous]-[arterial] x RBF) in response to altered renal perfusion pressure (RPP) within (90 mmHg) and below (65 mmHg) the range of RBF autoregulation. Dogs on normal Na+ diet (n=3), dogs kept 1 wk on low Na+ diet (n=6) and dogs treated acutely with kininase inhibitors CAPTOPRIL (C) and ε-amino caproic acid (εACA,n=7) were studied. Renal venous and arterial catheters and a loop to occlude the renal artery were placed via a flank incision in pentobarbital anesthetized dogs. Arterial and venous blood was sampled during a 30 min control period and following RPP reduction to 90 and 65 mmHg.

	KSR in pg/min/gm kidney wt		
GROUP	CONTROL	90 mmHg	65 mmHg
Normal Na+	0±0	36±22	43±22
Low Na+	666±208‡	372±89‡	940±294‡
C + εACA	0±0	76±30	108±56**

‡=p<0.01 vs normal Na+, ** = p<0.05 vs control.

The KSR was unchanged in normal Na+ dogs with reduced RPP. While low Na+ greatly increased KSR, reductions in RPP did not significantly alter KSR. With C and εACA, basal KSR was unaltered but KSR significantly increased at RPP below the range of RBF autoregulation. These data suggest KSR can be greatly enhanced by Na+ restriction. Kininase inhibition may unmask a role for local renal kinin generation when renal circulation is compromised.

ROLE OF EXTRACELLULAR CALCIUM IN VANADATE (VO_4) INDUCED VASOCONSTRICTION IN THE DOG. Julio E. Benabe, Manuel Cruz-Soto and Manuel Martínez-Maldonado. VA Center, Research and Medical Service San Juan, Puerto Rico.

We have previously shown that intrarenal infusion of vanadate in the dog produces severe vasoconstriction, decreased GFR, antinatriuresis and antidiuresis. These effects were shown to be inhibited by calcium antagonists such as verapamil and trifluoroperazine. To evaluate the role of extracellular calcium vanadate (0.5 ml/min) was infused in acutely thyroparathyroidectomized (TPTX) dogs. Serum calcium decreased from 8.84±0.30 mEq/l to 5.41±0.40 mEq/l (p<0.005). The results obtained were as follows:

	RBF	GFR	FE_{Na}
Control	156.4±30	19.1±4.1	7.7±2.1
VO_4	131.8±30*	15.2±3.1	9.3±3.7*
VO_4+Ca	76.8±19*	8.1±1.6*	13.3±3.2*

*0.005>p<0.05

Calcium increased from 5.41 mg% in the TPTX dogs to 9.72 mg% with the Ca^{++} + VO_4 infusion. In the TPTX dogs the effects of VO_4 on RBF and GFR are blunted. A natriuretic effect is unmasked. Re-establishing serum calcium to normal values elicits vanadate effects of vasoconstriction and decreased GFR but does not affect V and provokes further increments in FE_{Na}+. The data supports a critical role for calcium as mediator in vanadates vasoconstriction. Furthermore during hypocalcemia a natriuretic effect of vanadate is unmasked consistent with inhibition of tubular Na^+-ATPase. This effect persists and is enhanced with re-establishing serum Ca++.

MECHANISM OF SODIUM (Na^+) REGULATION OF GLOMERULAR ANGIOTENSIN (AII) RECEPTORS. A. BELLUCCI* and B.M. Wilkes,Div. of Nephrology/Hypertension, North Shore Univ. Hosp., Manhasset, N.Y.

AII binds to high affinity glomerular receptors to modulate glomerular filtration. These studies were undertaken to investigate the effects of Na^+ intake on glomerular AII receptor concentration. Rats (175-250g) were maintained on one of 3 diets for at least 5 days: high Na^+(H), normal Na^+(NL) and low Na^+ with daily Lasix (1 mg/kg, i.p.;LL). Rats were sacrificed by rapid decapitation, blood collected for AII measurements and cortical glomeruli isolated for equilibrium binding studies. Scatchard analysis revealed a striking increase in AII receptors in the H rats (NL, 953±41;H,1179±43 fmol/mg,p<.001) and decrease in the LL rats (779± 47 fmol/mg;NL vsLL,p<.025). Additional experiments were performed to determine whether Na^+ intake per se or circulating hormone regulates AII receptors: Additional rats (L) were placed on low Na^+ diet without Lasix; others (HA) were placed on high Na^+ diet but received a continuous AII infusion via an implanted minipump. Results (mean ± SE):

Group (N)	Receptor# (fmol/mg)	Plasma AII (pg/ml)	U_{Na^+} (mEq/24h)
LL (10)	779 ± 47	105 ± 12	0.22
HA (8)	823 ± 105	75 ± 15	15.2
L (5)	1023 ± 72	46 ± 8	0.01
NL (30)	953 ± 41	13 ± 2.5	0.94
H (21)	1179 ± 43	8.0 ± 1.1	7.66

There was a significant negative correlation (p<.05) between plasma AII and glomerular receptor number. Dietary Na^+ did not correlate with AII receptor concentration. We conclude that dietary Na^+ regulates glomerular AII receptors via changes in the circulating AII level.

PRE- AND POSTSYNAPTIC RESPONSES TO GRADED RENAL NERVE STIMULATION BEFORE AND DURING BETA-ADRENOCEPTOR BLOCKADE. M.L. Blair*, Y-H. Chen* and J.L. Izzo Jr. Depts. of Physiology and Medicine, University of Rochester, Rochester, New York.

Stimulation of presynaptic beta-adrenoceptors on noradrenergic nerve terminals is reported to augment norepinephrine release. We studied the effects of propranolol on renal norepinephrine (NE) efflux and renin secretion rate (RSR) during graded renal nerve stimulation in order to assess the functional importance of renal presynaptic beta-adrenoceptors in neural control of renin release. In 5 pentobarbital-anesthetized dogs, the renal nerves were transsected to remove tonic nerve activity and the distal ends stimulated (10-20 V, 0.5 msec) over the range of 0.3 - 5.0 Hz for consecutive 4 min periods. NE efflux and RSR were calculated as renal plasma flow times the veno-arterial hormone concentration difference. Basal NE efflux (-7.3 + 2.2 ng/min increased to -2.4 + 2.6, 1.7 + 4.7 and 34 + 18 ng/min at 0.3, 0.6 and 1.2 Hz respectively, with no change in renal blood flow (RBF). At 2.4 and 5.0 Hz, RBF decreased by 21 and 37%, but there was no further increase in NE efflux (36 + 12 and 21 + 6.4 ng/min, respectively). The pattern of RSR during nerve stimulation paralleled that of NE efflux at all frequencies. Propranolol (0.5 - 1.0 mg/kg + 0.4-0.5 mg/kg/hr IV, n=4) reduced the RSR response, but did not alter the effect of nerve stimulation on either NE efflux or RBF. Thus blockade with propranolol reveals functional evidence of post- but not presynaptic beta-adrenoceptors controlling renin release. Reduced NE efflux at the highest nerve stimulation rate was unexpected, and must be further investigated.

PROSTAGLANDIN ENDOPEROXIDE METABOLISM IN CONGENITAL UNILATERAL HYDRONEPHROTIC AND UNILATERAL URETERAL OBSTRUCTED RATS. F. Boineau, D. McNamara, M. McMullen-Laird, H. Lippton, J. Lewy, A. Hyman, and P. Kadowitz. Dept. of Pharmacology, Tulane Medical School, New Orleans, LA.

In order to determine whether or not the indomethacin reversible reduction in renal function reported in rats with congenital unilateral hydronephrosis (HN) is due to alterations in metabolism of prostaglandin endoperoxide PGH_2, we determined the metabolism of ^{14}C-PGH_2 by renal microsomes of rats with congenital HN, normal rats (NR) and those with unilateral ureteral obstruction (UUO). NR exhibited thromboxane synthetase (Txase) and PGE_2 isomerase. Congenital HN had Txase and PGE_2 isomerase similar to NR in both the contralateral and ipsilateral kidneys (CLK and ILK). In UUO: ILK-Txase activity fell at 24 hr, doubled at 48 and was 3-4 times NR at 72 hr after UUO; CLK-Txase fell at 24 hr and was the same as NR at 48 and 72 hr after UUO; PGE_2 isomerase in both ILK and CLK required reduced glutathione (GSH). The activity in CLK and ILK increased at 24 hr and was the same as NR at 48 and 72 hr after UUO. These data show: 1) NR has Txase activity; 2) UUO increases the Txase of the ILK but not the CLK and therefore the CLK may not be an adequate control for the ILK in UUO; 3) the increase in Tx formation in UUO can occur via mechanisms other than increased cyclooxygenase (CO) or phospholipase (PL) activity as previously reported; 4) unlike UUO, there is no alteration in PGH_2 metabolism in congenital HN therefore the indomethacin reversible reduction in renal function may involve CO or PL.

EFFECT OF REDUCED ALDOSTERONE METABOLITES IN THE TOAD BLADDER. Andrew S. Brem, Maryann Pacholski*, Christopher J. Kenyon*, and David J. Morris*. Brown University, Dept. of Pediatrics and Pathology, Providence, RI.

While the liver is a major site of aldosterone (aldo) metabolism, target tissues including rat kidney and toad bladder have been shown to transform aldo into various polar and reduced metabolites (J. Steroid Biochem 15:473, 1981). What role these metabolites play is at present unknown. The natriferic response of 3 pre-selected reduced metabolites, 5α DHA, 3α5β THA, and 5β DHA, were individually tested in the toad bladder. These dihydro and tetrahydro aldo metabolites were chosen initially since 5α DHA and 3α5β THA possess significant antinatriuretic activity in the ADx rat (1/10 and 1/50 of aldo) whereas 5β DHA has very little activity. Sodium transport was determined by the short circuit current technique (SCC). Aldo (10^{-7}M) was used as a control where appropriate. When placed in the serosal bath, 5α DHA (10^{-7}M) and 3α5β THA (10^{-7}M) both stimulated SCC. 5α DHA exerted a greater effect on SCC than 3α5β THA. Latent periods and time courses appeared similar to aldo, but the metabolites were less potent than aldo. 5β DHA appeared to have no effect on SCC. The reduced metabolites synthesized in the toad bladder have been chromatographed and indicate that at least one of the above compounds is made in this target tissue. Thus, not only are target tissues capable of reducing aldo directly, but some of these reduced metabolites have biologic activity. It is possible that enzymatic reduction of aldo in toad bladder cells may well play a role in mediating aldo stimulated Na transport.

NEPHRON SITE OF EFFECT OF NSAID'S ON SOLUTE EXCRETION IN MAN. D. Craig Brater, Polavat Chennavasin,* Shirley A. Anderson,* and Sming Kaojarern*. Univ. of Texas Health Science Ctr., Depts. of Pharmacol. and Internal Med., Dallas, Texas.

NSAID's decrease solute excretion when administered acutely to normal subjects. We performed clearance studies during water loading of 10 and during hydropenia in 8 additional volunteers to determine the nephron site of this effect using indomethacin and carprofen as inhibitors of prostaglandin (PG) synthesis. Their administration decreased fractional excretions of sodium, chloride, and volume. During water loading, fractional clearance of free water decreased from 0.13±0.04 to 0.09±0.03 and 0.06±0.02 with indomethacin and carprofen, respectively. However, fractional delivery of solute to the diluting segment decreased in parallel such that free water clearance corrected for delivery did not change with either drug; 86±3, 92±5, and 86±6%, respectively. Therefore, the decrement in solute excretion with administration of NSAID's occurs prior to the diluting segment. During hydropenia free water reabsorption relative to osmolar clearance increased from 0.59±0.13 to 0.70±0.04 ($P<0.05$). In both studies, neither markers of renal perfusion nor proximal nephron function changed with inhibition of PG synthesis. The data indicate that at the tubular level, NSAID's probably increase solute reabsorption at the medullary segment of the thick ascending limb. Consequently, a physiologic role of renal PG's at this nephron site is implied.

ANGIOTENSIN II (ANG II) BINDING SITES DIFFER IN RAT RENAL TUBULAR BASOLATERAL MEMBRANES (BLM) AND BRUSH BORDER MEMBRANES (BBM). Gail Brown* and Janice Douglas.* CWRU, Cleveland, Ohio (Intr. by Robert Eckel)

Rat renal tubular BBM contain specific Ang II binding sites as reported by us. The purpose of this study was to determine whether the contraluminal membranes, BLM, also contain Ang II binding sites, and to compare characteristics of these sites with those of the BBM. Membranes were prepared by differential-and/or isopycnic centrifugation in Percoll medium. Marker enzyme specific activities were enriched 9.7 (BLM, n=20) and 12 (BBM, n=33) fold. Data are means ± SEM: Steady-state binding of ^{125}I-Ang II was achieved at 10 min. with BBM and 30 min with BLM. The K_d was lower ($P<0.05$) in BLM (2.2 ± .2 nM, n=12) than in BBM (6.5 ± .9 nM, n=7). Binding site concentrations in BLM and BBM (929 ± 138 and 573 ± 36 fmol/mg protein) did not differ. Bound ^{125}I-Ang II at steady state was 61 ± 6% (BLM, n=3) and 57 ± 8% (BBM, n=4) intact, while free was 85% (BLM, n=2) and 46% (BBM, n=4) intact as determined by rebinding to fresh membranes. Cations affected binding kinetics in BLM and BBM similarly. Na+ (100 mM) decreased and Ca^{2+} (2-10 mM) increased K_d, while Mg^{2+} (1-4 mM in BLM and 10-25 mM in BBM) increased receptor site concentration. These data indicate the presence of specific receptors for Ang II of differing characteristics on BBM and BLM and suggest that there may be more than one mechanism by which Ang II affects Na+ reabsorption in tubular epithelial cells.

MECHANISM OF EFFECT OF DIPHENYLHYDANTOIN ON RENAL RENIN RELEASE. Pravit Cadnapaphornchai, Gayle Jones*, and Franklin D. McDonald. Department of Medicine, Wayne State University School of Medicine Detroit, Michigan.

Previous studies from our laboratory have shown that intrarenal arterial infusion of diphenylhydantoin (D) increased renin secretion rate (RSR). This rise in RSR was abolished by prior acute or chronic renal denervation. The present study was designed to examine the mechanism of renin release at a step(s) after the renal nerves. In 5 anesthetized, indomethacin-treated dogs, intrarenal infusion of D increased RSR from 131 ± 32 to 421 ± 106 ngAI/hr.min. ($p<.025$). GFR, renal blood flow (RBF) and blood pressure (BP) did not change significantly. $U_{Na}V$ increased from 6 ± 4 to 148 ± 2 uEq/min. ($p<.001$). In 5 dogs treated with Ca ionophore (A23187), D did not increase RSR significantly (150 ± 54 to 229 ± 70 ngAI/hr.min. GFR, RBF, $U_{Na}V$ did not change significantly but BP decreased from 153 ± 3 to 142 ± 3 mmHg ($p<.01$). In contrast, in 4 dogs treated with the vehicle for ionophore, D increased RSR from 188 ± 33 to 789 ± 126 ngAI/hr.min. ($p<.02$). GFR, RBF and BP did not change significantly. $U_{Na}V$ was 84 ± 22 uEq/min. before and 161 ± 30 after D ($p<.005$). These data suggest that D-stimulated renin release is mediated by the renal nerves. The step(s) after the renal nerve is/are not mediated by prostaglandins but appear to be mediated by changes in intracellular calcium.

HYPORESPONSIVE RENIN SECRETION IN THE SPONTANEOUSLY DIABETIC RAT. A.J.Cohen, D.M.McCarthy, and D.M. Clive, UMass Medical Center, Worcester, MA

Decreased plasma renin activity (PRA) and kidney renin content (KRC) have been demonstrated in alloxan- and streptozotocin-induced experimental diabetes but the potential nephrotoxicity of these agents makes these observations difficult to interpret. We studied renin secretion in the genetically diabetic BB/W rat, which develops spontaneous insulinopenia, hyperglycemia, and ketoacidosis. Diabetic (D) animals >100 days after onset of glycosuria were compared with age-matched, nondiabetic BB/W controls (C). PRA (ng/ml/hr) was measured after one week of oral sodium loading (SL) or deprivation (SD), following which the right kidneys were isolated and perfused for measurement of isoproteronol-stimulated renin release (RR,µg/g.k.w./min), and the left kidneys were removed for quantitation of KRC (ng/g.k.w.). % of KRC released per minute was calculated.

SD	n	PRA	KRC	RR	% KRC released
controls	9	9.1±1.8	54.0±11.8	95±8	.33±.14
diabetics	8	7.8±1.1	66.5±8.0	49±7†	.08±.01*
SL					
controls	11	1.1±0.2	16.2±2.9	22±8	.12±.02
diabetics	9	2.2±0.8	34.8±4.6†	15±4	.04±.01*

p(D vs C) * = <.05, † = <.005

As shown, PRA following either SD or SL was similar in D and C, unlike in chemically-induced diabetes. After SD, KRC was similar in D and C, whereas RR was considerably greater in C. In contrast, after SL, KRC was greater in D, but RR was similar in both groups. % KRC released was smaller in D than in C after both SD and SL. Although in D renin synthesis is equal to or greater than in C, RR is hyporesponsive to β-adrenergic stimuli.

EVIDENCE FOR GLOMERULAR PROSTAGLANDIN [PG]E-RECEPTORS IN RATS. A. Chaudhari* and M.A. Kirschenbaum, UCLA Dept. of Med., Los Angeles, CA.

Previous studies evaluating glomerular hemodynamics and function have suggested that PGE may exert some of its biologic effects through interaction with appropriate receptors. Recently, it has been shown that PGE_2 increases cyclic-AMP levels in isolated rat glomeruli [Wang and Kurokawa, Biochim Biophys Acta, 677:397-402, 1981] suggesting stimulation of adenylate cyclase. This observation supports the existence of PGE receptors in the glomerulus. The present study was performed to examine the characteristics of ^{3}H-PGE_1 binding to specific PGE-receptors. Rat glomeruli (>95% pure), isolated by a sieving method, were incubated with ^{3}H-PGE_1, 78 nM, at 37° C for 1 h. The tissue-bound ligand was separated from free ligand using a Millipore® filter. Non-specific binding was defined as binding which was not displaced by 125 µM PGE_1 and was 30-50% of the total binding. Nonspecific binding subtracted from total binding was interpreted as specific binding. Both PGE_1 and PGE_2 produced a dose-dependent inhibition of specific binding resulting in maximum inhibition of 88 and 60% respectively at a concentration of 1.25 µM. Following a 1 h incubation of glomeruli with ^{3}H-PGE_1, addition of 63 µM PGE_1 and an additional 30 min incubation resulted in displacement of approximately 50% of the prebound radioligand. Indomethacin, 25 and 100 µM, inhibited binding by 17 and 50% respectively thus ruling out interference by endogenously synthesized PGs. In summary, these data provide evidence for PGE-receptors in intact rat glomeruli and are consistent with the premise that PGE_2-induced cyclic AMP accumulation may be related to adenylate cyclase stimulation in these cells.

MECHANISMS OF RENIN INHIBITION AND RENAL VASOCONSTRICTION EFFECTS OF OUABAIN: DEPENDENCE ON CALCIUM. Manuel Cruz-Soto, Julio E. Benabe and Manuel Martínez-Maldonado. VA Center, Medical Service, San Juan, Puerto Rico.

Ouabain (OUA) induces natriuresis (Na), renal vasoconstriction (RVC) and inhibits renin secretion (RS). These studies were aimed at characterizing the role of cytosolic (Cyt.) calcium (Ca) on the renal effects of OUA. Experiments were performed on two groups of anesthetized dogs. All drugs were administered into the renal artery. In Group I (N=7) OUA (1 ug/kg/min) increased urine flow (V̇= 1.85±0.44 ml/min) and FENa (10.32±2.1%); both continued to rise until the end of exp. (80 min). The GFR and RS declined at 30 min and remained depressed thereafter. The renal blood flow (RBF) decreased from 109±20 to 79±7.7 ($p<0.02$), but spontaneously returned to baseline at 60 min. Blood pressure (BP) and hematrocrit (Hct) did not change. In Group II (N=6), the Ca channel blocker verapamil (100 µg/min) increased V̇ (4.68±0.52 ml/min), FENa (12.9 ±1.16%) and RBF (116±12 to 147±23 ml/min $p<0.02$), whereas decreased BP (113±0.5 to 103±6.8 $p<0.01$). The RS increased in 4 dogs and decreased in 2. There were no changes in GFR or Hct. Superimposition of OUA elicited further diuresis and Na, but no changes in GFR, RBF, BP or Hct. The RS increased in all dogs but one. These data demonstrate that blockade of Ca channels prevents the RVC and renin inhibition by OUA. Thus both effects are dependent upon entry of Ca into Cyt. The late spontaneous increase in RBF during OUA suggests that some intracellular mechanism operate to reduce Cyt Ca allowing vasodilatation.

PROSTAGLANDIN (PG)-VASOPRESSIN (AVP) INTERACTIONS IN SICKLE CELL NEPHROPATHY (SCN). P.E.de Jong,* A.W.Saleh,* D.de Zeeuw,* A.J.M.Donker,* G.K.van der Hem,* L.W.Statius van Eps. Dept.of Med. State Univ.Hosp. Groningen, the Netherlands.

There is indirect evidence that renal PG's do compensate for medullary defects in SCN. We suggested either a high AVP and/or an increased PGE_2 in SCN (Clin.Sci.63,53,1982) Therefore we studied plasma(P) and urinary(U) AVP and PGE_2 and $F_{2\alpha}$ excretions in 7 patients with SCN and 6 controls. AVP and PG's were measured with RIA, the latter after purification with HPLC. U osmolality(osm) decreased from 423 to 50 mOsm/kgH_2O in SCN and from 1026 to 55 mOsm/kgH_2O in controls. P_{osm} fell from 291 to 283 mOsm/kgH_2O in SCN ($p<0.02$) and from 289 to 284 mosm/kgH_2O in controls ($p<0.05$). Sodium excretion was similar in both groups. Mean and SD of AVP and PG measurements were:

		P_{AVP}	U_{AVP}	PG E_2	PG $F_{2\alpha}$	$E_2/F_{2\alpha}$
		pg/ml	pg/min	pg/min	pg/min	ratio
water deplete	SCA	11.2(1.4)	227(163)	436(183)	119(82)	6.5(6.4)
	Contr.	9.7(1.8)	159(70)	395(387)	545(258)	0.7(0.3)
	p	ns	ns	ns	<0.01	<0.01
water loaded	SCA	7.5(1.5)	29(11)	5680(3208)	893(514)	6.7(2.8)
	Contr.	7.8(1.0)	39(21)	2910(1619)	1312(513)	2.1(0.5)
	p	ns	ns	ns	ns	<0.01

For every mOsm decrease in P_{osm} a mean fall in P_{AVP} of 0.33 pg/ml was found both in SCN and Contr. We conclude that AVP production as well as PGE_2 excretion are normal in SCN, whereas $PGF_{2\alpha}$ excretion is found decreased. Therefore the $E_2/F_{2\alpha}$ ratio is higher in SCN. This suggests a relative increase in PGE_2 activity, which could be due to a defect in PGE_2-9-ketoreductase in sickle cell anaemia.

SODIUM BALANCE AS A DETERMINANT OF PGI_2 PRODUCTION BY ISOLATED RAT AORTA. Rainer Düsing, Rudi Scherhag*, Kilian Glänzer*, and Herbert J. Kramer. Medizinische Universitäts-Poliklinik Bonn, West Germany.

The present study investigates whether changes in the vascular synthesis of prostacyclin (PGI_2) participate in the altered vascular responsiveness during changes in NaCl balance. Two groups of 10 rats each were fed a diet low in NaCl with group I receiving distilled water and group II 0.9% saline as drinking water for 10 days. At the end of the dietary protocol, systolic arterial blood pressure was significantly lower in group I (101±2 mm Hg) as compared to group II (110±3 mm Hg; $p < 0.05$). Generation of PGI_2-like activity was determined in the animals' isolated aorta using a platelet aggregation bioassay following incubation in Tris buffer for 12, 15, and 30 min. During these incubation times, generation of PGI_2 was 37.5±2.8, 46.2±3.2, and 61.3±4.0 pmol/mg in group II and was significantly stimulated in group I to 50.2±2.5, 57.7±2.7, and 72.9±3.7 pmol/mg ($p < 0.01$), respectively. When added directly into the incubation buffer, angiotensin II and phenylephrine but not isoproterenol induced a dose-dependent stimulation of the release of PGI_2. Our results show that NaCl restriction decreases arterial blood pressure and stimulates the vascular synthesis of PGI_2-like activity possibly via angiotensin II and/or increased α-adrenergic activity.

INCREASED AFFINITY AND DENSITY OF GLOMERULAR ANGIOTENSIN II (ANG II) RECEPTORS FOLLOWS ANG II INFUSION IN RATS. Janice Douglas,* Carson White,* and Gail Brown.* Univ. Hosps., Cleveland, Ohio. (Intr. by Robert Eckel).

Low dose Ang II infusion has been demonstrated by us to increase the affinity of Ang II binding to smooth muscle receptors. This correlates with increased vascular sensitivity to Ang II. Since glomeruli like smooth muscle exhibit a contractile response to Ang II, we were interested in whether Ang II infusion affects glomerular receptors in a similar manner. Continuous infusion of Ang II with osmotic minipumps in male rats at a dose of 33 ng/kg/min resulted in a 4-fold increase in plasma Ang II and a 2-fold increase in aldosterone (Aldo). Isolated glomeruli were used in a radioreceptor assay for Ang II. The K_d decreased significantly from the control value of 6.7 X 10^{-10}M to 5.2 X 10^{-10}M ($P<0.02$, n=4) and the receptor concentration increased from 893 to 1221 fm/mg protein ($P<0.005$). To determine whether these changes were due to altered Ang II and/or Aldo blood levels, we infused Aldo alone and with Ang II. With a constant Aldo blood level, Ang II decreased the K_d by 20% from 6.03 X 10^{-10}M to 4.83 X 10^{-10}M ($P<0.005$, n=3). With a constant Ang II blood level, an increase in plasma Aldo caused a significant decrease in receptor concentration from 1221 to 909 fm/mg protein ($P<0.05$, n=4). These studies demonstrate direct regulatory influences by both Ang II and Aldo. The predominant effect of Ang II was to increase receptor affinity, while Aldo affected receptor density. Low dose Aldo increased, while high dose decreased receptor density. Ang II infusion should enhance sensitivity of the glomerular contractile response to Ang II.

DISSOCIATION OF RENIN FROM PROSTAGLANDINS IN PATIENTS WITH CIRRHOSIS. Murray Epstein, Meyer Lifschitz & Oscar Larios.* Depts. Med., Univ. of Miami Sch. of Med. & Univ. of Texas Health Sci. Ctr. & VA Med. Ctrs., Miami, FL & San Antonio, TX.

Most previous studies have suggested that prostaglandins (PGE_2 and PGI_2) are important factors involved in augmenting renin release from the kidney resulting in an increase in plasma renin activity (PRA). In this regard, PGI_2 has recently been proposed as being more important than PGE_2. Because cirrhotic patients often manifest marked alterations of the renin-angiotensin system, we chose to study this patient population in order to evaluate in a dynamic manner the relationship between PRA and prostaglandins E_2 and I_2. Since the central hypervolemia (CV) induced by water immersion (NI) has been shown to suppress PRA levels, NI afforded a unique opportunity to assess simultaneous changes in PGE, 6-keto $PGF_{1\alpha}$, and PRA in patients with decompensated cirrhosis. 6-keto $PGF_{1\alpha}$ (6-keto) was measured as an index of PGI_2 production. Twelve cirrhotic patients were studied during NI while in balance on a 10 mEq Na, 100 mEq K diet. Urinary PGE and 6-keto were measured hourly and PRA at 60-min intervals. During NI, PGE excretion increased from a pre-study mean of 4.0±1.4 to 12.1±2.0 ng/min during immersion ($p< 0.001$). Simultaneously, 6-keto increased from a pre-study mean of 3.2±0.6 to a peak of 9.1±1.2 ng/min ($p<0.001$). Concomitantly, PRA decreased from pre-study level of 2.0±0.6 ng/ml/hr to a nadir of 0.4±0.1 during hours 3 and 4 of NI ($p<0.05$). The observed dissociation of PGE and 6-keto from PRA suggests that PGE and PGI_2 may not constitute the major determinants of the activity of the renin-angiotensin axis in cirrhotic man.

IN VIVO PRODUCTION OF PROSTACYCLIN IN DAHL SALT-SENSITIVE AND SALT-RESISTANT RATS. Pierre Falardeau and André Martineau, (intr. by Vincentiu Béroniade), Clinical Research Institute of Montreal, Lab. on Prostaglandin Res., Montreal, Quebec, Canada.

Prostacyclin (PGI_2), a potent vasodilator synthesized by the blood vessels, has been postulated to play a role in hypertension, either as a cause or as an effect. The goal of the present study was to evaluate the in vivo production of prostacyclin in Dahl salt-sensitive (SSR) and salt-resistant (SRR) rats.

The 24 hour-urinary excretion of an endogenous metabolite of prostacyclin, dinor-6-oxo-$PGF_{1\alpha}$, was measured by combined gas chromatography-mass spectrometry and used as an index of the total production of prostacyclin by the animals (Fitzgerald, Brash, Falardeau and Oates, J. Clin. Invest. 68:1272-1276, 1981).

Following the administration of a high salt diet (8% sodium chloride, starting at 35 days of age), the levels of this metabolite markedly increased in the SRR (from 35 ± 6 ng/24h. at 35 days to 52 ± 4 and 56 ± 10 ng/24h. at 60 and 80 days, respectively), whereas they decreased slightly in the SSR (from 41 ± 7 ng/24h. at 35 days to 30 ± 6 and 28 ± 9 ng/24h. at 60 and 80 days, respectively). During the same period, the SRR remained normotensive (111 ± 8 mmHg, systolic blood pressure) while the blood pressure of the SSR increased gradually (to 142 ± 8 and 175 ± 20 mmHg at 60 and 75 days, respectively).

These results suggest that a relative deficiency in the biosynthesis of prostacyclin may play a role in the genesis of hypertension in the SSR following a pro-hypertensive stimulus.

ALDOSTERONE AND INSULIN STIMULATION OF K^+ TRANSPORT IN CULTURED KIDNEY CELLS (A6). M.L. Fidelman* and C.O. Watlington. Med. Coll. of Virginia-VCU, Dept's of Med. & Physiol., Richmond, Va.

A6 epithelia grown on permeable supports exhibit active Na^+ transport (short-circuit current, I_{sc}) responsive to aldosterone (A) and insulin (I). In this study A and I effects on basolateral to apical K^+ transport were evaluated separately and in combination. Epithelia were incubated for 24h with K^+-free medium on the apical side and varying K^+ concentration (2, 4.5, 7 and 9.5 mM) on the basolateral side. Apical K^+ accumulation (flame photometry) and final PD and I_{sc} were determined. In control series K^+ accumulation was a positive and near linear function of basolateral K^+ concentration. Hormonal stimulation of K^+ transport seemed to begin in 6h but most occurred in 6-24h. The range of stimulation of apical accumulation (6-24h) at the four basolateral K^+ concentrations was: I, 10-35%; A, 50-80%; and A+I, 150-225%. In each case increase in A+I was greater than the sum of A and I effects alone. A similar synergistic effect of A+I on final I_{sc} was observed. Network thermodynamic simulations show it to be highly unlikely that changes in K^+ transport produced by A and A+I are due solely to increased electrical gradients across a single membrane e.g. tight junctions. The EC_{50} for aldosterone stimulation of K^+ transport and I_{sc} in the presence of a fixed concentration of I was $1\text{-}3\times10^{-8}$M, the range previously reported for I_{sc} stimulation by A alone and its K_d' for nuclear binding. Aldosterone may stimulate transcellular K^+ transport by a receptor mediated mechanism in A6 cells. Insulin may play an important interactive role with aldosterone in transepithelial Na^+ and K^+ transport regulation.

ADH ACUTELY INCREASES URINARY PROSTAGLANDIN EXCRETION. Geza Fejes-Toth*, Aniko Naray-Fejes-Toth* and Jürgen C.Frölich. Institute of Clinical Pharmacology, Stuttgart, Germany

Although it is well-established that chronic treatment with antidiuretic hormone increases renal prostaglandin (PG) excretion the effects of short-term infusions are controversial. Therefore, in the present study the effect of acute administration of arginine-vasopressin (AVP) on urinary PG excretion was investigated in conscious Brattleboro (DI) rats and in water-diuresing Long-Evans (LE) rats. Water balance was kept constant during AVP infusion. AVP caused a significant, dose-related and reversible increase in urinary PG excretion within 20 min in both models. Similar results were obtained during the infusion of 1-deamino-8-D-arginine vasopressin in the DI rat. Normalization of hydropenia of DI rats by infusion of large amounts of hypotonic fluid or water loading in LE rats failed to elevate urinary PG excretion. In spite of a similar degree of water loading PGE_2 excretion, both basal as well as AVP-stimulated, was significantly lower in DI rats suggesting that chronic exposure to AVP exerts an effect on urinary PG excretion in addition to that seen after acute AVP administration. Blockade of the kallikrein-kinin system (KKS) with aprotinin as evidenced by complete suppression of urinary kallikrein activity, had no effect on the renal response to AVP. These results suggest that the acute enhancement of urinary PG excretion is not mediated by vasoconstriction, volume retention, induction of cyclooxygenase or by the renal KKS but rather indicate that ADH can increase renal PG synthesis through a more direct mechanism.

GLOMERULAR PROSTAGLANDIN SYNTHESIS LINKED TO PHOSPHATIDYLINOSITOL TURNOVER. Vaughn W. Folkert and Detlef Schlondorff. Albert Einstein College of Medicine, Dept. of Med., Bronx, New York.

Glomerular prostaglandins (PG) play an important role in controlling glomerular function. The mechanisms which control release of arachidonic acid (AA) and PG synthesis and whether AA is released via a phospholipase A_2 or C remain poorly defined.

To define which factors control AA release in isolated rat glomeruli and to identify the AA pool related to PG synthesis we used as tools calcium ionophore A23187, trifluoperazine (TFP), a calmodulin inhibitor, and mepacrine, a phospholipase inhibitor. Phospholipid turnover was measured by {^{14}C}AA and {^{32}P} orthophosphate incorporation and total phospholipid content by inorganic phosphorus content. PG were measured by RIA.

A23187 (2μM) significantly stimulated PGE_2 and $PGF_{2\alpha}$ synthesis, increased phosphatidylinositol (PI) turnover and decreased total PI content. A23187 had no significant effect on phosphatidylcholine (PC) or phosphatidylethanolanime (PE). TFP (10 to 100μM) progressively increased PGE_2 and $PGF_{2\alpha}$ synthesis and also increased the turnover of phosphatidic acid and PI and decreased PI content. However, TFP significantly inhibited PC and PE turnover and their total content was unaffected. Mepacrine (1mM) markedly inhibited PG synthesis and both PC and PE turnover but had no consistent effect on PI. These findings indicate that glomerular PG synthesis is linked to a specific AA pool in PI controlled by phospholipase C which is controlled by calcium but in a calmodulin independent manner. In contrast, PC and PE are controlled by a calcium-calmodulin dependent phospholipase A_2.

CONTROL OF RENAL HEMODYNAMICS, ELECTROLYTE EXCRETION AND ARTERIAL PRESSURE DURING ACUTE AND CHRONIC INTRARENAL INFUSION OF GLANDULAR KALLIKREIN. Joey P. Granger* and John E. Hall, Dept. of Physiology, Univ. of Miss. Med. Ctr., Jackson, MS.

The objective of this study was to examine the effects of acute and chronic intrarenal infusion of glandular kallikrein(GK), which releases endogenous kinins from kininogens, on control of renal hemodynamics, electrolyte excretion, and arterial pressure (AP). In 6 normal dogs, acute intrarenal infusion of GK(1 U/min) for 60 mins caused no significant changes in GFR while increasing renal blood flow from 174±15 to 281±31, 237±26, and 221± 25 ml/min after 10, 30, 60 mins of GK infusion, respectively. Calculated pre- and postglomerular resistances fell during acute GK infusion. Acute GK infusion increased urine volume(UV) by an average of 67±6% while causing no significant changes in urinary Na excretion ($U_{Na}\dot{V}$). In 6 conscious dogs, intrarenal infusion of GK (1 U/min) for 7 days caused no significant changes in AP, renal vascular resistance(RVR), GFR, or effective renal plasma flow. GK, however, did increase $U_{Na}\dot{V}$ by 66%, UV by 116%, and decreased $U_{osm}\dot{V}$ by 44% during the first 24 hrs of infusion. However, after 1 day of GK infusion UV, $U_{Na}\dot{V}$ and $U_{osm}\dot{V}$ returned toward control levels. Chronic GK infusion caused no significant changes in PRA or plasma aldosterone concentration. Results from these studies indicate that although increased intrarenal kinin formation can cause potent acute effects on RVR and UV, these effects wane within 24 hrs and do not result in a long-term decrease in AP. The lack of a chronic effect of GK on AP, UV and renal hemodynamics is not due to a compensatory response of the renin-angiotensin-aldosterone systems.

URINARY PROSTAGLANDIN E_2 (PGE_2) IS A POOR INDICATOR OF PGE_2 IN RENAL PAPILLA. MA Johnson*, L Tobian, T. Ferris, J Iwai*; Univ of Minnesota & Brookhaven Lab.

PGE_2 concentration in quick-frozen renal papilla is always twice higher in 15-week Dahl R rats than in 15-week Dahl S rats(18 rats/group): 42ng/100mg solids vs 17 on .3%NaCl diet with both groups normotensive($p<.01$); 80 vs 41 after 4 weeks of 4%NaCl diet($p<.05$); 78 vs 39 after 11 weeks of 4%NaCl diet ($p<.01$). However urinary PGE_2(ng/hour/kg body weight) was not significantly different between R & S rats in any of these three groups(6 vs 5 on .3% NaCl; 13 vs 12 on 4%NaCl for 4 weeks; 14 vs 12 on 4%NaCl for 11 weeks). The 60% reduction in PGE_2 concentration in pre-hypertensive S papillas suggested that this feature could serve to predict future hypertension in young rats or possibly in young humans. Thus in 5-week Dahl rats, papillary PGE_2 concentration averaged 184 in 8 R rats vs 62 in 8 S rats, a threefold difference in young rats ($p<.05$). Alas however, this marked difference could not be found in urinary PGE_2(ng/hr/kg body weight) in 5-week rats. Urinary PGE_2 averaged 28 in 20 R rats vs 35 in 21 S rats on normal chow(NS). After drinking a .01%methyclothiazide solution for 24 hours, urinary PGE_2 averaged 13 in 19 R rats vs 12 in 20 S rats(NS). After drinking .01%methyclothiazide in 1%saline for 24 hours, urinary PGE_2 averaged 19 in 12 R rats vs 14 in 14 S rats(NS). After drinking 1%saline for 24 hours, urine PGE_2 averaged 53 in 11 R rats vs 38 in 13 S rats(NS). During 30 min after s.c. furosemide, urine PGE_2 was almost identical in S & R rats. With all these maneuvers, no significant difference was noted; hence urinary PGE_2 was indeed a poor indicator of papillary PGE_2. It is not at all definite how PGE_2 in different renal areas relates to urine PGE_2.

DO PHYSIOLOGICAL LEVELS OF GLUCOCORTICOIDS AFFECT ELECTROLYTE TRANSPORT? John P. Hayslett, Rodolfo S. Martin* and Will J. Jones*. Dept. of Med., Yale School of Medicine, New Haven, CT.

Recent studies suggest that glucocorticoids (Gluc), at physiologic plasma levels, increase Na-K-ATPase activity, electrolyte transport and PD of the renal tubule and colon. All studies have employed synthetic agents, such as dexamethasone, administered by bolus injection and dose levels were determined from estimates of relative potency compared to endogenous adrenal hormones. Since plasma levels of synthetic hormones have not been measured and these substances bind to plasma proteins with low affinity, it seemed possible that observed transport changes resulted from the pharmacological action of Gluc. To examine this question studies were performed in adrenalectomized animals to determine the physiological plasma level of aldosterone (Aldo), administered by minipump, and corticosterone (Comp B), administered by implanted pellets. Transmural PD in colon was used as a marker for Aldo action. Physiologic levels were 3.8 ± 0.3 ng/dl of Aldo and 3.0 ± 0.2 μg/dl of Comp B. Chronic, stable levels of Comp B > 6 μg/dl increased colonic PD. Dexamethasone in a dose of 5 μg · 100 gm BW^{-1} · 12 hr^{-1} resulted in plasma levels of 3.8 ± 0.3 μg/dl for at least 3 hours and markedly increased colonic PD.

Chronic administration of synthetic hormones in amounts calculated to be physiological result in high transient plasma levels which are largely available to tissue receptor sites, compared to Comp B which is tightly bound to CBG (80%). The observed actions on transport epithelium therefore, may reflect a pharmacologic effect.

TYROSINE-STIMULATED DOPAMINE SYNTHESIS FAILS TO AFFECT URINARY ALDOSTERONE AND SODIUM RELATIONSHIP IN NORMAL WEIGHT AND OBESE WOMEN. Janice L. Johnston,* Alexander G. Logan, and G. Harvey Anderson*. Univ. Toronto, Dept. Nutritional Sciences and Medicine, Toronto, Ont. Canada.

The effect of tyrosine (tyr)-induced increases in dopamine (DA) and norepinephrine (NE) turnover on the relationship between aldosterone and sodium excretion was measured in normal weight and obese subjects adapting to a low sodium diet. In six normal weight controls, during four continuous days on a liquid diet, Ensure®, with an average intake of 57 meq sodium, urinary DA and NE metabolites were unchanged while urine sodium decreased until balance was achieved and aldosterone increased. The inverse relationship between urinary sodium and aldosterone was also maintained in six normal weight subjects even after tyr induced 9% increases in the urinary DA metabolite, homovanillic acid (HVA), and in the NE metabolites, vanilmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) (all $p<0.05$). Similarly, while tyr supplement- increased urinary HVA and VMA in nine obese subjects sodium and aldosterone remained inverse related. We conclude first that enhanced DA and NE synthesis and catabolism, as measured by urinary HVA, VMA and MHPG occur with no effect on the relationship between urinary sodium and aldosterone, second that DA is not natriuretic at low sodium intakes, third, that DA is not inhibitory of aldosterone secretion at low sodium intakes.

RENAL ROLE OF DOPAMINE 1 RECEPTORS IN SALT LOADING
P.A.Jose, R.D.Fildes*,R.A.Felder*, J.C. Pelayo & P.L.Calcagno. Georgetown Univ. Med. Ctr., Wash., D.C. & V.A. Hospital, San Diego, Calif.

We have reported that the prior administration of the dopamine (D) antagonist (anti) cis-flupenthixol (CF), to hydropenic rats attenuated the diuresis induced by Ringer's loading (RL). We examined further the effects of dopamine blockade on the renal response to RL by administering CF (Gr.I) to diuresing rats induced by RL. Since perfusion pressure, mm Hg,(PP) is decreased by CF, the stereo isomer transflupenthixol with minimal D anti properties was also administered in another group of rats (Gr.II) at comparably reduced PP by constricting the aorta above the renal arteries. The results (M±SEM) are tabulated:

		$\bar{P}P$	GFR[a]	V[b]	UNaV[c]	%FENa
RL alone	I.	113±6	1.0±.11	122±27	5.0±.91	3.3±.31
	II.	112±1	1.0±.05	153±18	4.9±.55	3.0±.17
5 ug/ kg/min	I.	96 ±8*	0.8±.15	18±8*	0.6±.30*	0.7±.35*
	II.	86 ±2*	0.9±.05	141±15	5.1±.62	3.5±.24
10 ug/ kg/min	I.	90 ±9*	0.7±.09	13±3*	0.7±.28*	0.7±.25*
	II.	79 ±1*	0.9±.04	132±14	5.8±.92	3.9±.30
Recovery	I.	91 ±5*	0.9±.07	38±6*	2.7±.55*	1.9±.34*
	II.	88 ±1*	0.9±.05	133±17	5.5±.73	3.8±.29

*p <.05 compared to RL alone(unpaired t test) n=6/Gr. a=ul/100 G/min; b=ul/min; c=uEq/100 G/min

Thus the attenuation of diuresis induced by RL is not due to changes in PP or GFR but rather to a direct tubular effect of CF. In additional studies we showed that D stimulated adenylate cyclase activity in renal basolateral membranes (EC_{50} of 10^{-6}M). CF inhibited the ability of D 10^{-4}M to stimulate adenylate cyclase (K_i of 10^{-7}M). Therefore D plays a significant role in the diuresis of RL via occupation of D_1 receptors.

PROSTAGLANDIN E_2 (PGE_2) IN RENAL PAPILLA INFLUENCES Na DEPENDENCE IN HYPERTENSION.
J Lange*, L Tobian, MA Johnson*, J Iwai*, Univ of Minnesota & Brookhaven.

Hypertension can have great variability with regard to Na dependence. Hypertension in Dahl S rats fed 8%NaCl can be completely prevented when drinking .01%methyclothiazide. In sharp contrast, drinking .01%methyclothiazide will not decrease the BP of Kyoto SHR whatsoever. Isolated kidneys from prehypertensive Dahl S rats excrete half as much Na as those from Dahl R rats. Isolated kidneys from SHR have no reduction in Na excretion whatsoever, compared to WKY rats. The PGE_2 concentration in renal papilla could partially account for this marked variation. PGE_2 concentration in quick-frozen renal papilla is always twice higher in 15-week Dahl R rats than in 15-week Dahl S rats(18 rats/group): 42 ng/100mg solids vs 17 on .3%NaCl diet($p<.01$); 80 vs 41 after 4 weeks of 4%NaCl diet($p<.05$); 78 vs 39 after 11 weeks of 4%NaCl diet($p<.01$). In 5-week Dahl rats, papillary PGE_2 averaged 184 in 8 R rats vs 62 in 8 S rats, a threefold difference($p<.05$). Contrastingly, 14 4-week borderline hypertensive SHR had an average papillary PGE_2 concentration of 65 vs 27 in 14 4-week normotensive WKY rats, an 140% increase($p<.001$). The reduced papillary PGE_2 in S rats is thought to enhance Na transport in ascending limb, collecting tubule & collecting duct, & reduce papillary plasma flow, thereby preventing rapid natriuresis and enhancing hypertension in S rats. SHR actually have a striking increase in papillary PGE_2 in conjunction with the complete inability of thiazide to lower their BP. Their kidneys, replete with PGE_2, can excrete Na so rapidly that thiazide offers no real enhancement.

EFFECT OF ACUTE POTASSIUM (K) INFUSION ON CORTICAL LOOP SEGMENT (LS) CHLORIDE (Cl) UPTAKE AND PLASMA RENIN ACTIVITY (PRA).
Kent A. Kirchner. Univ. of Mississippi Med. Ctr., Dept. of Med., Jackson, MS.

Both K and Na^+ loading suppress PRA via the macula densa (MD) mechanism. We proposed Cl is the signal for renin release via the MD. However, Cl administration is necessary for PRA suppression only during Na^+ and not during K loading. To determine if K induced changes in nephron Cl handling which could account for PRA suppression during K loading, the effect of 0.3M KNO_3 infusion on late proximal (LD) and early distal (ED) Cl delivery and PRA was examined by micropuncture in male Sprague-Dawley rats. To exclude effects of volume expansion or anion on the Cl uptake and PRA, control rats received an equal volume of 0.3M $NaNO_3$. MAP, plasma volume expansion, whole kidney GFR, LP and ED SNGFR were not different (p-NS) between groups. Plasma Cl concentration, LP tubule Cl concentration, and TF/UF Cl ratio were not different (p-NS) between groups. ED tubule Cl concentration (39.4±3.4 pEq/nl vs 25.0±1.8; $p<.001$; mean±SEM) and TF/UF Cl ratio (0.39±0.4 vs 0.24±0.2; $p<.005$) were greater following K than Na^+ infusion. K infusion decreased absolute (2026±143 pEq/min vs 3064±173; $p<.001$) and fractional (84±1% vs 90±1; $p<0.02$) superficial LS Cl reabsorption compared to Na^+ infused animals. PRA was less ($p<.001$) following K than Na^+ infusion (10.2±1.4 ng A_I/ml/hr vs 24.1±2.7). We conclude (1) K loading has no effect on LP tubule Cl handling and (2) K loading decreases superficial LS Cl reabsorption and increases Cl delivery to the ED tubule. These findings are consistent with the hypothesis that K suppresses PRA through increased MD Cl delivery.

ANTIGEN SIMILAR TO GLANDULAR KALLIKREIN IN PLASMA IS INCREASED IN DIALYSIS PATIENTS.
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Based on our prior animal work, the kidneys may remove glandular kallikrein from plasma. A radioimmunoassay was used to detect immunoreactive glandular kallikrein in human plasma (IRK). The antibody to human urinary kallikrein (HUK) was provided by Dr. J.V. Pierce, NIH, and cross-reactivity is not seen with plasma kallikrein or kininogen but is seen with both active urinary kallikrein and prokallikrein. Plasma was studied from normals (NL), patients with kidneys undergoing hemodialysis (PT) and bilaterally nephrectomized patients undergoing Hemodialysis (PT-NX). Antigen in plasma was not immunologically identical to the purified HUK standard. The results below show comparative values of antigen detected at 1:4 dilutions in NL and predialysis plasma.

Group	n	IRK ng/ml (x̄±SEM)
NL	9	19.3±4.4
PT	5	42.2±8.6
PT-NX	5	117.6±58.2

The NL were significantly different from the PT and PT-NX ($p<.05$). IRK in PT vs. PT=NX was not different. When plasma was diluted 1:16, antigen was not detected in any normals but was detected in the 5 PT-NX tested. Hemodialysis had no effect on the antigen levels.

Patients with chronic renal failure requiring hemodialysis have increased immunoreactive antigen similar to glandular kallikrein in plasma and the highest values were present in some bilaterally nephrectomized patients. This data supports a role for the kidney in the normal handling of glandular kallikrein in plasma.

ROLE OF ARGININE VASOPRESSIN (AVP) IN THE SUPPORT OF BLOOD PRESSURE IN POTASSIUM DEFICIENT (KD) CONSCIOUS RATS. S. Linas and M. Paller, University of Colorado, Denver, CO, and University of Minnesota, Minneapolis, MN.

AVP has been found to contribute to the maintenance of blood pressure (BP) in the rat. Since KD results in alterations in systemic hemodynamics the role of AVP in the control of BP was studied after 14-21 days of dietary KD. When KD and control (C) rats were allowed free access to water, plasma osmolality (301.4 vs 293.4 mOsm/kg; $p<.02$) and plasma AVP (3.5 vs 2.4 pg/ml; $p<.02$) were increased in KD animals. In order to determine the role of this increase in AVP in the maintenance of BP, BP was determined in rats made polydipsic (PD) by adding 2.5% glucose to the drinking water. In both C and KD rats, increased fluid intake resulted in an increase in urine output, and a decrease in urinary osmolality and a decrease in plasma AVP. While there was no change in BP in PD-C rats, BP fell from 103.9 in KD rats to 96 mmHg ($p<.05$) in PD-KD rats. In order to confirm that the decrease in plasma AVP caused the decrease in BP in KD rats, an AVP pressor antagonist was employed. Following the administration of the AVP pressor antagonist, there was no change in BP in C animals. In contrast, BP fell from 104.3 to 98.3 mmHg ($p<.05$) in KD rats. Moreover, there was no decrease in BP when the AVP antagonist was given to PD-KD rats with suppressed AVP levels. In summary, KD results in an elevation in plasma AVP as a consequence of hyperosmolality. Since maneuvers which decreased either the content or action of AVP resulted in a decrease in BP in KD, but not C rats, we conclude that AVP is important in the support of BP in the KD rat.

ELEVATED PLASMA NOREPINEPHRINE (NE) IN ACUTE RENAL FAILURE: ATTENUATION OF NE AND RENAL PROTECTION BY SPLENECTOMY. Anil K. Mandal. V.A. Medical Center and Medical College of Georgia, Augusta, Georgia.

We have shown that chronic splenectomy is protective against epinephrine (EPI)-induced acute renal failure (ARF) in dog. This report describes that the splenectomy attenuates circulating NE in providing renal protection. Plasma EPI and NE were measured (radioenzyme method) before (0 hr.), and during 1 hr. and 6 hr. intravenous EPI (4 mcg/kg/min for 6 hrs.) in dogs: intact (Group 1), chronic splenectomy (Group 2), splenectomy with autologous splenic tissue implants (Group 3), and chronic splenectomy infused with autologous splenic extract (Group 4). Five splenectomy animals received splenic extract alone (Group 5). Hourly urine volume, glomerular filtration rate, renal blood flow (flow probe), urinary sodium (U_{Na}), and urine osmolality were measured. Renal tissues were studied using light and transmission electron microscopy. While Groups 1, 3, and 4 showed significant ($P<.05$-$.01$) decrease of all renal function parameters and severe acute tubular lesions, Group 2 was protected. Group 5 showed a significant decrease ($P<.05$) of U_{Na} and mild tubular change. Plasma EPI and NE were significantly elevated ($P<.05$-$.001$) at 1 hr. and 6 hrs. in Groups 1, 2, 3 and 4. However, NE levels were significantly ($P<.01$-$.001$) lower in Group 2 than in Groups 1, 3 or 4. Plasma EPI and NE were not elevated in Group 5. In conclusion, the data indicate that a vasoactive material in the spleen potentiates EPI in producing ARF, but neither alone produces ARF in the splenectomized animal. Further, these data indicate a splenic function in modulating plasma NE.

THE IMPORTANCE OF AGE ON URINARY PROSTAGLANDIN E_2 EXCRETION IN NORMAL AND HYPERTENSIVE MEN. T. Mackenzie,* M. Johnson,* I. Sahhar,* S. Green,* E. T. Zawada, Jr., McGuire VA Medical Center and Med. College of Virginia, Richmond, Virginia.

Previous studies have shown reduced levels and total excretion of prostaglandins in a heterogenous population of patients with hypertension. The following study was undertaken to extend these observations to focus on the influence of age and blood pressure on renal prostaglandin E_2 production in males. Four groups of human subjects were studied: Group 1 consisted of 7 normal young male volunteers with a mean age of 28 ± 1 years (yrs). Group 2 consisted of 11 young male essential hypertensive patients with a mean of 32 ± 1 yrs. Group 3 consisted of 8 older non-hypertensive men with a mean age of 56 ± 1 yrs. Group 4 consisted of 25 older men with essential hypertension who had a mean age of 55 ± 2 yrs. The results are shown below:

Group	Age Range (yrs)	$UPGE_2v$(ng/day)	P values
1	23 - 33	865.6 ± 128.3	(N.S.)
2	24 - 39	588.0 ± 171.0	
3	49 - 63	993.9 ± 302.6	0.001
4	41 - 71	195 ± 42.6	0.005

Reduced PGE_2 excretion was seen in elderly essential hypertensives only. The reduction of PGE_2 could not be accounted for by normal aging alone.

Conclusion: Reduced renal prostaglandin production plays a significant role in the essential hypertension of the elderly males and, as yet, an unclear role in the hypertension of young males.

INTERRELATIONSHIPS BETWEEN NOREPINEPHRINE, PROSTAGLANDIN E AND SYSTEMIC BLOOD PRESSURE IN PREGNANT RABBITS. Inkee Min*, Peter Barone*, Ab Donker*, Eugene Cunningham, and Rocco Venuto. SUNY at Buffalo, Dept. of Medicine, Buffalo, New York.

During pregnancy the circulating level of the potent vasopressor angiotensin II (AII) is increased while the hypertensive response to exogenous AII is diminished. The possibility that these alterations are not limited to a single vasoconstrictor compound was explored in conscious pregnant (p) and non-pregnant (np) rabbits with permanent arterial and venous catheters. The peripheral arterial blood concentration of norepinephrine (NE) was 497.4 ± 95.3 pg/ml in p and 270.6 ± 22.0 pg/ml in np rabbits ($p<0.05$). The circulating prostaglandin E_2 (PGE_2) level was also higher before manipulation of the gravid animals (1430 ± 200 pg/ml in p versus 139 ± 29 pg/ml in np rabbits) ($p<0.01$). Exogenous NE was infused at eight incremental rates that ranged from 190 to 2500 ng/kg/min. The increase in mean arterial blood pressure observed in response to each dose of NE was always less in p than np rabbits ($p<0.01$). Following an initial NE infusion the animals were given 3 mg/kg of meclofenamate. PGE_2 levels and the responses to exogenous NE were re-examined. In p rabbits meclofenamate reduced PGE_2 by more than 60% and the pressor response to NE in these rabbits was no longer different from that observed in np rabbits. These studies demonstrate that both the changes in the blood levels of and the pressor response to NE and AII are similar during rabbit pregnancy. Enhanced synthesis or release of vasodilator prostaglandins appears to mediate the blunted hypertensive response to and the increased peripheral concentrations of NE.

ALDOSTERONE EFFECT ON URINARY PROSTAGLANDIN AND KALLIKREIN EXCRETION. Aniko Naray-Fejes-Toth*, Geza Fejes-Toth* and Jürgen C.Frölich. Institute of Clin. Pharmacol., Stuttgart, Germany

Although it is well-established that aldosterone may stimulate urinary prostaglandin excretion (E_{PG}) and urinary kallikrein excretion (E_{kall}) the contribution of changes in extracellular volume to these effects is unclear. In an attempt to distinguish between direct effects and those secondary to volume retention we investigated the influence of adrenalectomy and exogenous aldosterone administration on E_{PG} and E_{kall} during dietary manipulation of extracellular volume in Sprague-Dawley rats.

Adrenalectomy significantly decreased while aldosterone increased E_{PG} and E_{kall} both during low and high sodium intake. Aldosterone induced a prompt change in Na/K ratio, however, E_{PG} and E_{kall} increased gradually during the seven days of treatment. When the animals were forced into a negative sodium balance during the administration of aldosterone by reducing their Na-intake, E_{PG} and E_{kall} still increased but to a lesser extent than on a constant diet. In a control group of adrenalectomized rats receiving the solvent of aldosterone only, shifting of the diet to a low Na intake in itself caused a decrease of E_{PG} and E_{kall}. Administration of corticosterone failed to correct the changes evoked by adrenalectomy and did not reproduce the effects of aldosterone showing that the aldosterone effect was not mediated through glucocorticoid receptors.

It is concluded that although a change in sodium balance in itself influences E_{PG} and E_{kall}, aldosterone exerts an additional effect on these parameters which is independent of changes of the extracellular volume.

EFFECT OF ALDOSTERONE (ALDO) AND DEXAMETHASONE (DEX) ON Na TRANSPORT IN DISTAL RAT COLON. **R.D. Perrone, E.A.Alexander, H.H.Bengele and J.H. Schwartz. Thorndike Mem. Lab., Renal Section, Boston City Hospital and Depts. Med. & Physiology, Boston University Medical School, Boston, Mass.**

Aldo and Dex increase Na absorption by the rat colon but the mechanism of this action is unclear. To help clarify this effect we studied the terminal 2-3 cm of partially stripped colon obtained from rats treated continuously by osmotic minipump for 4-7 days with Aldo or Dex (12 µg/Kg/hr) or diluent (CONT). In vitro chamber techniques were utilized to measure short circuit current (SCC) and unidirectional Na^{22} flux (J_{Na}) during two 20 min periods before and during the mucosal addition of Amiloride (AM, 10 µM). We obtained the following data (µeq/hr/cm²):

	CONT	+AM	ALDO	+AM	DEX	+AM
SCC	1.6	1.2	4.3†ϕ	-0.4†ϕ	5.9ϕ	0.5ϕ
$J_{Na_{M-S}}$	5.2	6.0	12.0ϕ	7.6†ϕ	10.2ϕ	4.4

P < .05: † ALDO vs DEX: ϕ vs CONT

The decrease in $J_{Na_{M-S}}$ with AM was not different comparing Aldo and Dex. $J_{Na_{S-M}}$ (~ 4.5-5.0) was similar in all groups and unchanged after AM. We conclude that Aldo and Dex stimulate electrogenic, AM sensitive $J_{Na_{M-S}}$ in the distal rat colon. In control rats AM has no significant effect on J_{Na} or SCC. The effect of Aldo differs from Dex in that Aldo also stimulates a moiety of $J_{Na_{M-S}}$ that is not AM sensitive or accounted for by the SCC.

REDUCED PROSTACYCLIN PRODUCTION IS ASSOCIATED WITH CYCLOOXYGENASE-DEPENDENT RENAL FUNCTION IN CHRONIC GLOMERULONEPHRITIS. C. Patrono,* G. Ciabattoni,* F. Pugliese,* M. Manzi,* B.M. Simonetti* and A. Pierucci* (intr. by M.J. Dunn), Depts. of Pharmacology and Medicine, Catholic Univ. and Univ. of Rome, Italy.

Prostacyclin (PGI_2) is the major cyclooxygenase product in human glomeruli (Sraer et al., Prostaglandins 23:855,1982) for which a local modulatory role has been proposed (J.Clin.Invest.69:231,1982). We investigated renal PGI_2 and PGE_2 production, as reflected by urinary (U) immunoreactive 6-keto-$PGF_{1\alpha}$ and PGE_2 excretion, and plasma renin activity (PRA) in 19 female pts with biopsy proven chronic glomerulonephritis (CGN). Moreover, the cyclooxygenase dependence of renal function was examined by measuring RBF (para-aminohippurate clearance), GFR (creatinine clearance), C_{Na}, C_K during 3 consecutive weeks, before during and after treatment with a renal PG-inhibiting (ibuprofen: 1.2 g/d) or a renal PG-sparing (sulindac: 0.4 g/d) cyclooxygenase inhibitor. CGN pts had normal upright PRA levels (3.5± 2.5 ng/ml/h; Mean±SD), normal U-PGE_2 (8.9±4.3 vs 7.4±3.3 ng/h in 33 healthy women) and significantly ($p<0.01$) reduced U-6-keto-$PGF_{1\alpha}$ (2.5±1.0 vs 4.3±0.9 ng/h). Ibuprofen significantly reduced GFR to 72±7% of basal (88±25 ml/min), RBF to 66±8%, C_{Na} to 70± 10% and U-6-keto-$PGF_{1\alpha}$ to 23±10% (Mean±SD, n=9) on the 4th day of treatment. These changes were fully reversible by the end of the 3rd control week. In contrast, sulindac which failed to reduce U-6-keto-$PGF_{1\alpha}$ (2.5±1.0→2.3±0.8 ng/h, n=10) did not affect any of the measured renal parameters.
We conclude that in CGN pts reduced renal synthesis of vasodilator PGI_2 may represent a major pathogenetic mechanism underlying the cyclooxygenase dependence of renal function.

SITE OF ACTION OF THYROID HORMONE ON THE Na^+ TRANSPORT RESPONSE TO ALDOSTERONE IN CULTURED EPITHELIAL CELLS. R.D. Pratt and J.P. Johnson. Walter Reed Army Inst. of Res. Wash, DC

Thyroid hormone (T_3) has been demonstrated to inhibit the action of aldosterone (Aldo) on Na^+ transport in toad urinary bladder and rat kidney. We have examined the effect of T_3 on Aldo action and specific nuclear binding in cultured epithelial cells derived from the toad urinary bladder. In cell line TB6C addition of 5×10^{-8}M T_3 to culture media for up to 3 days results in no change in short circuit current (I_{sc}) or trans-epithelial resistance (R). This concentration of T_3 completely inhibits the maximal increase in I_{sc} in response to 10^{-7}M Aldo. The inhibition can be demonstrated with 18 hours preincubation with T_3 or with simultaneous addition of T_3 and aldo. ½ maximal concentration for inhibition of Aldo effect is approximately 5×10^{-9}M and no inhibition is seen with 10^{-10}M T_3. T_3 has no effect on cAMP stimulated I_{sc} in these cells. The effect of T_3 on nuclear binding of ^3H Aldo was examined using a filtration assay technique with data analysis by a least squares curve fitting program. Best fit was obtained with a two binding site model with $k'_{d1} = .82 (\pm .36) \times 10^{-10}$ and $k'_{d2} = 3.2 (\pm .60) \times 10^{-8}$M. ½ maximal concentration for Aldo stimulation of Na^+ transport in these cells is approximately 1×10^{-8}M. Analysis of aldo binding in cells incubated for 18 hours in 5×10^{-8}M T_3 showed $k'_{d1} = .15 (\pm .10) \times 10^{-10}$ and k'_{d2} 3.5 ($\pm$ .10) $\times 10^{-8}$M. We conclude that T_3 inhibits the action of aldo on Na^+ transport at a site after receptor binding.

EFFECT OF Mg ON RENIN, ALDOSTERONE, CORTISOL AND ACTH. M.I. Primera,*M. Rahman,*A. Molteni,* F. del Greco, and A. Quintanilla. Department of Medicine, VA Lakeside Medical Center and Northwestern University Medical School, Chicago, Ill.

We examined the effect of an acute intravenous infusion of MgCl (0.1 mmoles/min) on plasma renin activity (PRA) and plasma aldosterone, cortisol and ACTH concentration in dogs. Control values (C) are the mean of 3 control periods in a euvolemic state. Experimental values (Mg) are the mean of 2 periods, 100 to 140 min after beginning of Mg infusion. The following table presents the mean ± SEM of pertinent results obtained in 10 experiments:

	P_K mEq/L	PRA ng/ml/hr	Aldo. pg/ml	Cortisol ug/dl	ACTH pg/ml
C	3.31±0.1	5.89±0.92	197±45	13.7±1.5	141±19
Mg	3.29±0.2	4.81±0.88	278±47	22.4±2.6	166±17
p	NS	NS	<0.05	<0.001	NS

There was a significant increase in plasma aldosterone and cortisol. Mg appears to stimulate aldosterone directly since neither plasma K, PRA or ACTH increased. Since there is evidence suggesting that aldosterone enhances renal Mg excretion, the observed effect of Mg on aldosterone may correspond to a regulatory feedback mechanism. Cortisol increased without a concomitant elevation of ACTH, suggesting direct stimulation of steroidogenesis.

INTRARENAL CONVERSION OF ANGIOTENSIN I (AI) TO ANGIOTENSIN II (AII) AT NORMAL AND REDUCED RENAL BLOOD FLOW IN THE ANESTHETIZED DOG. Laszlo Rosivall*, J. Champion*, L.G. Navar, D. Rinder* and S. Oparil*. University of Alabama, Dept. of Physiol. and Biophys. and Dept. of Med., Birmingham, AL 35294.

Because of increasing evidence that AII formed within the kidney participates in local hemodynamic regulation and our recent demonstration that AII generation within the pulmonary capillary bed is altered by hemodynamic changes, we have examined intrarenal conversion of AI to AII under conditions of altered renal blood flow (RBF). Renal conversion (conv) of AI to AII was measured in 13 pentobarbital anesthetized dogs before and after reductions in renal perfusion pressure (RPP) by clamping the renal artery. Tracer doses of ^{125}I-AI (5-12 pmoles) were injected into the renal artery and ^{125}I-AI and ^{125}I-AII determined in renal venous effluent by high voltage paper electrophoresis. Renin secretion rate (RSR) and local generation of AI (AI-GR) were also determined. Results (mean ±SEM, *p<0.05):

RPP mmHg	RBF ml/min/g	RSR ng AI/g/hr	AI-GR ng AI/g/hr	% Conv
130±5	3.9±.4	2.2±.5	0.2±.1	27.4±3.9
58±2*	2.8±.3*	13.6±3.8*	1.4±.4*	27.2±3.6
35±1*	1.9±.3*	10.3±5.1*	.2±.9*	28.3±3.9

These data indicate that intrarenal conversion of AI to AII (during one passage through the kidney) is as high as 30% and is independent of RBF even under conditions in which RSR and AI-GR are increased severalfold. The data suggest that increases in RSR in the presence of reduced RBF are accompanied by increased generation of AI and AII in the kidney.

INFLUENCE OF CHRONIC BUN ELEVATION ON OSMOTIC-VASOPRESSIN RELATIONSHIPS IN DOGS. E.W.Quillen,Jr.* and A.W.Cowley,Jr.* (intr. by R.Roman). Med. Coll. of Wisc., Milwaukee, WI.

We have reported previously that the influence of changes in plasma osmolality on plasma arginine vasopressin (PAVP) was nearly abolished in anephric dogs maintained chronically with peritoneal dialysis (Kidney Int. 21:264, 1982). The present studies were performed to investigate the role of elevated blood urea nitrogen (BUN) in this reduction of osmotic-PAVP sensitivity, since BUN was elevated to 66 mg/dl in the study cited above. Dogs (n=8) were studied in the unanesthetized state. An osmotic forcing with i.v. distilled H2O and hypertonic NaCl was used to quantitate the relationship between POSM and PAVP both before and on days 6 and 7 of a one week continuous i.v. urea infusion (100gm/day in 1L H2O). The osmotic forcing maneuver was not associated with changes in mean left atrial or mean arterial pressure. Urea infusion increased BUN (5.7±1.4 to 59.3±3.4 mg/dl) and POSM (293.6±1.5 to 313.6±3.65 mOsm/kg) but plasma sodium (143.8±0.6 vs. 143.6±1.1 mEq/L), mean arterial pressure (103.9±4.0 vs. 100.9±3.0 mmHg) and PAVP (2.9±0.4 vs. 3.2±0.6 pg/ml) were not altered. The sensitivity (slope) of the POSM-PAVP relationships at the elevated BUN levels were not different from that determined in the same dogs prior to beginning the infusion (0.198 vs. 0.202 pg/ml/mOsm/kg). We conclude that chronic elevations of BUN do not alter either the steady state level of PAVP or the ability of PAVP to respond to changes in the osmolality of the extracellular fluid.

THE EFFECTS OF PROSTAGLANDINS (PGs) AND VASOPRESSIN (VP) ON CYCLIC AMP SYNTHESIS IN CULTURED RENAL PAPILLARY COLLECTING TUBULE (RPCT) CELLS. M. Sato* and M.J. Dunn. Case Western Reserve Univ. and Univ. Hosp., Dept. of Medicine, Cleveland, OH.

It has been proposed that PGE inhibits the water-permeability response to VP by inhibiting the stimulation of cAMP synthesis in the toad urinary bladder and the rabbit cortical collecting tubule. To examine the interactions of VP, PGs and cAMP, we assessed the effects of arachidonic acid (AA), PGE_2 and aspirin (ASA) on basal and VP-stimulated intracellular cAMP in cultured RPCT cells. RPCT cells were isolated from rat papillae after hypotonic lysis of other papillary cells and studied after 4 days growth in cell culture media. AA, 0.5 to 5μg/ml, and PGE_2, 1pM to 10μM, increased basal cAMP synthesis up to 2.5 and 4.0 fold, respectively, and ASA decreased cAMP by 50%. As shown in the table, AA (5μg/ml) or PGE_2 (0.1μM) had no inhibitory effect on VP-stimulated cAMP.

cAMP (fmole/μg protein/5 min) (mean ± SEM)

VP conc.	none	10^{-9}M	10^{-8}M	10^{-7}M
VP alone	22.3±1.6	30.6±2.5	74.2±4.6	98.5±6.3
+AA	37.1±1.5	63.7±2.4	112.4±4.2	161.4±9.8
+ASA	7.1±0.8	22.6±1.1	87.0±2.5	150.1±3.0
+PGE_2+ASA	11.2±0.9	29.8±1.2	97.4±2.0	166.3±18.9

Six hr. pretreatment of cells with AA or PGE_2 induced homologous desensitization of PG-sensitive adenylate cyclase, ie., AA or PGE_2-induced cAMP synthesis were suppressed to 35 and 18% of control without changes of VP-stimulated cAMP. We conclude that rat RPCT cells have both PG-sensitive and VP-sensitive adenylate cyclases. We find no evidence of PG interaction with the VP-stimulated adenylate cyclase.

PROSTAGLANDINS MODULATE ANGIOTENSIN II (ANG II)-STIMULATED GLOMERULAR CONTRACTILITY (G.C.). L.A. Scharschmidt*, M.J. Dunn and A. Norris*, Case Western Reserve Univ. and Univ. Hosp., Cleve. OH.

ANG II stimulates mesangial cell contraction and selectively stimulates mesangial PGE_2 synthesis which may modulate G.C. Therefore we studied ANG II-stimulated G.C., as a measure of mesangial contraction, and the effect of inhibition or stimulation of PGE_2 synthesis. Glomeruli were isolated from Sprague-Dawley rats, with and without indomethacin (4mg/kg) or meclofenamate (4mg/kg) pretreatment several hrs. before sacrifice. Glomeruli were immediately incubated in ANG II 10^{-9} through 10^{-14}M in cell culture media, with or without cyclo-oxygenase inhibitors (5µg/ml) or arachidonic acid (AA), 5µg/ml. After 30 min. the glomeruli were fixed in 2.5% glutaraldehyde. Glomerular planar surface area (GPSA) was measured by a Millipore particle measurement counter attached to a light microscope and television screen. There was an ANG II-induced G.C., with a significant decrease in GPSA starting at $5x10^{-13}$M ANG II, becoming maximal with a reduction in GPSA of 20% at 10^{-11}M ANG II. Pretreatment with cyclo-oxygenase inhibitors caused a shift to the left in the dose response curve, increasing the sensitivity to $5x10^{-14}$M. Coincubation of glomeruli with AA and ANG II shifted the dose response curve to the right, increasing the threshold dose of ANG II necessary for a significant reduction of GPSA to 10^{-11}. This was not a nonspecific effect, as incubation with linoleic acid had no modulating effect on GPSA. We conclude that prostaglandins reduce ANG II-induced G.C., presumably by inhibition of ANG II-mediated mesangial contraction.

EFFECT OF JAUNDICED HUMAN SERUM ON VASCULAR PROSTACYCLIN PRODUCTION BY RAT AORTA. E T Schroeder and P Hueber*, Department of Medicine, Upstate Medical Center, SUNY, Syracuse, New York.

Jaundiced serum increases vascular sensitivity to the pressor effect of catecholamines. To determine if this action is due to suppression of vascular production of the vasodilator prostacyclin (PGI), we compared the ability of sera from normal, jaundiced and other chronically ill (uremic) patients to stimulate PGI production in "exhausted" rat aorta incubated 1 hr. with serum, washed, then incubated 12 min. in buffer. PGI in buffer was estimated by RIA for 6 Keto $PGF_{1\alpha}$ (6KPGF). Tissue from four rats was used for each assay and the mean was compared to results from a single normal reference serum, run in parallel.

6KPGF production:		% of normal reference sample		
Normal	(n= 6)	94.7 ± 12	p<.001	
Jaund.	(n=17)	44.4 ± 4.1		p>0.1
Uremic	(n= 5)	90.5 ± 14	p<.001	

Addition of arachidonic acid (A.A.) to serum increased 6KPGF in all groups but did not alter differences. Suppression of PGI correlated closely with the level of serum lipid peroxides ($p < .01$). Jaundiced rat serum did not suppress PGI in this model. Conclusion: Jaundiced human serum is less effective than normal serum in stimulating PGI production by rat aorta. This is not due to lack of A.A., is correlated with serum lipid peroxide levels and may be species specific. Suppression of vascular PGI production may, in part, account for the ability of jaundiced serum to enhance the vasoconstrictive effect of catecholamines.

GLUCOCORTICOID-INDUCED SODIUM ABSORPTION IS NOT DUE TO CROSSOVER BINDING TO ALDOSTERONE (A) RECEPTORS (R). T.J. Schmidt, V. Kapoor and C.P. Bastl, Temple Univ. Health Sciences Center, Phila. PA.

Since there is significant binding of glucocorticoids(G) to AR a pure G effect on sodium absorption (Na Abs) and transmural PD has been questioned even when physiological doses of G such as dexamethasone (D) have been used. Another synthetic G, RU 26988 (11β-17β-dihydroxy-17α-propynyl-androsta-1,4,6-triene-3-one) has been used to study steroid R because its affinity for G R is high and it has negligible affinity for AR (relative affinity compared to A (0.7%/100%) (Endoc. Soc. LXIII 255, 1981, Abs). Using this "pure" G we demonstrated that G-induced Na Abs in colon is mediated through binding to a specific GR.

Competitive binding in colonic cytosol demonstrated that RU26988 displaced 88+1% of specifically bound [^{3}H] triamcinolone acetonide at 100X excess, indicating specific G binding sites in colon. RU 26988 or D (both at 10 µg/100 bw/d) was then administered to adrenalectomized rats (adx) for 1 day. Colonic Na Abs and transmural PD was subsequently measured during in vivo perfusion.

	Proximal Colon		Distal Colon	
	Na Abs+	PDmV	Na Abs+	PDmV
Adx	1.9 ± 1.6	1.9 ± 0.6	2.4 ± 1.4	11 ± 1
RU26988	14.1 ± 1.0*	4.6 ± 0.4*	16.7 ± 1.0*	22 ± 1*
Dex	15.0 ± 1.1*	6.0 ± 0.3*¶	20.0 ± 1.0*¶	33 ± 3*¶

(+µEq/min/g dry tissue, * p<0.001 compared to ADX ¶p<0.05 compared to RU 26988)

This study demonstrates that the majority of G induced Na Abs is mediated through binding to the GR and not as previously thought to the AR.

ARACHIDONATE METABOLISM VIA LIPOXYGENASE IN HUMAN AND MURINE GLOMERULI. Josée Sraer*, Raymond Ardaillou*, and Michel Rigaud* (intr. by Morris Schambelan). INSERM 64, HOPITAL TENON, PARIS, FRANCE.

The metabolites of arachidonic acid via the lipoxygenase pathway in murine and human glomeruli have been characterized. 1-^{14}C arachidonic acid was incubated with isolated glomeruli or glomerular fractions. The products were purified by HPLC, and identified both by comparison of their retention times with those of HETE standards and by gas chromatography-mass spectrometry. At low substrate concentrations (10-200 µM), rat glomeruli synthesized mainly 12-HETE, and only traces of 15-HETE. Human glomeruli synthesized less 12-HETE than rat glomeruli, but more 15-HETE. The synthetic rates of both compounds by human glomeruli were similar. No other HETE was detected in both species. The lipoxygenase products were stored within the glomeruli, and recovered mainly in the pellet after × 100,000g centrifugation of homogenized glomeruli. 12-HETE accumulation was linear over 15 min and 12-HETE production was linearly correlated with the amount of glomerular protein. 12-lipoxygenase activity was maximum at pH 7.5 (rat) or 9 (man) and at 40-45°C (both). The shapes of the velocity versus substrate concentration curves suggested a complex enzyme system for 12- and 15-lipoxygenases. Km values calculated at low substrate concentrations were for 15-lipoxygenase, 125 and 667 µM with murine and human glomeruli respectively, and for 12-lipoxygenase, 44 µM with the glomeruli of both species. This study demonstrates that the renal glomeruli metabolize arachidonic acid through the lipoxygenase pathway. Thus 12-, and 15-HETE may play a role as mediators of the glomerular inflammatory response during immune injury.

ANGIOTENSIN II STIMULATES PROSTAGLANDIN PRODUCTION BY ISOLATED HUMAN GLOMERULI. Rolf A.K. Stahl, Margit Paravicini, Ludwig Ritter, Peter Schollmeyer. Department of Medicine, University of Freiburg, F.R.G. (intr. by M.J. Dunn).
In vitro prostaglandin (PG) and thromboxane (TB) formation by human isolated glomeruli was evaluated. PGs were determined by direct radioimmunoassay (RIA) in the superfusion medium. The conversion of radiolabeled arachidonic acid (A.A.) to PGs and TB was also studied. Under basal conditions 6-keto-$PGF_{1\alpha}$ was the predominant PG produced by isolated human glomeruli determined by RIA. The pattern of relative abundance was 6-keto $PGF_{1\alpha} > PGE_2 > PGF_{2\alpha} > TB_2$ which is different to our observations in the rat. A.A. (10^{-5}M) stimulated glomerular formation of all measured PGs and TB. In the presence of AA angiotensin II in increasing doses (10^{-7}g/l-10^{-5}g/l-10^{-3}g/l) stimulated radioimmunoassayable PG and TB formation significantly. The effect was not observed under basal conditions. The conversion of radiolabeled A.A. to PGs and TB was also demonstrated. After TLC separation 6-keto-$PGF_{1\alpha}$ also was the major product. The study demonstrates PG and TB synthesis by isolated human glomeruli. The effect of angiotensin II suggests a physiologic interaction of both systems at the human glomerulus.

SITE OF ACTION OF ATRIAL NATRIURETIC SUBSTANCE (ANS). Boris Steipe*, Josephine P.Briggs and Jürgen Schnermann*. Department of Physiology, University of Munich, Munich, W-Germany.

Extracts of atrial muscle have been reported to produce natriuresis in rats (de Bold et al., Life Sci. 28,89,1981). To define its nephron site of action, ANS was extracted from rat atria by homogenization, boiling for 10 min and low speed centrifugation. Micropuncture was performed during infusion of extract at two dose levels, low(L) and high(H), corresponding to 3 and 6 atria/hour.

		CONTROL	EXPERIMENTAL	RECOVERY
V_{urine}	L	9.5 ± 2.8	17.2 ± 1.2*	4.4 ± 0.5
(µl/min)	H	6.4 ± 2.1	40.3 ± 2.5**	2.3 ± 0.4
$U_{Na}V$	L	0.06± 0.02	1.8 ± 0.14**	0.2 ± 0.003
(µM/min)	H	0.18± 0.08	6.0 ± 0.93**	0.13± 0.03
SNGFR	L	25.1 ± 0.9	27.9 ± 2.1	27.4 ± 1.1
(nl/min)	H	28.3 ± 1.7	38.2 ± 2.5*	20.7 ± 1.7
J_v prox	L	13.0 ± 0.6	13.8 ± 1.0	14.3 ± 0.6
(nl/min)	H	14.7 ± 1.0	18.2 ± 1.5	12.5 ± 1.0
J_v loop	L	7.9 ± 0.9	7.1 ± 1.1	7.9 ± 0.9
(nl/min)	H	8.6 ± 0.8	11.7 ± 1.3	4.4 ± 1.0
$[Cl]_{dist}$	L	35.7 ± 5.9	45.6 ± 5.2	40.2 ± 6.5
(mM/l)	H	44.2 ± 4.2	48.1 ± 1.8	52.3 ± 2.0

*p <0.05, **p <0.001 compared to control

Response of SNGFR to loop perfusion was reduced. We conclude: a) crude atrial extracts contain a potent natriuretic and diuretic substance; b) this substance causes a dose-dependent rise in SNGFR probably related to blunting of tubuloglomerular feedback responsiveness; c) proximal and loop fluid transport rates are not inhibited; d) transport inhibition is restricted to later nephron sites.

PROSTAGLANDIN FORMATION BY ISOLATED RAT GLOMERULI: ALTERATIONS IN GOLDBLATT HYPERTENSION. Rolf A.K. Stahl, Margit Paravicini, Ludwig Ritter, Peter Schollmeyer. Department of Medicine, Univ. of Freiburg, F.R.G. (intr. by M.J. Dunn).
Radioimmunoassayable prostaglandin (PG) and thromboxane (TB) formation by isolated glomeruli from normotensive (N), two kidneys one clipped Goldblatt-Hypertensive (GH) and unclipped normotensive Goldblatt rats (NG) was investigated. In vitro studies were done whithKrebs-Ringer-Buffer in the presence and absence of arachidonic acid (A.A.10^{-5}M), using a superfusion system. Biosynthetic capacity for PGs by glomeruli from high blood pressure exposed kidneys of the GH rats were significantly reduced when compared to PG formation by glomeruli from kidneys of N rats. Glomerular PG-production of clipped kidneys of the GH rats were highest of all studied animal groups. All differences were only significant in the presence of exogenous substrate. After removel of the clip and normalisation of the blood pressure in the NG animals the differences were no longer present. We conclude, that in vivo changes in renal perfusion, induced by clipping the renal arteries and elevation of systemic blood pressure alters in vitro glomerular PG production. The effect is reversible after removel of the clip and normalisation of blood pressure and renal perfusion.

MECHANISM OF DESENSITIZATION TO PARATHYROID HORMONE IN DIETARY HYPERPARATHYROIDISM. J. Tamayo*, E. Bellorin-Font, and K.J. Martin. Washington University School of Medicine, St. Louis, MO

These studies examine the effects of chronic mild elevations of endogenous parathyroid hormone (PTH) in vivo on the PTH receptor-adenylate cyclase system of canine kidney cortex. Hyperparathyroidism (HPT) was induced in normal dogs by feeding a diet low in calcium, high in phosphorus for a period of 6-9 weeks and resulted in a 2-3 fold increase in plasma C-terminal i-PTH. This degree of HPT is similar to that seen in patients with HPT and normal renal function. After 6-9 weeks basolateral renal cortical membranes were prepared for the study of the PTH receptor-adenylate cyclase system in vitro. The dietary HPT resulted in desensitization of the PTH responsive adenylate cyclase (AC) (Vmax 3863 ± 634 pmol cAMP/mg protein/30 min in HPT vs 5303 ± 348 in normal controls). The Kact (concentration of PTH required for half-maximal AC activation) was unchanged. However, PTH receptor binding was not different in the two groups of animals. Thus, dietary HPT resulted in an uncoupling of the PTH receptor-AC system. This defect was not corrected by guanyl nucleotides (GN) in vitro, and the effects of GN on PTH binding and AC activation appeared normal. NaF stimulated AC was reduced in the HPT animal (8285 ± 607 pmol cAMP/mg protein/30 min vs 10851 ± 247 in controls). These data indicate that desensitization of the PTH responsive AC system of canine kidney as a result of dietary HPT is due to a post-receptor defect, demonstrable by NaF activation, not corrected by GN, leading to abnormal PTH-receptor AC coupling.

EFFECT OF DEXAMETHASONE (DEX) ON RENAL POTASSIUM EXCRETION AND POTASSIUM TOLERANCE. K. Tyler*, R. DeFronzo, M. Bia. Yale Sch. of Med., New Haven, Ct.

We have previously shown that Dex enhances U_KV in adrenalectomized rats (Adx)(Endoc.111:882'82). To furthur characterize this effect,Dex, 10 µg/100 gm, was acutely administered to 3 groups of rats: Gp I- 12 unreplaced Adx; Gp II - 13 Adx maintained on 10 µg/100g Dex/day; Gp III - 9 Controls (C).After Dex, U_KV doubled in Gp I (1 ±0.1 to 2±0.3 µEq/min; p<.005) and increased by 59% in Gp II (.9±.1 to 1.4±.2 µEq/min; p<.05). In contrast, U_KV remained unchanged in C (1.2±.1 to 1±.2 µEq/min). The kaliuresis in Adx was associated with a decrease in plasma K (5.8 to 5.1 mEq/L; p < .01) and with a rise in both U_{PhosV} and $U_{Cl}V$. Creatinine clearance was unchanged. Six to eight rats in each group were also evaluated in an acute K loading study to evaluate K tolerance. Gp II rats received a stress dose of Dex, 50 µg/100g, before KCl loading. Following IV KCl (0.1 mEq/100g over 40 min), plasma K rose higher in Gp I vs C during all periods. Furthurmore, renal K clearance was markedly impaired vs C (577±90 vs 1104±120 µl/min; p<.01) as was the % of K dose excreted (38±8 vs 61±6; p<.01). In contrast, peak renal K clearance (942±60 µl/min) was similar to C in Gp II as was the% of K dose excreted (56±7). Extrarenal K disposal (ΔP_K/mEq K load retained) was impaired in both adrenalectomized groups. Conclusion:(1) the kaliuretic potential of Dex is influenced by prior exposure of renal receptor sites to glucocorticoid; (2)during KCl loading Dex, at high doses, improves renal K clearance but does not improve extrarenal K disposal in Adx. Whether more physiologic doses of Dex will also improve K tolerance remains to be tested.

MECHANISM OF RENAL VASODILATATION WITH CAPTOPRIL: ROLE OF RENAL INTERSTITIAL PROSTAGLANDINS. C.S. Wilcox, S. Swartz,* P. Dunckel,* V.J. Dzau,* G. Williams,* and A. Tanaka,*. Harvard Medical School and Brigham and Women's Hosp.,Boston, Mass.

In man, Captopril (C) inhibits angiotensin-converting enzyme (ACE) and increases systemic prostaglandin E_2 (PGE_2) levels. Since renal lymph (L) originates from renal interstitium and reflects local interstitial hormone levels, we measured ACE, angiotensin II (AII) and PGE2 in L during intrarenal infusion of C (0.6 mg/kg/h) to 8 salt-depleted dogs. This permitted us to study whether reduction in renal vascular resistance (RVR) with C is related to local renal release of PGE_2. C blocked ACE since ACE fell by -54±13% (p<.01) and AII fell by -45±12% (p<.02); with C, the dose of AI required to double RVR was increased 12-fold and therefore AI metabolism detected in L mirrored the physiologic effects of the hormone. With C, PGE_2 rose sharply (+68±16%; p<.01) without changing L flow indicating increased interstitial PGE2 production. Concomitantly, C increased renal blood flow (RBF) 29±6% (p<.01) and decreased RVR by 33±3% (p<.001). To determine the role of PG, Indomethacin (Indo) was given with C. This invariably increased RVR (p<.001) almost to basal levels (-11±5%, ns); ACE or AII in L were still depressed as with C alone but PGE_2 in L fell sharply; Indo alone did not change RBF. Overall, changes in RVR correlated with PGE_2 (r=-.74, p<.01) but not AII (r=.18,ns). In conclusion, hormones in L reflect the physiologic responses of the kidney. C-induced renal vasodilatation depends primarily on increased renal interstitial prostaglandin release and can occur independently of ACE inhibition.

MECHANISM FOR INCREASED RENIN RELEASE (RR) IN THE ADRENALECTOMIZED (ADX) RAT. William Welch*, Cobern Ott, and Theodore Kotchen. Univ. of Kentucky College of Medicine, Depts. of Physiology and Medicine, Lexington, Kentucky.

Inhibition of RR by NaCl may be related to absorptive Cl^- transport in the loop of Henle. To determine if increased RR in the Adx rat is related to altered loop function, we studied free water clearance (C_{H_2O}), urinary concentrating ability, and plasma renin concentration (PRC) in Adx rats, Adx rats treated with dexamethasone (Adx + Dex), and sham controls. During 7 days after Adx, Adx animals drank 0.9% NaCl. Despite a more positive Na^+ balance, PRC was higher (p<0.01) in Adx than in the other 2 groups and did not decrease with 0.9% NaCl infusion (31.2 units/ 30 min ± 9.6 SE to 30.4 ± 9.5). PRC was suppressed (p<0.01) by NaCl infusion in Adx + Dex (10.2 ± 2.4 to 4.1 ± 1.2) and sham (9.7 ± 0.9 to 2.6 ±0.5). In separate animals PRC decreased (p<0.01) in response to volume expansion with albumin in both Adx (42.6 ± 8.9 to 23.1 ± 6.1) and sham (10.2 ± 1.2 to 2.1 ± 0.7). In additional animals, C_{H_2O} was determined following hypotonic NaCl infusion and an ADH antagonist, $d(CH_2)_5$Tyr (Et)AVP. C_{H_2O} of Adx (0.64 ml/hr/100 gm ± 0.11) was less (p<0.001) than that of Adx + Dex (2.95 ± 0.20) and sham (3.50 ± 0.23). Maximum U_{Osm} was decreased (p<0.01) in Adx (1526 mOsm/Kg ± 99) compared to Adx + Dex (2119 ± 53) and sham (2117 ± 169). C_{H_2O} and maximum U_{Osm} of Adx + Dex did not differ from sham. Thus increased renin release in Adx may be related to impaired loop function. Glucocorticoid replacement restores both the loop defect and renin responsiveness to NaCl.

EFFECT OF VASOPRESSIN AND OTHER cAMP-INDUCING AGENTS ON CULTURED RABBIT NEPHRON SEGMENTS. Patricia D. Wilson* and Michael Horster* (intr. by R. Anderson). Univ. Co. Hlth. Sci. Ctr., Den., Co. and Univ. Munich, Munich, West Germany.

We have demonstrated that cultures of renal collecting tubule (CT) and thick ascending loop of Henle (TAL) cells can be used to examine cell growth responses to arginine vasopressin (AVP), isoproterenol (ISO), salmon calcitonin (SCT), parathyroid hormone (PTH) and dibutyryl (db) cAMP. In the present studies, individually microdissected rabbit nephron segments of defined anatomical origins were grown for 7 days as monolayers in vitro under strictly defined, low serum conditions. Cultures of TAL bound H^3-ouabain, suggesting the presence of Na-K-ATPase. Collecting tubular cells contained a cytoskeletal tonofilament-associated cytokeratin suggesting epidermal origin. Addition to the medium of agents causing an increase in cAMP decreased the proliferative growth of CT>TAL cultures, as measured by H^3-thymidine uptake (cpm/cell):

	Medullary (m)CT	Medullary (m)TAL
Control (3% FCS)	2.14±0.3	2.13±0.4
AVP (25µU/ml)	0.58±0.2	1.30±0.3
dbcAMP (0.5mM)	0.48±0.1	1.74±0.3
MIX (10^{-4}M)	0.76±0.2	1.04±0.2
dbcAMP+MIX	0.42±0.02	0.49±0.1

Cytochemistry demonstrated hormone responsiveness of adenylate cyclase in cortical (c) CT (AVP >ISO>SCT), MCT (ISO=SCT>AVP), cTAL (AVP=SCT>PTH), and in mTAL (AVP=SCT). We conclude that agents which increase cAMP suppress growth in primary cultures of CT and TAL cells. Moreover, this suppression occurs in a cell type-specific manner to AVP and other cAMP-inducing agents.

DIVERGENT RESPONSE OF RENIN AND BLOOD PRESSURE AT THE LACTATE THRESHOLD OF EXERCISE.
P.M. Zabetakis, G.W. Gleim,*M.H. Gardenswartz, E.E. DePasquale,* J.A. Nicholas,* M.F. Michelis. Section of Nephrology and Institute of Sports Medicine, Lenox Hill Hospital, New York, N.Y.

Having previously observed that mean arterial pressure (MAP) plateaus at a point during progressive dynamic exercise (PE), we studied the relationship of plasma renin activity (PRA, ng AI/ml/hr), lactate (La, mM/L), oxygen consumption (VO_2, L/min), heart rate (HR,bpm) and MAP (mm Hg) during PE to exhaustion in 8 healthy males. Workloads increased by 25 Watts every 4 minutes with blood sampled at 3 min of each load. Lactate threshold (LT) was defined as the point where a non-linear increase in La occurred. All variables increased significantly from rest to LT (* $p<.05$), while only MAP did not increase from LT to maximum exercise (Max) (** $p<.05$).

	REST	LT		MAX
VO_2	0.32±.04	*1.48±0.10	**	2.74±0.20
La	1.27±.06	*2.42±0.20	**	7.89±0.45
PRA	3.05±.90	*5.58±1.63	**	9.22±2.54
HR	78±2	*140±7	**	189±4
MAP	97±3	*112±2	N.S.	116±3

Despite a rise in PRA, La, and HR above the LT, there was a fall in the slope of MAP (-2.4 above LT vs. 11.5 below LT, $p<.001$) when plotted as a function of VO_2. The plateau in MAP begins at LT in the face of increasing amounts of La and the pressor hormone renin. Consequently, work above LT is associated with a divergent response in PRA and MAP.

Transplantation

INCREASED FREQUENCY OF PAINFUL CRISIS FOLLOWING CADAVER KIDNEY (CK) TRANSPLANT (TRANS) IN SICKLE CELL NEPHROPATHY (SS NEPH), Catherine Alcarez*, Seymour Ribot, Melvin G. Goldblat, Michael A. Grasso, and Hossein Eslami.* Newark Beth Israel Med. Ctr. and U. of N. J. School of Med. & Dent., Newark, N. J.

There is little documentation of the natural history of CK trans in SS neph. Experience with CK trans in 4 black patients (pts) with SS neph is reported. The pts were (1) male (M) age 14, (2) female (F) age 25, (3) M age 26 and (4) F age 23. The F age 23 had CK nephrectomy at 32 days post trans and is not considered further. The remaining 3, pts 1,2 & 3, continue with good allograft function at 13, 36, and 18 mos post trans. Prior to the start of MHD crises occurred 1, 6, and 0 times per year in patients respectively. While receiving MHD no painful crises were observed. During the year following CK trans patients experienced 2, 5, and 3 crises respectively, the earliest occurred 1 mo post CK trans. Units of packed RBC transfusions given during the year on MHD prior to CK trans for pts #1, 2 & 3 were 12, 9 and 19. All showed improved erythropoesis following CK trans. Transfusion requirement declined considerably following CK trans.

In conclusion: (1) CK trans is feasible for SS neph (2) SS crises, not observed during MHD may be anticipated after successful CK trans.

INHIBITION OF PROSTAGLANDIN (PG) PRODUCTION AND RENAL COOXIDATIVE DRUG METABOLISM BY ANTITHYROID DRUGS. **S. Zelman, N. Rapp,* M.B. Mattammal,* B.B. Davis, T.V. Zenser.* VA Medical Center, St. Louis, MO.**

Propylthiouracil (PTU) protects against the toxic effect of drugs such as acetaminophen. Cooxidation by prostaglandin endoperoxide synthetase (PES) is an important mechanism of renal oxidative drug metabolism. PTU and methimazole (MI) effects on renal PG production and cooxidative metabolism of ^{14}C-benzidine (B) were evaluated in renal inner medullary slices. Slices were incubated in KRB, 5% CO_2, buffer with 150 mM Arachidonic acid (AA). PTU and MI inhibited PGE_2 production in a dose dependent manner (PTU 3.0 mM 2.0±0.1 ng/mg wet tissue wt vs. control 5.6±0.5), (MI 3.0 mM 3.3±0.2 vs control 5.3±0.4). PTU exhibited a dose dependent inhibition of AA dependent covalent binding of B to tissue protein. In microsomes incubated for 2 min with AA 60 uM and B 50 uM, PTU 25 uM reduced AA dependent covalent binding of B (17.8±0.1 nmol/mg protein) vs. control (35.5±0.3) MI 10 uM similarly reduced covalent binding 24.7±0.1) vs. control (49.4±0.4). When ASA 1.0 mM or indo 0.1 mM were added, no B binding was detected. Thus all four drugs inhibited AA dependent covalent binding of B. However in other studies only PTU (200 uM) and MI (200 uM) completely inhibited peroxide (H_2O_2 200 uM) dependent binding. These results indicate a different mechanism of inhibition by PTU and MI than ASA and indo. Antithyroid drugs inhibit renal PG production and cooxidative drug metabolism.

T CELL SUBPOPULATIONS OF PERIPHERAL BLOOD AND THORACIC DUCT LYMPHOCYTES AS PREDICTORS OF REJECTION IN THORACIC DUCT DRAINAGE (TDD). J.D. Bell, G.D. Marshall,* B.A. Shaw*. Univ. of Texas Med. Br., Galveston, TX.

Cell-mediated rejection (CMR) of cadaveric renal transplants is a comparatively uncommon event following thoracic duct drainage (TDD). Analysis of T cell subpopulations in peripheral blood (PBL) and thoracic duct lymphocytes (TDL) of 14 patients who experienced no rejection were compared to 7 patients who had CMR within 6 months after renal transplantation. T cell subpopulations were analyzed by flow cytometry and fluoresceinated monoclonal antibodies directed toward surface antigens characteristic of most peripheral T cells (Leu1), T helper cells (Leu3), and T suppressor/cytotoxic cells (Leu2). Results were as follows:

	Leu1(%)	Leu3(%)	Leu2(%)	Leu3/Leu2
NO REJECTION				
PBL	20.8 ± 6.5	12.6 ±2.2	5.7 ± 1.5	2.19 ±0.45
TDL	42.4 ± 5.5 ($p<.05$)	27.1 ±4.1 ($p<.01$)	11.2± 2.0 (p=NS)	2.82 ±0.36 ($p<.05$)
REJECTION				
PBL	47.7 ± 6.1	35.6 ±6.1	24.0± 3.9	1.48 ± 0.12
TDL	40.4 ± 7.2 (p=NS)	30.5 ±8.5 (p=NS)	11.5± 1.4 ($p<.05$)	2.59 ± 0.57 (p=NS)

In addition, comparison of PBL subpopulations between the groups revealed a significantly higher percentage of Leu1 ($p<.01$), Leu3 ($p<.01$), and Leu2 ($p<.01$) in the rejection group. No significant differences in Leu3/Leu2 ratios or TDL subpopulations were noted between the two groups. These data show that TDD produces different levels of immunosuppression in patients drained for similar periods of time. These changes can be predictive of immune events that occur after transplantation.

MONOCLONAL OKT3 ANTIBODY THERAPY OF ACUTE RENAL ALLOGRAFT REJECTION: EFFECT ON LYMPHOCYTE FUNCTION. PA Bowen*, JH Helderman, LC Edwards*, and P Gailiunas. Univ. TX Hlth. Sci. Cntr., Dallas, TX.

The availability of monoclonal antibodies directed against subpopulations of human T-lymphocytes has stimulated interest in their use as immunosuppressive therapy for acute renal allograft rejection. Clinical studies to assess the efficacy of one such antibody, OKT3-PAN (directed against all mature peripheral T-cells) are underway. Although clinical data indicate that administration of αOKT3 clears the circulation of OKT3 + cells, in vitro studies have demonstrated that αOKT3 may not be lymphocytotoxic and may itself be a potent mitogen. We are participating in a multicenter trial of murine αOKT3 for treatment of acute renal cadaveric allograft rejection. Allograft rejection (AR) was reversed in 2/3 pts with αOKT3 (5 mg IV/d x 14 d) therapy. Post-transplant spontaneous blastogenesis (SB), as well as T-cell responsiveness in MLC and to the lectins PHA and ConA, were assessed prior to monoclonal therapy, and on day 3 and 12 of αOKT3. SB was not enhanced by αOKT3 nor was it altered during treatment. However, PHA and ConA responsiveness were markedly reduced on day 3 of αOKT3 treatment but returned to baseline by day 12. Donor-specific MLC during αOKT3 was equal to pre-treatment values. Summary: 1) αOKT3 was not observed to be mitogenic in patients treated for AR; 2)αOKT3 causes transient depression of lectin-responsive T-cell clones in these patients.

We conclude that the clinical efficacy of αOKT3 in therapy of AR may be mediated by deletion of PHA/ConA responsive lymphocyte subsets.

EVALUATION OF CELLULAR INFILTRATES IN HUMAN RENAL ALLOGRAFTS WITH MONOCLONAL ANTIBODIES AND HETERO-ANTISERA. Timothy Drevyanko,* and Louis Ercolani. VAMC. Depts. of Medicine and Pathology. Univ. of Iowa, Iowa City, IA.

Imbalances of suppressor/helper T cell populations occur in the peripheral blood of recipients following renal allograft transplantation. We questioned whether these imbalances are also reflected in renal allografts during clinical episodes suggestive of no rejection (NR), acute cellular R (ACR), hyperacute R (HR) or chronic R (CR). Renal biopsies during clinical episodes were examined by light and immunofluorescent microscopy for: cellular infiltrates utilizing OKT3, OKT4, OKT8, and OKM1 monoclonal antibodies (Orthoclone) and antithymocyte globulin (ATG)(Upjohn). Infiltrating cells were found to be increased during all types of R (n=20) as compared to NR (n=4). The ratio of OKT4/8+ cells in NR were similar to that found in peripheral blood. AR (n=6): OKT3+, 8+ cells were increased $P<0.001$ over OKT4+ and OKM1+ cells. CR (n=13): cells were B cells, plasma cells, fibroblasts and OKM1+. HR (n=1): cells were granulocytes and OKM1+. ATG+ cells were comparable to OKT3+ cells during all clinical episodes. These findings demonstrate patterns of cellular infiltrate may be useful in determining various types of human renal allograft rejection and suggest different subpopulations of mononuclear cells are involved in each. Previous studies demonstrating an increased ratio of OKT4/8+ cells during AR in renal recipients' peripheral blood may, in part, be explained by a selective depletion of OKT8+ cells from these patients' circulation which accumulate in the allograft.

EVALUATION OF INTRA-OPERATIVE VOLUME LOADING (VL) ON RETURN OF RENAL FUNCTION OF ICED STORED KIDNEYS IN THE DOG. R. Didlake,* S. Raju,* and K.A. Kirchner. Univ. of Mississippi Med. Ctr., Depts. of Surg. and Med., Jackson, Mississippi.

VL at the time of renal transplantation has been said to improve urine output and speed return of normal renal function. Controlled evaluation of this maneuver, however, has not been reported. To test this hypothesis, the effects of lactated Ringer's VL on the rate of return of renal function was determined in autotransplanted dog kidneys. The left kidney was removed and stored in Eurocollins at 4°C for 24 hrs. The right kidney was then removed and dogs randomized to either a low (L) (0-5 cm H_2O) or high (H) (15-20 cm H_2O) CVP group and the left kidney reimplanted. Systemic arterial pressure was maintained at 90-100 mm Hg in both groups by adjusting the anesthetic agent. Renal artery blood flow prior to wound closure was 97±3 ml/min (mean±SEM) in H CVP and not different (p-NS) from L CVP (95±5 ml/min). Peak serum creatinine was not different between groups (5.8±0.1 H CVP vs 6.0±0.2 mg/dl L CVP). Urine flow rate was greater ($p<0.05$) during the first 3 post-op days in H CVP. Return of renal function to pre-op level as determined by return to initial serum creatinine concentration and a fractional sodium excretion of less than 1% occurred more rapidly ($p<0.05$) in H CVP than L CVP (5±1 vs 18±3 days). We conclude VL speeds return of renal function in the transplanted dog kidney. The mechanism of this effect is not due to differences in initial renal perfusion pressure or renal blood flow but may be due to increased urine flow rate or solute excretion.

ADVERSE PRE-TRANSPLANT SENSITIZATION DETECTED BY ADCC. L. Ercolani, P. Mueller*, D. D. Nghiem*, and R. J. Corry*. VAMC, Depts. of Medicine and Surgery, Univ. of Iowa, Iowa City, IA.

Transfusion of blood products to recipients of cadaveric renal allografts prior to transplantation is associated with higher allograft survival. However, blood products may also adversely presensitize recipients to donor alloantigens as well. We questioned whether low levels of anti-donor antibodies detected in transfused patients by antibody dependent cellular cytotoxicity (ADCC) but not by complement dependent cytotoxicity (x-match) pre-transplant would adversely affect renal allograft survival. Forty adult recipients were studied. Purified donor T cells were used as ^{51}chromium labelled targets in the ADCC. Efficiency of 3rd party effectors was confirmed by pre-incubation of donor cells with antithymocyte globulin (Upjohn) as a positive control. One year graft survival was as follows:

	Successful	Failed	
ADCC (-)	26	1	P< 0.001
ADCC (+)	2	11	

Eleven of 40 patients (including a recipient of a double DrW match) were adversely pre-sensitized as detected by ADCC but not by the standard x-match. Blood transfusion was associated with higher graft survival, 70% in this group in contrast to our historical non-transfused patients with graft survival of 45%. However, these data also suggest very low levels of presensitization to donor antigens in some transfused recipients may adversely effect their long term renal allograft survival as well.

RENAL ALLOGRAFT SURVIVAL IN PATIENTS TRANSFUSED PERIOPERATIVELY ONLY. W.Fassbinder*, U.Frei*, G. Dathe*, D.Jonas*, W.Schoeppe*(intr.by F.K.Port). Depts.Nephrol.and Urol.;Univ.Hosp.;Frankfurt FRG

The beneficial effect of preoperative blood transfusions(BT) on kidney graft survival is well documented,however optimal timing of BT is subject to controversy.We investigated in a prospective study the role of perioperative BT. All patients (n=88) receiving their first cadaveric graft since 1979 were transfused perioperatively(i.e. 0-6 hours before transplantation) with 2 units of non-washed,unfiltered packed red cells. Prophylactic antirejection therapy consisted of azathioprine and prednisone only. Patients were divided into 2 groups:Group A was transfused perioperatively only and had no prior BT.Group B was transfused pre- and perioperatively.Actuarial patient and graft survival (in %) is given in the table:

Group	Survival	Time post-transplant(months) 3	12	18	24	36
A	patient	100	100	100	100	100
n=40	graft	78.6	75.8	75.8	75.8	75.8
B	patient	95.6	95.6	95.6	88.8	88.8
n=48	graft	89.1	85.5	76.0	69.1	69.1

In regard to mean age(39.9 vs 35.8 years) and average HLA-identities between donor and recipient both groups did not differ significantly. Graft survival has substiantially increased since introduction of perioperative BT. The data demonstrate well acceptable graft survival rates in patients transfused perioperatively only. In these patients undesired sensitization with cytotoxic antibodies is avoided,thus increasing their chance to get a compatible graft after a short waiting time.

A RANDOMIZED PROSPECTIVE STUDY OF DONOR SPECIFIC TRANSFUSION (DST) IN 1-HAPLO DISPARATE RELATED RENAL TRANSPLANTS (LRD-TP). P Gailiunas, JH Helderman, C Atkins, AR Hull, P Stastny and PC Peters. UTHSCD Dallas, Texas.

A number of centers routinely use DST, but no prospective randomized study has identified a means of selecting recipients for DST or the mechanism of its efficacy. In our study LRD recipients were randomly assigned [disregarding MLC stimulation index(SI)] to receive 200 ml donor blood x 3 at 3 wk intervals. TP followed within 1-3 mos. T cell(TXM+) but not B cell sensitization (BXM+) precluded TP. Recipients were T and B XM(-) before DST or TP. 24% became TXM(+) while 55% became BXM(+)(at 4,20 and 37°C). Those who were BXM+ but had -TXM have excellent graft function. 2-21 (mean 10.8) mo. graft survival is 54% (7/13) in non-DST controls (C)vs 91% (10/11) in DST's ($p<0.05$). SI's (mean + SEM) were:C(function)7.9± 1.3;C,(non-function)23.6±9.0; DST(preDST)20.7±7.5; DST(post-DST) 47.1±15.8; DST(T-sensitized,preDST value)47.2±19.7. In summary, 1)low MLC (SI ≤10) is associated with excellent graft outcome without DST;2) DST is associated with improved graft survival if baseline SI is >10 ≤ 40;3) DST results in a)227% increase in SI, b)55% incidence of B and 24% incidence of T cell sensitization, 4) B cell sensitization has no adverse effect on graft function; 5)High (>40) baseline SI is associated with T cell sensitization. We conclude that 1) Efficacy of DST is in part mediated by alloactivation of T cells (presumably suppressor) and/or enhancing B cell antibodies. 2)High MLC SI may predict probability of T cell sensitization by DST and consideration might be given to deliberate third party transfusions in such cases.

INCREASED RESOURCES REQUIRED FOR DIABETIC RENAL TRANSPLANTS. Eli A. Friedman, Tai Ping Shyh*, Monica M. Beyer, and K.M.H. Butt. Downstate Medical Center, Brooklyn.

To quantify resources needed for kidney transplants in type I diabetics, we analyzed the first year course of 30 consecutive diabetic recipients (8 live donor, 22 cadaver donor) performed after March 1979. As controls, sequential transplants in 30 nondiabetic adult recipients of the same donor types were used.

Diabetics and controls had the same mean age (38.5 yrs). There were 10 deaths (33%) and 5 lost grafts (17%) in diabetics compared with 6 deaths (20%) and 5 lost grafts (17%) in nondiabetics. Graft rejection and infection were the most common reasons for readmisssion accounting for 46% in diabetics and 39% in nondiabetics. Diabetics were hospitalized significantly longer (81.4±58.1 days vs 56.5±36.2 days, $p<.025$). Six diabetics (20%) were hospitalized for more than 130 days, 4 (13%) for over half the year. The longest nondiabetic hospitalization was 125 days. Total first year hospital costs were 139% as much per diabetic recipient ($51,248± $33,367) as for a nondiabetic ($36,894±$18,154). Living donor transplants were 69% more expensive in diabetics than in nondiabetics ($41,356 vs $24,489). The cost of cadaveric transplantation in a diabetic was a third greater (133%) than in a diabetic live donor transplant. Following initial discharge, diabetics were readmitted a mean of 1.9 times compared with a mean of 0.9 readmissions for controls.

We conclude that excessive dollar and personnel resources expended for each transplant in a diabetic must be considered in planning for their care. As the proportion of newly treated uremic patients who are diabetic rises above 25%, it is evident that the economic burden imposed by this disease is enormous.

FLOW CYTOMETRY ANALYSIS (FCA): A HIGH TECHNOLOGY CROSSMATCH. Garovoy, M.R., Rheinschmidt, M.A.*, Bigos, M.*, Perkins, H.*, Colombe, B.W.,* Salvatierra. O.* University of California, San Francisco, California.

We have examined whether Flow Cytometry can detect antibodies(Ab) which are missed by available lymphocyte cytotoxicity crossmatch techniques. Flow cytometry was performed utilizing a Becton-Dickinson FACS II with 5 watt argon laser on lymphocytes isolated from peripheral blood, incubated with 0.1 ml. serum at 22°C. for 30 min., washed and incubated with FITC conjugated anti-human Ig. Cytotoxicity crossmatches were performed using the modified Amos, long incubation and anti-globulin (kappa) techniques. The FCA histograms generated in normal human serum showed a low intensity peak consisting of T lymphocytes and a high intensity peak containing surface Ig positive cells. Antisera with HLA-Ab caused a shift of the T cell peak proportionate to the amount of Ab binding. Flow cytometry detected these Ab in serial dilution out to 1:4096 as compared to 1:32 by cytotoxicity. In recipients of donor-specific transfusions, FCA detected the development of Ab 4-6 weeks before cytotoxicity. The sera of several patients which produced B cell cytotoxicity shifted the T cell peak on FCA implying the presence of anti-HLA A,B,C antibodies rather than HLA-DR. Preliminary clinical studies have shown steroid resistant rejection episodes requiring ATG therapy in four recipients of donor-specific transfusions, and early graft loss in 4/4 cadaver recipients who were FCA positive, but cytotoxicity negative. In conclusion, crossmatch testing by FCA is a rapid and extremely sensitive assay for Ab, which promises to assume an increasingly important role in improving graft survival.

REVERSAL OF STEROID-RESISTANT RENAL ALLOREJECTION (SRR) BY PLASMALEUKAPHERESIS (PLP). MC Gelfand, GB Helfrich,* T Phillips*, P McCurdy,* GE Schreiner, Georgetown U, Wash, DC.

To date, hi dose steroids remain the primary tx to reverse AR. Virtually all tlpts undergoing steroid resistant rejection (SRR) are lost. Since both cellular and humoral reactants are involved in AR, we have txd SRR with PLP. 24 pts with SRR 6-90 d post-tplt were tx'd with PLP. During each PLP, one plasma vol. and a $\bar{m}$ of 10^9 WBC(90-95% lymph) were removed. Pts were tx'd qd for 5 d then qod for 5 tx (total of 10tx). 16(67%) of the 24 SRR pts had either complete (12) or partial (4) response to PLP. In responders, $\bar{m}$ s. creat was 1.5 ± 0.3 mg/dl. 10 pts are now > 1 yr post-tplt. In partial responders, $\bar{m}$ s. creat was 3.9 ± 0.3 mg/dl. All 5 pts with low (<10%) pre-tplt. PRA had complete response to PLP. While the precise mechanism of action of PLP is not known, PLP resulted in: 1) significant decrease in I-C from a $\bar{m}$ 5-fold elevation to WNL. 2) significant decrease in peripheral blood T cell levels from 72 to 26% and B cell levels from 25 to <5% (both $p < 0.01$). 3) Increase in circulating null (non-T, non-B) cell levels from 5 to 70% ($p<0.001$). 4) In 5 studies in 4 pts after at least 5 PLP txs, PLP resulted in a significant increase in the ratio of OKT8 (suppressor) to OKT4 (helper) cells from 1.1 to 2.3 ($p < 0.01$) thereby favoring suppressor T cell dominance. Complications were not significant. Thus, PLP enhances survival of kidney tplts undergoing SRR, possibly as a result of removal of I-C or reduction in a circulating lymph subpopulation, or by increasing the ratio of OKT8/OKT4 cells thus favoring suppressor cell predominance.

INTENSIVE PLASMA EXCHANGE (IPE) THERAPY OF ACUTE OLIGURIC RENAL TRANSPLANT REJECTION. JH Helderman, P Gailiunas, AI Sagalowsky* and PC Peters. Univ. of Texas Hlth. Sci. Cntr., Dallas, TX.

Oliguric transplant rejection places patients at high risk of early renal graft loss, generally related to humoral rejection. Plasma exchange has been shown to be of benefit in several antibody mediated diseases. Therefore, we studied the effect of IPE in early acute oliguric renal transplant rejection, a setting which may involve anti-transplant anti-bodies. 13 pts were studied: 10 with excellent early function (a mean serum creatinine 2.1 mg%); 3 with marginal early function but with spontaneous declines in serum creatinine and urine outputs >1,000 ml/24 hr in the immediate post-transplant period. All patients developed acute oliguric renal transplant rejection within 7 da of transplant. Pts were treated with 5 consecutive daily 4 liter plasma exchanges + 1 g/d of IV methylprednisolone X 4 days. 9 pts responded to this regimen within one week of plasma exchange with an increase in urine output and a concomitant decrease in serum creatinine. Although 7 of 13 pts (54%) retained renal function at one month, only 3 of 13 (23%) still had func tion at 4-13 months. Histologic evidence of severe humoral rejection was present in all lost grafts. A simultaneous group who received bolus steroids alone had a 63% graft survival (12/19). Conclusion: IPE + high dose bolus steroids has short term benefit in pts with acute oliguric transplant rejection but does not appear to improve the grave prognosis in these patients.

ERYTHROCYTOSIS IN RENAL TRANSPLANTATION. John W. Graves,* William E. Braun, Susan A. Rothmann,* Andrew C. Novick, Donald R. Steinmuller, and Debbie Protiva*. Cleveland Clinic Foundation, Cleveland, Ohio.

Erythrocytosis is an increasingly common complication of renal transplantation. We have retrospectively analyzed 301 patients (pts.) from Jan. 1978-Aug. 1982 to define the clinical implications of post renal transplantation erythrocytosis.

Twenty-two pts. with post transplantation erythrocytosis (Hct. >50% for more than 3 mos.) were identified with a mean follow-up of 18 mos. No episodes of acute rejection occurred, and 21 of the 22 pts. were without significant change in their serum creatinine. In the remaining pt. erythrocytosis had abated 24 mos. prior to graft deterioration. 18/163 pts. with their native kidneys developed erythrocytosis post transplantation as opposed to 4/138 with pretransplant nephrectomies. Three of the later group of pts. were females of 34-40 kg. body wt. Serum erythropoietin (Ep) was elevated in 8 of 10 pts. (mean 320 miu). The degree of Ep elevation did not correlate with hct., hypertension, smoking history, type of dialysis or average hct. on dialysis. Two pts. had thrombotic complications with hcts. >50%. Erythrocytosis is not associated with acute rejection but is more common in pts. with native kidneys in place. Ep production is abnormal in these pts. for reasons that are unclear but may relate to renal mass disproportionate to body mass.

LATE URETERAL OBSTRUCTION IN PEDIATRIC RENAL ALLOGRAFT PATIENTS RELATED TO URETERAL FIBROSIS AND STRICTURE. J.R. Ingelfinger, R.L. Teele*, R.H. Levey*, S. Rosen and A.J. Eraklis*, Children's Hospital Medical Center, Boston, MA.

Late onset (1-4 yrs post-transplant) asymptomatic ureteral obstruction was detected in 10 of 160 pediatric renal allograft recipients (6 cadaver; 4 LRD transplants) during routine ultrasonography or pyelography. In all patients (pts) earlier post-transplant (Tx) studies had been normal. Three pts had only mild obstruction with no loss of renal function over 1-3 years. Percutaneous balloon-catheter dilatation was unsuccessful in 2 pts prior to definitive surgery. Marked hydronephrosis in 7 pts necessitated surgical correction with cutaneous ureterostomy (1), ileal conduit (1), reconstruction with a segment of ureter (3), and lysis of periureteral adhesions (1). Following surgery renal function remained stable in these pts. A 7th pt requiring pyelostomy drainage eventually lost his allograft to fungal infection.

Multiple etiologies may be involved. Urinary sediment cytology demonstrated no viral cytopathic effects in any pts. Clinical rejection was absent at onset of hydronephrosis in all pts. Three pts. had previous Tx and an additional 4 had previous pelvic surgery. Thus, prior pelvic surgery and/or ureteral vascular injury may lead to ureteral stricture in Tx pts. Non-invasive ultrasonography appears sensitive to detect this anomaly and should be frequent in pediatric Tx pts long after Tx. Surgical management results in high salvage rate for such grafts.

PROTEINURIA AS AN EARLY MARKER OF RENAL TRANSPLANT REJECTION AND POOR STEROID RESPONSE. P. Jaber*, and E. Davidson*, and K. Lau. Univ. of Mich. Med. School, Ann Arbor, MI.

Although proteinuria is considered useful in diagnosing renal transplant rejection, previous studies provided no appropriate controls to define its sensitivity and specificity. Episodes of acute rise in serum creatinine (S_{creat}) were analyzed in 52 patients (pts) without knowledge of urine protein (U_{pro}) and categorized as: 1) non-rejection (ischemia, obstruction, ATN) (N=12); 2) steroid-sensitive rejection (N=26); and 3) steroid-resistant rejection (N=10). U_{pro} was low (<0.4 gm/d) and comparable in all pts except those with steroid-resistant rejection (1.73 gm/d), apart from any relationship to S_{creat}. With established azotemia, pts with rejection had more U_{pro} than non-rejectors (1.8 vs 0.4 gm/d), and if $\geq$0.25 gm/d was diagnostic of acute rejection with 85% sensitivity and 92% specificity. Further, a rise in U_{pro} often preceded the rise in S_{creat} during acute rejection. In all 17 of such pts (with serial data) one day prior to increased S_{creat}, U_{pro} was doubled, and on this day their U_{pro} was already higher than the non-rejectors' during azotemia. Pts resistant to steroid pulses were marked by sustained and heavier post-treatment proteinuria (2.2 vs 0.7 gm/d), which could not be solely attributed to increased S_{creat}. Pulsing before or during the rise in S_{creat}, as opposed to after, resulted in lower U_{pro} in the steroid responders (1.4 vs 3.0 gm/d). Four pts with prolonged (> 18 days) azotemia eventually recovered renal function (S_{creat} $\leq$2) without steroid pulses and, like the non-rejectors, had minimal U_{pro}. Their spontaneous improvement argued against empiric pulse therapy in similar pts. CONCUSION: Increased U_{pro} often preceded and was diagnostic of acute rejection and, if sustained, indicated poor steroid response.

THE SIGNIFICANCE OF PREFORMED CYTOTOXIC CELLS IN RENAL TRANSPLANTATION. T. Kovithavongs and J.B. Dossetor. Departments of Medicine and Laboratory Medicine, University of Alberta, Edmonton, Canada.

The detrimental effect of preformed cytotoxic antibody in kidney transplantation is well established. Evidence over the past several years suggests that the presence of cytotoxic cells as detected by lymphocyte mediated cytotoxicity (LMC) against the donor at the time of transplantation is also associated with graft failure (Transplant. Proc. 10:509, 1978). In this study, patients transplanted over the last 9 years who had a LMC crossmatch are reviewed to see whether the presence of cytotoxic cells is a contraindication to transplantation and whether the poor outcome can be reversed with high dose intravenous methylpredisolone (MP) given at the time of allografting. Of the 196 patients in this analysis, 19 gave evidence of preformed cytotoxic cells in the blood without co-existing cytotoxic antibody towards the donor. Seven of these did not receive MP and the outcome was, in general, poor. Thus, 2 grafts never functioned, 2 were rejected in 6 weeks, one had multiple rejection episodes and was eventually lost at one year, and only 2 had graft survivals longer than 2 years. In contrast,of the 12 LMC crossmatch positive recipients who received MP at the time of transplantation, only one was rejected at 6 weeks, the others have good functioning grafts despite delayed functioning of up to 3 weeks in 8 of them. Seven have no further rejections for over 2 years. It is concluded that the presence of preformed cytotoxic cells is not a contraindication to transplantation if cytotoxic antibody is not present but the patient must receive MP at the time of transplantation.

INTERVAL ALG THERAPY IN HIGH RISK CADAVERIC RENAL TRANSPLANT PATIENTS. Bruce Jarrell, James Burke, Shin Yang*, Robert Karsch*, Anatole Besarab. Thomas Jefferson University Hospital, Depts. of Surgery & Nephrology, Philadelphia, Pennsylvania.

We evaluated the efficacy of Anti-Lymphoblast Globulin (ALG) in the treatment of acute rejection. Thirteen patients at high risk for graft failure (4 diabetic, 5 with second or third renal transplants and 4 not responding to high dose methyl prednisolone--mean total dose 1.8 grams) were treated with a 30 day course of ALG (30 mg/kg/dose) given daily for 10 days, every other day for 10 days and every third day for 10 days. The outcome in these patients was compared with that found in a low-risk group (first transplants, 1 diabetic) who received daily prophylactic ALG (30 mg/kg/dose) for the first 2 weeks post-transplant. Azathioprine and prednisone were used as basic immunosuppression in both groups. Graft survival in the high risk group was 77% at 6 months and 56% at one year compared to 75% and 54% for the same intervals in the low risk group. The infection rate did not differ significantly in the two groups. Leukopenia and thrombocytopenia were frequent occurances with daily ALG therapy and required frequent dosage modification, whereas they were not a significant problem when the interval between ALG doses was increased. Development of a positive Coomb's test occurred within 3-4 days in all patients and within 6 hours in 1 patient. The positive Coomb's test persisted for 40-70 days. We conclude that interval therapy with ALG over 1 month is a safe and effective way of treating rejection in high risk cadaveric renal transplantation.

LFA-1,2,3: THREE UNIQUE HUMAN LYMPHOCYTE FUNCTION ASSOCIATED ANTIGENS. Alan M. Krensky,* Francisco Sanchez-Madrid,* Thomas McElligott,* Timothy Springer,* and Steven J. Burakoff.* Departments of Pediatrics and Pathology, Harvard Medical School, Boston, MA. (intro. by Warren E. Grupe).

Monoclonal antibodies (mAb) were generated against OKT4+, DR-specific cytotoxic T lymphocytes (CTL) (PNAS 79: 2365, 1982) to investigate the molecular basis of CTL mediated killing. Hybridomas were selected for 1) inhibition of cytolysis in a 4 hour ^{51}Cr-release assay, 2) immunoprecipitation of novel proteins, and 3) binding to T cells. Three new families of mAb which block CTL function were generated. LFA-1 immunoprecipitates two proteins of molecular weights 180K, 95K and blocks cytolysis by both OKT4+ and OKT8+ CTL as well as by natural killer (NK) cells. Both LFA-2 (50K, 35K) and LFA-3 (60K) inhibit OKT4+ CTL and OKT8+CTL, but not NK cells. Cytotoxicity is expressed as % specific release (%S.R.) in a 4 hr ^{51}Cr-release assay.

ANTIGEN	CYTOTOXICITY (%S.R.)		
SPECIFICITY	OKT4+CTL	OKT8+CTL	NK cells
Control	51	57	48
LFA-1	20	25	24
LFA-2	18	22	47
LFA-3	28	29	49

These mAb also block T cell proliferative responses to mitogens (PHA, Con A), alloantigens (mixed lymphocyte response), and microbial antigens (monilia, SKSD, viruses). Since CTL have been implicated in allograft rejection, these mAb may prove important in the manipulation of the immune response to transplants.

IMMUNOGLOBULIN (Ig) PRODUCTION IN RENAL TRANSPLANT (Tx) RECIPIENTS. David N.Landsberg* and Carl J. Cardella. Toronto Western Hospital, Toronto.

Substantial Ig deposition in the allograft is found in pts with chronic rejection but not in stable pts. To evaluate the relationship of B cell function to graft function in long term (≥2 yr post-Tx) renal Tx recipients, 11 stable pts (serum creat.=1.4±.4 mg%) and 4 pts with biopsy proven chronic rejection (serum creat.=7.1±1.6 mg%) were compared to 12 controls. Age, time post-Tx, dosage of immunosuppressive drugs and serum Ig levels were similar in both groups of pts. B cell function was assessed by using an ELISA assay to measure Ig produced per ml of supernatant by $2X10^6$ lymphocytes after 8 days of culture with and without the addition of 20 uL of pokeweed mitogen (PWM).

Unstimulated Ig production was similar in the 3 groups. After PWM stimulation controls produced 411±182 ng/ml of IgG and 635±420 ng/ml of IgM while stable pts produced 232±321 ng/ml of IgG ($p\leq.05$) and 217±322 ng/ml of IgM ($p\leq0.04$). After PWM stimulation pts with chronic rejection produced 1320±504 ng/ml of IgG and 1477±360 ng/ml of IgM which were both significantly greater than in stable pts ($p\leq0.0004$) and controls ($p\leq0.002$).

Stimulated B cells of pts with chronic rejection produce more Ig than normals and stable Tx pts; whereas stable Tx pts produce less Ig than normals. These studies suggest that there may be differences in either intrinsic stimulated B cell function or immunoregulatory T cells or both in pts with chronic rejection and in those with stable function. These differences are not reflected in serum Ig levels or in unstimulated cultures.

ROLE OF COLD-REACTIVE ALLOANTIBODIES IN CAUSING ALLOGRAFT MALFUNCTION (AM) IMMEDIATELY POST TRANSPLANT. P.I. Lobo*, B.C. Sturgill, W.K. Bolton, Univ. of Va. Med. Sch., Charlottesville, VA

Previous reports have demonstrated a strong relationship between the presence of cold reactive IgM alloantibodies in renal transplant recipients and AM. The present investigations were undertaken to determine if the presence of such alloantibodies (i.e. lymphocytotoxins and agglutinins) was associated with any histological abnormalities. Pre- and one hour post-transplant biopsies were analyzed from 49 cadaveric renal allografts that came from ideal donors and were subjected to "optimal" preservation. Firstly, development of segmental glomerular capillary lesions (and not severity of tubular abnormalities) correlated with post transplant AM. Intracapillary RBC aggregates and fibrin deposition were present in 71% of biopsies in the 21 allografts with AM, but only in 29% of biopsies in the 28 allografts with immediate function ($p<0.005$). Secondly, presence of cold reactive lymphocytotoxins (CL) correlated significantly with development of post-transplant AM and glomerular lesions ($p<0.01$). Of the 37 patients with CL, 60% developed RBC aggregation whereas in 12 patients with no CL, such lesions were present in just 9% of allografts. No correlation could be made between the glomerular lesions and perfusion preservation. We postulate that vascular injury leading to AM occurs in the cold and is immune mediated since recipients with CL also have cold reactive anti-endothelial cell antibodies and cold agglutinins. Warming the allograft after anastomosis reduces AM. This observation may explain why certain allografts function promptly, whereas others subjected to similar preservation methods do not.

POST-TRANSPLANT HYPERPARATHYROIDISM: DEMOGRAPHICS AND GROSS ANATOMY. B.S. Lenfesty,* C.D. Morris * and D.A. McCarron. Division of Nephrology, Oregon Health Sciences University, Portland, Oregon.

This investigation was intended to define the demographics and gross anatomy of post-transplant hyperparathyroidism (Tx-HPTH). Fifteen successful Tx recipients [mean (±SD) serum creatinine 1.5±0.1 mg/dl] underwent total parathyroidectomy (PTX) an average of 44±7.2 mos. after Tx. Individual gland volumes were measured at the time of PTX and forearm implantation.

Ten females and 5 males who were dialyzed for 20.5±7.3 mos before their Tx, composed the group. Eight had tubulo-interstitial (TI) and 7 chronic GN. Mean (±SEM) total gland size was 2.92±0.44 cm^3. Individual gland sizes were:

Mean±SEM	Lt Sup.	Rt Sup.	Lt Inf.	Rt Inf.
Vol. - cm^3	0.47	0.81	0.62	0.97
	±0.08	±0.22	±0.10	±0.20
% Total Vol.	17.0	25.8	24.1	33.0
	±2.5	±4.13	±3.77	±4.44

Both absolute volume and % of total volume of each gland was highly variable ($p<.001$) in each subject, i.e. hyperplasia did not result in uniform hypertrophy. No single site predominated, though, right-sided glands ($p<.03$) and inferior glands ($p<.04$) were larger. Both individual ($p<.002$) and total gland volume [3.88±.59 cm^3 TI vs 1.83±.34 cm^3 GN; $p<.02$) were greater in TI disease. Gland mass did not vary by sex, time post-Tx, time on dialysis, or serum creatinine.

In conclusion, post-Tx HPTH does not produce diffuse enlargement of all glands, as each subject exhibits marked heterogeneity of individual gland size. Tubulo-interstitial disease produces greater gland hypertrophy than does chronic GN.

FFFECTS OF CYCLOSPORIN A ON LIVER FUNCTION AND RENAL TISSUE IN KIDNEY TRANSPLANT RECIPIENTS. Rolf Loertscher*, Michael Mihatsch*, Felix Harder*, Gilbert Thiel* (intr. by Terry B. Strom,Boston) University of Basel, Basel, Switzerland.

Nephrotoxicity and hepatotoxicity are important side effects of Cyclosporin A (CyA) treatment. We analyzed graft biopsies and evaluated liver function in 28 patients treated with CyA alone and observed over a period of 2 weeks to 13 months. Treatment was initiated with 17 mg/kg and reduced on the basis of CyA serum through levels (TL) and/or histological findings. The presence of tubular vascuolization, and tubular inclusion bodies (TIB) are strongly associated with CyA treatment. 18 graft biopsies of CyA treated patients were compared with 20 biopsies of patients on azathioprine and prednisone. TIB numbers were assessed quantitatively. In CyA patients in 10 out of 18 biopsies TIB numbers were increased. Only 2 out of 20 control biopsies showed sparse TIB. 9 out of 28 patients showed elevated alk.p'tase values post-transplant. Their liver parameters were compared with CyA TL. After 20 days of treatment CyA TL peaked with a mean±SEM of 1220±293 ng/ml and decreased to 383±138 ng.ml until day 90. [Bilirubin] paralled these values with 37.9±10.3 umol/l and 12.0 3.7 umol/l after 20 and 90 days respectively. Alk.p'tase was increased to 187±30 UI (20d) and 194±40 U/l (90d). Differentiation of alk.p'tase isoenzymes by heat inactivation method revealed a bone specific ratio in 8 of these 9 patients. We conclude (1) that graft biopsies are helpful for detecting CyA induced tubular alterations, (2) that [bilirubin] is the best parameter for CyA dose adjustment in absence of CyA serum levels and (3) that in some patients a persistent elevation of alk.p'tase of unknown pathogenesis may develop.

BACTERIURIA AND PROTEINURIA IN DIABETIC KIDNEY TRANSPLANT RECIPIENTS. James M. Luciano*, K.M.H. Butt, and Eli A. Friedman. Downstate Medical Center, Brooklyn, New York.

To determine the effect of diabetes on the prevalence of bacteriuria and proteinuria in kidney transplant recipients 47 consecutive patients were studied 3 to 54 months (mean 24 months) posttransplant. Patients were excluded from study if undergoing a rejection episode or manifesting a febrile illness. The group included 21 insulin dependent diabetics, 14 of whom received cadaver grafts, while 7 received living related kidneys. Of these 21 diabetics, 6 had not evinced carbohydrate intolerance prior to transplantation and were therefore termed "steroid diabetics". As controls, a group of 26 adult kidney recipients, which included 18 cadaver grafts was chosen.

Diabetics were older (mean age 37 vs 30 yrs), predominantly male (70% vs 50%), but had equivalent renal function (CCr 67.3 vs 63.2 ml/min) compared to nondiabetics. Glucose control in diabetics was only fair (HbAlc 11.0% vs 6.4% in nondiabetics).

Only 2 (9.5%) patients, both diabetic had bacteriuria. Daily protein excretion was less than 130 mg in 3 patients (all nondiabetic), and >3.5g in 3 (6.4%) patients (1 steroid diabetic and 2 nondiabetics). The majority of patients (65% of nondiabetics, 80% of diabetics, and 33% of steroid diabetics) excreted between 0.14 and 1.0g protein/day. Median urinary B_2-microglobulin excretion comprised 1 to 2% of total protein excretion. Serum albumin levels were normal in all (3.9 to 4.4g/dl). None of the patients in any group had been nephrotic at any time.

It is concluded that neither bacteriuria nor nephrotic range proteinuria is typical of diabetic kidney transplant recipients after 2 years.

DISPARATE MIXED LYMPHOCYTE REACTIONS OF PERIPHERAL BLOOD AND THORACIC DUCT LYMPHOCYTES FROM RENAL TRANSPLANT PATIENTS. G.D. Marshall*, B.A. Shaw*, and J.D. Bell. Univ. Texas Med. Br., Galveston, TX.

Thoracic duct drainage (TDD) in preparation for cadaveric renal transplantation (CRT) has been shown to be associated with decreased cell-mediated immunity and lower incidence of cellular rejection. The mixed lymphocyte responses (MLR) of peripheral blood lymphocytes (PBL) were compared to those of thoracic duct lymphocytes (TDL) from 21 patients who had TDD for at least 4 weeks prior to CRT. PBL and TDL from each patient were tested in a 5 day one-way MLR against donor spleen cells. Results were expressed as counts per minute (cpm) or stimulation index (SI). Results demonstrated MLR of PBL were significantly less than TDL (PBL: cpm=1516 ± 779, SI=3.0 ± 1.2; TDL: cpm=10486 ± 3383, SI=13.4 ± 3.3, p = .002). When patients were grouped according to whether or not they had rejection episodes, the group that exhibited no rejection (n=14) had significant MLR differences between PBL and TDL (PBL: cpm=537 ± 222, SI=1.2 ± 0.1; TDL: cpm=8891 ± 2817, SI=11.9 ± 3.3, p = .002). In contrast, the group of patients who experienced rejection episodes (n=7) showed no significant differences between PBL and TDL responses in MLR (PBL: cpm=4259 ± 2718, SI=8.1 ± 3.8; TDL: cpm=17044 ± 10587; SI=17.4 ± 9.1, p = N.S.). PBL responses of the non-rejection group were significantly less than those of the rejection group (p = .004). There was no significant difference between TDL responses of the two groups. These data suggest that similar periods of TDD produce variable degrees of immunosuppression which may account for differences in the incidence of rejection in these patients.

HIGH RISK OF LEUKOPENIA (LEU) IN CADAVERIC RENAL TRANSPLANTS (TX): ROLE OF LITHIUM (Li). T. Manis, J. Stanchina,* J.H. Hong,* E.A. Friedman, and K.M.H. Butt. Downstate Medical Center, Depts. of Medicine and Surgery, Brooklyn, N.Y.

Li carbonate reduces septic complications of chemotherapy in malignancy. In renal TX, LEU increases septic risk and limits use of immunosuppression. Since June 1978 we nonrandomly treated 44 cadaveric TX recipients with Li for LEU (WBC < 5000 x 3 days). LEU was associated with acute rejection in 34/44 cases. Assessing current (7/82) status we found:

DATE OF TX:	6/78-4/82	5/81-4/82		
	LEU+Li	LEU+Li	LEU	No LEU
Function	12(27%)	3(23%)	7(50%)	25(63%)
No function	17(39%)	5(38%)	6(43%)	12(30%)
Dead	15(34%)	5(38%)	1(7%)	3(7%)
Total	44	13	14	40

Very poor outcome in Li patients suggested an adverse effect of Li, as by enhancing rejection. However, analysis of short-term outcome did not support this: while 7/44 (16%) of Li-treated died, and 11/44 (25%) lost function during Li-treatment hospitalization, 26/44 (59%) left hospital with functioning grafts, despite limited immunosuppression and existing acute rejection before Li in most. All 7 short-term deaths were septic, confirming the high-risk status of LEU patients. Our experience does not demonstrate a clear adverse effect of Li on rejection. A random prospective study is needed to demonstrate any positive effect on patient survival. LEU identifies a very high risk group in TX recipients: from 5/81-4/82, LEU patients constituted 27/67 (40%) of cadaver TX, but incurred 6/9 (67%) of deaths.

A PROSPECTIVE TRANSFUSION PROTOCOL USING HLA TYPED BLOOD DONORS. Douglas J. Norman, John M. Barry, William M. Bennett. Oregon Health Sciences University, Portland, Oregon.

Since January 1980, 41 previously untransfused prospective recipients of first cadaver renal allografts have been entered into a protocol calling for transfusion of 5 units of fresh (4 days old) packed rbcs from HLA typed blood donors. Blood donors were mismatched with each other and recipients for HLA-A and B antigens. The blood was given at two-week intervals and serum samples were collected and screened on a lymphocyte panel two weeks after each transfusion. The incidence of sensitization to <3%, 3-20%, 20-80% and >80% of the panel was 22% (n=9), 54% (n=22), 12% (n=5) and 12% (n=5) respectively. A total of 31 patients were transplanted and 21 of these received 5 units of HLA typed blood only (1 yr surv.=64±13.5%) while 10 received additional units of blood for clinical reasons (1 yr surv.=70±14.5%). Among the 21, 1-year graft survival was 75.2±15% in patients who were exposed to cadaver donor antigens 5 or more times from the blood donors and 47±22% in those exposed <5 times (p=.73). A significant association was found between peak serum cytotoxicity levels against the lymphocyte panel and graft survival. One year graft survival was 83±9% in 22 patients whose peak cytotoxic sera killed >2% of the lymphocyte panel and 30±17.5% in 9 patients whose peak cytotoxic sera killed <2% of the lymphocyte panel (p=.025). These data suggest that pretransplant exposure to cadaver kidney donor antigens is not detrimental to graft survival and the production of antibodies after transfusion may be an important determinant for allograft survival.

IMMUNE TOLERANCE INDUCED BY PLATELET TRANSFUSION IN RHESUS MONKEYS. Jung H. Oh, Harold M. McClure* and Elbert P. Tuttle. Dept. of Medicine and Yerkes Primate Center, Emory University, Atlanta, Georgia.

In an attempt to seek a means of modifying donor-specific immune responsiveness of prospective renal allograft recipients without causing presensitization, we examined the effect of platelet transfusion in rhesus monkeys.

A total of 19 monkeys were studied; 10 controls and 9 experimentals. The control animals were transfused with fresh whole blood (2 ml/kg) weekly twice. The experimental animals were pretreated with three weekly transfusions of fresh platelets (2×10^8/kg) before receiving whole blood from the same donors. Lymphocyte-free platelets were obtained by centrifuging an aliquot of whole blood at 500 g for 10 minutes.

All of the 10 control animals developed donor-specific lymphocytotoxic antibodies. In the experimental group, only 2 out of the 9 animals developed lymphocytotoxic antibodies following the platelet transfusion. When the 7 antibody-free animals were challenged with fresh whole blood, 5 animals remained negative. The difference in the frequencies of antibody formation between the control and experimental groups was statistically significant ($P < 0.01$), suggesting an induction of donor-specific tolerance by the platelet transfusion.

Thus, we speculate that pretransplant transfusion of platelets may have a beneficial influence on renal allograft survival without causing undue presensitization as contrast to the effect of whole blood.

SUCCESSFUL PEDIATRIC RENAL TRANSPLANTATION WITHOUT DONOR SPECIFIC TRANSFUSION (DST). Zoe L. Papadopoulou, Mary Ellen Turner, G. Baird Helfrich Georgetown University Medical Center, Dept. of Pediatric Nephrology and Renal Transplantation, Washington, D.C.

Recent publications strongly recommend deliberate DST therapy for living-related renal transplantation. Donor recipient sensitization occurred in 30-40% precluding the transplant. This study was done to identify factors other than DST which allow successful pediatric renal transplantation. Review of twenty-one consecutive pediatric living-related renal transplants from 1976 to date shows successful grafting in all recipients unrelated statistically to total number of blood transfusions before transplants; 11/21 patients received 1-5 transfusions, 5/21 received 6-10, 3/21 received more than 10 transfusions, and two received none. With a mean follow-up of 3.5 years, there was 100% patient and graft survival and excellent function in all renal allografts. ATG, routine splenectomy and purposeful transfusions were not utilized. Imuran, prednisone immunosuppression were used. One patient required plasmaleukapheresis for resistant allograft rejection. Because of the importance of LRD transplant to pediatric patients, we conclude that adoption of DST protocols may be detrimental in the pediatric transplant group because of donor sensitization.

WESTERN PACIFIC (WESTPAC) REGIONAL RENAL TRANSPLANT DONOR REFERRAL PROGRAM. Terrence J. O'Neil, Maj USAF, MC (Assoc. ACP), Clark Air Base, USAF Regional Medical Center Clark AB, Republic of the Philippines, Filoteo A. Alano, Executive Director, Kidney Center of the Philippines, the Medical City Greenhills, Metro Manila, John Cross, MD, PhD, Scientific Director Naval Medical Research Unit-2 (NAMRU-2) Manila.

Logistics problems of transporting viable leukocyte specimens from WESTPAC to Transplant Centers in the Continental US and Europe have hindered transplantation when the potential donors are in WESTPAC. A referral service is offered "at cost" to US military and International Civilian Colleagues whereby dialysis for the potential recipient and HL-A typing/cross-matching for recipients and donors could be provided in Manila and/or Clark Air Base. Initial screening examination of the best HL-A/cross-match donor could also be done. This is not a commercial enterprise. It is offered solely to facilitate living-related transplants for person who might otherwise have to settle for a cadaver transplant because of inability to arrange for family donor histocompatibility testing.

HISTOLOGICAL COURSE OF CHRONIC HEPATITIS B ASSOCIATED LIVER DISEASE IN RENAL TRANSPLANT PATIENTS. P.S. Parfrey*, R.D.C. Forbes, R.D. Guttmann. McGill University, Royal Victoria Hospital, Montreal, Canada.

Serial liver biopsies were performed on 22 of 25 patients who had surviving renal transplants for more than 1 year and who were HB_sAg positive. In 20 patients the initial biopsy was taken either pre or in the first year after transplantation. The last biopsy was obtained between 12 and 86 months (mean = 44 months) after transplantation. Eighty-five biopsies were examined. Mean SGOT, measured each year following transplantation, was calculated for each patient.

Five patients ultimately developed severe progressive chronic active hepatitis (CAH), 2 of whom died with hepatoma and 1 with hepatic failure. Mean SGOT for the group was 125 ± 79 (S.D.) u/l and mean duration of follow-up was 57 ± 33 months. CAH progressed at a variable rate in 7 patients, producing mild to moderately severe disease, and did not progress in 1 patient. Mean SGOT for the 8 patients was 76 ± 49 u/l and mean follow-up was 39 ± 18 months. Four patients developed chronic persistent hepatitis in whom little or no progression of liver disease occurred. Mean SGOT was 53 ± 11 u/l and mean follow-up was 45 ± 12 months. Five patients were HB carriers with viral particles in hepatocytes but no chronic inflammation. Mean SGOT was 49 ± 17 u/l and mean follow-up was 38 ± 24 months.

It is concluded that chronic hepatitis B associated liver disease in renal transplant patients often results in progressive CAH, which may predispose to hepatoma and hepatic failure.

EARLY GRAFT DYSFUNCTION: ITS IMPACT ON LONG TERM SURVIVAL AND AN ANALYSIS OF POSSIBLE ETIOLOGIC FACTORS. R.R. Riggio, M. Suthanthiran, J.S.Cheigh, W.T. Stubenbord*, R. Haschemeyer*, K.H. Stenzel, and A.L. Rubin. Rogosin Kidney Center, The New York Hospital-Cornell Medical Center, New York, New York.

Early graft dysfunction correlates with a decrease in long term transplant survival rates. When hemodialysis is indicated in the first post-operative week, the Cumulative Survival Rate at 1,2 and 3 years for cadaveric grafts falls from figures of 82±7%, 72±9% and 72±9%, respectively, to those of 41±5%, 38±5% and 29±6% (p=0.007). It was the purpose of this study, therefore, to retrospectively review and analyze data obtained on all donors used in this series of 137 first cadaver transplants performed at our Center. When selected donor variables such as creatinine (Cr) levels, ischemic times (IT) and sources of donor kidney were correlated with selected recipient variables suggestive of either the presence or absence of renal dysfunction, none bore a positive relationship. Thus, donor Crs above or below a value of 1.5mg/dl, ITs greater or less than 48 hours and whether we used local or imported kidneys bore no relationship to: a recipient's urine output on the first post-operative (PO) day; his Cr value on PO day 7, and his need for dialysis in the first week of transplantation. These findings indicate that within the context of the physiologic criteria of the donor evaluated here, none were clearly predictive of early renal dysfunction. Recipient-related factors, both of a physiologic and immunologic nature, may thus be responsible for this deleterious event observed at our Center.

REVERSAL OF ACUTE CADAVERIC RENAL ALLOGRAFT REJECTION WITH ADJUNCTIVE ATG TREATMENT. Simon Simonian,* Patricia Lyons,* Robert Chvala,* Charles Swartz, Gaddo Onesti and Stephen Bulova.* Hahnemann Med. Coll. & Hosp., Philadelphia, Pa.

In March 1981, a prospective randomized controlled study was initiated to determine the efficacy of ajunctive horse antithymocyte globulin (ATGAM-Upjohn, ATG), in the treatment of cadaveric renal allograft (CAD-T) rejections. We had previously fractionated and found to be effective the IgG fraction of antilymphocyte globulin (Salerni, Simonian et al, Surgical Forum 21:277-279,1970). As of March 1982, 20 patients met all requirements and were entered into the clinical trial. Group I, controls, (n=10) received standard treatment of rejection (ST) of intravenous methylprednisolone 15 mg/Kg q.d. x 3 doses. Group II (n-10) received methylprednisolone with addition of ATG (15 mg/Kg, I.V.) x 14 to 21 doses. Patient follow up ranges from 6 to 18 months: (Mean 1 year). The groups were comparable in age, preformed antibody, number of transfusions and histocompatibility matching. Side-effects of ATG were generally mild and reversible. At present, allograft survival in the controls is 40%, in patients treated with ATG it is 100%, p=.005. Mean serum creatinine in the ST group is: 1.7 mg/dl, in the ATG group it is: 1.3 mg/dl, p=.730. The mean creatinine clearance in the controls is 46 ml/min. and in the ATG group is 78 ml/min. p=.001. Patient survival is 100% (10/10) in each of the two groups. These data show that adjunctive ATG in CAD-T paients used for the treatment of allograft rejection results in a significantly higher allograft survival and allograft creatinine clearance without any increased patient mortality.

EARLY DIAGNOSIS OF REJECTION(REJ) AND DIFFERENTIATION FROM ATN BY SERIAL POST-TRANSPLANT(pTx) ^{99m}Tc-DTPA RENAL SCAN (RS). A Sagalowsky*, J McConnell*, S Lewis*, I Dawidson*, P Peters, C Atkins*, JH Helderman, and P Gailiunas. UTHSCD Dallas, Texas.

The diagnosis of renal transplant(Tx) REJ is usually made on clinical grounds when significant damage has presumably ensued. The diagnosis is particularly difficult during oliguric pTx ATN. To assess the sensitivity and specificity of radionuclide RS in detecting changes in renal blood flow(RBF) and function(RF) we prospectively obtained RS at days 1,4,7,10,14,21 and 28 pTx. RBF was assessed by:1) magnitude of renal vs iliac artery (IA) peak RBF;2) time to renal vs IA peak: 3) washout of nuclide, on computer-assisted scintigram. RF was assessed by the nephrogram and nuclide appearance in the collecting system. Clinical data were blinded to the radiologist and clinical decisions excluded interpretation of the RS. Deterioration from baseline RS was assumed to indicate REJ. Radiologic interpretation of the data from 62 RS in 15 patients were correlated with the clinical impression of 3 diagnoses 1) good function; 2) ATN or 3) REJ. χ^2 analysis revealed a correlation of $p < 0.0001$. There were no false negative RS during clinical ATN. Of 26 RS performed during clinical REJ 3(12%) were false negatives. 28 RS suggested the diagnosis of REJ. In 5 (18%) of these, RS predicted clinical REJ by 1-4 d. There were therefore, no false positives RS with respect to REJ. We conclude;1) that the ^{99m}Tc-DTPA RS is a sensitive and specific measurement of Tx function: 2)establishment of baseline RS(d.1 pTx) followed by serial RS provides an important adjunct in the diagnosis of Tx REJ.

LONG TERM FOLLOWUP AFTER STOPPING PREDNISONE (P) IN KIDNEY TRANSPLANT RECIPIENTS (KTR). T.I. Steinman, R.S. Brown, H.M. Yager, C.E. Zimmerman,* A.P. Monaco,* B.J. Ransil.* Beth Israel Hosp. & Harvard Med. Sch., Depts. Med. & Surg., Boston, MA

In a preliminary report we have previously demonstrated that P can be tapered to discontinuation in KTR. From 1973 through 1980 we have followed for ≥ 1 year 51 cadaver (C) and 32 living related donor (LRD) transplants (Tx) in 79 KTR. P taper was considered in 42 stable patients (24 LRD and 18 C) who had a serum creatinine (Cr) ≤ 2.0 mg/dl at 1 year post-Tx. This group was then followed 6 to 48 months after P taper. The average time required to taper P to discontinuation was 7 months, starting at a baseline dose of 10 mg. qd or 20 mg. qod. 7/42 pts could not be tapered off P because of a rise in Cr (Incomplete). Success is defined as remaining off P to the present with a stable Cr (30/42). Failure means a return to P once off; 2/42 failed for a rise in Cr and 3/42 for other reasons. 1/42 died of unrelated causes off P.

	Incomplete	Success	Failure
LRD	4/24	17/24	3/24
C	3/18	13/18	2/18

Azathioprine was continued in all KTR, but the average dose was lowered in every pt off P because of leukopenia and/or anemia. In addition to the obvious benefits of stopping P, we also noted weight loss, fall in BP requiring no or less antihypertensive treatment, and a lowering of serum cholesterol. No correlation was noted between HLA type, blood type, age, sex, etiology of renal failure, duration of time on dialysis, or kidney donor source and the ability to be tapered off P. P can be stopped in a majority of LRD & C KTR.

PRIMARY (1°) CYTOMEGALOVIRUS(CMV) INFECTION POST RENAL TRANSPLANTATION(RT). Donald Steinmuller, A. Novick, W. Braun, R. Cunningham, C. Buszta, Cleveland Clinic Foundation, Cleveland, Ohio.

Some centers have reported increased graft loss and patient mortality associated with 1° CMV infection post transplant. To assess this risk, all renal transplants performed from 1/79 to 2/82 were studied prospectively for the development of 1° CMV infection. A positive culture or fourfold increase in complement fixation antibody titer in a sero⊖ recipient was taken as indicative of a 1° infection. 209 transplants were studied - 153 cadaver, 56 living related donor (LRD). 55 (41-cadaver, 14-LRD) developed 1° CMV infection.

		Cadaver	LRD
1°CMV Infection	Graft survival - 6 mos	76%	93%
	Patient survival - 6 mos	93%	100%
Sero⊖ pre and post RT	Graft survival - 6 mos	65%	90%
	Patient survival- 6 mos	92%	96%

Donor specific transfusion(DST) was performed from 6 sero⊕ donors to sero⊖ recipients. 5 were subsequently transplanted and all developed symptomatic CMV infections post transplant. All infections resolved without sequellae and all have a functioning graft. One recipient was not transplanted due to the development of a positive cross match. He developed an asymptomatic increase in antibody titer. We conclude that potential transplant recipients who are sero⊖ for CMV can receive a kidney from a sero⊕ donor without risk of increased mortality or graft loss from a 1° CMV infection. DST and subsequent transplantation typically results in symptomatic 1° CMV infection post transplant, but also, does not influence graft or patient survival.

MORBIDITY IN LONG TERM SURVIVALS OF KIDNEY TRANSPLANTS. Luis Tapia, Manikkam Suthanthiran, Jhoong Cheigh, Kurt Stenzel, Robert Riggio, and Albert Rubin. Rogosin Kidney Center-The New York Hospital-Cornell Univ. Med. Ctr., New York, N.Y.

This is a retrospective analysis of 64 recipients of kidney transplants; 35 males, 28 females, mean age 41.2 years with a functioning graft (mean Cr.1.8±0.9 mg/dl) for an average time of 8.6 years. Forty three (67.2%) had one or more medical complications, and twenty one (32.8%) had no medical complications. Cadaveric recipients had more complications (69.9%) than living related recipients (30.1%) p=<0.01. The most common complications were: Hypertension (46%), Urinary Tract Infections (33%), Hepatitis (14%), Arteriosclerotic heart disease (14%), Diabetes Mellitus (6%), Aseptic bone necrosis (4%), malignancies (Endometrial Ca) one patient. The most common cause of hypertension was chronic rejection: 14 out of 20 patients with hypertension had "significant" proteinuria (mean 3 Gm/24 hr) and renal biopsies showed chronic rejection. Renal function deteriorated significantly: initial serum Cr.1.5±0.14 mg/dl, present Cr.3.1±0.4 mg/dl in a mean follow-up of 11 years in the chronic rejection with hypertension group. Renal function in the rest of the group with complications but without hypertension or proteinuria remained stable: initial serum Cr.1.5±0.2, present Cr. 1.6±0.4 mg/dl in a mean follow-up of 9 years. Urinary tract infections did not affect the renal function significantly: initial Cr.1.6±0.5 mg/dl, present Cr.1.7±0.9 and no scars were apparent by I.V.P. or sonogram. Prednisone therapy did not influence the incidence of complications, either as a mean dose of 10 mg a day or 20 mg every other day.

LYMPHOCYTE ACTIVATION IN RENAL ALLOGRAFT RECIPIENTS: ANALYSIS AT THE LEVEL OF PRODUCTION AND RESPONSE TO INTERLEUKIN-2. M. Suthanthiran, W.A. Kaye*, A.L. Rubin, A. Novogrodsky*, and K.H. Stenzel. Rogosin Kidney Center, The New York Hospital-Cornell Medical Center, New York, N.Y.

Included in the cascade of events pertinent to lymphocyte activation are the production of growth factors such as Interleukin-2 (IL-2), the generation of receptor sites (RS) for IL-2, and successful interaction between the RS and IL-2. Since such events are required for the ultimate realization of immune responses, we examined the integrity of various components of lymphocyte activation in renal allograft recipients (R). IL-2 production was determined by activating peripheral blood mononuclear cells (PBM) with PHA and activity assayed with an IL-2 dependent cytotoxic T lymphocyte line. PBM from R exposed to PHA were also incubated with IL-2 to determine their ability to respond to IL-2. Production of IL-2 by PBM from R was considerably impaired as compared to controls during stable periods. PBM from R also responded considerably less when exposed to IL-2. IL-2 activity, however, was quite similar to controls when PBM were obtained pre-transplantation, preceding rejection and during infection. Additional studies revealed that steroids were mainly responsible for the inhibition of IL-2 production. Our findings indicate that 1) inhibition of lymphocyte activation in R is accomplished by interference at multiple sites in the activation cascade, 2) determination of IL-2 production might serve as an index of in-vivo immune reactivity, and 3)steroids play a significant role in suppression of IL-2 production.

NATURAL KILLER CELL ACTIVITY IN RENAL ALLOGRAFT RECIPIENTS. P.A. Taufield*, R. Vogel*, K.H. Stenzel, A.L. Rubin, and M. Suthanthiran. Rogosin Kidney Center, The New York Hospital-Cornell Medical Center, New York, New York.

Several investigators have provided evidence linking Natural Killer (NK) cells to immune surveillance. We therefore examined NK activity in renal allograft recipients (R) with respect to successful graft function, rejection and infection Serial NK activity was determined during the first 6 weeks post-transplantation in 108 R. NK activity was determined with peripheral blood mononuclear cells from R as effector cells and ^{51}chromium labeled MOLT-4 or K-562 as target cells in a 3 hour cytotoxicity assay. Immunosuppressive therapy resulted in 50%, 60% and 86% suppression of PHA and Con A induced mitogen responses and NK activity, respectively. Detailed analysis of NK activity in 20 R revealed that 12 of 17 rejection episodes were preceded by an increase in NK activity. NK activity during rejection was 22±2% (mean±SEM) compared to 12±1% during periods of stable renal function ($p<.000001$). Specific chromium release was >15% in 24 of 27 assays performed during rejection, and was <15% in 71 of 84 assays performed during periods of stable graft function ($p<.00001$). NK activity was high during episodes of bacterial or viral infection (26±1%) or when azathioprine was discontinued. Steroid pulse therapy suppressed NK activity in all but one patient. Our findings indicate that 1.)NK activity is profoundly depressed in R and is extremely sensitive to manipulations in immunosuppressive therapy and 2.) serial determinations of NK activity accurately reflects *in vivo* immune responses pertinent to rejection and infection.

PROSPECTIVE DR-MATCHING IN CADAVERIC RENAL TRANSPLANTATION (TX). B.A. VanderWerf, T.M. Vyvial*, & L.J. Koep*. Phoenix Transplant Center, Good Samaritan Medical Center, Phoenix, Arizona.

Recent studies have indicated that matching for both DR antigens improves one year kidney survival to > 80% and patient survival to > 95%.

Since 1/81 we have evaluated the feasibility of transplanting only DR matched recipients. The limited number of DR combinations (45) made this possible. During 18 months kidneys used locally were for DR matched recipients only. Excess kidneys were preferentially offered to centers with DR matched recipients. Of 96 kidneys available, we transplanted or provided kidneys for 53 DR identical, 13 DR compatible, and 30 DR mismatched recipients. Over this period DR identical Txs rose from 27 to 68%. Despite using only 20% of our local kidneys, our Tx volume actually increased.

DR typing was repeated at the recipient center 21 times. Twice we identified a second antigen when a kidney with one antigen was received. No other discrepancies were found.

For 66 DR matched recipients, including 45% high risk pts, graft survival was 85% and patient survival 96% at one yr. For the 30 DR mismatched recipients graft survival was only 60% and patient survival 85% at one yr. DR identical and DR compatible results appeared the same. All 96 pts received preoperative blood transfusions.

We conclude that DR matching is superior to other matching. Furthermore, consistent typing results among different centers makes DR sharing possible. The advantage of DR matching might be extended to all cadaveric renal transplants if extensive sharing through cooperative networks could be realized.

REHABILITATION IN RECIPIENTS OF LONG-TERM RENAL ALLOGRAFTS. Matthew R. Weir*, Robert L. Kirkman,* Terry B. Strom, and Nicholas L. Tilney* Dept. of Medicine and Surgery, Brigham & Women's Hosp., Boston, MA.

A patient survey was undertaken at one institution to evaluate the quality of life in long-term renal transplant recipients. 217 recipients were followed from 5-20 years, with 33 late deaths. Of the survivors, 70% responded to the questionnaire. 89% described their health as good or excellent and only 11% as fair or poor, in spite of complications which affected 94% of patients. Most complications were chronic, began before the allograft had functioned 5 years, and included hypertension(46%), cataracts(24%), urinary tract infection(17%), osteonecrosis of a joint(17%) and malignancy(14%). Complete hospitalization data after 5 years was available in 89% of patients. The median number of days in the hospital per year was 1, the mean,4.85. 40% of the patients were not hospitalized after 5 years post-transplant. Leading causes for days in the hospital included: failed transplant(29%), infection(14%), bone disease(11.9%), atherosclerotic disease(10.4%), liver disease(6.1%) and psychiatric disease(4.7%). Job rehabilitation was excellent. 54% were employed full-time and another 24% half-time since transplantation. Only 12% were unable to work because of illness. 77% of patients were married, and 42 children were born to these patients post transplant. 52% of patients described their quality of life as improved after transplant,while 32% felt it was the same. Although a majority of patients demonstrated excellent rehabilitation, the risk of late morbidity and mortality persists.

PARATHYROID FUNCTION AFTER > FOUR YEARS OF STABLE CADAVARIC RENAL TRANSPLANTATION. Leslie Walter and Norman Bank. Montefiore Medical Center, Bronx, N.Y. 10467.

We assessed the long term effect of cadavaric renal transplantation (CRT) on parathyroid hormone activity and renal osteodystrophy. 17 patients with CRT were followed for four to thirteen years (mean 7.6 years). Serum creatinine in all 17 patients remained consistently below 2 mg% (mean 1.2 mg%). 6 (35%) of these patients have been noted to have persistently elevated C Terminal PTH (C-PTH) levels ranging from 545-3754 pg eq/ml mean 1142 pg eq/ml (normal <300 pg eq/ml). Despite the elevated C-PTH, serum calcium was 9.0-10.3 mg%, mean 9.8 mg% (normal 8.5-10.5 mg%) and serum phosphorus was 2.6-3.9 mg%, mean 3.3 mg% (normal 2.5-4.5 mg%). Alkaline phosphatase was 60-87 IU/L, mean 69 IU/L (normal 30-115 IU/L). Only one patient had an elevated calcium of 11 mg% and an elevated C-PTH of 2039. Her alkaline phosphatase was 87 IU/L. In all 17 patients bone X-rays revealed no evidence of subperiosteal reabsorption or osteitis fibrosa cystica.

It is concluded that parathyroid hyperplasia may persist for many years following stable cadavaric renal transplantation despite excellent renal function. Nonetheless, no evidence of excess parathyroid effect on Ca, PO_4, alkaline phosphatase or bone X-ray has been observed. One reason is possible receptor down regulation. Alternatively other factors which produce renal osteodystrophy may be corrected by CRF.

HERPES VIRUS INFECTIONS IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS. Robert A. Weiss*, Howard Trachtman*, Leonard Feld, Ira Greifer, Children's Kidney Center of the Hospital of the Albert Einstein College of Medicine, Bronx, New York.

Viral infections are an important cause of morbidity and mortality among renal transplant recipients. We report the clinical profile of all symptomatic herpes virus infections (N=26) in 65 pediatric patients who received 84 allografts from 1972-1982. Criteria for diagnosis were a four-fold rise in titer and/or viral isolation. The causative agents were CMV (65%), herpes simplex virus (27%), varicella-zoster (8%) and Epstein-Barr virus (8%). Viral infections were diagnosed more frequently after 1978 (18/26). Clinical findings included fever (77%), hepatitis (35%), thrombocytopenia (35%), pneumonitis (31%), leukopenia (27%) and graft loss (23%). Bacterial sepsis occurred in 2 patients, death in 3. ALG was administered to 30% of infected patients and 16% of uninfected patients. We conclude that 1) Clinical features and etiologic agents of viral infections are similar in pediatric and adult renal allograft recipients. 2) ALG use does not enhance the risk of viral infections (X^2 NS). 3) Risk of secondary bacterial infection is not high enough to warrant routine antibacterial chemoprophylaxis, except for patients with severe leukopenia

POST-RENAL TRANSPLANT (RT) ERYTHROCYTOSIS (EC): A REVIEW OF 53 CASES. Curtis G. Wickre, Douglas J. Norman, John M. Barry,* William M. Bennett. The Oregon Health Sciences University, Portland, Oregon.

A review of a large RT experience revealed a 17.3% incidence of EC defined as an hematocrit (Hct) $\geq$ 51% (nl=41-46%). Comparison was made with 49 control recipients (C) matched for RT function, time after grafting, age and sex. EC occurred 3-90 months after RT and persisted for 1-84 months. Average maximum Hct ± SD, despite being reduced by phlebotomy (Px) in some patients, was 54.8 ± 2.6% (range 51-62%). Age of onset was 10-59 years. No patients had coincidental thrombocytosis, leukocytosis, splenomegaly or chronic pulmonary disease.

In distinction to prior smaller series, EC occurred in patients with good RT function (creatinine = 1.62 ± .93 mg/dl), without RT rejection, RT artery stenosis or ureteral obstruction. RT prognosis in EC was excellent with 48/53 (91%) having well preserved RT function at death or last follow-up (mean = 41.5 months). Risk factors were smoking (p=.02), diabetes (p=.04) and rejection free post-RT course (p=.03). No factor evaluated independently of the others was significantly associated with EC. RT source, HLA mismatch, incidence of splenectomy, parathyroidectomy, immunosuppression, hypertension, liver enzyme abnormalities, and pre-RT dialysis and Hct were similar in EC and C.

In spite of aggressive therapeutic Px, 11 thromboembolic events (TE) occurred in EC, but none in C (p<.001). Awareness of EC and controlled evaluation of therapeutic modalities is recommended.

SUCCESSFUL KIDNEYS TRANSPLANTATION CORRECTS THE IMPAIRED BLOOD VISCOSITY OF CHRONICALLY HEMODIALYZED PATIENTS.
N.S.Zerefos,J.Boletis, Ch.Stathakis,J.Alexopoulos, E.Xefteri,N.Katsilabros and G.Daikos.University of Athens, Athens Greece.

The goal of our study was to evaluate the influence of successful kidney transplantation on the impaired blood viscosity of chronic dialysis (CD) patients. Blood viscosity values of 16 successfully transplanted (T) CD patients (pts),10 males (m) and 6 females (f),mean age 35,were compared to those observed in 27 CD pts,16 m and 11 f,mean age 37 and in 35 age matched controls (C). Blood viscosity (v) was measured by means of a Wells-Brookfield cone on plate rotational microviscometer at two different shear rates (SR);23 $sec^{-1}$and 230sec^{-1} and was converted to corrected blood viscosity(CBV) by using the regression equation from the semilogarithmic V-Hct relationship derived from the whole group studied.
The following Table shows CBV values in centipoises (cp), (mean ± the standard deviation):

SR	23 sec^{-1}	230 sec^{-1}
	CBV (cp)	CBV(cp)
T	8.01 ± 1.49	4.28 ± 0.34
CD	12.00 ± 3.10	5.14 ± 0.51
C	8.11 ± 1.32	4.46 ± 0.43

Statistically significant high values of CBV were observed only in the CD group when compared to both T and C individuals (P<0.01).

We conclude that successful kidney transplantation corrects the increased blood viscosity of chronically hemodialyzed patients.

THE RENIN DEPENDENCY OF HYPERTENSION FOLLOWING RENAL TRANSPLANTATION. E. T. Zawada, Jr., M. Goldman,* R. Reinitz,* S. Green,* M. Johnson,* D. Sica. McGuire VA Medical Center and Medical College of Virginia, Richmond, Virginia.

The saralasin test was performed in 12 renal transplant recipients to assess in a variety of situations the role of the renin-angiotensin axis in their hypertension. Intravenous titration of 1,5,10 and 20 Ug/kg/min was used. The results are shown below:

Patient	1	2	3	3	4	4	5
Saralasin test	+	+	+	-	-	+	-
Native Kidneys	+	-	+	+	+	+	+
Diagnosis	HNK	CRJ	ARJ	TAR	NG	CRJ	INF
Patient	6	7	8	9	10	11	12
Saralasin test	+	+	+	-	+	+	+
Native Kidneys	-	+	+	+	-	-	-
Diagnosis	TAS	TAS	HNK	NG	TAS	NWU	ARJ

HNK-hypertension in native kidneys; CRJ=chronic rejection; ARJ=acute rejection; TAR=treated acute rejection; NG=normal graft function; INF=accelerated graft deterioration with graft infarction; TAS=transplant artery stenosis; NWU=no work up.

Conclusion: 1) In a variety of clinical situations (10/12) renin mediated the hypertension. 2) The graft alone (solitary kidney) is sufficient to lead to renin-mediated hypertension (5/5). 3) Acute and chronic rejection is commonly associated with renin-dependent hypertension. 4) The saralasin test may not effectively discriminate transplant artery stenosis from other causes. 5) Though highly sensitive for renin-dependent hypertension post transplantation, there was a lack of specificity in this test as a screening measure for transplant artery stenosis.

STUDY OF A PATIENT WITH RECURRENT FOCAL GLOMERULAR SCLEROSIS (FGS) IN TWO RENAL ALLOGRAFTS (TXS)
Stephen W. Zimmerman, Dept. of Medicine, University of Wisconsin, Madison, Wisconsin

The pathogenesis of FGS is unknown. Serum from a patient with recurrent FGS was studied to determine the effect on proteinuria in rats. The effect of plasmapheresis (P) on proteinuria and renal function was also studied. A 37 y.o. man developed rapid recurrence of nephrotic syndrome with documented FGS in 2 cadaver renal txs. His first graft was removed after 31 mo. During this time his serum was studied. After heat inactivation, RBC heterophile antibody was absorbed with bovine kidney. Serum was then infused into the aorta of male hooded rats after a control period. Urine protein and rat albumin excretion, inulin clearance (GFR), and Na excretion were measured. Other nephrotic sera (N=11) and non-recurrent FGS sera served as controls. Significant increases in urine protein and rat albumin excretion were noted with infusion of test serum (N=9, P<.05) but not controls. Albumin excretion increased from .25 ± .09 mgm/hr. baseline to .47±.23 during infusion and .88±.61 immediately after. The increase in albumin excretion was not correlated with urine flow, GFR or Na excretion. Because of these results P was performed on 2 occasions after recurrent FGS was noted in the second tx. Three months after tx P resulted in a 50% decrease in urine protein excretion early with a subsequent increase. At 23 mo. when severe FGS was noted on biopsy proteinuria was unaffected by P. These data should be interpreted with caution, but are consistent with a possible serum proteinuric factor in this man.