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EFFECT OF LYMPHOCYTES FROM NEPHROTIC PATIENTS ON UPTAKE OF ^{35}S BY GLOMERULAR BASEMENT MEMBRANE
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Patients with idiopathic minimal lesion nephrotic syndrome (IMLS) have an increase in glomerular permeability to proteins due to the loss of fixed negative charges in the glomerular basement membrane (GBM). Heparan sulfate is the main compound carrying the anionic sites; SO_4 in this molecule represents an important source of the negative charges.

We have studied the effect of peripheral blood lymphocytes (PBL) from nephrotic patients on the incorporation of ^{35}S in rat GBM. ^{35}S uptake increased when glomeruli were incubated with lymphocytes from 10 patients with IMLS in relapse compared to simultaneous assays on 5 controls (2295 ± 634 cpm/mg glomerular weight, mean $\pm$ SEM, and 962 ± 248 cpm/mg, respectively) ($P < 0.02$).

^{35}S incorporation did not increase when glomeruli were incubated with PBL from seven patients with other glomerulopathies (1016 ± 503 cpm/mg) as compared to controls (1283 ± 642 cpm/mg) or from eight IMLS patients in remission (822 ± 435 cpm/mg) as compared to controls (1475 ± 608 cpm/mg). No significant differences in ^{35}S uptake were seen between glomerular cultures with and without control PBL.

These data suggest a role for PBL in the pathogenesis of IMLS, since the increased ^{35}S incorporation may be the result of increase catabolism of proteoglycans in the GBM. This may subsequently lead to decrease in anionic sites and increase in glomerular permeability.

HEMODYNAMIC EFFECT OF POST-ISCHEMIC ATP-MGCL₂. Karen M. Gaudio, Gunilla Thulin, Michael Kashgarian, Norman J. Siegel, Yale Univ. Sch. of Med., Dept. of Pediatrics and Pathology, New Haven, CT 06510.

The postischemic infusion of ATP-MgCl₂ is known to enhance recovery from acute renal failure. Since both ATP and MgCl₂ are vasoactive compounds and increase renal blood flow (RBF), the hemodynamic effects of this complex were determined by treating animals with either dopamine (15 $\mu\text{g/kg}$), ATP-MgCl₂ (25 μmoles) or normal saline (NS) after 45 min of renal ischemia. Inulin clearance (C_{in}) and renal blood flow (electromagnetic flow probe) were determined at 2 hrs and C_{in} and recovery of microinjected inulin (INR%) were evaluated 24 hrs after the injury.

At 2 hrs the infusion of dopamine resulted in a level of RBF (1.70 ± 0.09 ml/min/100gBW/kidney) and C_{in} (400 ± 44 $\mu\text{l/min/100gBW}$) that was similar to that in the ATP-MgCl₂ rats (1.73 ± 0.27 RBF, 404 ± 38 C_{in} and significantly ($P < 0.01$) greater than NS rats (0.80 ± 0.04 RBF, 78 ± 19 C_{in} ; $P < 0.01$). However, by 24 hrs the C_{in} in dopamine animals had not continued to improve (460 ± 25) and was similar to that in NS animals (388 ± 40). In contrast, C_{in} in ATP-MgCl₂ animals showed sustained and accelerated recovery (676 ± 28 ; $P < 0.01$). These differences resulted from improved integrity of the tubular epithelium as reflected by diminished backleak: INR $92 \pm 3\%$ ATP-MgCl₂; $45 \pm 5\%$ dopamine; $P < 0.01$.

This study demonstrates that the initial hemodynamic effect of ATP-MgCl₂ produces some improvement in C_{in} but the sustained salutary effect of this compound is related to the repair of sublethally injured tubular cells and restoration of cellular metabolism, which we have previously documented.

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IS "CLEAN CATCH" URINE COLLECTION NECESSARY? A COMPARISON OF A SIMPLIFIED METHOD OF COLLECTION AND TRADITIONAL CLEAN CATCH METHODS. Bernard G. Gauthier, Anne Dubin, Howard Berkenfeld, and Henry D. Isenberg. (Spon. by Philip Lanzkowsky) SUNY at Stony Brook and Schneider Children's Hospital of Long Island Jewish-Hillside Medical Center, Depts. of Medicine and Laboratories, New Hyde Park, New York.

Two methods of urine collection were studied in 109 children (45 boys, 65 girls, mean age 13.4 ± 4.7 years) who were toilet trained, not bedridden, and had no urological problems. On day 1, the patient was asked to void into a disposable paper cup. No other instructions were given. On day 2, traditional clean catch methods were followed using the Bard Clean Catch Rigid Funnel Urine Collector with Castile Soap Towlettes. The samples were immediately refrigerated and cultured within 2 hours. All samples were handled identically using the following procedures: urinalysis (Ames Labstix), office culture (Ames Microstix), microscopic examination of the sediment, Gram stain, and urine culture and colony count using a blood agar, selective enterococcus agar, staphylococcus 110 agar, and eosin methylene blue agar plates. There was no statistically significant difference in the colony counts of the urine or in the microscopic examination of the sediments.

We conclude that in children, urine can be collected for culture without any precautions. Our study suggests that there is no advantage in using traditional clean catch methods. This appears to be true in both boys and girls of all ages.

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DEVELOPMENT AND LOCALIZATION OF VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEIN TO THE BRUSH BORDER MEMBRANE IN NEONATAL RAT KIDNEY Wallace A. Gleason, Jr.,

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In order to investigate the role of vitamin D in renal tubular calcium reabsorption we measured vitamin D-dependent calcium-binding protein (CaBP) in renal homogenates and determined its localization in suckling and weanling rat kidney. One pup was taken from each of five litters at weekly intervals from 7-35 days post partum. CaBP was measured in kidney homogenates by radioimmunoassay and localized in freeze-substituted sections using an immunoperoxidase technique. We found only a 30% increase in CaBP in renal homogenates between 7 and 14 days, and none thereafter. We therefore studied two groups of four animals at 2 and 6 days of age and found a 104% increase between 2 and 6 days. Immunocytochemical studies revealed localization of the CaBP to the brush border of cells in the proximal tubule which was more pronounced with increasing age. The presence of vitamin D-dependent CaBP, a molecular marker of the vitamin D endocrine system in the brush border of the proximal tubule of rat kidney suggests that this is a site of vitamin D-dependent calcium transport analogous to that in the intestine. The developmental appearance and cellular localization noted in the kidney are, however, very different from that seen in the enterocyte, in which the CaBP is present at low levels until weaning, when it undergoes a five-fold increase, and rather than being a brush border protein, is widespread throughout the cytoplasm.

INCORPORATION OF $^{35}\text{SO}_4$ INTO GLOMERULAR BASEMENT MEMBRANE IS REDUCED IN AMINONUCLEOSIDE NEPHROSIS. Paul R. Goodyer, Feige Kaplan, and Bernard S.

Kaplan. McGill University, The Montreal Children's Hospital, Department of Pediatric Nephrology, Montreal, Quebec, Canada.

Physiologic and histochemical studies suggest that glomerular basement membrane (GBM) polyanion may be reduced in puromycin aminonucleoside nephrosis (PAN). We assessed the effect of PAN on incorporation of $^{35}\text{SO}_4$ (an index of GBM polyanion) and ^3H -proline (an index of general GBM synthesis) into GBM *in vitro*. Glomeruli were isolated from rat kidney at various times after a single dose of PA (15 mg/100 g body wt) and incubated with $^{35}\text{SO}_4$ and ^3H -proline for 18 hours at 37°C . SDS-PAGE analysis of GBM prepared by detergent extraction (4% DOC) indicated uptake of $^{35}\text{SO}_4$ mainly into a high molecular weight band ($\sim 250\text{K}$) of counts in two smaller bands (90K and 200K). One day after PA, incorporation (CPM/mg GBM prot) of $^{35}\text{SO}_4$ ($35 \pm 12\%$ of control*) and ^3H -proline ($55 \pm 15\%$ of control*) were reduced. Thereafter, recovery of ^3H -proline incorporation was rapid and exceeded controls ($190 \pm 10\%$ on day 3, $145 \pm 8\%$ on day 8) whereas $^{35}\text{SO}_4$ incorporation remained depressed ($22 \pm 10\%$ on day 3, $65 \pm 12\%$ on day 8). *In vivo* degradation of $^{35}\text{SO}_4$ ($T_{1/2} = 5.9$ days) and ^3H -proline ($T_{1/2} = 12.2$ days) labelled GBM in PA-rats was similar to controls ($T_{1/2} = 5.4$ and 12.5 days respectively). There was therefore a prolonged period in which the SO_4 /proline ratio of newly formed GBM was reduced ($< 50\%$ of normal). These findings suggest that from day 3-8 in PAN, there is production of GBM with reduced charge density. This may explain onset of proteinuria at 6-8 days. * $p < .01$.

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RENAL FUNCTION IN INFANTS < 1250 G (VLBW) IN THE FIRST THREE DAYS OF LIFE. Krishnamurthy C. Sekar, Kenneth L. Harkavy, Pedro A. Jose (Spon. by John W. Scanlon) Georgetown University, Columbia Hospital for Women, Division of Neonatology Washington, D.C.

Renal function has been measured extensively in larger and older preterm infants. We studied 16 VLBW infants during the first three days of life. Eight infants were studied with 50 mg/kg boluses of inulin followed by infusions of 0.1 mg/kg/min. The other eight received 100 mg/kg and 0.2 mg/kg/min, respectively. The mean inulin levels were 19.5 ± 5.96 mg/dl (mean $\pm$ SD) and 65.0 ± 59.8 mg/dl, respectively. Timed urines were collected for 4 to 7 hrs and inulin clearances (IN) calculated in standard fashion. An attempt was made to allow a daily weight loss of 1.5-3.5% of birthweight.

The gestational age was 28.5 ± 2.2 weeks and the birthweight, 990 ± 158 G. The results are not different between protocols and are combined.

Day	1	2	3
N	11	9	11
IV ml/kg/hr	4.8 ± 8	5.1 ± 6	6.0 ± 1.2
Urine ml/kg/hr	1.5 ± 9	3.3 ± 1.6	3.5 ± 1.4
IN ml/kg/min	$.27 \pm .23$	$.37 \pm .13$	$.46 \pm .44$
FeNa %	3.0 ± 2.9	5.0 ± 3.8	7.0 ± 5.9 *

The daily change in values was not significant. IN did not correlate with either urine flow nor IV rates. Individual IN values ranged from .039 to 1.54 ml/kg/min. 9 of 16 infants had average $\text{IN} > .3$ ml/kg/min. IN and FeNa in VLBW infants < 72 hours old are lower than in older infants.

*Fractional sodium excretion. One study coincident with furosemide treatment was omitted.