- 49 *Effect of Furosemide on Renal Glycolysis and Oxygen Uptake in Rats.* T.YOSHIDA, P.R.LEWY, L.E. VOYER, A.B.WARD, Y.Y.AL-UBAIDI and J. METCOFF, Dept. of Ped., Michael Reese Hosp. and Med. Center, Chicago, Ill.

Furosemide (F), a potent diuretic agent, is known to inhibit tubular Na^+ reabsorption. The mechanism of action of F is not known. Since Na^+ reabsorption is energy dependent, F may inhibit this process by interference with renal energy metabolism.

Normal, fed, 200 g Sprague-Dawley male rats were used in this study. Homogenates of renal cortex and medulla were incubated in a medium containing 5 mM glucose (G). Addition of F (100–200 $\mu\text{g}/\text{ml}$) strongly inhibited formation of lactate ($p < 0.005$), α -glycero-P ($p < 0.005$), and all glycolytic intermediates below 1,3-diP-glycerate ($p < 0.05$) in both tissues; fructose-6-P increased sharply; triose-P formation did not change. The QO_2 of renal cortex and medulla slices obtained from controls, or 1 h after IV injection of F (20 mg/kg), were measured in a Warburg respirometer. Endogenous QO_2 was increased in the F-group cortex and medulla ($p < 0.02$); addition of G (5 mM) enhanced QO_2 in control cortex and medulla ($p < 0.05$) but not in F-group cortex. However, in F-group medulla, a synergistic effect of F+G on enhancement of QO_2 was observed.

Decreased lactate formation from G is consistent with inhibited glycolysis or enhanced Krebs cycle activity; our data indicate both may play a role. Inhibition of glycolysis may be a direct effect of F, or may result from increased Krebs cycle activity [NEWSHOLME *et al.*, *Nature* 193: 270, 1962]. The increased CO_2 with F in renal slices suggests uncoupling of the usual relationship between Na^+ reabsorption and renal O_2 utilization.

- 50 *Increased Urinary Glomerular Basement Membrane Products in Pyelonephritis.* BORIS LUSTIK, WILLIAM T.KNIKER and CHARLES V.PRYLES, Depts. of Ped., State Univ. of N.Y., Downstate Med. Center, The Jewish Hosp. Med. Center of Brooklyn, and the Univ. of Texas Med. Sch., San Antonio.

Soluble glomerular basement membrane products (gbm-p) are excreted in urines of normal children, but increased excretion of these products occurs in various types of glomerular disease. [Abstracts, Soc. for Ped. Res., p.9, May 1969.] Since pyelonephritis is a disease in which glomeruli may be involved, gbm-p were searched for in the urines of such patients.

Twenty-four hour urine samples from 83 subjects were concentrated and examined by immunodiffusion against sheep anti-human gbm globulin. Slight to marked increases in gbm-p were found in patients with untreated pyelonephritis. In one of these patients the level of gbm-p exceeded the level found in 3 patients with acute glomerulonephritis. In 2 additional children with acute pyelonephritis the quantity of gbm-p excreted equaled that found in one of the patients with glomerulonephritis. Patients with treated pyelonephritis, cystitis, and congenital renal anomalies without infection excreted gbm-p in the range found of healthy controls.

Sterile urine samples from normal subjects were inoculated with a strain of *E. coli* (0-6) isolated from a patient with acute pyelonephritis, and tested by the same technique in order to rule out cross-reactivity with bacterial antigen. All these tests were negative. These

preliminary studies suggest: (1) that increased excretion of gbm-p may be a manifestation of glomerular involvement in acute pyelonephritis, and (2) that this technique might prove useful in the differential diagnosis of upper vs. lower urinary tract infection.

- 51 *Chemical and Immunologic Characteristics of Glomerular Basement Membrane Fragments in Rat Urine.* S.RAYMOND WONG, CLAUDIUS KULVINSKAS, DONALD B.KAUFMAN and RAWLE M.MCINTOSH, Univ. of California Med. Center, Los Angeles (introduced by Solomon Kaplan).

Glomerular basement membrane (GBM) is composed of a collagen-like component and a carbohydrate rich glycoprotein. GBM fragments have been found in the urine of normal and nephritic man and experimental animals. This study was designed to isolate, purify and chemically and immunologically characterize GBM fragments of rat urine.

A glycoprotein, the major protein in normal rat urine (MUP), was isolated by DEAE ion exchange chromatography and purified by gel filtration on Sephadex G 200. MUP was shown to be a carbohydrate rich glycoprotein with an amino acid content similar to the non-collagen component of GBM. Fluorescein conjugated antisera stained rat glomeruli in a linear pattern. The antisera could be absorbed with rat GBM, rat glomeruli, 8 M urea solubilized GBM but not by rat collagen or the collagen component of GBM. The glycoprotein is similar to albumin in molecular size. In experimental nephrosis, the excretion of MUP is increased and the content of hexose altered.

- 52 *Chemically Induced Polycystic Kidney Disease in the Newborn*¹. JOHN F.S.CROCKER² and ROBERT L.VERNIER, Univ. of Minnesota Hosp., Dep. of Ped., Minneapolis, Minn.

KIME *et al.*, DARMADY and others have shown that diphenylamine (DPA), a fungicide, causes diffuse cystic disease in mature rats fed the compound for 6 months to one year. We report the development of polycystic changes in kidneys of newborn rats born of the mothers a) fed DPA (2 ½% in diet), and b) tube fed 2 ml of 1% DPA in alcohol, for the last 6 days of pregnancy. Controls received diet without DPA or were tube fed alcohol. All 70 newborn in the experimental group showed a) cystic dilatation of the collecting ducts, and b) vacuolar degeneration of the proximal tubules. Micro-dissection studies of the abnormal tubules will be presented.

This new model of cystic disease in the newborn animals demonstrates the increased susceptibility of the neonatal kidney to DPA and provides approaches to improved understanding of the enigma cystic disease.

- 53 *Prospective Study of Nephritis Associated With a New Nephritogenic Type at Red Lake.* PATRICIA FERRIERI, ADNAN S.DAJANI, S.STEPHEN CHAPMAN, JONATHAN JENSEN, LEWIS W.WANNAMAKER, Univ. of Minn. Coll. of Med. Sci., Dept. of Ped., Minneapolis, Minn.

In July 1969 a study of the pathogenesis of impetigo was initiated at the Red Lake Indian Reservation. Serial (3 × weekly) cultures of nose, throat, normal

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² Fellow Canadian Arthritis and Rheumatism Society, and U.S. National Kidney Foundation.

skin (wrist, ankle, back) and lesions and weekly urinalyses were done on 38 children in 6 families. One month later a 43-year-old mother, her 5-year-old twins, and an unrelated child, not in the study, had onset of acute nephritis (AGN) within a 14-day period. Findings included hematuria (4/4), proteinuria (3/4), hypertension (3/4), low serum complement and C'3 (4/4), and elevated streptococcal antibodies (4/4). All exhibited impetigo, and lesions in 3 of 3 patients cultured before treatment yielded type 57 streptococci. Throat cultures were negative and no pharyngitis preceded onset. Latent periods for the twins were 19 and 22 days. Type 57 streptococci reappeared on the normal skin of both twins after penicillin therapy, resulting in recurrence of lesions in one. Over a 10-week period 704 cultures from the twins and 4 siblings yielded 297 (42%) positive for group A streptococcus, of which 294 were M-57. Lesions accounted for 42%, normal skin sites for 47% and nose and throat for 11%. Normal skin sites were positive for the M-57 strain in 4 of 6 children before development of lesions (mean 8 days). This strain was found in the respiratory tract (5 of 6) only after its recovery from normal skin and/or lesions. Impetigo with type 57 but no AGN occurred in 16 other children. This is the first report of the occurrence of type 57 nephritis in this country. Prospective studies document the skin as the initial site of infection in these patients with AGN. The nephritogenic strain was found on normal skin prior to the development of impetigo and reappeared after treatment.

- 54 *Kinetics of Peritoneal Dialysis in Children.* A.B. GRUSKIN and M. L. COTE, Dep. of Ped., Temple Univ. Sch. of Med. and St. Christopher's Hosp. for Children, Philadelphia, Pa. (introduced by V. H. Auerbach).

Peritoneal dialysis was performed in 7 uremic children (4 months to 18 years). Rates of diffusion of Na, K, Cl, PO₄, urea, creatinine, and uric acid were similar to adult diffusion curves. With the exception of uric acid diffusion, no age related differences were found. Peritoneal urea and creatinine clearances paralleled the rate of exchange of dialysis fluid. Actual urea clearance values were within the range of clearance values obtained in adults dialysed at similar rates. Data to compare creatinine clearances in adults to children is not available over the range of dialysis volumes utilized in their studies. Estimation of urea production based on the average daily rise in BUN revealed similar production rates per kg body weight in all ages.

These studies demonstrate no intrinsic age-related differences in the kinetics of peritoneal dialysis. Thus, there is no necessity to correct peritoneal clearances to either 70 kg body weight or 1.73 M², in order to compare children to adults. The greater 'efficiency' of peritoneal dialysis in children can be ascribed to a smaller body pool size in children relative to adults. Therefore, at a given clearance rate, the amount of material in the pool diminishes at a faster rate in children than in adults. Moreover, since peritoneal clearance increases as the rate of dialysis increases, no single clearance value for a given individual exists but rather a family of values related to the rate of dialysis. (Supported in part by NIH grants RR-5624, HD-2870 and FR-75.)

- 55 *Calorie Deficiency in Children on Chronic Hemodialysis and Some Effects of Calorie Supplements.* JAMES M. SIMMONS, DAVID M. PERIN, DONALD E. POTTER

and MALCOLM A. HOLLIDAY. Dept. of Ped., Univ. of California San Francisco Med. Center, and San Francisco Gen. Hosp., San Francisco, Calif.

Uremic children, including those on chronic hemodialysis, often exhibit mild lassitude, are wasted, and do not grow well. The absolute volumes of the total body water and extracellular fluid compartments of children compared with adults are small, necessitating strict dietary limitations of protein, sodium potassium and water. Observed cell mass is low and extracellular volume is high, confirming clinical impression of wasting and salt retention. Food record analyses of 10 children on hemodialysis 3 times weekly indicate that within the dietary restrictions calorie intakes were approximately 50% of ideal in the 5 small children (<110 cm in height and <6 years old), and 70% of ideal in the 5 larger children (>125 cm and >10 years old). Daily calorie supplements of from 40-55% of ideal intake were taken by 3 of the small children and 2 of the larger children, increasing their total calorie intake to 75-95% ideal. The heights of 9 of the children were measured monthly for no less than 4 months, and growth velocity computed from this. Growth velocity was compared to the 50th percentiles of growth increments in normal children of the same age and was expressed as a fraction of normal growth velocity for the period of observation. The 5 children given calorie supplements all grew at 0.9-1.7 of normal and the 4 non-supplemented children grew at 0.1-0.4 of normal. Protein intakes were all above recommended. These findings provide tentative evidence that calorie deficiency may be a factor in uremic growth failure, and that in the absence of other complications, some growth is possible when high density calorie supplements are provided.

- 56 *Growth After Renal Transplantation in Children.* RICHARD N. FINE, CARL M. GRUSHKIN, BARBARA M. KORSCH and ELLIN LIEBERMAN, Childrens Hosp. of Los Angeles and Univ. of So. Calif. Sch. of Med.

Growth (3-16 cm) has occurred in only 6 of 19 renal allograft recipients, aged 2 to 18 years, who have survived 6 to 36 months post-transplantation. At the time of transplantation 9 of 19 children were <10th percentile in height for their chronologic age; 5 were >10th percentile and 5 were >50th percentile. Growth has occurred in 5 of 6 children <12 years of age at the time of transplantation and in only 1 of 13 >12 years at transplantation. Eight of 12 children >12 years who did not grow after transplantation had fused distal femoral and/or proximal tibial epiphyses at the time of transplantation. The daily dose of prednisone was <15 mg at the time of growth in 5 of the 6 children who grew, whereas, all the children who did not grow were receiving >15 mg daily. No spectacular (>4 cm) catch-up growth occurred during the first 6 months after transplantation. The child who has survived the longest and has grown 16 cm in 36 months, required <8 mg of prednisone daily for the past 33 months. One 7 1/2-year-old boy grew 4 cm during the first 6 months after transplantation while receiving 10 mg of prednisone daily; chronic rejection occurred and no further growth has occurred during the next 18 months. Menstruation has occurred in 4 of 6 adolescent girls 4 to 12 months after transplantation; all had menses prior to transplantation. The lack of growth post-renal homotransplantation is re-