

**1231** IDIOPATHIC HYPERCALCIURIA (IH): A POTENTIAL CAUSE OF RENAL DISEASE IN CHILDREN WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM). John I. Malone, Saul Lowitt, John A. Duncan, Shirish C. Shah, Alfonso Vargas, and Allen W. Root. Univ. of S. Fla., Dept. of Pediatrics, Tampa, Fla.

Renal disease is a common complication of IDDM. The pathogenesis is believed to be microvascular and to increase with duration of IDDM. Hypercalciuria causes renal dysfunction and has been reported in children with IDDM. A group of children (157) with IDDM had urine and blood collected after a 10 hour fast. Thirty-seven age similar non-diabetic (ND) subjects were controls. Urine calcium (Ca), phosphate (PO<sub>4</sub>) and creatinine (Cr) were measured. The Ca/Cr and PO<sub>4</sub>/Cr ratios were calculated as an indicator of urinary excretion. IH (definition: Ca/Cr 2 SD>mean for ND) was found in 45 (29%) of the IDDM subjects (6% prevalence in ND children). PO<sub>4</sub>/Cr in IDDM (1.2±0.06) was greater than ND (0.72±0.08) p<0.002. Ca/Cr correlated with PO<sub>4</sub>/Cr in subjects with IDDM while no relationship was found in ND. Ca/Cr correlated both with coincident serum glucose (r=0.34, p<0.001) and glycosylated hemoglobin (r=0.25 p<0.001) but was not related to duration of diabetes or the current insulin dose. Both Ca/Cr and PO<sub>4</sub>/Cr correlated inversely with serum PO<sub>4</sub> in IDDM; serum PO<sub>4</sub> correlated directly with PO<sub>4</sub>/Cr but was not related to Ca/Cr in ND. PTH levels in ND and IDDM children were normal. Conclusion: 1) a defect in tubular reabsorption of Ca and P exists in IDDM that is related to blood glucose concentration; 2) the ion product of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> in the urine of (29%) IDDM children exceeds the solubility constant and is 3 times that of the ND children; 3) IH may be a cause, previously unrecognized, of some renal complications of IDDM.

**1232** BETA-GLUCURONIDASE DEFICIENCY: A MUCOPOLYSACCHARIDOSIS WITH CHONDROITIN SULFATURIA. Reuben Matalon, Ricardo V. Macias, Ulrich Diekamp, Minerva Deanching and Ira M. Rosenthal, University of Illinois at Chicago, Departments of Pediatrics and Pathology.

Beta-glucuronidase deficiency is an autosomal recessive mucopolysaccharidosis with variable clinical manifestations similar to the other mucopolysaccharidoses. The urinary excretion of acid mucopolysaccharides (AMPS) has not been very clear, with reports suggesting the excretion of dermatan, heparan and chondroitin sulfates. These suggestions were based primarily on electrophoretic mobility. This report deals with the isolation and characterization of the urinary AMPS from three unrelated patients with beta-glucuronidase deficiency. Urinary AMPS were isolated using gel chromatography with Sephadex G-25, followed by precipitation of the AMPS fraction with cetylpyridinium chloride. Dermatan sulfate was identified by the formation of a complex with CuSO<sub>4</sub>, the elution pattern from Dowex 1, X2Cl<sup>-</sup> and susceptibility to chondroitinases. Heparan sulfate was estimated by the determination of the N-sulfated hexosamine, a reaction specific for this compound. Chondroitin 4 and 6 sulfates were determined by the pattern of elution from Dowex 1 and digestibility with chondroitinase AC. Total AMPS isolated ranged from 18.4mg to 71.0mg/24h. The major fraction (>90%) was chondroitin 4/6 sulfate. Dermatan sulfate comprised <8% and heparan sulfate <2%. Gas chromatography/mass spectrometry of the uronic acids hydrolyzed from these fractions confirmed these findings. These studies indicate that chondroitin sulfaturia is a characteristic urinary finding of beta-glucuronidase deficiency.

**1233** SAFETY AND EFFICACY OF A NEW FORMULA FOR TREATMENT OF CHILDREN WITH PHENYLKETONURIA (PKU). Edward RB McCabe, Arlene Ernest, Ann Nord, and Linda McCabe (Spon. by Donough O'Brien). University of Colorado School of Medicine, Department of Pediatrics, Denver.

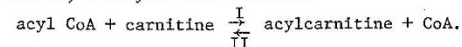
We tested the safety and efficacy of a new formula for PKU patients, the Milupa product, PKU-2. Ten children with classical PKU ranged in age from 1.13 to 11.33 years (x̄±SD = 4.48±3.57 years) at the beginning of the study. Baseline measurements were collected and patients were reevaluated after 4, 8 and 12 months on PKU-2. Growth was maintained, and serum phenylalanine and tyrosine concentrations remained essentially unchanged from baseline values throughout the study. Hematologic, protein-nutritional and hepatic parameters showed no physiologically significant differences when compared with baseline. Evaluation of trace metal status indicated that the mean plasma zinc values were at the lower limits of normal (68-110 mg/dl) at baseline (72±13), 4 (76±14) and 8 months (69±16), differing significantly from 0-time only at 12 months (56±24, p<0.002). Hair zinc differed significantly from baseline (156±56 mg/g) at all three reevaluations (127±66, 138±70, and 112±55), but all remained within the normal range (>105). No clinical evidence of zinc deficiency was noted throughout the study. Decreased zinc values have been observed in children with PKU treated with other formulas (Acosta et al, J Parent Ent Nutr 5:406, 1981). This may result from decreased bioavailability of zinc in these formulas (Casey et al, Pediatr 68:394, 1981). We conclude that the Milupa product, PKU-2, is safe and efficacious for the management of children with PKU.

**1234** RELIABILITY OF THE GUTHRIE BACTERIAL INHIBITION ASSAY (BIA) FOR PHENYLALANINE. Linda McCabe, Kathy Kuhlman, and Edward RB McCabe (Spon. by Donough O'Brien), Univ. of Colo. Sch. of Med., Dept. of Peds., Denver.

The BIA is the primary method used in newborn screening for phenylketonuria (PKU). To test the reliability of the BIA to differentiate known phenylalanine (phe) standards, seven subjects experienced with BIA for newborn screening measured growth diameters of 18 phe standards distributed randomly on 6 plates. These standards included 2,4,6,8,10,12,20 mg/dl. Each concentration was represented in duplicate or triplicate and no concentration was replicated on the same plate. Six subjects showed overlap between standards of different concentrations, and serial pairs of standards all showed some degree of overlap. Comparisons within individual subjects indicated 3 subjects with ≤ 1/3 and 4 subjects with > 1/3 to 2/3 overlap. Overlapping measurements were noted between each serial pair of standards and the frequency of overlap ranged from 5-51%. There were 8 serial phe pairs with both members of the pair appearing on the same plate; there was 43% overlap on one pair, 14% overlap on 3 pairs and no overlap on the remaining 4 pairs. We conclude that there is considerable overlap in measurements of diameters of bacterial growth using the BIA throughout clinically relevant concentrations. This may account for a portion of the false negative and false positive PKU screens. Our limited subject population showed wide variability in individual performances. A test of performance might be appropriate in selection of personnel involved in newborn screening by BIA.

**1235** CARNITINE ACYLTRANSFERASE II: INHIBITION BY KREBS CYCLE INTERMEDIATES. Kenneth McCormick, Vivian Mattson. (Spon. by Gilbert B. Forbes). University of Rochester Medical Center, Department of Pediatrics, Rochester, New York.

Carnitine acyltransferase (CAT), located on the mitochondrial inner membrane, catalyzes the reaction:



This enzyme transfers acyl CoA units to the enzyme-rich matrix.

The forward reaction (I), the pivotal rate-limiting step in fatty acid oxidation, occurs on the outer side of the membrane and is regulated by cytosolic malonyl CoA. The opposite reaction (II) has not been studied. After synthesizing <sup>14</sup>C-acylcarnitine, we investigated the modification of this reaction (II) by numerous intramitochondrial metabolites. The normal CAT (prepared from rat liver) rate for this reaction (II) was 18 ± 5 nmols/min/mg protein (mean ± S.E.; n = 7). Various CoA compounds, lactate, pyruvate, citrate and acetate did not affect this reaction at physiologic intramitochondrial concentrations (0.1 mM). However, the citric acid cycle intermediates fumarate, malate, and oxaloacetate were inhibitory (66 ± 9, 71 ± 8, 77 ± 8 percent of control, respectively; n = 7, p < .025). The attenuation of this reaction by these compounds may be salient to the overall regulation of hepatic ketogenesis, especially since glucagon alters the intracellular compartmentation of these metabolites.

**1236** INSULIN'S INTRACELLULAR MESSENGER: EFFECT ON MITOCHONDRIAL FATTY ACID OXIDATION. Kenneth McCormick, Margaret Williams, JoAnn Steinberg, David Levey, Eric Gottesman. (Spon. by Gilbert B. Forbes). University of Rochester Med. Center, Dept. of Pediatrics, Rochester, NY.

Recent studies have demonstrated the release from isolated crude plasma membranes of a putative undefined mediator(s) of insulin's molecular action. This messenger is generated after insulin-receptor binding. Rat liver particulate cell membranes (4 mg/ml prot) were incubated with physiologic [insulin] or saline; the mediator was prepared as described (Proc Natl Acad Sci, 1982; 79:3513). To study its effect on ketogenesis and pyruvate dehydrogenase (PDH), suspensions of either the crude lyophilized extract (C) or the ethanol-separated stimulatory (S)/inhibitory (I) fractions were added to freshly-prepared liver mitochondria or mitoplasts. Our mediator had similar effects on PDH as reported previously (above ref) and was <12,000 daltons. The S fraction (ethanol nonextractable) did not modify ketogenesis; however, both the undiluted and 1:10 C (data below) and I fractions (data not shown) were inhibitory (p < .025). Results are expressed as % inhibition of ketogenesis by mediator prepared from insulin (10<sup>-8</sup>M) vs saline-treated membranes (mean ± S.E.; n=12); the control mitochondria ketogenic rate was 1.8±0.3 nmol/min/mg prot.

Extract dilution:	0	1:10	1:100	1:1000
Inhibition (%) :	31±8	28±5	8±2	-5±6

Preliminary data suggest that the addition of ATP with insulin may facilitate the generation of mediator (? receptor or membrane phosphorylation). We conclude that insulin's mediator suppresses fatty acid oxidation in intact mitochondria.