

ALANINE UTILIZATION IN THE NEWBORN. Ronald L. Gutberlet, Jeffrey Quartner, Kulsum Merchant, Rupla Eshai, Marvin Cornblath and Pinar Ozand, Univ. of Maryland Sch. of Med., Dept. of Ped., Baltimore.

The role of alanine as a substrate and a gluconeogenic precursor were studied by measuring blood glucose, lactate, pyruvate, and  $\beta$ -OH butyrate responses to an oral alanine load (0.5 gm/kg) in 22 newborns and 4 adults. Tests were performed at 6 or 36 hours of age and responses followed for 2 or 4 hours. Fasting alanine values were highest in 6 preterm newborns (PNB) and 10 term newborns (TNB) at 6 hrs (.87 and .68 mμ/ml) but were the same as adults (.59 and .56 mμ/ml) in the TNB by 36 hrs. Although adults had a significant increase in blood alanine, no increase in glucose occurred. In contrast, a marked increase in glucose (36-137 mg%) occurred in 3 of 6 LGA and an SGA at 6 hrs. This was associated with sharp increases in alanine, lactate, and pyruvate levels. In all TNB, glucose increased an average of 12% at 36 hrs but in both TNB and PNB a variable response was found at 6 hrs. In all groups, pyruvate and lactate rose an average of 20-50% after alanine, while  $\beta$ -OH butyrate increased only at 6 hrs. The results suggest that alanine is utilized for gluconeogenesis in the neonate, but the consistent increase in pyruvate and lactate may reflect incomplete utilization and conversion of this amino acid to glucose. These studies were supported in part by Grants from the John A. Hartford Foundation and the NIH #03959-06 and 06291-03.

LACTIC ACIDOSIS DUE TO A DEFECT IN PYRUVATE METABOLISM IN 3 SIBLINGS. James C. Haworth, Thomas L. Perry and Shirley Hansen. Dept. of Pediatrics, University of Manitoba, and Dept. of Pharmacology, University of British Columbia, Canada.

3 infants are reported who were born to related North American Indian parents. Each infant developed metabolic acidosis and fasting hypoglycemia shortly after birth. Blood lactate and pyruvate were greatly increased in all infants and, in siblings 2 and 3, there was marked elevation of plasma alanine, proline and glutamic acid. Alanine and proline were also elevated in spinal fluid. Urinary pyruvate and  $\alpha$ -ketoglutarate were increased. The following causes of organic acidosis were excluded: liver glycogen disease, fructose diphosphatase deficiency, methylmalonic acidemia, propionic acidemia, isovaleric acidemia, butyric and caproic acid disease and  $\beta$ -methylcrotonylglycinuria. After infusion of U-<sup>14</sup>C alanine into one infant, the label was measured in glucose, lactate and expired CO<sub>2</sub> in approximately normal adult proportions, suggesting that gluconeogenesis pathways were intact. Normal activity of phosphoenolpyruvate carboxykinase was measured in fibroblasts. The biochemical abnormalities were not influenced by pharmacological doses of biotin, thiamine or prednisone. Siblings 1 and 2 died at 4 months of age. It is postulated that these siblings had an inherited defect in pyruvate metabolism, possibly at the level of the pyruvate dehydrogenase complex.

POMPE'S DISEASE WITH NORMAL LEUKOCYTE ACID  $\alpha$ -1,4-GLUCOSIDASE ACTIVITY: ENZYME AND GLYCOGEN IN TISSUES AND CULTURED CELLS. Virginia C. Hieber and Roy D. Schmicke. Univ. of Michigan Med. Sch., Dept. of Ped., Ann Arbor.

Pompe's disease is characterized biochemically by a generalized deficiency of acid  $\alpha$ -1,4-glucosidase activity. The fact that normal leukocyte activity is occasionally found in these patients has serious diagnostic implications and raises interesting questions in regard to the etiology, genetic expression, and therapy of this disease. In the patient studied, a normal level of acid  $\alpha$ -glucosidase activity was found in the lysosomal fraction of the leukocytes, but less than 5% of normal enzyme activity and an elevated 8.7% glycogen were detected in a muscle biopsy. Cadaver heart and liver contained 7.6% and 7.9% glycogen. Less than 2% of normal acid  $\alpha$ -glucosidase activity was measurable in these tissues.

Long-term cultured lymphocytes and fibroblasts contain 10.2% and 24.2% of normal activity respectively. These two cell cultures were compared to normal cultures with respect to enzyme-substrate affinity and isozyme patterns.

Radioisotope labeling showed the glycogen content of Pompe's fibroblasts to be twice that of normal cells. Chase experiments indicate that while all glycogen of normal cells has a half life of 4 days, only 50% of the glycogen in Pompe's fibroblasts turns over this rapidly. The remainder has a half life greater than 30 days. This stable glycogen component may be similar to the excess lysosomal glycogen found *in vivo* and may serve to test for corrective factors at the cellular level.

STUDIES ON KETOTIC HYPERGLYCINEMIA-INHIBITORS OF SERINE HYDROXYMETHYLTRANSFERASE. Chen K. Ho and Richard E. Hillman, Washington Univ. Sch. Med., St. Louis Children's Hosp., Dept. Ped., St. Louis, (Intr. by Philip R. Dodge).

Recently Hillman and Otto showed tiglic acid to inhibit glycine-serine interconversion in fibroblasts from a patient with  $\beta$ -ketothiolase deficiency and normal cells. In order to elucidate the mechanism of these phenomena, the effects of tiglyl CoA and related compounds on partially purified rabbit liver serine hydroxymethyltransferase (SHMT) were investigated. The rate of conversion of serine to glycine was measured as described by Schirch. Using SHMT 27.7 units/ml, serine 16mM, and dl-L-tetrahydrofolate 0.32mM, the concentration of tiglyl CoA and % inhibition (N=5, Mean  $\pm$  S.E.M.) of the enzyme activity were 2.5mM 15 $\pm$ 1.5, 5.0mM 28 $\pm$ 2.4, 7.5mM 40 $\pm$ 2.8, 10mM 41 $\pm$ 3.3. Isovaleryl CoA, n-valeryl CoA, crotonyl CoA and isobutyryl CoA also showed inhibitory effects, but at a lesser degree than tiglyl CoA. n-Butyryl CoA, propionyl CoA and CoA inhibited only slightly. Tiglate, isovalerate, n-valerate, crotonate, isobutyrate and  $\beta$ -methylcrotonate at a concentration of 30mM were incapable of inhibiting SHMT activity. Tiglyl CoA inhibition showed competitive kinetics with tetrahydrofolate, but not serine. The findings suggest that impaired glycine-serine metabolism in the ketotic hyperglycinemia syndrome may, at least in part, result from inhibition of SHMT by high intracellular concentration of Tiglyl CoA.

PSEUDOTUMOR HEPATIS IN TYPE I GLYCOGEN STORAGE DISEASE (VON GIERKE'S). R. Rodney Howell, Roger E. Stevenson, Robert L. Phyllyk and Robert J. Lull. The Univ. of Texas Med. Sch. at Houston, Program in Ped. and Genetics, Houston; and Brooke Army Med. Ctr., Hematology and Nuclear Med. Services, Fort Sam Houston, Texas.

Patients with liver glucose-6-phosphatase deficiency are at risk for developing hepatoma in adult life. Investigations during adolescence and early adult life indicate that these patients may have discrete filling defects as well as depressed isotope uptake on liver scan with technitium sulfur colloid but only mild abnormalities of liver related serum chemistries (Table). On hepatic angiograms in two patients, the filling defects appeared as vascular masses consistent with primary or metastatic malignancy; biopsy showed increased glycogen content but no changes of malignancy.

Age of Patients	14-28 Years
Liver Scans	Decreased liver function with patchy or discrete filling defects
SGOT	13-73 units
Bilirubin	0.2-0.9 mg/100 ml
Alkaline phosphatase	8.9-180 K. A. units
Total protein	7.4-8.7 gm/100 ml
Albumin	2.4-7.0 gm/100 ml

Other storage disorders affecting the liver which have been investigated to date have not shown similar abnormalities on liver scan, nor do they have the tendency for developing malignancy in later life.

GLUCOSE-INDUCED CALCURIARIA IN DOGS. Julian J. Irias, Thomas C. Lee, Makepeace U. Tsao, Walter H. Hamilton, and Larry Hein (Intr. by Robert E. Greenberg). Univ. Calif. at Davis, Sch. Med., Depts. of Ped., Physiol., Surg.

In human subjects, glucose (G) ingestion results in a prompt increase in urinary calcium excretion (CE). It has been suggested (Lindeman *et al.*, J Lab Clin Med 70:236, 1967) that G utilization by the kidney is involved in this calciuric effect, but no direct evidence is available in this regard.

In the present study, dogs fed G (1.5g/kg) had 1.5 to 5-fold increases in CE and 1.4 to 3-fold increases in urine calcium concentrations. Increased CE began within 30 minutes and continued during the 2 hours of observation. Plasma G did not rise above 111 mg/100ml, and there was no glycosuria. The dog thus appeared a suitable model for studying G-induced calciuria.

The possibility of a direct renal effect of G was investigated by infusing G into the left renal arteries of anesthetized hypophysectomized dogs. CE increased only with infusion rates great enough to cause glycosuria and marked natriuresis. Lower infusion rates did not affect CE.

These findings do not rule out a role for renal G metabolism in G-induced calciuria, since hormonal consequences of a G meal (e.g., insulin release) could affect renal carbohydrate metabolism. But an alternative possibility would be an indirect effect not involving renal G utilization.