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## RED CELL PHOSPHOFRUCTOKINASE [PFK] DEFICIENCY IN NEWBORN INFANTS: NORMAL SYNTHESIS OF AN UNSTABLE ENZYME.

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Cord blood erythrocytes from 9 term infants were separated by density gradient centrifugation into cohorts of intact red blood cells [RBC's] of progressively increasing density and compared with RBC's treated in a similar manner from 4 healthy adults. The rate of decline in activity of pyruvate kinase [PK], an age-dependent enzyme, was essentially the same in erythrocytes from term infants and adults, which demonstrated that there is a similar relationship in newborn RBC's between the rate of decline of PK activity and the progressive increase in RBC density that occurs with aging, that had previously been confirmed in adult RBC's. Thus, PK activity appears to be a useful parameter of RBC age in the gradient in the newborn as well as in the adult. This relationship permitted evaluation of the rate of decline of *in vivo* enzymatic activity. PFK activity was similar in the youngest fractions of adult and neonatal RBC's in the gradient. However, in contrast to the similar rate of decline of PK in RBC's from infants and adults, the rate of decline of PFK activity was significantly faster in neonates than in adults, suggestive of an accelerated rate of *in vivo* decay of PFK in newborn RBC's. The demonstration that PFK in cord erythrocytes has increased lability in the gradient suggests that the relative PFK deficiency in neonatal RBC's is secondary to normal synthesis of an unstable enzyme and raises the speculation that PFK in cord erythrocytes may be an isozyme [a "fetal PFK"].

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## FAMILIAL HYPERTRIGLYCERIDEMIA IN CHILDREN: DIETARY THERAPY. C.J.Gluck, M.J.Mellies, R.C.Tsang, P.M. Steiner. University of Cincinnati, College of Med., General Clinical Research Center, Cincinnati.

In 40 children with familial hypertriglyceridemia (FTG), 13±6 years old (X±SE), effectiveness of weight reduction and the NIH type IV diet was assessed. The children came from 34 kindreds where 1 propositus parent and at least 1 additional 1st or 2nd degree relative had FTG. Initially, 20 of the 40 children were obese (defined as ≥ the 95th percentile for weight, adjusted for height). For obese children, weight reduction programs were provided. For nonobese children and for children after weight reduction, NIH Type IV diet was provided, with 20% of the calories as protein, 40% as fat, 40% as carbohydrate, with a P/S of 1.5/1. Diet adherence and dietary re-instruction were checked every 2 months. After 6 months on diet, mean (±SE) triglycerides (TG) in the 40 children fell from 242±30 to 120±8, p<.001; TG were normalized (≤ 140 mg/dl) in 29 of 40 children. Plasma cholesterol fell from 195±7 to 182±6 mg/dl at 6 months, p<.05. Mean weight loss over the 6 month period was 1.2±1.4 kg, p>.1. Decrements in weight failed to correlate with decrements in TG, r=.212, p>.1. In 13 children, after 8-12 months on diet, TG fell from 290±86 (basal levels) to 149±32 mg/dl, p<.05, with TG ≤ 140 mg/dl in 8 of the 13. Obesity facilitates expression of FTG in children at genetic risk. Despite only modest weight reduction, and with use of the Type IV diet, TG levels can be normalized in most children with FTG. Sensitivity to dietary therapy emphasizes the importance of quantitating TG in children from kindreds with FTG.

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## NEONATAL HYPOBETALIPOPROTEINEMIA. C.J.Gluck, R.C. Tsang, M.J.Mellies, P.M.Steiner. Univ. of Cincinnati, Coll. Med., Gen. Clin. Res. Center, Cincinnati, Ohio.

Familial hypobetalipoproteinemia, FHB, characterized by low plasma total cholesterol (C) and low density lipoprotein cholesterol (C-LDL), rarely is accompanied by clinical findings, save reduced myocardial infarction morbidity-mortality, and longevity. Cord blood C-LDL quantitation and kindred study with longitudinal follow-up confirms neonatal diagnosis of FHB. To evaluate neonatal FHB, 11 kindreds were studied with hypoblipoproteinemic neonatal propositi. Cord blood C and C-LDL were quantitated in neonates in a lipoprotein study of 3000 unselected births. Kindreds with neonatal C-LDL ≤ the 2.5 percentile (10 mg/dl) were studied. Of the 11 kindreds selected, 4 generations vertically transmitted FHB was documented in 2. In 1 infant C-LDL was low at 1.5 years (19), but C-LDL was normal (96) in the other at .5 years. In 3 kindreds, 1 parent had primary hypoblipoproteinemia (C-LDLs 63,43,55 mg/dl), but 1st degree relatives had normal C-LDL, and C-LDL in the 3 infants at ages 2.5, 2, and 5 years was normal (89,93,96 mg/dl). Maternal C-LDL in 1 kindred was low-normal (65 mg/dl), the infant at 2 years retained low C-LDL (28); other family members were unavailable. In 1 kindred (cord blood C-LDL 9 mg/dl), 4 generations transmission of familial hyperlipoproteinemia was documented; the infant's C-LDL at 8.5 months was normal (79), high density lipoprotein C was elevated (101 mg/dl). In the other 4 kindreds, parental C-LDL was normal, C-LDL in later infancy (2 and 3 years) were normal (83,100,90,77 mg/dl). FHB, a dyslipoproteinemia associated with longevity, can be diagnosed by measurement of cord blood C-LDL and family study in at least 1 per 1500 births.

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## VITAMIN D RESISTANT HYPOPARATHYROIDISM - IMPAIRED RESPONSE TO 1,25 DIHYDROXYCHOLECALCIFEROL (1,25 DHCC)

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Resistance to vitamin D is an unusual, frustrating complication of hypoparathyroidism. Magnesium depletion, coincident malabsorption, hepatic or renal hydroxylation, and Ca<sup>++</sup> mobilization from bone are postulated mechanisms, but do not explain relative resistance to all vitamin D sterols, including 1,25 DHCC in three patients recently encountered at two institutions.

A.G., a 14 year old calciferol-resistant boy with idiopathic hypoparathyroidism, candidiasis and mild malabsorption, maintained for 4 years with oral Dihydroxycholesterol (DHT), 4-6 mg/day, suddenly became refractory to DHT in doses up to 15 mg daily. Oral 1,25 DHCC to 10 µg was ineffective once daily and unaltered by parenteral Mg SO<sub>4</sub>. A calcemic response to low dose (20-100 U.) PTH was observed. Response to 1,25 DHCC was achieved only with frequent administration (3 µg. q. 6 hours) + 30 Cm elemental Ca<sup>++</sup>/day. Two other hypoparathyroid patients, one surgical, have been found resistant to calciferol, DHT and ordinary doses of 1,25 DHCC. One responded to 5 µg once daily and has stabilized with 1-2 µg/day, while the other responds to a 5 µg daily dose only with IM parathormone, 20-40 U./day.

Plasma 1,25 DHCC (Dr. H. DeLuca) was normal in all 3 patients immediately following ingestion. Preliminary data in A.G. showing a rapid decline in plasma 1,25 DHCC suggest that accelerated sterol metabolism may account for his apparent "resistance".

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## HYPERAMMONEMIA ASSOCIATED WITH SEVERE PERINATAL ASPHYXIA. Ronald N. Goldberg, Luis A. Cabal, Joan E. Hodgman. Los Angeles County-Univ. of So. California

Med. Ctr. and Children's Hosp. of Los Angeles, Dept. of Peds. Although hyperammonemia has been associated with asphyxia in experimental animals, its role and clinical manifestations in the asphyxiated newborn have not been defined. We report 5 newborns with the following common features: gestational age greater than 40 wk. (4 > 42 wks.), evidence of fetal distress by severe fetal bradycardia lasting at least 10 min. in addition to the presence of meconium in the amniotic fluid, prolapsed umbilical cord or abruptio placentae. All had severe neonatal asphyxia (1 or 5 min. Apgar-5), CNS irritability and convulsions. Hyperthermia was found to be associated with ammonia elevations (185-960 mcg/100ml) as was hypertension, respiratory alkalosis and exaggerated cardiac vagal tone. Three infants survived and 2 died. The infants who died were comatose preterminally whereas 2 survivors showed improvement of their neurologic status concomitant with declining ammonia levels. Neurologic improvement was temporally related to exchange transfusion for hyperammonemia in 1 patient. Two survivors showed signs of CNS abnormality at 1 yr. followup. As high ammonia levels are felt to diminish the rate of oxidation in the brain by depletion of ketoglutarate, thereby influencing the Krebs cycle adversely, the accumulation of ammonia in the newborn can only aggravate the neurologic disorder. These infants suggest a clinical syndrome whose symptoms may be related to the ammonia level. The further identification of infants with this syndrome will be necessary to ascertain the role of hyperammonemia in asphyxia and its long term sequelae.

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## GLUCONEOGENESIS IN ISOLATED FETAL HEPATOCYTES, Robert Greenberg, William Howell, William Woodside, Depts. of Ped. &amp; Pharm., Univ. of New Mexico School of Medicine, Albuquerque, New Mexico.

The capacity for gluconeogenesis is absent from liver in the rat fetus, and develops rapidly after birth. Since the regulation of gluconeogenesis has been studied with greater precision in isolated liver cells from adult mammals, a method was developed for isolation of hepatocytes from the rat fetus, and for their use in determining factors involved in initiation of hepatic glucose production.

Fetal hepatocytes were isolated following *in situ* perfusion via the hepatic vein with collagenase in Ca<sup>++</sup> free buffer, followed by pre-incubation in 4% albumin-buffer and subsequent incubation with C<sup>14</sup>-pyruvate (2 mM). Fetal hepatocytes (21 day gestation) converted approximately 20% as much pyruvate to glucose, as compared to hepatocytes from fed adult rats (.39 ± .06 vs 2.63 ± .44 umoles pyruvate converted to glucose/hr/mg DNA). Glucagon (1.4 nM) increased the rate of gluconeogenesis in fetal hepatocytes by 100%, (.79 ± .12 umoles pyruvate converted), dibutyryl cyclic AMP (0.1 mM) by 94% (.76 ± .06) and oleic acid (0.64 mM) by 130% (0.69 ± .09). Glucagon and cyclic AMP enhanced glycogenolysis in isolated fetal hepatocytes.

Immediate extra-uterine adaptation is accompanied by an increase in the rate of glucagon secretion and lipolysis. Although other studies indicate transient neonatal impairment in hepatic response to glucagon, both glucagon and fatty acid oxidation may participate in the initiation of gluconeogenesis in the newborn period.