523 PRENATAL DIAGNOSIS OF HEMOGLOBIN-H DISEASE BY MOLECULAR HYBRIDIZATION. H.M. Koenig, T.S. Vedvick, M.S. Golbus, A.M. Dozy, and Y.W. Kan (spon. by W.L. Nyhan), Naval Regional Medical Center and University of California, San Diego, and University of California, San Francisco.

α-globin synthesis is controlled by 4 structural genes. The α-thalassemia syndromes are characterized by 4 states of increasing clinical severity: silent carriers, α-thal traits, HbH disease and Bart's hydrops fetalis which causes death between 30 and 40 gestational weeks. One, 2, 3, & 4 structural genes respectively are deleted in these syndromes. A Filipino mother with HbH disease and her husband with α-thal trait requested prenatal diagnosis because 1 of 2 previous pregnancies had been a Bart's hydrops fetalis. Fetal fibroblasts were obtained by amniocentesis during week 13 of gestation and placed in culture. α-cDNA was prepared with ^{32}P-deoxycytidine triphosphate from α-mRNA and reverse transcriptase. After 6 weeks of culture, enough fibroblast DNA was present to be hybridized with α-cDNA. Similar studies were conducted simultaneously on DNA from the parent's white blood cells. Results were compared to hybridization studies done on DNA from persons with a full spectrum of α-thalassemia syndromes. DNA from the fetus, the mother, and other persons with HbH disease hybridized 42-50% of the α-cDNA, indicating the fetus had HbH disease. Fibroblast chromosomes were xy, confirming their fetal origin. A male infant was born at term with moderate hepatosplenomegaly. Cord blood values were: Hb 12.8 g/dl, Rbc ct. 5.61×10^{12}/l, MCV 71 fl, Retic ct. 800×10^{9}/l, nucleated Rbc ct. 16×10^{9}/l, Hb Bart's 26%, and α/β+γ synthesis ratio 0.5; confirming diagnosis of HbH disease.

524 CLUSTERING OF CONGENITAL ATHYREOTIC CRETINISM C. Charlton Mabry and Dorothy R. Hollingsworth University of Kentucky, Dept. Pediatrics, Lexington

Congenital athyreotic cretinism (CAC) occurs in more than one sibling or relative on rare occasion. Heterogeneity with the existence of an autosomal recessive form has been evoked as the most plausible explanation, yet almost all cases occur sporatically without apparent cause. The Kentucky experience has been different in that a disproportionate number of CAC cases have come from the same rural community with two sets of affected siblings as first cousins.

Since 1963 we have cared for 25 infants and children (13M, 12F) with CAC, and most have come from distant communities. Six have been clustered in South Madison County, four being two sets of affected siblings who are first cousins. In addition, three older sibs of this kindred died in infancy and childhood with histories and photographs suggestive of CAC. All the Madison County families live within ten miles of each other, and all have lived in the community for generations. Additionally, two brothers with CAC have come from another county. There is no apparent consanguinity in any of the families, and identification of cretins in earlier generations is unreliable. The population is stable, homogenous and of English origin. Other thyroid disease occurred in second degree relatives in six families.

Geographic clustering and familial aggregation of CAC in rural communities with sparse population suggest a common pathogenic mechanism leading to failure of embryonic thyroid development. A Mendelian trait for the defect which is enhanced by additional genetic influences is the best explanation.

525 BEHAVIOR SYNDROME IN FEMALE LIPODYSTROPHY PATIENTS RECEIVING NEUROLEPTIC DRUGS. C. Charlton Mabry University Kentucky, Dept. Pediatrics, Lexington

Pimozide, an antipsychotic neuroleptic drug, administered to a young girl with generalized lipodystrophy (GLD) induced a beneficial effect (Pediat.Res.8:435,1974). When we administered pimozide to other lipodystrophy patients, the drug produced in affected females beyond puberty a mood change which prevented its continued use.

Pimozide in low dose (1-4 mg/day) to 5 postpubescent females (3 GLD, 2 atypical lipodystrophy) produced an immediate disabling mood change characterized by catalepsy, anxiety, and sedation. These changes were not the extrapyramidal side effects produced by pimozide in high dose (>40 mg/day). In one GLD patient, the neuroleptic agents trifluropromazine and haloperidol were administered separately in low dose with effects like that of pimozide. Continued use of pimozide in the original prepubescent GLD patient has shown limited beneficial effect; now that she is entering puberty, the behavior syndrome is developing. Two males with GLD (2 and 56 years) and one prepubescent female with partial lipodystrophy, have not developed the behavior syndrome on pimozide.

These observations indicate an aberrant neurohumoral etiologic mechanism in lipodystrophy and support the theory of defective hypothalmic dopamine metabolism. Absence of the behavior syndrome in a pre- and postpubescent male suggests a sex difference in hypothalamic dopamine metabolism. The findings are analogous to the induced behavior in rats injected intracerebrally with β-endorphin, a fragment of the pituitary hormone, β-lipotrophin.

526 SIALOGLYCOPROTEIN AND GLYCOLIPIDS IN CULTURED FIBROBLASTS FROM PATIENTS WITH CYSTIC FIBROSIS. Reuben Matalon and J.A. Cifonelli. Abraham Lincoln Sch. of Med. Dept. of Ped., Univ. of Ill. and Univ. of Chicago, Chgo. Ill.

Cystic fibrosis (CF) is an autosomal recessive disease affecting the exocrine glands and manifested by increased sweat electrolytes, viscous pancreas and lung secretions, leading to pancreatic and pulmonary insufficiency. Cultured skin fibroblasts from patients with CF and normal individuals were utilized for the study of glycoprotein and glycolipid metabolism. Cells were grown in modified Eagle's medium until confluency (2-3 weeks). Sialic acid content was determined in the CF fibroblasts and was compared to the normal controls. The levels of sialic acid were 3 to 8 fold increased in the CF fibroblasts. The incorporation of ^{14}C-acetate in the glycoprotein and glycolipid fractions of cultured CF and normal fibroblasts was determined. CF fibroblasts showed 2 to 10 fold increase of the label in the glycolipid and glycoprotein fractions. Chlostridium sialidase released 30% of the label indicating incorporation of acetate into sialic acid. N-acetyl groups from glycoprotein of normal and CF cells were estimated, and found to be 18.5% and 14.0% respectively. The O-acetyl groups in the normal cells represented 13% of the total radioactivity while in the CF fibroblasts only 2.5%. Methanolysis of the deacetylated residue showed the incorporation of label into glycolic acid and the methylglycoside of sialic acid. Labeled glucosamine, mannosamine, mannose, fucose and galactose showed similar incorporation in CF and normal cells. The increased levels of sialic acid and the incorporation of acetate by CF cells may afford a new tool for the study of CF in tissue culture.

527 PHENYLALANINE HYDROXYLASE ACTIVITY IN HUMAN TERM PLACENTA. Reuben Matalon, Minerva Deanching, and Parvin Justice. (Spon: by Ira M. Rosenthal) Abraham Lincoln Sch. of Med. Dept.,Ped., Univ. of Ill. Chicago, Ill.

Phenylketonuria (PKU) is an autosomal recessive aminoacidopathy caused by a deficiency of phenylalanine hydroxylase. PKU is the most common aminoaciduria which responds to dietary restriction of phenylalanine. The enzymic studies of PKU have been difficult to approach since phenylalanine hydroxylase activity has been found primarily in liver. Recently some phenylalanine hydroxylase activity has been reported in cultured human fibroblasts, but the activity seems very low. A search for a new source for phenylalanine hydroxylase was attempted using term human placentas. Slices of fresh placentas were washed with cold 0.15M NaCl and then incubated in serum free medium 199 to which repurified L-U-^{14}C-phenylalanine was added. The incubation was carried out for 4 h at 37° with constant stiring. The reaction was terminated by boiling then the material was hydrolyzed with 6 N HCl for 20 h and amino acid analysis was carried out. The conversion of phenylalanine to tyrosine was considered as an indication for phenylalanine hydroxylase activity. In repeated experiments 30%-70% of the labeled phenylalanine was recovered as tyrosine indicating presence of phenylalanine hydroxylase in placenta. Two dimensional thin layer chromatography was performed for the identification of radioactive tyrosine and similar ratios of 30%-70% conversion were observed. These data suggest that placental phenylalanine hydroxylase may be studied in pregnancies at risk for PKU, explore the enzymic differences of PKU variants and possibly used for prenatal detection.

528 EARLY TREATMENT OF COMPLETE ORNITHINE TRANSCARBAMYLASE DEFICIENCY WITH KETO ANALOGUES OF ESSENTIAL AMINO ACIDS. J. McReynolds, S. Mantagos, M. Walser, S. Brusilow, and L. Rosenberg. Dept. Human Genetics, Yale U. Sch. Med. and Depts. Pharm. and Peds., Johns Hopkins Med. Sch., New Haven, CT and Baltimore, MD

Complete deficiency of the urea cycle enzyme, ornithine transcarbamylase (OTC), causes hyperammonemia and death in the neonatal period. Past therapeutic attempts have been singularly unrewarding. We now report treatment of such a male infant (hepatic OTC activity 0.1% of control) from the second day of life with a dietary regimen in which a mixture of essential amino acids and their nitrogen free analogues supplements a protein-restricted milk-based formula. Milk protein (1 g/kg/d) is supplied by standard infant formula and caloric needs (130 Cal/kg/d) and trace elements by a synthetic, protein-free product. To this formula is added a mix consisting of threonine, histidine, lysine, tryptophan, and arginine, plus the keto analogues of valine, leucine, isoleucine and the hydroxy analogues of methionine and phenylalanine, each in a quantity exceeding the infant's daily requirements. On this regimen the child has grown normally to the present time (age 9 wks) and his blood ammonia concentrations are persistently within normal limits (<150 µg/dl). Balance studies have shown normal nitrogen retention for age and intake. Plasma amino acid concentrations are near normal with the exception of reduced values for the branched chain amino acids and arginine which necessitated dietary adjustment at age 6 wks. To our knowledge this child now has lived longer than any previously reported infant with OTC deficiency of this severity.