553 FALSE POSITIVE PRENATAL DIAGNOSIS OF GALACTOSEMIA. Stephen J. Sepe, Harvey L. Levy, Maurice J. Mahoney, Roseann Mandell and Won G. Ng. State Lab Inst., Mass. Dept. Public Health, Harvard Med. Sch., Mass. Gen. Hosp., Yale Univ. Sch. Med., Univ. of So. Calif. Sch. Med., Boston, New Haven and Los Angeles.

Amniotic fluid (AF) was obtained at 18 weeks gestation from a woman who had previously given birth to an infant with galactosemia. The AF cells were cultured and examined for activity of galactose-1-phosphate uridyl transferase (transferase) by UDPG consumption assay. No activity was detectable as compared to activities of 30.8 and 56.0 μmoles UDPG consumed/hr/10^9 cells in two AF cell control lines. After term delivery the infant was found to be heterozygous for galactosemia with erythrocyte transferase activities of 11.8 and 10.8 μmoles UDPG consumed/hr/gm Hb (nl 22 ± 3.7). He had normal galactose tolerance to milk ingestion. The AF cells from the proband were recultured and again had no detectable transferase activity by consumption assay. However, activity was detectable (2.4 μmoles UDPGal formed/hr/10^9 cells; nl 2.0 - 24.8) by a radioactive method. These same cells had normal galactokinase activity (1.32 mU/hr/10^9 cells; nl 0.61 - 1.30). The AF cell line from the proband most resembled fibroblasts but grew in a lacy pattern and never became confluent. 15 AF control lines had transferase activity of 10.1 - 34.9 by consumption assay. There was no correlation with cell type (epithelioid vs. fibroblast) or with state of confluency. Prenatal diagnosis for galactosemia may be falsely interpreted if the UDPG consumption assay is used and the fetus is heterozygous for galactosemia.

554 FUNCTIONALLY ABNORMAL α_2-MACROGLOBULIN (α_2M) IN CYSTIC FIBROSIS (CF). Emmanuel Shapira, Christine Martin* and Henry Nadler. Northwestern Univ. Med.Sch. Children's Memorial Hospital, Dept. of Pediatrics, Chicago.

Previous studies in our laboratory have demonstrated the absence of an α_2M-protease complex in activated plasma of patients with CF (Pediat. Res. 10:812, 1976). Recently we have documented decreased complex formation of CF α_2M with various endoproteases as compared to normal α_2M; CF heterozygotes gave intermediate values (Biochem. Biophys. Res. Commun. 71:864, 1976). This study was undertaken in order to further characterize the differences in the α_2M from CF patients as compared to normal controls. The kinetic properties of purified α_2M from 3 healthy donors were compared to those of α_2M from 3 patients with CF. The binding affinity of α_2M to bovine trypsin was determined from its inhibition of benzoyl-arginine ethyl ester hydrolysis by trypsin. A typical competitive inhibition was obtained with a KI value of 6×10^{-7} M for normal α_2M and of 3×10^{-6} M for CF α_2M. The Km value for α_2M-trypsin complex for this substrate was 5×10^{-4} M for normal α_2M-trypsin complex and 4×10^{-5} M for the CF α_2M complex. Upon incubation at 38° C, the normal α_2M-trypsin complex gradually regained partial activity towards high molecular weight substrate and susceptibility to soybean trypsin inhibitor inhibition. In contrast, this phenomenon could not be demonstrated with the CF α_2M-trypsin complexes.

These studies provide additional evidence that the α_2M in CF is functionally abnormal and provide an explanation for the presence of the various CF factors of a polypeptide nature.

555 β-MERCAPTOPYRUVATE SULFUR TRANSFERASE DEFICIENCY.THE ENZYME DEFECT IN β-MERCAPTOLACTATE CYSTEINE DISULFIDURIA. Vivian E. Shih, Miriam M. Carney, Lisa Fitzgerald and Virginia Monedlkova, Harvard Med. School, Mass. Gen. Hosp.,Dept. of Neurol., Boston, and Wrentham State School, Wrentham, Mass.

The disorder β-mercaptolactate cysteine disulfiduria was first identified in 1968 (Crawhall et al, Science 160: 419), and was thought to be an abnormality in cysteine metabolism. Current understanding of cysteine metabolism indicates that cysteine is mainly degraded to inorganic sulfate (60-80% total urinary sulfur). Thiosulfate constitutes <1% of the total sulfur output. In the latter case cysteine is first converted to β-mercaptopyruvate (βMP) by transamination. The transfer of sulfur of βMP to sulfite to form thiosulfate, or to other anions is mediated by βMP sulfur transferase. A deficiency of this enzyme activity could be the cause of β-mercaptolactate cysteine disulfiduria. βMP sulfur transferase activity was thus studied in the blood cells of the original patient. Hemolysates were prepared in 0.02M cysteamine, and 0.025M phosphate buffer, pH 7.4. The assay condition was modified from that described by Sörbo. Activity of βMP sulfur transferase averaged 2.70 mmoles thiosulfate formed/gmHb/hr (range: 2.27 to 3.53) in 9 control hemolysates but was not detectable in the hemolysate from the patient (<5% of control values).

Presumably the liver enzyme is likewise deficient in this patient and this defect causes accumulation of βMP which is excreted as β-mercaptolactate cysteine disulfide.

556 INHIBITION OF PURINE NUCLEOTIDE BIOSYNTHESIS BY AMMONIA: A POTENTIAL MECHANISM FOR PHYSICAL AND MENTAL RETARDATION IN CHRONIC HYPERAMMONEMIA. Stephen D. Skaper and Irwin A. Schafer Case Western Reserve University School of Medicine, Cleveland Metropolitan General Hospital, Department of Pediatrics, Cleveland, Ohio.

Enzyme deficiencies of the urea cycle and certain other inborn errors are associated with protein intolerance, hyperammonemia and retardation in both mental and physical development. We have studied the effect of ammonia on purine nucleotide biosynthesis to explore a possible mechanism that would relate chronic hyperammonemia to mental and physical retardation. Purine nucleotide biosynthesis in rat liver minces or homogenate was measured by following the incorporation of ^{14}C-Formate into total cellular purines (de novo) or ^{14}C-purine base into soluble nucleotides (salvage). Addition of 10-20mM NH_4Cl resulted in a 30-50% reduction in purine biosynthesis in both assays. The content of 5-phosphoribosyl-1-pyrophosphate (PRPP), an important intermediate in purine nucleotide biosynthesis was decreased 35-50% in liver homogenates incubated with 20mM NH_4Cl. Substitution of 5mM carbamyl phosphate (CP) for NH_4Cl resulted in a marked reduction of purine nucleotide biosynthesis both de novo (70% decrease) and from preformed bases (25-35% decrease). It is proposed that ammonia stimulates the production of CP by the mitochondrial CP synthetase, which in turn would stimulate de novo pyrimidine synthesis and result in a reduced tissue content of PRPP available for purine nucleotide biosynthesis. This work was supported in part by NIH grant #GM 07004.

557 ENZYME THERAPY: EVIDENCE FOR TWO DISTINCT RECEPTORS THAT MEDIATE UPTAKE AND CLEARANCE OF HUMAN β-GLUCURONIDASE. William S. Sly, Daniel T. Achord, C. Elliott Bell, Frederick E. Brot, and Arnold Kaplan, Washington Univ. Sch. Med., Depts. Ped., Med., Path., St. Louis Children's Hospital, St. Louis, Mo.

Specific pinocytosis of lysosomal enzymes by fibroblasts, initially recognized by Neufeld and co-workers, displays the selectivity and saturability expected for a receptor-mediated process. We have used β-glucuronidase uptake by deficient fibroblasts to study this process. Previous studies indicated that β-glucuronidase exhibits charge heterogeneity and that "high-uptake" forms of the enzyme are more acidic than poorly pinocytosed low-uptake forms. More recently, competitive inhibition of the uptake process has been demonstrated by certain hexoses, hexose phosphates, and yeast mannans which contain phosphate. The inhibitor studies, plus the observation that alkaline phosphatase treatment destroys the high uptake capacity of human platelet β-glucuronidase, suggest a novel receptor on fibroblasts that recognizes hexose phosphate on glycoproteins.

Low-uptake enzyme from placenta, though not recognized by fibroblasts, is cleared rapidly from rat plasma following infusion. Periodate treatment followed by borohydride reduction of the enzyme abolishes its rapid clearance. Clearance is inhibited by mannose terminal glycoproteins and free mannose. The enzyme localizes preferentially in hepatic Kupffer cells. Thus, Kupffer cells appear to have a receptor that recognizes mannosyl groups on low uptake enzyme that mediates its clearance. Identification of such cell-specific receptors is likely to be important to enzyme replacement therapy.

558 PROPERTIES OF HUMAN ADULT AND FETAL RED BLOOD CELL ARGINASE: A POSSIBLE DIAGNOSTIC TEST FOR ARGINASE DEFICIENCY. Elaine B. Spector, Stephen D. Cederbaum, and Betty Bernard (Spon. by E. Richard Stiehm), UCLA Sch. of Med., Depts. of Psych. and Ped., USC Sch. of Med., Dept. of Ped., Los Angeles.

Hyperargininemia due to arginase deficiency results in a syndrome of progressive neurological and intellectual deterioration and is inherited in an autosomal recessive manner. Arginase activity is deficient in liver and red and white blood cells. Its activity in normal skin fibroblasts is barely detectable and is undetectable in amniotic fluid cells.

Heparinized blood was obtained from five healthy fetuses between 14 and 20 weeks gestation at the time of therapeutic abortion by hysterotomy. Normal adult blood specimens were used as controls.

The specific activity for the fetal specimens was 0.35-5.20 mmoles urea/gm Hb/hr compared to 3.00 in adult samples. The pH maximum of one fetal and one adult blood with Mn++ activation was 9.5-10.0. In the fetal samples, the activity with Co++ as the divalent cation was 83% that of Mn++ at pH 7.5 and 44% at pH 9.5. The comparable figures for adult blood were 96% and 63%. Activity with Ca++ and Mg++ was minimal for all specimens at pH 9.5. The apparent Km for arginine at pH 9.5 with Mn++ was 14×10^{-3}M for two adult samples and ranged from $10-19 \times 10^{-3}$M for four fetal specimens.

Arginase in adult and fetal red cells may be specified by a single genetic locus. Therefore, fetal blood may be a suitable tissue for prenatal diagnosis of this disease.