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ACID LIPASE IN CULTURED AMNIOTIC FLUID CELLS: IMPLICATIONS FOR THE PRENATAL DIAGNOSIS OF WOLMAN'S DISEASE. Paul M. Coates and Jean A. Cortner
The Children's Hospital of Philadelphia, Joseph Stokes, Jr. Research Institute, Philadelphia, PA

Two pregnancies at risk for Wolman's disease (WD) were monitored by quantitative assays and electrophoresis of lysosomal acid lipase (LAL) in cultured amniotic fluid cells. Cells from case I had less than 5% of control LAL activity with ^{14}C-triolein as substrate, but showed 30-35% of control LAL activity when measured with either 4-methylumbelliferyl oleate or p-nitrophenyl myristate as substrate. Electrophoresis of cells from case I revealed the absence of band A of LAL. After termination of the pregnancy, results of the analysis of fetal tissues were consistent with the diagnosis of WD. Cells from case II had normal levels of LAL activity with all substrates tested and revealed a normal electrophoretic pattern of LAL. These results suggest that the use of synthetic substrates to quantitate LAL in the cultured amniotic fluid cells from a WD fetus may be misleading. On the other hand, electrophoresis of LAL from these cells readily reveals the deficiency of the A band and supports the diagnosis made using radiolabelled "natural" substrates. We recommend, therefore, that these two procedures, radioactive assay and electrophoresis, be employed for the prenatal diagnosis of WD. (Supported in part by NIH grant HL 18723, NF grant 6-80 and AHA grant 76-768).

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CYSTIC FIBROSIS SERUM FACTORS SEPARATED BY HIGH-PRESSURE VACUUM DIALYSIS AND COLUMN CHROMATOGRAPHY. James H. Conover and Elaine J. Conod. St. Vincent's Hospital and Medical Center of New York and New York University Medical School, Departments of Pediatrics, New York.

A limitation to the biochemical separation and subsequent isolation of the ciliary dyskinesia factor (CDF) from sera of Cystic Fibrosis (CF) patients is the sample size restriction. Treatment of CF serum IgG fractions, with high molarity solutions of guanidine hydrochloride(GHCl) has been reported necessary to dissociate low molecular weight substances. This treatment renders concentration of these substances difficult by usual methods. We have employed a reservoir apparatus to which is attached dialysis tubing, enclosed in a vacuum bottle. This permits the initial use of large volumes of CF samples for further chromatography. These CF samples are treated with 5M GHCl, added to the reservoir of the above apparatus and a pressure of .010 Torr applied. The dialyzed material is collected and desalted on G-15 Sephadex or applied to G-50 Sephadex in either a GHCl or an ammonium bicarbonate buffer system for subsequent fractionation and bioassay. We have isolated two major fractions by these methods whose biological activity does not agree with that of comparable fractions obtained by chromatography of PM-10 Amicon ultrafiltration of similar CF samples. It is our contention that treatment of CF serum-derived IgG with chemicals such as guanidine hydrochloride not only alters molecular stoichiometry but also affects the biological activity of the dissociated moieties.

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SUCCESSFUL SPLENO-RENAL SHUNT AND SPLENECTOMY IN TWO PATIENTS WITH ALPHA-1-ANTITRYPSIN DEFICIENCY. Edward A. Cutler, Carolyn A. Romshe, H. William Clatworthy, Juan F. Sotos. Depts. of Pediatrics and Surgery, College of Medicine, The Ohio State University & The Children's Hospital Research Foundation, Columbus, Ohio

The hepatitis associated with A1AT deficiency is no longer considered an invariably fatal disorder. For some patients portal hypertension may be the major determinant of survival. The few reports of porta-systemic venous anastomoses have shown poor results or uncertain outcome, and doubts exist as to whether such shunts should be performed. Two patients with A1AT deficiency (PiZZ) and associated portal hypertension, cirrhosis (proven by biopsy) and hypersplenism underwent spleno-renal shunt and splenectomy six years ago, and both are doing well, attending school, and have practically normal liver function. One of the patients has chronic severe headaches, diarrhea, exudative enteropathy (20% of Cr^{51}-labelled albumin recovered in stools in 4 days), sinusitis and hematuria, all uncommon in A1AT deficiency but likely related to the primary disorder. She also has a higher trypsin inhibitory capacity (0.45 mg trypsin inhibited/ml serum) than is generally reported in ZZ individuals. Based on the experience with these two patients, we conclude that A1AT deficiency is not a valid contraindication to the performance of a porta-systemic shunt.

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RAPID PRENATAL DIAGNOSIS OF FABRY'S DISEASE. Robert J. Desnick and Raman M. Reddy, Mt. Sinai School of Medicine, Division of Medical Genetics, New York City

A rapid (3 day) procedure has been evaluated for the prenatal diagnosis (Dx) of Fabry's disease, an X-linked lipidosis resulting from defective α-galactosidase A activity (α-gal A) and the accumulation of trihexosyl ceramide (GL-3). In 7 at-risk pregnancies Dx was based on 1) specific assay of thermolabile α-gal A, 2) α-gal A inhibition by myoinositol (Myo) and melibiose (Mel), and 3) GLC quantitation of GL-3 in amniotic fluid (AF). These data were supported by careful X and Y chromatin studies of uncultured amniotic cells (UAC) and subsequently by α-gal A and karyotype analyses in cultured (C) AC. Normal mean (range;n) α-gal A in AF, UAC and CAC were 0.93 nmoles/h/ml (0.50-1.3;10),1.76 nmoles/h/mg Pr (0.65-4.0;14), and 116.7 nmoles/h/mg Pr (58.1-210.0;10), respectively. Myo and Mel inhibited normal AF α-gal A activity by 50 and 85%, respectively. Normal mean AF GL-3 concentration was 0.35 nmoles/ml (0.18-0.46;5). Of the 7 pregnancies monitored, 1 affected hemizygote, 1 normal male and 2 heterozygous (het) and 2 normal female fetuses were diagnosed 3 days after amniocentesis. The affected fetus had no detectable α-gal A and 10X higher levels of GL-3 in AF, and was Y-chromatin positive. Each Dx was confirmed by enzyme and karyotype analyses in CAC. The affected pregnancy was terminated and the Dx documented by biochemical and ultrastructural studies. Postnatal studies confirmed the other Dx with the exception of the 2 het females who were not carriers by postnatal assay. These results indicate that the prenatal Dx of Fabry's disease can be made rapidly and reliably by these procedures.

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HUMAN HEMOGLOBIN IN VIVO SOMATIC CELL MUTATION ASSAY Richard Doherty and Elsa Cernichiari; Depts. of Pediatrics, Radiat. Biol./Biophysics, Obstetrics,Genetics Univ. of Rochester, Rochester, New York 14642

A codon-specific somatic cell mutation assay model system is being developed, using human erythrocytes and antisera which will distinguish human hemoglobins differing by a single amino acid residue. A goat anti-hemoglobin S antiserum ("anti-β^{6val})has been obtained which specifically distinguishes the single amino acid difference between HbS($\alpha_2\beta_2^{6val}$)and HbA($\alpha_2\beta_2^{6glu}$).Unabsorbed goat anti-HbS antiserum bound nearly equivalent amounts of HbS and HbA, but after absorption with an excess of HbA, was rendered specific for HbS. A quantitative radioimmunoassay for HbS has been developed which is highly specific and sensitive. With constructed mixtures of purified hemoglobins A and S, our current assay is capable of specifically detecting $<10^{-9}$ gram of hemoglobin S in the presence of more than a million-fold excess of hemoglobin A. We have documented equivalent sensitivity and specificity in human whole blood lysates. Results to date allow us to conclude that in "normal" AA individuals the sum of errors of replication (mutation) plus errors of expression(transcription plus translation)may be less than one molecule of Hb S per 10^7 molecules of HbA synthesized. We are currently determining whether there are detectable quantities of Hb S in bloods of AA individuals exposed to toxic environmental agents and whether these individuals differ significantly from unexposed populations

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BIOCHEMICAL AND IMMUNOLOGIC EVIDENCE OF A LIPOAMIDE DEHYDROGENASE COMPONENT WITHIN THE BRANCHED CHAIN ALPHA-KETOACID DEHYDROGENASE COMPLEX. Paul M. Fernhoff, Dean J. Danner, and Louis J. Elsas, II. Emory University School of Medicine, Dept. of Pediatrics, Division of Medical Genetics, Atlanta, Georgia 30322.

Branched chain alpha-ketoacid dehydrogenase (BCKAD) is located on the mitochondrial inner membrane and oxidatively decarboxylates the branched chain alpha ketoacids. It is presumably a multienzyme complex whose function is impaired in the inherited disorder Maple Syrup Urine Disease. However, neither the subunit composition nor the defective component in Maple Syrup Urine Disease has been identified. We have partially purified BCKAD from bovine liver mitochondria. It decarboxylates all 3 branched chain alpha ketoacids, has a maximum MW of 2.0×10^6 and minimum MW of 2.5×10^5 Daltons. It exhibits a typical flavoprotein spectra, and will reduce oxidized lipoamide with NADH as a substrate. To characterize this lipoamide dehydrogenase (LAD) immunologically, antibodies were produced against porcine LAD in rabbits and isolated by ion exchange chromatography. The monospecific IgG fraction completely inhibited porcine LAD activity at a 64:1 ratio. On Ouchterlony double diffusion gels a single precipitant band of identity was found between the porcine LAD and bovine BCKAD. We conclude that LAD is the flavoprotein subunit of the BCKAD multienzyme complex. The specific antibody to LAD can now be used to identify genetic defects in BCKAD and other mitochondrial dehydrogenase complexes. (Supported by NIH H.D. 08388)