GENETICS

481 DETECTION OF PYRUVATE CARBOXYLASE DEFICIENCY IN LEUKOCYTES AND FIBROBLASTS. Beth M. Atkin, Merton F. Utter, Mitchell B. Weinberg, and Neil R.M. Buist. Univ. Ore. Health Sci. Ctr., Div. Med. Genetics, Portland, Ore. and Case Western Reserve Univ., Dept. Biochem., Cleveland, OH.

Studies on a child with hepatic pyruvate carboxylase (PC) deficiency have led to the development of a practical assay for PC in cell-free leukocyte (WBC) and fibroblast preparations and to the first detection of PC deficiency in peripheral cells. This assay showed that the patient had about 2% (easily detectable in this assay) of normal values for PC in fibroblasts and undetectable PC in WBC. The assay is based on the fixation of $H^{14}CO_3^-$ into oxalacetate in the presence of citrate synthase. The assay is linear with time up to 90 min. with both WBC and fibroblasts and with protein up to 250 μg for WBC and 150 μg for fibroblasts. That the pyruvate-dependent $H^{14}CO_3^-$ fixation is due to PC is supported by the fact that citrate is the only radioactive product and that the activity in fibroblasts is inhibited by antibody prepared against liver PC. Normal values in fibroblasts (n=3) are 1.11 ± 0.08 nmole HCO_3^- fixed/min/mg protein (munits) and in WBC 0.089 munits ± 0.006 (SEM) for 3 adults and 2 children. The finding of little or no PC activity in WBC and fibroblasts in the patient provides strong evidence that the hepatic form of PC is identical with that of WBC and fibroblasts. Thus the assay for PC in peripheral cells should offer a valid diagnostic tool for the detection of hepatic PC deficiency. Also the assay in fibroblasts should make prenatal diagnosis and family studies of deficiencies of this enzyme possible for the first time.

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Abstract withdrawn

483 COMPLEX ANEUPLOIDY IN AN APPARENTLY NORMAL FEMALE Paul J. Benke and Joan Davies (Sponsored by Uwe Stave) Mailman Center and Department of Pediatrics, University of Miami School of Medicine, Miami, Fl.

A 35 year old woman was referred for genetic counseling after delivering a stillborn with multiple congenital anomalies. Thirty percent of peripheral blood chromosomes were aneuploid after PHA stimulation compared to less than 5 percent usually found. This increased to 48 per cent in skin fibroblasts after 6 months in tissue culture. Chromosome studies of the patient's two children, mother, father and two brothers were normal. G,D, and C group chromosomes were aneuploid, but not A, B or F. 13 of 25 clones from single cells grew up, and greater than 30 per cent aneuploidy was demonstrated in each one. These findings may be secondary to in vitro tissue culture conditions, and may not represent a defect in cell division in vivo. Mouse cells, for example, demonstrate high aneuploid rates in vitro. Nevertheless, these findings also may represent a new genetic mutation of mitotic cell division, perhaps in spindle fiber formation, and may be related to clinical aneuploid conditions such as trisomy chromosome syndromes.

484 QUANTITATION OF GM_1-β-GALACTOSIDASE (β-gal) CROSS REACTING MATERIAL (CRM) IN GM_1-GANGLIOSIDOSES (GM_1-gang). Yoav Ben-Yoseph*, Barbara K. Burton* and Henry L. Nadler. Northwestern Univ. Med. Sch., Children's Memorial Hospital, Department of Pediatrics, Chicago.

GM_1-gang are glycolipid storage diseases characterized by an accumulation of GM_1-ganglioside and a deficiency of β-gal activity. Antibodies evoked against purified A_1 isozyme of human liver were used to develop a sensitive radial immunodiffusion assay to quantitate the CRM of biological samples independent of their enzymic activity. β-Gal activities were determined in fibroblasts from controls and from patients with types 1 and 2 GM_1-gang and Krabbe's disease using 4 mu-β-D-galactoside, (^{3}H) lactosylceramide (assay I for "Krabbe's" enzyme, assay II for "GM_1" enzyme), and (^{3}H) GM_1-ganglioside. The CRM was determined and the ratio of GM_1 activity to CRM (CRM specific activity) was calculated.

In controls, the CRM was 0.31-0.43 μg/mg protein with a CRM specific activity of 512-588 nm/hr/μg. CRM of Krabbe fibroblasts and the β-gal activities were higher than those of controls resulting in CRM specific activity similar to controls. In GM_1-gang, type 1, normal quantities of CRM were found with about a 500-fold decrease in the CRM specific activity. Almost twice the normal quantities of CRM were found in GM_1-gang type 2 but the CRM specific activity was decreased only 100-fold. This assay provides new data regarding the molecular defect in GM_1-gang, types 1 and 2 and may serve as a tool for the study of other defects of β-gal.

485 ACID α-GLUCOSIDASE FROM NORMAL AND DEFICIENT CELLS. Nicholas G. Beratis, Gundula U. LaBadie, and Kurt Hirschhorn. Mount Sinai School of Medicine, Dept. Pediatrics; Inst. Basic Research in Mental Retardation, Div. Human Development and Genetics, New York, N. Y.

Acid α-glucosidase (AG) of 15% of mean control was found in cultured skin fibroblast (FB) of a patient with late onset AG deficiency. AG was purified from normal and from deficient FB by affinity chromatography. Placental AG was purified to homogeneity with CM-Sephadex C-50, ammonium sulfate precipitation, dialysis, Amicon filtration, affinity chromatography by Sephadex G-100 and DEAE-cellulose ion-exchange chromatography. Two activity peaks and a shoulder were eluted from DEAE-cellulose with a NaCl gradient. The two peaks and the shoulder had different specific activities and on acrylamide disc electrophoresis each gave one protein band with different mobilities corresponding to the enzyme activity. The pH optimum, Km, Vmax, electrophoretic mobility, thermal denaturation at pH 4.0 and 7.0, and inhibition by turanose, α-methylglucoside and trehalose were the same in purified wild type and mutant enzymes. A greater inhibition was found with all inhibitors when glycogen, rather than maltose, was the substrate. The Vmax of the placental enzyme was greater than that of the FB enzyme. Heating crude extracts from normal and deficient FB gave an initial increase in specific activity which was not present with purified enzyme. We are now producing antibodies against the enzyme to determine whether or not the amount of enzyme protein in deficient cells differs from that in normal cells.

486 MUTAGENESIS IN HUMAN LYMPHOID LINES. Arthur D. Bloom, Soja P. Bennett, Frank T. Nakamura, Sally Spence, and Keiichi Ohki. Coll. of Phys. & Surg., Columbia Univ., Dept. of Pediatrics, N. Y.

We are attempting to develop the human lymphocyte culture system for study of rates and mechanisms of chemically-induced mutation. Three loci are being examined: the hpt locus which specifies HGPRT synthesis; the as locus which specifies argininosuccinate synthetase (AS) synthesis; and the o locus which determines ouabain responsiveness. Mutation from $hpt^+ \rightarrow hpt^-$, using 6-thioguanine (6TG) selection, is being studied, as is mutation from $as^- \rightarrow as^+$, using high citrulline-low arginine medium, and $o^s \rightarrow o^r$, using 10^{-6}M ouabain. The tester line UM-21-5, of citrullinemic (as^-/as^-) origin, has, via selection, produced a line triply mutant (hpt^-, as^-, and o^r), as determined by growth in selective media and by HGPRT and AS specific activities. The Wi-L2 tester line, derived originally from a spherocytosis patient, has produced, by selection, an hpt^- and as^- derivative. Spontaneous mutation rates in Wi-L2 from $6TG^s \rightarrow 6TG^r (hpt^+ \rightarrow hpt^-)$, via fluctuation analyses, ranged from 0.1-0.3 X 10^{-7}/cell/generation. Using sister chromatid exchange (SCE) frequency as an indirect measure of mutagenicity, incubation of Wi-L2 in ethyl methanesulfonate (EMS), 50 μg/ml for 24 hours, resulted in a 3-fold increase in SCE's (mean in controls of 7.7 SCE's/metaphase vs. 21.9 in EMS-exposed cells). The mutation rates at these 3 loci after EMS mutagenesis are now being determined, as a model for demonstrating the feasibility of monitoring for chemical mutagens using these lymphoid lines.