GENETICS

IN UTERO TREATMENT OF METHYLMALONIC ACIDEMIA (MMA-EMIA) WITH VITAMIN B12. Mary G. Ampola, Tufts-New England Med. Ctr., Dept. of Ped., Boston and Maurice J. Mahoney, Eiichi Nakamura, Kay Tanaka, Yale Univ. Sch. of Med., Dept. of Human Genetics, New Haven, Conn. (Intr. by Sydney S. Gellis).

The question of whether Vitamin B12 can prevent fetal accumulation of methylmalonic acid (MMA) in the vitamin-responsive form of the disease was investigated for the first time. During the second pregnancy of a woman who had previously lost a child with presumed MMA-emia, sonicated amniotic fluid cells initially showed no activity of MM-CoA Mutase and no accumulation of the coenzyme 5'-deoxyadenosylcobalamin. Full activity of the enzyme was restored with the addition of this cofactor. Vitamin B12 in large doses was given to the mother from 32 wks. gestation to term. Maternal serum B12 levels reached 3,000-6,000 pg/ml (normal < 900) in the last 6 weeks. Cord blood B12 was over 19,000 pg/ml; maternal serum B12 taken simultaneously measured over 18,000. Instead of the anticipated progressive increase in maternal urinary MMA during the last 2 months of pregnancy, there was a decrease. Following birth, the infant's urinary MMA content was 67 μgm/mg creatinine (normal < 5.0), and has remained essentially the same for the first 5 weeks of life on a diet containing 1.5 gm protein/Kg/day; thus far supplemental B12 has not been necessary. Skin biopsy studies to confirm B12 responsiveness are nearing completion. It seems likely that administration of large doses of Vitamin B12 to mothers carrying fetuses with the B12 responsive form of MMA-emia can slow prenatal accumulation of MMA.

MOLECULAR GENETICS OF THE β-THALASSEMIA (β-THAL) SYNDROMES. E.J. Benz, Jr., B.G. Forget and D. Housman. (Intr. by D.G. Nathan). Children's Hospital Medical Center, and Harvard Medical School, Boston, Mass. 02115

Globin messenger RNA (mRNA) from reticulocytes of several patients with various β-thal syndromes was studied in two ways: I. partially purified globin mRNA preparations from β-thal reticulocytes were tested for their capacity to promote α and β^A globin chain synthesis in a heterologous cell-free system which preferentially synthesized β^A chains when incubated with normal human globin mRNA (β^A/α = 2-4). In this system, globin mRNA preparations from each of 10 patients with β^+-thal (β^A/α = 0.1-0.3); 2 patients with homozygous β^0-thal (β^A/α = 0); and 4 patients doubly heterozygous for β^0-thal and an abnormal hemoglobin (β^A/α = 0) reproduced in every case the defect in β chain synthesis occurring in their intact reticulocytes. II. The actual chemical amounts of β chain specific mRNA sequences (relative to α chain sequences) in the β-thal mRNA's were measured by re-annealing β-thal mRNA, in separate experiments, with synthetic radioactive DNA (cDNA) complementary to purified normal α chain specific or β chain specific mRNA. When measured by these hybridization techniques, β chain mRNA was reduced to 10-30% of normal in the 4 patients tested with β^+-thal and was absent in 4 patients with β^0-thal. All of the β-thal syndromes studied to date are thus characterized by β chain mRNA which is diminished (β^+-thal) or absent (β^0-thal) not only in terms of template activity but also in absolute chemical amounts.

HYBRIDIZATION OF ESTABLISHED IMMUNOGLOBULIN-PRODUCING CELLS. Arthur D. Bloom, Anita S. Wong, and Frank T. Nakamura. Univ. of Mich. Med. Sch., Depts. of Human Genetics and Ped.

To study the regulation of immunoglobulin (Ig) production in human lymphocytes and to link the genes for heavy and light chain synthesis to specific human chromosomes, we have developed methods for hybridization of the cells of established lymphocyte lines. Our first fusion involved an interspecific human lymphocyte (HGPRT$^-$, kappa chain producing) x hamster fibroblast (TK$^-$) cross; the second, an intraspecific human lymphocyte x lymphocyte cross, with both lines producers of gamma, mu, and kappa chains. In both instances, the lymphocytes were pre-treated with 0.01% trypsin prior to addition of Sendai virus. In the interspecific cross, kappa chain production was lost; in the intraspecific cross, in which the chromosomes of both parental cells were initially retained, gamma, mu, and kappa chain production were also retained, as determined by immunofluorescence. The lines resulting from the lymphocyte x lymphocyte hybridization were, however, unstable, and reverted to diploidy by 8 weeks post-fusion. We are currently attempting to establish and maintain a chromosomally stable intraspecific hybrid obtained from fusion of an Ig$^+$ and an Ig$^-$ cell line.

PARTIAL ARYL SULFATASE A (ASA) DEFICIENCY IN METACHROMATIC LEUKODYSTROPHY (MLD): A NEW VARIANT? Carol W. Booth* and Henry L. Nadler, Northwestern Univ. Med. Sch., Children's Mem. Hosp., Dept. of Pediatrics, Chicago.

MLD is a degenerative neurologic disease associated with a deficiency of ASA. A number of variants have been described differing primarily in age at onset. ASA levels are decreased to <15% of normal, no ASA is demonstrable on electrophoresis, and sulfatide accumulates to varying degrees. We report here a patient with onset of seizures at 6 mos. who at 5 yrs. had spasticity and absent reflexes with normal nerve conduction velocity, spinal fluid protein and gall bladder function.

WBC's and fibroblasts had 15% of normal ASA (MLD controls had 15% in WBC's and <5% in fibroblasts). Parents had 50% in both WBC's and fibroblasts, as did classic MLD heterozygotes. Electrophoresis revealed two bands of aryl sulfatase activity with decreased intensity of ASA. The pH optimum and K_m of the mutant ASA in fibroblasts were similar to normal. S^{35} sulfatide accumulation in the fibroblasts was abnormal and comparable to MLD. After 20 passages, the patient's fibroblasts' ASA had increased to 30% of normal and sulfatide accumulation was normal. In contrast, neither MLD nor control cells showed changes in ASA or sulfatide accumulation.

The clinical and laboratory observations suggest that this patient may represent a new variant of MLD and ASA deficiency. The partial deficiency of ASA, demonstrable on electrophoresis is similar to findings in Type III, G_{M2} gangliosidosis where residual hexosaminidase A behaves in a comparable fashion.

3-HYDROXY-3 METHYL GLUTARYL COENZYME A (HMG CoA) REDUCTASE ACTIVITY IN FIBROBLASTS FROM PHENOTYPIC HOMOZYGOUS TYPE II HYPERLIPOPROTEINEMIA (HHLPII). Jan L. Breslow, Samuel E. Lux, Duane R. Spaulding, Harvard Med. Sch., Children's Hosp. Med. Ctr., Dept. of Ped., Boston; Howard R. Sloan, NIH, Bethesda. (Intr. by Fred S. Rosen).

The activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, is repressed by serum in normal fibroblasts. We measured HMG CoA reductase by the conversion of [3-^{14}C] HMG CoA to mevalonate in confluent fibroblast cultures incubated for 24 hr in Eagle's MEM with and without 10% fetal calf serum (FCS). Five normals (NLS) and 4 patients with HHLPII were studied (TP, JaP, LH, LC: Levy et al., Ann. Int. Med. 77:267, 1972). HMG CoA reductase in NLS was 1.10 ± 0.53 (avg ± SD) units (pmol/min/mg protein) with FCS and 7.70 ± 3.78 units without FCS. HMG CoA reductase in HHLPII cells was 7.83 ± 3.69 units with FCS and 10.97 ± 4.80 units without FCS. Ratios of HMG CoA reductase activity with/without FCS equals 0.16 ± 0.07 in NL cells and 0.68 ± 0.31 in all 4 HHLPII cell lines (p 0.005). An intermediate value, 0.34 ± 0.09, was observed in one HHLPII cell line (LH) which differed significantly from the other 3 HHLPII values, 0.80 ± 0.26 (p 0.05), and from NL values (p 0.01). Conclusions: (1) HMG CoA reductase levels were not inhibited by serum in cells from 3 patients with HHLPII, which confirms and extends to new patients the observation of Goldstein et al. (PNAS 70:2804, 1973). (2) Genotypic variation is suggested by one patient, LH, a phenotypic HHLPII, who exhibited partial inhibition by FCS.

NIEMANN-PICK DISEASE, TYPE C: ABSENCE OF A SPHINGOMYELINASE ISOENZYME. John W. Callahan, Mary Khalil and J. Alexander Lowden. Research Inst., The Hosp. for Sick Children, Toronto, Canada.

Niemann-Pick disease exists in multiple forms. Two types (A and B) are characterized by sphingomyelin storage and markedly reduced levels of sphingomyelinase activity. A third form (Type C) also shows sphingomyelin storage but total enzyme activity is within normal limits. Since defects in multiple enzyme species could explain this data, extracts of human liver were subjected to isoelectric focusing in sucrose gradients (pH range 4-7). Two major (I and II) and two minor (III and IV) species of sphingomyelinase were resolved in normal liver. Recovery of enzyme activity was 70-80%. Isoenzyme I has a pI of 4.5-4.7, a pH optimum near 5.0 and a Km of 0.069 mM. The second major species (II) has a pI of 5.1-5.3, a pH optimum near 4.0 and a Km of 0.045 mM at pH 4.0. Both species are found at the corresponding isoelectric point when re-focused separately. Species III and IV have not been characterized. In liver from a known case of Niemann-Pick Type C, only one major isoenzyme (pI 4.6) was found. It refocused to the same isoelectric point and showed the same properties as isoenzyme I from normal liver. In preliminary experiments on brain, isoenzyme I was the major species present. We conclude that multiple species of sphingomyelinase exist in human tissues and the absence of species II in liver constitutes the genetic defect in Type C, Niemann-Pick disease. Supported by Provincial Health Grant PR 360C.