97 DIAGNOSIS OF LESCH-NYHAN HETEROZYGOTES USING PERIPHERAL BLOOD

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Although carriers of deficient genes for Lesch-Nyhan syndrome are detectable using cultured fibroblasts or hair roots, peripheral blood has been insufficient specimen for the diagnosis. We have established a method using peripheral T-cells for the diagnosis of the heterozygotes. Mononuclear cells from five mothers of Lesch-Nyhan boys were stimulated with phytohemagglutinin and then maintained in the medium containing T-cell growth factor. The growing viable T-cells were then exposed to 6-thioguanine. Most of the cells were killed by the treatment, but after 2-4 days, rapid growth of 6-thioguanine resistant cells was observed. T-cells from Lesch-Nyhan boys were totally resistant to 6-thioguanine and normal T-cells were sensitive. We estimated the percentages of HGPRT negative cells among T-cell growth factor-responsive T-cells from each heterozygote by comparing the growth curves for these cell populations with those for mixed cell populations containing enzyme-negative (Lesch-Nyhan's cells) cells and positive (normal) cells in various ratios. The Lesch-Nyhan mothers were estimated to contain 5-40% enzyme negative T-cells, indicating that mosaicism is present in T-cell populations in heterozygotes and the carrier state for Lesch-Nyhan syndrome is detectable using peripheral blood.

98 JAPANESE PATIENTS WITH PARTIAL APRT DEFICIENCIES ACCOMPANIED WITH 2,8-DIHYDROXYADENINE LITHIASIS SYNTHESIZE MUTANT APRT WITH ALTERED KINETIC PROPERTIES WHICH DOES NOT FUNCTION IN VIABLE CELLS

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Although all Caucacian patients with 2,8-dihydroxyadenine urolithiasis completely lack adenine phosphoribosyltransferase (APRT), more than half of Japanese patients with the same lithiasis only partially lack APRT. The degrees of APRT deficiencies in these Japanese patients are broadly the same as healthy heterozygotes for complete APRT deficiencies. We found that cultured T-cells from the Japanese patients with partial APRT deficiencies were, like those from the patients with complete APRT deficiencies but unlike the heterozygotes, more than 100-fold less sensitive to adenine analogs, 6-methylpurine and 2,6-diaminopurine, suggesting that APRT of these Japanese patients is non-functional in viable cells, although it functions normally in test tubes. Therefore, it is not APRT activity in test tubes but whether APRT is functional in viable cells that is important for clinical symptoms (lithiasis). The mutant enzymes from four separate families were found to have reduced affinities to PRPP but not to adenine, and at physiological concentrations of PRPP, normal but not mutant enzymes showed significant activities. The mutant enzymes contained several other altered characteristics in common possibly suggesting that they are coded by a common mutant allele.

99 PURINE NUCLEOTIDE METABOLISM IN HUMAN NEUROBLASTOMA CELL LINES.

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Purine nucleotide biosynthesis de novo, purine salvage pathways, interconversions and catabolism were studied in two human neuroblastoma cell lines generated from patients with localized (NUB-6) and metastatic (EW2) disease. Radioactive adenine and hypoxanthine were effectively incorporated into nucleotides. Adenine salvage exceeded markedly that of hypoxanthine salvage. AMP formed from adenine was mainly phosphorylated to ATP and only slightly dephosphorylated to AMP and GMP but also intensively dephosphorylated to inosine. Interconversion of IMP to AMP exceeded twice that of IMP to GMP. Adenosine and guanosine were also efficiently incorporated. Adenosine was mainly deaminated but some adenosine was also phosphorylated. Guanosine was markedly phosphorylized to guanine which was subsequently salvaged to GMP. GMP formed was then phosphorylated or deaminated with the same intensity. Nucleotide biosynthesis de novo from formate or glycine was very low compared to the salvage pathways. Hypoxanthine was the major catabolic product of ATP, and only minor amounts of inosine, adenosine and uric acid were found. The Addition of deoxycoformycin increased only slightly adenosine accumulation. Addition of tubercidin markedly induced ATP catabolism but only slightly affected adenosine salvage. Although overall fate of nucleoside metabolism is similar in both cell lines, activity of most purine pathways is 2-3 times higher in the metastatic line.

100 ISOLATION AND CHARACTERIZATION OF LEISHMANIA DONOVANI RESISTANT TO 5-FLUOROPYRIMIDINES.

Kiran J.Kaur and Buddy Ullman. Univ. KY Medical Center, Dept. of Biochemistry, Lexington, KY, U.S.A. From a mutagenized population of wildtype Leishmania donovani promastigotes, clonal isolates of mutant organisms were isolated in semi-solid agar containing 200μM 5-fluorouracil. These mutant organisms were cross-resistant to the cytotoxic effects of 5-fluorouridine but were just as sensitive to 5-fluorodeoxyuridine and methotrexate. Wildtype and mutant organisms, however, were equally capable of accumulating radiolabelled 5-fluorouracil, uracil, and uridine into anionic metabolites, suggesting a lack of a metabolic defect in the mutant organisms. Measurements of the intracellular nucleoside triphosphate levels did not indicate any abnormalities. Yet overnite incubations of wildtype cells with cytotoxic (50μM) concentrations of 5-fluorouracil markedly depleted pyrimidine nucleoside triphosphate pools without affecting ATP and GTP levels. In mutant organisms no such depletion of CTP and UTP were observed after similar incubations with 5-fluorouracil. Certain naturally occurring pyrimidines could protect wildtype cells from the cytotoxic effects of 5-fluorouracil. This biochemical genetic analysis of 5-fluorouracil-resistant Leishmania donovani suggests that 5-fluorouracil exerts its cytotoxic effects by inhibiting pyrimidine biosynthesis and that the mutant cells possess a genetically altered pyrimidine biosynthetic pathway.

101 ORGANIZATION OF THE HUMAN HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE GENE.

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The human hypoxanthine phosphoribosyltransferase (HPRT) gene has been isolated and characterized as a set of overlapping genomic clones spanning more than 30kb of the human X chromosome. Restriction endonuclease mapping and DNA sequence analysis of exon-intron boundaries show that the HPRT gene is split into nine exons whose boundary sequences are consistent with the established consensus splice site sequences and whose boundary locations are the same as those defined in the mouse HPRT gene. Transcription orientation and some intron sizes of genomic clones have been determined by heteroduplex analysis. Characterization of 5' flanking sequences shows that the promoter region lacks typical eukaryotic regulatory elements such as TATA and CCAAT boxes and is very rich in G and C residues. These characteristic features common to a number of housekeeping genes presumably have some role in the expression of the human HPRT gene.

102 GLIAL CELLS METABOLICALLY COOPERATE: A POTENTIAL REQUIREMENT FOR GENE REPLACEMENT THERAPY.

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Immunofluorescent-labeled glial cells are shown by radioautography to metabolically cooperate with fibroblasts. The observations of cooperation without cell contact and of incorporation gradients suggest that cooperation occurs through extracellular transport of radiolabeled adenine, adenosine or methylthioadenosine. The transfer of these purine compounds is supported by the quantitative loss of cooperation when the recipient cell is also deficient in enzymes required for adenine or adenosine salvage. The demonstration of glial cell cooperation provides impetus for current research toward gene replacement therapy for the neurologic symptoms of certain inborn errors of metabolism, such as Lesch-Nyhan syndrome.