145 CLASSIFICATION OF PURINE NUCLEOSIDE ANALOGS BASED ON MULTIPLE BIOLOGICAL AND BIOCHEMICAL PARAMETERS.

M. Jean Noujaim, George Zombor, J. Frank Henderson. Cancer Research Group, University of Alberta, Edmonton, Canada.

On the basis of a systematic study of the effects of growth inhibitory purine and purine nucleoside analogs on 29 biological and biochemical parameters, we have classified them into 12 major groups. Drugs whose toxicity is reversed by adenine and hypoxanthine are divided into 3 groups. In addition to reduction of formate incorporation, altered ribonucleotide pool sizes and inhibition of macromolecule synthesis, 1 group is metabolized to drug nucleotides whereas the 2nd group is not. The 3rd group has diverse effects. Drugs whose toxicity is not reversed by adenine and hypoxanthine and which demonstrate reduction of incorporation into nucleotides of both formate and hypoxanthine are sub-divided into 4 categories. The first 2 inhibit macromolecule synthesis with differences in drug nucleotide formation and cell volume. The latter 2 categories form drug nucleotides and show diverse effects on ribonucleotide pools and DNA, RNA and protein synthesis. Five groups remain. One markedly inhibits DNA synthesis, a 2nd involves IMP dehydrogenase inhibition, and a 3rd shows high GTP levels and marked reduction of formate and hypoxanthine incorporation. The last 2 groups, involving potentiation by deoxycoformycin or guanine, overlap with the groups already discussed. This study directs attention to promising analogs which warrant further biochemical and pharmacological investigation and perhaps clinical trial.

146 ON THE ROLE OF CYTIDINE DEAMINASE IN CELLULAR METABOLISM

Per Nygaard, University Institute of Biological Chemistry B, Sølvgade 83, 1307 Copenhagen K, DK

Cytidine deaminase (CDD) catalyzes the deamination of various cytosine nucleosides to their corresponding uracil nucleosides. Due to its ability to inactivate cytidine analogs, it has been a target of considerable interests. The levels of CDD differ greatly among the various organisms studied, in some organisms and cell types CDD appears to be absent.

In Enterobacteriaceae cytidine and deoxycytidine are used as carbon source, through the intermediate formation of uridine and deoxyuridine respectively. It is the pentose moiety which is catabolized. Uracil is utilized as pyrimidine source and excess uracil is excreted. When nucleosides are added to growing cells the synthesis of the nucleoside catabolizing enzymes is induced.

The role of CDD in mammalian cells has been less well defined. The activity of CDD has been measured in human white blood cells, leukemic cells and in various cell lines. Low enzyme levels have been found in immature cells. An inverse relationship with respect to adenosine deaminase levels is seen, indicating the absence of a coordinate regulation of purine and pyrimidine nucleoside deamination. The determination of CDD levels might prove useful in studies to identify maturating steps of human blood cells.

147 BONE MARROW TRANSPLANTATION IN LESCH-NYHAN DISEASE.

William L. Nyhan, Robertson Parkman, Theodore Page, Harry E. Gruber, Jagdeesh Pyati, Douglas Jolly and Theodore Friedmann. University of California, San Diego, Departments of Pediatrics and Medicine, La Jolla, California, USA and Children's Hospital of Los Angeles, Division of Research Immunology, Los Angeles, California, USA

Advances in molecular biology have led to interest in new approaches to the treatment of genetic disease, such as gene therapy. Bone marrow transplantation provides a model for the introduction of a cloned gene into the marrow of the patient. Results of bone marrow transplantation in disorders involving the CNS should provide information on pathogenesis and whether prevention or reversal of CNS abnormalities can be achieved by the provision of a source of enzyme peripheral to the CNS. A 22 year old with Lesch-Nyhan disease was subjected to bone marrow transplantation. He was prepared with 4 doses each of horse antithymocyte globulin, 20 mg/kg, busulfan 3 mg/kg and cyclophosphamide 50 mg/kg. He was given 5 x 10^8 bone marrow cells from an HLA identical brother. Methotrexate was given 10 mg/m^2 on 3 days posttransplantation to inhibit GVH, but also providing an in vivo equivalent of HAT medium to select against the patient's cells. Course was uneventful and uncomplicated by GVH. By day 60 the wbc were 14.3 and the platelets 200 x 10^3 per cmm and the hemetocrit 34.5. Prior to transplant the leucocyte activity of HPRT was 0.073 pm/min/µg protein and values obtained post transplant were 5.87 and 1.04. In rbc lysates pretransplant the activity was 2.4 mmol/min/ml packed rbc and data obtained 37 and 60 days post were 366 and 443. Radioautography of ficoll/hypaque separated lymphocytes stimulated with phytohemagglutinin documented the incorporation of ^{3}H-hypoxanthine and revealed no HPRT- cells.

148 DEFICIENCY OF ERYTHROCYTE TYPE ISOZYME OF AMP DEAMINASE IN HUMAN

Nobuaki Ogasawara, Haruko Goto, Yasukazu Yamada and Iwazo Hasegawa*

Institute for Developmental Research, Aichi Prefectural Colony, Department of Biochemistry, Aichi 480-03, and *Aichi Red Cross Blood Center, Nagoya 460, Japan

There are multiple tissue specific AMP deaminase isozymes in human. These are erythrocyte type (E_1, E_2), liver type (L) and muscle type (M). The deficiency of isozyme M has been reported.

Five individuals with complete deficiency of erythrocyte AMP deaminase have been discovered. The subjects had normal values for complete blood count and there was no evidence of hemolysis or hematological disorder. The ATP content was 50 % higher than normal control, but other purine metabolizing enzymes tested revealed normal levels of activity. AMP deaminase levels of mononuclear cells and platelets were normal, as suggested from the previous results of existence of L isozyme in these cells. Of the known AMP deaminase isozymes, E_2 is very similar to E_1 in immunological properties and kinetic properties. The subject with complete deficiency of erythrocyte AMP deaminase lacks both E_1 and E_2, indicating that these two isozymes are the product of the same gene.

From the familiar study, it is evident that the deficiency is inherited as an autosomal recessive trait. The frequency of heterozygote of mutant gene is approximately 1/30, resulting in one complete deficiency in about 3,600 population. This frequency is surprisingly high. Thus the erythrocyte AMP deaminase deficiency must be one of the most common enzyme deficiencies.

149 ANALYSIS OF MUTATION AT THE APRT LOCUS OF L-5178Y MOUSE LYMPHOMA CELLS.

UMNARJ PAERATAKUL AND MILTON W. TAYLOR, INDIANA UNIVERSITY, BLOOMINGTON, IN.

APRT heterozygotic (aprt$^{+/-}$) and homozygotic (aprt$^{-/-}$) mutants have been isolated following mutagenesis in the mouse lymphoma cell line L-5178Y (tk$^{+/-}$). Presumptive heterozygote (or hemizygote) can be differentiated from true homozygous mutants by poor growth in the presence of 75 uM 2,6 diaminopurine(DAP) in 0.37% agar. These cells form small colonies, and upon isolation were shown to be heterozygotes by APRT activity and immunoprecipitable material. The homozygotic APRT$^-$ mutants are fully resistant ot 75 uM DAP, and form colony of normal size. Using a mouse 3.2 Kb APRT probe, and Eco-RI digested wild type L-5178Y cell DNA, two hybridizing bands were detected by Southern blot analysis. These two bands represent the two APRT gene copies, each one differing by an Eco-RI site. Analysis of one "heterozygote" and one "homozygote" showed that they were actually hemizygous. As previously reported by us for Chinese hamster ovary cells, "mutants" at the APRT locus appeared to arise by loss of one of the APRT alleles with true mutation occuring at the remaining allele.

150 ^{18}FLOURODEOXYGLUCOSE PET SCANNING IN HPRT DEFICIENCY.

Thomas D. Palella, Richard D. Hichwa, Richard L. Ehrenkaufer, Jill M. Rothley, Mark A. McQuillan, Ann B. Young and William N. Kelley, Departments of Internal Medicine and Neurology, University of Michigan, Ann Arbor, MI, USA.

The Lesch-Nyhan syndrome (LNS) results from the virtually complete deficiency of hypoxanthine phosphoribosyltransferase (HPRT); partial deficiency of HPRT results in gout. The metabolic mechanisms for neurologic dysfunction in the more severe HPRT deficiency states remain unclear. Previous attempts to assess therapy for LNS also have been limited due to the lack of non-invasive measures of metabolic and neurochemical function in the CNS. In an effort to develop such measures, we have examined the local glucose metabolic rate (LGMR) using the positron emitter ^{18}Flourodeoxyglucose (FDG) with positron emission tomography (PET) scanning in 10 normal subjects and in four HPRT deficient individuals. One subject had partial HPRT deficiency with gout and no clinical neurologic disease. Two subjects had no detectable RBC HPRT and age-related, progressive neurologic dysfunction. The fourth subject had the classical LNS. LGMR in the basal ganglia was normal in the first subject without neurologic disease and reduced in the regions of the basal ganglia in both partially deficient subjects with neurologic disease. LGMR was severely reduced in the basal ganglia of the subject with LNS. From these initial studies we conclude that the severity of neurologic dysfunction appears to be proportional to the degree of HPRT deficiency and that PET scanning of the basal ganglia with ^{18}FDG may provide a sensitive non-invasive measure of metabolic derangement resulting from HPRT deficiency. This study suggests for the first time a characteristic abnormality in CNS metabolism unique to HPRT deficiency with neurologic dysfunction.