77 ARA-CTP METABOLISM FOLLOWING HYDROXYUREA OR METHOTREXATE TREATMENT IN HUMAN LEUKEMIA CELL LINES. Masaru Kubota, Tetsuya Takimoto, Toshiyuki Kitoh, Akihiko Tanizawa, Yuichi Akiyama, Yukio Kiriyama and Haruki Mikawa. Department of Pediatrics, Kyoto University, Kyoto, Japan.

Using human leukemia cell lines, we investigated the biochemical basis for the synergistic interaction between ara-C and hydroxyurea(HU) or methotrexate(MTX) In the cells of B cell phenotype, pretreatment with 1mM HU increased ara-CTP formation up to 2~10 fold, whereas it did not have significant effects on T cell lines. The phosphorylation of ara-A or deoxyadenosine to the corresponding triphosphates was also augmented by HU, indicating the activation of deoxycytidine(CdR) kinase in intact cells through HU treatment. As a possible mechanism, we propose the important role of de novo CdR production, since (i) the changes of pyrimidine dNTP pools by HU (the decrease in dCTP and increase in TTP) were equally seen in both cell lines; (ii) the excretion of CdR into the medium was much higher in B cell lines, which was significantly inhibited by HU. On the other hand, MTX(1μM) increased ara-CTP generation significantly in both cell lines, albeit it was less than 50%. These results suggest the cell type specific effectiveness of such combination chemotherapyin acute leukemia.

78 THE ISOLATION AND CHARACTERIZATION OF A PORCINE cDNA FOR URATE OXIDASE AND PHOSPHORIBOSYLPYROPHOSPHATE SYNTHETASE. CC Lee, X Wu, R Howell, RA Gibbs, AL Nelson, and CT Caskey. Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030 USA

Our laboratory has cloned 3 genes critical to uric acid synthesis in mammals. They include hypoxanthine-guanine phosphoribosyl transferase (HPRT), phosphoribosylpyrophosphate (PRPP) synthetase and urate oxidase. Rapid diagnostic methods have been developed which will enable the identification of point mutations in the HPRT gene which is associated with Lesch-Nyhan (L-N) syndrome in human patients.

We have succeeded in the isolation for a 2.3 kb cDNA for PRPP synthetase from pig. The DNA sequence determined from the coding region shows a high degree of homology with the cDNA sequence recently determined from rat.

The enzyme urate oxidase catalyzes the oxidation of uric acid to allantoin in all mammals except humans and certain primates. Recently, mice with complete HPRT deficiency have been identified. These animals display none of the neurological symptoms observed in human patients. This has raised the possibility that the absence of urate oxidase activity in man may contribute to the neurological symptoms observed in L-N patients. A 2.2 kb cDNA for urate oxidase has been isolated from a porcine liver λ cDNA library. Although humans demonstrate no urate oxidase activity, analysis of human genomic DNA has revealed the presence of homologous sequences in human. The loss of urate oxidase activity in human is probably due to a lack of gene transcription since a Northern analysis detects no urate oxidase mRNA in human liver.

79 DEGRADATION OF PURINE NUCLEOSIDES BY MITOCHONDRIAL ENZYMES OF BOVINE LIVER. Roger A. Lewis, Laura Link and Wilbert Chen. University of Nevada-Reno, Dept. of Biochemistry, Reno, NV 89557, U.S.A.

In recent years, it has been shown that mitochondria actively metabolize purine nucleosides. It is documented that both deoxyguanosine and deoxyadenosine are phosphorylated by mitochondrial enzymes. In the case of deoxyguanosine, intact mitochondria form dGMP, dGDP and dGTP. Herein we report that mitochondria are also involved in the degradation both deoxyguanosine and deoxyadenosine. When intact or fractured mitochondria are treated with deoxyadenosine, there is an immediate loss of the deoxynucleoside and a corresponding formation of deoxyinosine. A partially purified preparation of the mitochondrial adenosine deaminase activity was able to deaminate both adenosine and deoxyadenosine and a Lineweaver-Burk plot showed that an apparent Km for adenosine was 10μ M. AMP and dAMP were not substrates. When deoxyguanosine was added to either intact or fractured mitochondria it was transformed to guanine in addition to the above mentioned nucleotides. A partially purified preparation of mitochondrial purine nucleoside phosphorylase was shown to be active using inosine as substrate. An apparent Km of 6μ M was measured. These data indicate that mitochondria play a role in the catabolism of purine nucleosides and thus suggest new opportunities for the study of cellular nucleoside metabolism.

80 A STUDY OF ADENOSINE DEAMINASE AND ITS CONVERSION FACTOR IN HUMAN SERUM. Jeffrey T. Starkey, Cynthia Ma, and Pang Fai Ma, Ball State University, Center for Medical Education, Muncie, Indiana 47306.

A large and small molecular form of adenosine deaminase have been reported in advanced mammals, including man. The molecular weights of these enzyme forms are estimated to be 200,000 and 35,000, respectively, by gel filtration column chromatography. The small form can be reversibly converted into the large form in the presence of a conversion factor, a high molecular weight glycoprotein that is aggregated during this process. Previous studies have shown that the distribution of the two molecular forms of adenosine deaminase is tissue specific. The large form of the enzyme is predominant in human liver while the small form is predominant in human heart. It follows that the conversion factor is present in higher amounts within those tissues where the large form predominates. An attempt is made to study the conversion process and determine if the serum level of conversion factor is elevated in certain pathological conditions. This work was supported by a grant from the Indiana Delaware County Cancer Society.

81 ERYTHROCYTE PYRIMIDINE NUCLEOTIDE METABOLISM: 5'-NUCLOTIDASE HPLC ASSAY AND OBSERVATIONS ON THE ENZYME BEHAVIOR. A. Amici, F. Santini and G. Magni
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Pyrimidine 5'-nucleotidase catalyzes the hydrolysis of pyrimidine 5'-monophosphates to the respective nucleosides. The enzyme activity was determined using HPLC assay by a chromatografic separation on a 100x4.6 mm column containing 3 μm reversed-fase C-18 stationary phase with a 20x2.1 gard column containing 5 μm reverse-phase. The mobile phase was 100mM KH_2PO_4 solution pH 6.0 containing 6% methanol for the determination of cytidine and uridine or 14% methanol for the determination of thymidine. Products were identified from their retention times and quantified by standards. The complete chromatogram was developed within 3 min. In order to ascertain on the presence in the erythrocytes of multiple forms of the enzyme, as observed by others (1), crude hemolysate was passed through a TSK-DEAE 5PW column. The elution performed with a linear gradient from 0 to 0.2 M of KCl revealed two peaks of activity by using both CMP and TMP as substrates. However preliminary results indicated no difference in the susceptibility of both substrates as it could be expected from the observations made by other investigators (1). Characterizaton of the purified enzyme preparation (4400 fold) showed a molecular weight of 30,000, a pH optimum around 7.5 and an isoelectric point at 5.1. Kinetic analysis conducted using CMP as the substrate showed a Km value of 30 μM

(1) Hirono et all (1987) Br. J. Haemat. 65, 35-41

82 PURINE AND PYRIMIDINE METABOLISM OF NORMAL AND LEUKEMIC CELLS. Yolanda M.T. Marijnen, Dirk de Korte, Bert J.S den Breejen*, Dirk Roos and Albert H. van Gennip*, Central Lab. Netherl.Red Cross Blood Transf. Service, inc. the Lab. of Exp. and Clin. Immunology of the Univ. of Amsterdam, *Children's Hosp. "Het Emma Kinderziekenhuis", Amsterdam, The Netherlands

The flux through the purine and pyrimidine metabolism of normal lymphocytes, resting as well as proliferating, and lymphoblastic cell-line cells (MOLT-3) was measured by incubation of the cells with radioactive precursors of either "de novo" or "salvage" pathways. Tonsillar lymphocytes were taken as immature, slowly proliferating lymphocytes (2% in S phase) and PHA-stimulated lymphocytes as more actively proliferating lymphocytes (about 8% and 20% in S phase on the 2nd and 4th day after stimulation, resp.). The incorporation of the precursors in the purine and pyrimidine ribonucleotides was followed by a combination of anion-exchange HPLC and on-line radioactivity measurement.

Lymphoblastic cell-line cells incorporated 10 to 200 times more of the various precursors in the ribonucleotides, compared to both resting and proliferating normal lymphocytes. The low adenine:guanine ratio in leukemic cells was reflected in the incorporation pattern of the various precursors. To some extent, this was also true for the low uracil:cytosine ratio. The activities of the branch-point enzymes IMP dehydrogenase and CTP synthetase most likely determine the imbalance in the nucleotide pool of lymphoblastic cells and might provide targets for selective chemotherapy.