115 HUMAN PLACENTAL CYTOPLASMIC 5'-NUCLEOTIDASE: PURIFICATION AND MOLECULAR PROPERTIES.

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Purine nucleotide degradation in human tissues is highly regulated. Dephosphorylation of nucleoside 5'-monophosphates is the first committed and irreversible reaction of purine nucleotide catabolism. Recent studies indicate that cytoplasmic 5'-nucleotidase may have an important role in intracellular nucleotide degradation. We purified cytoplasmic 5'-nucleotidase from human placenta 8075-fold to a specific activity of 58.85 µmol/min/mg. The enzyme showed absolute requirement for magnesium with a Km of 6 mM and pH optimum from 7.4 to 9.0. CMP and UMP are preferred substrates. A large variety of purine, pyrimidine and pyridine compounds exert an inhibitory effect on enzyme activity. IMP, GMP and NADH produce almost 100% inhibition at 1.0 mM. Nucleoside di- and triphosphates are potent inhibitors. ATP and ADP are competitive inhibitors with respect to AMP and IMP as substrates with Ki values of 100 µM and 15 µM, respectively. Inorganic phosphate is a noncompetitive inhibitor with Ki values of 19 mM and 43 mM. The estimated molecular weight is 143,000 and the stokes radius is 46.1A. The subunit molecular weight is 76,000, suggesting that the enzyme is a dimeric protein. These data elucidate the nature of cytoplasmic 5'-nucleotidase enzyme and its role in purine nucleotide degradation in human tissues.

116 PURINE ENZYME ACTIVITIES AS MARKERS OF LYMPHOCYTIC DIFFERENTIATION: STUDIES OF LYMPHOCYTES FROM HORSES HORSES WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID).

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Foals with SCID lack both identifiable B lymphocytes and functional T lymphocytes. Morphological characterization of SCID lymphocytes showed them to be large granular lymphocytes (LGL), a morphology associated with natural killer (NK) cells in human and rat systems. Like NK and T lymphocytes, SCID LGL could be maintained in continuous culture in the presence of interleukin 2 (Il-2). The distribution of cell surface antigens and purine enzymatic activities were evaluated. Surface markers on fresh SCID LGL resembled normal peripheral blood lymphocytes (PBL) except for the pan-T marker (26%, SCID; 62%, Normal). Incubation of SCID and normal PBL with IL-2 for 2 weeks increased the percentage of cells carrying the lymphoid precursor (from 10% to 55%) and the monocytic (from 3% to 52%) markers. Normal PBL, however, lost the pan-T marker (from 62% to 2%) while no change was detected for SCID cells. Purine enzymatic activities (6 enzymes) for fresh SCID cells were 2 to 10 times higher than for normal PBL. When normal PBL were maintained in culture with IL-2 for several weeks, morphology and enzymatic activities had changed to those of fresh SCID LGL. These results suggest that LGL from SCID horses may be related to NK or T lymphocytes. (Supported by USPHS grant HD 08886.)

117 POST-MORTEM OXYPURINE CONCENTRATIONS IN THE CSF.

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Post-mortem oxypurine levels in the cerebrospinal fluid were determined in 77 forensic autopsy cases. The oxypurine concentrations ranged between 21.8 and 1051.2 µmol/l hypoxanthine, 4.8 and 620.7 µmol/l xanthine, and 6.3 and 1038.1 µmol/l uric acid. The mean values of hypoxanthine (413 µmol/l and xanthine (130 µmol/l) were about 100 times higher than normal in vivo CSF levels.
Lower values of hypoxanthine were found in cases of sudden death, e.g. traffic accident or myocardial infarction, and higher values in some cases of drug intoxication. There were neither any significant correlations between the hypoxanthine and the xanthine nor between the hypoxanthine or xanthine and the uric acid concentrations. It is assumed that the hypoxanthine level in CSF post mortem reflects the degree of hypoxia preceding death.

118 HYPOXANTHINE AND TETRAHYDROBIOPTERIN TREATMENT OF A PATIENT WITH FEATURES OF THE LESCH-NYHAN SYNDROME

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In previous studies on this patient of normal intelligence 6.9% of the normal HGPRT activity and an elevated Km for hypoxanthine in intact fibroblasts had been found. It was thought that perhaps a slight increase in HGPRT activity could alleviate his neurological symptoms. The patient was treated with hypoxanthine 3 x 1 g + allopurinol 3 x 100 mg daily for 3 months. Serum hypoxanthine and xanthine rose from 0.4 and 0.35 to 1.53 ± 0.11 and 1.19 ± 0.11 mg%, respectively. His 24h urinary excretion of hypoxanthine ranged between 1.34 and 2.02 g. His 24h urinary excretion of dopamine was on an average 25% below our reference values, and his serotonin excretion decreased at 50-70% of his baseline values. The patient felt quieter and suffered less from disturbances of equilibrium. The therapy had to be interrupted because of renal colics. Treatment with tetrahydrobiopterin had no effect on his neurological symptoms although his urinary dopamine excretion increased significantly.

119 THE "SWITCH-OFF" MECHANISM OF SPONTANEOUS RESOLUTION OF ACUTE GOUT ATTACK

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In order to investigate the intriguing problem of the spontaneous resolution of acute gout attack,we examined the hypothesis that occurrence of urate crystals size changes or dissolution by oxygen radicals and/or lysosomal enzymes released by stimulated PMNs represents the more likely "switch-off" mechanism. The absorption spectra and the concentrations of uric acid,allantoin and urea were determined before and after in vitro exposition of MSU crystals to O_2^- flow generated from photoreduction of riboflavin and O_2.The results showed a progressive dissolution of MSU crystals that started after 5 hrs of incubation under O_2^- flow and was completed after 20 hrs.A progressive decrease of uric acid and an increase of allantoin and urea concentration were simultaneously observed.These results were also confirmed by polarizing and electron microscopy and microcalorimetric techniques. Taking into account these observations,the spontaneous resolution of acute gout attack could be attributed to the dissolving effect on urate crystals by oxygen radicals released extracellularly by phagocytising PMNs.

120 RELATIONSHIPS BETWEEN LIPID AND PURINE METABOLISM: THE BEHAVIOR OF FATTY ACID IN THE PLASMA TRIGLYCERIDES OF GOUTY PATIENTS

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The composition of fatty acids in plasma triglycerides has been analyzed in 42 gouty patients and 22 controls, by extraction and subsequent gas-chromatography as previously described by Ciccoli et al. (1). The gouty patients present remarkable changes and, specifically, an increase in oleic acid, decreases in arachidonic and linoleic acids, which do not vary according to the age of the subjects and remained the same after a standard diet in which lipids were represented only by olive oil. The picture is similar to that observed both in diabetic and atherosclerotic patients, and we may therefore conclude that gout is another condition in which the ratio of essential fatty acids/monoenoic acid is affected; the relationship of this alteration with purine metabolism is not yet clear and is referred to the hormonal variations observed in gouty patients (2).

(1) Ciccoli L. et al. (1983) Lipids 18, 363-370
(2) Gregolini L. et al. (1983) Clin. Chim. Acta 130, 269-277