25 HYPOXANTHINE TRANSPORT THROUGH HUMAN ERYTHROCYTE MEMBRANES.

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The uptake of (8-^{14}C)hypoxanthine by human erythrocytes suspended in a phosphate-free medium has been studied. The erythrocytes freshly drawn from human healty donors were washed twice and resuspended in isotonic NaCl medium containing 1-30 uM labelled hypoxanthine to give a 50% hematocrit. At regular intervals, the cells were separated from the bathing medium by rapid centrifugation through a layer of dibutyl phthalate in order to determine hypoxanthine uptake. Under these conditions, more than 90% of labelled hypoxanthine taken up by the cells was present in the cytosol as such. The time course of hypoxanthine uptake behaved as a single exponential process, the relaxation time being reversely related to the temperature of the cell suspension (τ = 130 sec at 0°C). Hypoxanthine was not appreciably taken up by erythrocytes when the external site of the cell membranes was partially degested by proteinase K.

26 PRIMARY GOUT IN AGING PATIENTS

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The onset of primary gout in aging patients has been rarer in the past than it is today.
We observed late onset - over 65 years of age - of gout in 15 patients or in 14% out of 104 patients.
Comparative studies of clinic and physiopatological manifestations of these 15 patients revealed the following:
- prevalence of females was higher (26.5%), familial incidence of the disease was present only in 6%, overweight was uncommon (13%).
In most cases hyperuricemia was present (mean value 8.3 mg%), with significant reduction of uric acid clearance (mean value 6.7 ml/min) while uricosuria was normal (mean value 672 mg/24h.)
The site of initial attack was the first metatarsal falangeal joint (podagra) in 60%. Tophi were never found. Renal calculi were observed only in 2 cases (13%).
Cholesterol and tryglicerides mean levels were normal meanwhile significantly lower levels of HDL-Cholesterol and of Apo-A Lipoproteins were observed in patients with coronary heart disease (20%).
Hypertension was present in 33% of the patients.
In these patients levels of ionized calcium were significantly lower (mean value 2.04 mEq/l) than in patients with normal blood pressure (mean value 2.18 mEq/l).

27 HYPOXANTHINE UPTAKE BY ISOLATED BRAIN MICROVESSELS.

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Isolated bovine brain microvessels were used as _in vitro_ model of blood/brain barrier. In a phosphate-free Krebs Ringer medium at 37°C, (8-^{14}C)hypoxanthine is taken up by the microvessels and converted for about 45% in the corresponding nucleotide. The initial rate of the uptake is a hyperbolic function of hypoxanthine concentration in the suspending medium. Inorganic phosphate (up to 10 mM) decreases the apparent Km without affecting the apparent Vmax of hypoxanthine uptake. The presence of inorganic phosphate, which (in the absence of hypoxanthine) causes a raise in the ATP concentration in the endothelial cells, induces also an increase in the equilibrium levels of IMP and hypoxanthine within the cells, the value of the nucleotide /base ratio remaining approximately constant.

28 5'-DEOXY-5'-METHYLTHIOADENOSINE (MTA) PHOSPHORYLASE DEFICIENCY IN LEUKEMIA: GENETICS AND BIOCHEMICAL ASPECTS.

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MTA is produced in eukaryotic cells during the synthesis of polyamines from decarboxylated S-adenosylmethionine. The nucleoside is rapidly cleaved to adenine and methylthioribose-1-P by MTA phosphorylase (MTAse). We have assigned the gene _MTAP_ to chromosome 9pter->9q12 by enzymatic and electrophoretic analysis of somatic cell hybrids.

All normal tissues and non-malignant cell lines contain MTAse. However, several human leukemic cell lines are deficient in the enzyme, and 5 patients with acute lymphoblastic leukemia (ALL) have been shown thus far to lack MTAse in their malignant cells. Karyotypic abnormalities involving fragile site 9p21 occur in ALL with lymphomatous clinical features. One of 5 such patients studied prospectively lacked MTAse in her leukemic cells but not in normal blood cells at remission. No inactive enzyme protein has been detected by immunoadsorption among 7 leukemic lines tested. The MTAse deficient cell lines excrete MTA up to 0.32 nmol/hr/mg protein. In mice, the growth of MTAse deficient mutant lymphoma cells (but not MTAse positive wild type cells) causes plasma MTA to rise from undetectable levels to > 800 nM pre-terminally. Assay of plasma or urine MTA may thus prove useful to screen leukemic patients for MTAse deficient malignant cell clones.

29 GENETIC ANALYSIS OF DEOXYADENOSINE TOXICITY IN DIVIDING HUMAN LYMPHOBLASTS.

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The accumulation of deoxyadenosine (dAdo) and its metabolites may produce immunodeficiency in children who lack adenosine deaminase. The metabolism of dAdo in dividing human lymphoblasts, and the mechanism of dAdo toxicity, have aroused considerable controversy. To investigate this problem, we have selected stable mutant human lymphoblastoid cell lines resistant to the antiproliferative effects of dAdo and have compared their biochemical phenotypes. The dAdo-resistant mutants differed from wild type cells in one of three ways: (1) an increase in the activity of ribonucleotide reductase, (2) an increase in cytoplasmic nucleotidase activity, (3) a decrease in deoxycytidine kinase activity. All three genetic changes caused a secondary rise in _de novo_ deoxycytidine formation and excretion, and a reciprocal inability to phosphorylate dAdo and to form dATP. Thus, human lymphocytes can avert dAdo toxicity by increasing deoxycytidylate synthesis and degradation, as well as by decreasing deoxycytidine kinase levels. The net result in each case is an impaired functional capacity to phosphorylate deoxyadenosine.

30 MECHANISM OF ADENOSINE TOXICITY TO ADENOSINE KINASE DEFICIENT MAMMALIAN CELLS.

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Somatic cell genetics has been used to probe the mechanism of adenosine (Ado) toxicity to mammalian cells deficient in Ado deaminase and Ado kinase. Ado resistant clones of an Ado kinase deficient murine lymphoblastoid cell line (R1.1) were isolated and characterized. In deoxycoformycin supplemented medium, the mutant clones were 10-30 fold more resistant than parental cells to the anti-proliferative actions of Ado, 3-deaza-Ado, carbocyclic-Ado, adenine, 5'-methylthioadenosine, and several other Ado analogs. The mutants were normally sensitive to the toxic effects of deoxyadenosine and adenine arabinoside. Levels of S-adenosylmethionine (SAM) were 50 pmols/10^6 cells in the parental line, compared to 250-350 pmols/10^6 cells in the mutant. Methionine adenosyltransferase activity was 1.5-3.2 fold higher in the Ado-resistant cells than in parental lymphoblasts, and varied inversely with the medium methionine concentration. Ado induced the accumulation of equivalent amounts of S-adenosylhomocysteine (SAH) in both cell types. However, the SAH to SAM ratio in the Ado-resistant mutants never exceeded 0.1. These results show that (i) methionine adenosyltransferase is an inducible enzyme in mammalian cells, (ii) the toxic effects of Ado, adenine, and many Ado analogs, can be averted by increasing the velocity of SAM synthesis.