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ENZYMES OF PURINE NUCLEOTIDE METABOLISM IN MATURE RAT BRAIN NEURONS IN CULTURE.

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Several enzymes of purine nucleotide metabolism were assayed in mature (10-14 days old) rat brain neurons in culture:

Enzyme	Activity (nmol/hr/mg protein)	
AMP deaminase	443.7±189.7	(7)
AMP 5'-nucleotidase	269.7±143.3	(14)
Adenosine kinase	216.1±100.4	(10)
Adenosine deaminase	700.5±374.1	(6)
IMP 5'-nucleotidase	72.7± 18.9	(6)
Purine nucleoside phosphorylase	2358.7±356.7	(8)
Hypoxanthine-guanine PRT	19.7± 13.6	(25)
Adenine PRT	113.9± 86.2	(13)
Guanase	38.9± 20.3	(8)

No activity could be detected for xanthine oxidase and uricase. The cultures exhibited de novo nucleotide synthesis. The results indicate that purine nucleotides can be synthesized in the mature rat brain cultured cells by de novo and by salvage from hypoxanthine, adenine and adenosine. From the Vmax values of the enzymes and from the known Km values, it appears that the degradation of adenine nucleotides through deamination to IMP has a slightly better capacity than that through dephosphorylation to adenosine and that the capacity for adenosine salvage is better than that for its deamination. The absence of xanthine oxidase activity is in favor of hypoxanthine salvage to IMP, but the presence of guanase competes with the salvage of guanine.

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THE METABOLIC BASIS FOR GTP DEPLETION IN RED CELLS WITH PURINE SALVAGE DEFICIENCIES.

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This study was aimed at elucidation of the metabolic basis for the marked depletion of GTP in RBC of patients with the hereditary deficiency of HGPRT and PNP. RBC were preincubated with labelled Gu, Ad, or Hx, the cells washed and the distribution of labelling followed in nucleotides, nucleosides and bases over 3 h. 36% of the counts in Gu nucleotides (GuRN) were lost to IMP, Ino and Hx. The results of inhibition of IMP dehydrogenase by mycophenolic acid suggested that only a negligible proportion of these counts return normally to GMP. 15% of the counts in AdRN were lost through IMP to Ino and Hx, but were not detected in Gu derivatives All the counts originating from Hx remained in Hx derivatives. There was a marked degradation of newly formed IMP to Ino and Hx. The results suggest that in RBC, there is a substantial reduction of GMP to IMP, but that the fluxes from IMP or AMP towards GMP are negligible. Thus, RBC depend mainly on Gu salvage for the maintenance of GuRN pools. Accordingly, GuRN depletion is expected in RBC with deficiencies of the salvage enzymes. (Supported by the USA-Israel BSF).

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AFFINITY CHROMATOGRAPHY ON AMP-SEPHAROSE 4B FOR THE SEPARATION OF "HIGH Km" AND "LOW Km" 5'-NUCLEOTIDASES AND OTHER AMP METABOLIZING ENZYMES.

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AMP-Sepharose 4B has been widely used as a general ligand affinity chromatography for purification of AMP deaminase, 5'-nucleotidase, adenosine kinase and other adenine nucleotide metabolizing enzymes. Since these enzymes generally differ in their kinetic properties related to the values of Km for AMP and analogous compounds, it was assumed that there may be a specific elution pattern of some of the enzymes which would enable sequential elution from the column during a single run. Using 0.5 M NaCl, 10 mM ATP and 5 mM adenosine as eluting agents, it was possible to separate on AMP-Sepharose column AMP deaminase, "high Km" and "low Km" 5'-nucleotidase and adenosine kinase. Adenylate kinase, adenosine deaminase and nonspecific phosphatase did not bind to the column. Using human placental extract, AMP deaminase, "high Km" and "low Km" 5'-nucleotidase and adenosine kinase were purified 2.8, 2.9, 105 and 1240 fold, respectively. AMP deaminase and "high Km" 5'-nucleotidase were further separated using phosphocellulose column chromatography and the final purification was 227 and 143 fold, respectively. The specific activities of purified enzyme preparations were 9.1, 1.0, 0.4 and 0.5 umoles/min/mg protein of AMP deaminase, "high Km" 5'-nucleotidase and adenosine kinase, respectively. This approach provides a rapid method for initial purification of these enzymes from crude soluble extracts.

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MOLECULAR CLONING OF HUMAN UMP SYNTHASE

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In order to clarify the cause of hereditary orotic aciduria, UMP synthase (a multifunctional protein having both orotate phosphoribosyltransferase and OMP decarboxylase activities) was purified from normal human erythrocytes. The enzyme protein had a molecular weight of 51,000. Then a cDNA fragment of mouse OMP decarboxylase was isolated from a λgt11 mouse spleen cDNA library by the use of a synthesized oligonucleotide probe. Screening of a λgt11 human placenta cDNA library yielded positive clones which hybridized strongly to the mouse cDNA fragment. Analysis of the nucleotide sequence of the entire cDNA insert (1.7 kb) of one of the clones indicated that it contained an open reading frame (1,473 bp) encoding a protein with a molecular weight of 53,496. The deduced amino acid sequence of the 3'-half of the insert showed 89% homology with that deduced from Ehrlich ascites carcinoma OMP decarboxylase cDNA. Northern blot analysis revealed a presence of a single band of approximately 1.8 kb, which suggests that the cloned cDNA contains the whole message for human UMP synthase.

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ERYTHROCYTE ADENINE PRPP AVAILABILITY IN TWO TYPES OF APRT DEFICIENCY USING SILICON OIL METHOD.

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For the cause of 2,8-dihydroxyadenine urolithiasis, Japanese type adenine phosphorobosyltransferase (APRT) deficiency is common in Japan. Some homozygotes for the Japanese type APRT deficiency had no urolithiasis and were clinically healthy. APRT activity in erythrocyte from homozygotes of Japanese type APRT deficiency was at the same levels as the activity from the healthy heterozygotes for the complete APRT deficiency. For the diagnosis of Japanese type APRT deficiency we compared the erythrocyte adenine phosphoribosylpyrophosphate (PRPP) availability of Japanese type APRT deficiency with complete APRT deficiency using silicon oil method. Homozygote of Japanese type APRT deficiency showed 4.3±2.7% (mean ± standerd deviation) of adenine PRPP availability and every patient showed the almost same PRPP availability. Heterozygote showed 86.0±6.0% of adenine PRPP availability in comparing to those of normal subjects. These results supported the same mutation in Japanese type APRT gene. While in a Japanese family of complete APRT deficiency, adenine PRPP availability of homozygote was undetectable and that of heterozygote was normal low (54.3% of mean normal activity). The adenine PRPP availability of heterozygote of complete APRT deficiency was diagnostically different from that of homozygote of Japanese type APRT deficiency.

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CHARACTERIZATION OF NUCLEOSIDE TRANSPORT DURING LEUKEMIC CELL DIFFERENTIATION

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Changes in nucleoside transport during cellular differentiation were examined. The rate of nucleoside transport decreased drastically following differentiation induced by several agents in human promyelocytic leukemia HL-60 and T lymphoblastoid MOLT-4 cells.

The mutant cells deficient in deoxycytidine kinase (dCK) or hypoxanthine guanine phosphoribosyltransferase (HGPRT) revealed similar tendency.

Furthermore, the study using transport inhibitors, nitrobenzyl-6-thioinosine (NBTI) or dipyridamole (DP), showed that HL-60 cells had NBTI-insensitive transport sites. Such was retained after retinoic acid-induced maturation. The reduction of transport is, in part, considered to be a function of altered cell cycle distribution after differentiation induction.

These results suggest one possible biochemical basis for the decreased sensitivity to certain nucleoside analogs in more differentiated leukemia.