133 PURINE SALVAGE ENZYMES IN *TRICHOMONAS VAGINALIS*.

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Trichomonas vaginalis, a pathogenic protozoa, is incapable of de novo purine synthesis and is thus dependent on preformed purines. Unlike other protozoa, *T. vaginalis* is devoid of purine phosphoribosyltransfer activity but has both purine nucleoside phosphorylase (PNP) and purine nucleoside kinase activity. These enzymes comprise a potential route for the salvage of purines in this organism. Both enzymes have been purified and characterized.

The purified PNP (mol. wt. 95,000) catalyzed the synthesis and cleavage of guanosine, adenosine and inosine at maximal velocities (µmol/min/mg) of 590:360:240 and 81:16:390 respectively. K_m values ranged from 17-54 µM for these purine nucleosides and 21-25 µM for the purine bases. Initial velocity studies in both the synthetic and cleavage direction indicate a sequential mechanism for this enzyme.

As a result of the extreme lability of the nucleoside kinase, only a limited purification was possible. The purified enzyme (mol. wt. 16,000) had a specific activity of 34 nmol of GMP formed/min/mg. It was free of interfering activities and catalyzed the phosphorylation (K_m µM/Rel. V_{max}) of guanosine (1/100), adenosine (200/111) and inosine (20/67). This enzyme appears to be the only purine ribonucleoside kinase activity in extracts of this organism.

The finding that both of these enzymes catalyze reactions involving the common purine nucleosides, guanosine and adenosine, suggests that they may act as a coordinated set of enzyme activities to salvage purines and purine ribonucleosides.

134 EFFECTS OF 4'-THIOADENOSINE ON HL-60 HUMAN PROMYELOCYTIC LEUKEMIA CELLS.

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4'-Thioadenosine (4'-SAdo) is an adenosine analog with the bridge oxygen in the ribose replaced by a sulfur atom. 4'-SAdo is cytostatic to the HL-60 cells in concentrations up to 25 µM after four days, within which the HL-60 cells can be induced to differentiate in a dose-dependent manner, as judged by their ability to reduce nitroblue tetrazolium. 4'-SAdo is cytotoxic to the HL-60 cells at 40 µM after four days. The ability of 4'-SAdo to induce HL-60 differentiation may be related to its ability to inhibit potently S-adenosylhomocysteine hydrolase (AdoHcy hydrolase), leading ultimately to the accumulation of AdoHcy with an attendant inhibition of cellular transmethylation reactions. HL-60 cells treated with 4'-SAdo show a decrease in the levels of ADP + ATP, but without any change in GDP + GTP. Interestingly, two unknown nucleoside products appear in a dose-dependent manner in the HL-60 cells treated with 4'-SAdo.

135 5-AZA-2'-deoxycytidine synergic action with thymidine: kinetic interaction of its monophosphate and triphosphate with dCMP deaminase.

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5-AZA-CdR, an analogue of deoxycytidine, is an effective anti leukemic agent. The antineoplastic action of 5-AZA-CdR appears to be related to the inhibition of DNA methylation. This inhibition activates gene expression and induces cellular differentiaion. The metabolism of 5-AZA-CdR is important because changes in the intracellular pool of deoxynucleotides may modulate the action of nucleoside analogs. In fact Grant et al., (Cancer Res. 1982) have reported drug synergism between thymidine and 5-AZA-CdR in a human premyelocytic cell line and in our experiments on L1210 cells, a correlation between 5-AZA-CdR effect and DNA methylation has been found. We have investigated the kinetic interactions of 5-AZA-dCMP and its triphosphate with mammalian dCMP deaminase, an enzyme that plays an important role in deoxynucleotide metabolism by modulating the intracellular pool size of dCTP and dTTP. 5-AZA-dCMP is a substrate of the enzyme and is deaminated at a rate of about 100-fold lower than the natural substrate, dCMP. 5-AZA-dCTP is an allosteric activator which reverts the inhibitory effect of dTTP. The mechanism by which the antileukemic action of 5-AZA-CdR can be enchanced by thymidine is the inhibition of 5-AZA-dCMP deamination by dTTP.

136 SEX STEROIDS INFLUENCE ON URIC ACID BINDING TO HUMAN PLASMA PROTEINS.

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Uric acid binding to human plasma proteins has been studied in various physiological and paraphysiological conditions, including the following groups:

1-a group of children aged 4-10 years;
2-a group of healthy males;
3-a group of subjects treated with estrogen preparations for prostatic cancer;
4-a group of regularly menstruating females with normal cycle;
5-a group of post-menopausal females;
6-a group of pregnant females;
7-a group of females treated with oral contraceptives;
8-a group of females treated with estrogen preparations for post menopausal symptoms.

The results obtained demonstrate a significant correlation between the increase of plasma estrogen and the increase of the uric acid binding to human plasma proteins.

137 A SINGLE SYSTEM FOR THE EVALUATION OF PURINE AND PYRIMIDINE NUCLEOSIDES AND BASES TOGETHER WITH THEIR ANALOGUES IN BIOLOGICAL FLUIDS.

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Procedures for the HPLC analysis of nucleosides and bases have generally been designed to fulfil specific requirements. No single method exists capable of separating all the purines and pyrimidines found in the biological fluids of patients in the different inherited disorders in a single run and within a reasonable time. Because of a need for such a system an appropriate method has been devised.

The system has been in continuous use for over two years and three to four thousand samples have been analysed. It has proved reliable and reproducible throughout the life of many columns. Problems encountered during this period have resulted from artifacts and errors inherent to different isolation/extraction procedures. Pitfalls frequently noted have in the main been due to diet and/or drug metabolites with similar chromatographic behaviour to endogenous purines and pyrimidines. These problems are exaggerated in renal failure and during antibiotic therapy.

Data derived from the chromatography of body fluids of patients with various purine and pyrimidine defects, illustrate the adaptability of the system. The method is particularly applicable to the separation of endogenously derived adenosine based compounds. An abbreviated form of the system has been used successfully for enzyme assays and other in vitro studies.

138 CHANGES OF PURINE METABOLISM DURING DIFFERENTIATION OF RAT HEART MYOBLASTS

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Rat heart myoblasts (H9c2) were cloned and cultivated in Eagle's minimum essential medium supplemented with 10% FCS. Clones exhibiting high creatine kinase (CK) activity were investigated. Reduction of FCS to 2% for one week resulted in a differentiation of myoblasts towards myocytes. Surface morphology and ultrastructural changes of myoblasts and differentiated myocytes (transverse banding pattern) were investigated by means of electron and light microscopy.

Following enzyme activities (nmoles/mg prot./hr) were measured in myoblasts and myocytes:

Enzyme	Myoblasts	Myocytes
CK, EC. 2.7.3.2.	177 ± 77	1618 ± 655
5'-N, EC. 3.1.3.5.	719 ± 326	1638 ± 921
AMP-DA, EC. 3.5.4.6.	76 ± 32	788 ± 280
ADA, EC. 3.5.4.4.	38 ± 8	39 ± 8
PNP, EC. 2.4.2.1.	123 ± 47	157 ± 34
AK, EC. 2.7.1.20.	66 ± 28	69 ± 13
APRT, EC. 2.3.2.7.	60 ± 22	79 ± 15
HGPRT, EC. 2.4.2.8.	122 ± 86	147 ± 31