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D-XYLULOSE-INDUCED DEPLETION OF ATP AND Pi AND INCREASE IN PRPP IN ISOLATED RAT HEPATOCYTES.

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Xylitol provokes hepatic ATP catabolism by trapping P_i as glycerol 3-P (Woods & Krebs, Biochem. J. 134, 437-43, 1973). The dependence of the latter upon an increase in the NADH/NAD^+ ratio, due to oxidation of xylitol to D-xylulose (D-Xyl), raised the question whether D-Xyl depletes ATP. Incubation of hepatocytes with 5 mM D-Xyl decreased ATP by 80 % within 5 min and by 30 % with 5 mM xylitol. Intracellular P_i decreased by 66 % within 5 min with both compounds, but reincreased 3-fold faster and reached supraphysiological levels with D-Xyl. D-Xyl was used at the rate of 5 µmol/min/g of cells, i.e. 3-fold faster than xylitol. With D-Xyl, glycerol 3-P was barely modified but Xyl 5-P increased from 0.02 to 1.4 µmol/g of cells. The main cause of the ATP- and P_i-depleting effect of D-Xyl was an accumulation of sedoheptulose 7-P which increased from 0.3 to 8 µ mol/g after 10 min. Ribose 5-P increased from 0.03 to 0.50 µmol/g with D-Xyl, and to 0.16 µmol/g with xylitol. Ribose 1-P also accumulated, albeit extracellulary, because of release of purine nucleoside phosphorylase from damaged cells, acting on inosine which diffused out. D-Xyl increased PRPP from a basal value of 6 ± 1 nmol/g to 509 ± 72, as compared to 42 ± 11 with xylitol. It is concluded that the markedly higher PRPP level reached with D-Xyl results from a higher activity of PRPP synthetase, owing to a more marked increase of intracellular P_i and of ribose 5-P, combined with other, still unknown factors.

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MOLECULAR AND KINETIC PROPERTIES OF CYTIDINE DEAMINASE FROM HUMAN SPLEEN.

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Cytidine deaminase (EC 3.5.4.5) catalyzes the irreversible hydrolytic deamination of both cytidine and its nucleoside analogues including many antineoplastic agents. The enzyme has been purified from human spleen 2000-fold with respect to the crude extract. The procedure consists of heat treatment, anionic exchange on DEAE-52, hydrophobic interaction on TSK-Phenyl-5PW and an anionic exchange on Mono Q. The last two steps were performed using an FPLC system. The final enzyme preparation shows a microheterogeneity. The native molecular weight is about 50,000. Cytidine deaminase is strictly dependent on the presence of reducing agents for its activity. The enzyme displays linear kinetics. It shows the highest affinity for deoxycytidine among the various substrates tested. The enzyme is inhibited by the reaction products uridine and ammonia in a competitive fashion suggesting a rapid equilibrium random Uni-Bi kinetic mechanism. Furthermore, the enzyme is competitively inhibited by various compounds, such as 5,6-dihydrouridine, deoxyuridine, thymidine, thymine riboside and deoxyadenosine.

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PURINE METABOLISM IN NORMAL AND ITP PYROPHOSPHOHYDROLASE-DEFICIENT ERYTHROCYTES.

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The activity of ITP pyrophosphohydrolase (ITP-ase) was measured in erythrocyte lysates of 48 healthy volunteers. This group could clearly be divided into two different polulations. Mean activity was 48.3 ± 13.1 nkat/g Hb ($\bar{x}$ ± SD, n=38) in one population, and only one-fourth thereof in the other population (11.4 ± 4.3 nkat/g Hb, n=10). In two subjects, previously selected because of high levels of ITP in fresh or stored erythrocytes, the ITP-ase activity was 0.2 and 2.4 nkat/g Hb, respectively.

In normal erythrocytes incubated in phosphate-rich buffer (20 mM), the rate of conversion of allopurinol, hypoxanthine and guanine to their respective nucleotides followed a biphasic pattern. Initial "salvage rate" was 130 nmol/l/s, which leveled of after 15 min to about 50 nmol/l/s. Exactly the same pattern was observed in erythrocytes from ITP-ase deficient subjects. Routine liquid preservation of erythrocytes from both groups in CPD-SAGMAN (containing citrate, phosphate, dextrose, sodium chloride, adenine, glucose and mannitol) resulted in a slow decline in salvage rate (0,7 % /day). The salvage rate of AICA (aminoimidazolecarboxamide) and AICA-riboside was 8 and 40 nmol/l/s, respectively.

We conclude that, despite the fact that the energy consumption in the IMP-ITP cycle can amount to up to 10 % erythrocyte energy turnover in vitro, ITP-ase deficiency and lack of activity in the IMP-ITP cycle, does not influence purine salvage.

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MAST CELL ADENOSINE RECEPTORS, G PROTEINS, AND ADENYLATE CYCLASE INTERACTIONS.

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Forskolin, cholera toxin, and pertussis toxin may alter various aspects of cell function. Mouse bone marrow-derived mast cells exposed to micromolar concentrations of forskolin, an agent thought to directly activate adenylate cyclase, exhibited an inhibition of antigen-induced β-hexosaminidase (β-hex) release but retained the ability of adenosine to potentiate this release. Forskolin alone augmented resting mast cell cyclic AMP levels in a dose-dependent fashion, but adenosine enhanced cyclic AMP concentrations in both control and forskolin-treated cells. Cholera toxin, an agent that ADP-ribosylates and activates G_s proteins in many cell types, failed to alter β-hex release from antigen-challenged mast cells in the presence or absence of 10uM adenosine. Although cholera toxin caused an elevation of mast cell cyclic AMP levels, adenosine enhanced cyclic AMP to a similar extent in control and toxin-treated cells. Mast cells cultured with pertussis toxin, an agent that ADP-ribosylates the G_i protein in some cells, exhibited an attenuation of antigen-stimulated β-hex release and an inhibition of adenosine responsiveness as well. Although pertussis toxin did not alter resting mast cell cyclic AMP levels, high doses of the toxin inhibited the ability of adenosine to increase cyclic AMP. These data suggest that many agents that alter mast cell cyclic AMP concentrations fail to alter adenosine responsiveness. Pertussis toxin appears to be an exception to this finding, although its exact mechanism of action on mast cell adenosine receptors is uncertain.

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UTILIZATION OF 2'-DEOXYNAD FOR ADP-RIBOSE TRANSFER REACTIONS.

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Besides its function as a major electon acceptor, NAD is a substrate for numerous ADP-ribose transfer reactions that are important in the regulation of cell function. The poly(ADP-ribosyl)ation of nuclear proteins has been shown to modify chromatin structure. The mono(ADP-ribosyl)ation of elongation factor-2, catalyzed by diphtheria and psuedomonas toxins, has been demonstrated to block protein synthesis. The mono(ADP-ribosyl)ation of G proteins by bacterial toxins can critically modulate transmembrane signalling. We have developed a simple method to distinguish between mono(ADP-ribosyl)ation and poly(ADP-ribosyl)ation reactions in crude cell extracts. Radioactive 2'-deoxyNAD (dNAD) was prepared enzymatically from [2,8-^{3}H]-dATP and nicotinamide mononucleotide, and was purified by HPLC. Then dNAD and NAD were compared as substrates for purified poly(ADP-ribose) polymerase from calf spleen, and diphtheria toxin mono(ADP-ribose) transferase. The results showed that 2'-dNAD was an efficient substrate for the mono(ADP-ribose) transferase. The transfer of the dADP moiety of dNAD to elongation factor-2 inhibited protein synthesis in reticulocyte lysates. In contrast, dNAD was not a detectable substrate for poly(ADP-ribose) polymerase, under conditions where NAD was rapidly consumed. These results suggest that dNAD may be a useful tool for the investigation of specific cellular targets of eukaryotic and bacterial mono(ADP-ribosyl)ation reactions.

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THE MECHANISM OF 2-CHLORODEOXYADENOSINE-INDUCED CELL DEATH

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The in vitro cytotoxities of 2-chlorodeoxyadenosine (2-CldAdo) were tested using 35 different lines of human leukemia-lymphoma cells. 2-CldAdo was found to be broadly cytotoxic to human T- (EC_{50} value: 0.005-5.3 µM), B-(0.015-4,8 µM), myeloblatic (0.009-12 µM), and monocytic (2.6-5.4 µM) cell lines. The mechanism of cytotoxic action of 2-CldAdo in CCRF-HSB2 cells was investigated. We observed the 2-CldAdo-induced imbalance of intracellular deoxyribonucleoside triphosphate(dNTP) pool and subsequent double strand breaks in mature DNA, accompanied by cell death. When the cells were exposed to 2-CldAdo at 1 µM, an imbalabce of dNTP pool resulted: a depletion of dCTP, dATP and dGTP was observed. Within 4hr of treatment dCTP pool size became smaller than the lower limit of measurement. The loss of cell viability occurred at about 12 hr. Cycloheximide inhibited both the production of double strand breaks and 2-CldAdo-mediated cell death. We assume that the double strand breaks play an important role in the process of 2-CldAdo-mediated cell death and the intracellular dNTP imbalance is the trigger of these events.