19 IDENTIFICATION OF A PURINE-SPECIFIC 5'-NUCLEOTIDASE IN HUMAN ERYTHROCYTES. F. Bontemps, G. Van den Berghe & H.G. Hers. Laboratory of Physiological Chemistry, University of Louvain & International Institute of Cellular & Molecular Pathology, Brussels, Belgium.

In human erythrocytes, a pyrimidine 5'-nucleotidase has been characterized (Paglia & Valentine, Curr. Top. Hematol 3:75, 1980) but the enzyme(s) responsible for the dephosphorylation of AMP and IMP had hitherto not been identified. Haemolysates in 10 mM Tris-maleate buffer pH 7, incubated with 1 mM [¹⁴C] IMP, produced [¹⁴C] inosine at the rate of approx. 2 μmol/h per g of Hb. The enzymic activity was purified around 3000-fold in 3 steps: (1) batchwise binding to DEAE cellulose, followed by washing with 10 mM Tris-maleate pH 6.5 to remove Hb; (2) column separation from acid phosphatase and pyrimidine 5'-nucleotidase by elution with a NaCl gradient in the same buffer, which released the purine 5'-nucleotidase at 0.2 M NaCl; (3) adsorption on blue-sepharose, followed by elution with 2 M NaCl. The enzyme was Mg⁺⁺-dependent and displayed a broad pH optimum around pH 7. It was maximally active with IMP and hydrolysed GMP at 75 % and AMP at 10 % of the rate of IMP. Km for the three nucleotides was around 0.1 mM. At 0.25 mM AMP, the enzyme activity was 80 % inhibited by 5 mM Pi and stimulated 8-fold by 5 mM 2,3-bis-P-glycerate, half maximal stimulation being obtained at 0.5 mM of this compound. In contrast with rat liver cytosolic 5'-nucleotidase, the erythrocyte enzyme was not stimulated by ATP or GTP. Supported by FRSM.

20 PATHWAYS OF ADENINE NUCLEOTIDE CATABOLISM IN HUMAN ERYTHROCYTES. F. Bontemps, G. Van den Berghe and H.G. Hers. Laboratory of Physiological Chemistry, ICP & University of Louvain, Brussels, Belgium.

Adenine nucleotide catabolism in erythrocytes incubated for 4h under physiological conditions is very low as shown by a production of hypoxanthine (HX) of 4.2 ± 0.7 nmol/h per g of packed cells (mean $\pm$ SEM for n=8). It is not influenced by maximal inhibition of adenosine (Ado) deaminase by 1 μM deoxycytosine (dCF) and of Ado-kinase by 10 μM iodotubercidin (ITU) indicating that it proceeds by deamination of AMP, followed by dephosphorylation of IMP, and that there is no recycling between AMP and Ado. Suppression of glucose increases AMP 30-fold and the production of HX 20-fold. dCF decreases this production by 75 % and provokes an accumulation of Ado, demonstrating a dephosphorylation of AMP. ITU enhances this accumulation, indicating a recycling of Ado under glucose deprivation which reaches approx. 20 nmol/h per ml of cells. Alcalinisation of the incubation medium in the presence of glucose increases AMP 5-fold and the production of HX 15-fold. dCF has no effect on this production, demonstrating that it results from a deamination of AMP. ITU also evidences recycling of Ado under this condition, reaching 25 nmol/h per ml of cells. The 2-fold elevation of intracellular Pi during glucose deprivation and its 50 % decrease during alcalinisation, as well as experiments in which extracellular Pi was modified, indicate that the dephosphorylation of AMP is mainly responsive to variations of AMP, whereas its deamination is more sensitive to modifications of Pi. Supported by FRSM.

21 DECREASED PURINE AND METHIONINE SYNTHESIS IN PURINE NUCLEOSIDE TREATED T AND B LYMPHOBLASTS: REVERSAL BY HOMOCYSTEINE
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Adenine and guanine nucleosides and deoxynucleosides inhibit methionine synthesis in cultured human T and B lymphoblasts; the deoxynucleosides are more potent inhibitors than the corresponding nucleosides and T lymphoblasts are more sensitive than B lymphoblasts (J. Clin. Invest. 74:1262-1268, 1984). Since homocysteine largely reverses the methionine synthesis inhibition, it appears that the mechanism is through S-adenosylhomocysteine hydrolase inhibition with a consequent decrease in intracellular homocysteine synthesis. Because the 5,10-methylenetetrahydrofolate reductase reaction is functionally irreversible in vivo, reduced intracellular homocysteine production should lead to trapping of folates as 5-methyltetrahydrofolate and thus to decreased purine synthesis. This hypothesis was tested in cultured T and B lymphoblasts. To eliminate inhibition of the first reaction of the de novo pathway, cells were treated with azaserine and aminomidazolecarboxamide; thus, when incubated with [¹⁴C]formate only the last step of the pathway is measured. In the T lymphoblasts dAdo and dGuo inhibited purine synthesis significantly at concentrations less than 1 μM whereas it required greater than 3 μM of these deoxynucleosides to inhibit purine synthesis in the B lymphoblasts; at these low concentrations of dAdo and dGuo homocysteine fully reversed the decreased purine synthesis.

22 COBALAMIN INACTIVATION DECREASES PURINE AND METHIONINE SYNTHESIS IN CULTURED HUMAN LYMPHOBLASTS
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The megaloblastic anemia of cobalamin deficiency appears secondary to decreased methionine synthetase activity. Decreased activity of this enzyme should cause 5-methyltetrahydrofolate to accumulate intracellularly. However, only some of the clinical and biochemical manifestations of cobalamin deficiency can be explained by the methylfolate trap. Since nitrous oxide (N₂O) inhibits methionine synthetase activity by inactivating cobalamin, cultured lymphoblasts were treated with N₂O to create a model of cobalamin deficiency; 4 h of N₂O exposure reduced rates of methionine synthesis by 89%. Rates of purine synthesis were not significantly reduced by N₂O when folate and methionine were present at 100 μM in the medium; however, at the physiologic methionine concentration of 10 μM, N₂O decreased rates of purine synthesis by 33% and 57% in the presence of 100 μM folate and in the absence of folate, respectively. The dependency of rates of purine synthesis on methionine availability would be expected in cells with restricted methionine synthetic capacity because (a) methionine is the immediate precursor of S-adenosylmethionine, a potent inhibitor of 5-methyltetrahydrofolate synthesis; (b) methionine serves as a source of formate for purine synthesis; and (c) rates of purine synthesis are dependent on the intracellular availability of essential amino acids. It appears that cobalamin deficiency decreases purine synthesis by both methylfolate trapping and reduction of intracellular methionine synthesis.

23 HPLC PLASMA ADENOSINE ASSAY FOR USE IN CARDIAC STUDIES. Phyllis R. Brown, Mary Jo Wojtusik, Ronald Stuart and Henry Gewirtz

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To optimize an HPLC assay for adenosine (Ado) in plasma every step in the procedure was examined. The presence in the plasma of several drugs used in the cardiac studies interfered with the usual isocratic HPLC assay of Ado and necessitated the use of a step-gradient. Sample preparation procedures routinely used in biomedical studies were evaluated to determine lability of adenosine prior to chromatographic analysis. Factors investigated included the use of EHNA (an inhibitor of adenosine deaminase), dipyrindamole (an inhibitor of nucleoside transport) and EDTA (an inhibitor of ATPase and 5'-nucleotidase). Effects of deprotenization methods, time of analysis, minimum detectable limits and recovery of Ado from both whole blood and plasma were examined. Ado values in samples treated immediately with PCA were much lower than those in which the samples were collected in solutions of EHNA and dipyrindamole. Moreover, the latter samples showed substantial intra- and inter-animal variability. However, if EDTA was added to the sample solution, along with the EHNA and dipyrindamole, the concentrations of Ado in the plasma samples were similar to those obtained with the PCA. Precision studies showed marked improvement. Our results provide evidence that in the analysis of plasma for Ado, in vitro production as well as the catabolism of Ado must be stopped in order to obtain valid estimates of plasma Ado concentration.

24 THE USE AND ABUSE OF ALLOPURINOL IN RENAL FAILURE
THE TUMOUR LYSIS SYNDROME AND UROLITHIASIS.
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Despite widespread use, side effects of allopurinol have been rare. Problems arising in chronic renal failure (CRF) have been well documented, but necessity to reduce dosage in such cases is often ignored. Not so well known is the acute sensitivity to xanthine oxidase inhibition in situations of gross purine overproduction and the attendant risk of xanthine stones, or acute renal failure (ARF) from xanthine nephropathy.

To underline potential hazards and their avoidance, purine levels found in collaborative studies on allopurinol-treated patients are reported. 90% of referrals involved monitoring of plasma oxipurinol levels in CRF. Results frequently exceeded the desirable level of 100 μmol/l, with values as high as 310 μmol/l in those receiving 300 mg/day and still 60 μmol/l three days after stopping therapy in one case complicated by exfoliative dermatitis. One death from bone marrow aplasia had occurred prior to general awareness of the marrow depressant effects of sustained oxipurinol levels in CRF.

Four cases of ARF due to xanthine nephropathy occurred during therapy for conditions resulting in rapid cell lysis where plasma xanthine (normally <1 μmol/l) and oxipurinol levels of 650 μmol/l and 642 μmol/l, respectively, were recorded. Two Lesch-Nyhan patients developed xanthine stones and required careful monitoring. The results demonstrate the effects of allopurinol overdose which should be avoidable through knowledge of purine metabolism in the particular situation.