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METABOLISM OF GUANOSINE AND ITS C-8 SUBSTITUTED ANALOGUES IN HUMAN LYMPHOCYTE CYTOPLASM. N. Lawrence Edwards. Dept Med, Univ of FL and VAMC, Gainesville, FL and James J. Mond, USUHC, Bethesda, MD, USA.

The C-8 substituted guanine nucleosides are a new class of immunologically active purine analogues. Naturally occurring guanosine is immunosuppressive to B-cells. 8-Mercaptoguanosine (8-SGuo) and 8-bromoguanosine (8-BrGuo) have polyclonal B-cell activating properties in murine lymphocytes. 8-Aminoguanosine (8-NH Guo) has no immunologic effects on cells in culture but is a potent inhibitor of the purine catabolic enzyme, purine nucleoside phosphoylase. This study examined the metabolic fate of guanosine and the C-8 substituted analogues in dialyzed human splenic cytosol. Required cofactors for the involved enzyme systems were added to the cytosol. The metabolism of the ribonucleoside substrate to other purine forms was monitored by reverse-phase and anion exchange HPLC at 15 minute intervals for 2 hours. Guanosine declines linearly over 90 minutes with linear accumulations in guanine, xanthine, GTP and GDP when incubated at $37^{o}C$ in the presence of PRPP. 8-SGuo and 8-BrGuo remain essentially non-metabolized to other forms after 120 minutes. 8-NH Guo is rapidly metabolized to 8-NH guanine and a minor peak of 8-NH GDP appearing after 60 minutes. These preliminary studies in human spleen demonstrate that 2 C-8 substituted purine analogues (8-SGuo and 8-BrGuo) that stimulate B-cell proliferation and differentiation are not substantially metabolized by cytoplasmic purine enzymes. Conversely guanosine is extensively metabolized to both higher (GTP and GDP) and lower (guanine and xanthine) forms. These findings may help define the molecular basis for both immune-suppressive and immune-potentiating effects of guanosine and its C-8 substituted analogues.

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A MODEL OF GOUT NEPHROPATHY. B.T.EMMERSON, R. Axelsen and M. Cross. Department of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, 4102.

Although many factors contribute to renal disease in patients with gout, the common denominator appears to be the presence of urate crystals and the formation of micro-tophi in the renal interstitium. In order to examine this further, the reaction between crystalline urate and cultured renal cells was studied using Madin-Darby canine kidney cells (MDCK cells), grown in long-term culture. Exposure of these cells in monolayer to urate crystals induced defined reaction sites which increased to a peak at 24 hours and subsided subsequently. Crystals appeared to pass both through the cells in monolayer as well as between these cells. Scanning EM showed crystals which appeared to be enveloped by cell processes. Crystals were demonstrated beneath the epithelial monolayer in the heaped-up reaction sites. The cell crystal interaction was associated with significant release of lysosomal enzymes and to a lesser extent of cytosolic enzymes, the extent of lysosomal enzyme release being proportional to both crystal concentration and the duration of exposure.

In a medium with a lower sodium concentration and at a lower pH similar to that in the distal tubule, a comparable reaction could be demonstrated between MDCK cells and uric acid crystals. These studies confirm that there is an active reaction between distal nephron cells and intraluminal crystals of urate and uric acid which has the potential to induce renal damage.

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EVALUATION OF OXYPURINES BY HPLC IN LYMPHOPROLIFERATIVE DISEASES.

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The behavior of oxypurines has been evaluated in 20 normal subjects and in 20 patients affected by lymphoproliferative diseases (LPD). Plasma and 24 h urine were analyzed for uric acid, hypoxanthine and xanthine levels by high performance liquid chromatography (HPLC), according to a previously described procedure (1). 6 patients had myeloma, 5 lymphoma, 9 acute lymphocytic leukemia. Determinations were carried out before and after pharmacological treatment. There was an evident rise in oxypurines in the plasma and urine of all patients with representative patterns for each disease. Oxypurines were substantially reduced - although only slightly - after chemotherapy, and their behavior was not related to that of uric acid. The changes observed may be due either to an accelerated synthesis of purine nucleotides, to an enhanced degradation, or to release from the cells. These aspects are currently under investigation, as well the possibility of using this determination as marker for the disease.

(1) L.Lorenzi et al., Tumori 73, 289-294, 1987.

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BEHAVIOR OF OXYPURINES IN NORMAL SUBJECTS AFTER AN 8 DAY PURINE-FREE DIET.

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Seven normal adult volunteers (aged between 22 and 60 years) received a purine free diet for eight days (meat, fish, ham, salami and sausages, eggs, legumes, chocolate, wine, beer, superalcoholics, coca-cola, tea and coffee were rigorously excluded; noodles, rice, tomato, bread, cheese and sweets were allowed). Plasma and 24 h urine were analyzed both before and after the experiment for uric acid, hypoxanthine and xanthine content by high performance liquid chromatography (HPLC), as previously described (1). Urinary excretion of uric acid and xanthine was reduced, that of hypoxanthine rose: changes were more evident in plasma, where there was a highly significant rise ($P < 0.01$) in both oxypurines and lower values for uric acid. Results were surprising in part and interpretation was difficult. Increased purine catabolism, associated with a tendency of the body to spare oxypurines, provoked with a subsequent increase in exchange of oxypurines between tissues: the behavior of xanthine oxidase - which is sensitive to dietary changes and to purine intake: could partially explain the results.

(1) M.Lorenzi et al., Tumori 73, 289-294, 1987.

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EVIDENCE FOR "LOW Km" AND "HIGH Km" SOLUBLE 5'-NUCLEOTIDASES IN HUMAN TISSUES AND RAT LIVER. Józef Spychała, Vincente Madrid-Marina, and Irving H. Fox. The University of Michigan, Depts. of Int. Med. and Biol. Chem., Ann Arbor, Michigan, USA.

Three distinct 5'-phosphomonoesterase activities were isolated from soluble fractions of human placenta, cultured human T- and B-lymphoblasts and rat liver using AMP-sepharose 4B affinity chromatography and a sequence of specific eluting agents. We have defined these activities as "low Km" 5'-nucleotidase, "high Km" 5'-nucleotidase and nonspecific phosphatase. "High Km" 5'-nucleotidase was eluted with 0.5 M NaCl, "low Km" 5'nucleotidase was eluted with 10 mM ADP and nonspecific phosphatase was not retained on the column. The relative content of "high Km" and "low Km" activities in the tissues studied ranged from 5.5 to 264. The molecular weight of the "low Km" enzymes ranged from 72.5 to 209 kD, optimum pH ranged from 7.4 to 9.0, Km for AMP ranged from 7 to 15 and for IMP from 10 to 26 uM, respectively. ATP and ADP were inhibitors of "low Km" enzymes with the apparent Ki values of 55 to 100 and 8 to 20 uM, for ATP and ADP, respectively. The molecular weight of the "high Km" 5'-nucleotidases ranged from 182 to 210 kD, pH optimum was at 6.5, and Km for IMP was 0.3 to 0.5 mM and for AMP 1.0 to 9.4 mM. "High Km" enzymes were activated by ATP with $A_{0.5}$ values, measured at 20 uM IMP, of 1.7 to 2.3 mM. The ATP activation was substrate dependent. The data indicate that soluble "low Km" and "high Km" 5'-nucleotidase coexist in mammalian cells and fulfill different functions. These observations suggest a complex system for the regulation of AMP and IMP dephosphorylation.

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PURIFICATION AND PROPERTIES OF HUMAN T-LYMPHOBLAST DEOXYCYTIDINE KINASE. Nabanita S. Datta, Donna S. Shewach, Mary C. Hurley, Beverly S. Mitchell, and Irving H. Fox. The University of Michigan, Depts. of Int. Med. and Biol. Chem., Ann Arbor, Michigan, USA

Nucleoside kinases phosphorylate endogenous nucleosides and nucleoside analogs which are anticancer and antiviral drugs. dCytidine kinase was purified from cultured human T-lymphoblasts to a specific activity of 8 umol/min/mg protein and 92% purity. The molecular weight was 60 kD and the stokes radius was 32 A. The subunit molecular weight was 30.5 kD. dGuanosine, dadenosine and cytidine phosphorylating activities copurified with dcytidine kinase to final specific activities of 7.2, 13.5 and 4 umol/min/mg protein, respectively. This enzyme had apparent Km values of 1.5, 430, 500, 450 and 40 uM for dcytidine, dguanosine, dadenosine, cytidine and cytosine arabinoside, respectively. The pH optimum ranged from 6.5-9.0. Mg^{2+} and Mn^{2+} were the preferred divalent cations. ATP, GTP, dGTP, ITP, dITP, TTP and XTP were substrates for the enzyme. This highly purified enzyme will facilitate our delineation of the role for this enzyme in nucleoside or nucleoside analog phosphorylation and the structural basis for the regulation of this enzyme.