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ADENOSINE DEAMINASE ACTIVITY IN RECIPIENTS OF BONE MARROW FROM IMMUNODEFICIENT MICE HOMOZYGOUS FOR THE WASTED MUTATION. Erik H. Willis, Leonard D. Shultz, and Dennis A. Carson. Research Institute of Scripps Clinic, Department of Basic and Clinical Research, La Jolla, California, USA, and The Jackson Laboratory, Bar Harbor, Maine USA.

Mice homozygous for the mutation *wasted (wst/wst)* have been postulated to be a model for the form of human severe combined immunodeficiency disease (SCID) that is secondary to a genetic deficiency of adenosine deaminase (ADA). To test this hypothesis more critically, we transplanted marrrow from *wst/wst* and littermate control mice into lethally irradiated normal recipients. If there was an inherent ADA deficiency in the hematopoietic stem cells of the *wst/wst* mutant, the defect would be expected to be passed on to the irradiated marrow chimeras, resulting in decreased Vmax values for ADA, and/or changes in the Km for adenosine. However, the Vmax and Km values for ADA and adenosine, repsectively, in recipient's hematologic and non-hematologic tissues did not differ significantly from control values. In addition, we also found no significant differences in ADA activities between lymphoid and liver tissue from untreated 24-to-28-day-old *wst/wst* and littermate control mice. These results indicate that the *wasted* mouse is not a model for ADA deficiency and SCID.

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POTENT AND SPECIFIC INHIBITORS OF MAMMALIAN PHOSPHORIBOSYLPYROPHOSPHATE (PRPP) SYNTHETASE. **Randall C. Willis, L.D. Nord, Timothy S. Breen, and Steven S. Matsumoto. The Nucleic Acid Research Institute, Biochemistry, 3300 Hyland Avenue, Costa Mesa, CA 92626 U.S.A.**

The adenosine analogs 4-amino- and 4-methoxy-8-(D-ribofuranosylamino)-pyrimido[5,4-d]pyrimidine provide ID50 values against the human lymphoblast line WI-L2 of 0.18 μM and 0.05 μM, respectively. Cultured WI-L2 and *in vivo* mouse L1210 tumor lines selected for resistance to these analogs are characterized by a deficiency in adenosine kinase activity. These compounds completely inhibit de novo purine and pyrimidine biosynthesis as measured by [14C]-bicarbonate incorporation and reduce by > 85% the rate of incorporation into nucleotides of either [14C]-hypoxanthine, -adenine, or -orotate and by > 60% [14C]-nicotinamide into NAD; a requirement for PRPP is common to each of these incorporation pathways. In contrast the rates of incorporation of [14C]-adenosine, -5-aminoimidazole-4-carboxamide riboside and -uridine, which do not require PRPP for incorporation, are relatively unaffected. Treatment of cells with the 4-amino analog causes a 50% reduction in the PRPP pool and, in studies of inosine-driven, adenine incorporation by HPRT deficient cells, > 95% reduction in PRPP availability. Studies with the human erythrocyte PRPP synthetase indicate that at physiological phosphate concentrations, 1 - 5 mM, the monophosphate form of these adenosine analogs maintains the enzyme in an inactive form. At high phosphate concentrations, > 32 mM, the monophosphates of the analogs have no effect on the activity of PRPP synthetase.

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ATP-DEPENDENT MINERALIZATION OF HYALINE ARTICULAR CARTILAGE MATRIX VESICLES. John W. Rachow, Majeedul H. Chowdhurry, Robert L. Wortmann. Medical College of Wisconsin, and Veterans Administration Medical Center, Milwaukee, Wisconsin, USA.

One *in vitro* model of the chondro-osseous dysplasia (COD) seen in adenosine deaminase deficiency is characterized by depletion of chondrocyte ATP. If normal bone mineralization is an energy dependant process, low growth plate ATP concentration could be a partial basis for COD. Since cartilage matrix vesicles (MV) are involved in mineralization of growth cartilage and MV possess an ensemble of membrane bound purine nucleotide-processing enzymes including ATP pyrophosphohydrolase, 5'nucleotidase and alkaline phosphatase, we investigated whether ATP was required for MV mineralization.

MV were prepared by ultracentrifugation of collagenase-digested adult porcine hyaline articular cartilage. Mineralization experiments were conducted in inorganic phosphate (Pi)-free media containing 2.2mM ^{45}Ca at ph 7.6 and 37° C. Additions of ATP, AMP, Pi, αß-methylene ATP were made in various experiments. Mineralization was expressed as ^{45}Ca uptake in MV separated by 0.45 micron membrane filtration. Mineralization occured by 6 hours in the presence of 0.5-1.5 mM ATP or ßγmethylene ATP but not with stoichiometric added Pi alone. Mineralization was inhibited by 5 mM ATP. AMP alone only poorly supported mineralization, and no mineralization was observed with αß-methylene ATP.

ATP requirement for MV mineralization indicates the potential importance of this simple model in studing the mechanisms of defective bone-formation in COD.

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PURINE CATABOLIC ENZYMES IN HUMAN SYNOVIAL FLUIDS. Robert L. Wortmann, Judith A. Veum, and John W. Rachow. Medical College of Wisconsin and Veterans Administration Medical Center Milwaukee, Wisconsin USA.

Cartilage 5'nucleotidase (5NT) activity has been implicated in calcium pryophosphate dihydrate (CPPD) deposition [Arthritis Rheum 24:492,1981]. We measured synovial fluid (SF) 5NT and nucleotide pyrophosphohydrolase (NPPH), another cartilage ectoenzyme associated with CPPD deposition, in 173 patients with well-characterized arthropathies. A cytosolic enzyme, adenosine deaminase (ADA), and an ectoenzyme not associated with CPPD deposition, alkaline phosphatase (AP), were measured as controls.

	Enzyme Activity, mean±SD(n), nmol/hr/mg protein			
	NPPH	5NT	AP	ADA
gout	1.2±0.8(10)	1.7±1.3(7)	10.2±2.6(6)	11.6±9.9(6)
psuedogout	1.8±0.9(7)	2.5±1.0(5)	18.8±2.4(3)	10.4±5.2(3)
RA	1.6±0.9(33)	3.8±1.9(21)	14.7±3.0(6)	10.5±5.3(22)
osteoarthritis	2.0±1.5(34)	4.6±1.3(26)	13.0±4.7(6)	2.6±2.1(29)
CPPD	2.6±2.2(10)	4.7±2.0(4)	12.1±3.9(6)	3.7±2.4(8)
BCP	2.4±1.3(25)	5.5±2.2(19)	11.7±3.2(6)	2.3±1.7(24)
CPPD+BCP	3.1±3.2(27)	8.8±5.4(23)	11.9±3.7(9)	2.2±1.5(22)
MSS	2.5±0.6(20)	10.4±4.4(11)	11.2±6.4(6)	3.6±1.6(10)

BCP=basic calcium phosphate; MSS=Milwaukee shoulder syndrome

SF 5NT and NPPH activities are highest and ADA activities are lowest in joints with noninflammatory SF which contain calcium crystals (Wilcoxon rank-sum, $p < 0.05$). SF activities correspond with those reported in cartilage and suggest a specific association of elevated SF 5NT and NPPH activities with articular calcium crystal deposition.

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TWO DISTINCT TARGET SITES ON IMP DEHYDROGENASE IN CHEMOTHERAPY Yasukazu Yamada, Yutaka Natsumeda, Yasufumi Yamaji and George Weber Indiana University School of Medicine, Laboratory for Experimental Oncology, Indianapolis , Indiana, U.S.A.

The inhibitory mechanisms of ribavirin 5'-monophosphate, (RMP), SM-108 nucleotide (bredinin 5'-monophosphate, BMP) and thiazole-4-carboxamide adenine dinucleotide (TAD), the active forms of the antimetabolites, ribavirin, SM-108 and tiazofurin, were studied in IMP dehydrogenase purified to homogeneity from rat hepatoma 3924A. RMP and BMP as well as XMP inhibited competitively with IMP and noncompetitively with NAD^+. The inhibition by TAD was similar to that of NADH: uncompetitive with IMP and mixed type with NAD^+. Ki values for RMP (0.8 uM), BMP (0.11 uM) and TAD (0.13 uM) were markedly lower than those for XMP (136 uM) and NADH (210 uM), and also the Km for IMP (23 uM) and NAD^+ (65 uM). Thus, the drugs interact with IMP dehydrogenase with higher affinities than the natural metabolites, RMP or BMP with IMP-XMP and TAD with NADH site. Preincubation of the purified enzyme with RMP enhanced its inhibitory effect in a time-dependent manner. The enzyme was protected from inactivation by IMP or XMP but not by NAD or NADH. Ribavirin and tiazofurin together provided synergistic killing of hepatoma cells in clonogenic assays and the metabolic flux of de novo guanylate synthesis was also synergistically inhibited. The results provide a biochemical basis for combination chemotherapy with tiazofurin and ribavirin against the 2 different ligand sites of IMP dehydrogenase. (Supported by Outstanding Investigator Grant CA-42510 to G.W.)

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KINETIC PROPERTIES OF IMP DEHYDROGENASE PURIFIED FROM RAT HEPATOMA 3924A Yasukazu Yamada, Tadashi Ikegami, Yutaka Natsumeda and George Weber Indiana University School of Medicine, Laboratory for Experimental Oncology, Indianapolis, Indiana, U.S.A.

IMP dehydrogenase (EC 1.1.1.205), the rate-limiting enzyme of de novo GTP biosynthesis, a promising target for cancer chemotherapy, was purified about 5,000-fold to homogeneity from rat transplantable hepatoma 3924A. Sephacryl S-400 gel filtration showed that the molecular weight of the native enzyme protein was about 245,000. SDS electrophoresis indicated that the molecular weight of the subunits was about 60,000. Thus, the enzyme has a tetrameric structure. The double reciprocal plots for substrate dependence yielded Km values for IMP and NAD^+ of 23 and 65 uM, respectively. Excess NAD^+ caused substrate inhibition in the purified enzyme. The enzyme requires potassium ions; an apparent Km value for K^+ was 7.8 mM. Product-inhibition studies showed that XMP was competitive with IMP (Ki = 136 uM) and noncompetitive with NAD^+. NADH exerted uncompetitive inhibition with respect to IMP (Ki = 210 uM) and mixed type with NAD^+ (Ki(slope) = 290 uM). This inhibitory pattern suggests an ordered Bi-Bi mechanism for the enzyme reaction in which IMP binds to the enzyme first, followed by NAD^+. NADH dissociates from the ternary complex after rearrangement, and finally XMP is released. XMP interacts with the free enzyme and competes for the ligand site with IMP, while NADH binds to the enzyme XMP complex. (Supported by Outstanding Investigator Grant CA-42510 to G.W.)