109 THE BIOSYNTHESIS OF DEOXYGUANOSINE TRIPHOSPHATE (dGTP) IN HERPES SIMPLEX TYPE-1 (HSV-1) INFECTED VERO CELL TREATED WITH ACYCLOVIR (ACV) AND HYDROXYUREA (HU).

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Purine deoxynucleoside triphosphate pools increased dramatically in HSV-1 infected vero cells treated with ACV. This study quantitatively compares the contributions of the ribonucleotide reductase (RR) and deoxyguanosine salvage pathways for the biosynthesis of dGTP in HSV-1 infected cells. [^{14}C]Guanine introduced 1 hr after infection was readily incorporated into dGTP and HSV DNA. By 6-8 hrs after treatment with ACV at 100 μM, both dATP and dGTP pools increased 7 fold and [^{14}C]dGTP accumulated 32 fold over untreated infected controls. The increase in [^{14}C]dGTP, dGTP and dATP in the presence of ACV was prevented by HU at 10 mM, which inhibits RR. By prelabeling cellular DNA with [5'-^{3}H]deoxyguanosine prior to infection, it was possible to show that salvage of deoxyguanosine, derived from host DNA, was a minor pathway for dGTP syntheis during infection, and that HU had no effect on this process. Greater than 99% of the dGTP in infected ACV treated cells was derived from the guanine ribonucleotide pool through the RR reaction, as compared with <1% of the dGTP being derived from the salvage of endogenous deoxyguanosine formed from [5'-^{3}H]-labeled cellular DNA.

110 MULTIENZYME RNA SYNTHETASE COMPLEXES: METABOLIC CHANNELLING OF NUCLEOTIDE MONO- AND DIPHOSPHATE PRECURSORS INTO RNA

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The uptake of exogenous nucleoside tracers into RNA proceeds without equilibration of the tracer with the cellular ribonucleotide triphosphate pools. Kinetic analysis of the tracer progression into RNA suggests that the functional nucleotide RNA-precursor pool is very small, so that simple compartmentation into nuclear and cytoplasmic pools fails to account for these results. We have tested the hypothesis that RNA synthesis is dependent on a multienzyme complex (similar to the proposed "DNA replitase") that functions as a metabolic channel. Nuclear lysates of mouse lymphoma S49 cells and human HL-60 promyelocytic leukemia cells were separated on sucrose gradients (20-60%) and fractions analyzed for RNA polymerase and nucleotide kinase activities. At least three fractions with high molecular weights (> 10^6 Daltons) were identified that were capable of incorporating mono-, di- and triphosphates (U+C+A+G) into acid-insoluble macromolecules. The mono- and diphosphates did not seem to equilibrate with the triphosphate pools in the medium, which suggests metabolic channeling. Tracer incorporation was dependent on the presence of all four bases as their nucleotides in the medium, while DNA template was not required in these fractions. These results provide first evidence for the existence of multienzyme RNA synthetase complexes that preferentially accept distant precursors, i.e. nucleotide mono- and diphosphates. Supported by CA 34304.

111 DOSE DEPENDENCY OF THE URIC ACID LOWERING EFFECTS OF ALLOPURINOL WITH AND WITHOUT ORAL PURINES

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Healthy young volunteers received a purine-free formula diet for 18 days. Allopurinol (A) was given from day 11-18 in single oral doses of 125, 250, 375 and 500 mg/m^2 body surface to 4, 8, 4 and 8 subjects, respectively. In a second study 4 of the 8 subjects on 250 and 500 mg/m^2 took the same doses with the formula diet being supplemented by 4 g RNA/d. Maximum reduction of renal uric acid excretion was 55% and 70% during purine-free diet and RNA intake, respectively. On 250 and 500 mg/m^2 reduction of endogenous renal uric acid was 36 and 46%, while exogenous uric acid was reduced by 74 and 86%, respectively in the 4 subjects. Renal uric acid clearance was increased by A during purine-free diet, but was not during RNA loading. In a different series of experiments A was given alone first followed by additional purine intake. On this regimen uric acid excretion did not increase during purine ingestion in most instances. This suggests previous inhibition by A or stimulation by oral purines of xanthine oxydase to be a factor in determining the influence of A on exogenous uric acid production.

112 UNPROPORTIONAL INCREASE IN HEMOLYSATE OF THE ACTIVITY OF A MUTANT HPRT DURING HEMODIALYSIS

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A patient having been treated by hemodialysis because of gouty nephropathy since 1973 was diagnosed to have partial HPRT deficiency (19 nmol/mg proteinxh in hemolysate) in 1978. His grandson presented with acut renal failure at the age of 3 weeks in 1982. After an initial value of 31 nmol/mgxh after previous transfusion his enzyme activity consistently was below 1 nmol/mgxh. Intact red cell studies revealed a twofold activity in the propositus compared with his grandson (12.2 and 6.4 nmol/ml packed cells per h). In lysates of cultured fibroblasts both patients showed the same activity (0.8 nmol/mgxh). In both patients kinetic studies of the enzyme gave normal results and heat inactivation showed it to be more labile than normal controls. From family studies as well as activity in cultured fibroblasts it can be concluded that both patients inherited the same enzyme defect. Comparing the results in these two patients the twofold activity in intact red cells of the propositus can be attributed to a shortened half-life of his erythrocytes, whereas the fortyfold increase (range 20-60) in hemolysate is unexplained.

113 INHIBITION OF T CELL CYTOTOXICITY BY CYCLOSPORINE (CSA), ADENOSINE (ADO), AND AN INHIBITOR OF ADENOSINE DEAMINASE (ADA).

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The resistance of cytotoxic T cells (Tc) to CSA may be related to the way they handle purines such as Ado. Ado has previously been shown to be toxic to Tc. We tested the effect of CSA, Ado, and the ADA inhibitor EHNA on the cytotoxicity of IL-2 dependent cloned Tc cells derived from C57/BL6 mice directed against syngeneic targets bearing SV40 antigen. No effect was seen if Tc were treated only with Ado 10^{-3}M, EHNA 10^{-3}M or CSA 10 ug/ml or 25 ug/ml.

	% specific ^{51}Cr release		
E:T	10:1	3:1	
No Treatment	19.3 ± 2.0	31.1 ± 1.2	
Ado + CSA	21.7 ± 1.7	20.9 ± 1.3	
Ado + EHNA	17.2 ± 0.8	18.5 ± 1.1	
EHNA + CSA	24.8 ± 1.0	19.7 ± 3.1	
Ado + EHNA + CSA	11.5 ± 0.6	13.4 ± 0.8	$p < .05$

Significant inhibition of cytotoxicity occurred when Ado, EHNA, and CSA (25 ug/ml) were combined. Mild inhibition of cytotoxicity occurred when Ado was combined with EHNA. Marked inhibition occurred if Ado was added. These data suggest that if ADA is inhibited, CSA can potentiate the toxicity of Ado to cytotoxic T cell function. This may have relevance to antirejection therapy in organ transplantation.

114 A STUDY OF ADENOSINE DEAMINASES IN HUMAN SERA.

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Two different molecular forms of adenosine deaminase have been found in human tissues: the high molecular weight enzyme and the low molecular weight enzyme, designated as the A form and the C form respectively. These two enzyme forms are present in various proportions in different tissues. In previous studies, the two enzyme forms were separated and identified by gel filtration column chromatography and spectrophotometric measurements of enzyme activity in the elution fractions. A rapid and efficient method is needed to perform routine analysis of the enzyme form distribution, especially in samples containing little enzyme activity, and to study the interrelationship of the two enzyme forms. This study shows the development of the thin-layer gel filtration technique for the separation and the identification of the two molecular forms of adenosine deaminase in human tissue extracts. Possible clinical application of this technique and the enzyme system is discussed. This work was supported by a grant from the Indiana Delaware County Cancer Society.