7 ETHANOL INDUCED NUCLEOTIDE CATABOLISM IN MOUSE T LYMPHOBLASTOID CELLS. Jerzy Barankiewicz and Amos Cohen. Division of Immunology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

The effect of ethanol on nucleotide catabolism was studied in mouse purine nucleoside phosphorylase deficient (NSU-1) T lymphoblastoid cells. Cells were pre-incubated first with radioactive adenine to label intracellular ATP and then incubated with a range of ethanol concentration (0-5%). Changes in intracellular concentrations of nucleotides and amounts of nucleosides excreted into the medium were measured. It was found that ethanol induces ATP degradation which increased with increasing ethanol concentrations. However, no significant changes were found in ADP or AMP levels. Inosine was the major product of ATP catabolism excreted to the medium but small amounts of adenosine were also found. Inhibition of adenosine deaminase activity by deoxycoformycin increased only slightly the excretion of adenosine but had no effect on inosine production. This indicates that ethanol induced nucleotide catabolism proceeds mainly via AMP deaminase and IMP dephosphorylase reactions. Ethanol had no effect on the intracellular orthophosphate levels. On the other hand ethanol in low concentrations (below 1%) decreased adenine and uridine incorporation into nucleotides, and thymidine incorporation into DNA. The elevation of nucleotide catabolism together with the inhibition of nucleotide synthesis may significantly alter nucleotide availability for a number of cellular processes. It may therefore explain suppression of the blastogenic transformation and cause lymphopenia in acute alcoholism.

8 EFFECTS OF ADA INHIBITION ON T CELL DEVELOPMENT IN VITRO. Randall W. Barton, Univ. of CT Health Center, Pulmonary Division, Farmington, CT USA

The effects of an induced ADA deficient state on thymocyte development in vitro has been studied in fetal thymus organ culture. Culturing day 16 fetal thymus for 5 days results in the development of normal cortical and medullary thymocyte populations. Thymuses were explanted at various stages of fetal life, day 16 (thymocytes are all large, immature subcapsular cortical thymocytes), day 18 (cortical thymocytes predominate - medullary thymocytes undetectable), and day 20 (both cortical and medullary thymocytes detectable in normal proportions), and incubated at the lowest concentration of inhibitor (both EHNA and DCF were run in separate studies) that produced >90% enzyme inhibition plus varying concentrations of AdR. Control cultures contained AdR or medium only. The results are summarized as follows:

a) Day 16 cultures - little increase in cell number and no evidence of cortical or medullary thymocyte development.

b) Day 18 cultures - a decrease in cell number and no medullary thymocyte development.

c) Day 20 cultures - a decrease in cell number in the cortical thymocyte population with medullary thymocytes unaffected.

The day 16 and 18 thymocytes that were incubated in an ADA deficient state had the characteristics of subcapsular thymocytes: they were $Thy1^+$, TdT^+ and $RT\text{-}1^+$ (histocompatibility antigen). Thus, an induced ADA deficient state prevented the differentiation of subcapsular thymocytes into "mature" cortical and medullary thymocytes.

(Supported by NIH grants HD-17061 and HD-00554)

9 THE EFFECTS OF ADA INHIBITION ON B CELL DIFFERENTIATION IN THE RAT. Randall W. Barton, University of Connecticut Health Center, Pulmonary Division, Farmington, CT USA

We have examined the stage or stages of rat B cell development in vivo which are preferentially affected by an induced ADA deficient state. In normal rats two populations of B cells (surface and lymph node - sIg^+, $Thy1^+$ and sIg^+, $Thy1^-$. Lymphocytes which bear both sIg and Thy1 antigen are among the least mature members of the B lymphocyte lineage in the rat. In normal 4- to 5-week-old rats given daily i.p. injections of the ADA inhibitor, DCF, plus deoxyadenosine there was an approximate 2-fold reduction in total B cells in spleen and lymph node and virtually all remaining B cells were $Thy1^+$; in saline- or deoxyadenosine-treated rats approximately 40-50% of B cells were $Thy1^+$. Similarly, in irradiated rats that were given daily injections to induce an ADA deficient state the regeneration of $Thy1^-$ B cells was blocked. Virtually identical results were obtained when EHNA was substituted for DCF and when adenosine was injected instead of deoxyadenosine. We have compared the ADA specific activities in purified populations of $Thy1^-$ and $Thy1^+$ B cells and found that $Thy1^-$ B cells had 2- to 3-fold higher ADA activity. Thus, in contrast to the T lymphocyte lineage in which immature thymocytes were preferentially affected, an induced ADA deficient state specifically affected mature B lymphocytes.

(Supported by NIH grants HD-17061 and HD-00554)

10 ENHANCEMENT OF MTX CYTOTOXICITY BY URACIL ANALOGUES THAT INHIBIT CELLULAR dUTPase ACTIVITY. William S. Beck, George E. Wright, Neil J. Nusbaum, and Eric M. Isselbacher. Harvard Medical School, Massachusetts General Hospital, Department of Medicine, Boston, Massachusetts 02114, USA.

Ample evidence indicates that the cytotoxicity of MTX depends on an increase in [dUTP]/[dTTP], uracil misincorporation into DNA, and uracil excision without adequate repair. The result is extensive DNA fragmentation and cell death. In a survey of many cell lines we found notable differences in their levels of dUTPase activity. When 7 cell lines selected for their wide differences in dUTPase content were assayed for (1) misincorporation of uracil due to MTX, (2) sensitivity to growth inhibition by MTX, and (3) effect of MTX on [dUTP]/[dTTP], the data suggested an apparent inverse correlation between dUTPase levels and sensitivity to MTX. The evidence that low dUTPase level may be associated with higher MTX sensitivity led to a search for dUTPase inhibitors that would enhance the cytotoxicity of MTX. Studies were performed on 44 uracil analogues (mainly 5- and 6-substituted derivatives). Many inhibited dUTPase in cells and sonicates at concentrations of 0.25-3.0 mM. A few were effective inhibitors at 0.1 mM. The most interesting compounds did enhance MTX cytotoxicity as measured by cell growth, uracil misincorporation, and alkaline elution of DNA fragments. No uracil analogue tested inhibited purified dUTPase but added ATP increased inhibition of dUTPase by analogues in sonicates. Thus active inhibitors are probably nucleotide derivatives of uracil analogues.

11 PRPP SYNTHETASE SUPERACTIVITY IN LYMPHOBLAST LINES. M.A. Becker, M.J. Losman, D. Rimon, and M. Kim. Univ. of Chicago, Dept. of Medicine, Chicago, IL, USA.

Superactivity of PRPP synthetase (PS) is an X chromosome-linked disorder associated with gout and uric acid overproduction. Fibroblasts from affected males show PS superactivity and increased rates of PRPP and purine nucleotide synthesis. Kinetic aberrations underlying PS superactivity include: catalytic defects; regulatory defects; substrate (ribose-5-P) binding defects; and combined catalytic and regulatory defects.

We studied PS in B lymphoblasts derived from blood lymphocytes (PBL) of 6 males whose fibroblasts express the 4 classes of PS superactivity. Neither excessive PS activity nor increased rates of PRPP or purine synthesis were found in lymphoblasts from 3 patients with catalytic defects in fibroblasts, despite PS superactivity in PBL from these patients. Catalytic superactivity was also absent in lymphoblasts from a patient with combined defects in fibroblast PS. These cells and cells from 2 other patients expressed the specific regulatory and substrate binding defects found in fibroblast PS from the respective patients and also showed increased rates of PRPP and purine synthesis.

Immunoreactive PS differed <20% among lymphoblast lines, and PS absolute specific activities were those of normal fibroblast PS. Thus, neither reduced levels of mutant PS nor activation of normal PS explained selective expression of PS superactivity in lymphoblasts. Altered electrophoretic mobilities and thermal stabilities, found with some catalytically superactive fibroblast PS, were absent in lymphoblasts from the respective patients. The basis of selective expression of PS superactivity remains to be determined.

12 INHERITED PRPP SYNTHETASE SUPERACTIVITY DUE TO ABERRANT INHIBITOR AND ACTIVATOR RESPONSIVENESS, M.A. Becker, M.J. Losman, and H.A. Simmonds, Univ. of Chicago, Chicago, IL USA, and Guy's Hospital, London, England.

Superactivity of PRPP synthetase (PS) is associated with purine nucleotide and uric acid overproduction, usually manifested by early adult-onset gout. We studied fibroblasts and lymphoblasts from a male child (NB) with inherited uric acid overproduction, deafness, and retarded motor development, suspected to have a superactive PS (Simmonds et al. 1982. Lancet 2:68-70). Our studies confirm a defective enzyme altered in regulation of PS activity in response to noncompetitive inhibitors and to the activator, Pi.

Fibroblasts and lymphoblasts from NB show PRPP concentrations and generation and rates of all PRPP-requiring purine synthetic pathways, including purine synthesis de novo, which are increased at least 2-fold with respect to normal. In dialyzed fibroblast and lymphoblasts extracts, NB PS shows hyperbolic Pi activation due to increased maximal reaction velocity which is found only at <2 mM Pi. In chromatographed or partially purified enzyme preparations, Km's of normal and NB PS for substrates and Mg^{2+} are comparable, but the Pi concentration at which PS is half activated is 0.12 mM for NB PS and 0.45 to 0.60 mM for normal PS. In addition, inhibitory constants ($I_{0.5}$) for the competitive inhibitors ADP and 2,3-DPG are comparable for normal and NB PS, while NB PS is, respectively, 5- and 6-fold less responsive than normal PS to the noncompetitive inhibitors GDP and MMPR monophosphate. The association of increased rates of intracellular PRPP and purine synthesis with specific defects in the regulation of PS activity suggest that these PS regulatory mechanisms are of physiological importance in the control of PRPP and purine production.