187 BIOCHEMICAL BASIS FOR DEOXYADENOSINE AND 2-CHLORODEOXYADENOSINE TOXICITY TO RESTING HUMAN LYMPHOCYTES. Shiro Seto, Carlos J. Carrera, D. Bruce Wasson, and Dennis A. Carson. Scripps Clinic and Research Foundation, Department of Basic and Clinical Research, La Jolla, California.

Deoxyadenosine (dAdo) is toxic at micromolar concentrations to adenosine deaminase inhibited resting human peripheral blood lymphocytes. 2-chlorodeoxyadenosine (CdA), a metabolically resistant dAdo congener, exhibits similar properties. Four hours of exposure to dAdo or CdA induced the accumulation of strand breaks in the DNA of normal resting lymphocytes, as measured by a DNA unwinding assay. The DNA damage was followed by consumption of NAD, probably mediated by increased poly(ADP-ribose) synthesis. The addition of 1-5mM nicotinamide prevented the dAdo and CdA triggered fall in NAD levels, and rendered the resting lymphocytes resistant to the toxic effects of both compounds. Both dAdo and CdA inhibited the repair of radiation induced DNA damage in resting lymphocytes, by impeding DNA polymerization. CdA was 100 fold more potent than dAdo. These results suggest that (i) a slow rate of DNA polymerization is required to maintain DNA integrity in resting human lymphocytes, (ii) dAdo and CdA inhibit polymerization, and cause DNA strand breaks to accumulate, (iii) the strand breakage triggers poly(ADP-ribose) synthesis, and causes lethal NAD depletion.

188 CHARACTERIZATION OF ARABINOSYLGUANINE (AraG) RESISTANCE IN A LYMPHOBLASTOID CELL LINE. Donna S. Shewach and Beverly S. Mitchell, University of Michigan, Department of Internal Medicine, Ann Arbor, MI

AraG is a deoxyguanosine (dGuo) analog which exhibits selective cytotoxicity for T relative to B lymphoblasts. This drug is metabolized intracellularly to the 5'-triphosphate, araGTP, which is associated with the inhibition of DNA synthesis. In order to investigate the enzymes responsible for the selective araG metabolism, we have isolated an araG-resistant clonal cell line (designated line 24B3) from MOLT-4 T lymphoblasts which exhibits a 600-fold and 36-fold increase in IC_{50} values for araG and dGuo, respectively. In contrast, this cell line is only 3- to 4-fold less sensitive to arabinosylcytosine (araC) relative to unselected MOLT-4 cells. Following a 4 hr incubation with araG, dGuo and araC, the intracellular accumulation of the active metabolites araGTP and dGTP is decreased 8- and 9-fold, respectively, compared to only a 3-fold reduction in araCTP accumulation in the resistant cells. Similar decreases in nucleotide accumulation are observed in cell-free lysates of the 24B3 cell line. The degradation of araG is the same in both sensitive and resistant cell lines. In addition, the HL-60 promyelocytic leukemia cell line is 300-fold less sensitive to araG than are T lymphoblasts, and the ability of the promyelocytes to accumulate araGTP is similar to that of the 24B3 cell line. These data suggest that sensitivity to araG is mediated through a cell-specific kinase which is more selective for dGuo analogs than for a deoxycytidine analog such as araC.

189 OROTIC ACIDURIA ASSOCIATED WITH THE FORMIMINOTRANSFERASE/CYCLODEAMINASE DEFICIENCY Yoon S. Shin, S. Reiter*, O. Zelger, I. Brünstler and A. v.Rücker. Children's Hospital and Poliklinik*, University of Munich, F.R.Germany.

A 12 year-old girl with a long history of megaloblastic anemia, hypotonia and mental retardation was found to have a mild elevation of homocystine, orotic acid and orotidine and a marked increase in formiminoglutamic acid (FIGLU) in urine and plasma. Serum concentration of folate and vitamin B-12 was normal, and the activity of orotate phosphoribosyltransferase, thymidylate synthetase, methylene tetrahydrofolate reductase and methionine synthetase in fibroblasts was in the normal range. Upon histidine loading the urinary excretion of FIGLU was app. 100 above the normal range and decreased sharply by leucovorin administration. The result of assay of fromiminotransferase/cyclodeaminase showed a 30-50 % of normal range (70-150 nmol/min/g Hb, n=15), which is comparable with the cases of the deficiency described by Arakawa's group. An interesting observation, however, is the increased level of orotic acid in this patient. Although mild the consistently elevated urinary ortate, possibly due to an increased synthesis rather than a metabolic block leads us to a speculation that the highly reactive formimino group may be a precursor of orotic acid.

190 Z-NUCLEOTIDE ACCUMULATION IN ERYTHROCYTES FROM LESCH-NYHAN PATIENTS. Yechezkel Sidi & Beverly S. Mitchell. University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor, MI.

5-Amino-4-imidazolecarboxamide riboside (Z-riboside) is an intermediate in the purine de novo synthetic pathway which may be metabolized either to IMP in a folate-dependent reaction or to the corresponding Z-nucleotides. Accumulation of ZTP in microorganisms has been associated with depletion of folate intermediates and has been postulated to play a regulatory role in cellular metabolism. We have demonstrated the presence of Z-nucleotides in erythrocytes derived from 5 individuals with the Lesch-Nyhan syndrome. Erythrocyte folate levels were within the normal range in these individuals, although GTP and GDP levels were significantly reduced below those in normal control ($p<0.001$). Lesser amounts of Z-nucleotides were found in 3 individuals with partial deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase and in 2 individuals with other disorders of purine overproduction. In contrast, no Z-nucleotides were detected in 13 normal controls or in 3 individuals with gout on allopurinol therapy. We conclude that Z-nucleotide formation may result from markedly increased rates of de novo purine biosynthesis. Whether or not the further metabolism of purine intermediates plays any role in the pathogenesis of the Lesch-Nyhan syndrome remains unknown.

191 TRANSITIONAL URATE CRYSTALS: A FACTOR IN GOUTY ARTHRITIS? Peter A. Simkin and Richard S. Benedict. Dept. of Med., U. of Wa., Seattle, WA, USA.

Apparent crystal-negative effusions are sometimes seen in the early course of acute gouty arthritis. Also, newly-prepared urate crystals become less phlogistic after heating in vitro. A plausible explanation for these phenomena lies in the tendency for some solutes to precipitate first as unstable hydrated crystals which then evolve into more stable but less hydrated forms. Polyhydrated urate salts could be intensely phlogistic yet non-birefringent in polarized light. We examined this hypothesis after finding only needle-shaped, non-birefringent, intracellular objects up to 15 microns long in a gouty synovial fluid.

A supersaturated urate solution (500 mg/dl) was prepared in 0.1 N NaOH at 90°, allowed to cool, neutralized to pH 7.2 with HCl, and incubated at 37°. Serial observations and photographs of this solution revealed a mixed population of crystals including rod-like structures which appeared after 60 minutes and became more birefringent over time. This transition sometimes occurred uniformly, but more often progressed from individual foci within the crystal. Heating for 2 hours at 190° converted all crystals to a strongly birefringent form.

For 25 years, monosodium urate monohydrate has been accepted as "the gout culprit." It now seems possible, however, that one or more polyhydrated, transitional form(s) of urate may also participate in the acute inflammatory process. Further, such crystals may be missed by polarized light microscopy.

192 CORRELATIONS BETWEEN PURINE LEVELS,CLINICAL AND IMMUNOLOGICAL STATUS IN ADA DEFICIENCY. H. Anne Simmonds, Lynette D Fairbanks, George S. Morris, Diane R. Webster, Gareth Morgan,Roland J. Levinsky. Guy's Hospital & Institute of Child Health,London,United Kingdom.

Nine cases of adenosine deaminase (ADA) deficiency have been found amongst fifty infants investigated for immunodeficiency. Prenatal diagnosis identified a further two affected foetuses in six pregnancies at risk. Absence of ADA activity and high dATP levels in foetal blood,with low lymphoid cell numbers and few T-cells, indicated early intrauterine onset.

High dATP levels, accompanied by varying degrees of ATP depletion were also found in the red cells of the nine infants. Two late presenters had normal ATP levels, normal lymphocyte numbers and immunoglobulin levels, with some detectable ADA in mononuclear cells (PBMs). dATP was found in PBMs in only one case where heparinised blood contaminated with platelets was used. The novel finding in the two latest cases was the presence of ATP depletion accompanied by dATP accumulation in the platelets but not PBMs from defibrinated blood. This explains the earlier results and questions the possible significance for the immune response.

Deoxyadenosine was found in the urine in all cases.S-adenosylhomocysteine was not; adenosine was identified in some. The results suggest that the degree of ATP depletion may reflect the clinical status most closely,but that any therapeutic approaches are likely to be ineffective because of the early toxicity to T-lymphocyte precursors.