41 COMPARATIVE STUDY OF THYMINE AND URACIL METABOLISM IN HEALTHY PERSONS AND IN A PATIENT WITH DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY.

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Metabolism of thymine(T) and uracil(U) was investigated in 6 healthy volunteers(HV), and in a girl CW with dihydropyrimidine dehydrogenase(DHPD) deficiency and her mother JW and halfbrother HW. Three HV were loaded with T, the other three with U. CW, JW and HW were loaded with both. Dosis: 1 mmol/kg b.w. orally.
Plasma T on loading did not exeed 1.0 mM in the HV, but in CW and JW much higher values were found. In HW the concentrations were slightly above the highest values seen in the HV. The levels of 5-OH methyluracil(5-HMU) 2 hrs after loading ranged from 4-12 µM in the HV, from 21-34 µM in CW, JW and HW. Only in the HV and in HW significant and increasing amounts of plasma β-aminoisobutyric acid(β-AIB) were seen. Plasma U reached max.values from 0.87-1.0 mM. β-Alanine(β-Ala) was undectable in all plasma samples.
After loading the excretion(mmol/g creat/12hr) of T in HV, JW and HW was in the same range (5.2-17), but in CW it was much higher (40.2). 5-HMU was excreted in all cases (0.6-2.9); in CW 2.5 mmol/g creat/12hr). On loading the excretion (mmol/g creat/12 hr) of β-AIB was very high in the HV (18.9-21.3), intermediate in JW and HW (8.7 resp. 7.2) and low in CW (0.7). On loading with U, the excretion (mmol/g creat/12hr) of U was below 34.0 in the HV, JW and HW, but in CW it was 84.5. β-Ala was low in all urine samples (<0.08). These results show that T or U loading can be used for the diagnosis of DHPD-deficiency. Loading with T seems to be more discriminative than U in detecting heterozygotes. Moreover, β-AIB gave valuable additional information about the (residual) capacity of T-catabolism. 5-HMU and β-Ala provided no diagnostic information.

42 CHEMILUMINESCENT ASSAYS IN THE STUDY OF PURINE METABOLISM

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The measurement of purine nucleosides and bases in biological fluids is a particularly challenging problem because their concentrations are extremely low and very limited amount of the specimen is often available (e.g. paediatric specimens). Abnormal levels of these compounds are associated with inborn errors of purine metabolism, neoplastic diseases and conditions leading to a net ATP degradation. Xanthine oxidase-initiated isoluminol chemiluminescence, in the absence of peroxidase, has been employed in our laboratories for the assay of hypoxanthine/xanthine, guanine, guanosine, inosine and adenosine. Any purines lower in the catabolic sequence than that to be assayed are removed by a preincubation in the presence of appropriate enzymes. The assayed purine is converted to xanthine and/or hypoxanthine. Isoluminol chemiluminescence elicited by xanthine oxidase/hypoxanthine-xanthine system, is enhanced by adding p-iodophenol to the reaction mixture. The assay is rapid, specific, sensitive (1 nmol/liter) and suitable for the assay of purines in deproteinized biological fluids, provided that uric acid is removed by preincubation with uricase and that EDTA is added to the reaction mixture. Internal standardization is required. A very sensitive luminometric assay for the determination of xanthine oxidase activity in biological samples is also described.

43 HYPERURICEMIA, GOUT AND IDIOPATHIC ASEPTIC NECROSIS OF BONE

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Among 6997 patients admitted to the Institute of Rheumatology of the University of Rome 33 had aseptic necrosis of bone. One patient had polycytemia. In the remaining 32 patients no etiology could be established. Associated rheumatic diseases in the idiopathic group are shown in the table.

ASSOCIATED RHEUMATIC DISEASE	N. OF PATIENTS	N. OF PATIENTS WITH ASEPTIC NECROSIS
Rheumatoid arthritis	2047	10 (0.49%)
Connective tissue diseases	401	1 (0.25%)
Primary gout	358	4 (1.12%)
Osteoarthritis	1598	9 (0.56%)
None	8	8

The male:female ratio was 12:20 with a mean age of 50.2 years (range 10-82). Eight patients (6 with rheumatoid arthritis, 1 with progressive systemic sclerosis and 1 with asthma) received corticosteroids more or less regularly for at least 1 year before aseptic necrosis was diagnosed. One patient had diabetes mellitus, 6 had significant hypertension and 5 admitted excessive alcohol intake. Serum urate concentration has been determined in 27 of the 32 patients. Hyperuricemia was present in 4 of 19 women and in the four male gouty subjects. Affected bones in the gouty patients were the femoral head (2 patients), the II metatarsal and the lunate. In Kienbock's disease marked osteoporosis of the wrist was observed simulating a monoarthritis of different etiology.

44 CHARACTERIZATION OF GENOMIC DNA, mRNA AND ENZYME PROTEIN IN CASES OF HPRT-DEFICIENCY.

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Studies are being undertaken to define the molecular basis of the enzyme abnormality in a number of Australian patients with HPRT-deficiency. These studies include quantitation of HPRT enzyme protein by immunological techniques, as well as Southern and Northern blotting analysis of genomic DNA and RNA isolated from lymphocyte cultures. Cross-reacting material to normal HPRT antibody was detected in erythrocyte and lymphoblast cells in all but one patient with partial HPRT-deficiency.

Digestion of DNA with a number of restriction enzymes demonstrated a normal-sized HPRT gene in each patient. Normal HPRT-message was demonstrated in four patients with partial HPRT-deficiency. However two Lesch-Nyhan patients had abnormal message; one had reduced levels of HPRT-mRNA and the other a complete absence of HPRT-mRNA. These studies were aimed at selecting those patients with possible point mutations in the HPRT-coding region. Sequencing of HPRT-cDNA is proceeding in these patients.

45 RENAL EXCRETION OF PURINE BASES - EFFECTS OF PROBENECID, BENZBROMARONE AND PYRAZINAMIDE -

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Experiments were conducted which revealed the renal transport mechanism(s) of oxypurines by employing uricosuric agents and pyrazinamide. All specimens being drawn from healthy, normal subjects, the data showed that probenecid increased fractional uric acid, fractional hypoxanthine and fractional xanthine clearance, 3.97-, 1.52- and 2.31-fold, respectively, while benzbromarone increased fractional uric acid clearance 2.11-fold, decreased fractional xanthine clearance 0.48-fold and had no effect on fractional hypoxanthine clearance. In addition, pyrazinamide decreased both fractional uric acid clearance 0.35-fold and fractional xanthine clearance 0.44-fold but increased fractional hypoxanthine clearance 1.49-fold. These results suggest that the possibility of differing renal transport mechanisms among the purine bases, uric acid, hypoxanthine and xanthine.

46 DETECTION OF HPRT MUTATION IN A HUMAN STEM CELL BEFORE DIFFERENTIATION INTO T OR B CELLS

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We have been measuring the frequency of in vivo HPRT mutant T cells in the peripheral blood of atomic bomb survivors in Hiroshima. The mean frequency of mutant T cells in 30 survivors (5.2×10^{-6}) was significantly higher than that in 17 controls (3.4×10^{-6}) and was found to correlate with the radiation dose estimates. In the course of this study, extremely high mutant frequency (2×10^{-4}) was identified in one male survivor (59 y.o. radiation dose=199 rad). All the mutant colonies examined had the same chromosome aberration (20 q-) and the same alteration of DNA at the HPRT locus, indicating that all these mutants were derived from a single cell. However, the pattern of T cell receptor gene rearrangement was different between colonies. Furthermore, mutant B cells possessing the same aberration and HPRT gene alteration have also been cloned from this individual by using EB virus transformation. All these results indicate that all the mutant cells were derived from a single stem cell which can differentiate into at least T and B cells. Thus, a single mutational event in an undifferentiated stem cell produced a large number of mutant lymphocytes in periphery in the person studied here.