71 FAMILY STUDY OF HEREDITARY XANTHINURIA — DECREASED DUODENAL XANTHINE OXIDASE ACTIVITY AND INCREASED URINARY EXCRETION OF XANTHINE AND HYPOXANTHINE IN HETEROZYGOTES

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We studied two brothers with hereditary xanthinuria (xanthine oxidase deficiency) and their family members. The two brothers had extremely low concentrations of urate but markedly high concentrations of xanthine and hypoxanthine in plasma and urine. Xanthine oxidase activities were virtually absent in the duodenal mucosa. In their parents (presumed obligate heterozygotes), the activities of xanthine oxidase were about half that of normal subjects. Although plasma xanthine and hypoxanthine concentrations of the parents were normal, urinary xanthine and hypoxanthine excretions were significantly higher than those of normal subjects (xanthine, father 17.1 mg/g creatinine and mother 27.4 vs. normal controls 5.7 to 11.0; hypoxanthine, father 14.0 and mother 27.3 vs. controls 4.0 to 8.4). Similar changes in the metabolite concentrations were seen in at least 6 other relatives, suggesting they were heterozygotes. This study indicates that the presumed obligate heterozygotes of xanthine oxidase deficiency retained about half normal enzyme activities causing the partial metabolic blockage in vivo at this enzyme step.

72 RELATIONSHIP BETWEEN INTRAELLULAR dCTP/ARA-CTP RATIO AND CYTOTOXIC EFFECT OF ARA-C

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The dCTP/ara-CTP ratio seem to be one of very important factors in ara-C cytotoxicity. In confirmation of this hypothesis, we studied relations between cell growth and ratio of dCTP/ara-CTP in R.1.1. mice splenic cells, CCRF-CEM human T cell leukemic cells and ML-1 human myelogenous leukemic cells. (Method) Intracellular acid-soluble nucleotide were extracted with TCA, then neutralized with Freon and Amine. After periodate oxidation, deoxy-ribonucleotide were obtained. Intracellular ara-CTP and deoxy-ribonucleotide pools were analyzed by the HPLC method. (Result and Discussion) Growth of R.1.1. cells was inhibitted at concentration up to 1uM of ara-C. Intra-cellular dCTP and ara-CTP level in the R.1.1. cells exposure to 1uM of ara-C were 56.0 and 48.0pmol/10^6cells, respectively, whereas exposure to 10uM of ara-C, intra-cellular dCTP and ara-CTP level were 44.0, 351.0pmol/ 10^6cells, respectively. At the higher level of ara-CTP than of dCTP, ara-C significantly effected on the growth of R.1.1. cells. Same relation was shown in CCRF-CEM cells and ML-1 cells. These data suggested that ara-C had significant effects on the growth of cells at the higher level of ara-CTP than of dCTP. So the dCTP/ara-CTP ratio is one of very important factors in ara-C cytotoxicity.

73 IS HPRT-LIKE PROTEIN PRESENT IN LESCH-NYHAN PATIENTS?

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A complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity results in the Lesch-Nyhan syndrome. Of the 15 Lesch-Nyhan patients studied by Wilson et al. (1), HPRT cross-reacting material (CRM) was detected in lymphoblasts from only four (1.3%, 50%, 72% and 92% of control concentrations) whereas 12 had HPRT-specific mRNA. None of the three patients lacking mRNA had detectable CRM. We have studied CRM levels in haemolysates and lymphoblast lysates from two Lesch-Nyhan patients (R.W. and J.G). In the first series of experiments, normal CRM levels were measured and lysates from both patients gave strong precipitation lines in immunodiffusion analysis. In the second series, using a different HPRT preparation in the radioimmunoassay (RIA), very low CRM levels (0.5% and 0.1%) were obtained, although strong precipitation lines were still observed on Ouchterlony plates.

A possible explanation for these discrepancies lies in a change in structure of normal HPRT which we have observed on storage, leading to a reduction in binding affinity for the antibody. The enzyme used in the first series of CRM determinations had been stored for an extended period, whereas enzyme used in the second series was freshly prepared. This explanation implies that the protein present in Lesch-Nyhan patients binds to the antibody with a lower affinity than does the normal enzyme (see also ref.2). These studies suggest that HPRT-like protein may be present in a higher percentage of Lesch-Nyhan patients than previously thought and may be present even when HPRT-specific mRNA cannot be detected, as in patient R.W.

1. Wilson, J.M., Stout, J.T., Palella, T.D., Davidson, B.L., Kelley, W.N. and Caskey, C.T. (1986) J.Clin.Invest. 77:188-195.
2. Wilson, J.M., Baugher, B.W., Landa, L. and Kelley, W.N. (1981) J.Biol.Chem. 256:10306-10312.

74 RAT LIVER PHOSPHORIBOSYLPYROPHOSPHATE SYNTHETASE: EXISTENCE AS HETEROGENEOUS AGGREGATES AND IDENTIFICATION OF CATALYTIC SUBUNIT.

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Phosphoribosylpyrophosphate (PRPP), an important substrate in the synthesis of purine, pyrimidine and pyridine nucleotides, is synthesized by the catalysis of PRPP synthetase. The enzyme from human erythrocytes exists as various associated forms and is subject to complex regulation. However, the in situ regulation of PRPP synthesis is poorly understood. As an approach to the problem, we purified rat liver PRPP synthetase and partially characterized the complex physical nature of the enzyme. It was purified to a specific activity of 7.28 μmol/min/mg, the highest value so far reported for a mammalian enzyme, but still contained several components of 34 kDa, 38 kDa and 40 kDa in relative amounts of that order. In various usual chromatographies, these components were coeluted, suggesting that the heterogeneous aggregate is the native form of the enzyme. To identify the catalytic subunit, the enzyme was subjected to gel filtration on TSK G2000SW in 1 M $MgCl_2$, an agent to dissociate proteins. The enzyme appeared as a 68 kDa peak and the last quarter of the fractions contained only the 34 kDa species, with a definite catalytic activity. Other components in the aggregate appear to exert suppressive effects on the activity. Sequence analyses of N-terminal region and two tryptic peptides of the 34 kDa subunit, together with our cDNA cloning experiments (J.Biol.Chem., 262, 14867, 1987), revealed that the 34 kDa component consists of two homologous isoforms (PRS I and II).

75 EXERCISE-INDUCED ALTERATION OF ERYTHROCYTE GLYCOLYSIS ASSOCIATED WITH MYOGENIC HYPERURICEMIA

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Myogenic hyperuricemia is caused by increased production of uric acid secondary to enhanced release of inosine and hypoxanthine from exercising muscles into blood. This novel mechanism is evident in glycogenosis types VII (muscle phosphofructokinase deficiency), III (debranching enzyme deficiency), and V. In this study, we examined the effect of muscular exercise on the glycolysis of circulating erythrocytes in glycogenosis types VII and III. The concentration of erythrocyte 2,3-bisphosphoglycerate, which had decreased because of genetic partial deficiency of erythrocyte phosphofructokinase, was further decreased after prolonged bed rest in patients with type VII. Ergometric exercise rapidly increased fructose-1,6-P_2, dihydroxyacetone-P plus glyceraldehyde-3-P, and 2,3-bisphosphoglycerate in circulating erythrocytes. Similar changes were observed after exercise in patients with type III. The exercise-induced metabolic alteration of erythrocytes was reproduced in vitro by incubating normal erythrocytes in the presence of inosine. We conclude that physical activity affects glycolysis in erythrocytes in glycogenosis types VII and III, and that myogenic factors including inosine are responsible for this change.

76 ISOLATION AND CHARACTERIZATION OF HELA CELL MUTANTS RESISTANT TO AN ADENINE ANALOG 4-CARBAMOYLIMIDAZOLIUM-5'-OLATE (CIO).

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It is known that CIO is metabolized into bredinin-5'-monophosphate (Brd-MP) by adenine phosphoribosyltransferase (APRT) and that this metabolite is a specific inhibitor of IMP dehydrogenase. We have reported that mouse cell mutants resistant to the cytotoxicity of CIO have lost the enzyme activity (Cancer Res., 42, 4210, 1982). Recently, we isolated the same type of resistant mutants from mutagenized HeLa cells. In contrast, the HeLa cell mutants had a normal level of APRT and were cross-resistant to bredinin, a ribosylated form of CIO, but not to mycophenolic acid and virazole. These mutants showed a normal level of IMP dehydrogenase and the sensitivity to Brd-MP of the parental and mutant cell enzymes were similar. The mutants gradually reduced its resistance after passage without the drug for more than 2 months. We also found that in 10^5 g supernatants prepared from the mutants there existed a 28 k protein by SDS polyacrylamide gel electrophoresis and its amount was proportional to the degree of the resistance. These results suggest that this protein should be involved in the resistance observed in the HeLa cell mutants.