XANTHINE PHOSPHORIBOSYLTRANSFERASE IN MAN: RELATIONSHIP TO HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE.

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Since the original identification of enzymes capable of catalysing the formation of purine nucleotides directly from their respective purine bases in the presence of 5-phosphoribosyl-1-pyrophosphate (PRPP)(Korn, et al, 1955; Kornberg, Lieberman and Sims, 1955), these pathways have been shown to be of major importance to the economy of the organism (Seegmiller, Rosenbloom and Kelley, 1967; Kelley, et al, 1967). In bacteria four distinct purine phosphoribosyltransferase enzymes have been identified for the natural purine bases, adenine, hypoxanthine, guanine, and xanthine, by protein fractionation procedures (Flaks, 1963) and by analysis of bacterial mutants (Kalle, Gots and Abramson, 1960). Several apparent differences exist in mammalian cells. A single enzyme converts hypoxanthine and guanine to their respective ribonucleotides (Brockman, 1965; Kelley, et al, 1967). In addition, an enzyme converting xanthine directly to xanthylic acid has not been previously demonstrated in a mammalian system (Brockman, 1965). In the present study xanthine phosphoribosyl transferase (XPRT) activity has been demonstrated in human erythrocytes. The thermal stability of this enzyme as well as its deficiency in human mutants lacking hypoxanthine-guanine phosphoribosyltransferase (HGPRT) suggests that in man HGPRT and XPRT activity reside in the same enzymatic protein.

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METHODS. HGPRT (EC 2.4.2.8) and adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7) activities were assayed in dialyzed human erythrocyte hemolysates using a radiochemical method as previously described (Kelley, et al, 1967). XPRT was assayed in a similar manner except for the use of 0.5mM xanthine-8-C^{14} (1.73 mc/mM) as the purine base and 4 to 6 mg of hemolysate protein. After incubation for 3 hours at 38°C, the reactions were terminated by the addition of EDTA and rapid freezing. Twenty µl of the reaction mixtures was placed on 3 MM Whatman paper with freshly prepared xanthylic acid as carrier and the reaction products were separated from the purine substrate by high voltage electrophoresis in 0.05 M borate buffer pH 9.0 with 0.001 M EDTA at 4,000 volts for 30 minutes. Xanthylic acid was located by inspection of the paper under ultraviolet light and the appropriate area was cut out and counted in a liquid scintillation counting system at 60 percent efficiency. By this procedure xanthylic acid could be separated from guanylic, inosinic, and adenylic acids, xanthosine, guanosine, inosine, adenosine, guanine, xanthine, hypoxanthine, and uric acid. Less than 0.01% of the radioactivity present as XMP could be attributed to the conversion of IMP or GMP to XMP. Erythrocytes used as a source of the enzyme were prepared as previously described (Kelley, et al, 1967). Protein concentration was estimated by the method of Lowry, et al (1951).

RESULTS AND DISCUSSION. Pomales, Elion and Hitchings (1965) were able to demonstrate the incorporation of isotopic xanthine into nucleic acid purines in mice when the catabolism of this base was retarded by pretreatment with 4-hydroxypyrazolo-(3,4-d)-pyrimidine, a potent inhibitor of xanthine oxidase (Elion, et al, 1963). Lowy, Williams and London (1961) and Cook and Vibert (1966) have shown that xanthine is incorporated into GTP and ATP in rabbit reticulocytes and mature erythrocytes. Cook and Vibert have also demonstrated a similar conversion of xanthine to GTP and IMP in human erythrocytes. Although these studies provide evidence that xanthine can be utilized as a precursor for nucleotide formation in the mammalian

cell, they do not indicate whether the nucleotide is formed directly from xanthine by a purine phosphoribosyltransferase or indirectly with the intermediate formation of xanthosine by the sequential actions of a purine nucleoside phosphorylase and a kinase.

TABLE I

Phosphoribosyltransferase Activity

mμmoles/mg prot/hr

Subject	Xanthine	Hypoxanthine	Guanine	Adenine
Normal (32)*		103±18	103±22	31.2±6.0
W. K.	0.313	87	98	35.2
J.S.	<0.006	<0.02	<0.004	71.0
F.H.	<0.006	<0.02	<0.004	94.3
E.H.	0.339	88	92	6.6

* Number of subjects.

Dialysed human erythrocyte hemolysate (WK) which had normal phosphoribosyltransferase activity with hypoxanthine, guanine or adenine as substrate also catalyzed for the formation of xanthylic acid from xanthine (Table 1). This reaction required the presence of PRPP (complete = 1200 cpm; complete - PRPP = 97 cpm) and occurred in the absence of added ATP or ribose-1-phosphate indicating that the nucleotide was formed directly from the free base by the xanthine phosphoribosyltransferase reaction. Although this activity was quite low when compared to other purine phosphoribosyltransferases present in the erythrocyte, the level of activity is comparable to that observed for a pyrimidine phosphoribosyltransferase, orotic acid pyrophosphorylase, also present in human erythrocytes (Smith, Huguley and Bain, 1966)

XPRT activity has been assessed in hemolysates obtained from human mutants deficient in APRT and HGPRT (Table I). Subject E.H. had been previously shown to have a partial deficiency of APRT (Kelley, et al, in preparation). XPRT activity of the dialysed hemolysate obtained from this

subject was the same as the normal (W.K.) despite a reduction in APRT activity to 20% of normal (Table I). This suggests that the enzyme catalyzing the formation of xanthylic acid is separate from that responsible for the formation of adenylic acid. Dialysed hemolysates obtained from two unrelated subjects (J.S. and F.H.) with a complete deficiency of an apparently different purine phosphoribosyltransferase, HGPRT (Seegmiller, Rosenbloom and Kelley, 1967), however, have no detectable XPRT activity (<2% of normal). This

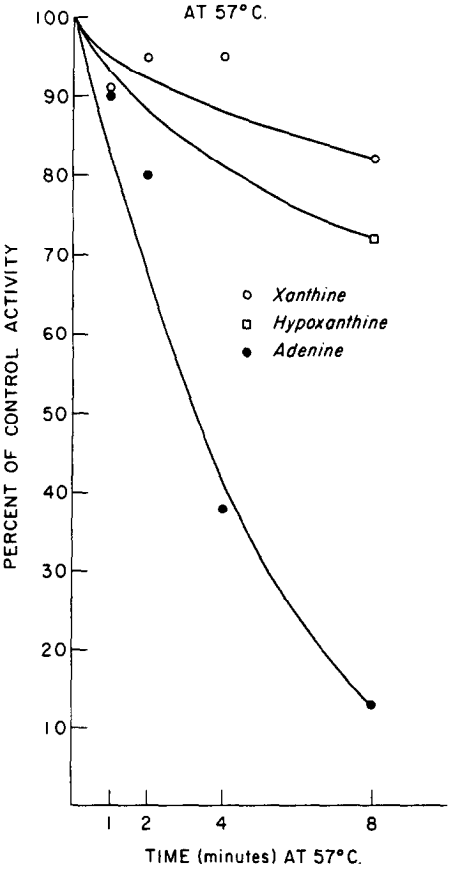

Figure 1a - Inactivation of phosphoribosyltransferase at 57°C with xanthine, hypoxanthine or adenine as the purine substrate. A dialysed hemolysate (W.K.) was diluted with 4 volumes of 0.01 M Tris buffer pH 7.4 and heated at 57°C for the times indicated. After cooling, the hemolysate was diluted ten fold with 0.01 M Tris buffer pH 7.4 for assay of hypoxanthine and adenine phosphoribosyltransferase activity while xanthine phosphoribosyltransferase activity was assayed without further dilution.

finding suggests that 1) the purine bases, xanthine, hypoxanthine and guanine, are converted to their respective nucleotides by the same enzyme in man or 2) that there has been a single mutation in these subjects leading to a deficiency of three separate enzymes. The latter possibility becomes tenable only if one postulates that a single protein component or subunit is common to each of these three enzymes or that three genetic sites are inactivated or repressed simultaneously although there is no data available at this time to support these hypotheses.

The possibility that a single enzyme is responsible for the conversion

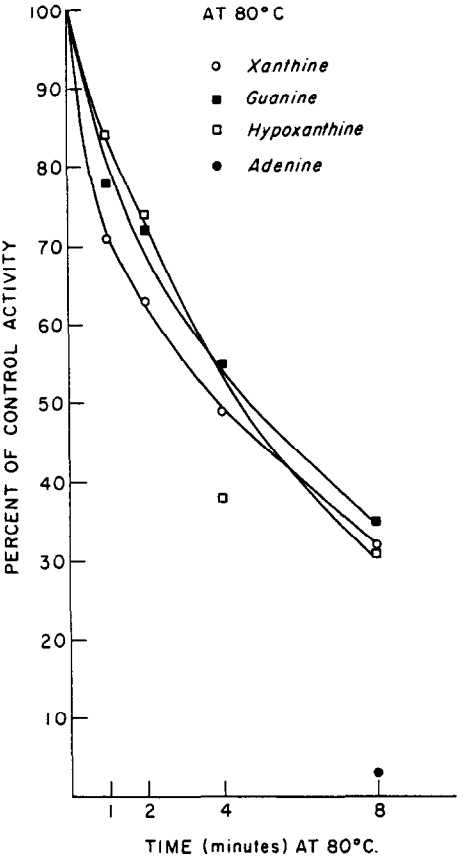

Figure 1b - Inactivation of phosphoribosyltransferase at 80°C with xanthine, hypoxanthine, guanine or adenine as the purine substrate. The dialysed hemolysate (W.K.) was treated in a manner similar to that noted in Figure 1a. The hemolysate was diluted ten fold after heating for assay of guanine phosphoribosyltransferase.

of xanthine, hypoxanthine and guanine to their nucleotides and that a separate enzyme catalyzes the formation of adenylic acid from adenine receives support from studies of the heat stability of these enzymes. As noted in Figure 1a, APRT is rapidly inactivated by heating at 57°C whereas there is little loss of activity for xanthine or hypoxanthine under these conditions. At 80°C (Figure 1b) the loss of activity for xanthine, hypoxanthine, and guanine is virtually the same and the rate of denaturation approximates that seen for adenine at the lower temperature.

These studies therefore demonstrate that an enzyme capable of catalyzing the formation of xanthylic acid directly from xanthine is present in mammalian tissue. Genetic evidence suggests that a single enzyme is responsible for the xanthine, hypoxanthine and guanine phosphoribosyltransferase activity observed. Studies of heat inactivation support this contention. A deficiency of this enzyme in the human is associated with an accelerated rate of purine biosynthesis de novo and the excessive production of uric acid, the ultimate end product of this pathway (Seegmiller, Rosenbloom and Kelley, 1967; Kelley, et al, 1967).

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