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Altered Purine and Pyrimidine Metabolism in Erythrocytes with Purine Nucleoside Phosphorylase Deficiency

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Purine and pyrimidine metabolism was compared in erythrocytes from three patients from two families with purine nucleoside phosphorylase deficiency and T-cell immunodeficiency, one heterozygote subject for this enzyme deficiency, one patient with a complete deficiency of hypoxanthine-guanine phosphoribosyltransferase, and two normal subjects. The erythrocytes from the heterozygote subject were indistinguishable from the normal erythrocytes. The purine nucleoside phosphorylase deficient erythrocytes had a block in the conversion of inosine to hypoxanthine. The erythrocytes with 0.07% of normal purine nucleoside phosphorylase activity resembled erythrocytes with hypoxanthine-guanine phosphoribosyltransferase deficiency by having an elevated intracellular concentration of PP-ribose-P, increased synthesis of PP-ribose-P, and an elevated rate of carbon dioxide release from orotic acid during its conversion to UMP. Two hypotheses to account for the associated immunodeficiency—that the enzyme deficiency leads to a block of PP-ribose-P synthesis or inhibition of pyrimidine synthesis—could not be supported by observations in erythrocytes from both enzyme-deficient families.

Key Words: purine nucleotide degradation; PP-ribose-P; deoxynucleosides; orotic acid; hypoxanthine-guaninine phosphoribosyltransferase.

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INTRODUCTION

A multiplicity of phenotypic expression is associated with inborn errors of purine metabolism. Profound central nervous system dysfunction, hyperuricemia, renal calculi, and gout accompany the complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (Lesch and Nyhan, 1964; Kelley *et al.*, 1967; Rosenbloom *et al.*, 1967; Kelley and Wyngaarden, 1972). Disorders of immune function are associated with a deficiency of adenosine deaminase (Giblett *et al.*, 1972; Meuwissen *et al.*, 1975) or purine nucleoside phosphorylase (Giblett *et al.*, 1975; Cohen *et al.*, 1976; Hamet *et al.*, 1977; Siegenbeek van Heukelom *et al.*, 1977; Stoop *et al.*, 1977; Edwards *et al.*, 1978; Gelfand *et al.*, 1978*a*; Rich *et al.*, 1979).

The absence of purine nucleoside phosphorylase causes biochemical features similar to the deficiency of hypoxanthine-guanine phosphoribosyltransferase. Both enzyme deficiencies are associated with purine overproduction (Lesch and Nyhan, 1964; Kelley *et al.*, 1967; Rosenbloom *et al.*, 1967; Kelley and Wyngaarden, 1972; Cohen *et al.*, 1976; Siegenbeek van Heukelom *et al.*, 1977; Edwards *et al.*, 1978) and both are characterized by elevated concentrations of erythrocyte PP-ribose-P (Fox and Kelley, 1971*b*; Cohen *et al.*, 1976; Siegenbeek van Heukelom *et al.*, 1977). These similar features may result from the two enzymes occurring in series in the purine catabolic pathway (Fig. 1).

Our recent studies of fresh erythrocytes from two families with purine necleoside phosphorylase deficiency and one patient with complete hypoxanthine-guanine phosphoribosyltransferase deficiency have afforded the opportunity to compare the biochemical changes of these two enzyme deficiencies. We have asked specific questions with reference to purine nucleoside phos-

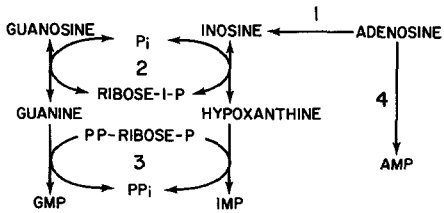

Fig. 1. Relationship of purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase in human erythrocytes. Purine nucleoside phosphorylase (reaction 2) and hypoxanthine-guanine phosphoribosyltransferase (reaction 3) represent sequential steps of nucleoside metabolism in human erythrocytes. The products of reaction 2 are the substrates for reaction 3. Adenosine may be either deaminated to inosine by adenosine deaminase (reaction 1) or phosphorylated to AMP by adenosine kinase (reaction 4). Deoxyadenosine, deoxyinosine, and deoxyguanosine are degraded to hypoxanthine and guanine by the same reactions as their ribonucleoside derivatives (Fox, 1978; Fox and Kelley, 1978).

phorylase deficiency: (1) Is disordered erythrocyte metabolism similar in purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase deficiencies? (2) Is there evidence for biochemical abnormalities in erythrocytes from a heterozygote for purine nucleoside phosphorylase deficiency? (3) Is there evidence for decreased PP-ribose-P synthesis and inhibition of pyrimidine synthesis as potential mechanisms for immunodeficiency in purine nucleoside phosphorylase deficiency? We have studied normal and enzyme-deficient erythrocytes to answer these questions.

MATERIALS AND METHODS

Adenosine, tetrasodium PP-ribose-P, inosine, IMP, ADP, ATP, deoxyadenosine, deoxyinosine, and deoxyguanosine were purchased from Sigma Chemical Company (St. Louis, Missouri). Adenine and hypoxanthine were purchased from Calbiochem (San Diego, California). EHNA, inhibitor of adenosine deaminase, was a gift from Dr. Gertrude Elion of Burroughs Wellcome Company (Research Triangle Park, North Carolina). From Amersham Corporation (Chicago, Illinois) we purchased [8-^{14}C]inosine (60 mCi/mM) and [U-^{14}C] adenine (270 mCi/mM). From New England Nuclear Corporation (Boston, Massashusetts), we purchased [8-^{14}C] adenosine (54.7 mCi/mM), [8-^{14}C] adenine (52 mCi/mM), and [carboxyl-^{14}C]orotic acid (41.25 mCi/mM).

Two brothers, ages 9 and 10 years (family 1), with purine nucleoside phosphorylase deficiency were hospitalized at the Hospital for Sick Children in Toronto. One child, age 5, with purine nucleoside phosphorylase deficiency (family 2) was hospitalized at the Children's Hospital in Chicago. The clinical features of these patients are described elsewhere (Edwards *et al.*, 1978; Gelfand *et al.*, 1978*a*; Rich *et al.*, 1979). Erythrocytes for *in vitro* studies of purine metabolism or enzyme assay were obtained by collecting fresh blood into heparin treated tubes at 4 C from three patients with purine nucleoside phosphorylase deficiency (families 1 and 2), one patient with a deficiency of hypoxanthine-guanine phosphoribosyltransferase, one heterozygote for purine nucleoside phosphorylase deficiency (family 2), and two normal subjects. Erythrocytes were spun at 1000*g* at 4 C for 5 min, and plasma was removed and frozen at −20 C. Erythrocytes were washed twice with cold 150 mM sodium chloride and were used immediately for *in vitro* studies or frozen at −70 C for enzyme assay.

In vitro studies of purine nucleoside phosphorylase-deficient erythrocytes were compared with normal and hypoxanthine-guanine phosphoribosyltransferase deficient erythrocytes. Erythrocytes were incubated in a 100 μl medium containing 2.5–20 μl erythrocytes, 7 mM glucose, 150 mM sodium chloride, 67 mM tris-HCl (*p*H 7.4), 4–6 mM disodium phosphate, and 0–1.25 mM nucleoside. The metabolism of 37 μM [8-^{14}C] adenosine or 41 μM [8-^{14}C]

inosine was studied by incubating the reaction mixture for 5–10 min according to methods previously described (Planet and Fox, 1976; Edwards *et al.*, 1978). Available PP-ribose-P was measured by adding 20 μM [8-^{14}C] adenine to the incubation medium (Henderson and Khoo, 1965; Planet and Fox, 1976; Edwards *et al.*, 1978). Free PP-ribose-P was assayed with a radiochemical method using [U-^{14}C] adenine (Fox and Kelley, 1971*a*). The rate of conversion of orotic acid to UMP in erythrocytes was quantitated by the release of $^{14}CO_2$ from [carboxyl-^{14}C]orotic acid (Edwards *et al.*, 1978; Fox *et al.*, 1978). Inorganic phosphate was quantitated according to the method of Chen *et al.* (1956). Hypoxanthine-guanine and adenine phosphoribosyltransferases, PP-ribose-P synthetase, adenosine deaminase, and purine nucleoside phosphorylase were assayed by previously described radiochemical methods (Kelley *et al.*, 1967; Fox and Kelley, 1971*a*; Van der Weyden *et al.*, 1974; Fox *et al.*, 1977). Protein was estimated by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as a standard. The concentrations of compounds used in these studies were based on the description provided by the manufacturer. Erythrocytes from each faimily were studied separately with a normal control for comparison.

RESULTS

Erythrocyte Enzyme Levels

Purine nucleoside phosphorylase was severely deficient with a value of 0.07% of control activity in the affected child from family 2, while the two brothers from family 1 were partially deficient with 0.45% of normal erythrocyte purine nucleoside phosphorylase activity. The mother of the enzyme-deficient child from family 2 had purine nucleoside phosphorylase activity which was 45% of normal. Normal erythrocyte purine nucleoside phosphorylase has a value of 2166 ± 576 nmol/hr/mg (Fox *et al.*, 1977). The patient with Lesch-Nyhan syndrome had a complete deficiency of erythrocyte hypoxanthine-guanine phosphoribosyltransferase and normal purine nucleoside phosphorylase.

Block at Purine Nucleoside Phosphorylase

Since the affected patients from both families had a measurable amount of purine nucleoside phosphorylase, the degree of block at purine nucleoside phosphorylase was estimated in intact cells by examining the conversion of inosine to hypoxanthine and the decrease of inorganic phosphate concentrations following the addition of inosine. Inosine and inorganic phosphate are substrates for purine nucleoside phosphorylase. Fresh erythrocytes were incubated with [8-^{14}C] inosine, and the formation of [^{14}C]hypoxanthine was quan-

titated (Table I). Normal erythrocytes converted 9–21% of inosine to hypoxanthine at 6.5 mM inorganic phosphate. The erythrocytes from the affected child in family 2 did not convert any inosine to hypoxanthine, indicating a virtually complete block at purine nucleoside phosphorylase. The erythrocytes from the two affected brothers from family 1 converted a small but substantial amount of inosine to hypoxanthine, indicating an incomplete block of purine nucleoside phosphorylase in the intact erythrocytes.

When inosine is converted to hypoxanthine, inorganic phosphate is utilized as a substrate to form ribose-1-phosphate. Patients with purine nucleoside phosphorylase deficiency demonstrate resistance to the inosine-induced decreased in inorganic phophate (Table I), indicating decreased utilization of inorganic phosphate to form ribose-1-phosphate.

Erythrocytes from the heterozygote of family 2 and the patient with Lesch-Nyhan syndrome were indistinguishable from normal erythrocytes in their capacity to form hypoxanthine from inosine or to utilize inorganic phosphate.

Biochemical Effects of the Enzyme Deficiency

Inhibition of PP-ribose-P formation and blockade of pyrimidine biosynthesis are two mechanisms suggested as potential bases for immunodeficiency in the purine enzyme deficiency states (Green and Chan, 1973; Ishii and Green, 1973; Planet and Fox, 1976; Snyder *et al.*, 1976; Fox *et al.*, 1978). These

Table I. Block at Purine Nucleoside Phosphorylase[a]

	Hypoxanthine formation[b] (% [8-^{14}C] inosine utilized)	Phosphate concentration[c] (% control value with inosine 1.25 mM)
Family 1		
Normal	9	42
PNP$^-$ (A)	0.5	92
PNP$^-$ (B)	0.8	91
Lesch-Nyhan syndrome	9	46
Family 2		
Normal	21	49
PNP$^-$	Not detectable	82
Heterozygote	24	41

[a] PNP$^-$, Purine nucleoside phosphorylase deficiency; heterozygote, heterozygote for purine nucleoside phosphorylase deficiency.
[b] Calculated for 2.5 μl erythrocytes incubated for 6 min.
[c] Concentration estimated following a 30-min incubation with 1.25 mM inosine. Control values ranged from 0.78 to 0.91 mM for study 1 and 1.55 to 1.95 mM for study 2.

hypotheses were testable in erythrocytes because they have the pathway for PP-ribose-P synthesis and the pathway for the formation of pyrimidine nucleotides from orotic acid. PP-ribose-P concentration in the erythrocytes from the affected child of family 2 was increased by 284% above the control value, while the PP-ribose-P concentration from the erythrocytes from the brothers from family 1 were increased slightly by 22% or 120%. The erythrocyte PP-ribose-P concentration in the heterozygote (family 2) was not increased, while the erythrocyte PP-ribose-P concentration in the Lesch-Nyhan patient was 2345% above the control value. Thus there was a modest increase in PP-ribose-P concentrations in erythrocytes in the patient from family 2.

The possibility that an accumulation of nucleosides in purine nucleoside phosphorylase deficiency might alter PP-ribose-P formation was further tested. The basis for this hypothesis is the ability of nucleosides to reduce intracellular concentrations of PP-ribose-P in human erythrocytes, human lymphoblasts, and *Escherichia coli* (Bagnara and Finch, 1973, 1974; Planet and Fox, 1976; Snyder *et al.*, 1976; Snyder and Seegmiller, 1976). Since PP-ribose-P is an essential substrate for pyrimidine biosynthesis *de novo*, purine biosynthesis *de novo*, and purine salvage pathways (Fox and Kelley, 1971*b*), decreased intracellular concentration of this compound could have profound metabolic effects. An estimation of the capacity to synthesize PP-ribose-P was assessed by the measurement of 'available PP-ribose-P.' Purine nucleoside phosphorylase deficient erythrocytes did not have a decrease of PP-ribose-P synthesis as measured under these conditions (Fig. 2). In erythrocytes from the affected brothers of family 1 there was a modest increase in baseline synthesis of PP-ribose-P, whereas the severely deficient erythrocytes from the affected child from family 2 synthesized large quantities of PP-ribose-P similar to the level seen in the cells from the patient with Lesch-Nyhan syndrome. Erythrocytes from the heterozygote subject from family 2 synthesized normal quantities of PP-ribose-P.

Adenosine or inosine 1.25 mM markedly inhibited PP-ribose-P synthesis by normal erythrocytes and erythrocytes from the heterozygote subject (Fig. 2). This reduction of intracellular concentrations of PP-ribose-P is caused by decreased synthesis of this compound as a result of the diminution of inorganic phosphate concentration by added nucleosides. In purine nucleoside phosphorylase deficiency the addition of inosine did not diminish PP-ribose-P levels and a decreased effect was evident with adenosine. This may be related to an inability to degrade inosine to hypoxanthine and the absence of a decrease in inorganic phosphate concentration (Planet and Fox, 1976).

Another hypothesis is that a block of pyrimidine nucleotide synthesis could result from the purine nucleoside accumulation found in purine nucleoside phosphorylase deficiency. The addition of adenosine to mammalian cells leads to (1) an accumulation of orotic acid, (2) a depletion of the pyrimidine

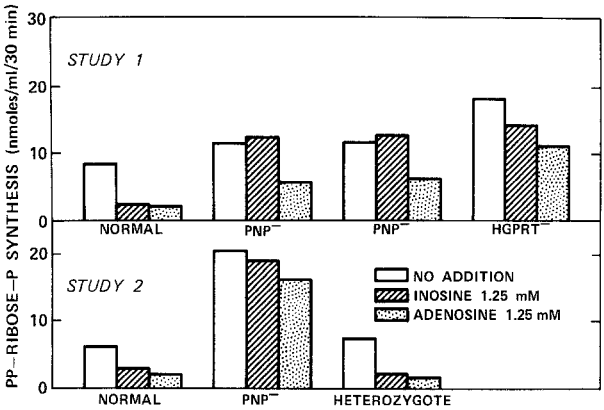

Fig. 2. PP-ribose-P synthesis in normal and enzyme-deficient erythrocytes. Fresh erythrocytes were obtained from normal subjects, patients with purine nucleoside phosphorylase deficiency (PNP^-), a patient with hypoxanthine-guanine phosphoribosyltransferase deficiency ($HGPRT^-$), and a heterozygote for purine nucleoside phosphorylase deficiency. Study 1 refers to experiments using erythrocytes from family 1 with 0.45% of normal purine nucleoside phosphorylase in the affected brothers, and study 2 refers to experiments on erythrocytes from family 2 with 0.07% of normal enzyme activity in the affected child. Enzyme-deficient erythrocytes are resistant to the inhibitory effects of added inosine and adenosine.

nucleotide pool, and (3) an expansion of the adenine nucleotide pool (Kaukel *et al.*, 1972; Green and Chan, 1973; Hilz and Kaukel, 1973; Ishii and Green, 1973; Snyder and Seegmiller, 1976). These observations imply that adenosine inhibits pyrimidine biosynthesis at a site between orotic acid and UMP synthesis. Recently a block of pyrimidine synthesis in purine nucleoside phosphorylase deficiency was suggested by a minute elevation of urinary orotic acid in two patients with a complete enzyme deficiency (Cohen *et al.*, 1977). Baseline measurements in our studies did not demonstrate a block in UMP synthesis in the erythrocytes with purine nucleoside phosphorylase deficiency (Fig. 3). In fact, the erythrocytes from the affected child of family 2 and from a patient with Lesch-Nyhan syndrome demonstrated a three- and five fold increase, respectively, in the baseline carbon dioxide release from orotic acid during its concersion to UMP. An increase in activity of orotate phosphoribosyltransferase and orotidylic decarboxylase occurs in erythrocytes from patients with Lesch-Nyhan syndrome (Beardmore *et al.*, 1973).

Purine nucleoside phosphorylase deficient erythrocytes demonstrated a relative resistance to the inhibitory effects of inosine and to a smaller extent adenosine on carbon dioxide release from orotic acid (Fig. 3). The block in the

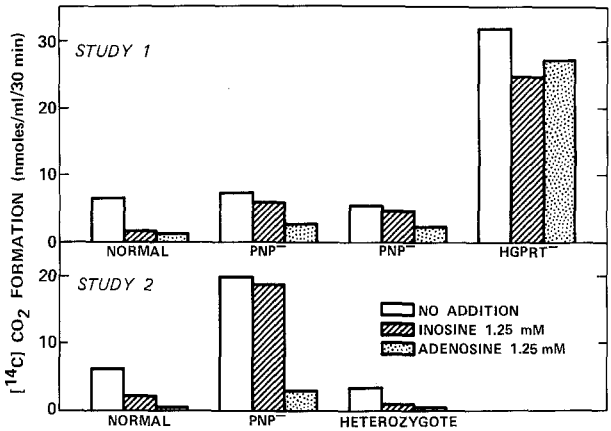

Fig. 3. Conversion of orotic acid to UMP in normal and enzyme-deficient erythrocytes. Studies were carried out as described in Fig. 2. The conversion of orotic acid to UMP was quantitated by the release of $^{14}CO_2$ from [carboxyl-^{14}C] orotic acid.

release of carbon dioxide from orotic acid resulted from a depletion of intracellular PP-ribose-P, a substrate for orotate phosphoribosyltransferase reaction (Fox *et al.*, 1978). The inhibitory effect of purine nucleosides on this pyrimidine pathway in normal erythrocytes and the relative resistance to this effect in hypoxanthine-guanine phosphoribosyltransferase deficient cells agree with reported observations (Fox *et al.*, 1978).

Effects of Deoxynucleosides

It has recently become evident that nucleotide derivatives of 2′-deoxynucleosides accumulate in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency (Cohen *et al.*, 1978*a,b*; Coleman *et al.*, 1978). These are believed to result from the increase of deoxyadenosine levels in adenosine deaminase deficiency and deoxyguanosine levels in purine nucleoside phosphorylase deficiency. Since a block of pyrimidine synthesis has been a prominent hypothesis used to explain immunodeficiency, it is of interest to consider whether these deoxynucleosides modify PP-ribose-P synthesis or the conversion of orotic acid to UMP. Using 2′-deoxynucleosides, there was a relative resistance to inhibition of PP-ribose-P synthesis and orotic acid conversion of UMP in purine nucleoside phosphorylase deficient erythrocytes with a severe enzyme deficiency (Table II).

Table II. Effect of Deoxynucleosides on PP-Ribose-P Synthesis and the Conversion of Orotic Acid to UMP in Erythrocytes (Studies on Blood from Individuals from Family 2)[a]

Inhibitor (1.25 mM)	Percent of value with no inhibitor		
	Normal	PNP⁻	Heterozygote for PNP⁻
PP-ribose-P synthesis[b]			
dAdenosine	34	60	25
dInosine	49	71	35
dGuanosine	51	75	39
Orotic acid conversion to UMP[c]			
dAdenosine	8	66	7
dInosine	46	65	33
dGuanosine	37	62	37

[a] PNP⁻ refers to purine nucleoside phosphorylase deficiency with 0.07% normal activity.
[b] See Fig. 2 for baseline values.
[c] See Fig. 3 for baseline values.

DISCUSSION

Purine nucleoside phosphorylase catalyzes the reversible phosphorolysis of guanosine, deoxyguanosine, inosine, or deoxyinosine to guanine or hypoxanthine. This reaction is of critical importance to purine degradation and has been detected in extracts from human erythrocytes, lung, liver, spleen, kidney, muscle, and fibroblasts (Edwards *et al.*, 1971; Wortmann *et al.*, 1979). This enzyme reaction precedes hypoxanthine-guanine phosphoribosyltransferase in the purine degradation pathway (Fig. 1). Hypoxanthine-guanine phosphoribosyltransferase catalyzes the transfer of the 5-phosphoribosyl moiety of PP-ribose-P to the 9 position of guanine, hypoxanthine, or xanthine to form GMP, IMP, or XMP, respectively. The enzyme is present in many tissues in man, with the highest specific activities in brain, placenta, gonads, erythrocytes, fibroblasts, and leukocytes (Rosenbloom *et al.*, 1967). The physiological role of the enzyme may be related to a salvage function, allowing the conservation of purine bases that would otherwise be further degraded to uric acid (Henderson, 1968).

The metabolic abnormalities of purine nucleoside phosphorylase deficiency resemble to a great extent the disorders seen in the deficiency of hypoxanthine-guanine phosphoribosyltransferase. Previous studies have demonstrated urinary purine overexcretion to a level resembling that seen in

patients with Lesch-Nyhan syndrome (Lesch and Nyhan, 1964; Kelley *et al.*, 1967; Rosenbloom *et al.*, 1967; Kelley and Wyngaarden, 1972; Cohen *et al.*, 1976; Siegenbeek van Heukelom *et al.*, 1977; Edwards *et al.*, 1978). As well, elevated erythrocyte PP-ribose-P concentrations have been described (Fox and Kelley, 1971*b*; Cohen *et al.*, 1976; Siegenbeek van Heukelom *et al.*, 1977). Our current studies of two families with purine nucleoside phosphorylase deficiency demonstrate that the erythrocytes from the patient with the more severe abnormality (family 2) resemble the erythrocytes from a patient with Lesch-Nyhan syndrome. Erythrocyte PP-ribose-P concentrations were elevated. The ability to synthesize PP-ribose-P by these erythrocytes and the production of carbon dioxide from orotic acid were also increased.

The biochemical basis for the metabolic similarities of these two enzyme deficiencies is related to the fact that the deficiency of purine nucleoside phosphorylase leads to a secondary relative deficiency of hypoxanthine-guanine phosphoribosyltransferase. With the lack of purine nucleoside phosphorylase activity, there is no production of hypoxanthine and guanine, substrates necessary for the hypoxanthine-guanine phosphoribosyltransferase reaction. With the lack of substrate for the reaction, hypoxanthine-guanine phosphoribosyltransferase is no longer active and is relatively deficient despite the fact that the enzyme specific activity is normal.

Despite these similarities in the biochemical alterations in deficiencies of purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase, there remain marked contrasts between these disorders. Although there is purine overproduction in both disorders, the net result is profoundly different. In hypoxanthine-guanine phosphoribosyltransferase deficiency there is accelerated oxidation of hypoxanthine to uric acid, as a result of an inability to reutilize this compound (Lesch and Nyhan, 1964; Kelley *et al.*, 1967; Rosenbloom *et al.*, 1967; Kelley and Wyngaarden, 1972; Edwards *et al.*, 1979). Uric acid is inert metabolically. Our current observations and previous data (Cohen *et al.*, 1976; Stoop *et al.*, 1977; Edwards *et al.*, 1978) indicate a block in the converson of nucleosides to uric acid in purine nucleoside phosphorylase deficiency. The net result is an accumulation of nucleosides and hypouricemia. Although inosine and guanosine appear not to be metabolized further when purine nucleoside phosphorylase is deficient, this is not the case with the 2′-deoxy derivatives of these compounds. Markedly increased concentrations of dGTP occur in erythrocytes from two previous patients with purine nucleoside phosphorylase deficiency (Cohen *et al.*, 1978*a*) and the patients currently reported (Wortmann *et al.*, 1979). It is believed that dGTP may be synthesized from accumulating deoxyguanosine and that this may be relevant to the observed disorder of cellular immunity. Accumulation of abnormal concentrations of intracellular nucleotides (Rosenbloom *et al.*, 1968; Brenton *et al.*, 1977; Nuki *et al.*, 1977) or altered immune function

(Seegmiller *et al.*, 1977; Gelfand *et al.*, 1978) does not occur in the complete deficiency of hypoxanthine-guanine phosphoribosyltransferase.

The erythrocytes from a subject with 45% of normal purine nucleoside phosphorylase activity (family 2) show no evidence of any alteration of metabolism. Previous studies had revealed a normal subject (Rich *et al.*, 1979). This contrasts with patients with this degree of enzyme deficiency in hypoxanthine-guanine phosphoribosyltransferase, who have evidence of uric acid overproduction (Fox, 1977).

There are a number of hypotheses which have been proposed to account for the immunodeficiency in purine enzyme deficiency states (Fox and Kelley, 1978; Fox, 1979). Inhibition of PP-ribose-P formation and a blockade of pyrimidine biosynthesis had appeared to provide an important molecular basis this association (Green and Chan, 1973; Ishii and Green, 1973; Planet and Fox, 1976; Snyder *et al.*, 1976; Fox *et al.*, 1978). The occurrence of orotic aciduria in two patients with purine nucleoside phosphorylase deficiency (Cohen *et al.*, 1977) supported this mechanism. However, there is no evidence for inhibition of PP-ribose-P formation or block of pyrimidine synthesis in purine nucleoside phosphorylase deficient erythrocytes in the current study or in enzyme-deficient cultured human fibroblasts (Fox, 1978). The observations suggest that other mechanisms may be more important. A block at ribonucleotide reductase, an accumulation of *S*-adenosylhomocysteine, or elevated concentrations of cyclic-3′,5′-AMP appear to be more viable mechanisms to explain the biochemical basis of immune dysfunction (Fox, 1979).

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