

De Novo Synthesis of Purine Nucleotides in Human Peripheral Blood Leukocytes

EXCESSIVE ACTIVITY OF THE PATHWAY IN HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE DEFICIENCY

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ABSTRACT Human peripheral blood leukocytes were studied for the presence and the regulatory properties of the pathway of de novo synthesis of purine nucleotides. The cells were found to incorporate the labeled precursors formate and glycine into purines. The rate of [^{14}C]-formate incorporation was decreased by several compounds known to inhibit purine synthesis by affecting the activity of glutamine phosphoribosylpyrophosphate (PRPP) amidotransferase, the first committed enzyme in the pathway, either through decreasing the availability of PRPP, a substrate for this enzyme, or through exerting inhibition on this enzyme.

PRPP availability in the leukocyte was found to be limiting for purine synthesis. Increased PRPP availability resulting from activation of PRPP synthetase by increasing inorganic phosphate (P_i) concentration caused acceleration of purine synthesis. On the other hand, no clear-cut evidence was obtained for the availability of ribose-5-phosphate in the leukocyte being rate limiting at physiological extracellular P_i concentration for PRPP generation, and thus for purine synthesis. However, the addition of methylene blue, which accelerates the oxidative pentose shunt that produces ribose-5-phosphate, resulted in acceleration of PRPP gen-

eration and of purine synthesis only when PRPP synthetase was largely activated at high P_i concentration. These results may be taken to suggest that ribose-5-phosphate availability is indeed not limiting for PRPP generation under physiological conditions.

Purine synthesis de novo was accelerated more than 13-fold in the leukocytes of two gouty patients affected with partial deficiency of hypoxanthine-guanine phosphoribosyltransferase, but was normal in the leukocytes of an obligate heterozygote for this enzyme abnormality.

The results demonstrate in peripheral human leukocytes the presence of the complete pathway of de novo synthesis of purine nucleotides and the manifestation in these cells of the biochemical consequences of hypoxanthine-guanine phosphoribosyltransferase deficiency, i.e., increased availability of PRPP and acceleration of purine synthesis de novo. The results indicate the usefulness of leukocytes as a model tissue for the study of purine metabolism in man.

INTRODUCTION

Peripheral blood cells, in particular erythrocytes, are commonly used for the study of normal and abnormal metabolic pathways. The use of the erythrocyte for the study of purine metabolism in man is beset with the difficulty that this cell lacks the de novo purine synthesis pathway (1). Fibroblast cultures provide an excellent model for this pathway but have two main shortcomings: the relatively long time needed to obtain a sufficient amount of cells and the expense involved in the maintenance of the cultures.

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The capacity of normal human peripheral blood leukocytes to carry on purine nucleotide synthesis *de novo* has not yet been conclusively assessed. Several investigators could not detect the complete pathway in normal leukocytes (2) or in lymphocytes (3), but demonstrated its presence in phytohemagglutinin-stimulated normal lymphocytes (3) and in leukemic cells (2, 3). In contrast, other investigators did demonstrate *de novo* purine synthesis in normal leukocytes and found it to be accelerated in various hyperuricemic conditions such as primary gout, chronic renal failure, and secondary hyperuricemia (4, 5).

In the present study, we bring evidence for the activity of the complete pathway of *de novo* purine synthesis in normal human peripheral blood leukocytes and characterize some regulatory properties of this pathway in these cells. In addition, we measured the rate of activity of purine synthesis *de novo* and the availability of 5-phosphoribosyl-1-pyrophosphate (PRPP),¹ an important regulator of the pathway, in leukocytes of patients with partial hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency. In accordance with earlier observations on cultured fibroblasts from such patients (6), PRPP availability and the rate of purine synthesis *de novo* were found to be increased.

METHODS

Control subjects and patients. Volunteer normal blood donors and various patients with normal purine metabolism and normal hematological values served as controls. The gouty patients, H. R. and A. R., both having partial HGPRT deficiency, and Ch. R., the obligate heterozygote for this enzyme abnormality, were subjects III-5, IV-6, and III-4, respectively, in the pedigree of the previously reported family (7). The serum uric acid level in patient H. R., was 7.3 mg/100 ml and urinary excretion of uric acid 2,040 mg/24 h. These values in patient A. R., were 11.0 mg/100 ml and 1,670 mg/24 h, respectively. HGPRT activities in the erythrocytes of patients H. R. and A. R., were 2.4 and 1.1% of the normal mean, respectively. Subject Ch. R. had normal erythrocyte HGPRT activity. Patient A. R. is being treated by the uricosuric drug sulfinpyrazone (Anturane; the patient is allergic to Allopurinol) and alkalization; patient H. R. is being treated with Allopurinol. Anturane was obtained from Geigy Pharmaceuticals, Ardsley, N. Y. In either patient, treatment was discontinued 1 day before blood was taken for study.

Chemicals. All radiochemicals were purchased from the Radiochemical Centre, Amersham, England. Purine bases, nucleotide, and other fine chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. Allopurinol and oxipurinol were a gift from Dr. G. B. Elion of the Wellcome Research Laboratories, Research Triangle Park, N. C. Growth medium was purchased from Grand Island Biological Co., (GIBCO) Grand Island, N. Y.

Separation of leukocytes. 20–30 ml of blood was drawn

¹Abbreviations used in this paper: HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PCA, perchloric acid; Pi, inorganic phosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; R-5-P, ribose-5-phosphate.

in the morning by venipuncture into tubes containing 4.5 ml of acid citrate dextrose (0.8% citric acid, 26.2% sodium citrate, and 2.45 dextrose). 1 vol of 3% polyvinylpyrrolidone in 0.9 NaCl solution was added to the blood which was left in ice for 30–60 min. The upper phase containing the leukocytes was separated and the residual erythrocytes in this phase were lysed by the addition of 2 vol of 0.87% NH₄Cl. The resulting leukocytes were washed two-three times and finally suspended in cold 0.9% NaCl solution. Cell concentration was measured by either counting or by determining cell volume by the microhematocrit method, and expressed accordingly.

Purine synthesis *de novo*. The rate of purine nucleotide synthesis *de novo* was studied in intact leukocytes by measuring the rate of incorporation of [1-¹⁴C]glycine or of [¹⁴C]formate into the cellular purines or into the total purines (cellular purines and purines excreted by the cells into the incubation medium), soluble in hot (100°C for 1 h) 1 N perchloric acid (PCA). The procedure was essentially that described by Martin and Owen (8) and that by Green and Martin (9) for cultured mutagenized hepatoma cells, and by Zoref et al. for cultured fibroblasts (10). In the standard procedure, 2×10^7 cells were suspended in fresh 2.5 ml Eagle's Minimal Essential Medium containing Earle's Balanced Salt Solution (GIBCO, F-15), 15% fetal calf serum, folic acid (50 µg/ml), penicillin (100 U/ml), streptomycin (100 µg/ml), mycostatin (25 µg/ml), and 20 µCi sodium [¹⁴C]formate (59 mCi/mmol). Inorganic phosphate (Pi) concentration of this incubation medium was measured and found to be 1.4 mM. Higher Pi concentrations were obtained by addition of K₂HPO₄, pH 7.4, to the specified concentration. The cell suspensions were saturated with 5% CO₂–95% air and incubated for 2 h in a shaking bath at 37°C. The effect of inhibitors was studied by their addition to the specified concentration and the effect of methylene blue was studied at 0.1 mM. For the determination of incorporation into cellular purines, the cells, after incubation, were washed five times with ice-cold saline and finally suspended in 0.5 ml of distilled water and subjected to freezing and thawing seven times. For the determination of incorporation into total purines, the whole cell suspensions (the cells in their incubation medium) were subjected to freezing and thawing. The following procedure was identical for both cellular and total purines. PCA was added to the cell lysates to a concentration of 1 N, and 0.2 µCi of [8-³H]-adenine (500 mCi/mmol) was added as an internal reference for recovery of purines. The tubes were thoroughly mixed and placed at 100°C for 1 h. The tubes were chilled, centrifuged, and the hydrolysate adjusted with water to a volume of 3 ml. To each tube 0.7 mg adenine was added as carrier and the purine bases were then precipitated as purine silver complex, washed, extracted in 0.1 N HCl by heating at 100°C for 1 h, and the radioactivity counted (10). The results were corrected for incomplete recovery of purines, utilizing the tritiated adenine as reference, and related to cell number. In all experiments, the recovery of tritiated adenine ranged between 40 and 60%. Up to 3 mM Pi at the incubation medium, labeling of the cellular purines and of the total purines in the leukocytes of all subjects studied, was linear with time for at least 4 h. At higher extracellular Pi concentration, the labeling of purines exhibited a tendency to increase with time, presumably due to the gradual increase in intracellular Pi concentration. For a fixed period of incubation, at all extracellular Pi concentrations, the labeling of purines was proportional to the number of cells, up to at least 6×10^7 cells/tube.

PRPP availability. Cellular PRPP availability for nucleotide synthesis was assayed by measuring the rate of [$8\text{-}^{14}\text{C}$]adenine or [$8\text{-}^{14}\text{C}$]hypoxanthine incorporation into the intact cell total nucleotide pool (soluble in cold 1 N PCA), essentially as described by Zoref et al. for cultured human fibroblasts (10). For the study of the effect of Pi concentration and of methylene blue on PRPP availability, 15–20 μl of packed leukocytes were suspended in 1.3 ml of growth medium (Eagle's medium, as described for the assay of purine synthesis de novo), containing 140 nmol of [$8\text{-}^{14}\text{C}$]adenine (10 $\mu\text{Ci}/\mu\text{mol}$). The cell suspensions were incubated in a shaking bath at 37°C for 30 min. The effect of methylene blue was studied at 0.1 mM and the Pi concentration specified was adjusted with K_2HPO_4 , pH 7.4. After incubation the tubes were put immediately in ice, 5 ml of ice-cold saline was added, the cells washed twice with ice-cold saline at 4°C, suspended in 0.5 ml of distilled water, and subjected to freezing and thawing 5 times. Protein and nucleic acids were precipitated by addition of PCA to 1 N and the total labeled nucleotides in the supernate were separated by thin-layer chromatography on microcrystalline cellulose, with butanol: H_2O : NH_4OH 25% (60:20:20:1, vol/vol) as solvent, as described by Zoref et al. (10), and counted. The reaction exhibited linearity with time for at least 45 min and at a fixed time period also with cell number, up to at least 30 μl packed cells. The results were related to 1 ml packed leukocytes. For the measurement of PRPP availability in control of HGPRT-deficient subjects, the same method was employed except that Pi concentration was constant (1.4 mM) and $1\text{--}2 \times 10^7$ cells were added to each tube. The incorporation of adenine and of hypoxanthine (each 140 nmol/tube, 10 $\mu\text{Ci}/\mu\text{mol}$) into the nucleotides in separate leukocyte samples from each subject were studied. The results were related to cell number. The incorporation of both labeled purine bases exhibited linearity with time up to 45 min and at fixed time period also with cell number up to at least 4×10^7 cells/tube.

Rate of activity of the oxidative pentose shunt. The rate of activity of the oxidative pentose shunt was assayed by the measurement of the release of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]glucose (11). 1×10^8 leukocytes were suspended in 2 ml of either phosphate-buffered saline or in growth medium (as for the synthesis de novo of purines), containing 5.5 μmol of [$1\text{-}^{14}\text{C}$]glucose (25 $\mu\text{Ci}/\text{mmol}$). Incubations were carried at 37°C for 4 h at 50 oscillations/min.

PRPP content. The PRPP content of leukocytes was determined essentially as described by Hershko et al (11) and by Fox and Kelley (12). Cells were prepared as described above and their concentration adjusted to approximately 20% by volume. Activated carbon (Darco S-60, Atlas Chemical Industries, Inc., Wilmington, Del.) was added and the cells subjected to freezing and thawing six times. After centrifugation at 15,000 g for 10 min, the supernate was treated once again with activated carbon, and a 200- μl sample of the charcoal-free supernate was subjected to heating at 85°C for 2 min, chilled, and centrifuged at 3,000 rpm for 10 min. The reaction mixture, a total volume of 200 μl , contained 100 μl of the heated supernate, 5 mM MgCl_2 , 0.3 mM [$8\text{-}^{14}\text{C}$]hypoxanthine (25 mCi/mmol), 100 μg of a partially purified human erythrocyte HGPRT (diethylaminoethyl cellulose extract treated for 2 min at 85°C to eliminate PRPP synthetase activity [13]) in 55 mM Tris-HCl buffer, pH 8.5. After incubation for 120 min, the reaction was stopped by the addition of 40 μl of 15% PCA in ice (14). [^{14}C]IMP in the protein-free supernate was separated using thin-layer chromatography, and counted

TABLE I
Proportionality of [$1\text{-}^{14}\text{C}$]Glycine and of [^{14}C]Formate
Incorporation into Leukocyte Purines with Respect
to Cell Number and Incubation Time*

Cell number	Time of incubation	^{14}C incorporation	
		[$1\text{-}^{14}\text{C}$]glycine	[^{14}C]formate
$\times 10^7$	h	dpm	
1	4	6,120	2,600
2	0.5		710
2	1	2,849	1,460
2	2	6,200	2,715
2	4	12,165	5,620
3	4	18,600	9,200
4	4	26,861	12,174
6	2		8,860

* The incorporation of [$1\text{-}^{14}\text{C}$]glycine (55 mCi/mmol, 25 $\mu\text{Ci}/\text{tube}$) and of [^{14}C]formate (59 mCi/mmol, 20 $\mu\text{Ci}/\text{tube}$) into cellular purines was measured. The conditions of the assay were as given in the Methods section. Pi concentration was 1.4 mM.

as described above. Controls in which PRPP was added to cell lysates before the heating stage revealed a recovery of at least 95%. The results were related to 1 ml packed cells.

RESULTS

Synthesis de novo of purine nucleotides. Human leukocytes were shown to incorporate [^{14}C]formate and [$1\text{-}^{14}\text{C}$]glycine into purines. Proportionality of the rate of incorporation of these precursors with respect to time of incubation and number of cells is demonstrated in Table I. The rate of incorporation of [^{14}C]formate, which was studied in greater detail, exhibited sensitivity to inhibition by several compounds known to specifically inhibit de novo purine nucleotide synthesis (Table II). The activity of glutamine-PRPP amidotransferase, the enzyme which catalyzes the first committed step of this pathway, is affected by these compounds by various mechanisms: through depletion of the enzyme substrate PRPP or by inhibition, either of the feedback or the competitive type.

Effect of Pi concentration on PRPP availability and purine synthesis de novo. Intracellular Pi concentration (15) in freshly separated blood leukocytes was found in our laboratory to range from 7 to 10 mM.³ At Pi concentration in the incubation medium higher than that, incubation of leukocytes resulted in a gradual increase in intracellular Pi concentration, reaching up to 19 mM after a 2-h incubation at 50 mM Pi. This increase in intracellular Pi concentration was associated with acceleration of PRPP generation and of de novo purine

³ The actual value may be lower, since under the acidic conditions of the assay hydrolysis of some organic phosphate esters may occur.

TABLE II
Inhibition of Purine Synthesis

Inhibitor	Concentration	Residual [^{14}C]formate incorporation into purines*	
		1.4 mM Pi^\dagger	50 mM Pi^\dagger
—	mM	% of control	
—	—	100	100
Hypoxanthine	0.2	50	80
Adenine	0.01	44	10
	0.05	35	0
Azaserine	1.0	33	
	5.0	20	
Allopurinol	0.5	43	
Oxipurinol	0.5	18	
Orotic acid	0.5	50	100

* Rate of incorporation was assayed into total purines as described under Methods. Each value represents the average of two determinations.

† Pi concentration in the medium.

synthesis (Figs. 1, 2, and 3). Cellular PRPP availability for nucleotide synthesis increased approximately fivefold upon increasing Pi concentration in the medium from 5 to 50 mM. Increase in Pi concentration from 1.4 to 5

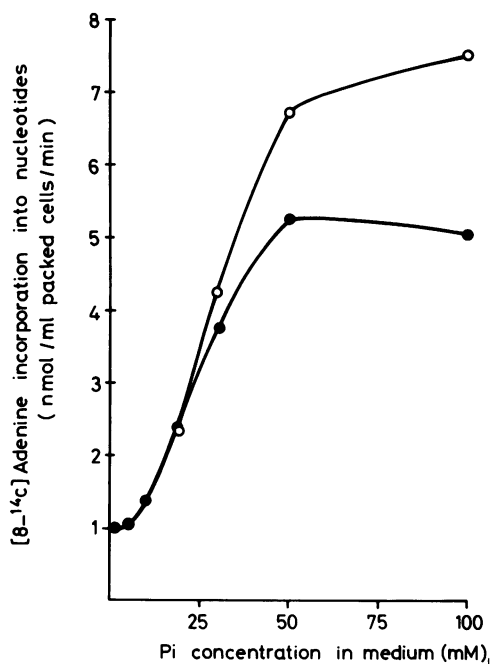


FIGURE 1 The effect of increasing Pi concentration in the incubation medium on the availability of PRPP in the leukocytes. PRPP availability was gauged by the rate of [$8\text{-}^{14}\text{C}$]adenine incorporation into intact cell nucleotides (see Methods). ●, In absence of methylene blue; ○, in presence of methylene blue (0.1 mM).

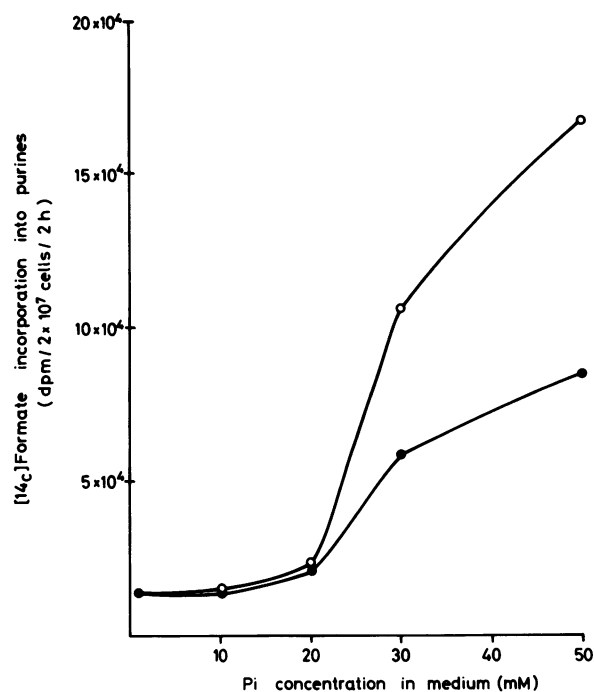


FIGURE 2 The effect of increasing Pi concentration in the incubation medium on the rate of purine synthesis de novo. The rate of purine synthesis de novo was gauged by measuring the rate of [^{14}C]formate incorporation into total purines (see Methods). ●, In absence of methylene blue; ○, in presence of methylene blue (0.1 mM).

mM and from 50 to 100 mM did not affect PRPP availability. Incubation at 1.4 mM Pi did not cause a significant change in PRPP concentration, but incubation

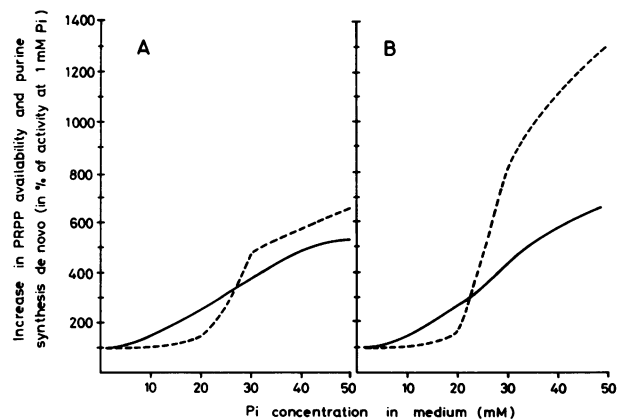


FIGURE 3 Relationship between PRPP availability and the rate of purine synthesis de novo in intact leukocytes. The curves were depicted on the basis of the data presented in Figs. 1 and 2. The increase in PRPP availability and in the rate of purine synthesis de novo is expressed in percent of the respective value at 1.4 mM Pi . (A), in absence of methylene blue; (B), in presence of methylene blue (0.1 mM). (—), PRPP availability; (---), rate of de novo purine synthesis.

TABLE III
Effect of Pi Concentration and Methylene Blue on
Leukocytes PRPP Content*

Conditions	Cellular content of PRPP
	nmol/ml packed cells
Fresh cells†	3.84±1.7
Cells after incubation, 30 min‡	
at 1.4 mM Pi	5.3±2.1
at 1.4 mM Pi + 0.1 mM MB	5.2±2.7
at 50 mM Pi	15.2±5.3
at 50 mM Pi + 0.1 mM MB	41.3±16

* Experiments were done in quadruplicates. Values represent mean±1 SD.

† Fresh cells analyzed after separation from whole blood, without incubation.

‡ Incubation conditions were as described under Methods for the study of the effect of Pi concentration and of methylene blue (MB) on PRPP availability.

in a Pi-rich medium (50 mM Pi) increased the PRPP content of the leukocytes, almost fourfold, $P < 0.01$, (Table III). The rate of purine synthesis de novo was accelerated more than fourfold when Pi concentration in the medium was increased from 10 to 50 mM (Fig. 2). Increasing the Pi concentration from 1.4 to 10 mM did not affect the rate of purine synthesis. KCl or K₂SO₄, at 50 mM each, did not affect purine synthesis de novo.

Effect of methylene blue on PRPP availability and purine synthesis de novo. Addition of methylene blue (0.1 mM) to either phosphate-buffered saline or to the growth medium similarly accelerated the rate of the activity of the oxidative pentose shunt by approximately fivefold, the release of ¹⁴CO₂ from [1-¹⁴C]glucose increasing from 1,740 to 8,831 cpm/10⁶ cells per 4 h (average values of four experiments; see Methods). Varying Pi concentration (1.4, 10, and 50 mM) did not affect the rate of the oxidative pentose shunt, both in presence and absence of methylene blue. Methylene blue (0.1 mM), when added to cells incubated at 1.4 mM Pi, did not cause a significant alteration in PRPP concentration, but when added to cells incubated at 50 Pi for 30 min, the content of PRPP increased by 2.7-fold, $P < 0.02$, (Table III). The effect of methylene blue on the availability of PRPP exhibited dependency on the Pi concentration in the medium; no effect was observed at Pi concentration lower than 25 mM, while the PRPP availability gradually increased, by approximately 50%, when the Pi concentration was increased to 100 mM (Fig. 1). In accordance with its effect on PRPP availability, methylene blue accelerated the rate of purine synthesis de novo only when Pi concentration in the incubation medium was 30 mM or higher (Fig. 2).

PRPP availability and purine synthesis de novo in

TABLE IV
PRPP Availability in Leukocytes

Subjects	Rate of incorporation into cellular nucleotides*	
	[8- ¹⁴ C]adenine	[8- ¹⁴ C]hypoxanthine
	pmol/10 ⁶ cells per min	
Control (20)‡	1.04±0.30 (0.71 – 1.67)	0.47±0.13 (0.27 – 0.76)
Partial HGPRT deficiency		
A. R.	1.93	0.25
H. R.	1.70	0.13

* Values represent for the control group mean±1 SD (range in parentheses), for the HGPRT-deficient subject the averages of triplicate determinations. Pi concentration was 1.4 mM.

‡ Number of subjects in parentheses.

leukocytes of gouty overproducers with partial HGPRT deficiency. The rates of incorporation of labeled adenine and of labeled hypoxanthine into the leukocyte nucleotides were measured in the leukocytes from the two subjects, H. R. and A. R., with partial deficiency of HGPRT (Table IV). The rate of incorporation of labeled adenine into their leukocyte nucleotides was significantly increased ($>$ normal mean + SD), indicating an increased availability of PRPP in these cells. In contrast, due to HGPRT deficiency, the measurement of the rate of incorporation of labeled hypoxanthine into the leukocyte nucleotides of these two patients gave the lowest values obtained in the series studied. However,

TABLE V
De Novo Synthesis of Purine Nucleotides in Leukocytes of
Patients with Partial HGPRT Deficiency

Subjects	Rate of [¹⁴ C]formate in- corporation into purines*
	dpm/2 × 10 ⁷ cells per 2 h
Control (26)‡	3,329±1,479
Partial HGPRT deficiency	
H. R.	44,620
A. R.	46,781
Obligate heterozygote for HGPRT deficiency	
Ch. R.	4,773

* Incorporation was assayed into total purines as described under Methods. Pi concentration was 1.4 mM. The value for the control group represents mean±1 SD; the rate in each subject was determined in duplicates or triplicates. The values for the HGPRT-deficient subjects and for the heterozygote for this enzyme deficiency represent averages of determinations in triplicates.

‡ Number of subjects in parentheses.

only in the leukocytes of one of these subjects (patient H. R.) was the rate of incorporation significantly low ($< \text{normal mean} - 2 \text{ SD}$).

The rates of incorporation of [^{14}C]formate into the total purines produced by the leukocytes of the two gouty patients with partial HGPRT deficiency and of an obligate heterozygote for this enzyme abnormality, Ch. R., were compared with that into normal cells, at 1.4 mM extracellular Pi concentration (Table V). In the leukocytes of the patients with partial HGPRT deficiency, the rate of incorporation of the labeled formate into purines exceeded the mean normal rate by approximately 13-fold, but in the leukocytes of the obligate heterozygote for HGPRT deficiency (subject Ch. R.) the rate of incorporation was normal. In the leukocytes of subject A. R., the rate of [^{14}C]formate incorporation was studied also at 50 mM Pi in the incubation medium. At this extracellular Pi concentration, the rate of labeled formate incorporation was further accelerated, being 2.8-fold the rate measured at 1.4 mM Pi in the incubation medium.

DISCUSSION

In the present study on purine synthesis in human peripheral blood leukocytes, cells from normal subjects were demonstrated *in vitro* to incorporate [^{14}C]formate and [^{14}C]glycine into purines. The rate of incorporation of [^{14}C]formate into the normal leukocyte purines was found to be markedly lower, by about 10-fold, than that found in a previous study (10) for normal human cultured fibroblasts under similar conditions. Although this difference may in part reflect variation in endogenous formate pool size in these tissues, the low rate of activity of *de novo* purine synthesis in normal leukocytes, as gauged by formate incorporation, may be the explanation for the disagreement between various investigators concerning the presence of this pathway in these cells (2-5).

The pathway of purine synthesis *de novo* consists of 10 consecutive steps, the first step being the formation of phosphoribosylamine from PRPP and glutamine catalyzed by the enzyme glutamine-PRPP amidotransferase, and ending with the formation of the purine nucleotide inosinic acid. Glycine is incorporated into the purine molecule in the second step, and formate is incorporated in the third and ninth steps in the pathway (16). The presence in normal leukocytes of the first step of this pathway is evident from the presently demonstrated sensitivity of the rate of [^{14}C]formate incorporation into the leukocyte purines to inhibition by several compounds, known to inhibit purine synthesis *de novo* by affecting the activity of glutamine-PRPP amidotransferase. The purine bases adenine and hypoxanthine are inhibiting, through conversion into their nucleotide

forms, reactions catalyzed by the respective phosphoribosyltransferase. These conversions have a combined inhibitory effect on the glutamine-PRPP amidotransferase: by consumption of its substrate, PRPP, and by feedback inhibition on this enzyme by the formed ribonucleotides (17). A similar mechanism has been suggested for the purine analogues allopurinol and oxipurinol (18), whereas orotic acid is presumed to decrease purine production by depleting cellular PRPP only (19). Azaserine, an analogue of glutamine, competitively inhibits the glutamine requiring enzymes, glutamine-PRPP amidotransferase and phosphoribosyl-formylglycineamidase synthetase, which catalyze the first and fourth reactions, respectively, in the *de novo* purine synthesis pathways (20). Strong additional evidence for the existence of the complete pathway of purine synthesis *de novo* in normal leukocytes is the present finding of increased purine production in the leukocytes of gouty patients with partial HGPRT deficiency, as will be further discussed below.

The inhibitory effect of the PRPP-depleting compounds on purine synthesis in the normal leukocytes demonstrates, in addition to verifying the validity of the method employed, the regulatory role of PRPP availability on the rate of activity of this pathway in these cells. The inhibition experiments demonstrate that purine synthesis *de novo* in the leukocytes is decelerated by depletion of cellular PRPP. The finding of the decreased inhibitory effects of orotic acid and of hypoxanthine on the rate of purine synthesis in leukocytes, when incubated at high extracellular Pi concentration, may be taken to suggest that these compounds inhibit purine synthesis *de novo* mainly through consumption of the common substrate PRPP. On the other hand, the observed increased inhibitory effect of adenine at high Pi concentration presumably indicates that adenine inhibits purine synthesis *de novo*, more by feedback inhibition exerted by its nucleotides on the amidotransferase than by consumption of PRPP. The experiments with artificially high intracellular Pi concentration (produced by incubation at high Pi concentration in the incubation medium), show the ensuing acceleration of the pathway to be due to increased PRPP availability. The latter conclusion is based on the known accelerating effect of increasing Pi concentration on the activity of PRPP synthetase, both in cell-free systems (12, 21) and in intact cells, such as erythrocytes (22), Ehrlich ascites tumor cells (23), and rat liver slices (24). Indeed, in the present study we could show that increasing intracellular Pi concentration resulted in activation of leukocyte PRPP synthetase, as evidenced by increased PRPP content and availability for nucleotide synthesis. That the acceleration effect of increasing Pi concentration on purine synthesis *de novo* in the leukocytes is due to this

augmentation of PRPP availability and not to a direct action of Pi on enzymes involved in the de novo synthesis pathway also follows from the known inhibitory action of Pi on the activity of glutamine-PRPP amidotransferase (25), which is the rate-limiting enzyme in this pathway. Furthermore, the importance of PRPP availability in the regulation of purine de novo synthesis is manifest in the sigmoidal relationship between the increase in the rate of purine synthesis and the availability of PRPP in the intact leukocyte. As can be seen in Fig. 3, both in the presence and the absence of methylene blue (which activates the oxidative pentose shunt, ensuing at certain Pi concentration in increased PRPP availability, as discussed below) the almost linear increase in PRPP availability is associated with a sigmoidal increase in the rate of purine synthesis de novo. These results reflect the known sigmoidal response of glutamine-PRPP amidotransferase to increasing PRPP concentration in cell-free systems in the presence of physiological inhibitors (25, 26).

The regulatory properties of the purine biosynthesis pathway in respect to PRPP availability, as characterized in the present study on normal leukocytes, are in agreement with earlier studies of other investigators who utilized cultured fibroblasts as a model (27, 28). In addition, the accelerating effect of increasing Pi concentration on purine synthesis de novo demonstrated in the present study in the normal and in HGPRT-deficient leukocytes, is compatible with the findings of Nuki et al. (29). These investigators showed that increasing Pi concentration in the incubation medium resulted in augmentation in PRPP content and in acceleration of the early steps of purine synthesis de novo, both in normal and in HGPRT-deficient cultured lymphoblasts. The normal plasma Pi concentration normally ranges between 0.75 and 2 mM. In some pathological conditions it may rise to 3 mM, but rarely to a higher level. Thus, the finding in the present study of absence of effect of Pi concentration below 10 mM on the rate of de novo synthesis of purines may be taken to suggest that alteration in plasma Pi concentration within the physiological range does not affect purine synthesis in the leukocytes. Whether plasma Pi concentration at the physiological range affects the rate of purine synthesis in other tissues has yet to be clarified.

The leukocyte model was found useful in the study of the relationship between the activity of the oxidative pentose shunt and purine synthesis de novo. The marked accelerating action of increasing Pi concentration on PRPP generation in the leukocytes is consistent with the possibility that, at the physiological intracellular Pi concentration (i.e., at intracellular Pi concentration prevailing in leukocytes incubated at Pi concentration within the physiological range of plasma Pi concentration),

the cellular availability of ribose-5-phosphate (R-5-P) is not limiting for PRPP generation. It is of interest in this connection that methylene blue, which activates the R-5-P producing oxidative pentose shunt by acting as an electron acceptor for NADPH, did not increase PRPP generation nor the rate of purine synthesis at the physiological extracellular Pi concentration. Only upon increasing Pi concentration, presumably through activating PRPP synthetase, the activation of the pentose shunt caused increased production of PRPP and acceleration of purine synthesis de novo. Although these results support the suggestion made above concerning the leukocyte R-5-P availability being saturating for PRPP generation, this could not be proven as yet conclusively.

HGPRT deficiency in the gouty patients A. R. and H. R. was manifest in decreased rate of hypoxanthine incorporation into the nucleotides of their leukocytes. The incorporation values found in the leukocytes of these patients, 53 and 27% of the normal mean, respectively, are in accordance with the values found previously in their erythrocytes, 23.6 and 20.2% of the normal mean (30). Thus the leukocyte incorporation values are in agreement with our previous finding on erythrocytes of partially HGPRT-deficient subjects; that in these cells the functional activity of the enzyme is much higher than that obtained by the determination of the specific activity of the enzyme, as measured in cell lysates at saturating concentration of the substrate PRPP. Similar results with HGPRT-deficient leukocytes were reported by Balis et al. (31). It is of interest that, despite the relatively small functional deficiency in HGPRT activity in the leukocytes of the studied patients with partial HGPRT deficiency, the biochemical consequences of the defect in these cells were obvious: PRPP availability was significantly increased and purine synthesis de novo was accelerated more than 13-fold. This discrepancy-accumulation of PRPP, despite substantial residual activity of HGPRT, could be adequately explained if the leukocyte contained an alternative, non-PRPP-consuming, salvage pathway for synthesis of inosinic and from hypoxanthine, such as catalyzed by nucleoside phosphorylase and nucleoside kinase in the anabolic direction. Nevertheless, this pathway could not be detected in human tissues such as cultured fibroblasts (32) and erythrocytes (30). Its presence in leukocytes has yet to be assessed.

The accelerated incorporation of [¹⁴C]formate into purines in the leukocytes of the gouty subjects with partial HGPRT deficiency is important in various aspects. Firstly, it is an additional confirmation for the presence of the complete pathway of de novo purine synthesis in normal leukocytes, since the mechanism established for excessive purine production in this en-

zyme abnormality operates through supplying the first-committed enzyme of the pathway, glutamine-PRPP amidotransferase, with increased concentration of its substrate PRPP, and, possibly in addition, by diminishing the feedback inhibition on this enzyme consequent to the presumed lower nucleotide level in the affected cells (6). Indeed, increased availability of PRPP in leukocytes of the HGPRT-deficient subjects has been verified in the present study in two affected subjects. Furthermore, the accelerated purine production in leukocytes of such patients, associated with the increased availability of PRPP, may also be taken as an additional evidence for the regulatory role of PRPP for purine synthesis in this tissue. The still further increase in the rate of purine synthesis *de novo* in patient A. R.'s leukocytes, when incubated at high 50-mM Pi, indicates that even the elevated cellular PRPP availability prevailing in the HGPRT-deficient leukocyte is not saturating for purine synthesis *de novo*.

Secondly, the finding of excessive *de novo* purine synthesis in the HGPRT-deficient leukocytes adds another cell to those in which this biochemical manifestation of the enzyme defect has been demonstrated: cultured fibroblasts (6) and lymphoblasts (29). It is noteworthy that in patient A. R. with partial HGPRT deficiency, the magnitude of acceleration of purine synthesis in his leukocytes, as compared to leukocytes from normal subjects, was similar to that found for his fibroblasts, being 11,000 cpm/mg protein per h as compared with the normal mean of 722 ± 354.5 cpm/mg protein per h, i.e., 12-fold the normal rate (the method used for the fibroblasts was essentially the same as described for leukocytes, except that the incorporation of the labeled formate was measured into the purines excreted by the cells into the incubation medium only).

The normal rate of purine synthesis found in the leukocytes of subject Ch. R., an obligate heterozygote for partial HGPRT deficiency (a sister and at the same time a mother of affected subjects), may be taken to indicate that this X-linked enzyme abnormality is not expressed in her leukocytes. This finding is compatible with that of Dancis et al. (33), who demonstrated the absence of mosaicism in the lymphocytes of obligate heterozygotes for HGPRT deficiency. It has been previously established that HGPRT deficiency is not expressed in erythrocytes of heterozygotes for the virtually complete enzyme deficiency (the Lesch-Nyhan syndrome), and that it is also not expressed or only partially expressed in the erythrocytes of heterozygotes for the partial enzyme deficiency (34). The presently found normal purine synthesis in the leukocytes of the heterozygote for the partial enzyme deficiency, together with our previous finding of normal HGPRT activity in subject Ch. R.'s erythrocytes (7), may indicate the presence in heterozy-

gotes of a similar expression of this inherited defect in these two cell series.

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