

Variations in Purine Metabolism of Cultured Skin Fibroblasts from Patients with Gout

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ABSTRACT Purine metabolism was studied in fibroblasts cultured from three patients with gout in an attempt to determine the biochemical bases of their disease. The rate of purine biosynthesis de novo was normal in one line of cells, but the rate of catabolism of adenine nucleotides to hypoxanthine and inosine was greatly increased. The rate of purine biosynthesis de novo was increased in two lines of cells, and this was associated with increased concentrations of 5-phosphoribosyl 1-pyrophosphate. Purine synthesis was also less sensitive than normal to feedback inhibition. The catabolism of inosinate synthesized de novo was increased.

INTRODUCTION

Unlike most "inborn errors of metabolism," the hyperuricemia characteristic of gout probably results from a wide variety of metabolic defects. Accelerated purine biosynthesis de novo is observed in many, but not all, cases of gout (1, 2), and this has diverse causes which are just begin-

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ning to become known. It has recently been shown that deficiencies of the enzymes glucose-6-phosphatase (EC 3.1.3.9) (3, 4) and hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) (5, 6) can lead to increased rates of purine biosynthesis de novo, but there still remain a group of patients with accelerated purine synthesis in whom such enzyme activities are normal.

The bases of hyperuricemia in those gouty patients with normal rates of purine biosynthesis de novo has been less studied, but impaired renal handling of uric acid is known or believed to be involved in some of these cases (1, 7-10).

Fibroblasts grown in tissue culture have been shown to reproduce some of the biochemical abnormalities observed in the gouty patients in which they originated. Thus, where gout is associated with loss of hypoxanthine-guanine phosphoribosyltransferase, both this enzyme defect (5) and accelerated purine biosynthesis (11) are found in cultured cells. This paper reports the results of studies on skin fibroblasts cultured from two individuals who have gout associated with accelerated purine biosynthesis de novo, but in whom no specific enzymatic defect is known, and one gouty patient with a normal rate of purine biosynthesis de novo who has been shown to have abnormal purine metabolism in vivo.

METHODS

Punch biopsies were removed from the upper arm skin of normal males of ages 15-23 and of gouty patients. Fibroblast cultures were derived and cultivated as monolayers in Eagle's medium supplemented with nonessential amino acids, fetal calf serum, and neomycin sulfate. Fibroblasts were harvested for biochemical studies by

trypsinization, washed with 0.154 M sodium chloride, and suspended in Krebs-Ringer phosphate medium, pH 7.4. Cultures of approximately the same stage of growth were used for all experiments. The growth rates of fibroblasts from normal and gouty individuals were comparable under the conditions used. These procedures are described in detail elsewhere (11).

The *PP*-ribose-*P* content of fibroblasts was measured by a modification of the method of Henderson and Khoo (12). The incorporation of radioactive precursors into perchloric acid-soluble and nucleic acid purines and purine derivatives was measured as described by Henderson, Paterson, Caldwell, and Hori (13). α -*N*-Formylglycinamide ribonucleotide (FGR) was extracted from cells with ethanol and purified by electrophoresis. These procedures are described in detail elsewhere (11).

Lines of fibroblasts were derived from three gouty patients whose daily excretions of uric acid and serum uric acid values, measured while on a purine-free diet, are given in Table I. The purine metabolism in vivo of these patients has been reported by Kelley, Rosenbloom, and Seegmiller (14). Erythrocyte hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase (EC 2.4.2.7) activities were normal in all cases (5 and unpublished observations), as were creatinine clearances (14).

RESULTS

The rate of the early and rate-limiting reactions of purine biosynthesis de novo was determined by measuring the incorporation of radioactive formate into an intermediate of this pathway, FGR, when the subsequent reaction was completely and irreversibly inhibited by the antibiotic azaserine (15). The data in Table I show that the B. P. and T. B. lines of fibroblasts, taken from gouty patients with accelerated purine biosynthesis de novo in vivo, also showed increased rates of this biosynthetic pathway in vitro. These data also demonstrate that this rate in R. McJ.'s cells was not appreciably different from that in fibroblasts from normal individuals, a result which is consistent with previous measurements of this pathway in this patient in vivo (14). Therefore experiments were performed to determine if the accelerated purine biosynthesis de novo observed in two lines of cells might be due to; (a) an increased rate of synthesis or concentration of *PP*-ribose-*P*; (b) decreased sensitivity of the pathway to feedback inhibition by purine ribonucleotides; and (c) changes in the rates of synthesis or interconversion of purine ribonucleotides which might lead to changes in the concentrations of potential feedback inhibitory ribonucleotides. Abnormalities in purine metabo-

TABLE I
Purine Biosynthesis De Novo

Cell line	Serum uric acid*	Urinary uric acid*	FGR
	<i>mg/100 ml</i>	<i>mg/24 hr</i>	<i>cpm</i>
Normal			1740 (range 1380-1980)
R. McJ.†	9.9	417	1620
B. P.§	9.7	1529	6470
T. B.	10.5	1241	6320

Fibroblasts, 8 mg, wet weight, were incubated in Krebs-Ringer phosphate medium, pH 7.4, in an air atmosphere for 1 hr at 37°C with 4 mM glycine, 20 mM glutamine, 5.5 mM glucose, 0.3 mM azaserine, and 1.52 mM formate-¹⁴C. The normal value is the mean of separate analyses of 14 flasks in seven experiments using fibroblasts derived from four different individuals. The other figures are the means of separate analyses of six flasks in three experiments. The average variation between analyses of duplicate flasks was less than 10%.

* Reference 9.

† NIH-01-74-04, age 58.

§ NIH-06-35-21, age 27.

|| NIH-01-61-64, age 49.

lism which might lead to an increased rate of synthesis of precursors of uric acid were also sought in R. McJ.'s cells.

Table II shows the concentrations of free *PP*-ribose-*P* in normal cells and in fibroblasts derived from two gouty patients with accelerated purine synthesis. This substrate is elevated approximately twofold in both cell lines. The rate of *PP*-ribose-*P* synthesis can be approximated by measurement of the rate of adenine ribonucleotide synthesis at saturating concentrations of radioactive adenine

TABLE II
PP-Ribose-P Concentrations in Fibroblasts

Cell line	<i>PP</i> -ribose- <i>P</i>	Adenine nucleotide synthesis
	<i>mμmoles/g</i>	<i>mμmoles/g</i>
Normal	0.853	4.02
B. P.	1.52	4.16
T. B.	1.34	3.95

Fibroblasts, 16 mg, wet weight, were incubated with 5.5 mM glucose, or with 5.5 mM glucose and 0.5 mM adenine-³H. The normal values are the means of separate analyses of six flasks in three experiments, with two separate lines of fibroblasts. Other figures are means of separate analyses of four flasks in two experiments. The average variation between analyses of duplicate flasks was less than 10%.

(16). Data for the "availability" of *PP*-ribose-*P*, estimated by this method, are also presented in Table II, and they indicate that cell lines from B.P. and T.B. synthesized this substrate at approximately normal rates. The cells with increased *PP*-ribose-*P* levels may, therefore, have been utilizing this compound at less than normal rates under the conditions used.

Although free *PP*-ribose-*P* analyses were done in the absence of added glutamine or purines so that the rate of utilization would be minimal, the interpretation of these results is complicated by the presence of endogenous supplies of, or synthesis of, glutamine. The relative amount of glutamine present in these cell lines therefore was estimated from the relative rates of FGR synthesis measured in the presence and absence of added glutamine. Endogenous glutamine supported 53% of maximal FGR accumulation in normal cells, 48% in B.P.'s cells, and 61% in T.B.'s cells. These differences, if significant, probably do not contribute significantly to the observed increase in *PP*-ribose-*P* levels in B.P.'s and T.B.'s cells.

Table III shows the relative sensitivity of the lines of cells studied to feedback inhibition of purine biosynthesis *de novo* by exogenously supplied adenine, hypoxanthine, and 6-methylmercaptapurine ribonucleoside. All three compounds exert their inhibitory effects only after their conversion to ribonucleotides by adenine phosphoribosyltransferase, hypoxanthine-guanine phosphoribosyltransferase, and adenosine kinase, respectively (15, 17-20). R. McJ.'s cells were only

TABLE III
Feedback Inhibition of Purine Biosynthesis De Novo

Additions	Concentration <i>mole/liter</i>	FGR inhibition %			
		Normal	R. McJ.	B. P.	T. B.
Adenine	10 ⁻³	81.8	70.6	51.2	60.9
	10 ⁻⁴	75.2	64.7	30.3	36.7
Hypoxanthine	10 ⁻³	63.4	51.3	44.0	32.4
	10 ⁻⁴	53.3	51.3	17.2	12.4
6-Methylmercaptapurine ribonucleoside	10 ⁻³	80.5	74.7	62.9	58.9
	10 ⁻⁴	80.0	69.8	20.5	34.6

Fibroblasts, 8 mg, wet weight, were incubated as described in Table I with various concentrations of adenine, hypoxanthine, or 6-methylmercaptapurine ribonucleoside. The normal values are the means of separate analyses of six flasks in three experiments, with two separate lines of fibroblasts. Other figures are means of separate analyses of four flasks in two experiments. The average variation between analyses of duplicate flasks was less than 10%.

TABLE IV
Interconversion of Purine Nucleotides in Fibroblasts

Precursor	Cell line	Acid-soluble		Nucleic acid	
		Adenine	Guanine	Adenine	Guanine
		<i>cpm/μg</i>	<i>cpm/μg</i>	<i>cpm/μg</i>	<i>cpm/μg</i>
Adenine	Normal	—	4110	1427	43.3
	R. McJ.	—	5760	1390	55.4
	B. P.	—	3876	1248	48.1
	T. B.	—	3482	1658	39.6
Guanine	Normal	1960	—	263	16.6
	R. McJ.	2370	—	302	17.2
	B. P.	1635	—	286	13.6
	T. B.	1990	—	202	21.4
Hypoxanthine	Normal	2041	83.6	54.7	5.3
	R. McJ.	2760	87.6	65.1	7.8
	B. P.	1835	72.4	48.8	5.1
	T. B.	2380	85.9	62.4	6.9

Fibroblasts, 16 mg, wet weight, were incubated with 5.5 mM glucose and either 0.5 mM adenine-¹⁴C, 0.5 mM hypoxanthine-¹⁴C, or 0.1 mM guanine-¹⁴C. Each figure is the mean of separate analyses of two flasks in one experiment. The average variation between analyses of duplicate flasks was less than 15%.

slightly less sensitive to feedback inhibition than was this reaction sequence in normal fibroblasts, and these results are compatible with the normal rate of purine biosynthesis *de novo* observed both *in vitro* and *in vivo*. However, it is also apparent that the cells from both B.P. and T.B. were considerably less sensitive to inhibition by all three compounds than were normal cells. Therefore, this could be a possible basis for the acceleration of purine biosynthesis *de novo* observed in these cell lines.

The metabolism of radioactive purine bases was then studied in order to measure the relative rates of other pathways of purine ribonucleotide metabolism. These data are shown in Table IV where it is seen that the rates of several of the purine ribonucleotide interconversion reactions were increased in the cells from R. McJ. Although the data are not shown here, it was determined that the total amount of nucleotides formed from adenine, guanine, and hypoxanthine were approximately the same in these cells as in normal fibroblasts. In contrast, the extent of conversion of purine bases to nucleotides, and that of the interconversion of the ribonucleotides, was apparently normal in most respects in the cells from B.P. and T.B.

A key intermediate in purine ribonucleotide metabolism is inosinate, not only because it is the central compound in the cycles of purine ribonucleotide interconversion and the end product of

purine biosynthesis de novo, but also because it is one of the immediate precursors of the hypoxanthine which is oxidized to uric acid, and whose overproduction leads to gout. The catabolism of inosinate to inosine and hypoxanthine therefore was measured when inosinate was synthesized either de novo or via adenine nucleotides. (Because these cells lack xanthine oxidase, the hypoxanthine formed was not converted to uric acid. Inosine was measured together with hypoxanthine because the inosine formed from inosinate or adenosine could not be distinguished from that formed from hypoxanthine and ribose-1-phosphate.) The data presented in Table V show that in R. McJ.'s cells the catabolism of inosinate formed de novo occurred at normal rates, as did the de novo process itself. In contrast, adenine was converted to hypoxanthine and inosine at very much higher rates in these cells than in normal fibroblasts. This is in contrast to cells of two normal individuals, cells from B.P. and T.B., two patients with gout of unknown origin, and cells from three patients with gout due to deficiency of hypoxanthine-guanine phosphoribosyltransferase, in which the rate of this process was always normal (11).

The conversion of adenine to hypoxanthine and inosine must take place via the previous conversion of adenine to adenylylate, since animal cells do not have either adenine deaminase or adenosine phosphorylase. Whether adenylylate is converted to these catabolic products via inosinate or via adenosine cannot be determined from the data presently available.

TABLE V
Purine Nucleotide Catabolism

Precursor	Hypoxanthine plus inosine			
	Normal	<i>cpm</i>		
		R. McJ.	B. P.	T. B.
Adenine	2440	49660	2890	2690
Formate	13800	12600	16900	18100

Fibroblasts, 8 mg, wet weight, were incubated either with 5.5 mM glucose, 4 mM glycine, 20 mM glutamine, and 1.52 mM formate-¹⁴C, or with 5.5 mM glucose and 0.5 mM adenine-³H. The normal values are the means of separate analyses of six flasks in three experiments, with two separate lines of fibroblasts. Other figures are means of separate analyses of four flasks in two experiments. The average variation between analyses of duplicate flasks was less than 10%.

The data in Table V also show that, as expected, an increased amount of the inosinate formed de novo in cells which have accelerated rates of this process broke down to inosine and hypoxanthine.

DISCUSSION

This study has shown that skin fibroblasts grown in tissue culture from gouty patients produce precursors of uric acid at increased rates. Cells from two patients in whom purine biosynthesis de novo was accelerated in vivo also have increased rates of this process in vitro, and derive increased amounts of hypoxanthine and inosine from the inosinate so produced. Fibroblasts from another individual have normal rates of purine biosynthesis, as does the patient in vivo, and in this case increased amounts of hypoxanthine and inosine are formed by an accelerated catabolism of adenine nucleotides.

Two changes were noted in the cells from B.P. and T.B. which could be responsible for accelerated purine biosynthesis de novo. These changes were an increased concentration of *PP*-ribose-*P* and a decreased sensitivity of this pathway to feedback inhibition. Increased *PP*-ribose-*P* levels have also been observed in fibroblasts whose accelerated purine biosynthesis was associated with deficiency of hypoxanthine-guanine phosphoribosyltransferase (11). To be relevant to the accelerated purine synthesis observed in vivo, this substrate would have to be a rate-limiting factor for this process, and there is some indirect evidence which is compatible with this idea.

Altered feedback inhibition of purine biosynthesis de novo has previously been suggested as a possible cause of gout (see 1). Because of the technical difficulties of growing 2–3 g of each type of cells, the concentrations of each of the various purine ribonucleotides which are or might be feedback inhibitors has not been measured. The normality of the isotope incorporation and interconversion data suggest, but do not prove, that no major changes in ribonucleotide pool sizes have occurred in the cells from B.P. and T.B. Likewise, there is no indication that the catabolism of at least adenine nucleotides has increased in these fibroblasts.

A second possible explanation for the apparent alteration of sensitivity of the purine biosynthetic pathway to feedback inhibition is that the target

of this inhibition, *PP*-ribose-*P* amidotransferase, is abnormal in the fibroblasts derived from gout patients. A similar observation has been made recently in an experimental tumor resistant to 6-methylmercaptapurine ribonucleoside and evidence for an altered amidotransferase was presented (21). Although the present data are more easily interpreted in this way, definitive assays of this enzyme and measurements of its sensitivity to purine ribonucleotides performed without the possibility that experimental manipulation has altered its sensitivity are required before a more positive conclusion can be reached on this point. It is to be noted that both patients responded normally *in vivo* to feedback inhibition of purine biosynthesis *de novo* by azathioprine 6-[(1'-methyl-4'-nitro-5'-imidazolyl)-thiopurine].

An increased rate of catabolism of adenine nucleotides was noted in cells from one gouty patient, and other findings in the same patient *in vivo* are compatible with the increased destruction of other nucleotides as well. Thus he is completely insensitive to feedback inhibition of purine biosynthesis by azathioprine (14), an effect which is believed to be mediated by 6-mercaptapurine ribonucleotide, and an increased rate of catabolism of azathioprine has been demonstrated in this patient (14). These abnormalities in purine metabolism *in vivo* lend support to the idea that this case of gout is not due merely to impaired renal handling of uric acid.

Although the rate of conversion of adenine to hypoxanthine and inosine was increased about 20-fold in R. McJ.'s cells, the actual amount of adenine involved in this process was only a few per cent of the total amount of adenine converted to nucleotides. Therefore, it is not surprising that little or no difference was detected in the amounts of nucleotides formed in normal and R. McJ.'s cells.

Attempts to find the enzymatic basis of increased nucleotide destruction in fibroblasts have so far been unsuccessful. Such studies are complicated by the fact that the identity of the enzyme(s) responsible for nucleotide catabolism under physiological conditions in intact cells is not known, nor is the relationship to intracellular compartmentation of their substrates (22), nor the factors which control their rates.

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