JCI The Journal of Clinical Investigation

Mutant feedback-resistant phosphoribosylpyrophosphate synthetase associated with purine overproduction and gout. Phosphoribosylpyrophosphate and purine metabolism in cultured fibroblasts.

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J Clin Invest. 1975;56(5):1093-1099. https://doi.org/10.1172/JCI108183.

Research Article

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Mutant Feedback-Resistant Phosphoribosylpyrophosphate Synthetase Associated with Purine Overproduction and Gout

PHOSPHORIBOSYLPYROPHOSPHATE AND PURINE METABOLISM IN CULTURED FIBROBLASTS

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ABSTRACT We have reported previously two siblings with gout and uric acid lithiasis associated with excessive purine production. In the erythrocytes of these patients, phosphoribosylpyrophosphate (PRPP) synthetase exhibited resistance to feedback-inhibition by normal cell constituents such as guanosine-5′-diphosphate (GDP) and adenosine-5′-diphosphate (ADP), resulting in superactivity of the mutant enzyme and consequently in increased PRPP content and availability for nucleotide synthesis. Erythrocyte PRPP content and availability were normal in the propositus' parents, his healthy brother and three sons, and they all had normal serum level and urinary excretion of uric acid, except for the mother who was hyperuricosuric. To further characterize this mutation we studied PRPP and purine metabolism in cultured fibroblasts of the affected family. PRPP synthetase in dialyzed lysates of fibroblasts from the propositus and his mother exhibited increased specific activity, more markedly at low inorganic phosphate concentration, and decreased sensitivity to inhibition by ADP and GDP. PRPP content and availability and the rate of *de novo* purine nucleotide synthesis were markedly increased in the fibroblasts of the propositus and to a lesser extent in the fibroblasts of his mother but were normal in the fibroblasts of the other family members investigated. The fibroblast studies demonstrate the following sequence of abnormalities: feedback-resistance of PRPP synthetase; superactivity of this enzyme in normal physiological milieu; increased availability of PRPP; and increased *de novo* synthesis of purine nucleotides. The pattern of inheritance of this disorder is compatible with both an X-linked recessive and autosomal dominant traits.

INTRODUCTION

Phosphoribosylpyrophosphate (PRPP)[1] synthetase catalyzes the formation of PRPP from ribose-5-phosphate and ATP. The enzyme is activated by inorganic phosphate (P_i) and inhibited by several cellular compounds such as adenosine-5′-diphosphate (ADP) and guanosine-5′-diphosphate (GDP) (1). PRPP is a substrate for PRPP-amidotransferase, the first uniquely committed enzyme in the *de novo* synthesis of purine nucleotides, and is a potent regulator of this pathway. In human tissue the concentration of PRPP is limiting for the PRPP-amidotransferase reaction (2). Furthermore, the velocity response of this reaction to PRPP concentration is sigmoidal in the presence of physiological inhibitors (3), a significant acceleration occurring in response to a relatively minor increase in the concentration of this substrate. Studies in vitro, utilizing cultured human skin fibroblasts, have demonstrated that methylene blue-induced increase of PRPP concentration accelerates purine synthesis *de novo* (4).

A preliminary report was presented at the Annual Meeting of the Israeli Association of Chemical Pathology, Rehovot, Israel, March 1974.

Professor de Vries is an Established Investigator of the Chief Scientist's Bureau, Ministry of Health.

Received for publication 6 February 1975 and in revised form 7 July 1975.

[1] *Abbreviations used in this paper:* APRT, adenine phosphoribosyltransferase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PCA, perchloric acid; P_i, inorganic phosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate.

In accordance with the above, it has been proposed that increased PRPP availability could be the common causal denominator in the accelerated *de novo* purine nucleotide synthesis associated with all known underlying enzymic abnormalities in man. Increased PRPP availability has been demonstrated in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency, complete in the Lesch-Nyhan syndrome, partial in some patients with metabolic gout (5), and has been suggested in glucose-6-phosphatase deficiency (glycogen storage disease type I) (6) and superactivity of glutathione reductase (7). Recently we reported a new familial enzyme abnormality associated with excessive purine production, gout, and uric lithiasis (8–10). PRPP synthetase in the eythrocytes of the two affected siblings exhibited feedback-resistance to inhibition by several cellular compounds such as GDP and ADP, resulting in superactivity of the enzyme in the normal physiological milieu and consequently in increased PRPP content and availability for nucleotide synthesis. Study of other family members revealed normal PRPP availability in their erythrocytes, and serum level and urinary excretion of uric acid were found to be normal, except in the mother who was hyperuricosuric.

Two additional families with gout associated with increased PRPP synthetase activity have been described subsequently by Becker et al. (11, 12). In these PRPP synthetase mutations the enzymes respond normally to the regulatory cofactors, and the structural alteration appears to involve the catalytic site of the enzyme only.

Cultured human fibroblasts possess the principal pathways of PRPP generation and, in contrast to the erythrocyte, also the complete pathways of purine metabolism, except for the last two steps of purine degradation catalyzed by the enzyme xanthine oxidase (13). In the present study, cultured skin fibroblasts from the family affected with the mutant feedback-resistant PRPP synthetase were utilized to characterize the metabolic abnormalities caused by the mutation and to obtain data concerning the genetic pattern of this disorder.

METHODS

Subjects. Propositus O. G., at present 38-yr-old, has a history of recurrent bilateral uric acid lithiasis since the age of 14 and of severe gouty arthritis since the age of 20. Since the age of 29 he has received Allopurinol with excellent response. When temporarily off treatment his serum uric acid rose to 13.5 mg/100 ml, and his 24-h urinary uric acid excretion increased to 2,400 mg. The excessive rate of urine production in this patient was verified by the measurement of [^{15}N]glycine incorporation into his urinary uric acid, amounting in 7 days to 3.32% of the administered dose, as compared to values of 0.12, 0.13, and 0.17% found in three normal subjects (14). The similarly affected brother (H. G.) of the propositus was not available for the present study. There was no urolithiasis or gout in other family members, nor abnormality in serum level or urinary excretion of uric acid, except in the propositus' mother who is hyperuricosuric (Table I). The case report of patient T. N. with Lesch-Nyhan syndrome, studied for comparison, was given elsewhere (15). Five healthy laboratory staff numbers served as controls.

Chemicals. [8-^{14}C]Adenine (59 mCi/mmol), [8-^{3}H]adenine (500 mCi/mmol), [8-^{14}C]hypoxanthine (59 mCi/mmol), [8-^{14}C]guanine sulphate (56 mCi/mmol), and Na [^{14}C]formate (59 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, England. Purine bases, nucleotides, and other fine chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other reagents were of analytical grade.

Cell growth. Biopsies (3 mm) were removed from the forearm skin of the propositus (O. G.), from his healthy brother (A. G.), his parents (father I. G., mother D. G.), three of his four sons (Al. G., Am. G., and Ra. G.), five normal unrelated subjects, and the patient (T. N.) with Lesch-Nyhan syndrome. The biopsies were minced, placed in Falcon Plastics (Oxnard, Calif.) sterile disposable bottles and flooded with Eagle's minimal essential medium containing Earle's balanced salt solution (Grand Island Biological Co., Grand Island, N. Y., F-15), 15% fetal calf serum, folic acid (50 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and mycostatin (25 μg/ml). The media were saturated with a 5% CO_2-95% air environment, and the bottles were stoppered and incubated at 37°C until outgrowth was apparent. The cells were then subcultered and serially propagated until they become confluent. Cells used were from the 4th–15th passage and from confluent monolayers. In the study of purine synthesis *de novo,* in which the excretion of labeled purines bases into the medium was determined, nonconfluent cultures were employed. Cultures for each experiment were selected to be from a similar passage.

Assay of PRPP synthetase. The fibroblasts were harvested by brief trypsinization, washed four times with saline, and suspended to the desired concentration in 4 mM potassium phosphate buffer. Fibroblast extracts were prepared by rapid freezing and thawing of the cells four times in dry ice and acetone. After centrifugation at 40,000 *g* for 25 min at 4°C, the supernate was dialyzed against 4,000 vol of 4 mM potassium phosphate buffer, pH 7.4, at 4°C for 5 h. PRPP synthetase was assayed essentially as described by Fox and Kelley (16). The reaction mixture, a total volume of 200 μl, contained 30–150 μg protein of the dialyzed cell lysate, 0.5 mM ATP, 0.5 mM ribose-5-phosphate, 5 mM magnesium chloride, and 2.5 mM mercaptoethanol in potassium phosphate buffer, pH 7.4, of the specified molarity. Incubation was carried for 15 min at 37°C. The PRPP was extracted by heat and determined as described (16), except that [8-^{14}C]guanine (80 μM) was utilized instead of [8-^{14}C]adenine. The labeled nucleotide was separated by thin-layer chromatography (TLC) on microcrystalline cellulose developed in butanol : methanol : H_2O : NH_4OH 25% (60 : 20 : 20 : 1 vol/vol) (17). The nucleotide spots were scraped off and counted in a liquid scintillation counter (Packard Tri-Carb model 3380, Packard Instrument Co., Downers Grove, Ill.). The results are expressed in relation to the milligrams protein in dialyzed fibroblast lysate. The time of incubation and the amount of lysate protein in the experiments were selected to be within the linear range of the reaction velocity. The inhibitory effect of ADP and GDP on enzyme activity was studied by the addition of these nucleotides into the incubation mixture in the concentration specified. In these experi-

TABLE I

Serum Level and Urinary Excretion of Uric Acid in Studied Subjects

Subject		Age	Wt	Uric acid*	
				Serum	Urine
		yr	*kg*	*mg/100 ml*	*mg/24 h*
Control subjects‡		20–45		<7.0	<800
Affected family					
Propositus	O. G.	38	95	13.5	2,400
Father	I. G.	68	78	6.7	880
Mother	D. G.	68	70	5.3	1,100
Healthy brother	A. G.	23	80	6.0	
Sons	Al. G.	16	61	5.2	361
	Am. G.	9	26	3.7	330
	Ra. G	10	26	3.7	300
	T. G.	4	16		220
Lesch-Nyhan syndrome	T. N.	7		11.5	570

* Values in the patients were obtained either before onset of treatment (T. N.) or after 10 days cessation of treatment (O. G.). Patients were on low purine diet and uric acid was determined by an enzymatic spectrophotometric method. The uric acid values given for the control subjects are the upper normal limit for normal subjects on normal home diet in our laboratory (colorimetric autoanalyzer method).

‡ Three females and two males.

ments the absolute activities of the PRPP synthetases of normal and mutant cell lysates were equalized by proper dilution.

PRPP content. Cells were harvested and washed as described above. 1.5–3.0 × 10^6 cells were suspended in 120 μl of distilled water and subjected to freezing and thawing four times. 100 μl of the cell lysate were transferred to a tube containing 100 μl of 10 mM EDTA in 10 mM Tris-HCl, pH 7.4, heated at 85°C for 2 min, chilled, and centrifuged at 3,000 rpm for 10 min. PRPP, extracted in the supernate, was assayed as described by Fox and Kelley (16). The reaction mixture, a total volume of 250 μl, contained 150 μl of the PRPP extract, 25 mM $MgCl_2$, 0.2 mM [8-^{14}C]hypoxanthine (25 mCi/mmol), 100 μg of partially purified human erythrocyte HGPRT (DEAE-cellulose extract treated for 2 min at 85°C to eliminate PRPP synthetase activity [18]) in 55 mM Tris-HCl buffer, pH 7.4. After incubation for 60 min, the reaction was stopped by the addition of 40 μl of 15% perchloric acid (PCA) in ice. [^{14}C]Inosine-5-monophosphate in the protein-free supernate was separated by TLC and counted as described above. Controls in which PRPP was added to cell lysates before heating revealed a recovery of at least 95%. The results are related to total cell protein.

PRPP availability. Cellular PRPP availability for nucleotide synthesis was assayed by measuring [^{14}C]adenine and [^{14}C]hypoxanthine incorporation into the intact cell total nucleotide pool. 1.0–2.0 × 10^6 washed trypsinized cells were suspended in 0.5 ml of fresh growth media containing 80 μM of [8-^{14}C]adenine or [8-^{14}C]hypoxanthine. The cell suspension were incubated in a shaking bath at 37°C for 15 min. After incubation the tubes were put immediately in ice, 10 ml of ice-cold saline was added, and the cells were washed five times with ice-cold saline, suspended in 0.5 ml of distilled water, and subjected to freezing and thawing four times. Protein and nucleic acids were precipitated by addition of 40 μl cold 42% PCA, and the total ^{14}C-labeled nucleotides were separated and counted as described above. A saturating concentration of the labeled purine bases was used, the reaction exhibiting linearity for at least 30 min. The results are related to total cell protein.

Purine synthesis de novo. The rate of purine nucleotide synthesis *de novo* was studied in intact fibroblasts by measuring incorporation of [^{14}C]formate into the total cellular nucleotide pool (soluble in cold 1 N PCA) and also into the total purines excreted into the incubation medium. The procedure was essentially that described by Martin and Owen (19) and Green and Martin (20) for cultured mutagenized hepatoma cells. For the study of incorporation of [^{14}C]formate into the total cellular nucleotide pool, 2.0–3.0 × 10^6 cells were suspended in fresh 10 ml growth medium containing 20 μCi sodium [^{14}C]formate. The cell suspensions were gassed with 5% CO_2-95% air and incubated for 2 h in a shaking bath at 37°. After incubation, the flasks were placed in ice and immediately centrifuged at 4°C, washed five times with ice-cold saline, and suspended in 0.5 ml of distilled water and subjected to freezing and thawing seven times. 0.5 ml 2 N PCA and 0.2 μCi of [8-^{3}H]adenine were added, and the tubes were throughly mixed and put in ice for 1 h. After centrifugation the purine nucleotides in the supernate were hydrolyzed at 100°C for 1 h. The hydrolysate was adjusted with water to a volume of 1.5 ml and chilled and 0.7 mg adenine was added to each tube as carrier. The purine bases were then precipitated as a purine-silver complex, washed, and assayed for radioactivity (19). Incorporation of [^{14}C]formate into the purines excreted by the cells into the growth medium was measured essentially as described by Green and Martin (20). 0.5–1.5 × 10^6 cells growing in nonconfluent monolayer in 75-cm^2 Falcon Plastics culture flasks were incubated for 6 h with 10 ml of fresh growth medium containing 10 μCi [^{14}C]formate. At the end of incubation, the purines in the total volume of the growth medium were

precipitated and counted (19). The results of both methods are corrected for incomplete recovery of purines, utilizing tritiated adenine as internal standard, and are related to total cell proein (21). In the fibroblasts of all subjects studied, labeling of purine nucleotides and of purines excreted into the medium was linear with time for at least 6 and 8 h, respectively, and for a fixed period of incubation was also proportional to the number of cells incubated.

RESULTS

Properties of PRPP synthetase. The mean activity of PRPP synthetase in dialyzed lysates of cultured fibroblasts from the propositus (O. G.) was significantly higher than that from the control subjects, the increase being more marked at the lower P_i concentration applied (2.95-fold, $P < 0.025$) than at the higher P_i concentration (1.27-fold, $P < 0.05$). The specific activity of PRPP synthetase in fibroblast lysates from the mother (D. G.) was significantly increased ($P < 0.05$) only at low P_i concentration (Table II). Furthermore, the enzyme in the dialyzed lysates of fibroblasts from the propositus (O. G.) and from his mother (D. G.) exhibited decreased sensitivity to feedback-inhibition by ADP and GDP, the residual activity of the mutant enzyme in the presence of the inhibitor being approximately two to fourfold that of the normal enzyme, the ratio depending on the lysate protein concentration employed for study (Table III).

PRPP content and availability. The mean PRPP concentration in the cultured fibroblasts of the propositus (O. G.) was 3.7-fold the mean PRPP concentration of normal cells, a value above the 99.5% confidence limit (> normal mean + 3 SD) (Table IV). Fibroblasts from the patient's mother (D. G.) contained approximately 150% the normal mean PRPP concentration (> normal mean + 2 SD). Fibroblasts of the patient with Lesch-Nyhan syndrome (T. N.) contained the highest PRPP concentration, being about fivefold the normal mean.

The rate of incorporation of labeled adenine and of labeled hypoxanthine into the total nucleotide pool of the intact cultured fibroblasts was taken to reflect the PRPP availability in the cells (Table IV). The rate of adenine incorporation into the nucleotides was accelerated 1.7 times normal in the fibroblasts of the propositus (O. G.) (> normal mean +3 SD) and about 1.4 times normal in his mother's (D. G.) fibroblasts (> normal mean + 2 SD). Fibroblasts from other family members, including the three studied sons of the propositus, exhibited normal rates of adenine incorporation. The rate of adenine incorporation into the nucleotides of the fibroblasts of the patient with Lesch-Nyhan syndrome (T. N.) was accelerated, being 1.6-fold the normal rate (> normal mean + 3 SD). Hypoxanthine incorporation into the nucleotides of the fibroblasts of the propositus (O. G.) and of his mother (D. G.), in comparison to the fibroblasts of the control subjects, was even more accelerated, being 2.6- and 2.1-fold the normal, respectively (> normal mean + 3 SD). On the other hand, hypoxanthine incorporation into the nucleotides of the fibroblasts cultured from the patient with Lesch-Nyhan syndrome (T. N.) was hardly detectable.

De novo synthesis of purine nucleotides. The rate of incorporation of [^{14}C]formate into the nucleotide pool of the fibroblasts was accelerated in the propositus (O. G.) by 3-fold, in his mother (D. G.) by about 2-fold and in the Lesch-Nyhan patient (T. N.) by approximately 10-fold the mean normal rate, all these values being above the normal mean + 3 SD limit. The rate of excretion into the growth medium of labeled purines by the fibroblasts during incubation with [^{14}C]-

TABLE II

Specific Activity of PRPP Synthetase in Dialyzed Fibroblast Lysates

P_i concentration	PRPP synthetase activity*		
	Control subjects	Propositus O. G.	Mother D. G.
mM	*nmol/mg protein/h*		
1.6	20 (7)	59 (4)	40 (1)
50.0	267 (8)	339 (6)	283 (2)

* Data refer to mean values. The number in parentheses indicates the number of experiments; each experiment in the controls was carried out on cells pooled from the five subjects.

TABLE III

*Sensitivity of PRPP Synthetase in Dialyzed Fibroblast Lysates to Feedback-Inhibition**

Inhibitor	Concentration	Activity of PRPP synthetase‡		
		Control subjects	Propositus O. G.	Mother D. G.
	mM	*nmol/h/tube*		
Low protein concentration				
—	—	0.416	0.436	
ADP	0.05	0.087	0.213	
	0.10	0.036	0.143	
GDP	0.05	0.133	0.213	
	0.10	0.095	0.205	
High protein concentration				
—	—	8.3	9.9	10.0
ADP	0.05	0.645	2.58	2.61
	0.10	0.408	0.84	0.98
GDP	0.05	1.30	5.79	6.02
	0.10	1.18	3.42	4.20

* P_i concentration in incubation mixture 1.6 mM.

‡ Values represent mean of experiments, three at the low enzyme activity (low protein concentration) and two at the high enzyme activity (high protein concentration). Each experiment in the controls was carried out on cells pooled from two to three subjects. The absolute activities of normal and mutant enzymes were equalized by proper dilution.

TABLE IV

*PRPP Content and Availability in Cultured Fibroblasts**

Subjects		PRPP content	Incorporation into cellular nucleotides	
			[8-^{14}C]Adenine	[8-^{14}C]Hypoxanthine
		nmol/mg protein	*pmol/mg protein/min*	
Control subjects‡		0.126±0.0768 (10)	106.81±24.51 (12)	56.8±8.92 (3)
Affected family				
Propositus	O. G.	0.466 (5)	188.0 (10)	141.0 (3)
Father	I. G.	0.062 (2)	91.9 (2)	
Mother	D. G.	0.329 (2)	157.8 (4)	124.0 (1)
Healthy brother	A. G.		128.3 (5)	
Sons	Al. G.		88.3 (1)	
	Am. G.		76.6 (1)	
	Ra. G.		109.1 (1)	
Lesch-Nyhan syndrome	T. N.	0.634 (2)	183.7 (4)	<1.0 (3)

* Values represent for control subjects mean ±1 SD, for all other subjects means only. Number of experiments is indicated in parentheses.

‡ Five subjects for PRPP content, six for adenine incorporation, and three for hypoxanthine incorporation.

formate was accelerated in the propositus (0. G.) by 15-fold, in his mother (D. G.) by 9-fold and in the Lesch-Nyhan patient (T. N.) by 10-fold the normal mean. All these values are above the normal mean + 3 SD limit. The fibroblasts of the patient's father (I. G.), healthy brother (A. G.), and three sons exhibited normal rates of purine synthesis *de novo* (Table V).

DISCUSSION

In this study we demonstrate the presence of mutant feedback-resistant PRPP synthetase in the fibroblasts of the propositus (O. G.) and of his mother (D. G.), members of a family affected with excessive purine production associated with gout and uric acid lithiasis. In the propositus and his similarly affected brother (H. G.) this mutation was previously detected in the erythrocytes. Similarly to the erythrocyte mutant enzyme (10), the PRPP synthetase in the propositus' fibroblasts exhibited resistance to inhibition by the purine nucleotides ADP and GDP. A similar resistance to inhibition was exhibited by the PRPP synthetase in lysates of the mother's (D. G.) fibroblasts. The finding of increased specific activity of PRPP synthetase in the dialyzed fibroblast lysate of the propositus, especially at high P_i concentration, appears inconsistent with the normal or even lower specific activity of the enzyme previously found

TABLE V

*De Novo Synthesis of Purine Nucleotides in Cultured Fibroblasts**

Subjects		Incorporation of [^{14}C]formate into cellular nucleotides	Excretion into medium of labeled purines synthetized from [^{14}C]formate
		cpm/mg protein/h	
Controls‡		2,785±684.66 (9)	722±354.55 (9)
Affected family			
Propositus	O. G.	9,536 (16)	11,187 (7)
Father	I. G.	1,542 (3)	548 (2)
Mother	D. G.	6,602 (5)	6,451 (3)
Healthy brother	A. G.	2,336 (4)	1,201 (3)
Sons	Al. G.	1,363 (1)	857 (1)
	Am. G.	1,155 (1)	1,383 (1)
	Ra. G.	2,524 (1)	690 (1)
Lesch-Nyhan syndrome	T. N.	26,398 (8)	7,500 (2)

* Values given are for controls mean ±1 SD, and for all other subjects means only; the number of experiments is indicated in parentheses.

‡ Five subjects.

in this subject's dialyzed hemolysates (9). The ratio of the specific activity of the mutant enzyme to that of the normal enzyme in dialyzed fibroblast lysate was higher at low than at high P_i concentration. In this respect the behavior of the mutant enzyme in the dialyzed fibroblast lysate of the propositus (O. G.) resembles that of the mutant enzyme in nondialyzed hemolysate from this patient (9). In the nondialyzed hemolysate the mutant enzyme exhibited, in comparison to the normal enzyme, a higher specific activity at P_i concentration lower than 2 mM but a normal specific activity at higher P_i concentration. This difference was shown to reflect the effect of physiological inhibitors, in the presence of which the normal enzyme activity exhibited a sigmoidal response whereas the mutant enzyme exhibited a hyperbolic response with increasing P_i concentration. Partial purification of the erythrocyte PRPP synthetase caused both the mutant and the normal enzyme to exhibit similar hyperbolic response to increasing P_i concentration, whereas on addition of inhibitors, such as ADP, the difference between the enzymes reappeared. Thus, it is possible that in the dialyzed fibroblast lysate, the observed higher specific activity of the propositus' PRPP synthetase is due to the presence in such preparations of inhibitors either in trace amount or enzyme-bound.

The feedback-resistance of the PRPP synthetase in the fibroblast of the propositus and his mother manifest in superactivity of the enzyme, resulted in increased cellular content and metabolic availability of PRPP. The metabolic availability of PRPP was assessed by measuring the incorporation of labeled adenine and of labeled hypoxanthine into the intact fibroblast total nucleotide pool. The rate of these incorporations is dependent on the activity of the respective phosphoribosyltransferase, as well as on the size of the cold intracellular pools of adenine and hypoxanthine. The latter parameters were not determined, but the specific activities of adenine phosphoribosyltransferase (APRT) and HGPRT were found normal in the lysate of the propositus' fibroblasts; being 238 and 106 nmol/mg protein per h, respectively, as compared to the normal range as determined in our laboratory, 200-300 nmol/mg protein per h for APRT and 100-180 nmol/mg protein per h for HGPRT (22). In view of the normal activity of the phosphoribosyltransferases and of the higher PRPP content demonstrated in the cells of the propositus and his mother, it seems most likely that the increased rate of adenine and of hypoxanthine incorporation into their fibroblast nucleotides, by 66 and by 149%, respectively, reflects increased metabolic availability of PRPP. Increased PRPP availability was also found in the fibroblasts of the Lesch-Nyhan patient (T. N.), but the underlying mechanism in these mutations is different, being caused in the PRPP synthetase mutation by increased PRPP synthesis and, in HGPRT deficiency by decreased PRPP utilization. The HGPRT deficiency in the fibroblasts of the Lesch-Nyhan patient (T. N.) was manifest also in the negligible incorporation of hypoxanthine into fibroblast nucleotides, similarly to what has been found by other investigators (23).

In this study we demonstrated accelerated *de novo* purine nucleotide synthesis in the fibroblasts of the propositus (O. G.) and his mother (D. G). Increased PRPP availability is the most likely explanation for the excessive rate of purine production in these two subjects. A similar mechanism is held to be operating in cells of patients with Lesch-Nyhan syndrome (5).

A marked difference was noted in the ratio between the rate of incorporation of [^{14}C]formate into the cellular nucleotides and that into the purines excreted into the medium by normal cells, cells with mutant PRPP synthetase, and HGPRT-deficient cells. This difference may be attributed to the different kinetics of purine nucleotide metabolism characterizing these various cells.

The pattern of transmission for this disorder has yet not been fully clarified. In the presently reported family, the father (I. G.), one brother (A. G.), and all four sons of the propositus were found normal by the clinical and biochemical criteria studied. On the other hand, the propositus' other brother (H. G.) as well as his mother (D. G.) were found to be affected. Whereas the biochemical and clinical manifestations of the defect in this brother (H. G.) were similar to that in propositus (O. G.), these manifestations in the mother (D. G.) were attenuated. She exhibited hyperuricosuria, but she was normouricemic and never suffered from gouty arthritis or kidney stones. No abnormality could be detected in her erythrocytes (8), but her fibroblasts exhibited increased PRPP content and availability and accelerated synthesis *de novo* of purine nucleotides, the increase above normal in these parameters ranging from 56-72% the increase exhibited by the fibroblasts from her affected son (O. G.). On the basis of the available data, the mother (D. G.) could be heterozygous for either X-linked recessive or autosomal dominant traits. An autosomal dominant trait has indeed been suggested for the family with mutant PRPP synthetase reported by Becker et al. (11).

If the mother is an heterozygote for X-linked recessive trait, then her fibroblast cultures should contain two cell populations, the normal and the mutant. On the other hand, if the mother is heterozygous for autosomal dominant trait, her fibroblast cultures should contain only the mutant cell population. Studies carried in our laboratory, aiming at the demonstration of two cell populations in the mother's cell cultures, by selecting

for the mutant cell, yielded negative results. The method utilized was essentially that described by Green and Martin (20). Addition to the fibroblast culture media of methylmercaptopurine ribonucleoside (0.2 mM), uridine (0.5 mM), and hypoxanthine (0.2 mM), caused the death of normal cells but allowed the survival of cells from the propositus (O. G.), which possess increased purine salvage capability. Under these conditions, the fibroblast cultures of the propositus' mother (D. G.) behaved similarly. This finding indicates the presence of a homogenous mutant cell population in the mother's fibroblast and thus supports the suggestion that the mutant PRPP synthetase in this family is transmitted as an autosomal dominant trait.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Mrs. H. Oelsner.

This work was supported in part by research grant 185 from the Israel-USA Binational Fund.

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