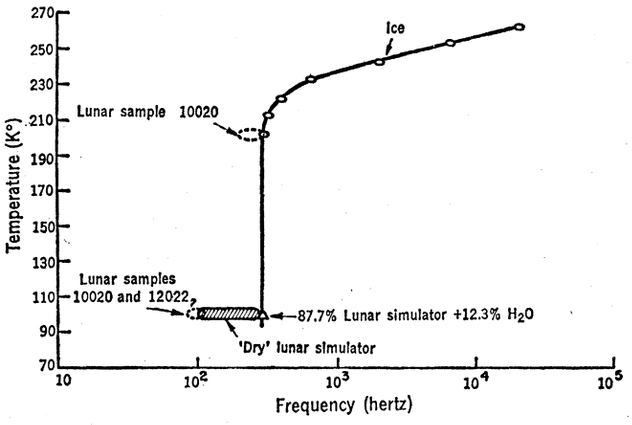

Fig. 3. Temperature plotted against the frequency at which the peaks of dielectric relaxation occur for ice (*12*), lunar simulator plus water, "dry" lunar simulator, and two lunar samples (*5*).

κ' and tan δ are shown as curves 5 (Figs. 1 and 2). If the same data are plotted as κ'' against κ' ($\kappa'' = \kappa'$ tan δ), they correspond to a Cole-Cole distribution (*10*) given by

$$\kappa^* = \kappa_\infty' + \frac{\kappa_0' - \kappa_\infty'}{1 + (j\omega\tau)^{1-\alpha}} \qquad (1)$$

with $\kappa_0' = 4.90$, $\kappa_\infty' = 2.57$, $\tau = 6.5 \times 10^{-4}$ second, and $\alpha = .39$. The symbol κ^* denotes the complex (relative) dielectric permittivity, κ_0' and κ_∞' are the values of the real part of κ^* at zero and infinite frequency. The imaginary part of κ^* is κ'', $j = (-1)^{1/2}$, ω is 2π times the frequency, τ is the generalized relaxation time, and α is a parameter that can vary between 0 and 1.

These results show that with increasing amounts of moisture the dielectric behavior of the sample, in the frequency range of 30 to 10^5 hetrz and at 100°K, eventually becomes dominated by ice. The broad peaks observed in curves 1 through 4 (Fig. 2) are interpreted as relaxations occurring in the frozen moisture remaining in the sample. The corresponding κ' curves are in agreement with the existence of such relaxations. However, the small κ' increments with decreasing frequency preclude its consideration as positive evidence. The vapor pressure of ice at 120°K has been estimated as 1.4×10^{-12} torr (*11*); therefore, the amount of water frozen at 100°K and 7.0×10^{-8} torr will remain in the sample as long as such conditions are maintained in the laboratory. Curves 3, 4, and 5 (Fig. 2) qualitatively establish that the sample losses are proportional to their ice contents. A quantitative evaluation of the amount of frozen moisture and its effect on κ' and κ'' is presently under way (*8*).

The existence of lunar permafrost is expected within the temperature range of 100° to 273°K (*4*). The results presented here represent the lower temperature limit. It is known that the relaxation peak for ice moves toward lower frequencies when the temperature decreases (*12*). In Fig. 3 I have plotted the ice temperature against the frequency at which the peak of the relaxation occurs. The ellipses represent data for ice (*12*); the triangle corresponds to the peak in curve 5 (Fig. 2). Extrapolation of the results for ice is in agreement with my results at 100°K.

Lunar samples 10020 and 12022 were contaminated by atmospheric moisture when the dielectric measurements were performed (*5*). Consequently, relaxation phenomena associated with the frozen contaminating water should be expected. The peaks observed for tan δ in those measurements have been incorporated in Fig. 3. The question mark by the lunar samples at 100°K indicates the possibility of the peak occurring at slightly lower frequencies.

In conclusion the evidence presented indicates that if permafrost exists in the moon between 100° and 213°K it will have a dielectric relaxation peak at approximately 300 hertz. If its temperature is between 213° and 263°K the relaxation peak will occur between 300 hertz and 20 khz. The frequency at which the relaxation maximum occurs may serve as a crude thermometer.

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Purine Overproduction in Man Associated with Increased Phosphoribosylpyrophosphate Synthetase Activity

Abstract. *In hemolyzates from red cells of two brothers with purine overproduction and gout, activity of phosphoribosylpyrophosphate synthetase is more than twofold greater than that measured in normal or other gouty individuals. The increased enzyme activity, which is also demonstrable in fibroblasts of the one patient tested, is associated with increased production of 5-phosphoribosyl-1-pyrophosphate by intact cells, an indication that the enzyme abnormality is the basis for the purine overproduction. This genetic abnormality is an example of an increased enzyme activity producing a disease state.*

Excessive purine synthesis de novo contributes to the hyperuricemia of a substantial portion of patients with gout (*1*). Progress has been made in identifying some of the specific biochemical and genetic factors responsible for derangements in this important regulated biosynthetic pathway (*2*). In addition to clarifying clinical syndromes, these studies have shed light on the nature of some of the factors involved in the normal mechanism regulating purine metabolism (*3*). Nonetheless, the derangements now known and associated with excessive purine production can account for only a small proportion of the cases of purine overproduction in man (*1, 4*).

We now report another distinct genetic and biochemical abnormality re-

sulting directly in purine overproduction as manifested clinically by gouty arthritis. Of particular interest is the finding that the regulatory aberration is a result of an increase in the activity of a specific enzyme, phosphoribosylpyrophosphate synthetase (E.C. 2.7.6.1).

5-Phosphoribosyl-1-pyrophosphate (PRPP), a precursor of the ribose phosphate moiety of purine, pyrimidine, and pyridine nucleotides, has been postulated to have a role in the regulation of the rate at which purine nucleotides are synthesized (*1, 5*). This high-energy sugar phosphate is synthesized from adenosine triphosphate (ATP) and ribose 5-phosphate in a reaction requiring magnesium and inorganic phosphate and catalyzed by PRPP synthetase. The PRPP formed is a substrate in the PRPP amidotransferase reaction which is the first reaction committed to purine synthesis de novo and which is probably rate-limiting in the pathway (*6*). PRPP is also a substrate in the phosphoribosyltransferase reactions, constituting the salvage pathway of purine nucleotide synthesis. The studies from which a regulatory role for PRPP has been suggested have shown that, in situations in which the intracellular concentration of PRPP is altered, there is a corresponding alteration in the rate of purine synthesis de novo (*3, 7–9*). Thus, in the case of severe deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT), a striking increase in PRPP concentrations in all tissues thus far examined is accompanied by an increase in the rate of purine synthesis in those cells possessing a complete pathway for purine synthesis de novo (*3, 7*). Conversely, depletion of intracellular PRPP is associated with decreased purine synthetic rates both in individuals administered allopurinol, adenine, or orotic acid (*8*), and in human fibroblasts in tissue culture incubated with nicotinic acid or orotic acid (*9*).

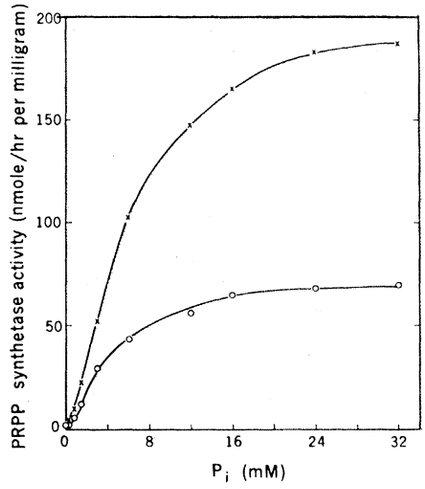

Fig. 1. Activity of PRPP synthetase in dialyzed hemozates from a normal subject (○—○) and patient H.B. (×—×) as a function of added inorganic phosphate (P_i). Enzyme assays were performed as described in Table 1, except that final inorganic phosphate concentrations were varied.

We have studied the synthesis of PRPP in a number of patients with gout and previously demonstrated overproduction of uric acid. Among these hyperuricemic individuals we have found increased PRPP synthetase activity as well as increased PRPP production in erythrocytes of two brothers (H.B. and T.B.), findings that were confirmed when fibroblasts cultured from one patient (T.B.) were examined. Daily excretion of uric acid in these patients, while maintained on a diet essentially free of purines, is 1050 and 1500 mg, respectively, compared to a normal value of less than 600 mg (*1*). As is shown in Table 1, in each of these patients, the rate of purine synthesis de novo as measured by the incorporation of [1-^{14}C]glycine into urinary uric acid (*1, 10*) is increased four- to fivefold beyond that seen in normal individuals. Activities of both HGPRT and adenine phosphoribosyltransferase (APRT) in the patients' erythrocyte lysates are normal.

The activity of PRPP synthetase (Table 1) measured in dialyzed hemolyzates from red cells of patients H.B. and T.B. by a modification of the method of Hershko *et al.* (*11*), is increased more than twofold beyond that seen in normal persons or in a large number of patients with gout (both with and without demonstrable purine overproduction), or in children with severe deficiency of HGPRT and the Lesch-Nyhan syndrome (*12*). Activity of PRPP synthetase is unaltered by administration of uricosuric drugs, allopurinol, or a purine-free diet. A brother of the affected patients (J.B.) as well as the son (B.B.) and wife (Y.B.) of patient H.B. have normal enzyme activities. The 16-year-old daughter (C.B.) of H.B. has activity of PRPP synthetase comparable to that of her father and uncle T.B. despite a normal concentration of uric acid in her serum and absence of history of joint symptoms or renal calculi. In this girl, however, the ratio of urinary uric acid to creatinine, a gross index of urate production (*1*), is slightly elevated. More extensive studies of this individual are needed.

The metabolic consequences of the increased activity of PRPP synthetase have been investigated further in erythrocytes and in cultured fibroblasts from one of the affected brothers. Table 2 shows that the PRPP synthetase activity is also increased three- to fourfold in the fibroblasts cultured from patient T.B., which nevertheless show a normal growth rate. The increased PRPP synthetase activity is associated with an increased intracellular concentration of PRPP (*13*) and an enhanced capacity

Table 1. Correlation of rate of purine synthesis de novo and PRPP synthetase activity. Cumulative [1-^{14}C]glycine incorporation into urinary uric acid over 7 days represents value corrected for extrarenal disposal of uric acid measured from simultaneous administration of [^{15}N]urate (*1*). The PRPP synthetase was measured by a modification of the two-step method of Hershko *et al.* (*11*). Hemolyzates were dialyzed for 4 hours against 8 m*M* sodium phosphate buffer, *p*H 7.4, with 1 m*M* EDTA and 5 m*M* reduced glutathione. The first step, in which PRPP was generated, was carried out at inorganic phosphate, adenosine triphosphate, and ribose 5-phosphate concentrations of 32.0 m*M*, 0.05 m*M*, and 0.35 m*M* respectively, and was terminated by addition of 200 μl of 0.1*M* EDTA. After deproteinization by heat, the PRPP concentration was determined as described by Wood *et al.* (*7*). For patients H.B. and T.B., enzyme activities represent the means of five separate measurements, while for other B. family members, two measurements were made.

Group	Rate of purine synthesis		PRPP synthetase in hemolyzates		
	Number studied	[1-^{14}C]Glycine incorporated (%)	Number studied	Activity (nmole hr^{-1} mg^{-1})	
				Mean	Range
Normal	3	< 0.4	16	68	41–98
Gout	5	0.2–1.9	24	66	37–85
HGPRT-deficient	2	3.1–4.7	4	70	53–97
B. family					
H.B.		1.2		183	160–193
T.B.		1.4		180	159–188
J.B.				87	79–95
B.B.				92	89–95
Y.B.				78	75–80
C.B.				190	185–195

to generate PRPP both in erythrocytes and in fibroblasts. [Generation of PRPP was estimated by the sum of the incorporation of [^{14}C]adenine into nucleotides and nucleic acids (*14*) and the alteration in intracellular PRPP content resulting from this adenine addition.] Furthermore, the intact fibroblasts of patient T.B. also showed an enhanced rate for the early reactions of purine synthesis as shown by the two- to threefold increase in the incorporation of [^{14}C]formate into the formylglycinamide ribonucleotide (*9*) which accumulates in the presence of the glutamine antagonist azaserine. Thus, the abnormally high enzyme activity can be demonstrated in a cell that is capable of purine synthesis de novo, and is associated with an increased rate of purine synthesis. The correlation of increased PRPP synthetase activity with increased production of PRPP by intact cells provides evidence that the PRPP synthetase abnormality shown in extracts manifests itself within the cells as well. Moreover, the association of increased PRPP production with purine overproduction strengthens the evidence for a regulatory role of PRPP in purine synthesis.

Previous studies of fibroblasts from patient T.B. have shown that their increased rate of purine synthesis de novo is relatively resistant to inhibition by adenine and hypoxanthine (*15*). We have confirmed this finding. Such an observation is compatible with either an increase in PRPP production as demonstrated here, or, as previously postulated (*15*), by an abnormality in the regulatory properties of PRPP amidotransferase, an enzyme activity which is inhibited in man by the purine mononucleotides (*16*). Measurement of the activity of PRPP amidotransferase and its sensitivity to purine nucleotide feedback inhibition has not yet been successful in fibroblasts. In human lymphoblasts, however, PRPP and purine nucleotides compete for binding to PRPP amidotransferase (*16*), providing a plausible model for both the stimulatory effect of increased PRPP concentration on purine synthesis and the diminished response to inhibition by purine nucleotides seen in fibroblasts of patient T.B.

Several mechanisms could account for the increased concentration of PRPP synthetase activity in our patients' cell extracts. The presence of abnormal concentrations of small molecule effectors can alter the activity of structurally normal enzyme either by direct activation (*17*), induction of new synthesis (*18, 19*), or stabilization (*19, 20*) of the catalytically active protein. These mechanisms imply an altered intracellular milieu as the primary event leading to increased enzyme activity. Conversely, primary alteration in protein structure could result in an increased rate of enzyme synthesis [as in the Hektoen variant of glucose 6-phosphate dehydrogenase or the E Cynthiana variant of pseudocholinesterase (*21*)], a decreased rate of enzyme degradation, or an enzyme of higher activity per molecule. Finally, primary alteration in the regulation of the synthesis of the enzyme could also explain increased activity. We have examined some of these possibilities with respect to the observed increased enzyme activity in our patients.

Table 2. Metabolism of PRPP and rate of purine biosynthesis in normal and B. family cells. The PRPP concentration and PRPP synthetase activity were determined as described in Table 1. The rate of purine synthesis in fibroblasts was estimated according to the method of Boyle *et al.* (*9*). Generation of PRPP was estimated as noted in the text. Incorporation of [^{14}C]-adenine into nucleotides and nucleic acids was measured as follows. To 500 μl of Krebs-Ringer phosphate solution (modified to contain 27.5 m*M* sodium phosphate buffer), containing either 1×10^6 to 2×10^6 fibroblasts or 25 μl of packed erythrocytes, 50 μl (2.5 μc) of [^{14}C]adenine was added to give a final adenine concentration of 40 μ*M*. After incubation for 60 minutes at 37°C, 20 μl of cold 42 percent perchloric acid was added; after removal of the supernatant layer, the precipitate containing DNA and RNA was washed with 5 percent trichloroacetic acid, collected on a Millipore filter, and counted. The supernatant layer was neutralized with 30 μl of 4.45*N* potassium hydroxide, and the radioactivity in the nucleotides, separated by chromatography on polyethyleneimine-cellulose paper in increasing concentration of sodium formate buffer, *p*H 3.4 (*29*), was counted. In the column headed "Normal," numbers in parentheses indicate the number of different individuals or cell lines tested. All values for normal cells are ranges except as indicated. All values for T.B. and H.B. are means of at least two separate experiments.

Item	Samples		
	Normal	T.B.	H.B.
Erythrocytes			
PRPP concentration (nmole per ml of packed cells)	2.8 ± 0.5* (24)	5.1	5.0
PRPP generation (nmole/hr per ml of packed cells)	127–140 (4)	200	234
Fibroblasts			
PRPP synthetase (nmole/hr per mg)	240–500 (8)	1450	
PRPP concentration (nmole/10^9 cells)	0.1–0.3 (12)	0.45	
Purine synthesis† (count/min per 10^6 cells)	1600–2100 (6)	4400	
PRPP generation (nmole/hr per 10^9 cells)	2.41–2.76 (4)	4.23	

* Standard deviation. † Formylglycinamide ribonucleotide accumulation.

The increase in PRPP synthetase activity is due to the enzyme itself. All routine enzyme measurements were made in extracts dialyzed to remove endogenous nucleotides, sugar phosphates, and other small molecule effectors. In addition, in experiments in which normal hemolyzates (both dialyzed and undialyzed) and those of the patients were mixed in various combinations and proportions, no activation of normal enzyme activity or inhibition of patients' enzyme activity was observed. Since inorganic phosphate is a potent activator of the enzyme, possible differences in intracellular inorganic phosphate concentrations were evaluated by direct assay (*22*). The intracellular concentration of inorganic phosphate in erythrocytes of patient T.B. (1.05 m*M*) was the same as that found in normal individuals (0.97 to 1.36 m*M*).

Substrate or product stabilization of otherwise normal enzyme is another possible explanation for increased enzyme activity. For example, in erythrocytes deficient in HGPRT, the activity of the enzyme APRT is increased, probably through the stabilizing effect of the increased PRPP concentration (*20*). Several lines of evidence make it unlikely that an analogous stabilization of PRPP synthetase activity can, by itself, explain the increased activity of this enzyme in these patients. First, Valentine and Kürschner (*23*) have demonstrated that the decrease in the activity of PRPP synthetase with increasing density of mature erythrocytes is slight. Stabilization of the enzyme in older erythrocytes, therefore, would not account for the more than twofold greater activity in hemolyzates from our patients, in whom, in addition, no evidence of a younger red cell population is apparent. Second, the concentrations of PRPP in our patients' erythrocytes are less than twofold normal, making stabilization of the enzyme by PRPP unlikely. Finally, T.B.'s increased PRPP synthetase activity is demonstrable in fibroblasts as well as in erythrocytes, whereas the increased APRT activity in HGPRT-deficient cells is found only

in erythrocytes and not in fibroblasts or in lymphoblasts (*7, 24*).

Whether the increased enzyme activity observed in our patients results from a structural alteration in the PRPP synthetase molecule or from an increase in amount of this enzyme in the cells remains to be determined. Identical patterns of inactivation of PRPP synthetase at 60°C were found in hemolyzates of normal subjects and of patient H.B. Measurement of affinity constants for ATP and ribose 5-phosphate in hemolyzates have shown no difference between normal and patient's enzyme for either substrate. The sensitivity of the patient's enzyme to inhibition by purine nucleotides does not appear altered from normal in studies with adenosine diphosphate or guanosine diphosphate as inhibitors.

The increased PRPP synthetase activity in these patients' cells is apparent at all concentrations of added inorganic phosphate (Fig. 1). This differentiates the abnormality in PRPP synthetase activity of our patients from that of the patient of Sperling *et al.* (*25*), in which increased enzyme activity was identifiable only at concentrations of inorganic phosphate up to 2 m*M*. The existence of at least two separate abnormalities in PRPP synthetase activity associated with purine overproduction implies that a range of separable defects in this activity may exist among patients with clinical gout, much as a variety of abnormalities in HGPRT have been discovered among patients with the Lesch-Nyhan syndrome (*26*).

Our studies, relating an increased enzyme activity with increased intracellular production of a regulatory substrate and concomitant overactivity of an entire pathway, demonstrate that disease states in man may result from genetic alterations leading to increased as well as diminished enzyme function. Increased activity of hepatic δ-aminolevulinic acid synthetase in acute intermittent porphyria has been postulated to represent an important factor in the pathogenesis of that disease (*27*). Studies of Strand *et al.* (*28*) indicate, however, that the increased activity of δ-aminolevulinic acid synthetase is secondary to a partial deficiency of uroporphyrinogen I synthetase, with resulting alterations in the intracellular concentration of negative feedback effectors or inducers of δ-aminolevulinic acid synthetase. The possibility of a similar mechanism to account for increased PRPP synthetase activity in our patients' cells cannot yet be dismissed.

The mode of inheritance of the PRPP synthetase abnormality discussed here remains to be defined. Transmission of the abnormality from father (H.B.) to daughter (C.B.) without detectable abnormality in the mother's (Y.B.) enzyme activity suggests dominant inheritance that is either autosomal or X-linked.

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DNA Content and DNA-Based Centromeric Index of the 24 Human Chromosomes

Abstract. *The chromosomes of two human males were identified by fluorescent banding, restained, and measured by scanning microscopy and computer analysis. The two variables, DNA content and DNA-based centromeric index, provided almost complete discrimination of chromosome types. Some chromosomes showed significant differences in DNA content between the men, and for one man two pairs of chromosomes showed significant differences between homologs.*

The fluorescent banding method of Caspersson *et al.* (*1*) allows every chromosome in selected human metaphase cells to be identified with assurance. After restaining such chromosomes with a DNA-specific procedure, we can measure their DNA content and DNA-based centromeric index using our techniques of quantitative image analysis. We present, in this first report on these combined methods, the chromosomal DNA contents from two normal human males and the centromeric index based on DNA content from six cells of one of the men.

Previously reported, generally standard, and newly developed procedures were combined into the following steps:

1) Blood samples from two adult males, BHM and DHM, who have no known genetic or medical disorder, were cultured and prepared for chromosome analysis by standard techniques (*2*).

2) The cells were stained with 0.0005 percent quinacrine hydrochloride and