Phosphoribosylpyrophosphate Synthesis in Cultured Human Cells

Abstract. *Phosphoribosylpyrophosphate (PRPP) concentrations and PRPP synthetase activity were studied in cultured human fibroblasts with control and mutant hypoxanthine-guanine phosphoribosylpyrophosphate (HPRT) activity. The PRPP concentrations increased 20- to 50-fold and PRPP synthetase activity 3-fold in cells from patients with the Lesch-Nyhan syndrome when aminopterin, an inhibitor of de novo purine synthesis, was added to the medium. Concentrations of PRPP and PRPP synthetase activity did not increase in control cells in medium containing aminopterin unless hypoxanthine was removed from the medium. Exposure of cells to cycloheximide, a protein synthesis inhibitor, prevented the induction of PRPP synthetase and the formation of high PRPP concentrations. Cells from a patient with a mutant HPRT with a high Michaelis constant for PRPP increased PRPP levels and PRPP synthetase activity to a new steady state when they were grown in medium containing aminopterin. Inhibitors of de novo purine synthesis, the presence of hypoxanthine in the medium, and mutant HPRT activity affect the regulation of PRPP levels and PRPP synthetase activity.*

Phosphoribosylpyrophosphate (PRPP) is a high-energy ribose-phosphate that is the initial substrate in the synthesis of purines and pyrimidines. Concentrations of PRPP are high in at least two human disorders associated with accelerated purine synthesis and increased concentrations of serum uric acid (*1*). In purine metabolism, PRPP is utilized by PRPP amidotransferase (E.C. 2.4.2.17), the first enzyme step in purine synthesis, by hypoxanthine-guanine phosphoribosyltransferase (E.C. 2.4.2.8; HPRT), which converts hypoxanthine from diet or purine catabolism to nucleotide, and by adenine phosphoribosyltransferase. Most of the hypoxanthine formed from catabolism of purines recycles to nucleotide, and HPRT plays an important role in the economy of purine metabolism in man (*2*). Because low PRPP levels may be limiting for purine synthesis de novo under certain conditions (*3*), and because nucleosides diminish PRPP production (*4*) and purine synthesis de novo, the regulation of PRPP metabolism affects the cellular synthesis of purines. Recently, reports by Reem (*5*) and by Martin and co-workers (*6*) suggest that PRPP synthetase activity is increased in cells from Lesch-Nyhan patients (*7*) and in mutant hepatoma cells that have almost no HPRT activity. An explanation suggested by Martin and co-workers (*6*) is that the HPRT chromosome locus is genetically bifunctional and may regulate PRPP synthetase activity. Because this hypothesis is not supported by other studies (*8*), and is relevant to the regulation of de novo purine metabolism, we investigated factors that affect PRPP levels and PRPP synthetase activity.

Control and Lesch-Nyhan fibroblast cells and an HPRT mutant cell strain were studied. The latter, derived from a patient (C.M.) with high concentrations of serum uric acid, has a mutant HPRT enzyme with a normal Michaelis constant (K_m) for hypoxanthine and an increased K_m for PRPP (*9*). Like Lesch-Nyhan cells, they grow in the presence of purine analogs such as 8-azaguanine, and like control cells, they grow in the presence of aminopterin, an inhibitor of de novo purine synthesis. In tissue culture, cells from C.M. demonstrate accelerated purine synthesis (*9*) which mimics the increased synthesis of uric acid in the patient (*10*). They also demonstrate markedly depressed utilization of extracellular hypoxanthine in spite of normal HPRT activity at saturating substrate concentrations (*11*). We present evidence that the growth of C.M. cells in the presence of aminopterin is achieved by increasing PRPP levels and PRPP synthetase activity to a new steady state. This enables this cell type to utilize its mutant HPRT. Our data demonstrate that by changing the conditions of the tissue culture media, both the PRPP concentrations and the PRPP synthetase activity increase in control cells in a manner previously reported for HPRT mutant cells (*5, 6*). The regulation of PRPP concentrations by PRPP synthetase activity appears to be a sensitive point in the control of purine metabolism in cultured cells.

All experiments were performed with cultured human fibroblasts (*12*) in tissue culture during the log phase of growth. Both PRPP synthetase activity and PRPP levels were determined as previously described (*13*) with the use of partially purified HPRT (*14*).

The activity of PRPP synthetase was slightly increased in Lesch-Nyhan cells grown in F10 medium with hypoxanthine, as shown in Fig. 1 ($P < .05$). Striking changes in PRPP metabolism were observed when aminopterin was added to the culture medium. Lesch-Nyhan cells, which cannot obtain purines from the medium for growth, demonstrated 20- to 50-fold increases in PRPP levels after 48 hours, as shown in Fig. 2A, and a threefold increase in PRPP

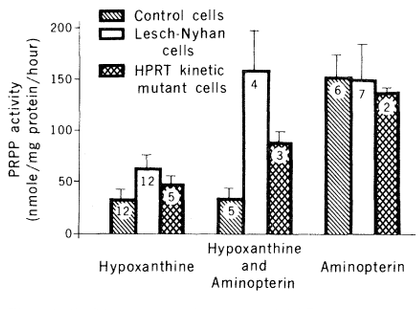


Fig. 1 (left). The activity of PRPP synthetase in control and HPRT mutant fibroblast cells. Enzyme activity was measured in cells in the log phase of growth after 48 hours of exposure to medium with 15 percent dialyzed fetal calf serum and (i) F10 media with hypoxanthine, (ii) F10 media with hypoxanthine and $5 \times 10^{-6}M$ aminopterin, and (iii) F10 media with aminopterin and no hypoxanthine. Numbers within the columns indicate the numbers of experiments conducted with four control cell strains, three Lesch-Nyhan cell strains, and a kinetic mutant cell strain. Values are means ± 1 standard deviation.

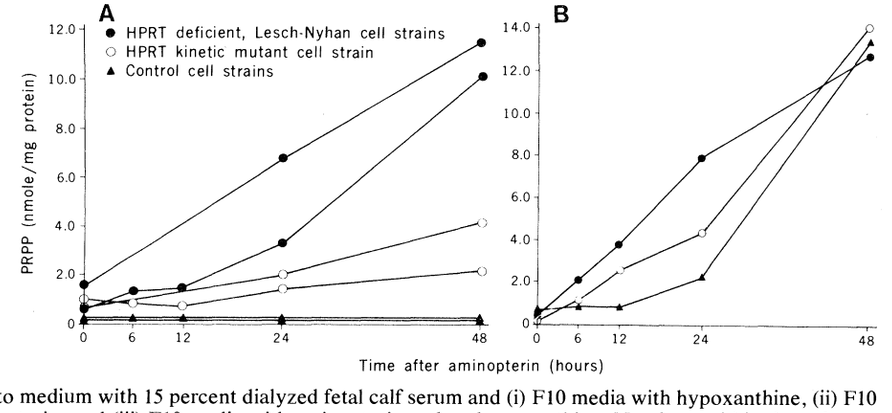


Fig. 2 (right). Concentrations of PRPP in control and mutant cells treated with $5 \times 10^{-6}M$ aminopterin. Cells were grown in 15 percent dialyzed fetal calf serum and F10 medium with hypoxanthine (A) or without hypoxanthine (B). Concentrations of PRPP were determined after exposure to aminopterin for various times.

synthetase activity (see Fig. 1). Increased PRPP synthetase activity decreased in Lesch-Nyhan cells by 72 hours, as shown in Fig. 3. At this time, toxic changes became evident, and the cells became detached from the culture dish after an additional 24 to 48 hours. The addition of adenine (5 mg/liter) to the medium prevented the toxic changes and the PRPP synthetase induction in aminopterin-treated Lesch-Nyhan cells.

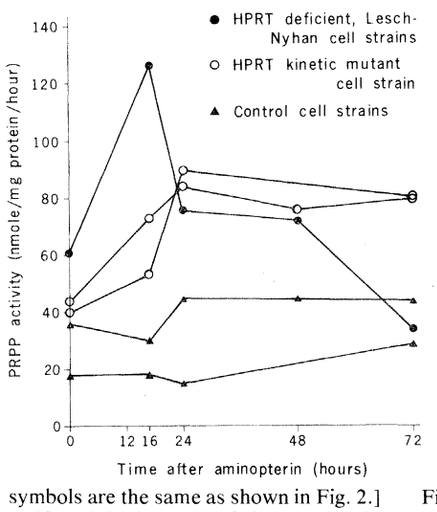
The concentration of PRPP and PRPP synthetase activity in control cells did not increase in hypoxanthine-containing media in the presence of aminopterin (see Figs. 1, 2A, and 3). However, in the cells from C.M., the PRPP concentration increased to a new steady state in the presence of aminopterin (Fig. 2A) by increasing PRPP synthetase activity (Figs. 1 and 3). Such an adjustment allows these cells to grow normally in the presence of aminopterin (*9*).

The accumulation of PRPP in Lesch-Nyhan cells in the presence of aminopterin might be secondary to induced PRPP synthetase activity, or might be due to a failure of these cells to utilize PRPP because of a pharmacologic block of de novo purine synthesis and a mutational block in HPRT activity. To distinguish between these possibilities, cells were grown in the presence of cycloheximide, a chemical that inhibits protein synthesis at a concentration of 1 μg/ml, but does not cause cell death.

Figure 4 shows that PRPP accumulation is largely prevented in control and Lesch-Nyhan cells when the induction of PRPP synthesis is inhibited by cycloheximide. These data suggest that PRPP synthetase induction and not failure of PRPP utilization is primarily responsible for the large increase in PRPP levels observed in Fig. 2. A difficulty in the interpretation of this experiment, however, is that two inhibitors of metabolism must be used, and these inhibitors may have other cellular effects.

Concentrations of PRPP and PRPP synthetase activity were measured in F10 medium with no hypoxanthine and dialyzed fetal calf serum. When aminopterin was added to HPRT kinetic mutant cells and control cells under these conditions, PRPP synthetase activity increased in both cell types in 48 hours to levels found in aminopterin-treated Lesch-Nyhan cells (Fig. 1). Concentrations of PRPP in both cell types increased to those found in aminopterin-treated Lesch-Nyhan cells (Fig. 2B). This experiment demonstrates that PRPP synthetase activity in control cells is induced when aminopterin is added to a medium that does not contain a purine source. It suggests that intracellular conditions affect PRPP synthetase activity and PRPP concentrations, and that the effects of HPRT gene mutations are indirect.

Concentrations of PRPP are altered by changes that affect cellular purine metabolism. Under normal conditions, with hypoxanthine in the medium, control cells preferentially utilize this formed purine, which can be converted to nucleotide at the energy cost of splitting a PRPP molecule. Synthesis of purines from simple amino acids requires the energy of at least four adenosine triphosphate molecules and one PRPP molecule and active folic acid methyl carriers. The K_m of control HPRT for PRPP is 0.2 mM (*14*), and the K_m of human PRPP amidotransferase for PRPP is 0.48 mM (*15*). When hypoxanthine is available to control cells, synthesis of nucleotide by HPRT activity is kinetically preferred at lower PRPP concentrations. We show in Figs. 1 and 2A that addition to aminopterin to control cells does not change PRPP concentrations or PRPP synthetase, and presumably PRPP is utilized by



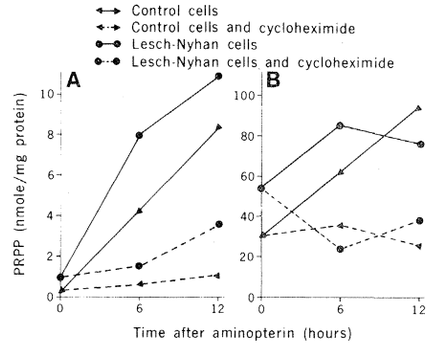


Fig. 3 (left). Activity of PRPP synthetase in control and mutant cells. Aminopterin ($5 \times 10^{-6}M$) was added to cells in 15 percent dialyzed fetal calf serum and F10 medium with hypoxanthine at time 0, and PRPP synthetase activity was assayed after various times. [The symbols are the same as shown in Fig. 2.] Fig. 4 (right). Effects of cycloheximide on control and Lesch-Nyhan cells. Cells were incubated for 6 hours in cycloheximide (1 μg/ml) and growth media. Concentrations of PRPP (A) and PRPP synthetase activity (B) were determined in cells grown with and without cycloheximide in F10 medium without hypoxanthine and with 15 percent dialyzed fetal calf serum.

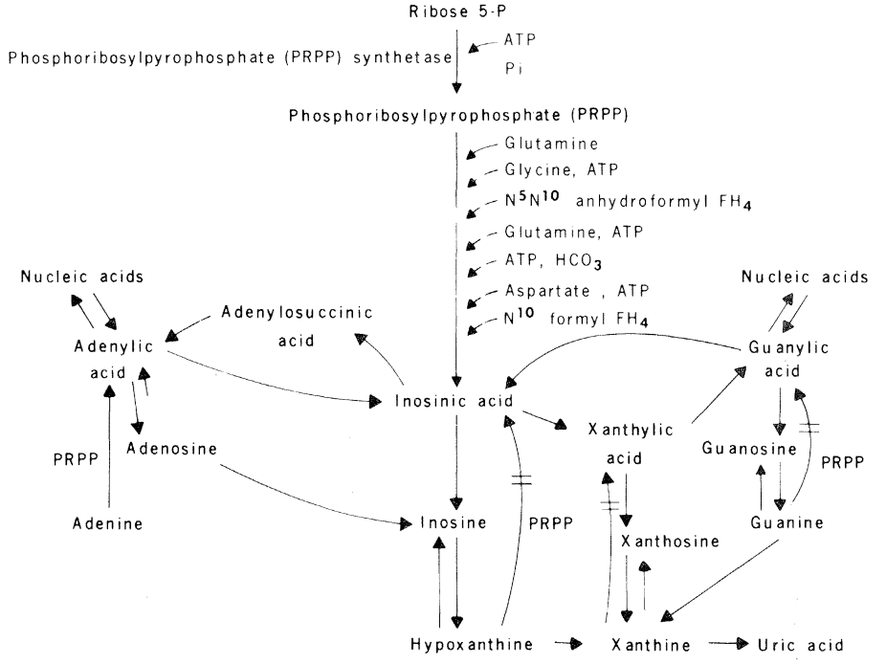


Fig. 5. Pathways in purine metabolism. The enzyme PRPP synthetase is inhibited by nucleotides and activated by inorganic phosphate. The Lesch-Nyhan mutation in HPRT is shown by the double bars. The PRPP amidotransferase reaction combines PRPP and glutamine. Aminopterin blocks formyl tetrahydrofolate (FH_4) steps in de novo purine synthesis.

the HPRT enzyme when hypoxanthine is present.

The growth rate of control and Lesch-Nyhan cells in tissue culture is the same (*9*). Presumably, net total synthesis of RNA and DNA by the two cell types is the same. Lesch-Nyhan cells, with almost no HPRT activity, require a mechanism to generate a higher concentration of PRPP because PRPP amidotransferase has a higher K_m for PRPP than HPRT.

Experiments with the HPRT kinetic mutant cells are interesting in this regard. The K_m of the mutant HPRT for PRPP is 1 m*M* (*9*) which is more than twice that of control PRPP amidotransferase (*15*). In C.M. cells, PRPP is utilized by PRPP amidotransferase and there is little function of the mutant HPRT (*11*). We have previously shown that these cells utilize mutant HPRT in the presence of aminopterin (*11*). In C.M. cells, PRPP synthetase activity rises to a new steady state (Figs. 1A and 3), and this generates the PRPP concentration shown in Fig. 2A, which in turn enables these cells to grow at normal rates in tissue culture (*9*). Pathways in purine metabolism are shown in Fig. 5.

These experiments do not examine the mechanism of PRPP and PRPP synthetase changes, or the relative importance of PRPP synthetase regulation versus PRPP amidotransferase regulation in the control of de novo purine synthesis. Most studies have been done with partially purified enzyme preparations and not with whole cells under physiologic conditions (*15*). Both regulatory enzymes may be important in the fine regulation of purine metabolism. It is interesting that the two enzymes have different relative responses to mono- and trinucleotide inhibitors and inorganic phosphate (*4, 15*). That there may be a dual regulation of purine metabolism can be inferred from the studies of Becker (*17*), who found that cells grown in inosine (hypoxanthine riboside) demonstrate an increase in PRPP at the same time that de novo purine synthesis is inhibited, presumably by nucleotide effects on PRPP amidotransferase. The studies presented here do suggest the existence of mechanisms that adjust the concentrations of cellular PRPP to those required for purine needs and cellular growth. PRPP levels depend on whether the cell utilizes HPRT, PRPP amidotransferase, or a mutant HPRT activity to obtain its purine requirements.

PAUL J. BENKE, DAVID DITTMAR
Mailman Center for Child Development, University of Miami School of Medicine, Miami, Florida 33152

References and Notes

1. This has been reviewed by M. A. Becker [*J. Clin. Invest.* **57**, 308 (1976)].
2. L. Sorensen, *ibid.* **49**, 968 (1970).
3. S. Brosch, P. Boer, B. Kupfer, A. deVries, O. Sperling, *ibid.* **58**, 289 (1976); S. D. Skaper, R. C. Willis, J. E. Seegmiller, *Science* **193**, 587 (1976).
4. G. Planet and I. H. Fox, *J. Biol. Chem.* **251**, 5839 (1976).
5. G. H. Reem, *Science* **190**, 1098 (1975).
6. D. W. Martin, Jr., and B. A. Maler, *ibid.* **193**, 411 (1976); L. H. Graf, Jr., J. A. McRoberts, T. M. Harrison, D. W. Martin, Jr., *J. Cell. Physiol.* **88**, 331 (1976).
7. The Lesch-Nyhan syndrome is an X-linked spastic neurologic disorder associated with accelerated purine metabolism and almost complete absence of HPRT activity.
8. F. M. Rosenbloom, J. F. Henderson, I. C. Caldwell, W. N. Kelley, J. E. Seegmiller, *J. Biol. Chem.* **243**, 1166 (1968); G. H. Reem, *Ciba Found. Symp.*, in press.
9. P. J. Benke and N. Herrick, *Am. J. Med.* **52**, 547 (1972); _____, A. Hebert, *J. Clin. Invest.* **52**, 2234 (1973). A case with similar biochemical findings has been described by J. F. Henderson, J. B. Dossetor, M. K. Dasgupta, and A. S. Russell [*Clin. Biochem.* **9**, 1 (1976)].
10. L. B. Sorenson and P. J. Benke, *Nature (London)* **213**, 1122 (1967).
11. P. J. Benke, N. Herrick, A. Hebert, *Biochem. Med.* **8**, 309 (1973); M. J. C. Holland, A. M. DiLorenzo, J. Dancis, M. E. Balis, T. F. Yu, R. P. Cox, *J. Clin. Invest.* **57**, 1600 (1976).
12. Human fibroblasts were grown from punch biopsies at 37°C in commercial F10 medium containing penicillin (100 unit/ml) and streptomycin (100 μg/ml) with 15 percent fetal calf serum previously dialyzed against phosphate buffered isotonic saline. The F12 medium without hypoxanthine was prepared by Gibco, Grand Island, N.Y. Cells were grown in plastic P-100 dishes, and PRPP levels and PRPP synthetase activity were determined in freshly harvested, nonconfluent cells.
13. The PRPP synthetase was assayed by a modification of the two-step procedure of A. Hershko, A. Razin, and J. Mager [*Biochim. Biophys. Acta* **184**, 64 (1969)]. Fibroblasts were disrupted by freezing and thawing three times; they were then mixed with Norit A charcoal, 10 mg/ml in 1 m*M* EDTA. The charcoal and cellular debris were removed by centrifugation at 10,000*g* for 15 minutes. The reaction mixture for step one consisted of 50 m*M* tris buffer, *p*H 7.4, 5 m*M* $MgSO_4$, 5 m*M* glutathione, 1 m*M* EDTA, 50 m*M* Na_2HPO_4, 0.1 m*M* adenosine triphosphate, 0.1 m*M* ribose-5-phosphate, and cell extract in a final volume of 0.25 ml. The PRPP formed in this step and the content of fibroblast extracts were assayed by a modification of the method of J. F. Henderson and M. K. Y. Khoo [*J. Biol. Chem.* **240**, 2349 (1965)]. The reaction mixture consisted of 55 m*M* tris buffer, *p*H 7.4, 5 m*M* $MgCl_2$, 10 nmole of [^{14}C]hypoxanthine (50.4 mc/mmole), cell extract, and partially purified HPRc in a total volume of 0.125 ml. The [^{14}C]inosine monophosphate formed was isolated by high-voltage electrophoresis in 0.05*M* sodium borate buffer, *p*H 9.0, and the addition of appropriate carrier compounds. For the PRPP synthetase assay, background PRPP levels were subtracted by using a blank reaction mixture which contained no ribose-5-phosphate.
14. T. A. R. Krenitsky, R. Papaioannou, G. Elion, *J. Biol. Chem.* **244**, 1263 (1969). Lower and higher values have been reported [see A. W. Murray, *Biochem J.* **104**, 675 (1967); J. F. Henderson, L. W. Brox, W. N. Kelley, F. M. Rosenbloom, J. E. Seegmiller, *J. Biol. Chem.* **243**, 2514 (1968)].
15. E. W. Holmes, J. A. McDonald, M. H. McCord, *J. Biol. Chem.* **248**, 144 (1973); J. F. Henderson, *Regulation of Purine Biosynthesis* (Monograph 170, American Chemical Society, Washington, D.C., 1972), pp. 62 and 163.
16. On the basis of a consideration of the kinetics in vitro and cellular nucleotide concentrations, C. M. Smith and J. F. Henderson [*Can. J. Biochem.* **54**, 341 (1976)] argue that purine nucleotides do not have an effect on PRPP aminotransferase activity in vivo.
17. M. A. Becker, *Biochim. Biophys. Acta* **43**, 132 (1976).
18. This work was supported by a Basil O'Connor grant from the National Foundation–March of Dimes.

6 May 1977; revised 9 August 1977

Aggressive Chemical Mimicry by a Bolas Spider

Abstract. *Mature female* Mastophora *sp. spiders attract prey with a volatile substance which apparently mimics the female sex attractant pheromone of the fall armyworm* Spodoptera frugiperda *(Lepidoptera). The rate of prey capture is similar to that of a conventional orb weaver of comparable body size.*

Bolas spiders of the genus *Mastophora* are descended from orb weavers, but their webs have become reduced to a sticky ball suspended on the end of a short vertical thread that is attached to a single horizontal line (*1*). They are found throughout the Americas, but, perhaps because of their cryptic habits, are rare in collections. Previous observations have shown that the spider hangs on the horizontal thread, and holds the vertical thread with one front leg (Fig. 1A), swinging the ball at passing insects. When the ball hits an insect, it sticks, and the spider then descends the line, paralyzes the prey, and feeds (*1*). Whether or not the spider is able to attract its prey from a distance has been uncertain (*2*). Evidence is presented here showing that this improbable and seemingly ineffective trapping method derives its success in *Mastophora* sp. (*3*) from the use of volatile substances that apparently mimic prey sex attractant pheromone (*4*).

Several types of evidence suggest the use of a chemical attractant. The prey always approached slowly from directly downwind of the spider (more than 100 observations) with their antennae extended (Fig. 1E), and often they made repeated passes at the spider. Occasionally it was possible to follow their flight several meters from the spider: typically the animal slipped 10 m or so downwind of the spider, made several wide and erratic arcs there, and began moving upwind, narrowing the arcs as it neared the spider. When a cardboard sheet was held just downwind of the spider to deflect the airflow and hide the spider from sight, prey continued to approach, arriving by way of the trailing edge (Fig. 2).

That prey were attracted to the spider rather than the ball was shown by experimentally removing the ball just after it