A mutant of *Salmonella typhimurium* with undetectable phosphoribosylpyrophosphate (PRPP) synthetase activity in vitro and abnormally low PRPP pools in vivo was identified by screening temperature-sensitive isolates by an autoradiographic procedure. The lack of PRPP synthetase activity in vitro and temperature-sensitive growth were shown to result from separate, but closely linked mutations mapping at 47 units on the *Salmonella* chromosome. Mutant cell extracts prepared by a variety of methods did not show any detectable PRPP synthetase activity, but material that was immunochemically cross-reactive with PRPP synthetase was detected by complement fixation analysis. A second mutant, isolated by localized mutagenesis, contained about half the PRPP synthetase and cross-reacting material of the parental strain.

**INTRODUCTION**

Phosphoribosylpyrophosphate (PRPP) synthetase (ATP:5'-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) catalyses an essential step in the *de novo* synthesis of histidine, tryptophan and the purine, pyrimidine and pyridine nucleotides. In addition, PRPP has been implicated in the uptake or utilization of exogenous nucleic acid bases and their nucleosides. The regulation of PRPP synthetase activity by end-product inhibition has been studied in detail (Sadler & Switzer, 1977; Switzer & Sogin, 1973), but other than the demonstration of repression by pyrimidines (Olszowy & Switzer, 1972; White et al., 1971) little is known about the control of the synthesis of PRPP synthetase in bacteria. Furthermore, it has been proposed that adenine and other bases are transported into *Escherichia coli* cells by a group translocation mechanism involving membrane-bound phosphoribosyltransferases and intracellular PRPP (Hochstadt, 1974; Hochstadt & Stadtman, 1971). Other investigators (Burton, 1977; Munch-Petersen et al., 1979; Page & Burton, 1978; Roy-Burman & Visser, 1975) have challenged this proposal, suggesting that PRPP is required for nucleotide formation after bases have entered the cell by other transport mechanisms.

The role of PRPP in transporting bases and nucleosides, as well as in other metabolic processes, and the biosynthesis of the enzyme could be studied in vivo if mutants with appropriate defects in PRPP synthetase were available. The isolation of PRPP synthetase mutants has proved to be difficult because PRPP is essential for the synthesis of several crucial metabolites, which are not readily available via PRPP-independent pathways. In this paper we describe the use of a screening procedure for the isolation of two mutant strains that have defective PRPP synthetase activities.

**METHODS**

*Strains and culture conditions.* Strains used in this study (Table 1) were derived from *Salmonella typhimurium* strain LT-2. Minimal medium was the E medium of Vogel & Bonner (1956) supplemented with 0.5% (w/v)
**Table 1. Bacterial strains used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT-2</td>
<td>Wild-type</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td>SU422</td>
<td>met</td>
<td>P. Sypherd</td>
</tr>
<tr>
<td>PS-1</td>
<td>met, temperature-sensitive</td>
<td>EMS mutagenesis of SU422</td>
</tr>
<tr>
<td>PS-2</td>
<td>Partially temperature-sensitive</td>
<td>Localized mutagenesis using LT-2</td>
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<td>proA</td>
<td>J. Ingraham</td>
</tr>
<tr>
<td>JL1210</td>
<td>pycrC1502 cdd-9 cod-8 udp-8 udk-6 udh-3</td>
<td>J. Ingraham</td>
</tr>
<tr>
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<td>thiD</td>
<td>SGSC</td>
</tr>
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<td>SGSC</td>
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<td>cysA</td>
<td>SGSC</td>
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<tr>
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<td>SGSC</td>
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<td>hisW3333</td>
<td>J. Brenchley</td>
</tr>
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<td>J. Roth</td>
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<td>TT184</td>
<td>pro(A or B)622::Tn10</td>
<td>J. Roth</td>
</tr>
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<td>TT946</td>
<td>put-810 Tn10</td>
<td>SGSC</td>
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<td>F-prime strains</td>
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<td>JL657</td>
<td>his-2461 aroC5 purF145/F'*32 dsd- purF+ aroC5+</td>
<td>J. Ingraham</td>
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<tr>
<td>SA2165</td>
<td>hisF1009 trpE2 metA22 xyl-1 ΔproAB47 rpsL201/F'*128 lac+ pro+ ataA+</td>
<td>K. E. Sanderson</td>
</tr>
</tbody>
</table>

* SGSC, Salmonella Genetic Stock Center.

Glucose and amino acids (50 μg ml⁻¹) as necessary. Nutrient broth and Penassay broth (Difco) served as rich media. cdd⁺ transductants were selected on the 007 medium described by Clark & Maaloe (1967) with 200 μg cytidine ml⁻¹ as the sole nitrogen source. Cultures were grown with vigorous aeration at 30 °C (permissive) and 42 °C (non-permissive). Growth was followed turbidimetrically with a Klett–Summerson colorimeter.

**Isolation of mutant strain PS-1.** Salmonella typhimurium strain SU422 (Met⁻) was mutagenized by treatment with 0.1% ethyl methanesulphonate (EMS) (Pfaltz and Bauer, Inc.) for 60 min at 30 °C in 0.1 M-Tris/maleate buffer, pH 6 (Roth, 1970). The mutagen was removed by washing and the cells (1% survivors) were grown for 8 to 10 h in rich medium at 30 °C to permit expression of mutations prior to shifting to 42 °C. After two or three doublings at 42 °C the cells were treated with 500 units of penicillin G (Lilly) and ml⁻¹ for 1 h. After a second cycle of penicillin enrichment for temperature-sensitive mutants, the survivors were scored by replica plating at 30 °C and 42 °C. Temperature-sensitive colonies, which occurred at a frequency of about 2%, were picked and restreaked.

The conditional mutants were screened for defects in PRPP synthetase by an autoradiographic method. A large loopful of cells (about 2 mg) from overnight growth on agar plates was picked from each mutant and lysed in 75 μl of 50 mM-potassium phosphate/triethanolamine buffer, pH 8, containing 25 mM-NaF, 5 mM-MgCl₂, 1 mM-EDTA and 1 mg lysozyme (Sigma) ml⁻¹. Samples (30 μl each) were incubated at either 30 °C or 42 °C for 1 h, then 30 μl of the assay mixture was added, and the incubations were continued at the same temperature for 1 h.

The assay mixture consisted of 50 mM-NaF, 10 mM-ribose-5-phosphate, 10 mM-ATP, 25 mM-MgCl₂, 50 μM [¹⁴C]adenine [0–1 μCi (3.7 kBq), Schwarz–Mann] and 5 μl of a solution of partially purified E. coli adenine phosphoribosyltransferase (1 mg ml⁻¹).

The reactions were terminated by spotting 10 μl samples on DEAE-cellulose paper strips (Whatman, DE81). Any [¹⁴C]AMP produced in the reaction depends on formation of PRPP from ATP and ribose-5-phosphate and is bound to the DEAE paper; unreacted [¹⁴C]adenine was removed by gently washing the strips twice for 15 min in 1 mM-Tris/HCl buffer, pH 8, containing 1 mM non-radioactive adenine. The DEAE paper was then dried and...
PRPP synthetase mutants of S. typhimurium

Fig. 1. Autoradiogram illustrating the identification of a mutant (PS-1) that is defective in PRPP synthetase (see Methods for description). APRTase denotes adenine phosphoribosyltransferase.

Exposed to X-ray film (DuPont Cronex 2DC) for 48 h. Figure 1 shows that a mutant strain, PS-1, with defective PRPP synthetase (column B) is easily distinguished from the parent strain (column A) by comparing spots at 42 °C. Differences at 30 °C are near background, perhaps due to lower activity of PRPP synthetase. Control experiments also show that the indicator reaction required the addition of ATP, ribose-5-phosphate and adenine phosphoribosyltransferase (columns C, D and E, respectively), although the reaction proceeds to some degree without these additions because of cellular pools. Reaction was almost completely inhibited by ADP (column F), a potent inhibitor of PRPP synthetase (Switzer & Sogin, 1973).

Isolation of mutant strain PS-2. After the genetic loci of the mutations in strain PS-1 were determined, strain PS-2 was isolated by localized mutagenesis (Hong & Ames, 1971) using strain LT-2 as the donor strain for growth of phage P-22, selecting for cold-insensitive transductants of strain JL250 (Brenchley & Ingraham, 1973) and screening these for PRPP synthetase defects by the autoradiographic procedure described above.

Assays and genetic methods. Protein in cell extracts was assayed by the Lowry method. PRPP synthetase was assayed by a modification of the 32P transfer method (Switzer & Gibson, 1978). The modification consisted of the use of [β-32P]ATP in place of [γ-32P]ATP, which resulted in much lower background radioactivity in blinks from which ribose-5-phosphate was omitted when crude extracts were assayed. PRPP synthetase was also assayed by a modification of the one-dimensional TLC procedure of Jensen et al. (1979) using polyethylenimine-impregnated plates (Macherey-Nagel Cel 300 PEI). The modifications were to use the assay conditions described previously (Switzer & Gibson, 1978), to remove nucleotides by charcoal treatment after the reaction but before spotting samples on the plates and to use 0.4 M-potassium phosphate, pH 7, in place of 0.85 M-phosphate, pH 3.4, as the developing solvent. Dried plates were autoradiographed by exposure to X-ray film. PRPP pools were determined by the coupled assay described previously (Sadler & Switzer, 1977).

Hfr crosses were done by the broth mating procedure of Sanderson et al. (1972) using a derivative of the mutant strain PS-1 resistant to 600 µg streptomycin ml⁻¹. Another derivative of mutant strain PS-1, which was resistant to both streptomycin and nalidixic acid (4 µg ml⁻¹), was isolated. This strain was used as a recipient for interrupted mating experiments, in which DNA replication in the donor strains was interrupted with nalidixic acid (Hane, 1971). These drug-resistant, spontaneous derivatives of strain PS-1 were isolated by plating 10⁴ cells per plate on selective media. Techniques for growing and conducting transduction with P22 but7, an integration-defective mutant of phage P22, were as described by Roth (1970). F-prime episome transfers were performed by mixing 0.1 ml of stationary cells of donor and recipient strains on selective plates. Since the F-primes used are of E. coli origin, it was not necessary to use recombination-deficient strains to maintain diploids (Riddle & Roth, 1972). Temperature-insensitive revertants were isolated in separate experiments by spreading 10⁸ to 10¹⁰ mutant cells per plate and incubating at 42 °C.

Immunological experiments. Antiserum to native PRPP synthetase was obtained by injecting a rabbit three times, at 7 d intervals, with 1 mg of homogeneous PRPP synthetase (Switzer & Gibson, 1978) emulsified with Freund's complete adjuvant. Antiserum was collected 1 week after the last injection. The immunoglobulin fraction was purified as described previously (Switzer et al., 1975) and salt was removed by dialysis. Specificity of antisera was examined by Ouchterlony gel diffusion and analysis of immunoprecipitates by SDS–PAGE (Crowle, 1960; Laemmli & Favre, 1973). Cell extracts for immunoprecipitation were prepared by sonication in 30 s bursts with intermittent cooling on ice. For immunoprecipitation of PRPP synthetase from crude extracts, 50 µg anti-PRPP synthetase antibody was mixed with 5 mg of crude cell protein and incubated first at 30 °C for 1 h and then at
4 °C for 16 h. Goat anti-rabbit IgG antibody (Miles Laboratories, 10 μg) was added to each tube and the tubes were incubated for 4 h at 4 °C. Precipitates were collected by centrifuging for 5 min at 9600 g in Beckman Microfuge tubes, washed twice with 0.5 M-potassium phosphate buffer, pH 7.5, containing 0-05 mM-NaCl, 0-1% (w/v) sodium deoxycholate and 0-1% Triton X-100, and analysed on 15% (w/v) polyacrylamide gels containing 0-1% sodium dodecyl sulphate (Laemmli & Favre, 1973). The microcomplement fixation technique described by Levine & Van Vunakis (1967) was also used for quantitation of PRPP synthetase antigen in crude extracts.

RESULTS

Characterization of mutant strain PS-1

The doubling time for strain PS-1 at 30 °C was 70 min, which is somewhat slower than for its parent strain SU422 (60 min). The growth of the mutant ceased within 1 h after a shift from 30 °C to 42 °C, and a majority of the cells lost viability within 4 h at 42 °C. Supplementation of the medium with histidine, tryptophan and guanosine, which might decrease the PRPP requirement of the cells, did not affect growth of the mutant at 42 °C. Addition of 1 mM-adenine, which is known to cause a sharp decrease in intracellular PRPP pools (Bagnara & Finch, 1974), did not inhibit the growth of the mutant at 30 °C.

Direct assays for the PRPP synthetase activity in cell extracts of strain PS-1 and its parent were conducted with the 32P transfer assay. The parent strain yielded an average specific activity of 0.08 μmol [min⁻¹ (mg protein)⁻¹] at 30 °C and 0.17 μmol [min⁻¹ (mg protein)⁻¹] at 42 °C, but, surprisingly, no significant activity above the background could be detected in extracts of strain PS-1 at either 30 °C or 42 °C. Mixing of mutant and parental strain extracts did not cause a significant decrease in the expected activity of the latter, which tends to exclude the presence of an inhibitory substance in the mutant extracts. PRPP synthetase activity in crude extracts was also assayed by the TLC method of Jensen et al. (1979) with modifications described in Methods. Even at 30 °C, extracts of strain PS-1 revealed <1% of the specific activity of the extracts of strain SU422 in a series of experiments with this assay. Since the growth of the mutant was nearly normal at 30 °C, it appeared that the loss of activity in the extracts resulted from extreme lability of the PRPP synthetase of strain PS-1 to preparation of cell extracts. Accordingly, we attempted to assay PRPP synthetase in mutant cells, which had been grown at 30 °C, with the [β-32P]ATP transfer assay at 20 °C, or at 30 °C in toluenized cells, in sonic extracts prepared in the presence of glycerol, EDTA and PMSF, in extracts containing 10 mM-Mg²⁺ and 5 mM-ATP and in extracts prepared by very gentle sonication, without centrifugation, or by lysozyme-EDTA lysis. No activity was found in any case. Other attempts to demonstrate formation of [14C]AMP from [14C]adenine in the coupled assay were also unsuccessful.

As a means of assessing the activity of PRPP synthetase in strain PS-1 at 30 °C and 42 °C in vivo, PRPP pools in the cells were measured. At 30 °C the PRPP levels in strain PS-1 ranged from 0.7 to 0.9 nmol (mg cell protein)⁻¹ which was only about 20% of the PRPP level in the parental strain at the same temperature. At 42 °C, the PRPP content of the parent dropped to one-half to one-third of the value at 30 °C. Similarly, the PRPP pools in strain PS-1 decreased from an average of 0.8 nmol mg⁻¹ at 30 °C to about 0.2 nmol mg⁻¹ after a shift to 42 °C. The fact that PRPP pools were much lower in strain PS-1 than in the parent strain is indicative of a defect in PRPP synthesis in vivo, but the observation that the PRPP pool did not decrease rapidly to undetectable levels at 42 °C does not support the suggestion that the PRPP synthetase in the mutant is thermolabile in vivo.

Immunoochemical characterization of strain PS-1

We were not able to detect protein that was immunoochemically cross-reactive with purified PRPP synthetase in extracts of strain PS-1 by direct or indirect immunoprecipitation procedures, but cross-reactive protein was detected by a more sensitive complement fixation
Fig. 2. Complement fixation with increasing antigen (cell extract) and fixed antibody concentration. (a) Anti-native PRPP synthetase antibody (0.5 µg) was titrated with crude extracts from strain SU422 (○) or strain PS-1 (▲). (b) Anti-native PRPP synthetase antibody (1 µg) was titrated with extracts as in a, except that the sample from strain PS-1 was from a 50% (NH₄)₂SO₄ precipitated fraction from the cell extract. Anti-denatured PRPP synthetase antibody (1 µg) was also titrated with the same extracts from strain SU422 (□) or strain PS-1 (△). Different complement preparations were used in a and b, so equivalence points are not directly comparable.

Genetic characterization and mapping of mutations in strain PS-1

Genetic experiments demonstrated that the temperature sensitivity and PRPP synthetase defectiveness in vitro of strain PS-1 result from two separate, but closely linked mutations.
Strain PS-1 was transduced to temperature insensitivity with phage P22 lysates prepared on strain LT-2. Of 17 transductants assayed for PRPP synthetase in vitro, 15 displayed wild-type activity (88% cotransduction) and two had no assayable activity. Temperature-insensitive transductants that still lack PRPP synthetase activity in vitro grew at slightly slower rates than strain SU422. Spontaneous temperature-insensitive revertants were isolated independently at a frequency of 1 in $10^8$ in several experiments. All of the revertants examined grew at rates slower than strain SU422 (80 to 90 min doubling times, as opposed to 60 min for strain SU422), so they were presumably not true revertants. None of the revertants tested contained assayable PRPP synthetase activity. These results confirm that the phenotype of strain PS-1 results from separate mutations. Because of the frequent appearance of revertants in cultures of strain PS-1, purification of the strain by replica plating was necessary at the start of experiments, especially after prolonged storage of the culture.

Introduction of the F'32 episome, which covers the 45 to 50 unit region (Fig. 3) into strain PS-1 restored both the ability to grow at 42 °C and the PRPP synthetase activity in vitro. This observation confirmed the general map location of the lesions in strain PS-1, showed the dominance of wild-type episomal alleles, and suggested that analogous genes occur at similar loci in E. coli. We were unable to demonstrate a gene dosage effect for PRPP synthetase activity in vitro in F'32/purF145 diploids as compared to the purF145 strain, which may indicate that the F'32 episome supplies a missing regulatory function. The partial diploids were able to act as F' donors as expected. As a control it was shown that the episome F'128 (Fig. 3) did not confer temperature insensitivity.

Broth mating and interrupted conjugation (Roth, 1970; Sanderson et al., 1972) using several Hfr donor strains (Fig. 3) and a streptomycin-resistant, nalidixic acid-resistant derivative of strain PS-1 as recipient showed that the temperature-sensitive lesion lies between 45 and 50 units (data not shown). Linkage of mutations in strain PS-1 with markers in the 45 to 50 unit area (Sanderson & Hartman, 1978) was determined by cotransduction (Table 2). The temperature sensitivity was 12% linked to cdd and 50% linked to hisW. No linkage was found to any other marker examined. Since cdd and hisW were found to be only weakly (2%) linked, we infer that the mutation maps between these two markers (Fig. 3). Similarly, P22
Table 2. Transductional mapping of the temperature-sensitive lesion of strains PS-1 and PS-2

<table>
<thead>
<tr>
<th>P22 donor</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>Unselected marker</th>
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</thead>
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<td>Cdd⁺</td>
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<td>HisW⁺</td>
<td>ts</td>
<td>205/411</td>
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<td>Thi⁺</td>
<td>ts</td>
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</tr>
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</tr>
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<td>hisW3333</td>
<td>HisW⁺</td>
<td>ts‡</td>
<td>125/125</td>
</tr>
</tbody>
</table>

ts, denotes temperature sensitivity.

*hisW3333 is a cold-sensitive mutant described previously (Brenchely & Ingraham, 1973).
† The ability to use cytidine as a sole source of nitrogen (Clark & Maaloe, 1967).
‡ Thirteen hisW⁺, temperature-sensitive transductants were tested for PRPP synthetase activity levels. All of them were found to have enzyme levels similar to the donor strain PS-2 [average specific activity of 0.04 μmol (min mg)⁻¹ compared with 0.07 μmol (min mg)⁻¹ for hisW3333 in crude extracts at 30 °C].

lysates from strain TT5371, in which a Tn10 transponon is 90% linked to hisW (J. Roth, personal communication), gave rise to transductants of strain PS-1 that were both tetracycline-resistant and temperature-insensitive with a frequency of 1 in 10⁶ plaque-forming units. When strains TT184 and TT946, in which Tn10 is incorporated at 7 units and 21 units, respectively (J. Roth, personal communication), were used as the source of P22 lysates, the frequency of double transductants was <1 in 10⁶. Five out of the six double transductants of the TT5371/P22 × PS-1 cross that were tested showed donor type PRPP synthetase activity in vitro. These results are consistent with the mapping data above linking the temperature sensitivity and PRPP synthetase defect in strain PS-1 to hisW.

As described above, temperature-insensitive derivatives of strain PS-1 that still had no assayable PRPP synthetase activity in vitro could be obtained by selecting revertants and by transduction. PRPP pools were determined at 30 °C and 42 °C in cultures of a PRPP synthetase mutant of each type. The PRPP pools in these strains were about 25% of the normal values at both growth temperatures and were similar to the values found in the temperature-sensitive parent strain. The result reinforces the conclusions that the PRPP synthetase defect and temperature sensitivity of PS-1 result from separate mutations and that the PRPP synthetase lesion is expressed in vivo, even though the strains are able to grow at nearly normal rates.

Characterization of strain PS-2

A second temperature-sensitive mutant, strain PS-2, with defective PRPP synthetase activity in vitro was isolated by localized mutagenesis (see Methods). Strain PS-2 grew with a doubling time of 80 min at 30 °C, as compared to 66 min for its parent strain, LT-2. The strain grew very poorly on agar plates at 42 °C and in liquid culture started with a small inoculum, but did not show a sharp decrease in growth rate when a culture was shifted from 30 °C to 42 °C. The specific activity of PRPP synthetase in extracts of PS-2 was about half of that of the parent strain when assayed at either 30 °C or 42 °C. The $K_m$ value for ATP, measured in an ammonium sulphate fraction from a crude extract, was about the same for the mutant and its parent. Both complement fixation assays and analysis of immunoprecipitates from radiolabelled cells indicated that strain PS-2 contained about half as much protein cross-reactive with PRPP synthetase as did strain LT-2 (data not shown). PRPP pools in
in vivo in strain PS-2 were essentially the same as in the parent strain (data not shown). Since strain PS-2 was isolated by localized mutagenesis, its lesions would be expected to map at 47 units. This was confirmed by the transduction experiment shown in Table 2.

**DISCUSSION**

Our major conclusion is that a mutation affecting PRPP synthetase activity in vitro and in vivo maps at 47 units on the *Salmonella* chromosome. It is clear from analysis of transductants and revertants that the phenotype of strain PS-1 results from two closely linked mutations. PRPP pools in the mutant are not obviously temperature-sensitive, but growth of the strain is. The lower PRPP pool in temperature-insensitive derivatives of PS-1 provides evidence that the PRPP synthetase defect is expressed in vivo, but the PRPP pool is not sufficiently lowered to yield a readily selectable phenotype. Thus, we were compelled to use the closely linked temperature sensitivity marker for genetic mapping.

The properties of strain PS-2 could also result from double mutation, although this has not been proven by genetic analysis. The PRPP synthetase of strain PS-2 was a little more temperature-sensitive in vitro in crude extracts than the wild-type enzyme, but the behaviour of PRPP pools in vivo indicated that the temperature sensitivity of growth did not result from this defect. The major conclusion we draw from the study of strain PS-2 is that multiple PRPP synthetase phenotypes result from mutations mapping at 47 units.

The PRPP synthetase of strain PS-1 appears to be functional at a reduced level in vivo but hyperlabile in vitro. These properties and the immunochemical characteristics of the mutant enzyme can be best explained by suggesting a defect in the structural gene for PRPP synthetase. The results can also be explained by postulating that another source of PRPP exists in vivo. No such pathway is known at present, and we have not been able to assay one in vitro.

It is also possible that PRPP synthetase formation requires participation of additional genetic loci. Recently, Jochimsen et al. (1980) reported the isolation of a mutant of *S. typhimurium* that had abnormally low PRPP pools and an apparently defective PRPP synthetase. The method of selection was quite different from the approach used in this paper. The mutation was mapped near *proAB* at 7 units, although further characterization is in progress (J. S. Gots, personal communication). P22 phage grown on the mutant described by Jochimsen et al. were able to transduce strain PS-1 to temperature insensitivity and normal PRPP synthetase activity in vitro, so it is clear that the PRPP synthetase lesions in the two strains are genetically separable. Using a similar approach to that of Jochimsen et al., Hove-Jensen & Nygaard have isolated an *Escherichia coli* mutant that contains a PRPP synthetase with altered kinetic properties; this mutation was found linked to *hemA* and *purB* near 25 min (P. Nygaard, personal communication). In the case of *Salmonella*, however, *hemA* and *purB* are unlinked genes. In each of these cases the properties of the PRPP synthetase activities in vitro suggest that the mutations lie in the structural gene for the enzyme. Yet the map locations are far from each other and from our locus for the mutation in strains PS-1 and PS-2. All available evidence indicates that *Salmonella* PRPP synthetase is composed of identical subunits (Schubert & Switzer, 1975), so it is unlikely that two or more genetic loci code for structural elements of the enzyme. The possibility that some of these loci represent regulatory genes or genes for modifying enzymes must be considered. Further genetic and biochemical characterization of all of the mutants is clearly required. The methods and results reported here should be valuable in future studies.

We are grateful to K. Gibson for valuable suggestions and for providing purified PRPP synthetase and to S. Rosenzweig for synthesizing [β-32P]ATP and performing PRPP pool measurements. We acknowledge Professor J. Cronan for helpful discussions of genetic experiments and Professor E. Voss for discussions of immunochemical
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experiments. Thanks are due to Drs P. Nygaard and J. S. Gots for communication of their results prior to publication and to Dr Gots for cultures of his mutant and parent strains.

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