

Metabolism of Exogenous Purine Bases and Nucleosides by *Salmonella typhimurium*

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Purine-requiring mutants of *Salmonella typhimurium* LT2 containing additional mutations in either adenosine deaminase or purine nucleoside phosphorylase have been constructed. From studies of the ability of these mutants to utilize different purine compounds as the sole source of purines, the following conclusions may be drawn. (i) *S. typhimurium* does not contain physiologically significant amounts of adenine deaminase and adenosine kinase activities. (ii) The presence of inosine and guanosine kinase activities in vivo was established, although the former activity appears to be of minor significance for inosine metabolism. (iii) The utilization of exogenous purine deoxyribonucleosides is entirely dependent on a functional purine nucleoside phosphorylase. (iv) The pathway by which exogenous adenine is converted to guanine nucleotides in the presence of histidine requires a functional purine nucleoside phosphorylase. Evidence is presented that this pathway involves the conversion of adenine to adenosine, followed by deamination to inosine and subsequent phosphorolysis to hypoxanthine. Hypoxanthine is then converted to inosine monophosphate by inosine monophosphate pyrophosphorylase. The rate-limiting step in this pathway is the synthesis of adenosine from adenine due to lack of endogenous ribose-1-phosphate.

The sequence of reactions responsible for the de novo synthesis of purine nucleotides in *Salmonella typhimurium* is well established (25, 45). The end products of the entire pathway, adenosine monophosphate (AMP) and guanosine monophosphate (GMP), are generated from a common intermediate, inosine monophosphate (IMP) through the intermediary formation of adenylosuccinate (succinyl-AMP) and xanthosine monophosphate (XMP), respectively (9, 23, 27, 31), as shown in Fig. 1.

In addition, GMP may be reduced directly to IMP by GMP reductase (EC 1.6.6.8; reference 28) and thereby serve as a precursor for AMP. Similarly, a pathway exists for the conversion of adenine nucleotides to IMP and thereby GMP. This latter pathway, however, has been shown to involve the prior formation of adenosine triphosphate (ATP), which, by a series of reactions in common with the histidine biosynthetic pathway (26, 32), is converted to aminoimidazole carboxamide ribotide (AICAR) and imidazoleglycerol phosphate (IGP). AICAR is an intermediate in the de novo synthesis of IMP, and IGP is a precursor of histidine. The first enzyme involved in the conversion of ATP to AICAR, phosphori-

bosyl-ATP pyrophosphorylase, is feedback inhibited by histidine (3, 30), and it has been shown that the conversion of adenine nucleotides to IMP through this pathway is completely blocked in cells growing in a histidine-supplemented medium (reference 26; Fig. 1).

Although free purine bases and nucleosides are not intermediates in the de novo pathway for purine nucleotide synthesis, they are readily incorporated into nucleic acids by enteric bacteria (5, 6). This indicates the existence of alternative pathways for purine nucleotide synthesis from preformed purine compounds.

Although the importance of purine nucleotide pyrophosphorylases for the utilization of exogenous purine bases is well established (8, 19, 20), much less is known about the metabolism of exogenous purine nucleosides and the physiological function of purine nucleoside phosphorylase (EC 2.4.2.1) and adenosine deaminase (EC 3.5.4.4) in the utilization of exogenous purine compounds for nucleotide synthesis.

Zimmerman and Magasanik have presented evidence for the existence of inosine kinase and guanosine kinase activities in *S. typhimurium* (46). Furthermore, they showed that exogenous adenine, in the presence of histidine, may be converted to guanine nucleotides through the inter-

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mediary formation of hypoxanthine (46). Studies with *Escherichia coli* have revealed that exogenously added adenosine is rapidly metabolized to inosine and hypoxanthine, which can be recovered from the medium (29). In addition, Peterson and Koch observed that the deamination of adenosine was tightly coupled to the uptake of this compound by cell suspensions of *E. coli* (40).

The purpose of the present work was to gain a more detailed insight into the metabolic reactions involved in the utilization of exogenous purines and purine nucleosides for nucleotide synthesis. Different purine-requiring mutants of *S. typhimurium* containing additional mutations in either adenosine deaminase (*add*) or purine nucleoside phosphorylase (*pup*) were constructed, and the ability of these mutants to grow on different purine sources was studied.

MATERIALS AND METHODS

Bacterial strains. The strains used throughout the present work were all derived from *S. typhimurium* LT2. They are listed together with their characteristics in Table 1.

Purine-requiring mutants. Purine-requiring mutants were isolated after treatment with *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine (NTG; from K&K Laboratories Inc., Plainview, N.Y.; reference 1) and penicillin counterselection in glucose minimal medium. Purine auxotrophic mutants were divided into three classes according to their growth requirement. (i) Mutants blocked in one of the 10 enzymes involved in the de novo synthesis of IMP (see Fig. 1) are satisfied in their entire purine requirement by either hypoxanthine, adenine, or guanine (*pur*). (ii) Mutants deficient in either succinyl-AMP synthetase (EC 6.3.4.4; *purA*) or adenylosuccinase (EC 4.3.2.2; *purB*), i.e., blocked in the conversion of IMP to AMP, have a specific adenine re-

TABLE 1. *Salmonella typhimurium* LT2 mutants used in this study

Strain	Derived from	Genotype	Specific activity ^a of			Nutritional growth requirements
			Purine nucleoside phosphorylase	Adenosine deaminase	Deoxyribomutase	
LT2 ^b		Wild type	125	31	59	None
DP2 ^b	LT2	<i>pyrA</i> 81	149	34	152	Arginine, uracil
DP65	DP2	<i>pyrA</i> 81, <i>pup</i>	6			Arginine, uracil
JH35	LT2	<i>pur</i> ^c				Hypoxanthine ^d
JH48	JH35	<i>pur</i> , <i>pup</i>	2			Hypoxanthine
JH100	JH48	<i>pur</i> , <i>pup</i> , <i>his</i>				Hypoxanthine, histidine
JH103	JH100	<i>pur</i> , <i>pup</i> , <i>hisG</i> 1102	<1			Hypoxanthine
JH108	JH48	<i>pur</i> , <i>drm</i>			1	Hypoxanthine
JH32	LT2	<i>purA</i> ^e	175	37		Adenine
JH40	JH32	<i>purA</i> , <i>add</i>	123	2		Adenine
JH65	JH40	<i>purA</i> , <i>add</i> , <i>pup</i>	3	2		Adenine
JH67	DP65	<i>pyrA</i> 81, <i>pup</i> , <i>guaB</i> ^f	1			Guanine, arginine, uracil
<i>hisG</i> 203 ^b		<i>hisG</i> 203				Histidine
<i>hisG</i> 1102 ^g		<i>hisG</i> 1102				None
KJ163	DP2	<i>pyrA</i> 81, <i>thyA</i> , <i>drm</i>				Arginine, uracil, thymine

^a Assayed as described in Materials and Methods. Specific activities are expressed as nanomoles of product formed per minute per milligram of protein. In the adenosine deaminase assay, the determination of protein was performed on sonic extracts of the cell suspensions.

^b Obtained from J. L. Ingraham. *hisG* 203 contains a deletion in the *hisG* gene.

^c Since the growth yield of this mutant on limiting amounts of purine is unaltered whether histidine is present or not, it may be concluded that the block in purine biosynthesis is located prior to the formation of AICAR. Significant amounts of purine supplement would be excreted as aminoimidazole carboxamide (AICA) riboside and lost for growth purposes if the mutation was in one of the two steps between AICAR and IMP. In the presence of histidine, however, this would be prevented (26).

^d Mutants with a nutritional requirement for hypoxanthine are able to satisfy this requirement by either hypoxanthine, adenine, or guanine.

^e The *purA* mutation (succinyl-AMP synthetase) may be distinguished from the other class of adenine-requiring mutants, *purB* (adenylosuccinase), by the fact that the latter cannot supply their GMP de novo. Adenylosuccinase catalyzes both the conversion of succinyl-AMP to AMP as well as the formation of AICAR from 5-amino-4-imidazole-*N*-succino-carboxamide-ribose. In contrast, *purA* mutants have an intact de novo pathway for GMP biosynthesis. The ability of JH40 (an *add* derivative of JH32; see equation 4) to grow on adenine plus histidine clearly indicates that JH40, and therefore JH32, is a *purA* mutant.

^f Besides guanine, only xanthine will satisfy the purine requirement of this strain, indicating that the mutation is in IMP-dehydrogenase (*guaB*) rather than in XMP-aminase (*guaA*).

^g Obtained from P. Hartman.

quirement (11). (iii) Mutants unable to convert IMP to GMP, i.e., lacking either IMP dehydrogenase (EC 1.2.1.14) (*guaB*) or XMP aminase (EC 6.3.4.1; *guaA*), have an absolute requirement for guanine (31).

JH40 (*purA*, *add*). From the *purA* mutant JH32, a strain deficient in adenosine deaminase (*add*) was obtained as follows. Portions of a mutagenized culture of JH32, phenotypically expressed in glucose minimal medium containing adenine, were spread on glucose minimal agar plates containing low concentrations of deoxyadenosine as the sole purine source (4 μ g/ml). JH32 is unable to grow on low amounts of deoxyadenosine due to the presence of adenosine deaminase, which deaminates deoxyadenosine rapidly to deoxyinosine (Table 2). Thus, from among the cells that grew on these plates, adenosine deaminase-negative mutants (*add*) were isolated.

Purine nucleoside phosphorylase-negative mutants (*pup*). *pup* Mutants were obtained by penicillin-counterselecting a mutagenized culture (NTG) in minimal medium containing a purine nucleoside as the sole source of carbon. Mutants unable to utilize adenosine but able to grow on uridine as the sole carbon source were assayed for purine nucleoside phosphorylase activity. The isolation of *pup* mutants in JH40 (*purA*, *add*) was carried out as follows. Five-hundred cells of a mutagenized culture (NTG) of JH40 were spread on glucose minimal agar plates containing a sufficient amount of deoxyadenosine (30 μ g/ml) to support full growth of the parent strain. In addition, they contained limiting amounts of adenine (0.3 μ g/ml). Since JH40 requires a functional purine nucleoside phosphorylase to utilize deoxyadenosine as an adenine source (see Fig. 2), microcolonies were picked and tested for growth on purine nucleosides as carbon sources.

JH103 (*pur*, *pup*, *hisG* 1102). The *hisG* 1102 mutation has been shown to make the cells resistant to the histidine analogue, 2-thiazole-alanine, due to a mutation in the first enzyme of the histidine biosynthetic pathway, phosphoribosyl-ATP pyrophosphorylase. The mutant enzyme is insensitive to feedback inhibition by histidine (43). As a result, cells harboring the *hisG* 1102 mutation overproduce histidine and excrete it into the medium.

Strain JH103 was constructed by phage P22-mediated transduction. The donor strain was *S. typhimurium* *hisG* 1102 and the recipient was JH100, a histidine-requiring derivative of JH48 (*pur pup*; see Table 1). *His*⁺ transductants were spotted on glucose minimal plates containing adenine plus 10⁷ cells of the indicator bacterium, *S. typhimurium* *hisG* 203. The *hisG* 203 cells were mixed into the agar just before pouring the plates. Transductants carrying the *hisG* 1102 mutation were easily distinguished, since a halo of secondary growth of the indicator bacterium (*hisG* 203) surrounded the colonies.

JH108 (*pur drm*) is a purine-requiring mutant with an additional block in phosphopentomutase (*drm*). Phosphopentomutase catalyzes the conversion of ribose-1-phosphate and deoxyribose-1-phosphate to the corresponding 5-phosphates (15). Since the structural gene for phosphopentomutase has been shown to be closely linked to the *pup* locus on the *Salmonella* linkage map (42), and since a *drm* mutant of *S. typhimurium* KJ163 (*pyrA* 81, *thyA*, *drm*) was available in

our culture collection, JH108 was constructed by co-transducing the *pup*⁺, *drm* characters from KJ163 into JH48 (*pur*, *pup*, *drm*⁺). Transductants capable of utilizing deoxyinosine as the sole purine source (*pup*⁺) but unable to grow on adenosine as a carbon source (*drm*) were isolated.

Growth conditions. Bacteria were grown in minimal medium (36) containing FeSO₄·7H₂O (0.25 mg/liter) and thiamine (1 μ g/ml). In most experiments, glucose (0.2%) was used as the carbon source; however, in a few cases 0.1 % of a purine ribonucleoside or deoxyribonucleoside replaced glucose.

Required nutrients were added to the medium in the following concentrations (unless otherwise indicated): ribonucleosides and deoxyribonucleosides, 30 μ g/ml; purine bases, 15 μ g/ml; histidine, 50 μ g/ml; arginine, 50 μ g/ml; and uracil, 10 μ g/ml. Cultures were grown on a reciprocal water-bath shaker at 37 C. Increase in the cell mass was followed at 450 nm in a Zeiss M4QIII spectrophotometer. One milliliter of a bacterial culture with an absorbancy at 450 nm of 1,000 contains approximately 4 \times 10⁸ cells or 0.2 mg dry weight.

Transduction. Bacteriophage P22 Int4 (44) was used for all transductions. Transducing phages were recovered from cells growing in nutrient broth (10). Recipient bacteria from a freshly outgrown broth culture (Nutrient Broth, Difco; 2 \times 10⁹ bacterial cells/ml) were mixed with transducing phages at a multiplicity of infection of 5 (JH103) or 1.5 (JH108), and incubated at 37 C for 10 min. The cells were centrifuged, washed, resuspended in broth, and plated on the appropriate selective plates.

Enzyme assays. Sonic extracts were used for determination of enzyme activities except in the case of adenosine deaminase, in which assays were performed with suspensions of washed cells. Sonic treatment was performed with an MSE 100-w Ultrasonic Disintegrator (Measuring & Scientific Equipment Ltd., London, S.W. 1, England). Portions (1 to 5 ml) of washed cell suspensions containing about 10¹⁰ cells/ml were sonicated for 60 sec at 0 C.

Purine nucleoside phosphorylase. Purine nucleoside phosphorylase was determined spectrophotometrically by coupling the reaction with xanthine oxidase (EC 1.2.3.2.) as described by Kalckar (17). The reaction mixture contained the following in 1 ml: potassium phosphate buffer, pH 7.1, 170 μ moles; inosine, 2 μ moles; 0.01 to 0.02 unit of xanthine oxidase; and cell extract corresponding to 4 \times 10⁸ cells. The reaction was carried out at 37 C in a thermostated cuvette, and the increase in absorbancy at 293 nm was followed in a Zeiss spectrophotometer. When the amount of product formed was calculated, an increase in molar absorbancy of 12.0 \times 10³ (21) was assumed.

Phosphodeoxyribomutase. Phosphodeoxyribomutase was assayed as described by Hammer-Jespersen and Munch-Petersen (12).

Adenosine deaminase. Adenosine deaminase was determined spectrophotometrically at 265 nm (18, 21). The reaction mixture contained in 1.0 ml: adenosine, 0.05 μ mole; tris(hydroxymethyl)aminomethane-chloride, pH 7.6, 25 μ moles; and approximately 10⁸ washed cells. The assay was carried out at 37 C in a thermostated cuvette, and the decrease in absorbancy at 265 nm was followed in a Zeiss spectrophotometer. In the

calculation of enzyme activity, a factor of 8.5×10^3 was used as the difference in molar extinction coefficient between adenosine and inosine (21).

Protein was determined by the method of Lowry et al. (24).

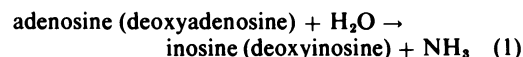
Determination of acid-soluble nucleoside triphosphate pools. Cells were grown exponentially for several generations in the presence of ^{32}P -orthophosphate ($3 \mu\text{Ci}/\mu\text{mole}$) and $[8\text{-}^{14}\text{C}]\text{adenine}$ ($14 \mu\text{Ci}/\mu\text{mole}$). Preparation of extracts as well as thin-layer chromatographic separation of the labeled nucleoside triphosphates have been described (38). The spots from the chromatograms corresponding to the purine nucleoside triphosphates were cut out and counted (38).

Radiochemicals. ^{32}P -orthophosphate was obtained from Atomenergikommissionens Forsøgsanl g (Risø, Denmark). $[8\text{-}^{14}\text{C}]\text{adenine}$ was purchased from the Radiochemical Centre, Amersham, England.

RESULTS

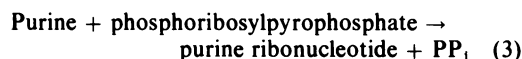
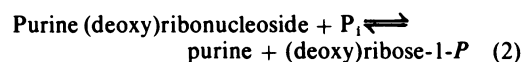
Metabolism of adenosine and deoxyadenosine.

Mutants of *S. typhimurium* blocked in one of the enzymes catalyzing the formation of AMP from IMP (*purA* or *purB*) require adenine for growth. The adenine requirement can be replaced by either adenosine or deoxyadenosine. However, as shown in Table 2, the growth yields on the nucleosides are extremely low. This is in accordance with observations made by Zimmerman and Magasanik (46) and is explained by the presence of adenosine deaminase, which catalyzes the deamination of both adenosine and deoxyadenosine (21, 22; equation 1). The products of the reactions, inosine and deoxyinosine, are unable to support growth of an adenine-requiring mutant. Accordingly, the adenine-requiring mutant JH40 (*purA*, *add*), which lacks adenosine deaminase, has a 10-fold increase in growth yield on adenosine and deoxyadenosine (Table 2) as compared with the parent JH32 (*purA*).



Two possible pathways for the conversion of purine ribonucleosides to the corresponding ri-

bonucleotides may be considered: (i) direct phosphorylation of adenosine to AMP, catalyzed by a kinase, and (ii) a pathway involving adenine as an intermediate, catalyzed by the sequential action of purine nucleoside phosphorylase (equation 2) and AMP pyrophosphorylase (references 19, 20; equation 3; Fig. 1).



Only equations 2 + 3 can account for the conversion of deoxyadenosine to AMP in JH40. To differentiate between these two alternatives for the conversion of adenosine to AMP, a mutation in purine nucleoside phosphorylase (*pup*) was introduced into JH40, yielding the triple mutant JH65 (*purA*, *add*, *pup*). As shown in Fig. 2, JH65, in contrast to JH40, is unable to satisfy its adenine requirement by adenosine or deoxyadenosine, whereas growth on adenine is normal. Thus, it may be concluded that exogenous adenosine and deoxyadenosine may be metabolized by two pathways in *S. typhimurium*, of which direct deamination constitutes the major route (90%). About 10% escapes deamination and is converted to adenine by purine nucleoside phosphorylase. Furthermore, the data show that *S. typhimurium* is unable to phosphorylate adenosine to AMP in vivo.

Metabolism of inosine and deoxyinosine. As indicated in Table 3, the purine-requiring mutant JH35 grows equally well on either hypoxanthine, inosine, or deoxyinosine as the sole source of purine. The only pathway by which deoxyinosine may fulfill the growth requirement of JH35 must be via degradation to hypoxanthine. The inability of JH48 (a purine nucleoside phosphorylase-deficient derivative of JH35) to utilize deoxyinosine (Table 3) agrees with this and also establishes that purine nucleoside phosphorylase is obligatory for this degradation.

Table 3 shows that JH48 (*pur*, *pup*) will grow

TABLE 2. Growth yield of *S. typhimurium* JH32 and JH40 on adenine, adenosine, and deoxyadenosine in the presence of histidine^a

Strain	Genotype	Growth yield after 16 hr					
		2 μg of adenine/ml		4 μg of adenosine/ml		4 μg of deoxyadenosine/ml	
		OD ₄₅₀	cells/m μmole	OD ₄₅₀	cells/m μmole	OD ₄₅₀	cells/m μmole
JH32	<i>purA</i>	0.940	2.5×10^7	0.150	0.4×10^7	0.107	0.3×10^7
JH40	<i>purA</i> , <i>add</i>	1.370	3.7×10^7	1.300	3.3×10^7	1.190	3.0×10^7

^a A 10-ml amount of minimal medium containing histidine (50 $\mu\text{g}/\text{ml}$), glucose (0.2%), thiamine (1 $\mu\text{g}/\text{ml}$), and the indicated amount of purines was inoculated with JH32 or JH40 (2×10^6 cells/ml). Turbidity was measured in optical density (OD) of 450 nm after 16 hr at 37 C.

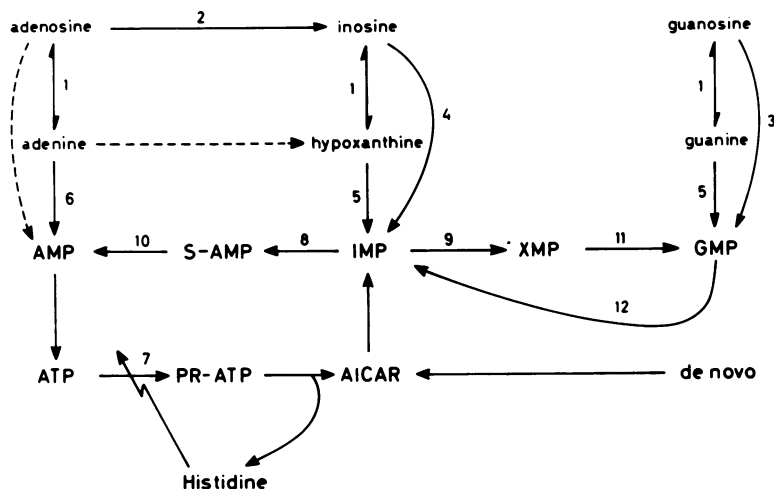


FIG. 1. Purine metabolism in *Salmonella typhimurium*. 1, Purine nucleoside phosphorylase; 2, adenosine deaminase; 3, guanosine kinase; 4, inosine kinase; 5, IMP (GMP) pyrophosphorylase; 6, AMP pyrophosphorylase; 7, phosphoribosyl-ATP pyrophosphorylase; 8, succinyl-AMP synthetase; 9, IMP dehydrogenase; 10, adenylosuccinase; 11, XMP aminase; 12, GMP reductase. Broken lines indicate reactions which do not occur. S-AMP, succinyl-AMP; PR-ATP, phosphoribosyl-ATP.

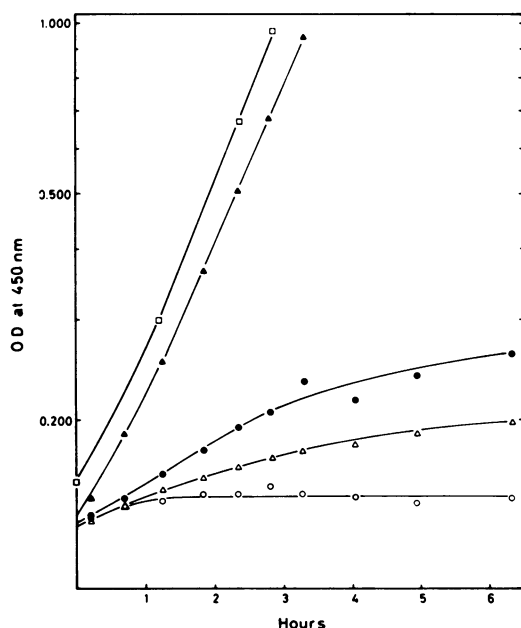


FIG. 2. Growth of JH65 on different purine sources. Cultures growing exponentially with adenine as the purine source were filtered and washed free of adenine. Cells were suspended in glucose minimal medium supplemented with thiamine (1 µg/ml) and the following purine sources: O, none; ▲, adenine (15 µg/ml); ●, adenosine (30 µg/ml); Δ, deoxyadenosine (30 µg/ml). The growth curve for JH40 on adenosine, from a parallel experiment, is added for comparison (□).

on inosine as the only purine source, suggesting the existence of inosine kinase activity (46). However, the low growth rate obtained indicates that this pathway is normally of minor importance for the utilization of inosine. This is further emphasized by the finding that, even under conditions where inosine only has to fulfill the requirement for guanine nucleotides (i.e., in the presence of inosine, adenine, and histidine; Table 3), the growth rate of JH48 is suboptimal.

Thus the majority of exogenously added inosine seems to be phosphorylated by purine nucleoside phosphorylase before it is converted to IMP. The finding that JH35 grows equally well on deoxyinosine and hypoxanthine (Table 3) shows, moreover, that the capacity of purine nucleoside phosphorylase is sufficient to ensure an adequate supply of hypoxanthine from deoxyinosine in a purine-requiring cell.

Metabolism of guanosine and deoxyguanosine. To test for the existence of guanosine kinase activity in *S. typhimurium*, a specific guanine-requiring mutant, JH67, defective in purine nucleoside phosphorylase (*guaB*, *pup*) was constructed. The ability of JH67 to grow on different guanine compounds is shown in Table 4. The results presented suggest that the cells contain guanosine kinase activity (46) and establish that the activity is not rate limiting for growth of a *gua* mutant on guanosine.

Pathways in adenine metabolism. In view of the apparent lack of adenosine kinase in *S. typhimurium*, the only pathway for the direct conversion

TABLE 3. Growth rate of *S. typhimurium* JH35 and JH48 on different purine sources^a

Strain	Genotype	Growth rate (doublings per hr) on					
		Hypoxanthine	Inosine	Deoxyinosine	Inosine + adenine + histidine	Adenosine	Deoxyadenosine
JH35	<i>pur</i>	1.11	1.03	1.00	1.00	1.05	1.05
JH48	<i>pur, pup</i>	1.11	0.41	0	0.88	0.38	0

^a Exponentially growing cultures were filtered, washed free of hypoxanthine, and suspended in minimal medium (4×10^7 cells/ml) containing glucose (0.2%), thiamine (1 μ g/ml), and the indicated purines in the following concentrations: hypoxanthine and adenine (15 μ g/ml), inosine, deoxyinosine, adenosine, and deoxyadenosine (30 μ g/ml), and histidine (50 μ g/ml). Growth was followed spectrophotometrically at 450 nm.

of adenine to AMP in this organism is by condensation with phosphoribosylpyrophosphate (PRPP) catalyzed by AMP pyrophosphorylase. Adenine may be converted to guanine compounds by two alternative pathways, one of which involves the first six enzymes of the histidine biosynthetic pathway (26, 32; cf. Fig. 1). The first enzyme of this pathway (i.e., phosphoribosyl-ATP pyrophosphorylase) is feedback controlled by histidine, and it has been shown that excess histidine is able to prevent the formation of AICAR and thereby IMP and GMP from ATP. Although the second route for the conversion of exogenous adenine to guanine compounds does not involve deamination at the nucleotide level (46), it has not been clearly established whether a direct deamination of adenine to hypoxanthine or a conversion of adenine to adenosine followed by deamination at the nucleoside level participates in this pathway.

To differentiate between these two alternatives, the growth of JH35 and its *pup* derivative, JH48, was followed in the presence of histidine. Table 5 shows that, in contrast to JH35, JH48 is unable to satisfy its purine requirement by adenine.

TABLE 4. Growth rate of *S. typhimurium* JH67 on different guanine compounds^a

Strain	Genotype	Growth rate (doublings per hr) on		
		Guanine	Guanosine	Deoxyguanosine
JH67	<i>guaB, pup, pyrA 81</i>	1.22	1.25	0

^a Exponentially growing cultures were filtered, washed free of guanine, and suspended in minimal medium (4×10^7 cells/ml) containing glucose (0.2%), arginine (50 μ g/ml), uracil (10 μ g/ml), thiamine (1 μ g/ml), and the indicated guanine compounds in the following concentrations: guanine (15 μ g/ml), guanosine and deoxyguanosine (30 μ g/ml). Growth was followed spectrophotometrically at 450 nm.

Since a purine-requiring mutant requires a functional purine nucleoside phosphorylase to utilize adenine as the sole purine source in the presence of histidine, it follows that *S. typhimurium* does not contain significant amounts of adenine deaminase activity. Thus, the histidine-insensitive conversion of adenine to IMP (or GMP) must involve ribosylation of adenine to adenosine followed by deamination to inosine.

Quantitative aspects of adenine metabolism.

The observation that the growth rate of JH35 in the presence of histidine and adenine is significantly decreased (Table 5) compared to growth on hypoxanthine led us to study the quantitative aspects of adenine utilization. Figure 3 shows the effect of increasing concentrations of adenine on the growth rate of JH35 in the presence (curve B) and absence (curve A) of exogenous histidine. In both cases adenine is growth limiting at concentrations below 100 μ g/ml. Moreover, it appears that the contribution from the histidine-controlled pathway (curve A minus curve B) is independent of the concentration of adenine in the range studied. Thus, the increase in growth rate

TABLE 5. Growth rate of *S. typhimurium* JH35 and JH48 on different purine bases in the presence of histidine^a

Strain	Genotype	Growth rate (doublings per hr) on		
		Hypoxanthine	Adenine	Adenine + guanine
JH35	<i>pur</i>	1.11	0.57	1.09
JH48	<i>pur, pup</i>	1.11	0	1.16

^a Exponentially growing cells were filtered, washed free of purines, and suspended in minimal medium (approximately 2×10^7 cells/ml) containing glucose (0.2%), thiamine (5 μ g/ml), histidine (50 μ g/ml), and the indicated purines in the following concentrations: hypoxanthine and adenine (15 μ g/ml), guanine (5 μ g/ml). Growth was followed spectrophotometrically at 450 nm.

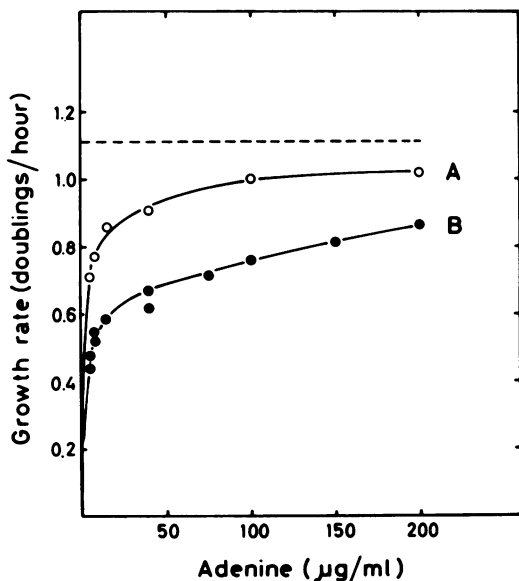


FIG. 3. Growth rates (doublings/hour) of JH35 as a function of the concentration of adenine in the medium. An exponentially growing culture was filtered, washed free of adenine, and suspended in glucose minimal medium supplemented with thiamine (1 $\mu\text{g/ml}$) and adenine at different concentrations. Curve A: no histidine added. Curve B: in the presence of histidine (50 $\mu\text{g/ml}$). Growth was followed spectrophotometrically at 450 nm. The broken line represents the growth rate of a culture growing on hypoxanthine as the sole source of purine.

observed with increasing adenine concentrations seems to be a result of a stimulation of the purine nucleoside phosphorylase-dependent pathway.

Since JH35 has a normal generation time on adenosine (Table 3), the rate-limiting step in the utilization of adenine is most probably the condensation of ribose-1-phosphate and adenine to yield adenosine, catalyzed by purine nucleoside phosphorylase. If so, simple mass action would explain the stimulation observed with increasing adenine concentrations.

An increase in the availability of the second substrate (i.e., ribose-1-phosphate or deoxyribose-1-phosphate; reference 42) would likewise be expected to force the reaction in the anabolic direction. Two ways of increasing the endogenous pool of ribose-1-phosphate or deoxyribose-1-phosphate may be envisaged. The first consists in adding either uridine or thymidine to the growth media. These compounds are readily degraded by inducible phosphorylases to the corresponding bases and ribose-1-phosphate and deoxyribose-1-phosphate, respectively. Table 6 indicates that addition of uridine or thymidine increases the growth rate of JH35 to normal levels, even when

TABLE 6. Effect of uridine and thymidine on the growth of *S. typhimurium* JH35 and JH108 in the presence of adenine and histidine^a

Strain	Genotype	Growth rate (doublings/hr) on			
		Adenine (5 $\mu\text{g/ml}$)	Adenine (100 $\mu\text{g/ml}$)	Adenine (5 $\mu\text{g/ml}$) + uridine	Adenine (5 $\mu\text{g/ml}$) + thymidine
JH35	<i>pur</i>	0.52	0.76	0.95	1.00
JH108	<i>pur, drm</i>	1.00	1.00		

^a Exponentially growing cultures were filtered, washed free of purines, and suspended in minimal medium (approximately 3×10^7 cells/ml) containing glucose (0.2%), thiamine (1 $\mu\text{g/ml}$), histidine (50 $\mu\text{g/ml}$), and the indicated amounts of purines. Uridine and thymidine were used in concentrations of 50 $\mu\text{g/ml}$. Growth was followed spectrophotometrically at 450 nm.

growing on low concentrations of adenine in the presence of histidine.

In enteric bacteria, ribose-1-phosphate and deoxyribose-1-phosphate are rapidly converted to ribose-5-phosphate and deoxyribose-5-phosphate, respectively, by phosphopentomutase (14, 15, 16). Therefore, a mutant lacking in phosphopentomutase activity (*drm*) would be expected to contain increased levels of ribose-1-phosphate and deoxyribose-1-phosphate which would enable the mutant to utilize exogenous adenine more effectively than a *drm*⁺ strain. Table 6 shows that a *pur, drm* double mutant grows equally well with either high or low concentrations of adenine in the presence of histidine.

An estimate of the contribution from the histidine-controlled pathway to the synthesis of guanine nucleotides from exogenous adenine was obtained as follows. The adenine-requiring *pup, add* mutant (JH65) was labeled with ³²P-ortho-phosphate and [8-¹⁴C]adenine both in the presence and in the absence of histidine. After several generations of exponential growth, the specific activities (¹⁴C counts/min/³²P counts/min) of the ATP and the GTP pools were determined (Table 7). Since JH65 lacks the purine nucleoside phosphorylase-dependent pathway, the results obtained indicate that only 50% of the GTP pool is derived from adenine via the histidine-controlled pathway, the rest being synthesized *de novo*. Therefore, it may be predicted that a purine-requiring strain, which in addition is blocked in either purine nucleoside phosphorylase or adenosine deaminase, will not be able to satisfy its requirement for guanine nucleotides when grown on adenine as the sole purine source. Figure 4A and B shows the growth of JH48 (*pur, pup*) and the parent JH35 (*pur*) on different purine sources. As

TABLE 7. Incorporation of 8-¹⁴C-adenine into the ATP and GTP pools of *Salmonella typhimurium* JH65^a

Strain	Genotype	Histidine (50 µg/ml)	ATP			GTP		
			¹⁴ C ^b	³² P ^b	Specific activity $\frac{^{14}\text{C}}{^{32}\text{P}}$	¹⁴ C ^b	³² P ^b	Specific activity $\frac{^{14}\text{C}}{^{32}\text{P}}$
JH65	<i>purA, pup, add</i>	—	1.689	1.754	0.96	165	342	0.48
		+	2.082	2.245	0.93	5	293	0.02

^a JH65 was grown exponentially for several generations in glucose minimal medium containing thiamine (1 µg/ml), [8-¹⁴C] adenine (9 µg/ml, specific activity: 14 µCi/µmole), ³²P-orthophosphate (specific activity: 3 µCi/µmole), and in one experiment histidine (50 µg/ml). Samples (5-ml) were harvested on Millipore filters and extracted with 0.3 M perchloric acid. The specific activities (¹⁴C/³²P) of the ATP and GTP pools were determined as described in Materials and Methods.

^b Expressed as counts per minute.

^c Obtained as the specific activity of GTP compared to that of ATP.

can be seen, very limited growth is observed with JH48 on adenine alone; moreover, the growth rate decreases continuously, eventually becoming zero after several hours. Since JH48 grows normally with either adenine plus guanine or adenine plus adenosine (or inosine), it may be concluded that the lack of growth on adenine alone results from a shortage in the supply of guanine nucleotides due to the inability of JH48 to catalyze the conversion of adenine to adenosine.

The characteristic growth behavior of JH48 on adenine (Fig. 4A) may be explained by the fact that this mutant can only synthesize GMP from exogenous adenine via ATP and AICAR. The pathway involved (see Fig. 1) is negatively controlled by histidine and produces one molecule of histidine for each molecule of GMP. These two properties of the pathway might be incompatible with steady-state growth since the supply of GMP will limit the growth rate (and thus protein synthesis). As a consequence, the endogenous concentration of histidine would be expected to rise, and this would then further restrict the supply of GMP. The chain of events would eventually lead to complete cessation of growth due to GMP starvation (Fig. 4A).

In accordance with this explanation, we have been able to show that the introduction into JH48 of a mutation that renders the histidine-sensitive enzyme (phosphoribosyl-ATP pyrophosphorylase) of this pathway insensitive to inhibition by histidine (*hisG* 1102; reference 43) results in a strain (JH103) which regains the ability to grow exponentially on adenine as the sole source of purine (Table 8).

DISCUSSION

Several years ago Zimmerman and Magasanik showed (46) that an adenine-requiring mutant of *S. typhimurium* exposed to totally labeled ¹⁴C-

adenosine incorporated both the purine and the ribose moieties of the nucleoside into nucleic acid AMP and GMP. The incorporation of the ribose moiety was 70% of that of the adenine. Based on these experiments, they concluded that *S. typhimurium* contains adenosine kinase activity. However, as pointed out by these authors, an alternative explanation involving cleavage of adenosine to adenine was not excluded. The ribose (ribose-1-phosphate) formed by this cleavage of adenosine must subsequently be utilized for PRPP synthesis in preference to the endogenously generated ribose.

In the present work, we show that adenine-requiring mutants of *S. typhimurium* (*purA*) carrying additional mutations in purine nucleoside phosphorylase (*pup*) and adenosine deaminase (*add*) are unable to utilize adenosine as an adenine source (Fig. 2). This shows that *S. typhimurium* does not contain appreciable amounts of adenosine kinase activity. Since a *purA, add* mutant grows normally on adenosine, it appears that the utilization of adenosine involves phospholytic cleavage of the nucleoside. Thus, our results support the alternative explanation offered by Zimmerman and Magasanik to explain their results.

In accordance with previous observations (46), our data (Tables 3 and 4) seem to indicate that *S. typhimurium* contains guanosine kinase as well as inosine kinase activities, although the latter seems to be of minor importance for the utilization of exogenous inosine (Table 3). Karlström (21a) recently showed that cultures of *E. coli* are unable to phosphorylate deoxyadenosine and deoxyguanosine to the corresponding deoxyribonucleotides. Thus, enteric bacteria are very limited in their capacity for purine nucleoside phosphorylation. To utilize these compounds for nucleotide synthesis, the cells must initially de-

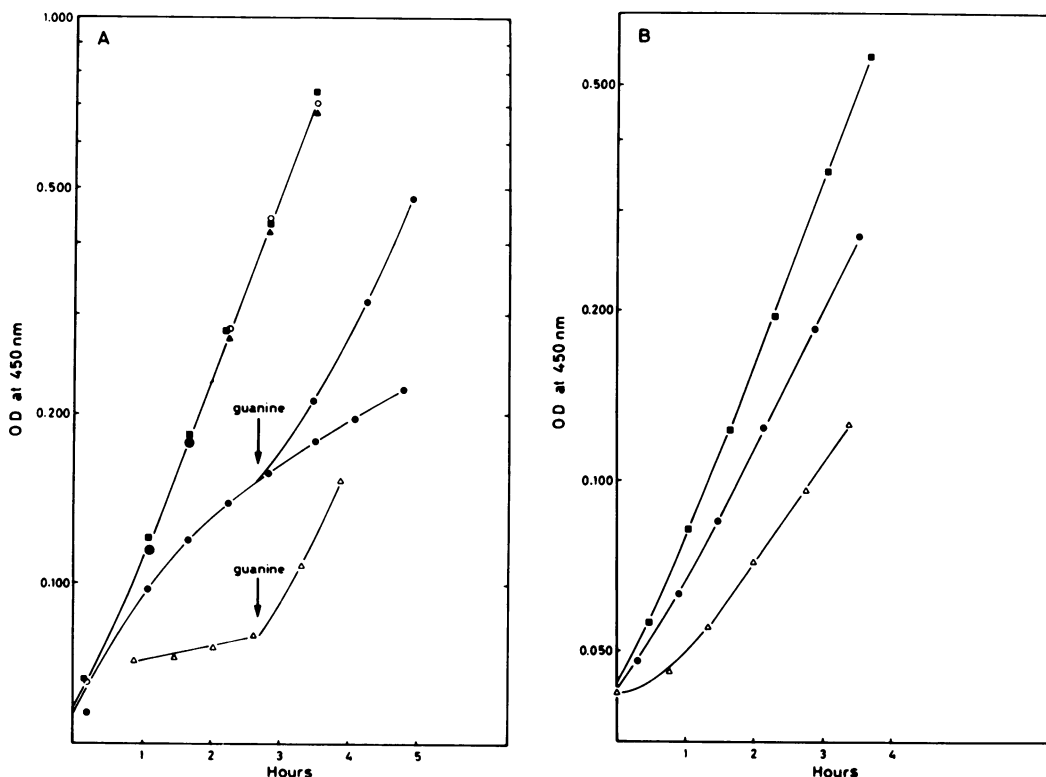
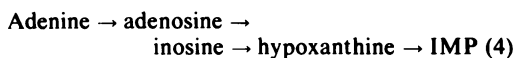


FIG. 4. Growth curves for JH48 (A) and JH35 (B) on different purine sources. Exponentially growing cultures were filtered and washed free of hypoxanthine. The cells were suspended in glucose minimal medium supplemented with thiamine (1 µg/ml) and the following purine sources: hypoxanthine, 15 µg/ml (■); adenine, 15 µg/ml (●); adenine, 15 µg/ml, plus histidine, 50 µg/ml (Δ); adenine, 15 µg/ml, plus guanine, 15 µg/ml (○); adenine, 15 µg/ml, plus adenosine, 30 µg/ml (▲). As indicated by the arrows, guanine (15 µg/ml) was added to two of the cultures during growth.

grade them to the free bases. The bases may subsequently be converted to the nucleotide level by condensation with PRPP, catalyzed by nucleotide pyrophosphorylases (see Fig. 1).

A single enzyme, purine nucleoside phosphorylase, seems to be responsible for the conversion of all purine nucleosides to the corresponding bases. This is borne out by the following observations. (i) Highly purified purine nucleoside phosphorylase from *S. typhimurium* catalyzes the phosphorylase of adenosine, inosine, and guanosine as well as the corresponding deoxyribonucleosides (42). (ii) Purine-requiring mutants of *S. typhimurium* lacking purine nucleoside phosphorylase are unable to utilize purine deoxyribonucleosides as the sole source of purines (Tables 3 and 4). (iii) Adenosine or deoxyadenosine will only serve as an adenine source for adenine-requiring mutants if they have a functional purine nucleoside phosphorylase (Fig. 2). In strains containing adenosine deaminase, however, adenine nucleosides are to a large extent deaminated before they are phosphorylated (Table 2).

The utilization of exogenous adenine for IMP(GMP) synthesis in the presence of histidine was shown by Zimmerman and Magasanik to involve the intermediary formation of hypoxanthine (46). This was used as evidence for the existence of adenine deaminase activity in *S. typhimurium*. However, the present finding that purine nucleoside phosphorylase is essential for this conversion (Table 5) indicates that the cells do not contain significant amounts of adenine deaminase. Deamination, therefore, takes place at the nucleoside level. Thus, the conversion of adenine to IMP (GMP) in the presence of histidine must occur as follows (equation 4):



Recently a *purB*, *add* double mutant of *E. coli* was isolated in this laboratory. Since this strain, grown in the presence of histidine, has an absolute requirement for both adenine and hypoxanthine, whereas its parent *purB*, *add*⁺ requires

TABLE 8. Growth rate of *S. typhimurium* JH48 and JH103 on different purine bases^a

Strain	Genotype	Growth rate (doublings per hr) on		
		Hypoxan- thine	Adenine	Adenine + histidine
JH48	<i>pur, pup</i>	1.11	0 ^b	0
JH103	<i>pur, pup, hisG 1102</i>	0.97	0.81	0.81

^a Exponentially growing cultures were filtered, washed free of purines, and suspended in minimal medium (approximately 3×10^7 cells/ml) containing glucose (0.2%), thiamine (1 μ g/ml), hypoxanthine or adenine as indicated (15 μ g/ml), and histidine (when added, 50 μ g/ml). Growth was followed spectrophotometrically at 450 nm.

^b Exponential growth was never obtained (see Fig. 4A).

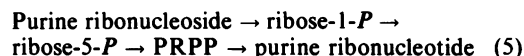
only adenine, it may be concluded that *E. coli* is also devoid of adenine deaminase (P. Nygaard, *personal communication*).

If a purine-requiring mutant is grown on adenine in the presence of histidine, the pathway outlined in equation 4 becomes rate limiting for growth (Fig. 3). However, an increase in the availability of either adenine or ribose(deoxyribose)-1-phosphate overcomes this limitation (Table 6), suggesting that the limiting step is the synthesis of adenosine (deoxyadenosine) from adenine, catalyzed by purine nucleoside phosphorylase. This was confirmed by the finding that the introduction of a mutation which renders JH35 unable to catabolize ribose(deoxyribose)-1-phosphate [i.e., a mutation in phosphopentomutase (*drm*)] results in a strain, JH108 (*pur, drm*), which grows with optimal growth rates on low concentrations of adenine even in the presence of histidine (Table 6). A similar dependency on substrate availability has been observed for the utilization of thymine by thymine-requiring mutants (*thy*) of *E. coli* and *S. typhimurium*. The initial reaction in the utilization of thymine is the thymidine phosphorylase-catalyzed conversion of thymine to thymidine. The introduction of a *thy* mutation results in mutants with a high thymine requirement (20 μ g/ml) for growth. From these strains, mutants which are capable of growing on low concentrations of thymine (2 μ g/ml) are easily obtainable. This new phenotype has been shown to be the result of an additional mutation in either phosphopentomutase (*drm*) or phosphodeoxyriboaldolase (*dra*; references 4, 7, 13, 33, 34).

As shown in Table 3 and equation 4, inosine derived either from the medium or generated from exogenous adenine is preferentially broken down to hypoxanthine before being utilized for

IMP synthesis. At first this seems unreasonable, since the cells contain inosine kinase activity. However, the finding by Munch-Petersen (35 and *personal communication*) that purine nucleoside phosphorylase as well as adenosine deaminase are released from cells of *E. coli* by osmotic shock (37) suggests that these enzymes are located close to the cell surface, presumably in the periplasmic space. The entire conversion of exogenous adenine to hypoxanthine, as outlined in equation 4, may therefore occur in a restricted region of the cells, close to the surface. Only in *pup* mutants, where the phosphorolysis of nucleosides is blocked by mutation, will inosine kinase be of significance for the utilization of inosine (Table 3).

Several lines of evidence were presented in the beginning of the present discussion, suggesting that the utilization of exogenous purine ribonucleosides involves phosphorolytic cleavage of the *N*-glycosidic bond, followed by a preferential utilization of the ribose-1-phosphate for PRPP and, thereby, purine nucleotide synthesis (equation 5).



Two recent observations support this further. (i) AMP-pyrophosphorylase of *E. coli* K-12 is located in the cell membrane (J. H. Ozer, Fed. Proc. 29:342, 1970). (ii) Phosphopentomutase, one of the enzymes presumably involved in the conversion of ribose-1-phosphate to PRPP (equation 5), is released from cells of *E. coli* by osmotic shock (12). Thus, at least three of the four enzymes involved in the pathway outlined in equation 5 seem to be localized in a restricted "compartment" close to the surface of the cell. It remains to be shown, however, that PRPP synthetase activity is also found either in the cell membrane or in the periplasmic space.

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