JOURNAL OF BACTERIOLOGY, Mar., 1966
Vol. 91, No. 3
Copyright © 1966 American Society for Microbiology
Printed in U.S.A.

Metabolism of Methylated Purines in *Escherichia coli*: Derepression of Purine Biosynthesis

SAMUEL H. LOVE AND CHARLES N. REMY

Departments of Microbiology and Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina

Received for publication 18 November 1965

ABSTRACT

LOVE, SAMUEL H. (Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N.C.), AND CHARLES N. REMY. Metabolism of methylated purines in *Escherichia coli*: derepression of purine biosynthesis. J. Bacteriol. **91**:1037–1049. 1966.—Various methylated purines were examined for their effects on growth of purine-requiring mutants of *Escherichia coli*, strains W-11 and B-96, and for their effects on purine biosynthesis. 6-Methylaminopurine and 6-methoxypurine stimulated the accumulation of purine precursor derivatives (ribosyl-5-aminoimidazole and ribosyl-5-amino-4-imidazole carboxamide) beyond their ability to support growth. Information obtained from in vivo and in vitro systems demonstrated that the metabolism of 6-methylaminopurine and 6-methoxypurine utilized identical pathways. The riboside derivatives are formed either by direct ribosidation via nucleoside phosphorylase or, indirectly, by dephosphorylation of the 5′-phosphoribosyl derivatives which are synthesized via adenylate pyrophosphorylase. Information obtained with the aid of strain W-11/DAP (lacking adenylate pyrophosphorylase) demonstrated that both pathways were important to the growing cells. Regardless of the metabolic pathway by which they are synthesized, the ribosyl derivatives are demethylaminated (demethylated) by adenosine deaminase to yield inosine. The final conversion of inosine to inosinic acid via the intermediate formation of hypoxanthine accounts for the net conversion of the methylated bases to inosinic acid. The utilization of the bases is sufficiently rate-limiting to cause derepression of the early enzymes required for the de novo synthesis of purine, thus accounting for the elevated accumulation of purine precursors originally observed.

Considerable information has been obtained concerning the synthesis of the methylated purines and pyrimidines, the minor bases, which are present in soluble ribonucleic acid (RNA), ribosomal RNA, and deoxyribonucleic acid (DNA) (8, 9, 11, 23, 33). The methyltransferase enzymes, which transfer the methyl group of *S*-adenosylmethionine to specific base moieties of a preformed polynucleotide structure, have been purified from bacterial sources (10, 15, 16). However, the precise function of the methylated bases has not been clarified (27, 32). By contrast, relatively little is known concerning the anabolism or catabolism of the free methylated purines beyond the reports that 6-methylaminopurine (6-MAP) per se is not incorporated into nucleic acids but, instead, undergoes demethylamination at the ribosyl-6-methylaminopurine (ribosyl-6-MAP) and 5′-phosphoribosyl-6-methylaminopurine (N^6-methyladenosine-5′-phosphate; 6-methyl-AMP) levels to yield inosine and inosinic acid, thus permitting the entry of 6-MAP into the normal metabolic pathway for nucleotide synthesis and interconversions (4, 5, 6, 28, 31).

The present studies were initiated to investigate the effect of various methylated purines on growth and de novo purine synthesis in the nondiscriminating, purine-requiring auxotrophs of *Escherichia coli*, strains W-11 and B-96. The accumulation of the intermediate in the de novo purine pathway which precedes the genetic block served as an index of purine synthesis. This report concerns the mechanisms by which specific purines, containing an extranuclear methyl group at position 6, support growth and stimulate de novo purine synthesis to an extent far beyond their growth promoting activity. Supporting data were obtained with the aid of additional mutants

and bacterial extracts. A preliminary report of this material has been presented (Love and Remy, Bacteriol. Proc., p. 94, 1965).

MATERIALS AND METHODS

Synthetic medium. The salts-glucose medium was a modification of that originally described by Gots and Chu (13) and contained (in grams): Na_2HPO_4, 6; KH_2PO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.1; NaCl, 1; NH_4Cl, 1; glucose, 2; and water to 1 liter. Glucose was autoclaved separately.

Organisms. The purine-requiring auxotrophs of *E. coli*, strains W-11, B-96, and B-96/1t, have been described (12, 19). The genetic blocks prevent the de novo synthesis of inosinic acid, which results in a nonspecific requirement for purines and a release of purine intermediates into the growth medium. Strains W-11 and B-96 accumulate ribosyl-5-aminoimidazole (ribosyl-AI) and ribosyl-5-amino-4-imidazole carboxamide (ribosyl-AIC), respectively. *E. coli* B-96/1t, which also accumulates ribosyl-AIC, is a double auxotroph derived from strain B-96 with trytophan as the second growth requirement. The isolation and properties of *E. coli* B/DAP, a mutant resistant to 2,6-diaminopurine and lacking adenylate pyrophosphorylase, has been reported (29). *E. coli* W-11/DAP was selected by culturing strain W-11 in the synthetic medium containing hypoxanthine (0.15 μmole/ml) and 2,6-diaminopurine (3 μmoles/ml). The resistant colonies were streaked on solid medium for isolation and subsequent verification of their resistance.

Aerobacter aerogenes, *Pseudomonas aeruginosa*, and *Alcaligenes faecalis*, obtained from the routine clinical laboratory (Baptist Hospital, Winston-Salem, N.C.), were cultured on tryptic soy broth at 37 C on a mechanical shaker for 12 hr prior to making extracts of the organisms.

Measurement of growth and the accumulation of purine intermediates. Growth was measured as a function of turbidity with the aid of a Klett-Summerson colorimeter fitted with a 540-mμ filter. De Long flasks (Bellco Glass Inc., Vineland, N.J.) were used in experiments requiring sequential measurements of turbidity changes. To determine whether the various methylated purines stimulated growth, had no effect, or were inhibitory for growth, strains W-11 and B-96 were grown in the synthetic medium containing a suboptimal amount of hypoxanthine (0.037 μmole/ml) supplemented with the individual methylated purines (0.06 μmole/ml), and incubated at 37 C for 16 hr on a mechanical shaker. The ratio of inoculum volume to incubation system was 1:100, except when otherwise indicated. The results were compared with those obtained on medium containing only suboptimal hypoxanthine.

The accumulation of ribosyl-AI or ribosyl-AIC was determined on samples of the culture supernatant fluids by the method of Bratton and Marshall (2). The molar extinction coefficients of the diazotized arylamines were assumed to be the same as those reported for their ribotide derivatives (7). Arylamine accumulation by these organisms was utilized as an index of purine synthesis (14). Since ribosyl-AI and ribosyl-AIC accumulate primarily at the end of the log phase of growth, the ability of the cells to utilize a purine supplement (low concentrations) usually results in a proportional increase in cell mass and subsequently a greater accumulation of ribosyl-AIC or ribosyl-AI as growth ceases.

Repression of purine-synthesizing enzymes. The variable potential of *E. coli* W-11, cultured in media containing different concentrations of purines, to synthesize ribosyl-AI was a reflection of the degree of repression of the purine-synthesizing enzymes (20). To determine the ability of the selected methylated purines or their metabolites to repress the formation of the enzymes involved in the early steps of purine synthesis, strain W-11 was cultured in the synthetic medium containing different concentrations of the methylated purines for 16 hr at 37 C. The cells were harvested, resuspended (0.5 mg/ml, dry weight) in unsupplemented synthetic medium, and reincubated at 37 C with shaking. The concentration of ribosyl-AI in the supernatant fluids was routinely determined after 4 hr, since the rate of synthesis was linear throughout this period and the extent of synthesis was near its maximum.

Feedback inhibition in nonproliferating cells. The ability of various purines to exert feedback inhibition of ribosyl-AIC synthesis in suspensions of strain B-96/1t was determined as previously described (12). The additional requirement for tryptophan by this organism maintained a nonproliferating system in the presence of purine supplements. Feedback inhibition of ribosyl-AI formation by purines in suspensions of strain W-11 was determined in the presence of chloramphenicol to provide a nonproliferating system. Chloramphenicol (7 μg/ml) prevented growth and the formation of β-galactosidase in strain W-11 without impairing the formation of ribosyl-AI. Therefore, the effects of purines on the chloramphenicol-inhibited cells involved neither growth nor protein synthesis.

Metabolic reactions in cell-free extracts. *E. coli* B and B/DAP were cultured in synthetic medium; this medium was supplemented with adenine (0.2 μmole/ml) for growth of strains W-11 and W-11/DAP. The cells were harvested, washed with 0.9% NaCl, disrupted either by ultrasonic vibration (Branson Sonifier, Branson Instruments, Inc., Stamford, Conn.) or grinding with alumina (Alcoa A-301), and extracted with 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer, *p*H 7.4. The cellular debris was removed by centrifugation for 30 min at 32,000 × *g*, and the soluble portion was recentrifuged for 30 min at 32,000 × *g*. The final supernatant fluid was used as such or dialyzed against 0.005 M Tris (*p*H 7.4) for 16 hr at 2 C. The various incubations utilizing these extracts were terminated by heating for 3 min at 100 C. After removal of the protein, samples were chromatographed (descending) with appropriate reference compounds on Whatman no. 1 paper employing one of the following: solvent A (*n*-butanol-glacial acetic acid-water, 58.1:15.7:26.2); solvent B [86% *n*-butanol plus NH_3 atmosphere (24)]; solvent C [isoamylalcohol-Na_2HPO_4 (3)]; or solvent D (isobutyric acid-NH_4OH-water, 61:1:33). The ultraviolet-absorbing areas were located with a Mineralight lamp; the C^{14}-containing components were located with a 4 π paper-strip scanner (model RSC 160, Atomic Accessories Inc., Valley Stream, N.Y.) and quan-

titated by counting segments of the paper strips directly in the Tri-Carb liquid scintillation counter (Packard Instruments Co., LaGrange, Ill.). The R_F values with solvent A were: 6-MAP, 0.70; ribosyl-6-MAP, 0.63; adenine, 0.61; adenosine, 0.51; hypoxanthine, 0.50; inosine, 0.34; 6-methyl-AMP, 0.19; adenosine-5′-phosphate (AMP), 0.12; inosinic acid, 0.09. The R_F values with solvent B (relative to 6-MAP) were: ribosyl-6-MAP, 0.74; adenine, 0.53; adenosine, 0.34; hypoxanthine, 0.17; inosine, 0.04.

Chemicals. 6-MAP, 6-methoxypurine, 1-methylinosine, adenine, hypoxanthine, guanine, xanthine, α-D-ribose-1-phosphate di (cyclohexylammonium), 4-amino-5-imidazole carboxamide ribonucleoside, and 6-methylaminopurine-*8-C*^{14} were from Calbiochem; 6-methylaminopurine riboside, 6-methoxypurine, 2-amino-6-methylaminopurine, 1-methylguanine, 2-methyladenine, 6-dimethylaminopurine, 1-methylinosine, 6-dimethylaminopurine riboside, and 2,6-diaminopurine were from Cyclo Chemical Corp., New York, N.Y.; adenosine-5′-phosphate, inosine-5′-phosphate, adenosine, inosine, and 5-phosphorylribose-1-pyrophosphate (magnesium) were from P-L Biochemicals, Inc., Milwaukee, Wis.; adenine-*8-C*^{14}, 2,6-diaminopurine-*2-C*^{14}, and hypoxanthine-*8-C*^{14} were from Isotopes Specialties Co., Burbank, Calif.; adenosine-5′-monophosphate-*8-C*^{14} was from Schwarz BioResearch, Inc., Orangeburg, N.Y.; and chloramphenicol was from Parke, Davis & Co., Detroit, Mich.

RESULTS

Growth and arylamine accumulation. The effects of various methylated purines on the growth of *E. coli* W-11 and B-96, and on the accumulation of the purine precursor derivatives, are illustrated in Table 1. The effects of the methylated bases were compared with those of the normally occurring nonmethylated purines and with that of 2,6-diaminopurine, a nonmethylated purine analogue.

Supplements of the normal nonmethylated purines supported growth of both organisms beyond the control values; the increase in cell population was reflected in an increase in ribosyl-AIC or ribosyl-AI. Diaminopurine stimulated the growth of strain B-96 but inhibited strain W-11; in both instances it caused an increase in arylamine. Of the methylated purines examined, 6-MAP, 6-methoxypurine (6-MOP), and 2-amino-6-methylaminopurine were of special interest, since they stimulated arylamine synthesis to an extent far beyond their ability to support growth. Strain W-11 was selected as the test organism to examine the mechanisms by which 6-MAP and 6-MOP were able to support growth and to stimulate ribosyl-AI synthesis, since the effects of these methylated bases, although similar in the two organisms, were more dramatic in strain W-11.

Growth and ribosyl-AI synthesis versus time. The stimulation of growth and ribosyl-AI synthesis by the methylated purine supplements were studied with respect to incubation time. The growth curves were normal and did not indicate an adaptation to the methylated purines (Fig. 1A). Ribosyl-AI synthesis during growth on the methylated purine supplements was initiated at the same time as in the controls, but the rates

TABLE 1. *Effect of methylated purines on growth and purine synthesis*

Purine supplement (0.06 μmole/ml)	Strain B-96*		Strain W-11	
	Turbidity† (540 mμ)	Ribosyl-AIC† (mμmoles/ml)	Turbidity† (540 mμ)	Ribosyl-AI† (mμmoles/ml)
Hypoxanthine	84	10.3	87	14.2
Guanine	93	19.8	81	17.1
Adenine	75	12.3	73	12.6
Xanthine	96	26.9	73	16.6
2,6-Diaminopurine	70	20.9	−24	36.6
1-Methylguanine	2	−2.9	7	3.8
2-Methyladenine	−2	−2.7	−3	−12.0
6-Methylaminopurine	65	25.9	70	93.3
6-Dimethylaminopurine	1	−0.5	11	8.6
2-Amino-6-methylaminopurine	1	−2.1	15	44.8
1-Methylinosine	18	5.7	24	8.9
6-Dimethylaminopurine riboside	2	0	3	0.8
6-Methoxypurine	48	33.1	26	77.0
Controls	(84)	(19.7)	(90)	(24.7)

* Contained 0.1% casein hydrolysate to increase growth and the yield of ribosyl-AIC.

† The controls, containing synthetic medium plus hypoxanthine (0.037 μmole/ml), are expressed in absolute values. The test systems contained the indicated purine supplements in addition to the hypoxanthine. The effects of the supplements on the turbidity and ribosyl-AIC or ribosyl-AI synthesis are expressed as differences between the absolute values of the control and test system.

and yields were much higher (Fig. 1B). The differences due to the methylated purines would be more magnified if the yield of ribosyl-AI were plotted versus the yield of cells.

Cell yield and ribosyl-AI synthesis versus purine concentration. Figure 2A illustrates the final growth response of strain W-11 when the methylated purines served as the sole source of purines in the synthetic medium. Hypoxanthine, which served as the normal control, was a more effective purine source for growth than were the methylated purines. Maximal growth was obtained on 0.15 μmole/ml of hypoxanthine, 0.2 μmole/ml of 6-MAP, and 0.4 μmole/ml of 6-MOP. The reduced cell yield per unit of methylated purine supplied to the cells was a reflection of the limited utilization of the methylated purines at a reduced growth rate. The growth rates on equimolar concentrations (0.15 μmole/ml) of 6-MAP, hypoxanthine, and adenine were nearly equal, but growth on 6-MOP was more linear with time (Fig. 3).

The yield of ribosyl-AI obtained during growth on the various concentrations of purines is illus-

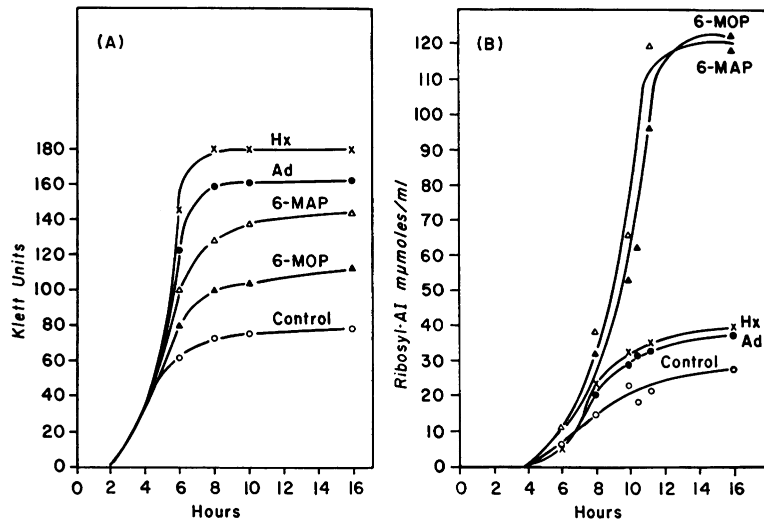

FIG. 1. *Growth of Escherichia coli W-11 and ribosyl-AI synthesis versus time. Abbreviations are: Ad, adenine; Hx, hypoxanthine; 6-MAP, 6-methylaminopurine; 6-MOP, 6-methoxypurine.*

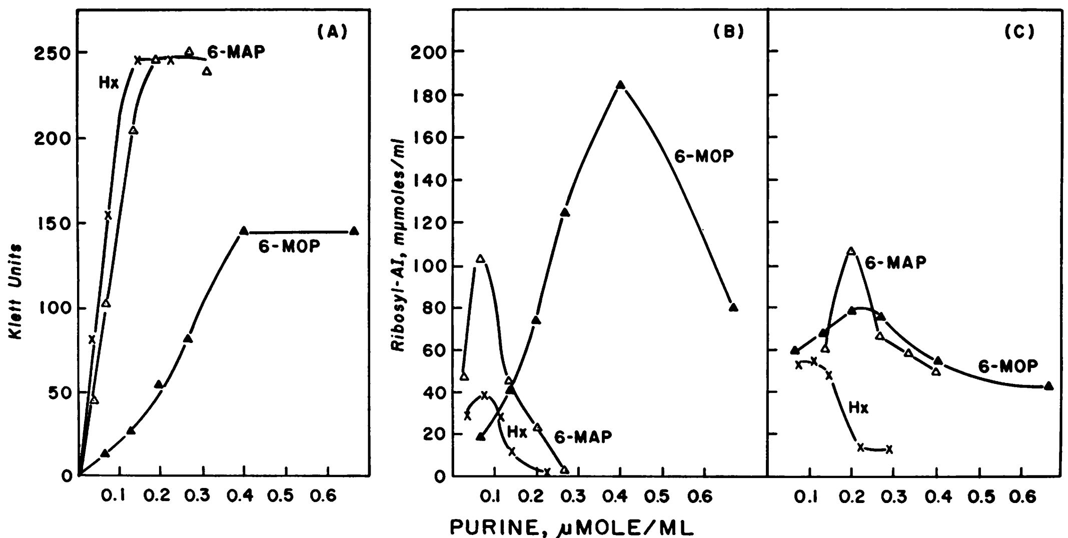

FIG. 2. *Growth of Escherichia coli W-11 (A), ribosyl-AI synthesized during growth (B), and repression of purine synthesis (C) versus purine concentration. (A and B) The organisms were grown in synthetic medium (100 ml) containing the individual purines (concentrations indicated) at 37 C for 16 hr with shaking. (C) The organisms were derived from (A). See Materials and Methods.*

trated in Fig. 2B. Concentrations of hypoxanthine and 6-MAP which were suboptimal for growth yielded maximal ribosyl-AI; the amount of 6-MOP which supported maximal growth also permitted maximal synthesis of ribosyl-AI, but maximal growth on 6-MOP was suboptimal as compared with that obtained on hypoxanthine or 6-MAP. The yield of ribosyl-AI was markedly reduced by high concentrations of purines and was inversely related to the ability of strain W-11 to utilize the purine in question.

Maximal accumulation of ribosyl-AI during growth of strain W-11 was observed when the purine source was limiting. Another means of limiting the available purines to the cells during growth is to supply them with 5'-AMP, which is utilized slowly in comparison with the purine bases or nucleosides (18). Maximal accumulation of ribosyl-AI occurred when strain W-11 was grown on 0.1 μmole/ml of 5'-AMP (Table 2). Even when 5'-AMP was supplied in excess of 0.4 μmole/ml, its limited utilization permitted the accumulation of 115 mμmoles/ml of ribosyl-AI.

Repression. The increased yield of ribosyl-AI during growth on the methylated purines (Fig. 2B) does not distinguish between derepression and lack of feedback inhibition; consequently, the cells grown on various concentrations of purines (Fig. 2A) were harvested, washed, and resuspended in the synthetic medium to measure their state of repression (Fig. 2C). In comparison with the normal pattern of repression obtained with hypoxanthine, the methylated purines were less effective in repressing de novo purine synthesis. At a concentration of 0.2 μmole/ml, hypoxanthine exerted maximal repression, whereas cells grown on an equivalent amount of the methylated purines were maximally derepressed. When 6-MAP was the purine source, the cells were maximally derepressed for purine synthesis on the same concentration of purine (0.2 μmole/ml) which supported maximal growth.

The possibility was considered that protein synthesis might occur to a very slight but select extent in the absence of exogenous purines and result in derepression during the 4-hr assay incubation period. Chloramphenicol would inhibit ribosyl-AI synthesis if derepression were occurring; however, ribosyl-AI was not inhibited by chloramphenicol (7 to 70 μg/ml). In some cases it actually stimulated ribosyl-AI synthesis, but without altering the patterns obtained. It was concluded that the observed differences in potential to synthesize ribosyl-AI were due to the degree of repression which existed prior to the assay procedure.

Feedback inhibition. The methylated purines were examined for their ability to serve, directly or indirectly, as feedback inhibitors of purine synthesis after different conditions of growth. In initial studies, *E. coli* B-96/1t previously cultured in tryptose broth was used as the reference system (Fig. 4A). Feedback inhibition occurred in the presence of the methylated purines, but not as well as in the presence of adenine, which was added as a control. Similar results were obtained with strain W-11 derived from tryptose broth (Fig. 4B). However, when the cells were intentionally derepressed by growth on suboptimal concentrations of either hypoxanthine

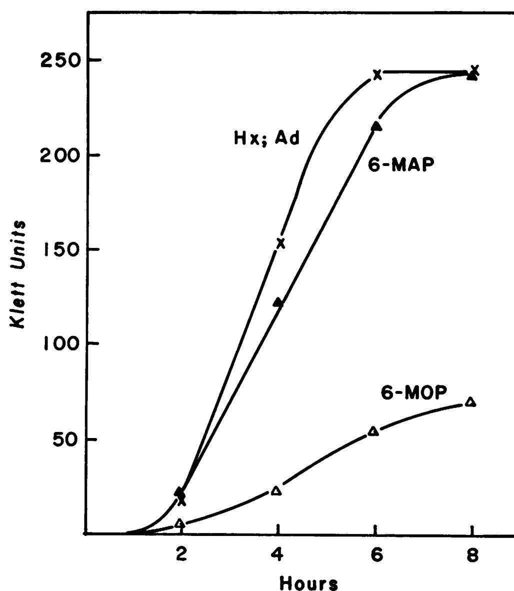

FIG. 3. *Comparative growth rates of Escherichia coli W-11 on methylated purines. Synthetic medium (15 ml) containing the individual purines (0.15 μmole/ml) received 1.66 ml of cell suspension as inoculum. Incubation conditions were unaltered.*

TABLE 2. *Ribosyl-AI synthesized during growth on 5'-AMP**

5'-AMP supplement	Cell turbidity	Ribosyl-AI synthesized
μmoles/ml		*mμmoles/ml*
0	5	—
0.1	148	172
0.2	177	159
0.4	195	115
0.8	195	121

* Incubation systems (10 ml) contained synthetic medium supplemented with 5'-AMP as indicated. Each flask was inoculated with 0.1 ml of cell suspension of strain W-11 and was incubated with shaking in 300-ml DeLong flasks at 37 C for 16 hr.

(0.11 μmole/ml) or 6-MAP (0.17 μmole/ml), higher concentrations were required to exert comparable feedback inhibition (Fig. 4C and D). The largest amount was required when the cells had grown on 6-MAP. In all cases, adenine was more inhibitory than 6-MAP, which in turn was more inhibitory than 6-MOP.

In vitro synthesis of 6-methyl-AMP. The incubation of 0.2 μmole of C^{14}-labeled adenine, hypoxanthine, 2,6-diaminopurine, or 6-MAP with 80 μmoles of Tris or phosphate buffer (*p*H 7.4), 5 μmoles of $MgCl_2$, 0.44 μmole of 5-phosphoribosyl-1-pyrophosphate (P-ribose-PP), and extracts of *E. coli* B (total volume of 0.5 ml) for 30 min at 37 C yielded the 5′-phosphoribonucleosides of each purine as the only significant C^{14}-labeled component. Essentially complete conversion of 6-MAP to 6-methyl-AMP was obtained by proper selection of enzyme concentration and time of incubation; however, the rate of nucleotide synthesis with 6-MAP was <10% of that with adenine. In contrast to these observations, extracts of *E. coli* B/DAP failed to show any evidence for ribonucleotide synthesis in regard to adenine, 6-MAP, and 2,6-diaminopurine, whereas the synthesis of inosinic acid from hypoxanthine was normal. The *p*H optimum for the analogues is significantly lower than the optimum of 7.4 for adenine; in the presence of 0.2 μmole of 6-MAP, 0.15, 0.16, 0.13, 0.12, and 0.08 μmole of 6-methyl-AMP were formed at *p*H 6.0, 6.5, 6.8, 7.4, and 8.0, respectively.

Extracts of strain W-11, but not strain W-11/DAP, likewise contained adenylate pyrophosphorylase capable of synthesizing 6-methyl-AMP from 6-MAP and P-ribose-PP. The additional formation of hypoxanthine, inosine, and inosinic acid by these extracts occurred as a result of the demethylamination of 6-MAP (see below).

Isolation and identification of 6-methyl-AMP. A large volume of heat deproteinized incubation medium (extract of *E. coli* B) was streaked (120 μliters/cm) as a narrow band on Whatman 3MM paper, and the 6-methyl-AMP was isolated by chromatography with solvent A. (The chromatography of heat deproteinized incubation mixtures containing Tris buffer may lead to an artifact when relatively large volumes are chromatographed. Tris buffer, but not phosphate buffer, causes the 6-methyl-AMP to appear to migrate as two overlapping spots. As the concentration of Tris on the paper is reduced, either by spotting small volumes or streaking large samples, the 6-methyl-AMP component migrates as a single, compact spot.) The narrow band of 6-methyl-AMP, located by C^{14} content, was eluted by descending chromatography with water, rechromatographed on Whatman no. 1 paper with the same solvent, and again eluted with water. The purity of the isolated 6-methyl-AMP was verified by paper chromatography employing solvent C (R_F: AMP, 0.72; 6-methyl-AMP, 0.82) and solvent D (R_F: AMP, 0.53; 6-methyl-AMP, 0.58), and by paper electrophoresis (Whatman 3MM) in 0.05 M ammonium formate (*p*H 3.6) and 0.05 M sodium borate (*p*H 9.2). After hydrolysis of the purified 6-methyl-AMP

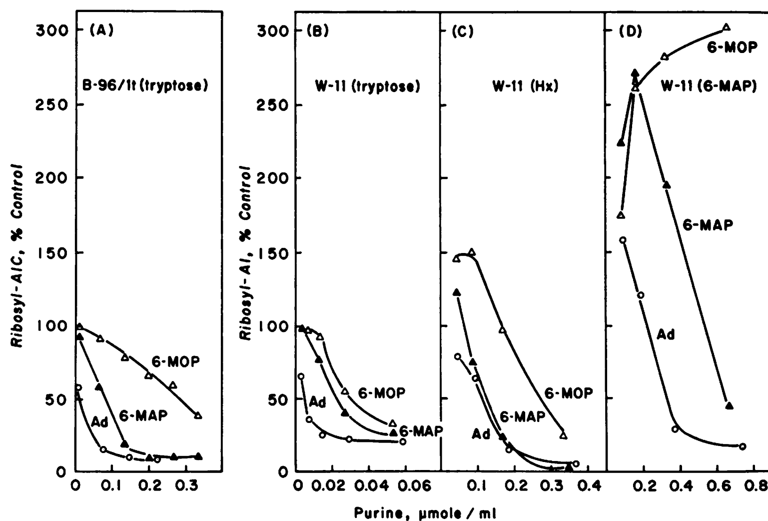

FIG. 4. *Feedback inhibition of purine synthesis. The test organisms and their previous culture media are indicated. See Materials and Methods for other conditions. (A) The yield of ribosyl-AIC in the control was 30 mμmoles/ml. The yields of ribosyl-AI in the controls were: (B) 9.5 mμmoles/ml; (C) 58 mμmoles/ml; (D) 63 mμmoles/ml.*

in 1 N HCl for 30 min at 100 C, 6-MAP was the only C^{14}-labeled component detected by chromatography.

Catabolism of 6-methyl-AMP. The conversion of 6-MAP to 6-methyl-AMP by extracts of *E. coli* B was almost complete within 30 min; thereafter, as 6-methyl-AMP was catabolized, the quantity of 6-MAP increased (Table 3). The synthesis of 6-methyl-AMP by extracts of strain W-11 occurred concurrently with the formation of inosinic acid and hypoxanthine owing to the presence of an active demethylamination system. The catabolism of 6-methyl-AMP, as well as inosinic acid, was reflected in an increase in hypoxanthine as the quantity of the ribonucleotides decreased with time. Similar results were obtained when exogenous AMP-C^{14} was incubated with the extracts (not illustrated). The pathway of catabolism appears to be via the dephosphorylation of the nucleotide, followed by the phosphorolysis of the resulting ribonucleoside.

In vitro synthesis of ribosyl-6-MAP. Complete conversion of 6-MAP to ribosyl-6-MAP was obtained when 0.2 μmole of 6-MAP-C^{14}, 1 μmole of ribose-1-phosphate, and 80 μmoles of Tris, *p*H 7.4 (final volume of 0.5 ml), were incubated with extracts of *E. coli* B or *E. coli* B/DAP for short periods. Since the nucleoside phosphorylase reaction is reversible (reaction 2, Fig. 5), the relative proportions of base to ribonucleoside actually obtained with a given enzyme concentration depended on time and the quantity of ribose-1-phosphate and phosphate present in the incubation mixture. When large volumes of extract or long incubations were employed, significant ribonucleoside synthesis occurred in the absence of exogenous ribose-1-phosphate, presumably because of the release of endogenous ribose-1-phosphate during the catabolism of nucleotide cofactors. Small amounts of inosine and hypoxanthine accumulated despite minimal demethylamination in extracts of *E. coli* B; the inclusion of 0.35 μmole of P-ribose-PP and 5 μmoles of $MgCl_2$ (in addition to ribose-1-phosphate) resulted in the elimination of inosine and hypoxanthine and a concomitant formation of inosinic acid via inosinate pyrophosphorylase. When both the nucleoside phosphorylase and nucleotide pyrophosphorylase reactions proceeded simultaneously in a given extract , a competition for 6-MAP, the common substrate, often limited the synthesis of 6-methyl-AMP or ribosyl-6-MAP, or both. The actual extent of inhibition depended on the relative activities of the two enzymes and the concentration of P-ribose-PP, ribose-1-phosphate, and 6-MAP.

Although extracts of *E. coli* W-11 and W-11/DAP likewise synthesized ribosyl-6-MAP, the measurable quantity of the ribonucleoside synthesized was greatly reduced owing to its rapid catabolism to inosine and hypoxanthine.

Demethylamination of 6-methylaminopurine.

TABLE 3. *Catabolism of ribonucleotides*

Time	Ribonucleotide concn*			
	Escherichia coli B		*E. coli* W-11	
	6-Methyl-AMP	Inosinic acid	6-Methyl-AMP	Inosinic acid
min				
15	88.3	0	48.6	14.5
30	100.0	0	55.0	19.7
60	98.5	0	38.2	17.3
120	68.5	0	11.0	13.2

* Incubation mixtures, containing 0.28 μmole of 6-MAP-C^{14}, 0.31 μmole of P-ribose-PP, 5 μmoles of $MgCl_2$, 80 μmoles of Tris buffer (*p*H 7.4), and either 6.5 mg of *E. coli* W-11 extract (prepared by grinding with alumina A-301) or 4.7 mg of *E. coli* B extract (prepared by sonic vibration) in a total volume of 0.50 ml, were incubated for the indicated incubation periods. The purine components of the deproteinized samples were separated by paper chromatography with solvent A. The quantity of ribonucleotides present at various time intervals is expressed as relative percentages, employing the maximal quantity of 6-methyl-AMP as 100%. These values represent the net result of synthesis and catabolism.

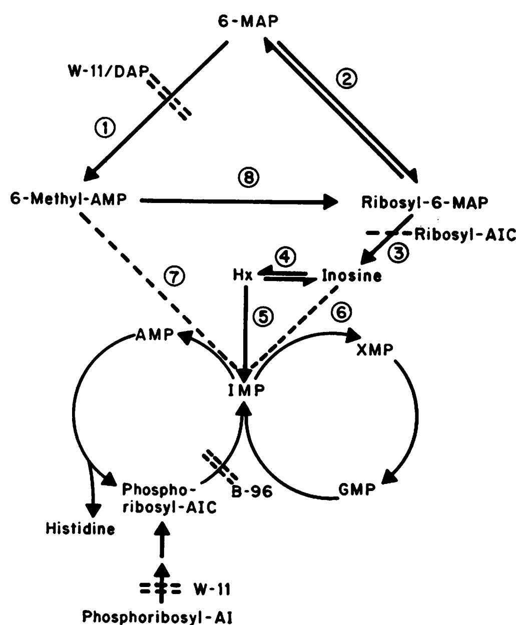

FIG. 5. *Purine interconversions.*

Unlike *E. coli* B and B/DAP, unsupplemented extracts (grinding with alumina) of *E. coli* W-11 and W-11/DAP actively converted 6-MAP to hypoxanthine derivatives (Table 4). As the enzyme concentration was decreased, the extent of demethylamination decreased, a larger proportion of the reaction product was present as inosine rather than as hypoxanthine, significant quantities of ribosyl-6-MAP were present, and the extent of total demethylamination became dependent on exogenous ribose-1-phosphate. Ribosyl-AIC, a competitive inhibitor of adenosine deaminase (18), markedly inhibited demethylamination in the absence or presence of ribose-1-phosphate. The presence of P-ribose-PP plus $MgCl_2$, in addition to ribose-1-phosphate, facilitated the conversion of hypoxanthine (product of the phosphorylase and deaminase reactions) to inosinic acid via inosinate pyrophosphorylase, thereby resulting in the net conversion of 6-MAP to inosinic acid (not illustrated). A time sequence study (Table 5) revealed fluctuations in the concentrations of the reaction products which occurred as the demethylation process proceeded toward completion. The first significant reaction product to appear was ribosyl-6-MAP, which reached its maximal concentration early in the incubation period and then declined steadily. Inosine increased at a slower rate than did ribosyl-6-MAP and reached its maximal concentration later. Hypoxanthine, the ultimate reaction product, increased at the slowest rate but, unlike the other products, continued to increase in concentration throughout the reaction period.

The data in Table 6 demonstrate that the demethylamination system of *A. aerogenes* has properties very similar to that of *E. coli*, including a requirement for ribose-1-phosphate and inhibition by ribosyl-AIC. By contrast, the demethylation of 6-MAP by extracts of *P. aeruginosa* and *A. faecalis* was unaffected by either ribose-1-phosphate or ribosyl-AIC, suggesting that demethylamination in these organisms occurs at the base rather than at the nucleoside level (not illustrated).

Inhibition of methylated purine utilization. The normal growth responses of strain W-11 toward adenine, hypoxanthine, 6-MAP, and 6-MOP are illustrated in Fig. 3. A similar comparison employing strain W-11/DAP demonstrated that adenine and hypoxanthine supported growth to the same extent as for strain W-11. By contrast, the response to 6-MAP and 6-MOP by strain W-11/DAP was 66 and 37%, respectively, of that exhibited by strain W-11. The inability of this organism to synthesize adenylate pyrophosphorylase represented a genetic block for one of the metabolic pathways employed in the utilization of 6-MAP and 6-MOP.

Ribosyl-AIC inhibited the growth of strain W-11 when the purine source was 6-MAP or 6-MOP, but no inhibition occurred with adenine as the purine source (Fig. 6A). Less ribosyl-AIC

TABLE 4. *Demethylamination of 6-methylaminopurine by extracts of Escherichia coli W-11/DAP*

Incubation*		Reaction products			
Supplement	Extract	6-MAP	Ribosyl-6-MAP	Inosine	Hypoxanthine
	mg	*mμmoles*	*mμmoles*	*mμmoles*	*mμmoles*
None	8.4	18.6	0.0	22.2	159.0
None	4.2	78.4	13.6	31.6	76.2
Ribosyl-AIC	4.2	147.5	43.0	2.8	7.0
None	2.1	93.0	39.0	38.0	30.0
Ribose-1-phosphate	2.1	0.0	0.0	79.7	120.5
Ribosyl-AIC	2.1	129.0	65.2	3.0	2.8
Ribose-1-phosphate + ribosyl-AIC	2.1	77.2	95.5	13.2	14.2

* The incubations contained 200 mμmoles of 6-MAP-C^{14} (0.75 μc per 0.2 μmole), 80 μmoles of Tris buffer (*p*H 7.4), and enzyme (as indicated) in a total volume of 0.5 ml; the supplements, 1.25 μmoles of ribosyl-AIC and 1 μmole of ribose-1-phosphate, were added as indicated. All samples were incubated for 20 min at 37 C.

TABLE 5. *Time sequence study of demethylamination*

Time	Reaction component* (mμmoles)			
	6-MAP	Ribosyl-6-MAP	Inosine	Hypoxanthine
min				
0	168	0	0	0
5	159	9	Trace	0
10	139	16	9	3
15	106	31	21	10
20	69	42	35	22
30	35	35	52	46
40	24	16	53	65
60	8	5	46	110

* The reaction mixture contained 1.34 μmoles of 6-MAP-C^{14}, 512 μmoles of Tris buffer (*p*H 7.4), and 10.8 mg of *Escherichia coli* W-11/DAP extract (prepared by grinding) in a total volume of 3.2 ml. At the stated time intervals, 0.4 ml of the reaction mixture was withdrawn and deproteinized; 25 μliters of supernatant fluid, plus the carriers, 6-MAP, ribosyl-6-MAP, hypoxanthine, and inosine, were chromatographed in solvent A. The data are presented as millimicromoles of the various components present in the 0.4-ml sample removed at the specified time periods.

TABLE 6. *Demethylamination by extracts of Aerobacter aerogenes*

Incubation*			Products (mμmoles)			
Supplement	Extract	Time	6-MAP	Ribosyl-6-MAP	Inosine	Hypoxanthine
	mg	*min*				
None	2.22	45	19.0	26.8	69.2	85.0
None	1.11	20	87.0	73.5	21.3	18.1
None	0.55	20	145.4	44.8	9.9	0.0
R-1-P†	2.22	45	0.0	0.0	166.8	32.2
R-1-P	1.11	20	0.0	71.8	128.0	0.0
R-1-P	0.55	20	0.0	128.0	71.8	0.0
R-1-P + ribosyl-AIC	1.11	20	0.0	175.8	24.3	0.0
R-1-P + ribosyl-AIC‡	1.11	20	0.0	133.5	66.7	0.0
Ribosyl-6-MAP	2.22	45	17.2	88.0	64.2	30.6

* The samples contained 200 mμmoles of 6-MAP-C^{14} (0.75 μc per 0.2 μmole), 80 μmoles of Tris buffer (*p*H 7.4), and extracts of *A. aerogenes* (prepared by sonic vibration) in a final volume of 0.5 ml; the supplements, 1.0 μmole of R-1-P, 1.25 μmoles of ribosyl-AIC, and 1 μmole of ribosyl-6-MAP, were added as indicated. The quantity of enzyme and the time of incubation were varied as indicated.
† Ribose-1-phosphate.
‡ A 0.25-μmole amount instead of 1.25 μmoles.

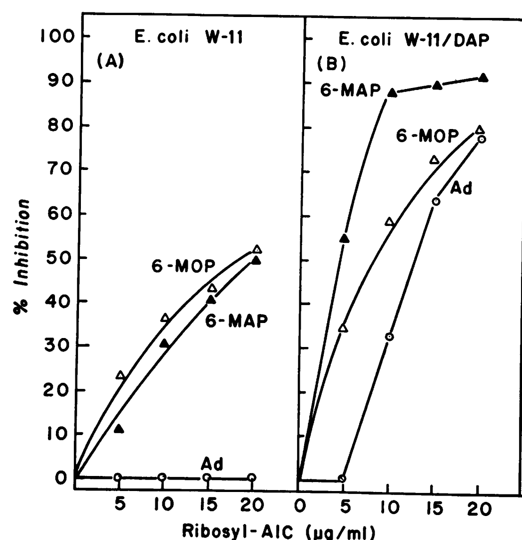

FIG. 6. *Inhibition of methylated purine utilization. Each incubation system (10 ml) contained synthetic medium supplemented with either 6-MAP (0.21 μmole/ml) or 6-MOP (0.33 μmole/ml), 0.33 ml of cell suspension, and ribosyl-AIC as indicated. The flasks were incubated at 37 C with shaking for 7.5 hr. In the absence of ribosyl-AIC, the control values, in Klett units, were: W-11 grown on adenine, 232; 6-MAP, 245; 6-MOP, 110; W-11/DAP grown on adenine, 238; 6-MAP, 170; 6-MOP, 92.*

was required to exert comparable inhibition of strain W-11/DAP (Fig. 6B). Contrary to the results obtained with strain W-11, ribosyl-AIC inhibited strain W-11/DAP when grown on adenine. The higher concentration (0.33 μmole/ml) of 6-MOP employed in this experiment to support adequate growth minimized the marked differences in the growth responses of strains W-11 and W-11/DAP originally observed with low concentrations (0.15 μmole/ml) of this compound.

Histidine (20 μg/ml) inhibited neither the growth nor the accumulation of ribosyl-AI by strain W-11 when 6-MAP served as the purine source.

DISCUSSION

To support growth of purine-requiring auxotrophs, methylated purines must enter the known scheme of purine interconversion at some point (Fig. 5). Various organisms, including *E. coli*, are able to carry out the net conversion of 6-MAP to hypoxanthine via a process of demethylamination, that is, the removal of CH_3NH_2 to yield the purine moiety hypoxanthine. Demethylamination by *E. coli* B and 15T⁻ occurs at the level of the ribonucleoside rather than the base (28). 6-Methoxypurine is similarly converted to hypoxanthine, presumably by the release of CH_3OH. 6-Methylaminopurine does not support growth of the adenine-requiring mutants, *E. coli* B-97 (28), or *Salmonella typhimurium* Ade-1 and Ade-12 (6); therefore, simple demethylation to yield an adenine derivative does not occur. The in vivo studies employing rat liver (5), Ehrlich ascites carcinoma (5), *E. coli* B (31), and *S. typhimurium* (6) have demonstrated quite clearly that the major pathway for the conversion of 6-MAP to polynucleotide adenine and guanine involves the conversion of 6-MAP to a hypoxanthine derivative (hypoxanthine, inosine, or inosinic acid). Regardless of the site of demethylamination, the ultimate hypoxanthine derivative

must be inosinic acid, which enters the de novo synthetic pathways for both AMP and guanylic acid.

The present report offers additional evidence for the general concept that the enzymes responsible for the metabolism of 6-MAP are those which normally utilize adenine. In regard to the actual site of demethylamination in *E. coli*, the irreversible nature of the de novo synthesis of AMP from inosinic acid (22) and the lack of evidence for either an AMP deaminase (35) or adenase (17) indicate that the only potential site of demethylamination (reaction 3, Fig. 5) is at the level of the ribonucleoside via adenosine deaminase (17, 18, 28). The histidine biosynthetic pathway serves as a major route for the conversion of AMP to inosinic acid in members of the Enterobacteriaceae; therefore, a consequence of feedback inhibition by histidine is the loss of the important route by which *E. coli* converts endogenous AMP to inosinic acid (22, 25). No evidence was obtained from the present studies to suggest that demethylamination of 6-MAP derivatives required participation of the enzymes involved in the de novo synthesis of histidine from AMP.

The process of demethylamination as observed in *E. coli* is characterized by a requirement for ribose-1-phosphate, the rapid formation of ribosyl-6-MAP as the initial reaction product (via ribose-1-phosphate and nucleoside phosphorylase), the accumulation of ribosyl-6-MAP when ribosyl-AIC is present to inhibit adenosine deaminase, and the accumulation of inosine as the initial demethylated reaction product. The adaptive nature of the responsible enzyme was demonstrated previously (17, 28). The phosphorolysis of inosine via nucleoside phosphorylase yields hypoxanthine which is converted to inosinic acid via inosinate pyrophosphorylase (reactions 4 and 5, Fig. 5); alternately, inosine may be converted directly to inosinic acid via a kinase (35) (reaction 6, Fig. 5). By either pathway, 6-MAP is converted to inosinic acid via inosine.

Ribosyl-AIC (a known inhibitor of adenosine deaminase) inhibits both the demethylamination of ribosyl-6-MAP in extracts of *E. coli* W-11 and W-11/DAP and the growth of the same organisms when either 6-MAP or 6-MOP serves as the sole purine source, providing evidence that the major route for the utilization of both methylated purines requires the demethylation of their respective ribonucleosides by adenosine deaminase. The key role of adenosine deaminase in the demethylation of 6-MAP and 6-MOP, and in the deamination of adenine by W-11/DAP, is similar to its central role in the utilization of exogenous AMP by purine-requiring mutants of *E. coli* B (18).

In contrast to the reactions proposed for *S. typhimurium* (4), the adenylate pyrophosphorylase enzymes of *E. coli* B and W-11 react with 6-MAP and 2,6-diaminopurine in addition to adenine, since resistance to 2,6-diaminopurine results in complete loss of activity toward the three bases. The nonspecificity of adenylate pyrophosphorylase eliminates the necessity of postulating the existence of a pyrophosphorylase specific for 6-MAP.

The formation of 6-methyl-AMP by *E. coli* W-11 does not represent a metabolic "dead end" since it is catabolized to hypoxanthine, probably via ribosyl-6-MAP (reaction 8, Fig. 5). The dephosphorylation of 6-methyl-AMP by *E. coli* alkaline phosphatase has been demonstrated (4). Thus, the formation of ribosyl-6-MAP, a prerequisite for demethylation, can occur either by direct ribosidation of the free base or by dephosphorylation of the ribonucleotide. As compared with *E. coli* W-11, the growth response of *E. coli* W-11/DAP (lacking adenylate pyrophosphorylase) is normal toward hypoxanthine and adenine, whereas growth on 6-MAP and 6-MOP was reduced by 34 and 63%, respectively. This observation, in conjunction with the fact that ribosyl-AIC was more inhibitory for W-11/DAP than for W-11, is interpreted to indicate that the synthesis of 6-methyl-AMP via adenylate pyrophosphorylase (reaction 1, Fig. 5) is an important pathway for the conversion of 6-MAP to hypoxanthine by serving as an additional pathway for the synthesis of ribosyl-6-MAP. Thus, the formation of 6-methyl-AMP may serve as an important "salvage" pathway for 6-MAP in *E. coli*, despite the lack of an adenylate deaminase in members of the Enterobacteriaceae (35). The direct demethylamination of 6-methyl-AMP via adenylate deaminase (reaction 7, Fig. 5) may constitute a major "salvage" pathway for mammalian systems (6). Thus, in the specific case of *E. coli*, adenosine deaminase has an indispensable role in the deamination (demethylamination) of methylated adenine derivatives. The present studies have not indicated the specific rate-limiting step(s) in the growing cell.

6-Methyl-AMP and ribosyl-6-MAP synthesis can be studied most conveniently under conditions of minimal demethylamination; consequently, ribosyl-AIC may be employed to minimize demethylamination and thus assure both a high concentration of 6-MAP, the substrate for both enzyme systems, and a simplification of the interpretation of the data by minimizing the formation of hypoxanthine, inosine, and inosinic

acid. Ribosyl-AIC does not significantly inhibit either the nucleoside phosphorylase or nucleotide pyrophosphorylase systems.

The great variations in metabolic potential among microorganisms require the realization that, since 6-MAP is an analogue of adenine, demethylamination may occur at the base, nucleoside, nucleotide, or any combination, depending on whether the particular organism possesses adenase, adenosine deaminase, or adenylate deaminase. The actual site of demethylamination depends on both the activity and specificity of enzymes such as nucleoside deaminases and phosphorylases as well as nucleotide phosphatases and deaminases. The site of demethylamination in *A. aerogenes* resembles that in *E. coli*, but it may occur at the level of the base in *P. aeruginosa* and *A. faecalis*. In contrast to the nearly equal growth rate of *E. coli* on adenine and 6-MAP, the latter supports only linear and suboptimal growth of the purine-requiring auxotroph Ade-11 of *S. typhimurium* LT_2 (6). Mammalian adenosine deaminase was reported to be inactive toward ribosyl-6-MAP (4); however, such differences in substrate specificity between mammalian and bacterial enzymes are neither unique nor unexpected. To obtain the ultimate proof of its role in the demethylamination of 6-MAP, adenosine deaminase is being purified from extracts of *E. coli* W-11.

The essential role of adenosine deaminase in demethylating 6-MOP is implied by the inhibitory effects of ribosyl-AIC on the growth of strain W-11 when 6-MOP serves as the purine source and the similar requirements for the demethylamination of 6-MAP and 6-MOP by nonproliferating cells (28). The potential role of adenylate pyrophosphorylase in synthesizing phosphoribosyl-6-MOP as an indirect route to ribosyl-6-MOP is suggested by the reduced growth of strain W-11/DAP, which lacks this enzyme, on 6-MOP. Consideration of the above suggests that 6-MOP is metabolized by the same pathways as 6-MAP.

The stimulation of ribosyl-AI synthesis, which occurs during growth on the methylated purines, could result from a derepression of enzyme formation, the limited ability of the methylated purines to give rise to effective feedback inhibition of purine synthesis, or from a combination of both. The methylated purines exert feedback inhibition in direct relationship to their ability to support growth; the extent of feedback inhibition is directly related to the degree of repression. Adenylic and guanylic acids, the metabolic end products of 6-MAP and 6-MOP, are the probable feedback inhibitors (26). Even though adenylate pyrophosphorylase converts 6-MAP and possibly 6-MOP to their respective ribonucleotides, the degree to which methylated ribonucleotides per se can act as pseudofeedback inhibitors of de novo purine synthesis (21) cannot be ascertained from these experiments.

The limited utilization of 6-MOP by strain W-11 is expressed by a low growth rate, reduced cell yield, and derepression of purine synthesis. The utilization of 6-MAP is less limited since the growth rate and cell yield closely approach that obtainable on an equimolar concentration of hypoxanthine or adenine, but the limitation is expressed by a high state of derepression for purine synthesis. Under these conditions of growth on limited available purine, effective feedback inhibition could not occur. Therefore, derepression of purine synthesis is the major factor contributing to the stimulation of ribosyl-AI synthesis during growth of strain W-11 on the methylated purines.

In highly derepressed cells (Fig. 4C and D), the lower concentrations of purines stimulate ribosyl-AI synthesis rather than exert the expected feedback inhibition. This phenomenon is thought to result from a replenishment of the nucleotide cofactors in cells depleted of purines.

Derepression of enzyme formation generally is accomplished by limiting the source of the end product to the cells. A convenient method with auxotrophs is to supply the cells with suboptimal concentrations of the required substrate or with an analogue which can be converted slowly to the end product. Cells which are derepressed for histidine synthesis can be obtained after growth on formylhistidine (1) or L-histidinol (30). Similarly, growth of purine-requiring auxotrophs on 5′-AMP or 3′-AMP relieves repression of inosinate dehydrogenase and xanthylate aminase, which are required for guanine synthesis (34). The limited utilization of 5′-AMP by *E. coli* W-11 stimulates the accumulation of ribosyl-AI, presumably by the same mechanism. Growth of *E. coli* W-11 on 6-MAP or 6-MOP provides another means of limiting the available purine and obtaining cells which are derepressed for the early enzymes required for purine synthesis. 6-MAP would be very useful for obtaining the derepressed purine-requiring mutants in high yield.

ACKNOWLEDGMENTS

We wish to acknowledge the capable technical assistance of Eleanor Green, and that of David Smith and John Scott who participated as Student Summer Research Fellows.

This investigation was supported by Public Health Service grants M-10699 and AI-04555 from the National Institutes of Health.

LITERATURE CITED

1. AMES, B. N., B. GARRY, AND L. A. HERZENBERG. 1960. The genetic control of the enzymes of histidine biosynthesis in *Salmonella typhimurium*. J. Gen. Microbiol. **22**:369–378.
2. BRATTON, A. C., AND E. K. MARSHALL, JR. 1939. A new coupling component for sulfanilamide determination. J. Biol. Chem. **128**:537–550.
3. CARTER, C. E. 1950. Paper chromatography of purine and pyrimidine derivatives of yeast ribonucleic acid. J. Am. Chem. Soc. **72**:1466–1471.
4. DUGGAN, D. E. 1963. The metabolism of 6-methylaminopurine in cell-free systems. Biochim. Biophys. Acta **68**:319–321.
5. DUGGAN, D. E., AND E. TITUS. 1962. The utilization of 6-methylaminopurine by mammalian tissues. Biochim. Biophys. Acta **55**:273–275.
6. DUGGAN, D. E., M. G. WEIGERT, W. E. GRIEB, AND E. O. TITUS. 1963. The metabolism of 6-methylaminopurine in *Salmonella typhimurium*. Biochim. Biophys. Acta **68**:519–525.
7. FLAKS, J. G., AND L. N. LUKENS. 1963. The enzymes of purine nucleotide synthesis *de novo*, p. 52–95. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 6. Academic Press, Inc., New York.
8. FLEISSNER, E., AND E. BOREK. 1962. A new enzyme of RNA synthesis: RNA methylase. Proc. Natl. Acad. Sci. U.S. **48**:1199–1203.
9. FLEISSNER, E., AND E. BOREK. 1963. Studies on the enzymatic methylation of soluble RNA. I. Methylation of the s-RNA polymer. Biochemistry **2**:1093–1100.
10. GOLD, M., AND J. HURWITZ. 1964. The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. V. Purification and properties of the deoxyribonucleic acid-methylating activity of *Escherichia coli*. J. Biol. Chem. **239**:3858–3865.
11. GOLD, M., J. HURWITZ, AND M. ANDERS. 1963. The enzymatic methylation of RNA and DNA. II. On the species specificity of the methylation enzymes. Proc. Natl. Acad. Sci. U.S. **50**:164–169.
12. GOTS, J. S. 1957. Purine metabolism in bacteria. V. Feedback inhibition. J. Biol. Chem. **228**:57–66.
13. GOTS, J. S., AND E. C. CHU. 1952. Studies on purine metabolism in bacteria. I. The role of *p*-aminobenzoic acid. J. Bacteriol. **64**:537–546.
14. GOTS, J. S., AND S. H. LOVE. 1954. Purine metabolism in bacteria. II. Factors influencing biosynthesis of 4-amino-5-imidazole-carboxamide by *Escherichia coli*. J. Biol. Chem. **210**:395–405.
15. HURWITZ, J., M. ANDERS, M. GOLD, AND I. SMITH. 1965. The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. VII. The methylation of ribosomal ribonucleic acid. J. Biol. Chem. **240**:1256–1266.
16. HURWITZ, J., M. GOLD, AND M. ANDERS. 1964. The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. IV. The properties of the soluble ribonucleic acid-methylating enzymes. J. Biol. Chem. **239**:3474–3482.
17. KOCH, A. L., AND G. VALLEE. 1959. The properties of adenosine deaminase and adenosine nucleoside phosphorylase in extracts of *Escherichia coli*. J. Biol. Chem. **234**:1213–1218.
18. KURAMITSU, H. K., S. UDAKA, AND H. S. MOYED. 1964. Induction of inosine 5′-phosphate dehydrogenase and xanthosine 5′-phosphate aminase by ribosyl-4-amino-5-imidazolecarboxamide in purine-requiring mutants of *Escherichia coli* B. J. Biol. Chem. **239**:3425–3430.
19. LOVE, S. H., AND J. S. GOTS. 1955. Purine metabolism in bacteria. III. Accumulation of a new pentose-containing arylamine by a purine-requiring mutant of *Escherichia coli*. J. Biol. Chem. **212**:647–654.
20. LOVE, S. H., AND B. LEVENBERG. 1959. Formation of 5-aminoimidazole riboside by *Escherichia coli*: evidence for its structure and metabolic relationship to the purines. Biochim. Biophys. Acta **35**:367–373.
21. MCCOLLISTER, R. J., W. R. GILBERT, JR., D. M. ASHTON, AND J. B. WYNGAARDEN. 1964. Pseudofeedback inhibition of purine synthesis by 6-mercaptopurine ribonucleotide and other purine analogues. J. Biol. Chem. **239**:1560–1563.
22. MAGASANIK, B., AND D. KARIBIAN. 1960. Purine nucleotide cycles and their metabolic role. J. Biol. Chem. **235**:2672–2681.
23. MANDEL, L. R., AND E. BOREK. 1963. The biosynthesis of methylated bases in ribonucleic acid. Biochemistry **2**:555–560.
24. MARKHAM, R., AND J. D. SMITH. 1949. Chromatographic studies of nucleic acids. I. A technique for the identification and estimation of purine and pyrimidine bases, nucleosides and related substances. Biochem. J. **45**:294–298.
25. MOYED, H. S., AND B. MAGASANIK. 1960. The biosynthesis of the imidazole ring of histidine. J. Biol. Chem. **235**:149–153.
26. NIERLICH, D. P., AND B. MAGASANIK. 1965. Regulation of purine ribonucleotide synthesis by end product inhibition. The effect of adenine and guanine ribonucleotides on the 5′-phosphoribosyl-pyrophosphate amidotransferase of *Aerobacter aerogenes*. J. Biol. Chem. **240**:358–365.
27. PETERKOFSKY, A., C. JESENSKY, A. BANK, AND A. H. MEHLER. 1964. Studies on the role of methylated bases in the biological activity of soluble ribonucleic acid. J. Biol. Chem. **239**:2918–2926.
28. REMY, C. N. 1961. Metabolism of 6-methylaminopurine: synthesis and demethylation by *Escherichia coli*. J. Biol. Chem. **236**:2999–3005.
29. REMY, C. N., AND M. S. SMITH. 1957. Metabolism of 2,6-diaminopurine: conversion to 5′-phosphoribosyl-2-methylamino-6-aminopurine by enzymes of *Escherichia coli*. J. Biol. Chem. **228**:325–338.
30. SMITH, D. W. E., AND B. N. AMES. 1964. Inter-

mediates in the early steps of histidine biosynthesis. J. Biol. Chem. **239**:1848–1855.

31. STARR, J. L. 1962. Studies on the methylation of soluble ribonucleic acid. I. Failure of the direct incorporation of 6-methylaminopurine. Biochim. Biophys. Acta **61**:676–680.
32. STARR, J. L. 1963. The incorporation of amino acids into "methyl-poor" amino acid transfer ribonucleic acid. Biochem. Biophys. Res. Commun. **10**:181–185.
33. STARR, J. L., AND R. FEFFERMAN. 1964. The occurrence of methylated bases in ribosomal ribonucleic acid of *Escherichia coli* K12 W-6. J. Biol. Chem. **239**:3457–3461.
34. UDAKA, S., AND H. S. MOYED. 1963. Inhibition of parental and mutant xanthosine 5′-phosphate aminases by psicofuranine. J. Biol. Chem. **238**: 2797–2803.
35. ZIMMERMAN, E. F., AND B. MAGASANIK. 1964. Utilization and interconversion of purine bases and ribonucleosides by *Salmonella typhimurium*. J. Biol. Chem. **239**:293–300.