Regulation of Synthesis of Serine Hydroxymethyltransferase in Chemostat Cultures of Escherichia coli*

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Serine hydroxymethyltransferase was synthesized as a constant fraction of total protein of Escherichia coli over a wide range of specific growth rates. This was observed in all strains when grown in glucose-limited chemostat cultures; in thymine-requiring mutants during thymidine-limited growth; and in met A and met B auxotrophs, defective in homocysteine biosynthesis, during methionine-limited growth. This behavior has been referred to by others as "metabolic control." In addition, the synthesis of serine hydroxymethyltransferase was subject to specific active control mechanisms, which responded to the needs of the cell for purine biosynthesis, methylation reactions, as well as to serine limitation. Under purine limitation, the rate of enzyme synthesis increased with decreasing growth rate, that is with increasing purine limitation. During methionine-limited growth of met E and met F auxotrophs (mutants unable to methylate homocysteine) the rate of enzyme synthesis increased with a decrease in specific growth rate from 0.65 to 0.30 h⁻¹ but declined with further decrease in growth rate. Under serine limitation the rate of enzyme synthesis remained proportional to the growth rate, but at a rate twice that observed in unrestricted or glucose-limited growth. When purines were added to unrestricted or glucose-limited cultures, the rate of enzyme synthesis decreased by 40%, but remained proportional to growth rate. Addition of methionine or serine alone had no effect.

Serine hydroxymethyltransferase (EC 2.1.2.1) catalyzes the transfer of carbon-3 of serine to tetrahydrofolate forming N⁵,N¹⁰-methylene-H₄folate and glycine. The resulting one-carbon adducts of H₄folate are utilized in the biosynthesis of purines, thymidine, methionine, and the formyl group of fMet-tRNA₉, providing up to 3% of the total carbon of the cell (1). In Escherichia coli growing on glucose minimal medium, most of these one-carbon units enter the folate pathway via the serine hydroxymethyltransferase reaction (2); but when purines, thymidine, and methionine are supplied in the medium the incorporation of one-carbon units into these products is reduced by 95% (3). Serine hydroxymethyltransferase may be regarded as the initial enzyme of a branched pathway with multiple products, subject to highly variable demand. Its regulation is likely to involve a complex set of controls.

In studying the regulation of synthesis of an enzyme in bacteria, possible regulatory effectors or their precursors are usually either added to the medium in presumed excess, or supplied to auxotrophic mutants at rates or quantities which are assumed to limit the growth rate, and any changes in enzyme levels measured. A number of such observations have been made on serine hydroxymethyltransferase in bacteria. When different products of the folate pathway were added to the growth medium, a 60 to 70% reduction in serine hydroxymethyltransferase activity was observed in various E. coli K12 strains (4-6). Starvation for purines resulted in elevated levels of serine hydroxymethyltransferase, in some of the purine auxotrophs of E. coli and Salmonella typhimurium, but not in the others (6, 7). The addition of serine to the growth medium caused some repression of the enzyme synthesis in Streptococcus faecium and S. typhimurium (7, 8). However, when serine-glycine auxotrophs of S. typhimurium and E. coli were starved for serine, no significant derepression of the enzyme was observed (6, 7). Folk and Berg (9) examined the levels of serine hydroxymethyltransferase in an E. coli mutant with an altered glycylic transfer ribonucleic acid synthetase, but found that this mutation had no significant effects on the levels. The enzyme levels were also unaffected in a prototrophic strain when glycine was added to the growth medium. However, Miller and Newman (6) have suggested glycine as an important corepressor of serine hydroxymethyltransferase synthesis.

Methionine is one of the major products of the folate pathway and is utilized for many important reactions in the cell (Fig. 1). The reported effects of methionine on the synthesis of serine hydroxymethyltransferase have also been contradictory. Mansouri et al. (10) observed a 2- to 18-fold increase in serine hydroxymethyltransferase activity in a methionine or cyanocobalamin auxotroph of E. coli 113-3 (met E) grown in the presence of low levels of methionine, but other workers (5, 7) were unable to repeat these observations. However, when the supply of methionine was limited by growth on D-methionine sulfoxide, Greene and Radovich (5) observed an increase in serine hydroxymethyltransferase levels in met E, but not in met A mutants of E. coli. Needle and Pizer (11) observed that serine hydroxymethyltransferase levels increased 1.5- to 2-fold in a met K strain of E. coli K12 and proposed that S-adenosylmethionine is the corepressor for its regulation. Different workers (5, 11, 12), have shown that a prototrophic met J strain when grown on glucose minimal medium had the same activity as the wild type, leading to the suggestion that the regulatory system for serine hydroxymethyltransferase is different from the one which controls the met regulon.

Among these results there is agreement that serine hydrox-
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**FIG. 1.** The relationship between serine hydroxymethyltransferase and biosynthesis and utilization of methionine in *E. coli*. The reaction numbered I is catalyzed by serine hydroxymethyltransferase enzymes catalyzing other reactions shown above are reviewed in Refs. 31 and 32. Gene symbols are from Ref. 33. Biochemical symbols are: MTA, methylthioadenosine; PUT, putrescine; SPD, spermidine; CYN, cystathionine; RIB-HCY, ribosyl homocysteine; remaining symbols are according to IUPAC guidelines. a, N6,N'6-methylene-H4folate is also utilized directly in the biosynthesis of thymidine and oxidized to N10-formyl-H4folate by an enzyme complex of methylene-H4folate dehydrogenase and methenyl H4folate cyclohydrolase (1). N'6-formyl-H4folate provides both carbons-2 and -8 of the purine ring, and the formyl group of met-tRNA (34). b, the substrate for non-B12 homocysteine transmethylase is 5-CH3-H4PtGlu (32).

...methytransferase synthesis is regulated by the end products of the folate pathway. However, the assignments of the roles of individual end products to their role as a corepressor or repressor are apparently contradictory. Many of the contradictions may result from the fact that the majority of studies have been carried out on bacteria growing in poorly defined physiological states. In particular, the studies of derepression of serine hydroxymethyltransferase have been made under conditions in which it was uncertain that any nutrient limitation had been achieved. In this study we have avoided these difficulties. Our principal results have been obtained from chemostat cultures of mutants of *E. coli* auxotrophic for products of the folate pathway and it is shown that in addition to the changing demands imposed by changing growth rate, serine hydroxymethyltransferase synthesis is subject to active control mechanisms which respond to the requirements for purine biosynthesis, and for methylation reactions, as well as to serine limitation.

**EXPERIMENTAL PROCEDURES**

**Organisms**—The bacterial strains used are listed in Table I. *E. coli* B (ATCC 23226) and *E. coli* Hfr 3000 (ATCC e252571 were obtained from American Type Culture Collection. *E. coli* 5073 and CSH70 were obtained from John Andrews of these laboratories. Purine auxotroph ML30-PUR103, serine glycine auxotroph ML30-SG40, methionine auxotroph ML30-M15, and thymidine auxotroph ML30-Thy16 were obtained by nitrosoguanidine mutagenesis of *E. coli* ML30. The remaining strains were kindly supplied by Dr. Ronald Green of Duke University.

**Growth Conditions**—The basal growth medium has been described (11). All cultures were grown under forced aeration at 30 °C. For batch cultures, 1.0 mg of glucose/ml (5.6 mM) was added, and where required, adenosine (0.15 mM), L-serine (0.2 mM), glycine (0.27 mM), thymidine (0.16 mM), methionine (0.13 mM), and vitamin B12, (0.0067 mM). Specific growth rates (λ, h⁻¹) for batch culture cells were calculated by linear regression of ln of optical density at 600 nm versus time.

The methods for growth of chemostat cultures have been described earlier (13). The concentration of limiting nutrient supplied in the chemostat reservoir was chosen to give a steady state cell concentration of 80 to 120 μg, dry weight, per ml. The concentrations used were: 0.015 mM (adenosine), 0.938 mM (L-serine), 0.025 mM (thymi-

**TABLE I**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Requirements</th>
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<td>ML30</td>
<td>Methionine</td>
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<tr>
<td>ML30-M15</td>
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<td></td>
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<tr>
<td>ML30-PUR103</td>
<td>Purines</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td>ML30-SG40</td>
<td>Serine or glycine</td>
<td>ser A</td>
<td></td>
</tr>
<tr>
<td>ML30-Thy16</td>
<td>Thymidine</td>
<td>thy A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>Wild type</td>
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<tr>
<td>K12</td>
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<td>arg E, thi</td>
<td>5</td>
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<tr>
<td>AB1932</td>
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<td>met E</td>
<td>5</td>
</tr>
<tr>
<td>MU104</td>
<td>Methionine, arginine, thiamine, thi</td>
<td>met F</td>
<td>5</td>
</tr>
<tr>
<td>RG350</td>
<td>Methionine, arginine, thiamine, thi</td>
<td>met A28, met F, met K86</td>
<td>Transductant of RG175 (5)</td>
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<tr>
<td>RG344</td>
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<td>met A28, met K86</td>
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<tr>
<td>CSH70</td>
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<td>met B, met K86</td>
<td>Formerly strain P4X (35)</td>
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<tr>
<td>RG109</td>
<td>Methionine, arginine, thiamine, thi</td>
<td>met K86</td>
<td>Spontaneous mutant (5)</td>
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* ND, not determined.
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dine), 0.13 mM (L-methionine), 4 x 10^{-11} M (vitamin B12), or 1.4 mM (glucose). In glucose-limited chemostat cultures of auxotrophic strains, the concentration of the required nutrient was the same as in the batch culture medium. Cultures were assumed to have reached a steady state when cell concentrations varied no more than 1% during one generation. Samples for measurement of serine hydroxymethyltransferase were removed from the chemostat either continuously, by collecting 100 to 200 ml of the effluent in a chilled flask containing ice, or by tipping the entire chemostat culture (500 ml) over ice. During continuous sampling, the optical density of the chemostat culture was monitored to ensure that the steady state was maintained. Chemostat cultures in which the growth of an auxotrophic mutant was limited by the concentration of the required nutrient provided extreme pressure for the selection of prototrophic revertants, and could seldom be maintained for longer than about 10 doubling times. The appearance of a significant number of revertants in a chemostat culture could, however, be readily detected, being marked by a sharp increase in the cell density and loss of the steady state. All results reported here were from cultures which had been growing in a steady state for at least one doubling before sampling, and if sampled by the continuous method, remained in the steady state during sampling and for at least one doubling thereafter.

Mutagenesis and Selection—Cells of E. coli ML30 were harvested during exponential growth and treated with N-methyl-N’-nitro-N’-nitrosoguanidine at a final concentration of 300 μg/ml for 15 min at 30 °C, according to the procedure of Adelberg et al. (14). After overnight growth on the required nutrient, and plating, methionine and adenine auxotrophs were obtained by direct selection. Thymidine auxotrophs were enriched by growth in the presence of thymidine and trimethoprim (15), followed by direct selection. To enrich for serine-glycine auxotrophs, the culture was filtered, the cells washed three times with basal medium, suspended in basal medium, and incubated at 30 °C for 60 min. Glucose (1 mg/ml) and penicillin (2000 units/ml) were added and incubation continued for 60 min. Penicillinase (2000 units/ml) was added, and after plating, mutants were obtained by direct selection.

Enzyme Assays—Serine hydroxymethyltransferase activity was assayed by measuring the formation of N5,N10-methylene-H4folate at 30 °C by the procedure of Taylor and Weisbach (16), except that the buffer was potassium maleate, 0.05 M, pH 7.2, and serine concentration in the reaction mixture was 2 mM.

To measure the specific activity of serine hydroxymethyltransferase, batch cultures (200 ml) were harvested by tipping over crushed ice; chemostat cultures were harvested as described above. The cells were washed twice by centrifugation at 3000 g for 15 min at 30 °C, according to the procedure of Adelberg et al. (14). After overnight growth on the required nutrient, and plating, methionine and adenine auxotrophs were obtained by direct selection. Thymidine auxotrophs were enriched by growth in the presence of thymidine and trimethoprim (15), followed by direct selection. To enrich for serine-glycine auxotrophs, the culture was filtered, the cells washed three times with basal medium, suspended in basal medium, and incubated at 30 °C for 60 min. Glucose (1 mg/ml) and penicillin (2000 units/ml) were added and incubation continued for 60 min. Penicillinase (2000 units/ml) was added, and after plating, mutants were obtained by direct selection.

Estimation of Rate of Enzyme Synthesis—According to the definition of balanced growth (19) the rate of synthesis of an enzyme may be given by

$$\frac{dE}{dt} = \lambda \cdot E$$

(1)

Where λ is the specific growth rate and E represents total units of the enzyme. The specific activity of the enzyme (S) is defined as units of enzyme/mass of cell protein (P); i.e. $S = E/P$. The ratio of total protein (P) to DNA (D) is constant at different growth rates (20), i.e. $P/D = C$. Substituting these relationships into Equation 1 gives;

$$\frac{dE}{dt} = \frac{1}{D} \cdot \lambda \cdot S \cdot C$$

(2)

Thus, according to Equation 2 the rate of serine hydroxymethyltransferase synthesis/gene in arbitrary units was estimated by multiplying enzyme specific activity by the specific growth rate.

RESULTS

Serine Hydroxymethyltransferase in Glucose-limited Chemostat Cultures—The specific activities of serine hydroxymethyltransferase in wild type E. coli ML30 were constant over a wide range of specific growth rates in glucose-limited chemostat cultures. The addition of serine, methionine, and thymidine in combination did not have any significant effect on the enzyme levels. However, the addition of adenine to these cultures resulted in about a 2-fold reduction in enzyme levels (Fig. 2A).

Serine Hydroxymethyltransferase Levels in Purine-limited Cells—The enzyme levels in a purine auxotroph were also constant at different growth rates in steady state glucose-limited chemostat cultures with an unrestricted amount of purines in the growth medium (Fig. 2B).

However, when purines were limited for the growth of this mutant in steady state chemostat cultures, the specific activity of the enzyme increased with decreasing specific growth rate, i.e. with increasing purine limitation. The addition of methionine, serine, glycine, and thymidine had no significant effect on the enzyme levels during purine-limited growth of this mutant.

Serine Hydroxymethyltransferase Activity during Serine Limitation—Serine-glycine auxotrophs grow more slowly on glycine than on serine. The results presented in the Miniprint with this paper show that the reduced growth rate on glycine is due to limitation of the supply of serine. A serine-glycine auxotroph ML30-SG40 was grown in serine- and glucose-limited chemostat cultures and in minimal media supplemented with serine or glycine and the specific activity of serine hydroxymethyltransferase determined. Results are shown in Fig. 2C. During serine limitation, the enzyme levels were significantly higher than in the cells grown in batch cultures and glucose-limited chemostat cultures with an unrestricted amount of serine. A decrease in specific growth rate from 0.64 to 0.57 h^{-1} caused the maximum derepression of enzyme specific activity observed in serine-limited cultures. The further decrease in specific growth rate due to increasing serine limitation had little effect on the enzyme specific activities.

The addition of purines to the serine-limited cultures resulted in a decrease in specific activity of serine hydroxymethyltransferase. However, the enzyme levels were still 2-fold more than the corresponding levels in purine-supplemented medium with an unrestricted amount of serine. The addition of methionine and thymidine had no significant effect on the enzyme levels in serine-limited cells (data not shown).

Serine Hydroxymethyltransferase Activity in Methionine-limited Cultures—The enzyme specific activities of the E. coli methionine auxotrophs stayed fairly constant over a wide range of growth rates in steady state glucose-limited chemostat cultures (Fig. 2D).

Similarly, during methionine limitation, the levels of serine

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1 Portions of this paper (including part of “Results” and part of “Discussion,” Fig. 5, and Tables 5–7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-581, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Control of Serine Hydroxymethyltransferase

Specific activities of serine hydroxymethyltransferase as a function of growth rate. A, glucose-limited chemostat cultures of E. coli ML30 with (○) no additional supplement; (△) with adenosine; (●) with serine, methionine, and thymidine. The points at the highest specific growth rates (0.55 to 0.62/h) were measured on cells from cultures in unrestricted balanced growth, and the remaining points are from measurements on steady state glucose-limited chemostat cultures. B, purine Auxotroph ML30-PUR103; adenosine-limited chemostat culture with (●) no additional supplement, (△) with unrestricted amounts of methionine, thymidine, and serine in medium; (○) measurements from steady state glucose-limited chemostat cultures with unrestricted amounts of adenosine in growth medium; batch cultures with (△) adenosine alone or with (○) adenosine, methionine, thymidine, and serine in growth medium. C, serine-glycine Auxotroph ML30-SG40; (●) serine-limited chemostat cultures; glycine-supplemented batch cultures with (○) no additional supplement; (△) unrestricted amounts of adenosine in the growth medium; serine-supplemented batch cultures with (△) no additional supplement, with (○) unrestricted amounts of adenosine in the growth medium; glucose-limited chemostat cultures with unrestricted amounts of serine and (△) no additional supplement and (●) unrestricted amounts of adenosine in the growth medium. D, glucose-limited chemostat cultures of methionine auxotrophs, MU104 (met E) with unrestricted amounts of (○) methionine or (●) vitamin B12 in the growth medium; (△) AB1932 and (△) ML30-M15 with unrestricted amounts of methionine in the growth medium. The points at high specific growth rates (0.6 to 0.65 h⁻¹) were obtained from unrestricted balanced growth and remaining points were from steady state glucose-limited chemostat cultures.

hydroxymethyltransferase in methionine auxotrophs ML30-M15 (met A), AB1932 (arg, met A, thi), and CSH70 (met B, thi, arg), which are defective in de novo homocysteine biosynthesis were also unchanged at different growth rates (Fig. 3A). The addition of adenosine, serine, and thymidine to the methionine-limited cultures of AB1932 and ML30-M15 had no significant effect on the enzyme levels. However, when these additives were added to batch cultures of these mutants they resulted in about 2-fold decrease in enzyme levels. In ML30-M15 the addition of adenosine alone produced similar reduction of enzyme levels in batch cultures but not in AB1932 (data not shown).

However, the enzyme levels in methionine auxotrophs RG350 (met F), Mu104 (met E), and RG344 (met A, met F, thi), mutants which are unable to methylate homocysteine, increased with decreasing growth rate, i.e. with increasing methionine limitation (Fig. 3, B and C). During methionine limitation, the addition of adenosine, serine, cysteine, glycine, and a mixture of amino acids had no effect on the enzyme specific activities. However, when adenosine was added to the methionine-supplemented batch culture, the enzyme levels in Mu104 (met E) were decreased by about 30%.

In E. coli there are two homocysteine transmethylases; one of them is inducible and is only present in vitamin B12 grown cells (Fig. 1). Mu104 (met E) which lacks a functional non-B12 transmethytransferase can grow on vitamin B12. The serine hydroxymethyltransferase levels in vitamin B12-limited chemostat cultures, and in vitamin B12-supplemented batch cultures were measured. Results in Fig. 3D show that a significant increase in serine hydroxymethyltransferase activity was observed in B12-limited cells, and the increase was a function of the decreasing growth rate.

Serine Hydroxymethyltransferase in Thymidine-limited Cells—Specific activities of serine hydroxymethyltransferase were determined in the thymidine auxotroph ML30-Thy16 grown in thymidine-limited and glucose-limited chemostat cultures, and in unrestricted balanced batch culture. Table II shows that no significant change in enzyme activities occurred under any of the growth conditions studied.

Serine Hydroxymethyltransferase Levels in Batch Cultures—Table III shows that growth in the presence of glycine, methionine, serine, and thymidine, individually, and in combination, did not have any significant effect on the specific activities of serine hydroxymethyltransferase in different strains of E. coli. In all except Hfr 3000, the addition of adenosine to the growth medium of E. coli strains caused a 40 to 60% reduction in enzyme-specific activities. However, when a combination of all these compounds was added to the growth medium of Hfr 3000, like other E. coli strains, a 60% decrease in enzyme levels was observed.

Trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine) by inhibiting dihydrofolate reductase (21) inhibits all reactions of the folate pathway. Inhibition of growth by trimethoprim is due to limitation of the rate of production of one or more of the products of the folate pathway, and has been shown to result in a 2-fold derepression of serine hydroxymethyltransferase in S. typhimurium (7). In E. coli ML30 a similar increase in enzyme levels in trimethoprim-supplemented growth medium was also observed (Table IV). However, this increase could be overcome by the addition of adenosine, serine, methionine, thymidine, and glycine to the growth medium. The addition of serine, methionine, thymidine, and glycine without adenosine had no significant effect on the enzyme levels, which suggests that it is the limitation of supply of purines in trimethoprim medium which results in the derepression of enzyme synthesis in E. coli. The limitation of glycine, thymidine, and methionine in these cultures apparently had no significant effect on the enzyme levels.

DISCUSSION

We have studied the regulation of serine hydroxymethyltransferase by measuring the specific activities of the enzyme
range of growth rates. This was observed in all strains of *E. coli* when grown in glucose-limited chemostat cultures; in thymidine-requiring mutants during thymidine-limited growth; and in *met A* and *met B* auxotrophs, defective in homocysteine biosynthesis, during methionine-limited growth. The rate of enzyme synthesis in these cultures was proportional to the growth rate and is represented by group A in Fig. 4. The synthesis of such proteins which are present at constant specific activity over a wide range of growth rates and physiological conditions, has been said to be under "metabolic control" (22, 23). Various mechanisms have been proposed to explain this type of control (24–26). The transcriptional and translational yields from the repressorless *trip* operon (27), the repressorless and unattenuated *trp* operon (28), the *lac* operon with different catabolite-insensitive *lac* promoters (23), and in the strains uncoupled for *his* operon and attenuated control (29) have been examined. The results have unanimously shown that the product of an operon, presumably freed of active controls, represents a relatively constant fraction of total cellular protein under a large variety of growth conditions. Metabolic controls may be characteristic of enzymes lacking specific active control mechanisms and the gene for serine hydroxymethyltransferase appears to be subject to this control.

However, our results also show that in addition to the apparent metabolic control, serine hydroxymethyltransferase synthesis is subject to active control mechanisms which respond to the needs of the cell for various end products of the folate pathway. Under serine limitation (Fig. 4, group B), the rate of enzyme synthesis remained proportional to the growth rate, but at a rate twice that observed in unrestricted or glucose-limited cultures. Addition of serine to the unrestricted or glucose-limited cultures had no effect on the rate of enzyme synthesis.

When purines were added to the unrestricted or glucose-limited cultures, the rate of enzyme synthesis decreased by 40%, but remained proportional to the growth rate (Fig. 4, group C). However, under purine limitation the rate of enzyme synthesis increased with decreasing growth rate, that is with increasing purine limitation (Fig. 4, group D). This strongly suggests that a product of the purine pathway is a repressor or corepressor of serine hydroxymethyltransferase.

The addition of methionine to unrestricted or glucose-limited chemostat cultures, and methionine limitation in *met A* and *met B* strains (Fig. 4, group A), had no significant effect on serine hydroxymethyltransferase synthesis. However, during methionine-limited growth of *met E* and *met F* auxotrophs, mutants unable to methylate homocysteine, and thus unable to either synthesize methionine *de novo* or to regenerate it after its use in methylation reactions, the rate of enzyme synthesis increased with a decrease in specific growth rate from 0.65, to 0.30 h⁻¹ and declined with further decrease in growth rate (Fig. 4, group E). During B12 limitation, the synthesis of the enzyme in the *met E* mutant followed a similar pattern, except that the initial increase in rate of enzyme synthesis with decreasing growth rate was more rapid (Fig. 4, group F).

These data suggest that it is the requirements of methionine for methylation reactions rather than for protein synthesis which control the synthesis of serine hydroxymethyltransferase. In a following paper (30), we have investigated this further and have found a high correlation between the ratio of homocysteine to S-adenosylmethionine and the rate of serine hydroxymethyltransferase synthesis during methionine limitation.

The synthesis of serine hydroxymethyltransferase in *E. coli*
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TABLE III

<table>
<thead>
<tr>
<th>Supplements*</th>
<th>ML30</th>
<th>B</th>
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<tr>
<td>G* Specific activity</td>
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<td>nmol/min/mg P</td>
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<td>0.72</td>
<td>40 ± 5 (3)</td>
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* Cells were grown on glucose minimal medium supplemented as indicated with glycine, L-methionine, thymidine, l-serine, and adenosine.

# Specific growth rate.

The values are mean of the number of measurements given in parentheses.

thus responds to the changing demands for one-carbon ad- ducts of H$_4$folate imposed by changing growth rate, to the requirements for purine biosynthesis and methylation reactions, as well as to serine limitation.

The results presented in this paper also suggest that these control mechanisms do interact in a complex manner. A synergistic effect of different end products of the folate pathway on the synthesis of serine hydroxymethyltransferase has been reported for some strains of S. typhimurium (7) and E. coli (5) and has also been observed by us in Hfr 3000 and AB1932. During serine limitation, the synthesis of serine hydroxymethyltransferase does respond to purines. The two controls may be functioning independently of each other as the addition of purines to serine-limited cultures resulted in a decrease in the rate of enzyme synthesis, however, the rate was still two times higher than the corresponding rate in purine-supplemented medium without serine restriction. During methionine limitation, the synthesis of the enzyme does not respond to purines, and likewise the addition of methionine to purine-limited cultures had no effect. This suggests that the regulation of synthesis of serine hydroxymethyltrans-

# FIG. 4. Rate of serine hydroxymethyltransferase synthesis/ genome as a function of specific growth rate. The data shown elsewhere in this paper was grouped into six major groups according to the pattern of expression of serine hydroxymethyltransferase; and for groups from A to D, regression parameters were calculated and lines were drawn by connecting the points. Group A, includes data from glucose-limited chemostat cultures for ML30, unsupplemented and with serine, methionine, and thymidine; glucose-limited chemostat cultures for serine glycine auxotroph ML30-SG40; glucose-limited cultures of methionine auxotrophs; methionine-limited cultures of ML30-M15 (met A), AB1932 (met A), CSH70 (met B); glucose- and thymidine-limited chemostat cultures for thymidine auxotroph ML30-Thy16. Group B, serine-limited cultures of ML30-SG40. Group C, glucose-limited chemostat cultures for ML30 supplemented with unrestricted amounts of adenine and for purine auxotroph ML30-PUR105. Group D, adenine-limited chemostat cultures for ML30-PUR105. Group E, methionine-limited cultures of MU104 (met E), RG350 (met F). Group F, vitamin B12- limited cultures of MU104 (met E).
ferase during methionine and purine limitation may be affected by a common corepressor or repressor.

REFERENCES

SUPPLEMENTAL MATERIAL TO
REGULATION OF SYNTHESIS OF SERINE HYDROXYMETHYLTRANSFERASE IN CHEMOSTAT CULTURES OF ESCHERICHIA COLI

Inhibitory Effects of a Common Corepressor on the Synthesis of Serine Hydroxymethyltransferase (SHMT) in Escherichia coli

Growth of a Bacterial Strain: Serine Hydroxymethyltransferase (SHMT)

N. O., and Maalbe, O., eds) Munksgaard, Copenhagen.

To determine the primary limitation the kinetic parameters of serine and glycine transport were examined. In Table 5 it is shown that both serine and glycine can be accumulated intracellularly to concentrations 18 to 188-fold in excess of the extra-cellular concentrations. However, although the

Values of the Nmax for transport determined from initial velocities are similar for both the extra and intra cellular, the maximum velocity for glycine transport is about 3-fold lower than that for serine transport. This is consistent with the maximal velocities for the transport of serine and glycine by E. coli membrane vesicles (5).

Values of the Nmax for transport determined from initial velocities are similar for both the exoergic and endoergic reactions. The maximum velocity for glycine transport is about 3-fold lower than that for serine transport. This is consistent with the maximal velocities for the transport of serine and glycine by E. coli membrane vesicles (5).
TABLE 6

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Km (mM)</th>
<th>Vmax (umol/min/mg protein)</th>
<th>Inter cellular Curr. (umol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>0.15</td>
<td>0.70</td>
<td>19.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.78</td>
<td>0.77</td>
<td>2.7</td>
</tr>
</tbody>
</table>

The values are means of the intracellular concentrations obtained from extra-cellular concentrations ranging from 0.15 to 6.53 mM; the number is given in parentheses.

P represents the total cell protein.

The apparent kinetic parameters for the serine hydroxymethyltransferase from S. ellii are shown in Table 6. When serine-hydroxymethyltransferase synthetase from Glycine and methionine H2Sulfate, not only is the maximum velocity significantly lower in the reverse direction than in the forward direction, but the values of apparent Km for both substrates in the reverse direction are higher compared to those of the corresponding values for the forward reaction. What should be noted is that the maximum velocity of serine hydroxymethyltransferase in both directions is higher than the maximum velocity for the transport of the amino acid used. In fact, the maximum velocity of serine hydroxymethyltransferase in the serine-furfural direction is several fold more than that for the transport of glycine. Some growth experiments show that reduced growth rates on glycine is due to serine limitation, the primary reasons for this limitation must be the slow rate of glycine transport.

TABLE 7

<table>
<thead>
<tr>
<th>Product</th>
<th>Uptake (umol/mg) at Specific Growth Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Protein and Purine from Glycine</td>
<td>10.0</td>
</tr>
<tr>
<td>C-1 Product</td>
<td>15.0</td>
</tr>
<tr>
<td>Serine from Glycine</td>
<td>8.0</td>
</tr>
<tr>
<td>Glycine from Serine</td>
<td>12.0</td>
</tr>
<tr>
<td>Total</td>
<td>56.0</td>
</tr>
</tbody>
</table>

References