JOURNAL OF BACTERIOLOGY
Vol. 88, No. 3, p. 611-619 September, 1964
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NUTRITIONAL AND REGULATORY ASPECTS OF SERINE METABOLISM IN *ESCHERICHIA COLI*

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Received for publication 11 April 1964

ABSTRACT

PIZER, LEWIS I. (University of Pennsylvania, Philadelphia), AND MARY L. POTOCHNY. Nutritional and regulatory aspects of serine metabolism in *Escherichia coli*. J. Bacteriol. **88**:611–619. 1964.—Growth studies with a serine auxotroph have demonstrated a relationship between the serine supplied and the extent of growth, which closely agrees with the value calculated from the reported chemical composition of *Escherichia coli*. Serine could be replaced by glycine and, to a limited extent, by L-threonine. Both exogenous serine and glycine regulate their own endogenous synthesis from glucose or fructose. The inhibition of endogenous synthesis of both amino acids by serine was greater than 90% with both carbon sources. Greater amounts of exogenous glycine were utilized for cell synthesis when fructose was the carbon source. Growth conditions affected the levels of phosphoglycerate dehydrogenase found in cell-free extracts. The highest levels were found in glucose-grown cells and the lowest in cells grown in a medium augmented with L-threonine, L-methionine, L-leucine, and DL-isoleucine. The levels of serine phosphate phosphatase were not altered by changes in growth conditions.

Although considerable information has been obtained on the uptake and metabolism of serine in *Escherichia coli* (Meinhart and Simmonds, 1955; Simmonds and Miller, 1957; Levine and Simmonds, 1960), the major biosynthetic pathway from glucose has only recently been established (Pizer, 1963; Umbarger, Umbarger, and Siu, 1963). Enzymes have been found in *E. coli* extracts which catalyse the oxidation of 3-phosphoglycerate to form hydroxypyruvate-phosphate, the transamination of hydroxypyruvate-phosphate to form serine-phosphate, and the dephosphorylation of serine-phosphate to yield serine. These reactions make up the "phosphorylated pathway" described in rat liver by Ichihara and Greenberg (1957). *E. coli* depends on this pathway for the biosynthesis of serine, because mutations which result in the loss of phosphoglycerate dehydrogenase lead to a growth requirement for serine.

Our experiments were performed in conjunction with studies on the enzymes of the biosynthetic pathway (Pizer, 1963) so that observations in cell extracts might be related to growing cells. The response of these enzyme levels to alterations in cell nutrition was studied to determine whether nutritional factors regulate serine biosynthesis.

MATERIALS AND METHODS

Bacteria and growth conditions. E. coli strains W and Wc⁻ were obtained from S. S. Cohen of the University of Pennsylvania, and *E. coli* strain Ws⁻ from S. Simmonds of Yale University. The bacteria were maintained on nutrient agar slants and were grown in a synthetic medium which had the following composition (in g per liter): $NaHPO_4$, 6; KH_2PO_4, 3; NH_4Cl, 1; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.005. Carbon sources and nutrients were sterilized by filtration and subsequently added to the sterile salts solution. Bacterial growth was measured turbidimetrically in a Klett colorimeter fitted with a no. 42 filter and was correlated with viable counts made on nutrient agar plates. The cultures were grown aerobically at 37 C.

Overnight cultures in synthetic medium were diluted 1:20 in fresh medium on the morning of the growth experiment. When the culture reached a cell concentration of approximately 2×10^8 cells per ml, it was chilled to 12 C and washed twice by resuspension in cold salts solution and by centrifugation. The cells were then resuspended in the salts solution to give a final concentration of 10^8 cells per ml. To each of a set of growth tubes, 9 ml of the cell suspension were added, followed by the carbon and energy source and other nutrilites according to the experimental design. The growth flasks were of the type described by Cohen and Barner (1955),

which allow turbidimetric measurements to be made in a side arm attached to the flask. When the essential nutrients were present, growth resumed without a detectable lag and proceeded in an exponential fashion.

Glucose or fructose was present in the medium at 1 mg/ml (5.5 mM), and growth was limited at approximately 10^9 cells per ml. Lactate was used at a final concentration of 11 mM. The enriched medium described by Novick and Maas (1961) was used with the following modifications: glycine, serine, cysteine, asparagine, biotin, and the purines were omitted, and arginine was added to give a final concentration of 100 μg/ml.

Preparation of cell-free extracts. Overnight cultures were diluted into fresh medium and were allowed to grow until they reached the middle of the exponential phase of growth (4 to 5 $\times$ 10^8 cells per ml). The cells were chilled and centrifuged, and the bacteria were disrupted by grinding with alumina. The disrupted cells were extracted with 0.05 M tris(hydroxymethyl)-aminomethane-chloride buffer (pH 7.5); 10 ml of buffer were used per gram (wet weight) of cells. Cell debris and alumina were removed by centrifugation at 15,000 $\times$ g for 15 min. Fractionated extracts were prepared from this supernatant fluid by streptomycin and ammonium sulfate precipitation (Pizer, 1963).

Chemical compounds and procedures. Total and inorganic phosphate were determined by the method of Bartlet (1959). Protein was measured by the method of Lowry et al. (1951). Radioactivity measurements were made by counting samples plated in stainless-steel planchets in a windowless flow counter (Nuclear-Chicago Corp., Des Plaines, Ill.). All samples were counted to 2,000 counts. C^{14}-phosphoglycerate was a gift from N. Pon. L-Serine-phosphate was synthesized by the method of Neuhous and Korkes (1958). All other materials were purchased from Calbiochem.

Enzyme assays. The capacity to synthesize serine-phosphate was measured by determining the C^{14}-serine-phosphate formed from C^{14}-phosphoglycerate. The details of this assay were previously published (Pizer, 1963). With the unfractionated extracts used in this study, duplicate assays agreed to within 10%, and the enzymatic activity in different extracts prepared under similar growth conditions varied from 15 to 20%. A unit of enzyme activity is defined as that amount of enzyme which catalyses the formation of 1 μmole of serine-phosphate per 30 min at 37 C. Enzyme assays contained a maximum of 0.3 enzyme units. Specific activities are in terms of enzyme units per milligram of protein, and are based on the average of two to five assays. L-Serine-phosphate phosphatase was assayed as described previously (Pizer, 1963). Specific activities are presented as enzyme units per milligram of protein.

Competition between exogenous amino acids (serine or glycine) and glucose and fructose. The method of isotopic competition described by Roberts et al. (1955) was utilized with the following modifications. Specific activities were used to measure the quantity of isotope incorporated into the compounds of interest. A uracil-requiring strain, Wc^-, was used instead of the wild-type organism to avoid the incorporation of glucose carbon into the pyrimidine ring. The precision of measuring the radioactivity in the methyl group of thymine was thereby improved. The methyl group of thymine was selected to represent "one carbon" units because of technical advantages offered in purification and measurement of specific activity.

The cells were grown as described previously with C^{12}-glucose or C^{12}-fructose to a Klett reading of about 60. They were then chilled, centrifuged, and washed with salts solution. After resuspension, samples were added to growth flasks containing medium at 37 C. The medium in each flask contained uracil (0.1 mg/ml) and uniformly labeled radioactive glucose or fructose (1 mg/ml) with specific activities of 36 $\times$ 10^3 counts per min per μmole. Flasks A and D had no further additions and served as control cultures. Flasks B and E had 84 mg of L-serine added, and flasks C and F had 60 mg of glycine added. The final volume of medium in each of the flasks was 200 ml. The initial Klett reading was 60; growth was stopped by chilling the cultures when the Klett reading reached 130. From the growth studies data, it was estimated that the serine added to flasks B and E was six times the quantity used by the cells during their doubling in mass. The procedure used for the fractionation of the cells into acid-soluble, lipid, nucleic acids, and protein has been described (Roberts et al., 1955).

The protein residues were hydrolyzed with 6 N HCl for 16 hr at 105 C. After removal of the HCl by placing the samples in a vacuum desic-

cator over KOH, the dinitrophenyl (DNP) derivatives of the amino acids were prepared. Samples of these DNP derivatives were separated by two-dimensional paper chromatography. The solvent for the first dimension, butanol-ammonia (Smith, 1958), was allowed to drip off the paper until the serine and glycine derivatives had moved about halfway down the paper. The solvent for the second dimension was 0.75 M potassium phosphate, pH 6.0 (Smith, 1958). The concentrations of the eluted DNP-amino acids were determined from their absorbancies.

The bases were liberated from the nucleic acids by hydrolysis in 6 N HCl for 3 hr at 105 C. These bases were separated and purified by paper chromatography. The spectral properties of the compounds eluted from the chromatographs were used to determine their purity and concentration. The chromatography involved the sequential use of the following solvents: isopropanol-HCl, *n*-butanol-ammonia, and isobutyrate-ammonia, for the purification of adenine, thymine, and uracil. The specific activities of the adenine and thymine determined after the isopropanol chromatography did not alter after chromatography in the *n*-butanol and the isobutyrate solvents (Smith, 1958). The specific activity of the uracil decreased after each chromatographing, indicating a radioactive impurity in this compound. The pyrimidines were then chromatographed with an ethyl acetate-formate solvent. The specific activity of the thymine did not alter, and the radioactivity of the uracil samples dropped to background.

For purification of guanine, use was made of its low solubility in water. The regions of the isopropanol-HCl chromatographs which contained the guanine were first eluted with a small volume of water. These eluates contained radioactive materials which contaminated the guanine. The guanine was then eluted from each of the papers with a larger volume of 0.01 N HCl, and the specific activities of these solutions were determined.

Results

Growth studies. These experiments were designed to provide information on the serine requirements for growth, and the compounds which replace serine. Glucose was used as the carbon source.

The growth of *E. coli* strain Ws⁻ was directly dependent on the quantity of serine added (0.01 to 0.15 mg/ml), although the initial rate of growth was independent of this factor (Fig. 1). From the growth response to measured amounts of serine, it was estimated that 10^{10} cells in the exponential phase of growth utilized approximately 23 μmoles of the amino acid.

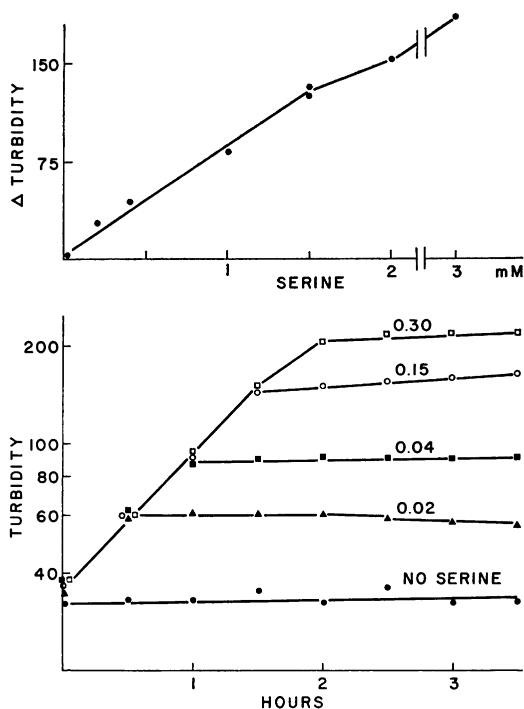

FIG. 1. *Growth response of Escherichia coli strain Ws⁻ to limiting L-serine. In the lower part of the figure the change in turbidity with time is shown for cultures supplied with different amounts of L-serine. The numbers on the curves give the initial L-serine concentration in mg/ml. The upper part of the figure shows a plot of the maximal change in turbidity against initial L-serine concentration. These data were obtained from the curves below.*

A number of compounds were tested to see whether they could replace the serine requirement of strain Ws⁻. Glycine was active but it was not used as well as serine, as indicated by the relative division times. The division time was approximately 50 min with L-serine as the growth factor, and was increased to 120 min when glycine was substituted. The glycine was present initially at 0.26 mg/ml. Doubling the glycine concentration did not increase the growth rate, but lowering the concentration reduced it.

When the culture was grown in a mixture of serine and glycine (0.04 and 0.28 mg/ml, respectively), the growth rate was initially that of a control culture containing only serine. When a Klett reading of 80 was reached, the serine was exhausted and growth stopped in the control containing only serine. In the culture initially containing both amino acids, growth continued, but now at the slower rate characteristic of a control culture growing with glycine.

These experiments show that L-serine and glycine can be interconverted, but that serine is the preferred growth factor. This preference may be one of entry into the cell or may be due to limitations in the rates of subsequent enzymatic reactions. The observation that the rate of growth with L-serine does not depend on high external concentrations, as does the rate with glycine, indicates that uptake of glycine could be limiting its ability to promote growth. Levine and Simmonds (1960), studying the serine-glycine relationships in an auxotrophic strain of K-12, showed that exogenous L-serine inhibited the uptake of C^{14}-glycine, but they did not find a diphasic character to the growth curve obtained with a mixture of L-serine and glycine.

Other compounds tested which did not support growth included the keto-acids, hydroxypyruvate, and glyoxylate, either at the concentration used for their amino acid analogues or at ten times these concentrations, and D-glycerate at ten times the normal serine concentration. *E. coli* strain Ws^- had the same growth characteristics when either fructose or lactate replaced glucose as carbon source. Growth of strain Ws^- occurred when the medium was augmented with 3 mM threonine. The rate of growth was linear rather than exponential; with either glucose, fructose, or lactate as the carbon source, the time required for the number of cells to increase from 10^8 to 2×10^8 per ml was approximately 190 min. It appears that L-threonine gives rise to L-serine at a rate which limits growth.

The rate of growth of the prototrophic strain of *E. coli* was measured in the presence and absence of exogenous serine. Glucose was the carbon source (1 mg/ml), and serine when added was at the final concentration of 0.3 mg/ml. In the absence of L-serine, the division time was 60 min, and in the presence of serine it was 50 min. The amount of growth was approximately 12% greater when L-serine had been added.

Competition experiments. The original experiments of Roberts et al. (1955) indicated that, in *E. coli* strain B, exogenous L-serine limited the endogenous synthesis of L-serine and glycine from glucose. They also observed that exogenous glycine only limited the incorporation of glucose carbon into glycine, but, when C^{14}-fructose or C^{14}-acetate were the carbon sources, glycine limited the incorporation of isotope into both L-serine and glycine. It was concluded that in glucose-grown cells L-serine is the precursor of glycine, and it was postulated that, when the cells are grown in fructose or acetate, metabolic pathways are altered so that L-serine is synthesized by a novel route. Experiments with cell-free extracts of *E. coli* strain W defined the serine pathway from glucose and showed that the initial enzyme in the pathway, phosphoglycerate dehydrogenase, is subject to feedback inhibition (Pizer, 1963). L-Serine is a more effective inhibitor than glycine by a factor of approximately 100. Competition experiments were performed with *E. coli* strain W to determine whether the feedback inhibition observed in cell-free extracts operates in growing cells, and to ascertain whether growth on fructose alters the serine-glycine relationship. These experiments were also designed to give quantitative information on the role played by L-serine and glycine in the synthesis of the purine nucleus and the methyl group of thymine (Table 1).

In the experiment with uniformly labeled C^{14}-glucose, the supply of exogenous serine reduced the formation of both serine and glycine from glucose to less than 5%. The presence of exogenous glycine reduced the conversion of glucose to serine to 48%, while 12% of the glycine in protein arose from glucose.

The specific activities of the nucleic acid bases presented in Table 1 provide information on their origin when considered in conjunction with amino acid data. In flask A where no competitor had been added, the specific activities in thymine, in which only the methyl carbon was radioactive, and the purines, in which five carbons were labeled, show that the radioactivity was 2,930 counts per min per μmole of carbon. This figure agrees within experimental error with the amino acid data. In flask B where nonradioactive serine was present to compete with the radioactive glucose, the specific activity of the thymine indicates that not more than 3% of that group was derived

TABLE 1. *Specific activities of nucleic acid bases, serine, and glycine**

Flask	Growth conditions	Serine	Glycine	Thymine	Adenine	Guanine
A	C^{14}-glucose	7,794	4,750	2,940	14,900	14,400
B	C^{14}-glucose plus C^{12}-serine	329	224	85	2,940	3,360
C	C^{14}-glucose plus C^{12}-glycine	4,020	592	1,030	5,620	5,620
D	C^{14}-fructose	7,455	4,310	2,000	12,000	—
E	C^{14}-fructose C^{12}-serine	166	121	34	2,800	—
F	C^{14}-fructose C^{12}-glycine	2,445	174	335	3,560	—

* The specific activities of the amino acids are averages of four independent determinations on the dinitrophenyl derivatives; expressed as counts per minute per micromole.

from glucose. The exogenous serine must supply 97% of the "one carbon" fragments which are used for thymine synthesis, with the remaining 3% coming most likely from the limited amount of endogenous serine. The specific activities of the purines allow for one atom of carbon to arise undiluted from the labeled glucose. Carbon-6 of the purine ring is derived from CO_2 (Roberts et al., 1955), and would be expected to have the same specific activity as does the glucose carbon. The remaining purine carbons must be unlabeled and therefore derived from the unlabeled serine. Positions two and eight are derived from one-carbon fragments, whereas positions three and four are derived from glycine. In flask C where nonradioactive glycine was present, 35% of the thymine methyl group was derived from glucose, probably via the carbon of serine. The remainder was derived from unlabeled glycine. The glycine contribution to "one carbon" units was greater than was expected from its contribution to serine. If the contributions of the purine specific activity expected from two "one carbon" fragments and one undiluted CO_2 molecule are taken into account, the radioactivity in position three and four should be 620 counts per min per μmole. This figure is in agreement with the data obtained for glycine.

In the experiment with uniformly labeled C^{14}-fructose, the specific activities in the compounds from control flask D were slightly lower than when C^{14}-glucose was used. This reflects less growth in the presence of C^{14}-fructose. When C^{12}-serine was present, the endogenous synthesis from fructose was reduced in both serine and glycine to approximately 2% of the control value. When C^{12}-glycine was present, the endogenous serine synthesis was reduced to 33% of the control and the endogenous glycine synthesis to 4% of the control; 83% of the methyl groups of thymine were derived from glycine. Exogenous serine efficiently limited the formation of both serine and glycine from glucose or fructose. Exogenous glycine, while reducing the incorporation of isotope from both carbon sources into glycine, did not limit serine synthesis from glucose as well as it did from fructose. The observation that serine can be synthesized from unlabeled glycine confirms the mutant work and indicates that glycine can readily give rise to "one carbon" units in this organism.

Effect of nutrition on enzyme levels. The enzymes responsible for L-serine biosynthesis were assayed in extracts of cells grown under different nutritional conditions. Initially, the effect of an exogenous supply of L-serine or replacement of glucose by fructose was tested. The capacity to synthesize serine-phosphate was lower in extracts from cells grown on fructose than in extracts of cells grown on glucose (Table 2). The relationship of this observation to the competition experiments described above will be discussed later.

Growth in the presence of 3 mM L-serine did not affect the enzyme levels of *E. coli* strains W, B, or K-12. Because the endogenous serine pool may repress the synthesis of these enzymes to a maximal extent, an attempt to reduce the size of this pool was made by accelerating growth in the absence of exogenous serine or its derivatives. Table 2 shows that growth rate was increased in the enriched lactate medium used but the enzymatic synthesis of serine-phosphate was lowered. Al-

TABLE 2. *Effect of nutrition on enzyme levels*

Growth conditions	Division time	Specific activity	
		Serine-phosphate formation	Serine-phosphate phosphatase
	min		
Glucose	55–60	3.04	2.3
Fructose	60	1.65	2.1
Lactate	80	1.50	1.9
Enriched lactate	40	0.50	1.7

TABLE 3. *Serine-phosphate synthesis in mixed extracts*

Extract from cells grown in		C^{14}-serine phosphate*
Glucose	Enriched lactate	
30†	—	1,980
60	—	3,900
60	—	4,200
90	—	5,220
—	60	720
—	160	1,260
60	80	4,380
60	160	4,440

* Specific activity: 17,500 counts per min per μmole.

† Expressed as micrograms of protein.

though it was not possible to relate enzyme levels to either serine supply or growth rate, this nutritional effect was investigated further. This is not a generalized effect on enzyme synthesis, because the last enzyme in the serine pathway, serine-phosphate phosphatase, possesses approximately the same specific activity in all the extracts examined. This enzyme was subsequently used as a control on the preparation of extracts.

Experiments were performed to determine whether an enzyme inhibitor was present in the extracts prepared from cells grown in enriched lactate medium. Mixtures of extracts from cells grown in glucose and enriched lactate medium were assayed for their capacity to synthesize serine-phosphate. The activity of the mixtures was compared with that in unmixed extracts (Table 3). Because the addition of an extract of cells grown in enriched medium did not reduce the enzyme activity in extracts of glucose-grown cells, enzyme inhibitors were not present in the extract of the cells grown in enriched medium. Fractionation of extracts from cells grown in the enriched medium failed to increase the specific enzymatic activity. These experiments make it unlikely that the reduced enzyme levels observed were due to readily dissociable enzyme inhibitors in the extract.

Two enzymes are responsible for the production of serine-phosphate, phosphoglycerate dehydrogenase, and serine-phosphate transaminase. Assays of these enzymes in unfractionated extracts are inaccurate and, in view of the pitfalls in comparing enzyme activity in extracts after fractionation, an indirect approach was made to the question of which enzyme limits the rate of serine-phosphate formation in the extracts of cells grown in enriched medium. A fractionated extract was prepared from *E. coli* strain Ws^- grown on glucose. This extract lacks the phosphoglycerate dehydrogenase, but has the normal amount of transaminase (Pizer, 1963). Supplementation of extracts of cells grown in enriched media with this transaminase preparation failed to increase serine-phosphate formation. It is concluded that phosphoglycerate dehydrogenase limits serine-phosphate formation.

To identify the compounds responsible for the reduction in enzyme activity, the enriched medium was modified by omitting components. Initially, several components, grouped together on the bases of chemical similarity, were omitted from the medium (Table 4). From these data, it is possible to eliminate the compounds in four of the six groups from a role in the regulation of enzyme levels because their absence from the medium did not increase enzyme activity. When either the group of aliphatic amino acids (leucine, isoleucine, valine, alanine) or methionine and threonine were omitted, the enzyme level increased, indicating that at least one amino acid from each group was responsible for the original reduction in enzyme activity. To determine specifically which of these six amino acids was involved, each was omitted in turn from a growth medium which contained the remaining five. The enzymatic activity in the extracts prepared from cells grown in these media was measured (Table 5). Alanine and valine were not involved in enzyme regulation, but the other four amino acids were required to minimize the enzyme level. When the medium was enriched with L-threonine, L-methionine, L-leucine, and DL-isoleucine, the

specific activity of serine-phosphate synthesis was 0.52, whereas it was 1.67 in the lactate-grown control. It appears that four amino acids are involved in the regulation of the biosynthesis of phosphoglycerate dehydrogenase. The lowest concentration of these amino acids which gives this effect has not been determined, but doubling the amino acid concentration failed to further reduce the enzyme levels. *E. coli* strains B and K-12 also showed this reduction in enzyme level when grown with these four amino acids.

DISCUSSION

Growth experiments with strain Ws⁻ established that the major biosynthetic pathway from glucose to L-serine is blocked as a result of the loss of phosphoglycerate dehydrogenase (Pizer, 1963). The slow growth which occurred when exogenous L-threonine was provided probably results from the conversion of threonine to glycine (Roberts et al., 1955). Endogenous threonine does not satisfy the serine requirement of strain Ws⁻, since growth did not occur when the exogenous supply of serine was exhausted. The failure of endogenous threonine to be converted to glycine probably reflects the maintenance of the threonine pool at a level too low for the functioning of the enzyme responsible. A major factor in regulating the size of the L-threonine pool is the feedback inhibition described by Wormser and Pardee (1958). Other strains would be expected to have different degrees of control and different threonine supplies, and could make more L-serine from this source. Simmonds and Miller (1957) reported that a serine-requiring strain of *E. coli* K-12 can obtain 14% of its glycine from glucose. This strain has the same enzymatic deficiency as does strain Ws⁻ (Pizer, *unpublished data*). When exogenous threonine is available, it can be concentrated in the cell to the point where its enzymatic conversion to glycine occurs.

In the absence of a secondary source of L-serine, the growth found with limited quantities of L-serine should reflect the minimal requirements for cell synthesis. Roberts et al. (1955) provided a table of the quantitative composition of *E. coli* strain B, and information on what compounds can derive their carbon from serine. Inspection of their data shows that approximately 12 μmoles of L-serine or compounds directly derived from it, approximately 9.4 μmoles of glycine or compounds derived directly from it, and approximately 11 μmoles of compounds derived from "one carbon" fragments are present in 10^{10} cells during the exponential phase of growth. Because each L-serine molecule can give rise to a glycine and a "one carbon" fragment, the total quantity of L-serine required is approximately 22 μmoles. The balance between the serine growth requirement, 23 μmoles per 10^{10} cells, and the quantity of compounds derived from it in the cell, indicates an efficient utilization of the exogenous supply of serine. Endogenously produced serine would be expected to be used with

TABLE 4. *Serine-phosphate synthesis in extracts of cells grown on modified enriched media**

Omitted compounds	Serine-phosphate synthesis†
Leucine, DL-isoleucine, L-valine, DL-alanine	0.92
L-Methionine, L-threonine	0.93
L-Histidine, L-arginine, L-lysine	0.52
L-Glutamate, L-aspartate	0.50
L-Tyrosine, L-phenylalanine, L-tryptophan, L-proline	0.53
Vitamins, uracil	0.54
None	0.50

* The enriched media described in Materials and Methods was modified by omitting the compounds indicated.

† Expressed as micromoles per milligram per 30 min.

TABLE 5. *Serine-phosphate synthesis in extracts of cells grown in amino acid-enriched media**

Omitted amino acids	Serine-phosphate synthesized†
L-Leucine	1.07
DL-Isoleucine	1.07
L-Methionine	0.77
L-Threonine	0.70
DL-Alanine	0.50
L-Valine	0.40
L-Valine, DL-alanine	0.52
All	1.67

* The medium was supplemented with the following six amino acids: L-leucine, DL-isoleucine, L-methionine, L-threonine, DL-alanine, and L-valine, at the concentrations used in the complete enriched medium.

† Expressed as micromoles per milligram per 30 min.

equal efficiency. This amino acid is of major importance as a biosynthetic intermediate, since the quantity of glucose carbon which passes through serine prior to incorporation into biosynthetic end products is approximately 15% of the total assimilated carbon. These calculations predict the absence of degradative reactions acting on L-serine. The reported failure of L-serine to induce L-serine deaminase (Pardee and Prestidge, 1955) is in agreement with this prediction.

The results of the competition experiments clearly show that the feedback inhibition of phosphoglycerate dehydrogenase by serine and glycine observed in cell-free extracts functions in the intact cell. In our experiments, specific activities of the compounds were used to measure the amount of isotope incorporated. This method offers advantages over the methods used in previous studies, because quantitative recoveries are not required and multiple determinations on each sample can be made. Previously, reliance was placed on the quantitative measurement of the material in the test sample. The use of specific activities appears particularly warranted with a compound like serine, which undergoes degradation during hydrolysis. When serine was the competitor, our results agreed with those of Roberts et al. (1955). However, when glycine was the competitor, our results differed from those reported. It was reported that when glucose was the carbon source glycine did not reduce L-serine synthesis, but did with 100% efficiency when fructose was the carbon source. Our results showed that glycine functioned as a competitor with both carbon sources, was more efficient when fructose was used, but still did not attain 100% efficiency. The ability of glycine to compete to different extents with different carbon sources may have several alternative explanations. Growth on fructose may initiate a novel biosynthetic pathway which is regulated by glycine; the enzymes made during growth on fructose may be more sensitive to feedback inhibition by glycine; or growth on fructose may lower the endogenous L-serine supply, making glycine a better competitor.

The initiation of a new pathway appears unlikely, because strain Ws$^-$, which is blocked in the pathway from phosphoglycerate to L-serine, maintains its nutritional requirement when grown on fructose. Measurements of the inhibition of the enzymes in the biosynthetic pathway by glycine showed that the phosphoglycerate dehydrogenase was inhibited to the same extent in extracts from glucose- and fructose-grown cells. The reduced levels of phosphoglycerate dehydrogenase in extracts of cells grown in fructose supports the hypothesis that growth on fructose lowers the endogenous L-serine synthesis. Other factors, such as reduction in phosphoglycerate concentration, might contribute to lowered endogenous L-serine synthesis. A direct measurement of the endogenous serine pool in cells grown under these different conditions would help answer this question.

The results of the competition experiments show that L-serine provides essentially 100% of both the glycine for purine synthesis and the "one carbon" fragments for thymine and purine synthesis. Studies with a serine-glycine auxotroph by Pitts, Stewart, and Crosbie (1961) showed that *2-C*^{14}-glycine was used for thymine and purine synthesis. The specific activities obtained indicate that one-carbon fragments were also obtained from glucose; but whether endogenous serine was their immediate precursor cannot be determined, since the specific activity of serine itself was not reported.

The observation that four amino acids not directly involved in the biosynthesis of L-serine regulate the level of phosphoglycerate dehydrogenase was unexpected and difficult to interpret in terms of a control mechanism beneficial to the cell. Possibly, this unusual regulation is connected with the role serine plays as a precursor to a large number of metabolites. The capacity for serine synthesis is reduced only when several amino acids are available to the cell and the medium is relatively rich. The other amino acids which play a major role in the synthesis of metabolic intermediates are glutamate and aspartate. Halpern and Umbarger (1960) showed that growth of *E. coli* on glutamate or casein hydrolysate reduced the level of glutamic dehydrogenase to 25 and 4%, respectively, of that found in glucose-grown cells. Whether specific components of casein hydrolysate were responsible for the reduction in the enzyme level was not investigated. It appears that the transaminase responsible for aspartate biosynthesis is also involved in the production of other amino acids (Rudman and Meister, 1953). If the transaminase levels are regulated by the cell, it is probable that several compounds are involved.

ACKNOWLEDGMENTS

We wish to thank N. Pon for the gift of C^{14}-phosphoglycerate, and S. S. Cohen and S. Simmonds for cultures of bacteria.

This investigation was supported by Public Health Service grant AI-03957.

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