EFFECT OF CULTURAL CONDITIONS ON GROWTH AND METABOLITE UPTAKE BY SERINE-GLYCINE AUXOTROPHS OF *ESCHERICHIA COLI*[1]

ELLIOT M. LEVINE[2] AND SOFIA SIMMONDS

Department of Biochemistry, Yale University, New Haven, Connecticut

Received for publication, May 3, 1962

ABSTRACT

LEVINE, ELLIOT M. (Yale University, New Haven, Conn.) AND SOFIA SIMMONDS. Effect of cultural conditions on growth and metabolite uptake by serine-glycine auxotrophs of *Escherichia coli*. J. Bacteriol. **84**:683–693. 1962.—A study was made of the effect of environmental conditions on the growth and metabolite uptake by *Escherichia coli* strain S/G (a serine-glycine auxotroph) and strain S (a serine-glycine auxotroph lacking a normal transport system for glycine). The induction in strain S of the glycine transport system, which occurs during growth in a glycine-containing medium, was prevented by L-asparagine; the "repression" appeared to result from the endogenous formation of glycine from asparagine (through homoserine and threonine) via a pathway in which serine was not an obligatory intermediate. The response of both strains to glycine appeared to be determined by the amount of L-serine or glycine present in the media (containing asparagine) in which the cells were grown prior to testing. Preliminary growth in the presence of low concentrations of exogenous glycine, which should produce cells with small endogenous "glycine-serine pools," favored the induction of a functional glycine transport system in cells of strain S. Cells of strain S/G grown under similar conditions subsequently showed an enhanced ability to take up C^{14}-labeled glycine. With both strains, preliminary growth conditions leading to the formation of large endogenous "glycine-serine pools" (e.g., growth in the presence of high concentrations of exogenous serine) produced cells exhibiting a glycine uptake rate that was not commensurate with the rate at which these cells grew on glycine. The growth response apparently depends not only on the ability of the cells to transport glycine but also on the size of the endogenous "glycine-serine pools"; inocula composed of cells having a limited capacity to accumulate labeled glycine showed significant growth in glycine-containing media if the cells had large "glycine-serine pools." The growth and uptake responses to glycylglycine appear to be governed, in part, by the same factors that control the responses to free glycine.

The phenotypic expression of genetically controlled blocks in microbial metabolism is governed largely by cultural conditions (Wagner and Mitchell, 1955). In bacterial mutants lacking constitutive mechanisms for the uptake of metabolites from the medium into the cell, the genetic deficiency often can be circumvented by changes in the composition of the culture medium. For example, the lack of an adequate transport system may be overcome by greatly increasing the exogenous concentration of the metabolite in question, which then may enter the cell by "free diffusion" rather than as a result of "active uptake" (Cohen and Monod, 1957; Levine and Simmonds, 1960).

The environmental conditions under which the cells are grown prior to testing also may play an important role in determining their uptake and growth characteristics. Thus, the growth response of "cryptic" galactose auxotrophs can be improved by preliminary growth on galactose, and such induction of "galactoside permease" is

[1] Some of the data in this paper are taken from a dissertation presented by Elliot M. Levine to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy.

[2] National Science Foundation Summer Graduate Teaching Assistantship Fellow, 1959; Predoctoral Research Fellow (EF-8532) of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, 1959 to 1961. Present address: Laboratory of Pharmacology and Toxicology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

inhibited by glucose (Cohen and Monod, 1957). Similar induction of the uptake system for tryptophan has been observed, but this induction is not inhibited by glucose (Boezi and De Moss, 1961).

The present communication deals with the effect of "preliminary growth conditions" on the subsequent metabolite uptake and growth responses of strain S and strain S/G, two serine-glycine auxotrophs of *Escherichia coli* strain K-12 (Meinhart and Simmonds, 1955*a*). Unlike strain S/G, strain S does not grow readily on exogenous glycine but it can be induced to do so by prolonged incubation in the presence of glycine (Simmonds and Miller, 1957). Recent studies on the uptake of glycine-C^{14} by strains S/G and S (using cells harvested from a serine-containing medium) provide direct support for the view that strain S lacks the constitutive glycine transport system present in strain S/G (Levine and Simmonds, 1960). Some of the factors controlling the induction of this amino acid transport system have now been studied in greater detail.

MATERIALS AND METHODS

Test compounds. Glycine-1,2-C^{14} was purchased from the Nuclear-Chicago Corp. (Des Plaines, Ill.); glycylglycine, labeled with C^{14}-glycine in either the NH_2-terminal residue or the COOH-terminal residue, was synthesized as described previously (Levine and Simmonds, 1960). The labeled amino acid and peptides were diluted with the corresponding unlabeled compounds to the specific activities indicated for each experiment. The authors are greatly indebted to Joseph S. Fruton for the unlabeled peptide and amino acids used.

Growth experiments with small inocula. The methods used were essentially those of Simmonds and Miller (1957). After sterilization in the autoclave, each Evelyn colorimeter tube (Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.) containing 10 ml of the "basal" medium (see below) and the amino acid supplement was inoculated by the addition of approximately 10^7 bacteria in 0.1 ml of a 50% glucose solution; the inoculum cells were taken from a 24-hr slant of "complete agar" (0.5% Difco Yeast Extract, 0.3% Difco peptone, and 1.5% Difco agar). Cultures were incubated on a shaker at 33 C, and growth was followed as a function of time by absorbancy measurements (Evelyn colorimeter, filter no. 540). The basal medium contained: NH_4Cl, 5 g; NH_4NO_3, 1 g; Na_2SO_4, 2 g; K_2HPO_4, 2.86 g; KH_2PO_4, 1.19 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; L-asparagine, 1.5 g (11.4 mmoles); "trace element solution" (Horowitz and Beadle, 1943), 1.0 ml; D-glucose, 5 g; total volume, 1 liter; pH 6.7. Glucose was added (as a sterile 50% aqueous solution) after sterilization of the remainder of the medium.

Growth and accumulation experiments with large inocula. As described in detail by Levine and Simmonds (1960), inocula were "prepared" by growing cells in the basal medium supplemented with L-serine or glycine ("preliminary growth medium") and harvesting during the logarithmic phase of the growth cycle. [In a few experiments, inocula were prepared by "successive passage" through two preliminary growth media. For example, cells were grown first in a medium containing serine until the culture was in the logarithmic phase (culture S); some cells from culture S were transferred, as a small inoculum, to a fresh medium containing glycine and allowed to grow until the second culture was in the logarithmic phase (culture S-G).] The cells were resuspended in fresh basal medium (suspension absorbancy, 0.21; approximately 10^9 cells per ml), the suspension was shaken at 33 C for 30 min, and 10-ml portions were then added to Evelyn colorimeter tubes containing either 0.2 ml of water (controls) or 0.2 ml of a solution of the appropriate test compound. The incubation was continued for 3 to 4 hr, and the absorbancy changes (growth) of the resultant cultures in "uptake media" were measured at intervals of 10 to 20 min.

The same procedure was used for the uptake (i.e., accumulation) experiments with labeled test compounds; at appropriate times, 1-ml samples of the cultures in the uptake medium were removed for collection of the cells and C^{14} analysis by the "Millipore method" of Levine and Simmonds (1960). For the reasons discussed by these authors, the isotope content of cells was calculated in terms of mμmoles (mμg-atoms) of amino acid- or peptide-carbon, and is given as the C^{14} content of the cells collected by filtration of 1 ml of culture. To compare cultures growing at significantly different rates, the C^{14} content of the cells is expressed as a function of the culture absorbancy at the time of sampling;

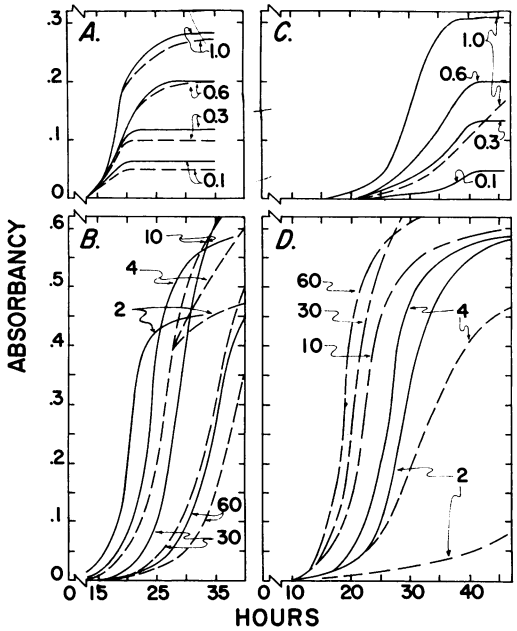

FIG. 1. *Representative growth curves for small inocula of strain S (dash lines) and strain S/G (solid lines). The basal medium was supplemented with* L-*serine (A and B) or glycine (C and D) at the initial concentrations (mM) of the amino acids indicated for each curve. In D, the curves for the two strains growing on 10, 30, and 60 mM glycine are identical.*

absorbancy in the range 0.21 to 0.31 is directly proportional to the dry weight (mg) of cells per ml of culture.

RESULTS AND DISCUSSION

Growth experiments with small inocula. In confirmation of earlier results (Meinhart and Simmonds, 1955*a*, *b*; Simmonds and Miller, 1957), it was again observed that the growth rates of both strains on serine at concentrations below 2 mM were essentially the same (Fig. 1*A*) but that the strains differed markedly in their growth rates on such low concentrations of glycine (Fig. 1*C*). This difference in response to glycine was evident at the 2 mM level, decreased at 4 mM glycine, and disappeared at levels of 10 mM or higher (Fig. 1*D*). For both strains, the lag periods in media containing 2 and 4 mM glycine (Fig. 1*D*) were longer than those characteristic of growth on equimolar concentrations of serine (Fig. 1*B*); at 10 to 60 mM, glycine appeared to stimulate the initiation of growth (Fig. 1*D*) while serine inhibited it (Fig. 1*B*). Although the reason for the bacteriostatic effect of very high serine concentrations is unknown, its presence in both strains S and S/G indicates that it probably has no bearing on their relative ability to grow on glycine.

The similarity in the growth responses of small inocula of strains S and S/G to glycine concentrations at or above 10 mM is not inconsistent with the hypothesis that strain S has a faulty glycine transport system. Large inocula of strain S, which also grow readily on high concentrations of glycine, still can not take up glycine when the amino acid is present at a low concentration, e.g., 0.3 mM (Levine and Simmonds, 1960). However, the initial rate at which glycine-C^{14} is accumulated by large inocula of strain S approaches that of strain S/G as the exogenous glycine level is raised from 0.3 to 6 mM. The ability to grow rapidly on extremely high metabolite concentrations shown by strain S resembles that shown by the "galactoside-permease" auxotroph of *E. coli* (Cohen and Monod, 1957), and probably reflects the greatly augmented role of simple diffusion in satisfying the growth requirement.

Effect of asparagine on growth response and glycine accumulation. The growth response of small inocula of strain S to low concentrations of glycine can be improved greatly by decreasing the L-asparagine in the basal medium from the usual concentration of 11.4 mM to 1.14 mM (Simmonds and Miller, 1957). Evidence has now been obtained to suggest that asparagine inhibits the initiation of growth by repressing the "induction" of a functional glycine-uptake system rather than by inhibiting the actual transport process itself. For example, a large inoculum of strain S was prepared in the usual basal medium (i.e., containing 11.4 mM asparagine) supplemented with 30 mM L-serine, and was then tested in an uptake medium devoid of asparagine and containing 0.3 mM glycine-C^{14} (specific activity, 3.78 count per min per mμmole). After 1, 2, and 5 hr, the amount of glycine-carbon in the cells from 1 ml of culture was 13, 12, and 14 mμmoles, respectively (the absorbancy of the culture did not increase significantly during the incubation period); these values are no greater than the values obtained when strain S was tested in uptake medium containing asparagine (see Fig. 3 of Levine and Simmonds, 1960). A similar experiment with strain S/G showed that glycine-carbon accumulated by cells

tested in the absence of asparagine was not significantly different from that found previously for cells exposed to asparagine. These results support the earlier suggestion (Simmonds and Miller, 1957) that the glycine transport system in strain S is "inducible" and the transport system in strain S/G is "constitutive."

It seemed possible that a product of asparagine metabolism, rather than the amide itself, might be the inhibitory compound. Because homoserine and threonine are formed from aspartic acid (Black and Wright, 1955), which should arise by deamidation of asparagine, the effects of the hydroxy amino acids were tested. Low concentrations of both compounds were found to inhibit the growth of strain S on glycine but not on serine (Table 1).

A logical explanation of this type of inhibition (i.e., repression of the formation of a functional glycine transport system) may be based on the following considerations. (i) Both strains S and S/G are blocked in the "major" pathway by which glucose-carbon is used for the biosynthesis of serine and thence of glycine, but can form some glycine from threonine which they can synthesize from glucose (Miller and Simmonds, 1957; Simmonds and Miller, 1957; Elliott and Simmonds, *unpublished data*). The latter ("minor") pathways for the endogenous synthesis of glycine from the 4-carbon amino acids and glucose of the medium does not supply sufficient glycine to support growth in the absence of exogenous glycine or serine. (ii) It is probable that the size of the endogenous "glycine pool" is very important in regulating the activity of the transport system: a very small pool might signal the need for a functional uptake system and so induce its formation *de novo* in strain S; a larger pool might obviate the necessity for an active uptake system and prevent its formation. (Alternatively, the controlling factor may be the pool size of a product of glycine metabolism.)

Under certain circumstances, the pool size may be large enough to prevent induction of the transport system but not sufficiently large to provide optimal amounts of glycine for rapid cell multiplication. Thus, because compounds like asparagine, homoserine, and threonine can serve as sources of endogenous glycine, they may, in effect, prevent the formation of the inducible glycine transport system in strain S and prolong the lag time of the growth cycle.

TABLE 1. *Growth response of strain S in various media*[a]

Supplement		$T_{0.05}$ in medium used[b]			
Compound	Concn	Glycine (1 mM)	Glycine (1.5 mM)	Serine (1 mM)	Serine (1.5 mM)
	mM	*hr*	*hr*	*hr*	*hr*
None		35[c]	28	16	17.5
L-Asparagine	11.4	81	55		14.5
	5.70	50			
	1.14	33			
DL-Homoserine	11.4	>119[d]			
	2.28	84	81	15.5	
	1.14		70		17
L-Threonine	11.4	56			18.5
	5.70	85			
	1.14	47[e]	52	13	
L-Aspartic acid	11.4	26[f]			15
	5.70	28[f]			
	1.14	35			
L-Alanine	11.4			17	
	1.14	>84[d]	>88[d]		

[a] L-Asparagine was omitted from the basal medium for these tests. Glycine and L-serine were present at the concentrations shown; in their absence, there was no visible growth.

[b] $T_{0.05}$, time required for the culture to attain an absorbancy of 0.05, is mainly a measure of the lag time prior to the onset of logarithmic growth (Simmonds and Miller, 1957).

[c] In the presence of 11.4 mM L-glutamic acid, $T_{0.05}$ was 28 hr.

[d] No visible growth at the time indicated, which is that of the last absorbancy measurement.

[e] Final absorbancy of culture was much greater than that for unsupplemented glycine; transfer of cells from final culture to fresh media indicated adaptation to growth on glycine (in the presence or absence of 11.4 mM asparagine) and to growth on threonine in the absence of both glycine and serine.

[f] Final absorbancy of culture was significantly greater than that for unsupplemented glycine; transfers to fresh media did not indicate adaptation to growth on either glycine or aspartate.

Although aspartic acid might be expected to inhibit strain S, the addition of high concentrations of aspartic acid to a glycine-containing medium (in the absence of asparagine) caused a decrease in the lag time and an increase in the extent of growth (Table 1). A high concentration of aspartic acid also stimulated growth on glycine in the presence of 11.4 mM asparagine but did not

reverse completely the inhibitory effect of the amide (e.g., the prolongation of $T_{0.05}$ was reduced by about 50% when 11.4 mM aspartic acid was present). These data suggest that exogenous aspartic acid is metabolized by a pathway different from that of exogenous asparagine, and cells exposed to high concentrations of exogenous aspartic acid probably amass much larger amounts of endogenous aspartic acid than do cells that derive their aspartic acid from exogenous asparagine. The uptake of large amounts of aspartic acid and the rapid conversion of much of the amino acid to fumaric acid (by the aspartase reaction) might account for the stimulating effect on growth. High concentrations of glutamic acid also stimulated growth on glycine in the absence of asparagine (Table 1), and glutamic acid could readily give rise to fumaric acid via the intermediate formation of α-ketoglutaric acid.

Of the other aliphatic amino acids tested (L-alanine, L-isoleucine, L-leucine, and L-valine), only alanine specifically inhibited the growth of strain S on glycine (Table 1). [The growth of strain S/G on glycine or serine was not inhibited by 11.4 mM L-asparagine, 11.4 mM L-threonine, or 1.14 mM L-alanine; 2.28 mM DL-homoserine had some inhibitory effect on the growth response both to glycine and to serine, but the prolongation of $T_{0.05}$ (from about 15 to about 26 hr) was small compared to that found with strain S in glycine-containing media (Simmonds, *unpublished data*).] The rapid deamination of alanine by strain S (Simmonds, *unpublished data*) would provide carbon which, like glucose-carbon, could be used for the endogenous synthesis of glycine. In this connection, it may be noted that the growth response of strain S to glycine is improved when the glucose (and ammonia) content of the asparagine-free basal medium is decreased (Simmonds and Miller, 1957). In terms of the hypothesis proposed above, this effect would be the result of a decreased biosynthesis of glycine within the cells.

Growth and accumulation experiments with large inocula. All our early work on metabolite accumulation had been done with cells prepared in a preliminary growth medium containing 30 mM serine (Levine and Simmonds, 1960). This study now has been extended to determine how changes in the serine concentration and replacement of serine by glycine alter the subsequent behavior of cells constituting a large inoculum.

Response of strain S to glycine. When the serine concentration in the preliminary growth medium was raised from 1 to 6 or 30 mM, the ability of the resultant inocula of strain S to grow on glycine showed a marked improvement (Fig. 2*A*, curves 1S and 6 or 30S). [Undoubtedly more serine *per se* was carried over as a "contaminant" in large inocula harvested from preliminary growth media initially containing 6 and 30 mM. The amount of the contaminant plus the endogenous pools of glycine or serine are insufficient to support culture growth because the absorbancy of control cultures (see Material and Methods) does not increase significantly (Levine and Simmonds, 1960); the presence in the uptake medium of as little as 0.1 mM serine, however, permits a rapid, but short-lived, increase in culture absorbancy.] Increasing the glycine level in the preliminary growth medium had just the reverse effect: the best growth response to low concentrations of glycine was obtained with inocula harvested from the preliminary medium containing 1 mM glycine (Fig. 1*A*, curves 1G, 2G, and 30G). This is similar to the "induced" utilization of glycine for growth observed by Simmonds and Miller (1957) in experiments with small inocula of strain S.

Further study of the induction (Fig. 2*B*) indicated that large inocula, prepared in a preliminary growth medium containing 1 mM glycine and harvested shortly after the initiation of visible growth, grew very poorly on glycine (curve G*) compared with inocula prepared in the same medium and harvested during logarithmic growth (curve G). Successive passage through preliminary media containing 1 mM glycine produced large inocula (curves G*-G and G-G) whose growth response to glycine was much improved, but still was not equal to that of strain S/G (curve S/G). [Although the dash curves G* and S in Fig. 2*B* indicate the absence of any "induction," parallel tests showed that the growth response to 1 mM glycine of a *small* inoculum of culture G* ($T_{0.05}$, as defined in Table 1, 42 hr) was much better than that of a small inoculum of culture S ($T_{0.05}$, 66 hr); both inocula grew equally well on 1 mM serine ($T_{0.05}$, 26 hr). Small inocula of culture G*-G responded equally rapidly to 1 mM glycine ($T_{0.05}$, 18 hr) and 1 mM serine ($T_{0.05}$, 17 hr), and may be considered to be "fully adapted."] Successive passage through media containing 1 mM serine did not have an induction effect (curves S and S-S). Nevertheless,

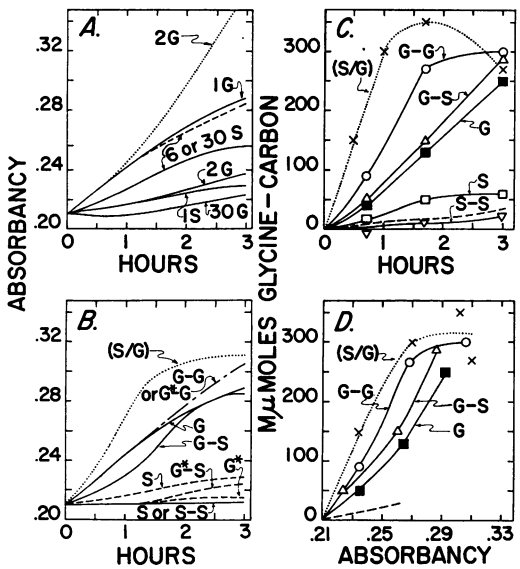

FIG. 2. *Response of large inocula of strain S to glycine. (A) Representative growth curves for uptake media containing glycine at 0.3 mM (solid lines), 1.5 mM (dash lines), and 3 mM (dotted line). Inocula prepared in preliminary growth media containing serine at 1 mM, 6 mM, or 30 mM (curves 1S and 6 or 30S) or containing glycine at 1 mM, 2 mM, or 30 mM (curves 1G, 2G, and 30G). (B) Representative growth curves for uptake media containing 0.3 mM glycine. Strain S inocula prepared in preliminary media containing 1 mM serine (S) or 1 mM glycine (G) or by successive passage through two preliminary media (see Materials and Methods): S-S, G-G, G*-G, G-S, and G*-S indicate cells obtained by growth in the presence of the first amino acid shown followed by transfer and growth in the presence of the second amino acid shown. G* designates inocula harvested from a preliminary culture when the absorbancy was 0.08; all other cultures had absorbancies of 0.16 to 0.20 at the time of harvesting or transfer to fresh preliminary media. Dash lines, experiment 1; solid lines, experiment 2. In experiment 2, the last absorbancy measurement for culture S was made at 1.5 hr. Dotted line (S/G), growth of strain S/G inoculum prepared in a medium containing 1 mM glycine. (C) Accumulation of glycine-carbon from uptake media containing 0.3 mM glycine (3.77 count per min per mμmole) by the strain S/G inoculum and strain S inocula of experiment 2 in B. Dash line, accumulation rate of strain S inoculum prepared in a medium containing 30 mM serine (Levine and Simmonds, 1960); other curves labeled to indicate inoculum source as in B. (D) Data from C replotted as function of culture absorbancy at time of sampling to correct for differences in growth rates. Curves labeled as in C. Data in C for inocula S and S-S not replotted because cultures showed no significant absorbancy changes (See B).*

once strain S became adapted to growth on glycine (curve G), the organism retained a large part of this ability after transfer and growth in a serine-containing medium (curve G-S).

Changes in the ability of strain S inocula to grow on 0.3 mM glycine were, in general, accompanied by analogous changes in their capacity to accumulate glycine-C^{14} (Fig. 2*C* and *D*). Two successive passages through the "1 mM glycine medium" produced cells whose rate of glycine-carbon accumulation (Fig. 2*D*, curve G-G) was almost equal to that of strain S/G (curve S/G). Moreover, cells prepared by growth on glycine (curve G) followed by growth on serine (curve G-S) did not lose their induced ability to accumulate glycine.

Because the C^{14}-values were relatively low, it was not possible to assess accurately the effect of raising the serine concentration of the preliminary medium from 1 mM (Fig. 2*C*, curves S and S-S) to 30 mM (dash line) on the subsequent accumulation of glycine by strain S. The data suggest that preliminary growth of inocula in the presence of 1 mM serine leads to an increased uptake of isotope per unit weight of cells, i.e., when allowance is made for the fact that the "1 mM serine-grown" inocula (in contrast to "30 mM serine-grown" inocula) did not grow during the uptake experiment.

The results of the growth and glycine accumulation studies are in accord with the hypothesis that strain S forms a functional transport system *de novo* after preliminary growth on low concentrations of exogenous glycine and that induction of the transport system is prevented by preliminary growth under conditions that lead to relatively large endogenous pools of glycine and serine. One would, therefore, expect preliminary growth in a medium containing 30 mM serine to produce cells that lack the capacity to take up glycine and, consequently, that fail to grow in the presence of the amino acid. Although such cells showed little ability to accumulate glycine-carbon (Fig. 2*D*, dash line), they were capable of growing slowly in the uptake medium (Fig. 2*A*, solid curve 30S). The apparent inconsistency between the relatively good growth response of inocula prepared on 30 mM serine and the poor

response of inocula prepared on 1 mM serine (Fig. 2*B*, curves S and S-S) can be explained if one assumes that, during the preliminary growth period and at the time of harvesting, the cells in the medium containing 30 mM serine have developed very large endogenous pools of glycine and serine. [The endogenous "glycine-serine pools" in such cells can be fed both by the "minor" pathway of glycine biosynthesis (from exogenous glucose or asparagine) and by the endogenous formation of glycine from exogenous serine.] Upon resuspension in the basal medium (containing asparagine and glucose) prior to the addition of glycine, the size of the pool is maintained by the reactions of the "minor" pathway of glycine synthesis. It is assumed also that cells become "adjusted" during preliminary growth to a metabolic state characterized by a given pool size and, after resuspension in a different type of medium, do not "readjust" to a different metabolic pattern with a different pool size during either the 30-min period prior to the addition of glycine to the basal medium or the subsequent 3-hr "uptake" period. (In most cases, the extent of cell multiplication during the uptake period is limited to one cell division by the low concentration of exogenous glycine or by the slow growth rate of the culture.) This pool is not large enough to support cell multiplication but, when glycine is added to the uptake medium, the small amount of amino acid entering the cells apparently is sufficient to raise (and maintain) the level of intracellular glycine to that required for the culture growth. On the other hand, cells prepared in a medium containing 1 mM serine would not have such a large unlabeled "glycine-serine pool." This difference in pool size probably explains why the nongrowing cultures S and S-S (Fig. 2*C*) showed a greater isotope accumulation per unit weight of cells than that found for the growing cultures of cells prepared on 30 mM serine (Fig. 2*C*, dash line). The observation that preliminary growth in the presence of 1 mM serine (as opposed to 1 mM glycine) did not lead to the formation of an active uptake system for glycine in strain S suggests that the presence of exogenous glycine is required for the induction of its transport system in this strain. A similar requirement for exogenous "substrate" is suggested by the data describing the metabolism of citrate by an *Aerobacter* auxotroph in which the endogenous formation of citrate apparently does not induce

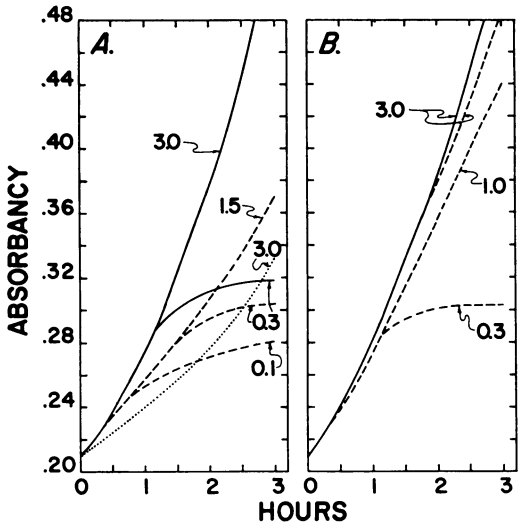

FIG. 3. *Representative growth curves for large inocula of strain S/G in uptake media containing glycine at concentrations (mM) indicated for each growth curve. (A) Inocula prepared in preliminary growth media containing* L-*serine at 30 mM (solid lines), 6 mM (dash lines), or 2 mM (dotted line). (B) Inocula prepared in media containing glycine at 30 mM (solid line) or 1 mM (dash lines). Solid line identical for initial 2-hr period to solid line "3.0" in A showing growth of inoculum prepared in medium containing 30 mM serine.*

the formation of the citrate transport system initially absent from cells grown on glucose (Davis, 1956).

The apparent failure to "deadapt" (i.e., produce a less active glycine transport system) by passage of a partially "adapted" strain S (Fig. 2*D*, curve G) through a serine-containing medium (curve G-S) is not entirely unexpected. Growth tests with small inocula indicate that two or more complete growth cycles in a serine-containing medium often are required for "deadaptation" (Simmonds and Miller, 1957; Simmonds and Levine, *unpublished data*). In experiment 2 of Fig. 2, cells from the preliminary culture used as the source of inoculum G were transferred and allowed to grow in serine-containing medium only until the resultant culture reached the logarithmic phase of the growth cycle before the response to glycine was again tested (curve G-S).

Response of strain S/G to glycine. As the serine concentration of the preliminary growth medium was raised from 2 to 30 mM, the resultant cells of strain S/G showed increasingly greater ability to

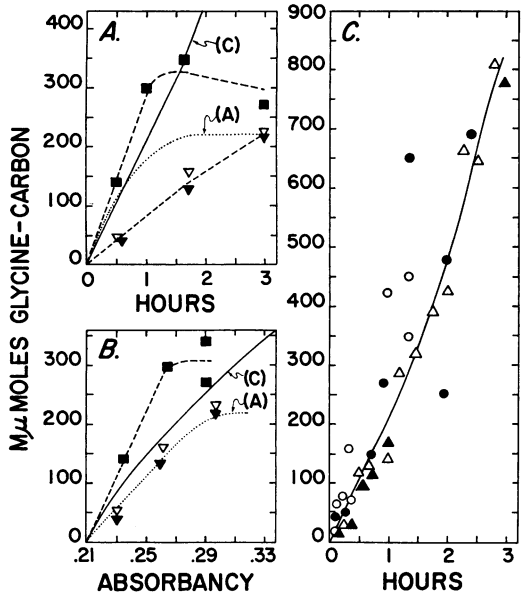

FIG. 4. *Accumulation of glycine-carbon by large inocula of strain S/G. (A) Accumulation from uptake medium containing 0.3 mM glycine-C¹⁴. Inocula prepared in preliminary growth media containing 1 mM glycine (■), 6 mM serine (▽ and ▼), or 30 mM serine (dotted curve A, data of Levine and Simmonds, 1960). Solid curve (C) reproduced from C (see below). (B) Data from A replotted as function of culture absorbancy at the time of sampling to correct for differences in growth rates (see Fig. 3); curves labeled and symbols used as in A. (C) Accumulation from uptake medium containing 3 mM glycine-C¹⁴. Inocula prepared in preliminary media containing 30 mM serine (△ and ▲) or 30 mM glycine (○ and ●); the two types of inocula grew at approximately the same rate. Specific activities of glycine-C¹⁴ used (count per min per mμmole): 4.27 (▽ and ▼); 4.45 (■); 6.67 (△ and ○); 13.3 (●); and 20.0 (▼).*

grow on glycine (Fig. 3*A*). The accumulation of glycine-carbon by inocula prepared in media containing 30 mM serine (Fig. 4*A*, curve A) also appeared to be faster than that by inocula prepared in media with 6 mM serine (triangles). However, when the uptake data were corrected for the differences in growth rates, no difference was detectable in the rates at which the two types of inocula accumulated glycine-carbon (Fig. 4*B*, curve A and triangles).

On the basis of the hypothesis discussed earlier in relation to the metabolism of strain S, strain S/G cells prepared in a medium containing 30 mM serine would have a larger endogenous "glycine-serine pool" than would cells prepared in a medium containing 6 mM serine; because the "30 mM serine-grown" cells would maintain their larger pool size during the subsequent growth and accumulation tests, they should, and did, show a faster rate of growth on glycine. One would expect that the "30 mM serine-grown" cells also should have a somewhat slower rate of glycine-carbon accumulation as a result of the larger amount of unlabeled glycine supplied from the "pool." Such a difference in C^{14}-accumulation was not evident. It should be noted, however, that the difference in growth rate was relatively small, and it may be calculated that the methods used were not sufficiently sensitive to detect the effect of this small difference on the glycine-C^{14} uptake rate.

The metabolism of strain S/G cells harvested from preliminary growth media containing 30 mM glycine also seemed to be "adjusted" to the maintenance of large endogenous pools of glycine and serine. Thus, preliminary growth in a medium containing 30 mM glycine produced cells whose initial growth response to glycine (Fig. 3*B*, solid curve) and transport system for glycine (Fig. 4*C*) appeared to be indistinguishable from those of cells prepared in the 30 mM serine medium.

Lowering the glycine concentration of the preliminary growth medium from 30 to 1 mM did not alter the subsequent growth response of large inocula to glycine (Fig. 3*B*). It may, however, cause a rise in rate at which glycine-carbon is accumulated by the cells, since the "1 mM glycine-grown" cells took up C^{14} present in the uptake medium as 0.3 mM glycine-C^{14} (Fig. 4*B*, dash line) at a faster rate than the "30 mM glycine-grown" cells took up C^{14} present in the uptake medium as 3 mM glycine-C^{14} (Fig. 4*B*, curve C). If these data also reflect the difference in the accumulation rates of "1 mM glycine-grown" cells and "30 mM glycine-grown" cells in uptake medium containing the same concentration of glycine-C^{14}, then preliminary growth media that have a low concentration of glycine produce cells with a more efficient glycine transport system than that of cells prepared in media containing a high concentration of glycine, and also than that of cells prepared in media containing serine at 30 mM (Fig. 4*B*, curves C and A) or at 6 mM (triangles). Thus, the activity of the constitutive transport system in strain S/G is influenced by the same factors that govern the formation of the

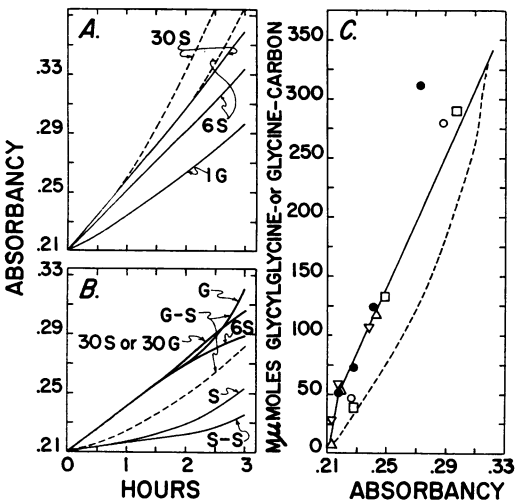

FIG. 5. *Response of large inocula to glycylglycine. (A) Representative growth curves for strain S/G in uptake media containing peptide at 1.5 mM (dash lines) and 0.3 mM (solid lines). Inocula prepared in preliminary growth media containing serine at 30 mM (curves 30S) or 6 mM (curves 6S) or glycine at 1 mM (curve 1G). (B) Representative growth curves for strain S in uptake media containing peptide at 0.3 mM (solid lines) and 0.15 mM (dash line). Inocula prepared in preliminary media containing glycine at 30 mM (30G) or 1 mM (G) or containing serine at 30 mM (30S), 6 mM (6S), or 1 mM (S), and inocula prepared by successive passage through two preliminary media containing 1 mM serine or 1 mM glycine as described for Fig. 2B. (Inocula for curves G, S, S-S, and G-S were from same preparations used in experiment 2 of Fig. 2B). (C) Accumulation of peptide-carbon from uptake media containing 0.3 mM labeled glyclyglycine. Points refer to strain S/G inoculum prepared in medium containing 1 mM glycine (●) and to strain S inocula prepared in media containing 1 mM glycine (○) or 1 mM serine (△) or by successive passage through such media as described for B above and designated there as S-S (▽) and G-S (□). Curves show rates of accumulation by inocula of strain S/G (solid line) and S (dash line) prepared in media containing 30 mM serine (Levine and Simmonds, 1960). Specific activities of dipeptides used: ●, 8.05 count per min per mμmole of glycyl(glycine-C^{14}); all other points, 6.54 count per min per mμmole of (glyceryl-C^{14})glycine.*

inducible transport system in strain S. Moreover, the growth responses to glycine by the various types of inocula of strain S/G and strain S suggest that the growth of these strains also is governed by the same factors (i.e., the relative activity of the "minor" pathway and size of the glycine pool to which the cells have become "adjusted" during the preliminary growth period, and the activity of the glycine transport system).

Response of strains S and S/G to glycylglycine. As reported by Levine and Simmonds (1960), neither strain suffers from an impaired glycylglycine transport system. Furthermore, the dipeptide is accumulated by a mechanism different from that which transports free glycine (Levine and Simmonds, 1960; Levine, 1961). Nevertheless, variations in the preliminary growth medium affect the subsequent response of large inocula of strains S and S/G to glycylglycine as well as to glycine.

Inocula of strain S/G showed faster growth rates on the dipeptide as the serine concentration of the preliminary growth medium was raised from 6 mM (Fig. 5*A*, curves 6S) to 30 mM (curves 30S). An analogous effect was found for strain S when the serine concentration of the preliminary medium was raised from 1 mM (Fig. 5*B*, curve S) to 6 mM (curve 6S), but further increase in the serine concentration did not produce inocula showing a faster initial rate of growth on glycylglycine (curve 30S). The growth on the dipeptide by inocula of strain S harvested from media containing 30 mM glycine was identical with that of inocula from the 30 mM serine medium (Fig. 5*B*, curve 30S or 30G). Thus, all preliminary growth media favoring the production of a large endogenous "glycine pool" produced cells of both strains S and S/G that grew rapidly on the dipeptide as well as on free glycine. This was to be expected since growth on the dipeptide involves its hydrolysis to glycine as the first step in its metabolism (Levine and Simmonds, 1960).

The growth tests also provided data suggesting that the observed response to glycylglycine involves the activity of the glycine transport system in addition to the synthetic activity of the "minor" pathway. For example, with strain S (Fig. 5*B*), cells prepared in a medium containing 1 mM glycine (which were "adapted" to grow on glycine and had a relatively efficient transport system but produced relatively little glycine by the "minor" pathway) grew on glycylglycine at the same rate as cells prepared on 30 mM glycine or serine (which lacked the ability to take up glycine rapidly but had large endogenous "glycine pools"). Likewise, the

"unadapted" cells of strain S prepared in the medium with 30 mM serine (Fig. 5*B*) grew on the dipeptide more slowly than did strain S/G cells (Fig. 5*A*) prepared in the same medium and possessing an adequate transport system for glycine. Furthermore, inocula of both strains prepared in media containing 1 mM glycine had the ability to transport glycine, and such inocula grew at the same rate in the presence of glycylglycine (compare curve 1G, Fig. 5*A*, and curve G, Fig. 5*B*). It should be noted, however, that, with strain S/G, inocula prepared in media containing 1 mM glycine (Fig. 5*A*, curve 1G) grew on the dipeptide more slowly than inocula prepared on 30 mM serine (curve 30S); presumably, the rate of growth depends less on the enhanced activity of the glycine transport system characteristic of the "1 mM glycine-grown" cells than it does on the extra glycine formed via the "minor" pathway in the cells with large "glycine-serine pools."

Although it seems unlikely that the transport system for glycine plays a direct role in the growth response to glycylglycine, preliminary data suggest that the rapid endogenous hydrolysis of glycylglycine to glycine leads to a loss or "leakage" of glycine from cells to the external medium (*see also* Levine and Simmonds, 1960). Consequently, the improvement in glycine transport will result in an enhancement in the over-all rate of growth on the dipeptide. A similar enhancement in the rate of accumulation of dipeptide-carbon also should result.

The rates of accumulation of carbon from glycylglycine were approximately the same for most of the inocula tested (Fig. 5*C*); the exceptions were (i) the strain S/G inoculum harvested from a medium containing 1 mM glycine, which appeared to maintain a very rapid accumulation rate for a longer time than other types of cells (solid circles), and (ii) the strain S inoculum harvested from a medium containing 30 mM serine, which accumulated peptide-carbon at a much slower initial rate (dash line). In the "1 mM glycine-grown" inoculum of strain S/G, the prolonged rapid rate of accumulation can be explained by an increased ability to transport glycine; in the "30 mM serine-grown" inoculum of strain S, the decreased accumulation of peptide-carbon per unit weight of cells would result from the large pool of unlabeled glycine contributed by the "minor" pathway coupled with the absence of a functional glycine transport system. Nevertheless, a comparison of the relatively good growth and uptake responses of strain S cells prepared on 1 mM serine to glycylglycine with the poor responses of such cells to glycine indicates that the role of the glycine transport system in the utilization of the dipeptide is, indeed, an indirect one.

It may also be noted that results obtained in a study of the response of strain S/G to L-serine (Levine and Simmonds, *in preparation*) are interpretable by a scheme analogous to that proposed for response of this strain to glycine. This strain grows readily on serine and has a constitutive uptake system for the amino acid (Levine and Simmonds, 1960), but variations in the preliminary growth medium seem to affect the subsequent response of the cells to serine. For example, cells prepared in preliminary growth media containing either 1 mM glycine or 30 mM serine grow on 0.3 mM serine at the same rate, but the cells prepared on 30 mM serine accumulate serine-carbon at a somewhat slower rate. The rapid growth of the cells prepared in the "high" serine medium apparently reflects the presence of a relatively large endogenous pool of serine and glycine, which is produced during the preliminary growth period and subsequently is maintained to a large extent by the endogenous production of serine from the glycine formed via the "minor" pathway from exogenous glucose and asparagine.

The data describing the response of strain S/G to serine reinforce the conclusions based on the experiments with glycine and glycylglycine. In sum, the observations illustrate the relatively greater importance of the intracellular environment of bacteria, as opposed to the extracellular environment, in the regulation of the metabolism of the cells.

ACKNOWLEDGMENT

This study was supported by a research grant (G-7586) from the National Science Foundation.

LITERATURE CITED

BLACK, S., AND N. G. WRIGHT. 1955. Intermediate steps in the biosynthesis of threonine, p. 591–600. *In* W. D. McElroy and H. B. Glass [ed.], A symposium on amino acid metabolism. The Johns Hopkins Press, Baltimore.

BOEZI, J. A., AND R. D. DE MOSS. 1961. Properties of a tryptophan transport system in

Escherichia coli. Biochim. et Biophys. Acta **49**:471–484.

COHEN, G. N., AND J. MONOD. 1957. Bacterial permeases. Bacteriol. Rev. **21**:169–194.

DAVIS, B. D. 1956. Relations between enzymes and permeability (membrane transport) in bacteria, p. 509–522. *In* O. H. Gaebler [ed.], Enzymes: units of biological structure and function. Academic Press, Inc., New York.

HOROWITZ, N. H., AND G. W. BEADLE. 1943. A microbiological method for the determination of choline by use of a mutant of *Neurospora*. J. Biol. Chem. **150**:325–333.

LEVINE, E. M. 1961 Metabolite uptake by serine-glycine auxotrophs of *Escherichia coli*. Thesis, Yale University, New Haven, Conn.

LEVINE, E. M., AND S. SIMMONDS. 1960. Metabolite uptake by serine-glycine auxotrophs of *Escherichia coli*. J. Biol. Chem. **235**:2902–2909.

MEINHART, J. O., AND S. SIMMONDS. 1955*a*. Serine metabolism in a mutant strain of *Escherichia coli* strain K-12. J. Biol. Chem. **213**:329–341.

MEINHART, J. O., AND S. SIMMONDS. 1955*b*. Metabolism of serine and glycine peptides by mutants of *Escherichia coli* strain K-12. J. Biol. Chem. **216**:51–65.

MILLER, D. A., AND S. SIMMONDS. 1957. The metabolism of L-threonine and glycine by *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **43**:195–199.

SIMMONDS, S., AND D. A. MILLER. 1957. Metabolism of glycine and serine in *Escherichia coli*. J. Bacteriol. **74**:775–783.

WAGNER, R. P., AND H. K. MITCHELL. 1955. Genetics and metabolism, p. 289. John Wiley & Sons, Inc., New York.