STUDIES ON THE CONTROL OF SERINE TRANSHYDROXYMETHYLASE ACTIVITY IN *ESCHERICHIA COLI*

by

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ABSTRACT

Variations in serine tranhydroxymethylase (STHM) activity as a function of the addition of certain nutrilites to the growth media of cells of Escherichia coli have been studied. STHM levels of activity are decreased (1) by high concentrations of glycine and (2) by a mixture of C_1 derived metabolites (histidine, methionine, thymidine, adenine and purines), each at usual moderate levels. Glycine at low concentrations had no effect but did increase the effect of the C_1 derived metabolites. It is concluded that the level of STHM activity is governed by the level of intracellular glycine. Concentrations of a C_1 intermediate may also be of importance. Implications of this finding in terms of C_1 metabolites in the management of the metabolic disease hyperglycinemia, have been briefly discussed.

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INTRODUCTION

Two pathways of serine biosynthesis are known, a phosphorylated and a non-phosphorylated pathway. Of these only the former appears to exist in Escherichia coli. Various reactions leading to the formation of glycine are also known, but in E. coli only one is of quantitative importance - the conversion of serine to glycine with the release of the β carbon atom of serine as a one-carbon fragment.

Glycine and serine are freely interconvertible both enzymatically and physiologically. An enzyme responsible for the conversion of serine to glycine, serine transhydroxymethylase (STHM), also operates in the reverse direction so that glycine can be converted to serine. Serine, glycine and the one-carbon fragment are all important in the synthesis of a number of cell constituents such as adenine, guanine, methionine, histidine or thymidine.

It is clear therefore that STHM plays a key role in a number of cellular reactions and its activity would be expected to be carefully controlled. This study is addressed to the elucidation of factors controlling the concentration of STHM in the cell with a view ultimately of defining the role of the enzyme in the provision of serine, glycine and one-carbon units.

An understanding of the role of the enzyme also entails an understanding of the general problems of serine,

glycine and one-carbon metabolism. For this reason the relevant literature is reviewed in the introduction. Evidence for the existence of two biosynthetic routes to serine will be considered first. It will be shown that the phosphorylated pathway is the predominant pathway in mammalian and bacterial cells though the non-phosphorylated pathway has also been described in both. Instances of tissues containing enzymes of both pathways will be discussed.

Evidence that serine and glycine are closely related will be considered next. The enzyme that interconverts serine and glycine, STHM, will be discussed in detail and some of its chemical characteristics noted.

Because serine, glycine and the one-carbon fragments are involved in the biosynthesis of several compounds, and several experiments in this thesis are based on this fact, the biosyntheses of the relevant compounds are indicated briefly.

Biosynthetic routes to serine

Serine can be derived either via a phosphorylated or non-phosphorylated pathway from a 3-carbon Embden - Meyerhof intermediate. The work of Ichihara and Greenberg, (1957) with rat liver, Bridgers, (1965) with mouse brain and Pizer, (1966) with cultured human polyploid KB cells and guinea pig liver cells provides evidence that in some

mammalian systems the phosphorylated pathway is the major route to serine. This pathway results in the enzymatic conversion of 3-phosphoglyceraldehyde to serine via an oxidation to phosphohydroxypyruvic acid and a transamination step between phosphohydroxypyruvic acid and glutamate to yield phosphoserine. Phosphoserine is finally dephosphorylated by a specific phosphatase (Equations 1-3).

$$\text{D-3-Phosphoglycerate} + NAD^+ \rightleftharpoons \text{3-Phosphohydroxypyruvic acid} + NADH + H^+ \quad (1)$$

$$\text{3-Phosphohydroxypyruvic acid} + \text{L-Glutamate} \rightleftharpoons \text{L-Phosphoserine} \quad (2)$$

$$\text{L-Phosphoserine} + H_2O \longrightarrow \text{L-Serine} + P_i \quad (3)$$

The phosphorylated pathway is also the major route to serine biosynthesis in microorganisms. In Salmonella typhimurium, Umbarger and Umbarger, (1962), Umbarger et al., (1963), Escherichia coli, Pizer, (1963), Pizer and Potochny, (1964), Umbarger et al., (1963) and Haemophilus influenzae, Pizer et al., (1969), at least 2 of the 3 enzymes, the transaminase and the phosphatase have been identified. From these bacterial systems many mutants unable to grow without serine or glycine have been isolated. This auxotrophy can be induced from a single mutational step and provides evidence that there can be only one significant pathway for the

biosynthesis of serine from a carbon source such as glucose.

On the other hand a non-phosphorylated pathway has also been described. Sallach (1956) and Willis and Sallach (1962) established that in beef liver, glycerate can be converted to serine via hydroxypyruvate. The reaction involves a transamination step between hydroxypyruvate and alanine (Equations 4-5).

D-Glycerate + NAD+ $\rightleftharpoons$ hydroxypyruvate + NADH (4)

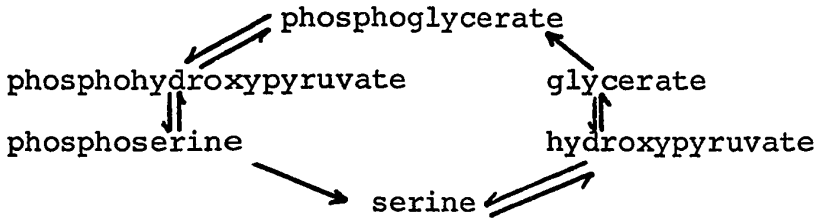
Hydroxypyruvate + L-Alanine $\rightleftharpoons$ L-Serine (5)

The same conversion of carbohydrate to glycerate, to hydroxypyruvate and eventually to serine was also shown in the bacterium Acetobacter suboxydans (Rohr and Chiari, 1964).

Umbarger, Umbarger and Siu (1963) reported that both pathways may function for serine formation in animal tissue. Furthermore while some tissues possess predominantly either the enzymes of the phosphorylated route or those of the non-phosphorylated one, other tissues are characterized by substantial levels of activity of all the enzymes of both pathways. Other studies have shown that both routes of serine biosynthesis are functioning in crude extracts of Neurospora crassa (Sojka and Garner, 1967). In this case the enzymes of the phosphorylated pathway are more active in glucose grown cells but the relative activities of the two pathways can be altered by changing the carbon source to fructose. Similarly Johnson, Walsh and Sallach (1964)

suggested that the biosynthetic route to serine shifts from the pathway involving the phosphorylated intermediates to one utilizing non-phosphorylated intermediates during development and growth of the rat.

Hepinstall and Quayle (1970) have shown that all 3 enzymes of the phosphorylated route are present in extracts of methane- or succinate-grown Pseudomonas AM1. These results plus those reported by Large and Quayle (1963) concerning a non-phosphorylated system, suggest that in Pseudomonas AM1 there exists two metabolic routes that interconvert phosphoglyceric acid and serine (Scheme 1).



Scheme 1. Serine metabolism in Pseudomonas AM1 (Harder and Quayle, 1970).

Serine may also be synthesized from glycine which suggests that serine and glycine are metabolically closely related. Goldsworthy et al. (1949) found that following the administration of either carboxyl- or methyl-labeled glycine to rats, high concentrations of ^{14}C were found in the serine as well as in the glycine of liver protein. The serine accounted for 35 - 43% and the glycine 45% of the

total ^{14}C. The conversion was estensive and extremely rapid. Siekevitz and Greenberg (1949) have further shown that in rat liver slices the conversion can occur both aerobically and anaerobically. Tissue slices incubated with ^{14}C-formate plus unlabeled glycine synthesized serine labeled with ^{14}C in the β-carbon. Thus serine is readily made from glycine in mammalian tissues.

The same relationship was indicated in bacteria as early as 1944 when Roepke et al. found that either serine or glycine could be used for the growth of a mutant of E. coli.

Biosynthetic routes to glycine

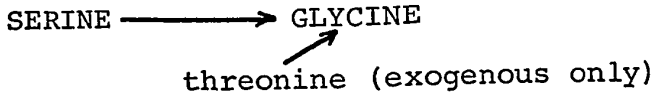
Although glycine can be made by several reactions; the cleavage of threonine (Roberts et al., 1955) (only exogenous threonine is effective in E. coli.), the breakdown of purine (Rabinowitz, 1960), the amination of glyoxylic acid (Meister, 1965), in E. coli the major pathway of glycine biosynthesis is via serine. This conversion of serine to glycine was first demonstrated by Shemin (1946), who fed serine labeled with ^{15}N in the amino group and ^{13}C in the carboxyl group to rats and guinea pigs. The isolated glycine was found to have the same $^{15}N/^{13}C$ ratio as the administered serine indicating that L-serine was converted into glycine by the splitting off of the β carbon atom. Roberts et al., (1955) also found that radioactive

glucose was not incorporated into serine or glycine when serine was given exogenously, whereas exogenously administered glycine could not prevent the incorporation of radioactive glucose into serine but only into glycine (Table 1).

Competitor added	Radioactivity of bacterial amino acids relative to control	
	Gly.	Ser.
none (control)	100	100
glycine	0	100
glyoxylic acid	100	100
threonine	50	100
serine	5	5

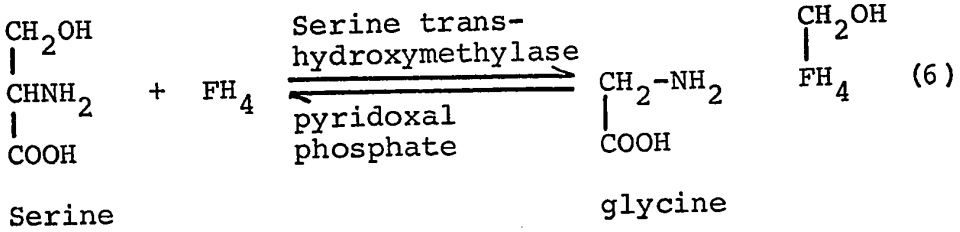
Table 1. Metabolism of radioactive glucose by E. coli: Effect of supplements on the incorporation of radioactivity into the amino acids (Roberts et al., 1955).

The results of this table can be summarized as follows:



The interconversion of serine and glycine has also been observed in intact plant tissues (Nath and McConnell 1960; Sinha and Cossins, 1964). Moreover Wilkinson and Davies (1960) have shown that extracts of plant tissues catalyze the interconversion by the same mechanism described for animal tissues. Thus it is clear that the reaction is entirely reversible. Not only can serine be synthesized from glycine but glycine can also be converted to serine.

Several investigators have described an enzyme capable of the interconversion (B. Wright, 1955; Alexander and Greenberg, 1955; Huennekens et al., 1957; Hafeti et. al., 1957 and Schirch and Mason, 1962). Alexander and Greenberg (1956) originally proposed the name serine aldolase to conform with the idea that aldolases liberate aldehydes from β-hydroxyamino acids (e.g. serine). The generally accepted name serine transhydroxymethylase (STHM) was first suggested by Kisliuk and Sakami (1955). The enzyme catalyzes the reaction shown in Equation 6:



(6)

L-serine is converted to glycine with the release of a β carbon as a C-1 fragment. That it is the β carbon which is released was described by Roberts et al., (1955) who found that serine and glycine isolated from E. coli cells grown with glucose-1- and glucose-2-^{14}C showed 50% labeling of serine from both carbon sources and of glycine from glucose-2-^{14}C,but only 5% labeling of glycine from glucose-1-^{14}C.

Various investigations were made concerning the chemical form in which the β carbon was liberated. Mitoma and Greenberg (1952) observed that neither formate nor formaldehyde but rather some derivative thereof, known as "active C1", was an effective precursor of the β carbon of serine. An involvement of folic acid was first suspected when it was shown that in folic acid deficient rats, formate was incorporated into serine at a reduced rate (Plaut et al., 1950). It was finally discovered that tetrahydrofolic acid was required as the cofactor in the interconversion (Blakely, 1954). Experimental evidence describing FH_4 in the cofactor role was provided by Kisliuk and Sakami (1954) who found that pigeon liver extracts inactivated by treatment with Dowex-1 chloride and dialyzed for 15-16 hours, were reactivated by the addition of FH_4, which was the only active factor in this system. Similar results have been reported in bacteria (Holland and Meinke, 1949). It was further shown that derivatives of folic acid may serve as

cofactors for the introduction of single carbon units into purines, pyrimidines and probably histidine (Gordon et al., 1948). The major form of this active one-carbon is of a methylene group linked to the 5th and 10th nitrogen of the pteridine residue of the coenzyme FH_4 (Figure 1).

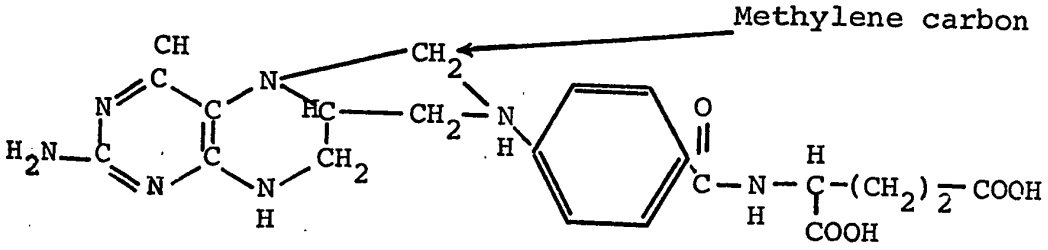


Figure 1. N^5,N^{10}-methylene tetrahydrofolic acid.

However FH_4 is not the only cofactor in the reaction. Liver homogenates from vitamin-B_6-deficient chicks gave evidence that pyridoxal phosphate was required as another cofactor(Deodhar and Sakami, 1953). These pyridoxine deficient chicks had a diminished ability to incorporate formate-^{14}C into serine. Lacelles and Woods, (1950) first observed the requirement of the B vitamin in bacteria, while studying serine synthesis in Streptococcus faecalis. Pyridoxal was needed for the optimal synthesis

of serine. Scirch and Mason, (1962) have reported that dialysis of a D-alanine - enzyme[1] solution gives an inactive enzyme, however the addition of pyridoxal phosphate to the dialyzed preparation could restore most of the activity. The compound removed by dialysis gave spectra characteristic of pyridoxamine phosphate.

Characteristics of serine transhydroxymethylase

STHM has been purified and its properties investigated both in a highly purified state and in crude extracts. A preparation of the enzyme was shown to be more than 95% pure by the criteria of starch gel electrophoresis and the ultracentrifuge. Its molecular weight was determined at 310,000 $\pm$ 8000 and its absorption maximum at 430mμ. 3.8 to 4.1 moles of pyridoxal phosphate were found bound per mole of enzyme. The site of attachment of pyridoxal phosphate appeared to be an α-amino group of a lysyl residue (Scirch and Mason, 1962).

STHM interacts with substrates other than serine and glycine. A rabbit liver preparation purified 300-400 times reacted with cysteine as shown by a decrease in the absorption peak of the enzyme from 430 to 330mμ on incubation with cysteine.

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[1] STHM has been shown in mammalian systems to form enzyme substrate complexes with glycine, serine, cysteine and D-alanine.

Schirch and Gross (1968) have further reported that STHM catalyzes the cleavage not only of L-serine, but also of L-threonine and DL-allothreonine (to glycine and acetaldehyde); the same reaction thought to be catalyzed by threonine and allothreonine aldolase respectively. The reactions are shown in Equations 7-8.

L-threonine $\rightleftharpoons$ glycine + acetaldehyde (7)

Allothreonine $\rightleftharpoons$ glycine + acetaldehyde (8)

FH_4 is not required as a cofactor. The evidence in support of a single enzyme catalyzing all three reactions includes a constant ratio of specific activities during the purification of the enzyme and the similarity of affinity constants for substrates and inhibitors. However, a mutant of E. coli known to lack STHM does appear to have threonine aldolase activity. This does not disprove the idea that STHM has threonine aldolase activity, but does indicate that a second threonine aldolase may also exist.

STHM was also partially purified from lyophilized cells of the bacterium Clostridium cylindrosporum (Uyeda and Rabinowitz, 1967). A pyridoxal free apoenzyme was prepared in the absence of cofactor. The activity of the enzyme was shown to be dependent on the addition of catalytic amounts of pyridoxal phosphate in addition to the substrates. However, no compound would substitute for l-serine in the enzyme reaction. D-serine, DL-hydroxymethyl-serine,

DL-methylserine and L-cysteine were without activity at up to 4 μ molar. No inhibition was observed with l-cysteine at 4 mM. The optimum pH was between 6.8 and 7.4. Studies with plants have shown that partially purified STHM from Nicotiana rustica has a pH optimum at 4.0. The reaction requires pyridoxal phosphate and is folic acid dependent (Prather and Sisler, 1966).

Nakano et al. (1968) found that isoenzymes of STHM occur in the soluble and mitochondrial fractions of rat liver. The same is true of ox, mouse, pig and rabbit. The soluble fraction was shown to have a sharp pH optimum at 8.0 while the mitochondrial enzyme had a broad pH optimum centering at 7.4. The mitochondrial enzyme was shown to be more stable than the soluble one. Nakano et al have also shown that there is no significant difference in the affinities of the substrates, serine and glycine, for the STHM's from both fractions. This fact and the ready reversibility of the reaction suggests that the direction of the reaction catalyzed by these isoenzymes in vivo is largely dependent on the availability of substrates in particular cell components. In a further paper Fujioka (1969) determined the molecular weights of the enzymes from the soluble fraction and the mitochondrial fraction by the sedimentation equilibrium method. These were found to be 185,000 and 170,000 respectively, both containing 4 moles of pyridoxal

phosphate per mole of enzyme. These molecular weights differ from Schirch and Mason's measurements but are in agreement with Mansouri et al., (1972) who found that the molecular weight of STHM from E. coli 113-3 was 170,000, but were not able to elucidate the significance of this difference. Fujioka (1969) attributed this difference to destruction of the enzyme by Schirch and Mason during the heat step involved in the purification method.

Biosynthesis of one-carbon (C_1) metabolites

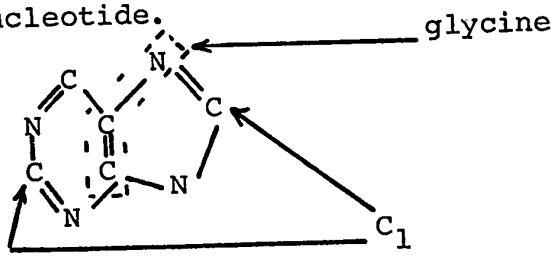
One carbon units are used for such diverse purposes in biosynthesis as the methyl group of methionine or thymidine, carbon atom 2 of the imidazole ring of histidine and the synthesis of purines. The most important source of the one carbon units is L-serine, although glycine itself may also act as a donor (glycine is broken down to NH_3, CO_2 and a C_1 fragment in the form of methylenetetrahydrofolic acid) (Sagers and Gunsalus, 1961). This will be dealt with in more detail in the discussion. Some indication of the role of serine, glycine and C_1 in these biosyntheses is given in the following paragraphs.

Biosynthesis of purine nucleotide

Purines are made from a complex series of reactions which involves the conversion of the intermediate 5-amino-4-imidazole carboxamide ribonucleotide (AICAR) to the purine

ring. The biosynthesis of the purine ring itself involves the incorporation of both glycine and one carbon groups; carbon 6 is derived from CO_2, carbon atoms 2 and 8 from C_1, and carbon atoms 4 and 5 and nitrogen 7 from an intact molecule of glycine (Buchanan and Hartman, 1959; Figure 2).

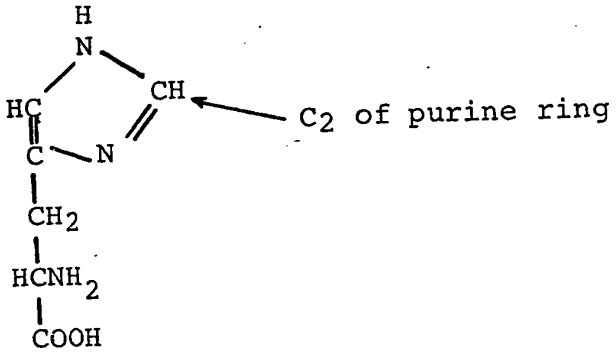
Figure 2. Purine nucleotide.



Biosynthesis of histidine

Histidine is also made from a series of complex reactions but for the purpose of this thesis what is important to note is that carbon atom 2 of the imidazole ring of histidine comes from carbon 2 of the purine ring and thus from C_1. This is shown in Figure 3.

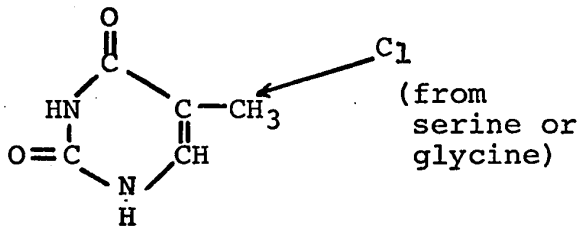
Figure 3. l-histidine.



Biosynthesis of thymidine

The biosynthesis of thymidine is accomplished by a set of reactions involving UMP, FH_4 and C_1 units. Again it is important to note only that a C_1 unit is added to the fifth carbon position of the pyrimidine ring (Reichard, 1959), and that the donor of the one-carbon methyl group is either serine or glycine (Elwyn and Sprinson, 1950, Figure 4).

Figure 4. Thymidine.



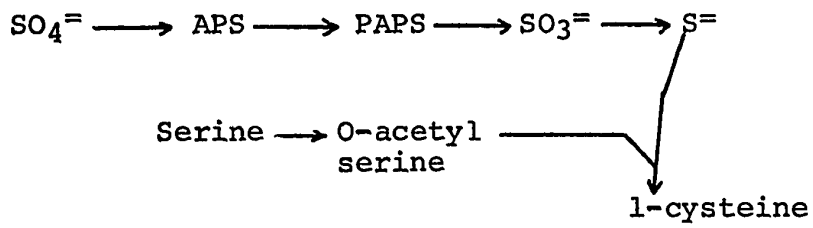
It is probable that the hydroxymethyl group from serine is first inserted into the fifth position of the ring by N10-hydroxymethyltetrahydrofolic acid and then reduced to the methyl form.

Biosynthesis of cysteine

In the enterobacteria, the biosynthesis of cysteine involves the carbon skeleton of serine and an inorganic sulfur atom. The sulfur as sulfate is activated with ATP followed by a reduction of phosphoadenosine phosphosulfate (PAPS) to sulfite and a further reduction of sulfite to sulfide. An enzyme O-acetyl serine sulfydry-

lase, catalyzes the reaction of sulfide with the serine derivative O-acetyl serine to give l-cysteine (Kredich and Tomkins, 1966). O-acetyl serine is made from acetyl-CoA and l-serine by serine transacetylase (Figure 5).

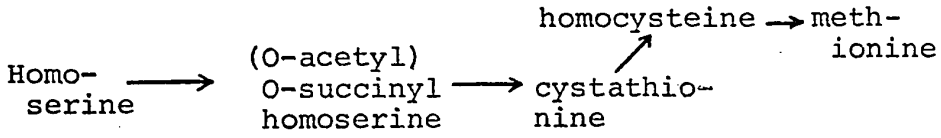
Figure 5. Biosynthesis of cysteine.



Biosynthesis of methionine

Homoserine is acylated in E. coli with succinyl-CoA to give either the acylated derivative O-succinyl- or O-acetyl homoserine. This derivative then acts with cysteine to produce the thioester cystathionine. Cystathionine in turn is hydrolyzed to homocysteine which is then methylated to give methionine (Cohen, 1968). The methyl donor is the β carbon of serine and the methyl carrier FH_4 (Figure 6).

Figure 6. Biosynthesis of methionine.



Regulation of biosynthesis

Organisms are able to control the flow of materials through the various metabolic pathways with great efficiency. This is done by altering the amount of enzyme present or by altering the efficiency of any given amount of enzyme. (Mechanisms are described in detail in the discussion of this thesis).

As a general rule a cell will not form endogenously, compounds with which it is provided exogenously. Thus Roberts *et al.* (1955) found with *E. coli* strain B that a supply of exogenous serine could inhibit the formation of serine *de novo*. The first enzyme of the pathway, phosphoglycerate dehydrogenase (PGA dehydrogenase) has been shown to be inhibited by serine and this provides a simple mechanism by which exogenous serine inhibits its own synthesis.

Various studies on the changes in the level and the activity of the enzymes of serine biosynthesis are reviewed in the following paragraphs. The discussion is then extended to possible mechanisms in the control of glycine and C_1 biosynthesis.

Control of serine biosynthesis

Control of serine biosynthesis is accomplished through the control of at least two enzymes specific to the

serine pathway. In E. coli and S. typhimurium serine was found to inhibit PGA dehydrogenase, the first enzyme in the conversion of phosphoglycerate to serine. Umbarger and Umbarger (1962) have lent support to this finding by showing that in S. typhimurium serine blocks the conversion of glucose and 3-phosphoglyceraldehyde to serine by bacterial extracts, but is unable to block the conversion of phosphohydroxypyruvate or phosphoserine to serine. In plants Slaughter and Davies, (1968) have also reported that PGA dehydrogenase from etoliated pea epicotyls is specifically inhibited by low concentrations of L-serine. There is some evidence that this dehydrogenase may be an allosteric enzyme. Sugimoto and Pizer (1968) have suggested that changes in the fluorescence emission and activation spectra of the enzyme which occur in the presence of serine, indicate that serine binding is accompanied by a conformational change in the enzyme. This is further supported by the work of Bridgers (1965) which describes the synthesis of serine from glucose in the mouse brain as stimulated by pyruvate perhaps through an allosteric effect with the dehydrogenase.

The question of control of serine biosynthesis has also been studied in animal tissues. In these cases the enzyme inhibited was not the first one but the third, phosphoserine phosphatase. Neuhaus and Byrne, (1958) have reported that while serine had no effect on the dehydrogenase reaction, it was found to inhibit

phosphoserine phosphatase activity. The phosphatase has also been shown as the point of control in human cultured cells (Pizer, 1964). Similarly Eagle et al. (1965) have described that the prior growth of cultured human cells in L-serine and glycine reduced their subsequent synthesis de novo. (i.e. one or more of the enzymes was repressed). The synthesis of glycine from serine was unaffected.

Fallon et al. (1966) have also shown that at least 2 of the serine enzymes, the PGA dehydrogenase and the phosphatase are affected in rat liver. Rats fed 2% casein diets for 7 days had a 5-20 fold increase of both enzymes as compared with rats fed a 25% casein diet. The addition of 1% cysteine to the 2% casein diet prevented the increase. Rats fed on an 88% casein diet have to depressed PGA dehydrogenase and phosphatase while the phosphoserine phosphatase was inhibited by L-serine.

In the study of the cultured human cells quoted above it was also shown that cells grown in the presence of serine had lower levels of the dehydrogenase and the phosphatase than cells grown in its absence and a lowered capacity for serine synthesis. The same experiments seen with the bacterial cell gave no evidence of repression.

Control of glycine biosynthesis

Some evidence on the control of glycine biosynthesis does exist but the situation can hardly be said to be resolved. The control of glycine biosynthesis can be approached in the same manner as that of serine biosynthesis. However, in this case only one enzyme is involved and action of the enzyme results in the formation of two products, glycine and one-carbon units. The controls might therefore be expected to be more complex.

One of the few studies dealing directly with the control of glycine biosynthesis was done by Folk and Berg (1970), who reported that mutant cells requiring glycine to grow, showed no detectable change in the level of STHM once they exhausted the exogenously provided glycine from the medium. In this case one would expect that depletion of the glycine concentration would result in derepression of STHM synthesis in an attempt to replenish the disappearing glycine pool. As a result Folk and Berg have suggested that since STHM is the centre of a number of crucial compounds (discussed previously) it may be that regulation of STHM involves additional inputs.

Using a similar approach to the problem Mansouri *et al.* (1972) have shown that methionine requiring *E. coli* 113-3 cells grown in the presence of low levels of methionine or cyanocobalamin have a 2 to 18 fold increase in specific

activity of STHM compared to extracts from cells grown in the presence of high concentrations of these compounds. No inhibition of STHM activity was observed when methionine or s-adenosylmethionine were added to the STHM assay. They have suggested therefore that the level of methionine in the growth medium may regulate the overall availability of one-carbon units. On the other hand Botsford and Parks (1969) have shown that methionine and s-adenosylmethionine are involved in the control of the formation and activity of STHM in crude cell free extracts of Saccharomyces cerevisiae (yeast). The enzyme was found to be sensitive to inhibition by both methionine and s-adenosylmethionine. L-ethionine, D-homocysteine, adenine, adenosine, D-methionine, S-adenosylhomocysteine, S-methyl-methionine and thymine failed to inhibit the enzyme consistently.

The question of end-product repression has also been looked at in bacteria and yeast. Folk and Berg (1970) observed that E. coli strain PB14a grown with high levels of glycine and serine (1 mg/ml) had its STHM activity reduced to about 1/2 the usual value. The authors considered this change to be small and stated that this did not indicate repression. The results in this thesis indicate that their result was misinterpreted. Taylor et al. (1966) have on the other hand shown that with an E. coli K12 auxotroph for L-methionine or Vitamin B_{12}, glycine alone has no

effect on the enzyme level. Yet Botsford and Park (1969) contend that yeast cells grown with 10 μM glycine have stimulated STHM levels to as much as 6 fold as compared to unsupplemented growth. It is obvious that very little has been resolved as far as the effect of glycine on the level of STHM is concerned.

Several other compounds have been tested for repressive action on STHM but again little attention has been drawn by the authors to the physiological significance of the results.

Albrecht et al. (1968) found that physiological additions of 5 and 15 μg/ml of serine were minimally repressive for STHM in the amethopterin resistant strain Streptococcus faecium var. durans A_k[1]. Similar results are presented in this thesis for an E. coli strain and their possible implications discussed.

Taylor et al. (1969) have shown a 15-30% decrease in STHM specific activity observed at all concentrations of guanosine greater than 2.5 X 10^{-4}M in a methionine-requiring E. coli strain 2276. A 40% decrease in STHM activity was noted with thymidine, glycine, guanosine and L-methionine added together. Neither histidine nor methionine alone had any effect on STHM synthesis. This particular paper will be

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[1] In this particular strain serine is the major product of glycine in glucose grown cells. The reverse is true of the E. coli strain.

discussed in detail later.

Finally Botsford and Park (1969) have also indicated that in yeast cells grown in medium supplemented with methionine, thymidine or purine, there was no appreciable effect on STHM concentration.

It appears that whetever information is available concerning the regulation of glycine metabolism in bacteria has not been synthesized. It was therefore the purpose of this study to investigate a possible regulating role for STHM in view of the fact that the reaction catalyzed by STHM not only provides serine and glycine but is also the most important source of one-carbon units necessary to synthesize a number of crucial compounds needed by the cell.

The preceeding discussion has concerned itself with two widely known mechanisms of control of enzyme activity (feedback inhibition and repression of enzyme synthesis) through evidence of others is now being reported (Holzer and Duntze, 1971).

The first of these, feedback inhibition, involves changes in the activity of a given amount of enzyme. The second, repression of synthesis, involves variations in the amount of enzyme actually present. The methods use in this thesis will detect the only variations of the second type - i.e. in the amount of enzyme actually present. Activity is

in all cases measured in extracts of cells taken from the mid-exponential phase of growth and assayed under optimal conditions with no inhibitor added. Thus the variations can only be in the number of active enzyme molecules present - i.e. due to repression and not due to feedback inhibition. However, because other controls on the number of enzyme molecules present are also known, it cannot be said with certainty that the mechanism is repression as opposed, for instance, to some chemical modification of the enzyme. Because feedback inhibition and repression are the best-known control mechanisms, they are described in detail in the discussion. The mechanism of change in activity is in any case irrelevant to the purpose of the thesis which is concerned principally with using data for analysis of the regulation of glycine and C_1 metabolism in *E. coli*.

METHODS AND MATERIALS

Synthesis of dl-Tetrahydrofolic Acid

Tetrahydrofolic acid was synthesized by a modification of the method of Davis (1968).

350 mg of 98.8% pure folic acid and 3 grams of sodium hydrosulfite were mixed for 30 minutes in an aluminum foil-wrapped flask with 90 ml of glacial acetic acid containing 3% 2-mercaptoethanol (used to prevent oxidation). During mixing, the flask was continually flushed with nitrogen (Union Carbide Canada Ltd., Toronto, Ont.). The mixture was then filtered inside a nitrogen-filled polyethylene glove chamber (I^2R, Chelttenham, Pa.) (Figure 7), into a 500-ml aluminum foil-wrapped flask with 200 ml of anhydrous ether containing 3% 2-mercaptoethanol. A whitish precipitate was formed in the ether. The flask was then removed from the polyethylene glove chamber and the following procedure was carried out under normal laboratory conditions.

The precipitate was centrifuged and washed 4 times with ether containing 3% 2-mercaptoethanol. The precipitate was then suspended in 25 ml of the ether containing 3% 2-mercaptoethanol and dried under vacuum in a pyrex brand dessicator covered with aluminum foil to keep out light.

The product was resuspended in water containing 1/10 M 2-mercaptoethanol and dissolved by adding 1N NaOH.

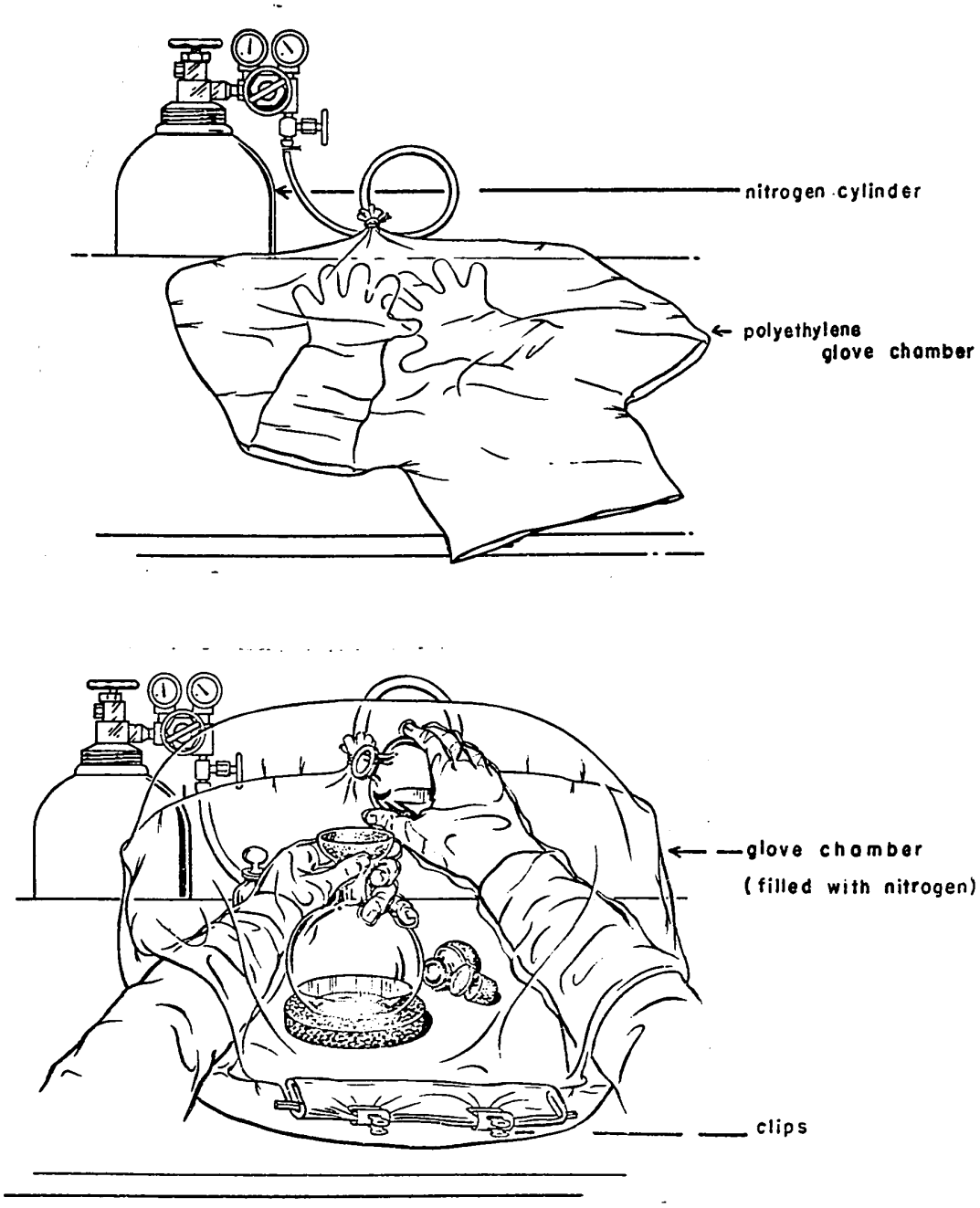


Figure 7. Diagram of a polyethylene glove chamber (reproduced from I^2R, Chelttenham, Pa.).

Convenient aliquots were then sealed under vacuum in 10 ml ampules (Wheaton Glass Co., Millville, N.J.) and stored in the dark between 0 and 4°C. These proved to be useful even after 3 months (sealed) and up to 1 hour when in contact with atmospheric O_2.

Chemical identity of the product was tested by determining the absorption spectrum in an ultraviolet recording spectrophotometer (Unicam Instruments Ltd., Cambridge, England, Model SP.800). An absorption maximum was seen between 294 and 297 mμ with a specific shoulder at 350 mμ (Figure 8). When allowed to stand in contact with the atmosphere the spectrum changed to one characteristic of dihydrofolic acid (Figure 8).

This method is identical to the Davis method except in the following respects: 3 grams of sodium hydrosulfite were used instead of 2 grams which in our system failed to reduce folic acid. 3% 2-mercaptoethanol was used instead of 2% to protect against reoxidation and careful handling of the preparation in subdued light.

Sources of Strains

Most experiments involved Escherichia coli K10, a prototrophic F+ strain of Escherichia coli K12 from the collection of E.B. Newman, who originally obtained it from A. Garen,(Dept. of Biology, Yale University, New Haven Conn.).

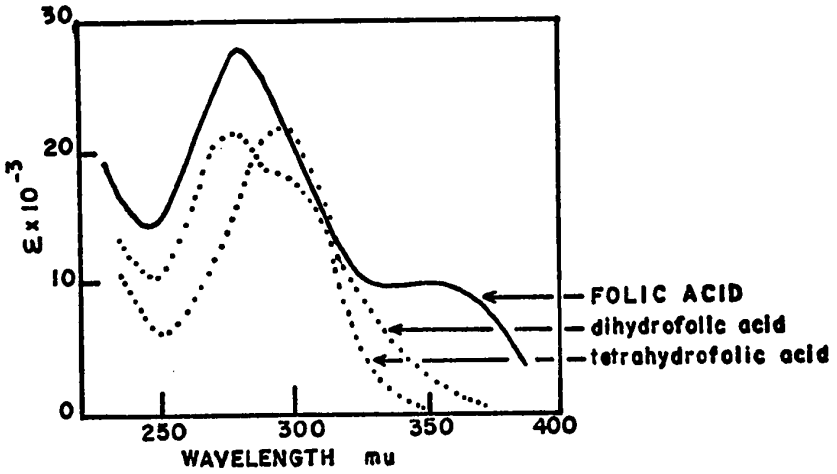


Figure 8. Absorption spectra of folic, dihydrofolic, and tetrahydrofolic acids (Stokstad and Koch, 1967).

Three auxotrophs LESV1, LESV2 and LESV3 were isolated from the above strain as described below. When grown in a glucose mineral salts medium (minimal medium) at 37°C, LESV1 showed an absolute requirement for purines, LESV2 for either serine or glycine and LESV3 for serine.

Escherichia coli JM140 was obtained from Dr. Jones-Mortimer (Dept. of Biology, University of Birmingham, England). This is a cysteine requiring auxotroph derived from E. coli 703, another prototrophic strain of E. coli K12, and known to carry a structural gene alteration at the cys P or Q locus which is responsible for the formation of sulfite reductase. A general account of this auxotroph is included in the text.

Strain AT2046, an auxotroph of E. coli K12 also came from the collection of E.B. Newman who originally acquired it from L.I. Pizer (University of Pennsylvania, Med. Sch., Phila., Pa.). This mutant lacks serine transhydroxymethylase (STHM) but is capable of growth in the presence of serine plus an amino acid or glycine. This is further characterized in the text.

All organisms were maintained in stock on slants of yeast tryptone agar at 4°C. The composition of this and other growth media are included below.

Media

Strains were grown in a liquid minimal medium containing 0.54% K_2HPO_4, 1.26% KH_2PO_4, 0.2% $(NH_4)_2SO_4$, 0.2% $MgSO_4 \cdot 7H_2O$ and 0.001% $CaCl_2$ at a pH of 6.4. All solutions were autoclaved at 15 lb/in^2 for 12 minutes. The carbon and energy sources, glucose, glycerol, succinate and sodium acetate were autoclaved separately and added to a final concentration of 0.2%. Other additions to the growth medium were made according to the requirements of the experiment as outlined in the text.

Cultures of all strains were kept by frequent transfer on slants of yeast tryptone agar containing 1% yeast extract, 1% tryptone, 2% agar and 0.25% K_2HPO_4. Glucose was added to a final concentration of 0.01%.

Plating experiments were done on simple minimal medium agar plates consisting of the basic medium described above and 2% agar. Glucose was sterilized and added separately to a final concentration of 0.2%.

Growth of Bacteria

Bacteria were grown at 37°C in a gyrotory water bath shaker (New Brunswick Scientific Co., N.J., Model G76) at approximately 180 rpm.

Cells from 8-hour yeast tryptone slant cultures grown at 37°C were inoculated into one liter Erlenmeyer flasks each containing 100 ml of fresh media and a single carbon and energy source.

All strains were grown overnight to mid-log phase. Growth in all cases is defined by an increase in the optical density. One generation equalled the time necessary for the optical density to double. Growth was followed by measuring the optical density of 2 ml portions of the cultures in a Klett-Summerson photoelectric colorimeter (Klett Mfg. Co. Inc., New York, N.Y., Model 800-3), using a blue, #42 filter (spectral range 400-465 mμ).

The cells were then chilled in ice water and salt ready to be harvested (see cell extract preparation).

Auxotrophic strains which required subculturing were grown with the appropriate supplement, harvested as described below and then resuspended in exactly the same volumes and optical density as the overnight cultures. These were then incubated for 4 hours at 37°C.

Cell Extract Preparation

400 ml lots of mid-log phase grown cells were centrifuged at 6000 rpm for 5 minutes at 0°C in a refrigerated centrifuge (International Equipment Co., Needham Hts., Mass., Model B-20) washed once with minimal medium without ammonium

sulfate and subsequently resuspended to 20% weight/volume in potassium phosphate buffer (pH 7.0) with 0.1% 2-mercaptoethanol. The yield in every case was approximately one gram wet weight. The cells were then disrupted by sonic oscillation with a 100 watt ultrasonic disintegrator (MSE Ltd., 25-28 Buckingham Gate, London S.W.1.).

The sonication was carried out as follows: The cells suspended in buffer were pipetted into a glass tube set in a glass jar packed with ice. A 1/2-inch probe was lowered well into the solution and the apparatus securely clamped. The suspension was then exposed to a voltage of 7 calibrated in microns for 90 seconds. The homogenate consisting of large debris, and a soluble fraction from the broken cells as well as unbroken cells was clarified by centrifugation at 8000 rpm for 18 minutes and the supernatant fluid collected for the enzyme assay. All assays were performed within 30 minutes of the preparation of the extract.

Isolation of Mutants

The isolation of auxotrophic mutants of *E. coli* was by the method of B.D. Davis (1949). This method is based on the fact that penicillin kills only growing cells. Wild type cultures that have been irradiated under ultraviolet light result in a mixed population consisting of a large

group of parental cells and a small number of mutants of various classes. This mixed group is allowed to divide a number of times in the presence of the growth factor for which auxotrophs are desired. The reasoning is as follows: bacteria have several copies of genetic information (i.e. several identical "chromosomes"). More often than not the mutagen has only affected one "chromosome" and the others still maintain the ability to synthesize the growth factor. Thus several divisions will allow the "chromosomes" to segregate such that daughter cells will have homogenous "chromosomes". Cells are then resuspended in a medium that lacks the specific growth factor. This ensures that any mutant requiring the additional metabolite will not be able to grow. Subsequently added penicillin will kill the prototrophs and various other mutant classes while the desired mutant which cannot grow will escape being killed. These can then be further characterized by plating on a defined agar. In principle the colonies should be largely those of the desired mutant. As an example of the general method used, the detailed technique used to select a purine requiring auxotroph is described as follows:

Cells were suspended in a 10-ml volume of sterile distilled water to an optical density of 200 , placed in a one inch deep petri dish and slowly rotated under an ultraviolet lamp (10 cm from the light source) (Universal

U.V. lamp, Gelman-Camag, Switzerland, Model 51402) at 250 mμ for 60 seconds to induce ultraviolet mutagenesis. .1 ml of the irradiated cells were then suspended in an aluminum foil wrapped 250 ml Erlenmeyer flask containing 20 ml of a glucose minimal medium supplemented with purines. Cells were grown at 37° to stationary phase and harvested by centrifuging at 6000 rpm for 5 minutes in a refrigerated centrifuge (International Equipment Co., Needham Hts., Mass, Model B-20). Both pellet and tube were carefully rinsed with sterile water by slowly rotating the centrifuge tube without disturbing the contents. The sterile distilled water was poured out, replaced by one ml of an unsupplemented glucose minimal medium in which the pellet was resuspended and then transferred to a 250 ml Erlenmeyer flask containing 20 ml of the same unsupplemented glucose minimal medium to give a final optical density reading of 30 O.D. units . In theory all cells except those showing an absolute requirement for an essential metabolite (e.g. purines) should grow.

These cells were grown at 37°C for 4 hours at which time 2000 units per ml of sodium penicillin sterilized by membrane filtration in a Swinney adapter (Gelman Instr. Co., Ann Arbor, Mich.) was added to the shaking culture. In one generation cell debris presumably from prototrophs killed by the penicillin was found floating in the suspension. The cells were then chilled, diluted and plated on the appropriate

agar to give approximately 50 colonies per plate, at 2 days incubation at 37°C. The cells which should all be auxotrophs were then replica plated and characterized according to their ability to grow on certain media. Further characterization was done in liquid media in 250-ml Erlenmeyer flasks fitted with side arms for direct reading in a Klett-Summerson photoelectric colorimeter using a number 42 filter.

Radioactive Assay

Serine transhydroxymethylase activity was assayed by a modification of the method of Folk and Berg (1970). This assay measures the rate of formation of $H^{14}CHO$ ($^{14}CH_2OH\text{-}FH_4$) formed from the hydrolytic cleavage of serine-3-^{14}C. The reaction mixture (.4 ml) was assembled in 12 X 75 mm Kimax tubes (Kimble Products, Toledo, Ohio) and consisted of the following: 0.05 ml of 75 mM potassium phosphate buffer (ph 7.4), 0.05 ml of 25 mM pyridoxal phosphate, 0.08 ml of 10 mM dl-tetrahydrofolic acid, 0.05 ml of 25 mM 2-mercaptoethanol, 0.07 ml distilled water and 0.05 ml enzyme extract diluted to various concentrations (to ensure linearity) in potassium phosphate buffer with 0.01 M 2-mercaptoethanol.

This was then preincubated at 37°C for 5 minutes at which time 0.05 ml of serine-3-^{14}C (specific activity

- 1.08 X 10^6 cpm/μm) was added and the mixture again incubated for 9 minutes at 37°C. The reaction was stopped by adding 0.3 ml of cold 1 M sodium acetate (pH 4.5). The tubes were then chilled in ice water for 5 minutes after which 0.2 ml of 1 M formaldehyde was added followed by 0.03 ml of 0.4 M 5,5-dimethyl-1,3-cyclohexanedione (dimedon) in 50% ETOH. (The formaldehyde equilibrates with the $^{14}CH_2OH$-FH_4 cleaved from serine-3-^{14}C and is then precipitated with 5,5-dimethyl-1,3-cyclohexanedione). The tubes were then heated for 5 minutes at 95°C and allowed to cool on standing for 10 minutes. The precipitates were then collected by vacuum filtration on millipore glass paper filters (Millipore Ltd., Montreal, Que. Model AP 200 2500) using pyrex microanalysis filter holders 1" X 6" (Millipore Ltd., Montreal, Que., Model XX10 025 00) (Figure 9). These were subsequently washed with 35 ml of cold distilled water and dried under a 300 watt - 150 volt heat lamp (Sylvania Electric (Canada) Ltd., Drummondville, Que.) for 15 minutes in a glass petri dish. The filters were then placed in 20 ml screw-top glass liquid scintillation vials (Amersham/Searle, Toronto, Ont. Model 3326) with 10 ml of .5% 2,5-diphenyloxazole and .02% p-Bis 2-(5-phenyloxazoly)-benzene (Packard Instrument Co., Inc., Downers Grove, Ill. 60515) in toluene and counted in a radioactive scintillation counter (Nuclear Chicago Co., Montreal, Que. Model Unilux II) with automatic calculator.

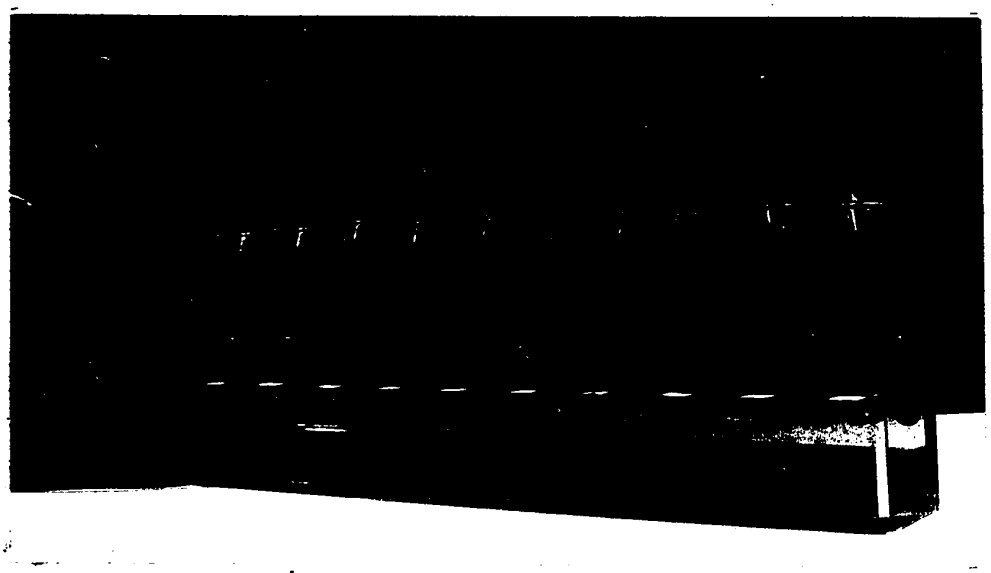


Figure 9. Photograph of the vacuum filtration box with with pyrex microanalysis filter holders used to collect the radioactive precipitate on glass paper filters.

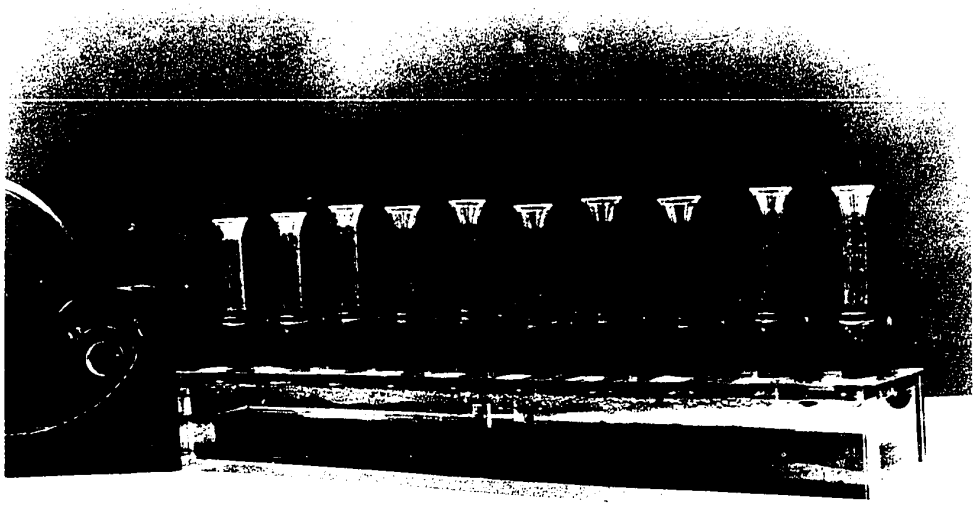


Figure 9. Photograph of the vacuum filtration box with with pyrex microanalysis filter holders used to collect the radioactive precipitate on glass paper filters.

Protein Determination

Protein was determined by the method of Lowry *et al.* (1951) using trypsin as a standard.

RESULTS

The purpose of this study was to grow cells of E. coli in the presence of a variety of metabolites and determine the specific activity of STHM in extracts of these cells. An interpretation of these results would then be made as to the probable relation of these metabolites to serine and glycine biosynthesis.

Such a study requires a valid assay of enzyme activity. Several methods of assaying STHM have been reported (Scrimgeour and Huennekens, 1962; Taylor and Weissbach, 1965; Folk and Berg, 1970), and the one selected, that of Folk and Berg is described in the methods section of this thesis. Part 1(a-e) of the results is devoted to a demonstration that the method, as used here, gives a reliable indication of the amount of enzyme activity present.

The validity of the assay having been clearly established, Parts 2 and 3 of the results deal with the effect of certain metabolites on the specific activity of the STHM in the cell. The metabolites used were chosen by virtue of their relation to serine and glycine metabolism as is explained in the presentation of the individual experiments.

Part 1: Validity of the Enzyme Assay Measuring STHM

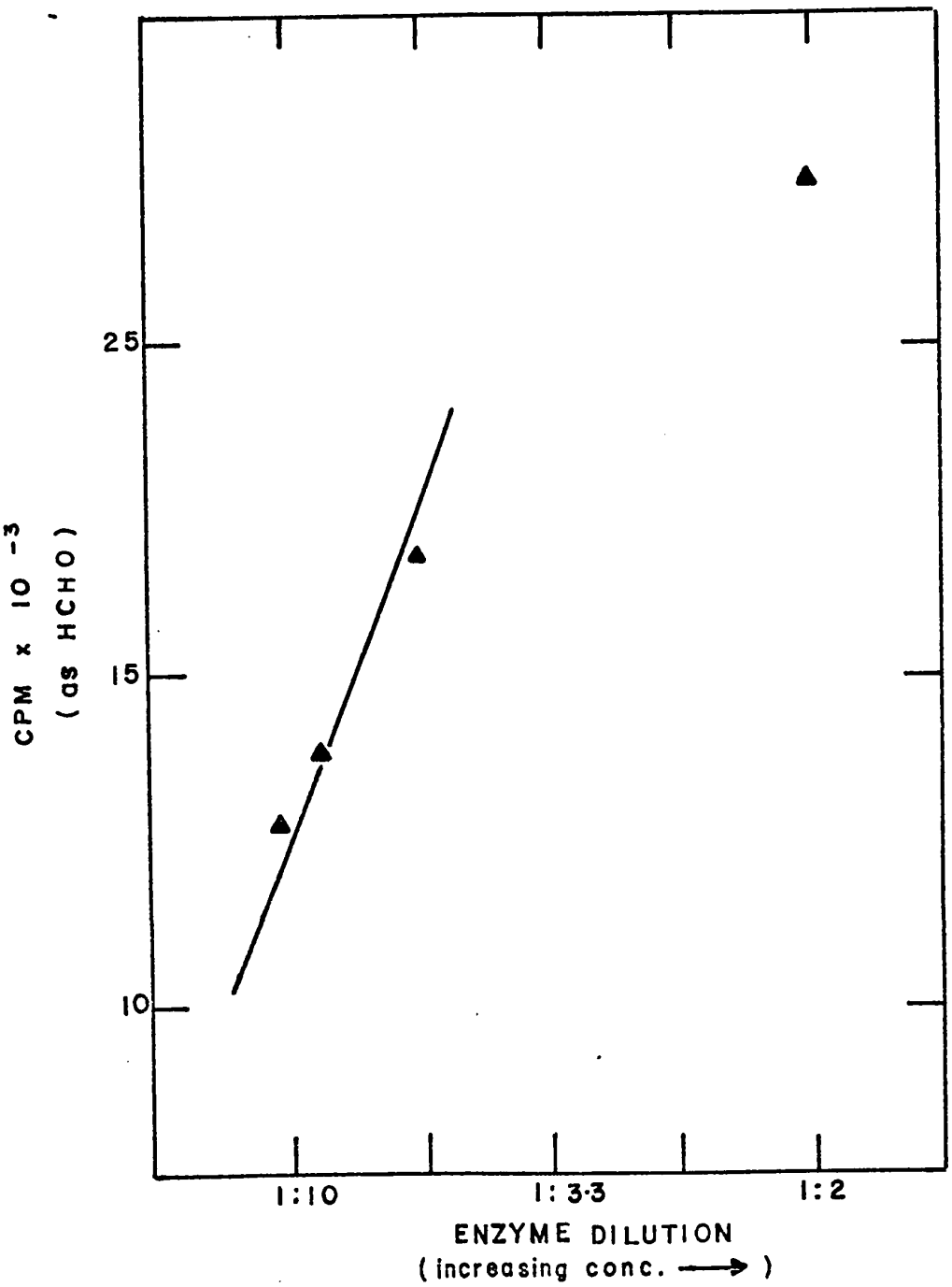
The presence of STHM is detected by the occurrence of the specific reaction it is known to catalyze - i.e. the conversion of serine-3-^{14}C to $^{14}CH_2OH$-FH_4. To know the amount of enzyme activity present, it is necessary to determine the extent of the reaction in a suitable time period. To be sure that the assay as carried out did in fact give an accurate measure of the amount of enzyme activity, the following studies were made.

Part 1a: Response to Enzyme Concentration

The reaction rate of an enzymatic reaction is generally found to increase with increasing enzyme concentration, provided that other conditions are optimal (Dixon and Webb, 1964). To test whether this was true for STHM, various dilutions of an extract of E. coli were incubated in duplicate for a standard 9 minute period (see below) with serine-3-^{14}C and the extent of the reaction determined as the number of counts precipitable as $^{14}CH_2OH$-FH. Figure 10 shows that the relationship between enzyme activity and CPM (counts per minute) was approximately linear at the three lowest enzyme concentrations studied.

Figure I0. The effect of dilution on STHM activity in an extract of E. coli KI0.

Cells of E. coli KI0 were grown in minimal medium supplemented with glucose added to a final concentration of 0.2%. An extract was made from mid-log phase cells as described in the methods. Dilutions of the extract, 1/3, 1/6, 1/8 and 1/12 were prepared in the same buffer used to make the extract. STHM activity was determined at each dilution as counts precipitable as $^{14}HCHO$ ($^{14}CH_2OH$-FH_4) after a 9 minute incubation period.


25
15
10
CPM x 10 -3
(as HCHO)
1:10
1:3·3
1:2
ENZYME DILUTION
(increasing conc. ⟶)

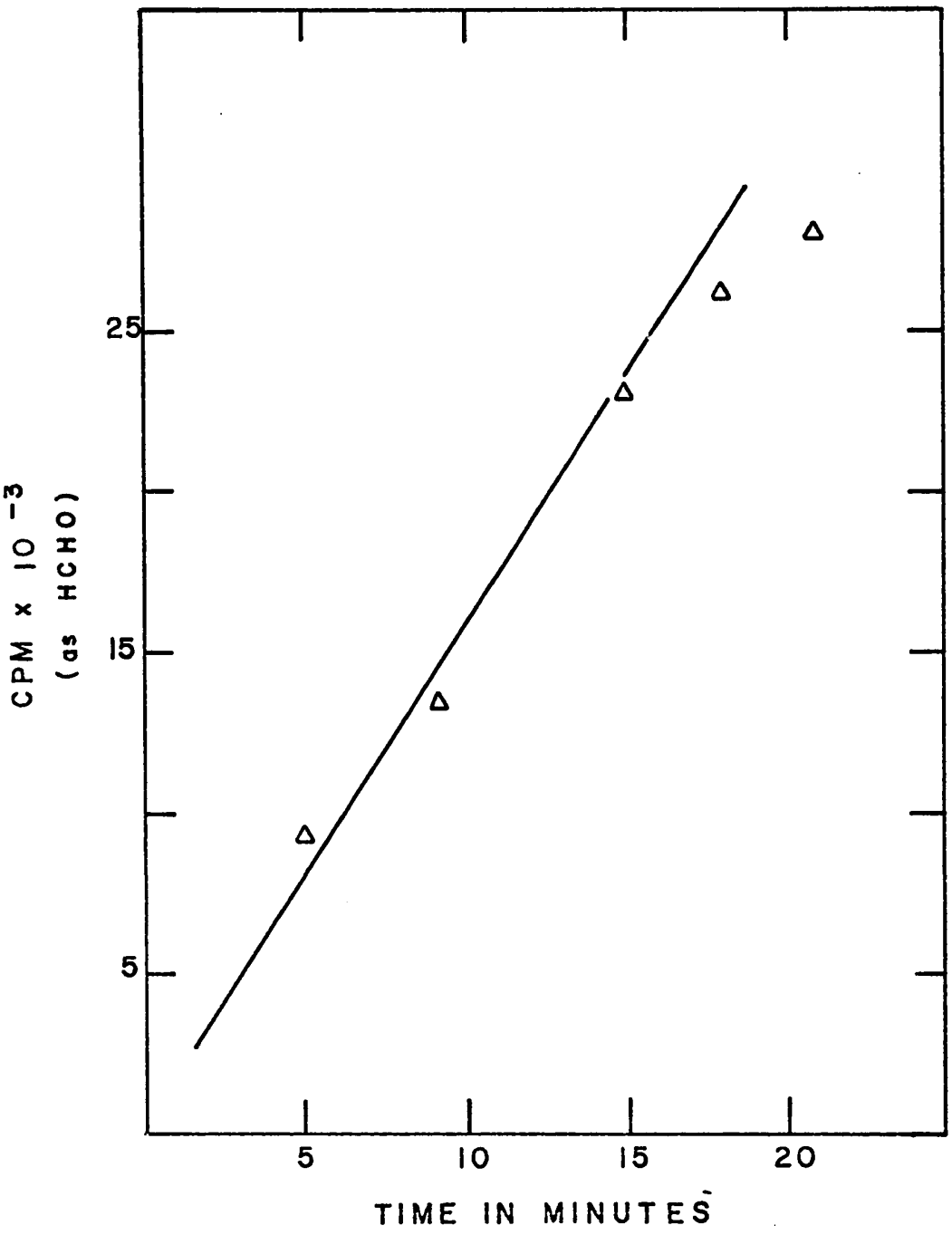
Part 1b: Enzyme Response to Time

For any enzyme assay the amount of substrate converted should be proportional to the time allowed for the reaction, at least for short time periods. The time chosen for a standard assay must allow enough time to permit a measurable amount of substrate to be formed. However, it must not be so long as to reduce the substrate concentration to less than a saturating level for the enzyme. Moreover the enzyme must be stable during the time period chosen (Dixon and Webb, 1964).

To test whether the extent of the STHM reaction was indeed a function of time, and to select a time period for further assays, replicate assays were incubated for 0, 5, 9, 18 and 21 minutes and the activity of $^{14}CH_2OH\text{-}FH_4$ formed was then determined (Figure 11). It can be seen that the reaction rate was proportional to time, at least for the first 15 minutes. A nine minute incubation period was chosen for the standard assay. This allowed a measurable amount of serine to be converted to $^{14}CH_2OH\text{-}FH_4$ with no appreciable deviation from linearity. The fact that the time selected was within the linear portion of the curve excluded the possibility that end product could be accumulating and inhibiting the enzyme. Appreciable inactivation of the enzyme during that period was also excluded by choosing the 9 minute incubation period.

Figure 11. The effect of incubation time on STHM activity

Cells of E. coli K10 were grown in minimal medium supplemented with glucose added to a final concentration of 0.2%. An extract was made from mid-log phase cells as described in the methods. Replicate assays were incubated for 0, 5, 9, 15, 18, and 21 minutes and STHM activity was reported as counts precipitable as $^{14}HCHO$ ($^{14}CH_2OH$-FH_4) at the end of each incubation period.


CPM x 10 -3
(as HCHO)
25
15
5
5
10
15
20
TIME IN MINUTES

Part 1c: Requirements of the Assay Measuring STHM Activity

STHM activity is reported to require the presence of two cofactors, tetrahydrofolic acid and pyridoxal phosphate. To test whether this requirement was also seen in our assay as performed here, extracts of cells of E. coli K10 were incubated with the complete reaction mixture as well as with mixtures from which either tetrahydrofolic acid or pyridoxal phosphate had been omitted. That both cofactors were necessary for the reaction can be seen in Table 2. Omission of pyridoxal phosphate reduced activity to 30%. Omission of tetrahydrofolic acid reduced activity to less than 1%. The extract itself is likely to contain appreciable amounts of pyridoxal phosphate, but very little FH_4 since the latter readily oxidizes to dihydrofolic acid (FH_2) when exposed to atmospheric oxygen. Moreover pyridoxal phosphate is reported to be tightly bound to STHM so that exogenous additions to the crude extract might not be necessary (Folk, 1972, personal communication).

Table 2: Requirements of the assay measuring serine transhydroxymethylase activity.

Assay Condition	($^{CPM}/_{9}$ min. incub.) X 10^{-3}	% Activity
Complete	15.0	100
Without pyridoxal phosphate	5.0	30
Without tetrahydrofolic acid	0.15	<1

Extracts from cells of E. coli K10 were prepared and assayed as described in the methods.

Part 1d: Reproducibility

The purpose of this work is to compare the activity of STHM in cells grown under different conditions. Having substantial evidence that the assay as used here is a valid measure of the amount of enzyme activity in a given extract, it was necessary to show that replicate extracts made from cells grown under the same conditions contained the same amount of activity. For this purpose cells of E. coli K10 were grown in minimal medium with glucose as a carbon source to mid log phase, extracts were made and the activity of STHM determined. Table 3 presents the results of seven such extracts, expressed as μmoles of $H^{14}CHO$ formed per hour per milligram of protein. It can be seen that the values obtained vary between 0.80 and 1.10 with a mean of 0.93. Confidence limits (95%) for the normal range of values gave an interval of 0.75 - 1.12. Results outside this range were taken to indicate regulation of STHM in cells grown in various environmental conditions i.e. values >1.12 could be considered as derepression, while those <0.75 could be taken to indicate repression.

Part 1e: A Comparison of the Activity of STHM in a Wild Type E. coli Strain K10 and an $STHM^-$ Strain AT2046

This assay appears to be both valid and reprodu-

Table 3: The activity of serine transhydroxymethylase in cells of *E. coli* K10.

Experiment	Specific Activity μmoles ^{14}HCHO/hr/mg protein
1	0.94
2	1.10
3	0.92
4	0.80
5	0.86
6	0.99
7	0.91
mean	0.93

Cells of *E. coli* K10 were grown in minimal medium supplemented with glucose added to a final concentration of 0.2%. Extracts were prepared and assayed as described in the methods.

cible. That it is actually STHM that was being assayed is inherent in the assay method. However, it was further verified by carrying out the assay with a strain of E. coli, AT2046, known to be deficient in the synthesis of STHM (Pizer, 1965). Extract of cells of strain AT2046 were made from cells grown for two days on serine supplemented glucose agar plates. From Table 4 it can be seen that the specific activity of the enzyme measured from cells of E. coli K10 grown to mid log phase in a glucose minimal medium was 93 times the specific activity found in cells of E. coli strain AT2046. It can therefore be concluded that the assay used is valid, reproducible and assays the activity which it is designed to assay.

Part 2: Effect of Glycine and One-Carbon Metabolites on STHM Activity

The conversion of serine to glycine illustrated in equation 6 is an important step in one carbon metabolism. The β carbon of serine may serve as a one-carbon donor in the biosynthesis of a number of diverse metabolites including methionine, histidine, thymidine, adenine or guanine (McGilvery, 1970). Thus STHM which catalyzes the reaction, occupies a strategic point in cellular metabolism. This consideration makes it a likely target for cellular control. In view of this, the following studies were undertaken in

Table 4: A comparison of the activity of STHM in a wild type *E. coli* strain K10 and a STHM$^-$ strain AT2046.

Strain	Experiment		Specific Activity μmoles ^{14}/HCHO/hr/mg protein
K10	*	mean	0.93
AT2046	1		0.01
	2		0.01
		mean	0.01

Extracts from the K10 strain were prepared from mid log phase liquid cultures. Extracts from the AT2046 strain were prepared from cells grown for 2 days on glucose agar plates supplemented with 100 μg/ml serine. Both were assayed as described in the methods section.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

order to clarify the role of this enzyme in the provision of serine, glycine and one-carbon units (C_1 units).

Part 2a: Effect of Glycine on STHM Activity

One of the simplest mechanisms in the control of glycine biosynthesis would be repression of STHM by products of the reaction. To see whether this repression did in fact occur, cells of E. coli K10 were grown to mid log phase on minimal medium supplemented by one of two glycine concentrations, 100 µg/ml or 3000 µg/ml. In 5 cultures at 100 µg/ml, the specific activity of STHM varied between 0.84 and 1.10 with a mean of 0.91 (Table 5). Thus at 100 µg/ml no repression of STHM by glycine was seen. However, two cultures grown with 3000 µg/ml glycine had markedly lower enzyme activity (0.39 and 0.41). Thus at high glycine levels, STHM is in fact repressed.

A concentration of 100 µg/ml glycine is known to be in excess of an autotroph's nutritional requirements (Newman and Magasanik, 1963). It is therefore surprising that such amounts had no effect. This might be understood however, in terms of a rate-limiting transport system for glycine. To examine this question in more detail a serine-glycine auxotroph, strain LESV2, was grown in the presence of various concentrations of glycine. One might then expect that any defect in the transport system for glycine would

Table 5: The effect of glycine on STHM activity in cells of *E. coli* K10.

Addition to the Growth Medium	Experiment	μmoles	Specific Activity ^{14}HCHO/hr/mg protein
glycine (100)	1		0.95
	2		0.86
	3		0.84
	4		1.10
	5		0.90
		mean	0.91
glycine (3000)	1		0.39
	2		0.41
		mean	0.40
none	*	mean	0.93

Cells were grown in minimal medium supplemented with glycine 100 μg/ml or glycine 3000 μg/ml. Other conditions were as described in the methods section.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

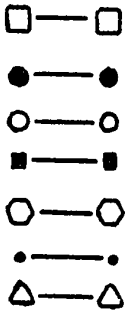
manifest itself in the growth rate of the cells, grown with glycine as a nutritional supplement. As can be seen from the curves in Figure 12, the rate of growth of this E. coli mutant is indeed related to the concentration of glycine. 100 µg/ml was not nearly as effective in promoting the growth of strain LESV2 as was 3000 µg/ml as can be seen from the fact that the doubling time for 100 µg/ml was 100 minutes as compared to 60 minutes for 3000 µg/ml. This indicates that the rate of entry of glycine is limiting and suggests an explanation for the fact that glycine does not repress at low extracellular concentrations and that the effect seen at high concentrations may be physiologically significant. This point will be further considered in the discussion.

Part 2b: Effect of One-Carbon Metabolites on STHM Activity

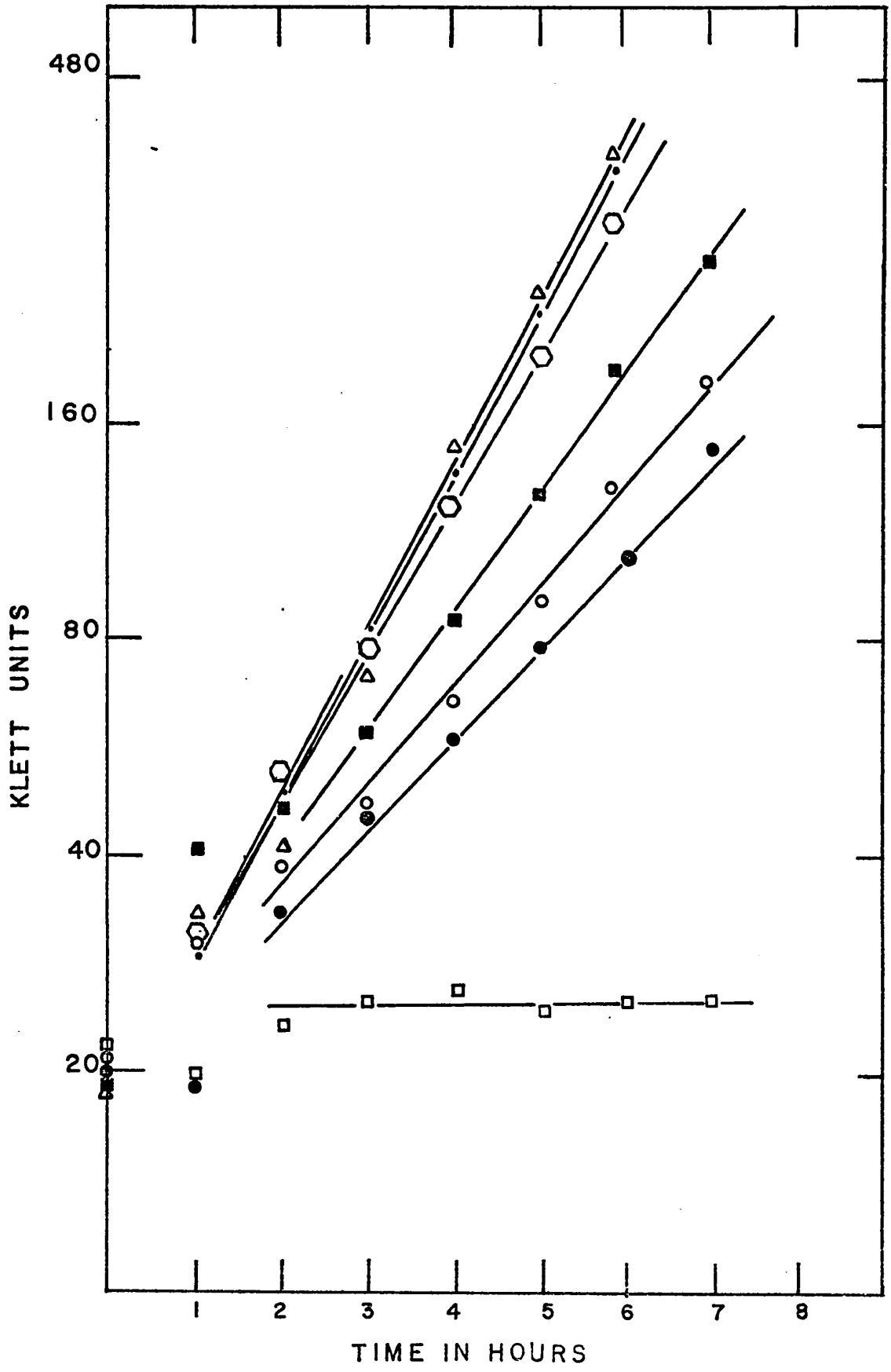
Glycine is not the only product of STHM. Serine is converted simultaneously to glycine and a one-carbon unit (C_1) carried by tetrahydroflic acid (FH_4). One could therefore ask whether adding C_1 to the growth medium would increase the C_1 pool and repress STHM activity, in the same way that adding glycine does. C_1 cannot be added as such. One could however, hope to increase the size of the pool of one-carbon derivatives of FH_4 by adding to the medium compounds derived from C_1. The compounds chosen were

Figure I2. The growth rate of a serine-glycine requiring auxotroph , E. coli strain LESV2 in response to various concentrations of glycine.

Cells pregrown to mid-log phase in minimal medium supplemented with 3000 µg/ml glycine were washed with minimal medium without nitrogen and resuspended into 250 ml side arm Erlenmeyer flasks containing minimal medium supplemented with glycine at the following concentrations: 50, I00, 200, 500, I000 and 3000 µg/ml. Glucose was added to a final concentration of 0.2%. Growth was followed in a Klett-Summerson photoelectric colorimeter using a blue #42 filter and plotted as a function of time. Other conditions were as described in the methods section.



minimal medium (no addition)
glycine 50 µg/ml
glycine I00 µg/ml
glycine 200 µg/ml
glycine 500 µg/ml
glycine I000 µg/ml
glycine 3000 µg/ml


480
160
80
40
20
KLETT UNITS
1
2
3
4
5
6
7
8
TIME IN HOURS

methionine,histidine,thymidine, adenine and guanine. This combination of compounds replaces folic acid in folate-deficient organisms and thus includes all the quantitatively important C_1 derived compounds (Newman and Magasanik, 1963). This mixture of compounds is referred to here as one-carbon metabolites (C_1 metabolites).

For this reason cells of E. coli K10 were grown with all the above compounds, each at concentrations that would be in excess of the requirements of a corresponding auxotroph. Formate was also added, in the hope of directly influencing the intracellular C_1 pool size. These cells were harvested in mid log phase growth. In extracts from two cultures prepared as usual, the specific activity of STHM varied between 0.38 and 0.39 with a mean of 0.385 (Table 6). It seems therefore that increasing the C_1 pool does result in the repression of STHM.

Part 2c: Effects of Glycine and One-Carbon Metabolites Together on STHM Activity

The results discussed above suggest that both end products of serine breakdown, glycine and C_1, are independently capable of repressing STHM. It was therefore desirable to examine what effect the two together might have on the activity of the enzyme. For this purpose cells of E. coli K10 were grown in 100 µg/ml glycine and the C_1

Table 6: Effect of glycine and one-carbon metabolites on STHM activity in cells of *E. coli* K10

Addition	Experiment	Specific Activity μmoles $^{14}HCHO$/hr/mg protein
l-met, l-his, thy ade, guan, Na form.	1	0.38
	2	0.39
	mean	0.385
l-met, l-his, thy ade, guan, Na form, gly (100).	1	0.33
	2	0.27
	3	0.30
	mean	0.30
l-met, l-his, thy, ade, guan, Na form, gly (100), l-ser, isol, val.	1	0.27
l-met, l-his, thy, ade, guan, Na form, gly (3000), l-ser, isol, val.	1	0.15
	2	0.15
	mean	0.15
none	*	mean 0.93

The media additions were present at the following concentrations: l-methionine (l-met) 50 μg/ml, l-histidine (l-his) 20 μg/ml, thymidine (thy) 20 μg/ml, adenine (ade) 20 μg/ml, guanine (guan) 20 μg/ml, sodium formate (Na form) 50 μg/ml, l-serine (l-ser) 200 μg/ml, isoleucine (isol) 100 μg/ml and valine (val) 100 μg/ml. Gly (100) refers to glycine 100 μg/ml, gly (3000) to glycine 3000 μg/ml. Other conditions were as described in the methods section.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

metabolites. The specific activity of STHM was measured between 0.27 and 0.33 with a mean of 0.30. This value was considerably lower than the value shown by cells grown with 100 µg/ml alone (0.93) or cells grown with the C_1 metabolites alone (0.385) and again suggests that a concentration of 100 µg/ml glycine enters the cell slowly. According to this explanation the simultaneous presence of C_1 metabolites avoids the diversion of glycine to either C_1 or purine synthesis and allows the maintenance of a higher intracellular glycine pool.

A similar experiment was performed in which serine was also added to the mixture of glycine (100 µg/ml) plus the C_1 metabolites. The result, a specific activity for STHM of 0.27 (compared to 0.30 with serine) indicated that serine had no effect under these circumstances (Table 6). Serine was also added to a medium containing glycine (3000 µg/ml) plus the C_1 metabolites. This resulted in the lowest specific activity for STHM seen (0.15) (Table 6). The difference between 0.15 and 0.30 is very likely due to the increased glycine concentration provided, rather than to the serine concentration, but this was not tested further.

Part 2d: Effect of Purines on STHM Activity

The mixture of 100 µg/ml and the C_1 metabolites

clearly results in repression of STHM activity. To test how much of the C_1 effect was due to purines, cells were grown with glycine 100 µg/ml and all the C_1 metabolites except adenine and guanine. As can be seen in Table 7, this resulted in a specific activity for STHM of 0.51. Thus the difference between the former experiment (glycine 100 µg/ml and all the C_1 metabolites - 0.30) and this one (glycine 100 µg/ml and all the C_1 metabolites except purines - 0.51) may be attributed to an effect of purines.

Since the presence of purines contributes to the repression to such an extent, one then might expect the absence of purine (i.e. starving the cells for purine) to increase the specific activity of STHM (derepress). The prototrophic K10 strain can make its own purine and therefore cannot be starved for purine. Thus in order to test this hypothesis a purine requiring auxotroph, strain LESV1, was isolated from strain K10, grown to mid log phase in a purine supplemented medium, subcultured (incubated) for 4 hours in a medium without purines and the specific activity of STHM measured before and after subculture. After the subculture the enzyme activity was 2.5 to 3.0 fold higher (1.3 to 1.9, mean 1.6) than before the subculture (0.51 to 0.63). It is clear therefore that purines contribute to the control of STHM both when in excess and when in limiting concentrations.

Table 7: Effect of purines on STHM activity in cells of *E. coli* K10 and a purine requiring auxotroph *E. coli* LESV1.

Strain	Addition to growth medium	Experiment		Specific Activity μmoles $^{14}HCHO$/hr/mg protein at mid-log	after subc.
K10	l-met, l-his, thy, Na form, gly (100)	1		0.46	---
		2		0.56	---
			mean	0.51	---
LESV1	ade, guan, [subculture in minimal medium (without ade, guan)]	1		0.63	1.9
		2		0.51	1.3
			mean	0.57	1.6
K10	l-met, l-his, thy ade, guan, Na form, gly (100).	**	mean	0.30	---
K10	none	*	mean	0.93	---

Compounds were added at the same concentrations as specified for Table 5. Cells of strain LESV1 were grown to mid log phase in a minimal medium supplemented with adenine and guanine, washed with minimal medium without nitrogen and subcultured for 4 hours in an unsupplemented minimal medium. Extracts were prepared and assayed as described in the methods.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

**Specific activity of STHM in K10 cells grown with glycine (100 μg/ml and the C_1 metabolites (including purines) From Table 6.

Part 2e: Effect of Starving the Cells for Glycine on STHM Activity

Since starving the cells for purines resulted in an increased specific activity for STHM, a similar effect was anticipated in cells starved for glycine. In order to test this the serine-glycine auxotroph, LESV2, isolated from strain K10, was cultured to mid-log phase in a glycine supplemented medium (3000 µg/ml) and then transferred to either a minimal medium without glycine orto a medium containing a limiting concentration of glycine (10 µg/ml). The enzyme was assayed both before and after subculturing. There was no change in the level of activity of STHM in either case (Table 8).

Strain LESV2 is an auxotroph that responds to serine or glycine. The mutation seems to be in the pathway for serine biosynthesis. In this experiment the cells were grown with glycine, and presumably obtained their serine from that glycine. When glycine was withdrawn, the pool first affected may have been the serine pool and not the glycine pool. Thus the expectation of an effect on STHM activity may have itself been unlikely.

The experiment which would exactly parallel the purine starvation experiment could only be done if there were two enzymes involved in glycine biosynthesis and STHM were the first of these. Then starving for glycine due to

Table 8: Effect of starving the cells for glycine on STHM activity in cells of a serine-glycine auxotroph, E. coli strain LESV2.

Addition to the Growth Medium	Experiment	specific activity µmoles ^{14}HCHO/hr/mg protein at mid-log	after subc.
gly (3000)	1a	0.64	---
none	1b	---	0.71
gly (3000)	2a	0.61	---
gly (10)	2b	---	0.69

Cells were grown to mid-log phase in minimal medium supplemented with glycine 3000 µg/ml (1a, 2a), washed in minimal medium without nitrogen and subcultured in either minimal medium (1b) or minimal medium supplemented with 10 µg/ml glycine (2b). Other conditions were as described in the methods section.

a block in the second enzyme should increase STHM activity.

Part 2f: Effect of Aminopterin on STHM Activity

The specific activity of STHM was increased up to 3 fold as a result of starvation for purines. Starving for glycine did not have the same effect. These experiments differ in that purine starvation might be expected to drain both the glycine and C_1 pools, whereas the effect of glycine starvation is harder to determine.

Aminopterin is a well known inhibitor of dihydrofolate reductase (Lehninger, 1970). In the presence of aminopterin, FH_2 cannot be reduced to FH_4. As cells grow and synthesize thymidine in the presence of aminopterin, FH_4 is oxidized to FH_2 and the FH_2 cannot be reduced again. Cells are thus starved for FH_4 and one might expect that STHM would be inactive and the cell would be starved for its products, glycine and C_1.

To test this, cells were grown to mid-log phase in minimal medium with glucose and subcultured with aminopterin (300 µg/ml) for 4 hours. That aminopterin was an effective inhibitor of growth was seen by its inhibition of an increase in optical density (O.D.) of the cells during subculture. However, no change in STHM activity was seen (Table 9).

If the cells were starved for C_1 in the presence

Table 9: Effect of aminopterin on STHM activity in cells of *E. coli* K10.

Addition to the Growth Medium	Experiment	O.D. at time of harvesting	Specific Activity μmoles $^{14}HCHO$/hr/mg protein at mid-log	after subc.
Aminopterin	1a	139	---	1.00
Aminopterin, l-met Na form.	1b	147	---	0.85
none	*	130	0.93	---

Cells were grown to mid-log phase in minimal medium, washed with minimal medium without nitrogen and subcultured for 4 hours in the presence of aminopterin 300 μg/ml (1a) or aminopterin 300 μg/ml, l-methionine 50 μg/ml and Na formate 500 μg/ml (1b). Other conditions were as described in the methods section.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

of aminopterin, this might have a generalized effect on protein synthesis. Protein synthesis is initiated in E. coli by the formylation of methionyl transfer ribonucleic acid (met-tRNA) (Adams and Capecchi, 1966). In the absence of C_1, STHM activity may be derepressed but if the absence of C_1 also inhibits protein synthesis, STHM would not be made. Thus, one might not expect to see any increase in STHM activity. In an attempt to avoid this problem, cells were subcultured into minimal medium containing methionine (50 µg/ml) and formate (500 µg/ml). As seen in Table 9 there was still no increase in the activity of STHM.

Aminopterin clearly did not have the anticipated result. This might be due to the fact that aminopterin grown cells are not in fact starved for FH_4 but are inhibited elsewhere. It could also be that initiation of protein synthesis is impaired and this impairment is not reversed by the addition of formate and methionine. The formation of formyl-methionyl-tRNA involves an interaction of ^{10}formyl-FH_4 and methionyl-tRNA (Marcker, 1965). Formate itself however, may not be a good donor under these circumstances.

Part 3: Effect of Serine and Related Metabolites on STHM Activity

Part 3a: Effect of Serine, Isoleucine and Valine on STHM Activity

Previous results (part 2a) show that STHM activity is repressed by growth in glycine. To test whether it is similarly affected by serine, cells of E. coli K10 were grown to mid-log phase in medium supplemented with serine (200 µg/ml), isoleucine (100 µg/ml) and valine (100 µg/ml). In three experiments values for STHM of 0.69, 0.57, and 0.65 were obtained. (Table 10). It seems therefore that the activity of STHM was slightly decreased.

Glycine was repressive at very high concentrations only (i.e. 3000 µg/ml). The need for these high concentrations has been explained in terms of a transport problem. No transport problem has ever been reported for serine. Indeed L-serine is known to be inhibitory at concentrations above 100 µg/ml (Cosloy and McFall, 1970) 100 µg/ml iso-

Table 10: Effect of serine, isoleucine and valine on STHM activity in cells of E. coli K10 and a serine-glycine requiring auxotroph E. coli LESV2.

Strain	Addition to Growth Medium	Expt.	Specific Activity μmoles ^{14}HCHO/hr/mg protein at mid-log	after subc.
K10	l-ser (200) isol, val.	1	0.69	---
		2	0.57	---
		3	0.65	---
		mean	0.63	---
K10	l-ser (350) isol, val.	1	0.69	---
LESV2	ser (200) isol, val.	1a	0.61	---
	none	1b	---	0.50
	l-ser (200) isol, val.	2a	0.61	---
	l-ser (10) isol, val.	2b	---	0.45
K10	none	*	0.93	---

Strain K10 cells were grown to mid-log phase in minimal medium supplemented with 200 or 350 μg/ml l-serine (l-ser) and isoleucine (isol) and valine (val) at 100 μg/ml each. Strain LESV2 cells were grown to mid-log phase in minimal medium supplemented with 200 μg/ml l-serine (1a, 2a) washed with minimal medium without nitrogen and subcultured for 4 hours either in minimal medium (1b) or minimal medium supplemented with 10 μg/ml l-serine and isoleucine and valine 5 μg/ml each (2b). Other conditions were as described in the methods section.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

leucine is added to counteract this inhibition. (Isoleucine is itself inhibitory in E. coli K10 and therefore valine 100 µg/ml is added to counteract isoleucine toxicity). However, to investigate this further, cells of E. coli K10 were grown with serine 350 µg/ml again with isoleucine and valine, 100 µg/ml each. This experiment was done only once and gave a value of 0.69 (Table 10). It appears therefore that the presence of serine (200 µg/ml) decreases the concentration of STHM in the cell somewhat and that increasing the concentration of serine has no further effect. Because isoleucine and valine were added, such effect as there is, may be due to those two. This could be examined by growing the cells with isoleucine and valine alone. However, this was not tested.

The effect of serine is small and it is difficult to say whether it is biologically significant. Therefore it was decided to try to alter the serine pool in other ways to see if STHM activity could be varied. This question was first approached by starving strain LESV2, the serine-glycine requiring auxotroph discussed in Part 2e, for serine. Strain LESV2 was grown to mid-log phase in serine, isoleucine and valine (200, 100 and 100 µg/ml respectively) and subcultured in the same medium without serine. No change in enzyme activity was seen when measured both before (0.61) and after (0.50) subculture. (Table 10). The same experiment was then repeated by subculturing in limiting amounts of serine (10 µg/ml), as a control against the possibility that in the first case protein synthesis

might have been totally inhibited. Again no change in enzyme activity was seen (0.61 before subculture as compared to 0.45 after subculture, Table 10).

Part 3b: Effect of Cysteine on STHM Activity

A direct analogy with the drain on the glycine and C_1 pools consequent on purine starvation could be made by starving a cysteine requiring auxotroph for cysteine and therefore draining the serine pool. For this reason cells of a mutant E. coli JM140 which is unable to reduce sulfate were grown to mid-log phase in a cystine* supplemented minimal medium (100 µg/ml) and subcultured in the absence of cystine. No change in enzyme activity was seen after subculture (0.71 before subculture as compared to 0.71 after subculture, Table 11).

The mutant studied here is unable to reduce sulfate. It therefore cannot synthesize SH groups and must be given them as cysteine. In the normal cell serine is converted via O-acetyl serine to cysteine and cysteine inhibits its own biosynthesis (Smith, 1971). When the cell is starved of cysteine, one would expect O-acetyl serine to accumulate due to the lack of the usual inhibition by the end product cysteine.

---

*Cysteine itself is highly toxic in E. coli. Therefore cysteine was provided instead (Smith, 1971).

Table 11: Effect of cystine on STHM activity in cells of a cysteine requiring auxotroph, E. coli strain JM140.

Addition to the Growth Medium	Experiment	Specific Activity µmoles $^{14}HCHO$/hr/mg protein at mid-log	after subc.
l-cystine	1a	0.71	---
none	1b	---	0.71

Cells were grown to mid-log phase in minimal medium supplemented with cystine 100 µg/ml (1a), washed with minimal medium without nitrogen and subcultured for 4 hours in unsupplemented minimal medium (1b). Other conditions were as described in the methods section.

Subculturing without cysteine in an attempt to drain the serine pool did not affect STHM activity. This may be because the serine pool is in fact not drained. The amount of serine converted to cysteine is small (Jones-Mortimer, 1972, personal communication) and so a drain on cysteine may not be quantitatively important. However, it may also be that STHM is simply not controlled by the level of the serine pool.

Part 3c: Effect of the Carbon Source on STHM Activity

It is well documented that glucose as well as other energy sources can suppress the formation of many inducible enzymes, a phenomenon known as catabolite repression (Magasanik, 1961). Most of the enzymes controlled in this way form part of catabolic (energy producing) enzyme systems. Indeed the concept is so general that the existence of a glucose effect can be taken as evidence that the enzyme affected is involved in a catabolic system. To determine whether there is an effect on STHM activity cells of E. coli K10 were grown to mid-log phase in minimal medium supplemented respectively with glycerol, succinate or sodium acetate (each at a final concentration of 0.2%) and the activity of STHM determined from extracts prepared as usual. As can be seen in Table 12, the activity of STHM is somewhat lower than that in extracts of glucose grown

Table 12: Effect of the carbon source on STHM activity in cells of *E. coli* K10.

Carbon Source	Experiment	Specific Activity μmoles ^{14}HCHO/hr/mg protein
glucose	*	0.93
glycerol	1	0.73
succinate	1	0.65
sodium acetate	1	0.60

Cells were grown to mid-log phase in minimal medium. The carbon sources, glucose, glycerol, succinate and sodium acetate were added at a final concentration of 0.2%. Other conditions were as described in the methods.

*Specific activity of STHM in glucose grown K10 cells from Table 2.

cells. It appears then that STHM does not play a catabolic role in the cell. The fact that the activity of STHM in these conditions is repressed is in itself surprising however, this aspect was not studied here.

Part 3d: Role of Serine in C_1 Production

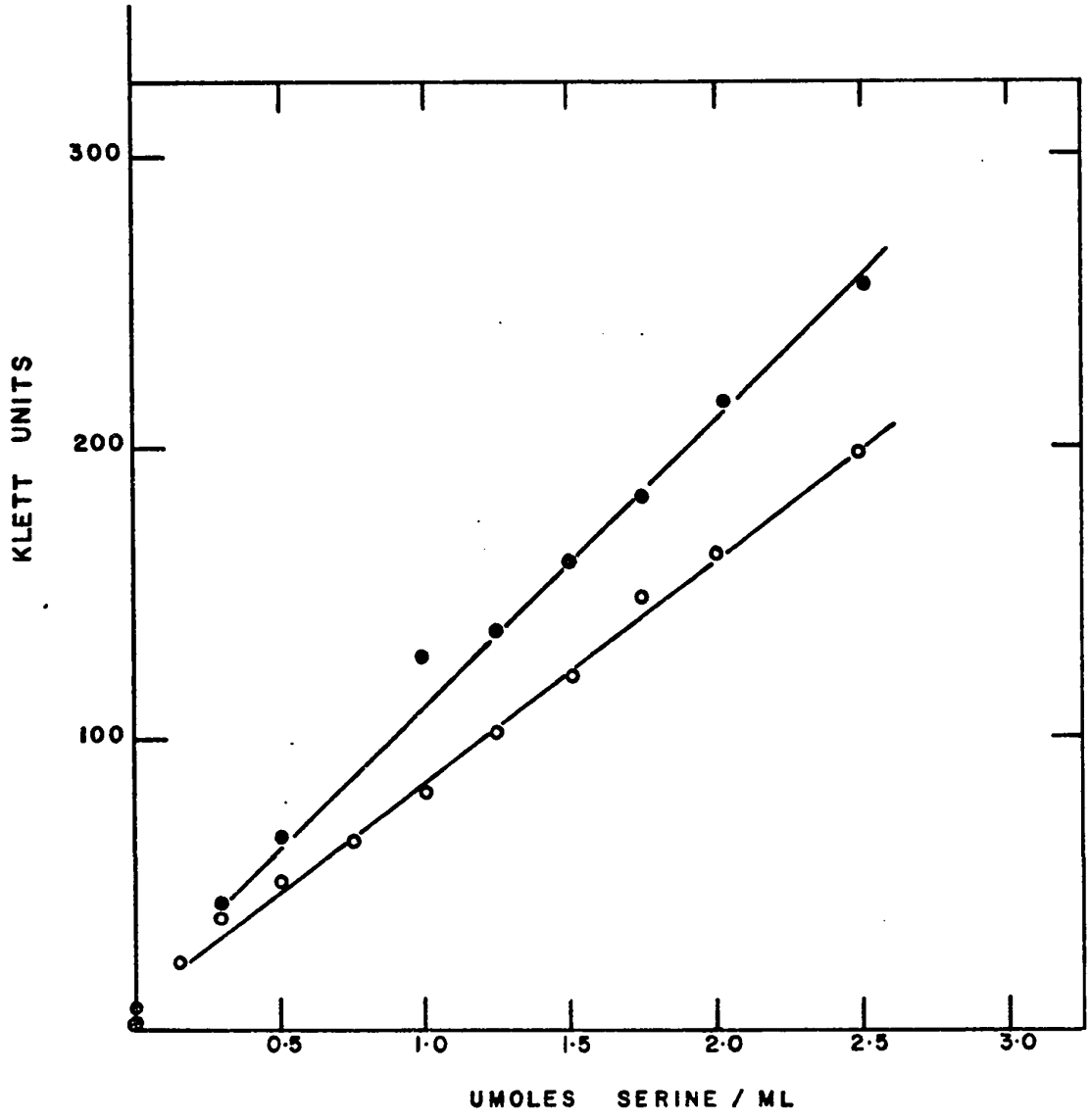
When cells of E. coli K10 are grown with glycine at high concentrations the specific activity of STHM is repressed. It thus appears that in these conditions, the need for C_1 units must be met from some other source than serine. In both strains K10 and LESV2, glycine may compensate for the needed C_1 units via the enzymes which cleave glycine to CO_2 and one carbon derivatives of FH_4 (glycine to C_1 enzymes). It was therefore of interest to try and isolate a strain unable to convert glycine to C_1 to see what effect such a mutation would have, if indeed it was not lethal to the strain. A strain LESV3 with this particular mutation was isolated from LESV2. LESV3 requires serine as LESV2 does. It does not however, grow in glycine supplemented media; it was indeed selected as being unable to grow on plates supplemented with glycine 3000 µg/ml. That it cannot convert glycine to C_1 has been shown by Newman et al. (1972 unpublished results).

Figure 13. A comparison of the amount of serine used per unit mass by the serine-glycine requiring auxotroph E. coli strain LESV2 and the serine requiring auxotroph E. coli strain LESV3.

The cells from strain LESV2 and LESV3 were previously grown to mid-log phase in minimal medium, washed and harvested as described in the methods and transferred to 250 ml Erlenmeyer side arm flasks containing minimal medium supplemented with l-serine at the following concentrations: 0, 16, 50, 75, 125, 150, 175, and 250 µg/ml. 0.2% glucose was added in all cases. Growth of the cultures was followed in a Klett-Summerson photoelectric colorimeter using a blue #42 filter. The final optical density was recorded once the cells had stopped growing (i.e. depleted their exogenous serine supply). This final optical density was plotted as a function of the serine concentration used.

LESV2 ●—●

LESV3 ○—○


KLETT UNITS
300
200
100
0
0·5
1·0
1·5
2·0
2·5
3·0
UMOLES SERINE / ML

The mutation affecting C_1 production from glycine is clearly not lethal. One might assume however, that in the absence of the glycine to C_1 enzymes, the pattern of serine metabolism might change. To test this, a dose response curve to serine was performed with both LESV2 and LESV3. Each strain was precultured in serine, isoleucine and valine, (250, 150 and 150 µg/ml respectively) and subcultured into a series of flasks containing 0, 16, 25, 50, 75, 100, 125, 150, 175, 200 and 250 µg/ml serine. The concentrations were chosen so as to limit growth according to the availability of serine. The cultures were grown to a constant optical density. The optical density reached at each concentration for each strain is plotted in Figure 13. It can be seen that in both cases, growth is proportional to serine, but the optical density reached is in all cases higher for strain LESV2 than LESV3.

The glycine to C_1 enzymes are thus not essential for growth. They do however, play some role in the efficient usage of serine.

LESV3 does not grow in media supplemented with glycine. Glycine has been shown to repress STHM synthesis (Part 2a). However, in the presence of serine, glycine 3000 µg/ml does not alter the growth rate of strain LESV3. The implications of this are considered in the discussion.

When the metabolism of serine is altered, is the activity of STHM altered too? To test this cells of strain LESV3 were grown to mid-log phase in minimal medium supplemented with serine (200 µg/ml), isoleucine (100 µg/ml) and valine (100 µg/ml) and then transferred to either a minimal medium without serine or to a medium containing a limited concentration of serine (10 µg/ml), isoleucine (5 µg/ml) and valine (5 µg/ml). It can be seen from Table 13 that the activity of STHM was higher in strain LESV3 (0.82, 0.80) but in the same range as strain LESV2 (0.61) while the activity of the enzyme after subculture was not increased but decreased (0.51, 0.61). This will be further considered in the discussion.

Table 13: Role of serine in C_1 production.

Strain	Addition to Growth Medium	Experiment	Specific Activity µmoles $^{14}HCHO$/hr/mg protein at mid-log	after subc.
LESV3	l-ser (200) isol, val.	1a	0.82	---
	none	1b	---	0.51
	l-ser (200) isol, val.	2a	0.80	---
	l-ser (10) isol, val.	2b	---	0.61

Cells of strain LESV3 were grown to mid-log phase in minimal medium supplemented with l-serine (l-ser) 200 µg/ml, isoleucine (isol) 100 µg/ml and valine (val) 100 µg/ml (1a, 2a) washed with minimal medium without nitrogen and subcultured for 4 hours in either minimal medium (1b) or in minimal medium with 10 µg/ml l-serine, 5 µg/ml isoleucine and 5 µg/ml valine (2b). Other conditions were as described in the methods section.

DISCUSSION AND CONCLUSIONS

Very little work has been done on the question of the regulation of serine transhydroxymethylase (STHM) concentration in the cell. Those experiments which have been done have all suffered from the same flaw in experimental design: - they have concentrated on the role of the enzyme in glycine formation only, and have not taken into account the fact that two products are formed and are used for different purposes in the cell. Because of this, the role of C_1 derived materials has been largely overlooked until the present study.

This discussion will begin with the specific question of the effect of glycine and one-carbon metabolites on the specific activity of STHM. One of the important ancillary questions is the problem of glycine transport which will be considered next. The discussion will then concern itself with whether the entire effect on STHM is mediated via the size of the intracellular glycine pool or whether the intracellular pools of both glycine and C_1 may have an effect.

It is known that STHM catalyzes a reversible reaction in vitro. Whether it is also reversible in vivo will be discussed next. The discussion will then consider the significance of mutants affected in serine biosynthesis in the overall interpretation of serine-glycine metabolism.

Because the enzyme is involved in C_1 production and the availability of C_1 units is important both in the above and subsequent interpretations, mention of the various other sources of C_1 will be made. The discussion is concerned principally with the regulation of glycine and C_1 metabolism in E. coli (i.e. regulation of STHM). Two of the more common control methods, feedback inhibition and repression of enzyme synthesis are reviewed here although the actual mechanisms have not been investigated. The discussion will then conclude with a reexamination of the results of this thesis for possible application in the design of a therapeutic method to counteract the metabolic disease, hyperglycinemia.

Growth of cells of E. coli K10 in the presence of 100 µg/ml glycine did not alter the specific activity of STHM. This lack of repression by glycine 100 µg/ml might therefore indicate that there is no repression of the enzyme by glycine. However repression need not be ruled out, since the absence of any effect may be explained in at least two other ways:

a) Glycine is not the only product of STHM. The cell also needs one-carbon groups (C_1) that are derived from the β carbon of serine. It might be then that glycine and C_1 are both needed to repress STHM.

b) The E. coli K10 cells may be less permeable to glycine than to other metabolites. This might make it necessary to use much higher external glycine concentrations to increase the intracellular glycine pool and thus repress STHM.

Suppose as suggested in (a) above, both products (glycine and C_1) are needed to alter the specific activity of STHM and that neither glycine nor C_1 alone would suffice to repress the enzyme. This might be a reasonable mechanism for if either one were repressive alone, its presence might interfere with the production of the other. For example the repression of STHM by glycine alone would decrease not only the amount of glycine made from serine, but also the amount of C_1 made from serine. Thus the cells, while well supplied with glycine, would not have their normal supply of C_1 units from serine. The same would be true of adding C_1 (C_1 metabolites) alone. The cells well supplied with C_1, would lack a normal supply of glycine from serine.

The results of an experiment where glycine (100 µg/ml) and C_1 (C_1 metabolites) were added together to the growth medium of cells of E. coli K10, showed that there was a significant decrease in the specific activity of STHM, compared to the specific activity seen with cells of E. coli K10 grown either with glycine (100 µg/ml) alone or without any addition to the growth medium. (The specific activity with

both glycine and C_1 was 0.30 as compared to 0.91 with glycine (100 µg/ml) alone or 0.93 without any addition).

In a further experiment however, it was shown that the C_1 metabolites alone in the growth medium could repress STHM. Cells of E. coli K10 grown in these conditions also had low specific activities for STHM (0.385) as compared to cells grown with glycine (100 µg/ml) alone (0.91) or without any addition (0.93).

It is clear then that even though glycine (100 µg/ml) alone cannot repress STHM, the C_1 metabolites alone can repress STHM and that a simple hypothesis such as (a) can be discarded here.

According to hypothesis (b), cells of E. coli K10 may be poorly permeable to low concentrations of glycine such as 100 µg/ml. In fact several strains of E. coli are known to have impaired transport (uptake) systems for glycine. For example Levine and Simmonds (1962) described two serine-glycine auxotrophs of E. coli K12 with different glycine uptake characteristics, S and S/G. Strain S/G could take up glycine from the medium normally. Strain S on the other hand was unable to transport glycine normally and as a result grew much more slowly than did strain S/G when supplied with the usual concentration of glycine, 75 µg/ml. However strain S did grow faster with higher concentrations of glycine in the growth medium Newman (1970) described

another serine-glycine auxotroph, E. coli strain HM100 with little or no ability for active transport of glycine and considered that glycine entered the cell by diffusion. The apparent division time decreased markedly with increasing glycine concentrations. This suggests that as the concentration of glycine given to strain HM100 is increased the amount diffusing into the cell similarly increases. On the other hand at lower concentrations of glycine, the amount diffusing into the cell is decreased and correspondingly the amount of intracellular glycine available to the cell is diminished.

An experiment described in this thesis (Results, Part 2a) has shown that a serine-glycine auxotroph, LESV2, isolated from strain K10, also has a transport problem for glycine. Again the apparent division time steadily decreased when the cells were grown with increasing concentrations of glycine. This therefore suggests that the K10 strain used in the previous experiments has the same glycine permeability problem.

Glycine at low concentrations (100 µg/ml) has no effect on STHM. However, cells grown in 3000 µg/ml glycine have markedly lowered specific activities for STHM (0.40), as compared to the specific activity seen in the glucose grown K10 strain (0.93). In fact the specific activity of STHM is significantly lower in K10 cells grown with 3000 µg/ml

glycine and the C_1 metabolites (0.15) than in cells grown with 100 µg/ml and the C_1 metabolites (0.30). Thus it is clear that there is some kind of transport problem for glycine in the K10 strain and that in order to repress STHM by glycine alone, it is necessary to use high concentrations of this amino acid.

Either glycine or C_1 alone can affect the activity of STHM. When both are present together, however, the effect is still greater. The simple explanation involving the obligatory coordinated action of two compounds has already been discarded. However, two other explanations can be considered for this additional effect:

a) The entire effect may be mediated via the size of the intracellular glycine pool alone.

b) The specific activity of STHM may be affected independently by the intracellular pool size of two compounds, that of glycine and that of some C_1 metabolite.

Suppose the entire effect is mediated via glycine. Why then is it that glycine 3000 µg/ml does not repress STHM to the lowest specific activity seen? It is suggested that even at this concentration cells of E. coli K10 are not flooded internally with glycine. Enough may enter to repress STHM to a specific activity of 0.40 and support a rapid growth rate, but the intracellular glycine pool may still be only slightly above growth limiting concentrations. With the addition of the C_1 metabolites, the internal glycine

may no longer be diverted to the synthesis of one carbon groups nor to make purine nucleotides (which draw one intact molecule of glycine from the internal pool per mole of purine synthesized). Thus it is conceivable that under these conditions the intracellular glycine pool size may be somewhat higher than when the cells are grown with glycine (3000 µg/ml) alone. This might account for the increased repression (i.e. 0.40 vs 0.15).

If the entire effect is in fact due to changes in the glycine pool size, one would wonder why the C_1 metabolites also substantially repress STHM from 0.93 (cells grown without any addition) to 0.385. It may be that when the C_1 metabolites are added alone, glycine is diverted to make neither C_1 nor purines and the intracellular glycine pool can increase to a concentration capable of repressing STHM (i.e. to a specific activity of 0.385). One might therefore expect there to be a higher specific activity for STHM than 0.385 when the cells are grown with all the C_1 metabolites except purines, since the intracellular glycine pool will not be diverted to make C_1, but will be required to supply glycine to make purines. However, this was not tested.

When the K10 cells are grown with the C_1 metabolites alone the specific activity of STHM is 0.385. When the C_1 metabolites are added together with glycine (100 µg/ml) the

activity of the enzyme is repressed even further (0.30), though glycine 100 μg/ml alone has no effect. Here again it is conceivable that the intracellular glycine pool is diverted to make neither C_1 nor purine when the K10 cells are grown with the C_1 metabolites alone, while the added 100 μg/ml glycine further increases the intracellular glycine pool size. Thus the specific activity of STHM is repressed even more in the presence of even the low concentration of glycine (100 μg/ml) which is inactive on its own.

In fact when purines are omitted from the mixture which includes glycine (100 μg/ml) and the other C_1 metabolites the specific activity of STHM is not nearly as low (0.51) as when the purines are present (0.30). The effect of purines can be understood in terms of an effect on the glycine pool and it may be that decreasing the need for glycine via the addition of purines has a more drastic effect on the glycine pool size than adding glycine directly to the medium, at least at low concentrations.

Most effects on the specific activity of STHM are explainable in terms of the intracellular glycine pool size. Can some of the effect be directly attributable to the intracellular pool size of some C_1 metabolite alone? This appears to be unlikely in view of the fact that when E. coli strain 2276 was tested with each of the metabolites separately, not one of them alone (with the exception of purine) could affect the specific activity of STHM (Taylor et al.,

1966). That purine was able to repress the specific activity of the enzyme when alone can be understood in terms of an effect mediated via the glycine pool as suggested above. It seems clear then that all effects discussed so far may be understood in terms of variations in the intracellular concentration of glycine.

It appears that in cells of E. coli K10 both the glycine and C_1 intracellular pools are easily varied by the exogenous addition of metabolites to the growth medium and indeed the preceeding experiments all deal with the effect of these additions on the specific activity of STHM. However, the intracellular pool size can also be varied by endogenous alterations which do not involve any initial exogenous additions to the growth medium.

For example, it is well known that glycine is used not only for protein synthesis as are all the amino acids, but also in purine biosynthesis and the manufacture of C_1 units (McGilvery, 1970). Suppose the synthesis of nucleic acid decreases in the cell. At the same time the intracellular purine pool size should begin to increase (since the purine nucleotides are no longer required to make nucleic acid). In turn as long as the intracellular purine pool size is high in the cell, neither glycine nor C_1 would be required to make the purine ring. The effect should be the same as adding purines exogenously. That is, glycine

will no longer be diverted to make purine either as glycine or a C_1 donor, thereby increasing the intracellular glycine pool size. Thus the glycine pool size may be altered not only by adding glycine and/or the C_1 metabolites exogenously but also by alterations in the endogenous demand for glycine.

A cell growing with no exogenous additions except glucose must have some size of a glycine pool. The specific activity of STHM in these conditions is 0.93. When glycine is added exogenously the specific activity of STHM is repressed. By the same reasoning as above but in an inverse direction, draining the intracellular glycine pool to a level even lower than that seen when the specific activity of STHM is 0.93 could conceivably lead to an increased activity (derepression) of STHM

This was tested by taking advantage of strain LESV1, a mutant isolated from E. coli K10 and unable to make its own purine. This mutant requires adenine or guanine to grow and when cultured in their absence, these cells continue to synthesize purine nucleotide precursors though they cannot make the actual compound needed. These precursors are then excreted into the growth medium. Thus the glycine and C_1 pools continue to drain in order to make the purine precursors to wherever the genetic block has been induced, at which point this precursor does not accumulate but is excreted from the cell.

Therefore unlike the first example which explains how the intracellular glycine and C_1 pools might increase through a strictly endogenous alteration it is assumed here in this case that both the glycine and C_1 pools decrease to lower levels than would exist in cells grown in an unsupplemented minimal medium.

The specific activity of STHM in LESV1 grown with purines was 0.56. This same strain when subcultured without purines and thus incubated under the precise conditions described above, had a significantly increased specific activity of STHM to 1.60 or approximately 3-fold greater. The specific activity of course is also significantly greater than that of cells of E. coli K10 grown without any additions (0.93). The value of 1.60 is a mean of 2 experiments done in this way. The specific activity in one of the experiments was recorded as high as 1.90 (Results, Table 7).

Thus the maximum production of STHM so far seen, at least in this particular mutant, appears to be when the intracellular purine concentration is low (i.e. when the intracellular glycine and C_1 pools are lowered considerably) and as the purine concentration is increased (i.e. as the level of the glycine and C_1 pools are increased), STHM is repressed.

The specific activity of STHM has also been shown to increase in cells starved of methionine (Mansouri et al.,

1972). In this work, a methionine requiring auxotroph, E. coli strain 113-3 was incubated with limiting concentrations of methionine and the specific activity of STHM shown to increase. The methyl group of methionine is derived from the C_1 pool. One might expect then that starving the cell of methionine would deplete the C_1 pool just as starving the cell for purine depletes the glycine and C_1 pools. This derepression by methionine starvation is however, different from derepression by purine starvation. In the purine case both the C_1 and glycine pools are drained. In the methionine case, only the C_1 pool is drained. This causes an increase in the specific activity of STHM and presumably an increase in the conversion of serine to glycine and C_1. Glycine should then accumulate and repress STHM. The fact that derepression is seen probably indicates that glycine does not accumulate and it seems likely then, that the enzymes converting glycine to C_1 are derepressed at the same time.

The activity of STHM in cells of E. coli K10 grown with glucose (unsupplemented medium) is 0.93. When various compounds are added to this glucose medium the environmental conditions are altered and the specific activity of STHM changes correspondingly. For example glycine 3000 µg/ml repressed the activity of STHM to 0.40. This implies that the amount of glycine and C_1 units made from serine also changes. In fact in different strains

of E. coli (as compared to strain K10 from which these strains were isolated), grown precisely in the same conditions, the activity of STHM is found to differ in accord with the "needs" of the cells for serine, glycine and one carbon units. For this reason this section of the discussion is concerned with an interpretation of these results as to the probable relation of these "needs" of the cells and the specific activity of STHM seen under these conditions.

The specific activity of STHM in cells of E. coli K10 grown with 3000 µg/ml glycine is less repressed than in cells grown with 3000 µg/ml glycine and the C_1 metabolites together (0.40 vs 0.15).

When the serine-glycine auxotroph strain LESV2 is also grown with 3000 µg/ml the activity of STHM is 0.61. This is different from the activity seen in strain K10 grown under the same conditions. One might ask therefore what difference there might be in the metabolic patterns of the two strains that could account for the difference in the STHM activities? The K10 strain grown on glycine 3000 µg/ml can make serine de novo and can use that serine to make glycine and C_1 units both. The LESV2 strain, isolated from the K10, cannot make serine de novo; when grown on 3000 µg/ml glycine it must use the glycine provided to make its serine. It therefore requires two molecules of

glycine for every molecule of serine (and tryptophan and cysteine) the cell makes. Thus more glycine is used per unit mass in the LESV2 strain than in the K10 strain. Both strains are identical except that LESV2 lacks one of the enzymes of serine biosynthesis which if present could convert glucose to serine. Indeed both strains have the same cell membrane, the same entry problems for glycine and therefore glycine enters the LESV2 strain at the same rate it enters the K10 strain. But the rate of entry of glycine has been considered earlier as a limiting factor in growth of serine-glycine auxotrophs exposed to glycine in the growth medium. If strain LESV2 as described above uses glycine faster than K10 and the entry of glycine is rate-limiting for growth, then one could expect the intracellular glycine pool size to be lower in LESV2 than in K10. That the specific activity of STHM is higher in LESV2 (0.61) than in strain K10 (0.40) both grown with 3000 µg/ml glycine alone, is thus understandable.

A similar comparison can be made between strains K10 and LESV2 grown with serine (200 µg/ml). In this case both cells have the same specific activity for STHM. Again the K10 strain can make its serine de novo. Strain LESV2 unable to make serine de novo, must use whatever serine is provided in the growth medium. Now there is no reason to suspect the cell has any difficulty in transporting serine across the cell membrane. Roberts et al (1955) have shown

that the presence of serine in the growth medium inhibits the de novo synthesis of serine in the prototrophic E. coli K12 strain. When grown with serine in the medium therefore, both K10 and LESV2 rely on exogenous serine and make little or none de novo. One could expect therefore that the size of the serine pool would be similar in K10 and LESV2 as long as serine was present in the medium and that they would both show the same specific activity for STHM.

Though strains K10 and LESV2 have the same specific activity for STHM when grown with 200 µg/ml l-serine (0.61 vs 0.63), strain LESV3 (the serine-requiring auxotroph) has a different specific activity for STHM at 0.81. This particular strain isolated from strain LESV2 also cannot make serine de novo, since it carries the same mutation as LESV2. LESV3 also cannot make C_1 units from glycine and therefore cannot grow on glycine (Newman et al, 1972 unpublished results). Like strain LESV2 it must use whatever serine is available to make glycine and C_1 units both. Apparently this second mutation results in the different specific activity seen for STHM in this strain. Now one would expect the specific activity of STHM to remain constant at approximately 0.6 as long as exogenous serine is available in the medium. The experiments which are discussed here are all done with cultures grown on serine at 200 µg/ml. This concentration is in excess of the require-

ments for most serine-glycine auxotrophs and in any case for strain LESV2 (Figure 13). But it is not in excess of the requirement of strain LESV3. It is thus conceivable that at the time of assaying the cells, the intracellular serine pool of LESV3 could well have been depleted and that may be why the specific activity for STHM is increased.

The preceeding appears to be reasonable. However, if the above argument is correct, then starving the cells for serine should derepress STHM. Yet the specific activity of STHM in cells of strain LESV3 starved of serine (0.55) isn't even as high as the level of activity in the same cells grown with serine (0.81), much less higher. The most that can be said is perhaps the methodologies are different. Cells depleting serine slowly as with the LESV3 strain may increase the specific activity for STHM more readily than when the cells are abruptly transferred to a medium containing little or no serine. The cells may not be able to adapt to such sudden changes. Once again it may be that these cells because they are without glycine, are unable to make protein. Thus there is no observed increase in the specific activity of STHM. The same could be true of strain LESV3 (the 2 step serine-requiring auxotroph isolated from strain LESV2) starved for serine. The specific activity of STHM may therefore not be able to derepress because of the reasons given above. The other

possibility is that the reaction is not reversible *in vivo* in the *E. coli* cell, at least under conditions where it is not necessary to be so, and in these experiments described here, one might expect the enzymes of the phosphorylated pathway to be derepressed and not STHM.

It is clear from the preceeding discussion that the specific activity of STHM in cells of *E. coli* is regulated in accordance with environmental conditions. The activity responds in particular to variations in the availability of glycine and one carbon groups. The exact regulatory factors are not known but can be explained in terms of the intracellular glycine pool size or in terms of both the glycine and C_1 pool sizes together, but not in terms of the size of the C_1 pool alone.

The action of STHM has so far been considered in converting serine to glycine and one carbon groups. It is however, a reversible enzyme at least *in vitro* in *E. coli* and one could ask whether glycine and one carbon groups are converted to serine *in vivo* in cells of *E. coli* grown in minimal medium, or whether this pathway is used by cells only when no other alternative is possible (for example a serine-glycine auxotroph grown on glycine). If serine is made exclusively in *E. coli* K10 by the phosphorylated pathway described in the introduction of this thesis,

one would suppose that the cell might make serine starting from phosphoglyceric acid and glycine from serine, but not serine from glycine.

The specific activity of STHM in cells of E. coli grown in physiological concentrations of l-serine is repressed, however, repression is slight and in any case may not be due to serine since isoleucine and valine were also added. Higher concentrations of serine had no further effect. These results are not easily explained since unless serine is also made from glycine via STHM one would not expect STHM to be repressed.

If the repression seen here were biologically significant and if glycine and C_1 were converted to serine in significant amounts in the E. coli cell, one might therefore expect that lowering the intracellular serine pool (in a manner analogous to the purine example described above) would result in an increase in the specific activity of STHM. Again an attempt was made to lower the intracellular serine pool size by an entirely endogenous alteration using a cysteine requiring auxotroph, E. coli strain JM140. (The carbon skeleton of cysteine is derived solely from serine). In this case no derepression of STHM activity was observed. However, it may be that very little serine is converted to cysteine and thus the intracellular serine concentration may not drop significantly (Jones-Mortimer, 1972, personal communication).

With strain LESV2 (the serine-glycine auxotroph) grown in serine was starved for serine (again a direct analogy to growing strain LESV2 with glycine and starving for glycine) the specific activity also failed to increase significantly.

At the start of the discussion it was suggested that if glycine repressed STHM the cell would be starved for one carbon groups. It is now clear that glycine does repress STHM while the cell continues to grow normally. One might then ask what the source of C_1 units is in the presence of glycine?

The normal donor of C_1 units in the E. coli cell appears to be serine via STHM (McGilvery, 1970). However, if high concentrations of glycine repress STHM, the need for C_1 must be met from some other source. Another principal donor is glycine by a less well understood series of reactions (Blakely, 1969). If this were the only other source, one would expect the enzymes of this pathway to be present in higher concentrations in the presence of glycine. This is in accord with the evidence of Newman and Magasanik (1963) that these glycine to C_1 enzymes do vary with environmental conditions and are induced by glycine and repressed by high levels of C_1. In fact it was previously suggested that the reason STHM was not fully repressed when grown with 3000 µg/ml glycine was because

much of the C_1 units did indeed come from glycine itself and therefore the intracellular glycine pool size could not remain as high as one might expect with this particular concentration of glycine.

However alternative C_1 sources may also exist in E. coli such as the conversion of pyruvate to formate (Blakely, 1969) or the conversion of serine by a pathway not involving STHM (Newman et al., 1972 unpublished results).

The glycine to C_1 enzymes may provide a source of C_1 in glycine repressed E. coli cells. However, strain LESV3 does not have all these enzymes and thus cannot convert glycine to C_1. It therefore must rely on some other source besides glycine to donate C_1. Not a great deal of eivdence can be presented in this thesis in favor of this suggestion; however, it has been shown that strain LESV3 which cannot use glycine to make C_1 also cannot utilize its serine as well as strain LESV2 which can use its glycine to make C_1 units. Since this is the only difference between the two strains, it appears that the ability of LESV2 to convert glycine to C_1, increases the efficiency of the utilization of serine. Serine or some derivative thereof may therefore be a donor.

In this thesis, the level of STHM activity is reported for cells grown in a variety of conditions. The variations in the level of activity are interpreted in terms of possible regulation mechanisms, e.g. the effect of the pool size of various compounds on the level of activity of STHM.

For purposes of discussion, it has been assumed that we are dealing with repression of enzyme synthesis. In the next few pages two of the more common control methods, feedback inhibition and repression of enzyme synthesis, are discussed in detail. The data in this thesis are consistent with the model of repression described, i.e. repression of STHM synthesis by glycine, but the mechanism actually used by the cell has not been investigated.

Mechanisms Regulating Enzyme Activity in Bacteria

The chemical composition of the E. coli cell is not absolutely fixed. If the cellular environment changes, i.e., if there are changes in the culture medium, then the type and number of some of their molecules also change. In general this response to environment is as economical as possible and the cell does not produce material which is already available under particular conditions. This integration of the cell with its environment implies the existence of sensitive mechanisms which control the rate of

synthesis of the various molecules. Such controls have in fact been described. The two best known control mechanisms are 1) regulation of the quantity of enzyme formed (repression-derepression) and 2) regulation of the activity of a given quantity of enzyme (inhibition).

Feedback Inhibition

A given amount of enzyme may function at different rates depending on the availability of substrates and the presence of inhibitors. One of the commonest controls which modifies the activity of a given amount of enzyme is a mechanism known as end-product inhibition. This works on the principle that the final product of a biosynthetic series inhibits the first enzyme specific to that series by reversibly combining with it. Thus in a series $A \xrightarrow{1} B \xrightarrow{2} C \xrightarrow{3} D$ the final product D inhibits the first enzyme (1) and thus no A is converted to B, or indeed C or D. Thus in the presence of appreciable concentrations of D, no further synthesis of D can take place.

An example of this is seen in histidine biosynthesis. The first enzyme in this pathway, phosphoribosyl pyrophosphate-ATP pyrophosphorylase converts ATP and 5-phosphoribosyl 1-pyrophosphate to N^1-(5'-phosphoribosyl)-ATP and pyrophosphate. This enzyme however, does not function in the presence of histidine (Figure 14). Though histidine

is in no way directly involved in the reaction catalyzed by this enzyme, the enzyme is structured in such a way that it cannot function when histidine is bound to it. This assures that in the presence of histidine no further histidine will be made (Martin, 1963).

A similar feedback inhibition of STHM by glycine or C_1 or even serine is conceivable. However, this was not tested for in this thesis.

End-Product Repression

The end-product of a biosynthetic pathway may act to prevent further synthesis of enzyme(s). This second control on biosynthesis reduces the amount of given enzyme(s) in the biosynthetic pathway by a mechanism known as end-product repression. The principle in this case is that the end-product of a biosynthetic sequence prevents (represses) the synthesis of the enzyme(s) specific to that sequence. Unlike end-product inhibition described above where there is a direct interaction between the end-product and the enzyme, control by repression involves an interaction between the end-product and the machinery that makes the end-product (Lehninger, 1970).

Again referring to the histidine example, *E. coli* cells growing in a medium without histidine contain all the enzymes necessary for the biosynthesis of histidine.

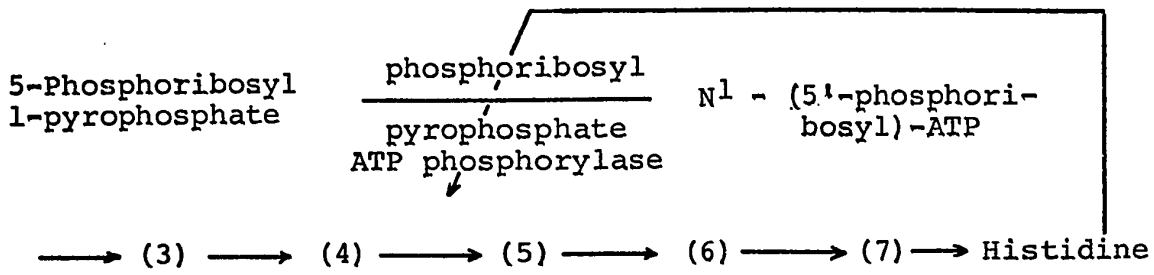


Figure 14. Feedback inhibition: The end product histidine inhibits phosphoribosyl pyrophosphate-ATP pyrophosphorylase - the first enzyme in the reaction sequence leading to histidine. As a result no further synthesis of any of the precursors of histidine and thus histidine itself can take place (i.e. subsequent enzymes in the pathway have no substrates with which to interact). Once histidine is used up the inhibition is released and synthesis continues.

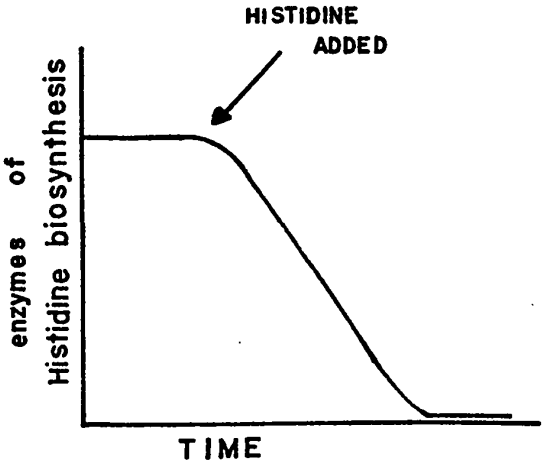


Figure 15. Repression of enzyme synthesis: When histidine is added to a growing culture of E. coli further synthesis of the histidine biosynthetic enzymes stops. With subsequent generations these enzymes are diluted out. Once the histidine is used up new synthesis of these enzymes will resume (DeBusk, 1968).

However, when the growth medium is supplemented with histidine the corresponding biosynthetic enzymes decrease until they are entirely missing (Figure 15 , Debusk, 1968). The end-product which decreases the formation of an enzyme is known as a co-repressor (e.g. histidine is the co-repressor of the histidine biosynthetic enzymes (Watson, 1970).

For the same reasons when glycine is added to the growth medium of cells of E. coli K10 its corresponding biosynthetic enzyme (STHM) is repressed. Thus it appears that this particular mechanism would be consistent with the findings of this thesis and that glycine acts as the co-repressor of STHM. The actual details of what could be considered a model for repression by glycine is discussed next.

Mechanism of End-Product Repression

That the actual formation of an enzyme is prevented in repression is clear from studies on the amount of enzyme contained by cells grown in different conditions (Martin, 1963). However, the mechanism of this inhibition of enzyme synthesis remained unclear until Jacob and Monod (1961) elaborated their general hypothesis of repression.

It is known of course that a DNA molecule, by virtue of the sequence of its component nucleotides, contains all the information for specifying the structure of the proteins

of the cell. The synthesis of any given protein is accomplished by mRNA molecules being transcribed from the relevant DNA sequence. The rate of formation of a given enzyme then, will depend on the rate at which its corresponding DNA sequence is transcribed into mRNA.

The Jacob and Monod hypothesis invokes three DNA sites in order to explain the controls on mRNA and enzyme synthesis (Figure 16, Watson, 1970). The first site, the structural gene site, determines the amino acid sequence of an enzyme (i.e. it determines the enzyme structure but not the amount of enzyme formed). The amount of enzyme actually formed depends on how often this first site is transcribed into mRNA. The transcription only occurs when the site next to the structural site, the operator site, is free (i.e. transcription is initiated at a free operator site). The third site, the regulator, codes for repressor molecules. These repressors are made at all times and are free in the cytoplasm. When the repressor combines with the co-repressor (the end-product of the pathway) the combined form binds to the operator site (thus the operator is no longer free) and prevents transcription. This in turn prevents any further synthesis of the enzyme. To continue with the histidine example, the enzymes specific to the histidine biosynthetic pathway are specified by structural genes which can be repressed by the end-product histidine. The repressor molecule made by the regulatory gene combines

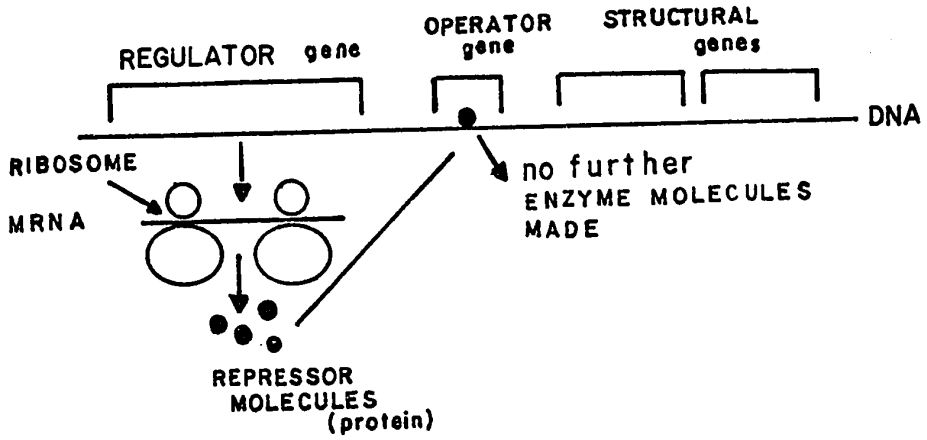


Figure 16. The interaction of repressor and operator controlling the synthesis of E. coli proteins: The structural genes provide the code for the synthesis of specific proteins. The regulatory gene codes for the synthesis of 'repressors' which may be active or inactive depending on whether they are attached to the co-repressor. The active repressor (i.e. repressor-co-repressor complex) can bind to the operator gene site on the chromosome and turn off the transcriptional duties of the structural genes. As a result no further enzyme molecules of that particular type are made.(Watson, 1970).

with the co-repressor histidine to form a repressor-co-repressor complex, which in turn combines with the operator site. Because the operator site is occupied, transcription of the structural genes and thus enzyme synthesis is prevented (Lehninger, 1970).

The fact that glycine represses STHM suggests that STHM is specified by a particular sequence of DNA consisting of a regulatory, operator and structural gene site responsible for coding for the enzyme. Glycine may combine with a repressor molecule made by the regulatory gene for STHM to form a repressor - co-repressor complex which in turn combines with the operator site to shut off synthesis of the enzyme.

Any one pathway can be controlled by either repression or feedback inhibition or both of these mechanisms. Though they have the same final effect the mechanisms differ considerably in the time it takes for their effect to be seen. Mechanisms of enzyme regulation that act at the level of enzyme synthesis (repression) require a considerable time period before an effect on the enzyme level can be seen. Once enzyme synthesis is prevented, several generations of growth are necessary before the enzyme level decreases through succeeding dilutions as each new cell is produced. E. coli whose division time is approximately one hour would therefore require a period of several hours

to attain maximal repression. On the other hand inhibition of enzyme activity (end-product inhibition) affects the enzyme product pool with no significant time lag. As soon as the enzyme interacts with the end-product, the enzyme can no longer interact with the substrate. Hence there is almost an immediate response to a change in the environment.

Most microorganisms control their metabolism closely through these and other mechanisms. However, there are several examples of microorganisms which lack one or the other control, one of which has been described by Kinoshita et al. (1957) who found that the mutant Micrococcus glutamicus could regulate its entire metabolism normally except for the biosynthesis of glutamate. This one defect resulted in the entirely inefficient use of glucose - up to 50% of which was diverted to make glutamic acid, which is normally not used by the cell in such high quantities. It can be assumed that there is no feedback mechanism to turn off excess glutamic acid synthesis. This is clearly detrimental to the bacteria. It is however, advantageous in the commercial production of monosodium glutamate as a flavor-enhancing agent both for the food industry and for table use.

These are not the only controls known in bacteria. Enzyme inhibition and repression of enzyme synthesis are

both negative control methods. Other positive control systems have been described, of which the arabinose operon is the most thoroughly studied (Englesberg, 1971). However, because STHM synthesis can be explained by a negative feedback model, it was the only model discussed here.

A Possible Application in the Design of A Therapeutic Method

Hyperglycinemia is an inborn error of amino acid metabolism (Ando et al. 1968). Patients suffering from this disease have high levels of glycine in the blood, urine and cerbrospinal fluid and this may result in mental retardation. The non-ketotic form is due to the absence of non-function of the enzyme system responsible for the conversion of glycine to NH_3, CO_2 and hydroxymethyl-FH_4, (glycine to C_1 enzymes). This conclusion is supported by the demonstration of Ando et al. (1968) that in patients suffering from this disease, the first carbon of glycine was very slowly converted to CO_2 while there was almost no conversion of the second carbon to the β carbon of serine.

Since hyperglycinemic organisms cannot meet their C_1 requirements from glycine (i.e. glycine cannot participate as a C_1 donor) the conversion of serine to glycine proceeds at an accelerated rate. Consequently glycine accumulates.

deGroot et al. (1970) tried to find diets that would result in lowered levels of plasma glycine. Whereas plasma glycine levels remained elevated even on serine and glycine free diets, they were lowered by the administration of high doses of methionine. They proposed that methionine in the diet itself provides C_1 units since in mammals methionine is well known to be a methyl donor when metabolized to cysteine. Since the C_1 units are provided by methionine there is less necessity to convert serine to glycine (i.e. to make C_1 from the β carbon of serine) and consequently, glycine does not accumulate.

deGroot et al. also suggested that histidine may be used as a replacement for methionine; however, they point out that caution must be taken when raising the plasma levels of histidine since this may lead to hyper-histidinemia, also associated with mental retardation. Recently Benevenga and Harper (1970) have reviewed the fact that methionine is one of the most toxic amino acids. As little as 1.5% of methionine included in a low protein diet decreased the growth rate of rats while higher levels resulted in serious damage to the tissues.

It is therefore interesting to speculate on another and perhaps a better means of controlling the metabolic accumulation of glycine in hyperglycinemics. One possible method would be to replace methionine or histidine with

either purines or thymidine. These compounds might be less toxic while still having the same effect. One could also approach this problem by administering purines, methionine and histidine either all together or in various combinations. The use of such compounds each at low non-toxic concentrations might make it unnecessary to use any one compound at high, toxic concentrations.

SUMMARY

Serine transhydroxymethylase (STHM) catalyzes the hydrolytic cleavage of l-serine to form glycine and C_1 derivatives of N^5,N^{10}-methylene tetrahydrofolic acid. Glycine is used by the cell for protein synthesis, purine biosynthesis and to a certain extent as a source of C_1 units. C_1 derivatives of tetrahydrofolic acid (FH_4) are involved in the biosynthesis of a variety of metabolites (C_1 metabolites) including histidine, methionine, thymidine and purines.

Thus it appears that STHM plays a key role in a number of cellular reactions and its activity would be expected to be carefully controlled in accordance with the cells' needs for its products. To investigate controls on the activity of this enzyme cells of Escherichia coli K10 were grown in the presence of various combinations of the above compounds and the specific activity of STHM determined in extracts of these cells. Cells grown with glucose as a carbon source (i.e. with no addition to the growth medium) had specific activities for STHM of 0.93 (μmoles $^{14}HCHO$/hr/mg protein). Glycine alone at low concentrations (100 μg/ml) had little effect on the specific activity of the enzyme (0.91). However cells grown with glycine alone at high concentrations (3000 μg/ml) had markedly repressed specific act-

ivities for STHM (i.e. 0.40). This repression by glycine at high rather than low concentrations can be explained in terms of a transport (uptake) problem for glycine in the E. coli cell.

Cells grown with the C_1 metabolites alone again had sinificantly repressed levels of activity for STHM (0.385). Glycine (100 or 3000 µg/ml) in combination with the C_1 metabolites repressed STHM further than either glycine or the C_1 metabolites alone. (0.30 and 0.15 respectively).

The specific activity of STHM could also be derepressed when cells of E. coli strain LESV1 were starved for purines (i.e. glycine and C_1). Attempts at derepressing the activity of the enzyme by starving directly for glycine were unsuccessful.

In the above experiments the activity of STHM responds to either glycine or C_1 in the growth medium and the entire effect may be explained by the -ize of the intracellular glycine pool. An additional effect of the intracellular pool size of C_1 has not been excluded.

The reaction is readily reversible in vitro. If the enzyme also converts glycine and C_1 to serine in vivo, one might expect some repression by the 'end product' serine. Slight repression of STHM activity has in fact been seen. However attempts at showing derepression of STHM in cells starved of serine again were unsuccessful. It is concluded that there is no evidence that the level of STHM activity

varies with respect to the availability of its product in the direction of glycine conversion to serine.

The activity of the enzyme has also been determined in mutants affected in the area of serine-glycine metabolism. The results of these experiments have been interpreted in terms of the cells' overall requirements for serine, glycine and one-carbon groups and a suggestion made as to a possible application of this data in medical practice.

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