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The Synthesis of Vitamin B_6 by some Mutant Strains of *Escherichia coli*

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SUMMARY: *Escherichia coli* B166 synthesizes as much vitamin B_6 when growing on serine or glycine plus glycolaldehyde as does its parent (*E. coli* B) on unsupplemented medium. Suspensions of strain B166 harvested after growth on serine + glycine plus glycolaldehyde, and incubated with glucose and NH_4^+, only synthesize vitamin B_6 when glycolaldehyde is added; serine + glycine increases the synthesis. Strain 22-99 produces more vitamin B_6 when grown on glycolaldehyde plus serine + glycine than when it is grown on the amino acids alone, while, depending upon the conditions of growth, vitamin B_6 synthesis by suspensions is absolutely or partially dependent on serine + glycine plus glycolaldehyde. However, serine + glycine plus glycolaldehyde have little effect on the biosynthesis of vitamin B_6 by wild-type *E. coli* during growth, or in washed suspensions.

The growth of certain mutants of *Escherichia coli* (strains B166 and 22-99), which respond suboptimally to vitamin B_6, is more rapid and extensive when serine or glycine is also provided. The same organisms grow in the absence of added vitamin when supplied with either glycine or serine plus glycolaldehyde (Morris & Woods, 1959).

Serine or glycine plus glycolaldehyde would be able to replace vitamin B_6 for *Escherichia coli* B166 if they were the sole products of function of the vitamin when growth was taking place in an ammonium + glucose medium. This is most unlikely; an alternative explanation is that the mutant is able to synthesize an amount of the vitamin which is sufficient to satisfy all normal functions except those of synthesizing serine, glycine and glycolaldehyde. A third possibility is that glycolaldehyde and serine or glycine are concerned in the biosynthesis of vitamin B_6 by *E. coli*; the present work provides some evidence that this may be true for the mutants *E. coli* B166 and 22-99.

METHODS

Organisms. *Escherichia coli* PA15, a mutant strain requiring L-serine or glycine, was obtained from Dr Barbara Wright. The other *E. coli* auxotrophs were those used by Morris & Woods (1959).

Growth. The media, conditions of growth, and the method of assessment of growth were as described previously (Morris & Woods, 1959).

Suspensions. Organisms were harvested after maximal growth on chemically-defined media and washed twice with 0·1 M-phosphate buffer containing 4×10^{-4} M-$MgSO_4$ before final suspension in the same buffer containing the test substrate. When it was desired to deplete the organisms of endogenous substrates a suspension of organisms (equiv. to 5 mg. dry wt./ml.) in 50 ml. of the above buffer was rocked in a large ⊥-tube at 37° for 2–3 hr. before

re-harvesting and washing. Test incubations were with rocking for not more than 8 hr. at 37° in ⊥-tubes of 19 mm. diameter holding 10 ml. of suspension.

Extraction and assay of vitamin B_6. This was carried out by the method described by Morris, Hughes & Mulder (1959), using *Saccharomyces carlsbergensis* 4228*c*.

Miscellaneous. DL-Serine and glycine were frequently used in equimolar mixture referred to as serine + glycine, the molar concentration being given in terms of the total amino nitrogen. 'Activated glucose' refers to solutions of glucose in 0·04M-phosphate buffer (pH 7·4) autoclaved at 115° for 7 min. All other chemicals were as described by Morris & Woods (1959).

RESULTS

The total amount of vitamin B_6 synthesized by wild-type *Escherichia coli* during growth on a simple glucose + ammonium + salts medium (G2) increased commensurately with growth and did not significantly decrease thereafter, though the distribution of vitamin between organism and culture medium changed. The proportion of vitamin present in the organisms was high during the logarithmic phase of growth and then decreased rapidly at the onset of the stationary phase (Fig. 1). The decrease in internal vitamin B_6 after growth ceased could be accounted for by its increase in the culture medium. In a comparison of vitamin B_6 synthetic abilities, it is therefore insufficient to assay only the bacterial content of vitamin, for this will vary with the age of the culture.

The synthesis of vitamin B_6 *by mutant strains of* Escherichia coli *which respond to vitamin* B_6 *or to serine + glycine plus glycolaldehyde*

Synthesis of vitamin B_6 *during growth.* Production of vitamin B_6 by mutant B166 growing on medium G2 supplemented with serine + glycine plus glycolaldehyde or activated glucose, was quantitatively similar to that of its parent strain (*Escherichia coli* B) growing on the unsupplemented basal medium. The results given (Table 1*a*) are in terms of the total vitamin B_6 content of the culture (organisms plus medium) at full growth. There was no increase in vitamin B_6 when the media were supplemented with casein hydrolysate, a mixture of purines and pyrimidines, and vitamins other than B_6.

Escherichia coli 22-99 grows optimally on serine + glycine plus glycolaldehyde and less well on serine + glycine alone (Morris & Woods, 1959). More vitamin B_6 was synthesized when the organism was grown on glycolaldehyde plus serine + glycine than when it was grown on the amino acids alone (Table 2*a*).

Synthesis of vitamin B_6 *by suspensions.* After growth of *Escherichia coli* B166 in the presence of 10^{-6}M-pyridoxine only about half of this compound was recoverable as assayable vitamin B_6; there was, however, complete recovery when a washed suspension was incubated aerobically with the same concentration of pyridoxine for 8 hr. at 37°. It was therefore possible to study vitamin B_6 synthesis by suspensions of this mutant.

Suspensions of strain B166 harvested from growth media containing pyridoxine, synthesized little vitamin B_6 when subsequently incubated in phosphate buffer containing Mg^{++}, NH_4^+, glucose, serine + glycine and glycolaldehyde. Organisms grown in the absence of added vitamin B_6 were able to synthesize the vitamin in much better yield. The magnitude of the synthesis

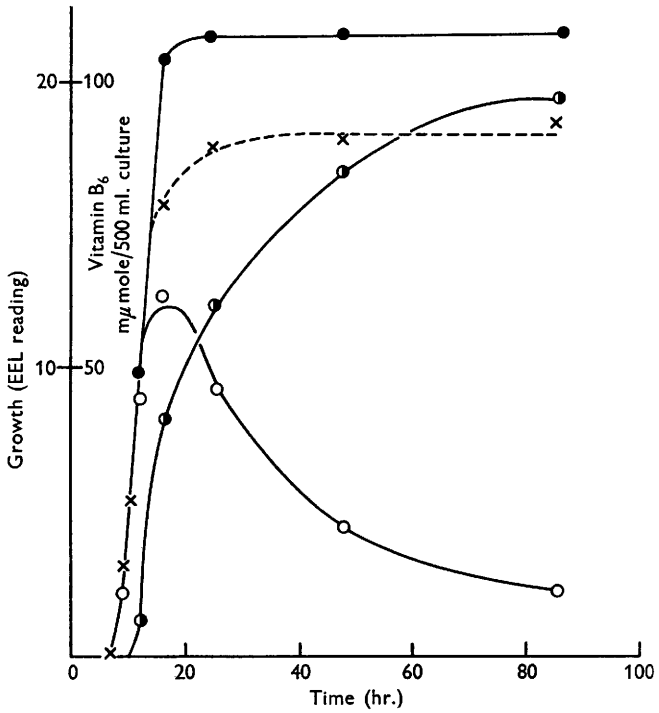

Fig. 1. Distribution of synthesized vitamin B_6 between organisms and culture medium during growth of *Escherichia coli* strain 15 on medium G2. Growth of organisms (× – – – ×), vitamin B_6 content of organisms (○) and of culture medium (◑) giving total synthesis (●).

differed from experiment to experiment even though great care was taken to grow, harvest and treat each batch of organisms identically. When such washed suspensions were supplied with glucose plus NH_4^+ some synthesis of vitamin B_6 always occurred, but this was invariably increased by the addition of glycolaldehyde. With serine + glycine and glycolaldehyde as sole substrates, some vitamin B_6 was formed, though not as much as when glucose was also present. The basal synthesis from glucose plus NH_4^+ might be due to the carry-over of some precursor(s) formed by the organisms during their growth on serine + glycine plus glycolaldehyde—the substrates whose effect on vitamin B_6 biosynthesis was to be tested. In further experiments, suspensions were incubated in buffer alone before use, in the hope of removing such endogenous material. Organisms so depleted were unable to synthesize vitamin B_6 from glucose plus NH_4^+ alone, but did so when glycolaldehyde (10^{-3}M) was added. Serine + glycine increased the synthesis but NH_4^+ was still required for

maximum vitamin production (Table 1*b*). The yield of vitamin B_6 again differed from batch to batch of organisms though all showed an absolute requirement for glycolaldehyde for any synthesis of the vitamin. Little synthesis occurred in the absence of glucose, which may serve both as a source of energy and of another compound which would otherwise have to be provided by endogenous metabolism.

Table 1. *Synthesis of vitamin* B_6 *by* Escherichia coli B166

(*a*) *During growth.* Medium G2 supplemented as shown with serine + glycine 10^{-3}M; glycolaldehyde 10^{-3}M; activated glucose 10 mg./ml. Cultures (500 ml.) were incubated aerobically for 40 hr. at 37°.

Supplements	Vitamin B_6 synthesis (mμmole pyridoxine/g. dry wt.) Prototroph (strain B)	Auxotroph (strain B166)
None	465	No growth
Serine + glycine plus		
activated glucose	400	255
glycolaldehyde	470	420

(*b*) *By washed suspensions.* Depleted, washed organisms (equiv. 6 mg. dry wt./ml.) grown on medium G2 with serine + glycine and glycolaldehyde (10^{-3}M each) were suspended in phosphate buffer (0·1M; pH 7·0), supplemented as shown with glucose 1 % (w/v); NH_4^+ as $(NH_4)_2SO_4$ 5×10^{-2}M; serine + glycine 10^{-3}M; glycolaldehyde 10^{-3}M. Incubated for 6 hr. at 37°.

Supplements	Vitamin B_6 biosynthesis (mμmole pyridoxine/g. dry wt.) Experiment (*a*)	(*b*)	(*c*)	(*d*)
None	0	0	0	0
Serine + glycine + glycolaldehyde	—	—	—	4
Glucose plus				
serine + glycine	20	0	0	0
glycolaldehyde	20	0	0	0
glycolaldehyde + serine + glycine	320	140	220	120
Glucose + NH_4^+	10	0	0	0
Glucose + NH_4^+ plus				
glycolaldehyde	—	100	180	140
glycolaldehyde + serine + glycine	—	—	370	210

Since good growth of strain 22–99 took place on medium G2 with serine + glycine alone, it was considered possible that suspensions of the organism so grown might not show an obligatory requirement for glycolaldehyde for vitamin B_6 synthesis. Such suspensions proved capable of synthesizing vitamin B_6 to some extent from glucose plus NH_4^+. However, the synthesis was still further improved by the addition of glycolaldehyde. Suspensions of depleted organisms grown on serine + glycine plus glycolaldehyde synthesized vitamin B_6 only when provided with serine + glycine plus glycolaldehyde (Table 2*b*).

Table 2. *Biosynthesis of vitamin* B_6 *by* Escherichia coli 22-99

(*a*) *During growth.* Supplements (10^{-3}M) to medium G2 were as shown and the cultures (50 ml.) were incubated aerobically (with rocking in large ⊥-tubes) at 37° until maximum growth was achieved.

Supplements	Growth (EEL reading)	Total vitamin B_6 synthesis (mμmole pyridoxine/g. dry wt.)
None	0	0
Serine + glycine	21	180
Serine + glycine + glycolaldehyde	28	424

(*b*) *By washed suspensions.* Depleted, washed organisms (equiv. 6 mg. dry wt./ml.) grown on medium G2 with *A*, serine + glycine; *B*, serine + glycine plus glycolaldehyde, suspended in phosphate buffer (0·1 M, pH 7) with supplements as shown in concn. given in Table 1. Incubated for 6 hr. at 37°.

	Vitamin B_6 synthesis (mμmole pyridoxine/g. dry wt.) in presence of			
	NH_4^+		Serine + glycine	
Other additions	*A*	*B*	*A*	*B*
Glycolaldehyde	0	0	0	70
Glucose	75	0	60	0
Glucose + glycolaldehyde	135	0	180	180

Table 3. *Vitamin* B_6 *synthesis by* Escherichia coli *DW during growth at* 25°

Medium G2 was supplemented as shown with alanine, methionine, serine + glycine and glycolaldehyde (10^{-3}M each); amino acid mixture AA-SG in 1/10 dilution to give final individual amino acid concn. of 5×10^{-4}M. Cultures (50 ml.) were incubated aerobically at 25° until best growth was achieved

Supplements	Growth (EEL reading)	Total vitamin B_6 synthesis (mμmole pyridoxine/g. dry wt.)
None	0	0
DL-alanine + DL-methionine	26	33
AA-SG	22	28
AA-SG + serine + glycine + glycolaldehyde	23	73

Vitamin B_6 *synthesis by a temperature-sensitive vitamin* B_6 *auxotroph*

Strain DW, although showing an absolute requirement for vitamin B_6 at 37°, may be grown at 25° in the presence of any mixture of amino acids containing DL-alanine and DL-methionine; the amount of vitamin B_6 then synthesized is less than one-tenth of that formed by prototrophic strains (Table 3). When grown on medium G2 supplemented with (*a*) an amino acid mixture not including serine or glycine (AA-SG), or (*b*) the same mixture with 10^{-3}M each of serine + glycine plus glycolaldehyde, only a little more vitamin B_6 was produced under the latter conditions (Table 3).

Vitamin B_6 *synthesis by wild-type* Escherichia coli

Glycolaldehyde and serine + glycine (both at 10^{-3} M) alone or together had little effect on the amount of vitamin synthesized during growth of *Escherichia coli* strains 15 and B either on glucose-containing medium (G2) or on the lactate medium (L) (Table 4*a*). Although best synthesis was in fact obtained by growth in the presence of a mixture of all these substances, the increase is of doubtful significance.

Serine + glycine and glycolaldehyde (10^{-3} M, each) also did not increase the production of vitamin B_6 by washed suspensions of *Escherichia coli* B above that obtained from glucose plus NH_4^+ (Table 4*b*). Addition of serine + glycine plus glycolaldehyde to the original growth medium had no effect on subsequent vitamin synthesis by harvested organisms.

Table 4. *Biosynthesis of vitamin* B_6 *by wild-type strains of* Escherichia coli

(*a*) *During growth.* Cultures (50 ml.) were incubated aerobically at 37° until best growth was achieved.

	Total vitamin B_6 synthesis (mμmole pyridoxine/g. dry wt.)		
Organism	Strain 15		Strain B
Basal medium	G2	L	L + glucose
Other additions			
None	454	450	450
Serine + glycine + glycolaldehyde	570	452	600

(*b*) *By washed suspensions of strain* B. Washed organisms (7·5 mg./ml.) grown on medium G2, suspended in phosphate buffer (0·1 M, pH 7·0) supplemented with Mg^{++}, and other supplements as shown, glucose 1 % (w/v); NH_4^+ as $(NH_4)_2SO_4$ 5×10^{-2} M; serine + glycine 10^{-3} M; glycolaldehyde 10^{-3} M. Incubated for 8 hr. at 37°.

Supplements	Vitamin B_6 synthesis (mμmole/g. dry wt.)
None	0
Glucose + NH_4^+	340
+ serine + glycine + glycolaldehyde	340

Vitamin B_6 *synthesis of a serine + glycine mutant of* Escherichia coli

It is conceivable that no effect of serine + glycine was demonstrable with prototrophic strains because these organisms are fully capable of synthesizing these amino acids from glucose plus NH_4^+. *Escherichia coli* PA15 was therefore studied as a representative mutant which is incapable of *de novo* synthesis of serine and glycine. When this organism was grown with suboptimal concentrations of glycine the amount of vitamin B_6 synthesized (expressed in terms of amount/g. dry wt. organism) was actually greater than during maximal growth in the presence of an excess of glycine, while vitamin B_6 synthesis from glucose plus NH_4^+ by washed suspensions of organisms so grown was not increased by an excess of glycine (Table 5).

Table 5. *Biosynthesis of vitamin* B_6 *by the serine-glycine mutant* Escherichia coli *PA* 15

(*a*) *During growth.* Medium G 2 (50 ml.) supplemented with glycine as shown. Incubated aerobically with rocking in ⊥-tubes at 37° until best growth was achieved.

Glycine concn. (M)	Growth (EEL reading)	Total vitamin B_6 synthesis: Concn. (M)	Total vitamin B_6 synthesis: (mμmole/g. dry wt.)
5×10^{-2}	66	10^{-6}	500
10^{-2}	60	$1{\cdot}1 \times 10^{-6}$	565
5×10^{-3}	35	$1{\cdot}1 \times 10^{-6}$	1100
2×10^{-3}	15·5	$5{\cdot}5 \times 10^{-7}$	1550
10^{-3}	6	$2{\cdot}8 \times 10^{-7}$	1870
5×10^{-4}	3	$1{\cdot}4 \times 10^{-7}$	1870

(*b*) *By washed suspensions.* Washed depleted organisms (equiv. 6 mg. dry wt./ml.) grown on medium G 2 with 10^{-3} M-glycine (a growth-limiting concentration), suspended in phosphate buffer (0·1 M, pH 7·0) supplemented as shown with glucose 10^{-1} M; NH_4^+ as NH_4Cl 10^{-1} M glycine 10^{-2} M; glycolaldehyde 10^{-3} M. Incubated aerobically for 6 hr. at 37°.

Supplement	Vitamin B_6 synthesis (mμmole/g. dry wt.)
None	0
Glucose + NH_4^+	800
Glucose + NH_4^+ +	
glycine	720
glycolaldehyde	800
glycine + glycolaldehyde	960

DISCUSSION

Serine and glycine have some sparing action on the vitamin B_6 requirement of *Escherichia coli* B 166 which is manifested in a somewhat decreased pyridoxine requirement and increased resistance to inhibition by 4-deoxypyridoxine or isoniazid in the presence of these amino acids (Morris & Woods, 1959). However, it does not appear that this can be the sole explanation of all their effects upon the organism. It is unlikely that the replacement of vitamin B_6 is due solely to the establishment of conditions in which there is a reduced requirement for the vitamin for the following reasons: (*a*) serine + glycine plus glycolaldehyde have no marked pyridoxine-sparing action with auxotrophs M 2 and DW; (*b*) glycolaldehyde alone has no pyridoxine-sparing effect; (*c*) the amount of vitamin B_6 synthesized by strain B 166 when growing on serine + glycine plus glycolaldehyde equals that produced by the parent strain in unsupplemented medium. The last finding immediately suggests that serine + glycine and glycolaldehyde may be concerned with the actual synthesis of vitamin B_6 by this mutant.

It is probable that the growth of mutant DW at 25°, when vitamin B_6 synthesis is less than one-tenth that of wild-type strains, is a consequence of the metabolic block being incomplete at this temperature. In the presence of certain essential products of its function (in this instance, amino acids,

particularly D-alanine and L-methionine) the decreased amount of vitamin that is now synthesized is sufficient to fulfil all other growth requirements.

That the situation with strains B166 and 22-99 is different from that in strain DW is confirmed by the results obtained with washed suspensions. These support the view that glycolaldehyde (and possibly serine + glycine as well) is concerned with the actual synthesis of the vitamin by the first two strains. However, it cannot be concluded that even glycolaldehyde is an actual reactant in the synthesis of vitamin B_6 by these mutants; it may act indirectly either by sparing a normal intermediate or by inhibiting a deleterious reaction. The role of the serine or glycine is confused by their ability also to accelerate and enhance growth of strains B166 and 22-99 in the presence of added pyridoxine (Morris & Woods, 1959), an action which cannot be explained by their possible participation in a sparing or synthesis of vitamin B_6. This stimulation of growth might be due to conversion of the vitamin into a more active form, or to increased permeability of the organisms to added vitamin. Yet maximal growth in the absence of glycine or serine was not obtained even in the presence of very high concentrations of all known forms of the vitamin. It is possible that interference with vitamin B_6 biosynthesis in the organism may be only one consequence of the presence of an incomplete metabolic block which also controls synthesis of serine and glycine.

No confirmation of a function of serine, glycine and glycolaldehyde in vitamin B_6 biosynthesis has been possible with prototrophic strains. Some restriction of vitamin B_6 biosynthesis must operate even in the wild-type organism and it is not inconceivable that the amount of synthesis normally attained *de novo* from glucose plus NH_4^+ cannot be surpassed when precursors are provided which, even if directly on the route of biosynthesis, are rather remote from the final product.

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