WIJESUNDERA, S. & WOODS, D. D. (1960). *J. gen. Microbiol.* 22, 229-241

Suppression of Methionine Synthesis in *Escherichia coli* by Growth in the Presence of this Amino Acid

By S. WIJESUNDERA* AND D. D. WOODS

Microbiology Unit, Department of Biochemistry, University of Oxford

SUMMARY: The ability of washed suspensions of *Escherichia coli* Y 44 (which requires histidine and *p*-aminobenzoic acid for growth) to synthesize methionine from homocysteine was decreased almost to zero when the organism was harvested from a growth medium containing methionine itself. There was a similar effect, though in no case quite so severe, with eleven other strains of the organism. In further experiments with strain Y 44 the effect was found to be specific to methionine; the presence during growth of no other amino acid tested decreased subsequent synthesis by more than 20 %. Ability to synthesize methionine was lost and regained on single successive cultivations in the presence and absence of the amino acid; there was also no evidence from viable counts on agar media $\pm$ methionine for a genetic change. 'Inactive' organisms (methionine-grown) were not activated by changing the nature of the precursor of the methyl group nor by adding heated extracts of yeast or of 'active' organisms. There was no evidence that 'inactive' organisms produced an inhibitor of methionine synthesis. It is concluded that growth with methionine leads to the non-production of an enzyme(s) concerned directly in the addition of the one-carbon unit to homocysteine.

In the course of a study of the role of *p*-aminobenzoic acid in the synthesis of methionine from homocysteine by suspensions of certain strains of *Escherichia coli* it was discovered incidentally that growth in the presence of methionine greatly decreased the ability of the harvested organisms to synthesize that amino acid; a brief report of these results was made at the time (Wijesundera & Woods, 1958). Using basically the same test system, Cohn, Cohen & Monod (1958) independently obtained essentially similar results. This was one of the earliest cases described of suppression of the production of enzymes by the ultimate product of their activity. A number of other cases have since been described (see reviews by Pardee 1959*a, b*) and the term enzyme repression has been suggested by Vogel (1957) to describe the phenomenon. It represents one of the two mechanisms (Umbarger & Brown, 1958) whereby ultimate products of metabolic pathways may exert a 'negative feed-back control' of their own formation; the other mechanism is inhibition of the actual activity of enzymes by such products. In view of the considerable current interest in regulation of cell metabolism it seemed of interest to record our earlier observations in detail.

Burgeois, Wiame & Lelouchier-Dagnelie (1960) have recently obtained evidence, based entirely on the kinetics of growth with different types of inocula, that methionine may similarly repress the formation of enzymes concerned in its synthesis by *Proteus morganii*.

* Present address: Department of Biochemistry, University of Ceylon, Colombo 8, Ceylon.

METHODS

Organisms and media. Most of the work was carried out with *Escherichia coli* strain Y 44, a double mutant which requires both histidine and *p*-aminobenzoic acid for growth. The organism was obtained from Dr E. L. Tatum; stock cultures were maintained on tryptic meat agar slopes, transferred monthly, incubated 18 hr. at 37° and stored at 4°.

The basal medium (*A*) for the preparation of washed suspensions was the glucose + salts medium of Davis & Mingioli (1950), except that the glucose was added to the rest of the medium before autoclaving at 115° for 10 min. The required growth factors (*p*-aminobenzoic acid, 10^{-6} M; DL-histidine, 2×10^{-4} M) were also added (when needed) to the other constituents before autoclaving.

In certain experiments a number of other strains of *Escherichia coli* (both phototrophs and auxotrophs) were used; their sources and growth requirements are given in Table 4 with the results obtained. Growth was again on medium *A* supplemented with the appropriate growth factor; unstable substances (e.g. pyridoxal) were separately sterilized by Seitz-filtration.

Measurement of mass of organisms. Extent of growth and the dry weight of suspensions of organisms was assessed with an E.E.L. photoelectric colorimeter (Evans Electroselenium, Harlow, Essex, England) using 6 mm. tubes and a neutral density filter; uninoculated medium or water was used to obtain the zero setting. Within the range used (0–20) there was a linear relationship between instrument reading and dry weight of organism; a reading of 15 was equivalent to 0.5 mg. dry wt. organism/ml. with strain Y 44 and was closely similar with other strains.

Preparation of washed suspensions. Growth medium (100 ml.) in Roux bottles was autoclaved for 10 min. at 115° and inoculated with 0.1 ml. of a 2- to 50-fold dilution of a 24 hr. culture on the same medium. The size of the inoculum varied with the different strains (and supplements to the medium) and was so adjusted as to obtain cultures in the logarithmic phase of growth (0.07–0.17 mg. dry wt. organisms/ml.) after 12–15 hr. of incubation. The organisms were centrifuged out, washed once on the centrifuge with the culture volume of 0.067M-phosphate buffer pH 7.4 and finally suspended in 0.133M buffer at twice the concentration (0.5 mg. dry wt./ml.) desired in the solution used for testing methionine synthesis.

Reaction mixture for methionine synthesis. The test system for methionine synthesis was based on the experiments of Gibson & Woods (1960). The washed organisms were suspended at a final concentration of 0.25 mg. dry wt./ml. in 2.5 ml. volumes of solution *MS* which contained: DL-homocysteine (0.006M), DL-serine (0.01M), glucose (0.02M), *p*-aminobenzoic acid (10^{-6} M), cobalamin (10 µmg./ml.) and 0.067M-phosphate buffer, pH 7.4. Serine and cobalamin were included because, although not essential, they increase methionine synthesis by most strains of *Escherichia coli* (Gibson & Woods, 1960). *p*-Aminobenzoic acid was added since it is a growth requirement for strain Y 44 with which most of the work was done and would be required

absolutely for methionine synthesis if this organism were harvested (as it sometimes was) from media deficient in this factor. When not so required the presence of *p*-aminobenzoic acid had no adverse effect on methionine synthesis. The suspensions in solution *MS* were incubated in upright tubes (125 × 16 mm.) for 16 hr. at 37°. Pilot experiments showed that methionine synthesis with suspensions of strain Y44 was linear over this period of time. In each experiment there was a control with organisms heated to 100° for 5 min. before adding to solution *MS*.

After incubation tubes were placed in a boiling water bath for 10 min. to stop the reaction and to release soluble constituents of the organisms. The supernatant liquids after centrifuging were used for the assay of methionine and for other experiments. Results are expressed throughout in terms of μ m-mole L-methionine formed/mg. dry wt. organism/hr.

Estimation of methionine. The microbiological assay with *Leuconostoc mesenteroides* P60 as described by Gibson & Woods (1960) was followed exactly. This organism gives no response with D-methionine, or to precursors of methionine (cysteine, homoserine, cystathionine, homocysteine) which were sometimes present in the material under assay. Typical experimental samples gave dose/response curves superimposable on that given by pure methionine.

Chemicals. Solutions of DL-homocysteine were prepared and standardized as described by Gibson & Woods (1960). L-Cystathionine was isolated from the mycelium of *Neurospora crassa* (strain H-98A) by a method based on that of Horowitz (1947). The L- and D-isomers of methionine were commercial specimens.

The cobalamin used was 'Anacobin' (British Drug Houses Ltd., London, England). Pyridoxal was a gift from Dr K. Folkers; stock solutions (2×10^{-2} M) were sterilized by Seitz-filtration. Members of the folic acid group were as described by Lascelles, Cross & Woods (1954).

Phosphate buffers were prepared from stock solutions of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and KH_2PO_4 .

Chromatography. Descending chromatograms were developed on Whatman no. 1 paper with 'butanol-acetic acid' (the upper layer of a mixture of *n*-butanol, glacial acetic acid and water in the proportion (by vol.) of 4:1:5). The detecting agent (for amino acids) was 0.1% (w/v) ninhydrin in *n*-butanol. The method was used both to confirm the presence of methionine in experimental samples and to check the purity of certain amino acids.

Viable counts. The method used was based on the colony count technique of Miles & Misra (1938). Serial tenfold dilutions of the cultures were made in basal medium *A*; those selected for counting contained 800 or less organisms/ml. Standard drops (0.025 ml.) were deposited on sectors of plates containing 15 ml. of 2% (w/v) washed agar (Adelberg & Meyers, 1953) in medium *A* containing *p*-aminobenzoic acid (10^{-6} M) and DL-histidine (2×10^{-4} M); DL-methionine when added was 10^{-3} M. The plates were previously dried 3 hr. at 40° to ensure that the drops soaked in within 25 min. of deposition; incubation was for 20 hr. at 37°. Sufficient colonies (about 400) were counted to decrease the random sampling error to $c. \pm 10\%$.

RESULTS AND DISCUSSION

Effect of growth on methionine on the ability of Escherichia coli Y 44 to synthesize methionine

Lampen, Jones & Roepke (1949) found that a *p*-aminobenzoic acid auxotroph (strain 273/884) of *Escherichia coli* grew on a medium devoid of *p*-aminobenzoic acid when provided with thymine, purines and a mixture of certain amino acids. Strain Y 44, which also requires *p*-aminobenzoic acid for growth, was found in the present work to behave similarly; the composition of the supplements used (*E* and *F* of Gibson & Woods, 1960) is shown in Table 1.

Suspensions of organisms harvested after growth in the presence of *p*-aminobenzoic acid synthesized methionine well in solution *MS* (condition I, Table 1). When *p*-aminobenzoic acid was replaced for growth by the supplements there was insignificant synthesis of methionine by the suspensions whether the amino acids were supplied as the synthetic compounds or as the L-isomers in the form of casein hydrolysate (conditions II and III, Table 1). The main

Table 1. *Effect of growth medium on the synthesis of methionine by suspensions of Escherichia coli Y 44*

Medium *A* was supplemented as shown. Cultures were harvested at a density of 0.1 mg. dry wt./ml. Test for methionine synthesis by suspensions as in Methods.

Supplements: (a) Casein hydrolysate, equivalent to 1.5 g. casein/l. (b) Amino acids (mg./l.); DL-methionine (10), glycine, DL-serine, DL-threonine, DL-histidine, DL-lysine and DL-tyrosine (each 40). (c) Purine/pyrimidine (mg./l.); thymine (80), uracil, adenine, guanine and xanthine (each 5). (d) DL-Histidine (2×10^{-4} M). (e) *p*-Aminobenzoic acid (*p*-AB) (10^{-6} M).

Con- dition	Supplement to medium <i>A</i>					L-Methionine formed (μ m-mole/mg. organism/hr.)
	Casein hydrolysate (a)	Amino acids (b)	Purine/ pyrimidine (c)	Histidine (d)	<i>p</i> -AB (e)	
I	—	—	+ / —	+	+	63–94*
II	—	+	+	+ / —	+ / —	< 1
III	+	—	+	+ / —	+ / —	< 1
IV	—	Methionine only	—	+	+	1

+, present; —, absent; + / —, either present or absent.

* Range of values from over twenty experiments.

part of the effect was traced to methionine itself; the presence of this amino acid alone at 6.6×10^{-5} M in the growth medium almost completely suppressed synthesis by the harvested organisms (condition IV, Table 1). *p*-Aminobenzoic acid was necessarily present for growth under these conditions, i.e. in the absence of the other components of the replacement mixture.

In these experiments the organisms had been harvested during logarithmic growth; the result was not changed when older or younger cultures were used.

Two types of organisms may thus be obtained at will. Those able to synthesize methionine from the components of solution *MS* will in future be referred to as 'active' organisms and were harvested after growth on medium

A + *p*-aminobenzoic acid + histidine. 'Inactive' organisms were obtained from the same medium supplemented with 10^{-3} M-DL-methionine.

Quantitative aspects. The effect of adding methionine to the growth medium was just perceptible at 10^{-6} M and became almost maximal at 10^{-4} M (Fig. 1). In a large number of experiments with 10^{-3} M DL-methionine the % decrease in methionine synthesis varied from 90 to 100. Towards the end of this work the values were more often at the lower end of this range; the strain appeared to have undergone a minor variation and full inhibition was not recovered in substrains derived from single colony isolations.

Stereo-specificity. A specimen of D-methionine showed approximately one-hundredth the activity of L-methionine (Fig. 1). However, on assay with *Leuconostoc mesenteroides* P 60 the specimen showed an apparent L-methionine content of 2.5%. The effect of the D-isomer might thus have been due to this contamination. On the other hand, it may have intrinsic activity, since D-methionine has been shown to be converted to the L-isomer by suspensions and cell-free enzyme preparations of *Escherichia coli* (Karunairatnam & Wijesundera, 1955, 1956).

Fig. 1. Methionine synthesis by suspensions of *Escherichia coli* strain Y 44 grown in the presence of L-, D- and DL-methionine. Growth was in medium *A* + *p*-aminobenzoate (10^{-6} M) + DL-histidine (2×10^{-4} M) and methionine L- (∇), D- ($\bullet$) or DL- ($\circ$) at concentrations indicated. (Concn. of DL-compound given in terms of L-isomer.) Synthesis of methionine by harvested organisms measured as in Methods.

Specificity of the effect

Amino acids. Amino acids (usually the DL-compounds) were added singly to the growth medium and the organisms harvested when the cultures had reached approximately the same density (about 0.1 mg. dry wt./ml.) All the amino acid specimens used for the results given in Table 2 were free of methionine as judged by chromatography on paper and by microbiological assay with *Leuconostoc mesenteroides* P 60.

The only amino acids (apart from methionine) which lead to significant

suppression of methionine synthesis by suspensions (Table 2) were DL-valine, DL-alanine and DL-aspartic acid, and even here the effect was small (15–20 % suppression). The still smaller effects (4–10 %) given by certain other amino acids were not regarded as significant in view of the known variation in activity of 'active' organisms derived from different cultures (Table 1).

The case of L-leucine. A similar effect of methionine on the methylation of homocysteine has been reported by Cohn *et al.* (1958) who also found L-leucine to cause a 50 % decrease in methionine formation. In the present work one specimen of L-leucine gave considerable suppression (42 %), but this was traced

Table 2. *Effect of various amino acids during growth on the activity of harvested organisms*

Escherichia coli Y44 grown on medium A + histidine (2×10^{-4} M) + *p*-aminobenzoate (10^{-6} M), with the further addition of the test amino acid at 10^{-3} M. Cultures harvested at equal density (about 0.1 mg. dry wt./ml.) Control organisms grown without added amino acid. Test for methionine synthesis by suspensions as in Methods.

Activity in synthesizing methionine expressed as percentage increase (+) or decrease (–) compared with control.

Amino acid added growth medium	Change in ability to synthesize methionine as compared with control (%)	Amino acid added to growth medium	Change in ability to synthesize methionine as compared with control (%)
DL-Alanine	– 15	DL- and L-Leucine	0 to – 10*
DL- α -Aminobutyric acid	0	DL-Lysine	– 10
L-Arginine	– 7	DL-Methionine	– 90 to – 100*
DL-Aspartic acid	– 15	DL-Norvaline	+ 40 to + 60*
DL-Ethionine	– 10	DL-Ornithin	0
DL-Glutamic acid	0	DL-Phenylalanine	– 4
Glycine	+ 40 to + 50*	DL-Proline	0
DL-Histidine	– 10	DL-Serine	0 to – 10*
DL-Homoserine	0	DL-Threonine	– 8
L-Hydroxyproline	0	DL-Tryptophan	0
DL-Isoleucine	– 8	DL-Tyrosine	– 5
		DL-Valine	– 18

* Range derived from three or more experiments.

to the presence of 3.3 % L-methionine as impurity (by microbiological assay). Similarly, suppressions of 25 % and 38 %, respectively, by certain specimens of DL-leucine and DL-ethionine were also shown to be due to contamination with methionine.

Enhancement of methionine synthesis. Addition of glycine or DL-norvaline to the growth medium enhanced methionine synthesis by the harvested organisms by about 50 %. This effect was not further investigated; Gibson & Woods (1960) found synthesis by suspensions of a serine/glycine auxotroph of *Escherichia coli* to be markedly increased when growth was on glycine rather than serine.

Precursors of methionine. There is strong presumptive evidence (reviewed by Davis, 1955) that the pathway of methionine synthesis in *Escherichia coli* proceeds by a condensation of cysteine with homoserine to yield cysta-

thionine which is then degraded to homocysteine. Addition of these precursors of methionine to the growth medium, however, had no effect on the synthesis of methionine from homocysteine by the harvested organisms (Table 8). Growth in the presence of a mixture of the specific components of the reaction mixture for methionine synthesis (homocysteine, serine, *p*-aminobenzoic acid, cobalamin) led to a slight decrease of ability to form methionine (Table 8). This may be an effect of methionine itself formed from readily available substrates during the early stages of growth. Cystathionine or homocysteine (each 10^{-3} M) when added as well as methionine to the growth medium did not overcome the effect of the last-named amino acid.

Table 8. *Effect of precursors of methionine during growth on the activity of harvested organisms*

Escherichia coli Y 44 grown in medium A plus required growth factors with the supplements listed. Methionine synthesis of harvested organisms tested as in Methods.

Supplement to growth medium (M)	L-Methionine formed (μ m-mole/ mg. dry wt. organism/hr.)
L-Cysteine (10^{-3})	78
L-Cystathionine (10^{-3})	75
DL-Homocysteine (10^{-3})	73
DL-Methionine (10^{-3})	< 1
None	75
DL-Homocysteine (6×10^{-3}) + DL-serine (10^{-3}) + cobalamin (7×10^{-9})	60

Tests with other strains

Eleven other strains of *Escherichia coli* were tested for the effect of methionine during growth on the subsequent ability to synthesize methionine; ten were auxotrophs with various growth factor requirements, the other was a prototroph. The additional presence of methionine in medium A usually caused rather more rapid growth, but since this varied in degree with different strains the cultures were harvested when they reached similar optical densities rather than after a standard time of growth. A standard concentration of 10^{-3} M-DL-methionine (which suppresses activity of strain Y 44 almost completely) was chosen.

In every case (Table 4) there was considerable decrease of ability to synthesize methionine as compared with the controls grown without methionine (range 74–94 %). In no case, however, was the effect quite so severe as with strain Y 44. Strain M200 (a cobalamin auxotroph) was grown by Gibson & Woods (1960) in the presence of methionine in order to obtain cobalamin-deficient organisms; the effect is less marked with this organism (74 %) than with most of the others and would presumably have been less still with the lower concentration of methionine (1.3×10^{-4} M) used by those authors.

Table 4. *Repression of methionine synthesis in other strains of Escherichia coli*

Medium *A* supplemented with growth factors indicated at the concentration stated; DL-methionine (10^{-3} M) also added for methionine-grown organisms. Test for methionine synthesis by suspensions as in Methods.

Strain*	Growth supplement		L-Methionine formed (μ m-mole mg. dry wt. organism/hr.) when grown with methionine		% Decrease due to methionine
	Substance	Concn. (M)	Absent	Present	
15/9/518	None	—	78	12	84
273/384	<i>p</i> -Aminobenzoate	10^{-7}	50	8	84
48/34	<i>p</i> -Aminobenzoate	10^{-7}	81	6	93
M200	Cobalamin	7×10^{-10}	47	12	74
22/99	Pyridoxal	10^{-7}	109	6	94
D/W	Pyridoxal	10^{-6}	94	17	82
B166	Pyridoxal	2×10^{-7}	94	24	74
B170	Pyridoxal	2×10^{-7}	81	16	80
55/5	DL-Serine	10^{-3}	50	6	88
159/11	DL-Tryptophan	2×10^{-4}	51	6	88
73	Adenine and guanine	7×10^{-4} each	50	5	90
Y 44	<i>p</i> -Aminobenzoate and DL-histidine	10^{-6} 2×10^{-4}	78	< 2	> 98

* The sources of the strains used were: 48/34, M200, 22/99, 55/5 and 159/11 from Dr B. D. Davies; B166 and B170 from Dr J. Gots; D/W (derived from M154-59L) from Dr E. Work; 15/9/518 and 273/384 (A.T.C.C. nos. 9723 and 9723a) from Dr R. Roblin.

Nature of the effect

Genotypic or phenotypic change. It was first necessary to establish whether or not growth in the presence of methionine had caused a permanent and inheritable change in the organism. Organisms grown in the presence of methionine were used as inoculum for further cultures grown in the presence and in the absence of methionine; the activities of the harvested organisms were examined. This procedure was repeated serially four times (Fig. 2). The ability to synthesize methionine was lost or regained completely by single subcultivations in media with and without methionine, respectively. Although unlikely, it was still possible that a few surviving representatives of the respective genotypes, able or not to synthesize methionine, had survived the passages and were selected out during subsequent growth in the absence and presence of methionine, respectively. Alternatively, non-synthesizing mutants might arise continuously and be selected out. Viable counts were therefore made of typical suspensions of 'active' and 'inactive' organisms on agar plates, with and without methionine (Table 5); there was no significant difference between any of the counts. Assuming any 'active' organisms still present in 'inactive' suspensions to have the normal degree of activity, it would not have been expected that more than about 5% of the organisms present in the 'inactive' suspension could have grown in the absence of methio-

nine; however, within the experimental error all the organisms grew. It was concluded therefore that the loss of ability to synthesize methionine is a purely phenotypic change.

Dependence on growth. 'Active' organisms were harvested from growth without methionine and incubated in buffered glucose with and without methionine before testing in the usual way for ability to synthesize the amino

Fig. 2. Effect of successive subcultivations of *Escherichia coli* Y 44 in the presence or absence of methionine on the synthesis of methionine by suspensions. Growth was in medium A plus *p*-aminobenzoate (10^{-6} M) and DL-histidine (2×10^{-4} M) with methionine added (----) or omitted (—). [75] etc. indicates activity of harvested suspensions (μ m-mole L-methionine formed/mg. dry wt. organism/hr.). 'Inactive' or 'active' organisms were used to inoculate succeeding cultures as indicated.

Table 5. Viable counts of 'active' and 'inactive' organisms in media with and without methionine

'Active' and 'inactive' organisms (strain Y 44) obtained by growth in medium A plus required growth factors without and with DL-methionine (10^{-3} M) respectively. Cultures harvested at 0.17 mg. dry wt./ml., and organisms washed once on the centrifuge with medium A. Suitable dilutions counted (see Methods) on agar medium with and without methionine. Activity in synthesizing methionine checked as usual.

Organisms	L-Methionine formed (μ m-mole/mg. dry wt. organism/hr.)	Viable count (organisms $\times 10^{-6}$ /ml. original culture) on counting medium with methionine	
		Absent	Present
'Inactive'	5	$2.50 \pm 12\%$	$2.80 \pm 18\%$
'Active'	98	$2.09 \pm 14\%$	$2.53 \pm 18\%$

acid. There was no loss of activity on incubation with methionine (Table 6). Indeed the only change was a slight comparative decrease in activity on incubation without methionine; it is possible that methionine protects the enzyme system from slight inactivation. It appears therefore that the enzyme systems, once formed, are stable and that loss of activity by the organisms is not due to the formation of a stable complex between the enzyme and methionine. In view of the relatively small amounts of methionine synthesized it was not possible for technical reasons of assay to investigate directly whether methionine

itself at relatively high concentrations inhibited the formation of methionine by 'active' organisms. However, there was no inhibition by quantities up to five times those normally formed in synthesis experiments; the total recovery of methionine from tests with 'active' organisms in solution *MS* was the sum of that added plus that formed in controls without added methionine (Table 7).

Table 6. *Stability of harvested 'active' and 'inactive' organisms*

'Active' and 'inactive' organisms (*Escherichia coli*, strain Y44) obtained as in Table 5. Suspensions (0.25 mg. dry wt./ml.) were first incubated (6 hr., 37°) in 0.066M-phosphate buffer (pH 7.4) containing glucose (0.1M) and the supplements shown. Organisms re-harvested and tested for methionine synthesis as usual.

Supernatants 'A' and 'I' were obtained by heating cultures of 'active' and 'inactive' organisms to 100° for 10 min. and centrifuging.

Type of organism	Supplement to first incubation mixture	Final activity (μ m-mole L-methionine formed/mg. dry wt. organism/hr.)
'Active'	None	51
	DL-Methionine (10^{-3} M)	63
	Supernatant 'I'	69
'Inactive'*	None	<1
	Supernatant 'A'	6

* Original activities of untreated organisms were (μ m-mole L-methionine formed/mg. dry wt. organism/hr.): 81 ('active') and < 1 ('inactive').

Table 7. *Recovery of methionine added to the synthesis system*

'Active' and 'inactive' organisms obtained as in Table 5. DL-Methionine added in quantities stated (in terms of the L-isomer) to solution *MS* for tests of methionine synthesis.

Type of organism	L-Methionine (μ -mole/mg. dry wt. organism)		% Added L-methionine recovered
	Added	Recovered	
'Active'	0.0	1.0	—
	0.9	2.0	105
	4.5	5.4	97
'Inactive'	0.0	< 0.02	—
	0.9	0.8	89
	4.5	4.2	93
	10.0	9.4	94

'Active' organisms were not inactivated by incubation with heated culture filtrates of 'inactive' organisms. However, 'inactive' organisms acquired very slight activity on incubation with heated culture filtrates of the 'active' type (Table 6); this may have been due to slight growth occurring during this incubation in the absence of methionine. Finally, harvested 'inactive' organisms remained 'inactive' when incubated in glucose without methionine (Table 6). It was concluded provisionally that the effect of methionine is on the formation of an enzyme or enzymes affecting directly or indirectly its own synthesis rather than on the activity of those enzymes once formed.

Possible destruction of substrates or product. It remained possible that 'inactive' organisms had acquired an ability either to destroy methionine as rapidly as formed or to metabolize by competitive pathways the major substrates (serine and homocysteine) required for its synthesis. Methionine added to suspensions of 'inactive' organisms in solution *MS* in amounts up to ten times that synthesized by 'active cells' was recovered almost completely (Table 7). 'Inactive' organisms were incubated in solution *MS* and the organisms were removed by centrifuging. 'Active' organisms were then suspended in the supernatant liquid and the incubation continued; half as much methionine was formed as when fresh solution *MS* was used. Failure to synthesize methionine by 'inactive' organisms cannot therefore be due to removal of homocysteine and serine by other reactions.

When 'inactive' organisms were incubated in solution *MS* it was noted that, although no methionine was formed, as judged by microbiological assay, a ninhydrin-positive spot with an R_f value in butanol+acetic acid slightly greater than methionine itself appeared on paper chromatograms. This material was not identified, but it appeared to be similar if not identical to material observed by Gibson & Woods (1960) with auxotrophs of *Escherichia coli*, again in conditions when methionine was not synthesized (lack of cobalamin or *p*-aminobenzoic acid).

Table 8. *Methionine synthesis by mixtures of 'active' and 'inactive' organisms*

'Active' and 'inactive' organisms were obtained as in Table 5. Both were used in solutions *MS* (test for methionine synthesis) at concn. of 0.25 mg. dry wt./ml.

Type of organism	L-Methionine formed (μ m-mole/mg. dry wt. 'active' organism/hr.)
'Active'	75
'Inactive'	6
'Active' + 'inactive'	70

Possible production of inhibitors. The synthesis of methionine by 'active' organisms was not affected by the concurrent presence of an equal quantity of 'inactive' organisms in the synthesis system (Table 8). The failure of the 'inactive' organisms cannot therefore be due to the production of a freely permeable inhibitor.

Question of one-carbon donor. Failure to synthesize methionine might be due to failure to activate homocysteine or to inability to use the normal source of the one-carbon unit. A number of possible alternate ultimate sources of the methyl group were substituted for serine in solution *MS* in tests with 'inactive' organisms; glycine, formate, glyoxylate, glycollic acid, threonine, homoserine, histidine and tryptophan all gave negative results.

Folic acid and other cofactors. There is strong evidence (Gibson & Woods, 1960) that *p*-aminobenzoic acid must first be converted to some form of folic

acid before it is effective as cofactor for methionine synthesis by *Escherichia coli*. The possibility that 'inactive' organisms were failing to bring about such conversion was tested by techniques similar to those of Lascelles & Woods (1952); both 'active' and 'inactive' organisms were, however, equally proficient in synthesizing factors active for the growth of *Lactobacillus casei* and *Leuconostoc citrovorum*. Pteroylglutamic acid and its N^5 -formyltetrahydro derivative had no effect when added to solution *MS*.

'Inactive' organisms were not made active by altering the concentrations of *p*-aminobenzoic acid and cobalamin in the reaction mixture or by adding pyridoxal, pyridoxal phosphate, *p*-hydroxybenzoic acid, yeast extract or combinations of all the factors so far mentioned. Furthermore, heat-treated extracts of 'active' organisms did not activate methionine-grown organisms. It is unlikely therefore that the inability of 'inactive' organisms to methylate homocysteine can be attributed to absence of a necessary cofactor.

Conclusions. All the evidence so far obtained is compatible with the view that when the organism is grown in the presence of methionine it loses the ability to form some enzyme or enzymes concerned in the over-all methylation of homocysteine. Preformed enzymes are not lost and the process appears to be bound to growth. It is likely that the enzyme which is not produced is one concerned with the utilization of homocysteine rather than with the provision of the one-carbon unit which becomes the methyl group of methionine; a deeper analysis must await experiments with isolated cell-free enzymes. Loss of the ability to form the enzyme(s) might be due to the prevention of the formation of the enzyme-forming system or to a specific inhibition of its activity.

One of us (S.W.) was indebted to the University of Ceylon for a post-graduate scholarship during the period of this work. We are grateful to several colleagues (see Table 4) and especially Professor B. D. Davis for providing strains of organisms. The work was aided by a grant from the Rockefeller Foundation to the Department.

REFERENCES

- ADELBERG, E. A. & MEYERS, J. W. (1953). Modification of the penicillin technique for the selection of autotrophic bacteria. *J. Bact.* **65**, 348.
- BURGEAIS, S., WIAME, J. M. & LELOUCHIER-DAGNELIE, H. (1960). Étude du 'rétro-contrôle' de synthèses d'enzymes par des acides aminés au cours de la croissance de *Proteus morganii*. *Biochim. biophys. Acta* (in the Press).
- COHN, M., COHN, G.-N. & MONOD, J. (1953). L'effet inhibiteur spécifique de la méthionine dans la formation de la méthionine-synthase chez *Escherichia coli*. *C.R. Acad. Sci., Paris*, **236**, 746.
- DAVIS, B. D. (1955). Intermediates in amino acid biosynthesis. *Advanc. Enzymol.* **16**, 247.
- DAVIS, B. D. & MINGIOLI, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B₁₂. *J. Bact.* **60**, 17.
- GIBSON, F. & WOODS, D. D. (1960). The synthesis of methionine by suspensions of *Escherichia coli*. *Biochem. J.* **74**, 160.
- HOROWITZ, N. H. (1947). Methionine synthesis in *Neurospora*. The isolation of cystathionine. *J. biol. Chem.* **171**, 255.

- KARUNAIRATNAM, M. C. & WIJESUNDERA, S. (1955). The utilization of D-methionine by *Escherichia coli*. *Proc. Ceylon Ass. Advanc. Sci.* p. 88.
- KARUNAIRATNAM, M. C. & WIJESUNDERA, S. (1956). Vitamin B₆ and the conversion of D-methionine to L-methionine. *Proc. Ceylon Ass. Advanc. Sci.* p. 44.
- LAMPEN, J. O., JONES, M. J. & ROEPKE, R. R. (1949). Mutant strains of *Escherichia coli* unable to synthesize *p*-aminobenzoic acid. *J. biol. Chem.* **180**, 423.
- LASCELLES, J., CROSS, M. J. & WOODS, D. D. (1954). The folic acid and serine nutrition of *Leuconostoc mesenteroides* P60 (*Streptococcus equinus* P60). *J. gen. Microbiol.* **10**, 267.
- LASCELLES, J. & WOODS, D. D. (1952). The synthesis of 'folic acid' by *Bacterium coli* and *Staphylococcus aureus* and its inhibition by sulphonamides. *Brit. J. exp. Path.* **33**, 288.
- MILES, A. A. & MISRA, S. S. (1938). The estimation of the bactericidal power of blood. *J. Hyg., Camb.* **38**, 732.
- PARDEE, A. B. (1959*a*). Mechanisms for control of enzyme synthesis and enzyme activity in bacteria. In *Regulation of Cell Metabolism*, p. 295. Ed. G. E. W. Wolstenholme & C. M. O'Connor. London: J. and A. Churchill.
- PARDEE, A. B. (1959*b*). The control of enzyme activity. In *The Enzymes*, ed. P. D. Boyer, H. Lardy & K. Myrbäck, 2nd ed., p. 681. New York: Academic Press Inc.
- UMBARGER, H. E. & BROWN, B. (1958). Isoleucine and valine metabolism in *Escherichia coli*. VII. A negative feed back mechanism controlling isoleucine biosynthesis. *J. biol. Chem.* **233**, 415.
- VOGEL, H. J. (1957). Repression and induction as control mechanisms of enzyme biogenesis: the 'adaptive' formation of acetylornithinase. In *The Chemical Basis of Heredity*, ed. W. D. McElroy & B. Glass, p. 276. Baltimore: Johns Hopkins Press.
- WIJESUNDERA, S. & WOODS, D. D. (1958). The effect of growth on a medium containing methionine on the synthesis of this amino acid by *Bacterium coli*. *Biochem. J.* **55**, ciii.

(Received 20 July 1959)