METHIONINE GENES AND ENZYMES OF *SALMONELLA TYPHIMURIUM*

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1. INTRODUCTION

THE combined genetical and biochemical studies of large numbers of similar auxotrophs and other mutants of *Salmonella typhimurium* LT-2 initiated by Demerec and his associates (Demerec, Blomstrand and Demerec, 1955; Demerec and Demerec, 1955; Hartman, 1956) has yielded, amongst other things, much information on genetic fine structure (Demerec and Hartman, 1959), the arrangement of genes with respect to functions (Hartman, Loper and Šerman, 1960) and the control of protein synthesis (Ames and Hartman 1963). One of the most striking features of this work has been the demonstration of close linkage or clustering of genes concerned with related functions (Demerec and Demerec, 1955; Glanville and Demerec, 1960; Ames and Hartman, 1963; Margolin, 1963) which is compatible with the operon model for the control of protein synthesis (Jacob and Monod, 1961) and its modifications (Ames and Martin, 1964).

Preliminary work with less than 50 methionine mutants of *S. typhimurium* LT-2 led to the recognition of five genes concerned with imprecisely defined steps in the biosynthesis of methionine (Glover, 1958; Smith, 1961). Only two of these genes were close enough to be co-transduced (Smith, 1961). The clustering of methionine genes therefore appeared to be very limited and it was thought desirable to investigate the system further. As about 200 more methionine mutants became available they, and those previously used, were investigated genetically and biochemically with the aim of identifying new genes, investigating as far as possible the enzymic deficiencies of all methionine genes, analysing the fine structure of each of them and relating the results of these studies to the location of the genes on the bacterial chromosome. This work was aided greatly by the precise definition of the pathway of methionine biosynthesis firstly in *Escherichia coli* (Wijesundera and Woods, 1962; Rowbury and Woods, 1964; Woods, Foster and Guest, 1965) and then in *S. typhimurium* (Foster, personal communication; Flavin, Delavier-Klutchko and Slaughter, 1964; Rowbury, 1964*a*). The mapping studies using Hfr conjugation techniques were carried out elsewhere (Sanderson and Demerec, 1965).

The system of nomenclature and terminology is the same as that used previously (Smith, 1961) except that mutant symbols have been

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modified to coincide with the alterations of Sanderson and Demerec (1965, table 7).

2. MATERIALS AND METHODS

Bacteria, phage and media. All of the 243 different methionine (*met*) mutants used (table 1) were derived either from wild type or auxotrophic mutants of *Salmonella typhimurium* strain LT-2. Forty-four (*met2-68* inclusive) had been used previously (Smith, 1961, table 1) and 199 (*met69-422* inclusive) were newly isolated either as spontaneous mutants or following X-ray, ultra-violet, 2-aminopurine or diethyl sulphate induction. One hundred and ninety-five of the new mutants were obtained from Dr M. Demerec, Brookhaven National Laboratory, Upton, Long Island, New York, one from Professor Stocker, Lister Institute, Chelsea Bridge Road, London (*met422*, originally SL 582) and three in this department (*met419*, *420* and *421*).

The maintenance of stock cultures, the preparation and centrifugation of liquid cultures of bacteria, the testing of the growth responses of mutants to methionine intermediates, the demonstration of cross-feeding between mutants and the preparation and assay of suspensions of phage PLT-22 (P2-2) were as previously described (Smith, 1961). The media used were also the same except that Oxoid Nutrient Broth and Nutrient Agar (NA) were used as additional nutrient media and the minimal medium (MM) was supplemented when required with the following additional growth factors at a final concentration of 20 μg./ml.: DL-allo cystathionine, L-arginine HCl, L-histidine HCl, L-proline and L-tyrosine.

Heat sensitivity. This was tested by streaking loopfuls of two saline suspensions containing approximately 2×10^8 and 2×10^4 orgs./ml. respectively onto two plates of minimal agar (MA) or appropriately supplemented MA medium and incubating one plate at 37° C. and the other at 25° C. Observations were made during four days incubation. Mutants were designated heat sensitive if the growth of both inocula after two days incubation was clearly visible and similar to that of wild type at 25° C. but negligible at 37° C.

Transduction. In addition to a standard transduction technique for *met* mutants previously described (Smith, 1961) a method was devised for the rapid detection of complete and abortive transduction (*i.e.* recombination and complementation) between large numbers of mutants. It was similar to that of Hartman, Hartman and Šerman (1960). Up to 9 crosses could be carried out on a Petri plate of 9 cm. diameter. Overnight broth cultures of the recipient strains were centrifuged and resuspended in 1/10th volume of broth (*c.* 2×10^{10} orgs./ml.) and 0·1 ml. quantities spread evenly on the surface of MA plates which had been dried for three or four days at 37° C. When the inocula had soaked in, drops of donor phage preparations containing *c.* 1×10^{10} particles were placed on the surface of the plates with Pasteur pipettes. They each covered an area of 1-1·5 cm. diameter. These plates were then incubated for 48 hours at 25° C. or 37° C., depending upon the *met* mutants used. Transduction of *met* mutants with a wild type donor or between mutants of different *met* genes gave 40-100 recombinant colonies per area and that between mutants of the same *met* gene 0-20. The diameter of the colonies varied from 0·5-2 mm. Minute colonies of 0·035-0·05 mm. diameter resulting from abortive transduction could also be detected with a binocular stereoscopic microscope ($\times 25$ magnification). They were more easily visible after keeping the plates for a further 24 hours at room temperature.

3. RESULTS

(i) *The gene deficiencies of new mutants*

The gene deficiencies of most of the 199 new methionine mutants were identified (table 1) on the basis of their growth responses to known intermediates in the biosynthesis of methionine and the frequency of transduction between them and representative mutants of the 5

TABLE 1

Methionine (met) *mutants of* Salmonella typhimurium

metA

Mutant	Other markers	Mode of origin	Reversion Index	Other properties
7	*cysA21*	u/v	o	dl
10	*cysB12*	u/v	+	
15		u/v	o	dl
18		sp	o	lys
19	*tryA8*	sp	+	hs
21	*tryA8*	sp	+	
22	*tryB2*	sp	o	dl
40	*purG2*	sp	+	
43	*purE11*	sp	o	dl
44	*purE11*	sp	+	
48		sp	+	
53		sp	+	hs
54		sp	+++	
55		sp	+	
69		sp	+	
73		sp	o	
89		sp	+	
94		u/v	+	
98		sp	+	hs
99		u/v	+	hs
105		sp	+	
159		sp	+	
177	*araq*	sp	+	
200	*araq*	*2ap*	+	
201	*araq*	*2ap*	+	
215	*araq*	*2ap*	+	
222	*araq*	*2ap*	++	
229		2ap	+	
240		2ap	+	
247		2ap	+	
253		2ap	+	
303	*araq*	X	+	
309	*araq*	X	o	dl
313	*araq*	X	+	
321	*araq*	X	+	
322	*araq*	X	++	
326	*araq*	X	+	
329	*araq*	X	+	
332	*araq*	X	+	
339	*araq*	X	+	
351		sp	++	
388		2ap	+	
391		2ap	++	
414		2ap	+	

metB

Mutant	Other markers	Mode of origin	Complementation group	Reversion index	Other properties
6		u/v	G	+	
16	*cysB16*	sp	A	++	
17	*cysB16*	sp	A	++	
20	*tryA8*	sp	A	+	
23		sp	K	+	
34	*purG2*	sp	K	+	
36	*purG2*	sp	A	o	dl
39	*purG2*	sp	C	+	
41	*purE11*	sp	L	+	
45	*purE11*	sp	G	+	

TABLE 1—(Continued)

metB					
Mutant	Other markers	Mode of origin	Complementation group	Reversion index	Other properties
49		sp	A	+	
66		sp	A	+	
78		sp	I	++	
81		sp	A	+++	
85		sp	F	++	
87		sp	K	+	
91		u/v	A	+	
92		u/v	A	+	
93		u/v	A	++	
160	*ser*	sp	K	+	
178	*ara7*	sp	H	+	
194	*ara9*	2ap	B	+++	hs
202	*ara9*	2ap	A	+	
203	*ara9*	2ap	E	+++	
210	*ara9*	2ap	K	+	
211	*ara9*	2ap	A	+	
212	*ara9*	2ap	K	+	
214	*ara9*	2ap	A	+	
216	*ara9*	2ap	A	+	
218	*ara9*	2ap	A	o	
219	*ara9*	2ap	K	+	
220	*ara9*	2ap	J	+	
221	*ara9*	2ap	K	+	
223	*ara9*	2ap	K	+	
228		2ap	K	+	
231		2ap	A	+++	
238		2ap	A	++	
239		2ap	K	+++	
300	*ara9*	X	A	+	
301	*ara9*	X	D	+	
302	*ara9*	X	K	+	
305	*ara9*	X	K	+	
307	*ara9*	X	A	+	
311	*ara9*	X	A	+	
315	*ara9*	X	A	+	
317	*ara9*	X	A	+	
320	*ara9*	X	A	+	
323	*ara9*	X	A	+	
324	*ara9*	X	C	+	
330	*ara9*	X	J	+	
333	*ara9*	X	A	+	
335	*ara9*	X	A	+	
340		des	A	+	
342		des	A	++	
345		2ap	B	+++	hs
347		2ap	K	+	
350		2ap	K	+	
363		2ap	K	+	
364		2ap	K	+	
369	*hisD23 gal50HfrA*	des	K	+	
380		2ap	A	+	
382		2ap	G	+	
393		2ap	K	+	
394		2ap	K	+	
395		2ap	F	+	
401		2ap	K	+	
402		2ap	J	+	
406	*hisD23 gal50Hfr21*	des	A	+	
408		2ap	A	++	
411		2ap	K	++	

TABLE 1—(Continued)

metC

Mutant	Other markers	Mode of origin	Complementation group	Reversion index	Other properties
30		sp	A	+	hs
32	*purG2*	sp	A	++	
35	*purG2*	sp	A	+++	
37	*purG2*	sp	F	++	
50		sp	A	+	
52		sp	B	++	
56		sp	A	++	
58		sp	A	+	
65		sp	A	+	hs
71		sp	A	++	
79		sp	A	+	
86		sp	A	+	
88		sp	A	+	
103		sp	A	++	
104		sp	E	+	
125		sp	A	+	
193	*ara9*	2ap	A	+	
195	*ara9*	2ap	F	++	
233		2ap	C	+	
251		2ap	C	+	
354	*ara9*	2ap	A	++	
356	*ara9*	2ap	A	+++	
359		2ap	A	++	
360		2ap	A	++	hs
372	*cysdl519*	2ap		+	lys
373	*cysdl519*	2ap	A	+++	
374	*cysdl519*	2ap	A	+++	
376	*cysdl519*	2ap	A	+	
377	*cysdl519*	2ap	A	+	
378	*cysdl519*	2ap		+	
379	*cysdl519*	2ap	A	+	hs
386		2ap	A	+	
399		2ap	G	+++	
409		2ap	A	++	
415		2ap	A	+++	
416		2ap	D	+	
417		2ap	A	+++	

metE

Mutant	Other markers	Mode of origin	Complementation group	Reversion index	Other properties
2	*cysC7*	u/v	I	+	
47		sp	I	o	dl
51		sp	I	++	
60		sp	I	+++	
61		sp	I	+	
68		sp	I	++	
70		sp	I	++	
80		sp	I	+	
82		sp	I	+	
84		sp	I	++	
196	*ara9*	2ap	I	++	
197	*ara9*	2ap	II	+++	
204	*ara9*	2ap	I	+	
205	*ara9*	2ap	I	+	
206	*ara9*	2ap	I	+	
217	*ara9*	2ap	I	+	
225		2ap	I	+++	

TABLE 1—(Continued)

metE

Mutant	Other markers	Mode of origin	Complementation group	Reversion index	Other properties
227		2ap	I	+	
230		2ap	I	+	
235		2ap	II	+++	
237		2ap	I	+	
243		2ap	I	++	
245		2ap	I	++	
246		2ap	I	+	
248		2ap	I	+	
249		2ap	I	+	
250		2ap	I	++	
338	*ara9*	X	I	++	
343		des	I	+	
344		2ap	I	+	
346		2ap	I	+	
348		2ap	I	+	
353	*ara9*	2ap	I	+	
355	*ara9*	2ap	I	+	
361		2ap	I	++	
368	*hisD23* *gal50HfrA*	des	I	+	
370	*argF118*	des		+++	
381		2ap	I	+++	
383		2ap	I	+++	
384		2ap	I	+	
385		2ap	I	+	
387		2ap	II	+++	
390		2ap	I	++	
396		2ap	II	+++	
400		2ap	I	+++	
403		2ap	I	+	
404	*purD55*	des	II	+++	
405	*tyr3*	des	I	+	
410		2ap	II	+++	
412		2ap	I	+	
413		2ap	I	+	
420		sp	II	+++	

metF

Mutant	Other markers	Mode of origin	Reversion index	Other properties
31	*purG2*	sp	+	
59		sp	+++	
64		sp	+++	
95		u/v	++	
96		sp	+	
100		sp	++	
101		sp	+	
176	*ara9*	sp	+	
185		sp	o	dl
226		2ap	+++	
232		2ap	+++	
236		2ap	+++	
241		2ap	+++	
242		2ap	o	
244		2ap	++	
304	*ara9*	X	++	
306	*ara9*	X	++	
308	*ara9*	X	++	

TABLE 1—(Continued)

metF				
Mutant	Other markers	Mode of origin	Reversion index	Other properties
310	*ara9*	X	+	
318	*ara9*	X	+	
375	*cysdl519*	2ap	+++	
392		2ap	+++	
397		2ap	+++	
418		2ap	+++	
422		sp	+	
metG				
Mutant	Other markers	Mode of origin	Reversion index	Other properties
319	*ara9*	X	+++	
371	*cysdl519*	2ap	+++	
419		sp	+++	
421		sp	+++	
Non-transducible				
Mutant	Other markers	Mode of origin	Reversion index	Other properties
97		sp	+	
102		sp	+	
299	*ara9*	X	++	hs
312	*ara9*	X	+	
314	*ara9*	X	+	
316	*ara9*	X	+	
325	*ara9*	X	+	
327	*ara9*	X	+	
334	*ara9*	X	+	
336	*ara9*	X	o	
337	*ara9*	X	+	

Other nutritional requirements, *arg* = arginine; *cys* = cysteine; *his* = histidine; *pur* = purine; *try* = tryptophan; *tyr* = tyrosine.

Inability to ferment sugars, *ara* = arabinose; *gal* = galactose.

Hfr = high frequency recombination male strain.

Mode of origin, 2ap = induction with 2 aminopurine; des = induction with diethyl sulphate; sp = spontaneous; u/v = induction with ultra violet light; X = induction with X-rays.

Reversion index (average number of revertant colonies per $2\text{-}3 \times 10^9$ bacteria plated on MA medium) o = none; + = above 0-5; ++ = 5-50; +++ = above 50.

Other properties, dl = deletion; hs = heat sensitivity; lys = lysogenic.

All mutants were obtained from Dr M. Demerec, Department of Biology, Brookhaven National Laboratory, Upton, Long Island, New York, U.S.A., except *metF422* (SL582) supplied by Professor B. A. D. Stocker, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1, and *metE420*, *metG419* and *metG421* which were isolated in this department.

known *met* genes *A*, *B*, *C*, *E* and *F* (Smith, 1961). One hundred and twenty-four of these mutants responded to homocysteine (including 28 which were leaky at 37°C.), 47 to vitamin B12 and 28 to methionine alone. The results of the transduction tests using the modified rapid technique indicated that those responding to homocysteine comprised 30 *metA*, 58 *metB* and 28 *metC* (leaky) mutants and that 46 of the 47 that responded to B12 were *metE* mutants. Twenty-two of those responding to methionine only were *metF* mutants.

Thus the gene deficiencies of 184 of the 199 new mutants were clearly identified. Of the remaining 15 mutants 11 could not be used in transduction experiments as they were not transducted by phage

TABLE 2

Met *mutants arranged according to their mode of origin* (*from Table 1*)

Mode of origin of mutants	Numbers of mutants of each gene						Totals
	metA	*metB*	*metC*	*metE*	*metF*	*metG*	
Spontaneous	19	18	16	10	9	2	74
Induced: ultra violet	5	4	0	1	1	0	11
Induced: X-ray	9	14	0	1	5	1	30
Induced: 2-aminopurine	11	31	21	35	10	1	109
Induced: diethyl sulphate	0	4	0	5	0	0	9
Totals	44	71	37	52	25	4	233

propagated on any *met* mutant used or wild type and phage could not be propagated on them. The reasons for this are largely unknown. Only one of these mutants (*met102*) appeared to be lysogenic using wild type *S. typhimurium* as an indicator. The other 4 mutants were transduced at about the same frequency by *metA*, *B*, *C*, *E* and *F* phage suspensions. Transduction between them was either not detected or occurred at a much lower frequency. Also, they were all slightly leaky at 37°C., showed high reversion frequencies and, as recipients in transduction yielded 10-100 times more recombinants with wild type donors than other *met* mutants. They were considered to be mutants of a new gene designated *metG*.

(ii) *The relationship between mode of origin and site of methionine mutation*

If the methionine mutants whose gene deficiencies can be identified are arranged according to their mode of origin (table 2) it is seen that no *metC* and very few *metE* mutants appear to be induced by X-rays (and u/v light, although the numbers are low) and the incidence of *metE* mutants and, to lesser extent, *metB* mutants is greatly increased if 2-aminopurine is used as a mutagen. Nitrous acid would appear to have an affect similar to 2-aminopurine at least with respect to *metE* mutants, because recent investigation revealed 20 of them among 28

met mutants isolated after induction with this mutagen. (Miss F. Pettit, personal communication.)

Reconstruction experiments to determine the effect of penicillin enrichment on the survival of small numbers of *metA*, *B*, *C*, *E* and *F* bacteria in mixed cultures with large numbers of X-irradiated wild type bacteria revealed no significant differential loss of viability between the different *met* mutants (M. Stern, personal communication). This would suggest that the absence of *metC* mutants and low numbers of *metE* mutants following X-irradiation was not a penicillin enrichment artefact.

(iii) *The pathway of methionine synthesis*

There was previously some doubt whether cystathionine was an intermediate in methionine synthesis in *S. typhimurium* (Smith, 1961). The preparation of cystathionine used stimulated an equally slow growth response of *metA*, *B* and *C* mutants. As the medium supplemented with the cystathionine used was found to contain small quantities of homocysteine after incubation, this growth response was considered to be insignificant. However, DL-allo cystathionine (Calbiochem) elicited a clear and rapid growth response from *metA* and *B* mutants, a much slower response from *metC* mutants, which are leaky anyway, and no response at all from *metE*, *F* or *G* mutants. The cystathionine growth responses of the *metA* and *B* mutants were still slower than that of these and the *metC* mutants to either homocysteine or methionine suggesting a deficiency in permeability for cystathionine. These results were confirmed in quantitative experiments in which the generation times and lag period of representative mutants of all 6 *met* genes were measured in the liquid glucose-lactate salts minimal medium of Szulmajster and Woods (1960) supplemented with either cystathionine, homocysteine or methionine (Dr M. Foster, personal communication). It was therefore concluded that cystathionine was an intermediate in methionine synthesis in this organism and that the *metA* and *B* genes controlled biosynthetic steps before this substance and the *metC* gene a step between it and homocysteine.

The enzymic deficiencies of representative mutants of each of 5 of the *met* genes have been identified (fig. 1). The *metA* mutants lack homoserine *O*-transsuccinylase and are unable to convert homoserine and succinate to *O*-succinylhomoserine and *metB* mutants, lacking cystathionine synthetase, cannot synthesise cystathionine from this intermediate and cysteine (Rowbury, 1964*a*, *b*) and so accumulate small quantities of *O*-succinylhomoserine (Rowbury, 1964*a*). The *metC* mutants lack cystathionase and are thus unable to convert cystathionine to homocysteine (Dr Rowbury, personal communication). The methylation of homocysteine to methionine can be achieved in two ways as in *E. Coli* (Guest, Friedman and Foster, 1962). One involves the direct transfer to homocysteine of the methyl group from

Enzymes:—
metA—homoserine *O*-transsuccinylase.
metB—cystathionine synthetase.
metC—cystathionase.
metE—N^5-methyltetrahydropteroyltriglutamate-homocysteine methylase.
metF—N^5N^{10}-methylene tetrahydrofolate reductase.

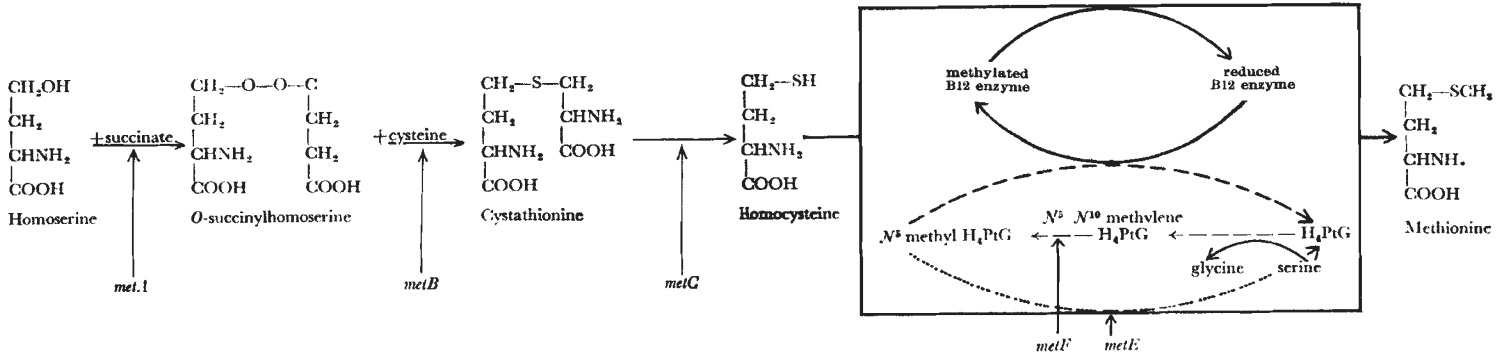

FIG. 1.—The pathway of methionine biosynthesis in *Salmonella typhimurium*. .... → H_4PtG_3 only; — — — → either H_4PtG_1 or H_4PtG_3.

a methyl derivative of the folic acid cofactor tetrahydropteroyltriglutamate (N^5-methylH_4PtG_3) and the other an indirect transfer from the same substance or the monoglutamate form (N^5-methylH_4PtG_1) indirectly via an enzyme containing vitamin B12. The *metE* mutants lack N^5-methyltetrahydropteroyltriglutamate-homocysteine transmethylase, and are unable to carry out the direct methylation. As the amount of vitmain B12 synthesised by *S. typhimurium* is inadequate for the indirect pathway to function, the methionine requirement of *metE* mutants can be overcome by supplying vitamin B12 (Cauthen and Foster, unpublished). The intracellular supply of N^5-methyl-H_4PtG_1 or N^5-methylH_4PtG_3 is maintained by the methylation of H_4PtG_1 or H_4PtG_3 in two stages. Firstly, N^5N^{10}-methyleneH_4PtG_1 or N^5N^{10}-methylene H_4PtG_3 is formed by the transfer of the hydroxymethyl group from serine by serine hydroxymethyl transferase (glycine being formed in the reaction) and secondly, these compounds are reduced to either N^5-methylH_4PtG_1 or N^5-methylH_4PtG_3 (Guest, *et al.*, 1962). The *metF* mutants are unable to carry our this reduction as they are deficient in N^5N^{10}-methylenetetrahydrofolate reductase (Foster, unpublished).

Syntrophism tests between *metG* mutants and representative *metA*, *B*, *C*, *E* and *F* mutants using the replica plating method (Smith, 1961) revealed cross feeding only between *metG* and *C* mutants, *metC* organisms predominating in the areas of syntrophic growth. However, as *metC* mutants are leaky at 37° and leaky methionine mutants respond to much lower levels of utilisable growth factors than non-leaky mutants (Smith, 1961), this cross feeding reaction was considered to be of doubtful significance. Subsequent studies (Foster, personal communication) showed that *metG* mutants do not appear to have a specific enzyme deficiency. They exhibit very low *metC*, *E* and *F* enzyme activity and also probably much reduced *metA* and *B* activity.

(iii) *The fine structure of each met gene*

The fine structure of the *met* genes was studied by determining the intragenic recombination and complementation behaviour of each mutant of each gene using the rapid transduction technique exclusively. At the same time the residual growth characteristics and reversion frequency of each mutant were also observed. A reversion index based on the average number of revertants per $2-3\times10^9$ bacteria plated on the selective medium used in each experiment is recorded in table 1. The possible heat sensitivity of each mutant was tested separately.

In complete and abortive transduction experiments the controls comprised homologous crosses, crosses between each donor and a non-methionine recipient and between each recipient and a wild type donor. Mutants were considered not to recombine only if the original cross, its reciprocal and repetition of the cross in both directions in

triplicate failed to yield recombinants. Complementation between mutants was considered to occur only if the first observation of abortive transduction could be detected on repeating the cross and, in most cases, in its reciprocal. When mutants appeared not to revert, 0·1 ml. quantities of × 10 concentrated washed overnight cultures were spread on to each of at least 5 appropriately supplemented MA medium plates and incubated for a minimum of two days. Colonies appearing after this time were picked and streaked on to similar MA medium to determine whether they possessed wild type growth characteristics. If a mutant did not revert and also failed to recombine with mutants which themselves recombined with each other, it was considered to be a multisite (deletion) mutant. If mutants showed the same recombination and complementation pattern in crosses, failed to recombine with each other and possessed similar residual growth characteristics and reversion frequencies, their sites of mutation were considered to be identical.

The results of these experiments will be considered gene by gene:

1. *metA*. Forty-two of the 44 *metA* mutants were used in intragenic transduction experiments. The use of *metA10* was impossible because it yielded very low numbers of recombinants even in intergenic crosses and *metA18* could not be used because it appeared to be lysogenic. All possible crosses were carried out between 38 (*metA7-339* inclusive) of these 42 mutants: those involving the remaining four (*metA351-414* inclusive) were on a more restricted scale.

Five mutants *metA7*, *22*, *15*, *43* and *309* behaved as deletions. The failure of *metA43* to recombine with any other *metA* mutant indicated that it was likely to be a deletion for the whole gene. Although *metA7* failed to recombine with any of the other deletions, it recombined with one point mutant (*metA229*) which failed to recombine with *metA43* so the extent of its deleted region was considered to be less than that of *metA43*. Also *metA15* recombined with both *metA22* and *309* but these two mutants did not recombine with each other. The occurrence of point mutants which recombined with either *metA22* or *309* in addition to those recombining with neither suggested that these two deletions did not overlap completely.

An unambiguous linear deletion map of the *metA* gene in which overlapping lines indicate failure to recombine, cannot be constructed but one of the 8 possible maps appears in fig. 2. In it 8 regions are defined but there are 7 other possible orders for these regions. They are: abcfedgh, adefcbgh, afedcbgh, habcdefg, habcfedg, hadefcbg and hafedcbg. The reality of these regions whatever their order is supported by considering presence or absence of recombination between *metA* point mutants and the deletions. Twenty-one of these 37 point mutants could be assigned to specific regions; 5 to region b, one to d, 5 to e, 9 to F and one to h. The remaining 16 mutants showed patterns of recombination characteristic of regions a, c or g. Also failure of recombination between point mutants was mainly confined to those

whose sites of mutation were in the same region or the adjacent regions d, e and f.

Observations of the reversion frequencies and residual growth characteristics of mutants failing to recombine indicated that the sites of mutation of each of the pairs *metA21* and *388*, *98* and *99*, *201* and *414* and *247* and *253* were probably identical. No abortive transduction (complementation) was detected between any of the *metA* mutants. Four of them, *metA19*, *53*, *98* and *99* were heat sensitive (table 1).

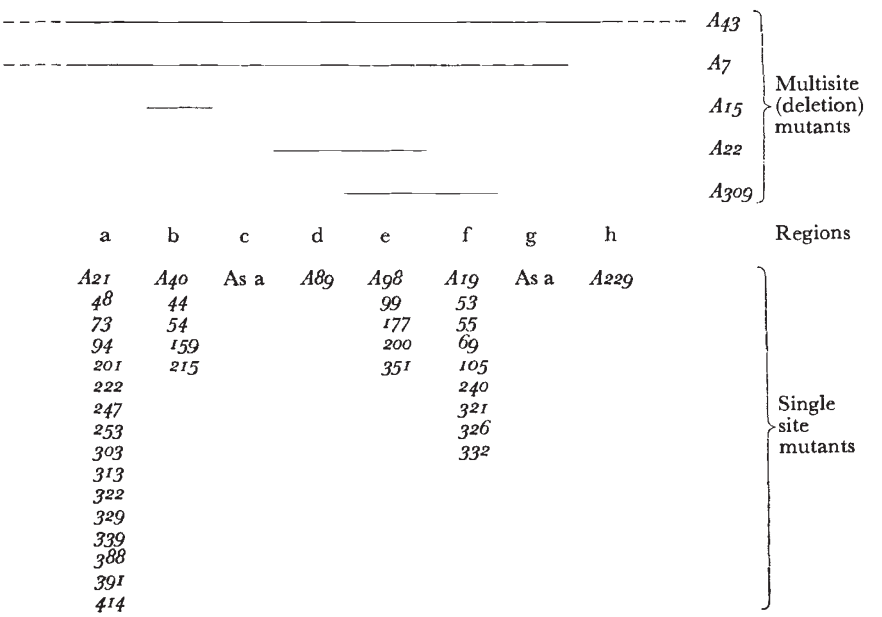

FIG. 2.—A deletion map of the *metA* gene.

2. *metB*. All of the 70 *metB* mutants were used in intragenic transduction experiments although results obtained with 6 of them (*metB81*, *194*, *239*, *345*, *408* and *411*) were rather unreliable either because of their high reversion frequencies or heavy residual growth characteristics or both.

One mutant (*metB36*) was completely stable and failed to recombine with any other *metB* mutant, thus lending support to the previous suggestion that it was a deletion mutant (Smith, 1961). It is likely to be a deletion for the whole *metB* gene. Most other mutants recombined with each other. Consideration of the similarities of reversion frequency and residual growth characteristics of those that did not, indicated that the three mutants *metB16*, *17* and *93*, and each of the three pairs *metB39* and *324*, *92* and *340*, and *393* and *394* were identical site mutants.

Some intragenic abortive transduction occurred between *metB* mutants. Although the numbers and size of the minute colonies was

not necessarily the same in each cross, a complex pattern of complementation was apparent and is summarised in fig. 3. The mutants fell into 12 groups. One group (A) comprised 31 mutants which failed to complement any other *metB* mutant. The other 39 mutants could be divided into 11 groups (B-L) on the basis of their patterns of complementation. Ten of these groups comprised one to three mutants and the other (K) 22 mutants (see also table 1). A complementation matrix was constructed which could be expressed as an unambiguous linear map in which overlapping lines indicate failure to complement (Catcheside, 1960).

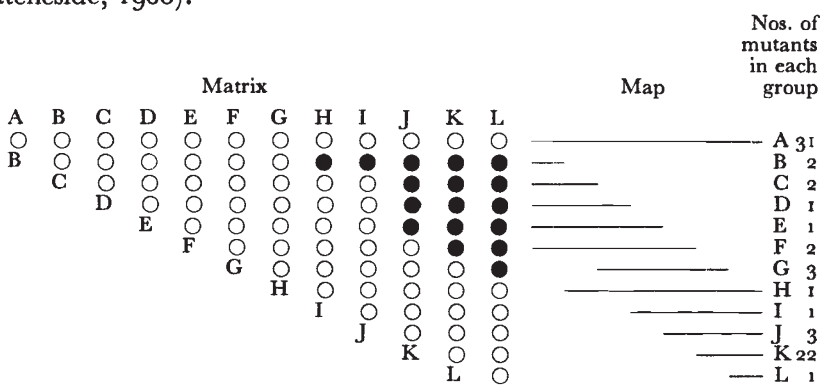

FIG. 3.—Complementation between *metB* mutants. ○ = no complementation; ◐ = poor complementation; ● = complementation. Overlapping lines in the map indicate failure to complement (after Catcheside, 1960).

Two mutants, *metB194* and *345*, the only representatives of complementation group B, were heat sensitive. In addition, these were the only *metB* mutants to exhibit mucoid colony morphology when growing slowly at 20° on MA medium. This was found to be an indirect result of the mutation to methionine requirement in these two mutants probably related to an intracellular imbalance of nitrogen and polysaccharide metabolism during their slow growth on MA medium (Childs, 1964).

3. *metC.* Mutants of this gene were difficult to study as all were leaky at 37°. In using the spot transduction technique incubation for three days at 25°, where the strains involved were not heat sensitive, facilitated much easier detection of complete and abortive transduction.

Only one of the 37 *metC* mutants could not be used. This was *metC378* whose residual growth on MA medium was too great to permit the detection of recombinants when it was used as a recipient. All of the remaining 36 mutants were crossed with each other. Three (*metC50*, *58* and *386*) had some of the characteristics of deletions in that they failed to recombine with other mutants which themselves recombined. However, each of these strains reverted at a very low frequency of *c.* 10^{-10} so that the construction of a deletion map was

not attempted. Three mutants, *metC30*, *379* and possibly *360*, were identical site mutants.

Observations of intragenic abortive transduction suggested that each of 35 of the *metC* mutants could be assigned to one of 7 complementation groups (fig. 4 and table 1). One group (A) comprised 27 mutants which failed to complement any *metC* mutants. The patterns of abortive transduction between the remaining 8 mutants permitted their arrangement into 6 further groups (B-G). A matrix expressing these data and the unambiguous linear map constructed from it appear in fig. 4.

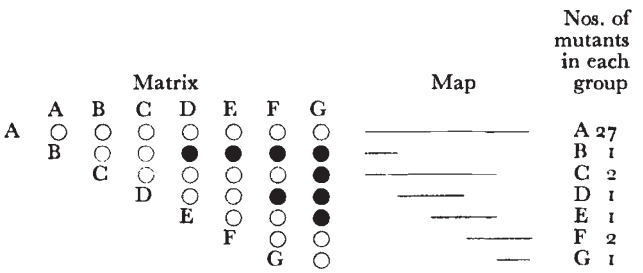

FIG. 4.—Complementation between *metC* mutants. ○ = no complementation; ○ = poor complementation; ● = complementation. Overlapping lines in the map indicate failure to complement (after Catcheside, 1960).

Four *metC* mutants (*30*, *65*, *360* and *379*) were heat sensitive. All were in complementation group A and two or three of them were probably identical site mutants.

4. *metE*. The transduction experiments with the 52 mutants of this gene were carried out in two stages. The first involved making all possible crosses between the 28 mutants from *metE2-338* inclusive and results indicated that none of these mutants were deletions although *metE47* failed to revert. Abortive transduction was detected in some crosses and each mutant could be assigned to one of two groups (I or II). All 26 mutants of group I complemented both the group II mutants (*metE197* and *235*) and vice versa. No complementation occurred between mutants of the same group.

In the second stage the remaining 24 mutants (*metE343-420* inclusive) were each transduced with two group I mutants (*metE47* and *204*) and the two group II mutants. None of these 24 mutants possessed the reversion and transduction characteristics of deletions. Eighteen fell into complementation group I, 5 into group II and one (*metE370*) could not clearly be assigned to a complementation group because of difficulty in detecting minute colonies using it as a recipient, even in crosses in which wild type was donor. Thus a total of 44 *metE* mutants fell into complementation group I and 7 into group II (table 1) giving an overall complementation pattern quite different from that of either the *metB* or *metC* mutants.

It was interesting to note that the frequency of complete transduction between mutants of different complementation groups was often as much as four times that between mutants of the same group. Also

the average reversion frequency from group II mutants was at least 10 times greater than that for group I mutants.

All *metE* mutants appeared to arise from mutation at different sites and none was heat sensitive.

5. *metF*. The results of all possible crosses between the 25 *metF* mutants showed that one mutant, (*metF185*) was a deletion and seven mutants failed to recombine with it (*metF64*, *100*, *232*, *244*, *304*, *306* and *422*). Only *metF 101* and *242* appeared to be identical site mutants, although the high reversion frequencies of *metF59*, *64*, *226*, *241*, *392*, *397* and *418* made recombination between these mutants difficult to detect. No intragenic abortive transduction was observed and no *metF* mutant was heat sensitive.

6. *metG*. When used as recipients in transduction *metG* mutants all yielded recombinants at a frequency 10-100 times that obtained with mutants of other *met* genes in both inter and intragenic crosses. This necessitated the use of unconcentrated recipient broth cultures of them in transduction experiments using the rapid technique. However, their leakiness and high reversion frequency still made scoring of recombinant colonies and observation of minute (abortive transduction) colonies difficult so that the results obtained with *metG* mutants may be unreliable.

In intragenic transduction experiments involving all possible crosses in both directions *metG419* and *421* behaved similarly. They both recombined with and complemented *metG319* and *371* although neither interaction occurred between them. On the other hand recombination and complementation was detected between *metG319* and *371*. It was concluded that *metG419* and *421* were identical-site mutants and that it was likely that the complementation pattern of the *metG* was complex. No *metG* mutant was heat sensitive.

(iv) *Attempted detection of linkage between* metG *and other* met *genes*

If bacterial genes are very closely linked they may be co-transduced. It was previously shown in donor phenotype selection experiments that of the five genes *metA*, *B*, *C*, *E* and *F* only *metB* and *F* were co-transducible (Smith, 1961). These experiments were extended to see if the *metG* gene was closely linked to the *metA*, *B*, *C* or *E* genes (the growth response pattern of *metF* and *G* mutants is the same thus precluding donor phenotype selection). All of at least 2000 recombinant colonies from each of the crosses between *metG319* and *metA7*, *B66* and *C30* plated on MA+homocysteine medium and between the same strain and *metE2* plated on MA+vitamin B12 medium were wild type. This indicated no co-transduction of the *metG* and either the *metA*, *B*, *C* or *E* genes.

4. DISCUSSION

(i) *The genes and enzymes concerned with methionine biosynthesis*

The numbers of methionine mutants of *Salmonella typhimurium* studied has been expanded from 46 (Smith, 1961) to 243. Results

obtained support the existence of the 5 methionine genes originally identified (*metA*, *B*, *C*, *E* and *F*) and have revealed another (*metG*). Parallel work on the enzyme deficiencies of similar *Escherichia coli* mutants and representative *S. typhimurium* mutants (Rowbury, 1964*a*, *b*; Rowbury and Woods, 1964; Wijesundera and Woods, 1962; Woods, Foster and Guest, 1965; Foster, personal communication) has permitted the firm establishment of the same pathway of methionine synthesis in these two organisms (fig. 1). It was particularly satisfactory that work with the *metA*, *B* and *C* mutants of *S. typhimurium* supported the existence of *O*-succinylhomoserine as a precursor of cystathionine and that the enzyme deficiencies of the *metE* and *F* mutants (N^5-methyltetrahydropteroyltriglutamate-homocysteine methylase and N^5N^{10}-methylene tetrahydrofolate reductase respectively) were compatible with the postulated two pathways of methylation of homocysteine. The recent isolation of mutants of *S. typhimurium* unable to methylate homocysteine indirectly via the enzyme containing vitamin B12 lends further support to this postulate (Childs, unpublished). Because the *metA*, *B*, *C*, *E* and *F* mutants possess specific enzyme deficiencies it is likely that these genes are structural (Jacob and Monod, 1961). As *metG* mutants do not possess a specific enzyme deficiency but show a low level of *metA*, *B*, *C*, *E* and *F* enzyme activity the function of the *metG* gene may be regulatory.

Results of the intragenic complementation tests between mutants of the five structural genes indicate something of the nature of their enzymes. The absence of complementation between mutants of the *metA* and *F* genes, suggests that homoserine *O*-transsuccinylase and N^5N^{10}-methylene tetrahydrofolate reductase are monomers. On the other hand, the complex complementation patterns of the *metB* and *C* genes, which can be expressed in the form of linear maps, suggest that cystathionine synthetase and cystathionase each comprise more than one identical polypeptide subunit (Fincham, 1962) and are perhaps multimers (Crick and Orgel, 1964). As *metE* mutants can be assigned to either one of two complementation groups and the frequency of recombination between mutants of the different groups was higher than that between mutants of the same group, it is likely that the *metE* gene is composed of two functional units or cistrons (Benzer, 1957) and that N^5-methyltetrahydropteroyltriglutamate-homocysteine methylase comprises two different polypeptide subunits.

It has recently been found that *metE*I and *E*II mutants appear to be biochemically identical except that they have different growth characteristics in minimal medium supplemented with vitamin B12: *metE*I mutants commence growth immediately but *metE*II mutants exhibit a lag period of 4-5 hr. (Miss S. Cauthen, personal communication). This is difficult to explain because in the presence of adequate vitamin B12 the indirect methylation of homocysteine by the unrelated B12 enzyme (fig. 1) should proceed quite normally if the direct pathway is non-functional whatever the reasons for the deficiency.

Although the *metA* deletion map (fig. 2) did not permit the precise location of the *metA* point mutants, it was unfortunate that similar maps of the *metB* and *C* genes facilitating at least some comparison with their complementation maps (figs. 3 and 4) could not be constructed.

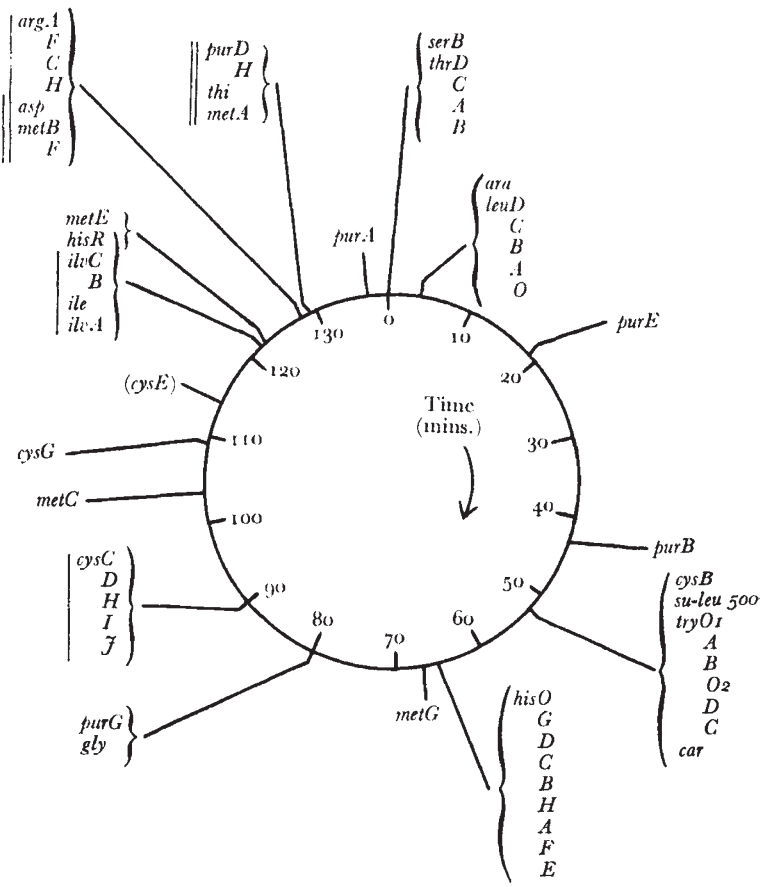

FIG. 5.—Part of the circular linkage map of *Salmonella typhimurium*. (After Sanderson and Demerec, 1965).

The time scale on the inside of the circle is based on the time of entry of the different genes during *Hfr* conjugation. } = transducing fragment; gene order and orientation known. [} = transducing fragment; gene order known, orientation unknown ‖} = transducing fragment; gene order and orientation unknown. ( ) approximate position.

The symbols are those used in table 1 with the following additions:—

1. nutritional requirements, *asp* = aspartate or glutamate; *gly* = glycine; *ile* = isoleucine; *ilv* = isoleucine-valine; *leu* = leucine; *ser* = serine or glycine; *thi* = thiamin; *thr* = threonine.
2. *car* = carbohydrate utilisation.
3. *su-leu500* = suppressor of *leu500*.

(ii) *Mutagen susceptibility of the* met *genes*

As so little is known about the mechanism of X-ray induced mutation speculation on any special properties of the *metC* and *E* genes, which show equal insusceptibility to that mutagen (table 2), is impossible. It is unlikely that there is a common explanation for the

absence of ultra-violet and X-ray induced mutants of the *metC* gene as the mechanism of induced mutation by non-ionising irradiation is probably different from that of ionising irradiation.

Concerning the relatively greater incidence of 2-aminopurine and nitrous acid induced mutations of the *metE* gene, Margolin (1963) also noted a similar effect in a study of 156 leucine (*leu*) auxotrophs of *S. typhimurium*. Of the four *leu* structural genes I, II, III and IV (later labelled *leuA*, *B*, *C* and *D*—see fig. 5) the relative incidence of mutants of gene I was much greater following the use of 2-aminopurine, 5-bromouracil and nitrous acid as mutagens compared with that using X-rays or no mutagen. This could mean that AT⇌GC transitions (Freese, 1959) occur more frequently in the *metE* and *leu*I genes than in other *met* and *leu* genes.

(iii) *The location of the* met *genes on the linkage map of* Salmonella typhimurium

Part of the linkage map of *S. typhimurium* based on cotransduction and *Hfr* conjugation data (Sanderson and Demerec, 1965) is reproduced in fig. 5. It can be seen that although the *metA*, *B*, *F* and *E* genes are located within an arc equivalent to about 1/20th of the total map they could not be regarded as clustered in the way that the histidine (*his*), tryptophan (*try*), leucine (*leu*) and threonine (*thr*) genes are. Also the *metC* and *G* genes are located in quite different regions of the map. The *met* gene locations are therefore somewhat similar to those of the purine (*pur*) and cysteine (*cys*) genes in that some are co-transducible and others widely separated.

(iv) *The control of methionine biosynthesis*

Although the studies of the methionine mutants of *S. typhimurium* are in their early stages, some consideration of the likelihood of an operon model (Jacob and Monod, 1961; Ames and Martin, 1964) for the mode of control of methionine biosynthesis in this organism is permissible:—

(1) Is there any suggestion that functionally related methionine structural genes are clustered together with a regulatory (operator) gene at one end of the cluster? Only *metB* and *F* are cotransducible, *metA*, *C* and *E* are quite separate. Operator mutants of the O^0 type (Jacob and Monod, 1961) amongst the *metB* and *F* mutants would fail to complement all mutants of both genes if *metB* and *F* comprised the whole or part of an operon. In further abortive transduction tests none of the 31 non-complementing *metB* mutants (group A, fig. 3) failed to complement *metF308*, *392* and *418* and none of the 25 *metF* mutants failed to complement *metB39*, *340*, *345*, *363* or *382*.

(2) What evidence is there that the relationship between the rates of synthesis of some or all of the methionine enzymes is constant *i.e.* that repression, if it occurs, is coordinate? Methionine certainly represses the synthesis of all the known enzymes of the methionine

pathway in *S. typhimurium* except the cystathionase (Foster, personal communication; Rowbury, 1964*b* and personal communication) but it is not known if this repression is coordinate.

(3) Is it likely that there is a cytoplasmic repressor substance acting upon one or more methionine genes whose synthesis is controlled by a gene located away from the operon(s)? A specific enzyme is not associated with the *metG* gene, the mutants of which exhibit a low level of activity of all the methionine enzymes and map close together away from the other methionine genes (fig. 5). They could be repressor mutants producing a repressor with a reduced affinity for the methionine operator(s).

It is concluded that at present there is no immediately obvious compatibility with an operon model for the control of methionine biosynthesis in *S. typhimurium*. Further information is being obtained through studies of the mutants unable to carry out the indirect methylation of homocysteine (Childs and Smith, unpublished) and mutants of wild type whose growth is resistant to inhibition by analogues of methionine (Lawrence, personal communication). Some of the latter mutants may be of the derepressed or O^c type (Jacob and Monod, 1961). It is hoped to investigate the possibility of coordinate repression of the methionine enzymes by methionine and to attempt their purification.

5. SUMMARY

Correlation of the results of growth response and transduction studies using 243 spontaneous or induced methionine mutants of *Salmonella typhimurium* with parallel detailed biochemical investigation of the enzymic deficiencies of a selected few of these mutants led to the recognition of 5 structural genes (*metA*, *B*, *C*, *E* and *F*) and probably one regulator gene (*metG*). Transduction analysis of all the mutants of each gene was carried out. A deletion map of the *metA* gene could be constructed but it was ambiguous. No interallelic complementation (abortive transduction) occurred between the mutants of the *metA* and *F* genes, but the *metE* mutants fell into two complementation groups and the *metB*, *C* and *G* mutants into 12, 7 and 3 groups respectively. Unambiguous complementation maps of the *metB* and *C* genes could be constructed. The *metG* gene was not co-transducible with either the *metA*, *B*, *C* or *E* genes. This, and the results of previous transduction experiments, was in accordance with the separate location of all but the *metB* and *F* genes on the linkage map of *S. typhimurium*. The establishment of the pathway of methionine biosynthesis, the nature of the genes and enzymes concerned, and the relative susceptibility of methionine genes to the action of different mutagens are discussed and the mode of control of methionine biosynthesis considered.

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