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Positive Control of Sulphate Reduction in *Escherichia coli*

ISOLATION, CHARACTERIZATION AND MAPPING OF CYSTEINELESS MUTANTS OF *E. COLI* K12

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To determine to what extent the biosynthesis of cysteine in *Escherichia coli* resembles that in *Salmonella typhimurium*, the following experiments were performed. (1) Mutants of *E. coli* K12 deficient in the biosynthesis of cysteine were isolated. (2) These mutants were classified by nutritional and biochemical criteria; some mutants lacked a single enzyme of sulphate reduction, other mutants appeared to lack two or more enzymes. (3) The genetic map predicted from the biochemical data alone is shown to be incorrect, and an alternative map, consistent with the genetic data, is proposed for the *cys* mutants of *E. coli*.

As far as possible, *Escherichia coli cys* mutants have been described by the same symbols as the analogous mutants of *Salmonella typhimurium* (Table 1) (Dreyfuss & Monty, 1963; Sanderson, 1967).

Pasternak, Ellis, Jones-Mortimer & Crichton (1965) showed that the repression of the enzymes of sulphate activation and reduction in *Escherichia coli* is nearly, but not quite, co-ordinate. Mizobuchi, Demerec & Gillespie (1962) showed that, in the closely related organism *Salmonella typhimurium*, the *cysE* gene (mutations in which prevent growth with sulphide as the sole sulphur source) and the *cysC*, *cysD* and *cysH* genes (mutations in which prevent growth with sulphate as the sole sulphur source but allow the utilization of sulphite) could not be co-transduced by bacteriophage P22. Yet Ellis, Humphries & Pasternak (1964) stated that mutants of *E. coli* blocked between sulphide and cysteine lacked the ability to synthesize PAPS† when grown under conditions known to cause de-repression of the relevant enzymes in prototrophic strains.

Therefore in *E. coli*, either the arrangement of the *cys* genes differs from that in *S. typhimurium* in such a way as to allow simultaneous deletions (or polarity mutations) of the two functions, or the deficiency in the cysteineless mutants described by Ellis *et al.* (1964) is of a more complex nature.

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† Abbreviations: PAPS, adenosine 3′-phosphate 5′-sulphatophosphate; APS, adenosine 5′-sulphatophosphate.

The present experiments were carried out to distinguish between these hypotheses: this paper describes the isolation, characterization and mapping of cysteineless mutants of *E. coli* K12.

MATERIALS AND METHODS

E. coli *K12 strains.* Strain 701 (F^+, prototroph) and its *cys* derivatives strains JM37, JM39, JM40, JM41, 11B and 13A were kindly supplied by Dr C. A. Pasternak. [Strain JM41 is the same strain as 12C (Wheldrake & Pasternak, 1965).] Strain 703 (F^-, prototroph) was kindly supplied by Professor R. C. Clowes. Other strains used are described in Table 2. The *cys* mutant strains JM13, JM14, JM15, JM22, JM29 and JM32 were derived from strain 703, and strains JM59–66, JM70–74, JM77, JM85–93 and JM96–97 were derived from strain PA309.

Table 1. *Enzyme deficiencies associated with* cys *mutants of* E. coli

The same symbols for loci are used as for the analogous mutants of *S. typhimurium* (Dreyfuss & Monty, 1963; Sanderson, 1967).

Mutant locus	Enzyme deficiency
cysA	Sulphate permease
cysB	Pleiotropic
cysC	APS kinase
cysD	ATP sulphurylase
cysE	Apparently pleiotropic (serine transacetylase)
cysG	Sulphite reductase
cysH	PAPS reductase
cysP	Sulphate permease and sulphite reductase
cysQ	Sulphite reductase

Construction of hybrid strains. Strain JM116 was constructed by infecting strain JM15 with the F′ *lac* of strain 240. The construction of strain JM107 is described in the following paper (Jones-Mortimer, 1968).

Chemicals. *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine was a gift from Dr J. D. Childs. Benzylpenicillin and streptomycin sulphate were obtained from Glaxo Laboratories Ltd. (Greenford, Middx.). Broth no. 2 was obtained from Oxoid Ltd. (London, E. C. 4). Maltose was obtained from May and Baker Ltd. (Dagenham, Essex). All other chemicals were as described by Pasternak (1962), Ellis *et al.* (1964) or Pasternak *et al.* (1965). Membrane filters were obtained from Oxoid Ltd. Kodirex X-ray film was used.

Growth of bacteria. Strains were grown either as described by Pasternak *et al.* (1965) with GSH as the sulphur source, or with cystine as the sulphur source, in which case the cells were harvested, resuspended in fresh sulphur-free medium and incubated for 2–3 hr. before being reharvested. The minimal medium was supplemented with L-amino acids (histidine and arginine as the hydrochlorides) (0·004%, w/v) and thiamine hydrochloride (0·0004%, w/v) when required. When medium of a known composition was not required, bacteria were grown in 2·5% (w/v) Oxoid no. 2 broth supplemented with L-cystine (0·01%, w/v). The medium was solidified by the addition of 1·7% (w/v) agar. The cultures were grown aerobically at 37°. Exponential-phase cultures (for mating) were obtained by subculturing an overnight broth culture by diluting it 1:100 with fresh medium and incubating it for 1¾ hr. Such cultures contained about 10^8 cells/ml.

The harvesting and disruption of the cells and the preparation of the supernatant fraction were carried out as described by Pasternak *et al.* (1965). Protein concentration was measured as described by Pasternak (1962). The growth response of mutants to various sulphur compounds was tested by the procedure of Ellis *et al.* (1964).

Assay of enzymes. Incorporation of [^{35}S]sulphate into whole cells was measured by the method of Britten & McClure (1962). The enzyme system catalysing the synthesis of PAPS (or APS) from sulphate and ATP was assayed as described by Pasternak (1962). PAPS reductase was assayed by the method of Pasternak *et al.* (1965) except that 0·1 M-tris–HCl buffer, pH 8, was used instead of sodium phosphate buffer, pH 7. Sulphite reductase was assayed by the method of Ellis (1964). All values quoted are the means of two or more determinations.

Isolation of mutants. Cysteineless mutants of strain 703 were isolated by replica-plating (Lederberg & Lederberg, 1952) after treatment of the wild-type with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine by the procedure of J. D. Childs (personal communication).

Cysteineless mutants of strain PA309 were obtained as follows: 15 ml. of an exponential-phase broth-grown culture of strain PA309 was harvested and resuspended in isoosmotic phosphate buffer (3 g. of KH_2PO_4, 7 g. of K_2HPO_4, 4 g. of NaCl and 0·2 g. of $MgCl_2,6H_2O$ in 1 l. of water). The cell suspension, in an open Petri dish, was irradiated 20 cm. below a Hanovia u.v. lamp (slit dimensions 5 cm. × 4 cm.) for 3·5 min. (A viable-cell count indicated that one cell in 10^5 survived.) Broth (15 ml.) was added to the cell suspension, which was distributed into 15 tubes and allowed to grow overnight. About 10^7 cells from each culture were inoculated into fresh supplemented minimal medium, with sulphate as the sulphur source, containing benzylpenicillin

Table 2. E. coli *auxotrophs*

Strain	Streptomycin	Sex	Genetic markers: *thr*	*leu*	*pro*	*trp*	*his*	*arg*	*met*	*cys*	*ilv*	*thi*	*lac*	*xyl*	*gal*	*mal*	Obtained from
PA309	r	F–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Broda 1	s	Hfr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	W. Hayes
Broda 10	s	Hfr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
240	s	F′ *lac*+/Hfr	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
712	r	F–	+	+	+	–	–	+	–	+	+	+	–	–	–	–	
YS-57	r	F′ *cys B*+	+	+	–	–	–	+	+	+	+	+	–	–	–	–	I. P. Crawford via K. Sanderson
AB1621*	r	F–	+	+	+	+	+	+	+	+	+	–	+	+	+	–	E. A. Adelberg via J. D. Childs
AB2271	r	Hfr	+	–	+	+	+	+	+	+	+	–	–	+	–	+	
K10 *cys*	s	Hfr	+	+	+	+	+	+	+	*CHPQ*	+	+	+	–	–	–	S. I. Alikhanian
S21	r	F–	+	+	+	+	–	+	+	*CHPQ*	+	+	–	–	–	–	
JM73	r	F–	+	–	+	+	–	+	+	*P*	+	+	–	+	+	–	Ultraviolet irradiation of strain PA309
JM104	r	F–	+	+	+	–	–	–	+	*P*	+	(–)	–	–	–	–	Hybrid of strains JM73 and Broda 1
JM107	r	Hfr	+	+	+	?	?	+	+	*P*	–	(–)				+	Hybrid of strains JM104 and AB2271

* Strain AB1621 is also *mil ara*.

Table 3. *Growth response of cysteineless mutants of* E. coli *to different sulphur sources*

Cultures were grown at 36° in minimal medium (supplemented as required) with the sulphur sources indicated. +, Good growth on the sulphur source employed; −, no significant growth.

	Growth response				
Sulphur source ... / Strains	Sulphate (10 mM)	Sulphite (0·85 mM)	Sulphide (0·42 mM)	Cystine (0·85 mM)	Nutritional class
Wild-type	+	+	+	+	
JM41, JM96	−	+	+	+	*ACDH*
JM13, JM14, JM37, JM40, JM63, JM72, JM73, JM77, 11B, 13A	−	−	+	+	*BGPQ*
JM15, JM39, JM70	−	−	−	+	*E*

Table 4. *Enzyme concentrations in supernatant fractions of cysteineless mutants of* E. coli

Undisrupted cells were assayed for sulphate permease activity. For assay procedures see the Materials and Methods section. ND, Not determined.

	Enzyme activity (mμmoles of product/min./mg. of protein)				
Strain	Sulphate permease	PAPS synthetase	PAPS reductase	Sulphite reductase	Biochemical class
701	+	0·31	0·185	6·6	*cys*$^+$
703	+	1·3	ND	5·85	*cys*$^+$
PA309	+	2·2	0·53	5·8	*cys*$^+$
	Enzyme activity (% of wild-type activity)				
JM41	+	34*	ND	40	*C*
JM96	+	125	3	60	*H*
JM13	−	0·2	ND	0·5	*B*
JM14	−	0·3	ND	0·5	*B*
JM37	ND	0·1	8	1	*B*
JM63	−	0·2	4	1	*B*
13A	ND	0·2	ND	1	*B*
JM72	+	80	37	2	*GQ*
JM73	−	71	60	1	*P*
JM77	+	59	145	1	*GQ*
JM40	ND	27	ND	1	*GPQ*
11B	ND	39	ND	1	*GPQ*
JM15	−	1·5	ND	0·5	*E*
JM39	−	0·1	8	1	*E*
JM70	−	0·5	32	1	*E*

*APS, not PAPS, made.

(0·12 mg./ml.) and incubated at 37° for about 48 hr. (Davis, 1948). About one cell in 10^4 survived. About 20–100 cells were plated on supplemented minimal medium containing GSH (0·125 mM) and [^{35}S]sulphate (5 μc, 0·01 mM). The plates were incubated at 37° for 48 hr. The colonies were taken up from the surface of the agar on sterile filter-paper disks. The plates were reincubated for 24 hr. Annular colonies appeared surrounding the spots from which colonies had been removed by the filter paper. (Colonies of normal shape were contaminants.) The filter-paper disks were dried at 185° for 30 min., which caused the colonies to go brown and hence become easily visible. Radioautographs were made of the filter-paper disks (20 hr. exposure). The radioautographs were compared with the filter-paper disks and with the reincubated plates. Colonies that did not correspond to intense black spots on the radioautographs were selected and tested for their ability to utilize sulphate as the sole sulphur source. Each mutant obtained was reisolated as a single colony from a broth plate.

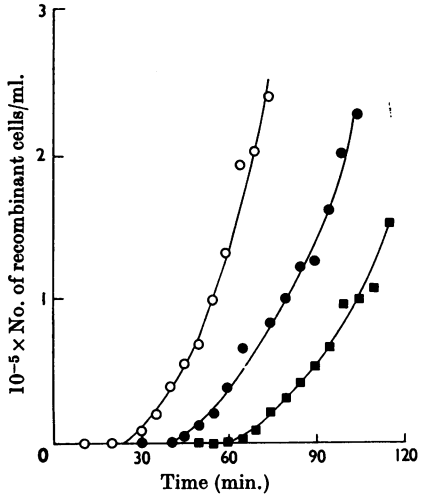

Fig. 1. Kinetics of transfer of markers from strain Hfr AB2271 to strain JM73 (F⁻ *cysP*). The strains were mated as described. Mating was interrupted at the times shown, and suitably diluted samples were plated on selective medium to be scored for recombinants: ○, *mal*⁺ recombinants; ●, *cys*⁺ recombinants; ■, *his*⁺ recombinants.

Table 5. *Times of entry of markers (with strain AB2271 as donor) into mutants of* E. coli

Recipient strain	Biochemical classification of *cys* locus	Times of entry (min.)	
		xyl⁺	*cys*⁺
JM15	*E*	—	12·5
JM70	*E*	15	15
JM72	*G(Q)*	14	18
JM73	*P*		41·5

Mating experiments. (*a*) Interrupted mating (Hayes, 1957). About 5×10^7 exponential-phase cells of the donor strain (0·5ml.) were mixed with about 5×10^8 exponential-phase cells of the recipient strain (4·5ml.) in a 50ml. conical flask, and incubated in a water bath at 37° with gentle shaking. At intervals, 0·05ml. samples were withdrawn, diluted to 5ml. with iso-osmotic phosphate buffer, and blended for 1min. with a Whirlimixer, to interrupt mating. When required, the cell suspension was diluted further, and 0·1ml. samples were spread on the selection plates. The plates were incubated at 37° for about 44hr., before the scoring of the recombinant clones.

(*b*) Plate mating. Lawns of about 10^8 male cells from an overnight culture were spread on selection plates, and recipient cells streaked on these lawns. The plates were incubated as above, and the recombinants scored.

RESULTS

Nutritional classification. Mutants were tested for their ability to grow with sulphite or sulphide as the sole source of sulphur. Any culture that grew was checked for reversion on plates containing sulphate as the sulphur source. The results of this experiment are given in Table 3. These results show that the mutants can be divided into three classes: *ACDH*, able to utilize both sulphite and sulphide as the sole source of sulphur; *BGPQ*, able to utilize sulphide but not sulphite as the sole source of sulphur; *E*, unable to utilize either sulphite or sulphide as the sole source of sulphur. Most of the mutants tested fell into class *BGPQ*.

Biochemical classification. The ability of mutant cells to bind (or transport) sulphate, and the ability of extracts of mutant cells to catalyse the reactions involved in the activation and reduction of sulphate, were measured. The results of these experiments are given in Table 4, which shows that the nutritional classes *ACDH*, *BGPQ* and *E* may be subdivided.

Class *ACDH*. Class *C*, strain JM41 lacks APS kinase only; class *H*, strain JM96 lacks PAPS reductase only.

Class *BGPQ*. Class *B*, strains JM14 and JM63 lack all the enzymes measured; class *GQ*, strains JM72 and JM77 lack only sulphite reductase; class *P*, strain JM73 lacks sulphite reductase and the ability to bind sulphate.

Class *E*. Strain JM15 lacks all the enzymes measured; strain JM70 lacks all the enzymes except PAPS reductase, which is present in decreased concentration; this PAPS reductase activity is not an artifact, since it is repressed during growth on cystine to less than 10% of its de-repressed concentration (mutants of this class lack the enzyme serine transacetylase; Jones-Mortimer, Wheldrake & Pasternak, 1968).

Genetic mapping

Sexduction. In an experiment to map some of the *cys* mutants of strain 703, they were mated with strain YS-57, which carries the F′ *cysB*⁺ episome. Mating was interrupted after 30min. The progeny were tested for their ability to grow on unsupplemented minimal medium with sulphate as the sulphur source. The *pro* and *his* loci of the male were used for contraselection. The mating mixtures contained $2{\cdot}4 \times 10^7$ cells of strain YS-57/ml. and about 2×10^8 cells of the recipient strains. Strain JM13 gave $3{\cdot}2 \times 10^6$ *cys*⁺ progeny/ml., strain JM14 $7{\cdot}1 \times 10^6$/ml. and strain JM15 3×10^4/ml.

Interrupted mating. In an experiment to map the *cysP* gene, cells of strain JM73 were mated with the male strain AB2271. Samples were taken at 5min. intervals. The *ilv* locus of the male strain was used for contraselection. The kinetics of chromosome transfer are shown in Fig. 1. The times of entry for the markers were: *mal*⁺, 25min.; *cysP*⁺, 41·5min.; *his*⁺, 55–60min.

Strains JM15, JM70 and JM72 were also mapped by interrupted mating with strain AB2271 as the donor. The times of entry of the *xyl*$^+$ and *cys*$^+$ markers into these strains are given in Table 5.

Plate mating experiments. (*a*) F$^-$ *cys* mutants (strain S21 and mutants derived from strain 703 or PA309). F$^-$ *cys* mutants were mated with the male strains JM107 (derived from strain JM73 and carrying the same *cysP* locus), JM116 (derived from strain JM15 and carrying the same *cysE* locus) and YS-57 (carrying the *cysB*$^+$ gene on an episome). No *cys*$^+$ colonies were obtained in the absence of a male strain. The results of this experiment are given in Table 6.

(*b*) F$^+$ *cys* mutants (derived from strain 701) and strain K10 *cys*. These mutants were mated with the F$^-$ strains JM14, JM15, JM72 and JM73. No *cys*$^+$ colonies were obtained in the absence of a female strain. The results of this experiment are given in Table 7.

Order of the cysE, mtl *and* xyl *loci by a three-point cross.* Exponential-phase cells of strains JM116 (F′ *lac/cysE str*^{s}) and AB1621 (F$^-$ *xyl mtl thi str*^{r}) were mixed in the proportions indicated in Table 8 and collected on membrane filters. The membranes were placed on pre-warmed broth plates and incubated at 36° for 2hr. (Matney & Achenbach, 1962). The cells were resuspended in 5ml. of buffer,

Table 6. *Genetic classification of F$^-$* cys *mutants of* E. coli

Strain JM32 resembles strain JM30; strain JM60 resembles strain JM59; strains JM64, JM65, JM66, JM71, JM74, JM77, JM85, JM90, JM91 and JM93 resemble strain JM62; strains JM89 and JM97 resemble strain JM86. ++++, Confluent growth; +++, nearly confluent growth; ++, about 10 recombinants/cm. streak; +, about 2 recombinants/cm. streak; −, no recombinants; ND, not determined.

		Yield of *cys*$^+$ cells with male strains			
	F$^-$ strain	YS-57 F′ *cysB*$^+$	JM107 *cysP*	JM116 *cysE*	Possible loci
Controls	JM14	++++	+++	+++	*B*
	JM15	+	+++	−	*E*
	JM73	+	−	+++	*P*
Derived from strain 703	JM22	++	+++	−	*E*
	JM29	+	+++	+	*E*
	JM30	++++	+++	+++	*B*
Derived from strain PA309	JM59	+	++	+++	*P, Q*
	JM61	+	+	+++	*P, Q*
	JM62	+	+	ND	*P, Q*
	JM63	++++	+++	+++	*B*
	JM69	+	−	+++	*P, Q*
	JM70	+	+++	+	*E*
	JM72	+	+++	+++	(*G*)
	JM86	+	++	ND	*P, Q*
	JM96	+	+	ND	*P, Q* (*H*)
Derived from strain K10 *cys*	S21	+	+	+++	*P, Q*

Table 7. *Genetic classification of F$^+$* cys *strains (derived from strain* 701)

++++, Confluent growth; +++, nearly confluent growth; ++, about 10 recombinants/cm. streak; +, about 2 recombinants/cm. streak; −, no recombinants; ND, not determined.

	Yield of recombinants with F$^-$ strains				Yield of *cys*$^+$ colonies	
F$^+$ *cys* strain	JM14 *cysB*	JM15 *cysE*	JM72 *cysG*	JM73 *cysP*	YS-57 F′ *cysB*$^+$	Possible loci
JM37	−	++	+	−	++++	*B*
JM38	−	+++	+++	++	ND	*B*
JM39	+++	−	++	++	ND	*E*
JM40	+++	+++	+++	+	ND	*CHPQ*
JM41	+++	+++	+++	+	ND	*CHPQ*
K10 *cys*	+++	+++	+++	+	ND	*CHPQ*

Table 8. *Progeny of the cross JM116/AB1621*

	No. of parent cells/ml.		No. of str^r recombinant cells/ml.					
Column no. ...	1	2	3	4	5	6	7	8
	JM116 *cysE* F′ *lac*$^+$	AB1621 *xyl* *mtl* *str*^{r}	*mtl*$^+$	*xyl*$^+$	*mtl*$^+$ *cys*$^+$	*mtl*$^+$ *cys*$^+$ *xyl*$^+$	*xyl*$^+$ *cys*$^+$	*xyl*$^+$ *cys*$^+$ *mtl*$^+$
Expt. no.	($\times 10^{-7}$)	($\times 10^{-7}$)	($\times 10^{-5}$)	($\times 10^{-5}$)	($\times 10^{-3}$)	($\times 10^{-3}$)	($\times 10^{-3}$)	($\times 10^{-3}$)
1	2·2	6·9	12	6	25	9·0	68	9·7
2	2·1	9·5	12	6	24	8·2	41	5·5

and diluted 1:10. The cell suspension was blended in the Whirlimixer for 1 min., and 0·1 ml. samples were plated on medium supplemented with thiamine and streptomycin, with sulphate as the sulphur source and either xylose or mannitol as the carbon source. The cell suspension was also diluted 1:10 again and 0·1 ml. samples were plated on to medium supplemented with thiamine and streptomycin, with cystine as the sulphur source, and either xylose or mannitol as the carbon source. The plates were incubated at 36° for 48 hr., and the recombinants scored. The *cys*$^+$ *xyl*$^+$ and *cys*$^+$ *mtl*$^+$ colonies were replicated on to plates supplemented with thiamine and streptomycin, with sulphate as the sulphur source and mannitol and xylose respectively as the carbon source, to test for the inheritance of the other fermentation marker. The replica plates were incubated at 36° for 24 hr. and the colonies scored.

The results of this experiment, which was carried out in duplicate, are summarized in Table 8. Comparison of columns 3 and 4 of Table 8 shows that there are more *mtl*$^+$ recombinants than *xyl*$^+$.

These results would be obtained either if the *mtl*$^+$ locus were nearer the origin of the donor than the *xyl*$^+$ locus, or if the *xyl*$^+$ locus were nearer the streptomycin-sensitivity locus used for contra-selection. Both assumptions lead to the same gene order: 0–*mtl*–*xyl*–*str*.

The probability of a cross-over between *mtl*$^+$ and *cysE*$^+$ is $(25 \times 10^3)/(12 \times 10^5) = 2\%$.

The probability of a cross-over between *xyl*$^+$ and *cysE*$^+$ is $(6{\cdot}8 \times 10^4)/(6 \times 10^5) = 11\%$.

Assuming that the probability of a cross-over between two points is proportional to the distance between them, the distance *mtl*–*cysE* is less than the distance *xyl*–*cysE*. Therefore the gene order –*mtl*–*xyl*–*cysE*– is impossible.

If the gene order is –*mtl*–*cysE*–*xyl*–, and if two cross-overs are more likely than four, 2% of the *xyl*$^+$ *cysE*$^+$ recombinants will be *mtl*$^+$, whereas if the gene order is –*cysE*–*mtl*–*xyl*–, 2/11 (= 18%) of the *xyl*$^+$ *cysE*$^+$ recombinants will be *mtl*$^+$. Comparison of columns 7 and 8 in Table 8 shows that 14% of the *xyl*$^+$ *cysE* recombinants are *mtl*$^+$. Therefore the gene order –*cysE*–*mtl*–*xyl*– is more likely.

This gene order predicts that the proportion of *mtl*$^+$ recombinants that are *xyl*$^+$ should not depend on whether the *cysE*$^+$ or *cysE* allele is inherited, provided that there is no interference. Comparison of columns 5 and 6 in Table 8 shows that 35% of the *cys*$^+$ *mtl*$^+$ recombinants are *xyl*$^+$, and comparison of columns 3 and 4 shows that not more than 50% of the total *mtl*$^+$ recombinants can be *xyl*$^+$. This result is in reasonable agreement with the gene order proposed.

DISCUSSION

The phenotypes of the mutants investigated are compatible with the pathway for sulphate activation and reduction (Pasternak *et al.* 1965). No mutants of class *A* (lacking sulphate permease only) or of class *D* (lacking ATP sulphurylase only) were isolated in *E. coli* K12, though mutants of each class are known in *E. coli* 9723 (C. A. Pasternak, unpublished work; Wheldrake & Pasternak, 1965).

Besides those mutants that lack only one enzyme there are three classes, *B*, *E* and *P*, that lack two or more of the enzymes. If it is assumed that these pleiotropic mutants result from deletions or polar mutations in the chromosome, the results allow the prediction of a map of the *cys* loci. The mapping data show that neither the *cysB* nor the *cysE* gene is linked to any of the other *cys* genes (Fig. 2).

The results of the sexduction experiment show that the *cysE*$^+$ gene is not carried on the episome of strain YS-57. Thus it is not linked to the *cysB*$^+$ gene, so the hypothesis that mutants showing the *B* and *E* phenotypes are deletion or polarity mutants of different extents is incorrect.

The interrupted-mating experiments show that the *cysE* locus is quite closely linked to the *xyl* locus of strain PA309, and that the *cysG* and *cysP* loci lie between the *xyl* and *malA* and the *malA* and *his* loci respectively. The plate-mating experiments show that the *cysC*, *cysH* and *cysQ*

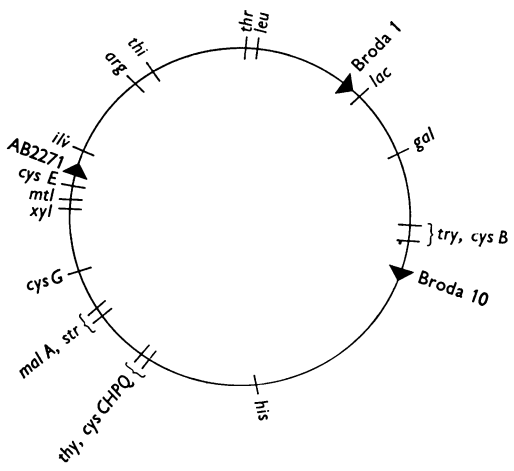

Fig. 2. Positions of *cys* loci on the genetic map of *E. coli* K12.

loci are closely linked to the *cysP* locus. Though neither class *B* nor class *E* of pleiotropic mutants can be explained by the deletion or polarity hypothesis, this type of explanation has not been ruled out for the *cysP* mutants.

The results with strains K10 *cys* and S21 (a derivative of K10 *cys*) show that the *cysP* locus is closely linked to the *cys* locus in these strains. But Alikhanian, Iljina, Kaliaeva, Kameneva & Sukhodolec (1966) have shown that the *cys* locus of strains K10 *cys* and S21 is closely linked to the *thy* locus. It seems therefore that in *E. coli*, as in *S. typhimurium*, the main group of structural genes for the cysteine biosynthetic pathway is linked to the *thy* locus.

The three-point cross of the *cysE*, *mtl* and *xyl* loci confirms the result of Taylor & Thoman (1964, Fig. 6), but indicates that the gene order shown on their map (Taylor & Thoman, 1964, Fig. 7) is incorrect.

Table 6 shows that only one of the *cys* mutants examined failed to give any recombinants with strain JM107, even though the test for recombinants was not very stringent. Thus it seems likely that *E. coli* does not give rise to *ditto* deletions of the *cys* genes as *S. typhimurium* does (Demerec & Ohta, 1964).

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