Transductional Analysis of Arginineless Mutants in *Proteus mirabilis*

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SUMMARY

A genetic map of eight structural genes involved in arginine synthesis in *Proteus mirabilis* strain 13 was obtained by transduction. Genes *argE*, *C*, *B*, *G*, *H* form a cluster linked to the gene *cysE*, the *argD* gene is linked to a locus which controls resistance to 1 mg./ml. streptomycin, *argF* is linked to a pyrimidine marker, while the *argA* gene could not be co-transduced with other markers. The clustering and order of *argE*, *C*, *B*, *H*, the linkage of *argD* to *str-r* and the non-linkage of *argF* and *argA* to other arginine genes are features shared by *P. mirabilis*, *Escherichia coli* and *Salmonella typhimurium*. *Proteus mirabilis* differs from the other organisms in that *argG* is included in the *argE*, *C*, *B*, *G*, *H* cluster. This cluster is closely linked to *cysE* and not to methionine markers. *ArgF*, which is the gene for the sixth step in the pathway, is linked to a pyrimidine marker while in *E. coli* this step is governed by two genes, one of which (*argI*) is linked to *pyrB*.

INTRODUCTION

In *Proteus mirabilis* strain 13 (Coetzee & Sacks, 1960*b*) the arginine pathway consists of eight enzymes which can be altered by single-step mutations (Prozesky, 1967). These enzymic steps are the same as those in *Escherichia coli*, and are outlined in Fig. 1. To allow comparisons between gene maps the nomenclature is that of Glansdorff (1965, 1967) and Prozesky (1967) but the equivalent designations used for *Salmonella typhimurium* (Sanderson & Demerec, 1965) and strains of *E. coli* (Vogel, Bacon & Baich, 1963; Vogel & Bacon, 1966) are given in Fig. 1. In *E. coli* K-12 the loci of seven structural arginine genes are distributed over four regions of the chromosome (Maas, 1961; Maas & Maas, 1962). An eighth gene specific for step *D* (Fig. 1) has been identified in *E. coli* W and is situated in another region of the chromosome (Vogel, Bacon & Baich, 1963). An additional locus *argI* for the sixth step (Fig. 1) was investigated by Glansdorff, Sand & Verhoef (1967) in *E. coli* K-12. They found *argI* situated separately from the other *arg* genes and linked to *pyrB* (Taylor & Thoman, 1964). Masters & Pardee (1965) found a mutation which results in a block at this step closely linked to *pyrB* in *Bacillus subtilis*.

The regulation of these scattered genes has been the subject of many investigations (see Baumberg, Bacon & Vogel, 1965) and led to detailed examination of the *argE*, *C*, *B*, *H* cluster in *E. coli* K-12 by Glansdorff (1965, 1967) and Sand & Glansdorff (1967), who concluded that they could constitute an operon (Jacob, Perrin, Sanchez & Monod, 1960). Other workers (Demerec *et al.* 1960; Sanderson & Demerec, 1965; Armstrong, 1967) found the same arrangement of the corresponding genes in

Salmonella typhimurium. Baumberg, Bacon & Vogel (1966) found a pleiotropic mutation situated near this cluster which caused alterations in the regulation of genes *argC*, *B*, *H* but not those of *argE* or the unlinked *argD* gene. They concluded that the *argE* and *argH* genes in the cluster are repressed separately although the *argE*, *C*, *B*, *H* genes are closely linked. Sand & Glansdorff (1967) described a polar *argE* mutant which influences the enzyme levels of *argH* in the cluster. They decided that the cluster could still be one operon.

The spatial arrangement of the eight structural genes of the arginine pathway of *Proteus mirabilis* was investigated in an attempt to resolve some aspects of this regulation problem as well as to contribute in general to genetic knowledge of *Proteus mirabilis* (Coetzee *et al.* 1966).

Fig. 1. Pathway of arginine synthesis. A–H represent enzymic steps in *Proteus mirabilis* and *Escherichia coli* κ -12 (Prozesky, 1967; Glansdorff, 1965) although step D mutants are only known in *E. coli* w. The corresponding designations for these steps in the same sequence are B, C, H, G, A, D, E, F for *E. coli* w (Vogel & Bacon, 1966) and *Salmonella typhimurium* (Sanderson & Demerec, 1965).

METHODS

Media. The minimal medium was that used by Prozesky (1967). Growth factors were added to it (100 $\mu\text{g./ml.}$) for selection of auxotrophic donor-type transductants: L-arginine hydrochloride, L-ornithine monohydrochloride, uracil, L-cysteine hydrochloride or DL-methionine (Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.). Difco SS agar or minimal agar containing 1 mg./ml. streptomycin sulphate was used for selection of streptomycin-resistant transductants. The broth was that of Coetzee & Sacks (1960a).

Bacteria. *Proteus mirabilis* strain 13, a streptomycin-resistant mutant of this strain *str-r1* (the mutant 13 *str-r* of Coetzee & Sacks, 1960b) and auxotrophic derivatives of these organisms were used (Table 1). The biochemical characteristics of the arginineless mutants have been described (Prozesky, 1967). The two pyrimidine-requiring mutants *pyr-3* and *argF5pyr-2* have the same requirement for uracil as the mutant *argF3pyr-1str-r-1* which was originally named *AC3Ur* (Prozesky & Coetzee, 1966). The blocks in the pyrimidine pathway of these mutants have not been investigated enzymically but their growth requirements and linkage by transduction to the *argF* gene have been established (Prozesky & Coetzee, 1966). The cysteineless and methionineless mutants were obtained from Mr W. O. K. Grabow (Grabow & Smit, 1967). The *cysE* enzymic blocks of the selected double mutants *argE1cysE5str-r-1* and *argH1cysE3* were also determined by these workers. Double arginineless mutants for three-point crosses were selected and characterized according to Prozesky & Coetzee (1966) and Prozesky (1967). Streptomycin-resistant mutants resistant to 1 mg./ml. streptomycin were selected by the method of Coetzee & Sacks (1960b). Strains were maintained on agar slopes at 4° and incubation temperature was 37°.

Transducing phage. The general phage techniques were those of Adams (1959). Lysates of phage 34/13 (Coetzee & Sacks, 1960b) with plaque-forming titres of 5×10^9 to 2×10^{10} were prepared by an agar-layer technique (Prozesky, de Klerk

Table 1. *Strains used for transduction analysis*

Genotype	Enzyme blocks (Fig. 1)	Reaction to strepto- mycin 1 mg./ml. *	Phenotype	Reference
13 (wild-type)	—	s	proto }	Coetzee & Sacks,
<i>str-r-1</i>	—	r	proto }	1960 <i>b</i>
Arginineless mutants				
Growth response with:				
<i>argA1str-r-1</i>	<i>argA</i>	r	orn, cit, arg	Prozesky, 1967
<i>argA2</i>	<i>argA</i>	s	orn, cit, arg	
<i>argA2str-r-2</i>	<i>argA</i>	r	orn, cit, arg	
<i>argA3</i>	<i>argA</i>	s	orn, cit, arg	
<i>argA3str-r-3</i>	<i>argA</i>	r	orn, cit, arg	
<i>argA4str-r-1</i>	<i>argA</i>	r	orn, cit, arg	
<i>argA5</i>	<i>argA</i>	s	orn, cit, arg	
<i>argA5str-r-4</i>	<i>argA</i>	r	orn, cit, arg	
<i>argA6</i>	<i>argA</i>	s	orn, cit, arg	
<i>argA6str-r-5</i>	<i>argA</i>	r	orn, cit, arg	
<i>argB1</i>	<i>argB</i>	s	orn, cit, arg	
<i>argB2</i>	<i>argB</i>	s	orn, cit, arg	
<i>argC1</i>	<i>argC</i>	s	orn, cit, arg	
<i>argD1</i>	<i>argD</i>	s	orn, cit, arg	
<i>argD1str-r-6</i>	<i>argD</i>	r	orn, cit, arg	
<i>argD2</i>	<i>argD</i>	s	orn, cit, arg	
<i>argD2str-r-7</i>	<i>argD</i>	r	orn, cit, arg	
<i>argD3</i>	<i>argD</i>	s	orn, cit, arg	
<i>argD3str-r-8</i>	<i>argD</i>	r	orn, cit, arg	
<i>argD4</i>	<i>argD</i>	s	orn, cit, arg	
<i>argD5</i>	<i>argD</i>	s	orn, cit, arg	
<i>argD5str-r-10</i>	<i>argD</i>	r	orn, cit, arg	
<i>argD6</i>	<i>argD</i>	s	orn, cit, arg	
<i>argD6str-r-11</i>	<i>argD</i>	r	orn, cit, arg	
<i>argE1str-r-1</i>	<i>argE</i>	r	orn, cit, arg	
<i>argE2</i>	<i>argE</i>	s	orn, cit, arg	
<i>argE3str-r-1</i>	<i>argE</i>	r	orn, cit, arg	
<i>argF1str-r-1</i>	<i>argF</i>	r	cit, arg	
<i>argF2str-r-1</i>	<i>argF</i>	r	cit, arg	
<i>argF3str-r-1</i>	<i>argF</i>	r	cit, arg	
<i>argF4</i>	<i>argF</i>	s	cit, arg	
<i>argF5</i>	<i>argF</i>	s	cit, arg	
<i>argG1</i>	<i>argG</i>	s	arg	
<i>argG2</i>	<i>argG</i>	s	arg	
<i>argH1</i>	<i>argH</i>	s	arg	
<i>argH2</i>	<i>argH</i>	s	arg	
<i>argH3</i>	<i>argH</i>	s	arg	
<i>argC1argH4</i>	<i>argC + argH</i>	s	arg	
<i>argE1argH5str-r-1</i>	<i>argE + argH</i>	r	arg	
<i>argB1argH6</i>	<i>argB + argH</i>	s	arg	
Assorted mutants				
Growth response with:				
<i>pyr-3</i>	n.d.	s	ura	See Methods
<i>argF3-pyr-1-str-r-1</i>	<i>argF</i>	n.d.	arg, cit + ura	Prozesky & Coetzee, 1966
<i>argF5-pyr-2</i>	<i>argF</i>	n.d.	arg, cit + ura	See Methods
<i>cysE-448</i>	<i>cysE</i>	s	hcys, cys, met	Grabow & Smit, 1967
<i>argE1-cysE5-str-r-1</i>	<i>argE + cysE</i>	r	orn, cit, arg + hcys, cys, met	See Methods
<i>argH1-cysE3</i>	<i>argH + cysE</i>	s	arg + hcys, cys, met	See Methods

* r = resistant; s = sensitive; orn = ornithine; cit = citrulline; arg = arginine; ura = uracil; hcys = homocysteine; cys = cysteine; met = methionine; proto = prototroph; n.d. = not done.

& Coetzee, 1965) on the wild-type organism and all mutants. Lysates were sterilized with chloroform and stored at 4°. Small numbers of phage 13vir (Prozesky *et al.* 1965) were sometimes present in transducing lysates. The effect of these virulent phages on the transduction frequency was tested in reconstruction experiments as follows: Phage 13vir was prepared by the method of Prozesky *et al.* (1965). Dilutions of the 13vir lysates were made in a lysate of transducing phage 34/13 and samples of the mixed lysates were then used to transduce two auxotrophs to prototrophy.

Transduction. This procedure has been described (Prozesky & Coetzee, 1966). Phage lysate (1 ml.) was mixed with 1 ml. of a 16 hr broth culture of recipient bacteria (about 2×10^9 /ml.) and incubated without shaking. After adsorption for 15 min. the bacteria were spun down, the pellets resuspended in 0.9% (w/v) NaCl and various dilutions plated. Phage sterility controls as well as controls in which recipients were treated with lysates prepared on homologous and wild-type bacteria respectively were included. Results were read after 48 hr.

Selection of transductants. Wild-type transductants were selected on minimal medium and auxotrophic donor-type transductants on appropriately supplemented minimal medium (Lennox, 1955). Plates with donor-type transductants were replicated to minimal medium for scoring (Lederberg & Lederberg, 1952) and read after 24 hr. When streptomycin resistance was the unselected marker, prototroph colonies on minimal medium were replicated to minimal medium with streptomycin. In crosses between *argG* or *argH* recipients and *argB*, *C* or *E* mutant class donors the master plate technique (Glansdorff, 1965) was used to obviate feeding of the donor-type recombinant classes by the background growth of the recipient (Prozesky, 1967).

Linked transduction. Linked transduction of arginine genes with other markers was demonstrated by the use of arginineless mutants as donors with various mutant recipients and selection for *arg*-donor-type transductants (Demerec *et al.* 1956). Non-replicating colonies were picked off into broth, purified and tested for inheritance of the donor-type by auxanography. Results which suggested linked transduction were confirmed by reverse crosses with selection for the opposite donor-type.

Genetic mapping. Two-point transductions were performed by crosses of single mutants in all combinations. The number of colonies on control plates of recipients treated with homologous phage were subtracted from those on test plates. Three-point linkage tests were done with the mutants in seven of the genes where linkage to external markers was found. In three-point experiments double mutants with linked markers were crossed with single mutants for the determination of the order of the genes and the mutations order by donor-switching (Glansdorff, 1965). The crossovers involved in the formation of wild-type transductants in the respective crosses have been discussed and illustrated by Clowes (1960) and Glansdorff (1965). The sequence of the three markers involved in the crosses can be derived from the ratio of the number of transductants from a cross to the number obtained from the reverse cross. A low ratio is indicative of an unequal number of crossovers while a ratio of about unity means that the same number of crossovers are involved in the two crosses. A similar method with selection for *arg*⁺, *str-r* transductants was used to map the *argD* mutations with *str-r* as the linked marker. Relative map distances were determined by a ratio test with a linked marker (Lennox, 1955; Gross & Englesberg, 1959). This ratio is expressed as 'wild-type transductants per total transductants' in per cent or with the *argD* and *str-r* markers as '*str*⁻, *arg*⁻ transductants per *str-r* transductants' in per cent.

The *argA* mutants were mapped by a ratio test with the unlinked *str-r* marker. Streptomycin-resistant mutants were selected from each of the *argA* mutants and crossed with the other streptomycin-sensitive mutants in the gene. Selection was made separately for prototrophy and streptomycin-resistance. The number of prototrophs was then compared with the number of streptomycin-resistant transductants from the same cross.

Purity of transductant clones. This was checked in two crosses: 50 *arg*⁺ colonies from each of the transductions *argE1cysE5str-r-1* × *argH1* and *argH1cysE3* × *argE1* were tested for the *cysE* marker by auxanography.

RESULTS

Mutants. The streptomycin-resistant mutants are resistant to 1 mg./ml., exhibit no additional auxotrophy (Sanderson & Demerec, 1965) and are linked to the *argD* gene (see below). These mutants correspond to the *str-A* mutants of Sanderson & Demerec (1965). The co-transduction findings (Table 10) indicate that the streptomycin mutants are situated in a single locus. The auxotrophic mutants were obtained with the use of MnCl₂ (Prozesky, 1967) and behave like revertable point mutations except for mutant *argD4* which may be a multisite mutant. No true deletions (Hartman, Loper & Serman, 1960) were encountered.

Table 2. *Effect of phage 13vir on transduction frequency*

Titre of transducing phage in transducing lysates was constant. Results are the mean of two experiments. Selection was for wild-type transductants.

Cross 1 Cross 2	Recipient	Donor
	<i>argE1</i> × 13 (wild-type)	<i>argB1</i> × <i>argH1</i>
Titre of phage 13vir in transducing lysate (p.f.u./ml.)	Number of transductants	
	Cross 1	Cross 2
0	621	136
5	615	144
10	632	131
50	643	131
1 × 10 ³	592	139
0.5 × 10 ³	630	148
1 × 10 ³	657	145
0.5 × 10 ⁴	606	133
1 × 10 ⁴	612	123
0.5 × 10 ⁵	584	117
1 × 10 ⁵	568	105
0.5 × 10 ⁶	540	92

The effect of virulent phage on the transduction frequency. There was a significant decrease in the yield of transductants (Table 2) when transducing lysates contained more than 1 × 10⁴ plaque-forming units of phage 13vir per/ml. Lysates used in this investigation never contained more than 50 plaque-forming units (p.f.u.) of phage 13vir/per ml.

Purity of transductant clones. No mixed clones were encountered and the incidence of such colonies was taken to be negligible (Glansdorff, 1965).

Linkage of other markers to arginine genes. Linked transduction of an *argF* and a pyrimidine marker has been described (Prozesky & Coetzee, 1966). In crosses between arginineless mutants and representative cysteineless and methionineless mutants of Grabow & Smit (1967) donor-type transduction occurred only with the *cysE* and *argB*, *C*, *E*, *G* and *H* markers. Streptomycin resistance could only be co-transduced with the *argD* marker. The *argA* mutants are not linked to any other marker (also see Prozesky & Coetzee, 1966). Seven of the eight arginine genes could thus be mapped with linked markers.

Two-point crosses for preliminary mapping. Results of an experiment are given in Table 3. Small numbers of transductants were obtained from intragenic crosses or crosses between sites in closely linked genes. Mutants *argF4* and *argH2* whether used as recipients or donors always yielded larger numbers of transductants than other mutants. Mutants *argA1* and *argD4* on the other hand were poor recipients with average donor capacities. The efficiency of plating of phage 34/13 on these four mutants is unity. Mutant *argF4* is slightly 'leaky' but has a low spontaneous reversion rate (Table 3). Numbers of colonies on control plates which form a column diagonally across Table 3 show that the reversion rate of the mutants is low except in the case of *argG2*. This mutant was retained in the series as it and *argG1* are the only representatives of their class. The bold figures in Table 3 delineate genes and lines indicate linked genes. Indications of gene arrangement were obtained from these findings. The *argB* and *argC* mutants yield very small numbers of transductants in reciprocal crosses and were taken to be closely linked. Crosses between *argE* and *argH* mutants give rise to more transductants than other members of the *argB*, *C*, *E*, *G*, *H* group and were expected to be at the extremities of the cluster.

Three-point linkage tests with the argB, C, E, G, H mutants. Double mutants with known *argB*, *C* or *E* mutations and additional *argH* mutations (Table 1) were used in reciprocal experiments with single *argB*, *C*, *E*, *G* or *H* mutants. Results are given in Table 4. The ratios of the numbers of transductants obtained in the reciprocal crosses fall into two groups, namely 0-0.2 and 0.6-1.3. The gene order established is *argE-C-B-G-H* and the relative positions of some of the mutants are

$$\begin{array}{ccccccc} \textit{argE1-E2-C1-B1-B2-G1-H5-H2-H4-H1.} \\ \textit{(E3)} & & \textit{(G2)} & & \textit{(H3)(H6)} \end{array}$$

Mutants in parentheses are assigned to the same positions occupied by those above them.

Three-point linkage tests with the argE, C, B, G, H mutants and external cysE markers. Two double mutants, *argE1cysE5* and *argH1cysE3*, were used in donor-switching experiments. Results of a typical experiment are given in Table 5. One group of ratios ranged from 0.5 to 0.8 and the other from 0 to 0.2. The mutant order above was confirmed in that all the single *arg* mutations as well as *cysE448* mapped between *cysE3* and *argH1* and all the mutations except *cysE448* mapped to the 'right' of *argE1* and *cysE5*. The final map is given in Fig. 2.

Relative distances within the argE, C, B, G, H gene cluster. The results of the ratio test with ornithine-requiring *argE*, *C* or *B* donors and arginine-requiring *argG* or *argH* recipients (Glansdorff, 1965) are given in Table 6. Results confirm the marker order arrived at above and also indicate the positions of mutants which could not be placed with certainty. By this test the position of *argH2* seems to be farther to the

Table 4. Three-point transductions with the *argB*, *C*, *E*, *G*, *H* mutants

Cross		Prototroph transductants obtained	Ratio <i>a/b</i>	Mutant order
Recipient	Donor			
(a) <i>argB1argH6</i> × <i>argE1</i>		8	1.3	<i>argE1 argB1 argH6</i>
(b) <i>argE1</i> × <i>argB1argH6</i>		6		
(a) <i>argB1argH6</i> × <i>argE2</i>		4	0.8	<i>argE2 argB1 argH6</i>
(b) <i>argE2</i> × <i>argB1argH6</i>		5		
(a) <i>argB1argH6</i> × <i>argE3</i>		11	1.1	<i>argE3 argB1 argH6</i>
(b) <i>argE3</i> × <i>argB1argH6</i>		10		
(a) <i>argB1argH6</i> × <i>argC1</i>		2	0.7	<i>argC1 argB1 argH6</i>
(b) <i>argC1</i> × <i>argB1argH6</i>		3		
(a) <i>argB1argH6</i> × <i>argB1</i>		0	—	— — —
(b) <i>argB1</i> × <i>argB1argH6</i>		0		
(a) <i>argB1argH6</i> × <i>argB2</i>		0	0	<i>argB1 argB2 argH6</i>
(b) <i>argB2</i> × <i>argB1argH6</i>		2		
(a) <i>argB1argH6</i> × <i>argG1</i>		1	0.1	<i>argB1 argG1 argH6</i>
(b) <i>argG1</i> × <i>argB1argH6</i>		23		
(a) <i>argB1argH6</i> × <i>argG2</i>		4	0.1	<i>argB1 argG2 argH6</i>
(b) <i>argG2</i> × <i>argB1argH6</i>		46		
(a) <i>argB1argH6</i> × <i>argH1</i>		6	1.5	<i>argB1 argH6 argH1</i>
(b) <i>argH1</i> × <i>argB1argH6</i>		4		
(a) <i>argB1argH6</i> × <i>argH2</i>		1	0.1	<i>argB1 argH2 argH6</i>
(b) <i>argH2</i> × <i>argB1argH6</i>		17		
(a) <i>argB1argH6</i> × <i>argH3</i>		0	0	<i>argB1 argH3 argH6</i>
(b) <i>argH3</i> × <i>argB1argH6</i>		5		
(a) <i>argE1argH5</i> × <i>argE1</i>		0	—	— — —
(b) <i>argE1</i> × <i>argE1argH5</i>		0		
(a) <i>argE1argH5</i> × <i>argE2</i>		0	0	<i>argE1 argE2 argH5</i>
(b) <i>argE2</i> × <i>argE1argH5</i>		11		
(a) <i>argE1argH5</i> × <i>argE3</i>		0	0	<i>argE1 argE3 argH5</i>
(b) <i>argE3</i> × <i>argE1argH5</i>		2		
(a) <i>argE1argH5</i> × <i>argC1</i>		2	0.1	<i>argE1 argC1 argH5</i>
(b) <i>argC1</i> × <i>argE1argH5</i>		20		
(a) <i>argE1argH5</i> × <i>argB1</i>		1	0.1	<i>argE1 argB1 argH5</i>
(b) <i>argB1</i> × <i>argE1argH5</i>		12		
(a) <i>argE1argH5</i> × <i>argB2</i>		3	0.2	<i>argE1 argB2 argH5</i>
(b) <i>argB2</i> × <i>argE1argH5</i>		17		
(a) <i>argE1argH5</i> × <i>argG1</i>		4	0.1	<i>argE1 argG1 argH5</i>
(b) <i>argG1</i> × <i>argE1argH5</i>		73		
(a) <i>argE1argH5</i> × <i>argG2</i>		2	0.1	<i>argE1 argG2 argH5</i>
(b) <i>argG2</i> × <i>argE1argH5</i>		31		
(a) <i>argE1argH5</i> × <i>argH1</i>		3	0.8	<i>argE1 argH5 argH1</i>
(b) <i>argH1</i> × <i>argE1argH5</i>		4		
(a) <i>argE1argH5</i> × <i>argH2</i>		32	0.6	<i>argE1 argH5 argH2</i>
(b) <i>argH2</i> × <i>argE1argH5</i>		53		
(a) <i>argE1argH5</i> × <i>argH3</i>		4	0.7	<i>argE1 argH5 argH3</i>
(b) <i>argH3</i> × <i>argE1argH5</i>		6		
(a) <i>argC1argH4</i> × <i>argE1</i>		11	0.7	<i>argE1 argC1 argH4</i>
(b) <i>argE1</i> × <i>argC1argH4</i>		16		
(a) <i>argC1argH4</i> × <i>argE2</i>		24	0.8	<i>argE2 argC1 argH4</i>
(b) <i>argE2</i> × <i>argC1argH4</i>		31		
(a) <i>argC1argH4</i> × <i>argE3</i>		23	0.7	<i>argE3 argC1 argH4</i>
(b) <i>argE3</i> × <i>argC1argH4</i>		35		
(a) <i>argC1argH4</i> × <i>argC1</i>		0	—	— — —
(b) <i>argC1</i> × <i>argC1argH4</i>		0		
(a) <i>argC1argH4</i> × <i>argB1</i>		0	0	<i>argC1 argB1 argH4</i>
(b) <i>argB1</i> × <i>argC1argH4</i>		4		
(a) <i>argC1argH4</i> × <i>argB2</i>		1	0.1	<i>argC1 argB2 argH4</i>
(b) <i>argB2</i> × <i>argC1argH4</i>		9		
(a) <i>argC1argH4</i> × <i>argG1</i>		2	0.1	<i>argC1 argG1 argH4</i>
(b) <i>argG1</i> × <i>argC1argH4</i>		18		
(a) <i>argC1argH4</i> × <i>argG2</i>		3	0.1	<i>argC1 argG2 argH4</i>
(b) <i>argG2</i> × <i>argC1argH4</i>		24		
(a) <i>argC1argH4</i> × <i>argH1</i>		3	0.6	<i>argC1 argH4 argH1</i>
(b) <i>argH1</i> × <i>argC1argH4</i>		5		
(a) <i>argC1argH4</i> × <i>argH2</i>		0	0	<i>argC1 argH2 argH4</i>
(b) <i>argH2</i> × <i>argC1argH4</i>		3		
(a) <i>argC1argH4</i> × <i>argH3</i>		0	0	<i>argC1 argH3 argH4</i>
(b) <i>argH3</i> × <i>argC1argH4</i>		7		

'right' than indicated by the previous test. Like the *Escherichia coli* mutant *argC2* (Glansdorff, 1965), *argH2* yields more wild-type transductants than other mutants and can only be accurately mapped by three-point transductions which correct for the high rate of transduction to prototrophy.

Relative distances of the argE, C, B, G, H mutations from the linked external marker cysE448. These results are presented in Table 7 and confirm the order derived above. Map distances appear slightly smaller than in the previous ratio test (Table 6). This

Table 5. *Three-point transductions with the argE, C, B, G, H mutants and the external marker cysE*

Cross		Prototroph transductants obtained	Ratio a/b	Mutant order
Recipient	Donor			
(a) <i>argE1cysE5</i> × <i>cysE448</i>		3	0.1	<i>cysE5 cysE448 argE1</i>
(b) <i>cysE448</i> × <i>argE1cysE5</i>		66		
(a) <i>argE1cysE5</i> × <i>argE1</i>		0	—	— — —
(b) <i>argE1</i> × <i>argE1cysE5</i>		0		
(a) <i>argE1cysE5</i> × <i>argE2</i>		2	0.7	<i>cysE5 argE1 argE2</i>
(b) <i>argE2</i> × <i>argE1cysE5</i>		3		
(a) <i>argE1cysE5</i> × <i>argE3</i>		2	0.5	<i>cysE5 argE1 argE3</i>
(b) <i>argE3</i> × <i>argE1cysE5</i>		4		
(a) <i>argE1cysE5</i> × <i>argC1</i>		11	0.7	<i>cysE5 argE1 argC1</i>
(b) <i>argC1</i> × <i>argE1cysE5</i>		16		
(a) <i>argE1cysE5</i> × <i>argB1</i>		16	0.8	<i>cysE5 argE1 argB1</i>
(b) <i>argB1</i> × <i>argE1cysE5</i>		20		
(a) <i>argE1cysE5</i> × <i>argB2</i>		10	0.6	<i>cysE5 argE1 argB2</i>
(b) <i>argB2</i> × <i>argE1cysE5</i>		16		
(a) <i>argE1cysE5</i> × <i>argG1</i>		185	0.6	<i>cysE5 argE1 argG1</i>
(b) <i>argG1</i> × <i>argE1cysE5</i>		302		
(a) <i>argE1cysE5</i> × <i>argG2</i>		152	0.7	<i>cysE5 argE1 argG2</i>
(b) <i>argG2</i> × <i>argE1cysE5</i>		212		
(a) <i>argE1cysE5</i> × <i>argH1</i>		64	0.5	<i>cysE5 argE1 argH1</i>
(b) <i>argH1</i> × <i>argE1cysE5</i>		120		
(a) <i>argE1cysE5</i> × <i>argH2</i>		180	0.6	<i>cysE5 argE1 argH2</i>
(b) <i>argH2</i> × <i>argE1cysE5</i>		304		
(a) <i>argE1cysE5</i> × <i>argH3</i>		96	0.5	<i>cysE5 argE1 argH3</i>
(b) <i>argH3</i> × <i>argE1cysE5</i>		184		
(a) <i>argH1cysE3</i> × <i>cysE448</i>		0	0	<i>cysE3 cysE448 argH1</i>
(b) <i>cysE448</i> × <i>argH1cysE3</i>		21		
(a) <i>argH1cysE3</i> × <i>argE1</i>		3	0.1	<i>cysE3 argE1 argH1</i>
(b) <i>argE1</i> × <i>argH1cysE3</i>		54		
(a) <i>argH1cysE3</i> × <i>argE2</i>		3	0.1	<i>cysE3 argE2 argH1</i>
(b) <i>argE2</i> × <i>argH1cysE3</i>		43		
(a) <i>argH1cysE3</i> × <i>argE3</i>		8	0.2	<i>cysE3 argE3 argH1</i>
(b) <i>argE3</i> × <i>argH1cysE3</i>		37		
(a) <i>argH1cysE3</i> × <i>argC1</i>		2	0.1	<i>cysE3 argC1 argH1</i>
(b) <i>argC1</i> × <i>argH1cysE3</i>		34		
(a) <i>argH1cysE3</i> × <i>argB2</i>		0	0	<i>cysE3 argG1 argH1</i>
(b) <i>argB2</i> × <i>argH1cysE3</i>		29		
(a) <i>argH1cysE3</i> × <i>argG1</i>		5	0.1	<i>cysE3 argG2 argH1</i>
(b) <i>argG1</i> × <i>argH1cysE3</i>		85		
(a) <i>argH1cysE3</i> × <i>argH1</i>		0	—	— — —
(b) <i>argH1</i> × <i>argH1cysE3</i>		0		
(a) <i>argH1cysE3</i> × <i>argH2</i>		0	0	<i>cysE3 argH2 argH1</i>
(b) <i>argH2</i> × <i>argH1 cysE3</i>		17		
(a) <i>argH1cysE3</i> × <i>argH3</i>		0	0	<i>cysE3 argH3 argH1</i>
(b) <i>argH3</i> × <i>argH1cysE3</i>		7		

Table 6. Distances between *argE*, *C*, *B*, and *argG* and *H* mutantsValues are the mean of two experiments. Mutant *argG2* was not used because of its high reversion rate.

Cross		Transductants: prototrophs/total	%
Recipient	Donor		
<i>argG1</i> × <i>argE1</i>		83/779	10.7
<i>argG1</i> × <i>argE3</i>		91/900	9.9
<i>argG1</i> × <i>argE2</i>		60/854	7.0
<i>argG1</i> × <i>argC1</i>		62/1150	5.4
<i>argG1</i> × <i>argB1</i>		59/1184	5.0
<i>argG1</i> × <i>argB2</i>		31/803	2.6
<i>argH3</i> × <i>argE1</i>		82/481	19.1
<i>argH3</i> × <i>argE3</i>		211/1148	18.4
<i>argH3</i> × <i>argE2</i>		128/810	15.8
<i>argH3</i> × <i>argC1</i>		177/1308	13.5
<i>argH3</i> × <i>argB1</i>		127/975	13.0
<i>argH3</i> × <i>argB2</i>		68/631	10.8
<i>argH2</i> × <i>argE1</i>		412/970	42.5
<i>argH2</i> × <i>argE3</i>		811/2102	38.6
<i>argH2</i> × <i>argE2</i>		734/2331	32.4
<i>argH2</i> × <i>argC1</i>		318/1112	28.7
<i>argH2</i> × <i>argB1</i>		542/1993	27.2
<i>argH2</i> × <i>argB2</i>		228/1155	19.7
<i>argH1</i> × <i>argE1</i>		160/828	19.3
<i>argH1</i> × <i>argE3</i>		163/878	18.6
<i>argH1</i> × <i>argE2</i>		127/802	15.8
<i>argH1</i> × <i>argC1</i>		116/847	13.7
<i>argH1</i> × <i>argB1</i>		126/960	13.1
<i>argH1</i> × <i>argB2</i>		64/611	10.6

Table 7. Distances between *argE*, *C*, *B*, *G*, *H* mutants and the *cysE448* marker

Values are the mean of two experiments.

Cross		Transductants: prototrophs/total	%	Average
Recipient	Donor			
<i>argE1</i> × <i>cysE448</i>		186/238	78.2	77.6
<i>cysE448</i> × <i>argE1</i>		143/186	76.9	
<i>argE3</i> × <i>cysE448</i>		196/246	79.7	78.2
<i>cysE448</i> × <i>argE3</i>		190/248	76.6	
<i>argE2</i> × <i>cysE448</i>		208/258	80.6	80.1
<i>cysE448</i> × <i>argE2</i>		156/196	79.6	
<i>argC1</i> × <i>cysE448</i>		252/307	82.1	81.6
<i>cysE448</i> × <i>argC1</i>		366/452	81.0	
<i>argB1</i> × <i>cysE448</i>		183/228	80.3	82.1
<i>cysE448</i> × <i>argB1</i>		188/224	83.9	
<i>argB2</i> × <i>cysE448</i>		289/350	82.6	83.7
<i>cysE448</i> × <i>argB2</i>		260/307	84.7	
<i>argG2</i> × <i>cysE448</i>		395/452	87.4	84.8
<i>cysE448</i> × <i>argG2</i>		192/234	82.1	
<i>argG1</i> × <i>cysE448</i>		101/117	86.3	87.5
<i>cysE448</i> × <i>argG1</i>		265/299	88.6	
<i>argH2</i> × <i>cysE448</i>		452/482	93.8	93.9
<i>cysE448</i> × <i>argH2</i>		378/402	94.0	
<i>argH3</i> × <i>cysE448</i>		219/256	85.6	89.2
<i>cysE448</i> × <i>argH3</i>		358/386	92.8	
<i>argH1</i> × <i>cysE448</i>		339/368	92.1	91.6
<i>cysE448</i> × <i>argH1</i>		244/268	91.0	

discrepancy is possibly caused by different efficiencies of pairing in different regions of the chromosome (Pritchard, 1960).

Donor-switching experiments with the argF and pyr markers. Two double mutants *argF3pyr-1* and *argF5pyr-2* were crossed with the single *argF* and *pyr-3* mutants. Results (Table 8) indicate that *pyr-1pyr-2* and *pyr-3* are situated on the same side of the *argF* gene and that the order of mutants in this linkage group is

$$\text{pyr-3-pyr-1-argF4-argF3-argF1-argF5-argF2.} \\ (\text{pyr-2})$$

The two ratio groups indicative of the number of crossovers that occurred ranged between 0.004 and 0.57-1.78.

Table 8. *Donor-switching transductions with the argF and pyr markers*

Cross		Prototroph transductants	Ratio <i>a/b</i>	Mutant order
Recipient	Donor			
(a) <i>argF3pyr-1</i> × <i>pyr-3</i>		7	0.70	<i>pyr-3 pyr-1 argF3</i>
(b) <i>pyr-3</i> × <i>argF3pyr-1</i>		10		
(a) <i>argF3pyr-1</i> × <i>argF4</i>		74	0.03	<i>pyr-1 argF4 argF3</i>
(b) <i>argF4</i> × <i>argF3pyr-1</i>		2364		
(a) <i>argF3pyr-1</i> × <i>argF3</i>		0	—	— — —
(b) <i>argF3</i> × <i>argF3pyr-1</i>		0		
(a) <i>argF3pyr-1</i> × <i>argF1</i>		4	0.57	<i>pyr-1 argF3 argF1</i>
(b) <i>argF1</i> × <i>argF3pyr-1</i>		7		
(a) <i>argF3pyr-1</i> × <i>argF5</i>		10	0.83	<i>pyr-1 argF3 argF5</i>
(b) <i>argF5</i> × <i>argF3pyr-1</i>		12		
(a) <i>argF3pyr-1</i> × <i>argF2</i>		23	1.78	<i>pyr-1 argF3 argF2</i>
(b) <i>argF2</i> × <i>argF3pyr-1</i>		13		
(a) <i>argF5pyr-2</i> × <i>pyr3</i>		10	0.91	<i>pyr-3 pyr2 argF5</i>
(b) <i>pyr-3</i> × <i>argF5pyr-2</i>		11		
(a) <i>argF5pyr-2</i> × <i>argF4</i>		132	0.04	<i>pyr-2 argF4 argF5</i>
(b) <i>argF4</i> × <i>argF5pyr-2</i>		3250		
(a) <i>argF5pyr-2</i> × <i>argF3</i>		0	0	<i>pyr-2 argF3 argF5</i>
(b) <i>argF3</i> × <i>argF5pyr-2</i>		14		
(a) <i>argF5pyr-2</i> × <i>argF1</i>		0	0	<i>pyr-2 argF1 argF5</i>
(b) <i>argF1</i> × <i>argF5pyr-2</i>		5		
(a) <i>argF5pyr-2</i> × <i>argF5</i>		0	—	— — —
(b) <i>argF5</i> × <i>argF5pyr-2</i>		0		
(a) <i>argF5pyr-2</i> × <i>argF2</i>		4	0.80	<i>pyr-2 argF5 argF2</i>
(b) <i>argF2</i> × <i>argF5pyr-2</i>		5		

Map of argF sites as determined with the pyr-3 marker. Results of crosses between *pyr-3* and the *argF* mutants are given in Table 9. The order determined by donor-switching was confirmed apart from the position of *argF4*. This mutant behaves like *argH2* mentioned previously and can only be mapped by three-point tests.

Three-point crosses between argD and str-r markers. Double mutants with five known *argD* and *str-r* markers (Table 1) were crossed with five single *argD* mutants in donor-switching experiments (Table 10). Selection was made for *arg⁺str-r* transductants. Two ratio classes (0.003 and 1.3-2.3) were again identified. Results indicate that the *str-r* mutations are on the same side of the *argD* gene and that the mutant order within this linkage group is *str-r-argD5-argD2-argD1-argD6-argD3*.

Relative distances in the argD-str-r linkage group. Results of a ratio test with *str-r1* as donor and *argD* mutants as recipients are given in Table 11. Mutant *argD4*, which could not be mapped previously because of its poor recipient capacity (Table 3), could now be placed. These results confirm the order derived from donor-switching experiments excepting the position of *argD2*. This mutant behaves like *argH2* and *argF4*. The map of this linkage group is presented in Fig. 2.

Table 9. *Distances between argF mutants and the pyr-3 marker*

Values are the mean of two experiments.

Cross		Transductants: prototroph/total	%	Average
Recipient	Donor			
<i>argF4</i> × <i>pyr-3</i>		6600/12900	51.2	45.0
<i>pyr-3</i> × <i>argF4</i>		2277/5870	38.8	
<i>argF3</i> × <i>pyr-3</i>		52/2464	2.1	2.0
<i>pyr-3</i> × <i>argF3</i>		38/2208	1.8	
<i>argF1</i> × <i>pyr-3</i>		62/2142	2.9	2.6
<i>pyr-3</i> × <i>argF1</i>		46/1988	2.3	
<i>argF5</i> × <i>pyr-3</i>		193/5116	3.8	3.6
<i>pyr-3</i> × <i>argF5</i>		101/2965	3.4	
<i>argF2</i> × <i>pyr-3</i>		101/2035	5.0	4.8
<i>pyr-5</i> × <i>argF2</i>		103/2242	4.6	

Order of argA sites as determined with the non-linked str-r marker. Results of a typical experiment are given in Table 12. Mutants *argA1* and *argA4* were derived from *str-r-1* and could only be used as donors in these experiments. Results obtained were used to construct a 'best-fit' map (Hartman *et al.* 1960), which is presented in Fig. 2.

The arginine gene maps of Proteus mirabilis, Escherichia coli and Salmonella typhimurium. These are presented in Fig. 3. The clustering of *argE*, *C*, *B*, *H* in that order (*E. coli* K-12), the linkage of *argD* to *str-r* (*E. coli* w) and the non-linkage of *argF* and *argA* to the other arginine genes are common characteristics. The *argG* gene is included in the *argE*, *C*, *B*, *G*, *H* group in *P. mirabilis* and this cluster is closely linked to *cysE* not to *met* genes as in the other organisms. The *argF* gene is linked to a *pyr* marker in *P. mirabilis* like the additional gene for the same enzymic step *arg1* in *E. coli* K-12 (Glansdorff, Sand & Verhoef, 1967; Taylor & Thoman, 1964).

DISCUSSION

Double mutants constructed by transduction (Glansdorff, 1965) would have been preferable to the selected double mutants used here for the reason that the second mutations in selected double mutants cannot be used in simple ratio tests because they are not available separately. Phage 34/13 causes lysogenic conversion (Coetzee, 1961) which results in non-adsorption of homologous phage. Despite the use of low multiplicities of infection and antiserum to phage 34/13 (Coetzee & Sacks, 1960*b*) transductants were invariably lysogenized and could not be re-transduced. Attempts to cure the transductants (Zinder, 1958) were also unsuccessful (Prozesky, unpublished results).

Abortive transduction with the system used here has not been reported (Coetzee & Sacks, 1960*a*; Coetzee, de Klerk & Maré, 1963; Coetzee, 1963). Minute colonies

Table 10. *Three-point transductions with the argD and str-r markers**

Cross		Transductants <i>arg</i> ⁺ , <i>str-r</i>	Ratio <i>a/b</i>	Mutant order
Recipient	Donor			
(a) <i>argD1</i>	× <i>argD5str-r-10</i>	0	0	<i>str-r-10 argD5 argD1</i>
(b) <i>argD5str-r-10</i>	× <i>argD1</i>	14		
(a) <i>argD2</i>	× <i>argD5str-r-10</i>	0	0	<i>str-r-10 argD5 argD2</i>
(b) <i>argD5str-r-10</i>	× <i>argD2</i>	9		
(a) <i>argD3</i>	× <i>argD5str-r-10</i>	3	0.1	<i>str-r-10 argD5 argD3</i>
(b) <i>argD5str-r-10</i>	× <i>argD3</i>	21		
(a) <i>argD5</i>	× <i>argD1str-5r-10</i>	0	—	— — —
(b) <i>argD5str-r-10</i>	× <i>argD5</i>	0		
(a) <i>argD6</i>	× <i>argD5str-r-10</i>	2	0.2	<i>str-r-10 argD5 argD6</i>
(b) <i>argD5str-r-10</i>	× <i>argD6</i>	13		
(a) <i>argD1</i>	× <i>argD2str-r-7</i>	3	0.2	<i>str-r-7 argD2 argD1</i>
(b) <i>argD2str-r-7</i>	× <i>argD1</i>	13		
(a) <i>argD2</i>	× <i>argD2str-r-7</i>	0	—	— — —
(b) <i>argD2str-r-7</i>	× <i>argD2</i>	0		
(a) <i>argD3</i>	× <i>argD2str-r-7</i>	0	0	<i>str-r-7 argD2 argD3</i>
(b) <i>argD2str-r-7</i>	× <i>argD3</i>	17		
(a) <i>argD5</i>	× <i>argD2str-r-7</i>	17	1.6	<i>str-r-7 argD5 argD2</i>
(b) <i>argD2str-r-7</i>	× <i>argD5</i>	11		
(a) <i>argD6</i>	× <i>argD2str-r-7</i>	5	0.3	<i>str-r-7 argD2 argD6</i>
(b) <i>argD2str-r-7</i>	× <i>argD6</i>	20		
(a) <i>argD1</i>	× <i>argD1str-r-6</i>	0	—	— — —
(b) <i>argD1str-r-6</i>	× <i>argD1</i>	0		
(a) <i>argD2</i>	× <i>argD1str-r-6</i>	7	1.4	<i>str-r-6 argD2 argD1</i>
(b) <i>argD1str-r-6</i>	× <i>argD2</i>	5		
(a) <i>argD3</i>	× <i>argD1str-r-6</i>	4	0.2	<i>str-r-6 argD1 argD3</i>
(b) <i>argD1str-r-6</i>	× <i>argD3</i>	22		
(a) <i>argD6</i>	× <i>argD1str-r-6</i>	0	0	<i>str-r-6 argD1 argD6</i>
(b) <i>argD1str-r-6</i>	× <i>argD6</i>	6		
(a) <i>argD1</i>	× <i>argD6str-r-11</i>	6	1.5	<i>str-r-11 argD1 argD6</i>
(b) <i>argD6str-r-11</i>	× <i>argD1</i>	4		
(a) <i>argD3</i>	× <i>argD6str-r-11</i>	1	0.2	<i>str-r-11 argD6 argD3</i>
(b) <i>argD6str-r-11</i>	× <i>argD3</i>	5		
(a) <i>argD5</i>	× <i>argD6str-r-11</i>	4	1.3	<i>str-r-11 argD5 argD6</i>
(b) <i>argD6str-r-11</i>	× <i>argD5</i>	3		
(a) <i>argD6</i>	× <i>argD6str-r-11</i>	0	—	— — —
(b) <i>argD6str-r-11</i>	× <i>argD6</i>	0		
(a) <i>argD1</i>	× <i>argD3str-r-8</i>	72	1.8	<i>str-r-8 argD1 argD3</i>
(b) <i>argD3str-r-8</i>	× <i>argD1</i>	40		
(a) <i>argD2</i>	× <i>argD3str-r-8</i>	10	1.3	<i>str-r-8 argD2 argD3</i>
(b) <i>argD3str-r-8</i>	× <i>argD2</i>	8		
(a) <i>argD3</i>	× <i>argD3str-r-8</i>	0	—	— — —
(b) <i>argD3str-r-8</i>	× <i>argD3</i>	0		
(a) <i>argD5</i>	× <i>argD3str-r-8</i>	82	2.0	<i>str-r-8 argD5 argD3</i>
(b) <i>argD3str-r-8</i>	× <i>argD5</i>	41		
(a) <i>argD6</i>	× <i>argD3str-r-8</i>	46	2.3	<i>str-r-8 argD6 argD3</i>
(b) <i>argD3str-r-8</i>	× <i>argD6</i>	20		

* *argD4* was not used in this investigation as it is a poor recipient with average donor capacity.

resembling abortive transductant colonies were sometimes observed in this investigation but attempts to prove them so (Ozeki, 1956; Nishioka, Demerec & Eisenstark, 1967) did not yield reproducible results and this problem is being investigated further.

Proteus mirabilis, *Escherichia coli* and *Salmonella typhimurium* have guanine + cytosine molar contents of 38–41%, 50% (Hill, 1966) and 50–52% (Marmur, Falkow & Mandel, 1963) respectively. Notable genetic differences between *P. mirabilis* and the other two organisms were expected. The different linkage of the *argE*, *C*, *B*, *G*, *H* cluster found here may mean that the placing of this cluster differs in *P. mirabilis* and *E. coli*. However, we are looking at the genetic map of *Proteus* through the keyhole of

Table 11. Distances between *argD* mutants and the *str-r-I* marker

Values are the mean of two experiments

Cross		No. transductants		Ratio	%
Recipient	Donor	<i>arg</i> ⁺ , <i>str-r</i>	<i>str-r</i>	<i>str-r-arg</i> ⁺ , <i>str-r</i> / <i>str-r</i>	
<i>argD4</i> × <i>str-r-I</i>		108	1208	1100/1208	91.2
<i>argD5</i> × <i>str-r-I</i>		185	2355	2170/2355	92.1
<i>argD1</i> × <i>str-r-I</i>		447	5719	5272/5719	92.2
<i>argD6</i> × <i>str-r-I</i>		310	5132	4822/5132	94.0
<i>argD3</i> × <i>str-r-I</i>		411	5828	5417/5828	94.7
<i>argD2</i> × <i>str-r-I</i>		134	2628	2494/2628	94.9

Table 12. Mapping of the *argA* mutants with prototroph transductant numbers normalized with respect to *str-r* marker transduction in the same crosses

Recipient*	Donor	No. transductants		Ratio	Average
		Prototroph	<i>str-r</i>	<i>arg</i> ⁺ / <i>str-r</i> %	
<i>argA2</i> × <i>argA1str-r-I</i>		2	484	0.41	
<i>argA3</i> × <i>argA1str-r-I</i>		36	543	6.63	
<i>argA5</i> × <i>argA1str-r-I</i>		59	915	6.45	
<i>argA6</i> × <i>argA1str-r-I</i>		47	851	3.53	
<i>argA2</i> × <i>argA4str-r-I</i>		4	139	2.88	
<i>argA3</i> × <i>argA4str-r-I</i>		12	285	4.21	
<i>argA5</i> × <i>argA4str-r-I</i>		8	227	3.52	
<i>argA6</i> × <i>argA4str-r-I</i>		1	198	0.51	
<i>argA2</i> × <i>argA2str-r-2</i>		0	186	—	
<i>argA3</i> × <i>argA2str-r-2</i>		20	306	6.54	6.45
<i>argA2</i> × <i>argA3str-r-3</i>		8	126	6.35	
<i>argA2</i> × <i>argA5str-r-4</i>		6	138	4.35	5.86
<i>argA5</i> × <i>argA2str-r-2</i>		23	312	7.37	
<i>argA2</i> × <i>argA6str-r-5</i>		9	165	5.45	5.41
<i>argA6</i> × <i>argA2str-r-2</i>		16	298	5.37	
<i>argA3</i> × <i>argA3str-r-3</i>		0	288	—	
<i>argA5</i> × <i>argA3str-r-3</i>		2	207	0.97	1.35
<i>argA3</i> × <i>argA5str-r-4</i>		5	289	1.73	
<i>argA6</i> × <i>argA3str-r-3</i>		4	203	1.97	1.50
<i>argA3</i> × <i>argA6str-r-5</i>		3	294	1.02	
<i>argA5</i> × <i>argA6str-r-5</i>		1	286	0.35	0.43
<i>argA6</i> × <i>argA5str-r-4</i>		1	197	0.51	
<i>argA6</i> × <i>argA6str-r-5</i>		0	226	—	

* *argA1str-r-I* and *argA4str-r-I* were not used as recipients.

transduction and no firm conclusions as to the orientation of the linkage groups can be reached. The spatial arrangements of the arginine genes themselves are similar in *P. mirabilis*, *E. coli* and *S. typhimurium*. This may be necessary for the function and regulation of these genes and could be an evolutionary trait. Mutants with defective control of arginine synthesis have been isolated in *P. mirabilis* strain 13 (Prozesky, in

Fig. 2. Map of mutations in the four linkage groups. Mutants in brackets were not mapped by all methods or gave inconclusive results. The tentative positions assigned to such mutants are based on results obtained from donor-switching experiments except for mutant *argG2*, where results of the ratio test were used. The relative distances in linkage group I are those estimated with *cysE448* as the linked reference marker. In linkage group IV only the order of mutants was established and relative distances are tentative.

preparation) and it will be of interest to know whether the control of the *argG* gene in *P. mirabilis* and *E. coli* differs. In *E. coli* this gene is not included in the *argE*, *C*, *B*, *H* cluster and is distantly linked by transduction to the regulator gene *argR* (Jacoby & Gorini, 1967). It may also be of taxonomic interest to know whether other members of the *Proteus-Providence* group have the same arrangement and linkage relationships of their arginine genes as *P. mirabilis* (Coetzee, Smit & Prozesky, 1966).

Fig. 3. The arrangement of *arg* genes in the linkage maps of *Escherichia coli* strains K-12 and W and *Salmonella typhimurium* compared to the arrangement of these genes in the four linkage groups mapped by transduction in *Proteus mirabilis* strain 13. The orientation of the *Proteus mirabilis* linkage groups and their positions relative to one another are unknown. The *Escherichia coli* maps are given according to Glansdorff, Sand & Verhoef (1967) and Vogel & Bacon (1966) for strains K-12 and W respectively. The map for *Salmonella typhimurium* is that of Sanderson & Demerec (1965). Maps are not drawn to scale. *arg* = arginineless; *str-r* = streptomycin-resistant; *cys* = cysteineless; *pyr* = pyrimidineless; *try* = tryptophanless; *his* = histidineless; *met* = methionineless; *thr* = threonineless.

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