TOPOGRAPHY OF COTRANSDUCIBLE ARGININE MUTATIONS IN ESCHERICHIA COLI K-12

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THE sequence of enzymatic steps specific for arginine biosynthesis in *Escherichia coli* is outlined in Figure 1. In mutants of *E. coli* K-12, the synthesis of any one of the enzymes involved in this pathway, except acetylornithine transaminase (step D), can be altered by single-step mutation (GORINI, GUNDERSEN and BURGER 1961; MAAS 1961; VOGEL 1961). Mutants blocked in step D are known among derivatives of the W strain only (VOGEL, BACON and BAICH 1963). The results of mapping experiments, performed by conjugation techniques with arginine (arg) mutants of *E. coli* K-12 (for abbreviations see Figure 2), have shown that the loci of the seven known structural genes are distributed among four regions of the bacterial chromosome (Figure 2) (LAVALLE, JACOB and MAAS, *see* MAAS 1961; MAAS 1961; MAAS and MAAS 1962). Four of them (*argB*, *C*, *E* and *H*) lie in the same region, together with two methionine markers and one locus which is most probably the structural gene of a phosphoenolpyruvate carboxylase (VANDERWINKEL, LIARD, RAMOS and WIAME 1963). These markers are cotransducible by the temperate bacteriophage 363 (LAVALLE *et al.*). An opportunity was thus provided to extend our information about the genetic control of arginine biosynthesis; knowledge of the spatial arrangement of the four arginine loci might throw some light on the mechanism of repression in this pathway.

We shall describe the mapping by transduction of the *B*, *C*, *E* and *H* loci. The significance of the results for studies dealing with control mechanisms in arginine biosynthesis will be briefly discussed.

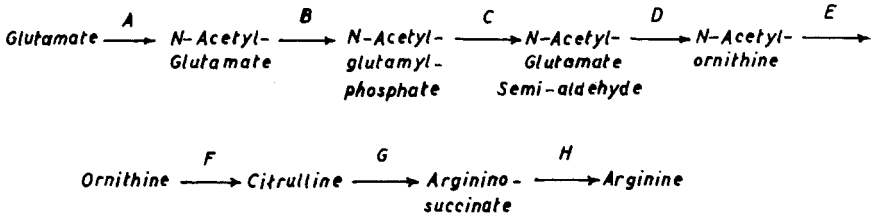

FIGURE 1.—The biosynthesis of arginine in *E. coli*. The capital letters refer to the known enzymic steps.

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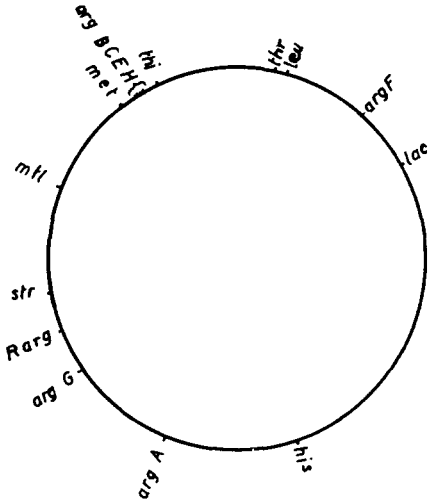

FIGURE 2.—Map of *arg* loci in *E. coli* K-12. Abbreviations: *arg*=arginine; *glu*=glucose; *his*=histidine; *leu*=leucine; *lac*=lactose; *met*=methionine; *mtl*=mannitol; *thi*=thiamine; *thr*=threonine; *str*=streptomycin; *T6*=phage T6; *r*=resistant; *s*=sensitive; λ=phage λ; (λ+)=lysogeny for phage λ; /λ=phage λ not adsorbed; Hfr=high frequency male donor; F⁻=recipient, female; *Rarg*=regulatory gene of arginine biosynthesis.

MATERIALS AND METHODS

Genetic terminology: The genetic terminology follows the rules proposed by DEMEREC (1956). Similar mutants of independent origin were designated by numbers following the abbreviation corresponding to their particular defect. To designate mutations which were defined by metabolic studies and genetic localization, a capital letter was included in the symbol. For *arg* mutants, the letters refer to defined enzymatic steps in the biosynthesis of arginine; for example, mutants *argC-1* and *C-2* are two independently isolated organisms blocked in the conversion of acetyl-glutamyl-phosphate to acetyl-glutamate semi-aldehyde. The features of the strains are given in Table 1. The symbol "orn" refers to the phenotype of strains whose requirement can be satisfied

TABLE 1

List of strains used

Strain	*thr-leu*	*his*	*thi*	*met*	*arg* Genotype	*arg* Phenotype	*glu*	*T6*	λ	*str*	Sex
P10	—	+	—	+	+	+	+	*s*		*s*	Hfr
3OSOMA4	+	+	—	+	*argB-1*	orn	+	*s*	(λ⁻)	*s*	Hfr
P4XSB167	+	+	+	*met-1*	*argC-1*	orn	+	*s*	(λ+)	*s*	Hfr
P4XSB170	+	+	+	*met-1*	*argC-2*	orn	+	*s*	(λ+)	*s*	Hfr
P4XSB166	+	+	+	*met-1*	*argE-1*	orn	+	*s*	(λ+)	*s*	Hfr
P4XSB145	+	+	+	*met-1*	*argH-1*	arg	+	*s*	(λ+)	*s*	Hfr
3OSOMA2	+	+	—	+	*argH-3*	arg	+	*s*	(λ⁻)	*s*	Hfr
PA342	—	—	—	+	*argH-2*	arg	*glu-2*	*s*	(/λ)	*r*	F⁻
PA214	—	—	—	+	+	+	*glu-1*	*s*	(/λ)	*s*	F⁻
J5-3	+	+	+	*met-2*	+	+	+	*s*	(λ⁻)	*s*	F⁻

For abbreviations, see Figure 2 legend.

with ornithine (*argB*, *C*, *E* mutants); "arg" refers to the phenotype of *argH* mutants, which strictly require arginine.

The methionine mutations used in this work (*met-1* and *met-2*) have not yet been identified enzymatically. *met-1* strains can grow on homocysteine or methionine; the *met-2* defect leads to a specific requirement for methionine.

glu-1 and *glu-2* are two independently isolated mutants which originally were classified as glutamate-dependent, because they grew on glucose + glutamate, but not glucose alone (WOLLMAN and JACOB 1959). Since glutamate can be replaced by C3 or C6 metabolites related to the Krebs cycle, it seems preferable to designate the mutation as glucose minus, keeping the original symbol. The lack of glucose utilization for growth results most probably from the absence of phosphoenolpyruvate carboxylase (VANDERWINKEL *et al.* 1963). Such a defect has been discovered recently by THEODORE and ENGLESBERG (1962) in a similar mutant of *Salmonella typhimurium*; this metabolic deficiency makes the cell unable to use carbohydrates as sole source of energy and biosynthetic material. Succinate was used as growth substrate for the *glu* mutants.

Phage strains: The strain of the phage 363 used in this work was obtained through the courtesy of F. JACOB.

Bacterial strains: All strains are derived from *E. coli* K-12. Table 1 lists their characteristics. The *arg* mutants have been defined by growth tests, syntrophism experiments, enzymatic studies (WIAME, BOURGEOIS and MAAS, see MAAS 1961) and preliminary mapping by conjugation techniques (LAVALLE, JACOB and MAAS; see MAAS 1961). We are indebted to W. K. MAAS for strains 30SOMA2 and 4, to F. JACOB for strains PA214, PA342, P10 and J5-3, and to F. RAMOS for determinations of the enzymatic defects in the *argB* and *C* mutants. The strain P4XSB166 (*argE*) strongly feeds *argB* and *C* mutants, since it excretes the utilizable precursor acetyl-ornithine; this fact explains the growth on minimal medium of an *argB* or *C* recombinant class in transductions involving as recipient an *argE* mutant, and as donor, phage grown on *argB* or *C* strains. *argH* strains do not feed other *arg* mutants; *argC* strains do not feed the *argB* mutant.

In order to perform three-point tests, we needed a collection of double mutants. In addition to the (*met-1*, *arg*$^-$) mutants already available (*glu*$^-$, *arg*$^-$) organisms were obtained by appropriate transductions. For example, from the transduction (recipient *glu-2*, *argH-2*) $\times$ (donor *argB-1*), we isolated a (*glu-2*, *argB-1*) recombinant; this strain could be distinguished from the other recombinants by its ability to grow on succinate and ornithine (or arginine). (*met*$^+$, *arg*$^-$) strains were obtained by transduction of (*met*$^-$, *arg*$^-$) organisms to methionine independence with a *met*$^+$ donor.

Media: The minimal medium (132), which was used for the selection and scoring of genetic recombinants, has the following composition: KH_2PO_4 3 g; K_2HPO_4 7 g; $(NH_4)_2SO_4$ 1 g; $MgSO_4 7H_2O$ 0.1 g; Na citrate $2H_2O$ 0.5 g; $MnSO_4$ 10^{-6}M; Fe citrate 10^{-6}M; H_2O: 1 l. For solid medium: 1.8 percent Difco agar.

The final concentration of the carbon source (glucose or succinate) was 0.5 percent. Other requirements were added at the following concentrations: DL-threonine: 200 μg/ml; DL-leucine: 160; L-histidine: 100; L-arginine: 100; L-methionine: 50; thiamine: 1. Streptomycin was used at the final concentration of 200 μg/ml.

The composition of complex media is: *Liquid broth* (869): Difco Bacto-tryptone 1 percent; Difco yeast extract 0.5 percent, glucose 0.1 percent, NaCl 0.5 percent; *Medium for phage experiments* (857): Difco Bacto-peptone 1 percent; Liebig meat extract 0.1 percent; NaCl 1 percent; adjusted to neutrality with NaOH 1N; Difco agar 1.5 percent; *Soft agar:* medium 869 supplemented with 0.6 percent Difco agar.

Culture conditions: Cells taken from overnight broth cultures were inoculated into fresh broth and grown with agitation at 37°C to a concentration of about 5×10^8 cells/ml, these cultures being in the exponential phase of growth. Unless otherwise specified, cell suspensions of this concentration were used in the experiments.

Mating conditions: Exponentially grown cells were mixed in a 150 ml Erlenmeyer flask, using 0.25 ml of Hfr cells and 4.75 ml of F$^-$ cells. The mating mixture was incubated at 37°C with gentle shaking. The mating was interrupted at intervals with phage T6 (HAYES 1957). Efficiency of killing by the phage was about 5×10^3 at a multiplicity of 100. After adsorption

of T6, the suspensions were diluted ten times in minimal medium and 0.1 ml plated on each selective medium. Controls of the F⁻ and Hfr parents to test for the pertinent properties were prepared from cultures of the same concentration.

Transduction procedure: 4 to 5 $\times$ 10^8 cells were mixed in broth with 5 $\times$ 10^8 phage particles, in a final volume of 1.0 ml. $Ca(NO_3)_2$ was added to the suspension at a final concentration of 0.001M. Adsorption of the phage was allowed to take place during 15 minutes at 37°C without agitation. The mixture was then centrifuged, the pellet resuspended in minimal medium and aliquots plated at suitable dilutions on selective media. The dilutions were determined on the basis of the average yield of recombinants obtained in pilot experiments for transducing single auxotrophs to prototrophy. This yield was about 5 $\times$ 10^{-5} recombinants per particle of plaque forming phage, in a range of multiplicity from 0.2 to 1.0. Controls were run without phage and with phage grown on the recipient strain.

Phage stocks: These were prepared by the technique of confluent lysis on agar (SWANSTROM and ADAMS 1951). Owing to the difficulties frequently encountered in the application of this empirical method to the preparation of high titer phage suspensions, the details of the manipulation are given below. In our hands, the method gave suspensions with titers between 5 $\times$ 10^9 and 5 $\times$ 10^{10} plaque forming particles per ml.

Cells were grown in broth to a concentration of 5 $\times$ 10^8 cells/ml (cells lysogenic for lambda were then centrifuged and resuspended in the same volume of prewarmed broth). $Ca(NO_3)_2$ was added at the final concentration of 0.001M. Aliquots of 0.75 ml were distributed in small tubes kept in a 37°C bath. Each sample was mixed with one or two drops of a phage suspension containing about 1 $\times$ 10^8 particles per ml. After 20 min, 1.5 ml of liquid soft agar was mixed with the content of each tube. The mixture was plated in prewarmed standard Petri dishes containing 10 to 15 ml of phage medium. These plates had to be prepared the day before, in order to have a suitable water content. Satisfactory multiplication of the phage was usually obtained after 4 to 6 hours at 37°C. The semiliquid lysates were then collected, centrifuged twice and kept at 4°C in screw-capped tubes with a drop of chloroform. The stocks retained sufficient activity for at least ten months.

Titration of phage suspensions was carried out by mixing 0.75 ml of a broth culture of *E. coli* C600 (363 and lambda sensitive) containing about 2.5 $\times$ 10^8 cells/ml and supplemented at the time of mixing with $Ca(NO_3)_2$ 0.001M final, with an aliquot of a suitable dilution of the phage in $MgSO_4$ 0.01M; the mixture was incubated 20 min at 37°C, and plated with soft agar on thick layers of phage medium (60 to 70 ml per plate). The plates had to be incubated at least 24 hours at 37°C for accurate counting of the plaques.

Replica plating of recombinants: Recombinant colonies which were to be scored for unselected markers were streaked in patches onto master plates containing the same medium as that on which they arose. This procedure eliminates the recombinant classes which grew because of feeding by background bacteria. The master plates were replicated onto the test media by the method of LEDERBERG and LEDERBERG (1952).

Purity of recombinant colonies: Purity of transductants was checked in two instances; 40 arg+ recombinant colonies, from the transduction (*met-1, argC-1*) $\times$ (*argE-1*), were tested for the *met* genotype; the same was done with 32 arg+ recombinants from the transduction (*glu-2, argH-2*) $\times$ (*argC-1*), with respect to *glu* genotype. In neither case did we find any mixed colonies. However, the scoring of several hundred recombinants obtained from the latter experiment and distributed among four classes (glu⁻, orn⁻; glu⁻, orn+; glu+, orn⁻; glu+, orn+) yielded 1 percent of mixed colonies. These contained about 50 percent of each of the two more frequent classes, i.e., (glu⁻, orn+) and (glu+, orn⁻). These can be recognized in other, similar transductions, by growth on succinate + ornithine, succinate alone, glucose + ornithine, and barely perceptible growth on glucose alone. They could readily be distinguished from (glu+, orn+) recombinants which grew well on all four media. The constituents of these mixed colonies were scored as separate recombinants, although their omission would not have changed the significance of the results.

RESULTS

Evidence from the literature indicates the sequence: *mtl—met-1—met-2—argH—thi* (JACOB and WOLLMAN 1961). We have confirmed and extended these data by the following experiments.

(1) The Hfr strain P10 (*arg*$^+$, *glu*$^+$, *met*$^+$ *str*^{s}, *T6*^{s}), which transfers the sequence *thi—met—mtl* in the first 30 minutes of mating at 37°C, was crossed with a F$^-$ strain which is *argH-2*, *glu-1*, *met-1*, *str*^{r}, *T6*^{r}. This organism was obtained by successive transductions with appropriate donors from the strain PA342 (*glu-2*, *argH-2*, *str*^{r}, *T6*^{s}). The mating was interrupted at intervals with phage T6, and aliquots plated on media devised for the selection of (*arg*$^+$, *str*^{r}), (*glu*$^+$, *str*^{r}) and (*met*$^+$, *str*^{r}) recombinants. *argH*, *glu-1* and *met-1* wild-type alleles entered F$^-$ cells between the 8th and the 9th minute of mating at 37°C; the curves for their time of entry are practically superimposible and are not given here. The genetic constitution of recombinants taken at early times (see Table 2) clearly shows an increase of met$^+$ recombinants among the arg$^+$ class, and a less pronounced increase among the glu$^+$ class. On the other hand, the proportion of glu$^+$ and arg$^+$ recombinants among the met$^+$ class does not exhibit more than 14 percent variation. The *met-1* marker is thus distal to *argH-2* and *glu-1* Despite the strong linkage joining *argH-2* and *glu-1*, we may consider that the smaller increase of met$^+$ recombinants among glu$^+$ than arg$^+$ recombinants, is evidence for the location of *glu-1* between *argH-2* and *met-1*. The probable order of the markers is thus: *met-1—glu-1—argH-2*.

(2) This conclusion is strengthened by the results of a three-point transduction which involved the (*met*, *glu*, *argH*) strain as recipient, and phage grown on a wild-type strain as donor (see Table 3). The rare (met$^+$, glu$^-$, arg$^+$) recombinants appear as a four-point crossover class, and point to the predicted order. As expected from the previous experiment, the difference in size between the (met$^+$, glu$^-$, arg$^-$) and (met$^-$, glu$^-$ arg$^+$) classes indicates stronger linkage between *argH-2* and *glu-1*, than between *glu-1* and *met-1*.

(3) The position of the *met-2* locus between *met-1* and *glu-1* was confirmed by two-point transductions. In crosses in which two of these markers were in opposition, one in the recipient genome, one in the donor, their distance could be

TABLE 2

Localization of the met-1, glu-1 *and* argH-2 *markers by interrupted mating*

Time of interruption (min)	Percent of unselected Hfr markers among recombinants					
	arg$^+$ *str*^{r}		*glu*$^+$ *str*^{r}		*met*$^+$ *str*^{r}	
	glu$^+$	*met*$^+$	*arg*$^+$	*met*$^+$	*glu*$^+$	*arg*$^+$
8	90	35	86	44	64	63
10	97	49	98	53	66	65
12	92	62	92	60	72	72

Genetic analysis of recombinants selected at early times from a phage T6 interrupted mating between Hfr P10 prototroph *str*^{s}, *T6*^{s} and F$^-$ *argH-2*, *met-1*, *glu-1*, *str*^{r}, *T6*^{r}. Each figure was obtained from the analysis of 100 to 200 recombinants by replica plating.

TABLE 3

Localization of the met-1, glu-1 *and* argH-2 *markers by transduction*

Classes of recombinants	Crossing-over regions*	Genetic constitution of recombinants	
		arg+	*met*+
arg+ *met*+ *glu*−	1–2–3–4	7	4
arg+ *met*+ *glu*+	1–4	205	154
arg+ *met*− *glu*+	2–4	197	. .
arg+ *met*− *glu*−	3–4	71	. .
arg− *met*+ *glu*−	1–2	. .	287
arg− *met*+ *glu*+	1–3	. .	33

Genetic analysis of *arg*+ and *met*+ recombinants from the transduction: recipient *met-1, glu-1, argH-2* × donor prototroph.

* Crossing-over regions as shown in parentheses if order is: (1)—*met-1*—(2)—*glu-1*—(3)—*argH-2*—(4).

estimated by the value of the ratio: *number of prototroph recombinants/number of prototrophs + donor-type recombinants.* Prototrophs were scored by replica plating on minimal medium, after transfer onto master plates of the colonies appearing on a medium permitting growth of both classes of recombinants. The results are given in Table 4. They confirm the order: *met-1—met-2—glu-1* and show the distances to be additive between the loci investigated.

Linkage studies: By the method used in the last experiment, the distances between each *arg* marker and either *met-2*, or *glu-1*, could be estimated (Table 4). All of them exhibit closer linkage to *glu-1* than to *met-2*. Moreover, they seem to be located on the right side of *glu-1*, in the sequence: *met-2—glu-1*

TABLE 4

Distance estimates between arg, met *and* glu *markers*

Cross*	Prototrophs/ Total scored	Percent		Cross	Prototrophs/ total scored	Percent	
met-2 × *met-1*	21/185	11.4		*argH-1* × *argB-1*	1/120	0.8	
met-2 × *glu-1*	79/152	52.0	47.7	*argH-1* × *argC-1*	11/120	9.2	
glu-1 × *met-2*	87/200	43.5		*argH-1* × *argC-2*	67/254	26.4	
glu-1 × *met-1*	68/124	54.9		*argH-1* × *argE-1*	11/158	7.0	
argB-1 × *met-2*	50/83	60.2		*argB-1* × *glu-1*	153/987	15.4	
met-2 × *argC-1*	114/200	57.0		*argC-1* × *glu-1*	75/711	11.6	
met-2 × *argC-2*	127/200	63.5		*glu-1* × *argC-2*	53/213	24.9	
argC-2 × *met-2*	435/588	73.8	70.9		38/124	30.6	32.1
	246/352	69.9		*argC-2* × *glu-1*	208/593	35.1	
met-2 × *argE-1*	85/200	42.5		*argE-1* × *glu-1*	8/192	4.2	
met-2 × *argH-2*	107/160	66.9		*glu-1* × *argH-1*	29/157	18.4	
argH-2 × *met-2*	69/144	47.9	57.9	*glu-1* × *argH-2*	26/164	15.8	15.7
argH-3 × *met-2*	117/200	58.5		*argH-2* × *glu-1*	24/154	15.6	
				argH-3 × *glu-1*	183/998	18.3	

* The recipient is listed first, the donor second. The results were obtained by replica plating, after transfer onto master plates. Selective medium when the donor is *met-1* = glucose homocysteine; *met-2* = glucose methionine; *glu-1* = succinate; *argB, C* or *E* = glucose ornithine; *argH* = glucose arginine.

—*arg*, with the possible exception of *argE-1*, which is strongly linked to *glu-1*; indeed, the distance *met-2—glu-1* estimated from difference values involving crosses with *arg* markers is roughly constant (about 40), slightly less than the direct estimate of the *met-2—glu-1* interval (47). Further evidence for the clustering of the *arg* markers was obtained from linkage determinations between *argH-1* and the other *arg* markers (Table 4). Special mention must be made of *argC-2*, which exhibits lower linkage with *met-2* or *glu-1* than the other markers, and thus seems to be situated further to the right. Since three-point test experiments have shown that *argC-2* is situated in the middle of the sequence formed by the *arg* loci, this marker is characterized by an abnormally high frequency of crossing over in its vicinity. This effect appears whether *argC-2* belongs to the recipient or the donor genotype, and is less pronounced with more distant reference markers (compare values obtained for *glu-1* and *met-2* in crosses with *argC-2* and other *arg* markers); the effect also seems to occur on both sides of *argC-2:* to the left with *glu-1* and *met-2*, to the right with *argH-1*. *argC-2* is thus similar in these respects to some of the *his* mutants studied by HARTMAN (1956). These features point clearly to the necessity of three-point transductions, in order to derive the order of the mutations.

The frequency of crossing over between the sites of mutations concerned with different enzymatic steps can be estimated on the basis of the average (and quite constant) yield of recombinants obtained when *arg* mutants are transduced to prototrophy (5×10^{-5}/phage) and the linkage values of Table 3. This frequency is found to vary from 10^{-6} to 5×10^{-6}. The frequencies of crossing over observed between mutations concerned with the same enzyme extend from 10^{-7} (*argH-1*, *argH-2*), (*glu-1*, *glu-2*), to 10^{-6} (*argC-1*, *argC-2*). Mutations affecting the same function seem thus to be clustered in the same region. Attempts to derive the complementation pattern of *arg* mutants by abortive transduction have so far been unsuccessful.

Three-point tests: The results listed in Table 4 clearly show that *glu-1* and all the *arg* markers lie on the same side of *met-1*. Taking advantage of that point, we have mapped *glu* and *arg* mutations with respect to *met-1* by three-point tests. Pairs of crosses involving two *arg* mutants, or one *arg* and one *glu* mutant, were performed. Figure 3a gives the possible arrangements of the markers involved in one pair of crosses in different coupling phases, when *met-1* is in the recipient strain. If the order is *met-1—a—b*, and *a* the donor marker, (a^+, b^+, met^+) recombinants are the result of a quadruple crossing over; if the donor marker is *b*, the same class arises from a double crossing over. The value of the ratio (a^+, b^+, met^+)/(a^+, b^+) is thus expected to be lower if *a* rather than *b* is contributed by the donor. Opposite predictions can be made if the disposition is *met-1—b—a*. Thus, comparison of the values obtained for this ratio in pairs of transductions in different coupling phases can be used to derive the order of the genes. In two instances, reciprocal transductions were performed, by switching, making the donor of one of the experiments the recipient in the other (Figure 3b); then, the ratio (a^+, b^+, met^+)/(a^+, b^+) obtained from the transduction involving a *met-1* strain as recipient, was compared with the ratio (a^+, b^+, met^-)/

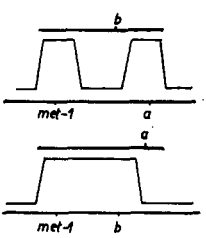

FIGURE 3a.—Possible arrangements of the markers *a* and *b* involved in one pair of crosses in different coupling phases, using a *met-1* recipient strain. Broken lines represent crossing over responsible for the formation of (a^+, b^+, *met*+) recombinants.

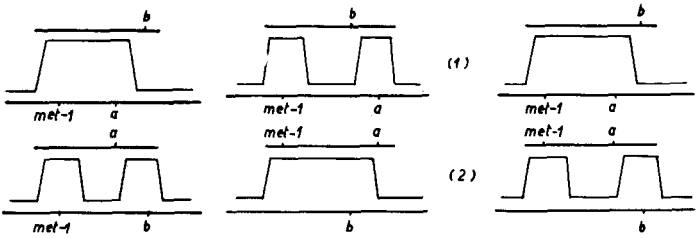

FIGURE 3b.—Possible arrangements of the markers *met-1*, *a* and *b* in reciprocal crosses. Broken lines indicate the origin of (a^+, b^+, *met*+) recombinants in Cross 1 and of (a^+, b^+, *met*−) recombinants in Cross 2.

(a^+, b^+) obtained when this last strain was taken as donor in the reciprocal experiment.

Table 5 gives the results obtained by this method; they support the sequence

TABLE 5

Order of glu *and* arg *markers with respect to* met-1

Cross No.	Strains*	Ratio (*arg*+ *met*+)/*arg*+†	Percent	Order
1	*met-1 glu-1* × *argE-1*	106/206	51.5	*met-1 glu-1 argE-1*
2	*met-1 argE-1* × *glu-1*	35/147	23.7	
3	*met-1 argC-1* × *argE-1*	30/190	15.8	*met-1 argE-1 argC-1*
4	*met-1 argE-1* × *argC-1*	33/ 72	45.8	
5	*met-1 argC-2* × *argE-1*	76/282	27.0	*met-1 argE-1 argC-2*
6	*met-1 argE-1* × *argC-2*	101/256	39.0	
7	*met-1 argC-2* × *argH-1*	122/318	38.4	*met-1 argC-2 argH-1*
8	*met-1 argH-1* × *argC-2*	70/312	22.4	
9	*met-1 argC-1* × *glu-2*	$\frac{arg^+\ glu^+\ met^+}{arg^+\ glu^+}$ 1/ 20	5.0	*met-1 glu-2 argC-1*
10	*glu-2* × *met-1 argC-1*	$\frac{arg^+\ glu^+\ met^-}{arg^+\ glu^+}$ 23/ 76	30.3	
11	*met-1 argE-1* × *glu-2*	$\frac{arg^+\ glu^+\ met^+}{arg^+\ glu^+}$ 8/ 42	19.0	*met-1 glu-2 argE-1*
12	*glu-2* × *met-1 argE-1*	$\frac{arg^+\ glu^+\ met^-}{arg^+\ glu^+}$ 48/114	42.1	

* See Table 4.
† Except Nos. 9, 10, 11, 12.

met-1—glu-1—argE-1—argC-2—argH-1, and point to the location of *glu-2* and *argC-1* on the left and right side of *argE-1*, respectively.

These results were confirmed and completed by the following experiments. In three-point tests which involve a (*glu*, *argH*) mutant as recipient, and as donor each of the other *arg* markers one at a time, the distribution of the classes should be compatible with the order *glu—arg—argH*. These experiments could also provide information about the location of *argB*. Table 6 shows the results of such experiments. The rare occurrence of prototroph recombinants recovered from each transduction agrees with the predicted order. Moreover, the relative frequencies of exchange occurring in regions 2 and 3 (compare the 2–4 and 3–4 classes in Table 6) are compatible with the arrangement established with respect to *met-1*. Indeed, the closer the *arg* marker has been found to the *glu* locus, the less crossing over occurs in region 2 and the more in region 3. It is clear also that the size of the 2–4 class, (glu^- orn^-) recombinants, is too large, at least when *argC-2* or *B-1* is the donor marker, to be derived from a quadruple crossover; this excludes the order *argB, C—glu—argH-2*. Thus, the *argB* locus also seems to lie between *glu-2* and *argH-2*, probably between *argC-2* and *argH-2*.

The last step in the mapping of *arg* markers has involved pairs of crosses between *arg* and *glu* mutants, with recipient strains carrying the *glu-2* marker. The results of such transductions were expected to confirm the order of *arg* loci which had been established in similar experiments with respect to *met-1*. In two instances, comparisons were made between reciprocal crosses rather than transductions in different coupling phases; in the first one, the ratio $(arg^+, glu^+)/(arg^+)$ obtained from the transduction (*glu-2*, *argH-2*) × (*argH-1*), was compared with the ratio $(arg^+, glu^-)/(arg^+)$ derived from the transduction (*argH-1*) × (*glu-2*, *argH-2*); in the second one, evidence for the order of *glu-1*, *glu-2*, and *argH-2* was obtained by comparison of the ratio $(glu^+, arg^+)/(glu^+)$ given by (*glu-2*, *argH-2*) × (*glu-1*), with the ratio $(glu^+, arg^-)/(glu^+)$ obtained from (*glu-1*) × (*glu-2*, *argH-2*).

TABLE 6

Three-point test analysis of the glu argH *region*

Recombinant classes	Crossover regions*	Genetic constitution of *argH*+ recombinants						
		Donor *arg* marker						
		argE-1 a	*argE-1* b	*argC-1* a	*argC-1* b	*argC-2*	*argB-1* a	*argB-1* b
argH+ *glu*+ *orn*+	1-2-3-4	6	9	2	13	16	1	3
argH+ *glu*+ *orn*−	1-4	234	588	87	317	385	209	511
argH+ *glu*− *orn*+	3-4	25	59	11	43	48	6	15
argH+ *glu*− *orn*−	2-4	11	17	5	30	62	23	68

Genetic analysis of the transductions: recipient *glu-2 argH2* × donor *argB, C* or *E*.

* Crossing-over regions as shown in parentheses if order is: (1)—*glu-2*—(2)—*argC*$\begin{matrix}B\\E\end{matrix}$—(3)—*argH2*—(4).

argB, C or *E* recombinants are designated as *orn*−. The letters a and b refer to separate experiments.

TABLE 7

Order of arg *and* glu-1 *markers with respect to* glu-2

Cross No.	Strains*	Ratio $(arg^+ glu^+)/arg^+$†	Percent	Order
1	*glu-2 argE-1* × *argC-1*	163/180	90.6	*glu-2 argE-1 argC-1*
2	*glu-2 argC-1* × *argE-1*	18/224	8.0	
3	*glu-2 argC-1* × *argC-2*	126/221	57.0	*glu-2 argC-1 argC-2*
4	*glu-2 argC-2* × *argC-1*	43/223	19.3	
5	*glu-2 argC-2* × *argB-1*	154/320	48.2	*glu-2 argC-2 argB-1*
6	*glu-2 argB-1* × *argC-2*	48/191	25.1	
7	*glu-2 argB-1* × *argH-2*	69/101	68.2	*glu-2 argB-1 argH-2*
8	*glu-2 argH-2* × *argB-1*	32/200	16.0	
9	*glu-2 argC-2* × *argH-2*	101/201	50.2	*glu-2 argC-2 argH-2*
10	*glu-2 argH-2* × *argC-2*	28/128	21.8	
11	*glu-2 argE-1* × *argH-2*	202/223	90.2	*glu-2 argE-1 argH-2*
12	*glu-2 argH-2* × *argE-1*	9/117	7.7	
13	*glu-2 argH-2* × *argH-1*	$\frac{arg^+ glu^+}{arg^+}$ 72/109	66.1	*glu-2 argH-2 argH-1*
14	*argH-1* × *glu-2 argH-2*	$\frac{arg^+ glu^-}{arg^+}$ 4/ 32	12.5	
15	*glu-2 argH-2* × *glu-1*	$\frac{glu^+ arg^+}{glu^+}$ 43/ 90	47.8	*glu-1 glu-2 argH-2*
16	*glu-1* × *glu-2 argH-2*	$\frac{glu^+ arg^-}{glu^+}$ 11/ 68	16.2	

* See Table 4.
† Except Nos. 14, 15, 16.

The data are given in Table 7; they confirm and complete the previous results, giving without ambiguity the sequence shown on Figure 4.

DISCUSSION

The *argB, C, E* and *H* loci are clustered in a small region close to *glu*. Because few mutants have so far been studied, it is not possible to decide if these genes are adjacent to each other. However, a comparison of linkage values obtained in other metabolic pathways studied by transduction for markers occupying the extremities of a cluster of genes, with the values obtained for the most distant *arg* loci considered in this study (*argE* and *argH*), favours the hypothesis that the cotransducible *arg* loci are arranged on the chromosome without discontinuity. (GROSS and ENGLESBERG 1959: arabinose mutants of *E. coli*; YANOFSKY and LENNOX 1959: tryptophan mutants of *E. coli*; DEMEREC and HARTMAN 1956:

FIGURE 4.—Map of the *met, glu* and *arg* alleles (approximately to scale).

tryptophan mutants of Salmonella; HARTMAN 1956: histidine mutants of Salmonella; SMITH-KEARY and DAWSON 1963: leucine mutants of Salmonella). Further studies with a larger number of *arg* mutants will help to clarify this point; in particular, the occurrence of deletion mutants extending over more than one locus (which have not yet been discovered in this system), should be investigated.

The results presented here might be useful for understanding of regulation of enzyme synthesis in the arginine pathway. Several examples are known involving the clustering of genes concerned with a specific repressible or inducible sequence of enzymatic steps in one genetic unit of coordinated expression (operon: JACOB, PERRIN, SANCHEZ and MONOD 1960). Studies on the regulation of arginine biosynthesis in *E. coli* have shown that arginine represses the synthesis of the enzymes involved in its pathway (evidence reviewed by GORINI *et al.* 1961; MAAS 1961; VOGEL 1961). The existence of a repressor, which would be able, when activated by arginine, to suppress the synthesis of messenger RNA by the structural genes of the biosynthesis or to inhibit its translation, has been postulated (ibidem). This hypothesis has found support in the discovery of at least one regulatory gene, which is presumed to produce the repressor (ibidem) and further support has been acquired recently by the demonstration that the wild-type allele of this gene is dominant over mutant alleles carried by non-repressible strains. (MAAS and CLARK 1964; MAAS, MAAS, WIAME and GLANSDORFF 1964). However, the mechanism of action of the postulated repressor is still an open question. The operon hypothesis assumes that repression occurs either at the site where the genetic transcription is supposed to begin (the operator locus) or at the level of translation, on the RNA specified by the operator. This hypothesis would lead to postulate that each isolated structural gene concerned with the arg pathway is under the control of one operator. From the topographical study presented in this paper, we may ask if the cluster formed by the *B*, *C*, *E* and *H* loci suggests the existence of a single operon for these genes. If so, the situation of the arginine pathway would resemble the one which has been described and revised recently for pyrimidine biosynthesis (BECKWITH, PARDEE, AUSTRIAN and JACOB 1962; TAYLOR, BECKWITH, PARDEE, AUSTRIAN and JACOB 1964).

The results reported here induce thus further studies, both genetic and physiological; in particular, it should be investigated if the synthesis of the four enzymes specified by *argB*, *C*, *E* and *H* is coordinately repressed. Work has been undertaken in this direction.

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SUMMARY

Mutations affecting the synthesis of the second, third, fifth and eighth enzyme of arginine biosynthesis in *E. coli* have been mapped by transduction experiments,

involving determination of the order of the genes and the distance between them. The mutations investigated seem to be grouped in a cluster of adjacent loci, which could form an operon.

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