# THE GENETIC MAP OF ESCHERICHIA COLI K-12

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OF the several known methods for locating the positions of genetic markers on the single linkage group of *Escherichia coli*, the one most convenient and satisfactory for mapping long chromosomal segments is the interrupted conjugation technique described by WOLLMAN, JACOB and HAYES (1956). This method uses strong mechanical agitation to sever the conjugation tube that unites mating cell pairs. As a consequence, the unilateral transfer of chromosome from the donor, or Hfr, conjugant cell to the recipient, or F⁻, partner is terminated. Since the donor cell injects its chromosome at a constant speed and with a specific orientation, periodic interruption of transfer permits a determination of the time when a given genetic marker of the donor first becomes evident in the recipient cell. The time intervening between the transfer of two markers is a measure of the distance between them; hence a genetic map based on time units can be constructed by this method.

No single Hfr strain can be used to map the entire chromosome because the speed of transfer, which is constant during injection of the first one third to one half of the chromosome, apparently decreases later on (JACOB and WOLLMAN 1958). This limitation is offset, however, by the fact that Hfr strains may have different "points-of-origin"; that is, they may transfer different chromosomal regions initially during conjugation (JACOB and WOLLMAN 1957). The unique points-of-origin of three very high frequency (Vhf) donor strains described by TAYLOR and ADELBERG (1960) made it possible to extend genetic mapping over the full length of the chromosome. These and other donor strains have been used extensively since that time to assign precise map locations to the growing catalogue of genetic markers in *E. coli*, and one of the objectives of this report is to present a detailed genetic map based on new data from both this laboratory and many others as well. Several improvements in experimental technique have brought greater accuracy to the basic mapping method. Consequently, this report will also include some revisions of previously published mapping data, plus a revised estimate of the total length (in time units) of the map of *E. coli*.

## MATERIALS AND METHODS

*Bacteria and phages:* The bacteria referred to in this paper were all originally derived from *E. coli* K-12. The properties of Vhf donor strains AB311, AB312, and AB313 were described by TAYLOR and ADELBERG (1960). Hfr strain AB259 was originally designated Hfr 3000 and it has the properties of strain Hfr H (WOLLMAN, JACOB and HAYES 1956). Hfr strain AB261 (originally

Hfr P4X-6) was described by ADELBERG and BURNS (1960). Vhf strain AB451 is similar to AB261 except that it requires threonine, leucine and thiamine for growth instead of methionine. The number of polyauxotrophic F⁻ recipient strains used in this study was too large to permit listing of their full genotypes. Strain numbers of the various F⁻ stocks and the auxotrophy markers used in the crosses are given in the tables of results. Stocks of phage T6 were made from a wild-type strain given to us by A. D. HERSHEY. The male-specific RNA phage strain MS-2 isolated by A. J. CLARK (unpublished) was used to selectively kill donor bacteria. Transducing phage P1kc (LENNOX 1955) was given to us by S. E. LURIA.

*Media:* The synthetic minimal medium used for the selection of genetic recombinants and the broth medium used for routine cultivation of bacteria were described by ADELBERG and BURNS (1960).

*Cultural and mating conditions:* Bacterial stocks were maintained either on broth agar slants or as lyophilized cultures. Parental strains were reisolated from single colonies and checked for their genetic markers a few days prior to their use in crosses. Approximately $10^8$ bacteria from overnight broth cultures were inoculated in 10 ml of fresh broth and incubated at 37°C with aeration until the cultures reached midexponential phase, or a titer of about $5 \times 10^8$ cells per ml. Crosses were started by mixing the parental cultures in appropriate volume ratios to give final titers of about $2 \times 10^7$ donor cells and $4 \times 10^8$ F⁻ cells per ml. The mating mixtures were gently agitated at 37°C for 5 min to allow formation of specific pairs and then diluted, as described by DE HAAN and GROSS (1962), into 100 ml of fresh prewarmed broth to prevent, or at least reduce, further pair formation. The mixtures were diluted 1:100, 1:200, or 1:500, depending on the fertility of the individual crosses; in all cases, the highest dilution which would produce adequate numbers of recombinants was used. The one-liter Erlenmeyer flasks containing the diluted cells were left undisturbed in a water bath at 37°C for the remainder of the experiment.

*Interruption of mating:* Samples (2.0 ml) were removed from the flask at 2 to 5 min intervals and transferred into 15 × 100 mm metal test tubes; these were attached to a Lourdes homogenizer fitted with a Model 15-ATT rotary knife assembly. Cell pairs were separated by blending the samples for one minute at a transformer setting of 30. The rotary knife was cleaned between samples by running it in several large volumes of sterile water. Duplicate 0.1 ml samples of the blended material were plated on various minimal media to select for different classes of genetic recombinants. Exconjugant donor cells were killed after the blending to prevent them from forming new mating pairs on the plates. The genotypes of the parental strains determined the mode of killing. For example, the selective plating media contained 200 $\mu$g/ml of streptomycin to kill the donors in crosses between streptomycin-sensitive donors and streptomycin-resistant F⁻ strains. Streptomycin-resistant donor strains were killed with phage T6 if the F⁻ parent was T6-resistant, or with phage MS-2 if the F⁻ was T6-sensitive. (We are indebted to S. FALKOW for suggesting the phage MS-2 method to us.) The blended samples were mixed with 1.0 ml of broth containing about $2 \times 10^{10}$ phages, incubated 10 min at 37°C to allow adsorption, and then plated on selective media. Growth of phage-resistant mutants of the donor strains was prevented by omitting required growth factors of the donor parents from the selective media.

## RESULTS

*The genetic maps of donor strains AB261 and AB451:* Strain AB261 was first crossed to F⁻ strain AB1133 to measure the time of entry of the familiar reference markers, *proA*, *thr* and *arg* (the genetic symbols used in this paper are defined in Table 6). A plot of the number of recombinants found in successive blended samples versus the time of blending is shown in Figure 1A. Note that the transfer curve of each selected marker has a low initial slope that is followed by a steeper secondary slope. This is in marked contrast to the transfer curves originally described by WOLLMAN *et al.* (1956) and later confirmed by many others. Their results showed that the number of recombinants increased linearly with time,

and the resulting straight line plots were readily extrapolated to the time axis to give unambiguous times of entry for selected donor markers. Upward-breaking curves were encountered in about 25 percent of the crosses performed in this study. Their occurrence was not correlated with the use of particular selective and mating conditions or specific recipient strains in the crosses; it did appear, however, that certain donor strains produced these anomalous curves more frequently than other strains. Comparison of the times of entry obtained by extrapolating either the first or the second slopes to the time axis showed that the former values varied less than the latter in duplicate experiments. This suggested that the early recombinants, though they represented only a small proportion of the ultimate yield, were nonetheless the significant ones for measuring the time of entry of donor markers. Additional evidence to support this view came from the following cross.

The locus of *purD* relative to the reference loci *proA* and *arg* was determined in crosses of donor AB261 to F⁻ strain AT1380. The results, illustrated in Figure 1B, show that the *proA*⁺ marker first enters zygotes at 5.5 minutes, *purD*⁺ at 23 minutes, and *arg*⁺ at 23.5 minutes, as measured from the intercepts of the first slopes. On the other hand, the distance between *purD* and *arg* appears to be five times greater (2.5 minutes) if the second slopes are extrapolated to the time axis. It is known from the studies of LENNOX (1955) and DEMEREC *et al.* (1958) that the frequency of joint transfer of two closely linked markers in phage-mediated transduction varies inversely with the distance between markers. Moreover, the joint transduction of loci separated by more than 1.5 minutes during conjugation at 37°C is extremely rare. It was therefore expected that the *purD* and *arg* loci would be cotransduced if they were only 0.5 minutes apart and that

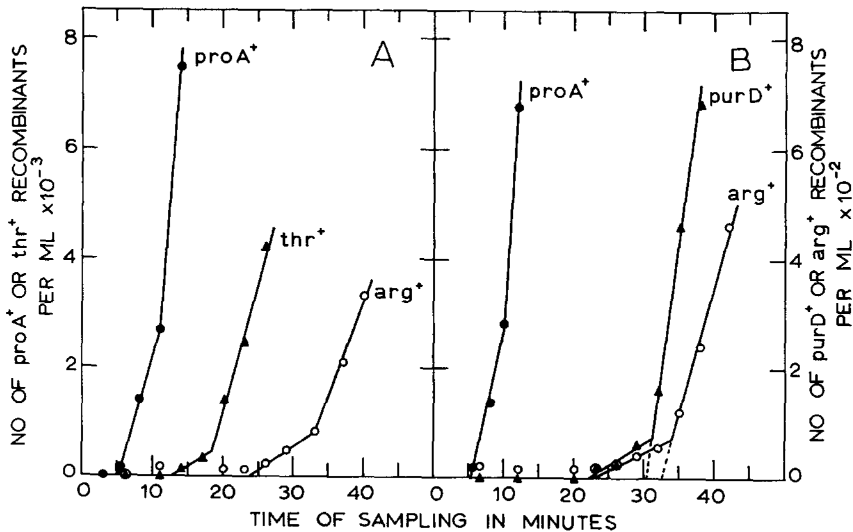


FIGURE 1.—Kinetics of chromosome transfer by donor strain AB261. A. Kinetics for a cross to recipient strain AB1133. B. Kinetics for a cross to recipient strain AT1380.

they would not be measurably cotransduced if they were separated by 2.5 minutes. In transduction crosses of strain AT1380 with phage P1 grown on a prototrophic donor strain, the *purD*+ and *arg*+ markers were incorporated jointly in 35 to 40 percent of recombinants selected for the inheritance of either marker alone. This relatively high frequency of cotransduction fits in well with the close linkage originally inferred from the "first slope" time of entry data, and it also rules out the 2.5 minute distance obtained from the second slopes. We conclude that the second slopes of transfer curves do not provide a reliable means for determining the distances between chromosomal markers.

The locus of one additional marker, *pyrB*, was determined in crosses between F- strain AT1385 and donor strain AB451, which has the same point-of-origin and transfers its chromosome in the same orientation as strain AB261. Breaks in the slopes of the transfer curves were not observed in these crosses, and the times of entry of reference markers *proA* and *arg* were identical to those obtained with strain AB261 (Table 1). A map of the region analyzed with strains AB261 and AB451 is presented in Figure 2. The map distances between loci were computed from the data of Table 1 as the average time interval separating the transfer of pairs of donor markers, one member of the pair being a reference locus and the other an unknown. Thus, *proA* and *pyrB* are separated in time by an average 12.0 minutes in two experiments, *proA* and *arg* are separated by an average 18.3 minutes in four experiments, and so on.

*The genetic map of donor strain AB259:* Strain AB259 was crossed to five different F- recipients to determine the loci of *proB*, *purE*, *lys* + *met*, *aroA*, and *purB* relative to the reference loci *leu* or *proA* and *gal*. The time of entry data obtained in duplicate trials of each cross are recorded in Table 2. Strain AB259 was also used in one experiment to map the *pyrA* locus; the results of this cross

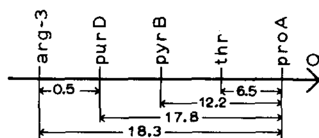


FIGURE 2.—Map of the chromosomal segment transferred by donor strains AB261 and AB451. The symbol O indicates the point-of-origin and the arrowhead shows the direction of chromosome transfer by the donors. The average map distances between loci are given in minutes.

TABLE 1

*Time of entry of markers transferred by strains AB261 and AB451*

| Recipient parent strain No. | Donor parent strain No. | Selected markers of the donor parent | | | | |
|---|---|---|---|---|---|---|
| | | *proA* | *thr* | *pyrB* | *purD* | *arg-3* |
| AB1133 | AB261 | 5.5 | 12.0 | ... | ... | 24.0 |
| AT1385 | AB451 | 5.0 | ... | 17.0 | ... | 23.0 |
| | | 5.5 | ... | 17.5 | ... | ... |
| AT1380 | AB261 | 4.5 | ... | ... | 22.5 | 23.0 |
| | | 5.5 | ... | ... | 23.0 | 23.5 |

TABLE 2

*Time of entry of markers transferred by strain AB259*

| Recipient parent strain No. | Selected markers of the donor parent | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *thr* | *pyrA* | *leu* | *proA* | *proB* | *purE* | *lys+met* | *gal* | *aroA* | *purB* |
| AT2213 | 7.25 | 7.5 | 7.75 | ... | ... | ... | ... | ... | ... | ... |
| AT2217 | ... | ... | 8.0 | ... | 15.5 | ... | ... | ... | ... | ... |
| | ... | ... | 8.0 | ... | 16.0 | ... | ... | ... | ... | ... |
| AT2270 | ... | ... | ... | 14.0 | ... | 18.5 | ... | 24.0 | ... | ... |
| | ... | ... | ... | 15.0 | ... | 19.5 | ... | 24.0 | ... | ... |
| AT2036 | ... | ... | ... | 15.5 | ... | ... | 22.0 | 25.0 | ... | ... |
| | ... | ... | ... | 14.5 | ... | ... | 21.5 | 23.5 | ... | ... |
| AB1321 | ... | ... | ... | 14.0 | ... | ... | ... | 23.5 | 27.5 | ... |
| | ... | ... | ... | 15.0 | ... | ... | ... | ... | 29.0 | ... |
| AB1325 | ... | ... | ... | 15.0 | ... | ... | ... | 24.0 | ... | 32.5 |
| | ... | ... | ... | 15.0 | ... | ... | ... | 24.0 | ... | 32.0 |

(Table 2) confirmed the recent observation of BECKWITH *et al.* (1962) that the locus of *pyrA* lies between the closely linked *thr* and *leu* loci.

The map of the region extending from *leu* through *gal* to the *purB* locus (Figure 3) was constructed from the data of Table 2 in the manner previously described. The sequence of markers on this segment was in most cases clearly discernable from the time of entry data alone. One exception was the *proB* locus, which had a time of entry close to that of *proA*. The gene sequence *leu–proA–proB* shown in Figure 3 was independently confirmed, however, by the observation that donor strain AB261 injects the *proA* locus first during chromosome transfer, the *leu* locus next, and the *proB* locus last. The *proB* locus is apparently distinct from a third proline locus, *proC*, which is transferred late by Hfr AB261 and is separated from *thr* by 11.5 minutes (N. M. SCHWARTZ, personal communication).

*The genetic map of donor strain AB312:* The loci of *pyrE*, *metE* and *metB* were mapped in the six interrupted-mating experiments recorded in Table 3. The markers are all located on a short segment of the chromosome between the *mtl* and *arg* reference loci. The time of entry data indicate a gene sequence of *mtl–metE–metB–arg* (Figure 4), which agrees with other maps of this region constructed by JACOB and WOLLMAN (1961) and by PITTARD, LOUTIT and ADELBERG (1963); however, the average distance between *mtl* and *arg* is 25 to 30 percent shorter than the distance given in the earlier maps. The close proximity

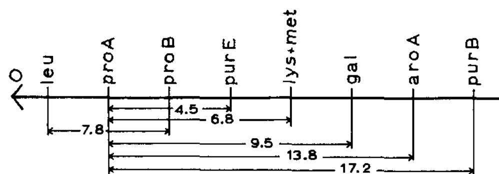


FIGURE 3.—Map of the chromosomal segment transferred by donor strain AB259.

TABLE 3

*Time of entry of markers transferred by strain AB312*

| Recipient parent strain No. | Selected markers of the donor parent | | | | | |
|---|---|---|---|---|---|---|
| | *malA* | *mtl* | *pyrE* | *metE* | *metB* | *arg-1* |
| AB1376 | 13.0 | 19.5 | 20.0 | ... | ... | 26.0 |
| | 13.0 | 19.0 | 19.5 | ... | ... | ... |
| AT2132 | 14.5 | 20.5 | ... | 22.0 | ... | 27.5 |
| | 16.0 | 22.0 | ... | 24.0 | ... | 29.5 |
| AB356 | 15.0 | 20.5 | ... | ... | 25.0 | 25.5 |
| | 14.5 | 20.5 | ... | ... | 26.0 | 27.0 |

of *pyrE* to *mtl* that was found in the AB312 × AB1376 crosses did not agree with the data of BECKWITH *et al.* (1962), which placed the *pyrE* locus in a different chromosome segment close to the *gal* loci. The reconciliation of these conflicting observations is described elsewhere (TAYLOR *et al.*, 1964).

*The genetic maps of donor strains AB313 and AB311:* Eleven markers in the region extending from *mtl* through *malA* to *his* were mapped with strain AB313, using *xyl*, *malA*, or *his* as reference loci. Note that three of the F⁻ recipients listed in Table 4 carry the *his-1* marker and that seven other recipients carry a different histidine marker, *his-4*. It was expected that *his-1* and *his-4* would be closely linked and would therefore be usable as a single reference locus common to both sets of recipient strains. This assumption was based on the knowledge that all the *his* loci of *Salmonella typhimurium*, a bacterium whose linkage map is highly similar to that of *E. coli* (SANDERSON and DEMEREC 1964), are clustered in a single small region (HARTMAN *et al.*, 1960). The interrupted matings of strain AB313 nevertheless indicated a rather large difference in the apparent times of entry of *his-1*$^+$ and *his-4*$^+$ (see Table 4). The mean time of entry in four experiments was 40.5 minutes for *his-1*$^+$ and 47.0 minutes in 12 experiments for *his-4*$^+$, the mean difference being 6.5 minutes.

As a further check on this result, the two *his* markers were mapped again with strain AB311, a donor that is isogenic with strain AB313 except that it transfers the *his* loci early during conjugation (TAYLOR and ADELBERG 1960). These crosses showed that *his-1*$^+$ and *his-4*$^+$ now had nearly identical times of entry; moreover, there was no significant difference in the distance between each of the two *his* markers and a *tryB* reference marker (see Figure 5 and the first two crosses in Table 5). These observations confirmed our original expectation that the two *his* alleles would be closely linked. The conflicting results obtained with these two donor strains can be explained on the hypothesis that strain AB313

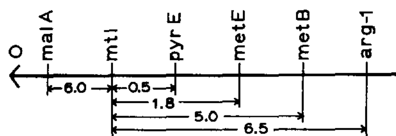


FIGURE 4.—Map of the chromosomal segment transferred by donor strain AB312.

TABLE 4

*Time of entry of markers transferred by strain AB313*

| Recipient parent strain No. | Selected marker of the donor parent | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mtl* | *cysA* | *xyl* | *malA* | *argE* | *serA* | *lys* | *pheA* | *tyr* | *cysC* | *aroC* | *purC* | *pheB* | *aroB* | *his-1* | *his-4* |
| AB442 | ... | 9.0 | 9.5 | 15.0 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | ... | 10.5 | 11.0 | 16.0 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| AT2272 | 9.0 | ... | ... | 14.5 | 18.5 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | ... | ... | ... | 16.0 | 20.5 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| AT2090 | ... | ... | ... | ... | ... | 22.0 | ... | ... | ... | ... | ... | 35.0 | ... | ... | 40.5 | ... |
| AT2034 | ... | ... | ... | ... | ... | 24.0 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 48.0 |
| AT2002 | ... | ... | ... | ... | ... | ... | 25.0 | ... | ... | ... | ... | ... | ... | ... | ... | 47.0 |
| | ... | ... | ... | ... | ... | ... | 25.5 | ... | ... | ... | ... | ... | ... | ... | ... | 48.0 |
| AT2092 | 9.0 | ... | ... | 14.0 | ... | ... | ... | 29.5 | ... | ... | ... | 35.0 | ... | ... | 40.0 | ... |
| | 9.5 | ... | ... | 15.0 | ... | ... | ... | 29.0 | ... | ... | ... | 34.5 | ... | ... | 40.5 | ... |
| AT2273 | ... | ... | ... | 14.5 | ... | ... | ... | ... | 29.0 | ... | ... | ... | ... | ... | ... | 45.0 |
| AT2024 | ... | ... | ... | ... | ... | ... | ... | ... | 31.0 | ... | ... | ... | ... | ... | ... | 46.0 |
| | ... | ... | ... | ... | ... | ... | ... | ... | 30.5 | ... | ... | ... | ... | ... | ... | 47.5 |
| AB1322 | ... | ... | 10.5 | ... | ... | ... | ... | ... | ... | 33.0 | ... | ... | ... | ... | ... | 49.0 |
| | ... | ... | ... | ... | ... | ... | ... | ... | ... | 34.0 | ... | ... | ... | ... | ... | 47.0 |
| AB444 | ... | ... | 10.0 | 14.5 | ... | ... | ... | ... | ... | ... | 34.0 | 34.5 | ... | ... | 41.0 | ... |
| | ... | ... | ... | 16.0 | ... | ... | ... | ... | ... | ... | 34.5 | 35.5 | ... | ... | ... | ... |
| AT2022 | ... | ... | 10.0 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 35.0 | ... | ... | 46.0 |
| AB1320 | ... | ... | 10.5 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 36.5 | ... | 46.0 |
| | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 37.0 | ... | 46.5 |
| | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 38.0 | ... | 47.5 |

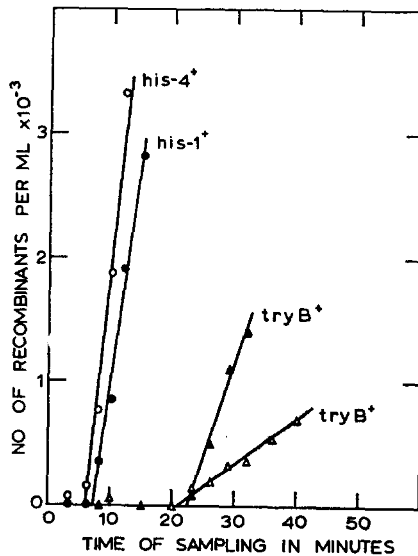


FIGURE 5.—Kinetics of chromosome transfer by donor strain AB311 in crosses to recipient strain AT2121 (solid circles and triangles) and to recipient strain AT1372 (open circles and triangles).

TABLE 5

*Time of entry of markers transferred by strain AB311*

| Recipient parent strain No. | Selected markers of the donor parent | | | | |
|---|---|---|---|---|---|
| | *his-1* | *his-4* | *aroD-5* | *aroD-6* | *tryB* |
| AT2121 | 5.5 | ... | ... | ... | 19.5 |
| | 7.0 | ... | ... | ... | 22.0 |
| AT1372 | ... | 6.5 | ... | ... | 21.0 |
| | ... | 6.0 | ... | ... | 21.0 |
| AB1359 | ... | 6.0 | 12.0 | ... | ... |
| | ... | 6.5 | 12.5 | ... | ... |
| AB1360 | ... | 6.0 | ... | 12.5 | ... |
| | ... | 5.5 | ... | 11.5 | ... |

carries a transposition of one histidine locus while strain AB311 does not. Since it is technically difficult to determine the exact genetic basis for the average difference of 6.5 minutes in the time of entry of these markers, it becomes necessary to choose one or the other as the reference point for constructing the linkage map of AB313. The time of entry of *his-1*$^+$ seemed the logical choice because (1) it is the more reproducible one experimentally and (2) it is the one referred to in several earlier genetic maps. Consequently, the interlocus distances computed from the data in Table 4 were corrected by a factor of minus 6.5 minutes in those crosses where *his-4* was the only reference marker available.

Maps of the regions analyzed with strains AB313 and AB311 are shown in Figure 6. The 26 minute interval found between the *malA* and *his* loci is close to the 29 to 30 minute figure reported by JACOB and WOLLMAN (1961). On the other hand, the *his* and *try* loci are separated by only 14.6 minutes in crosses with AB311; this amounts to nearly a 50 percent reduction over former estimates of the length of this segment.

Note in Figure 6 that certain of the markers independently mapped with AB313

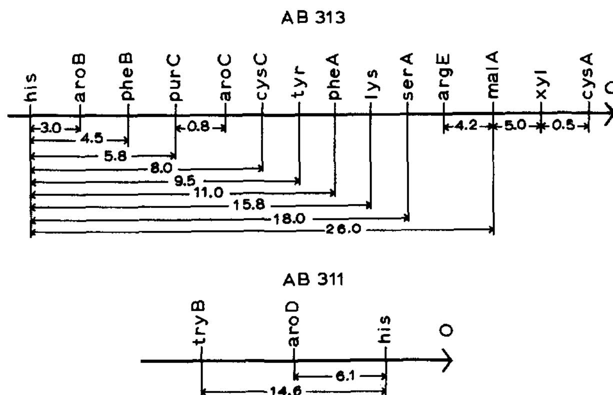


FIGURE 6.—Maps of the chromosomal segments transferred by donor strains AB313 and AB311.

(e.g., *pheA, tyr, cysC*) have closely similar times of entry. It should be emphasized that time of entry differences of only 1 or 2 minutes in separate experiments are usually too small to permit unequivocal ordering of loci on the chromosome. This is specially true for markers far from the chromosomal point-of-origin, because extrapolations to the time axis become progressively less accurate with increasing distance from the origin. The inferred sequence for some of the closely linked markers shown in Figure 6 must therefore be considered as tentative until such time that more detailed mapping data become available.

*The full genetic map of E. coli:* It is now generally recognized, from both genetic evidence (JACOB and WOLLMAN 1961; TAYLOR and ADELBERG 1960) and autoradiographic studies (CAIRNS 1963), that the chromosome of *E. coli* has a closed or circular structure. The circular map presented in Figure 7 was con-

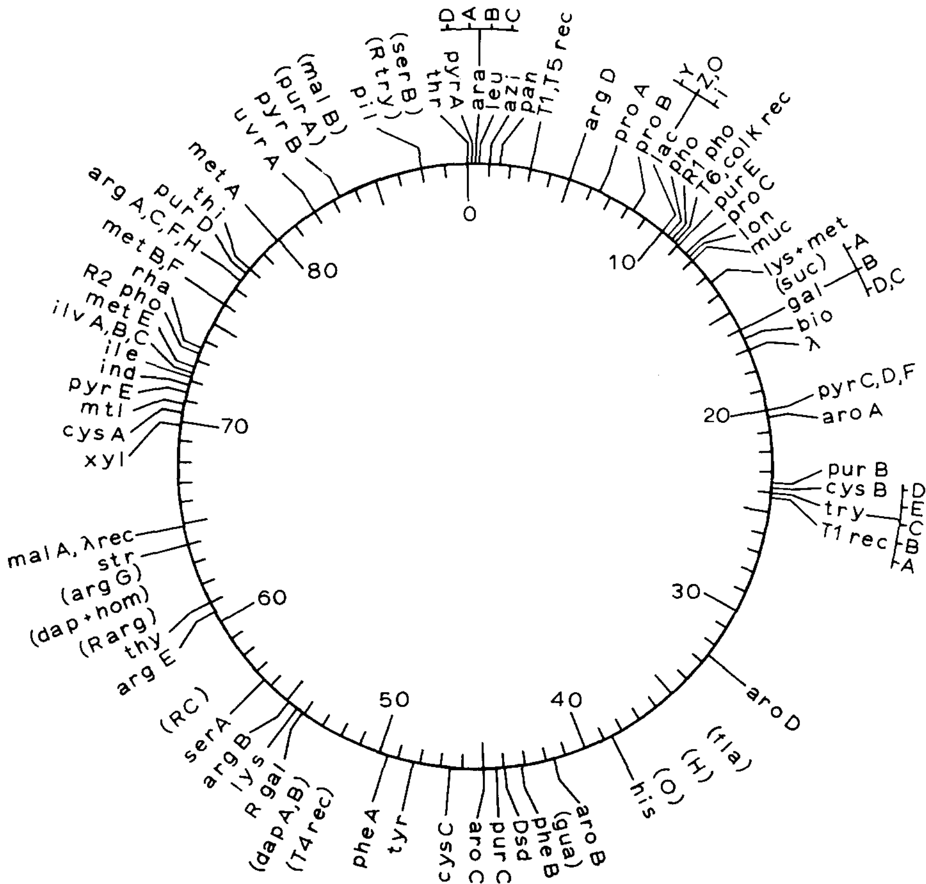


FIGURE 7.—Genetic map of *E. coli*, drawn to scale. The complete map is constructed from the partial maps shown in Figures 2, 3, 4 and 6, and from additional sources given in Table 6. A key to the genetic symbols appears in Table 6. The map is graduated in one-minute intervals (89 minutes total) and numbered at 10-minute intervals to facilitate computation of interlocus distances. Markers enclosed in parentheses are only approximately mapped at the positions indicated. The exact sequence of markers in crowded regions is not always known (see text).

structed by joining together the individual map segments of the six donor strains described in the preceding paragraphs. For this purpose, the *arg-3* marker that was mapped with strain AB261 and the *arg-1* marker that was mapped with donor AB312 were treated as if they occupied a single locus on the genetic map, although they are actually alleles of two separate but closely linked loci. The combined segments cover the full length of the chromosome, except for the short region between the *gal* and *try* loci; the gap was closed by including in Figure 7 the 8-minute interval given by JACOB and WOLLMAN (1961) for this segment. The over-all length of the map, in time units, is 89 minutes.

The most recent complete map of *E. coli* appeared in the monograph of JACOB and WOLLMAN (1961, p. 165). However, new mapping data have accumulated rapidly since that time. In an effort to bring the chromosome map of *E. coli* up to date, we have included much of this new information in Figure 7. References to the individual sources of published and unpublished mapping data are given in Table 6. There has also been marked progress in identifying the biochemical functions determined by these gene loci. For this reason, we have also indicated wherever possible the specific enzymes associated with the genetic markers listed in Table 6.

Table 6 is not a complete list of all the known loci of *E. coli*; it is limited instead to those loci which have been mapped with reasonable accuracy either by time of entry experiments, by transduction, or by linkage analyses in uninterrupted Hfr × $F^-$ crosses. Even so, the available data are often too limited to permit a precise ordering of all the loci in crowded regions of the map. For example, the exact order of 11 single and complex loci in the segment from *xyl* to *metA* (see (Figure 7) is known, but the correct positions in this sequence of four additional markers—namely *ile*, *R2 pho*, *rha* and *thi*—are not known, even though each has been independently mapped in this region by its time of entry during conjugation.

## DISCUSSION

Interrupted-mating experiments performed with several donor strains showed that the number of recombinants found in successive blended samples does not always increase uniformly as a function of time. In several experiments, the number of recombinants increased slowly at first, then more rapidly some 5 to 15 minutes later. Independent tests suggested that extrapolation of the initial slopes of the resulting anomalous transfer curves provides the most accurate time of entry data and that extrapolation from the steeper second slopes generally tends to exaggerate the distance between loci.

Reexamination of data obtained in previous experiments revealed that the early recombinants were undetected in some instances; in others, they were so few in number that they were thought to be prototrophic revertants rather than recombinants. Previous mapping data were thus often based on extrapolations from second slopes, and this may account for the consistent trend toward diminution of interlocus distances that typifies most of the new mapping data. As a consequence of this trend, the total length in time units of the *E. coli* linkage

group was reduced to about four fifths of its previously estimated length—89 minutes versus 108 minutes (JACOB and WOLLMAN 1961) or 111 minutes (TAYLOR and ADELBERG 1960).

The increased sensitivity of the present method for detecting early recombinants is largely due to two simple changes in experimental procedure. First, the blended samples were plated directly, without benefit of washing or further dilution in buffer to reduce the amount of broth transferred to the agar plates. Under these conditions, the number of recombinants recovered per donor cell in the mating mixtures was 10 to 50 times greater than in previous experiments where cells were diluted in buffer prior to plating. GROSS (in DE HAAN and GROSS 1962) reported that small amounts of broth enhance the recovery of genetic recombinants from zygotes on minimal agar plates. This effect may well account for the increased yields of recombinants. The second modification was the blending of samples at closely spaced intervals of 2 or 3 minutes. The shallow initial slopes of the upward-breaking transfer curves would not have been detected in many experiments had the intervals between samples been extended to 5 or more minutes.

Anomalous transfer curves similar to the ones shown in Figure 1A and 1B have also been observed consistently in intergeneric crosses between Shigella Hfr donors and *E. coli* recipients (H. SCHNEIDER and S. FALKOW personal communication). Although the physical basis for this phenomenon is still unknown, a plausible explanation for the broken slopes can be derived from a comparison of the kinetics of chromosome transfer in crosses with Hfr strains AB261 and AB451. Recall that AB261 and AB451 are identical in direction of transfer and in point-of-origin, but that the former produces transfer curves having two slopes whereas the latter produces single slope curves. The slopes generated by the secondary rise in $pro^+$ and $arg^+$ recombinants in the AB261 crosses were equal, within the limits of experimental variation, to the slopes of the corresponding transfer curves obtained in AB451 crosses. Thus, both donor strains ultimately transfer these markers to recipients with equal efficiency, and they differ only in that the bulk of the recombinants formed in the AB261 crosses do not appear until after a delay of 5 to 15 minutes. This, together with the observation that the delay is shortest for early markers and progressively longer for later markers (see Figures 1A and 1B), suggests that the majority of AB261 donor cells inject their chromosomes at a slower speed than AB451 donors. The shallow initial slopes which extrapolate to the same time of entry values as the corresponding AB451 transfer curves (Table 1) would then be produced by a minority population of AB261 donors that inject at the same speed as strain AB451. Although other interpretations are not excluded by the present data, the notion of variable rates of chromosome transfer in different cells of a mating Hfr population does satisfactorily account for the shape of the anomalous transfer curves. Moreover, these observations complement the earlier studies of DE HAAN and GROSS (1962), who showed that certain other variations in the kinetics of chromosome transfer could also be best explained on the basis of different rates of transfer among individual donor cells.

TABLE 6

*List of chromosomal markers of* E. coli

| Key to genetic symbols* | | Activity affected | References | |
|---|---|---|---|---|
| | | | Biochemical studies | Genetic mapping |
| *araD* | arabinose | L-ribulose 5-phosphate 4-epimerase | 43 | 43 |
| *araA* | arabinose | L-arabinose isomerase | 43 | 43 |
| *araB* | arabinose | L-ribulokinase | 43 | 43 |
| *araC* | arabinose | unknown | 30 | 43 |
| *argB* | arginine | N-acetylglutamate synthetase | 62 | 46,47,27,62 |
| *argC* | arginine | N-acetyl-γ-glutamokinase | 62 | 46,47,27,62 |
| *argH* | arginine | N-acetylglutamic-γ-semialdehyde dehydrogenase | 62 | 46,47,27,62 |
| *argG* | arginine | acetylornithine-δ-transaminase | 62 | 46,47,27,62 |
| *argA* | arginine | acetylornithinase | 62 | 46,47,27,62 |
| *argD* | arginine | ornithine transcarbamylase | 62 | 46,47,27,62 |
| *argE* | arginine | argininosuccinic acid synthetase | 62 | 46,47,27,62 |
| *argF* | arginine | argininosuccinase | 62 | 46,47,27,62 |
| *aroA,B,C* | aromatic amino acids and vitamins | shikimic acid to 3-enolpyruvylshikimate-5-phosphate | 55 | . . |
| *aroD* | aromatic amino acids and vitamins | biosynthesis of shikimic acid | . . | . . |
| *azi* | azide | resistance or sensitivity to sodium azide | . . | 37 |
| *bio* | biotin | | | 65 |
| *cysA* | cysteine | unknown | | . . |
| *cysB* | cysteine | 3′-phosphoadenosine 5′-phosphosulfate to sulfide† | 19 | 67 |
| *cysC* | cysteine | sulfate to sulfide; four known enzymes† | 19 | . . |
| *dapA* | diaminopimelic acid | dihydrodipicolinic acid synthetase | 14 | 14 |
| *dapB* | diaminopimelic acid | N-succinyl-diaminopimelic acid deacylase | 14 | 14 |
| *dap + hom* | diaminopimelic acid + homoserine | aspartic semialdehyde dehydrogenase | 14 | 14 |
| *Dsd* | D-serine | D-serine deaminase | . . | 52 |
| *fla* | flagella | | . . | 53 |

| Key to genetic symbols* | | Activity affected | References | |
|---|---|---|---|---|
| | | | Biochemical studies | Genetic mapping |
| *galA* | galactose | galactokinase | 40 | 11,12,4 |
| *galB* | galactose | galactose 1-phosphate uridyl transferase | 40 | 11,12,4 |
| *galD* | galactose | uridinediphosphogalactose 4-epimerase | 40 | 11,12,4 |
| *galC* | galactose | operator mutants | . . | 11,12,4 |
| *gua* | guanine | | . . | . . |
| *H* | H antigen | flagellar antigen | . . | 53 |
| *his* | histidine | ten known enzymes and an operator† | 6 | 7,29 |
| *ile* | isoleucine | threonine deaminase | . . | 54 |
| *ilvA* | isoleucine + valine | $\alpha$-hydroxy $\beta$-keto acid reductoisomerase† | 63 | 54 |
| *ilvB* | isoleucine + valine | $\alpha,\beta$-dihydroxyisovaleric dehydrase† | 8 | 54 |
| *ilvC* | isoleucine + valine | transaminase B | 2 | 54 |
| *ind* | indole | tryptophanase | . . | 23 |
| λ | prophage λ | | . . | 37 |
| *lac Y* | lactose | galactoside permease | 35 | 37,35 |
| *lac Z* | lactose | $\beta$-galactosidase | 35 | . . |
| *lac O* | lactose | operator mutants | 35 | . . |
| *leu* | leucine | three known enzymes and an operator† | 39 | 37,49 |
| *lon* | long form | filament formation and radiation sensitivity | 33,3 | 33,3 |
| *lys* | lysine | diaminopimelic acid decarboxylase | . . | . . |
| *lys + met* | lysine + methionine | unknown | 9 | . . |
| *λrec,malA* | λreceptor and maltose | maltose permease and resistance to phage λ | 64 | 42 |
| *malB* | maltose | probably amylomaltase | 64 | . . |
| *metA* | methionine | synthesis of the succinic ester of homoserine† | 21 | 54 |
| *metB* | methionine | succinic ester of homoserine + cysteine to cystathionine† | 21 | 54 |

TABLE 6—Continued

*List of chromosomal markers of* E. coli

| Key to genetic symbols* | | Activity affected | References: Biochemical studies | References: Genetic mapping |
|---|---|---|---|---|
| *metF* | methionine | probably 5,10-methylene tetrahydrofolate reductase | 41 | 54 |
| *metE* | methionine or cobalamin | unknown | .. | .. |
| *mtl* | mannitol | probably mannitol dehydrogenase | .. | .. |
| *muc* | mucoid | regulation of capsular polysaccharide synthesis | 50 | 50 |
| *O* | O antigen | somatic antigen | .. | 53 |
| *pan* | pantothenic acid | | .. | 18 |
| *pheA,B* | phenylalanine | | .. | .. |
| *pho* | phosphatase | alkaline phosphatase | 45 | 20 |
| *pil* | pili (fimbriae) | | .. | 48 |
| *proA* | proline | | .. | .. |
| *proB* | proline | supports syntrophic growth of *proA* mutants | .. | .. |
| *proC* | proline | supports syntrophic growth of *proA* mutants | .. | 57 |
| *purA* | purine | adenylosuccinic synthetase | 26 | .. |
| *purB* | purine | adenylosuccinase | 25 | .. |
| *purC,E* | purine | 5-aminoimidazole ribotide (AIR) to 5-aminoimidazole 4-(N-succinocarboxamide) ribotide | 15 | .. |
| *purD* | purine | biosynthesis of AIR | .. | .. |
| *pyrA* | uracil + arginine | carbamate kinase | .. | 10 |
| *pyrB* | uracil | aspartate transcarbamylase | .. | 10 |
| *pyrC* | uracil | dihydroorotase | .. | 10 |
| *pyrD* | uracil | dihydroorotic acid dehydrogenase | .. | 10 |
| *pyrE* | uracil | orotidylic acid pyrophosphorylase | .. | .. |
| *pyrF* | uracil | orotidylic acid decarboxylase | .. | 10 |
| *R arg* | repressor | arginine repressor | .. | 47,27 |
| *R gal* | repressor | galactose repressor | 12 | 12 |

| Key to genetic symbols* | | Activity affected | References: Biochemical studies | References: Genetic mapping |
|---|---|---|---|---|
| *R1 pho, R2 pho* | repressor | alkaline phosphatase repressor | 20 | 20 |
| *R try* | repressor | tryptophan repressor | .. | 38 |
| *RC* | RNA control | regulation of RNA synthesis | .. | 5 |
| *rha* | rhamnose | utilization of D-rhamnose | .. | .. |
| *serA* | serine | 3-phosphoglycerate dehydrogenase | 61 | .. |
| *serB* | serine | phosphoserine phosphatase | 61 | .. |
| *str* | streptomycin | resistance, sensitivity, or dependence on streptomycin | .. | 44 |
| *suc* | succinic acid | | .. | .. |
| *T1,T5 rec* | phage receptor site | resistance to phages T1 and T5 | .. | 37 |
| *T1 rec* | phage receptor site | resistance to phage T1 | .. | 67 |
| *T6, colK rec* | phage and colicine receptor site | resistance to phage T6 and colicine K | .. | 22,31 |
| *T4 rec* | phage receptor site | resistance to phage T4 | .. | .. |
| *thi* | thiamine | | .. | 48 |
| *thr* | threonine | | .. | 37 |
| *thy* | thymine | thymidylate synthetase | .. | 34 |
| *tryA* | tryptophan | tryptophan synthetase, A protein | 16 | 67 |
| *tryB* | tryptophan | tryptophan synthetase, B protein | 16 | 67 |
| *tryC* | tryptophan | indole 3-glycerolphosphate synthetase | 24 | 67 |
| *tryE* | tryptophan | anthranilic acid to anthranilic deoxyribulotide | 58 | 51 |
| *tryD* | tryptophan | 3-enolpyruvylshikimate 5-phosphate to anthranilic acid | .. | 67 |
| *tyr* | tyrosine | | .. | .. |
| *uvrA* | ultraviolet radiation | reactivation of UV-induced lesions in DNA | 32 | 32 |
| *xyl* | xylose | utilization of D-xylose | .. | .. |

* Established systems of genetic nomenclature are retained wherever possible, except that capital letters beginning with the letter A are arbitrarily assigned to functionally related gene loci which do not conform to the system of bacterial genetic nomenclature proposed by DEMEREC (1963).

† Denotes enzymes controlled by the homologous gene loci of *Salmonella typhimurium*.

The incorporation of mapping data from many independent sources into a single scale drawing of the genetic map results in a marked grouping of loci into short crowded regions (Figure 7). Note for example the clusters around the *leu, lac, try, purC, lys, str,* and *ilv* loci. In most of these crowded regions, the exact sequence of loci on the chromosome is at best only partially determined. It is expected, therefore, that some of the sequences shown in Figure 7 will be revised when more refined mapping data become available. On the other hand, it is also expected that this detailed map will be useful both as a guide for further mapping studies in *E. coli* and as a reference point for future comparisons of the *coli* map with the chromosome maps of other bacterial species.

## SUMMARY

The loci of 34 markers were determined by the interrupted conjugation technique. Of these, 20 were markers hitherto unmapped by this method. The map distances between certain pairs of loci were found to be 10 to 50 percent shorter than in older published maps. As a consequence, the over-all length of the genetic map in time units was reduced to 89 minutes, or about four fifths of its former length. Anomalies in the kinetics of chromosome transfer were discovered in some of the crosses, and their importance in interpreting the results of interrupted mating experiments was discussed. In addition, recent mapping data from many laboratories were compiled; a revised map based on them shows the positions of 100 gene loci.

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