CHROMOSOME FRAGMENTS PARTICIPATING IN TRANSDUCTION IN SALMONELLA TYPHIMURIUM[1]

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IN the experimental transduction system involving *Salmonella typhimurium* and bacteriophage PLT-22 (ZINDER and LEDERBERG 1952), a number of instances of joint transduction of two (or more) markers have been observed (STOCKER *et al.* 1953; DEMEREC *et al.* 1954; DEMEREC *et al.* 1955). Thus it appears that the genetic material transferred by a phage particle can include more than one gene. Moreover, it has been found that only certain combinations of Salmonella markers can be transduced jointly: such markers are assumed to be linked on the chromosome. LENNOX (1955), working with a different system (*Escher*-ella markers can be transduced jointly: such markers are assumed to be linked genes in recombination experiments can be jointly transduced. It therefore seems reasonable to assume that the material transferred by a phage particle is itself a short fragment of the bacterial chromosome, as suggested originally by STOCKER and his co-workers.

The present study is concerned with the problem of whether or not the fragments carrying a given marker are uniform, that is, all of the same length and composition. The results reported here were obtained from observations of two different types of products appearing in transduction experiments made with auxotrophic markers, namely, (1) minute colonies, resulting from abortive transduction, which will be described in detail in a later section, and (2) large colonies, resulting from genetic recombination through transduction. The latter process will be referred to in this paper as "complete transduction," in contrast to "abortive transduction."

It has been thought that the genetic recombination involved in complete transduction results from crossing over between the chromosome of the recipient bacterium and a fragment of donor chromosome brought in by a phage particle (DEMEREC and DEMEREC 1956; LEDERBERG 1956; cf. MORSE *et al.* 1956); an even number of crossovers would be required to obtain a viable recombinant. The hypothesis that the material whose introduction results in abortive transduction is a fragment of chromosome has been discussed in detail by LEDERBERG (1956) and STOCKER (1956); their observations do not conflict with this hypothesis, and the additional information to be presented here supports the idea. A further assumption, based on the above hypothesis, will be made as a working premise,

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viz., that the chromosome fragments whose introduction results in complete transduction (through crossing over) have the same size distribution as those concerned in abortive transduction.

MATERIALS AND METHODS

S. typhimurium LT-2 (ZINDER and LEDERBERG 1952) was the basic strain used in the experiments; it will be referred to as wild type. The auxotrophic strains were all derivatives of LT-2, and all were sensitive to the temperate phage PLT-22 (ZINDER and LEDERBERG 1952), which was the vector in the transduction experiments. The symbols (see DEMEREC 1956b) and growth requirements of the auxotrophic markers are listed below. Capital letters designate different loci within each category of requirement; numerals identify individual mutant strains, which are numbered in the order in which they were isolated.

(Since this paper was written, a uniform nomenclature for auxotrophic mutants has been proposed (Microbial Genetics Bulletin No. 16), and the symbols now in use for the mutants listed are as follows: adenine, *ade*; purine-plus-thiamine, *adth;* guanine, *gua*; histidine, *his*; methionine, *met*; and serine, *ser.*)

adC-2, *adC-7*, and *adE-11:* require adenine. (Guanine, hypoxanthine, and xanthine also satisfy this requirement.)

ad-3: requires adenine. (This mutant, although obtained in a single cycle of the penicillin screening procedure, behaves genetically as though it had undergone mutation at each of the loci *adA* and *adB;* see YURA 1956.)

athA-2, *athA-4*, *athB-6*, *athC-5*, *athC-14*, and *athD-12:* require one of the purines plus thiamine.

cysA-1, *cysB-12*, and *cys-5:* require cystine. (Mutant *cys-5*, although obtained in a single cycle, behaves genetically as a double mutant, *cysA-5 cysC-80;* see HOWARTH 1958).

guA-1: requires guanine.

hiA-38, *hiB-22*, *hiD-34*, and *hiD-39:* require histidine.

meA-22, *meB-45*, *meC-50*, and *meF-31:* require methionine.

proA-24 and *proA-46:* require proline.

se-1 and *se-5:* require serine.

tryA-8, *tryB-2*, and *tryD-10:* require tryptophan.

(For more details regarding these mutants, see DEMEREC *et al.* 1955, 1956.) The double-mutant strains were combinations of these markers, and their phenotypes were additive. They will be symbolized *tryA-8 cysB-12*, *athC-5 proA-46*, and so forth.

The minimal medium (M) contained K_2HPO_4, 10.5 gm; KH_2PO_4, 4.5 gm; $MgSO_4$, 0.05 gm; $(NH_4)_2SO_4$, 1.0 gm; sodium citrate, 0.47 gm; glucose, 2.0 gm; demineralized water, 1000 ml. For enriched minimal medium (EM), 0.01 percent dehydrated nutrient broth was added. Both media contained 1.5 percent agar for plate culture. The concentrations of various supplements added to the media were: adenine sulfate (A), 20 μg/ml; thiamine (B_1), 10 μg/ml; cysteine (C), 20 μg/ml; guanine (G), 20μg/ml; proline (P), 30 μg/ml; $Na_2S_2O_3.5H_2O$ (S), 1

mg/ml; tryptophan (T), 20 μg/ml. The supplemented media will be designated by combinations of the abbreviations shown above; for example, MT, minimal medium plus tryptophan; EMGP, enriched minimal medium plus guanine and proline.

In transduction experiments a suspension of the recipient bacteria was infected with phage grown on the donor bacteria in nutrient broth (for *cys* mutants, 20 μg/ml cysteine were added). After incubation at 37°C for ten minutes in broth or saline, to allow adsorption to take place, 0.1 ml of the suspension was plated on suitable medium (usually in triplicate). The number of infected cells plated was adjusted in each experiment to give a desirable number of colonies on the plates. Multiplicity of infection was always between one and ten (usually around five), to avoid lysis. Colonies were counted after one or two days' incubation at 37°C. The "minute colonies" were counted with a microscope at 10×–20× magnification (see DEMEREC and OZEKI 1958). Formulas for transduction experiments are written *strain* (recipient) × *strain* (donor).

When it was necessary to isolate phage-sensitive clones descended from transduced cells, a mutant strain (phage *H-4* of ZINDER) was substituted for the wild type PLT-22.

RESULTS

Abortive transduction among linked markers: It had been observed (OZEKI 1956) that, in certain transduction experiments with auxotrophic mutants of *S. typhimurium* LT-2, "minute colonies" appeared in addition to the large colonies expected as a result of complete transduction. Analysis of the mechanism of formation of such minute colonies showed that it is apparently similar to the mechanism responsible for the formation of "trails" in the transduction of motility in Salmonella—namely, abortive transduction (STOCKER *et al.* 1953). Evidence indicates that in abortive transduction the fragment introduced into a recipient cell fails to be incorporated into its genome, and does not duplicate, but persists in a functional state in one of the descendants through a number of generations (see also STOCKER 1956; LEDERBERG 1956). The formation of minute colonies when auxotrophic recipient cells are plated on minimal medium requires the presence in some of these cells of genetic fragments containing the wild type allele (functional unit) corresponding to the mutated locus in the recipient strain. Consequently, the appearance of minute colonies on a plate that does not contain the growth-factor requirement corresponding to the marker being studied is a sign of the transfer of the wild type allele of that locus by the transducing phage particle (OZEKI 1956; DEMEREC and OZEKI 1958).

To extend the above described observations, the formation of minute colonies was examined in experiments with double-mutant recipients whose two markers are linked in transduction. The double mutants *tryD-10 cysB-12* and *tryA-8 cysB-12* were used, since linkage among these three markers has been clearly demonstrated (DEMEREC and Z. HARTMAN 1956). MS served as "minimal" medium for these two-marker mutants because *cysB* mutants accumulate the

growth factor for any *try* mutant on M or EM medium, and the addition of thiosulfate suppresses this accumulation without supporting the growth of *cysB-12* cells and without inhibiting the growth of wild type cells. One set of results obtained in the experiments with *tryD-10 cysB-12* is shown in Table 1. Similar results were obtained with *tryA-8 cysB-12* as recipient.

TABLE 1

Abortive transduction with tryD-10 cysB-12 *recipient bacteria. Figures represent the numbers of minute colonies per plate (average of three plates) after 18 hours of incubation at 37°C. Figures in parentheses are the numbers of large colonies (results of complete transductions) on the same plates*

Medium	Donor			
	tryD+ cysB+	*tryD-10 cysB+*	*tryD+ cysB-12*	*tryD-10 cysB-12*
MS*	4700 (122)	0 (0)	0 (0)	0 (0)
MT	4300 (601)	4100 (399)	0 (0)	0 (0)
MC	4000 (355)	0 (0)	2350 (135)	0 (0)

* MS served as "minimal" medium in this experiment (see text).

The formation of minute colonies on MS plates required the presence in recipient cells of fragments containing both wild type alleles, *tryD*$^+$ and *cysB*$^+$, whereas the formation of minute colonies on MT and MC plates required the presence of only one of these two wild type alleles. It appears that most, if not all, of the fragments carrying the *tryD* locus also carried the *cysB* locus, and vice versa; for the numbers of minute colonies observed on MS, MT, and MC were not significantly different within any given set of experiments with *tryD*$^+$ *cysB*$^+$ (wild type) as donor. This conclusion is supported by results of experiments (see Table 5) in which *tryD-10*, *cysB-12*, and *tryD-10 cysB-12* bacteria were all infected with the same phage preparation from a wild type donor; the numbers of minute colonies were similar.

These results suggest two possibilities concerning the nature of the chromosome fragments participating in abortive transduction: (1) the fragments are long in relation to the distance between the *tryD* and *cysB* loci, and the chromosome of donor cells is disrupted at random; or (2) the fragments are short, but chromosome fragmentation does not occur in a random manner and the fragments concerned always or almost always include both the *tryD* locus and the *cysB* locus.

Since the *try-cysB* mutants are the only linked double mutants showing abortive transductions that we have isolated so far, the same technique could not be applied to other regions of the chromosome to extend the observations described above. However, similar results have been obtained in experiments involving *athD-12* and *athB-6*. These two genes are rather closely linked, about 85 percent donor type and 15 percent wild type being recovered in *athD-12* (recip.) × *athB-6* (donor) tests. In abortive-transduction experiments with *athD-12* as recipient, smaller minute colonies (less than half the diameter) were observed on M (or

EM) plates when *athB-6* was donor than when wild type was donor. The numbers of minute colonies appearing in the experiments were approximately the same with the two donors, and the size distribution curves were in both cases unimodal (the modes of these two curves differed by a factor of two). This result suggests that all or almost all fragments carrying *athD*$^+$ also carry *athB-6* or *athB*$^+$. The reduction in size of minute colonies when the *athB-6* marker is present has not been explained.

The formation of minute colonies has also been tested with unlinked double mutants as recipients, that is, double mutants from which stable prototrophs had not been obtained in experiments with wild type donor bacteria. Ten different unlinked double mutants were tested with phage grown on wild type bacteria. They were *cysA-5 cysC-80*, *ad-3* (*adA adB*), *athA-2 meF-31*, *adE-11 meB-45*, *hiB-22 tryA-8*, *tryB-2 meA-22*, *adE-11 hiA-38*, *athC-5 proA-46*, *athA-2 hiD-34*, and *cysB-12 proA-24*. With most of the strains no minute colonies were observed on minimal plates. With some, minute colonies did appear; but in every case they were shown to be the result of abortive transduction at one locus, the other locus being "leaky."

These results show that the relationship (linkage or its absence) among auxotrophic markers inferred from observations of complete transduction is maintained in abortive transduction. It furthermore appears that, in the only two systems testable, pairs of genes which are inferred to be linked are generally transferred together in abortive transduction. These observations, together with the fact that in complete transduction linkage between markers whose phenotypes are not closely related is very rare, suggest that the fragments participating in transduction are generally short, and support possibility (2) above, rather than possibility (1). In this they also agree with the results of an earlier analysis by DEMEREC and Z. HARTMAN (1956) of complete transduction of *try cysB* double mutants by wild type (Table 2), which showed that the number of two-marker

TABLE 2

Average numbers of colonies per experiment of the different transduction classes recovered in experiments with try cysB × try$^+$ cysB$^+$. *Media used: M=minimal; MT=minimal with tryptophan added; MC=minimal with cysteine added (From DEMEREC and Z. HARTMAN 1956)*

Recipient	M	MT		MC	
	try$^+$ *cys*$^+$	*try*$^+$ *cys*$^+$	*try cys*$^+$	*try*$^+$ *cys*$^+$	*try*$^+$ *cys*
tryA-8 cysB-12	150	123	224	261	312
tryB-2 cysB-45	253	234	935	213	272
tryD-10 cysB-12	336	337	1,512	351	877
tryD-11 cysB-18	143	141	523	193	391

transductions was always smaller than either of the single-marker classes, and thus indicated that the genetic lengths of the regions beyond these two genes on a fragment are not greater than the distance between them.

Reciprocal transduction: Only a few combinations of recipient and donor strains are known in which donor-type recombinants can be recovered in both directions of reciprocal experiments. One such combination, *athC-5* and *guA-1* has been studied in detail.

Linkage of the three loci *adC*, *athC*, and *guA* has been reported previously (DEMEREC *et al.* 1956). Results of reciprocal transductions among *adC*, *athC*, and *guA* mutants, presented in Table 3, suggest that the order of these loci is *adC–athC–guA*. Usually, numbers of colonies obtained in different transduction experiments cannot reliably be compared, since frequency of transduction is affected by many factors that are difficult to control. However, the ratios of wild type (one-marker) to donor type (two-marker) transductions in different experiments can probably be compared. It was originally thought that, the chromosomes of donor bacteria being disrupted into small fragments in a random way, these ratios simply reflected the distance between the two markers involved: the closer the markers the higher the probability that both donor markers would be incorporated. On this hypothesis, the ratio of wild type to donor-type transduction would always be the same for a particular pair of markers, in whichever direction the transduction was carried out, because the distance between the two markers and the mean length of the transducing fragments would be the constant factors. But in the present case the ratios varied according to the direction of transduction. For instance, with *guA-1* as recipient and *athC-5* as donor, the ratio of wild type to donor-type was 2.9 to 1, but with *athC-5* as recipient and *guA-1* as donor the ratio was 0.9 to 1, as shown in Table 3. To find out whether the difference in the ratios was due to the difference in the wild type class or to the difference in the donor-type class, the following experiments were done.

A spontaneous proline-requiring mutant of *athC-5* (designated *athC-5 proA-*

TABLE 3

Results of reciprocal transduction experiments with adC, athC, *and* guA *mutants. Figures represent average numbers of colonies per plate (based on three plates), expressed as wild type/donor type. Figures in parentheses show the ratios of wild type to donor type. Number of infected bacteria per plate, about* 2×10^8*; multiplicity of infection, about five. Medium used was enriched minimal supplemented where possible with a growth factor supporting the multiplication of donor-type bacteria without allowing growth of the recipient cells*

	Donor				
Recipient	*adC-2*	*adC-7*	*athC-5*	*athC-14*	*guA-1*
adC-2	0/–	3/–	181/–	163/–	156/–
adC-7	4/–	0/–	196/–	130/–	189/–
athC-5	322/38	311/49	0/–	10/–	155/170
	(8.5)	(6.3)			(0.9)
athC-14	267/42	251/46	11/–	0/–	144/129
	(6.4)	(5.5)			(1.1)
guA-1	426/12	490/13	508/174	540/154	0/–
	(35.5)	(37.7)	(2.9)	(3.5)	

46) was first isolated. Then the *proA-46* marker was combined with *guA-1* by infecting *athC-5 proA-46* with phage from *guA-1* and isolating the *athC*$^+$ *guA-1 proA-46* recombinant (nonlysogenic). To analyze the relation between frequencies of complete transductions at the *athC-guA* region and at the *proA* region, both two-marker strains, *athC-5 proA-46* and *guA-1 proA-46*, were infected with phage grown on wild type bacteria. As shown in Figure 1, a linear relation was observed between frequencies of transduction in the two regions within a certain range of multiplicity of infection. Similar results were obtained with *adC-7 proA-46* (nonlysogenic), isolated in the transduction *athC-5 proA-46* (recip.) × *adC-7* (donor).

Thus corrections can be made for numbers of transductions at the *athC-guA* region in different experiments on the basis of the numbers of colonies resulting from complete transductions from *proA-46* to *proA*$^+$ in the same experiments. This method was applied to the results of reciprocal experiments between *athC-5* and *guA-1* (Table 4). As the table shows, the frequencies of transductions to the donor type (two-marker transductions) were similar in the two experiments, whereas the frequencies of one-marker transductions were significantly different. As a result, the ratios of one-marker to two-marker transductions are different for the two directions of the reciprocal experiments. These experiments were repeated several times, using different phage preparations, with similar results; two sets of data are given in Table 4. The difference in ratios may be explained on the assumption that all transducing fragments carrying the *athC-guA* region are approximately identical, and that the position of a marker on the fragment has an important effect on the probability of its incorporation into the recipient chromosome. Figure 2 is a schematic representation of the positions of the *athC-5* and *guA-1* sites on the transducing fragments, based on the values presented in Table 4. In class c of the table, the similar values obtained for the reciprocal experiments 1 and 2 are explained on the basis that in both cases crossing over in the X and in the Z portion of the fragment was required for incorporation of the *athC* and *guA* loci; and the difference in values in class b is explained on the basis

TABLE 4

Reciprocal transduction experiments with linked markers. Each figure represents the actual number of colonies on a total of three plates. Figures in parentheses are corrected values (per 1000 colonies in column a). (a). Transduction from proA-46 *to* proA$^+$. *(b). One-marker transduction: expt. 1, from* athC-5 guA$^+$ *to* athC$^+$ guA$^+$; *expt. 2, from* athC$^+$ guA-1 *to* athC$^+$ guA$^+$. *(c). Two-marker transduction: expt. 1, from* athC-5 guA$^+$ *to* athC$^+$ guA-1; *expt. 2, from* athC$^+$ guA-1 *to* athC-5 guA$^+$

Expt.	Recipient	Donor	a	b	c	b/c
1	*athC-5 guA*$^+$ *proA-46*	*athC*$^+$ *guA-1 proA*$^+$	1072 (1000)	167 (156)	182 (169)	0.93
			684 (1000)	134 (196)	138 (202)	0.97
2	*athC*$^+$ *guA-1 proA-46*	*athC-5 guA*$^+$ *proA*$^+$	2,149 (1000)	1,315 (612)	399 (186)	3.29
			724 (1000)	454 (628)	144 (198)	3.16

that experiment 1 involved the X and Y portions whereas experiment 2 involved Y and Z. Thus the difference in ratios shown in Table 4 (see also Table 3) can be explained on the assumption that the Z portion is longer than the X portion in

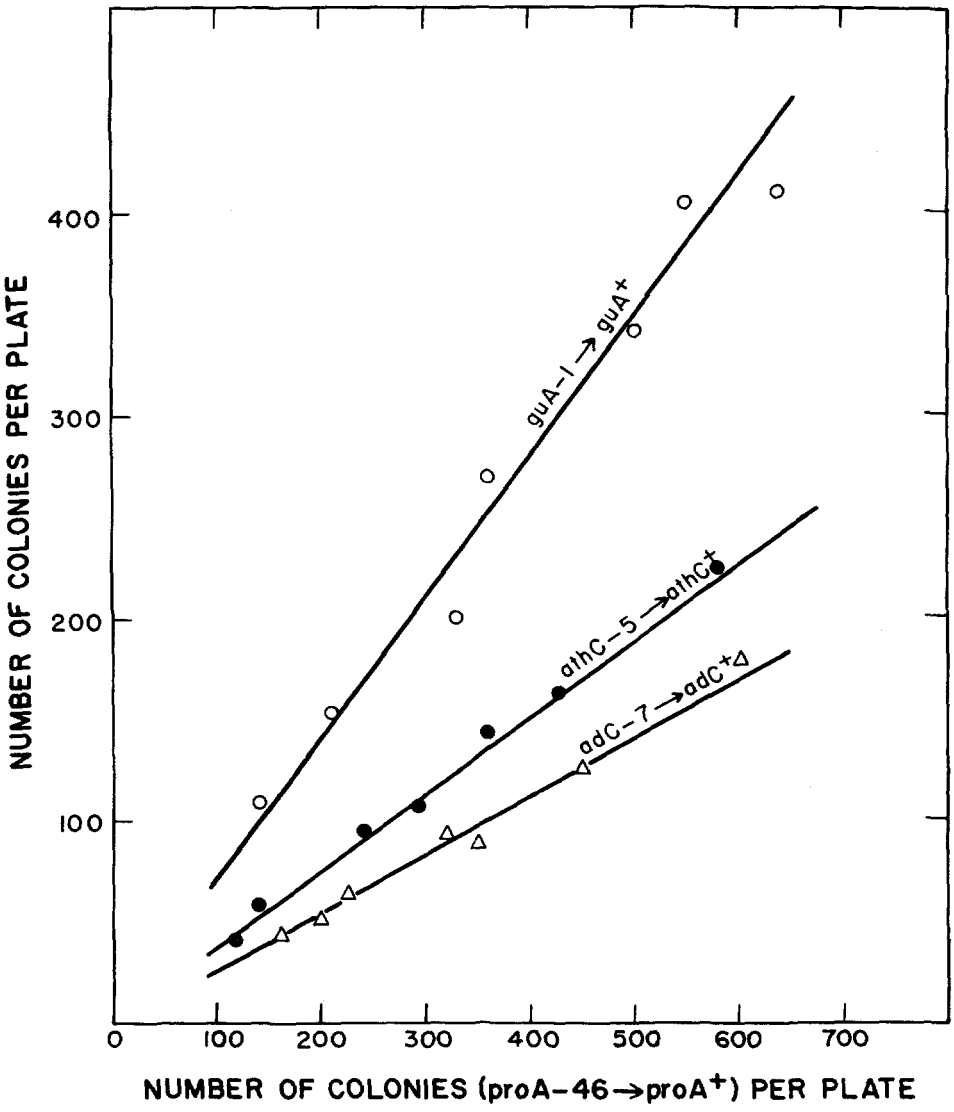

FIGURE 1.—Relationships between frequencies of complete transduction occurring at the *adC–athC–guA* region and the *proA* region. Data were obtained in the following transduction experiments: *guA–1 proA–46* (recip.) × wild type (donor), platings on EMG and EMP media; *athC–5 proA–46* (recip.) × wild type (donor), platings on $EMAB_1$, and EMP; *adC–7 proA–46* (recip.) × wild type (donor), platings on EMA and EMP. To obtain a range of numbers of colonies per plate, the number of bacteria plated was varied from 10^8 to 2×10^8, and the multiplicity of infection from one to ten.

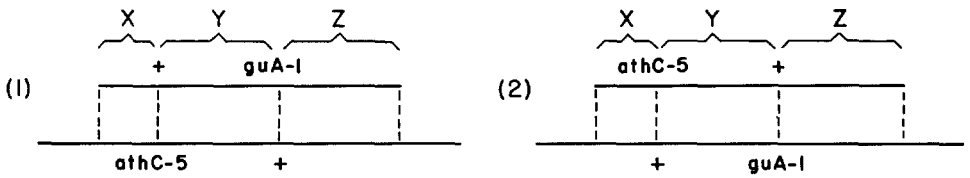

FIGURE 2.—Schematic representation of locations of the genetic markers in the *athC-5–guA-1* reciprocal transductions. (1) and (2) correspond to experiments 1 and 2 in Table 4. Lower line represents the bacterial chromosome, upper line the transducing fragment. X, Y, and Z: relative distances between genetic markers and terminal points on the transducing fragment.

Figure 2; that is, the fragmentation of the donor chromosome does not occur in a random way.

When *athC-5* is infected with phage grown on wild type bacteria, the incorporation of the *athC-5*+ marker into a viable recombinant requires one crossover in the X portion and one in the Y or Z portion, so that the probability of incorporation, in cells which have received the chromosome fragment carrying *athC-5*+, is $X(Y + Z)$. Consequently, the ratio of frequencies(f) of transduction to prototrophy in *athC-5* and in *guA-1* recipients with wild type donor is expressed as $f\ athC/f\ guA = X(Y + Z)/Z(X + Y)$. The relative values of X, Y, and Z may be estimated from one set of the b/c ratios in Table 4, as follows: $Y/Z = 0.93$, and $Y/X = 3.29$; accordingly $X/Y/Z = 0.93/3.05/3.29$; whence $f\ athC/f\ guA = 1/2.23$. Similar calculations, based on the data from further tests of the same kind as those recorded in Table 4, gave ratios of f *athC* to f *guA* in the range of 1/1.6 to 1/2.5. The ratios observed in the experiments described in the next section fell within the range that agreed well with these calculated values. The agreement between predicted and experimental values lends further support to the hypothesis that the ends of transducing fragments are predetermined.

As mentioned above, the order of the loci *adC*, *athC*, and *guA* is *adC–athC–guA*; that is, *adC* lies in the X portion of Figure 2. In experiments with *adC-7* and *athC-5*, donor-type recombinants could be scored only when *adC-7* was the donor. In this experiment the ratio of wild type to donor-type transduction was 6.3 to 1 (Table 3); by an argument similar to that above it is inferred that *adC-7* lies at a point such that its distance from the left end of the transducing fragment (Figure 2) is 1/6.3 of its distance from *athC-5*. The predicted value of $f\ adC/f\ athC$ is 0.15/1, and the experimental one is 0.77/1 (see next section). In this case the experimental value for *adC* is about five times higher than the expected one, although there is still a tendency for the frequency of complete transduction to decrease towards the end of the fragment. This disagreement might be explained by assuming the existence of some mechanism that would increase the probability of incorporation of the marker *adC-7*+. It is possible, however, that the method used for calculating the distances between markers and the ends of fragments was inaccurate.

The results presented in Table 2 for the one-marker (*try*+ or *cysB*+) and two-marker (*try*+ and *cysB*+) classes, in transduction experiments involving various

combinations of *try* and *cysB* as recipients and the wild type as donors, show the same type of differences between the frequencies of the two single-marker classes as has already been described for transduction at the *athC-guA* region. In the case of *tryA-8 cysB-12*, the difference between the one-marker classes may not be significant, since the variation in numbers of prototrophs (two-marker class) on the different types of plates indicates that some complicating factor may have been present.

Relative frequencies of transduction among linked markers: Relative frequencies of complete transductions of *adC-7*, *athC-5*, and *guA-1* markers by phage grown on wild type bacteria were measured in two different ways. (1) The marker *proA-46* was used as a standard, as described above; the results are shown in Figure 1. (2) Strains carrying a single marker—*adC-7*, *athC-5*, or *guA-1*—were infected with equal amounts of phage from the same wild type donor preparation (Table 5). The two sets of data are in agreement, and indicate the following relative frequencies of complete transduction:

$$f\ adC\text{-}7\ :\ f\ athC\text{-}5\ :\ f\ guA\text{-}1 \simeq 1\ :\ 1.3\ :\ 2.5$$

This agreement between results obtained by different methods seems to indicate that the difference in frequencies of complete transduction among these three markers is not due to physiological differences among the recipient cells with respect to adsorption rate, residual growth rate, or the like.

On the other hand, the frequencies of abortive transduction for these markers were similar. Accordingly, the ratios of abortive to complete transduction varied inversely with the frequency of complete transduction (Table 5).

Relative frequencies of transduction among unlinked markers: Since frequencies of transduction of different markers have been known to vary over a wide range (order of 10^{-5} to 10^{-7} per phage), it may be of some interest to compare frequencies of complete transduction and ratios of abortive to complete transduction for several unlinked markers (Table 6). The results are different from those obtained with linked markers; there is no inverse relation between the frequency of complete transduction and the ratio of abortive to complete

TABLE 5

Relative frequencies of abortive and complete transduction of linked markers in three different chromosome regions. Recipient strains whose markers are linked are grouped together. Donor, wild type. Platings on minimal medium. AT/P=number of minute colonies per 4×10^8 phage particles; CT/P=number of large colonies per 4×10^8 phage particles; AT/CT=ratio of abortive to complete transductions

	Recipient							
	adC-7	*athC-5*	*guA-1*	*tryD-10*	*cysB-12*	*tryD10 cysB-12*	*se-1*	*se-5*
AT/P	1,120	1,240	1,030	3,810	3,430	3,070	1,440	1,260
CT/P	81	105	190	346	503	136	160	152
AT/CT	13.7	11.8	5.4	11.0	6.8	22.5	9.0	8.3

TABLE 6

Relative frequencies of transduction among unlinked markers. Donor, wild type; platings on minimal medium. AT/P=numbers of minute colonies per 4 × 10⁸ phage particles; CT/P=numbers of large colonies per 4 × 10⁸ phage particles; AT/CT= ratio of abortive to complete transductions

	Recipient					
	hiD-39	*athA-4*	*proA-46*	*cysA-1*	*meC-50*	*athD-12*
AT/P	18,880	7,160	1,760	1,700	900	480
CT/P	1,988	664	376	188	64	25
AT/CT	9.5	10.8	4.7	9.0	14.0	19.2

transduction among unlinked markers. This will be discussed in the following section.

DISCUSSION

The results presented indicate that the fragments participating in abortive transduction at one region of Salmonella chromosome, which has been closely examined, always carry a restricted but constant group of markers within which simultaneous complete transduction of markers can be demonstrated. Moreover, in any tests made so far, unlinked markers (so inferred from observations of complete transduction) have never been carried jointly by a fragment participating in abortive transduction. These observations suggest that in abortive transduction, as well as in complete transduction, the transfer of genes and not the products of genes is involved. A similar case has been reported by LEDERBERG (1956), who observed the uniform coupling of a locus (for formation of flagella) with another linked locus (for flagellar antigen type) in fragments participating in abortive transduction.

Fragments concerned in complete transduction, in two different regions tested, have been shown to have a nonrandom distribution of markers with relation to the ends of the fragment. Since all, or almost all, markers appear to be readily transducible, it seems probable that the chromosomes of donor bacteria are disrupted into small fragments having a more or less definite size and distribution of markers. Once the chromosome is broken down in this regular way, the fragments produced can be picked up by phage particles and transferred to recipient cells.

Other explanations of the uniformity of the fragments are also possible, however, and cannot be excluded. It is known that in *Escherichia coli* all those particles of lambda phage which are capable of bringing about transduction of the *Gal* loci are defective, lacking a particular region of the phage chromosome (ARBER *et al.* 1957). This suggests the possibility that, in Salmonella transduction also, phage particles can carry only particular regions of the bacterial chromosome, which have an affinity for or genetic homology with particular parts of the phage genome. Some evidence of partial genetic homology between phage PLT-22 and *S. typhimurium* LT-2 has been reported by GAREN and ZINDER (1955).

The hypothesis introduced here, that the ends of transducing fragments are

predeterminate, may explain the following cases. As was mentioned earlier, the *ad-3* mutant was obtained in a single cycle of the penicillin screening procedure (LEDERBERG 1950) but behaves genetically as though it had undergone mutation at each of the loci *adA* and *adB*, although these loci are unlinked as far as can be judged from (complete) transduction experiments (YURA 1956). Similarly, *cys-5*, although obtained in a single cycle of isolation, behaves as a double mutant, *cysA cysC*; and no linkage between the *cysA* and *cysC* loci has been demonstrated by transduction (HOWARTH 1958). Since there is no double mutant, isolated in a single cycle of isolation and requiring two different amino acids, in DR. DEMEREC's collection of bacterial mutants including more than five hundred auxotrophs of *Salmonella typhimurium*, there is almost no chance of obtaining a mutant having two independent mutations at two adenine (or two cystine) loci during a single cycle of penicillin screening. It may, therefore, be suggested that in these mutants a mutation extends over a certain length of chromosome, covering at least a part of each of the loci involved, as has been reported in several other instances in this organism (DEMEREC 1956a; CLOWES 1958). This implies that two functionally related loci *adA* and *adB* (or *cysA* and *cysC*) are closely linked on the chromosome, as has been reported in many other instances by DEMEREC (1956a). The discrepancy between this consideration and the conclusion arrived at by the transduction experiments (absence of linkage) suggests that there is a point, in a region involving *adA* and *adB* (or *cysA* and *cysC*), where breakage of the chromosome prior to transduction usually occurs. In this relation a case reported by CLOWES (1958) may be considered. Two cystine-requiring mutants of *S. typhimurium*, *cys-48* and *cys-50*, classified as *cysD* mutants by phenotype, behaved in transduction tests as though not linked to any other markers of the *cysD* group. It is, of course, difficult to decide whether or not *cys-48* or *-50* belongs to the *cysD* locus on these grounds alone. However, since in Salmonella there is a strong tendency for genes controlling related reactions to be grouped close together on the chromosome (DEMEREC 1956a), this case may be thought of as another example suggesting predeterminate fragmentation of chromosomes.

The differences in frequencies of complete transduction observed among linked markers seem to reflect differences in the distances between the markers and the ends of the fragment. The frequencies of abortive transduction are roughly the same among these linked markers. Unlinked markers show more striking differences in complete-transduction frequencies, which are paralleled, although very roughly, by their frequencies of abortive transduction (see Tables 5 and 6). Thus it appears that the frequency of complete transduction of a given marker is affected not only by the probability of its incorporation but also by the availability of the region containing it. ZINDER (1955) has shown that phage populations obtained at various times after infection of donor cells have different capacities to transduce given markers. This variability presumably reflects differences in the availability of fragments carrying these markers at different times during the course of phage growth, and may indicate that disruption of the donor chromosome occurs in some sequential manner.

SUMMARY

Pairs of markers which can be simultaneously transduced (complete transduction), and which are inferred to be linked, are always (or almost always) present together in the fragments participating in abortive transduction of one or the other marker. Other, unlinked, markers, according to tests made so far, are never carried by a fragment participating in abortive transduction of one marker.

In transduction experiments A^- B^+ (recip.) × A^+ B^- (donor) and the reciprocal A^+ B^- (recip.) × A^- B^+ (donor), the frequencies of complete transduction of the two single markers, A^+ and B^+, are not the same whereas the frequencies of two-marker transduction (A^+B^- or A^-B^+) are about equal in the two reciprocal combinations. These results may be explained by the hypothesis that complete transduction is the consequence of crossing over between the chromosome of the recipient and a transduced fragment of donor chromosome of uniform composition, the probability of incorporation (complete transduction) for any particular marker varying according to its position in relation to the ends of the fragment.

Among linked markers, the frequency of occurrence of complete transduction varies, whereas frequencies of abortive transduction for these markers are similar. Among unlinked markers, the differences in frequency of complete transduction are much greater, and the frequencies of abortive transduction roughly parallel those of complete transduction.

The hypothesis that the chromosomes of donor bacteria are broken up into fragments of predeterminate composition prior to transduction is considered.

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