HIGH NEGATIVE INTERFERENCE OVER SHORT SEGMENTS OF THE GENETIC STRUCTURE OF BACTERIOPHAGE T4[1, 2]

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THROUGH the development of highly specialized selective techniques, it has recently become possible to do genetic experiments which may ultimately permit a quantitative correlation between genetic material and chemical structure. The *r*II mutants of bacteriophage T4 are especially well suited for such studies, since there is a large number of genetically recombinable markers which fall into two distinct functional genes on the linkage map of this organism (BENZER 1955, 1957). If attempts to relate "genetic length" to "molecular length" are to be successful, and if the markers are to be profitably employed in radiological genetics studies (DOERMANN and CHASE 1958; KRIEG 1957), it is of utmost importance to establish unambiguously whether map linearity exists even within the individual functional units.

During the course of crosses designed to test linear order within functional units, an unexpected result was encountered. A high degree of negative interference, or a positive correlation of recombination events, was observed and was found to increase as the map intervals under study were decreased. Similar observations have been reported among h^+ mutants in phage (STREISINGER and FRANKLIN 1956) and adenine-requiring mutants in *Aspergillus nidulans* (PRITCHARD 1955). At the present time, the *r*II mutants of phage provide most favorable material for a systematic and thorough investigation of this phenomenon. It is possible to decide conclusively that the aberrantly high coefficients of coincidence obtained in these experiments are the result of multiple recombinations in short segments of the genetic structure. They cannot be accounted for by the hypothesis of gene conversion.

MATERIALS AND METHODS

Phage and bacterial strains: The *Escherichia coli* bacteriophage T4, specifically the variety now called T4B (the strain used by BENZER 1955, 1957), was used in all experiments reported in this paper. The authors are indebted to DR. SEYMOUR BENZER for kindly supplying the wild type phage and the ten *r*II mutants which were used in this study. The *r*II mutants used are distributed over nearly

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the entire length of the two functionally distinguishable segments (cistrons, see BENZER 1957) of the *r*II region thus far identified. Except for two mutants, all markers satisfy two technical criteria: (1) they have low transmission coefficients, and (2) they have finite reversion indices (BENZER 1955) of less than 10^{-6}. A reversion index of 10^{-6}, or less, assures that the stock will not contribute a background of r^+ in the crosses, while a *finite* reversion rate eliminates from use mutants which appear to be long "deletions" (BENZER 1955, 1957) and to exhibit peculiar genetic properties (DOERMANN and CHASE 1958). The two exceptions, r_{145} and r_{168}, are "stable": that is, reversions to r^+ have not been observed in samples of up to 10^9 particles, but according to BENZER (1957) neither seems to show a mapping anomaly, and by cross-reactivation (CR) tests, r_{145} is indistinguishable from the other mutants used in this study (DOERMANN and CHASE 1958).

The bacterial strains are all *E. coli*. Strains B and S/6 are those described by DOERMANN and HILL (1953). B was used as the host in all crosses, and S/6 as the host in the preparation of phage stocks and for assay of the total yields from crosses. Strain K12(λl), obtained from DR. DAVID R. KRIEG, was used for the selective assay of r^+. Streptomycin resistant strains B/s, obtained from DR. DOROTHY FRASER, and K12(λ)/s, obtained from DR. S. E. LURIA, were used in the replica-plating technique (described below) to isolate double-*r*II phages.

Media: "Plain" broth was used as the medium for crosses and for the growth of all bacterial cultures except S/6. It contains 8 gm Difco Nutrient Broth and 5 gm NaCl per liter demineralized water.

HERSHEY's nutrient broth was used for the growth of cultures of S/6 and for the preparation of stocks. It contains, in addition to the ingredients in plain broth, 5 gm Bacto-peptone and 1 gm glucose per liter.

The bottom-layer agar for plates is composed of 12 gm Bacto-agar, 13 gm Bacto-tryptone, 8 gm NaCl, 2 gm sodium citrate dihydrate, and 1.3 gm glucose per liter demineralized water. The top layer contains 6 gm Bacto-agar, 10 gm Bacto-tryptone, 8 gm NaCl, 2 gm sodium citrate dihydrate, and 3 gm glucose per liter. Freshly poured plates were not incubated before use but were left overnight at room temperature, since it was found that the efficiency-of-plating (e.o.p.) of r^+ on K12(λl) is lower on dry plates.

Preparation of stocks: Phage stocks were prepared by essentially the same procedure described by DOERMANN and HILL (1953). All stocks were made using S/6 as the host cell, because it was discovered, early in the course of the experiments, that the use of stocks made on K12S [a host which is nonselective for r^+ (BENZER 1955)] results in up to 80 percent loss of infected bacteria at multiplicities as low as four per bacterium, and up to 95 percent loss of infected cells at multiplicities of 15, which is the average total multiplicity desired in crosses to map markers. This phenomenon is not observed when S/6-grown phages are used at comparable, and even higher multiplicities, many of which exceeded 15 per cell in these crosses. Stocks were made at 30°C (instead of 37°C), since

growth at the lower temperature proved to result in both appreciably higher titers of phage and lower reversion indices.

Double- and multiple-*r*II strains were isolated by a replica-plating technique especially developed for this purpose. The basic method, originally devised by LEDERBERG and LEDERBERG (1952) for bacterial colonies, was modified for use with phage plaques by BAYLOR, HURST, ALLEN, and BERTANI (1957). Further modifications of the procedure have made it possible to distinguish T4 plaques of different single- and multiple-*r*II composition in the progeny from crosses and to isolate the double- and multiple-mutant genotypes. The discovery by BENZER (1955) that T4r^+ can grow in K12(λ) but that *r*II mutants cannot, and the selective techniques developed by him, form the basis of the method. In crosses between nonallelic *r*II's the recombinant *rr* is phenotypically indistinguishable from the two parents when the progeny of the cross are plated on B or S/6. The new procedure involves replicating from B plates to K12(λ) plates, and is based on the fact that two nonallelic *r*II's, when both infect the same bacterium, will, under certain conditions, multiply and produce r^+ recombinants. The r^+ from an infected cell of this type will multiply and produce a plaque on K12(λ). On the basis of this information, the following procedure was devised: A "parent" plate is seeded with B and approximately thirty phages from a mixture (for example, the progeny from a cross $r_a \times r_b$, which contains r_a, r_b, r^+, and $r_a r_b$). This parent plate is incubated 18 to 24 hours at 37°C. For each parent plate to be tested, two "replica" plates are prepared. To the top layers of both are added two drops of a solution (5 mg/ml) of dihydrostreptomycin sulfate [to prevent growth of B which will be transferred from the parent plate (BERTANI 1951)] and three drops of a one to 50 mixture of B/s and K12(λ)/s. In addition, one of the replica plates is seeded with 10^8 r_a phages and the other with 10^8 r_b phages. All three plates are immediately refrigerated for about two hours (or more) to harden the top layer and prevent peeling during the transfer process. After this interval, a sterile *silk* velvet is stamped lightly onto the parent plate and transferred onto one replica plate. A second piece of velvet must be used to print onto the second replica plate, in order to prevent transfer of the seeding phages from one replica plate to the second. The replica plates are incubated overnight and examined for spots. Phages from an r_a plaque on the parent plate, when transferred to the r_a replica plate, cannot form any r^+ recombinants; but on the r_b plate they can, since they infect bacteria which are already infected with r_b. A large, clear spot will be formed on the r_b plate, but not on the r_a plate. In this manner, all four genotypes, three of which are indistinguishable on the parent plate, can be differentiated: the r^+ spots on both replica plates; r_a spots on the r_b plate only; r_b spots on the r_a plate only; and $r_a r_b$ spots on neither replica plate. In a quantitative test of the replica-plating method, with an artificial mixture of the three phages, r_{205}, r_{271}, and $r_{205} r_{271}$, 835 plaques were tested by replication. The results were in statistical agreement with the numbers expected.

To isolate the double-*r*, one finds on the parent plate a plaque which does not spot on either replica plate. The phage from such a plaque is suspended in broth,

replated, and then retested by replication. If it proves to be a correct identification, a stock is made and is then further tested by backcross to each of the component single *r*'s. The method permits the selection of double-*r*II's in a manner which is practical, and thus makes it possible to obtain double mutants composed of very closely linked markers.

Experimental techniques: The procedure for genetic crosses has been somewhat modified from the methods described by DOERMANN and HILL (1953) and by HERSHEY and ROTMAN (1949) in order that it should conform as nearly as possible to the methods used in the CR experiments with the same markers (DOERMANN and CHASE 1958). Aerated overnight cultures of bacteria (B) are diluted 1 to 1000 into fresh broth and aerated 2 hours and 20 minutes at 30°C, then concentrated by centrifugation to a final titer of 2×10^8 per ml, and put into the adsorption tube, with aeration. KCN (M/500 in the bacterial suspension) is added 2.5 minutes prior to infection of the cells with phage. An equal volume of the approximate phage mixture containing 1.4×10^9 of each parent per ml (a multiplicity of 7 of each type) is added to the adsorption tube at time (t) = 0. At t = 3.5, an aliquot is taken for an assay of unadsorbed phage; and at t = 4, anti-T4 serum is added to neutralize the remaining unadsorbed virus. Addition of serum to the adsorption tube, rather than dilution into a tube containing serum, makes it possible to introduce another dilution step away from the serum, and thus to assay the yield of r^+ at a higher total-yield concentration than was possible by the DOERMANN and HILL (1953) method. At t = 9, the growth tube dilutions are made and an aliquot plated for the count of infected bacteria. At t = 90 (after complete lysis), the progeny are plated (at appropriate dilutions) for the total yield on S/6 and for the r^+-recombinant yield on K12(λl). It was learned during the course of the experiments that the adsorption mixture (containing KCN and antiphage serum) must be diluted to a concentration of 2×10^{-4} or lower before the end of the latent period. Postlysis platings from tubes which originally contained higher concentrations of infected cells showed recombination values which were twice those obtained by plating from greater dilutions. The interpretation of this observation remains obscure.

Phage assays were made by the usual agar-layer technique described by HERSHEY and ROTMAN (1949). Plating bacteria were always overnight, aerated broth cultures grown at 30°C. Bacterial assays were made by spreading 0.05 ml on the plate. All plates were incubated 18 to 24 hours at 37°C.

EXPERIMENTAL RESULTS

Calculation of the data: As discussed in detail by DOERMANN and HILL (1953), both the relative multiplicity of the two parents and the total multiplicity of phages per bacterium may affect the estimate of the recombination value given by a cross. Since the allele ratios in the yield are not easily measured, multiplicities were determined from the total adsorbed phage distributed according to the ratio of *input* titers. Experiments were rejected when the ratio of parental multi-

plicities was less than 0.67, or when the average multiplicity of either parent was less than three. Of the 275 individual experiments included in this study, 34 were discarded because they did not meet these criteria. The experiments which will be discussed are, therefore, all of the kind which give maximum and comparable recombination values. The number of experiments per cross ranged from one to 22; all crosses, excepting those of the type $r_a r_b \times r_c$ (to be discussed below), have been done at least twice with acceptable multiplicities.

The recombination value is determined by multiplying the titer of r^+ by two (assuming that the *rr* recombinant not scored is equally frequent), dividing the product by the total-phage titer, and multiplying the frequency thus obtained by 100. Concurrent with most of the experiments, a control of the e.o.p. of r^+ on K12(λl) and *r*II on S/6 was done, using the count on B as a standard. The ratio of the e.o.p. on K12(λl) to the e.o.p. on S/6 in 40 comparisons averaged 0.79, with 95 percent confidence limits of 0.74 and 0.84 based on the statistics of a normal distribution. Thus, the determinations of r^+, which are made from K12(λl) platings, are, on the average, only 79 percent as efficient as the estimates of the total phage populations, which are made from S/6 platings. All the data have been corrected for this error by dividing r^+ frequencies by 0.79.

In the analysis of results where multiple recombinations are involved, the observed values (O) are compared with calculated values (C). The latter are the products of the individual recombination values. The calculation of O/C for each type of cross is detailed with the appropriate tabulation of the data.

In crosses where very low frequencies of recombinants are expected (e.g., crosses requiring double or triple recombination events), it is necessary to be certain that the observed r^+ progeny are really the result of the type of exchange under consideration and not the consequence of lower order recombination events. The latter could arise from matings involving single-*r* contaminants in the *rr* stocks, presumably due to reversion at one locus or the other. A maximum estimate of the r^+ frequency for each locus of the *rr* stock has been made. It is based on the number of r^+ recovered in the backcrosses of the stock. From the r^+ frequency in the backcross yield is subtracted one half the reversion index of the single-*r* parental stock (because the stock represents only one of the two parents in the backcross). The remaining r^+ are the most which could have arisen from recombination between the backcross parent *r* and the concealed single-*r* particles contributed by the presumptive *rr* stock. Dividing this value by the frequency of r^+ observed in the two-factor cross $r \times r$ gives a maximum estimate of the frequency of phage with r^+ at one locus.

For all crosses in which O/C is greater than one, the data have been analyzed to determine the percent of the observed recombinants which could have arisen from matings involving such single-*r*'s in the *rr* stocks. The calculation is done independently for each *r* locus. The maximum frequency of recombinants which could derive from this source is the sum of the frequencies calculated for each locus. An approximation is made by multiplying the maximum estimate of the frequency of *r* at a locus by the frequency of recombination which is obtained

from the appropriate cross in which the locus under analysis is wild type. Corrections for input multiplicity and mating kinetics (VISCONTI and DELBRÜCK 1953) roughly cancel one another. If used, they reduce slightly the upper limit of lower order recombinants. Since the greatest correction indicated by these tests changes the observed r^+ frequency by only 2.0 percent, such correction of the data was not made.

Crosses: Since the experiments to be discussed are not easily visualized until one is familiar with the genetic material involved, an artificial symbolism will be employed. All crosses to be discussed involve *r*II markers, and five types of experiments (diagrammed in Figure 1) have been done with various combinations of single- and multiple-*r* parents. The *r* markers will be given the subscripts a, b, c, d, *etc.*, the alphabetical order indicating their order from left to right on the map (Figure 2). When no symbol is given, the wild type allele (r^+) is indicated. Thus, a cross of the type $r_{168}r_{320} \times r_{271}$, where r_{271} lies outside the interval

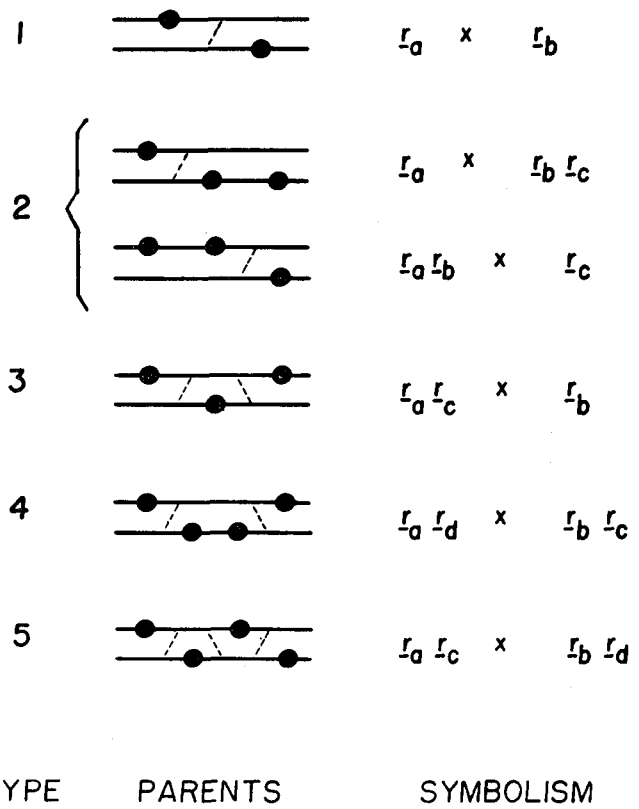

FIGURE 1.—Types of crosses between the *r*II mutants in T4B included in this study. Each cross is represented by two horizontal lines which indicate the linkage structures of the two parents. The solid circles indicate the positions of *r* mutants, and the dotted lines between the linkage structures represent the recombination events which are required to produce the only recognizable recombinant, namely wild type. The accompanying symbolism is used in the text.

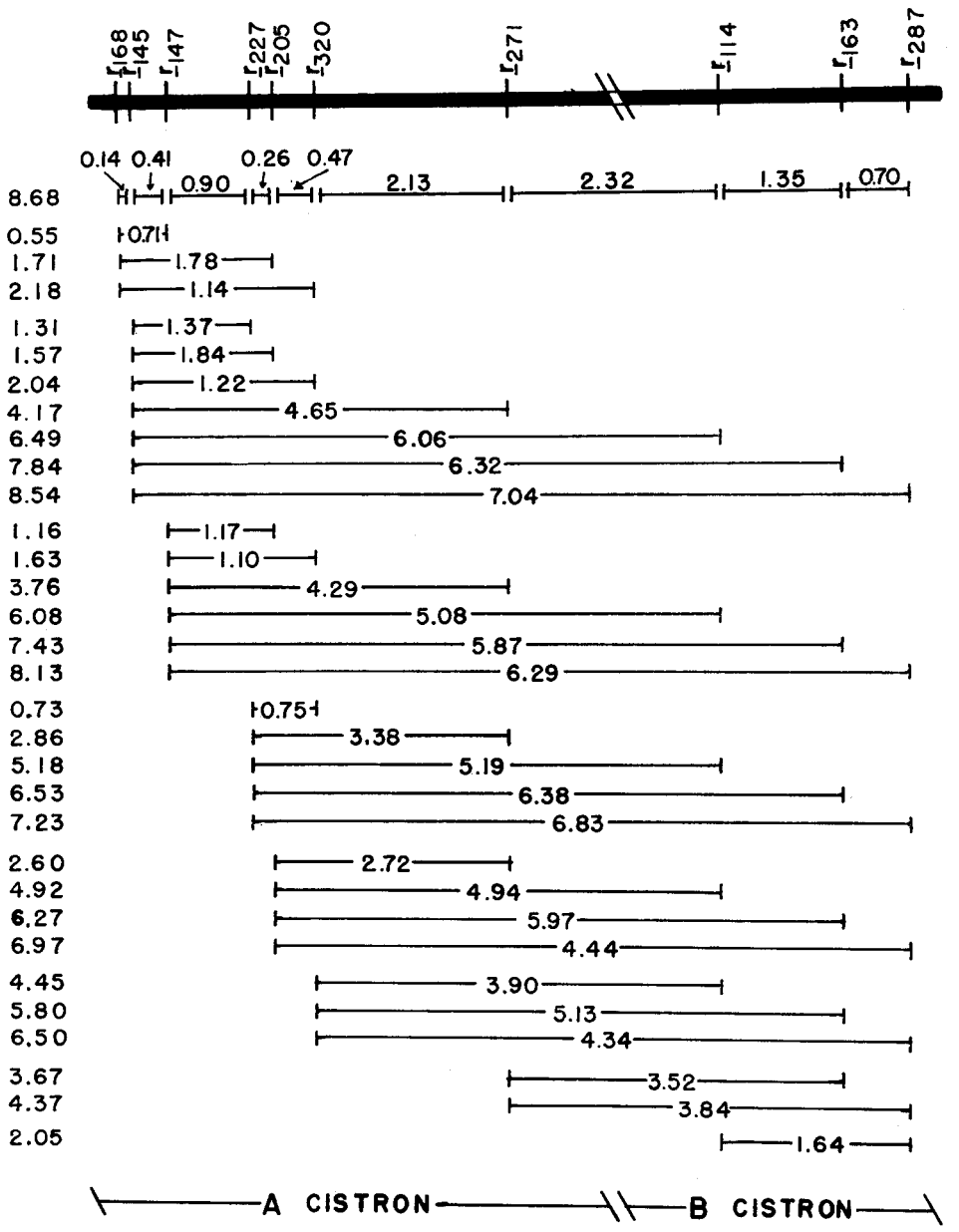

FIGURE 2.—Map of the *r*II region of T4B. The average recombination values are shown for all two-factor crosses which meet the selective criteria. The numbers in the left-hand column represent the sums of the shortest map intervals and are to be compared with the corresponding recombination values for more distant markers given below the map.

r_{168} to r_{320}, will be designated as $r_a r_b \times r_c$; or, another example, the crosses of the type $r_{168}r_{271} \times r_{320}r_{163}$, in which the left-to-right order is r_{168}, r_{320}, r_{271}, r_{163}, will be designated by $r_a r_c \times r_b r_d$. The wild type is the only phenotypically distinguishable recombinant in the progeny. Thus, in this symbolism (Figure 1), crosses of types 1 ($r_a \times r_b$) and 2 ($r_a r_b \times r_c$ or $r_a \times r_b r_c$) require one recombination event; crosses of types 3 ($r_a r_c \times r_b$) and 4 ($r_a r_d \times r_b r_c$) require two recombination events; and crosses of type 5 ($r_a r_c \times r_b r_d$) need three recombination events to yield the recognizable wild type.

From the results of 110 two-factor ($r_a \times r_b$) experiments, the map of the *r*II region of T4B has been constructed (Figure 2). When the markers are arranged in the order given, the various intervals are roughly additive. In 12 of the 31 comparisons, the value obtained by adding the component intervals is less than that obtained from the cross between the two terminal markers for the overall interval being considered; in 19 cases, it is greater. The 31 comparisons are not, of course, independent of one another, and consequently additivity cannot be evaluated precisely. It is also difficult to assess the error introduced into additivity considerations by nonrecombinant heterozygotes in intercistron crosses. Phages of that type usually make a plaque on K12(λ) which would be scored as a recombinant (EDGAR 1958a).

Three-factor crosses of both types were done originally to check the map order obtained from the two-factor crosses. In these crosses, the phenomenon of high negative interference was first noticed. The data from 52 three-factor experiments of the type $r_a r_c \times r_b$ (requiring a double recombination event to produce r^+) are presented in Table 1 and Figure 3a. The crosses are arranged according to the recombination value for the markers r_a and r_c. The high order of negative interference (high O/C) apparent in these crosses indicates a positive correlation between recombinations in adjacent regions. The value of O/C is inversely related to the map interval between the terminal markers r_a and r_c. It is fairly independent of the length of that interval in the range from 2.7 to 7.0 units (O/C = 4.6 to 7.8 in 19 crosses). For intervals less than 2.7 (1.8 to 0.7 units), the factor rises sharply (O/C = 14 to 31 in six crosses), showing that the phenomenon is most pronounced over very short regions of the linkage structure.

The observation of high negative interference should be apparent also in three-factor crosses of the type $r_a r_b \times r_c$ (or $r_a \times r_b r_c$). In $r_a r_b \times r_c$ crosses, wild type is produced by a single recombination event between r_b and r_c, and with no accompanying single or odd number of recombinations permitted in the region between r_a and r_b. The positive correlation between recombinations in adjacent intervals, however, predicts that the observed frequency of wild type will be reduced from what is expected on the basis of the two-factor cross $r_b \times r_c$. In the three-factor experiment, the recombination event between r_b and r_c will frequently be accompanied by a second exchange between r_a and r_b, and this will convert the r^+ into an r. The data from 21 three-factor crosses of this type are summarized in Table 2. They are arranged according to the recombination values between r_a and r_b. All but one of the crosses show a smaller proportion of r^+

TABLE 1

Three-factor T4BrII crosses in which two recombination events are required to produce a wild type recombinant
$[r_a r_c \times r_b]$

Cross	Recombination values for terminal markers	Recombinants (percent)			
		Calculated (C)*	Observed (O)	Maximum correction†	O/C
$r_{145}r_{287} \times r_{147}$	7.0	0.025	0.18	0.000069	7.2
$r_{145}r_{287} \times r_{227}$	"	0.093	0.45	0.00010	4.8
$r_{145}r_{287} \times r_{205}$	"	0.082	0.43	0.00014	5.2
$r_{145}r_{287} \times r_{320}$	"	0.053	0.28	0.00010	5.3
$r_{145}r_{287} \times r_{271}$	"	0.18	0.88	0.00030	4.9
$r_{145}r_{287} \times r_{114}$	"	0.091	0.53	0.00038	5.4
$r_{145}r_{287} \times r_{163}$	"	0.044	0.30	0.00038	6.9
$r_{227}r_{163} \times r_{205}$	6.4	0.015	0.070	0.000024	4.6
$r_{147}r_{287} \times r_{227}$	6.3	0.060	0.31	0.00018	5.1
$r_{147}r_{287} \times r_{205}$	"	0.052	0.34	0.00022	6.5
$r_{320}r_{163} \times r_{271}$	5.1	0.075	0.52	0.000027	6.9
$r_{320}r_{163} \times r_{114}$	"	0.052	0.39	0.000020	7.5
$r_{205}r_{287} \times r_{320}$	4.4	0.020	0.093	0.00017	4.6
$r_{205}r_{287} \times r_{271}$	"	0.10	0.55	0.00084	5.3
$r_{205}r_{287} \times r_{114}$	"	0.081	0.49	0.0015	6.0
$r_{205}r_{287} \times r_{163}$	"	0.042	0.26	0.0018	6.3
$r_{320}r_{114} \times r_{271}$	3.9	0.049	0.39	0.000060	7.8
$r_{271}r_{163} \times r_{114}$	3.5	0.031	0.23	0.000059	7.2
$r_{205}r_{271} \times r_{320}$	2.7	0.010	0.065	0.00021	6.5
$r_{145}r_{205} \times r_{147}$	1.8	0.0047	0.079	0.000042	17.
$r_{145}r_{205} \times r_{227}$	"	0.0035	0.068	0.000028	20.
$r_{145}r_{227} \times r_{147}$	1.4	0.0036	0.050	0.000023	14.
$r_{147}r_{205} \times r_{227}$	1.2	0.0022	0.039	0.000050	17.
$r_{227}r_{320} \times r_{205}$	0.75	0.0012	0.020	0.000019	16.
$r_{168}r_{147} \times r_{145}$	0.71	0.00057	0.018	0.000025	31.

* The calculated frequency, expressed in percent, is the product of the recobination values from the crosses $r_a \times r_b$ and $r_b \times r_c$, assuming the coefficient of coincidence to be one.

† The correction which represents the calculation of the maximum frequency of recombinants which may come from r contaminants in the rr stock is done in the following manner: For locus r_a, it is the product of the frequency of r_a^+ in the $r_a r_c$ stock times the frequency of recombinants in the cross $r_b \times r_c$. For the locus r_c, the corresponding calculation is made. The maximum frequency of r^+ from this source is the sum of the two calculated maximum frequencies and is expressed in percent for direct comparison with the observed value.

than expected on the basis of the two-factor mapping experiments. There is some indication that O/C is inversely related to the map value between r_a and r_b, but this cannot be demonstrated statistically. The mean of O/C is 0.76 with a standard deviation of 0.16. The standard error of the mean is 0.035, which shows that, with 99 percent confidence limits, the mean value falls significantly below one. Therefore, these crosses, also, indicate a positive correlation between recombinations in adjacent regions.

Four-factor crosses which require a double recombination event should also produce an unexpectedly high yield of wild type. Furthermore, crosses of the type

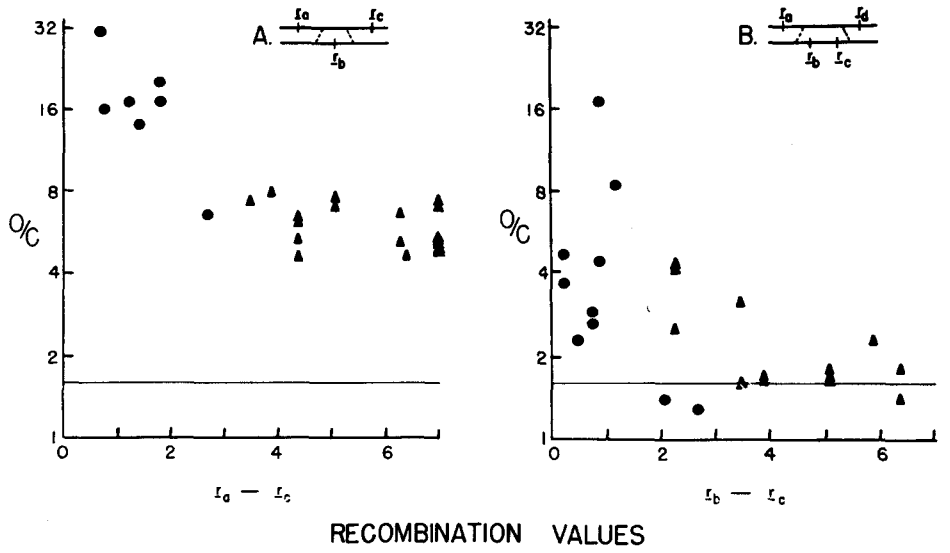

FIGURE 3.—Relationship of high negative interference (O/C) to map distance. Data are plotted from three- and four-factor crosses requiring double recombinations to yield wild type. The circles represent values from intracistron crosses and the triangles from intercistron crosses. The horizontal lines at 1.6 represent the factor expected from mating kinetics (STAHL personal communication).

A. Data from Table 1.

B. Data from Table 3.

$r_a r_d \times r_b r_c$ can, in principle, measure the length of the map interval over which the correlation may be observed. It has been shown above that O/C increases as the intervals studied are decreased. Extrapolation of that observation predicts that O/C should decrease in the crosses $r_a r_d \times r_b r_c$ as the r_b *to* r_c interval becomes longer. Enlarging the r_b to r_c value separates more and more the two regions in which recombination must occur to produce a wild type particle. Data from 48 four-factor experiments of this type are given in Table 3 and Figure 3b. The crosses are arranged according to the recombination value from the cross $r_b \times r_c$. While the results here are not so clear-cut as in the three-factor experiments, at least qualitative agreement with prediction is indicated. Theoretically, a negative interference introduced by mating kinetics (VISCONTI and DELBRÜCK 1953) would give O/C equal to 1.6 (STAHL personal communication). Figure 3b indicates fair agreement with this expectation, except at shorter map intervals, where the anticipated excess is observed. The data are obviously too variable for an estimation of the length of the interval over which correlated recombination events are observable.

It may be added here that, in locating a point on the abscissa in Figures 3a and 3b, an error may be introduced when the decisive interval includes the intercistron region. EDGAR (1958a) has shown that nonrecombinant heterozygotes have

TABLE 2

Three-factor T4BrII crosses in which a single recombination event is required to produce a wild type recombinant [$r_a r_b \times r_c$]

Cross	Recombination values for markers of *rr* parent	Recombinants (percent)		
		Calculated (C)*	Observed (O)	O/C
$r_{147}r_{287} \times r_{145}$	6.3	0.38	0.25	0.67
$r_{320}r_{163} \times r_{205}$	5.1	0.45	0.30	0.67
$r_{320}r_{163} \times r_{287}$	"	0.66	0.45	0.68
$r_{205}r_{287} \times r_{147}$	4.4	1.1	0.79	0.70
$r_{320}r_{114} \times r_{205}$	3.9	0.45	0.26	0.58
$r_{271}r_{163} \times r_{205}$	3.5	2.7	1.8	0.69
$r_{271}r_{163} \times r_{320}$	"	2.1	1.3	0.65
$r_{271}r_{163} \times r_{287}$	"	0.67	0.52	0.77
$r_{205}r_{271} \times r_{287}$	2.7	3.7	2.8	0.75
$r_{271}r_{114} \times r_{205}$	2.3	2.7	2.4	0.88
$r_{271}r_{114} \times r_{320}$	"	2.1	1.9	0.90
$r_{271}r_{114} \times r_{163}$	"	1.3	1.2	0.92
$r_{271}r_{114} \times r_{287}$	"	1.6	1.9	1.2
$r_{320}r_{271} \times r_{205}$	2.1	0.46	0.34	0.75
$r_{320}r_{271} \times r_{227}$	"	0.73	0.59	0.80
$r_{145}r_{205} \times r_{320}$	1.8	0.46	0.27	0.58
$r_{145}r_{227} \times r_{168}$	1.4	0.14	0.12	0.85
$r_{147}r_{227} \times r_{145}$	0.90	0.40	0.34	0.84
$r_{147}r_{227} \times r_{205}$	"	0.25	0.20	0.78
$r_{227}r_{320} \times r_{145}$	0.75	1.4	0.59	0.44
$r_{227}r_{205} \times r_{320}$	0.26	0.47	0.41	0.88

*The calculated frequency, expressed in percent, is the recombination value obtained in the cross $r_b \times r_c$ multiplied by one minus the recombination value in the cross $r_a \times r_b$.

a high probability, on K12(λ), of making plaques, which would here be scored as recombinants. The over-all effect is to exaggerate the length of the intercistron region, especially where the adjacent markers involved are close together.

The final series of crosses to be considered is composed of four-factor crosses, $r_a r_c \times r_b r_d$, which require three recombination events to produce a wild-type phage particle. Each of five such crosses has been made twice. The averaged data are presented in Table 4. The O/C values found in such crosses are uniformly greater than in comparable three-factor crosses. Further analysis of the data (Table 5) shows that the positive correlation between two recombination events may be extended to at least three. After correction for the negative interference in two of the three regions concerned, any remaining correlation in recombination events has been called "residual correlation." These factors range from 2.9 to 14.6, well above the factor 2.1 which is presumably the maximum anticipated from mating kinetics. (STAHL personal communication). Therefore, not only double, but also higher-order exchanges occur over a small area of the linkage structure in unexpectedly high frequency.

DISCUSSION

Linearity and additivity at the level of the cistron: The experiments presented in this paper were originally undertaken because of the importance of investigating whether mutants in a given cistron can be arranged in a linear order. While BENZER's (1955, 1957) experiments suggest that the *r*II mutants fall in linear order on the linkage structure, his conclusions are based on the results of two-factor experiments. In the present paper, also, it may be seen (Figure 2) that, if the loci are arranged in the order shown, the values obtained from two-factor crosses between adjacent markers, within a set of three, are generally smaller

TABLE 3

Four-factor T4BrII crosses in which two recombination events are required to produce a wild type recombinant

$[r_a r_d \times r_b r_c]$

Cross	Recombination values for inside markers	Recombinants (percent)			
		Calculated (C)*	Observed (O)	Maximum correction†	O/C
$r_{145}r_{287} \times r_{227}r_{163}$	6.4	0.0090	0.016	0.000071	1.8
$r_{147}r_{287} \times r_{227}r_{163}$	"	0.0058	0.0079	0.00014	1.4
$r_{145}r_{287} \times r_{147}r_{163}$	5.9	0.0027	0.0062	0.000026	2.3
$r_{145}r_{287} \times r_{320}r_{163}$	5.1	0.0081	0.014	0.000063	1.7
$r_{147}r_{287} \times r_{320}r_{163}$	"	0.0073	0.013	0.00017	1.7
$r_{205}r_{287} \times r_{320}r_{163}$	"	0.0031	0.0055	0.00011	1.8
$r_{145}r_{287} \times r_{320}r_{114}$	3.9	0.019	0.032	0.000074	1.7
$r_{205}r_{287} \times r_{320}r_{114}$	"	0.0073	0.013	0.00012	1.7
$r_{145}r_{287} \times r_{271}r_{163}$	3.5	0.031	0.051	0.00023	1.6
$r_{205}r_{287} \times r_{271}r_{163}$	"	0.018	0.057	0.00063	3.1
$r_{145}r_{287} \times r_{205}r_{271}$	2.7	0.069	0.086	0.00019	1.3
$r_{145}r_{287} \times r_{271}r_{114}$	2.3	0.074	0.19	0.00030	2.5
$r_{205}r_{287} \times r_{271}r_{114}$	"	0.043	0.18	0.00069	4.1
$r_{320}r_{163} \times r_{271}r_{114}$	"	0.028	0.12	0.000065	4.3
$r_{145}r_{287} \times r_{320}r_{271}$	2.1	0.046	0.064	0.000099	1.4
$r_{145}r_{287} \times r_{147}r_{205}$	1.2	0.018	0.15	0.000068	8.4
$r_{145}r_{287} \times r_{147}r_{227}$	0.90	0.028	0.12	0.000067	4.4
$r_{145}r_{205} \times r_{147}r_{227}$	"	0.0010	0.017	0.000013	17.
$r_{145}r_{287} \times r_{227}r_{320}$	0.75	0.059	0.17	0.00011	2.9
$r_{145}r_{287} \times r_{227}r_{205}r_{320}$	"	0.059	0.15	0.00013	2.6
$r_{145}r_{287} \times r_{205}r_{320}$	0.47	0.079	0.18	0.00017	2.3
$r_{145}r_{287} \times r_{227}r_{205}$	0.26	0.061	0.29	0.00017	4.7
$r_{147}r_{163} \times r_{227}r_{205}$	"	0.053	0.19	0.000055	3.7

* The calculated frequency, expressed as percent, is the product of the recombination frequencies from the crosses $r_a \times r_b$ and $r_c \times r_d$ multiplied by one minus the recombination frequency for $r_b \times r_c$.

† The correction which represents the calculation of the maximum frequency of recombinants which may come from *r* contaminants in the *rr* stocks is done in the following manner: For the locus r_a, it is the product of the maximum estimate of the frequency of r_a^+ in the $r_a r_d$ stock times 0.76 of the frequency obtained from the cross $r_c \times r_d$. (0.76 is the mean O/C obtained from crosses of the type $r_b r_c \times r_d$.) For the locus r_d, a corresponding calculation is made. For the locus r_b, it is the product of the maximum estimate of the frequency of r_b^+ in the $r_b r_c$ stock times the recombination frequency from the cross $r_a r_d \times r_c$. For the locus r_c, a corresponding calculation is made. The maximum frequency of r^+ from this source is, therefore, the sum of the four upper limits which have been calculated, and is expressed in percent for direct comparison with the observed value.

TABLE 4

Four-factor T4BrII crosses in which three recombination events are required to produce a wild type recombinant

$[r_a r_c \times r_b r_d]$

Cross	Recombination value for inside markers	Recombinants (percent)			
		Calculated (C)*	Observed (O)	Maximum correction†	O/C
$r_{320}r_{114} \times r_{271}r_{163}$	2.3	0.00066	0.020	0.000061	31.
$r_{145}r_{205} \times r_{147}r_{287}$	1.2	0.00021	0.010	0.000071	50.
$r_{145}r_{227} \times r_{147}r_{205}$	0.90	0.0000098	0.0019	0.000020	198.
$r_{145}r_{227} \times r_{147}r_{287}$	"	0.00024	0.011	0.00011	46.
$r_{147}r_{205} \times r_{227}r_{163}$	0.26	0.00013	0.0071	0.000026	53.

* The calculated frequency, expressed in percent, is the product of the recombination frequencies from the crosses $r_a \times r_b$, $r_b \times r_c$, and $r_c \times r_d$, assuming the coefficient of coincidence to be one.

† The correction which represents the calculation of the maximum frequency of recombinants which may come from r contaminants in the rr stocks is done in the following manner: For the locus r_a, it is the product of the maximum estimate of the frequency of r_a^+ in the $r_a r_c$ stock times the recombination frequency in the cross $r_b r_d \times r_c$. For the locus r_d, a corresponding calculation is made. For the locus r_b, it is the product of the maximum estimate of the frequency of r_b^+ in the $r_b r_d$ stock times 0.76 of the recombination frequency from the cross $r_c \times r_d$ (0.76 is the mean O/C obtained from crosses of the type $r_a r_c \times r_d$). For the locus r_c, a parallel calculation is made. The maximum frequency of r^+ from this source is, therefore, the sum of these four upper limits, and is expressed in percent for direct comparison with the observed value.

than those obtained from crosses between nonadjacent markers. A stronger argument than this, however, comes from the results of three-factor crosses (Tables 1 and 2), which are the conventional genetic test for linearity. The order of the loci as shown in Figure 2 gives the most reasonable coefficients of coincidence (O/C) for all the three-factor tests done, even those in which the three markers are very close together. Linear order in the cistron is, therefore, firmly established.

It seems worthwhile to discuss here the calculations made by BENZER (1957) in his attempt to correlate quantitatively the number of genetic map units with the number of nucleotide pairs in the DNA molecule. His experiments indicated that map intervals, as measured by two-factor crosses, are not additive; that is, when he summed the shortest individual map distances between *r*II markers, he obtained a value which was several fold greater than the value obtained from crosses between terminal markers of the *r*II region. To correct for such discrepancies, he multiplied the total map length by four. The data in Figure 2 indicate that BENZER's factor is far too high.

One reason for apparent lack of additivity, which applies to either set of data, is that intercistronic crosses give recombination values [if measured by plating on K12(λ)] which are higher than the actual length of the map interval (EDGAR 1958a). If one examines the data (Figure 2) from intercistronic crosses only, it is seen that, in 15 out of 17 cases, the sum of the individual intervals is higher than the value obtained from the cross between the terminal markers of the region being considered. If, however, one considers only intracistronic crosses, the reverse is true. In 10 out of 14 possible comparisons, the sum of the individual in-

tervals is lower than the observed value from the cross of terminal markers. These results seem to bear out the predictions from EDGAR's data. Correction for the effect of the intercistron region would, therefore, improve additivity.

An additional reason which may account for the extreme deviation from additivity in BENZER's experiments is based on a technical difference in methods used. BENZER uses parental stocks which are prepared with K12S as the host cell. As mentioned above, the present authors find that this procedure can result in 80 percent loss of infected bacteria in crosses with a total multiplicity as low as four. BENZER uses an average total multiplicity of six in his mapping experiments. If he loses any infected cells, he probably selectively loses those with higher multiplicities. The actual multiplicities in his experiments may then be much lower than three of each parent, and this could well result in recombination values widely deviant from the true values, producing an unusually high variance in

TABLE 5

Residual correlation in $r_a r_c \times r_b r_d$ *crosses*

Cross	Recombination frequencies (percent) J*	K†	L‡	Residual correlation¶ Region concerned	J/KL	Corresponding O/C§
$r_{320}r_{114} \times r_{271}r_{163}$	0.020	0.39	1.35	r_c—r_d	3.8	7.2
	0.020	0.23	2.13	r_a—r_b	4.4	7.8
$r_{145}r_{205} \times r_{147}r_{287}$	0.010	0.079	4.44	r_c—r_d	2.9	6.5
	0.010	0.34	0.41	r_a—r_b	7.2	17.
$r_{145}r_{227} \times r_{147}r_{205}$	0.0019	0.050	0.26	r_c—r_d	14.6	17.
	0.0019	0.039	0.41	r_a—r_b	11.9	14.
$r_{145}r_{227} \times r_{147}r_{287}$	0.011	0.050	6.83	r_c—r_d	3.2	5.1
	0.011	0.31	0.41	r_a—r_b	8.7	14.
$r_{147}r_{205} \times r_{227}r_{163}$	0.0071	0.039	5.97	r_c—r_d	3.0	4.6
	0.0071	0.070	0.90	r_a—r_b	11.3	17.

* J = Observed value (Θ) from table 4.
† K = Observed value (O) from table 1.
‡ L = Observed value (O) from figure 2.
¶ Residual correlation for each cross is:

for region $r_c - r_d$:

$$\frac{100J}{KL} = \frac{O_{(r_a r_c \times r_b r_d)} \times 100}{O_{(r_a r_c \times r_b)} \times O_{(r_c \times r_d)}}$$

for region $r_a - r_b$:

$$\frac{100J}{KL} = \frac{O_{(r_a r_c \times r_b r_d)} \times 100}{O_{(r_b r_d \times r_c)} \times O_{(r_a \times r_b)}}$$

§ Corresponding O/C for region $r_c - r_d$ is O/C for cross $r_b r_d \times r_c$, and for region $r_a - r_b$ is O/C for cross $r_a r_c \times r_b$ (Table 1).

BENZER's estimate of the map intervals. A greater deviation from additivity may, of course, be found in studies with many markers which are much closer together than those used for the data presented here. The authors of this paper believe, however, that the correction factor cannot be as high as a factor of four, and is probably less than 1.5.

The concept of switch areas: In addition to the demonstration of linearity and additivity, the experiments permit analysis of high negative interference. Multiple recombination events over short regions of genetic structure have been previously described in phage (STREISINGER and FRANKLIN 1956; EDGAR 1956, 1958b), in *Aspergillus nidulans* (PRITCHARD 1955), in *Neurospora crassa* (DE SERRES 1956), and in *Drosophila melanogaster* (STURTEVANT 1951). An idea which has proved useful in thinking about negative interference in phage is one similar to the model proposed by PRITCHARD (1955). His "effective pairing segments" would be directly comparable to the "switch areas" which, in the present model, are assumed to include all recombination events. The switch areas occur randomly throughout the genome, and, since they are short, represent only a fractional part of the genome. Recombination must, then, have a much higher probability of occurring in a given physical or genetic length of switch area than in an equivalent length of average genome. When markers are, on the one hand, far apart, the chance of multiple recombination being observed depends largely on the probability that switch areas will occur in the appropriate intervals. In this circumstance, any interference would be a function of the effect of one switch area on another. On the other hand, the chance of multiple recombination between markers which are sufficiently close together to be encompassed in a single switch area depends both on the probability of the intervening region being included in a switch area and on the probability of recombination per unit length of switch area. High negative interference will, consequently, become more pronounced as the markers under study are located closer and closer together, since their chance of inclusion in a single switch area will thereby be increased.

The mechanism proposed has, then, two main parameters: (1) the average length of switch areas; and (2) the probability of a recombination event per unit switch area. A mathematical formulation of the switch theory is being constructed by BARRICELLI (personal communication) and will be presented in a separate paper. The data from all types of crosses reported in this paper are qualitatively accounted for by such a model. On the hypothesis of switch areas, crosses of the type $r_a r_b \times r_c$ are expected to yield fewer recombinants than would be predicted from the two-factor mapping experiments alone. This expectation is borne out by the data in Table 2. The other three types of multifactor crosses should yield O/C values much greater than unity. Again the expectation is borne out (Tables 1, 3, and 4). Finally, it is expected that the deviation of O/C from unity will become more pronounced as the map intervals under consideration become shorter, since the chance is thereby increased that the entire interval under consideration will be included in a single switch area. It has already been

seen that the three-factor crosses of the type $r_a r_c \times r_b$ (Figure 3a) agree with prediction, and the four-factor crosses of the type $r_a r_d \times r_b r_c$ (Figure 3b) appear to show a similar trend.

If the hypothesis that all recombination events take place in switch areas is tentatively accepted, consideration of the two types of four-factor crosses permits an estimate of the frequency of recombination events per unit length of switch area. The frequency of wild type phage from the cross $r_a r_c \times r_b r_d$ may be represented by x and the frequency from the cross $r_a r_d \times r_b r_c$ by y. Further, the plausible assumption can be made that the wild type recombinants are produced from a single switch area which overlaps the crucial interval, rather than from two adjacent switch areas. Under these conditions, wild type phage is, in both types of cross, produced from switch areas which cover the central interval (from r_b to r_c) completely. Then 2x represents the fraction of cases in which a recombination did occur in the central interval in addition to one in each of the neighboring intervals, and 2y the fraction in which a recombination did not occur. Continuing, $2x/(2x \times 2y)$ is the frequency of recombination events in a switch area of the length measured by the map interval r_b to r_c. If the resulting number is divided by the number of map units in the central interval, the frequency of recombination events per map-unit length of switch area is found. Calculations of this type are given in Table 6 for five comparisons in which the central interval (M) varies from 0.26 to 2.32 map units. Thus, while two-factor mapping experiments give a probability of recombination of 0.01 per map unit, the probability of recombination within such a length of switch region would be 0.06 to 0.14 or more.

The tacit assumption was also made in these calculations that the probability of recombination is unifrom over the entire length of the switch area. If one assumes further that a single switch area is involved for the entire span between terminal loci, then experiments of the types considered can test the assumption of uniformity, since it predicts that the estimates of the probability of recombination per unit switch area should be independent of the length of the interval r_b to r_c. The results presented are not extensive enough to allow a conclusion on this point, especially in view of the high variance introduced into the calculations because each one involves three independent experimental determinations. Nevertheless, it should be added that the data suggest clustering of recombination events even within switch areas, since the cases involving a shorter central interval show higher probability of recombination per map unit than those where the r_b to r_c distance is longer.

Inadequacy of gene conversion: A number of investigators (M. B. MITCHELL 1955a, 1955b, 1956; H. K. MITCHELL 1957; ST. LAWRENCE 1956; ST. LAWRENCE and BONNER 1957; LINDEGREN 1953, 1955; LINDEGREN and LINDEGREN 1956), on the basis of observations with ascomycetes, have invoked the notion of "gene conversion" to explain their results. Gene conversion has been defined by LINDEGREN (1955) as "the interaction ... between the dominant and the recessive allele in a heterozygote, resulting in transformation of one or more dominant

TABLE 6

Frequency of recombination events per hundred map units of length in the switch area

Crosses compared	M*	Recombinants (percent) 2x†	Recombinants (percent) 2y‡	Frequency per 100 map units $\frac{1}{M}[2x/(2x + 2y)]$
$r_{320}r_{163} \times r_{271}r_{114}$ *vs.* $r_{320}r_{114} \times r_{271}r_{163}$	2.32	0.0203	0.117	6.4
$r_{145}r_{287} \times r_{147}r_{205}$ *vs.* $r_{145}r_{205} \times r_{147}r_{287}$	1.16	0.0104	0.150	5.6
$r_{145}r_{205} \times r_{147}r_{227}$ *vs.* $r_{145}r_{227} \times r_{147}r_{205}$	0.90	0.00193	0.0174	11.
$r_{145}r_{287} \times r_{147}r_{227}$ *vs.* $r_{145}r_{227} \times r_{147}r_{287}$	0.90	0.0112	0.120	9.5
$r_{147}r_{163} \times r_{227}r_{205}$ *vs.* $r_{147}r_{205} \times r_{227}r_{163}$	0.26	0.00711	0.193	14.

* M is the map distance from r_b to r_c (Figure 2).
† 2x is twice the observed percentage of wild type from the cross $r_a r_c \times r_b r_d$ (Table 4).
‡ 2y is twice the observed percentage of wild type from the cross $r_a r_d \times r_b r_c$ (Table 3).

alleles into the corresponding recessive allele, or vice versa." If events of this type occur in phage, they are unsatisfactory as an explanation of the present data. The following arguments seem to be conclusive against a simple gene conversion [or more properly here, "muton" (BENZER 1957) conversion] model to account for the excess of wild types which is observed in the crosses with closely linked *r*II markers.

It may be assumed that a coefficient of coincidence of unity exists for the true multiple recombinants, and that any excess over expectation arises from gene conversion. It may further be assumed that the two-factor mapping crosses ($r_a \times r_b$) give wild type principally from true recombination events. Evidence for this assumption comes from the observation that the map intervals are approximately additive, a result which would not occur if gene conversion rates approached the map values. With the two assumptions, one can make an approximation of the rate of gene conversion for the central marker in the three-factor crosses $r_a r_c \times r_b$. This is done in Figure 4. For example, the rate of gene conversion for r_{227} in four estimates ranges from 0.0036 to 0.00037, and for r_{205}, from 0.0035 to 0.00019. Further, the change in rate of conversion is directly related to the expected double recombinant frequency, indicating that a higher rate

of conversion is necessary when a higher frequency of double recombinants is expected. The requirement that the rate of gene conversion must vary in this way by a factor 10 in one case and 18 in another renders the simple hypothesis untenable.

Another fairly conclusive argument can be made from comparison of the two types of four-factor crosses which include the same markers. It must first be established that the presumed rates of conversion for the various markers are similar, a conclusion which can again be reached on the basis of the data in Figure 4. It is seen that none of the points deviates widely from the line drawn, even though in any particular range of expected double recombinant frequency, various r's have been tested. It is clear, therefore, that the presumed rates of gene conversion for the different markers are similar. If the excess of observed wild types, corrected for the expected multiple recombinations, in the cross $r_a r_c \times r_b r_d$ is compared with that from the cross $r_a r_d \times r_b r_c$, the two values should be equal, because in either cross, two gene conversions are required to produce a wild type phage. Table 7 shows that the excess is always greater in the type of cross $r_a r_d \times r_b r_c$, the factor ranging from 4.2 to 20 in the five comparisons.

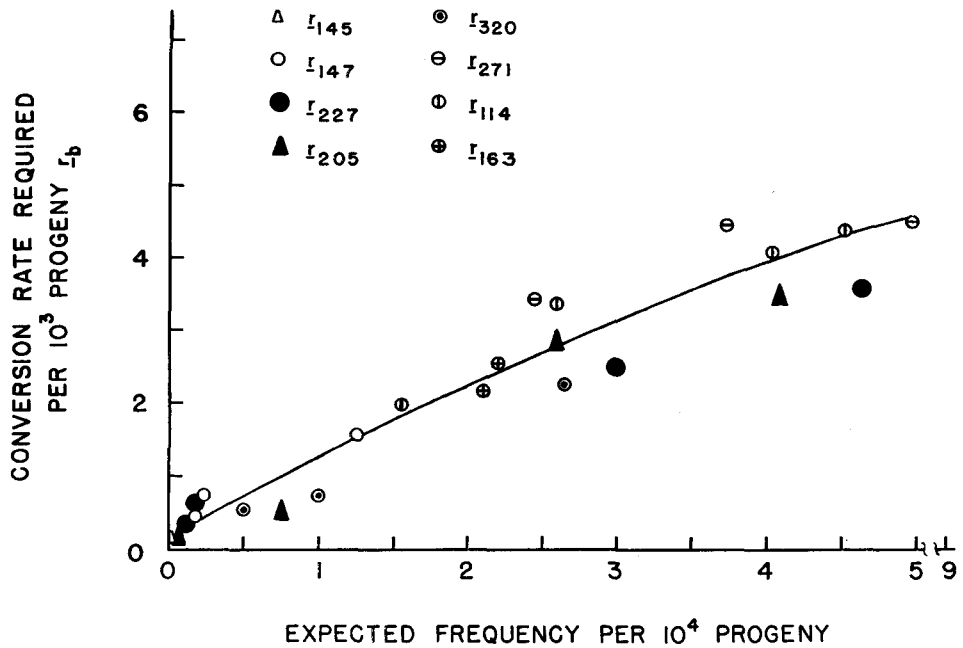

FIGURE 4.—Presumed gene (muton) conversion rates for the r_b markers in $r_a r_c \times r_b$ crosses. The ordinate gives the rate of gene conversion (r_b to $r+$) required to account for the excess of wild type phage found. This rate is determined by subtracting the expected frequency (abscissa) of true $r+$ recombinants (1/200 of the calculated recombination percentage, C) from the observed frequency of $r+$ (1/200 of the observed recombination percentage, O) and multiplying the value thus obtained by two to determine the rate per progeny r_b. Mating kinetics will introduce only negligible alterations in these calculations. Data are from Table 1.

TABLE 7

Comparison of presumed gene (muton) conversion rates for double conversions in two types of four-factor crosses

Cross	Frequency of wild-type			Relative excess†
	O*	C*	O − C	
$r_{320}r_{163} \times r_{271}r_{114}$	0.00055	0.00014	0.00041	4.2
$r_{320}r_{114} \times r_{271}r_{163}$	0.00010	0.0000033	0.000097	
$r_{145}r_{287} \times r_{147}r_{205}$	0.00075	0.000090	0.00066	13.
$r_{145}r_{205} \times r_{147}r_{287}$	0.000050	0.0000010	0.000049	
$r_{145}r_{205} \times r_{147}r_{227}$	0.000085	0.0000050	0.000080	8.4
$r_{145}r_{227} \times r_{147}r_{205}$	0.0000095	0.000000049	0.0000095	
$r_{145}r_{287} \times r_{147}r_{227}$	0.00060	0.00014	0.00046	8.5
$r_{145}r_{227} \times r_{147}r_{287}$	0.000055	0.0000012	0.000054	
$r_{147}r_{163} \times r_{227}r_{205}$	0.00095	0.00026	0.00069	20.
$r_{147}r_{205} \times r_{227}r_{163}$	0.000036	0.00000065	0.000035	

* Values from Tables 3 and 4, multiplied by 1/200.
† (O − C) from first cross ($r_ar_d \times r_br_c$) divided by (O − C) from the second ($r_ar_c \times r_br_d$).

Thirdly, all the data taken together show that the events which give rise to the excessive wild types occur in clusters. It seems unnecessary to invoke a new, and in this case untenable, mechanism, i.e., gene conversion, to account for the results. Clustering of recombination events seems to be a much more plausible explanation.

Occurrence of high negative interference in other organisms: Considerable data are being accumulated which suggest that high negative interference may be a general phenomenon. The interpretation suggested here may be extended to explain apparently aberrant results in higher organisms. STURTEVANT's (1951) data with the fourth chromosome of *Drosophila melanogaster* seem, at least superficially, to be a case of similar results which may be understood on a switch mechanism. The studies of DEMEREC (1928) on the reddish-alpha character of *D. virilis* might also involve this type of model. His "reversions" seem to be associated with a high frequency of crossing over in the reddish-scute region of the X chromosome.

The extension of the present interpretation seems justified especially in view of the work of PRITCHARD (1955) with *Aspergillus nidulans* and DE SERRES (1956) with *Neurospora crassa*, both of which show high negative interference in short intervals. The studies of ST. LAWRENCE (1956; ST. LAWRENCE and BONNER 1957), also with *N. crassa*, suggest a similar situation. The data mentioned by DE SERRES are particularly indicative. He observed that recovery of adenine-independent progeny from intercistron crosses showed intense negative interference, but that linkages with outside markers remained as expected. Among adenine-independent progeny from *ad-3* × *ad-3* intracistron crosses, however, the outside markers assorted almost randomly. This is what would be predicted

by the crosses described here, which show that negative interference gets more intense as the markers involved are closer and closer to one another. PRITCHARD has already explained his data in this way; it seems unnecessary to postulate a non-crossover mechanism to account for either DE SERRES' or ST. LAWRENCE's results.

The observation of gene conversion which has not been given an explanation on a recombination model is the occurrence within a tetrad of three copies of one allele and only one of the other. In *N. crassa*, a thorough study of such aberrant recombination of pyridoxine mutants has been undertaken by M. B. MITCHELL (1955a, 1955b, 1956) and H. K. MITCHELL (1957). They wish to invoke "a mechanism which is different and distinguishable from that of crossing over" (H. K. MITCHELL 1957). Such an interpretation may, in this case also, be unnecessary. If one can accept a copy choice kind of model in which effective pairing sites are the regions of copy choice, the unusual ratio may be easily accounted for. It is unnecessary to assume that copying takes place simultaneously and at equal rates throughout the pairing segment. Equally plausible is the notion that copying on one strand may sometimes precede replication on the homologue. Then, in the closely paired region, an allele, already copied, may by a switch from the other strand, be copied again, giving rise to the 3:1 ratio. Since the phenomenon is postulated to occur in a pairing segment, it is expected to be strongly correlated with crossing over, as observed.

The same argument may be applied to cases of gene conversion in *Saccharomyces cerevisiae*. Many of the examples of conversion cited by LINDEGREN (1953, 1955) may be explained by the occurrence of supernumerary mitoses (WINGE and ROBERTS 1954) and other types of abnormal segregation patterns (EMERSON 1956). Those which remain (LINDEGREN and LINDEGREN 1956) can be interpreted as occasional mistakes in copy-choice. This does not require the existence of two different phenomena, but only some revision of ideas about the mechanism of recombination, and the latter may well become necessary with the advent of selective methods which permit detailed scrutinization of short map intervals.

SUMMARY

The experiments reported in this paper form a detailed descriptive analysis of high negative interference over short distances on the linkage structure of bacteriophage T4B. Seven A-cistron and three B-cistron *r*II mutants were used in a variety of combinations in two-, three-, and four-factor biparental crosses. The results firmly established the existence of linearity within the individual functional units of the *r*II region. The data indicate, further, that the observed excess (assuming coefficients of coincidence of one) of double- and triple-recombinations is the result of multiple-recombination events in short segments of the genetic structure, and that the frequency of such events is inversely related to the length of the map interval being studied. That the excess wild-type recombinants are due to reversions or to gene conversion is unambiguously ruled out. A

model is presented which accounts for all the observations here and may be applied also to explain apparently aberrant results in several other organisms.

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