RECOMBINATION IN PHAGE T2: ITS RELATIONSHIP TO HETEROZYGOSIS AND GROWTH

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WHEN genetic recombination was discovered in bacteriophage (HERSHEY and ROTMAN 1948) it was natural to think of the process in the same way that one thought of recombination in higher organisms. This type of approach has met with varying degrees of success. Linkage groups have been established with roughly additive map distances between markers and three-factor crosses can be understood in terms of double cross-overs. However, there is a considerable body of evidence on the increasing recombinant frequency with time after infection and on three-parent crosses (see DOERMANN 1953) which makes it impossible to understand a phage cross (i.e., the output of a single, plurally infected bacterium) in terms of a single mating event.

The repeated mating theory of VISCONTI and DELBRUCK (1953) explains all of the accumulated data on phage genetics except those involving the phenomenon of heterozygosis (HERSHEY and CHASE 1951; HERSHEY 1953b). In this theory, a mixedly infected bacterium is considered as a population mixture of randomly mating particles, which are in a noninfective state designated as vegetative. The infected cell is assumed to have a pool of vegetative phage in which there is a continual approach to genetic equilibrium by successive matings. This theory has the advantage of introducing no postulates about the basic mechanism of recombinant production that have not already been used in the genetics of higher organisms.

The experiments and calculations reported here arose from consideration of models of recombination in phage in which the production of recombinants is treated as a special case of vegetative phage replication. The requirement that the mechanisms be similar to those which seem to apply in higher organisms was relaxed. But it is of course necessary that any new theory make the same predictions as that of VISCONTI and DELBRUCK where the latter have successfully explained the experimental data.

This paper will be divided into three parts; first, the results of experiments designed to investigate the nature of the heterozygotes; second, calculations which indicate that heterozygote formation provides a mechanism for the production of all of the observed recombinants and, third, speculations on the implications of those findings for the theory of recombination and replication in phage.

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REVIEW OF PREVIOUS DATA ON HETEROZYGOTES

The data of HERSHEY and CHASE (1951) can be summarized as follows:

1) A heterozygote is defined as a free virus particle that produces two types of progeny when it infects a sensitive bacterial cell. In crosses of r^+ by r (rapid lysis mutant) they are scored by the mottled plaques which they form; these contain a mixture of r^+ and r particles. Heterozygous particles recognized by this mixed progeny will be called mottlers.

2) The five different markers tested in crosses $r \times r^+$ all produced about 2% heterozygotes recognizable as mottlers. A cross $h^+ \times h$ (host range mutant) also produced 2% plaques on sensitive bacteria which segregated to form h and h^+ progeny. Thus apparently any marker will be heterozygous in about 2% of the progeny of an equal input cross.

3) The frequency of the heterozygotes for a single marker is constant at 2% whether the cells are lysed prematurely, at the end of the normal latent period, or lysis is delayed. This is to be contrasted with the proportion recombinants which, for closely linked markers, increases by a factor of 4 to 6 when lysis is delayed (LEVINTHAL and VISCONTI 1953).

4) When the two markers are unlinked or distant, heterozygosis occurs almost independently in each; only about 3% of the heterozygotes show the effect for both markers.

5) When two markers are closely linked double heterozygosis is more frequent; for the markers h and r_{13} about 75% of the r mottlers are also heterozygous for h.

Two conclusions are suggested by these data. First, the heterozygous region of the chromosome is short. Its length is on the average greater than the $h\ r_{13}$ distance and less than the $h\ r_7$ distance. The rest of the chromosome may be homozygous diploid or hemizygous. Second, the heterozygotes are not reproduced as such inside the bacterial cell since if they did one would expect a cumulative increase in the fraction of the progeny showing heterozygosis as the burst size increases with delayed lysis. Also, the progeny of a heterozygous particle show only the same incidence of heterozygosis as do the progeny of mixedly infected bacteria.

Possible alternatives

Two conceptions of a partial duplication or partial diploidy of a haploid chromosome suppose (I) a small piece attached to the side of a normal chromosome or (II) partial overlapping of two pieces of different parental chromosomes, as shown in figure 1a. The equivalent alternatives with complete diploidy are shown in figure 1b. Either of those possibilities is consistent with the data of HERSHEY and CHASE, but they can be distinguished in a three-factor cross. On model I the markers on opposite sides of the duplication would be contributed by the same parent, and on model II they would be contributed by unlike parents. Thus in a three-factor cross ($A\ B\ C \times A^+\ B^+\ C^+$) heterozygotes at the B locus should be $A\ C$ or $A^+\ C^+$ on model I but $A\ C^+$ or $A^+\ C$ on model II.

There are, however, two obstacles to realizing this idealized experiment. 1) We do not yet have three different, separately recognizable markers on one linkage group in phage and 2) when the first mature intracellular phage appears many recombinants have already been formed, so even the first mature heterozygotes formed will not have been made by combining a pure population of $A\,B\,C$ and $A^+\,B^+\,C^+$. They will have been formed by the mixed population of parental type vegetative phage and recombinants.

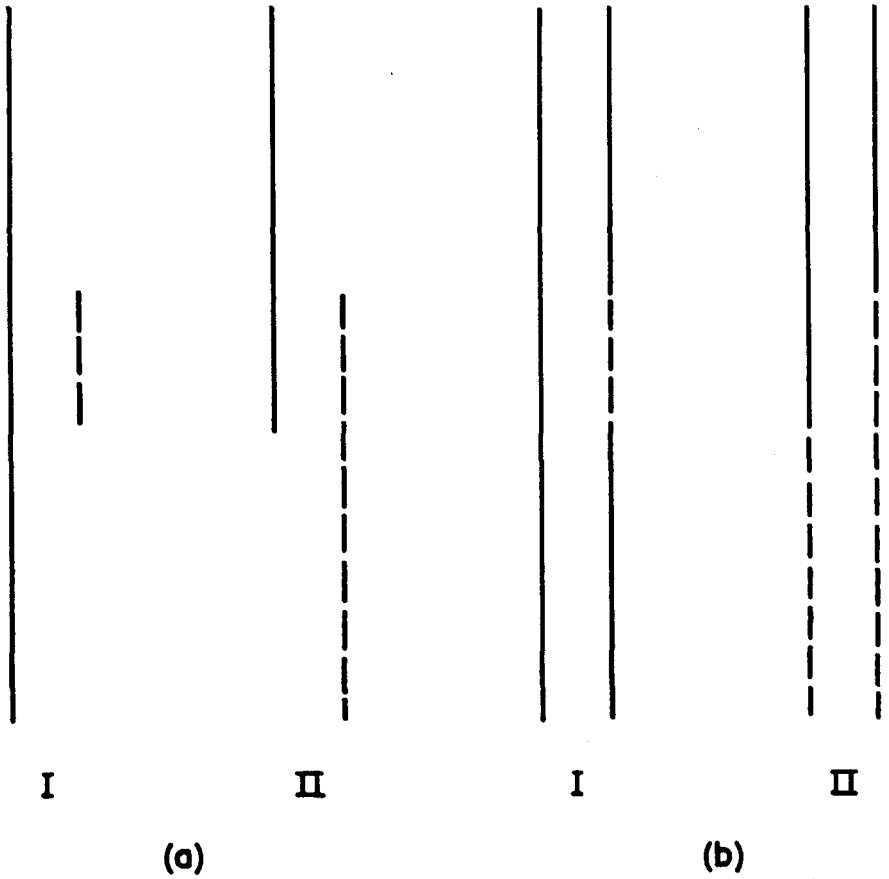

FIGURE 1.—Possible models of the heterozygotes consistent with the data of HERSHEY and CHASE. Solid lines represent the chromosomes from one parent and dotted those from the other parent.

The first difficulty is avoided by crossing $h\,r_2{}^+\,r_7 \times h^+\,r_2\,r_7{}^+$. These markers are on the same linkage group and in the order shown. In two-factor crosses the recombination frequency between h and r_2 is 16% and between r_2 and r_7 8%. But r_2 and r_7 are mutually epistatic, that is, a virus particle carrying any r factor will produce an r type plaque. Thus any mottler from this cross must contain both $r_2{}^+$ and $r_7{}^+$ as well as one of the r genes. The four possible segregation patterns for the r heterozygotes in this cross are 1)

$r_2^+ r_7^+/r_2 r_7^+$ (r_2 heterozygotes); 2) $r_2^+ r_7^+/r_2^+ r_7$ (r_7 heterozygotes); 3) $r_2 r_7^+/r_2^+ r_7$ and 4) $r_2^+ r_7^+/r_2 r_7$. Only the first two of these will lead to mottled plaques. The third will not produce mottling, as too few r^+ will be formed by recombination after plating. This was shown by HERSHEY and CHASE as well as in these experiments by the fact that bacteria infected with several r_2 and r_7 phage yield only a negligible number of mottlers when plated before burst. The fourth class can be ruled out by HERSHEY's finding that the mottled plaques in $h\,r \times h^+\,r^+$ crosses never segregated to form the two recombinants. We can now consider how the h character will be distributed in the two classes of mottlers on the two models for the heterozygotes shown in figure 1. It can be seen from figure 2 that mottlers of type 2), that is, r_7 het-

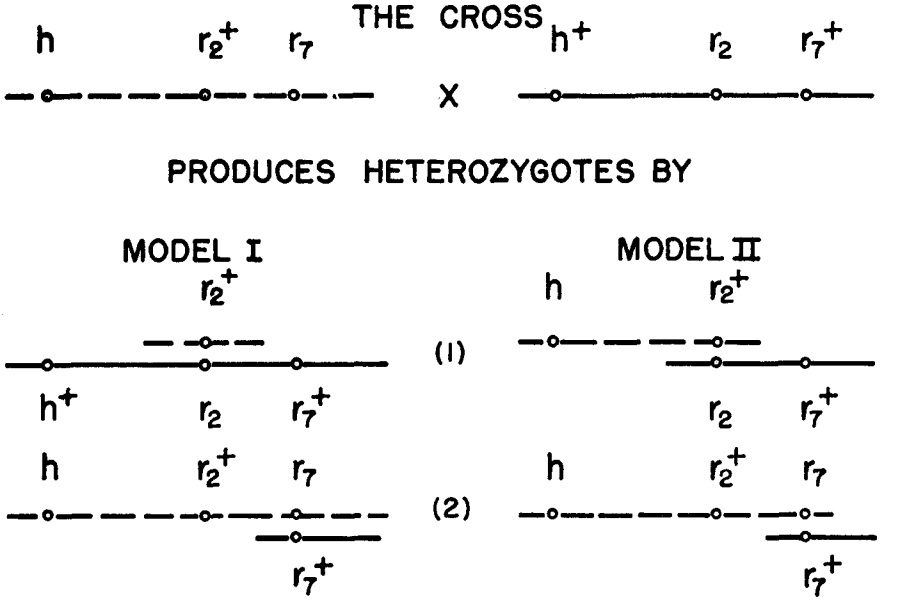

FIGURE 2.—Structure of the r_2 (above) and r_7 (below) heterozygotes from the cross $h\,r_7 \times r_2$ considering Models I and II of figure 1.

erozygotes, will have the h marker in either model, but the type 1) mottlers, that is r_2 heterozygotes, will have the h^+ character in model I and h in model II. Since Hershey showed that heterozygotes for r_2 are as frequent as those for r_7, we would expect on model I to find 50% of the mottled plaques containing h and on model II 100% containing h if there had been no recombination between the markers before the heterozygotes were formed.

We will now determine how these conclusions are modified by the fact that the vegetative pool contains recombinant particles as well as parentals. If we designate the total frequency of the recombinants between h and r_2 as m and the frequency between r_2 and r_7 as n, then to first order in m and n (that is considering only single crossovers) the frequency of parental genotypes in the population is $1 - (m + n)$. In table 1 are listed the two parental and four

TABLE 1

Genotypes in the vegetative pool in a bacterium equally infected with hr_7 and r_2 phage. m is the recombinant frequency between h and r_2 and n is that between r_2 and r_7. All calculations assume m and n are small compared to 1.

Type	Frequency
A) $hr_2^+r_7$	½ (1 − (m + n))
B) $h^+r_2r_7^+$	½ (1 − (m + n))
C) $h^+r_2^+r_7$	½ m
D) $hr_2r_7^+$	½ m
E) $hr_2^+r_7^+$	½ n
F) $h^+r_2r_7$	½ n

Heterozygotes formed by "collisions" of above types, showing frequency and host range as calculated on model II.

Interacting types	Heterozygous for	h character	Frequency × 4 to first order in m and n
A × B	r_2	h	1 − 2(m − n)
A × D	r_2	h	m
B × C	r_2	h^+	m
B × E	r_2	h	n
B × E	r_2	h^+	n
A × B	r_7	h	1 − 2(m − n)
A × D	r_7	h	m
A × E	r_7	h	2n
B × C	r_7	h^+	m

Thus $hr_2r_2^+ = 1 - (m + n)$ and $hr_7r_7^+ = 1 - m$

recombinant types which could be produced by a single crossover or its equivalent, with their frequencies given in terms of the parameters m and n. If we consider only the results for premature or normal lysis, m and n are both small compared to one and we can neglect products of two such small quantities. In the lower part of table 1 are listed the results of such first order calculations for the frequency of *h* in the mottlers assuming only that the probability of a given type of pairing (or mating) is proportional to the product of the two population frequencies. The conclusion of these first order calculations is that the fraction of the r_2 mottlers showing *h* (designated $h\,r_2\,r_2^+$) is $[1 - (m + n)]$ and $h\,r_7\,r_7^+ = 1 - m$.

EXPERIMENTAL METHODS AND RESULTS

Crosses were made between $h\,r_2^+\,r_7$ and $h^+\,r_2\,r_7^+$ and the mottled plaques tested for their *h* character. If the majority were *h* it would indicate that the r_2 heterozygotes are recombinants between *h* and r_7.

All of the crosses were done using mutants of the phage T2H and the host bacterium *Escherichia coli* strain H. The plating was done with strains S and 2bc (S/T2) as the sensitive and resistant bacteria, respectively. All of these strains were originally obtained from HERSHEY and have been described by him (HERSHEY and ROTMAN 1949). The methods of adsorption and plating were those described by ADAMS (1950) as modified by VISCONTI and DEL-

BRUCK (1953) and LEVINTHAL and VISCONTI (1953). Premature lysis was produced by dilution into a chilled buffer containing M/100 KCN (DOERMANN 1952). In each cross the multiplicity of infection was measured by assaying for uninfected bacteria before burst. The infected bacteria were plated before burst to check for unadsorbed phage. The progeny of the crosses were assayed on strain S, sensitive to both *h* and h^+. On this indicator the average burst size and one class of recombinants, namely r^+, could be scored, and mottled plaques could easily be distinguished. All mottled plaques were counted and those not in contact with any other plaques on the plate were picked and suspended in buffer. The phage from each of the mottled plaques was then diluted and replated on mixed indicator plates with S and *2bc*. These plates gave an additional check on the selection of mottlers since they showed both r^+ and *r* plaques; these plates were scored as to host range. For each cross

TABLE 2

Three-factor crosses.

Cross and condition	1 Input multiplicity	2 Average burst size	3 h in total progeny (percent)	4 r^+ in total progeny (percent)	5 Mottlers in total progeny (percent)	6 Mottlers with h observed (percent)	7 Mottlers with h expected (percent)	8 Mottlers with h expected (percent)
$hr_7 \times r_2$ premature lysis	8	1	47 ± 2.5	2.9 ± .2	1.3 ± .2	80 ± 2.7 226 tested	85	80
$hr_7 \times r_2$ normal lysis	7	100	45 ± 3.5	4.2 ± .4	1.5 ± .2	73 ± 4.2	79	72
$hr_7 \times r_2$ inhibited lysis	8	350	47 ± 2.5	14 ± 1	1.2 ± .2	57 ± 5	~55	54
$hr_2 \times r_7$ premature lysis	7	4	53 ± 2	3.0 ± .3	1.4 ± .2	29 ± 4	15	20

The fraction of h among the mottlers as calculated from model II is given in columns 7 and 8. The value expected from model I is given in column 3. No correction for negative interference has been made in column 7. The expected values corrected for negative interference (from experimental value of negative interference) are given in column 8.

about 120 mottled plaques were tested in this way and scored for host range, and the following data were thus obtained: 1) input multiplicity, 2) average burst size, 3) fraction of *h* in total progeny, 4) fraction r^+ in total progeny, 5) fraction of mottlers among the total progeny, and 6) fraction of *h* among the mottlers. The expected value of the fraction *h* among the mottlers was calculated for model II from 4) by making use of the fact that for these markers m = 2n and averaging $h\ r_2\ r_2^+$ and $h\ r_7\ r_7^+$. For model I the fraction of *h* among the mottlers would be equal to the fraction of *h* among the total progeny. The standard errors of the experimental data are calculated for sampling error alone. In the case of the percent *h* among the mottlers the sampling is believed to be unbiased since the scoring is unambiguous and the mottlers tested are picked from single indicator plates on which the host range character cannot be detected.

The results of three crosses $h\ r_7 \times r_2$ and one $h\ r_2 \times r_7$ are shown in table 2.

It can be seen that the results are in agreement with the expected values from model II and in disagreement with the expected values from model I. The significance of this agreement will be discussed below and we will now take up one further experiment.

It was assumed in the calculation of table 2 that the r_2 heterozygotes are as frequent as the r_7's. This point could be checked only by backcrossing r segregants from the mottlers with known r_2 and r_7 stocks.

The pooled phage from five r plaques from each mottler was back crossed with both r_7 and r_2 stocks. Standard procedures were used except that the inputs of the phage under test were only controlled to about a factor of three, and the final platings were done on plates crowded with r so that even a few r^+ plaques could be easily scored.

TABLE 3

Results of back crosses.

	Observed	Expected from	
		Model I	Model II
Percent r_2 heterozygotes among mottlers	42 ± 5%	50	50
Percent h among r_2 heterozygotes	75 ± 6	18	82
Percent h among r_7 heterozygotes	88 ± 4	88	88

The results of back crosses to determine the r character of 106 mottled plaques. The types obtained were as follows: 54 r_7h; 34 r_2h; 11 r_2h^+; 7 r_7h^+. The mottled plaques were obtained from the cross $hr_7 \times r_2$ premature lysis. No correction was made for the effect of negative interference.

The r phage from 106 mottled plaques in the progeny of the premature lysis $h\ r_7 \times r_2$ cross were tested by two back crosses each and the r character determined. Thus a true three-factor cross was done and the results so obtained are given in table 3. As can be seen, the results are in agreement with the expected values of model II.

INTERPRETATION OF THE EXPERIMENTS

The experimental results can be summarized as follows: 1) In a three-factor cross particles which are heterozygous for the middle marker, r_2, are generally recombinants for the end markers h and r_7, 2) as the lysis is delayed and the recombinant frequency increases, the fraction of the heterozygotes showing recombination for the end markers decreases, approaching 50% as genetic equilibrium is reached, 3) there is fair agreement between the observed percent h among the r mottlers and the value expected from the overlap model.

However, the problem of calculating the fraction of the heterozygotes created by parents which are themselves recombinants is the same as the problem of calculating the fraction of double crossovers among the class of particles having at least one crossover. VISCONTI and DELBRUCK (1953) investigated the apparent negative interference which one finds in this case. They pointed out that

in selecting the class with at least one crossover one is selecting phage which have been involved, on the average, in more mating processes. The calculations of this effect of inhomogeneity cannot be made accurately since there are unknown sources of variation among different bacterial cells. However, the correction can be made in our case using the experimental results on negative interference. These corrected values are shown in column 8 of table 2.

THE RELATIONSHIP OF HETEROZYGOSIS TO RECOMBINATION

We have demonstrated that a phage particle heterozygous in a certain region will produce progeny which are recombinants for markers on opposite sides of this region. But the question immediately arises as to whether these are the only intermediates that produce recombinants. There does not seem to be any way of answering this question rigorously but we can determine that this mechanism is a sufficient one. That is, we can calculate the number of recombinants which would be produced by the heterozygotes alone. As will be seen, they account, at least approximately, for the number of recombinants observed and by the principle of conservation of hypothesis we conclude that *all* recombinants in phage are produced from the heterozygotes.

We know from the fact that the types of heterozygotes produced change with time that new heterozygotes are continually being formed from whatever population exists in the vegetative pool. But from the fact that the percentage of heterozygotes does not increase with time, we conclude that the heterozygous condition is not reproduced as such. This assumption concerning the internal progeny of the heterozygotes is in agreement with findings of free phage. The progeny of a cross with two percent mottlers will have less than one mottler in a thousand after one cycle of growth under conditions of single infection in sensitive bacteria. Thus we conclude that heterozygosis occurs for any marker in two percent of all new vegetative particles in an equally infected bacterium, but these heterozygotes produce progeny which are haploid (or homozygous) for the marker investigated.

We will introduce some parameters necessary to describe the overlapping partial diploids. We will call the average length of the overlap piece L. The position of the overlap will be characterized by the distance, X, of its center from one end of the chromosome and we will call the probability that its center fall between X and $(X + dx)$, $P(X)dx$. That is $P(X)$ is the probability per unit length that the center of an overlap occurs at X. Using this notation we find that the probability of any part of an overlap region covering a particular gene is

$$\int_{-L/2}^{L/2} p(x)\, dx$$

but this is just the probability of finding heterozygosis in a particular marker which Hershey found to be two percent for all markers tested. The above integral will be independent of X if $P(X)$ and L are constants and under these conditions the integration can be performed to yield the relationship

$$PL = 2\% \qquad (1)$$

Let D hr_{13} be the distance between the genes h and r_{13} measured in the same units as L and X. Then the ratio of L to Dhr_{13} can be calculated from the fraction of the r_{13} mottlers which are also heterozygous in h, which HERSHEY measured to be 75%. Since the range of X which will produce double heterozygotes is (L − D) out of a total range L which will put r_{13} on the overlap we have:

$$L = 4\,Dhr_{13} \tag{2}$$

If we consider a population in the vegetative pool consisting entirely of parental types, then recombinants will be produced between any two genes if they are separated by the overlap. Let R be the number of recombinants and N the total number of particles in the pool. Then

$$\frac{dR}{dN} = PD \tag{3}$$

where D is the distance between the genes. This expression correctly takes into account the end effects of structures like those shown in figure 3 if we assume that the progeny of the heterozygotes in the pool are distributed in the same way as to genetic type as are the progeny of the heterozygous free phage. This means that a structure like the one in figure 3 is counted as half of a recombinant for h and r_{13} since half of its progeny will be recombinants while it is counted as a full recombinant for h and r_7.

When the population in the pool has both parental types and recombinants we must modify (3) to take into account the fact that the process which makes a recombinant heterozygote from two parental types will make a parental type from two recombinants. Thus only the "collisions" between parental types will produce recombinants and the number of these is proportional to $(N - R)^2$ while the number of "collisions" between recombinants which can lead to the production of parents will be proportional to R^2. "Collisions" between one recombinant and one parental type cannot change the genetic distribution. We must also consider that the recombinants are able to reproduce their own kind and presumably at the same rate as the total population. Finally, equation (3) can be rewritten as

$$\frac{dR}{dN} = \frac{R}{N} + PD\,\frac{(N-R)^2}{N^2} - PD\,\frac{R^2}{N} = \frac{R}{N}(1 - 2PD) + PD \tag{4}$$

The solution to this equation for the recombinant frequency is

$$\frac{R}{N} = \frac{1}{2}\left[1 - \left(\frac{N}{N_0}\right)^{-2PD}\right]$$

where N_0 is the number of particles at infection when $R = 0$. If we write N/N_0 in terms of number of generations, and n, as $N = N_0 e^n$, then the solution becomes

$$\frac{R}{N} = \frac{1}{2}(1 - e^{-2PDn}) \tag{5}$$

This equation can be compared to equation (10b) of VISCONTI and DELBRUCK (1953) which for an equal input cross reads

$$\frac{R}{N} = \frac{1}{2}(1 - e^{-mP}) \tag{6}$$

where m is the number of rounds of random mating and P is the linkage. For unlinked markers P = ½ and the effective m for the normal cross must be 5 to account for the observed approach to genetic equilibrium. In our case heterozygotes could be formed only from some sort of a " collision " between opposite types, but half of the " collisions " will be identical particles. Furthermore, any model we have been able to construct would require the same assumption as that made by VISCONTI and DELBRUCK, namely only half of the

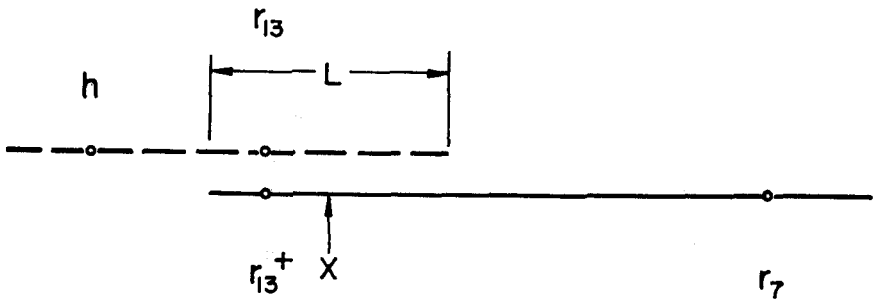

FIGURE 3.—The genetic structure of a particle heterozygous for r_{13}. The progeny of this heterozygote would be half $h\,r_{13}\,r_7$ and half $h\,r_{13}{}^{+}\,r_7$. That is, half of the progeny would be recombinants for h and r_{13} and all would be recombinants for h and r_7.

" collisions " between opposite types will yield recombinant type heterozygotes. Thus the maximum value of PD would be one-quarter and our equation (5) becomes identical to that of VISCONTI and DELBRUCK with m = n. From this we can conclude that the number of generations n in this treatment should have the same value as the number of rounds of mating m in the VISCONTI-DELBRUCK theory.

Now that we know the value of n for the normal cross and we know the value of PD for the $h\,r_{13}$ distance from equations (1) and (2), we can calculate the recombinants produced by this mechanism. From (1) and (2) we have $PDhr_{13} = 0.5$ percent, and with m = 5

$$\left(\frac{R}{N}\right)_{hr_{13}} = \frac{1}{2}(1 - e^{-.005 \times 5 \times 2}) = 2.5 \text{ percent}$$

which is to be compared to the value of 2.6 ± 0.3 percent observed in the normal cross (LEVINTHAL and VISCONTI 1953). Thus within the accuracy of the experiments the heterozygotes and their progeny account for the observed recombinants.

DISCUSSION

In the preceding calculations it has been shown that the heterozygotes are the intermediates in the production of recombinants in phage. These calcula-

tions did not require any detailed specification of a model, but before considering the models which are consistent with the findings, the assumptions used so far will be explicitly listed and discussed.

Assumptions used in the calculations

1) The basic ideas of the VISCONTI-DELBRUCK theory concerning population interaction are correct. That is, the phage interact (i.e., "mate" in a very general sense) in a pool of vegetative, noninfective particles within the bacterium. The " mating " is considered random both as to partner and time and there is a random and irreversible extraction from the pool to form intact nonmating phage particles. The justification of these hypotheses was discussed by VISCONTI and DELBRUCK and was based on the results of two- and three-factor crosses and on the gradual approach to genetic equilibrium with time.

2) The production of the heterozygotes involves some sort of an interaction between two unlike vegetative phages which, as seen from the experiments, leads to a particle with an overlapping structure.

3) The heterozygotes do not reproduce their own kind but produce progeny at the usual rate in the vegetative pool and the distribution as to type of these progeny is the same as that found for the heterozygous free phage. As stated above, this assumption is based on the constancy of the heterozygote frequency in the bursts after delayed lysis and its reduction after one cycle of growth in new bacteria.

4) The overlap segment is equally likely to occur at any point on the chromosome. This assumption is justified by HERSHEY's finding that the probability of heterozygosis is the same for the five markers tested. It is also assumed that the overlaps have a distribution in length such that few of them are shorter than one-quarter of the mean length. However, even if this were not true, it could only raise the number of recombinants from this mechanism by a small amount.

Implications of these findings for possible models

It has been very difficult to construct a model of mating in which two formed chromosomes come together to produce an overlapping heterozygote without correlating the mating with replication. The only scheme which seems possible is the one shown in figure 4 (a) and its equivalent in 4 (b). In this model we must assume that the unwanted ends are disposed of by an unknown process. It is not a very satisfactory model since it requires that unusually large amounts of chromosomal material be " thrown away," and that we introduce several other very artificial assumptions. For instance, unless we assume some special restriction we might expect to find structures of the type shown in figure 4 (c), but they would have been observed even if they had occurred in only a few percent of the heterozygotes. No simpler model seems to result from considering the heterozygotes as completely diploid.

Obviously the real motivation behind the use of this type of model is that it is similar to those which have been used successfully in higher organisms;

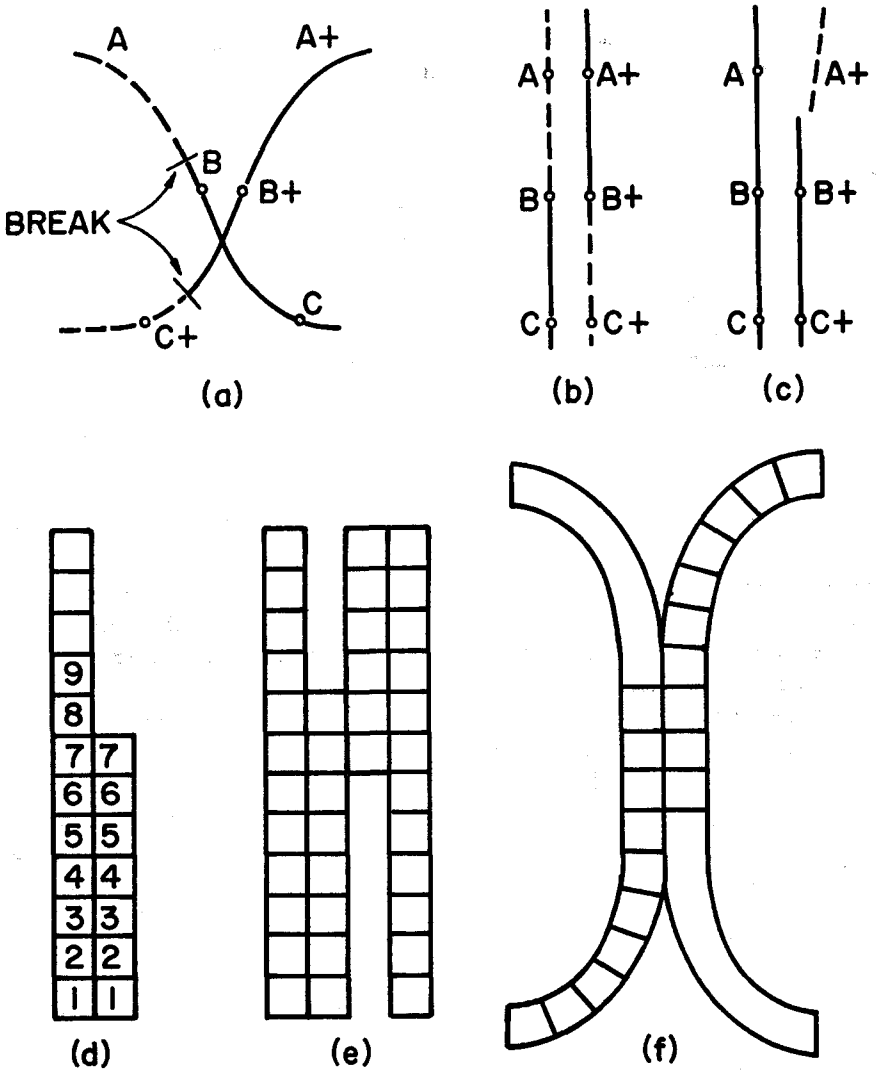

FIGURE 4.—(a) and (b) show methods of formation of the heterozygotes by breakage of formed chromosomes. The dotted portions must be thrown away. The type of structure shown in (c) must be ruled out by some new hypothesis. (d) shows the lateral zipper like model with the half completed daughter on left. (e) shows two parents on the outside and a newly formed heterozygote on the inside. (f) shows how the heterozygote (shown cross-hatched) could be formed above two paired parents. The daughters of the heterozygotes would be formed by replication on one side or the other.

but it is necessary to modify the usual concepts to such an extent that there does not seem to be any gain in simplicity by starting with the more usual mechanisms. No discussion has been given of models involving duplications in the usual sense, that is, linear insertions, since it does not seem possible to explain the segregation patterns in this way.

We will now consider the model which led to these experiments. In it we

assume that the recombinants, or in this case heterozygotes, are not constructed out of pieces of the previously formed chromosomes, but are formed *de novo* under the control of two previously completed structures.

We start with a model of replication involving lateral aggregation in a zipper-like fashion (see a suggestion by STURTEVANT quoted in HERSHEY and ROTMAN (1949) also BELLING (1933). This is pictured schematically in figure 4 (d) where one of the previously completed structures shown with solid lines is acting as a parent for a half-completed daughter. We assume that the structures consist of a linear array of sub-units and that the replication is the assembling of the sub-units in an order which is identical to that of the parent. We postulate some kind of an attachment between the end sub-unit and a free one which is identical to it, and then the attachment of a unit like the second, etc. If we think of the phage, it is likely that the problem of genetic replication is the same as that of the molecular replication of desoxyribosenucleic acid (HERSHEY and CHASE 1953). The specific attachment might be enzymatic (DOUNCE 1953) or some kind of direct physical or chemical force between identical or complementary molecules (WATSON and CRICK 1953). In order to establish replication of the lateral zipper type we must postulate some sort of a specific attachment which occurs sequentially. Thus in figure 4 (d) the next sub-unit added would be in the number 8 position. We must also postulate some effect which causes the two units to separate after the daughter is completed.

With the two assumptions that are used in constructing a model of replication we can explain the production of the heterozygotes without major modifications of the scheme. The phage DNA molecules are extremely long and thin. They are even long compared to the dimensions of the bacterium (LEVINTHAL and FISHER 1953) so that if any of the specific attachment remains in the completed structures we would expect to find many of them lying with homologous units in apposition. If replication starts with the paired structures as parents, we would expect it to start on opposite ends of the two in about half the cases. When the two partially completed daughters meet there will be a specific attachment between them. If we postulate that this cross attachment stops the process, then the structure formed has all of the characteristics of the heterozygotes.

If one accepts the hypothesis that DNA is the genetic material of phage then the structure of this molecule proposed by WATSON and CRICK (1953a) immediately suggests a specific model for the chromosomes of the heterozygotes. There are two intertwined complementary chains in this proposed DNA structure. Every adenine in one chain is attached to a thymine molecule in the other by a pair of hydrogen bands; and every guanine will similarly be attached to a cytosine (in the T2 virus a 5-methyl cytosine) in the complementary chain. In any structure of this type the genetic information must be contained in the sequence of the nucleotides, and in the WATSON and CRICK structure this information is contained twice, since the order of the nucleotides in one uniquely determines the order in the other. This suggests that the DNA

of the heterozygotes have a structure which can be represented schematically by figure 1 b II. The two lines represent the two chains of one molecule, and the solid and dotted portions refer to the parental particles which determine the order of the nucleotides. In the overlap region the complementarity breaks down, but since phage particles are generally heterozygous for only one or two markers the lack of complementarity would probably not be significant for the molecular structure.

This type of model is fundamentally different from normal diploidy since the two chains will not necessarily reassort randomly. We consider (WATSON and CRICK 1953b) a model of replication in which the two chains first separate at one end, and then each of the chains acts as a template for the formation of its complement. The new partial structure would be stable and might tend to break the next bonds of the parental structure. This process would then continue in a zipper-like fashion until two molecules were completed. If this mechanism does apply to the heterozygotes we would have segregation on the first replication and the heterozygotes would not reproduce themselves.

The problem of the formation of the heterozygotes still remains and even if we consider the WATSON and CRICK mechanism of replication the discussion given above concerning *de novo* information seems to apply. There does not seem to be any way in which breakage of already formed molecules could produce heterozygotes of the required structure without introducing very artificial assumptions. The lateral attachment of partially formed complements, although complicated by the helical nature of the structure, seems to present a reasonable possibility.

Several additional predictions follow from the type of model used here. Recombinants are not produced reciprocally by the heterozygotes. The $A^+ B$ recombinants are statistically, but not individually, related to the $A B^+$ recombinants. In single burst experiments HERSHEY and ROTMAN (1949) showed that there is no correlation between the numbers of the reciprocal recombinants from single bacteria although they are equal in the mass culture. VISCONTI and DELBRUCK (1953) explained this lack of correlation by the randomizing effect of growth and extraction from the vegetative pool following mating, but without an excessively large pool it is not clear that these randomizing influences would be sufficient. Another prediction, which has not as yet been testable, is that no chromosomes are broken down during mating. In particular, the chromosomes of the input particles must reappear unchanged in the progeny.

It was shown in the interpretation of the increase of recombinants in lysis-inhibited cultures (LEVINTHAL and VISCONTI 1953) that if we assume a connection between the production of recombinants (i.e., mating) and replication, we can then calculate the size of the vegetative pool from the total burst size. This connection seems to be more strongly indicated by the nature of the heterozygotes; and the number of rounds of mating, m, becomes the number of generations in the vegetative pool. From the rate of increase in recombinants we estimated the rate of growth to be about one generation every two minutes.

This time should be approximately equal to the washout time for the P^{32} in the DNA pool if the vegetative phage are DNA molecules. HERSHEY's (1953a) measurements of this quantity are in approximate agreement with the estimate from the genetic data.

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SUMMARY

In three-factor crosses with T2 phage it was found that particles selected for heterozygosis of the middle marker were generally recombinants for the end markers. This suggests a model for the heterozygotes that involves a small overlapping piece which can be considered as a partial diploid section of a haploid chromosome. Knowing that the heterozygotes lead to recombinants, it is possible to calculate the number of recombinants which would be contributed by this mechanism alone. The calculated value is in agreement with the observed number and it is concluded that heterozygotes are the intermediates in the production of recombinants in phage. In a discussion of the implications of these results it is suggested that the simplest explanation is one in which the heterozygotes are formed *de novo* by the cooperative production of a daughter by two parental structures.

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