EXCHANGE OF DNA IN THE RECOMBINATION OF BACTERIOPHAGE λ

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Genetic recombination in λ at the molecular level studied in the preceding paper[1] can also be studied by means of the following experimental system:

Mutants of λ are known[2] which differ among themselves and differ from the wild type by their densities. Density-gradient centrifugation permits the physical separation of phages of different densities. For two well-studied mutants at least, it was found that the differences in densities with respect to the wild type are due to genetic markers which can be localized on the genetic map of λ. These density markers behave in crosses as does any other marker. Thus we have described the marker *b*2 in the region *m*5–*h* of the linkage group of λ, and in the present work we shall also make use of another character called *b*5, situated in the *c* region. No phenotypic mixing occurs with these markers, that is to say that the density of the progeny phages, in a cross involving *b*2 and *b*5, corresponds strictly to their genotype.

In a cross the density differences are additive (within the experimental error) for the recombinants. In other words, a cross $b2b5^+ \times b2^+b5$ (both phages are less dense than the wild type) yields recombinants of wild type density $b2^+b5^+$ and recombinants *b*2*b*5 less dense than either of the parents. When the progeny of such a cross is centrifuged in a density gradient, four bands appear, two of which correspond to the parental densities and two new ones containing only recombinants.

We have made use of this system to see if there was a physical exchange of DNA in genetic recombination. We labeled the DNA of one of the infecting parents with P^{32} and measured the distribution of P^{32} among the progeny. The presence of P^{32} in phages different in density from the labeled parent, particularly in the recombinant phages, shows that recombination involves a physical exchange of DNA. These experiments also show that breakdown and reutilization of the parental infecting DNA is probably negligible.

Material and Methods.—Media: Difco Tryptone 1% with 0.5% NaCl; *M* 9 to which 0.5% of Difco amino acids was added, called here *M* 9 AA; Difco Neopeptone 1% with 0.5% NaCl; and adsorption medium, 0.01 *N* $MgSO_4$ in distilled water.

Bacterial strains: W3110 (Lederberg) C 600 (Appleyard[3]) and derivatives of W3110 made lysogenic for λ wild type and λ *b*5.

Phages: $b2^+b5^+$ "wild type" (Kaiser[4]) having a buoyant density in CsCl of 1.508 gm cm^{-3}
*b*2*b*5$^+$ (1.491 gm cm^{-3})
$b2^+b5$ (1.501 gm cm^{-3})
*b*2*b*5 (1.483 gm cm^{-3})

The mutation *b*2 has been described elsewhere.[2] The phage λ*b*5 was found in a stock of λ wild type. The original λ*b*5 mutant carried another marker besides *b*5, giving its plaques a small size; this morphological marker was eliminated by crossing the mutant with λ wild type. The marker *b*5 is in the region *c* and confers on the phage carrying it an immunity different from that of λ. Thus λ*b*5 forms plaques on bacteria lysogenic for λ$b5^+$ and inversely λ$b5^+$ forms plaques on bacteria lysogenic for λ*b*5. We have also shown that (1) λ*b*5 crosses with λ$b5^+$, (2) it carries the

marker $b2^+$, (3) it has the same host range as λ, and (4) it is located on the bacterial chromosome at the λ site, or very close to it.

Clear mutants of λ*b*5 can be isolated and one of them, *b*5*c*, was used in the crosses to be described here. A clear mutant,[5] c_1 of $\lambda b5^+$ and the morphological marker *mi* were also used. The positions of these different markers on the genetic map of λ are given in Figure 1.

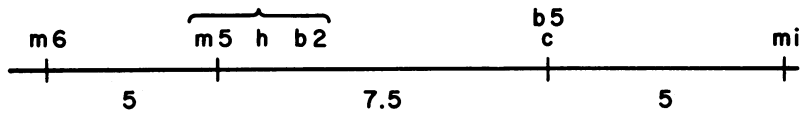

FIG. 1.—Genetic map of phage λ.

The phage λ*b*2*b*5 was obtained in crosses of $\lambda b2b5^+$ with $\lambda b2^+b5$.

Preparation of phase stocks marked with P^{32}: Bacteria W3110 were grown in neopeptone medium to a density of 2×10^9 cells/ml. The bacteria were infected with phage at a multiplicity of about 0.01 phage per bacterium. After allowing 10 min for adsorption, the infected bacteria were diluted about 10 times in the radioactive medium (containing 1% Difco Neopeptone, 0.5% NaCl, 0.003% gelatin, 2% glycerol, and P^{32} in the form of orthophosphate in dilute HCl solution to obtain an activity of about 1 mc/mgP). The pH of the "hot" medium was adjusted to a value of 7 by the addition of NaOH. After 5 hr of growth and lysis, the stocks were freed of most of the contaminating P^{32} by three successive centrifugations at low and high speeds and resuspended in a protective medium containing glycerol and gelatin. The stocks made in this fashion contained only about one P^{32} atom per phage. The P^{32} stocks were used within 48 hr after their preparation. Control experiments utilizing the blendor technique of Hershey and Chase[7] (1952) have shown that most of the P^{32} adsorbed onto fresh bacteria is injected into them.

Gradient centrifugation: The method of gradient centrifugation and the assay of the phage distribution in the centrifuge tube by drop collection has been described elsewhere.[6] The Spinco preparative centrifuge was used with its swinging bucket rotor (SW39) and run at 22,000 rpm. The drops were collected in separate tubes containing 0.2 ml of Tryptone broth. An aliquot of 0.1 ml from each tube was put on planchettes for the measurements of the P^{32} content. Then convenient dilutions of the remaining volume were made, to determine the phage concentration in each tube. When crosses involved the markers *b*5 and $b5^+$, platings were made on bacteria carrying λ*b*5 and $\lambda b5^+$, respectively, and the numbers obtained were corrected for the plating efficiency relative to C600. (The efficiency of plating was usually about 0.80 for λ*b*5 on 3,110 lysogenic for $\lambda b5^+$.)

Genetic crosses: The crosses described were made in bacteria W3110. Cultures grown to a titer of 2×10^9 bacteria/ml were centrifuged and the bacterial pellets resuspended in adsorption medium and aerated at 37° for 1 hr. After this treatment, the bacteria were infected with an approximately equal number of both parental types of phages, the total multiplicity varying between 5.5 and 6.5 phages per bacterium in the different experiments. Ten minutes at 37° were allowed for adsorption (more than 95% adsorption) and the nonadsorbed phages as well as the small amount of free P^{32} still contained in the phage stocks were removed by centrifugation. The pellet of infected bacteria was resuspended at a titer of 2 to 4×10^8 cells/ml in fresh culture medium (some crosses were made in tryptone broth and some in *M* 9 AA), and the suspension was aerated at 37°. Time for lysis was between 70 and 150 min in different experiments. The lysates were centrifuged at low speed to eliminate bacterial debris, then at high speed to concentrate the phages and eliminate the free P^{32} in the supernatant. The phage pellets were resuspended in fresh medium and centrifuged a second time at high speed. Finally they were resuspended in a mall volume of Tris buffer at pH 7.6 before being diluted in CsCl for the density gradient centrifugation.

Results.—Crosses were made with the markers *b*2 and *b*5 in both coupling and repulsion. In each cross, one of the parents was labeled with P^{32}; experiments involving all of the four possible combinations of genotypes and P^{32} label have been carried out.

1. *Controls:* To show that during the growth of the phage of a single genetic

density type no other density mutants arise in amounts which would complicate the results of the crosses and that the presence of P^{32} does not affect the density distribution, the crosses $b2b5cP^{32} \times b2b5c$ and $b2^+b5^+cP^{32} \times b2^+b5^+c$ were first performed. The essential data concerning these are found in Table 1.

TABLE 1

Parents I	Parents II	Multiplicities of infection I	Multiplicities of infection II	Yield	Medium	Total transfer
$b2b5cP^{32}$	$\times b2b5c$	3.0	2.9	120	*M* 9 AA	0.43
$b2^+b5^+cP^{32}$	$\times b2^+b5^+c$	3.4	3.4	43	Tryptone	0.22

The density analyses by gradient centrifugation are shown in Figures 2 and 3. One sees that, essentially, no new densities appear in these crosses.‡ As has been pointed out,[1] the shape and width of the band obtained by collecting drops cannot be trusted if the band has been formed at or after drop number 40. Thus, although in general the bands of phages of lower densities seem to be wider than those of higher densities, no significance is to be attached to this fact.

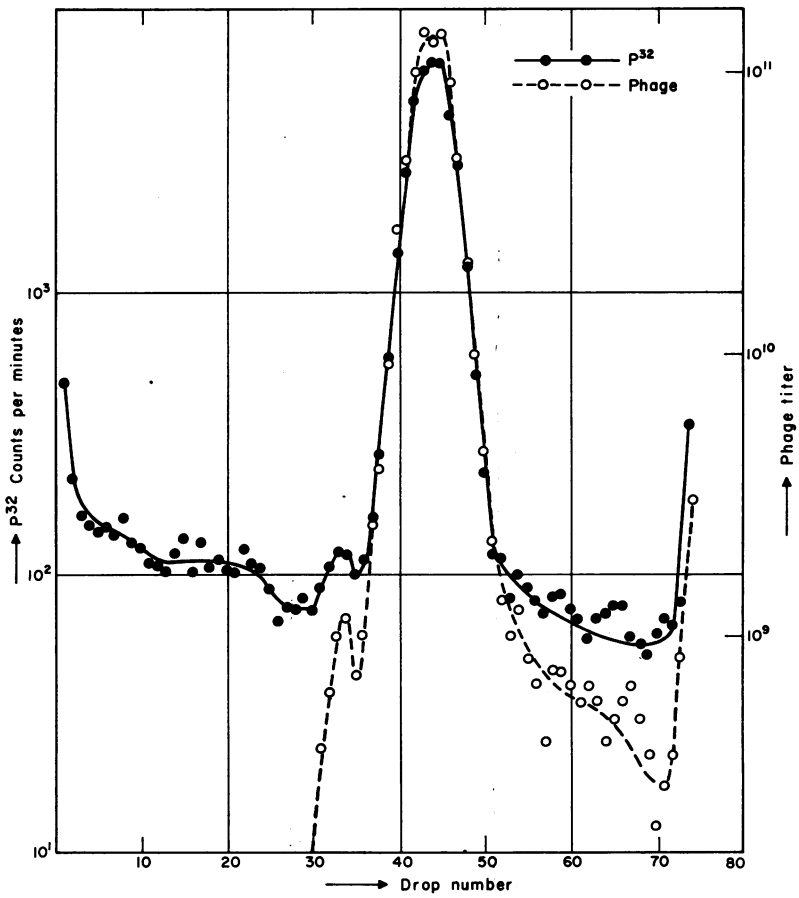

FIG. 2.—Control cross $\lambda b2b5cP^{32} \times \lambda b2b5c$. The two curves give, respectively, the titer of the progeny phage and the P^{32} contained in the drops collected after centrifugation.

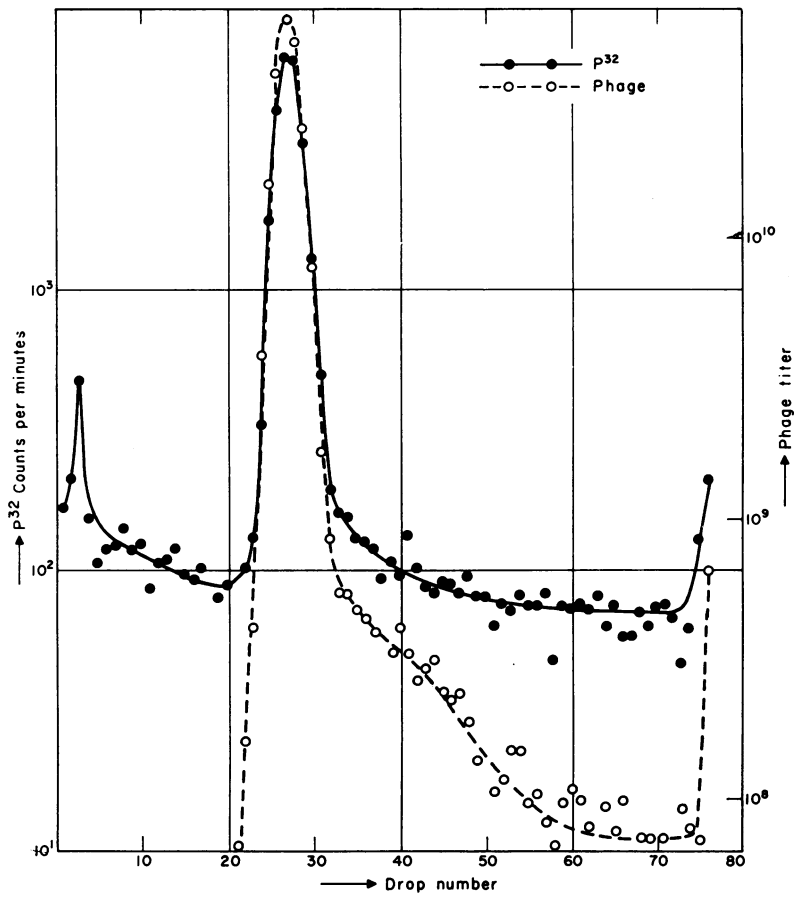

FIG. 3.—Control cross $\lambda b2^+b5^+cP^{32} \times \lambda b2^+b5^+c$. The two curves give, respectively, the titer of the progeny phage and the P^{32} contained in the drops collected after centrifugation.

The P^{32} is concentrated in the phage bands except for a background of radioactivity which appears in all the crosses. This background could be due to unfinished phage particles§ which burst and liberate their DNA when placed in the CsCl solution.

One concludes from the data of Figures 2 and 3 that the multiplication of the phages *b2b5* or $b2^+b5^+$ does not produce phages of a density different from that determined by their genotype.

2. *Transfer of P^{32}:* The amount of transfer of P^{32} from infecting particles to the progeny depends very much on the medium in which the crosses are performed. For instance in *M* 9 AA medium, lysis is delayed and a large part of the P^{32} remains in unlysed bacteria which can be centrifuged down at low speeds. At least 30 per cent and as much as 60 per cent of the introduced P^{32} can thus be lost. In these conditions, 30 to 50 per cent of the P^{32} is transferred to progeny particles adsorbable to bacteria and the remaining part of the P^{32} is found in the supernatant, probably in the form of free DNA or unfinished phages.

In tryptone broth, lysis is fairly complete but the phage yield is low and the

transfer is only about 20 per cent. As much as 75 per cent of the P^{32} can be found in the supernatant.

These data, based on many experiments, apply only when the lysing culture contains more than 2×10^8 bacteria/ml.

3. *Exchange of P^{32} in crosses of phages of different densities:* The data concerning the crosses $b2b5cP^{32} \times b2^+b5^+c$ and $b2^+b5^+cP^{32} \times b2b5c$ are found in Table 2 and the analysis of the progeny in the density gradient are represented in Figures 4 and 5. One sees in the figures that phages of two new bands of intermediate densities appear; they are the recombinants $b2^+b5$ and $b2b5^+$.

TABLE 2

Parents I	Parents II	Multiplicities of infection I	Multiplicities of infection II	Yield	Medium	Total transfer
$b2b5cP^{32}$	$\times b2^+b5^+c$	3.2	3.0	90	*M* 9 AA	0.52
$b2^+b5^+cP^{32}$	$\times b2b5c$	3.2	2.8	46	Tryptone	0.21

We have made use of the difference in immunity between $b5$ and $b5^+$ to obtain a better separation of the phage bands. The phages of each drop were plated on two indicator strains, one lysogenic for $\lambda b5$ and the other for $\lambda b5^+$: the plaques formed on the first strain give the count of $\lambda b5^+$ only while the second strain gives $\lambda b5$ only. Both curves were corrected for the plating efficiency with respect to the common indicator strain, UV-irradiated C600.

It will be noted in Figures 4 and 5 that the two recombinants do not appear in equal frequency. The $b2b5^+$ phage is two to three times as frequent as the reciprocal recombinant $b2^+b5$. We interpret this as meaning that $cb2b5^+$ has a selective advantage over the other phages involved in the crosses. When other markers such as *m*6, *mi*, etc. are associated with *c*, these phages do not have a selective advantage and behave in a "normal" fashion. Obviously, the advantage of $cb2b5^+$ gives an erroneous value of 20 per cent for the recombination frequency between $b2$ and $b5$. This same frequency is 10 per cent when the phages carry the markers mentioned above, the presence of which eliminates the selective advantage of $cb2b5^+$.

All the crosses mentioned in this paper have also been performed without the P^{32} label. The absence of P^{32} does not alter the recombination frequencies.

To make sure that no phenotypic mixing with respect to density had taken place in the crosses, the phages from the central drop of each peak were passed through one cycle of multiplication at low multiplicity of infection on bacteria W3110. The progeny was then centrifuged in the gradient along with additional phage to provide appropriate density markers. None of the four lysates obtained in this way contained more than 1 per cent of phages of a density different from that of the band from which they had been chosen.

P^{32} is found in all four types of progeny phages, i.e., in the two parents and in the two recombinants, but its distribution among the different types is very unequal. The largest part of the P^{32} remains with the parental genotype which brought it into the cross. The two recombinants contain approximately equal quantities and the progeny of cold parental genotype receives relatively little P^{32}. Although there are differences in the details of the distribution of P^{32} among the progeny depending on the parent which carried the P^{32} (see Fig. 6 for the other crosses $b2b5^+P^{32} \times$

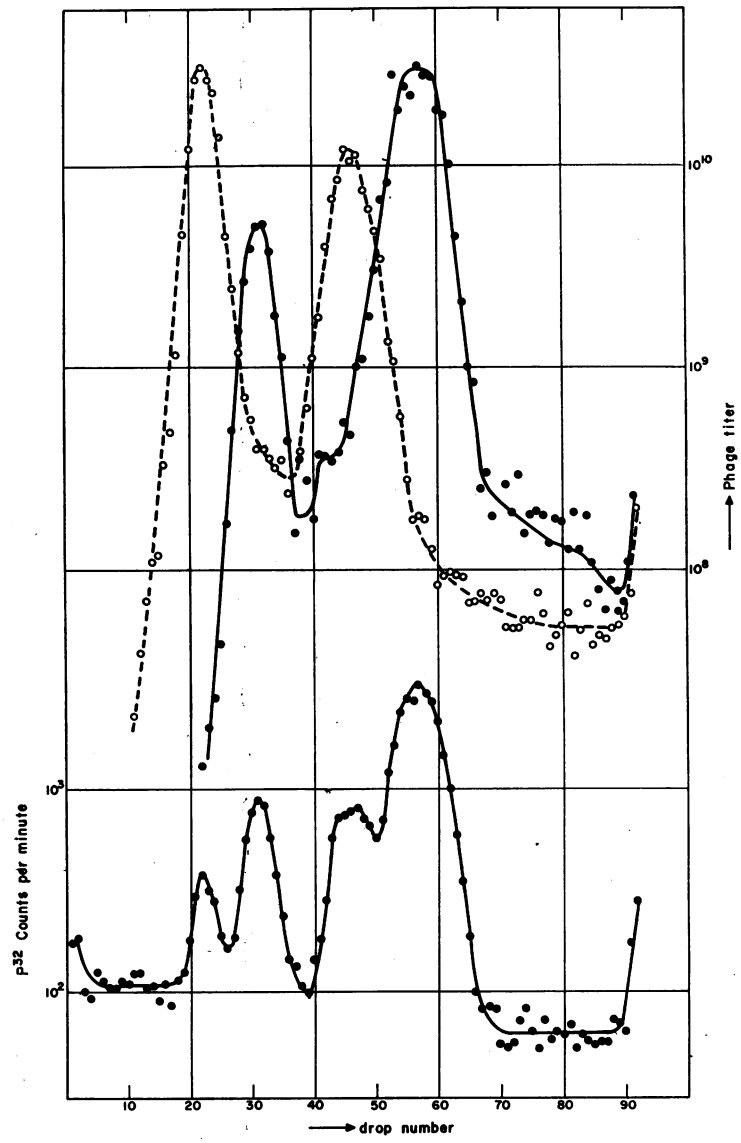

FIG. 4.—Cross $\lambda b2b5cP^{32} \times \lambda b2^{+}b5^{+}c$. The upper curves give the titer of the progeny phage in the drops collected after centrifugation. The peaks starting from the left represent the phage of the following genotypes $b2^{+}b5^{+}$, $b2^{+}b5$, $b2b5^{+}$, and $b2b5$. Since $\lambda b5$ and $\lambda b5^{+}$ have different immunities, selective indicator strains carrying the prophages $\lambda b5$ and $\lambda b5^{+}$ can be used to separate these two genotypes: on the indicator carrying $\lambda b5$, one obtains the measurements given by the interrupted line; on the other indicator, the continuous line. The lower curves give the P^{32} (in counts per minute) contained in the drops.

$b2^{+}b5$ and $b2b5^{+} \times b2^{+}b5P^{32}$) the general distribution of P^{32} is always that described above.

To measure the total amount of P^{32} in each band, an approximate extrapolation was made for each peak and the area under each band measured (subtracting the background activity). These measurements are given in Table 3.

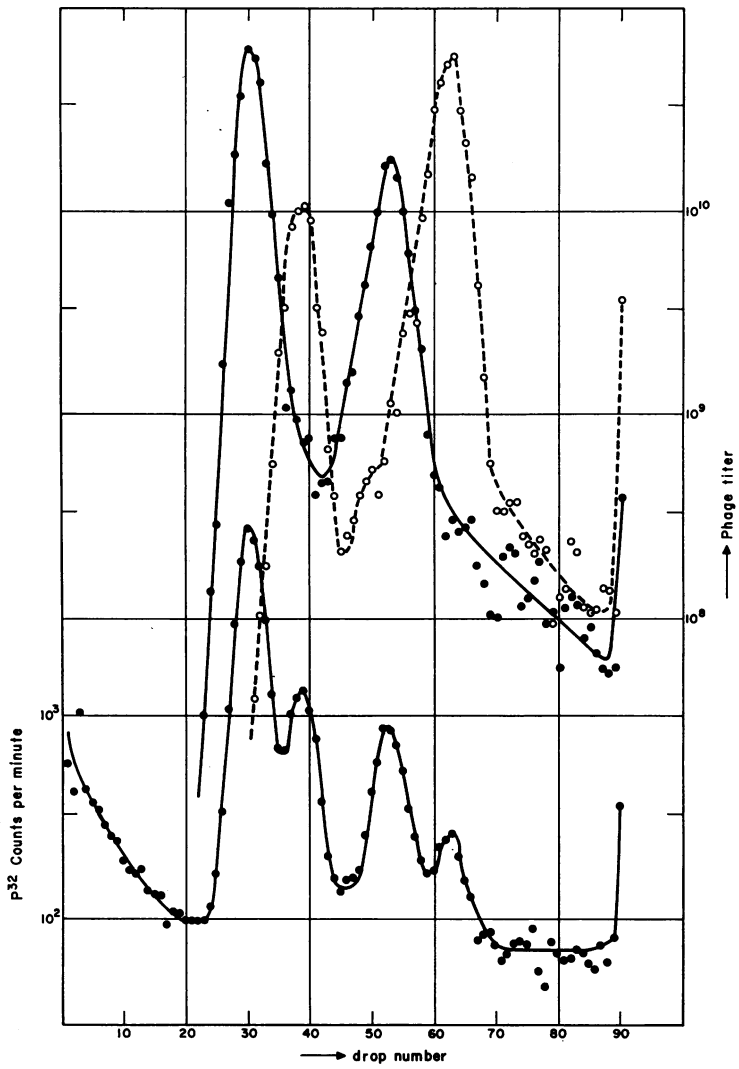

FIG. 5.—Cross $\lambda b2^{+}b5^{+}cP^{32} \times \lambda b2b5c$. The upper curves give the titer of the progeny phage contained in the drops collected after centrifugation. The lower curves give the P^{32} contained in the drops. For the details see the legend of Fig. 4.

TABLE 3

Cross	Radioactivity* in the Different Bands of Phage $b2^{+}b5^{+}$	$b2^{+}b5$	$b2b5^{+}$	$b2b5$
I. $b2b5cP^{32} \times b2^{+}b5^{+}c$	1,171	4,071	4,308	26,035
II. $b2^{+}b5^{+}cP^{32} \times b2b5c$	35,100	5,285	4,245	998
III. $b2b5^{+}cP^{32} \times b2^{+}b5c^{+}$	1,303	303	8,311	1,194
IV. $b2^{+}b5c^{+}P^{32} \times b2b5^{+}cmi$	6,810	52,540	(2,524)	4,753

* As described in the text, the radioactivity, in counts per minute, was measured in each band of phages by adding all the counts under the band, the shape of the band being extrapolated from the curves giving the number of phages in each band. The background of radioactivity was subtracted as well as the background counts of the counter.

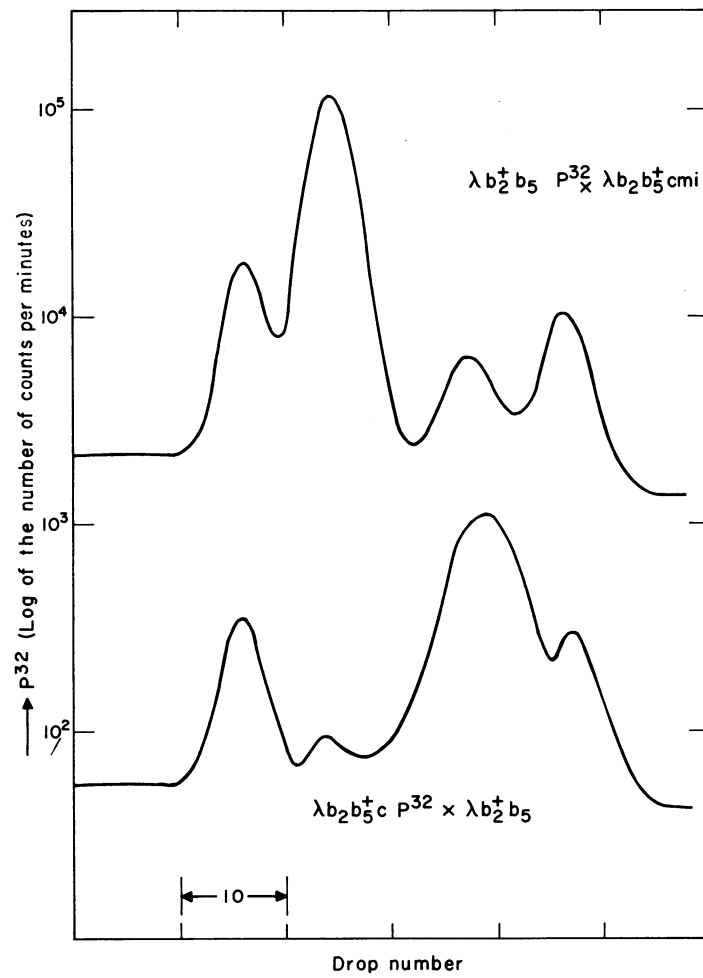

FIG. 6.—P^{32} contained in the drops, after centrifugation, of the crosses $\lambda b2^+b5P^{32} \times \lambda b2b5^+cmi$ (upper curve) and $\lambda b2b5^+P^{32} \times \lambda b2^+b5$. The positions of the peaks of the two curves do not quite coincide because the total number of drops collected in the two experiments were not equal. The logarithm of the number of counts per minute in the different drops is plotted as a function of the drop number. The upper curve has been arbitrarily displaced vertically.

In spite of the approximations involved, we estimate these measurements to be correct within 4 per cent except for the band $b2b5^+$ of the last cross of Table 3. For this cross, the "cold" parent band $b2b5^+$ is in the tail of the "hot" parent and a large correction must be applied. The error, in this case, may be as much as 50 per cent.

Discussion.—The experiments described above were designed to answer the following question: Are recombinant phage chromosomes made entirely of newly synthesized material or do they contain some of the original parental DNA material? A definite answer is given by our experiments: the recombinants *do* contain a large amount of the material from at least one of the infecting parents. Thus a second question can be asked: is the transferred material due to breakdown of

parental DNA and reutilization by the newly synthesized phage? If such were the process of transfer, one would expect to find at least as much P^{32} in the progeny with the genotype of the cold parental phage as in the recombinants. This is clearly not the case. The total amount of P^{32} in the cold parental progeny is only about 12 per cent of the total amount of P^{32} in the two recombinants; thus the parental DNA is transferred preferentially to the recombinants.

The simplest model accounting for these results is that recombination in λ occurs by breaks of the recombining chromosomes. This model allows one to calculate the relative amount of P^{32} brought to the progeny phages having the genotype of the cold parent by crossovers *outside* of the markers *b*2 and *b*5. Assuming that the amount of P^{32} in a chromosome is proportional to the genetic length and that there is only one break and knowing the position of *b*2 and *b*5 (see Fig. 1), one finds that the two recombinant genotypes (crossovers *between* *b*2 and *b*5) should contain 8 times more P^{32} than the progeny of the cold parental genotype. The data of the first three crosses (the data of the fourth cross cannot be used for the reason given above) given for the ratio of the total amount of P^{32} in the recombinant to that contained in the cold parental genotype the value 8 ± 1.

The good agreement between the estimated and the observed ratio leads us to think that most, if not all, of the P^{32} is transferred by crossovers and not by breakdown and reutilization. This estimate is based on the fact that crossovers are rare in λ and that double crossovers can be neglected. It may be that further studies of the mechanism of breaks will need a more detailed analysis of our data.

All these considerations are based on the total amount of P^{32} found in the recombinants and the cold parental genotypes, respectively. They are thus *independent of the mating theory.*

The nature of the density markers *b*2 and *b*5 has so far been left unspecified. These markers simply gave a definite phenotype to the phages harboring them, and in a cross they behaved like any other genetic marker. If one takes into account the fact that $b2^+$ and probably $b5^+$ contain more DNA than *b*2 and *b*5, the data of Table 3 contains the information necessary to calculate, under certain assumptions, the amounts of the supplementary DNA. If one assumes (1) that the extra DNA of each marker is situated at the location of the marker itself, and (2) that the presence of the extra DNA does not significantly alter the genetic exchanges between the homologous sections of the two genomes, the different relative amounts of P^{32} brought into the recombinant by a $P^{32}b2^+b5^+$ and by a $P^{32}b2b5$ phage lead to the following results: λ $b2^+b5$ contains 17 per cent, and λ $b2b5^+$ contains 6 per cent more DNA than λ *b*2*b*5. The assumption that the density difference between these phages is due entirely to their DNA content would have given, according to the formula (1) of Weigle *et al.*,[6] the values 17 per cent and 7 per cent, respectively.

Conclusions.—In genetic crosses between phage λ of different densities, the recombinants can be physically separated from the parents. When one of the parents is labeled with P^{32} and the genetic markers are far apart, most of the P^{32} transferred to the progeny of genotypes different from that of the hot parent is found in the recombinants. Breakdown and reutilization of the parental material is probably negligible.

The simplest model accounting for these facts is one in which the recombinants are formed by breaks of the two parental materials.

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‡ The small band appearing on the dense side of *b*2*b*5 in Fig. 2 contains only 0.2% of the phages; it is not due to an error in collecting the drops, for if the phages are centrifuged again in the density gradient, these phages appear at the same density; however, after one cycle of multiplication they give progeny phages of the density *b*2*b*5. The reason for this behavior is unknown.

§ Observed in λ lysates by E. Kellenberger in the electron microscope.

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ON THE CLASS NUMBER OF REAL QUADRATIC FIELDS

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THEOREM.—*Let p be a prime $\equiv 1 \pmod 4$, h the class number of the real quadratic field $R(\sqrt{p})$, $\epsilon = [(t + u\sqrt{p})/2] > 1$ its fundamental unit. Then,*

$$\left(\frac{p-1}{2}\right)! \equiv (-1)^{(h+1)/2}\frac{t}{2} \pmod p. \tag{1}$$

Proof: From Dirichlet's classical formula we derive

$$\sqrt{p}\,\epsilon^h = \prod_n (1 - \theta^n), \tag{2}$$

where $\theta = e^{2\pi i/p}$ and n runs over the numbers with $0 < n < p$ and $(n/p) = -1$, where (x/p) is Legendre's symbol of quadratic residuacity. Working with integers of $R(\theta)$, we note

$$\sqrt{p} = \left(\frac{p-1}{2}\right)!\,(1-\theta)^{(p-1)/2} \pmod{(1-\theta)^{(p+1)/2}}, \tag{3}$$

$$\prod_n (1 - \theta^n) = (1-\theta)^{(p-1)/2} \pmod{(1-\theta)^{(p+1)/2}}. \tag{4}$$

(1) follows from (2), (3), and (4). Our result supplements formulae of Ankeny-Artin-Chowla in *Ann. Math.*, 1952, p. 479.

I am grateful to Professor A. Selberg for noticing an error in my original argument. Paromita Chowla has checked the formula in many special cases. See L. J. Mordell, *Amer. Math. Monthly* (Feb. 1961) for a similar result for primes $p \equiv 3 \pmod 4$.