Effects of Ionizing Radiation on Cross-Reactivation of T1 Bacteriophage

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INTRODUCTION

Genetically marked bacteriophage inactivated by radiation are still able to contribute markers to the progeny of mixed infection with active phage of a differing genotype. This phenomenon is termed 'cross-reactivation' and has been studied by several workers, notably Watson (1), Luria (2), Stent (3), Doermann et al. (4), Jacob and Wollman (5), and Stahl (6). Their findings indicate that radiation damage is localized on the genetic material of the phage and leaves part of the genome still functional. Stahl's results, obtained with phage T4 inactivated by the decay of incorporated P32, indicate that P32 decay can damage a considerable portion of the genome.

The experiments to be reported below represent an attempt to measure the size and shape of the functional part of the genome remaining after exposure of the phage to ionizing radiation. Under certain specific conditions, the kinetics of the loss of function of biological materials, when exposed to varying doses of different types of ionizing radiation, give a good estimate of the size and shape of the physical structure associated with the particular function. The method has been reviewed by Pollard et al. (7). T1 phage was used in these experiments for the following reasons: (1) It is able to withstand drying without being inactivated. This makes it suitable for radiation studies. (2) It is amenable to genetic analysis (Bresch, 8; Bresch and Mennigmann, 9). (3) The genetic information is apparently carried exclusively on its DNA (Christensen and Tolmach, 10). (4) Some of its properties have already been investigated with the radiation technique (e.g., Fluke and Pollard, 11; Pollard and Setlow, 12).

When these experiments were begun, it was not known that cross-reactivation of T1 was possible, and the first objective of the work was to demonstrate that the process could occur. A preliminary account of this demonstration has been pub-
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lished (13). The second objective was to utilize cross-reactivation as a means of detecting the functional portions of the genome.

MATERIALS AND METHODS

The phages employed were wild-type T1, along with a host-range mutant T1h and four plaque-type mutants, T1, p1, p2, p3, and p4. The bacterial stocks used consisted of E. coli strains B, B1, and S. A strain S-p isolated from the stock of S was also used.

Mutant stocks were broth lysates of E. coli B obtained by inoculating young log-phase bacteria with mutant phage picked from a young plaque of the appropriate mutant type appearing on agar plates. The lysates, consisting of $5 \times 10^9$ infectious particles/ml, were centrifuged to remove bacterial debris and stored in the refrigerator, where they appeared to retain their initial titer for more than a month. The bacteria usually were log-phase cultures grown with constant agitation at 37° C in 0.8 % nutrient broth.

The plating media were as follows: Bottom layer agar; Bacto-agar, 10 gm; Bacto-tryptone, 13 gm; NaCl, 1 gm; distilled H2O, 1 liter. Top layer agar: Bacto-agar, 6 gm; Bacto-tryptone, 10 gm; NaCl, 1 gm; glucose, 3 gm; distilled water, 1 liter. The low concentration of agar in the overlay medium resulted in large T1 plaques, enabling the plaque-type mutants to be scored with greater certainty.

The procedure followed in carrying out phage irradiations was as follows: 5/100-ml aliquots from a T1 stock were deposited onto clean glass cover slips 1 cm in diameter, ten such cover slips being mounted on a brass bombardment plate. The samples were vacuum-dried at room temperature, and the dry samples were given varying doses of ionizing radiation. The setup for cyclotron irradiation using charged particles has been described (7). The samples were then resuspended in broth and stored in the refrigerator until assayed. The bombarded samples appeared to be as stable in the refrigerator as the original stocks.

For X-irradiation, a G.E. Maxitron 250-kv machine was used, operated at 250-kv peak voltage and 30-ma tube current, with no filtration of the beam except that due to the tube itself. The target-to-sample distance employed was 12.3 cm. Under these conditions, the dose rate at the sample as measured with a Victoreen 250-rionization chamber was 2740 r/min. Bacterial irradiations were carried out by placing 2 ml of a broth culture of E. coli B in an uncovered 60-mm petri dish and exposing it to X-rays, aliquots of $\frac{1}{10}$ ml being extracted periodically for assay.

The procedure for cross-reactivation was as follows: Irradiated phage at the multiplicity of infection not exceeding 0.3 and active phage of a different genotype

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3 We are indebted to Dr. N. Melechen of the Department of Genetics, Carnegie Institute of Washington, Cold Spring Harbor, New York, for sending us the stock containing p2, and to Dr. Ethel Tessman of the Department of Nuclear Studies, Cornell University, Ithaca, New York, for p1 and p4, called by her 2E and 21B, respectively.
("carrier phage") at a multiplicity of 3 to 4 were attached to cells of *E. coli* B, in the presence of KCN, which inhibits phage development. In most experiments the final concentrations in the absorption tube were approximately as follows: bacteria, $1 \times 10^8$ cells/ml; KCN, $2 \times 10^{-3} M$; irradiated phage, $5 \times 10^6$ particles/ml; active phage, $1 \times 10^9$ particles/ml. The multiplicities used were arranged such that, if the phage were distributed among the bacteria randomly, less than 5% of the infected bacteria had more than one phage of the irradiated type, and also less than 5% of the infected bacteria had no carrier phage. The cells were left in the absorption tube at $30^\circ C$ until attachment of the phage to the cells was 70% to 80% complete, at which point the unattached phage were removed either by centrifugation or by using anti-T1-serum.

The samples were then diluted an appropriate factor, and suitable aliquots were either plated before bursting against an appropriate bacterial strain (infective-center assay) or else distributed among a large number of tubes, such that each tube contained, on the average, one bacterium capable of releasing active phage (single-burst assay). After 90 minutes at room temperature, the tubes of the single-burst assay were plated in the usual way.

Plates were incubated overnight at room temperature and then scored for the number and type of plaques present.

In each experiment, determinations were made of the number of infected bacteria, the number of unattached phage, and the number of surviving bacteria. The bacterial concentration in the original bacterial stock was determined by colony counts on agar plates.

In each cross-reactivation experiment, controls were run in which the carrier phage had been replaced by an equal quantity of broth. A control containing carrier phage but no irradiated phage was also run.

It may be noted that the number of carrier phage considerably outnumbers the number of irradiated phage present. This is especially true for the heavily irradiated phage, where the carrier phage outnumber the viable phage remaining in the irradiated sample by several powers of 10. To prevent the plaques of the irradiated phage on the plates from being swamped by carrier plaques, it was usually arranged that the irradiated phage carried the host-range (h) marker, and the final plating was done either on *E. coli* B/1 alone or on a mixture of B and B/1. Since only phage carrying the h marker are able to form plaques on *E. coli* B/1, the presence of the irradiated T1h phage could be detected readily.

When a marker other than h was irradiated, plating was done on *E. coli* S-p, derived from strain S. On this bacterial strain the plaque-type mutants form smaller plaques than on strain B, and in particular, T1p1 forms very small plaques with a low plating efficiency. Wild-type plaques, on the other hand, are of nearly normal size on strain S-p and can be scored in the presence of many p-mutant plaques with much greater ease than when *E. coli* B is used as the plating bacterium.
RESULTS

Detection of Cross-Reactivation

The results of the first series of experiments which showed the presence of cross-reactivation of T1 are shown in Fig. 1. With deuteron-bombarded T1h as the minority parent and T1p as the active majority (carrier) parent, the upper curve was obtained for the number of plaques appearing on B/1. For the controls, where broth was added instead of carrier, the lower curve was obtained. The difference between these two curves at higher doses may be attributed to the occurrence of cross-reactivation. To verify this finding, the single-burst assay was utilized.

![Graph showing the number of infective centers plated on bacteria B/1 resistant to T1p, but not to T1h, when T1h has been irradiated by varying numbers of deuterons. The lower curve was found when only T1h was used to infected, the upper when T1h and T1p were used in a mixed infection. The unirradiated T1p cross-reactivates some of the irradiated T1h, giving an increased number of infective centers.](image)
The variation of the number of bursts showing the h marker (upper line) and showing hp2 recombinants (lower line) with the dose of protons. The upper curve has two components, one which is less sensitive and which has the same sensitivity as the recombinant, and a more sensitive component. In the absence of carrier phage the less-sensitive component is not observed.

Tlh was irradiated with protons having a linear energy transfer (LET) of 60 ev/100 A, and Tlp2 was used as the carrier phage. The results are given in Fig. 2. The upper curve shows the variation with radiation dose of the number of bursts after mixed infection which show the presence of the h marker, relative to the number for no dose. This curve shows a change in slope with increasing dose. If carrier is not present, the change in slope does not occur. The lower curve is a plot of the survival as a function of dose of those bursts which show the presence of hp2 recombinants, relative to the total number showing the h marker for no dose (i.e., relative to the dose control of the upper curve). The h marker was detected by plating on E. coli B/1, which is resistant to Tlh+. Thus, of the four possible types
of phage—hp₂⁺ (irradiated parent), h₊p₂ (carrier), and the recombinants hp₂ and h₊p₂—only the irradiated parent hp₂⁺ and the recombinant hp₂ can form plaques on *E. coli* B/1. The h₊p₂⁺ (wild-type) recombinants do not form plaques and so were not scored in this experiment.

In computing the number of single bursts containing the h marker, and the number containing hp₂ recombinants, the usual assumption was made that these bursts were distributed among the tubes of the single-burst assay according to the Poisson formula.

The change in slope of the upper curve of Fig. 2 represents an increase in the survival of the irradiated phage in the presence of active carrier phage, since this change in slope does not occur if the carrier phage are absent. Thus cross-reactivation is indeed occurring, and the point where the curve changes slope represents the point where cross-reactivated phage predominate in the progeny bursts.

The second part of the upper curve, which represents those bursts containing cross-reactivated survivors, also shows a definite slope, which is the same as the slope of the curve for those bursts showing recombinants.

This implies that cross-reactivation is accomplished through a similar mechanism to that which leads to the appearance of recombinants, since both processes show a similar sensitivity to radiation damage. Also, if the recombinants in the progeny of unirradiated phage were formed by a different mechanism to those arising after cross-reactivation, one would expect the lower curve of Fig. 2 also to show some sort of change in slope with increasing dose.

The exponential dose-survival curves of Fig. 2 may be fitted with the expression \( \ln(N/\text{No}) = -SD \), where \( N/\text{No} \) is the fraction of the irradiated phage sample which retains some specific function after receiving a radiation dose of \( D \) particles/cm². Physically, it represents the “cross section,” or sensitive area, presented to the beam of radiation by that structural element of the dried phage particle which is associated with the function being studied. Thus, from the curves of Fig. 2, cross sections may be calculated for the two functions involved, namely, the infectivity, which is the ability of the phage to multiply independently, and cross-reactivation, defined as the ability of the phage, even though it cannot multiply independently, to donate its markers to the progeny of mixed infection with active phage. The data of Fig. 2 yield a value of 0.6 ± 0.1 for the ratio of the cross section for cross-reactivation to the cross section for infectivity. This suggests that the physical area of the total radiation-sensitive structure involved in the cross-reactivation process is 0.6 that of the total radiation-sensitive structure involved in infectivity.

*Dependence of Cross Sections on Linear Energy Transfer (LET)*

Varying the LET of the charged particles used in the irradiation gives further information concerning the size and shape of radiation-sensitive structures. The
rate of energy loss of the particles was varied by placing aluminum foils of known thicknesses in the path of the beam in front of the phage sample. These foils reduce the energy of the particles incident on the sample and so increase their rate of energy loss. The effect of this procedure on the cross sections obtained for infectivity and cross-reactivation is shown in Fig. 3. The curves both rise in the same manner for increasing energy loss. Over the whole range, the cross sections for the cross-reactivated phage appear to be about 60% of the cross sections for infectivity, indicating that the ratio of cross sections is essentially independent of the nature and rate of energy loss of the radiation employed as an inactivating agent. These results were obtained by irradiating T1h with deuterons and α-particles from the Yale cyclotron, and with protons from the cyclotron at Brookhaven National Laboratories. Here the infective-center assay was used instead of the single-burst assay; i.e., the infected bacteria were plated directly, before bursting, against E. coli B/1, and the plaques scored as to plaque type. These plaques represent mixed bursts and may contain both parental and recombinant phage types. It proved possible from the plaque morphology to score the plaques unequivocally for the presence or absence of recombinant-type phage, and the survival curves thus obtained and previously reported (13) are identical with those of Fig. 2 obtained by the single-burst method. Since the single-burst assay is very tedious, the infective-center assay was used whenever possible. The latter technique is in essence the same as that used by Stent (3) in doing cross-reactivation of T2 after inactivation by the decay of incorporated $^{32}$P.

In the above experiments, only one class of recombinants, the $h_{p2}$ types, was scored. There is the possibility that the other class of recombinants, the $h+_{p2}^+$...
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(wild-type) phage, is affected differently by radiation. To test this possibility, the infective-center assay was carried out, but instead of plating the infected bacteria on *E. coli* B/1 alone a "mixed indicator" of two parts B/1 to one part S-p was used. On this indicator, phage not possessing the h marker form cloudy plaques owing to the presence of the resistant B/1 bacteria, and wild-type recombinant plaques may be detected fairly easily even amid many carrier plaques.

The results obtained for the h+p2+ recombinants were a good deal less reliable than those obtained for the hp2 recombinants, owing to the possibility of back-mutation to the wild type on the part of the parental phage. However, the survival curve obtained for the phenotypically wild-type recombinants led to a cross section which was the same fraction of the infectivity cross section as was obtained for the other recombinant class, i.e., 0.6 ± 0.1.

In addition, the number of wild-type recombinants was scored as a function of dose of radiation delivered to a mutant phage other than T1h. With deuterons having a LET of 240 ev/100 A, first T1p1 was irradiated and T1p2 used as the carrier, then T1p2 was irradiated and T1p1 used as the carrier. A ratio of cross sections of 0.6 ± 0.1 was obtained in both cases, indicating that this ratio is not dependent on the particular marker irradiated. It is also not dependent on the particular mutant phage used as the carrier. When T1h was irradiated, the same ratio was obtained when each of T1p2, p2, p3, and p4 was in turn the carrier. The ratio of cross sections also appears to be insensitive to the multiplicity of infection.

**Recombination Frequency**

The cross-reactivation process leads to a considerable increase in the fraction of bursts showing the presence of the h marker which also show the presence of hp2 recombinants. Figure 4 shows a plot of this fraction (designated R.F. for "recombinant fraction") as a function of radiation dose, from the data of Fig. 2. Since only phage bearing the h marker and not the h+p2+ phage were scored in this experiment, a true recombination frequency could not be measured directly. A true recombination frequency can be found by making use of the equation of Visconti and Delbrück (14). If we set X as the number of recombinants of the hp2 type, the total number present will include h+p2+ and will be 2X. If U and Z are the numbers of majority and minority parents, respectively, in the progeny, then after an average of m matings we have, by measurement, the frequencies of a parental type $a_{22}^{(m)}$ and a recombinant type $a_{12}^{(m)}$ as

$$a_{22}^{(m)} = \frac{Z}{U + Z + 2X}; \quad a_{12}^{(m)} = \frac{X}{U + Z + 2X}$$

Now the definition of recombination frequency is

$$\frac{100(2X)}{U + Z + 2X} = 200a_{12}^{(m)}$$
Fig. 4. The *recombinant fraction*, the ratio of plates showing hp₂ to the plates showing h, plotted as a function of the dose delivered to the h parent. An approximate correction to give the usual *recombination frequency* can be made by multiplying by a factor of 5.

whereas the recombinant fraction used is a measure of the number of bursts containing the hp₂ recombinant divided by the number of bursts showing the h marker. Since the average number of recombinants per burst (*Bhp₂*) was measured, and the average phage showing the h marker per burst (*Bh*), the “recombinant fraction” can be related to the quantities above by the relation

\[
\frac{B_{hp₂}}{B_{h}} \text{ (R.F.)} = \frac{X}{Z + X}
\]

Now equations 10b in Visconti and Delbrück’s paper give

\[
a_{22}^{(m)} = \frac{1}{4} [(1 - f)^2 + (1 - f^2)e^{-mp}]
\]

\[
a_{12}^{(m)} = \frac{1}{4} [(1 - f^2) + (1 - e^{-mp})]
\]

where \( f = 2a_{11} - 1 = 1 - 2a_{22} \), \( a_{11} \) being the fraction of majority and minority parental phage in the original infection, and \( p \) the probability that a recombination between the two factors occurs during the mating of two particles in the pool. Now

\[
\frac{X}{Z + X} = \frac{a_{12}^{(m)}}{a_{22}^{(m)} + a_{12}^{(m)}}
\]
and by using the two equations above we can find the ratio of \( \frac{2X}{Z + U + 2X} \) to \( \frac{X}{Z + X} \) to obtain the result \( \frac{X}{Z + X} \left/ \frac{2X}{Z + U + 2X} \right. = 1 - f. \)

If we define \( ma \) as the average number of majority parent per mixedly infected cell, and \( mb \) as the average number of minority parents per cell, we have

\[
\begin{align*}
\alpha_{11} &= \frac{ma}{ma + mb} \\
\alpha_{22} &= \frac{mb}{ma + mb}
\end{align*}
\]

If \( M = \frac{ma}{mb} = \alpha_{11}/\alpha_{22} \), then \( f = \frac{(M - 1)}{(M + 1)} \). Thus we can "correct" the recombinant fraction to give the usual recombination frequency. In Fig. 4, an approximate recombination frequency can be obtained by multiplying the recombinant fraction by a constant factor of 5.

**Test for Phenotypic Mixing**

The cross-reactivation experiments were done under conditions in which phenotypic mixing would be expected to occur as found by Novick and Szilard (15). If the extent of its occurrence were for some reason dependent on the dose of radiation delivered, then the ratio of cross sections observed would be erroneous. This was tested by irradiating Tlh with X-rays, reactivating it with Tlp2 carrier, and plating the infected bacteria before bursting on E. coli B/1 and on the "mixed indicator" of B/i plus S-p.

Those phage which have suffered phenotypic mixing, i.e., which are genotypically h+ and phenotypically h, or vice versa, will form plaques on the mixed indicator but not on B/1 alone. A sample of the results obtained is given in Table I.

In the table \( Nh \) represents the total number of plaques showing the h marker,

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>PHENOTYPIC MIXING IN NORMAL AND IRRADIATED SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Number of plaques</td>
</tr>
<tr>
<td>No. lethal hits</td>
<td>7.5 lethal hits</td>
</tr>
<tr>
<td>Carrier present</td>
<td></td>
</tr>
<tr>
<td>(1) B/1 alone Nh</td>
<td>103 ( \times 10^5 )</td>
</tr>
<tr>
<td>( Nh_{p2} )</td>
<td>15 ( \times 10^4 )</td>
</tr>
<tr>
<td>(2) Mixed indicator Nh</td>
<td>206 ( \times 10^5 )</td>
</tr>
<tr>
<td>( Nh_{p2} )</td>
<td>20 ( \times 10^5 )</td>
</tr>
<tr>
<td>No carrier</td>
<td></td>
</tr>
<tr>
<td>(3) B/1 alone Nh</td>
<td>249 ( \times 10^4 )</td>
</tr>
<tr>
<td>(4) Mixed indicator Nh</td>
<td>209 ( \times 10^5 )</td>
</tr>
</tbody>
</table>
and $N_{hp_2}$ represents the total number of plaques showing the presence of $hp_2$ recombinants. A "lethal hit" is that dose which under the same conditions reduces the infectivity of the phage to 37% of its initial value.

The values of Table I are typical of those found in a cross-reactivation experiment. The difference between (1) and (2) can be attributed to phenotypic mixing. This amounts to approximately 50%, indicating that about half of those bursts which contain the h genotype show only the h+ phenotype and so cannot yield plaques on B/1. Though phenotypic mixing is apparently occurring, however, it should have no effect on the survival curves, since the fraction of bursts showing only the h+ phenotype appears not to have changed markedly even after a radiation dose equivalent to 8 lethal hits per phage particle has been delivered to the sample.

**The Effect of Irradiating the Bacteria**

To see if irradiating the host bacteria had any effect on the cross-reactivation process, samples of log-phase *E. coli* B grown in broth at 37°C with constant agitation were given a dose of X-rays and then used to carry out the infective-center

![Graph](image)

**Fig. 5.** A plot of the burst-size distribution for all phage bearing the h marker. The accumulated number of bursts, $Y_x$, is plotted versus the burst size, $x$. The relationship $xY_x = \text{constant}$ is obeyed for the cross-reactivated irradiated h markers and can be interpreted to mean that the multiplication of these is exponential after the first completed form is made.
assay for cross-reactivation. For a given dose to the bacterium, it was found that varying doses delivered to the free h mutant had the same effect on infectivity and cross-reactivation as was found if the bacteria were not irradiated. The survival curves were similar to those of Fig. 1 and showed a ratio of cross sections of 0.6 ± 0.1, for any dose of X-rays delivered to the bacteria, up to the maximum dose used, which was equivalent to 6 phage lethal hits. This result is in contrast to the results found by Tessman (16) for ultraviolet-inactivated T1, where a change in the slope of the survival curve followed after irradiation of the host bacteria.

**Burst-Size Distributions**

The single-burst analysis which provided the results shown in Fig. 2 also yielded some data concerning the burst-size distributions for irradiated phage which have undergone cross-reactivation. In Fig. 5 is shown the burst-size distributions obtained for those phage showing the presence of the h marker. The upper curve is for unirradiated phage, and the lower curve is for a radiation dose such that cross-reactivation predominates, i.e., below the bend in the upper curve of Fig. 2. The effect on the burst-size distribution is considerable, although the mean burst size does not change appreciably, as is shown in Table II. In view of the shift in distribution, the geometric mean burst size would appear to be more significant. This point has been discussed by Doermann et al. (4) and Stahl (6). These authors have also discussed the possible significance of the shift in the distributions.

In Fig. 6 is shown the burst-size distributions for the hp\text{2} recombinants. The mean burst sizes are given in Table II. It will be noted that the upper curve, for no dose, has an arithmetic mean burst size of 1.7. If the distribution about this mean is calculated from the Poisson formula, the dotted curve is obtained.

The lower curve of Fig. 6 is for a dose delivered to the h parent such that cross-reactivation predominates.

The type of plot shown in Figs. 5 and 6 was first used by Luria (17) in a study of the burst sizes of spontaneous mutants arising during the infectious cycle. If

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>MEANS OF BURST SIZES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class</strong></td>
<td><strong>Burst size</strong></td>
</tr>
<tr>
<td>(1) Any phage carrying the h marker:</td>
<td></td>
</tr>
<tr>
<td>h parent unirradiated</td>
<td></td>
</tr>
<tr>
<td>h parent irradiated</td>
<td></td>
</tr>
<tr>
<td>(2) hp\text{2} recombinants:</td>
<td></td>
</tr>
<tr>
<td>h parent unirradiated</td>
<td></td>
</tr>
<tr>
<td>h parent irradiated</td>
<td></td>
</tr>
</tbody>
</table>
the method of reproduction of a new phage form is exponential, then the relation between the number, \( x \), of new forms in a burst and frequency, \( y_x \), of such bursts is

\[
y_x = \frac{mN}{x}
\]

where \( m \) is a probability of formation of a new form, and \( N \) is the total number in the population. For the special case of exponential multiplication, \( x = 2^k \), where \( k \) is an integer, and for the accumulated number, \( Y_x \), of bursts with \( x \) or more new forms we find \( Y_x = 2mN/x \). Then the product \( xY_x \) should be a constant. This appears on a log \( Y_x \) versus \( x \) plot, as is given in Fig. 5, as a straight line with a downward slope of 45°. Luria used this expression to analyze the number and size of clones of mutants and concluded that multiplication is exponential after the formation of a mutant. Since this same clonal distribution is obtained for cross-reactivated phage, a similar interpretation would appear to be applicable.

It will be noticed that the mean burst sizes in these experiments were very small. Attempts to increase the mean burst size by varying the experimental conditions met with little success. It is believed that the small bursts are at least partly due to
the fact that the plaque-type mutants used as carrier phage all form small plaques compared to the wild type and so would be expected to have smaller burst sizes.

**Linkage Distances**

An estimate of the linkage distances between the five markers utilized was obtained by means of two factor crosses. The linkage map obtained is shown in Fig. 7. Since the ratio of cross sections under study was found to be independent of which particular marker was present in the carrier phage, it was felt to be sufficient to demonstrate that the particular plaque-type markers used did actually represent a range of linkage distances, and beyond this point no special pains were taken to construct an accurate linkage map.

**DISCUSSION**

It is reasonable to assume that radiation interferes with the cross-reactivation process by damaging the DNA of the phage, probably by fragmenting the DNA fiber. P$^{32}$ suicide and ionizing radiation appear to be similar in this respect, as shown by Stent and Fuerst (18) and Stahl (6). Ultraviolet light appears to inflict a different sort of damage. The data obtained by varying the LET of the radiation beam supports the hypothesis of DNA damage. The fact that the curves of Fig. 3 are straight lines over the entire range of LET employed implies that the radiation-sensitive region is very long and thin. Otherwise, the cross-section curves would have leveled off at some maximum value. The curves of Fig. 3 are compatible with the known dimensions of the DNA macromolecular fiber.

The fact that the ratio of the cross section for cross-reactivation to the cross section for infectivity is a constant over a wide range of LET shows that the two functional activities have a common structural basis. That is, if the loss of infectivity is due to radiation damage to the DNA of the phage, then so is the loss of the ability to be cross-reactivated.

The value obtained for the ratio of cross sections, 0.6 ± 0.1, may be interpreted as representing that fraction of the functional DNA of the phage which must remain undamaged in order for cross-reactivation to occur. To postulate a reason why the value should be as high as 0.6 is a more difficult matter. A similarly large cross section was found by Stent (3) for cross-reactivation of T2 and T4 after inactivation by the decay of incorporated P$^{32}$. Stahl (6) found similar results. Stent has put forward two hypotheses to explain his cross-reactivation data:

1. The disappearance of a genetic locus from the progeny may be due to damage
to the locus itself, or to the section of the linkage group of which this locus forms a part.

2. The damage may occur elsewhere on the genome, but the mechanism of the cross-reactivation process, whatever it may be, fails to separate the locus from the damage, so the locus is not seen.

Both these hypotheses assume that the damage is to the genetic material of the phage.

The relatively large size of the cross section for cross-reactivation would seem to rule out the possibility that damage is to the locus itself. Results such as those of Benzer (19) would indicate that a locus is much smaller than these sizes. On the other hand, as Stent (3) has pointed out, if the second hypothesis is correct, one would expect the probability of elimination of a marker to be dependent on those experimental conditions which affect the cross-reactivation mechanism. If this is a mechanism similar to that involved in the process of genetic recombination, the survival curves should be strongly influenced by the multiplicity of infection and the linkage distance between the markers used. This was not found to be the case.

It would therefore appear that the size of the cross section for cross-reactivation represents the size of a section of the linkage group of which the locus in question forms a part. It is possible that this large linkage group is in fact the large single molecule of DNA that has been detected in T2 by Levinthal (20), which is apparently passed on intact from parent to progeny. If this were so, it would mean that damage to this molecule prevents cross-reactivation of the phage carrying it.

It should be stressed, however, that one cannot, from our observations, rule out any of several alternative explanations for the observed cross section for cross-reactivation. For example, it is possible that radiation impairs the mechanism for injection of DNA into the cell, so that the observed cross section may be a reflection of the inability of part of the damaged genome to enter the cell.

As was mentioned earlier, the fact that the survival curve for the recombinants appearing after cross-reactivation shows no change in slope over a wide range of radiation dose suggests that there is only one mechanism for the formation of recombinants, or, at least, if there are two or more mechanisms, that they all have the same radiation sensitivity. This means that the process of cross-reactivation is such that it allows the normal mechanism for the formation of recombinants to operate.

Even though the mechanism for the formation of recombinants may not change, however, the kinetics of their formation is certainly influenced by the cross-reactivation process. This influence is reflected in the shift in the burst-size distribution of the recombinant phage from a nearly random distribution of burst sizes to a clonal distribution, and in the increase in recombination frequency that is observed. In the progeny of mixed infection with active and irradiated phage, recombinant types appear to have some sort of selective advantage over nonrecombinants.
To explain this, Stahl (6) has assumed that, in the case of P32 suicide, the genome of T4 is fragmented and the fragments are incorporated into the replicating carrier phage, thus forming an increased number of recombinants. It is difficult to see how this model can be made compatible with the observed survival curves obtained for cross-reactivated phage. One would expect the survival curves to be of the "multiple-hit" type and to show a considerably smaller cross section than is observed.

A similar model was put forward by Jacob and Wollman (5) on the basis of their work on the effect of ultraviolet on phage λ. In view of the apparent difference in mechanism of action of ultraviolet and ionizing radiation (18), one must exercise caution in comparing their effects. One can at least say, however, that the cross-reactivation processes in T1, T4, and λ do appear to be qualitatively similar.

As is the case for the multiplicity reactivation process as observed by Dulbecco (21) and Harm (22), it would seem that no satisfactory hypothesis has yet been put forward to explain the survival curves obtained for cross-reactivated phage.

SUMMARY

Cross-reactivation has been shown to occur for mutants of T1 bacteriophage that have been inactivated by exposure in the dry state to various kinds of ionizing radiation. A host-range mutant and four different plaque-type mutants were used. The ability of the irradiated phage to be cross-reactivated has been found to have a sensitivity to ionizing radiation which is 0.6 ± 0.1 of the sensitivity observed for the ability of the phage to undergo independent multiplication. This ratio appears to be insensitive to the experimental conditions used. The results may be interpreted as indicating that about 60% of the material associated with the ability to multiply independently is involved in the cross-reactivation process. The possible significance of this finding is discussed.

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