ULTRAVIOLET IRRADIATION OF BACTERIOPHAGE DURING INTRACELLULAR GROWTH

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In the course of attempts to produce mutations in bacteriophage by ultraviolet irradiation of bacteria infected with the phage, we found that suppression of the ability of an infected bacterium to liberate phage required higher doses than either sterilization of noninfected bacteria or inactivation of free phage. The doses required to suppress phage liberation varied in the course of the interval between infection and liberation, during which intracellular phage growth takes place. These observations were in agreement with the fact that phage multiplication can take place in bacteria recently sterilized by irradiation (Anderson, 1944; Rouyer and Latarjet, 1946). They suggested that the observed effect of radiation on the infected bacteria might depend on inactivation of intracellular phage. Variations of this effect might then reflect the changes in number and properties of phage particles during intracellular growth—that is, in the course of processes that lead to the production of over 100 phage particles from each infected bacterium (Delbrück, 1946). An analysis of the changes in ultraviolet sensitivity during the period of intracellular growth could then be expected to supply information on the mechanism of growth.

The rate of ultraviolet inactivation of free phage is a simple exponential function of the dose of radiation (one-hit inactivation, see Latarjet and Wahl, 1945). If inactivation of the individual intracellular phage particles followed the same function, and if the ability of a bacterium to liberate phage depended on the survival in it of at least one active particle, then the number of intracellular particles per bacterium at the time of irradiation should influence the rate of suppression of phage liberation. Instead of an exponential one-hit inactivation curve, as for free phage, we should find for the infected bacteria a multiple-hit curve, the number of hits reflecting the number of active particles present at the time of irradiation.

The feasibility of this analysis was suggested by some preliminary observations of this type by Anderson (1944). His data, which he kindly discussed with us, seemed to indicate a shift from one-hit to multiple-hit type of curve in the inactivation curves for phage-infected bacteria during intracellular phage growth.

The process of intracellular phage growth—in particular, of the kinetics of phage production—has so far escaped every attempt at clarification made either by breaking down infected bacteria or by electron microscopy. This problem is of

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such fundamental interest that we considered it worth while to explore thoroughly the new lead which ultraviolet irradiation seemed to offer. The results of this investigation are discussed in the present paper.

MATERIAL AND TECHNIQUE

Escherichia coli, strain B, and bacteriophage T2 were used. Some experiments with bacteriophage T7 and with *Escherichia coli*, strain B/r, were also done. Bacteria and phages were grown in an ammonium glucose phosphate buffer medium with salt complements, chosen for its transparence to ultraviolet light. In this medium *Escherichia coli* B grows with a generation time of 40 minutes at 37 C; phage T2, in the presence of a standard young culture of B, has a latent period of 21 minutes between infection and the beginning of liberation; liberation is complete 35 to 40 minutes after infection; the average phage yield per bacterium is 100 to 150. Phage T7 has a latent period of 13 to 14 minutes, complete liberation before 25 minutes, and a yield of 40 to 60. The values for time of liberation and phage yield are less reproducible in our medium than in nutrient broth.

The experimental technique for irradiation of growing phage is as follows: All suspensions are maintained in a water bath at 37 C. The desired amount of phage lysate (titer measured by plaque count) is introduced into an aerated bacterial culture containing 5 to 10 $\times$ 10^7 bacteria per ml, as assayed by viable count. After allowing a definite short time for phage adsorption (generally 30 to 60 seconds), a sample of the mixture is diluted in a solution of antiphage serum capable of inactivating in 30 to 60 seconds all the remaining free phage. This does not affect the course of phage growth inside the infected bacteria (Delbrück, 1945*b*). A further heavy dilution in serum-free medium, to give a serum concentration without appreciable absorption of ultraviolet, yields a suspension of bacteria part or all of which are infected by phage. The proportion of infected bacteria and the average number of phage particles adsorbed per bacterium can easily be regulated by varying the initial proportions of phage and bacteria. When only a fraction of the bacteria is infected, each infected bacterium will adsorb only one phage particle ("single infection"). When most or all bacteria adsorb several phage particles, we speak of "multiple infection."

The number of infected bacteria is measured by plaque count, by plating a sample on agar with an excess of sensitive bacteria. Each infected cell gives one plaque up to the time when phage liberation begins. The infected bacteria, as numbered by plaque count during the latent period, will hereafter be defined as "infective centers."

Samples of the final highly diluted suspension of infective centers are taken at intervals and exposed to radiation within 45 seconds from the time of sampling. The time of irradiation is always given as the time when exposure begins, although some exposures lasted as long as 80 seconds. The irradiated samples are immediately assayed to determine the proportion of infective centers still capable of producing plaques. When the phage yield is to be determined, the samples are quickly returned to a temperature of 37 C and assayed at intervals,

a nonirradiated sample which has undergone the same manipulations as the irradiated ones being used as a control.

The source of radiation was a General Electric germicidal lamp, of the low-pressure type; 80 per cent of its ultraviolet output consisted of wave length 2,537 A. As this radiation is especially efficient in bacterial sterilization and phage inactivation (Gates, 1934), more than 95 per cent of the effects were due to it. We could therefore consider our ultraviolet beam as almost monochromatic. Its intensity was measured by comparison with the beam from a similar lamp calibrated in absolute units by Dr. A. Hollaender, and it was recalibrated at frequent intervals.

In most experiments the samples were exposed at a distance of 56 cm from the bulb and received a uniform flux of 16 ergs $\times$ mm^{-2} $\times$ sec^{-1} for the wave length 2,537 A. Variations in intensity, when desired, were obtained by varying the distance from the bulb, the dependence of intensity upon distance having been determined by suitable photoelectric measurements.

The samples were irradiated in open dishes 5 cm in diameter. The depth of the suspensions was about 1 mm, the bacterial concentration not higher than 10^4 per ml. Under such conditions, no mutual screening of bacteria took place, and the dose was uniform throughout the sample.

RESULTS

Killing of noninfected bacteria and of free phage.[3] The survival curve of B in synthetic medium was determined down to a survival of 10^{-4}, using clear, non-screening suspensions. The results (curve 1, figure 1) show that the killing is an exponential function of the dose. As observed by Witkin (1946), a change in the slope of the curve appears for survivals less than 10^{-2}, as if 1 per cent of the bacteria had higher resistance.

The survival curve of T2 was determined down to a survival of 10^{-5}, and proved to be a regular exponential function of the dose (curve 2, figure 1). Our results agree to within 2 per cent with those previously obtained in Paris by Latarjet and Wahl (1945) using a calibrated beam of 2,537 A. This agreement shows the reliability of spectrophotometric ultraviolet measurements in absolute units and the value of phage inactivation as a biological test for estimating the intensity of monochromatic ultraviolet light. The survival of the more resistant phage T7 is given in curve 4 of figure 1.

Evidence that killing of infective centers is due to killing of intracellular phage. This evidence was brought out by several observations. First, if killing of an infective center results from direct action on the phage, the killing curve for single-infected bacteria immediately after infection, before any growth takes place, will be very similar to that of free phage. The only expected difference will be a slight increase in resistance due to possible screening of the phage particle by a layer of bacterial protoplasm. The killing curve for B single-

[3] The term "killing" is used for convenience throughout this paper. The more precise expression would be, in the case of bacteria, "sterilization"; in the case of free phage, "inactivation"; and in the case of infected bacteria, "loss of ability to liberate active phage."

infected with T2, two minutes after infection (curve 3, figure 1), is actually very similar to that of free phage. That this is not a fortuitous coincidence is shown by the existence of the same relation for the more resistant phage T7 (curve 5, figure 1).[4]

Further evidence was obtained by using, instead of strain B, a mutant strain B/r, derived from B and much more resistant than B to radiation (Witkin, 1946).

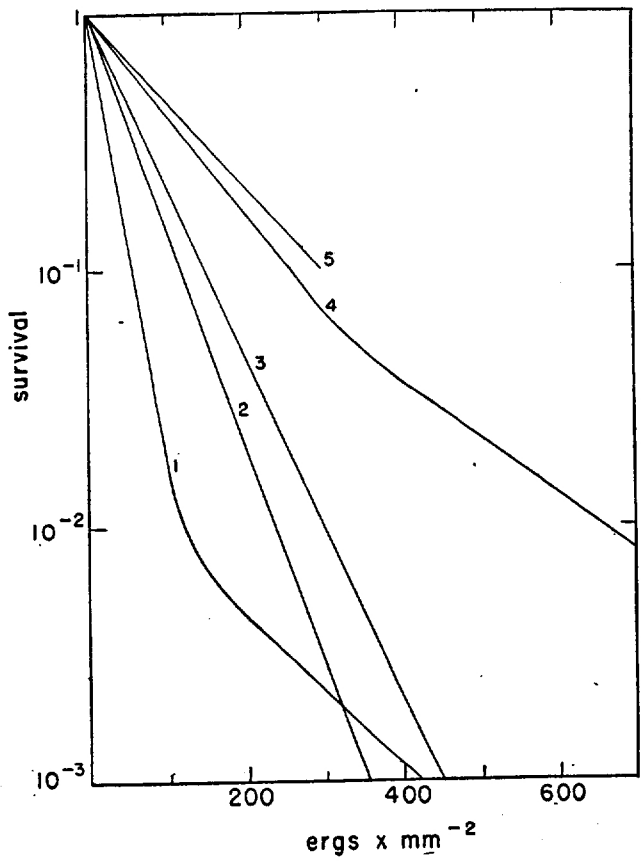

FIG. 1. SURVIVAL CURVES

(1) *Escherichia coli*, strain B. (2) Phage T2. (3) T2 infective centers, 2 minutes after single infection. (4) Phage T7. (5) T7 infective centers, 3 minutes after single infection.

The infective centers for T2 still showed the same resistance. The radiosensitivity of the bacterium itself, therefore, does not seem to determine the sensitivity of the infective centers.

Moreover, as will be seen later, multiple infection leads to "multiple-hit" survival curves for the infective centers, with multiplicity closely corresponding

[4] Further experiments with phage T7, similar to those described in the following sections for phage T2, were hampered by technical difficulties, mainly concerned with the use of concentrated antiphage serum, and will not be discussed in this paper.

to the average number of phage particles adsorbed per bacterium. We can conclude, therefore, that killing of infective centers results from killing of intracellular phage, at least at the beginning of the latent period.

Survival curves of infective centers in the case of single infection. Figure 2 shows the results of a typical experiment in which one out of every two or three bacteria was infected with one T2 phage particle. The suspension of infective centers

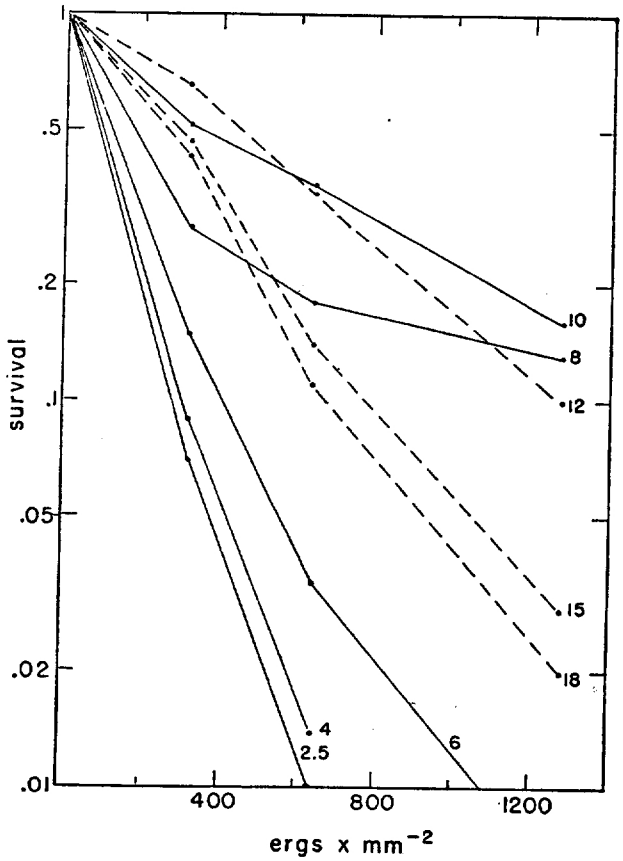

FIG. 2. SURVIVAL CURVES OF T2 INFECTIVE CENTERS AT VARIOUS TIMES

Data from a single experiment. The numbers indicate the time of irradiation (minutes after infection).

was irradiated at various times (2.5, 4, 6, 8, 10, 12, 15, and 18 minutes) after infection. At each time three doses (320, 640, and 1,280 ergs $\times$ mm^{-2}) were given in 20, 40, and 80 seconds, respectively. The curves give, in semilogarithmic co-ordinates, the survival of infective centers as a function of the dose at each time. They illustrate the regularity of the results within one experiment.

A large number of comparable experiments were performed, in which the proportion of infected bacteria was between $\frac{1}{2}$ and $\frac{1}{10}$. Doses from 50 to

1,180 ergs $\times$ mm^{-2} were given at various times. Altogether, 242 experimental values were thus collected, covering almost every minute of the latent period for a variety of doses. Values for the same dose given at the same time displayed some degree of variability, and were averaged graphically. The averages were used to construct the set of curves in figure 3, (a) and (b), in which survival is plotted in logarithmic scale as a function of the dose. The results can be described as follows:

The resistance of infective centers to radiation increases progressively during the first part of the latent period, up to 11 minutes. Early in this period (at

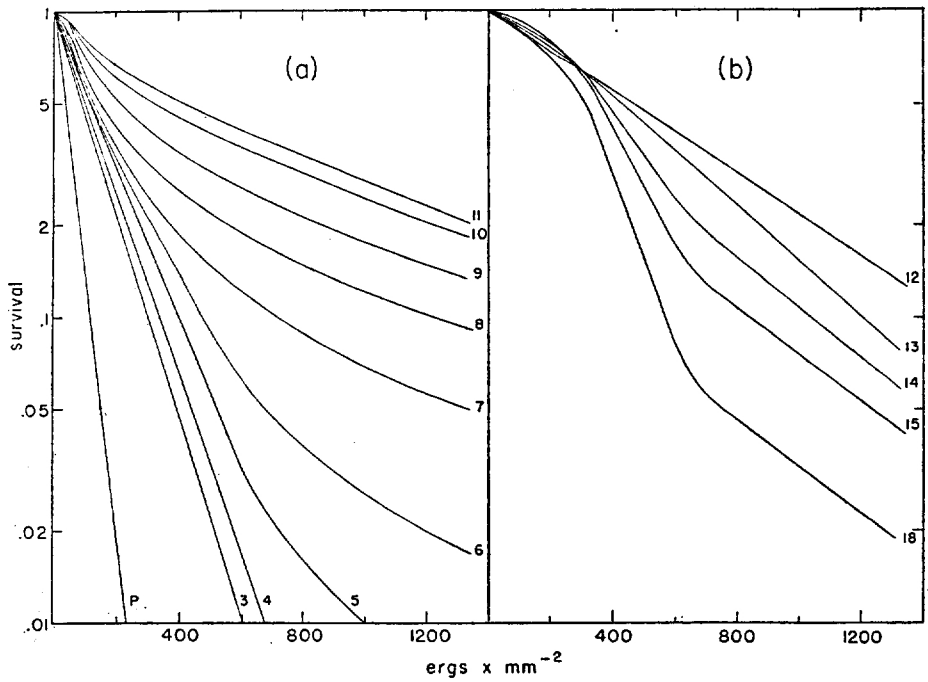

FIG. 3. SURVIVAL CURVES OF T2 INFECTIVE CENTERS

Averages of all the data from experiments with single infection, 1 out of 2 to 10 bacteria being infected. (a) Early times; (b) late times. The numbers indicate the time of irradiation. Curve *P* is the survival curve for free phage.

3 and 4 minutes) the survival curves are almost exponential, showing a slight increase in resistance with time. From 5 minutes on, however, survival for high doses increases more rapidly than for low doses, so that the successive curves show increasing amounts of upward concavity. From 7 minutes on, a slight downward concavity appears in the upper part of the curves, making them deviate more and more from simple exponentials.

After 11 to 12 minutes the general trend changes. Although the downward concavity for low doses becomes more pronounced, resistance to high doses drops progressively with time, until after 15 to 18 minutes the survival curves become more and more of the multiple-hit type (see figure 5). A discussion of these curves will be given in a later section.

Dependence of the survival curve for single infection on the proportion of infected bacteria. In the preceding experiments, in which 1 out of 2 to 10 bacteria was infected, the fluctuations of individual results were slight and nonsystematic. Significant deviations appeared, however, in experiments in which we infected only 1 out of 25 to 150 bacteria by introducing smaller amounts of T2 lysate into the bacterial culture. It is important to remember that in both cases we

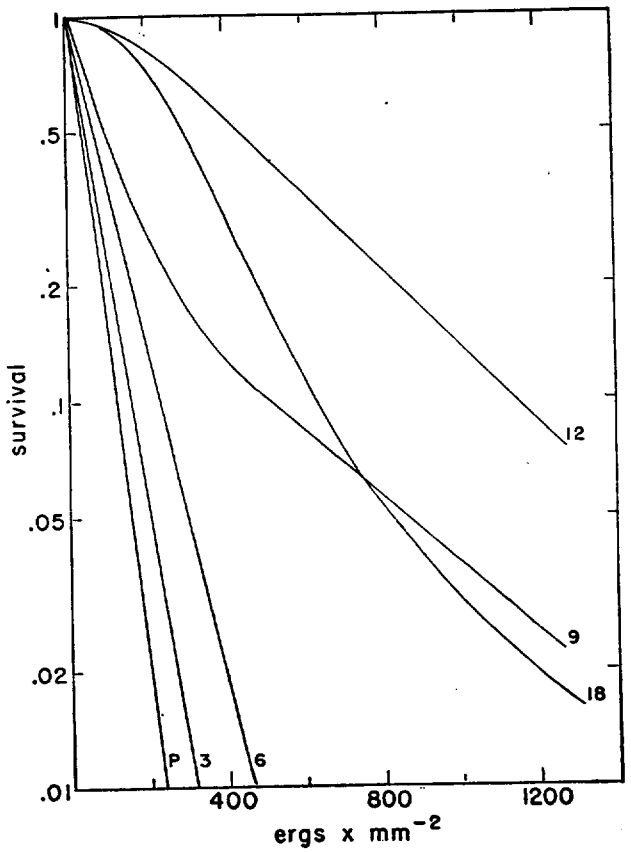

FIG. 4. SURVIVAL CURVES OF T2 INFECTIVE CENTERS

Averages of all the data from experiments with single infection, 1 out of 25 to 150 bacteria being infected. The numbers indicate the time of irradiation. Curve *P* is the survival curve for free phage.

have single infection of practically all of the infected bacteria. The probability that any one bacterium will adsorb two or more phage particles is very low whenever the average number of particles adsorbed per bacterium is much less than one. Length of the latent period and yield of phage per bacterium are the same in both cases.

Numerous experiments, performed with low proportions of infection, gave consistent results. Altogether, 153 experimental values were collected and averaged; the survival curves are given in figure 4. Comparison of figures 3 and 4 shows the following facts:

Both sets of curves show the same trends, with an early increase in resistance and, for high doses, a late decrease. Moreover, from 12 minutes on, the curves in the two sets are practically identical. Striking differences are present, however, in the survival curves for early times. When the proportion of infected bacteria is low, the sensitivity to radiation in the first minutes is greater, and the increase in resistance progresses more slowly. It appears that some process responsible for the changes is delayed. For example, the survival curve for 6 minutes is still practically exponential, similar to that for 4 minutes in figure 3. The delay is compensated for by a swift increase after 6 to 7 minutes, so that by 12 minutes no difference remains.

What causes the more rapid increase in resistance in cases with a high proportion of infected bacteria? Experiments under a variety of conditions excluded the possibility of any influence of either the concentration of antiserum or the time during which the bacteria were exposed to it. Since experiments with low and high proportions of infection were comparable in every respect except for the amount of phage lysate introduced, we must conclude that the difference between the two cases is due to the presence in the lysate, and adsorption by the bacteria, of some other material besides the active phage. With larger amounts of lysate, enough of the unknown active material is probably introduced to exert its effect on part or all of the infected cells and change the rate of increase in resistance. The same phenomenon was found for all the lysates of phage T2 tested.

As the survival curves are the same for proportions of infection between 1:150 and 1:25, it is likely that in these cases the changes in resistance during the latent period are due to the action of the active phage only.

Phage yield from irradiated infective centers. In a number of experiments we measured the yield of phage from the infected bacteria irradiated at various times with different doses. Irradiation at any time produces a definite decrease in yield, between 20 and 50 per cent. No systematic dependence on the dose or the time of irradiation was found during most of the latent period. Infected bacteria irradiated at 15 minutes or later, however, yield only 10 to 20 per cent as much phage as the control. No appreciable delay in phage liberation is caused by the irradiation.

These results seem to indicate that, after irradiation, phage growth can continue in those bacteria where some active phage remains. If irradiation takes place very late, the surviving phage will have less time left to grow, and the yield will accordingly be smaller.

Multiple infection. We have presented evidence that ultraviolet killing of infective centers results from inactivation of the intracellular phage. The survival curve for single infection at very early times is close to the exponential curve for the survival of free phage. In the case of multiple infection each bacterium adsorbs several phage particles. If only one of these particles could penetrate the cell and grow (mutual exclusion, Delbrück, 1945c) the survival curves would be similar to those for single infection. If, however, several particles can grow in the same bacterium, the survival curves will be affected.

The survival function for single particles is $y = e^{-\alpha D}$, where y is the fractional

survival, D the dose, and α a constant defining sensitivity. If there are n active particles per bacterium, all with the same sensitivity, and if an infected bacterium is counted as one infective center as long as one phage particle at least remains active, the survival function of the infective centers will be

$$y = 1 - (1 - e^{-\alpha D})^n. \tag{1}$$

The corresponding set of curves for various values of n is given in figure 5.[5]

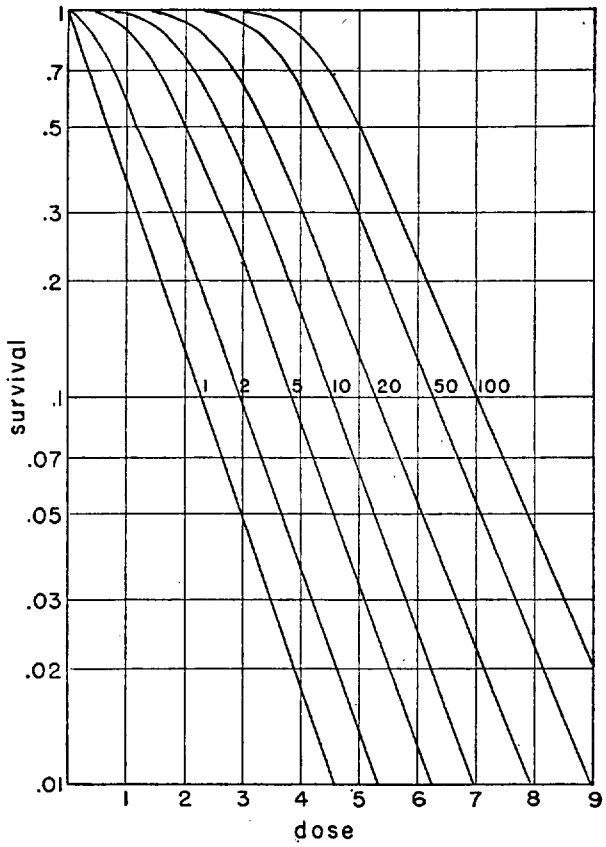

FIG. 5. THEORETICAL SURVIVAL CURVES CORRESPONDING TO FORMULA (1)

Dose in arbitrary units. The numbers on the curves refer to the corresponding values of n in the formula.

When we irradiated bacteria multiple-infected with phage T2, we found that from the earliest times (4 minutes after infection) the survival curves were very

[5] The actual multiplicity of infection must vary from cell to cell within a culture around the average value n. Assuming a Poisson distribution, Dr. M. Delbrück derived the following expression:

$$y' = 1 - e^{-ne^{-\alpha D}} \tag{1'}$$

The suvival curves calculated according to formula (1') are very similar to those calculated from formula (1) and shown in figure 5.

different from those obtained for single infection, and of a definite "multiple-hit" type. Several experiments were performed, giving, in all, 96 consistent values. These could not be averaged, as in the case of single infection, because the multiplicity of infection varied from one experiment to another. Figure 6 shows the results of some individual experiments.

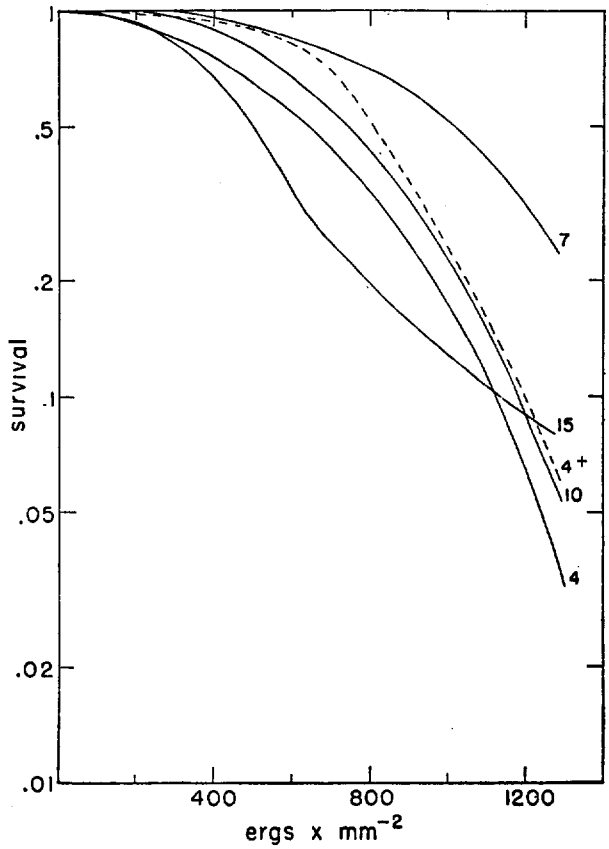

FIG. 6. SURVIVAL CURVES OF T2 INFECTIVE CENTERS, MULTIPLE INFECTION

Each curve represents a separate experiment. The solid lines refer to experiments with multiplicity of about 5 phages per bacterium. The broken line (+) refers to an experiment with a multiplicity of about 15. The numbers on the curves indicate the time of irradiation.

A comparison with the theoretical curves of figure 5 shows that the shape of the experimental curves for early times is similar to that of the theoretical curves for values of n corresponding to the multiplicity of infection. An increase in multiplicity of infection causes a shift in the curve toward higher values of n. For example, the shape of the curve for 4 minutes, multiplicity 5, is similar to that of the theoretical curves for 4 to 5 hits, whereas the curve for 4 minutes, multiplicity 15, is more similar to a 15- or 20-hit curve. The multiplicity of infection is necessarily a roughly estimated quantity and may vary rather widely from cell to cell in the same culture. Better agreement with theoretical curves is

scarcely to be expected. The results clearly indicate, however, that most or all of the infecting phage particles remain active inside the bacterium and can participate in the process of growth.

The survival curves for multiple infection at 4 minutes (figure 6), compared with those for single infection (figures 3 and 4), show resistance higher than can be explained on the basis of the multiplicity alone. For example, at 4 minutes, for multiplicity of 5, the dose leaving 50 per cent survival is 750 ergs $\times$ mm^{-2} as compared with 85 ergs $\times$ mm^{-2} for single infection at the same time. The ratio of the doses is 9. Comparison of the curves for $n = 1$ and $n = 5$ in figure 5 shows a theoretical ratio of 3. The discrepancy indicates that the resistance of the individual particles in the case of multiple infection is higher than in single infection. This is probably due to the fact that in experiments with multiple infection the bacteria come in contact with higher concentrations of phage lysate—that is, with greater amounts of the unknown component which, as previously discussed, plays a role in increasing the resistance of intracellular phage.

In experiments with multiple infection the resistance of the infective centers increases fast, reaches a maximum between 6 and 7 minutes (instead of 11 to 12 minutes as for single infection), then decreases. Thus it seems that for multiple infection the growth process arrives earlier at the stage at which the trend of the changes in resistance is reversed.

The magnitude of the increase in resistance between 4 and 7 minutes, while the survival curve maintains its multiple-hit shape, seems to indicate that all the infecting particles actually participate in the growth process, rather than that one particle grows and the others are capable only of replacing it if it should be inactivated.

The decrease in resistance late in the latent period makes the survival curves at 15 minutes and later resemble closely those for single infection. This agrees with the known fact that the end results of phage growth are the same for single and multiple infection, as regards both duration of the latent period and phage yield per bacterium (Delbrück and Luria, 1942).

DISCUSSION

What information concerning the mechanism of phage growth can be derived from these experiments? We shall first consider some of the possible events in the growth processes and see how our results agree with the corresponding expectations.

In the case of single infection, we could suppose that in every bacterium phage multiplies at the same rate, producing identical particles capable of further multiplication. We have seen that in newly infected bacteria radiation acts by direct inactivation of intracellular phage. If the survival of an infective center after irradiation depended on the survival of at least one active particle, the survival curves should become multiple-hit curves, similar to the theoretical curves of figure 5, corresponding to progressively higher values of n as growth proceeds. The curves for successive times would reveal the number of active

particles per bacterium at each time. Figures 3 and 4 clearly show that the process of phage growth does not fit this simple picture.

Let us consider what happens in the case of single infection during the first 11 to 12 minutes. The main change is a progressive increase in the resistance of infective centers to radiation. The survival curves show a progressively increasing upward concavity (figures 3 and 4), the opposite of what would be given by any multiple-hit phenomenon. We must, however, keep in mind that whatever phage multiplication takes place inside the bacteria is likely to proceed at varying rates, so that at any one time there must be bacteria with widely different numbers of particles. In fact, Delbrück (1945*a*) observed a wide distribution of the phage yields from individual infected bacteria. It was thought that the survival curve might reflect the variability in phage multiplication. We tried, therefore, to calculate a distribution of the number of phage particles that would account for our experimental curves. This calculation led to very improbable assumptions. In particular, the upward concavity of the curves could only be explained by extremely bimodal distributions. The survival curve at 9 minutes (figure 4), for instance, would require a mixture of 80 to 90 per cent cells containing 1 to 5 particles and 10 to 20 per cent containing 50 to 100 particles per cell.

Since high doses require a longer time of exposure (up to 80 seconds), it was thought that the upward concavity of the curves might be due to the fact that part of the dose was received when phage growth had already reached a later stage. This possibility was ruled out by experiments with high intensity and short exposures, which gave the same results.

We consider as the most likely interpretation that during the early part of the latent period most of the change in the survival curves is caused not by phage multiplication, but by a progressive increase in the resistance of the individual particles. A change in the ultraviolet sensitivity of phage particles during intracellular growth is a novel feature, but hardly a surprising one if we think of the complicated processes that must take place inside the infected cell.

The simplest mechanism by which the increase in resistance could be brought about would be the accumulation, around the particles, of some ultraviolet-absorbing material. Let us consider, for example, the curves for 3 and 10 minutes in figure 3. The doses giving 50 per cent survival are 70 and 290 ergs $\times$ mm^{-2} respectively (ratio = 4). A survival of 20 per cent results from doses of 190 and 1,220 ergs $\times$ mm^{-2}, respectively (ratio = 6.4). If no phage multiplication took place, this increase in resistance of about 4 to 6 times—the variation being due to the different shape of the survival curves—could be explained by the accumulation between 3 and 10 minutes of enough material around the phage to absorb 4 to 6 times more radiation. Such an increase in resistance could be provided, for example, by a layer of about 200 mμ of a substance having an extinction coefficient of 30,000 cm^{-1}, the approximate value for nucleic acids. If some phage multiplication takes place, it will in itself produce some increase in resistance; the amount of screening material required will be less than calculated above. Indeed, the slight deformation of the initial part

of the survival curves after 7 to 8 minutes (figure 4) is probably an indication that some multiplication has started by this time.

According to the "screening" hypothesis, the change in sensitivity of the intracellular phage would be apparent rather than real. This hypothesis may be too naïve, however. It is easy to imagine other mechanisms that may affect the intrinsic sensitivity of the phage particle. Phage inactivation may, for example, be caused by absorption of radiation in any one of a number of chemical structures within the particle, and the growing phage may contain fewer of these "vital spots." Also, the probability that absorption in a certain structure results in inactivation may vary during growth as a result of changes in chemical reactivity. There exists strong evidence (Pirie, 1946) that the state of virus particles inside the host cell may actually be quite different from that of free particles.

In the following discussion we adopt the hypothesis of accumulation of absorbent materials. Most of the considerations would still be valid if changes in sensitivity resulted from any of the other mechanisms discussed above.

The upper concavity of the survival curves is probably due to large fluctuations in the rate of evolution of the growth process in different cells. These fluctuations are in agreement, and possibly in causal relation, with the previously mentioned variability in phage yield per bacterium.

The dependence of the resistance of the infective centers on the absolute amount of phage lysate with which the bacteria have been in contact has indicated that some other component of the lysates, besides the active phage, influences the early rise in resistance. We have no information yet about the nature of this component. Studies on its sedimentation in the ultracentrifuge, its heat resistance, and other properties may clarify its nature.

Sensitivity of the infective centers to high doses of radiation begins to increase 12 minutes after infection for single-infected bacteria and 8 minutes for multiple-infected ones. Since the sensitivity to low doses does not increase, the survival curves become more and more of the multiple-hit type. We can imagine that, as the end of the latent period approaches, phage multiplication takes place in most bacteria, while the screening material disappears, possibly being used up in phage reproduction. These two processes could account qualitatively for the shape of the survival curves at late times.

It is also likely that the presence of many particles inactivated by radiation may interfere with the ability of the remaining ones to carry the process of phage liberation to its successful completion. Thus, the apparent increase in sensitivity at late times may be partly due to failure to count as infective centers some bacteria still containing active phage but unable to liberate it.

On the basis of our results we may propose a very tentative and probably crude picture of the sequence of events in intracellular growth of phage T2. After one or more phage particles have penetrated into a bacterial cell, an ultraviolet-absorbing material, possibly needed for phage building, accumulates and intercepts part of the incident radiation. Additional stimulus for the accumulation of this material can be supplied by some other component present in phage

lysates besides the active phage itself. The accumulation of screening material seems to vary widely from cell to cell, and this variation may be partly responsible for the variability of phage multiplication in different cells, reflected in the variability of phage yield. Phage multiplication is apparently under way 7 minutes after infection, and as it proceeds the amount of screening material around the phage particles begins to diminish. In the last part of the latent period most bacteria probably contain large numbers of particles. The distribution of these numbers is probably connected, not only with the distribution of burst sizes, but also with the variability of the time of lysis for individual cells.

This picture seems to be in agreement with some preliminary results of cytological studies of the growth of phage T2 (Luria and Palmer, unpublished). The first reaction to phage infection is seen as a disruption of the Giemsa- and Feulgen-positive "nuclear" bodies (see Robinow, 1945) and migration of their material toward the periphery of the cell. The amount of stainable material in the cell then increases rapidly until the whole cell, somewhat enlarged, becomes stained in a fine granular way. If this "nuclear" material is assumed to contain a large proportion of nucleic acid, the amounts of it visible in stained preparations are more than sufficient to account for the increase in apparent phage resistance during growth.

The apparent changes in sensitivity of the individual phage particles during the latent period make it impossible to attempt calculation of the rate of phage multiplication from the results of ultraviolet irradiation of infective centers. If our interpretation of the changes in phage sensitivity as due to protection by nonphage material is correct, it might be possible to avoid this difficulty by using X-rays instead of ultraviolet light. Even for X-rays, however, the analysis of the survival curves may be rendered very complicated by the large fluctuations expected in the rate of phage multiplication within individual cells.

The results of our experiments with multiple infection indicate growth of most or all of the infecting particles. The principle of mutual exclusion, according to which only one of several particles adsorbed by the same cell can grow, seems not to hold for particles of the same phage strain. Evidence to the contrary, derived from experiments on interference between a phage and one of its mutants (Luria, 1945), was not too conclusive, in view of the fact that infection with particles of the two types was not simultaneous, and deserves reconsideration.

It appears that mutual exclusion always takes place between particles of unrelated phages (Delbrück and Luria, 1942; Delbruck, 1945*c*), is somewhat limited between particles of related phages (Delbrück and Bailey, 1946), and does not occur between particles of the same strain. The similarity of the yield of phage in cases of single and multiple infection is likely to be due, not to mutual exclusion, but to the amount or rate of formation of some substrate which limits phage multiplication.

SUMMARY

Ultraviolet irradiation of *Escherichia coli*, strain B, infected with bacteriophage T2 showed that, immediately after infection, suppression of the ability to liberate phage results from inactivation of the intracellular phage.

The sensitivity of the infected bacteria was studied during the 21-minute interval between infection and lysis. In the first 12 minutes, the infected bacteria show a rapid increase in resistance, apparently due to increased resistance of the intracellular phage particles. This is possibly caused by accumulation of ultraviolet-absorbing material around the phage. At later times the resistance of infected bacteria to high doses of radiation decreases. This is interpreted to indicate that, as phage multiplication proceeds, the apparent sensitivity of the intracellular phage particles returns to higher values.

A quantitative study of phage multiplication by an analysis of the survival curves of infected bacteria is made impossible by these changes in sensitivity of the individual phage particles during growth, and by the presence of wide fluctuations, in the course of phage growth, among individual infected cells.

Phage lysates appear to contain, besides the active phage itself, some other component which influences the course of the intracellular phage growth as manifested in the changes in ultraviolet sensitivity described above.

In case of infection of a bacterial cell with more than one particle of phage T2, analysis of the survival curves shows that several particles can grow in the same host cell.

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