INTRACELLULAR GROWTH OF BACTERIOPHAGE STUDIED BY ROENTGEN IRRADIATION

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The experimental studies carried out on virus particles extracted from infected cells and tissues supply information concerning only the particles that have withstood the extraction treatments. The state of virus particles inside the host cell may be quite different from that of free particles (Pirie, 1946). Moreover, studies of extracts can yield only "static" data, such as facts concerning morphology, chemical constitution, serological properties, etc. They give no approach to virus physiology and disclose nothing about the behavior of the infective virus inside its host. In particular, the key problem of virus multiplication eludes such methods, and this makes it necessary to devise new techniques for the observation of the virus in its natural state.

Electron microscopy has already permitted the observation of virus particles within tissue cultures, whose cells are thin enough to provide a fair transparency to the electrons of 50 to 80 kv. (Claude, 1947). Certain biochemical studies of infected cells also supply information about the behavior of the virus (Cohen, 1947).

A new method, using radiation, was recently suggested by Luria and Latarjet (1947)[1] and applied to the study of bacteriophage T2. The results which were obtained by means of ultraviolet irradiation showed that the method deserved to be extended, and that x-rays might lead to progress along the new path that radiation seemed to offer. In particular, it appeared that one could hope to obtain some answers to the following basic questions: (*a*) What happens to a virus particle once it has been absorbed by a cell? Does it remain as a unit? (*b*) If it remains as a unit, and if multiplication starts from this unit, is it possible to count the number of units present in a given cell at any one time during multiplication, and to obtain thereby a picture of the kinetics of multiplication?

After referring briefly to the theoretical and experimental basis of the method, and the first results reported in Paper I, this paper will describe some new experiments performed with x-rays on the same bacteriophage, and will discuss the results in respect to the two main lines pointed out above.

I

Method

The method, already described in Paper I, is based on the following considerations.

[1] Hereafter this paper will be referred to as Paper I.

(*a*) One bacteriophage is inactivated by x-rays or by short ultraviolet rays according to an elementary photochemical process initiated by a single quantum of radiation. This is proved by the fact that if one irradiates a homogeneous population of free phage, the survival ratio is a logarithmic function of the dose of radiation. With semilogarithmic coordinates, the survival curve is a straight line (Fig. 2). Inactivation is produced either by a single ionization (x-rays) within some sensitive part of the particle (Wollman and Lacassagne, 1940; Holweck *et al.*, 1940) or by a single short ultraviolet photon (Latarjet and Wahl, 1945).[2]

(*b*) After having received a high dose of radiation, an infected bacterium is still capable of supporting phage growth and of liberating the active particles which it contains. It is therefore possible, by plating an irradiated infected bacterium with an excess of sensitive bacteria, to check whether it still liberates some active virus. (Virus growth within cells sterilized by radiation appears possible also in the case of animal viruses, such as Shope's papilloma virus—Friedewald and Anderson, 1943.)

(*c*) Evidence was given in Paper I that radiation suppresses the infective power of an infected bacterium (let us call the latter an "infective center"), by directly inactivating the virus that it contains. Immediately after single infection (one particle per bacterium), before any multiplication takes place, the survival curve of the infective centers is very similar to that of the extracellular virus, and is unrelated to that of the bacterium. In the case of multiple infection (several particles per bacterium) the survival curve of the infective centers is a "multiple-hit" curve with an initial plateau whose multiplicity closely corresponds to the average number of phage particles adsorbed per bacterium. The same feature was observed with x-rays (Fig. 1).

The method therefore consists in infecting a homogeneous bacterial population with a known average number of particles of a given virus, and in irradiating it with increasing doses at a given time during the growth process (latent period); then in determining, for each dose, the survival of the infective centers, and in drawing the survival curve. As the radiation acts directly upon the intracellular virus, such a curve, whose shape is related to the number of virus units actually present in every cell and to their individual sensitivity to radiation, might supply some information about the condition of the intracellular virus at the time of irradiation.

The growth of virus T2 in *Escherichia coli*, strain B, was first studied with ultraviolet rays (Paper I). Survival curves were drawn from minute to minute throughout the 21 minute latent period. Soon after infection, the resistance to radiation of the infective centers increased continuously either because of some structural change of the virus or because of the formation of some ultraviolet-absorbing material in the host which accumulated around the virus and protected it against radiation. Later on, probably after 7 minutes, multiplication seemed to play a rôle in the increase in

[2] For more details about the radiobiological method, see Lea, D. E., The Action of Radiation on Living Cells, Cambridge University Press, 1945, or Latarjet (1946).

resistance. Still later, after about 12 minutes, the resistance of the infective centers to high doses of radiation decreased, as if, as phage multiplication proceeded, the apparent sensitivity of the virus particles returned to higher values. But a quantitative analysis of the survival curves was made impossible chiefly by the changes in sensitivity of the individual particles during growth. If the latter were actually due to changes in ultraviolet-absorbing material of the host, the use of x-rays would remove this obstacle. As a matter of fact, rapid synthesis of protein and nucleic acid occurs in *E. coli* infected with T2 virus (Cohen, 1947), which might greatly influence

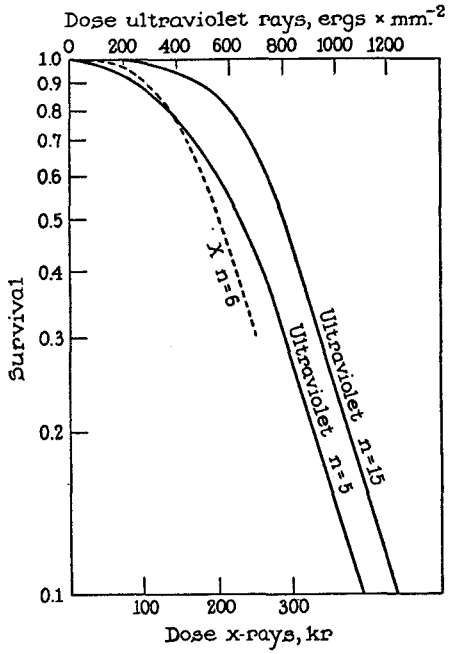

FIG. 1. Survival curves at 5 minutes in the case of multiple infection system, with ultraviolet rays and x-rays.

the ultraviolet experiments, whereas x-ray absorption might remain unchanged. The use of x-rays would therefore eliminate this disturbing factor.

II

Technique of X-Ray Experiments

As in Paper I, *Escherichia coli*, strain B, and bacteriophage T2 were used. Both were grown in a synthetic medium (see Paper I) to which 10 per cent of ordinary nutrient broth was added. In this medium at 37°C., in the presence of a young growing culture of strain B, whose division time is 21 minutes, T2 has a latent period of 21 minutes between the time of infection and the beginning of liberation. Liberation is almost complete 35 minutes after infection; the average phage yield per bacterium is 130. In order to get more time for giving high doses of radiation, some

experiments were carried out at 29°C., a temperature which lengthens the latent period to 43 minutes, while keeping the yield unchanged.

The technique for irradiation of growing phage was the same as that described in Paper I. Powerful specific anti-T2 rabbit serum was used after infection to eliminate free phage (see Appendix).

Because of the high resistance of phage to x-rays, very large doses of radiation had to be given, and, as the main purpose was to draw the survival curve almost from minute to minute during the short latent period, these high doses had to be given in very short exposures. The x-ray set-up was thus devised so as to reach intensities as high as possible with a beam hard enough to penetrate the samples without much loss. The source was a Holweck-Beaudouin tube with a water-cooled molybdenum target. The operating voltage was 33 kv., and the amperage could reach 45 ma. The output, filtered with 0.05 mm. aluminium, included the K_α lines of molybdenum (0.71 A) and some continuous background. The average absorption was that corresponding to 0.95 A, the energy absorbed in a water layer 2 mm. in thickness being about 50 per cent.

Samples of infected bacteria were placed close to the window of the tube in small plastic dishes 17 mm. in diameter and 2 mm. in depth. With an amperage of 43 ma. in the tube, the intensity of the incident beam on the surface of the sample was 90,000 roentgens per minute. A surface dose of 1000 r left in the sample an average of 1.14×10^{15} ionizations per ml., a dose equivalent to an incident dose of 715 r on a non-absorbing layer. In the following paragraphs all dosages will be expressed in such effective doses, *i.e.* in number of ionizations per volume unit in the sample; 1000 r will mean 1.6×10^{15} ionizations per ml. In the conditions described above, these 1000 r correspond to 1400 r at the surface, as indicated by a standard ionization chamber.[3]

Protection against Indirect Action of X-Rays.—When one applies high doses of x-rays to biological material suspended in aqueous media, one must deal in general with some indirect action which interferes with the direct action due to the radiant energy absorbed within the biological material itself. This indirect action is due to photochemical effects upon the medium and liberation of toxic substances such as activated O_2, H_2O_2, H^+, OH^-. These effects have been observed in many instances with viruses suspended in aqueous media (Friedewald and Anderson, 1941; Luria and Exner, 1941; Bonét-Maury, 1941; Lea *et al.*, 1944).

In the present work, such indirect action must be carefully avoided, for the method described above is based on the assumption that phage inactivation is due to direct absorption of radiation by the phage itself. Fortunately, virus can be somewhat protected against indirect action by the presence of various substances, which, acting as buffers, fix the by-products of irradiated water.

As a preliminary step, the behavior of T2 was carefully studied. The inactivation of free T2 follows an exponential rate (Fig. 2),[4] but in any aqueous medium indirect

[3] Calibration was done by Dr. M. Frilley, using the standard large chamber of the Institute of Radium.

[4] $D_{0.37}$ = 34 kr. The sensitive volume (target) is equivalent to a sphere 33 mμ in diameter.

action appears when the dose exceeds 100 kr.[5] From that dose on, the survival curve shows a downward concavity. Addition of 10 per cent of ordinary broth counteracts this action until doses of 250 to 300 kr are reached. For this reason broth was added to the synthetic medium in the present experiments. Moreover, the cytoplasm was also expected to protect the intracellular phage. As a matter of fact, no detectable indirect action appeared to disturb the survival curves of infective centers until doses above 400 kr were reached.

III

Experimental Results (Single Infection)

The present work was devoted chiefly to the case of single infection, which appeared more appropriate for providing a fair picture of virus growth. In order to reduce mutual reactivation of inactive particles within the same cell, which may transform single infection into multiple infection (Luria, 1947), only a small fraction of the bacteria were infected with a small amount of phage lysate (low single infection); in general, 7×10^6 phage particles were added to 7×10^7 growing bacteria (per ml.), and the time of contact was reduced to 1.5 minutes. Then, antiserum was added which inactivated almost all non-adsorbed virus. After suitable dilution, a sample was taken at a given time and irradiated. Survival was determined by immediate plating on agar with an excess of strain B, and plaque count. In general, the highest doses given at 37°C. did not exceed 3 minutes of exposure. The question arose whether during such an exposure the infective centers continue to evolve normally or not; that is, whether they are "fixed," by the radiation, in their state at the beginning of the exposure. As will be observed later, the growth system evolves so rapidly at certain times of the latent period that the results of exposures 3 minutes long would be meaningless in the case of non-stabilization of the system. This question was previously considered in the case of ultraviolet rays (Paper I), and it was found that the same dose given either in a few seconds or in an exposure as long as 2 minutes yielded the same results. Moreover, with x-rays, even the dose usually delivered in 1 minute (about 60 kr) disturbs the growth process to such an extent that the latent period is lengthened to 35 minutes, and the individual yield is reduced to a few particles. For these two reasons it was considered that the results given by a 1 to 3 minute exposure were apt to show the situation at the beginning of irradiation. However, when higher doses had to be given, the experiment was carried out at 29°C.

The individual results obtained in successive experiments with a certain dose given at a certain time show considerable variation because of difficulties in maintaining constant all biological and physical factors. Their wide variation makes it necessary to increase the number of individual experiments and to

[5] The symbol kr will be used for kiloroentgen.

average the results. The great number of values required to draw the whole set of survival curves made it advisable to use a graphic average rather than to

TABLE I

Sets of Experimental Data Obtained at Three Different Times during the Latent Period

Time	Dose	Survival
	kr	*per cent*
5 min.		
	20	70
	40	30–46
	56	15–23–20–28
	66	34–20
	114	11–8
	134	17–12
	170	4–5
	200	7–0.2
	230	3–4
	270	2–0.2
10 min.		
	28	94–93–96
	43	85
	57	86–91–90–90–97–91–97
	114	78–67–93–95–92–96
	170	68–56–65
	230	64–39–45–75–41
	340	11–21–18–20–25
	460	4–6–5–5–5
18 min.		
	28	100–102–96–94–95–97
	40	86–84–104–82–95–107–92
	48	98–86
	60	92–80–82–80–90–98–85–98–86
	96	69–78
	115	70–87–79
	130	57
	170	29–47
	230	13
	285	7–5

calculate for each single point a numerical average, whose standard deviation would be very uncertain.

Preliminary results were recently published (Latarjet, 1947), based on about 200 experimental values. They showed clearly some new features in the growth process which could lead to a tentative hypothesis for this process. But consideration of these results showed that such an hypothesis depended basically

on some details of the curves, and that these details had to be worked out more thoroughly. A new series of experiments was therefore undertaken, particularly with high doses at low temperature. Altogether, 280 data were recorded. As the standard deviation has not been determined accurately for each point, the dispersion of the individual results and the precision of the curves are illustrated by the data in Table I, which gives, as examples, the complete sets of data for three curves. Finally, all results obtained in the case of low single infection were grouped and averaged, and the corresponding survival curves

TABLE II

Average Values of All Experimental Results at Various Times during the Latent Period (Low Single Infection)

The numbers express in per cent the survival of x-rayed infective centers as a function of the radiation dose.

Dose	Extracellular phage	Time of irradiation, *min.*							
		4, 5, 6	8	9	10	11.5	13	15	18
kr									
25	56	56	70	76	98	99	100	99	99
50	30	30	47	60	94	98	100	98	98
75	17	17	32	52	90	95	99	96	92
100	9	10	22	48	85	91	98	92	82
125	5	7.6	17	43	80	88	94	78	68
150	2.8	5.5	13	40	74	83	83	58	50
200	0.8	2.9	7.5	35	58	70	47	29	23
250	0.24	1.5	4.3		42	53	24	14	10
300	0.09				27	36	13	7	4.3
350					15	22	7	3.5	
400					9	14	3.5		
450					5.5	8.5	1.9		

drawn (Table II and Fig. 2). A survey of these results and curves leads us to divide the latent period into three periods:

First Period: First 7 Minutes.—The first characteristic feature is that the resistance of the infective centers to radiation remains constant during the first 6 to 7 minutes, and equal to that of the extracellular virus, at least for low doses. Table III gives as an example the results of an experiment in which a dose of 56 kr was given from minute to minute. For doses higher than 80 kr, *i.e.* for survival below 10 per cent, the remaining infective centers at 5 and 6 minutes appear to be a little more resistant than the extracellular virus, as if the process which, from 7 minutes on, increases the resistance in most of the bacteria had started earlier in some of them.

Second period: from 7th to 13th Minute.—Around 7 minutes (Table III) a slight increase in resistance appears, which becomes more marked as time goes

on. The survival curves at 8 and 9 minutes display this general increase in resistance, with an upward concavity and no plateau; *i.e.*, no feature of multiplicity (compare with Fig. 1). On the other hand, at 10 minutes an entirely

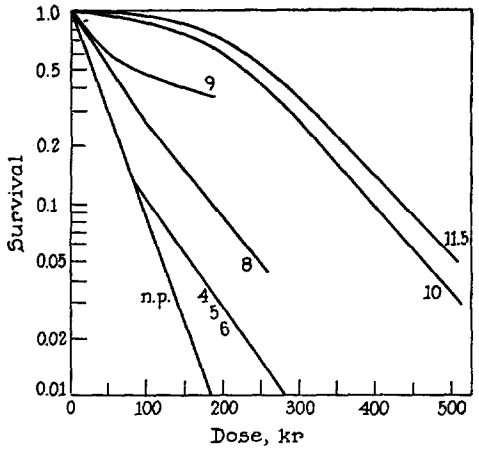

FIG. 2 *a*

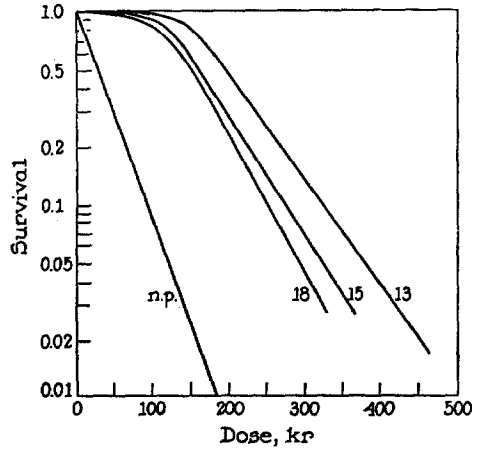

FIG. 2 *b*

FIG. 2. Survival curves of low single infective centers. (*a*) From 4 minutes to 11.5 minutes; (*b*) from 13 minutes to 18 minutes. Curve *n.p.* is that of extracellular T2.

new picture appears, with a greatly increased resistance, a downward concavity, and some kind of an initial plateau. At 11.5 minutes these new features are even more marked, at which time the resistance of the infective centers to high doses of radiation has reached its maximum.

Third Period: from 13 Minutes to Burst.—At 13 minutes the survival curve is definitely of a multiple-hit type. It shows a long plateau, a downward concavity, and finally, a straight portion. Such a curve can be analyzed for multiplicity (see Discussion). From this time on, the survival curve keeps its general shape, but the sensitivity to radiation increases slowly until the end of the latent period. At 18 minutes, the straight part of the curve is parallel to the curve of extracellular phage.

It should be pointed out that the general picture obtained with x-rays resembles closely that obtained with ultraviolet (Fig. 3). The differences will be emphasized in the Discussion.

IV

Comparison of Low and High Single Infections

It was found with ultraviolet rays (Paper I) that the amount of phage lysate introduced into the bacterial suspension influences the resistance of the infective

TABLE III

Survival during the First Period, after a Dose of 56 Kr

Time of irradiation	Survival
min.	*per cent*
(Extracellular virus)	24.5
4	24
5	23
6	24.5
7	33
8	55

centers to radiation during the first 10 to 12 minutes, even in the case of single infections. The greater the amount, the faster the resistance increases. New experiments with x-rays confirmed what had been found with ultraviolet rays.

The usual bacterial suspension containing about 7×10^7 bacteria per ml. was infected with 5×10^7 T2 per ml. The number of infective centers was about 3.5×10^7, indicating that about 1 bacterium out of 2 (high single infection) was infected, whereas in the case of low single infection 1 out of 10 to 20 bacteria was infected. In both cases, a majority of the infected bacteria were "single infected," and all other experimental conditions were the same, the only difference being the amount of phage lysate introduced.

Fig. 4 shows the survival curves obtained in the case of high single infection during the first 10 minutes of the latent period, and compares them to some of the low single curves of Fig. 2. Here again the process which increases the resistance of the infective centers starts earlier in the case of high single infection, the difference between the two cases being 2 to 3 minutes. For instance,

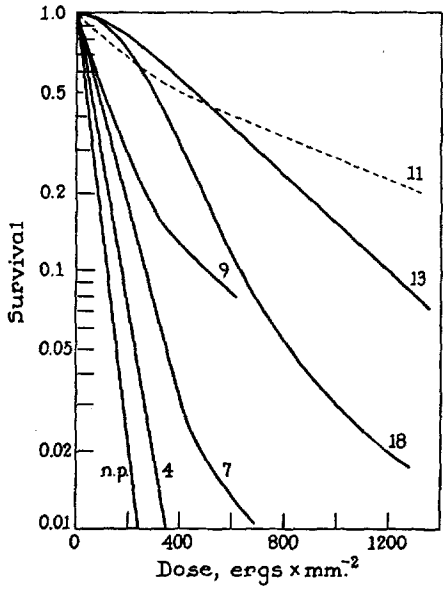

FIG. 3. Survival curves of low single infective centers in the case of ultraviolet irradiation (from Paper I).

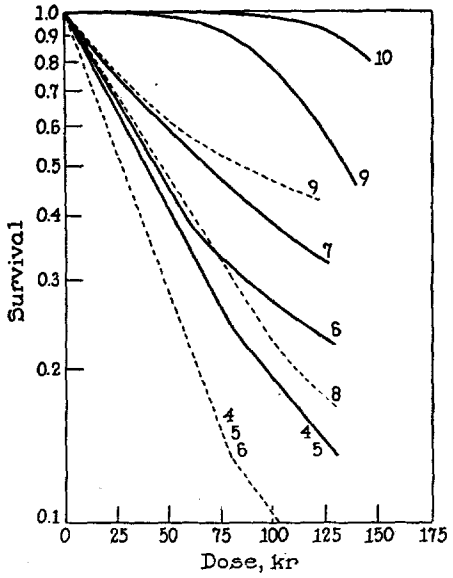

FIG. 4. Survival curves during the first 10 minutes, in the case of high single (plain curves) and low single (dotted curves) infections.

at 9 minutes the high single curve is already of the multiple-hit type, and the 10 minute curve is similar to the 13 minute low single curve. It seems that, in the case of high single infection, the first period is very much reduced, and the second one ends around 10 minutes. From that time on, there is no more difference between low and high single curves.

DISCUSSION

The main purpose of the present work, as already pointed out in Paper I and in the introduction of the present paper, is to clarify the fundamental problem of virus growth, whose importance may be related not only to viruses but also to specific cellular constituents such as microsomes, plastids, genes, and enzymes. It was believed that the knowledge of the kinetics of growth, *i.e.* the variation with time of the number of virus particles in a given cell, would provide the first clue to the problem, and radiation was used chiefly as a possible tool for counting the average number of virus particles within the infected cells of a homogeneous population at any one time between infection and lysis.

If one assumes that the growth process yields only replicas of the initial virus particle, and that it is the same in all cells of the population, the survival curves after irradiation can be theoretically drawn as a function of the number of particles actually present in each cell (Fig. 5; for explanation, see Paper I). A comparison of these theoretical curves with the experimental ones should permit estimations of the number of particles per cell at any one time. On the other hand, if the experimental curves differ significantly from the theoretical ones, one or both preceding assumptions would be ruled out and some new feature could be disclosed in the growth process.

It should be noted that phage multiplication is likely to proceed at varying rates, leading to a wide distribution of the phage yields from individual infected bacteria (Delbrück, 1945). Not all bursts occur at the same time; some cells yield few particles whereas others yield several hundreds. This wide distribution might somewhat distort the curves from their theoretical shape.

Before discussing the preceding results with x-rays, one should keep in mind the chief significance of the theoretical curves drawn in semi-logarithmic coordinates. A straight line means a one-hit killing process.[6] As killing of an infective center results from the inactivation of the infective virus (see section I *c*), a straight line means the presence of only one virus particle per cell. The presence of several identical particles per cell leads to multiple-hit curves (Fig. 1). Such curves (Fig. 5) display an initial plateau whose length is related to the number of particles, and a straight part which is parallel to the one-hit curve. The common slope of all straight parts defines the radiosensitivity of the individual particle: the greater the slope, the higher the sensitivity. In

[6] In the present case of infected bacteria, the term "killing" is used for convenience, and means loss of ability to liberate active phage.

general, x-ray sensitivity of virus particles is related to their size, at least to the size of a special sensitive volume of their body: the greater this volume, the higher the sensitivity. Therefore, if the size of the particle increases, the slope of the survival curve may be expected to increase.

We shall now try to interpret the curves obtained in the case of low single infection (Fig. 2 *a* and *b*) during each of the three periods into which these curves lead us to divide the latent period.

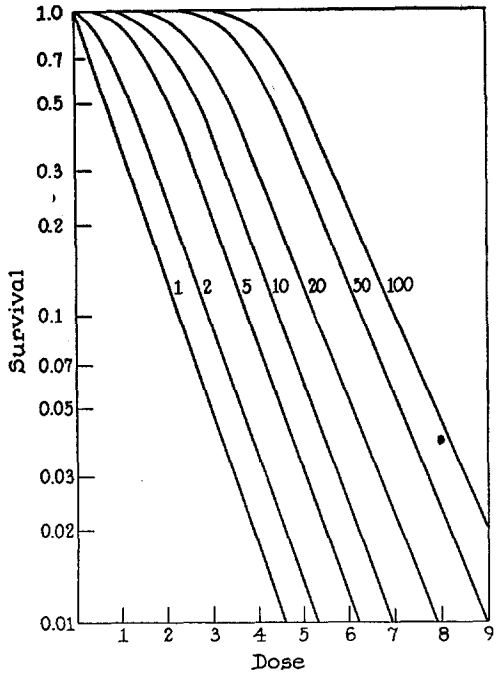

FIG. 5. Theoretical survival curves for different values of the multiplicity, from 1 to 100.

First Period: Bacterial Synthesis.—During the first 7 minutes, the survival curve of the infective centers remains almost identical with that of the extracellular virus. This means that: (*a*) the infective virus particle remains within the cell in such a state that its x-ray sensitivity is unchanged; it remains genetically intact, whether broken into subunits or not; (*b*) this virus remains unique; no multiplication takes place during this period.

The ultraviolet curves (Fig. 3) show that, during this time, the resistance of the infective centers to ultraviolet radiation progressively increases. It was suggested that this increase was due to bacterial synthesis of a large amount of ultraviolet-absorbing material (Paper I). The x-ray curves confirm this hy-

pothesis by ruling out a possible change in the radiosensitivity of the virus particle itself.

The chief phenomenon that occurs during the first 7 minutes seems to be the bacterial synthesis of ultraviolet-absorbing material probably devoted to the building of future particles. McFarlane (1947) has shown that epidermal cells infected with vaccinia virus are very rich in proteins with the same antigenic constitution as the proteins of the virus. Cohen and Anderson (1946) and Hook *et al.* (1946) found that virus T2 contains about 35 per cent of desoxyribose nucleic acid. From Cohen's experiments (1947), the synthesis of this acid in the infected cells starts after 7 minutes only. The previous syntheses might then be concerned mainly with protein material.

The first alterations following infection affect the host and not the virus, a fact which may explain the interference phenomenon by changes in the host functions. Working on ultraviolet-irradiated influenza virus, Henle and Henle (1947) reached the same conclusion that the first step after adsorption of the virus onto the host cell would "lead to changes in the host cell which alter its function and exclude other viruses from entering."

Second Period: Multiplication.—Around 7 minutes, the first critical change occurs: a process starts which increases the x-ray resistance of the infective centers, without changing essentially the shape of the survival curves, which remains without any plateau for about 2 minutes (8th and 9th minutes), proving that the multiplicity is still equal to 1. The infective particle, still unique, undergoes some changes which affect its resistance to radiation. The responsible process appears highly heterogeneous within the population (hence the upward concavity of curves 8 and 9, and the break of curves 4 to 6 indicating that in a number of cells the process starts before 7 minutes). Such heterogeneity may be connected with that in individual yields after lysis.

This unknown process is favored by a constituent of the phage lysate different from the active particles themselves, for it goes faster in the case of high single infection, and even faster in multiple infection.

It has been shown with ultraviolet rays, and confirmed with x-rays, that, in the latter case, several virus particles (one does not know yet how many) are capable of growing in the same bacterial cell, a fact which is in accord with Hershey's observation (1946) that bacteria simultaneously infected with T2 wild type and its *r* mutant yield both types. Both latent period and yield are the same as in the case of single infection, although the first steps of the growth process are accelerated, the end of the second period occurring around 11 instead of 13 minutes.

Between 9 and 10 minutes a new phenomenon transforms the survival curve into a multiple-hit type one, with a definite plateau and a straight part whose slope is far weaker than that of the extracellular virus curve. This seems to indicate that: (*a*) some units are multiplying very fast and are already numer-

ous at 10 minutes; (*b*) these units are more resistant to radiation (probably smaller) than the extracellular particle. The multiplicity increases rapidly and reaches its maximum around 13 minutes, with 100 to 150 virulent units whose x-ray sensitivity is about half that of the initial particle.

Thus multiplication itself seems to proceed for about 4 minutes (9th to 13th minute), and the multiplying units appear less sensitive to x-rays (probably smaller) than the extracellular virus particle.

Third Period: Maturation of Virus Particles.—At 13 minutes, a second critical change occurs: multiplication stops. The number of units has reached its maximum and will not change any further. From this time on, general sensitivity of the infective centers increases until, at the very end, a situation is reached in which an average of 100 to 150 particles are present per cell, displaying the same radiosensitivity as that of the oroginal particle in its free state. This last phase of the growth process is probably devoted to the maturation of the new particles.

These results confirm those obtained with ultraviolet rays, and add some new details to the tentative and crude picture of the sequence of events in T2 growth which was given in Paper I and can now be drawn as follows: After one virus particle has penetrated into a bacterial cell, some ultraviolet-absorbing material, probably needed for virus building, accumulates. Additional stimulus for this phenomenon can be supplied by some other component present in phage lysates besides the active phage itself. For about 7 minutes the infective particle remains unique and genetically intact, its resistance to x-rays being constant. Between 7 and 9 minutes, the particle undergoes some changes which increase this resistance. The whole process so far varies widely in speed or magnitude from cell to cell, and this variation may be partly responsible for the variability of virus multiplication in different cells, reflected in the variability of virus yield. At about 9 minutes, multiplication starts and continues until, about the 13th minute, an average of 100 to 150 infective units are present per cell. The multiplying units are more resistant to x-radiation than the liberated virus; they probably are smaller. In the last part of the latent period, the new infective units undergo some changes (probably grow in size) until, at the very end, they appear similar to the extracellular virus.

As far as the nature of the multiplying units and the rate of multiplication are concerned, nothing as yet can be said. It was impossible to draw, between 9 and 13 minutes, as many survival curves as were needed to get a picture of the kinetics of growth. The present work fails to tell whether the rate is linear, logarithmic, or otherwise; whether multiplication proceeds by some "template mechanism" (linear rate) or by self-duplication of all units behaving like higher microorganisms (logarithmic rate). It fails therefore to answer the fundamental question mentioned in the introduction. But it shows that the multiplying

units are somewhat different from the infective particle, and strongly suggests that they are smaller. It does not rule out Luria's idea, based on his discovery of cross-reactivation between ultraviolet-inactivated T2 particles within the same host cell (1947), according to which the infective particle would break into genetic loci which might reproduce with a certain degree of independence, and then be utilized in the building of new particles.

The present crude picture of virus T2 growth reveals the whole process as highly dependent upon bacterial metabolism. Multiplication starts only after some specific material has been synthesized by the cell, and possibly stops upon exhaustion of some substrate, which plays the rôle of a limiting factor. Bacterial synthesis may continue during the period of multiplication, but it also may be curbed when a certain number of new units are formed. Early deviation of normal bacterial synthesis indicates that, in the competition among cellular elements for enzymes and energy, the infective particle appears as a winner. For instance, it stops normal synthesis of ribose nucleic acid (Cohen, 1947) and the capability of the cell for enzymatic adaptation (Monod and Wollman, 1947). One can imagine that at a certain stage of the growth process, the cell is disturbed to such an extent that it is no longer capable of building the material needed by the multiplying units.

At the time of infection, when viruses come into contact with growing bacteria, the cells are in widely varying stages of their growth; *i.e.*, of their synthetic potentiality. Since the latter seems to be involved in virus growth, this could account, at least partly, for the wide distribution of the individual yields.

SUMMARY

Growing *Escherichia coli* infected with bacteriophage T2 was x-rayed during the 21 minute latent period which elapses between infection and lysis of the cells. Survival curves of the infected bacteria were determined almost from minute to minute; they disclosed the following facts which are related to the process of phage growth:

During the first 7 minutes, the infective virus particle remains in the cell unique and genetically intact. The host cell synthesizes some ultraviolet-absorbing material probably devoted to building future particles. From the 7th to 9th minute the x-ray resistance of the virus particle increases, probably because of some internal change. Then, multiplication starts and is completed at about the 13th minute, when an average of 130 virulent units is present per cell, displaying an x-ray resistance twice as high as that of the extracellular virus particle. From 13 minutes to the end, the new units progressively recover the x-ray sensitivity of the extracellular virus.

Nothing can be said about either the rate of multiplication between 9 and 13 minutes, or the nature of the multiplying units, except that they are more radiation-resistant (probably smaller) than the extracellular virus.

The first steps of the growth process are favored by an unknown component of the lysate, different from the active particles.

Several particles can grow in the same host cell.

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APPENDIX

A New Method for Obtaining Powerful Antiphage Rabbit Sera

In the experiments with ultraviolet rays (Paper I) we used an anti-T2 rabbit serum prepared according to Delbrück's technique (1945), which consists of two courses of subcutaneous injections of a high titer lysate. Each course consisted of five injections of 5 ml., given twice a week, and there was an interval of about 1 month between courses. With T2 virus the best serum we obtained in this manner inactivated 99 per cent of free particles in 30 seconds, when diluted tenfold. With smaller phage particles, such as T7, the results were far less satisfactory, probably because the injections provide a much smaller amount of antigenic proteins.

A striking improvement was achieved with both T2 and T7 by (*a*) using intravenous injections in the marginal vein of the rabbit's ear; and (*b*) using a new schedule of injections which was suggested to me by Dr. J. Loiseleur.

High titer filtered lysates in synthetic medium were used (1 to 5 $\times$ 10^{10} particles per ml.). A first course was given of ten subcutaneous injections of 2 ml. twice a week. After an interval of 1 month, a second course of intravenous injections was given with the same lysate:

Day	Amount of lysate injected
	ml.
1	0.5
4	0.5
6	0.5
8	1
11	1
13	2
15	2
21	Bleeding

Bleeding was done aseptically by direct intracardiac punction, 20 cc. of blood being withdrawn. This punction was withstood well by the rabbit and could be repeated later on.

Activity.—The T2 antiserum, diluted 50-fold, reduced the titer of a lysate from 1 $\times$ 10^{10} particles per ml. to 5 $\times$ 10^4 in 30 seconds (inactivation of 2 $\times$ 10^5), and achieved complete inactivation of this lysate in 1 minute.

The T7 antiserum gave the same rate of inactivation when diluted tenfold. If compared with the preceding sera, the present ones are from 10^3 to 10^4 times more active.

Storage.—In order to avoid contamination, a small crystal of thymol was added to the serum. Almost insoluble, it remains indefinitely, and the small amount dissolved prevents practically all contamination without affecting the activity. Such a saturated solution of thymol, if diluted fivefold, does not kill T2, T7, or B bacteria in 5 minutes. Under the usual conditions, complete inactivation of the free particles is obtained before any danger of the toxic action of thymol on the phage or on the bacteria.

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