THE PROPERTIES OF X-RAY INACTIVATED BACTERIOPHAGE[1]

II. Inactivation by Indirect Effects

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The rate at which X-ray irradiation inactivates bacteriophage particles, that is, destroys their ability to reproduce, has been shown to depend on the medium in which the virus particles are suspended (Luria and Exner, 1941). Phage suspended in a solution of inorganic salts is inactivated at a faster rate than in nutrient broth, and the more rapid inactivation is interpreted as being due largely to chemical agents produced in the surrounding medium by ionizations outside the phage. Some components of the nutrient broth must act as protective substances. The chemical basis for the protective action has been analyzed by several workers, among them Dale (1942), Luria and Exner (1941), and Latarjet and Ephrati (1948), who found that small concentrations of many substances such as tryptophan, thiourea, glutathione, and gelatin give complete protection. It is generally believed that the protective nature of these substances is due to their competing with the virus particles for the toxic agents produced in the medium.

In the presence of an excess of most protective substances there remains a residual nonprotectable inactivation, whose rate is independent of the temperature during irradiation and of the intensity of the irradiation. The nonprotectable inactivation is generally considered as due to a direct effect of X-rays, that is, to acts of absorption of X-ray energy within the phage particles (Luria and Exner, 1941). In support of the idea of "direct" action is the finding that the X-ray sensitivity of different phages in nutrient broth increases with the particle size as determined by ultrafiltration (Wollman and Lacassagne, 1940; Luria and Exner, 1941). The logarithm of the survival ratio is inversely proportional to the dose; the particle size could be estimated relatively accurately by assuming that every cluster of ionization produced within a particle resulted in inactivation. Wollman, Holweck, and Luria (1940) found that a given amount of ionization was less effective if produced by alpha particles than by X-rays, as expected if inactivation resulted from ionizations produced within the phage particles. Ionizations produced by alpha particles are more closely packed than those produced

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by X-rays, so that, for every inactivation, several ionizations will be wasted within the particle.

Recent experiments by Doermann (1951) reveal a complication in this picture. Cysteine, added in concentrations of the order of 0.01 M to nutrient broth or gelatin solutions, reduces the rate of inactivation of phage T2H (Hershey, 1946) to about one-half the rate generally considered to correspond to the direct effect (hyperprotection). This result may be interpreted as suggesting that some of the direct effect, although caused by radiation energy absorbed within, or in the immediate vicinity of, the geometric domain of the virus particle, is mediated through agents that react secondarily with the essential virus material and can be prevented from doing so by cysteine. Hydration water may be the source of such agents. It is, of course, not excluded that cysteine acts directly on the virus particles to modify their response to radiation energy delivered directly on virus material proper.

Since the "direct" and the "indirect" effects are supposedly produced, at least in part, by different mechanisms, we might expect that the inactive particles produced by these two types of agents would differ in their properties. In an earlier paper (Watson, 1950) the properties of phage inactivated by the direct effect were described. A similar study of the properties of phage inactivated by "indirect" agents, presented in this paper, shows the existence of more than one type of indirect inactivating agents, distinguishable from the properties of the inactive particles. The inactive phage produced by indirect agents differs in its properties from phage inactivated by direct effect. This gives further evidence in favor of the distinction between direct and indirect effects. A study of the properties of phage inactivated in broth in the presence of cysteine (hyperprotected phage) would be greatly desirable.

MATERIALS AND METHODS

Coli-phages T1 to T7 and their common host *Escherichia coli*, strain B (Demerec and Fano, 1945), were used in all experiments. The general methodology has been summarized by Adams (1950). The phage stocks used were lysates in "Difco" nutrient broth (with 0.5 per cent NaCl) or in the synthetic medium M-9, of the following composition: Solution A—KH_2PO_4 3 g; $MgSO_4$ 0.2 g; NH_4Cl 1 g; anhydrous Na_2HPO_4 6 g dissolved in 900 ml of distilled water. Solution B—4 g of glucose dissolved in 100 ml of distilled water. Solutions A and B are sterilized separately and mixed in a 9:1 ratio before use. The pH of the final solution is 7.0. Unless otherwise indicated, all experiments were done at 37 C.

The X-ray tube at Indiana University was operated under 200 KV peak and 20 mA. Its calibration has been described (Watson, 1950). The intensity was 1,330 roentgen per minute. For the photoreactivation experiments, the light source consisted of two parallel fluorescent lamps, 40 watts each, at a distance of 20 cm from the exposed material.

EXPERIMENTAL RESULTS

Under any condition of irradiation, some direct effect damage must always be present. It is impossible to obtain irradiated phage damaged only by indirect

chemical agents. The contribution of the direct effect to inactivation, however, becomes small when we irradiate phage suspended in a medium relatively free of protective substances, for example, in the form of purified suspensions obtained from lysates in M-9 medium by washing the phage several times by differential centrifugation and resuspending it in M-9 solution A. When such a purified stock is irradiated and assayed for survivors immediately after irradiation, the inactivation curve of figure 1 (broken line) is obtained. Inactivation,

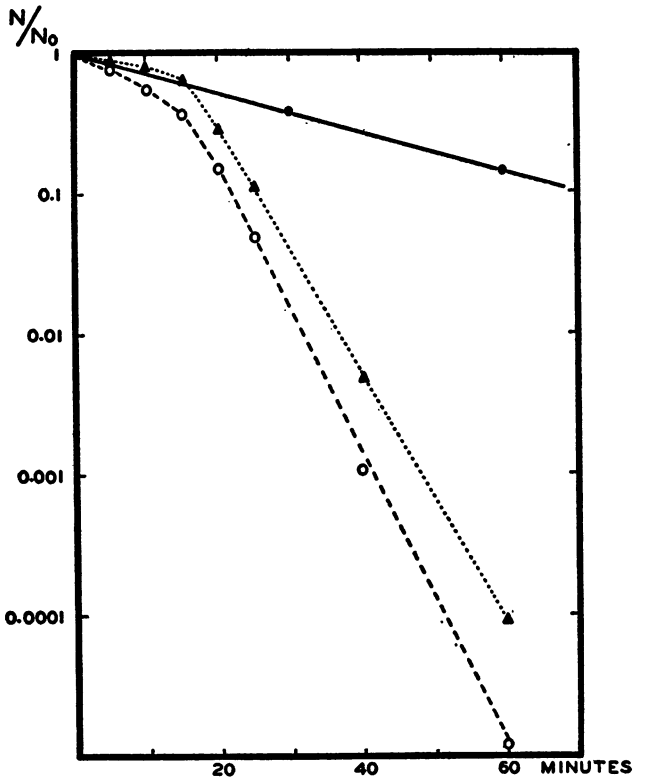

Figure 1. Inactivation of purified T2 phage suspended in M-9 solution A. N/No = proportion of active phage particles after irradiation. The X-ray dose is expressed in minutes of exposure. The broken line gives the inactivation curve due to both the direct effect and the indirect effect. The inactivation curve due to the indirect effect only (dotted line) is obtained by substracting from the total inactivation (both direct and indirect) an amount corresponding to the direct inactivation (solid line).

however, does not stop after cessation of irradiation; the irradiated samples show a progressive decrease in titer with time. The inactivation due to indirect agents, which occurs during irradiation, will be called the "indirect effect", while the inactivation occurring after the irradiation has ceased will be called "after effect". Addition of an equal amount of nutrient broth to an irradiated sample eliminates all after effect. Phage inactivated by after effect only is obtained by adding purified phage to irradiated solutions.

Both the indirect effect and the after effect show a strong temperature depen-

dence, the rate of inactivation increasing with temperature. In addition, the agents causing the after effect are not completely stable since irradiated solutions show a steady decay in phage-inactivating power with time. The experiments reported hereafter indicate that the agents responsible for most of the indirect effect differ from those responsible for the after effect.

Inactivation by Indirect Effect

Survival curve. Figure 1 shows the survival curve of a purified T2 stock exposed to X-rays at room temperature (about 22 C). Inactivation is due to direct and indirect effects only, since broth was added to the irradiated samples immediately after irradiation, to prevent after effects. The amount of direct inactivation expected from various X-ray doses (Watson, 1950) was subtracted from the total inactivation to give the inactivation curve due to indirect action only (figure 1). Dilution of the purified stock in M-9 solution A, to decrease the amount of residual protective substances that may still be present in purified preparations, caused no increase in inactivation rate, suggesting that any residual impurities in the stock were not limiting the rate of inactivation. The possibility remains, however, that impurities in the synthetic medium itself were the rate limiting factor.

Addition to the purified stock of tryptophan, thiourea, or histidine at concentrations 10^{-3} M or higher resulted in complete protection from indirect action.

In contrast to exponential inactivation of phage by direct effect, the inactivation curve for the indirect effect shows a downward bend. This shape observed earlier by Alper (1948) for phage T1 might reflect either a progressive accumulation of an inactivating agent in the medium during irradiation or a cumulative damage to the phage particles, with inactivation resulting upon reaching a threshold. To differentiate between these possibilities the following experiment was performed.

A T2 suspension in broth was diluted 1:100 into M-9 solution A, whereby the concentration of protective substances was reduced enough to permit a relatively large amount of "indirect" inactivation. The diluted sample was irradiated with X-rays for 40 minutes, an exposure that gave a survival corresponding to the beginning of the steeper portion of broken curve in figure 1. Broth was then added to the irradiated sample, after which addition the titer of the surviving phage remained constant, indicating that any stable inactivating agent in the medium was neutralized. After 4 hours, the sample was again diluted 1:100 into M-9 solution A and given a second 40 minutes' exposure to X-ray. If the downward bend in the inactivation curves was largely the result of accumulation of inactivating agents, the second dose of irradiation should be just as effective as the first. If, however, we are dealing with a cumulative damage to the phage, the second exposure should be more effective. The results of such an experiment, shown in table 1, clearly indicate that the second irradiation was more effective, a result that favors the hypothesis of cumulative damage.

Adsorption of inactive phage by bacteria. The fact that indirect inactivation is due to a chemical agent acting on the surface of the phage suggested that the

accumulation of damage resulting in inactivation might be caused by gradual change in the portion of the phage surface responsible for adsorption onto susceptible bacteria. Thus, we might expect to find that the inactive particles have a reduced adsorption capacity and that some of the residual active particles also may show such a reduced ability to adsorb. In what follows we use the term "adsorption" to mean irreversible phage adsorption, which is probably a second step in phage interaction with bacteria, following a reversible adsorption recently described (Puck *et al.*, 1951).

The adsorption of phage was studied using heat killed bacteria, which enable us to measure adsorption uncomplicated by virus multiplication (Schlesinger, 1932; Watson, 1950). The heat killed bacteria were prepared from broth cultures of bacteria in the logarithmic phase of growth by heating for 1 hour at 65 C. No viable bacteria remain, and all ability to support phage growth is lost, but

TABLE 1

Two-step indirect inactivation of T2

A T2 stock in M-9 was diluted 1:2 in broth, then 1:100 in M-9 solution. The diluted sample was irradiated for 40 min, then diluted 1:2 into broth. After 4 hr, this sample was diluted 1:100 in M-9 solution A and again irradiated for 40 min. For both irradiations, the phage survival was determined at 20 and 40 min intervals.

	DURATION OF EXPOSURE	TOTAL PHAGE SURVIVAL	SURVIVAL CORRECTED FOR INACTIVATION BY DIRECT EFFECT
	min		
First irradiation	0	1.00	1.00
	20	0.43	0.84
	40	0.105	0.40
Second irradiation	0	1.00	1.00
	20	0.174	0.34
	40	0.0172	0.064

the ability to adsorb phages T2, T4, T5, and T6 remains unchanged. The adsorption capacity of the active survivors of phage irradiated in M-9 solution A was tested by measuring the decrease in active phage in a mixture with heat killed bacteria due to irreversible union of phage with the bacteria. The results, shown in figure 2, indicate that the active survivors do have a reduced adsorption rate; furthermore, this rate decreases with increasing X-ray dose.

The adsorption ability of the inactive phage cannot be measured by titrating the free particles after contact with bacteria, since the phage cannot reproduce. Instead, we determined the ability of the inactive particles to interfere with adsorption of active phage by coating the bacterial surface (Schlesinger, 1932; Watson, 1950). For phage T2, about 250 to 300 particles must be adsorbed to bacteria in the logarithmic phase before the ability of the bacteria to adsorb T2 or T6 disappears.

Phage T2 inactivated by indirect effect was added to heat killed bacteria at a ratio of 250 inactive particles per bacterium. After allowing either 2 or 20 hours

for adsorption, phage T6 was added at a ratio of 1 particle to 200 bacteria. The residual T6 was measured at various intervals by plating on bacteria of strain B/2, resistant to phage T2. The results, shown in table 2, indicate that the inactive particles were much less effective in coating the heat killed bacteria than the control phage unexposed to the indirect effect. The rate of T6 adsorption, however, was slightly decreased by the presence of the inactive particles, indicating a partial coating of the bacteria, corresponding to the adsorption of 15 to 20 per cent of the inactive particles. The reduced adsorption of inactive T2 is not merely due to a slowing down of the adsorption rate since an increase of the period of preincubation of T2 with heat killed bacteria from 2 to 20 hours hardly changes the rate of adsorption of T6 added later. In all probability, a

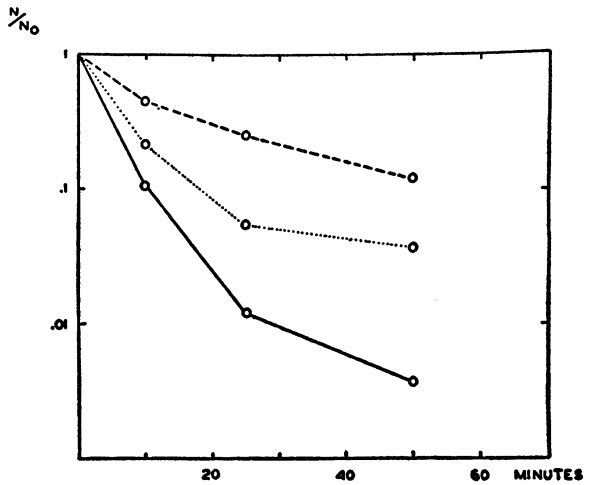

Figure 2. Adsorption of the residual active T2 survivors by heat killed bacteria following indirect inactivation. N/No = proportion of unadsorbed phage after various intervals of incubation with heat killed bacteria. The solid line is the adsorption curve for the control active phage. The dotted line is the adsorption curve for the residual active phage after 40 minutes of irradiation. The broken line is the curve for the residual active phage after 60 minutes of irradiation.

majority of the inactive particles have completely lost the ability to be adsorbed by host cells.

In summary, exposure to the indirect effect results in a gradual loss in adsorption ability, eventually resulting in complete loss. Since a fraction of the inactive particles can be adsorbed by host cells, inactivation is not entirely due to loss of adsorbability. The reduction in adsorption makes it difficult to test for other phases of the interaction of the inactive particles with the bacteria since the failure to observe the property may simply be due to insufficient adsorption.

Inactivation by Irradiated Media—After Effect

The continued inactivation of irradiated purified stocks after cessation of irradiation indicated that the irradiation had produced relatively stable chemical agents capable of inactivating phage. Various solutions were irradiated at 5 C,

brought to 37 C, and inoculated with phage, which was then assayed after incubation. The results for various solutions commonly employed in phage work are

TABLE 2

Prevention of adsorption of active T6 by T2 inactivated by the indirect effect

The concentration of heat killed bacteria was 2×10^7 cells per ml. The survival of irradiated phage T2 was 10^{-3}. Platings were done on B/2, so that only T6 would form plaques.

TUBE NO.	CONTENTS AFTER THE FIRST INPUT	TIME OF ADDITION OF T6, 0.005 PHAGES PER BACTERIUM	PLAQUE COUNT OF T6 AT VARIOUS TIMES			
			0	30 min	60 min	90 min
		hr				
1	Active T2 plus HKB,* 250 phages per bacterium	2	321	290	246	238
2	Active T2 plus HKB,* 250 phages per bacterium	20	317	306	305	271
3	Irradiated T2 plus HKB, 250 phages per bacterium	2	308	152	68	37
4	Irradiated T2 plus HKB, 250 phages per bacterium	20	322	296	102	54
5	Broth plus HKB	2	308	126	42	14
6	Broth plus HKB	20	310	125	54	15

* HKB = heat killed bacteria.

TABLE 3

Inactivation of T4 by irradiated solutions

The solutions were irradiated at 5 C for 30 min (40,000 *r*). Before the addition of phage, they were brought to 37 C. A T4 stock in M-9 was diluted 1:10^4 in phosphate buffer M/15, pH 7, containing 10^{-3} M Mg^{++}. One-tenth ml of this phage dilution was added to 0.9 ml of various irradiated solutions. The mixtures were incubated for 60 min at 37 C, then assayed for phage survival.

TUBE NO.	IRRADIATED SOLUTION	SURVIVAL AFTER 60 MINUTES' INCUBATION
1	Phosphate buffer M/15, pH 7, plus Mg^{++} 10^{-3} M	0.80
2	M-9 solution A plus Mg^{++}, pH 7	0.45
3	T1 lysate in M-9 medium plus Mg^{++}	0.025
4	T2 lysate in M-9 medium plus Mg^{++}	0.063

given in table 3. It is clear that all irradiated solutions inactivate phage but differ in the amount of inactivation.

The reason for the differences in inactivating capacity remains largely un-

known, because the complex differences between the various solutions have not been adequately investigated. The difference between M-9 solution A and phosphate buffer is the easiest to analyze since M-9 solution A contains some inorganic salts ($MgSO_4$, NH_4Cl) in addition to phosphate buffer. Each salt was added to the buffer separately and tested. It was found that $MgSO_4$ had no effect, while both NH_4Cl and NaCl markedly increased the inactivating ability of irradiated buffer. The influence of these salts is not on the radiochemical reaction itself since their effect is the same whether they are added to phosphate buffer before or after irradiation.

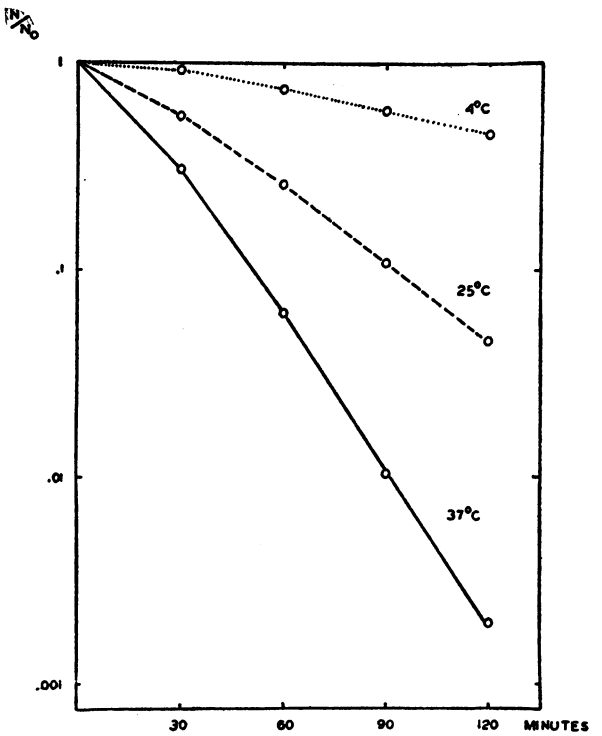

Figure 3. Inactivation of phage T2 by an irradiated T1 lysate (40,000 *r*) as a function of the time of exposure to the irradiated lysate and of the temperature. N/No = proportion of active phage particles after various intervals of incubation with the irradiated lysate.

Among the irradiated solutions so far tested, phage lysates in M-9 medium are the most effective inactivating agents. These lysates consist of M-9 medium, bacterial debris, and phage material. How the bacterial debris and the phage increase the inactivating capacity of the medium is unknown. Due to their powerful inactivating ability, it proved convenient to use such irradiated lysates to prepare the inactive phage used in the study of the biological properties of phage inactivated by after effect.

Survival curve. The survival curve of T2 suspended in irradiated T1 lysates (figure 3) is not exponential but bends downward. Since the toxic agents are

slightly unstable, losing about 30 per cent activity in 2 hours at 37 C, the downward concavity is partly compensated for, and the true dose-effect curve would show an even sharper downward bend. A similar bend is also shown by the inactivation of T4 in irradiated T2 lysates. The sensitivity of T2 and T4 to irradiated solutions is quite similar, possibly a little higher for T4. As shown in figure 3, the inactivation rate increases rapidly with increasing temperature.

Biological properties. The experiments were done with phage inactivated by irradiated T1 lysates. Many of the inactive particles retain the ability to adsorb on sensitive bacteria and kill them. This can be demonstrated by mixing bacteria with inactive phage and determining their survival. One particle is sufficient to kill a bacterium, so that if the fraction of surviving bacteria is e^{-x}, x is the average number of killing particles adsorbed per bacterium. The fraction of inactive particles that retain the killing ability decreases with increasing degree of inactivation (see table 4) and is similar for phages T2 and T4.

TABLE 4

Bacterial killing ability of T2 inactivated by irradiated T1 lysates

Phage T2 was introduced into an irradiated T1 lysate. At various intervals samples were diluted into broth, to stop further after effect, and assayed for phage survival. The bacterial killing ability of the inactive phage was determined by mixing the inactive phage with bacteria for 10 min, after which the bacteria were plated to determine the number of bacterial survivors.

PERCENTAGE OF T2 PARTICLES THAT ARE ABLE TO REPRODUCE	PERCENTAGE OF PARTICLES THAT CAN KILL BACTERIA
100	100
25	91
9	63
2	53
0.2	38
0.02	30

Luria (1947) found that phages T2, T4, T5, and T6 inactivated by ultraviolet light can give multiplicity reactivation, consisting in the production of active phage in a fraction of the bacteria infected with two or more inactive particles. We tested for this type of reactivation in phage inactivated by after effect of X-rays. Various dilutions of inactive T2 were mixed with bacteria, and adsorption was allowed to occur. Before lysis, the mixtures were diluted and samples were plated with an excess of sensitive bacteria; the number of bacteria that liberated active phage was determined. The results, illustrated in table 5, indicate that the number of bacteria yielding active phage is greater than the number of bacteria infected with the residual active phage. The frequency of reactivation (the ratio between the number of bacteria that liberate active phage, corrected for residual active, and the number of multiple infected bacteria) is a function of the number of inactive particles per bacterium and increases with increasing multiplicities. Less reactivation occurs with phage from samples in which the survival was lower and in which the inactive particles had presumably received

more damage. Phages T2 and T4 show approximately equal amounts of multiplicity reactivation. For corresponding survivals, phage inactivated by irradiated lysates shows a greater amount of reactivation than phage inactivated by the direct effect of X-rays (Watson, 1950) but much less than phage inactivated by ultraviolet (Luria, 1947).

Dulbecco (1950) observed reactivation of phage inactivated by ultraviolet light upon exposure to visible light in the presence of bacterial cells (photoreactivation). We tested for photoreactivation of phage inactivated by irradiated lysates by plating phage with bacteria and immediately incubating the plates under fluorescent lights. No reactivation was found, even though the dose of visible light was sufficient to cause maximal reactivation of phage inactivated by ultraviolet.

TABLE 5

Reactivation of T2 inactivated by an irradiated T1 lysate as a function of the multiplicity of infection

The residual active fraction of phage was 10^{-3}. Each mixture contained a constant amount of bacteria and various amounts of inactive phage.

MIXTURE NO.	PHAGE INPUT*	MULTIPLICITY OF INFECTION*	(a) BACTERIA WITH RESIDUAL ACTIVE PARTICLES	(b) BACTERIA WITH TWO OR MORE INACTIVE PARTICLES	(c) BACTERIA THAT LIBERATE ACTIVE PHAGE	(d) EXCESS (c) − (a)	RATIO (d)/(b)
1	3.2×10^8	3.2	2.8×10^6	8.2×10^7	1.38×10^7	1.1×10^7	0.134
2	1.6×10^8	1.6	1.4×10^6	4.9×10^7	4.98×10^6	3.5×10^6	0.071
3	8×10^7	0.8	7.0×10^5	2.1×10^7	1.8×10^6	1.1×10^6	0.052
4	4×10^7	0.4	3.5×10^5	6.6×10^6	5.8×10^5	2.3×10^5	0.035

* Only particles able to kill are included in these calculations. For every killing particle there are 3 nonkilling ones.

In summary, phage is inactivated by exposure to media previously irradiated with X-rays. At relatively low survivals, a large fraction of the inactive particles can still adsorb on the host bacteria and kill them. This is in marked contrast to the situation with phage inactivated by the indirect effect of X-rays, where the adsorbing ability, and thereby also the killing ability, is lost rapidly. Multiplicity reactivation of the inactive particles occurs, but no photoreactivation has been observed.

DISCUSSION

Phage inactivated by previously irradiated solutions has different biological properties than phage inactivated by the total indirect agent(s) present during irradiation of purified phage suspensions. It is thus probable that more than one type of indirect agent exists, one type being unstable and not persisting following cessation of irradiation, the other relatively stable and detectable after irradiation. The unstable agent(s) inactivates the phage largely by destroying its ability to adsorb on bacteria. On the contrary, the more stable agent(s) usually inactivates in a manner that allows adsorption of the inactive particles. Simple calcula-

tions show that the amount of stable toxic products produced by irradiating M-9 solution A is insufficient to account for more than 10 per cent of the indirect effect resulting from irradiation of purified stocks in the same medium.

The nature of the two types of indirect agents is not known. The short lived agents may be the highly reactive radicals produced during irradiation of water (Weiss, 1944). Preliminary experiments with phage inactivated by H_2O_2 showed many similarities with phage inactivated by irradiated media, thus suggesting that H_2O_2 may be one of the stable toxic products persisting after irradiation. Other peroxides might also be involved.

TABLE 6

The properties of bacteriophage T2 inactivated by X-rays and ultraviolet light

INACTIVATING AGENT	RATE OF INACTIVATION	ADSORPTION	BACTERIAL KILLING ABILITY	PHOTOREACTIVATION	MULTIPLICITY REACTIVATION
Direct effect of X-rays	Exponential	Normal	Lost at a rate $\frac{1}{3}$ the rate of inactivation	+	+
Indirect effect of X-rays	Cumulative damage	Greatly reduced	Uncertain because of poor adsorption	−	Uncertain because of poor adsorption
After effect of X-rays	Cumulative damage (?)	Slightly reduced	Lost at slow rate	−	++
Ultraviolet light*	Nearly exponential	Normal	Normal	++++	++++

(?) Experimental data inconclusive.

* Data from Luria (1947) and Dulbecco (1950).

This study in no way attempts to present a comprehensive picture of the action of poisons produced by radiation on bacteriophage. Its interest is mainly that it demonstrates the existence of at least two types of indirect agents produced by radiation. Any unitarian interpretation of indirect effects is, therefore, an over simplification. We have no evidence as to the role of the various toxic agents in indirect effects of ionizing radiation observed with other biological systems. The complexity of the radiochemistry of such relatively simple systems as virus particles suggests that indirect effects in different cases are probably due to a variety of reactive chemicals produced by the irradiation. This is especially suggested by the observation that crude phage lysates in synthetic medium are more effective sources of inactivating agents than the medium itself.

It is interesting to compare the properties of phage inactivated by the indirect agents with those of phage inactivated by the supposedly direct adsorption of energy in the particles (direct effect). Such a comparison, summarized in table 6, shows important differences among phage particles inactivated by X-rays under

various conditions. The distinction between direct and indirect effects, previously based on considerations involving only the rate of inactivation, is validated by the finding of different properties produced by these types of damage. Table 6 also includes the properties of phage inactivated by ultraviolet light, which produces a distinctive type of inactive particle; most noticeable is the large amount of photoreactivation. Whether this is connected with the specific adsorption of ultraviolet by the nucleic acid fraction of the phage cannot yet be decided.

The results also show that multiplicity reactivation, which was only known to take place with ultraviolet inactivated phage and to a very slight extent with phage inactivated by the direct effect of X-rays, also occurs with phage inactivated by treatment with chemicals, such as those present in an X-ray irradiated medium.

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SUMMARY

The inactivation of bacteriophages of the T group by X-rays was investigated under conditions where indirect effects are prevalent, and the properties of the inactive viruses were studied. Indirect inactivation is caused by at least two different agents or groups of agents. One, short-lived, is detectable only by its action during actual exposure of phage to radiation; the other, relatively stable, is detected by the persistence of its effect after irradiation. A variety of factors in the composition of the medium influence the amount of inactivation. Phages of the T2 group, inactivated by the short-lived indirect agent, exhibit a reduced ability to be adsorbed by bacteria; the active survivors are also adsorbed at a reduced rate. The same phages, inactivated by the stable indirect agent, show an adsorption ability only slightly reduced, and many of the inactive particles that are adsorbed are still able to kill the host bacteria and to be reactivated in multiple infected bacteria. No photoreactivation has been observed. All types of inactive phage particles produced by indirect effects of X-rays differ in their properties from phage inactivated by the direct effect of X-rays or by ultraviolet light.

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