THE EFFECT OF ULTRAVIOLET LIGHT ON THE PRODUCTION OF BACTERIAL VIRUS PROTEIN*

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Hershey and Chase discovered in 1952 (1) that it is the deoxyribonucleic acid (DNA) and not the protein of bacteriophage T2 which is responsible for the initiation of phage reproduction within an infected bacterial cell. The phage DNA, therefore, appears to have at least two roles in the synthesis of the progeny bacteriophage particles: one is to cause its own, several hundredfold replication and the other is to induce the formation of phage-specific protein. The present work is an attempt to study the second of these roles by examining to what extent and how long after infection the integrity of the phage DNA is required for the successful synthesis of that phage-specific protein whose presence can be detected by virtue of its ability to react with antiphage serum (2, 3). For this purpose, the effect of ultraviolet light (UV) irradiation on the appearance of protein possessing phage antigenicity inside T2-infected bacterial cells has been studied at various stages of phage development.

Materials and Methods

Bacteriophage T2r$^+$ and its host *Escherichia coli*, strain B(H), were used in this study.

Tris-glucose medium (4) contains per liter 2 gm. glucose, 5.4 gm. NaCl, 3 gm. KCl, 1.1 gm. NH_4Cl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1 M tris-HCl buffer (tris-hydroxymethyl amino methane) adjusted to pH 7.2 at 37°C., 20 mg. phosphorus (as KH_2PO_4), and 6 mg. sulfur (as Na_2SO_4). In this medium, the latent period of T2 is 22 minutes at 37°C.

The general methods of bacterial culture, preparation of phage stocks and antisera, and assay of infectivity used in this study were those described by Adams (5).

Determination of Phage Antigen.—T2-antigenic protein has been defined here as that protein which can be precipitated specifically with anti-T2 rabbit serum. The amount

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of protein so precipitated was determined by measurement of the radioactivity in precipitates (3) formed by the addition of anti-T2 serum to S^{35}-labelled lysates, according to the following procedure: The crude radioactive lysate was first dialyzed in the cold against adsorption buffer (1) (containing 0.003 M KCN and 20 mg./ml. chloramphenicol to inhibit bacterial growth) and then centrifuged at low speed (1,300 × *g* for 20 to 30 minutes) to remove large cell debris. The supernatant of this low speed centrifugation was assayed for infective phage and total radioactivity and will be referred to as "supernatant S" in the following. Approximately 10^{12} UV-killed T2 per ml. were added to supernatant S as carrier, a fixed amount of anti-T2 rabbit-serum, which had been exhausted repeatedly for any bacterial antibodies with a mixture of intact and broken *coli* cells, was then introduced, and the mixture incubated first for 1 or 2 hours and then refrigerated overnight (6). The precipitate which had formed was sedimented by low speed centrifugation (850 × *g* for 13 minutes), and washed three times with adsorption buffer. Radioactive counts on the precipitate were made in a windowless gas flow counter on dry samples mounted in plastic planchets.

Two groups of control experiments were carried out to test the specificity of such precipitates. In one of these, UV-killed carrier T3 bacteriophage particles (serologically unrelated to T2) were added to various supernatants S and precipitates formed by addition of strong anti-T3 serum. In the second type of control, UV-killed carrier T2 phages were added to cultures of S^{35}-labelled, uninfected bacteria which had been disrupted by sonic oscillation and precipitates formed by addition of strong anti-T2 serum. Both types of control precipitates were found to contain about 5 per cent of the total radioactivity of the mother liquor in each case. The radioactivity in these control precipitates thus represents a background of non-specific protein which is carried down together with the antigen-antibody complexes and for which a correction must be made in the calculation of the specific radioactivity of the specific precipitates from the supernatants S in the experiments reported below. It was not possible to reduce this background further either by a preliminary dialysis of supernatant S against 15 per cent mercaptoethanol (which should have prevented unspecific binding of radioactive peptides to the serum proteins through divalent metal linkages or disulfide bonds (7)), or by employing for precipitation only the γ_2-globulin fraction of the exhausted anti-T2 serum instead of the whole serum (8). The *specific* radioactivity contained in the precipitates, S_ϕ, was then estimated according to the relation

$$S_\phi = \frac{S_{ppte} - r \cdot S_{total}}{1 - r}$$

in which S_{ppte} and S_{total} represent respectively the radioactivity of the precipitates and of supernatant S and $r = 0.05$, being the fraction of the total S^{35} found to be precipitated non-specifically in the control experiments.

UV irradiations were carried out in dim light with a 15 watt Westinghouse "sterilamp" at a distance of 80 cm. During the irradiations, the suspensions of phage or infected cells were placed on a watch glass set over cracked ice (9). Incubations after UV irradiation were always carried out in dim light to minimize any possible photoreactivation.

Experimental Results

Sulfur Content of T2.—In the work reported below, the presence of phage protein has been detected by introducing radiosulfur, S^{35}, into its sulfur-containing amino acids. In order to determine the total amount of sulfur per T2 particle, the following experiment was carried out.

A number of cultures of bacteria growing in tris-glucose medium labelled with 2 to 8 mc. of S^{35} per mg. total sulfur were infected with a multiplicity of 5 T2 particles per cell and lysed by addition of M/100 KCN 100 to 200 minutes after the start of phage growth at 37°C. The lysates, which contained from 80 to 100 progeny per infected bacterium, were mixed with UV-inactivated carrier T2 particles and the phages isolated from the lysates by three cycles of alternate high-speed (10,000 *g* for 40 minutes) and low-speed (1,300 *g* for 20 minutes) centrifugations (6). The infective titer of the purified suspensions as well as the amount of S^{35} precipitable by anti-T2 serum or adsorbable to sensitive cells was assayed and from these data and from the specific activity of the growth medium the amount of sulfur per infective T2 unit calculated.

This experiment indicated that each infective T2 particle contains $2.4 \pm 0.3 \times 10^{-12}$ μg. of sulfur, in agreement with the range of values obtained by Hershey and Chase (1).

Phage Protein Synthesis in Normal Infection.—The kinetics of formation of S^{35}-labelled phage antigenic protein in bacteria infected with T2 bacteriophage was studied by breaking open the infected cells at various stages of intracellular phage development and assaying the radioactivity precipitable by anti-T2 serum by a method similar to that employed for T4 by Maaløe and Symonds (3).

A culture of bacteria was grown to a density of 2×10^8 cells/ml. in tris-glucose medium containing from 2 to 8 mc. S^{35} per mg. total sulfur, washed, resuspended in tris-buffered saline (glucose-free tris medium), and starved for 30 minutes at 37°C. (9). The culture was then infected with a multiplicity of 5 to 7 T2 phages per cell, and, after allowing 5 minutes at 37° for phage adsorption, the infected bacteria were separated from unadsorbed free phage by centrifugation, resuspended in tris-glucose medium containing the original concentration of S^{35}, and incubated at 37°C. At various times after the beginning of phage development, the infected cells were lysed by addition of M/100 KCN and (in some experiments) a high titer of UV-killed $T2r^+$ phage particles (10). These lysates were then analyzed for their content of infective phage progeny and antigenic protein.

The result of a typical experiment is presented in Fig. 1, on which the amount of T2 antigen per infected cell recovered at different times of phage development has been plotted in multiples of the amount of sulfur per T2 phage particle. It can be seen from the curve labelled "total antigen" that phage antigenic protein makes its intracellular appearance about 10 to 12

minutes after the start of phage development and then rises rapidly in amount until a level of about 75 phage units per cell has been attained by the 40th minute. The ratio of the amount of antigen to infective progeny particles is seen to be very high at the conclusion of the eclipse period and falls rapidly to a much lower value as more mature progeny particles appear. These results agree very well with the observations of Maaløe and Symonds (3) on the appearance of T4 antigen and indicate the presence of non-infective phage

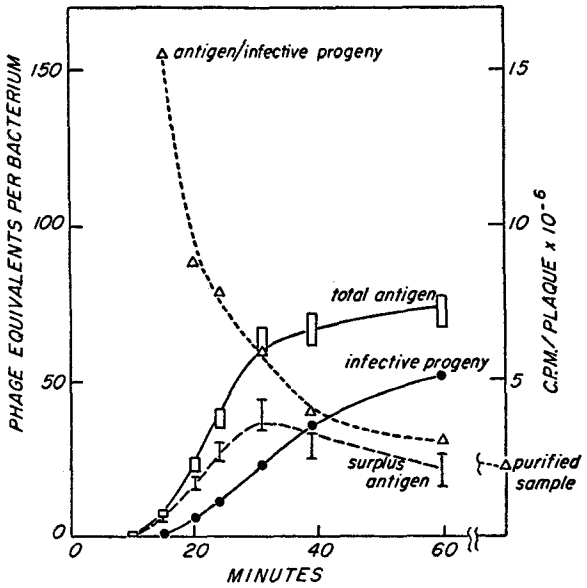

FIG. 1. Amounts of S^{35}-labelled antigen and infective progeny (left ordinate) and antigenic S^{35}-radioactivity per infective progeny (right ordinate) found in T2-infected bacteria at various times after the onset of phage development. "Purified sample" refers to the S^{35} activity per infective phage found upon isolation by differential centrifugation of the T2 particles of a late lysate of this experiment.

antigens within the infected cell. The amount of such non-infective or "surplus antigen" present at various times can be estimated by subtracting from the amount of total antigen the product of the number of infective progeny times the amount of sulfur per infective unit. The results of such calculations are also indicated in Fig. 1, where it may be seen that the surplus antigen appears to reach a constant level at 40 phage units per bacterium between 20 and 40 minutes after the onset of phage development.

As in the case of T4, these non-infective antigenic particles are lighter than the infective, mature phage particles and can be separated from the latter by differential centrifugation (3). Less than 10 per cent of such non-infective

antigens, however, were found to be adsorbable to sensitive bacteria, in contrast to the finding of Maaløe and Symonds that non-infective T4 antigen adsorbs to bacteria as readily as infective phage. In our case, non-infective protein resembles more the phage protein "doughnuts" (2), which do not adsorb to sensitive cells.

Phage Protein Synthesis after Infection with UV-Inactivated Phage.—Although there occurs neither production of infective progeny nor any synthesis of phage DNA after infection of bacteria with UV-inactivated T2, some protein still continues to be synthesized in such cells (11). In order to determine now whether the protein formed under these conditions is phage protein or

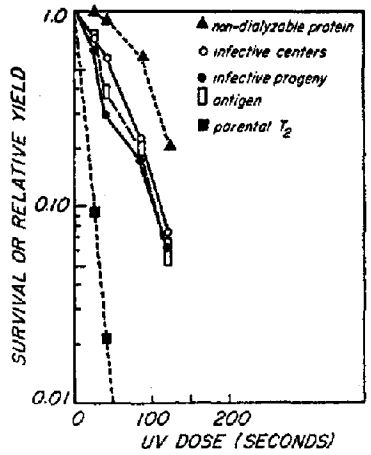

FIG. 2. Survival as infective centers and relative yield 70 minutes after the onset of phage development of infective progeny, S^{35}-labelled antigen, and S^{35}-labelled non-dialyzable protein of bacteria multiply infected with T2 particles which have received various doses of UV. The survival of the free parental T2 is also shown.

not, we have tested its precipitability with anti-T2 serum. For this purpose, non-S^{35}-labelled bacteria were infected at a multiplicity of 6 phages per cell with stocks of T2 which had received various doses of UV. The infected bacteria were then transferred to tris-glucose medium labelled with S^{35} and incubated at 37°C. for 70 minutes before being lysed by KCN. The lysates were assayed, as above, for infective progeny and for total non-dialyzable as well as antigenic S^{35}-labelled protein. The results of this experiment are presented in Fig. 2, on which the ratio of the yield of these substances formed after infection of bacteria with phage having received various doses of UV to the yield found after infection with unirradiated phage is plotted against the UV dose. Also shown in Fig. 2 is the relative survival with UV dose of the free parental T2 and of the multiply infected bacteria as infective centers when

plated before burst. (The greater resistance of the infective centers than of the parental phage to UV inactivation is due to multiplicity reactivation (12).) It is seen in Fig. 2 that the survival of the infective centers as well as the yields of infective progeny and phage antigen formed by the 70th minute after the start of phage development all decrease in the same way with the UV dose to which the parental T2 phages have been exposed before infection. It can be concluded from these results that only those UV-irradiated T2 phages and hence only those parental DNA structures can induce the synthesis of phage antigenic protein which are also able to give rise to infective progeny. The fact that the non-dialyzable protein synthesized after infection decreases more slowly with UV dose than the infective centers confirms the observation of previous workers that some protein synthesis still proceeds in bacteria infected with phages unable to give rise to any infective progeny.

Phage Protein Synthesis after UV Irradiation during the Eclipse.—The effect of UV on phage antigen production has now been examined after irradiation of T2-infected bacteria, rather than of free parental phage, at various stages of intracellular phage development. Multiply T2-infected bacteria growing in non-labelled tris-glucose medium were irradiated with different doses of UV at different times during their eclipse period and S^{35} added to the growth medium after irradiation. After further incubation at 37°C. for 60 minutes, the infected bacteria were lysed by KCN and the lysates assayed as above for the amount of infective progeny phage and S^{35}-labelled antigenic protein. The results of this experiment are shown in Fig. 3, on which the ratio of the amounts formed after UV-irradiation to those formed in the aliquot which had received no UV, are plotted as a function of UV dose. It is seen in Fig. 3, first of all, that UV irradiation of those infected bacteria in which no phage development had been allowed to proceed ($t = 0$) eliminated the formation of infective centers, phage antigen, and infective progeny in a manner similar to UV irradiation prior to infection of free T2 subsequently used to infect non-irradiated bacteria at a comparable multiplicity (*cf.* Fig. 2). At later stages of phage development, *i.e.* during the first 11 minutes after infection, the infective centers are seen to grow increasingly refractory to UV inactivation, in agreement with observations of Luria and Latarjet (13) and Benzer (9). Simultaneously, the UV sensitivity of phage antigen formation appears to decrease to the same extent. By the 16th minute after the start of phage development, when the UV sensitivity of the infective centers is seen to have increased once more, phage antigen formation has experienced a further stabilization towards UV inactivation. The relative amount of infective progeny formed, however, is seen to have experienced little change in UV sensitivity throughout this period. A striking disbalance between the amount of phage antigen and infective progeny can therefore be achieved by irradiating T2 infected bacteria at a stage later than 15 minutes after the start

of phage development. For example, the data presented in Fig. 3 indicate that the ratio *phage antigen/infective progeny* is ten times greater than normal in the aliquot irradiated for 210 seconds 16 minutes after the onset of phage development.

It is possible that the great increase in surplus antigen formed after UV irradiation at late stages of the eclipse period actually represents structurally intact progeny phages which are non-infective because they harbor non-infective DNA possessing UV lesions. If this were true, such surplus antigen should be adsorbable to sensitive bacteria, in contrast to the surplus antigen

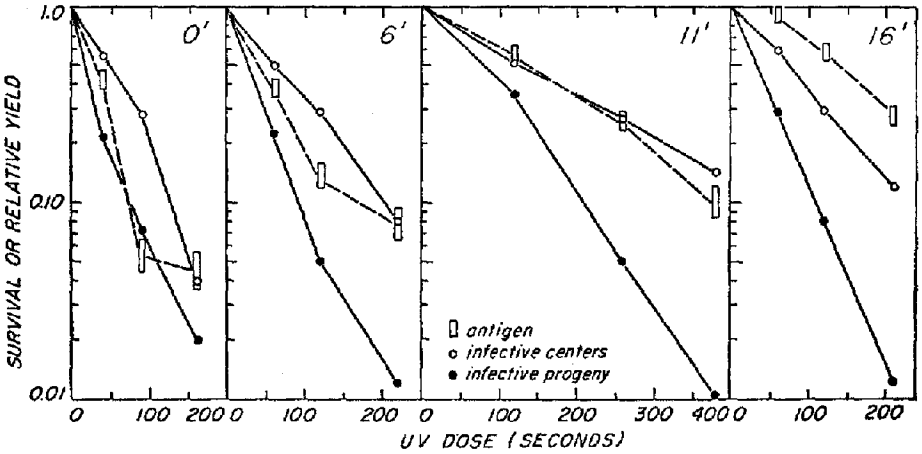

FIG. 3. Survival as infective centers and relative yield of infective progeny and S^{35}-labelled antigen 60 minutes after exposure to various doses of UV of bacteria multiply infected with T2. Each panel represents a culture in which phage development has been allowed to proceed for a certain number of minutes before UV irradiation.

formed in the course of normal infection. We have accordingly tested the adsorbability of the antigenic S^{35} in a lysate of bacteria irradiated at the 15th minute after the onset of phage growth and found that only 15 per cent of the antigenic S^{35} could attach itself to sensitive cells. The bulk of the surplus antigen in such lysates, therefore, appears to be in some form other than morphologically intact but non-infective progeny.

In order to test whether the great relative increase in surplus antigen after UV irradiation late in the eclipse reflected the possibility that once phage reproduction has proceeded beyond a certain point, the phage antigen synthesizing mechanism becomes more stable against UV irradiation than the synthesis of phage DNA, the following experiment was carried out. Bacteria were multiply infected with T2, as above, incubated in non-labelled tris-glu-

cose medium at 37°C. for 15 minutes, and irradiated with a UV dose of 150 seconds, which permitted survival of 20 per cent of the infective centers. Radiosulfur, S^{35}, or radiophosphorus, P^{32}, was then added to two different samples of the irradiated culture. The infected bacteria were incubated further at 37°C. and aliquots lysed from time to time by addition of KCN. The amounts of infective progeny and S^{35}-labelled phage antigen in the lysates were then assayed as above, while the amount of P^{32}-labelled DNA synthesized

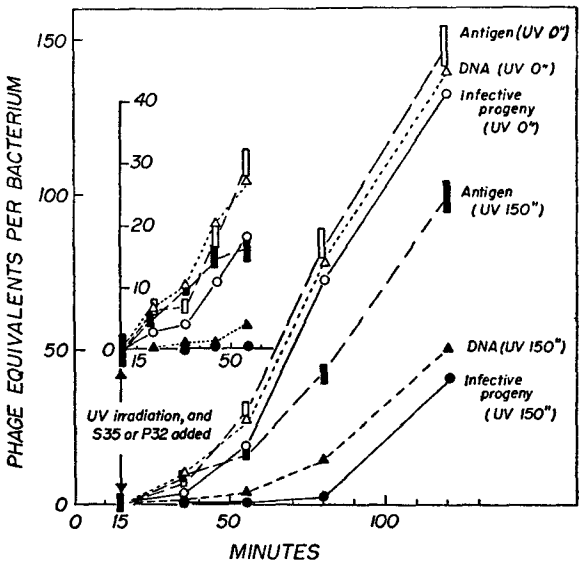

FIG. 4. Amounts of P^{32}-labelled DNA, S^{35}-labelled antigen, and infective progeny found in T2-infected bacteria at various times after the onset of phage development in a culture to which radioisotopes were added at the 15th minute. At that point, one part of the culture was UV-irradiated (UV 150″) while another remained unirradiated (UV 0″). Insert presents early points with expanded ordinate.

after irradiation was determined by the extraction technique of Morse and Carter (14). Control assimilation experiments in which the same amounts of radiosulfur or radiophosphorus were added 15 minutes after infection to infected bacteria not irradiated with UV were carried out at the same time. The results of this experiment are presented in Fig. 4, where the amounts of S^{35}-labelled antigen and P^{32}-labelled DNA per infected cell have been expressed in multiples of the amount of sulfur (2.4×10^{-12} μg.) and phosphorus (2.3×10^{-11} μg.) (15) per infective T2 particle. It is seen, first of all, that in the non-irradiated control, the amounts of infective progeny, phage antigen, and DNA formed subsequently to 15 minutes increase hand in hand with each other. In the UV-irradiated culture, on the other hand, formation of infective progeny as

well as of DNA is almost completely arrested for some 60 minutes after irradiation, after which time their synthesis resumes. Phage antigen, however, continues to be formed at almost a normal rate for the first 20 to 30 minutes after UV irradiation, after which time it proceeds at a somewhat reduced rate.

Thus it is evident that by the 15th minute after the onset of phage development the reactions necessary for phage protein production have indeed become more refractory to inactivation by UV than the processes of phage DNA synthesis. After that time, furthermore, there appears to be no direct relation between the rates of phage DNA and phage protein synthesis, since protein synthesis can take place at an almost normal rate while DNA synthesis is arrested. This finding complements previous observations that at this same late stage of the eclipse period phage DNA synthesis can proceed after phage protein synthesis has been suppressed by addition of inhibitors or removal of required amino acids (16–18).

DISCUSSION

The present experiments show that at the outset of T2 phage infection the two functions of the DNA of the parental phage, *i.e.* causing its own reduplication and inducing the synthesis of phage protein, are equally sensitive to inactivation by UV, suggesting that the integrity of quantitatively equivalent parts of the phage DNA is required for the successful initiation of both reactions. After the synthesis of phage-specific protein has proceeded to the point at which the first phage antigen makes its intracellular appearance (*cf.* Fig. 1), the protein-synthesizing mechanism has become very much more resistant to UV irradiation than the DNA-synthesizing mechanism, so that phage antigen can continue to be formed at a high rate even after UV irradiation at some late stage in phage development has abolished further DNA synthesis (*cf.* Fig. 4). Two possibilities can be considered for the reason why during the eclipse period phage antigen formation becomes more resistant to UV irradiation than DNA synthesis, depending on whether one envisions a *direct* or an *indirect* control of protein formation by the phage DNA throughout the eclipse period. If the control is *direct*, then there may occur a change of state or structure of the phage DNA at the onset of its replication during the eclipse period, so that UV lesions on the vegetative DNA do not interfere as much with its protein- synthesizing functions as with its self-duplication. If the control is only *indirect* then there may occur a transfer of the "information" pertaining to the specificity of the phage antigen from the parental DNA to another substance which is more resistant to UV irradiation than DNA, *e.g.* ribonucleic acid, protein, or ribonucleoprotein, and which can still direct the synthesis of antigenic protein after UV irradiation has destroyed the parental DNA. Although it is difficult to decide between the two alternatives on the basis of the available evidence, the recent

demonstration of the synthesis of an essential, non-phage–antigenic protein (16–18) and of a special ribonucleic acid (19) at the onset of T2 and T4 reproduction is in good agreement with the second hypothesis of *indirect control* of the synthesis of the antigenic phage protein by the DNA of the infecting phage.

SUMMARY

The amount of phage-specific protein in T2-infected bacteria growing in a medium containing radiosulfur, S^{35}, has been studied by measuring the radioactivity in specific antiphage serum precipitates of lysates. In the course of normal infection, non-infective phage antigen has been found to make its first intracellular appearance shortly before the end of the eclipse period, in agreement with the findings of Maaløe and Symonds with phage T4. No such phage antigen is produced either in bacteria infected with UV-inactivated T2 or in T2-infected bacteria whose survival as an infective center has been destroyed by UV irradiation during the early stages of the eclipse period. If the infected bacteria are UV-irradiated only at later stages of the eclipse period however, then phage antigenic protein continues to be synthesized in those infected cells in which DNA synthesis and, *a fortiori*, production of infective progeny have been almost completely suppressed. It is concluded from these results that once the mechanism for formation of phage-specific protein has been established within the infected cell under the influence of the parental DNA, synthesis of phage-specific protein can continue independently of the synthesis of phage DNA. The possibility that the phage DNA controls the specificity of the phage protein indirectly through substances other than DNA is discussed.

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