Effect of Infection with Phage Lambda on the Synthesis of Protein, RNA and DNA in *Escherichia coli*

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SUMMARY

Delay in deoxyribonucleic acid (DNA) synthesis after use of ultraviolet radiation to induce *Escherichia coli* lysogenic for phage λ was due to the irradiation procedure; the same delay was found in non-lysogenic bacteria exposed to the same dose of radiation. After infection with phage λ_c or λ_v in 0.02 M-MgSO₄, DNA synthesis began without delay when complete medium was added. During vegetative development of phage in induced bacteria, 81–89 % of the DNA synthesized was accounted for in the phage progeny, indicating that host DNA synthesis is much diminished and may be completely inhibited. Mature phage particles arose soon after the appearance of serum blocking power (SBP) and endolysin activity. Induced bacteria synthesized up to 15 times more SBP than was incorporated into complete phage particles: the excess SBP was not sedimented at 40,000 g (unlike phage particles), but about half was sedimented at 90,000 g, the rest remaining in the supernatant fluid. Whilst protein and RNA synthesis in infected or induced bacteria was initially similar to that in the control, there was a marked decrease of synthesis in the second half of the latent period.

INTRODUCTION

The extent to which host-cell metabolism is affected by the vegetative development of bacteriophage has been studied almost exclusively in *Escherichia coli* infected with the T series of coliphages. Thus in bacteria infected with phage T₂ or T₄, there are profound effects on the net synthesis of ribo- and deoxyribo-nucleic acids (Cohen, 1948), and early in the latent period several new enzymes must be produced before the synthesis of specific phage components can take place (see Flaks, Lichtenstein & Cohen, 1959; Bessman, 1960). Analogous studies of the events during the latent period in cells infected with temperate bacteriophage (i.e. bacteriophage which can exist in the lysogenic state) have until recently received comparatively little attention and most of the data concerned *Bacillus megaterium* (Siminovitch & Rapkine, 1952). When *B. megaterium* lysogenic for phage 1 was induced by ultraviolet (u.v.) radiation, there was little change in the rate of synthesis of ribonucleic acid (RNA) and protein. On the other hand, no net synthesis of deoxyribonucleic acid (DNA) was detected during the first part of the latent period. Since after infection of *E. coli* with phage T₂ there is also a lag period before DNA synthesis is resumed (Cohen, 1948), it was of interest to know whether the lag observed in the induced organisms was a direct effect of the onset of phage development, or only a secondary and indirect effect brought about by the irradiation

procedure. The exposure of the non-lysogenic parent *B. megaterium* to the same dose of u.v. radiation was in fact found to produce no lag in the synthesis of DNA (Siminovitch & Rapkine, 1952). However, with *E. coli* it has long been known that exposure to u.v. radiation causes inhibition of DNA synthesis, and the period of this inhibition is directly related to the dose of radiation (Kelner, 1953). In the present work, a study has been made of the effect of vegetative phage development on host-cell metabolism first in *E. coli* lysogenic for wild-type phage λ after induction by u.v. radiation, and secondly in *E. coli* after infection with phage mutants λ_c and λ_v . The latter are mutants of phage λ which have lost the ability to lysogenize *E. coli*; phage λ_c can infect and develop in bacteria which are not lysogenic for phage λ , whilst phage λ_v (virulent inducer mutant) will infect and develop in *E. coli*, whether or not the bacteria are lysogenic for phage λ (Jacob & Wollman, 1954). Recently Séchaud (1960) showed that when starved *E. coli* organisms were infected with phage λ_c in the presence of MgSO_4 and then transferred to complete medium, there was a delay of about 8 min. before net DNA synthesis was resumed. Most, if not all, of this period appeared to be required for events concerned with the injection of the phage DNA into the host cell.

METHODS

Organisms. *Escherichia coli* K12 strains K112 and c600, and the phages λ_{22} (wild-type) and the two mutants λ_{gc} and λ_{gv} (large plaque-forming mutants) have been described by Jacob & Wollman (1954, 1956*b*) and Fry (1959).

Growth media and assay methods. In general the media and plating procedures were based on those of Adams (1950). Peptone broth (PB) contained (g./l.) 10, Oxoid peptone; 1, Lab. Lemco; at pH 7. Tryptone broth (TB) contained (g./l.) 20, Oxoid tryptone; at pH 7. Phage stocks were stored and diluted in medium PB. For plating, medium PB was supplemented with 1 % NaCl and agar (Parke-Davis) as required (1% for the bottom layer of plates for phage and viable counts; 0.7 % for the small tubes in which samples were mixed before plating). *Escherichia coli* strain c600 was used as indicator organism in the phage assays.

Preparation of phage stocks. Stocks of phage λ_{22} , prepared by the induction of *Escherichia coli* (λ_{22}) and concentrated by centrifugation (see Fry, 1959), were stable for several months at 4° and contained about 1×10^{12} active particles/ml. Small stocks of phage λ_{gv} and λ_{gc} were prepared from plates by the confluent lysis technique and these were used to infect cultures (1 l.) of *E. coli* c600 in the log phase of growth (5×10^8 bacteria/ml.) at a multiplicity of 5 λ phage/cell. Cultures were incubated until lysis was complete, and then any whole bacteria and cell debris removed. The phage particles were collected by centrifugation (as for phage λ_{22}) and the pellet suspended in a small volume of medium PB.

Ultraviolet irradiation. An Hanovia Chromatolite low-pressure Hg lamp (Hanovia Ltd., Slough, England) without the filter was placed 75 cm. from the sample (100 ml.), which was rocked gently to and fro in an enamel dish (32 $\times$ 28 cm.). Irradiations were done in a dark room and in these conditions a dose of 60 sec. was just sufficient for the optimal induction (i.e. 90 % or more) of *Escherichia coli* K112 (λ_{22}): this dose allowed 30 % survival of non-lysogenic coli K112 (measured as ability to form colonies) and 45 % survival of phage λ_{22} (measured as ability to form plaques).

Preparation of samples and the determination of protein, RNA and DNA. Samples of cultures were taken at the required times and 1 ml. used for the determination of growth (in terms of turbidity), whilst 6 ml. were rapidly mixed with 0.6 ml. 50 % trichloroacetic acid (TCA). After standing overnight at 4° the precipitates were collected by centrifugation and washed once with 5 ml. 5 % TCA. Each precipitate was then suspended in 2 ml. 5 % TCA and heated at 90° for 30 min. in a water bath; double aluminium foil caps on the tubes prevented evaporation. After centrifugation the supernatant fluids were carefully removed and kept for the assay of RNA and DNA. Residues were dissolved in N-NaOH and 0.5 ml. samples were used for the assay of protein by the method of Lowry, Rosebrough, Farr & Randall (1951). Each sample received 5 ml. of the reagent prepared from 50 ml. 2 % Na_2CO_3 and 1 ml. 0.5 % CuSO_4 in 1 % NaK tartrate, followed after 10 min. by 0.5 ml. Folin-Ciocalteu reagent in N-HCl (British Drug Houses Ltd). After standing for 1 hr at 37°, the absorption of the blue colour was determined at 500 $\text{m}\mu$ (blue photo cell). A standard protein solution was prepared from bovine plasma albumin fraction V (Armour Pharmaceutical Co. Ltd., Eastbourne) in N-NaOH. For the determination of RNA, a suitable sample of the hot TCA extract in a total volume of 1.5 ml. 5 % TCA was mixed with 1.5 ml. of 0.5 % FeCl_3 in concentrated HCl and 0.15 ml. 10 % orcinol in absolute ethanol, and then heated at 100° for 30 min. (Schneider, 1945). After cooling, the absorption of the green colour was read at 660 $\text{m}\mu$ (red photocell). D-Ribose (L. Light, Colnbrook, Bucks.) served as the standard, and results are expressed in terms of μg . apparent ribose. DNA was determined by the method of Burton (1956). Samples (1 ml.) of the hot TCA extract were each mixed with 0.2 ml. of 3 N- HClO_4 and 1.2 ml. of Burton's acetaldehyde-Dische reagent. After overnight incubation at 37°, the blue colour was read at 600 $\text{m}\mu$ (blue photocell). 2-Deoxy-D-ribose (L. Light, Colnbrook, Bucks.) served as the standard and results are expressed in terms of μg . apparent deoxyribose. (The word 'apparent' is used because only the ribose and deoxyribose bound to purine nucleotides reacts in these procedures).

Determination of DNA content of phage λ_{22} . The phage particles were collected as a pellet by centrifugation for 1 hr at 40,000 g , the supernatant fluid removed and the pellet extracted overnight with 2 % tryptone solution. Insoluble material was removed (8000 g for 15 min.) and then the phage suspension centrifuged at 105,000 g for 1 hr. After removing the supernatant fluid as completely as possible, the pellets were extracted overnight with 0.05 M phosphate buffer (pH 7) containing 0.01 M- MgSO_4 and insoluble material removed (8000 g for 15 min.). The supernatant fluid was assayed for phage particles and 1 and 2 ml. samples used for the determination of DNA. The purified phage preparation contained 3.8×10^{11} particles/ml. (from mean of four plates; standard deviation $\pm 0.2 \times 10^{11}$) and 8.14 μg . apparent deoxyribose/ml. (from mean of 3 determinations; standard deviation ± 0.41), giving 2.14×10^{-11} μg . apparent deoxyribose/particle of phage λ_{22} .

Assay of serum blocking power (SBP). The methods used were based on those of DeMars (1955) and Jacob & Wollman (1956). Samples of the cultures were stored in ice until they were placed in the ultrasonic generator (2 min. exposure in a 1 kW. Mullard generator). The disintegrated suspensions were stored at 0° until required (usually overnight). Samples (0.2 ml.) containing the SBP material to be assayed were mixed with 0.8 ml. of 1.25×10^{-4} dilution of phage antiserum (neutralization

value, κ , = 200) and incubated for 6 hr at 37°. The test phage (λ_{gv}) was then added (0.2 ml. containing about 2×10^7 particles) and the mixture incubated for a further 3 hr. before plating appropriate dilutions with the indicator organism, strain $\kappa 112$ (λ_{22}). Standard curves were prepared by using stock phage suspensions.

Assay of endolysin. The method used was based on that of Jacob & Fuerst (1958). The lytic activity of the suspensions of bacteria disrupted by ultrasonic treatment was tested against bacterial suspensions prepared as follows. Cells of *Escherichia coli* $\kappa 112$ were harvested from growing cultures (5×10^8 organisms/ml.), washed once with distilled water and resuspended in 0.1 M ethylenediaminetetra-acetate (EDTA) at pH 8.3. After 5 min. at 37°, the bacteria were collected by centrifugation, washed and suspended in distilled water. Such bacterial suspensions (optical density, E , = 1.0) could be kept at 0° for 48 hr. Endolysin activity was determined by adding the sample (1.5 ml.) to 1.5 ml. of the washed bacterial suspension and the optical density read at intervals at 610 m μ (blue photocell). When endolysin is present, the optical density at first decreases linearly for a period, and then less rapidly to a plateau value, after which there is little or no further change with time. The rate of change in optical density ($\Delta E/\text{min.}$) over the initial linear portion of the graph was taken as a measure of the amount of endolysin in the sample.

Determination of viable counts. The sample was diluted in 0.9 % NaCl solution and then plated by the soft agar layer technique on to PB agar plates.

Measurement of turbidity. Measurements of optical density, E , were made at 610 m μ (blue photocell).

Spectrophotometry. All measurements were made in a Unicam spectrophotometer Model SP 600 with 1 cm. glass cells.

Experimental procedure. Large-scale cultures were grown in conical flasks (150 ml. medium TB/1 l. flask) shaken in a water bath at 37°. Cultures were normally harvested when they reached 5×10^8 bacteria/ml. When the bacteria were to be u.v.-irradiated, they were collected by centrifugation and resuspended at an optical density of about 0.6 in 0.9 % NaCl solution containing 0.1 vol. of medium PB or TB (i.e. about 6×10^8 bacteria/ml.). The suspension was divided into two parts; one was irradiated and the other kept as the control. Within about 2 min. of irradiation, 100 ml. of each suspension were placed in a 1 l. flask, 20 ml. 10 % tryptone at 65° added (to bring the culture to 37° quickly), and then shaken in a water bath at 37°.

For infection with phage λ_{gc} or λ_{gv} in the growth medium, cultures were grown to 5×10^8 bacteria/ml., MgSO_4 added to a final concentration of 0.02 M, and then infected with a high-titre phage stock (multiplicity 10 particles/bacterium). For infection in the absence of growth medium, the culture was centrifuged and resuspended in 0.02 M- MgSO_4 ; a small volume of high-titre phage suspension was then added, and after 15 min. at 37° the bacteria were collected by centrifugation and resuspended in warm 2 % tryptone medium.

All percentages of solutes are w/v and all incubations were done at 37° unless otherwise stated.

RESULTS

Effect of induction of Escherichia coli lysogenic for wild-type phage λ_{22}

When the induced bacteria were incubated in complete medium, the turbidity of the culture increased for about 70 min. (phase of residual growth) and then lysis began (Fig. 1). Although for the first few minutes the rates of protein and RNA synthesis were the same in the induced and control (unirradiated) bacteria, the rates in the induced culture eventually became slower than in the control culture (Fig. 2).

Fig. 1. Growth of *Escherichia coli* K112 (λ) and the synthesis of DNA after the induction of phage development by ultraviolet radiation. Experimental procedure described in Methods (p. 416). Samples were taken for the determination of turbidity (○, ●) and DNA (□, ■): closed symbols for irradiated culture and open symbols for control (unirradiated). Initially 5.6×10^8 bacteria/ml. and 96 % were induced.

In the induced bacteria, the net synthesis of DNA was completely inhibited during the first 30 min. of the latent period (Figs. 1, 2). After this lag, synthesis was resumed and eventually became faster than in the unirradiated bacteria (Fig. 1). Similar experiments were done with the non-lysogenic parent strain. By comparing Figs. 2 and 3 it can be seen that the changes in DNA, RNA and protein synthesis which occurred in the induced bacteria were similar to the changes produced in the non-lysogenic bacteria by the same dose of radiation as used in the induction experiments. It was therefore concluded that the lag in DNA synthesis and the falling off in the synthesis of RNA and protein observed in the induced organisms were due to the effects of the irradiation on the general metabolic processes of the bacteria and were not a reflexion of events concerned with phage development.

*Comparison of amount of DNA synthesized with DNA present
in phage progeny*

An attempt was made to determine how much of the DNA made after the induction of *Escherichia coli* $\kappa 112$ (λ) was phage DNA. The base composition of DNA from phage and host are the same (Lwoff, 1953; Kaiser & Hogness, 1960), so there is no direct way of following the synthesis of phage DNA as distinct from host DNA. However, the phage yield can be determined, and, when the DNA content of a phage λ

Fig. 2

Fig. 3

Fig. 2. The synthesis of protein (○, ●), RNA (△, ▲) and DNA (□, ■) in *Escherichia coli* $\kappa 112$ (λ) during the first half of the latent period after induction of phage development by ultraviolet radiation (closed symbols for irradiated culture and open symbols for control culture). Experimental procedures as for Fig. 1. Initially 3.8×10^8 bacteria/ml. and 89 % were induced.

Fig. 3. The synthesis of protein (○, ●), RNA (△, ▲) and DNA (□, ■) in non-lysogenic *Escherichia coli* $\kappa 112$ after exposure to ultraviolet radiation (closed symbols for irradiated culture and open symbols for control culture). Experimental procedure and dose of ultraviolet radiation as for Fig. 2. Initially 3.9×10^8 bacteria/ml. and 77 % were killed.

particle is known (see Methods, p. 415), the amount of DNA present in the phage progeny can be compared with the total amount of DNA synthesized in the culture. Experiments with induced bacteria (Table 1) showed that the DNA estimated to be present in the phage yield accounted for an appreciable part (between 81 and 89 %) of the DNA synthesized after irradiation. Hence the synthesis of host DNA was at least very much decreased during the vegetative development of phage λ ; perhaps no synthesis took place at all and the excess DNA was phage DNA which had not

been incorporated into complete phage particles (see similar experiments with phage T₂ by Hershey, 1953, and Hershey & Burgi, 1956). One of the main difficulties in this type of experiment is to measure the phage yield. If this is done by assay of the bulk culture when lysis is complete then some of the phage particles will have been lost by adsorption to cell debris and unlysed bacteria and thus the estimate of the phage yield will be inaccurate. If to avoid this the practice of Ellis & Delbruck (1939) is followed and a sample of the culture is diluted several thousand times before lysis begins, the assumption has to be made that the phage yield/bacterium in the diluted culture is the same as in the bulk culture from which samples for the estimation of DNA are obtained. The figures in Table 1, Expt. 1, show how great the difference was between phage yield as determined by direct assay of the bulk culture and the value obtained from the diluted culture.

Table 1. *Synthesis of DNA and the production of phage particles after the induction of Escherichia coli κ 112 (λ) by ultraviolet radiation*

Bacteria were harvested from cultures in the log phase of growth, resuspended, irradiated and, after the addition of warm medium, incubated at 37° (details as in Methods, p. 416). After 40 min., a sample was diluted 6×10^5 times in tryptone broth (at 37°) and also incubated with shaking. A sample of the diluted culture was immediately plated to determine the number of induced bacteria. After 120 min. a sample of the diluted culture was incubated for a further 30 min. in TB + 0.02 M-KCN (Doerman, 1952; Weigle & Delbruck, 1951), and then assayed for phage particles. Figure in parentheses shows phage particles found in sample of bulk culture taken at 120 min. and incubated in TB + 0.02 M-KCN for 30 min. The phage particles present at the time of irradiation were determined after incubating a sample of the unirradiated bacteria in broth + 0.02 M-KCN + a drop of chloroform for 30 min. In Expt. 1, at time 0 min. 5.9×10^8 bacteria/ml., and 93 % were induced; in Expt. 2, 8.6×10^8 bacteria/ml. and 93 % were induced; in Expt. 3, 6.7×10^8 bacteria/ml., and 90 % were induced. All results are expressed per 6 ml. of culture. DNA content of phage 2.14×10^{-11} μ g. apparent deoxyribose/particle (Methods, p. 415).

	Total apparent deoxyribose (μ g./6 ml. culture).		DNA synthesized (μ g. apparent deoxyribose) (B - A)	Phage (particles/6 ml.) At		Apparent deoxyribose in phage (μ g.) (C)	% of DNA synthesized in phage (C $\times$ 100) (B-A)
	At			At			
	0 min.	120 min.		0 min.	120 min.		
	(A)	(B)					
(1)	8.26	28.58	20.32	5.4×10^4	7.7×10^{11} (2.5×10^{11})	16.47	81
(2)	11.52	42.40	30.88	3.8×10^6	1.2×10^{12}	25.62	83
(3)	9.54	24.50	14.96	—	6.2×10^{11}	13.26	89

Effect of infection of Escherichia coli κ 112 with phage λ_{gc}

Infection with phage λ_{gc} or λ_{gv} can be done in a medium which does not support growth, e.g. in 0.02 M-MgSO₄ alone, or in the growth medium itself supplemented with 0.02 M-MgSO₄. The former conditions enable the majority (90 %) of the bacteria to be infected, and, when they are subsequently transferred to the growth medium, phage development will begin in all the bacteria at the same time. This procedure is preferred since, when organisms are infected in the growth medium, unless infection of the population as a whole is very rapid the events which occur in the infected bacteria will tend to be blurred by those in non-infected bacteria. After exposure to phage λ_{gc} in 0.02 M-MgSO₄, 95 % of the bacteria were infected, and there

was little residual growth when they were placed in broth (though the non-infected bacteria grew without lag). One reflexion of this was the small amount of protein synthesis (Fig. 4*a*). The synthesis of DNA occurred without delay in both infected and non-infected bacteria, but the rate of synthesis in infected bacteria was somewhat more rapid during the latter part of the latent period (Fig. 4*b*). In the experiment reported, no net synthesis of RNA was detected, although the amount of RNA in the control bacteria increased steadily (Fig. 4*b*). Lysis of the infected bacteria began at about 35 min.

Fig. 4. Growth of *Escherichia coli* K112 and the synthesis of protein, RNA and DNA after infection with phage λ_{gc} in the presence of 0.02 M-MgSO₄ alone. A log-phase culture was harvested, suspended in 0.02 M-MgSO₄ (5.7×10^8 bacteria/ml.) and divided into two parts. One part received a small volume of phage λ_{gc} (8.5×10^8 particles/ml.) in 0.02 M-MgSO₄; the other part was mixed with MgSO₄ alone. After 15 min. at 37°, the cells were harvested and resuspended in warm TB medium, and samples taken at intervals for the determination of (a) turbidity (○, ●) and protein (▽, ▼) and (b) RNA (△, ▲) and DNA (□, ■). Closed symbols for infected culture and open symbols for control culture.

When bacteria in the log phase of growth were infected in the growth medium with phage λ_{gc} there was an appreciable amount of residual growth before lysis began after about 30 min (Fig. 5*a*). Total protein synthesis in the infected bacteria was less than in the control bacteria (Fig. 5*a*), and again there was no lag in the synthesis of DNA, the rate of synthesis of which during the latent period was greater than in the uninfected organisms (Fig. 5*b*). The synthesis of RNA in the infected bacteria was much reduced (Fig. 5*b*).

Effect of infection of Escherichia coli K112 with phage λ_{gv}

As in the experiments with phage λ_{gc} , infection was brought about in 0.02 M-MgSO₄ or in growth medium. When the former procedure was used, the rate of growth of the infected bacteria was less than that of the control bacteria, which grew without lag when resuspended in the tryptone medium (Fig. 6). Phage λ_{gv} , like phage λ_{gc} , markedly decreased the synthesis of protein and RNA in the infected

bacteria. There was no delay in the synthesis of DNA and, even though lysis of the culture began at 30 min., the amount of DNA present continued to increase for about 30 min. at a faster rate than in the control culture, implying that the rate of synthesis in bacteria still unlysed was extremely high (Fig. 6). When infection in the growth medium was studied, and DNA estimated at 5 min. intervals, no lag in the synthesis of DNA was observed and the rate of synthesis was always greater than in the uninfected bacteria.

Fig. 5a

Fig. 5b

Fig. 6

Fig. 5. Growth of *Escherichia coli* κ 112 and the synthesis of protein RNA and DNA after infection of a growing culture with phage λ_{gc} . A log-phase culture (5.7×10^8 bacteria/ml.) in TB was divided into two parts. One part received phage λ_{gc} plus MgSO_4 (final concn. 0.02 M and 8.0×10^9 phage/ml.) and the other MgSO_4 alone. After 15 min., 98 % of the cells were infected. Samples were taken for the estimation of (a) protein (∇ , $\blacktriangledown$) and turbidity ($\circ$, $\bullet$); and (b) RNA ($\triangle$, $\blacktriangle$) and DNA ($\square$, $\blacksquare$). Closed symbols for infected culture and open symbols for control culture.

Fig. 6. Growth ($\circ$, $\bullet$) of *Escherichia coli* κ 112 and the synthesis of DNA ($\square$, $\blacksquare$) after infection with phage λ_{gc} in the presence of 0.02 M MgSO_4 alone. Experimental procedure as for Fig. 4. Initially, 5.0×10^8 bacteria and 1.0×10^{10} phage/ml. in 0.02 M MgSO_4 ; 95 % of the bacteria were infected. Closed symbols for infected culture and open symbols for control culture.

Synthesis of phage-specific proteins

During the vegetative development of phage new proteins may be formed in the host organism. Some of these are enzymes concerned with the synthesis of specific phage components (e.g. the new DNA polymerase in *Escherichia coli* infected with phage T_2 ; Aposhian & Kornberg, 1962), whilst others will be the phage components themselves (e.g. head protein, tail fibres). Specific methods are not yet available for identifying and assaying many of these phage proteins, but two may be studied, namely serum blocking power and endolysin. Serum blocking power (SBP) is the name given to antigenic material in the tail of a phage particle which combines with antibody in phage antiserum and as a result of this combination the phage can no longer adsorb to sensitive bacteria. Endolysin is the name given to a lytic enzyme, similar to lysozyme, which appears in bacteria during the vegetative development of phage and which is regarded as being the same as the lytic enzyme in the tails of mature phage particles.

The time course of the appearance of SBP and endolysin was determined after the induction of λ phage development in *Escherichia coli* κ 112 (λ_{22}). Since only small amounts of these materials were to be expected in the early part of the latent period, 100 ml. samples of the induced culture were quickly cooled in an ice-salt

mixture. The bacteria were then collected by centrifugation at 0°, suspended in 10 ml. of medium PB, and ultrasonically treated. The same disintegrated suspensions were tested for SBP, endolysin activity and plaque-forming units. The assay for plaque-forming units underestimates the number of mature phage particles present in the bacteria at the time of sampling, since ultrasonic treatment has a lethal effect on some of the phage particles (ultrasonic treatment causes a decrease in the titre of a phage suspension). The experimental procedure was standardized as far as possible, and it was assumed that the proportion of phage which survived would be of the same order in each sample, and that the measured phage titres would reflect changes in the number of mature phage particles in the bacteria.

Table 2. *Time course of the appearance of SBP, endolysin and phage particles during onset of the induced vegetative development of phage λ*

An exponential culture of *Escherichia coli* K112 (λ) was harvested, resuspended, induced and reincubated in the normal way (Methods, p. 416). At time 0 min., 4.5×10^8 bacteria/ml.: 92% were induced. Samples (100 ml.) were taken at intervals and quickly cooled. The bacteria were collected by centrifugation, suspended in 10 ml. PB and then subjected to 5 min. ultra sound. Each disintegrated sample was assayed for SBP, endolysin activity and plaque-forming units. Other samples were taken at intervals to determine turbidity and hence follow residual growth and lysis of induced culture. Temperature during endolysin assay was 22–23°.

Time after induction (min.)	SBP (phage equivalents/ml.)	Endolysin activity (ΔE/min.)	Plaque-forming units (per ml.)
0	6.3×10^7	0.001	—
15	6.0×10^7	—	6.5×10^5
20	6.0×10^7	0.001	4.9×10^5
25	5.9×10^7	0.03	2.4×10^5
30	6.6×10^7	0.05	1.2×10^6
35	7.6×10^7	0.20	3.8×10^6
40	7.7×10^7	0.40	3.9×10^6

A small degree of spontaneous induction occurs naturally in a culture of *Escherichia coli* (λ), and it is to be expected that the sample taken immediately after u.v.-irradiation would show small amounts of SBP, endolysin and mature phage particles (Table 2). In the experiment reported, endolysin activity began to increase 25 min. after induction, whilst SBP and mature phage showed an increase 30 min. after induction. Since the method for the detection of endolysin is more sensitive than that for SBP, it is not possible to conclude unequivocally that endolysin formation precedes the production of SBP. However, it does seem that the first appearance of these proteins is followed within 5 min. by the formation of the first mature phage particles. In this experiment, lysis began between 52 and 55 min. after incubation of the induced cultures began. In previous experiments (see Fig. 1), lysis normally began at about 60–65 min. The difference was probably due to the time taken to set up the larger amount of culture required in the present experiment. In later experiments the total amount of SBP in the final lysate of an induced culture (105 min. after induction) was as much as 15 times the number of plaque-forming units; that is to say the SBP antigen appears to be produced in great excess as compared with the number of mature phage particles which are eventually assembled. When the phage particles were removed from such a lysate by centrifuga-

tion at 40,000 *g* for 90 min., a large part of the excess SBP remained in the supernatant fluid (Table 3). However, after this first centrifugation, the recovery of phage particles and SBP was only about 50 % of that initially present. In spite of all precautions, and the use of media which contained 0.001 % gelatin and working at 4°, this result was consistently obtained in five experiments. Evidently high-speed centrifugation caused inactivation of some of the SBP and phage particles. Part of the losses of phage particles were probably due to aggregation of particles in the pellet and failure to disperse them completely when resuspended in fresh medium. Kaiser & Hogness (1960) encountered difficulties in the quantitative recovery of λ phage from crude lysates. After the removal of the majority of the phage particles, the amount of SBP in the supernatant fluid was about 18 times greater than the number of phage particles detected by plaque counts. About a quarter of this SBP remained in the supernatant fluid after centrifugation at 96,000 *g* for 90 min., whilst about half was found in the pellet. The SBP remaining in the supernatant fluid probably represents molecules of antigenic phage material which was not incorporated into organized structures at the time of lysis of the host bacteria, whilst the SBP in the pellet is probably composed of phage tails and/or fibres; compare the experiments of Franklin (1961) with lysates from *E. coli* B infected with phage T₂.

Table 3. *Distribution of serum blocking power and phage particles after differential centrifugation of lysates from induced Escherichia coli K112 (λ)*

Escherichia coli K112 (λ) was grown, harvested, induced (200 ml. containing 1.9×10^8 bacteria/ml.) and reincubated with nutrient medium in the usual way (Methods, p. 416) until lysis was complete (105 min.). After removal of cell debris etc. by 5000 *g* for 20 min., the lysate was centrifuged in the Model L Spinco centrifuge. Pellets were suspended in the same volume of TB as the original supernatants and 'insoluble' material removed at 5000 *g* for 15 min.

	Phage (particles/ml.)	SBP (phage equivalents/ml.)
Lysate	3.01×10^{10}	1.5×10^{11}
Supernatant after 40,000 <i>g</i> for 90 min.	2.34×10^9	4.3×10^{10}
Suspension of 40,000 <i>g</i> pellet	1.55×10^{10}	1.60×10^{10}
Supernatant after 96,000 <i>g</i> for 90 min.	6.23×10^7	1.1×10^{10}
Suspension of 96,000 <i>g</i> pellet	7.91×10^8	2.25×10^{10}

DISCUSSION

In the experiments reported here, no lag was observed in the synthesis of DNA following infection of *Escherichia coli* with phage λ_{gc} or λ_{gv} . Moreover, the lag in DNA synthesis in induced bacteria was the same as in bacteria of the non-lysogenic parent strain which had received the same dose of u.v. radiation. This implies that the length of this lag was due to an effect of the irradiation on a process common to lysogenic and non-lysogenic bacteria. These results therefore contrast with those obtained with *Bacillus megaterium* where, although there was a delay in DNA synthesis after induction of the lysogenic strain, there was no delay after u.v.

irradiation of the non-lysogenic parent (Siminovitch & Rapkine, 1952). Séchaud (1960) has studied the effect of infection of *E. coli* with phage λ_c . In her experiments the synthesis of DNA in the bacteria was temporarily inhibited by starvation (incubation in 0.01 M-MgSO₄) or by starvation and subsequent u.v. irradiation. When such bacteria were returned to a growth medium, there was a lag before DNA synthesis was resumed. This lag was reduced to about 8 min. when the bacteria were infected with phage λ_c before their return to complete medium. The majority of the adsorbed phage did not inject their DNA into the bacteria until the latter were placed in the growth medium. Injection of the phage DNA took about 4 min. and another 4 min. elapsed before DNA synthesis was resumed. Injection of the phage DNA only when the host organism is in a growth medium appears to be a phenomenon associated with starved bacteria and has not been observed with freshly harvested bacteria. It would therefore seem that the lag in DNA synthesis observed by Séchaud was due to changes brought about by the treatment of the bacteria before infection with phage. We have avoided using starved bacteria, because the primary effects of starvation on cellular metabolism are still unknown and also because starvation by incubation in the absence of sources of nitrogen, carbon and energy profoundly alters the size and composition of the ribonucleo-protein particles of a bacterial cell (Bowen, Dagley & Sykes, 1959), especially when the starvation medium contains 0.01 M-MgSO₄ (Dr J. Sykes, personal communication). Such particles play an important role in the synthesis of protein and perhaps RNA (see McQuillen, 1961). Starvation procedures may also alter the size and integrity of host DNA molecules, and thus diminish or inhibit DNA and RNA synthesis.

Irrespective of whether the development of phage λ took place as a result of induction (Fig. 1) or after infection in MgSO₄ (Figs. 4*a*, 6) or in the growth medium (Fig. 5*b*), the synthesis of DNA occurred at a greater rate than in the control bacteria. When the amount of DNA estimated to be present in the phage progeny is compared with the net amount of DNA synthesized in the culture (Table 1), it appears that, during vegetative phage development, the synthesis of host DNA is at least very much decreased and perhaps completely inhibited. This is in agreement with the conclusions of Séchaud (1960) from experiments with phage λ_c . Since the bacterial DNA and phage DNA are of identical composition, chemical analysis cannot be used to determine whether only one type or both types of DNA are made during the latent period. If host-cell DNA synthesis is in fact markedly diminished or inhibited during the development of phage λ , a unique control mechanism would seem to be operating whereby the DNA polymerase is primed for the synthesis of phage DNA, but for some reason the bacterial DNA can no longer act as primer for the synthesis of more host DNA. In phage T₂-infected *Escherichia coli*, the phage initiates the production of enzymes which lead to the removal of one of the substrates (deoxycytidine triphosphate) required for making host DNA (Zimmerman & Kornberg, 1961). No such mechanism can operate in bacteria infected with phage λ since the phage and bacterial DNA are composed of the same four deoxyribonucleotides. It may be suggested that the onset of the vegetative development of phage λ causes disorganization or modification of the host DNA such that the latter can no longer act as a primer for the polymerase.

With regard to effects on the synthesis of RNA, the most notable result was seen

with bacteria which had been infected with phage λ_{gc} in the presence of MgSO_4 : in such bacteria, net RNA synthesis was completely inhibited (Fig. 4*b*). Much less extreme effects were observed with induced bacteria (Fig. 2) and bacteria infected in the growth medium with phage λ_{gc} (Fig. 5*b*) or in MgSO_4 with phage λ_{gv} ; in these experiments RNA synthesis was similar to that in the control bacteria for the first third of the latent period and only later became slower so that, at the commencement of lysis, total RNA was somewhat less than half that in the control cultures. The effect of phage development on net protein synthesis was similar to these effects on RNA synthesis, and, for example, became more noticeable in the latter half of the latent period in bacteria infected with phage λ_{gc} (Fig. 4*a*), where the rate fell to about 30–40 % of that in the control culture.

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REFERENCES

- ADAMS, M. (1950). Methods of study of bacterial viruses. *Meth. med. Res.* **2**, 1.
- APOSHIAN, H. V. & KORNBERG, A. (1962). The polymerase formed after T_2 bacteriophage infection of *Escherichia coli*. *J. biol. Chem.* **237**, 519.
- BESSMAN, M. J. (1960). The enzymology of normal and virus infected *Escherichia coli*. In *The Cell Nucleus. Faraday Soc. Symp.* p. 187.
- BOWEN, T. J., DAGLEY, S. & SYKES, J. (1959). A ribonucleoprotein component of *Escherichia coli*. *Biochem. J.* **72**, 419.
- BURTON, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**, 315.
- COHEN, S. S. (1948). The synthesis of bacterial viruses. 1. The synthesis of nucleic acid and protein in *Escherichia coli* B infected with T_2 bacteriophage. *J. biol. Chem.* **174**, 281.
- DEMARS, R. I. (1955). The production of phage-related materials when phage development is interrupted by proflavine. *Virology*, **1**, 83.
- DOERMAN, A. (1952). Intracellular growth of bacteriophages. 1. Liberation of intracellular bacteriophage T_4 by premature lysis with another phage or with cyanide. *J. gen. Physiol.* **35**, 645.
- ELLIS, E. L. & DELBRUCK, M. (1939). The growth of bacteriophage. *J. gen. Physiol.* **22**, 365.
- FLAKS, J. G., LICHTENSTEIN, J. & COHEN, S. S. (1959). Virus induced acquisition of metabolic function. II. Studies on the origin of the deoxycytidylate hydroxymethylase of bacteriophage-infected *Escherichia coli*. *J. biol. Chem.* **234**, 1507.
- FRANKLIN, N. C. (1961). Serological study of tail structure and function in coliphages T_2 and T_4 . *Virology*, **14**, 417.
- FRY, B. A. (1959). Conditions for the infection of *Escherichia coli* with lambda phage and for the establishment of lysogeny. *J. gen. Microbiol.* **21**, 685.
- HERSHEY, A. D. (1953). Nucleic acid economy in bacteria infected with bacteriophage T_2 . II. Phage precursor nucleic acid. *J. gen. Physiol.* **37**, 1.
- HERSHEY, A. D. & BURGI, E. (1956). Genetic significance of the transfer of nucleic acid from parental to offspring phage. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 91.
- JACOB, F. & FUERST, C. R. (1958). The mechanism of lysis of phage studied with defective lysogenic bacteria. *J. gen. Microbiol.* **18**, 518.
- JACOB, F. & WOLLMAN, E. L. (1954). Étude génétique d'un bactériophage tempéré d'*Escherichia coli*. 1. Le système génétique du bactériophage λ . *Ann. Inst. Pasteur*, **87**, 653.

- JACOB, F. & WOLLMAN, E. L. (1956*a*). Recherches sur les bactéries lysogènes défectives. 1. Déterminisme génétique de la morphogénèse chez un bactériophage tempéré. *Ann. Inst. Pasteur*, **90**, 282.
- JACOB, F. & WOLLMAN, E. L. (1956*b*). Sur les processus de conjugaison et de recombinaison chez *Escherichia coli*. 1. L'induction par conjugaison ou induction zygotique. *Ann. Inst. Pasteur*, **91**, 486.
- KAISER, A. D. & HOGNESS, D. S. (1960). The transformation of *Escherichia coli* with desoxyribonucleic acid isolated from bacteriophage λ_{dg} . *J. molec. Biol.* **2**, 392.
- KELNER, A. (1953). Growth, respiration and nucleic acid synthesis in ultraviolet-irradiated and in photoreactivated *Escherichia coli*. *J. Bact.* **65**, 252.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin reagent. *J. biol. Chem.* **193**, 265.
- LWOFF, A. (1953). Lysogeny. *Bact. Rev.* **17**, 269.
- MCQUILLEN, K. (1961). Ribosomes and the synthesis of proteins. *Progr. biophys. Chem.* **12**, 67.
- SCHNEIDER, W. C. (1945). Phosphorus compounds in animal tissues. 1. Extraction and estimation of deoxypentose nucleic acid and of pentose nucleic acid. *J. biol. Chem.* **161**, 293.
- SÉCHAUD, J. (1960). Développement intracellulaire du coliphage lambda. *Arch. Sci. phys. nat., Genève*, **13**, 428.
- SIMINOVITCH, L. & RAPKINE, S. (1952). Biochemical modifications in lysogenic *Bacillus megaterium* 899 (1) after induction with ultra violet light. *Biochim. biophys. Acta*, **9**, 478.
- WEIGLE, J. J. & DELBRUCK, M. (1951). Mutual exclusion between an infecting phage and a carried phage. *J. Bact.* **62**, 301.
- ZIMMERMAN, S. B. & KORNBERG, A. (1961). Deoxycytidine di- and tri-phosphate cleavage by an enzyme found in bacteriophage infected *Escherichia coli*. *J. biol. Chem.* **236**, 1480.