Effect of Ultraviolet Radiation and the Induction of Vegetative Reproduction of Phage Lambda on the Ultracentrifuge Pattern of Extracts of *Escherichia coli*

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SUMMARY

After ultraviolet irradiation, the growth of *Escherichia coli* (non-lysogenic and lysogenic for phage λ) resulted in diminution of the 8S component of water extracts of the bacteria and the loss of its characteristic spike. In the induced lysogenic bacteria, 8S material was synthesized in the second half of the latent period and the characteristic shape of the boundary was regained. The 8S material was free DNA of relatively high molecular weight and these changes were correlated with alterations in the rate of DNA synthesis in the irradiated bacteria. The synthesis of ribosomal material was found to continue during the development of phage λ . After log phase bacteria had stood for a short time in saline, extracts prepared in tris buffer + 0.01 M-magnesium acetate showed that an 85S component developed. The latter was replaced by slower material (73S) when the bacteria (non-lysogenic and lysogenic, irradiated or un-irradiated) resumed logarithmic growth in broth. This conversion may reflect a configurational change in the 73S component in environments which do not support growth.

INTRODUCTION

Siegel, Singer & Wildman (1952) studied the effect of infection with bacteriophage T₂ on the high molecular weight components of the host *Escherichia coli*. Extracts from the infected bacteria were examined in the ultracentrifuge and it was found that the main effect of virus infection concerned the material with a relatively low sedimentation coefficient (of the order of 10S) and containing deoxyribonucleic acid (DNA). The ultracentrifuge diagrams also appeared to show changes in components with higher sedimentation coefficients (29S, 40S), i.e. in the ribonucleoprotein (RNP) particles present in the extracts. These studies were made before the factors which influence the size and therefore the sedimentation coefficients of such particles had been studied. In this respect it is now known that the concentration of Mg²⁺ is especially important. In extracts made in water or dilute phosphate buffer the RNP particles mainly sediment at 29S and 40S (Schachman, Pardee & Stanier, 1952; Dagley & Sykes, 1956, 1959) whilst, in the presence of 0.01 M-Mg²⁺, particles which sediment at $(S_{20,w})_0 = 70$ predominate (Tissières, Watson, Schlessinger & Hollingworth, 1959).

As part of a programme devoted to phage lambda (λ) and its host *Escherichia coli* an investigation has been made of the effect of vegetative reproduction of 'wild-

type' phage λ on the components which can be detected in cell-free extracts of the host bacteria by analytical ultracentrifugation. Induction of phage development in bacteria lysogenic for phage λ was here achieved by ultraviolet irradiation.

METHODS

Organisms and culture conditions. *Escherichia coli* strain $\kappa 112$ and the wild-type phage lambda (λ_{22}) were from the collections of Drs F. Jacob and E. Wollman of the Pasteur Institute, Paris. Organisms were grown with aeration at 37° in a nutrient broth medium containing (per litre, w/v) Oxoid tryptone (20 g.) and Lab. Lemco (2 g.) at pH 7.

Irradiation procedure. Cultures were harvested in the mid-logarithmic phase of growth, and then suspended in sterile distilled water at an extinction reading in the range 0.3–1.0 (equivalent to approximately 3×10^8 to 1×10^9 bacteria/ml.). In the experiments where extracts were subsequently made in tris buffer + 0.01 M-magnesium acetate the bacteria were resuspended in 0.9% NaCl. Samples (120 ml.) of the suspension were irradiated for 60 sec. in a sterile enamel dish (11 in. $\times$ 14 in.) rocked gently 75 cm. below the ultraviolet lamp (an Hanovia Chromatolite low pressure Hg lamp without the filter). The irradiation of 2 l. or more of suspension took an appreciable time, but control experiments showed that residual growth, percentage induction and phage yield per induced cell were the same in samples transferred to growth medium at 37° immediately after irradiation and in those kept at room temperature for 60 min. before the addition of broth and incubation at 37° . In the experimental conditions used here, the latent period, i.e. the time from the addition of broth until the onset of lysis and the release of mature phage particles, was about 72–75 min. (Fig. 3a). Concentrated nutrient broth (10 times usual strength) at 100° was added to the irradiated suspension (200 ml./l.) to help raise the temperature quickly to 37° . The cultures were then aerated in glass bottles immersed in a water bath at 37° . Silicone antifoam emulsion RD (Hopkin and Williams Ltd., England) was added to prevent foaming.

Preparation of extracts. The bacteria were collected by centrifugation and the pellet transferred to a Hughes press (Hughes, 1951) and crushed. The crushed bacteria were extracted for 30–60 min. at 4° with either distilled water or 0.001 M-tris buffer (pH 7) containing 0.01 M-magnesium acetate. After centrifuging the extracted cell paste at 24,000g for 45 min. to remove whole bacteria and insoluble debris, the supernatant fluids were collected and diluted with the appropriate solvent (i.e. water or tris + magnesium acetate buffer) so as to contain 10 mg. protein/ml. In this manner variations in other macromolecular components could be viewed against a constant protein background in the analytical ultracentrifuge. After storage overnight in ice, the extracts were examined next day in the Spinco ultracentrifuge (model E with schlieren optics) at room temperature. In each individual experiment, photographs taken at the same time interval after reaching speed (50,740 rev./min.) were taken at the same bar angle. Sedimentation coefficients for the components visible in crude extract sedimentation diagrams are expressed in Svedberg (S) units and are uncorrected values. It is well known that the 40 and 29S components reported here for crude extracts have ($S_{20,w}$)₀ values of 50 and 30, respectively, in homogeneous solution (Tissières *et al.* 1959). Area

measurements were made with a planimeter on enlarged photographs taken from comparable diagrams; results are recorded in arbitrary units and have been corrected for radial dilution and differences in bar angle.

Treatment of extracts with enzymes. Extracts and enzymes dissolved in buffer were pre-incubated separately for 10 min. at 37° and then a sample of each mixed together, incubated for the required time and then plunged into crushed ice. Each extract was treated immediately before its addition to the ultracentrifuge cell. In control systems, extracts were incubated with the same amounts of buffer but containing no enzyme. Crystalline ribonuclease (Sigma Chemical Co., St Louis, U.S.A.) was dissolved in the tris + magnesium acetate buffer described above; crystalline deoxyribonuclease (Worthington Biochemical Corp., U.S.A.) was dissolved in 0.05 M-phosphate buffer (pH 7).

Chemical estimations. Protein was estimated by the biuret method (Gornall, Bardawill & David, 1949) with bovine serum albumin (Fraction V, Armour Pharmaceutical Co. Ltd., England) as a standard. DNA was estimated by the method of Burton (1956) with 2-deoxy-D-ribose (L. Light, Colnbrook, England) as standard, and RNA by the method of Schneider (1945) with D-ribose (L. Light, Colnbrook, England) as standard. Results are expressed in terms of 'apparent deoxyribose' or 'apparent ribose', since only the sugar residues bound to purines react in these procedures.

Preparation of samples for determination of protein RNA and DNA. The procedure followed that described by Fry & Gros (1959).

Turbidity of bacterial suspensions. Measurements were made at 610 m μ (with a blue photo-cell).

Spectrophotometry. A Unicam spectrophotometer SP 600 with cells of 1 cm. light path was used.

Ultrasonic treatment. An M.S.E. 60 W. ultrasonic disintegrator fitted with a titanium probe was used and throughout the sonic treatment the extract was cooled in ice.

RESULTS

Ultracentrifugal analysis of cell-free extracts prepared in water

The cell-free extract from unirradiated *Escherichia coli* lysogenic for phage λ gave an ultracentrifuge diagram with components sedimenting at 4, 8, 29 and 40S (Fig. 1a); the overall picture was no different from that obtained from the non-lysogenic parent strain (Fig. 1e). Immediately after irradiation (i.e. before transfer to growth medium) the ultracentrifuge pattern of extracts of the lysogenic bacteria (Fig. 1b) appeared to be the same as for the unirradiated bacteria. Irradiation thus produced no immediate change directly visible in the photographs. However, measurement of boundary areas did reveal a small decrease in the concentration of the 40S component (compare pre- and post-u.v. treatment data in Table 1). Since the bacteria were suspended in water for the irradiation procedure, this result was probably not due to the irradiation as such but to a slow loss of magnesium ions from the bacteria, and, as a result, part of the 40S material became unstable. Dagley & Sykes, 1959, reported a similar phenomenon.

When the irradiated lysogenic bacteria were returned to growth medium, samples taken after 30 and 60 min., showed significant changes in the ultracentrifuge pattern of the extracts. After 30 min., the 8S component had diminished,

Fig. 1. Ultracentrifuge patterns of extracts from non-lysogenic and lysogenic *Escherichia coli* K12. All photographs were taken 16 min. after reaching 50,740 rev./min. Sedimentation is to the right. From left to right in each diagram the sedimenting boundaries have uncorrected sedimentation coefficients of 4, 8, 29 and 40 S.

and the characteristic spike was absent. On the other hand, in the 60 min. sample this component was again prominent, its characteristic shape had been restored, and, if anything, the spike was more pronounced (Figs. 1*c* and *d*). Siegel *et al.* (1952) concluded that the 8S component in ultracentrifuge diagrams of bacterial extracts was mainly, if not entirely, free DNA. In our experiments, incubation of the extracts with deoxyribonuclease for 3 min. at 37° abolished the 8S boundary but had no effect on the other components (Fig. 2*a*). Similarly, when the extracts were treated ultrasonically for 1 min., the 8S spike disappeared and the 40 and 29S

Fig. 2. Effect of (a) deoxyribonuclease and (b) ultrasonic treatment on the ultracentrifuge pattern of water extracts of induced bacteria harvested after 60 min. in broth. For (a), final concentration of enzyme 9 $\mu\text{g./ml.}$ and incubated 3 min. at 37°: picture taken 16 min. after reaching 50,740 rev./min.; bar angle 55°. For (b), sonic treatment for 1 min.; picture taken 16 min. after reaching 50,740 rev./min.; bar angle 55°.

components were again unaffected (Fig. 2*b*). Ultrasonic treatment has been shown to disorganize and break large DNA molecules into smaller pieces (Doty, McGill & Rice, 1958). In view of these observations and those of Siegel *et al.* (1952), it seems reasonable to conclude that the 8S component observed in our experiments was free DNA of high molecular weight (perhaps partially depolymerized by the extraction procedures employed). Furthermore, these results show that the presence or absence of a spike sedimenting at 8S in the ultracentrifuge diagrams was an indication of the amount of polymerized DNA in the system. Inspection of the ultracentrifuge diagrams from bacteria 30 and 60 min. after induction (Fig. 1*a-d*), and area measurements of the 40 and 29S components (Table 1), lead to the conclusion that ribosome synthesis continued during the induced development of the temperate phage λ . This result is to be contrasted with the situation in *Escherichia coli* strain B infected with the coliphage T_4 , where ribosome synthesis came to a halt immediately after infection (Brenner, Jacob & Meselson, 1961).

The observed changes in the ultracentrifuge diagrams reflected the changes in the synthesis of protein, RNA and DNA in the induced bacteria (Fig. 3). The synthesis of DNA was inhibited during the first 30 min. of the latent period of phage development. On the other hand, protein synthesis continued and in consequence the

DNA:protein ratio fell (from 9.0×10^{-3} to 6.7×10^{-3}), and, as would be predicted, this was shown in the ultracentrifuge picture as a diminution in the 8S component (Fig. 1c). In the second half of the latent period, DNA synthesis was resumed at an accelerating rate and became faster than in the control (unirradiated) bacteria (Fig. 3b; Waites & Fry, 1964). At the same time there was a falling off in protein synthesis so that the DNA:protein ratio increased (from 6.7×10^{-3} to 7.8×10^{-3}) and this was correlated with the very pronounced 8S component in the bacteria 60 min. after induction (Fig. 1d).

Table 1. *Amounts of 40S and 29S ribonucleoprotein components in water extracts of lysogenic and non-lysogenic Escherichia coli*

The bacteria were harvested, irradiated as required, and subsequently incubated in broth as described in the Methods (p. 232). Samples were taken at the times shown and water extracts prepared. Area measurements were made with a planimeter on enlarged photographs of comparable ultracentrifuge diagrams; results are recorded in arbitrary units and have been corrected for radial dilution and differences in bar angle.

Experiment	Sample time (min.)	Corrected area in arbitrary units	
		40S	29S
Lysogenic culture, unirradiated	0	428	253
	20	460	273
	40	525	285
	60	460	360
Lysogenic culture, 60 sec. u.v.-irradiation	Pre-u.v.	482	357
	Post-u.v.	467	357
	30	583	457
	60	610	437
Non-lysogenic culture, 120 sec. u.v.-irradiation	Pre-u.v.	404	242
	Post-u.v.	352	238
	30	260	202
	60	296	239
Non-lysogenic culture, 60 sec. u.v.-irradiation	Pre-u.v.	420	—
	Post u.v.	386	—
	30	477	—
	60	441	—

To determine whether the observed changes in the ultracentrifuge patterns were due to the induced development of phage λ , it was necessary to consider the results obtained in two types of control experiment involving: (a) exponential growth of the (unirradiated) lysogenic bacteria, and (b) the effect of irradiation on the non-lysogenic parent strain. A culture of *Escherichia coli* lysogenic for phage λ was prepared as described in Methods (except that it was not irradiated) and samples were taken at intervals for chemical analysis and the preparation of extracts for ultracentrifugal analysis. In the first 60 min. after addition of broth, whilst the DNA:protein ratio fell slowly from 9.1×10^{-3} to 8.4×10^{-3} , the RNA:protein ratio rose from 1.2×10^{-1} to 1.6×10^{-1} at 40 min. and fell to 1.3×10^{-1} at 60 min. These chemical data could be correlated with deductions made from the ultracentrifugal diagrams (Fig. 1i, j and k) and area measurements of the 40, 29 and 8S components. There was a marked increase in the 40 and 29S ribonucleo-

protein components (Table 1), a result in agreement with previous workers (Wade & Morgan, 1957; Dagley & Sykes, 1958) who showed that this is a feature of exponential growth. Area measurements of the 8S component lack precision, because of the pronounced self-sharpening and incomplete resolution of the boundary from the 4S peak. None the less, consistent with the chemical analysis, the area of the 8S component slowly decreased during the 60 min. incubation period.

It is known that exposure of non-lysogenic bacteria to u.v. radiation can lead to complete inhibition of DNA synthesis for a period depending upon the amount of radiation received (Kelner, 1953). It was therefore of interest in the present

Fig. 3. Changes in (a) turbidity (○, ●) and protein (△, ▲), and (b) DNA (□, ■) and RNA (▽, ▼) after the induction of phage development in *Escherichia coli* K112 (λ). Initially 5.6×10^8 bacteria/ml. and 96% were induced. Solid symbols refer to irradiated bacteria and open symbols to unirradiated control culture.

context to study the effect of u.v. radiation on the ultracentrifuge diagrams of non-lysogenic *Escherichia coli* strain K112, since, whilst Billen & Volkin (1954) have studied the effect of irradiation with X-rays, there appeared to be no comparable experiments with u.v. radiation. The parent non-lysogenic *E. coli* strain K112 was therefore grown and treated in exactly the same manner as the lysogenic strain, and extracts prepared immediately before and after irradiation and after 30 and 60 min. in the growth medium (Fig. 1e-h). The bacteria were irradiated for 120 sec., after which only 4% survived, judged by their ability to form colonies. This dose of radiation was chosen because it gave a population analogous to a fully induced lysogenic culture in which phage development took place in most of the bacteria (90-95%). Once again there was no apparent difference in the ultracentrifuge diagrams of extracts from the bacteria just before and immediately after irradiation (Fig. 1e, f). However, after the irradiated bacteria had been incubated in complete medium for 60 min., area measurements of the ultracentrifuge diagrams (Fig. 1e-h) revealed a decrease in the concentration of the 40S and 8S

components to 73 and 56% of their respective initial (pre-u.v. treatment) values (Table 1). In contrast to the lysogenic strain, chemical analysis showed that DNA synthesis was not resumed during the first 45 min. after irradiation and it was only just beginning after 60 min. (presumably because of the growth of the few surviving bacteria); this explains why the characteristic 8S spike in the ultracentrifuge diagrams was not regained. Since the synthesis of protein and RNA continued at a decreased rate the concentration of the 40S component did not decrease so sharply as the 8S component (by 27% as compared with 44%) during the 60 min. period of the experiment. When the bacteria received u.v. radiation for 60 sec. (the dose used in the induction experiment), 65% were killed; as regards the DNA component, the results were essentially the same as when the bacteria received 120 sec. radiation. The synthesis of DNA was inhibited for 30 min., and with continued protein synthesis the DNA:protein ratio decreased from an initial value of

Fig. 4. Ultracentrifuge patterns of extracts of lysogenic *Escherichia coli* $\kappa 112$ (λ) made in tris + 0.01 M-magnesium acetate buffer: (a) 0 min. before induction by u.v.-irradiation, (b) 30 min., and (c) 60 min. after irradiated cells mixed with tryptone broth and incubated, 8 min. after reaching 50,740 rev./min.; bar angle 30°. In (a) the sedimenting boundaries (reading from left to right) have uncorrected sedimentation coefficients of 28, 44, 58 and 85S and, in (b) and (c), 28, 44, 58 and 73S.

8.5×10^{-3} to 5.5×10^{-3} after 60 min., and the 8S spike again disappeared from the ultracentrifuge diagrams. Ribonucleic acid synthesis continued, the RNA:protein ratio increasing from an initial value of 8×10^{-2} to 1.4×10^{-1} after 60 min., and there was a simultaneous increase in the 40S and 29S RNP components (Table 1). These observations are therefore in general agreement with those of Kelner (1953) for u.v.-irradiated *E. coli* strain B/r.

Ultracentrifugal analysis of extracts prepared in tris + magnesium acetate buffer

When tris buffer + 0.01 M-magnesium acetate was used to prepare extracts of *Escherichia coli* strain $\kappa 112$ (lysogenic for phage λ and non-lysogenic) the ultracentrifuge diagrams (e.g. Fig. 4a) showed components with uncorrected sedimentation coefficients of 28, 44, 58 and 85S (in addition to the 4S boundary due to soluble proteins). These components probably correspond to RNP particles which in

homogeneous solution have ($S_{20,w}$)₀ coefficients of 30, 50, 70 and 100S, respectively, as for example in the experiments of Tissières & Watson (1958). Extracts prepared from lysogenic bacteria before and immediately after u.v.-irradiation again showed no difference but after 30 min. in broth the fastest component (85S) was replaced by one sedimenting at an appreciably slower rate (73S) (Fig. 4*b*). This change was apparently not attributable to a change in the viscosity of the extract since the sedimentation coefficients of the other components were essentially unaltered. A similar slower component was also seen in extracts from bacteria harvested 60 min. after induction (Fig. 4*c*) and in extracts of the non-lysogenic parent strain, with or without prior irradiation, after 60 min. growth in tryptone medium. The reason for this change is unknown but was clearly not connected with phage development or the irradiation *per se* received by the bacteria. The faster 85S (uncorr.) component was observed to develop progressively during periods of restricted growth (i.e. while the bacteria were standing in saline during the irradiation procedure) and it was replaced by the slower 73S (uncorr.) boundary when growth was resumed (Fig. 4*b*, *c*). Bacteria harvested in the mid-logarithmic phase and crushed before suspension in saline gave extracts identical in ultracentrifuge pattern to Fig. 4*c*. Although the 28, 44, 58, 73 and 85S components were not isolated and analysed, they all appeared to contain RNA, since incubation of the extracts for 10 min. at 37° with ribonuclease (final concentration 30 µg./ml.) abolished the 28, 44, 73 and 85S components. The 58S component survived this treatment but was partially degraded after 60 min. incubation. Ribonuclease treatment had the same effect on all extracts irrespective of the history of the bacteria from which they were prepared. It therefore seems likely that these boundaries were due to ribonucleoprotein particles as described by Tissières *et al.* (1959).

DISCUSSION

The experiments described here suggest that the induction of temperate phage development by u.v.-irradiation of a lysogenic strain was accompanied by alterations in the RNA and DNA metabolism in the host-phage system. Although Siegel *et al.* (1952) did not make any claims to this effect, their ultracentrifuge diagrams of extracts from phage T₂-infected *Escherichia coli* appeared to indicate that phage development might cause changes in the RNP particles of bacteria. By using more refined labelling and density-gradient techniques Brenner *et al.* (1961) concluded that no new ribosomes were made during the reproduction of phage T₄ in the infected bacteria. The present experiments lead to the conclusion that during the vegetative development of a temperate phage the synthesis of ribosomal material continued. Whilst this stands in contrast to the T-even phage systems it would seem to be consistent with the observation that in induced lysogenic bacteria a limited amount of growth (phase of residual growth) takes place during the latent period of phage development (e.g. Fig. 3*a*). Comparison of the ultracentrifugal and chemical analyses of the induced system with those of the irradiated non-lysogenic control indicates that the observed differences are not simply attributable to the effect of u.v.-irradiation. The inhibition of bacterial DNA metabolism by u.v.-irradiation reported by Kelner (1953) is confirmed here for the irradiated non-lysogenic strain, but in the irradiated lysogenic strain there were additional effects due to the initiation of phage development. In the latter case DNA metabolism was briefly inhibited

and then resumed at an accelerated rate as revealed by chemical analysis (see also Waites & Fry, 1964) and the changes in the 8S DNA component in the ultracentrifuge diagrams. It seems reasonable to ascribe these changes to vegetative development of the temperate phage. When extracts were made in tris buffer + magnesium acetate the bacteria were harvested from cultures in the mid-logarithmic phase of growth and then immediately suspended in saline. This procedure apparently initiated the progressive loss of the slower 73S (uncorr.) component and the appearance of a faster sedimenting species (85S, uncorr.). This was an unexpected change since McCarthy (1960) reported that a similar transition (85S corr. to 100S corr.) was characteristic of *E. coli* passing from the logarithmic phase of growth to the stationary phase. Hence one interpretation of the observations reported here is that simple resuspension of log-phase bacteria in saline rapidly initiated their transformation to a stationary phase. In agreement with McCarthy (1960) we also found that the 73S (uncorr.) RNP component was characteristic of tris buffer + Mg acetate extracts prepared from *E. coli* in the log phase. The reasons for these transitions are unknown but McCarthy (1960) and Horowitz & Weller (1964) have suggested that they may result from a configurational change in the particles in environments of various Mg^{2+} concentrations.

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