SOME OBSERVATIONS ON THE MORPHOLOGICAL CHANGES IN *E. COLI* ACCOMPANYING INDUCTION BY ULTRAVIOLET LIGHT

by

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INTRODUCTION

Some strains of lysogenic bacteria undergo induction when exposed to certain agents. This phenomenon was first described by LWOFF, SIMINOVITCH AND KJELDGAARD[1] who exposed *B. megatherium* to ultraviolet light of wavelength 2537 A, and also to X-rays. The cultures increased in opacity for about 50 minutes following irradiation, by which time they had increased in opacity by a factor of between 2 and 4 compared with that at the time of irradiation. Lysis then began, and was complete after a further 20 minutes. When the individual bacteria or chains of bacteria were observed in microdrops, they were seen to divide or form septa before lysing. This was put forward as accounting for the increase in optical density.

WEIGLE AND DELBRUCK[2] showed that *E. coli* K12 was inducible with ultraviolet light in small doses. Using 80 seconds of irradiation, more than 95% of the bacteria burst after a latent period of 60 minutes. As in the case of *B. megatherium*, there was a marked increase in opacity during the latent period, which they found, by microscopical observation, to be due to an increase in length and thickness of the bacteria. No cell division was observed during this period. Immediately before lysis the cells swelled up to a spherical shape, and then burst, forming a mass of debris.

Using the electron microscope, LABAW, MOSLEY, AND WYCKOFF[3] studied the morphology of *E. coli* B following irradiation of the organisms with ultraviolet light, and also the morphology of the moribund bacteria after infection with virulent bacteriophage.

It was thought that interesting information would be obtained if the morphological changes observed by WEIGLE AND DELBRUCK by light microscopy could be observed by means of the electron microscope. This brief report presents the observations.

MATERIALS AND METHODS

A mutant strain of K12 designated Y10 by LEDERBERG[4] was used in this work. It is "marked" by virtue of its requirement of the addition of threonine, leucine and thiamine to the basal medium. Otherwise it is identical with the "wild" type K12, being lysogenically infected with λ and having similar induction characteristics. The advantage of using such a mutant lies in the ease with which the purity of the culture can be checked, before and after use. Occasional inducible non K12 strains were encountered before this technique was introduced.

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Media

All growth was carried out in PYLP medium. Its composition is:

Evans peptone	10 g
Yeastrel	3 g
Lab Lemco	5 g
Pronutrin	10 g
Sodium chloride (AR)	5 g
Distilled water	1 litre
pH adjusted to 7.4	

All induction was carried out in 56 buffer modified slightly from the original formula of Dr. J. MONOD of the Pasteur Institute:

Potassium dihydrogen phosphate	13.6 g
Sodium sulphate	2.0 g
Magnesium sulphate (crystals)	0.2 g
Calcium nitrate	0.01 g
Ferrous sulphate (crystals)	0.0005 g
Potassium hydroxide approximately	9 ml of 10 *N*
Adjusting the pH to 7.0	
Distilled water	1 litre.

Ultraviolet light source

A G.E.C. germicidal 30 watt tube giving 80–90 % emission at 2537 A was used. It was calibrated by constructing a killing curve for T2 situated 1 metre from the lamp. Later Dr. LATARJET of the Pasteur Institute confirmed the value of 10 ergs/sq. mm/sec by the use of his direct reading device[5].

Bacterial growth

All growth rates were measured in specially designed opacity tubes which could be shaken in a thermostatically controlled water bath at 37° C, and then taken out and inserted in a "Spekker" photometer (Hilger and Watts Ltd., London) at regular intervals.

Cultures were grown for 4 hours at 37° C, the tubes being continuously gently agitated. The cultures were then sedimented by centrifugation, resuspended in buffer, and irradiated in a dish of such a size that the fluid was between 1 and 2 mm deep. The dish was agitated gently to keep the fluid in motion. In order to obtain workable quantities of material for electron microscopy, the culture was irradiated for a standard time, and then 2 ml samples were dispensed into opacity tubes containing 8 ml PYLP. The opacity was then measured and one sample immediately spun down and resuspended in buffer to provide the "zero time" sample for electron microscopy. The remainder were shaken at 37° C in the dark. Opacity readings were taken every 30 min, while every hour one sample was removed, spun down and resuspended in buffer. Each of these samples for electron microscopy was washed twice in buffer, fixed overnight in Osmic acid and washed twice in distilled water. A drop of suspension was allowed to dry on a "formvar" covered specimen mounting grid, shadowcast with gold alloy at $\tan^{-1} \frac{1}{4}$ to the plane of the grid[6] and then examined in the electron microscope using a 60 kilovolt electron beam. For each dose of ultraviolet light, four samples were examined: zero time, *i.e.* immediately following irradiation; one hour after, corresponding to the maximum opacity prior to lysis; two hours after, when lysis was complete, and three hours after, when the opacity had begun to increase again. The process was repeated with no irradiation to provide control material.

RESULTS AND DISCUSSION

Fig. 1 shows the induction curves of Y10 after zero, 60 sec, 120 sec, and 180 sec irradiation with ultraviolet light. A further induction curve was plotted for 90 sec irradiation, but this proved to be so nearly identical in all respects to that for 120 sec that it is omitted.

Electron micrographs of the control series of samples were indistinguishable from one another (Fig. 2) and were observed as short dense bacilli. In the irradiated series, the samples corresponding to zero time were practically indistinguishable from the control material, but in some cases they showed less rigidity and density than the control series (Fig. 3).

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It must be admitted in this connection that a short time elapsed after irradiation before the samples were resuspended in buffer, owing to the time taken in ascertaining the opacity and sedimenting the organisms in the centrifuge. Thus zero time is probably more accurately described as 5 mins at room temperature after irradiation.

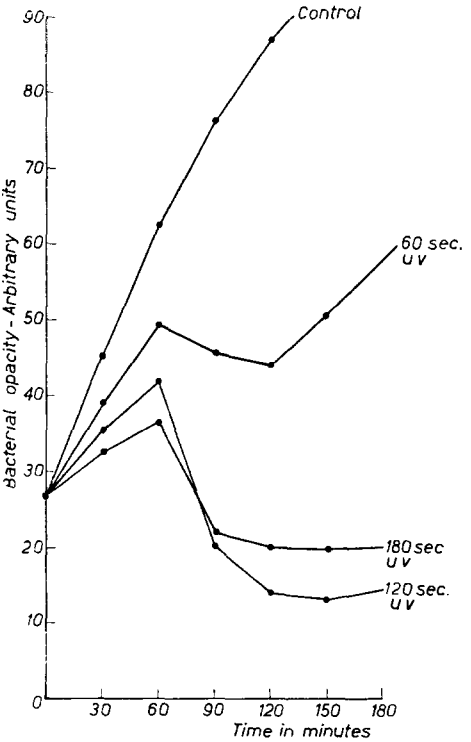

Fig. 1. Induction curves of Y10 for different doses of ultraviolet irradiation.

Samples irradiated for 60 sec show production of long forms of the bacterium after 1 hour, with little further change in the 2 and 3 hour samples. Only a small amount of debris is present at all the three times (Fig. 4). Samples irradiated for 120 sec, however, after 1 and 2 hours' incubation show many long forms of great length which often extend across one square on the specimen support ($\sim 50\ \mu$) (Fig. 5). Debris is present in the 1 hour sample, (Fig. 6), indicating that lysis has begun, but the 2 hour sample is almost free of debris (Fig. 5), presumably because the centrifugation was not powerful enough to sediment debris from completely lysed organisms. Three hours after incubation shows little perceivable reduction in the proportion of long forms, but 5–7 hours shows a substantial decrease in this proportion, and in 24 hours the long forms have largely disappeared. Samples irradiated for 180 sec again show many long forms, but debris is presents in small quantities in the 1, 2 and 3 hour samples, indicating that lysis is not confined to the short period observed when 120 sec irradiation is used (Fig. 7). Long forms are still present after 3 hours incubation. In all cases where debris is present it resembles collapsed spheres.

In the long forms, "necks" may often be observed, indicating that the process of cell division has, at least, begun. Owing to the well-known tendency of bacteria—and indeed many small particulate preparations—to aggregate when prepared in the manner described, it is most difficult to distinguish between inhibited cell divisions and cells that have simply aggregated. Often, these inhibited divisions appear to be bent at an angle (Fig. 4) and on occasions pictures have been obtained which could be taken to indicate an organic junction between two bacteria to form a T (Fig. 8). Although this observation is regarded with reserve it is felt that the possibility of its representing a real phenomenon cannot be ignored.

Despite the production of a titre of 10^{11}, no bacteriophage can be seen adhering to the debris or the bacteria.

DISCUSSION

The electron microscope has demonstrated that induction of *E. coli* K12 by ultraviolet light produces longer forms than was suggested by the light microscopical studies

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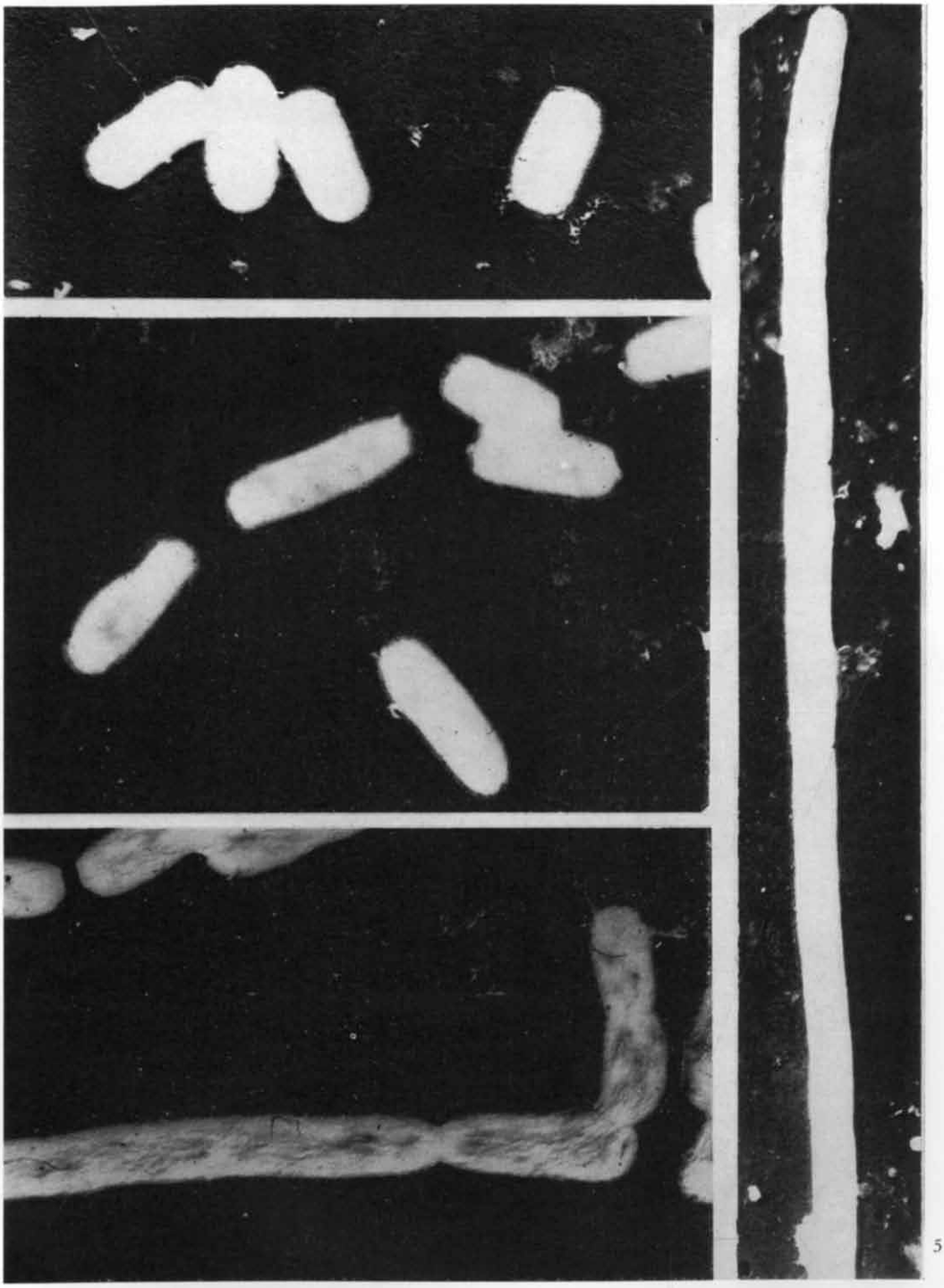
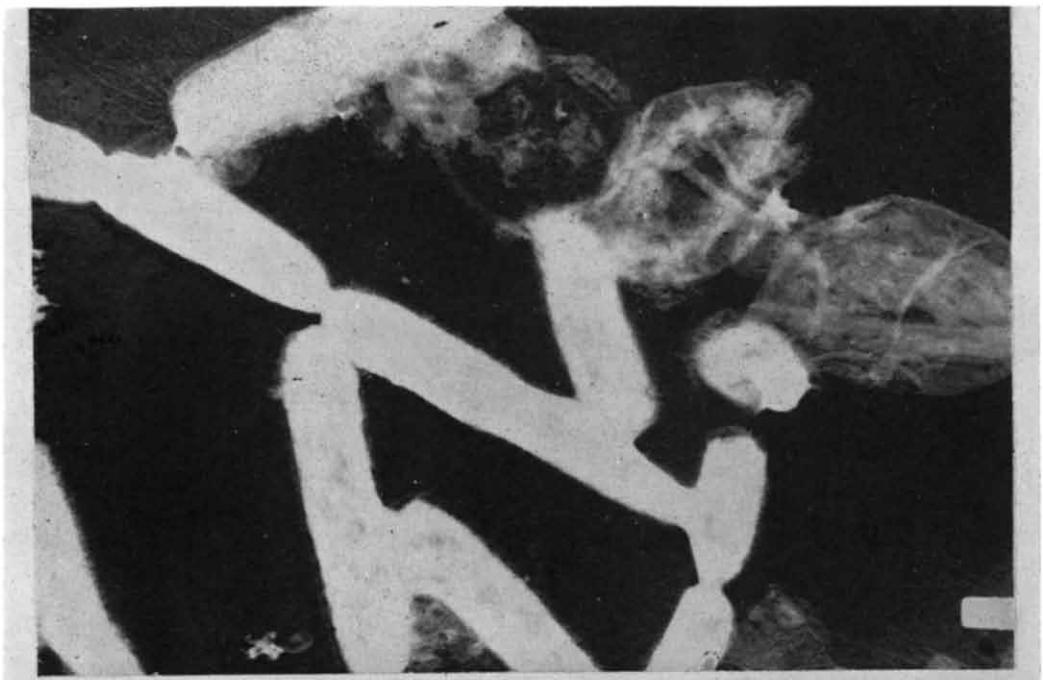


Fig. 2. Control bacteria grown 2 hours in dark. × 13,000.
Fig. 3. Bacteria immediately following 60 sec irradiation with ultraviolet light. × 13,000.
Fig. 4. Bacteria 2 hours after 60 sec irradiation. An inhibited division bent in a right angle is seen. Little debris is present. × 13,000.
Fig. 5. Bacteria 2 hours after 120 sec irradiation. Part only of a long form. Little debris is present. × 9000.



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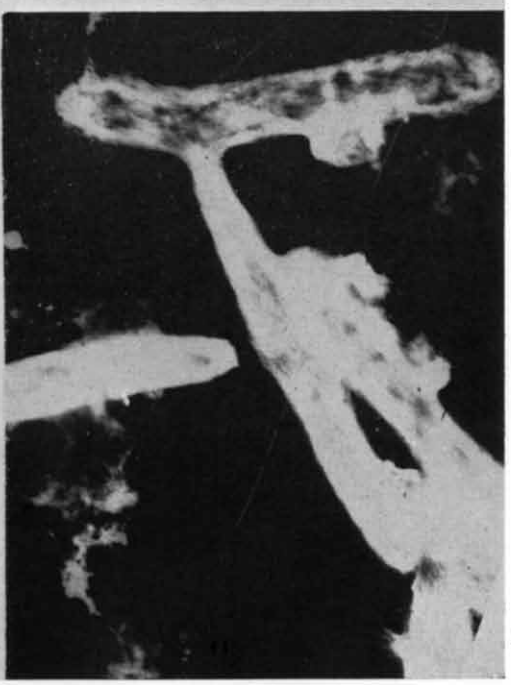

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Fig. 6. Bacteria 1 hour after 120 sec irradiation. Debris resembling collapsed spheres is seen attached to bacteria. Inhibited cell divisions also are seen. × 13,000.
Fig. 7. Bacteria 3 hours after 120 sec irradiation. Considerable debris is still present. × 13,000.
Fig. 8. Bacteria 2 hours after 120 sec irradiation. An apparent "T" form. The cell contents appear continuous across the "T" junction. × 13,000.

of WEIGLE AND DELBRUCK[2]. The presence of collapsed spherical skins is in agreement with their observation that the bacteria swell up into spheres before bursting. The observation that long forms are present in the 2 and 3 hour incubation samples which have had the optimum ultraviolet dose (120 sec) suggests that the long forms represent the survivors, and the forms that do not proliferate sufficiently in this manner swell up and burst.

The absence of bacteriophage adhering to the debris or bacteria is in agreement with the observations of WEIGLE AND DELBRUCK[2] that λ is not adsorbed onto K12 at a measurable rate.

ACKNOWLEDGEMENTS

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SUMMARY

The morphological changes in *E. coli* K12 accompanying induction by ultraviolet light have been studied by electron microscopy. Long forms have been observed, and in some cases debris consisting of collapsed spherical skins. No bacteriophage was observed adhering to the debris or bacteria.

RÉSUMÉ

Les modifications morphologiques subies par *E. coli* K12 à la suite d'une exposition à la lumière ultraviolette ont été étudiées au microscope électronique. Il apparaît des formes allongées, et, quelquefois, des débris de parois sphériques éclatées. Il n'y a pas de bactériophage visible adhérant aux débris ou aux bactéries.

ZUSAMMENFASSUNG

Die morphologischen Veränderungen, die die Einwirkung von ultraviolettem Licht auf *E. coli* K12 begleiten, wurden elektronenmikroskopisch untersucht. Es wurden lange Formen und in einigen Fällen Detritus, der aus kollabierten, kugligen Häuten besteht, beobachtet. Es wurde nicht beobachtet, dass Bakteriophagen dem Detritus oder den Bakterien anhaften.

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