FURTHER OBSERVATIONS ON THE ELECTRON MICROSCOPE CHANGES FOLLOWING INDUCTION OF *E. COLI* K12

by

R. H. GORRILL AND C. E. CHALLICE

The Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School London (England)

In a previous paper[1] it was shown that induction of lysogenic *E. coli* K12 was accompanied by the production of many long forms of bacterial cell together with debris resembling collapsed spheres. These spheres are of interest as they have not been recorded when bacteria are lysed by virulent bacteriophage. This paper records our investigation into the relationship between sphere production and induction.

In order to carry out this work use was made of the fact that *E. coli* K12 can be cured of lysogenicity and then becomes an indicator for the phage lambda. Such cured strains have been used to determine the position of the prophage in the bacterial chromosome by crossing cured with lysogenic cultures[2,3].

It should be stated that all the cured strains used in this work were either obtained from Dr. E. L. WOLLMAN of the Pasteur Institute, Paris or produced by one of us (RHG) during his tenure of an Anglo-French exchange bursary at the Pasteur Institute. Cured strains of K12 can usually be reinfected with phage λ and returned to their original lysogenic state. During the process of curing a number of strains are isolated carrying a mutant of λ. Amongst these mutants some are no longer inducible in the sense that irradiation is not followed by lysis although they often show a rise in free phage count under these conditions. As well as being non-inducible some also show a difference in plaque morphology. That the K12 host is still infected with a λ phage and not a contaminant is shown by the fact that the cultures are immune to λ but are sensitive to the virulent mutant described by JACOB AND WOLLMAN[4]. Using these various strains of K12 it was possible to examine the effect of ultraviolet light on cured strains, reinfected strains and strains carrying a mutant of λ. This has allowed us to demonstrate the close relationship between induction and sphere formation.

METHODS

All technical methods were as described in the earlier paper[1]. Bacterial strains used were:

(a) K12 S a cured culture of wild type K12.

(b) K12[1] a reinfected culture of K12 S.

(c) W677[1] and[7] two cured strains of W677.

(d) W705 SR21 carrying a mutant of λ, not inducible showing a small increase in free phage on irradiation, and giving a tiny plaque.

(e) *E. coli B.* as representative of a non lysogenic coliform which has been previously studied under the electron microscope[5].

References p. 86.

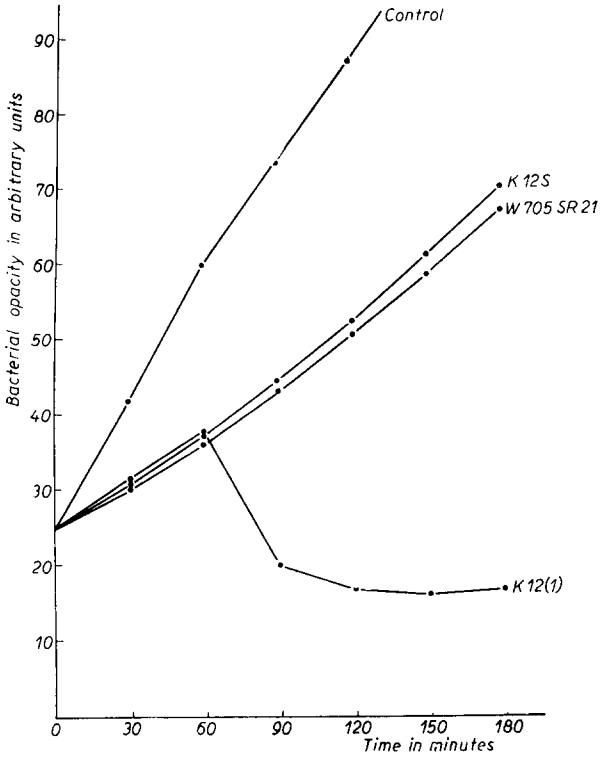

Fig. 1. Growth curves of K12S, K12(1) and W705 SR21 following 120 sec UV irradiation.

RESULTS

In Fig. 1 is shown the growth curves of K12 S, K12[1] and W705 SR21 following 120 sec irradiation with UV light. The curves have been reduced to a common starting density. The control curve is representative of all three strains growing in the medium used. As will be seen neither K12 S or W705 SR21 were inducible while K12[1] behaved as did Y10 in the earlier paper[1]. *E. coli B.* and W677[1,7] have been omitted for clarity but they had a growth curve similar to K12 S.

Electron microscopical examination of the cured strains and of *E. coli B.* following irradiation showed a considerable proportion of long bacillary forms but at no time were any collapsed spheres seen. Two illustrations of these

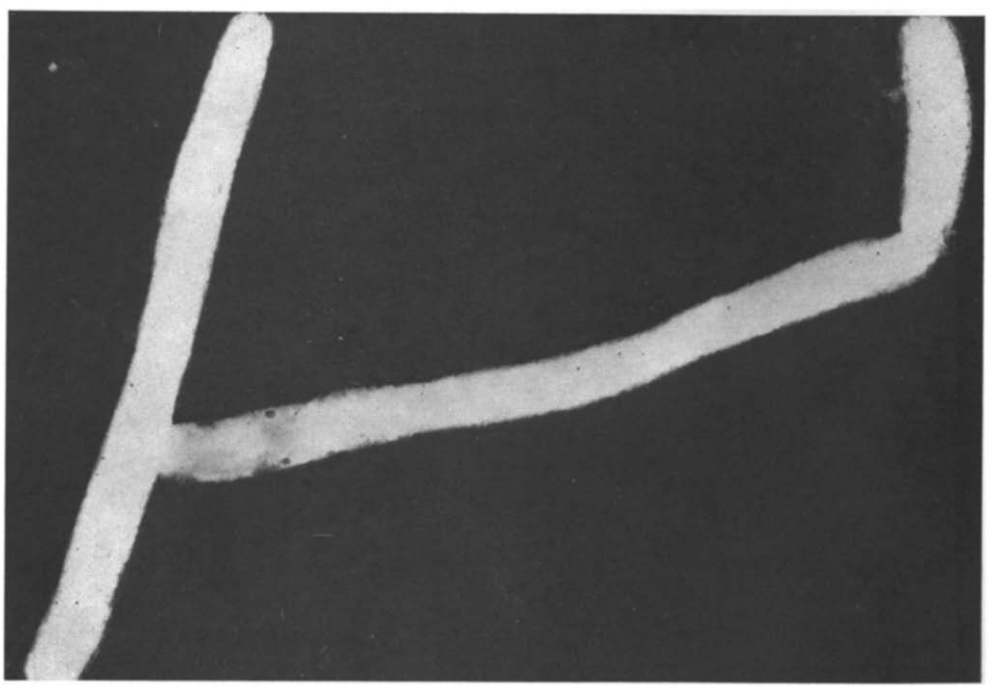

Fig. 2. K12 S two hours after 180 sec irradiation × 12,000.

References p. 86.

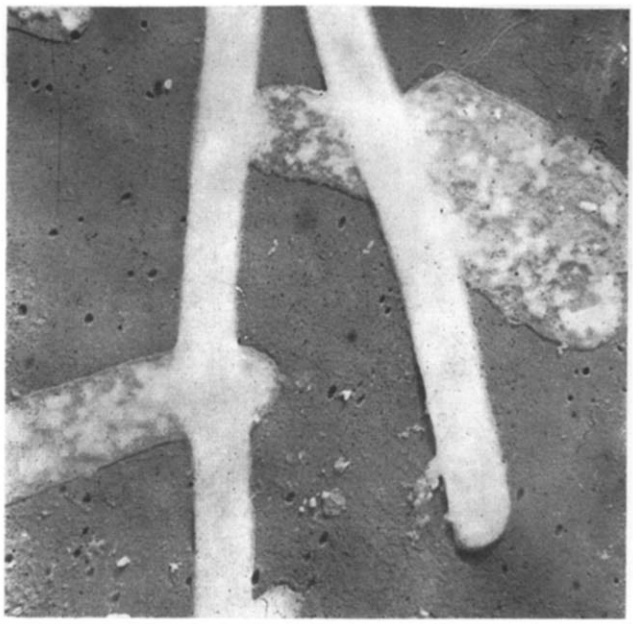

Fig. 3. *E. coli B.* three hours after 120 sec irradiation × 11,000.

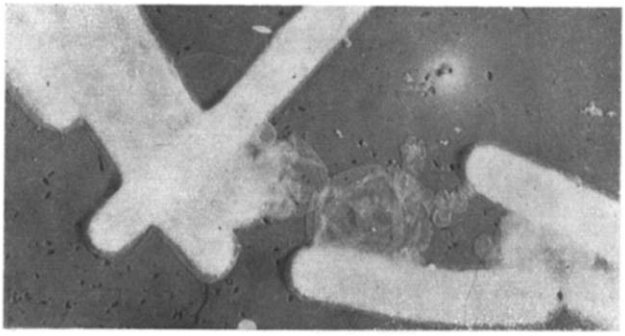

Fig. 4. K12(1) one hour after 120 sec irradiation × 11,000.

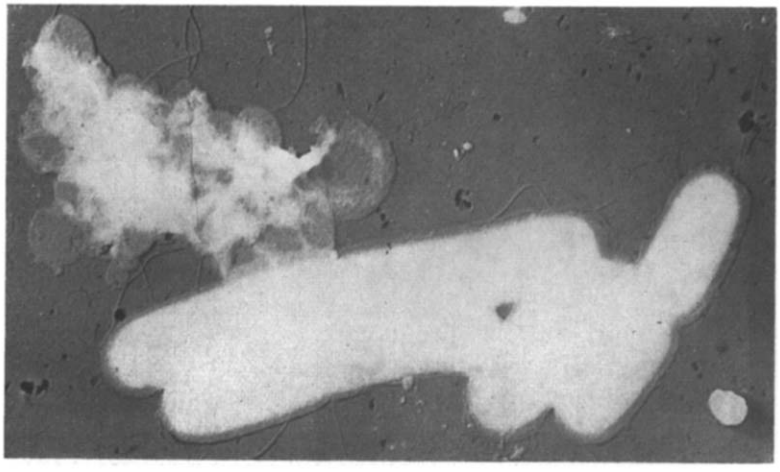

Fig. 5. K12(1) two hours after 120 sec irradiation × 14,000.

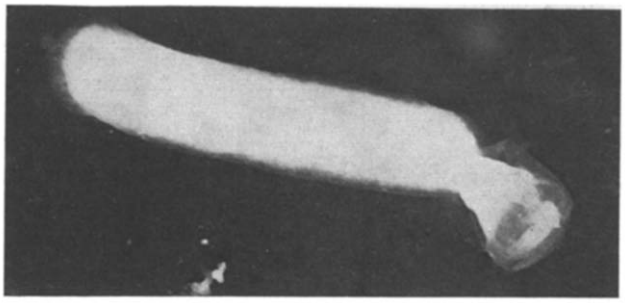

Fig. 6. W705 SR21 two hours after 60 sec irradiation × 16,000.

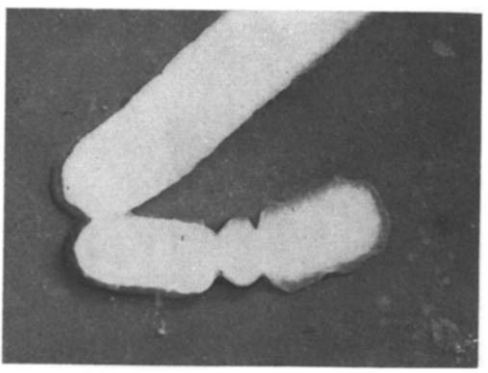

Fig. 7. W705 SR21 two hours after 180 sec irradiation × 15,000.

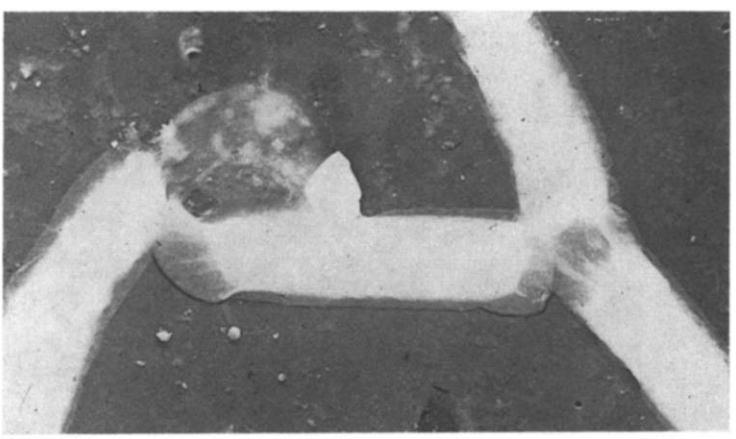

Fig. 8. W705 SR21 one hour after 180 sec irradiation × 15,000.

strains are given; Fig. 2 shows K12 S two hours after 180 sec of irradiation while Fig. 3 shows *E. coli B.* three hours after 120 sec irradiation.

When the irradiation specimens of K12[1] were examined it was found that the appearance was very similar to that recorded earlier[1]. Thus Fig. 4 shows a specimen 1 hour after 120 sec irradiation while Fig. 5 shows the appearance seen 2 hours after the same dose. With K12[1] as with Y10 120 sec irradiation gave the best pictures. The debris contains besides the large collapsed spheres smaller structures which may be likened to foam.

Finally the appearance of W705 SR21 was examined and some rather unusual

References p. 86.

pictures were seen. As well as the usual long forms some bacteria showed evidence of swelling at the ends, Fig. 6 (2 hours after 60 sec) and some showed a spherical structure in the middle, Fig. 7 (2 hours after 180 secs). Fig. 8 shows the nearest that was seen to a collapsed sphere in this strain (1 hour after 180 sec).

DISCUSSION

It will be seen that in this investigation the appearance of spheres in the debris is only found after induction. They are not produced by the same organism rendered non-inducible nor in normally non-inducible strains.

It is not possible from these pictures to determine the nature of the spherical object but it is possible that it represents the outer layer of a bacterium which has for some reason been greatly distended at the time the specimen was made. This suggests that before the bacteria burst to liberate the mature phage particles the control of the osmotic barrier is lost, the cell distends and normally bursts and that the forms we see are those in that state at the time the specimen was fixed.

The origin of the foam is obscure and one can postulate that it represents some inner structure of the bacterium which has been similarly distended.

The structures seen in W705 SR21, coupled with the fact that this strain is not inducible but does have an increase in phage count following irradiation, leads one to suggest that they are modified cellular disintegration.

The pictures support the view that it is the mechanical bursting of the cells at induction and not the presence of a prophage that leads to sphere formation.

ACKNOWLEDGMENTS

One of the authors (R.H.G.) is indebted to the Ciba Foundation for the award of an Anglo-French Exchange Bursary and to Drs. LWOFF and WOLLMAN of the Pasteur Institute for their help and encouragement.

SUMMARY

Irradiation of cured strains of *E. coli* K12 gave pictures similar to that of *E. coli B*. When the bacteria were rendered lysogenic the appearance changed and was marked by collapsed sphere formation. Strains of K12 carrying a mutant of phage λ gave an intermediate picture.

RÉSUMÉ

L'irradiation de souches guéries de *E. coli* K12 donne des images semblables à celles de *E. coli B*. Quand on a rendu les bactéries lysogènes, l'apparence change et ce changement se signale par la formation de sphères. Des souches de K12 portant un mutant de phage λ présentent un aspect intermédiaire.

ZUSAMMENFASSUNG

Die Bestrahlung von geheilten Stämmen von *E. coli* K12 ergab ähnliche Bilder wie diejenige von *E. coli* B. Wenn die Bakterien lysogen gemacht wurden, änderte sich ihr Aussehen und auf den Bildern wurden Kugeln sichtbar. Stämme von K12, die einen Mutant des Phagen λ enthielten, ergaben Bilder, welche zwischen den zwei erstgenannten Arten die Mitte hielten.

REFERENCES

1 C. E. CHALLICE AND R. H. GORRILL, *Biochim. Biophys. Acta*, 14 (1954) 482.
2 E. M. LEDERBERG AND J. LEDERBERG, *Genetics*, 38 (1953) 51.
3 E. L. WOLLMAN, *Ann. Inst. Pasteur*, 84 (1953) 281.
4 F. JACOB AND E. L. WOLLMAN, *Cold Spring Harb. Symposium Quant. Biol.*, 18 (1953) 101.
5 L. W. LABAW, V. M. MOSLEY AND R. W. G. WYCKOFF, *Biochim. Biophys. Acta*, 5 (1950) 327.

Received August 25th, 1954