

# CHROMATIN STAINING OF BACTERIA DURING BACTERIOPHAGE INFECTION<sup>1</sup>

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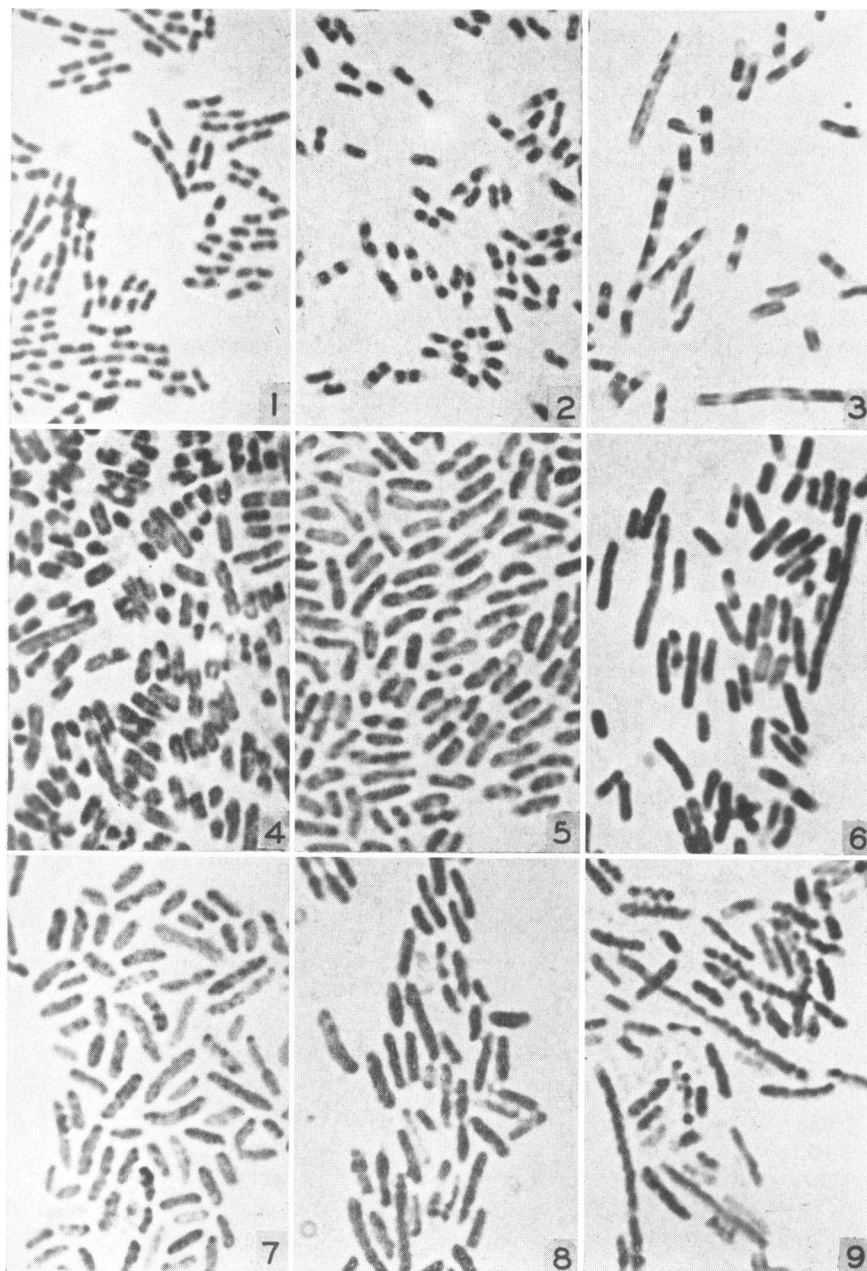
Infection of bacteria with bacteriophage, leading to bacterial lysis, must be accompanied by changes in cell organization that should be recognizable by cytological examination. Since bacteria contain desoxyribosenucleic acid (DNA) localized in discrete "chromatinic bodies," and since DNA is one of the major components of all phages thus far analyzed, chromatin stains should yield valuable information. Preliminary work on this problem (Luria and Palmer, 1946) was considered worth extending with improved technique (Robinow, 1944). A brief report of observations similar to those described in the present article has been published by Beumer and Quersin (1947). A different appearance of the chromatinic material in uninfected and lysogenic strains of *Bacillus megatherium* has also been described (Ehrlich and Watson, 1949). Boyd (1949) has observed specific morphological changes in *Escherichia coli* B after infection with phages of the T group by means of phase-contrast microscopy, a method that unfortunately yields no cytochemical information.

## MATERIALS AND TECHNIQUES

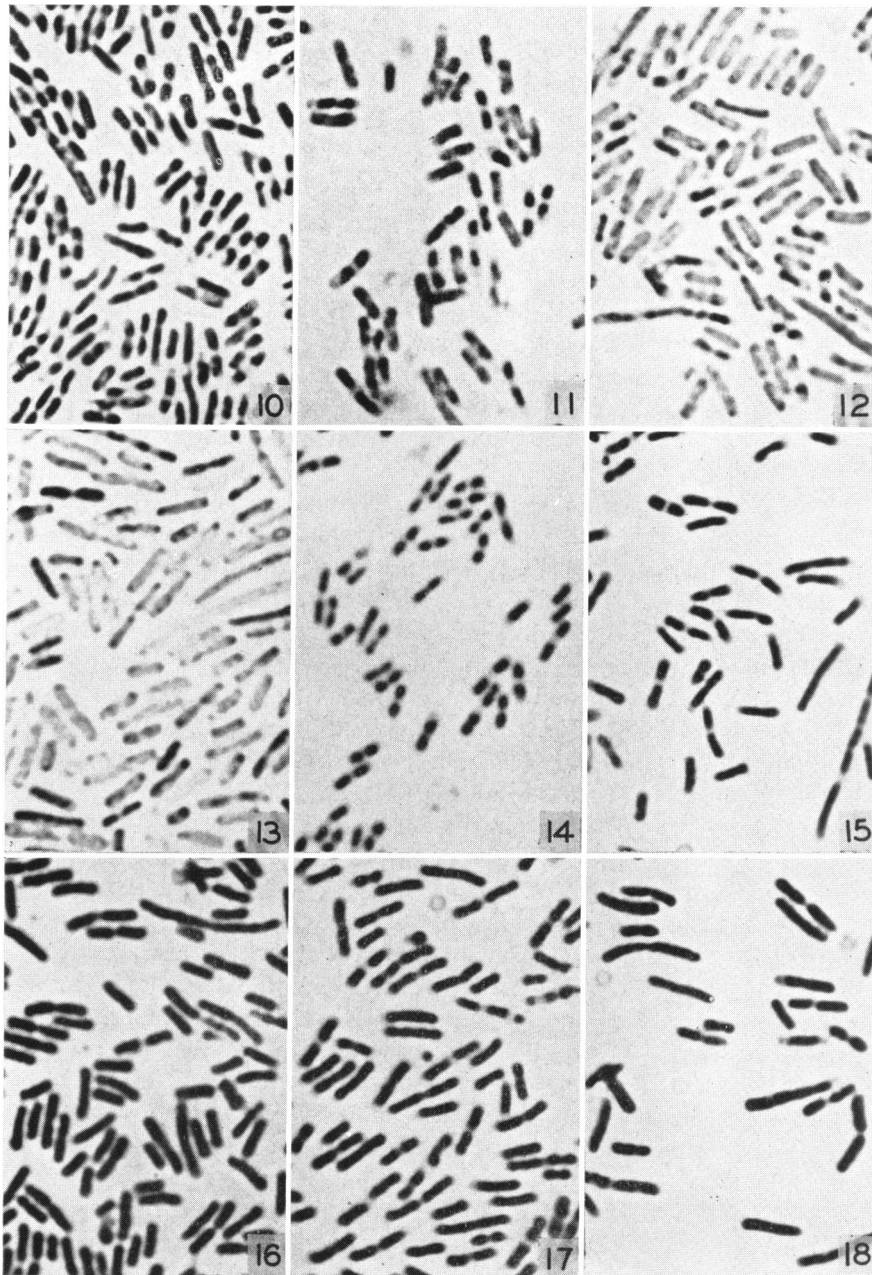
*Cultures.* *Escherichia coli*, strain B, and the phages of the T group were used (Delbrück, 1946). All observations on infected bacteria were made on mixtures of bacteria (in the late logarithmic phase) with phage in 4- to 8-fold excess (multiple infection, with less than 5 per cent uninfected cells). Most cells became infected within 2 minutes after adding phage. All cytological observations were made on cells from cultures in Difco nutrient broth kept at 37 C. Ultraviolet (UV) inactivated phages were treated as described by Luria and Dulbecco (1949).

*Fixation and staining.* Samples were removed at intervals, and 2 volumes of fixative fluid were added to give the following final concentrations: formalin 10 per cent (3.7 per cent HCOH),  $K_2Cr_2O_7$  2 per cent. After 20 minutes the cells were collected by centrifugation, washed once in water, and resuspended in a drop of water. This was spread on a plate containing 2 per cent agar in water; impression preparations were made on slides, which were immediately placed in 70 per cent alcohol. The slides were then washed in water, placed in 1 N HCl at 60 C for 7 minutes, washed in cold phosphate buffer m/15, pH 7.0, stained in

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Figures 1 to 9. 1. *Escherichia coli* strain B from a 130-minute culture in nutrient broth; viable titer =  $10^8$  cells per ml. Magnification,  $2,400\times$ . 2. Cells + active phage T2, 3 minutes after infection. 3. The same, 5 minutes after infection. 4. The same, 7.5 minutes after infection. 5. The same, 10 minutes after infection. 6. The same, 15 minutes after infection. 7. The same, 20 minutes after infection. This photograph was printed on a lower contrast paper, in order to show the granular aspect of the chromatin at this time. 8. The same, 25 minutes after infection. 9. The same, 30 minutes after infection.



*Figures 10 to 18. 10. Cells + irradiated phage T2, 5 minutes after infection. 11. The same, 7 minutes after infection. 12. The same, 15 minutes after infection. 13. The same, 30 minutes after infection. 14. Cells + active phage T1, 2.5 minutes after infection. 15. The same, 5 minutes after infection. 16. The same, 12 minutes after infection. 17. Cells + irradiated phage T1, 15 minutes after infection. 18. The same, 25 minutes after infection.*

Giemsa solution (National Aniline Company, stock solution diluted 1:10 in buffer) for 20 to 40 minutes, washed in water, and mounted in water by sealing under a cover slip with paraffin. Preparations of this type fade after 1 to 5 days; photographs, when desired, are made within 24 hours after staining.

After lengthy trials the foregoing technique, which completely avoids drying of the cells, proved the most reproducible and satisfactory.  $O_5O_4$  could not be used as a fixative because it produces premature lysis if added to a suspension of phage-infected bacteria several minutes after infection. Controls using either the Feulgen staining technique or DeLamater's basic fuchsin method (1948) gave preparations similar to those obtained with the Giemsa method, but less suitable for photography. Photographs were taken on "panatomic X" plates (Eastman Kodak) at a magnification of 1,100  $\times$ , enlarged in printing to 3,300  $\times$ , then reduced to 2,400  $\times$  in the accompanying figures.

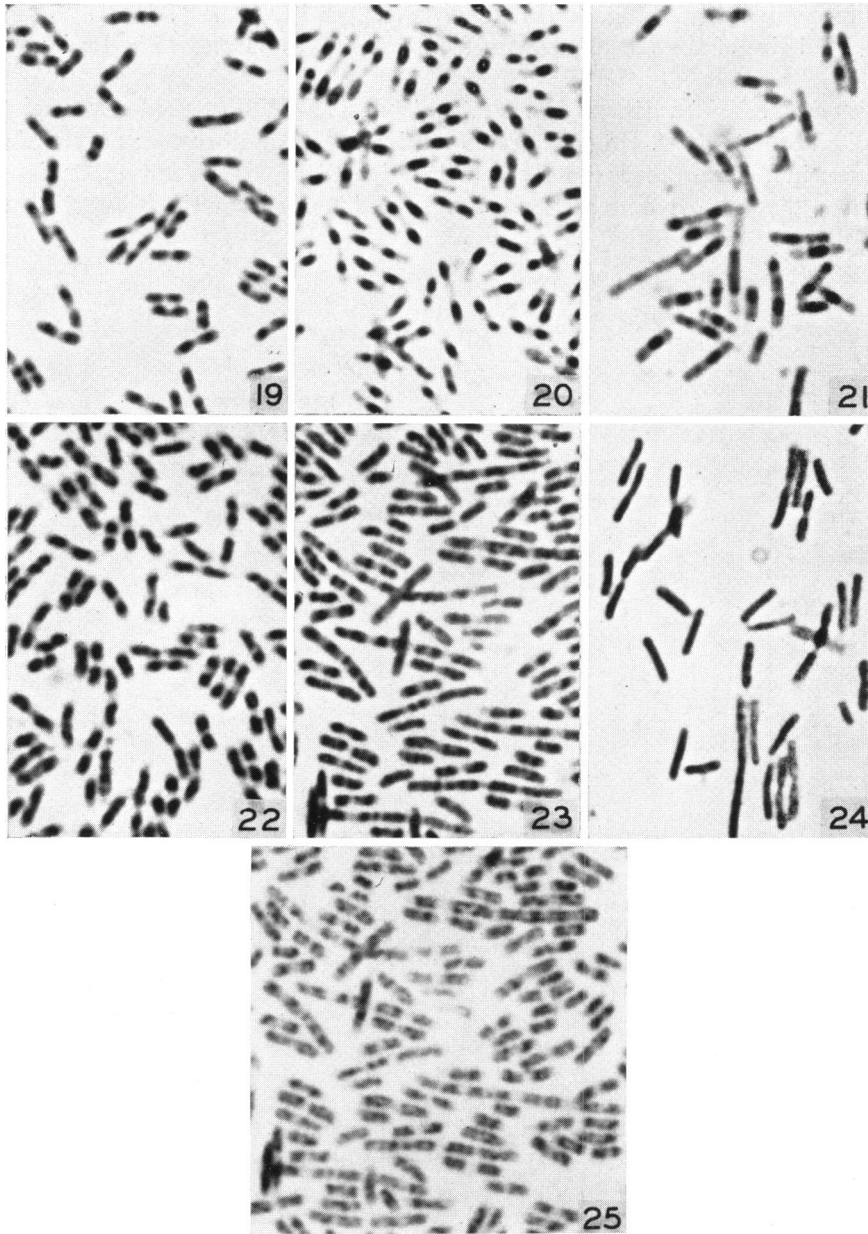
#### RESULTS

*Phages T2 and T2r, active (figures 2 to 9).* Samples were observed at intervals of 2 to 60 minutes after infection. The first visible modification consists in an alteration of the chromatin bodies, which 5 minutes after infection are changed into peripheral masses. The cells begin to swell and, after 7 to 9 minutes, to fill up with fine granular chromatin. After 15 to 20 minutes, the chromatin collects in coarser granules. After the beginning of lysis (21 minutes), most cells show somewhat jagged edges—possibly due to adsorption of phage liberated by other cells. Most cells persist for a long time (lysis inhibition) and show coarser chromatin granules. Lysed cells are generally not observed, probably because they are eliminated in preparing the sample for observation.

*Phages T2 and T2r, UV-irradiated (figures 10 to 13).* A UV dose giving a survival of  $10^{-8}$  was used; with multiplicities of infection of 3 to 5, about 5 per cent bacteria liberate reactivated phage (Luria, 1947). The cytological manifestations are the same as with active phage up to 7 or 8 minutes, but the peripheralization of the chromatin is not followed by filling up with granular chromatin. In the swollen cells the amount of chromatin progressively diminishes, and after 20 to 30 minutes the cells appear almost devoid of it; thereafter they remain unchanged for hours. These cells still stain normally with the usual basic dyes, if not hydrolyzed in HCl. A minority of the cells—probably those in which reactivation takes place—exhibit the same changes as cells infected with active phage, although somewhat delayed. Similar changes occur in cells infected with X-ray-irradiated phage as with UV-irradiated phage.

*Phages T4 and T6.* These phages cause cytological changes not readily distinguishable from T2, with the exception that the irregular cell outline following the initial lysis occurs somewhat later, in agreement with the longer latent periods (24 and 26 minutes, respectively). After 30 minutes or later, many cells show a peculiar bipolar concentration of chromatin.

*Phage T1, active (figures 14 to 16).* No change is noticeable for 3 or 4 minutes after infection; then, with little swelling, the cells become rapidly filled with chromatin up to the time of initial lysis (13 minutes). The last cells observable



*Figures 19 to 25.* 19. Cells + active phage T7, 5 minutes after infection. 20. The same, 13 minutes after infection. 21. The same, 17 minutes after infection. 22. Cells + irradiated phage T7, 5 minutes after infection. 23 and 25. The same, 13 minutes after infection. The photograph in figure 25, covering the same field shown in figure 23 but taken under different conditions of illumination, happened to show almost as much detail as is visible in the actual preparation, and is, therefore, presented for purposes of comparison. 24. The same, 30 minutes after infection.

before complete lysis (figure 16) show no surface irregularity, possibly due to the small size of the phage particles (compare figures 11 and 15 in Luria *et al.*, 1943).

*Phage T1, UV-irradiated (figures 17 and 18).* The UV dose used gave a phage survival of  $4 \times 10^{-4}$ . No lysis occurs nor is any phage liberated; nevertheless, chromatin filling proceeds exactly as with active T1. If observed after 30 minutes or later, the cells are a little enlarged and still full of chromatin (figure 18).

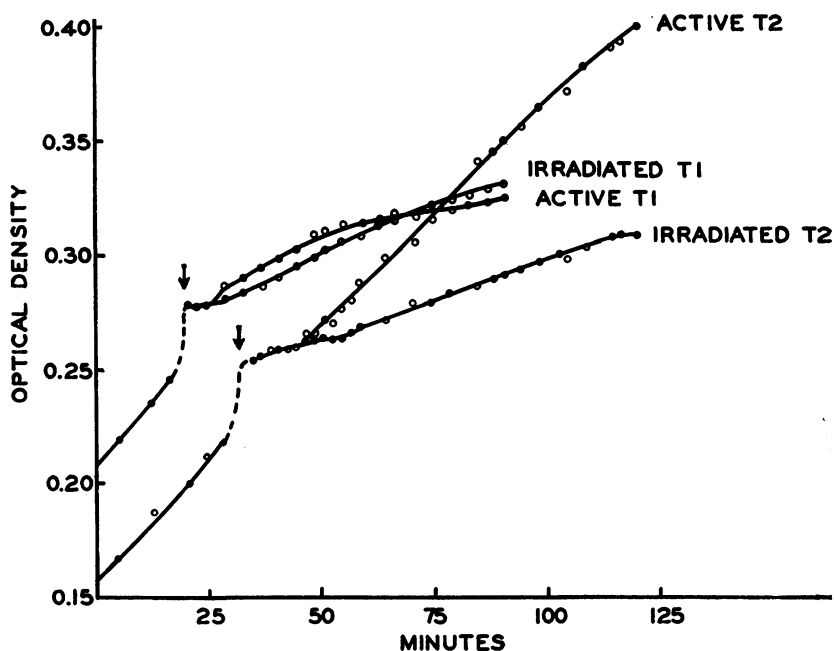


Figure 26. The optical density (at wave length  $260 \text{ m}\mu$ ) of cultures of *E. coli* B infected with phage, as a function of the time of incubation at  $26^\circ \text{C}$ . The arrows mark the times of phage addition. The UV doses used gave survivals of less than  $10^{-8}$  for T2, of about  $10^{-4}$  for T1. No measurable lysis took place with phage T2 (lysis inhibition), and the turbidity measured at wave length  $420 \text{ m}\mu$  remained constant from the time of infection on. With active phage T1 lysis begins 30 minutes after infection. The optical density at  $260 \text{ m}\mu$  was corrected for turbidity by subtracting the optical density measured at  $420 \text{ m}\mu$ . Since this correction neglects the dependence of light scattering on wave length, it causes a deformation of the curves for which the turbidity changes during the experiment. The values for active phage T1 are, therefore, only reliable for times up to about 50 minutes before lysis begins.

*Phage T7, active (figures 19 to 21).* Very typical changes are observed: the chromatin bodies are first deformed, then, in 6 or 7 minutes, converge into one large central mass of chromatin, which gives the cell a swollen, spindle-shaped profile. Lysis seems to leave some recognizable cells without chromatin (figure 21).

*Phage T7, UV-irradiated (figures 22 to 25).* Remarkably enough, cells infected with irradiated phage T7 ( $10^{-4}$  survival), after the early changes in chromatin shape, become filled with chromatin, like T1-infected cells, without any sign of the central clumping produced by active T7.

*Spectrophotometric tests.* The filling of cells with chromatin after infection with inactive T1 or T7, whereas inactive T2 causes chromatin disappearance, suggested an increase in DNA content of the cells in the former cases. Confirmation of the nucleic acid nature of the stainable material was sought by following in a Beckman spectrophotometer the absorption spectrum of cells after infection with phage T1 in a synthetic medium.<sup>2</sup> This technique has been proposed by Racker and Adams (1947).

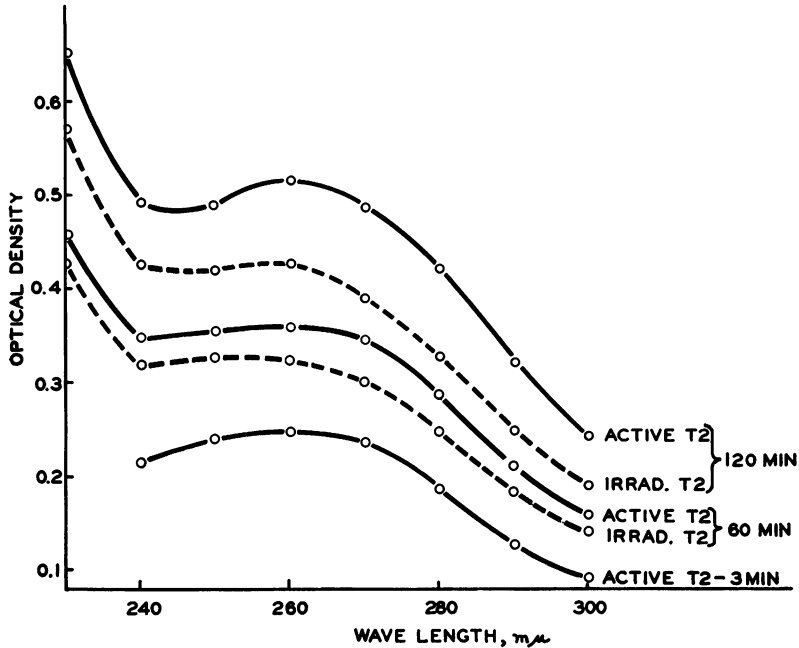


Figure 27. The optical density of two cultures of *E. coli* B infected, one with active and the other with irradiated T2, as a function of the wave length. The figures at the right end of each curve give the time in minutes after infection. Cells infected with active or irradiated T2 had the same optical density 3 minutes after infection. No change in turbidity took place during the experiment. The values for the optical density are not corrected for turbidity and should not be compared with those given in figure 26.

Bacteria in the logarithmic growth phase were placed in the quartz cell of the spectrophotometer kept at 26 C. The suspensions were stirred at 2-minute intervals, and absorption measurements at wave lengths 2,600 and 4,200 A were made every 2 minutes. In some cases, the absorption spectrum between 2,200 and 3,000 A was measured at 100-A intervals every 10 minutes. Measurements at 4,200 A were used as a test for turbidity. After a few minutes without an increase immediately following infection, the absorption coefficient at wave length 2,600 A increased linearly with time. Turbidity remained constant up to

<sup>2</sup> Medium M-9: KH<sub>2</sub>PO<sub>4</sub>, 3.0 g; Na<sub>2</sub>HPO<sub>4</sub>, 6.0 g; NH<sub>4</sub>Cl, 1.0 g; MgSO<sub>4</sub>, 0.1 g; NaCl, 0.5 g; glucose, 4.0 g; water, 1,000 ml.

the moment of lysis for active phage, and for several hours for inactive phage. The linear increase was about the same for active and irradiated phage T1, when correction was made for the change in turbidity upon lysis produced by the active phage (figure 26).

A difficulty in interpreting this result as a confirmation of the nucleic acid nature of the stainable material accumulating in cells infected with inactive T1 arises from the fact that after infection with inactive phage T2 also there is a noticeable rise in UV absorption (figures 26 and 27), although, according to Cohen (1949), no DNA synthesis takes place. It is possible that part of the increased UV absorption may be due to an increase either in ribonucleic acid or in purines and pyrimidines without nucleotide synthesis. Chemical analyses should throw more light on this question.

*Experiments with disrupted cells.* It seemed possible that irradiated phages T1 and T7, which cause chromatin increase, produced some intracellular active phage, which failed to be liberated because of the absence of lysis. We attempted to reveal such intracellular phage by sonic disruption.

We first repeated and confirmed Doermann's experiments (1948) on premature lysis of bacteria infected with phage T4, using the [KCN + T6] technique, and of bacteria infected with phage T7 (instead of Doermann's T3), using sonic vibration after the removal of free phage by differential centrifugation at 5 C. Premature lysis late in the latent period liberated active phage in amounts very similar to those found by Doermann.

Next we looked for the release of phage activity upon sonic disruption of bacteria infected with irradiated T1 or T7. Phage T1 is 50 per cent inactivated by 5 minutes of sonic treatment, and the values were corrected accordingly. In all cases, the increases in phage titer upon sonic disruption of cells infected with inactive phages only corresponded to a small fraction of the phage yield from the few bacteria infected with the phage particles that had survived irradiation. If, upon disruption, as few as 2 per cent of the bacteria infected with irradiated phage had liberated even one active phage per bacterium, our experiments could have detected it. It seems likely, therefore, that no active phage is obtainable from such bacteria, in spite of the increase in chromatin.

#### DISCUSSION

Our cytological observations confirm the remarkable specificity of the structural changes that follow infection of a given bacterial strain with different phages. Any interpretation of our observations is limited by the limited knowledge of the properties and organization of the nuclear apparatus of bacteria. Within the limits in which it is not marred by artifacts, the cytological evidence suggests, particularly in the case of T7, that phage production is a more spatially organized process than, for example, the growth of bacteria in a test tube. This only focuses once more the attention of the phage worker on the phage-infected cell as an integrated whole, structurally as well as metabolically. It seems well established (Cohen, 1949) that cells of *E. coli* B, infected with active phages of the T-even group, contain and utilize more or less unchanged the enzymatic



machinery of the bacterium, but fail to synthesize new specific bacterial components, such as adaptive enzymes (Monod and Wollman, 1947). All detectable syntheses seem directed toward phage production. The cytological picture offers for this situation a formal explanation, which is in agreement with current theories of gene action. If we assume that chromatinic bodies are homologous with the cell nucleus, the quick disruption of the nuclear apparatus of the bacterium by the T-even phages suggests that the bacterial genes are replaced by the phage genes as the directive agents for the specificity of the proteins formed thereafter. It is of interest that inactive T-even phages, which also suppress bacterial growth (Luria and Delbrück, 1942) and enzymatic adaptation (Luria and Gunsalus, unpublished), also disrupt the nuclear apparatus of bacteria. These inactive phages, which block, among other things, the synthesis of new enzymes, without affecting those already present, may offer an interesting tool for enzymatic studies. Since cells infected with phage T1 do not show clear signs of nuclear disruption, it should be interesting to investigate what syntheses take place in such cells.

The relative rapidity at which nuclear changes take place upon infection with T2, T7, and T1 is paralleled by, and may causally be related to, the relative "interfering" power of these phages, T2 being a stronger competitor than T7, and T7 than T1. Bacteria infected with inactive phage—unless reactivation takes place—fail to produce any active phage recognizable by cell disruption. Some syntheses probably go on, however, as shown for phages T1 and T7 by the increase in chromatin, for phage T2 by the increase in UV absorption. It seems unjustified to speculate at the present time on the relation of these syntheses to the activity of undamaged portions of the UV-inactivated particles.

#### SUMMARY

Cytological observation of *Escherichia coli* stained for chromatin during bacteriophage infection reveals changes specific for different phages. Disruption of the chromatinic bodies of normal bacteria by phages of the T-even group is followed by swelling of the cells and filling up with granular chromatin (phage nucleoprotein?) if the phage is active, by fading away of the chromatin if the phage is inactivated by ultraviolet light. Other phages, when inactivated, still cause an accumulation of chromatin. No active phage could be obtained by disrupting bacteria infected with inactive phage.

#### REFERENCES

- BEUMER, J., AND QUERSIN, L. 1947 Etude microscopique de la lyse bactériophagique par coloration des noyaux bactériens. *Compt. rend. soc. biol.*, **141**, 1280-1282.
- BOYD, J. S. K. 1949 Development of bacteriophage in *Escherichia coli* B. *Nature*, **164**, 874-875.
- COHEN, S. S. 1949 Growth requirements of bacterial viruses. *Bact. Revs.*, **13**, 1-24.
- DELAMATER, E. D. 1948 Basic fuchsin as a nuclear stain. *Stain Tech.*, **23**, 161-176.
- DELBRÜCK, M. 1946 Bacterial viruses or bacteriophages. *Biol. Rev. Cambridge Phil. Soc.*, **21**, 30-40.
- DOERMANN, A. H. Intracellular growth of bacteriophage. *Carnegie Inst. Wash., Year-book No. 47*, 176-182.

- EHRlich, H. L., AND WATSON, D. W. 1949 Lysogenesis of *Bacillus megatherium*. *J. Bact.*, **58**, 627-632.
- LURIA, S. E. 1947 Reactivation of irradiated bacteriophage by transfer of self-reproducing units. *Proc. Natl. Acad. Sci. U. S.*, **33**, 253-264.
- LURIA, S. E., AND DELBRÜCK, M. 1942 Interference between bacterial viruses. II. Interference between inactivated bacterial virus and active virus of the same strain and of a different strain. *Arch. Biochem.*, **1**, 207-218.
- LURIA, S. E., DELBRÜCK, M., AND ANDERSON, T. F. 1943 Electron microscope studies of bacterial viruses. *J. Bact.*, **46**, 57-77.
- LURIA, S. E., AND DULBECCO, R. 1949 Genetic recombinations leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles. *Genetics*, **34**, 93-135.
- LURIA, S. E., AND PALMER, J. L. 1946 Cytological studies of bacteria and of bacteriophage growth. *Carnegie Inst. Wash., Yearbook No. 45*, 153-156.
- MONOD, J., AND WOLLMAN, E. 1947 L'inhibition de la croissance et de l'adaptation enzymatique chez les bactéries infectées par le bactériophage. *Ann. inst. Pasteur*, **73**, 937-956.
- RACKER, E., AND ADAMS, M. H. 1947 Annual report of the Long Island Biological Laboratory.
- ROBINOW, C. F. 1944 Cytological observations on *Bact. coli*, *Proteus vulgaris* and various aerobic spore-forming bacteria, with special reference to the nuclear structures. *J. Hyg.*, **43**, 413-423.