# AN ELECTRON MICROSCOPE STUDY OF λ BACTERIOPHAGE DEVELOPMENT IN CELLS OF THE INDICATOR STRAIN

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There are many unresolved questions related to the intracellular development of phages in lysogenic bacteria (after induction) and in the cells of various indicator strains. In particular, it is unknown what differences occur in the developmental dynamics of phages within lysogenic cells following induction with different actions, or in what way development is changed when radioprotective substances are present in the medium. It is also unclear whether or not the character of the indicator strains influences the dynamics of intracellular development of moderate phages, since it is known that the type of indicator strain changes other properties of these phages in a substantial manner.

The purpose of this work was to study the ultrastructure characteristics of development for the λ bacteriophage in cells of the indicator strain, E. coli C-85.

## EXPERIMENTAL METHOD

As the subjects of the investigation, we used λ phage and 8-hour cultures of the indicator strain, E. coli C-85. The electron microscope was used to study sections of bacteria, prepared 5, 10, 20, 40, 55, 65, and 120 min after their infection with phage (infection plurality 1:4), and their subsequent cultivation in meat-peptone bouillon at 37°. In addition, we investigated sections of normal cells. Fixation and imbedding of the bacteria in methacrylate was carried out according to the schema described earlier [1,3].

Ultrathin sections were obtained with the aid of the LKV ultramicrotome, and also by the use of glass and diamond knives.

The sections were collected on disks of copper mesh, coated with a Formvar coating. Inspection and photographing of the preparations was carried out on the UÉMB-100 electron microscope.

## EXPERIMENTAL RESULTS

Preliminary study of the ultrathin sections of non-infected bacteria from the indicator strain, E. coli C-85, showed that the structure of these sections was characteristic of that seen in sections of normally functioning bacterial cells. There were also no changes or supplementary structures in the sections of bacteria fixed immediately after, or 5 min after, the addition of phage to the culture.

Supplementary structures did appear in the later intervals of fixation of the infected cells.

In the ultrathin sections of the bacteria, 10 min after the addition of phage, we observed electron-optically dense osmiophilic formations, which, as noted above, were not observed in analyzing the normal bacterial cells. These structures were quite varied in form and topography, and their quantity in the sections was unequal. Basically, they appeared in the cytoplasm, nearer to the cytoplasmic membrane than to the nuclear apparatus. These formations were distributed either singly along the surface of the section, or in small groups. We did not observe any differences in the cell walls and cytoplasmic membranes, as compared with the analogous components in normal cells, in any of the sections investigated. There were also no changes noted on the part of the cytoplasm or the nuclear apparatus.

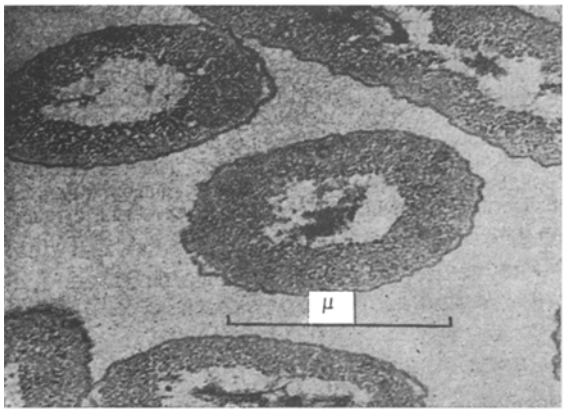


Fig. 1. Ultrathin sections of the bacteria, prepared 20 min after infection with phage. Magnification 33,000 ×.

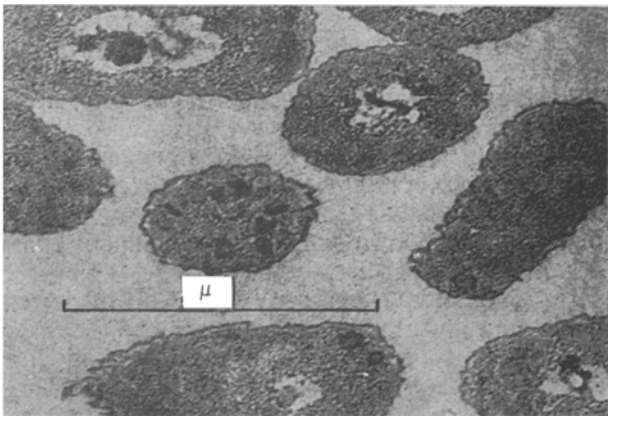


Fig. 2. Ultrathin sections of the bacteria, prepared 40 min after infection with phage. Magnification 46,000 ×.

Twenty minutes after addition of the phage to the bacteria, we observed the osmiophilic formations in their sections in greater number, and their dimensions were also increased. The development of these oval, osmiophilic formations, which presented as synthesizing phage particles, was not reflected on the structure of any of the cell components (Fig. 1).

Investigating the ultrathin sections of the bacteria 40 min after infection with phage, we noted further increase in the number of phage particles per section. Basically, they were distributed in the cytoplasm of the bacteria, singly or in groups (Fig. 2).

Marked changes in the sections were noted when we studied the preparations prepared 55 min after infection of the bacteria (Fig. 3). A large number of the cells were converted into round, ball-like formations, or formations of

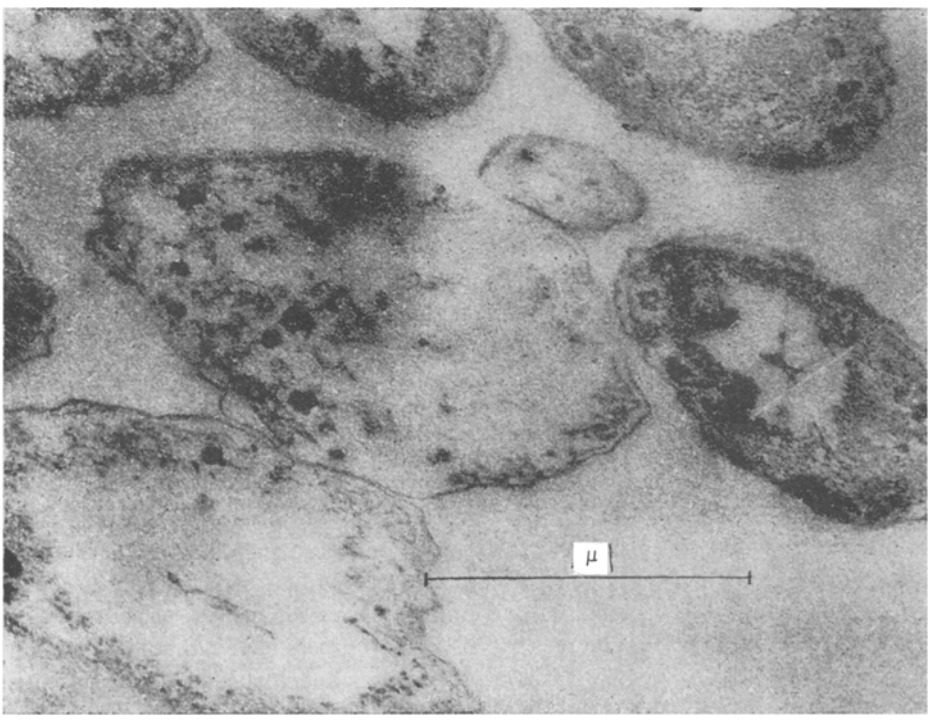


Fig. 3. Ultrathin sections of the bacteria, prepared 55 min after infection with phage. Magnification 48,000 ×.

indefinite form. In the sections of these cells we now observed partial or complete disintegration of the nuclear apparatus, while the developed phage particles appeared in the form of oval, osmiophilic formations, surrounded by extremely fine, concentric structures. The number of phage particles reached 25 or more per section. This period corresponded to the end of the latent period in the development of the phage particles.

Analysis of the ultrathin sections of bacteria prepared 65 and 120 min after initiation of the infection showed the same pictures as were noted when we studied the preparations prepared after 55 min.

Thus, studying the ultrathin sections of bacteria from the indicator strain, E. coli C-85, fixed at a varying interval after the addition of λ phage and subsequent cultivation at 37° in meat-peptone bouillon, made it possible to trace the basic stages in the intracellular development of a moderate phage. According to the results obtained, the first osmiophilic formations, varying in density and form, are observed in the preparations 10 min after the addition of the phage. On comparing these results with the data on the intracellular development of virulent phages [2,4], it is easy to see that the times of appearance of the first phage structures are the same in both cases. Differences are observed only in the structure of the nuclear apparatus in connection with the development of the phage particles. While, with development of the virulent phages, changes in the structure of the cell components begin with disruption of the nuclear apparatus, in the case of moderate phages the nuclear apparatus does not undergo visible changes up until the end of the latent period, and the phage particles develop, for the most part, in the cytoplasm. Changes in the structure of the nuclear apparatus, noted in the sections of the ball-like cells, are not related to development of the moderate phages, but are apparently the result of changes in the form of the cells, precursing their lysis.

Differences in the intracellularly developing virulent and moderate phages, pertaining to the nuclear apparatus of the bacterial cell, appear to be a reflection of differences in the biological properties of these phages. Finally, our results were obtained from investigating the indicator strain of the intestinal bacillus, belonging to the strains of Group C. Comparing them with the data on the development of λ phage in indicator strain cells of intestinal bacillus belonging to the K-12 group of strains [5], one finds no essential differences caused by using the different strains.

More complete judgements on the dynamics of intracellular development of the moderate phages, and the role of the nuclear structures in this case, can be advanced after studying lysogenic cells induced by one of the actions that leads to disruption of the equilibrium in the prophage-bacteria system.

## SUMMARY

The work deals with intracellular development of the lambda bacteriophage in the cells of the indicator strain, E. coli C. As demonstrated, bacteriophages appeared in the cells 10 min after the infection of the latter. The sizes, the density, and the number of bacteriophages depended on the period of development. No destruction of the nuclear apparatus was evident during the process of moderate bacteriophage development.

## LITERATURE CITED

1. B. A. Mishchenko, Byull. éksper. biol., 8, 63 (1962).
2. A. P. Pekhov, Byull. éksper. biol., 12, 75 (1959).
3. A. P. Pekhov, Electron Microscope Investigation of Bacteria and Phages [in Russian], Moscow (1962).
4. E. Kellenberger, A. Ryter, and J. Séchaud, J. biophys. biochem. Cytol., 4, 671 (1958).
5. J. Séchaud, Arch. siences, 13, 427 (1960).

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**

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