THE INTRACELLULAR DEVELOPMENT OF TEMPERATE PHAGES

REPORT 1. THE STUDY OF THE ULTRASTRUCTURE OF LYSOGENIC BACTERIA

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The result of the interaction between biological properties of phages and bacteria will differ in accordance with their nature. In addition to processes of destruction of bacteria and of liberation of the newly formed phage particles into the medium, in some types of interaction the phages do not destroy the bacteria, but merely lysogenize them. Lysogenized bacteria retain the ability to produce phage for an indefinite period, not differing in this respect from nonlysogenized bacteria. Phages which do not cause lysogenization of bacteria but actually destroy them have been called "virulent," and typical examples of these are the phages of the T-group. Phages with the property of lysogenizing bacteria have been called "temperate" phages.

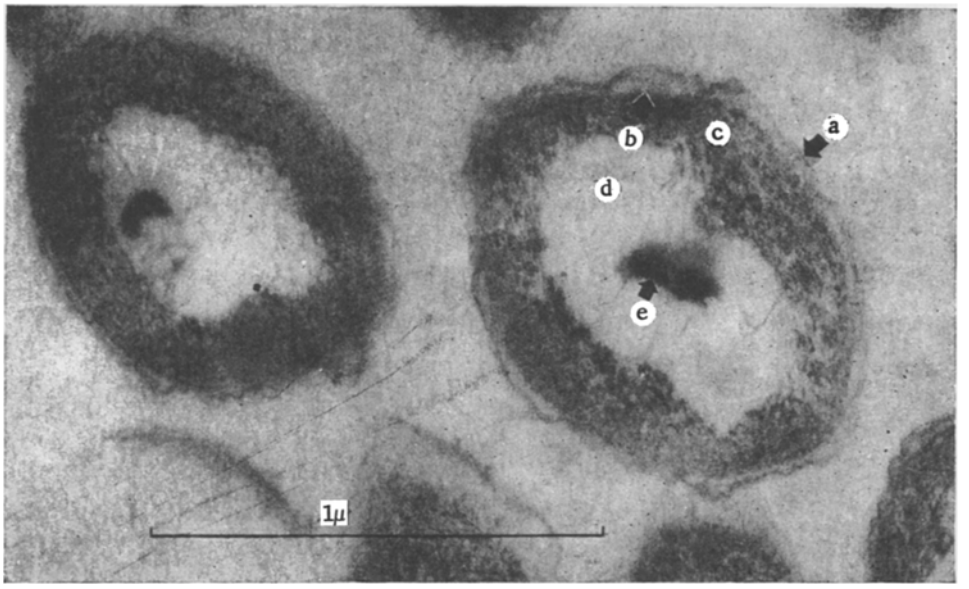

Fig. 1. Transverse sections of cells of E. coli K-12 (λ). a) Cell wall; b) cytoplasmic membrane; c) finely granular cytoplasm; d) nuclear vacuole; e) chromatin band. Magnification 80,000 ×.

The direct study of the intracellular development of phages has become possible with the introduction of electron microscopy and the technique of ultrathin sections into research practice, making it possible to identify the details of the fine structure of bacteria [2,4,7]. These methods have been used to study the intracellular development of virulent phages and their fine structure [1,3,5,6]. The results of these studies prompted the investigation of the intracellular development of the temperate phages.

When contemplating the study of the submicroscopic features of the intracellular development of the temperate phages, we thought it desirable at first to investigate the ultrastructure of the lysogenic bacteria. We needed to know whether the cells of the lysogenic bacteria possess any special features not found in the cells of nonlysogenic bacteria. The problem was tackled in this way because lysogenic bacteria spontaneously produce a few phage particles.

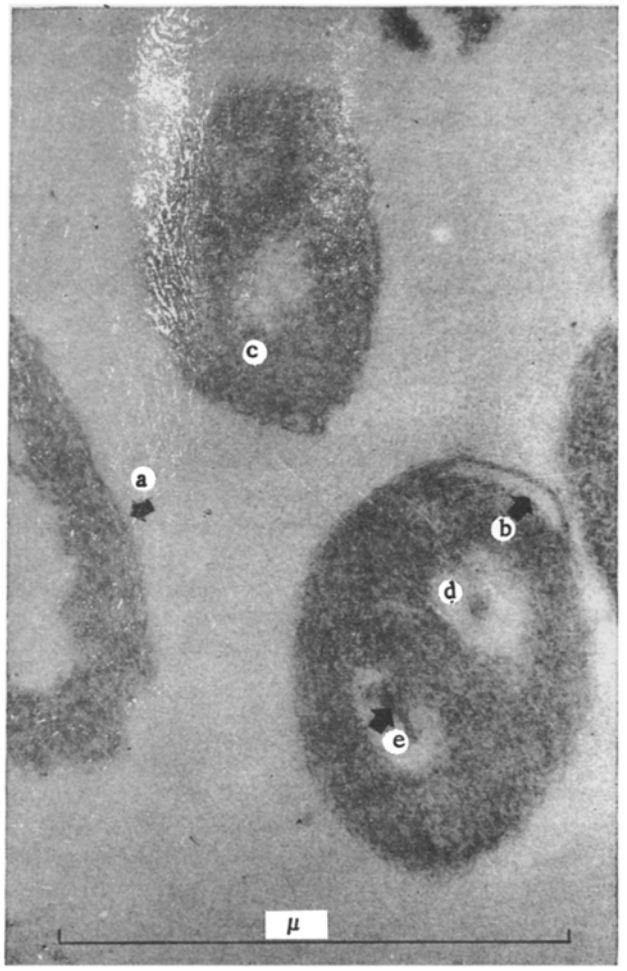

Fig. 2. Oblique sections of cells of E. coli C-27. a-e) As in Fig. 1. Magnification 90,000 ×.

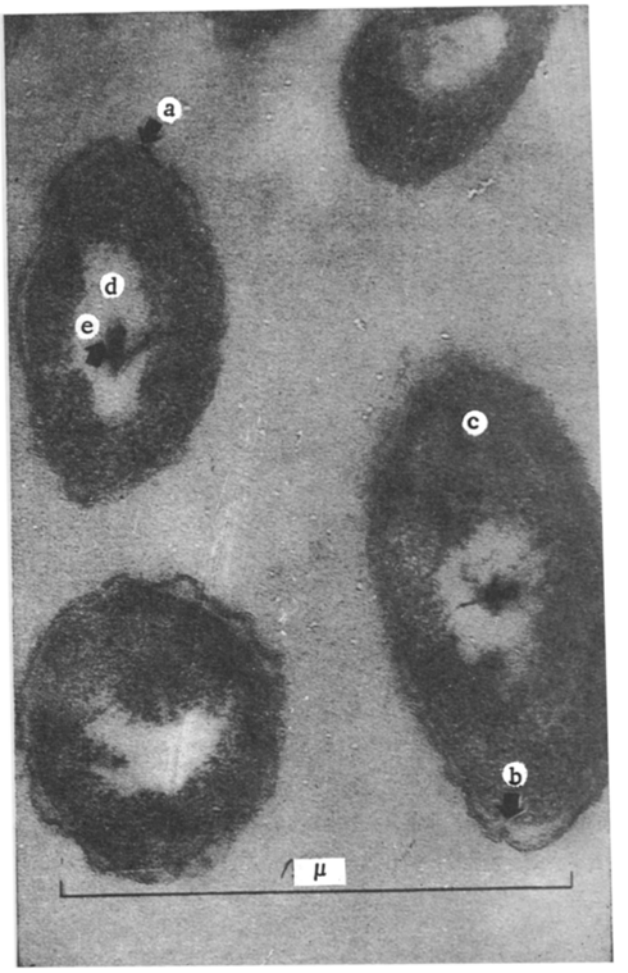

Fig. 3. Transverse sections of cells of E. coli PA-678. a-e) As in Fig. 1. Magnification 90,000 ×.

In this report we describe the results of an electron-microscopic study of ultrathin sections of the cells of all the best-known strains of lysogenic bacteria of the coliform group.

EXPERIMENTAL METHOD

The material used in the investigation consisted of ordinary and serial ultrathin sections of the cells of 18-h cultures of strains of E. coli C-27, E. coli C-87, and E. coli B-15 lysogenic for phage P2, and of strains of E. coli K-12, E. coli W-3110, and E. coli C-18 lysogenic for phage λ. As nonlysogenic objects we investigated ultrathin sections of cells of 18-h cultures of E. coli B, E. coli PA-678, and E. coli HfrH.

The fixation and embedding of the bacteria in methacrylate in order to obtain ultrathin sections followed the scheme used by A. P. Pekhov [1]. This scheme enables the structures to be preserved and sections of the test objects to be obtained showing sufficient contrast.

Ultrathin sections were cut with the LKV ultramicrotome. The knives were made from mirror glass, 5 mm thick. The sections were assembled on copper grids, covered with a collodion or "formvar" film, and examined under a Soviet UEMB-100 electron microscope.

EXPERIMENTAL RESULTS

Cell wall. In all the preparations the cell wall lay uniformly in contact with the subjacent structures. In many cases, as a result of the manipulations, in some strains the cell wall was separated from the underlying structures, giving absolute proof of its differentiated character (Figs. 1-3).

Analysis of the photomicrographs of the ultrathin sections of both the lysogenic and the nonlysogenic bacterial cells showed that the cell wall consists of two principal layers. The thickness of the cell wall was 100-200 A.

Cytoplasmic membrane. This cell component was found between the cell wall and the cytoplasm, and was an osmiophilic structure, firmly and directly applied to the protoplast. In contrast to the cell wall, the cytoplasmic membrane did not consist of separate layers. A space was present between the cell wall and the cytoplasmic membrane.

Cytoplasm. In all the preparations the cytoplasm was composed of small granules. The outlines of the individual granules of which the cytoplasm was formed were so well defined that they could be measured. The average diameter of the cytoplasmic granules was about 100-200 A. The preservation of the fine, granular character of the cytoplasm facilitated the proper fixation of the bacteria.

During our investigations of many ultrathin sections we never once observed the large cytoplasmic granules which certain writers regard as equivalent to mitochondria. Nevertheless, in some sections of both lysogenic and nonlysogenic bacteria, areas were seen in the cytoplasm possessing marked osmophilia. These osmiophilic areas differed very sharply in their shape, structure, and density from the phage particles observed by the above-cited writers when studying sections of bacteria inside which T_2 phage particles developed. We are inclined to regard these osmiophilic areas of the cytoplasm as lipid inclusions (lipid drops).

Nuclear structures. The boundaries between the finely granular cytoplasm and the nuclear structures were very well defined in all the electron photomicrographs. Although the investigation was carried out with a high degree of resolution, nevertheless no membranes could be found which might have enveloped the nuclear apparatus. The nuclear apparatus of the bacteria of all the studied strains consisted of a unique type of vacuole, situated in the center of the cytoplasm, and composed of substances readily permeable to electrons, in which an electron-optically dense chromatin band was included. In the cells which were evidently sectioned in the stage of preparation for division, two vacuoles were found in each cell (Fig. 2).

The results thus show that the structure of the cytoplasmic elements of the bacteria studied was identical, and that no additional structures whatever were present in the sections of the lysogenic bacteria. This demonstrates that the lysogenic and nonlysogenic coliform bacteria are indistinguishable as regards their ultrastructure.

SUMMARY

The ultrastructure of the best-known lysogenic bacterial strains E. coli K-12 (λ), E. coli C-27, E. coli B-15, E. coli C-87, E. coli W.3110, and E. coli C-18 was studied with the aid of electron microscopy and a technique of ultrathin sections. A parallel study was made of sections of nonlysogenic strains of bacteria (E. coli B, E. coli PA-678, and E. coli HfrH.). There were no morphological differences between the lysogenic and nonlysogenic bacteria. No additional structures were revealed in the sections of lysogenic bacteria.

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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.
