THE SUBMICROSCOPIC STRUCTURE OF Pasteurella pestis EV

B. A. Mishchenko and S. I. Kharlampovich

Laboratory of Genetics of Microorganisms (Head, Doctor of Biological Sciences A. P. Pekhov), Institute of Experimental Biology (Director, Professor I. N. Maiskii) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 3, pp. 63-65, March 1963
Original article submitted June 28, 1962

As a result of the widespread use of electron microscopy in conjunction with the technique of ultrathin sections, not only can the general characteristics of bacterial cells as a whole be defined, but the submicroscopic structure of many of the bacteria, their internal organization, and the topographical features of the structures discovered within them can also be studied in detail.

We found no reference in the literature to the study of the ultrastructure of Pasteurella pestis EV, although we consider that investigations of this type would help towards solving such problems as the existence of the isolated nuclear apparatus and the discrete structures of the cytoplasm of this microorganism. Details of the fine structure of the cell walls are also of considerable interest.

In order to examine these problems we used the technique of ultrathin sections. In the present paper we describe the results of the study of the electronmicroscopic structure of P. pestis.

EXPERIMENTAL METHOD

We used an 18-hour agar culture of P. pestis EV to study the intact cells under the electron microscope. The culture was suspended in physiological saline and applied to a collodion film, after which it was dialyzed for 50 min, dried in air, and dusted with chromium. We also investigated ultrathin sections of an 18-hour culture of the same microorganisms, grown in Martin's broth at pH 7.2. The sections were cut by means of a type LKB ultratome.

The microorganisms were fixed and embedded in metacrylate in accordance with the scheme used by A. P. Pekhov [2]. The knives were made from mirror glass, 5 mm thick. The sections were fixed to a copper grid, covered with a collodion film. The intact cells and ultrathin sections were examined with a Soviet UEMB-100 electron microscope.

EXPERIMENTAL RESULTS

Before going on to make a detailed study of the submicroscopic structure of P. pestis EV, we made a general investigation of the intact cells of the particular strain used. The results of these observations showed that the length of the bacterial cells was 1.5-4.5 μ and their thickness 0.6-1 μ. The mean dimensions of the microorganisms were 2.4 × 0.7 μ.

Morphologically, the bacterial cell was shaped like a rod with rounded ends and slightly convex sides (Fig. 1). At one pole, or more frequently at both opposite poles, electron-optically dense cylindrical, dome-shaped, or spherical structures were observed. The center of the cell contained cytoplasm with a nodular relief. The cell wall, forming a dense zone around the bacterial cell, had the appearance of a continuous band with an uneven surface.

The most complete picture of the structure of P. pestis EV was obtained by means of ultrathin sections.

The cell wall. The investigation of a large number of sections showed that the cell wall lies evenly in contact with the subjacent structures throughout its length. In individual cases, as a result of manipulations during the preparation of the ultrathin sections the detachment of the wall from the subjacent structures could be observed in limited areas or throughout its length, and the wall presented a wrinkled appearance (Figs. 2,a and 3,a) Analysis of high-resolution photographs showed that the cell wall consists of two principal layers. Measurements showed that the average thickness of the cell wall is about 100 A, of which 30 A is accounted for by the outer layer, 30 A by the layer facing the cytoplasmic membrane, and 40 A by the interstitial space.

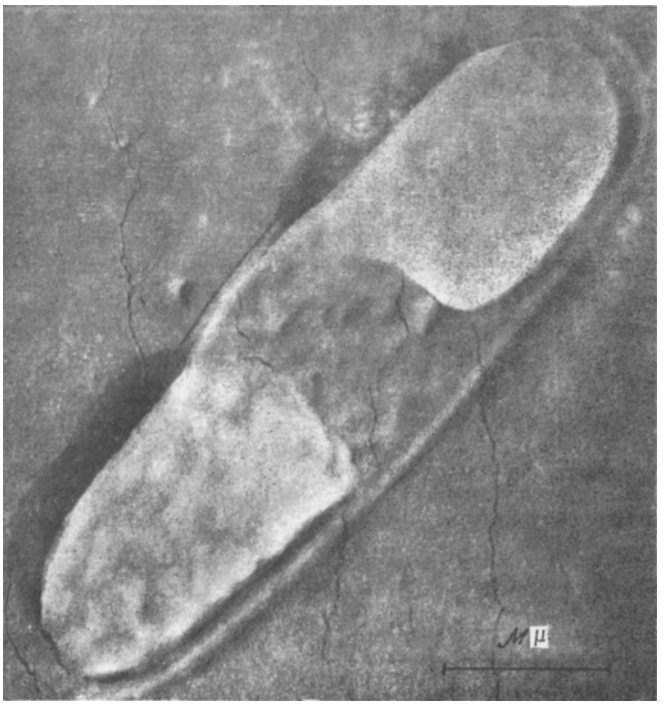

Fig. 1. Intact cells of P. pestis EV. Dusted with chromium. Magnification 35,000×.

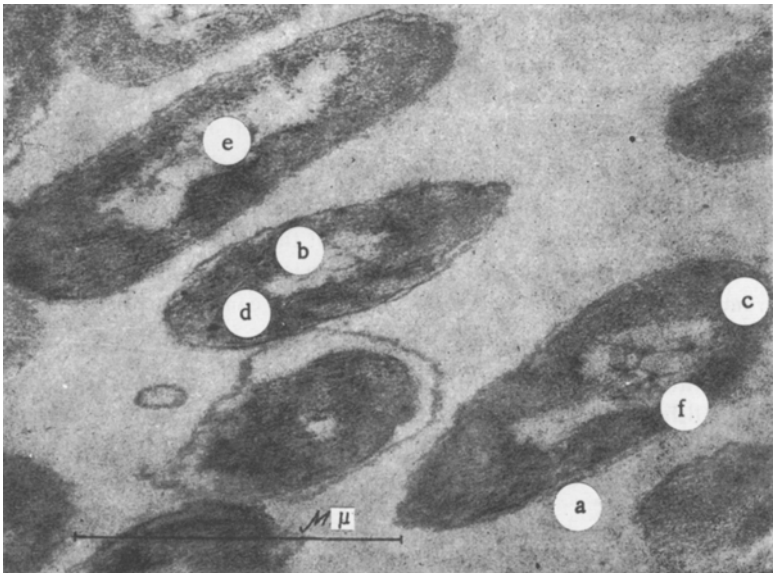

Fig. 2. Longitudinal sections of cells of P. pestis EV. a) cell wall; b) cytoplasmic membrane; c) finely granular cytoplasm; d) granules of nutrient substances; e) nuclear vacuole; f) chromatin substance. Magnification 52,000×.

The cytoplasmic membrane. Next to the cell wall, and in close contact with the protoplast, lies the cytoplasmic membrane (Figs. 2,b and 3,b), consisting of a relatively dense, osmophilic material, nonstratified in character. The average thickness of the cytoplasmic membrane is about 70 A.

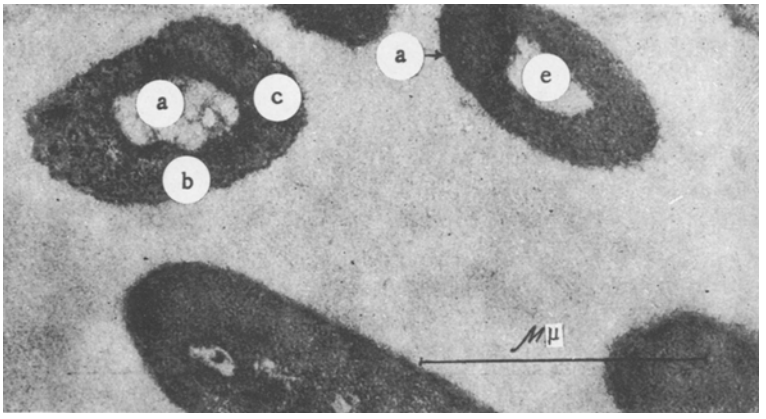

Fig. 3. Transverse sections of cells of P. pestis EV. a) Cell wall; b) cytoplasmic membrane; c) finely granular cytoplasm; d) granules of nutrient substances; e) nuclear vacuole; f) chromatin substance. Magnification 56,000×.

Cytoplasm. Investigation of large numbers of photographs showed in every case that the cytoplasm was of a finely granular structure (Figs. 2,c and 3,c). In this case the average diameter of the granules is about 100 A. In various parts of the cytoplasm there were structures with marked osmiophilia (Figs. 2,d and 3,d), which we are inclined to regard as inclusions of concentrated nutrient substances (lipoproteins, glycoproteins, etc.). In no case could could be detected any isolated areas of cytoplasm, demarcated by an osmiophilic cytoplasmic layer, the existence of which has been reported in other species of microorganisms by certain workers, who regard them as equivalent to the mitochondria [1, 5, 6, 8, 9].

Nuclear apparatus. In all the sections of the bacterial cells, in the center of the cytoplasm was situated a clearly demarcated substance, readily permeable to electrons (Figs. 2,e and 3,e). In this particular substance was incorporated the electron-optically dense chromatin substance (Figs. 2,f and 3,f), resembling an astrocyte in its shape. The presence of a clearly outlined membrane serving to contain the nuclear substance could not be demonstrated.

Hence, on the basis of electron-microscopic investigations of an 18-hour culture of a vaccine strain of P. pestis EV, it has been shown that the principal topographical structures of the bacterial anatomy are: 1) the cell wall, composed of several layers; 2) the cytoplasmic membrane, firmly surrounding the protoplast; 3) the finely granular cytoplasm with cytoplasmic granules situated atone or both poles; and 4) a central, distinct nuclear apparatus.

SUMMARY

An electronographic examination was done of the fine structure of the cell in the 18-hour culture of the P. pestis EV vaccine strain. As established, the main topographic structures of bacterial anatomy are: the cellular wall with a stratified structure; the cytoplasmic membrane, which closely embraces the protoplast; the microgranular cytoplasm with cytoplasmic granules located on one or both of the poles and a centrally located nuclear apparatus.

LITERATURE CITED

1. V. I. Biryuzova. Doklady Akad. Nauk SSSR 135, 6, 1519 (1960).
2. A. P. Pekhov. Byull. éksper. biol., 12, 75 (1959).
3. A. P. Pekhov. Vestn. Akad. Med. Nauk SSSR, 5, 43 (1960).
4. J. R. G. Bradfield. In: E. T. C. Spooner, B. A. D. Stocker (Ed). Bacterial Anatomy. Cambridge, 1956, p. 296.
5. G. Drews, Arch. Mikrobiol., 1960, Bd. 35, S. 53.
6. W. Niklowitz, Zbl. Bakt., I. Abt. Orig., 1958, Bd. 173, S. 12.
7. M. R. J. Salton, Bact. Rev., 1961, v. 25, p. 77.
8. C. Shinohara, J. Suzuki, K. Fukushi, et al., Electron Microscopy [Russian Translation], 1959, v. 7, N 2-3, p. 63.
9. K. Tokuyasu and E. Yamada, J. biophys. biochem. Cytol., 1959, v. 5, p. 123.