

GROWTH AND DEVELOPMENT OF BACTERIA IN CONNECTION WITH THE PHENOMENON OF BACTERIOPHAGY

COMMUNICATION 2

ELECTRON MICROSCOPE STUDY OF PHAGE FILTRATES OF MICROBES OF THE INTESTINAL GROUP

A. P. Pekhov

From the Institute of Experimental Biology (Professor I. N. Maisky, Director) AMS USSR, Moscow)

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N. N. Zhukov-Verezhnikov)

The interest in electron microscopic study of phage lysates and phage filtrates of bacteria has been established by very substantial circumstances.

As shown by the investigations of Raettig [4] and others, phage lysis of bacteria in a number of cases can take place without the formation of phage. With this, various formed elements of bacterial origin are found in the phage lysate. Raettig [3, 4], for example, described small formed elements, along with phage particles, measuring 12 m μ , which he named lysomes and which he thinks are formed from cellular membranes.

The data presented are significant not only in the study of the biology of bacteriophages, inasmuch as the majority of the authors indicated believe that phage particles are built of these elements, but are also extremely important for developing some idea of the morphology of filterable forms produced during phage lysis of bacteria, especially since this has hardly been studied.

Assuming that the accumulation of new experimental materials is beneficial in the one, as well as in the other instance, we conducted electron microscopic investigations of phage filtrates of microbes of the intestinal group, the results of which are the subject of the present communication.

EXPERIMENTAL METHODS

Phage lysates of strains 1321 and 1322 of Bact. coli aerogenes were filtered through asbestos filters.

Preparations for electron microscopy were prepared by placing small drops of phage filtrate on collodion films. Salt crystals were removed by dialysis for 2-2½ hours at room temperature. Finished preparations were shadowed with chromium to increase contrast.

EXPERIMENTAL RESULTS

Ten phage filtrates, checked for sterility by inoculation into meat-peptone broth and meat-peptone agar with subsequent incubation at 36° for 10 days, were subjected to electron microscopic investigation.

Despite the fact that the quantity of phage particles in the filtrate was relatively large (10^8), certain other morphological elements rather than phage particles predominated in the samples of filtrates studied.

Flat, oval elements, provisionally named "small plates," were principally encountered in the field of vision. In many instances, the "small plates" appeared as formations of bulging horse-shoe-like or irregular form, differing among themselves in size. Occasionally, well-defined outlines of fully disrupted cells were encountered in the field of vision, and also electron-dense formations, approximating phage heads in their dimensions (Fig. 1).



Fig. 1. "Small plates" and residue of lysed cell. Magnification $\times 36,500$.

In some preparations, accumulations of essentially homogeneous oval formations, also resembling phage heads in form, were found. The presence of long shadows behind these formations indicates their considerable depth of volume (Fig. 2).

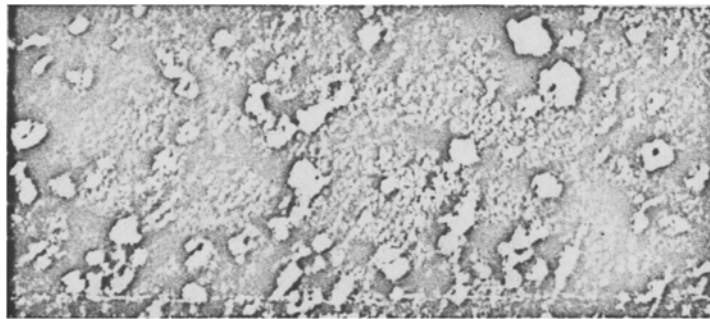


Fig. 2. Oval formations resembling phage heads in form. Magnification $\times 35,000$.

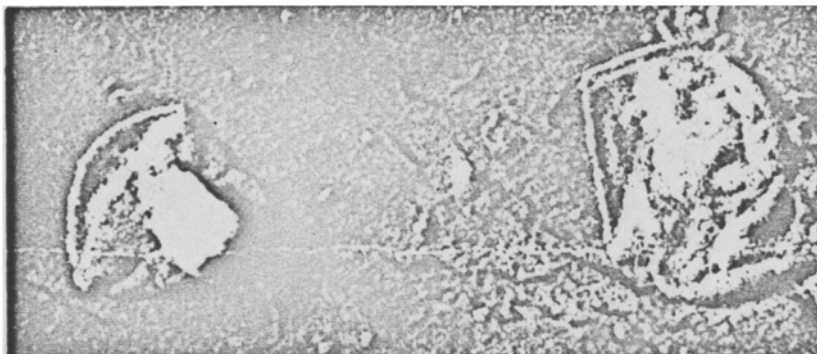


Fig. 3. Parts of cells with dense granules. Magnification $\times 37,800$.

In many of the preparations, the presence of larger elements, representing halves of microbial cells, was noted; some of these had dense granules (Fig. 3). We observed similar granules, which are designated as mitochondria by many authors, in earlier work in the process of vital studies of phage lysis of *Bact. coli* cells [1].



Fig. 4. Disrupted cell with flagellum. Magnification $\times 36,000$.

In several cases, disrupted bacteria with a partially preserved flagellar apparatus were found (Fig. 4). Fragments of flagella in the form of short segments or very long sections occupying almost the entire field of vision were also found in other preparations. Finally, in single filtrates were seen little-damaged bacterial cells with clearly distinguishable internal structure but devoid of flagella (Fig. 5) and, consequently, differing somewhat in their outward appearance from normal cells of the colon bacillus.



Fig. 5. Little-damaged cell without flagella. Magnification $\times 40,500$.

The discovery of microbial cells in the filtrates has a direct relationship to the method of filtration and the selection of filters. As is well known, in the 1930's, Chamberlain L₃ candles were principally used for filtration, excluding the passage of bacteria. In recent years, asbestos filters are chiefly used. For this reason, the results of our experiments, showing that bacteria can still be found in the filtrates in separate cases, must be taken into account in evaluating these filtrates with regard to the subject of the appearance in them of filterable forms. In any case, the assurance that the isolated secondary cultures develop from filterable forms, rather than from cells passing into the filtrate obtained with the aid of an asbestos SF filter, must involve the prior assumption of granular or some similar elements before cells are formed.

SUMMARY

The results of electronic microscopic study is presented of phage filtrates (tested for sterility) of 2 strains of *Bact. coli aerogenes*. Various elements of bacterial origin, different in size and form, as well as half cells with granules and intact microbe cells with or without partially or completely preserved flagellar antigen were revealed.

It is emphasized that one has to be very careful in working with the filterable forms of microbes since the cells may sometimes pass through asbestos filters "S. F." and be found in the filtrate.

LITERATURE CITED

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* Original Russian pagination. See C.B. Translation.