THE EFFECT OF BACTERIOPHAGIA ON THE GROWTH AND DEVELOPMENT OF BACTERIA

COMMUNICATION 1. ON CYTOPLASTMIC GRANULES OBSERVED IN BACTERIA DURING PHAGOCYTOSIS

A. P. Pekhov

From the Institute of Experimental Biology (Director - Prof. L. N. Malsky), AMS, USSR Moscow.

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Recently, much attention has been given to the study of the morphological organization of bacteria, and to their granular, cytoplasmic elements particularly. This question is especially interesting in connection with the morphological changes occurring in bacterial cells due to bacteriophagic action.

In this article, we present the results of observations concerned with the peculiarities of the cytoplasmic granules appearing in the course of bacteriophagic lysis.

EXPERIMENTAL METHODS

Bacteriophagic lysis was observed on typical cells of a Bact. coli aerogenes culture, strain 1321.

To observe what happened to individual microbes attacked by the phage and put in conditions favorable to development, we used the method of phase-difference microscopy during the life of the cell; the preparations used in this method were prepared by the method described by V. L. Troitsky and Z. G. Pershina [4].

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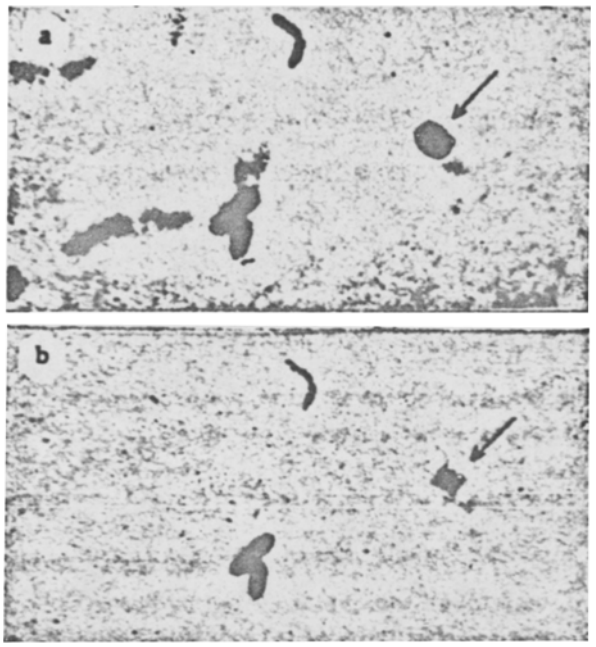
To observe what happened to individual microbes attacked by the phage and put in conditions favorable to development, we used the method of phase-contrast microscopy during the life of the cell; the preparations used in this method were prepared by the method described by V. L. Troitsky and Z. G. Pershina [4].

We used suspensions of Bact. coli aerogenes in a specific bacteriophage (titer from 10-6 to 10-7 according to Appelman), with a concentration of 1 million microbes per ml, as source material for the preparations. Instead of a heating process, we used a special incubator to maintain a stable temperature of 36°; the phase-contrast microscope with the preparation was placed in this incubator during the experiments. After finding corresponding fields of vision, the bacteria cells contained in them were designated by numbers on a chart made to scale on the field of vision in order to facilitate observation. This way of computing the results made it possible to observe several cells simultaneously, for, in most cases, the cells were lysed rather quickly.

EXPERIMENTAL RESULTS

Twelve experiments were conducted, in which we observed 183 microbes during bacteriophagic lysis. One hundred and fifty-nine of these lysed at intervals of 40 minutes–6 hours after observation began; rapid lysis was preceded by the formation of spherical, fusiform and other forms. Four cells lysed by means of a slow and even hyalinization of the protoplasm, until the original contours of the cells were lost, which occurred 8-20 hours after the experiment began. In this article, we shall not give a detailed description of the lyses and the preceding morphological changes; we shall only note that there were no cells in this group with any discernable structural differentiation in the protoplasm which might lead to the formation of granules. When the remaining 20 of the phage-attacked cells were examined, granules were found which were characterized by the following features. In 12 of these 20 cells, the appearance of the granules was connected with the hyalinization of the

protoplasm, which continued for 2-20 hours after the preparation was placed in the incubator. In some cases, the size of the bacteria did not change, and, by the end of the experiment, these cells were characterized by light protoplasm and granules. In other cases, the cells lost their contours completely due to the continuing, "thaw-like" hyalinization of the protoplasm, but the contours were replaced by isolated granules. The amount and distribution of the granules varied greatly. In 3 cells, there was 1 granule in the center or on one of the poles; in 8 cells, there was 1 granule on each of the poles; and, finally, there was 1 cell with 6 cytoplasmic granules, two on the poles, and four in different parts of the protoplasm. In the phase-contrast microscope, the granules looked like dense, rounded formation equal to or slightly smaller than the bacteria in diameter.



Zeitraffer microfilm showing phagocytosis of bacteria. Phase-contrast.
a) spherical form (arrow) of enteric bacillus before bacteriophagic lysis; b) granule (arrow) remaining after bacteriophagic "explosion" of enteric bacillus cell.

In some specimens, we also recorded protoplasm hyalinization and granule differentiation after a preliminary formation of gigantic forms (4 cells) which were 2-3 times as wide and 5-6 times as long as ordinary cells of this type of bacteria. And finally, in 4 cells, granule differentiation began with protoplasm hyalinization and was completed by the inflation or elongation of the specimens, followed by a lytic "explosion". After such a lytic reaction, the granules remained in the same place occupied by the cells before.

The preservation of the granules after the cell had lysed by instantaneous "explosion" was also observed when no previous granule differentiation had been seen in the cytoplasm. In this case, the cells only slightly lost their contrastability and, after forming a sphere or spindle, "burst," leaving granules behind them. This latter observation was recorded by a Zeitraffer microfilm in the AMS USSR department of microfilm scientific documentation (see Figure). The figure shows frames illustrating a bacterial cell of spherical shape before the "explosion," and the granule remaining after the lysis of the cells.

DISCUSSION OF RESULTS

One must take into account the literary data when evaluating the results we obtained.

In 1934, N. N. Zhukov-Verezhnikov and V. A. Friauf [1] established that the effect of a bacteriophage on typhoid fever bacillus causes formations to appear in the central portion of the cell, which the authors treated as typical nuclei in view of the fact that they contained peculiar figures of division. Other authors [3, 16] confirmed this observation.

However, the appearance of nuclear formations in the bacterial cells was apparently not the only manifestation of bacteriophagic influence.

In 1949, Boyd [8], conducting phase-contrast microscopy of enteric bacteria cells attacked by a bacteriophage, discovered peculiar, dense zones on the poles of the bacteria which he believed to be protoplasmic thickenings caused by the division of the phage in the central portion of the cells. Heden [13] observed similar pictures in phase-contrast and ultraviolet microscopes. In his opinion, the protuberant polar granules were accumulations of phagic corpuscles.

Later on, these granules, located at the poles or in other portions of the cytoplasm, began to be considered mitochondria. A similar opinion had existed earlier [10, 15], but was not confirmed until later by works of researchers who had ceased to indentify these elements with nucleoids (nuclei) in the form of single, but complex, organelles [6, 7].

The chemical properties of bacterial mitochondria were defined at the same time [20].

These granules have now been discovered in many bacteria and are considered to be definite cytoplasmic structural elements, which are the centers of coordinated enzymatic oxidizing-reducing reactions in the cytoplasm [5, 9, 11, 12, 17, 18, 19, 21, 23, 24, 25].

We propose that the granules which we found, like the granules found earlier in sound-treated microbes of a different type [2], are analogous to mitochondria, since there are indications that mitochondria of bacteria are more resistant to the bacteriophage, antibiotics and to a series of other influences [12, 24]. The nuclear substance evidently disappears in phagocytosis. Of course, this proposition must be experimentally confirmed. However, our data show that the resistance of the granules to the phage is of a strictly individual character. Only 20, or about 11%, of the 183 enteric bacillus cells contained the cytoplasmic granules. It is at present difficult to explain the reasons for this phenomenon. One can only suggest that it is explained by the activity of the phage corpuscles adsorbed into the surface of the cells as well as by the individual properties of the bacterial cells. The heterogenicity among the lytic reactions of the microbe and the difference degrees of heterogenization of their protoplasm in the differentiation and liberation of granules must be regarded in the same way.

From our observations and from the literary data, one can conclude that the theory regarding bacteriophagic lysis as a phenomenon ending in the complete destruction of bacterial morphological organization must be more accurately defined, since, in a definite number of cases, some cytoplasmic elements survive.

We are not concerned in this article with the fact that filtrable forms of bacteria remain capable of life after lysis, as that is a question which needs further study.

SUMMARY

In vivo dynamics of phagocytosis in 183 Bact. coli aerogenes strain 1321 were studied by phase-contrast microscopy. The formation of solid cytoplasmic granules was noted in 20 cells, and in some cases the granules could be seen after lysis of the bacterial cells. Microfilms revealed granules after lysis even in cells in which no protoplasmic differentiation was noted before "explosion." It is probable that these granules are identical with mitochondria, and possess an individual resistance to the phage.

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