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A Study of Living Microorganisms in an Electron Microscope by the Gas-Microchamber Method.

by I. G. Stoyanova and T. A. Nekrasova

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Until recently the investigation of the dynamics of morphological changes in microorganisms during their process of evoltuion has been possible only in the light microscope (1). But because some links of the indicated process remained beyond the range of the light microscope, interpretation of the observable phenomena was greatly hampered. Attempts to penetrate into the inaccessible areas of the phenomena with the aid of the electron microscope also failed to achieve the desired goal (2). The gas-microchamber method (3), which was developed as a result of a study of the interaction of electrons with biological preparations, allowed us to accomplish such conditions for observing living microorganisms in the electron microscope UEMB-100, whereupon they remain viable. As an example of the use of this method we present the results received with cells of B. mycoides.

It was observed that young cells that had not attained normal size by the time of the inspection in the electron microscope continued to grow after their placement into a fresh nutrient medium. The increase of the cells in volume occurred at the expense of the manifold growth; with this the portions of the cells that had been freshly formed were more transparent for the electrons than those formed earlier (fig 1).

It was observed that the clustering of the cells, which begins after the completion of their growth and which leads to a formation of large accumulations (which are sufficiently well studied in a light microscope), occurred as a result of an interaction that developed between the protoplasts of the cells not only when they were in contact with each other but also when they were situated at some distance from each other. A tendency to establish contacts during one of the life cycles of the bacteria was displayed in various ways, depending on the surrounding conditions. If the cells can move around freely, then the contact is established by means of simply touching. With this the protoplasts of the touching cells draw together, moving from the central position that they had occupied toward the contiguous wall (fig 2a). If the cells are situated at an angle to each other, as occurs in the accumulations that appear as clusters, then the protoplast is located along the diagonal (fig 3a). In analyzing the location of the protoplasts in the cells of a

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cluster, it is possible to establish that not in a single one of them are they separated, but are always in contact with the protoplasts of the adjacent cells. This can be easily detected in the newly formed accumulations (fig 2a), in contrast to the older and somewhat deformed ones (fig 3a) where this picture occurs less clearly. If the free movement of the cells is impeded (by drying of the MPA, or by drying the cells on a film at the period when their grouping occurs), then contact is established anyway, either by a considerable lengthening of the contacting cells (so that there are even disruptions in their exterior form, figs 2b,c), or by means of a cytoplasmic threadlike formation that originates in the protoplast of one cell and grows in length in the direction of the other until it reaches its protoplast (figs 2d, e). A swelling is formed at the point where the thread makes contact with the protoplast. Within the cell the thread has the form of a cone, the apex of which is toward the exterior. The diameter of the thread is constant along its entire length, but is altered by a skip at the line of the cellular membrane. The length of the threads reaches 20μ in isolated instances, and their diameter ranges from 0.04 to 0.1μ. The cellular contact is of a selective nature.

As a result of the cellular interaction during a certain period of their life cycle, the culture seems to consist entirely of clusters. The processes in the clusters that have formed take various courses, depending on the conditions of existence and the age of the cells. It was observed that with aging of the cells the clusters clarify around the edges, the space occupied by the protoplast is decreased and a strong granulation of the protoplast occurs. The protoplast is almost undetected in the older cells of an accumulation - the cell becomes transparent to electrons and is filled with minute particles (up to 0.01μ in diameter) and groupings of them, which are particularly well seen on the outer edge of the cell where they form a dark border. In transferring such a cluster from agar onto a film, some of the particles break away and remain on the film.

If the clusters being transferred to fresh nutrient media are no older than 72 hours (fig 4a) the development of the cells takes place in the following manner. Offshoots grow out of the protoplasts that go into the bacterial cluster. These offshoots pass freely through the cellular membrane, extend to a length of several microns and increase in diameter, reaching, in many cases, the diameter of a normal specimen (fig 2e, 4b). Inthe transparent (to electrons) portions of the offshoot it is possible to see that minute particles, 0.007-0.02μ, and conglomerations of them pass from the cell into the offshoot, whereupon a portion of them are excreted from the end of the offshoot. The offshoot gradually takes on the form of a long serpentine body that later separates from the bacterium. After separating, the serpentine form continues to live independently, splitting subsequently into bacterial cells, each of which will grow in length and split in its own turn. If the division of the serpentine form is not complete, then the freshly formed bacteria has the form of a chain that is connected by a common membrane. The substance excreted by the protoplast of the bacterial cell has various form that depends on many conditions. In certain instances it is excreted in the form of a cloudy formation whose diameter is approximately equal to that of the bacterium, or smaller, and the length reaches several tens of microns. There are a large number of minute particles detected in this formation, which are analogous to those

described above. In other instances an offshoot originates from the protoplast. This offshoot has either a form of a collapsed, very thin cover that tapers at the ends, or is uniform in diameter (fig 4b) and swellings sometimes appear on it. This event in the life of the bacteria has various modifications that are dependent on many causes, first of all on the homology of the r..e of the processes taking place in the cell.

With a transfer of an old cluster (10-15 day old) into a fresh nutrient medium (MPB) the rate of the processes occurring in the cells strongly increases so that during the first 20-40 minutes significant changes are already observed in them: the exterior contour of some areas of the cluster ceases to reflect the exterior features of the component bacteria because the mucus, the excretion of which is intensified by this, smoothes over the tortuous line of the cluster's border, which is composed by the edges of the individual cells (fig 3b). The amount of mucus fluctuates in a wide range, depending on the conditions in which the bacteria are situated. As a whole the cluster takes on a smooth, streamlined surface. Simultaneously with this process there also occurs a gradual granulation of the protoplast: first of all, 4-8 large granules with rather indistinct outlines are formedin the cell; these keep their position in the portion of the cell where they originate; later they break up into a large number of minute particles, up to 0.01μ in size, which keep separate, disengage from each other, and can move freely. When a break occurs in the membrane the inner contents of the cell pour out and the particles leave the cells.

But the process of protoplast granulation, the rupture of the membrane and the discharge of the cell's contents do not occur simultaneously in all of the cells. As a rule the membranes are destroyed first in those cells where the granulation of the protoplast has occurred earliest. It is possible to determine that the discharging substance has little viscosity by the character of the flow of the cell's inner content when the membrane is broken. There is a large quantity of minute particles found in the fluid flowing from the cell. These are similar to those which fill the offshoots. The particles are found both in the areas where the fluid flowed and in the space between the membrane and the remains of the protoplast in the cell. The minimal particle size that can be observed with the given method of investigation was 0.07 - 0.01μ. After swelling, these particles grew into young cells. These young cells were at first much smaller in size than the adult specimen. In the areas of the cluster where the bacteria had partially granulated and the minute granules retained their position in the maternal cell, young bacteria began to grow directly in the maternal cell. In the same areas of the cluster where the process of granulation had only begun there were no young growing cells. If the granules left the formation then bacteria grew from them outside of the maternal cell. As a result of this the cell, which had lost its inner content as a consequence of a rupture of the membrane, ceased to exist as an individual specimen and in its place there appeared a large quantity of minute forms - cellular regeneration occurred.

What attracts attention is that the small developing cells are not connected to the maternal cell or to each other, but grow independently. In a transfer of young cells to a fresh nutrient medium, they merely continue

their growth without exhibiting tendencies towards interaction at this stage of their development. The young cells glide around without touching each other. One should particularly note the tendency of both the minute particles that form in the maternal cell as well as the young cells that began their growth within it, to leave the maternal cell. The young cells are transparent to electrons and their internal structure is accessible to observation in the electron microscope.

Thus, the advent of the gas-microchamber method has enabled us to observe in the electron microscope, living bacterial cells, their growth, the interaction between them that causes the development of clusters, and in the clusters the formation of minute particles that transform into bacterial cells. It has also enabled us to compare the development of irradiated and normal cells.

In conclusion the authors express their gratitude to Yu. M. Kushnir for the interest he has exhibited in their work.

Footnote: The physico-technical bases of the gas-microchamber method will be described in another work.

Literature Cited

1. Imshenetskiy, A. A.: Bacterial structure, M.- L., 1940; Peshkov, M. A.: Bacterial Cytology, M.- L., 1955
2. M. von Ardenne, and H. Friedrich-Freksa: Naturwiss., 29, 523 (1941); M. von Ardenne: Zs. techn. Phys., 20, 239 (1940); Naturwiss., 29, 521 (1941); Abrams, J. M., and McBain, J.: Appl. Phys., 15, 607 (1944)
3. Stoyanova, I. G., and Mikhaylovskiy, G. A.: Biofisika, 4, 483 (1959) Stoyanova, I. G.; Nekrasova, T. A.; and Biryuzova, V. I.: D. A. N., 130, 195 (1960).

Illustrations

Fig. 1. Electron-optical image of living bacterial cells. B. mycoides in a gas microchamber, V = 65 kilovolts. a - from a 24 hour old culture; b - the same cells after being in MPB for 1 hour, 20 minutes.

Fig. 2. Electron-optical image of living bacterial cells, B. mycoides, in a gas microchamber, a - from an 144-hour culture; b - from a 50-hour culture; c - the same cells after being in MPB for 20 minutes; d - from a 48-hour culture; e - the same cells after being in MPB for 4 hours.

Fig. 3. Electron-optical image of living bacterial cells, B. mycoides, in a gas microchamber, a - from a 288-hour culture; b - the same cells after being in broth for 40 minutes.

Fig. 4. Electron-optical image of living bacterial cells, B. mycoides, in a gas microchamber, a - from a 72-hour culture; b - the same cells after being in broth for 40 minutes.