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

COMMUNICATION VII. FURTHER INVESTIGATIONS OF THE NUCLEAR STRUCTURES OF BACTERIA AND OF THE INFLUENCE OF PHAGE THEREON

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In a previous communication we gave experimental findings relating to the nuclear structures of cells of Escherichia coli aerogenes, studied by means of the electron microscope and microchemical analysis, and we put forward certain views on the general structure of the nuclear apparatus of bacteria [3].

In the present communication we describe the results of further submicroscopic investigations of the finer points of the nuclear structures and of their connection with the division of bacteria, and we also give data on the effect of phage on these structures.

EXPERIMENTAL METHODS

The investigations were conducted on ordinary and ultrafine serial sections of cells of 4-hour cultures of E. coli B., grown under aerobic conditions, and also on sections of cells of the same cultures of E. coli B, but infected with T2 phage to an infection coefficient of 1:1.3. The preparations were made 5 minutes and 2 hours after the beginning of infection, which was interrupted by cooling of the cultures. In selecting these time intervals after infecting the bacteria with phage, we were profiting from previous experience [2] that the study of lysis due to bacteriophage after successive intervals of time presents no advantages when the trend of the changes is being determined, for not all cells react in the same way to infection with phage in the course of time. The widest range of morphological changes in the E. coli cells is observed 1½–2 hours after the beginning of infection, when different morphological forms preceding lysis may be found [2].

In preparing the sections the fixation and embedding of the bacteria in methacrylate were done according to a scheme which differed slightly from that used in the previous work [3], namely:

room temperature,	0.1% OsO_4 (pH = 7.2–7.4)	– 30 minutes	(not changed)
" "	0.7% OsO_4 (pH = 7.2–7.4)	– 2 hours,	" "
cold.	70% alcohol	– overnight	" "
room temperature,	95% alcohol	– 2 hours	" "
" "	absolute alcohol	– 2 hours	" "
" "	mixture of methacrylates, catalyst, and alcohol (absolute) in eq. vols.	–30 minutes	(not changed)

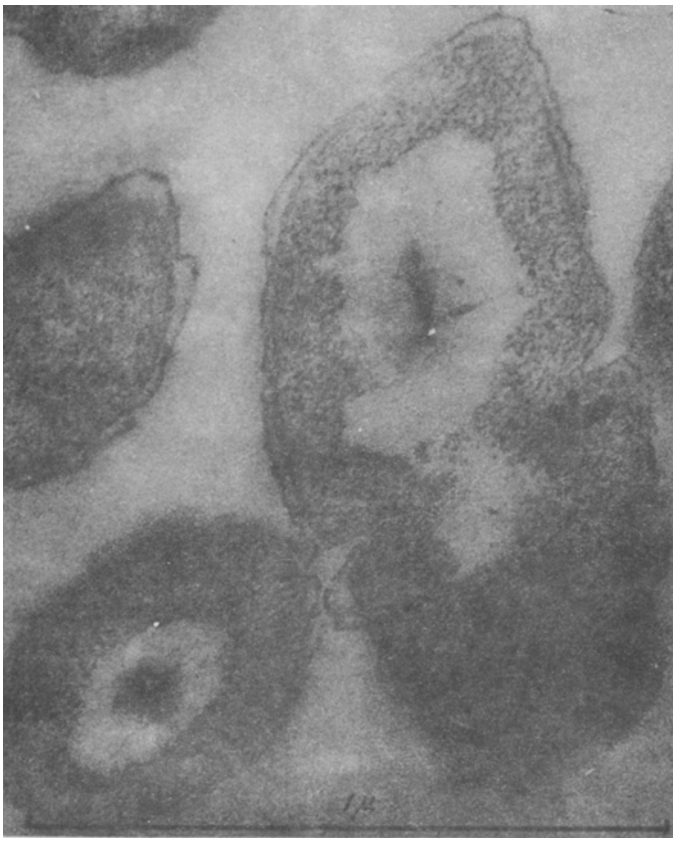

Fig. 1. Longitudinal and transverse sections of E. coli B. cells with nuclear structures. Magnification 123,000.

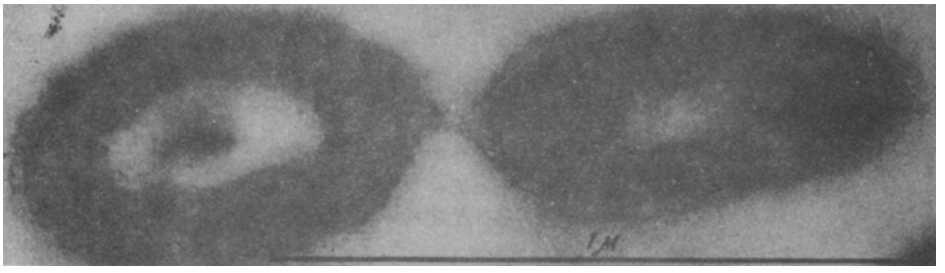

Fig. 2. Section through an almost completely divided E. coli B, cell. Magnification 123,000.

room temperature, mixture of methacrylates and catalyst – 3 hours (changed every hour)
48–48°. . . . . mixture of methacrylates and catalyst – 48 hours (in capsules).

Besides methacrylates, araldite was used for embedding of some specimens after dehydration [7] according to the following scheme:

room temperature, mixture of araldite mixture and absolute alcohol in equal volumes – 30 minutes (not changed).
48°, araldite mixture – 3 hours (changed every hour)
48°, " " – 1 hour (changed every 30 minutes)
48°, araldite mixture with catalyst – 1½ hours (changed every 30 minutes)
48°, araldite mixture with catalyst – 30 hours (in capsules).

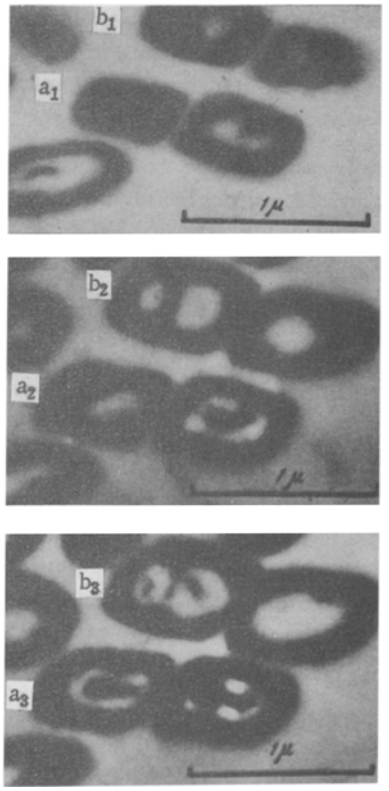

Fig. 3. Serial sections of E. coli B.cells in process of division (a, b). Magnification 36,000.

We thus had at the same time specimens embedded in methacrylates and araldite after the same methods of fixation and dehydration. The order of fixation and embedding adopted allowed the structures to be preserved and enabled sections to be obtained which were suitable for high resolution electron microscopy.

Serial sections were obtained by means of a Sjostrand ultramicrotome. The knives were of glass. Moderate and high resolution electron microscopy was undertaken .

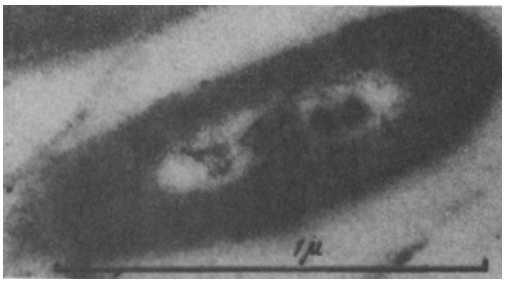

Fig. 4. Sections of E. coli B.cells and their nuclear structures, modified by the action of phage. Magnification 81,700.

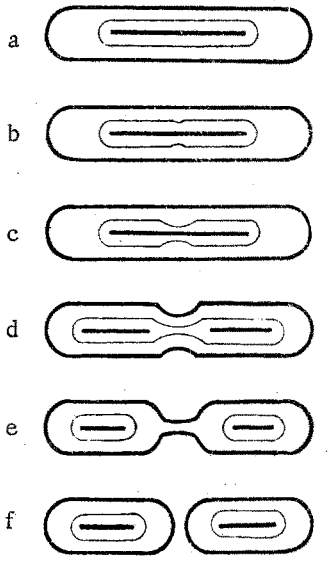

Fig. 5. Scheme of division of a bacterial nucleus. a) Resting cell; b) beginning of division of the nucleus; c) continuation of division of the nucleus; d) division of the chromatin band and beginning of indrawing of the membrane; e) end of division of the nucleus; f) division of the cell completed.

EXPERIMENTAL RESULTS

As in the experiments with ultrathin sections of E. coli aerogenes, we obtained photographs of ultrathin sections of cells of E. coli B., in which we observed the same peculiarities in the structures of the nuclear formations. Different methods of embedding gave the same results from this point of view.

Fig. 1 shows longitudinal and transverse ultrathin sections of E. coli B.cells. Despite the fact that the observations were made at high resolution of the microscope, we were unable to demonstrate the presence of membranes enveloping the nuclear formations in these sections, whereas at this resolution the double cell membranes showed up very well. The borders between the fine granular cytoplasm and the nuclear contents showed up very clearly, however, on all the films. This enabled some idea to be obtained of the area occupied by the nuclear structures in relation to the total area of the bacterial cell.

The study of many photographs of sections showed that the dimensions of the dense, longitudinal chromatin bands were not the same in all the cells. With a thickness on cross section of roughly 120 to 720 A, in individual sections these bands reached 5000 A and more in length. In none of the sections were these bands observed to begin directly from the borders with the cytoplasm; usually their ends were situated at a short distance from this border. Attention was drawn to the structure of the chromatin bands. On the films they had the character of continuous structures with no signs of splitting up into smaller elements.

The character of the nuclear formations in the dividing bacterial cells could also be examined in the sections. In the cells in which the process of division was very clearly expressed, as may be seen, for example, in Figs. 1, 2, and 3 (serial sections a_1, a_2 and a_3), nuclear substance was present in the form of light zones in which were included dense bands one by one in each part of the dividing or almost completely divided cells, in which only a small band was left of the cell membrane. It could be seen from the serial sections, on the other hand, that the process of division of the nuclear formations begins before signs of division are seen in the cell membrane and cytoplasm. Moreover, division of the nuclear formations, as can readily be seen in Fig. 3 (serial sections b_1, b_2 and b_3) took place in the newly formed cells at a time when the process of division of the original cell was not yet complete. These observations thus show that division of nuclear structures begins and ends sooner than the division of the bacterial cell as a whole to which they belong.

Electron microscopy of ultrathin sections of E. coli B cells infected with T2 phage, 5 minutes after the beginning of infection, i.e., 5 minutes after the addition of phage particles to the bacteria, revealed no appreciable changes in the nuclear apparatus of the bacteria. Such changes were readily demonstrated in preparations of bacteria which were prepared 2 hours after the beginning of infection. In a field of vision of such preparations were seen sections of cells of different shapes, beginning with some in which the changes in the external shape were hardly noticeable and ending with others which were converted into oval and spherical forms, usually preceding lysis.

The first thing to be observed from a study of the photographs of the sections was that the character of the nuclear structures in the sections of those cells that were unchanged or only slightly changed showed essential differences from the character of these structures in the sections of the oval and spherical cells. In the sections of unchanged or only slightly changed cells the light zones of nuclear substance were completely intact, although newly formed phage particles were to be found there. In shape they corresponded to the nuclear zone of cells not subjected to infection (Fig. 4). The chromatin bands were also preserved, although on account of a decrease in their density in relation to electrons, they were difficult to make out at this moment against the background of the cytoplasm.

In the cells in which the process of change in the external form was clearly expressed, light zones in the form of well defined central areas were absent; in the sections they had the appearance of small areas of ill-defined shape, scattered over the whole surface (see Fig. 4). It was characteristic that there were far fewer of these areas in the sections of the spherical cells, and their borders were more indistinct. Chromatin bands, as observed in the light zones of the nuclear substance of normal bacteria, were not found here. Thus, if the morphological changes in bacteria preceding lysis were far advanced, almost complete disintegration of the nuclear material was observed.

In selecting the test object for the present investigation, our aim was to choose a strain of E. coli which would have been most thoroughly studied in this direction, so that comparable results would be available for the formation of conclusions and for their discussion. We considered that E. coli strain B answered this purpose.

Our findings in relation to the structure of the nuclear apparatus of E. coli B were in complete agreement with the results of our previous experiments [3] in which observations were made on the nuclear apparatus of E. coli aerogenes, and with the conclusions drawn therefrom, and also with the data on the nuclear structures of E. coli B, published abroad [8, 10], where this strain of E. coli has been the subject of extensive cytomorphological study in recent years.

The use of serial ultrathin sections has revealed new and finer details of the structure of the nuclear apparatus and given additional evidence in support of the views which we have developed: In our opinion the nuclear apparatus of bacteria consists of distinctive vacuoles situated in the central part of the internal contents of the cells, and filled with material readily permeable to electrons, containing dense chromatin bands which are elongated in a longitudinal direction [3].

Like other authors [5, 9], we were unable to detect a membrane of the nuclear apparatus, although its absence is not yet finally settled. The presence of such a well-defined border between the nuclear structures and the cytoplasm, and also the possible use of a microscope with a higher resolving power (we worked with resolution of the order of 15 A) provide a basis for further research in this direction.

The data described above on the division of the nuclear apparatus of bacteria undoubtedly indicate the leading role of this process in the division of the bacterial cell as a whole, just as takes place in the division of higher forms of cells. From an analysis of the morphological pictures of the dividing nuclei in serial ultrathin sections of bacteria it may be concluded that the division of the nuclear structures, which initiates the division of the bacterial cell, corresponds in general outline to the scheme illustrated in Fig. 5. It can readily be seen that this scheme may be defined as amitosis. This conclusion disagrees with that of De Lamater [6], who considers, on the basis of optical and microchemical observations, that the nucleus of bacteria divides by karyokinesis.

The electron microscope pictures of the changes in the nuclear apparatus of bacteria under the influence of phage, which are described here, fully agree with the microchemical findings reported in the literature on this subject [1, 4, 11]. In conjuntion with the latter, our material shows that during infection of bacteria with phage, the destructive changes begin in the nuclear structures. Discrete nuclear structures are no longer visible in the changed bacterial cells before undergoing lysis.

The results of our experiments thus demonstrate the leading role of the nuclear structures both in processes connected with division of the bacterial cell and in the destructive changes in the bacterial cell resulting from phage infection.

In conclusion the author expresses his gratitude to Professor Sjostrand for his technical advice in the performance of this research, and also to laboratory assistant Cayland for technical assistance (from the Laboratory of Biological Ultra-structures, Karolinska Institute, Stockholm, Sweden).

SUMMARY

Electron microscopy of serial ultrathin sections of E. coli B cells revealed division of bacterial nuclear structures which may be regarded as being amitotic in character. Destructive changes which take place in phage infection of bacteria commence in the nuclear structures. Data on the nuclear apparatus and the effect of phage thereon demonstrate the leading role of the nuclear structures not only in processes connected with multiplication, but also in cases of destructive changes occuring in bacterial cells as the result of phage infection.

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*See C. B. Translation.