MICROBIOLOGY AND IMMUNITY

ELECTRON MICROSCOPIC ANALYSIS OF INFLUENCE OF HIGH FREQUENCY SOUND ON BACTERIA SUIPESTIFER

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Ultrasonic waves with a frequency modulation of 20 to 1,000,000 kilocycles have been extensively employed in biology. By means of ultrasound having the capacity to produce instant mechanical ruptures of cells and cellular structures, microbiologists subject microbial cells to destruction and disintegration [1, 2].

However, the influence on micro-organisms of high frequency modulations i.e., modulations below 10-20 kilocycles has been the subject of very little study.

The object of the present work was an electron-microscopic study of the fine morphological changes in the cells of Bact. suipestifer, subjected to the influence of sound modulations of high frequency in the order of 8000 cycles.

EXPERIMENTAL METHODS

A suspension of an 18 hour agar culture of the strain Bact. suipestifer No. 10 in physiological saline containing about 2 billion microbial bodies per ml was subjected to the influence of ultra sonic modulations for 10 minutes at a temperature of 20-30°C and a modulation frequency of 8000 cycles. A magnetostriction sound generator served as source of the modulations. After this the microbial suspension was dissolved in physiological saline to produce a perceptible opalesence and applied in the form of small drops on a collodion film. In order to remove the crystals of NaCl from the preparations they were subjected to dialysis for 25 minutes at a temperature from 4 to 5°C. For the purpose of contrasting the objects the dessicated preparations were dusted with chromium. The original microbial suspension not subjected to the influence of sound served as control.

EXPERIMENTAL RESULTS

By means of electron-microscopy we found significantly marked changes in the morphological features of the Bact. suipestifer cells subjected to the influence of ultrasonics.

Usually the cells of Bact. suipestifer, cultured on agar, have the appearance of homogenous electron-optically dense rod-shaped bacteria (Fig. 1).

In the field of vision of the preparations prepared from a microbial suspension subjected to sound, there was almost a complete absence of whole, unchanged microbial cells.

The majority of morphological elements observed were in the form of rod shaped bacteria with lucid protoplasm and with differentiated, electronically dense, large granules scattered in ones, twos, (at the termini) or in threes (along the longitudinal axis of the bacteria). The shape of the granules varied: from regular oval shapes with smooth or with broken edges to a stretched or indefinite shape, as is shown in Fig. 2. We very often noted cells split in half. Every such half had at the terminus a dense granule.

Fig. 1. Bact. suipestifer. Original 18 hour agar culture. x 28,000 (electron microscopy).

In a number of cases we did not succeed in finding a membrane and the microbes presented essentially "bare" lucid protoplasm with electron-optically dense granules, occasionally with their edges emerging beyond the confines of the protoplasm. The magnitude of the granules observed was not uniform: their diameter was equal sometimes to half and other times to the entire diameter of the bacteria, and the presence of shadows appearing as a result of dusting with chromium indicated their considerable volumetric depth.

Alongside these elements which still retained the general appearance of rod-shaped bacteria, in the field of vision, complete bacterial destruction was also encountered.

In many cases the differentiated granules whose diameter did not exceed that of the vegetative forms of Bact. suipestifer were ejected from the microbial cells constituting smooth or broken volumetric formations of different size, distributed against a granulated background with fragments of flagella (Fig. 3).

Such a discovery in the microbial suspensions subjected to sound, i.e. the ejection of the granule from the bacterial cells, raises the question of the true nature of these formations, since some authors are inclined to consider them as mitochondria [3].

The flagellate apparatus, which is transversely striated in the vegetative cells of Bact. suipestifer, as a

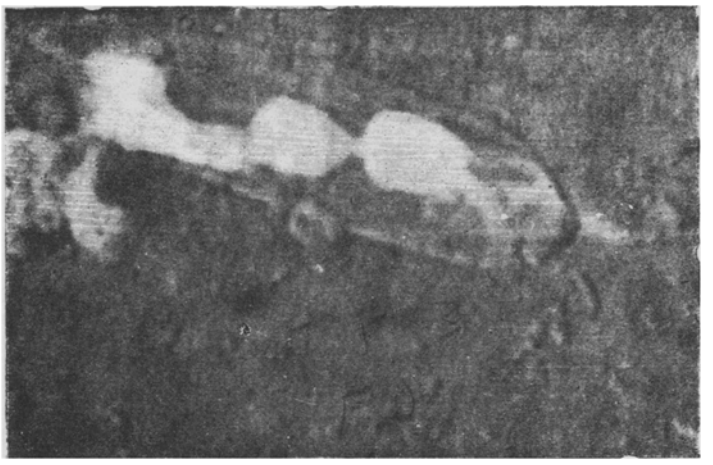

Fig. 2. Bact. suipestifer with lucid protoplasm and dense granules after ultrasonic influence. x 30,000.

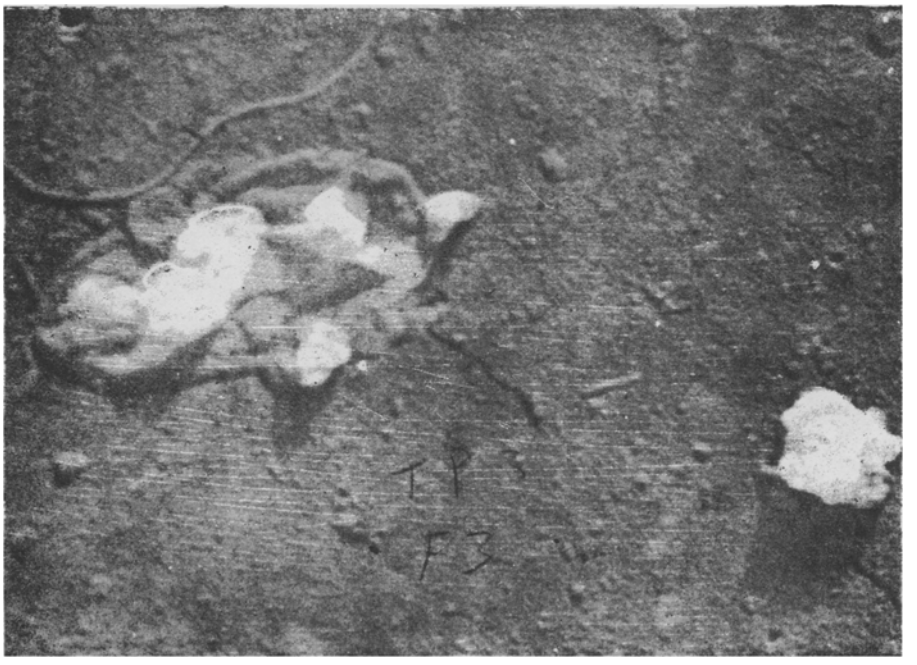

Fig. 3. Ultrasonic destruction of Bact. suipestifer cells and ejected granule, (right). x 32,000.

rule was destroyed by tearing itself in individual segments of differing length along the diameter of the flagella (see Fig. 3). No perceptible increase or decrease in the dimensions of the cellular elements after ultrasonic influence were found.

LITERATURE CITED

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