NOTES

ELECTRON MICROGRAPH OF AN ULTRATHIN SECTION OF *BEGGIATOA*

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The colorless sulfur bacterium, *Beggiatoa*, on which Winogradsky based his concept of chemolithotrophy, is described as a large, filamentous, colorless sulfur bacterium, with sulfur granules accumulating inside the cells when growth takes place in a natural environment in the presence of hydrogen sulfide. When wet mounts of *Beggiatoa* are viewed by light microscopy, the sulfur granules can be seen readily but the trichomes appear to lack definite cross walls. However, staining brings out the cross walls, whereas phase microscopy may or may not (Scotten and Stokes, Arch. Mikrobiol. **42**:353, 1962).

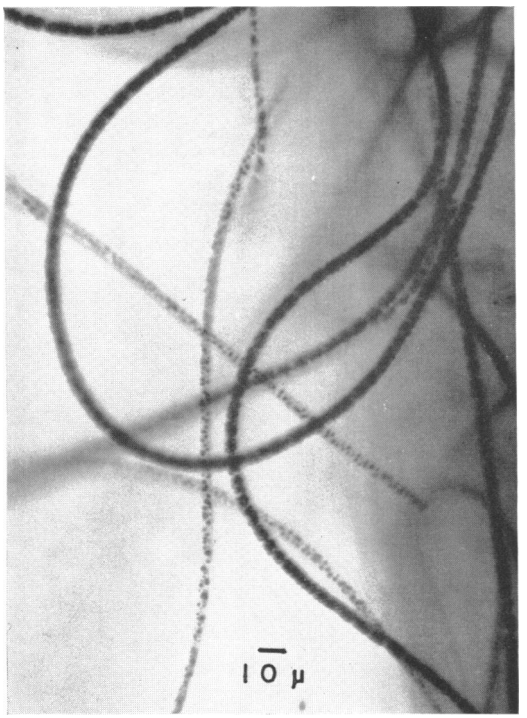

FIG. 1. *Photomicrograph of a wet mount of Beggiatoa showing highly refractive sulfur granules in the trichomes.*

If *Beggiatoa* are cultured in the laboratory under heterotrophic conditions, sulfur granules in the trichomes become very sparse. To demonstrate sulfur granules for an electron micrograph, an enrichment culture of *Beggiatoa* was grown at room temperature in Cataldi's [Rev. Inst. Bacteriol. (Buenos Aires) **9**:393, 1940] hay infusion medium using an inoculum obtained from a sulfur well located in Nebraska City, Neb. After tufts of *Beggiatoa* had developed, they were immediately immersed in 10% formalin solution for 10 min. Since *Beggiatoa* are delicate and lyse extremely readily, the formalin treatment was necessary. The tufts of *Beggiatoa* resembled those described by Johnson and Baker (J. Cellular Comp. Physiol. **30**:131, 1947; Plate 1, Fig. 1, 2, and 3) and Faust and Wolfe (J. Bacteriol. **81**:99, 1961; Fig. 2 and 3). The trichomes of our enrichment culture were filled with numerous highly refractive sulfur granules (Fig. 1). Formalin-treated tufts of *Beggiatoa* were washed in sterile tap water several times to rid the *Beggiatoa* of extraneous microorganisms as much as possible. The *Beggiatoa* were fixed according to the method described by Palade (J. Exptl. Med. **95**:285, 1952), dehydrated through an acetone-water series, and embedded in Vestopal W according to Ryter and Kellenberger's (J. Ultrastruct. Res. **2**:200, 1958) method. Ultrathin sections were cut by use of a Porter-Blum microtome and then stained with saturated uranyl acetate (Watson, J. Biophys. Biochem. Cytol. **4**:475, 1958). The electron micrograph was taken with an R.C.A. EMU-2D fitted with a Canalco condenser aperture.

Although Scotten and Stokes (Arch. Mikrobiol. **43**:353, 1962) could not demonstrate a cell wall

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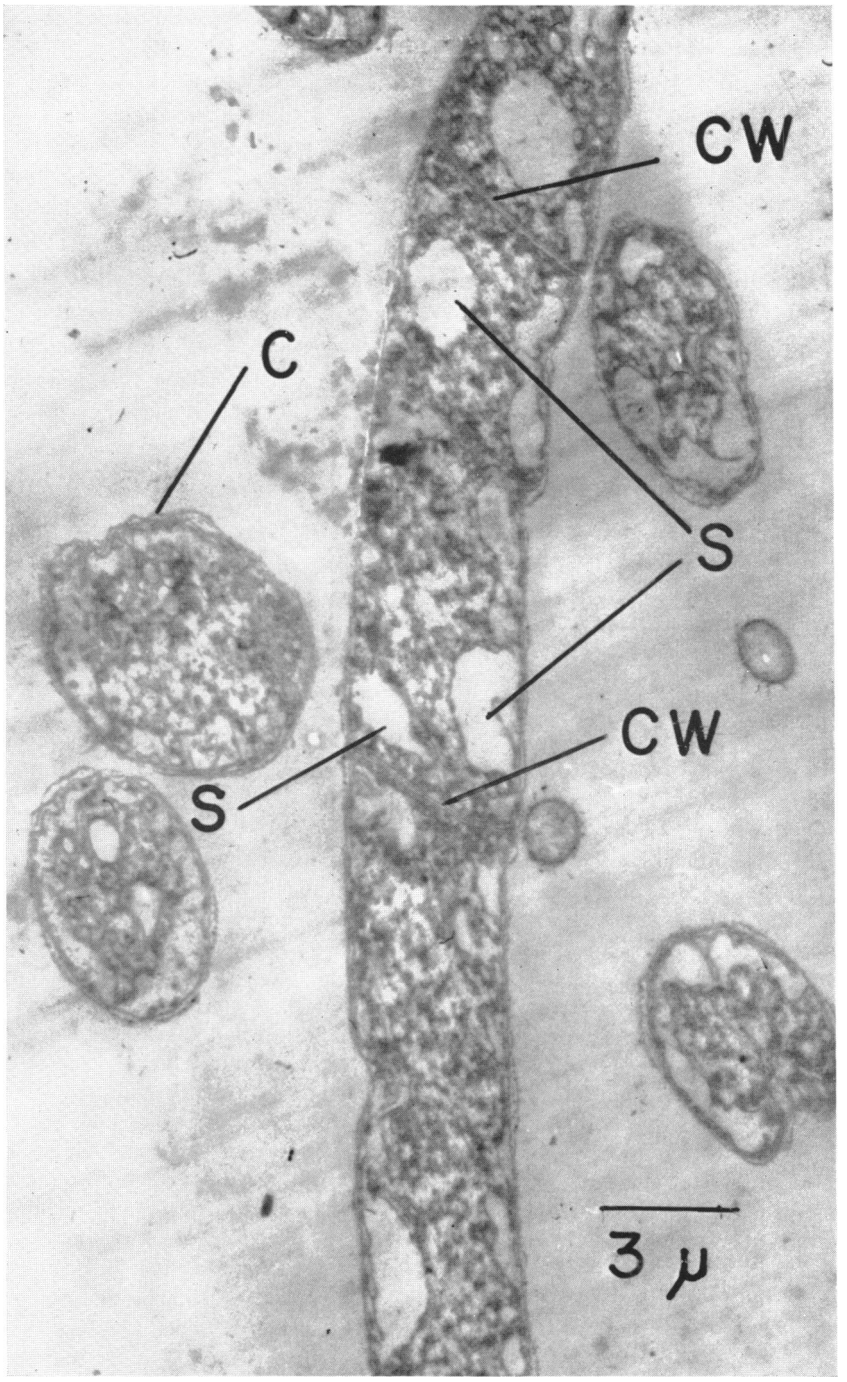

FIG. 2. *Electron micrograph of an ultrathin section of Beggiatoa. Note the two dense lines (C) surrounding the cells, sulfur granules (S), and cross walls (CW).*

by staining methods, it appears that the inner and outer dense lines surrounding the cell in Fig. 2 represent the cell membrane and cell wall, respectively. In this case the cell wall would be quite thin, which may be the reason why Scotten and Stokes could not demonstrate a cell wall.

Sulfur granules can also be noted, and more than one may occur in any given cell of the trichome as shown in the various cells of the trichomes of *Beggiatoa* (Fig. 2). Observations in this laboratory suggest that no set number of sulfur granules occur within any cell of the trichome. We have observed from one to seven sulfur granules per cell by phase microscopy. Although the cross walls can not be seen in the trichomes in Fig. 1, there appears to be no orderly arrangement of sulfur granules. The clear areas in the trichomes of Fig. 2 are the spaces in which sulfur was formerly located.

Scotten and Stokes observed discrete, ovoid or spherical nuclear bodies about two-thirds the diameter of the trichome, occuring singly or in pairs, within each cell of the trichome when treated with 1 N HCl and Giemsa stain. Likewise we have observed distinct nuclear bodies by using identical procedures. When the cells are not treated with HCl, the entire cells take up the basic dye, which suggests that the cells possess cytoplasmic ribonucleoproteins. No distinct nuclear bodies are shown in Fig. 2, which suggests that cytoplasmic ribonucleoproteins have masked the discrete nuclear bodies.

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SIMPLIFIED TUBE METHOD FOR THE PREPARATION OF H ANTIGENS[1]

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The preparation of H antigens requires actively motile forms of bacteria, since H antigen is associated with the flagella. To insure maximal development of H antigens, bacteria are grown in a semisolid medium. The most active bacteria move rapidly through the medium and are transferred for a second or third passage. There are various methods used for passage of the motile bacterial forms through semisolid medium. The upper part of the medium in regular test tubes may be inoculated and the growth harvested when it reaches the bottom of the tube. In U-shaped tubes, one side is inoculated and growth progresses to the other side. Another method utilizes a small tube, open at both ends, inserted in the center of a regular tube of medium. Inoculation is accomplished through the bore of the small inner tube. Bacteria grow down through the inner tube and up to the surface of the outer medium.

In our work with H antigens of *Escherichia coli*, a tube (Fig. 1) was devised that is simple to prepare, to use, and to clean. Glass tubing having an outside diameter of 15 mm was cut to 125-mm lengths. One end of the tubing was plugged with a no. 0 solid rubber stopper. This served as the bottom end of the tube. After adding the proper amount of semisolid medium, the tubes were covered with metal caps and sterilized at 15 psi (121 C) for 20 min. The metal caps have "fingers," thus giving a firm grip, but are still loose enough to prevent blowing off while being autoclaved. Tubes of the type described have been in daily use over a period of 8 months. The rubber stoppers have not loosened and no leakage has occurred.

The dimensions of the tube can be varied with appropriate variation in the size of the rubber stoppers. Since rubber stoppers have a tapered form, the widest part of the stopper should not interfere with insertion of the plugged tube in a rack.

The medium is inoculated at the upper surface, and growth can be transferred when it reaches the bottom simply by removing the stopper. Once the

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