ATTACHMENT OF *THIOBACILLUS THIOOXIDANS* TO SULFUR CRYSTALS

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ABSTRACT

SCHAEFFER, W. I. (Rutgers, The State University, New Brunswick, N.J.), P. E. HOLBERT, AND W. W. UMBREIT. Attachment of *Thiobacillus thiooxidans* to sulfur crystals. J. Bacteriol. **85**:137–140. 1963.—Electron micrographs of replicas of sulfur crystals before and after attack by *Thiobacillus thiooxidans* show that the microorganisms erode the crystal in the area immediately adjacent to the cell. When there are many cells, the entire crystal surface appears eroded.

Thiobacillus thiooxidans readily oxidizes insoluble sulfur, but, aside from the fact that a direct contact between the organisms and the sulfur is necessary, the means by which sulfur is attacked is not known. We had earlier supposed that there was an orientation of the cells at the surface of the sulfur (Vogler and Umbriet, 1941) and that the sulfur was dissolved in a fat globule existing in the cell (Umbreit, Vogel, and Vogler, 1942). However, more recent studies (Newburgh, 1954; Starkey, Jones, and Frederick, 1956) have shown that *T. thiooxidans* may be grown on shaking machines, and we have been able to grow it in violently agitated fermenters; thus the contact between the sulfur particle and the microorganism must be more stable than originally supposed. We were therefore interested to see what the surface of sulfur looked like after bacterial attack, to determine whether we could find any physical reason for the stability of the sulfur-bacteria contact.

MATERIALS AND METHODS

Sulfur crystals of appropriate size were obtained by dissolving rolled sulfur in warm carbon disulfide; the sulfur crystallized by slow evaporation of the solvent. The crystal surface was then replicated by allowing a 5.0% solution of collodion in amyl acetate to flow over the crystal surface and harden.

This replica was then loosened at the edge and stripped off with forceps, and a carbon replica was made by evaporating carbon at a normal incident angle. This carbon replica was shadow-cast with germanium on an angle of 26°, and the original collodion was dissolved in acetone. The shadow-cast carbon replica was photographed in a RCA EMU-2 or EMU-3 electron microscope. The crystal was then placed in a sterile inorganic culture medium (Umbreit et al., 1942), and the medium was inoculated with *T. thiooxidans*, using essentially sulfur-free cells. The cells were obtained from fluid cultures, which had been grown on shaking machines, centrifuged, and washed. Both low and high inocula were used. The only source of sulfur in the medium was the large single sulfur crystal. This system was then incubated at room temperature for 1 week under stationary conditions (to avoid damaging the crystals). The crystal was then removed, washed in distilled water, dried, and the same surface replicated as previously. It was found that the first replica did not give a picture of the surface, but only cells. A second replica, and sometimes a third, was necessary before all the cells clinging to the surface had been removed and the crystal surface itself was replicated. These replicas were shadowed and photographed in the same manner as the original replicas.

RESULTS AND DISCUSSION

Figures 1 and 2 show the surface of the same sulfur crystal before (Fig. 1) and after (Fig. 2) attack by the organism. Extensive etching has taken place. Further, areas approximating the shape and size of individual cells have been eroded. Figures 3 and 4 are at lower magnification and show two different crystals: one (Fig. 3) exposed to a low inoculum of cells, in which eroded positions presumably previously occupied by individual living cells can be seen, and the other (Fig. 4) a crystal exposed to a high inoculum of cells, which gives the appearance of a contour map. No areas are free of erosion. Figure 5 (comparable with Fig. 2) shows that the eroded areas

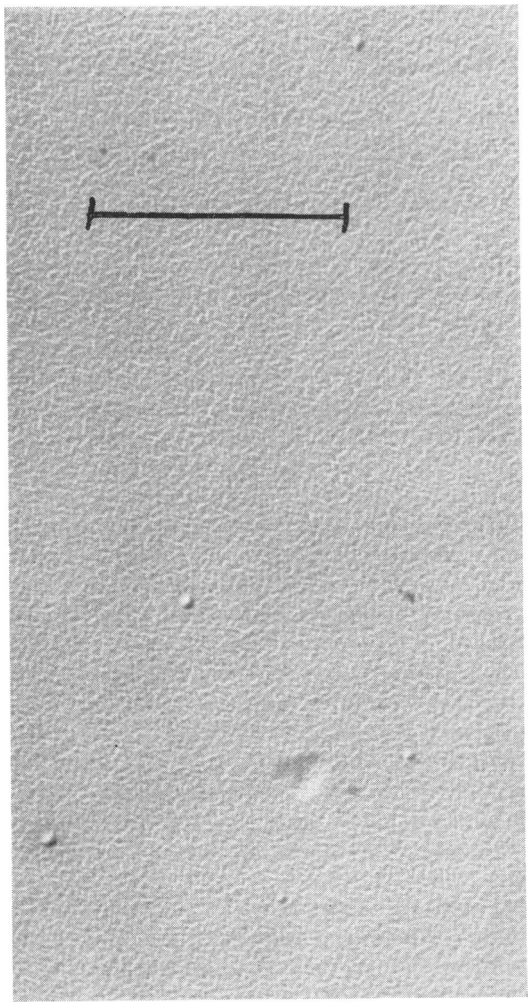

FIG. 1. *Surface of sulfur crystal prior to exposure to the bacteria. The line in all figures indicates 1 μ.*

are indeed occupied by microorganisms; Figure 6 (comparable with Fig. 4) shows that, with a large inoculum, considerable areas of the crystal are completely covered with the microorganism. Both Figures 5 and 6 are replicas in which cells attached to the sulfur crystal at the time it was removed from the culture vessel are stripped off with the replica. In Fig. 5, the "spots" are probably due to extremely minute quantities of collodion softened by the solvent but not completely removed.

From these results it appears evident that individual cells erode portions of the crystal immediately adjacent to them. When a large inoculum is used, the entire surface erodes in a somewhat uneven fashion. However, there is no evidence of holes bored into the sulfur of sufficient depth to explain the stability of the bacteria-sulfur contact to high turbulence. The nature of the attachment must therefore be chemical rather than physical.

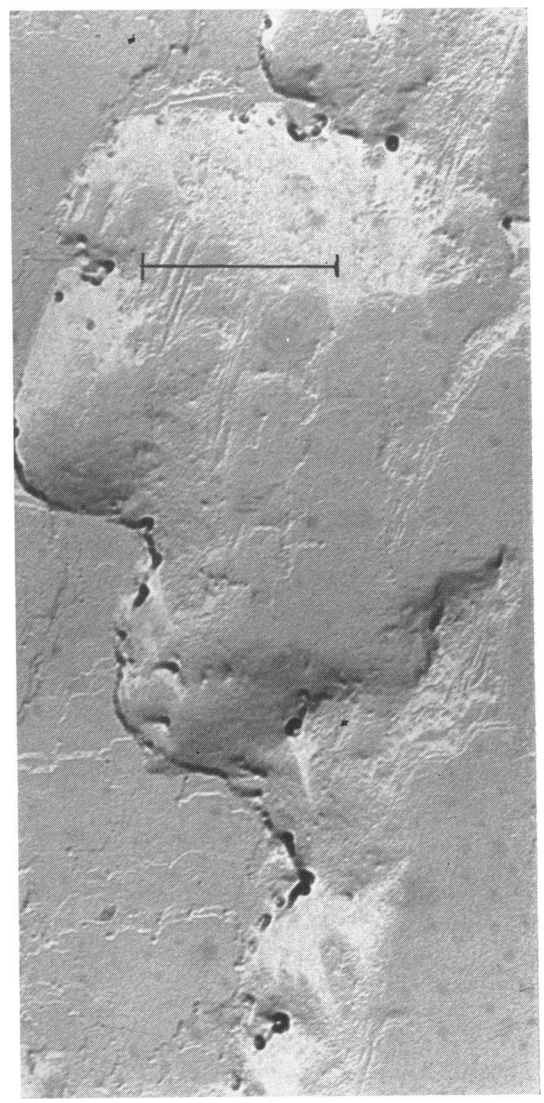

FIG. 2. *Surface of sulfur crystal after exposure to the bacteria. This is the same sulfur crystal shown in Fig. 1, and the same crystal face has been replicated after incubation with Thiobacillus thiooxidans for 1 week.*

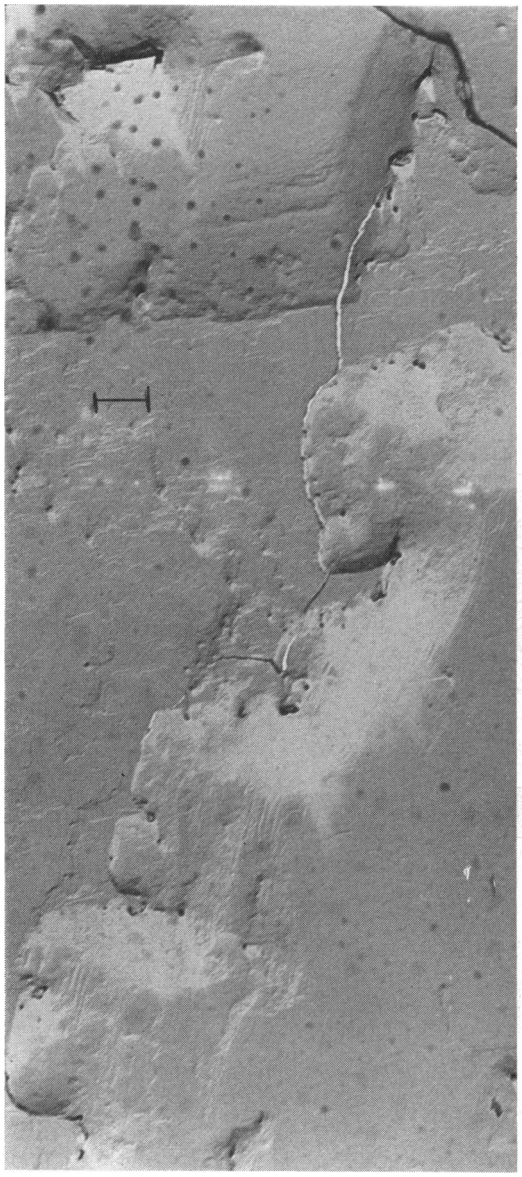

FIG. 3. *Surface of another crystal of sulfur after incubation with low inoculum of bacteria. Low inoculum of Thiobacillus thiooxidans, 7 days of incubation.*

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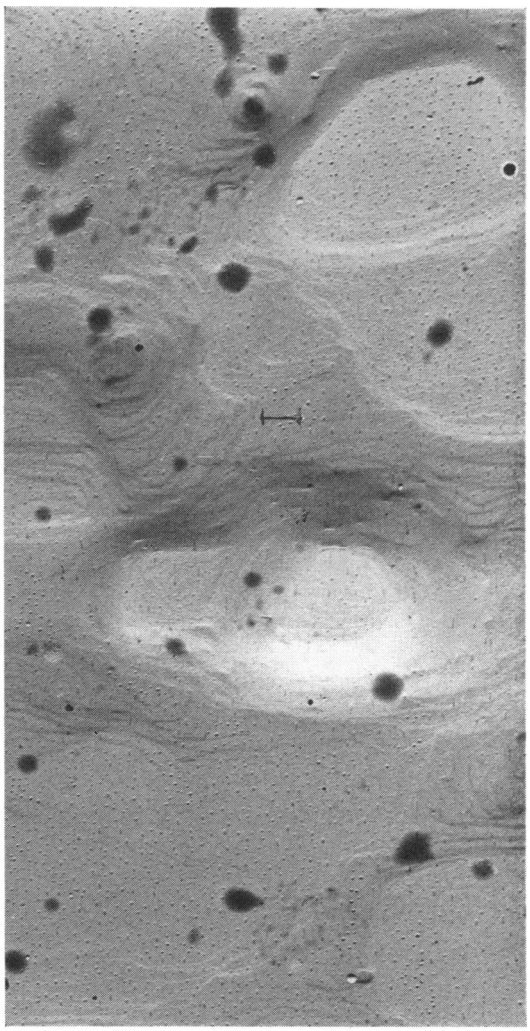

FIG. 4. *Surface of sulfur crystal after incubation with high inoculum of bacteria. High inoculum of Thiobacillus thiooxidans, 7 days of incubation.*

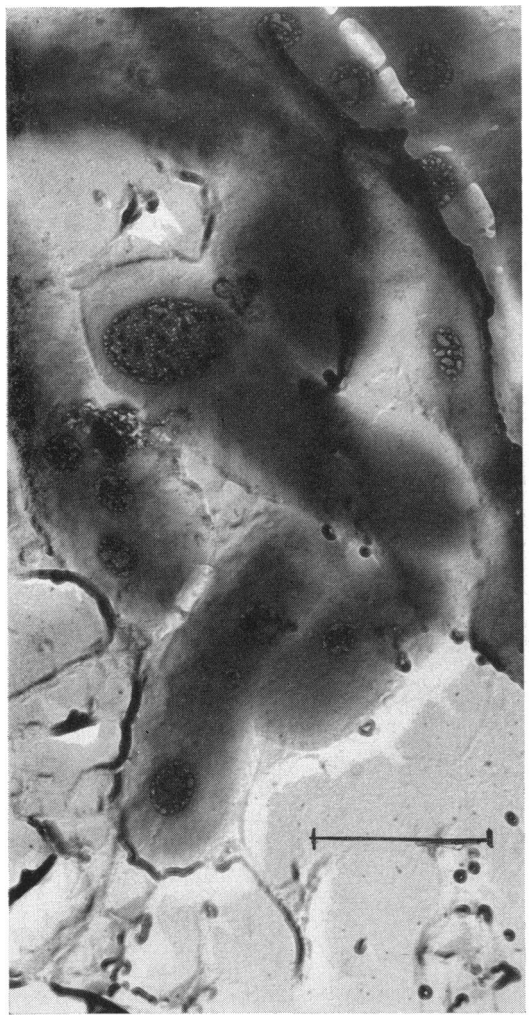

FIG. 5. *Replica with cells attached from sulfur crystal with low inoculum of bacteria.*

FIG. 6. *Replica of the cells attached from sulfur crystal with high inoculum of bacteria.*

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