

A STUDY OF THIOBACILLUS THIOOXIDANS WITH THE ELECTRON MICROSCOPE

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Thiobacillus thiooxidans, an obligate autotrophic sulfur-oxidizing bacterium, has received considerable attention because of the interest and importance attached to a knowledge of its physiology. In the original description of the organism (Waksman and Joffe, 1922) was included a photomicrograph the essential features of which are reproduced from our own cultures in figure 1. We have recently had the opportunity to examine pure cultures of this microorganism in the RCA electron microscope, using distilled water suspensions prepared and micrographed by methods previously described (Umbreit *et al.* 1941, Marton 1941). These electron micrographs (figures 2 through 6) seem to relate so directly to the original photomicrograph that we have felt that they would be of interest to bacteriologists even though the interpretations we have placed upon the evidence at hand may be modified by future study. We have repeatedly noted the forms found in these micrographs in countless preparations studied in the light microscope by a variety of staining methods. Their observation in the electron microscope illustrates, we believe, the profound effect which the latter instrument will exert upon bacteriology.

OBSERVATIONS

The cells observed seem to fall into three general types with transition stages sometimes evident. These are:

TYPE I (1 by 0.5μ); stainable with alcoholic crystal violet (Waksman and Joffe, 1922) or better with crystal violet followed by iodine (Umbreit *et al.*, 1941). Under staining procedures they frequently exhibit a "dipolar" appearance (fig. 1, *a*). It has recently been shown (Umbreit *et al.*, 1941) that these dipolar bodies consist of a highly unsaturated fat and are involved in the process of sulfur oxidation. The electron micrographs (*a* in figs. 2, 3, 4, 5) show the same type of cell as relatively opaque to the electron beam. The dipolar appearance noted in the stained cells is not evident in the electron micrographs. This is due to either of two possibilities; one, that the electron beam does not distinguish between the fat and other cell constituents, or, two, that the high vacuum in the microscope has evaporated the fat from the cell. Since the fat is quite volatile this would be a definite possibility and the slightly opaque granules noted in some cells of figures 2 and 3 might be regarded as residues from the evaporation.

TYPE II (2-3 by 0.5μ); evident in the photomicrograph in which it appears as a relatively homogenous cell of elongated form (fig. 1, *b*). Such cells might arise by a delay in cell division as seems to have been the case in certain forms noted (see *b* in figs. 2 and 3). But in others (figs. 5 and 6) a more complex structure is noted. In figure 6, particularly, the internal spiral structure, whose nature and function are unknown, is strikingly evident.

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All of these longer types are relatively opaque to the electron beam and stain almost as readily as the Type I cells.

TYPE III ($1-1.5$ by $0.5-0.8\mu$) do not stain readily (fig. 1, *c*) and are much less opaque to the electron beam. We would regard types labeled *c* in figures 2 and 3 as representatives of this type and *ac* in figure 4 as a transition form between Type I and Type III.

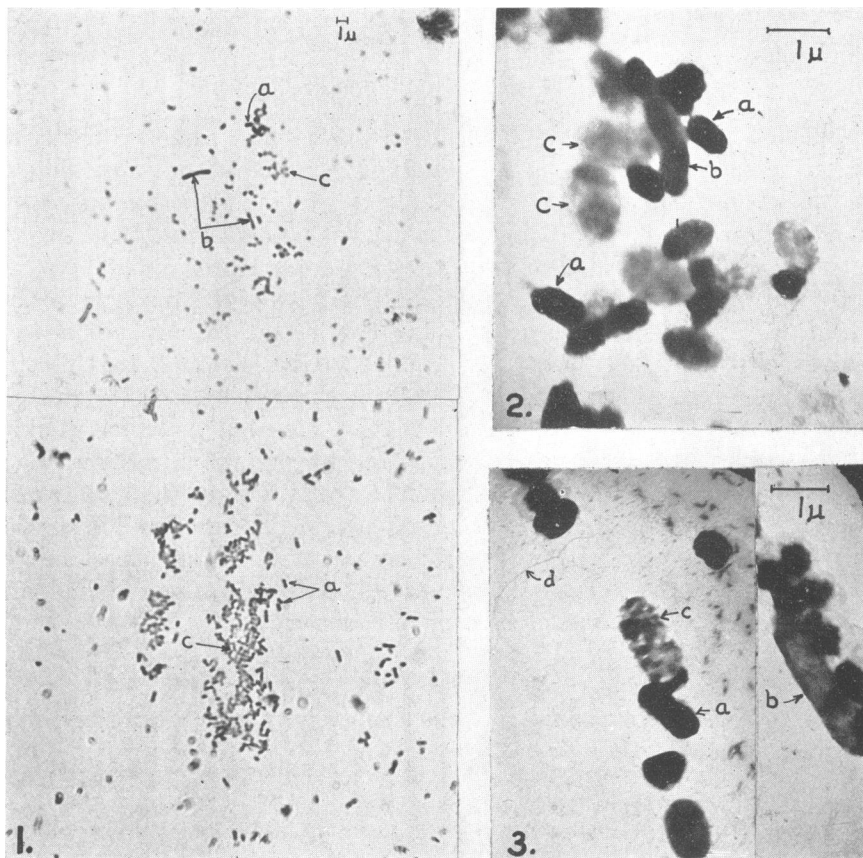


FIG. 1. THIOBACILLUS THIOOXIDANS, IN LIGHT MICROSCOPE, STAINED WITH CRYSTAL VIOLET

Top—three day culture. Bottom—twenty day culture. Original $900\times$. Enlargement $2\times$. Total magnification $1800\times$.

FIG. 2. THIOBACILLUS THIOOXIDANS, UNSTAINED, IN ELECTRON MICROSCOPE. $12,000\times$

FIG. 3. AS FIGURE 2

In addition, flagella may be noted (*d* in figs. 3 and 6) but it is probably of some significance that most of the cells are nonflagellated. In figure 4 there is a faint halo around certain cells; this we believe to be the bacterial cell-wall away from which the opaque, black, protoplasm has shrunk. (Mudd *et al.*, 1941). We regard the exceedingly faint "halo" extending for about a micron around the cells in figure 6 as an artifact developed in the preparation of the specimen.

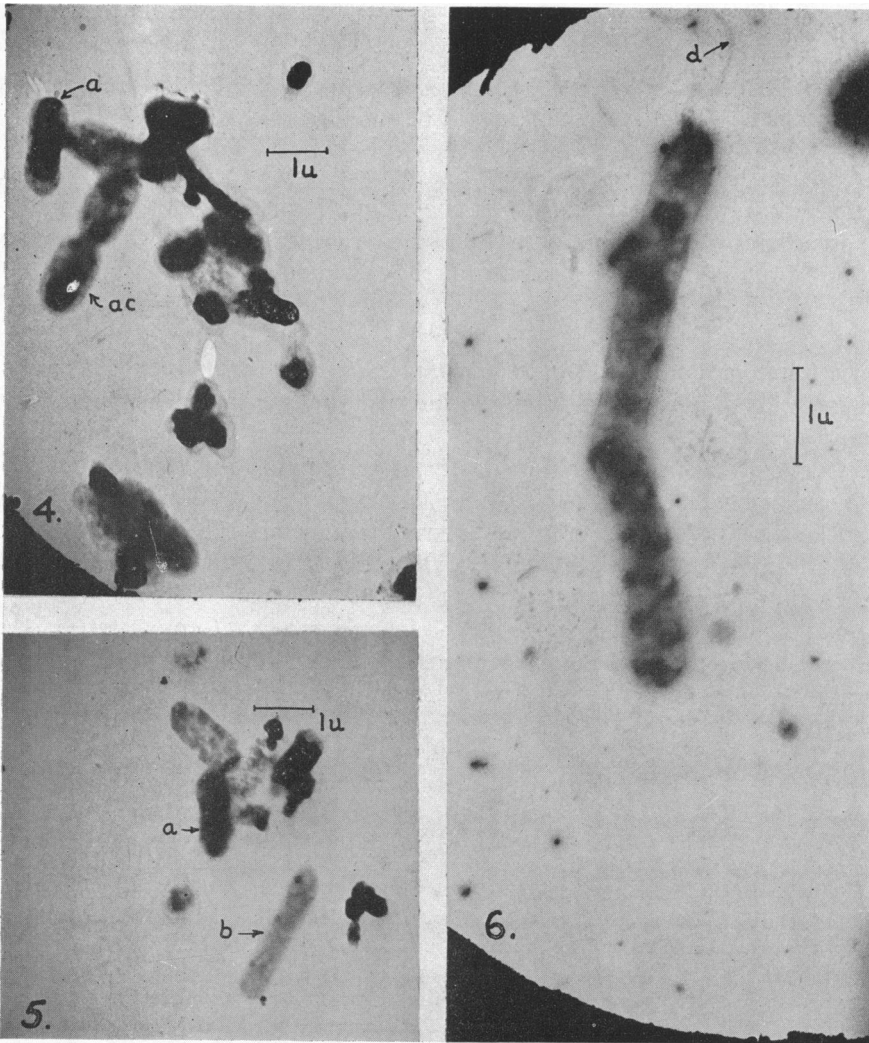


FIG. 4. AS FIGURE 2

FIG. 5. AS FIGURE 2

FIG. 6. THIOBACILLUS THIOOXIDANS, UNSTAINED, IN ELECTRON MICROSCOPE. 19,000 X

INTERPRETATION

It is obvious that any interpretation of the nature of the structures observed in these cells is decidedly premature and we therefore advance none. However, it may be of value to point out that a culture of *T. thiooxidans* is not a homogeneous entity but contains cells of all stages of growth, of all degrees of nutrition, and under environmental conditions (particularly low pH) which are decidedly detrimental to other forms. In the course of its growth on sulfur the

organism synthesizes a reserve storage product which enables it to live in the absence of oxidizable sulfur (Vogler, 1941). This supply of reserve food is sufficient for a considerable period of survival but will not last indefinitely. When it has been used up the cell dies and undergoes a slight autolysis. One might regard cells of Type I as those having a high reserve of storage products and those of III as being dead cells whose storage products had been utilized. Further work is necessary to determine whether this explanation is suitable. The structures observed in figure 6 present an interesting problem in bacterial cytology and their function and nature are matters for further study.

SUMMARY

Electron micrographs of *Thiobacillus thiooxidans* reveal that the cells possess a thin cell-wall differentiated from the internal protoplasm. The cells may be roughly divided into three categories:

1. Oval cells containing so much internal matter that they appear opaque to the electron beam.

2. Elongated cells containing less internal matter and exhibiting a wide variety of structures, granules, vacuoles, and even spirals.

3. Oval cells containing very little matter.

Flagella, which are about 17 $m\mu$ wide, occur only rarely. Comparisons of these micrographs with those of the light microscope are made and the possible physiological interpretations of the observed structures are briefly discussed.

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