

## SOME OBSERVATIONS ON THE FINE STRUCTURE OF A THERMOPHILIC, ACIDOPHILIC ALGA

WALTER G. ROSEN and KENNETH A. SIEGSMUND. From the Department of Biology, Marquette University, Milwaukee, Wisconsin

*Cyanidium caldarium* is a thermophilic, acidophilic alga with a cell diameter of *ca.* 3  $\mu$ . It has generally been assigned to the Cyanophyta (blue-green algae) but on the basis of morphology, pigment content, and life history, Hirose (2) suggested that it should be assigned to the Rhodophyta (red algae). *Cyanidium* grows, respire, and photosynthesizes optimally at pH 2.0 to 3.0, and at 55°C. (1). Because of its small size, disputed taxonomic position, and remarkable physiological characteristics, the fine structure of this organism is of particular interest.

The cells were grown in a mineral nutrient medium containing 2 per cent glucose at pH 2.0 (liquid) or 5.8 (agar slants) at a light intensity of 150 to 250 foot-candles. Cells were fixed for 30 minutes in an unbuffered solution of 5 per cent  $\text{KMNO}_4$  at room temperature. Fixation was also attempted with a variety of procedures employing buffered osmium tetroxide. The material was dehydrated in alcohol at room temperature and embedded in an Araldite mixture containing 5 per cent plasticizer. The plasticizer was added to permit centrifugation of the cells after Araldite

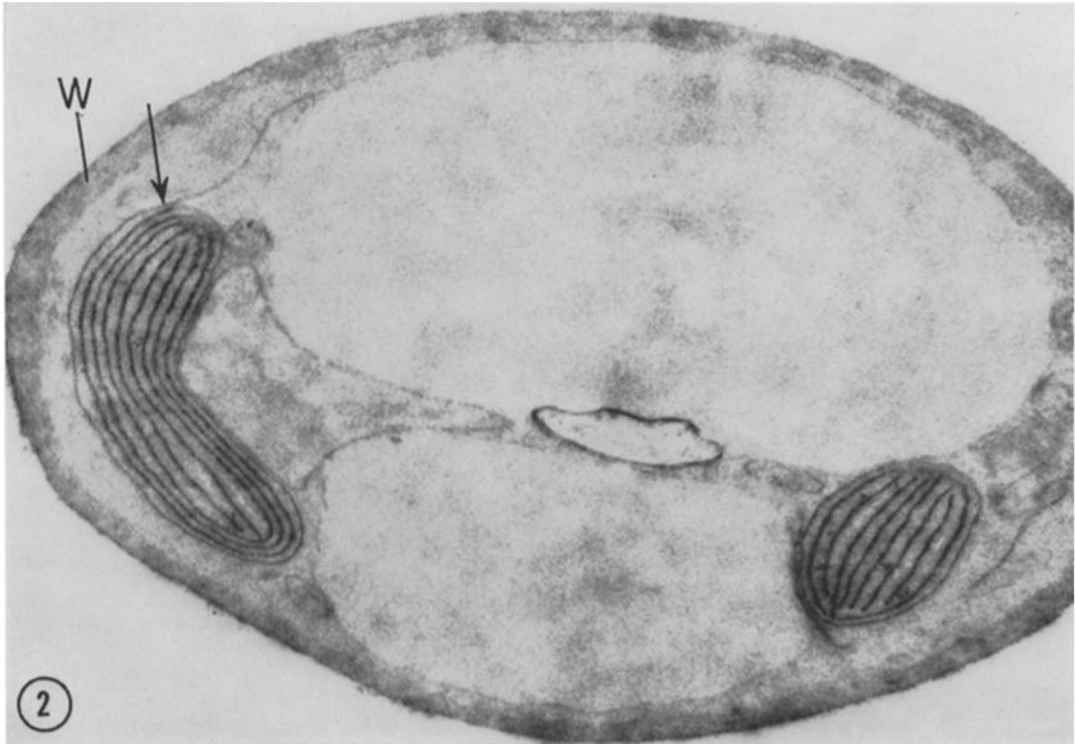
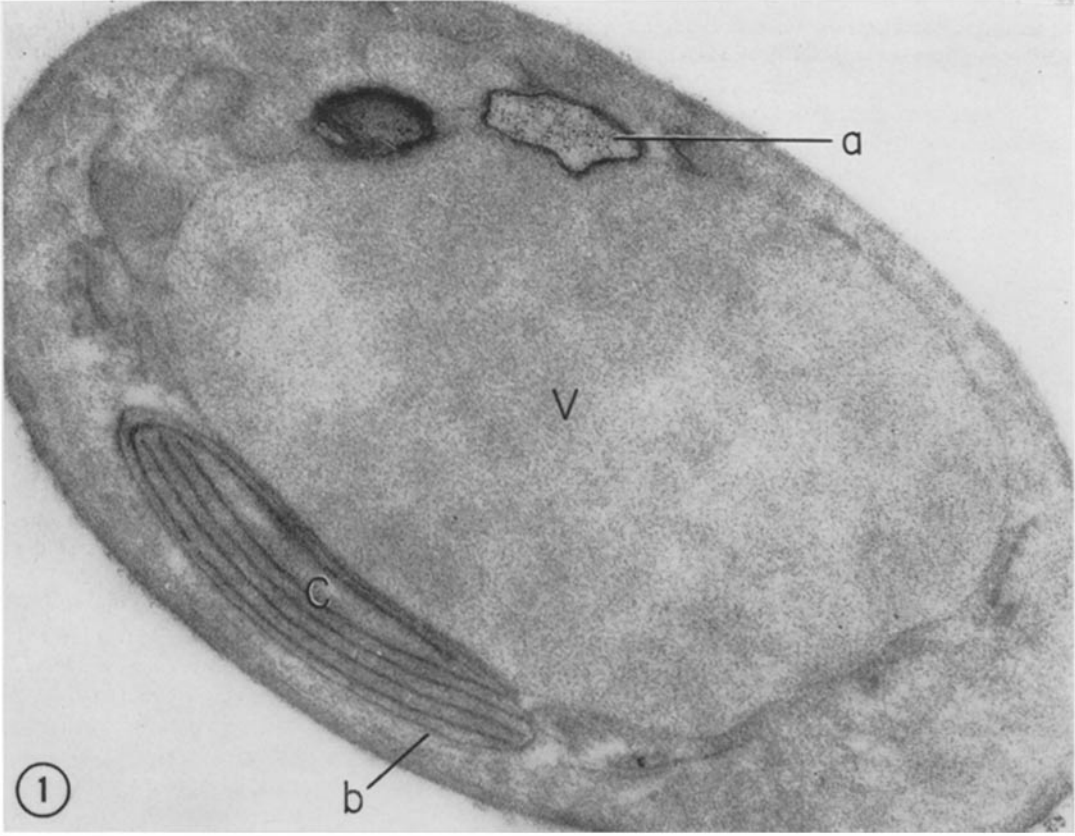
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### FIGURE 1

Electron micrograph of a thin section of *Cyanidium caldarium* showing single chloroplast (C). A large vacuole (V) occupies the central portion of the cell. A small irregular shaped body seen at (a) may represent the nucleus. A single outer membrane (b) can be seen surrounding the chloroplast.  $\times 30,000$ .

### FIGURE 2

A section presumably cut through opposite ends of the same chloroplast. A dense layer representing the cell wall (W) can be seen surrounding the cell. The outer lamellae of the chloroplast appear to be entire. At the arrow the outer membrane of the chloroplast and the vacuolar membrane appear to be continuous.  $\times 26,000$ .



infiltration. Sections were cut on a Porter-Blum microtome, mounted on uncoated copper grids, and examined in an RCA EMU-3c electron microscope.

The best osmium fixation was obtained with the method of Kellenberger, *et al.* (4) (Fig. 6). However, while this method produces excellent fixation of *Euglena* (Siegesmund and Rosen, unpublished), with *Cyanidium* it resulted in a more granular cytoplasm and a chloroplast with more irregular lamellae than did fixation with permanganate.

The electron micrographs show a cell wall about  $0.05 \mu$  in thickness (Fig. 1). The cytoplasm is most frequently seen as a thin layer near the periphery of the cell; a large vacuole usually occupies the central region (Figs. 1 and 2). Unidentified membranes, and bodies which may be small mitochondria, are seen in the cytoplasm of mature cells but are more evident in the endospores.

Hirose reported that each cell contains a single parietal chloroplast. Our micrographs reveal one to three laminated bodies per vegetative cell. Examination of whole cells with the light microscope, as well as electron microscope study of numerous sections cut at various angles through the chloroplasts, indicates that the cells generally contain a single sausage-shaped chloroplast which

may be sufficiently curved so that two or even three sections through the same plastid are sometimes encountered (Fig. 2). Some chloroplasts have been observed which appear to be dividing, usually in cells undergoing endospore formation (Fig. 4).

The chloroplasts appear to be about  $0.5 \mu$  in width and may extend up to half the diameter of the cell. It is particularly interesting to note that the chloroplast is bounded by a single limiting membrane, *ca.* 30 A, in contrast to the double membrane reported in other plant chloroplasts (3). It was noted, in one case, that this membrane and the vacuolar membrane appeared to be continuous (Fig. 2).

The limiting membrane encloses a parallel array of lamellae, each composed of a pair of adjacent membranes *ca.* 30 A thick and joined at their ends. The distance between the membranes comprising a lamella is *ca.* 85 A, and the distance between adjacent lamellae is *ca.* 560 A. The outermost one or two lamellae frequently appear to be continuous and resemble two layered sacs (Fig. 2) while the inner ones resemble an array of two layered plates (Figs. 1 to 3, and 5).

Hirose reported that the cells contain densely aggregated Feulgen-positive material in a body which he termed a nucleus. By staining with aceto

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FIGURE 3

Cross-section through a chloroplast showing lamellae.  $\times 46,000$ .

FIGURE 4

Endospore formation in *Cyanidium caldarium*. A chloroplast (*a*) in one of the endospores appears to be undergoing division.  $\times 10,500$ .

FIGURE 5

A higher magnification of a portion of the chloroplast showing lamellae.  $\times 120,000$ .

FIGURE 6

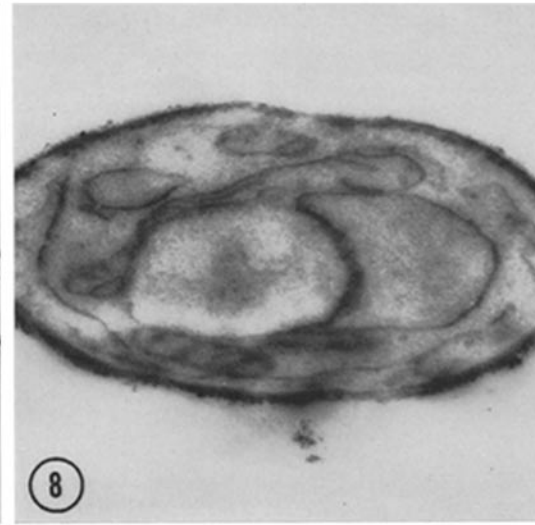
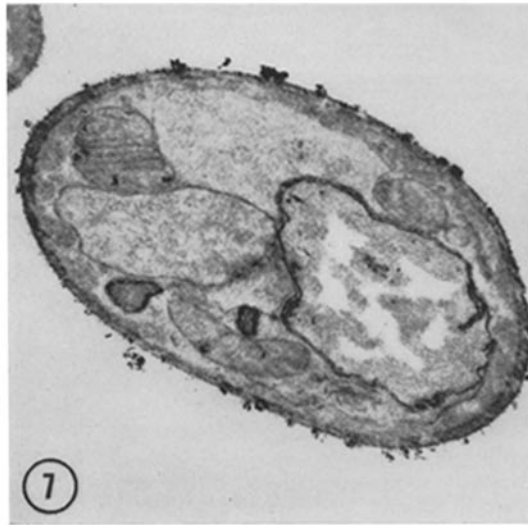
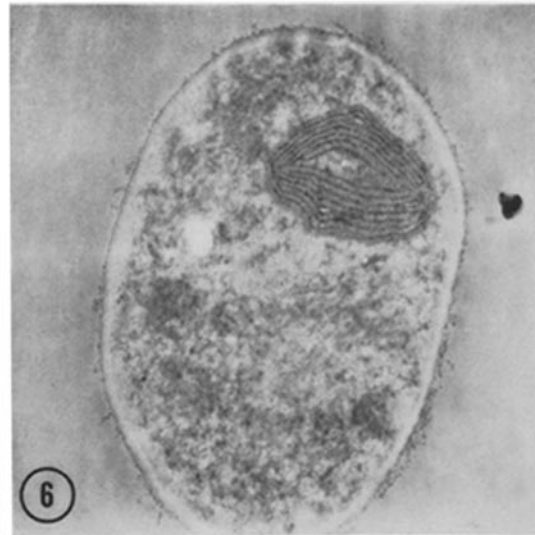
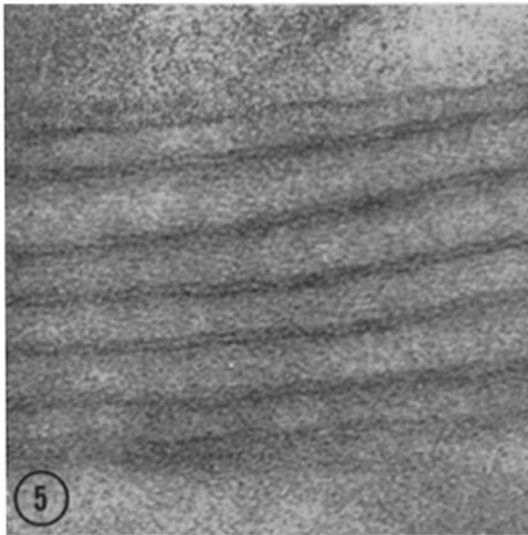
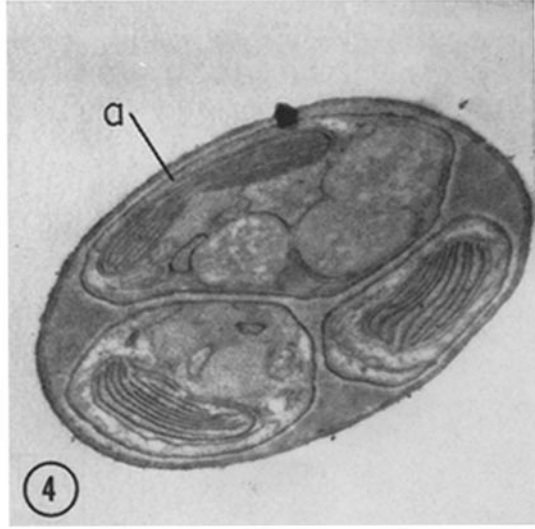
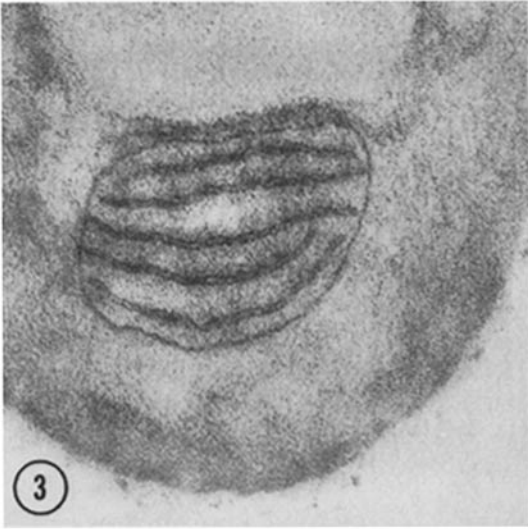
A thin section of *Cyanidium caldarium* fixed in 2 per cent osmium tetroxide.  $\times 9,500$ .

FIGURE 7

A thin section of *Cyanidium caldarium* cultured in the dark. Note the increased frequency and density of cellular inclusions.  $\times 10,000$ .

FIGURE 8

A thin section of *Cyanidium caldarium* cultured on a medium containing streptomycin  $\times 12,000$ .



carmine we were able to observe a similar small body within each vegetative cell and within each endospore. A body about 0.3  $\mu$  in diameter and containing granular material can be frequently seen in the electron micrographs and is presumed to be the nucleus (Fig. 1).

*C. caldarium* reproduces by the formation of endospores. We have observed up to eight endospores per mother cell (Fig. 4). Each endospore contains a single small chloroplast which appears to have been produced by division of the chloroplast of the mother cell.

Growth at 38°C. results in irreversible loss of the photosynthetic apparatus in some strains of *Euglena* (6), whereas photosynthetic rate in *Cyanidium* is maximal at 55°C. Streptomycin (SM) causes the irreversible loss of chlorophyll and chloroplasts from *Euglena gracilis* (7-9) and also inhibits chlorophyll accumulation in higher plant tissue (10, 11).

We have examined cells from *C. caldarium* cultures which were raised in the dark or treated with SM. Cultures placed in the dark lose their chlorophyll gradually, appearing pale green after ca. 23 days and pale yellow after ca. 33 days. Electron micrographs of cultures which had been in darkness for 98 days show an absence of the typical chloroplast. The presence of membranous structures the same size as the chloroplast, some of which may contain double membranes or lamellae (Fig. 7), suggests that the chloroplast membrane and segments of lamellae may be retained during growth in the dark. This is in contrast to the report that no trace of chloroplast lamellae can be observed in dark-grown *Euglena* (12).

Cells which had been cultured for 14 days on medium containing 1500 mg./l SM (as streptomycin sulfate) were pale yellow in color and upon electron microscope examination showed absence of chloroplasts (Fig. 8). We have not been able to determine whether the ultrastructural changes induced by SM treatment and by dark growth are identical. Endospore formation also appeared to have been affected by SM treatment; single endospores within mother cell walls were frequently seen. In both dark-grown and streptomycin-treated cultures cytoplasmic membranes appeared to be more numerous and distinct. Detailed studies of the effects of SM, darkness,

heat, and other factors on chloroplasts will be reported in subsequent papers.

*Cyanidium caldarium* differs from the blue-green algae in that it possesses what appears to be a discrete nucleus and its chloroplast consists of parallel lamellae bounded by a single membrane. However, its fine structure appears less complex than that of the even smaller alga, *Chromulina pusilla*, which has been shown to possess a flagellum, a mitochondrion, and a pyrenoid-containing chloroplast with a double-layered outer membrane (5).

The *Cyanidium* chloroplast appears to be transitional between the "primitive" photosynthetic apparatus of the photosynthetic bacteria and blue-green algae, and the more elaborate chloroplasts of higher algae and vascular plants.

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