Fine Structure of an Unusual Cytoplasmic Inclusion in the Diatom Genus, *Rhopalodia*

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With 7 Figures

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Introduction

Rhopalodia gibba O. Mueller and *R. gibberula* O. Muell. are motile, heavily silicified, freshwater diatoms. According to K l e b a h n (1896) the vegetative morphology of *R. gibba* was studied by P f i t z e r in 1869, who described in it anomalous cytoplasmic inclusions which he called "s p h ä r o i d i s c h e K ö r p e r." These bodies were usually found in pairs next to the nucleus and were distinctly different from the oil bodies in staining properties. All of the cells examined seemed to possess at least one of the bodies at all times. They multiplied by elongating slightly and then constricting; their multiplication did not necessarily coincide with diatom cell division.

K l e b a h n (1896) studied sexual reproduction or auxospore formation in *R. gibba* and, in addition to the nuclear changes, followed the spherical bodies, since they were also stained in his preparations. He recorded no change in their appearance throughout the entire process. K l e b a h n noted that vegetative cells contained 1 to 4 of them and reluctantly referred to them as pyrenoids, remarking that they seemed to be outside of the chloroplast. H e i n z e r l i n g (1908) refuted K l e b a h n's suggestion because the staining properties were in some cases distinctly different from those of any other diatom pyrenoid. Although he stated that the s p h ä r o i d i s c h e n K ö r p e r were not pyrenoids, H e i n z e r l i n g had no idea of their significance in the cellular activities of *R. gibba*. F r i t s c h (1945) accepted them as pyrenoids. In the present paper we are reporting on the nature of these inclusions as observed in thin sections with the electron microscope.

Materials and Methods

R. gibba and *R. gibberula* were collected locally and grown in culture as previously described (Drum 1963). Specimens were fixed 4 hours in 1% OsO_4 containing Ca^{++}, buffered at pH 6.1, or overnight in cold phosphate-buffered 3% glutaraldehyde, postfixed 3 hours in 1% OsO_4 (Sabatini et al. 1963) buffered with veronal acetate at pH 7.4 and containing Ca^{++}. Specimens were embedded in Epon (Luft 1961), sectioned with a Du Pont diamond knife, mounted in an LKB Ultratome and examined in an RCA EMU 3 F electron microscope after staining with uranyl acetate. Organisms were examined in wet mounts under oil immersion and also from slides of Feulgen stained material (Bowen 1956).

Observations

Inclusions

The inclusions are located in approximately the center of the diatom cell (Figs. 1 to 3). They are ellipsoid to ovoid in shape, 4 to 6 μ wide, 5 to 7 μ long and are frequently surrounded by a clear area 50 to 400 mμ wide. They are separated from the diatom by a cytoplasmic membrane of the diatom (Figs. 1, 3, and 7). No connections with the diatom cytoplasm have been recognized. The inclusions have a five-layered wall: the outer dense layer, 12 mμ thick, is occasionally resolved into two layers, each 5 mμ thick. Next are a less dense layer, 6 mμ thick, a 4 mμ dense layer followed by another less dense 6 mμ layer and finally an inner dense layer 6 mμ thick (plasma membrane) (Figs. 5 and 6). Lamellae (flattened vesicles) about 16 mμ wide and 25 mμ apart extend from near the inner layer and occasionally traverse the body. They tend to parallel each other and are more numerous near the periphery of the inclusion (Figs. 4, 5, and 7). The central portion is characterized by an amorphous appearance (Fig. 7). Moderately electron dense circular profiles, 50 to 60 mμ in diameter, resembling oil droplets from diatom (Drum and Pankratz 1964) and other algal chloroplasts (Gibbs 1962), occur singly between lamellae (Figs. 3 and 7). Also, 18 to 22 mμ granules occur in aggregations between the lamellae (Fig. 5).

Diatom protoplasm

The nucleus is a flat discoidal structure appressed to the chloroplast and is limited by a typical nuclear envelope containing pores (Figs. 4 and 5). The Chloroplast surrounds most of the central cytoplasm (Figs. 4 and 5).

Fig. 1. Longitudinal section of diatom, *Rhopalodia gibba*, which shows the inclusion (*I*) near the center of the cell. The pyrenoid (*P*) lies within the chloroplast. ×3,500 (Glutaraldehyde-OsO_4).

Fig. 2. Transverse cross section through central region of *R. gibberula*, which shows the position of two inclusions (*I*), the flattened nucleus (*N*), the pyrenoid (*P*), and the vacuole (*V*). ×7,000 (OsO_4).

Fig. 3. Inclusion from *R. gibberula* contains lamellae (*L*) which are closed vesicles. Oil droplets (*OD*) occur between them. ×30,000 (Glutaraldehyde-OsO_4).

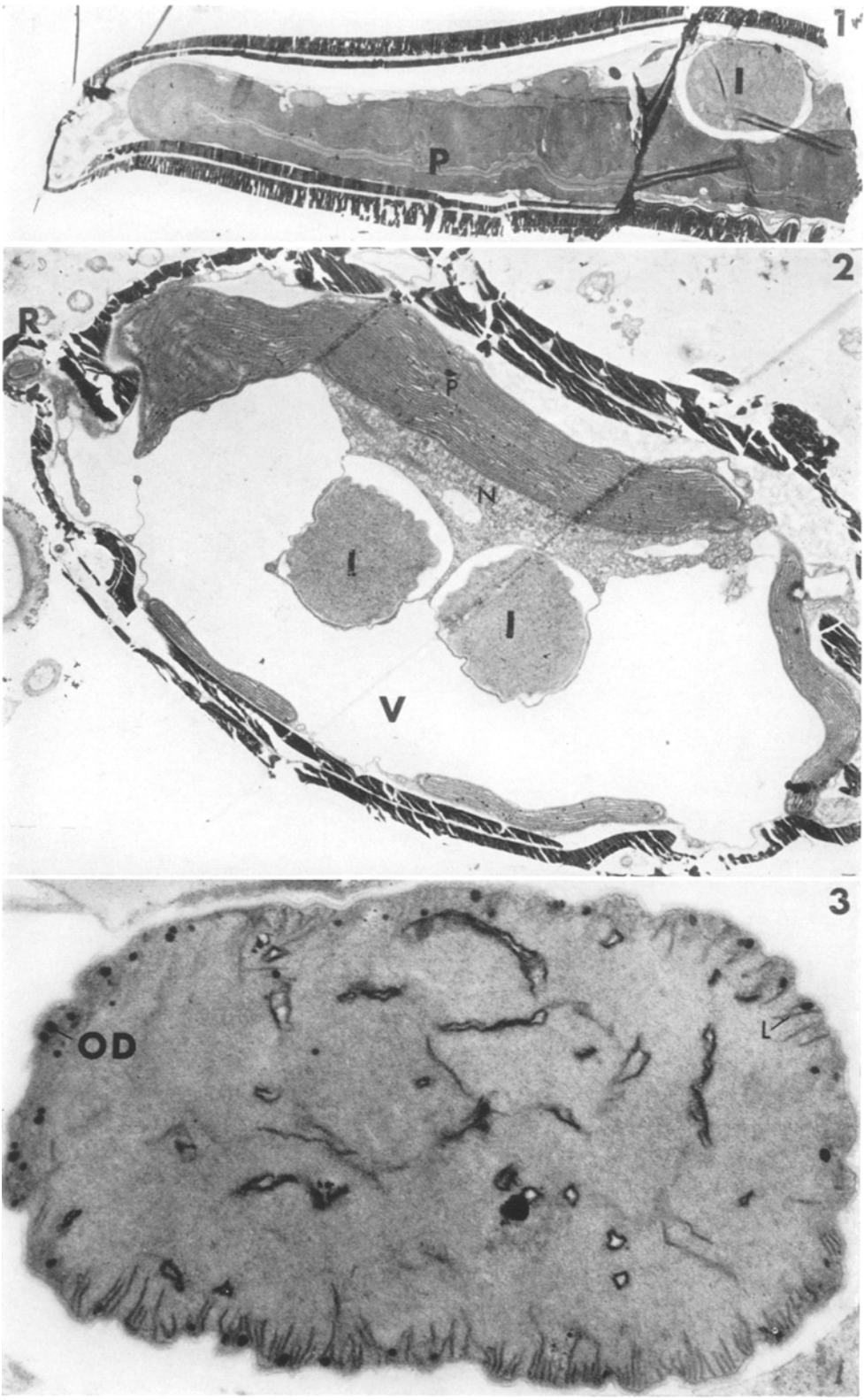

Fig. 1—3.

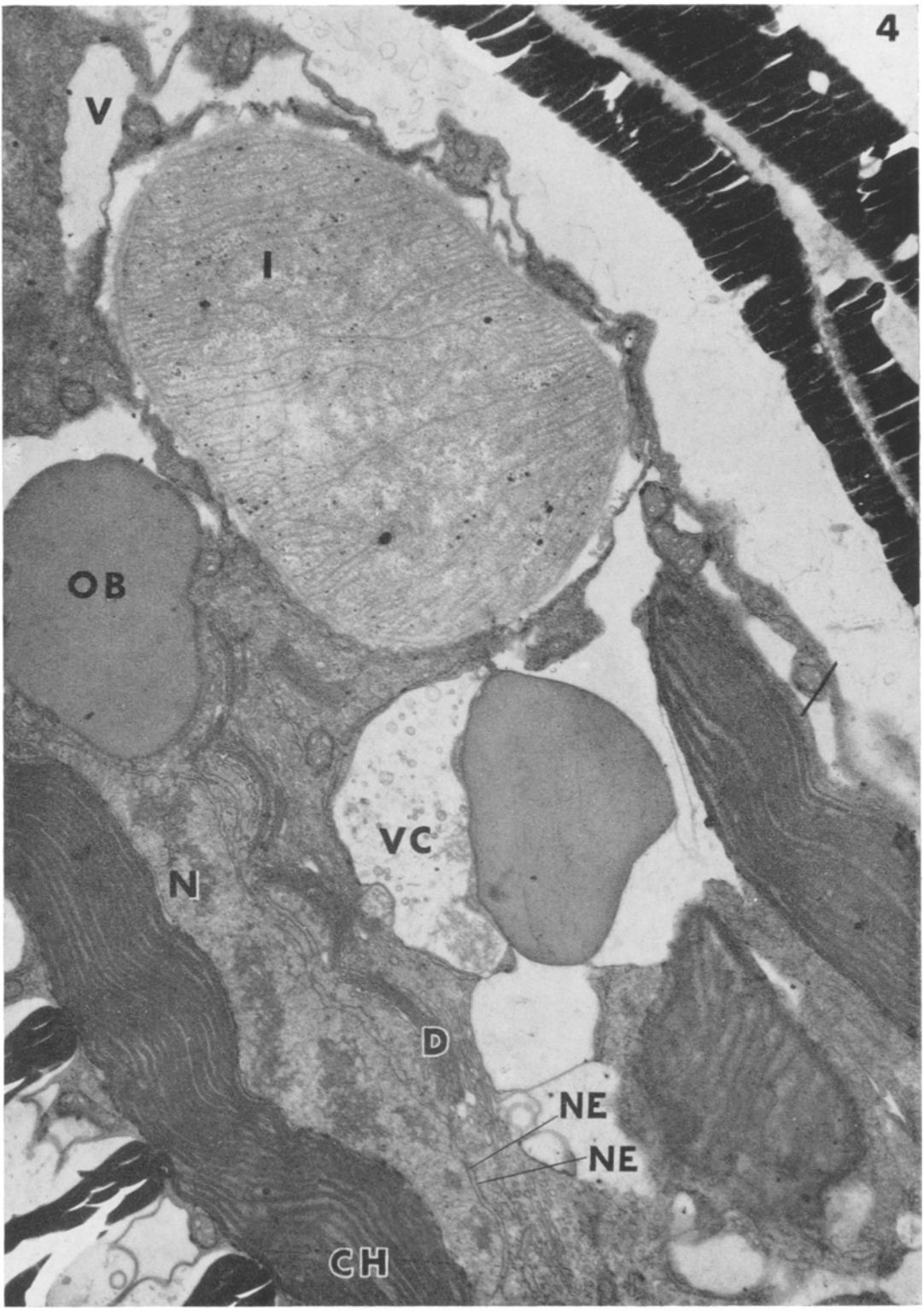

Fig. 4. Legend see page 147.

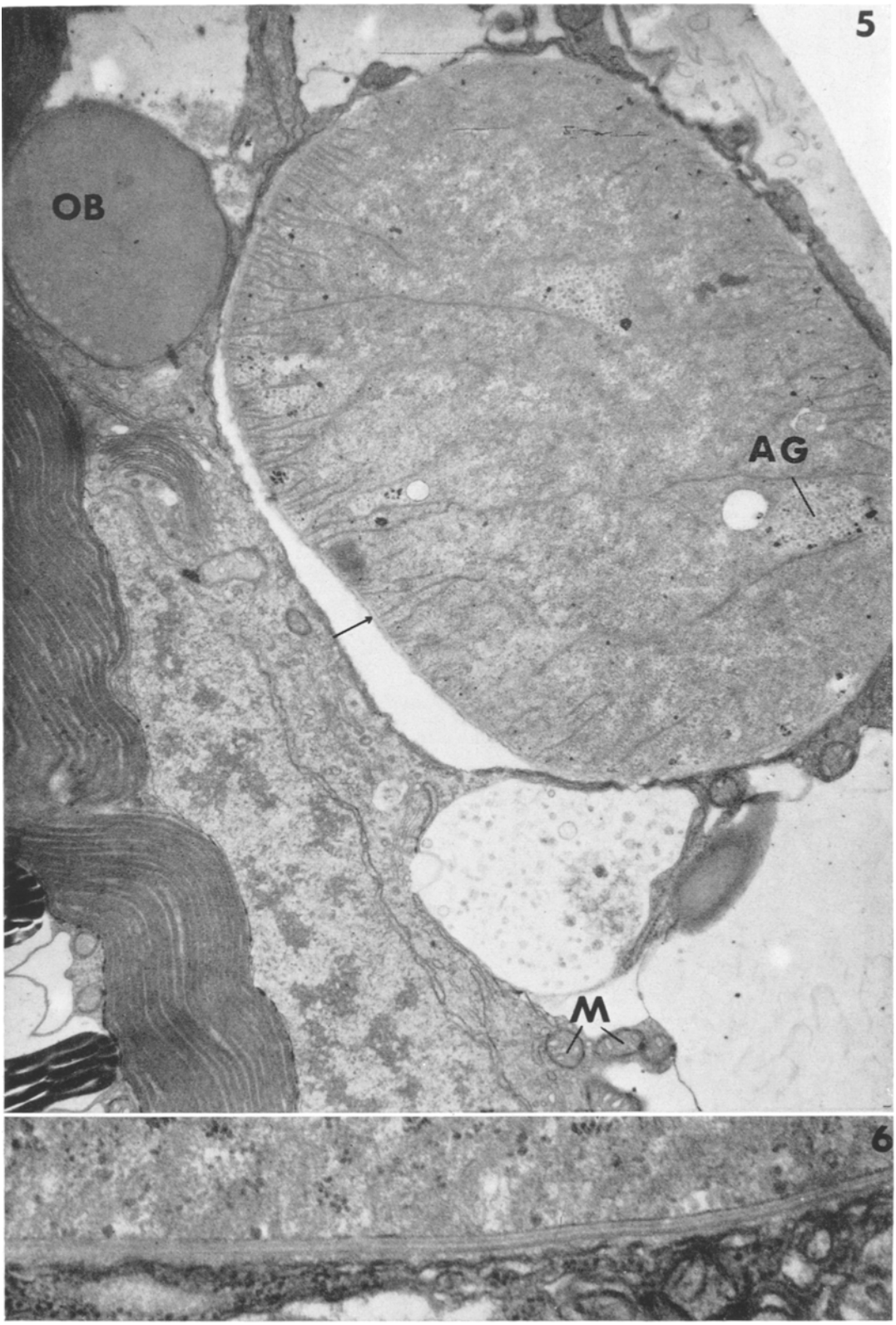

Fig. 5 and 6. Legend see page 147.

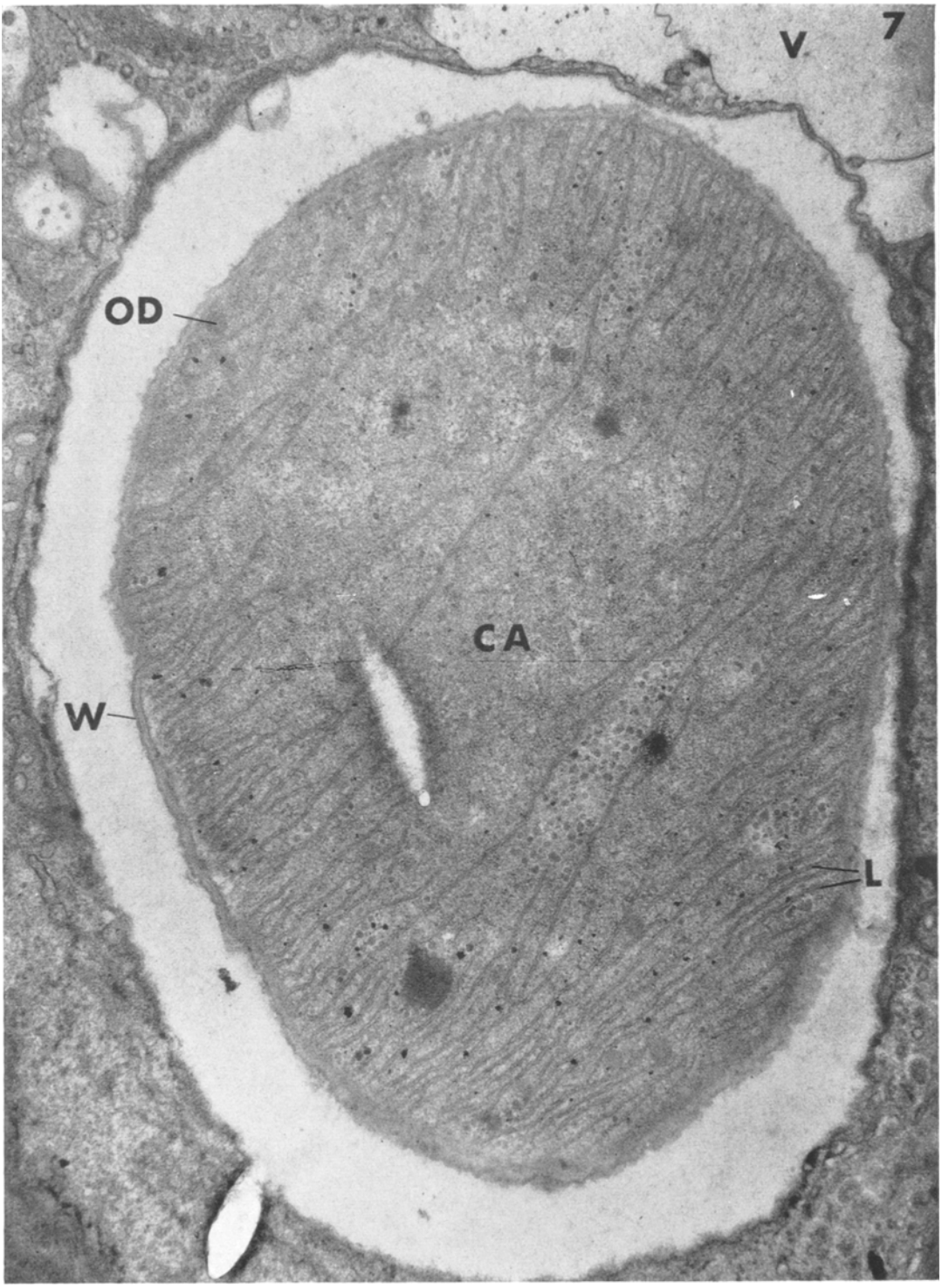

Fig. 7.

The inclusions can be distinguished from the diatom nucleous after Feulgen staining for DNA; the former are uniformly pink, whereas the nucleus has a granular appearance. The granular appearance of the nucleus was reported by Klebahn from haematoxylin preparations, and this may correspond with the denser areas of the nuclei seen in our electron micrographs (Fig. 5). The dictyosome complex is perinuclear (Figs. 4 and 5) as in other diatoms (Drum and Pankratz 1964). The vesicular complex (Drum and Pankratz 1964) contains mostly small circular profiles. The pyrenoid is an elongated-flattened, ellipsoidal structure 60 to 100 μ long found entirely within the chloroplast (Figs. 1 and 2) and is similar to other diatom pyrenoids (Drum and Pankratz 1964). Oil bodies are uniformly electron dense structures (Figs. 4 and 5). Mitochondria are sausage-shaped, 2 to 8 μ long, 0.2 to 0.3 μ wide, and occur throughout the cell (Fig. 5).

Discussion

We feel that the inclusions described from the diatoms, *Rhopalodia gibba* and *R. gibberula,* are either unique organelles or intracellular organisms. Their occurrence in each diatom may support the organelle idea; if such were the case their function remains unknown. If they are intracellular organisms, they resemble neither bacteria nor fungi but are similar to blue-green algal cells. Points of similarity include the wall structure, lamellae, interlamellar granules, and mode of multiplication (Pankratz and Bowen 1963; Ris and Singh 1961). In the living diatom cell they are readily distinguishable from the other cell constituents.

A blue-green alga, *Richelia intracellularis* has been reported living within the centric marine diatom, *Rhizosolenia sp.* (Desikachary 1959).

Key to Labelling

AG = 18 to 22 mμ inclusion granules; *CA* = amorphous central area of inclusion; *CH* = Diatom chloroplast; *D* = dictyosome; *I* = inclusion; *L* = 14 to 16 mμ wide inclusion lamellae; *M* = mitochondria; *N* = nucleus; *NE* = nuclear envelope; *OB* = amorphous oil body; *OD* = oil droplet; *P* = pyrenoid; *R* = raphe fissure; *V* = vacuole; *VC* = vesicular complex; *W* = inclusion wall.

Fig. 4. Central cytoplasm of *R. gibba* here contains one inclusion (*I*), several perinuclear dictyosomes (*D*), nucleus (*N*), vesicular complex (*VC*), two amorphous oil bodies (*OB*), and portions of the vacuole (*V*) and the chloroplast (*CH*). ×24,000 (Glutaraldehyde-OsO_4).

Fig. 5. Several aggregations of 18 to 22 mμ particles (*AG*) occur between the lamellae in this inclusion of *R. gibba.* Five-layered wall structure of the inclusion is shown at arrow. Mitochondria (*M*) and an oil body (*OB*) are also shown. ×27,000 (Glutaraldehyde-OsO_4).

Fig. 6. Inclusion wall section which shows five-layered structure, 35 to 40 mμ thick. ×66,000 (OsO_4).

Fig. 7. The central area (*CA*) of the inclusion from *R. gibba* is amorphous. The clear area around the inclusion is usually seen in both electron and light microscope preparations. The inclusion lamellae (*L*) and wall (*W*) are also noted. ×50,000 (Glutaraldehyde-OsO_4).

Intracellular blue-green algal symbionts have also been described in other unicellular organisms (Hall and Claus 1963). The relationship between the diatom and its blue-green alga-like inclusion is obscure, although nitrogen fixation may be involved (Fogg 1953), since the diatoms thrive on media without any added nitrogen source.

Compared to previous electron microscope studies of both diatoms and blue-green algae, our observations suggest that the inclusion is not a pyrenoid but possibly a modified coccoid blue-green alga living inside of at least two species of *Rhopalodia*. To date we have been unable to either isolate them from the diatoms or to grow the diatoms without them.

Summary

1. Ellipsoidal to ovoid inclusions in the diatoms *Rhopalodia gibba* and *R. gibberula* were examined in the electron microscope.

2. The inclusions are 4 to 6 microns wide, 5 to 7 microns long and separated from the diatom cytoplasm by a five-layered wall.

3. Lamellae about 16 mμ wide and 25 mμ apart occur near the periphery, with oil droplets and aggregations of 18 to 22 mμ particles occurring between them.

4. They are neither pyrenoids nor oil bodies.

5. They may be unique cell organelles whose function is unknown or possibly modified coccoid blue-green algae.

6. The cytoplasm of the two species of *Rhopalodia* resembles that of other diatoms.

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