THE CYTOPLASMIC FINE STRUCTURE
OF THE DIATOM, *NITZSCHIA PALEA*

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ABSTRACT

The cytoplasmic fine structure of the motile, pennate diatom, *Nitzschia palea* was studied in thin sections viewed in the electron microscope. The cells were fixed in OsO_4, embedded in methacrylate, and immersed in 10 per cent hydrofluoric acid (HF) for 36 to 40 hours to remove the siliceous cell wall prior to sectioning. The HF treatment did not cause any obvious cytoplasmic damage. The dictyosome complex is perinuclear, and located only in the central cytoplasm. Mitochondria are sparse in the central cytoplasm, but abundant in the peripheral cytoplasm, and fill many of the transvacuolar cytoplasmic strands. Characteristic, amorphous oil bodies fill certain cytoplasmic strands and probably are not leucosin. The pyrenoid appears to be membrane limited, and oil droplets are found adjacent to the pyrenoid. The pyrenoid of another diatom, *Cymbella affinis*, is also membrane-limited. The membrane limiting the pyrenoid may be a composite of the terminal portions of chloroplast discs, facilitating rapid movement of photosynthate into the pyrenoid matrix, where the characteristic oil droplets may be formed. Carinal fibrils are found singly in each carinal pore, and may be involved in the locomotion of *Nitzschia palea*.

Early cytological work employing staining techniques indicated that diatoms possess a discrete nucleus, nucleoli, chloroplasts, pyrenoids, mitochondria, vacuoles, oil droplets, and frequently bodies of unknown composition or function (3, 16, 18, 23). Initial electron microscope observations of the cytoplasmic structures of diatoms (6, 10, 31) were mostly restricted to small portions of the chloroplast. The chloroplasts of the diatoms examined were internally similar or identical with those found in the algae referred to by Sager and Palade (33) as "non-grana–containing algae;" this observation has been discussed by Gibbs (13).

The siliceous cell walls of diatoms have made them difficult subjects for thin-sectioning (8). In a paper published in 1851, Bailey (1) described a method for studying the structure of the siliceous frustule. He dissolved the silica slowly with dilute hydrofluoric acid (HF), presuming that the thinnest areas would dissolve first. In addition, he observed that the protoplast did not seem to be affected by the action of the hydrofluoric acid.

A technique has been developed for removing the silica from diatoms after embedding in methacrylate (9). The silica is removed prior to sectioning by immersing the entire block in dilute HF. The embedded material is darkened by the HF treatment, indicating that some reaction may have taken place; however, no damage to the fine structure was observed that could be attributed to the action of the HF.

Members of the diatom genus *Nitzschia* are found in both fresh-water and marine environments (34). Certain representatives from both environments are easily maintained in pure culture (24), including *N. palea*, which has been an experimental organism in several laboratory studies (2, 17, 36). A knowledge of the cytoplasmic fine structure of *N. palea* may permit a better understanding
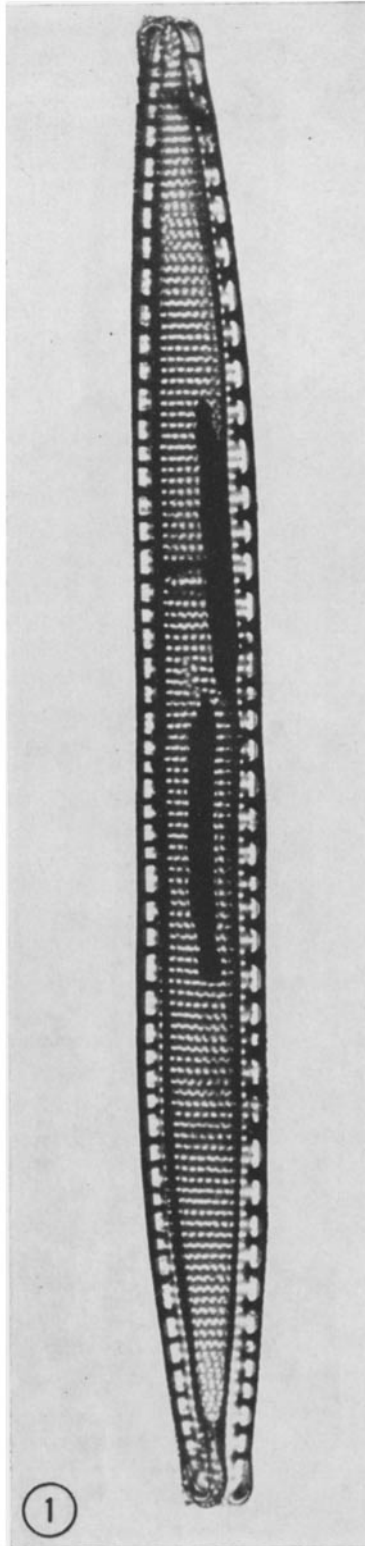
of the observations made during investigations on this organism.

Included in the original description of the method for removing silica (9) were the preliminary observations of the non-siliceous fine structure of *N. palea*. More extensive observations on the fine structure of *N. palea* have been made and are reported here; this paper is concerned especially with the dictyosomes, mitochondria, oil bodies, pyrenoids, and locomotion.

MATERIALS AND METHODS

A clone of the motile, pennate diatom, *Nitzschia palea*, was grown and subcultured on a chemically defined agar medium (Pringsheim's *Micrasterias* medium, see reference 30) in a growth chamber at 20°C, 400 foot candles, with 12-hour days and nights.

The cultured organisms were fixed *in situ* on the surface of the agar at room temperature for 40 minutes, using 1 per cent veronal acetate–buffered osmium tetroxide solution with 0.002 M $CaCl_2$, pH 7.4; they were then washed twice with deionized H_2O, and dehydrated with 30-minute changes of 30, 50, 75, and 95 per cent, and four changes of absolute ethanol. The organisms were embedded in methacrylate (three parts methyl, two parts butyl), and 1 per cent benzoyl peroxide (w/v): polymerization was done in an oven at 60°C for 26 hours. The blocks were coarsely trimmed to expose the specimen mass and placed in 10 per cent

Key To Labeling

Ab, abutment of two chloroplasts	*M*, mitochondrion
	N, nucleus
CF, carinal fibril	*Ncl*, nucleolus
CH, chloroplast	*NE*, nuclear envelope
CHd, chloroplast disc	*O*, overlap
CHM, chloroplast membrane	*OB*, oil body
	OD, oil droplet
CP, carinal pore	*P*, pyrenoid
CS, cytoplasmic strand	*PM*, pyrenoid membrane
Cv, circular vesicle	*Sp*, special vesicle
D, dictyosome	*Tu*, tubule
Ev, evagination	*V*, vacuole
K, keel	

Figure 1   An electron micrograph of the siliceous frustule or cell wall of the diatom, *Nitzschia palea*. × 5000.
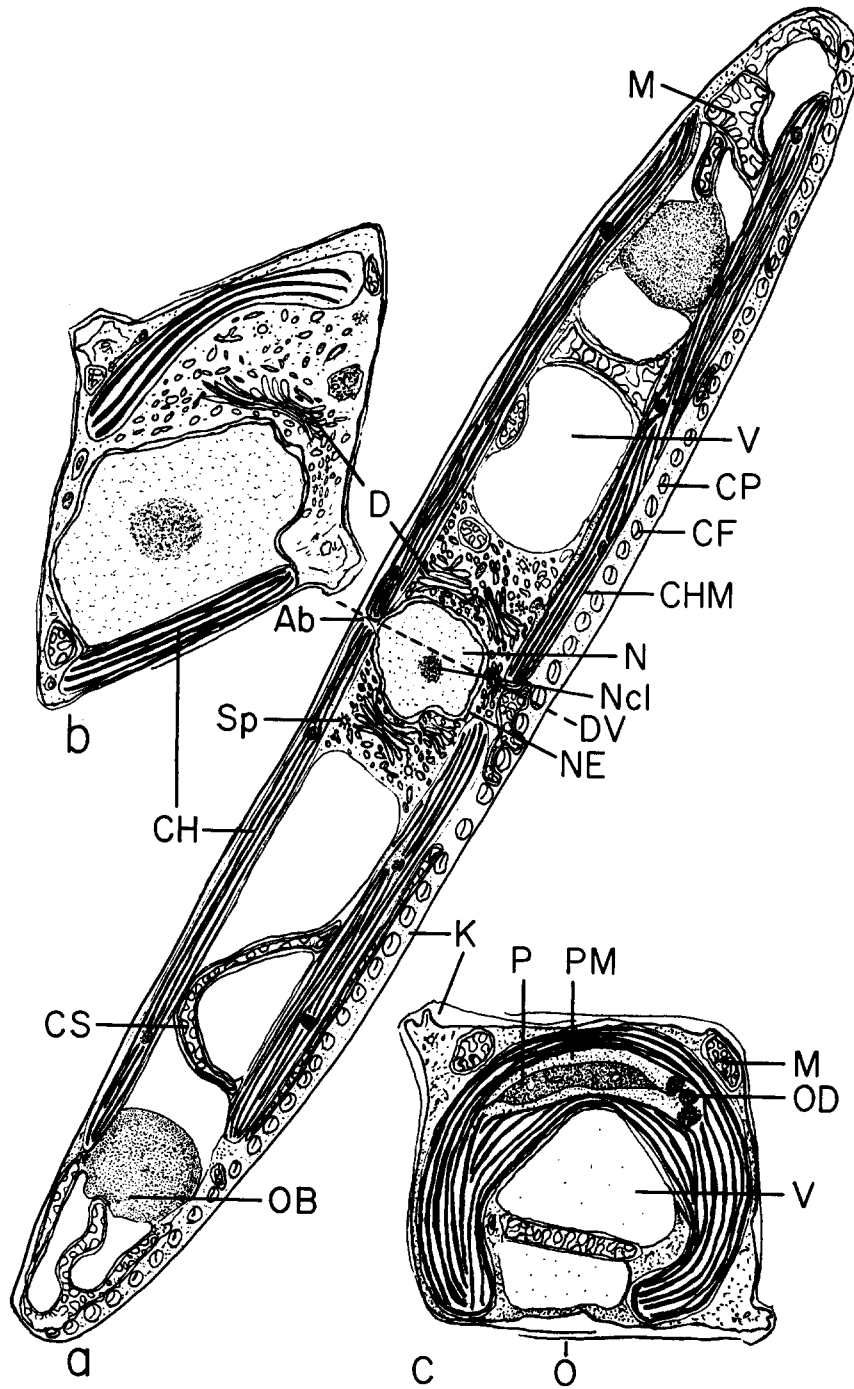
FIGURE 2 Schematic diagrams of three sections through the protoplast of *N. palea*: (a) a longitudinal section, (b) a transverse cross-section through the central cytoplasm, and (c) a transverse cross-section through the pyrenoid region of a chloroplast.

HF for 36 to 40 hours at room temperature to remove the siliceous portion of the cell wall. The treated blocks were washed several times with deionized H_2O and allowed to dry for 36 hours under a hood to remove residual HF prior to sectioning. HF was used after embedding to avoid any potential structural distortion that might have resulted from the removal of the siliceous frustule (19). Sections were cut on an LKB ultramicrotome, mounted on parlodion-coated copper grids, stained with $KMnO_4$, and covered with a thin layer of methacrylate (32) prior to examination in an RCA EMU-3F electron microscope.

Cymbella affinis, another motile, pennate diatom from a different family, was not cultured, but was collected locally and treated as above except in 12-ml centrifuge tubes, and spun down at 1200 RPM before each change.

Although HF-treated methacrylate blocks softened slightly, the material sectioned well and presented no unusual problems. Epon, however, softened much more during the HF treatment, becoming semifluid and unsuitable for sectioning.

Light microscope observations of wet mounts of *N. palea* included examination for motility, and *in vivo* staining with FerroVer (Hach Chemical Company, Ames, Iowa), a powdered orthophenanthroline iron reagent. About 100 mg of Ferro-Ver was added to four large drops of a dense suspension of agar free *N. palea*.

OBSERVATIONS

An electron micrograph of the siliceous frustule is shown in Fig. 1, which illustrates the general shape of the cell. In sectioned material this frustule was removed by HF. All of the cytoplasm lies within the frustule, as is pictured in a diagram of a longitudinal section in Fig. 2 *a*; a cross-section through the central cytoplasm is diagrammed in Fig. 2 *b*, and Fig. 2 *c* is a cross section showing the region of the chloroplast that contains the pyrenoid.

Longitudinal sections through the central cytoplasm are shown in Figs. 3 to 5. The nucleus (*N*) usually contains one or two nucleoli (*Ncl*) and extends across the central cytoplasm, lying adjacent to the respective membranes of the two chloroplasts (*CHM*) at points *I* and *II* in Figs. 3 to 5.

The dictyosomes (*D*) are loosely grouped around the nucleus and are located exclusively in the central cytoplasm. In addition to the discrete stacks of flattened vesicles seen in Figs. 3 to 5, the adjacent vesicular components are considered to be part of the dictyosome complex which fills most of the central cytoplasm not occupied by the nucleus. The vesicular profiles (*Cv*) about 50 mμ in diameter, that are found in the space between the dictyosomes and the nuclear envelope, seem to be continuous with either of these structures, but no single profile has been observed that is continuous with both. Distinct from the dictyosome vesicles are circular profiles 40 to 50 mμ in diameter (*sp*) that are bordered by a concentric ring of smaller circular profiles 11 to 12 mμ in diameter, as seen in Fig. 3. They may be examples of rough surfaced endoplasmic reticulum.

There are two parietal chloroplasts in *N. palea* (3, 4), one in each half of the cell as divided by the dotted line *DV* in Fig. 2 *a*. Each chloroplast extends from the points of mutual abutment (*Ab*), in the center of the cell, to within 2 or 3 microns of the respective end of the cell. The two chloroplasts approach each other, as in Fig. 5 at point *Ab*, but have not been observed to join. The chloroplast discs (*CHd*) are usually in groups of three. The average disc width is 20 mμ. As seen in Fig. 9, the chloroplast in each half of the cell is parietal to a separate vacuole (*V*). In most sections, as in Fig. 4, the chloroplasts each seem to be limited by a double membrane (*CHM*), but in Fig. 5 a triple membrane is evident. The third element may be a cytoplasmic membrane. Evaginations (*Ev*) occasionally extend from the chloroplast into the central cytoplasm (Fig. 3) but have not been observed in other parts of the cell. Throughout the chloroplasts are oil droplets 100 to 200 mμ in diameter, seen in Figs. 5, 8, and 9 (*OD*).

Mitochondria (*M*) are found in the central cytoplasm, but the greatest amount of mitochondria is found in other parts of the cell. The peripheral cytoplasm between the chloroplasts and the cell membrane is usually filled with mitochondria, as seen in Fig. 6. The transvacuolar cytoplasmic strands (*CS*) are also usually filled with mitochondria that are limited by double membranes

FIGURE 3 Longitudinal section through the central cytoplasm which shows the prominent nucleus (*N*) and the dictyosomes (*D*) which occupy most of the central cytoplasm. The section also cuts through one of the eccentric keels (*K*), showing the carinal pores (*CP*) each with a single carinal fibril (*CF*). $\times$ 54,000.
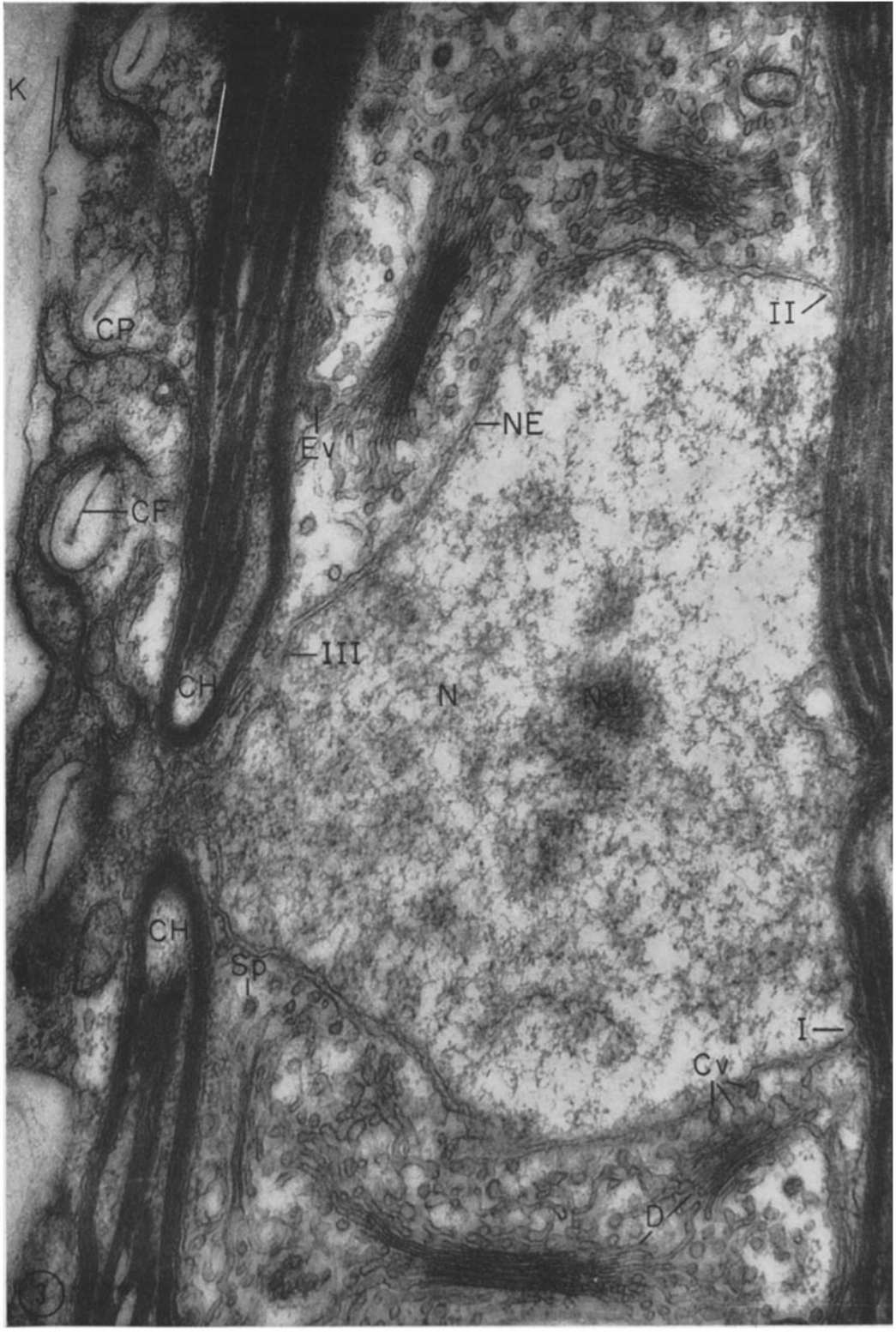
and contain the characteristic extensions of the inner membrane. These extensions are tubular structures and appear as vesicles in cross-section, as seen in Figs. 6, 7, and 9. Some portions of the cytoplasmic strands are enlarged to enclose large amorphous oil bodies (OB), such as those seen in Figs. 6 and 7.

A single discoidal pyrenoid (P) is located in each chloroplast. The pyrenoid profile in Fig. 8 is membrane-limited (PM) and filled with a dense granular matrix that appears more dense than the adjacent chloroplast matrix. The pyrenoid matrix is homogeneous except for four small tubules (Tu). The tubules have not been observed to be connected to the chloroplast lamellae. The membrane around the pyrenoid is 7 mμ thick and does not seem to be continuous with any of the nearby chloroplast disc membranes. The greatest accumulation of oil droplets in the chloroplasts occurs in the vicinity of the pyrenoid, but oil droplets have not been observed in the pyrenoid itself; this observation agrees with that of Gibbs (15). However, a point of contact between the pyrenoid membrane and an adjacent oil droplet can be seen at point C in Fig. 8.

The pyrenoid of the other diatom species examined, Cymbella affinis, is larger than that of N. palea, and is seen in Fig. 10. Here the chloroplast discs butt against the pyrenoid membrane and are sometimes continuous with it (see insert). In Fig. 10 the pyrenoid is bisected by two distended chloroplast discs.

Members of the diatom genus Nitzschia possess two canal-like raphes, one in each valve (7, 20). These extend longitudinally the length of the cell in diagonally opposed eccentric keels (K) that are seen in cross-section in Fig. 9. In the sides of each canal are openings called carinal pores (12). The longitudinal section through the central cytoplasm seen in Fig. 3 includes a portion of one of the keels, which is external to the chloroplasts. The section cuts through five carinal pores (CP), four of which are partially transversed by a single fibril (CF)

14 mμ in diameter. These pores, each with a single fibril, occur along the entire lengths of both keels, and have not been observed in other portions of the cell. Mitochondria are frequently found in the cytoplasm adjacent to the carinal pores. No structures which could be interpreted as cilia or flagella have been observed.

The overlap (O) of the two halves of the cell wall can be seen in Figs. 7 and 9.

DISCUSSION

Central Cytoplasm

The central cytoplasm is, primarily, the location of the nucleus and the closely associated dictyosome complex. It is probable that the dictyosomes are a single structure, or a group of connected structures composed of a variety of vesicular elements. They may be the "double rods" observed in light microscope studies (21, 23). The nuclear envelope does not appear to possess discrete pores such as those described by Marinos (27). Whether or not the nuclear envelope merges with the chloroplast membrane at points I and II in Figs. 3 to 5 cannot be ascertained from the figures presented. Similar relationships have been described and discussed by Gibbs for other algal groups (14).
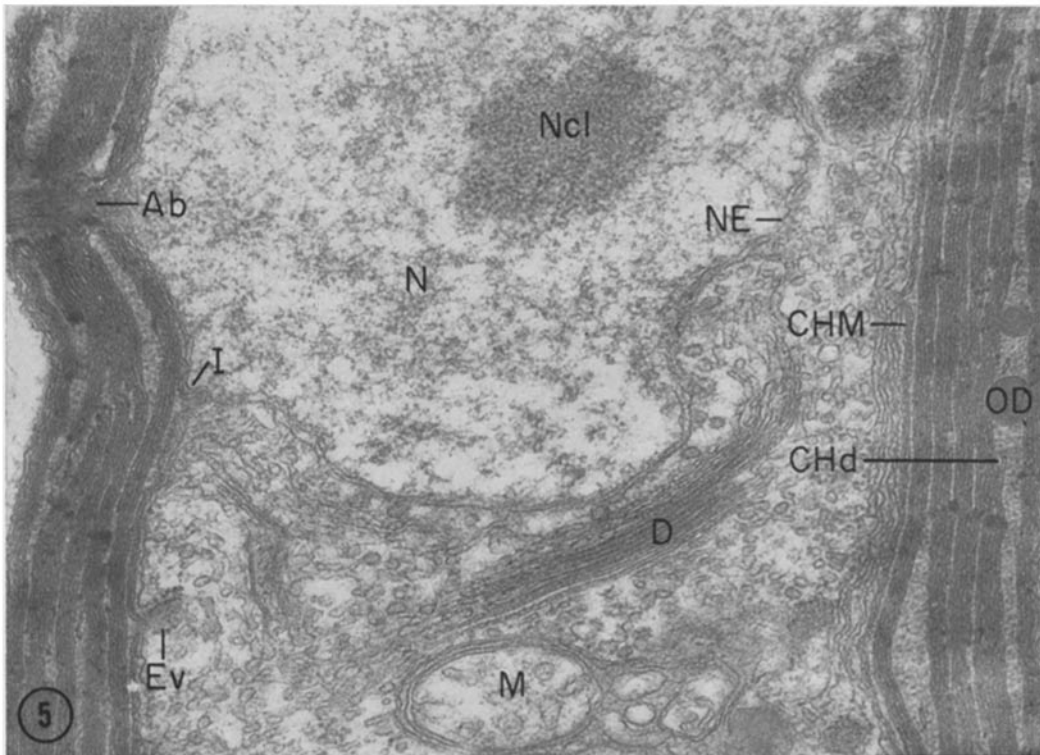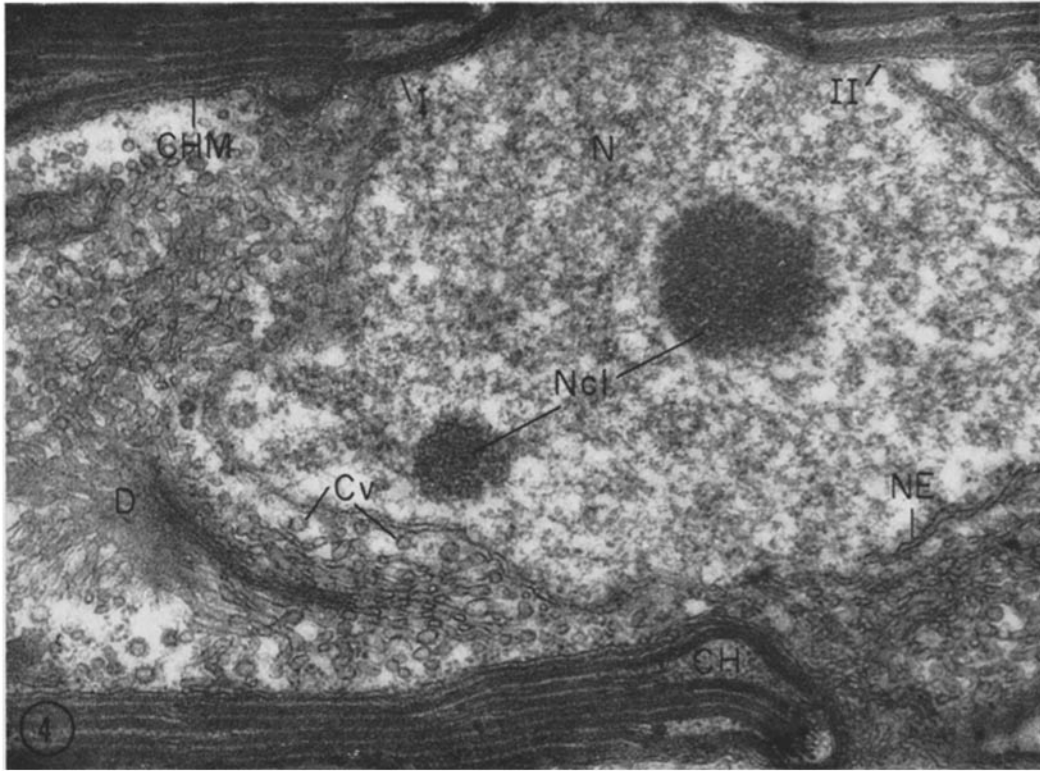
Mitochondria

The presence of numerous mitochondria in the peripheral cytoplasm may be responsible for certain staining phenomena observed in the light microscope. Smith (35) stained diatoms with KCN to show the presence of iron in the layer immediately beneath the cell wall. A similar qualitative test performed by the author on N. palea, using FerroVer, indicated a relatively high concentration of iron in the same layer, as evidenced by a bright orange girdle. This may be due in part to an abundance of protein-bound iron in enzyme systems located in peripheral mitochondria.

Guilliermond (16), in stained preparations of

FIGURE 4 The central cytoplasm of N. palea, showing a large dictyosome (D), and many associated vesicles in the adjacent cytoplasm. The chloroplasts appear limited by a double membrane (CHM). × 47,000.

FIGURE 5 A portion of the central cytoplasm showing a mitochondrion (M), and the abutment (Ab) of the two chloroplasts; an oil droplet (OD) occurs in one of the chloroplasts. × 54,000.

Navicula sp. (a motile, pennate diatom), showed presumed mitochondria to be more abundant in the vicinity of the vacuole than in the central cytoplasm; these mitochondria, as those observed here, may have been located in transvacuolar cytoplasmic strands.

Oil Bodies

Large oil bodies are frequent in both cultured and wild diatoms, and are considered to be an accumulation of food reserve that diminishes if the diatoms are kept in the dark for extended lengths of time (34). Their characteristically dense, amorphous appearance in electronmicrographs is probably sufficient evidence that they are not comparable to the leucosin bodies or reservoirs that have been observed in other algae (15, 25). Leucosin has been considered to be one of the food storage materials of some diatoms (22), and is reportedly dissolved out by fixation and embedding procedures similar to the ones used by the author (15). The location of oil bodies may be predetermined by the location of special areas of the cytoplasmic strands that become inflated with food reserve during periods of active photosynthesis. Light microscope studies of *N. palea* (4) indicate that the oil bodies shown in Figs. 6 and 7 are typically located.

The oil droplets in the chloroplasts appear to have the same density as the larger oil bodies, but the compositional identity of the two types of structures is unknown.

Pyrenoid

Recent work (11) has strongly indicated that oil is not the primary photosynthate in diatoms, but accumulates by means of secondary reactions that seem to occur very rapidly. Evidence is given in Fig. 8 at point C which shows that the pyrenoid may be directly involved in the accumulation of secondary reaction products. The tubules in the pyrenoid matrix are presumably extensions of adjacent chloroplast discs (15), and may thus be the source of primary photosynthate which enters the pyrenoid directly from the chloroplast discs; synthesis and/or accumulation of secondary products may occur in the pyrenoid matrix, and form the characteristic droplets as they leave the pyrenoid.

The membrane that borders the pyrenoid may be a composite of the terminal portions of chloroplast discs. This consideration is based upon the observation that the chloroplast discs which butt against the membrane occasionally merge with it; these discs thus become continuous with the interior of the pyrenoid. This would presumably permit rapid movement of primary (and/or secondary) products of photosynthesis directly into the pyrenoid.

Membrane-limited pyrenoids have not been observed in other algae, and Gibbs' micrograph of a pyrenoid of *Nitzschia angularis* does not appear to be membrane-limited (15). The presence of membrane-limited pyrenoids in diatoms may be both metabolically and phylogenetically significant, depending upon the frequency with which they can be demonstrated in both diatoms and other algal groups. All of the pyrenoids observed in diatoms prepared according to the above method have been observed to be membrane-limited.

Locomotion

The efforts of many observers over more than a century of careful thought and study have not solved the problem of diatom movement (5). Many explanations of movement have been offered (8, 28, 37), but none are universally acceptable. Diatoms seem to move without the aid of either

FIGURE 6 Longitudinal section through a distal portion of the cell, showing two lobes of the chloroplast (*CH*), with a large amorphous oil body (*OB*) located in a cytoplasmic strand (*CS*) between them; the peripheral cytoplasm and some of the cytoplasmic strands are filled with mitochondria (*M*). × 28,000.

FIGURE 7 Longitudinal section through the end of the cell; adjacent to the distal end of one of the chloroplasts (*CH*) is a large amorphous oil body (*OB*) that is joined by cytoplasmic strands (*CS*) containing mitochondria (*M*). Point *O* shows the overlap of the two halves of the cell wall. × 47,000.

cilia or flagella, and movement is generally considered to be restricted to those forms which possess a raphe.

The structures (carinal fibrils, *CF*) seen in the carinal pores in Fig. 3 are too small to be observed in living specimens, and considerable caution is necessary in any attempt to consider their possible role in diatom movement. The possibility exists that they (*CF*) may be localized extensions of the cell membrane, but there is no evidence that satisfactorily shows that they ever extend completely across a pore. If they do not function as direct organs of locomotion, then they may be part of a system of membranous valves which control the movements of water in the raphe canal, and, conceivably, the movements of the diatom.

Contractile Vacuoles

Pascher (29) has recorded and discussed the occurrence of a contractile vacuole in *Nitzschia hantzschiania*, but no structures have been observed in *N. palea* that resemble the contractile vacuoles seen in electron micrographs of other algae (26).

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FIGURE 8   Cross-section of the chloroplast of *N. palea* containing a profile of the pyrenoid (*P*) which is bounded by the pyrenoid membrane (*PM*), and filled with a dense granular matrix. An oil droplet (*OD*) lies next to the edge of the pyrenoid at point *C*. × 98,000.

FIGURE 9   Cross-section illustrating the parietal character of the chloroplast (*CH*), which surrounds the vacuole (*V*), and contains several oil droplets (*OD*). × 40,500.
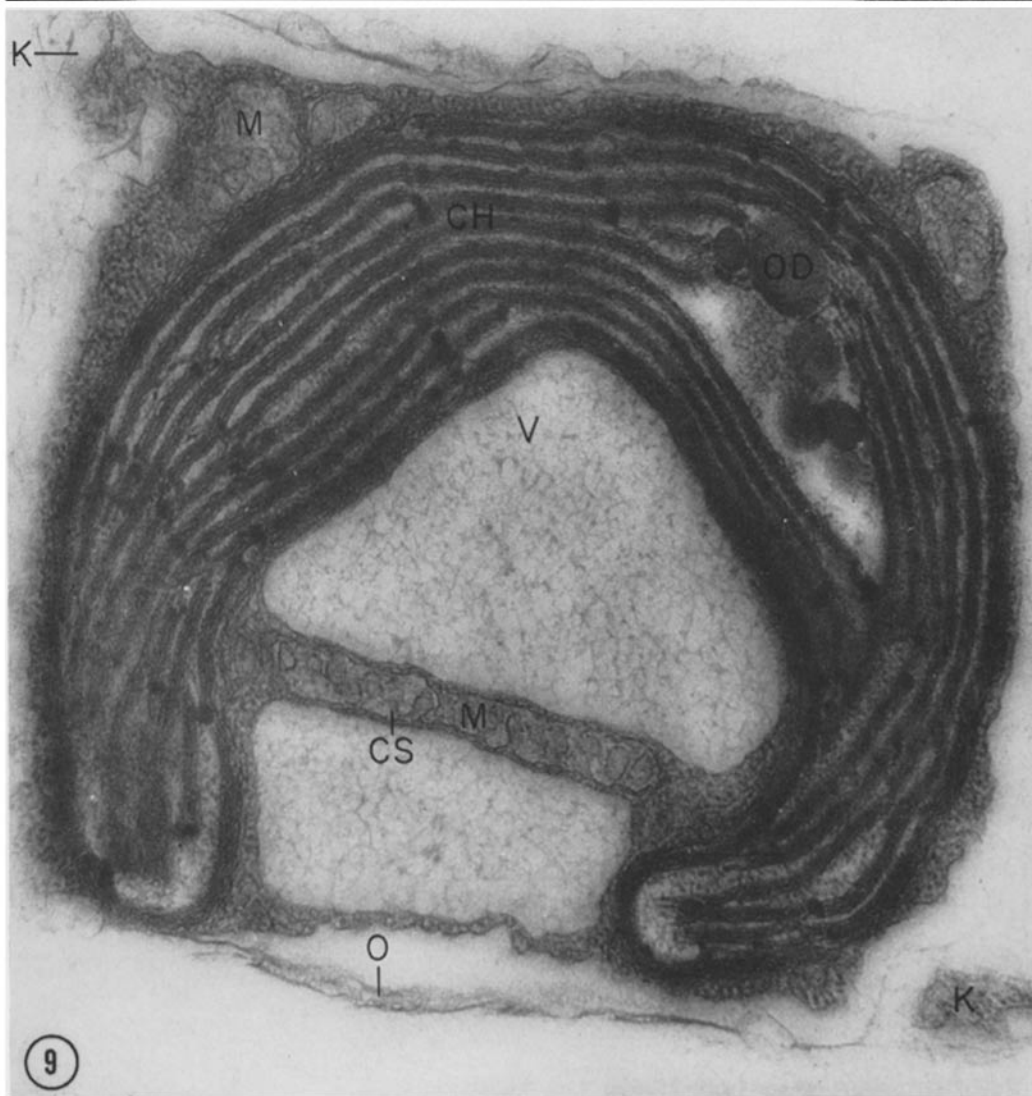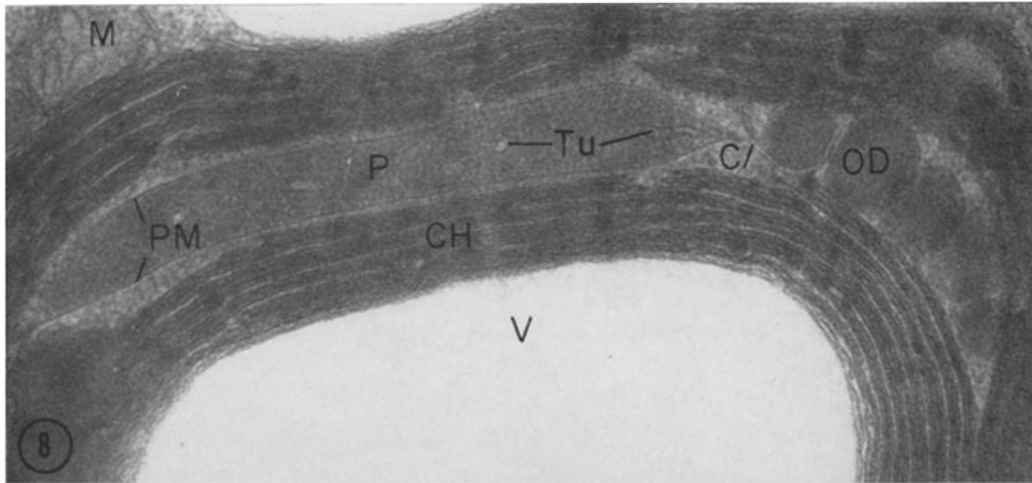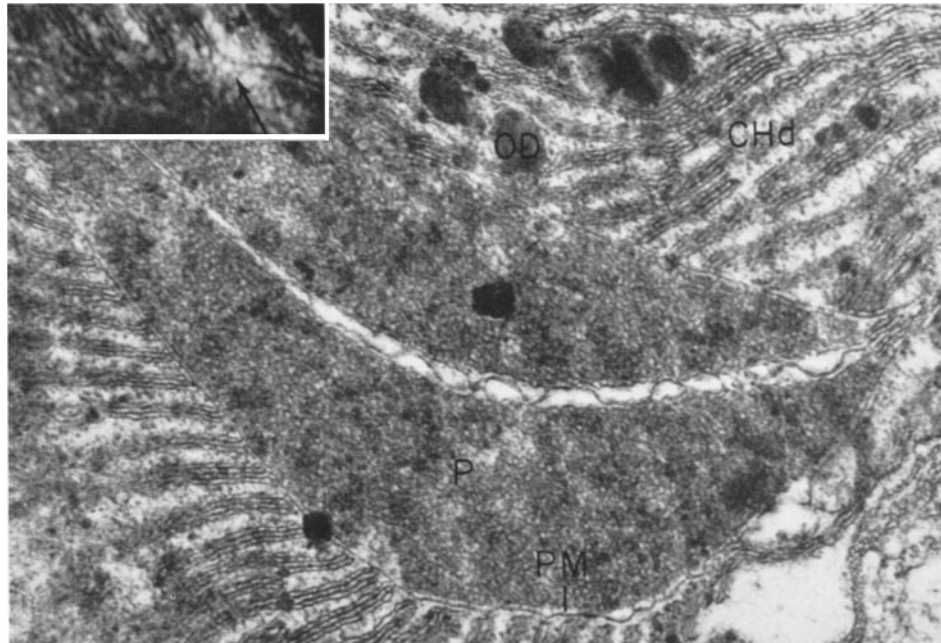
FIGURE 10 Section through the chloroplast of another diatom, *Cymbella affinis*, which contains a profile of the pyrenoid (*P*). The pyrenoid is membrane-limited (*PM*), and the chloroplast discs (*CHd*) butt against the pyrenoid membrane and often merge with it. × 40,000. The insert shows a chloroplast disc merging with the pyrenoid membrane. × 98,000.

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