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Cell and Lorica Fine Structure of the Chrysomonad Alga, *Dinobryon sertularia* Ehr. (Chrysophyceae)*

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Summary. The fine structure of wild living organisms of the chrysophycean alga, *Dinobryon sertularia*, has been studied after fixation with glutaraldehyde. A description of the cell body as revealed from serial sections is given. The similarity of the cellular organization to that of the related genus, *Ochromonas*, is emphasized. In addition, the microfibrillar arrangement of the lorica has been studied in negative staining. Possible modes of lorica formation are discussed, especially with the evidence presented that the mid region of the cell body is in direct, although perhaps transitory, contact with the lorica and shows a variety of vesicles suggesting secretory membrane flow.

Dinobryon is a pelagic chrysomonade genus living in ponds and lakes. The cell is individually surrounded by a vase-like lorica shell. The conical ends of the younger loricae are fixed to the inner side of the openings of the older loricae thus forming bushy colonies (Klebs, 1893; Fott, 1971), which in our material consisted of up to 15 individuals.

Its unique and characteristic lorica has made *Dinobryon* one of the classic objects in phycological research. In particular, the phycologists were intrigued by the question of how such a delicate "cellulose containing lorica" is formed (Klebs, 1893). As early as 1893 Klebs reported according to his light-microscopical observations: „*Allmählich scheidet nun mit ihren breiten Seiten die Zelle neuen Zellstoff aus, die Hülse wächst, während die Zelle selbst immer höher steigt*" ("gradually the cell secretes new cellulose with its broad sides, and the lorica is growing while the cell itself is ascending"). This observation is still a provoking one and is in obvious contrast to the majority of the current (not experimentally proven) cytological concepts of cellulose formation on the outer surface

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of the plasma membrane or in the cell wall (e.g., Northcote and Pickett-Heaps, 1966; Mühlethaler, 1967; Barnett and Preston, 1970; Northcote, 1971; Bowles and Northcote, 1972).

Electron microscopic information on the ultrastructure of this algal genus, however, is still very scarce. Except for some studies on the dried loricae using metal shadow cast (Karim and Round, 1967; Kristiansen, 1969, 1972a, b; Hilliard, 1971) there exist only two studies on the algal body itself by Joyon (1963) and Wujek (1969), both based on fixations with $KMnO_4$ or OsO_4. It has been the aim of the present study to give a description of the cell-lorica relationship of wild living *Dinobryon* as revealed in serial sections after glutaraldehyde fixation. In addition, we have demonstrated the fine structure of the lorica wall in negative staining.

Materials and Methods

The algae were collected from the lake Titisee, Black Forest (Germany), altitude 846 m, in the end of May. At this time of the year, *Dinobryon sertularia* is one of the most common algae of the near surface plancton of that lake. Fixations were performed with planctontic slurry upon collection, at the shore, either simultaneously with 2% glutaraldehyde and 1% OsO_4 (20 min at 4°C, for details see Franke *et al.*, 1969) or sequentially with 2% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2, 3 mM $CaCl_2$ (30 min, 0°C) followed, after repeated washing with cold buffer, by 2% OsO_4 in 0.05 M sodium cacodylate buffer (2 h at 0°C). Further washes and dehydration and embedding procedures were performed as usual (e.g., Franke *et al.*, 1972). Thin sections were cut in series at different levels of the lorica with a Reichert OmU 2 ultramicrotome. Sections were poststained with uranyl acetate and lead citrate according to the conventional procedures. The Siemens Elmiskop I A was used for electron microscopic observation.

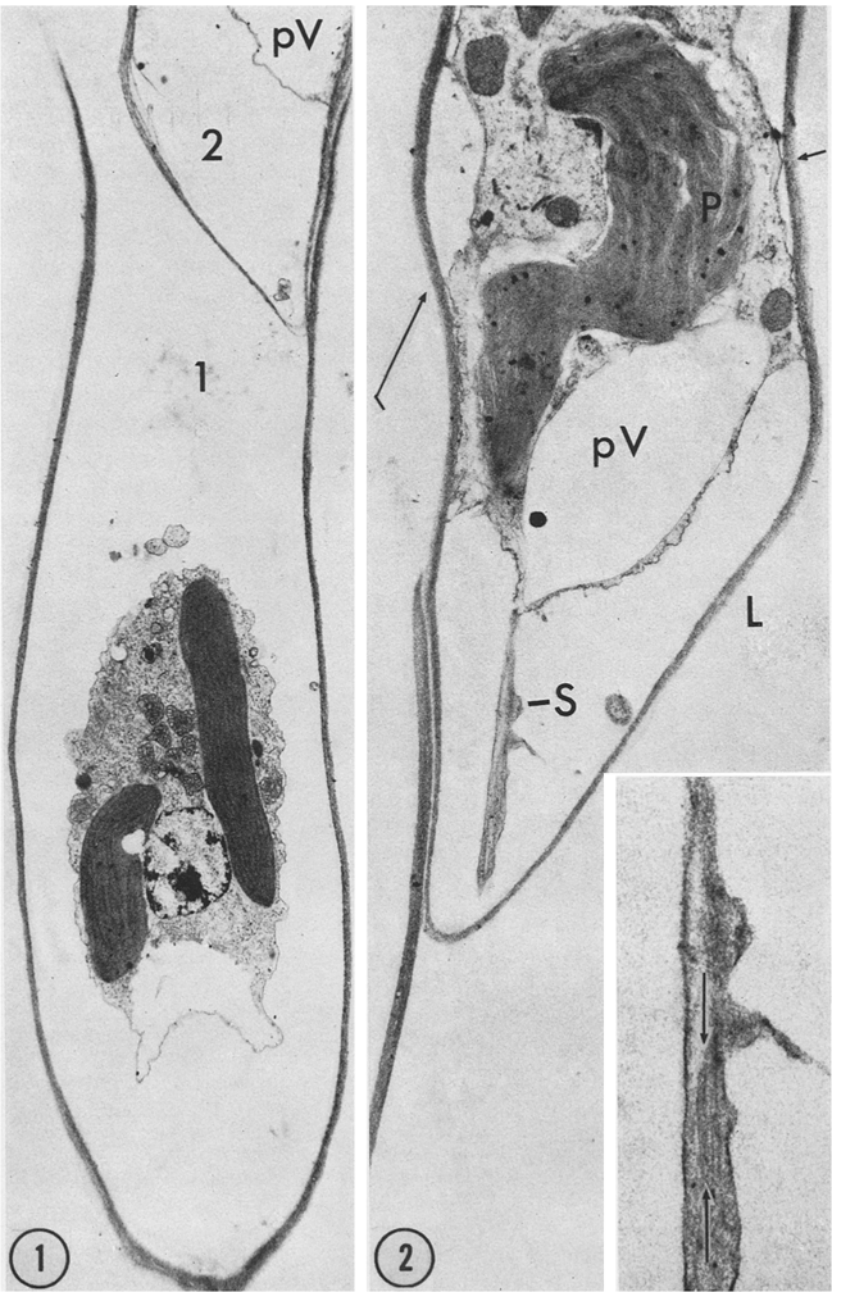
For better observation of lorica structure negative staining of whole loricae was performed immediately upon collection. The samples were pipetted onto formvar coated grids and were negatively stained with 2% phosphotungstic acid, adjusted to pH 7.2 with NaOH.

Results

Cellular Organization

Longitudinal median sections through the cell demonstrate the cytoplasmic organization of *Dinobryon* an dits position within the vase-like lorica (Figs. 1 and 2).

Figs. 1 and 2. Survey electron micrographs showing the *Dinobryon* cell in longitudinal sections and its position relative to the lorica. A daughter lorica (No. *2*) is attached near the opening of the old lorica (No. *1*) in the upper right corner of Fig. 1. The basal stalk (*S*) shows microtubular profiles (inset in Fig. 2, arrows). In addition, the cell is in contact with the lorica at many protrusions of the plasma membrane in a belt-like equatorial region (arrows). The zone of attachment of the young lorica (*L*) to the old lorica is visible in the left lower part of Fig. 2. *P* plastid; *pV* large posterior vacuole. Fig. 1, ×600; Fig. 2, ×10000, inset in Fig. 2, ×27000



Figs. 1 and 2

Fig. 2 demonstrates clearly that there are two regions at which the cell body, *i.e.* its plasma membrane, is in contact with the lorica, namely at the *basal stalk* as well as in the *belt-like mid zone* which is characterized by a somewhat ruffled surface appearance. The basal connection via the stalk has already been noted by Klebs (1893), Pascher (1913), Oltmanns (1922), (see also Krieger, 1930; Karim and Round, 1967; Pringsheim, 1963; Fott, 1971) and is also found in the cup-shaped loricae of the related chrysophycean genus, *Ochromonas* (e.g., Schnepf *et al.*, 1968; Schnepf and Kramer, 1969; Kramer, 1970, 1972). In our material the stalk was approximately 4 micra long but it might be capable of elongation, contraction or twisting, this being important for movements of the cell within the lorica (up- and down movements within the lorica according to Klebs, 1893; Pascher, 1913; Oltmanns, 1922; Karim and Round, 1967; cell rotations according to Pascher, 1913; Krieger 1930; Pringsheim, 1963). The electron microscope shows microtubules and slender cisternae to be the major constituents of the stalk (Fig. 2, inset). A similar organization has also been reported for the stalk of *Ochromonas malhamensis* by Schnepf and Kramer (1969), Kramer (1970, 1972).

The *Dinobryon* cell is principally ovoid, with a long axis of ca 10 μm, and a short one of 4—6 μm. The daughter cell lorica is always attached to the mother lorica with a zone of lateral attachment of ca 4 μm length in which individual lorica material fibers appear to be continuous between the two loricae, thus perhaps providing the coherence of the colony (Figs. 1 and 2, see also below).

The *nucleus* (average diameter 2.5 μm) shows one prominent nucleolus (Figs. 1 and 3). Dense chromatin ("heterochromatin") masses are identified at the nuclear envelope and this material is continuous with the perinucleolar heterochromatin (Fig. 3). A similar distribution has been noted in the light microscope by Joyon (1963) in OsO_4-haematoxyline stained cells of *Dinobryon*, but is not seen in his electron micrographs. The nuclear envelope appears as double membrane cisterna with normally structured nuclear pore complexes (e.g., Franke, 1970; identified in tangential section in Fig. 7).

The two *chromatophores* are of different size, the smaller one being posteriorly located, the larger one appearing as a band extending for about 2/3 of the cell body axis (Figs. 1 and 3).

Fig. 3. Details of a *Dinobryon* cell in median longitudinal section in a partial magnification from Fig. 1. The two flagella (arrows) at the anterior end of the cell are seen in cross section. The nucleus (*N*) in the posterior half of the cell is surrounded by the two plastids (*P*) and a cluster of mitochondria (*M*). The posteriorly located large vacuole (*pV*) is supposed to contain in vivo the reserve polysaccharide. In some regions plasma membrane invaginations suggesting exo- or pinocytotic processes can be detected (arrowheads). Magn. × 16000

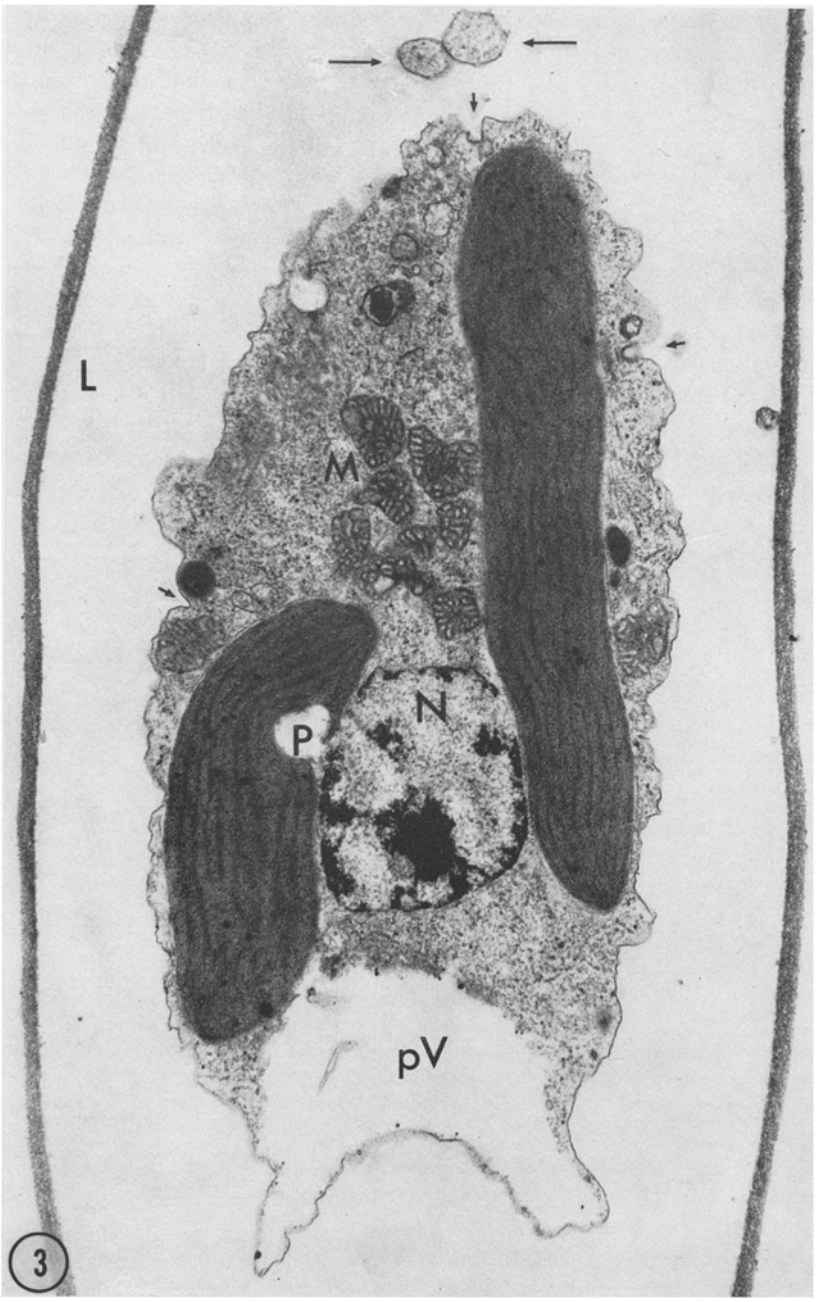


Fig. 3

There exists a clear spatial relationship between the nucleus and the chromatophores in that the nucleus is situated in a groove constituted by the plastids. This relàtionship is maintained by the luminal continuity between the nuclear envelope, the periplastidal cisternae, and the periplastidal reticulum (for terminology see Gibbs, 1962, 1970; Falk and Kleinig, 1968), a feature characteristic not only for *Chrysophyta* but for *Chromophyta* and *Euglenophyta* in general (Gibbs, 1970). The thylakoidal stacks consist of three discs each (e.g., Fig. 10, inset) and extend preferentially lengthwise through the plastidal stroma, with occasional interdigitations and bifurcations of the individual thylakoids (e.g., Figs. 3, 10 and 14) as also reported for other *Chrysophyceae* and related *Chromophyta* families (Gibbs, 1962, 1970; Falk, 1967; Falk and Kleinig, 1968). Lipid droplets are occasionally discernable in the stroma. An interplastidal *eyespot* (stigma) is located at the anterior end of the larger chromatophore (Figs. 13 and 14). It consists of rows of electron dense globules of somewhat variable size (Joyon, 1963; Wujek, 1969).

The *endoplasmic reticulum* (ER) is not very elaborate in *Dinobryon*, except for the periplastidal cisternae. Rough ER is mostly found in the vicinity of the nucleus (Figs. 4, 13 and 14), sometimes in aggregates resembling an „ergastoplasm" (Fig. 13). On the other hand, free non-membrane-bound polyribosomes in their typical helical formations are very conspicuous in the cytoplasm (Fig. 9).

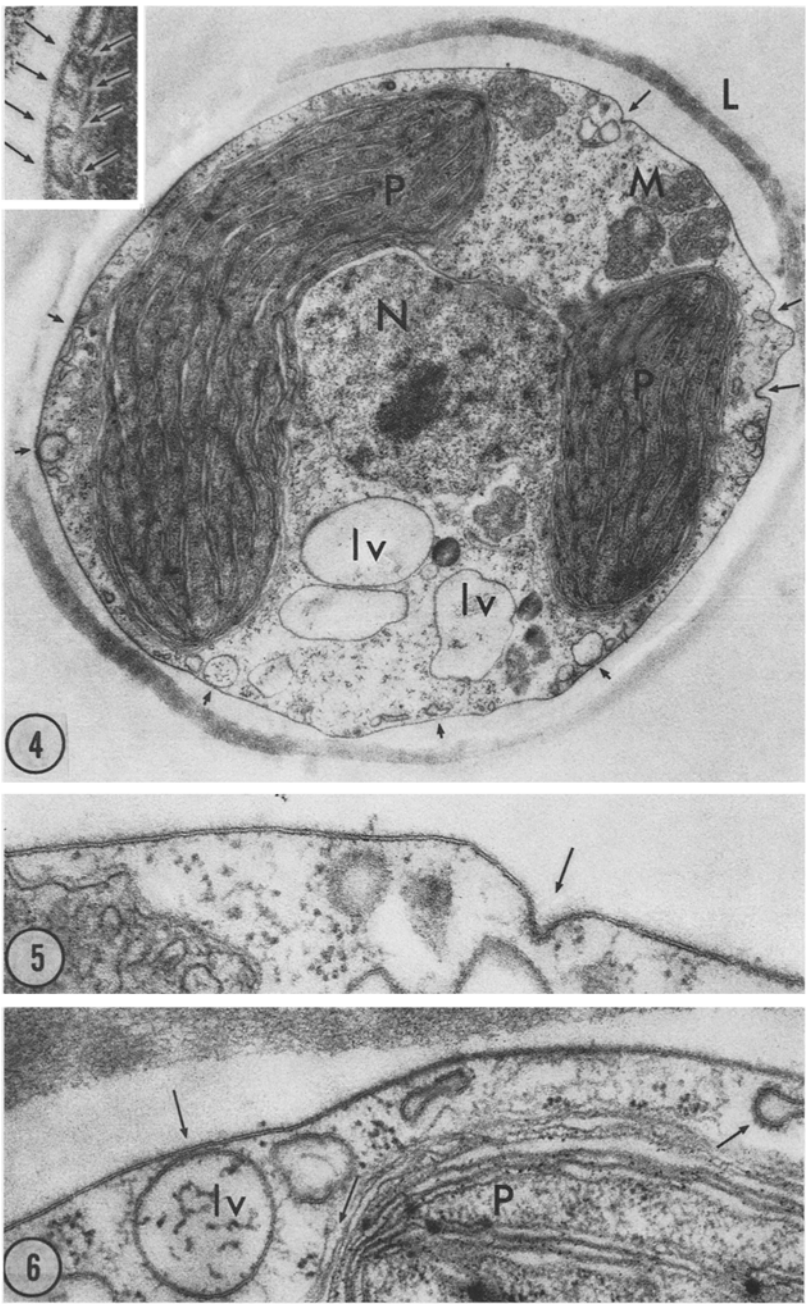
The internal phase of the *mitochondria* (diameter from 0.3 to 0.6 μm) is densely filled with tubular structures (e.g., Figs. 3, 11 and 12) and thus does not show any obvious deviations from other chrysophycean mitochondria (Leedale *et al.*, 1965).

At various places in the cytoplasm, free *lipid droplets* are observed (Figs. 3, 10 and 13) which do not reveal a surrounding membrane. Wujek

Fig. 4. Cross section of cell and lorica in the equatorial region. This region is characterized by the occurence of large smooth vesicles (*lv*). The arrows demonstrate the frequency of invaginations of the plasma membrane and the arrowheads denote subsurface vesicles lying underneath the plasma membrane. In the cytoplasmic space between plasma membrane and periplastidal cisterna cross sections of microtubular structures are visible (inset; arrows). Magn. × 15000; inset, × 72000

Fig. 5. The plasma membrane of *Dinobryon* reveals a clear dark-light-dark pattern. The arrow denotes an invagination. Magn. × 60000

Fig. 6. Higher magnification of the region near the plasma membrane. A coated ("bristle coat") vesicle is visible near the plasma membrane (arrow in the right). A large smooth vesicle (*lv*) containing somewhat granulofibrillar material is immediately subjacent to the plasma membrane (left arrow). The periplastidal cisterna is recognized in the center of this micrograph (arrow). Magn. × 60000



Figs. 4—6

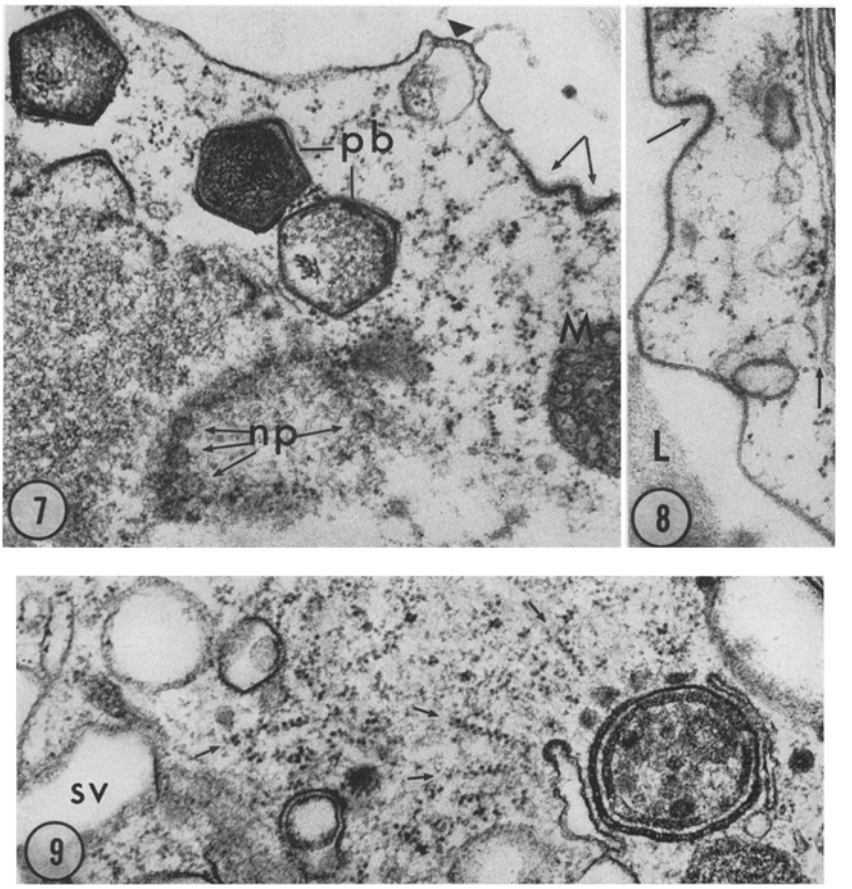


Fig. 7. Dense polygonal bodies (*pb*) are frequent in *Dinobryon*. This figure shows also tangential sections of nuclear pores (*np*) with central granules. The arrows in the upper right denote an area of the plasma membrane invaginations with regularly spaced dense knobs at the internal face (bristle coat appearance) and diffuse dense material on the external side. The triangle points to cross-bridges between a smooth vesicle and the plasma membrane. Magn. × 38000

Fig. 8. Cell periphery of *Dinobryon*. The periplastidal cisterna membrane is clearly revealed (arrow in the right) and shows ribosomes on its outer surface. A typical "coated" invagination of the plasma membrane is demonstrated by the left arrow. Note the contact between lorica (*L*) and plasma membrane in the very left. Magn. × 50000

Fig. 9. An unidentified spherical body is frequently found in the anterior third of the cell body. Electron dense globules, small vesicles as well as smooth surfaced cisternae are seen to build up this body. Helices of free polysomes are frequent in this region as well (arrows). *Sv* smooth vesicle. Magn. × 43000

(1969) has described a close association of such oil droplets with the mitochondria. Joyon (1963) found such storage lipid droplets to be enriched in the posterior region of the cell (also Pringsheim, 1963).

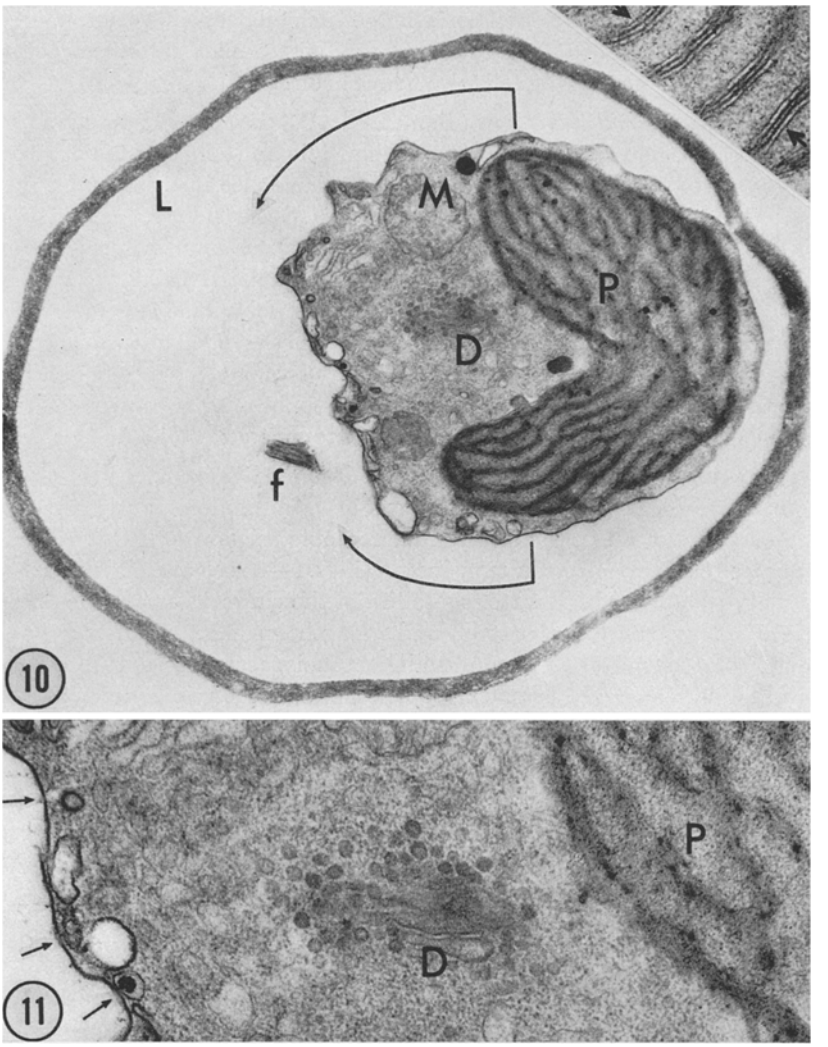
Dense polygonal inclusions (diameter ca 0.3 μm) are clustered in the equatorial region of the cell (Fig. 7). Their role and function is not clear. Joyon (1963) has described somewhat similar dense inclusions and suggested that they were endosymbiotic bacteria. Since endocytotic ingestion of food (phagotrophy) has been described in *Dinobryon* (e.g., Pringsheim, 1963; Wujek, 1969) and also in the closely related *Ochromonas* (e.g. Stoltze *et al.*, 1969) and other chrysomonads (Klebs, 1893; for review see Pringsheim, 1963; Allen, 1969) it is conceivable that such inclusions could be derived from ingested food particles. It is not clear in our sections whether these inclusions occur free in the cytoplasm or are surrounded by a vesicle membrane and thus represent "autophagic vacuoles".

The *large posterior vacuole* has been described since Klebs (1893) to contain the chrysolaminarin ("Leukosin"), a predominantly β-(1-3)-linked polyglucan. In the electron microscopic preparations this vacuole appears relatively electron translucent (Figs. 1—3; see also Joyon, 1963; Wujek, 1969). The high water solubility of the chrysolaminarin has been described by van Stosch (1952) and Pringsheim (e.g., 1963) who have made some chemical tests confirming the polysaccharide nature of this reserve substance. It is not clear whether the electron dense material associated with the inner side of the membrane of the large vacuole (Fig. 3) is a less soluble remnant of the reserve substances or is of a different chemical nature.

As all heterokontous algae, *Dinobryon* possesses two *flagella*, a long whiplash flagellum with mastigonemes ("flimmer", Figs. 1, 10 and 14) and a shorter naked flagellum (Figs. 1 and 3). The flagellar bases reveal the usual nine outer triplets without central microtubules in cross sections, whereas the flagella show the typical "nine plus two" pattern (Figs. 3 and 12).

The *Dinobryon* cell contains only one large, even light microscopically visible (Joyon, 1963) *dictyosome* (central diameter 1 μm) situated in the anterior third of the cell in a slightly eccentric parabasal position (Figs. 10—13). The stack is composed of up to 12 dictyosomal cisternae from which, in their marginal parts, many vesicles appear to pinch off (Figs. 10—13). Most of these dictyosome derived vesicles have an electron dense content. Frequently, the entire dictyosome appears to be embedded in a field of vesicles (Fig. 12). This great number of vesicles suggests a very high secretory activity of the dictyosome in *Dinobryon* (compare also Joyon, 1963; Wujek, 1969).

Several types of vesicles are found in the *Dinobryon* cytoplasm: (i) *Small smooth* surfaced *vesicles* with (Fig. 11) or without electron dense



Figs. 10 and 11. Section through the anterior third of the cell. In this region the cell shows a clear dorsoventrality with the secreting face here oriented to the left (left from the bar, as indicated by the arrows). An oblique flagellar section (*f*) is also identified in the free space between cell body and lorica. Note the bifurcations of the thylakoidal bands in the plastid (*P*). The inset in Fig. 10 shows in a partial magnification the band association of three thylakoids per band. Details of the dictyosome (*D*) show the abundance of small smooth vesicles, many of them with dense contents, in the dictyosome periphery, suggesting blebbing from the margins of the dictyosomal cisternae. Arrows denote regions in which vesicles have accumulated underneath the cell surface. Fig. 10, × 12000; inset in Fig. 10, × 36000; Fig. 11, × 30000

Fig. 12. Serial section to Fig. 10. The blebbing of different types of vesicles from the dictyosomal margin is clearly demonstrated in this section. Note again the larger smooth vesicles lying underneath the plasma membrane. An aggregate of small tubular elements is found between the plastid and a mitochondrion (arrow). Magn. × 48 000

contents (Fig. 12). (ii) *Large smooth vesicles* are especially numerous in the region near the flagellar base (Fig. 14) and in the equatorial region (Fig. 4). They have been hypothetically interpreted by Joyon (1963) as primitive "explosive vesicles" *i.e.* for extrusion in response to irritation. Many of these vesicles are found immediately underneath the plasma membrane suggesting a "pile up" (Figs. 4—9, 14). Similar structures are also found in the equatorial region. It cannot be said at this moment which vesicles

correspond to those forming the pulsating vacuoles described for *Dinobryon* by various authors (e.g. Klebs, 1893; Fott, 1971). Some of the larger smooth surfaced vesicles contain electron-opaque, fibrillar or granular

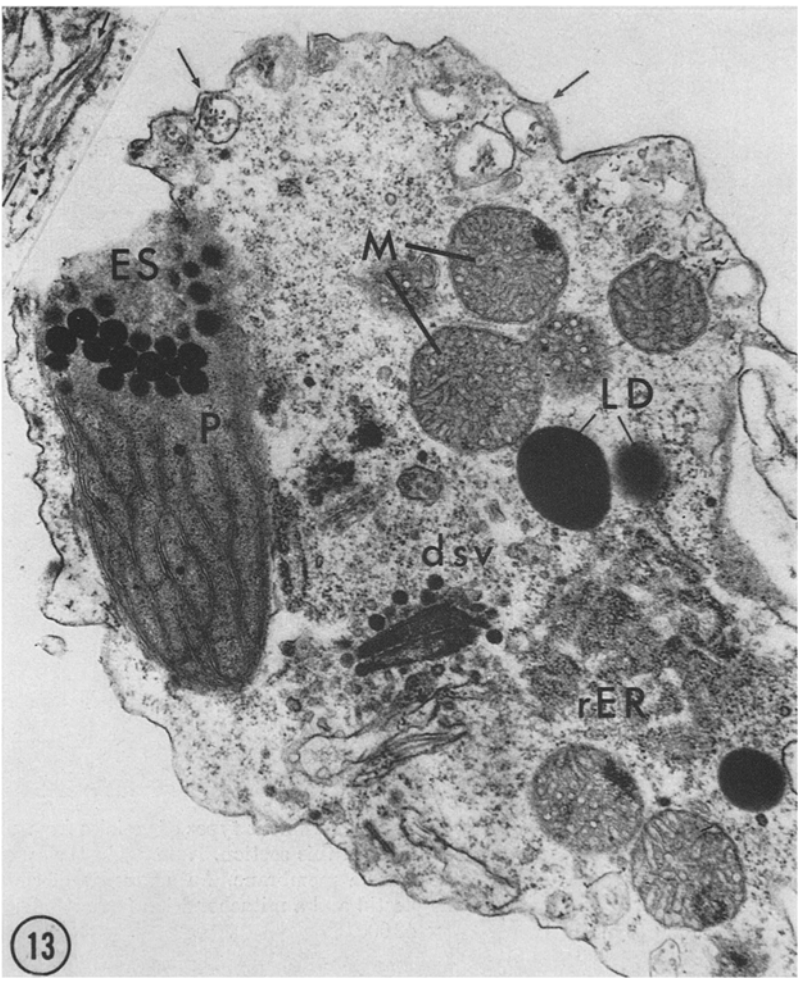


Fig. 13. Oblique section through the anterior half of *Dinobryon*. A vesicle aggregate ("dense smooth vesicles", dsv), perhaps representing a modified or marginally sectioned dictyosome, is found near a cluster of rough endoplasmic reticulum (*rER*). Large free lipid droplets (*LD*) are also frequent in the cytoplasm. The eyespot (*ES*, "stigma") consisting of regular arrays of dense globules is included in the anterior part of the plastid. The short arrows point to subsurface vesicles with granulofibrillar contents. The inset shows some of the large smooth vesicles containing tubular profiles indicative of assembly stages of mastigonemal elements. Magn. × 21000; inset × 46000

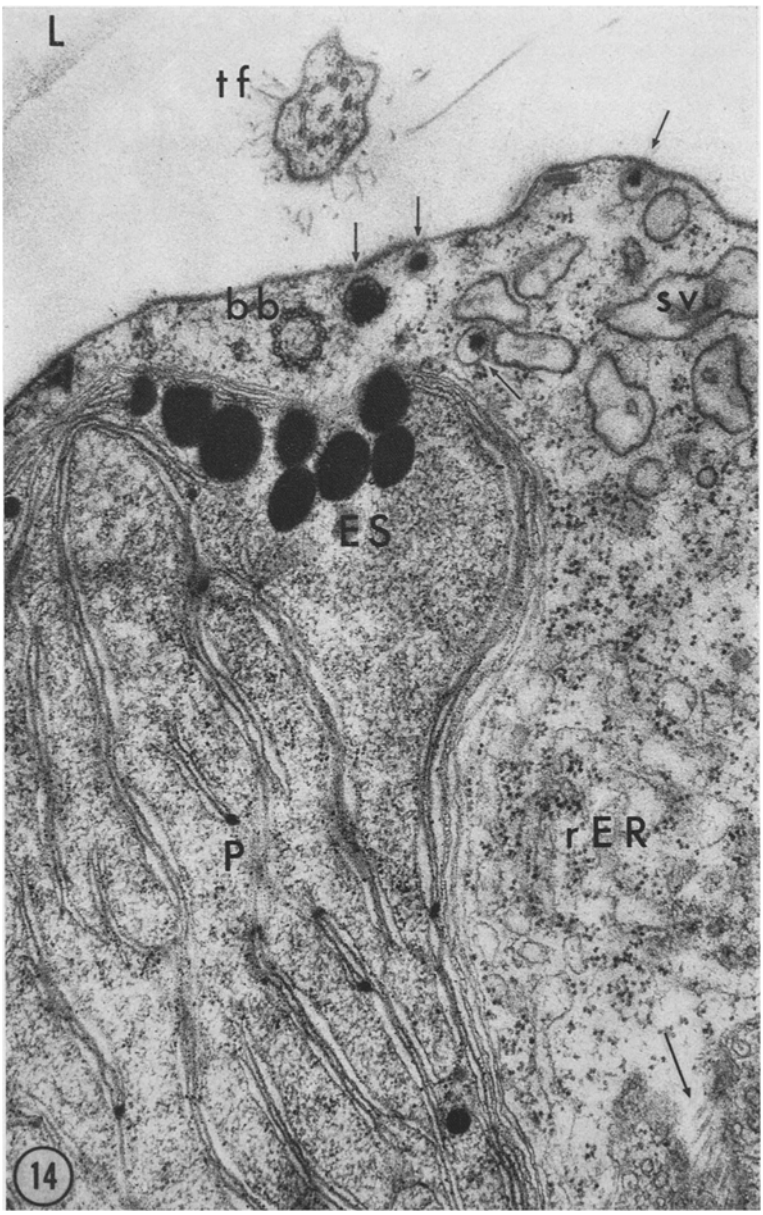


Fig. 14. Anterior end of the cell in a somewhat oblique section. The cross section of the tinsel flagellum (*tf*) with attached mastigonemes and a basal body are identified. Numerous large smooth vesicles (*sv*) are found near the plasma membrane in this region. Some of these vesicles contain electron dense aggregates (short arrows). Note the periplastidal rough ER (*rER*). A series of tubular elements is seen at the outer membrane of a mitochondrion (long arrow in the lower right). *ES* eye spot; *L* lorica. Magn. × 48000

material (e.g. Figs. 6, 7, 14) of unknown chemical nature whereas others are homogeneously filled with diffuse very densely staining masses (Fig. 14). (iii) "*Coated vesicles*" are also found frequently, especially in the cell cortex (e.g. Fig. 6), but also near the Golgi apparatus. (iv) A special *spherical body* (Fig. 9) made up of an aggregation of flattened concentric cisternae and small smooth "vesicles" both more or less filled with an electron dense substance, has repeatedly been observed as surrounding dense particles (possibly also representing a tight aggregate of very small vesicles 0.1 to 0.3 μm in diameter). These associations may be homologous to a dictyosome. (v) *Vesicles or inflated cisternae containing small tubular structures* (Fig. 13, inset) are also commonly found and are identical in structure to the "mastigoneme-containing" vesicles known in other *Chromophyta* and lower aquatic fungi (for phycological reviews see Bouck 1969, 1971; Gibbs, 1970; Leedale *et al.*, 1970; Heath *et al.*, 1970).

The *plasma membrane* revealed, more than all the other membranes in this cell, a conspicuous trilamellar "unit membrane" pattern with a mean thickness of 90 Å (Figs. 5, 6 and 8). At many sites, especially in the equatorial (girdle) region, the plasma membrane is in contact with the lorica (Figs. 2 and 3) and shows small indentations suggesting formation of endocytotic vesicles (Figs. 4—8). At these sites the membrane appearance is characteristically altered in that the outer face is associated with a coat of diffuse, electron dense material, perhaps of glycoproteinaceous character (e.g., Figs. 5, 7 and 8). Moreover, the electron opacity of the membrane itself is greatly enhanced at these sites (Figs. 5, 7 and 8), and the inner membrane face is coated with regularly spaced electron dense knobs in the mode which is typical for the outer surface of "coated" (or bristle coat) vesicles. It cannot be decided whether these surface membrane sites are involved in the formation of the coated vesicles which are so frequently seen in the cortical zone of the cell (see above), or *vice versa*, whether they represent sites of fusion of (secretory) coated vesicles with the plasma membrane, *i.e.* sites of input of new membrane material into the existing plasma membrane.

Apart from flagella and flagellar bases and the stalk, *microtubules* were especially frequent immediately underneath the plasma membrane ("cortical microtubules") which occasionally suggested lateral cross-bridging with the plasma membrane (Fig. 4, inset). Both *small tubular* and *microfilamentous structures* were also identified in association with the surfaces of organelle surrounding membranes, *i.e.* in periplastidal and perimitochondrial regions (Figs. 12—14).

Lorica Structure

The "cellulose containing" lorica (Klebs, 1893) of *Dinobryon* is composed of microfibrils of widths between 20 and 200 Å. This is recognized

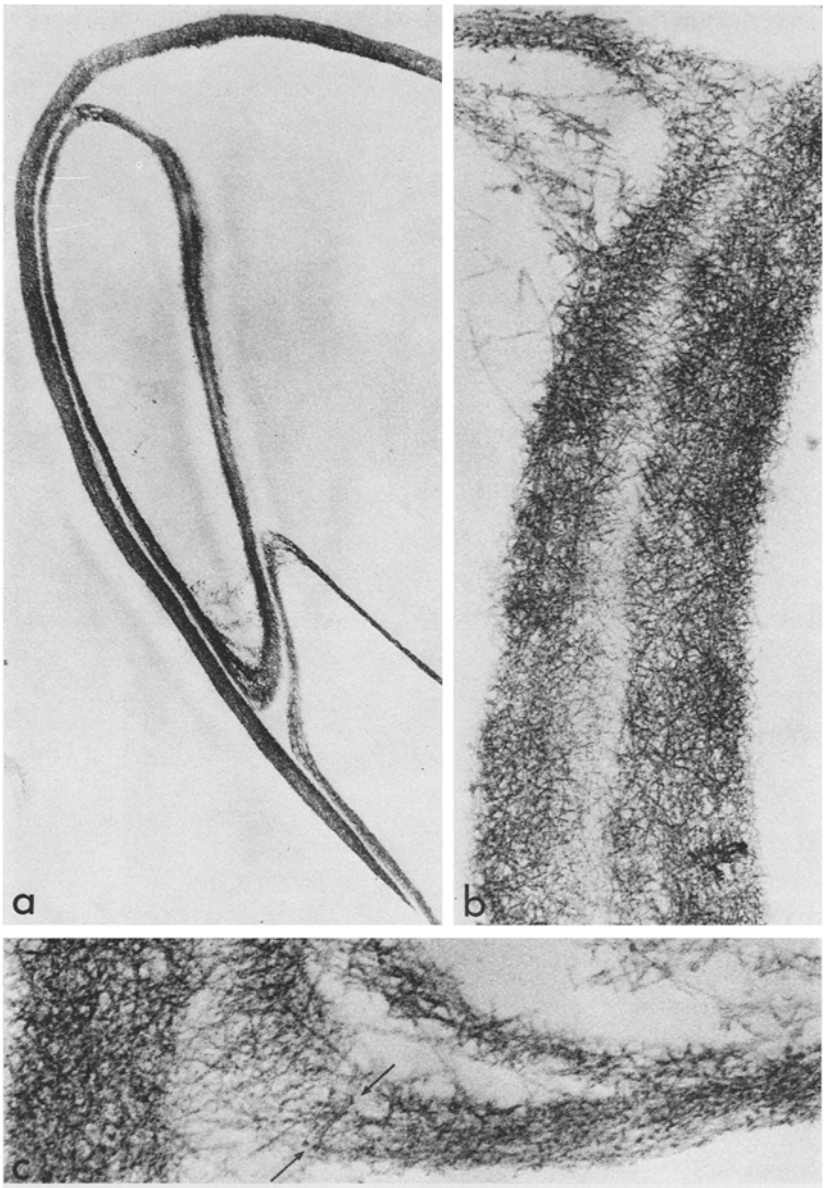


Fig. 15a—c. Details of lorica fine structure as revealed in thin sections. a shows two young loricae attached to an old lorica with their basal part. Note that the young loricae show several layers of fibrillar material but are still thinner than the mother lorica. b is an oblique section of a zone of attachment of two loricae clearly demonstrating the fibrillar elements and suggesting that some fibrils run from one lorica into the next one. c is a higher magnification demonstrating that the fibrils are coated by electron dense, positively stained material so that sometimes small "tubular" structures are revealed (arrows). a, × 12000; b, × 50000; c, × 80000

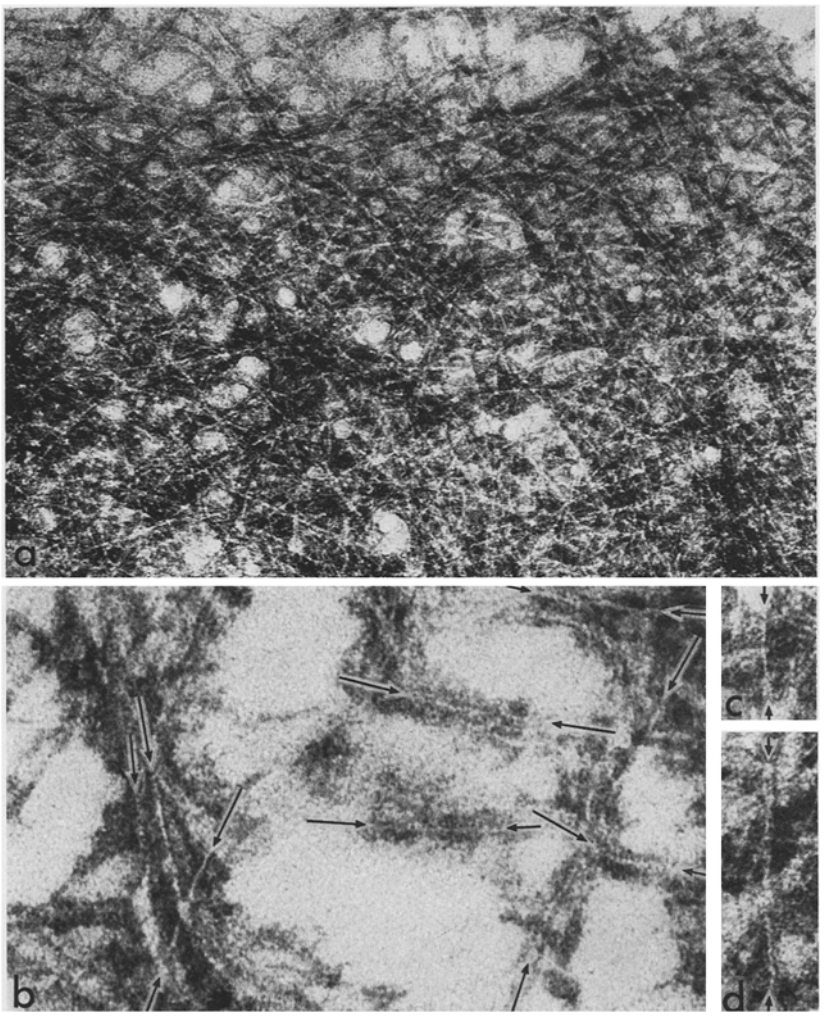


Fig. 16a—d. Details of the lorica fine structure as revealed by negative staining. Only in the region of the lorica "mouth" the fibril layer is thin enough so that its fibrillar composition can be resolved. a presents details of the region near the edge of the lorica mouth in which the fibrils form loops winding up and down. The fibrils are negatively stained but there exists also positively staining amorphous material between the fibrils. At very high magnification (b—d) the finest negatively stained fibrils may frequently reveal a "beaded" appearence due to the granularity of the PTA stain. The smallest fibrils (diameter ca. 20 Å) appear not to be very rigid. They have a tendency to form lateral associates, *i.e.* ribbons. a, × 120000, b—d, all × 200000

in metal shadow cast (Karim and Round, 1967; Belcher, 1968; Kristiansen 1969, 1972a, b; Hilliard, 1971) as well as in sections (Fig. 15) and in negatively stained preparations (Fig. 16 a—d). The marked positive staining of the fibrils in the ultrathin sections suggests that they do not exclusively consist of cellulose, but also contain noncellulosic material of a higher stainability, preferentially located on the surface of the cellulosic fibrils. This leads to the appearance of small "tubular" structures (Fig. 15c), a situation which is very similar to what has been described in *Glaucocystis nostochinearum* by Schnepf (1965). Positively stained material is also visible as diffuse amorphous material in between the cellulosic fibrils (Fig. 15c). Such interfibrillar material may represent the acidic exopolysaccharides as were described by Kauss (1968) for the related genus *Ochromonas*. Details of the fibrillar network are best visible in the negative staining preparations in the region of the lorica "mouth" (Fig. 16a). The fibrils show an interwoven random orientation except at the very margin of the lorica where the fibrils appear to form loops as has also been noted after shadow cast (Karim and Round, 1967; Belcher, 1968; Kristiansen, 1969, 1972a, b; Hilliard, 1971). We have measured the fibril dimensions in our negatively stained preparations (see also Pfisterer, 1970). There is a frequency peak of the smallest fibrils with diameters of 20—25 Å. Smaller diameters are also occasionally observed. However, the beaded appearance of such thin fibrils in negative stain (Fig. 16 b—d; for discussion see Franke and Falk, 1968; Franke and Ermen, 1969) makes it very difficult to obtain precise values. The majority of fibrils consists of aggregates of diameters of up to 200 Å which are often seen to fray out at the ends into individual components. These aggregates appear as broad ribbons and sometimes can be seen to be twisted, showing the small edge of the ribbon (compare also Franke and Ermen, 1969; Kramer, 1970). For the small fibrils it cannot be clearly decided whether the minimum diameter value given above represents the small edge of the ribbonlike fibril only or is a true diameter of an isodiametric fibril. In the zone of attachment of two subsequent lorica generations, fibrils can sometimes be seen to run from one lorica to another. Therefore, one has to discuss the possibility of a direct structural continuity of the lorica walls. Nevertheless, this interlorical linkage is very labile against e.g. ethanol (Klebs, 1893) and fixations (own observations) and is possibly mostly due to the noncellulosic components of the loricae.

Discussion

The present study extends the electron microscopic information on the structural organization of *Dinobryon* as documented in the work of Joyon (1963) and Wujek (1969), and shows, due to the improved fixation with glutaraldehyde, various novel structural details. This progress is

particularly noticeable with all nucleoproteinaceous, glycoproteinaceous or purely proteinaceous structures, including the microtubules, microfilaments and mastigonemes. Taken together, our results emphasize the close relationship of the *Dinobryon* cell body to that of *Ochromonas*, the best investigated genus among the Chrysophyceae (see, e.g., Gibbs, 1970; Bouck, 1971). The cortical microtubules which seem to run in a meridional orientation may, by linkages to each other as well as to the adjacent plasma membrane and periplastidal ER, increase the stability of the cell cortex, essentially similar to the organization in other algae (Kiermayer, 1968) and higher plants (e.g., Cronshaw, 1967; Robards, 1968). This may be a general structural principle in cellular cortices (for discussion see, e.g. Franke, 1971).

A conspicuous feature in our *Dinobryon* material is the abundance and variety of vesides, part of which seem to have originated from the highly active dictyosome. Since such vesicles may be involved in endocytotic or exocytotic membrane flow processes it seems likely that the vesicle richness reflects the special physiological status of our material rather than being a permanent structural feature of *Dinobryon* as such. Our micrographs strongly suggest a high membranogenetic and secretory activity, in particular in the upper hemisphere of the cell body.

The unique and typical structure of *Dinobryon* is its large vase-shaped lorica which allows the formation of the characteristic branching colonies. There is some evidence that this lorica and also the small basal cup of *Ochromonas*, (Syn. *Poteriochromonas*), contain cellulosic fibrils as suggested by the chlor-zinc-iodide-reaction, the solubility in copper tetramine complexes (Cuoxam), the alkali resistance of the fibrils, and the electron microscopic appearance of the microfibrils (Klebs, 1893; Pascher, 1921; Schnepf *et al.*, 1968; Kramer, 1970, 1972; and this study). Among the *Chrysophyta*, examples for the use of cellulosic fibers as the main structural polysaccharide for various types of wall construction have been shown in the scales of the haptophycean genera *Pleurochrysis* and *Hymenomonas* (e.g., Brown *et al.*, 1970; Franke and Brown, 1971; Herth *et al.*, 1972; Brown *et al.*, 1973), in the loricae of *Dinobryon* and *Ochromonas* (Klebs, 1893, Schnepf *et al.*, 1968), and in the sac cell walls of various Xanthophyceae, *Tribonema* and *Botrydium* (Falk, 1967; Falk and Kleinig, 1968; Pfisterer, 1970). A comparison of the cell wall structure in these three different construction types shows that they are composed of fibrils of similar minimal dimensions and exhibit similar details such as a tendency to ribbon shape formations and some rigidity as demonstrated by the frequency of kinking or cracking sites (e.g., Brown *et al.*, 1969, 1970; Pfisterer 1970; Franke and Brown, 1971; Kramer, 1970, 1972, Brown *et al.*, 1973) and similar resistances to alkali and weak acids (Brown *et al.*, 1969, 1970; Pfisterer, 1970; Herth *et al.*, 1972; Kramer, 1972; Brown

et al., 1973). In the case of *Pleurochrysis* it has been shown that such fibrillar material is produced within the cisternae of the Golgi apparatus and is then secreted exocytotically (Brown, 1969; Brown *et al.*, 1969, 1970; Brown *et al.*, 1973), and it has further been suggested that cellulosic material might be secreted by a similar process in other cell wall types as well, including those of higher plants, with the modification that in such cases the crystallization of the macromolecules takes place after secretion in the extracellular space (Brown *et al.*, 1970; Herth *et al.*, 1972). It is conceivable that the *Dinobryon* lorica material is produced in a similar way. We do not think that in *Dinobryon* the stalk surface is primarily involved in fibril formation as this has been suggested for *Ochromonas* by Kramer (1970, 1972). Our electron microscopic study shows, in contrast to those of others (Joyon, 1963; Wujek, 1969), but confirming the early light microscopic studies of Klebs (1893), Pascher (1913) and Krieger (1930), that the cell body of *Dinobryon* attaches to the lorica wall over a relatively broad equatorial region. This is also a region in which vesicle flow activity is observed and, therefore, there seems nothing contradictory to the hypothesis that the lorica material is secreted by vesicle flow and added to the lorica in its mid region. To this extent our electron microscopic observations lend fine structural support to Klebs' early idea on lorica formation quoted in the Introduction.

The various types of vesicles which are so abundant in *Dinobryon* could be involved in both, endocytotic uptake of material and exocytotic secretion. It may well be that some of them are involved in secretion of cell wall substances, perhaps including structural polysaccharides, which are finally incorporated into the lorica. We have no idea at the moment which vesicle type is preferentially involved in such secretory processes but none of them can be *a priori* excluded from such a function. This holds even for the "coated vesicles" which on the one hand have been shown in many animal systems to be clearly involved in endocytosis (e.g., Roth and Porter, 1964; for a recent discussion of the literature see Lagunoff and Curran, 1972), but in other systems, especially in plants, can as well be demonstrated as participating in secretion and formation of the plasma membrane, most convincingly during cell plate formation (Hepler and Newcomb, 1967; Schnepf, 1969; Whaley *et al.*, 1971).

Hypothetically we envisage extending the ideas of Klebs, the formation of the lorica as following, a pottery principle. New material is added to the lorica in the equatorial region by secretion in the plasma membrane attachment zones, and is symmetrically distributed through rotational movements of the algal body which is basally anchored to the lorica bottom by the stalk. Similar hypotheses have repeatedly been suggested by other students of *Dinobryon* (Pascher, 1913; Krieger, 1930; Pringsheim, 1963; Karim and Round, 1966; Wujek, 1969; Kristiansen, 1969).

As the fibril pattern of the loricae is species-specifically different (Karim and Round 1967; Kristiansen 1969, 1972a, b) there must exist a high degree of genetic control of the movements leading to the ordered deposition of fibrils in the lorica and a fine system of maneuverability of the cell movements during lorica formation.

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