

STRUCTURE AND DEVELOPMENT OF THE CHLOROPLAST IN CHLAMYDOMONAS

I. THE NORMAL GREEN CELL

BY RUTH SAGER*, PH.D., AND GEORGE E. PALADE, M.D.

(From *The Rockefeller Institute for Medical Research*)

PLATES 148 TO 156

(Received for publication, December 10, 1956)

INTRODUCTION

The recent development of electron microscopy has provided the means for a reexamination of many unsolved problems in biology. In general, it is not yet possible to determine the identity or arrangement of individual molecules in biological structures with the electron microscope. However, the next level of organization, at which molecules aggregate to form fibers, membranes, and particles ranging in size from 20 to 200 A, can be systematically studied. By examining structures in this size range, not only can a wealth of new descriptive information (of immediate application in the analysis of function and of morphogenesis) be obtained, but also tactics may be more readily devised for analyzing the ultimate molecular organization of the cell.

With these considerations in mind, we have undertaken to investigate the structure of the chloroplast, a cytoplasmic organelle of considerable biological interest, which is also particularly amenable to electron microscopy. The study of the fine structure of the chloroplast is obviously of value in the analysis of the photosynthetic process. Indeed, separable steps in photosynthesis may be correlated with particular structures and with various degrees of structural integrity of the chloroplast which could be detected by electron microscopy in mutant strains with heritably damaged chloroplasts and in cell or chloroplast fractions. The high concentration of insoluble chlorophyll carotenoid pigments and tightly packed, preferentially oriented "lamellae" within the chloroplast invites analysis of the molecular organization of these structures and of their relation to photosynthesis; some speculations on this subject are already in the literature (19, 54, 76).

The chloroplast may also be used in the analysis of hereditary mechanisms. It is a complex cytoplasmic organelle which can be heritably altered in many different ways without killing the organism which carries it. The specific nature of these alterations can then be studied by morphological and biochemical techniques. In addition, by genetic methods of progeny analysis it is possible

* Present address: Department of Zoology, Columbia University, New York.

to distinguish in some organisms between damage resulting from gene mutation, and that resulting from alterations in a non-chromosomal element, perhaps in the chloroplast itself.

Thus the chloroplast represents a system with which one may try to answer questions relevant to photosynthesis, as well as other fundamental questions, *e.g.*: whether intracellular structures arise exclusively from preexisting structures of the same sort; whether or to what extent structures are determined by specific hereditary factors; and whether these factors are nuclear or cytoplasmic. To restate these questions in current terms, one may ask where in the cell and in what form is the information stored which determines intracellular organization, and how is it utilized.

The green alga *Chlamydomonas* was chosen for this study for a number of reasons. Of most importance, mutant strains with damaged chloroplasts can be investigated, because the organism can be grown heterotrophically in the dark. The inheritance of the chloroplast damage can be analyzed, because the organism has a sexual life cycle, which can be readily controlled under laboratory conditions (58). Genetical investigations have established the Mendelian pattern of inheritance of several mutations affecting the chloroplast (57). In addition, the existence of a cytoplasmic pattern of heredity in this organism has also been demonstrated (56). *Chlamydomonas* contains a single large chloroplast, and a wealth of other cytoplasmic structures which can be adequately preserved and studied in the electron microscope (59).

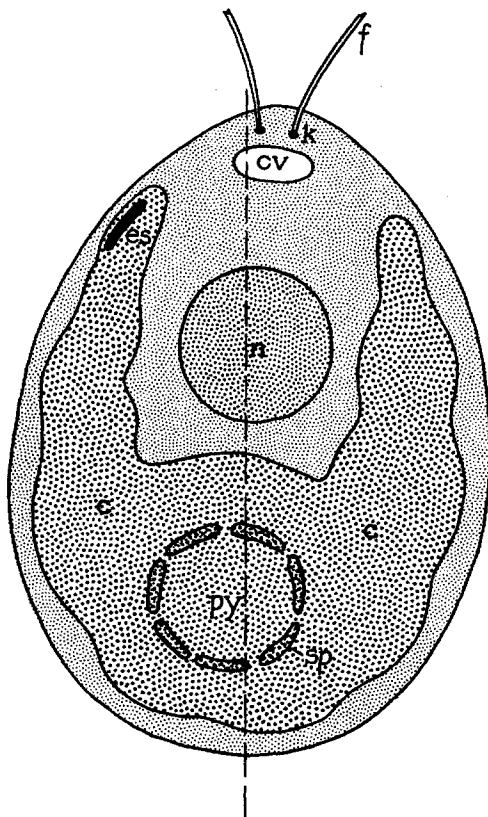
The general plan of this investigation has been to analyze the structure of the normal green strain and to compare it with that of a number of mutant, non-green strains. In this paper, the structure of the normal green cell will be described, with special emphasis upon the chloroplast and its relation to other membranous and granular systems of the cytoplasm. Subsequent papers in this series will concern the alteration of these structures following mutation or various treatments.

Materials and Methods

Chlamydomonas reinhardi, the unicellular heterothallic green alga employed in this study, is classified in the Chlorophyceae, the basic stock from which the higher plants as well as the more complex algae have presumably evolved (66). As a flagellate, *Chlamydomonas* is also classified among the primitive protozoa. This dual classification mirrors quite well the cytological findings to be presented; the organism contains a typical plant cell organ, the chloroplast, embedded in a cytoplasm similar in organization to that of animal cells.

For a full description and survey of the genus, the reader is referred to the comprehensive monograph by Gerloff (17). The strains used in this investigation were derived from two mating strains of *Chlamydomonas reinhardi* obtained from G. M. Smith as C 137+ and C 137-. As with most algae, *Chlamydomonas* exhibits considerable variation in morphology with culture conditions. All descriptions in this paper refer to cells grown in the light in liquid culture, on a roller tube apparatus, and harvested in the late logarithmic phase of growth by centrifugation (58).

For fixation, sedimented algae were resuspended in 1 per cent OsO_4 buffered with 0.028 M Na veronal-0.028 M Na acetate (41) at various pH's in the range 7.0 to 8.5. In a small number of experiments, the osmolar concentration of the fixative was raised by adding sucrose (final sugar concentration: 0.1 M).



TEXT-FIG. 1. Diagrammatic sketch of *Chlamydomonas* as seen in the light microscope. The chloroplast is marked *c*, the eyespot *es*, the pyrenoid *py*, and the starch plates surrounding the latter *sp*. The nucleus is indicated by *n*, the contractile vacuole, by *cv*, one of the two basal bodies by *k*, and one of the two flagella by *f*.

The fixation was carried through either at room temperature or at 0°C ., for 2, 4, or 24 hours. Thereafter the specimens were washed in the same concentration of buffer, dehydrated rapidly in a graded series of ethanols, impregnated with *n*-butyl methacrylate, and finally embedded in the same resin by polymerization catalyzed by 2,6-dichlorobenzoyl peroxide at $45\text{--}47^{\circ}\text{C}$. (40).

Sections, 200 to 500 A in thickness, were cut with a Porter-Blum microtome (52), and mounted on grids coated with either formvar or carbon films (73). They were examined, without removing the embedding plastic, in an RCA-EMU 2b microscope and micro-

graphed at magnifications ranging from 4000 to 16,000 \times . Higher magnifications were obtained by photographic enlargement of the electron micrographs.

After a long fixation at room temperature, the membranous structures of the cell appeared in general satisfactorily preserved, but other structural elements (nucleus, pyrenoid) showed evidence of extensive extraction. A short fixation or a fixation carried out at 0°C. largely prevented the extraction but did not result in a satisfactory preservation of membranous structures, as indicated by the frequency of retractions, lamellar fractures, and membrane discontinuities in chloroplasts. The best results were obtained with OsO_4 buffered at pH 8.5. No appreciable improvement was obtained in the preservation of chloroplasts by adding sucrose to the fixative solution.

OBSERVATIONS

I. Light Microscopy

The organism shown diagrammatically in Text-fig. 1 is ovoid in shape, averaging during the logarithmic phase of growth $6 \times 8 \mu$ in diameter. Each cell is ensheathed in a plasma membrane, surrounded by a cellulose cell wall, and exterior to that, a capsule containing polysaccharides which continually diffuse from motile cells into the medium, but which accumulate to considerable thickness around non-motile cells.

Within the cytoplasm of the posterior two-thirds of the cell, there is a single green cup-shaped chloroplast. Embedded centrally within the posterior part of this organelle, is the pyrenoid, a spherical body approximately 2μ in diameter, surrounded by a number of starch-containing plates. The pyrenoid itself appears structureless; whether or not it contains chlorophyll cannot be discerned, since it is surrounded by green chloroplast material. In *Chlamydomonas reinhardi* starch is stored in two locations. The first starch formed is stored in the plates which surround the pyrenoid, but under conditions of starch accumulation, small granules appear scattered throughout the rest of the chloroplast as well. Under certain conditions these granules may become so large as to distend greatly the plastid and indeed the entire cell. The eyespot lies within the chloroplast at its anterior edge, appressed against the cell membrane so that it protrudes as a small bulge. It is a deep orange, carotenoid-containing body about one micron long, which is plate-like as seen from above, and rod-shaped in side view.

The anterior third of the cell body contains the nucleus, and two contractile vacuoles. There are two anterior flagella, each about 10μ long.

II. Electron Microscopy

Fig. 1 is an electron micrograph which shows a sectioned alga at 15,000 \times diameter, the plane of the section being that shown as *a-a* in Text-fig. 1. Because of the large size and characteristic location of the larger cytoplasmic organelles of *Chlamydomonas*, little difficulty is encountered in correlating images obtained by light and electron microscopy. The chloroplast, for instance,

can be easily identified on account of its relatively large size, its posterior location within the cell body, and its U-shaped profile. The micrograph shows that the chloroplast consists of packed, dense membranes interspersed with granular material and dense, homogeneous bodies, the starch grains. A continuous chloroplast membrane surrounds all these structures. In this section the chloroplast is represented not by a single profile, but by four. This apparent multiplicity results from deep indentations in the rim of the cup-shaped organelle. In sections, each undulation of the rim may appear as a separate profile.

The pyrenoid is identified in Fig. 1 by its size and location. It appears as a differentiated region of the chloroplast, and is found to be non-membranous in structure, separated from the laminated material of the chloroplast by a ring of starch plates. The eyespot is not present in this section; it is located just beneath the chloroplast membrane and usually consists of two plates of dense granules, as in Fig. 7. The nucleus is found in the concavity of the chloroplast.

In addition to these organelles, expected and recognized on the basis of light microscope studies, the cytoplasm contains other structural elements, some of them very similar to structures described in animal cells. These include the mitochondria, the endoplasmic reticulum, the small granular component of the cytoplasm, and stacks of agranular cisternae (dictyosomes or Golgi apparatus). Finally, a number of large vacuoles and various small structures of constant occurrence but unknown nature are found in the cytoplasm.

A more detailed description of these structures follows.

A. Cell Membranes:

As seen in Figs. 2 to 4, and 11, the cytoplasm is bounded by a thin, dense, and continuous membrane which is about 100 A in thickness and which in some instances (Fig. 4) shows evidence of stratification (two dense layers each of ~30 A separated by a light intermediary layer). At the anterior pole the membrane evaginates to cover each of the two flagella (Figs. 2 and 3). Around the rest of the cell it usually follows the general curvature of the body and only rarely shows small invaginations or evaginations (Figs. 2 and 11).

Outside of the plasma membrane there is a continuous layer of less dense, usually homogeneous material, ~400 A thick, which corresponds to the cellulose wall described in light microscopy (Fig. 11). At the anterior pole of the cell, the wall thickens into a plate perforated by two tunnels for the flagella. This plate frequently shows some evidence of stratification and occasionally a faint layering can also be detected in other parts of the cell wall. The observation is consistent with the findings of Preston and his collaborators (*cf.* 53) who demonstrated the existence of distinct layered "lamellae" of

cellulose fibrils in the cell wall of other algae. Indirect evidence for the fibrillar nature of the cell wall in species related to *Chlamydomonas* has also been published (30). The cellulose wall is found either closely applied on the plasma membrane (Figs. 2 and 3) or separated from it by a more or less wide space (Figs. 4 and 11) occupied by a material of variable, usually light density, an indication that the two structures are independent.

Finally, the outside of the cell wall is covered by another layer of dense material which corresponds to the capsule seen in the light microscope. The capsular layer is of variable thickness (from 200 to 600 Å) and of characteristic texture: it appears as a fine fibrillar felt with a frayed outer surface and occluded, dense, irregular particles (Figs. 2, 4, and 11). The frayed appearance may be correlated with the fact that capsular material continually diffuses into the medium.

After a long fixation at room temperature, the plasma membrane frequently shows breaks and discontinuities, the material of the cell wall is largely extracted, and the fibrillar structure of the capsule accentuated (Figs. 10 and 17).

B. Flagella:

The flagella each arise near the anterior end of the cell from a basal body clearly visible in the phase contrast microscope. The electron micrographs show that the two basal bodies are in fact linked together, (Figs. 2 and 3). An array of dense parallel rods or fibers is found closely applied on the anterior side of this junction (Fig. 2).

As seen in cross-section, each flagellum consists of an outer membrane enclosing ten pairs of fine tubules of which one is centrally located and nine are disposed at regular intervals in a peripheral ring. In each peripheral pair the tubules are in close apposition; in the central pair they are separated by a narrow space. The diameter of the flagellum is ~ 250 m μ and each tubule is about 15 to 18 m μ . The outer membrane of the flagella is continuous with the plasma membrane of the cell.

The flagellar organization in *Chlamydomonas* is similar to that found in all plants and animals thus far examined by electron microscopy (35, 12). In the corresponding literature the fine tubules of the flagellum are usually described as fibers or filaments.

C. The Chloroplast:

The chloroplast is bounded by an envelope consisting of two membranes, each ~ 50 Å in thickness, usually disposed parallel to one another at a spacing of ~ 60 Å (Fig. 4). In rare instances the membranes diverge, the space between them becoming wider and somewhat less regular (Fig. 5). Occasionally the outside membrane appears to be continuous with smooth surfaced profiles of the endoplasmic reticulum (Fig. 4) or of the dictyosomes.

Within the chloroplast the following structures are found: (1) the lamellae, (2) a matrix containing granular material, (3) starch grains, (4) the pyrenoid with its tubules, and (5) the eyespot.

1. *Lamellae*.—Most of the chloroplast volume is occupied by regularly disposed lamellae. They occur throughout the organelle (Fig. 10) interspersed with zones containing fine granular material (Figs. 4, 5, and 10) and occasionally with large homogeneous bodies which correspond to the starch grains seen in the light microscope (Figs. 4, and 10). No lamellar structures are found within the pyrenoid.

A more detailed analysis shows that the lamellae occur in pairs, each pair forming a closed system, to be referred to as a disc. The discs appear to be uniformly flattened vesicles about 200 Å thick. Their actual shape, whether circular, ellipsoidal, or irregular is not known. In sections their apparent diameter varies considerably, ranging from 0.4 μ to 2 μ and averaging about 1 μ . It follows that each disc spans just a part of the chloroplast space (Figs. 5 and 6). The discs are disposed into stacks of two (Figs. 4 and 11) to twenty (Figs. 4 to 6) units, averaging about eight units per stack. The chloroplast space is filled with a mosaic of such stacks with irregular interpositions of matrix and granular material. In any given section, the stacks appear to be sectioned at different angles (Figs. 6 and 11), an indication that they are irregularly oriented throughout the cup-shaped chloroplast. The disc thus appears to be the basic unit, and the stack of discs an intermediary unit in the organization of the chloroplast.

The "two" lamellae of each disc constitute a limiting membrane, which is dense, smooth, continuous, and thin, measuring 55 to 65 Å in thickness in normal sections. Within the membrane there is a homogeneous material of rather low density. The width of the intradisc space varies from one specimen to another, ranging from 40 Å to as much as 700 Å. The over-all thickness of the disc may vary therefore from 150 to 800 Å. Frequently the interdisc spaces within a stack are narrower (\sim 100 Å) and more regular than the intradisc spaces. This observed variation in spacing may reflect the situation in the living cell or may result from preparative procedures. In unsatisfactorily fixed specimens, the discs are swollen, their limiting membrane frequently showing breaks, and the inter- and especially intradisc spacing is highly irregular. It should be noted, however, that variation in spacing is also found in specimens which, by other criteria, appear satisfactorily fixed.

Each disc has an apparently continuous rim of dense material, appearing in normal sections as a dense spot (\sim 140 Å). The discs are attached by this rim to one another or to the chloroplast envelope (Fig. 5). Frequently, however, the rim is free in the chloroplast matrix. Exceptionally, discs belonging to different stacks are connected by a single thin lamella, comparable to the "Trägerlamellae" described in *Aspidistra* by Steinmann and Sjöstrand (69).

2. *The Chloroplast Matrix.*—The stacks of discs are embedded in a continuous matrix of relatively low density, containing two types of granules. One type, measuring 70 to 100 $m\mu$ in diameter, consists of a dense, apparently homogeneous material, and probably represents lipide inclusions (Figs. 5, 9, and 11). Other granules are much smaller, 10 to 15 $m\mu$, of lower density, and frequently arranged in chains or clusters (Figs. 4 and 5). They resemble the small dense particles found in the surrounding cytoplasm. The matrix surrounding the stacks of discs is continuous with the relatively light material which occupies the interdisc spaces within each stack. This "intrastack" matrix is, however, free of granules and particles (Figs. 5 and 6).

After a long fixation at room temperature, the density of the matrix is reduced, and consequently the granules and particles appear in better contrast. (Compare Fig. 5 with Figs. 4 and 11). The lamellar material of the chloroplast is better preserved by a long fixation. After a short fixation, retraction spaces between discs and chloroplast envelope, as well as fracture and disruption of chloroplast lamellae, are frequent.

3. *Starch Grains.*—Relatively large bodies (100 x 400 $m\mu$) of elongated form, corresponding in size and shape to the starch grains seen in the light microscope, occur embedded in the matrix of the chloroplast among the stacks of discs (Figs. 4 and 10). They have the same fine structure and react in the same ways to the various preparative procedures as do the starch plates of the pyrenoid described below.

4. *The Pyrenoid.*—The pyrenoid is a spherical body with a diameter of 1.5 to 2 μ . It consists of finely granular, tightly packed material of medium density surrounded by a discontinuous shell of plate-like bodies which correspond to the starch plates seen in the light microscope (Fig. 11). A thin, continuous layer of chloroplast matrix separates the starch plates from the lamellar material of the chloroplast. Matrix material continuous with this layer also occupies the spaces between the starch plates, thus establishing contact with the dense core of the pyrenoid (Fig. 11). In certain favorably oriented sections, the regions between the starch plates appear as radially disposed bands (Figs. 1, 12 to 14); in three dimensions they probably form a continuous "wall-work" around the starch plates. Although no membrane separates the matrix material from the pyrenoid core, the difference in density between the two zones is great and the transition from one to the other is abrupt (Fig. 11).

In sections, small profiles of circular, oval, and elongated shape are found in the core of the pyrenoid and between the starch plates. They are limited by a thin, dense membrane, which bears irregular thickenings (200 to 300 Å) on its internal surface (Fig. 10 inset). The lumina of these profiles are occupied by a homogeneous material of light density. Favorably oriented sections (Fig. 9) as well as serial sections (Figs. 12 to 14) indicate that: (a) the profiles

belong to a network of tubules (diameter: 400 to 600 Å) embedded in the dense material of the pyrenoid core; and (b) the network is connected with the lamellae of the surrounding chloroplast by tubules located in the matrix that separates the starch plates. The membrane limiting the tubules is probably continuous with the limiting membrane of the discs, but conclusive evidence on this point is still lacking.

The starch plates measure 0.5 to 1.5 μ in length and 0.1 to 0.3 μ in thickness and have a fine granular texture. Their density, although variable, is usually high. Occasionally denser bodies of polygonal outline, presumably crystals, are found embedded within the starch plates. Similar bodies occur in the other starch grains scattered throughout the chloroplast (Fig. 11).

After a long fixation, most or all of the dense granular material of the pyrenoid core is lost. A light substance, containing a few dense particles, is left behind, and consequently the network of pyrenoid tubules appears in sharper contrast. No difference in density remains between the chloroplast matrix and the pyrenoid core under these conditions (compare Figs. 1, 10, and 10 inset with Figs. 9 and 11). The material of the starch plates is frequently entirely lost, the starch apparently being replaced by embedding plastic. Starch grains elsewhere in the chloroplast react in the same way (Figs. 1, 5, and 6). A short fixation preserves both the pyrenoid material and the starch.

5. *The Eyespot*.—The eyespot consists generally of two, sometimes of three curved plates, concentrically disposed in a rather constant location, near the anterior edge of the chloroplast, just beneath the limiting membranes of the chloroplast and of the cell. Each plate is composed of a single layer of dense, spherical, uniform bodies about 100 to 140 $m\mu$ in diameter. As shown in Fig. 7, the eyespot plates are embedded in the chloroplast matrix in a definite relationship with the envelope and the discs: just beneath the chloroplast envelope lies one plate of granules, followed by a disc, a second plate of granules, and another disc. Fig. 8 is an oblique section showing that in the plane of the plate, the granules are tightly packed and have a hexagonal profile. Each granule consists of a dense core and a less dense peripheral shell with no evidence of a surrounding membrane. The granules are separated by narrow spaces. In none of the sections studied, was there any evidence of fibrous connections between these granules and any other region of the cell.

D. Mitochondria:

Examples of mitochondrial structure are seen in Figs. 4 and 19. Both circular (200 to 300 $m\mu$ diameter) and elongated profiles are found, often in the same cell. From the relative frequencies of these profiles it can be inferred that most of the mitochondria of this organism are rod-shaped or filamentous. Branching mitochondria are sometimes encountered. The mitochondria are not randomly dispersed throughout the cytoplasm. Some are located between

the chloroplast and cell membrane (Figs. 4, 5, 10, and 11), and a few are scattered in the concavity of the chloroplast around the nucleus (Fig. 16). At the periphery of the mitochondrial profiles there are two generally parallel membranes, each ~ 50 A thick separated by a narrow space (50 to 80 A). The mitochondria have a system of internal folds similar to the cristae mitochondriales described in animal cells (42). The folds are of laminated form, measure from 160 to 250 A in thickness, are relatively few in number (about 10 cristae per micron length of mitochondrial profile), and are irregularly distributed throughout the organelle so that in a given profile they can be seen both in full face and in side view (Fig. 18). In favorable sections, it can be seen that the dense layer of the cristae is continuous with the inner mitochondrial membrane.

E. Membranous Systems of the Cytoplasm:

Two distinct membranous systems are found in the cytoplasm of *Chlamydomonas*: one consists of interconnected tubules and vesicles, most of them associated with small dense particles, and corresponding in morphology and location to the endoplasmic reticulum of animal cells (49, 45); the other is represented by tightly packed stacks of smooth surfaced cisternae located in the centrosphere region, and resembling the Golgi apparatus (7) or dictyosomes (8) of animal cells.

1. *Endoplasmic Reticulum.*—This system is represented, in sectioned material, by profiles ranging in size from 50 to 150 $m\mu$, consisting of a thin, limiting membrane and light contents, with no observed internal structure. The membranes of most of these elements are associated with small dense particles of 100 to 150 A in diameter (Fig. 23). Examination of numerous sections at various incidences, as well as serial sections, indicates that the profiles described belong to spherical vesicles, tubules, and cisternae, interconnected at least in part in a continuous network. This network is in general randomly oriented. The endoplasmic reticulum is concentrated mostly in the anterior third of the organism, with only a few elements scattered between the chloroplast and the cell membrane. The system does not penetrate the chloroplast or pyrenoid.

The arrangement of small dense particles on their supporting membranes is revealed particularly well in sections cut very obliquely through cisternal elements, thus allowing a full-face view of the limiting membrane. In most instances the distribution of particles appears random, but occasionally a strikingly regular pattern is encountered consisting of paired linear arrays of particles (Figs. 23 and 24). Other patterns, like the circles, spirals, and rosettes described in various types of mammalian cells (43), have not been found in *Chlamydomonas*.

In addition to the particles attached to the membrane of the endoplasmic

reticulum, a relatively large number of similar particles are found freely scattered throughout the cytoplasm, individually, in chains, and in clusters (Figs. 11, 23, and 24).

2. *Dictyosomes*.—Arrays of elongated, smooth surfaced profiles limited by a dense membrane (~ 70 A) and characterized by narrow lumina (50 to 80 A), parallel orientation, and tight packing (70 to 80 A spacing), are found in the vicinity of the nucleus (Fig. 15). Oblique sections indicate that the profiles represent cisternal elements, *i.e.* relatively large, flat vesicles (Fig. 16). In three dimensions, the arrays correspond to stacks of tightly packed cisternae. The ends of these cisternal elements are frequently dilated into vesicles of various sizes (30 to 100 $m\mu$) (Figs. 15 and 23) and occasionally ampullar dilatations are also encountered in the mid-region of the profiles (Figs. 16 and 22). Frequently the arrays are surrounded by swarms of smooth surfaced, circular, or oval elements representing vesicles (30 to 50 $m\mu$) which may either pinch off from, or coalesce with, the stacked cisternae (Fig. 15).

In their general appearance, these stacks of cisternae resemble structures described in many animal cells as the Golgi apparatus (7, 22 and 65) or the agranular reticulum (47). In their tight packing and usual absence of large vacuolar elements, they resemble more closely the dictyosomes found in invertebrate cells (8, 20 *c*) and in other protozoa (16). In *Chlamydomonas*, there are one to four such dictyosomes (each $\sim 1 \times 0.3 \mu$) per section around the nucleus, usually associated with other membranous structures such as the agglomerations of small, smooth surfaced elements already mentioned. Another associated structure is represented by a composite vacuole $\sim 150 m\mu$ in diameter, limited by a single thin membrane and containing a number of small ($\sim 30 m\mu$) vesicles embedded in a light matrix (Fig. 8). Such compound vesicles have been previously noted in such cells as perikarya (47), tracheal epithelium (55), and were found to be exceedingly numerous in oöcytes (67). As yet no centrioles have been observed in the vicinity of the dictyosomes in *Chlamydomonas*.

3. *Connections between the Membranous Systems*.—Connections between the various membranous systems of the cell are frequently encountered. They are particularly evident between the endoplasmic reticulum and the dictyosomes. In the immediate vicinity of the latter one frequently finds large irregular vesicles or dilated cisternae of dual character. Their limiting membrane is smooth where it faces the dictyosomes, and bears small, attached particles on the rest of their perimeter (Figs. 16, 21, and 22).

A few instances of apparent connection between the chloroplast envelope and smooth surfaced membranes of the centrosphere region, and of the endoplasmic reticulum (Fig. 4) have been encountered. Connections have also been found between the endoplasmic reticulum and the nuclear envelope (Fig. 16), and between the dictyosomes and the large vacuoles described below.

F. Other Cytoplasmic Structures:

Large (0.5 to 1 μ) vacuoles are frequently found lying between the nucleus and the concave surface of the chloroplast. They vary in number from two to eight per section, and apparently represent a constant structural feature of the organism. They are distinguished by the highly dense and polymorphic bodies they contain, usually embedded in a matrix as light as the embedding plastic (Fig. 20). Their appearance suggests that *in vivo* they are filled with water surrounding a solid conglomerate.¹

The two contractile vacuoles, clearly seen with phase contrast optics, have not been satisfactorily identified in electron micrographs, although the vacuole *v* in Fig. 1 may represent this organelle.

G. The Nucleus:

The nucleus is located in the concavity of the chloroplast and usually appears as a spherical or ovoid body of $\sim 2 \mu$ in diameter. The nucleoplasm is relatively dense and contains a fine granular material embedded in an amorphous matrix. The center of the nucleus is occupied by a large ($\sim 0.5 \times 0.9 \mu$), dense, ovoid nucleolus (Figs. 15 and 20) which is found only rarely in contact with the nuclear envelope. Within the nucleolus there are regions of local differentiation. The periphery consists of tightly packed particles 100 to 150 A in diameter, and the center contains particles of finer grain, and areas which appear as light inclusions.

The nucleoplasm is surrounded by a nuclear envelope consisting of two membranes (each 60 to 70 A) separated by a narrow space (~ 80 A). The envelope is perforated by pores of relatively large size (~ 500 A) and apparently irregular distribution. Some of these pores appear open; others are provided with a dense diaphragm. Oblique sections indicate that the pores are circular (Fig. 16).

The outer nuclear membrane bears dense (100 to 150 A) particles attached to the surface facing the cytoplasm. Tangential sections of the nuclear membranes reveal no orientation of granules associated with the pores, in contrast with the annuli which have been observed on the nuclear membranes of many other organisms (15). A few instances have been observed of continuity between the outer nuclear membrane and elements of the endoplasmic reticulum. Thus the nuclear envelope is part of a cytoplasmic membranous system and could be described as a perinuclear cisterna (72).

A long fixation at room temperature results in the complete removal of the nucleolar mass and in the extraction of most of the nucleoplasm. Only the nuclear envelope and a few threads and granules are left behind (Figs. 1 and 16).

¹ The contents of these vacuoles are metachromatic, as revealed by staining with azure B (Himes and Sager, unpublished).

DISCUSSION

It has been the purpose of this study to describe a normal green strain of the alga *Chlamydomonas reinhardi*, as seen in the electron microscope, and thereby to provide a basis for characterizing (in subsequent articles) the structural alterations which occur in non-green mutants.

In the present paper the observations made on the chloroplast and its components, the rest of the cytoplasm, and the nucleus of normal green *Chlamydomonas* will be discussed in the light of available information on plant and animal cells in general and on algae in particular.

*Chloroplast**Lamellar Organization:*

In the normal green alga the chloroplast appears as a complex, highly organized body. It contains not only stacks of lamellae, which are the presumed sites of photosynthesis, but also localized differentiated regions such as the eyespot, associated with phototaxis, and the pyrenoid (and other non-lamellar regions) associated with starch synthesis and storage. The dominant structural feature of the plastid in *Chlamydomonas* is its division into subunits, a disposition which confirms and extends information already available from studies of the chloroplasts of other organisms.

Evidence from Light Microscope Studies.—The existence of an internal structure in chloroplasts in general has been discussed in numerous studies published during the last 70 years by many investigators and recently reviewed by Rabinowitch (54), Weier and Stocking (74), Frey-Wyssling (13), Mühlethaler (39), and Granick (19). Already in the 1880's these plastids were described as containing dark "grana" surrounded by a lighter "stroma" (38). Since that time many different hypotheses of chloroplast organization have been proposed and contested, a situation resulting in part from the limited resolving power of the light microscope. Nonetheless, two important features were revealed by light microscope studies: (1) the presence of grana in some if not all chloroplasts; and (2) the birefringence of chloroplasts, suggesting a laminar arrangement.

The early descriptions of grana by Meyer (38) and others, although contested by advocates of chloroplast homogeneity (*cf.* 39), were given strong support by the work of Heitz (24, 25), who photographed grana in the chloroplasts of living cells of a large number of plants. Other investigators, however, found no grana in certain higher plant chloroplasts; similarly among the algae, some chloroplasts appeared granular and others did not. Thus grana were demonstrated in some material, but questions as to their universality, importance, and nature remained unresolved.

Evidence of laminar arrangement within the chloroplast came especially from studies with the polarizing microscope. Following Scarth's original observations (60), the birefringence and dichroism of chloroplasts were investigated (*cf.* 54, 13, 19) in numerous studies in which the existence of a laminar type of submicroscopic organi-

zation was postulated as an explanation for the form birefringence shown by this organelle.

Evidence from Other Electron Microscope Studies.—Electron microscope studies of the chloroplast of a number of organisms have recently appeared in the literature. Table I

TABLE I
Comparison of Chloroplast Dimensions from Electron Microscope Studies

	Diameter of a lamella	Disc diameter	Average number of discs per stack	References
	A	μ		
<i>Microorganisms</i>				
1. <i>Chlamydomonas</i>	50	0.98	8	
2. <i>Chlorella</i>	50			1
3. <i>Closterium</i>	80 \pm 20			32
4. <i>Euglena</i>	240*	6.5	21	75
5. <i>Fucus</i>				34
Egg	40-80	1.4-3.3	16-20	
Vegetative cell		2.9-4.0	16-28	
Old vegetative cell		4.0-8.0	24-34	
6. <i>Nitella</i>	40			37
7. <i>Poteriochromonas</i>	390*	3.3	10	75
8. <i>Spirogyra</i>	70			68
	80 \pm 20			32
9. <i>Synechrococcus</i>		0.22		5
10. <i>Rhodospirillum</i>		0.11		48
<i>Higher Plants</i>				
1. <i>Aspidistra</i>	65		20-30	69
	60-80	0.3-0.6	15-60	33
2. <i>Beta</i>		0.3-0.6	15-60	31
3. <i>Spinacia</i>		0.6		18
4. <i>Tulip</i>		0.6		2
5. <i>Zea</i>	35	0.3-0.4	2-60	26
6. <i>Nicotiana</i>	70	0.32		6

* In these an entire disc was considered as a lamella.

summarizes their salient findings and compares them with those reported here. The outstanding structural feature of all the chloroplasts thus far examined is the presence of tightly and regularly stacked membranes of high density and fairly uniform thickness (50 to 80 A). These membranes are usually associated in pairs designated as "double membrane discs" by Steinmann and Sjöstrand (69). The term lamella, or

lamina has been extensively but inconsistently used in chloroplast descriptions; it has been applied either to a single membrane, or to a pair of membranes, *i.e.*, a disc, or finally to other associations of membranes due to preparative artefacts. This inconsistency explains in part the wide variation in the thickness ascribed to the chloroplast lamellae in various studies.

In the chloroplasts of higher plants the discs are grouped into stacks which correspond to the grana seen in the light microscope.² It is noteworthy that the dimensions of these grana are quite uniform, not only within individual plants, but also among all the higher plants so far observed. In particular, the diameters of the grana have been found to vary only from 0.3 to 0.6 μ . There is greater variation in the number of discs per stack, both within and between species, a range of 2 to 60 having been reported. There is no evidence of a membrane surrounding the granum.

Again confirming the observations of light microscopists, the chloroplasts of the algae so far studied are not subdivided into grana (with the exception of *Chlamydomonas*, to be discussed below). Rather, the paired membranes run uninterruptedly across the chloroplast from end to end (1, 75).

In the shade plant *Aspidistra*, Steinmann and Sjöstrand (69) have described, in addition to the lamellae of the discs, other thinner lamellae which are located in the intergrana spaces and connect the discs of adjacent grana. The authors assume that these structures represent the "Trägerlamellae" postulated by Strüger (*cf.* 39). Similar connecting membranes have been seen in the young leaves of *Zea* (26), where they are about half as frequent as the paired membranes of the discs, and in *Nicotinia* and *Lemna*, where they are infrequent.³ The irregularity of their presence suggests that the "Trägerlamellae" play no essential functional role in photosynthesis. They may be connected with the formation of discs or may represent a device for maintaining the alignment of grana observed in certain chloroplasts.

Evidence from the Present Study.—Certain features of chloroplast organization clearly seen in *Chlamydomonas* appear to be already of wide occurrence. Indeed, they may prove to be basic structural features for photosynthetic plastids in general.

The Discs.—Of particular importance is the recognition of the disc as the basic structural unit of the chloroplast. The electron micrographs of *Chlamydomonas* show that the chloroplast membranes are not only paired but also that the membranes of each pair are joined at their ends to form closed sys-

² In an as yet unpublished dissertation, brought to our attention by Rabinowitch (*cf.* 54), Vatter has described chloroplast morphology and development in electron micrographs of osmium-fixed, thin sectioned maize seedlings. Chloroplasts of mesophyll cells contain stacks of discs arranged in grana and are similar in organization to plastids of other higher plants, while plastids of the specialized bundle sheath cells have no grana, and resemble algal chloroplasts in which the lamellae extend uninterruptedly across the chloroplast. The organization of lamellae into closed discs is found in both types of maize plastids, as it is in all other plant chloroplasts so far studied.

Vatter's description of chloroplast development and the morphology of mutant strains is particularly relevant to the next paper of this series and will be considered there.

³ G. E. Palade, unpublished observations.

tems which resemble uniformly and extremely flattened vesicles. A similar organization is seen in all published micrographs of chloroplasts in which the material is well enough preserved and the resolution sufficiently high so that the fusion points of the paired membranes and the enclosed intradisc space can be seen. The existence of discs or paired membranes has already been noted in published studies (26, 33, and 69) but in our view, the significance of this structure has not been fully appreciated.

The presence of discs in the chloroplasts of those few higher plants which have so far been examined in the electron microscope provides only a limited basis for generalization. However, with the demonstration of chloroplast discs in *Chlamydomonas*, an alga phylogenetically far removed from higher plants, it is now possible to postulate that the disc may represent a general and pervasive feature of chloroplast organization. As such the disc may well be of key functional significance in the photosynthetic process, a subject to be discussed further below.

A thickened rim at the disc periphery clearly seen in *Chlamydomonas* in which it frequently serves as a zone of attachment to the chloroplast membrane and to adjacent discs, has not been noted in the chloroplasts of other organisms.

Flattened vesicles similar in their general morphology to the chloroplast discs are a recurrent feature in the organization of other cytoplasmic systems. They have been described as cisternae (45) in the endoplasmic reticulum, and as cisternae (44) or "Golgi lamellae" (7) in the membranous system of the centrosphere region. *A priori* it may be assumed that they represent a device for insuring a large surface to volume ratio, and thus facilitate exchange and minimize diffusion problems. Even the formation of stacks by the orderly arrangement of such flattened vesicles is frequently encountered in other cytoplasmic systems (*e.g.*, preferentially oriented parts of the endoplasmic reticulum, dictyosomes). However nowhere is the stacking so regular and so extensive as in the photosensitive organelles such as the chloroplasts, the outer segments of the retinal rods (63), and possibly the myeloid bodies of the pigmented retinal epithelium (51). One may ask whether in these organelles the stacking may be essential for function.

Imperfect Segmentation into Grana:

As already pointed out, in the higher plants the grana consist of stacks of discs; and in the non-grana-containing algae, the discs run from one end of the chloroplast to the other. In *Chlamydomonas* the single, large, cup-shaped chloroplast contains many irregularly oriented stacks of discs which resemble the grana of higher plants, but exhibit much greater irregularity in size and in orientation. It would appear that the chloroplast of *Chlamydomonas* represents a stage in evolution transitional between the non-grana-containing

chloroplast typical of the algae, and chloroplasts of the higher plants with their usually uniform and regularly disposed grana. It is interesting to note that despite the range of stack sizes in *Chlamydomonas*, their average diameter does correspond rather closely to the diameter of grana in higher plants; perhaps this is an optimal dimension for photosynthetic efficiency.

Limiting Membranes:

Although the existence of a limiting membrane around the chloroplast is still questioned in certain studies, published micrographs of well preserved material clearly demonstrate the presence of such a membrane. In favorably oriented sections of *Chlamydomonas* it is further evident that two closely apposed membranes⁴ surround the organelle, thus forming a chloroplast envelope similar in its general construction to, but different in detail from the nuclear envelope described by Watson (72). The chloroplast appears, therefore, to be surrounded by a relatively vast cisterna.

Connections have been observed between the chloroplast envelope and the membranous systems of the cytoplasm. The doubleness of the chloroplast membrane is probably related to these anastomoses. Their presence and the existence of a cisterna in between the two membranes of the chloroplast makes possible the transfer of materials between the vacuolar systems of the cytoplasm and the plastid without direct contact with either the cytoplasmic or the chloroplast matrix. Secondly, the double membrane may be important in the process of lamellar formation, particularly if the lamellae arise by invagination of the inner membrane. It is of interest that both chloroplasts and mitochondria are surrounded by two successive membranes, and it is possible that in both organelles this disposition represents a common solution to similar problems. In view of past speculations in the cytological literature concerning relations between chloroplasts and mitochondria (21), it should be pointed out that despite the structural similarity mentioned, there is no evidence of any developmental relation between the two organelles in *Chlamydomonas*.

Chloroplast Matrix:

The light microscopists who described grana also suggested the term "stroma" for the material found in the intervening spaces (*cf.* 54). The concept of stroma as distinct from grana is no longer tenable, for the electron micrographs show clearly that the material in the non-lamellar, intergrana regions of the chloroplast is continuous with the material that occupies the interdisc spaces within the grana. It is proposed, therefore, to abandon the term "stroma" and to speak instead of a continuous matrix which surrounds and penetrates the grana, and in which the discs are embedded.

⁴ The finding confirms evidence already published (69, 26) on chloroplasts of higher plants.

There are, however, localized differences within the chloroplast matrix, as illustrated by the occurrence of dense granules (presumably lipide) and small particles (ribonucleoprotein?) in the intergrana regions, and by their absence in the interdisc spaces.

There is obviously an important distinction to be drawn between the matrix and the material within the discs. These two "phases," frequently of different density, are separated from one another by the membranes of the discs. Evidence of difference between these phases comes from the observation that swelling of the chloroplast frequently leads to irregular intradisc spacing, but close and regular spacing of the interdisc regions.

Functional Significance of the Lamellar Discs:

One may ask whether the disc, as the smallest structural unit within the chloroplast, has a special functional significance. Three aspects of the question will be considered here: (1) the membranes as energy-conducting structures containing closely packed and regularly disposed pigment molecules; (2) the disc as a "photosynthetic unit"; and (3) the disc as a permeability barrier.

The fact that the chlorophyll-containing bodies of all organisms, including photosynthetic bacteria, have a laminar structure itself suggests strongly that the lamellae may be essential for photosynthesis. Further support for this view comes from the recent evidence of energy transfer between pigment molecules in photosynthesis. Light absorbed by phycocyanin and phycoerythrin in the blue-green and the red algae is transferred to chlorophyll (4); and in *Chlorella*, a representative of the green algae, there is evidence of energy transfer from chlorophyll to chlorophyll (3). The energy transfer systems implied by these findings require close and precise disposition of pigment molecules in a continuous solid phase, such as a membrane, or lamella, through which energy transmission could occur. As suggested some years ago by Szent-Gyorgyi (70), energy transfer by free flowing electrons not bound to particular molecules may represent an important biological principle exemplified by chloroplast organization.

One may next inquire whether the disc itself represents some sort of photosynthetic unit. Emerson and Arnold (10) showed with *Chlorella* that the maximum number of CO₂ molecules reduced was some 2500 times less than the number of chlorophyll molecules present and postulated the existence of a "photosynthetic unit" represented by the minimum number of chlorophyll molecules per photosynthetic event. By using the model of chlorophyll packing and the assumptions made by Wolken and Schwertz (76) it can be computed that the maximum number of chlorophyll molecules per disc is 2.4×10^6 for spinach, a quantity considerably higher than the photosynthetic unit of Emerson and Arnold. However, if the amount of chlorophyll per disc could be varied experimentally, it might be possible to determine the minimum number of chlorophyll molecules per disc required for photosynthetic activity.

Another possible role of the disc is that of a permeability barrier. The process of photosynthesis may require the localized accumulation of high concentrations of certain diffusible metabolites or of soluble enzymes, and if so, the discs would appear to

represent suitable structures for this purpose. As an example, the carboxylating enzyme involved in photosynthetic CO₂ reduction is reportedly located within intact chloroplasts, but appears in soluble form in the supernatant after chloroplast homogenization (14). The observation suggests that the enzyme is located in one of the two "phases" separated by the disc membranes (the matrix or the disc content) and is released into the medium upon injury to the chloroplast envelope or the disc membranes.

The Pyrenoid:

The pyrenoid is a differentiated region of the chloroplast, found in most green algae and in the primitive moss *Anthoceros*, but not in higher plants. It has been considered an organelle concerned with starch synthesis since it has a high protein content and is generally associated with starch grains. Some algae, including the diatoms and some dinoflagellates, contain pyrenoids but do not form starch; there the pyrenoid appears to be associated with lipide storage.

In *Chlamydomonas* the pyrenoid contains no lamellae and therefore presumably no chlorophyll; it is characterized by a network of tubules embedded in a matrix of dense, finely granular material. The matrix of the pyrenoid is continuous with the matrix of the chloroplast; and the tubules are connected with the discs of the surrounding plastid. These tubules may represent a drainage system, conducting products of photosynthesis from the lamellae to a region specialized for starch synthesis. Apparently the pyrenoid is not an obligate system, for starch is also stored in other regions of the plastid, between stacks of discs. However, the first starch formed is stored in the plates surrounding the pyrenoid, and only under conditions of accumulation do starch grains also appear elsewhere.

Descriptions of pyrenoids of several algae as seen in the electron microscope have recently appeared in the literature. The pyrenoids described in *Euglena* (75), and in *Spirogyra* and *Closterium* (32) appear to contain lamellae continuous with those of the chloroplast but differently disposed. In *Chlorella* the situation is comparable to that found in *Chlamydomonas* since Albertsson and Leyon (1) have reported that the corresponding pyrenoid is a dense amorphous body, which is separated from the laminae of the chloroplast by starch plates and in which a tubular element has been noted.

Impressed by the fact that higher plants have grana but not pyrenoids, whereas the algae possess pyrenoids but not grana, Leyon has suggested (32) a functional and phylogenetic relationship between these two plastid components. The situation encountered in *Chlamydomonas* which has in the same chloroplast partly differentiated grana, a well developed pyrenoid, as well as extrapyrenoidal deposits of starch, may be used as a basis for a different interpretation according to which the grana represent an advanced organization

of chloroplast discs, whereas the pyrenoid is a primitive organ of starch synthesis whose function is gradually taken over by certain interdisc regions of the chloroplast. In this interpretation the evolutionary changes in the two systems are not functionally related. Evolutionary steps in pyrenoid disintegration have been described (27) in a comparison of different genera of *Anthoceros*, which show a range of types from a homogenous pyrenoid to complete dispersal throughout the chloroplast.

The Eyespot:

The eyespot of *Chlamydomonas* is a highly differentiated region of the chloroplast, consisting of two or sometimes three plates of hexagonally packed granules in a constant location, sandwiched between peripheral discs, and therefore lying in the interdisc matrix of the chloroplast. In each plate the granules are so closely packed that they constitute a continuous solid barrier to light penetration. No fibrous connections have been found thus far between the granules of the eyespot and any other part of the cell. Signals from this organ must presumably then be transmitted by chemical means.

Chlamydomonas is phototactic, the eyespot being considered the photoreceptor organ of the cell. However, Hartshorne (23) found that an "eyeless" mutant of *C. reinhardi*, having no eyespot detectable by light microscopy, was nonetheless phototactic, though very much less so than the normal strain. He suggested that the eyespot might not be the unique site of all photoreceptor activity.

Measurements of the action spectrum of phototaxis in *Chlamydomonas* and other algae (29, 36) reveal peak activity in a carotenoid-absorbing region. In *Haematococcus*, a close relative of *Chlamydomonas*, and in *Euglena* the eyespot pigment has been identified as astaxanthin, a pigment previously found only in invertebrates (71). Apparently carotenoids have been associated with photoreception from early evolutionary times.

Other Cytoplasmic Structures

Mitochondria

The mitochondria of *Chlamydomonas* are rod-shaped organelles which resemble those of animal tissues (42, 64) in being surrounded by two membranes and in containing plate-like structures or cristae which protrude into the interior of the organelles. In favorably oriented sections it is apparent that the cristae are continuous with the inner mitochondrial membrane.

The cristae mitochondriales of *Chlamydomonas* differ from those of animal cells in the randomness of their orientation. They are not lined up perpendicular to the long axis of the mitochondrion, and consequently most mitochondrial profiles contain cristae in both normal and oblique section (face- and end-on views). The mitochondria of normal green algae contain fewer cristae than do those of most animal tissues. However, preliminary observations of mutant strains and of normal green cells grown

under various nutritional conditions have revealed differences in the number of cristae per unit length of mitochondrial profile apparently correlated with the physiological state of the cells. This finding is consistent with the variation reported in the number of mitochondrial cristae from tissue to tissue in animal material (42).

The plate-shaped cristae of *Chlamydomonas* resemble those described in *Euglena* (75) both in shape and in randomness of orientation, but they differ from the villous projections found in ciliates such as *Paramecium* (61), *Tetrahymena*, and *Tokophrya* (62).

Membranous Systems of the Cytoplasm:

The cytoplasm of animal cells is permeated by a network of membrane bound cavities described under the name of endoplasmic reticulum (49, 45). Recent investigations have shown that several local differentiations can be recognized within this system, one of them resulting from the close association of the membrane limiting the cavities of the reticulum with small dense particles of ribonucleoprotein (46).

Another membranous system distinguished by its preferential location in the centrosphere region, the lack of associated particles, and the tight packing of its cisternal elements has been identified as the Golgi apparatus of the classical cytological literature (7, 22, 65). In invertebrate material and in protozoa, structures of similar morphology have been identified (8, 16, 20 *b*) with the dictyosomes described in light microscopy. These structures differ from the Golgi apparatus of vertebrate material primarily in the absence of associated large vacuoles. In other respects, the morphological similarities between the stacks of cisternal elements found in all types of cells so far investigated, has led to the hypothesis (20 *b*) that all these structures are homologous, a reasonable view, but one as yet without supporting evidence of functional identity.

In animal cells vesicles of intermediate type and points of fusion between the endoplasmic reticulum and the Golgi complex have been repeatedly noted and the hypothesis was advanced that the two systems are not independent but represent regional differentiations within a common network of membrane-bound cavities (44).

In the case of *Chlamydomonas* it has been a simple matter to distinguish the two membranous systems which differ in distribution and arrangement. One system can be identified with the endoplasmic reticulum of animal cells on account of the size and shape of its profiles, its location throughout the cytoplasm, and the frequent presence of particles attached to its membranes. The other system, represented by tightly packed stacks of smooth surfaced cisternae, is largely restricted to the perinuclear region. (*Chlamydomonas* lacks a distinct centrosphere region.) On purely morphological grounds there is an evident similarity between these membranous structures and those found in the centrosphere region of animal cells. The principal differences lie in the small size of the stacks, the higher regularity in the disposition of the component cisternae, and the absence of associated large vacuoles. In these respects the formations encountered in *Chlamydomonas* are highly reminiscent of the dictyosomes described in invertebrate material (8, 20 *c*) and in certain protozoa (16).

In addition to the dictyosomes described in certain flagellates (*cf.* 20 *a*) a few other notations in the literature may refer to this organelle. In cells fixed with osmium-containing fixatives and stained with haematoxylin, a cone-shaped fibrous mass near

the centrosome and spreading over the nuclear membrane has been described by Kater in *Chlamydomonas nasuta* (28), by Elliott in *Haematococcus* (9), and by Entz in *Polytoma* (11). This material was not identified, but was considered by Kater and by Elliott to be extraneous to the neuromotor system, which was the focus of interest in the mentioned studies.

Elements of intermediate appearance and instances of direct continuity between the two membranous systems described were frequently encountered in *Chlamydomonas*. In addition, continuities of limiting membranes and contents were found between the endoplasmic reticulum on one side, and the nuclear and chloroplast envelopes on the other. Elements of the dictyosomes were also found in continuity with the chloroplast envelope and with the membranes surrounding the large cytoplasmic vacuoles. Whether the various membrane-bound structures of the cytoplasm be regarded as one system or several, the existence of anastomoses and intergradations shows that these structures feed into one another. In this sense they may be considered as the circulatory system of the cytoplasm.

The Nucleus and Nuclear Membranes:

In rapidly fixed preparations of *Chlamydomonas* the nucleus is found to contain a large, centrally located, dense nucleolus, which consists in part of a mass of ~ 150 A particles. Similar particles have been observed to comprise the nucleoli of other cells and to occur free or attached to the elements of the endoplasmic reticulum in the cytoplasm. Moreover, it has been suggested (50) that the nucleolar particles are homologous with those of the outer nuclear membrane and of the cytoplasm, and that they may even move from one site to another. In *Chlamydomonas* the nucleolar and cytoplasmic particles respond differently to fixation. After a short fixation all are well preserved, but after a long fixation (24 hours) the small nucleolar granules are completely extracted and lost from the cell, while those associated with the outer nuclear membrane and with the endoplasmic reticulum remain apparently unaltered. The observation suggests that there may be an important chemical difference between the nucleolar and cytoplasmic particles.

The nuclear envelope of *Chlamydomonas* resembles those of other cells (72), in consisting of two membranes, the outer one being covered with dense granules and the inner one being smooth. This double membrane is provided with many perforations or pores which may be covered by a thin diaphragm. In other organisms, each pore has been found to be surrounded by a definite ring of granules (15). In *Chlamydomonas*, however, no evidence of such annuli has been observed thus far in favorably oriented sections of the nuclear envelope.

SUMMARY

The cytoplasmic organization of a normal green strain of the alga *Chlamydomonas reinhardi* has been investigated with the electron microscope using thin sections of OsO₄ fixed material.

The detailed organization of the chloroplast has been of special interest. The chloroplast, a cup-shaped organelle, surrounded by a double membrane, consists of: (1) discs about 1 micron in diameter, considered to represent the

basic structural unit of the chloroplast, and each composed of a pair of membranes joined at their ends to form a flat closed vesicle; the discs are grouped into stacks resembling the grana of higher plants; (2) matrix material of low density in which the discs are embedded; (3) starch grains; (4) the pyrenoid, a non-lamellar region associated with starch synthesis, and containing tubules which connect with the lamellae; (5) the eyespot, a differentiated region containing two or three plates of hexagonally packed, carotenoid-containing granules, located between discs, and associated with phototaxis.

In addition to the chloroplast, the cytoplasm contains various membranous and granular components, including mitochondria, endoplasmic reticulum, and dictyosomes, identified on the basis of morphological comparability with structures seen in animal cells. The nucleus, not investigated in detail in this study, contains a large, granular nucleolus and is surrounded by a nuclear envelope which is provided with pores and exhibits instances of continuity with the endoplasmic reticulum of the cytoplasm.

BIBLIOGRAPHY

1. Albertsson, P. A., and Leyon, H., *Exp. Cell Research*, 1954, **7**, 288.
2. Algra, L., Beijer, J. J., Van Iterson, W., Karstens, W. K. H., and Thung, T. H., *Biochim. et Biophysica Acta*, 1947, **1**, 517.
3. Arnold, W., and Meek, E. S., *Arch. Biophysic. and Biochem.*, 1956, **60**, 82.
4. Blinks, L. R., *The role of Accessory Pigments in Photosynthesis, in Autotrophic Microorganisms*, Cambridge, University Press, 1954.
5. Calvin, M., and Lynch, V., *Nature*, 1952, **169**, 455.
6. Cohen, M., and Bowler, E., *Protoplasma*, 1953, **42**, 414.
7. Dalton, A. J., and Felix, M. D., *Am. J. Anat.*, 1954, **94**, 171.
8. Dalton, A. J., and Felix, M. D., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 79.
9. Elliott, A. M., *Arch. Protistenk.*, 1934, **82**, 250.
10. Emerson, R., and Arnold, W., *J. Gen. Physiol.*, 1932, **16**, 191.
11. Entz, G., Jr., *Arch. Protistenk.*, 1918, **38**, 324.
12. Fawcett, D. W., and Porter, K. R., *J. Morphol. and Physiol.*, 1954, **94**, 221.
13. Frey-Wyssling, A., *Submicroscopic Morphology of Protoplasm and Its Derivatives*, New York, Elsevier Publishing Co., 1948, 155.
14. Fuller, R. C., *Chemistry Division Quarterly Report, University of California Radiation Laboratory Report, UCRL 2932, March 30, 1955*, 32.
15. Gall, J. G., *Exp. Cell Research*, 1954, **7**, 197.
16. Gatenby, J. B., Dalton, A. J., and Felix, M. D., *Nature*, 1955, **176**, 301.
17. Gerloff, J., *Arch. Protistenk.*, 1940, **94**, 311.
18. Granick, S., and Porter, K. E., *Am. J. Bot.*, 1947, **34**, 545.
19. Granick, S., *Plastid structure, Development and Inheritance, in Encyclopedia of Plant Physiology*, (W. Ruhland, editor), Berlin, Julius Springer, 1955, **1**, 507.
- 20 a. Grassé, P. P., *Compt. rend. Acad. sc.*, 1956, **242**, 858.
- 20 b. Grassé, P. P., and Carasso, N., *Nature*, 1957, **179**, 31.

- 20 c. Grassé, P. P., Carasso, N., and Favard, P., *Ann. des sc. naturelles. Zool., et biol. animale*, 1956, **18**, 339.
21. Guilliermond, A., *Cytoplasm of the Plant Cell*, Waltham, Massachusetts, Chronica Botanica, 1941.
22. Haguénau, F., and Bernard, W., *Arch. anat. micr. et morphol. exp.*, 1955, **44**, 27.
23. Hartshorne, J. N., *New Phytologist*, 1953, **52**, 292.
24. Heitz, E., *Ber. vol. Ges.*, 1936, **54**, 362.
25. Heitz, E., *Planta*, 1936, **26**, 134.
26. Hodge, A. J., McLean, J. D., and Mercer, F. V., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 605.
27. Kaja, H., *Protoplasma*, 1954, **44**, 136.
28. Kater, J., McA., *University of California Pub. Zool.*, 1929, **33**, 125.
29. Leob, J., and Wastenays, H., *J. Exp. Zool.*, 1916, **20**, 217.
30. Lewin, R. A., Owen, M. J., and Melnick, J. L., *Exp. Cell Research*, 1951, **2**, 708.
31. Leyon, H., *Exp. Cell Research*, 1953, **4**, 371.
32. Leyon, H., *Exp. Cell Research*, 1953, **6**, 497.
33. Leyon, H., *Exp. Cell Research*, 1954, **7**, 265.
34. Leyon, H., and von Wettstein, D., *Z. Naturforsch.*, 1954, **9b**, 471.
35. Manton, I., *Nature*, 1953, **171**, 485.
36. Mast, S. O., *J. Exp. Zool.*, 1917, **22**, 471.
37. Mercer, F. V., Hodge, A. J., Hope, A. B., and McLean, J. D., *Australian J. Biol. Sc.*, 1955, **8**, 1.
38. Meyer, A., *Das Chlorophyll Kern*, Leipzig, Felix, 1883.
39. Mühlethaler, K., *Internat. Rev. Cytol.*, 1955, **4**, 197.
40. Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bureau Standards*, 1949, **43**, 183.
41. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
42. Palade, G. E., *J. Histochem. and Cytochem.*, 1953, **1**, 188.
43. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 59.
44. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.
45. Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100**, 641.
46. Palade, G. E., and Siekevitz, P., *J. Biophysic and Biochem. Cytol.*, 1956, **2**, 171.
47. Palay, S. L., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.
48. Pardee, A. B., Schachman, H. K., and Stanier, R. Y., *Nature*, 1952, **169**, 282.
49. Porter, K. R., *J. Exp. Med.*, 1953, **97**, 727.
50. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2**, 346.
51. Porter, K. R., *The Harvey Lectures*, 1955-1956, **51**, 175.
52. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117**, 685.
53. Preston, R. D., and Kayper, B., *J. Exp. Bot.*, 1951, **2**, 247.
54. Rabinowitch, E. L., *Photosynthesis*, New York, Interscience Press, 1945, I; 1956, II, **2**.
55. Rhodin, J., and Dalhamn, T., *Z. Zellforsch. u. mikr. Anat.*, 1956, **44**, 345.
56. Sager, R., *Proc. Nat. Acad. Sc.*, 1954, **40**, 356.
57. Sager, R., *Genetics*, 1955, **40**, 476.
58. Sager, R., and Granick, S., *Ann. New York Acad. Sc.*, 1953, **56**, 831.
59. Sager, R., and Palade, G. E., *Exp. Cell Research*, 1954, **7**, 584.

60. Scarth, G. W., *Quart. J. Exp. Physiol.*, 1924, **14**, 115.
61. Sedar, A. W., and Porter, K. R., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 583.
62. Sedar, A. W., and Rudzinska, M. A., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 331.
63. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42**, 15.
64. Sjöstrand, F. S., and Rhodin, J., *Nature*, 1953, **171**, 30.
65. Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7**, 415.
66. Smith, G. M., *Fresh-water Algae of the United States*, New York, McGraw-Hill Book Company, 2nd edition, 1950.
67. Sotello, J., and Porter, K. R., unpublished observations.
68. Steinmann, E., *Exp. Cell Research*, 1951, **3**, 367.
69. Steinmann, E., and Sjöstrand, F. S., *Exp. Cell Research*, 1955, **8**, 15.
70. Szent-Gyorgyi, A., *Science*, 1941, **93**, 609.
71. Wald, G., *Vitamins and Hormones*, 1953, **1**, 195.
72. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
73. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 183.
74. Weier, T. E., and Stocking, C. R., *Bot. Rev.*, 1952, **18**, 14.
75. Wolken, J. J., and Palade, G. E., *Ann. New York Acad. Sc.*, 1953, **56**, 873.
76. Wolken, J. J., and Schwertz, F. A., *J. Gen. Physiol.*, 1953, **37**, 111.

EXPLANATION OF PLATES

PLATE 148

FIG. 1. Longitudinal section of *Chlamydomonas*, showing a general view of the internal organization of the cell. The anterior-posterior diameter, which appears the shortest in this figure, is actually the longest diameter of the living or fixed cell. In this section the shape has been deformed by knife pressure during microtomy.

The nucleus appears at *n*, the chloroplast is represented by four distinct profiles at *c*₁, *c*₂, *c*₃, and *c*₄; and the pyrenoid at *py*. Starch plates and starch grains, almost completely extracted, are marked *sp* and *s* respectively. The cytoplasm contains a number of mitochondria (*m*), a dictyosome (*dy*), elements of the endoplasmic reticulum (arrow), and a lipide inclusion (*l*). The large vacuole of irregular outline marked *v* is probably a contractile vacuole. The cell membrane can be seen at *cm*.

Specimen fixed for 24 hours at room temperature in 1 per cent OsO₄ buffered at pH 8.5. × 15,000.

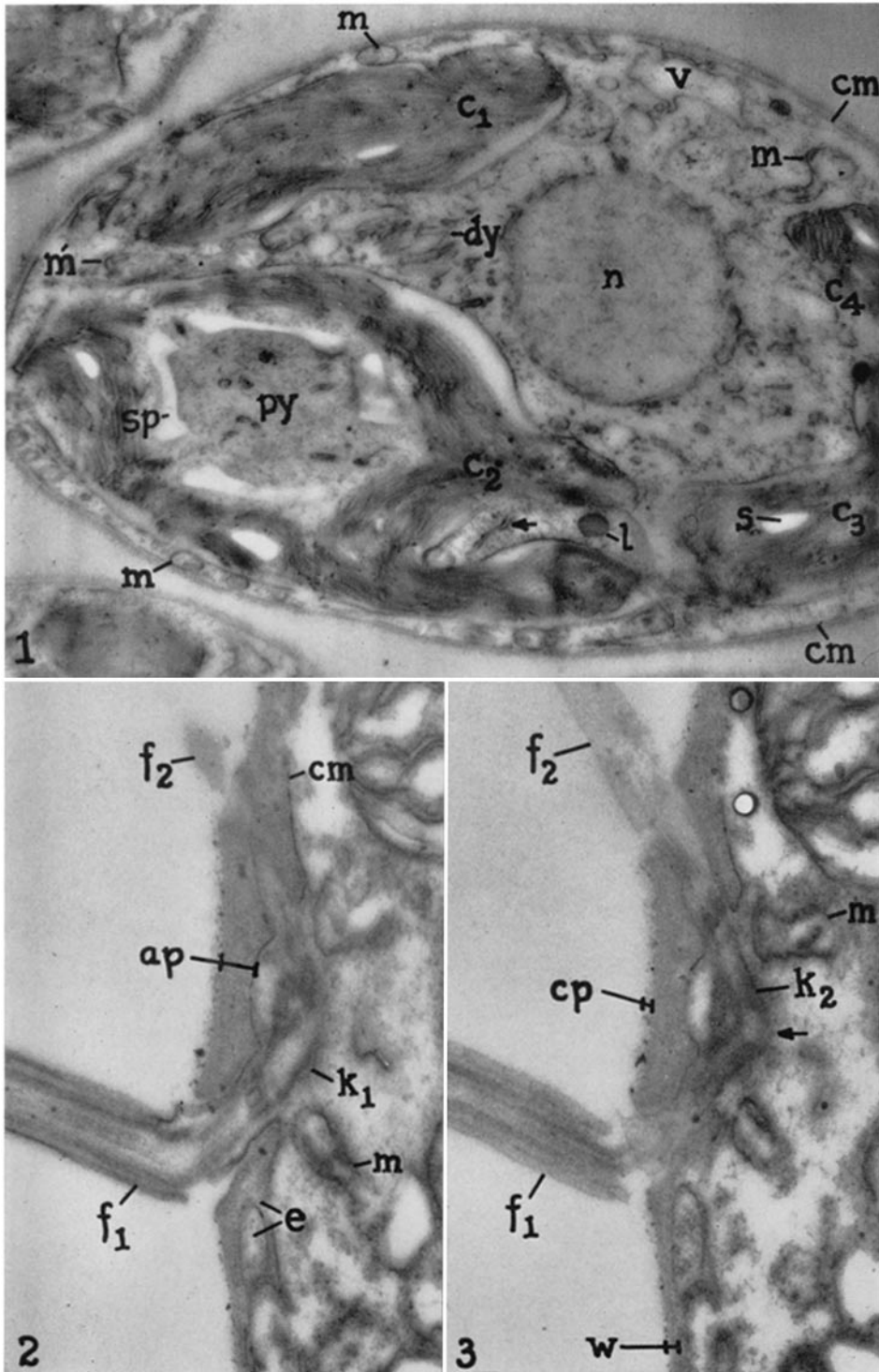
FIGS. 2 and 3. Two consecutive sections through the anterior pole of the cell. The first section (Fig. 2) cuts longitudinally through one of the flagella (*f*₁) and its basal body (*k*₁) and only grazes the second flagellum (*f*₂). The latter, together with the corresponding basal body (*k*₂), appears more clearly in Fig. 3. Note that the two basal bodies are joined (arrow) and that a bundle of dense fibrils is located on the anterior side of this junction.

Mitochondrial profiles in the immediate vicinity of the basal bodies are marked *m*.

The continuity of the cell membrane (*cm*) with the membrane covering the flagella is clearly visible for *f*₁ in Fig. 2. Small invaginations and evaginations of the cell membrane appear at *e*. Interruptions in the cell membrane result from unsatisfactory preservation.

The cell wall can be seen at *w*; its thickened anterior plate (*ap*) is provided with tunnels through which the flagella pass. In this cell, the capsular layer (*cp*) is relatively thin; it has a spongy structure with included dense particles of irregular size.

Short fixation (2 hours) at room temperature. × 35,000.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 149

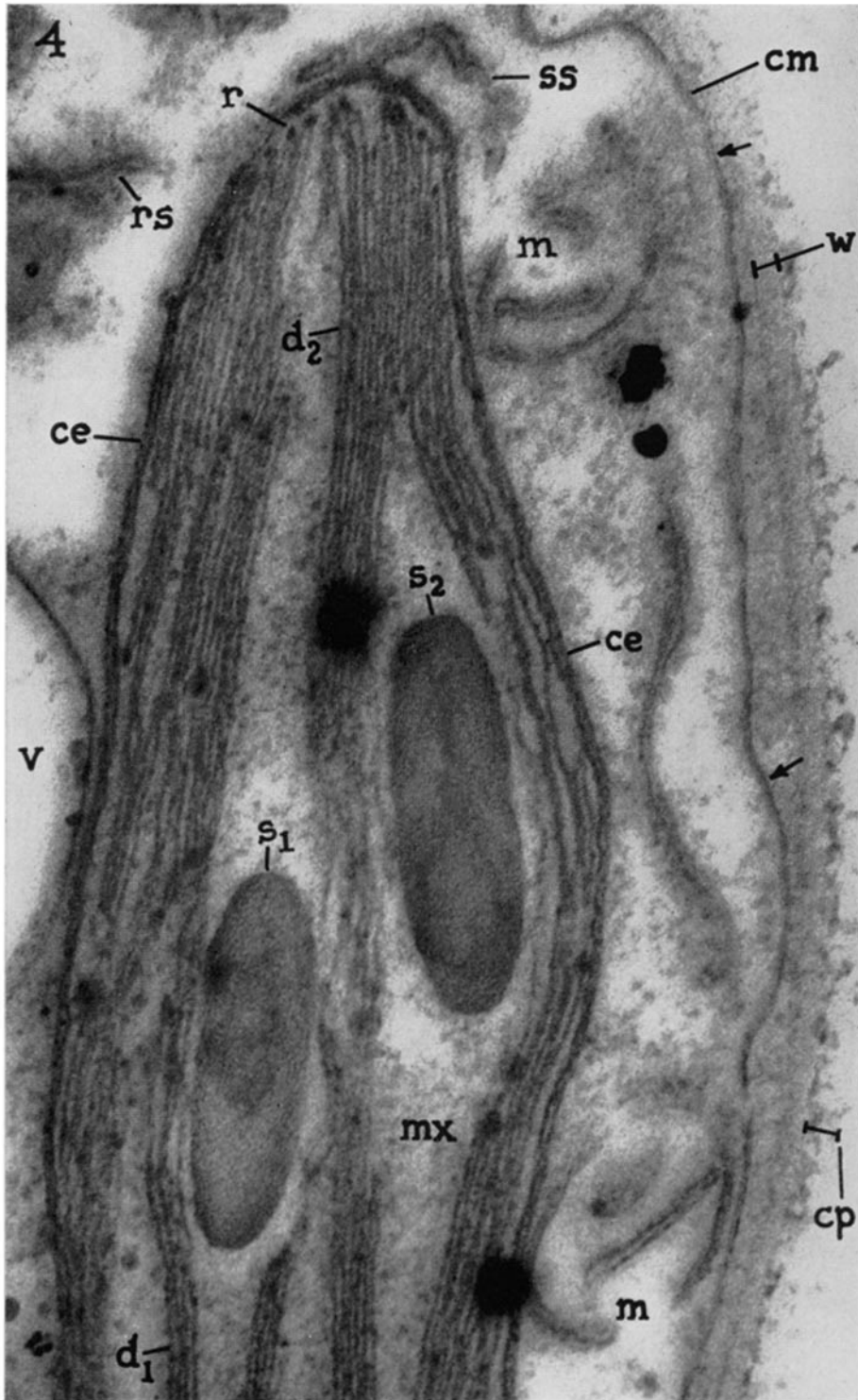
FIG. 4. Small field at the cell periphery showing one edge of the cup-shaped chloroplast.

The chloroplast envelope (*ce*) shows clearly the presence of two membranes separated by a narrow space. The interior of the plastid is occupied by a number of discs (*d*₁, *d*₂) which occur in stacks of two (for *d*₁) to eight (for *d*₂) units. The thickened rim of the discs can be seen at *r*. The space between the discs is filled by the relatively light chloroplast matrix (*mx*) in which are embedded small dense particles. The oblong profiles marked *s*₁ and *s*₂ represent starch grains. They have a fine, granular texture and show local variations in density.

In the cytoplasm that surrounds the chloroplast appear two mitochondrial profiles (*m*), part of a large vacuole (*v*), and profiles of the endoplasmic reticulum (*ss*, *rs*). The profile marked *ss* is apparently continuous with the chloroplast envelope.

The cell membrane (*cm*) shows evidence of stratification in many places (arrows). Between it and the cell wall (*w*) there is a narrow space of irregular depth filled by a relatively light material. The capsule (*cp*) appears as an uneven spongy layer outside the cell wall.

Fixation: 2 hours at room temperature in 1 per cent OsO₄ buffered at pH 8.5. The surface of the section is contaminated by a few dust particles (one under the upper *m*, another to the left of *s*₂). × 130,000.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 150

FIG. 5. Relatively large field at the periphery of a cell showing part of the chloroplast and the surrounding cytoplasm which contains mitochondria (*m*), smooth (*ss*) and rough surfaced (*rs*) elements of the endoplasmic reticulum and small, dense particles apparently freely dispersed in the cytoplasmic matrix.

The chloroplast profile shows: the chloroplast envelope (*ce*) with the two component membranes diverging at long arrow; stacks of discs or rudimentary grana (*g*₁, *g*₂, *g*₃, *g*₄) which appear cut normally for *g*₁ and *g*₂, obliquely for *g*₃, and very obliquely for *g*₄; intergrana spaces filled with a light matrix (*mx*) containing a dense particulate material; "empty" vacuoles (*s*) left behind by extracted starch grains; and dense homogeneous granules (*l*) presumed to be lipid inclusions.

Unsatisfactory preservation accounts for: discontinuities in the cell membrane and chloroplast envelope; retraction spaces between the latter and the grana; and irregular swelling of the intradisc spaces.

Note that the interdisc spaces are in general narrower (*g*₁, *g*₂) and more uniform (*g*₁) than the intradisc spaces; that the fragmentation of the laminar material into grana is incomplete—discs passing from one granum to another are indicated by short arrows; and that many discs in the grana *g*₁ and *g*₂ appear to be attached by their thickened rims to the chloroplast envelope.

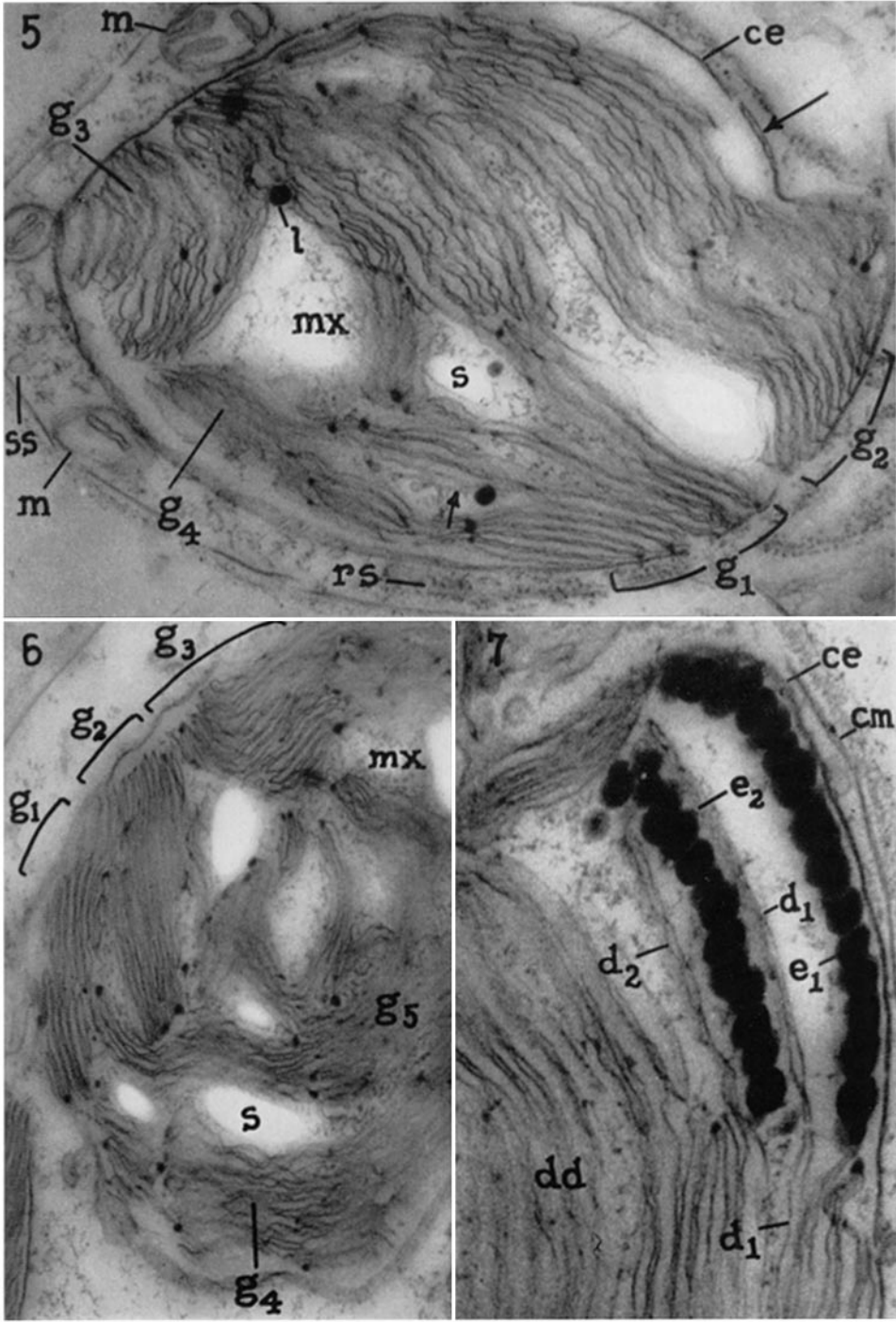
Fixation: 24 hours at room temperature in 1 per cent OsO₄ buffered at pH 8.5. Extraction during the long fixation has removed the material of the cell wall, the starch grains, and most of the chloroplast and cytoplasm matrix. As a result the chloroplast discs appear in good contrast. × 45,000.

FIG. 6. The chloroplast profile in this figure shows to advantage the arrangement of the chloroplast discs into a number of well individualized stacks or grana (*g*₁, *g*₂, *g*₃, *g*₄, *g*₅) which appear oriented at random in respect to each other. Some of these grana are normally sectioned (*g*₁), others are cut at various degrees of obliqueness (*g*₄, *g*₅). Note changes in the orientation of the discs within the same granum (*g*₂, *g*₄). The finding indicates that the granum as a whole is bent or twisted, owing perhaps to the movements of the cell. An "empty" vacuole left behind by an extracted starch grain is marked *s* and a region filled with chloroplast matrix *mx*.

Fixation: as for specimen in Fig. 5. × 37,000.

FIG. 7. Normal section through the eyespot. The cell membrane can be seen at *cm*, the chloroplast envelope at *ce*, and the stacked discs of the chloroplast at *dd*. Two rows of extremely dense, relatively large granules appear at *e*₁ and *e*₂; *e*₁ is situated between the chloroplast envelope and a disc (*d*₁); *e*₂ between two adjacent discs (*d*₁ and *d*₂).

Fixation: as for specimen in Fig. 5. × 65,000.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 151

FIG. 8. Oblique section through the eyespot. The favorable incidence of the section reveals the high degree of order with which the eyespot granules are arranged in the plate. Hexagonal packing is clearly visible around the granule marked with a cross. A fault in the lattice appears in the second vertical row to the right. Each granule has in section a hexagonal profile, and appears to possess a dense core (*cr*). Narrow regular spaces occur between the packed granules.

The chloroplast discs, the cell membrane, and the capsular layer—all obliquely sectioned—appear at *dd*, *cm*, and *cp* respectively.

Fixation: 24 hours at room temperature in 1 per cent OsO₄ buffered at pH 8.5 × 47,000.

FIG. 9. Part of the pyrenoid and of the surrounding chloroplast discs. The pyrenoid consists of a mass of finely granular, relatively dense material (*py*) in which are embedded tubular structures which appear normally, obliquely, and longitudinally sectioned at *t*₁, *t*₂, and *t*₃ respectively. Local thickenings can be seen on the walls of these tubules. A starch plate is seen at *sp* in close apposition to the pyrenoid mass. A corona of relatively light chloroplast matrix (*mx*) surrounds the pyrenoid and associated starch plates.

The chloroplast discs appear sectioned normally at *d*₁, and obliquely at *d*₂. Note that in this chloroplast the discs are predominantly disposed in pairs (arrows) with the interdisc spaces poorly resolved.

The chloroplast matrix contains dense granules (*l*), presumably lipide droplets, and small, dense particles (at *p*).

Note that the dense walls of the pyrenoid tubule *t*₃ appear to be in continuity (*x*) with a group of chloroplast lamellae.

Short fixation at room temperature. × 55,000.

FIG. 10. The pyrenoid and its surroundings as seen in a medial section.

The pyrenoid contains a number of obliquely (*t*₁) or longitudinally (*t*₂) sectioned tubules and is surrounded by four partially extracted starch plates (*sp*). Bands or "trabeculae" (*tr*) of light material separate the starch plates and connect the pyrenoid with the rest of the chloroplast. Stacks of chloroplast discs (*dd*) interspersed with finely granular matrix and starch grains (*s*) are disposed all around the starch plates.

The chloroplast envelope (*ce*) is separated from the cell membrane (*cm*) by a thin shell of cytoplasm which contains mitochondria (*m*), elements of the endoplasmic reticulum (*rs*), and small, dense particles (*p*).

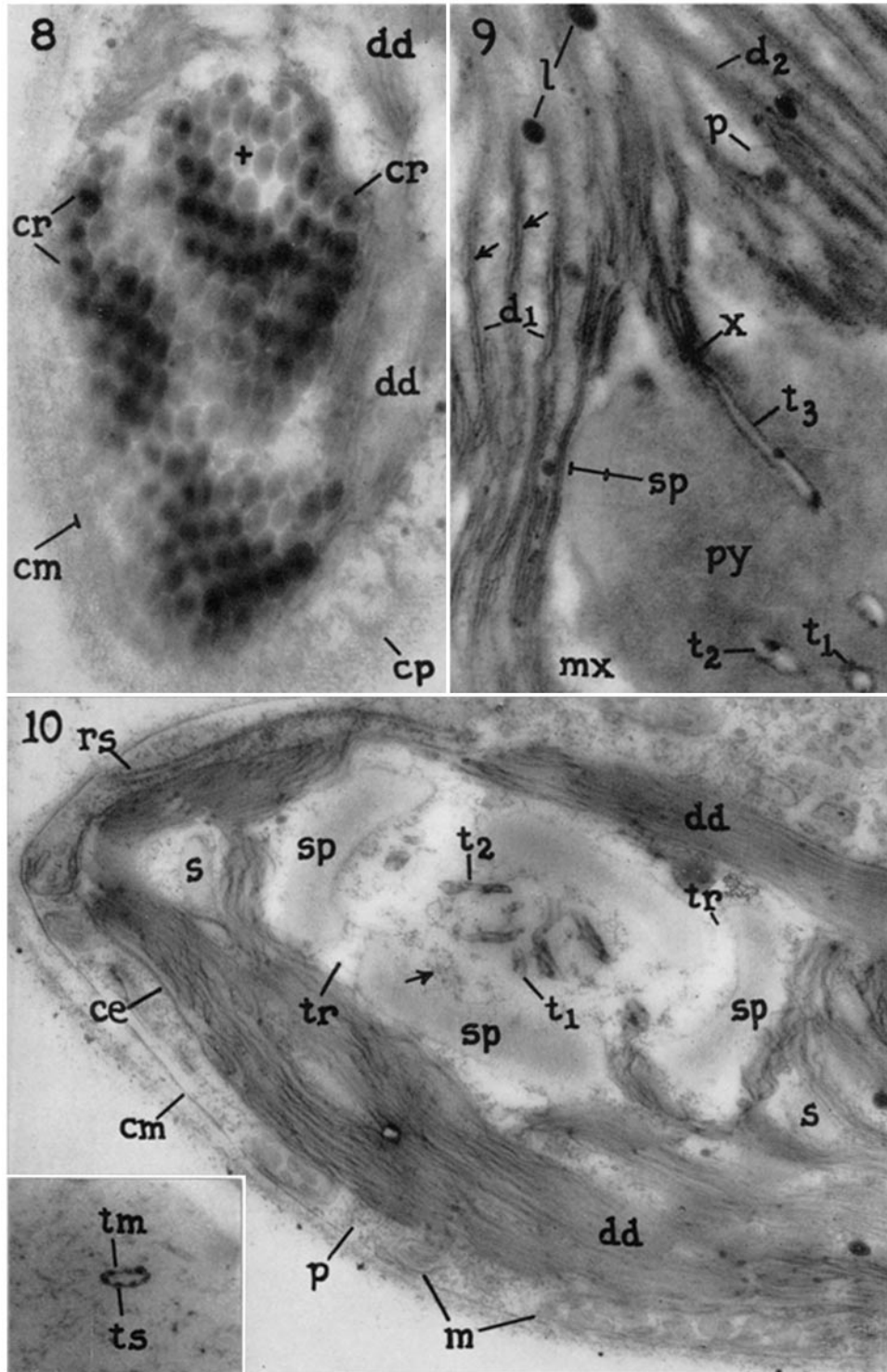
Fixation: as for specimen in Fig. 8. A comparison with Figs. 9 and 11 indicates that most of the pyrenoid material has been extracted during the long fixation and only a fine fibrillar and granular residue has been left behind. × 36,000.

FIG. 10 (insert). Structural details of a pyrenoid tubule.

The micrograph shows clearly the limiting membrane of the tubule (*tm*), its thickenings (*ts*) that protrude mostly towards the lumen, and the light content of the latter.

The fine granules and filaments around the tubule represent the residue after the extraction of most of the pyrenoid material.

Fixation: as for specimens in Figs. 8 and 10. × 50,000.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 152

FIG. 11. The pyrenoid and its surroundings as seen in a medial section of unextracted material.

The pyrenoid (*py*) is a mass of dense material of fine granular texture in which are embedded a number of tubular structures. The latter appear either normally (*t₁*), or obliquely (*t₂*, *t₃*) sectioned. The oblique sections reveal that the wall thickenings of the pyrenoid tubules are actually long strands or ridges of dense material protruding into the lumen.

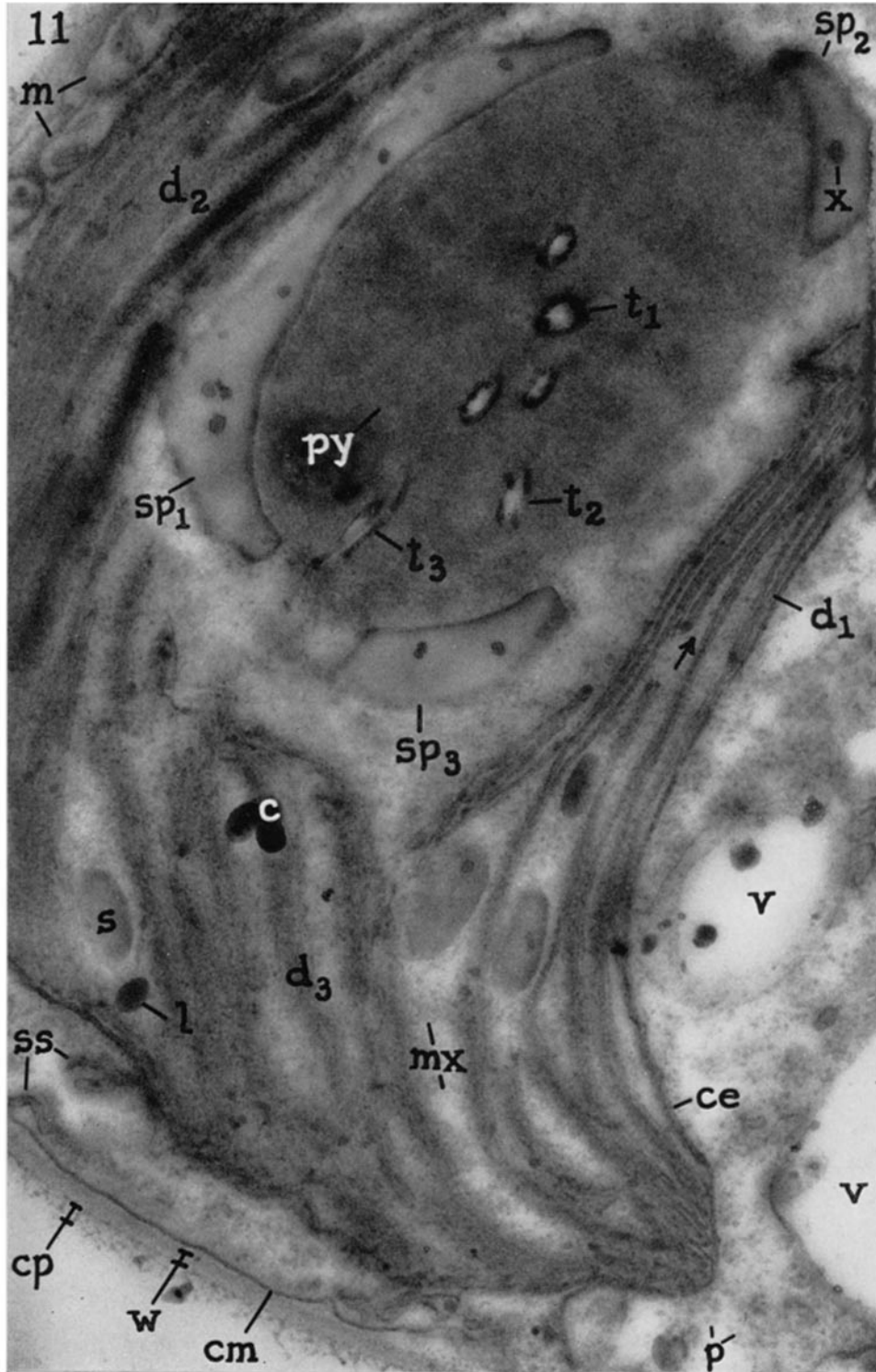
Three starch plates (*sp₁*, *sp₂*, *sp₃*) can be seen closely apposed to the pyrenoid. Note that their material, homogeneous at the present level of resolution, shows an increase in density in the immediate vicinity of the pyrenoid. Note also that a narrow light region separates *sp₁* from the pyrenoid. Dense bodies with polygonal outline (*x*) can be seen in the starch plates and starch grains elsewhere in the chloroplast.

The pyrenoid and the starch plates surrounding it are separated from the rest of the chloroplast by a shell of relatively light material similar in appearance to, and continuous with, the chloroplast matrix. Note the broad zone of contact of the matrix shell with the pyrenoid between the starch plates *sp₂* and *sp₃*. It indicates that in three dimensions the connecting bands of the pyrenoid (*cf.* Fig. 10) are actually long and relatively narrow septa.

The chloroplast discs surrounding the pyrenoid complex are cut normally at *d₁*, and obliquely at *d₂* and *d₃*. Individual chloroplast discs with narrow, uniform intradisc spaces can be clearly seen to the left of *d₁*. In this case, there is more variation in the size of the interdisc than in that of the intradisc spaces (*cf.* Fig. 5). The chloroplast matrix (*mx*) is relatively dense and contains starch grains (*s*), dense granules (*l*)—presumably lipide, and small, dense particles. Note that the matrix is continuous with the material occupying the interdisc spaces (arrow). The chloroplast envelope is marked *ce*.

The cytoplasm around the chloroplast contains small dense particles (*p*), elements of the endoplasmic reticulum (*ss*), large vacuoles (*v*) containing irregular aggregates of high density, and mitochondria (*m*) concentrated between the chloroplast and the cell membrane (*cm*). The latter shows a few invaginations (to the right of *cm*) and is covered by a continuous cell wall (*w*) which in turn is coated by the spongy layer of the cell capsule (*cp*). A particle of dust contaminating the surface of the section is marked *c*.

Fixation: as for specimen in Fig. 4. $\times 62,000$.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 153

FIGS. 12, 13, 14. Three serial sections through the same pyrenoid.

Comparison of these sections indicates that the dense structures seen in the pyrenoids: (1) are of cylindrical form (strands or tubules); (2) join in a reticulum; and (3) are connected with the laminae of the chloroplast by elements (*cn*) located between the starch plates.

Two tubules, one cut transversely (x_1, x_2, x_3), the other obliquely (y_1, y_2, y_3) can be readily followed from section to section. Junctions of pyrenoid tubules are indicated by arrows.

Long fixation at room temperature. $\times 15,000$.

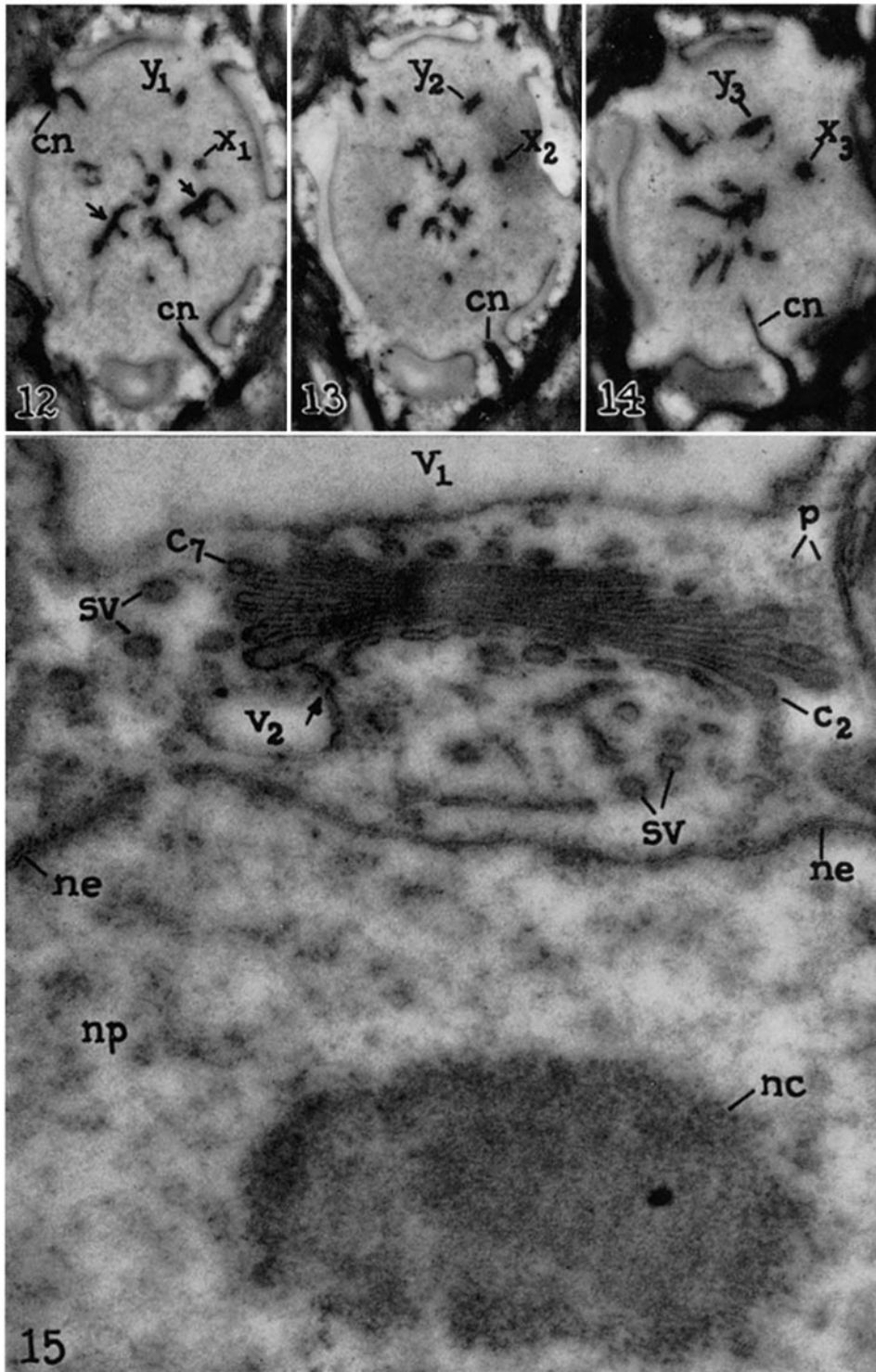
FIG. 15. A dictyosome and part of the nucleus in an unextracted cell.

The nuclear envelope can be seen at *ne*; the rather light nucleoplasm (*np*), which contains small clusters of fine granules, contrasts with the dense, well packed mass of the nucleolus (*nc*). The latter consists of a shell of small dense particles ($\sim 150 \text{ \AA}$) surrounding a dense core of finer texture.

The normally sectioned dictyosome appears as a stack of nine cisternae (many of them with dilated ends) surrounded by a number of small vesicles (*sv*). Two cisternae are marked c_2 and c_7 . The first cisterna (below c_2) is interrupted or fenestrated in many places. Part of a large vacuole can be seen at v_1 ; at v_2 occurs a smaller vacuole apparently connected with an element of the dictyosome (arrow).

Small dense particles (*p*) can be seen in the cytoplasm surrounding the dictyosome, but no particles are found on the cisternae or in the intervening matrix within the dictyosome.

Short fixation at room temperature. $\times 82,000$.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 154

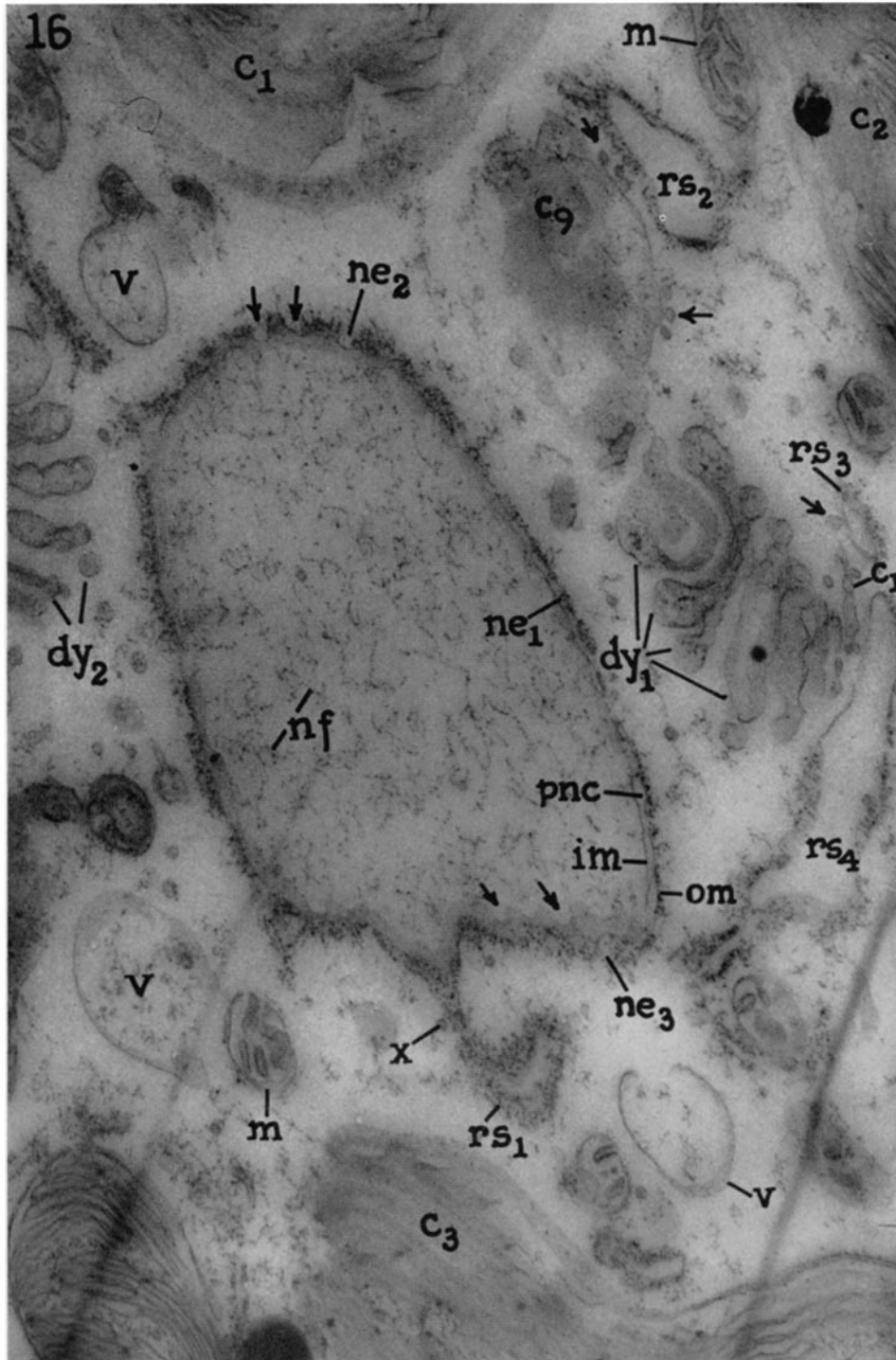
FIG. 16. A general view of the perinuclear region in an extensively extracted cell.

The nucleus has lost all of its nucleoplasm except for the fine beaded filaments marked *nf*. The two membranes of the nuclear envelope are clearly seen in normal (*ne*₁) and oblique section (*ne*₂, *ne*₃). In normal section, the envelope shows a smooth inner membrane (*im*) and a particle-covered outer membrane (*om*) enclosing the perinuclear cisterna (*pnc*). In oblique section, the envelope shows in part the outline of the nuclear pores (arrows) either in the outer (*ne*₂), or in the inner membrane (*ne*₃). A connection of the perinuclear cisterna with an element of the endoplasmic reticulum (*rs*₁) appears at *x*. A whole dictyosome (*dy*₁), and part of a second (*dy*₂) are present. In *dy*₁ the small cisterna (*c*₁) at the extreme right has been sectioned normally; those to the left are cut at increasing degrees of obliqueness. The last cisterna (*c*₃) appears almost in full-face view and shows small vesicles in continuity (arrows) with its main body. Three elements of the endoplasmic reticulum (*rs*₂, *rs*₃, *rs*₄) are found in the immediate vicinity of the dictyosome, in parallel alignment with its marginal cisternae. The limiting membrane of the elements marked *rs*₃ and *rs*₄ is free of attached particles, where it faces the cisternae of the dictyosome, and covered by such particles on the rest of their perimeter. A small vesicle of the dictyosome group is in continuity (arrow) within the element *rs*₃.

A few large vacuoles (*v*), some with ruptured membranes and fine granular content, and a number of mitochondria (*m*) can also be seen in the perinuclear region.

Parts of chloroplast profiles (*c*₁, *c*₂, *c*₃) appear at the periphery of the field.

The extensive extraction of the nucleoplasm and of the cytoplasmic matrix is due to the long fixation at room temperature. The two dense parallel bands in the lower part of the figure are folds in the supporting membrane. $\times 40,000$.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 155

FIG. 17. Small field at the anterior pole of the alga.

The cell membrane appears at *cm*, the cell wall at *w*, the capsular layer at *cp*, and a flagellum, already in the tunnel of the anterior plate, at *f*. The section has missed the corresponding basal body. A group of mitochondrial profiles (*m*) can be seen in the immediate vicinity of the implantation of the flagellum.

Elements of the endoplasmic reticulum (*ss*), small dense particles (*p*), and part of a vacuole (*v*) containing irregular masses of highly dense material are present in the rest of the field. Unidentified small dense granules (*x*) are scattered throughout the cytoplasmic matrix.

Long fixation at room temperature. $\times 23,000$.

FIG. 18. Small field in the perinuclear region.

The nucleus is seen at *n*, the nuclear envelope at *ne*, and smooth surfaced profiles, which may belong to a dictyosome, at *ss*. A group of compound vesicles (*cv*₁ to *cv*₆) appear in the center and the lower part of the figure. The group illustrates the various appearances taken by such structures: (1) the simplest form—a limiting membrane surrounding a collection of small vesicles—can be seen at *cv*₂ and *cv*₃; (2) a vesicle connected with an elongated, smooth surfaced profile at *cv*₁; and (3) a vesicle with an invaginated wall (arrow) at *cv*₄. The latter appearance suggests that the small inner vesicles are produced by the invagination of the limiting membrane of the larger vesicle. Compound vesicles are usually found in the vicinity of dictyosomes.

Long fixation at room temperature. $\times 47,000$.

FIG. 19. Mitochondrial profiles in the thin layer of cytoplasm between the cell membrane and the chloroplast. The cell membrane and its coatings (*cp*) as well as the chloroplast envelope (*ce*) have been obliquely sectioned.

The two mitochondrial membranes can be seen in normal section on the right side of profiles *m*₁ and *m*₂ and in oblique section on the left side of profile *m*₃. The cristae mitochondriales appear also in normal (*cr*₁) and oblique (*cr*₂) section. The crista at *cr*₃ seems to be entirely included in the thickness of the section.

Small dense particles (*p*) are present in large numbers in the cytoplasmic matrix.

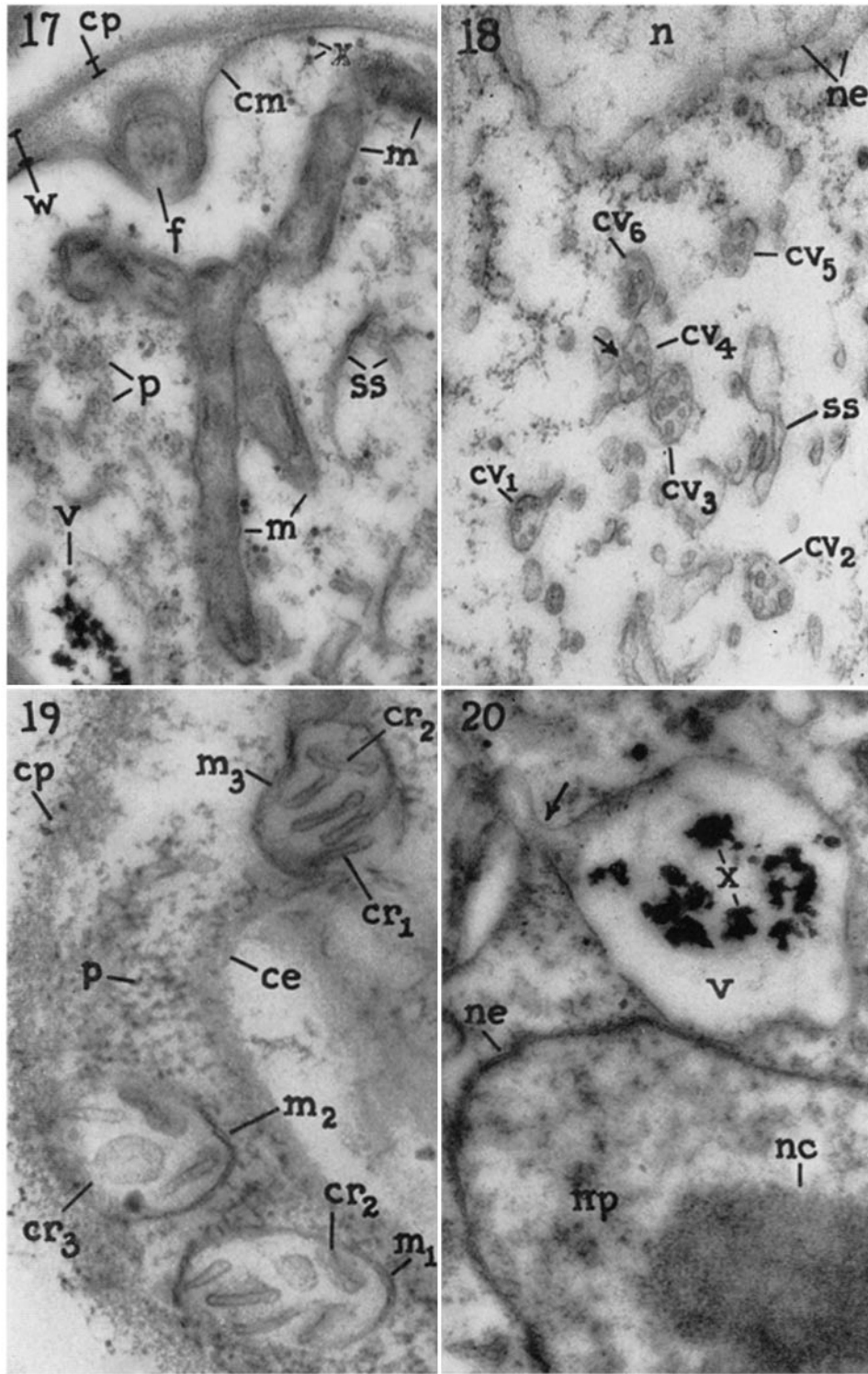
Long fixation at room temperature. $\times 65,000$.

FIG. 20. Small field showing part of the nucleus and a large vacuole.

The nuclear envelope is marked *ne*, the nucleoplasm *np*, and the nucleolar mass *nc*. The latter consists of an agglomeration of small dense particles.

The vacuole (*v*) has an irregular outline with an elongated extension in the upper left corner (arrow). Irregular masses (*x*) of high density and angular outline (crystals?), embedded in a very light matrix, form the characteristic content of such vacuoles.

Short fixation at room temperature. $\times 50,000$.



(Sager and Palade: Chloroplast in *Chlamydomonas*)

PLATE 156

FIG. 21. Field in the central region of the algal body showing a group of profiles belonging to the endoplasmic reticulum. The profiles rs_1 represent a typical rough surfaced segment of the reticulum. The profiles rss_1 and rss_2 represent intermediary or "transition" forms. Their limiting membrane is partly free of and partly covered with small dense particles. Some vesicles of the type usually found around the dictyosomes are in continuity (arrows) with the profile rss_1 . Similar vesicles occur in the immediate vicinity of the profile rss_2 .

A mitochondrial profile with randomly disposed cristae can be seen at m and part of a chloroplast profile at c . $\times 30,000$.

FIG. 22. Small field showing part of a dictyosome and elements of the endoplasmic reticulum in the perinuclear region of a cell.

The cisternae of the dictyosome (c_1 , c_2) show irregular dilatations of their lumina and are accompanied by a few small vesicles (sv). The three profiles representing the endoplasmic reticulum (rss_1 , rss_2 , rss_3) probably belong to a single large cisternal element of mixed or intermediary appearance: their limiting membrane is generally free of particles where it faces the dictyosome, and bears attached particles where it faces the rest of the cytoplasm. Two small vesicles of the type found around the dictyosome are in continuity (arrows) with the profiles rss_1 and rss_2 . Appearances like those illustrated in Figs. 16, 21, and 22 probably represent regions of continuity between the dictyosomes and the endoplasmic reticulum. $\times 50,000$.

FIG. 23. Field in the perinuclear region of *Chlamydomonas* showing a dictyosome (dy) in oblique section, a number of smooth surfaced profiles (ss) of various sizes and shapes, and a few typical, rough surfaced profiles (rs_1 , rs_2). The smooth surfaced profiles (ss) are concentrated at the lower end of the dictyosome and probably belong to the smooth surfaced parts of the endoplasmic reticulum.

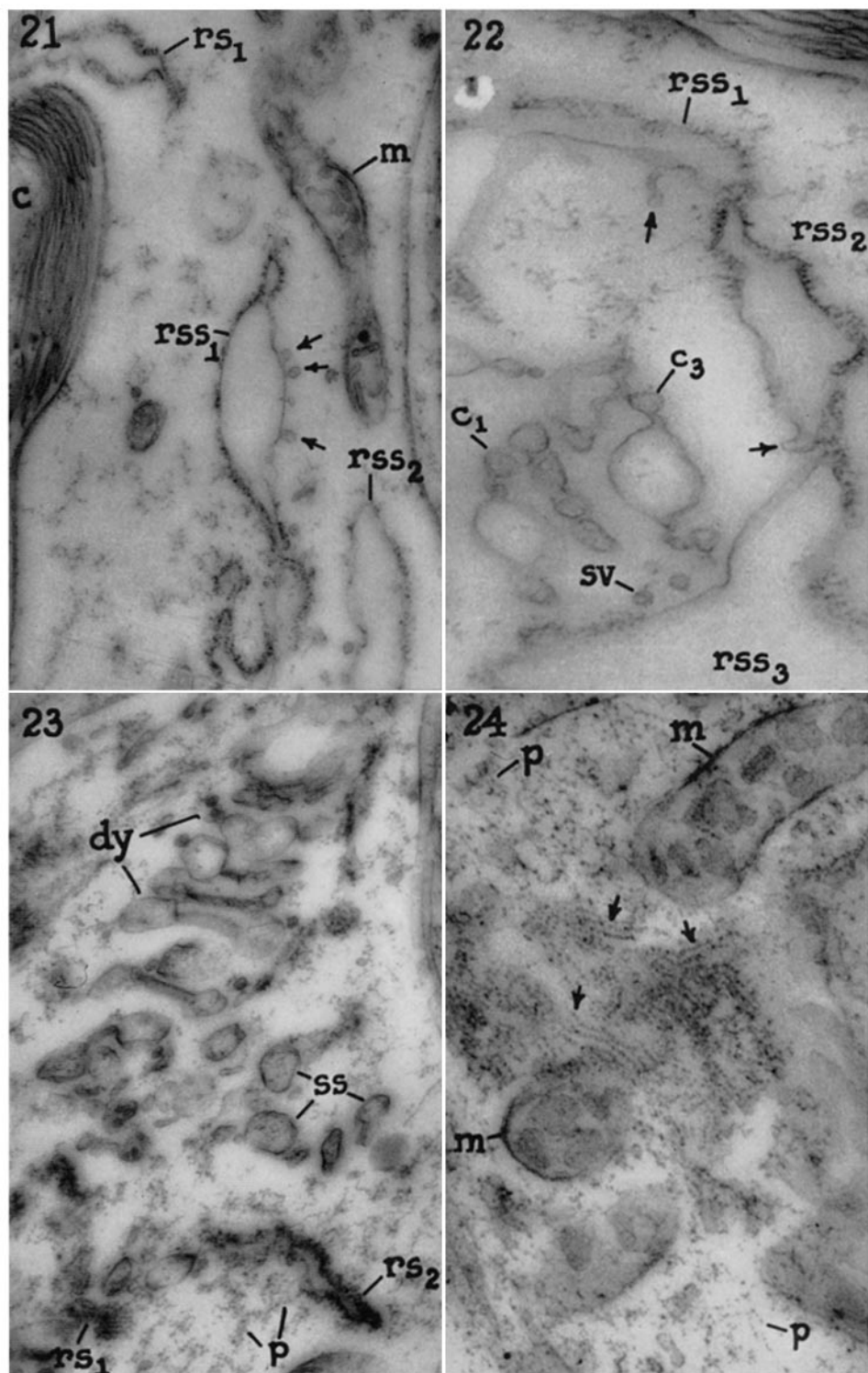
Note the regular arrangement of the particles attached to profile rs_1 , and the numerous small dense particles (p) scattered in chains or in small clusters throughout the cytoplasmic matrix. $\times 40,000$.

FIG. 24. Micrograph of a section that grazes the surface of a large cisternal element of the endoplasmic reticulum and thus reveals the patterns formed by the attached particles on the membrane limiting the cisterna.

The predominant pattern is represented by two parallel rows of particles (arrows). The membrane under the particles appears as a poorly outlined region of slightly higher density than the surrounding cytoplasmic matrix.

Two mitochondrial profiles (m), with many cristae in full-faced view, and numerous small dense particles (p), scattered in twisted chains or in clusters, occur in the rest of the field. $\times 45,000$.

Fixation for the specimens in Figs. 21 to 24: 24 hours at room temperature in 1 per cent OsO_4 buffered at pH 8.5.



(Sager and Palade: Chloroplast in *Chlamydomonas*)