STUDIES ON THE COMPARATIVE PHYSIOLOGY OF *CHARA AUSTRALIS*

II. THE FINE STRUCTURE OF THE PROTOPLAST

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Summary

The fine structure of the internodal cells of *C. australis* in the linear phase of cell expansion is described from electron micrographs of thin sections of osmium- and permanganate-fixed material. The static picture obtained is basically similar to that of the parenchyma cells of higher plants as established by electron-microscope observations. A unit volume of protoplast has the same fine structure in cells ranging from 1 cm to many centimetres in length, and contains cell wall, plasmalemma, tonoplast, endoplasmic reticulum, golgi bodies, microsomes, mitochondria, chloroplasts, nuclei, and other inclusions. This constant structure may account for the constant metabolism per unit volume of protoplast during the linear phase of development. A brief discussion of the possible significance of this picture of the structure of the internodal protoplast to the functional activity of the cell is given.

I. INTRODUCTION

Other investigations in this series (reported and in preparation) have led to the conclusion that a unit volume of the protoplast in the cells of *Chara australis* is similar in gross structure (Peebles, Mercer, and Chambers 1964), respiratory metabolism, and nitrogen status, for cells ranging in length from 1·0 to 20·0 cm. In the work on respiratory metabolism, electron-microscopic examination of the mitochondrial fractions isolated by differential centrifugation showed that these fractions, while containing 50% mitochondria by bulk, were heterogeneous mixtures of chloroplast fragments, microsomal particles, cytoplasmic membranes, and cell debris, thus demonstrating that the intact protoplast must have an elaborate fine structure, possibly similar to that of the higher plants. These findings suggest that information about the fine structure of the protoplast may be of considerable interest in our understanding of the apparent constancy of the metabolism of the internodal cell during cell expansion, and in comparative studies of the physiology of the internodal cell and parenchyma cells of higher plants.

II. METHODS

Single internodal cells of *C. australis* var. *nobilis* R. Br. of known serial number on the shoot were washed with tap water. Those in which protoplasmic streaming

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was observed microscopically were fixed for 15 min at 0–5°C in acetate–veronal-buffered 2% osmium tetroxide (with a total molarity adjusted to 0·2M with sodium chloride), followed by 6 hr at 25–28°C or 12–24 hr in cooler weather at 16–20°C. Satisfactory penetration and fixation could not be obtained at low temperatures. Cells sometimes kept their green colour for 3 days in 2% osmium solution at 0–5°C. The cells were found to be sufficiently fixed after they had turned dark brown, but before they had become jet black. Overfixed cells suffered loss of detail and were difficult to section.

With potassium permanganate fixation (Luft 1956) penetration was very rapid even at low temperatures, the solution reaching the vacuole within 15 sec. Comparison of available data on the penetration of other inorganic ions into the vacuole of these cells suggests that permanganate must penetrate by oxidizing its way through the various barriers to diffusion within the cell. Consequently it destroys a great deal of the fine structure, possibly leaving only the more resistant membranes and organelles.

Following fixation, the specimens were washed for 1 hr in 6–10 changes of 0·2M sodium chloride if osmium was used, or tap water in the case of potassium permanganate, and then dehydrated by immersion for at least 10 min in each of 10, 20, 30, 40, 50, 60, 70, 80, 95, and 100% ethanol, followed by soaking overnight in one of the three changes of 100% ethanol. Transference to a monomer mixture (containing 20% methyl methacrylate, 80% butyl methacrylate, and 2% benzoyl peroxide as a catalyst) was through at least one change of monomer–absolute alcohol mixture. The monomer was changed after 2 hr, and then the specimens soaked in fresh monomer overnight at room temperature before a final brief change. Specimens were embedded in partially prepolymerized monomer in gelatin capsules, and final polymerization carried out in ultraviolet light.

III. Light Microscope Observations

Peebles, Mercer, and Chambers (1964) have described in detail the structure of the protoplast during the linear phase of elongation as observed with the light microscope. A brief recapitulation is given here to serve as a basis for the interpretation of the electron micrographs.

In the living protoplasts the chloroplasts are arranged in regular files and embedded in a gel-like layer, the ectoplasm, adjacent to the cell wall. The ectoplasm merges on the inner side into a sol-like region, the endoplasm, which streams rapidly and follows a helical path around the cell, but there is no definite interface separating the two layers. During streaming the interface between the endoplasm and the vacuole appears to be in a continuous wavy motion. Except for a few free-floating plastids the endoplasm is devoid of chloroplasts, but contains numerous mitochondrial-sized bodies, nuclear fragments, and amorphous inclusions. The ground matrix of the endoplasm is not homogeneous but consists of regions, some discrete, others vague, of differing optical density. Some of the discrete regions are probably clumps of nuclei (Plate 1, Figs. 1–3).

IV. Electron Microscope Observations

(*a*) *General Topography*

As may be seen from the low-power electron micrographs (Plates 2 and 3), the structure of the protoplast is very complex, but the basic features correspond with those observed by means of the light microscope in living internodal cells. These include the cell wall, the layer of chloroplasts adjacent to the wall, occasional free-floating plastids, amorphous inclusions, nuclei, mitochondria, and, in addition, because of the greater resolution of the electron microscope, golgi bodies, endoplasmic reticulum, cytoplasmic membranes, cytoplasmic vacuoles bordering the central vacuole, and cytoplasmic vesicles.

(*b*) *Details of Fine Structure*

(i) *Mitochondria*

The mitochondria are up to 1 μ in length (av. 0·5–0·6 μ), slightly narrower than long, and with a very irregular arrangement of cristae which arise, as in the higher plants, from the inner dense member of the membrane. No truly rod-shaped or branched mitochondria were seen, but serial sections would be necessary to prove their presence or absence. The mitochondrial membrane has a triple-layered structure with two dense zones (each 50–75 Å) separated by a slightly wider electron-lucent layer (60–100 Å). Within the mitochondria, the region between the cristae is filled with a matrix which, in some preparations, contains small dense granules similar to the ribosome particles of the nucleus, nucleolus, and the endoplasmic reticulum (Plate 4, Fig. 1).

In a subsequent paper the authors will show that particulate preparations, isolated from *C. australis* by differential centrifugation and containing approximately 50% by volume of mitochondria, possess a number of steps of the normal tricarboxylic acid cycle plus the cytochrome-mediated electron transport chain. Thus the mitochondria of *C. australis* appear to be very similar in dimensions, fine structure, and function to the mitochondria of higher plants.

(ii) *Nucleus and Nucleolus*

The nuclear bodies vary greatly in size and shape (2–18 μ long), and frequently are deeply lobed and ridged (Plate 3; Plate 4, Fig. 2). They occur randomly in the endoplasm. The nuclear membrane appears as two dense zones, each 70–75 Å wide, separated by a clear region varying from 80 to 120 Å in width. The outer dense layer and the middle clear zone have occasionally been seen to be continuous with these layers in the endoplasmic reticulum. In some preparations gaps occur in the nuclear envelope. If the gaps are not artefact, direct connections exist between the nucleoli and nucleoplasm and that region of the cytoplasm lying between the endoplasmic reticulum. When fixed in osmium the ground mass of the nuclei consists of numerous small dense granules very similar to, but in greater concentration than, those seen in the cytoplasm. One or more regions of each nucleus contains a very dense mass of these granules in what appears to be a non-membrane-bounded region. Frequently these dense granular zones or "nucleoli" are adjacent to or have a prolongation to

the nuclear membrane (Plate 3). The granules have the same dimensions (140 Å, range 70–200 Å) as those described by La Fontaine (1958*a*, 1958*b*) in *Vicia* roots, and are interpreted as ribosomes. When fixed in potassium permanganate, the "nucleoli" and the granular nucleoplasm, while still visible, are not so clearly discernible, and the granule component of the cytoplasm is destroyed, as would be expected if they are ribosome particles. Light and ultraviolet microscope observations of living cells show a heterogeneous collection of bodies moving with the streaming cytoplasm (Plate 1). Some of these are of the dimensions of the bodies identified as nuclei in the electron micrographs and hence are likely to be nuclei.

The nuclei of the internodal cell have many features in common with the nuclei of higher plants, including the double nuclear membrane, nucleoplasm, nucleolar areas, and microsomal particles. However, they are very much larger, and far more extensively indented and deeply lobed. Whether these features indicate a basic difference between the two groups is a matter for speculation. The nuclear cycle in the internodal cell is not well understood, and has been described variously as degenerate mitosis, nuclear fragmentation, and amitosis. In Part 1 (Peebles, Mercer, and Chambers 1964) amitosis was favoured because chromosomes and division figures were never observed despite the large increase (to *c.* 1000) in Feulgen-positive bodies during the expansion of the internode (to *c.* 4 cm). The deeply lobed character of the nuclei allows the possibility of fragmentation of the nuclear body and amitosis, but because of the universality of mitosis further work is needed before this conclusion can be accepted.

(iii) *Chloroplasts*

In osmium-fixed preparations the chloroplast is bounded by a distinct membrane which has two dense zones, each about 50 Å wide, separated by a slightly wider clear zone (50–70 Å) (Plate 5, Fig. 3). The outer dense layer is very occasionally seen to be continuous with the dense zones of the endoplasmic reticulum while the inner dense zone is sometimes (probably more frequently) continuous with the lamellae system (Plate 5, Fig. 3, arrow). In some chloroplasts the lamellae tended to aggregate into closely packed regions, possibly grana (Plate 5, Fig. 2). The lamellae in the grana regions are triple-layered structures consisting of two dense zones approximately 30 Å wide, separated by a clear zone of about the same thickness. They tend to occur in pairs which are frequently fused at the ends to form a closed, disk-like structure enclosing a clear zone approximately 80–100 Å wide (Plate 5, Fig. 2). In the non-grana regions the lamellae occur singly. A matrix, the stroma, occurs between the single lamellae, and probably between the disk-like paired lamellae of the grana regions. In some preparations the stroma contains small dense granules similar in size and density to the ribosome-type granules in the cytoplasm, but far less common (Plate 4, Fig. 1). Starch grains occur in the stroma and distort the orientation of the lamellae. Potassium permanganate fixation (15 sec–30 min) proved unsatisfactory and frequently starch grains were all that remained to indicate the position of the chloroplasts (Plate 6, Fig. 3). The observations confirm the structure of the chloroplast of *C. australis* as described by Mercer (1955), McLean (1956), and Hodge, McLean, and Mercer (1956), but are more extensive.

Structurally the chloroplasts of *C. australis* resemble those of higher plants in consisting of a complex system of lamellae separated by a stroma and enclosed within a chloroplast membrane. However, in common with the chloroplasts of other green algae the system of lamellae is not differentiated into distinct grana and intergrana regions, although the regions of highly oriented lamellae could be considered as rudimentary grana.

As no photosynthetic measurements were made it is not known how closely the photosynthetic pattern of *Chara* resembles that of the higher plants. Clendenning and Gorham (1952) have shown that some features of the photosynthesis in *Nitella* are the same as in higher plants and that starch is an end-product formed in the stroma. Consequently it seems likely that both structurally and functionally the chloroplasts of *C. australis* resemble those of the higher plants.

(iv) *The Membrane Systems*

(1) *Plasmalemma.*—No definite membrane structure has been identified at the external cytoplasmic boundary adjacent to the cell wall in osmium-fixed cells (Plate 6, Figs. 1 and 2). A distinct plasmalemma membrane is found after permanganate fixation, but its structure is not well preserved (Plate 6, Figs. 3 and 4). In osmium-fixed cells the cytoplasm appears to merge with the wall. If separated from the wall, both the wall and cytoplasm have ragged edges and no distinct boundary. In the same preparations the membranes of the chloroplasts, mitochondria, reticulum, and tonoplast are well preserved indicating that the absence of an external membrane is not likely to be due to poor fixation.

(2) *Tonoplast.*—A definite tonoplast membrane is apparent after both osmium and permanganate fixation (Plate 2; Plate 6, Fig. 3). It is a continuous single membrane. Any triple-layered regions have invariably been due to the juxtaposition of adjacent membranes of the cytoplasmic vacuoles (Plate 2). In most preparations large cytoplasmic vacuoles and numerous small vesicles occur in close contact on one side with the tonoplast and merge into the bulk cytoplasm on the other. The cytoplasmic vacuoles appear to be limited to the region of protoplasmic streaming. Discontinuities in the membrane (Plate 3) which apparently allow a mingling of small cytoplasmic vacuoles with the central vacuolar region may have been due to the methods of preparation.

(3) *Endoplasmic Reticulum.*—The reticulum is extremely well developed and permeates both the ectoplasm and endoplasm (Plates 2, 3, 7, and 8). The shape of the profiles varies enormously; parallel and twisted or branching tubules, and oval, elliptical, spherical, or flattened vesicles, small and large, are common. The reticulum is interpreted as an interconnected system of flattened vesicles which only appear as two parallel, electron-dense zones (or membranes) separated by an electron-lucent layer when lying with their narrow edge parallel to the plane of the section. When their broad edge is parallel to the plane of sectioning the electron-dense zones appear as moderately dense patches scattered through the cytoplasm. The dense zone varies in thickness but appears to be of approximately similar dimensions to the layers of the membranes of the organelles to which the reticulum is sometimes attached. Mostly the membranes lack closely associated ribosomal granules.

An interesting feature occasionally observed is the tendency for the reticulum to have a parallel orientation and to be more tightly packed in the region of the ectoplasm adjacent to the stationary chloroplast layer. In the deeper regions of the endoplasm the reticulum is more loosely packed and the profiles more spherical in outline.

(4) *Golgi Zones.*—Each golgi body consists of a series of flattened cisternae (usually about 6) which appear to merge into vesicles at their margins but are not interconnected (Plate 4, Fig. 1). Continuity of the golgi zones with the reticulum has not been observed, but the aggregation of vesicles at the edges of the bodies may be evidence of a direct connection between the two. In osmium-fixed preparations the cisternae consist of two dense zones about 0·8 μ long and up to 150 Å wide, separated by a clear zone up to 200 Å wide, and each platelet separated by about 300 Å. (In young cytoplasm, not illustrated in the present paper, the platelets are only 80–90 Å apart, and the two dense zones are each about 30 Å, separated by a clear zone of 80 Å.) Structurally the golgi zones of the internodal cell are indistinguishable from those of the higher plants.

Thus, apart from the uncertainty about the plasmalemma, the membrane system (cell membranes, reticulum, and golgi regions) of the internodal protoplast in cells ranging in length from one to many centimetres resembles the membrane system of the very much smaller protoplasts of the parenchyma cells of the higher plants.

(v) *The "Microsomal" Granules*

Small dense granules, 60–200 Å in diameter, are common in most osmium-fixed preparations. Their distribution is interesting. A few are associated with the membranes of the reticulum, but mostly these membranes are agranular. A much higher concentration occurs in the cytoplasmic matrix where they appear to be scattered more or less randomly, such as occurs in the meristematic cells of the higher plants. They also occur to a small extent in the mitochondria and chloroplasts, and are very common in the nucleoplasm and highly concentrated in the nucleolar regions (Plate 3; Plate 4, Fig. 2). It is not known whether these granules in different regions of the cell are similar biochemically. The granules are of the same dimensions and electron density as the ribosome particles of the higher plant cell.

(vi) *The Cell Wall*

In osmium-fixed preparations, sections approximately in the transverse plane of the wall appear to have a fine microfibrillar structure, embedded in which are numerous small bodies of slightly greater density in the electron beam. The microfibrils appear to lie in alternating groups of greater or less density. The very dense regions sometimes running parallel to the microfibrils and sometimes cutting across these and radiating out from the cytoplasm (Plate 2; Plate 6, Figs. 1 and 2) are probably due to folds in the section. These folds, however, very likely occur in regions of weakness which may correspond with extensions of the cytoplasm into the cell wall itself—similar to those reported in *Hydrodictyon* cell walls by Northcote, Goulding, and Horne (1960).

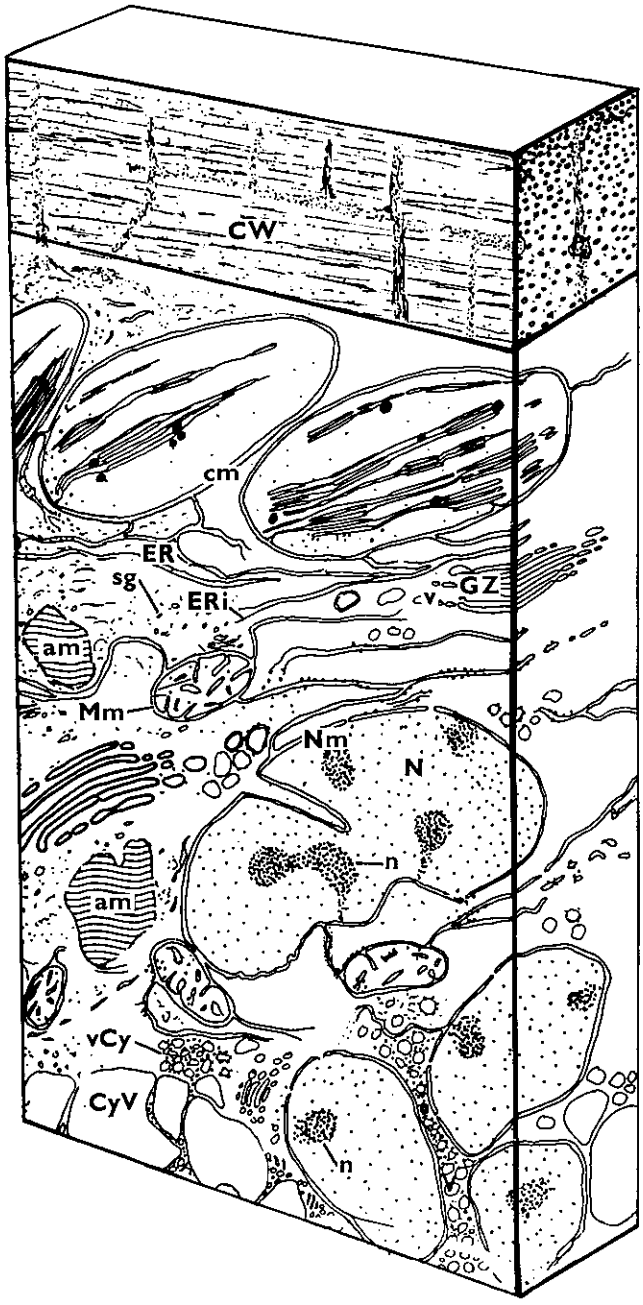

Fig. 1.—Suggested spatial relationships of the organelles of the cytoplasm in an internodal cell of *Chara australis*. The endoplasmic reticulum (*ER*) occasionally appears to be continuous with the chloroplast membrane (*cm*) and more frequently with the nuclear membrane (*Nm*) and the

[*For continuation see opposite page.*]

(vii) *Inclusions*

Irregular shaped, often deeply lobed, comparatively electron-dense, structureless bodies are commonly seen in the cytoplasm (Plate 3). Some of these probably correspond with the ninhydrin-positive bodies found in living cells, and as they react with both osmium and permanganate they are probably proteinaceous in composition. Similar bodies have not been reported for cells of the higher plants.

V. Discussion

(*a*) *Structural Relationships in Fixed Cells*

The static spatial relationship of the organelles and cytoplasm of the fixed internodal cell in the linear phase of cell elongation of *C. australis*, as deduced from the electron micrographs, is summarized diagrammatically in Figure 1. The nuclear membrane is continuous at occasional intervals with the endoplasmic reticulum which appears linked occasionally with the mitochondrial membrane and hence to the mitochondrial cristae. Also very occasionally the reticulum is continuous with the chloroplast membrane, and therefore with the chloroplast lamellae. Thus the membranes of the nucleus, endoplasmic reticulum, mitochondria, and possibly also the chloroplasts appear to be part of the same continuous membrane system. No conclusive observations on the relationships of the membranes of the golgi bodies to the reticulum have been obtained, but the vesicular structures at the ends of golgi regions suggest that the two may be part of the same system. The ramification of this continuous membrane system divides the cytoplasm into two regions, one enclosed within the membranes—"the intra-endoplasmic space", and the other lying outside the membranes—"the cytoplasmic matrix". If the occasional membrane connections between chloroplast and reticulum and between mitochondria and reticulum are real connections, and not artefacts, the intra-endoplasmic space is in contact at a

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[Fig. 1 *Continued.*]

mitochondrial membrane (*Mm*). The nucleus (*N*) is granular, frequently deeply lobed, and contains more densely granular non-membrane-bounded regions, the nucleoli (*n*). The endoplasmic reticulum is interpreted as a branching, flattened, ribbon-like structure which only has its characteristic triple-layered appearance when the section is cut at right angles to the broad plane. Occasionally it twists into a different plane (*ERi*) and so appears to merge with the cytoplasm. Sometimes its position may still be traced by following the direction of the associated small dense granules (*sg*). The endoplasmic reticulum appears to form a continuous membrane-bounded system with a number of organelles—the intra-endoplasmic reticulum compartment. Cytoplasm outside this region has been called the cytoplasmic matrix. The most conspicuous organelle apparently associated with this region is the golgi apparatus (*GZ*). This structure appears to produce vesicles (*v*) at its margin, some of which may develop into mitochondria. In more mature cells these vesicles frequently form a vesiculate cytoplasm (*vCy*) which tends to become closely associated with the tonoplast region of the cytoplasm. Amorphous bodies (*am*) (protein?) are also scattered through the cytoplasm. Numerous larger cytoplasmic vacuoles (*CyV*) which may prove to be continuous with the endoplasmic reticulum are also associated with this region of the cytoplasm. The cell wall (*CW*) is known from other work to consist of approximately transverse-running microfibrils and our micrographs suggest that it may be permeated by branching cytoplasmic strands.

number of areas with the space between the double membranes and cristae of the mitochondria, but not with the mitochondrial matrix. It is also in contact with the chloroplast double membrane and lamellae, but possibly not with the stroma.

The cytoplasmic matrix is in contact with the nucleoplasm through pores in the nuclear membrane. The microsomal particles appear to be confined mainly to the "continuum" of the nucleoplasm, nucleoli, and cytoplasmic matrix, but are more concentrated in the nucleoli. Pores in the chloroplast and mitochondrial membranes through which the cytoplasmic matrix would be in contact with the stroma and the mitochondrial matrix have not been observed.

The relationship of the tonoplast to the cytoplasmic membrane system is not understood, but most observations suggest that the tonoplast–vacuole is a discrete unit which, therefore, forms a third major region of the protoplast. In some electron micrographs there are indications that the cytoplasmic vacuoles and cytoplasmic vesicles could be expanded regions of the reticulum (similar to the expanded ends of golgi bodies). The discontinuities in the tonoplast which appear to be associated with the cytoplasmic vacuoles and cytoplasmic vesicles may indicate that the tonoplast is structurally related to the membranes of the cytoplasmic vacuoles. Contact between the central vacuole and the cytoplasm could involve the cytoplasmic vacuoles which form a continuous border adjacent to the tonoplast.

The structural relationship between the external surface of the cytoplasm and the rest of the cytoplasm is not obvious. If observations were confined to osmium-fixed cells the cytoplasm would appear to be without a definite external membrane as it appears to extend into and merge with the cell wall. Such a structural arrangement has been postulated by Bennett and Rideal (1954) to account for certain physical properties of the internodal cell of *Nitella*. In contrast a definite plasmalemma is obvious in permanganate-fixed cells. This is consistent with the findings of other investigators on the electrical properties and permeability of the internodal cell of *C. australis* (Walker 1960; Hope and Walker 1961). The possibility that the absence of an external membrane after osmium treatment was due to poor fixation or overfixation seems improbable because all other membranes were well preserved. The indistinctness of the plasmalemma after osmium fixation is also seen in the higher plant cell.

Obviously the static picture obtained for fixed cells must be modified to fit in with the observations on living internodal cells. One interesting difference is that the electron micrographs do not show the clear differentiation into ectoplasm and endoplasm which is such a striking feature of living internodal cells, although in some electron micrographs the membranes of the reticulum do lie parallel to the cell wall in the ectoplasm and at random and more loosely packed in the endoplasm. An absence of differentiation into two zones is typical of the appearance of cells (under the light microscope) in which streaming has been suspended through excess polyvalent ions, low temperature, or shock, etc. In the living state the endoplasm undergoes sol–gel transformations. Possibly during fixation the endoplasm reverts to the gel state, and so fails to preserve the structural orientations which exist in the fluid-like state.

Another problem concerns the relationship between the static picture of the electron micrographs and the labile structure of the streaming cytoplasm. For instance it is difficult to reconcile a continuity of membranes and organelles when the organelles obviously move and rotate during cyclosis. The nuclei and mitochondria in living cells may be seen to be in vigorous motion, yet in the fixed state their membranes appear continuous with the membranes of the reticulum. Further, if the membranes of the reticulum form a continuous network, as is suggested by the electron micrographs, and if this is a permanent but plastic structure in the living state, the moving part of the endoplasm may be the cytoplasmic matrix which flows around a more or less stationary membrane network.

The electron micrographs of cells of different serial numbers indicate that protoplast structure does not alter during the linear phase of cell expansion. The fine structure of a unit volume of the protoplast appears identical in all cells from a few to many centimetres in length. This conclusion is identical with that reached by Peebles, Mercer, and Chambers (1964) from light-microscope studies on the gross structure of the cell, namely that the gross structure is similar in cells from 1 to 20 cm in length. A constant fine structure may result in a constant metabolism. Each increment of protoplast added during growth apparently has the same concentration of chloroplasts, mitochondria, and fine structure as that already present. Consequently metabolism per unit of protoplast is likely to be the same for all cells in the linear phase of growth; alternatively a constant structure may be the result of a constant metabolism.

The static picture of the internodal cell (Fig. 1) is essentially similar to the static structure of the cells of the higher plants (Buvat 1958; Mercer 1960; Whaley, Mollenhauer, and Leech 1960), with the possible exception of the large and possibly interconnecting vacuoles and small vesicles which border the tonoplast in the internodal cell. Vacuoles and vesicles are common in parenchyma cells, but they tend to occur at random throughout the cytoplasm. Two interesting conclusions follow: first, adaptation to either terrestial or aqueous environments has not resulted in the evolution of basically different cytoplasmic systems at this structural level, and secondly, structure appears to be a conservative feature of organisms, since very large differences in phylogenetic position apparently have little effect on the fine structure of the protoplast. As far as cytoplasmic structure is concerned the internodal cell, apart from its extraordinary size and coenocytic condition, can be regarded as basically similar to the parenchyma cells of the higher plants.

(*b*) *Cytoplasmic Structure and Function*

The electron-microscope data demonstrate that the cytoplasm of the internodal cell is a highly complex polyphasic system which is differentiated into three major compartments by membranes: the cytoplasmic matrix, the internal phase of the endoplasmic reticulum, and the central vacuole. In *Chara* the endoplasmic reticulum is extremely well developed, extending throughout the cytoplasm from tonoplast to plasmalemma as an extensively divided vacuolar system and, as judged from area measurements of electron micrographs, may occupy about half the volume of

the cytoplasm, the remainder being matrix. This complexity of structure and the labile polymorphic behaviour and dynamism of cytoplasm as demonstrated by its very active streaming suggest that the cell and cytoplasmic membranes and the internal phases must be continuously altering in shape, form, and extent in the living cell, and results in a continuously changing system of interfaces of comparatively enormous area adjacent to the vacuole and throughout the protoplast. Clearly, certain aspects of the physiological activity of the internodal cell should be re-examined in the light of this newer picture of the submicroscopic structure of the protoplast. A few of these are considered below.

(i) *Protoplasmic Streaming*

Recent experimental work on velocity gradients within the streaming cytoplasm of *Chara* led Kamiya and Kuroda (1956, 1957) to conclude that the site of the generation of the driving force in cyclosis is the zone between the stationary chloroplast layer and the endoplasm, supporting an earlier suggestion of Ewart (1903). The electron micrographs could favour this view. If, as suggested in the previous section, the reticulum lies parallel to the cell wall in the ectoplasm and more random in the endoplasm, the localization of cyclosis to the endoplasm may have a submicroscopic structural basis. The oriented membranes of the reticulum by some contractile process may be responsible for the motive force of cyclosis and possibly the sol-like properties of the endoplasm may result from the more open packing of the reticulum in this region. Alternatively, the orientation of structure observed may simply reflect the shearing effect of streaming cytoplasm adjacent to a stationary layer, and hence have no significance to the mechanism of cyclosis.

(ii) *Ion Uptake, Permeability, and Electrical Properties*

On the basis of equilibration times, ion-uptake studies have indicated three physiological compartments in the internodal cells of the Characeae, a compartment with a very short half-time, a compartment with a half-time of about 1 hr, and a compartment with a half-time exceeding 1000 hr (MacRobbie and Dainty 1958). Presumably these compartments have a morphological reality, and should be identified by electron microscopy.

If the plasmalemma forms a continuous boundary sharply separating the cytoplasm from the wall, four morphological compartments may be described, namely the wall, the cytoplasmic matrix, the intra-endoplasmic space, and the vacuole, and one might expect four physiological compartments. Alternatively, the plasmalemma may not separate sharply the cytoplasm and wall. This is suggested by the vagueness of the cytoplasm–wall boundary of osmium-fixed cells and by the presence of an electron-dense substance in the cell walls which has a qualitative amino acid pattern similar to that of the bulk cytoplasm (Chambers and Mercer, unpublished data). If this situation exists, three morphological compartments may be described, the wall plus the cytoplasmic matrix, the intra-endoplasmic space, and the vacuole, and one might expect three physiological compartments. One consequence of this structure is that the initial equilibration of a solute with the cell would involve

penetration into the cytoplasmic matrix as well as the wall. Subsequently the active uptake of the ions may involve the membranes of the reticulum, or the more highly differentiated golgi regions. There is indirect evidence implicating the golgi bodies and endoplasmic reticulum in secretory processes in animal cells, and Mercer and Rathgeber (1962) have demonstrated a striking correlation between the form of the endoplasmic reticulum and the secretion of sugars in nectaries of higher plants.

Other physiological studies on members of the Characeae have shown an initial uptake of labelled ions (K^+, Pb^{2+}) by "granules" in the cytoplasm (Mullins 1940; Wernstedt 1944). Wernstedt centrifuged intact cells and sedimented "granules" which contained about 80% of the radioactive lead without causing visible damage to the cells which retained turgor and the capacity for cyclosis. Unfortunately at that time techniques were not available for identifying the granules. However, if one accepts a widely held view based on the ionic relationships of isolated mitochondrial suspensions from higher plants, they could have been mitochondria. Robertson *et al.* (1955) and Robertson (1958) suggest that mitochondria are sites of ion accumulation. This view may not apply to the Characeae since at least four "granules" can be recognized in the cytoplasm, the mitochondria, nuclei, golgi bodies, and vesicles of the reticulum. As each of these structures may have sedimented under the centrifugal forces employed by Wernstedt, there is an equal possibility that the golgi bodies or the larger cytoplasmic vesicles were the granules in which the concentration of radioactive lead occurred. Electron micrographs of sections of mitochondrial pellets from *C. australis* (Chambers and Mercer, unpublished data) show that at least half the volume of the pellet consists of aggregates of non-mitochondrial membrane fractions which would have been derived from reticulum and golgi bodies. If these fragments continue to function after isolation, the salt-uptake properties of mitochondrial suspensions may result from the membrane fragments and not the intact mitochondria.

Thus there are reasons for drawing attention to the possible role of the golgi bodies and membranes of the reticulum in salt accumulation in the Characeae. The golgi bodies might concentrate solute within their cisternae, and the vesicles which "bud" from the edges of the cisternae may contain a solution of the solute. Subsequently the vesicles may migrate to the tonoplast and release the solution into the vacuole through the pinocytotic activity of the tonoplast. Alternatively a transfer of solute may occur via the internal phase of the reticulum. Certainly, vesicles of similar dimensions to those associated with the golgi bodies are also associated with the tonoplast.

Apart from these hypothetical considerations arising from the intricate fine structure of the protoplast, the presence of numerous membranes throughout the protoplast may complicate the interpretation of cell permeability and cell electrical phenomena by confusing the value of the quantities in permeability equations. What meaning, for instance, can be given to surface area, thickness and number of diffusion barriers, and diffusion pathways in a protoplast containing an anastomozing system of membranes in addition to the major cell membranes, when even these are extensively convoluted structures?

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VII. References

Bennett, M. C., and Rideal, E. (1954).—Membrane behaviour in *Nitella*. *Proc. Roy. Soc.* B **142**: 483–96.

Buvat, R. (1958).—Recherches sur les infrastructures du cytoplasme, dans les cellules du méristème apical, des ébauches foliaires et des feuilles developpées *d'Elodea canadensis*. *Ann. Sci. Nat. Bot.* **18**: 121–61.

Clendenning, K. A., and Gorham, P. R. (1952).—Intracellular distribution of assimilated C^{14} in briefly exposed aquatic plants. *Arch. Biochem. Biophys.* **37**: 56–71.

Ewart, A. J. (1903).—"On the Physics and Physiology of Protoplasmic Streaming in Plants." (Oxford.)

Hodge, A. J., McLean, J. D., and Mercer, F. V. (1956).—A possible mechanism for the morphogenesis of lamellar systems in plant cells. *J. Biophys. Biochem. Cytol.* **2**: 597–608.

Hope, A., and Walker, N. A. (1961).—Ionic relations of cells of *Chara australis* R. Br. IV. Membrane potential differences and resistances. *Aust. J. Biol. Sci.* **14**: 26–44.

Kamiya, N., and Kuroda, K. (1956).—Velocity distribution of the protoplasmic streaming in *Nitella* cells. *Bot. Mag., Tokyo* **69**: 544–54.

Kamiya, N., and Kuroda, K. (1957).—Cell operation in *Nitella*. II. Behaviour of isolated endoplasm. *Proc. Imp. Acad. Japan* **33**: 201–5.

LaFontaine, J. G. (1958*a*).—A particulate component found in nucleoli of *Allium cepa* and *Vicia faba*. *J. Biophys. Biochem. Cytol.* **4**: 229–30.

LaFontaine, J. G. (1958*b*).—Structure and mode of formation of the nucleolus in meristematic cells of *Vicia faba* and *Allium cepa*. *J. Biophys. Biochem. Cytol.* **4**: 777–84.

Luft, J. H. (1956).—Permanganate—a new fixative for electron microscopy. *J. Biophys. Biochem. Cytol.* **2**: 799–801.

MacRobbie, E. A. C., and Dainty, J. (1958).—Ion transport in *Nitellopsis obtusa*. *J. Gen. Physiol.* **42**: 335–53.

McLean, J. D. (1956).—An investigation of the structure of the plant cell with special reference to the surfaces. Ph.D. Thesis, University of Sydney.

Mercer, F. V. (1955).—The water relations of plant cells. *Proc. Linn. Soc. N.S.W.* **80**: 6–29.

Mercer, F. V. (1960).—The submicroscopic structure of the cell. *Annu. Rev. Pl. Physiol.* **11**: 1–24.

Mercer, F. V., and Rathgeber, N. (1962).—Nectar secretion and cell membranes. In Proc. Fifth Int. Congr. Electron Microscopy, Philadelphia.

Mullins, L. J. (1940).—Radioactive ion distribution in protoplasmic granules. *Proc. Soc. Exp. Biol., N.Y.* **45**: 856–8.

Northcote, D. H., Goulding, K. J., and Horne, R. W. (1960).—The chemical composition and structure of the cell wall of *Hydrodictyon africanum* Yaman. *Biochem. J.* **77**: 503–8.

Peebles, M. J., Mercer, F. V., and Chambers, T. C. (1964).—Studies on the comparative physiology of *Chara australis*. I. Growth pattern and gross cytology of the internodal cell. *Aust. J. Biol. Sci.* **17**: 49–61.

Robertson, R. N. (1958).—The uptake of minerals. *Handb. PflPhysiol.* **4**: 243–79.

Robertson, R. N., Wilkins, M. J., Hope, A. B., and Nesztel, L. (1955).—Studies in the metabolism of plant cells. X. Respiratory activity and ionic relations of plant mitochondria. *Aust. J. Biol. Sci.* **8**: 164–85.

WALKER, N. A. (1960).—The electrical resistance of the cell membranes in a *Chara* and a *Nitella* species. *Aust. J. Biol. Sci.* **13**: 468–78.

WERNSTEDT, A. (1944).—On the absorption of lead into the cells of *Nitella*. *Acta Bot. Fenn.* **32**: 3–8.

WHALEY, W. G., MOLLENHAUER, H. H., and LEECH, J. H. (1960).—The ultrastructure of the meristematic cell. *Amer. J. Bot.* **47**: 401–49.

EXPLANATION OF PLATES 1–8

PLATE 1

Figs. 1–3.—Light microscope photomicrographs taken on very high speed film (Agfa Record ASA = 1200) of portions of living cells which were actively streaming at the time of the photograph. Note the stationary rows of chloroplasts (*c*) which lie in the "gel" layer of the cytoplasm. Beneath the chloroplast layer can be seen the moving cytoplasm, containing particles of various sizes, some much larger than the plastids. Figure 3 shows two such large particles (*N*) moving parallel with one of the helical clear regions (*h*) of the cell. Cytoplasm and particles above and below the clear zone in Figure 3 were moving in the opposite direction (arrows). × 1500 for each figure.

PLATE 2

Electron micrograph of a transverse section of a mature internodal cell. The cell wall (*CW*) appear to be a mass of microfibrils running approximately in the same plane as the section (i.e. transversely or in a low spiral). The dark regions in the wall are folds in the section. No distinct cytoplasmic membrane can be seen between the wall and the cytoplasm. The chloroplasts (*c*) mainly lie in the cytoplasmic layer adjacent to the wall. Each plastid is membrane-bounded and contains numerous lamellae some of which are grouped into grana-like structures. The cytoplasm in the chloroplast zone appears to be denser and more homogeneous than that in the inner zone. The inner layer of cytoplasm is vesiculate and appears to contain numerous organelles, the mitochondria (*M*), the golgi zone (*GZ*), and the endoplasmic reticulum. Nearer the vacuole the cytoplasm is permeated by large cytoplasmic vacuoles (*CyV*) each surrounded by a membrane and a layer of cytoplasm. The membrane separating the cytoplasm from the vacuole (*V*) appears here as a single layer, the tonoplast (*t*). Osmium-fixed. × 11,600.

PLATE 3

Transverse section of an internodal cell which was 1·5 cm in length (position of cell 2). On the lower left is the chloroplast layer which runs parallel to the cell wall (not visible in this micrograph). Each plastid is bounded by a triple-layered membrane (*cm*). The chloroplast lamellae (*cl*) in places are aggregated together as grana (*G*). The space between the lamellae is occupied by an undifferentiated granular matrix, the stroma (*sr*). Careful examination of the cytoplasm in the region near to the plastids shows an endoplasmic reticulum (*ER*) tending to run parallel to the plastids. The boundary of this part of the reticulum (indicated by arrows) may separate the stationary region in which the chloroplasts lie from the region of cyclosis. The inner cytoplasmic region appears to be composed of a large number of organelles ranging from the minute electron-dense granules to the relatively large nuclei (*N*) bounded by their triple-layered membrane (*Nm*) and supporting a number of non-membrane-bounded bodies which may be nucleoli (*n*). Between these extremes in size are a number of other organelles, the golgi bodies (*GZ*) with their characteristic plate-like structure, the mitochondria (*M*), the amorphous bodies (*am*) frequently bounded by a discontinuous dense zone, the numerous vesicles (*v*) which together with the small dense granules make up the cytoplasmic matrix (*Cy*). Separating the cytoplasm from the vacuole (*V*) is a region of cytoplasmic vacuoles (*CyV*). Each of these cytoplasmic vacuoles is bounded by a membrane. The cytoplasm appears to be continuous between each vacuole and also between the single-layered tonoplast (*t*) and each adjacent cytoplasmic vacuole. Osmium-fixed. × 13,000.

PLATE 4

Fig. 1.—Typical osmium-fixed cytoplasm in the main region of streaming showing mitochondria (*M*), golgi body (*GZ*), endoplasmic reticulum (*ER*), small dense granules (*sg*), vesicles (*v*), and what was a free-moving chloroplast bounded by a chloroplast membrane (*cm*) and containing lamellae (*cl*) and stroma (*sr*) which also has small dense granules present (arrow). ×40,000.

Fig. 2.—A portion of a deeply lobed nuclear fragment; the nuclear membrane (*Nm*) is occasionally perforated by pores which would give direct connection of the nucleoplasm to the cytoplasmic matrix (*Cy*). The nucleoplasm is composed of numerous small dense granules which are occasionally aggregated into very dense regions which may be nucleoli (*n*). Osmium-fixed. ×17,500.

PLATE 5

Fig. 1.—Typical golgi structure (*GZ*) seen in mature internodal cell when fixed in 2% potassium permanganate for 5 hr. A portion of the nuclear membrane (*Nm*) perforated by pores (*P*) is also shown. A secretory granule (*Gr*) filling a vesicle associated with the golgi body is visible. Apart from these apparently more resistant membranes and structures the permanganate has failed to preserve the details of the cytoplasmic matrix or of the nucleoplasm which are seen in osmium-fixed cytoplasm. ×40,000.

Fig. 2.—Portion of a chloroplast from cell 2 showing the aggregation of lamellae (*cl*) into a grana-like region (*g*). The lamellae appear to be paired. In the grana-like region each lamella is made up of three zones—two dense layers separated by a very narrow less-dense region. In the intergrana regions many of the lamellae appear as a single dense zone. Osmium-fixed. ×100,000.

Fig. 3.—A highly magnified peripheral portion of a chloroplast showing in at least two places (arrows) the continuity of the inner dense layer of the chloroplast membrane (*cm*) with the chloroplast lamellae (*cl*). The cytoplasm (*Cy*) contains numerous small dense granules (*sg*) and fragments of endoplasmic reticulum (*ER*). Osmium-fixed. ×140,000.

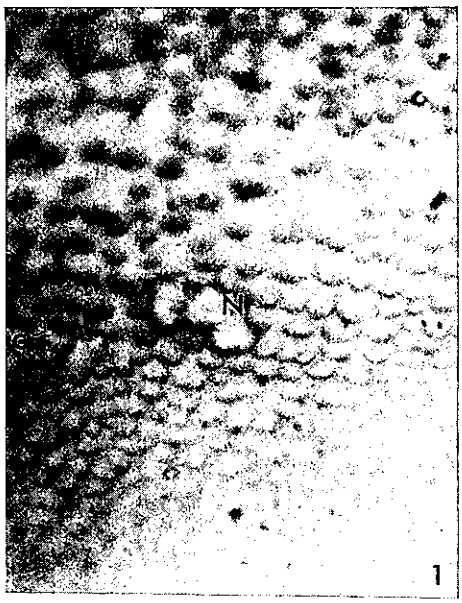
PLATE 6

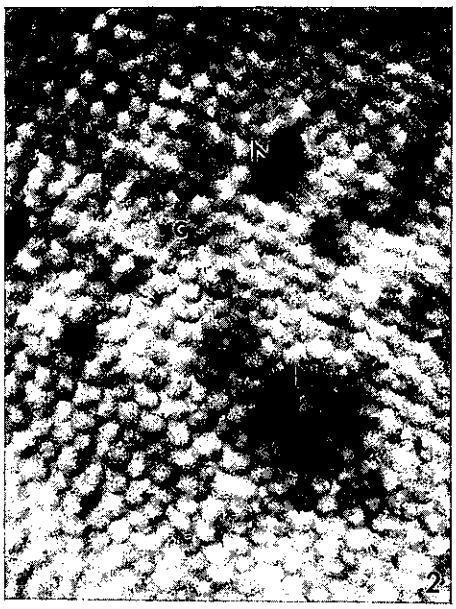
Figs. 1 and 2.—Transverse sections of an internodal cell showing relationship of the outer layers of the cytoplasm to the cell wall (*CW*) in osmium-fixed cells. Cytoplasm is shown in contact with the wall in Figure 1. The same cell as in Figure 1 is shown in Figure 2 but at a point where the cytoplasm has been torn away from the wall prior to sectioning. The absence of a distinct membrane in these two figures together with the ragged remains of cytoplasm attached to the wall in Figure 2 supports the view that the outer layers of the cytoplasm permeate the microfibril structure of the wall. The dark folds in the wall are probably weak points which we believe are caused by pores filled with cytoplasm. These traverse most of the wall but do not seem to be in direct connection with the external environment. ×30,000.

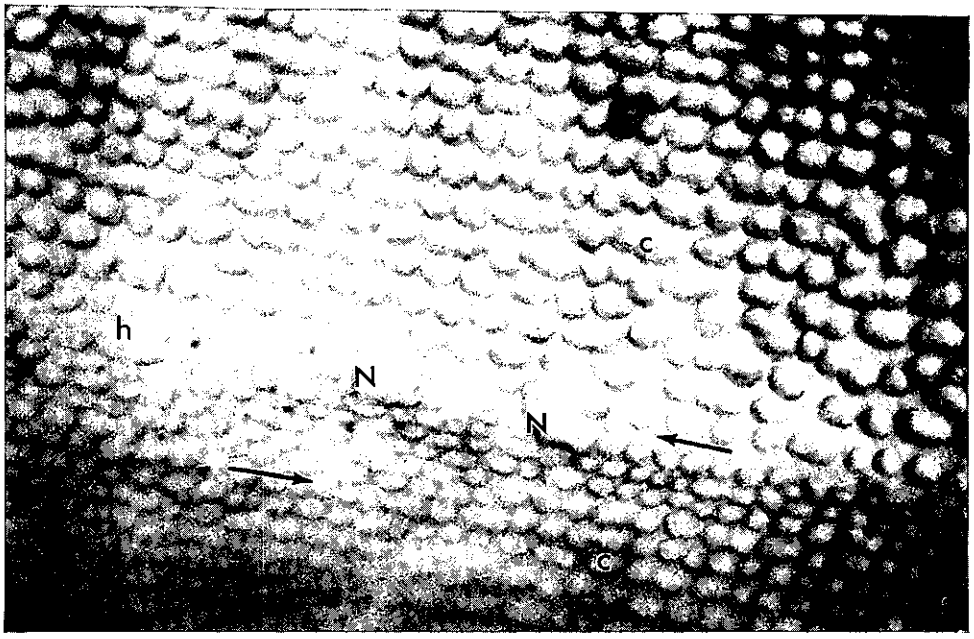
Fig. 3.—Transverse section of an internodal cell in cell 2 position fixed in 2% potassium permanganate for 5 hr. The cytoplasm has separated from the cell wall (not shown in this micrograph). The outer layer of the cytoplasm (*Cym*) may represent a cytoplasmic membrane. Plastid structure has been very poorly preserved. Swollen starch grains (*st*) appear as dark amorphous structures surrounded by oxidized remains of the chloroplasts (*c*). Nuclear bodies can be seen (*N*). The tonoplast (*t*) separating the vacuole (*V*) from the cytoplasm has remained intact. ×1000.

Fig. 4.—Nodal and internodal cell fixed for 5 hr in 2% potassium permanganate. A separating cell wall (*CW*) between adjacent nodal (left) and internodal cells shows pitting (*P*). The cytoplasm appears to be bounded by a cytoplasmic membrane (*Cym*) which may have been composed of two dense zones; the one nearer the cytoplasm (*Cy*) has been more severely oxidized by the permanganate than has the layer near the wall which is still almost continuous. The layer adjacent to the cytoplasm appears to have fragmented and pieces have curled away from the intact layer (arrows). Other membranous structures in the cytoplasm (*Cy*) show a similar tendency to swell and become disrupted. ×15,000.

COMPARATIVE PHYSIOLOGY OF CHARA AUSTRALIS. II







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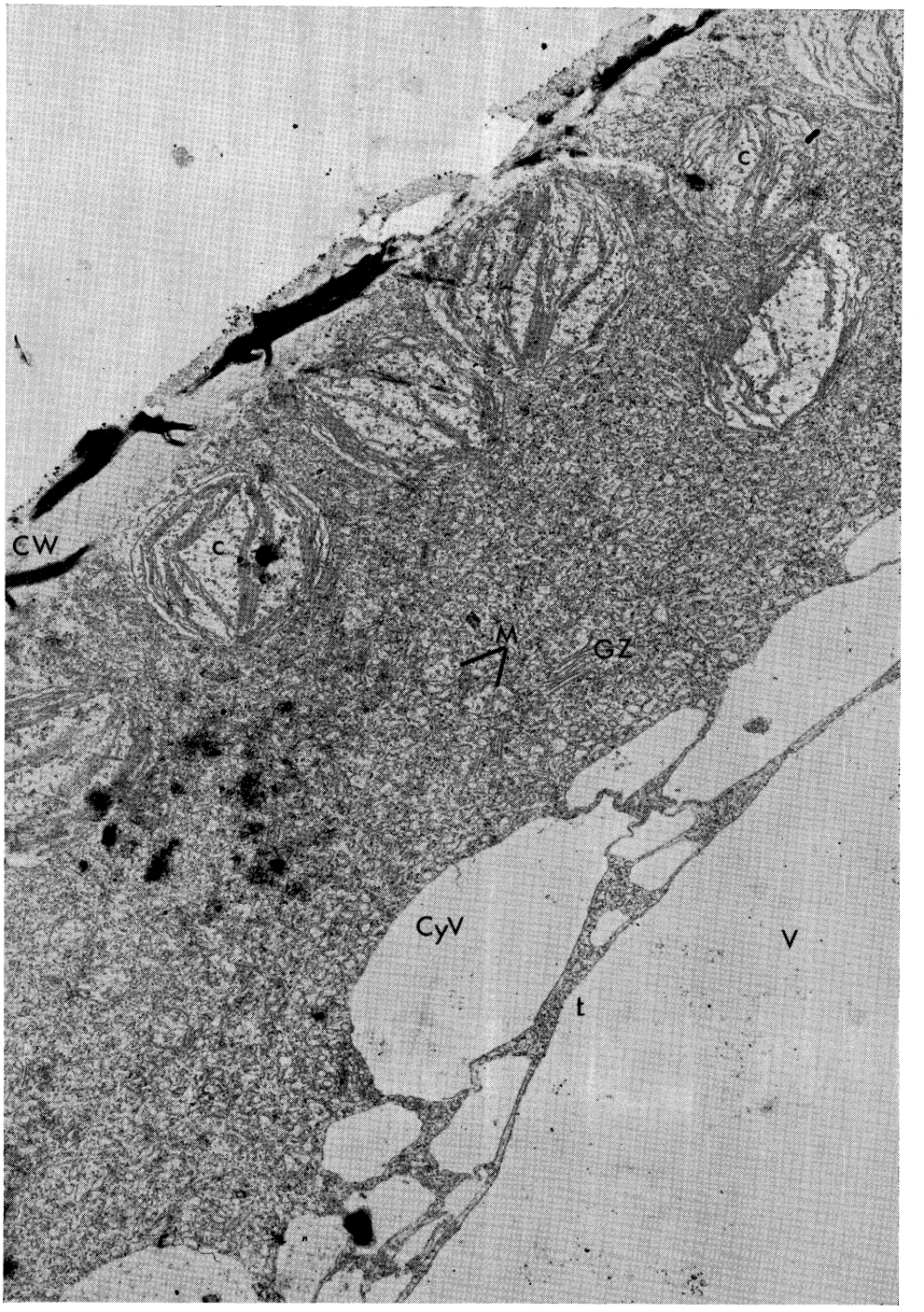

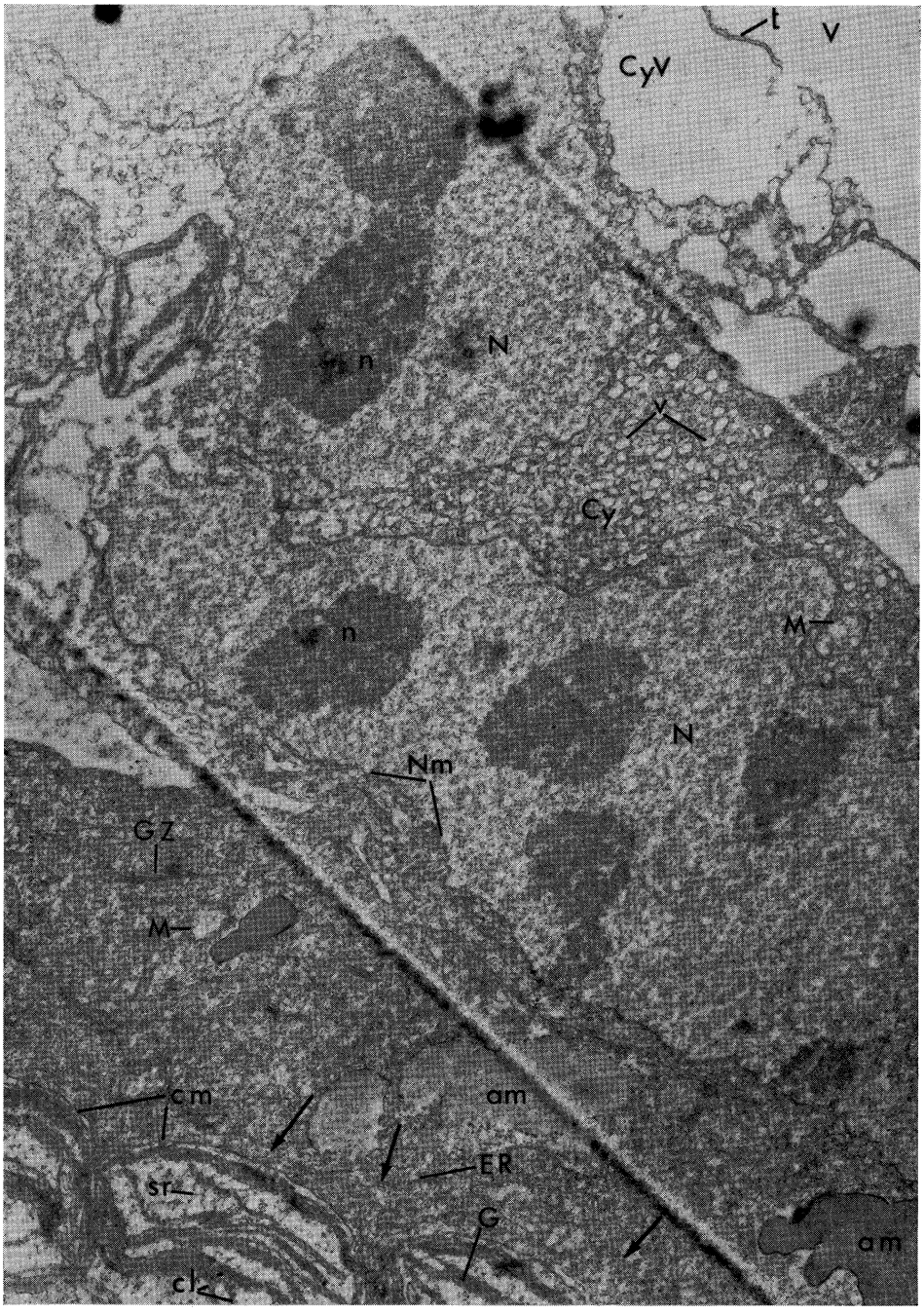
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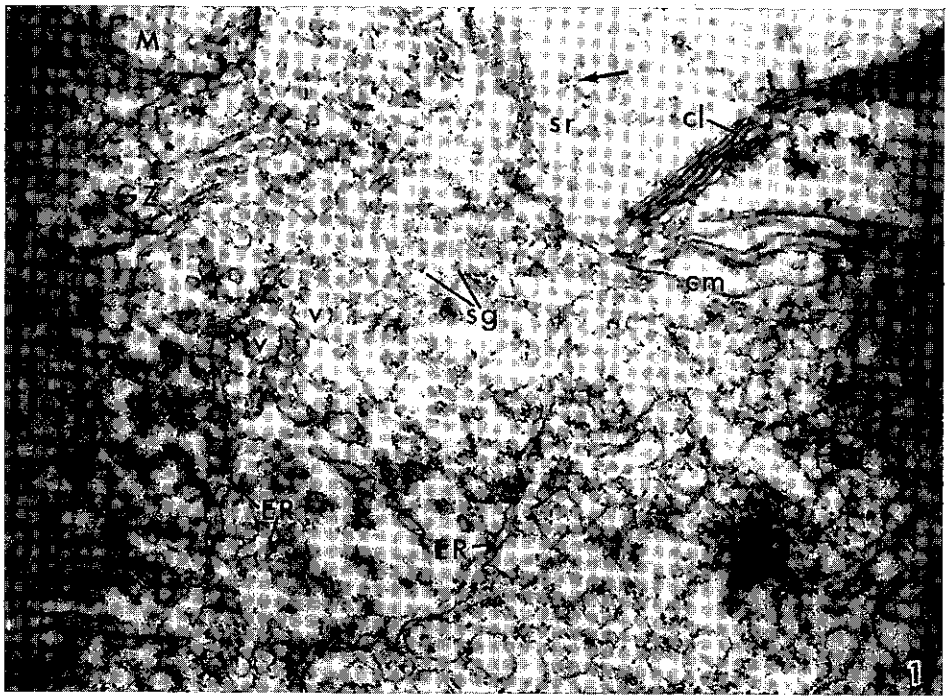

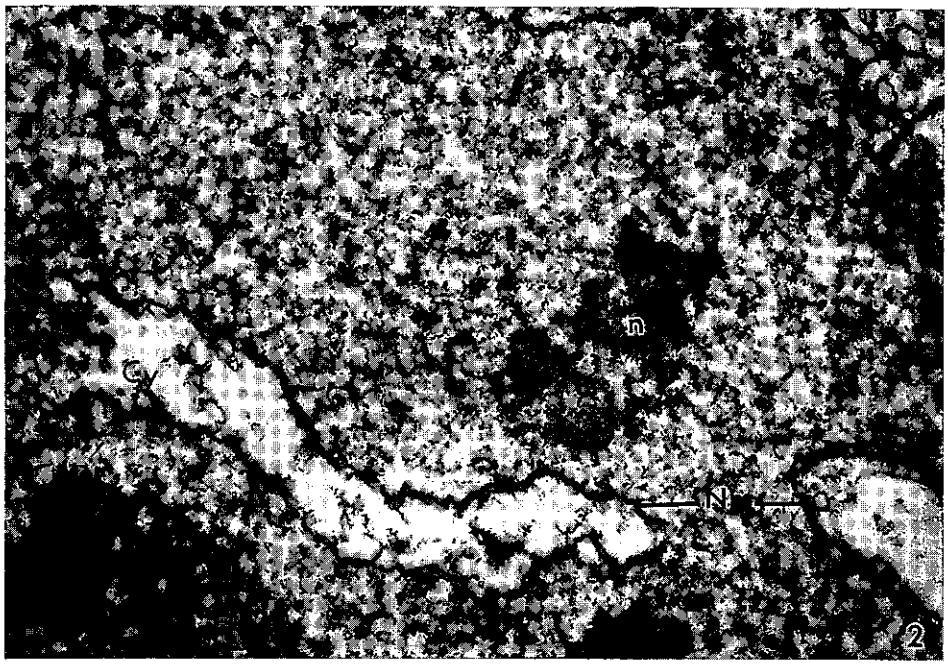

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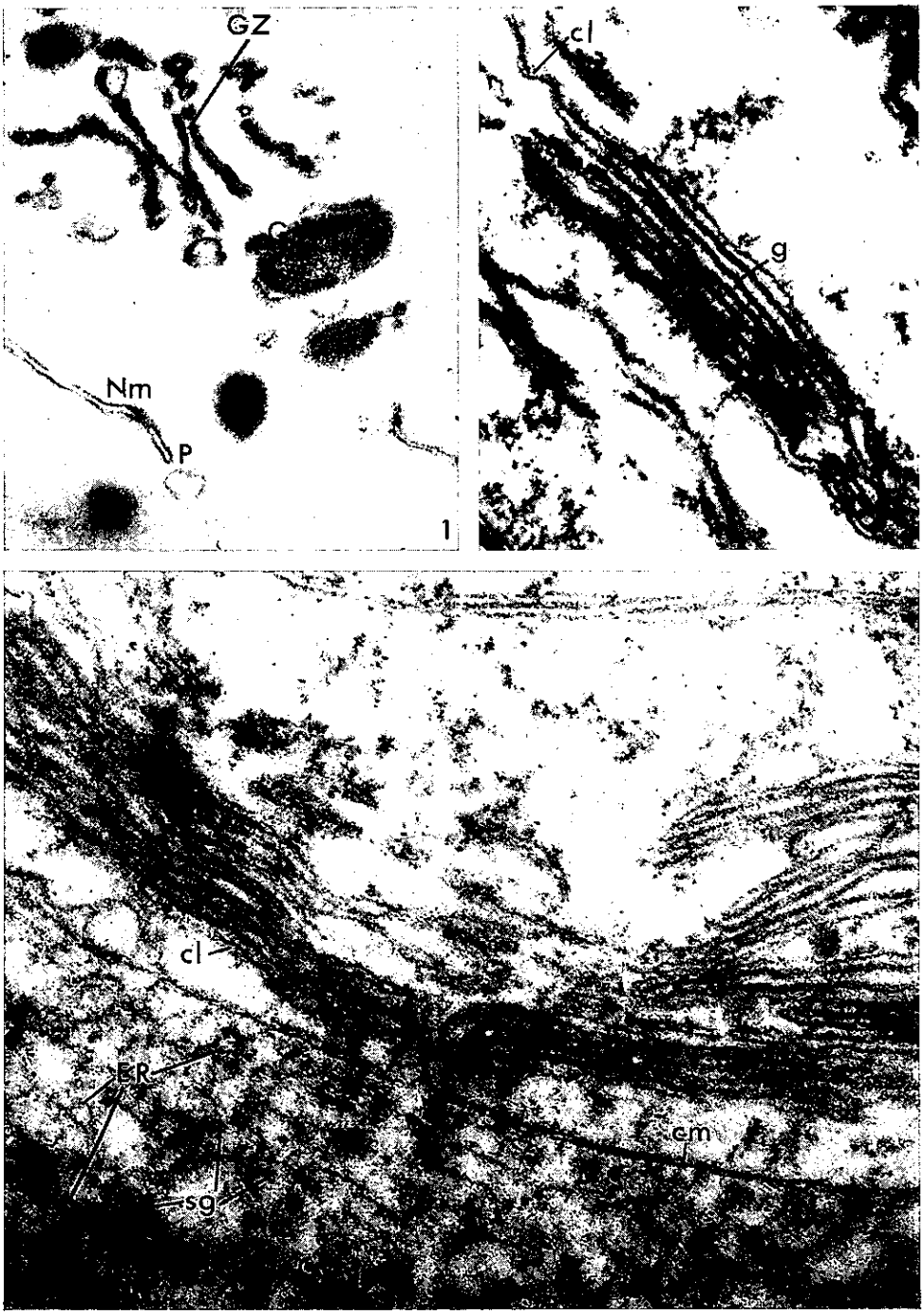

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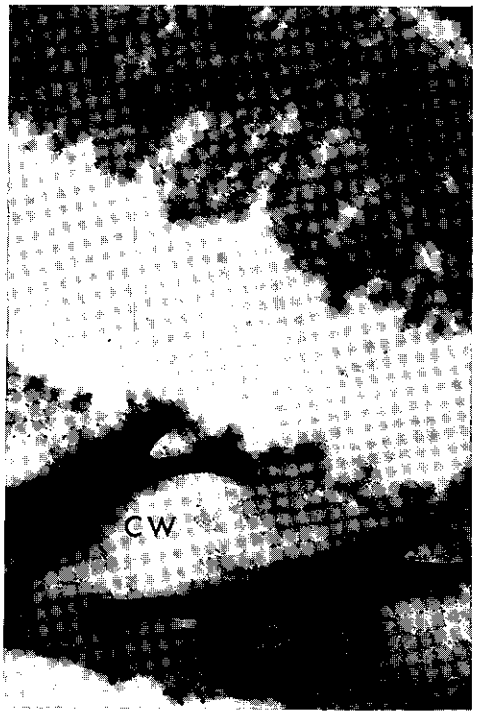

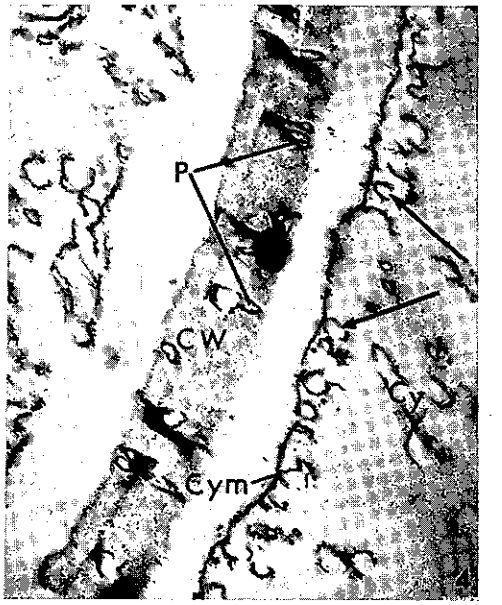

COMPARATIVE PHYSIOLOGY OF CHARA AUSTRALIS. II


N
cm
ER
sg
M
ERi

PLATE 7

Transverse section of osmium-fixed cytoplasm of internodal cell during elongation stage. The nucleus (*N*) is bounded by an irregular membrane (*Nm*) which in at least one place (arrow) appears to merge with the membrane of a mitochondrion (*M*). The cytoplasm contains numerous vesicles (*v*) and small dense bodies. Two golgi zones (*GZ*) can be seen with their characteristic plate-like lamellae terminating in vesicles. The tonoplast (*t*) separating the cytoplasm from the vacuole appears to be a single layer. × 23,000.

PLATE 8

Transverse section of cell 2 showing details of the endoplasmic reticulum. Numerous small dense granules (*sg*) can be seen to be associated with the reticulum (*ER*), each strand of which appears to be composed of two dense regions approximately 50–75 Å wide separated by a wider clear region approximately 60–100 Å wide. The dense zones of the chloroplast membrane (*cm*) appear to be of similar dimensions to the dense zones of the endoplasmic reticulum but the clear zone is much narrower in the plastid membrane. A nuclear body (*N*) also has a triple-layered membrane, the middle region of which in places (arrow) appears to be continuous with the endoplasmic reticulum. A mitochondrion (*M*) also has this triple membrane structure which may be continuous with the endoplasmic reticulum. Moderately dense patches (*ERi*) are interpreted as being endoplasmic reticulum with the broad edge parallel to the plane of the section. The irregular appearance is due to the wavy nature of the reticulum. Osmium-fixed. × 80,000.