

AN ULTRASTRUCTURAL STUDY OF PERIDINIUM TROCHOIDEUM  
WITH SPECIAL REFERENCE TO THE THECA AND ITS FORMATION

by

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## ABSTRACT

Freeze-etching and thin sectioning were used to examine the fine structure of the marine dinoflagellate Peridinium trochoideum (Stein) Lemm. Among the cytoplasmic inclusions described were three typical dinoflagellate organelles: a dinocaryotic nucleus with condensed interphase chromosomes, chloroplasts with thylakoids associated in groups of three, and trichocysts contained in membranous sacs. In addition to the above, dictyosomes, cytoplasmic membrane systems, fibrous bodies and 'segregated bodies' were observed and described. Upon examining the gross morphology of the theca, it was found that thecal age could be determined by the extensiveness of sutures, pits, and blisters which became predominant on the external thecal membranes with age. Characteristically, older cells sometimes had continuous, deeply scored sutures with adjacent 'marginal suture bands' and intercalary bands. Individual thecal plates of mature cells were not completely enclosed within membrane sacs as commonly assumed and some adjacent plates were found to be continuous. Four membrane systems were found to be associated with the thecal plates: the thecal membrane and outer plate membrane systems lay above the plates, the inner plate membrane and plasmalemma lay below. Sutures were formed by infolding of the outer plate membrane between adjacent plates. With the exception of the plasmalemma, the membranes associated with the plates displayed membrane asymmetry. In the mature, thecate, non-dividing cells, densely staining inclusions termed 'pro-thecal bodies' were found to be distributed throughout the cytoplasm. Before ecdysis, each amorphous prothecal body transformed into many vesicles each of which contained fibrous material in an electron transparent matrix. It appeared that the vast number of vesicles

so formed may have increased the cell's osmotic pressure enough to initiate ecdysis. At ecdysis, the thecal plates and overlying membranes were lost and the new wall was formed by deposition of material from prothecal bodies at the protoplast surface. The newly formed wall was continuous over the protoplast and no plates existed as such. Pores, however, were present. The sutures, when first formed were shallow and discontinuous.



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## INTRODUCTION

For the most part, classification of thecate dinoflagellates is based on the particular shape and arrangement of cell wall plates which form the theca\* of a given genus or species. Since non-thecate forms cannot be classified on the basis of thecal morphology, they are usually classified according to cell shape.

Recently, Dodge and Crawford (19) have established a potentially valuable system for the ultrastructural categorization of dinoflagellate cell surface layers. Thus non-thecate and thecate forms can be classified ultrastructurally using the same comparative system. The simplest degree of organization exists in cells which possess a single layer of flattened vesicles at the cell surface. Progressive degrees of organization are based primarily on the acquisition of rudimentary plates within the vesicles and subsequent thickening and elaboration of such plates in heavily thecate forms. Their survey shows that when plates occur, each plate is fully enclosed by a membrane sac.

If one assumes that thecate forms arose from non-thecate forms, the most obvious sequence would appear to be via the establishment of wall material within surface vesicles similar to those found in Amphidinium (17) or Oxyrrhis (19). In other words, a phylogenetic sequence of plate development may be reflected by the phenetic sequence of thecal structure beginning from the simple non-thecate forms to the elaborate thecate forms. This premise is based on the assumption that plates of the thecate forms are enclosed by membrane sacs or vesicles. Although one might expect to

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\* The term theca is used to describe both the thecal plates and the associated membranes collectively.

be able to follow the development of such plates within these vesicles, to date there is no electron microscopic evidence of such thecal ontogeny. On the contrary, there are reports which describe young thecate dinoflagellates as having continuous, undifferentiated walls (7,25).

To complicate the matter, the sequence of events leading to daughter cell formation in dinoflagellates can be quite variable. In a few thecate dinoflagellates an ecdysial stage may occur whereby the theca is lost either prior to or after cytokinesis. For instance, in Peridinium westii (35) cytokinesis takes place inside the parent theca prior to ecdysis, whereas in Gyrodinium cohnii (Crypthecodinium cohnii) (25) and Gonyaulax polyedra (22) cytokinesis occurs within a cyst after ecdysis. Forms such as Pyrodinium bahamense (7) and Peridinium triquetrum (6) do not undergo ecdysis. Instead, each of the daughter cells retains one-half of the parent theca and subsequently reforms the missing half. The latter is most common within the group.

As Braarud (6) has shown, the theca of Peridinium trochoideum (Stein) Lemm. is lost completely prior to cytokinesis and each of the daughter cells must form an entirely new theca. When living material of P. trochoideum is observed, it becomes apparent that cell wall formation takes place rapidly. This would suggest that a great deal of wall material or its precursors are probably synthesized and stored prior to cytokinesis.

With light microscopy, it is difficult to follow the development of the theca. The close association of the newly formed wall material on the plasma membrane with the underlying cytoplasmic matrix and inclusions obscures the delicate details of plate differentiation. Examination of thecae of fully developed cells in situ is also difficult for the

same reason and therefore, gross details of the plates, their sutures, and pores have had to be determined from dissociated thecae, often stained. Thecal structure has been used as an important criterion in the recognition of species in the Dinophyceae. Consequently, the added detail provided by the electron microscope should result in a more complete understanding of this important dinoflagellate structure.

Electron microscopic studies on dinoflagellate cell walls have in the past been accomplished by shadow-casting (14), negative staining (31), or thin sectioning techniques (26). Adequate assessment of the in vivo topography of the thecal plates is difficult to obtain using these techniques. To obtain a three-dimensional representation of the cell wall from electron micrographs of thin sections, a large number of serial sections must be taken from both tangential and cross-sectional planes through the theca. Shadow-casting and negative staining techniques require chemical cleansing and drying of the wall which may destroy or distort some of the three-dimensional features and details.

In this study, freeze-etching and thin sectioning techniques were used to elucidate the fine structure and formation of the theca of the marine dinoflagellate P. trochoideum (Stein) Lemm. Freeze-etching enables three-dimensional reliefs to be obtained from living or fixed material using physical rather than chemical methods (32).



### Materials and Methods

Peridinium trochoideum (Stein) Lemm. was obtained from the Culture Collection of Algae, Indiana University (Collection Number LB 1017) and was grown in Chihara medium (10) supplemented with soil-water extract. Cultures were maintained at 20-25°C. and were illuminated with fluorescent light for 16 hrs. per 24 hr. period.

The majority of material for freeze-etching was frozen directly in the culture medium. However, some material was fixed for 1 hr. in 2.5% glutaraldehyde buffered with sodium cacodylate (pH. 6.8) prior to freezing.

The freeze-etching technique was similar to that described by Moor (32) and was performed on a Balzers BA 360M Freeze-Etching unit. Freezing was accomplished by placing a suspension of material in a 3 mm. gold support cup, immersing the cup in liquid Freon 22 (cooled in liquid nitrogen) and then quickly transferring it to liquid nitrogen. Deep fracturing was achieved by taking low speed cuts across the material. Cutting and sublimation were performed at -100°C. and sublimation time was 1 min. at  $3 \times 10^{-6}$  Torr. pressure. The etched material was shadowed with platinum/carbon and subsequently strengthened by carbon evaporation. The replica was released directly in 70% sulphuric acid and after 1 hr., it was transferred to distilled water. The replica was then placed in sodium hypochlorite for a further 1 hr. to remove remaining debris. After thorough washing in distilled water, the replica was picked up and mounted on a Formvar coated grid.

Material for thin sectioning was fixed in one of two ways: (a) for 1 hr. in a combination of 2.5% glutaraldehyde and 2.5% formaldehyde

(from paraformaldehyde) in 0.1M sodium cacodylate buffer, pH 6.8. 2% sucrose (w/v) was added to adjust the osmolarity of the fixative to that of the growth medium; (b) for 1 hr. at 4°C. with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 6.8. 10% DCMU (v/v) was added to the fixative to inhibit oxygen evolution during fixation. After post-fixation with 1% OsO<sub>4</sub> in cacodylate buffer for 1 hr., the cells were concentrated by gentle centrifugation and embedded in 1% agar (w/v). The agar-embedded cells were dehydrated using a graded series of ethanol and infiltrated with increasing concentrations of Spurr's embedding medium (40) in ethanol or Maraglas (4) in propylene oxide. Finally, the agar-embedded cells were cured in 100% resin. Sections were cut on a Porter-Blum MT-1 Ultramicrotome using a du-Pont diamond knife and were post stained with saturated uranyl acetate in 70% methanol and lead citrate (36). Both sections and replicas were observed with a Zeiss EM 9A electron microscope.

Wall material for light microscopy was obtained by collecting thecae shed from cells which had been left in hypotonic distilled water overnight. Micrographs of thecae and living material were obtained using a Zeiss Photomicroscope with phase contrast or Nomarski interference illumination systems.

## OBSERVATIONS

ULTRASTRUCTURE OF THE CYTOPLASM AND  
ITS INCLUSIONS

Among the most common cytoplasmic inclusions observed with the electron microscope in cells of P. trochoideum are chloroplasts (C, fig. 1), starch grains (SG, fig. 4), nuclei (N, fig. 4), perinuclear fibrous bodies (PFB, fig. 9), cytoplasmic fibrous bodies (CFB, fig. 11), dictyosomes (D, fig. 12), 'segregated bodies' (SB, fig. 14), mitochondria (M, fig. 14), and trichocysts (T, fig. 16).

Within the chloroplasts of P. trochoideum it is possible to distinguish the associated thylakoids (TL) and two distinct stromal regions - the dense stroma (DS) and sparse stroma (SS) (fig. 1). The dense stroma appears to occupy the regions between thylakoid lamellae (fig. 1) while the sparse stroma occupies the central region of the chloroplast (fig. 2). Particulate components of both stromal types occur in equal density. It appears that the sparse stroma is less densely stained because it lacks a moderately dense amorphous component which is prevalent in the dense stroma. Fibrils (F, fig. 2) similar to DNA fibrils of chloroplasts (2, 38) and mitochondria (3) also exist in the sparse stromal region. In preliminary fluorescent studies, chloroplasts of P. trochoideum showed a positive fluorescence for DNA.

As expected, the thylakoids of P. trochoideum chloroplasts are associated in closely appressed groups of three (fig. 3). This is similar to other dinoflagellate plastids (16). The extent of the association, however, is not constant and it is not uncommon to see pseudo-grana of various sizes within a given length of thylakoid surface. Discontinuities

of single thylakoid lamellae are marked in figure 3 by arrows. Dodge has made similar observations in Aureodinium pigmentosum (15).

The nucleus (N, fig. 4) is a typical dinocaryotic type with nucleolus (NU), double membrane envelope (NE), and interphase chromosomes which remain condensed and coiled. Within the chromosomes (fig. 5) it is possible to detect two directions of coiling, (coils parallel to the chromosomal longitudinal axis are marked by black arrows, coils normal to the longitudinal axis are marked by white arrows). The chromatin of the chromosomes readily resembles the sparse stroma of chloroplasts (cf. figs. 2 and 6).

The nucleolus (NU, fig. 6) appears to contain equal amounts of fibrous and granular material. Except for small nucleolar 'vacuoles' (arrows, fig. 6) the staining density is relatively uniform.

Although the nuclear envelope has never been observed to totally break down, there are stages when the envelope has many large gaps (fig. 7). At such times, the nucleus is surrounded by many membranous vesicles (NV, fig. 7) which might have arisen from the nuclear envelope, and/or may contribute to its completion.

An unusual feature of the nucleus is the large dilations of the perinuclear cisternum (PE, fig. 8) within which is sometimes found a perinuclear fibrous body (PFB, figs. 9 and 10). At a point where the nuclear envelope is expanded (arrow, fig. 9), the inner nuclear membrane (INM) remains around the nucleoplasm while the outer nuclear membrane (ONM) expands to accommodate the fibrous body and its granular matrix.

In other instances fibrous bodies occur within membrane bound inclusions in the cytoplasm (CFB, figs. 11, 13, and 14). Both the nuclear and cytoplasmic fibrous bodies appear identical to fibrous bodies found in Peridinium westii (31), Woloszynskia micra (26), and Symbiodinium microadriaticum (23). Their origin and function are unknown.

In close proximity to the nucleus lie two other organelles. Dictyosomes are usually found in the juxtannuclear cytoplasm orientated with the distal face (secreting face) towards the nucleus (D, figs. 12 and 13). Neither the dictyosome nor the extensive membrane system often located with it appear to be directly involved in any readily recognizable or detectable function during wall formation. The second structure associated with the nucleus has been termed a 'segregated body' (SB, fig. 14). It is always located immediately below the nucleus at the antapical pole of the cell. There is no apparent delimiting membrane surrounding it, yet it is distinctly segregated from the cytoplasm and not an extension of it (fig. 15). It contains fibrous material (FB) similar to that observed in nuclear and cytoplasmic fibrous bodies and spherical globules (GL), the periphery of which stains denser than the center. Both fibrous bodies and globules are contained within an amorphous matrix which appears to have a somewhat dense center and less dense border, (arrows, fig. 15). The function of this inclusion is not known.

The last organelle to be discussed is the trichocyst. These organelles have the capacity to eject from the cell. Ejection, however, has never been observed in cultures of P. trochoideum. In cross section the organelles appear as crystalline square cores,  $0.20\ \mu$  in diameter, bounded by a single membrane (TS) which forms a sac around the core (fig. 16).

In longitudinal view, fibers can be seen extending from the pointed apical tip of the core to the trichocyst sac (TF, figs. 17 and 18). Trichocyst sacs do not appear to be within thecal pores (P, as seen in figure 17). In most cases trichocysts are spatially related to the pores but the sacs and pores are independent. Examination of the pore shows that it is an apparently isolated structure consisting of a canal through the plate material (W) and a semi-spherical, membranous, pore plug occupying the canal (figs. 19 and 20). The aperture of the canal is ridged on the outer wall surface (R, fig. 20) and when the plate (W) is observed in surface view both the ridged aperture and pore plug can be identified (fig. 21). The average pore diameter in P. trochoideum is  $0.12 \mu$ .

#### ULTRASTRUCTURE OF THE THECA

Excluding encystment, Peridinium trochoideum has a relatively simple life cycle (6). Mature cells are usually pear shaped and have short apical spines (fig. 22). In living cells, the girdle is easily observed but individual plates are rather difficult to discern. During ecdysis, the theca is broken along the girdle and the protoplast becomes slightly enlarged and somewhat spherical (fig. 23). It is interesting to note that the protoplast appears to have already acquired an appreciably thick surface layer. Although Braarud (6) observed a hyaline excretion between the protoplast and theca during earlier studies on P. trochoideum, such an event was never observed in the present clone obtained from Indiana. After freeing itself from the theca, the naked protoplast swims about for some time and in doing so acquires an elongate shape (fig. 24). Later, the flagella are shed and the protoplast assumes a resting stage (fig. 25).

A slight constriction in the midregion is the first indication of cytokinesis; subsequently a distinct cleavage furrow is formed (fig. 26). After separation, the young daughter cells appear non-motile, spherical, thick walled, and undifferentiated (with respect to thecal plates) (fig. 27).

The epitheca of mature cells of P. trochoideum is composed of three series of plates<sup>1, 2</sup> two of which are visible in ventral view (fig. 28). The apical plates 1', 2', and 4' (four plates comprise this set) are located around the apex while the precingular plates 1", 2", and 7" (seven plates comprise this set) are adjacent to the girdle. The small, narrow apical closing plate (CP) lies anterior to the first apical plate (1') between plates 2' and 4'. What has been previously described as a horn (27) appears to be two spines (H) which arise from the anterior end of the second and fourth apical plates and extend upwards on both sides of the apical closing plate parallel with the cell's central axis. Actually, the spines are not elaborately tapered (fig. 29), rather they appear acute due to the perspective rendered by the fracture plane. Normally, an apical pore exists in the apical closing plate (12), but in some cells it is not observed (fig. 30).

The two flagella originate on the ventral side from a single flagellar pore (FP) situated in the deeply excavated girdle (G, fig. 28). The girdle is left-handed, that is, the girdle forms a descending spiral with the right end of the girdle lying below the left (fig. 29). The transverse flagellum lies in the encircling girdle whereas the longitudinal flagellum lies in the sulcus (SL, fig. 29), a groove situated directly beneath the first apical plate, parallel to the axis of locomotion. The sulcus tends to broaden towards the cell antapex. There are no antapical spines or horns.

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<sup>1</sup>Notation according to Kofoed, ref. 24.

<sup>2</sup>Plate formula same as given by Lebour, ref. 27.

Plates from both the epitheca and hypotheca can be identified from the dorsolateral view of the cell shown in figure 31. An intercalary plate of the epitheca, 2a (three plates comprise this set), lies above the third and fourth precingular plates. The third apical plate which lies above the intercalary plate cannot be seen since the anterior end of the epitheca is embedded in the matrix. The position of the cell, however, allows the first antapical plate, 1''' (two plates comprise this set) to be seen lying beside the first postcingular plate, 1'', and below the second and third postcingular plates, 2'', and 3'' (five plates comprise this set).

As a rule, the girdle is subdivided into three plates demarcated by sutures. The sutures are distinct from the ridges since they are depressed in the wall rather than protruding from it (see S, fig. 32). The first suture of the girdle ( $S_1$ ) lies beneath the first precingular plate, 1'' (figs. 28 and 29), and the second ( $S_2$ ) is found adjacent to the junction of the second and third precingular plates, 2'' and 3'' (fig. 31). The third ( $S_3$ ) is found beneath the seventh precingular plate, 7'' (fig. 29). The girdle does not have extensive lists, but the anterior edge is more deeply ridged than the posterior (figs. 1 and 4).

Although the size variation of cells in a culture is due to unequal fission during cell division, aging also accounts for differences in cell size and certain changes in cell morphology. In general, young cells which acquire a theca soon after maturity are pear-shaped (fig. 28), but growth leads to the expansion of the cell into an almost ellipsoid shape (fig. 31) prior to division. Hence, the shape of the cell can be used to independently assess thecal age. The texture of membranes



covering the thecal plates also indicates the developmental stage of the theca. A young but relatively well-developed theca has surface membranes which have a relatively smooth exterior surface (fig. 28), whereas an older theca shows a striking alteration of the surface. The outer surface membranes of the plates become highly undulated, pitted, and blistered (B, figs. 31 and 32). There are, however, certain regions on the surface of older thecae that are free of pitting. These regions appear as small 'plaque-like' areas scattered over the thecal surface (PL, figs. 33 and 34).

In old cells, sutures are sometimes bordered by a distinctive 'marginal suture band' (MB, fig. 31, and 33). The band width is fairly constant for any one cell but the width varies among cells with thecae of different ages. Older cells usually have wider bands, 0.38 - 0.5  $\mu$  wide. The band represents a slightly elevated region of the theca adjacent to the recessed suture (MB, fig. 32 and 33). Intercalary bands are also present in some older thecae and appear on the opposite side of the suture as the marginal suture band (IB, fig. 31). Since they are not striate or pronounced they cannot be readily observed with the light microscope.

#### ULTRASTRUCTURE OF THE THECAL PLATES AND ASSOCIATED MEMBRANES

One striking external feature of both mature and old cells is the relative discontinuity of plate demarcation (sutures) at the thecal surface (arrows, figs. 34 and 35). This is due to the overlying plate membranes. The thecal membrane is the outermost membrane and it covers

the entire cell (TM, figs. 36, 37, and 40). It can be seen that the thecal membrane (fig. 36) must be slightly invaginated at the surface immediately above plate junctions to form what is recognized as a suture (S, fig. 35).

Intercalary bands have not been detected in thin sectioning; however, marginal suture bands appear as thickened regions at the edges of two overlapping plates (fig. 36). In some cases the plates may bear a ridge (R, fig. 36). The plate material is electron transparent and hence appears nondescript.

Closely appressed to and below the thecal membrane is the outer plate membrane (OPM, figs. 36 and 40). The outer plate membrane will be defined as the membrane which lies immediately above the plate. In-folding of such a membrane between adjacent plates occurs within each suture (S, figs. 36 - 41). It is quite clear from figure 37 that when sutures between plates are incomplete, the outer plate membrane reflects on itself and thus does not extend completely around the plate. For the most part, the outer plate membrane does not surround plates even when the plates are separate (arrow, fig. 38). Possible attachment or continuity of the suture with a membrane beneath the plate has been observed only once (arrow, fig. 39). It can be proposed therefore, that such a membrane system does not completely enclose each plate in a sac-like manner. Instead, the plates generally appear to be flanked by two separate membrane systems, the outer plate membrane immediately above and the inner plate membrane immediately below (IPM, figs. 38 - 40).

The inner plate membrane cannot be regarded as the plasmalemma. The plasmalemma, defined herein, (PM, figs. 38 and 40) lies below the inner plate membrane and delimits a narrow band of cytoplasm at the protoplast periphery. This cytoplasmic band is the result of extensive vacuolation (V) of the parietal cytoplasm and is a common characteristic

in mature cells of P. trochoideum. Furthermore, the plasmalemma is relatively thin, whereas the inner and outer plate membranes (and the thecal membrane) are not only thicker but are also asymmetric (figs. 40 and 41); the latter are about  $150\text{\AA}$  thick with partitions of  $75/50/25\text{\AA}$  whereas the plasmalemma is a symmetrically  $100\text{\AA}$  thick. In cells with mature thecae, the thicker partition of both the inner and outer plate membranes is always found closest to the plate (figs. 40 and 41). Where sutures exist, the outer plate membrane infolds and pairs. The two thinner partitions become appressed and form a central pairing line thus giving the suture an apparent pentapartite appearance (S, fig. 41).

The fractured face shown in figure 42 reveals all four membrane systems. Both the thecal membrane and outer plate membrane possess regions of pitting. The plate material has a relatively smooth surface. The underlying inner plate membrane has a reticulate surface whereas the plasmalemma appears slightly reticulate and undulated.

Since the four membrane systems can be recognized from their location and morphology in freeze-etched preparations, it is now possible to further demonstrate the relationship between the suture and the underlying plate membrane. In figure 43, both the plasmalemma and inner plate membrane appear to be continuous over the cytoplasm even at plate junctions. This supports the observations from thin sectioned material (figs. 37, 38, and 40) that there is no connection between the outer and inner plate membrane systems.

Figures 44 and 45 reveal the plate-side view of the inner plate membrane. If each plate were surrounded by a plate membrane one would

expect a fracture below the plate and above the inner plate membrane to reveal sutures. Instead, no sutures, tears, or membrane projections occur on the membrane immediately below the plate. The membrane, however, does exhibit distinct scars (SC). These scars may represent places where the membranes of the inner and outer membrane systems may once have been closely associated or even possibly attached.

#### ULTRASTRUCTURE OF WALL FORMATION

In addition to the inclusions described previously one finds large osmiophilic bodies distributed circumferentially around the nucleus (PB, fig. 46). These inclusions play an important role in the formation of the wall, and will be referred to as 'prothecal bodies.' In mature cells, these structures are, for the most part, fairly amorphous, although it is common to find the structure permeated by varying amounts of membrane (MC, fig. 47).

Prior to ecdysis, the prothecal bodies of the mature cell undergo transformation, at which time the almost wholly amorphous inclusion becomes traversed with increasing amounts of membrane (fig. 48). Subsequently, the prothecal body becomes almost totally composed of loosely packed flattened vesicles (fig. 49). Further transformation results in the formation of vesicles whose limiting membranes and contents are assumed to be derived from the prothecal body (see PV, fig. 57). Since the vesicles originate from prothecal bodies they will be called prothecal vesicles.

As prothecal vesicles migrate to the surface of the protoplast, an amorphous substance can be seen to accumulate between the plasmalemma and the inner plate membrane (arrow, fig. 50). After migration of the prothecal vesicles to the plasmalemma, the deposition of material

across the plasmalemma becomes extensive resulting in the formation of a cushion of new wall material (WM) between the plasmalemma and the inner plate membrane (fig. 51). The cell usually undergoes ecdysis at this stage and the thecal membrane, outer plate membrane, and plates are lost. The prothecal vesicles constitute a substantial part of the peripheral cytoplasm of cells at this stage (fig. 52).

Prothecal vesicles contain little, if any, of the amorphous component found in the prothecal body; instead, they contain loosely intertwined fibrous material (figs. 51 and 52). It is probably the incorporation of this material together with the prothecal vesicle membrane that gives the new cell wall a moderately dense staining characteristic at this stage of development (fig. 53).

During cytokinesis, light microscope observations indicate that new cell wall material is readily discernible, especially at the isthmus of the dividing cell (WM, fig. 54). If plasmolysis is induced at this stage, the protoplast, with a definite new wall layer (W), becomes separated from the overlying inner plate membrane of the parent cell (IPMp, fig. 55). The new wall material appears to be reasonably flexible at this time and remains closely associated with the underlying protoplast.

Electron microscope observations of the same stage indicate that an appreciable amount of wall material has been laid down at the protoplast surface (W, fig. 56), thus confirming the light microscope observations. The prothecal bodies have decreased in number by this stage but prothecal vesicles are still abundant in the peripheral cytoplasm adjacent to the new wall (PV, fig. 56). Wall formation takes place

very rapidly at the isthmus where, during late cytokinesis, the development of prothecal vesicles from prothecal bodies is very evident (fig. 57). Eventually, the prothecal vesicles become distributed in two bands across the isthmus (arrows, fig. 58). Their subsequent fusion and maturation results in the separation of the parent protoplast into daughter halves and the eventual formation of their new walls.

The wall material at the separation point appears as a very definite band (W, fig. 59) and is not nearly as fibrous as that observed in earlier stages (cf. fig. 53). There is no underlying inner plate membrane at this stage. After cytokineses and/or during early maturation, each of the daughter cells acquires an inner plate membrane (IPM, fig. 60). This membrane is asymmetrical and has its thicker partition adjacent to the wall similar to the inner plate membrane of a mature cell. The membrane appears to originate in a zone between the plasmalemma and the wall and although it is difficult to establish, it appears that the new inner plate membrane is derived from moderately electron opaque material associated with the wall (arrows, figs. 59 and 60). This material probably represents aggregates of membrane components derived from the prothecal vesicles.

Although the new inner plate membrane is now established, the outer plate membrane and thecal membrane are still absent. Some of the membrane covering the wall at this time is new and some represents inner plate membrane of the parent cell (IPMp, fig. 60). The

latter can be recognized because it has its thinner partition adjacent to the wall - the result of wall deposition between it and the plasmalemma. It should be made clear that both the outer plate membrane and thecal membrane of mature cells have their thicker partitions adjacent to the wall as described previously.

Each young daughter cell of P. trochoideum thus possesses a cell wall which is continuous but undifferentiated. That is, there are no individual plates or sutures (fig. 61). Prothecal bodies observed in young daughter cells appear similar to those of young, mature cells. There are no signs of the amorphous component being traversed with membrane although membrane components can be seen adjacent to some amorphous inclusions (MC, fig. 61).

Examinations of a young cell with a developing theca shows that both sutures and pitted regions are lacking, but a fully developed pore (P), identical to that on the mature theca, (P. fig. 28), is present on the smooth surface (fig. 62). Figure 63 illustrates an intermediate stage of plate morphogenesis as defined by the formation of the suture. At this stage, the sutures (S) are shallow, and very discontinuous (arrows). Again pores are present and although pitted regions can be observed on the thecal surface, they are not as distinct as in the mature thecae shown in figures 32 and 42.

## DISCUSSION

## CYTOPLASM AND ORGANELLES

With the possible exception of the segregated body, none of the organelles described in the observations are unique to Peridinium trochoideum.

The chloroplasts with their thylakoids associated in groups of three are typical for the group (16). What is not common however, is the pseudogranal associations between thylakoids of varying size. The stromal differentiation is another elaboration of chloroplast morphology. With two types of stroma, dense and sparse, it seems likely that each type may possibly have different yet probably linked functions. On the basis of preliminary fluorescent studies and chloroplast ultrastructure of plastid genophores (2, 38) it is suggested that the sparse stroma may contain DNA.

The nucleus of P. trochoideum can be described as a dinocaryotic nucleus (46) on the basis of having coiled and condensed interphase chromosomes. It would appear from the ultrastructure of the chromosomes that the chromatin fibers lack the histone and residual protein complex common to larger eucaryotic chromatin fibers (11, 13, 30, 37). Unlike the nucleolus of eucaryotic cells (animal cells specifically), the nucleolus of P. trochoideum is relatively simple and lacks the three detectable regions of the nucleolus referred to as the pars amorpha, pars fibrosa, and pars chromosoma (21).

Although the nucleoplasm is typical for a dinocaryotic nucleus, the envelope displays characteristics which tend to suggest a possible nucleo-cytoplasmic relationship common to a nocticaryotic



envelope. The nocticaryotic nucleus, as defined by Zingmark (46), has been described by Afzelius (1) as having no interphase chromosomes and having nucleo-cytoplasmic communication through blebbing of the nuclear envelope. The vesicle-associated stage observed in P. trochoideum could possibly be an analogous type of nucleo-cytoplasmic communication since not only are there large gaps in the nuclear envelope but the vesicles themselves may be agents of transport. The presence of nucleo-cytoplasmic communication by the envelope of a dinocaryotic nucleus has been reported by Dodge and Crawford for Gymnodinium fuscum (18). In P. trochoideum there are also large dilated areas between the inner and outer nuclear membranes where fibrous bodies are observed. Nuclear associated fibrous bodies have also been described by Dodge for Aureodinium (15) and by Taylor for a symbiotic marine dinoflagellate (42). These fibrous bodies have also been observed as membrane bound inclusions in the cytoplasm of P. trochoideum and are very similar, if not the same as, structures observed in Peridinium westii (31), Wolosynskia micra (26) and Symbiodinium microadriaticum (23). There is a possibility, therefore, that cytoplasmic fibrous bodies might arise from the perinuclear fibrous bodies which are released from the nuclear envelope at some stage. Other nuclear envelope elaborations have been described by Kofoed and Swezy (24) in Gyrodinium corallinum and G. virgatum where the outer part of the nucleus appears as a thin alveolar layer of ellipsoidal vacuoles and by Taylor (45) who described perinuclear structural elements in Gonyaulax pacifica.

The trichocysts observed in P. trochoideum are morphologically similar to those described in other species (5, 18, 25, 31) but are smaller in diameter. Whereas reports from the above sources describe trichocyst diameters of 0.2 to 0.4  $\mu$ ., the largest found in P. trochoideum were 0.2  $\mu$ . Trichocyst fibrils similar to those described by Bouck and Sweeney (5) and Messer and Ben-Shaul (31) were seen attached to the trichocyst core. The anterior end of the trichocyst sac containing the core and fibrils was not continuous with the pore plug but appeared to be terminal beneath the wall at the plasma membrane as in P. westii (31). This situation is somewhat different to that described for Gonyaulax and Prorocentrum where the trichocyst sac is situated in the wall adjacent to the plate membrane which overlies the pore (5). In P. trochoideum the trichocyst sac is situated below the pore in the wall which is covered by the plate membrane and the thecal membrane. These membranes must be punctured during the release of the shaft. Although it was not observed, the trichocyst sac may project into the canal prior to discharge.

The thecal pores through which the shafts are ejected have an average diameter of 0.12  $\mu$  which is much smaller than pores (0.2 - 0.3  $\mu$ ) described in P. westii (31). However, this can be related to the corresponding trichocyst diameter of the two species. Assuming the discharged trichocyst shafts are of smaller diameter than the resting or charged form (31), no problem should arise during exit of striate rods of P. trochoideum through a 0.12  $\mu$  pore.

## THECAL MORPHOLOGY

From the observations, it is clear that surface morphology of the thecal membranes of P. trochoideum reflects its stage of development. Smooth surfaces are characteristic of young cells; with aging, pits, blisters, sutures, marginal suture bands, and inter-calary bands become progressively differentiated. Pores, on the other hand, are formed very early in the ontogeny of the thecae.

Blistering at the cell surface has been demonstrated in Cryptocodium cohnii (Seligo) Javornicky<sup>3</sup> where it is evident that the outer thecal membrane and inner plate membrane become separated from the wall (25). It is uncertain, however, whether this is a natural occurrence analogous to the blisters on the surface of P. trochoideum.

Both marginal suture bands and intercalary bands occur on thecae of P. trochoideum. Each is discrete in morphology; the marginal suture band is a distinctive elevated region at the plate margin adjacent to the suture, whereas the intercalary band is not elevated and borders the suture on the opposite side to the marginal suture band. The intercalary band is generally twice the width of the marginal suture band, however both surface features appear as a function of aging. Some investigators (29, 34) believe that the intercalary band represents a region of growth. The marginal suture band may also fulfill the same function.

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3

This taxon was referred to as "Gyrodinium cohnii (Schiller)" (sic).

## PLATES AND ASSOCIATED MEMBRANES

Compared to other species in the genus, the cell wall material of Peridinium trochoideum takes up little, if any, stain and therefore appears quite nondescript. In both P. westii (31) and P. cinctum (19, 22) the wall material stains appreciably and one can easily detect its fibrillar nature. The multilayered cyst wall of Pyrocystis spp. (41) has been examined using freeze-etching technique and the fibrillar nature of the wall is quite evident. As shown in the observations, the mature wall of P. trochoideum shows no fibrils either in thin sectioned or freeze-etched material.

Dodge (19) has shown that plates of Heterocapsa triquetra possess two or more sides which bear ridges; the remaining sides have tapered flanges. This plate characteristic was also suggested as common in P. trochoideum. However, from the current investigation, it appears that in P. trochoideum such a characteristic may occur only when a marginal suture band is present. In most cases, plate overlap occurs without any elaboration.

In this work, a distinction has been made between the two separate membrane systems which are in contact with the plates. The outer plate membrane was defined as that membrane which lies between the thecal membrane and the plate and the inner plate membrane as lying between the plate and the plasmalemma. The reason for defining two separate plate membranes is based on the following observations. Firstly, each plate was not completely enclosed nor totally separated by one continuous membrane. Secondly, the membrane immediately under the plates was shown by thin sectioning and freeze-etching to be

continuous over the protoplast. Finally, a cytologically well defined membrane surrounding each plate in a manner similar to that observed by Dodge (19) in Wolozynskia coronata, Ceratium hirundinella, and Peridinium cinctum could not be shown.

Only once was the association of the outer plate membrane and the inner plate membrane observed to form a plate enclosure. Because of this, it is believed that the outer plate membrane may have been continuous with the inner plate membrane at one time, only to become dissociated from it during maturation. The vestigial point of association is probably represented by the scars observed on the inner plate membrane.

Whether the outer plate membrane is continuous with the inner plate membrane or whether it infolds in association with a suture, apparently only the thicker partition of the membrane lies adjacent to the plates.

The organization of the theca closely resembles that of Cryptocodium cohnii (25) in which the outer plate membrane was reported to form sutures by infolding. The following diagram illustrates the relationship between the various membrane systems and thecal plates of P. trochoideum.

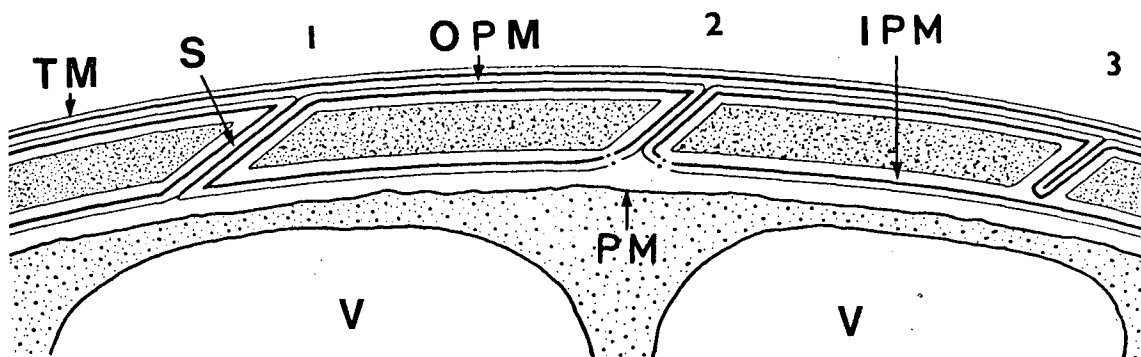


Diagram 1.

The asymmetric thecal membrane (TM) is the outermost membrane and is continuous over the entire cell. The outer plate membrane (OPM) may at the earliest stages of thecal ontogeny surround each plate (region 1). However, lack of association between the outer plate membrane (OPM) and inner plate membrane (IPM) is definitely predominant in most cells (region 3). If one assumes that the outer plate membrane and inner plate membrane are one and the same during early development, then an intermediate stage of separation must exist (region 2). In either case, the thicker partition of the membrane(s) always lies adjacent to the plate. Sutures (S) have a typical pentapartite appearance as a result of apposition of pairing membranes. The plasmalemma (PM) is a relatively thin symmetrical membrane containing the cytoplasm. In most cells a parietal cytoplasmic band is a result of vacuolation (V) at the cell periphery.

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Most of the results indicate that continuity between outer and inner plate membranes is lacking in mature cells. It is not likely that disruption at fixation causes the breakage of a membrane which would otherwise enclose a plate. It would be difficult to justify the fact that such a membrane would be subject to breakage at exactly the same point in every cell observed.

The last membrane to be discussed is the plasmalemma. The question of determining and defining the plasmalemma may involve a semantic problem. Dodge contends that the outermost membrane of the dinoflagellate is the plasmalemma (19). If this is the case, then at ecdysis the plasmalemma of P. trochoideum will be lost and an inner membrane must therefore be designated the plasmalemma. This problem of redefining a plasmalemma is superfluous in P. trochoideum if the plasmalemma is defined as that membrane which is in direct contact with the cytoplasm. It is, unlike the other membranes associated with the plates, symmetrical.

#### FORMATION OF THE WALL

The prothecal body in non-dividing cells was shown to be an electron dense, amorphous body. Prior to ecdysis, membrane components appear and traverse throughout the amorphous component of the body and there is a marked decrease in stainability. At this stage, the prothecal bodies appear identical to the "atypical plastids" described in Crypthecodinium cohnii (25). At ecdysis the amorphous component of the prothecal body is almost gone, being replaced by membranes which later form prothecal vesicles. The essence of this transformation appears to be the probable production of some soluble, electron transparent component from the amorphous material of the prothecal body. Such a conversion results in the formation of prothecal vesicles, the consequence of which possibly increases the osmotic pressure within the cell. Thus, at ecdysis such an increase

in pressure inside the protoplast may be the active force effecting the rupture of the theca. Although Braarud (6) believes that ecdysis in P. trochoideum occurs as the result of an excretion of hyaline substance between the protoplast and theca, no such event occurred in the clone of P. trochoideum from Indiana.

After escape from the theca, the protoplast proceeds through cytokinesis. Having lost the plates and overlying membranes, it is surrounded by the inner plate membrane and plasmalemma of the parent cell. The plasmalemma (as defined herein) is the only stable, wall-associated membrane retained during the cell cycle.

It is assumed that the wall material is derived from prothecal bodies, packaged in prothecal vesicles, and deposited by the latter at the site of the new wall. It is most interesting that Bursa (8, 9) has observed discharges of ectoplasmic colloid from Woloszynskia, Gyrodinium, and Peridinium which are capable of differentiating into membrane and plate structures. Indeed, if the ectoplasmic material were prothecal vesicles or their counterparts one might expect such a phenomenon to occur.

When the wall is formed, material is deposited between the plasmalemma and the inner plate membrane of the parent cell. There are no sutures and hence no discernible plates at this stage and the wall exists as a continuous sphere over the protoplast. This situation confirms the fact that the inner plate membrane is continuous. Similar studies have shown that Pyrodinium bahamense (7), Crypthecodinium cohnii (25), and Gonyaulax polygramma (44) also exhibit a stage in which wall material is present but apparently not differentiated into plates.



In P. trochoideum the first plate-membrane system to become established after wall formation is the inner plate membrane system. The thicker partition of this membrane lies, as expected, adjacent to the wall. Formation of individual thecal plates is presumed to take place concurrently with the formation of outer plate membrane and thecal membrane systems. It seems quite probable that the membrane components for these systems exist in the undifferentiated wall as a consequence of the total incorporation of prothecal vesicles into the wall. Indeed if a prothecal vesicle were to remain in the wall as such, it could well form a pore plug. This could explain the presence of pores in very young thecae and eliminates the necessity of invoking a dissolution of wall material in the formation of a pore after wall deposition has taken place. In Prorocentrum, it has been suggested that the first wall covering is complete (14) and it was assumed that dissolution of wall material resulted in pore formation.

Sutures are not present on young thecae but apparently develop some time after wall material has been laid down. Taylor (43) has found from light microscopy that young thecae of Gonyaulax tamarensis with little or no surface markings do not break along expected suture lines but fragment into odd shaped pieces of wall material. When sutures in P. trochoideum first appear they are shallow and quite discontinuous. To fully understand the process of suture formation it will be necessary to follow the development of the two overlying membranes of the wall (the thecal and outer plate membrane) and their subsequent contribution in subdivision of the wall into distinctly shaped plates. Presumably the mechanism for control over plate shape

must lie within the cell and hence it would appear logical to expect active sites at the plasmalemma and/or inner plate membrane that determine the format for all plates types. However, it must be recognized that wherever incomplete sutures have been observed, dissolution of wall material appears to have been initiated on the external face of the wall.

Undoubtedly plate formation is an extremely complicated process, especially if one were to attempt to explain the process from the basic genetical control systems to the actual physical outcome of determining plate demarcation.

## PLATES AND EXPLANATIONS

## LEGEND

B	=	blister	NV	=	nuclear vesicles
C	=	chloroplast	ONM	=	outer nuclear membrane
CFB	=	cytoplasmic fibrous body	OPM	=	outer plate membrane
CH	=	chromosome	P	=	pore
CP	=	apical closing plate	PB	=	prothecal body
D	=	dictyosome	PE	=	perinuclear extension
DL	=	dense layer	PFB	=	perinuclear fibrous bod
DS	=	dense stroma	PL	=	plaque
F	=	fibrils	PM	=	plasmalemma
FB	=	fibrous body	PP	=	pore plug
FL	=	folds	R	=	ridge
FP	=	flagellar pore	S	=	suture
G	=	girdle	SB	=	segregated body
GL	=	globules	SC	=	scar
H	=	spine	SG	=	starch grain
IB	=	intercalary band	SL	=	sulcus
INM	=	inner nuclear membrane	SS	=	sparse stroma
IPM	=	inner plate membrane	T	=	trichocyst
IPMp	=	parent cell inner plate membrane	TF	=	trichocyst fibers
M	=	mitochondria	TL	=	thylakoids
MC	=	membrane component	TM	=	thecal membrane
N	=	nucleus	TS	=	trichocyst sac
NE	=	nuclear envelope	W	=	wall
NU	=	nucleolus	WM	=	wall material

Figure 1. Chloroplasts (C) showing thylakoid lamellae (TL) in associations of three and dense (DS) and sparse (SS) stromal regions.

x 21,600

(inset) x 36,600

Figure 2. Sparse stroma showing fibers (F) believed to be DNA.

x 73,200

Figure 3. Thylakoids (TL) in associations of three. The margins of some of the small thylakoids are marked by arrows.

x 154,000

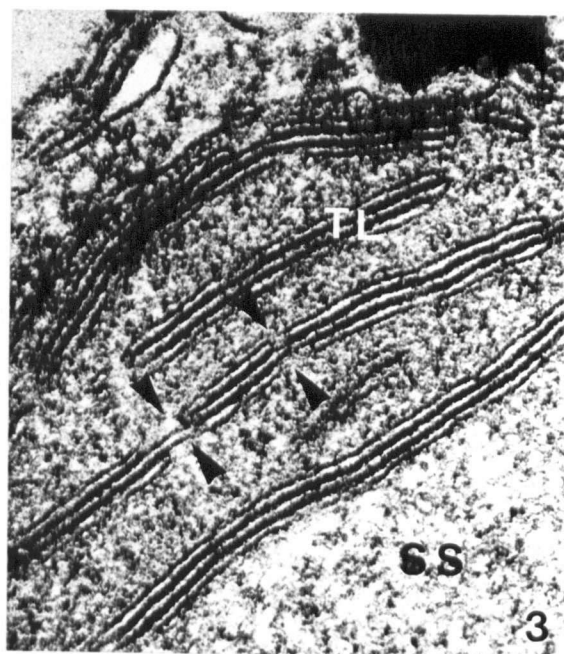
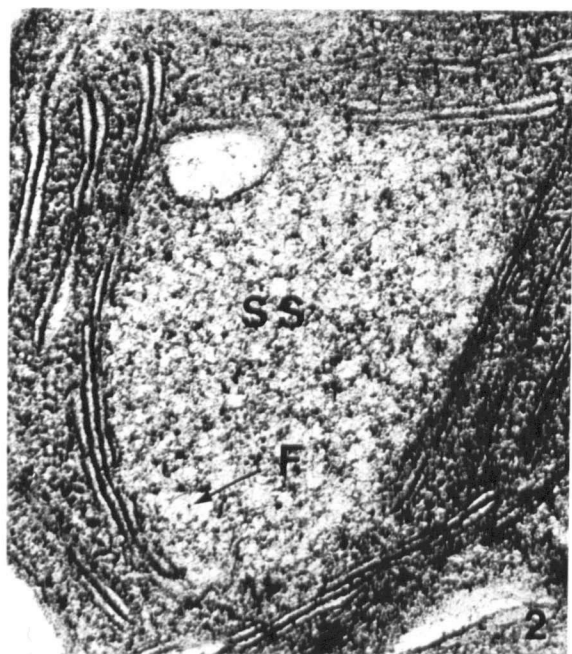
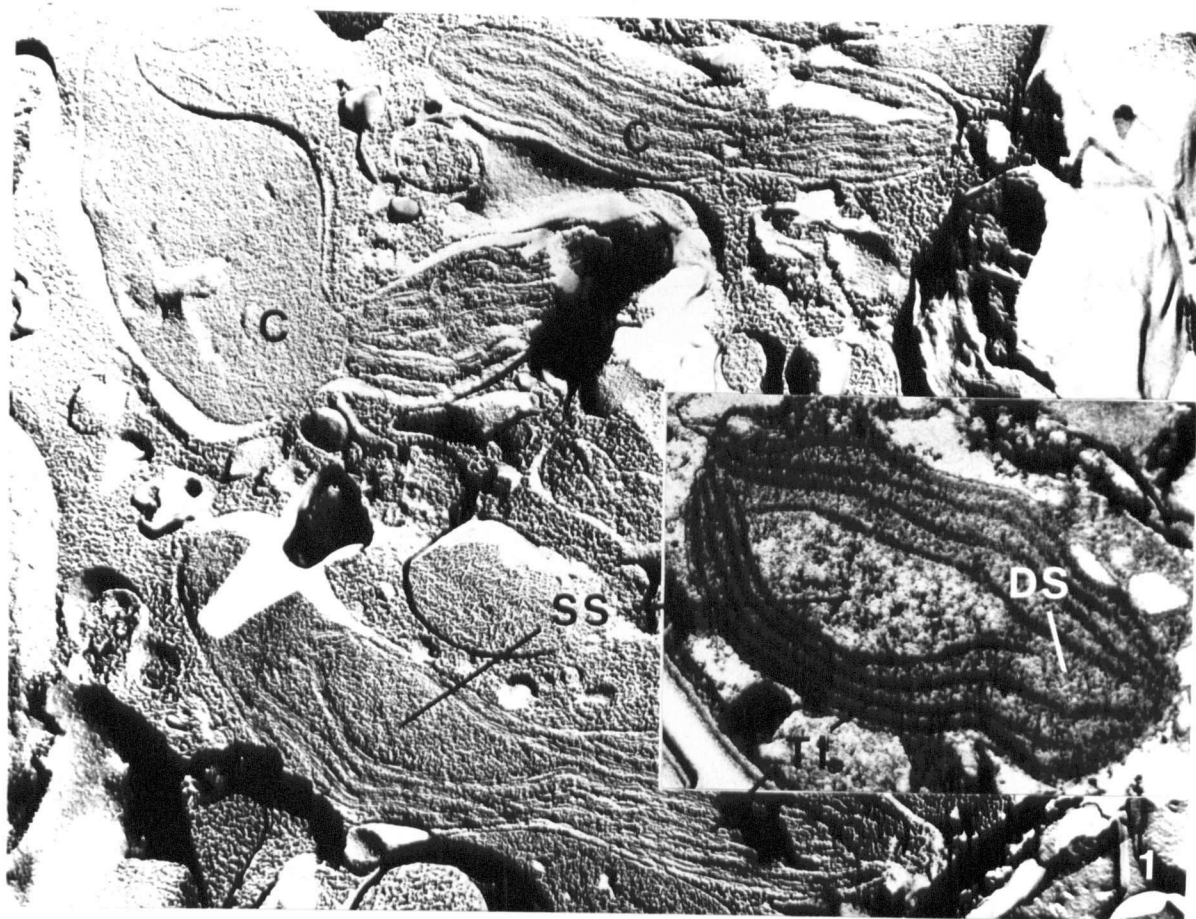


Figure 4. Typical mesocaryotic nucleus (N) with coiled and condensed interphase chromosomes (CH), nucleolus (NU), and double membrane envelope (NE).

x 9,700

Figure 5. Interphase chromosomes (CH) showing two directions of coiling: coils parallel to the chromosome longitudinal axis (black vertical arrows) and coils normal to the chromosome longitudinal axis (white horizontal arrows).

x 59,000

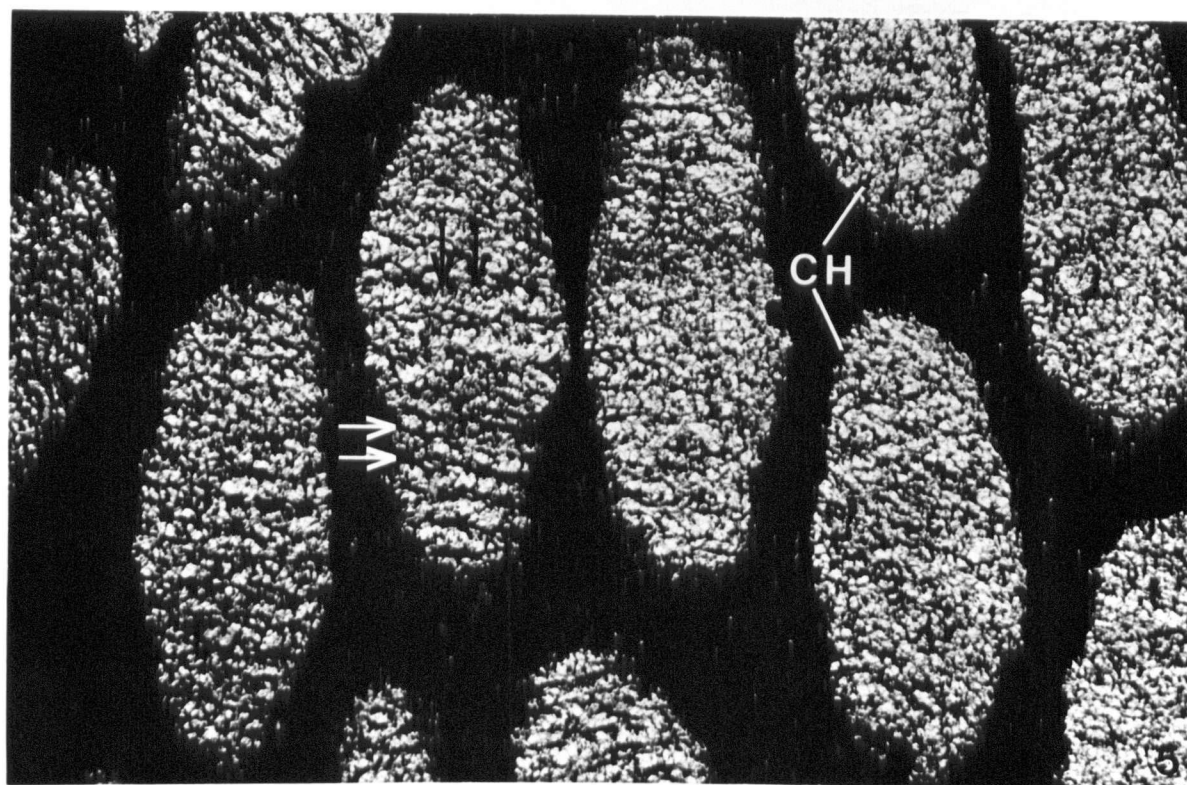
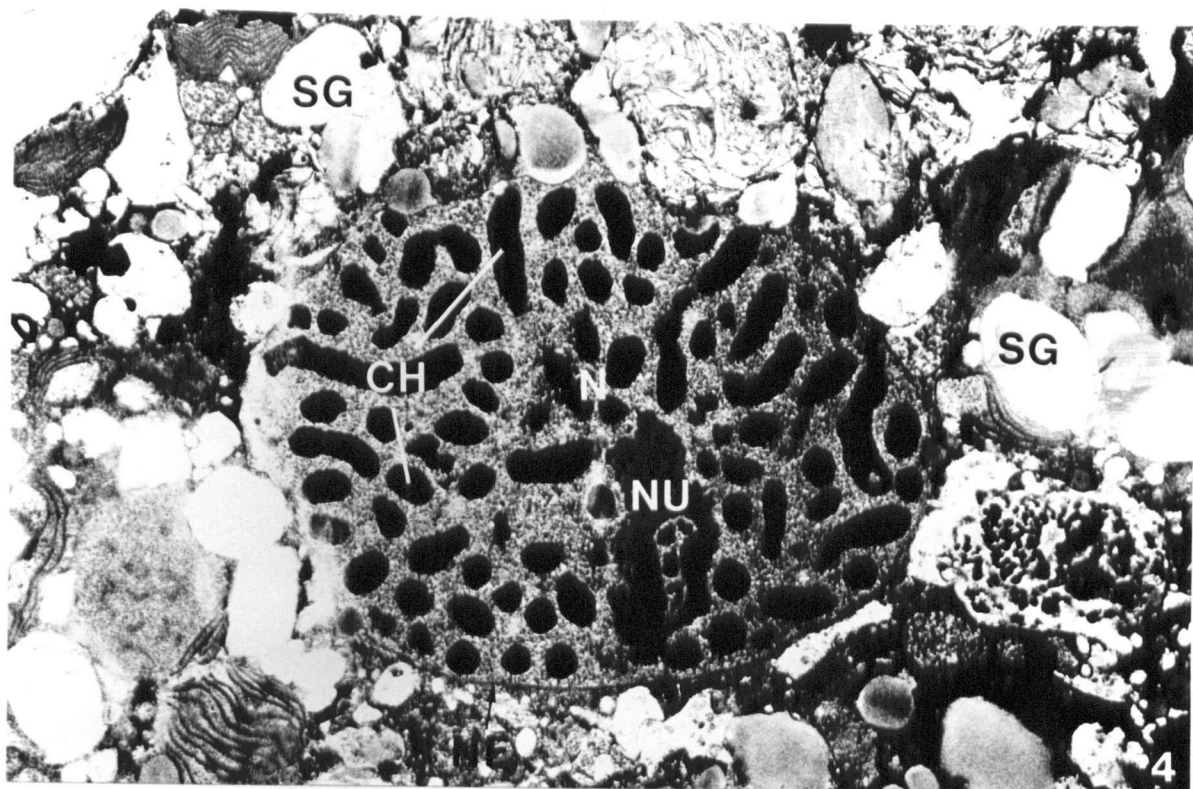


Figure 6. Fibro-granular nucleolus (NU) with small clear nucleolar vacuoles (arrows).

x 41,800

Figure 7. Partial breakdown of nuclear envelope (NE) and association with nearby vesicles (NV).

x 57,000



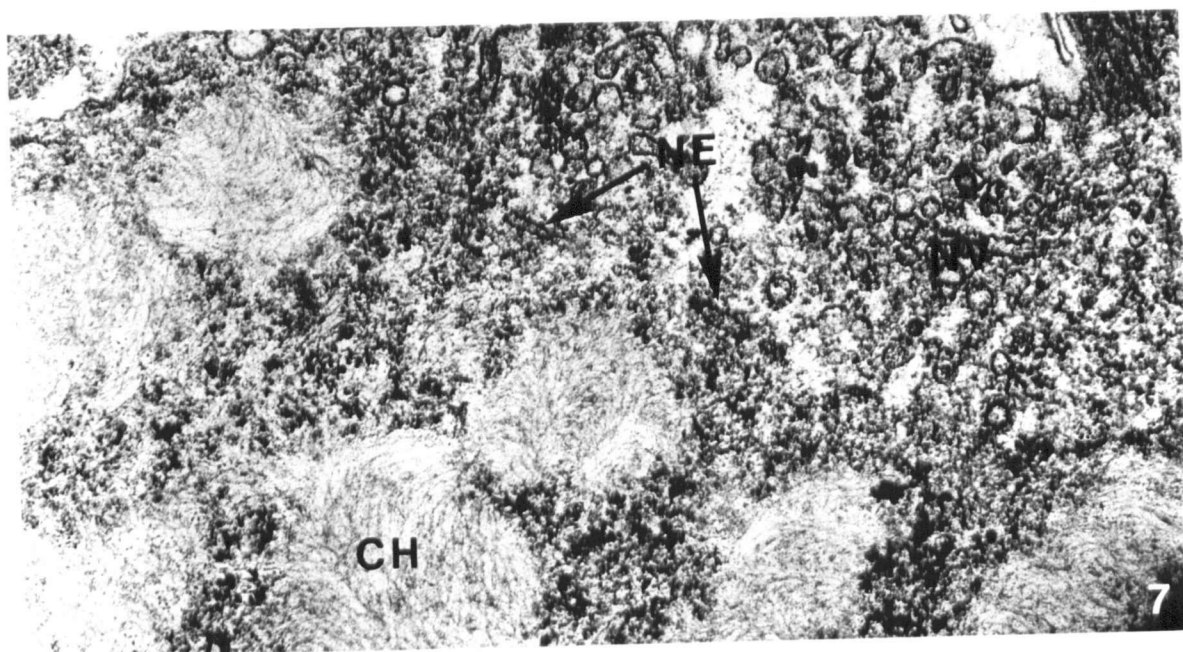
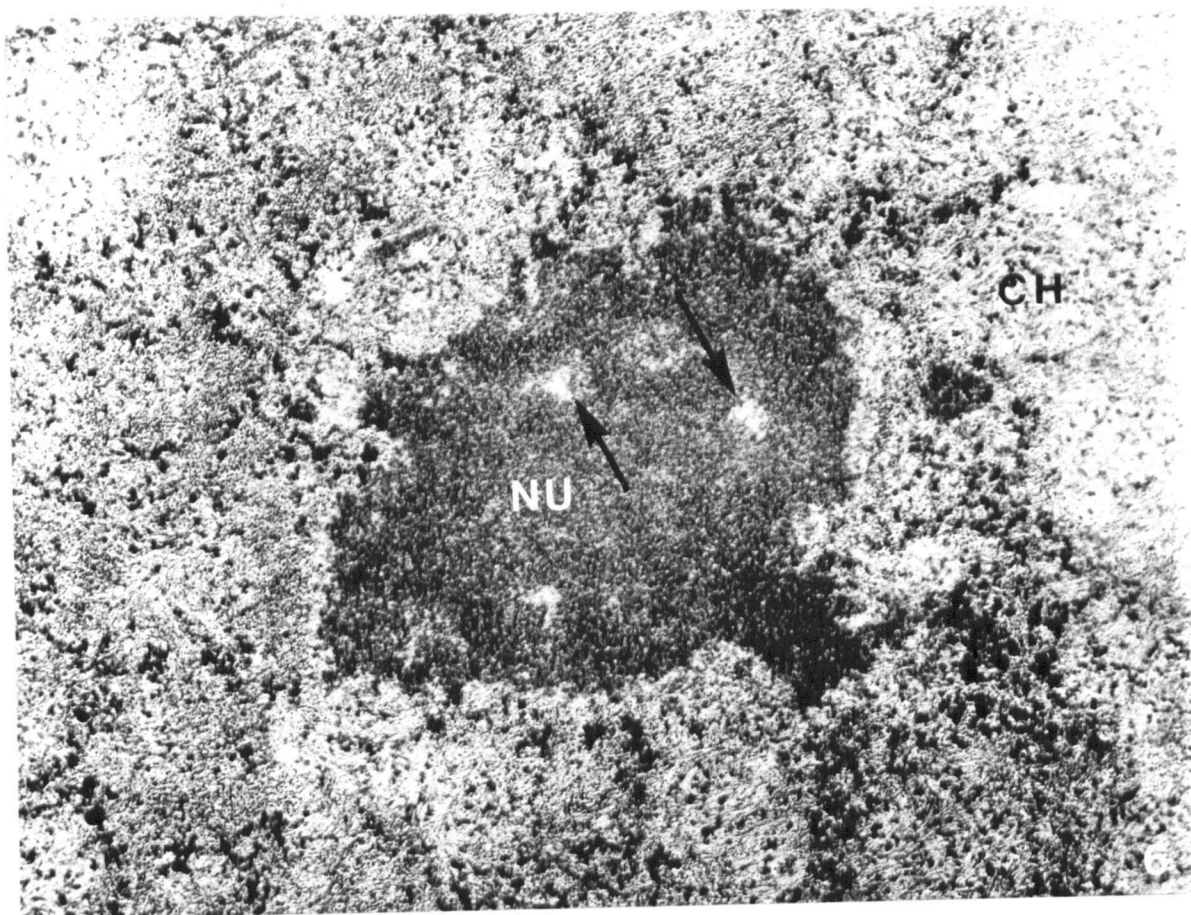


Figure 8. Extension of outer nuclear membrane around fibrogranular matrix of the perinuclear extension (PE).

x 17,000

Figure 9. Perinuclear fibrous body (PFB) within perinuclear extension. Note separation of inner (INM) and outer (ONM) nuclear membranes (arrow.)

x 57,000

Figure 10. Perinuclear fibrous body (PFB) apparently in close association with the nuclear material (CH).

x 57,000

Figure 11. Cytoplasmic fibrous body (CFB). The fibers do not occupy the entire inclusion.

x 12,100

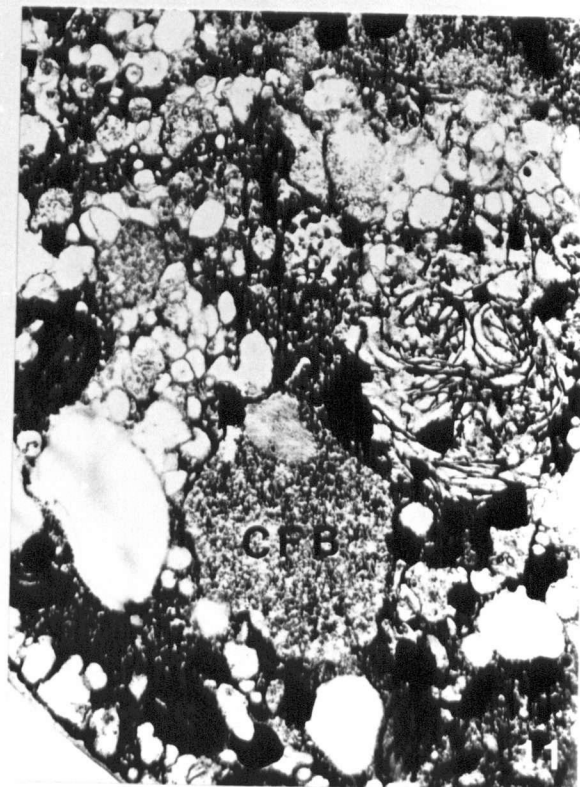
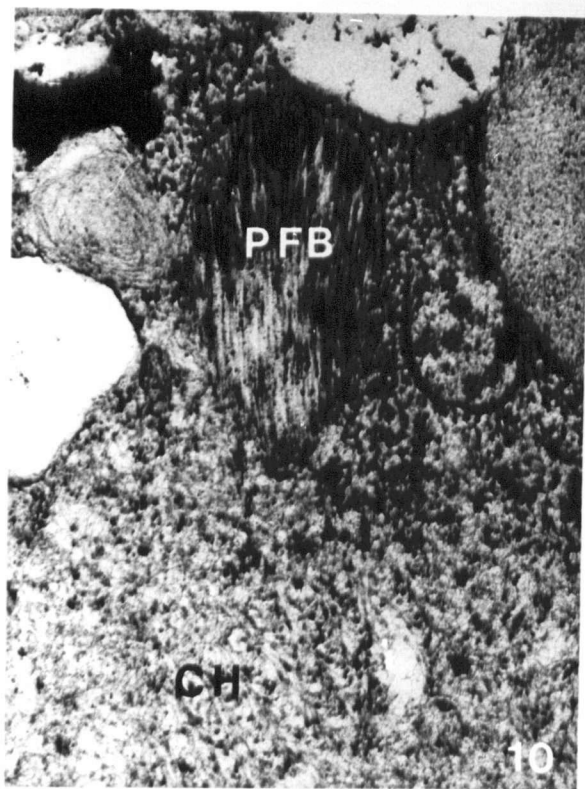
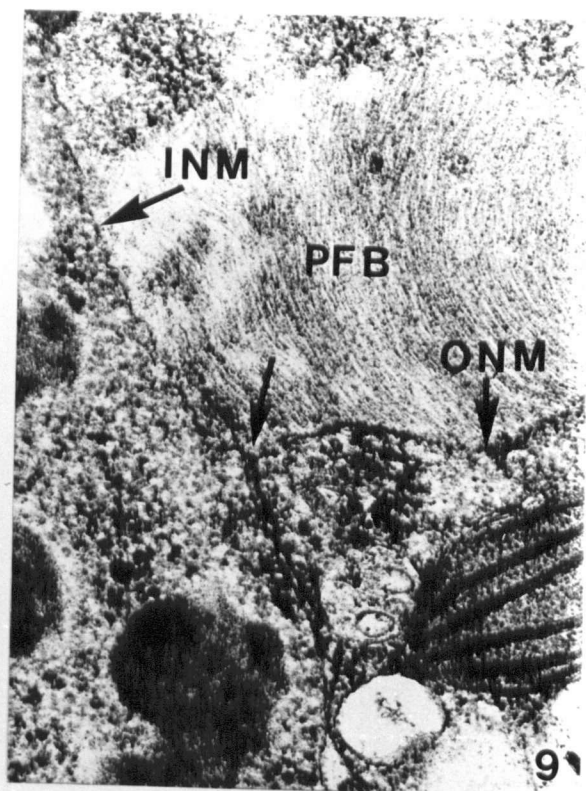


Figure 12. Dictyosome (D) with distal (secreting) face towards nucleus. (N)

x 28,500

Figure 13. Dictyosome (D), cytoplasmic fibrous body (CFB), and complex membrane system adjacent to nucleus.

x 25,500

Figure 14. Location of segregated body (SB) just below nucleus (N) at antapical end of cell.

x 18,000

Figure 15. The segregated body contains fibers (FB) similar to those in nuclear and cytoplasmic fibrous bodies and globules (GL) with dark staining peripheries. These are embedded in an amorphous matrix which consistently has a large central dense region and a less dense periphery (arrows).

x 21,600

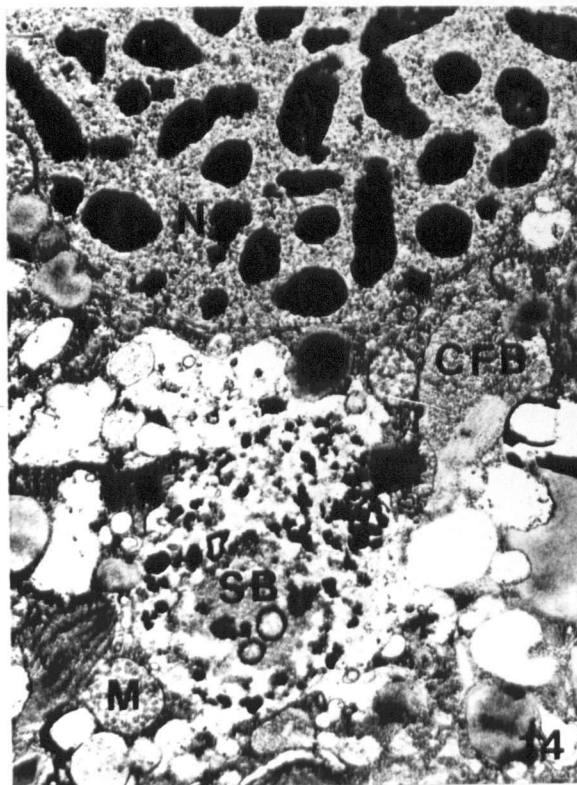
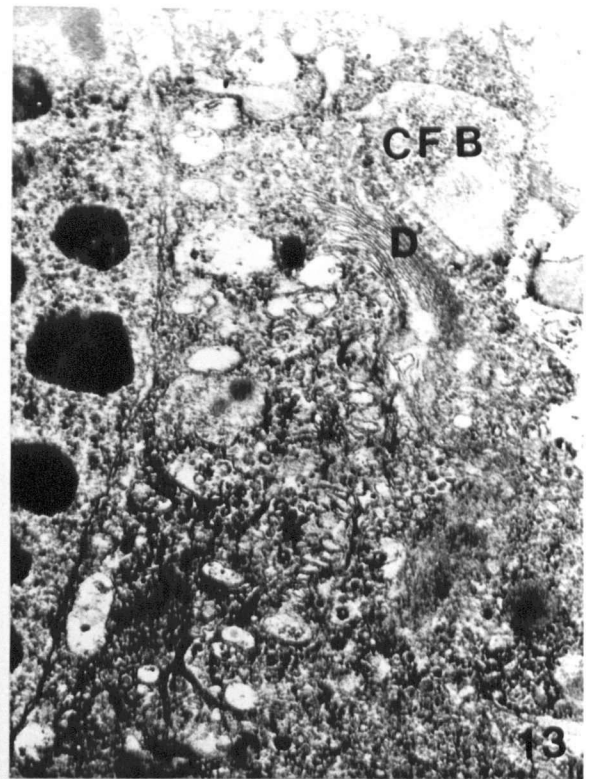


Figure 16. Trichocysts (T) in cross section appear as square, crystalline structures bounded by a single membrane (TS). The crystalline lattice of the trichocyst core is easily seen.

x 59,000

Figure 17. A trichocyst (T) can be seen dislocated from a pore (P) and lying adjacent to the cell membrane beneath the wall (W). Trichocyst fibers (TF) can be seen at the core tip. The overlapping wall plates are separated by a suture (S).

x 35,000

Figure 18. Association of trichocysts (T) with the trichocyst sac. The trichocyst (T) has fibers (TF) extending from its core to the top of its sac.

37,800

Figures 19 and 20. A continuous pore plug (PP) is present in the wall indicating that it exists as an independent structure from the trichocysts.

x 60,000

x 132,000



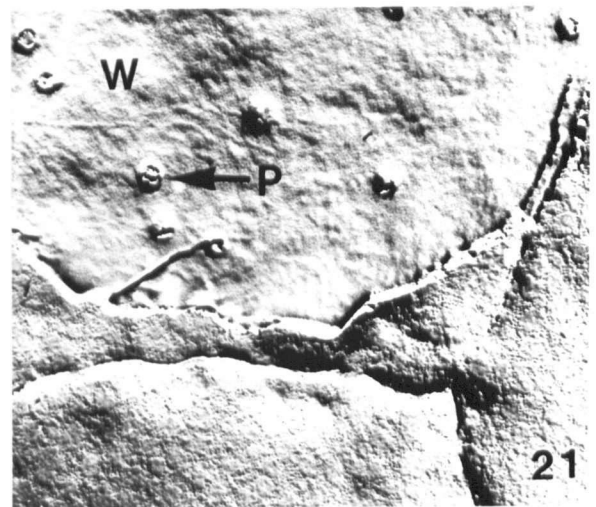
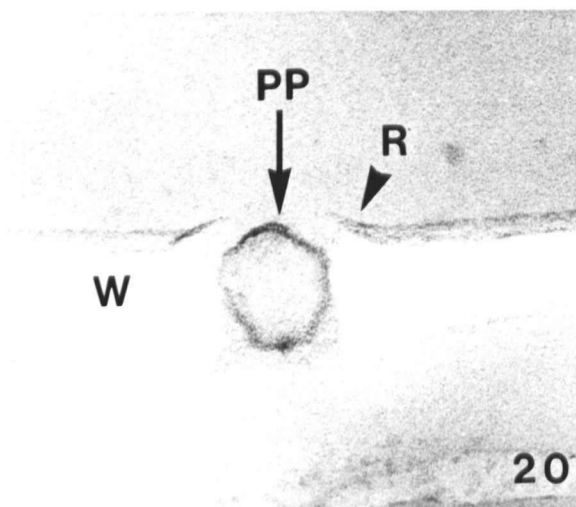
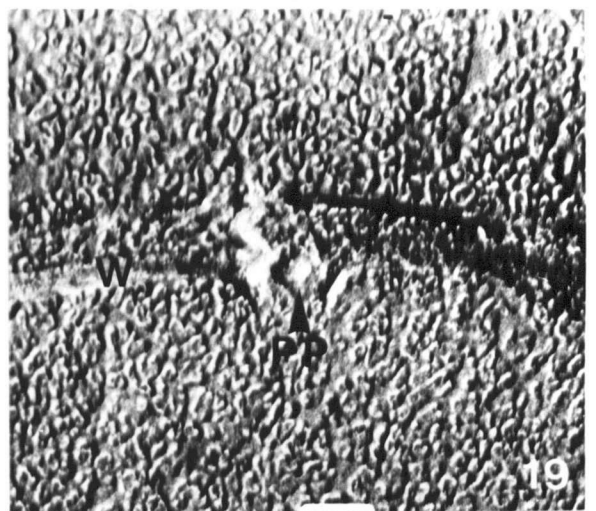
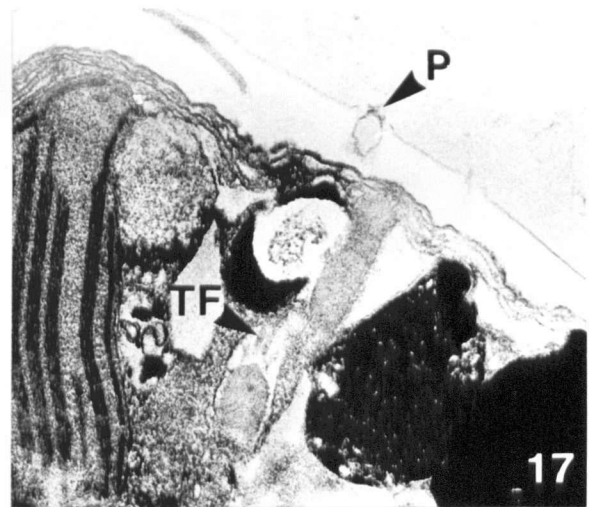
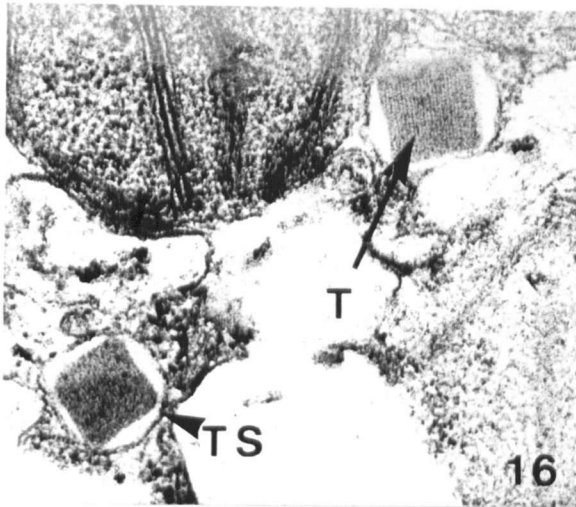


Figure 22. Thecate cell of P. trochoideum.

x 940

Figure 23. Cell undergoing ecdysis. Note relative thickness of the  
protoplast surface layer.

x 940

Figure 24. Free-swimming, elongate, naked protoplast. (Flagellar  
structures enhanced photographically).

x 940

Figure 25. Resting protoplast after loss of flagella.

x 940

Figure 26. Cell undergoing cytokinesis. Note the presence of  
wall material across the isthmus.

x 940

Figure 27. A newly formed daughter cell.

x 940



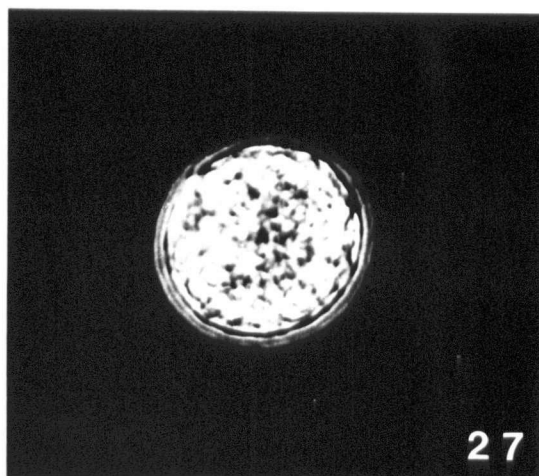
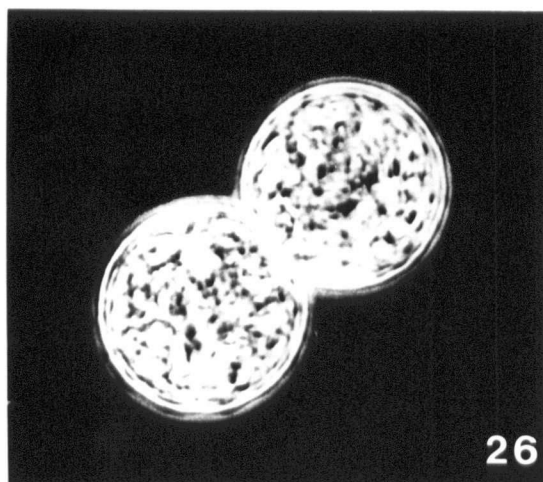
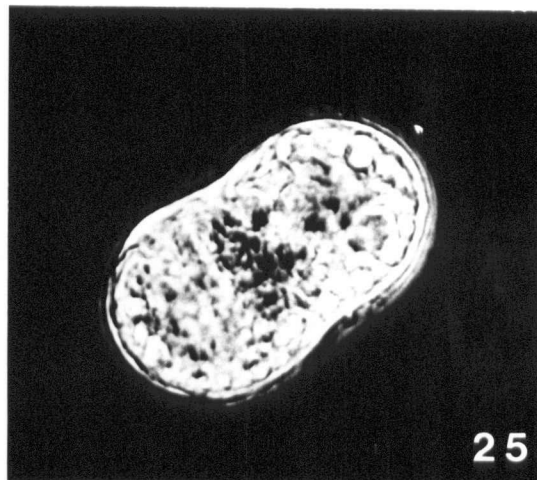
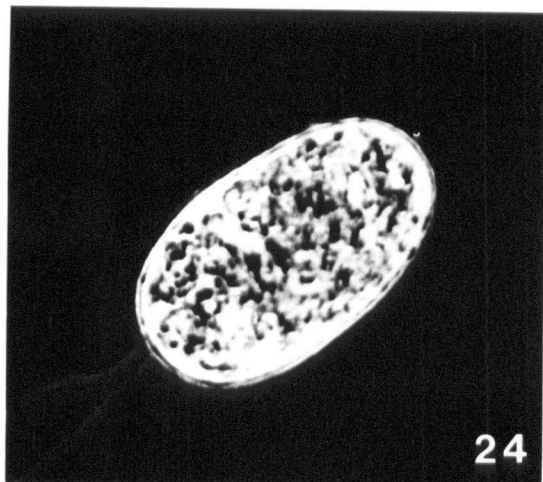
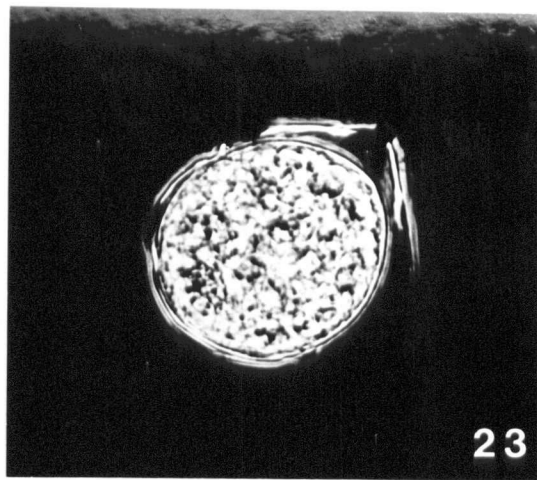
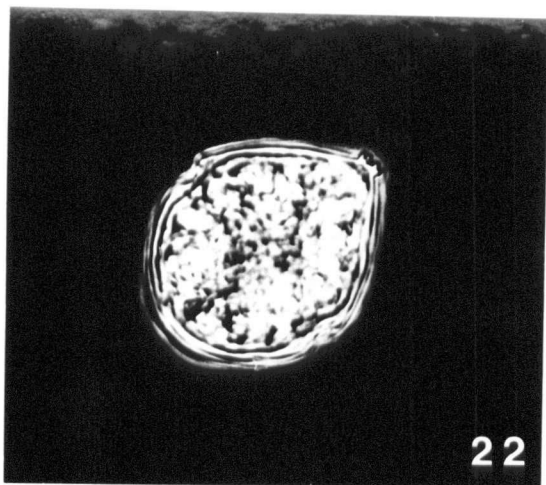


Figure 28. Ventral view of the epitheca and girdle (G) of P. trochoideum showing apical (1', 2', 4'), precingular (1'', 2'', 7''), and a girdle plate (G) demarcated by a suture ( $S_1$ ). The apical spines (H) arise from the second and forth apical plates (2' and 4') which border the apical closing plate (CP). The single flagellar pore (FP) lies in the girdle (G) immediately below the first apical plate (1').

x 12,000

Figure 29. Light micrograph (Nomarski interference) showing the ventral view of an empty theca. To the left of the flagellar pore (FP) is the first girdle suture ( $S_1$ ) and below the pore and first apical plate (1') is the sulcus (SL).

x 1,200

Figure 30. Phase contrast micrograph of an empty theca showing the absence of the apical pore in the apical closing plate (CP). The thecal plates correspond to those seen in the freeze-etched material in figure 28.

x 1,200

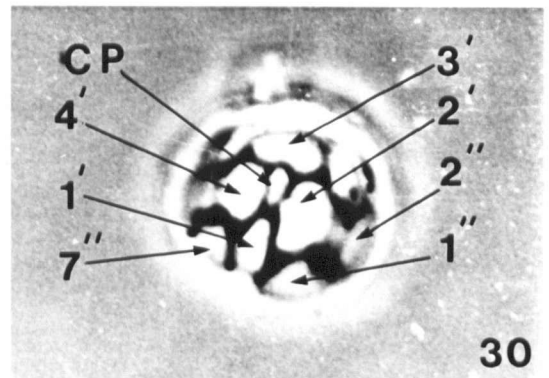
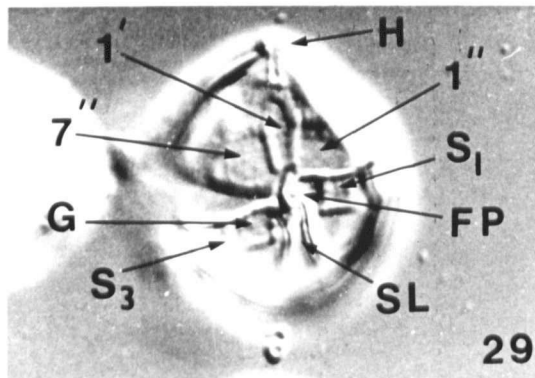
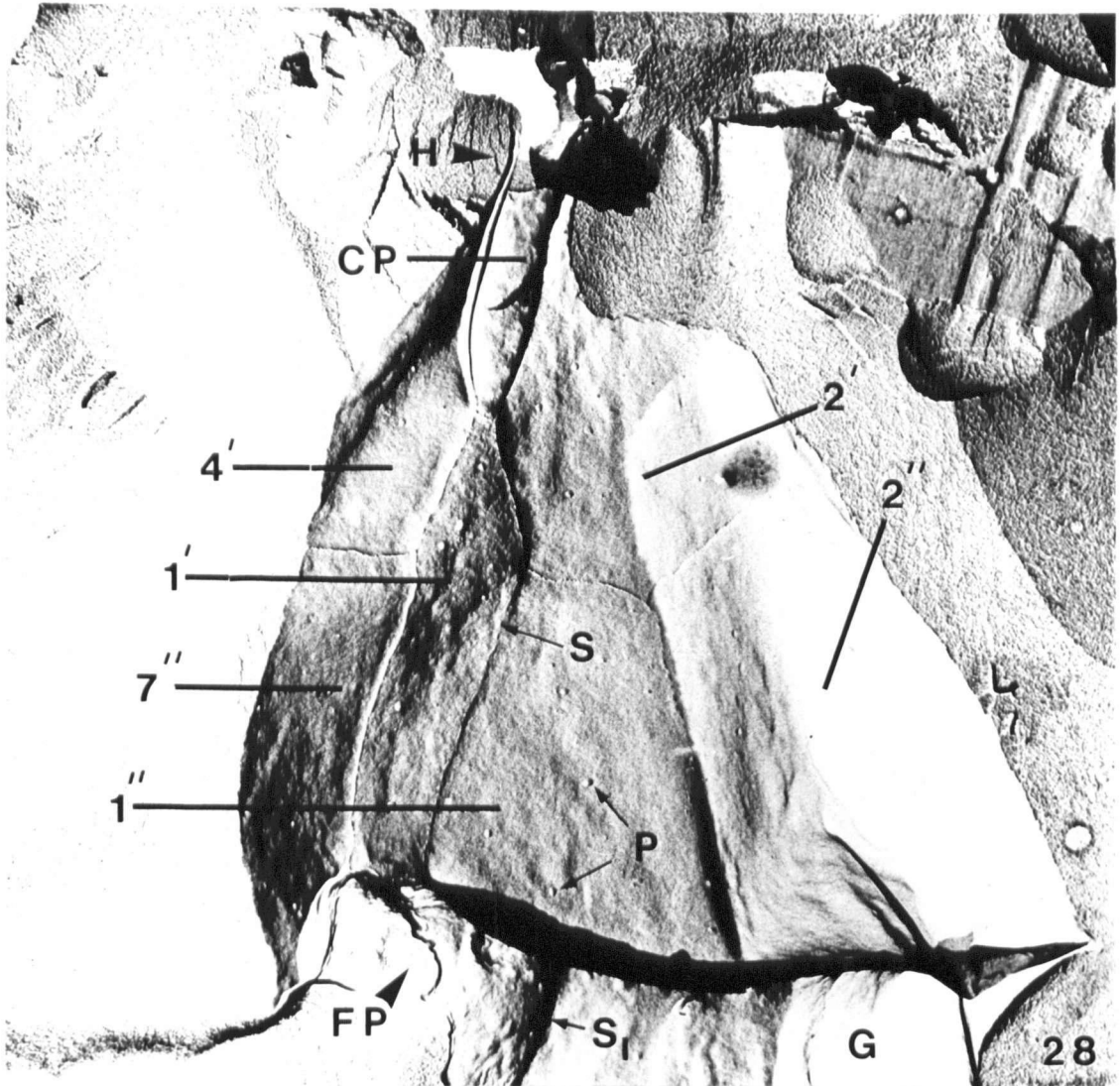


Figure 31. View of the left side of an older theca showing plates of the epitheca and hypotheca: intercalary (2a), precingular (2'', 3''), postcingular (1''', 2''', 3'''), and antapical (1'''). Pores (P), blisters (B), and folds (FL) can be seen on the thecal surface. Only sutures (S) of the epitheca and hypotheca have an accessory marginal band (MB). The folds are artifacts probably derived by centrifugation of cells. The intercalary band (IB) is fainter and wider than the marginal suture band. It lies on the opposite side of the suture than the latter.

x 9,500

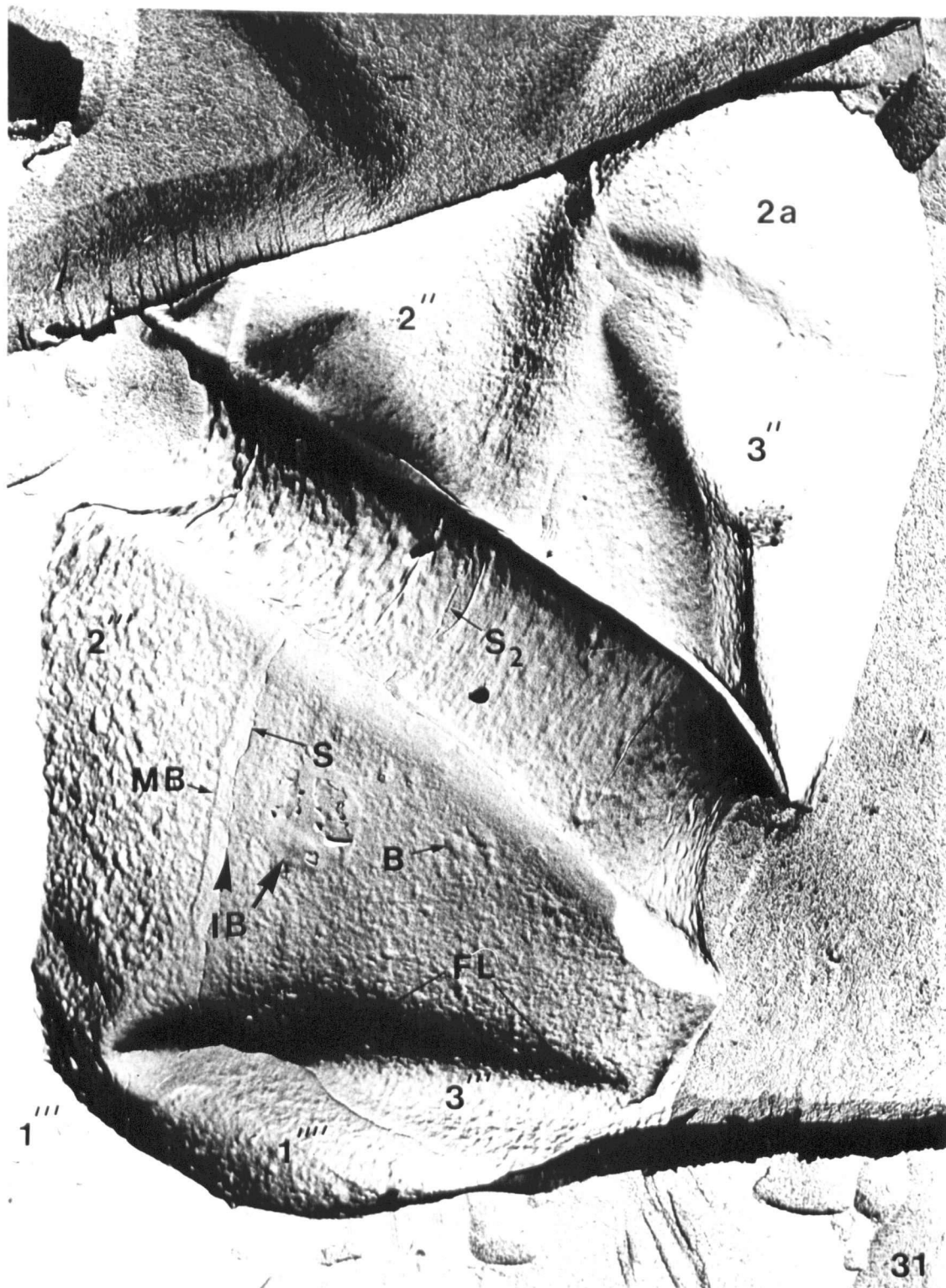
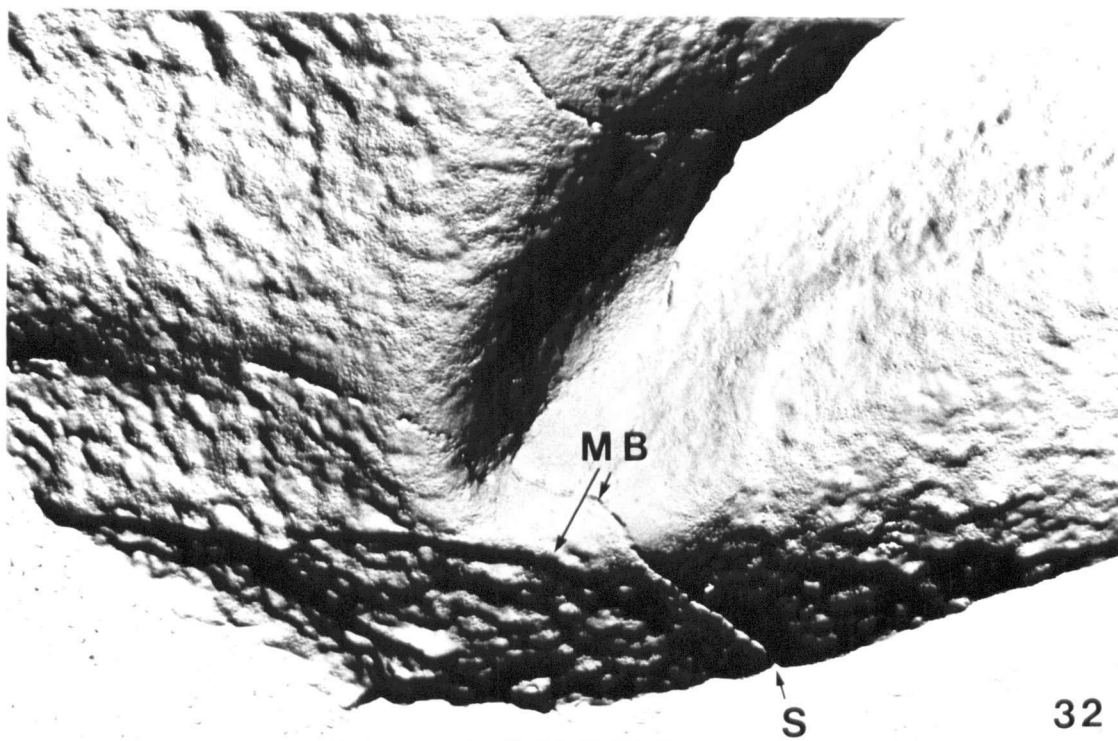


Figure 32. The rough thecal membrane surface is scored by a suture (S) which is associated with a marginal suture band (MB). Note that the suture (S) is depressed into the wall and the marginal band (MB) represents a slightly elevated part of the theca.

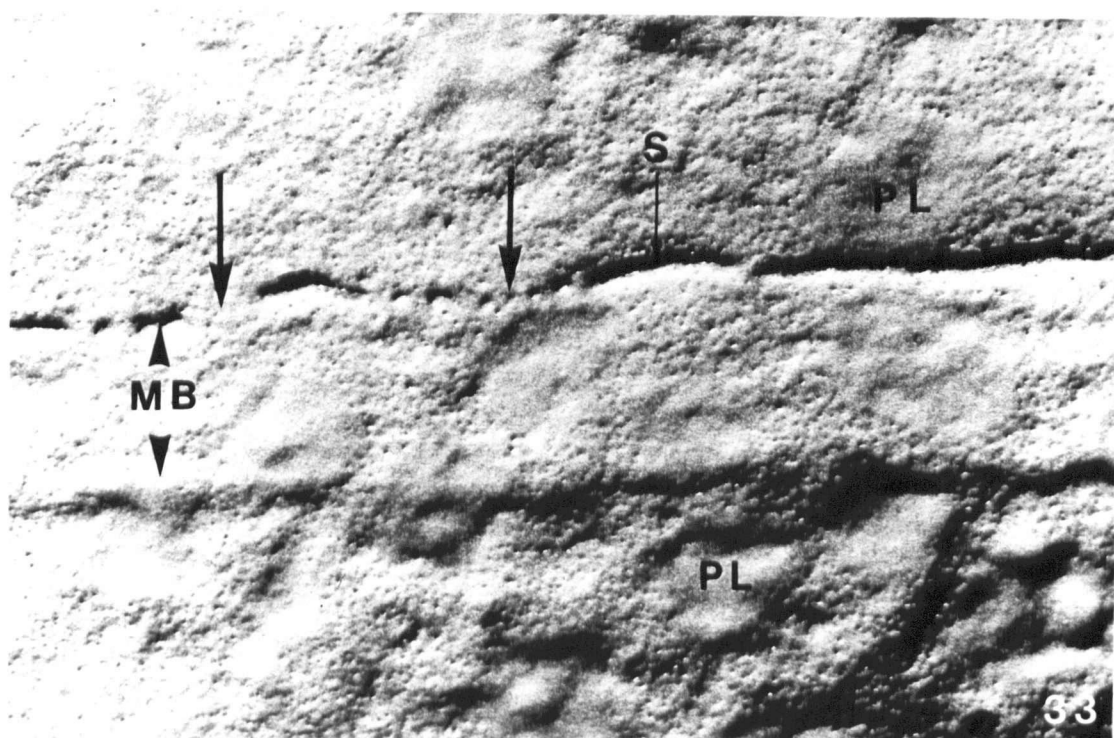
x 30,000

Figure 33. Arrows indicate points at which the sutures (S) are discontinuous. Note that the marginal band (MB), however, still maintains a continuous belt  $0.36 \mu$  in width regardless of suture inconsistency. Plaque-like areas (PL) free of pits are scattered randomly on the surface.

x 82,800



32



53

Figure 34. Portion of a mature thecal surface showing sutures (S), pores (P), circular plaques (PL), and pitting. Where the thecal membrane is continuous over the plates the sutures cannot be detected (arrows).

x 21,000

Figure 35. Portion of an old thecal surface showing the marginal suture band (MB) adjacent to the suture (S). Blistering (B) is common on older thecae.

x 14,000

Figure 36. Cross section through the marginal suture band showing the thickening of both plates along the length of the suture (S). The plate on the right bears a ridge (R). The nondescript wall (W) is covered by the outer plate membrane (OPM) and thecal membrane (TM). The inner plate membrane (IPM) lies below the wall. Note the absence of a connection between the inner plate membrane and the suture.

x 68,000

Figure 37. Cross section through the marginal suture band at a point where adjacent thecal plates are continuous.

x 68,000



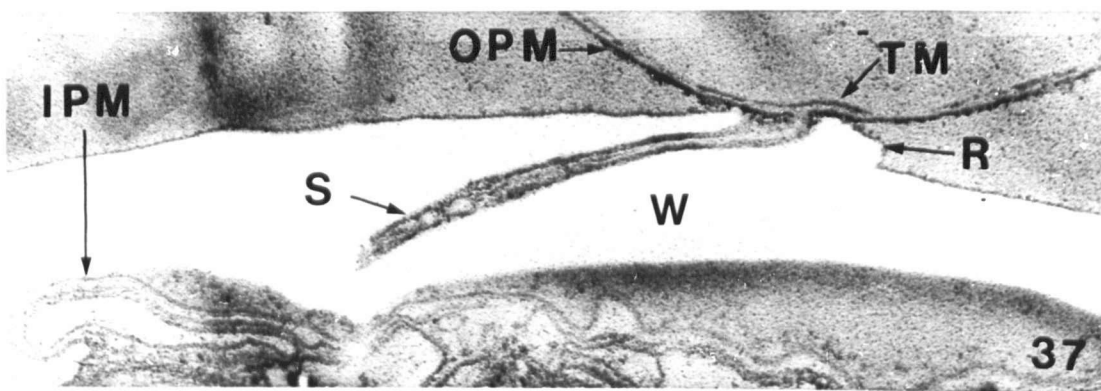
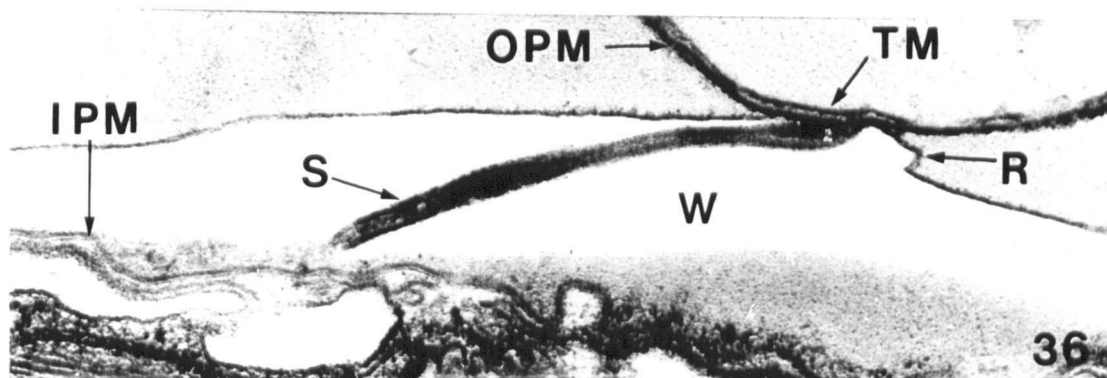
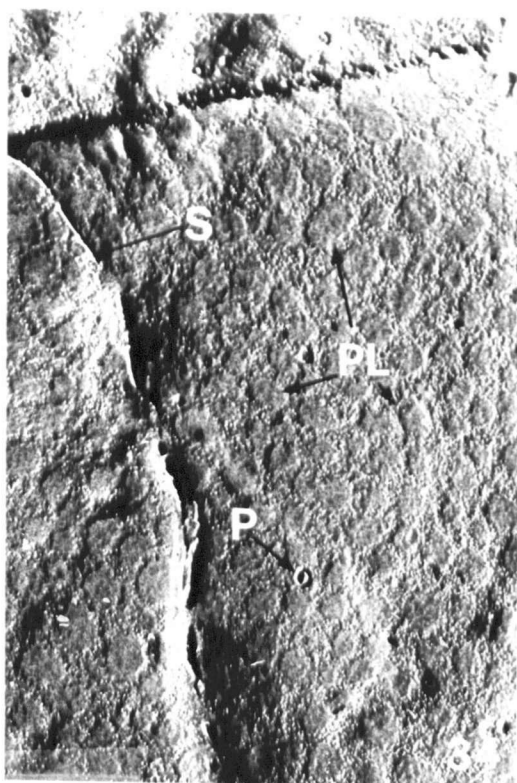


Figure 38. The outer plate membrane appears to terminate at the inner side of the overlapping plates. Although there is no connection between the suture (OPM) and the inner plate membrane (IPM), dense material is located between the two systems (arrow). The plasmalemma (PM) contains a narrow parietal cytoplasmic band which results from vacuolation (V) at or near the cell periphery.

x 66,000

Figure 39. The suture (S) appears to be attached to the inner plate membrane (IPM) by means of a foot (arrow). Note that only the terminal portion of the foot appears to be attached.

x 40,200

Figure 40. The thicker partition of the continuous asymmetric inner plate membrane (IPM) lies adjacent to the thecal plates. Note that there is no disruption of the inner plate membrane along its length. One would expect such a membrane to be disrupted at the point where a suture might be attached.

x 110,000

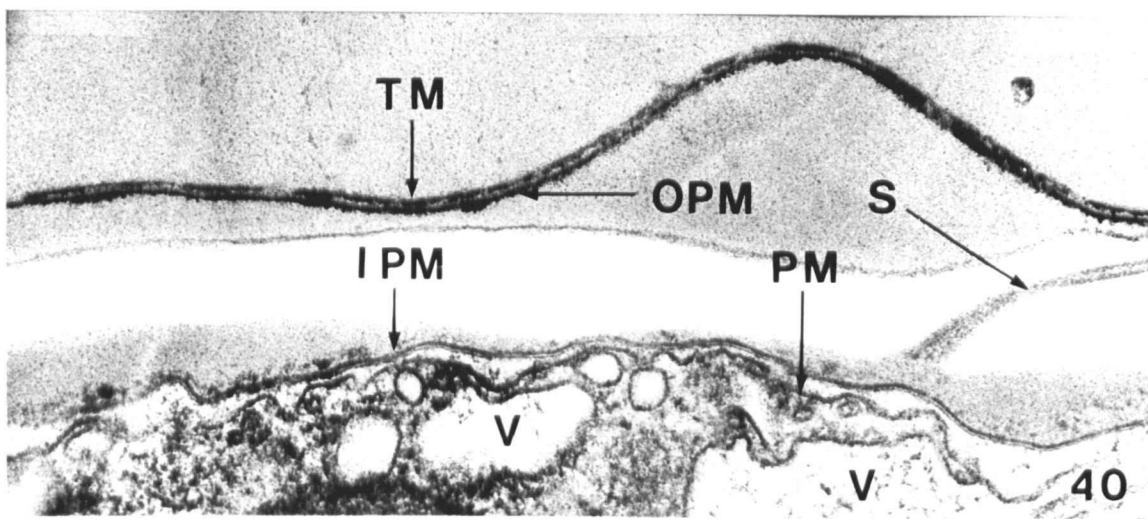
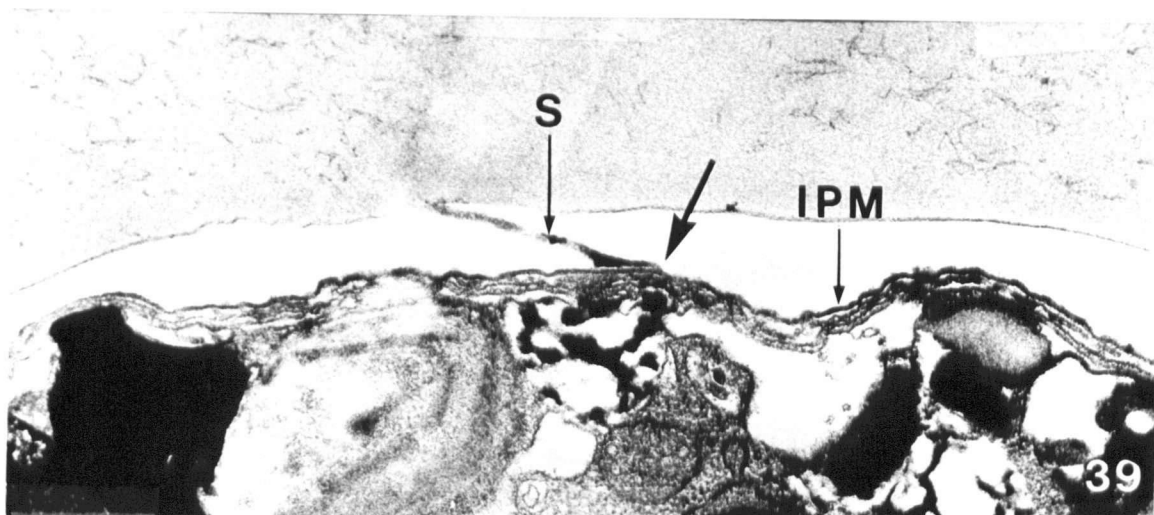
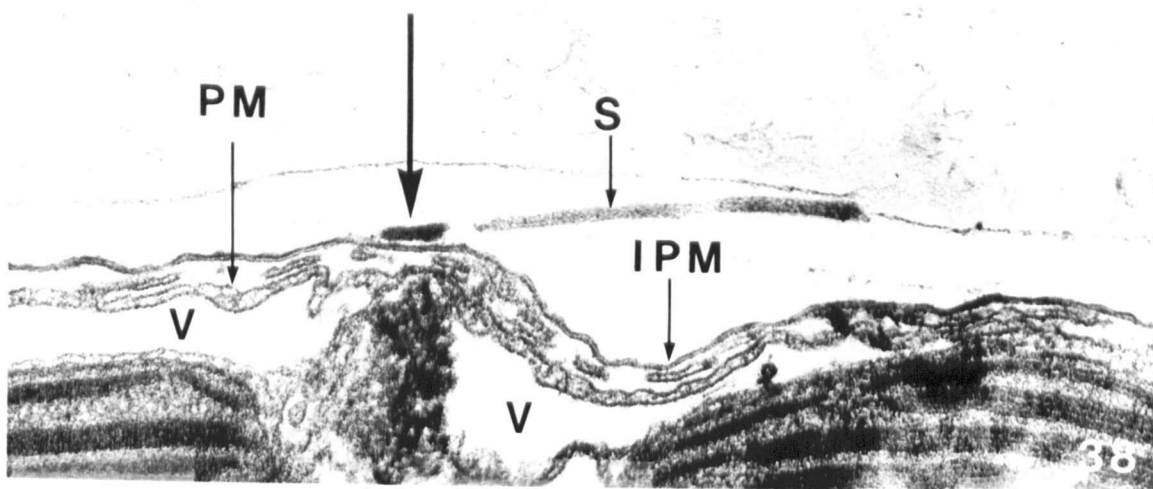


Figure 41. Thin section through a complex junction of four thecal plates.

The asymmetric thecal (TM) and plate membranes (OPM) are about 150 $\text{\AA}$  thick having partitions of 75/50/25 $\text{\AA}$ . The sutures (S) appear pentapartite as a result of the apposition of two outer plate membranes. The thicker partition of the outer plate membrane always lies adjacent to the plate (W).

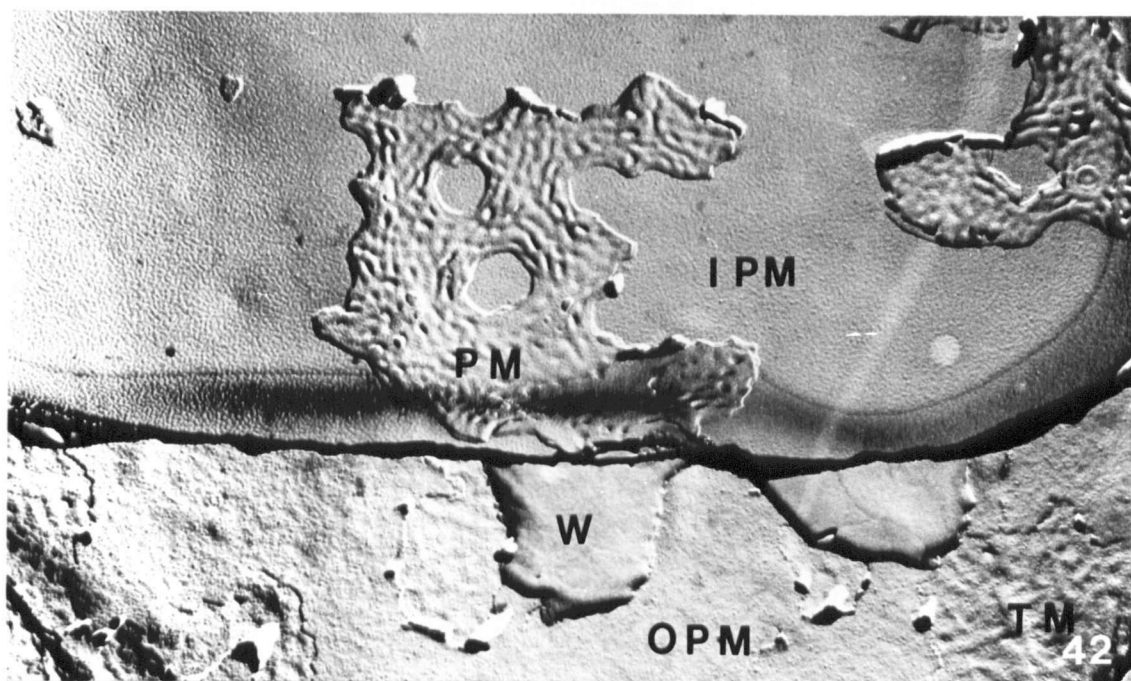
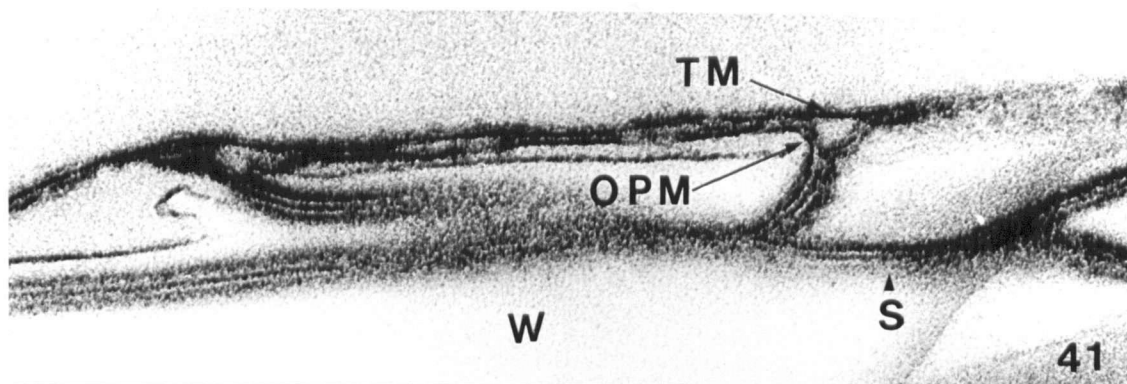
x 144,000

Figure 42. Fractured surface showing the thecal plates (W) and the four associated membranes. The thecal membrane (TM) and outer plate membrane (OPM) are pitted; the inner plate membrane (IPM) slightly reticulate, and the plasmalemma (PM) appears undulated.

x 14,200

Figure 43. The plasmalemma (PM) and inner plate membrane (IPM) appear to be continuous over the protoplast and independent of the overlying suture (S).

x 23,200



Figures 44 and 45.      Continuity of the inner plate membrane (IPM) over the protoplast. The outer surfaces of the inner plate membranes bear scars (SC) which may represent sites of previous association with sutures = outer plate membrane.

x 15,360

x 18,600

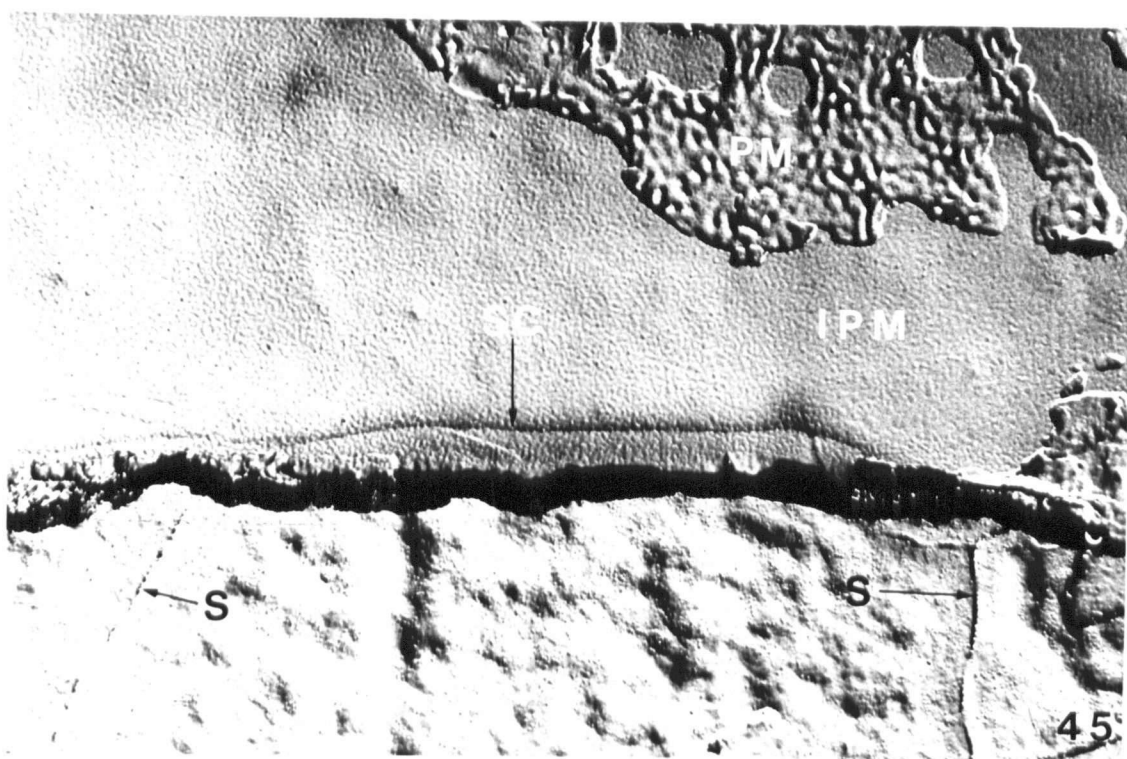
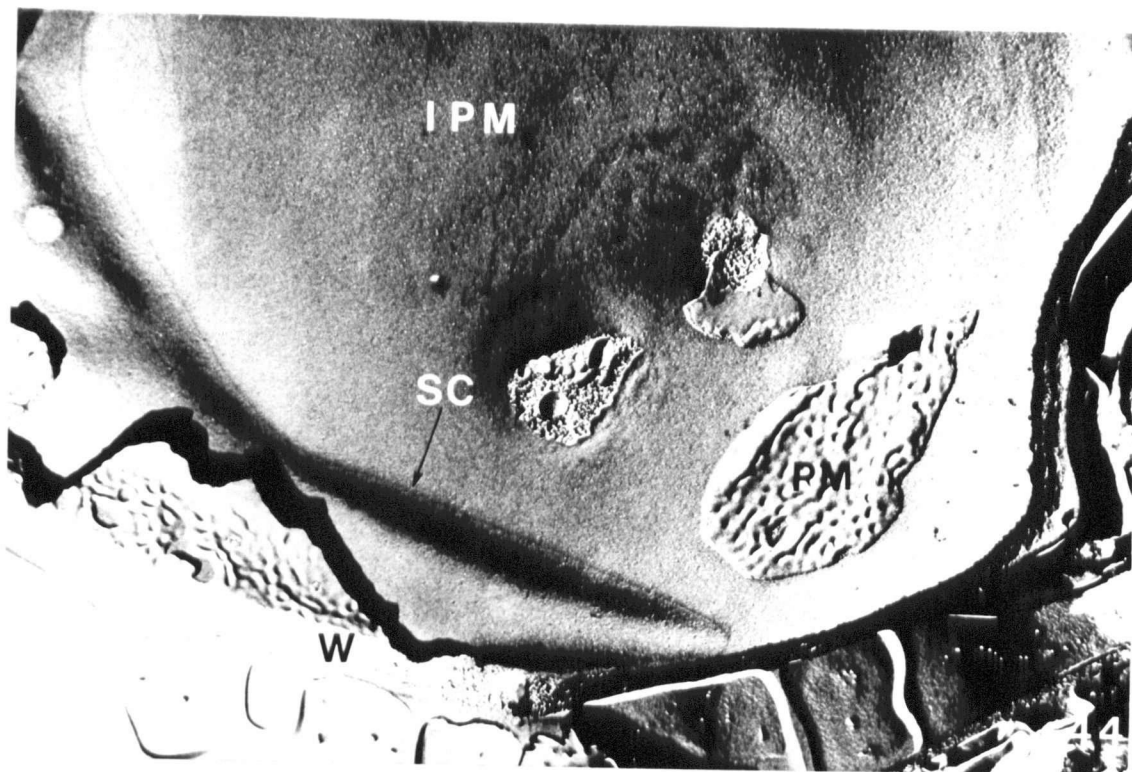


Figure 46.      Densely staining prothecal bodies (PB) as they appear in non-dividing mature cells.

x 12,000

Figure 47.      An older cell of P. trochoideum. The prothecal body (PB) is composed of a membranous component (MC) and an amorphous component (AC). Prothecal vesicles (PV) can be seen forming at the left.

x 22,200

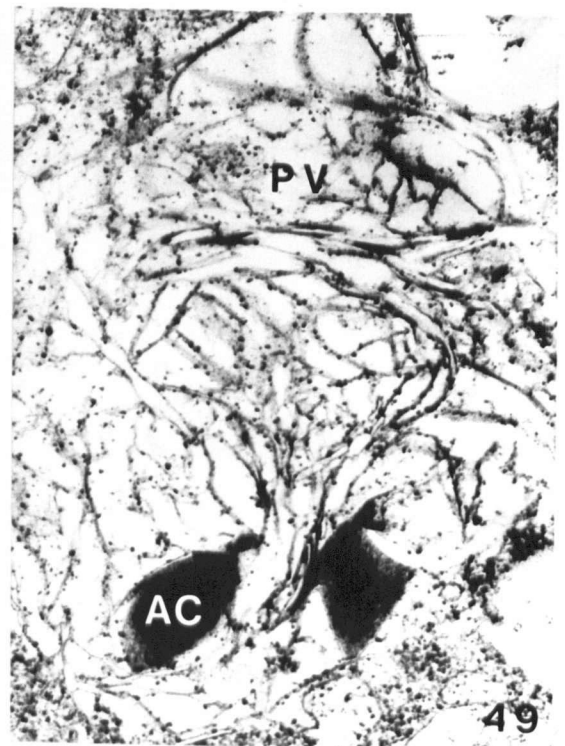
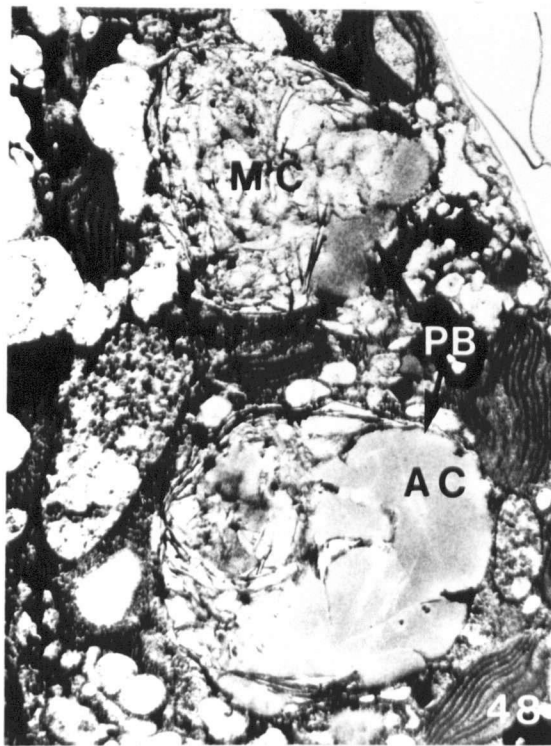
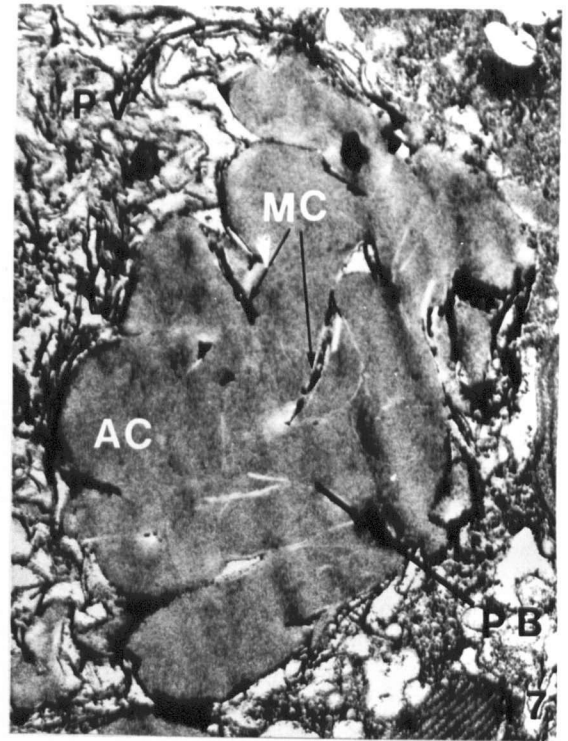
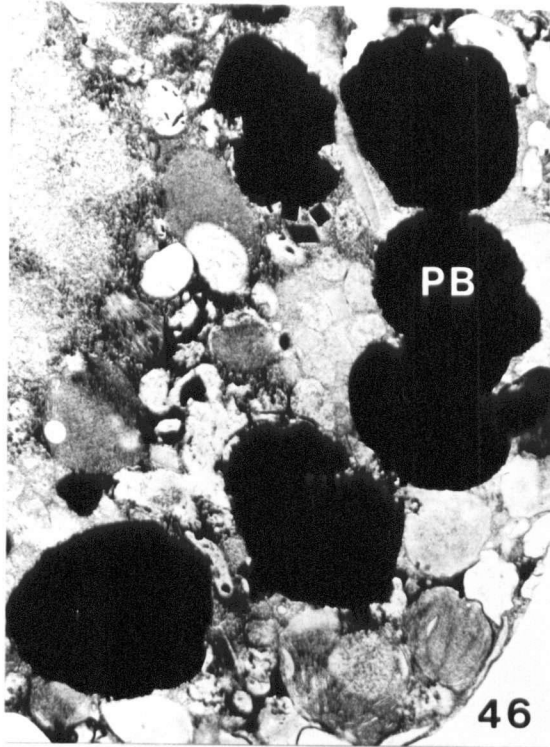
Figure 48.      Moderately staining prothecal bodies (PB) as they appear prior to ecdysis. The prothecal body at the top has progressed to a further state of differentiation than the one below. Note the difference in relative amounts of amorphous (AC) and membrane components (MC).

x 13,500

Figure 49.      Flattened prothecal vesicles (PV) from a prothecal body in which the amorphous component (AC) has substantially diminished.

x 15,000





Figures 50 and 51. Progressive stages showing the accumulation of wall material between the plasmalemma (PM) and the inner plate membrane (IPM).

x 47,500

x 25,000

Figure 52. Incorporation of prothecal vesicles (PV) into the wall. The fibrillar material in the vesicles and the membrane of the vesicles account for the moderate staining characteristics of the wall.

x 15,600

Figure 53. Fibrous nature of the newly formed cell wall (W). Prothecal vesicles (PV) can be seen entrapped within the wall.

x 25,000

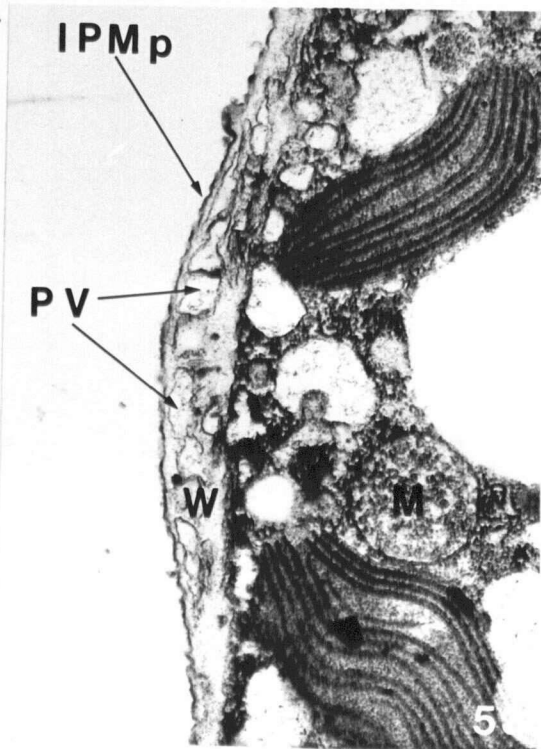
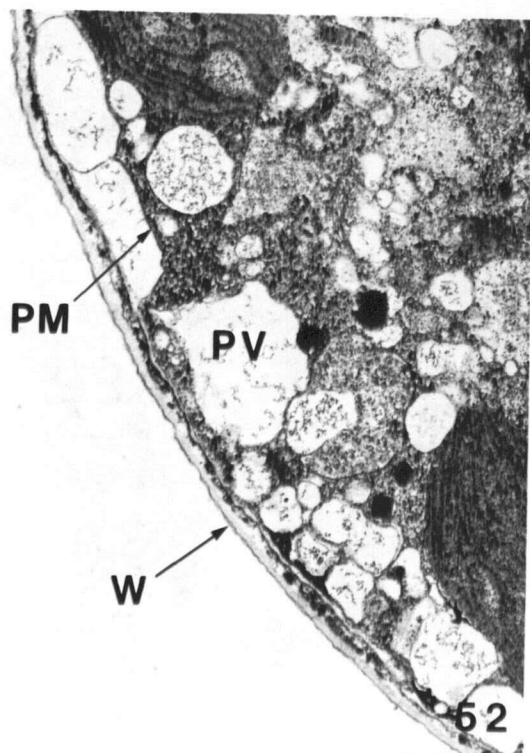
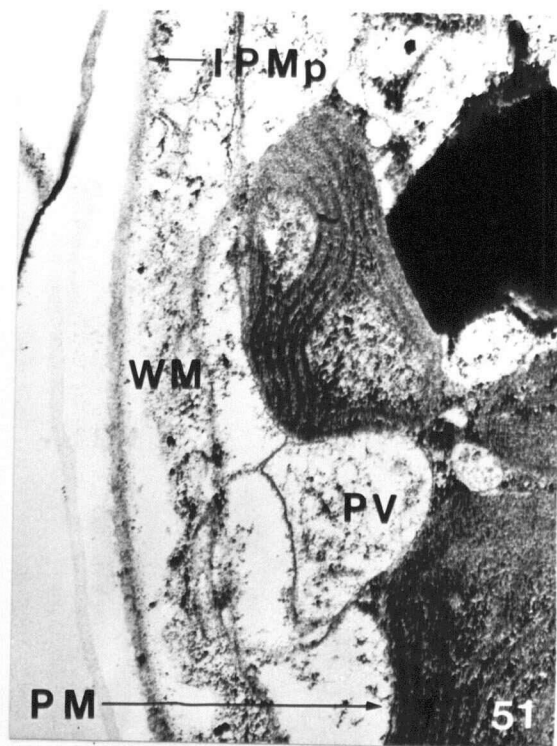
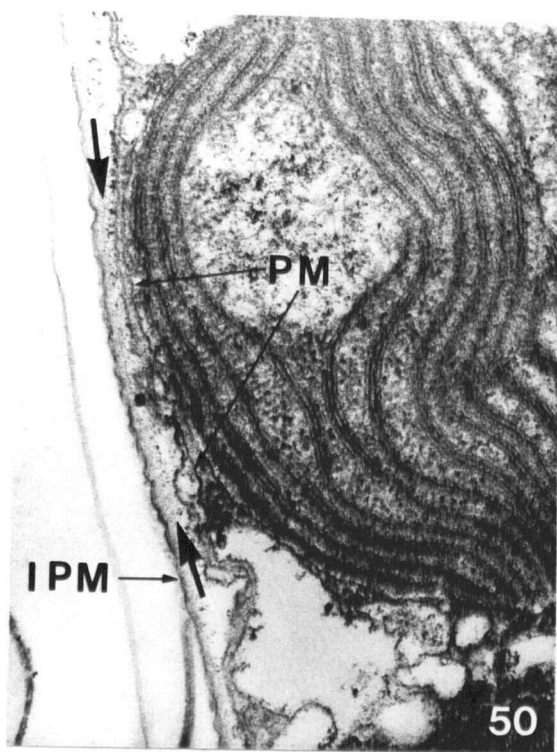


Figure 54. Nomarski interference micrograph of a dividing cell.

Note the accumulation of wall material (WM) across the cleavage furrow (arrow).

x 1,500

Figure 55. Phase contrast micrograph of a plasmolysed dividing

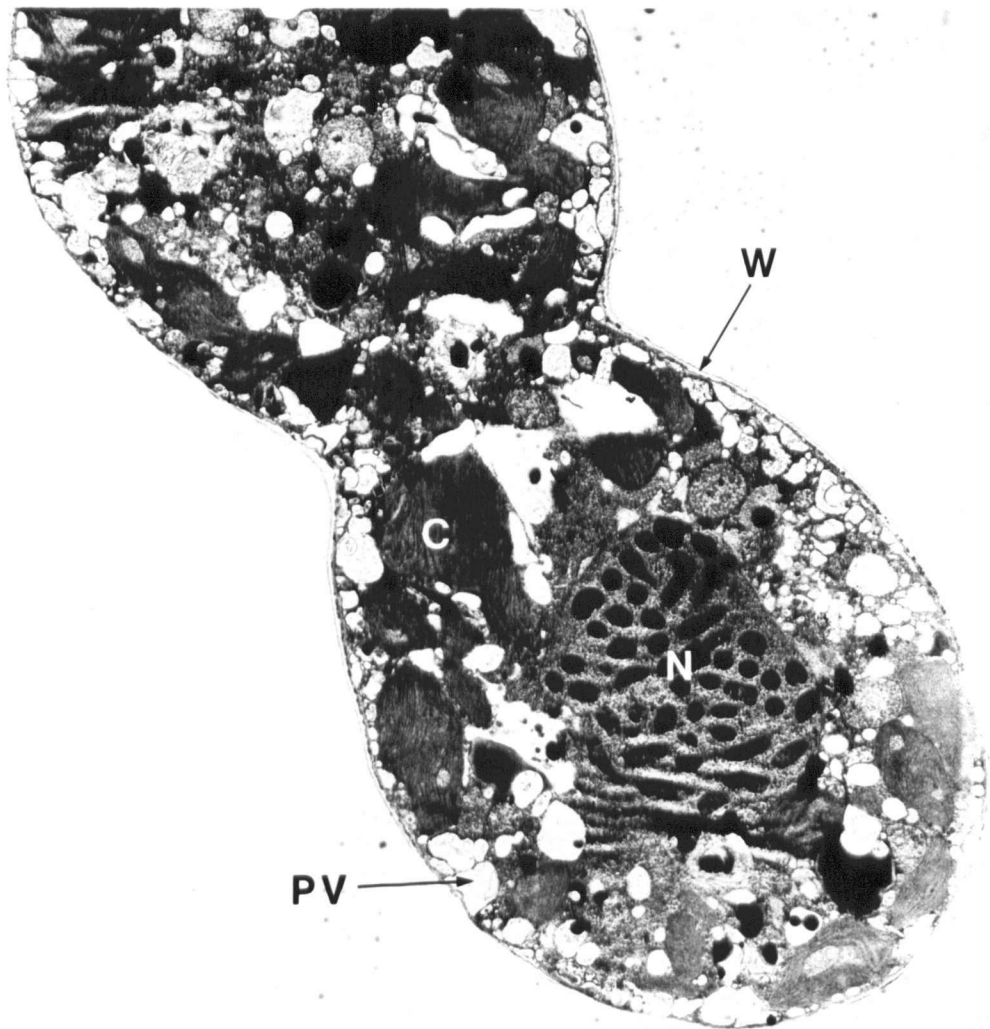
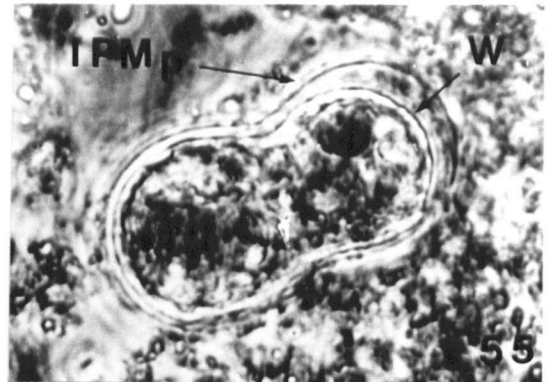
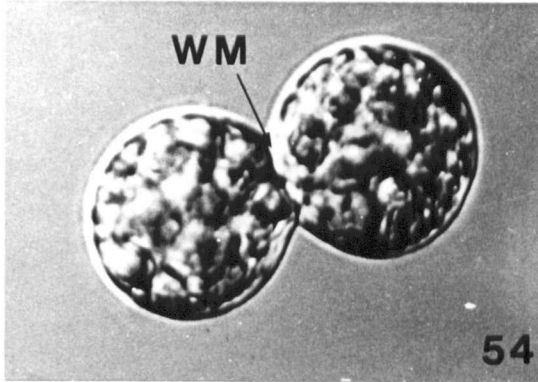
cell. Note the separation of the wall (W) from the overlying inner plate membrane of the parent cell (IPMp).

x 1,100

Figure 56. Electron micrograph of a dividing cell similar to that

stage shown in figure 54. The cell has a relatively thick wall (W). Note the prothecal vesicles (PV) lining the protoplast periphery.

x 5,600



56

Figure 57. Formation of prothecal vesicles (PV) from a prothecal body (PB) at the isthmus of a dividing cell. One of the vesicles is being released (arrow).

x 39,600

Figure 58. Isthmus of a cell in late cell division. The prothecal vesicles have aligned in two rows across the isthmus (arrows). These rows represent the sites where the two new cell walls of the daughter cells will be formed prior to complete separation.

x 25,800

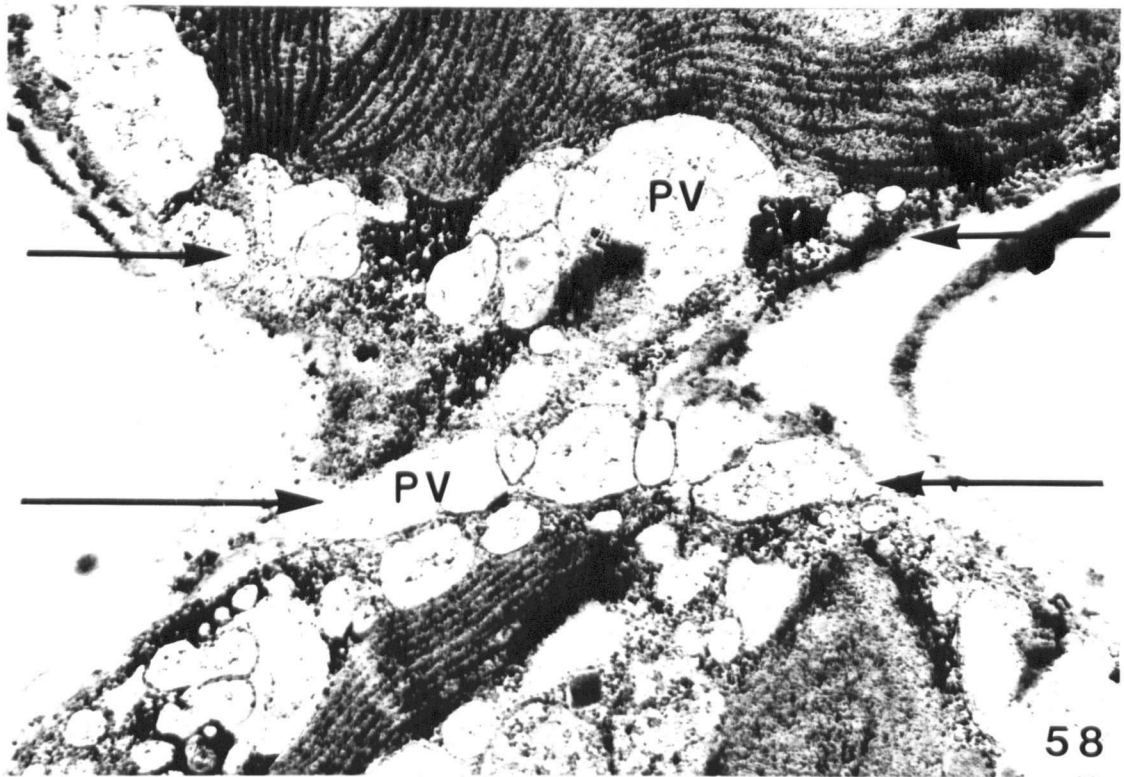
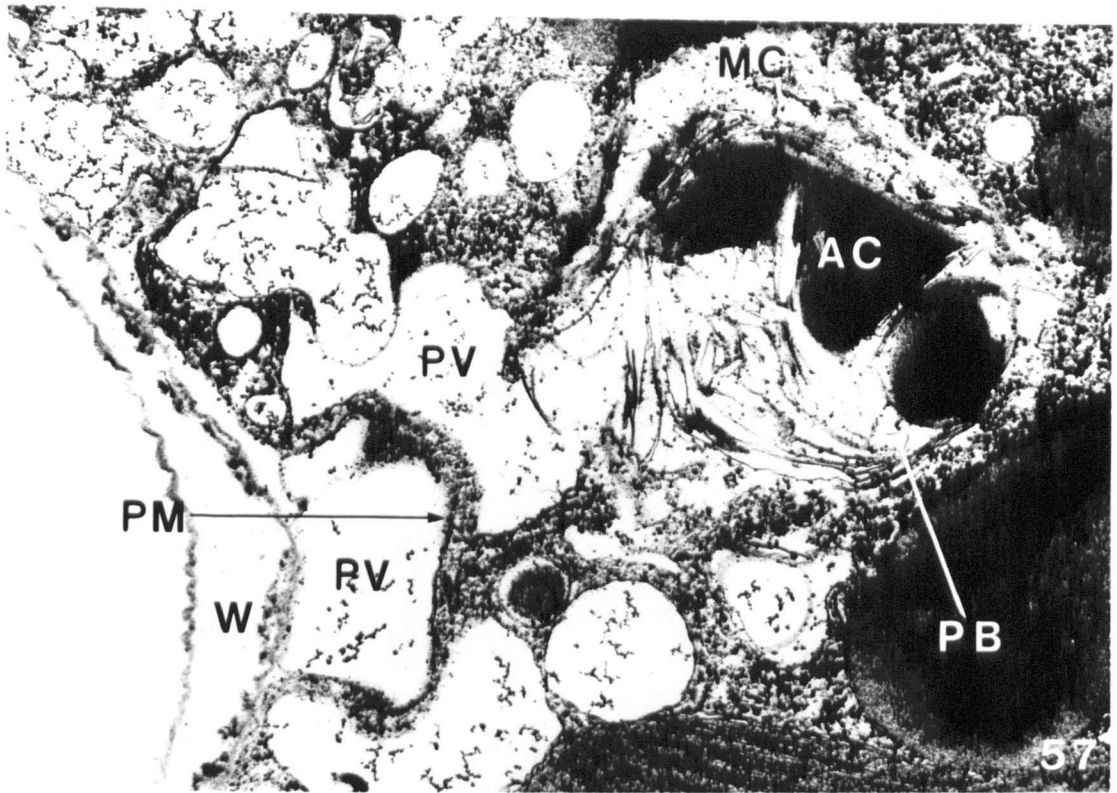


Figure 59. Small link between daughter cells. Note the definite layer of wall material present at this site (W). Below the lighter wall layer (W) lies a denser layer (DL) which appears to be sandwiched between the wall and cytoplasm.

x 57,000

Figure 60. Presence of a new inner plate membrane (IPM). The membrane appears to have arisen within the dense layer (DL) beneath the wall. Note membrane fragments (arrow) within the wall which may be involved in membrane formation. The parent cell inner plate membrane (IPMp) is still present.

x 104,500



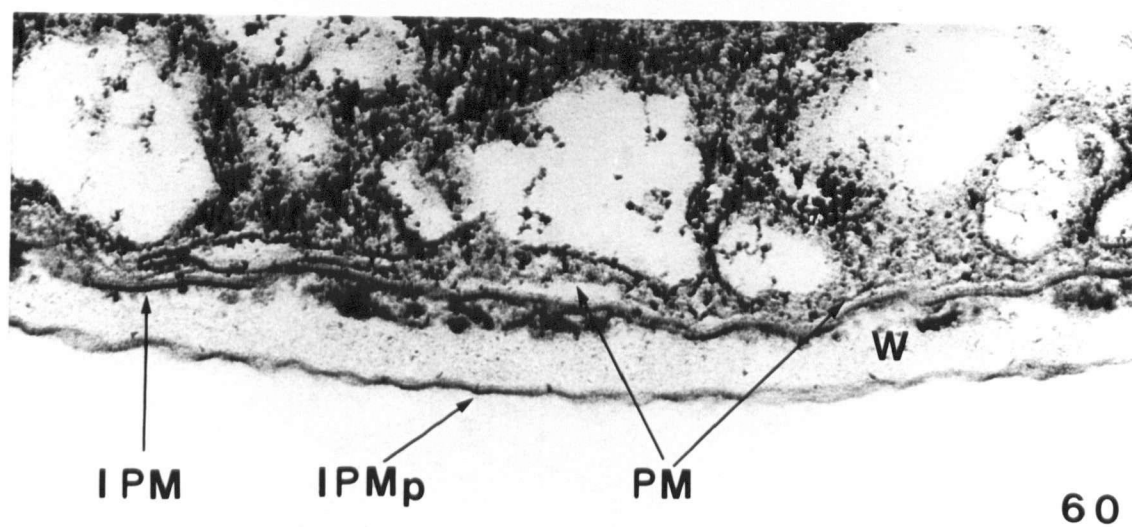
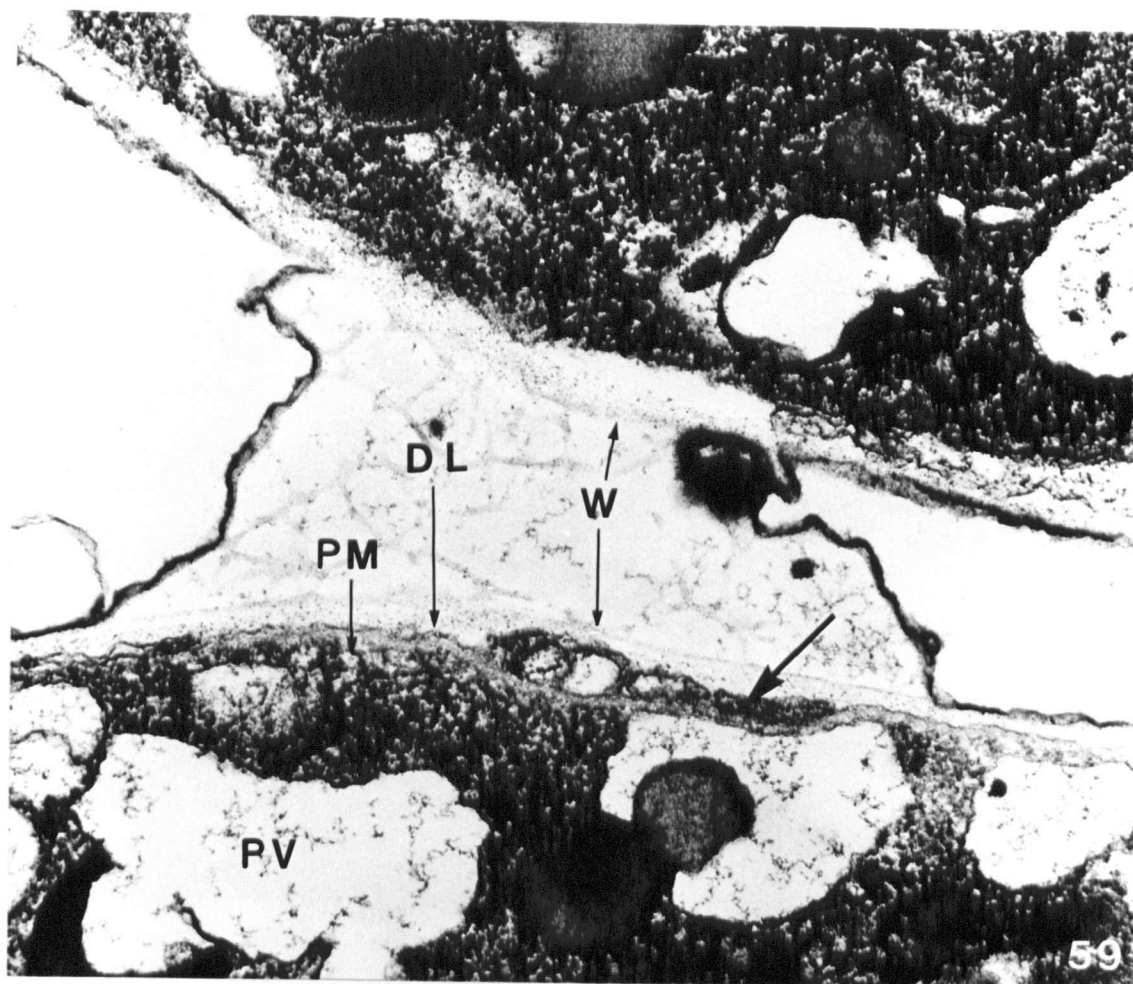


Figure 61. Two daughter cells nearly separated. The wall material appears to cover the entire protoplast without interruption - that is, there are no plates or sutures present at this time.

x 6,190

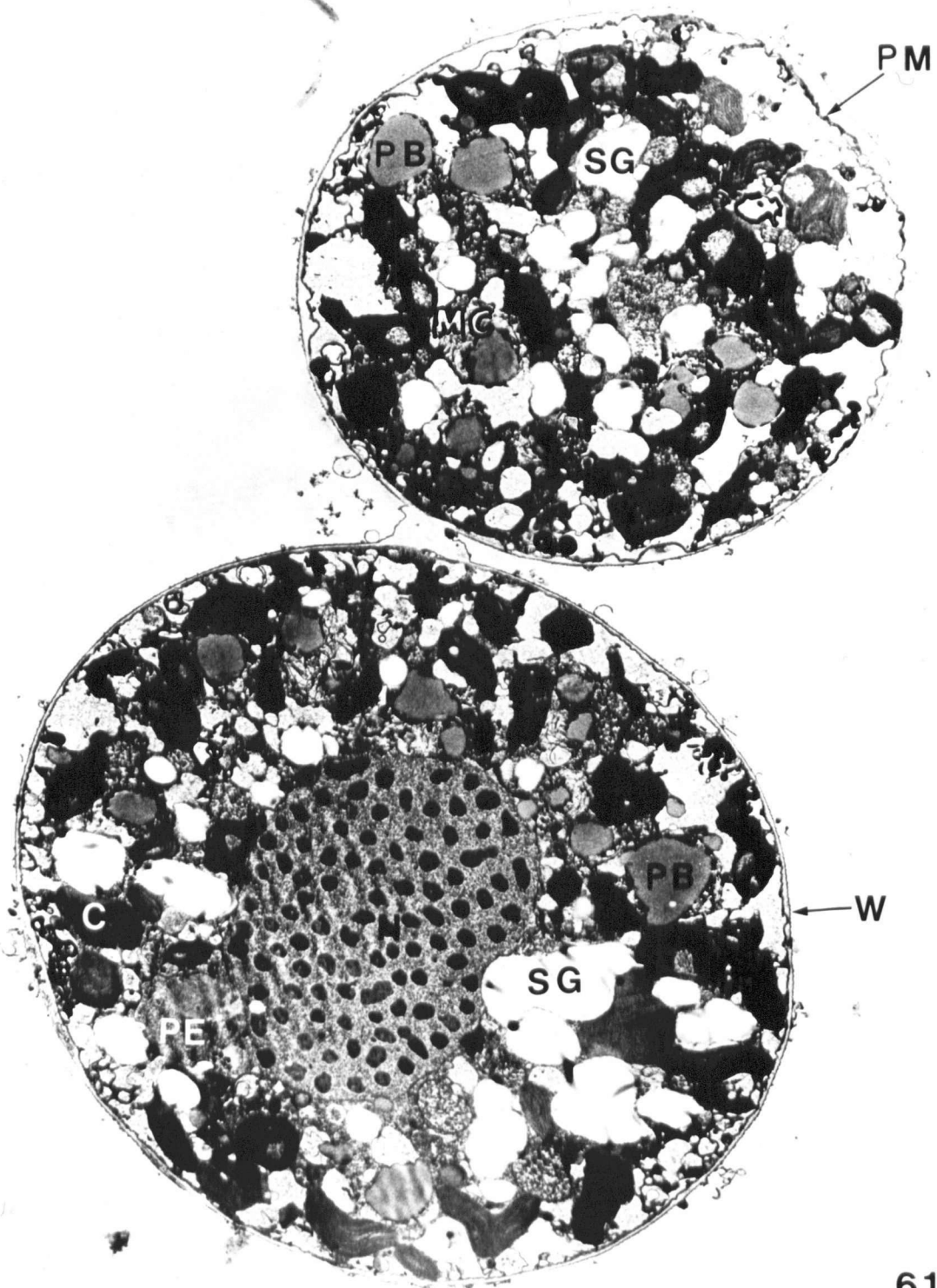
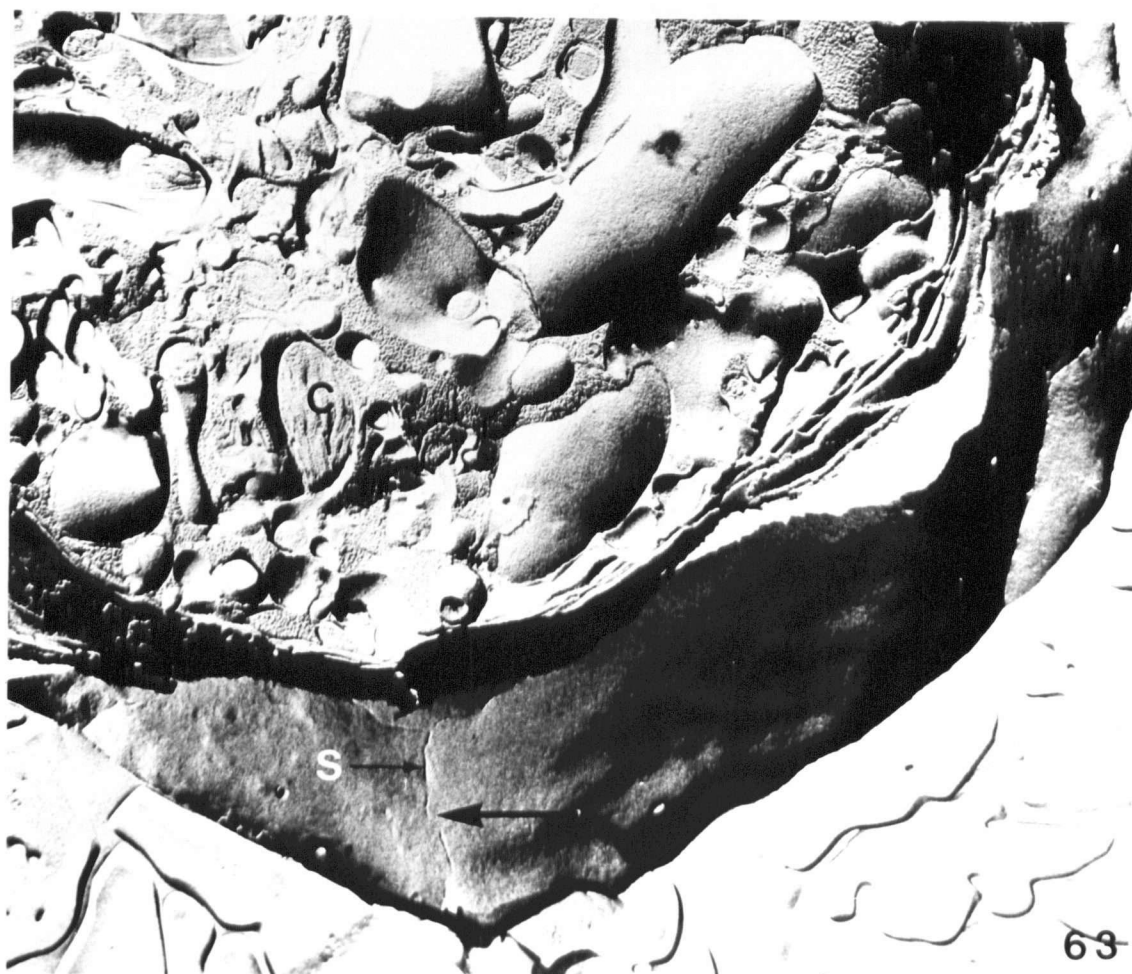
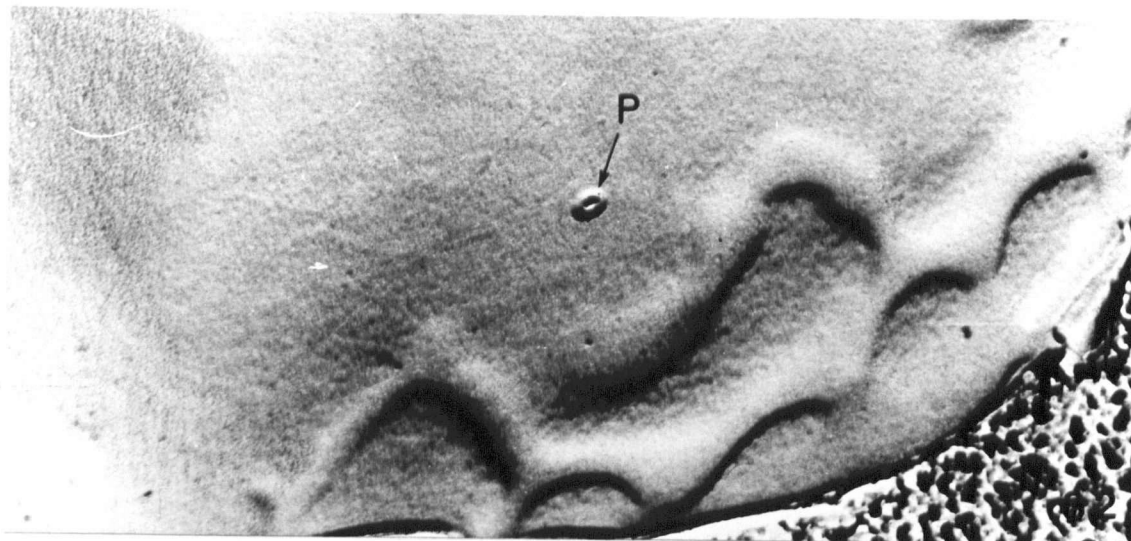


Figure 62. An undifferentiated theca which lacks fully delineated plates. The cell surface is relatively free of pits. The folds probably arose during centrifugation of the cells. Note the presence of the fully developed pore (P) with its ridge.

x 25,000

Figure 63. Initial stages in the differentiation of the sutures. The sutures (S) are still not deeply scored into the wall and are not continuous (arrows).

x 15,000



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## APPENDIX

CHIHARA MARINE MEDIUM

To 1000 cc. filtered\* sea water add:

materials	quantity
minor elements (see below)	2 cc.
$\text{NaNO}_3$	0.200 g.
$\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$	0.025 g.

## Minor Elements Solution

To 1000 cc. distilled water add:

materials	quantity
EDTA - $\text{Na}_2$	3.0000 g.
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.0800 g.
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.1200 g.
$\text{ZnCl}_2$	0.0150 g.
$\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$	0.0030 g.
$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	0.0012 g.
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.0500 g.
$\text{H}_3\text{BO}_3$	0.6000 g.

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\* millipore filter; pore diameter 0.22 $\mu$ .