Intercellular Attachment in the Epithelium of *Hydra* As Revealed by Electron Microscopy*

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ABSTRACT

In *Hydra* adjacent epithelial cells are bound firmly to each other by desmosomes of a type not described in detail hitherto. The most prominent feature of these desmosomes is the presence of a series of parallel lamellae which bridge the intercellular space and connect the two apposed cell surfaces directly. These structures, here termed intercellular attachment lamellae, display two peaks of density about 50 A apart. These dense lines appear in some instances to be continuous with the outer dense components of the plasma unit membranes of the attached cells.

The presence of prominent lamellae in intercellular attachments is sufficiently distinctive to deserve special terminology; accordingly, the term *septate desmosome* is proposed. It is noted that *septate desmosomes* may have been seen in other animals in instances where published electron micrographs show cross-striations or prominent connections in regions of intercellular attachment.

It is suggested that *septate desmosomes* in *Hydra*, in addition to binding cells firmly to each other, form barriers to the movement of water into intercellular spaces and thus help to protect the organism's internal environment.

Observations on the use of phosphotungstic acid for improving contrast in materials embedded in epoxy resins are also recorded.

INTRODUCTION

The manner in which cells in multicellular organisms are attached to one another permits mobility while maintaining relative cell positions. The structural nature of the cell surfaces and interposing substances which make such a relationship possible are poorly understood. Light microscope studies prior to 1930 suggested that in epithelia restricted regions of firm cellular adhesion provide the necessary topographical stability, permitting much of the cell surfaces to slide against adjacent cells relatively easily (review by Schaffer, 1927). Recent studies with the electron microscope have revealed at many cell surfaces localized specializations which have been interpreted as regions of firm cellular attachment (Porter, 1956; Sjöstrand, 1954; Selby, 1955; Horstmann and Knoop, 1958; Fawcett, 1958; Odland, 1958). For many tissues this concept supersedes the older idea of a homogeneous intercellular cementing substance binding cells together over their entire area of mutual contact (see von Recklinghausen, 1862).

It is notable that specialized cellular attachments have been described in detail to date only in vertebrate tissues. The purpose of this communication is to report the presence in *Hydra* of intercellular attachments which differ in fine structure from the types previously recognized.

The fine structure of *Hydra* attachments is set forth in detail and is compared with intercellular attachments described from other tissues. Attempts are made to interpret this special morphology in terms of function.

Before proceeding to the descriptive portion of this paper a brief review of the history of our knowledge

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of intercellular attachments seems fitting. This review may serve to clarify concepts of cellular relationships which have led to a confused terminology in the current literature.

Theodore Schwann, in his classical presentation of the cell theory (1839), recognized the possibility that processes of adjacent animal cells might fuse to establish cytoplasmic bridges. The histological literature is filled with subsequent reports of so-called *"cellular bridges"* occurring in a wide variety of tissues. The studies of Ogneff, 1892; Muscatello, 1895; Kolossow, 1892; Paladino, 1890, and Kultschitsky, 1887 may be cited as examples. This field was capably reviewed by Carlier in 1895 and by Studnička in 1929. The first suggestion that there might be local structural specializations of a presumed general intercellular cementing substance came from Schrön's investigations on epidermis presented in 1863 (published, 1865). This notion was confirmed independently by Schultze (1864) and Bizzozero (1864) one year later. In 1870 Bizzozero made the classic observation that although the prickles of adjacent epidermal cells were apposed end-to-end to form bridge-like structures, the cytoplasm of the two cells was not continuous in these regions. In addition he recognized a densely staining node at the junction of the two cytoplasmic extensions. He interpreted such nodes as representing adhesive plates to which each cell contributed. Dense nodes of a similar appearance subsequently were found on *"cellular bridges"* seen in other tissues (Studnička, 1897, 1929). In 1920 Schaffer applied the term *desmosome* to these nodes. In 1892 Heidenhain noted bands of densely staining material between the lateral surfaces of columnar epithelial cells near their free luminal border. These dense bands were later termed *Schlussleisten* or *terminal bars* by Bonnet (1895). Schaffer (1927) noted the structural similarity of epidermal desmosomes and terminal bars and postulated the formation of terminal bars from a linear arrangement of desmosomes. Schaffer was also of the opinion that many so called *"cellular bridges"* described earlier really represented localized zones of intercellular attachment and that the appearance of bridges was the result of tissue shrinkage during specimen preparation. Although some of Schaffer's contemporaries (Bonnet, 1895; Zimmermann, 1911) maintained that the dense staining of terminal bars and desmosomes was due to an accumulation of extracellular cementing substance, Schaffer supported Bizzozero's original contention that they were surface specializations of the two apposed cells and were bipartite in origin. The remarkable observations of Bizzozero and Schaffer have been very largely confirmed by recent electron microscope investigations (Porter, 1956; Selby, 1955; Horstmann and Knoop, 1958; Fawcett, 1958; Odland, 1958). It should be pointed out, however, that Schaffer was not convinced of the presence of desmosomes in non-epithelial tissues. In this view he seems to have been in error. (See Sjöstrand, 1954; Fawcett and Selby, 1958; Fawcett, 1958; Shoenberg, 1958). Recent conclusions by Fawcett (1958) that all cellular attachments so far described have fundamental structural similarities, were apparently arrived at independently, primarily on the basis of electron microscope observations, since Fawcett does not cite Schaffer's work.

It would appear from the above review that the term *desmosome*, as used by Schaffer and by Fawcett, is most appropriate in referring to intercellular attachments as seen with the light microscope and with the electron microscope. The term is derived from the Greek word *desmos*, meaning a bond, ligament, or fastening. Thus, the term means literally a "bonding body" or a "fastening body." It will be employed interchangeably with *intercellular attachment* in this present paper.

Materials and Methods

Specimens of *Pelmatohydra oligactis* (Pallas) and *Chlorohydra viridissima* (Pallas) were collected from local ponds and maintained in laboratory aquaria filled with pond water. Copepods and other small crustacea obtained at the time of collection provided sufficient food to maintain the cultures for considerable periods.

Preparation of the tissues for electron microscopy was as follows. One to several living animals were transferred by pipette with a small amount of fluid to 2 ml. shell vials. After the animals were fully expanded, the vials were flooded with 1 ml. of ice cold 4 per cent OsO_4 adjusted to pH 7.2–7.5 with acetate-veronal buffer.[1] The length of fixation varied from 15 minutes to 5 hours. The temperature of the fixative was maintained at 0°C. for short fixations but was permitted to warm to room temperature after the first hour in the longer procedures. After fixation the tissue was washed briefly in distilled water and dehydrated rapidly in increasing concentrations of ethanol. Some specimens were treated with phosphotungstic acid to enhance contrast (5 to 10 minutes in 0.05 per cent to 0.5 per cent PTA in the absolute alcohol of the dehydration series). Infiltration with methacrylate monomer was accomplished in 1½ to 2 hours with 3 changes of 10 per cent methyl methacrylate in *n*-butyl methacrylate, the final change of monomer containing 2 per cent benzoyl peroxide. Tissues were placed in gelatin capsules and polymerization was carried out at 50–55°C. in an oven. Some material was also embedded in "araldite" epoxy resin by a method modified from that of Glauert and Glauert (1958) by Dr. John Luft.

[1] The author is indebted to Dr. Neil Merrillees, now of the Department of Anatomy, University of Melbourne School of Medicine, Melbourne, Australia for suggesting this method of fixation.

Sections were cut with glass knives on a Porter-Blum ultramicrotome and selected for observation at the microscope according to interference colors in the range of light gold to grey. Sections were picked up on copper grids coated with a thin carbon film and examined with an RCA EMU 2C or a Siemens Elmiskop I. The RCA EMU 2C was fitted with a compensated 50 μ objective aperture (Canalco stigmator) and operated with a specially designed external power supply.

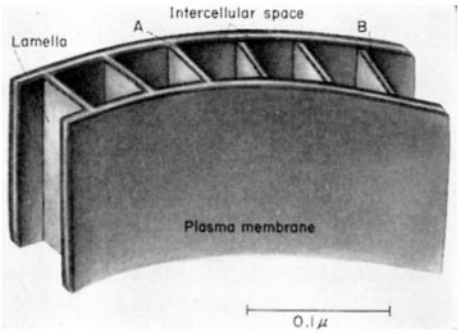
OBSERVATIONS

General.—In typical regions, the body wall of *Hydra* consists of two epithelial layers separated by an acellular meso-lamella (Hyman, 1940). The epidermis is made up of three readily discernible cell species, the epitheliomuscular cell (which comprises the majority of the population), the interstitial cell and the nematocyte. The epitheliomuscular cells, which, in *Hydra,* are sometimes referred to as supporting cells, have in their basal regions well developed muscle processes which are oriented parallel to the longitudinal body axis. Interstitial cells, which are interspersed between the bases of epitheliomuscular cells, are believed to be undifferentiated cells capable of developing into any of the somatic cells as well as into germ cells (Hyman, 1940; Brien and Reniers-Decoen, 1955). In the tentacles and oral region the epidermis is liberally supplied with nematocysts or stinging capsules. These are formed within nematocytes and are contained therein until discharged. Nematocysts are not recoverable after discharge and are continuously replaced. This is accomplished by a migration of differentiating nematocytes from the basal region of the epidermis into definitive surface positions (Hyman, 1940).

The entoderm or gastrodermis, is composed primarily of glandular and nutritive cells; flagellar extensions of both glandular and nutritive cells are important in creating water currents essential for the operation of the polypoid hydrostatic skeleton (see Hyman, 1940). The basal regions of many gastrodermal cells bear myofilaments oriented to form a layer of circular muscle. Large intracellular vacuoles occur in both epithelial layers. The cellular relationships thus far described are illustrated in Fig. 1, which is a low magnification electron micrograph of *Chlorohydra viridissima* in transverse section. Although sensory cells and nervous tissue have been described in *Hydra* (Hyman, 1940; Hadzi, 1909), they have not been identified in electron micrographs.

Desmosomes.—The plasma membrane and the limiting membranes of intracellular vesicles of *Hydra* are characterized by two dense lines about 55 A apart (peak to peak density) separated by a layer of lesser density (Figs. 2 to 6, and 8). This configuration corresponds to the unit membrane as described by Robertson (1957). The two components of these membranes in *Hydra* are not of equal density (Figs. 2, 3, and 3 *a*). Such membrane asymmetries have been noted by A. J. Hodge and J. D. Robertson in verbal presentations, but the present literature survey has not revealed publications on this subject. Ordinarily, where two plasma membranes lie adjacent to each other, the unit membranes of the respective cells are separated by a less dense region 150 to 200 A in width. Most of this narrow intercellular region has no recognizable structure, but contains sufficient material to produce an image density above that representing the embedding medium alone.

In both body layers near the places where intercellular contact areas intersect the surfaces of the cells exposed to the surrounding waters, one can discern regions of increased density which are localized to the outermost one-fifth to one-tenth of intercellular contact areas. These are interpreted as intercellular attachments or desmosomes (Fig. 1, *a*). Since these structures are visible in all planes of section, it is presumed that they form a complete belt around the epithelial cells much like terminal bars in higher organisms (Dahlgren and Kepner, 1930). The apparent position of the desmosomes varies somewhat according to the degree of expansion at the time of fixation. These observations were made on well expanded individuals. The intercellular regions at these desmosomes are about 150 to 200 A wide and show a characteristic structural organization. A series of oriented parallel lamellae arranged perpendicular to the apposed cell surfaces spans the intercellular region, connecting the two cell surfaces directly (Figs. 2 to 5). The number of lamellae in each desmosome is difficult to determine accurately because of differences in appearance due to the plane of section. In actual counts the number has varied from about 50 to nearly 200. These lamellae, which are here described in detail for the first time, are called intercellular attachment lamellae. The lamellar nature of these oriented structures can be seen in Fig. 4, which is a longitudinal section through a portion of an epidermal desmosome. In many instances the individual lamellae show the characteristic features of Robertson's "unit membrane," displaying two peaks of density about 50 A apart (Figs. 3, 3 *a*, and 4). There is also an indication from some electron micrographs, as in Figs. 3 and 3 *a*, that the two peaks of density characterizing the attachment lamellae may be continuous with the outer dense



TEXT-FIG. 1. A three-dimensional drawing illustrating the main features of a septate desmosome of *Hydra*. The two plasma membranes are connected to each other by parallel lamellae which divide the intercellular space into a series of compartments. Two interpretations of the relationships of the lamellae to the cell surfaces are represented. One possibility, indicated by the three lamella to the left (*A*), shows the outer dense components of the two plasma unit membranes to be reflected away from the cell surfaces and to contribute to the two dense components of the lamellae. A second possibility, illustrated by the three lamellae to the right (*B*), shows the lamellae to be adherent to the two cell surfaces, but so arranged that two dense outer components of the respective plasma membranes are not in direct structural continuity with those of the lamella. The situation designated by (*B*) is also consistent with an arrangement of the lamellae in which continuity with the plasma membranes is discontinuous. In view of this possibility and the apparent continuity of the lamellae as shown in Figs. 3 and 3 *a* the interpretation designated by (*A*) is deemed to be the more probable.

components of the two apposed plasma membranes. A diagram showing the relations of the attachment lamellae to the two cell surfaces is shown in Text-fig. 1.

Transverse lamellae, such as those found in the desmosomes of *Hydra* described above, have not been recognized as a prominent and constant feature of any previously described desmosomes, even where comparable techniques of preparing tissues were employed. There are suggestions, however, that a similar structural arrangement may exist in some other desmosomes. For example, transversely oriented striae already have been reported in desmosomes of rat epidermis (Horstmann and Knoop, 1958) and in localized portions of the intercalated disc of amphibian and mammalian cardiac muscle (Sjöstrand, Andersson-Cedergren, and Dewey, 1958). The function of the intercalated disc as a region of intercellular attachment was recognized by Sjöstrand and Andersson (1954) but has since been stated more explicitly (Selby, 1955; Fawcett and Selby, 1958). The presence of cross-striations in desmosomes, as described in the above instances, was not reported as having any special functional significance related to the mechanism of attachment. Possibly the striations may not be closely related to the attachment lamellae of *Hydra*.

Prominent transverse connections have also been observed recently in desmosomes located in the lamellar sheath of earthworm giant nerve fibers (Hama, 1959), in the sea anemone (Grimstone *et al.*, 1958) and in sea urchin embryos (Balinsky, 1959). Although these authors do not describe the connections as being septate, they do appear to be comparable to *Hydra* attachment lamellae. In addition, comparative studies during the present investigation have revealed similar structures in the desmosomes of the gut of the flatworm (*Dugesia tigrina* = *Planaria maculata*, *Leidy*).[2]

These findings suggest that two distinct types of desmosomes may exist, distinguishable from each other with the electron microscope by the presence or absence of transverse lamellae. The desmosomes of *Hydra*, which have prominent transverse lamellae, therefore will be referred to tentatively as *septate desmosomes*. The term *septate* conveys no precise concept of function but does indicate the definitive relationships of the lamellae as they divide the intercellular space into a series of compartments (see Text-fig. 1).

One of the characteristic features of most vertebrate desmosomes described to date is the convergence and anchoring of oriented intracytoplasmic filaments (tonofibrils, tonofilaments) on the attachment zone. These filaments appear to insert into a region of increased density, thought

[2] It is of further interest that Fernández-Morán (1958) in a study of the insect compound eye recently described within retinula cells peculiar intracellular structures which he designated *ultratracheoles*. These ultratracheoles consist of two dense lines about 150 A apart, connected by cross-striae recurring every 150 to 200 A. Although Fernández-Morán favors the view that these structures are direct tubular extensions of tracheoles, he presents no conclusive evidence for this interpretation. In view of their remarkable similarity to epithelial septate desmosomes of *Hydra*, the possibility that these *ultratracheoles* represent specializations of the retinula cell plasma membrane seems to be worthy of consideration.

to be a special thickening of the plasma membrane, which is termed the *attachment plaque* by Odland (1958). In the septate desmosomes of *Hydra* there is a condensation of a finely fibrillar material subjacent to the plasma membrane. Although this has some similarity to the dense attachment plaques of vertebrate desmosomes, it does not appear to be formed by a thickening of the plasma membrane itself. Furthermore, localized groups of oriented intracytoplasmic filaments have not been identified in association with septate desmosomes of *Hydra*.

Distinct plasma membranes, resolvable as unit membranes throughout the entire attachment region, bound each cell without interruption, thus indicating that there is no cytoplasmic continuity between adjacent cells at desmosomes in *Hydra*. This is in agreement with the observations of Porter (1956) and with all subsequent investigations on the fine structure of desmosomes.

The relationships of the lamellae of septate desmosomes to the plasma membranes of the attached cells deserves further comment. As already noted, some especially favorable electron micrographs (see Figs. 3 and 3 *a*) show continuity of the dense lines of the lamellae with the outer dense lines of the two apposed plasma membranes. Although this continuity has been seen only rarely and may not represent the true situation, there is a distinct possibility that such a continuity really exists. Since only the outer component of the bipartite plasma membranes appears to be associated with the formation of the lamellae, the two peaks of density in the lamellae may not show the same asymmetries as the two peaks of density seen in the plasma membranes (see Figs. 2, 3, and 3 *a*). It should be mentioned also that the dense lines of the lamellae appear to be somewhat closer together than the two components of the plasma membranes, but it is not certain whether the difference in dimensions is statistically significant.

An alternative view, that the lamellae are not continuations of the outer dense lines of the plasma membranes, also should be considered. It is conceivable that a specialized arrangement of material such as a sulfated muco-polysaccharide could be intimately associated with the cell surfaces and could precipitate in a crystalloid form under appropriate conditions. There is no direct evidence to support such a conjecture, but Meyer (1957) has recently suggested that sulfated muco-polysaccharides may be involved in the formation of specific attachment regions between cells. Present evidence does not permit drawing final conclusions on the question of the structure and relationships of the attachment lamellae to the cell surfaces in septate desmosomes (see Text-fig. 1).

Septate desmosomes are most prominent in the epidermis of *Hydra*, but they are present also near the luminar border of gastrodermal cells, as illustrated in Figs. 1 and 6. It is noteworthy that no desmosomes have been detected attaching interstitial cells to adjacent cells. This situation can be correlated with the subsurface position of the interstitial cells and their ability to migrate between epithelial cells (Hyman, 1940; Brien and Reniers-Decoen, 1955).

The use of phosphotungstic acid (PTA) as an electron stain enhances membrane contrast and thus is helpful in demonstrating intercellular relationships in *Hydra* (Figs. 3 to 5, 7, and 8). PTA is not requisite for demonstrating attachment lamellae, however, as can be seen in Figs. 2 and 6, where fixation in buffered osmium tetroxide alone was used. Consequently, the lamellae cannot be regarded as precipitation artefacts induced by treatment of the tissues with PTA.

Regarding the use of PTA for staining in electron microscopy, the following observation is worthy of note. Treating tissues with PTA prior to embedding in methacrylate frequently results in sectioning difficulty and produces coarse precipitation of material in the background cytoplasm. One of the criteria for determining good fixation currently employed in electron microscopy is the presence of finely dispersed granulation in the nucleus and cytoplasm (Palade, 1952, 1956). There are several possible explanations for the appearance of cytoplasmic clumping with PTA treatment, but this phenomenon is probably related to the acidity and to the precipitating action of PTA on proteins. Assuming this interpretation to be correct, subjecting well fixed tissue to PTA could weaken or alter some of the bonds contributing to tissue stability in dehydration and embedding. As a consequence, the tissues might be more easily damaged during embedding, sectioning, and under electron bombardment. It has been noted in the present study that if tissues treated with PTA are embedded in epoxy resin (Fig. 7), the appearance of clumping in the cytoplasm is greatly reduced. It is presumed that the type of polymerization and the degree of cross-linking in the epoxy resin is responsible for the better preservation. The contrast of PTA treated tissues makes focusing easy even after epoxy embedding.

DISCUSSION

It now seems clear that the dense staining of vertebrate desmosomes (including terminal bars) by iron hematoxylin and other classical techniques (von Recklinghausen, 1862; Heidenhain, 1892) is largely due to localized cytoplasmic and membrane differentiation rather than to accumulations of an extracellular cementing substance as proposed by Zimmermann (1911) and others. Electron microscope studies on vertebrate desmosomes have confirmed the presence of an array of intracytoplasmic filaments (first described by Ranvier in 1879) and have demonstrated that the filaments end in a dense attachment plaque, the latter being a specialized part of the plasma membrane (Odland, 1958). Thus Bizzozero (1870) and Schaffer (1927) were correct in their interpretation of desmosomes as bipartite structures formed from cytoplasmic specializations of the apposed cells but not involving cytoplasmic continuity between these cells.

Although *Hydra* has been extensively studied with the light microscope (Kleinenberg, 1872; Hadzi, 1909; Mueller, 1950; Brien and Reniers-Decoen, 1955; see Hyman, 1940, for additional literature) and has been investigated recently by electron microscopy (Hess, Cohen, and Robeson, 1957; Bouillon, Castiaux, and Vandermeersche, 1958 *a*, 1958 *b*; Chapman and Tilney, 1959; Slautterback and Fawcett, 1959), intercellular attachments have not been described in these animals previously. The lack of well developed attachment plaques and associated cytoplasmic filaments probably account for the failure to recognize septate desmosomes in *Hydra* earlier. It should be pointed out that desmosomes in *Hydra* must be organized so as to permit relatively easy modulation during nematocyte migrations and other cell movements which accompany asexual budding and regeneration, the capacity for which is developed to a remarkable degree in these organisms. A somewhat impermanent mode of structural interlacing and anchoring of the cell and its internal components may be essential for this degree of mobility.

The complex cellular specializations comprising the light microscopists' intercellular bridges, while interesting, do not demonstrate the actual mechanism of cellular cohesion. Birbeck and Mercer (1957) and Fawcett (1958) have recognized this fact in earlier publications but the point needs further emphasis. Although these cytoplasmic specializations serve to stabilize the cytoarchitecture to focal points at the cell surface, the features of the external surfaces of plasma membranes and of the region between determine the actual cohesion of the cells. The electron microscope has revealed various patterns of density in this narrow intercellular region (Porter, 1956; Selby, 1955; Horstmann and Knopp, 1958; Fawcett, 1958; Odland, 1958). As yet, it has not been possible to interpret these densities in relation to the mechanism of attachment. The appearace of transverse lamellae in septate desmosomes provides a geometrical arrangement, which, at least, on cursory observation, would seem well fitted for binding two cells together. It is well to point out, however, that density in electron micrographs cannot be interpreted directly in terms of bonding strength. Thus the dense lamellae in septate desmosomes are not necessarily direct visual evidence of a cohesive mechanism. If the lamellae are continuous with the plasma unit membranes of the apposed cells, as seems apparent in Figs. 3 and 3 *a*, this suggests a high protein and phospholipid content. Wislocki (1951) and Romanini (1954) present evidence from histochemical studies that there are increased amounts of lipid and phospholipid in desmosomes from a variety of sources. Puchtler and Leblond (1958), in more detailed histochemical studies on intestinal epithelium have concluded that terminal bar desmosomes of this tissue are composed primarily of protein. From the point of view of structural strength, one could conjecture that protein might be a major constituent of an attachment mechanism. It is hoped that further investigation of *Hydra* septate desmosomes will provide additional information on the chemistry of this type of intercellular attachment.

The position and general configuration of *Hydra* septate desmosomes resembles that of terminal bars in columnar epithelium (see Schaffer, 1920). According to Zimmermann (1911) and Dahlgren and Kepner (1930), terminal bars function for intercellular attachment and as a barrier to intercellular passage of ambient substances from the intestinal lumen. That desmosomes may function as a permeability barrier is a concept of considerable physiological significance for *Hydra*. Schlieper proposed in 1930 that osmotic regulation in *Hydra* consisted of a process of actively transporting water across the cell membranes, notwithstanding the lack of morphological evidence for active water extrusion in these organisms. In 1933 Palmhert reported experiments in which he meas-

ured O_2 consumption of *Hydra* placed in solutions of different tonicity. Palmhert's conclusions were that osmotic resistance in *Hydra* was not due to active metabolic work but was due to some colloid chemical property of the cell surfaces which reduced permeability to water. The lack of demonstrable connection of metabolism with osmoregulation in *Hydra* led physiologists more recently (Prosser *et al.*, 1950) to postulate that there must be some substance present in the pond water which keeps permeability to water low. From the considerations presented in this paper, it would appear that Palmhert's conclusion may be basically sound and that the permeability barrier of *Hydra* is inherent in the structure of the organism itself. It seems likely that water may be prevented from entering freely the exposed cell surfaces because of special permeability properties of the plasma membrane in this region. In addition, it appears that septate desmosomes may provide a series of barriers separating intercellular regions from the external medium. Thus it is possible that septate desmosomes in *Hydra* may have an important role in defending the intercellular environment of the organism. This conjecture is supported by the fact that solid materials in transit have not been observed between epithelial cells in the vicinity of desmosomes, but have always been located within intracellular vacuoles or vesicles. This applies particularly to the gastrodermis where food material is engulfed and retained within vacuoles and where intracellular digestion occurs (Hyman, 1940). It is apparent, therefore, that the concepts of vesicular transport proposed by Bennett (1956) are relevant also for *Hydra*. There is no evidence at present that a permeability barrier imposed by desmosomes would restrict the uptake of nutrients, though such a barrier may be necessary for the maintenance of an internal cellular environment.

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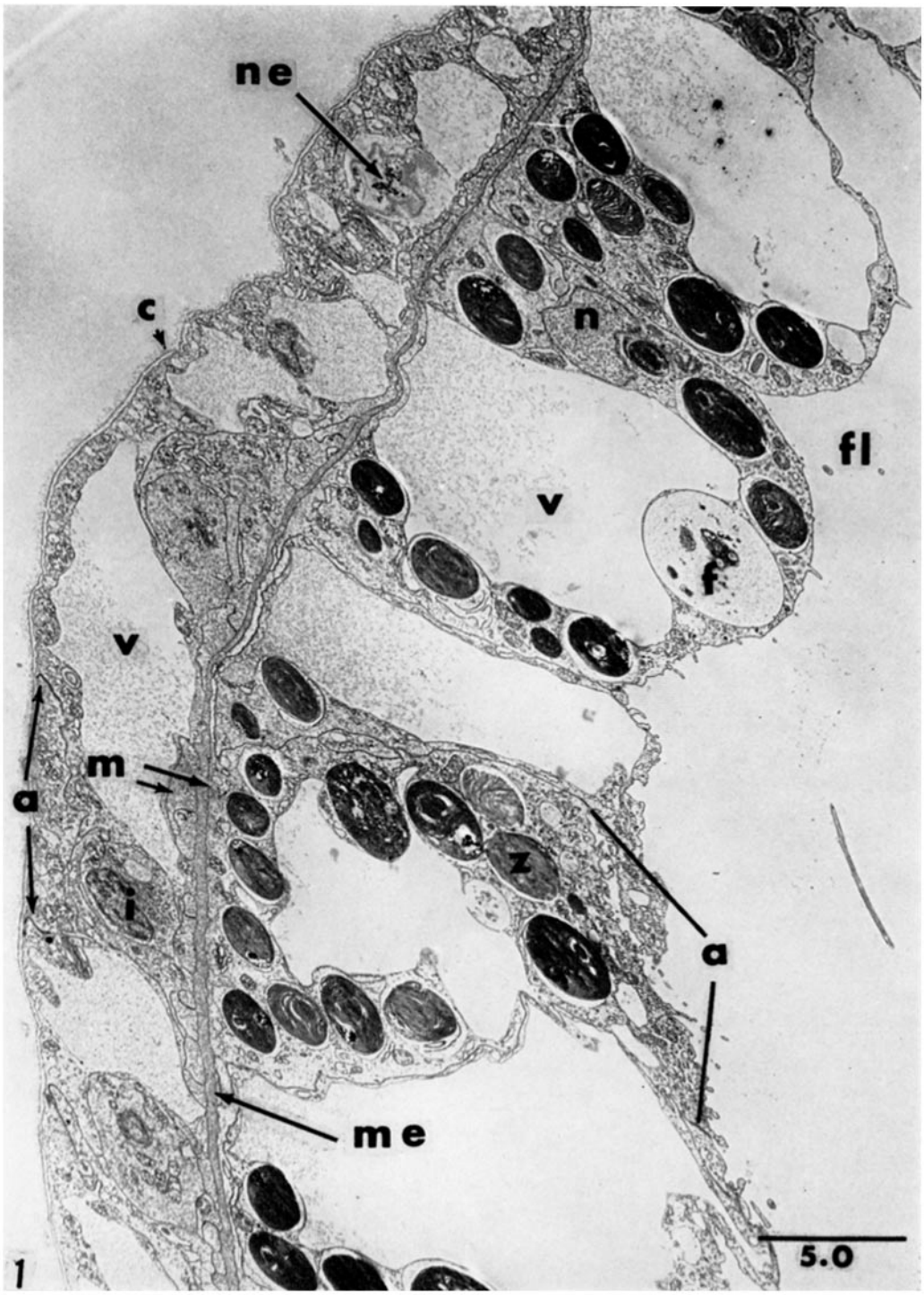
Explanation of Plates

Plate 160

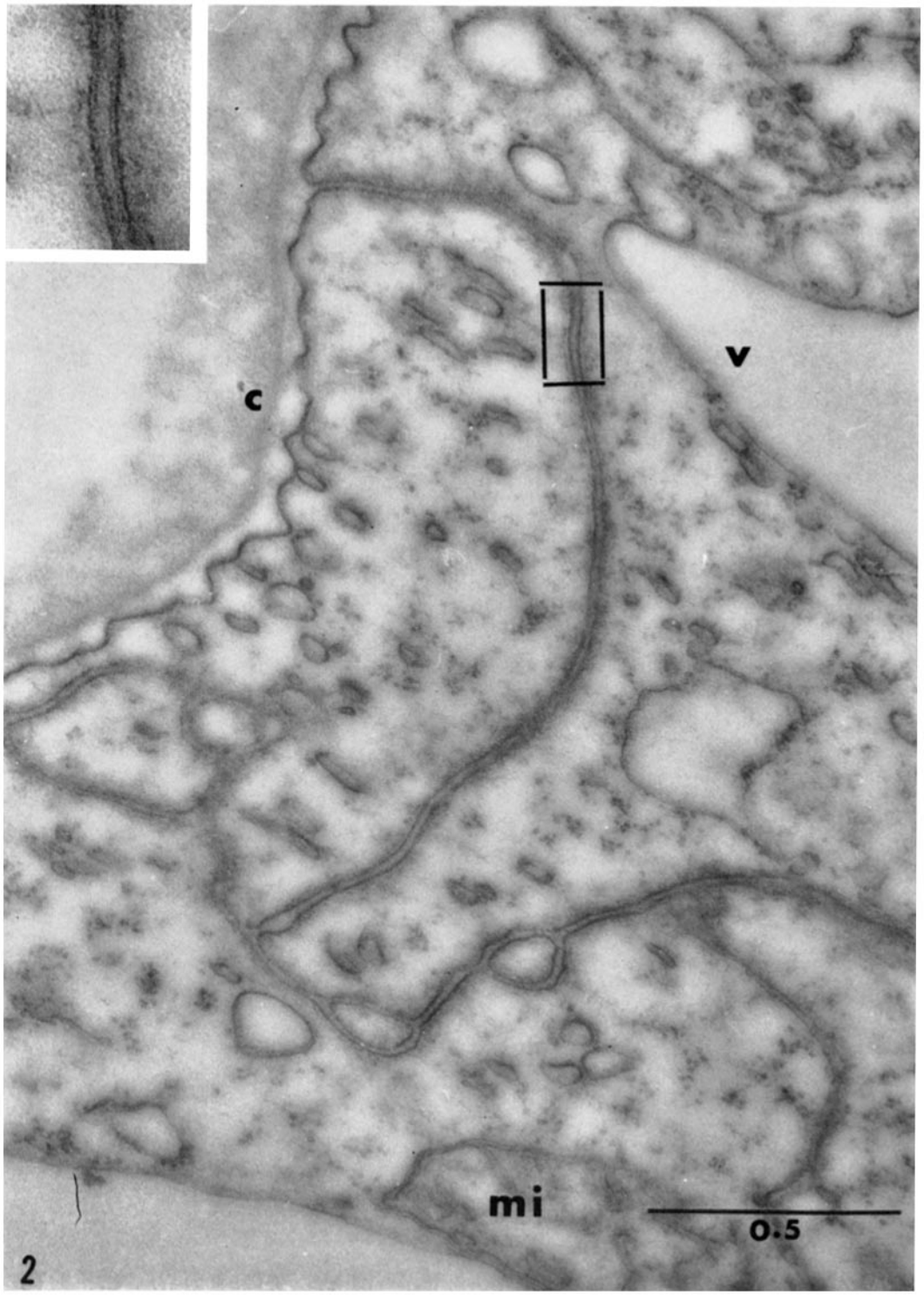
Fig. 1. Low magnification electron micrograph of a segment of body wall of *Chlorohydra viridissima*. The thinner layer of epithelium on the left is the epidermis. The mesolamella (*me*) separates the epidermis from the gastrodermis, whose cells contains symbiotic green algae (*z*). The gastrodermal cells have very irregular borders facing the gut lumen and show evidence of vesiculation and occasional food vacuoles (*f*). Cross-sections of two flagellae are seen at *fl*. Large intracellular vacuoles (*v*) occur in both epithelial layers. Musculature (*m*) appears in discrete packets in the epidermis (cross-section) and is seen only as a diffuse increase in density in the gastrodermis (longitudinal section). In the epidermis there is a nematocyst within a nematocyte (*ne*). One also sees what is perhaps an interstitial cell (*i*) and regions of increased density (*a*) which correspond to the attachment areas. Note the thin cuticle (*c*) covering the epidermis and the nucleus of a gastrodermal cell at *n*. The epidermal cells have a flattened appearance, indicating good relaxation of the specimen. Fixation, 5 hours in buffered osmium tetroxide. × 4,400.



(Wood: Septate desmosomes)

PLATE 161

FIG. 2. High magnification electron micrograph of *Pelmatohydra oligactis* in the peripheral region of the epidermis. Note the continuous cuticle (*c*) at the body surface on the left, the intracellular vacuole (*v*), and the mitochondrion (*mi*). The junction of at least three cellular processes is represented by the complex membrane relationships in the lower and central portions of the picture. Two peaks of density corresponding to Robertson's unit membrane may be seen in some portions of the plasma membranes. The region outlined, seen at higher magnification in the inset, shows the unit membrane structure clearly. Note the differences in density of the inner and outer components of the two unit membranes. Note the faint striations between apposed surface membranes near the top of the picture. Fixation, 4 hours in buffered osmium tetroxide. × 78,000. Inset, × 205,000.

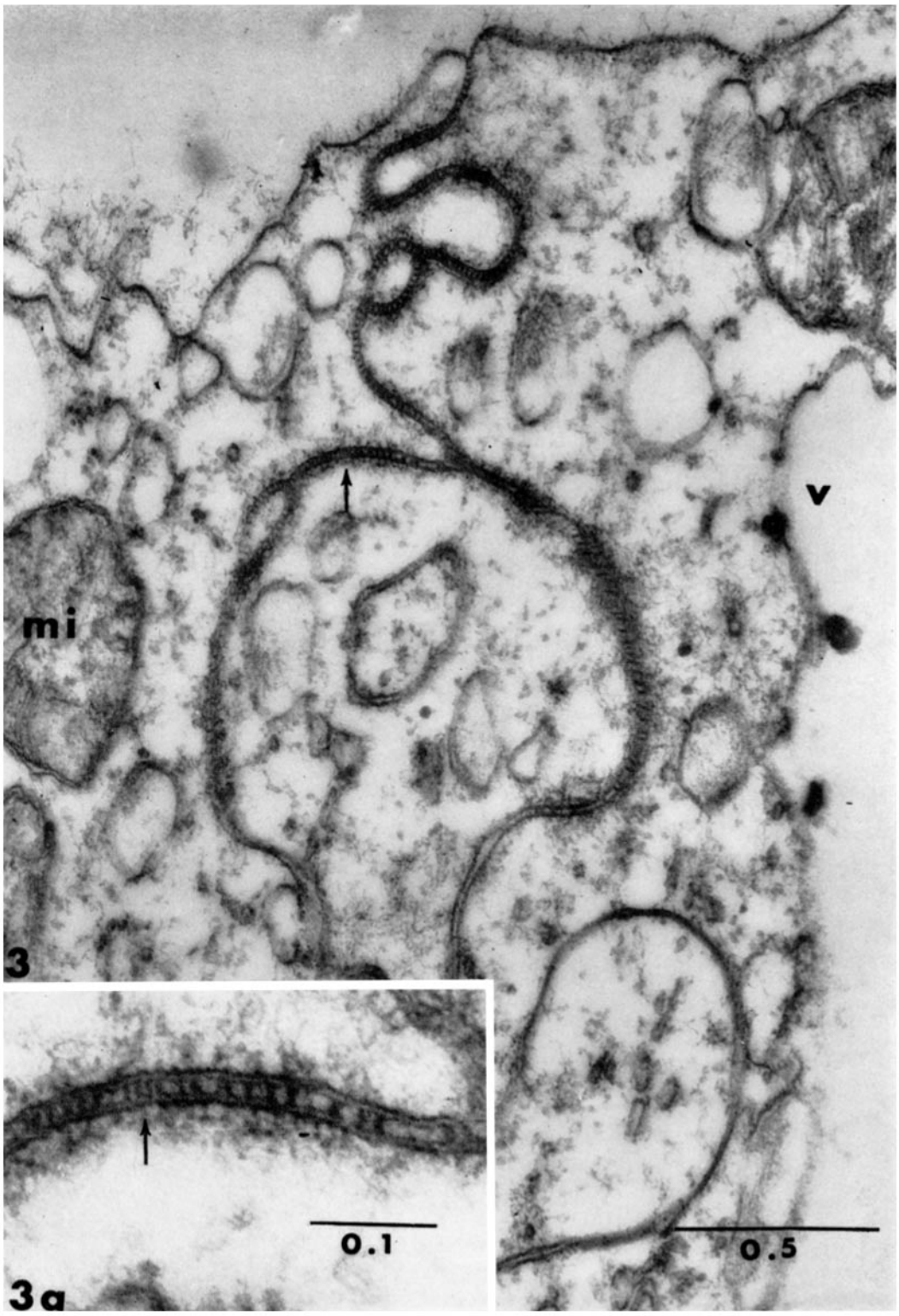


(Wood: Septate desmosomes)

PLATE 162

FIG. 3. An electron micrograph showing a transverse section through a desmosome in the epidermis of *Pelmatohydra oligactis*. The outer surface of the body is seen at the top. Note the intracellular vacuole (*v*) and the mitochondrion (*mi*). The attachment lamellae are particularly well demonstrated in this micrograph. At the arrow the image suggests that the lamellae are continuous with the outer dense component of the plasma unit membranes of the respective cells. Specimen fixed 30 minutes in buffered osmium tetroxide and treated 5 minutes with 0.5 per cent PTA in absolute alcohol. × 63,000.

FIG. 3 *a*. Photographic enlargement of the area in Fig. 3 indicated by the arrow, showing the relationship of the attachment lamellae to the respective plasma membranes. × 200,000.

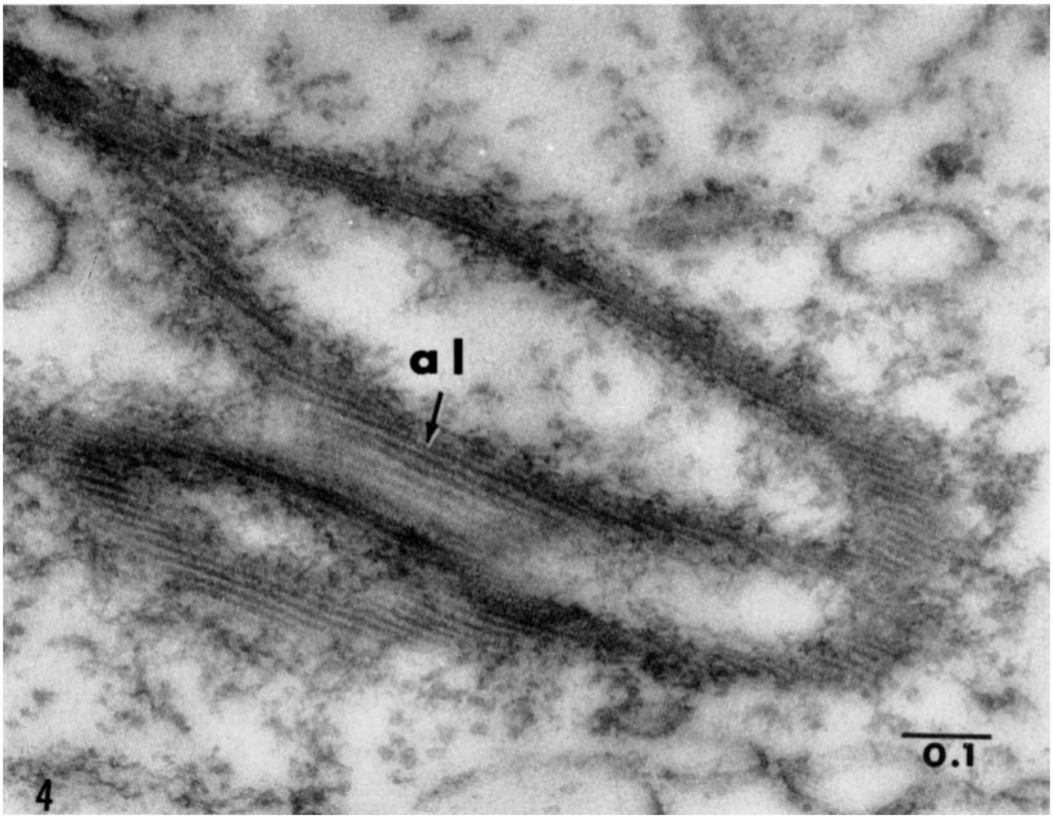


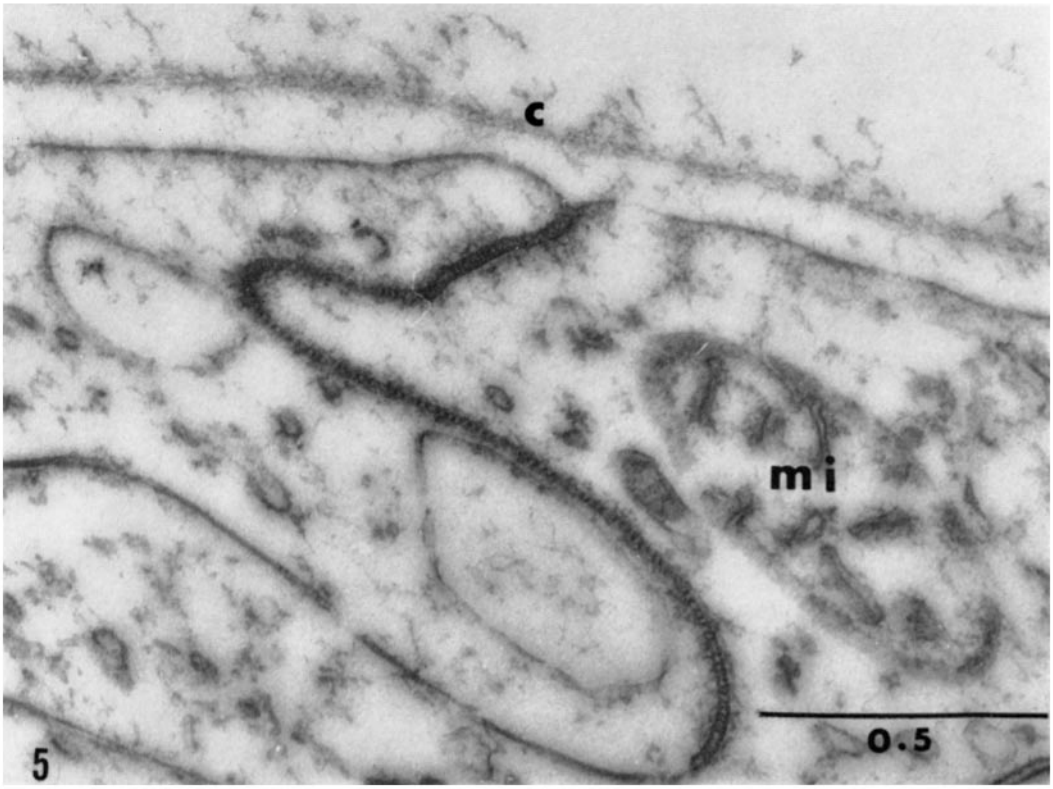
(Wood: Septate desmosomes)

PLATE 163

FIG. 4. A longitudinal section through a portion of a septate desmosome in the epidermis of *Pelmatohydra oligactis*. Note the regular arrangement of the attachment lamellae (*al*). Specimen prepared as that shown in Fig. 3. Micrograph taken with Siemens microscope. × 120,000.

FIG. 5. A transverse section through a desmosome in the epidermis of *Chlorohydra viridissima*. The cuticle (*c*) covering the outer surface of the body is seen at the top of the micrograph. Note the mitochondrion (*mi*), somewhat damaged in preparation, and the prominent attachment lamellae. Fixation, 15 minutes in buffered osmium tetroxide. Treated with 0.05 per cent PTA in absolute alcohol for 10 minutes. × 78,000.

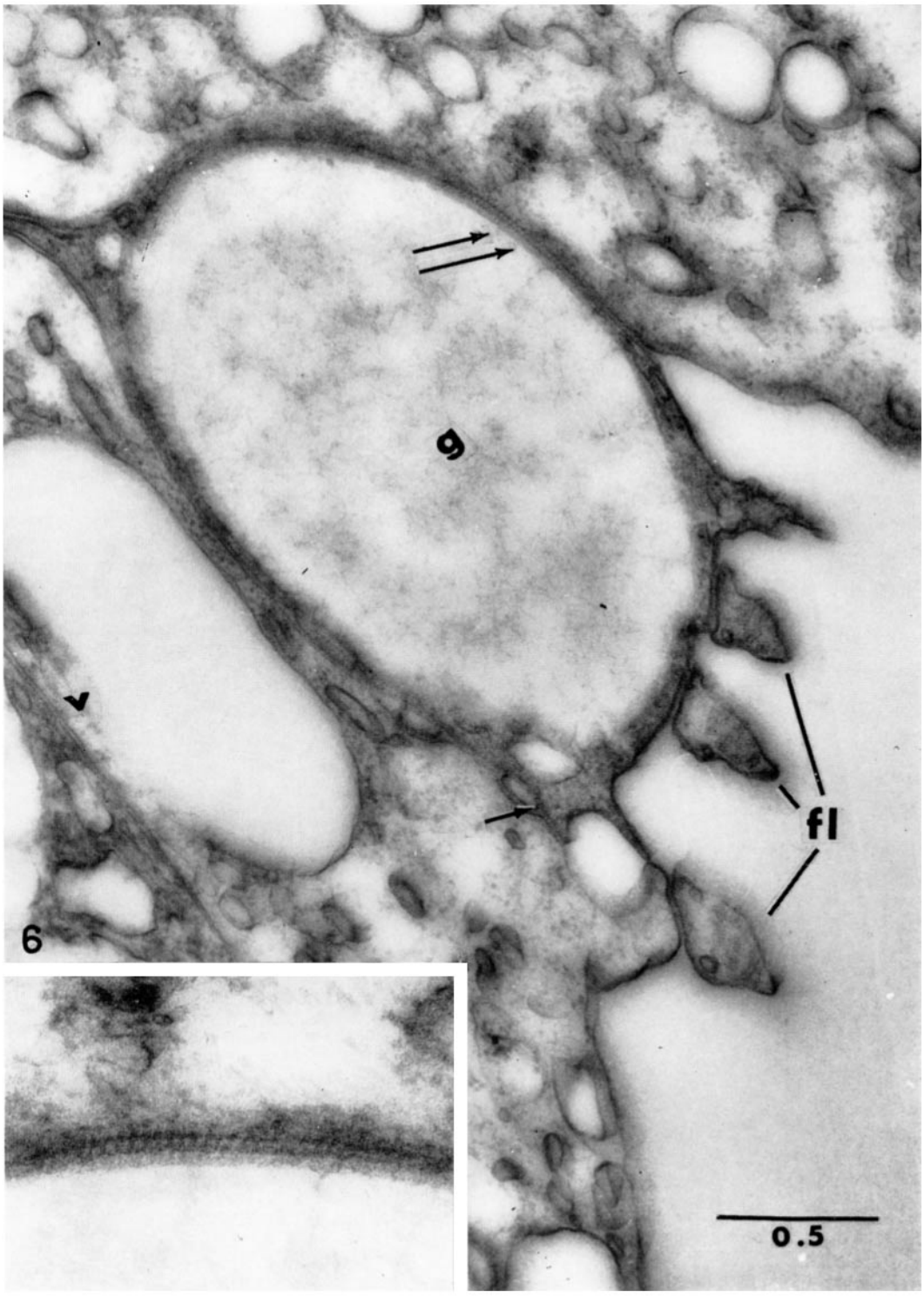




(Wood: Septate desmosomes)

PLATE 164

FIG. 6. Section through the luminal end of gastrodermal cells of *Pelmatohydra oligactis*. The digestive cavity appears at the right, with cross-sections of flagellae (*fl*) showing dimly the arrangement of internal tubule-like filaments in a pattern of nine peripheral pairs and one central pair. The large body (*g*) in the center of the picture with a slightly filamentous matrix is presumed to be a secretory granule of one of the spumeuse gland cells. Note also the intracellular vacuole (*v*). At the arrows the intercellular contact areas show striations which, on closer examination, are seen to represent the attachment lamellae of epidermal desmosomes (see inset). Such desmosomes can be detected under low magnification as stretches of specially dense membrane (Fig. 1, *a*). Fixation, 4 hours in buffered osmium tetroxide. × 50,000. The inset is a photographic enlargement of the region marked with a double arrow. × 110,000.

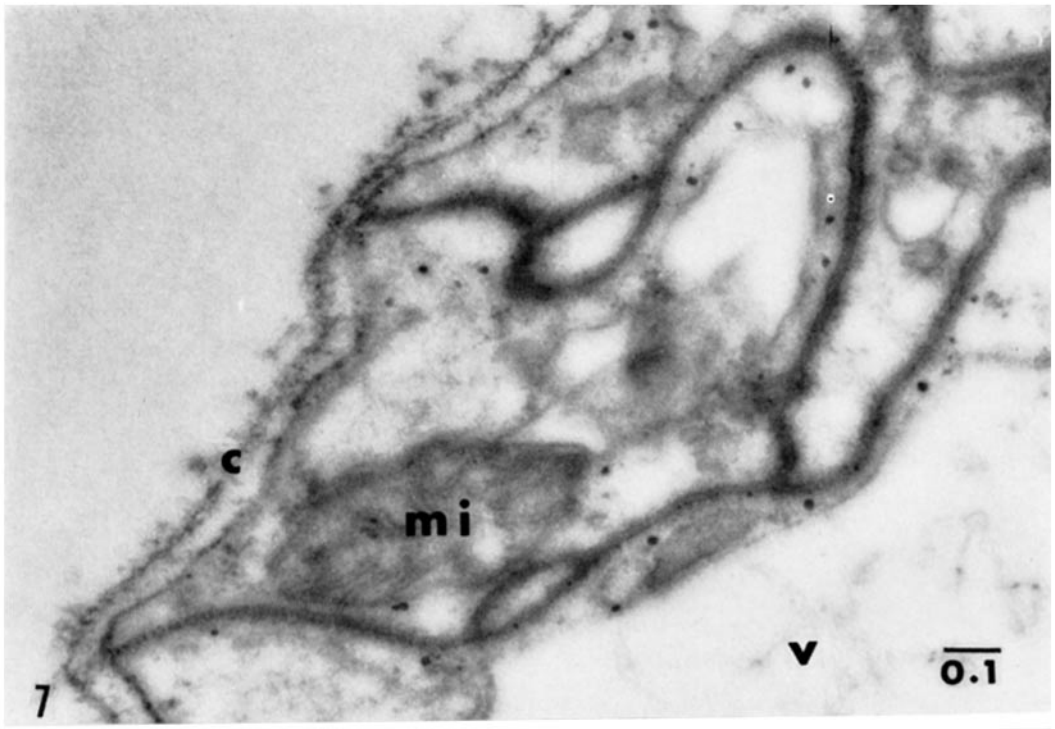


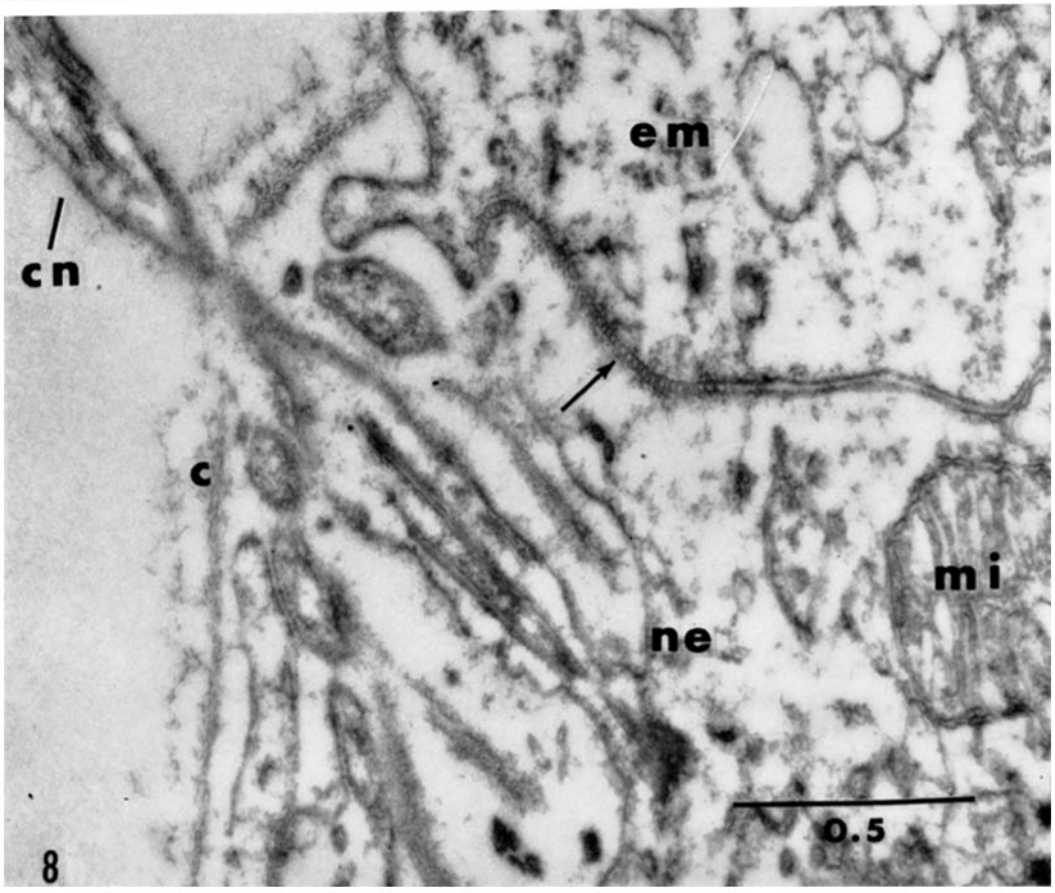
(Wood: Septate desmosomes)

PLATE 165

FIG. 7. Section through outer epidermis of *Chlorohydra viridissima* showing regions of intercellular contact. This micrograph was taken from material fixed 15 minutes in buffered osmium tetroxide, exposed to 0.1 per cent PTA in absolute alcohol for 10 minutes, and embedded in "araldite" epoxy resin. Note the cuticle (*c*), the intracellular vacuole (*v*), and the well preserved mitochondrion (*mi*). Note also the lack of clumping in the background cytoplasm. The attachment lamellae are not so clearly defined as in methacrylate-embedded material. This is a consequence of the lower specimen contrast. × 78,000.

FIG. 8. Section through the epidermis of *Pelmatohydra oligactis* at the base of a cnidocil, which functions as a trigger for a nematocyst. The cnidocil (*cn*) is a specialization of the nematocyte (*ne*), most of which is not visible in this picture. Note the cuticle (*c*), the mitochondrion (*mi*), and the clumping of the background cytoplasm. At the arrow is a desmosome attaching the nematocyte (*ne*) to the adjacent epitheliomuscular cell (*em*). This desmosome shows prominent attachment lamellae. Specimen prepared as described for Fig. 3. × 64,000.





(Wood: Septate desmosomes)