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Histological and Ultrastructural Studies of the Basal Disk of *Hydra**

I. The Glandulomuscular Cell

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Summary. The glandulomuscular cells of *Hydra* are located exclusively in the basal disk. They are derived from epithelio-muscular cells which have been forced proximally. Light and electron microscopical studies show that prior to their destruction and elimination, the transformed epithelio-muscular cells (i.e. the glandulomuscular cells) undergo certain striking morphological and physiological changes. Golgi complexes and elements of rough E. R. increase remarkably in activity, and individually or jointly produce at least six types of morphologically different droplets. One additional type of droplet is thought to originate from neighboring digestive cells. Although the chemical nature of the individual droplets is uncertain, it is known that some are Alcian blue and PAS positive and contain hyaluronic acid. These evidences suggest the presence of an acid mucopolysaccharide material, the adhesive agent which attaches the animal to a substrate. The myonemes contain thick (200 Å in diameter) and thin (60 Å in diameter) filaments as in epithelio-muscular cells. There are also filaments of intermediate sizes and large fibers (770 Å in diameter). The myonemes are oriented radially with respect to the aboral pore and therefore in addition to contributing to the contraction and relaxation of the body column, they apparently regulate the opening and closing of the aboral pore. Although there is no evidence to substantiate the mechanism for transformation of epithelio-muscular cells to glandulomuscular cells as well as cell death of the latter cell types, these problems are discussed briefly.

Key words: Glandulomuscular cells — *Hydra* — Basal disk — Light and electron microscopy.

Introduction

The basal disk is the most proximal region of the *Hydra* polyp. As such it is the area of the animal which attaches to any type of substrate. It is also the region containing the aboral pore through which undigested material and cellular debris may be egested and one of the extremities at which cells are sloughed off the animal. The basal disk, like all other regions in the polyp, contains two cellular layers, the epidermis and the gastrodermis, separated by an acellular mesoglea.

The epidermis of *Hydra* is composed chiefly of epithelio-muscular cells. These cells are found in all regions of the animal from the tips of the tentacles to the basal disk. Regardless of their location, all epithelio-muscular cells have certain basic structural characteristics in common. The basal regions contain myonemes

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which are oriented parallel to the longitudinal axis of the animal. The apical cytoplasm contains mucous droplets which are released periodically in order to maintain the external mucous covering. The center of the cell contains the nucleus, vacuoles and cytoplasmic organelles such as rough endoplasmic reticulum, Golgi complexes and mitochondria.

It has been shown that epithelio-muscular cells in the growth region of the animal undergo frequent mitosis (Burnett, 1966). These cells are pushed distally into the tentacles and proximally into the body column. The cells which are located in the tentacles form myonemes and secrete mucous droplets but eventually they die and are sloughed off at the extremities. Epithelio-muscular cells forced proximally along the body column also secrete mucous droplets and elaborate longitudinal myonemes. As the proximal movement progresses they are eventually positioned in the basal disk. Before these cells are discarded, however, they undergo certain morphological and physiological changes and produce acid-mucopolysaccharide droplets not found elsewhere in the epidermis (Burnett, 1966). As a result of the increased synthetic activities in the formation of numerous droplets these cells are referred to as glandulomuscular cells (Burnett, 1966; Hyman, 1940; Lentz, 1966).

The ultrastructure of the secretory droplets in glandulomuscular cells has been studied by Philpott, Chaet and Burnett (1966). These investigators described three types of droplets, designated Classes I, II and III in *Hydra pirardi*. It was suggested that two types of these droplets may represent the same droplets in different stages of formation. In some preliminary studies of the glandulomuscular cells of four species of *Hydra*, a variety of morphologically different droplets was observed. Some were identical to those described by the above authors. Others were vastly different and unlike any other type of secretory droplet seen in this organism.

In addition to the glandulomuscular cells with their heterogeneous secretory droplets, cnidoblasts and interstitial-like cells were observed. It has been reported that these latter cell types are normally not present in the basal disk. Also, nerve cells which are frequently described as forming a nerve ring in the basal disk, were seen in relatively few numbers in electron microscopical studies. These preliminary findings prompted a detailed morphological investigation of the entire basal disk. The results are present in this series of papers.

Materials and Methods

Hydra oligactis, *Hydra pseudoligactis*, and *Hydra pirardi* were used in these studies. The animals were raised by a modified method of Loomis and Lenhoff (1956). They were fed daily on brine shrimp and then starved for 24 hours prior to preparation for microscopy. Animals attached to culture dishes were detached by directing a gentle stream of culture medium at their bases. They were removed carefully and prepared for light and electron microscopy.

Light Microscopy. Animals were fixed in Zenker or Bouins fluid and prepared routinely for histological studies. Sections (5 μ) were stained with toluidine blue and others with alcian blue (Pease, 1961). Hyaluronic acid was detected by incubating sections in bovine testis hyaluronidase for periods up to three hours at 37° C. These sections were stained in 0.5–1.0% toluidine blue. Thin sections (approximately 1 μ) were prepared from tissues embedded for electron microscopy and stained in 1% toluidine blue or 0.5% methylene blue.

Electron Microscopy. Animals were fixed for one hour in cold 3–6% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.3. During fixation, the animals were excised at

the distal peduncular region in order to allow for proper orientation within the plastic. Tissues were washed in a buffered sucrose solution (in 0.1 M sodium cacodylate) at 4°C and then post-fixed for 30–45 minutes in cold 1% osmium tetroxide containing sucrose. Some tissues were prepared with the latter fixative only. The fixed tissues were dehydrated rapidly in alcohol and embedded in Maraglas. Sections were cut with glass or diamond knives on a Huxley microtome and mounted on carbon-coated Formvar-filmed grids. The sections were stained with uranyl acetate followed with lead citrate. Sections were examined in a RCA 4 electron microscope.

Observations

Light Microscopy

Toluidine Blue Staining. The basal disk and the most proximal portion of the peduncle are shown in Fig. 1 a. The central portion of the basal disk epithelial cells reveals a slight purple color while the peripheral regions are faintly bluish-green. The latter coloration is due to the presence of numerous small droplets. Similarly located peripheral droplets in the epithelio-muscular cells of the peduncle and throughout the epidermis of the animal reveal a deep blue color (Fig. 1 b). The dramatic difference in the staining of these droplets signifies the junction between the peduncle and the basal disk. The slightly-stained purple nuclei are located in the central cytoplasm and are recognizable by their prominent nucleoli.

Alcian Blue Staining. Sections stained with alcian blue and counterstained with hematoxylin and eosin show certain striking features of the basal disk epithelial cells (Fig. 1 c). The dense cytoplasm contains numerous droplets especially in the peripheral one-third of most cells. Few of these droplets are seen in the most proximal peduncular cells while they are lacking or not recognizable in other epithelial cells throughout the body column. Most of the conspicuous nuclei are located mainly in the central region of the epithelium while other nuclei, usually smaller and denser are situated closer to the peripheral areas where elimination occurs. Surrounding the outer limits of the epithelial cells is a thin layer of slightly blue staining material. In some preparations the blue coloration is also seen within the apical one-third of the cells (Fig. 1d). Although the intensity of the blue stained material is only slight, it resembles the more deeply stained materials in mucous cells of the gastrodermis. These are the only cell types stained in this manner.

Hyaluronidase Treatment. Sections incubated in hyaluronidase and then stained in alcian blue were used to determine the presence of hyaluronic acid in the basal disk epithelium. Fig. 1e shows that although there is a reduction in the number of droplets in the epithelium as compared to those seen in the alcian blue controls, there are still many droplets in the apical one-third of the cells. These droplets range from light green to fairly intense blue and are present only in the basal disk epithelium.

Toluidine Blue (1 μ plastic sections). The most conspicuous feature is the presence of numerous dense blue droplets occupying mostly the apical one-third of the cells. The cytoplasm and nuclei are moderately stained with a bluish-purple color. Due to the thinness of sections prepared in this manner, many more vacuoles are seen as compared to other methods of preparation. The gastrodermal cells and the acellular mesoglea at the basal disk show certain peculiarities as compared

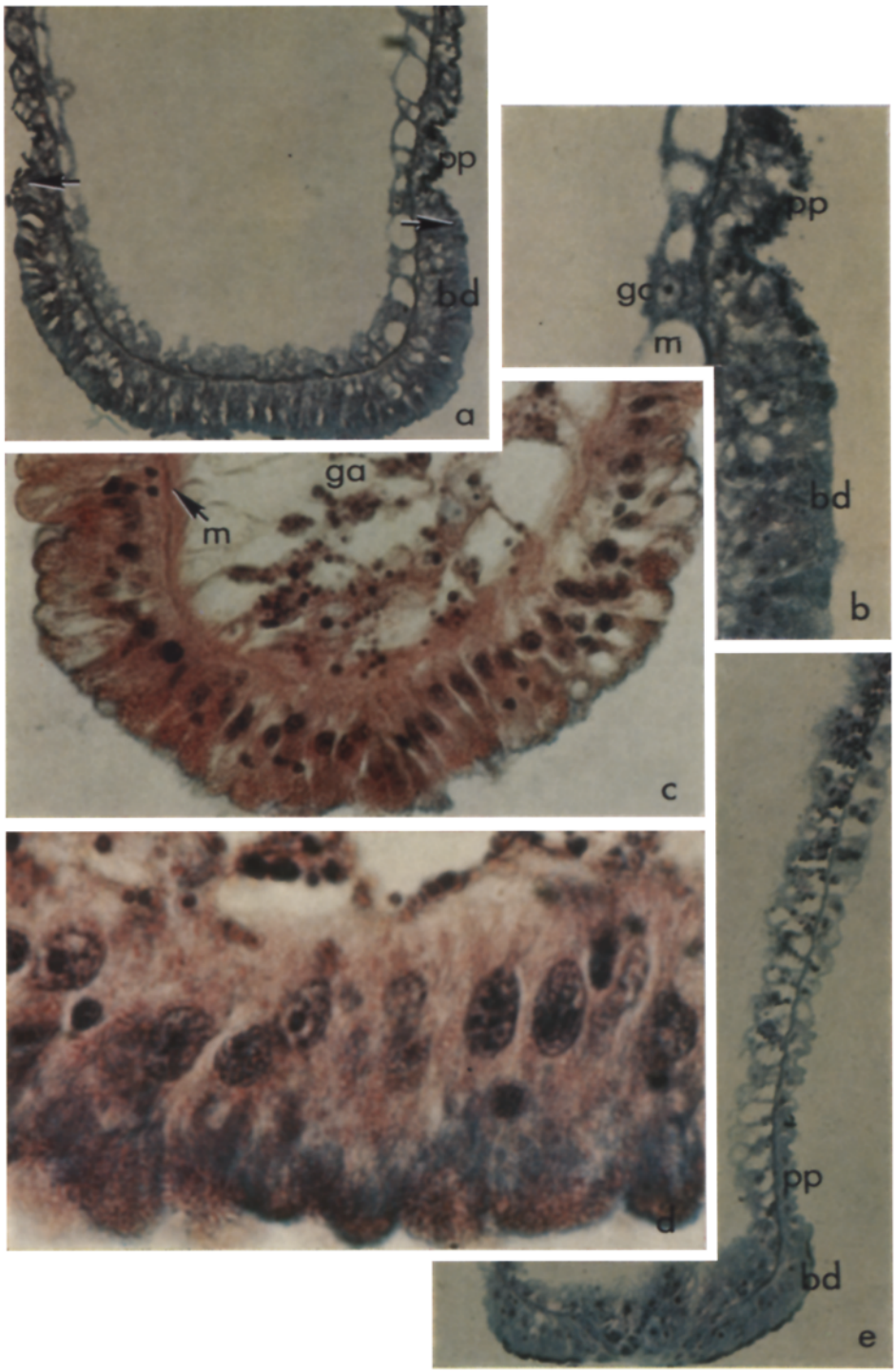

Fig. 1 a—e

to the same structures in other body regions. These studies will be presented in other papers.

Electron Microscopy

Portions of the basal disk epithelium are shown in Figs. 2 and 3. Although all the cells are glandulomuscular cells, there are several other cell types in the basal disk. These will be described later. The basal region of the glandulomuscular cells which contain intact myonemes are located immediately adjacent to the mesoglea (Fig. 2). The central region of the cell contains the nucleus, several droplets, Golgi complexes, rough endoplasmic reticulum, mitochondria and vacuoles of various sizes. The apical part of the cells, that is, the region which attaches the animal to a substrate contains numerous droplets of diverse types (Fig. 3).

In some species this apical region bulges into the surrounding medium as though in the process of extruding large portions of the cells. In other species, the droplets are located along the periphery of deep cytoplasmic folds. The lateral surfaces are attached to each other by septate desmosomes. All the organelles mentioned previously may also be present in this region, as well as several vacuoles and nuclei of cells which are being discarded. Although the nuclei of glandulomuscular cells are located usually in the center of the cells, they may be observed along the peripheral limits as the cells are pushed proximally. These nuclei, unlike those of epithelio-muscular cells throughout the body column, are most often highly irregularly shaped and always contain a conspicuous nucleolus (Fig. 2).

Droplets. Seven different types of droplets are recognized in glandulomuscular cells. These differences are based purely on variations in their morphology. For convenience, the types of droplets are designated Types I to VII. It is not known whether each cell contains all seven types of droplets, but it appears from our observations that there are at least five types in each cell.

Type I: The first type of droplet is shown in Fig. 4. They may be located immediately adjacent to the plasma membrane or deeper in the cytoplasm. These

Fig. 1a—e. Light micrographs of the peduncular and basal disk regions. a) Toluidine-blue stained proximal peduncle (*pp*) and basal disk (*bd*). Note the drastic difference in staining intensity of peripherally located droplets of the peduncle and base (arrows). *H. pseudoligactis* b) Higher magnification of the transitional region between the peduncle (*pp*) and basal disk (*bd*) seen in Fig. a. The droplets in the former region are stained intensely and are confined to the periphery of the cells. The droplets in the latter region are much less stained and occupy the apical one-third to one-half of the cells. Gastrodermis (*ga*), mesoglea (*m*). *H. pseudoligactis.* c) Alcian blue and H and E stained basal disk showing numerous droplets in the apical cytoplasm. Most of the nuclei are centrally located while smaller and denser nuclei are closer to the periphery. Note the thin layer of slightly blue staining material surrounding the outer limits. Gastrodermis (*ga*), mesoglea (*m*). *H. oligactis.* d) Alcian blue and H and E stained basal disk. The nuclei, some of which contain conspicuous nucleoli, are centrally located. Note that some of the droplets within the cells are alcian blue positive (blue color). *H. littoralis.* e) Hyaluronidase treated and alcian blue stained proximal peduncle (*pp*) and basal disk (*bd*). Most of the droplets have disappeared from the basal disk. There is still a densely stained layer around the periphery of the cells. The peduncle is virtually unstained. *H. littoralis*

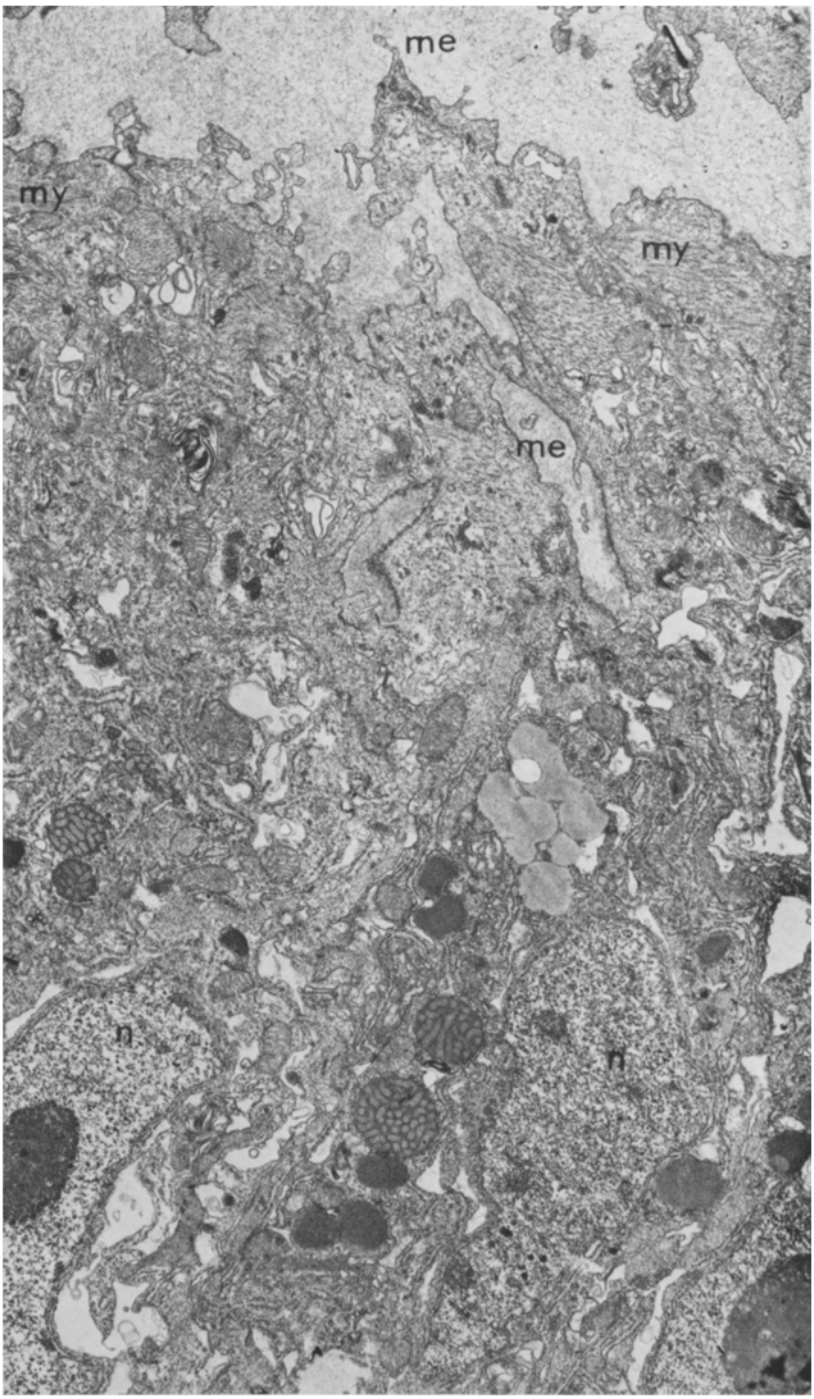

Fig. 2. Low magnification micrograph showing the basal and central regions of glandulomuscular cells. The myonemes (*my*) are located immediately adjacent to the mesoglea (*me*). Nucleus (*n*) $\times 5000$. *H. oligactis*; Glutaraldehyde and osmium

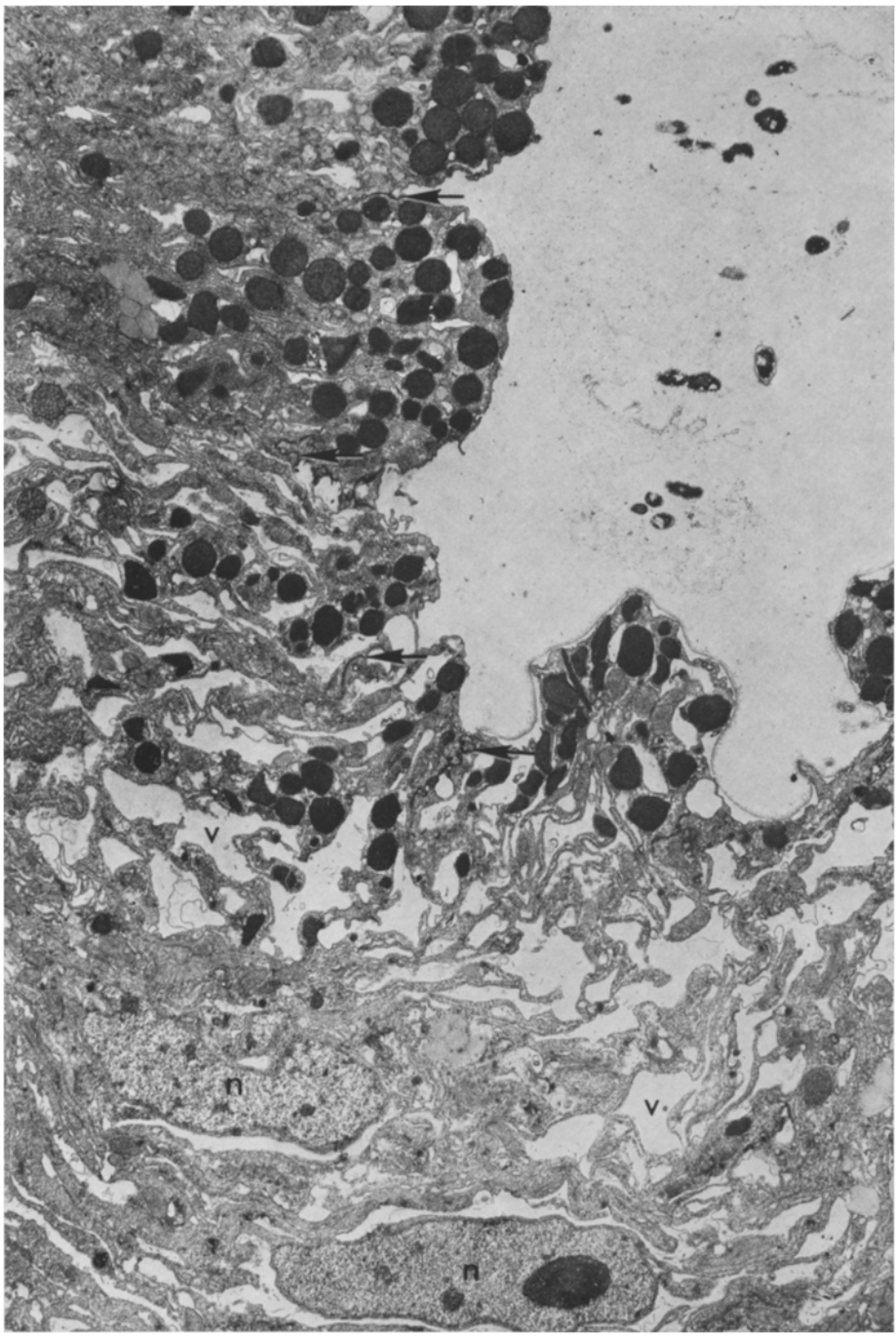

Fig. 3. Low magnification micrograph of the apical regions of glandulomuscular cells containing several droplets and vacuoles (*v*). Two nuclei (*n*) are near the point of extrusion. ×5000. Junctions (septate desmosomes) are seen at arrows. *H. oligactis*; Glutaraldehyde and osmium

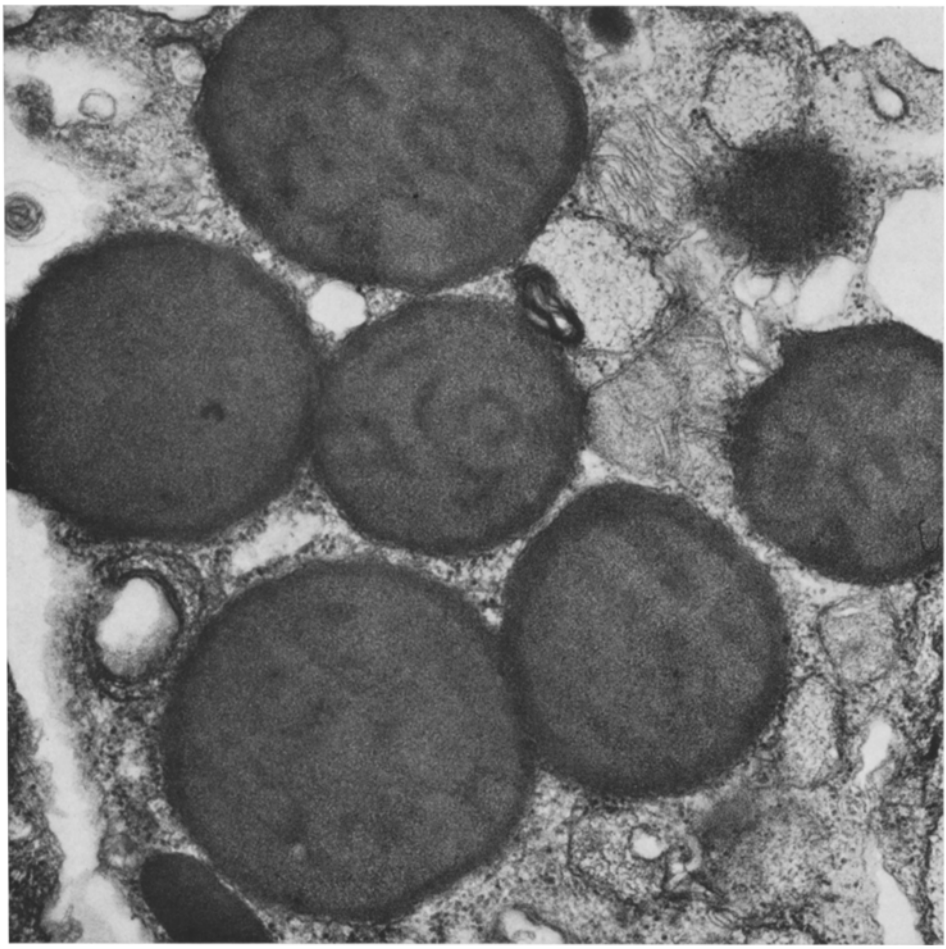

Fig. 4. Dense spherical membrane-bounded droplets (designated Type I droplets) are composed of minute granules. The droplets are approximately 1.25 μ in diameter. Some droplets contain a thin layer of slightly higher density around the periphery. In other droplets, regions of two densities are apparent. ×40600. *H. oligactis*; Glutaraldehyde and osmium

membrane-bounded droplets are spherical or oval, measuring up to 1.25 μ in diameter. Sometimes two or more of the smaller variety fuse to produce droplets of a more elongated form. These electron dense droplets are composed of a finely granular material and in general resemble the mucous droplets located in the apical region of epithelio-muscular cells. Higher magnification reveals that most of the droplets are not homogeneous. They may contain a dense core surrounded by a thin layer of slightly higher density, or may contain a random arrangement of both materials.

Type II: This type of droplet reveals a variety of shapes and sizes, and ranges up to 0.9 μ in diameter (Fig. 5). They are membrane-bounded and are composed

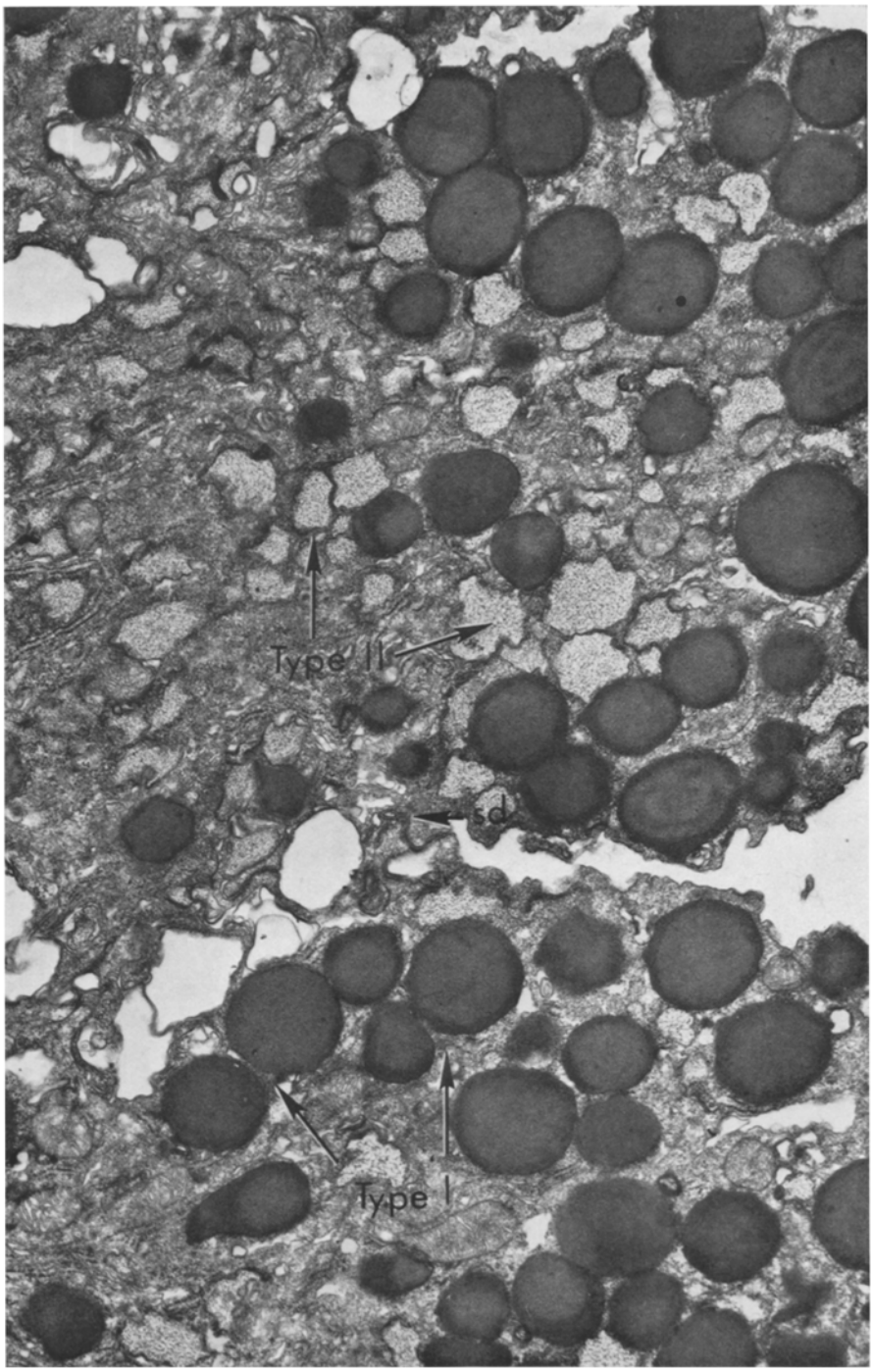

Fig. 5. Type I and Type II droplets located in the apical cytoplasm. Type I droplets are similar to those described in Fig. 4 except that several seem to be in the process of being fused. Type II droplets are membrane-bounded, composed of a fibrillar material of low density and range up to 0.9 μ in diameter. Smaller droplets apparently fuse to form the larger types. Septate desmosome-*Sd.* $\times$ 16000. *H. oligactis*; Glutaraldehyde and osmium

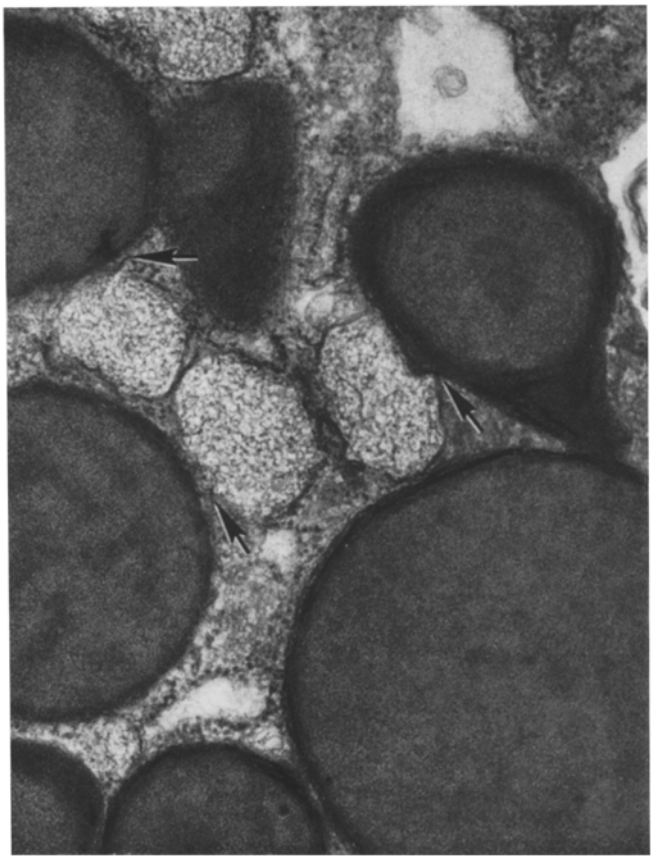

Fig. 6. Fusion (arrows) of Type I and Type II droplets. Some of Type I droplets reveal variations in density suggesting the beginning of Type III droplets. × 40600. *H. oligactis*; Glutaraldehyde and osmium

of a fibrillar material of low density which gives them an overall electron-lucent appearance. The presence of identical materials within the rough E. R. membranes, and the formation of vesiculated rough E. R. from which most ribosomes have disappeared, suggest that Type II droplets may originate solely from rough E. R. Some of the newly-formed droplets fuse to produce larger droplets. Fusion also occurs between Type I and Type II droplets (Figs. 6 and 7), presumably to form the third type of droplet.

Type III: This type of droplet exists in various forms, due perhaps to the different stages of their formation from Type I and Type II droplets (Figs. 8 and 9). The mature droplets are located in the apical cytoplasm and sometimes in close proximity to the plasma membrane. The first indication of their formation is shown in Figs. 6 and 7. The membranes surrounding Types I and II droplets appear to fuse, or at least, are in extremely close contact. Following this contact, the original dense Type I droplets assume various densities and reorganization

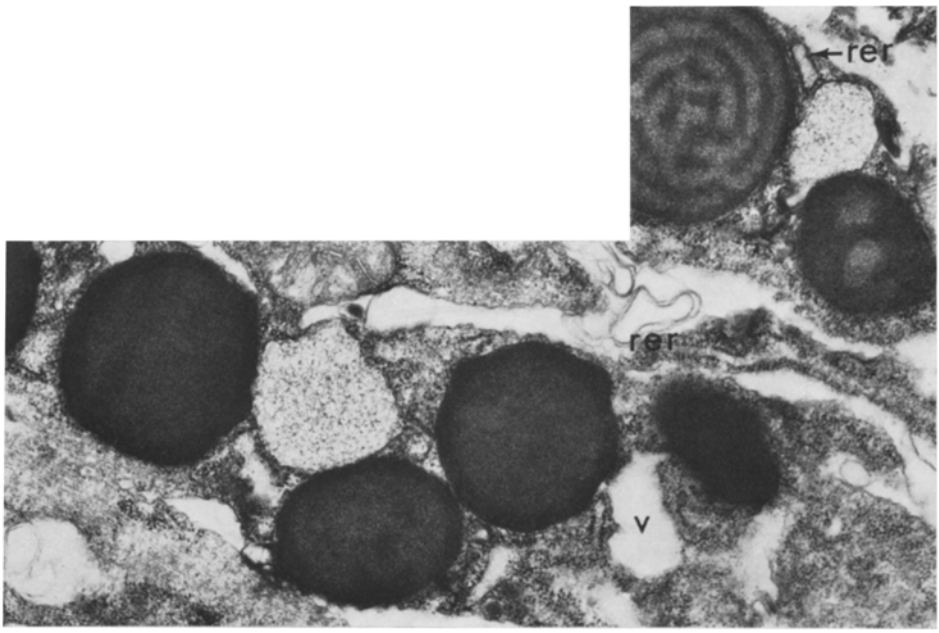

Fig. 7. Fusion (arrows) of Type I and Type II droplets apparently forms Type III droplets. The droplet at the extreme right probably represents the early stage of Type III droplet. Note the similarity in contents of Type II droplet and the rough E. R. (*rer*). Some of the empty vacuoles (*v*) are probably the result of depleted Type II droplets. × 26400. *H. oligactis*. Glutaraldehyde and osmium

of their contents occurs. The contents apparently continue to be reorganized until the mature membrane-bounded droplets are formed (Figs. 8 and 9). The original Type II droplets appear to be almost or completely transferred from their membranes during the formation of the Type III droplets. This is evidenced by the presence of several empty vacuoles of the same size and similarly located as Type II droplets (Figs. 7 and 8). The mature droplets are spherical or oval and range up to 1.5 μ in diameter. The internal contents are composed of alternating dense and moderately dense bands of very finely granular materials.

Type IV: This type of droplet is located always in the apical cytoplasm and sometimes the surrounding membranes are fused to the plasma membrane (Fig. 10). They are of various shapes and sizes, ranging from elongated forms (1.9 × 0.15 μ) to slightly oval types (1.2 μ in diameter). Their contents are extremely dense and finely granular. Within this material are regions of higher density, arranged circularly or linearly, depending on the shape of the droplet. Type IV droplets are similar morphologically to some of the droplets in the epithelio-muscular cells of the peduncular region. Accordingly, it might be assumed that these droplets are not synthesized in the glandulomuscular cells, but like Type I droplets, are present in the original epithelio-muscular cells.

Type V: This type of droplet, averaging 1.0 μ in diameter, may be located in the central cytoplasm among various cell organelles or anywhere in the apical

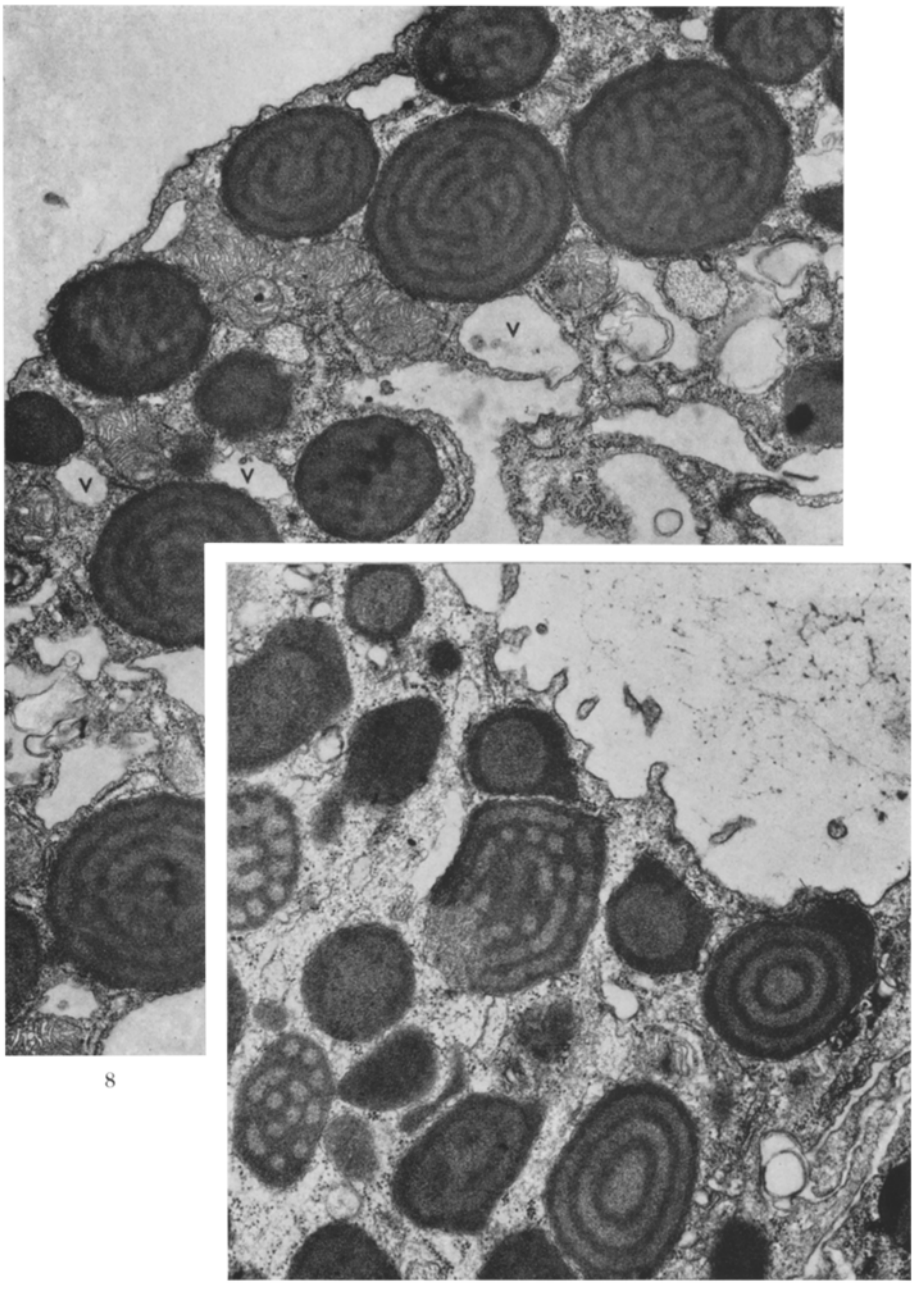

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Fig. 8. Type III droplets and empty vacuoles (*v*), the latter possibly derived from Type II droplets. These spherical droplets containing two densities and composed of minute granules are sometimes arranged alternately. They are membrane-bounded and range up to 1.5 μ in diameter. × 22000. *H. oligactis*; Glutaraldehyde and osmium

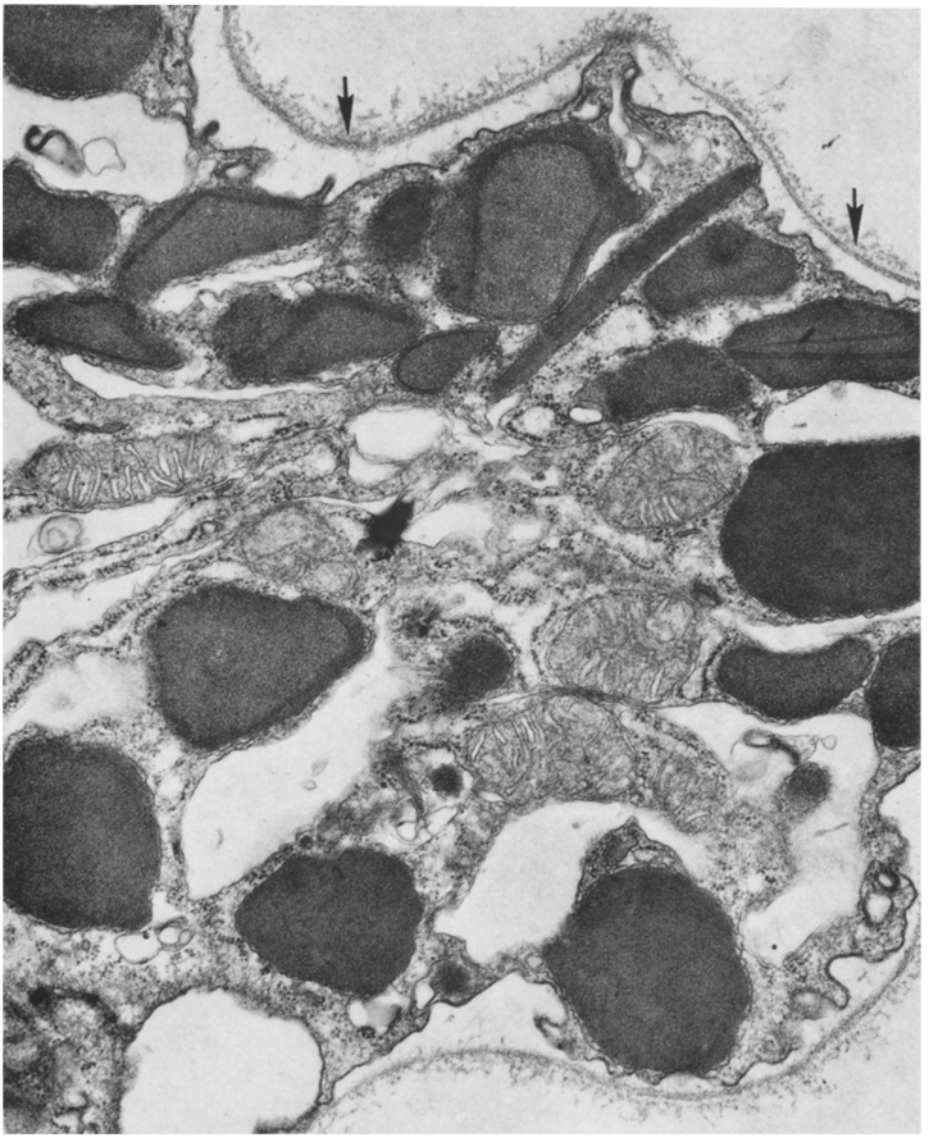


Fig. 10. Type IV droplets assume various sizes and shapes from elongated forms ($1.9 \times 0.15\ \mu$) to somewhat oval types ($1.2\ \mu$ in diameter). They are membrane-bounded and their contents are extremely dense and finely granular. Regions of higher density, arranged linearly or circularly appear within the homogeneous materials. $\times 26400$. Note the secretory material (arrows). *H. oligactis*; Glutaraldehyde and osmium

Fig. 9. Type III droplets probably representing the more mature variety. Two droplets show precise alternating bands of different densities. Other droplets, sectioned in a different plane show what may be the forming structured layers. $\times 26400$. *H. oligactis*; Glutaraldehyde and osmium

one-half of the cell. The membrane surrounding the mature droplets reveals several indentations, indicative of the completed fusion of small vesicles (Fig. 11). This process is illustrated at greater advantage in some immature droplets in which the periphery of the droplets has a rosette appearance (Inset, Fig. 11). The contents of the mature droplets are composed of a dense granular material. Circular or elliptical areas of low density (approximately 700 Å in diameter) are scattered throughout the dense material.

The formation of these droplets appears to involve the participation of Golgi complexes and rough E. R. (Figs. 11 and 12). Several small vesicles, derived from the Golgi membranes, fuse with blebs from the rough E. R. This fusion results in the formation of droplets and continues until the mature droplets are formed.

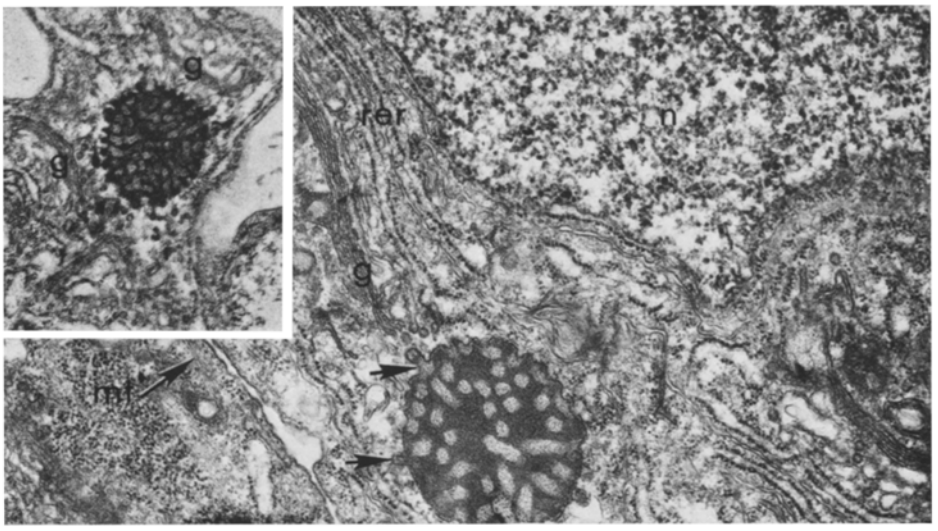
Type VI: Droplets of this type are approximately 1.3 μ in diameter (Fig. 13). They may be located anywhere from the central region of the cytoplasm to the peripheral limits of the cell. The membranes surrounding the droplets are irregular, due to the fusion of several small vesicles. The droplets themselves are composed of a moderately dense, finely granular matrix. Many circular or oval regions of low density (approximately 700 Å in diameter) occupy most of the droplets (Fig. 13). Golgi complexes and rough E. R. are apparently responsible for the synthesis of these droplets.

Type VII: Strictly speaking, this type of droplet should not be classified in a similar manner as all the other droplets which are products of the cells. For example, Type I and Type IV droplets are synthesized in the original epitheliomuscular cells and all the other droplets (Types II, III, V and VI) are formed in the transformed cells (glandulomuscular cells). The presence of these droplets, however, deserves a brief mention. They may be located anywhere in the cell, from immediately adjacent to the myonemes to the apical cytoplasm (Fig. 14). Individual droplets range up to 1.3 μ in diameter, but usually, the smaller spherical ones fuse together to form larger irregularly-shaped droplets. These are the only type of droplet which does not contain a limiting membrane. They are of low density, homogeneous in nature and resemble lipid droplets. The presence of similar droplets in the basal disk digestive cells (Fig. 14) and the occasional appearance of the same type of droplets in the mesoglea, suggest that these droplets are actually derived from digestive cells and are of a lipid nature.

The structure of glandulomuscular cell droplets depends in large measure on the species of hydra studied and the method of fixation. The latter factor concerns the use of double-fixation with glutaraldehyde and osmium tetroxide or osmium tetroxide alone. In all cases, double-fixation provides a much better preservation of the droplets.

The description of the ultrastructure of glandulomuscular cells and the various droplets presented above were made from *Hydra oligactis*. The structure of the cells in *Hydra pirardi* is similar to that of the previous species, except that the apical regions of the basal disk epithelium and the glandulomuscular cells themselves contain deep folds (Fig. 15). This condition may even be exaggerated depending on the state of the animal, that is, whether it is relaxed or contracted.

The droplets occur principally along the limits of the plasma membrane, although in other preparations, numerous droplets are located throughout the



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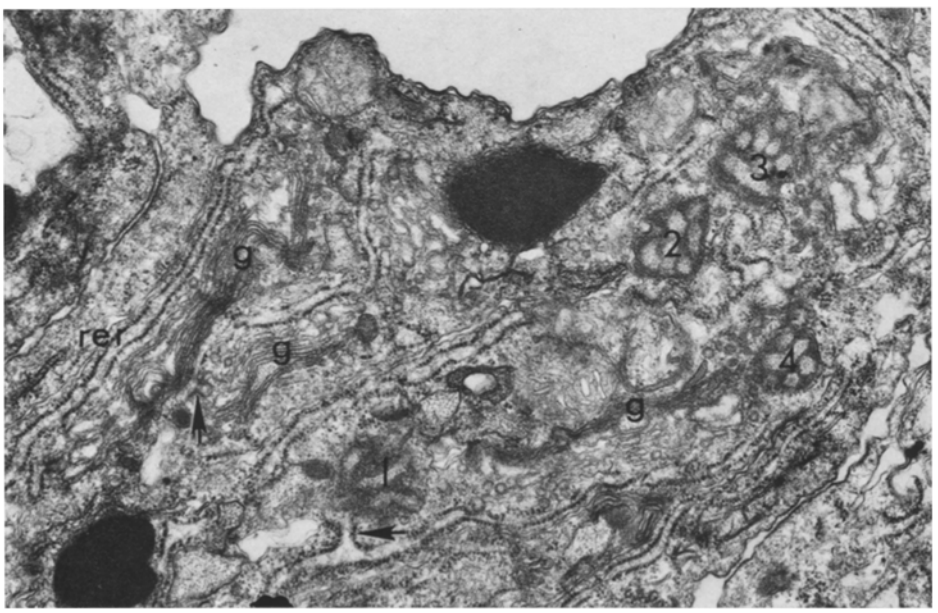

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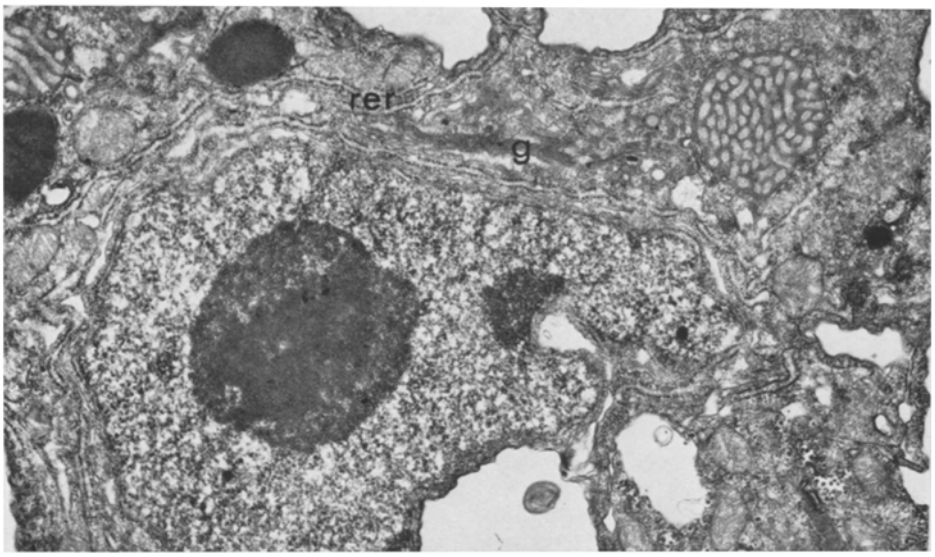
Fig. 11. Type V are membrane-bounded and average 1.0 μ in diameter. Note the irregular contour of the surrounding membrane and the attachment of a small vesicle (arrow). The contents are composed of a dense, finely granular material with circular or elliptical areas (about 700 Å in diameter) scattered throughout the dense matrix. Golgi complexes (*g*), rough E. R. (*rer*), microtubules (*mt*), nucleus (*n*). × 26400. *H. oligactis*; Glutaraldehyde and osmium. Inset: Type V droplet showing the irregular contour of its periphery during the intermediate stage of formation. Golgi complexes (*g*); × 14000. *H. oligactis*; Glutaraldehyde and osmium

Fig. 12. Golgi complexes (*g*) and rough E. R. (*rer*) are intimately associated during the formation of Type V droplets. Numerous small vesicles from both organelles and blebs (arrows) from rough E. R. fuse to form the early Type V droplets (*1*, *2*, *3*, and *4*). × 26400. *H. oligactis*; Glutaraldehyde and osmium



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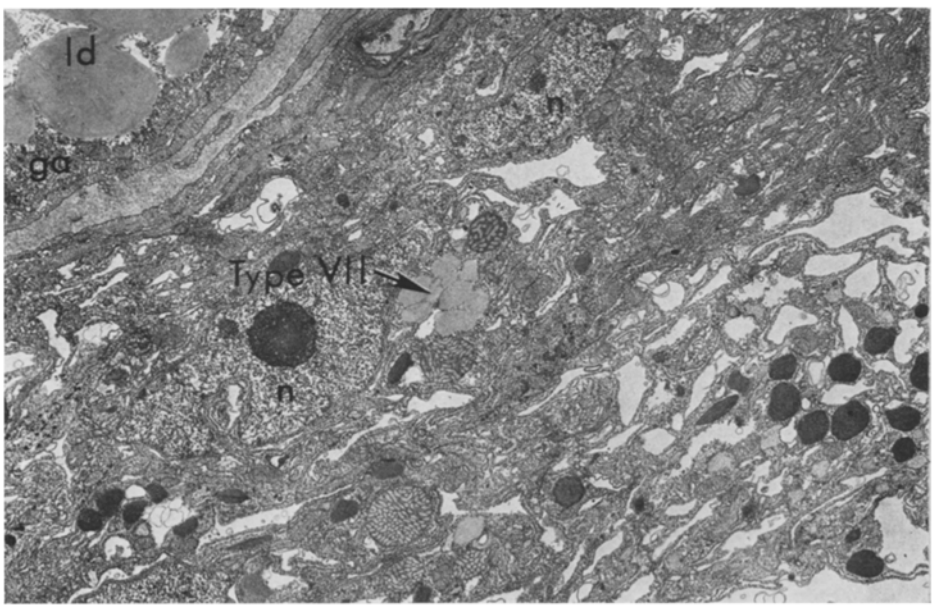

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Fig. 13. Type VI are membrane-bounded droplets, average 1.3 μ in diameter. Golgi complexes (*g*) and rough E. R. (*rer*) are closely associated during the formation of these droplets. The droplets are composed of a moderately dense, finely granular material in which are many elongated regions (700 Å in diameter) of low density. The latter areas consist of granular and fibrillar materials. × 16000. *H. oligactis*; Glutaraldehyde and osmium

Fig. 14. Type VII droplets may exist singly or as an accumulation of smaller droplets (up to 1.3 μ in diameter). They are the only droplets which are not membrane-bounded and the only type not synthesized in the cells. The similarity of their low density with the larger lipid droplets (*ld*) of the gastrodermis (*ga*) and the appearance of identical droplets in the mesoglea indicate that Type VII droplets are probably derived from the gastrodermis. Nucleus (*n*). × 5000. *H. oligactis*; Glutaraldehyde and osmium

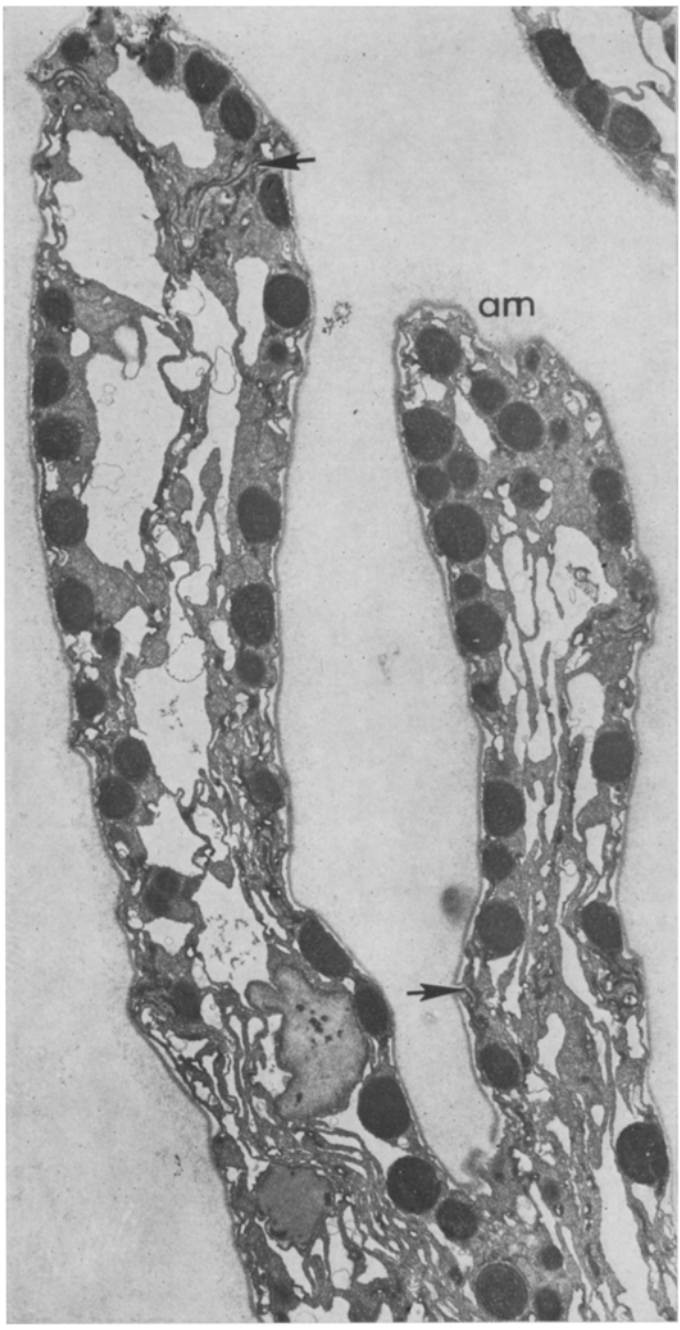

Fig. 15. Apical basal disk epithelium and glandulomuscular cells in some species sometimes contain deep folds. The droplets are located peripherally along the plasma membrane. Several large vacuoles are present in the same region. Note the adhesive material surrounding the cells (*am*) and junctions (arrows) which are the septate desmosome type. ×5700. *H. pirardi*; Glutaraldehyde and osmium

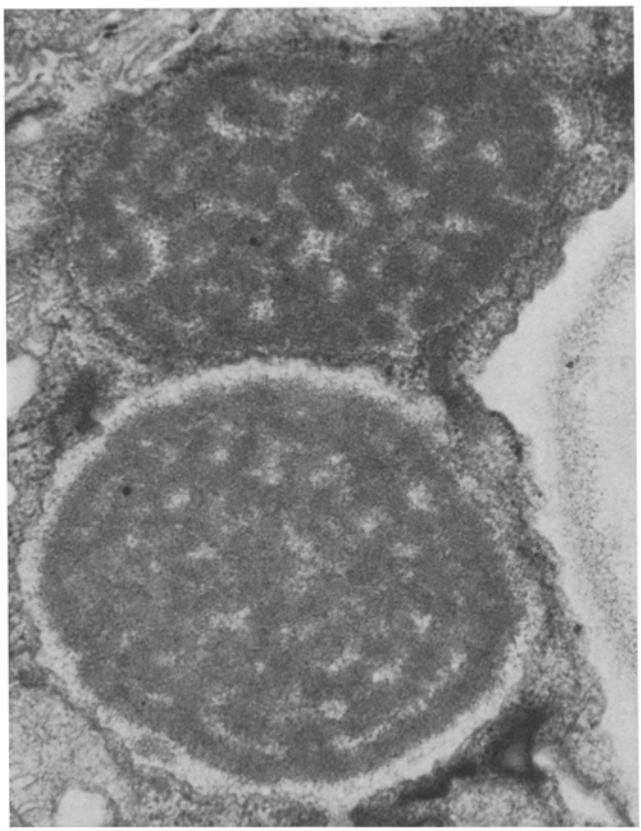

Fig. 16. Higher magnification of the same droplets in Fig. 15. They are membrane-bounded and approximately 1.4 μ in diameter. The droplets are of a dense, granular material with unorganized areas of lesser density. Fibrillar components are also observed in the lighter areas. These are comparable to Type III droplets (Figs. 8 and 9). Note the concentration of the filamentous materials surrounding the cell. ×40800. *H. pirardi*; Glutaraldehyde and osmium

apical one-fourth of the cytoplasm. The droplets are spherical or oval, membrane-bounded and are approximately 1.4 μ in diameter. Most of the droplet is of a dense, granular material with unorganized areas of lesser density (Fig. 16). Granules and fibrillar materials are located in the latter areas and especially in the clear region between the periphery of the droplet and the surrounding membrane. These droplets are considered to be Type III droplets.

The droplets shown in Figs. 15 and 16 were photographed from animals (*Hydra pirardi*) which were fixed in glutaraldehyde and post-fixed in osmium tetroxide. Examination of the droplets from animals (same species) fixed only in osmium tetroxide reveals wide, clear spaces around the droplets, receded or ruptured membranes and several "empty" spaces in the droplet material. Similar conditions exist in *Hydra oligactis* fixed by both methods mentioned above.

Myonemes: The basal regions of glandulomuscular cells containing the myonemes are similar to epithelio-muscular cell bases. The most basal cytoplasm is free of myonemes but occasionally such cell organelles as mitochondria, rough E. R. and ribosomes are present (Fig. 17). This region of the cytoplasm usually contains several projections which extend into the mesoglea, sometimes approaching similar extensions from the gastrodermal cells. The mesoglea, on the other hand, frequently makes deep insertions among the epidermal cells. The observations on the mesoglea will be reported later. On the side of the plasma membrane facing the mesoglea there are occasional patches of extremely thin, closely-packed filaments approximately 1100 Å in height (Inset, Fig. 17). They are oriented perpendicularly to the mesoglea and may be considered as "anchor" filaments.

It was indicated earlier that the apical regions of the glandulomuscular cells are attached to adjacent cells by means of septate desmosomes. The same type of junction is also present between adjacent epithelio-muscular cells along the body column. In the myoneme-containing regions, however, the cells form junctions which have been described as structurally similar to intercalated discs found in vertebrate cardiac muscle (Haynes *et al.*, 1968, Fig. 18). The interdigitating membranes of adjacent cells are separated by a space of approximately 200 Å wide. On the inner surfaces of the junctional membranes is a dense material through which the filaments of the myonemes pass.

The myonemes are cylindrical or slightly flattened structures with a diameter of 1–2 μ (Figs. 17 and 18). The filaments composing the myonemes of epithelio-muscular cells have been described as being of two types: (1) thick filaments (200 Å in diameter) and, (2) thin filaments (60 Å in diameter; Haynes *et al.*, 1968). Both types of filaments are also present in the glandulomuscular cell myonemes, but in addition, there are filaments of intermediate sizes (Figs. 17 and 18). Occasionally a few extremely dense fibers (up to 770 Å in diameter) appear among the filaments. These fibers closely resemble certain epithelio-muscular cell extensions which have been observed in the mesoglea (Davis and Haynes, 1968).

Most of the filaments in some cells are oriented along the longitudinal axis of the myonemes. Microtubules appear frequently among the filaments and are similarly aligned (Fig. 17). The myonemes themselves are oriented radially with respect to the aboral pore. The filaments in other cells are not so orderly arranged but instead, assume a disorganized appearance (Fig. 18).

Scattered among the filaments and generally in the extreme basal cytoplasm are certain droplets which have never been described previously in the glandulomuscular cells or their epithelio-muscular cell precursors. The droplets are dense, membrane-bounded and range from 800–1100 Å in diameter (Figs. 17 and 18). They bear a striking resemblance to neurosecretory droplets of neurosecretory and neurosensory cells (Davis *et al.*, 1968; Davis, 1969). These two types of nerve cells are in close proximity to the glandulomuscular cells.

The proximal movement of the glandulomuscular cells eventually results in their dislodgment and death. The myonemes of the cells at the aboral pore or close to this area begin to degenerate such that none of the typical filaments are recognized (Fig. 19). The thin 1100 Å long anchor filaments also begin to deteriorate and eventually become indiscernible. At the same time the junctions which once held the myonemes together disappear, thus providing an unrestricted route from

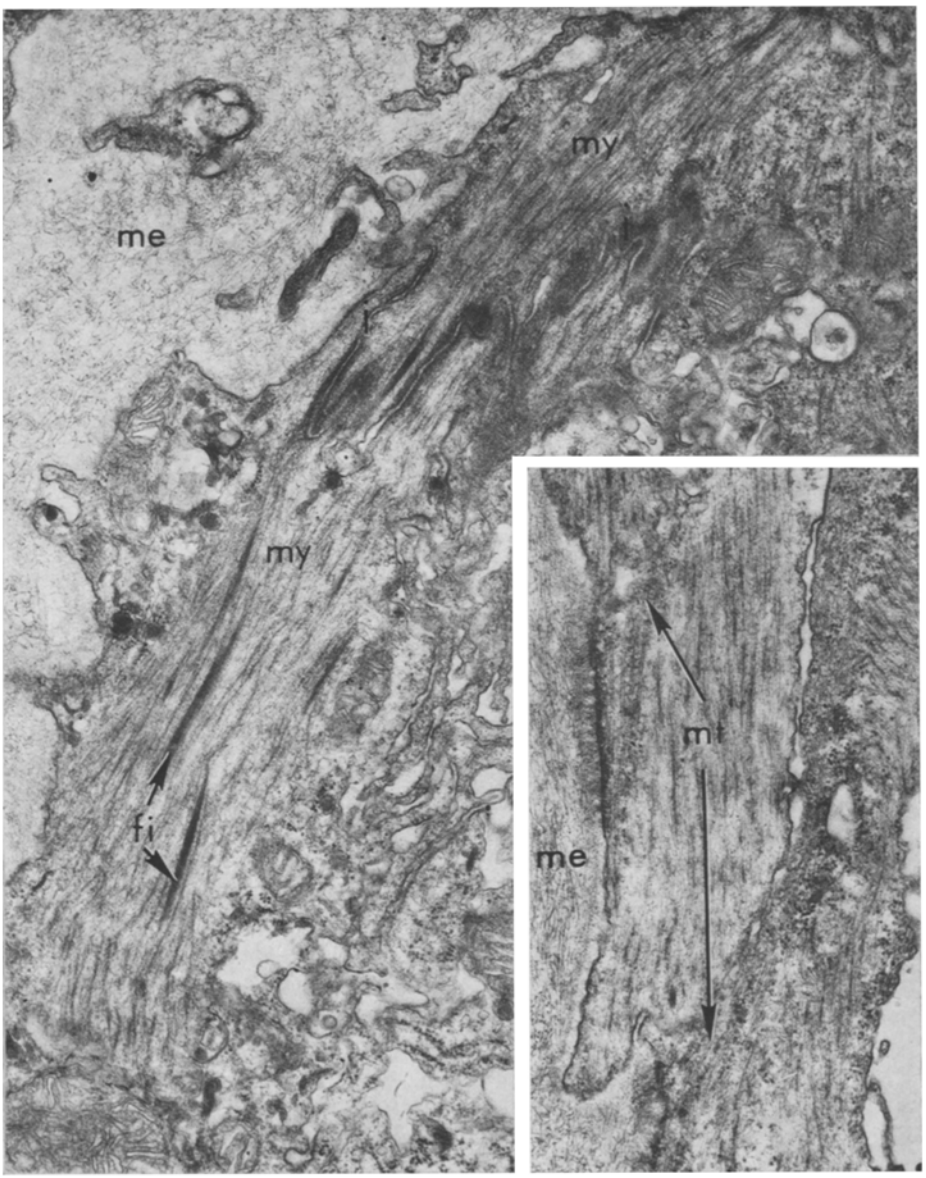

Fig. 17. Myonemes (*my*) in the basal cytoplasm (adjacent to the mesoglea, *me*) of two adjacent cells. The interdigitating membranes at the ends of the myonemes form junctions (*j*) described as being similar to intercalated disks of vertebrate cardiac muscle. The junctional membranes are separated by a 200 Å wide space. There are thick (200 Å in diameter) and thin (60 Å in diameter) parallel filaments, similar to those in epithelio-muscular cell myonemes. Filaments of intermediate sizes and large fibers (*fi*; 770 Å in diameter) are also present. These fibers have not been observed among the typical filaments of epithelio-muscular cell myonemes. Note the peculiar appearance of dense, membrane-bounded droplets (800–1100 Å in diameter) located near the filaments and in some cases near the plasma membrane. These droplets are strikingly similar to neurosecretory droplets. ×26400. *H. oligactis*; Glutaraldehyde and

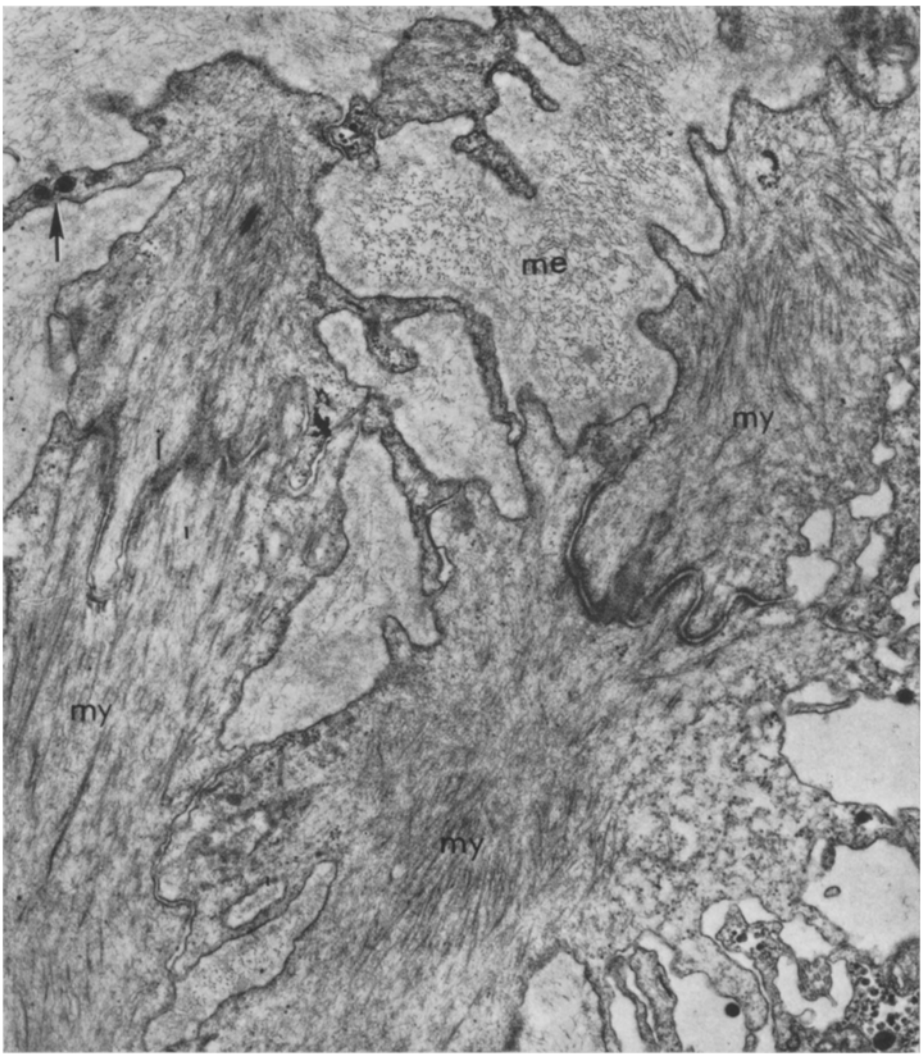

Fig. 18. Myonemes (*my*) and specialized junctions (*j*) between myonemes. Long cytoplasmic processes extend into the mesoglea (*me*). Note that most of the filaments assume a disorganized appearance. Dense, membrane-bounded droplets, 800 Å in diameter, are located in the thin cytoplasmic process (arrow) extending into the mesoglea × 26400. *H. oligactis*; Glutaraldehyde and osmium

osmium. Inset: Myonemes showing thick and thin filaments with microtubules (*mt*) among them. Note the thin fibers (1100 Å in height) oriented perpendicularly from the myonemal plasma membrane and extending into the mesoglea (*me*). × 26400. *H. oligactis*; Glutaraldehyde and osmium

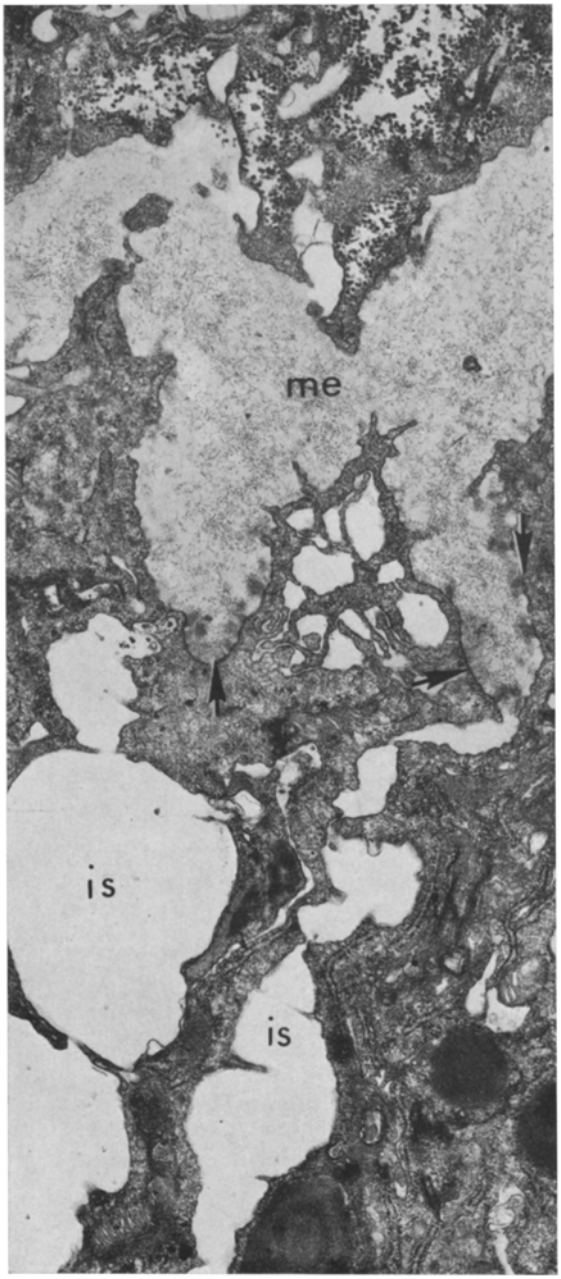

Fig. 19. Degenerative changes of glandulomuscular cells involve loss of myonemal junctions such that there is an unobstructed passage from the mesoglea (*me*) to the enlarged intercellular spaces (*is*). The 1100 Å filaments (arrows) also begin to be resolved. The thick and thin filaments are no longer recognizable. Gastrodermis (*ga*). × 16200. *H. oligactis*; Glutaraldehyde and osmium

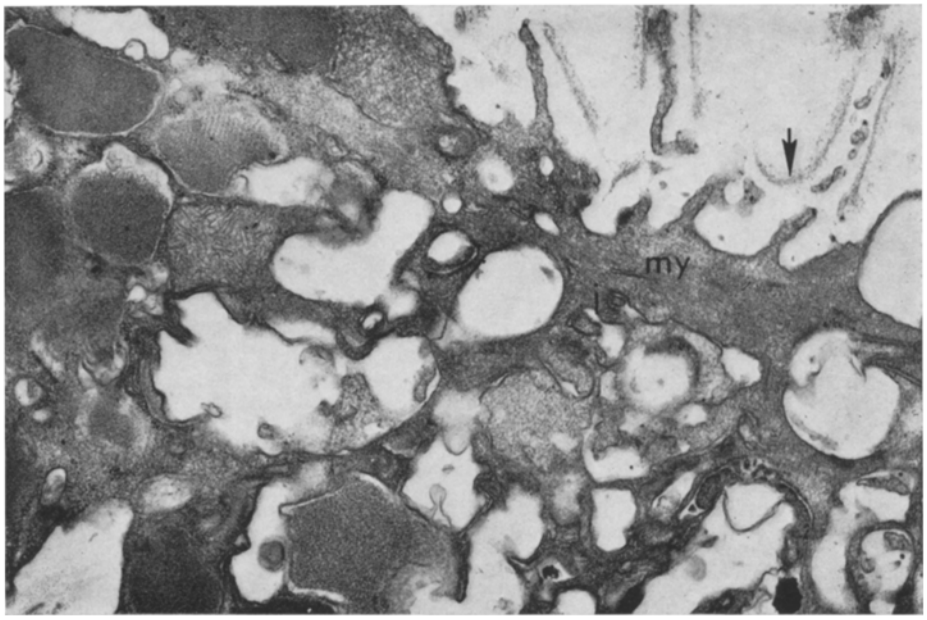

Fig. 20. Myoneme (*my*) which has been forced from its original position (adjacent to the mesoglea) to the other extreme position, i.e. adjacent to the adhesive secretion (arrow) of the basal disk. Some of the specialized myonemal junctions (*j*) are still present. Note that the thick filaments and large fibers have disappeared. The remaining filamentous-like material may be degenerating thin filaments. ×26400. *H. oligactis*; Glutaraldehyde and osmium

the mesoglea to the lateral intercellular spaces. These spaces become extremely large and with the formation of several vacuoles, the cells acquire an "empty" appearance (Fig. 19). The nuclei and cytoplasmic components also undergo striking changes as evidenced by the appearance of pycnotic nuclei and degradation of all cell organelles. An interesting observation is the appearance of intramitochondrial crystals. Similar types of crystals were reported in the epitheliomuscular cells only of regenerating and sexual animals (Davis, 1968). These observations were interpreted as a response to cellular damage or as a mechanism for storing materials temporarily. Their appearance in these cells may be as a result of the former conditions.

The destruction of all glandulomuscular cells does not follow the same pattern as described above. In some instances, the myoneme-containing regions still attached to neighboring myonemes, are distinguishable at the extreme limits of the animal where the adhesive secretion is located (Fig. 20). The thick filaments disappear and the remaining filamentous-like material may be the degenerating thin filaments.

Discussion

The epithelio-muscular cells of the gastric column are gradually forced proximally to the basal disk. During this sojourn, the cells are transformed into

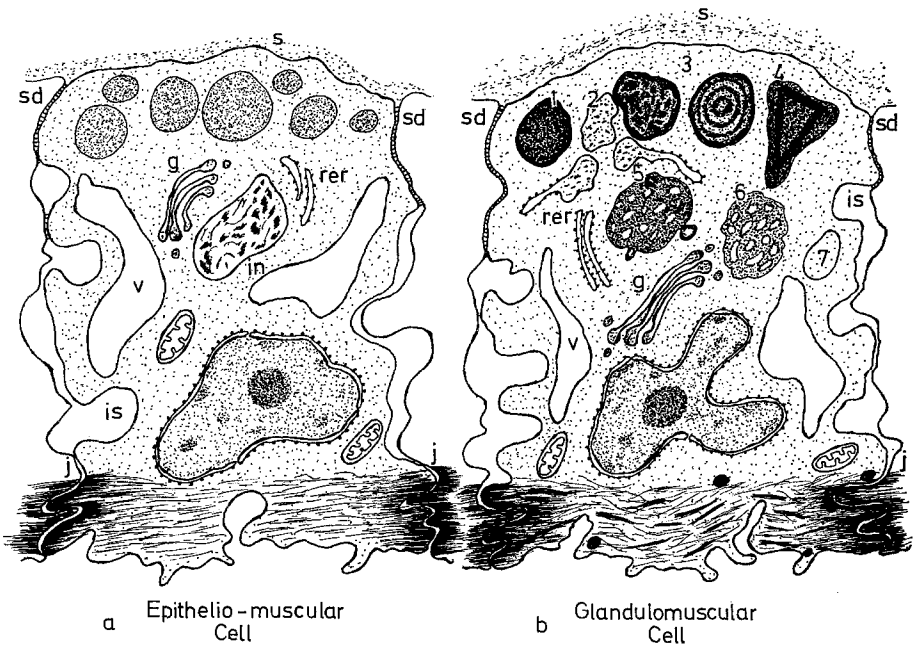

Fig. 21a and b. Diagram to illustrate some of the basic similarities and differences between the typical epithelio-muscular cell and the glandulomuscular cell. a) Epithelio-muscular cell contains only mucous droplets of various sizes located along the apical margins of the cell. The basally located nucleus may be oval or slightly irregularly-shaped. The filaments of the myonemes are generally of two types: i) thin filaments (60 Å in diameter) and ii) thick filaments (200 Å in diameter). They are parallel to the longitudinal axis of the animal. b) Glandulomuscular cells contain numerous droplets which occupy the apical one-third to one-half of the cells. There are at least seven types of droplets (numbered 1–7), one of which may have some similarities to the mucous droplets seen in epithelio-muscular cells. The increase in number and types of droplets is reflected in the striking increase in elements of rough E. R. and Golgi complexes. The nucleus of these cells is generally irregularly-shaped and contains a conspicuous nucleolus. In addition to the same two types of muscle filaments observed in epithelio-muscular cells, there are filaments of intermediate sizes as well as larger (770 Å) fibers. The filaments vary from being parallel to the longitudinal axis of small; dense membrane-bounded droplets (800–1100 Å in diameter) are seen often among the filaments. In both cases, the individual cells are attached to their neighboring cells by septate desmosomes (*sd*) in the apical region and junctions (*j*), described as similar to intercalated disks in the myonemal regions. Vacuoles (*v*); intercellular spaces (*is*); Golgi complex (*g*); rough E. R. (*rer*); Inclusion (*in*); Secretory material covering the apical region (*s*)

glandulomuscular cells (Fig. 21). Evidences for this transformation are presented in light and electron microscopic observations. Each cell type is easily distinguishable on the basis of its morphology. One problem, however, concerns the lack of evidence for "transitional" type cells at the junction between the peduncle and basal disk. Studies are presently underway in a further attempt to determine the presence of "transitional" cells in the early stage of transformation.

The considerable concentration of droplets in these cells of the basal disk, which is the region of the animal that attaches to a substrate, suggests that the droplets provide the adhesive secretion for attachment. The droplets seem to be of different chemical compositions. The toluidine blue stained material shows a drastic line of demarcation between the droplets of the peduncle and the remainder of the body column with those of the basal disk cells. It is suggested, therefore, that few of the typical mucous droplets of epithelio-muscular cells are present in glandulomuscular cells. The light micrographs also indicate that some droplets are alcian blue positive, contain hyaluronic acid and according to Philpott *et al.* (1966) some are PAS positive. These evidences suggest the presence of an acid mucopolysaccharide, the adhesive agent, not formed elsewhere in the epidermal cells (Burnett, 1966; Philpott *et al.*, 1966).

The electron microscopical studies indicate that the glandulomuscular cells may contain as many as seven morphologically different droplets. Furthermore, except for the lipid droplets which appear to be derived from the neighboring digestive cells, all the droplets are synthesized in the cells. The synthesis involves the activities of the Golgi complexes and rough E. R., which are highly developed as compared to similar organelles in the precursor epithelio-muscular cells. In one instance, the droplets (Type II) appear to be formed exclusively from the rough E. R.

Type I droplets resemble closely the mucous droplets of the epithelio-muscular cells. This structural resemblance suggests that Type I droplets may not be the result of "new" synthesis in glandulomuscular cells. Instead, it is possible that such droplets were present and retained during the transformation of epithelio-muscular cells to glandulomuscular cells. In the latter case, these droplets could be modified chemically such that even though there is a degree of structural similarity, the chemistry of the droplets is different. We believe this suggestion to be the most plausible explanation in view of the fact that although numerous sections have been examined, there was not a single indication of their formation as was observed for other droplets. It should be mentioned, however, that their synthesis in epithelio-muscular cells involves the participation of Golgi complexes and rough E. R.

Evidence for the synthesis of Type IV droplets has not been observed and therefore may also be considered as having been derived from precursor epithelio-muscular cells of the peduncle. The similarity of these droplets to some droplets in the peduncular cells may only be on a structural basis. The chemical compositions of the droplets may be different.

The description presented for Type V and Type VI droplets indicate that there are several similarities in so far as their location, structure and origin are concerned. They differ in that Type VI droplets are larger, contain only a moderately dense, granular matrix and many more areas of low density. However, the similarities are sufficiently distinctive to suggest that both types of droplets may be slight variations of one basic type.

The myonemes and their component filaments are similar to those of epithelio-muscular cells. However, in addition to thick (200 Å in diameter) and thin (60 Å in diameter) filaments (Haynes *et al.*, 1968), glandulomuscular cells contain filaments of intermediate sizes and large fibers (ranging up to 770 Å in diameter)

which give the appearance of greater filament development. These myonemes, so long as their filaments and junctions remain intact, are apparently capable of contraction which aids in the shortening of the body column. Their contraction and relaxation may also function in opening and closing the aboral pore.

The glandulomuscular cells are considered as the end point of the proximally directed epithelio-muscular cells of the growth region. According to Burnett (1966), epithelio-muscular cells of the above region divide frequently and elaborate longitudinal myonemes and mucous droplets. When these cells reach the peduncular region they lose the ability to divide, and ultimately in the basal disk, they are transformed into glandulomuscular cells prior to being discarded. Certain questions arise concerning the mechanism which initiates the process of transformation and also the degenerative activities of glandulomuscular cells even before they are sloughed off the basal disk.

At the present time, these questions are not easily answered from a factual point of view, and therefore we can only resort to speculative interpretations. The process of transformation may be related in some way to the presence of nerve cells in the peduncle and basal disk. As will be demonstrated in the second paper of this series, the above regions are highly concentrated with nerves, many of which contain neurosecretory droplets. This concentration indicates that cells in these regions are in extremely close contact with nerve cells. Furthermore, the location and orientation of many nerve cells are such that neurites containing neurosecretory droplets are immediately adjacent to the myonemes. This intimate spatial relationship could possibly explain the appearance of small, dense membrane-bounded droplets in the glandulomuscular cells, particularly in the myonemes. These droplets are morphologically indistinguishable from neurosecretory droplets, and although it is tempting to consider both droplets as one and the same, there is not sufficient data to warrant this conclusion. There are some evidences, however, to indicate that the small dense droplets are not synthesized by the glandulomuscular cells. Instead, they are seen in the intercellular spaces and are phagocytized at the base of the glandulomuscular cells especially near the myonemes. Once the droplets are incorporated into the cell, they apparently remain preferentially near the plasma membrane. Their ultimate fate is uncertain.

Previous studies on *Hydra* have suggested that neurosecretory materials may control growth and cellular differentiation (Burnett, 1966; Davis *et al.*, 1968; Davis, 1969; Lentz, 1966). It seems conceivable, therefore, that the mechanism of transformation of peduncular epithelio-muscular cells into glandulomuscular cells may be regulated to some extent by neurosecretion. Finally, the factors which initiate cell death in the basal disk are not understood. It is generally believed that normal glandulomuscular cells are sloughed off the basal disk, and eventually die. Inasmuch as several cells may be lost in this manner, the present study indicates that many, if not most glandulomuscular cells die *in situ* and ar *then* discarded.

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