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Ultrastructural Evidence for the Presence of Nerve Cells in the Gastrodermis of *Hydra*

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Summary. Two types of nerve cells, namely, neurosensory and neurosecretory cells have been identified and described in the *gastrodermis* of *Hydra pseudoligactis*. The morphological criteria used for the identification of gastrodermal nerves are based on those presented previously for epidermal nerves. The third type of nerve cell in the epidermis, ganglionic cells, was not observed in these studies. The distribution, function and origin of gastrodermal nerve cells are discussed briefly.

Key-Words: Gastrodermal nerves — *Hydra pseudoligactis* — Electron microscopy.

Introduction

Most of the morphological studies concerning the nervous system of *Hydra* have been limited to epidermal nerve cells. This is probably due to the fact that the epidermal nerve plexus is larger, contains many nerve cells and the nerve cells composing the plexus are larger in size. It has been shown that the epidermis contains three types of nerve cells: ganglionic, neurosensory and neurosecretory cells (Burnett and Diehl, 1964a; Burnett, Diehl and Diehl, 1964; Lentz and Barrnett, 1965; Davis, Burnett and Haynes, 1968). The ultrastructure of mature cells is fairly-well known and criteria for their identification have been presented (Davis, Burnett and Haynes, 1968).

The origin of epidermal nerves has been the subject of several investigations. Ultrastructural studies confirm earlier histological findings that at least two types of nerve cells (ganglionic and neurosensory) originate from interstitial cells (Lentz, 1965; Davis, 1969; Davis 1971). It has been assumed that the third type of nerve cell—the neurosecretory cell—also arises solely and independently from interstitial cells.

Little is known about gastrodermal nerve cells, except that brief references have been made as to their existence (Hyman, 1940; Lentz, 1966; Burnett and Diehl, 1964a). Virtually nothing is known about the types of nerve cells, their origin, location, distribution, possible concentration in specific areas, and especially their function. This paper presents a preliminary report on the definite existence of certain types of gastrodermal nerves, their ultrastructure and possible origin.

Materials and Methods

The animals used in these studies were normal non-budding *Hydra pseudoligactis*. They were cultured by a modified method of Loomis and Lenhoff (1956). All animals were starved for 24 hours prior to fixation. They were fixed for periods up to 1 hour in cold 3–6% glutar-

* With the technical assistance of Linda M. Bookman.

aldehyde buffered with 0.1 M sodium cacodylate at pH 7.3 (Sabatini, Bensch and Barrnett, 1963). During fixation, short segments were excised from the hypostome, mid-gastric regions and basal disk. Tissues were washed in buffered sucrose solution (in 0.1 M sodium cacodylate) and then post-fixed for 30–45 minutes in cold 1% osmium tetroxide containing sucrose (Caulfield, 1957). The fixed tissues were dehydrated rapidly in alcohol and embedded in Maraglas (Spurlock, Kattine and Freeman, 1963).

Sections were cut with glass or diamond knives on a Cambridge ultramicrotome and mounted on carbon-coated Formvar-filmed grids. The sections were stained with uranyl acetate (Watson, 1958) followed by lead citrate (Reynolds, 1963). Sections were photographed with a RCA-4 electron microscope.

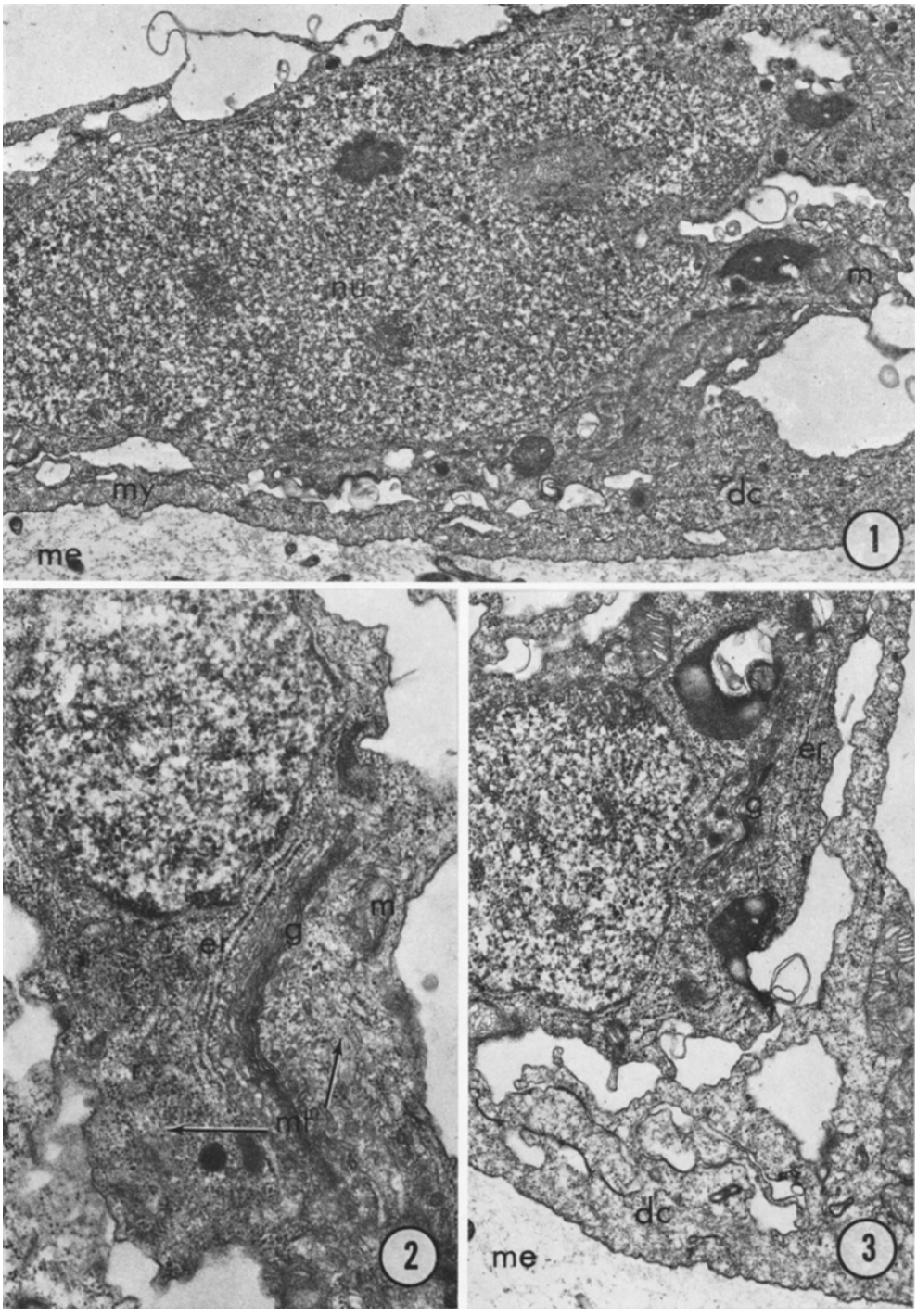
Observations and Results

The identification of gastrodermal nerves is based primarily on the structural criteria used for the identification of epidermal nerves. It is recognized, however, that it is entirely possible that some gastrodermal nerve cells may exhibit certain peculiar characteristics and therefore are not readily identified as nerves. This is only a slight possibility because uncertainties of cell identification are minimal due to the presence of only a few cell types in this layer. The gastrodermis is composed mainly of digestive, gland and mucous cells. A fourth cell type, namely, basal reserve cells, is also found in this species. These cells are similar in some respects to interstitial cells of the epidermis. They contain numerous free ribosomes, a few mitochondria and sparse segments of rough endoplasmic reticulum.

Cells identified as immature and mature neurosecretory cells are shown in Figs. 1–4. The nucleus of the immature cell (Fig. 1) is finely granular and homogeneous in appearance. The cytoplasm contains numerous ribosomes, few small droplets (900–1300 Å in diameter), Golgi complexes in various stages of development, few mitochondria and sparse segments of rough endoplasmic reticulum (Figs. 1–3). Larger dense droplets (0.5–1.0μ in diameter) typical of epidermal nerve cells are also present. A mature neurosecretory cell is shown in Fig. 4. The nucleus reveals dense chromatin material dispersed throughout the nucleoplasm. The cytoplasm contains several active Golgi complexes, secretory droplets (1100 to 1600 Å in diameter), microtubules and the same organelles described in the less mature cell (Fig. 1). The location of these cells is similar to that of their epidermal counterparts, in that, they lie immediately adjacent to the myonemes. In this case, however, the myonemes are the circular myonemes of the digestive cells (Fig. 1). In all instances which have been observed, the neurosecretory cells are surrounded mostly by digestive cells and occasionally are observed in close association with other nerves.

The second type of nerve cell observed in the gastrodermis is the neurosensory cell. Figs. 5 and 6 show two young cells located at the base of digestive cells. In

Figs. 1–3. Portions of immature neurosecretory cells located immediately adjacent to the circular myonemes (*my*) of digestive cells (*dc*). Fig. 1. The nucleus is finely granular and a prominent nucleolus is usually present (*nu*). The cytoplasm contains ribosomes, few mitochondria (*m*), small dense droplets (900–1300 Å in diameter). Mesoglea–*me*. 15000×. Fig. 2. Developing Golgi complex (*g*) and rough endoplasmic reticulum (*er*), microtubules (*mt*), mitochondria (*m*) and ribosomes (*r*) in neurite. 28600×. Fig. 3. Active Golgi complex (*g*) during the formation of secretory droplets, rough endoplasmic reticulum (*er*) and dense bodies. Digestive cell–*dc*; Mesoglea–*me*. 21000×



Figs. 1–3

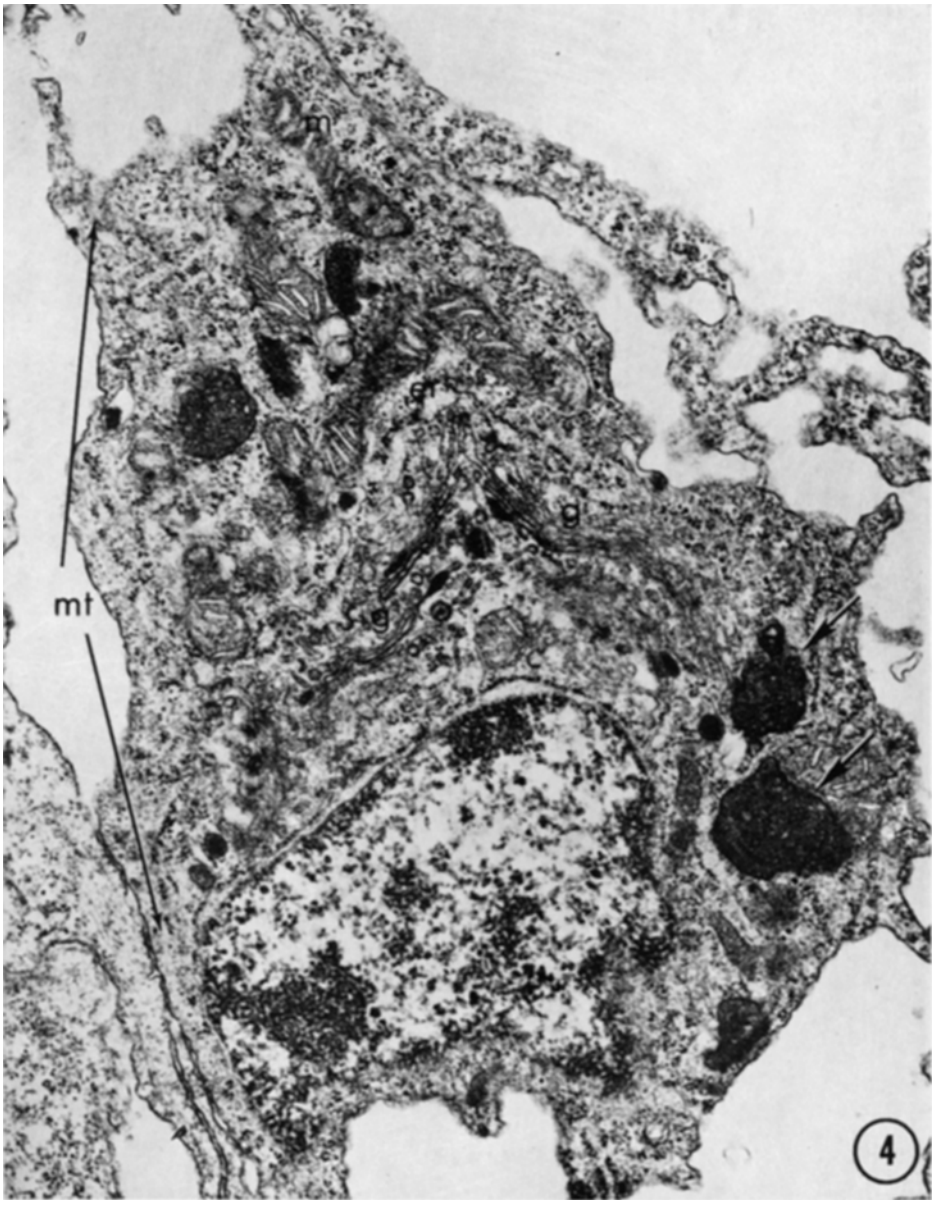


Fig. 4. Mature neurosecretory cell. The nucleus, displaced toward the periphery of the cell, contains dense clumps of scattered chromatin material. Active Golgi complexes (*g*), short segments of rough endoplasmic reticulum (*er*), microtubules (*mt*), mitochondria (*m*), small dense droplets (1100–1600 Å in diameter), larger dense droplets (arrows) and ribosomes in the cytoplasm. 22200×

one instance (Fig. 6) the nerve cell penetrates through the bases of digestive cells and lies immediately adjacent to the mesoglea. This represents an unusual observation since epidermal nerve cells have not been seen bordering the mesoglea.

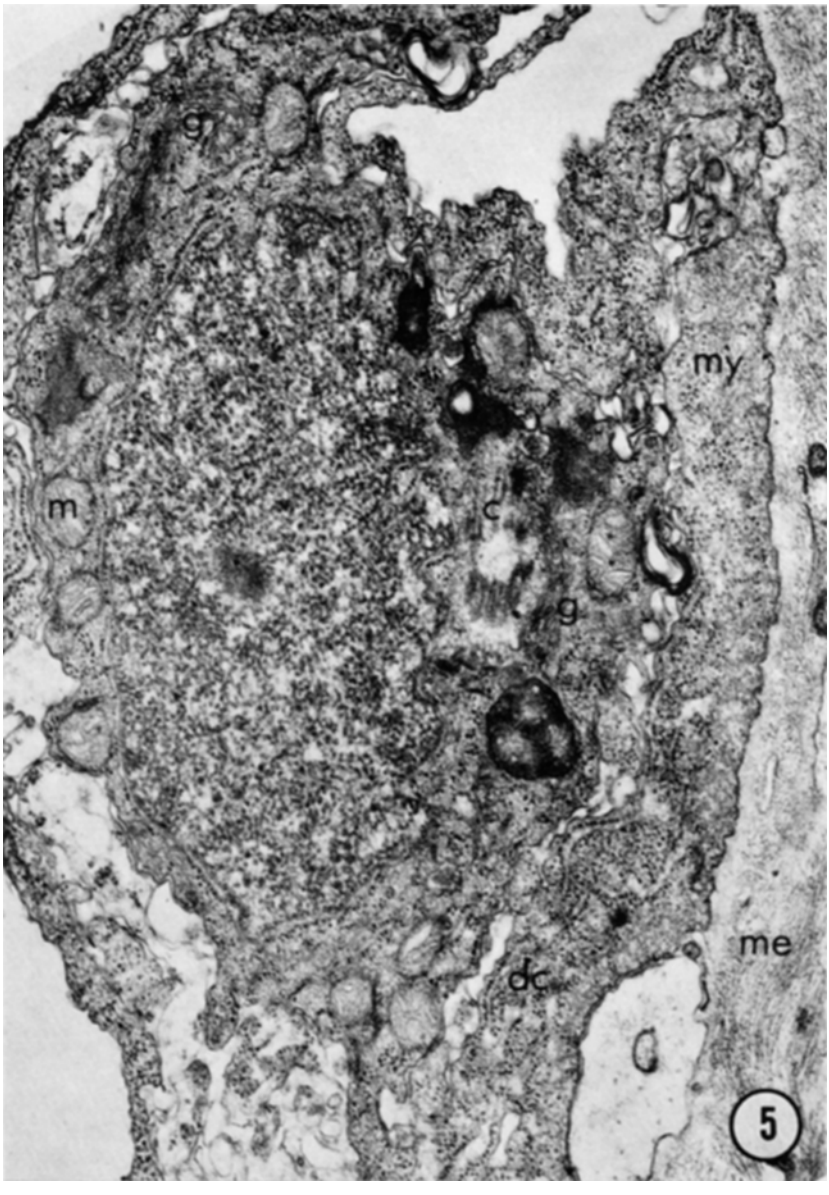


Fig. 5. Young neurosensory cell located immediately adjacent to the myonemes (*my*) of digestive cells (*dc*). The cytoplasm contains few mitochondria (*m*), Golgi complexes (*g*), ribosomes and a cilium (*c*) which has been sectioned near its base. Large irregularly-shaped droplets are also present. Mesoglea–*me*. 29800×

The nucleus of these cells is still relatively homogeneous. As the cells mature, the nucleus assumes a more condensed appearance as a result of scattered clumps of dense chromatin material. The cytoplasm contains free ribosomes, few mitochondria, small Golgi complexes and short segments of rough endoplasmic reticulum. A cilium, typical of epidermal neurosensory cells is also present (Fig. 6).

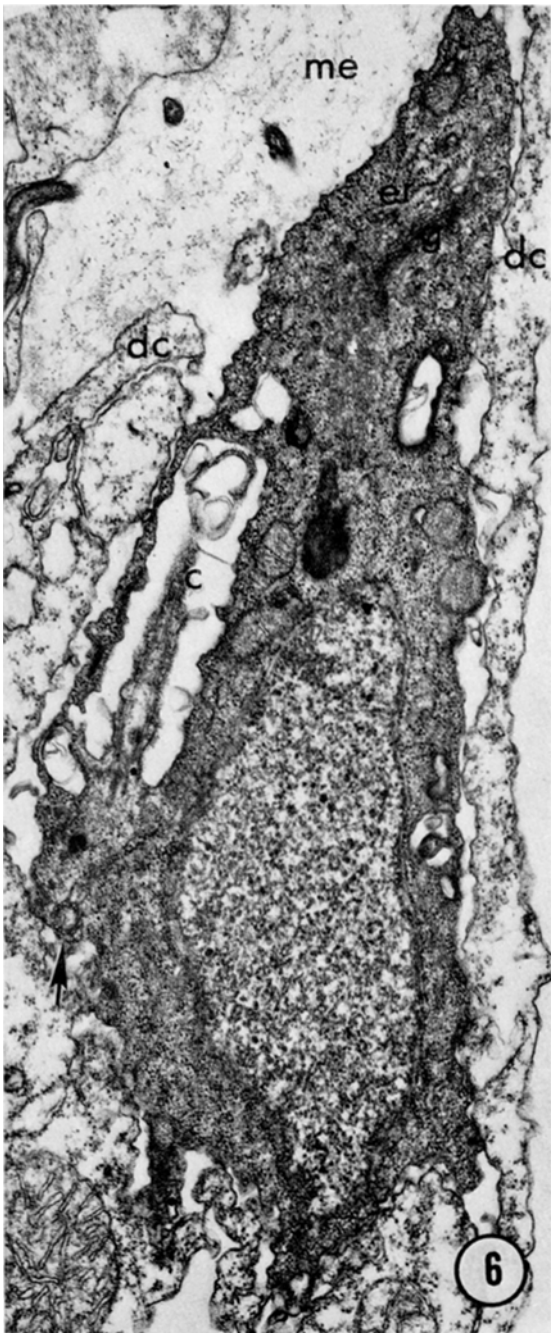


Fig. 6. Young neurosensory cell similar to that described in Fig. 5. The cilium (*c*) and a centriole (arrow) are more clearly observed. Note that a cytoplasmic extension of the neurosensory cell lies between the bases of digestive cells (*dc*) and is therefore immediately adjacent to the mesoglea (*me*). Golgi complex–*g*; rough endoplasmic reticulum–*er*. 22200×

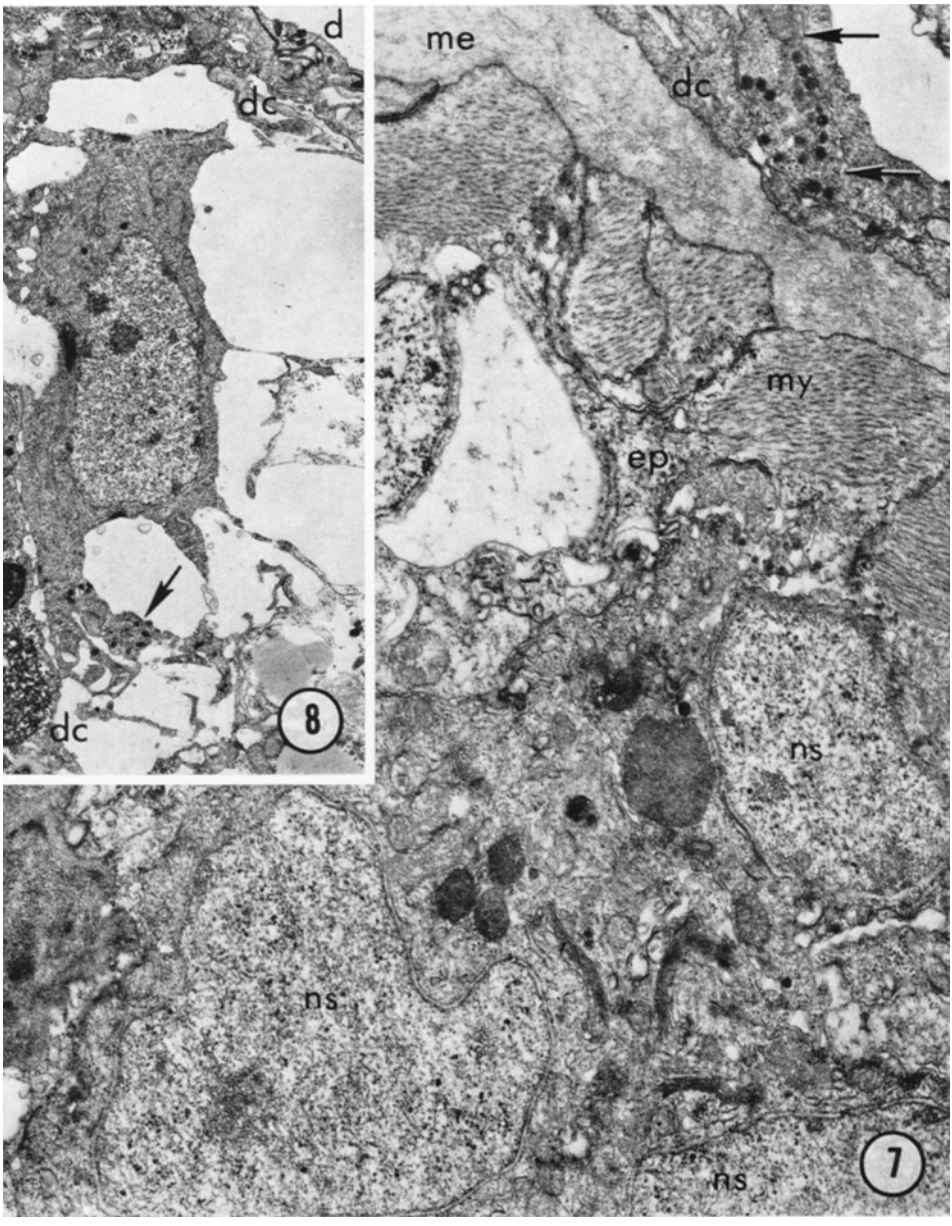
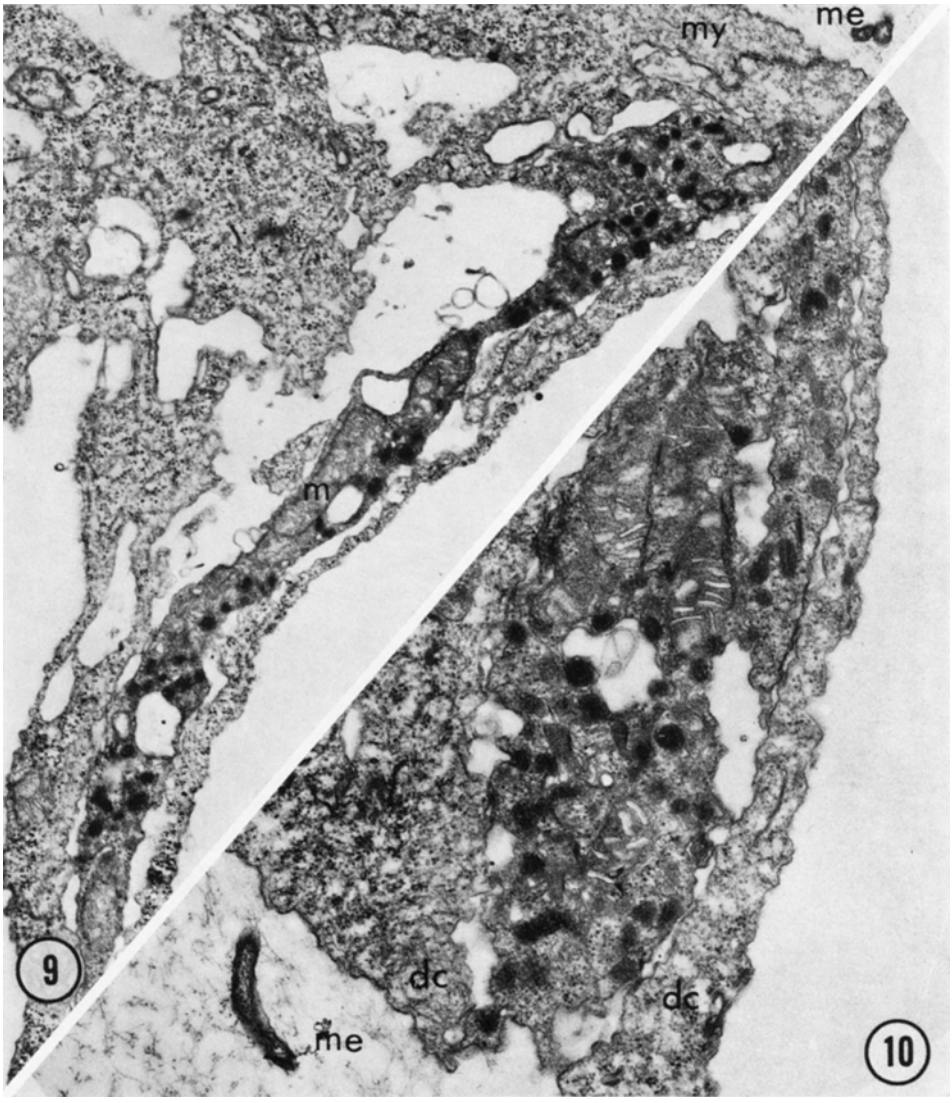


Fig. 7. Three neurosecretory cells (*ns*) located immediately adjacent to the myonemes (*my*) of epithelio-muscular cells (*ep*). The bases of digestive cells (*dc*) are seen on the other side of the mesoglea (*me*). Note especially the neurite of a gastrodermal nerve cell (arrows). It contains several dense membrane-bounded droplets (1000 Å in diameter), microtubules and a mitochondrion. This section was taken from the hypostome. 15400×

Fig. 8. Young gastrodermal nerve cell and nerve cell and neurite of another nerve cell (arrow) containing membrane-bounded droplets 1300 Å in diameter). They are surrounded by digestive cells (*dc*). Digestive cavity–*d*. This section was taken from the basal disk. 7300×



Figs. 9 and 10. Neurites of gastrodermal neurosensory and/or neurosecretory cells. Fig. 9. Long neurite extending for a distance of approximately 9.0 μ. It contains dense membrane-bounded droplets (700–1100 Å in diameter), mitochondria (*m*) and ribosomes. Myonemes of digestive cell–*my*; Mesoglea–*me*. 21400×. Fig. 10. Neurite containing dense droplets (1000–1400 Å in diameter), ribosomes and mitochondria lying between the bases of two digestive cells (*dc*). Note that it borders directly the mesoglea (*me*). 28600×

The third type of nerve cell found in the epidermis is the ganglionic cell. It is not known whether these nerve cells are also present in the gastrodermis, since our preliminary observations fail to identify them.

Although nerve cells are found throughout the epidermis, there are certain areas in which they are concentrated, that is, at the bases of tentacles, hypostome

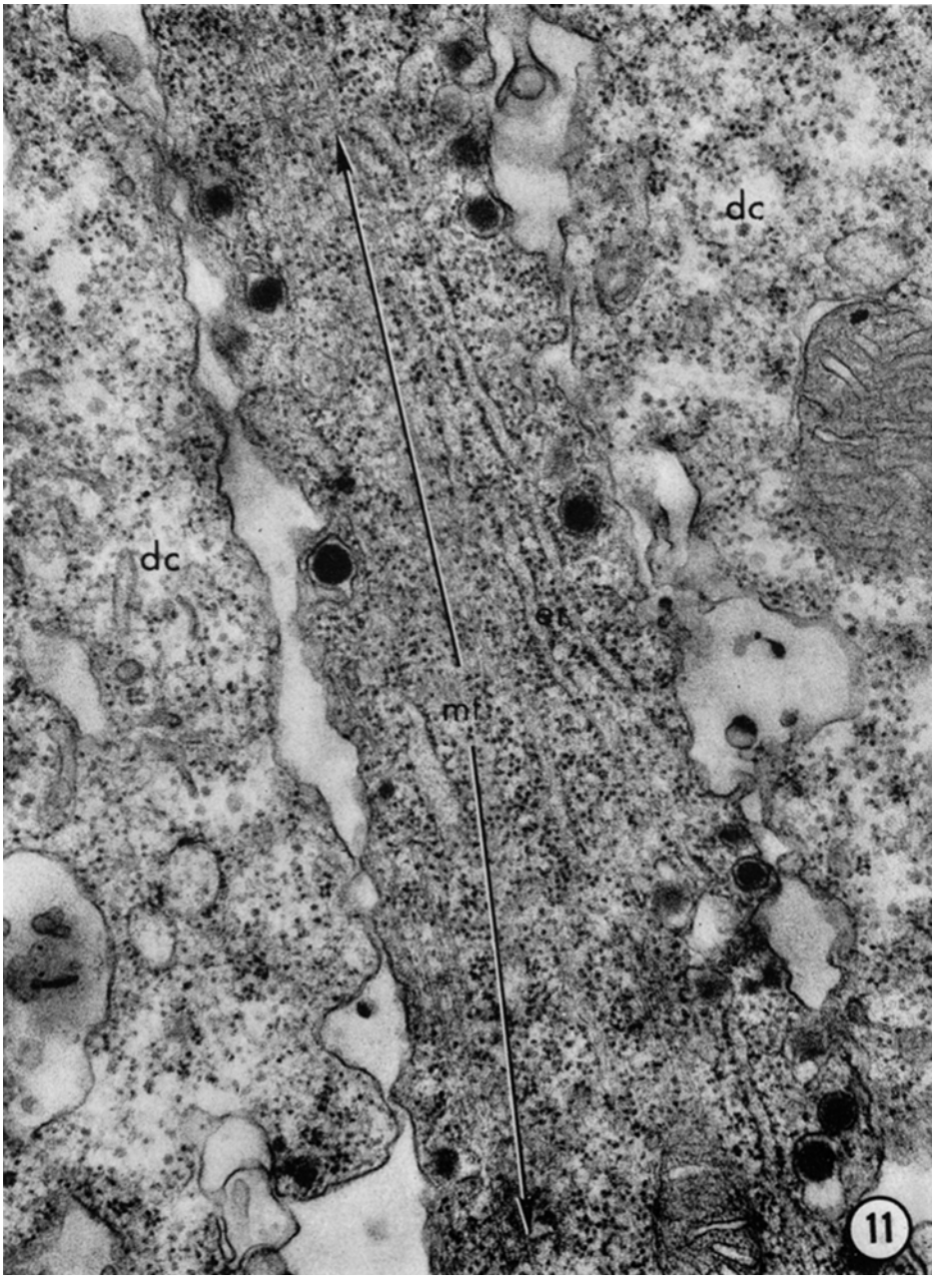
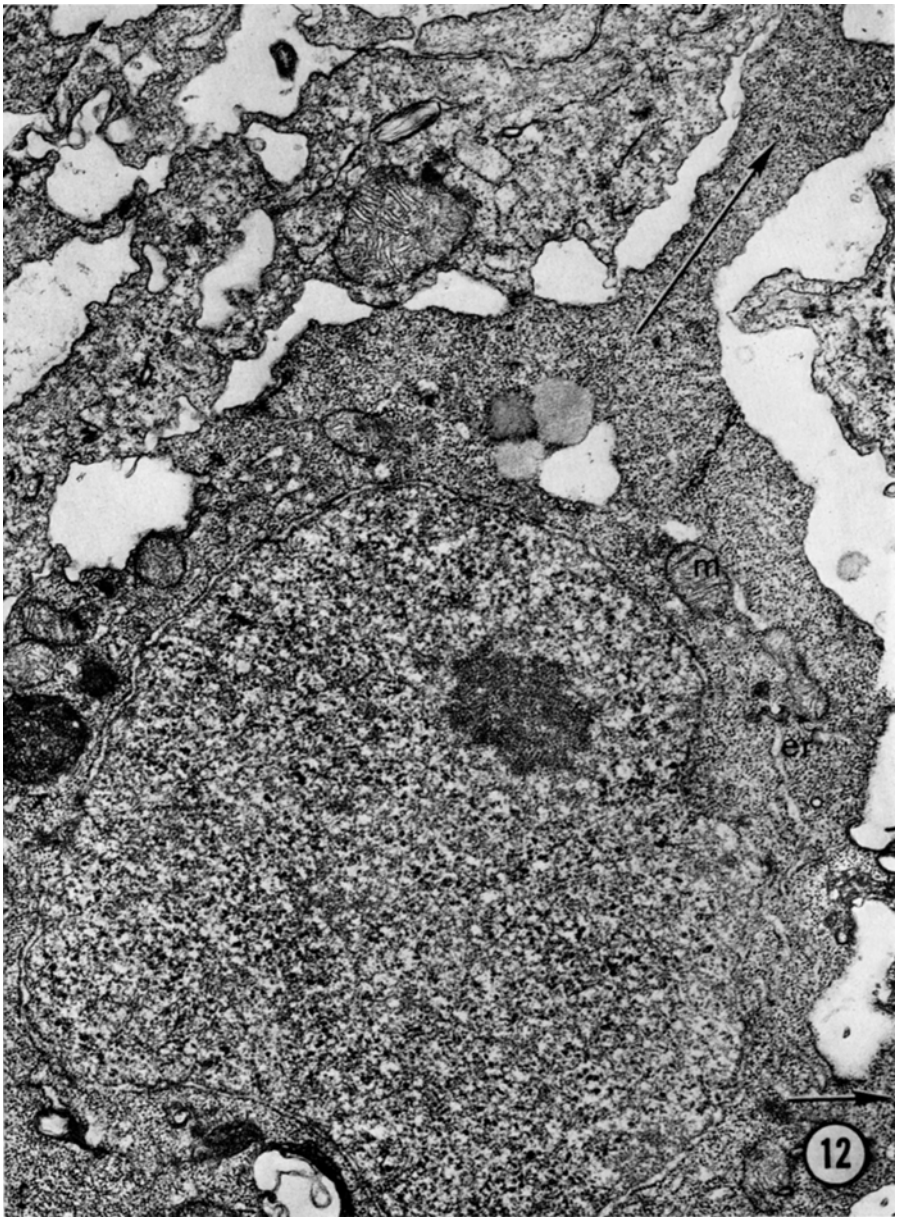


Fig. 11. Neurite closely resembling epidermal neurites lying between two digestive cells (*dc*). The membranes surrounding the dense droplets (1100–1700 Å in diameter) are seen clearly. Microtubules (*mt*) oriented parallel to the long axis of the neurite, rough endoplasmic reticulum (*er*) which appears to be more prevalent than in epidermal neurites, ribosomes and mitochondria are present. 34000×



Figs. 12–15. Different profiles of basal reserve cells some of which may have begun differentiation into other cell types. Fig. 12. Basal reserve cell containing a centrally located nucleus with a prominent nucleolus. Ribosomes are the most predominant cytoplasmic structure. Few mitochondria (*m*), occasional segments of rough endoplasmic reticulum (*er*), droplets (about 0.4 μ in diameter) of moderate density and larger, dense droplets (0,7 μ in diameter) similar to those seen in neurosecretory and neurosensory cells are in the cytoplasm. Long, narrow cytoplasmic extensions (arrow) are also observed. 22200×. Fig. 13. Basal reserve cell located adjacent to the myonemes (*my*) of a digestive cell. Note that the cell has two processes (arrows). Mitochondria and ribosomes represent the most conspicuous structures. Mesoglea–*me*; epithelio-muscular cell–*ep*. 15400×

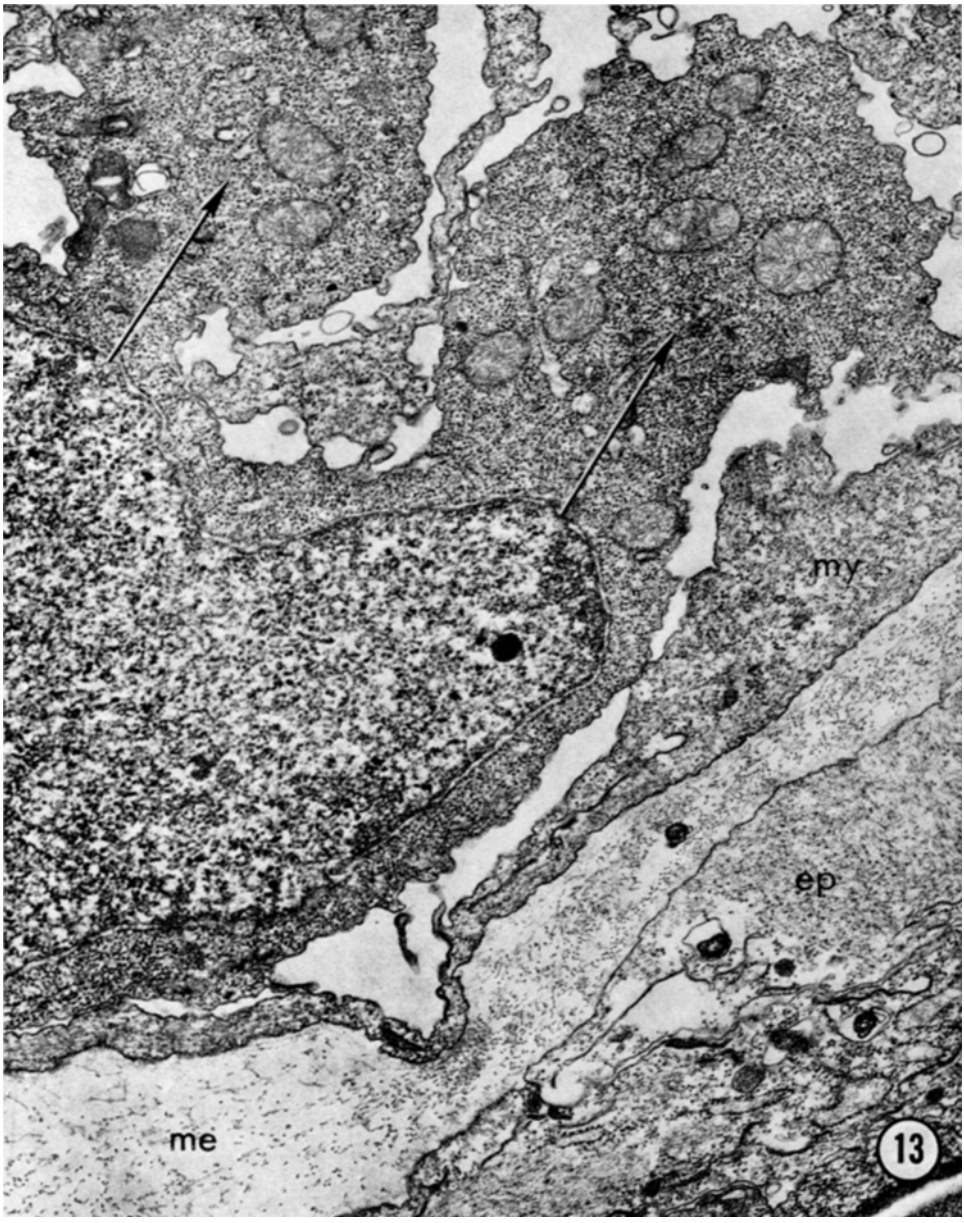
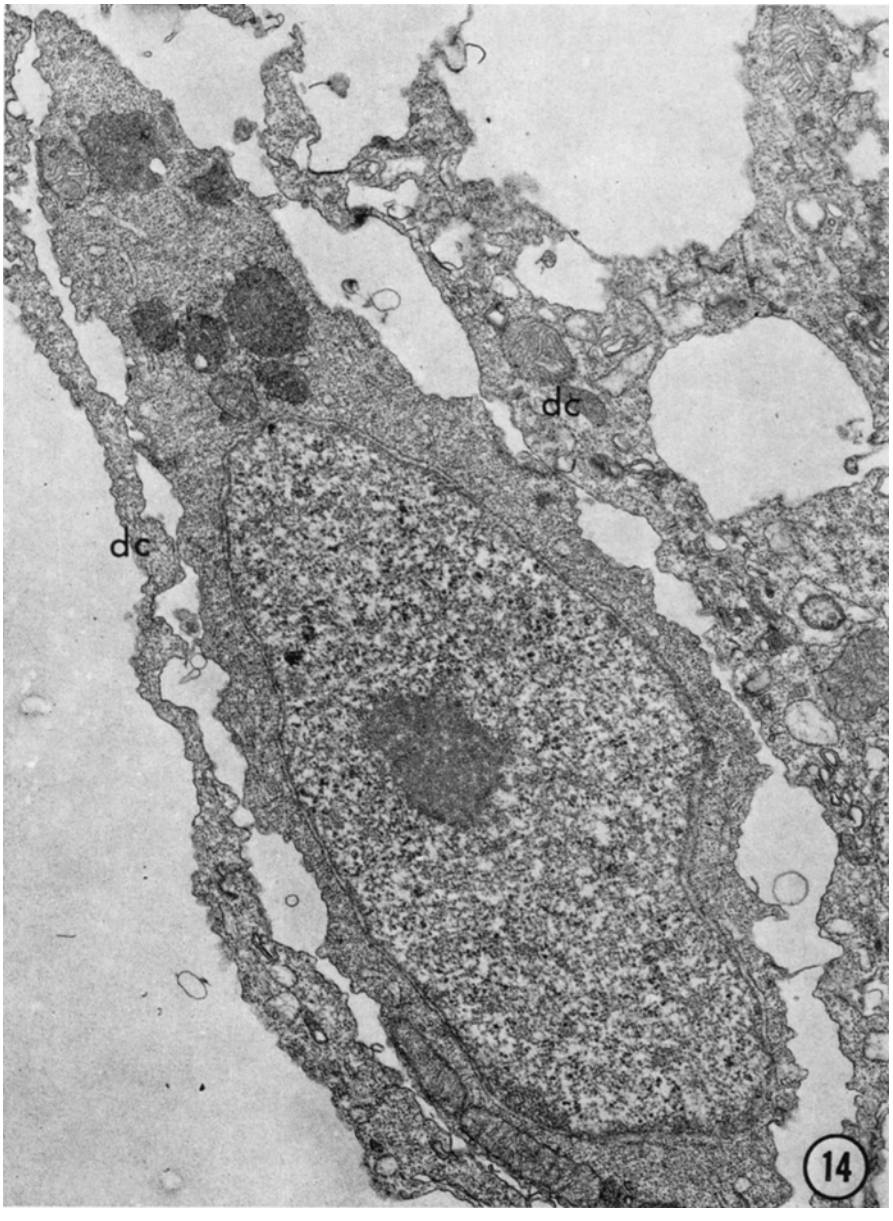


Fig. 13

and basal regions. It is not known whether there is definitely a similar distribution and concentration of nerves in the gastrodermis. It has been observed, however, that in the epidermis of the hypostome where neurosecretory cells accumulate, gastrodermal neurosecretory cells are also seen in the identical region (Fig. 7). Also, neurosecretory cells occur in the basal disk, but these cells contain fewer secretory droplets than those in the hypostome. Neurosecretory cells are also



Figs. 14 and 15. Fig. 14. Slightly elongated basal reserve cell completely surrounded by digestive cells (*dc*). The oval-shaped nucleus contains a prominent nucleolus. Numerous ribosomes, few mitochondria and large dense droplets (up to 0.8 μ in diameter) are present in the cytoplasm. 15400×. Fig. 15. Basal reserve cell with three cytoplasmic extensions (only one of which is shown; see arrow). The cell is surrounded partly by digestive cells (*dc*) and also by neurites of neurosecretory and/or neurosensory cells (*ns*). The cytoplasm is typical of epidermal interstitial cells. 15400×

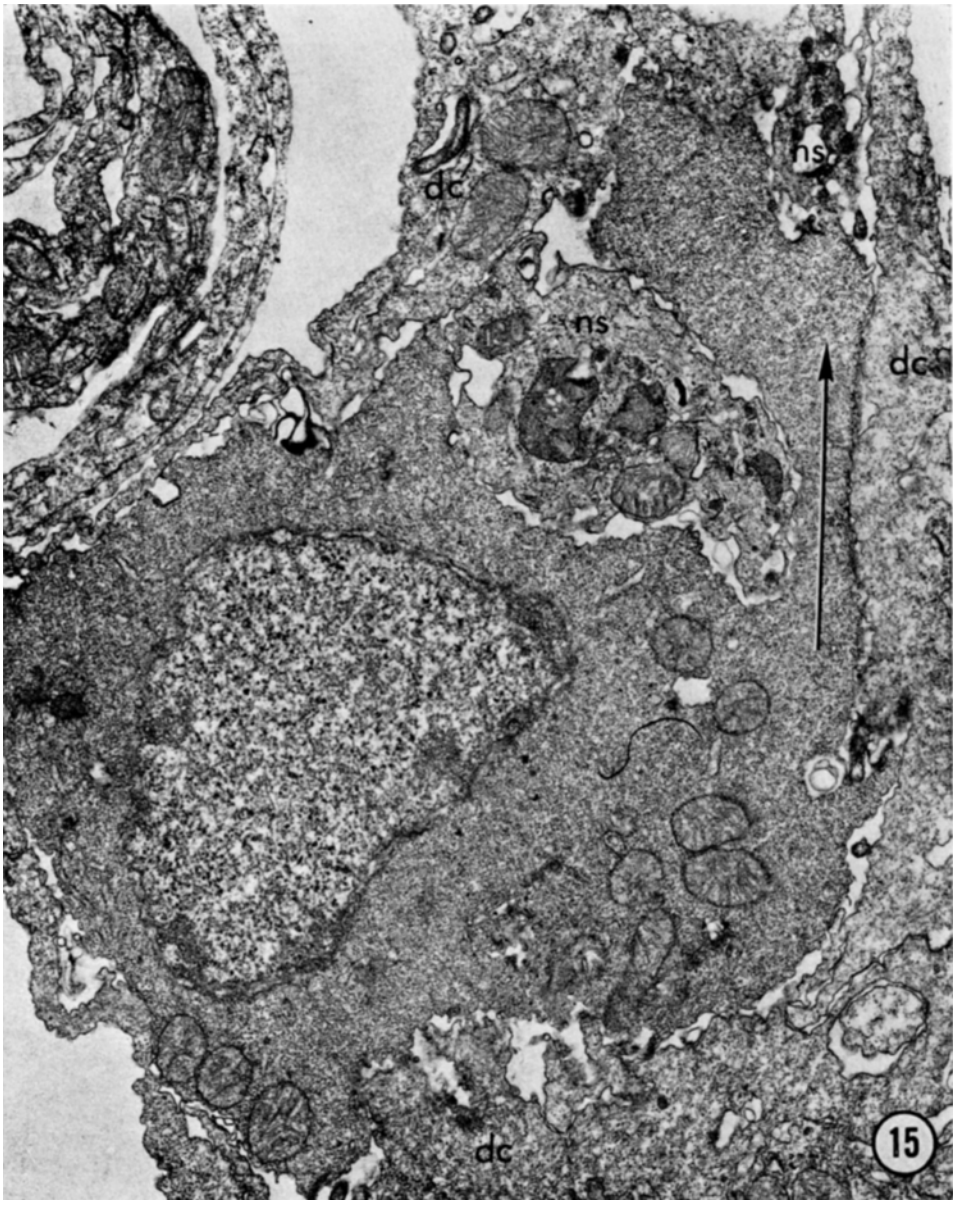


Fig. 15

found in the gastrodermis of the basal disk, and as in the case of their epidermal counterparts, they contain few secretory droplets (Fig. 8).

Neurites of epidermal neurosecretory cells and neurosensory cells are similar structurally and at the present time cannot be identified as belonging to a specific cell type unless the cell bodies are also included in the sections examined. The

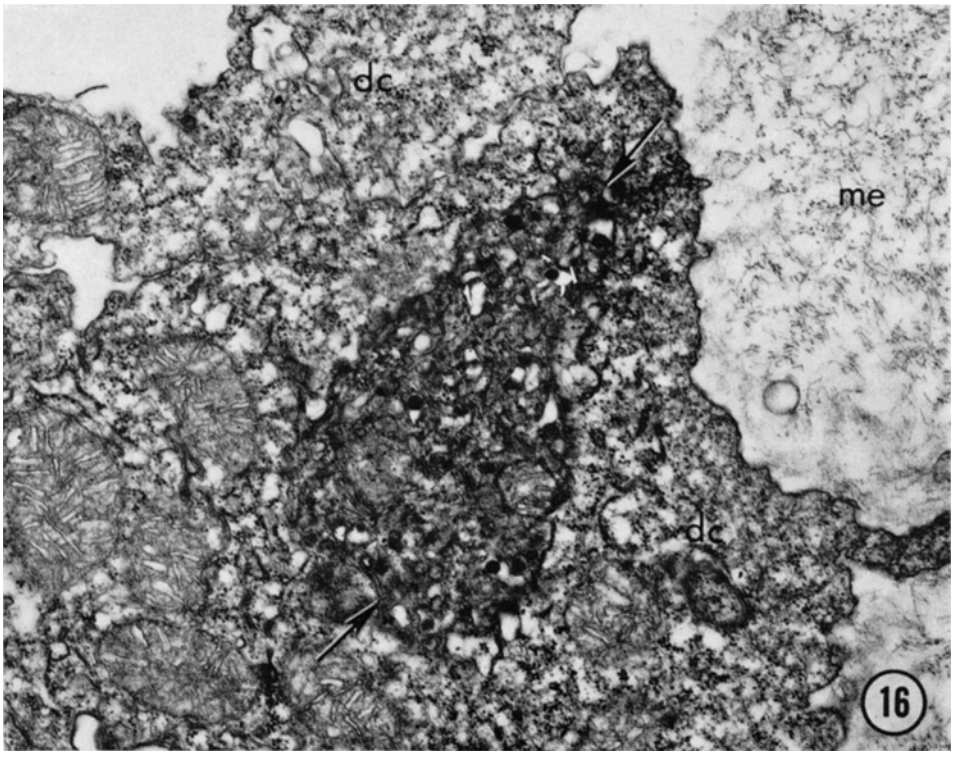


Fig. 16. Portion of a degenerating neurite in the gastrodermis (arrows). It contains dense secretory droplets (700–900 Å in diameter), mitochondria and membranous debris. Digestive cell–*dc*; Mesoglea–*m*. 28600×

same difficulty is true for gastrodermal nerve cells. Figs. 9–11 show neurites which may be from neurosecretory and/or neurosensory cells. They are similar to epidermal neurites in that they contain membrane-bounded neurosecretory droplets (ranging from 700–1700 Å in diameter) of various densities, microtubules oriented parallel to the long axis of the neurite, few mitochondria and ribosomes. Some of the gastrodermal neurites contain more rough endoplasmic reticulum than is normally observed in epidermal neurites (Fig. 9). Another peculiarity is that some neurites approach the limits of the mesoglea (Fig. 10) and therefore contribute to the mesogleal boundary.

The origin of gastrodermal nerve cells appears to involve basal reserve (or basophilic) cells. These cells are located immediately adjacent to myonemes at the bases of digestive cell (Figs. 12–13) or may lie deeper within the gastrodermis (Figs. 14–15). Accordingly, they are surrounded completely by digestive cells (Figs. 12–14) or partly by digestive cells and other nerve cells (Fig. 15). The nucleus is usually centrally located and contains a prominent nucleolus (Figs. 12, 14). The cytoplasmic contents resemble those of interstitial (epidermal) cells, in that there are numerous free ribosomes, few mitochondria and short segments of

rough endoplasmic reticulum. In addition, droplets of various densities (approximately 0.7μ in diameter) are also present in the cytoplasm (Figs. 12, 14). Although these cells have been designated as basal reserve cells, they may represent actually early stages of differentiating cells. This may be assumed from the appearance of long, narrow, single cytoplasmic extensions and also cells which contain two or more such processes (Figs. 12–15).

As in the cases of epidermal nerves, gastrodermal nerves die and are sloughed off apparently at the extremities, that is, at the mouth, tips of the tentacles and basal disk. A portion of a degenerating neurite is shown in Fig. 16. It contains secretory droplets (700–800 Å in diameter) typical of neurosecretory and neurosensory cells, mitochondria and dense membranous debris.

Discussion

This preliminary report has presented ultrastructural evidence for the existence of nerve cells in the gastrodermis of *Hydra*. The identification of these nerve cells is based on the ultrastructural criteria used for recognizing epidermal nerves. Using these criteria, two types of nerve cells are identified—neurosecretory and neurosensory cells which are structurally indistinguishable from their epidermal counterparts. These cells are also located in a similar position as most epidermal nerves, that is, they are observed immediately adjacent to the myonemes. In this case, however, the myonemes are circularly arranged and constitute the basal portions of digestive cells. As a result of this location, they are surrounded mostly by digestive cells and partly by other nerve cells.

It is entirely possible that the third type of nerve cell found in the epidermis—the ganglionic cell—also exists in the gastrodermis. The present report, however, has not confirmed their existence. The question arises as to the presence of other nerve cells which may be structurally dissimilar to epidermal nerves. The possibility seem unlikely in view of the fact that the gastrodermis contains only a few cell types, all of which have been identified histologically and ultrastructurally.

The definite existence of gastrodermal nerve cells raises other important questions. For example, are gastrodermal nerve cells concentrated in the hypostome and basal disk as are epidermal nerves? Are there connections between the epidermal and gastrodermal nerves? What are the functions of the gastrodermal nerves? What is the origin of the gastrodermal nerves? Some of these questions cannot be answered with any degree of certainty at the present time and others will be approached with only limited evidence.

Gastrodermal nerves are present in the hypostome and basal disk (Figs. 7, 8). In several instances, nerve cells from both cell layers of the hypostome are observed in the same micrograph (Fig. 7). Occasionally, single nerve cells were seen in the remaining body regions. According to Hyman (1940), there are indications of a gastrodermal plexus, but that this plexus contains fewer and smaller cells than the epidermal plexus. If the latter part of the above statement is correct, then this may explain the difficulty in studying and identifying gastrodermal nerve cells and the present uncertainty as to whether there is a similar distribution and concentration of nerve cells in the gastrodermis as in the epidermis.

It has been suggested that, at least in some types of coelenterates, epidermal and gastrodermal nerve cells extend into the mesoglea, contact each other and therefore establish some degree of communication among all nerve cells (Hyman, 1940). It is uncertain whether this situation occurs in *Hydra*. Studies on the epidermal nerve indicate that nerve cells of this layer are always surrounded by the myonemes of the epithelio-muscular cells (Lentz, 1966; Davis, Burnett and Haynes, 1968). None of these investigations showed nerve cells immediately bordering or traversing the mesoglea. Unlike the epidermal nerves, gastrodermal nerve cells extend to the limits of the mesoglea (Figs. 6, 10). Although the present evidence does not show any continuities through the mesoglea, it would seem that if such structural conditions do exist, the gastrodermal nerve cells may be primarily involved.

It has been suggested that nerve cells control growth and cellular differentiation in *Hydra* (Burnett and Diehl, 1964b; Davis, Burnett and Haynes, 1968; Lentz, 1966). These functions, however, are ascribed generally to epidermal nerves. The precise function of gastrodermal nerves is not known. Since growth and cellular differentiation also occur in the gastrodermis, it may be that some of the functions of the nerves are involved in the control of these processes.

Finally, the origin of gastrodermal nerve cells should be considered. It has been shown that two of the three types of epidermal nerves (ganglionic and neurosensory cells) arise exclusively from interstitial cells, and it has been assumed that neurosecretory cells also have a similar origin (Lentz, 1966; Davis, 1969; Davis, 1971). Reference was made earlier concerning the structural similarity between interstitial cells and basal reserve (or basophilic) cells found in the gastrodermis. This similarity, together with the fact that both cell types are capable of division and differentiation into other cell types (Rose and Burnett, 1968, 1970) allows one to consider the basal reserve cells as embryonic cells. Accordingly, the basal reserve cells seem to be the most likely cell type from which gastrodermal nerve cells originate.

It is clear from the foregoing discussion that although certain gastrodermal nerve cells have been identified ultrastructurally, little is known concerning other possible types of nerve cells, their distribution, function, origin and association with epidermal nerves. The evidences presented in this paper for the definite existence of gastrodermal nerves will require future studies in better understanding the nervous system of *Hydra*.

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