The Development of the Cnidoblasts of Hydra

An Electron Microscope Study of Cell Differentiation*

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ABSTRACT

The general histological organization of Hydra is reviewed and electron microscopic observations are presented which bear upon the nature of the mesoglea, the mode of attachment of the contractile processes of the musculo-epithelial cells, and the cytomorphosis of the cnidoblasts. Particular attention is devoted to the changes in form and distribution of the cytoplasmic organelles in the course of nematocyst formation.

The undifferentiated interstitial cell is characterized by a small Golgi complex, few mitochondria, virtual absence of the endoplasmic reticulum, and a cytoplasmic matrix crowded with fine granules presumed to be ribonucleoprotein. These cytological characteristics persist through the early part of the period of interstitial cell proliferation which leads to formation of clusters of cnidoblasts. With the initiation of nematocyst formation in the cnidoblasts, numerous membrane-bounded vesicles appear in their cytoplasm. These later coalesce to form a typical endoplasmic reticulum with associated ribonucleoprotein granules. During the ensuing period of rapid growth of the nematocyst the reticulum becomes very extensive and highly organized. Finally, when the nematocyst has attained its full size, the reticulum breaks up again into isolated vesicles.

The Golgi complex remains closely applied to the apical pole of the nematocyst throughout its development and apparently contributes to its enlargement by segregating formative material in vacuoles whose contents are subsequently incorporated in the nematocyst. The elaboration of this complex cell product appears to require the cooperative participation of the endoplasmic reticulum and the Golgi complex. Their respective roles in the formative process are discussed.

INTRODUCTION

The fresh-water polyp, Hydra, kills and captures its prey by means of *nematocysts*, small nettles or darts, which are fired when adequately stimulated. These projectiles penetrate and poison the victim, or immobilize it by entanglement until the tentacles can introduce it into the mouth. The nematocysts consist of a capsule, closed by an *operculum* and provided with a trigger device called the *cnidocil*. Coiled in the interior of the capsule, there is a tube or filament which may be smooth surfaced or armed with barbs. Upon discharge the operculum opens and the filament is suddenly thrust out either to pierce the cuticle of the prey or to enwrap its appendages. These complex organs are characteristic of coelenterates and some seventeen types have been described which differ in size, shape, armament, and mode of action (38). Four kinds are said to occur in the fresh-water polyp which is the subject of this investigation (12).

Since these expendable missiles are lost in feeding, they must be continually replaced. The new nematocysts are formed within special cells, the *cnidoblasts*, which occur in clusters that are widely distributed in the ectoderm. These cells, in turn, arise from small undifferentiated *interstitial cells* lodged between musculo-epithelial cells near the base of the epithelium. The present paper defines the cytological characteristics of the interstitial

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cells and follows their differentiation into cnidoblasts, placing particular emphasis upon the structural changes in the cell organelles during nematocyst formation.

Materials and Methods

Inability to obtain uniform fixation in small blocks of tissue excised from vertebrate embryos and the difficulty of sampling comparable areas of the same organ at successive stages of development, often constitute serious obstacles to the electron microscopic study of cell differentiation. Hydra, however, is unusually favorable material for such studies because of its small size, its simple histological organization, and the ease with which it can be grown under controlled conditions. The animal can be fixed and processed intact, thus avoiding mechanical damage incurred in cutting small blocks. Almost any section through the body wall will show undifferentiated interstitial cells and numerous clusters of cnidoblasts containing nematocysts in various stages of formation. The fact that the eight to sixteen cells, which comprise each cnidoblast cluster, develop synchronously, insures observation of multiple samples of each stage of differentiation.

The majority of the observations reported here are derived from electron micrographs of *Pelmatohydra oligactis*, but *Chlorohydra viridissima* and *Hydra littoralis* were also studied with similar results. By way of providing materials, mass cultures of Hydra were maintained by the methods of Loomis (20, 21) and fed on larvae of *Artemia salina*. In preparation for electron microscopy, individuals, isolated in petri dishes or small vials, were fixed whole for 2½ hours in 1 per cent OsO_4 buffered with veronal acetate to a pH of 7.5, washed very briefly, and dehydrated in graded concentrations of ethanol. The fixed organisms were then infiltrated in *n*-butyl methacrylate monomer containing 1 per cent luperco as catalyst; polymerization was carried out overnight in a 50°C. oven. Sections, showing yellow or golden interference colors, were cut on a Porter-Blum microtome (28) and examined with an RCA EMU-3B or a Siemens Elmiskop II microscope. Micrographs were taken at original magnifications of 1,000 to 10,000 and enlarged photographically to the desired final magnification.

OBSERVATIONS

General Body Organization:

The body of hydra can be divided into five regions for convenience in description. The *head* consists of the mouth and hypostome crowned by a single row of radially arranged tentacles which vary in number from five to nine. Below the head there is a slightly constricted *neck* segment and this is followed by a fusiform expansion that marks the *stomach*. From there the column in some species narrows slightly to form a definite *stalk*, while in others it terminates directly in the expanded *pedal disc* which serves to attach the polyp to its substrate. The body wall is composed of two epithelia, the *gastroderm* and the *ectoderm*, arranged base-to-base but separated by a gelatinous acellular layer, the *mesoglea*. Both cellular layers have a pseudostratified appearance, but none of the terms commonly used in the classification of epithelia accurately describes the association of mixed cell types found in the body wall of hydra. *Musculo-epithelial* cells predominate in both the ectoderm and gastroderm and their columnar cell bodies make up the greater part of the thickness of these epithelia. At their bases the cells are drawn out into long contractile processes which run perpendicular to the vertical axis of the cell body, but parallel to the mesoglea to which they adhere. Both epithelia are highly vacuolated. The gastroderm cells in the upper two-thirds of the column or body of the organism have a highly irregular free surface and contain clear vacuoles of varying size. After the organism feeds, the bodies of these cells become crowded with lipide droplets and dense masses of ingested material in various stages of intracellular digestion (Fig. 1). The ectodermal cells have a rather smooth surface which is coated with an amorphous layer of material, probably mucopolysaccharide. They contain multiple clear vacuoles, but are relative free of other inclusions except for occasional irregularly shaped hyaline masses which appear to be degenerating nematocysts (Fig. 3). Individual *mucous* and *zymogenic cells* are located between the columnar portions of the gastrodermal musculo-epithelial cells. The *interstitial cells* and *cnidoblasts* occupy a similar position between the musculo-epithelial cells of the ectoderm. The former occur singly or in pairs (Fig. 1) near the base of the epithelium; the latter occur in compact clusters of four to sixteen cells.

The contractile foot processes of the ectodermal musculo-epithelial cells run longitudinally with respect to the column, whereas those of the gastroderm are disposed circumferentially. Thus, in an electron micrograph of a longitudinal section of the body wall (Fig. 1), the bases of the gastroderm cells appear narrow, while those of the ectoderm are long (Fig. 2). In a micrograph at higher magnification, showing the contractile processes on either side of the mesoglea (Fig. 3), bundles of delicate myofilaments are seen running longitudinally in the ectodermal cell; whereas in the gastroderm cell

the transverse sections of these bundles appear only as ill defined areas with a density greater than the surrounding cytoplasmic matrix. The individual filaments are not resolved. Where contractile processes of musculo-epithelial cells meet end-to-end, their area of contact is enlarged by overlap or by deep interdigitation of their surfaces (Fig. 4). The membranes at these junctions are thickened as they are at desmosomes, terminal bars, and other sites of firm adherence between the cells of vertebrate tissues. The myofilaments terminate in a condensation of cytoplasm on the inner aspect of the membranes in much the same way that the myofilaments of cardiac muscle end at the intercalated discs (6).

The mesoglea consists of a loose feltwork of randomly oriented fine filaments (~50 A) embedded in an amorphous matrix of low density (Fig. 2). Many dense granules of fairly uniform size (~300 A) are dispersed in the matrix. These show regional variations in their abundance and seem to be material in transit across the mesoglea rather than an integral part of this layer. Identical granules are found *in* the gastroderm cells and *between* the cells of the ectoderm. On the basis of Best's carmine staining, previous investigators have reported the occurrence of glycogen in the epithelial cells and in the mesoglea (41) and we have confirmed this finding in histological sections stained with the periodic acid-Schiff reaction. A strong reaction was observed also in certain of the gastroderm cells and in the mesoglea and the reaction in the cells was abolished by amylase digestion while that in the mesoglea was distinctly diminished. The residual staining of the mesoglea after digestion of glycogen is attributed to a mucopolysaccharide component. Moreover, the granules observed in electron micrographs resemble, in size and density, particles previously described in certain glycogen-rich vertebrate tissues (6). These granules found in hydra are, therefore, tentatively interpreted as a particulate form of glycogen which is formed in the gastroderm, thence traverses the mesoglea, and accumulates in the extracellular spaces of the ectoderm.

Although a simple *nerve net* has been described for Hydra (8), it is notoriously difficult to demonstrate either by silver or methylene blue techniques, and the fragmentary reticular patterns usually obtained are open to other interpretations. We are not yet prepared to deny the existence of a primitive nervous system in Hydra, but no cells identifiable as neural elements have been found in the present electron microscopic study.

The Interstitial Cells:

The interstitial cells have been the object of much study because a number of investigators believe that they retain their embryonic pluripotentiality and play an important role in budding and in regeneration of a whole organism from a small fragment (2). The possibility that these cells may be pluripotent is not contested here, but under the conditions of the present study it was not evident that they have the capacity to differentiate into any cell type other than cnidoblasts. Our interest in them is therefore confined at present to an investigation of their role as a reservoir of undifferentiated cells that provide for continual replacement of the nematocysts lost in feeding.

The interstitial cells occur singly or in pairs and are usually situated near the base of the ectodermal epithelium. In stained preparations examined with the light microscope they are small in size, exhibit intense cytoplasmic basophilia, and possess a large vesicular nucleus with an unusually prominent nucleolus. In electron micrographs, the nucleus is somewhat variable in shape but usually shows no deep infolding or lobulation (Fig. 7). At low magnifications the nuclear content appears homogeneous except for the prominent nucleolus, but at higher power it is found to be composed of fine granules dispersed in a matrix of low density (Fig. 8). At least two categories of granules can be distinguished on the basis of their size and density. Those comprising the nucleolus are dense, sharply defined, 80 to 120 A in diameter, and are closely aggregated in a compact mass showing no clear evidence of organized arrangement. Granules of the same size and density also occur in lower concentration throughout the karyoplasm where they intermingle with ill defined granules of somewhat lower density that range in size from 100 to 280 A. It is difficult to determine whether these latter are, in fact, discrete granules or flocculent aggregates of smaller filamentous subunits. The denser particles (80 to 120 A) found in the nucleolus and elsewhere in the karyoplasm are presumed to be ribonucleoprotein. The chemical nature of the other, less dense particulate component is not known, but is probably in part desoxyribonucleoprotein. The nucleus is bounded by a pair of parallel membranes separated by a space 100 to 150 A wide. At certain points on the circumference of the nucleus the two membranes are fused around

the margins of small circular areas 200 to 300 A in diameter. These local specializations of the nuclear membrane seem to correspond to the structures, in other cell types, that have been called "nuclear pores" by other investigators (37).

The rod-shaped mitochondria are sparse, randomly distributed in the cytoplasm, and display an internal organization that conforms to Palade's (25) original description of the fine structure of this organelle. The mitochondrial matrix appears amorphous and is slightly more dense than the ground substance of the surrounding cytoplasm (Figs. 7 and 8). The cristae mitochondriales are, for the most part, parallel in their orientation, and those originating from one side tend to alternate with those projecting from the opposite side. Some seem to branch and some seem to extend completely across the mitochondrion.

The Golgi complex is located near the nucleus and is relatively small (Fig. 7). It consists of flattened sacs in parallel array, clusters of small spherical vesicles, and a few larger vacuoles. Its organization is therefore very similar to the Golgi complex of vertebrate cell types described by Dalton and Felix (4). The cytoplasm of the interstitial cells may contain one or more small lipide droplets, but is usually devoid of other inclusions.

The cytoplasm of highly basophilic cell types examined heretofore with the electron microscope has usually been found to be permeated by a system of membrane-bounded tubules, vesicles, and cisternae that constitute the endoplasmic reticulum (29). The basophilia of such cell types has been shown to reside in small 150 A granules of ribonucleoprotein that adhere in large numbers to the outer surfaces of the membranes bounding the endoplasmic reticulum (26). In the interstitial cells of Hydra, this system of cytoplasmic membranes is either entirely lacking or is represented by a very few, widely scattered vesicles. Nevertheless, small granules are present in great abundance and are uniformly distributed throughout the cytoplasm (Fig. 8). These cytoplasmic granules (100 to 120 A) appear to be identical to those found in high concentration in the nucleolus and in lesser numbers elsewhere in the karyoplasm. Except for their slightly smaller size, they resemble the ribonucleoprotein granules of vertebrate tissues.

The interstitial cells go through an initial phase of proliferation which results in the formation of clusters of four to sixteen early cnidoblasts. During this period there is little further structural differentiation. It is of particular interest that in the mitotic divisions that give rise to these cnidoblast clusters, karyokinesis proceeds normally but cytokinesis is arrested at a stage when the advancing cleavage furrow reaches the spindle remnant. The persisting filaments of the achromatic figure subsequently disappear, but a cylindrical cytoplasmic connection between the cells remains, surrounded by an annular thickening of their common plasma membrane (Fig. 5). All of the cells within the same cluster of early cnidoblasts are presumed to be united by intercellular bridges, as has been shown to be the case in the spermatid clusters in the germinal epithelium of the testis (3). The origin, fine structure, and significance of these protoplasmic connections are considered in greater detail in a separate paper (7). In addition to the intercellular bridges that arise by incomplete cytokinesis, there are communications of another type between early cnidoblasts, wherein the membranes of adjacent cells are simply incomplete along part of their surface of contact. Around the margins of the hiatus the membranes of the two cells are continuous with one another, but show no local thickening or other specialization at the site of their confluence (Fig. 5). How these simple defects in the boundary between cells are related to the bridges described above is not known. Indeed, were it not for their frequent occurrence in well fixed material, one would be inclined to interpret them as artifacts of specimen preparation. Their mode of formation remains obscure.

Early Cnidoblasts:

The mitotic divisions of the interstitial cells result in a reduction in the volume of cytoplasm. The clusters of early cnidoblasts are as a result made up of small polygonal cells with relatively large eccentrically placed nuclei. With the onset of differentiation, and shortly before the end of the period of proliferation, there is a noticeable increase in the concentration of ribonucleoprotein granules in the karyoplasm while those of the cytoplasm remain about as abundant as before. The mitochondria, although unchanged in number, show a slight increase in size and in the number of their cristae. The Golgi complex, on the other hand, enlarges markedly and forms within one of its vacuoles the primordium of the nematocyst (Fig. 6). Concurrently with these changes in the major organelles, numerous isolated vesicles appear in the cytoplasm where only a few small

ones were present before (Fig. 9). These are the first prominent elements to arise in the evolution of an endoplasmic reticulum. In subsequent development they increase in number, elongate, and apparently coalesce to form a more or less continuous system of channels that pursue a meandering course through the cytoplasm (Fig. 10). Although the majority of the ribonucleoprotein granules continue to be diffusely distributed in the cytoplasm, an increasing number of them become associated with the membranes of the newly elaborated reticulum.

The earliest nematocyst identified in the present study is a membrane-limited, pear-shaped object enclosed in a thick capsule, except at its apex, where it is bounded only by the thin, limiting membrane. The Golgi complex forms a conspicuous cap over this apical pole (Fig. 6). Early in its development the interior of the nematocyst is occupied by fine textured material of low density composed of granular or filamentous units extending down in size to the limits of our resolution. Later, irregularly shaped dense granules 200 to 400 A in diameter appear in the apical matrix (Fig. 6) and gradually spread downward into the interior of the nematocyst. They are distributed singly or clumped in coarse aggregates of varying size (Figs. 11, 12, and 17).

More Advanced Cnidoblast:

In more advanced stages of the cnidoblast, the growing nematocyst displaces the nucleus to one side and the modifications of the cell organelles described above become more evident. The mitochondria are definitely larger than before, but there seems to be no significant change in their number. Further enlargement of the Golgi complex is also apparent. However, the most striking transformation associated with this period of rapid nematocyst growth is the increase in amount and complexity of the endoplasmic reticulum (Figs. 11 and 12). The previously described tubular profiles become much more extensive and develop broad, flat expansions or cisternae which tend to be arranged parallel to each other and to the cell surface (Figs. 13 and 17). The ribonucleoprotein particles that were formerly distributed uniformly in the cytoplasmic matrix become concentrated on the membranes of the reticulum. The interior or lumen of this system of intercommunicating channels is translucent to electrons, suggesting either that it contains a substance of very low density or, more likely, that its contents are not preserved by osmium and are lost in the course of specimen preparation. Examples of continuity between the membranes of the reticulum and the outer nuclear membrane or the smooth surfaced Golgi membranes are common. Occasionally, the membranes bounding tubular or cisternal profiles of the reticulum are confluent with the membrane surrounding the nematocyst. It is probable that the existence of such open communications of the lumen of the endoplasmic reticulum, the lumen of the Golgi complex, and the lumen of the perinuclear cisterna may have great significance for the coordination of the functions of these organelles, and for the distribution of intermediate products in the intense synthetic activity associated with the formation of the nematocyst.

Sizeable crystals of unknown molecular species occur sporadically within the reticulum of the cnidoblasts of *Chlorohydra viridissima.* They are not characteristic of any particular stage of differentiation and are not a constant feature of this cell type, but when found in one cnidoblast they are usually present also in the other members of the same group. The crystals are usually rectangular or rhomboidal in section; they measure from one to three microns on a side, and are presumed to be protein in nature. They are closely invested by a membrane bearing ribonucleoprotein granules on its outer surface, and are therefore believed to reside in expansions of the endoplasmic reticulum rendered angular by growth of the crystal. On rare occasions, such crystals are also found in the space created in similar fashion between the two membranes which enclose the nucleus. As previously stated, this space may communicate with the lumen of the endoplasmic reticulum. Because of this relationship and the resemblance of the perinuclear space to the broad flat lacunae in the cytoplasm, it is sometimes referred to as the perinuclear cisterna (37). The observation of similar crystals in both sites in the cnidoblasts lends indirect support to the concept that the space between the nuclear membranes is functionally, as well as structurally, analogous to the cisternae of the endoplasmic reticulum.

It has proved surprisingly difficult to identify correctly the early developmental stages of the four different types of nematocyst possessed by Hydra. Therefore, to avoid possible error and confusion, we shall not name the kinds of nematocyst whose early development we are describing until further study has resolved our present doubts concerning their identity. It should be made clear

that the present paper has, thus far, dealt with the development of only two of the four recognized types. In these, the cytological changes observed in the cnidoblasts are so similar that the above description applies equally well to both. In the differentiation of the other two types, the changes are somewhat similar, but the development of the endoplasmic reticulum is far less extensive and the participation of the Golgi complex in the enlargement of the nematocyst is not so apparent.

The two kinds of nematocyst whose early development is described here can be distinguished from one another by their shape and the character of their capsule. One is flask- or retort-shaped, and possesses a capsule that is smooth in contour and of uniform thickness and density (Fig. 12). The other, less bulbous and more elongate in form (Fig. 11), has an inhomogeneous capsule exhibiting a narrow inner zone of low density and a wider outer zone of higher density. In longitudinal sections the inner aspect of its capsule has a distinctive scalloped contour due to the presence of irregular corrugations that run circumferentially with respect to the long axis of the nematocyst.

In a sense, the nematocyst can be looked upon as a highly organized secretory product of the cnidoblast. The part which the Golgi complex takes in its formation is similar to the role this organelle plays in the elaboration of the secretory granules of various glandular cells and in the formation of the acrosome of the spermatozoan. Throughout the period of growth of the nematocyst the cap-like Golgi complex remains closely applied to its apical pole. The portion of the Golgi cap overlying the thin upper margin of the capsule generally consists of parallel arrays of smooth surfaced flat vesicles of which a few may be distended to form elliptical vacuoles. That portion of the complex which overlies the delicate limiting membrane at the end of the nematocyst consists, for the most part, of a dense aggregation of minute tubular and vesicular elements (Figs. 15 to 17). In addition, larger, thin-walled vacuoles of irregular outline are often seen in this region in close apposition to the nematocyst membrane. A gradation of densities is noted in the contents of these vacuoles, some appearing empty, others having an homogeneous content of low density, and others containing material identical in appearance to that found in the interior of the nematocyst. The membrane that bounds a vacuole of the latter kind is often observed to be continuous with the limiting membrane of the nematocyst in a manner which strongly suggests that the vacuole and the nematocyst were in process of coalescing when immobilized by the fixative (Fig. 17). Thus it seems likely that the Golgi complex participates in growth of the nematocyst by segregating and concentrating material in vacuoles which then fuse with the nematocyst and contribute their contents to its enlargement.

When the body of the nematocyst has reached its definitive size, further growth of the apical region results in the formation of a long tapering process which is coiled in the surrounding cytoplasm. This structure apparently corresponds to the *external tube* described by some of the earlier investigators. Because of its meandering course in the cytoplasm it may be transected at several levels by the same thin section (Figs. 12 and 14). The cross-sections are round, bounded by a thin membrane, and contain a fine-grained matrix like that in the interior of the nematocyst proper. The term *external tube*, which implies a hollow structure, is not a particularly appropriate designation for this solid-appearing, tendril-like process, but there is, nevertheless, inadequate reason for changing a term already firmly engraved in the literature of light microscopy. During the progressive elongation of this process, the Golgi complex is carried farther and farther away from the nematocyst proper. Thus, in more advanced cnidoblasts, the Golgi membranes are found in the peripheral cytoplasm surrounding the growing end of the *external tube* (Fig. 12). It is presumed that at a still later stage this long process is inverted and drawn into the capsule to form the *internal tube* of the mature nematocyst, but to date, no intermediate stages in the process of inversion have been observed in electron micrographs and a description of these events in nematocyst maturation must await further study.

Late Cnidoblast:

In late cnidoblasts whose nematocysts have attained full size, the nucleus is displaced to the periphery and deformed to crescentic shape. The karyoplasm contains relatively few of the small granules believed to be ribonucleoprotein, but the larger granules are abundant and tend to be aggregated in clumps near the nuclear membrane. The nucleolus is unchanged. The cytoplasmic matrix continues to be rich in ribonucleoprotein granules and contains a few lipide droplets. The mitochondria are less numerous than before, their matrix is more dense, and the cristae more sparse

than in cnidoblasts still actively enlarging their nematocysts. The Golgi complex has regressed and now consists of a compact aggregation of flattened vesicles and small vacuoles that is no longer in close juxtaposition to any part of the nematocyst. The endoplasmic reticulum, previously represented by a continuous system of tubules and cisternae, has broken up again into a population of isolated vesicles with relatively few ribonucleoprotein granules adhering to their surface (Fig. 14). In the further regression of the reticulum that occurs in still later stages, these vesicles diminish in size and number. In very late cnidoblasts a few large vacuoles may appear and it is believed that these may arise by coalescence of the small vesicles derived from fragmentation of the endoplasmic reticulum.

The cytoplasmic organelles thus undergo a noticeable regression after the nematocyst has attained its maximum size, but at a time when its internal differentiation is still far from complete. The formation of the internal tube, the elaboration of its basal armament, the differentiation of the operculum and of the cnidocil apparatus, all appear to take place without the participation either of the Golgi complex or an organized endoplasmic reticulum. How the complex morphogenetic events in internal differentiation are determined and controlled, when seemingly isolated from the rest of the cell by the thick nematocyst capsule, defies explanation at present. Further details of the structure of the mature nematocysts will be the subject of a future paper.

DISCUSSION

Examination of the ectoderm of Hydra with the electron microscope has permitted us to define the characteristic submicroscopic structure of an undifferentiated and possibly pluripotential cell—the *interstitial cell*. Its transformation into the cnidoblasts and the subsequent differentiation of these cells has provided an unexcelled opportunity to follow a sequence of changes in cytoplasmic organelles associated with the elaboration of a highly complex cell product. We believe that the observations reported here have significance that goes beyond the specific problem of the development of the nematocyst. When considered in relation to the results of other electron microscope studies on proliferating and differentiating cells, the present findings add considerably to our understanding of the interplay of cytoplasmic ribonucleoprotein particles, the membranes of the endoplasmic reticulum, and the Golgi complex in the synthetic activities of cells in general.

The origin of the nematocyst has long been a matter of debate. A number of the early workers thought that its primordium was formed within the nucleus (24). Others believed that it arose in the cytoplasm, but from granules extruded from the nucleus (22, 34). In the past two decades the majority of investigators who have concerned themselves with this problem seem to have considered the nematocyst to be a product of the cytoplasm and have implicated the chromidial substance, the Golgi apparatus, or both, in its formation (39, 40). On the other hand, Schlottke (30), who carried out meticulous cytological studies on Hydra, concluded that the Golgi apparatus of the cnidoblast was not specifically related to the developing nematocyst. The present electron microscope investigation strongly supports the view that the Golgi complex is intimately involved in nematocyst formation. The formative material seems to accumulate within a vacuole which grows in volume by incorporation of small vesicles formed in the adjacent Golgi complex, a morphogenetic process which closely parallels the events responsible for the formation of the acrosome during mammalian spermatogenesis (3, 5). The participation of the Golgi complex in acrosome formation and in nematocyst development seems to be merely a special manifestation of the general function of this organelle in the formation of secretory products by cells.

It is obviously not the Golgi complex alone that is involved in the development of the nematocyst, for the most striking cytological changes observed in the course of cnidoblast differentiation are not in the Golgi complex but in the endoplasmic reticulum. It will be recalled that this organelle, which is all but absent in the interstitial cells, appears in the form of a few distended tubular elements late in the period of proliferation that precedes the differentiation of these cells as cnidoblasts. With the onset of nematocyst formation these canalicular elements increase greatly in extent and become expanded into broad, flat cisternae disposed in parallel array. When the growth of the nematocyst is at its height, the endoplasmic reticulum attains a complexity comparable to that of vertebrate plasma cells, acinar cells of the pancreas, and other cell types engaged in extremely active protein syntheses. Finally, when the nematocyst has reached its maximum size, the reticulum undergoes a notable regression, breaking up

into isolated vesicles which progressively diminish in number. This sequence of events clearly suggests that a highly organized endoplasmic reticulum is not essential during the preliminary period of cell multiplication or in the final period of maturation of the nematocyst. On the other hand, the intervening phase of active synthesis of nematocyst material apparently requires the presence of a continuous system of membrane-limited channels permeating the cytoplasm and communicating with the Golgi complex.

Of particular interest in relation to the synthesis of proteins, are the changes in the abundance and distribution of the small (120 A) particles believed to be ribonucleoprotein. In the undifferentiated interstitial cell these granules are very numerous and widely dispersed in the cytoplasm. They maintain this distribution throughout the period when the cells are dividing mitotically to form clusters of early cnidoblasts. It is reasonable to infer that the high concentration of ribonucleoprotein particles in the cytoplasmic matrix at this time is related to the synthetic activity involved in the production of new protoplasm. For this, the presence of cytoplasmic membranes is apparently unnecessary. With the cessation of cell division and onset of nematocyst formation the endoplasmic reticulum makes its appearance and the ribonucleoprotein granules become concentrated on its membranes. The coincidence of this change in distribution of ribonucleoprotein particles with the change in cell function suggests that when the granules are associated with membranes, they are no longer concerned with the production of new cytoplasm, but are probably involved in synthesis of a protein-rich cell product.

Although the changing relationships of granules and membranes in the course of cnidoblast differentiation are particularly striking, they are not different in kind from those previously reported for a variety of other tissues. Porter (29) and Palade (26) early drew attention to the sparse reticulum and high concentration of ribonucleoprotein particles in embryonic and rapidly dividing cells. Howatson and Ham (11) later observed a paucity of membranes and a profusion of granules in the cytoplasm of embryonic liver and in hepatoma cells, and they generalized, as we have here, that the free granules are related to synthesis of cytoplasmic protein for growth while those adhering to membranes are characteristic of the differentiated state and are related to synthesis of specialized secretions. Munger's (23) observations on the development of the exocrine pancreas are consistent with this conclusion. He found a highly granular cytoplasm in the early stages, but noted the association of granules with the newly formed ergastoplasmic membranes when differentiation of typical acinar structure began. Closely parallel to the changing conditions in the developing cnidoblasts of Hydra are the cytological changes during regeneration of amputated amphibian limbs described by Hay (9). There, the dedifferentiated cells of the proliferating blastema possess an abundance of cytoplasmic ribonucleoprotein particles, but few cytoplasmic membranes. During their redifferentiation into cartilage cells and their subsequent elaboration of extracellular matrix, the cytoplasmic membranes increase greatly in extent and become studded with granules. Finally in the mature chondrocyte the reticulum undergoes a partial regression.

These parallel observations invite the speculation that the combination of ribonucleoprotein granules with the cytoplasmic membranes is required for the elaboration of a protein-rich cell product. Such a conclusion would be in accord with the hypothesis recently advanced by Siekevitz and Palade (32, 27) that, in the pancreas, the enzymes secreted are synthesized by the attached ribonucleoprotein particles, and are transferred across the limiting membrane into the cavities of the endoplasmic reticulum. Our findings suggest also that the intermediate products collect in the reticulum and are channeled to the Golgi complex where they are first segregated in a form that is visible in electron micrographs. The concept that the interior of the reticulum is a site of accumulation and possibly an avenue of transport for cell products, is conjectural, but it derives indirect support from biochemical studies on incorporation of labelled leucine into the contents of the microsomes (32) and from recent electron microscopic observations on frozen-dried pancreas (33) which show that the lumen of the reticulum, which usually appears empty, is, in fact, filled by a substance of considerable density that is evidently lost with routine methods of specimen preparation.

One of the controversial points in the formation of the nematocyst has been the position of the tube during development. Jickeli (14, 15), in 1882, stated that the tube of all the nematocysts of Hydra develops, at least in part, outside of the capsule and invaginates secondarily. On the other hand, Bourne (1), in 1887, contended that the external tubes observed by Jickeli represented

immature nematocysts that had been discharged prematurely by the action of the fixative. These two points of view have persisted down to the present. Schneider (31), Iwanzoff (13), Jones (16, 17), and many other able investigators allied themselves with Jickeli, but as many others (35, 36, 40, 38), saw the external tube and were convinced that it resulted from premature discharge of the nematocyst. In electron micrographs an external tube is regularly seen on two of the types of nematocysts. In these it appears to develop as an apical prolongation of the nematocyst. It is supposed that it inverts secondarily, but the intermediate stages in the process of inversion have not been seen. This aspect of nematocyst development is in need of further study. The possibility that the extracapsular tubes observed in the cnidoblasts are due to premature firing caused by the fixative is considered highly improbable because the cytoplasm of the cnidoblast shows no evidence of disorder such as might be expected if the nematocyst had fired. Furthermore, the nematocyst types under discussion are precisely those in which there is a consistent close apposition of the Golgi complex to the tube. It is unlikely that this relationship could be established by chance in the premature discharge of the nematocyst into the cytoplasm of the cnidoblast. It may be that some of the existing confusion as to the presence or absence of an external tube is due to the fact that both mechanisms exist, some types forming an external tube which is later inverted, while other types apparently develop the tube and its armament entirely within the capsule. Certainly, in one type of nematocyst observed in Hydra no external tube is seen at any stage of development.

In recent years Kepner and coworkers (18, 19) have revived an earlier suggestion that the tube of the stenotele does not preexist within the capsule, but is formed at the time of discharge by extrusion and solidification of a glutinous fluid. This has been denied in a previous electron microscope study by Hess *et al.* (10), who found, as we have, that in the stenotele the tube is already present in the interior of the capsule before discharge of the nematocyst.

Bibliography

1. Bourne, G. C., The anatomy of the Madreporarian coral *Fungia*, *Quart. J. Micr. Sc.*, 1887, N.S., **27,** 293.
2. Brien, P., and Reniers-Decoen, M., La signification des cellules interstitielles des hydres d'eau douce et le probleme de la réserve embryonnaire, *Bull. biol. France et Belgique*, 1955, **89,** 258.
3. Burgos, M. H., and Fawcett, D. W., Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids of the cat, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 287.
4. Dalton, A. J., and Felix, M., Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis—*in situ*, in homogenates and after isolation, *Am. J. Anat.*, 1954, **94,** 171.
5. Fawcett, D. W., and Burgos, M. H., Observations on the cytomorphosis of the germinal and interstitial cells of the human testis, *in* Ageing of Transient Tissues. A Ciba Foundation Symposium, (G. E. W. Wolstenholme and Elaine C. P. Millar, editors), London, J. & A. Churchill Ltd., 1956, **2,** 86.
6. Fawcett, D. W., and Selby, C. C., Observations on fine structure of the turtle atrium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 63.
7. Fawcett, D. W., Ito, S., and Slautterback, D., The occurrence of intercellular bridges in groups of cells exhibiting synchronous differentiation, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 453.
8. Hadzi, J., Über das Nervensystem von Hydra, *Arb. zool. Inst. Wien*, 1909, **17,** 225.
9. Hay, E., The fine structure of blastema cells and differentiating cartilage cells in regenerating limbs of *Amblystoma* larvae, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 583.
10. Hess, A., Cohen, A. I., and Robson, E. A., Observations on the structure of hydra as seen with the electron and light microscopes, *Quart. J. Micr. Sc.*, 1957, **98,** 315.
11. Howatson, A. F., and Ham, A. W., Electron microscope study of sections of two rat liver tumors, *Cancer Research*, 1955, **15,** 62.
12. Hyman, L. H., The Invertebrates. Protozoa through Ctenophora, New York, McGraw-Hill Book Co., 1940.
13. Iwanzoff, N., Über den Bau, die Wirkungsweise, und die Entwickelung der Nesselkapseln der Coelenteraten, *Bull. Soc. Imp. Nat. Moscou*, 1896, **10,** 95.
14. Jickeli, C. F., Ueber *Hydra*, *Zool. Anz.*, 1882, **5,** 491.
15. Jickeli, C. F., Über den histologischen Bau von *Eudendrium* und *Hydra*, *Morphol. Jahrb.*, 1882, **8,** 373.
16. Jones, C. S., The place of origin and the transportation of cnidoblasts in *Pelmatohydra oligactis*, *J. Exp. Zool.*, 1941, **87,** 457.
17. Jones, C. S., The control and discharge of nematocysts in *Hydra*, *J. Exp. Zool.*, 1947, **105,** 25.
18. Kepner, W. A., Reynolds, B. D., Goldstein, L., and Taylor, J. H., The structure, development.

and discharge of the penetrant of *Pelmatohydra oligactis*, *J. Morphol.*, 1943, **72,** 561.

19. Kepner, W. A., Reynolds, B. D., Goldstein, L., Britt, G., Atcheson, C., Zielinski, Q., and Rhodes, M. B., The discharge of nematocysts of the *Hydra* with special reference to the penetrant, *J. Morphol.*, 1951, **88,** 23.
20. Loomis, W. F., The cultivation of Hydra under controlled conditions, *Science*, 1953, **117,** 565.
21. Loomis, W. F., Environmental factors controlling growth in *Hydra*, *J. Exp. Zool.*, 1954, **126,** 223.
22. Moroff, H. N., Entwicklung der Nesselzellen bei Anemonia. Ein Beitrag zur Physiologie des Zellkerns, *Arch. Zellforsch.*, 1909, **4,** 142.
23. Munger, B., A light and electron microscopic study of cellular differentiation in the pancreatic islets of the mouse, *Am. J. Anat.*,1958, **103,** 1.
24. Murback, L., Beiträge zur Kenntnis der Anatomie und Entwicklung der Nesselorgane der Hydroiden, *Arch. Naturgesch.*, 1894, **1,** (60), 217.
25. Palade, G. E., The fine structure of mitochondria, *Anat. Rec.*, 1952, **114,** 427.
26. Palade, G. E., A small particulate component of the cytoplasm, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
27. Palade, G. E., and Siekevitz, P., Liver microsomes, an integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.
28. Porter, K. R., and Blum, J., A study of microtomy for electron microscopy, *Anat. Rec.*, 1953, **117,** 685.
29. Porter, K. R., Electron microscopy of basophilic components of cytoplasm, *J. Histochem. and Cytochem.*, 1954, **2,** 346.
30. Schlottke, E., Zellstudien an Hydra. II. Die Cytoplasmakomponenten, *Z. mikr. anat. Forsch.*, 1931, **24,** 101.
31. Schneider, K. C., Histologie von *Hydra fusca*, mit besonderer Berücksichtigung des Nervensystems, *Arch. mikr. Anat.*, 1890, **35,** 321.
32. Siekevitz, P., and Palade, G. E., A cytochemical study of the pancreas of the guinea pig. III. *In vivo* incorporation of leucine-1-C^{14} into the proteins of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 557.
33. Sjöstrand, F. S., and Baker, R. F., Fixation by freezing-drying for electron microscopy of tissue cells, *J. Ultrastruct. Research*, 1958, **1,** 239.
34. Vignon, P., Introduction à la Biologie Expérimentale; Les Êtres Organizés, Activités Instincts, Structure, *Encycl. Biol.*, Paris, P. Lechevalier, 1930, **1.**
35. Wagner, G. On some movements and reactions in *Hydra*, *Quart. J. Micr. Sc.*, 1905, **48,** 585.
36. Wagner, J., Recherches sur l'organization de *Monobrachium parasiticum Mery*, *Arch. Biol.*, 1890, **10,** 273.
37. Watson, M. L., The nuclear envelope. Its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 257.
38. Weill, R., Contribution a l'étude des cnidaires et de leurs nematocysts, *Trav. Stat. zool. Wimereux*, 1934, **10** and **11,** 1934.
39. Will, L., Uber die sekretorischen Vorgange der Nesselkapselbildung der Coelenteraten, *Sitzungsber. Abhandl. naturforsch. Ges. Rostock*, 1910, **11,** N.S., 41.
40. Will, L., Die Bildung der Nesselkapseln von *Physalia*, *Sitzungsber. Abhandl. naturforsch. Ges. Rostock*, 1926, **1,** 8.
41. Yoder, M. C., The occurrence, storage and distribution of glycogen in *Hydra viridis* and *Hydra fusca*, *J. Exp. Zool.*, 1926, **44,** 475.

Explanation of Plates

Legend

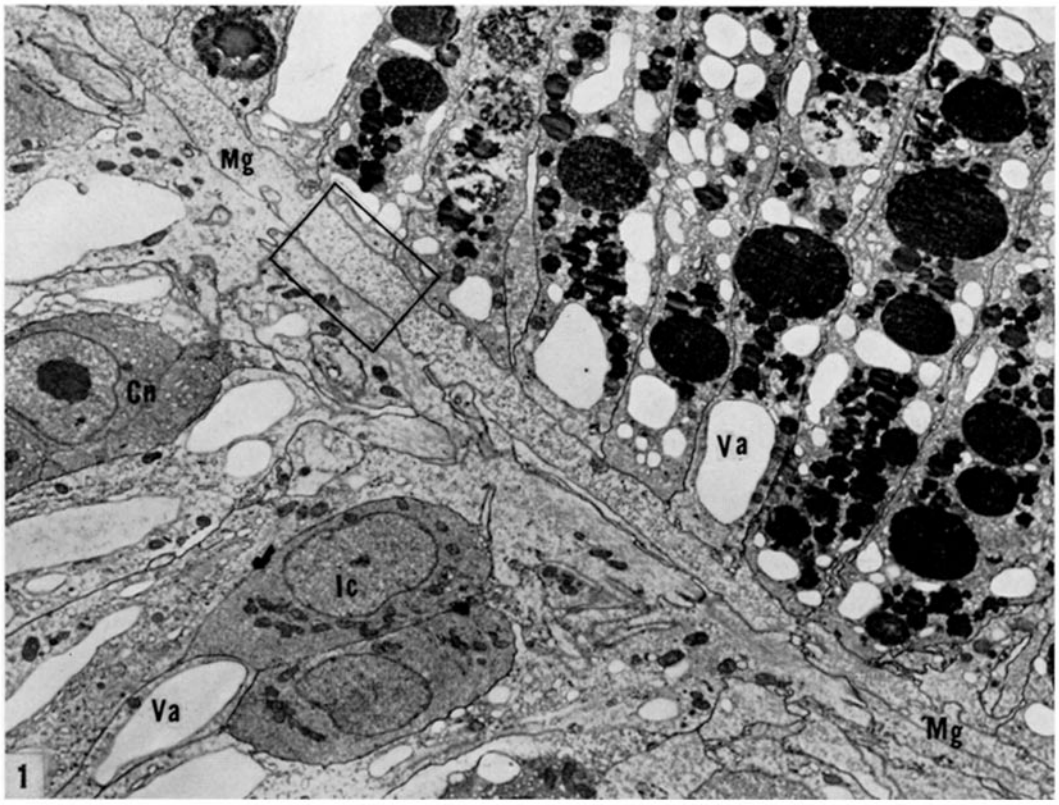
Ce, Centriole.
Cn, Cnidoblast.
Cr, Crypt in ectodermal surface.
Er, Endoplasmic reticulum.
E.T., External tube of nematocyst.
Gl, Particulate glycogen.
G.C., Golgi complex.
Hy, Hyaline inclusion in ectoderm.
IB, Intercellular bridge.
Ic, Interstitial cell.
J, Junction between cell processes.
M, Mitochondrion.
Mf, Myofilaments.
Mg, Mesoglea.
Nc, Nucleus.
Ncl, Nucleolus.
Nm, Nematocyst.
Va, Vacuole.
Vs, Vesicles.

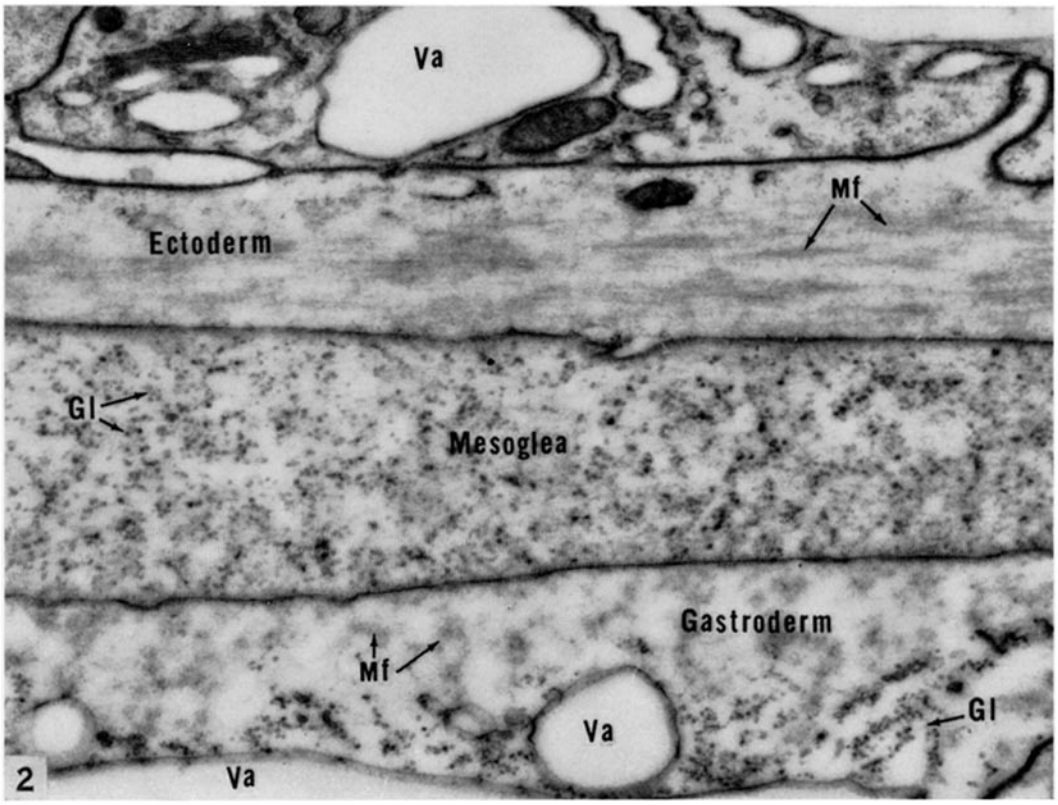
PLATE 193

FIG. 1. A low power electron micrograph of the body wall of *Hydra oligactis*. The mesoglea (*MG*) runs diagonally across the figure, with the gastroderm at the upper right and the ectoderm at the lower left. The columnar musculo-epithelial cells of the gastroderm contain many lipide droplets, clear vacuoles (*Va*), and vacuoles containing dense ingested material in various stages of intracellular digestion. The ectodermal musculo-epithelial cells are also vacuolated and lodged between them are interstitial cells (*Ic*) and clusters of cnidoblasts (*Cn*). An area such as that enclosed by the rectangle is presented at higher magnification in Fig. 2. × 4000.

FIG. 2. An electron micrograph of the mesoglea and the contractile foot processes of the musculo-epithelial cells of the adjacent ectoderm and gastroderm. For orientation see rectangle in Fig. 1. The long axis of the contractile processes of the ectoderm and gastroderm are oriented at right angles to one another. Thus, in this figure bundles of delicate myofilaments (*Mf*) are cut longitudinally in the ectoderm and appear in transverse section in the gastroderm as ill defined areas of slightly greater density than the surrounding cytoplasm.

The mesoglea consists of extremely fine filaments randomly oriented in an amorphous matrix of low density. It also contains conspicuous granules (*Gl*) that appear to be identical to granules found in the gastroderm cells and between the cells of the ectoderm. These are tentatively interpreted as particulate glycogen which arises in the gastroderm and traverses the mesoglea to reach the ectoderm. × 22,000.



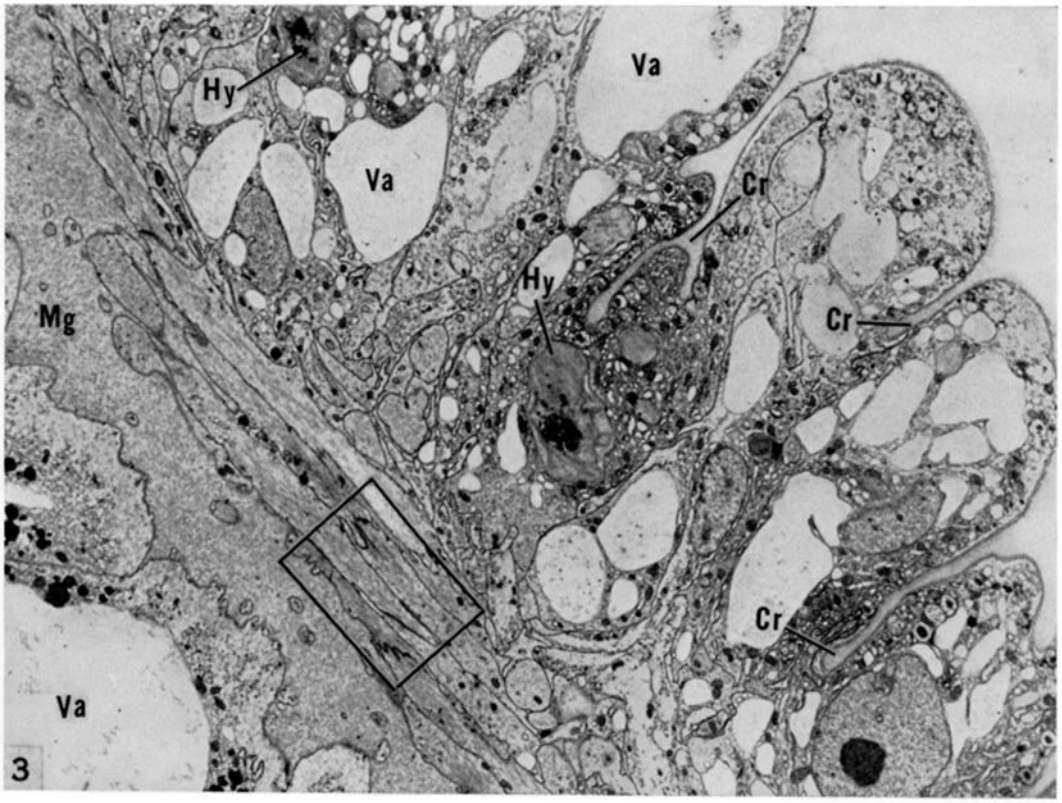


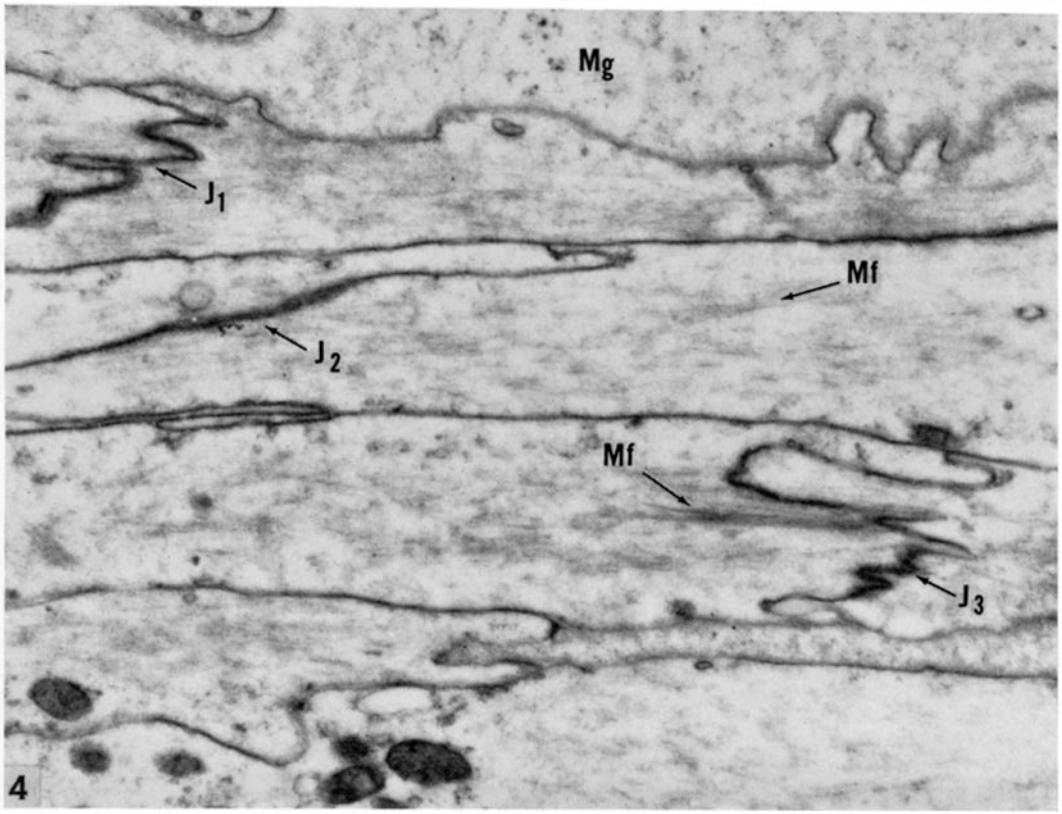
(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 194

FIG. 3. A section through the lower part of the column of Hydra. The ectoderm is at the upper right, endoderm at the lower left. The mesoglea (*Mg*), cut obliquely, appears unusually wide. Both of the epithelia in this region are highly vacuolated (*Va*). The ectoderm has a scalloped surface with branching crypts (*Cr*) extending into the epithelium between cells or groups of cells. Some of the cells contain hyaline inclusions (*Hy*) of irregular outline with dense granular central masses. These appear to be degenerating nematocysts. The rectangle encloses the parallel foot processes of several musculo-epithelial cells which are depicted at higher magnification in Fig. 4. × 25,000.

FIG. 4. An electron micrograph of an oblique section passing through the contractile processes of several adjacent musculo-epithelial cells. For orientation, see rectangle superimposed on Fig. 3. Of particular interest is the configuration of the junction between contiguous processes. To insure firmer union between contractile processes their area of contact is increased by overlap (J_2) or by interdigitation (J_1 and J_3). The myofilaments (*Mf*) terminate in a dense cytoplasmic substance immediately subjacent to the plasma membranes of the adjoining cells. The appearance of these junctions is strongly reminiscent of the intercalated discs of cardiac muscle. × 16,500.



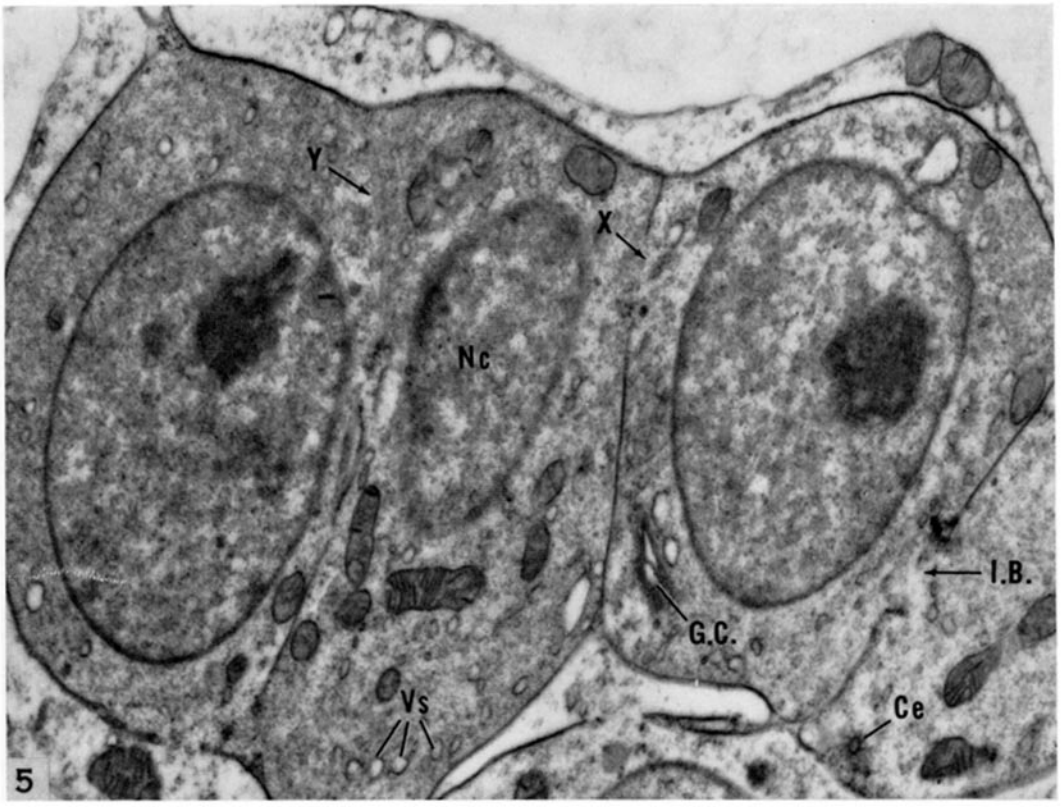


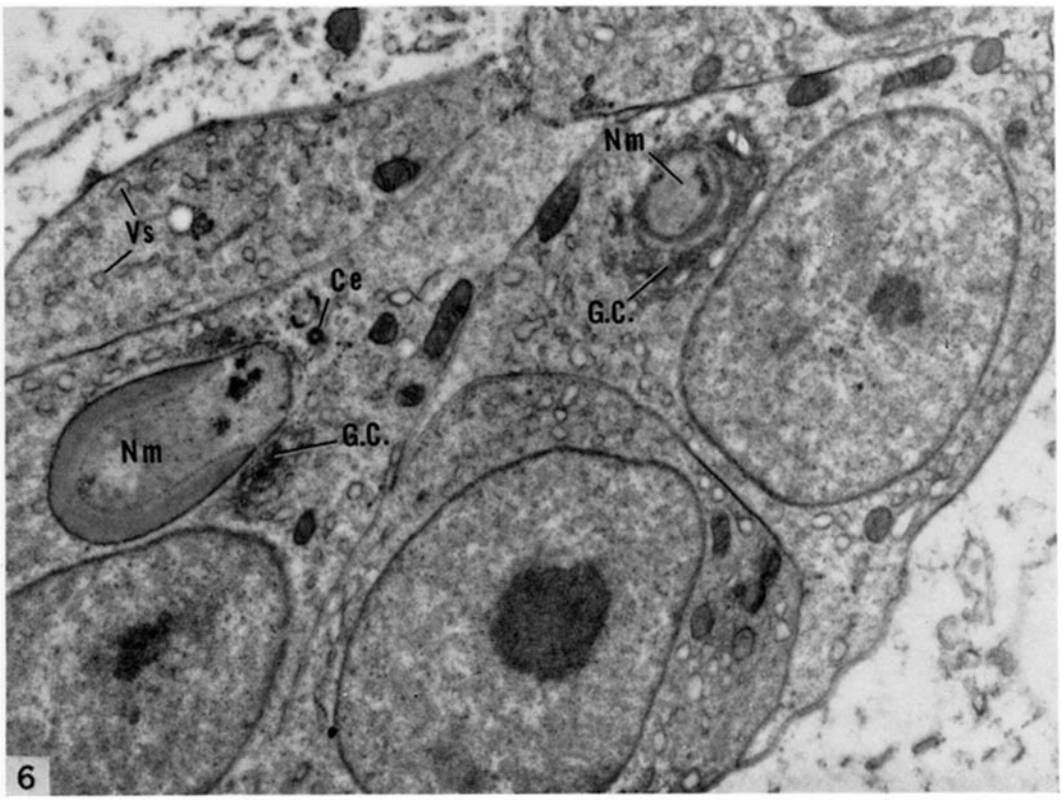
(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 195

FIG. 5. A cluster of very early cnidoblasts formed by the mitotic division of an interstitial cell. In the proliferation of the interstitial cells cytokinesis is incomplete so that the groups of four to sixteen cnidoblasts that result from their division remain connected by sizeable intercellular bridges (*IB*) that form around the spindle remnant and persist after resorption of the filaments. One intercellular bridge is visible at the right of this figure (*IB*). At other places (see at *X*) the cytoplasm of adjacent cells appears to be in continuity through simple defects in the limiting membranes of adjacent cells. These openings do not seem to be artifacts. The boundary between cells may also be indistinct (see at *Y*) when the plane of section is oblique to the cell surface. The cell at the lower right has a centriole (*Ce*) in its peripheral cytoplasm. × 12,000.

FIG. 6. A group of early cnidoblasts, two of which have been cut in a plane passing through their nematocyst (*Nm*). The one at the left cut longitudinally shows a moderately thick capsule except at the apical end where it is enclosed only by a thin membrane. Capped over this end is the Golgi complex (*G.C.*) and nearby is a centriole (*Ce*). The nematocyst at the right has been cut transversely through its apical end which appears to be surrounded by Golgi membranes. Note that in these cnidoblasts which have begun to form nematocysts the endoplasmic reticulum is represented by many isolated vesicles. × 11,000.



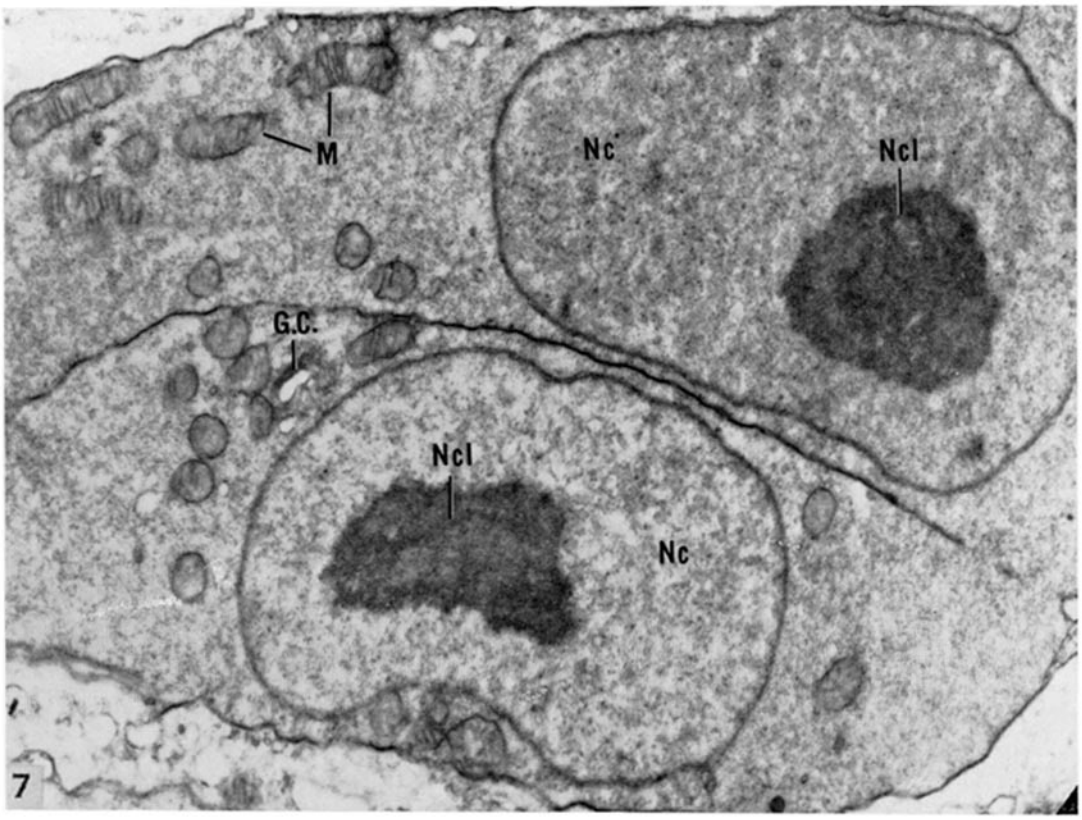


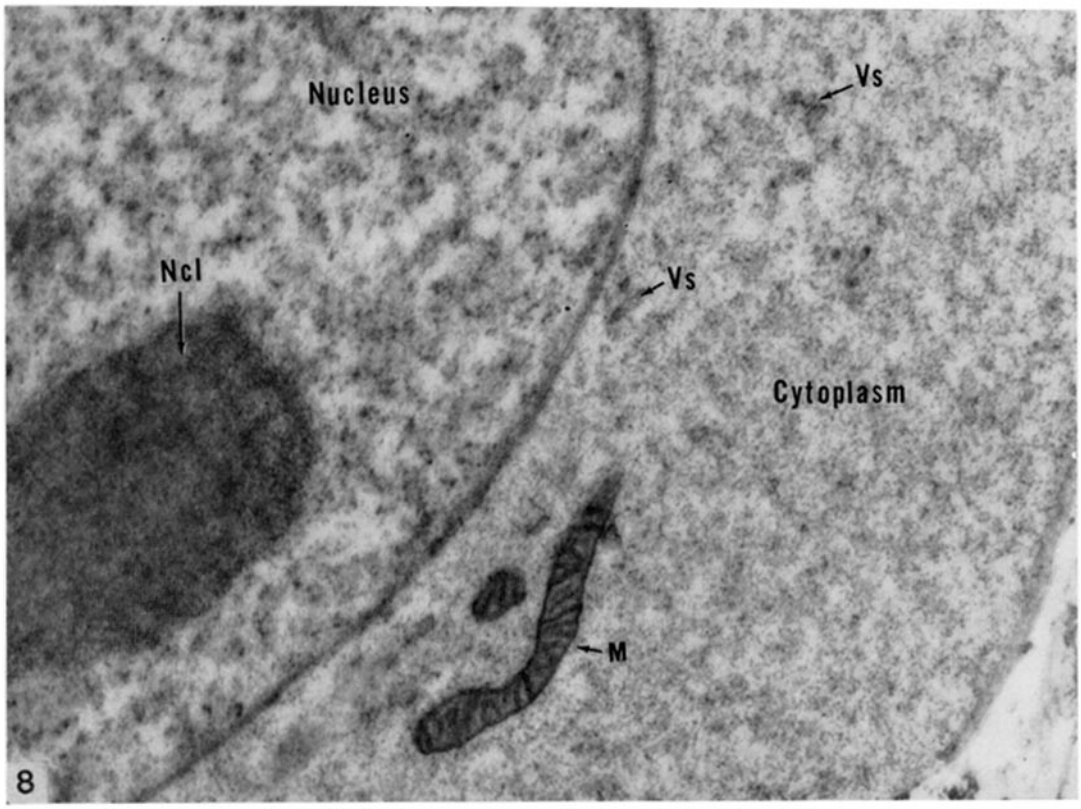
(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 196

FIG. 7. A pair of interstitial cells from the ectoderm of Hydra. They are small cells with a conspicuous nucleolus (*Ncl*) and finely granular cytoplasm containing a few mitochondria (*M*) and a small Golgi complex (*G.C.*). Note particularly the absence of the endoplasmic reticulum. × 13,500.

FIG. 8. An interstitial cell at higher magnification showing the granular character of the karyoplasm and cytoplasm. Fine granules (100 to 120 A) are present in great numbers in the cytoplasm. Similar granules, presumed to be ribonucleoprotein, also make up the bulk of the nucleolus (*Ncl*) and are found in lower concentration elsewhere in the karyoplasm. In addition to these, there are coarser aggregates that appear to be composed of fine granules or filaments of lower density than the ribonucleoprotein granules. The endoplasmic reticulum is represented only by widely scattered minute vesicles (*Vs*). × 36,500.



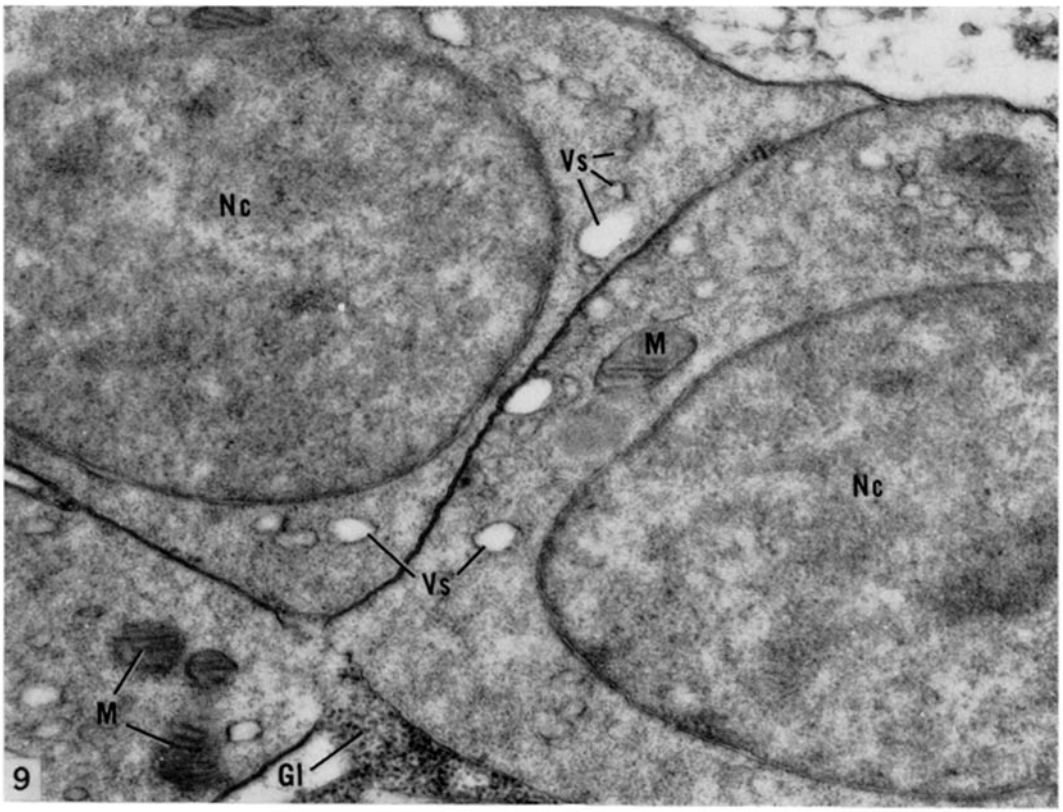


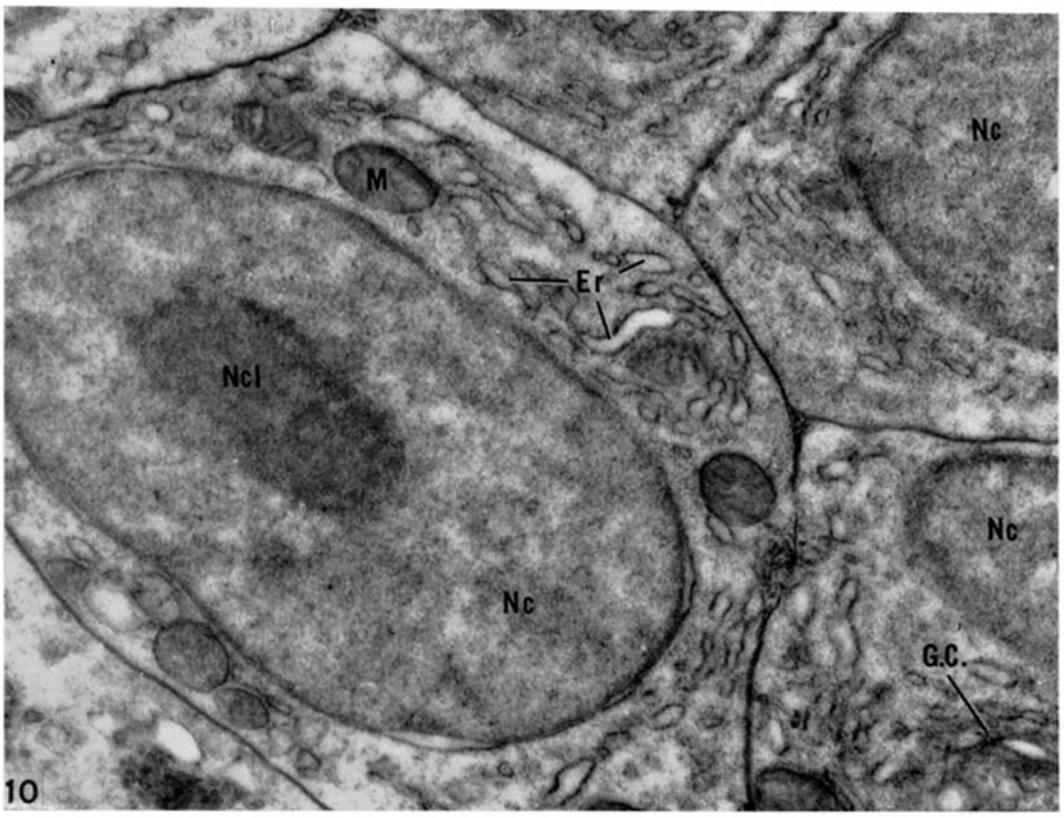
(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 197

FIG. 9. Portions of three early cnidoblasts at higher magnification. With the onset of differentiation, isolated vesicles (*Vs*) appear in the cytoplasm in increasing numbers. Some have ribonucleoprotein granules adhering to their membranes. These vesicles apparently represent an early stage in the development of a continuous endoplasmic reticulum. Between cells at the bottom of the figure is an accumulation of granules (*GI*) believed to be glycogen. × 27,000.

FIG. 10. A group of cnidoblasts slightly more advanced in their differentiation. The vesicular structures in the cytoplasm are more numerous than before and their profiles are now elongated, suggesting that isolated vesicles have coalesced to form a more or less continuous endoplasmic reticulum (*Er*) consisting of tubules and cisternae. The mitochondria (*M*) appear larger than in the interstitial cells, but no more numerous. × 24,000.



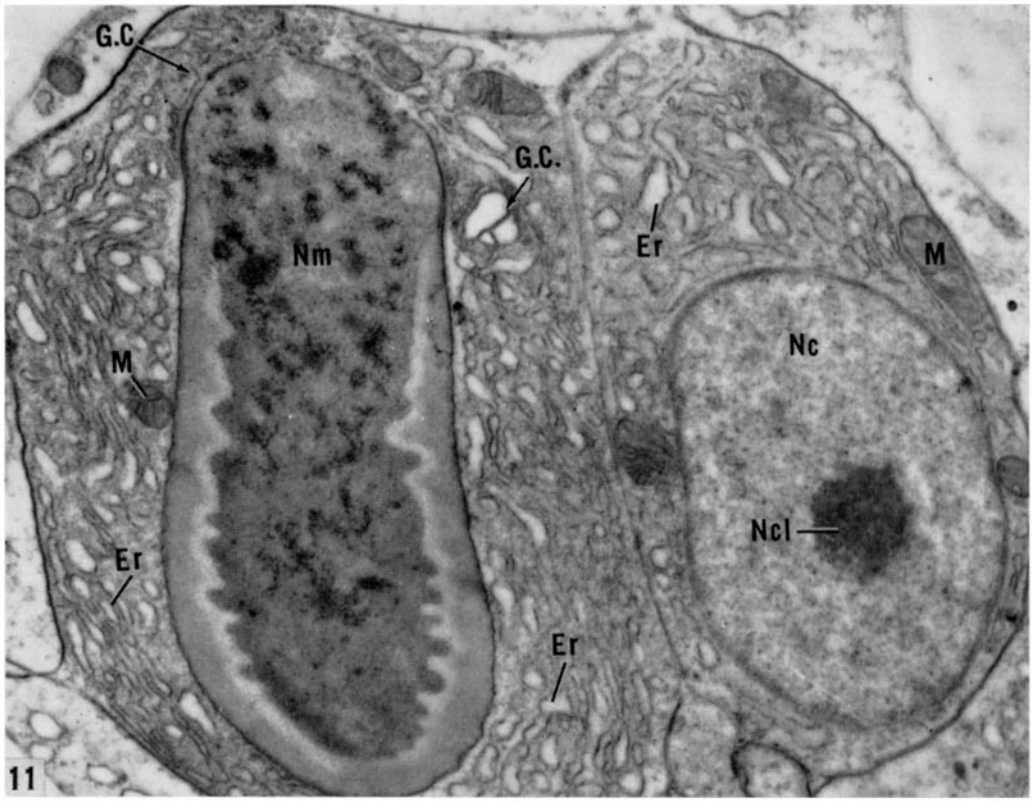


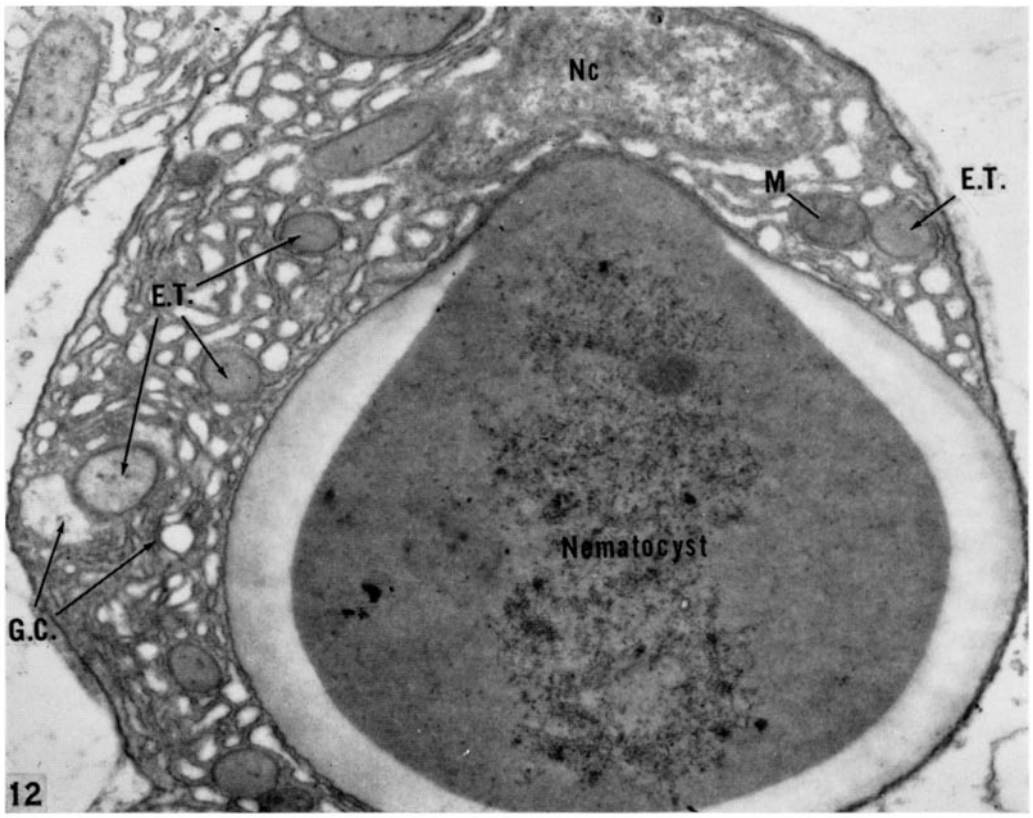
(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 198

FIG. 11. A pair of more advanced cnidoblasts. The section passes through the nematocyst (*Nm*) of one and through the nucleus (*Nc*) of the other. At this stage of rapid growth of the nematocyst there is a very extensive development of the endoplasmic reticulum (*Er*). The type of nematocyst shown here is elongated and cylindrical in form. The less dense, inner aspect of its capsule is irregularly corrugated, with alternating ridges and grooves that run circumferentially with respect to the long axis of the nematocyst. The capsule encloses an amorphous matrix of appreciable density in which are conglomerations of denser filaments and granules. The capsule is open at the apex and here the contents of the nematocyst are bounded only by the thin membrane that encloses the entire structure. The Golgi complex (*G.C.*) forms a cap over the apical end of the nematocyst where the thick capsule is lacking. × 12,000.

FIG. 12. A cnidoblast forming a nematocyst of a different type. This kind is flask- or retort-shaped and the capsule is homogeneous in density and smooth on its inner surface. A long slender process which projects from the apex of the nematocyst is coiled around it in the cytoplasm. This corresponds to the *external tube* described by earlier workers. Because of its meandering course in the cytoplasm, it (*E.T.*) is transected at eight different levels by the section illustrated here. At the left side of the figure one of the cross-sections presumed to be near the tip is surrounded by the Golgi complex (*G.C.*) which has been displaced farther and farther from the end of the nematocyst as a result of the elongation of the external tube. × 13,000.



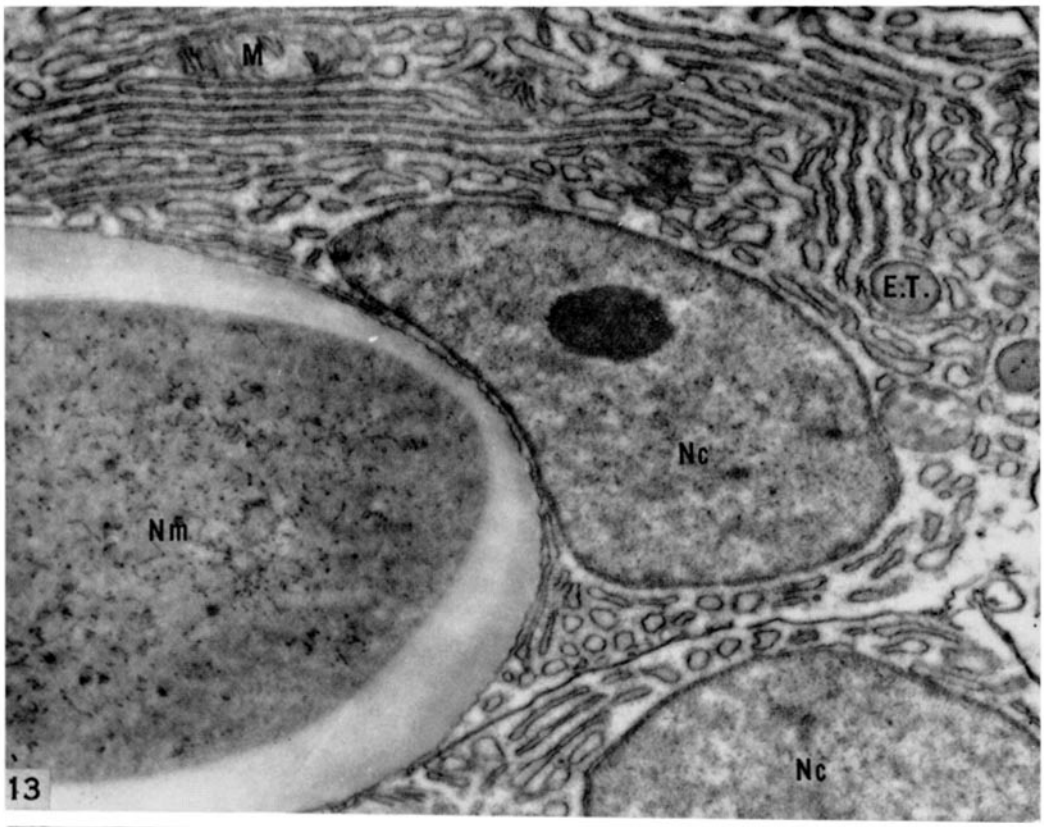


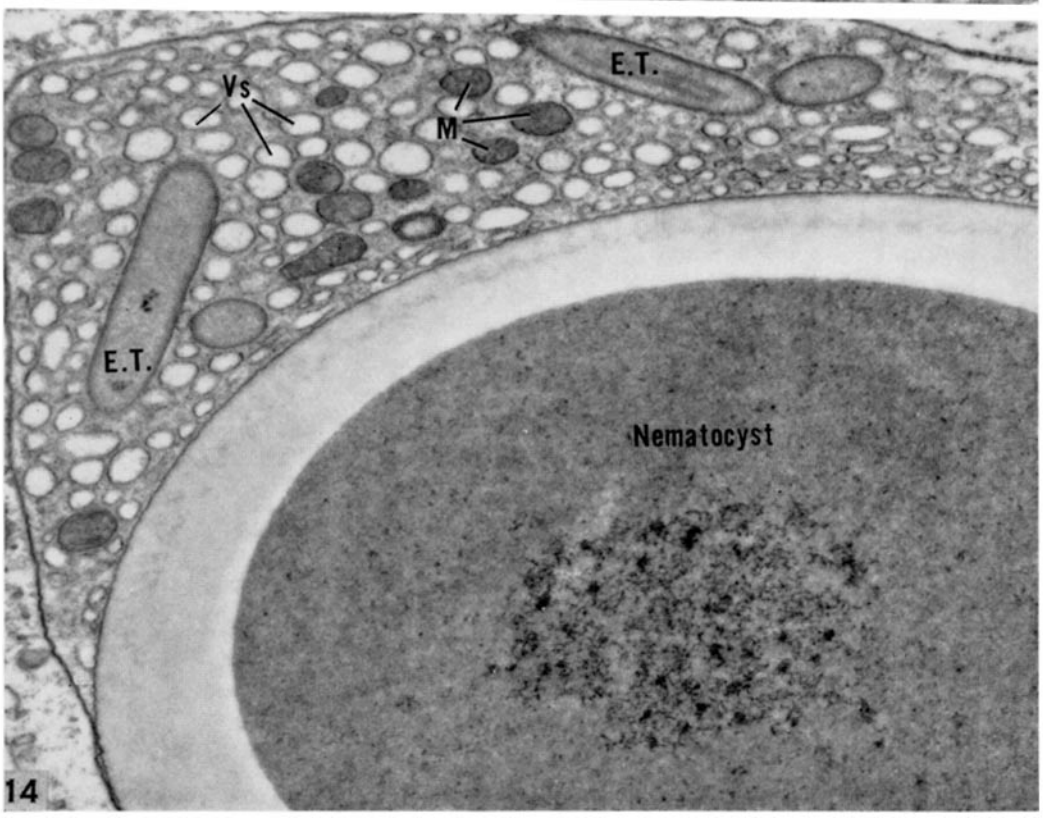
(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 199

FIG. 13. A portion of an advanced cnidoblast demonstrating that, at the height of the synthetic activity associated with nematocyst formation, the endoplasmic reticulum becomes as extensive as that of pancreatic or other active secretory cells. Notice the development of dense filaments in nematocyst matrix. × 15,500. (Fawcett, D. W., *in* Developmental Cytology, (D. Rudnick, editor), New York, Ronald Press, 1959.)

FIG. 14. A portion of a late cnidoblast whose nematocyst has attained its maximum size but has not completed its internal differentiation. The endoplasmic reticulum appears to have undergone some degree of regression, breaking up again into isolated vesicles (*Vs*). The mitochondria (*M*) seem to have diminished in size and their matrix is more dense than in the earlier, more active stages in the cytomorphosis of the cnidoblast. The nematocyst still has an external tube (*E.T.*). × 12,000.



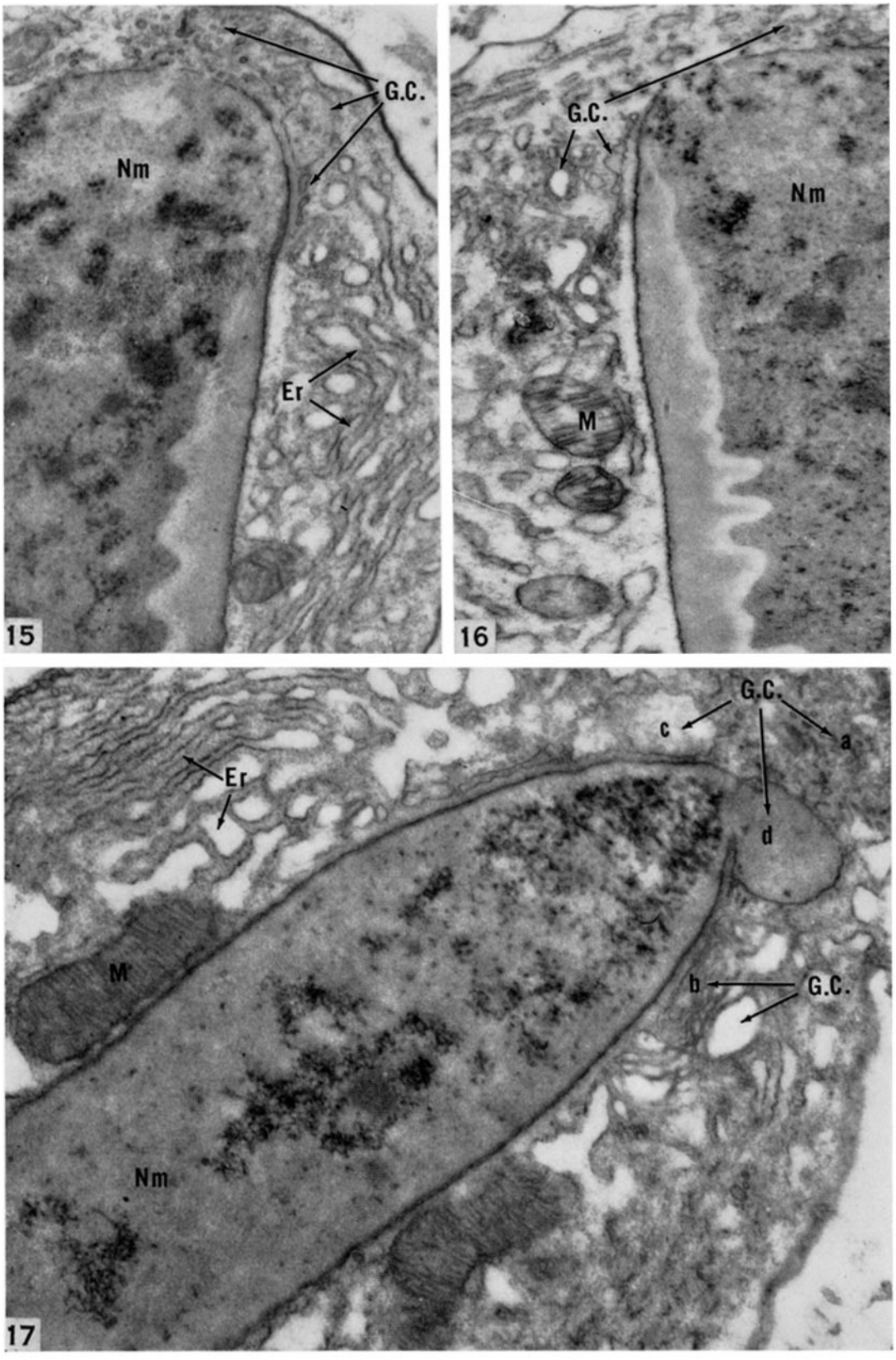


(Slautterback and Fawcett: Development of cnidoblasts)

PLATE 200

FIG. 15 and 16. Corresponding areas of the apical portion of two similar nematocysts presented here to illustrate the constant and intimate relation of the Golgi complex to this region. Many small vesicular and tubular elements of the complex are found in the cytoplasm over the end of the nematocyst. Toward the sides are larger vesicles, some of which appear empty, whereas others contain material similar to the amorphous matrix in the interior of the nematocyst. × 21,000.

FIG. 17. The thick proximal portion of the external tube of an advanced nematocyst showing the Golgi complex *G.C.*) still closely associated with its growing tip. At *a* is an aggregation of minute vesicles and tubules; at *b* are parallel arrays of membranes; at *c* a sizeable vacuole with a content of low density; at *d* a Golgi vacuole which has a content closely resembling that of the external tube with which it appears to be coalescing. × 21,000.



(Slautterback and Fawcett: Development of cnidoblasts)