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A FINE-STRUCTURAL STUDY OF EMBRYONIC AND LARVAL DEVELOPMENT IN THE GYMNOBLASTIC HYDROID *PENNARIA TIARELLA*

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Investigations of development in hydrozoans have consisted for the most part of descriptive studies of normal development using light microscopy and general histological staining procedures. Specific histochemical methods have been employed, however, to monitor macromolecular changes during development of *Pennaria* (Cowden, 1965) and *Hydractinia* (Edwards, 1973). In addition, Edwards (1975) has used autoradiographic methods to investigate changes in nucleic acid and protein synthesis during development of *Hydractinia*.

Few studies on hydrozoans other than *Hydra* have extended observations to the fine-structural level. For certain Anthozoa, the planulae (Lyons, 1973; Vandermeulen, 1974; Chia and Crawford, 1977) and adults (Goreau and Philpott, 1956; Lyons, 1973; Chia and Crawford, 1977) have been so described. Fine-structural studies of polyps of Hydrozoa have been limited to analyses of cell types in *Campanularia* (Brock, 1970), the epidermis of *Cordylophora* (Jha and Mackie, 1967), and mechanoreceptors of *Coryne* (Tardent and Schmid, 1973). There has been no detailed account of fine-structural changes during development of a hydrozoan, although the surface coat of the planula of *Corydendrium* has been described (Glätzer, 1970); and limited use was made of the electron microscope in a comparative study of development in gymnoblastic hydroids (Van de Vyver, 1967).

The vast amount of new information gleaned from ultrastructural analyses of *Hydra* (for a review, see Burnett, 1973) suggested the potential value of examining at the fine-structural level embryonic and larval development in a hydrozoan more typical than *Hydra*. The marine hydroid *Pennaria tiarella* was chosen for such a study because of the morphological and histochemical information already available (Cowden, 1965; Summers and Haynes, 1969; Martin and Edwards, 1974).

Materials and Methods

Colonies of *Pennaria tiarella* were collected from wharf pilings near the Bermuda Biological Station in July, 1973. Similar collections were made in August, 1975, at the Duke Marine Laboratory, Beaufort, North Carolina. Fronds from several mature male and female colonies were placed together in large finger bowls of Millipore-filtered sea water. The bowls were placed in the dark at 6:00 PM, and at 9:00 PM early cleavage stages were collected.

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Embryos were fixed at selected stages of development. The series collected in 1973 included the 2-hour cleavage, the 8-hour gastrula, and the 13-, and 16-hour planula stages. The series collected in 1975 was more complete, and included 2-, 4-, 6-, 8-, 10-, 12-, and 16-hour stages. Specimens were fixed for one hour at room temperature in 2% glutaraldehyde in 0.1 M sodium cacodylate-buffered sea water, pH 7.0, containing 1.5% sucrose and 0.01 M $CaCl_2$ (modified from Anderson and Personne, 1970). The embryos were rinsed in 0.1 M sodium cacodylate-buffered sea water, pH 7.0, containing 5% sucrose and 0.01 M $CaCl_2$ for three 10-minute changes at room temperature. They were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate, pH 7.0, for one hour, followed by a 30-minute rinse in 0.1 M buffer. The specimens were dehydrated in an ethanol series, infiltrated with propylene oxide, and embedded in an Epon-Araldite mixture. In the 1975 collection, a complete developmental series was also fixed for light microscopic study both in Clark's 3:1 ethanol-acetic acid fixative and in 10% neutral buffered formalin, dehydrated in an ethanol series and embedded in Paraplast Plus paraffin.

Specimens fixed for electron microscopy were sectioned with glass knives on a Porter Blum MT2-B ultramicrotome. Sections were placed on 200-mesh copper grids, stained in 3% uranyl acetate followed by 0.2% lead citrate (Veneable and Coggeshall, 1965), and examined with a Zeiss EM9S-2 electron microscope. Plastic sections 0.18 to 0.36 μ thick from a region of the block adjacent to that used for thin sections were mounted on glass slides and stained with 0.5% toluidine blue in 1% sodium borate for examination with the light microscope.

At each stage examined, paraffin sections 10 μ thick were utilized to obtain histochemical information and to make comparisons with results reported by other investigators. Paraffin sections were mounted with Mayer's albumin, hydrated through a series of alcohols in the usual manner, and stained.

General histological staining procedures included Mallory's Triple Stain, hematoxylin and eosin, and hematoxylin and Gray's Double Contrast Stain (Gray, 1958). Himes' Triple Stain (Thompson, 1966) was used to provide an overview of localization of deoxyribonucleic acid (DNA), protein, and mucopolysaccharide/glycogen.

Histological procedures for nucleic acids included toluidine blue, pH 4.0, with control slides incubated in hot trichloroacetic acid (TCA), deoxyribonuclease (DNAse), or ribonuclease (RNAse) according to the protocol of Thompson (1966); the nucleal Feulgen for deoxyribonucleic acid, with TCA or DNAse pretreatment of control slides (Thompson, 1966); and azure B for ribonucleic acids (RNA) with RNAse pretreated sections used as control slides (Swift, 1955). The periodic-acid-Schiff (PAS) procedure with α-amylase-treated control slides was employed for the localization of mucopolysaccharides (Thompson, 1966); and Best's carmine with α-amylase-pretreated control slides was used to identify sites of glycogen deposits (Thompson, 1966). The specific stain for the localization of protein was 1-fluoro-2, 4-dinitrobenzene coupled with β-naphthol with control slides pretreated with trypsin (Thompson, 1966).

In 1973, the living organisms were photographed with an Olympus photomicroscope just prior to their fixation. Paraffin sections were photographed with a Leitz Ortholux photomicroscope.

Results

Two- to six-hour stages (cleavage)

From paraffin sections, it is obvious that at two hours post-fertilization, the blastomeres are of unequal size. In general, the outer blastomeres are smaller than the inner ones, and mitotic figures are more numerous peripherally. Cell nuclei stain positively with specific stains for DNA and RNA. Nuclear staining properties remain unchanged during development.

The cytoplasm contains both acidophilia and basophilia. Acidophilia is indicated by a positive reaction to fast green, aniline blue, and napthol yellow S. Positive staining with dinitrofluorobenzene-β-naphthol suggests that this acidophilic component includes protein. Basophilia is demonstrated by staining with hematoxylin. Staining of the cytoplasm with azure B, eliminated by RNAse pretreatment, indicates the presence of cytoplasmic RNA. The cytoplasm of the outer blastomeres stains more intensely with the specific stains for protein and RNA than does the cytoplasm of the central blastomeres.

In 2-hour embryos fixed in 10% neutral buffered formalin, the peripheral cytoplasm appears distinctly granular. The granules are acidophilic, as indicated by a strong affinity for such acid stains as fast green, aniline blue, Gray's Double Contrast Stain, and naphthol yellow S. That the acidophilia includes protein is indicated by staining of the granules with dinitrofluorobenzene-β-naphthol, employed as a specific stain for protein. The granules are also positive for periodic-acid-Schiff (PAS) and negative for Best's carmine, indicating that they contain mucopolysaccharide, but not glycogen. The absence of nucleic acid is indicated by the consistently negative response of the granules to staining with nuclear Feulgen, azure B, and toluidine blue.

The granules are not preserved in Clark's 3 : 1 fixative. The difference in preservation of the granules between those embryos fixed in formalin and those fixed in Clark's alcohol-acetic acid mixture is suggestive of a phospholipid component of the granules, since that is a class of biological macromolecules known to be both soluble in alcohol and rendered insoluble by formalin (Thompson, 1966; Galigher and Kozloff, 1971). A basophilic component of the granule, demonstrable by staining with hematoxylin, may represent this phospholipid, although acid mucopolysaccharides are also basophilic.

A thin glycocalyx on the free surface of each blastomere is positive for acid dyes and dinitrofluorobenzene-β-naphthol, an indication of a protein component. Positive staining with PAS reflects the presence of mucopolysaccharide and/or glycogen. Staining of the glycocalyx with Best's carmine, reduced in intensity by pretreatment with α-amylase, reveals that this surface coat does contain glycogen. Areas of PAS-positive material are also present between adjacent blastomeres.

In electron micrographs of 2-hour embryos, microvilli are arranged in a sparse brush border at the cell surface (Figs. 1 and 2a). Also detectable is a fibrillar-like substance, *ca.* 1.25 μ thick, coating the surface of the embryo. Ovoid, electron-dense, membrane-bound granules with diameters ranging from 0.4 to 0.8 μ are located directly beneath and in close association with the cell surface membrane (Fig. 2b). At this and later stages, the granules, which are easily identifiable in

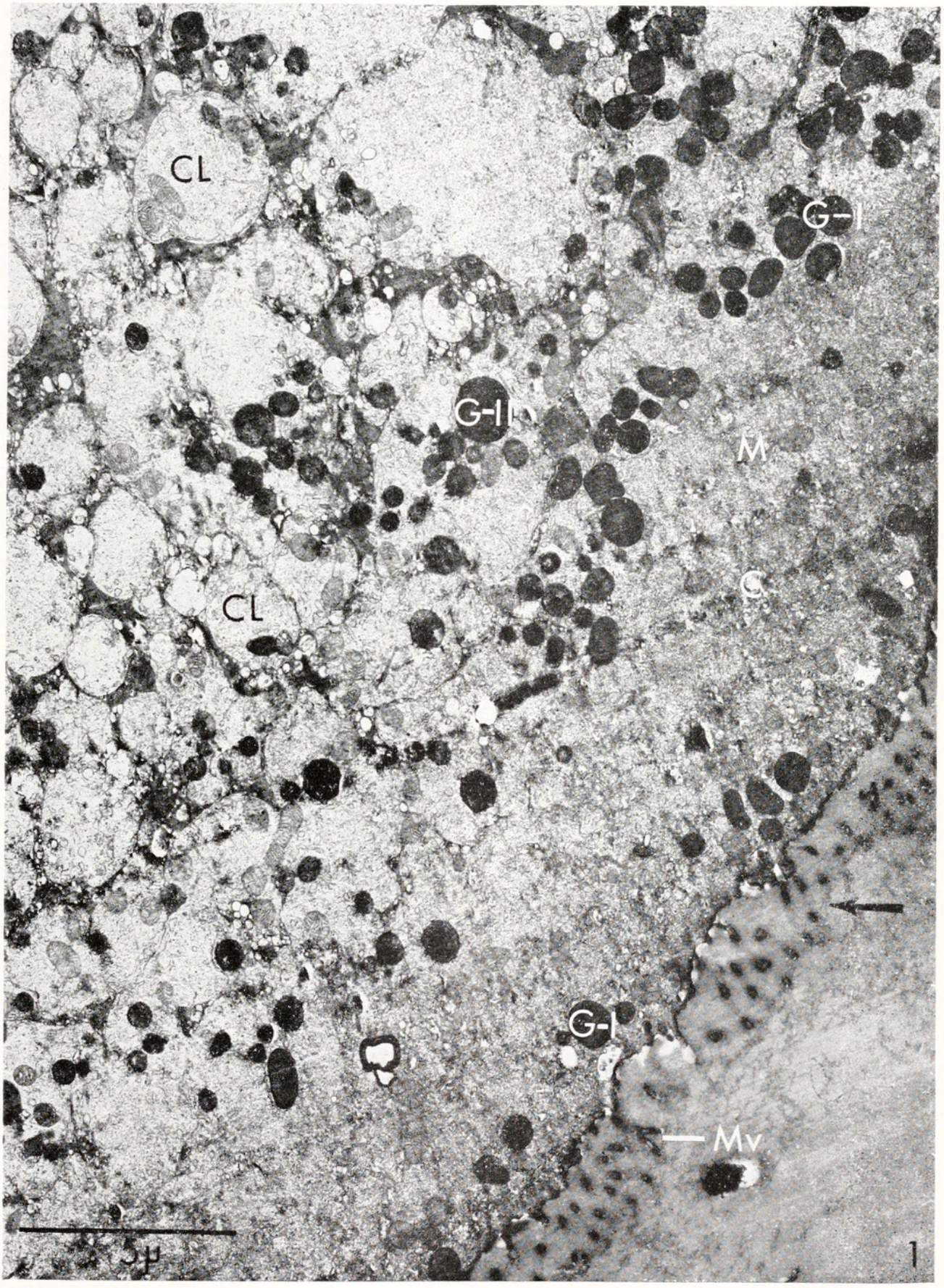


FIGURE 1. In the 2-hour stage a fibrillar-like substance (arrow) is associated with the microvilli. C represents cortex; CL, cytoplasmic lobule; G-I, Type I granule; G-II, Type II granule; M, mitochondrion; and Mv, microvillus.

thick plastic sections, are identical in size, shape, and location to the granules seen at the light microscopic level. These will be referred to as Type I granules.

A granule-free cortex, *ca.* 8 to 10 μ thick, separates the Type I granules at the surface from a band of similar granules occurring more interiorly (Fig. 1). Mitochondria with an unusual morphology are found scattered throughout the cortical region (Figs. 1 and 2c). These mitochondria have an extremely electron-dense matrix, and thick cristae measuring *ca.* 0.18 μ by 0.04 μ. Small, electron-dense bodies, *ca.* 0.02 μ in diameter, are found in the mitochondrial matrix (Fig. 2c).

Interior to the cortex of the blastomeres there occur anucleate, membrane-bound cytoplasmic lobules, measuring *ca.* 2 μ in diameter (Fig. 2d), which are identical in morphology and location to cytoplasmic lobules which can be seen in thick plastic sections. Lobulation of the cytoplasm is not discernible in paraffin sections, however. The cytoplasmic lobules are separated from one another by a homogeneous, electron-dense ground substance. The spheres contain the atypical mitochondria described above, Type I granules, an occasional Golgi body, free ribosomes, and short segments of granular endoplasmic reticulum. The organelles are dispersed in what appears to be a random arrangement within the cytoplasmic lobules. Large granules (Fig. 2e), *ca.* 0.8 μ in diameter, are found in some of the cytoplasmic lobules. These electron-dense Type II granules are characterized by the presence of numerous small, electron-lucent areas. No such granules can be detected in the cortical region.

Between two and four hours post-fertilization, morphological changes occur. In thick plastic sections, the surface of the 4-hour embryo appears ruffled. Microvilli have increased in length. The presence of surface vacuoles suggests that endocytosis or exocytosis is occurring. Type I granules have increased in number and have begun to accumulate at the surface in wedge-shaped pockets, *ca.* 1.5 to 2.0 μ in diameter. The circumferential granule-free cortical zone detected earlier is no longer present. Metachromatic granules (Type II), *ca.* 0.6 μ in diameter, appear in the central regions of the embryo. Some of the nuclei possess centrally located nucleoli. The nucleoli are more abundant in the inner blastomeres than in the outer ones.

At the fine-structural level, a few surface cilia can be seen. In the more peripheral cells the homogeneous, electron-dense ground substance found between cytoplasmic lobules at the 2-hour stage has disappeared, although the compartmentalizing membranes delimiting the cytoplasmic lobules still occur (Fig. 3a). Mitochondria increase in number, but retain the atypical morphology described above. In the cytoplasmic lobules (Fig. 3b), numerous segments of granular endoplasmic reticulum appear coalesced at the periphery of the spheres, located just beneath the limiting membrane. Free ribosomes, atypical mitochondria, and Golgi bodies occur in the lobules. Type I granules, first detected at the 2-hour stage, are present both in the cytoplasmic lobules and at the embryo surface. Numerous intercalations of the plasma membranes between adjacent blastomeres can be seen (Fig. 3a).

With the electron microscope it can be seen that by six hours of development the spherical intracellular organization of the cytoplasm into lobules is less obvious (Fig. 4a). Type I granules occur in increased numbers in aggregates near the surface. Type II granules are present in the deeper portions of the embryo. Microvilli are numerous and regularly arranged along the surface (Fig. 4b).

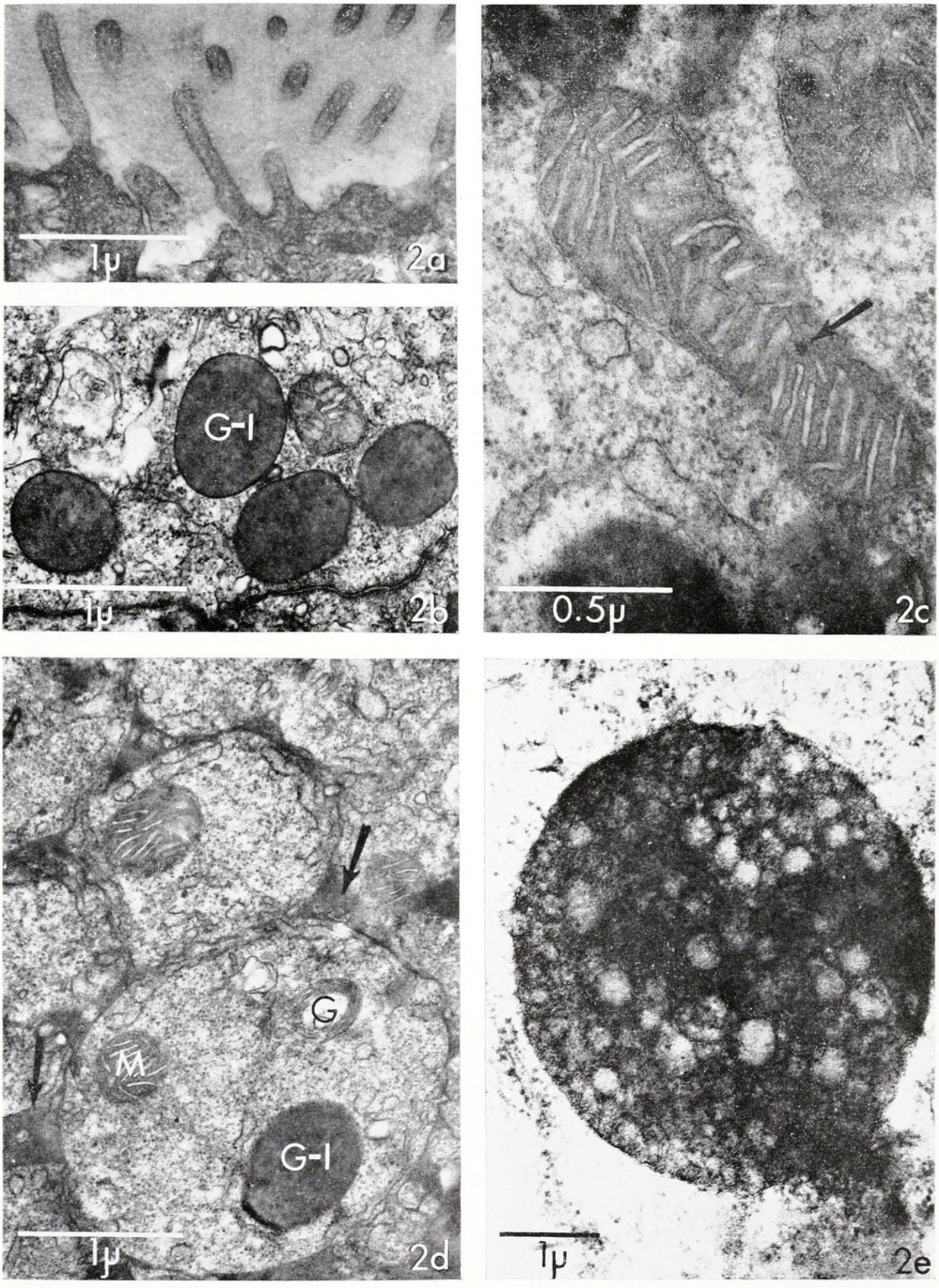


FIGURE 2. Embryos at the 2-hour cleavage stage possess: a. microvilli and a surface coat; b. Type I granules; c. mitochondria with electron-dense bodies; d. cytoplasmic lobules separated by an electron-dense ground substance (arrows); and e. Type II granules within cytoplasmic lobules. G represnts Golgi body; G-I, Type I granule; and M, mitochondrion.

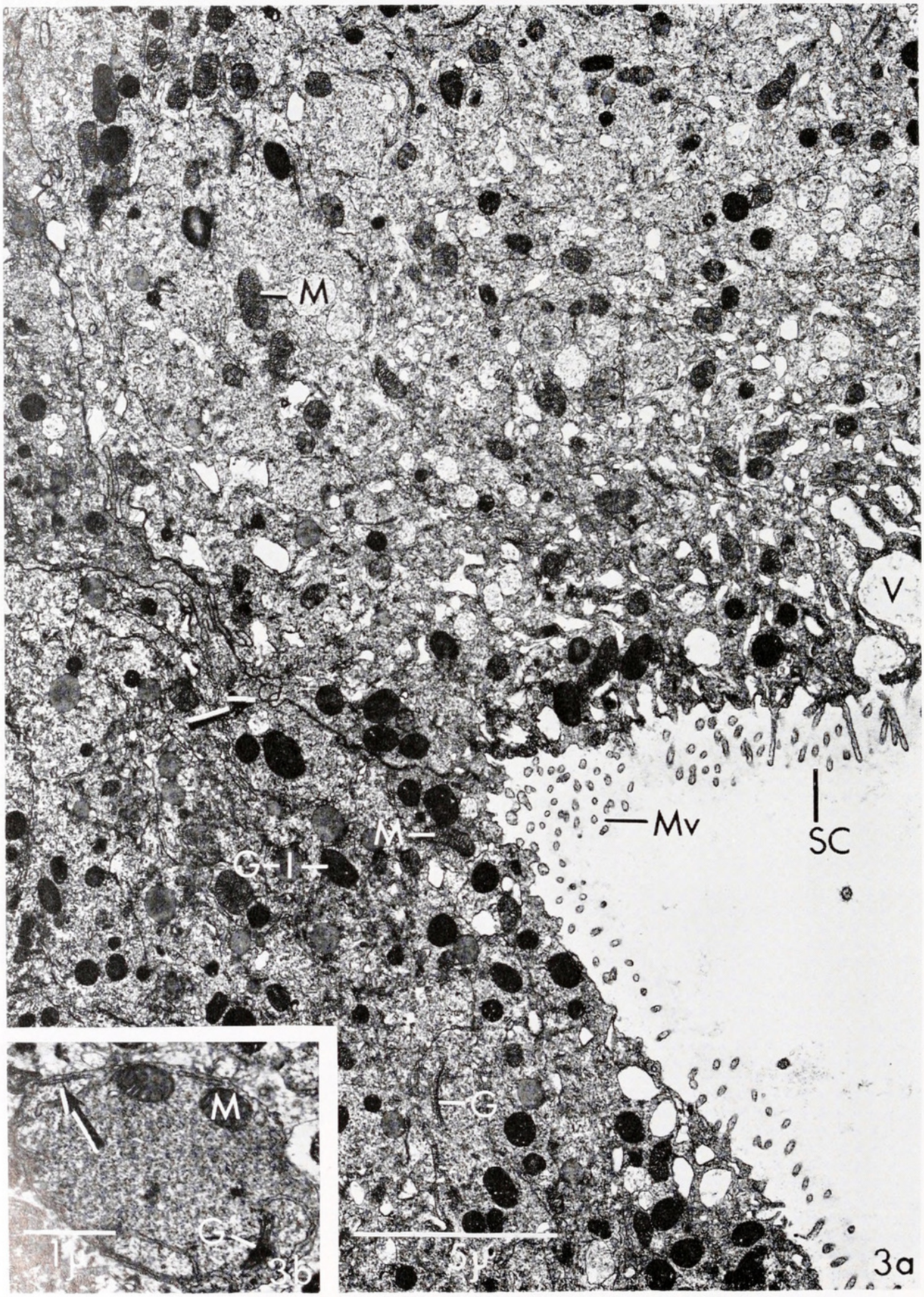


FIGURE 3. At the 4-hour cleavage stage: a. intercalations of plasmalemma between adjacent cells are abundant (arrow); and b. cytoplasmic lobules contain segments of granular endoplasmic reticulum coalesced beneath the limiting membrane of the lobule (arrow). G represents Golgi body; G-I, Type I granule; M, mitochondrion; Mv, microvillus; SC, surface coat; and V, vacuole.

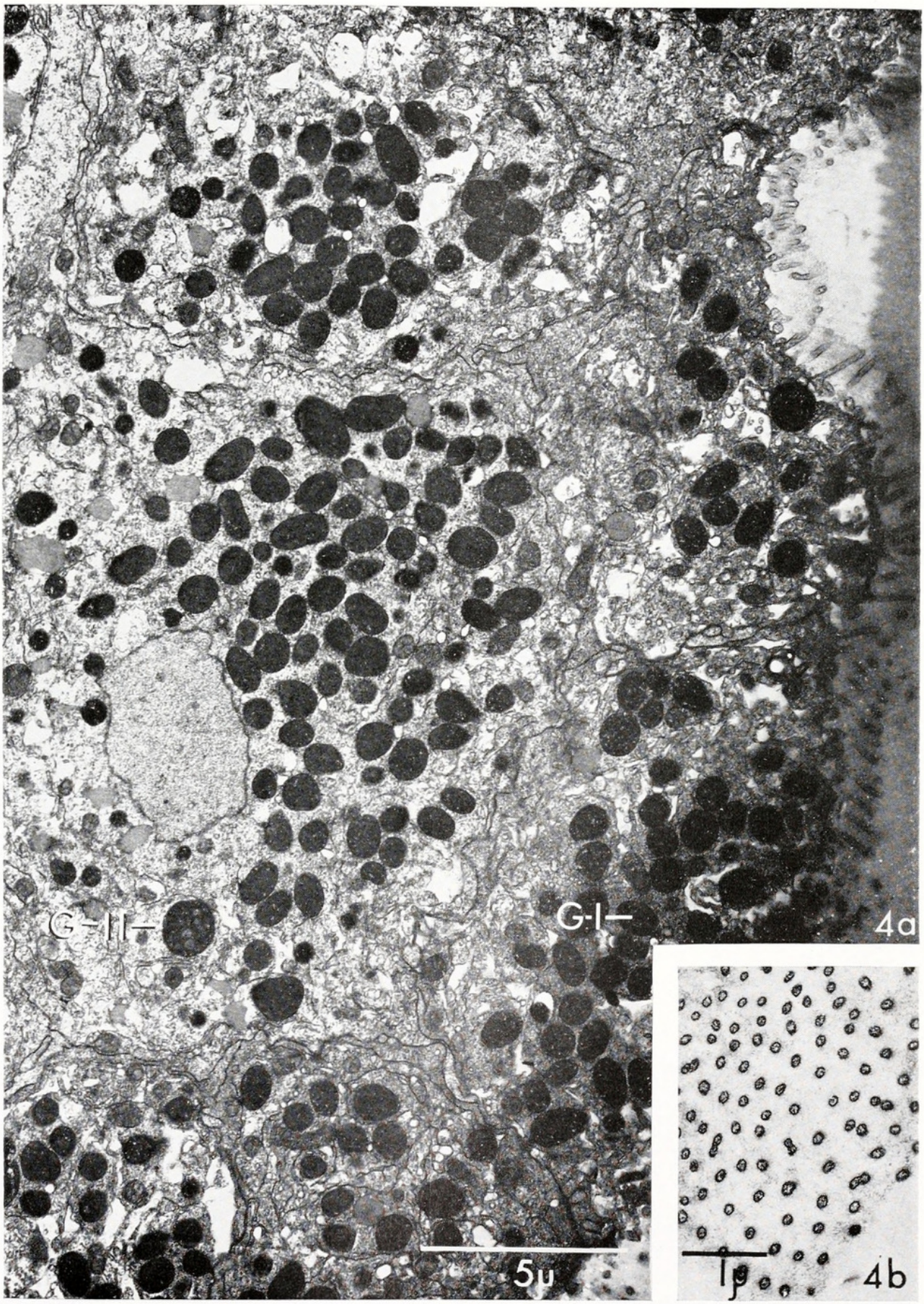
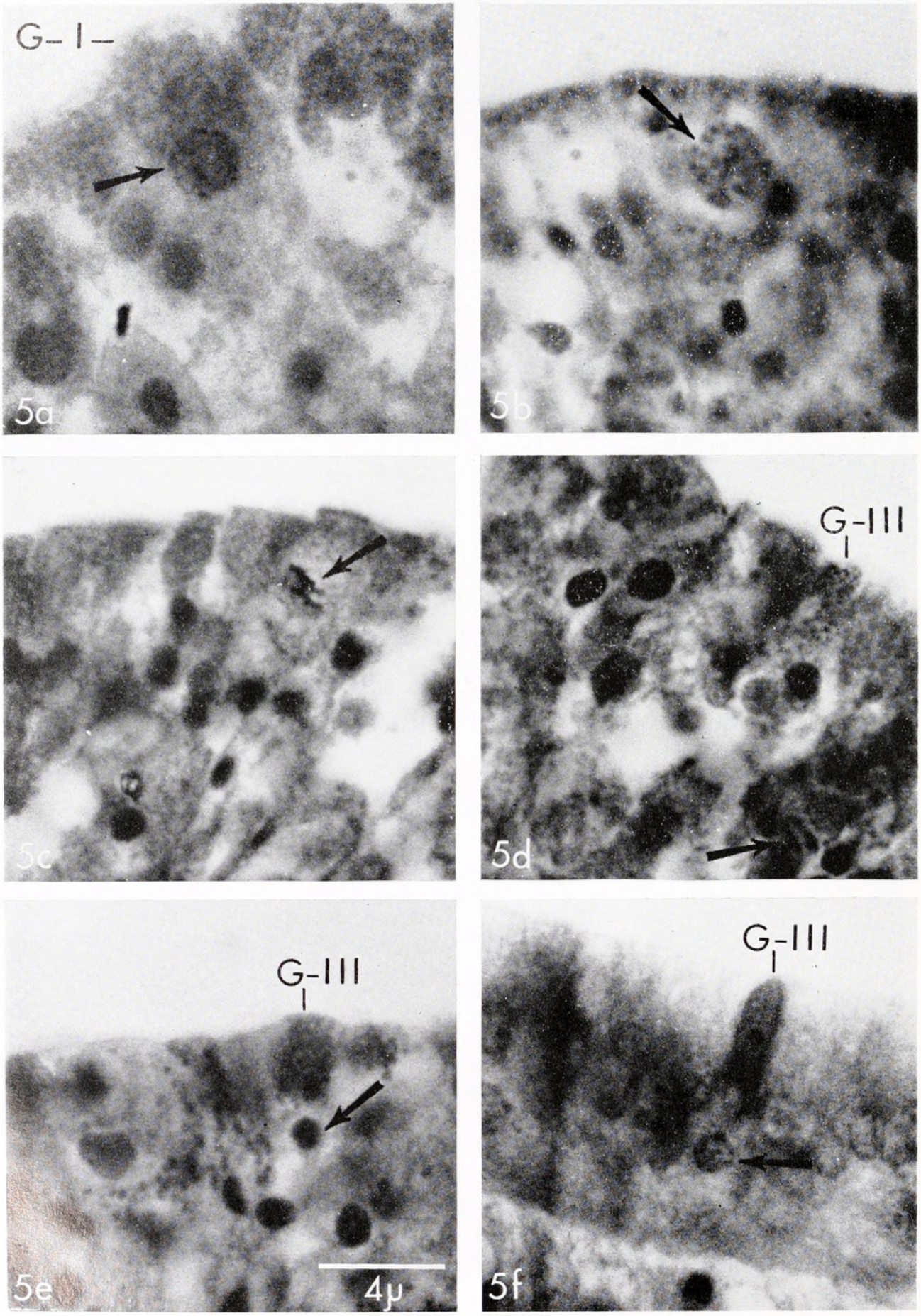


FIGURE 4. By six hours post-fertilization: a. the cytoplasmic lobules have disappeared; and b. microvilli are uniformly distributed over the surface of the cells. G-I represents Type I granule; and G-II, Type II granule.


G–I–
5a
5b
5c
G-III
I
5d
G-III
I
5e
4μ
G-III
I
5f

Eight-hour stage (post-gastrulation)

Gastrulation is completed by the formation of an acellular mesoglea (Cowden, 1965). The mesoglea can be detected in paraffin section at the 8-hour stage, although it is too thin to be analyzed histochemically. At the fine-structural level, it appears relatively undeveloped, measuring *ca.* 0.2 μ in thickness. Few, if any, fibers can be detected in the mesoglea at this stage.

Concomitant with the appearance of the mesoglea, differentiative events can be detected in the ectoderm. By eight hours, numerous mitotic figures are seen in the extreme apical portion of the ectoderm (Fig. 5a–d). Following division, the cytoplasm of these cells contains mucous granules which stain strongly with Best's carmine. The Best's carmine-positive granules (Type III) occur in discrete pockets, with the nucleus of the cell located at the proximal tip of the pocket (Fig. 5e and f). With the electron microscope, the Type III granules, which have an average diameter of *ca.* 0.4 μ, appear as membrane-bound structures with an electron-lucent matrix (Fig. 7e).

The apical portions of the differentiating epithelio-muscle cells (Fig. 6), which constitute the majority of the cells, are little changed from the cells of the pregastrulation embryo. The microvilli, which are *ca.* 1.0 to 1.5 μ in length, have an unusual distribution at this stage, however, in that they occur in pairs, diverging at an acute angle from a common basal region (Fig. 7a and b). These pairs appear to be uniformly distributed at intervals of *ca.* 1 μ over the surface of the cells. Epithelio-muscle cells possess a single cilium (Fig. 7c) with the associated ciliary structures characteristic of cnidarians (for a review, see Chapman, 1974). Each cilium arises from a knob-like cytoplasmic projection which is *ca.* 0.2 μ in diameter. The apical portion of the basal body extends into this evagination. At the basal plate there is a constriction of the plasmalemma surrounding the cilium to a diameter of *ca.* 0.16 μ, above which, in the region of cilium proper, the diameter increases to *ca.* 0.23 μ. The constriction gives the cilium the stalked appearance typical of the cilia of many cnidarians (Jha and Mackie, 1967; Lyons, 1973; Vandermeulen, 1974; Chia and Crawford, 1977).

Large numbers of Type I granules occur peripherally, interspersed among numerous electron-lucent vacuoles (Fig. 6). Mitochondria are also a conspicuous feature of the apical cytoplasm. More internally, Golgi complexes and segments of granular endoplasmic reticulum predominate. Nucleoli are first detected in the peripheral cells at this stage.

In at least some of the cells, the cytoplasm is divided by two closely apposed membranes into parallel compartments (Fig. 7d). At intervals, the membranes separate to form electron-lucent vacuoles. Within the cytoplasmic compartments,

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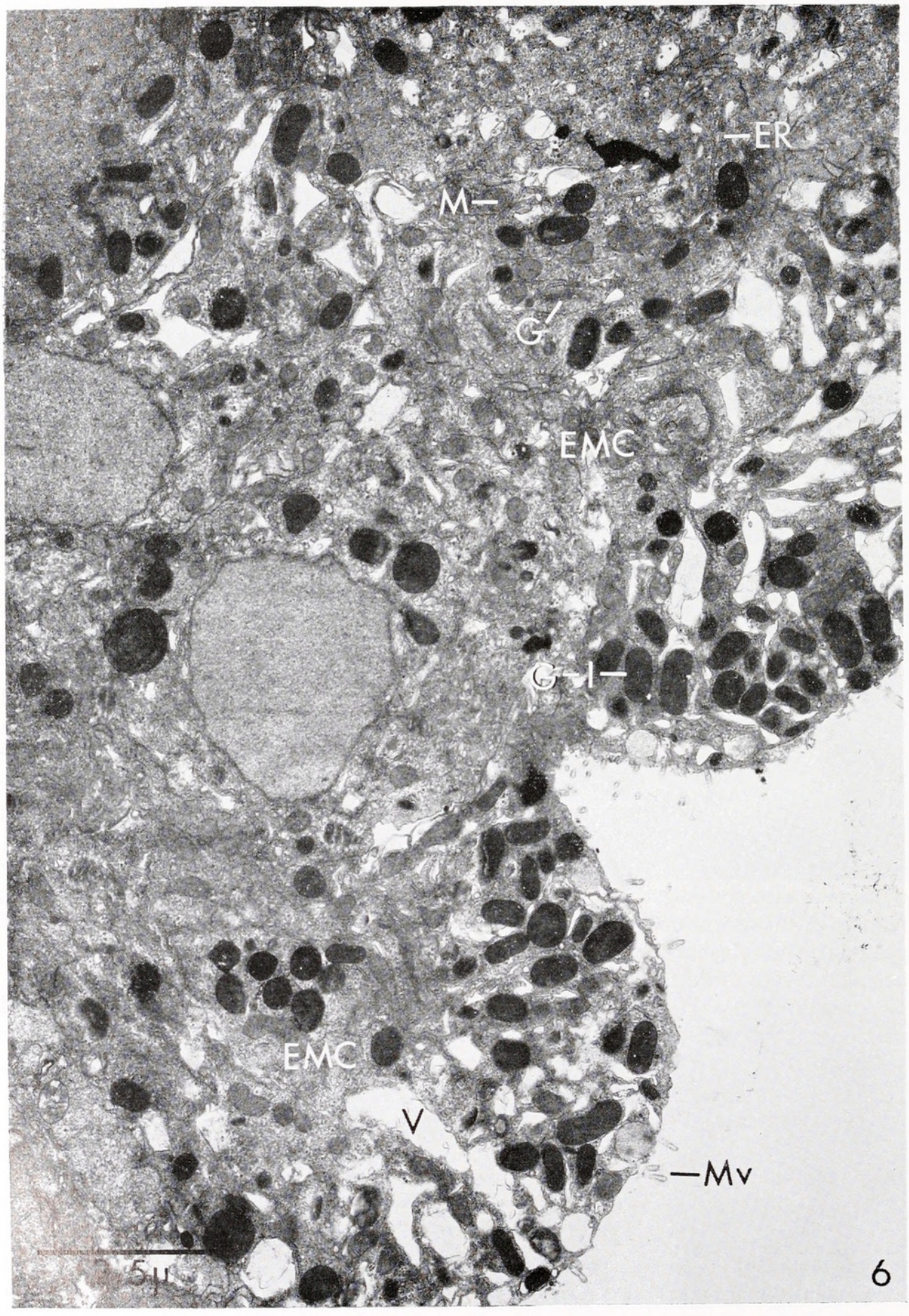
FIGURE 5. In light micrographs of the 8-hour stage, mitotic figures are detected in the apical portion of the ectoderm. The cytoplasm of these cells subsequently contains a third type of granule, Type III, which stains strongly with Best's carmine. a. The spherical cell contains fast green-positive Type I granules. The nucleus of the cell is indicated by the arrow. b. Spherical cells in prophase (arrow), c. metaphase (arrow), and d. telophase (arrow) can be detected. A third type of granule, Type III, appears in the ectoderm following division of the spherical cells. e. During differentiation of the mucous cell, the nucleus (arrow) lies at the proximal tip of the pocket of granules, and f. as development proceeds, the nucleus (arrow) becomes basally located. G-I represents Type I granule; and G-III, Type III granule.



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there frequently occurs a single cisterna of granular endoplasmic reticulum. Golgi complexes are often associated with and oriented parallel to the longitudinal compartments. Type I granules and mitochondria are scattered throughout the cytoplasm in these areas.

Type II granules are seen in the lower basal portions of the epithelio-muscle cells. The basal portions of the epithelio-muscle cells have begun to differentiate into foot processes which abut on the mesoglea, but these processes are incompletely differentiated in that myonemes cannot be detected.

Sixteen-hour stage (planula)

By 16-hours of development, the mesoglea has increased in size and complexity. It is *ca.* 0.3 μ thick and is composed of tiny, mesh-like fibers dispersed in an amorphous ground substance.

Three cell types can be detected at the 16-hour stage (Fig. 8). Especially numerous near the anterior end of the planula are mucous cells containing Type III granules, which first appeared at the 8-hour stage, and which stain positively with both Best's carmine and PAS and negatively with fast green (Fig. 8a and b). The pockets of granules extend more deeply into the ectoderm than was the case in the earlier stages; and the nuclei, located at the proximal ends of the pockets, lie in the basal half of the ectoderm (Fig. 8a). One or two large, clear, refractile vacuoles (Fig. 8a) often occur in association with these nuclei.

By 16 hours, epithelio-muscle cells are well-developed. The cells are columnar, extending from the free surface of the embryo to the mesoglea. The nuclei are elongated and are located medially with respect to the long axis of the cell. The nuclei are often surrounded by small unstained vacuoles. Type I granules, unchanged histochemically (Fig. 8a and b) and morphologically (Fig. 9) from those described for earlier developmental stages, heavily infiltrate the apical portion of the cells. The cytoplasm contains numerous small, electron-lucent vacuoles near the apex; short, sparse strands of granular endoplasmic reticulum; mitochondria; and free ribosomes. In the basal regions of these cells, large Type II granules can be seen (Fig. 10a).

Basal foot-processes of epithelio-muscle cells abut on the surface of the mesoglea at this stage, but myonemes have not completely formed. Atypical mitochondria are scattered throughout these foot processes.

The third cell type, the nongranular cell, first detected histochemically at the 10-hour stage, shows a strong affinity for dinitrofluorobenzene-β-naphthol, aniline blue, and Gray's Double Contrast Stain (Fig. 8c). These cells are evenly distributed around the periphery of the embryo. The nuclei are elongated and are slightly more basally located than are the nuclei of the epithelio-muscle cells.

The nongranular cell as it appears by electron microscopy is shown in Figures 9 and 10. The cells are characterized by having an unusual type of cytoplasmic compartmentalization in the area surrounding the distal half of the nucleus and

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FIGURE 6. The apical portion of the 8-hour postgastrulation embryo is characterized by the presence of electron-lucent vacuoles and Type I granules near the surface. EMC represents epithelio-muscle cell; ER, endoplasmic reticulum; G, Golgi body; G-I, Type I granule; M, mitochondrion; Mv, microvillus; and V, vacuole.

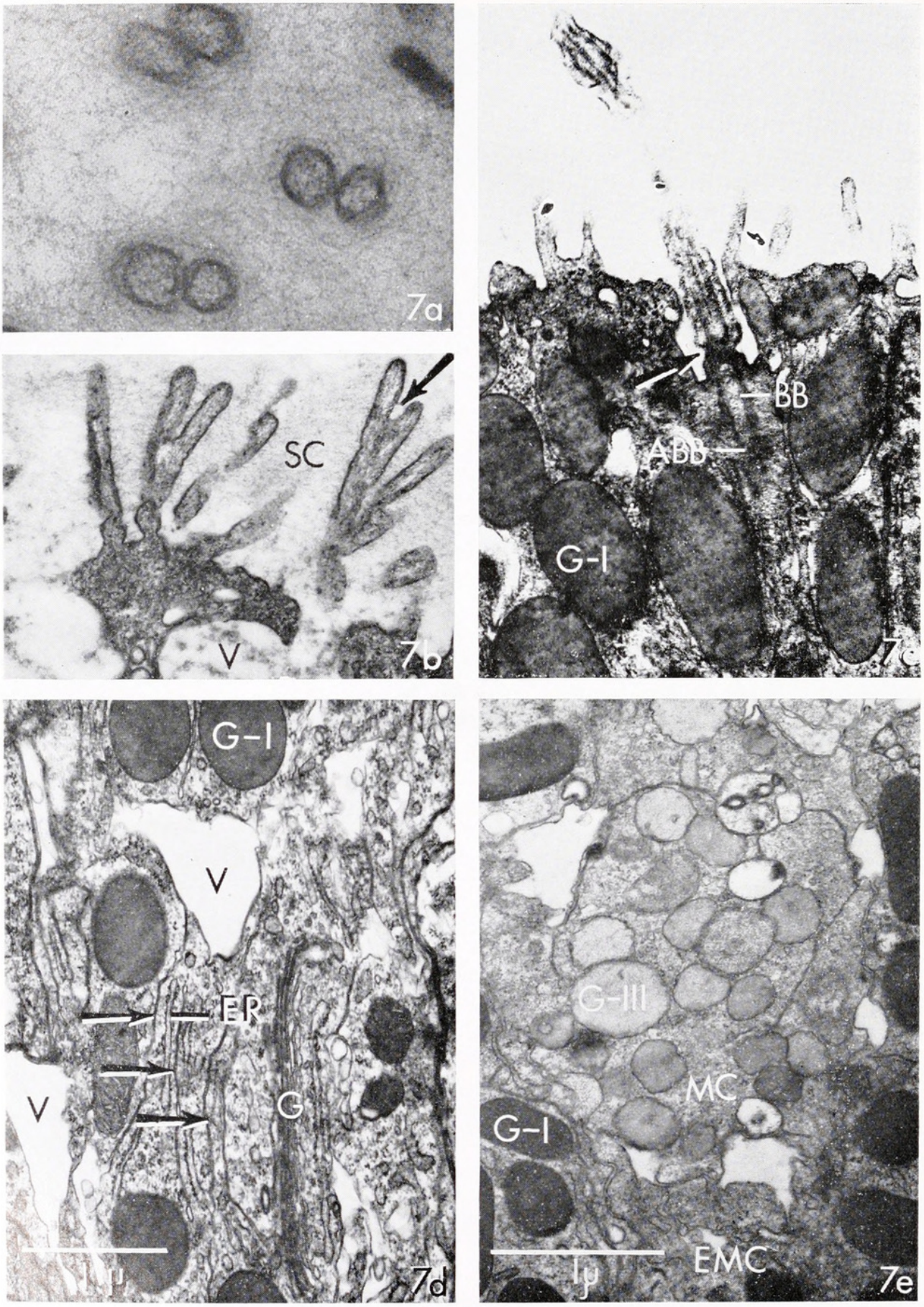

FIGURE 7. Several features characterize the 8-hour embryo. Microvilli occur in pairs as can be seen a. in transverse section, and b. in longitudinal section. The two microvilli

extending *ca.* 16 μ toward the apex of the cell. In this region, cell membrane oriented parallel to the long axis of the cell divides the cytoplasm into long, slender compartments (Fig. 10a). Each compartment typically contains either a single segment of granular endoplasmic reticulum or a series of segments aligned end-to-end. The endoplasmic reticulum is swollen at intervals to give a beaded appearance (Fig. 10b). Electron-dense accumulations *ca.* 0.1 μ in diameter occur within the cisternae of the endoplasmic reticulum (Fig. 10c). The membrane responsible for compartmentalization is modified from the typical unit membrane found elsewhere in the cell. That the modification may involve fusion of adjacent unit membranes is indicated by areas in which the unusual membrane is continuous with two discrete unit membranes (Fig. 10d). The cytoplasmic organization resembles that described in certain cells at the 8-hour stage (compare Figs. 7d and 10b).

These cells are also characterized by the presence along their entire lengths of electron-dense particles measuring only *ca.* 0.08 μ in diameter (Fig. 10e). The particles are not delimited by membrane; rather, they are surrounded by a halo of extremly electron-lucent material. The particles may occur singly, but as many as eight have been seen within a single area of electron-lucent cytoplasm. The non-granular cells also contain Type II granules near the mesoglea.

Fewer intercalations between adjacent ectodermal cells can be observed at 16 hours. Gap junctions *ca.* 0.1 μ in diameter can be detected, however.

Discussion

The cytoplasmic organization of the pregastrulation embryo of a hydrozoan has never been reported. Electron micrographs of very early cleavage stages of *Pennaria* have revealed the presence of microvilli uniformly distributed over the surface of the embryo. The fibrillar material shown in the present study to be associated with the microvilli closely resembles the mucous coat of *Hydra* described at the fine-structural level by Davis (1973). The fibrillar material presumably represents then the morphological equivalent of the PAS-positive surface coat first demonstrated in embryos of *Pennaria* by Cowden (1965).

The time of the first appearance of cilia has not been reported previously for *Pennaria*. Their detection in the present study at the 4-hour stage suggests that ciliogenesis in *Pennaria* is precocious relative to other hydrozoans, since, among most hydrozoans studied to date, cilia never appear prior to gastrulation (Van de Vyver, 1967). It should be noted, however, that in the present study the first cilia, which were present in small numbers, could not be detected with light microscopy at the 4-hour stage. This suggests that what appears to be precocious development of cilia in *Pennaria* may reflect instead the sensitivity of the method used to examine the embryo.

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are seen to share a common basal region (arrow). c. Cilia, present on the epithelio-muscle cells, have a constriction of the plasmalemma at the basal plate (arrow). d. The cytoplasm of certain cells is divided by fused membranes into parallel, longitudinal compartments (arrows) containing single cisternae of granular endoplasmic reticulum. e. Mucous granules occur in pockets in the ectoderm. ABB represents accessory basal body; BB, basal body; ER, endoplasmic reticulum; EMC, epithelio-muscle cell; G, Golgi body; G-I, Type I granule; G-III, Type III granule; MC, mucous cell; SC, surface coat; and V, vacuole.

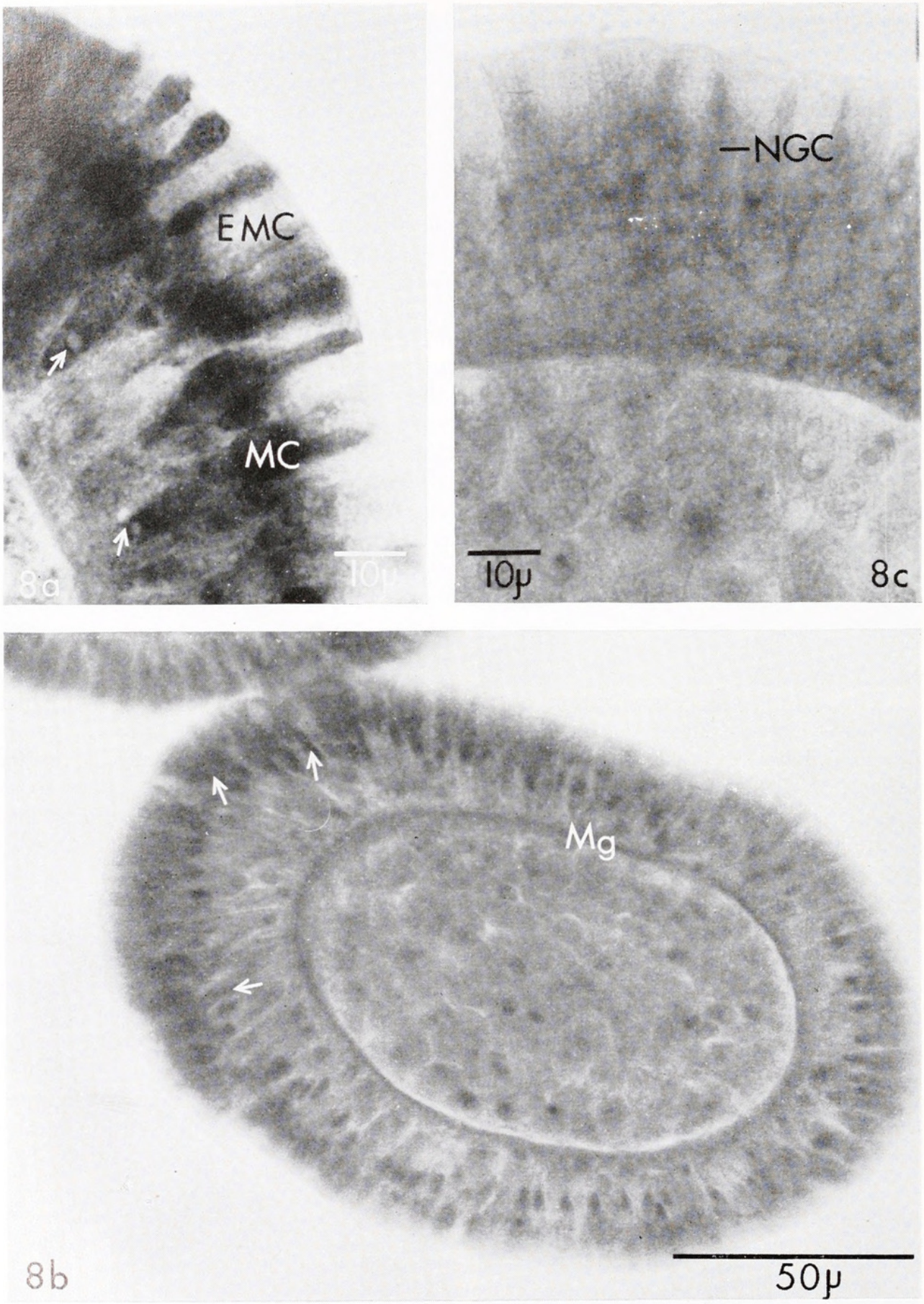

FIGURE 8. Histochemical analyses of the 16-hour planula reveal the presence of three cell types: epithelio-muscle cells, mucous cells, and nongranular cells. a. Stained with Best's

In addition to providing new information concerning surface structures of the pregastrulation blastomeres, electron microscopic examination has revealed an unusual organization of their internal cytoplasm. The cells at the very early stages were seen to contain spherical, membrane-delimited lobules separated from one another by an amorphous ground substance which disappeared by the 4-hour stage. Since the ground substance was not discernible in paraffin sections, its composition is not known.

The origin of the cytoplasmic organization of certain cells following gastrulation into parallel compartments delimited by fused unit membrane and containing a single cisterna of endoplasmic reticulum may be reflected in the organization of the pregastrulation blastomeres. The cytoplasmic lobules seen in the 2-hour stage persist following the disappearance of the ground substance. Their internal structure becomes highly organized with a single cisterna of granular endoplasmic reticulum occurring along a portion of the periphery of the lobule. Fusion of membranes of adjacent lobules, together with elongation of the lobules as the blastomeres elongate, could result in the unusual cytoplasmic compartmentalization described for later developmental stages. It is also possible that fusion of membranes of adjacent lobules might effect elongation. In this regard, it should be mentioned that microtubules, which are often found in association with elongating cells, were not detected in elongating cells of *Pennaria,* even though the usual precautions were taken to insure their preservation.

The membrane-bound, electron-dense Type I granules, which were present in large numbers in the earliest developmental stages examined, and which persisted during larval development, have not been previously reported for *Pennaria.* That these granules were not detected in the study by Cowden (1965) can be attributed to the fact that they were not preserved in Clark's 3 : 1 fixative which was employed in that study. The Type I granule demonstrated in formalin-fixed material in the present study has some of the staining properties that were apparently interpreted as general cytoplasmic staining by Cowden. In that study it was reported that cytoplasmic levels of PAS-positive and azure B-positive materials were relatively high during early cleavage. The same results were obtained in the present study in embryos fixed in Clark's solution, and no Type I granules could be discerned. In formalin-fixed embryos, however, it appears that the PAS-positive material is associated with discrete granules. The azure B-positive material, on the other hand, is associated with the general cytoplasm and not with the Type I granule.

Large, heterogeneous vitelline granules have been demonstrated in the eggs and developing embryos of *Hydractinia, Clava,* and *Cordylophora* (Van de Vyver, 1964, 1967), but from the descriptions and micrographs presented these bear no resemblance to the small, homogeneous Type I granules which occur in *Pennaria.* They

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carmine, the ectoderm shows two cell types. Epithelio-muscle cells are Best's carmine-negative, whereas mucous cells are Best's carmine-positive. The pockets of mucous granules extend more deeply into the ectoderm than was the case in earlier stages. The nuclei, located basally, often have clear, refractile vacuoles associated with them (arrows). b. The mucous cells (arrows) are also PAS-positive, as is the mesoglea. c. A third cell type evenly distributed around the periphery of the embryo, and first detected at the 10-hour stage, stains strongly with dinitrofluorobenzene-β-napthol. EMC represents epithelio-muscle cell; MC, mucous cell; Mg, mesoglea; and NGC, nongranular cell.

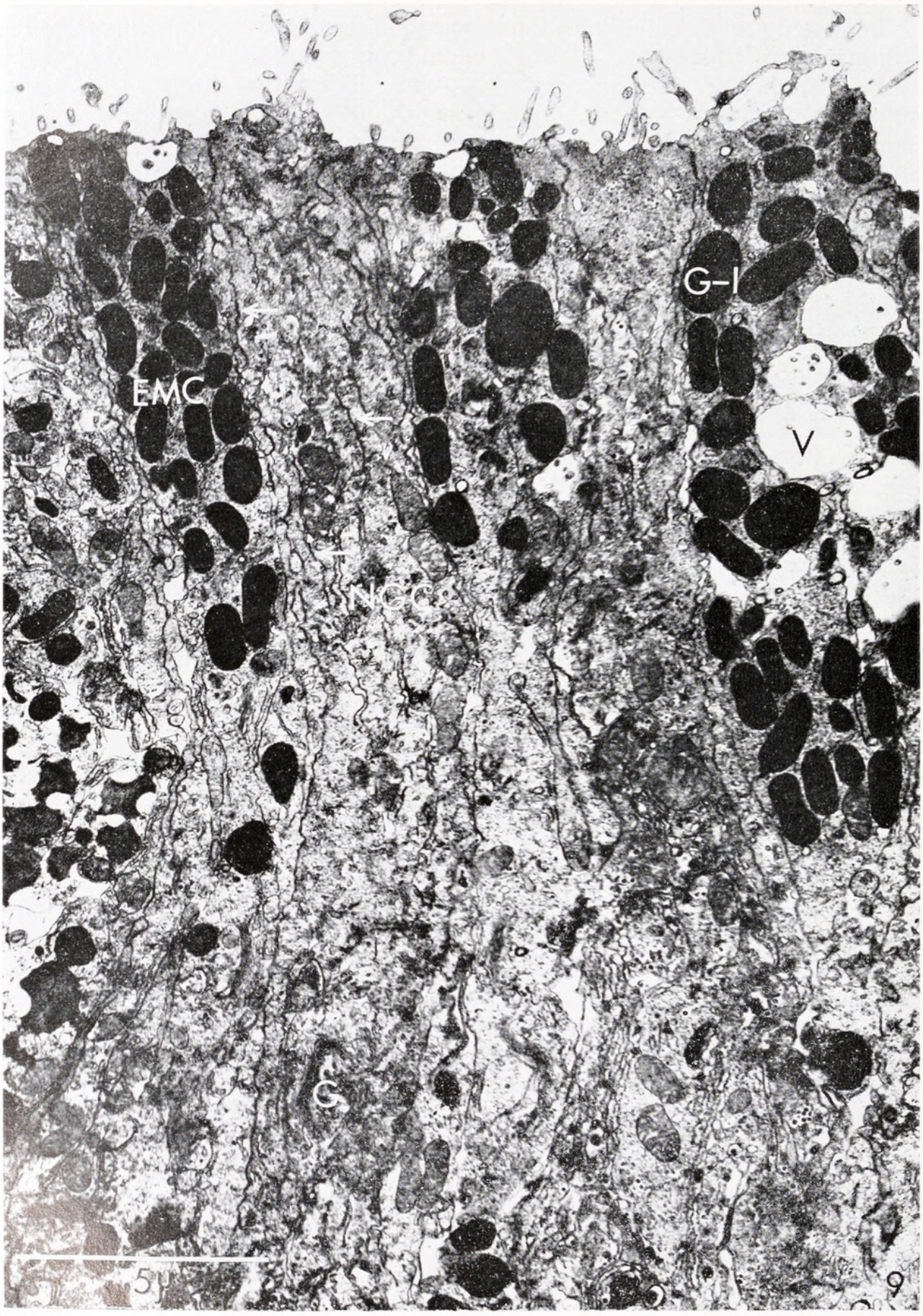

FIGURE 9. Sections of the ectoderm reveal differences between the apex of the nongranular cell and the epithelio-muscle cell. The nongranular cells contain long segments of

do resemble in size and electron density the degenerating mitochondria shown in a micrograph of *Hydractinia* by Van de Vyver (1967). In this regard, according to Van de Vyver (1967), Ephrussi has described for embryos of *Clava* a process of formation of lipid reserves for the embryo through modification of mitochondria. Although there is a similarity in size between mitochondria and Type I granules in *Pennaria,* it is unlikely that the granules represent degenerating or modified mitochondria, since no granules intermediate between mitochondria and Type I granules have been observed at any stage of development in *Pennaria.* Moreover, a function of the Type I granule as a food reserve appears unlikely, since, in contrast to the fate of the yolk granules described by Van de Vyver (1967), there is no significant decrease in their number in the epithelio-muscle cells during the course of larval development.

Haynes (1973) described the epithelio-muscle cell of *Hydra* as a developmentally polarized cell, with the apical region modified for secretory activity, and has suggested that large, electron-dense secretory droplets located apically are involved in the production of the surface coat. In the larvae of *Pennaria,* the epithelio-muscle cells are polarized and a surface coat does exist, even at the earliest stages examined. Type I granules occur apically, are also present at the earliest stages examined, and exhibit some of the staining properties of the surface coat. Despite these facts, it should be pointed out that there was never any morphological evidence that membranes of the Type I granule fuse with the plasmalemma, and therefore no evidence for a secretory role for the Type I granule. Moreover, although a well-developed surface coat is associated with mature polyps of *Pennaria,* Type I granules have never been observed in the polyp (unpublished observations). The presence of these granules throughout embryonic and larval development and their absence in the adult suggest that they play a role in metamorphosis. The possibility of such a function is currently being investigated.

Ectodermal mucous cells have been described for the planulae of many hydrozoans, including *Pennaria* (Cowden, 1965; Summers and Haynes, 1969). The mucous cells of *Pennaria* appear to be identical histochemically and morphologically, as well as in their ontogeny, to the mucous cells which function during the attachment of the planulae in all of the hydrozoans examined by Van de Vyver (1967). The origin of these cells from the products of mitotic activity at the apex of the ectoderm has not been reported previously.

It has been assumed that prior to migration of nematoblasts and interstitial cells from the endoderm only two cell types, the epithelio-muscle cell and the mucous cell, occupy the larval ectoderm of hydrozoans, although Van de Vyver (1967) has detected a second mucous cell type in the planula of *Coryne.* Examination of thick and thin plastic sections of the 16-hour planula of *Pennaria* has revealed the presence of an additional cell type, the nongranular cell. The cell is extremely difficult to detect in paraffin sections, even when it is known to exist from ultrastructural studies, and is clearly identifiable only in sections stained with dinitrofluorobenzene-β-naphthol. That the cell is not simply a degranulated

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granular endoplasmic reticulum (arrows). Epithelio-muscle cells, containing Type I granules, possess numerous vacuoles near the surface. EMC represents epthelio-muscle cell; G Golgi body; G-I, Type I granule; NGC, nongranular cell; and V, vacuole.

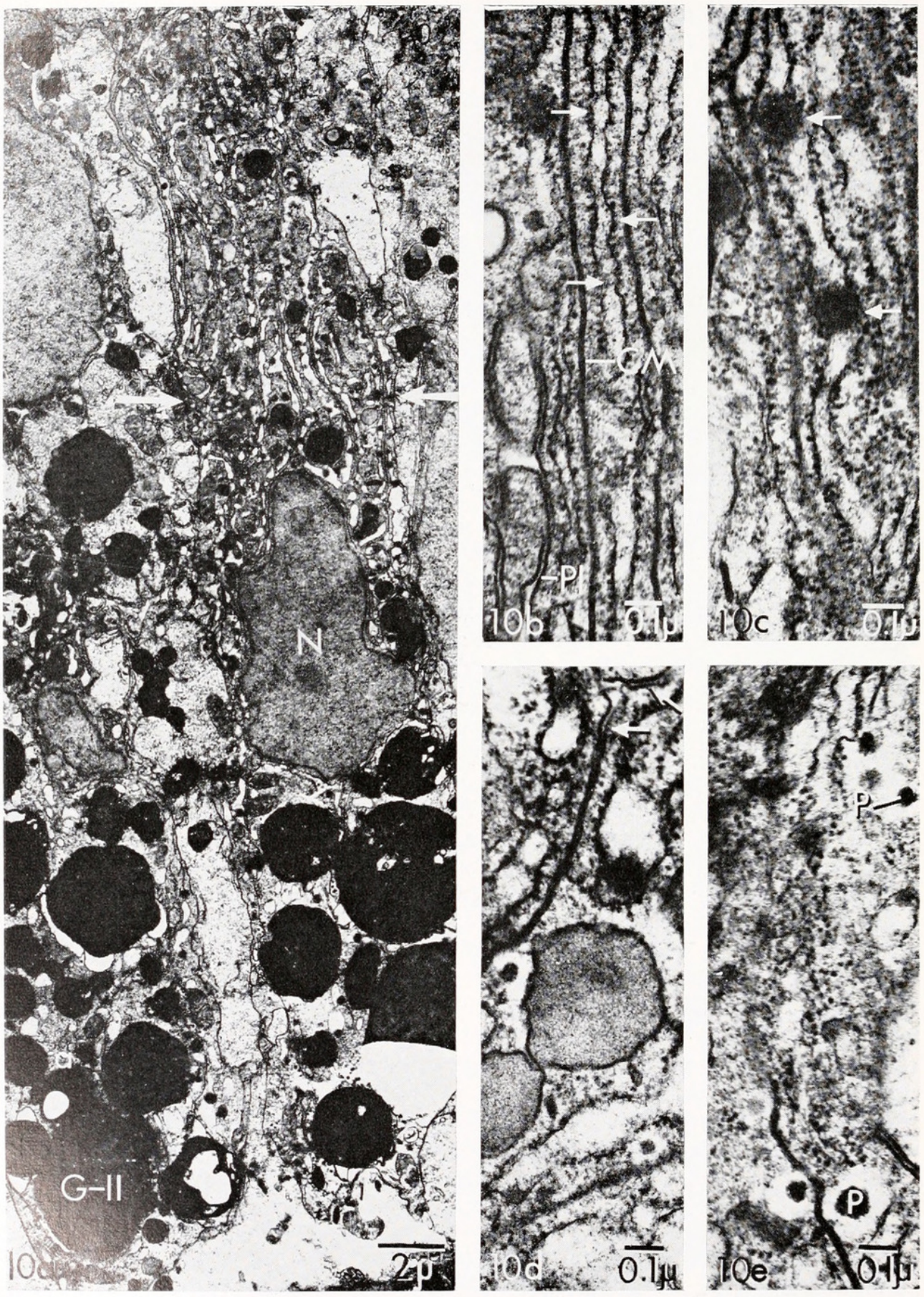

FIGURE 10. Sections of the medial and basal portions of the ectoderm demonstrate the features of the nongranular cell. a. An unusual cytoplasmic compartmentalization (arrows)

epithelio-muscle cell is indicated not only by its unique staining properties, but also by its distinctive fine-structural morphology. The nucleus is more electron-dense than that of the epithelio-muscle cell and often contains two nucleoli. The granular endoplasmic reticulum, located in longitudinal compartments distal to the nucleus, is swollen at intervals to surround spherical, electron-dense accumulations which also occur in membrane-bound vesicles in the adjacent cytoplasm. In addition, the cytoplasm contains numerous small, electron-dense particles surrounded by an electron-lucent zone. The vesicles and particles are never observed in the epithelio-muscle cells of *Pennaria*. Comparisons of the cytology of the nongranular cell of the planula of *Pennaria* with cell types described in *Hydra* (Davis, 1973; Westfall, Yamataka and Enos, 1971; Westfall, 1973) suggest that the cell most closely resembles a sensory cell, a possibility that is currently being investigated.

Summary

1. The pregastrulation blastomeres contain electron-dense granules which become localized after gastrulation in the apices of the developing epithelio-muscle cells and persist throughout larval development. The cytoplasm of the blastomeres is organized into anucleate, membrane-delimited lobules. The lobules, which persist until six hours of development, come to contain a single, peripherally located cisterna of granular endoplasmic reticulum. Microvilli are present at the earliest stages examined and persist throughout development. Cilia are first detected at four hours.

2. Gastrulation, marked by the appearance of the mesoglea, occurs between six and eight hours of development. Basal foot processes of epithelio-muscle cells are detected by eight hours, but myonemes cannot be detected until later in development.

3. Immediately following gastrulation, mucous cells begin their differentiation from dividing cells located near the apex of the ectoderm. During their differentiation, the cells elongate toward the mesoglea.

4. By 16 hours post-fertilization, a third cell type can be detected in the ectoderm. The cell, which contains no granules, has an unusual cytoplasmic organization in which fused membranes divide the cytoplasm into parallel compartments containing a single cisterna of granular endoplasmic reticulum.

5. The findings of the present study are correlated with those of previous studies of development in *Pennaria* and other hydroids. The possible functional roles of the Type I granules, the cytoplasmic lobules, and the nongranular cell are discussed.

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is seen in the area surrounding the distal half of the nucleus. b. The granular endoplasmic reticulum within the compartments is swollen at intervals to give a beaded appearance (arrows). Note the thickness of the compartmentalizing membrane relative to that of the endoplasmic reticulum and the plasmalemma. c. Electron-dense accumulations (arrows) are present within the cisternae of the endoplasmic reticulum. d. An atypical membrane is responsible for compartmentalization. That the modification of the membrane may involve fusion of two adjacent unit membranes is indicated by the occurrence of regions in which the specialized membrane is continuous with two unit membranes (arrows). e. Electron-dense particles, not bounded by membrane, occur along the entire length of the nongranular cell. CM represents compartmentalizing membrane; G-II, Type II granule; N, nucleus; P, particle; and Pl, plasmalemma.

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