Ultrastructure of the Lamellated Cytoplasmic Inclusions in Schwann Cells of the Marine Nematode, *Deontostoma californicum*

I. A. SIDDIQUI[1] AND D. R. VIGLIERCHIO[2]

Abstract: Electron microscopy of the photoreceptors in the marine nematode, *Deontostoma californicum*, revealed numerous lamellated inclusions in the Schwann cells ensheathing the lateral cephalic nerves. Immediately after the axons from the modified bipolar neurons of the photoreceptors enter the lateral nerves, these spherical-to-oval lamellated bodies are observed in the surrounding Schwann cell cytoplasm. These previously undescribed Schwann cell inclusions, approximately 500 nm long and 320 nm in diameter, are lamellated and characterized by the presence of an electron-dense stalk-like process, 80–280 nm long. The lamellated inclusions are bound by a single limiting membrane, 6–7 nm thick, which shows occasional interruptions. The internal structure of the inclusions is characterized by the presence of electron-dense lamellae or bands, 11–16 nm thick, which assume various complex patterns ranging from arrays of parallel linear densities to a reticulate appearance. In addition, the nematode Schwann cell cytoplasm contains the usual organelles, gliosome- and lysosome-like inclusions. Their relationship with lipofuscin pigments is briefly discussed. *Key Word:* Neuroglia.

Microbodies, cytosomes, or other electron-dense bodies have been observed by a number of investigators in the cytoplasm of various cell types (11). Palay and Palade (13) noted dense bodies in the nerve cells of rats. Subsequently, inclusion bodies with varying internal structure and shape were noted both in the axoplasm and glial (Schwann cell) cytoplasm of several animals (9, 15, 19). Schlote and Hanneforth (19) recorded several types of inclusion bodies in the neurons and glial cells of the gastropod, *Helix pomatia*, many of which possessed internal lamellae, spaced approximately 6 nm apart.

The fine structure of the cockroach gliosomes, measuring 0.5 to 4.0 μ in diameter and possessing whorls of membranes inside, was first described by Pipa *et al.* (15). Gliosomes are reported to be distinct from mitochondria in their cytochemical stainability (14). In contrast, Srebro (22) concluded that gliosomes were abnormal mitochondria. In a recent study on the development of gliosomes in cat neuroglia, Hashimoto (10) observed that during the first 4 to 10 days after birth many astrocytic mitochondria enlarged, their cristae dispersed peripherally in a finely homogeneous matrix, and finally transformed into gliosomes. The possibility of gliosomes being related to lipofuscin or "old-age" pigments has also been suggested (15).

The occurrence of inclusion bodies with membranous arrays or lamellated whorls has been observed in a wide variety of cell types. Lamellated inclusions with "fingerprint-like" patterns were reported from the sixth abdominal ganglion of crayfish (23). Degenerative changes in the peripheral nervous system in several animals are known to be accompanied by varying degrees of myelin fragmentation, resulting in the formation of lamellated inclusions (1), or myelin droplets (8). In particular, the occurrence of lipofuscin or "old-age" pigments accumulat-

Received for publication 1 December 1970.

[1] Nematologist. Present address: Bureau of Plant Pathology, California Department of Agriculture, Sacramento, California 95814.

[2] Department of Nematology, University of California, Davis, 95616. Thanks are due to Drs. A. R. Maggenti, D. J. Raski and R. W. Timm for their valuable discussions and criticisms of this manuscript. Grateful appreciation is also expressed to Miss Janie Woods for technical assistance.

ing in complex bands, hexagonal arrays, or membranous whorls in animal and human neuroglia and axons has been noted in several studies (2, 4, 7, 18). The terms "neuroglia" and "Schwann cells" are used interchangeably in the literature, just as astrocytes and oligodendrocytes of the central nervous system are homologous to the Schwann cells of the peripheral nervous system. With this consideration in view, the name "satellite cell" for both cell types has been suggested by Causey (3).

To date, studies on nematode neuroglia have dealt primarily with their gross morphology. Recently, Wright (24) described the neuron-glial relationship in *Trichurus myocastoris*. However, the occurrence of lamellated bodies in nematode neuroglia or Schwann cells has not yet been reported. During our studies on the fine structure of photoreceptors in the marine nematode, *Deontostoma californicum*, numerous lamellated inclusions were observed in the Schwann cell cytoplasm ensheathing the lateral nerve axons (20). Examination revealed that these inclusions differ from the normal cytoplasmic inclusions of the Schwann cells. A brief report concerning the occurrence of these inclusions in *D. californicum* was reported elsewhere (21). This paper describes the fine structure of the lamellated bodies and discusses their possible relationship to other Schwann cell inclusions.

MATERIALS AND METHODS

Adult *D. californicum* were obtained from holdfasts of the brown alga, *Laminaria digitata*, off Dillon Beach, California. The nematodes were fixed in 5% glutaraldehyde buffered with 0.1 M phosphate at *p*H 7.1 and postfixed in 1% osmium tetroxide. A detailed description of the tissue preparation for electron microscopy has appeared elsewhere (20). An Epon 812-Araldite 6005 mixture was used for tissue embedding. Sections were cut on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate (16), and examined with the RCA EMU-3E or G electron microscope.

OBSERVATIONS

In *D. californicum*, the neural elements in the peripheral nervous system consist of bundles of unmyelinated axons ensheathed by one or more Schwann cells which are in turn surrounded by a layer of fibrous connective tissue or neural lamella (Fig. 1, 3). The lateral cephalic nerve at the level of the ocelli measures 0.6 μ in diameter. Axons and nerve fibers are ensheathed individually or in groups of two to five by the Schwann cells. The axoplasm consists of neurofilaments, microtubules, bundles of dense fibrils, a few mitochondria and several irregularly distributed vesicles (Fig. 5). Each microtubule measures 16 nm in diameter. The axon-Schwann membranes, each 7 nm thick, are separated by a less-dense zone 18 nm wide. Occasionally, the axons are completely ensheathed by a fibrous layer, 30–160 nm thick, which resembles the neural lamella (Fig. 3, 5).

The cytoplasmic inclusions, here called "lamellated bodies" for descriptive purposes, occur in the Schwann cells ensheathing the axons and nerve fibers of the lateral cephalic nerve that innervates the photoreceptors and lateral cephalic papillae in *D. californicum* (Fig. 1). The Schwann cells surrounding the other four cephalic nerves and the pair of amphidial nerves lack these inclusions. The number of the lamellated bodies is greatest in the Schwann cells in the ocellar region; the axoplasm does not contain these inclusions. In both longitudinal and transverse sections, the lamellate bodies display spherical to oval profiles measuring from 200 to 640 nm along the major axis and from 200

to 420 nm along the minor axis. They did not display any specific orientation in the cytoplasm. Approximately 16–33% of the lamellated bodies sectioned sagittally showed an electron-dense, stalk-like process, 80–280 nm long (Fig. 2–4).

The lamellated inclusions are bounded by a single limiting membrane, 6–7 nm thick. Occasionally, the limiting membrane presents a scalloped appearance and is interrupted, thus enabling its internal interlamellar matrix to be continuous with the Schwann cell cytoplasm (Fig. 2, 5). In a lamellated body sectioned obliquely in the region of the stalk-like process, there is an uneven accumulation of electron-dense material along the limiting membrane (Fig. 2, 8).

The internal structure of the sectioned lamellated body is variable, ranging from parallel bands to a reticulate or honeycomb pattern (Fig. 2–4). The lamellae or bands, 11–16 nm thick, run parallel across the width and are separated by low electron density interstices, 20 nm wide. Occasionally, the lamellae appear to run along two or more planes, thus resulting in profiles that display both parallel bands and a reticulate pattern (Fig. 2, 3). The term "lamella" is used here in a less restrictive sense to designate an electron-dense band which does not necessarily represent a unit membrane. Here the lamellae are often suggestive of bands resulting from the accumulation of a finely granular material (Fig. 5, 8). Many inclusion bodies, however, show profiles of a diffused reticulate pattern, either because their internal structure is not caught fully in the plane of sectioning, or because it has not yet fully developed (Fig. 3, 8). In some inclusion bodies the parallel lamellae extend partially into the body while the rest is filled with a finely granular matrix (Fig. 5). Such bodies are usually observed in close apposition to the lysosome-like inclusions and may be interpreted as a transitional form. The lamellated bodies of varying sizes and a mitochondrion in the same vicinity shown in Fig. 4 illustrate the differences in gross morphology and substructure. The lamellated bodies differ from mitochondria in possessing: (i) a single limiting membrane; (ii) an electron-dense, stalk-like process at one end; (iii) several configurations of lamellae traversing the matrix either in arrays of parallel bands or a reticulate pattern.

The Schwann cell cytoplasm, in addition to the lamellated bodies described above, contains bundles of gliofilaments, microtubules, a few mitochondria, smooth endoplasmic reticulum, the Golgi apparatus, vesicles of varying sizes, and two additional types of inclusion bodies with a granular matrix (Fig. 5–7). These inclusion bodies differ from the lamellated bodies discussed earlier, lacking internal lamellae. Both of these inclusion bodies are spherical to oval and possess a single limiting membrane, 6–7 nm thick. They can be distinguished from each other on the basis of size and degree of matrix granulation.

The first of these bodies measures 0.2–0.4 μ in diameter and is filled with an amorphous matrix (Fig. 5). Morphologically, it resembles the lysosomes described by de Duve (5). It is usually observed in close association with the lamellated bodies and the matrix is similar to the amorphous areas of those lamellated bodies with a partially developed lamellation.

The second inclusion body is larger in size, measuring 0.7–1.0 μ in diameter, and possesses a highly granular matrix. Occasionally an accumulation of dense, coarsely granular material is observed along the limiting membrane (Fig. 6, 7). The membrane is often interrupted or has arm-like extensions which allow its granular substance to merge with the cytoplasm (Fig. 6, 7). These bodies

resemble the inclusions described as gliosomes from cockroach neuroglia (15).

Many vesicles, 18 nm in diameter and bounded by a single membrane, are present in the vicinity of gliosome-like inclusions. The Schwann cell cytoplasm is rich in gliofilaments, occurring in bundles instead of readily distinguishable filaments, and running parallel to the long axis of the cell (Fig. 2, 5).

DISCUSSION

The fine structure of the lamellated bodies of the Schwann cell cytoplasm described here indicates they differ from mitochondria and gliosomes. The lamellated bodies are characterized by a single limiting membrane, lack of crista-like invaginations in any plane of sectioning, presence of an electron-dense stalk-like process, and the arrangement of electron-dense material in parallel bands or a reticulate pattern. None of these characteristics are known in mitochondria of either vertebrate or invertebrate animals (12). Furthermore, the lamellated bodies here are too numerous to be considered an abnormal or obliquely sectioned mitochondrion. Thus, the possibility of the lamellated bodies being precursors of, or derived from mitochondria, appears remote.

There are conflicting reports in the literature on the fine structure and origin of gliosomes. Pipa *et al.* (15) observed gliosomes in cockroach neuroglia with a single limiting membrane, which at places appeared to be interrupted. On the contrary, several other studies report that gliosomes are bounded by paired limiting membranes with invaginations of the inner membrane resembling mitochondrial cristae (9, 10, 22). They concluded that gliosomes are either "abnormal" mitochondria or derived from mitochondria. Although the gliosomes in cockroach neuroglia are bounded by a single membrane and occasionally show whorls of concentric lamellae (15), the presence of stalk-like processes and characteristic configurations of lamellae in the nematode inclusion bodies distinguish them from those gliosomes.

Several observations have been made during this study which may suggest how these inclusion bodies are formed. Perhaps the most plausible mechanism involves the lysosome-like bodies, observed in close association with the lamellated inclusions. It is suggested that the amorphous matrix of lysosome-like bodies undergoes a series of alterations such that the lipid residues or the particles rich in such residues form arrays of parallel or reticulate lamellae. As the nematode ages, the matrix of these bodies transforms into structures which upon fixation show lamellate or reticulate substructures and localized areas of high electron densities. The presence of profiles with only diffused reticulations suggests that such a method of formation of the lamellated bodies is possible. Similar observations were made by de Duve and Wattiaux (6), who suggested that the appearance of membranous whorls was indicative of the late stages of lysosomes; they further hypothesized that lipofuscin pigments are the result of a progressive alteration in the lipid residues in lysosomes that eventually assume the form of membranous arrays or whorls of lamellae.

The occurrence of lipofuscin pigments has been reported in axons, capillary endothelium, neuroglia and Schwann cells of both invertebrate and vertebrate animals, especially in aged individuals (2, 4, 7, 17, 18). Two contradictory hypotheses have been advanced on the origin and functional significance of lipofuscin granules (18): according to the first, the pigment is considered an insoluble terminal product of nerve or glial cell metabolism, which accumulates with age; according to the second, it is considered a

storage substance involved in normal cellular physiology. The lysosomal origin of lipofuscin pigments has been implied by results of several studies (6, 7, 17, 18). Cytochemical and structural similarities between lipofuscin pigments and lysosomes were noted by Samorajski *et al.* (17, 18). They concluded that the formation of electron-dense lamellae in lipofuscin pigments represented a specific stage in the transformation of phospholipids. In a similar study, Few and Getty (7) observed an increase in lipofuscin granules with a corresponding decrease in lysosome numbers. The possibilities of the lipofuscin pigment originating from the Golgi apparatus (2) and mitochondria (4) have also been raised.

The sequence of transformations suggested here in leading to the formation of lamellated bodies in *D. californicum* Schwann cells is consistent with the observations of Samorajski *et al.* (18) and de Duve and Wattiaux (6). Functional implications of the association of these inclusion bodies only with Schwann cells ensheathing the lateral nerve axons and their maximum occurrence immediately outside the sensory cell of the nematode ocelli (20) are not yet clear. When information on the ultrastructure of the innervations of photoreceptors in other nematode species is available, the significance of this association can be resolved. It would be of interest to ascertain whether these lamellated bodies are also present in the larval stages of *D. californicum*. In our opinion, cytochemical analysis is another requisite to the understanding of the functional significance of the lamellated bodies.

Literature Cited

1. Blümcke, S. and H. R. Niedorf. 1966. Electron microscopic studies of Schwann cells during the Wallerian degeneration with special reference to the cytoplasmic filaments. Acta Neuropathol. 6:46–60.
2. Bondareff, W. 1957. Genesis of intracellular pigment in the spinal ganglia of senile rats. An electron microscope study. J. Gerontol. 12:364–369.
3. Causey, G. 1960. The Cell of Schwann, E. & S. Livingstone Ltd., Edinburgh. 120 pp.
4. Duncan, D., D. Nall and R. Morales. 1960. Observations on the fine structure of old age pigment. J. Gerontol. 15:366–372.
5. Duve, C. de. 1959. Lysosomes: a new group of cytoplasmic particles. p. 128–159. *In*: T. Hayashi (ed.) Subcellular Particles, The Ronald Press Co.
6. Duve, C. de and R. Wattiaux. 1966. Functions of lysosomes. Ann. Rev. Physiol. 28:435–492.
7. Few, A. and R. Getty. 1967. Occurrence of lipofuscin as related to aging in the canine and porcine nervous system. J. Gerontol. 22:357–368.
8. Finean, J. B. and A. L. Woolf. 1962. An electron microscope study of degenerative changes in human cutaneous nerve. J. Neuropathol. Exp. Neurol. 21:105–115.
9. Gray, E. G. 1959. Electron microscopy of neuroglial fibrils in the cerebral cortex. J. Biophys. Biochem. Cytol. 6:121–122.
10. Hashimoto, P. H. 1969. Electron microscopic study on gliosome formation in postnatal development of spinal cord in the cat. J. Comp. Neurol. 137:251–266.
11. Hruban, Z. and M. Rechcigl, Jr. 1969. Microbodies and related particles: morphology, biochemistry, and physiology. Int. Rev. Cytol., Suppl. 1. 296 pp.
12. Lehninger, A. L. 1964. The mitochondrion; molecular basis of structure and function. W. A. Benjamin, New York. 263 pp.
13. Palay, S. L. and G. E. Palade. 1955. The fine structure of neurons. J. Biophys. Biochem. Cytol. 1:69–88.
14. Pipa, R. L. 1961. Studies on the hexapod nervous system. III. Histology and histochemistry of cockroach neuroglia. J. Comp. Neur. 116:15–26.
15. Pipa, R. L., R. S. Nishioka and H. A. Bern. 1962. Studies on the hexapod nervous system. V. The ultrastructure of cockroach gliosomes. J. Ultrastruct. Res. 6:164–170.
16. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208–212.
17. Samorajski, T., J. R. Keefe and J. M. Ordy. 1964. Intracellular localization of lipofuscin age pigments in the nervous system. J. Gerontol. 19:262–276.

18. SAMORAJSKI, T., J. M. ORDY AND J. R. KEEFE. 1965. The fine structure of lipofuscin age pigment in the nervous system of aged mice. J. Cell Biol. 26:779–795.
19. SCHLOTE, F. W. AND W. HANNEFORTH. 1963. Endoplasmatische Membransysteme und Granatypen in Neuronen und Gliazellen von Gastropodennerven. Z. Zellforsch. 60: 872–892.
20. SIDDIQUI, I. A. AND D. R. VIGLIERCHIO. 1970. Ultrastructure of photoreceptors in the marine nematode *Deontostoma californicum*. J. Ultrastruct. Res. 32:558–571.
21. SIDDIQUI, I. A. AND D. R. VIGLIERCHIO. 1970. The fine structure of the lamellated, cytoplasmic inclusions in nematode Schwann cells. Xth Int. Nem. Symp. European Soc. Nematol., Pescara, Italy, 7–9. (Abstr.).
22. SREBRO, Z. 1965. The ultrastructure of gliosome in the brains of amphibia. J. Cell Biol. 26:313–322.
23. UCHIZONO, K. 1962. The structure of possible photoreceptive elements in the sixth abdominal ganglion of the crayfish. J. Cell Biol. 15:151–154.
24. WRIGHT, K. A. 1970. Some neuron-hypodermis relationships in the parasitic nematode, *Trichuris myocastoris* Enigk. J. Nematol. 2:152–160.

FIGURE LEGENDS

FIG 1. Lateral cephalic nerve (LN), a longitudinal view, slightly posterior to the ocellar region showing the distribution of the lamellated bodies (Lb) in the Schwann cell cytoplasm (Sch). The nerve runs parallel to the esophagus (E), which possesses many elongated mitochondria (Mi) and myofilaments (My). On the outside, the noncontractile region (NR) of the body wall musculature with numerous mitochondria projects into the body cavity, the pseudocoelom (P). × 7,800.

FIG. 2. A portion of a Schwann cell from the lateral cephalic nerve. The spherical to oval profiles of the lamellated bodies (Lb), some with an electron-dense stalk-like process (S), are present. The lamellated inclusions show three main patterns of internal lamellation; (1) parallel arrays of electron-dense bands, 11–16 nm wide, (2) reticulate or honeycomb pattern of the same lamellar thickness, and (3) a combination of the two patterns. Note the occasional discontinuities in the limiting membrane of the lamellated body (arrows). The cytoplasm contains numerous bundles of gliofilaments (GF) and microtubules (Mt). × 25,000.

FIG. 3. Transverse section of the lateral cephalic nerve showing a Schwann cell (Sch) and several axons (Ax). The Schwann cell cytoplasm contains numerous lamellated bodies (Lb), a few mitochondria (Mi), bundles of gliofilaments (GF), and the Golgi apparatus (Go). Note the variation in the internal structure of the lamellated bodies and their transitory forms. The Schwann cell sheaths enclose the axons (Ax) and a layer of fibrous connective tissue or neural lamella (NL) envelopes the Schwann cell. Basement membrane (BM) of the esophagus (E) is visible at lower right. × 22,000.

FIG. 4. Lateral cephalic nerve showing several peripherally located axons (Ax) ensheathed by the Schwann cell processes, which are in turn enveloped by neural lamellae (NL). Several lamellated bodies (Lb) with varying internal lamellations are present in the Schwann cell cytoplasm (Sch). Arrows point to a lamellated body with electron-dense lamellae arranged concentrically (1) and a partially lamellated internal structure (2). Note the differences in gross morphology and substructure between a mitochondrion (Mi) and a lamellated body (Lb). The mitochondrial features of a paired limiting membrane and internal cristae are visible; these distinguish them from the lamellated bodies which possess a single limiting membrane and no crista-like invaginations. Bundles of gliofilaments (GF) and lipid inclusions (L) and microtubules (Mt) are present. At lower right is a portion of the esophagus (E). × 25,000.

FIG. 5. A portion of a Schwann cell from the lateral cephalic nerve ensheathing an axon (Ax). Lamellated body (Lb_1) with arrays of parallel bands traversing its width and a transitional form (Lb_2) with bands extending only partially are present. Note the discontinuity in the limiting membrane of the lamellated body (arrow). In close apposition to the lamellated bodies are the inclusions which appear to be lysosomes (Ly). The Schwann cell cytoplasm contains bundles of gliofilaments (GF) and microtubules (Mt). Mitochondria (Mi), microtubules (Mt), bundles of dense filaments (DF) and vesicles (V) are also present in the axoplasm. × 64,000.

FIG. 6. A portion of a Schwann cell from the lateral cephalic nerve surrounded by neural lamella (NL). Profiles of lamellated bodies (Lb) with a reticulate but diffused pattern of an electron-dense material are present. The inclusions with a uniformly granular matrix and larger in size (0.7 to 1.0 μ in diameter) than the lamellated bodies appear to be the gliosomes (G). Note the single limiting membrane of a gliosome sending out a pseudopodium-like extension (arrows) to contain an unidentified inclusion (X). Many small vesicles (V), 18 nm in diameter, and bounded by a single membrane, and gliofilaments (GF) are present in the vicinity of gliosome-like inclusions. × 64,000.

FIG. 7. A gliosome-like inclusion (G) bounded by a single, triple-layered membrane with occasional discontinuities is present in the Schwann cell cytoplasm (Sch). Arrow points to the extension of the gliosome limiting membrane. Inclusion at upper left appears to be a degenerate mitochondrion (X). × 64,000.

FIG. 8. A portion of a Schwann cell from the lateral cephalic nerve showing the differences between the limiting membranes and substructure of a mitochondrion (Mi) and the lamellated bodies (Lb). Profiles of lamellae in the latter appear to be formed by the accumulation of a finely granular material and do not represent a unit membrane organization as in mitochondria. Electron-dense zones (X) are the stalk-like processes of the lamellated bodies sectioned in different planes. The structure at upper left is identified as a large vesicle (V). × 88,000.

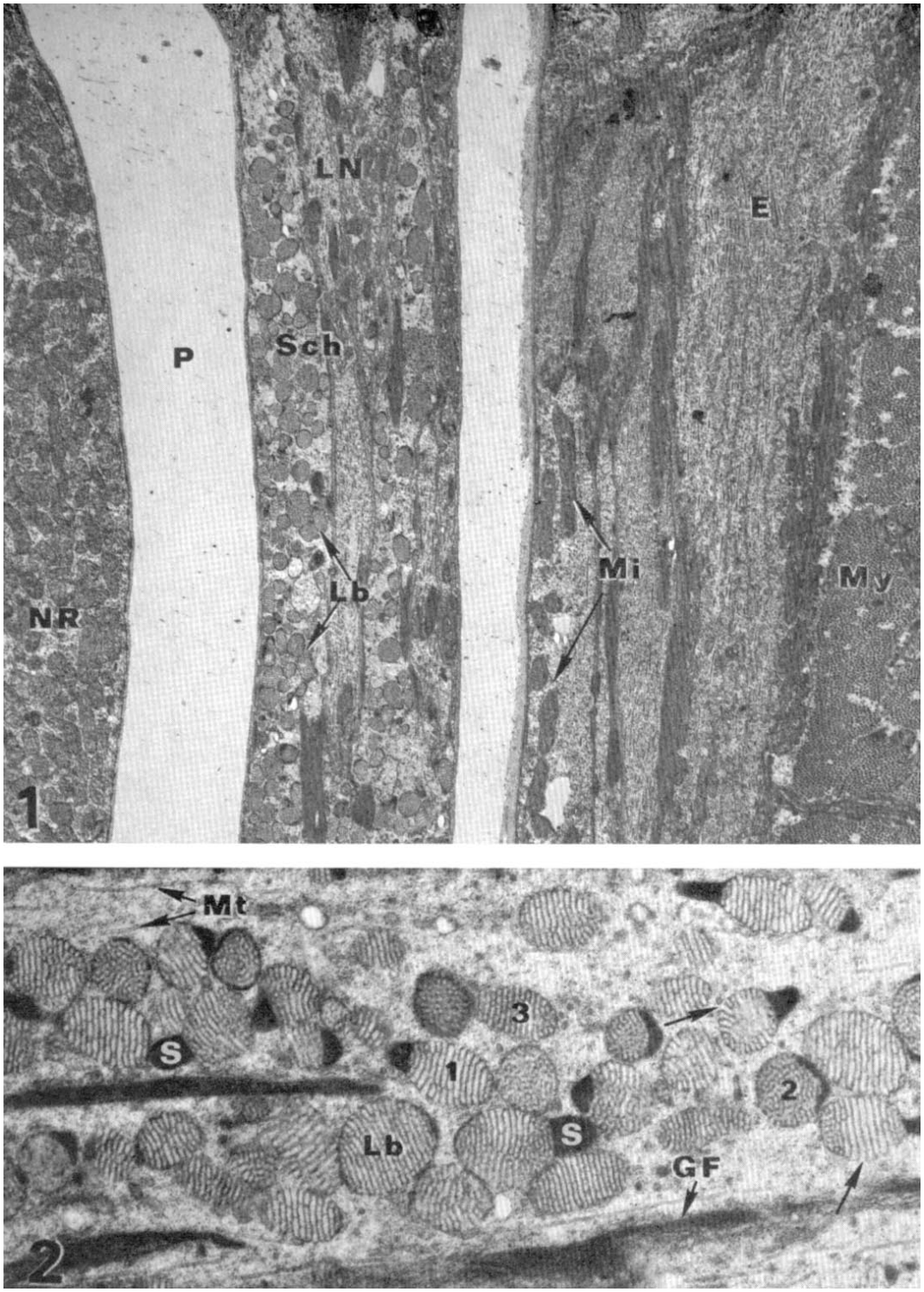

FIG. 1–2

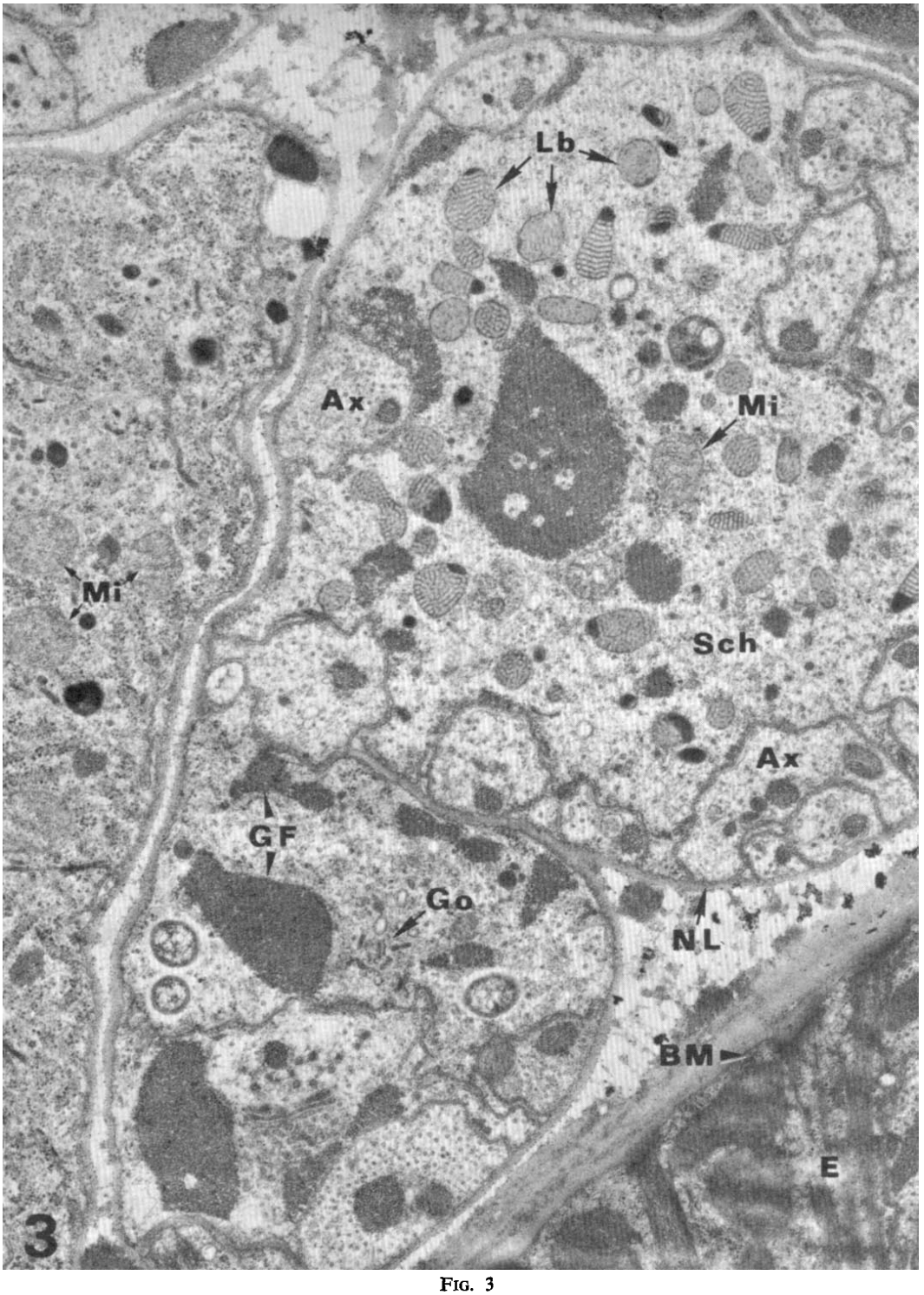

FIG. 3

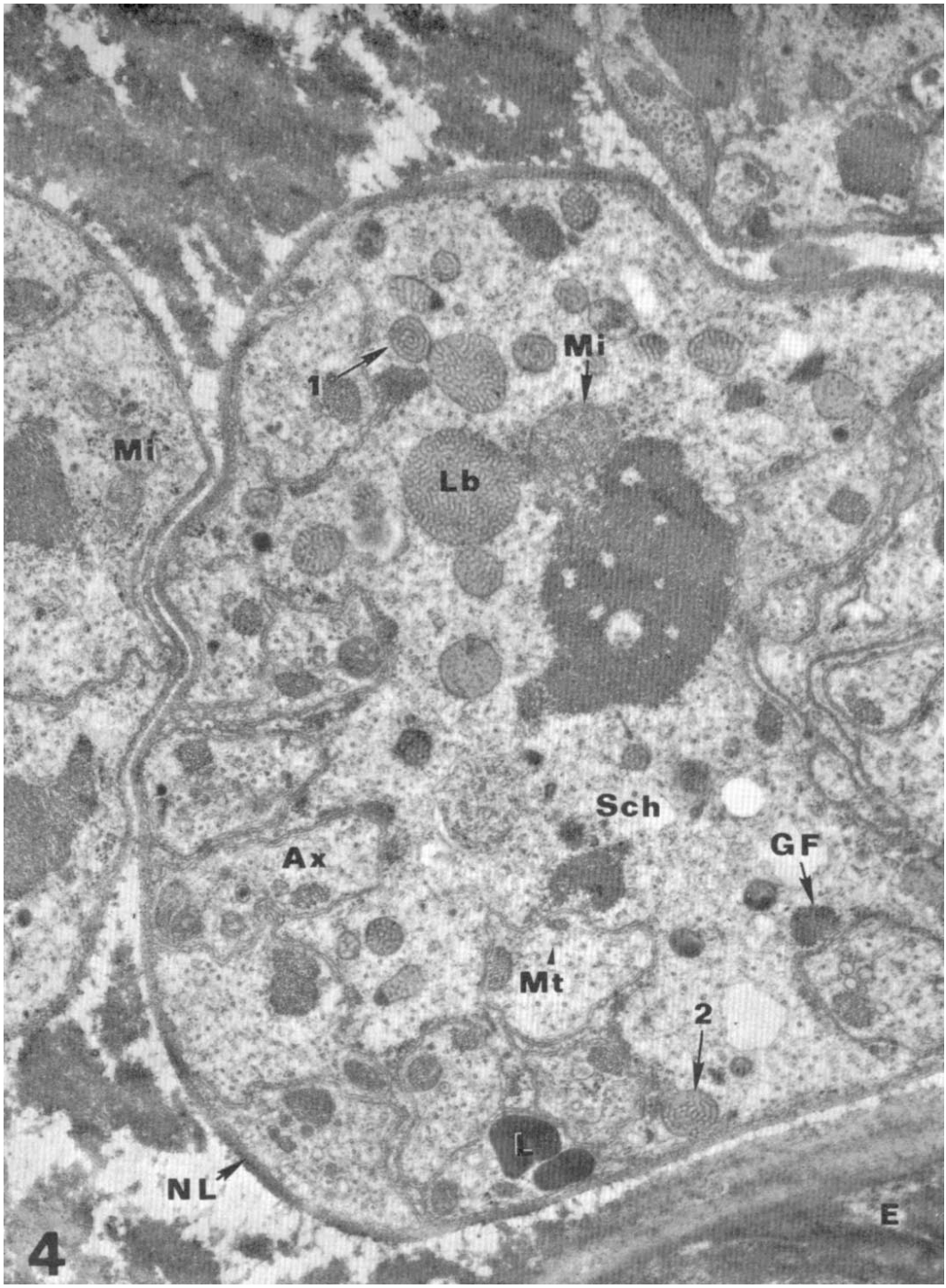

FIG. 4

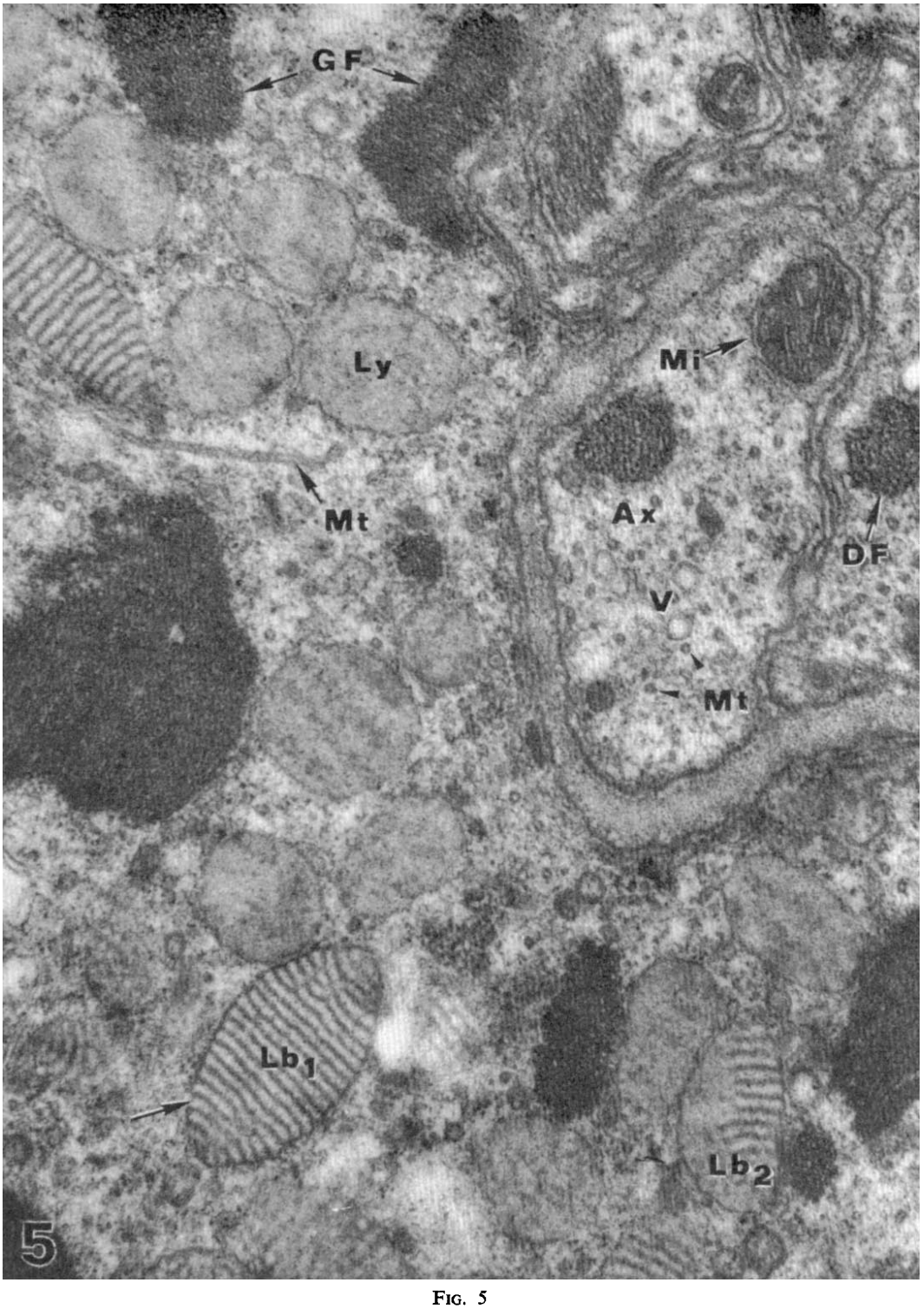

FIG. 5

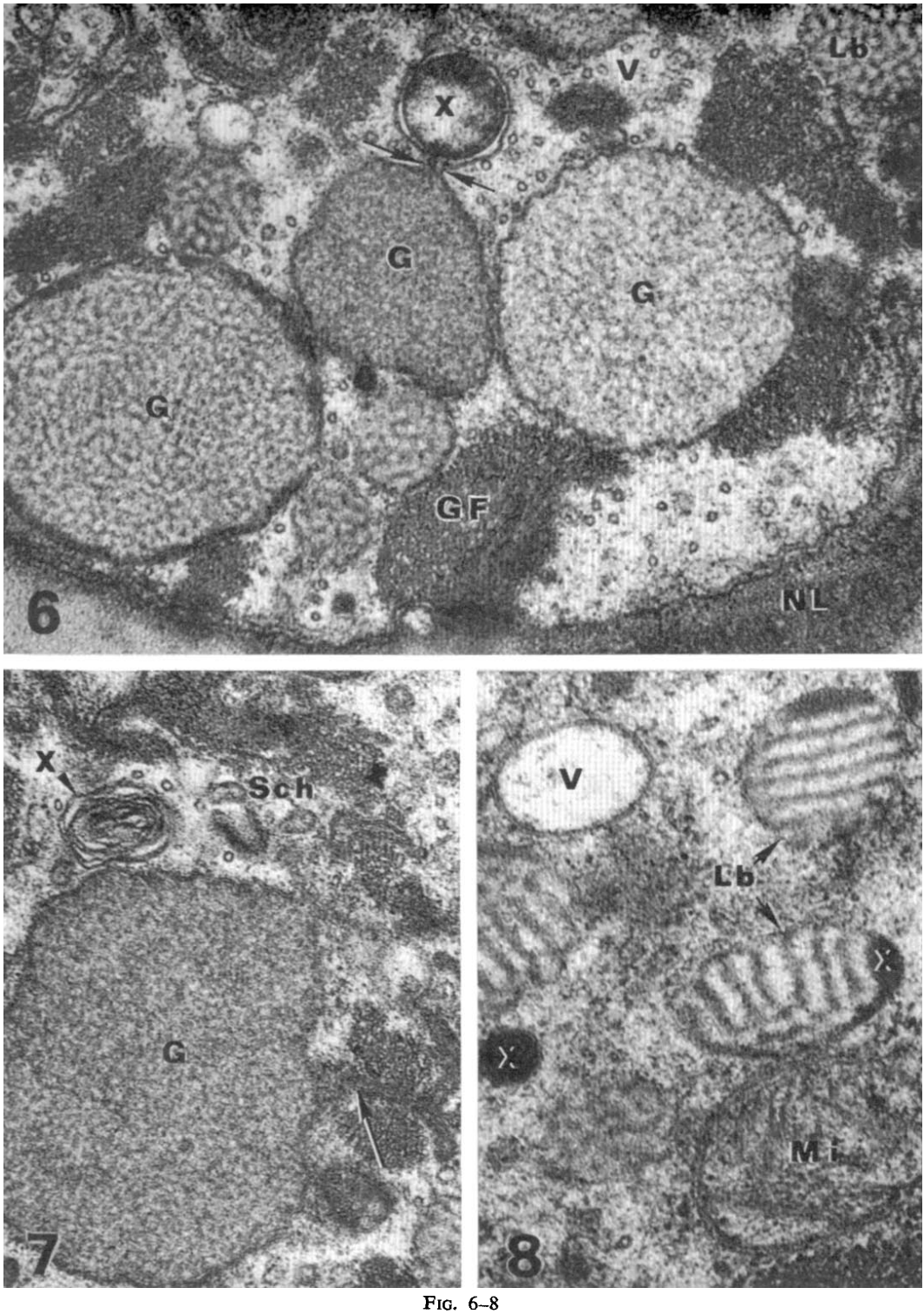

FIG. 6–8