

THE FINE STRUCTURE OF NEURONS*

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In the special cytology of the neuron, four major problems have persisted since the end of the nineteenth century. They are concerned with the structure of the Nissl bodies, the Golgi apparatus, the neurofibrillae, and the synapse. Solutions for these problems have been sought with each new cytological technique as it has been introduced. The resulting heritage of conflicting evidence and interpretation may be illustrated by the history of the first problem; *i.e.*, the structure of the Nissl bodies. Soon after their primary description by Nissl (40), in tissues fixed in alcohol and stained with methylene blue, Held (29, 30) carried out a series of histochemical studies which led him to conclude that the Nissl bodies were precipitates of material originally dispersed uniformly throughout the living cytoplasm. Attempts by Held and others to visualize the Nissl bodies in fresh or living neurons were all unsuccessful, and, moreover, the appearance of the Nissl material was found to vary with the fixative employed. Hence the opinion became general that the Nissl bodies were artifacts of fixation (8). In 1933 this concept was seriously questioned by Bensley and Gersh (5), who demonstrated by means of frozen-dried preparations that the Nissl substance of the nerve cell "is actually discontinuous and is actually segregated in the form of definite groups which in the aggregate constitute the Nissl bodies." Twenty years later, however, the problem is still not resolved, for some investigators maintain that no discontinuous Nissl pattern is revealed by ultraviolet light (34) in living neurons examined in tissue culture, whereas others have been able to discern Nissl bodies in supravitaly prepared neurons by means of phase-contrast microscopy (50). Similar controversies have distinguished the discussion of the Golgi apparatus, the neurofibrillae, and the synapse.

The impasse reached is, of course, largely due to the limited resolving power of the light microscope and to its inadequate sensitivity to the slight differences

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in density that exist among the various components of the cytoplasm. In spite of considerable effort, these limitations have been only partially reduced by the application of ultraviolet light, darkfield, and phase-contrast illumination. Further information on the structure of the neuron can be obtained by means of the electron microscope, which, because of its higher resolving power and its greater sensitivity to differences in density, may be expected to yield much toward the solution of the problems mentioned above. In moving into the domain of the electron microscope, however, one must abandon a valuable advantage; namely, the opportunity to compare living cells with fixed preparations. As is well known, the electron microscope can be used only on fixed material, a limitation which is undoubtedly serious but unavoidable. Irrespective of these instrumental problems, fundamental physical limitations preclude, in any case, the traditional parallel study of living and fixed material beyond a certain limit, set at approximately 0.1μ . In the "submicroscopic"¹ realm proper, one must therefore rely exclusively upon indirect evidence for judging the fidelity of an electron microscope image to the structure of the living cell. Such indirect evidence may be intrinsic to the preparation, as provided by the general continuity of protoplasmic structure (57) and the lack of precipitation, shrinkage, or swelling (42). Alternatively, this indirect evidence may be extrinsic and comparative as provided by consistency with the results of polarized light microscopy (65), x-ray diffraction (23), or cytochemistry (44). In general the indirect evidence thus far adduced commends the electron microscopic image obtained after careful fixation as being at least an acceptable approximation of the living organization of protoplasm, if not actually an arrested form of it. Consequently the limitation imposed upon an electron microscopic study of the neuron by the necessity of examining only fixed material appears to be an obstacle that may be satisfactorily surmounted by the investigator's interpretative ability. Actually such a study can yield valuable information from which much can be inferred about the organization of living neurons.

An electron microscope study of the neuronal cytoplasm appears, therefore, to be justified; actually it has been repeatedly attempted in the last few years. For instance, Pease and Baker (53), Beams *et al.* (3), Haguenu and Bernhard (26), and Hartmann (27, 28) have reported upon the structure of the perikaryon with particular reference to the Nissl bodies, neurofibrillae, mitochondria, and the nuclear membrane. In addition, numerous workers have studied the axoplasm of peripheral nerves, especially from invertebrate sources (18, 19, 21, 22, 31, 36, 62, 63). However, recent advances in preparatory techniques (39, 42, 56) warrant a reinvestigation of the neuronal cytoplasm, especially because the evidence and the conclusions of the studies mentioned conflict in many respects. The present paper reports a study of the perikarya of representative neurons from peripheral ganglia and the central nervous system of

¹ Referring to the light microscope.

the rat. Particular attention has been paid to the Nissl bodies and other cytoplasmic structures. The fine structure of the synapse and other intercellular relationships are the subject of a separate paper (48).

Material and Methods

Material for this study consisted of intramural (myenteric plexus of Auerbach), sympathetic, and dorsal root ganglia, medulla oblongata, and cerebellar cortex, obtained from living 2- to 3-month-old rats.

Fixation.—For the study of perikarya in the myenteric plexus, the intestinal wall was fixed in 1 per cent OsO₄ buffered at a slightly alkaline pH (7.3–7.5) according to a technique already published (42). For specimens consisting predominantly or exclusively of nervous tissue, a modified procedure was employed. Preliminary experience demonstrated that satisfactory fixation of such specimens could be obtained only when they were fixed *in situ* while their blood circulation was still going on. When the tissues were first excised and then immersed in fixing fluid, as in the original procedure, the microscopic changes in the cells, such as swelling of mitochondria and endoplasmic reticulum, and generalized precipitation of the protoplasm, were comparable to those found in tissue removed and fixed 20 minutes after the death of the animal.

In the present modification, the animal was anesthetized with sodium pentobarbital injected intraperitoneally, and the ganglia were carefully exposed by dissection. Injury to blood vessels and nerves was carefully avoided during removal of the overlying tissues. A fresh solution of 1 per cent osmium tetroxide in acetate-veronal buffer (pH 7.3–7.5) was dripped from a pipette onto the exposed ganglion, which was then excised and transferred to a drop of fresh osmium tetroxide solution upon a sheet of dental wax. Extraneous tissue was trimmed away and the ganglion was sliced into four to six small pieces with the aid of a new razor blade. The fragments were transferred by means of a strip of filter paper to a bottle containing the same fixative. Usually the animal was still alive at the end of this procedure so that a second ganglion could be removed from the other side or from a different level.

Satisfactory fixation of the medulla oblongata and the cerebellar cortex was obtained by injecting a 3 per cent solution of osmium tetroxide in acetate-veronal buffer (pH 7.3–7.5) into the fourth ventricle after removal under anesthesia of the posterior arch of the atlas and most of the occiput. The floor of the fourth ventricle was then excised with sharp scissors and transferred to a drop of fresh fixative on a wax plate. The facial colliculus was located and sliced into small pieces less than a millimeter in any dimension. Blackened parts of the cerebellar cortex were similarly cut into small fragments. The fragments of tissue were thereafter immersed in fresh buffered osmium tetroxide for 1 to 2 hours.

Dehydration and Embedding.—At the end of the fixation period the tissue blocks were rinsed with buffer of the same pH, and rapidly dehydrated by passage through a graded series of ethanol, 35 per cent, 50 per cent, 70 per cent, 80 per cent, 95 per cent, and 100 per cent for 30 minutes in each solution. Three changes of absolute ethanol were used. The specimens were then transferred to a mixture of equal parts of absolute ethanol and *n*-butyl methacrylate, thereafter to pure methacrylate changed twice, and finally to methacrylate containing 2 per cent luperco CDB² as catalyst, each change for 30 minutes. The tissues were then embedded in *n*-butyl methacrylate containing 2 per cent catalyst in No. 00 gelatin capsules at 47°C. for 12 to 18 hours.

Microtomy.—Thin sections of these blocks were cut with glass knives on the "improved" microtome designed by Porter and Blum (56). Suitable sections were selected immediately

² Containing 50 per cent 2,4-dichlorobenzoyl peroxide.

after cutting according to their interference colors. Silver sections (~ 20 to 40μ thick) were found most satisfactory.

Electron Microscopy.—The sections were picked up on collodion-coated copper grids, and without removal of the embedding medium were examined in the electron microscope (RCA model EMU-2b or Philips EM-100) at original magnifications of about 3,000 to 10,000. For study the electron micrographs were photographically enlarged 4 to 6 times.

OBSERVATIONS

In light microscopy, a typical, living or freshly isolated neuron is conventionally represented as a large multipolar cell which has a granular cytoplasm and a vesicular nucleus, containing a single prominent nucleolus. After fixation, special stains reveal in the cytoplasm the presence of basophil masses or Nissl bodies, numerous mitochondria, and fine, long filaments or neurofibrillae.

Nissl Bodies.—The appearance of the Nissl bodies is illustrated by the photomicrograph in Fig. 1, which shows a perikaryon belonging to a dorsal root ganglion fixed in buffered OsO_4 (pH 7.4) and embedded in *n*-butyl methacrylate. The section used for this photomicrograph was approximately 1μ thick and was stained in 1 per cent thionine after removing the embedding plastic with benzene. The staining was accordingly the same as in the current technique for the demonstration of Nissl bodies, but the fixation and embedding were different; instead of the conventional fixation in formalin or alcohol followed by paraffin embedding, the material was fixed and embedded as is usually done for electron microscopy. In spite of this difference, the irregular cytoplasmic masses stained by thionine (*nb*, in Fig. 1) have the general appearance of the Nissl bodies in such cells after the conventional treatment. Nissl bodies are therefore present in the cytoplasm of cells fixed in buffered (pH 7.4) OsO_4 and can be expected to appear in one form or another in electron micrographs.

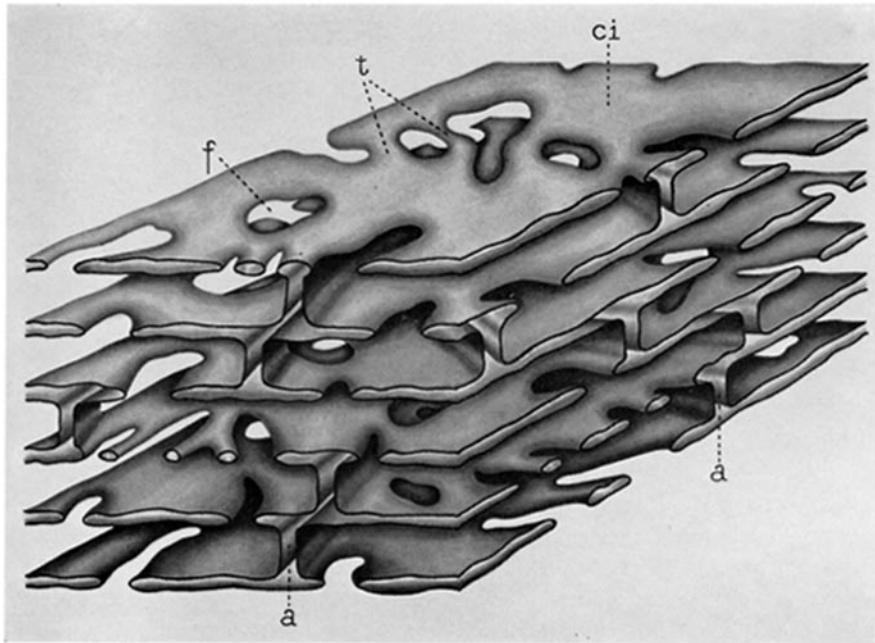
Fig. 2 shows an electron micrograph of a thin section cut from the same block as the section shown in Fig. 1. At this relatively low magnification the cytoplasm of a perikaryon appears to be crowded with formed elements, the most prominent of which are large irregular masses of relatively dense material. In form and distribution these masses are equivalent to the Nissl bodies encountered in stained preparations, as appears clearly from a comparison with Fig. 1. The correlation between the light microscope and the electron microscope images of these formations is therefore easily and satisfactorily established.

In electron micrographs, the Nissl bodies are not separated by membranes or sharp boundaries from the rest of the cytoplasm. Some of them appear as isolated bodies, whereas others merge with one another through broad or narrow bands of the same character (Figs. 2 and 7). Although the masses within a single perikaryon vary considerably in size, the range of variability is more or less characteristic for each type of neuron. In neurons of the sympathetic ganglion, for instance, the Nissl bodies are generally smaller than those in

dorsal root ganglion cells and especially smaller than those in motor neurons. In all these respects the electron microscope merely confirms previous light microscope observations.

New information, however, was obtained about the structure of the Nissl bodies at a lower dimensional level. For instance, two different component elements are readily seen in the masses that represent the Nissl bodies of sympathetic ganglion cells (Figs. 3 and 6), dorsal root ganglion cells (Figs. 4 and 7), Purkinje cells (Fig. 5), and motor neurons (Fig. 8). The first component is represented by numerous membrane-bound profiles that vary in shape from circular to oblong. Their limiting membrane measures 6 to 7 $m\mu$ in thickness, and their lumina vary in diameter from 30 to 50 $m\mu$ with occasional dilatations of 100 or 250 $m\mu$. Their content is of variable but generally light density and appears homogeneous at the present level of resolution. Frequently these profiles are arranged in parallel rows which are more or less regularly spaced at intervals of 80 to 200 $m\mu$ (Fig. 8). Branching profiles are frequent, and occasionally anastomoses between profiles of adjacent rows are encountered. In size and general configuration these elements are similar to those identified as profiles of the endoplasmic reticulum in previous studies (46, 49) in which a large variety of cell types was examined in sections. As demonstrated by these studies, such profiles represent sections through small spherical vesicles, tubules, and large flattened vesicles, designated as cisternae; all these elements are usually connected in a continuous tridimensional network, forming a finely divided vacuolar system. The examination of numerous sections at various incidences through the Nissl bodies of representative neurons, as well as the examination of a few series of successive sections, indicated that the profiles found in the Nissl bodies belong also to vesicles, tubules, and cisternae connected together in a continuous, complex network (Text-fig. 1). Similar profiles were encountered in the rest of the cytoplasm, where they appeared much less numerous and were sparsely scattered. The Nissl bodies seem, therefore, to represent regions in which, in contradistinction to the situation in the rest of the cytoplasm, the endoplasmic reticulum is denser. In certain neurons the endoplasmic reticulum displays in the Nissl bodies a considerable degree of orientation, similar to that described for the basophil cytoplasm of exocrine cells in the pancreas and other glands (49, 68). This orientation consists in the parallel layering at fairly regular intervals of reticular sheets (49), which in sections appear as parallel arrays of profiles. The individual rows forming these arrays may be bent, rippled, or even curved, while still maintaining their general parallelism. The predominance of elongated profiles in these arrays indicates that the corresponding reticular sheets contain numerous cisternae. In some rows the profiles are so long and the interruptions so few and narrow, that the corresponding reticular sheet could be more appropriately described as a large fenestrated cisterna. All possible intermediate forms are encountered

between this appearance and that of a simple reticular sheet which shows in sections as a row of circular, oval, and oblong profiles with numerous and



TEXT-FIG. 1. Diagrammatic representation of the highly oriented type of endoplasmic reticulum usually encountered in the Nissl bodies of motor neurons.

The construction is characterized by a piling of two-dimensional reticula, or reticular sheets (6 sheets in this diagram), each reticular sheet being made up of large, flattened vesicles, described as cisternae (*ci*), connected by tubules (*t*) and strings of small, spherical vesicles. Some cisternae are fenestrated (*f*). Anastomoses (*a*) connect adjacent reticular sheets and, together with occasional branchings (not shown in this diagram; see Fig. 8 for one), maintain the continuity of the endoplasmic reticulum even in such highly oriented forms.

The diverse forms under which the endoplasmic reticulum appears in sections can be obtained by passing planes of section through the diagram at various angles of incidence.

For a complete representation of a Nissl body, the surface of the reticular sheets should be studded with small granules and similar granules should be scattered in the cytoplasmic matrix in between the elements of the endoplasmic reticulum.

A tridimensional representation of the agranular reticulum would resemble this figure with the exception that the distance between cisternae in adjacent reticular sheets would be only one-fourth the distance shown above.

relatively wide interruptions. Text-fig. 1 represents diagrammatically the configuration and the arrangement of the endoplasmic reticulum in a Nissl body; it shows the cisternae and tubules, their tiered disposition, and the anastomoses connecting adjacent reticular sheets. The Nissl substance is thus

pictured as containing a continuous system of membrane-bound cavities, representing a closely meshed and sometimes highly oriented endoplasmic reticulum.

The second component characterizing the Nissl bodies is a variety of minute, punctate, or rod-like particles, 10 to 30 $m\mu$ in diameter, which are arranged in rows along the outer surfaces of the membranes of the endoplasmic reticulum, or in clusters in the intervening ground substance of the cytoplasm (Figs. 6, 8 to 11). Sections passing very obliquely through the elements of the endoplasmic reticulum and exposing their limiting membranes over relatively large areas (Fig. 11) provide a full-faced view of the aggregatory patterns of the granules lying upon the membranes. Such sections show that the particles are disposed in circles, loops, single and double rows, and spirals of various dimensions, but the most common pattern is a rosette of 5 to 7 closely clustered granules (Figs. 9 and 10). In the perikarya the granules are preferentially associated with the masses of endoplasmic reticulum found in the Nissl bodies; as a rule they are absent or rarely present in the cytoplasmic spaces between these bodies.

According to the classic investigations of Nissl (40), the different types of neurons contain characteristic types of basophil masses. In electron micrographs of thin sections, differences in size and shape are evident between the Nissl bodies of motor and sensory neurons, but no fundamental difference was observed in their fine structure. Irrespective of the functional specialization of their neurons, all Nissl bodies were found to consist of endoplasmic reticulum and small granules in close association. There are, however, noticeable differences in the degree of orientation exhibited by the profiles of the endoplasmic reticulum in different types of neurons. In motor neurons, for instance, they display the greatest degree of orientation thus far encountered in Nissl bodies. This orientation is characterized by parallel, more or less regular, layering as described above and illustrated by Fig. 8 (motor cell). The opposite extreme is represented by a reticulum disposed at random, which in section appears as a collection of completely disarrayed profiles, as shown in Fig. 5 (Purkinje cell in the cerebellar cortex). Intermediate degrees of orientation are illustrated in Figs. 3 and 6. In Fig. 6 (sympathetic ganglion cell) rows of slightly elongated profiles are still discernible. The scarcity of elongated profiles indicates that the corresponding reticular sheets have few cisternae. Regular piling is evident in only a few places. In Fig. 3 (sympathetic ganglion cell) elongated profiles are still predominant, but their parallel arrangement is disturbed in many places. Such differences are, however, more quantitative than qualitative. Highly oriented reticula occur commonly in motor cells, but are occasionally encountered also in sensory and even in sympathetic ganglion cells, as indicated by Figs. 4 and 14. The same situation obtains conversely for randomly oriented reticula. This variability in the orientation of the endoplasmic reticulum, even within the same cell, indicates that the configuration of these elements is

probably unstable, changing continually under the influence of streaming movements in the cytoplasm and the metabolic transformations in the cell.

In the perikarya of the myenteric plexus (Auerbach), the Nissl bodies are small in size and their endoplasmic reticulum usually shows a moderate degree of orientation. As in other Nissl bodies, small granules are present in large numbers on the membranes of the reticulum and in the surrounding cytoplasm.

Small neurons, like the granule cells of the cerebellar cortex, are thought not to contain any Nissl substance (59), but electron micrographs reveal the presence of membrane-bound profiles with associated granules similar to those found in other neurons. The profiles occur isolated or in small clusters. As the film of cytoplasm around the nuclei of these small cells is barely discernible in the light microscope, it is not surprising that Nissl substance is not ordinarily recognized in them.

Agranular Reticula.—In all neurons examined, a second system of membrane-bound elements occurs within the cytoplasm. In sections, this system is represented by closely packed profiles, which have narrower lumina than those of the endoplasmic reticulum in the Nissl substance and which have no granules either attached to or associated with them (Figs. 12 and 13). The membranes limiting the profiles of this agranular system are about 10 m μ thick, and the lumina have an average width of about 20 m μ in their undilated portions. Dilations, moreover, are relatively infrequent, and the elements of this system appear to withstand to some degree conditions, like anoxemia and slightly acid pH, which induce swelling of the endoplasmic reticulum and the mitochondria. The localized dilatations which do occur are usually restricted to the ends of elongated profiles (Fig. 13).

Like the profiles of the endoplasmic reticulum in the Nissl substance of many neurons, the profiles of the second, or agranular reticulum display a considerable orderliness in their arrangement. In effect, for them, random orientation seems to be unusual. In sections they appear as closely packed bundles of profiles lying parallel to one another, usually at regular intervals. The spacing between profiles varies from 20 to 100 m μ , but is usually less than 50 m μ , an interval smaller than that of similar arrays in the endoplasmic reticulum. In these bundles, the elongated profiles usually predominate, some of them stretching over distances of a micron or more (Fig. 13), but circular profiles, sometimes in long rows, also appear among the elongated ones, occasionally alternating with them, as in Fig. 12. As in the endoplasmic reticulum, branchings and anastomoses of elongated profiles are frequent in this system (Figs. 4 and 13). Around the orderly arrays described, tightly but disorderly packed clusters of circular profiles of the same diameters are occasionally encountered (Figs. 12 and 13). A less common formation, apparently related to the agranular reticulum, is a vesicular structure (Fig. 13) containing circular profiles which may represent villous invaginations from the surface membrane of this vesicle.

Vesicles with both evaginations and invaginations of this type have been found. Such structures differ from mitochondria by having a simple limiting membrane.

These agranular profiles, of various shapes and clustering patterns, are not distributed uniformly throughout the cytoplasm, but occur in masses scattered here and there without any obvious preferential location. Each mass usually consists of a few straight or bent bundles of elongated profiles at the ends of which are usually sprays of circular profiles. In its appearance, the agranular reticulum of perikarya is similar to structures described in other cells as the Golgi apparatus (16).

Examination of a considerable number of sections at various incidences and of a few sets of serial sections, as well as comparisons with similar formations in other cell types, indicates that these tightly packed profiles correspond in three dimensions to piles or packets of broad, flattened, shallow vesicles similar to the cisternae of the endoplasmic reticulum. The cisternae are also frequently fenestrated, as indicated by the presence of rows of circular and elongated profiles; they may, in addition, arborize into smaller, sometimes tubular, units, and apparently fragment at their periphery into swarms of vesicles. To a large extent, therefore, the basic arrangement of this system is like that of the endoplasmic reticulum in the Nissl substance, with the exceptions that the cisternae are shallower, the packing is tighter, and dense granules are absent both from the surfaces of the membranes and from the intervening cytoplasmic matrix. Because of this similarity in architecture and because of the absence of granules, this second membranous system is designated as an *agranular reticulum*. In sections, the elements of the agranular reticulum are aggregated into masses apparently independent from one another, like the endoplasmic reticulum of the Nissl bodies. Although it is possible that in this system, also, the masses form a continuous meshwork throughout the cytoplasm, no direct proof of such continuity is available from the present study.

The analogous configuration of the agranular reticulum and the endoplasmic reticulum is emphasized by the occurrence of intermediate forms, which can be found upon careful examination of the micrographs. These appear as tubular profiles between the two systems which are beset with granules on the outer surface of one wall and not on the other, or at one end and not at the other (Fig. 12).

A peculiar, concentrically laminated, membranous structure (Figs. 14 and 15), possibly related to the agranular reticulum, has been found most commonly in the cytoplasm of sympathetic ganglion cells. In sections, these bodies appear very much like a slice of an onion. They are constructed of imbricated, elongated profiles, comparable to the scales of an onion, closely packed together with the intervening cytoplasmic matrix compressed between them into narrow bands, 40 $m\mu$ or less in thickness. In Figs. 14 and 15 at least 8

"scales" can be easily counted in the outer portion of the structure, where the lumina are somewhat dilated (possibly artifactitiously). The more centrally placed membranes are difficult to distinguish from one another (particularly in Fig. 15) because of the increasing obliquity of the section in relation to the plane of the membranes. As Fig. 15 shows, aggregates of fine granules often occupy the cores of these onion-like corpuscles. The long narrow profiles are closely apposed and no granules lie between them. But upon the outermost profile and at the free ends of some profiles, numerous granules appear. In three dimensions, these corpuscles may be pictured as whorls of shallow cisternae, more loosely packed at the periphery than in the center.

In both Figs. 14 and 15, the onion-like corpuscles are immersed in Nissl substance. These topographical associations suggest a close relationship between the whorled agranular membranes and the endoplasmic reticulum of the Nissl bodies. The similarity between these corpuscles and the whorled structures in the pancreatic acinar cell described by Weiss (68) will be discussed later.

Mitochondria.—In the cytoplasm intervening between Nissl bodies and sometimes embedded in the Nissl substance, lie numerous filamentous, rod-like, or spherical mitochondria (Figs. 2, 3, and 16). Branching and bent forms also occur frequently in all types of neurons examined. The mitochondria of neurons possess the characteristic internal structure described for mitochondria of many other cell types (43, 44, 60, 64). They possess an outer smooth membrane and an inner folded membrane that forms cristae projecting into the interior of the organelles (Fig. 16). The cristae are usually of the conventional shelf-like type, extending more or less at right angles to the long axis of the mitochondrion. In some mitochondria, however, the cristae lie parallel to the long axis, thereby producing a strikingly regular pattern, and finally in others the projections of the inner membrane assume a villous form, appearing in some sections as circular profiles within the mitochondrial matrix.

Neurofilaments.—Fine, long threads, 60 to 100 A in diameter and of indefinite length, traverse the cytoplasmic matrix between masses of Nissl substance and other organelles (Figs. 3 and 7). Frequently they criss-cross one another in all directions and in all planes, but sometimes they course in one predominant direction either in loose bundles or as independent strands. Although they can be followed for only short distances in thin sections, they evidently form a loose feltwork of fibrils throughout the cytoplasm. The filaments appear to be smooth and homogeneous at the resolution obtained. Morphologically they are similar to those described in peripheral nerves by Schmitt and Geren (63), Fernández-Morán (22), Maxfield (36), and De Robertis and Thornburg (18). Presumably, they represent the structures which, after aggregation and encrustation by metals, result in the neurofibrillae visible with the highest powers of the light microscope.

Dense Inclusions.—Dense rounded bodies (350 to 660 $m\mu$ in diameter) are dispersed throughout the cytoplasm of the neuron in large numbers. Some of them are homogeneous bodies without a limiting membrane and are considered, therefore, to be lipid droplets. Others possess a limiting membrane and a heterogeneous content which exhibits a fine, regular, grainy structure, or small vacuoles, or, finally, poorly defined lumps of dense material (Figs. 3 and 14).

Nucleus.—As no systematic study of the nucleus was made in this investigation, only a few incidental observations will be reported here. The double nuclear membrane, noted by Hartmann (27, 28), is clearly demonstrated in Figs. 12 and 14. The first, or inner, nuclear membrane, about 130 A thick, is smooth, whereas the second, or outer, membrane, about 75 A thick, presents fine undulations and tubular extensions into the cytoplasm. In other cell types, e.g. the pancreatic acinar cell, the outer nuclear membrane is encrusted with fine granules, but in the neuron this membrane is consistently free of them. As Figs. 12 and 14 show, the two nuclear membranes are separated by a light space about 200 A wide. These dimensions are of the same order of magnitude as those reported by Hartmann (27).

At irregular intervals (marked by arrows in Fig. 12) the two nuclear membranes abruptly fuse for short distances (280 to 360 A). At some of these points the nuclear covering seems to be discontinuous. Configurations of this type may actually represent perforations or apertures in the nuclear membranes such as have been reported³ for the nuclei of amphibian oocytes (13), salivary gland cells of *Chironomus* (1), and Rous sarcoma cells (24). Bretschneider (12) reports that pores, 700 A in diameter, are regularly arranged in the nuclear membranes of spinal ganglion cells, but Hartmann (27) was unable to find clear evidence of such discontinuities.

The nucleolus, which is so prominent in light microscope images of the nucleus of the neuron, appears in electron micrographs of osmium-fixed cells as a tangle or convoluted mass of dense and thick filaments as described by Borysko and Bang (10) and Bernhard *et al.* (7). At higher magnifications these filaments appear as dense aggregations of fine granules, about 10 $m\mu$ in diameter, frequently disposed in linear and rather parallel arrays. Similar granules have been demonstrated in the nucleoli of myoblasts by Porter (55). No membrane surrounds the nucleolar mass.

DISCUSSION

As imaged in the electron microscope, the crowded cytoplasm of the neuron contrasts sharply with the relatively open cytoplasm of many other cell types. As this compact appearance stems largely from the extensive meshwork of

³ Recently M. L. Watson has described similar discontinuities in the nuclear membranes of pancreatic exocrine cells (*Biochim. et Biophysic. Acta*, 1954, **15**, 475).

Nissl substance, the neuron resembles, even at the electron microscope level, certain protein-secreting glandular cells, such as those of the pancreatic acini and the salivary glands. This similarity has long been evident from light microscopic studies, particularly since the realization that the cytoplasmic basophilia of both neurons and glandular cells is due to the same class of substances, *i.e.* pentose nucleoproteins (5, 9, 14, 33).

Comparison of Figs. 1 and 2 provides a close correlation between the electron microscope image and the classical light microscope appearance of the Nissl bodies. As already mentioned, the sections in these figures were cut from the same block of tissue, which had been fixed in buffered (pH 7.4) OsO_4 and embedded in *n*-butyl methacrylate as in the usual procedure for electron microscopy. A comparison of Fig. 1 with conventional light microscope representations of Nissl bodies indicates that the preparative procedure for electron microscopy provides a light microscope image which, in this particular respect, is similar to the one yielded by the usual technique for demonstrating Nissl bodies (fixation in alcohol or formalin followed by paraffin embedding). The dense, composite masses revealed by the electron microscope in Fig. 2 are equivalent to the stained Nissl bodies in Fig. 1, as proved by similarities in their distribution, size, and shape. It must be added that the examination of unstained sections in the phase-contrast microscope showed that the Nissl bodies are denser than the rest of the cytoplasm, a finding that further increases the area of agreement between light and electron microscope images. Evidently, therefore, a satisfactory correlation can be established between light and electron microscope findings concerning the Nissl bodies in fixed preparations.

Like the basophil substance or ergastoplasm of glandular cells, the Nissl substance is a composite material constructed of endoplasmic reticulum and fine granules, both of which have been revealed by electron microscopy (45, 49, 54). The first component of the Nissl substance appears to be part of the general endoplasmic reticulum of the neuron. The reticulum extends throughout the entire cytoplasm, but is considerably more condensed within the area of the Nissl bodies than in the rest of the cell. These condensations not only determine the size and shape of each Nissl body, but also constitute a membranous framework upon which the other components are arranged. In many types of neurons, the meshes of the endoplasmic reticulum are distributed at random in three dimensions (Fig. 5), but in all neurons, and especially in the large motor neurons, the endoplasmic reticulum may display a distinctly orderly arrangement within the Nissl bodies. This orientation, which is evident in Fig. 8 and represented diagrammatically in Text-fig. 1, consists of a layering of reticular sheets, at more or less regular intervals. Each sheet is a reticulum developed predominantly in two dimensions and comprising tubules, strings of vesicles, and numerous large and flat cisternae. Even in such highly ordered

forms as the Nissl bodies of motor neurons, the continuity of the reticulum persists, as indicated by frequent branches and anastomoses between layers.

The second component of the Nissl substance is represented by small granules disposed in patterned arrays either in close contact with the outer membranes of the endoplasmic reticulum or scattered in the intervening matrix. This matrix may be considered as a third component of the Nissl substance. It is evident, therefore, that although the Nissl bodies are differentiated parts of the cytoplasm, they are continuous with it by virtue of the continuity of the endoplasmic reticulum and the matrix. No interface or membrane separates them from the rest of the cytoplasm. In this respect, as well as in general architecture, they are comparable to the ergastoplasm of glandular cells. The only differences lie in (a) a different intracellular distribution, (b) a lesser degree of preferred orientation of the endoplasmic reticulum, and (c) an apparently greater concentration of fine granules within the areas of the Nissl bodies.

The foregoing description of the Nissl substance does not agree with that given by other recent electron microscopic studies of nerve cells. Pease and Baker (53) and Hartmann (27), for instance, were unable to identify any cytoplasmic structure with the Nissl bodies in cells which they believed were optimally preserved. Beams *et al.* (3) described the Nissl bodies as irregular, relatively dense, vacuolated bodies. However, both Beams *et al.* (3) and Hartmann (27) observed in a few cells "bundles of filaments" or "submicroscopic cytoplasmic fibrils," which they correlated with the filaments identified by Dalton *et al.* (17) and Bernhard *et al.* (6) as the basophil substance of the cytoplasm in hepatic and pancreatic cells. The filaments or fibrils found by Beams and by Hartmann are probably related to the highly oriented type of endoplasmic reticulum and represent either elongated profiles or compressed bands of intervening matrix. According to the observations of Haguenu and Bernhard (26), the Nissl bodies consist of dense granules, 70 to 140 μ in size. Because no "filaments" appeared in their preparations, the French authors felt that the ultrastructure of the Nissl substance differed from that of the ergastoplasm in glandular cells. The dense granules described by them may represent aggregates of the fine granular component reported in the present paper. At variance with their conclusion, the essential correspondence between the fine structures of ergastoplasm and Nissl substance has already been emphasized above.

It is interesting that Nissl (40) recognized the composite nature of the substance named for him, although he could not have resolved either of its components. He considered that the basophil bodies consisted of fine elementary granules cemented together by an achromatic substance. A comparable view was held by de Quervain (58), van Gehuchten (25), and Ramón y Cajal (59). Presumably the granules described by these authors correspond to clumps

of the small granules disclosed in the present study. The achromatic substance, which Ramón y Cajal described as a network or perhaps a system of alveoli, may correspond to swollen elements of the endoplasmic reticulum or to retraction spaces among the aggregated granules.

Although the observations reported in this study do not concern directly the configuration of the Nissl substance in living perikarya, they have enough bearing on this problem to deserve discussion as to whether and to what extent they reflect the situation *in vivo*. The opinion proposed by Held (29, 30), that the basophil substance is diffusely dispersed throughout the living cytoplasm, has been recently upheld by Koenig and Feldman (34) who state that "ribonucleoprotein is homogeneously distributed in the cytoplasm of living nerve cells." This opinion conflicts with the observations of Weimann (67) and Yamada (71), who examined unfixed, freshly prepared neurons in the ultraviolet microscope. In a previous study of perikarya freshly isolated from the hypothalamus of rabbits, Palay and Wissig (50) demonstrated parallel linear striations in the Nissl areas, an appearance which is probably a manifestation of the oriented type of endoplasmic reticulum described for Nissl bodies in the present paper. At variance with Koenig's findings, the observations mentioned would indicate therefore that *in vivo*, as after fixation, the Nissl substance is disposed in discontinuous masses, a view which is also supported by the evidence adduced by Bensley and Gersh (5) on the basis of frozen-dried material. If the opinion of Held and his followers were correct, an originally diffuse material must be redistributed into distinct masses during fixation by precipitation or coagulation. Such phenomena should lead to gross and easily recognizable artifacts such as retraction, discontinuity of structure, irregular clumping, and disorderly appearance of the cytoplasm. All of these features, however, are actually absent or minimal in satisfactorily preserved material as illustrated by most of the electron micrographs in this study. Their general appearance would suggest rather that no major redistribution has occurred during fixation. Moreover, in other similarly prepared cell types, such as the acinar cells of the parotid and the pancreas, electron microscopy reveals no discrete, irregular clumps of basophil material, as in nerve cells, but rather a homogeneous mass extending throughout the basal half of the cell from one border to another. Such observations suggest again that the factor determining the gross appearance of the basophil material in electron microscope preparations is not the fixation, but the original distribution *in vivo*.

The present study has revealed the existence of two components in the Nissl substance, one of them described as a small granular component. Recent investigations (45) have suggested that this small granular component is responsible for the staining affinities of the Nissl substance and of basophil cytoplasm in general. Under such circumstances, it may be argued that fixation affects only the small granular component without disturbing the distribution of the endoplasmic reticulum. It may be proposed, for instance,

that the granules, homogeneously dispersed in the living cytoplasm, are precipitated during fixation on available surfaces such as those of the endoplasmic reticulum. Although this hypothesis would reconcile the conflicting evidence derived from ultraviolet, light, and electron microscopy, a number of findings militate against it. Such is the case, for instance, with the absence of the granules from other available surfaces, *e.g.* the cell membrane and the mitochondrial membrane; the presence of "free" granules in the cytoplasm of many cell types fixed by similar methods; the existence in many cell types of segments of the endoplasmic reticulum free of granules; and the fact that the granules form special aggregatory patterns which seem to differ to a certain extent from one cell type to another. Moreover, the presence of small granules at the surface of "microsomes" isolated from other cell types (66) suggests that the association between the endoplasmic reticulum and the fine granules exists before fixation and is not materially influenced by it.

In conclusion, the majority of the findings agree with an uneven distribution of the Nissl material *in vivo* and recommend the electron microscope image of the Nissl bodies as an acceptable approximation of the situation in living perikarya. Admittedly this conclusion cannot be reconciled with the findings of Koenig and Feldman (34), who showed, as already mentioned, that the cytoplasm of whole, living perikarya in tissue culture is characterized by diffuse and even absorption in the ultraviolet. It should be commented that diffuse absorption does not necessarily mean diffuse distribution of absorbing material. Uneven distribution, though present, might be lost through superimposition of absorbing masses or through unsatisfactory focusing.

Agranular Reticulum.—Like the endoplasmic reticulum, the second system of membrane-bound cavities described in this paper apparently consists of broad, flattened cisternae, which arborize into tubules and fragment into smaller vesicles at their extremities. This membranous system differs from the endoplasmic reticulum of the Nissl bodies in three important respects. (1) Although arranged in piles like the elements of the endoplasmic reticulum, the cisternae of the agranular reticulum are more closely packed together. The distance between neighboring elements in the pile is only one half to one fourth the interval between the piled elements of the endoplasmic reticulum. (2) The broad cisternae are more shallow than those of the endoplasmic reticulum by one third or more, and cisternal dilatations are also smaller and less frequent. (3) The fine granular component is not associated with this membranous system. However, most of these distinguishing characteristics are only quantitative in nature, and the general architecture of the agranular reticulum is similar to that of the endoplasmic reticulum. This analogy, as well as the presence of intermediate forms already described, warrants the speculation that the two membrane systems may be related. Perhaps the agranular reticula are precursors of the endoplasmic reticulum in the Nissl substance.

Whorled structures similar to the onion-like corpuscles, described above as

possibly related to the agranular reticulum, have been encountered by Weiss (68) in the basal cytoplasm of the pancreatic acinar cells of animals recovering from fasting. He considered them as centers of formation of the ergastoplasm. Such relations must still be considered as merely speculative, so far as the nerve cell is concerned. Perhaps a study of the fine structure of nerve cells recovering from chromatolysis may elucidate this problem.

The identification of the agranular reticulum with any previously described cytoplasmic organelle is hazardous at this time. Unlike the Nissl substance, its shape, constitution, and distribution provide no certain clues to its appearance in the light microscope. Tight piles of similar profiles have been found in the centrosphere regions of many other cells (46, 49). In electron micrographs of epithelial cells of the epididymis, Dalton and Felix (16) have recently described comparable membrane systems, which they identify as the Golgi substance. They recognize three morphological components—large vacuoles, paired lamellae, and small granules. Probably, the lamellae of the epididymal cells correspond to the membranous elements of the agranular reticulum in the neuron, but large vacuoles and small granules have not been seen in association with these membranes in the neuron. Comparable structures have been identified as the Golgi apparatus in the cells of the proximal convoluted tubule (60) and of the adenohipophysis (61, 20).

Our own observations on neurons fixed in fluids with a pH between 6.0 and 7.0 indicate that the agranular reticulum is less susceptible to swelling or vacuole formation than either the endoplasmic reticulum or the mitochondria. The influence of lower pH's has not been explored. As is known, the Golgi apparatus has been repeatedly considered a fixation artifact (51, 2) in the production of which a low pH has been implicated (47). Under these circumstances reexamination of this problem by means of the electron microscope is needed before identifying the structures mentioned with the Golgi apparatus. This suggestion seems particularly pertinent in view of the fact that the original description of the Golgi apparatus and related structures was derived from studies of the neuron. At this time we feel that a simple descriptive term, such as *agranular reticulum*, is desirable until such a study has been made.

Neurofibrillae.—The long controversy over the preexistence of neurofibrillae in the living neuron need not be detailed here (see Parker (52) for review of literature). On the basis of studies of living neurons in tissue culture by Weiss and Wang (70) and by Levi and Meyer (35), most textbooks of histology accept the neurofibrillae as true entities. Yet, it is by no means certain that these investigators really saw neurofibrillae (32). Murnaghan (37) found no visible fibrils in living spinal ganglion cells and demonstrated only densely packed mitochondria in the positions presumed to be occupied by neurofibrillae. Murray and Stout (38) reported that no structures interpretable as neurofibrillae could be seen in living sympathetic ganglion cells. Recent studies with the aid

of the phase-contrast microscope have also failed to reveal neurofibrillae in fresh or living neurons (50, 32). Nonetheless, the birefringence of the cytoplasm in perikarya and especially in axons is sufficient basis for assuming that it has a longitudinally oriented submicroscopic structure (4, 15, 18). Exceedingly thin filaments which could account for the birefringence have been seen by nearly all investigators who have studied sections of nerve with the electron microscope (63, 62, 19, 3, 21, 22, 18). Pease and Baker (53) regarded the fibrils they found as frank artifacts, "a felt-work of precipitated protein," which only indicated a longitudinally oriented colloidal organization of the axoplasm, an opinion shared by Hess and Lansing (31). Recently Maxfield (36) has isolated fine beaded filaments from the axoplasm of squid giant nerves and has shown that they are capable of undergoing a reversible decrease in axial ratio and particle weight resembling depolymerization.

The electron micrographs presented in this paper demonstrate fine filaments, about 100 Å in diameter and of indefinite length, which occupy the interspaces among Nissl bodies. These filaments are too small to be resolved by the light microscope, but side-to-side aggregations of them could be resolved, especially if encrusted with silver after application of the usual histological procedures for revealing neurofibrillae.

Correlation of Structure and Function.—Although a full evaluation of the functional significance of the structures described in this paper must await further biochemical and cytochemical analysis, some relations they suggest deserve consideration. From this point of view, the most striking morphological feature of the neuron is the tremendous accumulation within its cytoplasm of small granules associated with a well developed endoplasmic reticulum. The same type of association is found in the ergastoplasm of glandular cells, a type of cytoplasm known for its intense basophilia, high RNA content, and prevalence in cells which sustain an intense protein production (11, 14). In the nerve cell this activity is implicit in the chromatolysis which it undergoes in response to intense stimulation or injury (33, 9) and to certain types of generalized stress such as cold (41). In the chromatolytic cycle such changes are usually visualized because of modification in the RNA metabolism, but they are paralleled by corresponding changes in the protein metabolism of the cytoplasm. The rapid regeneration of axons after section and the peculiar damming up of axoplasm proximal to a ligature, as described by Weiss and Hiscoe (69), are also reflections of a continuous and rapid protein synthesis in the perikaryon. The exact relationships of the components of the Nissl substance to the detailed processes of protein production remain unknown, but the fact that its structure is the same as that of the ergastoplasm in glandular cells means that future analyses of this process in such readily available cells as those of the pancreas and liver can be profitably applied to the nerve cell. When these correlations have been made, the prevalence of an extensive agranular reticulum, particularly the onion-like

whorls, may fit into the reproductive cycle of a system of structures that is essential for the orderly process of protein metabolism.

SUMMARY

1. Thin sections of representative neurons from intramural, sympathetic and dorsal root ganglia, medulla oblongata, and cerebellar cortex were studied with the aid of the electron microscope.

2. The Nissl substance of these neurons consists of masses of endoplasmic reticulum showing various degrees of orientation; upon and between the cisternae, tubules, and vesicles of the reticulum lie clusters of punctate granules, 10 to 30 $m\mu$ in diameter.

3. A second system of membranes can be distinguished from the endoplasmic reticulum of the Nissl bodies by shallower and more tightly packed cisternae and by absence of granules. Intermediate forms between the two membranous systems have been found.

4. The cytoplasm between Nissl bodies contains numerous mitochondria, rounded lipid inclusions, and fine filaments.

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BIBLIOGRAPHY

1. Bahr, G. F., and Beermann, W., *Exp. Cell Research*, 1954, **6**, 519.
2. Baker, J. R., *Quart. J. Micr. Sc.*, 1944, **85**, 1.
3. Beams, H. W., van Breeman, V. L., Newfang, D. M., and Evans, T. C., *J. Comp. Neurol.*, 1952, **96**, 249.
4. Bear, R. S., Schmitt, F. O., and Young, J. Z., *Proc. Roy. Soc. London, Series B*, 1937, **123**, 505.
5. Bensley, R. R., and Gersh, I., *Anat. Rec.*, 1933, **57**, 369.
6. Bernhard, W., Haguenu, F., Gautier, A., and Oberling, C., *Z. Zellforsch. u. mikr. Anat.*, 1952, **37**, 281.
7. Bernhard, W., Haguenu, F., and Oberling, C., *Experientia*, 1952, **8**, 58.
8. Bielschowsky, M., in *Handbuch der mikroskopischen Anatomie des Menschen*, (W. von Möllendorff, editor), Berlin, Springer, 1928, **4**, pt. 1, 38.
9. Bodian, D., and Mellors, R. C., *J. Exp. Med.*, 1945, **81**, 469.
10. Borysko, E., and Bang, F. B., *Bull. Johns Hopkins Hosp.*, 1951, **89**, 468.
11. Brachet, J., *Chemical Embryology*, New York, Interscience Publishers, Inc., 2nd edition, 1950, 229-249.
12. Bretschneider, L. H., in *International Review of Cytology*, (G. H. Bourne and J. F. Danielli, editors), New York, Academic Press, Inc., 1952, **1**, 305.
13. Callan, H. G., and Tomlin, S. G., *Proc. Roy. Soc. London, Series B*, 1950, **137**, 367.
14. Caspersson, T. O., *Cell Growth and Cell Function, a Cytochemical Study*, New York, W. W. Norton and Co., Inc., 1950.
15. Chinn, P., *J. Cell. and Comp. Physiol.*, 1938, **12**, 1.
16. Dalton, A. J., and Felix, M. C., *Am. J. Anat.*, 1954, **94**, 171.

17. Dalton, A. J., Kahler, H., Striebich, M. J., and Lloyd, B., *J. Nat. Cancer Inst.*, 1950, **11**, 439.
18. De Robertis, E., and Thornburg, W., cited by De Robertis, E., Nowinski, W., and Saez, F., *General Cytology*, Philadelphia, W. B. Saunders Company, 2nd edition, 1954, 425-426.
19. Duncan, D., and Antes, L., *Texas Rep. Biol. and Med.*, 1950, **8**, 329.
20. Farquhar, M. G., and Rinehart, J. F., *Endocrinology*, 1954, **54**, 516.
21. Fernández-Morán, H., *Exp. Cell Research*, 1950, **1**, 309.
22. Fernández-Morán, H., *Exp. Cell Research*, 1952, **3**, 282.
23. Finean, J. B., Sjöstrand, F. S., and Steinmann, E., *Exp. Cell Research*, 1953, **5**, 557.
24. Gaylord, W., 1954, personal communication.
25. van Gehuchten, A., *Anatomie du système nerveux de l'homme*, Louvain, Uystpruyt, 2nd edition, 1897, 241-243.
26. Hagnenau, F., and Bernhard, W., *Exp. Cell Research*, 1953, **4**, 496.
27. Hartmann, J. F., *J. Comp. Neurol.*, 1953, **99**, 201.
28. Hartmann, J. F., *Anat. Rec.*, 1954, **118**, 19.
29. Held, H., *Arch. Anat. u. Physiol., Anat. Abt.*, 1895, 396.
30. Held, H., *Arch. Anat. u. Physiol., Anat. Abt.*, 1897, 205.
31. Hess, A., and Lansing, A. I., *Anat. Rec.*, 1953, **117**, 175.
32. Hughes, A., *J. Anat.*, 1954, **88**, 192.
33. Hydén, H., *Acta physiol. scand.*, 1943, **6**, Suppl. 17, 1.
34. Koenig, H., and Feldman, D., *J. Histochem. and Cytochem.*, 1954, **2**, 334.
35. Levi, G., and Meyer, H., *Anat. Anz.*, 1937, **83**, 401.
36. Maxfield, M., *J. Gen. Physiol.*, 1953, **37**, 201.
37. Murnaghan, D. P., *Anat. Rec.*, 1941, **81**, 183.
38. Murray, M. R., and Stout, A. P., *Am. J. Anat.*, 1947, **80**, 225.
39. Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bureau Stand.*, 1949, **43**, 183.
40. Nissl, F., *Neurol. Zentr.*, 1894, **13**, 676.
41. Nurnberger, J. I., *Assn. Research Nerv. and Ment. Dis.*, 1953, **32**, 132.
42. Palade, G. E., *J. Exp. Med.*, 1952, **96**, 285.
43. Palade, G. E., *Anat. Rec.*, 1952, **114**, 427.
44. Palade, G. E., *J. Histochem. and Cytochem.*, 1953, **1**, 188.
45. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 59.
46. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, in press.
47. Palade, G. E., and Claude, A., *J. Morphol.*, 1949, **85**, 71.
48. Palade, G. E., and Palay, S. L., *Anat. Rec.*, 1954, **118**, 335.
49. Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100**, 641.
50. Palay, S. L., and Wissig, S. L., *Anat. Rec.*, 1953, **116**, 301.
51. Parat, M., *Arch. anat. micr.*, 1928, **24**, 73.
52. Parker, G. H., *Quart. Rev. Biol.*, 1929, **4**, 155.
53. Pease, D., and Baker, R. F., *Anat. Rec.*, 1951, **110**, 505.
54. Porter, K. R., *J. Exp. Med.*, 1953, **97**, 727.
55. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2**, 346.
56. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117**, 685.

57. Porter, K. R., and Kallman, F., *Exp. Cell Research*, 1953, **4**, 127.
58. de Quervain, F., *Virchows Arch. path. Anat.*, 1893, **133**, 481.
59. Ramón y Cajal, S., *Histologie du système nerveux de l'homme et des vertébrés*, Paris, A. Maloine, 1911, **1**.
60. Rhodin, J., *Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney*, Stockholm, Nordiska Bokhandeln, 1954.
61. Rinehart, J. F., and Farquhar, M. G., *J. Histochem. and Cytochem.*, 1953, **1**, 93.
62. Rozsa, G., Morgan, C., Szent-Györgyi, A., and Wyckoff, R. W. G., *Biochim. et Biophysic. Acta*, 1950, **6**, 13.
63. Schmitt, F. O., and Geren, B. B., *J. Exp. Med.*, 1950, **91**, 499.
64. Sjöstrand, F. S., *Nature*, 1953, **171**, 30.
65. Sjöstrand, F. S., *J. Cell. and Comp. Physiol.*, 1953, **42**, 15.
66. Slaughterback, D. B., *Exp. Cell Research*, 1953, **5**, 173.
67. Weimann, W., *Z. ges. Neurol. u. Psychiat.*, 1925, **98**, 347.
68. Weiss, J. M., *J. Exp. Med.*, 1953, **98**, 607.
69. Weiss, P., and Hiscoe, H. B., *J. Exp. Zool.*, 1948, **107**, 315.
70. Weiss, P., and Wang, H., *Anat. Rec.*, 1936, **67**, 105.
71. Yamada, E., *Kyushu Mem. Med. Sc.*, 1953, **4**, 25.

EXPLANATION OF PLATES

PLATE 17

FIG. 1. Photomicrograph of a section through a dorsal root ganglion cell.

The cytoplasm contains characteristic small, irregularly shaped Nissl bodies (*nb*), some fine granules, and a homogeneous unstained matrix. The nucleus appears to be clear except for a large, round, dense nucleolus (*nl*) and a weakly staining mass of chromatin. Capsule cells (*c*) surround the perikaryon.

The ganglion was fixed for 2 hours in 1 per cent osmium tetroxide buffered with veronal-acetate (pH 7.4), and embedded in *n*-butyl methacrylate. The section, about 1 μ thick, was stained with thionine after the methacrylate had been removed by immersion in benzene. \times 2,400.

FIG. 2. Electron micrograph of a section through a dorsal root ganglion cell from the same block as the section shown in Fig. 1.

The section passes through the nucleus (*n*) without touching the nucleolus. Small, irregular Nissl bodies (*nb*), mitochondria (*m*), and lipid droplets (*l*) can be seen in the cytoplasm. At *c* is the nucleus of a capsule cell and at *a*, an axon leaves a neighboring perikaryon. \times 4,600.

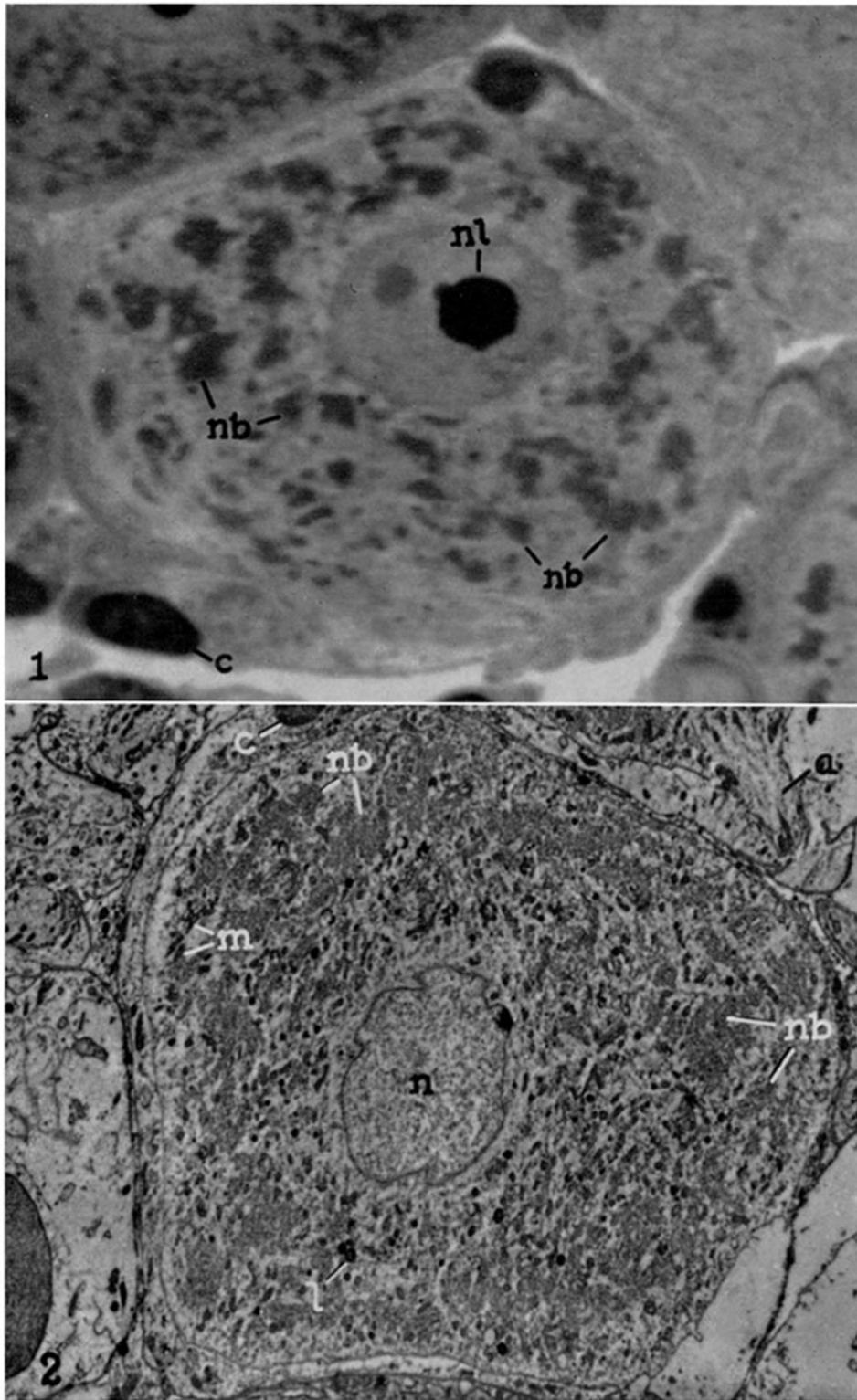


PLATE 18

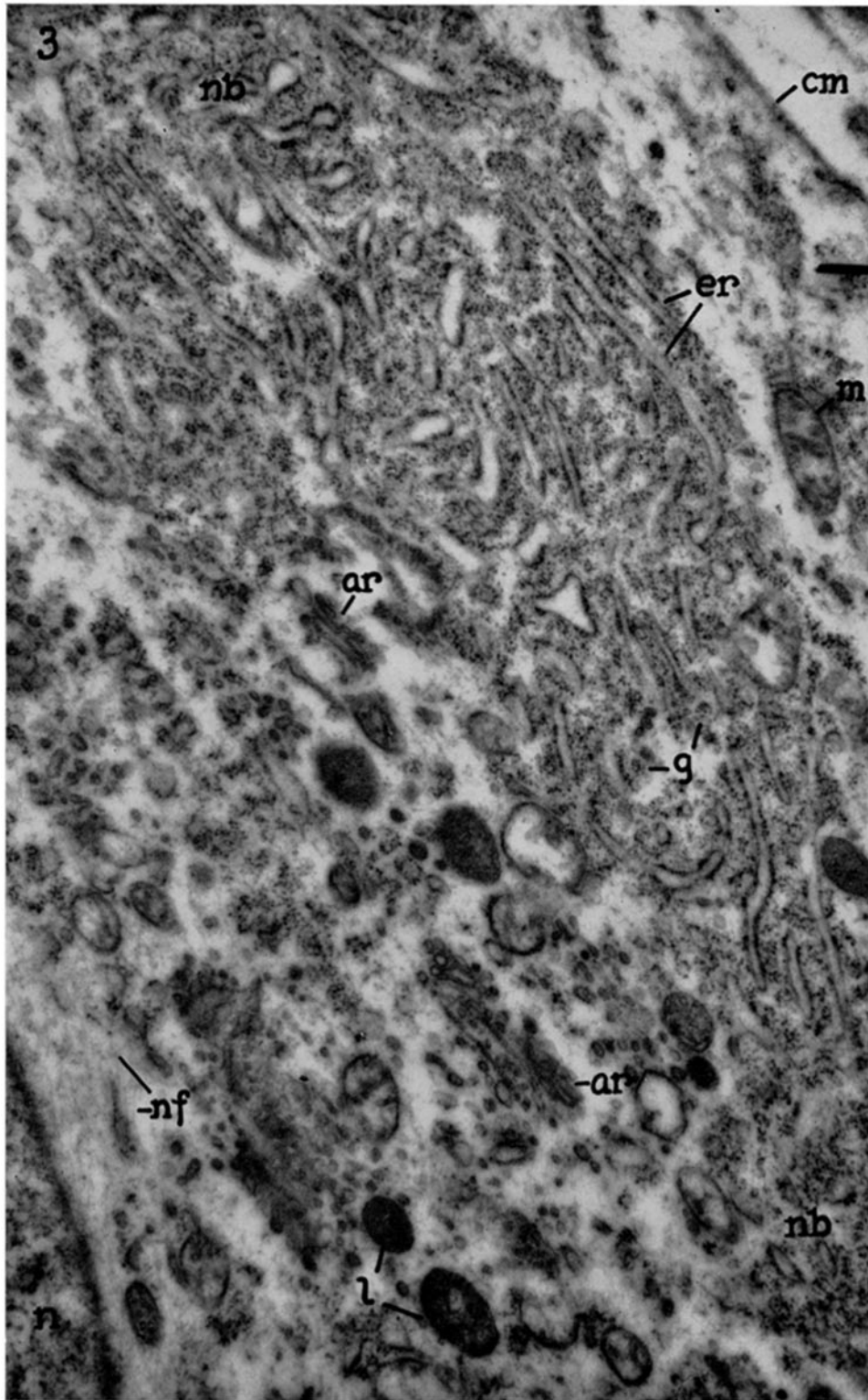
FIG. 3. Electron micrograph of a relatively large field in the cytoplasm of a perikaryon from the superior cervical ganglion. A small part of the nucleus appears at *n*, and the cell membrane at *cm*.

A large Nissl body (*nb*) occupies a diagonal field that stretches from the upper left, to the lower right corner. The electron micrograph shows that the Nissl substance is a composite material constructed of a framework of endoplasmic reticulum (*er*) among the meshes of which lie clusters of fine, punctate granules (*g*). Smaller masses of similar material can be seen between the Nissl body and the nucleus.

Mitochondria (*m*), and dense bodies (*l*), presumably lipid inclusions, are scattered throughout the cytoplasm.

Several masses of agranular reticulum (*ar*) can also be identified.

Near the nucleus appears a collection of fine, long filaments (*nf*) identified as neurofilaments. $\times 34,200$.



(Palay and Palade: Fine structure of neurons)

PLATE 19

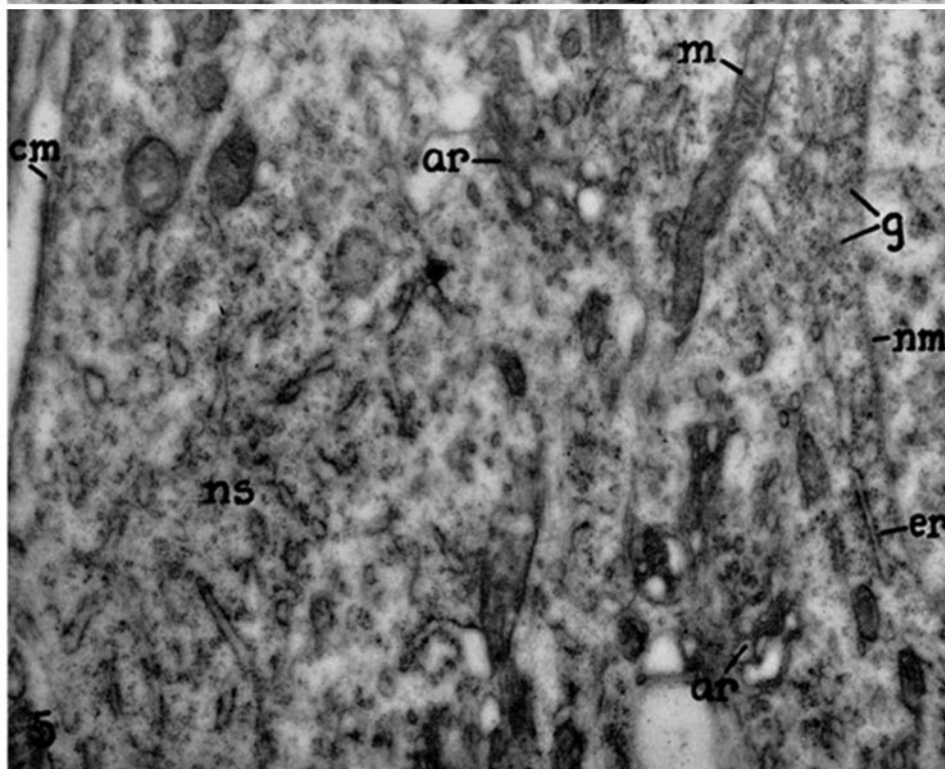
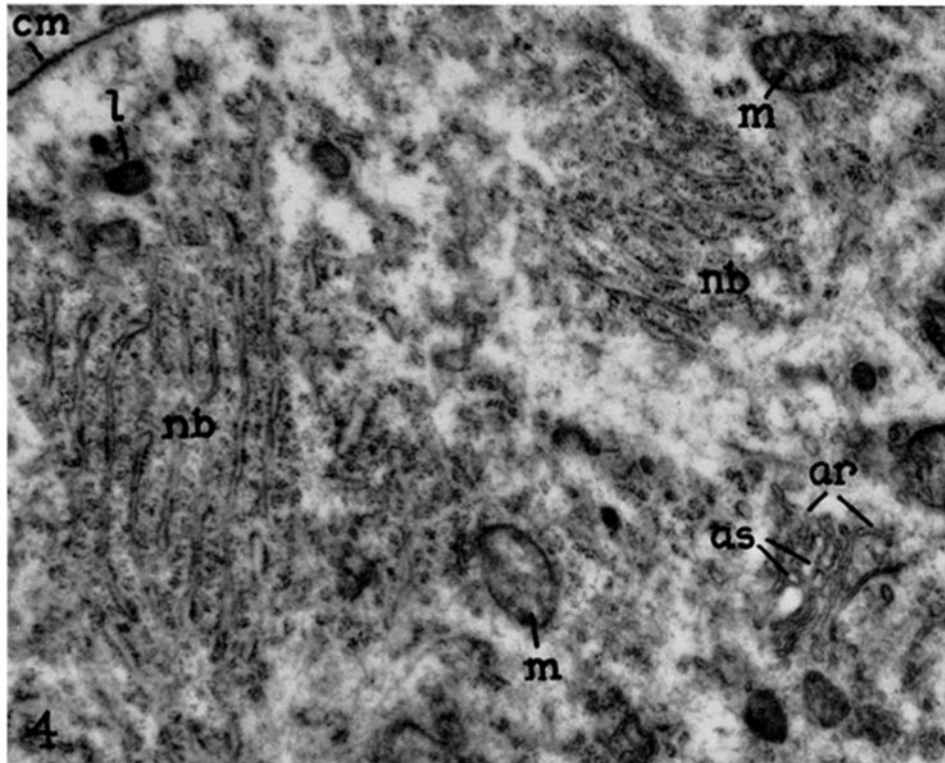
FIG. 4. The electron micrograph shows part of the cytoplasm of a dorsal root ganglion cell.

The cell membrane appears at *cm*, mitochondrial profiles at *m*, lipid inclusions at *l*, and Nissl bodies at *nb*. Note the parallel arrangement of the elongated profiles in each Nissl body and the numerous granules scattered around and among them.

A small mass of agranular reticulum can be seen at *ar*. Its elongated profiles are, in general, parallel to each other. In addition, they appear connected by a number of transverse anastomoses (*as*). $\times 30,200$.

FIG. 5. Electron micrograph of a section through a Purkinje cell in the cerebellar cortex.

The nuclear membrane (*nm*) is visible along the right margin of the picture, while the cell membrane (*cm*) lies along the left margin. Nissl substance (*ns*) is dispersed throughout the cytoplasm with relatively small interspaces occupied by mitochondria (*m*) and agranular reticulum (*ar*). The continuity between Nissl bodies is more obvious in Purkinje cells than in other large neurons, and the arrangement of the endoplasmic reticulum within the Nissl substance is much less orderly. Notice the concentration of endoplasmic reticulum (*er*) and fine granules (*g*) close to the nuclear membrane, equivalent to the characteristic nuclear cap, or perinuclear basophil cytoplasm of Purkinje cells. The agranular reticulum (*ar*) in this cell has an unusually large number of dilated segments. $\times 25,500$.



(Palay and Palade: Fine structure of neurons)

PLATE 20

FIG. 6. The electron micrograph shows the edge of a Nissl body in a perikaryon of the superior cervical ganglion.

The endoplasmic reticulum appears as a relatively tightly packed collection of circular (*c*), oval (*o*), and elongated (*e*) profiles clearly arranged in rows in only a few places. This appearance indicates an intermediate state between highly oriented (as in Fig. 8) and randomly oriented (as in Fig. 5) endoplasmic reticulum within the Nissl bodies. Fine granules are arranged in rows (*g₁*) upon the membranes and in clusters (*g₂*) between membranes. A filamentous mitochondrion (*m*) lies along the margin of the Nissl body. $\times 38,600$.

FIG. 7. Electron micrograph of a section through the cytoplasm of a dorsal root ganglion cell.

Small Nissl bodies (*nb*) are separated from one another by zones of cytoplasm containing mitochondria and fine, long filaments (*nf*) which in some places appear to have a preferred orientation. The filaments represent the neurofilaments of the perikaryon.

Some of the Nissl bodies are interconnected by elongated profiles (*e*) sometimes disposed in a recognizable reticulum (*r*). Most of these profiles are free of granules and some of them are continuous with tightly packed elements (*ar*) belonging apparently to the agranular reticulum. As such, they may represent transition forms between the agranular reticulum and the endoplasmic reticulum usually found in Nissl bodies. $\times 39,200$.

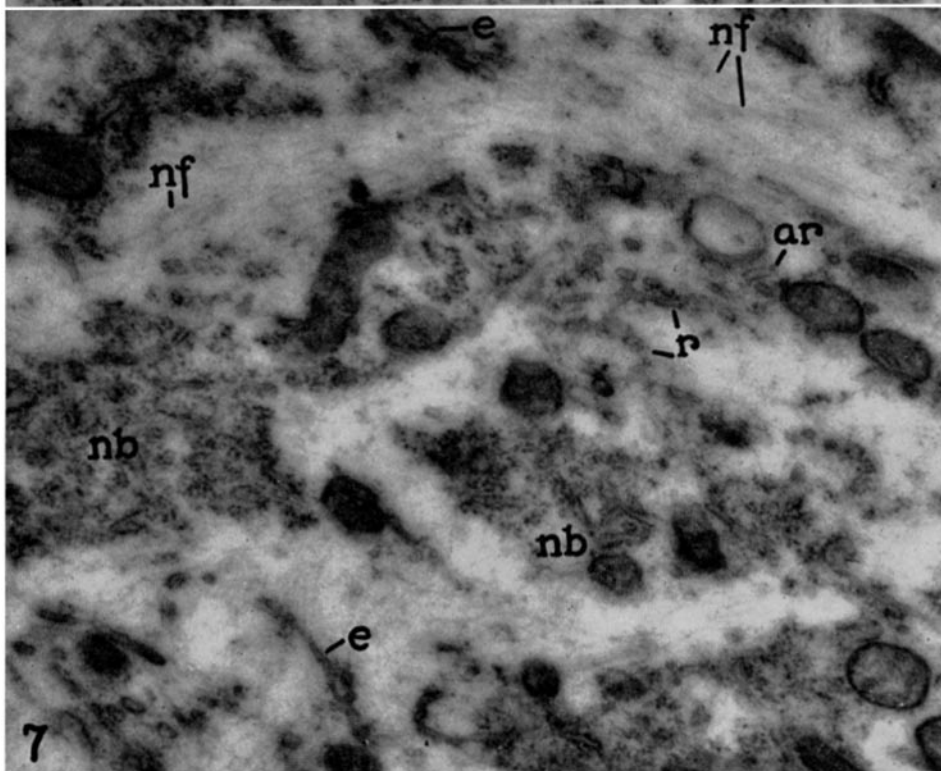
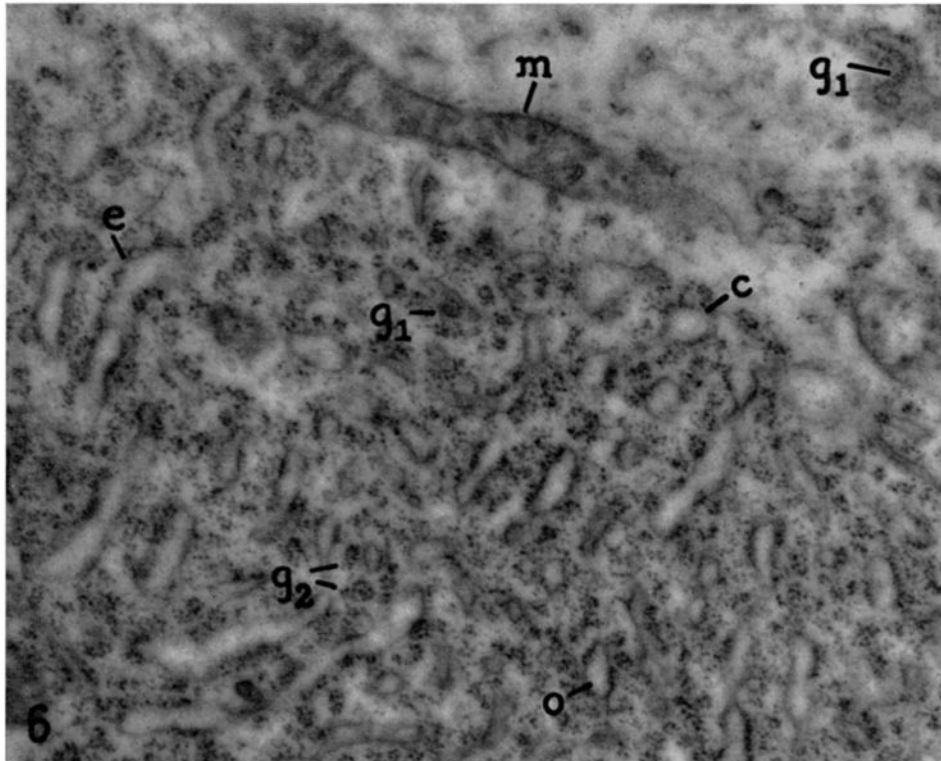
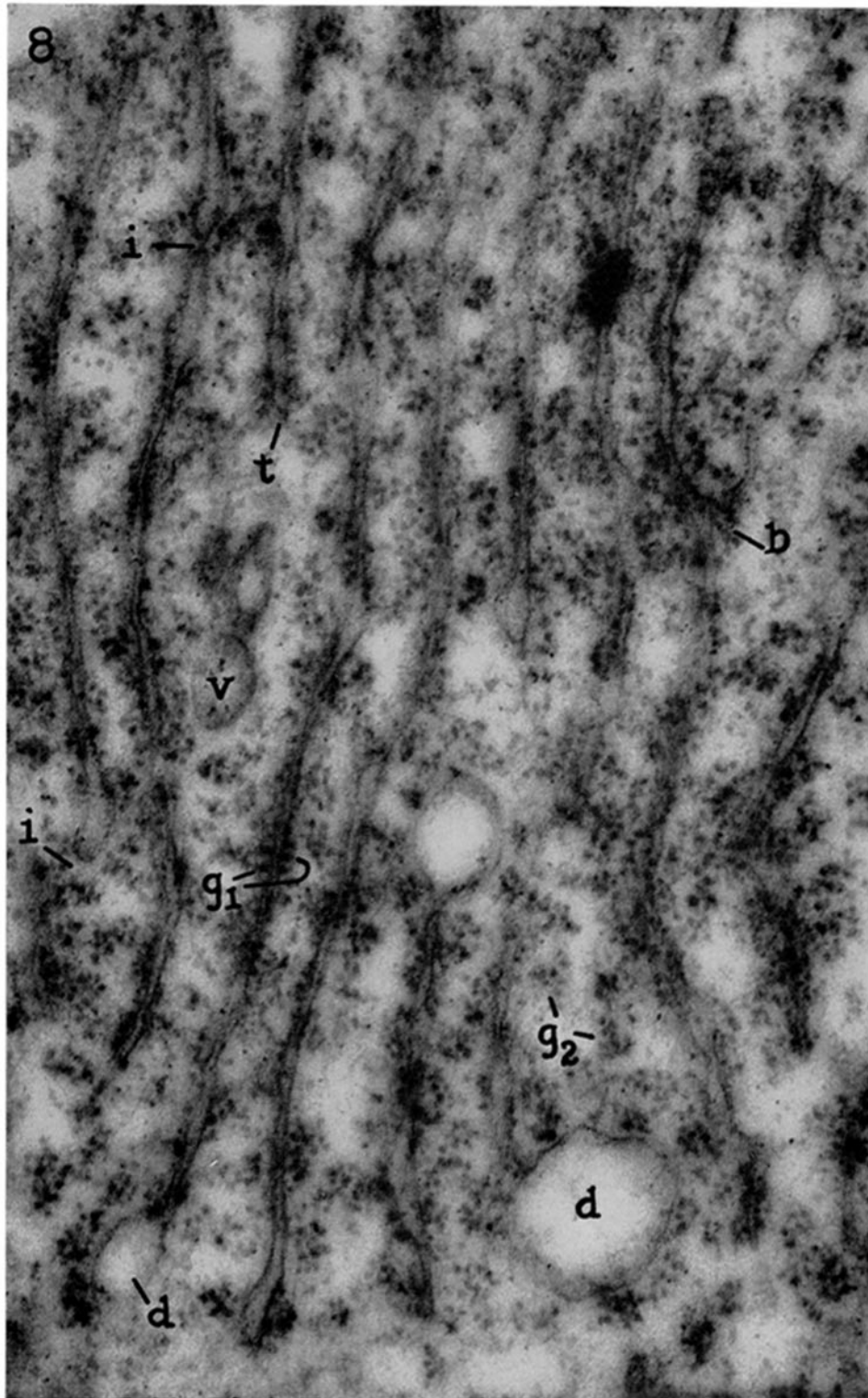


PLATE 21

FIG. 8. Electron micrograph of a section through a Nissl body of a motor neuron in the abducens nucleus (medulla oblongata).

Elongated profiles (some of them longer than 2μ) predominate in the endoplasmic reticulum of this Nissl body. They are disposed in rows that show few and narrow interruptions (*i*). In general the rows are arranged parallel to one another but their parallelism is disturbed in some places by the ending of some rows (*l*) and by branchings (*b*). The prevalence of elongated profiles indicates that cisternal elements predominate in the reticular sheets of this highly oriented type of endoplasmic reticulum. The interruptions mentioned may correspond to fenestrae or to branchings of these cisternae into smaller units. Most of the elongated profiles in the picture end in relatively large dilatations (*d*). A few apparently isolated vesicles (*v*) are found in between the other profiles.

The thin membrane limiting the various profiles is clearly visible; its outside surface is covered in some places by small, dense granules which occasionally appear disposed at regular intervals in short rows (g_1). Numerous other granules of similar appearance are scattered in the cytoplasmic matrix in between the profiles (g_2).
 $\times 84,200$.



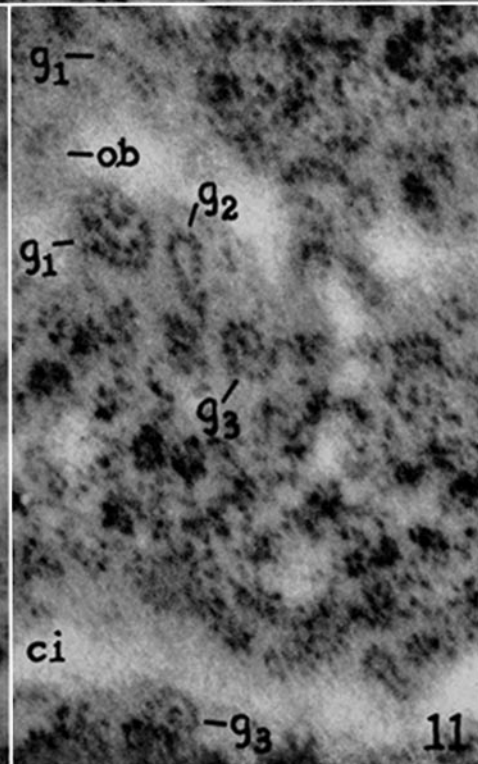
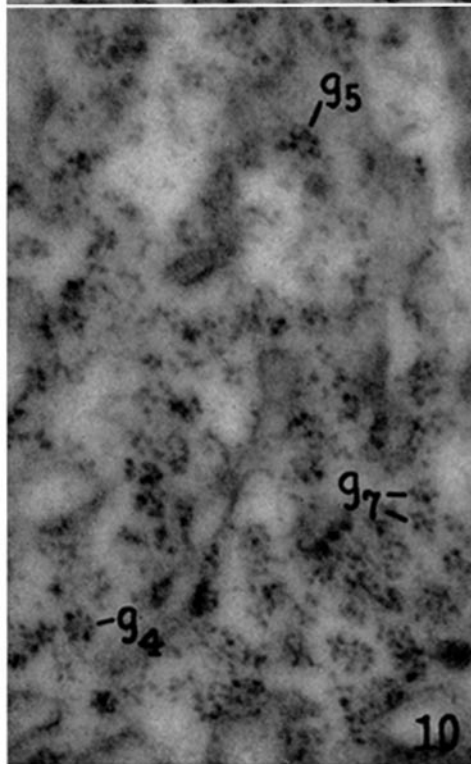
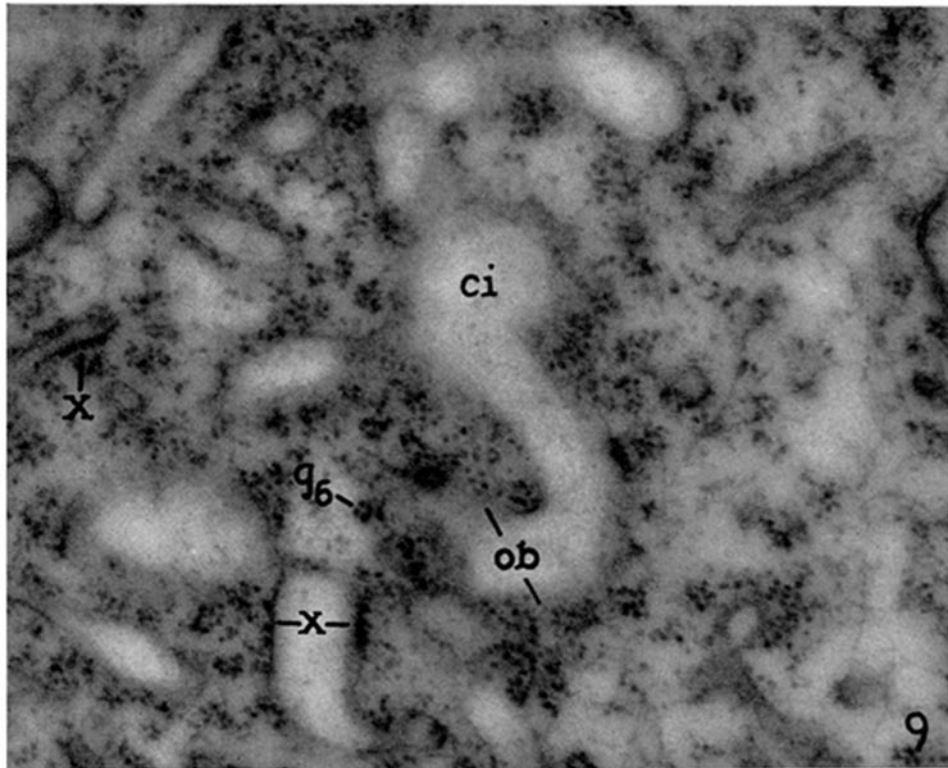
(Palay and Palade: Fine structure of neurons)

PLATE 22

FIGS. 9 to 11. These electron micrographs show limited fields in the Nissl bodies of sympathetic ganglion cells (superior cervical ganglion). They illustrate the relationship between the endoplasmic reticulum and the small granules.

The elements of the endoplasmic reticulum, mostly large cisternae (*ci*), have been sectioned perpendicularly in some places where they show a thin limiting membrane with granules "attached" on its outside surface (*x*). In other places, the section passes obliquely through the cisternal elements of the reticulum as indicated by their wide lumina and especially by the appearance of their limiting membrane, which shows as a relatively wide, poorly outlined band of low density (*ob*). Such bands offer an almost full-faced view of the membrane, and demonstrate to advantage the patterns formed on its surface by the small granules. The latter are frequently disposed at regular intervals in linear series, which in turn form parallel, double rows (*g*₁), loops (*g*₂), and spirals (*g*₃).

In addition to the granules "attached" to the membrane of the endoplasmic reticulum, numerous granules of similar size and density are scattered, sometimes in profusion (Fig. 11), in the intervening matrix where they frequently appear to be disposed in rosettes (*g*₄), circles (*g*₅), tetrads (*g*₆), or apparently irregular clusters (*g*₇). Fig. 9, $\times 66,300$. Fig. 10, $\times 72,100$. Fig. 11, $\times 85,700$.



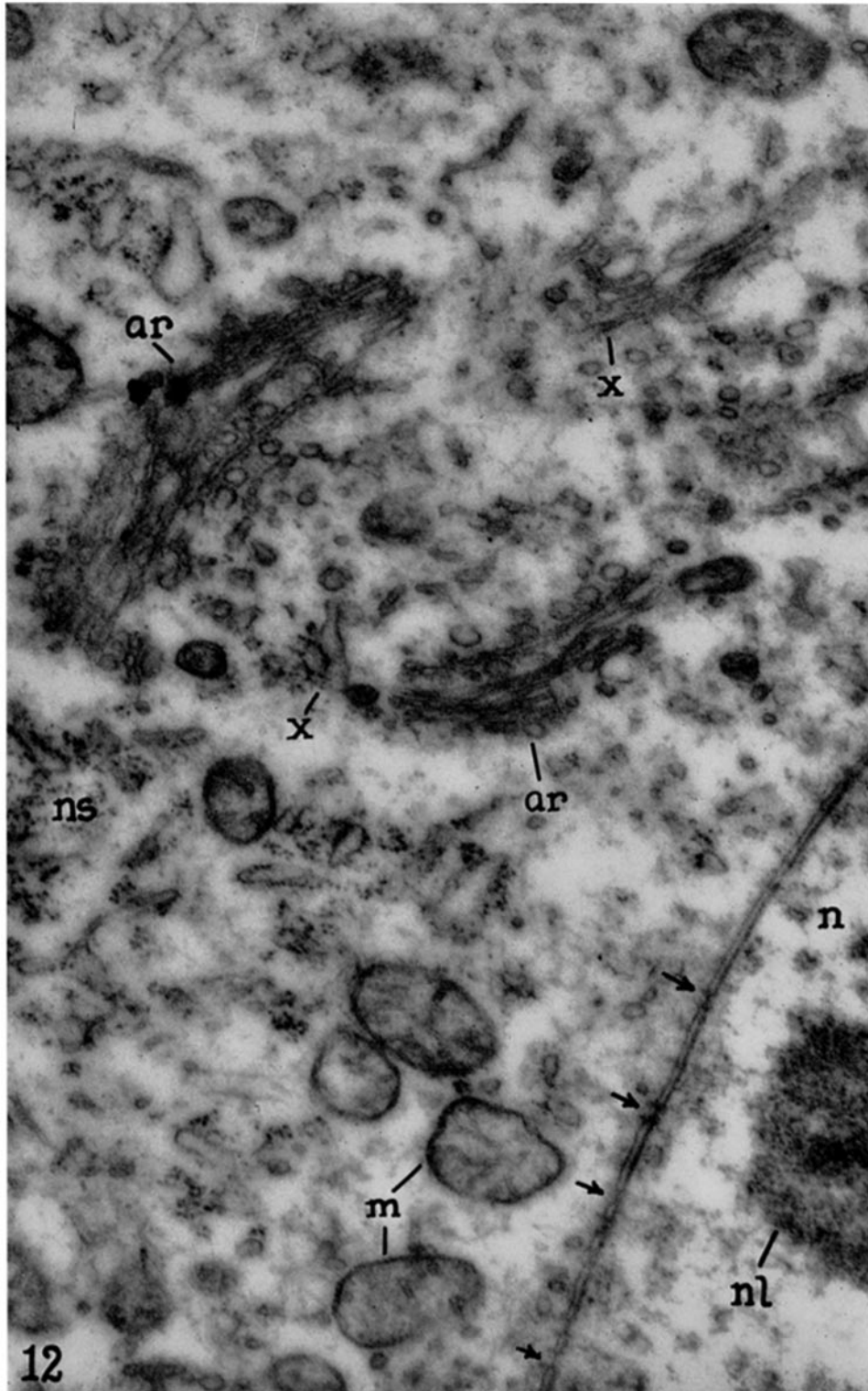
(Palay and Palade: Fine structure of neurons)

PLATE 23

FIG. 12. Electron micrograph of a section through a perikaryon of a dorsal root ganglion.

A portion of the nucleus (*n*) appears in the lower right corner. The nucleolus (*nl*) consists of finely granular particles arranged in closely packed rows. The nucleoplasm is separated from the cytoplasm by a double membrane which is fenestrated at several points (arrows). Near the nucleus lies a large mass of agranular reticulum (*ar*) composed of tightly packed, predominantly elongated profiles. Note in the upper left bundle the alternation of elongated profiles with rows of circular profiles.

At the points indicated by *x*, elements of the endoplasmic reticulum studded with granules appear to merge with profiles of the agranular reticulum. Numerous mitochondria (*m*) and small masses of Nissl substance (*ns*) are distributed through the remaining cytoplasm. $\times 49,000$.



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PLATE 24

FIG. 13. Electron micrograph of a section through a mass of agranular reticulum in the cytoplasm of a perikaryon from a dorsal root ganglion.

The mass, disposed in a roughly elliptical outline, is formed mainly of elongated profiles packed parallel to one another at relatively small intervals. Such profiles correspond to shallow cisternae which only occasionally show dilatations (*d*) of their lumina. In addition to elongated profiles, the mass contains rows of circular and oval profiles (*r*) which correspond to simple, reticular sheets. The formation marked *rs* is probably such a reticular sheet which appears in full-faced view because its plane coincides with the plane of the section.

Note that swarms of small, circular profiles (*s*) surround the ends of the bundles of elongated profiles and that similar circular profiles are scattered in the part of the cytoplasm outlined by the mass of agranular reticulum.

At *v* there appears a particular vesicular formation limited by a single membrane and filled with small, circular profiles which may represent membrane invaginations. A similar formation can be seen at *v'*.

Mitochondria are marked *m*, and a small Nissl body *nb*. $\times 39,200$.

PLATE 25

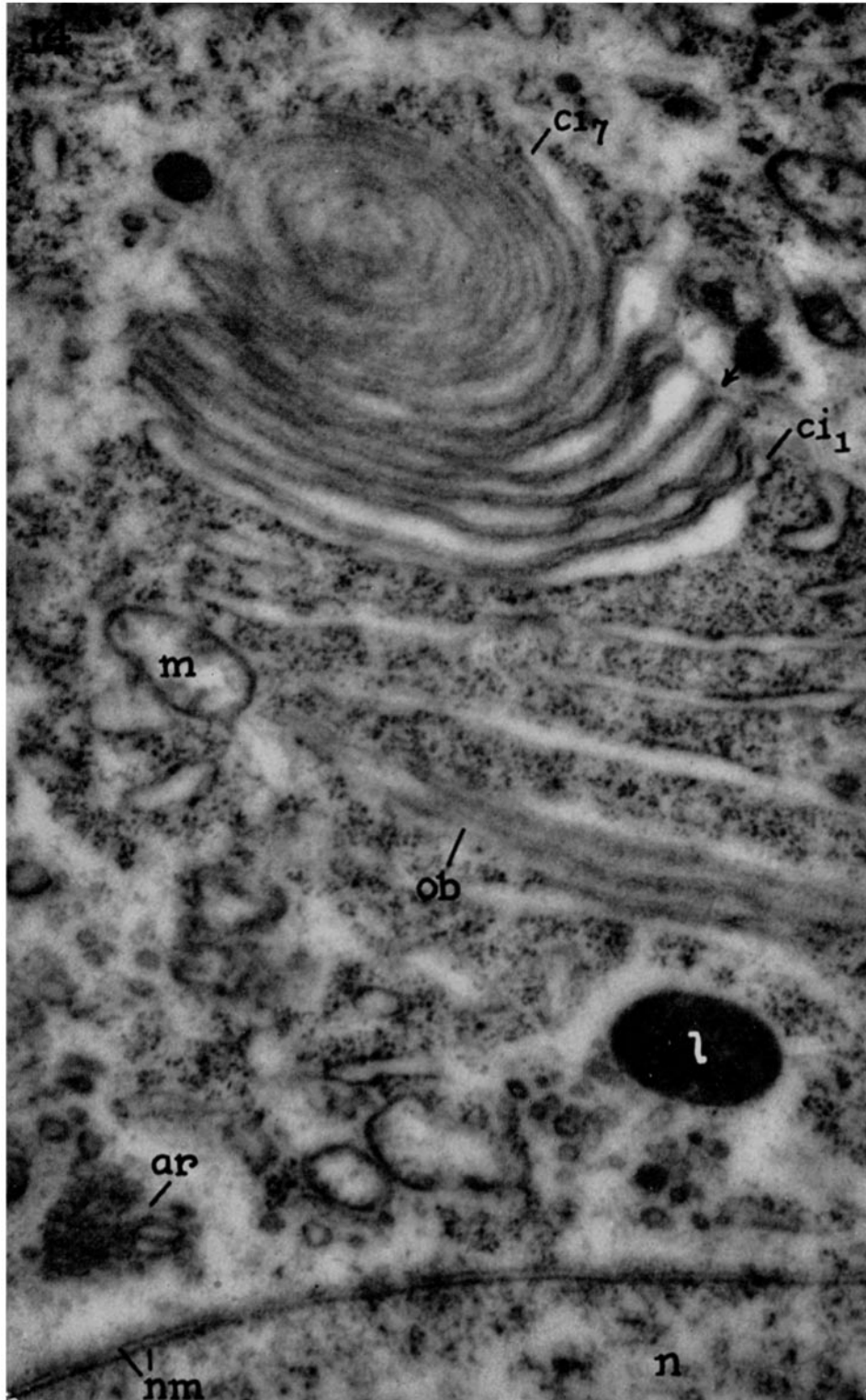
FIG. 14. Electron micrograph of a section through a whorl-like structure in the cytoplasm of a sympathetic perikaryon (superior cervical ganglion).

This structure, which is embedded in Nissl substance of usual composition, consists of concentrically arranged, tightly packed, flattened vesicles or cisternae. The most peripheral cisterna (ci_1) is perpendicularly sectioned as indicated by the thinness and high density of its limiting membrane. Its lumen, like the lumen of the six following cisternae, is dilated and filled with a light, homogeneous material. The following cisternae are cut at progressively lower angles. Because of the obliqueness of the section and because of the small spacing of the cisternae, their limiting membranes appear partly superimposed in the electron micrograph which shows in orthogonal projection all structural elements contained in the thickness of the section. This partial superimposition explains the dense, central strips within the lighter bands that separate adjacent cisternae; an appearance that shows to advantage between the cisternae 3 and 4 (arrow) and 6 and 7.

Note that the limiting membranes of the cisternae in the whorl-like structure are generally free of small granules; such granules encrust only the peripheral side of the cisterna marked ci_1 and the right end of the cisterna ci_7 .

A few obliquely sectioned cisternae without attached granules appear at ob , interrupting the continuity of a Nissl body. Although differently arranged, these cisternae are similar to the ones in the whorled structure described.

The electron micrograph shows also dense inclusions (l), presumably lipid in nature, a bundle of profiles belonging to the agranular reticulum (ar), mitochondrial profiles (m), and part of the nucleus (n), limited by a double membrane (nm). $\times 51,500$.



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PLATE 26

FIG. 15. The electron micrograph shows a whorl-like structure in a perikaryon of a superior cervical ganglion.

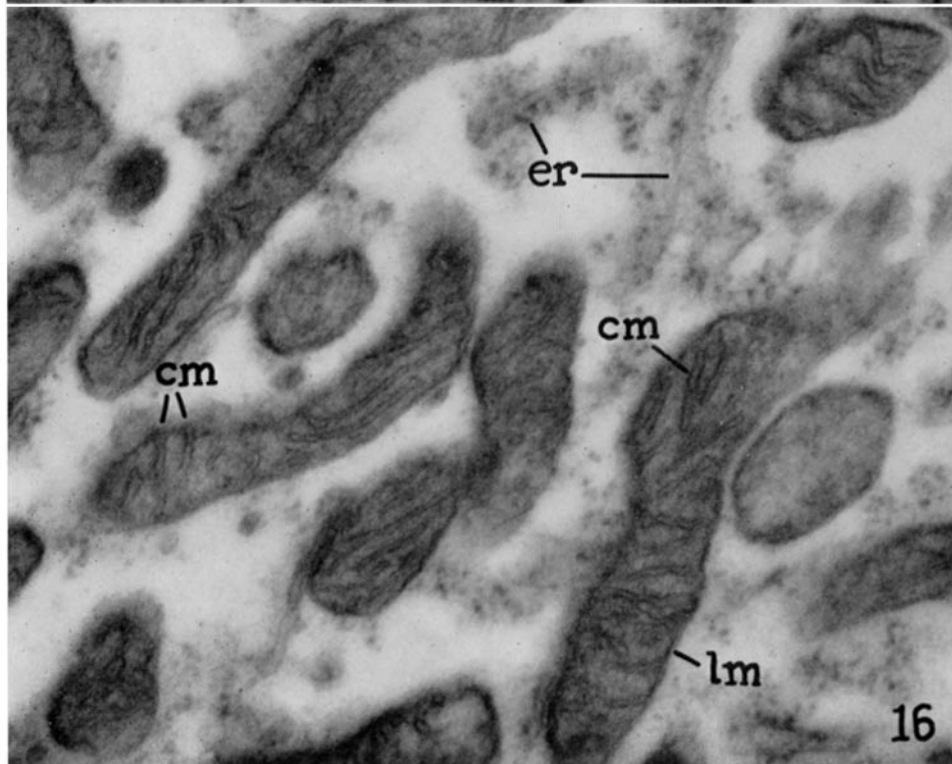
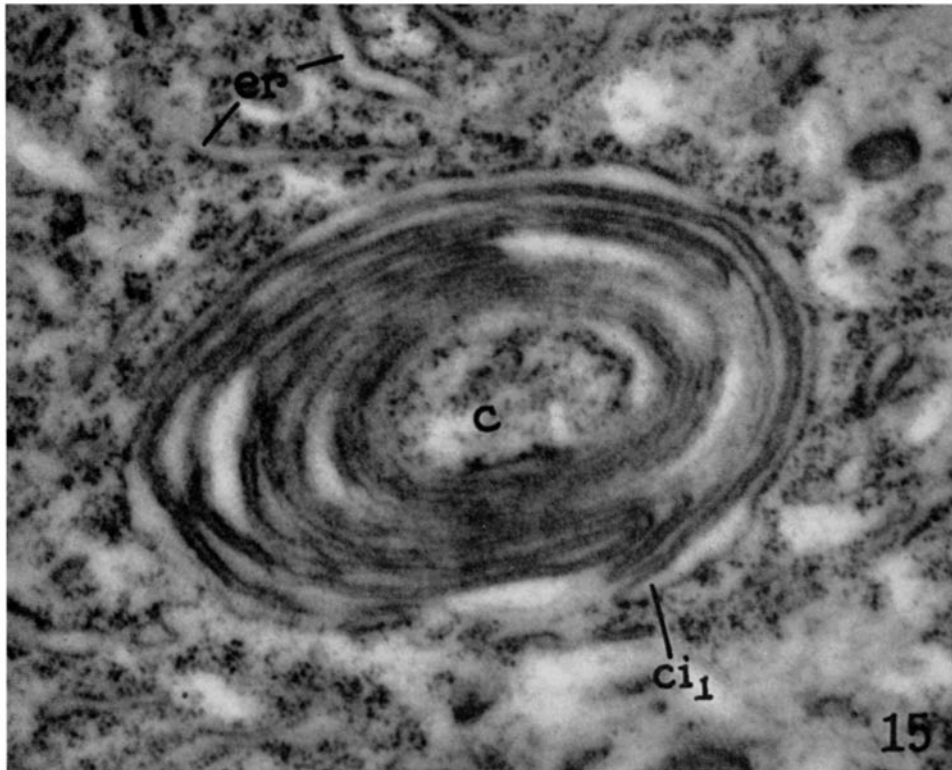
As in Fig. 14, the structure appears embedded in a Nissl body and consists of a large number of cisternae disposed concentrically and separated from one another by narrow bands of relatively dense cytoplasm. The limiting membrane of each cisterna is free of granules wherever apposed to the membrane of another cisternal element, but appears covered with dense, small granules when in contact with Nissl substance, as in the case of cisterna *ci*₁. In this respect, the profile of this cisterna partly resembles the profiles of the endoplasmic reticulum (*er*) usually found in Nissl bodies.

Small, dense granules are also present in the core (*c*) of this whorl-like structure. $\times 50,400$.

FIG. 16. Electron micrograph of a small field in a perikaryon of the myenteric plexus (Auerbach), in the wall of the small intestine.

The numerous mitochondrial profiles found in this field show the characteristic structural features of mitochondria; namely, an outer, limiting membrane (*lm*) and numerous cristae mitochondriales (*cm*) which may be considered as folds of an inner membrane. Note that in some mitochondria the cristae are disposed parallel to the long axis of the organelles.

A few profiles of the endoplasmic reticulum (*er*) with associated small granules are present among the mitochondria. $\times 74,500$.



(Palay and Palade: Fine structure of neurons)