STUDIES ON THE FINE STRUCTURE OF ULTRACENTRIFUGED SPINAL GANGLION CELLS

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ABSTRACT

The following structures were observed in electron micrographs of the mouse spinal ganglion cells: Nissl bodies composed of both aggregated rough-type, largely oriented, membranes of the endoplasmic reticulum and discrete particles; short rodlike mitochondria with well-developed transverse, obliquely or longitudinally arranged cristae, and a relatively typical Golgi complex. The components of ultracentrifuged ganglion cells (400,000 times gravity for 20 minutes) are stratified, the layers appearing in the order of their decreasing density as follows: (1) A microsomal or ergastoplasmic layer which may be further divided into three sublayers without sharp boundaries, namely, a discrete particle layer, a layer of discrete particles and highly distorted membranes of the endoplasmic reticulum, and a layer composed of relatively intact, but stretched membranes of the endoplasmic reticulum and discrete particles. (2) Mitochondria constitute a relatively broad layer. They are sometimes stretched; however, they retain most of their fine structure. The stratified nucleus is found within the mitochondrial layer. (3) A relatively wide layer of tightly packed vesicles. (4) At the centripetal end, resting against the cell membrane, are a few lipid vacuoles. A comparison is made between the ultrastructure of the stratified layers *in situ* and those described by others in differentially ultracentrifuged homogenates.

Experiments utilizing sufficiently high centrifugal force to cause displacement and stratification of the microscopically visible contents of cells (eggs) were first made by Gurwitsch (21). This study and those which followed (*cf.* 39) demonstrated that the displaced components and inclusions were stratified in the order of their relative densities, a condition which did not markedly injure the egg or interfere with its normal development. The experiments of Harvey (26) further demonstrated that stratified *Arbacia* eggs may be pulled into nearly equal halves and the halves into quarters, each of which when fertilized develop into normal plutei, indicating that none of the formed elements is essential to early development in this form (27). In addition, the centrifuge has been used in the study of "specific organ forming substance," "surface active substance," polarity and bilaterality, viscosity, and the distribution of enzymes (*cf.* 3, 27, 29, 32). The experiments on the distribution of enzymes in cells, utilizing both centrifugation and histochemistry, were not sufficiently specific, except in a relatively few instances, to link given enzymes or their precursors to specific morphological components. Although these studies were limited to cells of relatively

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large size and low viscosity, such as amoeba and certain invertebrate eggs, nevertheless they helped to prepare the way for modern fractionation studies (*cf.* 32).

In the meantime, the ultracentrifuge which generates forces thousands of times gravity, was developed and adapted for biological work (56). It was widely used in the determination of molecular weights, particularly of artificial and native colloids (56), and it was surprising to find that *Ascaris* eggs and certain tissue cells could withstand a centrifugal force of nearly a million times gravity without killing them or presumably disrupting the molecular organization of the cytoplasm (5). Finally, it was Bensley and Hoerr (9), Claude (13, 14), Hogeboom, Schneider, and Palade (31), and others who found that it was possible under certain conditions to homogenize and ultracentrifuge cell fractions into layers suitable for chemical analysis. At about the same time, the development of the electron microscope and the methods for fixing (41) and thin sectioning of tissue (37, 40) made it possible to identify ultramicroscopic particles both within the cell (*cf.* 42–44, 48, 53) and in ultracentrifuged fractions of cells (*cf.* 43, 44, 47, 51, 52), a condition which allowed for the coupling of certain enzyme systems with specific morphological structure (43, 49, 51, 52). Although electron microscope examinations of cell fractions are now routine, few studies have been made with the electron microscope on tissue cells that have been ultracentrifuged, and Schneider (49) has recently pointed out that one of the possible functions of such a study would be to serve as a kind of control for electron microscope studies of cell fractions. Therefore, it seemed desirable to investigate this problem and pay especial attention to the relative densities of the stratified layers, to effects, if any, on the fine structure of the cytoplasmic components, and to compare the submicroscopic morphology of the stratified layers *in situ* with those obtained by others after cell homogenization and fractionation.

MATERIAL AND METHODS

Spinal ganglia from anesthetized young adult mice were dissected and fixed immediately in buffered (pH 7.3) osmium tetroxide. These are referred to as "normal" cells. Other ganglia were placed in a dish of Ringer's solution; some were left as controls while others were transferred in Ringer's solution to the rotor of an air-turbine ultracentrifuge (7) and spun at speeds generating a centrifugal force of approximately 400,000 times gravity for a period of 20 minutes. Both control and centrifuged ganglia were then fixed by Delaney's (18) method for study of the Nissl bodies, Regaud's method for study of the mitochondria, and Palade's (41) buffered (pH 7.3) osmium tetroxide method for study of all the above named structures by the electron microscope (RCA EMU 3D and Siemens Elmiskop I).

DESCRIPTION

Light Microscope Observations

The cells of the spinal ganglion vary considerably in size (20 to 60 μ) and are, for the most part, irregularly spheroidal in form. Their cytology as revealed by the light microscope is well known (*cf.* 2, 4); hence, we have not included figures of the control spinal ganglion cell nor extensive reference to the massive literature on this subject.

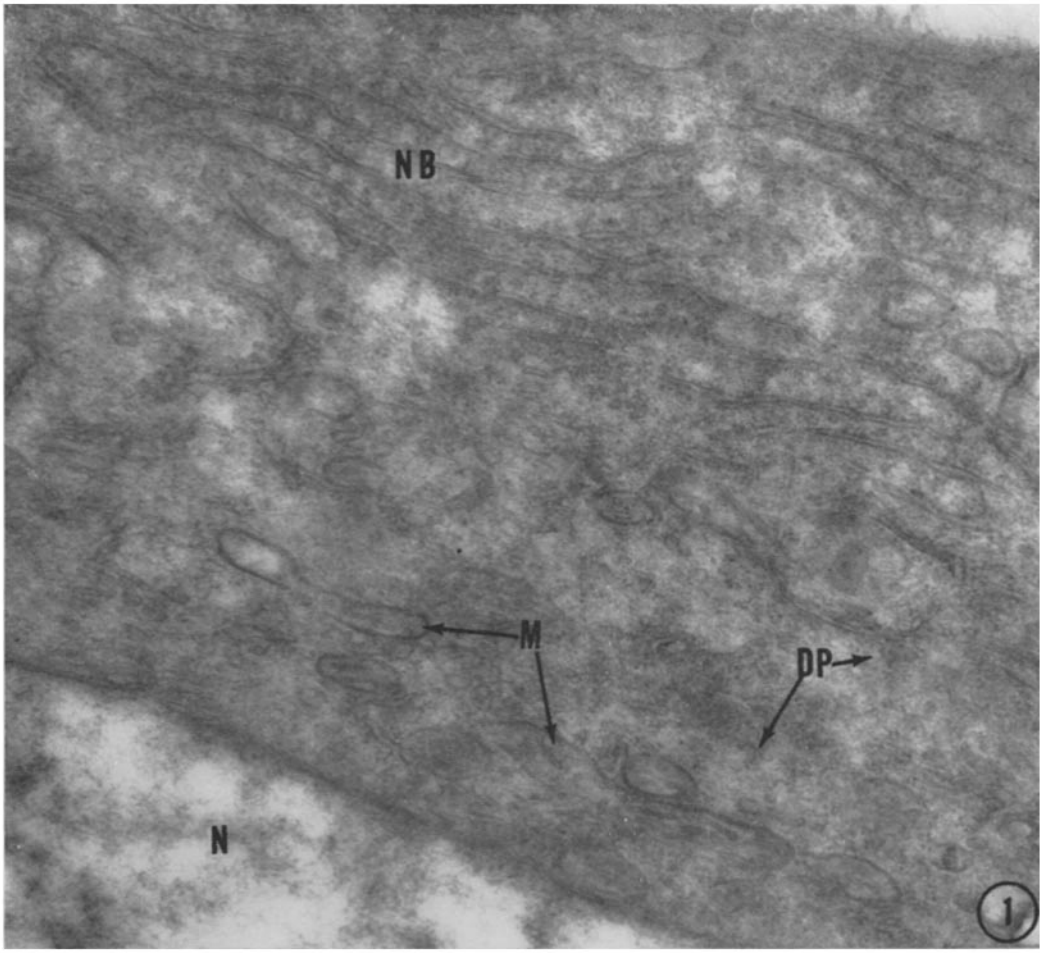
Notwithstanding the fact that spinal ganglion cells are relatively viscid (19), their cytoplasmic components and inclusions may be displaced and stratified in the ultracentrifuge. Beams and King (4) found that the basophilic-staining Nissl bodies were displaced to the centrifugal end of the cell (Fig. 5, NS); the nucleus was likewise stratified from the centrifugal to the centripetal end as follows: nucleolus, chromatin and nuclear sap (enchylema) (Fig. 5). Some of the nuclear sap

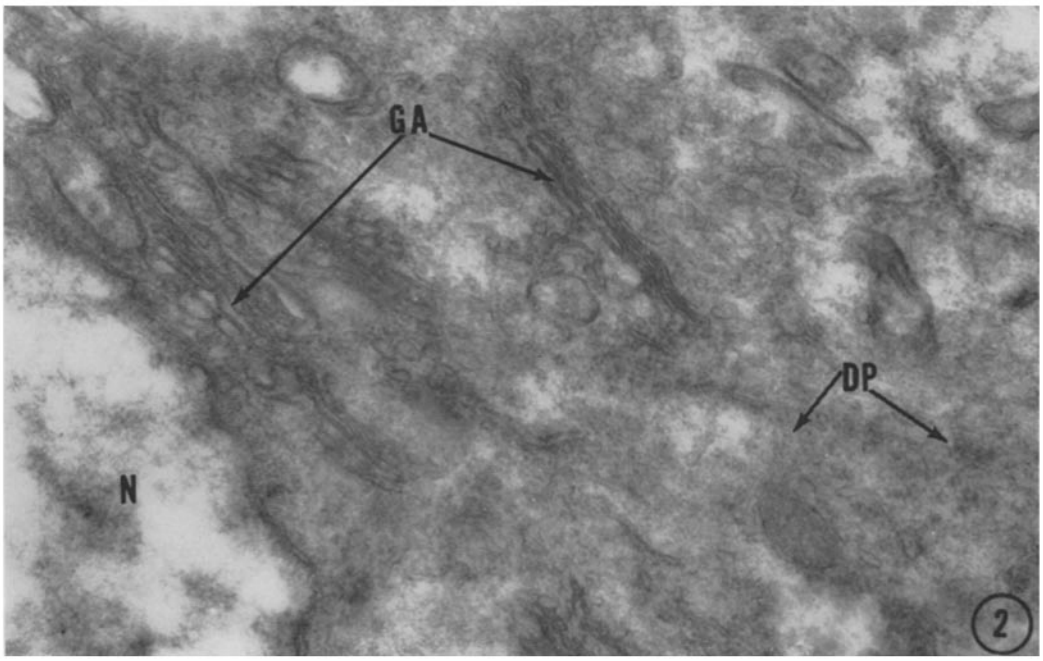
FIGURE 1

Nissl body (*NB*) of "normal" neuron showing both oriented, rough-type membranes of the endoplasmic reticulum and discrete particles (*DP*). Mitochondria appear at *M* and the nucleus at *N*. (× 53,000)

FIGURE 2

Section through Golgi complex *GA* of "normal" cell. Note discrete particles (*DP*). The nucleus is shown at *N*. (× 53,000)


NB
M
DP
N
1


GA
DP
N
2

probably extends throughout the nucleus, but it is more concentrated at the centripetal end because other materials have largely displaced it from the other regions.

The degree of stratification and compactness of the layers within the ultracentrifuged neuron will vary depending upon the strength of the centrifugal force applied and the duration of its action.

Electron Microscope Studies

"Normal" Neuron: We have found the structure of the spinal ganglion cell to be essentially like that reported by Palay and Palade (45). The Nissl bodies (*i.e.*, aggregates of ergastoplasm) are irregularly shaped and variously distributed within the cytoplasm. They are made up of rough-surfaced membranes of the endoplasmic reticulum plus discrete particles.[1] The particle-studded profiles of the endoplasmic reticulum are rather long and slender and show a considerable degree of preferred orientation in the main mass of the Nissl body (Figs. 1 and 3, NB). At the border of the Nissl bodies and extending into the cytoplasm between them are often observed scattered and unorganized membrane profiles of this structure, as well as a considerable number of discrete particles; hence, most but not all of the ergastoplasmic materials are limited to the Nissl bodies.

The short rodlike mitochondria are relatively numerous and possess well-defined, transversely and longitudinally arranged cristae; they are more or less evenly distributed throughout the cytoplasm between the Nissl bodies (Figs. 1 and 3, M). The Golgi material is readily identifiable by its agranular membranes and associated vesicles (Figs. 2 and 3, GA). The membranes are disposed parallel to one another and are spaced closer together (about 90 to 200 A apart) than are those of the endoplasmic reticulum (about 500 to 700 A apart). We have found no evidence which suggests that the endoplasmic reticulum, mitochondria, or Golgi material are, in any way, morphologically interrelated.

The nucleus (Figs. 1 and 2, N) has a distinctly double membrane, first observed in the nerve cell by Hartmann (25).

The ganglion cells in Fig. 3 are in close proximity to each other and reveal the intervening structure between them. At CM in the lower perikaryon is a single plasma membrane which is in juxtaposition to the membrane of its satellite (SC); this condition is repeated for the upper ganglion cell, and separating the satellites of these cells is a layer of dense, apparently homogeneous material identified as intercellular cement (Fig. 3, IC).

Control Neuron: Neurons allowed to stand in Ringer's solution for 20 minutes before fixing, show relatively little change in their ultrastructure (Fig. 4). In general, the fixation of these cells seems more variable than in the "normal" cells. Some were considered well fixed whereas others showed the cytoplasm to be slightly more vacuolated and the mitochondria somewhat more swollen than in the "normal" cells. There seems little change in the structure of the endoplasmic reticulum from that occurring in the "normal" cell (Fig. 4, NB).

Ultracentrifuged Neuron: As pointed out by Beams and King (4), the neurons of a given ganglion may vary considerably in their degree of stratification, a condition probably due, in part, to their variation in size and viscosity, rather than to an appreciable variation in density of the cellular materials. The cells usually become somewhat elongate in the direction of the centrifugal force,

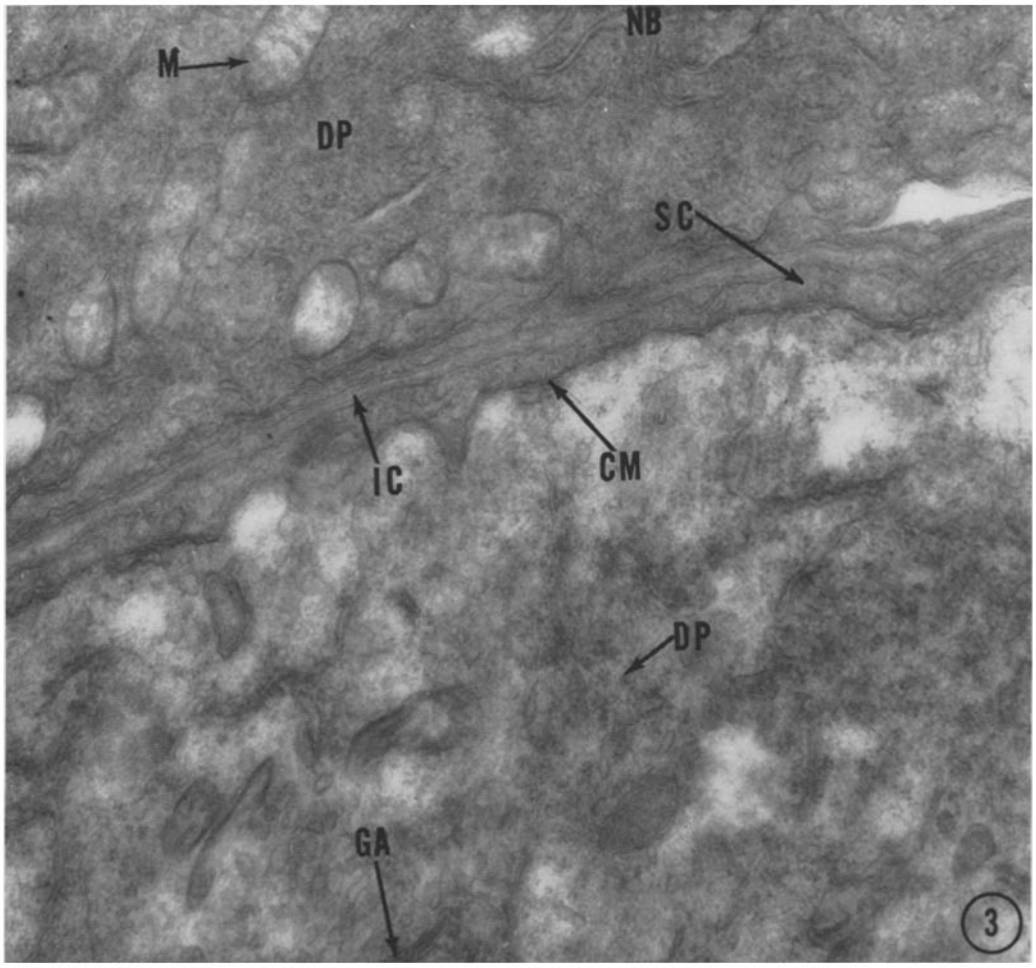
[1] The word particle is used here to designate the submicroscopic granular component of the Nissl or ergastoplasmic substance, *i.e.*, both "attached" and unattached to the membranes of the endoplasmic reticulum. These were first described by Palade (42) and have been referred to variously as microsomes, Palade granules, RNP granules, ribosomes, and particles.

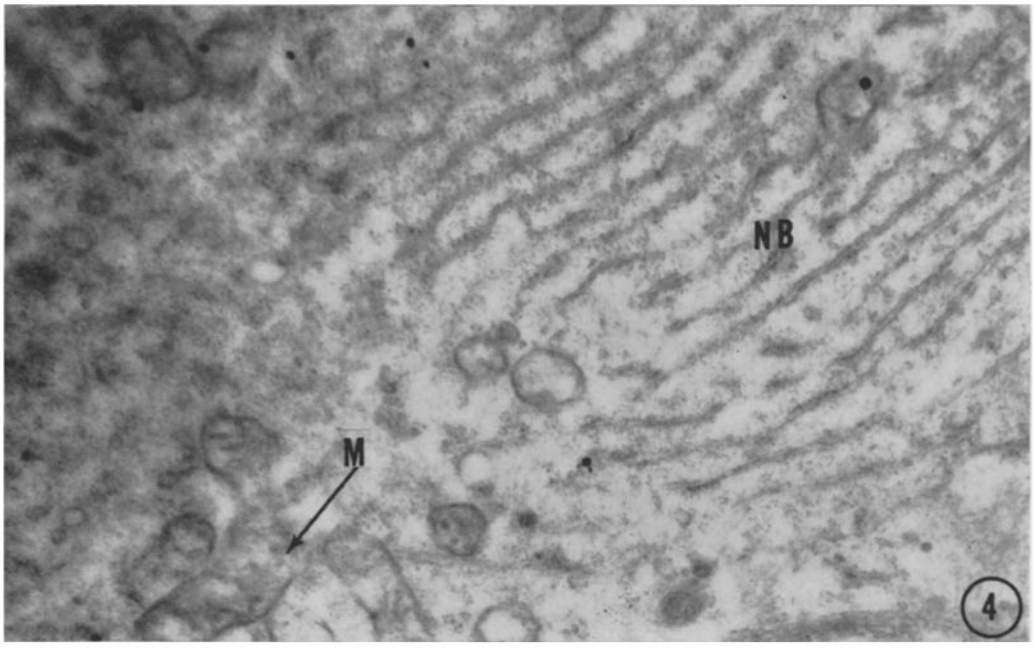
FIGURE 3

Portions of two neurons depicting mitochondria (*M*), discrete particles (*DP*), Golgi complex (*GA*), cell membrane (*CM*), extension of satellite cell (*SC*), and intercellular cement (*IC*). (× 52,000)

FIGURE 4

Control neuron showing portion of Nissl body (*NB*) and mitochondria (*M*). (× 24,000)


NB
M
DP
SC
IC
CM
DP
GA
3


NB
M
4

and when they are sectioned at right angles to their stratification (Fig 6), the following layers may be recognized extending from the centrifugal to the centripetal ends as follows.

1. *Microsomal Layer.* A relatively thick (occupying nearly one-fourth of the cell volume) and tightly packed layer consisting of particle bearing membranes of the endoplasmic reticulum and discrete particles (Fig. 6, ML). This layer contains few circular or oval microsomal vesicles and in this respect differs from the usual microsomal layer of homogenates. However, like the microsomal fraction of pancreatic brei (44) it may be divided further into more or less characteristic sublayers; these are without sharp boundaries and are arranged in order from the centrifugal end as follows. First, there is a densely packed, relatively homogeneous layer of discrete particles (Fig. 6, ML_1, and Fig. 7). This layer appears to be composed mainly of displaced particles normally situated in contact with the membranes of the endoplasmic reticulum and in the cytoplasm between them (Figs. 1, 2, 3, DP). Undoubtedly some of the particles have been dissociated from the membranes by the direct action of the centrifugal force; others have probably been freed by a dissolution of the membranes induced by some unknown secondary effects of the ultracentrifuging. Short segments of aligned particles (arrows in Fig. 7) are sometimes seen, and these probably represent portions of the particle-studded membranes undergoing dissolution. The dense aggregates of particles observed here may also be related to the breaking down of the rough-type membranes of the endoplasmic reticulum (Fig. 7). (Compare this layer with the postmicrosomal fractions of certain homogenates, 44.) Secondly, the sublayer two (Fig. 6, ML_2, and Figs. 8 and 9) is composed of discrete particles like that of Fig. 6, ML_1, and some distorted cisternae of the endoplasmic reticulum (Fig. 8, C). Fig. 9 is also of layer ML_2 (Fig. 6), but of a different cell and at a higher resolution than that of Fig. 8. This preparation shows the particles (150 to 200 A in diameter) to be stretched and flattened, yet still distinctly defined (SP). Observed here also are elements of the endoplasmic reticulum (ER) and small granules (30 to 50 A in diameter), which appear to be somewhat more concentrated on or within the particles than elsewhere (DSP). However, they are probably artifacts. Thirdly, sublayer ML_3 (Figs. 6 and 10) is located just below the mitochondria (Fig. 10, M) and contains numerous, somewhat elongate rough-surfaced membranes of the endoplasmic reticulum (ER), in addition to numerous unattached particles (DP). In some regions of this sublayer, as may also occur in sublayer ML_2, the elements of the endoplasmic reticulum appear to be free of particles; however, this condition may be the result of stretching and partial dissolution of the membranes, rather than of a detachment of the particles by direct action of the centrifugal force. In any case, the evidence indicates that the constituent particles are tightly "attached" to the membranes, since many of them are not detached by the high centrifugal force or by their displacement through the cytoplasm. In general, this sublayer shows less damaging effects of the ultracentrifuging than do sublayers M_1L and ML_2.

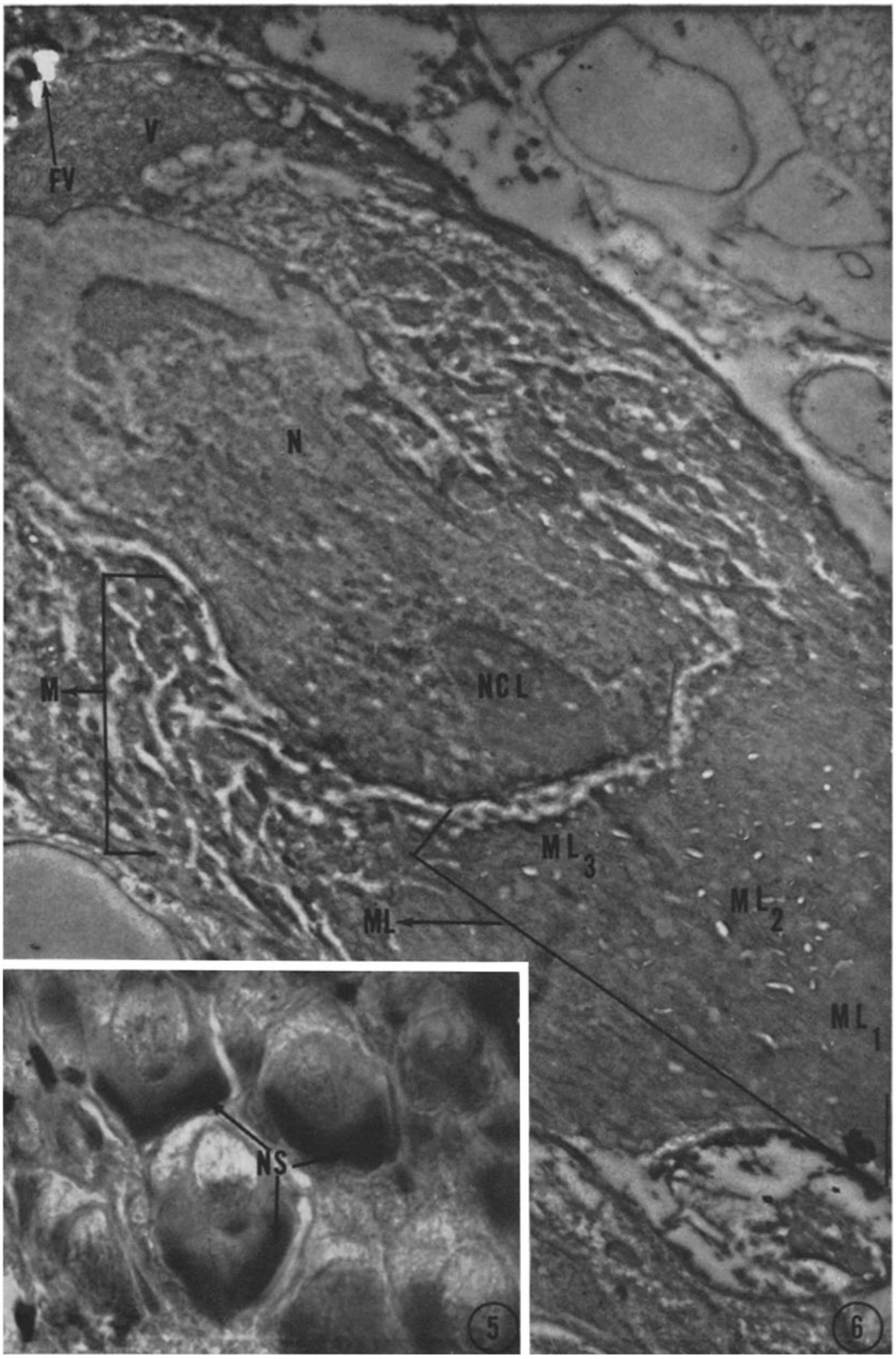
2. *Mitochondrial and Nuclear Layer.* Centripetal to the compact endoplasmic reticular or micro-

FIGURE 5

Ultracentrifuged, fixed, and stained neuron of the rat showing Nissl substance (*NS*) displaced to centrifugal end of cell. Nucleus also shows stratification (after Beams and King, 4). (× 800)

FIGURE 6

Electron micrograph of neuron ultracentrifuged as in Fig. 5. Note following stratified layers: microsomal layer (*ML*); this layer may be further divided into sublayers ML_1, ML_2, and ML_3; mitochondria layer (*M*), including the stratified nucleus (*N*) and displaced nucleolus (*NCL*); vacuolar layer (*V*) and a portion of the lipid layer (*FV*). (× 10,000)


FV
V
N
M
NCL
ML3
ML2
ML
ML1
NS
5
6

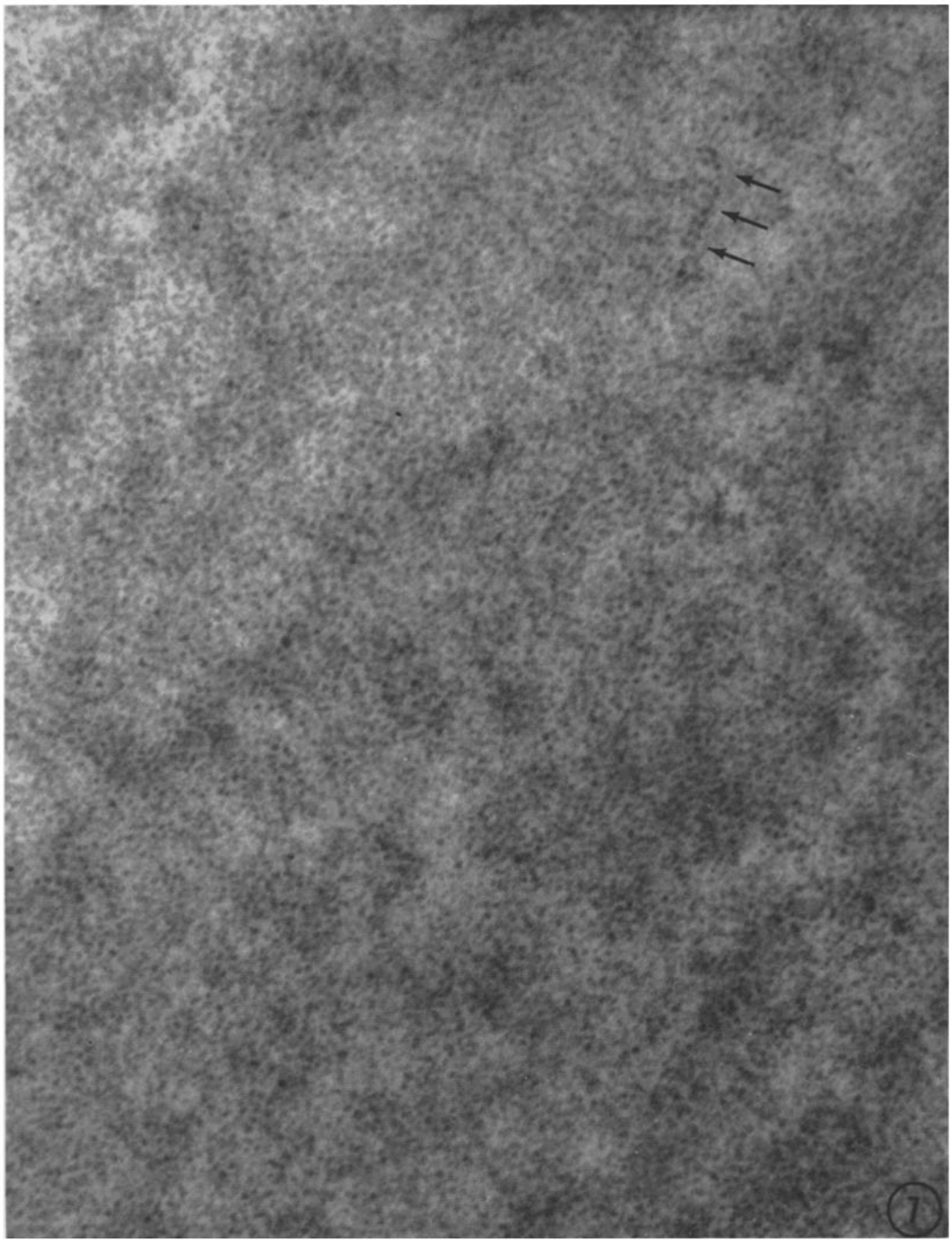


FIGURE 7

Higher magnification of sublayer ML_1 (Fig. 6). Note compact discrete particles and a few segments of aligned granules (arrows). (× 80,000)

somal layer is a relatively broad one containing the nucleus and mitochondria (Fig. 6, N and M). The latter are sometimes stretched with their cristae generally oriented parallel to their long axes, a condition probably enhanced by the stretching process. In the ground cytoplasm between the mitochondria are groups of particles (Fig. 11, DP) which failed to be completely displaced; this condition is no doubt due, in part, to the interference of their movement provided by the less readily displaced mitochondria. As in Fig. 5, the nucleus is stretched with the nucleolus thrown to the centrifugal end (Fig. 6, NCL), the chromatin occupying a relatively wide middle zone and a clear area appearing at the centripetal end.

3. *Vacuolar Layer.* Centripetal to the layer bearing the mitochondria and nucleus is a highly vacuolated layer, the larger vacuoles being located near the centripetal side of the layer (Figs. 12 and 13). The vacuoles vary from about 0.03 to 0.3 μ in diameter. They are tightly packed and surrounded by a meshwork of membranous-appearing structures which are embedded in a thin layer of cytoplasmic matrix. Since it is known that the Golgi material is usually lighter than most cellular materials, both within (3, 11) and without the cell (35), save for lipids, it seems reasonable to assume that part, at least, of the meshwork material of this layer may represent highly distorted membranes of the Golgi complex. Some of it may also be due to sections through or near the surface of the vacuolar membranes. The larger vesicles have probably been built up by displacement and coalescence of smaller ones, including those of the Golgi complex.

4. *Lipid Layer.* At the extreme centripetal end of the cell are found a few large lipid vacuoles (Figs. 12 and 13, FV). This material is obviously the lightest component of the cell, and the large vacuoles were undoubtedly formed during the ultracentrifuging process by the displacement and coalescence of small, dispersed lipid droplets. The large vacuoles appear empty, save for some unidentified, irregularly shaped dense bodies; these may be pigment. Apparently, the displaced lipid material has either not been sufficiently fixed to render it insoluble in the dehydrating solutions, or it was pulled completely out of the cell by the strong centripetal force (*cf.* 28).

Surrounding the large lipid vacuoles are well-defined membranes and a layer of cytoplasm more homogeneous than that surrounding the vesicles. At the extreme centripetal side of the lipid vacuoles may be seen a portion of the cell membrane (Fig. 12, CM) which displays considerable tensile strength and is capable of extensive stretching before rupture.

DISCUSSION

The nature of the Nissl bodies has long been a subject of controversy; that is, do they represent formed bodies in the living cell or are they gross artifacts formed by the methods used to demonstrate them (*cf.* 8, 15, 34)? Evidence derived from studies on the cytochemistry (8), ultracentrifugation (4), and phase-contrast microscopy (17) of nerve cells supports the view that Nissl bodies are real, an opinion which has been supported, for the most part, by electron microscope studies. Beams *et al.* (6) observed in electron micrographs of nerve cells groups of dense, oriented fibrils which they compared to the filaments of the basophilic substance observed in hepatic cells by Dalton *et al.* (16). In all probability these represent poorly preserved endoplasmic reticulum of the Nissl bodies. Pease and Baker (46) reported that Nissl bodies are probably artifacts; Hartmann (25) and Schultz, Maynard, and Pease (50) did not see them in electron micrographs of neurons. However, the first critical electron microscope study of the Nissl bodies was that of Palay and Palade (45). Their results support the view that Nissl bodies are real and that they are composed of both a rough-type endoplasmic reticulum and discrete particles. This view has been substantiated by others (1, 12, 22, 30, 55) and in this paper.

That the ergastoplasm of intact somatic cells could be displaced and stratified was clearly demonstrated by Beams and King (4), Claude (13, 14), Monné (38), and others. Work on the fine structure of centrifuged intact cells, mainly of eggs, has also revealed a displacement and stratification of the ergastoplasm (20, 36). Bernhard, Gautier, and Rouiller (10) studied, with the electron microscope, centrifuged liver cells and observed a displacement of the ergastoplasm (endoplasmic reticulum) to the centrifugal half of the cell, but little detail of its structure is here revealed. For a review of the literature relative to the effects of centrifugal force on cells in general, refer to Beams (3) and Harvey (27).

Upon ultracentrifuging, the order of stratification of the different materials *in situ* of the nerve cells differs from that occurring in differentially ultracentrifuged homogenates as described by others. For example, in a pellet of homogenate from the pancreas, the cell fragments and nuclei constitute the centrifugal layer, followed in sequence by layers of zymogen granules, mitochondria, and fragments of the endoplasmic reticulum (44). Some of this is probably due to a difference in the types of cells studied, to a difference in intensity of the centrifugal force applied, and to a difference in density of the suspending medium.

It has recently been suggested "that the particles do not exist in the intact, living cells but are formed rapidly with any change in the internal milieu of the cell, such as occurs at homogenization or at the relatively slow penetration of the material by osmium solution" (24). This view advanced by Sjöstrand and Baker (54) and Hanzon, Hermodsson, and Toschi (24) is based on their observations that no ribonucleoprotein particles are present in electron micrographs of freeze-dried cells of the rat pancreas. The results herein described would seem to bear upon the problem of whether or not the particles are present in the living cell. However, it should be emphasized that it is unknown whether or not the ganglion cells were killed by the relatively high centrifugal force used in our studies. In any case, it is well known that other types of cells will survive an even higher centrifugal force than that employed here (*cf.* 3). Hence, if the ribonucleoprotein particles are induced by the ultracentrifuging, this phenomenon does not result in the death of the cell, and it would necessarily have to be a reversible process. Furthermore, it seems unlikely that soluble ribonucleoprotein dispersed in molecular form would be displaced more rapidly than the larger components of the cell. Accordingly, we interpret these results as providing some evidence in support of the view that the ribonucleoprotein particles are real and not artifacts.

In some preparations a small particulate material is seemingly aggregated upon or within the particles. The nature of these bodies is unknown; they are probably artifacts. However, attention is called to them here because of the remote possibility that they may represent a subcomponent of the particle. Some evidence exists which indicates that the particles are not homogeneous (42, 57) and that they may possess subunits (23, 33).

It is of interest that few microsome vesicles with "attached" particles appear in ultracentrifuged intact cells. This is in marked contrast to the condition of the microsomal fraction of differentially centrifuged brei where most of the endoplasmic reticulum may appear in this form (43, 44, 51, 52). However, after treatment of pancreatic homogenate with low concentrations of Na deoxycholate, the microsomal fraction may be subfractionated by differential centrifugation into a heavy fraction of intracisternal granules, an intermediate fraction of discrete particles which is comparable to the heavy sublayer herein described, and a light fraction representing the deoxycholate soluble component of the microsomes (51, 52).

The mitochondria which stratify into a relatively broad layer *in situ* are less dense than the ergastoplasm, and they do not appear to vary so much in density as they appear to do in centrifuged *Arbacia* eggs (36). Probably some fragmentation of the mitochondria occurs, but this is not evident in our electron micrographs. However, we have observed, in unpublished work, that the mitochondria of ultracentrifuged liver cells appear as ghosts; *i.e.*, all their contents are removed, except for the membranes and cristae.

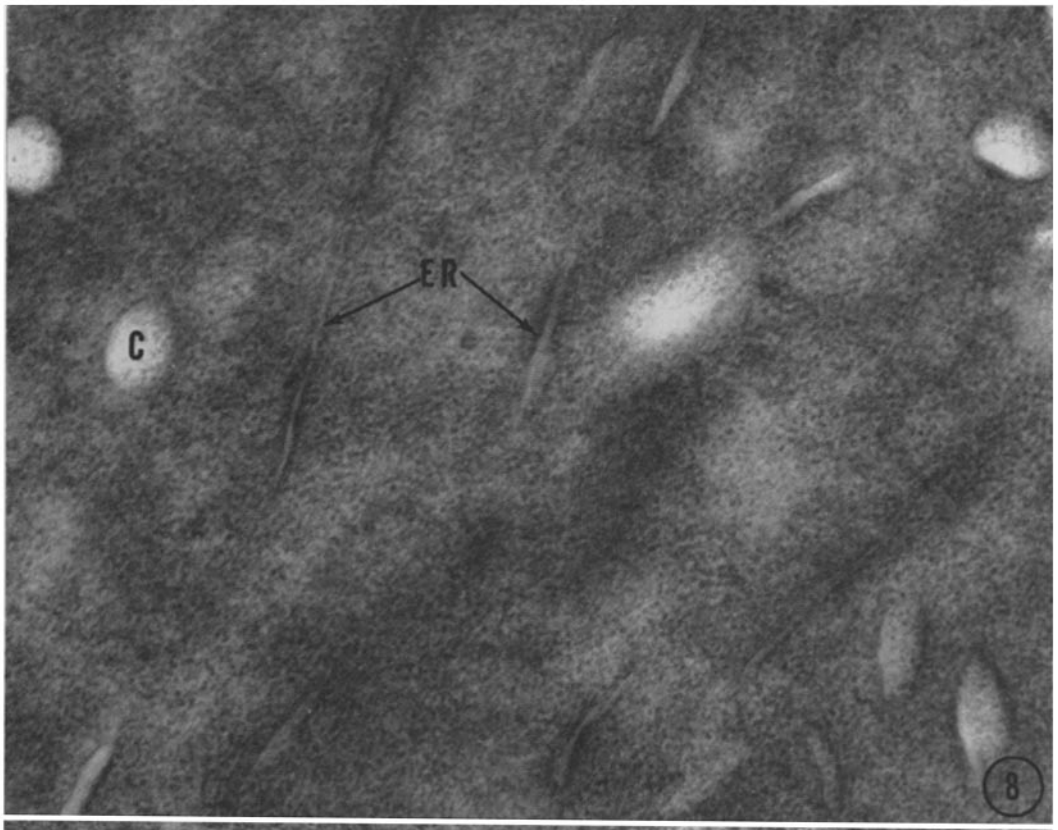
A further point deserving comment is the origin

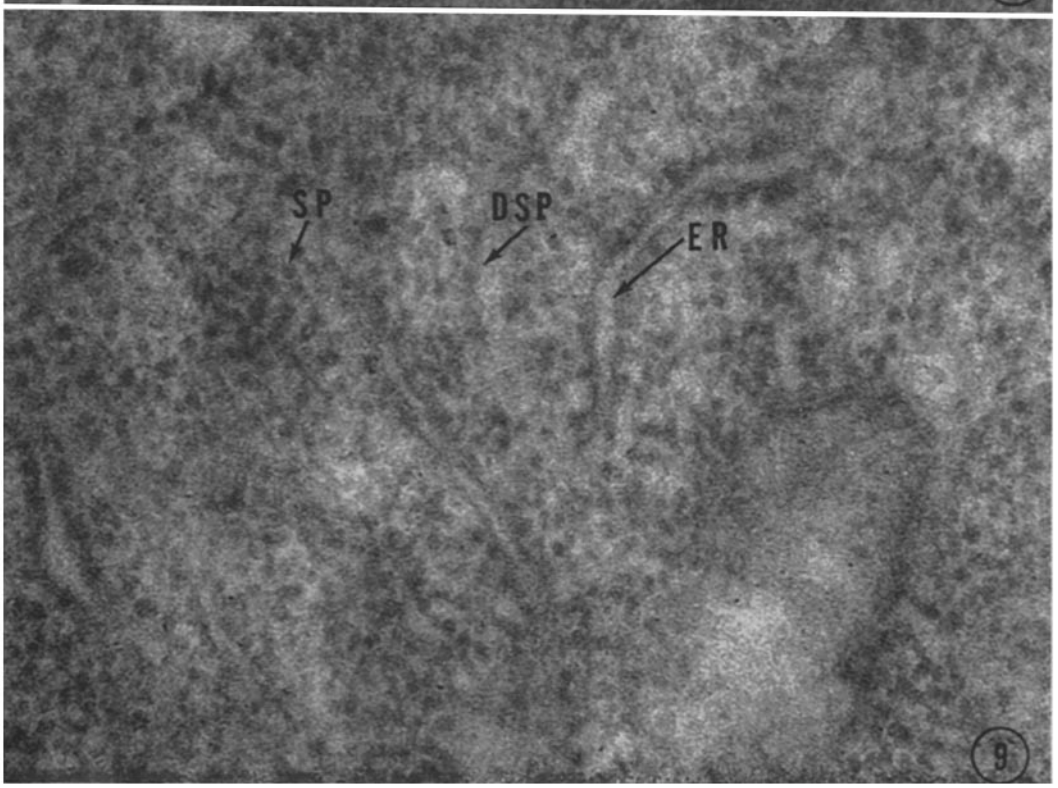
FIGURE 8

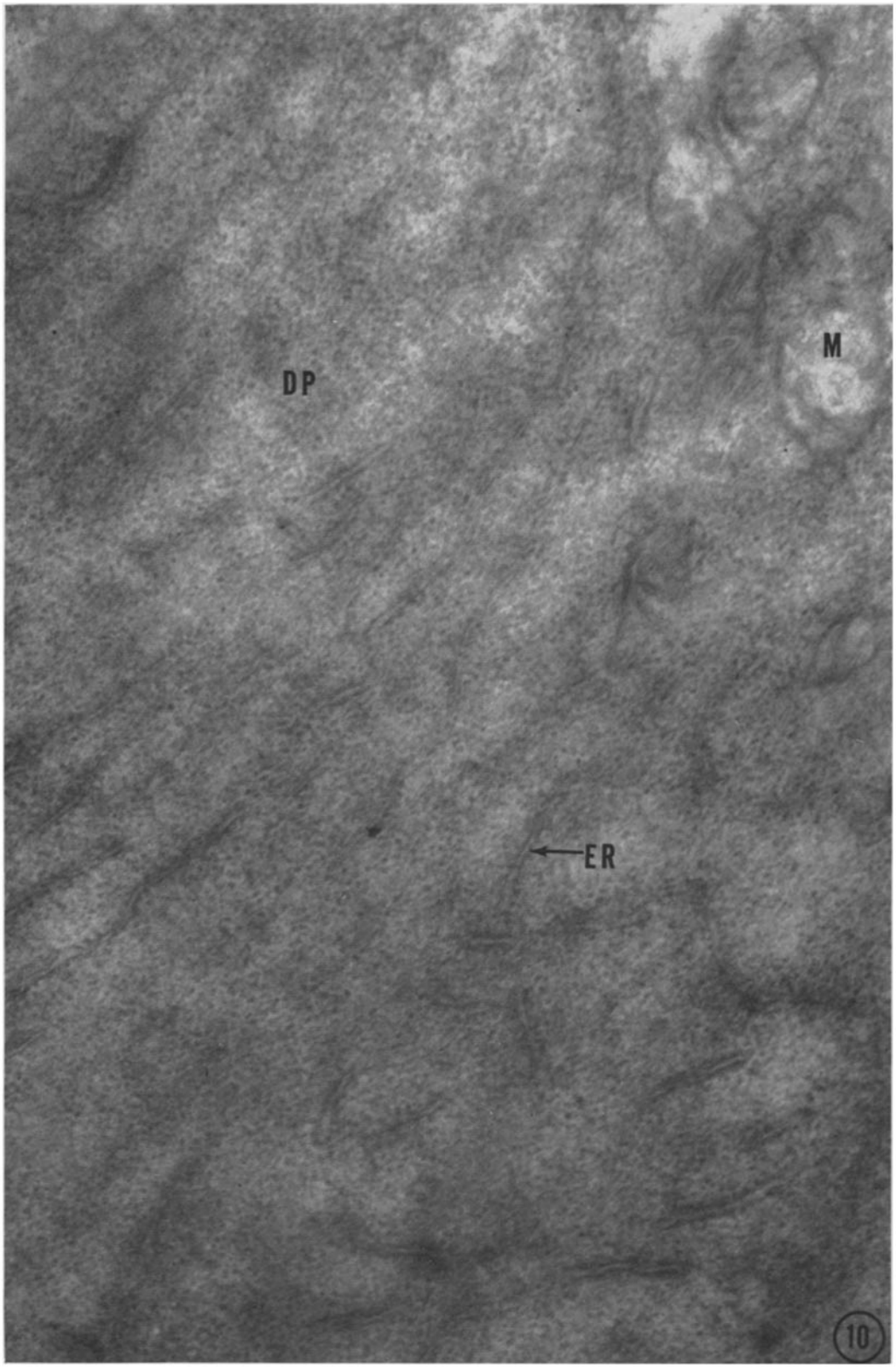
Higher magnification of sublayer ML_2 (Fig. 6). Here, in addition to discrete particles are found distorted cisternae (*C*) and damaged membranes of the endoplasmic reticulum (*ER*). (× 70,000)

FIGURE 9

Higher magnification of same sublayer as in Fig. 8. Note fine particulate material (*SP* and *DSP*) and remnants of the endoplasmic reticulum (*ER*). (× 140,000)


ER
C
8


SP
DSP
ER
9


DP
M
ER
10

of the vesicular layer. This layer undoubtedly constitutes what light microscopists have referred to in centrifuged eggs as the hyaline layer (39). It is a relatively broad, light layer. When compared to the "normal" or to the control cell, vesicles of this number and order of magnitude are not observed; thus many of them seem to have arisen during the process of centrifugation. They are not microsomal vesicles, *i.e.*, fragmented portions of the endoplasmic reticulum; neither does this layer contain ribonucleoprotein particles. Instead, they have probably been derived from the displacement and coalescence of smaller vesicles, some of which have not been identified; others perhaps represent the vesicular elements of the Golgi material.

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FIGURE 10

Layer ML_3 (Fig. 6). In addition to packed discrete particles (*DP*), a number of intact but stretched membranes of the endoplasmic reticulum are present (*ER*). Centripetal to this layer is the centrifugal side of the mitochondrial layer (*M*). (× 70,000)

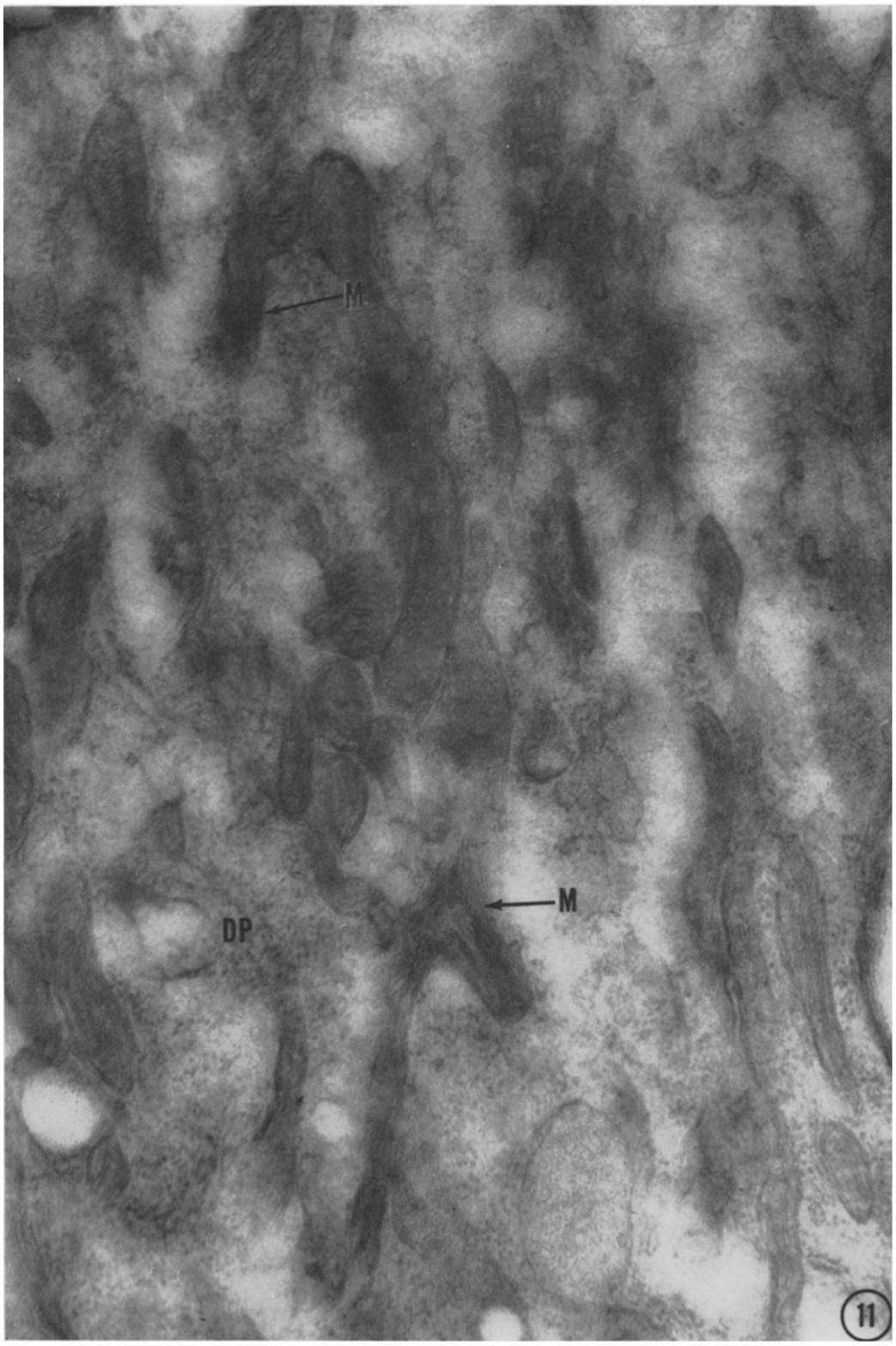
M
M
DP
11

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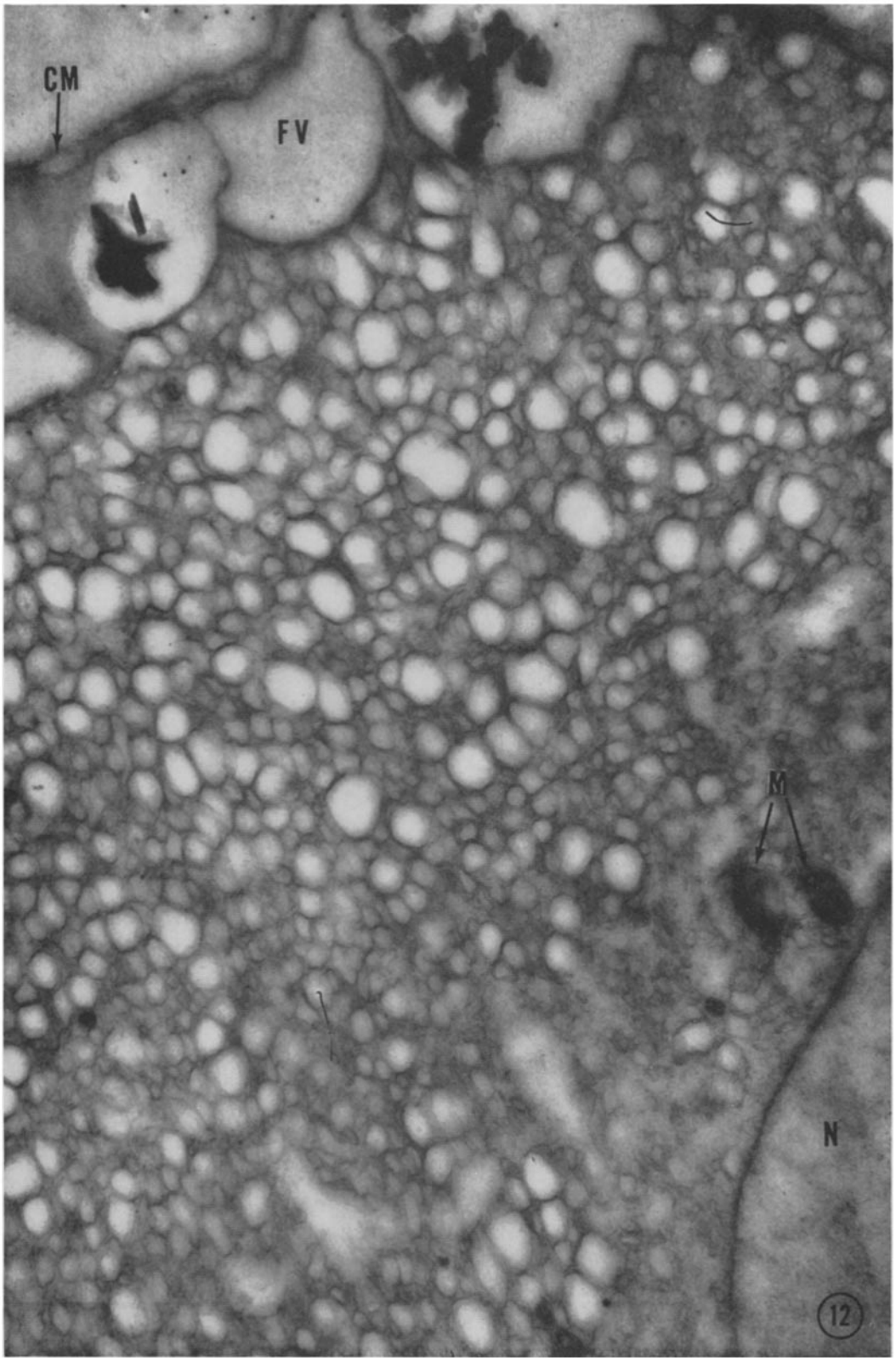
FIGURE 11

Mitochondrial layer (*M*, Fig. 6). Some of the mitochondria appear stretched with their cristae mostly oriented in their long axes. Aggregates of discrete particles are also present (*DP*). (× 65,000)

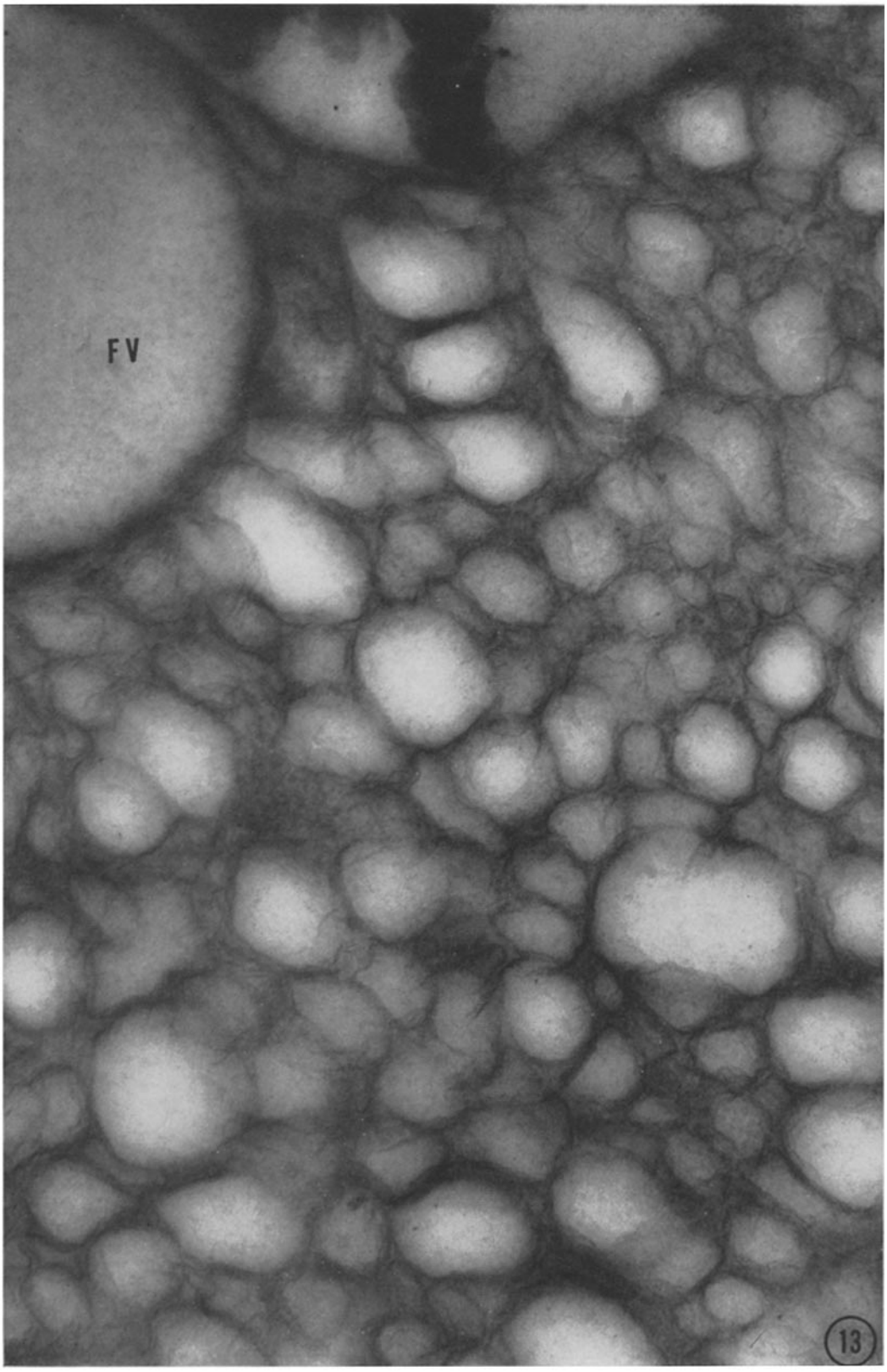
FIGURES 12 and 13

Vacuolar layer (*V*, Fig. 6). This layer is centripetal to the mitochondria (*M*) and nucleus (*N*) (Fig. 6). The vesicles appear empty and are surrounded by a cytoplasmic matrix. Centripetal to the vesicular layer are lipid vacuoles (*FV*) (*FV*, Fig. 6). The irregular dense masses within the vacuoles are unidentified; they may be pigment. (Fig. 12, × 35,000; Fig. 13, × 70,000)


CM
FV
M
N
12

FIGURES 12 and 13 (*continued*)

Vacuolar layer (*V*, Fig. 6). This layer is centripetal to the mitochondria (*M*) and nucleus (*N*) (Fig. 6). The vesicles appear empty and are surrounded by a cytoplasmic matrix. Centripetal to the vesicular layer are lipid vacuoles (*FV*) (*FV*, Fig. 6). The irregular dense masses within the vacuoles are unidentified, they may be pigment (Fig. 12, × 35,000; Fig. 13, × 70,000)


FV
13