LYSOSOMES AND GERL IN NORMAL AND CHROMATOLYTIC NEURONS OF THE RAT GANGLION NODOSUM

ERIC HOLTZMAN, ALEX B. NOVIKOFF, and HUMBERTO VILLAVERDE

From the Department of Pathology, Albert Einstein College of Medicine of Yeshiva University, New York

ABSTRACT

The rat ganglion nodosum was used to study chromatolysis following axon section. After fixation by aldehyde perfusion, frozen sections were incubated for enzyme activities used as markers for cytoplasmic organelles as follows: acid phosphatase for lysosomes and GERL (a Golgi-related region of smooth endoplasmic reticulum from which lysosomes appear to develop) (31–33); inosine diphosphatase for endoplasmic reticulum and Golgi apparatus; thiamine pyrophosphatase for Golgi apparatus; acetycholinesterase for Nissl substance (endoplasmic reticulum); NADH-tetra-Nitro BT reductase for mitochondria. All but the mitochondrial enzyme were studied by electron microscopy as well as light microscopy. In chromatolytic perikarya there occur disruption of the rough endoplasmic reticulum in the center of the cell and segregation of the remainder to the cell periphery. Golgi apparatus, GERL, mitochondria and lysosomes accumulate in the central region of the cell. GERL is prominent in both normal and operated perikarya. Electron microscopic images suggest that its smooth endoplasmic reticulum produces a variety of lysosomes in several ways: (*a*) coated vesicles that separate from the reticulum; (*b*) dense bodies that arise from focal areas dilated with granular or membranous material; (*c*) "multivesicular bodies" in which vesicles and other material are sequestered; (*d*) autophagic vacuoles containing endoplasmic reticulum and ribosomes, presumably derived from the Nissl material, and mitochondria. The number of autophagic vacuoles increases following operation.

INTRODUCTION

Chromatolysis, the process that ensues in the perikaryon following interruption of its axon, long has been of interest to cytologists and cell pathologists (see reviews in references 1–6). Although the process has been analyzed by many techniques including electron microscopy (7–12), it seemed likely that a study of the intracellular digestive system, lysosomes and associated structures, (14–15) would lead, in time, to a fuller understanding of cytological processes within neurons.

In the present study, marker enzyme activities and electron microscopy have been used to study lysosomes and other cytoplasmic organelles in normal and chromatolytic neurons. The results draw attention to the variety of lysosomes in perikarya and emphasize the likely role of Golgi-associated smooth endoplasmic reticulum (GERL) in the formation of lysosomes. They suggest further that changes in chromatolytic neurons shed light on ribonucleoprotein turnover in normal neurons.

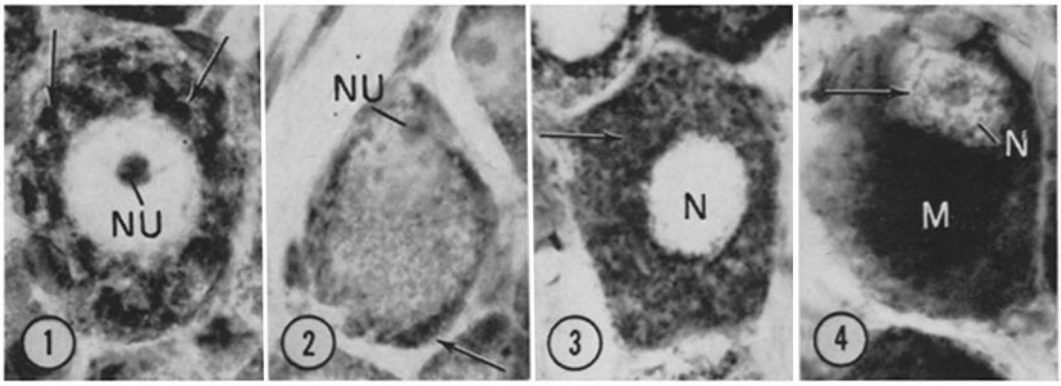
MATERIALS AND METHODS

The right vagus nerves of ether-anesthetized female Sprague-Dawley rats (180–280 g) were sectioned about 0.5–1.0 cm below the nodosal ganglion. No attempt was made to approximate or suture the severed ends. This operative procedure leaves intact a small laryngeal branch of the vagus nerve. Probably as a result of this, some 10–20% of neurons in the ganglion of the operated animal appear normal. These cells serve as "internal controls" in addition to the controls mentioned below with which changes in chromatolytic neurons can be compared.

The animals were sacrificed from 6 hr–46 days after vagotomy. They were anesthetized with Nembutal and perfused through the ascending aorta, first with warm, then with cold (5°C) 3% glutaraldehyde (see reference 66) buffered at pH 7.4 with either 0.1 M cacodylic acid with 0.25% calcium chloride (16, 17) or 0.1 M phosphate (18). The descending aorta was clamped shortly after perfusion was started. After 20–30 min the right nodosal ganglion was removed and fixation was continued by immersion in glutaraldehyde at 5°C. All fixation was carried out in a 5°C coldroom.

Unincubated tissues for fine structure studies generally were fixed by immersion for 1½–3 hr, but occasionally longer periods up to about 20 hr were used. They then were rinsed for a few minutes in the appropriate buffer without glutaraldehyde but with added 0.2 M sucrose, and postfixed for 2–3 hr in 1% osmium tetroxide buffered either with 0.1 M phosphate containing glucose (19), for material fixed with phosphate-buffered glutaraldehyde, or with Veronal-acetate-sucrose (20, 21), for tissue fixed with cacodylate-buffered glutaraldehyde. After post-fixation the material was dehydrated in ethanols and embedded in Araldite 506 (Ciba) by the method of Luft (13).

Cacodylate-buffered glutaraldehyde fixation was used for enzyme cytochemistry. The time of immersion fixation was 30–45 min. The tissues then were rinsed for 45 min or longer in cacodylate-buffer



Unless otherwise noted, all tissues were fixed in cacodylate-buffered glutaraldehyde. Electron micrographs are from sections stained with both uranyl and lead. Figs. 1–12 are light micrographs from frozen sections. For acid phosphatase preparations, CMP was the substrate for all preparations but that in Fig. 45, for which β-glycerophosphate was used. The incubation times are indicated in parentheses.

FIGURE 1 Neuron from an unoperated ganglion, stained with methyl green–pyronin. The pyronin stains the Nissl material (arrows) and the nucleoli (*NU*). Methyl green staining of the chromatin is too light to show in the photograph. × 1,000.

FIGURE 2 Neuron from a ganglion 7 days after operation. In the cytoplasm most of the pyronin-stained material is seen as a peripheral band (arrow). The nucleus is eccentric and shows a prominent nucleolus (*NU*). × 1,100.

FIGURE 3 Neuron from an unoperated ganglion, incubated for NADH-TNBT reductase activity (10 min). The numerous mitochondria, one of which is indicated by the arrow, are colored by the formazan. The nucleus is seen at *N*. × 1,200.

FIGURE 4 Neuron from a ganglion 7 days after operation, incubated for NADH-TNBT reductase activity (10 min). The mitochondria (*M*) are segregated to the center of the cell. A few individual mitochondria may be seen (arrow) in the thin layer of cytoplasm over the eccentric nucleus (*N*). × 1,200.

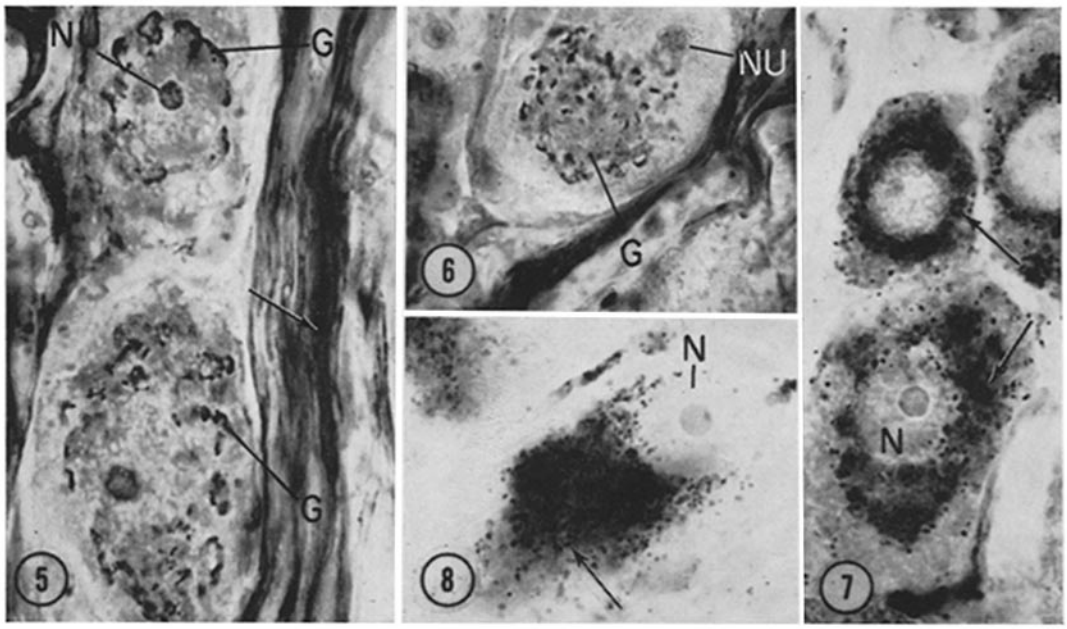


FIGURE 5 Neuron from an unoperated ganglion, incubated for TPPase activity (60 min). Much reaction product is seen in the Golgi apparatus (*G*) and moderate amounts in the nucleoli (*NU*). Alongside the perikarya reaction product is seen in an unmyelinated nerve fiber (arrow) (see reference 39). × 1,100.

FIGURE 6 Neuron from a ganglion 7 days after operation, incubated for TPPase activity (60 min). The Golgi apparatus (*G*) is segregated to the center of the cell. The nucleolus (*NU*) is lightly stained. × 1,000.

FIGURE 7 Neuron from an unoperated ganglion, incubated for acid phosphatase activity (45 min). Reaction product is present in numerous granules, the lysosomes, and in GERL (arrows). The upper cell shows a characteristic tendency of the GERL of the smallest neurons to be concentrated in a narrow zone around the nucleus. Larger cells, like the lower one in this figure, often show a somewhat more dispersed distribution. The nucleus is seen at *N*. × 1,100.

FIGURE 8 Neuron from a ganglion 4 days after operation, incubated for acid phosphatase activity (45 min). The lysosomes and GERL have become segregated to the center of the cell (arrow) while the nucleus (*N*) is seen at the periphery. × 1,100.

with 0.2 M sucrose. Frozen sections were cut at 10 μ for light microscopy and 40 μ for electron microscopy.

Some material also was fixed with cold formaldehyde-calcium for enzyme cytochemistry (22). The initial perfusion lasted 30 min. It was followed by overnight immersion at 5°C.

The frozen sections were incubated for demonstration of the following enzymes[1] (for electron microscopy, 5% sucrose was added to the media): acid phosphatase, with the Gomori medium with β-glycerophosphate (23) or CMP (24) as substrate; inosine diphosphatase and thiamine pyrophosphatase, with the Novikoff-Goldfischer medium (25); acetylcholinesterase, using Karnovsky's medium (26); NADH-TNBT reductase, substituting tetraNitro for Nitro BT in the medium of Novikoff (27).

The reductase activity was used only in frozen sections for light microscopy, while the other incubations were done for both light microscopy and electron microscopy.

Methyl green-pyronin staining was done according to the procedure of Taft (28).

[1] The following abbreviations are used: CMP, 5'-cytidylic acid; IDPase, inosine diphosphatase; TPPase, thiamine pyrophosphatase; NADH-TNBT reductase, reduced nicotinamide adenine dinucleotide-tetranitro blue tetrazolium reductase.

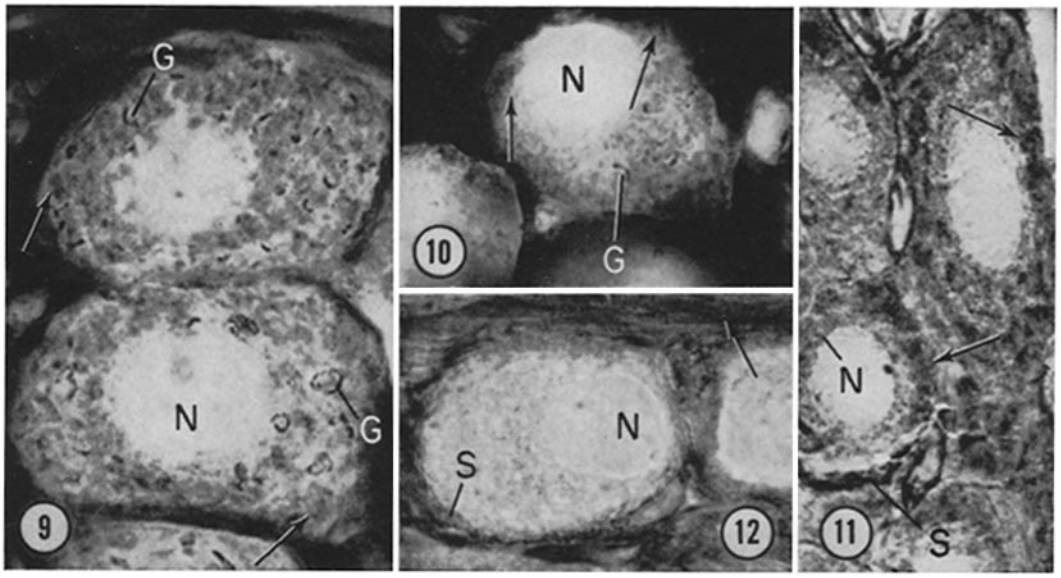


FIGURE 9 Neurons from an unoperated ganglion, fixed in formaldehyde-calcium and incubated for IDPase activity (40 min). Reaction product in the perikarya is present in the Golgi apparatus (*G*) and in the Nissl substance (arrows). *N*, nucleus. × 1,100.

FIGURE 10 Neurons from a ganglion 4 days after operation, fixed in formaldehyde-calcium and incubated for IDPase activity (60 min). The Golgi apparatus has become segregated to the center of the cell (*G*) and the Nissl substance to the periphery (arrows). Reaction product at the border of the neuron (see Fig. 9) is present at the sheath cell–perikaryal interface but the possibility of diffusion from adjacent unmyelinated fibers and blood vessels has not been eliminated (see reference 39). The eccentric nucleus is seen at *N*. × 1,000.

FIGURE 11 Neurons from an unoperated ganglion, incubated for acetylcholinesterase activity (120 min). Reaction product is present in the Nissl substance (arrows) and in the nuclear envelope (*N*). Reaction product, due to unspecific cholinesterase (see text), is present at the sheath cell–perikaryal interface (*S*). × 1,100.

FIGURE 12 Neuron from a ganglion 7 days after operation, incubated for acetylcholinesterase activity (120 min). No reaction product occurs in the perikarya. However, the sheath cell–perikaryal interface (*S*) and unmyelinated fibers (arrow) (see references 39, 82) continue to show activity of the unspecific cholinesterase. The eccentric nucleus is seen at *N*. × 1,000.

All enzyme reactions were carried out at 37°C, with the exception of the acetylcholinesterase which was done at 0°C.

The 10-μ sections and sample 40-μ sections were mounted in glycerogel for examination in the light microscope, after brief treatment with ammonium sulfide, for the phosphatases. The remaining 40-μ sections were postfixed for 30 min in Veronal-acetate–sucrose-buffered 1% osmium tetroxide, then embedded in Araldite as above.

Material from animals 6 and 12 hr, 1, 2, and 4 days, and 1, 2, 3, 4 and 6 wks after operation was studied by light microscopy. Five or more ganglia were studied in the electron microscope for each of the following four groups: 12 hr–1 day; 2–4 days; 5–7 days; 13–26 days following surgery. Ganglia from unoperated animals and the contralateral ganglia from operated rats served as controls for both electron microscopy and light microscopy. It should be noted that the cells from both operated and unoperated ganglia are from animals anaesthetized with Nembutal and that 20–60 seconds elapsed between opening of the chest cavity and initiation of the perfusion of fixative.

As enzyme controls, the frozen sections that were examined in the light microscope and electron microscope were incubated as follows. For the phosphatases, the media used were complete except for the substrates (23). For acid phosphatase, incubation also was performed in the full medium to which 0.01 M

NaF was added (23). 10^{-5} M BW 62C47 (Burroughs-Wellcome & Co., Inc., Tuckahoe, N.Y.) was added to the medium to inhibit acetylcholinesterase without inhibiting unspecific cholinesterase. To inhibit unspecific cholinesterase, RO 20683 (Hoffman-LaRoche, Inc., Nutley, N.J.) was used.

Thin sections were cut with glass knives on a Porter-Blum MT-1 microtome and were mounted on naked grids. They were stained either with a saturated solution of uranyl acetate in 50% ethanol (29), lead citrate (30) or, most often, with uranyl followed by lead. Micrographs were taken with an RCA EMU 3B or 3H microscope at 100 kv and initial magnifications of 2000–19,000.

RESULTS

Light Microscopy

UNOPERATED GANGLIA: Light microscopy of unoperated ganglia shows a more or less symmetrical distribution around the nucleus of the major organelles of the neurons although some variation from cell to cell does occur (Figs. 5, 7). The symmetrical distribution is shown for the Nissl substance in Fig. 1, for the mitochondria in Fig. 3, and for the Golgi apparatus in Fig. 5, as seen in methyl green-pyronin, NADH-TNBT reductase and TPPase preparations, respectively. Bodies containing demonstrable acid phosphatase are distributed similarly, as may be seen in Fig. 7. These include lysosomes and larger bodies similar to the GERL described previously in dorsal root ganglia (31–33) as a special region of smooth endoplasmic reticulum. As is true in the dorsal root perikarya, the areas occupied by GERL in the nodosal ganglia neurons are ellipsoidal or roughly spherical and appear to fit within the meshwork of the Golgi apparatus (compare Fig. 5 and Fig. 7). Electron microscopy, described below, confirms this impression.

IDPase activity is demonstrated in the Golgi apparatus and, unlike other neurons we have studied (34, 35), in the Nissl substance (Fig. 9). Activity in the Nissl substance is demonstrated best in formaldehyde-calcium–fixed tissues, but some activity also is present after glutaraldehyde fixation. Acetylcholinesterase reaction product is seen in the Nissl substance, the nuclear envelope, and the sheath cell-perikaryal interface (Fig. 11) (see references 36–40).

OPERATED GANGLIA: After the axons[2] have been sectioned, the distribution of organelles is changed drastically. Initial changes are visible within 24 hr, and by 4–7 days the alterations have reached a peak. The altered distribution of organelles persists, at least in part, for the longest intervals we have studied, 4–6 wk.

In chromatolytic neurons, the cell nuclei (Figs. 2, 4, 6, 8, 10, and 12) and most of the pyronin-stained material (Fig. 2) are seen at the periphery of the cells. In contrast, mitochondria (see references 7, 43) (Fig. 4), lysosomes (see references 6, 44, 45), and GERL (Fig. 8) are located toward the center. The Golgi apparatus also appears at the center of the cell (Fig. 6) rather than segregating to the periphery as has been reported for some other neurons (46–48).

IDPase reaction product is seen (Fig. 10) in the Golgi apparatus in the center of the cell and in the Nissl substance at the periphery. Although traces of activity remain in some neurons, in most perikarya little or no acetylcholinesterase activity is demonstrable (Fig. 12) (see references 49, 50).

In both normal and chromatolytic neurons, deposition of precipitate at all sites within the perikarya is abolished by omission of substrates from the incubation media or by inclusion of inhibitors. However, as expected, the acetylcholinesterase inhibitor (BW 62C47) does not prevent staining of the sheath cell–perikaryal interface (Figs. 11, 12); this indicates that the enzyme here is an unspecific cholinesterase (see reference 39). As in dorsal root ganglia (39), the sheath cell–perikaryal interface but not the perikaryon also has demonstrable activity (abolished by RO 20683) when butyrylthiocholine is used as substrate in place of acetylthiocholine.

Electron Microscopy

Electron microscopy confirms the light microscope observations as to enzyme localizations and over-all changes in organelle distribution. Other authors have described the changed distribution of mitochondria and the disruption of the rough endoplasmic reticulum of the Nissl substance in the center of the cell (7–11). Most of our attention will be devoted to the lysosomes, GERL, and Golgi apparatus.

UNOPERATED GANGLIA: In unoperated neurons, GERL is seen as a region of agranular membranes associated closely with the Golgi apparatus and showing reaction product in acid

[2] See Bodian (41) for a discussion of terminology relating to axons and dendrites.

phosphatase-incubated preparations (Fig. 13). While we refer to it as smooth endoplasmic reticulum, it may be continuous with the Golgi apparatus. Numerous vesicles of the type called "coated" or "alveolate" (52) are attached to the GERL (Fig. 19) or lie nearby, apparently unattached. The coat of the delimiting membrane shows radial projections. Some of the coated vesicles show reaction product in ganglia incubated for acid phosphatase activity (Fig. 20). The coated vesicles fulfill the cytochemical criteria for lysosomes: membrane-delimited cytoplasmic bodies with acid hydrolase activity (see reference 53).

Many other lysosomes are present in unoperated neurons, often in association with GERL. These include large numbers of dense bodies containing grains (Fig. 43) and "crystalline" or membranous material. "Multivesicular bodies" also are observed (Figs. 23, 40); and, on occasion, autophagic vacuoles (14, 24, 55–57) are encountered. The latter are identifiable by the organelles they contain: sometimes mitochondria (Fig. 21) but more commonly ribosomes and fragmenting endoplasmic reticulum (Fig. 23). Their delimiting membrane appears thickened and irregular. Further discussion of these various lysosomes will be deferred until consideration of the operated ganglia.

Electron microscopy shows reaction product for IDPase activity in the Golgi apparatus and in the endoplasmic reticulum of GERL and Nissl substance (Fig. 17). None is seen in the nuclear envelope. In acetylcholinesterase preparations reaction product is observed in the endoplasmic reticulum of both nuclear envelope and Nissl substance (Fig. 18).

OPERATED GANGLIA: Chromatolytic neurons were identified in the electron microscope by the peripheral position of the nucleus and the altered distribution of the other organelles. Only those cells were studied in which sufficient portions of nucleus and cytoplasm had been included in the plane of section to indicate that the nuclei were peripheral.

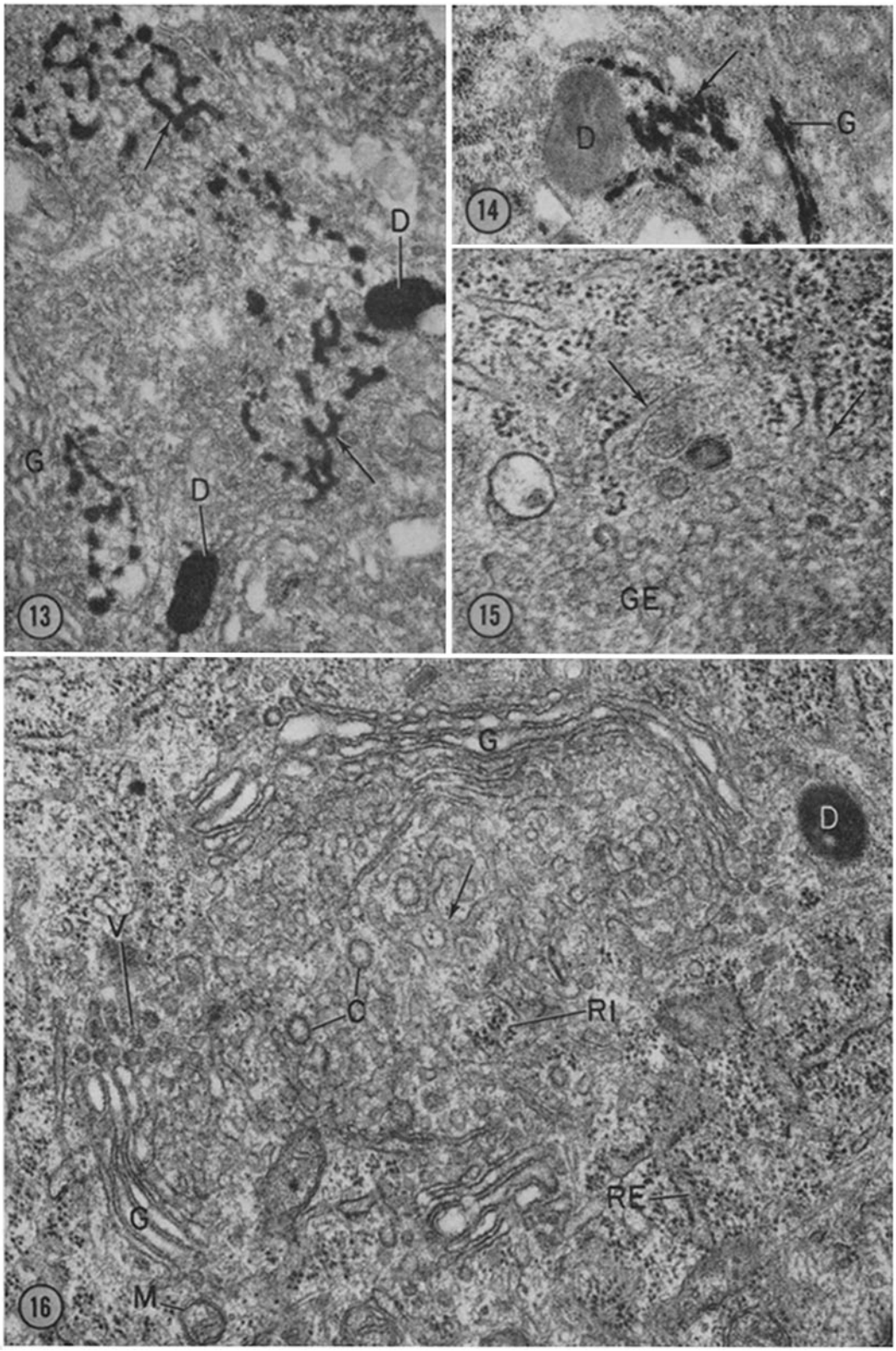
GERL continues to be prominent for the first few days after operation (Figs. 16, 27). As in unoperated preparations (see reference 31), connections between rough and smooth endoplasmic reticulum are seen often in the GERL area (Figs. 15, 27), and clusters of ribosomes occasionally are found within the meshwork of smooth endoplasmic reticulum (Fig. 16). Lysosomes of the types described for unoperated ganglia also are found associated with GERL in chromatolytic neurons. Of special interest are the autophagic vacuoles which are seen relatively infrequently in neurons from unoperated preparations. Their number increases considerably, especially during the first day or two after operation (Figs. 22, 24–26). Mitochondria within autophagic vacuoles are much more common in chromatolytic than in normal neurons. As in neurons from unoperated

FIGURE 13 Portion of a neuron from an unoperated ganglion, incubated for acid phosphatase (30 min). Reaction product is present in the dense bodies (*D*) and in GERL (arrows; see Figs. 16, 19, 27). None is seen in the Golgi saccules (*G*). × 33,500.

FIGURE 14 Portion of the Golgi area from a neuron of an unoperated ganglion, incubated for IDPase activity (55 min). Reaction product is seen in two saccules of the Golgi apparatus (*G*) and in GERL (arrow). None is seen in the dense body (*D*). × 38,000.

FIGURE 15 Portion of a neuron from the same ganglion shown in Fig. 16. Connections between rough endoplasmic reticulum and smooth endoplasmic reticulum (*GE*) are indicated by the arrows. × 51,000.

FIGURE 16 Portion of a neuron from a ganglion 1 day after operation. The Golgi apparatus (*G*) borders the fenestrated smooth endoplasmic reticulum (arrow) of GERL. Coated vesicles (*C*) and dense bodies (*D*) are seen in this area, as is frequently the case. Note the clusters of ribosomes (*RI*) within the GERL meshwork. A mitochondrion is seen at *M*, and rough endoplasmic reticulum and free ribosomes of the Nissl substance at *RE*. At *V*, Golgi vesicles are seen next to Golgi saccules (*G*) possibly dilated as a preparative artifact. The relations between GERL and Golgi saccules, with which it may prove to be continuous, are discussed in reference 15. × 38,000.


D
G
D
13
D
G
14
GE
15
G
D
V
C
RI
RE
G
M
16

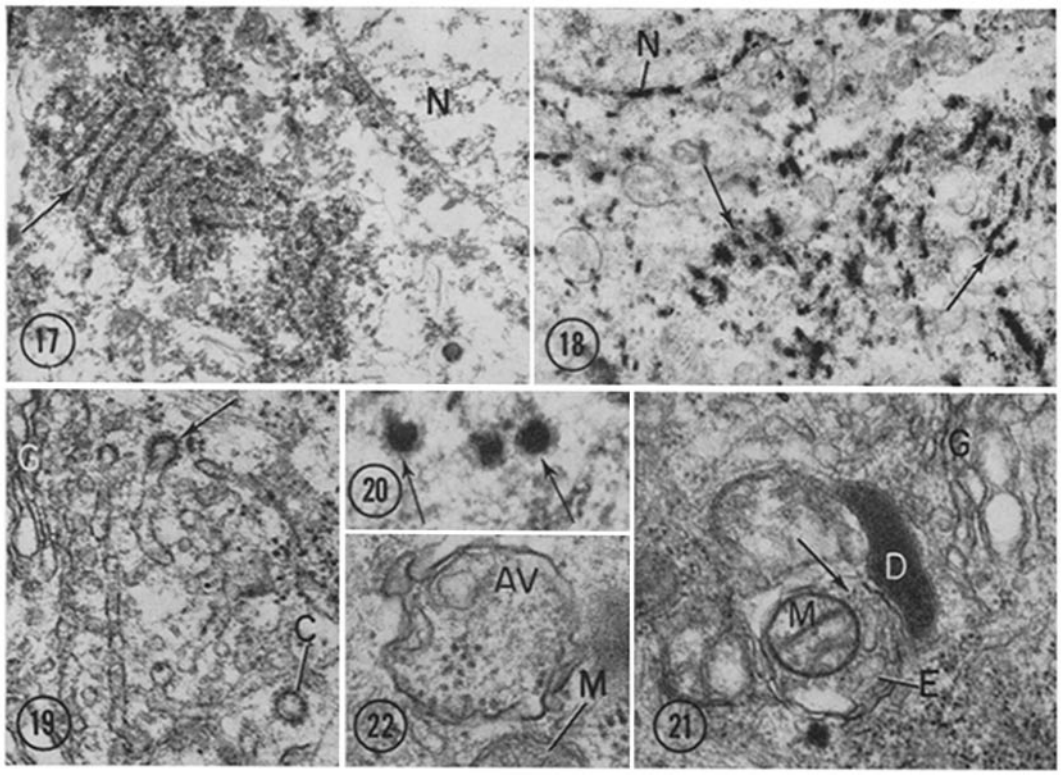


FIGURE 17 Portion of a neuron from an unoperated ganglion, fixed in formaldehyde-calcium and incubated for IDPase activity (60 min). Reaction product is present in the rough endoplasmic reticulum of the Nissl substance (arrow). None is seen in the nuclear envelope (*N*). × 14,500.

FIGURE 18 Portion of a neuron from an unoperated ganglion, incubated for acetylcholinesterase activity (105 min). Reaction product is present in the rough endoplasmic reticulum (arrows) and the nuclear envelope (*N*). The thin section was stained only with uranium. × 14,500.

FIGURE 19 Part of the GERL of a neuron from an unoperated ganglion. A coated vesicle (arrow) with a number of projections radiating from its surface is continuous with smooth endoplasmic reticulum. The projections are separated, at their tips, by 20–40 mμ. Another coated vesicle is seen at *C*, and portions of Golgi saccules at *G*. (See also Fig. 16.) × 45,000.

FIGURE 20 Three coated vesicles in the GERL region of a neuron from an unoperated ganglion, incubated for acid phosphatase activity (30 min). Reaction product is present within the vesicles. Note the projections of the coats of the vesicles indicated by the arrows (cf. Fig. 19). × 69,000. See also Fig. 58 in reference 15.

FIGURE 21 Portion of a neuron from an unoperated ganglion. In the GERL region an autophagic vacuole contains a mitochondrion (*M*), endoplasmic reticulum (*E*), and ribosomes (arrow). A dense body is seen at *D*, and portions of Golgi saccules at *G*. × 60,000.

FIGURE 22 Portion of the GERL region of a neuron from a ganglion two days after operation. Fixed in phosphate-buffered glutaraldehyde. An autophagic vacuole containing ribosomes is seen at *AV*. Part of a mitochondrion is seen at *M*. × 55,500.

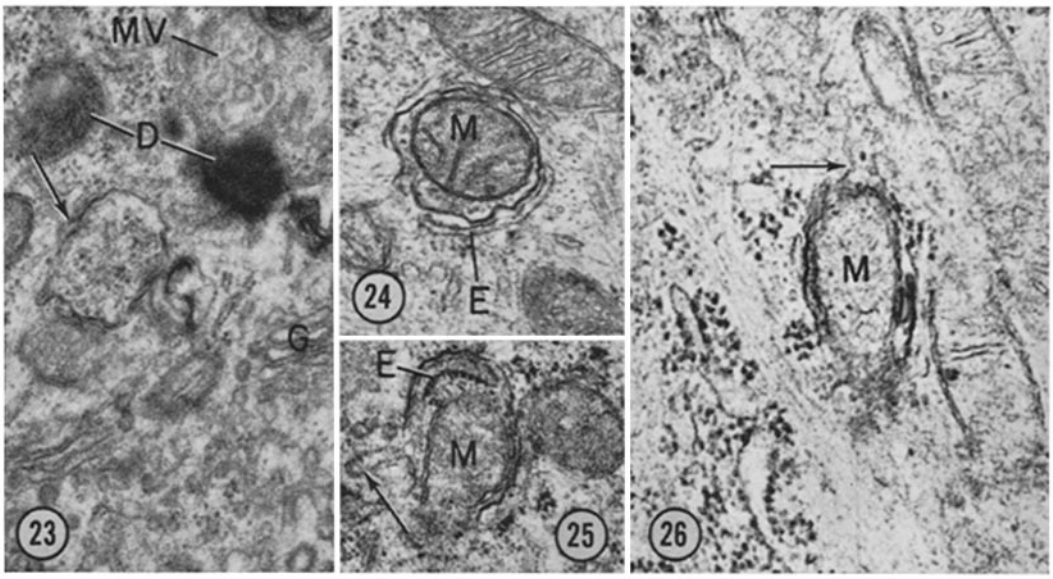


FIGURE 23 Portion of a neuron from an unoperated ganglion. Fixed in phosphate-buffered glutaraldehyde. Part of the Golgi apparatus is seen at *G*; the rest of the field is occupied by GERL. The arrow indicates an autophagic vacuole containing ribosomes present in the GERL region. Its delimiting membrane in some places is double (arrow), suggesting that it arises by the close apposition or "compaction" of two or more membranes (see Fig. 22, 24 and reference 58). A multivesicular body is seen at *MV*, and two dense bodies at *D*. × 45,500.

FIGURE 24 Portion of a neuron from a ganglion 7 days after operation. A mitochondrion (*M*) is present within an autophagic vacuole. Endoplasmic reticulum (*E*) is seen close to the surface of this vacuole. × 37,500.

FIGURE 25. Portion of a neuron from a ganglion 5 days after operation. Fixed in phosphate-buffered glutaraldehyde. This may be an autophagic vacuole in the process of formation. Thickened membranes (see Fig. 23 and reference 58) continuous with smooth endoplasmic reticulum (arrow) appear to be surrounding a cisterna of rough endoplasmic reticulum (*E*) and a mitochondrion (*M*). × 38,000.

FIGURE 26 Portion of a neuron from a ganglion 2 days after operation. Fixed in phosphate-buffered glutaraldehyde. This may be an autophagic vacuole in the process of formation. A membrane continuous with smooth endoplasmic reticulum (arrow) appears to be sequestering a mitochondrion (*M*). × 47,000.

ganglia, autophagic vacuoles also contain ribosomes and endoplasmic reticulum. Their delimiting membrane is thickened and often is composed of two or more distinct membranes (Fig. 23). Connections between these membranes and smooth endoplasmic reticulum sometimes are observed (Figs. 25, 26; see reference 58).

While they cannot be related specifically to chromatolysis, a number of other observations made on chromatolytic neurons should be noted. Increase or decrease in the size, number, or distribution of dense bodies, multivesicular bodies, and coated vesicles is difficult to assess in electron micrographs because of the large numbers present initially. However, information was obtained relating to the modes of origin of these bodies.

The numerous multivesicular bodies present in the neurons before and after operation contain small vesicular and tubular elements of varying morphology (Figs. 27, 28, 32, 34, 36). The delimiting membrane often is seen attached to smooth surfaced tubular elements of the endoplasmic reticulum of GERL (Figs. 27, 34, 37) (see reference 34). As reported by others (59–62), portions of the surface of the multivesicular bodies frequently show a coating (Figs. 28, 38). In our

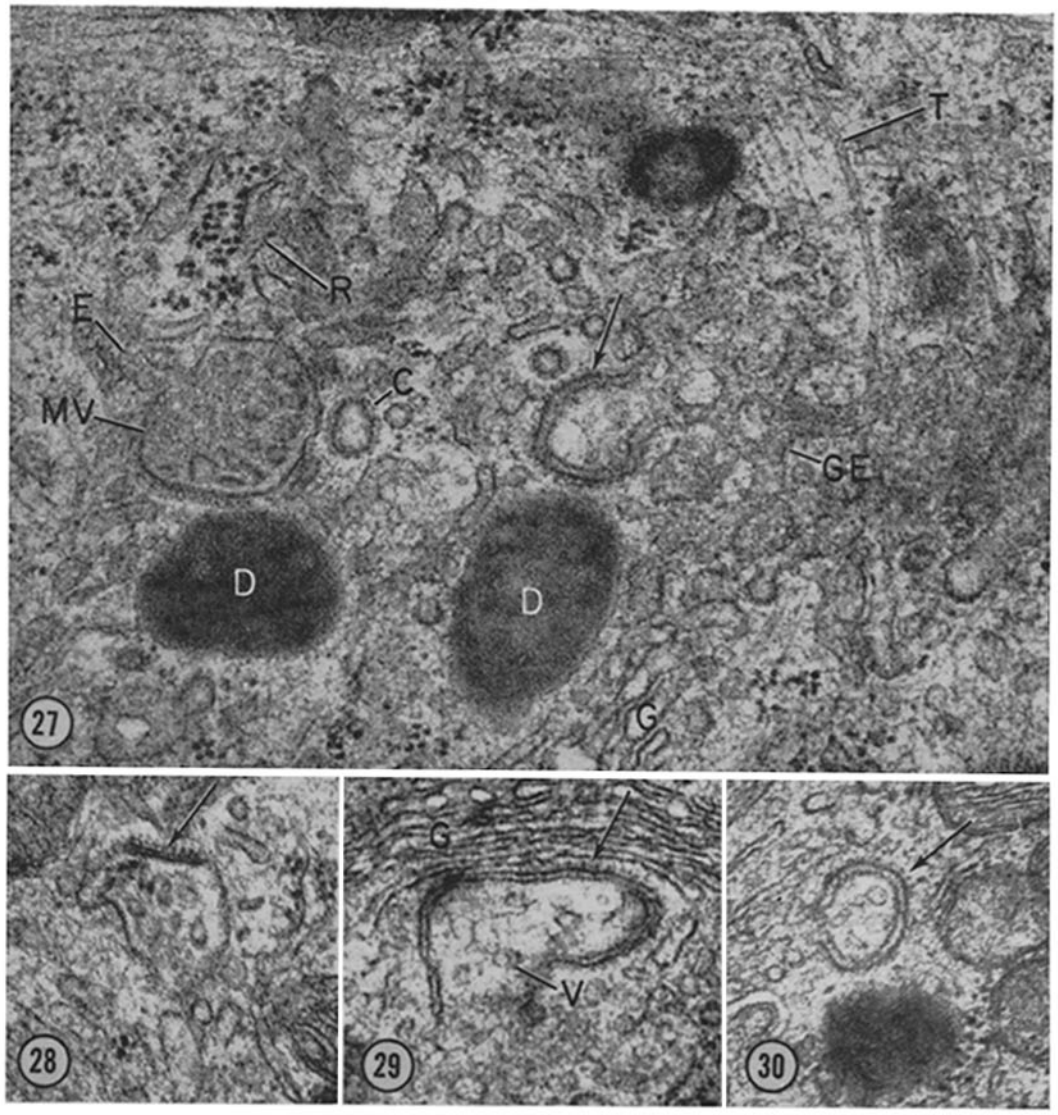


FIGURE 27 Portion of a neuron from a ganglion 1 day after operation. Most of the field is occupied by GERL. Note the fenestrations of the endoplasmic reticulum (*GE*). The arrow indicates a region of this endoplasmic reticulum that shows a surface coating with short projections. At *MV* a multivesicular body is seen attached to smooth endoplasmic reticulum (*E*). A coated vesicle is present at *C*, dense bodies at *D*, continuity of rough and smooth endoplasmic reticulum at *R*, and a microtubule at *T*. *G*, Golgi saccules. × 59,500.

FIGURE 28 Portion of a neuron from a ganglion one day after operation. A multivesicular body is seen in the center of the field. A portion of its surface (arrow) shows a coating with short projections spaced 20–30 mμ apart. This coating is similar to that seen on the endoplasmic reticulum (see Figs. 27, 29–31, 35). As is often the case, the delimiting membrane of the coated region (arrow) consists of two or more distinct layers. × 44,000.

FIGURE 29 Portion of a neuron from a ganglion 2 days after operation. Fixed in phosphate-buffered glutaraldehyde. Coated agranular membranes appear (arrow) as if surrounding a group of vesicles or tubules (*V*) probably derived from endoplasmic reticulum. Golgi saccules are indicated by *G*. × 62,000.

FIGURE 30 Portion of a neuron from a ganglion 5 days after operation. Fixed in phosphate-buffered glutaraldehyde. Coated endoplasmic reticulum (arrow) appears as if surrounding small vesicles and tubules (see Fig. 29). × 51,000.

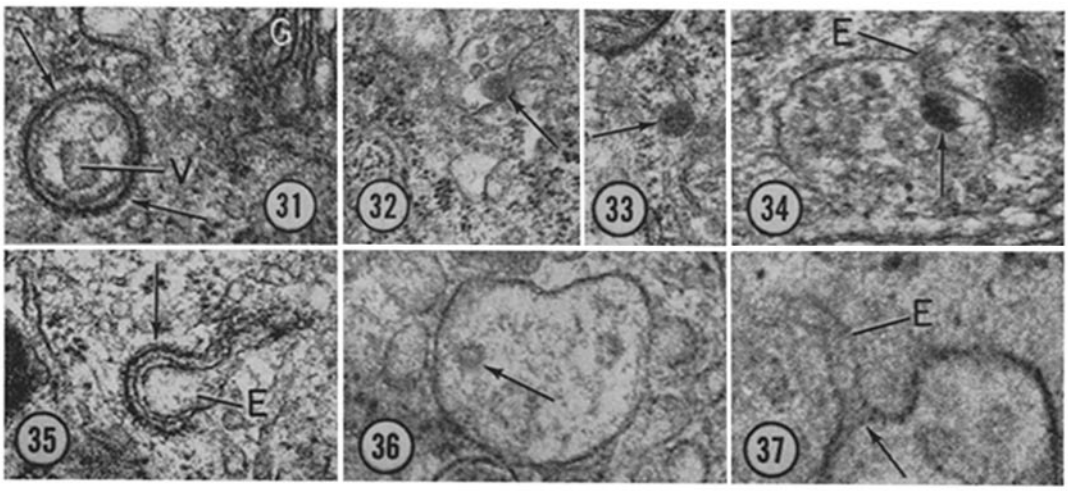


FIGURE 31 Portion of a neuron 2 days after operation. Fixed in phosphate-buffered glutaraldehyde. Coated endoplasmic reticulum (arrows) appears as if surrounding vesicles or tubules (*V*) that resemble dilated smooth endoplasmic reticulum (see Fig. 35). Golgi saccules are seen at *G*. × 55,000.

FIGURE 32 Portion of a neuron from a ganglion 5 days after operation. Fixed in phosphate-buffered glutaraldehyde. A multivesicular body is seen in the upper part of the field. Within it several vesicles are seen and a larger granule (arrow) like those seen free in the cytoplasm (see Fig. 33). × 32,000.

FIGURE 33 Cytoplasm of a neuron from the same ganglion shown in Fig. 32. The arrow indicates a cytoplasmic granule like that seen within the multivesicular body of Fig. 32. × 32,000.

FIGURE 34 Portion of a neuron from a ganglion one day after operation, showing a multivesicular body containing a granule (arrow). The delimiting membrane of this multivesicular body is continuous with the smooth endoplasmic reticulum indicated by *E*. × 45,000.

FIGURE 35 Portion of the GERL of a neuron 4 days after operation. A region of coated endoplasmic reticulum (arrow) seems to be surrounding a dilated region of smooth endoplasmic reticulum (*E*). × 55,000.

FIGURE 36 Portion of a neuron from a ganglion 4 days after operation. Most of the field is occupied by a multivesicular body in which one vesicle (arrow) shows projections on its surface like those seen on the coated vesicles of GERL (see Fig. 19). × 60,000.

FIGURE 37 Portion of a neuron from an unoperated ganglion. The thin section was stained only with lead. The delimiting membrane of a multivesicular body is continuous with a cisterna of smooth endoplasmic reticulum (*E*) (see Figs. 27, 34). The presence of the membrane indicated by the arrow may indicate that the delimiting membrane arises by flattening and close apposition (compaction, see reference 58) of two or more membranes (see Fig. 28), perhaps the membranes of a cisterna of endoplasmic reticulum. However, serial sections are needed to rule out possible optical artifacts like the superimposition of two images of the same membrane cut at different levels of the section. × 100,000.

material, this often appears as a series of short projections perpendicular to the surface (Fig. 28). A similar coating may be seen (Figs. 27, 29, 35) on regions of the smooth endoplasmic reticulum in GERL.[3] Such regions of coated endoplasmic reticulum frequently appear as if surrounding other bits of endoplasmic reticulum and vesicles (Figs. 29–31, 35). Similar observations have been made on dorsal root ganglia, both normal ganglia

[3] Although the short projections are regularly present, the appearance of the coating on multivesicular bodies and endoplasmic reticulum (and coated vesicles) is variable. Sometimes (see upper arrow in Fig. 31) a continuous line is seen with or without the

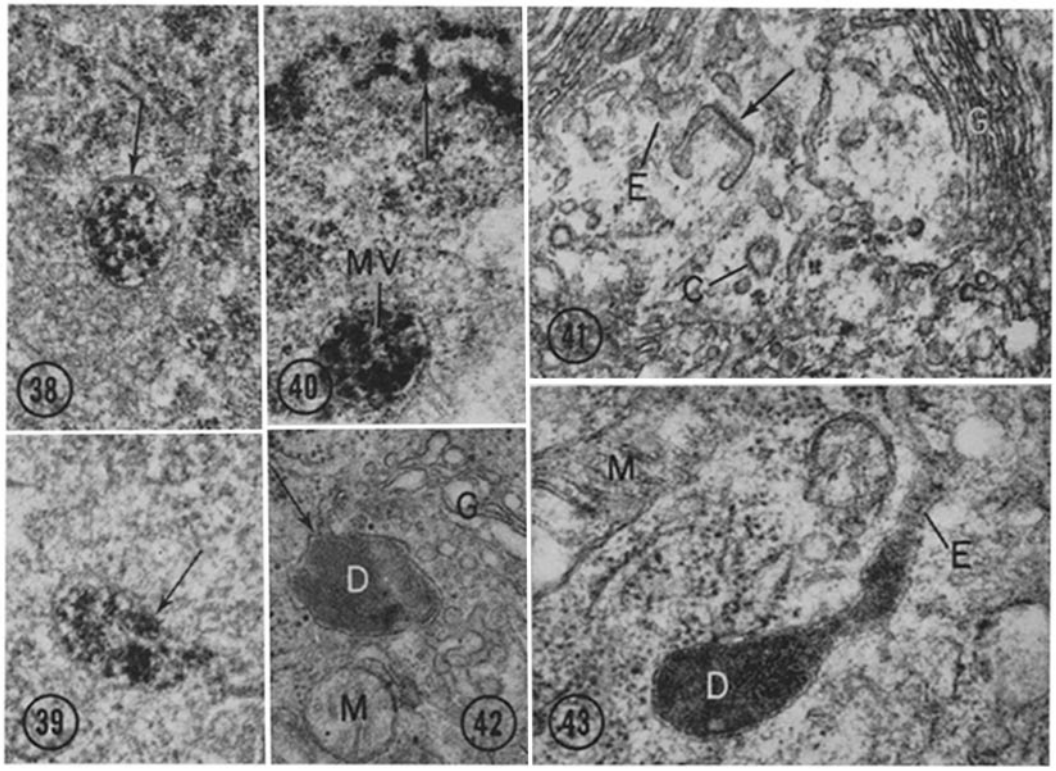


FIGURE 38 Portion of a neuron from a ganglion 2 days after operation, incubated for acid phosphatase (34 min). Reaction product is seen within a multivesicular body which shows a coating on part of its surface (arrow). × 46,000.

FIGURE 39 Another multivesicular body from the same preparation shown in Fig. 38. Reaction product (arrow) is seen within small tubules or vesicles inside the multivesicular body × 79,000.

FIGURE 40 Portion of a neuron from an unoperated ganglion, incubated for IDPase activity (55 min). Reaction product is present in GERL (arrow) and within a multivesicular body (*MV*) (see Fig. 14). × 38,500.

FIGURE 41 Portion of the GERL region from a neuron of a dorsal root ganglion of a rat injected intraperitoneally, twice in the 3 hr prior to fixation, with malononitrile (4.6 mg total; see reference 87). Fixed with cacodylate-buffered glutaraldehyde followed by phosphate-buffered osmium tetroxide. A structure that is probably a multivesicular body in the process of formation is indicated by the arrow. (See Fig. 28). Coated endoplasmic reticulum appears as if forming a cuplike structure. A region of smooth endoplasmic reticulum of GERL is seen at *E*, a coated vesicle at *C*, and Golgi saccules at *G*. × 38,500.

FIGURE 42 Portion of a neuron from a ganglion 5 days after operation. Fixed in phosphate-buffered glutaraldehyde. The arrow indicates continuity of the delimiting membrane of a dense body (*D*) with endoplasmic reticulum of GERL. A mitochondrion is seen at *M*, and saccules of the Golgi apparatus at *G*. × 36,500.

FIGURE 43 Portion of a neuron from an unoperated ganglion, showing a dense body (*D*) in the GERL region. The dense body is continuous with smooth endoplasmic reticulum (*E*) containing dense material of similar nature. A mitochondrion is seen at *M*. × 53,500.

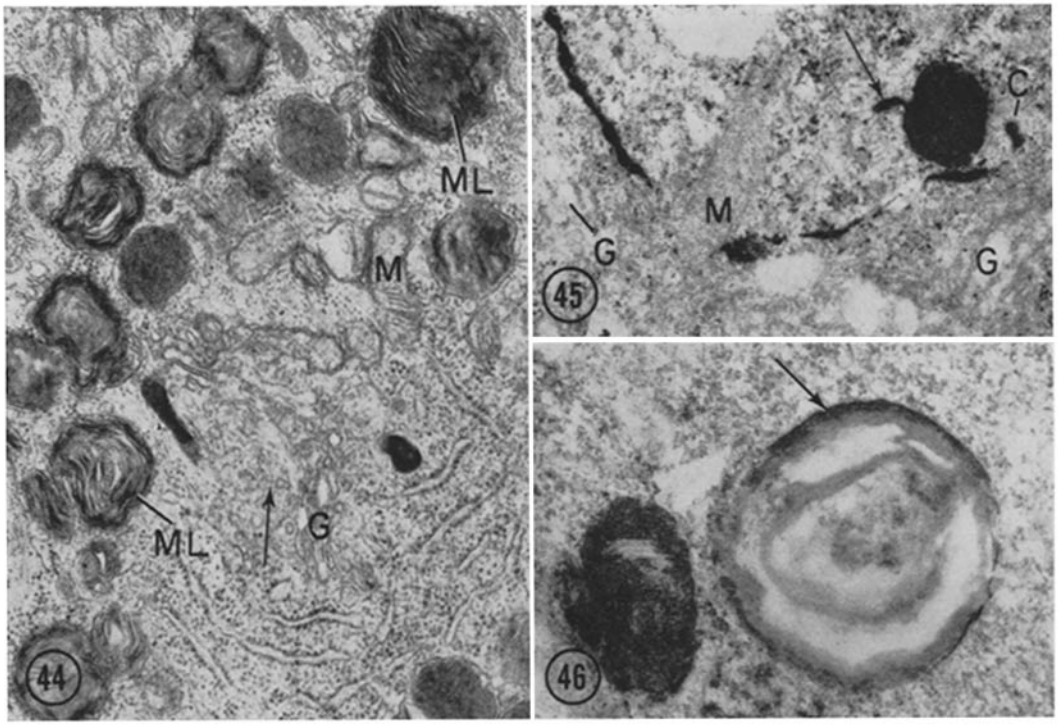


FIGURE 44 Portion of a neuron from a ganglion 5 days after operation. Fixed with phosphate-buffered glutaraldehyde. Many multilamellated bodies are present (*ML*). Part of the Golgi apparatus is seen at *G*, and part of GERL is seen at the arrow. A mitochondrion is present at *M*. × 28,000.

FIGURE 45 Portion of a neuron from an unoperated ganglion, incubated for acid phosphatase (30 min). Reaction product is seen in a dense body, in endoplasmic reticulum of GERL with which it is continuous (arrow), and in a coated vesicle (*C*). The Golgi apparatus (*G*) and mitochondrion (*M*) show no reaction product. Some unspecific deposits of lead phosphate are seen throughout the field. × 33,000.

FIGURE 46 Portion of a neuron from a ganglion 13 days after operation, incubated for acid phosphatase (36 min). Reaction product (arrow) is seen in a large multilamellated body. The body to the left may be a similar lysosome sectioned near its surface, or else a dense body. × 59,500.

and malononitrile-treated ganglia. In addition, cuplike structures are sometimes seen (Fig. 41), occasionally with a few vesicles within the delimiting cisterna.

In addition, we have encountered within multivesicular bodies occasional coated vesicles (Fig. 36) (see reference 63) and membrane-bounded granules of types found free in the cytoplasm fairly frequently (Figs. 32–34) (see references 64, 65, 76). In sections from tissue incubated for acid phosphatase (see references 53, 63, 65–67) or IDPase, reaction product is seen in the multivesicular bodies as well as in GERL[4] (Figs. 14, 38–40). In at least some cases reaction product for both these enzymes is associated chiefly with the vesicles and not the matrix (Figs. 38–40).

Continuities of dense bodies with endoplasmic reticulum are observed frequently, before and

projections, and occasionally images are encountered suggesting flattened cisternae such as those described for multivesicular bodies by Palay (62). Whether this reflects differences in plane of section or preparative procedures or the existence of different types of coats remains to be determined.

[4] In both acid phosphatase and IDPase preparations some multivesicular bodies are seen without reaction product. Thus we can not yet say whether the same multivesicular body has both enzyme activities.

after operation (Figs. 42, 43, 45). Sometimes, as in Fig. 43, the images obtained suggest formation of the dense body by focal dilatation of endoplasmic reticulum which becomes filled with dense material (see references 31, 34).

After 4–5 days, an increasing proportion of the cells shows, in addition to the other types of lysosomes, many large bodies with multilamellated structure (Fig. 44) that contain reaction product in preparations incubated for demonstration of acid phosphatase (Fig. 46). At this time the Golgi apparatus and GERL become less prominent than in the first few days after operation (Fig. 44).

DISCUSSION

Its ability to integrate phenomena of such varying morphological aspects is, we believe, one of the most useful aspects of the lysosome concept. It is still too early to specify in all cases the specific physiological roles of different lysosomes and of the related structures such as GERL which, together with the lysosomes, constitute an intracellular digestive apparatus (14). However, the variety of lysosomes in perikarya described in this report and in axons and Schwann cells described earlier (66) is remarkable.

It is self-evident that the study of pathological conditions, such as chromatolysis, will permit a better understanding of the capabilities of neurons in normal physiology and in response to injury. Thus the increase, during chromatolysis, in the number of autophagic vacuoles in which Nissl material commonly is sequestered suggests that these lysosomes are involved in metabolism of nucleoproteins and other macromolecules in a situation involving remodeling of the neuron preparatory to renewed growth. The regular presence of autophagic vacuoles in neurons from unoperated ganglia may reflect participation of these bodies in normal nucleoprotein turnover as well. From this point of view chromatolysis would represent an intensification of normal cellular mechanisms. Autophagic vacuoles also have been seen in the proximal portion of injured axons (66, 69) and in perikarya subjected to radiation (70) or prolonged incubation in vitro (71).

As do previous studies (31, 72), the present observations on the nodosal ganglion emphasize the role of Golgi-associated smooth endoplasmic reticulum, GERL, in the origin of lysosomes. They are consistent with the origin of the dense bodies as focal dilatations of GERL (31, 72), and they add some new information regarding the properties of the coated vesicles found in this area of the neuron and the sequestration of cytoplasmic structures by smooth endoplasmic reticulum.

Association of smooth endoplasmic reticulum with the delimiting membranes of autophagic vacuoles, as described above, also has been seen in other cells (58). Our observations are consistent with the suggestion made in a previous report (58) that the membrane surrounding these vacuoles arises by flattening and close apposition or "compaction" of the membranes of a cisterna of endoplasmic reticulum.

Multivesicular bodies also show continuities with the smooth endoplasmic reticulum. At present, "multivesicular bodies" are too ill defined to permit generalization. There may be different types that originate in different ways (see references 14, 42, 68, 73–77, 83, 84). Our observations suggest that the delimiting membranes of some of these bodies arise from GERL. Possible mechanisms include the formation of cuplike structures by the folding of a region of the smooth endoplasmic reticulum to include bits of cytoplasm or the dilatation and infolding of a portion of the reticulum. Some of the inner vesicles of multivesicular bodies may represent fragments of smooth endoplasmic reticulum or vesicles from the GERL region. Others appear to arise within the multivesicular body from infolded portions of the delimiting membranes (see references 42, 68, 74).

The similarity of the inner vesicles of the multivesicular bodies to those seen in the Golgi region has been noted previously (42, 68, 74–76, 84). It also has been suggested that some of the bodies develop from material originating at the cell surface (61, 73, 86). Cuplike and multivesicular bodies are also seen near the neuronal surface. It is possible that some have moved into the GERL region.

On the basis of their occasional content of recognizable cytoplasmic material, it may be appropriate to think of some of the multivesicular bodies in these neurons as a type of autophagic vacuole. Both multivesicular bodies and autophagic vacuoles containing mitochondria, ribosomes, or endoplasmic reticulum apparently are formed by sequestration of cytoplasm within a delimiting membrane derived from or closely associated with endoplasmic reticulum. Whether

a primary function of the multivesicular bodies is degradation of cytoplasmic structures, as is thought to be the case for autophagic vacuoles, remains to be seen (see references 65, 90).

The large multilamellated lysosomes found in the later stage of chromatolysis probably include residual bodies derived from the autophagic vacuoles and multivesicular bodies. Both autophagic vacuoles and multivesicular bodies often are seen with a dense internal matrix (see references 58, 64, 65, 78); these may be transitional forms. Multilamellated bodies also may arise de novo or by transformation of preexisting dense bodies (see reference 79). Although occasionally dense bodies and multilamellated bodies are seen grouped at the periphery of the perikarya, in axons, and at the region of origin of the axon, the ultimate fate of these lysosomes has not been studied.

To our knowledge, the presence of acid phosphatase activity in coated vesicles has not been reported previously. Taken together with the presence of a delimiting membrane, this activity satisfies the basis for tentative identification of these bodies as lysosomes (see reference 53). There is no evidence to suggest a relationship of these vesicles to the more peripherally situated "coated" vesicles described in other neurons (61, 80) and occasionally seen in neurons of the nodosal ganglion.

Although in these perikarya a substantial proportion of the lysosomes forms from GERL, some of the dense bodies and autophagic vacuoles appear to originate from smooth endoplasmic reticulum at some distance from the Golgi apparatus. This is also the case in peripheral nerves and other cells where lysosomes form from smooth endoplasmic reticulum (66, 81). The continuities of rough and smooth endoplasmic reticulum, known to occur in neurons since the studies of Palade and Palay (see references 54 and 85), may provide a pathway for the transport of lysosomal enzymes from their site of synthesis to the lysosomes. The mechanisms controlling the sites of formation and the nature of the lysosomes formed by a given portion of endoplasmic reticulum at present are completely unknown. It may be that observations of morphologically distinct regions of endoplasmic reticulum, like the coated smooth endoplasmic reticulum described in this report, will ultimately provide a clue to these mechanisms.

This work was supported by an American Cancer Society, Inc. postdoctoral fellowship (PF-297) to Eric Holtzman and by research grants from the U.S. Public Health Service (CA-06576) and the Damon Runyon Memorial Fund (DRG-872XT) to Alex B. Novikoff. Dr. Novikoff is a recipient of a Public Health Service Research Career Award (5-K6-CA-14,923) from the National Cancer Institute.

We wish to thank Regina Forschirm for preparation and incubation of the frozen sections, Arline Albala for help with the electron microscopy, Jack Godrich for preparation of the photographic prints, and Doctors Sidney Goldfischer and Luis Biempica for invaluable discussions and suggestions.

Portions of this material have been presented before (88–90).

Received for publication 31 August 1966.

REFERENCES

1. Ramon Y Cajal, S. 1928. Degeneration and Regeneration of the Nervous System. R. M. May, translator and editor. Oxford University Press, London. 2 volumes.
2. Bielchovsky, M. 1932. *In* Cytology and Cellular Pathology of the Nervous System. W. Penfield, editor. Hafner Publishing Co., Inc., New York. 1:145.
3. Nicholson, F. M. 1923. *Arch. Neurol. and Psych.* 11:680.
4. Hyden, H. 1960. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press Inc., New York. 4:215.
5. Greenfield, J. G., and A. Myer. 1963. *In* Greenfield's Neuropathology. The Williams & Wilkins Co., Baltimore. 2nd edition. 1.
6. Bodian, D., and R. C. Mellors. 1945. *J. Exptl. Med.* 81:469.
7. Andres, K. H. 1961. *Z. Zellforsch. Mikroskop. Anat.* 55:49.
8. Pannese, E. 1963. *Z. Zellforsch. Mikroskop. Anat.* 60:711.
9. Porter, K. R., Bowers, M. B., and students. 1963. *J. Cell Biol.* 19:56A.
10. Evans, D. H. L., and E. G. Gray. 1961. *In* Proceedings of the Anatomical Society of Great Britain and Ireland. Taylor & Francis Ltd., London. 71.
11. Smith, K. R. 1961. *J. Comp. Neurol.* 116:103.
12. Hudson, G., A. Lazarow, and J. F. Hartmann. 1961. *Exptl. Cell Res.* 24:440.
13. Luft, J. H. 1961. *J. Biophys. Biochem. Cytol.* 9:409.

14. De Duve, C., and R. Wattiaux. 1966. *Am. J. Physiol.* **28**:435.
15. Novikoff, A. B. 1967. Enzyme Localization and Ultrastructure of Neurons. *Progr. Brain Res.* In press.
16. Sabatini, D. D., K. Bensch, and R. J. Barnett. 1963. *J. Cell Biol.* **17**:19.
17. Webster, H. de F., and G. H. Collins. 1964. *J. Neuropathol. Exptl. Neurol.* **23**:109.
18. Fahimi, H. D., and P. Drochmans. 1965. *J. Microscop.* **4**:737.
19. Millonig, G. 1962. *In* Proceedings of the Fifth International Congress for Electron Microscopy. S. S. Breese, editor. Academic Press Inc., New York. **2**:8.
20. Palade, G. E. 1952. *J. Exptl. Med.* **95**:285.
21. Caulfield, J. B. 1957. *J. Biophys. Biochem. Cytol.* **3**:827.
22. Baker, J. R. 1946. *Quart. J. Microscop. Sci.* **87**: 441.
23. Gomori, G. 1952. Microscopic Histochemistry. University of Chicago Press, Chicago.
24. Novikoff, A. B. 1963. *In* Ciba Foundation Symposium on Lysosomes. A. V. S. de Reuck and M. P. L. Cameron, editors. Little, Brown and Company, Boston. 36.
25. Novikoff, A. B., and S. Goldfischer. 1961. *Proc. Natl. Acad. Sci. U. S.*, **47**:802.
26. Karnovsky, M. J. 1964. *J. Cell Biol.* **23**:217.
27. Novikoff, A. B. 1963. *In* First International Congress of Histochemistry and Cytochemistry. Pergamon Press, Inc. Oxford. 465.
28. Taft, E. B. 1951. *Exptl. Cell Res.* **2**:312.
29. Watson, M. L. 1958. *J. Biophys. Biochem. Cytol.* **4**:475.
30. Reynolds, E. S. 1963. *J. Cell Biol.* **17**:208.
31. Novikoff, A. B. 1967. Lysosomes in nerve cells. *Progr. Brain Res.* In press.
32. Novikoff, A. B. 1964. *Biol. Bull.* **127**:358.
33. Novikoff, A. B., N. Quintana, H. Villaverde, and R. Forschirm. 1964. *J. Cell Biol.* **23**:68A.
34. Novikoff, A. B., E. Essner, and N. Quintana. 1964. *Federation Proc.* **23**:1010.
35. Novikoff, A. B., E. Essner, S. Goldfischer, and M. Heus. 1962. *Symp. Intern. Soc. Cell Biol.* **1**:149.
36. Tennyson, V. M., M. Brzin, and P. E. Duffy. 1965. *J. Cell Biol.* **27**:105A.
37. Torack, R. M., and R. J. Barrnett. 1967. *Exptl. Neurol.* **6**:224.
38. Fukuda, T., and G. B. Koelle. 1959. *J. Biophys. Biochem. Cytol.* **5**:433.
39. Novikoff, A. B., N. Quintana, H. Villaverde, and R. Forschirm. 1966. *J. Cell Biol.* **29**:525.
40. Taxi, J. 1961. *Bibliotheca Anat.* **2**:73.
41. Bodian, D. 1962. *Science.* **137**:321.
42. Hicks, R. M. 1966. *J. Cell Biol.* **30**:623.
43. Kumamoto, T., and G. H. Bourne. 1963. *Acta. Anat.* **55**:255.
44. Barron, K. D., and S. Sklar. 1961. *Neurology.* **11**:866.
45. Fisher, A. W. F., and S. D. Sutherland. 1965. *J. Anat.* **99**:585.
46. Penfield, W. G. 1920. *Brain.* **43**:290.
47. Moussa, T. A. 1956. *La Cellule.* **57**:319.
48. Barron, K. D., and T. O. Tungbay. 1964. *J. Neuropathol. Exptl. Neurol.* **23**:368.
49. Koenig, H. L. 1965. *Arch. d'Anat. Microscop.* **54**:937.
50. Chacko, L. W., and J. A. Cerf. 1960. *J. Anat.* **94**:74.
51. Roth, T. F., and K. R. Porter. 1964. *J. Cell Biol.* **20**:313.
52. Palay, S. L. 1963. *J. Cell Biol.* **19**:89A.
53. Novikoff, A. B. 1961. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press Inc., New York. **2**:423.
54. Dallner, G., P. Siekevitz, and G. E. Palade. 1966. *J. Cell Biol.* **30**:73, 97.
55. De Duve, C. 1963. *In* Ciba Foundation Symposium on Lysosomes. A. V. S. de Reuck and M. P. L. Cameron, editors. Little, Brown and Co., Boston. 1.
56. Swift, H., and Z. Hruban. 1964. *Federation Proc.* **23**:1026.
57. Ashford, T. P., and K. R. Porter. 1962. *J. Cell Biol.* **12**:198.
58. Novikoff, A. B., and W.-Y. Shin. 1963. *J. Microscop.* **3**:187.
59. Pappas, G. D., and D. P. Purpura. 1961. *Exptl. Neurol.* **4**:507.
60. Bunge, R. P., M. B. Bunge, and E. R. Peterson. 1965. *J. Cell Biol.* **24**:163.
61. Rosenbluth, J., and S. L. Wissig. 1964. *J. Cell Biol.* **23**:307.
62. Palay, S. L. 1963. *J. Cell Biol.* **19**:54A.
63. Brandes, D., and E. Anton. 1966. *Lab. Invest.* **15**:987.
64. Palay, S. L. 1960. *Anat. Record* **138**:417.
65. Smith, R. E., and M. Farquhar. 1966. *J. Cell Biol.* **31**:319.
66. Holtzman, E., and A. B. Novikoff. 1965. *J. Cell Biol.* **27**:651.
67. Barka, T. 1963. *J. Am. Med. Assoc.* **183**:761.
68. Merker, H. J. 1965. *Z. Zellforsch. Mikroskop. Anat.* **68**:618.
69. Blumke, S., and H. R. Niedorf. 1965. *Beitr. Pathol. Anat. Allgem. Pathol.* **131**:38.
70. Forssmann, W. G., H. Tinquely, J. M. Posternak, and C. Rouiller. 1966. *Z. Zellforsch. Mikroskop. Anat.* **72**:325.
71. Forssmann, W. G., and Rouiller, C. 1966. *Z. Zellforsch. Mikroskop. Anat.* **70**:364.

72. NOVIKOFF, A. B., P. ROHEIM, and N. QUINTANA. 1966. *Lab. Invest.* **15**:27.
73. NICANDER, L. 1966. *J. Ultrastruct. Res.* **14**:424.
74. ERICCSON, J. L. E. 1964. *Acta. Pathol. Microbiol. Scand. Suppl.* **168**:1.
75. MEEK, C. A., and S. BRADBURY. 1963. *J. Cell Biol.* **18**:73.
76. TRUJILLO-CENOZ, O. 1962. *Z. Zellforsch. Mikroskop. Anat.* **56**:649.
77. SOTELO, J. R., and K. R. PORTER, 1959. *J. Biophys. Biochem. Cytol.* **5**: 327.
78. GORDON, G. B., L. R. MILLER, and K. G. BENSCH. 1965. *J. Cell Biol.* **25**:41.
79. PICK, J. 1965. *J. Cell Biol.* **26**:335.
80. ANDRES, K. H. 1964. *Z. Zellforsch. Mikroskop. Anat.* **64**:63.
81. LANE, N. J., and A. B. NOVIKOFF. 1965. *J. Cell Biol.* **27**:603.
82. SCHLAEPFER, W. W., and R. M. RORACK, 1966. *J. Histochem. Cytochem.* **14**:369.
83. PASTEELS, J. J., and E. DE HARVEN. 1963. *Arch. Biol. (Liege).* **74**:415.
84. REBHUN, L. I. 1960. *Ann. N. Y. Acad. Sci.* **90**:357.
85. PALAY, S. L., and G. E. PALADE. 1955. *J. Biophys. Biochem. Cytol.* **1**:69.
86. FARQUHAR, M. G., S. L. WISSIG, and G. E. PALADE. 1961. *J. Exptl. Med.* **113**:1.
87. ANDERSON, A., and V. L. VAN BREEMAN. 1958. *J. Biophys. Biochem. Cytol.* **4**:83.
88. HOLTZMAN, E., A. B. NOVIKOFF, H. VILLAVERDE, and R. FORSCHIRM. 1966. Proceedings of the 17th Annual Conference of the Histochemical Society, Atlantic City, April 1966. *J. Histochem. and Cytochem.* **14**:813.
89. HOLTZMAN, E., and A. B. NOVIKOFF. 1966. Abstracts of Symposium on Electron Microscopy and Cytochemistry, Leiden, The Netherlands, May 1966. *J. Histochem. and Cytochem.* **14**:748.
90. HOLTZMAN, E., and A. ALBALA. 1966. *J. Cell Biol.* **31**:49A.