Zeitschrift für Zellforschung 60, 711—740 (1963)

Institute of Human Anatomy (Laboratory of Neurocytology "P. Rando") University of Milaw
(Director: Prof. A. BAIRATI)

INVESTIGATIONS ON THE ULTRASTRUCTURAL CHANGES OF THE SPINAL GANGLION NEURONS IN THE COURSE OF AXON REGENERATION AND CELL HYPERTROPHY

I. CHANGES DURING AXON REGENERATION

By

ENNIO PANNESE*

With 19 Figures in the Text

(Received March 20, 1963)

Introduction

One of the characteristics of nerve cells is their poor adaptability to variations in the environmental conditions. This is reflected in the properties which have led to their inclusion in BIZZOZERO's class of "perennial elements" (i.e., early cessation of mitotic activity setting a fixed limit to the number of cells, early and irreversible differentiation, limited power of regeneration) and is also demonstrated by a series of other aspects, particularly apparent in the nerve cells of the central nervous system: e.g., much greater sensitivity to oxygen deficiency than that of other cell lines, occurrence of degenerative processes after section of the axon or interruption of the afferent connections (transneuronal degeneration), etc.

However, some types of neurons, namely those connected directly with the periphery (motor neurons and nerve cells of the sensory ganglia), show a greater capacity for self-regulation and adaptation, as evidenced by their reaction to axon division. This lesion, which deprives the neuron of a part of its cytoplasm and interrupts its connections with the peripheral field of innervation, is followed nearly always, in the mentioned types of nerve cells, by regeneration of the proximal stump of the axon. Besides, when the axon is severed and simultaneously its peripheral field of innervation is removed, the successive regeneration of the axon and of the peripheral territory appear strictly correlated the one with the other; therefore, the neuron undergoes hypertrophy when the peripheral field of innervation is increased.

The structural modifications occurring in the perikaryon after sectioning of the axon, have been studied extensively by light microscopy since the last decade of the past century (NISSL, 1892, 1894, 1896; LUGARO, 1896, 1897; MARINESCO, 1896, 1904; FLEMMING, 1897; VAN GEHUCHTEN, 1897; BIELSCHOWSKY, 1908; MARCORA, 1908; etc.).

Such studies have shown that interruption of the axon is readily followed by a series of structural changes which characterize the *reaction phase*, i.e., a) chromatolysis, b) modifications of the neurofibrils, c) swelling of the perikaryon, and d) displacement of the nucleus towards the periphery of the cell.

* Aknowledgement. The Author is greatly indebted to Miss A. BERTOLASI for drawing Figs. 1 and 19, and to Dr. phis. S. DE PETRIS for the help given in the statistical evaluation of the data.

The reaction phase is normally followed by a *phase of repair*, which usually starts 20—25 days after the lesion and lasts for a variable period of time. During this period the swelling of the perikaryon diminishes, the nucleus resumes its central position and the Nissl substance is restored in the cytoplasm. Many nerve cells exhibit a great increase in Nissl substance, hence their perikaryons stain intensely with basic dyes. Many of the nerve cells connected with the periphery regain their normal structural characteristics through such a series of stages.

Light microscopy, however, is inadequate to reveal the more intimate details of the changes occurring in nerve cells following axon severance. A deeper insight may be gained by electron microscopy (ANDRES, 1961; EVANS and GRAY, 1961; SMITH, 1961; CERVÓS-NAVARRO, 1962) or with the use of biochemical and histochemical technics (BODIAN and MELLORS, 1945; HOWE and MELLORS, 1945; HEINZEN, 1947; HOWE and FLEXNER, 1947; GEREBTZOFF and VANDERMISSEN, 1956; BRATTGÅRD et al., 1957; FISCHER et al., 1958; SCHWARZACHER, 1958; FRIEDE, 1959; BRATTGÅRD and HYDÉN, 1960; HYDÉN, 1960; MIANI et al., 1961).

In the present research, the analysis has been focused on the behaviour of nerve cells of the spinal ganglia of Sauria after tail amputation to test the degree of adaptability of neurons connected directly with the periphery. Under these conditions, a morphological study can be made, in the same type of cells, of the consequences of the severance and of the consecutive regeneration of the axon as well as of the changes pertaining to the hypertrophy of the perikaryon. A description will be given here only of the first set of structural changes. The hypertrophy of the perikaryon and the conclusions which can be drawn from a comparison of the two series of facts will be dealt with in a subsequent paper.

Materials and methods

The distribution of spinal nerves in the tail of the lizard *(Lacerta muralis)* is such, that section of the tail severs the majority of the axons running in three consecutive pairs of spinal nerves (Fig. 1). The sensory and motor nerve cells of these spinal nerves are particularly well suited for the study of the structural changes in the perikaryon which depend (a) on the section of the axon as well as (b) on the hypertrophy which the cells mentioned undergo as a consequence of the increase of their new peripheral field of innervation, viz. the whole regenerated part of the tail.

Mainly for practical reasons, our attention was focused on the spinal ganglia, which represent an excellent object for experimental studies (LEVI-MONTALCINI and LEVI, 1943). The spinal ganglia are in fact discrete organs with a rather simple architecture; the fairly regular shape of their nerve cells permits easy quantitative evaluations. Moreover, the techniques of fixation for electron microscopy give much more reproducible results in spinal ganglia than in the central nervous system, thus ensuring a better utilization of the material.

Eighteen specimens of adult *Lacerta muralis*, captured in July, were subjected to tail amputation and were then sacrificed 2, 4, 7, 14, 21, 30 and 60 days later. In the specimens sacrificed 7 to 21 days after amputation, the stump of the tail shows only a slight distal convexity, in those sacrificed after 30 days a small outgrowth is apparent, and after 60 days the regenerated part of the tail measures roughly 1 cm in length.

The last pair of spinal ganglia cranial to the plane of amputation, was removed for study. Spinal ganglia taken from levels not affected by the tail amputation and regeneration were used as controls.

The isolated ganglia were fixed in 2% OsO_4, buffered at pH 7.2 with veronal-acetate, and in Carnoy's fluid. The material fixed in OsO_4, was embedded in methacrylates or in araldite, sectioned with the Porter-Blum and LKB ultramicrotomes and examined with a Siemens Elmiskop II electron microscope. Uranyl acetate was used on several occasions to stain either sections or tissue blocks during dehydration.

In most cases sections 0.5 to 1.0 μ thick were cut adjacent to the ultrathin ones, mounted in glycerol or in Canada balsam and examined at polarized light (Leitz-Ortholux supplied with a xenon lamp) or stained with toluidin blue. Thus, the same perikarya could be studied with the electron and the optical microscopes.

The ganglia fixed in Carnoy's fluid were embedded in paraffin and the sections were stained with the Nissl method or used for histochemical tests. Some sections were treated at 37° C or 55° C for 60 min with 1/5,000 ribonuclease[1] dissolved in distilled water or in a buffered solution at pH 7.0, were stained with 0.5% toluidin blue (at pH 4.7 for 90 min), then kept for 5 min in an ammonium molybdate solution, according to the method suggested by Lison (1953). Other sections were stained with the Feulgen method.

Mitochondrial counts were carried out on electron micrographs. Since the distribution of mitochondria is not uniform even in control nerve cells, counts were carried out only on equatorial sections of the cells passing through the nucleus. Each section was photographed and the negative enlarged to a final magnification of × 10,000. All the mitochondrial profiles in the section were counted; their number ranging from one to ten or eleven hundred. The area of the perikaryon (nucleus excluded) was then measured. The number of mitochondrial profiles per square micron of section was obtained by dividing the total number of profiles in a perikaryal section by the area of the latter in microns. From this value it is difficult to estimate the number of mitochondrial profiles per unit volume, without severe errors of calculation. Nevertheless, the value per square micron of section is sufficiently indicative of mitochondrial frequency for the comparison of various nerve cells.

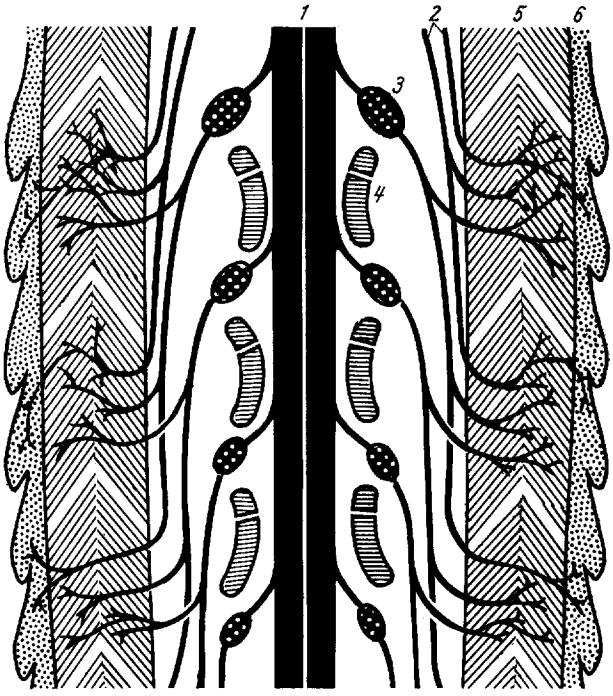

Fig. 1. Distribution of spinal nerves in a portion of a lizard's tail Each pair of spinal nerves supplies three adjacent tail segments; the spinal ganglia decrease in size in cranio-caudal direction. *1* spinal cord, *2* spinal nerves, *3* spinal ganglia, *4* vertebrae, *5* muscle, *6* skin

To determine whether the size of mitochondria varied in different nerve cells, the perikaryal sections selected for mitochondrial counts were photographed again and the negatives enlarged to × 22,500. The length of all the mitochondrial profiles in each section, was measured on the enlarged micrographs. A mean value per perikaryal section was calculated from the above measurements. The values thus obtained are certainly lower than the real mean length of the mitochondria, but they permit to make a rough comparison between various nerve cells. Since in the cells whose preservation appeared to be excellent, the thickness of the mitochondria does not vary appreciably in different nerve cells, the average length was taken as indicative of mitochondrial size in each cell. All the numerical data were subjected to statistical analysis.

[1] Salt and protease free crystalline ribonuclease (General Biochemical Inc.).

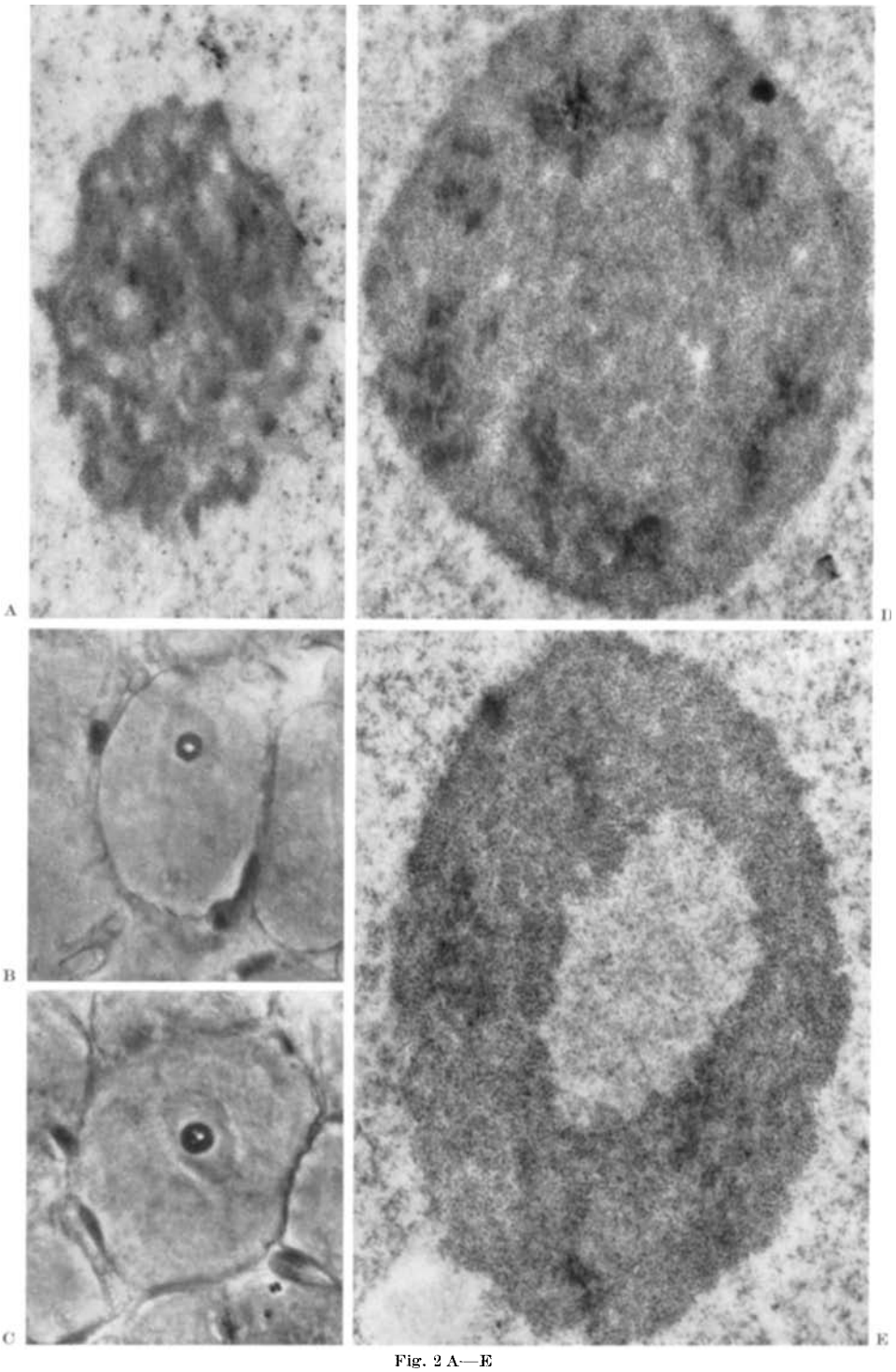

Fig. 2 A—E

Observations

Many control nerve cells, belonging to the spinal ganglia not affected by the operative procedure, were examined. The structure of these cells corresponds closely to that already described for nerve cells of mammalian spinal ganglia.

All the spinal ganglia of the sectioned nerves contained some nerve cells which did not show any structural modifications. Obviously, these were nerve cells whose axons had escaped section, either because they ramified and ended

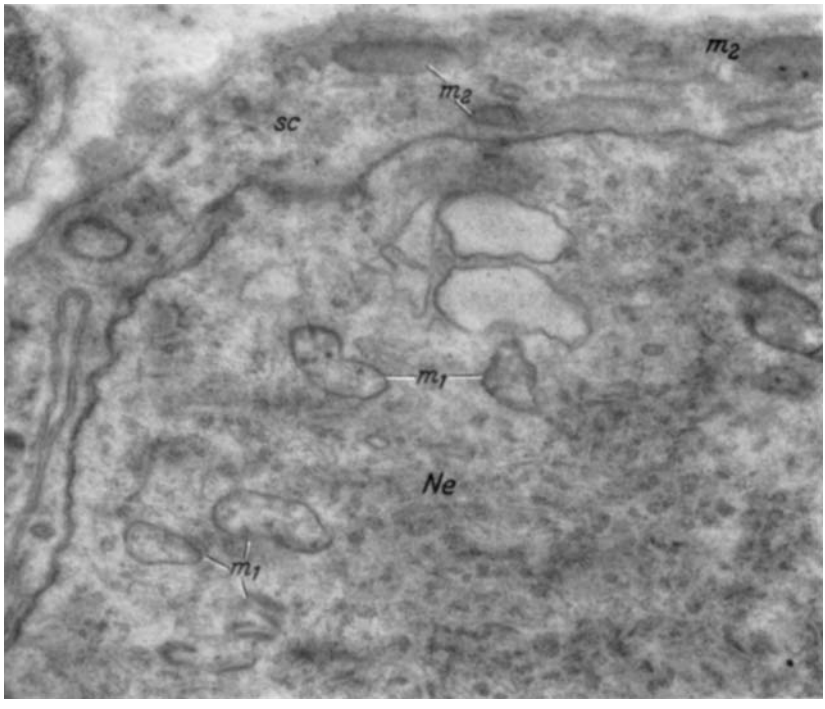

Fig. 3. Spinal ganglion, 2 days after nerve section. Araldite embedding, electron micrograph (22,500 ×). The mitochondria (m_1) of the nerve cell (*Ne*) are slightly swollen, their matrix is rather electron transparent; the mitochondria (m_2) of the satellite cells (*sc*) retain their normal structure

within the ganglia, or because they branched in the immediate vicinity of the ganglia. The following account is related, therefore, only to nerve cells showing structural modifications.

2 and 4-day stage after tail amputation. Two days after section of the axon only the nucleolus and the mitochondria shows some changes.

The nucleolus (Fig. 2) is often enlarged in comparison to that of control nerve cells: the maximum diameter is ca. 3 μ instead of 2,5 μ, and the other diameters are modified proportionally. The mean nucleolar increase in volume is about 60%.

Fig. 2 A—E. Spinal ganglia after nerve section. Modifications in the structure and size of nerve cell nucleoli. A, D, E: araldite embedding, electron micrographs (22,500 ×); B, C: phase contrast photomicrographs (850 ×). A: nucleolus of a control nerve cell; D: nucleolus of a nerve cell after axon section, showing the appearance described as (α) in the text; B, C, E: nucleoli of nerve cells after axon section, containing light "vacuoli" (described as β in the text)

The nucleolus shows also some structural changes. In phase contrast it often appears as a very dark ring circling a lighter central core (Fig. 2 B, C). After treatment with basic dyes, the peripheral region shows intense basophilia while the central core appears more lightly stained. The basophilia disappears after treatment with ribonuclease. The nucleolus is uniformly Feulgen-negative.

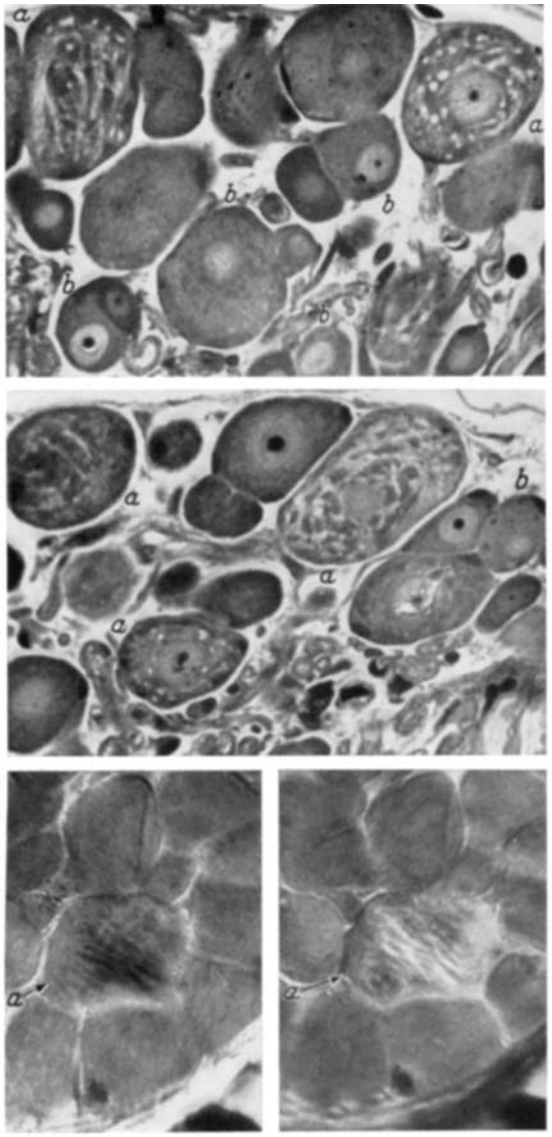

Fig. 4 A—D. Spinal ganglia, 7 days after nerve section. A, B: photomicrographs, Nissl staining (600 ×); C: photomicrograph at polarized light (Brace compensator) (500 ×); D: the same as C, but the position of the Brace compensator has been changed (500 ×). *a* perikarya containing stainless streaks, birefringent at polarized light; *b* chromatolytic nerve cells

With the electron microscope the nucleolus shows one of the following two aspects: (α) In some cases, it is made up of a very large number of electron opaque granules ca. 150—200 Å thick, tightly and uniformly packed (Fig. 2 D). (β) In other cases, the nucleolus consists of an electron opaque "cortex" and a lighter central core, the two being separated by a fairly sharp boundary. The outer cortical layer is composed of closely packed granules, 150—200 Å in diameter; the lighter central core consists of a few electron opaque granules, 300 Å in diameter, embedded in an electron transparent matrix (Fig. 2 E).

The lighter central region, described under (β), may be interpreted in two alternative ways: it may be due to an invagination of the nucleolar surface, or to a vacuole of the nucleolus proper. The latter hypothesis seems the more probable because the nucleolar outline appeared always very regular and no large indentation is apparent by optical and electron microscopy. Moreover, no evidence was ever found of a continuity between the lighter core and the nucleoplasm.

The mitochondria appear slightly swollen, their matrix being more electron transparent than that of mitochondria in control nerve cells. It appears unlikely

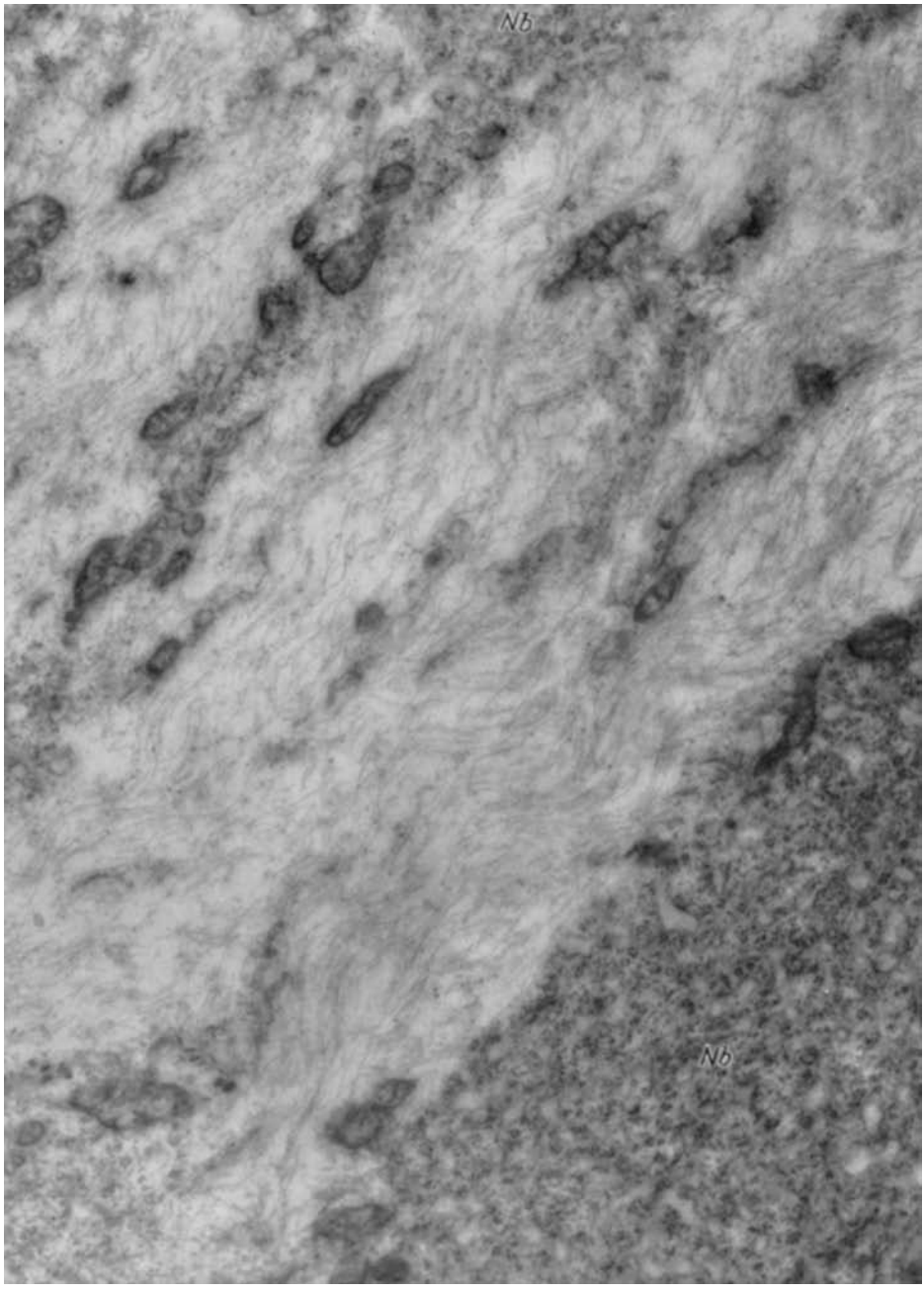

Fig. 5. Spinal ganglion, 7 days after nerve section. Methacrylate embedding, electron micrograph (22,500 ×). Part of the cytoplasm of a ganglion cell (described as *a* in the text) containing many neurofilaments and Nissl bodies (*Nb*). The Nissl bodies show a normal structure. The bundle of neurofilaments corresponds to one of the birefringent bands, which do not stain with the Nissl method

that the mitochondrial swelling may depend on technical artifacts, since the mitochondria of satellite cells enveloping the perikarya display a normal structure (Fig. 3).

While the volumetric and structural modifications of the nucleoli last a long time, the mitochondrial alterations regress so rapidly, that they can no longer be observed at the 7-day stage.

7-day stage after tail amputation. NISSL stained sections[1] examined with the light microscope revealed normal nerve cells and two groups of nerve cells showing markedly different modifications (Fig. 4). About 12% of the ganglion cells (indicated with *a*) are characterized by the presence in their cytoplasm of stainless, birefringent streaks arranged in various directions. A second, larger group of nerve cells (*b*) is typically chromatolytic. This group is represented by cells in which the process of chromatolysis appears more or less intense. Some can hardly be distinguished from the normal nerve cells; therefore it is difficult to give an exact percentage of these elements.

In the nerve cells (*a*), the stainless streaks appear rather transparent at the electron microscope and consist of bundles of neurofilaments embedded in a homogeneous electron transparent material (Fig. 5). The neurofilaments, 70—80 Å in diameter, appear wavy and arranged in a fairly orderly manner (Fig. 6). All the other cytoplasmic structures (i.e., endoplasmic reticulum, ribosomes, mitochondria, Golgi complexes, vesicular bodies, pigment granules) lie in basophilic areas of cytoplasm intervening between the bundles of neurofilaments (Fig. 5). It must be stressed that the endoplasmic reticulum in these nerve cells is as well developed as in normal nerve cells.

Table 1. *Mean number of mitochondrial profiles per square micron of section in control nerve cells and in nerve cells following axonal section*

	A	*B*	*C*
Control nerve cells	1.42	± 0.562	
Nerve cells 7 days after axonal section*	1.00	± 0.365	$t = 2.98$; $0.001 < P < 0.01$
Nerve cells 21 days after axonal section*	0.88	± 0.184	$t = 3.35$; $0.001 < P < 0.01$

A = average number of mitochondrial profiles per square micron of section; B = standard deviations; C = the t-values are those for the standard error test for small samples (Student distribution); P indicates the probability of this value being exceeded in random sampling; t and P values on each line refer to a comparison between the mean value on that line and the mean value of the first one.

* Mean values referring to all the cells at each stage.

Mitochondria are scanty among the neurofilaments and among the profiles of the endoplasmic reticulum, more numerous at the periphery of Nissl bodies. On the whole, their number is nearly the same in the perinuclear and in the peripheral regions of the perikaryon. In these nerve cells the average number of mitochondrial profiles is less than one (0.67) per square micron of section (cf. Table 2).

[1] The different structural aspects of nerve cells, observed under the present experimental conditions, will be designated in the text and figures with letters of the alphabet. This is not an attempt at a classification, but is intended only to render easier and quicker the cross-references between text and figures or the various sections of the text.

Nerve cells (*b*) show conspicuous changes of the profiles of the endoplasmic reticulum and of the ribosomes (Figs. 7, 8, and 9). Structures of the ergastoplasmic type are pratically absent[1]. Remnants of the ergastoplasm, represented by 3 or 4 short cisternae with an irregular outline in parallel arrangement, are found occasionally in restricted areas (Fig. 9). The endoplasmic reticulum consists essentially of membrane-bound vesicular elements of various dimensions (400 to 4.000 Å ca. in diameter) (Figs. 8, 9, and 10). Communications between vesicles are an extremely rare finding; hence it seems reasonable to suppose that the majority of vesicles represent closed units. Usually the vesicles contain an electron transparent material in which minute granules of low density are embedded. Ribosomes attached to membranes are scanty, and free ribosomes abundant in the cytoplasmic matrix (Fig. 9). Only a few ribosomes are clustered in rosette formations; the majority appear evenly distributed.

[1] In the present and in the next paper the term "endoplasmic reticulum" will be used to indicate cisternae arranged at random, limited by a membrane studded with ribosomes. The term "ergastoplasm" will be confined instead to cisternae arranged in an orderly pattern, which may be considered a special type of the endoplasmic reticulum.

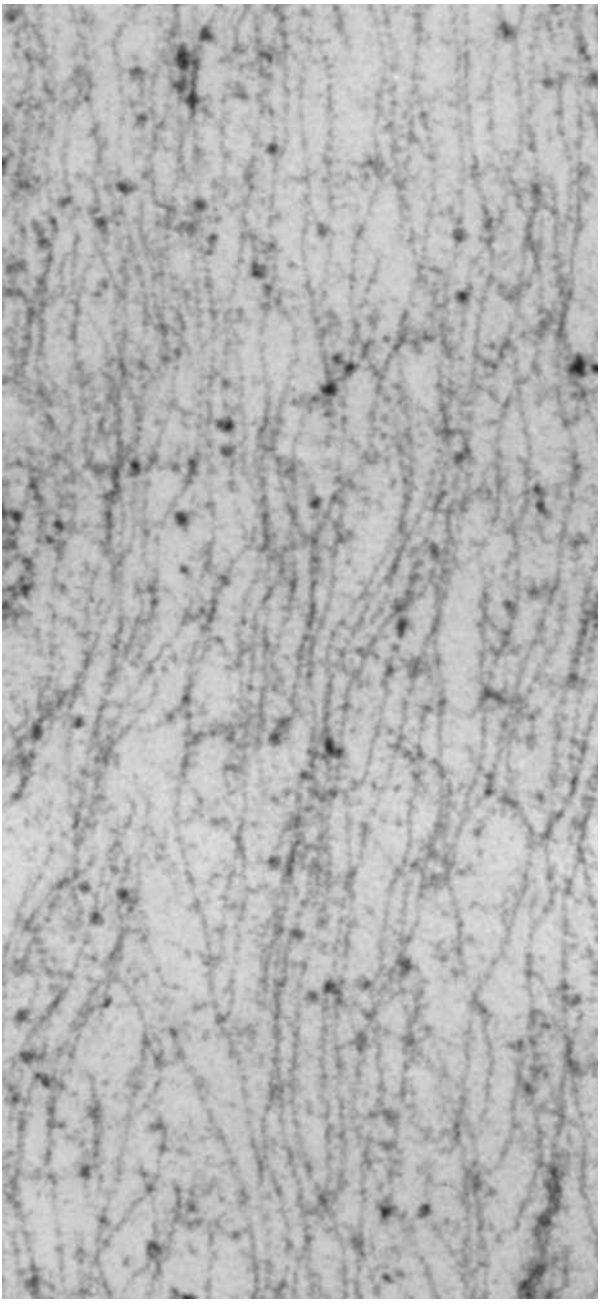

Fig. 6. Spinal ganglion, 7 days after nerve section. Methacrylate embedding, electron micrograph (100,000 ×). Bundle of neurofilaments in the perikaryon of a neuron (described as *a* in the text)

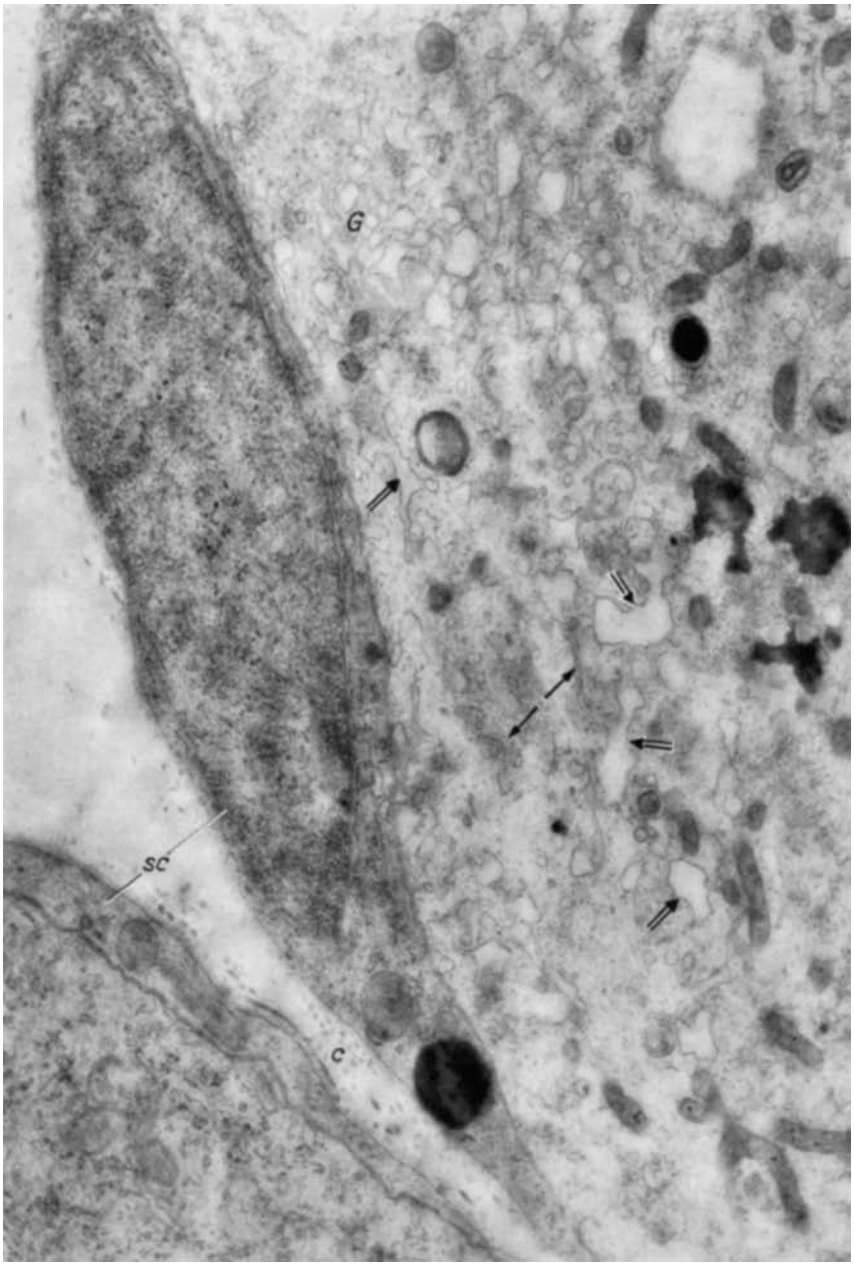

Fig. 7. Spinal ganglion, 7 days after nerve section. Araldite embedding, electron micrograph (22,500 ×). The ganglion cell on the right shows early indications of chromatolysis. Some cisternae of the endoplasmic reticulum retain their normal structure (single arrows), others are irregularly enlarged (double arrows). Swollen vesicles of the Golgi complex (*G*). The ganglion cell at the lower left retains a normal structure. *c* collagen; *sc* satellite cells

Some nerve cells show transitional characters between the normal elements of the endoplasmic reticulum (cisternae and tubules) and the closed vesicles mentioned, represented by irregular swellings of cisternae and tubules (Fig. 7)

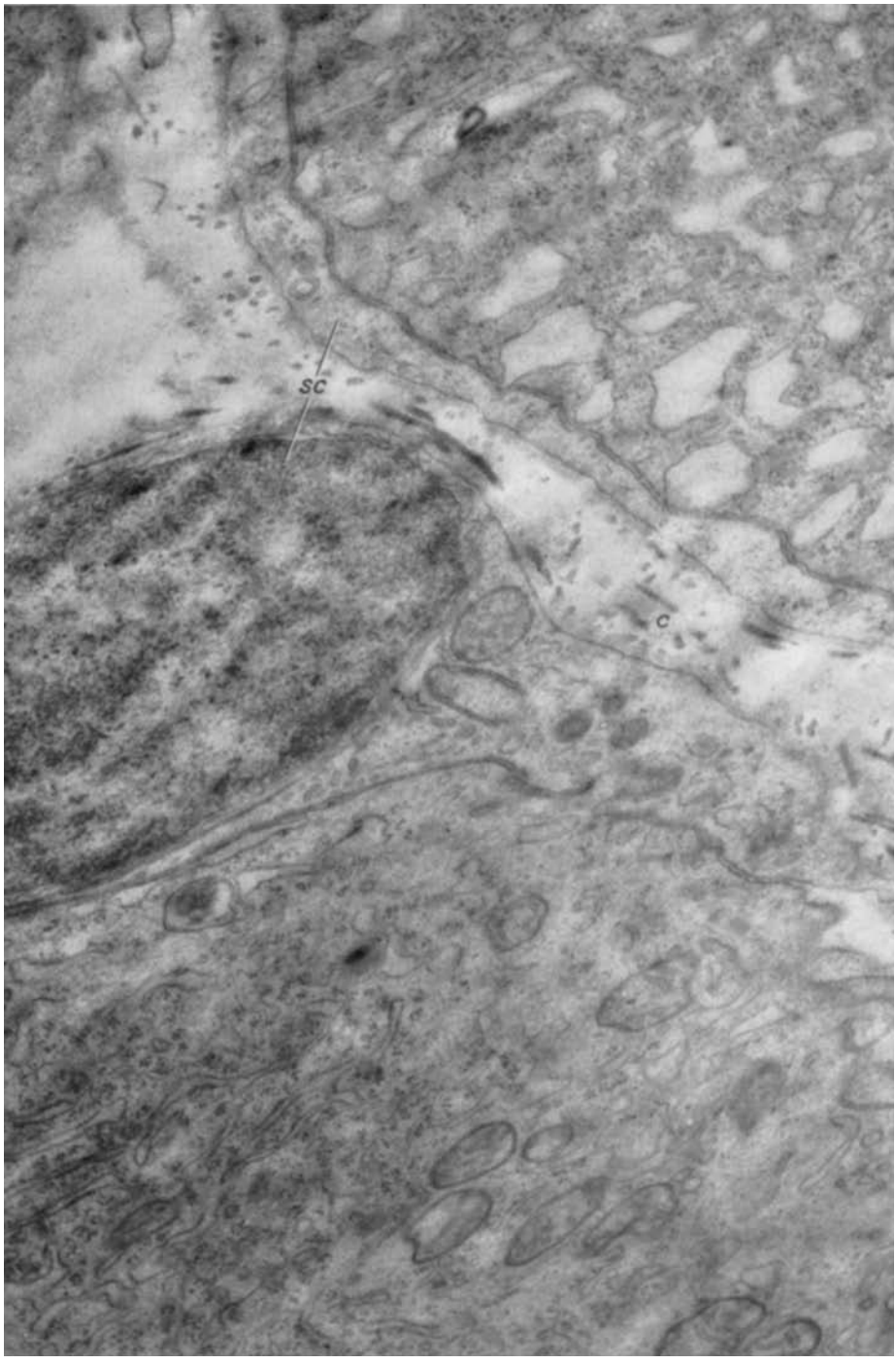

Fig. 8. Spinal ganglion, 7 days after nerve section. Araldite embedding, electron micrograph (30,000 ×). Two adjacent neurons: one chromatolytic (upper right), with enlarged cisternae of the endoplasmic reticulum and ribosomes mostly free, the other (lower part) normally structured. *c* collagen; *sc* satellite cells

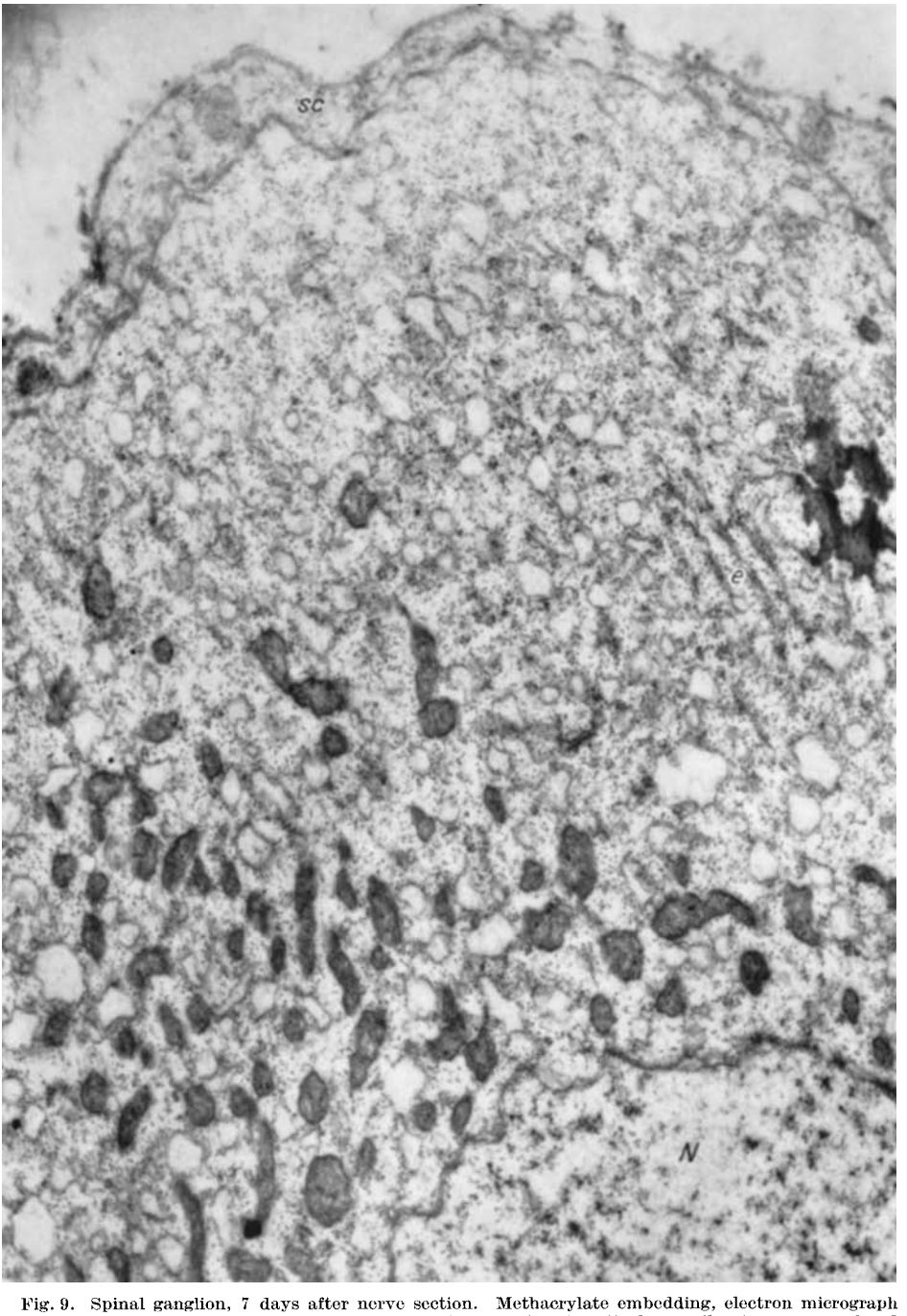

Fig. 9. Spinal ganglion, 7 days after nerve section. Methacrylate embedding, electron micrograph (22,500 ×). Part of a chromatolytic ganglion cell: endoplasmic reticulum built of numerous closed vesicles, ribosomes mostly free, and mitochondria concentrated in the perinuclear area. *e* remnants of the ergastoplasm; *N* nerve cell nucleus; *sc* satellite cell

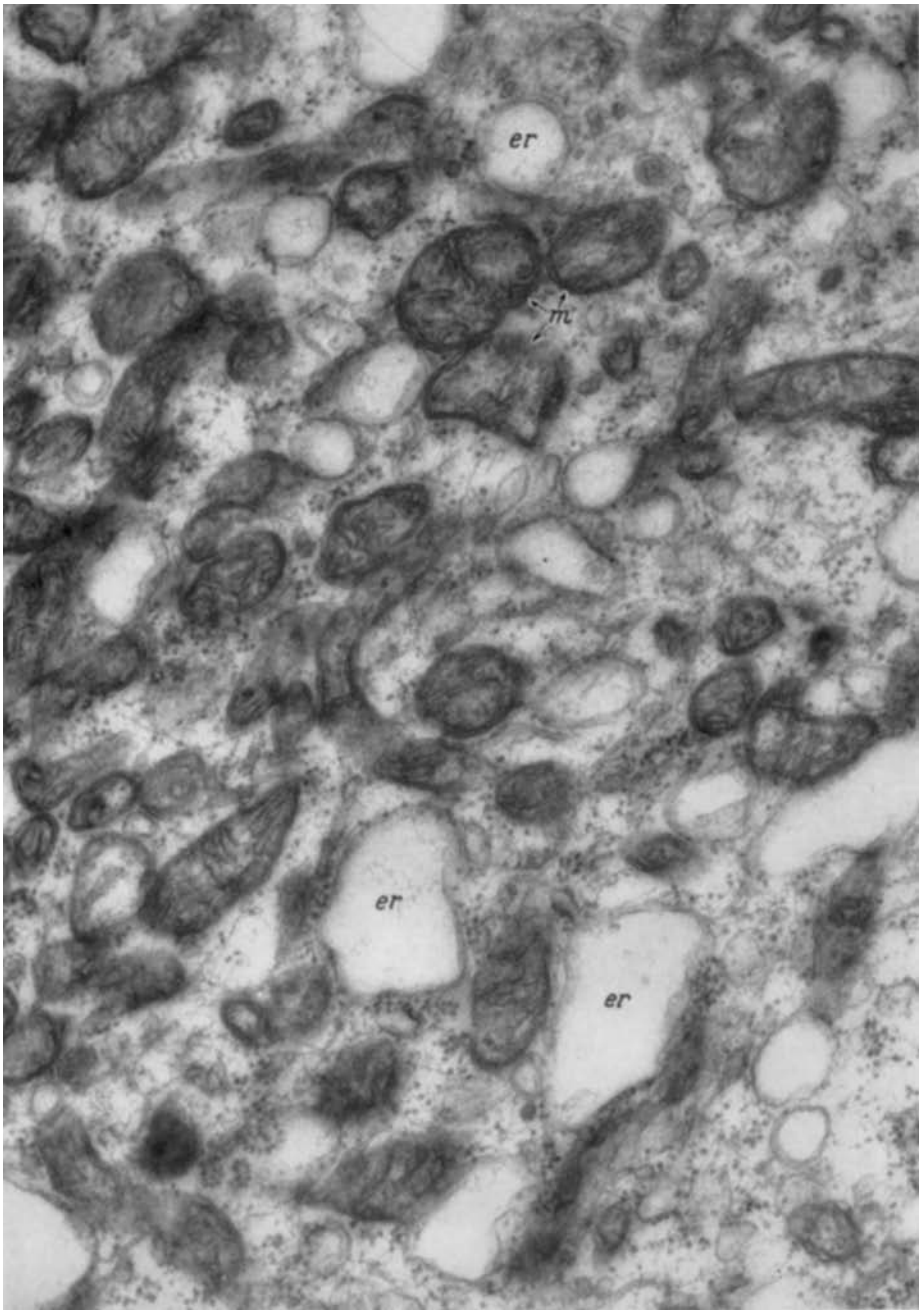

Fig. 10. Spinal ganglion, 14 days after nerve section. Methacrylate embedding, electron micrograph (45,000 ×). Part of the perinuclear cytoplasm of a chromatolytic ganglion cell, containing numerous mitochondria (*m*) and closed vesicles of the endoplasmic reticulum (*er*)

Such structure suggests that the closed vesicles, found in chromatolytic nerve cells, arise by swelling and fragmentation of cisternae and tubules.

Table 2. *Number of mitochondrial profiles per square micron of section in nerve cells 7 days after axonal section*

	A	B	C
Nerve cells (*a*) with bundles of neurofilaments	0.67	±0.131	
Chromatolytic nerve cells (*b*) with uniform distribution of mitochondria	0.98	±0.310	$t = 2.38$; $0.02 < P < 0.05$
Chromatolytic nerve cells (*b*) with mostly perinuclear distribution of mitochondria	1.30	±0.330	

A = average number of mitochondrial profiles per square micron of section; *B* = standard deviations; *C* = the *t*-value is that for the standard error test for small samples (Student distribution); *P* indicates the probability of this value being exceeded in random sampling. The nerve cells have been referred to with the letters *a* and *b* as in the text.

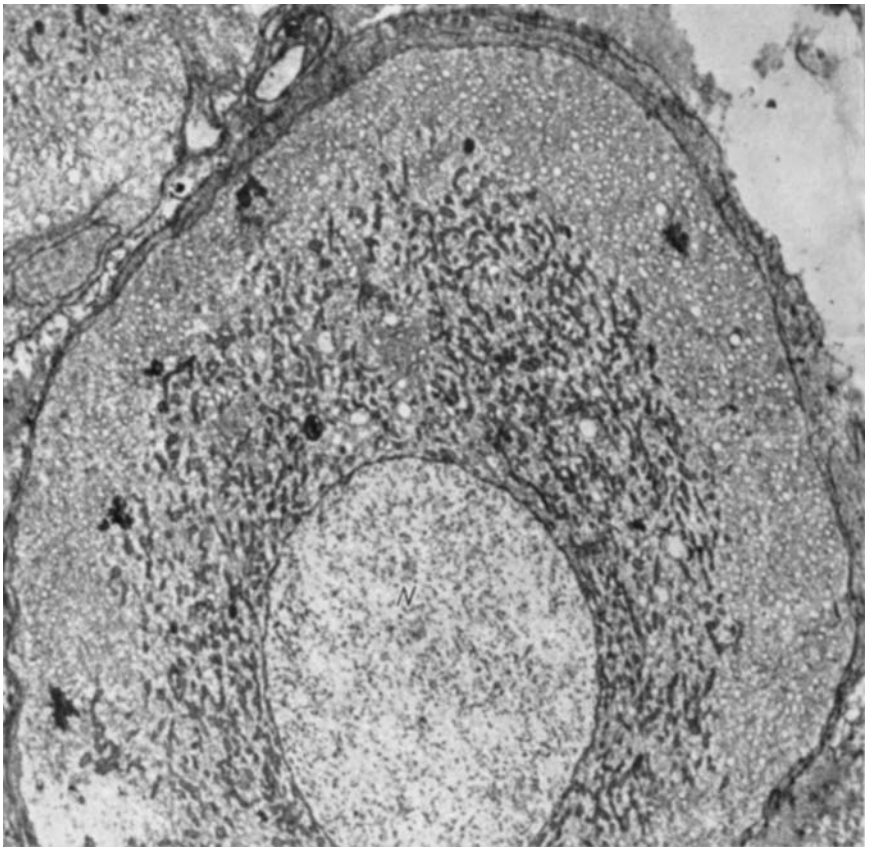

Fig. 11. Spinal ganglion, 7 days after nerve section. Methacrylate embedding, electron micrograph (4,500 ×). Part of a chromatolytic ganglion cell: marked concentration of the mitochondria around the nucleus and numerous closed vesicles of the endoplasmic reticulum. *N* nucleus

Neurofilaments are hardly detectable in the perikaryon of chromatolytic cells.

In some chromatolytic nerve cells, the same number of mitochondrial profiles is found in the perinuclear and in the peripheral cytoplasm; in these cells, the

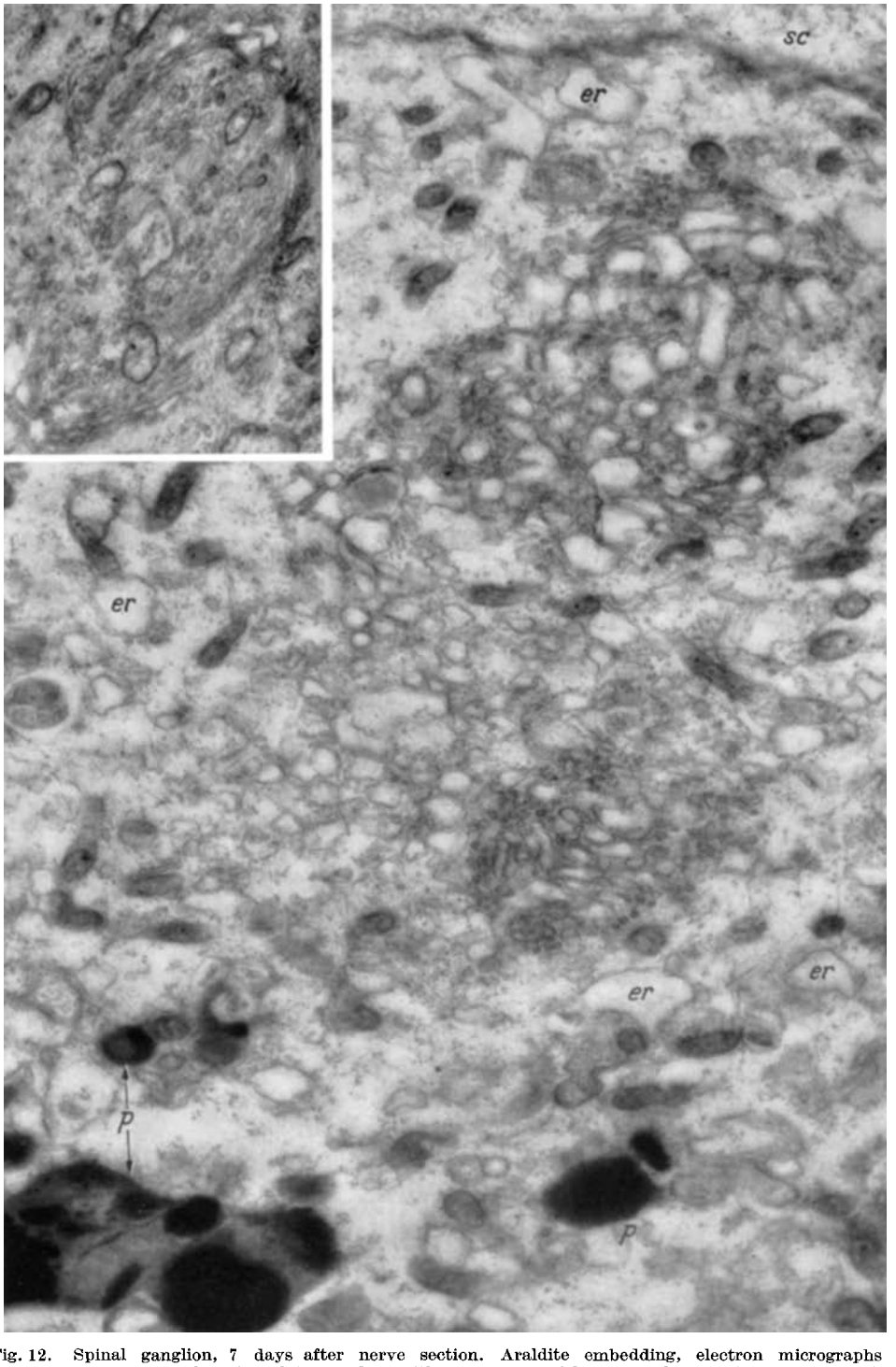

Fig. 12. Spinal ganglion, 7 days after nerve section. Araldite embedding, electron micrographs (22,500 ×). Greatly enlarged Golgi complex with enlarged vesicles in a chromatolytic nerve cell. In the inset, at the same magnification, a Golgi complex of a control ganglion cell. *er* closed vesicles of the endoplasmic reticulum; *p* pigment; *sc* satellite cell

average number of mitochondrial profiles per square micron of section is close to 1 (0.98, cf. Table 2). In other chromatolytic nerve cells, the mitochondria are

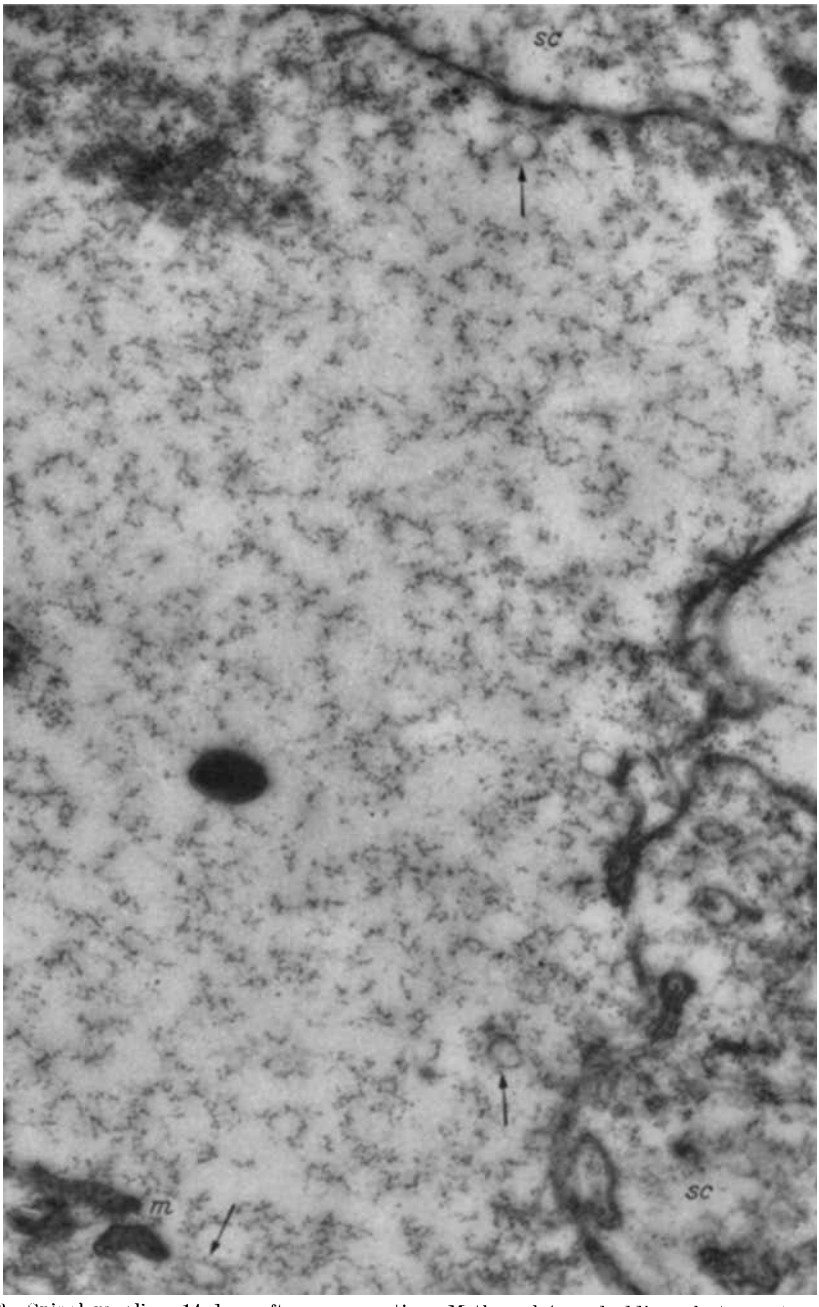

Fig. 13. Spinal ganglion, 14 days after nerve section. Methacrylate embedding, electron micrograph (30,000 ×). Part of the cytoplasm of a ganglion cell probably undergoing degeneration. The neuronal cytoplasm contains very few closed vesicles of the endoplasmic reticulum (arrows), some mitochondria (*m*), electron opaque granules (ribosomes?), and a very fine submicroscopic network. *sc* satellite cells

more numerous in the perinuclear region (Figs. 10 and 11); the peripheral cytoplasm is nearly free of mitochondria. The average number of mitochondrial

Fig. 14. Spinal ganglion, 7 days after nerve section. Methacrylate embedding, electron micrograph (5,700 ×). An element enwrapped by satellite cells, filled with filaments and containing also some mitochondria (*m*), similar to the "ghost cells" described by ULE (1962) (cf. text, p. 730). *Ne* nerve cells; *sc* satellite cell

profiles per square micron of section is greater than 1 (1.3, cf. Table 2), close to the value found in normal nerve cells (1.42, cf. table 1). The difference between the two mean values reported above (0.98 and 1.30) is significant (cf. Table 2).

In the nerve cells whose axon had been severed, all the Golgi complexes appear larger than in normal cells, their cisternae and vesicles obviously swollen (Fig. 12). From the comparison of equatorial sections passing through the nucleus of nerve cells of similar size, it appears that the number of Golgi complexes at this stage is ca. 25% lower than that of normal nerve cells. Of course, this value is merely indicative.

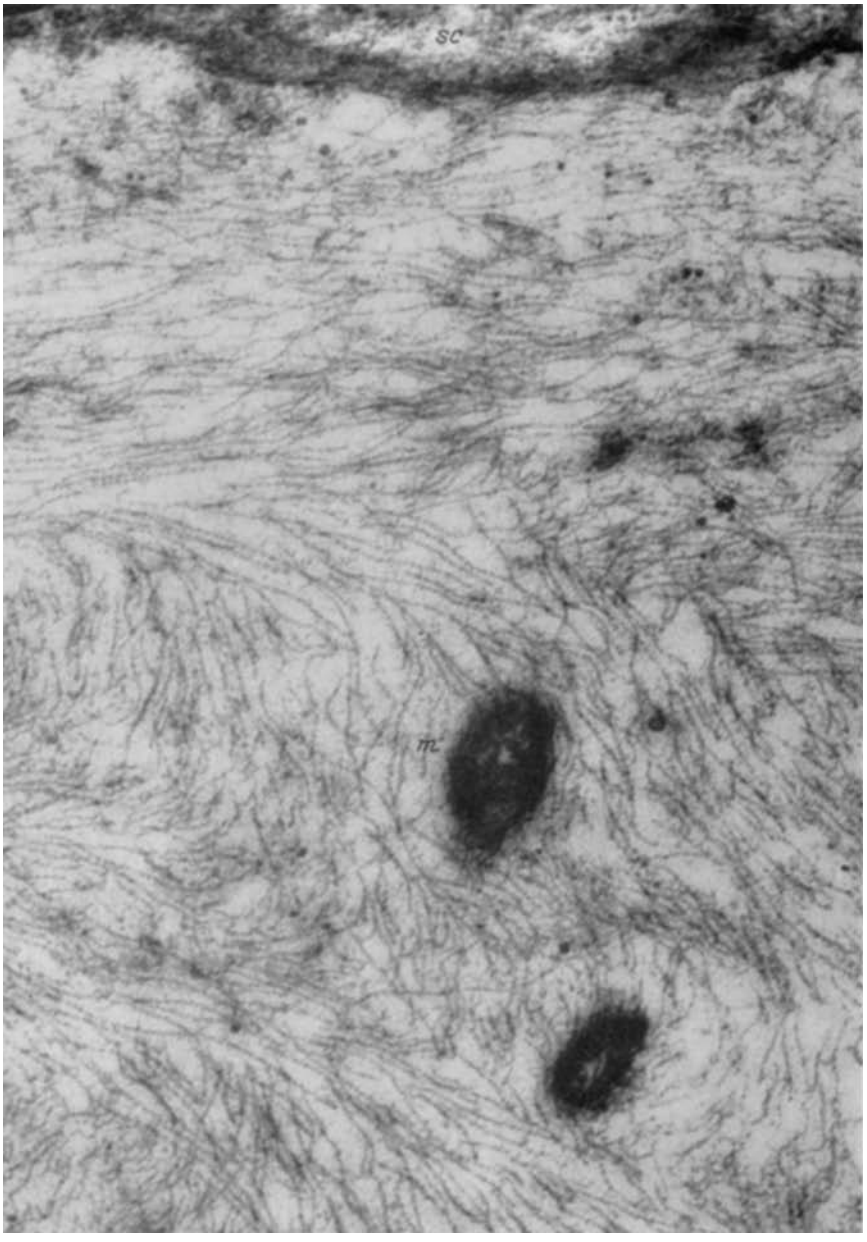

Fig. 15. Spinal ganglion, 7 days after nerve section. Methacrylate embedding, electron micrograph (60,000 ×). Part of the element shown in Fig. 14 at a higher magnification. *m* mitochondrion; *sc* satellite cell

In the perikaryon of some chromatolytic cells, the vesicles of the endoplasmic reticulum are very scarce. In these cells the perikaryon contains only a few ribosomes distributed at random, among which a fine submicroscopic network is apparent (Fig. 13); the latter is a rare finding in the perikaryon of normal nerve

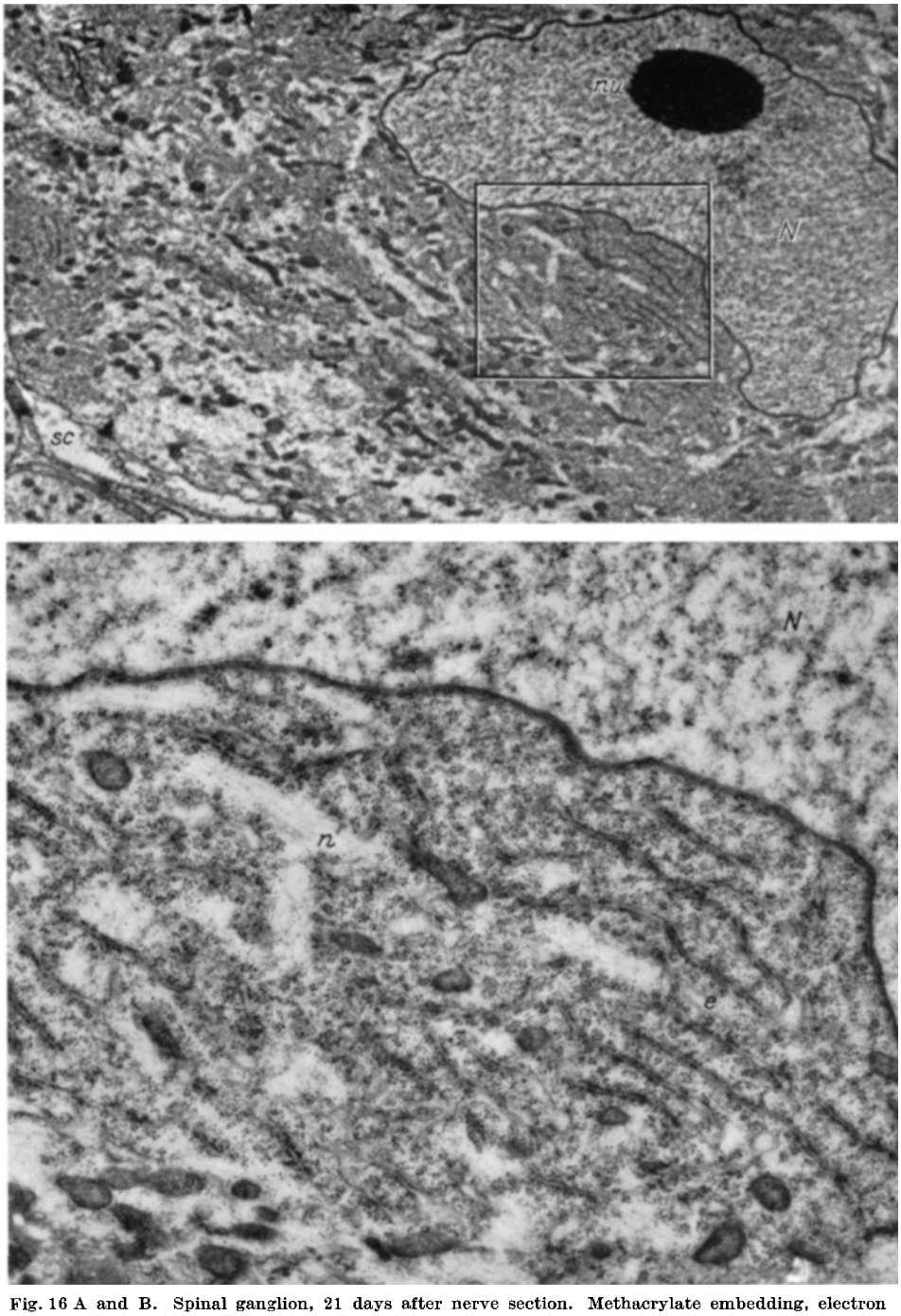

Fig. 16 A and B. Spinal ganglion, 21 days after nerve section. Methacrylate embedding, electron micrographs (A 5,000 × ; B 22,500 ×). Part of the perikaryon of a neuron (*c*) in the phase of restoration after chromatolysis. The nucleolus (*nu*) appears modified. The surface of the nucleus (*N*) shows a marked infolding and the perinuclear cytoplasm contains ergastoplasm (*e*) and numerous ribosomes. Fig. B corresponds to the area outlined in Fig. A. *n* neurofilaments; *sc* satellite cell

cells. This structural pattern might represent the initial stage of degenerative processes.

In a spinal ganglion, 7 days after division of the axon, a moderately electron-transparent formation entirely enveloped by a thin sheath of satellite cells has

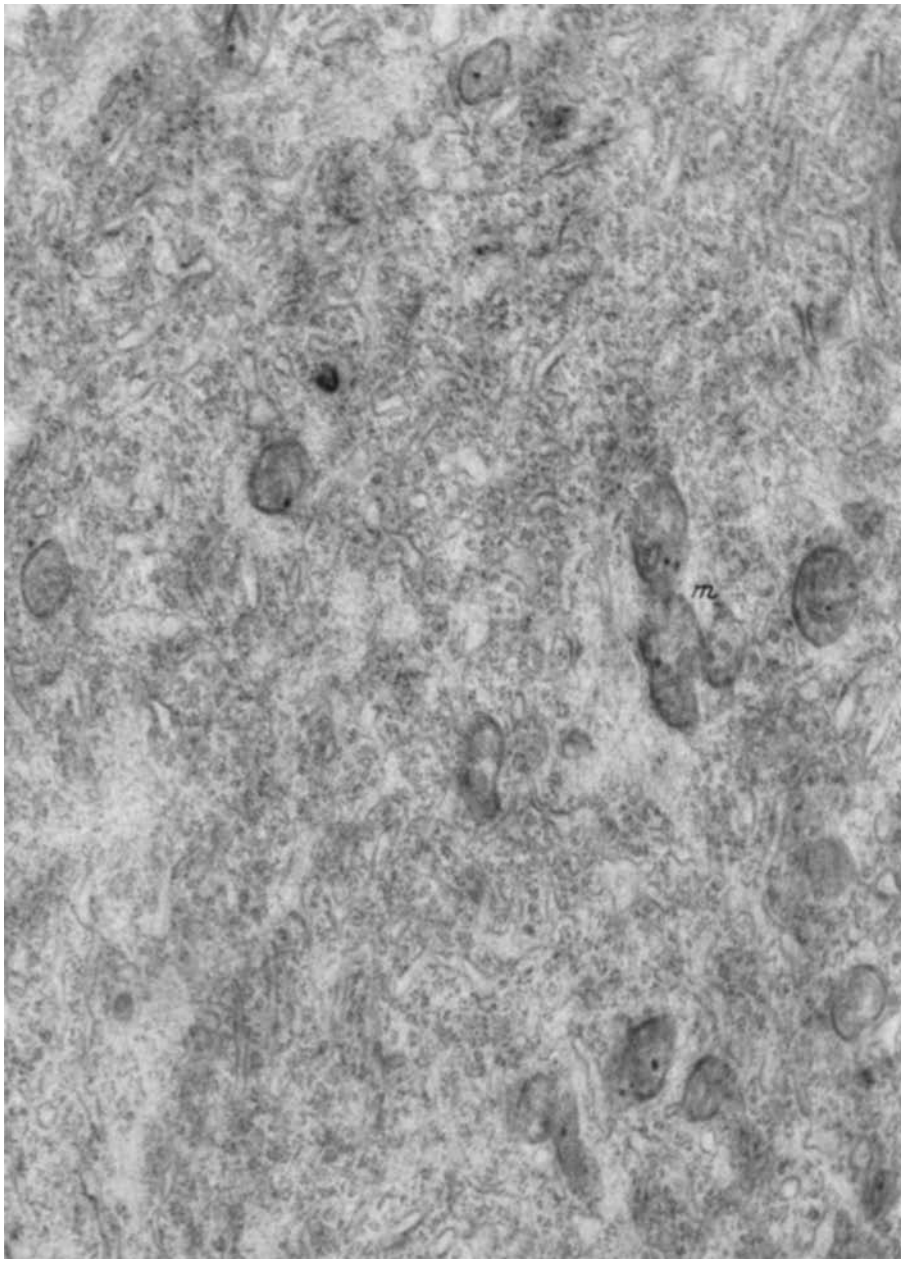

Fig. 17. Spinal ganglion, 21 days after nerve section. Araldite embedding, electron micrograph (30,000 ×). Part of the cytoplasm of a ganglion cell described as (*d*) in the text: numerous cisternae of the endoplasmic reticulum and many ribosomes. *m* mitochondria

been observed (Fig. 14); it contains a large number of filaments embedded in an amorphous electron transparent material, and a few mitochondria (Fig. 15). The filaments appear long, wavy, arranged at random and of the same thickness as the neurofilaments of the normal nerve cells. Also the mitochondrial structure

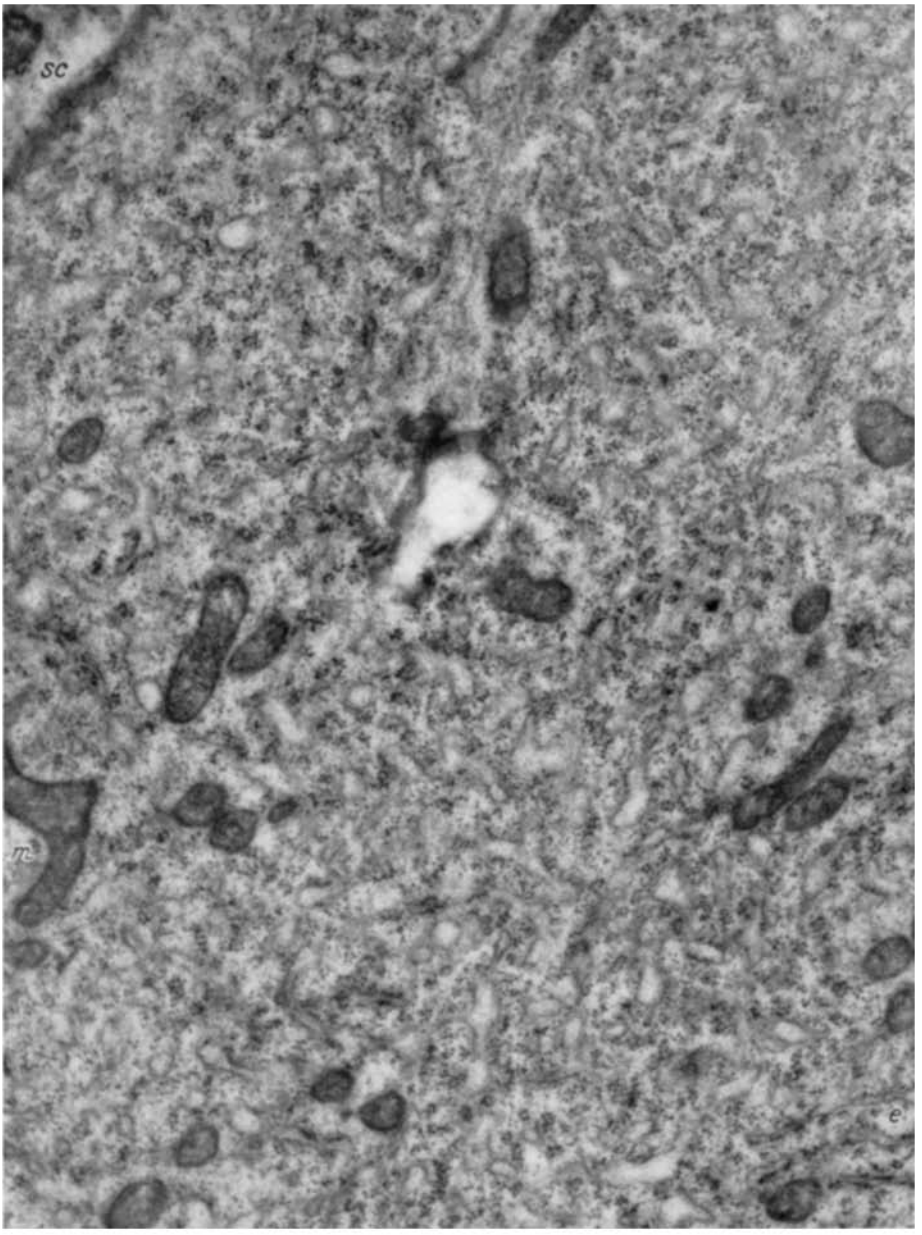

Fig. 18. Spinal ganglion, 21 days after nerve section. Methacrylate embedding, electron micrograph (30,000 ×). Part of the cytoplasm of a cell described as (*d*) in the text: numerous cisternae of the endoplasmic reticulum and many ribosomes. *e* ergastoplasm; *m* mitochondrion; *sc* satellite cell

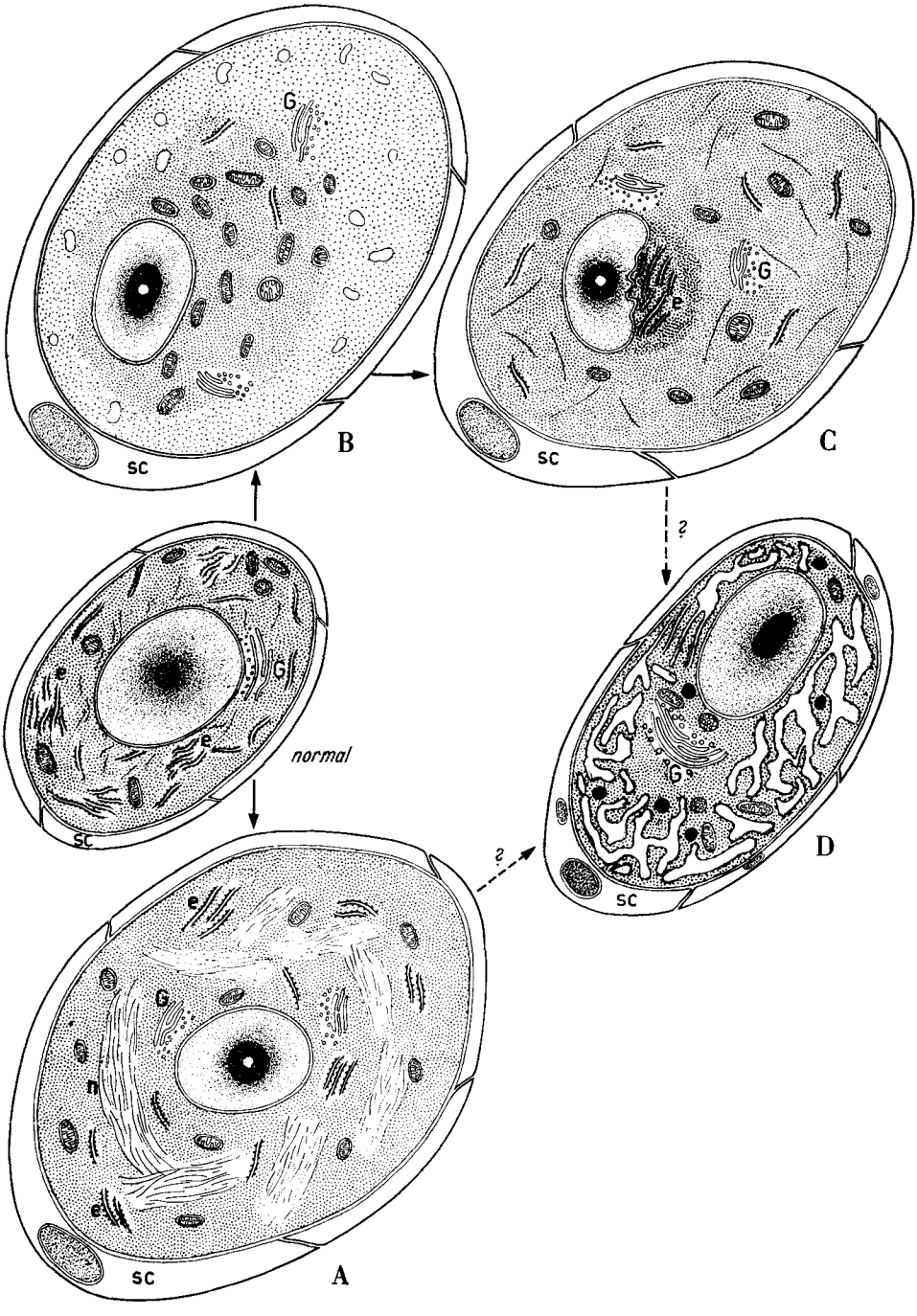

Fig. 19 A—D. Diagram of the main changes undergone by the nerve cells of spinal ganglia after axon section. Normal: nerve cell with a normal structure; A: neuron with large bundles of neurofilaments in the perikaryon; B: chromatolytic nerve cell; C: nerve cell regaining its normal structure after chromatolysis; D: nerve cell whose cytoplasm shows intense basophilia. The nerve cells have been referred to with the letters A to D as in the text. *e* ergastoplasm; *G* Golgi complex; *n* neurofilaments; *sc* satellite cells

appears normal. The mentioned structure always with the same characteristics was followed in several serial sections.

14-day stage after tail amputation. Light and electron microscopic patterns of affected spinal ganglia are essentially similar to those observed in the previous stage. However, the number of nerve cells (*a*) appears lower than in the preceding stage: only 5% ca. of all the ganglion nerve cells.

21 and 30-day stage after tail amputation. In the light microscope no (*a*) nerve cells are detectable; (*b*) cells, (*c*) cells with an intense perinuclear basophilia, and (*d*) cells whose cytoplasm appears basophilic throughout are found. In the electron microscope, chromatolytic nerve cells (*b*) are easily recognizable, on account of the severe alterations of the endoplasmic reticulum.

In nerve cells (*c*) part of the nuclear envelope is often infolded and indented (Fig. 16). Structural differences are apparent in the peripheral and in the perinuclear areas of these cells, respectively. The cytoplasmic region adjacent to the folded part of the nuclear envelope is filled with a great number of free ribosomes and ergastoplasmic structures (Fig. 16); the peripheral region of the perikaryon contains, instead, scattered tubular profiles of the endoplasmic reticulum, ergastoplasmic structures being entirely absent. The free ribosomes are clustered into rosettes, as commonly observed in normal nerve cells, and neurofilaments are either isolated or packed in small bundles (Fig. 16).

The intensely and evenly basophilic cytoplasm of nerve cells (*d*) observed with the electron microscope appears to contain a highly developed endoplasmic reticulum and a large number of free and membrane-attached ribosomes (Figs. 17 and 18). The endoplasmic reticulum consists of numerous branching and intercommunicating tubular profiles arranged at random.

Twenty-one days after tail amputation, the mitochondria in all nerve cells are evenly distributed in the perinuclear and in the peripheral regions of the cytoplasm. The mean number of mitochondrial profiles per square micron of section is less than 1 (0.88, cf. Table 1).

At this stage all the Golgi complexes are again larger than in normal nerve cells. The number of Golgi units in perikaryal sections of equivalent size is nearly identical in nerve cells whose axon had been severed to that in normal nerve cells.

60-day stage after tail amputation. This stage is characterized by a marked increase in the percentage of cells exhibiting a normal ultrastructure and by a definite reduction in the number of chromatolytic nerve cells (*b*). Moreover, in the few nerve cells which still show chromatolysis at this stage, the changes in the endoplasmic reticulum are limited to small areas of the cytoplasm. Nerve cells with basophilic cytoplasm previously described as (*c*) and (*d*) are also present.

A further class of cells observed at this stage will not be described here as they represent most likely ganglion cells undergoing hypertrophy. Accordingly, they will be described in a subsequent paper.

Considerations and discussion

It is likely that the ultrastructural modifications in the perikaryon of ganglion cells, described in the present paper, depend on the interruption and the early stage of regeneration of the axon. This assumption is supported by the results of the light-microscopic controls, long accepted as indications of cell reaction

to axon severance. The ultrastructural changes could not be attributed to cell hypertrophy because one month after amputation the tail regeneration is so modest that it might not possibly elicit an appreciable enlargement of the peripheral field of innervation, a prerequisite for the establishment of ganglion cell hypertrophy. The peripheral field of innervation of the ganglion cells begins to show a significant increase only at a later period and it continues to enlarge very slowly for a fairly long time afterwards. Under these conditions, the structural modifications of the perikaryon due to interruption and regeneration of the axon, may be easily distinguished from the alterations which depend on the enlargement of the peripheral field of innervation.

After tail amputation only a few nerve cells of the ganglion maintain their normal ultrastructure; probably the axon of these cells escaped section because it ended in the ganglion itself, or in tissues of the tail lying cranial to the level of amputation. The majority of the ganglion cells, however, show alterations, some of which are similar in all the nerve cells (1), while others affect only a part of the cell community (2a and b).

1) All the nerve cells whose axon was severed undergo an increase in the size of their perikaryon and a slight swelling of the mitochondria (Fig. 3): the matrix of the latter becomes rather electron transparent. The mitochondrial modifications last a short time and disappear by the 7th day after injury; the perikaryal swelling, instead, lasts for a longer period. The last event probably depends on uptake of water, and is likely to determine the reduction in the number of mitochondria and of the Golgi complexes observed after severance of the axon. The reduction in the number of the organelles mentioned could not be due to their disappearance since mitochondrial remnants were never observed and the Golgi complexes appeared actually hypertrophic. The decrease in the number of mitochondria was previously observed by MARINESCO and TUPA (1922), but not by HARTMANN (1947, 1948, 1952) and HUDSON et al. (1961).

2) Two groups of ganglion cells (*a* and *b*) show structural modifications whose characters is distinctly different.

a) Nerve cells with large bundles of neurofilaments in the perikaryon. In a group of ganglion nerve cells (*a*) which represent the 12% at the 7-day stage, large bundles of filamentous structures become apparent in the cytoplasm (Figs. 5 and 6), while in normal cells the filaments are never arranged in large bundles. It is hard to decide whether we are dealing here with bundles of newformed filaments or with a rearrangement and packing of preexisting structures. At any rate, the presence of a conspicuous amount of filamentous structures appears to be in agreement with the observations of VAN GEHUCHTEN (1897), DONAGGIO and FRAGNITO (1904), CAJAL (1928) and YOUNG (1932); the latter authors maintain that in the perikaryon of cells whose axon had been divided the neurofibrils (which are presumably composed of neurofilaments) are more evident than in normal cells in microscopical preparations. No significant alterations in the endoplasmic reticulum are apparent in this group of nerve cells (Fig. 5): the Nissl bodies are present and their structure is normal, they stand out even more neatly as they lie between lighter regions of the cytoplasm. The ultrastructural changes in these nerve cells regress fairly rapidly: 14 days after injury, the number of affected nerve cells has fallen to 5% and they are no longer found after 21 days.

The striking prominence of neurofilaments in nerve cells (*b*), and their disappearance from nerve cells undergoing chromatolysis after section of the axon, may perhaps find an explanation in the suggestion advanced by MAXFIELD and HARTLEY (1957), and SCHMITT (1957). According to these authors, the protein component of neurofilaments in normal nerve cells would be found partly in a disperse state and partly organized into filaments, which could be rather unstable structures. Changes in the pH or in ionic strength would be sufficient to decompose the long protein filaments into globular molecules; this process would be reversible. These characteristics of the protein of the neurofilaments, may help also in the interpretation of the rapid increase or decrease in the amount of the neurofilaments. Unfortunately, too little is known on the factors which may modify this hypothetical equilibrium between dispersed and aggregated states of the protein.

b) Chromatolytic nerve cells. A second, larger group of nerve cells, is characterized by profound changes in the endoplasmic reticulum and neurofilaments (Figs. 7, 8, 9 and 10). The endoplasmic reticulum is quantitatively reduced and its structure less complicated; these modifications correspond to the chromatolysis revealed by light microscopy. Neurofilaments are no longer visible in the perikaryon, although they should stand out more neatly because of the scarce density of the cytoplasm.

In some chromatolytic nerve cells, the mitochondria are assembled preferentially in the perinuclear area (Fig. 11), while in others they are scattered also in the peripheral cytoplasm, as in normal nerve cells. Since the average number of mitochondrial profiles per square micron of section is higher in the former than in the latter cells, and the difference between the two mean values appears statistically significant (cf. Table 2), it may be concluded that the gathering of mitochondria in the perinuclear region is coincident with an increase in their number. The displacement of these organelles is not accompanied, instead, by significant changes in their length (cf. Table 3). The mentioned data

Table 3. *Average length of mitochondrial profiles in control nerve cells and in nerve cells following axonal section*

	A	*B*	*C*
Control nerve cells	0.345	± 0.038	
Chromatolytic nerve cells (*b*) with uniform distribution of mitochondria .	0.460	± 0.065	$t = 1.76$; $0.10 < P < 0.20$
Chromatolytic nerve cells (*b*) with mostly perinuclear distribution of mitochondria	0.400	± 0.055	

A = average length (in micra) of mitochondrial profiles; *B* = standard deviations; *C* = the *t*-value is that for the standard error test for small samples (Student distribution); *P* indicates the probability of this value being exceeded in random sampling. The nerve cells have been referred to with the letter *b* as in the text.

seem consistent with the hypothesis of CAUSEY and HOFFMAN (1955), HOFFMAN and GRIGG (1958), and BRANDT and PAPPAS (1959), according to whom mitochondria arise from the nuclear membrane. In the present study, however, mitochondria in contact with the nuclear membrane were exceptionally observed, and no evidence was found of a derivation of mitochondria from the nuclear membrane.

In the past, chromatolysis has been often interpreted as a regressive process, i.e., as the expression of either an initial stage of degeneration, or a secondary response of the nerve cell to loss of part of its process. More recently, however, biochemical and histochemical investigations have demonstrated in chromatolytic nerve cells an increase in the activity of many enzymes and a raised rate of protein and lipid synthesis. These facts are not consistent with the original interpretation.

The increased synthesis of proteins and lipids which occurs in the perikaryon after section of the axon is probably related to the regeneration of the axon stump. It is very likely, in fact, that the proteins and lipids synthesized in the perikaryon, are transported along the axon and used for the growth of the new process. These facts are probably analogous to those occurring during normal growth of the axon; WEISS and HISCOE (1949) emphasized the existence of a flow of materials from the perikaryon down to the axon during the normal growth of the axon itself.

On the basis of the available data, chromatolysis occurring in spinal ganglion neurons following severance of the axon may be interpreted not as a degenerative process, but rather as an expression of a process of repair chiefly related to the regeneration of the axon. Further studies are required, however, to ascertain whether chromatolysis induced by chemicals or nerve cell fatigue may also be interpreted in analogous manner. During chromatolysis, the spinal ganglion neuron of the lizard cannot display its specific function because it lacks peripheral connections; all the activity of the nerve cell seems directed to the production of the materials necessary for the regeneration of the lost part.

In the chromatolytic nerve cells under study here a marked reduction in the membranes of the endoplasmic reticulum, not in the number of ribosomes was observed. This is consistent with data suggesting that free ribosomes are related to synthesis of cytoplasmic protein for cell growth, while those attached to membranes are related to synthesis of specialized protein molecules (MUNGER, 1958; SLAUTTERBACK and FAWCETT, 1959; HAY, 1963). These conclusions are also in agreement with observations on embryonic cells, in which proteins are actively synthesized, in spite of the nearly complete absence of the membranes of the endoplasmic reticulum (PALADE and PORTER, 1954; HOWATSON and HAM, 1955; PALADE, 1955).

The results of the present research suggest that a certain analogy could be established between the chromatolytic nerve cells under study and neuroblasts. The chromatolytic nerve cells and the neuroblast both have a poorly developed endoplasmic reticulum, no neurofilaments and no ergastoplasm; their ribosomes are mainly free; both cells are engaged in the synthesis of new protoplasm. In the spinal ganglion neurons the process of chromatolysis which accompanies the regeneration of the axon, seems thus to represent a temporary return of the nerve cell to a structural and metabolic condition analogous to that of the normally growing neuron. This is not the sole example of a cell undergoing well detectable changes during the initial stages of its regenerative activity. Structural changes analogous to those observed in chromatolytic nerve cells have also been described in other tissues. During regeneration of amputated amphibian limbs HAY (1958) observed, in fact, that the dedifferentiated cells of the proliferating blastema contain a large number of ribosomes, but only few cytoplasmic mem-

branes. The latter increase greatly and become studded with granules during the redifferentiation of the cells.

The changes undergone in the cells during the process of regeneration may fit into the general view according to which growth and differentiation are to some extent antagonistic processes.

Recovery after chromatolysis. The duration of the morphological changes occurring in sequence in chromatolytic perikarya, varies from cell to cell. In some cells they have allready disappeared 21 days after section of the axon; at this stage in comparison with the 7-day stage, there is in fact a percentual decrease in chromatolytic nerve cells and an increase in cells with normal structure. In a few nerve cells, however, chromatolysis is still apparent, though limited to small regions of the perikaryon, 60 days after injury.

When the process of chromatolysis is over, most of the nerve cells gradually resume their normal structure; only a very few undergo degenerative phenomena. In this regard, the behaviour of peripheral ganglion cells is different from that of the neurons of the central nervous system; in the latter a much higher percentage of cells degenerate after section of the axon. Degeneration in the peripheral ganglion cells is characterized by a progressive depletion of the vesicles of the endoplasmic reticulum and of the ribosomes; thus the citoplasm, now almost completely devoid of structures, appears electron transparent (Fig. 13).

After chromatolysis, the normal structure of the cells is reestablished through the following sequence of morphological steps (Fig. 16): (α) the nuclear membrane undergoes infolding and indentation; therefore, the surface of the nucleus is increased, and this might favour the exchanges between nucleus and cytoplasm; (β) ergastoplasmic structures reappear, and ribosomes are crowded in the perinuclear region; (γ) tubular profiles of the endoplasmic reticulum are found in the peripheral region of the perikaryon, and (δ) neurofilaments become apparent.

The changes undergone by nerve cells after chromatolysis in the present study are analogous to the modifications observed by HYDÉN (1943) and by C. and O. VOGT (1946, 1947) with the light-microscope; namely, infolding of the nuclear membrane and condensation of RNA in the perinuclear cytoplasm, mainly close to the nuclear infolding. The present electron microscopic investigation shows that only this region of the cytoplasm incloses ergastoplasmic structures and shows the greatest concentration of ribosomes (Fig. 16).

According to DEANE and PORTER (1960), cytoplasmic basophilia is not only related to ribosome-bound RNA but also to the content of free RNA; it is possible, therefore, that a high concentration of free RNA occurs around the nucleus of cells regaining their normal structure after chromatolysis.

The mentioned data stress once more the importance of the nucleus in the production of ribonucleoproteins and shows that, after chromatolysis, ergastoplasmic structures begin to develop in the perinuclear region, where the amount of ribosomes and free RNA is greater.

The changes in size and structure of the nucleolus (Fig. 2) observed in the present study, probably reflect its participation in the processes occurring in the nerve cell during and after chromatolysis and in particular in the increased synthesis of proteins. Although many details are still debatable, several authors agree that the nucleolus plays an important role in the synthesis of RNA and, indirectly, in the synthesis of cytoplasmic proteins (CASPERSSON, 1950; BRACHET,

1952). In this regard, many authors support the hypothesis that the nucleolus of nerve cells takes part in the formation of Nissl bodies (EINARSON, 1933; HYDÉN, 1943, 1947, 1960; HAMBURGER and HYDÉN, 1945, 1949; LA VELLE, 1951, 1956).

During and after chromatolysis, a central, more electron transparent area may appear in the nucleolus (Fig. 2 C, D, E); this core probably corresponds to the "vacuoles" observed with the light microscope by BARR and BERTRAM (1951) and by LINDSAY and BARR (1955) during chromatolysis, immediately before restoration of the Nissl substance. Also C. and O. VOGT (1946, 1947) reported that, under various conditions, the hypertrophy of nerve cell nucleoli is accompanied by the appearance of "vacuoles" within the latter.

Changes in the nucleolar ultrastructure, analogous to those observed in the present study, have also been observed occasionally e.g. in giant cells (BIRD, 1961), in amoebae (VICKERMAN, 1962) and in hepatic cells (ANDREW, 1962). This structural modification may be an expression of increased function.

Twenty-one days after section of the axon, some nerve cells show intense basophilia of the entire perikaryal cytoplasm and a highly developed endoplasmic reticulum (Figs. 17 and 18). It may be supposed that such nerve cells have recovered from chromatolysis. In fact, these neurons first appear when the percentage of chromatolytic nerve cells is decreasing. Indeed, the presence of hyperchromatic nerve cells after chromatolysis has often been reported.

The aspect shown in Fig. 14, observed only in one case 7 days after nerve section, is quite similar to that described by ULE (1962) in the spinal cord and spinal ganglia of rats suffering from experimental neurolathyrism. It appears nearly identical to ULE's Fig. 9 (1962), interpreted by this author as a grossly swollen axon of a degenerating nerve cell. Possibly the aspect reported in the present study may be related to axon degeneration; however, the singleness of the finding does not allow any definite conclusions.

References

ANDRES, K. H.: Untersuchungen über morphologische Veränderungen in Spinalganglien während der retrograden Degeneration. Z. Zellforsch. **55**, 49—79 (1961).

ANDREW, W.: An electron microscope study of age changes in the liver of the mouse. Amer. J. Anat. **110**, 1—18 (1962).

BARR, M. L., and E. G. BERTRAM: The behaviour of nuclear structures during depletion and restoration of Nissl material in motor neurons. J. Anat. (Lond.) **85**, 171—181 (1951).

BIELSCHOWSKY, M.: Über den Bau der Spinalganglien unter normalen und pathologischen Verhältnissen. Ein Beitrag zur Kenntnis der Regenerationsvorgänge an Ganglienzellen und Nervenfasern. J. Psychol. Neurol. (Lpz.) **11**, 1—38 (1908).

BIRD, A. F.: The ultrastructure and histochemistry of a nematode-induced giant cell. J. biophys. biochem. Cytol. **11**, 701—715 (1961).

BODIAN, D., and R. C. MELLORS: The regenerative cycle of motor neurons with special reference to phosphatase activity. J. exp. Med. **81**, 469—489 (1945).

BRACHET, J.: The role of the nucleus and the cytoplasm in synthesis and morphogenesis. Symp. Soc. exp. Biol. **6**, 173—200 (1952).

BRANDT, P. W., and G. D. PAPPAS: Mitochondria. II. The nuclear-mitochondrial relationship in Pelomyxa carolinensis. Wilson. J. biophys. biochem. Cytol. **6**, 91—96 (1959).

BRATTGÅRD, S. O., J. E. EDSTRÖM and H. HYDÉN: The chemical changes in regenerating neurons. J. Neurochem. **1**, 316—325 (1957).

—, and H. HYDÉN: (1960), quoted by HYDÉN (1960).

CAJAL, S. R.: Degeneration and regeneration of the nervous system. New York: Haffner 1928.

— Histologie du système nerveux de l'homme et des vertébrés. C. S. I. C., Madrid, 1952 (I. ed. 1909).

CASPERSSON, T.: Cell Growth and Cell Function. New York: W. W. Norton & Co. Inc. 1950.
CAUSEY, G., and H. HOFFMAN: Cytoplasmic synthesis in nerve cells. Brit. J. Cancer **9**, 666—673 (1955).
CERVÓS-NAVARRO, J.: Elektronenmikroskopische Befunde an Spinalganglienzellen der Ratte nach Ischiadikotomie. Proc. IV. Intern. Kongr. f. Neuropath. **2**, 99—104 (1962).
DEANE, H. W., and K. R. PORTER: A comparative study of cytoplasmic basophilia and the population density of ribosomes in the secretory cells of mouse seminal vesicle. Z. Zellforsch. **52**, 697—711 (1960).
DONAGGIO, A., e O. FRAGNITO: Lesioni del reticolo fibrillare endocellulare nelle cellule midollari per lo strappo dello sciatico e delle relative radici spinali. Riv. sper. Freniat. **31**, 383-386 (1904).
EINARSON, L.: Notes on the morphology of the chromophil material of nerve cells and its relation to nuclear substances. Amer. J. Anat. **53**, 141—175 (1933).
EVANS, D. H. L., and E. G. GRAY: Changes in the fine structure of ganglion cells during chromatolysis, in Cytology of nervous tissue, p. 71—74. London: Taylor & Francis Ltd. 1961.
FISCHER, J., Z. LODIN and J. KOLONŠEK: A histoautoradiographic study of the effect of section of the facial nerve on the uptake of methionine-S^{35} by the cells of the facial nerve nucleus. Nature (Lond.) **181**, 341—342 (1958).
FLEMMING, R. A.: The effect of "ascending degeneration" on the nerve cells of the ganglia. Edinb. med. J. **43**, 279—283 (1897).
FRIEDE, R. L.: Transport of oxidative enzymes in nerve fibers; a histochemical investigation of the regenerative cycle in neurons. Exp. Neurol. **1**, 441—466 (1959).
GEHUCHTEN, A. VAN: (1897), quoted by CAJAL (1909).
GEREBTZOFF, M. A., and L. VANDERMISSEN: Etude de la relation spatiale entre acétylcholinestérase et récepteur de l'acétylcholine. Ann. Histochim. **1**, 221—229 (1956).
HAMBURGER, C. A., and H. HYDÉN: Cytochemical changes in the cochlear ganglion caused by acoustic stimulation and trauma. Acta oto-laryng. (Stockh.), (Suppl.) **61**, 1—89 (1945).
— — Production of nucleoproteins in the vestibular ganglion. Acta oto-laryng. (Stockh.), (Suppl.) **75**, 53—81 (1949).
HARTMANN, J. F.: Changes in mitochondrial content of motor nerve cell bodies following axone section. Anat. Rec. **97**, 390 (1947).
— Mitochondria in nerve cell bodies following section of axones. Anat. Rec. **100**, 49—59 (1948).
— Electron microscopy of motor nerve cells following section of axones. Anat. Rec. **118**, 19—33 (1954).
HAY, E. D.: The fine structure of blastema cells and differentiating cartilage cells in regenerating limbs of Amblystoma larvae. J. biophys. biochem. Cytol. **4**, 583—592 (1958).
— The fine structure of differentiating muscle in the Salamander tail. Z. Zellforsch. **59**, 6—34 (1963).
HEINZEN, B.: Acid phosphatase activity in transected sciatic nerves. Anat. Rec. **98**, 193—207 (1947).
HOFFMAN, H., and G. W. GRIGG: An electron microscopic study of mitochondria formation. Exp. Cell Res. **15**, 118—131 (1958).
HOWATSON, A. F., and A. W. HAM: Electron microscope study of sections of two rat liver tumors. Cancer Res. **15**, 62—69 (1955).
HOWE, H. A., and J. B. FLEXNER: Succinic dehydrogenase in regenerating neurons. J. biol. Chem. **167**, 663—671 (1947).
—, and R. C. MELLORS: Cytochrome oxydase in normal and regenerating neurons. J. exp. Med. **81**, 489—500 (1945).
HUDSON, G., A. LAZAROW and J. F. HARTMANN: A quantitative electron microscopic study of mitochondria in motor neurones following axonal section. Exp. Cell Res. **24**, 440—456 (1961).
HYDÉN, H.: Protein metabolism in the nerve cell during growth and function. Acta physiol. scand. (Stockh.), (Suppl.) **17**, 1—136 (1943).
— Protein and nucleotide metabolism in the nerve cell under different functional conditions. Symp. Soc. exp. Biol. **1**, 152—162 (1947).
— The neuron. In: The cell (J. BRACHET and A. E. MIRSKY ed.), vol. IV, p. I, p. 215—323. New York and London: Academic Press Inc. 1960.
LA VELLE, A.: Nucleolar changes and development of Nissl substance in the cerebral cortex of fetal guinea pigs. J. comp. Neurol. **94**, 453—473 (1951).
— Nucleolar and Nissl substance development in nerve cells. J. comp. Neurol. **104**, 175—205 (1956).

LEVI-MONTALCINI, R., et G. LEVI: Recherches quantitatives sur la marche du processus de différenciation des neurones dans les ganglions spinaux de l'embryon de poulet. Arch. Biol. (Liège) **54**, 189—206 (1943).

LINDSAY, H. A., and M. L. BARR: Further observations on the behaviour of nuclear structures during depletion and restoration of Nissl material. J. Anat. (Lond.) **89**, 47—62 (1955).

LISON, L.: Histochimie et cytochimie animales. Paris: Gautier-Villars 1953.

LUGARO, E.: Sulle alterazioni delle cellule nervose per mutilazione parziale del prolungamento nervoso. Riv. Pat. nerv. ment. **1**, 432—435 (1896).

— Sulle alterazioni delle cellule nervose dei gangli spinali in seguito al taglio della branca periferica o centrale del loro prolungamento. Riv. Pat. nerv. ment. **1**, 457—470 (1896).

— Sul comportamento delle cellule nervose dei gangli spinali in seguito al taglio della branca centrale del loro prolungamento. Riv. Pat. nerv. ment. **2**, 541—543 (1897).

MARCORA, F.: Di una fine alterazione delle cellule nervose del nucleo di origine del grande ipoglosso consecutiva allo strappamento ed al taglio del nervo. Boll. Soc. med.-chir. Pavia **22**, 134—137 (1908).

MARINESCO, G. (1896), quoted by CAJAL (1909).

— Recherches sur la structure de la partie fibrillaire des cellules nerveuses à l'état normal et pathologique. Rev. neurol. **12**, 405—428 (1904).

—, et A. TUPA: Recherches histo-pathologiques sur les mitochondries. C. R. Soc. Biol. (Paris) **87**, 292—293 (1922).

MAXFIELD, M., and R. W. HARTLEY: Dissociation of the fibrous protein of nerve. Biochem. biophys. Acta (Amst.) **24**, 83—87 (1957).

MIANI, N., A. RIZZOLI and G. BUCCIANTE: Metabolic and chemical changes in regenerating neurons II. J. Neurochem. **7**, 161—173 (1961).

MUNGER, B.: A phase and electron microscope study of cellular differentiation in the pancreatic acinar cells of the mouse. Amer. J. Anat. **103**, 1—33 (1958).

NISSL, F.: Über experimentell erzeugte Veränderungen an den Vorderhornzellen des Rückenmarks bei Kaninchen mit Demonstration mikroskopischer Präparate. Allg. Z. Psychiat. **48**, 675—682 (1892).

— Über ROSINS neue Färbemethode des gesamten Nervensystems und dessen Bemerkungen über Ganglienzellen. Neurol. Zbl. **13**, 98—106, 141—144 (1894).

— Über die sogenannten Granula der Nervenzellen. Neurol. Zbl. **13**, 676—685, 781—789, 810—814 (1894).

— Über die Veränderungen der Nervenzellen nach experimentell erzeugter Vergiftung. Neurol. Zbl. **15**, 947—949 (1896).

PALADE, G. E.: A small particulate component of the cytoplasm. J. biophys. biochem. Cytol. **1**, 59—68 (1955).

—, and K. R. PORTER: Studies on the endoplasmic reticulum. I. J. exp. Med. **100**, 641-656 (1954).

SCHMITT, F. O.: The fibrous protein of the nerve axon. J. cell. comp. Physiol. **49** (Suppl. 1), 165—174 (1957).

SCHWARZACHER, H. G.: Der Cholinesterasegehalt motorischer Nervenzellen während der axonalen Reaktion. Acta anat. (Basel) **32**, 51—65 (1958).

SLAUTTERBACK, D. B., and D. W. FAWCETT: The development of the cnidoblasts of Hydra. An electron microscope study of cell differentiation. J. biophys. biochem. Cytol. **5**, 441—452 (1959).

SMITH jr., K. R.: The fine structure of neurons of dorsal root ganglia after stimulating or cutting the sciatic nerve. J. comp. Neurol. **116**, 103—107 (1961).

ULE, G.: Zur Ultrastruktur der „Ghost-cells" beim experimentellen Neurolathyrismus der Ratte. Z. Zellforsch. **56**, 130—142 (1962).

VICKERMAN, K.: Patterns of cellular organisation in Limax amoebae. Exp. Cell Res. **26**, 497—519 (1962).

VOGT, C., and O. VOGT: Ageing of nerve cells. Nature (Lond.) **158**, 304 (1946).

— — Eine neurohistologische Beleuchtung der Nucleolusfunktion. Biol. Zbl. **65**, 61—69 (1946).

— — Lebensgeschichte, Funktion und Tätigkeitsregulierung des Nucleolus. Ärztl. Forsch. **1**, 8—14, 43—50 (1947).

WEISS, P., and H. B. HISCOE: Experiments of the mechanism of nerve growth. J. exp. Zool. **107**, 315—395 (1949).

YOUNG, J. Z.: On the cytology of the neurons of Cephalopods. Quart. J. micr. Sci. **75**, 1—47 (1932).

Prof. dott. ENNIO PANNESE,
Istituto di Anatomia Umana dell'Università, Milano (Italia), Via Mangiagalli 31