THE RESPONSE OF VENTRAL HORN NEURONS TO AXONAL TRANSECTION

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ABSTRACT

The morphological changes induced in the frog ventral horn neurons by axonal transection have been studied with the electron microscope. During the first 2 wk after axotomy the neuronal nucleus becomes more translucent and the nucleolus becomes enlarged and less compact. The cisternae of the granular endoplasmic reticulum vesiculate and ribosomes dissociate from membranes. Free ribosomes and polysomes are dispersed in the cytoplasmic matrix. Neurofilaments and neurotubules are increased in number. These structures appear to be important in the regeneration of the axon. It is proposed that neurotubules, neurofilaments, and axoplasmic matrix are synthesized by the free polyribosomes in the chromatolytic neuron. By the fourth postoperative week, the neurons show evidence of recovery. The cytoplasm is filled with profiles of granular endoplasmic reticulum and many intercisternal polysomes. The substances being manufactured by the newly formed granular endoplasmic reticulum are not clearly defined, but probably include elements essential to electrical and chemical conduction of impulses. The significance of these observations in respect to recent studies of axoplasmic flow is discussed.

INTRODUCTION

Transection of the axon of a ventral horn neuron deprives the cell of a large part of its cytoplasm and interrupts its field of innervation. In an attempt to regenerate the axon, the neuron undergoes morphological and biochemical changes which have been the subject of investigation since the studies of Franz Nissl (1) in 1892. As visualized by light microscopy (2, 3), the nerve cell reaction includes swelling, displacement of the nucleus to an eccentric position, hypertrophy of the nucleolus, disruption of the neurofibrillar network, changes in the size and position of the Golgi apparatus, and dissolution of the Nissl bodies (chromatolysis). Although several reports of electron microscope observations on chromatolytic ventral horn cells have been published (4, 5), recent ultrastructural (6–10), histochemical (11), and radioautographic (6, 12) studies have dealt with ganglionic neurons. In 1963 Porter et al. (13) reported preliminary observations which indicated that frog ventral horn cells showed chromatolysis after transection of the sciatic plexus. These neurons were capable of regenerating functional axons. Over the past two years, this experimental model has been studied with light and electron microscopy, radioautography, and histochemistry in an attempt to understand neuronal injury, regeneration, and death.

MATERIALS AND METHODS

Specimens for this study were taken from adult male and female frogs, *Rana pipiens*, which weighed 30–60 g

at time of sacrifice. After the frogs were anesthetized with tricaine methanesulfonate (MS 222, Sandoz, Inc., Hanover, N.J.), the 7th, 8th, and 9th spinal nerves were transected at the same level in each animal. An attempt was made to approximate the nerve stumps. Postoperatively, the frogs were not able to move the hind limb on the side of the transection, but eventually some animals showed complete functional recovery.

The material for this report is derived from more than fifty animals sacrificed at 2, 4, 6, 7, 8, 9, 12, 14, 16, 17, 21, 28, 35, 42, 55, and 70 days after the nerve section. Animals to be sacrificed were anesthesized with cold MS 222. A catheter was inserted into the ventricle and passed into the conus arteriosis. Perfusion of cold-blooded Ringer's solution was continued until the articles over the ventral abdominal wall became transparent, at which time perfusion of fixative was begun. The fixative contained 1% formaldehyde, 2% glutaraldehyde, 1% acrolein in 0.1 M cacodylate buffer, pH 7.4, with 3% sucrose and trace amounts of $CaCl_2$. The perfusion lasted 1.5 hr. The tissue was allowed to remain *in situ* for several hours, after which the lower spinal cord, nerves, and leg muscles were dissected. This material was rinsed briefly in 0.1 M cacodylate buffer (pH 7.4) with 10% sucrose. All specimens for electron microscopy were postfixed for 1.5 hr in 1% OSO_4 in 0.1 M cacodylate buffer. The spinal cord was then cut into smaller segments which were subsequently dehydrated, embedded in Epon, sectioned at 0.5–1 μ, and stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate before examination with Philips 200 and 300 electron microscopes. In an accompanying series, spinal cords of animals sacrificed at similar time intervals were fixed, dehydrated, embedded in paraffin, and cut first in cross-section and then longitudinally so that the total ventral horn cell population of both sides could be compared in the same fields and in three planes. These sections were cut at 7 μ and stained with cresyl violet, Luxol fast blue (du Pont de Nemours and Company, Inc., Wilmington, Del.) and Bodian's stain for axons.

OBSERVATIONS

Morphology of the Normal Frog Ventral Motor Neuron

The neurons injured by axonal transection are in the lumbosacral enlargement of frog spinal cord. There are usually four to eight large ventral horn neurons present in each 7 μ cross-section. The nerve cell body (Fig. 1 A) appears elongate or triangular. The nucleus is oval and in the central part of the cell. There is focal clumping of chromatin in the nucleoplasm (Fig. 1 A). The nucleolus is usually single and measures 2–4 μ in diameter. Small, dense nuclear bodies are sometimes present in the nucleoplasm. In the cytoplasm, Nissl bodies are basophilic, irregularly shaped, and separated from one another by clear channels.

The fine structure of normal frog vental horn neurons resembles that of other animals (14, 15). The nuclear chromatin is composed of granular (250–350 A) and filamentous (70–150 A) aggregates (Fig. 2 A). The nucleolus appears as a moderately compact, spherical mass of darkly stained granules (150–200 A) and fine filaments (50 A) in a tightly woven skein (nucleolonema). The Nissl material is made up of aggregates of the granular endoplasmic reticulum (Fig. 3). Ribosomal particles stud the outer surfaces of the cisternae. Free ribosomes and polysomes are present in the intercisternal regions. Nissl bodies are separated from one another by translucent regions traversed by neurofilaments (100 A) and neurotubules (250 A). Elements of the agranular endoplasmic reticulum are continuous with the granular endoplasmic reticulum. Golgi complexes, dense bodies, and mitochondria are usually at the periphery of the Nissl bodies. The Golgi apparatus appears as stacks of three to six flattened cisternae arranged in parallel, curved arrays. The outer convex face is sometimes bordered by portions of the endoplasmic reticulum partially denuded of ribosomes. Simple, alveolate and dense-cored vesicles are present about the arms and in the hilus of the concave face. Lysosomes are commonly present near Golgi complexes. Membrane-limited dense bodies contain granular elements, stacked and whorled membranes, and amorphous material.

EXPERIMENTAL RESULTS

The response of injured neurons is divisible into four phases: (*a*) a latent period (0–4 days) during which no cytologic alterations are noted, (*b*) a phase (4th day to 3–4 wk) characterized by nucleolar hypertrophy and chromatolysis during which axonal regeneration is taking place, (*c*) a phase (second month) during which Nissl substance is reconstituted and the animals begin to show some return of neurologic function, and (*d*) return of neuron to normal.

First Week

In the light microscope, morphological alterations are first observed in ventral horn cells 4 days

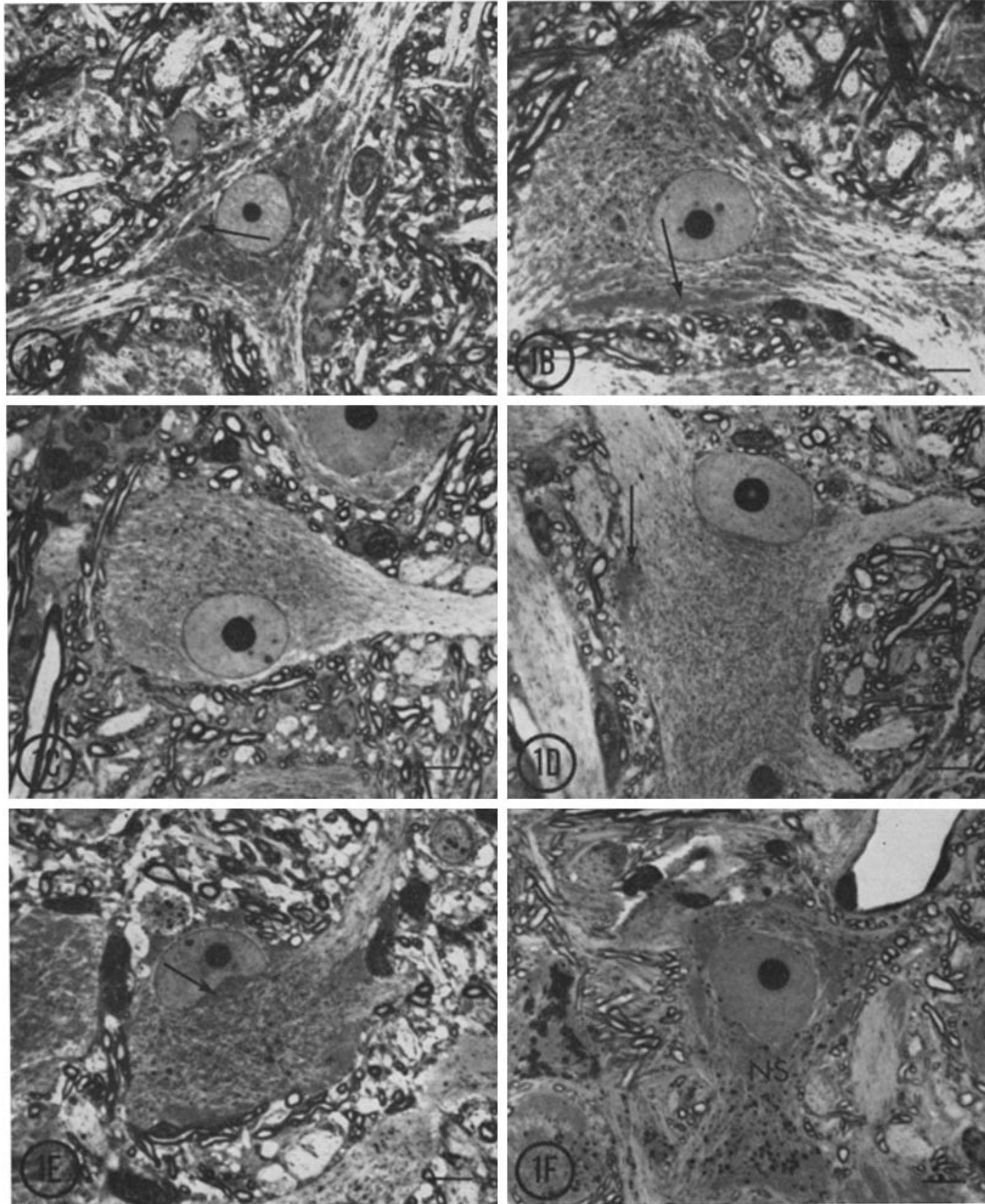


FIGURE 1 Light micrographs of frog ventral horn neurons in Epon sections stained with toluidine blue. Scale 10 μ. All magnifications, $\times$ 576. (A) The normal neuron has a triangular shape, mottled nucleus, dense nucleolus, and Nissl bodies (arrow) in cytoplasm. (B) 6 days after axotomy: the neuron, nucleus, and nucleolus are increased in size. The nucleoplasm shows several nuclear bodies. The Nissl bodies have begun to break down in the cytocentrum, but persist at the periphery (arrow). (C) 9 days postaxotomy: the perikaryon is round and the nucleus is eccentric. The nucleoplasm is translucent and contains nuclear bodies. The nucleolus is large. The Nissl material is finely dispersed. (D) 14 days postaxotomy: the neuron is large with an eccentric nucleus. Punctate basophilic material fills the cytoplasm; Nissl substance is present at the edge of the cell (arrow) and the juxtanuclear area. (E) 35 days postaxotomy: the nucleus appears mottled. Note basophilic elements in cytoplasm. A nuclear cap is evident (arrow). Dense bodies are present. (F) 42 days postaxotomy: The nucleus is mottled and the nucleolus large. Nissl substance (*NS*) is abundant and confluent. There are many dense bodies in the cytoplasm.

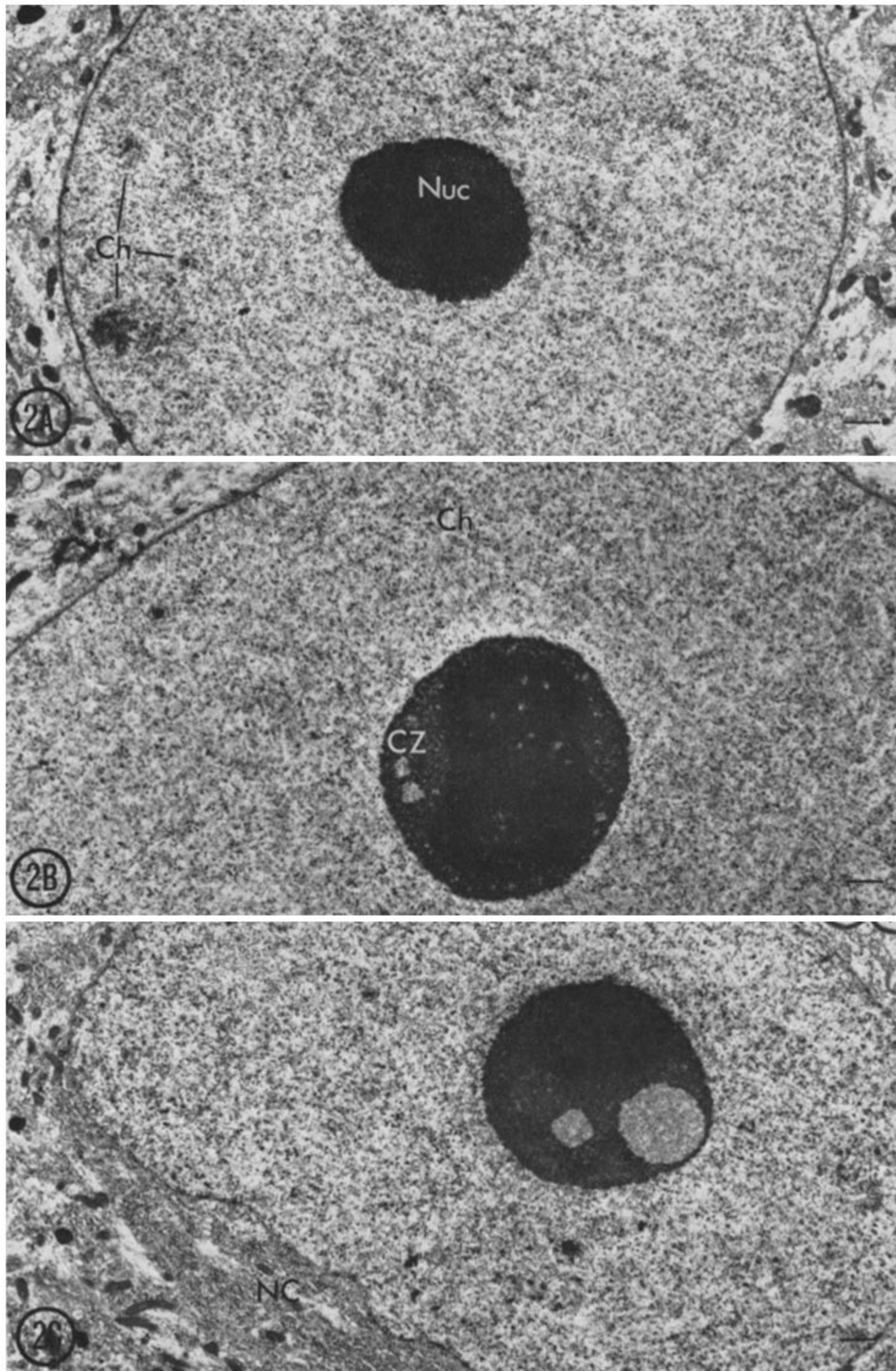
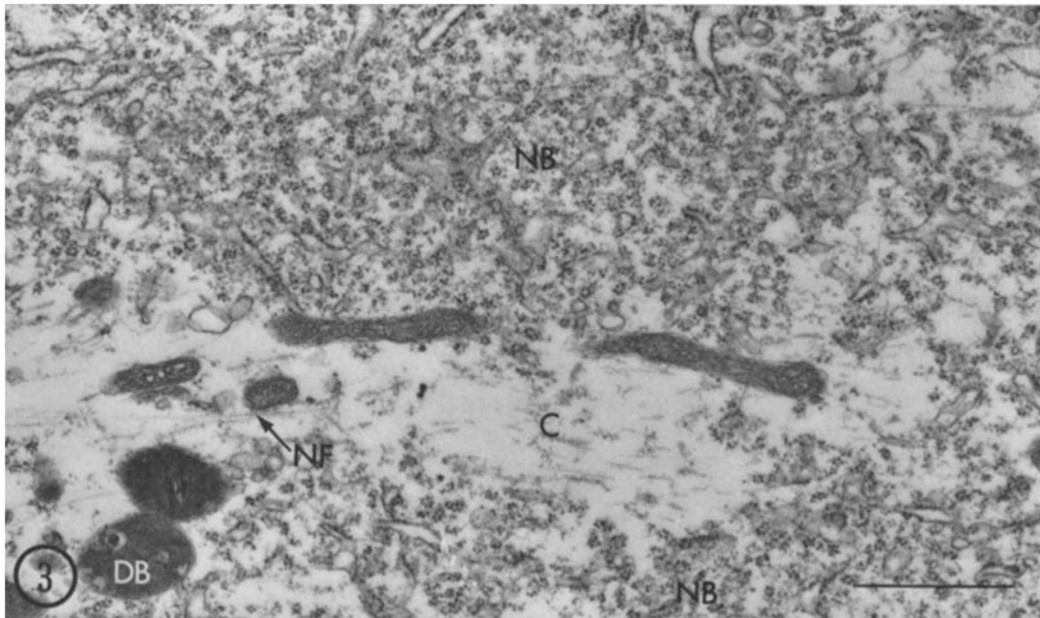


FIGURE 2 (A) Nucleus from control neuron showing moderately compact nucleolus (*Nuc*). Note that nuclear chromatin (*Ch*) is heterogeneous and focally clumped. Compare with Figs. 2 B and 2 C which are of same magnification. Scale 1 μ. × 5220. (B) Portion of nucleus from neuron 14 days postaxotomy. The chromatin (*Ch*) is less clumped than the control nucleus. The nucleolus is enlarged and shows a spongy cortical zone (*CZ*). × 5520. (C) Neuron 42 days after axonal transection. The nucleolus remains large. Nucleolar inclusions are present. There are abundant parallel cisternae of the granular endoplasm reticulum in the nuclear cap (*NC*). × 5520.



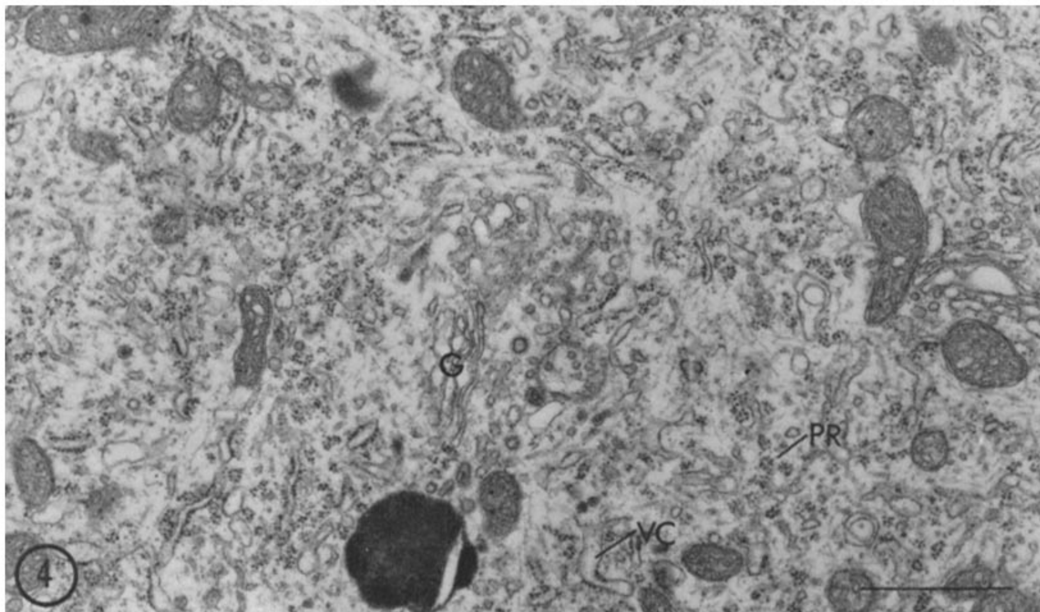


FIGURE 3 Portion of cytoplasm of control neuron. The Nissl body (*NB*) is composed of irregularly branching cisternae of granular endoplasmic reticulum. At periphery of Nissl body are other organelles, e.g., mitochondria and dense bodies (*DB*). Clear channels (*C*) containing neurofilaments (*NF*) separate Nissl bodies. Scale 1 μ. $\times$ 21,350.

FIGURE 4 Portion of cytoplasm from axotomized neuron. The granular endoplasmic reticulum is no longer aggregated in discrete Nissl bodies. Vesiculated cisternae (*VC*) have few ribosomes on membrane. Ribosomes are free and in polysomal (*PR*) formations. Neurotubules and neurofilaments course through cytoplasm. Note mitochondria. Golgi apparatus (*G*). 8 days after axonal transection. Scale 1 μ. $\times$ 20,400.

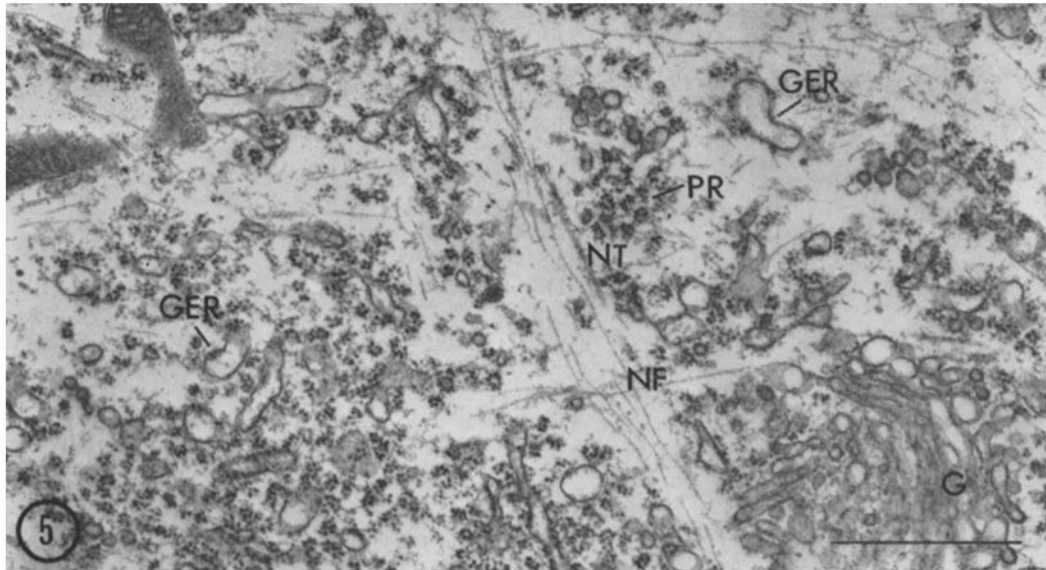


FIGURE 5 High magnification of portion of Nissl body 14 days after axotomy. The cisternae of granular endoplasmic reticulum (*GER*) are vesiculated and many ribosomes are dissociated from their membranes. Neurotubules (*NT*) and neurofilaments (NF) pass through fragmenting reticulum. Golgi apparatus (*G*). Polyribosomes (*PR*). Scale 1 μ. × 25,300.

after transection of the axon. The nucleolus enlarges. 5–7 days after injury, the neuron increases in size and becomes spheroidal. The nucleus is eccentric and its concave side faces the cytocentrum. Nissl bodies in the cytocentrum break down and are replaced by small basophilic particles (Fig. 1 B). Nissl material accumulates at the periphery of the cell and, in some neurons, at the hilus of the nucleus (nuclear cap) (4, 13).

By electron microscopy, the nuclei of operated neurons show dispersed chromatin. The nucleolus is hypertrophied. Small nuclear bodies are observed. In the cytocentrum, the cisternae of the granular endoplasmic reticulum vesiculate and ribosomes dissociate from their membranes. Free ribosomes and polysomes are present in the cytoplasmic matrix. The granular endoplasmic reticulum is intact at the periphery of the cell and in the hilus of the eccentric nucleus. Golgi complexes, present near the vesiculated cisternae of the granular endoplasmic reticulum, do not appear to change significantly during the early phase of chromatolysis.

Second Week

The operated neurons are increased in size. The nuclei are enlarged and more translucent (Fig. 1 C). The nucleoli are hypertrophied (6–8 μ) (Figs. 1 C and 2 B). Nuclear bodies are commonly present. The cytocentrum is filled with finely divided basophilic particles, the residua of Nissl bodies (Figs. 1 C and 1 D).

By electron microscopy, the nucleoli exhibit increased numbers of intensely staining, ribosome-like granules which, in some regions, are so densely aggregated that further delineation is difficult. There is an increase in the size of the spongy cortical zone of the nucleolus. This zone appears to be penetrated by nucleoplasm (Fig. 2 B). Nucleolar inclusions are made up of accumulations of fine fibrillogranular elements. Nuclear dense bodies are composed of granular aggregates and fibrillar material. The major ultrastructural alterations in the cytoplasm consist of shortening and vesiculation of cisternae accompanied by detachment of ribosomes from the membranes of endoplasmic reticulum (Figs. 4 and 5). There is a dispersal and dilution of the ribonucleoprotein particles (free ribosomes and polysomes) as the Nissl bodies break up and the cell volume enlarges. Numerous neurofilaments and neurotubules course through the cytoplasmic matrix (Figs. 6 and 7). The paths which they occupy further partition the fragmented Nissl masses. Golgi complexes are

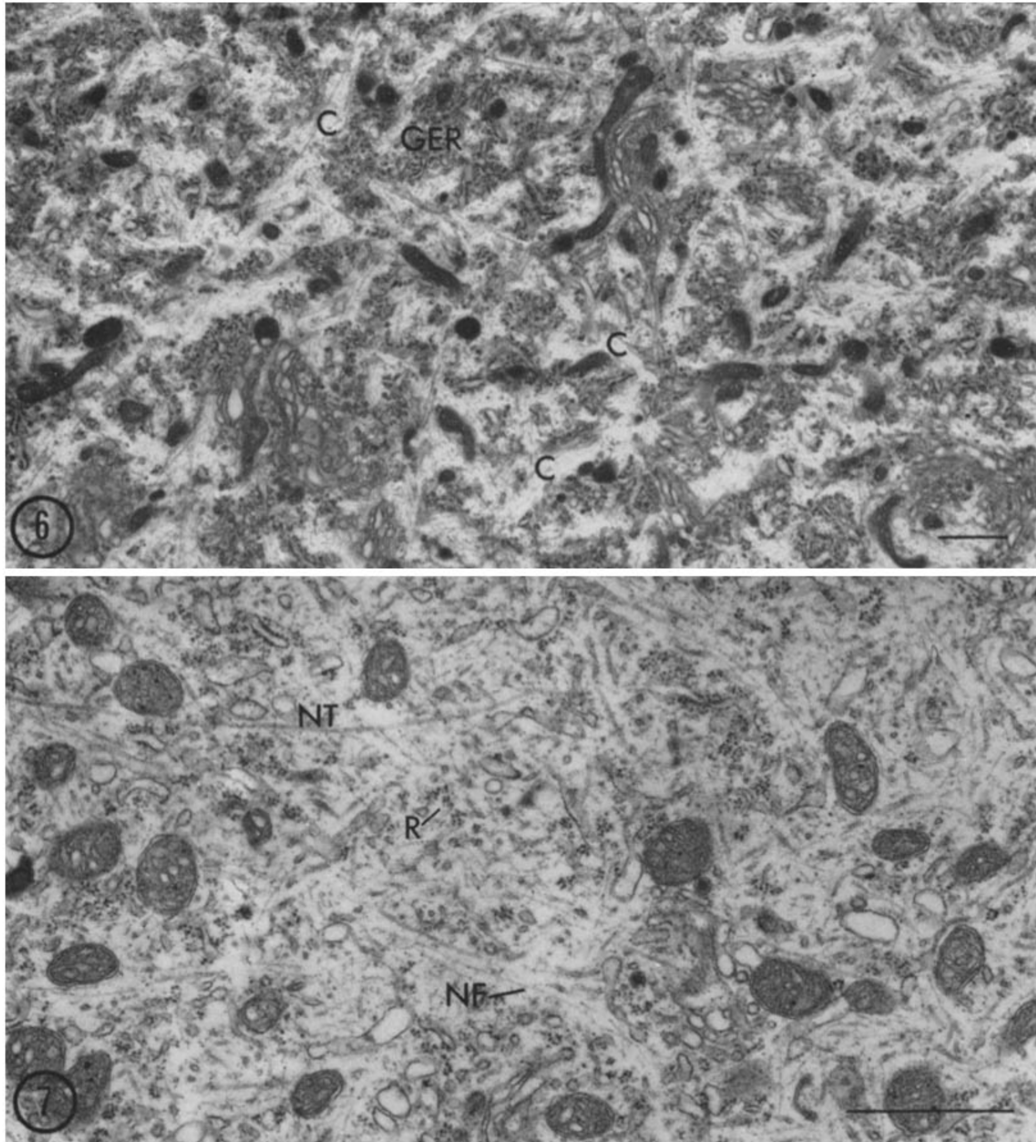


FIGURE 6 Low magnification electron micrograph of motor neuron 14 days after axonal transection. The components of the Nissl body (GER) are divided into small islands by many channels (*C*) containing neurofilaments and neurotubules. Scale 1 μ. × 8820.

FIGURE 7 High magnification of cytoplasm of chromatolytic neuron 12 days after axotomy. Ribosomes (*R*) are scattered. Note abundant neurofilaments (*NF*) and neurotubules (*NT*). Scale 1 μ. × 22,250.

present in regions where the granular endoplasmic reticulum is in the process of lysis. The forming face of the Golgi apparatus is sometimes adjacent to cisternae of the endoplasmic reticulum which are partially denuded of ribosomes (Fig. 5). Mitochondria, which commonly have an oval configuration, are no longer at the margins of Nissl bodies, but are distributed throughout the cytoplasm.

Third and Fourth Weeks

Chromatolysis reaches a peak in the third week after axonal section (Fig. 1 D). The nucleus, nucleolus, and perikaryon attain their greatest dimensions at this time. The nucleoli show loosening of the nucleolonema (Fig. 2 B), dense granular elements, and inclusions of fine fibrillogranular

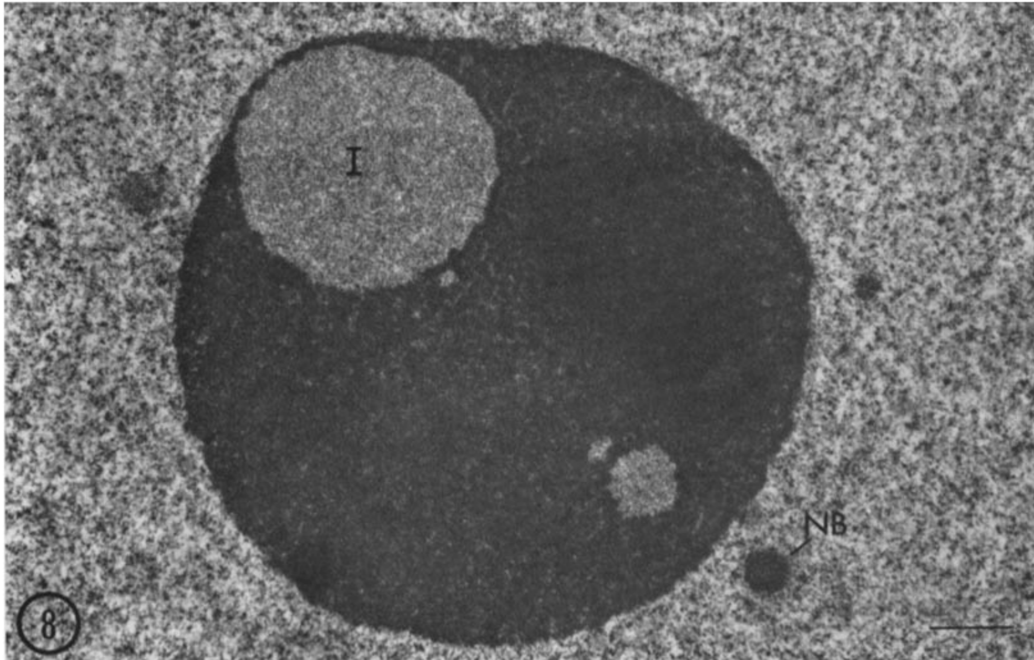


FIGURE 8 Portion of nucleus of motor neuron 21 days postaxotomy. The cortical zone of the nucleolus is spongy in appearance. Note nucleolar inclusion (*I*), the content of which differs from that of the nucleoplasm. Nuclear bodies (*NB*) are present. Scale 1 μ. × 10,930.

material (Fig. 8). During the height of chromatolysis, the cytoplasm contains many free ribosomes and polysomal clusters. Channels containing neurofilaments, neurotubules, and cytoplasmic matrix divide fragmented granular endoplasmic reticulum into small islands (Fig. 8). This ultrastructural arrangement accounts for the pattern of chromatolysis visualized by light microscopy. Elements of the granular endoplasmic reticulum are present at the margins of the cell (Figs. 1 D and 9). Golgi complexes are increased in number and linear dimension (Fig. 10). They are surrounded by an increased number of vesicles, lysosomes, and dense bodies.

Second Month

During the second month the neuron shows evidence of recovery. The perikaryon becomes smaller and the nucleus appears mottled (Fig. 1 E). The nucleolus remains large. Basophilic material accumulates in the nuclear hilus and cytocentrum (Fig. 1 E). The channels which usually subdivide these elements into Nissl bodies are thin and tenuous, thus creating a confluent Nissl substance (Fig. 1 F).

Viewed in the electron microscope (Fig. 2 C), the nuclear chromatin is clumped in the granular and filamentous aggregates similar to that of the control neurons. The nucleolonema is more compact. Nucleolar inclusions are present in some neurons. The granular endoplasmic reticulum is abundant. The nuclear cap (Figs. 1 E and 2 C) is composed of granular endoplasmic reticulum in the form of numerous long, roughly parallel cisternae studded with ribosomes (Fig. 11). In the cytocentrum, circular and spiral polyribosomes are conspicuous. The many polysomes present between the profiles of granular endoplasmic reticulum account for the confluent appearance of the Nissl substance (Fig. 12). Although mitochondria and dense bodies are occasionally trapped in these aggregates of granular endoplasmic reticulum, it is more common to find them, along with Golgi complexes, neurofilaments, and microtubules, in those regions not occupied by hypertrophic Nissl material. Dense bodies are abundant.

DISCUSSION

A major portion of axonal protein is synthesized in the perikaryon and transported distally (16,

17). It has been estimated that a cell body exports its own weight of axoplasm in less than 1 day (18). The ratio of axoplasm to somatoplasm has been estimated at 250 for motor neurons of the rhesus monkey (19). Therefore, the ventral horn cell loses a substantial portion of its cytoplasm when its axon is transected. The loss of axoplasm is registered in the cell body (20) and initiates a series of changes in the morphology and metabolic activities of the neuron. Using the metabolites at its disposal the neuron must replace lost axoplasm, supply protein for the regenerating neurite, and

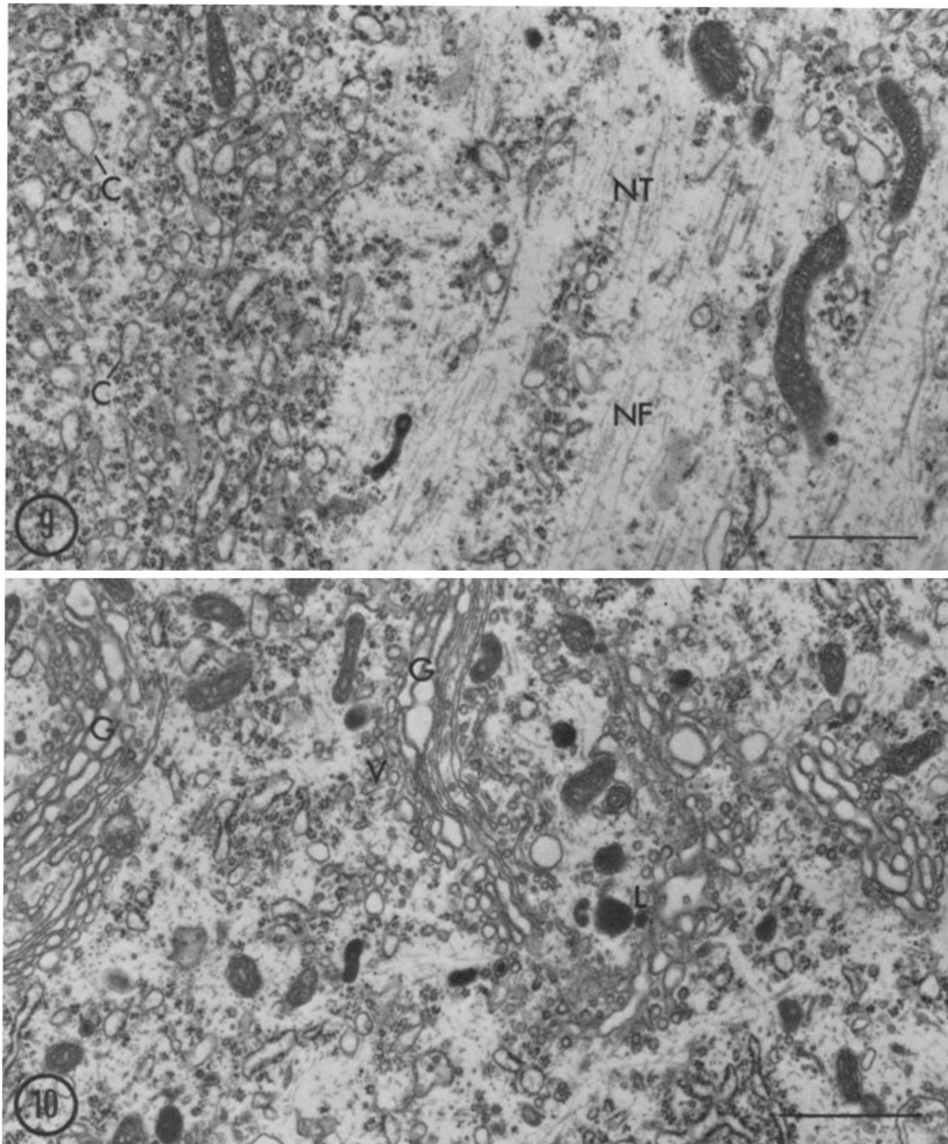


FIGURE 9 Electron micrograph of periphery of neuron 14 days after axotomy. Cisternae (*C*) show vesiculation. Ribosomes have dissociated from membrane. Note neurotubules (*NT*) and neurofilaments (*NF*) in region adjacent to cytocentrum. Scale 1 μ. $\times$ 17,080.

FIGURE 10 Portion of neuron 16 days after axotomy. Golgi complexes (*G*) are enlarged and appear increased in number. Golgi vesicles (*V*) and lysosomes (*L*) are prominent. Scale 1 μ. $\times$ 19,000.

once reinnervation occurs, synthesize elements necessary for nerve conduction and chemical transmission. The nucleolar hypertrophy, chromatolysis, and restoration of Nissl substance which ensue after axonal transection are morphological expressions of these changes in biochemical priorities.

Although several new observations are described in the present report, the alterations in axotomized ventral horn cells are similar to those recently

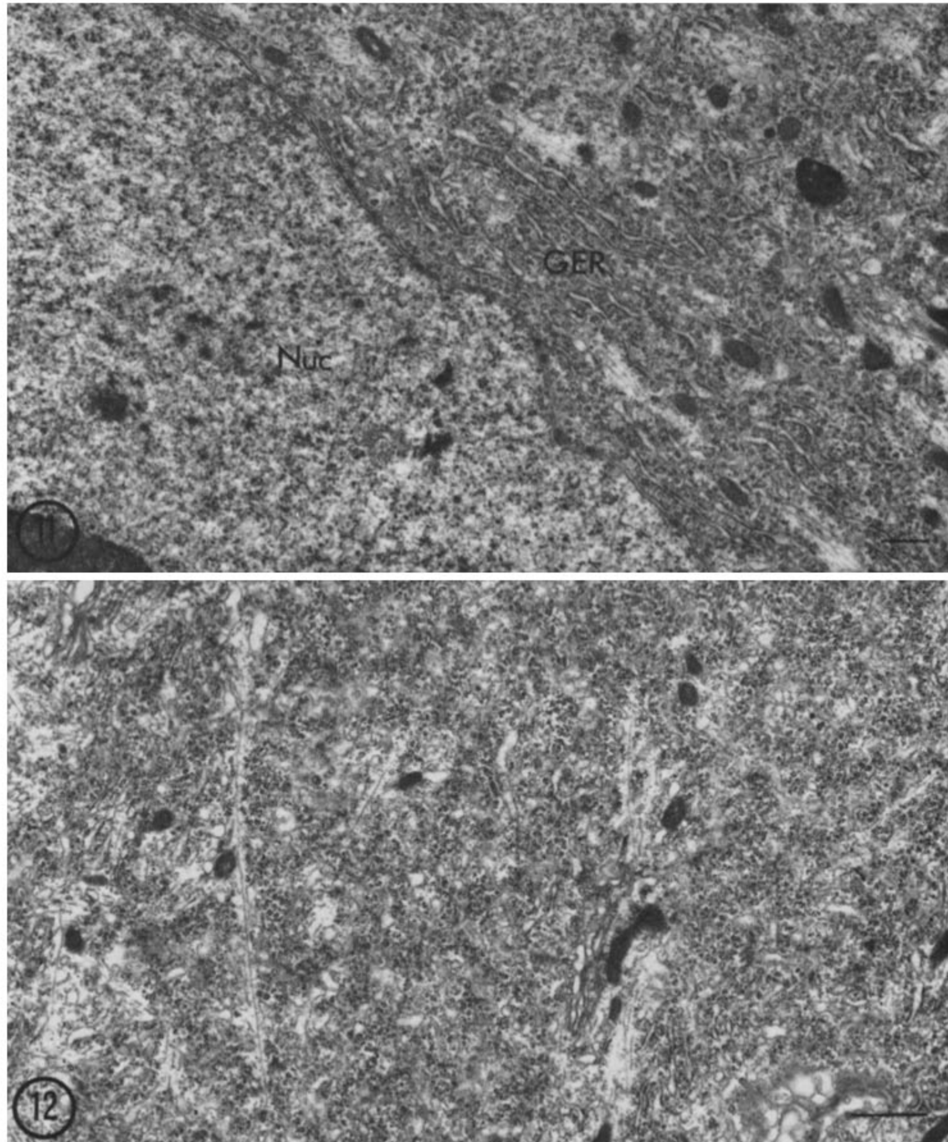


FIGURE 11 High magnification of nucleus (*Nuc*) and juxtanuclear area as shown in Fig. 2. The granular endoplasmic reticulum (*GER*) shows parallel cisternae in the region of nuclear cap. Many polysomes are present between cisternae. Scale 1 μ. × 6270.

FIGURE 12 Low-power electron micrograph of cytoplasm of motor neuron 42 days after axotomy. There are abundant polysomes both free and attached to membranes. Large masses of these elements produce the confluent Nissl substance shown in Fig. 1 F. Scale 1 μ. × 9850.

reported in ganglionic systems (6, 7). Ultrastructural changes occur in the nucleus, nucleolus, and several cytoplasmic organelles. The nucleus becomes enlarged, eccentric, and translucent. The nucleolus hypertrophies and its cortical zone is penetrated by nucleoplasm. The cisternae of the granular endoplasmic reticulum vesiculate and ribosomes dissociate from membranes. Free ribosomes and polysomes are dispersed throughout the cytoplasmic matrix. Neurofilaments and neurotubules are numerous. Golgi complexes and lysosomes increase in number, size, and complexity. In the recovery phase, profiles of the granular endoplasmic reticulum and intercisternal polysomes are abundant. Dense bodies are commonly present in the recovered neurons.

Nuclear and Nucleolar Alterations

During the first two postoperative weeks the nucleoplasm appears more finely dispersed, resulting in a lessened electron opacity. This modification of the chromatin pattern may represent changes in the state of DNA templates necessary for the replication of complementary RNA strands. After axonal injury, changes in RNA production are initiated and are transmitted to the cytoplasm so that axoplasm can be restored. The concept that RNA transcription is essential in the initiation of chromatolysis is supported by the observation that actinomycin D, which inhibits DNA-dependent RNA synthesis, prevents the appearance of retrograde cell reaction in neurons of the reticular formation (21).

As in some ganglionic systems (7, 22), nucleolar hypertrophy is detectable in ventral horn neuron within a few days of axotomy. In our material this nucleolar enlargement is accompanied by changes in the appearance of the nucleolus. These alterations reflect the role played by this structure in meeting the increased requirements for protein synthesis (23–25). Since ribosomal RNA is synthesized in the nucleolus, it is probable that nucleolar hypertrophy after axotomy is associated with an increased production of RNA necessary to restore lost axoplasm. In a high resolution radioautographic study (26) of RNA synthesis in the nucleolus of *Chironomus thummi*, label was found over chromosomal material extending into the nucleolus, suggesting that ribosomal RNA synthesis takes place at these chromosomal sites. Isotopic label was transposed from the pars fibrosa to the pars granulosa of the nucleolus. Subsequently, RNA migrated to the cytoplasm where functional ribosomes were formed. Similar events may begin where nucleoplasm penetrates the spongy nucleolus of the chromatolytic neuron.

Ribosomes and Endoplasmic Reticulum

The most striking cytologic effect of axonal transection is the disruption of Nissl bodies, hence the term chromatolysis. In our material the lysis of Nissl bodies begins in the cytocentrum shortly after the appearance of the changes in the nucleus and nucleolus. In chromatolytic neurons a large proportion of the ribosomes are free or in polysomal formations. It is probable that the production of new axoplasm is taking place on these polyribosomes. The increased numbers of neurofilaments and neurotubules in the cytoplasmic matrix surrounding the finely divided ribonucleoprotein elements lend credence to this hypothesis. The neuron actively engaged in regenerating lost axoplasm resembles the developing neuroblast which has a high concentration of cytoplasmic ribosomes but a poorly developed endoplasmic reticulum (27). On the basis of their study of maturing neurons of the chick, Eschner and Glees (28) suggested that free ribosomes and polysomes synthesized ground substance and structural elements in the developing neuronal process. It is not surprising that this pattern appears when axoplasm is in demand as it is after axonal transection.

These early changes in the nucleolus and granular endoplasmic reticulum conform well with biochemical studies of axonal reaction. During chromatolysis there is an increase in incorporation of labeled nucleosides and amino acids per neuron and an increase in the protein content per cell (29, 30). Protein production seems to begin around the time the ribosomes have begun to dissociate from membranes. During recovery the ribonucleoprotein content of the cell increases to about twice the normal amount and then returns to basal levels.

After regeneration of axoplasm, the synthetic machinery must assume additional functions. This is reflected in the morphologic changes which appear during recovery. The Nissl substance reappears in the cytocentrum and the nuclear hilus (late nuclear cap). The cytoplasm is filled with arrays of granular endoplasmic reticulum and many intercisternal polysomes. Biochemical studies have shown that the ribonucleoprotein

content is increased at this time (30). The neuron has new priorities and it is likely that the ribosomes attached to membranes have different functions than the free ribosomes and polysomes present during chromatolysis. Close association of ribosomes with the membranes of the reticulum appears to be necessary when the product of synthesis is to be exported from the perikaryon. It is of interest that during embryogenesis there is a progressive increase in membrane-bound ribosomes as the neuron grows into mesodermal tissues (27). Thus, the reappearance of Nissl substance can be considered a recapitulation of developmental sequences.

It is probable that the newly formed granular endoplasmic reticulum produces substances and structures to be transported down the regenerated axon. However, functional neuroeffector contacts are not essential for reformation of granular endoplasmic reticulum. Abundant Nissl material was sometimes present in animals in which there was no evidence of reinnervation. Although the nature of the products being synthesized remains under active study, it is probable that acetylcholinesterase is one of these substances. Acetylcholinesterase reaction product is formed in the Nissl substance and the nuclear envelope (11). Presumably, this enzyme is synthesized near the cisternae and then transported to sites of activity. In conformity with this concept is the observation that the acetylcholinesterase accumulates in the axon proximal to sites of sciatic nerve crush. Schwarzacher (31) showed that acetylcholinesterase activity disappeared from the cell body after axotomy, only to reappear when reinnervation had been achieved.

Our findings suggest that nonmembrane-bound ribosomes of the chromatolytic neuron synthesize cytoplasmic proteins necessary for regeneration of axoplasm. During the later stages the reformed granular endoplasmic reticulum produces materials important for functional recovery.

Neurofilaments and Neurotubules

During the second postoperative week neurofilaments and neurotubules appear to be increased in number. These organelles occupy clear channels which pass through the fragmenting Nissl bodies and partition the ribosomes. The pattern of neurotubules and neurofilaments in a field of ribosomes resembles that present in the developing neuroblast during the phase of axonal outgrowth (27). Using high resolution radioautography to study incorporation of leucine-^{3}H in normal neurons, Droz and Koenig (32) were not able to determine whether the label was contained in neurofilaments, neurotubules, or cytoplasmic matrix. On the basis of concentration of leucine-^{3}H grains over neurofilamentous bundles, they suggested that protein subunits seem to be synthesized and transported from perikaryonal ribosomes. The observations reported in this paper support the hypothesis that neurofilaments and neurotubules are synthesized by nonmembrane-bound polyribosomes which are prominent during the chromatolytic phase.

Golgi Apparatus and Lysosomes

In both control and injured neurons there is a close topographical relationship between Golgi complexes and lysosomes. At the peak of chromatolysis the Golgi complexes are enlarged and are more numerous in regions where the granular endoplasmic reticulum is in the process of fragmentation. The number, size, and complexity of the lysosomes increase during the third to sixth week. Studies (11, 33) have shown that neuronal lysosomes possess a variety of enzymes which participate in the breakdown of endogenous tissue elements. The changes in Golgi zones and lysosomes may be due to an increased demand for enzymes needed to catabolize cellular components not essential for regeneration. Although the Golgi complexes return toward normal during the recovery phase, large dense bodies, which contain amorphous, finely granular and membranous material, persist well into the third postoperative month.

Relationship between Chromatolysis and Axoplasmic Flow

In 1948, Weiss and Hiscoe (34) formulated the concept that the neuron is continuously forming new cytoplasm, some of which enters the axon and travels along its length. That the primary source of nerve protein is the nerve cell body is confirmed by isotope experiments (16, 18, 19, 35–38). The new proteins, elaborated on perikaryonal ribosomes, are transferred down the axon as fast and slow components (35, 36, 38). The slowly transported proteins are associated with axoplasmic matrix. Neurofilaments and microtubules (35) may be part of the slow component (35).

These proteins would be necessary for the renewal of axoplasm. The rapidly moving component, which arrives at nerve endings within a few hours of synthesis, is attached to particulate material and has a slow turnover. It has been proposed that the fast axoplasmic proteins are impoıtant in the transfer of certain metabolites from the cell body to the synaptic endings. Neurosecretory granules may be one constituent of the system (35, 38).

The findings present in this report suggest that the ribosomes of the chromatolytic neuron synthesize slowly moving protein (matrix, neurotubules, neurofilaments) needed for axonal growth. However, during recovery, the ribosomes of the newly formed Nissl substance are involved in the manufacture of rapidly transported elements important in cytoplasmic streaming and chemical transmission.

Although neurons have only one life to live, they possess considerable regenerative capacity during their lifetime. Axotomy induces profound cytological and biochemical alterations in the injured neuron. The changes documented in this report are interpreted as a recapitulation of the biological processes of growth and differentiation. The present experiments form the basis for further studies in this laboratory on cell injury, regeneration, and death in the central nervous system. Knowledge gained from these studies should provide some understanding of the nature of the central nervous sytem response to axonal transection, and, by analogy, to more complex experimental abnormalities of the nervous system and to their human counterparts.

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