# DETECTION OF NEUROFILAMENTS IN THE PERIKARYON OF HYPERTROPHIC NERVE CELLS

ENNIO PANNESE. From the Institute of Human Anatomy, Laboratory of Neurocytology "P. Rando," University of Milan, Italy

Researches carried out with the use of the polarizing and electron microscopes have evidenced that submicroscopic filamentous structures, which have been called neurofilaments, are present in the nerve axoplasm. Analogous filamentous structures have been detected in the perikaryon by electron microscopy, but only in restricted areas of the cytoplasm. No structures comparable to the neurofibrillar network of the silver-stained preparations have been described in the perikaryon of nerve cells studied with polarized light. It is, therefore, of some interest to report that filamentous structures have been seen, both by polarized light and electron microscopy, in the perikaryon of sensory ganglion cells undergoing hypertrophy, as shown in the present research.

It has long been known that in reptiles after tail amputation there is regeneration of the skin, muscles, and cartilaginous skeleton but not of nerve cells. The last three pairs of spinal ganglia left *in situ* cranial to the plane of amputation supply the sensory innervation of the regenerating tail. Terni (3, 4) showed that during tail regeneration the neurons of the mentioned ganglia undergo an increase in size which corresponds to the increase in size of the peripheral field of innervation. Starting with this evidence, we carried out parallel studies, with polarized light and electron microscopy, of the changes in the submicroscopic structure of the sensory ganglion cells that undergo hypertrophy during various stages of tail regeneration in specimens of the common lizard (*Lacerta muralis*).

The spinal ganglia were fixed in 2 per cent $OsO_4$ buffered at pH 7.4 with veronal-acetate, embedded in methacrylate or Araldite and sectioned with the Porter-Blum ultramicrotome. Sections 0.5 to 1 $\mu$ thick, mounted in glycerol or in Canadian balsam, were examined with polarized light under a Leitz Ortholux microscope supplied with a xenon lamp. Ultrathin sections, contiguous to the previous ones, were examined with a Siemens Elmiskop II electron microscope. Results obtained by the two techniques were compared (1).

Fig. 1 demonstrates the general appearance of the perikaryon in nerve cells of thoracic ganglia which have been used as controls. Under the electron microscope the neurofilaments appear scattered among the other perikaryal structures without any evident orientation: they occur both isolated and in small bundles. In the above cells only the nuclear membrane sometimes shows birefringence by polarized light; no birefringent structures can be observed in the cytoplasm.

Fig. 2 *A* demonstrates the general appearance of the perikaryon of hypertrophic ganglion cells. Medium-dense areas are separated from each other by more or less wide strips of low density. The cytoplasmic organelles (mitochondria, Nissl bodies, Golgi complexes) lie in the denser areas; the more transparent regions contain only neurofilaments embedded in an electron-permeable amorphous material. The 60 to 70 A thick sinuous neurofilaments do not show a structural periodicity along their axis (Fig. 2 *D*); they look very similar to the neurofilaments already described by various authors in the nerve axoplasm. The neurofilaments of the cell in Fig. 2 *A* are rather loosely arranged in the perikaryon and lie, in general, parallel to its surface. They may build a sort of cape which envelops the nucleus but is not in direct contact with it; thus, in equatorial sections (Fig. 2 *A*) they appear as a typical ring-shaped structure. When strictly corresponding areas of the perikaryon are examined under polarized light, birefringent bands can be seen (Figs. 2 *B*, *C*). The characteristics of the birefringence are identical to those described by Thornburg and De Robertis (5) and by Bairati and Palay (2) in the axoplasm of the CNS fibres and of peripheral axons, namely, the sign of birefringence is positive. It appears, therefore, that the filamentous structures of the perikaryon of the hypertrophic nerve cell are neurofilaments similar to those known to be present in the nerve axoplasm.

In the present experiment it was observed that only the small and medium-size ganglion cells hypertrophy, *i.e.*, those up to roughly 4,000 $\mu^3$ in volume; the larger cells do not show any modification.

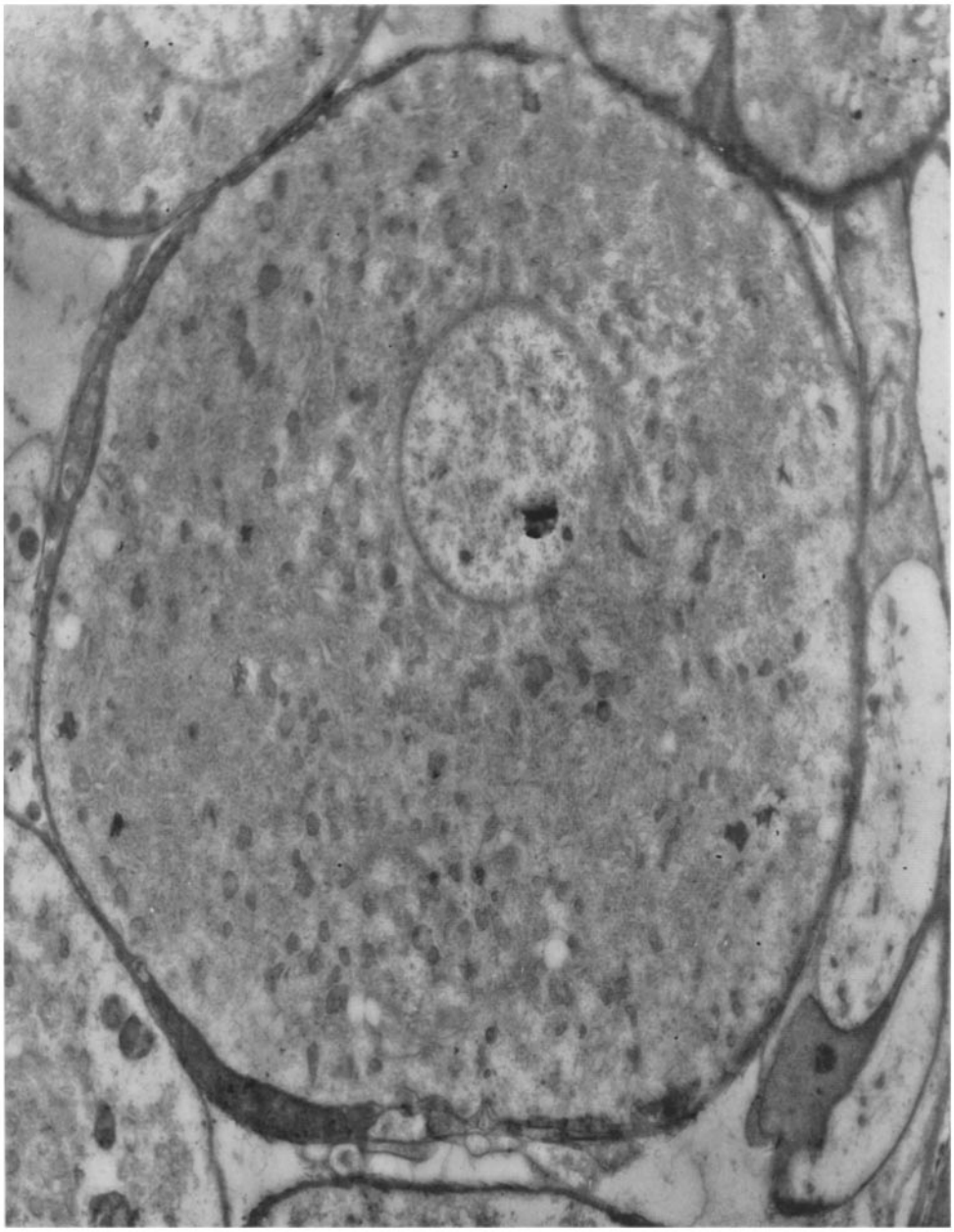

FIGURE 1

Nerve cell of a thoracic ganglion of *Lacerta muralis* used as control. Electron micrograph showing the general appearance of the perikaryon. No birefringent structures can be observed in the perikaryal cytoplasm of this cell when studied with polarized light. × 6,000.

The origin of these structural aspects of nerve cells undergoing hypertrophy will be discussed elsewhere. One basic question is whether in the hypertrophying neurons there is an increase in the number of the neurofilaments or simply a rearrangement of the cytoplasmic organelles which renders the preexisting neurofilaments more manifest. The former hypothesis would

seem the more feasible. It must be stressed, however, that under conditions in which bands of oriented neurofilaments become apparent in the electron microscope a birefringence is detectable in corresponding areas of the perikaryon, thus permitting the demonstration of these structures merely by optical microscopy without resort to silver staining methods.

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FIGURE 2

Hypertrophying nerve cell of a spinal ganglion of *Lacerta muralis*.

FIGURE 2 *A*

Electron micrograph showing the general appearance of the perikaryon. In the perikaryal cytoplasm there are strips of low density which appear as a ring-shaped structure surrounding the nucleus. They consist of neurofilaments embedded in an electron-transparent material. × 6,000.

FIGURE 2 *B*

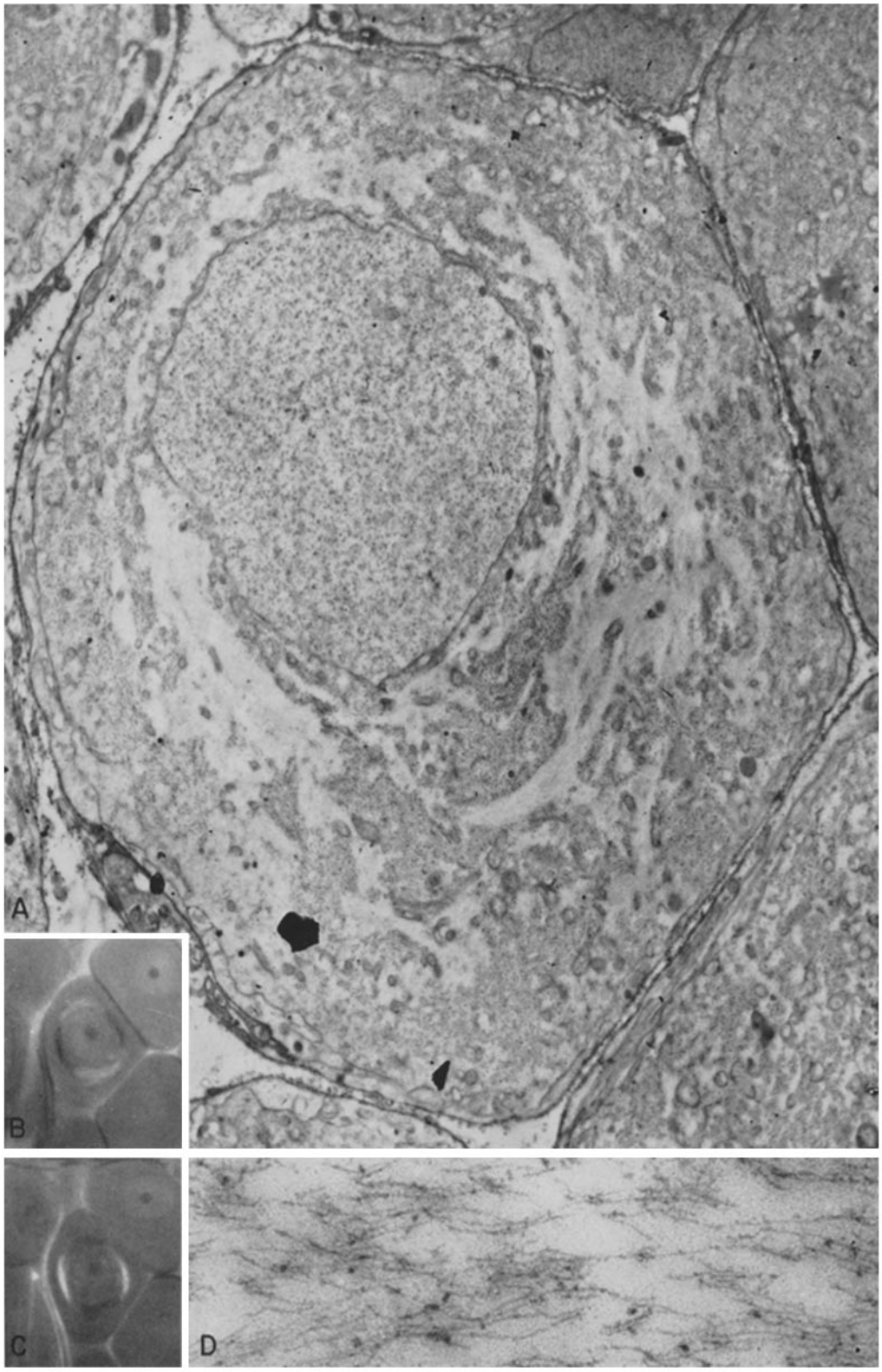
Polarized light photomicrograph (Brace compensator) of a 1 μ thick section, contiguous to the ultrathin one of Fig. 2 *A*. A birefringent ring surrounding the nucleus can be seen in the perikaryon. The birefringent ring corresponds strictly to the strips of low density of Fig. 2 *A*. × 650.

FIGURE 2 *C*

The same as Fig. 1 *B*, but the position of the Brace compensator has been changed. × 650.

FIGURE 2 *D*

Electron micrograph at greater enlargement showing the neurofilaments in the perikaryon of a hypertrophying nerve cell. × 75,000.


A
B
C
D