Changes in features of degenerating primary sensory neurons with time after capsaicin treatment

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Summary. Capsaicin (50 mg/kg) was injected into new born mice and 5 and 12 h, and 1, 2, 3, and 5 days later, their lumbar dorsal root ganglia (DRG) with the nerve roots were fixed by immersion. The morphological changes which ensued with time after treatment were examined by light and electron microscopy. The findings were as follows: (a) rapid degeneration of certain smaller B-type neurons, indicating their prompt death, was seen 5 h after treatment. Later, accumulated neurofilaments appeared in larger B-type neurons. Fissures of the cytoplasm and cell fragmentation were also observed as particular features of degeneration. Finally, these degenerating neurons, destined to die, appeared as small round figures with a disorganized nucleus. Severely degenerated neurons were seen throughout the survival time after treatment, but seemed to be most numerous after 2—3 days. (b) Three days after treatment the Nissl substances of large A-type neurons appeared dispersed, forming ring-like bundles in the periphery of cells. Cytoplasmic rupture and large membrane-bound spaces with fine granular or fibrillar materials, indicating peripheral cytolysis, were also conspicuous. Some of these cells showed severe degeneration clearly leading to cell death. The A-type neurons began to degenerate later than the B-type neurons. (c) Satellite cells showed an increased amount of electron-opaque cytoplasm that contained large vacuoles and neuronal cell debris. Mitotic figures were increased in satellite cells 3 days after treatment. (d) Unmyelinated axons in the dorsal root of mice treated with capsaicin became enlarged with accumulation of neurofilaments, synaptic vesicles or various kinds of vesicles, multivesicular bodies amd mitochondria. Numerous dense lamellar bodies appeared in the unmyelinated axons within DRG 3 days after treatment, but were scarcely seen in the dorsal roots. Degeneration of the myelinated fibers increased with time. Interestingly, capsaicin

seemed to have both a direct and indirect action on DRG neurons: its direct action induced rapid degeneration of the smaller neurons, whereas its indirect action induced relatively slow degeneration of the larger neurons, causing chromatolytic changes similar to those induced by periphal nerve axotomy. The injury to DRG neurons due to the indirect action seemed to be induced retrogradely.

Key words: Capsaicin — Degeneration — Chromatolytic change — Primary sensory neuron — Mouse

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In neonatal animals, capsaicin, an ingredient of red pepper, causes irreversible damage of most small B-type primary sensory neurons. As capsaicin selectively destroys chemosensitive primary sensory neurons, Jancsó and Kiraly [11] classified it as a "sensory neurotoxin". The neuropharmacological or neurophysiological effects of capsaicin have been reviewed [4, 22]. One feature of degeneration caused by capsaicin [6, 12, 14, 17] is degeneration of mitochondria; i.e., their swelling and disruption of their cristae in dorsal root ganglion (DRG) neurons. Recently capsaicin has also been reported to destroy some large A-type neurons as well as B-type neurons in the lumbar DRG of mice [10]. However, little is known about the morphological process of degeneration of DRG neurons after capsaicin treatment. In this study we first examined the histological changes of DRG with time after injection of capsaicin into mice. Then we compared the features of degeneration induced by capsaicin with that induced by other drugs, peripheral nerve axotomy, and some neuropathies. The possibility that capsaicin primarily affects nerve terminals and that this effect is followed by retrograde degeneration of DRG neurons is discussed.
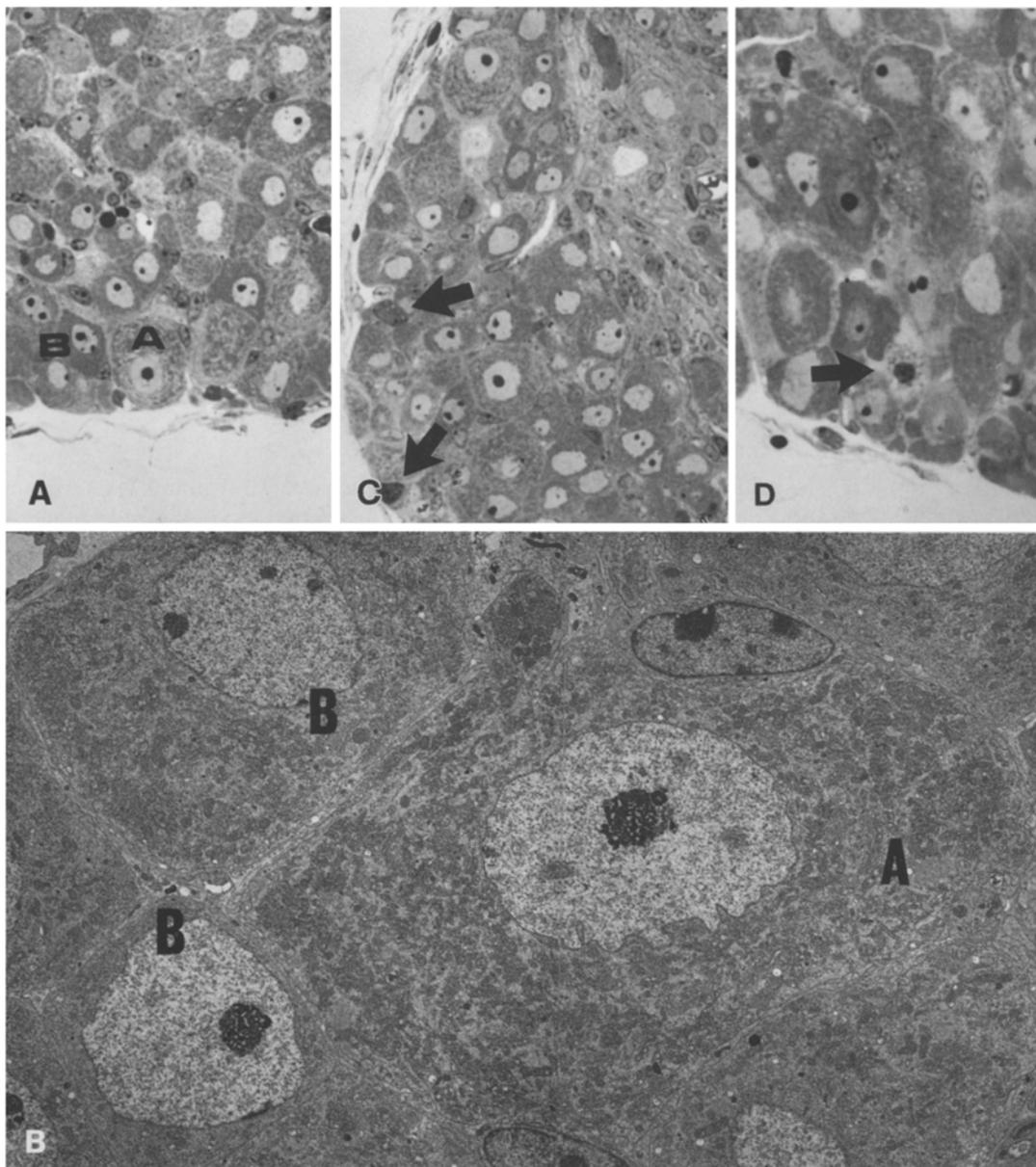

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Fig. 1A–D. Control and degenerated DRG neurons after injection of vehicle and capsaicin (CAP), respectively. **A** Normal large A-(type) cells (**A**) and small B-(type) cells (**B**) 5 h after injection of vehicle. ×400. **B** Ultrastructural appearance of normal A and B cells 5 h after injection of vehicle. ×2,500. **C** Degenerated neurons (*arrows*) 5 h after injection of CAP. ×400. **D** A severely degenerated B-cell (*arrow*) with an electron-dense nucleus and indistinct cytoplasm due to cytolysis (12 h after CAP injection). ×800

Materials and methods

In all, 21 new born mice including 9 controls were examined. Capsaicin (Sigma) dissolved in the vehicle described by Jancsó [12] was injected s.c. at a dose of 50 mg/kg into test mice of 2 days old. Six control mice received an injection of vehicle only and 3 control mice were not treated and examined on days 2, 5 and 8 after birth. After 5 and 12 h, and 1, 2, 3, 5 days, 2 animals of each were anesthetized with ether, and the lumbar DRG with the ventral and dorsal roots were excised. The specimens were promptly fixed in a mixture of 2.5% glutaraldehyde and 1.2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. The specimens were then washed with buffer, and postfixed in 1% OsO_4 in buffer. They were then processed by

the routine procedure and finally embedded in Epon. Serial 1-μm sections of the entire DRG from animals at each time were stained with toluidine blue for examination of changes of DRG neurons and satellite cells by light microscopy. Ultrathin sections of several areas of the DRG and dorsal roots were stained with saturated uranyl acetate and lead citrate, and examined in a Hitachi H-500 electron microscope.

Results

Types of DRG neurons were classified as described by Andres [2]. That is, neurons with large light neuroplasm and conspicuous Nissl substances were classi-
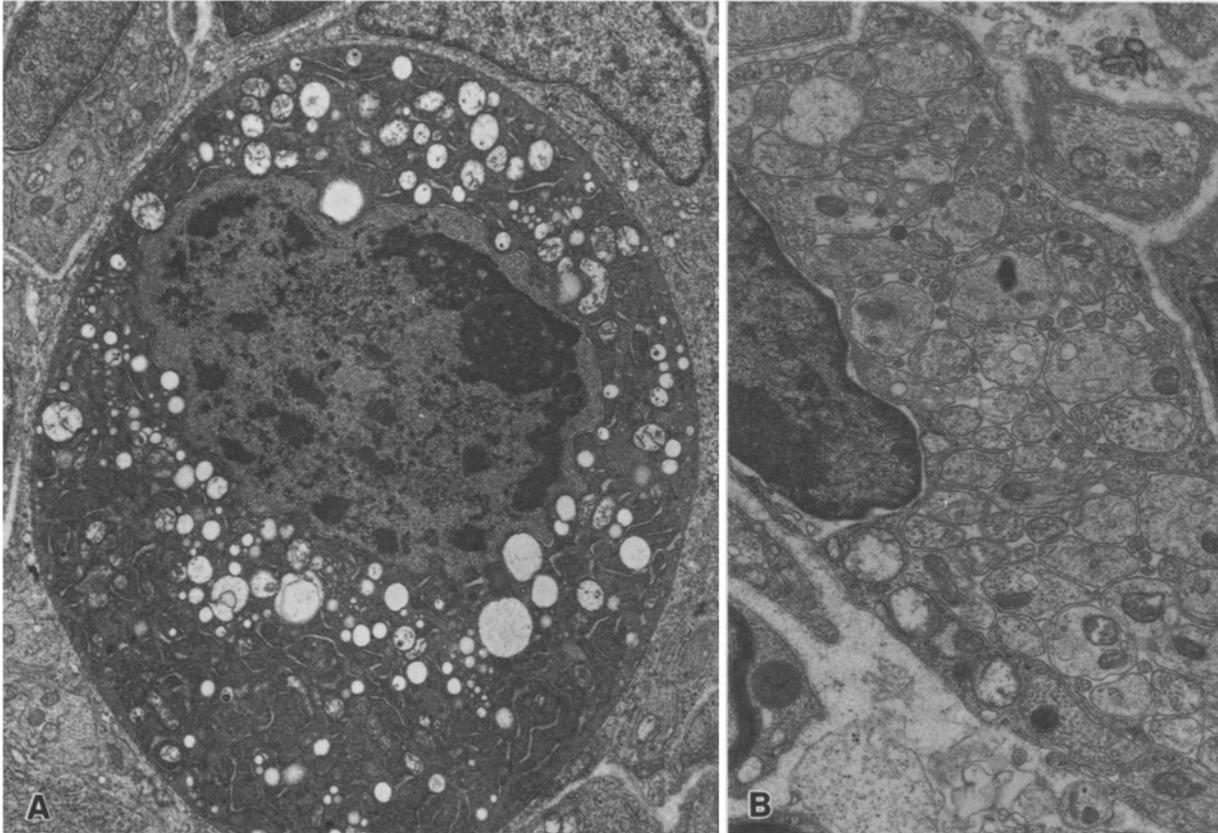
Fig. 2A, B. Ultrastructural appearance of neurons 5 h after CAP injection. **A** A rapidly degenerating B-cell with electron-dense cytoplasm packed with vesicles of various sizes and an altered nucleus. ×8,200. **B** Note the dorsal root unmyelinated axons showing no marked changes. ×15,300

fied as A-type (A-cells) and those with small dark neuroplasm with evenly destributed Nissl substances were classified as B-type (B-cells) (Fig. 1 A, B).

Light microscopic observations

Five hours after treatment. Some small B-cells had become very dark, indicating severe degeneration (Fig. 1 C).

Twelve hours after treatment. A severely degenerated neuron with a disorganized nucleus, showing cytolysis, was observed (Fig. 1 D).

Day 1 − 2 after teatment. The cytoplasm of some of the degenerated B-cells was ruptured (see Fig. 4 B). A gray band was observed around the nucleus of B-cells (see Fig. 4 A).

Day 3 after treatment. The number of severely degenerated B-cells with abnormal cytoplasm was increased. Nissl substances of A-cells had become peripherally localized, forming a ring-like structure (see Fig. 6 A). Mitotic figures were frequently seen in satellite cells in the soma (see Fig. 6 A).

Day 5 after treatment. The number of B-cells showing degenerated features was decreased. Fissures were seen in the cytoplasm of A-cells, but the nucleus and cytoplasm of these cells appeared intact. Some severely degenerated A-cells with an abnormal nucleus and marked cytolysis could be seen (see Fig. 7 A). Frequently, empty spaces devoid of cytoplasm were seen in peripheral part of the A-cells (see Fig. 7 B).

Ultrastructural observations

Five hours after treatment. Smaller B-cells (diameter < 15 μm) with an electron-dense appearance were seen to contain various types of closely packed round vesicles, randomly arranged cisternae of endoplasmic reticulum and a degenerating nucleus (Fig. 2 A). No other changes were seen in DRG neurons. The dorsal root unmyelinated axons showed no characteristic changes (Fig. 2 B).

Twelve hours after treatment. A mass of accumulated neurofilaments appeared around the nucleus. In the dorsal roots, some unmyelinated axons were enlarged and contained degenerated mitochondria, synaptic
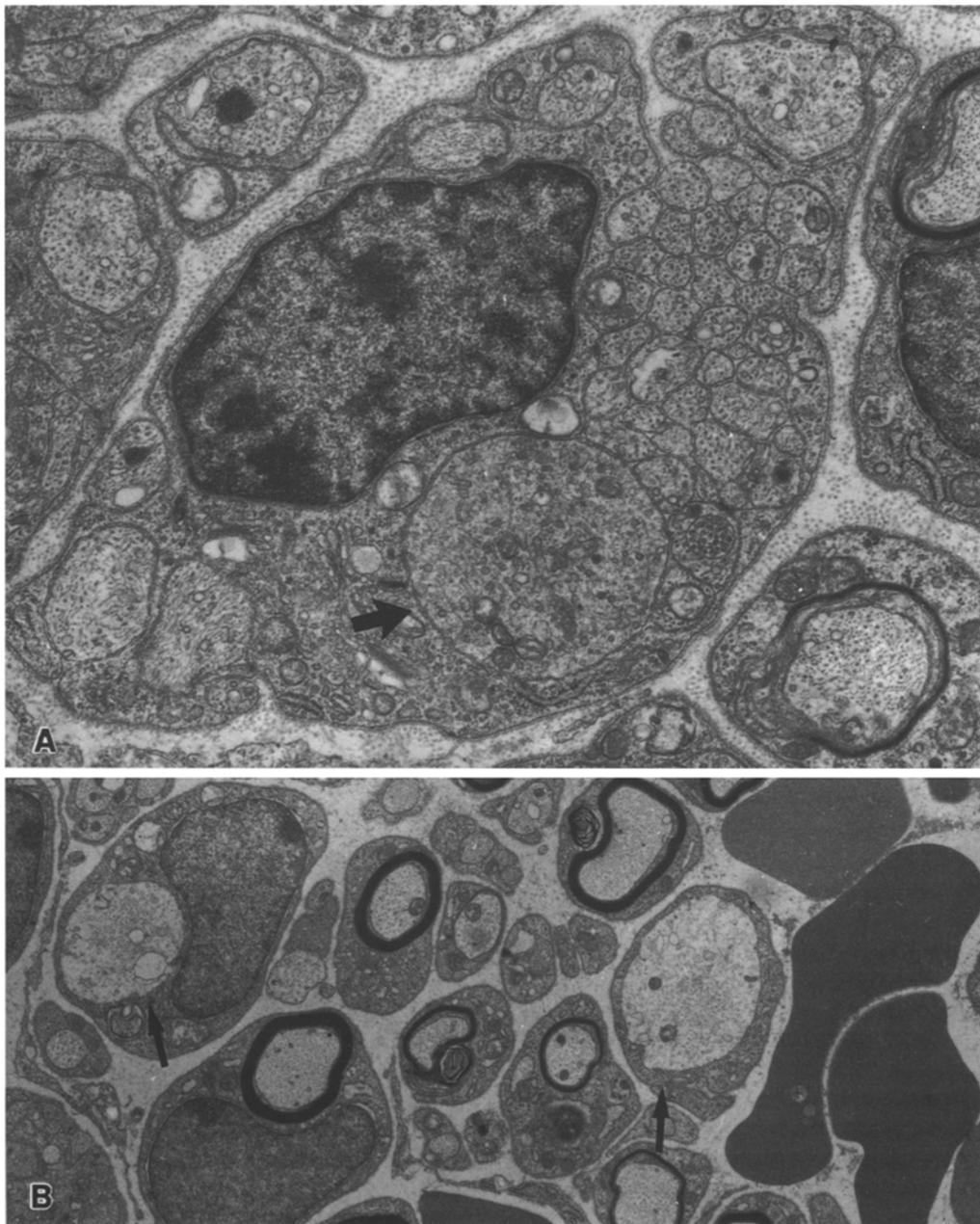
Fig. 3A, B. Features of degeneration in the dorsal root 12 h (**A**) and 1 day (**B**) after CAP injection. **A** An enlarged unmyelinated axon (*arrow*) containing synaptic vesicles, degenerated mitochondria and vesicles but little cytoskeleton. × 16,000. **B** Enlarged unmyelinated axons (*arrows*), showing central accumulation of neurofilaments. × 6,800

vesicles, and disorganized neurotubules and neurofilaments (Fig. 3A).

Day 1 after treatment. Accumulation of the neurofilaments was marked in B-cells of 13 to 20 μm diameter (Fig. 4C). Fissures were seen in the cytoplasm of affected B-cells, occasionally associated with cell fragments (Fig. 4C). Centrally located neurofilaments were observed in the enlarged unmyelinated axons of the dorsal roots (Fig. 3B). In a few myelinated fibers,

the myelin was disrupted and protruded into the axoplasm.

Day 2 after treatment. The B-cells showed more advanced stages of degeneration. Most of these degenerating cells were surrounded by satellite cells with electron-lucent cytoplasm and large vacuoles (Fig. 5A). The number of enlarged unmyelinated axons in the DRG had increased. While these axons contained degenerated mitochondria, synaptic ves-
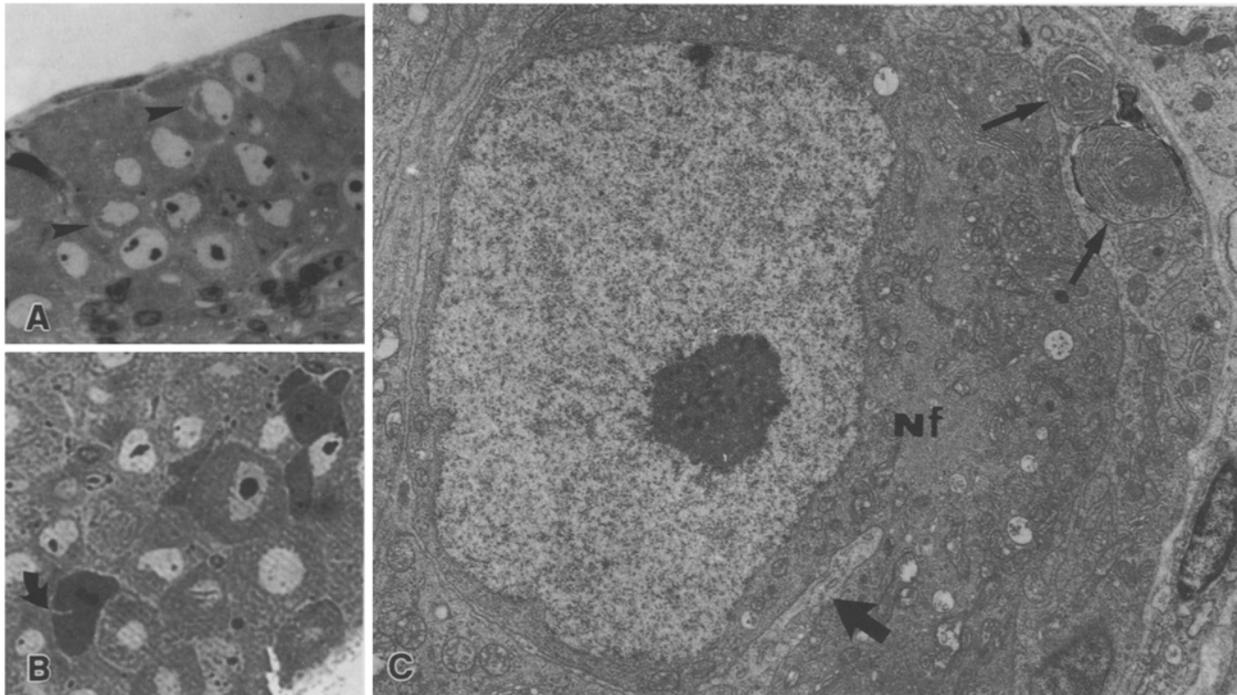
Fig. 4A — C. Features of degeneration 1 day after CAP injection. **A** Cap-like gray bands (*arrowheads*) near the nuclei of B-cells seen by light microscopy. × 800. **B** A cytoplasmic fissure (*arrow*) of a degenerating B-cell seen by light microscopy. × 800. **C** Ultrastructure of a degenerating B-cell with a cytoplasmic fissure (*large arrow*), accumulated neurofilament (*Nf*) corresponding to the cap-like band and cell fragments (*small arrows*). Note that the cytoplasmic fissure may represent the intrusion of a process of the satellite cell into the neuron. × 9,100

icles, various other vesicles and multivesicular bodies, some axons had become scanty in appearance because of degradation of the accumulated vesicles, indicating destruction of the axoplasm (Fig. 5B).

Day 3 after treatment. Fissures of the cytoplasm were frequently seen in degenerated B-cells. The rough endoplasmic reticulum in A-cells showed peripheral dispersion corresponding to the ring-like structure seen by light microscopy (Fig. 6B). Numerous unmyelinated axons in the DRG had become enlarged and included closely packed dense lamellar bodies (DLB) and multivesicular bodies (Fig. 6C). In the dorsal roots, no accumulation of DLB was seen, but slightly enlarged axons showing disruption of the cytoskeleton were observed in Remak bundles.

Day 5 after treatment. Most remaining B-cells had no abnormal neurofilament bundles, but some cells still contained small round granular materials derived from the nucleolus, densely packed mitochondria, and neurofilament bundles (Fig. 9A). These severely degenerated cells, which were dying, were always surrounded by satellite cells, but not macrophages. Some A-cells had a fissure in their cytoplasm, but their nuclei were normal. A-cells in a more advanced stage of degeneration had an eccentric nucleus with a partially

destroyed envelope, and closely packed degenerated mitochondria (Fig. 8). Frequently, the peripheral cytoplasm of A-cells contained fine granular or fibrillar material enclosed in a membrane (Fig. 7C). As the degeneration proceeded, the fine granular or fibrillar materials disappeared and large empty spaces were formed between satellite cell processes and the neuroplasm as the result of cytolysis. The unmyelinated axons including DLB were reduced in number. Thus, disorganization of the axoplasmic organelles was conspicuous in the DRG (Fig. 9B). The number of unmyelinated axons ensheathed by Schwann cells was markedly decreased in the dorsal roots. The features of disrupted myelin and myelinated axoplasm increased in the dorsal roots (Fig. 9C). Many macrophages had appeared and these engulfed degenerated nerve fibers.

Discussion

In this study we found that at all times after capsaicin treatment the number of degenerating neurons relative to the number of intact neurons was unexpectedly small. We also found that degeneration of B-cells started before that of A-cells for some unknown reason. Furthermore, some B-cells degenerated rap-
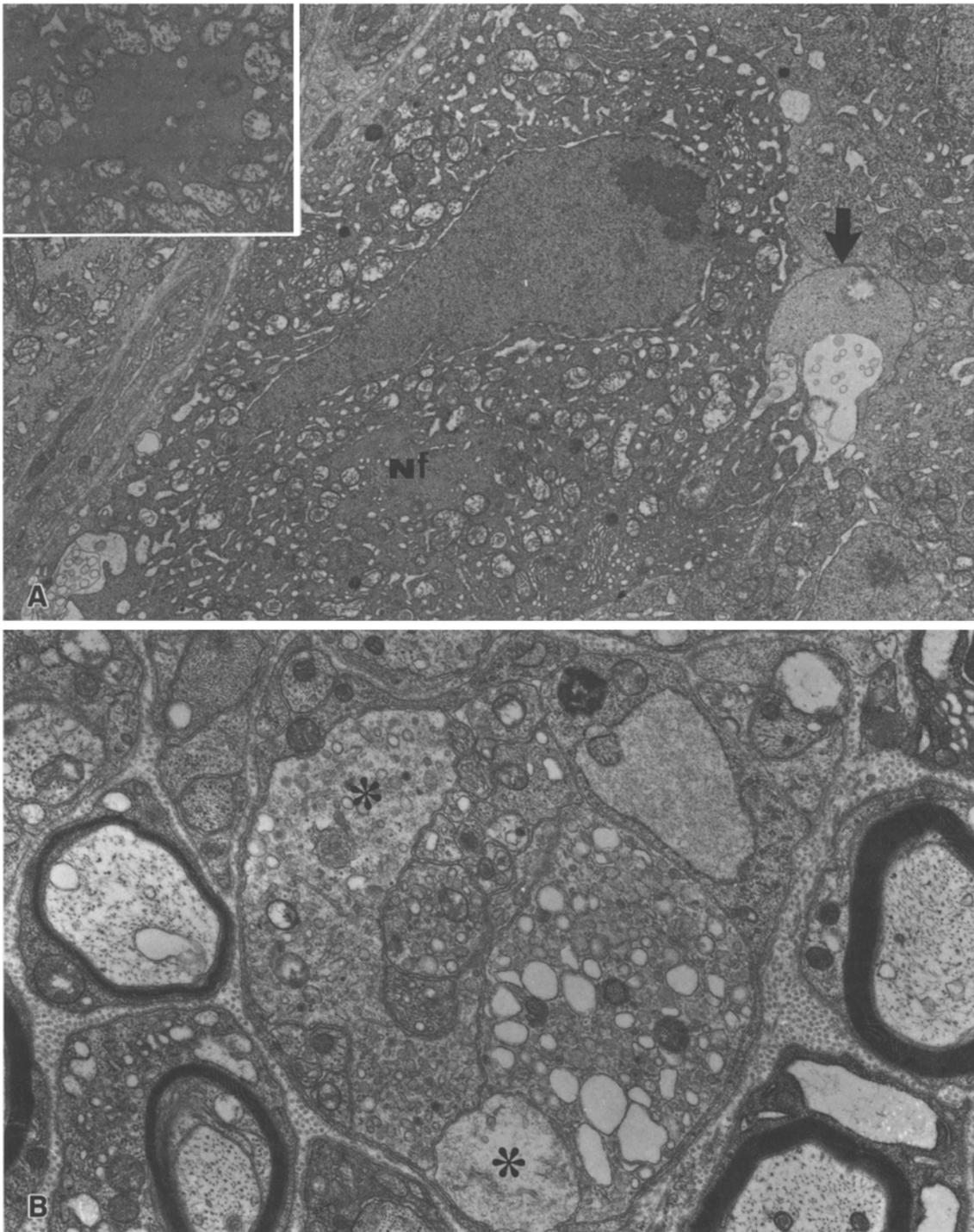
Fig. 5A, B. Features of degeneration 2 days after CAP injection. **A** A more advanced degenerating B-cell with an eccentric nucleus. Note accumulated neurofilaments (*Nf*) among the numerous degenerated mitochondria. A homogeneously electron-lucent satellite cell (*arrow*) containing large vacuoles is seen outside the degenerated neuron. × 8,600. The inset shows accumulated neurofilaments. × 12,000. **B** Enlarged unmyelinated axons in the DRG. The axons contain synaptic vesicles, degenerated mitochondria, and various types of vesicles. Some of these axons appear homogeneous due to destruction of organelles (*asterisks*). × 16,700
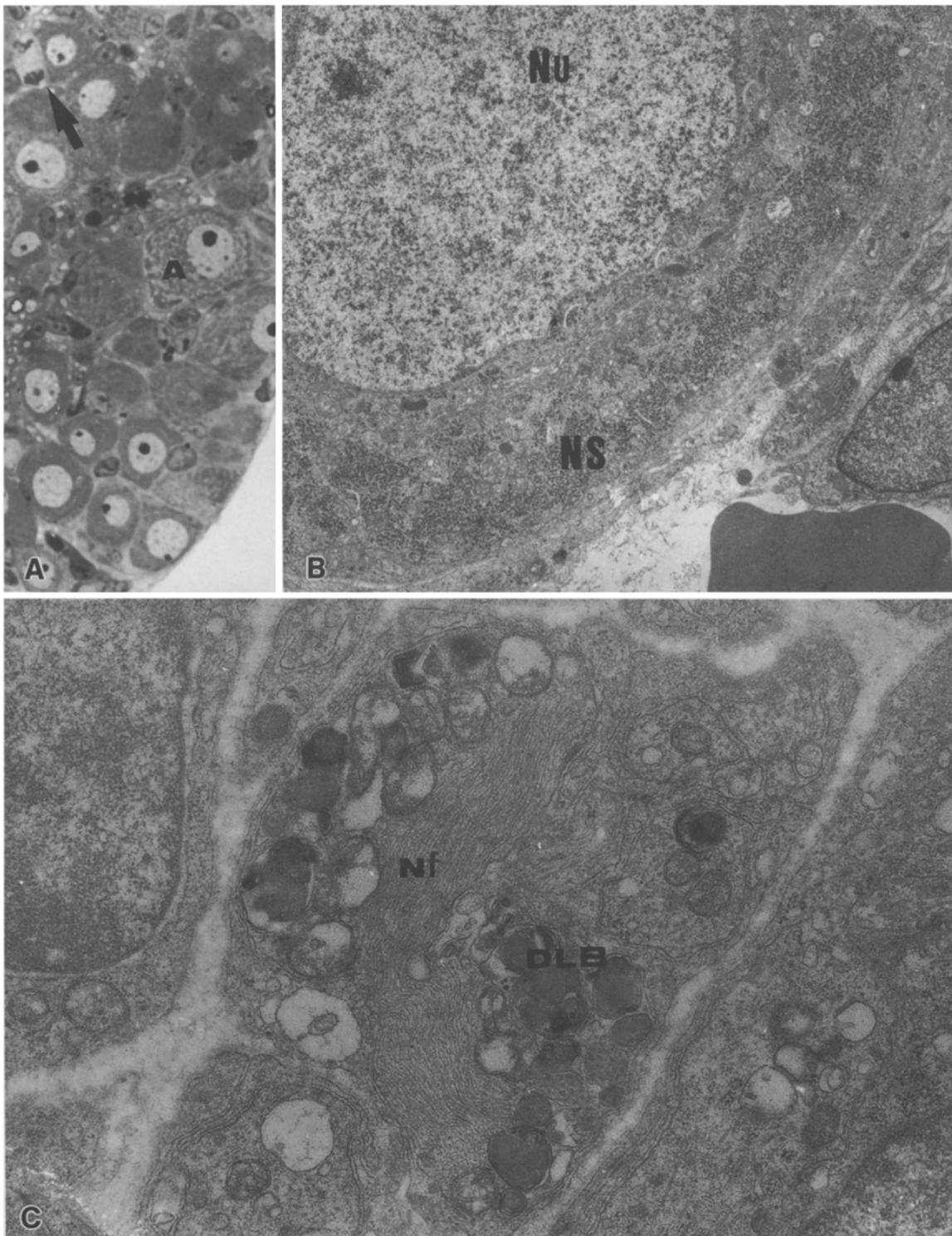
Fig. 6A – C. Features of degeneration 3 days after CAP injection. **A** An A-cell (*A*) showing peripherally located Nissl substances seen by light microscopy. The arrow indicates a mitotic figure in a satellite cell. × 800. **B** Ultrastructure of the peripherally located Nissl substances (*NS*), showing perinuclear chromatolysis. *Nu*: Nucleus. × 7,700. **C** An enlarged unmyelinated axon in the DRG containing numerous dense lamellar bodies (*DLB*) separated from accumulated neurofilaments (*Nf*). × 25,500
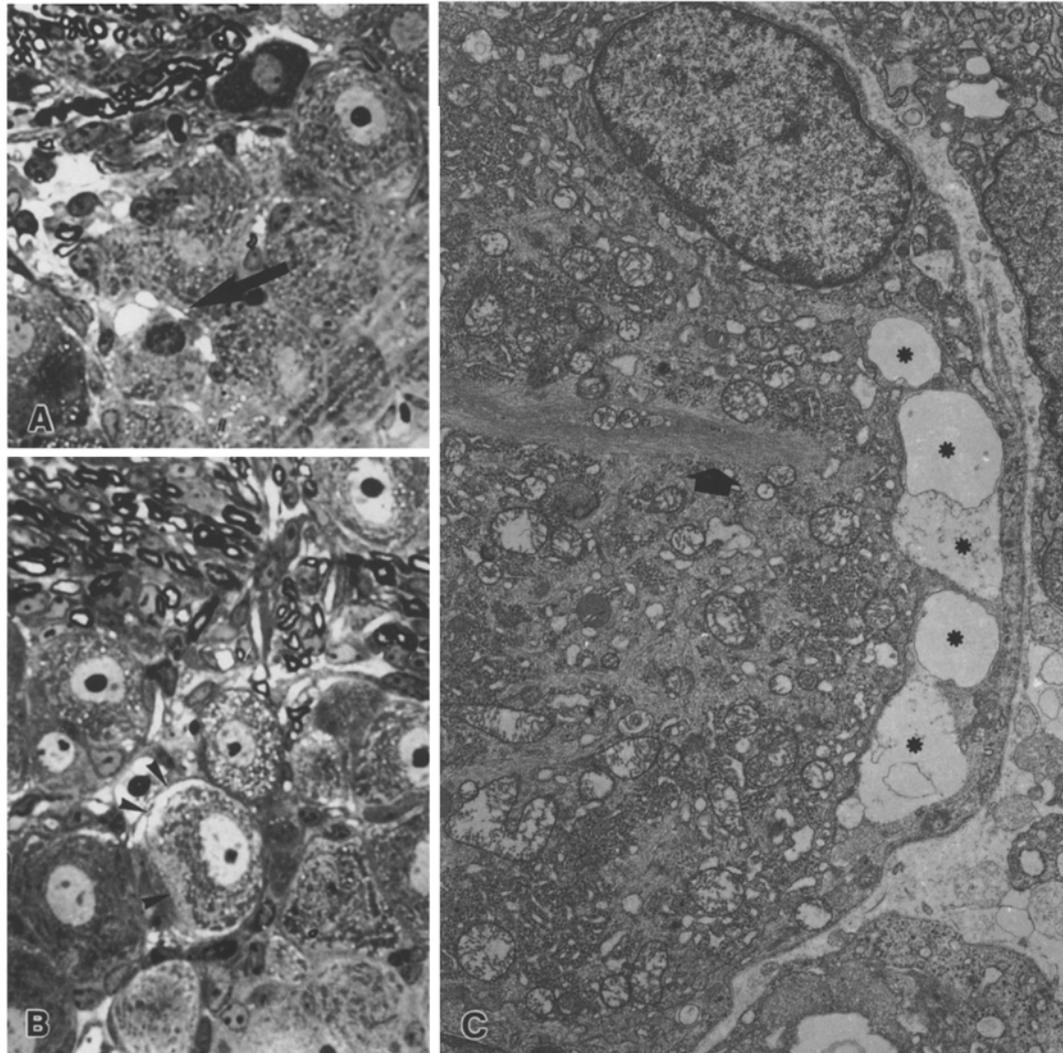
Fig. 7 A — C. Features of degeneration of A-cells 5 days after CAP injection. **A** A severely degenerating A-cell (*arrow*) showing nuclear alteration and marked cytolysis. × 800. **B** Space (*arrowheads*) devoid of cytoplasm. × 800. **C** Peripheral vacuoles (*asterisks*) bounded by a membrane between satellite cell processes and the neuroplasm, corresponding to the peripheral empty spaces seen by light microscopy. Note the abnormal neurofilament bundles (*arrow*). × 8,200

idly, whereas others degenerated later. For that reason, neurons in various stages of degeneration were seen throughout the observation time after treatment. We did not measure the number of degenerating neurons quantitatively at each observation time, but degeneration of B-cells seemed to be maximal 2 — 3 days after treatment.

Degeneration of the soma

The effects of capsaicin in causing eccentricity of the nucleus, mitochondrial degeneration, and dilation of the cisternae of the endoplasmic reticulum have been reported by others [6, 12, 14, 26]. Several additional features of degeneration were observed in this study. These included neurofilament accumulation, neuronal

cell fissure and fragmentation, A-type cell degeneration, and changes of satellite cells.

There are reports that neurofilament accumulation or proliferation in sensory and motor neurons is a special feature of degeneration associated with peripheral nerve axotomy [7, 16, 28, 29], the normal process of degeneration of chick embryos [19], chemically induced polyneuropathy [21], necrosis in Sprawling mutant mice [23, 24], administration of vincristine and vinblastine [25], and intoxication by pyridoxine [18] or aluminum [5]. Thus, induction of neurofilament accumulation is not a unique effect of capsaicin. As aggregation of neurofilaments was seen even in severely degenerated neurons, we consider that this phenomenon is related to cell degeneration, rather than regeneration as claimed by Zelená [30].
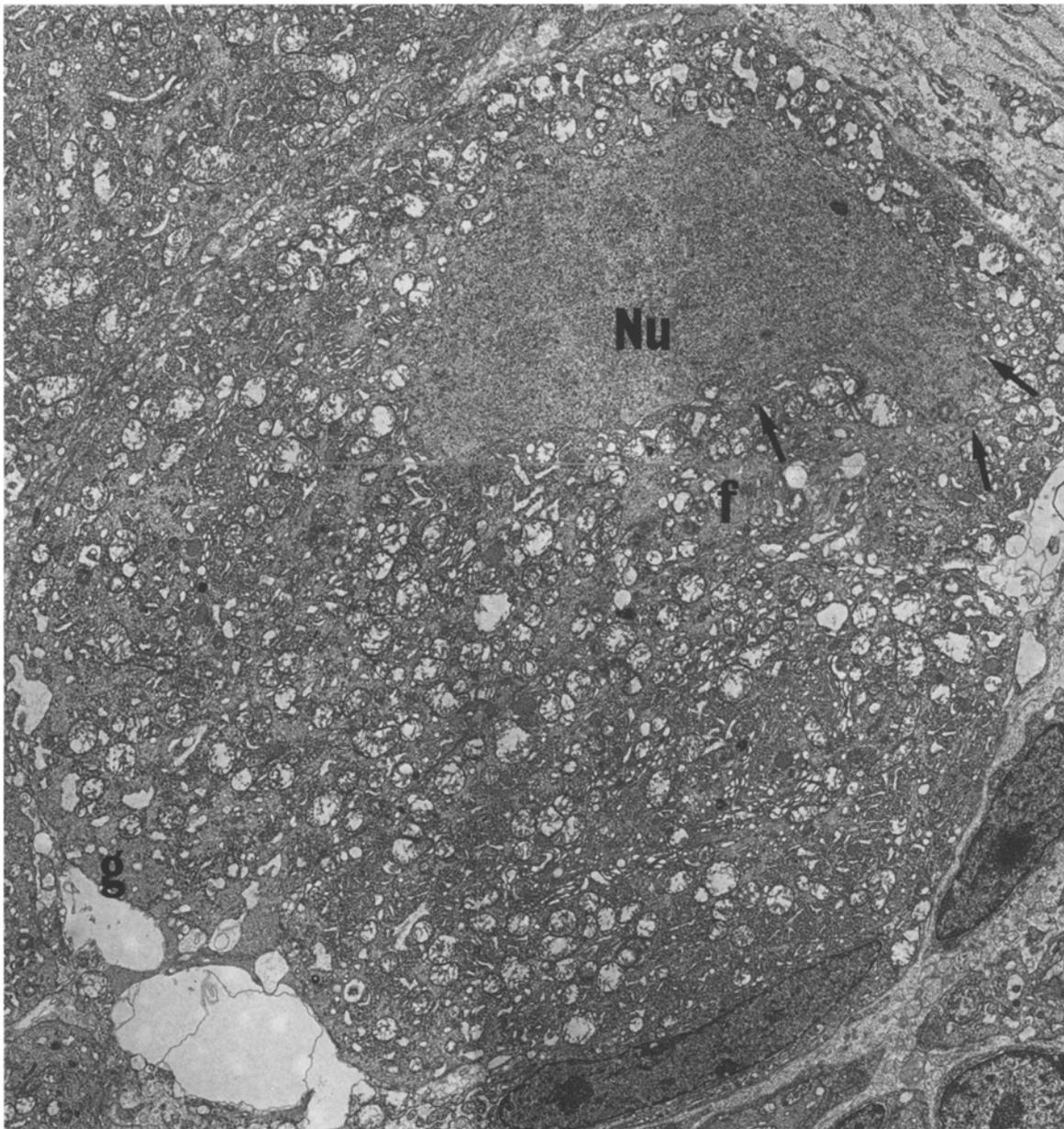
Fig. 8. A markedly degenerated A-cell showing nuclear eccentricity (*Nu*), and accumulation of degenerated mitochondria 5 days after CAP injection. Note the partially destroyed nuclear envelope (*arrows*) and granular (*g*) or fibrillar (*f*) materials in the peripheral neuroplasm. Boundary between adjacent neurons has become indistinct. × 5,100

Fissure and fragmentation of B-cells was frequently seen. We assume that cytoplasmic fissure leads to cell fragmentation and a round shrunken profile. The ultrastructural features of these severely degenerated neurons, such as their abnormally condensed nucleolus, were very similar to those of trigeminal ganglion neurons after infraorbital nerve section [1]. Cell fragmentation produced by axotomy has been reported [28], but the cytoplasmic fissure such as that seen in the capsaicin-treated neurons has not been reported previously. Cytoplasmic fissures may be a

peculiar feature of degeneration of sensory neurons induced by capsaicin.

As we have reported [10], capsaicin undoubtedly affects large A-cells, inducing dispersion of the Nissl substances and their location in a peripheral ring, with perinuclear chromatolysis. By 5 days after treatment, the periphery of A-cells appeared as a wider space without cytoplasm but containing fine granular or fibrillar materials. Later, clear empty spaces bounded by a membrane were seen, which correspond to the large membrane-bound cisternae reported by Prineas
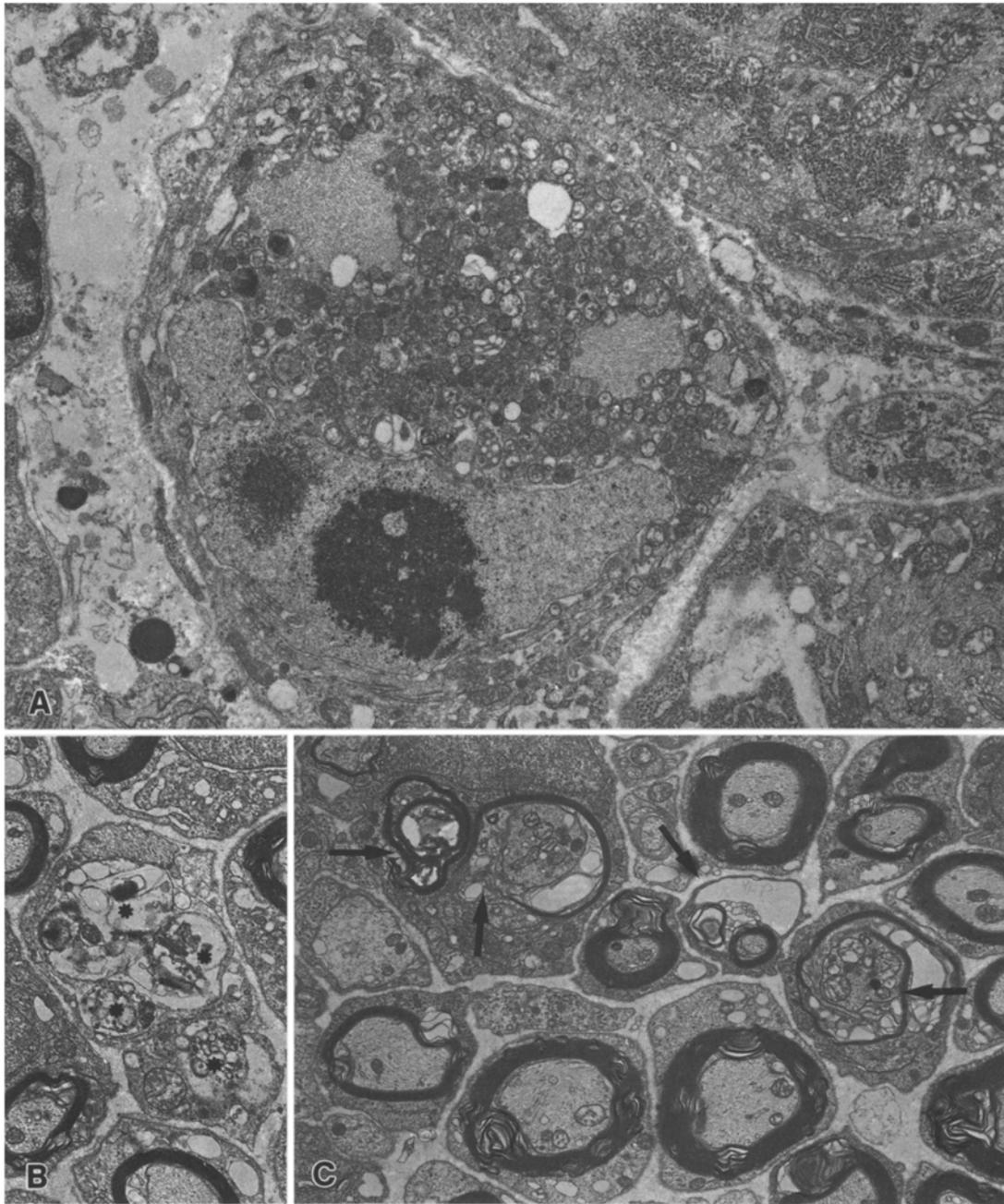
Fig. 9A — C. Features of degeneration of the DRG (**A, B**) and dorsal root (**C**) 5 days after CAP injection. **A** A small round degenerated neuronal cell with an irregular shaped nucleus, closely packed degenerated mitochondria and a mass of accumulated neurofilaments. × 8,600. **B** Completely degenerated unmyelinated axons (*asterisks*) including variously disorganized axoplasmic organelles. × 9,400. **C** Disruptions of the myelinated fibers (*arrows*). × 9,400

[21]. This cytolytic peripheral area is very similar to that observed by Andres [3] in rat DRG neurons after transection of the sciatic nerve and dorsal root, although he did not described its ultrastructure. Besides the changes described above, A-cells also showed cytoplasmic fissure. A-cells in later stages of degeneration with a disrupted nuclear envelope and degenerated mitochondria were probably dying.

Changes of satellite cells

During normal development, most neuronal cells of mouse DRG are surrounded by satellite cells on day 2 after birth. After capsaicin treatment, electron-lucent satellite cells were seen surrounding degenerating neurons. These satellite cells often contained fragmented cytoplasm of degenerated neurons or large

vacuoles. At later stages of neuronal degeneration, the satellite cells contained round shrunken neuronal cells with granular nucleolar material. Numerous mitotic satellite cells were seen on day 2−3 after capsaicin treatment. There are several reports of satellite cell proliferation caused by peripheral nerve axotomy [3, 15], although this was not observed by Aldskogius and Arvidsson [1]. Mitotic figures were rarely seen in satellite cells in normal and control DRG, so capsaicin activated satellite cell proliferation.

Degeneration of nerve fibers

Enlargement of some of the dorsal roots unmyelinated axons was observed during the early stages of degeneration induced by capsaicin. These axons all contained synaptic vesicles with dense cores, clear vesicles of various sizes, multivesicular bodies, degenerated mitochondria and accumulated neurofilaments. These features closely resembled the degenerated axons in mouse hereditary sensory neuropathy (Dystonia musculorum) reported by Janota [13]. In addition, the enlargement of unmyelinated axons in new born mice treated with capsaicin is comparable with the swelling of axons with rapid loss of cytoskeleton in adult guinea pig sensory nerves observed by Papka et al. [20]. Judging from the accumulation of axoplasmic organelles, the swelling of axons is probably due to inhibition of axoplasmic transport by capsaicin. On day 3 after treatment enlarged unmyelinated axons containing closely packed DLB and accumulated neurofilaments were seen in capsaicin-treated DRG. After 5 days the characteristic features of degeneration within DRG decreased, presumably in association with marked reduction of unmyelinated axons in Schwann cells. The number of myelinated axons showing destruction of the axoplasm gradually increased with time after treatment. Loss of myelinated axoplasm coincides with reduction of some of the myelinated fibers by capsaicin.

Certain smaller B-cells showed rapid degeneration within 5 h after treatment. These cells showed no chromatolytic changes, and no enlarged unmyelinated axons were found in the dorsal root 5 h after treatment. Therefore, capsaicin seems to act directly on these smaller B-cells. Moreover, the present study showed that capsaicin induced chromatolytic changes of larger B-cells and certain A-cells. Retrograde axonal transport dependent on exogenous nerve growth factor (NGF) is impaired in sensory nerve terminals treated with capsaicin [27]. These findings provide support for the Grafstein's claim [8] that chromatolytic change is elicited by drugs inducing block of axonal transport. Hiura and Sakamoto [9] found that after destruction of the tips of neurites with capsaicin, growth cones are regenerated when

the preparations are transferred in culture medium without capsaicin. This finding suggested that capsaicin primarily affects the nerve terminals. Thus, it is conceivable that capsaicin first acts on the central or peripheral terminals of the primary sensory neurons, and consequently blocks retrograde axonal flow.

In short, capsaicin seems to exert different actions on neurons of different sizes; it has a direct action on smaller neurons causing their rapid degeneration but an indirect action on larger neurons resulting in their slower degeneration.

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