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Ultrastructure of 6-Aminonicotinamide (6-AN)-induced Lesions in the Central Nervous System of Rats

III. Alterations of the Spinal Gray Matter Lesion with Aging

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Summary. Following a single i.p. injection of 6-AN (10 mg/kg), the anterior horn cells of 20- and 25-month-old rats increased more in size and recovered slower from chromatolytic changes than those of 3-month-old rats. Neurofilamentous hyperplasia of the perikarya was more prominent in aged rats; proliferated neurofilaments were arranged in thick parallel bundles.

In the acute stage, reactive and degenerative changes of glial and mesenchymal elements were more conspicuous in 3-month-old rats; however, they disappeared by day 14 with prominent proliferation of hypertrophic astrocytes.

The older rats showed less intensity and slower progression of these changes; sponginess and swelling of the astrocytic cytoplasm were still observed at day 14.

Our results suggest that these age-dependent changes in the response to neurotoxins are not only induced on the neuron without mitotic phenomena after birth, but also on neuroglial cells. Furthermore, an alteration or reduction in the support of the neuron augments its intensified and delayed susceptibility to neurotoxins.

Key words: 6-Aminonicotinamide – Aging – Spinal cord – Neurons – Neuroglia

Introduction

During the aging process of the organism, the neuron shows continuous accumulation of lipofuscin granules in the perikaryon (Hasan and Glees 1973; Glees and Gopinath 1973; Sekhon and Maxwell 1974; Johnson and Miquel 1974) and disorganization of the normally well-organized laminated pattern of the rough endoplasmic reticulum (Hasan and Glees 1973; Johnson and Miquel 1974) with (Andrew 1959; Cammermeyer 1963a; Sekhon and Maxwell 1974) or without a decrease in Nissl bodies (Hinds and McNelly 1978; Vaughan and Vincent 1979). Nuclear invaginations are frequently observed (Hasan and Glees 1973; Johnson and Miquel 1974).

Neuroglial cells proliferate progressively with aging and no consensus has been reached with regard to cell typing. In aging rats, the number of glia increases and neuroglial cells are often engorged with heterogenous material (Vaughan and Peters 1974). The combined cell population of astrocytes and oligodendroglia in the cerebral cortex (Brizzee et al. 1968), the population of astrocytes in the hippocampus (Landfield et al. 1977) and the volume fraction of astrocytic processes in gracile and cuneate nuclei (Fujisawa and Shiraki 1978) increase progressively with aging in rats. These age-related changes may influence alterations in the selective vulnerability of the CNS to neurotoxins.

We reported previously (Horita et al. 1980) that a single i.p. injection of 6-aminonicotinamide (6-AN) (10 mg/kg) produced severe pathological lesions in the CNS of rats. With aging, the lesions extended from the spinal gray matter, dentate nuclei, and brain stem nuclei through the limbic structures and striatum to the cerebral cortex. In all rats examined, irrespective of their ages, we noted lesions of the spinal gray matter; they consisted of chromatolysis of the anterior horn cells and other changes, including sponginess of the neuropil and a gliomesodermal reaction (Horita et al. 1978).

In the present study we investigated the structural changes that take place with aging in the cells of the rat cervical and lumbar gray matter. We also discuss the role of age-related changes in the vulnerability of nervous cellular elements, such as neurons and astroglia.

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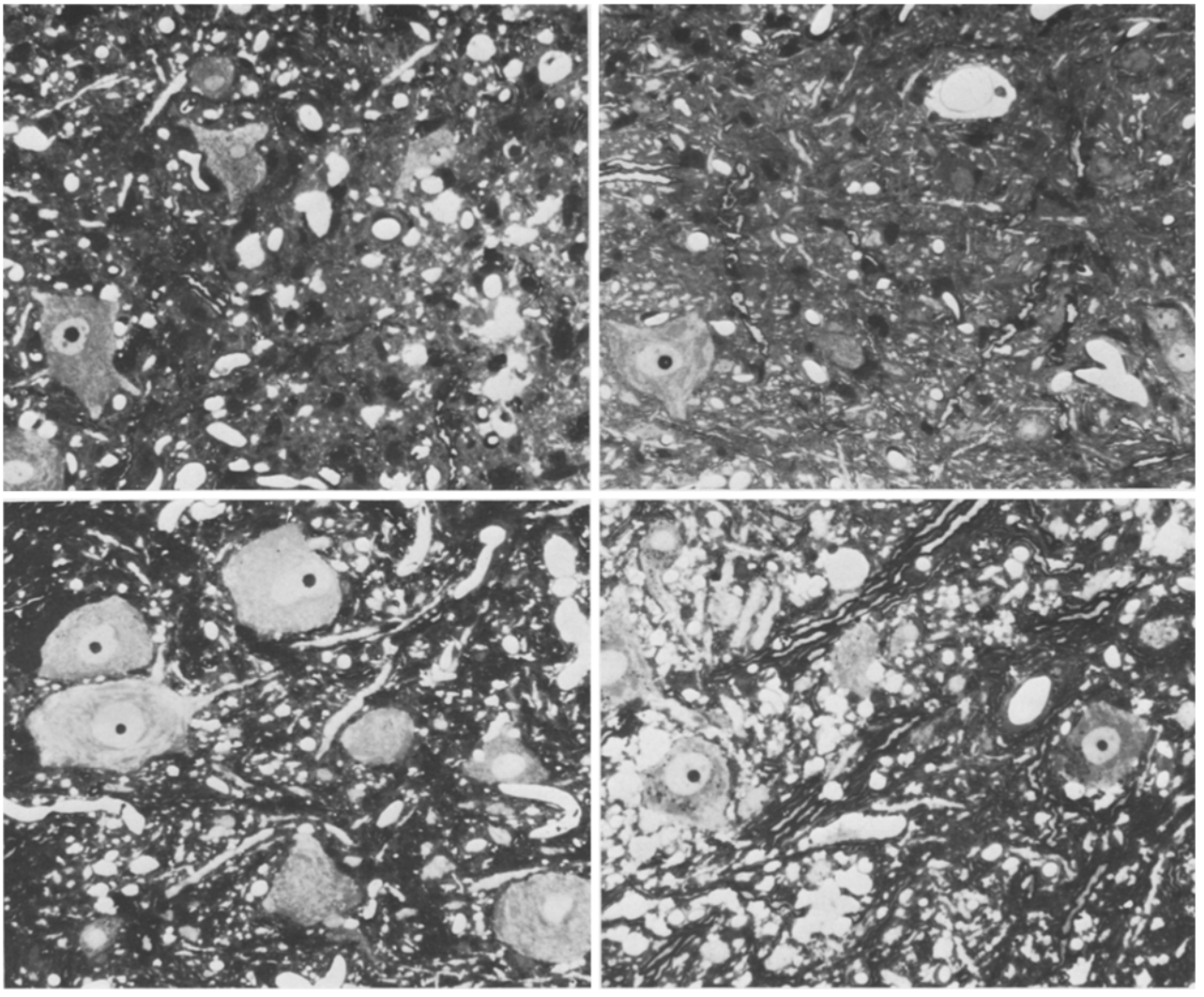


Fig. 1. (*Upper left*): 3-month-old rat. Anterior horn of lumbar cord, 5 days after 6-AN administration. The necrotic lesion of the central gray matter is almost completely occupied by degenerating tissue debris, and the spaces are filled with fluid and phagocytes. Anterior horn cells show chromatolytic change. Toluidine blue stain. ×300. (*Upper right*): 3-month-old rat. Anterior horn of lumbar cord, 14 days after 6-AN administration. Spongy appearance and dilatation of the extracellular spaces has disappeared and an anterior horn cell has recovered from chromatolytic change with hyperchromasia of the perikaryon. Mild proliferation of phagocytes is noted. Toluidine blue stain. ×300. (*Lower left*): 20-month-old rat. Anterior horn of lumbar cord, 5-days after 6-AN administration. Anterior horn cells show chromatolytic change. The perikarya are larger and contain many coarse bundles of neurofibrils. Some macrophage proliferation and spongy appearance due to vacuoles in the gray matter are noted. Toluidine blue stain. ×300. (*Lower right*): 20-month-old rat. Anterior horn of lumbar cord, 14 days after 6-AN administration. Sponginess, in some areas more prominent than at day 5, is noted. Toluidine blue stain. ×300

Materials and Methods

Wistar rats, raised under controlled conditions (Horita et al. 1980), were divided into three groups of 3-, 20-, and 25-month-old animals. They received a single i.p. injection (10 mg/kg) of 6-AN in physiologic saline (5 mg/ml). Two rats from each group were killed 3, 5, and 7 days after 6-AN treatment; three or four were killed after 14 days. Two rats in each age group, served as the controls; they were i.p. injected with physiologic saline and killed 7 days later. All rats were anesthetized with Nembutal and perfused through the left cardiac ventricle with a mixture of 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.4). The cervical and lumbar gray matter was dissected from the perfusion-fixed nervous system, embedded in Epon 812 (Horita et al. 1978, 1980) and 1 μm thick sections, stained by toluidine blue, were examined under a light microscope. Ultrathin sections were cut with an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a JEM 100 B electron microscope. Brain tissue, perfused with the above aldehyde mixture, was embedded in paraffin and stained with hematoxylin-eosin (HE), the Klüver-Barrera- or the Bodian method for light microscopy.

Results

Light-microscopic Findings

In the spinal gray matter of 3-month-old rats, chromatolysis of anterior horn cells, frequent necrosis of

internuncial cells, petechial hemorrhages, marked proliferation of microglial cells, and reactive and degenerative changes of neuroglial cells were noted (Fig. 1, upper left). These changes were most severe 5 and 7 days after 6-AN administration and had disappeared by day 14, except for mild proliferation of microglial cells (Fig. 1, upper right). There were many astrocytes manifesting hypertrophic change.

In 20- and 25-month-old rats, the neuronal perikarya exhibiting chromatolytic change tended to be larger in diameter; they contained coarse neurofibrils. In the acute stage, reactions of glial and mesenchymal elements were less conspicuous than in 3-month-old rats, however, there was no difference with respect to the petechial hemorrhages (Fig. 1, lower left). The spongy appearance, due to astrocytic swelling and mild proliferation of phagocytes, was still noted on day 14 (Fig. 1, lower right).

Electron-microscopic Findings

The anterior horn cells of 20- and 25-month-old control rats showed continuous accumulation of lipofuscin granules and indentations of the nuclear envelope. In some neurons, the normally well-organized laminated patterns of the rough endoplasmic reticulum appeared to be poorly defined and the cytoplasm was often occupied by lipofuscin granules and slightly increased neurofilaments. In addition, 25-month-old rats rarely exhibited dystrophic axonal change in laminae VIII and IX of Rexed. Neuroglial cells did not manifest any apparent change except for a slight increase in the number of microglial cells, many of which were engorged with heterogenous materials.

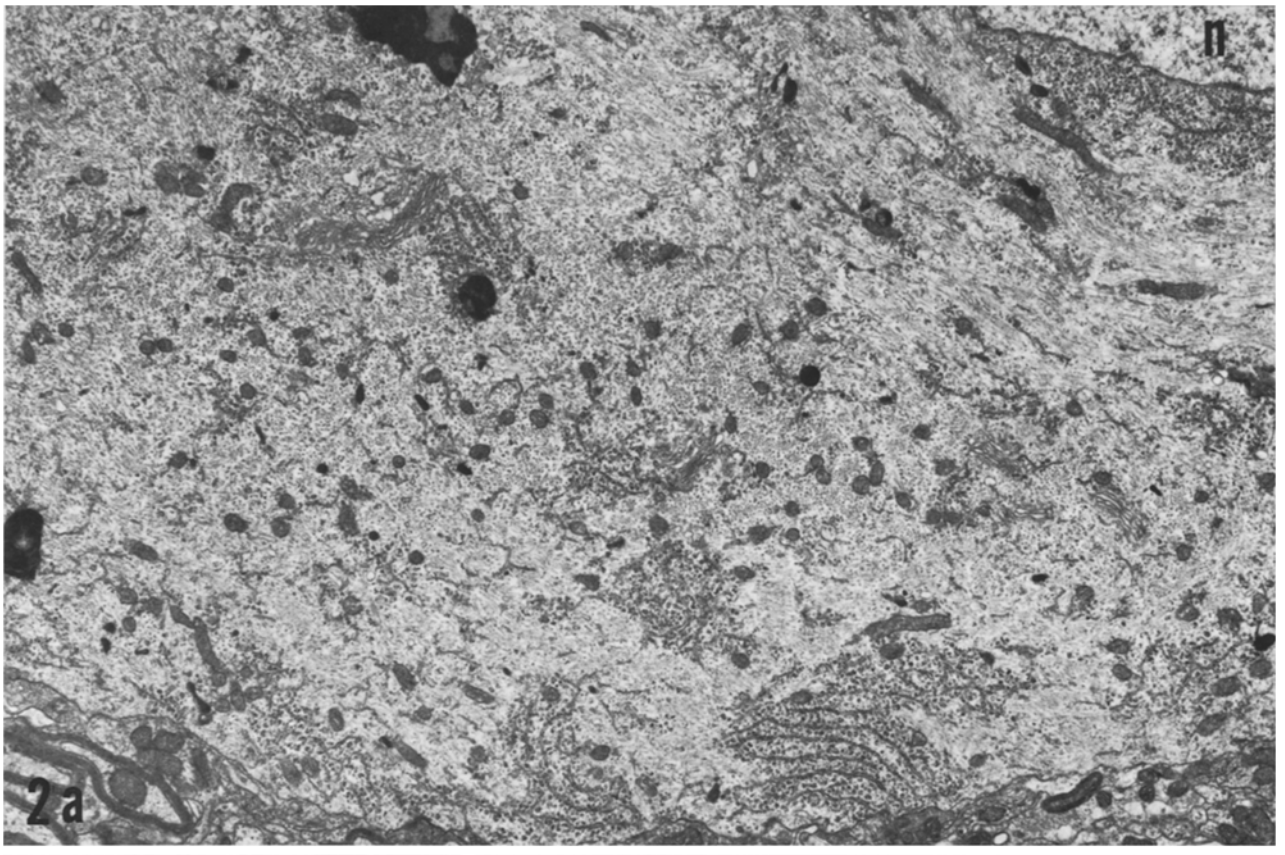
Following the administration of 6-AN, the majority of anterior horn cells showed typical chromatolysis without obvious axonal damage. In 3-month-old rats, the rough endoplasmic reticulum disappeared from the central portion of the perikaryon and the cytocentrum was filled with many mitochondria, dense bodies, and other organelles. Proliferated neurofilaments were disarranged and ran at random among the other accumulated organelles. This change was prominent at 3, 5, and 7 days after 6-AN treatment. At day 14, recovery from these changes, the restoration of the nucleus to the cell center and several islands composed of aggregated masses of abundant rough endoplasmic reticulum, was observed.

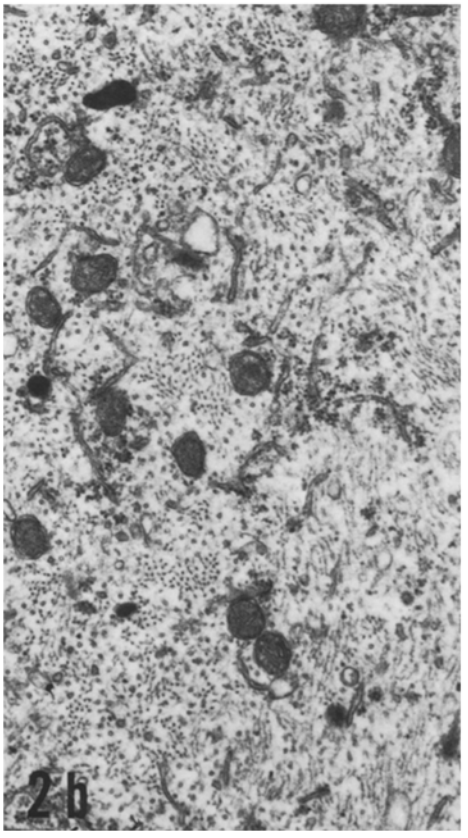
In 20- and 25-month-old rats, marked neurofilamentous hyperplasia was noted in many of the chromatolytic neuronal perikarya (Fig. 2a). The rough endoplasmic reticulum was markedly reduced in volume and dispersed along the nuclear membrane and the cell periphery as aggregated masses of irregularly dispersed cisterns. There were a few tubules of endoplasmic reticulum, smooth or surrounded by ribosomes (Fig. 2b). Proliferated neurofilaments were more prominent at day 5; they were arranged in thick parallel bundles. The neuron showed a shift of the nucleus towards the cell periphery; marked indentation of the nuclear membrane was frequently seen (Fig. 3). In 25-month-old rats, the chromatolytic perikaryon was occupied more frequently with proliferated neurofilaments; other organelles were less densely packed than in 3-month-old rats and there was no apparent change in the accumulated lipofuscin granules (Fig. 4). By day 14, the rough endoplasmic reticulum had reappeared in the perikaryon and the nucleus had returned to the central portion of the cell body. The chromatolytic change, however, persisted partially and the nuclear membrane showed remarkable invagination in some neurons.

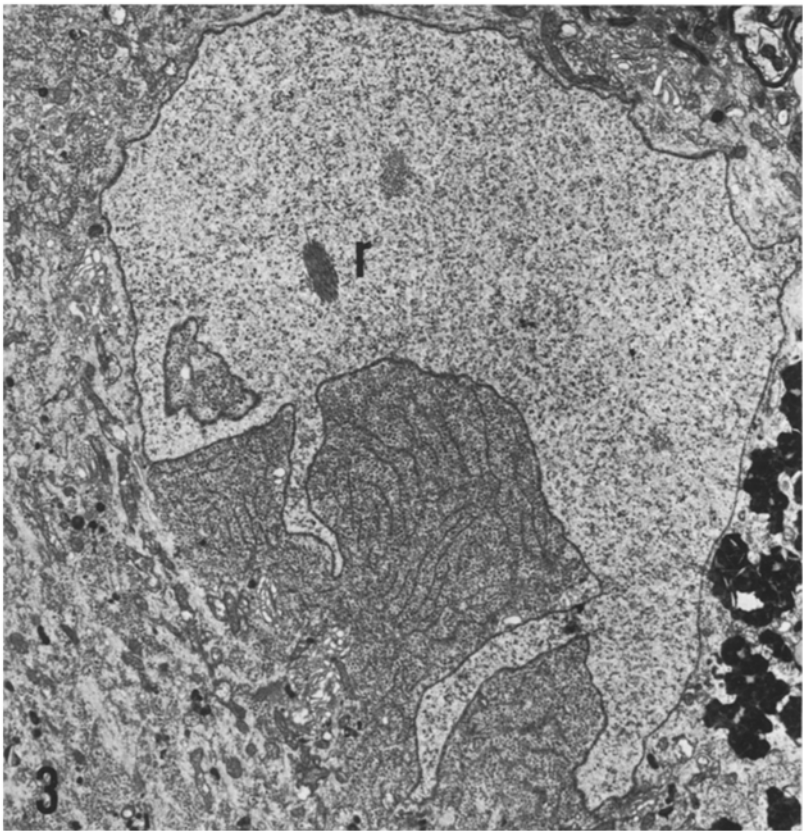
In the acute stage, reactive and degenerative changes of glial cells and proliferation of phagocytes were most prominent in 3-month-old rats. In these animals, the disappearance of these acute changes and proliferation of large astrocytes with multiple or multilobular nuclei in thin section and numerous organelles, such as gliofilaments, dense bodies, and rough endoplasmic reticulum were noted at day 14. The older rats, on the other hand, showed slower progression of these changes and they did not exhibit severe degenerative changes of neuroglial cells, nor obvious dilatation of the extracellular space in the acute stage. The spongy appearance, due to astrocytic swelling, was still observed at day 14. Some astrocytes were packed with regularly arranged endoplasmic reticulum and degenerated organelles (Fig. 5a, b); others were packed with many vacuoles which often contained glycogen granules and gliofilaments (Fig. 6). Astrocytes whose cytoplasm contained more prominent vacuoles and degenerated organelles, showed nuclear buddings characteristic of the degenerating nucleus (Fig. 7). In the same region, hypertrophic astrocytes with numerous gliofilaments and two separate daughter cells with apposing cytoplasmic membranes were frequently observed; the nuclei of most of these astrocytes were oval (Fig. 8). Examination of thin sections revealed a few astrocytes with multiple or multilobular nuclei.

Discussion

In normal aging rats, the anterior horn cells in the cervical and lumbar cords showed increasing accumulation of lipofuscin granules in the perikaryon and frequent indentations of the nuclear envelope. In addition, the rough endoplasmic reticulum was poorly defined and neurofilaments were observed in slightly increased numbers among other organelles.







Figs. 2–3

Following the administration of 6-AN, the majority of anterior horn cells manifested typical chromatolysis without apparent axonal damage. The perikaryon appeared to be increased in size at days 3, 5, and 7; return to the normal size began at day 14. The anterior horn cells of 20- and 25-month-old rats showed a more prominent increase in size and less remarkable recovery from chromatolytic changes than those of 3-month-old rats. Neurofilamentous hyperplasia of chromatolytic perikarya was more marked in aged rats; proliferated neurofilaments were arranged roughly in thick parallel bundles.

There are some disagreements about the manifestation of chromatolytic changes in young and old animals (Nandy 1972; Torvik 1976). LaVelle and LaVelle (1958), however, reported that after facial nerve avulsion, embryonal hamsters showed acute neuronal degeneration without chromatolysis, that hamsters less of than 20 days of age showed chromatolysis without perikaryal enlargement, and that only hamsters older than 25 days exhibited central chromatolysis of the adult type with perikaryal enlargement. Cammermeyer (1963b) emphasized that the reactive change after transection of rabbit facial nerve was more conspicuous and the recovery process less pronounced in older (2 or more years of age) than in younger animals (6 months of age), although the sequence of these changes was similar. These results, including our present findings, may suggest that the perikaryal appearance of chromatolytic neurons may vary depending on age.

Hyperplasia of neurofilaments, which was more pronounced in aging animals, was a conspicuous morphological change in some chromatolytic neurons, although variations in the intensity of chromatolytic changes were present and not all anterior horn cells manifested proliferation of neurofilaments. This may be ascribable to a difference in the cell types (Cammermeyer 1963b; LeVay et al. 1971; Zelená 1971) and the chromatolytic stages. It is generally accepted that axonal reactions bring about increased proliferation of neurofilaments in the neuronal perikaryon. According to Torvik and Skjörten (1971), hyperplasia occurred especially in neurons undergoing degeneration and ultimately death via chromatolysis, although Barron et al. (1975) did not agree with this view. An increase in neurofilaments is also induced by cold acclimation in lizard brain stem neurons (Potter et al. 1975). Moreover, this phenomenon has been reported in various pathologic conditions following the i.p. injection of β-, β'-iminodipropionitrile (IDPN) (Chou and Hartmann 1965) and the intracranial inoculation of aluminum salts (Klatzo et al. 1965; Terry and Penã 1965) and mitotic inhibitors, such as colchicine, Vinca alkaloids, podophillotoxin (Wiśniewski et al. 1968, 1970) and maytansine (Ghetti 1979).

The neurofilament may be involved in the movement of substances in the neuron, although its role in

Fig. 2. a Twenty-month-old rat. Anterior horn cell of lumbar cord, 5 days after 6-AN administration. The perikarya of chromatolytic neurons are crowded with proliferated neurofilaments. The rough endoplasmic reticulum is reduced in volume and present as aggregated masses of irregularly arranged cisterns at the cell periphery and along the nuclear membrane. Golgi complexes are seen in the central portion of the perikaryon. *n:* nucleus. ×7,600. **b** Higher magnification of the cell in **a**. Filamentous elements are almost completely composed of neurofilaments. Among them are a few tubules of endoplasmic reticulum, smooth or surrounded by ribosomes, and mitochondria. ×21,000

Fig. 3. Twenty-five-month-old rat. Anterior horn cell of lumbar cord, 3 days after 6-AN administration. The nuclear membrane of the chromatolytic neuron is irregularly indented and the perikaryon, entrapped with nuclear processes, contains abundant rough endoplasmic reticulum. Note the intranuclear rod, consisting of a single bundle of filaments, in the nucleus. *r:* intranuclear rod. ×5,100

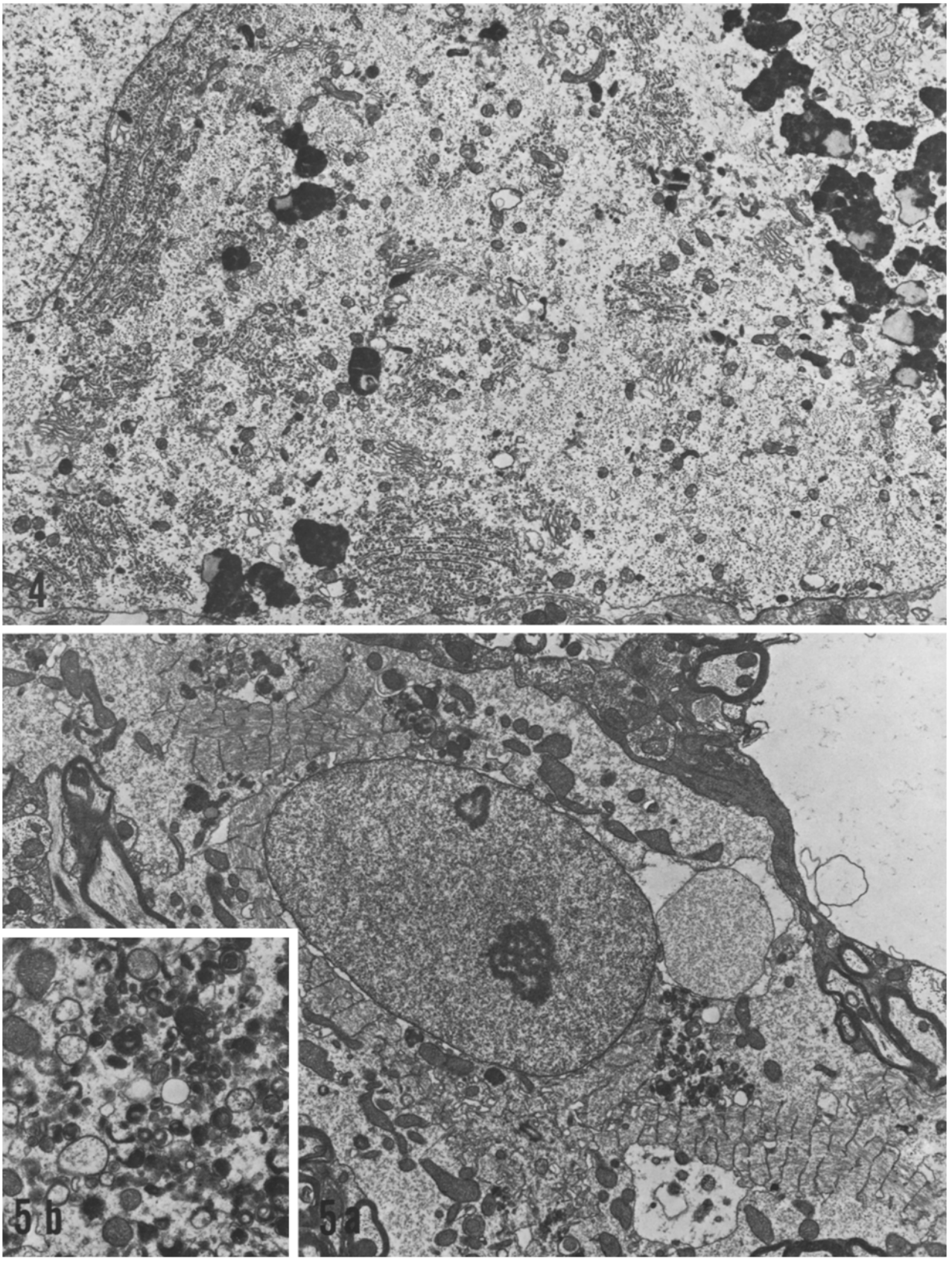
Fig. 4. Twenty-five-month-old rat. Anterior horn cell of cervical cord, 5 days after 6-AN administration. The perikaryon, exhibiting chromatolysis, is filled with many proliferated neurofilaments. Other organelles are less dense than in 3-month-old rats. The accumulated lipofuscin granules manifest no apparent change. ×8,500

Fig. 5. a Twenty-month-old rat. Lumbar gray matter, 14 days after 6-AN administration. The astrocytic cytoplasm is swollen. The perinuclear cytoplasm contains many organelles, such as lysosomes and mitochondria, intermittently arranged smooth endoplasmic reticulum and gliofilaments which cross the smooth endoplasmic reticulum at a right angle. The periphery is filled with moderately electron-opaque amorphous material. In some areas, dilatation of the endoplasmic reticulum is observed. ×6,300. **b** Twenty-month-old rat. Lumbar gray matter, 14 days after 6-AN administration. Higher magnification of the cytoplasm of a different astrocyte manifesting a similar change. There are many dense bodies, mitochondria and vacuoles surrounded by a double membrane. ×12,600

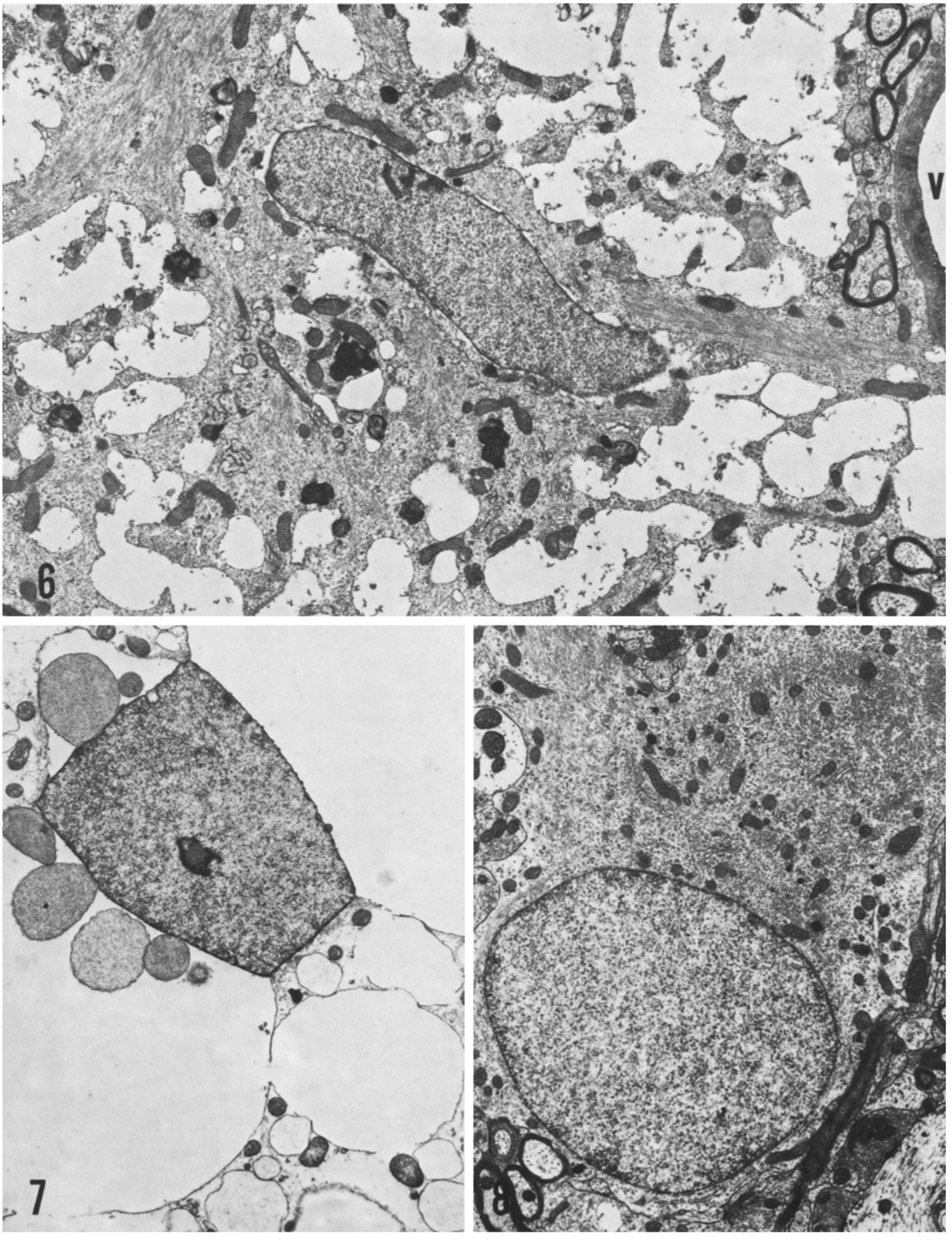
Fig. 6. Twenty-month-old rat. Lumbar gray matter, 14 days after 6-AN administration. The swollen astrocytic cytoplasm contains a moderate amount of proliferated gliofilaments, mitochondria and vacuoles which often include glycogen granules. *V:* blood vessel. ×7,600

Fig. 7. Twenty-month-old rat. Lumbar gray matter, 14 days after 6-AN administration. The degenerating astrocyte contains many vacuoles, probably derived from endoplasmic reticulum, and a few nuclear buddings in the dilated perinuclear space. ×7,600

Fig. 8. Twenty-five-month-old rat. Lumbar gray matter, 14 days after 6-AN administration. The astrocyte exhibits hypertrophic change with numerous gliofilaments and mitochondria in the cytoplasm. The nucleus is almost oval. The cytoplasm of another hypertrophic astrocyte is contiguous in the lower portion of this figure. ×8,500



Figs. 4–5. (Legends see p. 231)



Figs. 6–8. (Legends see p. 231)

chromatolytic change is obscure. The flow rate of fast axonal transport, which is inhibited by colchicine and Vinca alkaloids, is reduced in senescent animals (Geinisman et al. 1977). The rate of slow axonal flow, in which the neurofilament is normally carried (Hoffman and Lasek 1975), is lower in old than young rats (Komiya 1980). The increased production of fibrous organelles with aging has been frequently observed in human neuropathology, such as Alzheimer's disease, the Parkinson-dementia complex in Guam and Pick's disease, although the ultrastructure of these organelles is not identical with that of neurofilaments. These observations indicate that neurofilamentous hyperplasia in the neuron may be augmented in the aged, resulting in increased susceptibility to, or a reduction in the capacity to adapt to metabolic stress, such as is induced by 6-AN administration. Furthermore, this type of hyperplasia may occur in various pathologic conditions, irrespective of age.

In control rats, neuroglial cells showed only a slight age-related change. In the acute stage following the administration of 6-AN, the reactive and degenerative changes of glial and mesenchymal elements were more conspicuous in 3-month-old rats; they had disappeared by day 14 with prominent proliferation of hypertrophic astrocytes. On the other hand, 20- and 25-month-old rats manifested less intensive changes and a slower progression of the acute changes. The sponginess and swelling of the astrocytes at day 14 appeared to be partially due to degeneration, because the astrocytes contained many degenerated organelles and nuclear buddings (Bucher 1971).

According to biochemical studies of Ferrendelli et al. (1971), there is no obvious, or at most a slight change in some cerebral metabolic processes with aging. However, under conditions of greater energy demand such as spreading cortical depression (Sylvia and Rosenthal 1979), the in vivo capacity of the energy-producing system is decreased in aged, compared to younger rats. The reaction of hepatic glycogen phosphorylase following the electrical stimulation of the ventromedial hypothalamic nucleus is decreased, and the restoration of glycogen associated with the activation of glycogen synthetase after stimulation of the lateral hypothalamic nucleus is more delayed in aged than in young rats (Shimazu et al. 1978). The response of the brain dopaminergic neurotransmitter system to a single electroconvulsive shock is more diminished in older rats (McNamara et al. 1977). These data suggest that aging brings about a reduction and delay in the ability to adapt to external stimuli and that in older animals, pathologic manifestations occur under less stress situations.

The relationship between neuroglial cell change and aging has not been sufficiently elucidated. The rate of axonal degeneration and removal of degenerating debris in optic nerves after eye enucleation is faster in kittens than in adult cats (Cook et al. 1974). The occurrence of neuronophagia and satellitosis, in which primarily astrocytes and oligodendroglia participate, has been observed in the aged human central nervous system (Andrew and Cardwell 1940; Brownson 1956). The degree of neuronophagia in starving mice was greater in younger ones and the process of neuronophagia in normal senescent mice was much slower than in starving animals (Andrew 1939). These results suggest that age-dependent changes in the response to neurotoxins are not only induced on neurons without mitotic phenomena after birth, but also on neuroglial cells (Vernadakis et al. 1979) and that a change or reduction in the support of the neuron augments its intensified and delayed susceptibility to neurotoxins.

Based on the present and previous observations, we agree with Cammermeyer (1963b) who proposed that investigations should be performed on animals of known ages to correctly evaluate the cellular changes in response to external stress.

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