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Peripheral Nerve Abnormalities in Aging Rats

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Abstract. The sciatic/tibial/plantar nerve complex of normal aged rats displays striking morphological changes that are most pronounced distally. Nerve fiber abnormalities include: (a) large numbers of axonal glycogenosomes, mitochondria, dense membranous bodies, and decorated particles; (b) adaxonal Schwann cell processes sequestering portions of axoplasm; (c) swollen demyelinated and remyelinated axons, some encircled by supernumerary cellular processes; and (d) collagen pockets, denervated Schwann cell columns, and empty basal laminae. Abnormalities a and b were encountered with increasing frequency on descent through the tibial and plantar nerves. Abnormalities b, c, and d were found in lateral and medial plantar nerves, where they were associated with an enlarged endoneurial space. Found in animals kept in cages with smooth or wire-mesh floors, the incidence of these changes increased with advancing age. They are attributed to trauma and ischemia from chronic pressure on the plantar nerve.

INTRODUCTION

There is an increasing use of experimental animals for lifetime studies to examine the chronic effects of low-level exposure to a toxic substance, to reproduce a slowly developing human metabolic disease, or to study the effects of biological aging on the nervous system. Such studies require a detailed understanding of nerves from age-, sex-, and weight-matched control animals in order to isolate normal changes acquired with advancing age from an induced neuropathological lesion. During our work with control Sprague-Dawley rats, it has become apparent that pathological changes in peripheral nerves develop as a function of advancing age, and that structural abnormalities mimicking those reported in toxic/metabolic conditions are regularly encountered in normal animals (16). The purpose of the present paper is to document the ultrastructural abnormalities found in sciatic, tibial and, most especially, plantar nerves of aged rats.

MATERIALS AND METHODS

Although several hundred control rats between 3 months and 2.5 years of age have been examined, this report focuses on six CD-CRL:COBS CD (SD)BR 500 to 850 g male rats aged at least 2 years. During their lifetime, 3 animals were housed on wire-mesh floors, and 3 on smooth floors. Animals were anesthetized with sodium barbitone containing heparin, the chest and heart opened, and perfused via a cannula inserted into the

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aortic arch with 4% paraformaldehyde for 30 sec followed by 5% glutaraldehyde for 10 min, each fixative in a 0.1 M phosphate buffer. Multiple levels of the sciatic, tibial, medial, and plantar nerves were removed from both hindlimbs, post-fixed in 2% Dalton's chrome osmium solution, dehydrated stepwise, immersed in propylene oxide, infiltrated with Epon, placed into molds, and hardened by heating stepwise in ovens at 37°C, 45°C, and 60°C over a 2-day period. One-micrometer sections were cut from hardened blocks of tissue, stained with 1% toluidine blue, and examined by bright-field microscopy. Thin sections of selected areas were stained with uranyl acetate followed by lead citrate, and examined by transmission electron microscopy.

RESULTS

General

Morphological abnormalities were evident in all nerves examined (except the tibial nerve branches to the calf muscles), regardless of whether animals had

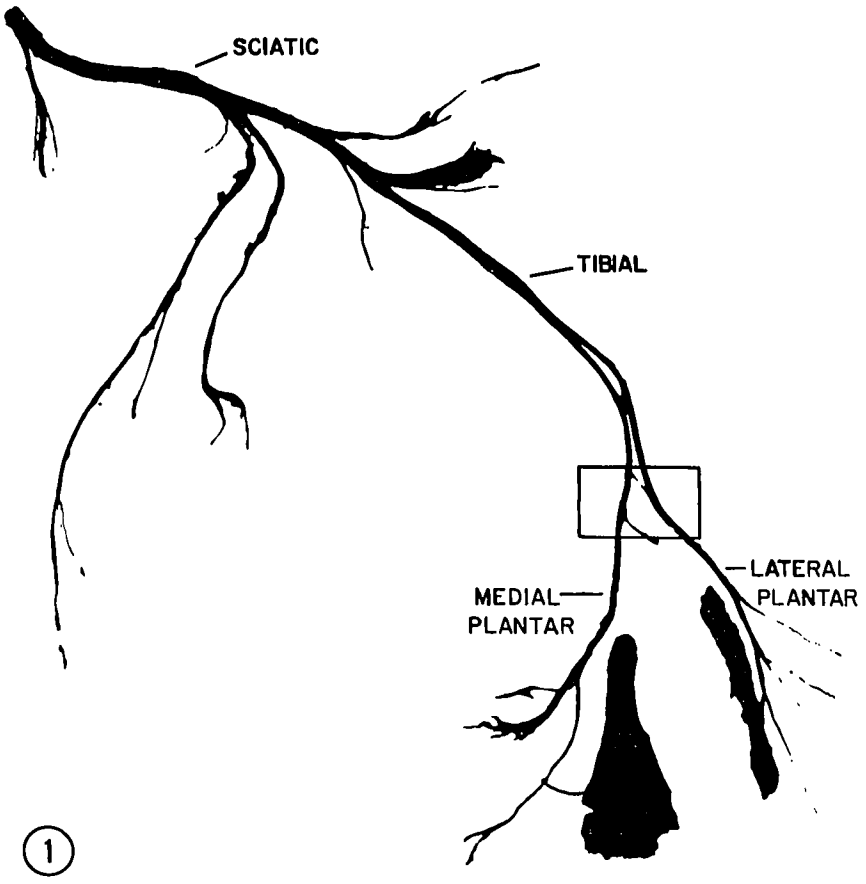


Fig. 1. Gross dissection of the sciatic/tibial/plantar nerve complex and some associated intrinsic hindfoot muscles of the rat. Box indicates region of maximum abnormality.

been housed on wire-mesh or on smooth floors. Examination of sciatic, tibial, and plantar nerves of animals 3 months to 2.5 years of age demonstrated that both the degree and proximal spread of abnormality increased as a function of age (and body weight). Aberrant findings were encountered with increasing

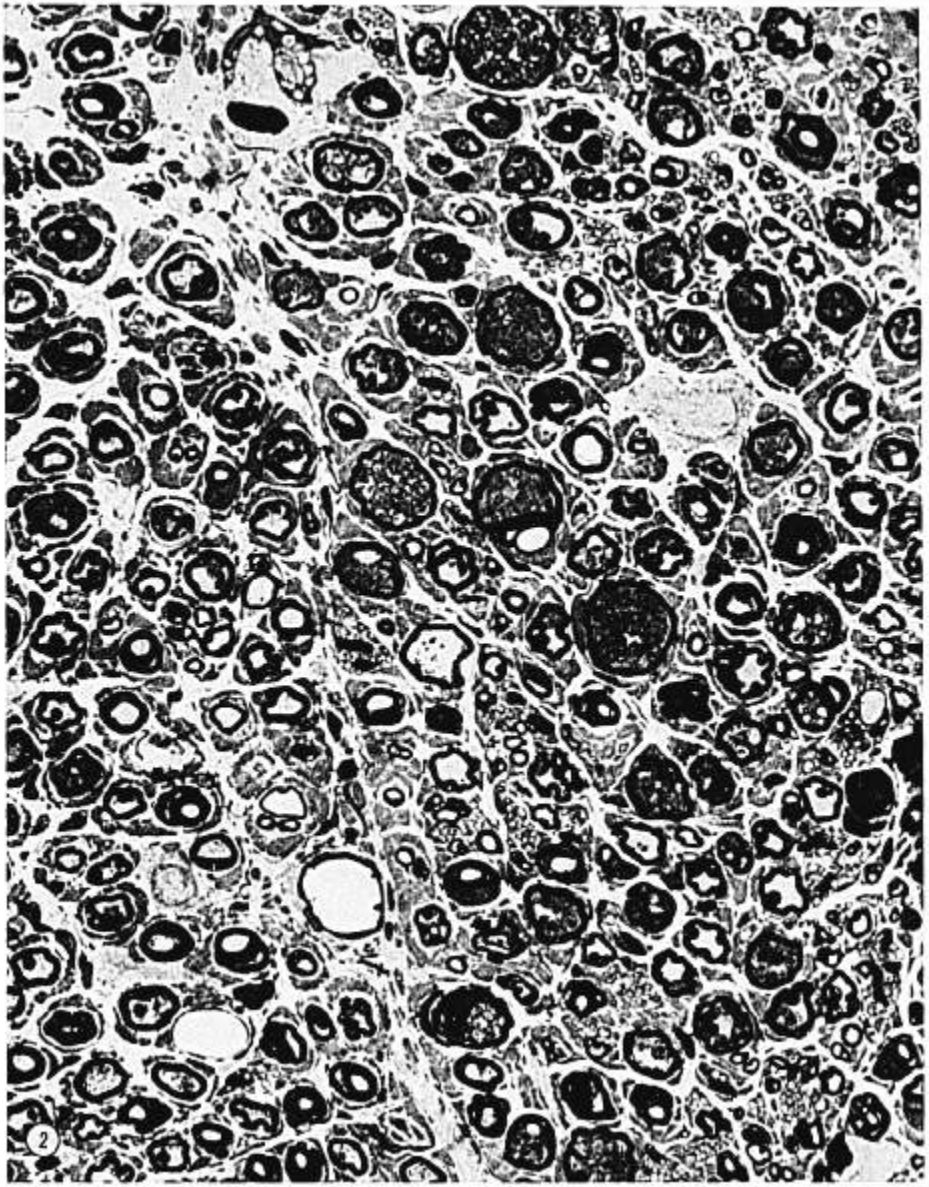


Fig. 2. Cross section of the plantar nerve at the heel of an animal aged over 2 years. Swollen myelinated nerve fibers containing spherical metachromatic structures are segregated to one side. Contorted and infolded fibers are prominent. Bright-field micrograph of a 1-μm epoxy section stained with toluidine blue. ×570.

frequency on descent from the sciatic to the tibial nerve in the lower hindlimb, and were most prominent in the lateral and medial plantar nerves. The following description applies to the plantar nerves at the heel (Fig. 1).

Light Microscopy

The majority of plantar nerve fibers displayed contorted and infolded myelin sheaths. Several myelinated fibers contained spherical, metachromatic bodies which obliterated the axonal compartment. Some of these fibers were markedly enlarged and frequently segregated within the nerve fascicle (Fig. 2). Grossly swollen demyelinated axons were common in some nerves. Remyelinated and regenerated nerve fibers were occasional findings. Degenerating fibers were rarely identified, although fiber loss was sometimes evident. Blood vessels were patent and did not display atherosclerotic change. The endoneurium contained large amounts of connective tissue, scattered mast cells, Renaut bodies, and fibroblasts, and was sometimes edematous.

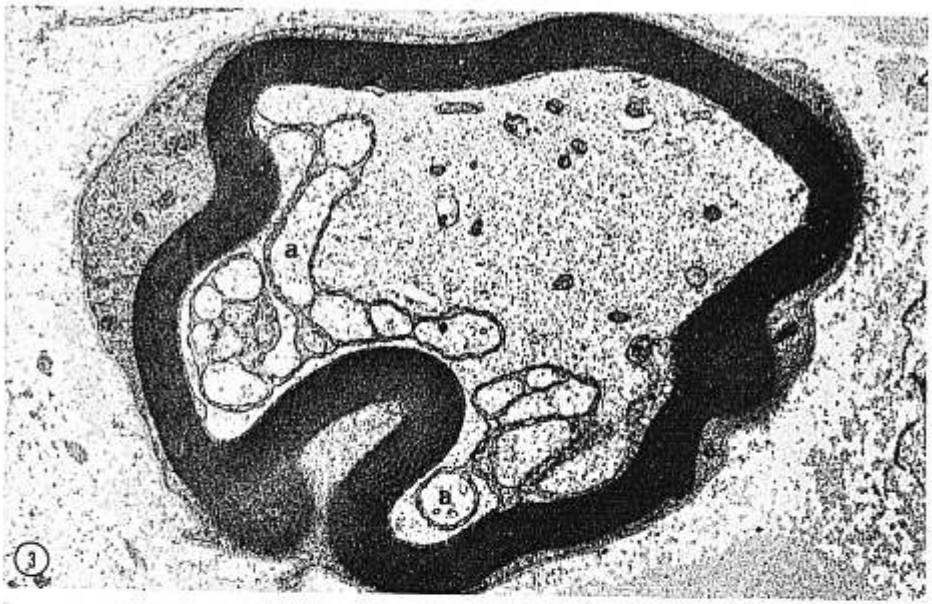
Electron Microscopy

Myelinated fibers: Fibers with a corrugated surface and deeply infolded myelin sheath were common. Unusual Schwann cell features included fluting of the external border of the abaxonal compartment, focal enlargements of adaxonal cytoplasm containing masses of fine filaments or microtubules, and tenuous adaxonal processes invaginating the axon. These adaxonal Schwann cell ingrowths frequently sequestered small portions of abnormal axoplasm (Fig. 3). Abnormal axonal organelles were encountered in the following decreasing order of frequency: (a) single or multiple membrane-bound collections of glycogen granules (glycogenosomes), often occupying a large proportion of the axon (Fig. 4), (b) numerous mitochondria, (c) decorated particles, and (d) excessive amounts of smooth endoplasmic reticulum. Degenerate mitochondria and dense bodies were sequestered in two axonal sites: (a) internodally, where they were often compartmentalized by adaxonal Schwann cell ingrowths, and (b) paranodally, within a circumferential rim of axoplasm separated from the central axon by an inflected myelin sheath.

Grossly swollen demyelinated axons contained masses of decorated particles, groups of neurofilaments, and scattered neurotubules (Fig. 5). Thinly remyelinated fibers were also found. Both types of abnormal fiber were often associated with encircling processes of Schwann cells or fibroblasts, or both, and redundant basal lamina. Myelinated fibers close to the perineurium were sometimes individually enveloped by a single cellular process of perineurial type (Fig. 6).

Unmyelinated fibers: Normal and abnormal configurations of unmyelinated fibers were found. The latter included fibers with redundant cytoplasmic processes, reduplicated basal lamina, or encircling supernumerary Schwann cell processes. Axons sometimes contained clusters of small vesicles and a few dense bodies. Accumulations of neurofilaments were not seen.

Endoneurial compartment: Collagen and elastin fibers were prominent. Empty folds of basal lamina, Schwann cell collagen pockets (Fig. 4), and over-



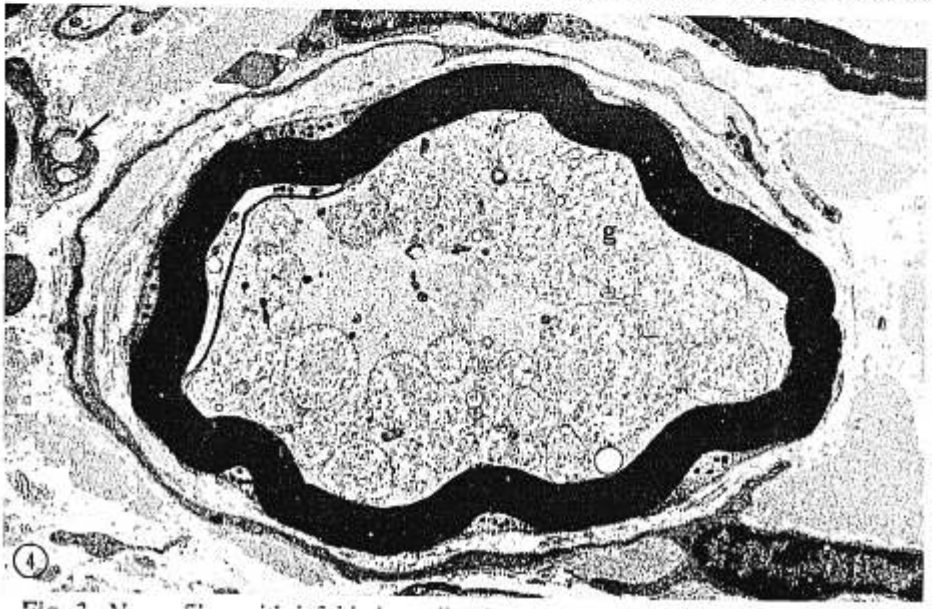


Fig. 3. Nerve fiber with infolded myelin sheath and network of adaxonal Schwann cell processes sequestering portions of axoplasm (*a*). This figure and Figures 4–6 are electron micrographs of thin epoxy cross sections stained with uranyl acetate followed by lead citrate. ×15,500.

Fig. 4. Swollen myelinated fiber, partially surrounded by supernumerary Schwann cell processes, containing a large number of axonal glycogenosomes. Arrow indicates Schwann cell collagen pockets. ×8,500.

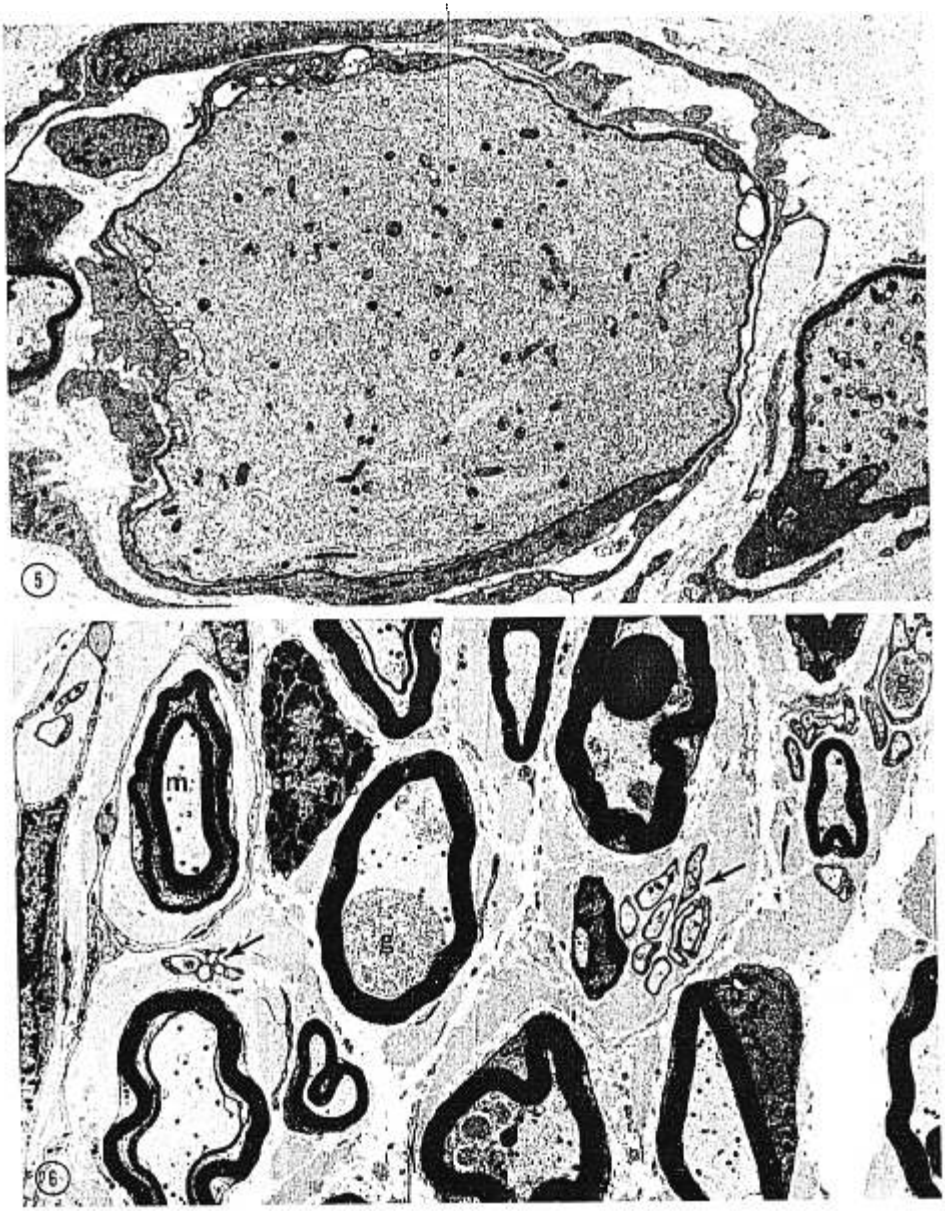


Fig. 5. Massively swollen, thinly myelinated axon containing a large number of decorated particles, mitochondria, clumps of neurofilaments and scattered neurotubules. The axon is partially surrounded by fibroblast processes. A thinly myelinated nerve fiber is visible on the right. ×6,750.

Fig. 6. Low-power field close to the perineurium (to the left) illustrating myelinated and unmyelinated (arrows) fibers with glycogenosomes (g), a mast cell, several normal-appearing unmyelinated fibers, and a myelinated fiber (m) surrounded by a cell of perineurial type. Enlargement of this area revealed several redundant folds of basal lamina and numerous endoneurial elastin fibers. ×4,400.

lapping plates of Schwann cells suggested some nerve fiber loss. Actively degenerating nerve fibers or classical bands of Bungner were not encountered. Endoneurial cells other than fibroblasts or mast cells were sometimes found adjacent to nerve fibers or blood vessels.

DISCUSSION

The development of abnormalities in hindlimb nerve fibers of aging experimental animals has been reviewed by Spencer and Ochoa (37). Sciatic and peroneal nerves may contain a few fibers that undergo Wallerian degeneration or display internodal myelin bubbles as a prelude to segmental demyelination (5, 13, 14, 22, 41, 42). Tibial nerves develop widespread changes, including segmental demyelination, axonal degeneration, and regeneration (31, 33). Plantar nerves of aged guinea pigs display localized demyelination and remyelination, bulbous deformation, myelin corrugation, and, occasionally, Wallerian degeneration (9, 10, 25). Such changes have been attributed to biological aging or nutritional deficiency (22), a dying-back process (42), possible neuronal loss (33), and to a distal nerve pressure lesion (9, 10, 25, 33). The present observations of nerve fiber alterations predominantly localized to plantar nerves is consistent with the latter view. However, in contrast to the pressure lesion in the guinea pig, which only develops when animals are housed on wire-mesh flooring (9, 10, 19), the rats in this study and those examined by Sharma, Bajada, and Thomas (33) acquired the lesion while living on smooth floors. Furthermore, the axonal abnormalities in rats are not restricted to the plantar nerves, but spread to affect more proximal regions of affected nerve fibers. That the more proximal axonal changes are associated with the plantar nerve lesion is evident from the absence of these changes in tibial nerve fibers supplying the calf musculature. These nerve fibers, therefore, provide the only region of the tibial/plantar nerve complex where the effects on distal peripheral nerves of chronic toxic/metabolic disease (37), or of biological aging, can be seen unimpeded by the effects of the focal plantar nerve lesion described here.

Many of the pathological changes found in the plantar nerve of the rat have been reported in chronic nerve pressure lesions: focal demyelination and remyelination, axonal degeneration and regeneration, and internodal contortion and nodal inflection of the myelin sheath (24). Decorated axoplasmic particles (origin unknown), excessive axonal mitochondria and smooth endoplasmic reticulum, and Schwann cell adaxonal ingrowths that sequester and remove axonal debris are common accompaniments of chronic axonal disease (39, 40). The relative roles of chronic pressure and of other factors, such as biological aging, in the production of these changes is impossible to assess (37).

The presence of spherical bodies in axons produced a striking metachromasia in semi-thin sections stained with toluidine blue. Ultrastructurally, these bodies consisted of collections of electron-dense particles, indistinguishable from glycogen granules (30), enclosed within a single membrane. Axonal glycogenosomes of this type have been described in the sciatic nerves of diabetic mice (36) and alloxan-induced diabetic rats (27). Powell et al. (28) made a quantitative study of the glycogenosomes in control and diabetic animals, found them to

be twice as frequent in the latter, and attributed the increase to the diabetic process. An alternative explanation for these observations is that diabetic animals are more susceptible to pressure injury of the plantar nerves because of the increased size of the nerve that results from changes in endoneurial blood vessel permeability to proteins (32) and increased endoneurial fluid retention (20, 21). Sima and Robertson (35), however, were unable to confirm abnormal vascular permeability in several models of experimental diabetes, and Gabbay (11) failed to detect osmotic swelling of peripheral nerves in rats treated with streptozotocin.

Axonal glycogenosomes appear to develop from mitochondria and accumulate in several toxic/metabolic states (38). Although they are usually regarded as pathological structures, their accumulation in large numbers in the absence of axonal breakdown may be more consistent with a physiological role. Certainly, in frog spinal ganglia, neuronal glycogen is a normal constituent which increases in the winter when the animal hibernates (43). This observation led Berthold (2) to suggest that glycogen represents a specialized energy store that allows the animal to adjust to anaerobic energy metabolism during the hibernation period. He proposed the existence in nervous tissue of a close relationship between mitochondria and carbohydrate turnover, and noted that crustacea can utilize glycogen to support impulse activity. Viewed from this perspective, glycogen accumulation in toxic/metabolic states might provide the axon with a mechanism for rapid mobilization of energy reserves for the maintenance of axonal transport and electrical conduction. In chronically traumatized plantar nerves, such as those described in the present study, the deposition of axonal glycogenosomes could represent a physiological response of the axon to an ischemic/anoxic state created by poor blood circulation at the site of pressure. Buja et al. (4) reported a similar accumulation of intramitochondrial glycogen in cardiac muscle cells during periods of anoxia, and suggested that three factors were responsible for this phenomenon: (a) solubilization of the enzymes of glycogen metabolism during the anoxic period; (b) increased permeability, due to anoxic damage of the outer mitochondrial membrane, and (c) diffusion of the solubilized enzymes of glycogen synthesis into the mitochondria, with subsequent formation of glycogen. Several other authors have proposed a relationship between ischemic or hypoxic states and glycogen accumulation in nervous tissue (3, 18), and suggested that the accumulation of glycogen reflects decreased oxidative tissue metabolism (6, 34).

It is apparent that the abnormalities encountered in the sciatic, tibial, and plantar nerves of normal aging rodents must be taken into account when evaluating the results of a long-term experiment. These changes can be added to the list of other age-associated abnormalities that occur in peripheral neurons and myelinating cells: loss of anterior horn cells (44), accumulation of lipofuscin (23), dystrophic changes in the terminals of centrally directed (8, 17, 26, 29) and peripherally directed axons (7), and the formation of scattered myelin-bubbles in sciatic nerves and spinal roots (1, 12, 15, 22, 42).

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