

Ultrastructural Lesions of Pyridoxine Toxicity in Beagle Dogs

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Abstract. Three adult Beagle dogs given pyridoxine hydrochloride orally at a dose of 150 mg/kg body weight/day for about 100 days developed ataxia and had spastic, dysmetric leg movements. Ultrastructural alterations in the dorsal funiculus of the spinal cord were degeneration and loss of axons and myelin, and secondary changes of the myelin sheaths. Possible pathogenic mechanisms of pyridoxine neurotoxicity are discussed.

Pyridoxine (pyridoxol) is one form of vitamin B₆, a group of water-soluble, structurally related compounds that also includes pyridoxal and pyridoxamine. These forms are about equally active in supporting animal life and are readily interconvertible [2]. Pyridoxal kinase converts these compounds to the phosphorylated coenzyme form.

Pyridoxine deficiency has been studied and reviewed extensively [4, 12, 18]. Deficiency of vitamin B₆ may be caused by absence of this vitamin in the diet or by compounds that interfere with the vitamin. Signs of deficiency in man and in animals include decreased basal metabolic rate and weight gain, microcytic hypochromic anemia, and neurologic abnormalities.

The recent interest in megavitamin therapy has resulted in the use of large therapeutic doses of pyridoxine for a variety of conditions. The toxicity of pyridoxine is low [17] and, possibly because of a lack of practical significance until now, it has received little study. The subcutaneous lethal dose of pyridoxine hydrochloride for fifty percent of rats is 3.7 g/kg body weight [17]. Affected rats had tonic convulsions and impairment of righting reflexes. The acute and subacute toxicities of excess pyridoxine hydrochloride in dogs resulted in clinical disease characterized by ataxia, and degenerative histologic lesions in spinal and trigeminal ganglia, dorsal roots, the dorsal funiculus, trigeminal nerve fibers and some fascicles of peripheral nerves [1, 7, 11]. Sensory denervation of the plantar lumbrical muscle spindles has been reported with pyridoxine neuropathy in the rat [9].

This report extends the study of pyridoxine neurotoxicity to the fine-structural changes in the central nervous system.

Materials and Methods

Three adult Beagle dogs were given pyridoxine hydrochloride (Roche Chemical Division, Hoffman-LaRoche, Inc., Nutley, N.J.) orally in gelatin capsules, 50 mg/kg body weight/day for the first week, 100 mg/kg body weight/day for the second week, and 150 mg/kg body weight/day from the 15th day to the end of the experimental period, about 100 days. A fourth Beagle dog served as a control.

The dogs were anesthetized with thiopental and the vasculature perfused with a 2% glutaraldehyde-2% paraformaldehyde phosphate-buffered fixative solution via intracardiac cannulation. Following whole-body fixation, necropsy was done. One-millimeter, quartered cross sections of the spinal cord at the levels of the second cervical and fifth lumbar spinal cord segments were collected for ultrastructural examination. The sections were washed in a phosphate buffer and then were transferred to phosphate-buffered osmium tetroxide for post-fixation osmification. The tissues then were dehydrated in a series of graded acetone solutions and infiltrated and embedded in epon. Areas of the dorsal funiculus, primarily the fasciculus gracilis, were selected for ultrastructural examination from 1- μ m sections stained with azure II-methylene blue. Thin sections of selected areas were picked up on 300- or 400-mesh uncoated copper grids, stained with uranyl acetate followed by lead citrate, and examined and photographed in an electron microscope.

Results

Neurologic disease characterized principally by proprioceptive defects developed in each pyridoxine-treated dog and occurred first in two dogs during the fourth week and in the third dog during the eighth week of administration. The dogs had spastic, dysmetric leg movements and lacked apparent sense of motion or position of the legs. Histologic alterations, limited to the nervous system, were degenerative changes in the dorsal funiculus, dorsal spinal roots, some fascicles of peripheral nerves, the spinal tracts of the trigeminal nerves and trigeminal nerve fibers. Minimal degenerative changes characterized by central chromatolysis of neurons and a few nodules of Nageotte occurred in dorsal spinal ganglia.

Ultrastructurally, the degenerative lesion in the dorsal funiculus was characterized by degeneration of axons, loss of axons, collapse of myelin sheaths, degeneration and loss of myelin, and astrocytic scarring. The most common alteration of axons was contraction with granular, electron-dense axoplasm, without any recognizable organelles (fig. 1). Many nerve fibers had no axon within the myelin sheath (fig. 2). In a few axons, the degenerative changes were mild and consisted of loss of microtubules and neurofilaments with deposition of flocculent electron-dense material. Occasionally, the mildly degenerate axons had accumulations of mitochondria, some containing electron-dense material. Membranous axonal proliferations formed large vesicular structures or appeared as smooth endoplasmic reticulum in some axons (fig. 3).

Alterations in myelin sheaths were associated with axonal changes. The myelin sheath and its inner tongue of oligodendrocytic cytoplasm often were morphologically normal in nerve fibers that had degenerated axons or axonal loss. Myelin sheaths frequently were collapsed around contracted degenerated axons or the space left after axon loss. Some myelin sheaths without an axon were dilated (fig. 4).

Astrocytic processes were large and numerous, and nearly filled the spaces between

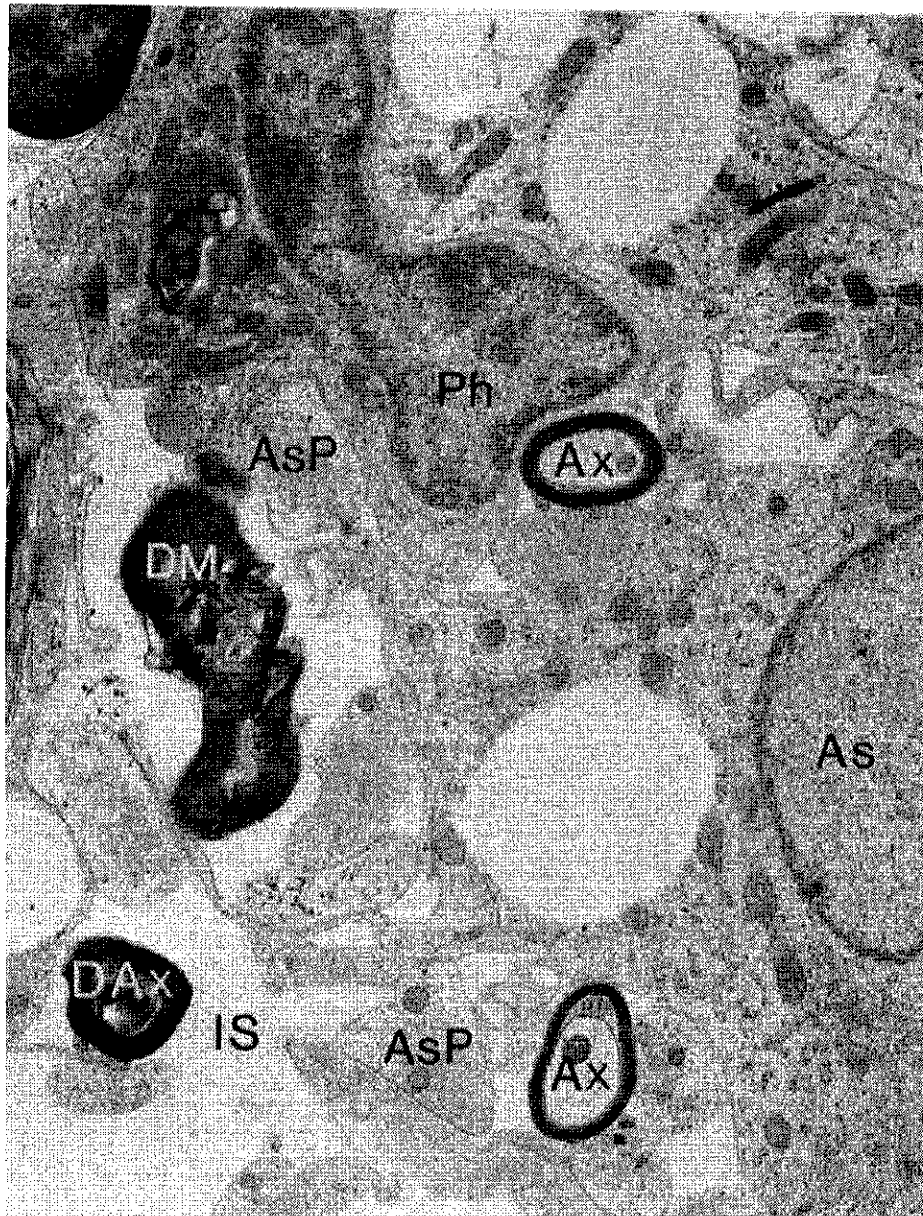


Fig. 1: Fasciculus gracilis (second cervical spinal cord segment): Severe loss of nerve fibers, a few remaining normal axons (Ax), contracted axon with granular electron-dense axoplasm within myelin sheath (DAX), degenerating myelin (DM), astrocyte (As), astrocytic processes (AsP) and increased interstitial space (IS). Phagocyte (Ph) in interstitial spaces between astrocytic processes. Lead citrate and uranyl acetate.

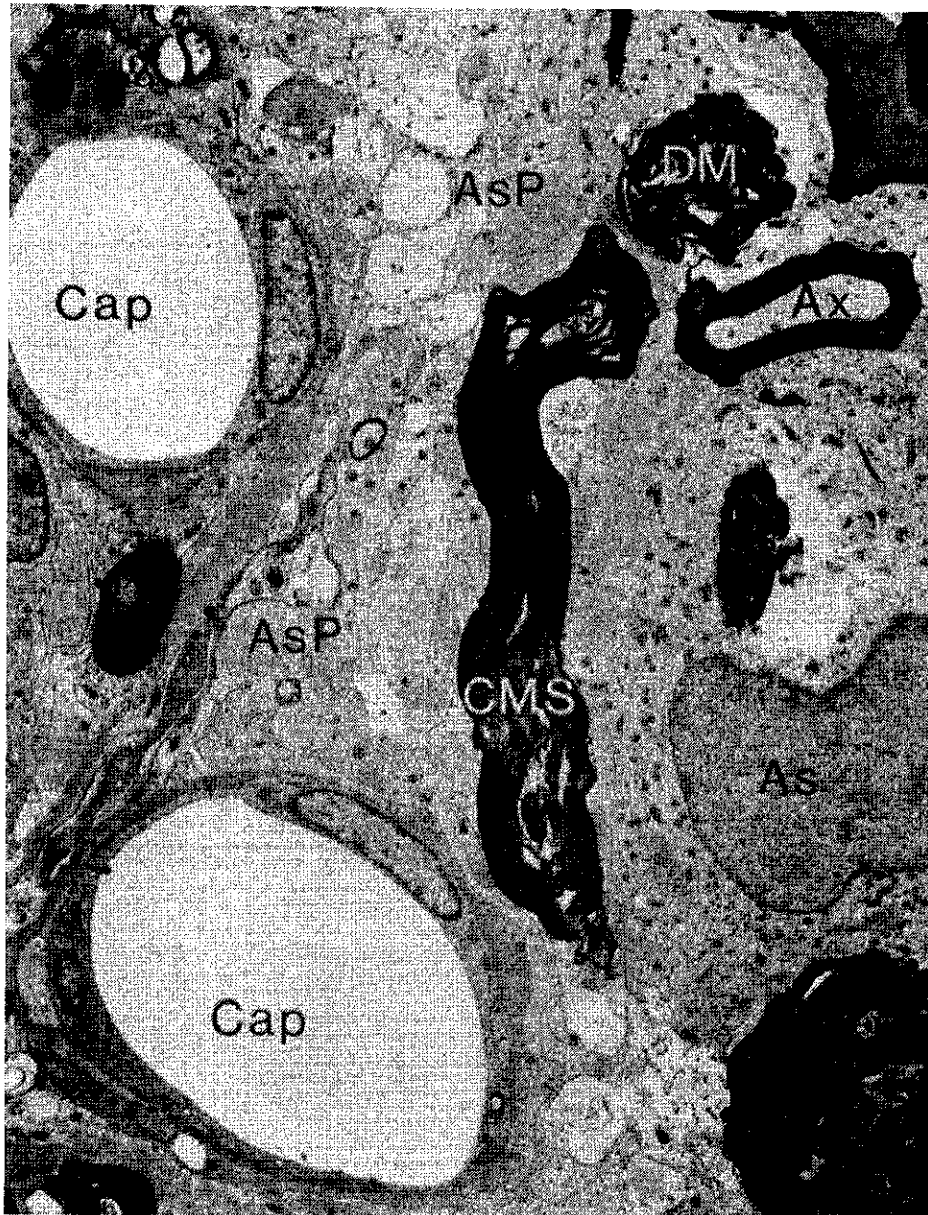


Fig. 2: Fasciculus gracilis (second cervical spinal cord segment): Few normal axons (Ax). Astrocytes (As) with abundant cytoplasm and numerous astrocytic processes (AsP) separate collapsed myelin sheaths (CMS) and degenerating myelin (DM). Capillaries (Cap) normal. Lead citrate and uranyl acetate.

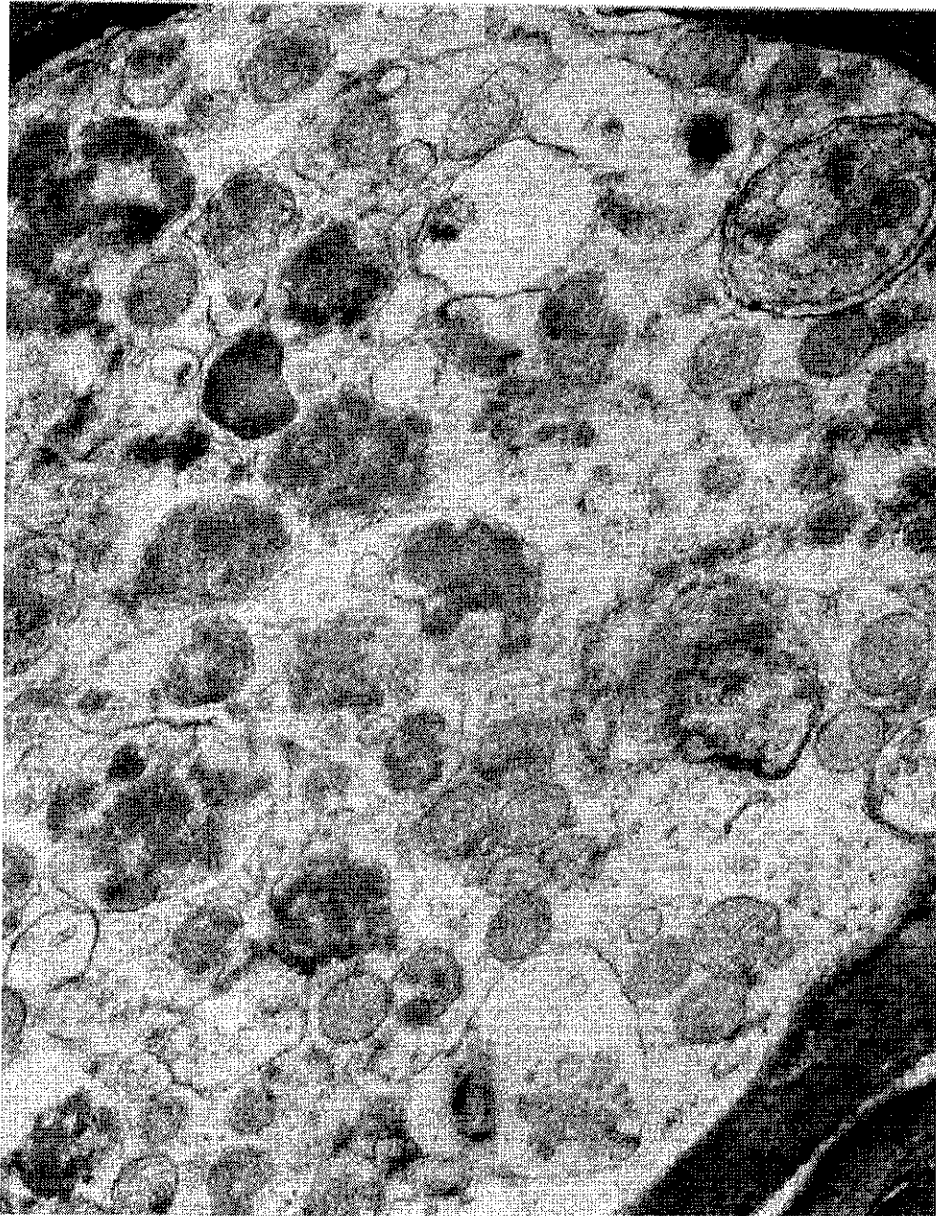


Fig. 3: Fasciculus gracilis (second cervical spinal cord segment): Accumulations of mitochondria, some with electron-dense matrices, membranous proliferations and electron-dense bodies in axoplasm of axon. Neurofilaments and microtubules replaced by flocculent electron-dense material. Lead citrate and uranyl acetate.

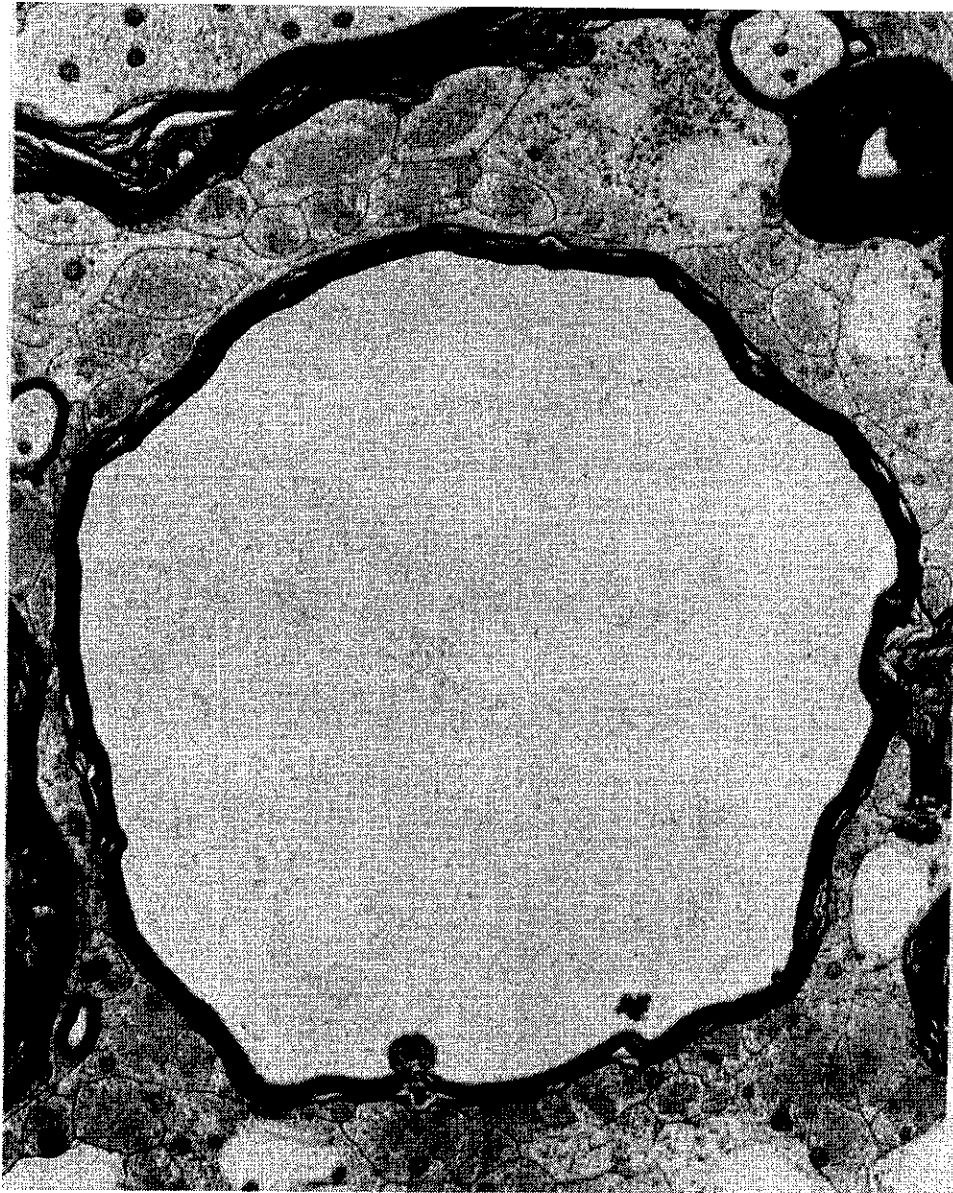


Fig. 4: Fasciculus gracilis (second cervical spinal cord segment): Central nerve fiber increased in diameter, has no axon; myelin sheath attenuated. Lead citrate and uranyl acetate.

collapsed myelin sheaths and degenerated myelin (fig. 1, 2). The cell bodies of astrocytes were enlarged and occasionally contained some myelin debris. The few phagocytes present usually were in the interstitial space. Vascular and connective-tissue elements were ultrastructurally normal.

Discussion

Axonal degeneration like that in our dogs, characterized by loss of neurofilaments and microtubules, accumulations of floccular electron-dense material and granular debris or of dense bodies, mitochondria, and membranous proliferations, is a common reaction to a variety of insults and occurs at some stages in wallerian degeneration [6]. Both morphologically normal nerve fibers and nerve fibers in various stages of degeneration were present in affected white tracts of our dogs, but no lesions of the myelin sheath were found without an axonal lesion. The severe degenerative changes of the myelin sheath were associated with loss of the axon. It was concluded that pyridoxine produced axonal degeneration followed by nonspecific secondary degenerative changes in myelin.

The degenerative changes induced by excess pyridoxine were located primarily in the nerves and fiber tracts that contain the processes of spinal and trigeminal ganglia, but may involve the neuron cell bodies of these ganglia as well [1, 7, 11]. Other compounds such as methyl mercury and doxorubicin (adriamycin) have induced neurologic lesions with the same distribution as in our dogs. The neuropathy produced by these compounds involved primarily neurons of the spinal and trigeminal ganglia, and the degenerative changes have a distribution selective for dorsal roots, the dorsal funiculus, and axons in certain peripheral nerves. With methyl mercury and doxorubicin toxicities, the distribution of the lesions was considered to be caused by the ability of the compounds to cross the blood-brain barrier. In the spinal and trigeminal ganglia, parts of the peripheral nervous system, the barrier is incomplete, thus the concentrations of the compounds could be great enough to produce a primary neuronal lesion [16]. Pyridoxine, in this subacute toxicity study, produced alterations that differed from those described for doxorubicin and methyl mercury; with pyridoxine, the neuronal body alterations were minimal as compared to those caused by methyl mercury and doxorubicin.

Studies of the various forms of vitamin B₆ in dogs have been limited; however, some findings in other species may apply to dogs. In the rabbit, vitamin B₆, principally in the non-phosphorylated forms, crosses the blood-brain and blood-cerebrospinal fluid barriers and enters the brain by a saturable process or processes [13, 14, 15]. In the rat, high dietary vitamin B₆ resulted in high plasma concentrations, but concentrations in the brain were not increased significantly [3, 10]. Slightly elevated vitamin B₆ concentrations were found in some, but not all, samples of brain from dogs given 200 mg/kg body weight/day of pyridoxine [11]. Because they are outside of the blood-brain barrier, neurons of the spinal and trigeminal ganglia in dogs given excess pyridoxine may be exposed to concentrations of pyridoxine greater than those available to central nervous system neurons protected by the blood-brain barrier.

Considerable similarity exists between the acute toxic effects produced by anti-vitamin B₆ compounds and by excess pyridoxal, one form of vitamin B₆. It has been proposed that both vitamin B₆ excess and deficiency produced their effects by a mechanism of decreased concentrations of pyridoxal phosphate [5]. Isoniazid is

presumed to produce its toxic effects by the same mechanism [8]. Thus, it is possible that excess pyridoxine produced axonal injury through the inhibition of pyridoxal phosphate, but the lesions would be limited by the blood-brain barrier to the processes of neuron cell bodies exposed to higher concentrations of the vitamin.

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