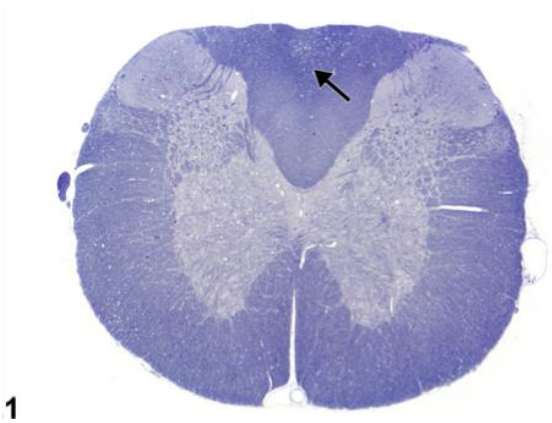
NTP Nonneoplastic Lesion Atlas

Brain – Axonopathy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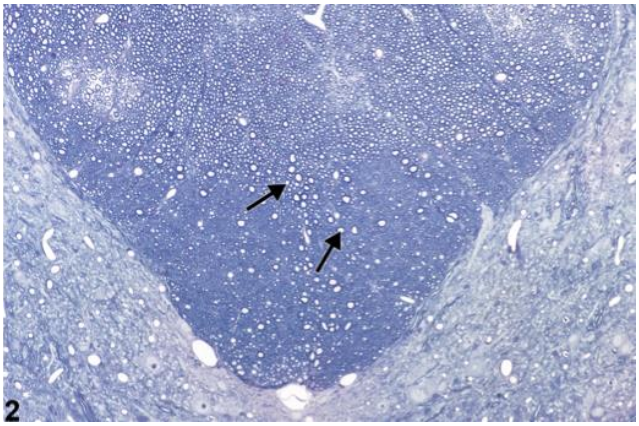
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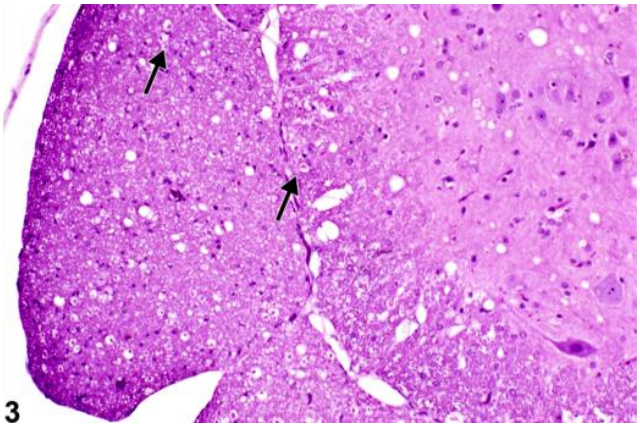
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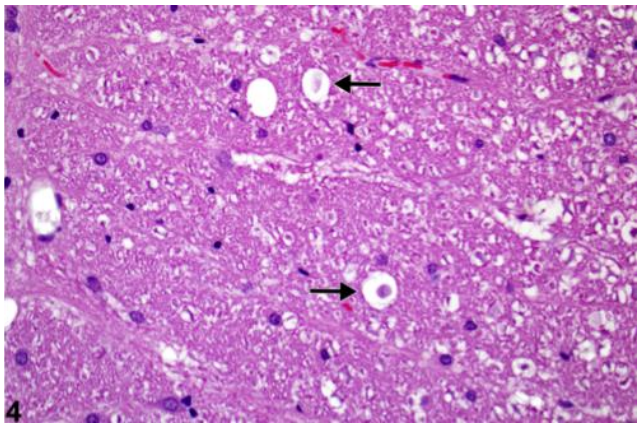
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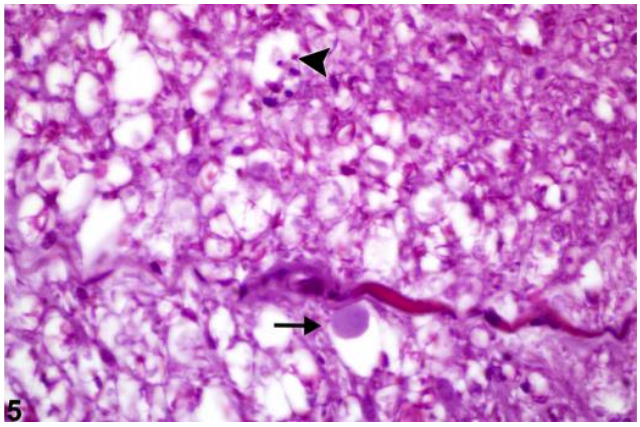
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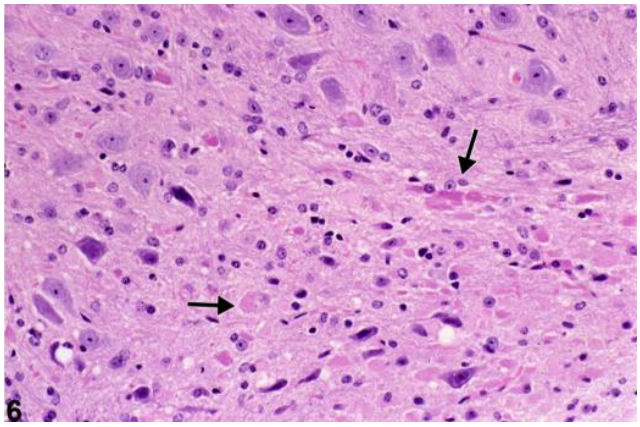
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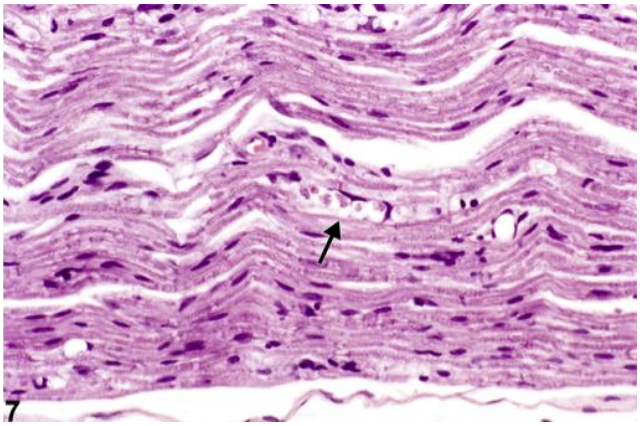
Brain – Axonopathy



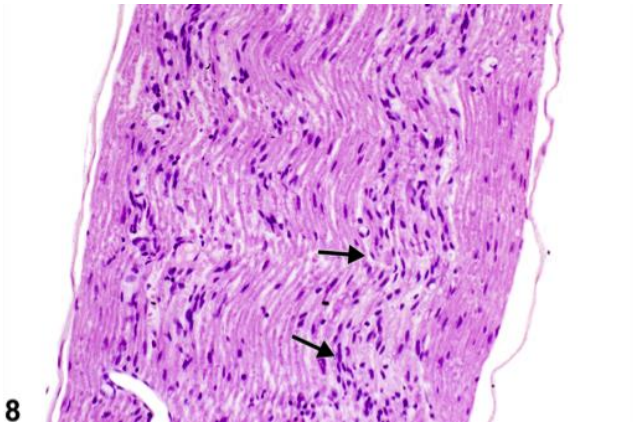
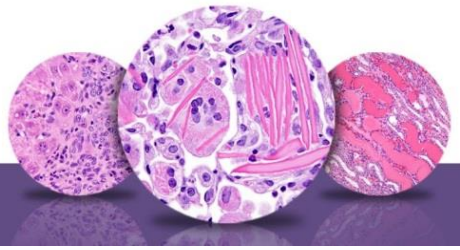


Figure Legend: Figure 1 Spinal cord axonopathy in a male F344/N rat from a subchronic study. The arrow in this toluidine blue–stained section indicates the subtle pallor of the dorsal funiculus in which axonopathy is present. **Figure 2** Toluidine blue–stained section in a male F344/N rat from a subchronic study. Note that the axons (arrows) are distended but still surrounded by myelin sheaths. **Figure 3** Spinal cord section in a male B6C3F1 mouse from a subchronic study. Note that some axons are swollen (arrows) and that the tissue is vacuolated from loss of axons and their myelin sheaths. **Figure 4** Cervical cord section in a female Sprague Dawley rat from a chronic study. The swollen appearance of some degenerate axons is clearly visible (arrows). **Figure 5** Spinal cord section in a female F344/N rat from a subchronic study. The arrow indicates a large swollen axon, and the arrowhead locates the presence of a degenerate macrophage occupying the space of a former axon. **Figure 6** Axonal spheroids (arrows) in brain parenchyma in a male B6C3F1 mouse from a subchronic study. **Figure 7** This longitudinal section of nerve in a female B6C3F1 mouse from a subchronic study shows an example of axonal fragmentation referred to as Wallerian-type degeneration (arrow). **Figure 8** Increased cellularity created by proliferating Schwann cells (arrows) as a response to sciatic nerve axonopathy in a male B6C3F1 mouse from a subchronic study. These linear arrays are known as bands of Büngner.

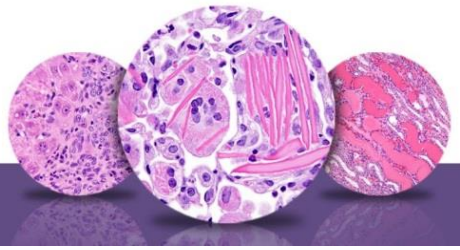
Comment: “Axonopathy” is a generic term describing a variety of lesions of the axon occurring in brain, spinal cord, and peripheral nerve. Care must be taken at low magnifications to ensure that the criteria for axonopathy (axonal swelling, fragmentation, loss, etc.) are not disregarded



as artifactual vacuolation. Its presence in defined regions of brain and spinal cord and frequent symmetrical distribution are useful clues to its genuine nature. Close examination of all regions of white matter and nerve tissue is required to correctly discover and interpret this type of lesion. Age-related axonopathy occurs commonly in the cauda equina, ventral spinal nerve roots, ventral and lateral spinal funiculi, sciatic and brachial nerves, and lower brainstem. Caution should be given to interpretation of spinal and radicular (nerve root) axonopathy in aged rats since it may be a result of senescence and has an incidence of 75-90% in rats 24 or more months of age. In specialized toxicologic-neuropathologic studies, use of special axonal stains such as Bielschowsky silver impregnation and de Olmos amino cupric silver to highlight the normal axon and axonal lesions, respectively, may be helpful in delineating the severity and distribution of these often subtle lesions.

Figure 1 shows spinal cord at low magnification demonstrating the appearance of axonopathy in the dorsal proprioceptive funiculi, in this case the fasciculus gracilis. Note, at this magnification, that the lesion is represented by subtle vacuolation (arrow) of a focal area ordinarily populated by normal ascending axons. Damage to the axons in this zone leads to swelling of both the axon and its myelin sheath, thus creating a dilated space. This image uses toluidine blue stain. Figure 2 is a higher-magnification toluidine blue–stained section of spinal cord showing axonal swelling in the fasciculus gracilis in a subchronic toxicity study. Note the many distended axons (arrows), most still surrounded by their myelin sheath. There is a prominent reduction in myelinated fibers, particularly in the deepest parts of the funiculi, making the swollen myelinated axons in these regions stand out in relief as large ”vacuoles.”

Figure 3, Figure 4, and Figure 5 depict swollen axons. In Figure 3, note the slightly enlarged occasional swollen axons (arrows) at the center of dilated spaces formerly occupied by a normal, compact lamellar myelin sheath. Many of the dilated spaces are empty because the axon has already undergone disintegration, lysis, and phagocytosis by macrophages. The space appears empty because the use of fat solvents in tissue processing has removed any remaining complex lipids, which made up the myelin sheath.



Brain – Axonopathy

Figure 4 represents swollen degenerate axons at higher magnification (arrows), where the diameter of the distended space surrounding them is approximately four times larger than the space around adjacent, apparently normal axons. Figure 5 represents spinal funicular axonopathy with detail of a swollen axon, a so-called spheroid (arrow). Note the pyknotic macrophage in a vacuole representing a later stage of axonal degeneration characterized by axonal phagocytosis (arrowhead). In most cases of axonal degeneration, identifiable macrophages are usually necrotic, presumably from the effects of myelin lipid phagocytosis and cytokine release during the axonal degenerative process. Apoptosis of macrophages results when they are loaded with free cholesterol and triacylglycerol. In this high-magnification hematoxylin and eosin–stained image, the large swollen axon has an amphophilic appearance. This is a good example of a spheroid at an early stage of axonal swelling that precedes axonal disintegration.

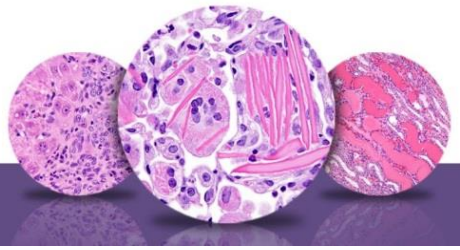
Figure 6 shows eosinophilic spheroids and oval to elongated structures (arrows) that represent injured axons in the brain sectioned in various planes, thus affecting their appearance. The presence of swollen axons in the brain is not generally accompanied by dramatic distension of the myelin sheath such as those seen in spinal cord and peripheral nerves. Note also that there is a mild increase in a mixed population of glial cells in the region, part of the associated inflammatory response.

Figure 7 shows axon degeneration with a typical “digestion chamber,” containing axonal fragments (arrow). In this hematoxylin and eosin–stained image of a longitudinal section of the sciatic nerve, the typical appearance of axonal degeneration referred to as Wallerian degeneration is evident. The term “Wallerian degeneration” is best reserved to describe axonopathy in peripheral nerve; however, similar changes can be seen in spinal cord and brain. Augustus Waller, in 1850, introduced the criteria for axonopathy in peripheral nerve from his sequential studies of experimental nerve crush injury. The distal nerve, particularly after crush, is characterized by progressive axonal swelling and, approximately 36 hours after crush, by fragmentation of the fiber, Schwann cell nuclear hypertrophy, infiltration of macrophages, and the formation of so-called digestion chambers. In Figure 7 the digestion chamber contains

several fragments of degenerate axon surrounded by a clear space, containing lipid breakdown products and macrophages. The enlarged nuclei of Schwann cells can be seen in adjacent nerve tissue.

Figure 8 shows sciatic nerve axon degeneration with reactive hyperplasia and hypertrophic nuclei of Schwann cells (arrows). These linear band-like proliferative Schwann cells are known as bands of Büngner. They are formed as a response to axonal injury when Schwann cells lose their axonal complement. The remaining basement membrane forms endoneurial tubes, important in the guidance of regenerating proximal axonal sprouts. Schwann cells and blood-borne macrophages are important in phagocytosis of fragmented axons to clear endoneurial tubes for axonal regeneration. The bands of Büngner should not be interpreted as inflammatory fibrosis but, rather, as a part of the reinnervation process subsequent to significant axon degeneration in peripheral axons.

Recommendation: In NTP studies, the term “axonopathy” is preferred for all forms of axonal injury. This lesion should be diagnosed in control and treated animals with its anatomic subsite location. Severity grade should be included with criteria, allowing a decision on its treatment-related significance to be based on incidence and severity. Searching diligently for tangible criteria of axonopathy is important in confirming the genuine nature of the lesions at several stages of their evolution. In NTP studies where focal vacuolar lesions in white matter are of uncertain pathogenesis to the study pathologist, or in studies where the lesion cannot be differentiated from demyelination or intramyelinic edema, the lesion should be diagnosed as white matter vacuolation. The narrative should describe features including whether the lesion is focal, unilateral, or bilateral. This should be followed by special evaluation by a neuropathologist.



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