

# Non-proliferative Lesions of the Nervous System in Rats

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## INTRODUCTION

This guide provides a simplified nomenclature for non-proliferative pathologic changes occurring in the rat nervous system and is primarily based on histopathologic findings in hematoxylin and eosin (H&E) stained tissue sections from Sprague-Dawley (SD) and Fischer 344 (F344) rats. It is important to recognize that toxicologic evaluation of the nervous system is currently performed on immersion-fixed tissues from standard toxicity tests and on perfusion-fixed tissues from special neurotoxicity tests. The latter are required under certain regulatory guidelines and involve tissue preparation in both paraffin and plastic (53). Reviews concerning the role of neuropathology in these special neurotoxicity tests are available (4, 16, 24, 33, 41). Since toxicologic evaluation of the nervous system is done with various histologic procedures under different testing circumstances, photographs in this guide include examples from several types of histologic preparations in order to illustrate changes most appropriately.

Non-proliferative changes in the nervous system that are toxicologically significant and related to the administration of test materials must be differentiated from changes that are spontaneous or artifactual. Spontaneous non-proliferative changes in the central and peripheral nervous system of laboratory rats used for

toxicology studies are common (2, 5, 12, 17, 22, 26, 27, 34, 39, 40, 54). The majority are degenerative changes in the central nervous system (CNS) which begin to appear at around six months of age (22). Traumatic or compressive-type changes in the peripheral nervous system (PNS) of guinea pigs (1, 21) are also observed in rats (O'Donoghue, unpublished data). In addition to spontaneous changes, it is important to recognize that artifactual changes can result from tissue handling (8, 23, 45), processing (37, 56) and perfusion (10, 42, 44).

## MICROSCOPIC ANATOMY OF THE NERVOUS SYSTEM

Neurons, the fundamental unit of the nervous system responsible for signal processing, consist of a cell body (soma or perikaryon) and cell processes (axons and dendrites). A single neuron may have one (unipolar) or two (bipolar) axons and many dendrites. Axons larger than 1-2  $\mu\text{m}$  are covered usually by a myelin sheath. The myelin sheath is formed from cell processes derived from oligodendroglial cells (CNS) or Schwann cells (PNS) that envelop the axon. In the PNS, a Schwann cell will form one myelin internode around one axon. In the CNS, a given oligodendroglial cell may form many myelin internodes around multiple axons. Other glial cells include astrocytes, which provide a supporting matrix for growth, development, and maintenance of the CNS, and microglia, which migrate into the CNS from the blood as monocytes and are the principal phagocytic cells in the CNS. The ventricular system of the CNS is lined by

ependymal cells, and the cells of the choroid plexus are important as a source of cerebrospinal fluid (CSF). The brain is surrounded by the meninges (pia mater, arachnoid, and dura mater). The leptomeninges (pia mater and arachnoid) more closely envelop the brain and will be visible in standard histologic preparations.

## MORPHOLOGY

The following diagnostic terms are grouped according to whether the primary change affects the axon, nerve cell body, myelin sheath, glia, or multiple components. This classification is based on changes in primary target sites that frequently occur with toxic damage to the nervous system (29, 43, 41, 47). General references to basic pathologic processes in the nervous system are available (14, 29, 31, 51).

### Axon

#### *Axonal Degeneration (Figures 1-2)*

An axon separated from its cell body will undergo degenerative changes from its proximal end toward the periphery (i.e., Wallerian degeneration or dying-forward degeneration). Axons which are injured but not separated from their cell body will also undergo degenerative changes. In this instance, axonal degeneration often begins distally (near the axon terminal) and progresses centrally toward the cell body in a "dying-back" manner (9). When nerve fibers undergo degeneration, the axon and its myelin sheath break down and appear as a short string of round to oval fragments (myelin ovoids or digestion chambers) which undergo phagocytosis.

Axonal degeneration is commonly observed in histologic sections of the spinal cord of rats >15 months of age. Degenerating fibers can be found in the dorsal and ventral horns, the intermediate gray matter, and in the fasciculus gracilis at cervical, thoracic, and lumbar levels (40).

#### *Axonal Swelling (Axonal Dystrophy) (Figures 3-6)*

In a number of conditions related to alterations in axoplasmic flow, axonal swelling is observed. Weiss and Hiscoe (55) first described this phenomenon following ligation of peripheral nerves. Axonal swelling is also observed in a number of toxic neuropathies, with some chemicals (e.g., methyl *n*-butyl ketone) causing very large swelling ("giant" axonal swelling). In longitudinal profile, the swellings appear as eosinophilic fusiform or torpedo-shaped structures. In cross-section, they may not be apparent unless the swellings are common, particularly large, or hyperchromatic. A distinguishing feature of swollen axons viewed in cross section is a reversal of the normal myelin thickness to axon diameter ratio. With certain exceptions (46), axons of larger caliber will have thicker myelin sheaths (15). When an axon enlarges, its

myelin sheath will become thinner in proportion to the increase in axonal diameter. Thus the myelin sheath of an enlarged axon may be thinner than the myelin sheaths of adjacent smaller axons.

The term *axonal dystrophy* should be used to describe swelling of distal axonal processes in the gracile and cuneate nuclei and rostral fasciculi of rats, as early as 6 months of age (22). Swollen axons found in the gracile and cuneate nuclei originate from dorsal root ganglion cells (18). Age-associated axonal swelling can also be seen in the spinal trigeminal nucleus, the rostral ends of the fasciculus gracilis and fasciculus cuneatus, and rarely in the grey matter of the spinal cord (22, 30). Swollen preterminal axons appear as eosinophilic spheroids within the boundaries of medullary nuclei. Swollen axons occur at a young age and with a greater frequency in rats treated with chemicals causing dying-back neuropathy (3).

#### *Axonal Atrophy (Figure 7)*

Axonal atrophy results in decreased average axonal diameter and increased interaxonal space in peripheral nerves. These features are best observed in cross section and are most apparent when axonal atrophy occurs as a generalized response to chemical treatment. Axonal atrophy can also occur as a focal feature in the region of an axon adjacent to an enlarged axon or one which is constricted. It is best demonstrated in plastic-embedded sections or in sections stained with Luxol Fast Blue to demonstrate myelin. Axonal atrophy should be differentiated from endoneurial edema, which involves an increase in interaxonal space without significant reduction of axonal diameters.

## NERVE CELL BODY

#### *Lipofuscin Accumulation (Figure 8)*

Lipofuscin granules appear within the cytoplasm of neurons and glial cells and are associated with normal aging in rats (32). The granules are typically yellow-brown, but may also appear to be greenish. Small amounts of lipofuscin can appear in neurons as early as 3 months of age. In aged animals, lipofuscin can occupy up to 50-60% of the perikaryon. Special stains to identify the granules more specifically include Schmorl's or carbol fuchsin stains. Exposure to chemicals such as acetyl ethyl tetramethyl tetralin can lead to increased amounts of lipofuscin within the CNS (49).

#### *Chromatolysis (Figure 9)*

Axonal damage, if it is severe and close to the proximal end of the axon, may lead to a reaction in the neuronal cell body referred to as chromatolysis. The term chromatolysis has been used synonymously with the terms "central chromatolysis" and "axonal reaction"

(14). Neurons displaying *central chromatolysis* become swollen and their nuclei move toward the margin of the cell. The Nissl substance becomes pale and may partially disappear. Neurons which undergo a degenerative process may be smaller than normal and show dissolution of the peripheral Nissl substance. In this latter situation, the term *peripheral chromatolysis* is preferred to differentiate it from central chromatolysis. Peripheral chromatolysis is also considered to be a stage of recovery from central chromatolysis.

### ***Neuronal Necrosis (Figure 10)***

Necrosis of neurons occurs after exposure to several different neurotoxins, including several heavy metals and drugs (19, 47). Necrotic nerve cell bodies are usually brightly eosinophilic in paraffin sections stained with hematoxylin and eosin. Identification of a necrotic cell as a neuron depends on being able to identify cell types based on cellular morphology or location. Other terms such as ischemic cell change and homogenizing cell change have been used to describe the same process; however, these are not recommended for toxicologic pathology (14). The nerve cell body is usually shrunken and angular as a result of cytoplasmic collapse. In the latter stages of degeneration, when karyorrhexis or karyolysis are present, necrotic neurons may be indistinguishable from other cell types at the light microscopic level. In addition, a glial cell response may be evident around necrotic cell bodies, particularly if several days have elapsed since exposure to the injurious agent. These are likely reactive astrocytes and microglia; however, definitive identification of these cells requires special staining procedures (20).

Necrosis is a relatively broad term that has traditionally been used to indicate the presence of cell death. As such, use of this term also encompasses neurons undergoing programmed cell death, or apoptosis. It should be noted that the current use of the terms necrosis and apoptosis is somewhat controversial and will have to be clarified in the future.

### ***Neuronophagia (Figure 11)***

When necrosis of neurons occurs acutely, the degenerating cell body may be surrounded by phagocytic microglial cells. Neuronophagia is more commonly associated with the encephalitides, but it may be seen in other situations where the chemotactic properties of microglia are active. Neuronophagia should be differentiated from perineuronal satellite cells which are resident oligodendroglia that surround otherwise normal neurons.

### ***Nerve Cell Loss (Figure 12)***

Loss of ventral motor horn cells has been reported to be associated with aging in rats (36). Nerve cell loss is sometimes difficult to establish as a morphologic

diagnosis because precise knowledge of the anatomical site is required. However, there are some anatomic sites where absence of neurons will be readily apparent (i.e., hippocampus, cerebellum, and cerebral cortex), particularly if attention is paid to the nerve cell density and normal anatomy. When neuronal death results from intermittent or chronic exposure to a toxicant, slight gliosis in the region and absence or reduced number of neurons may be the only features apparent at the end of the study.

Nerve cell loss can involve neuronal populations other than those directly affected by neurotoxicants. There are a number of growth factors which are responsible for the continued health and maintenance of neurons. Neurons require continued synaptic input for long term maintenance. Loss of these inputs can result in atrophy and eventually loss of neurons. This type of loss is referred to as trans-synaptic degeneration. Neurons undergoing this change become smaller in size but typically do not show evidence of chromatolysis.

## **MYELIN SHEATH**

### ***Myelin Folding (Figures 13-14)***

Myelin folding is a normal process that is increased in peripheral neuropathies. The normal myelin sheath is not smooth, but rather has periodic invaginations or folds which extend into the adjacent axoplasm. Invaginations of Schwann cells and oligodendroglia appear to provide a mechanism for the sequestration and removal of organelles from PNS and CNS axons (48). In cross-sections, myelin folding distorts the round profile of the axon. In longitudinal sections, the folds appear as multiple extensions of the sheath into the axon. While folding can be observed in both the CNS and PNS, it is much easier to observe in the PNS. In peripheral neuropathies, the frequency of folding is increased so that longitudinally sectioned axons appear to have an irregular or corrugated surface. When myelin folds are numerous they may be incorrectly interpreted as myelin degeneration (ovoids or digestion chambers) in longitudinal section.

### ***Intramyelinic Edema (Figures 15-16)***

The accumulation of fluid within the myelin sheath leads to splitting of the myelin along the intraperiod line. In H&E sections, fluid accumulation appears as clear spaces or bubbles within the myelin sheath. In heavily myelinated regions of the CNS, affected myelin will stain less intensely than normal with H&E. Phagocytic cells may be observed within edematous regions if fragmentation and degeneration of myelin accompanies the accumulation of fluid.

### ***Demyelination (and Remyelination) (Figures 17-20)***

Demyelination can be primary or secondary. Because identification of target cells is important when evaluating changes induced by chemical agents, it may be necessary to differentiate primary demyelination resulting from pathologic processes involving the Schwann cell or myelin sheath from secondary demyelination resulting from axonal degeneration. The process of differentiating primary from secondary demyelination involves using microscopic techniques which can demonstrate the absence of significant axonal degenerative changes in the presence of demyelination. In addition to special staining procedures, other techniques (e.g. electron microscopy, nerve fiber teasing) are usually required to make a clear distinction between types of demyelinations. In the PNS, nerve fiber teasing may be necessary to distinguish segmental from diffuse forms of primary demyelination.

The microscopic appearance of demyelination is highly dependent on the stage of the condition. Myelin ovoids (also called digestion chambers) are the swellings that result from disruption and fragmentation of the myelinated axon. These are characteristic during the early degenerative phase, but are not a prominent feature in later stages when remyelination predominates. Demyelination secondary to axonal degeneration will generally be characterized by the presence of myelin ovoids that are focally distributed among normally myelinated axons. In contrast, the chronic phase of a condition where the Schwann cell is the primary target may be characterized at the light microscopic level by a preponderance of non-myelinated or hypomyelinated fibers. Such nerves stain more lightly with H&E or Luxol Fast Blue. Although myelin ovoids may be present at early stages of Schwann cell degeneration, ovoids are usually not a predominant feature until later in the process when the myelin sheath itself breaks down. Remyelination or attempts at remyelination may predominate in later stages of demyelination, resulting in variable myelin thickness along the same axon.

## **GLIA**

### ***Gliosis (Figure 21)***

Gliosis is a common response to nervous system injury. The term refers to a general increase in the number of astrocytes and/or microglial cells and their processes. In H&E stained sections, areas undergoing gliosis will show increased numbers of glial nuclei and perhaps increased eosin staining in the background. In toxicologic pathology, the term gliosis is usually used to indicate an increase in astrocytes due either to an increase in the size and number of astrocytic processes per cell (astrocytic hypertrophy or astrogliosis) or to an increase in the absolute number of astrocytes (astrocytic hyperplasia or astrocytosis).

Astrocytic hypertrophy may be gemistocytic (enlarged cell bodies with prominent eosinophilic cytoplasm) or pilocytic (swollen cell processes that form a dense, hyalinized network). Phosphotungstic acid-hematoxylin stain (PTAH) or immunohistochemical stains for glial fibrillary acidic protein (GFAP) may be used to enhance the morphologic appearance and detection of reactive astrocytes. An increase in the number of microglial cells is termed *microgliosis*. This reactive change can be detected and confirmed by a variety of histochemical and immunohistochemical procedures (50, 52).

## **Multiple Components**

### ***Epidermoid Cyst (Figure 22)***

Epidermoid cysts are uncommon in the CNS and arise from embryological remnants of ectoderm becoming enclosed in the developing brain or spinal cord. These cysts are lined by stratified squamous epithelium and contain keratinaceous debris in their lumen (35).

### ***Hemorrhage (Figure 23)***

Hemorrhage is the antemortem escape of blood from the vasculature. In rats, hemorrhage can occur as an agonal event in animals dying from a variety of causes. This type of hemorrhage typically occurs in the Virchow-Robin space forming a collar around the vessel. Hemorrhage which occurs at earlier time points is characterized by pale erythrocytes with blurred cellular outlines, and may be associated with the accumulation of blood pigments in phagocytic cells. Artifactual hemorrhage in the brain, spinal cord, or cerebrospinal spaces is common when the nervous system is not handled carefully.

### ***Hydrocephalus (Figure 24)***

Hydrocephalus is the expansion of the ventricles with cerebrospinal fluid. This condition can arise spontaneously and appear as an incidental finding in younger animals, or can be acquired, most commonly as a secondary response to the pressure effects of pituitary tumors (28). The degree of ventricular distention can vary and may be difficult to confirm definitively in mild cases. Perfusion fixation often results in minimal to mild ventricular distention.

### ***Mineralization (Figure 21)***

Foci of mineralized tissue are occasionally found in the brains of older rats, especially in the thalamus (7). Age-related mineralization is often associated with blood vessels or the choroid plexus and typically lacks a glial reaction. However, mineralized foci can also be associated with lesions causing extensive tissue destruction in rats. These areas frequently have a localized glial

reaction. Bone chips pushed into the parenchyma during removal of the brain should not be confused with mineralization.

### ***Infarction (Figures 25-26)***

Infarction is cell death resulting from obstruction of blood supply to the tissue. The vascular occlusion is usually not seen, but its presence can be reasonably inferred by the presence of an area of necrosis limited to the tissue supplied by the affected vessel. Infarctions less than 24 hours old appear as sharply demarcated areas which have reduced eosinophilia due to cytoplasmic vacuolation and which have pyknotic nuclei. After several days macrophages invade and replace the normal parenchyma (38). Old infarcts often contain many cystic spaces and are surrounded by glial and vascular reactions (6). Hemorrhage sometimes occurs in infarcted zones, especially at the margins. A modifier such as "hemorrhagic" may be used when hemorrhage is a prominent feature of an infarct.

### ***Necrosis (Figures 27-28)***

Necrosis is a general diagnostic term used to denote destruction of neural tissue due to a variety of etiologic factors or when the etiology is uncertain. Necrosis is preferred to malacia as a histopathologic term, since malacia refers to the softening of areas of liquefactive necrosis in the CNS. The morphologic features of necrosis may be similar in some respects to those described above for infarction (i.e., pyknotic nuclei and rarefaction of neuropil due to vacuolation), but a vascular distribution is lacking. Other features of necrosis, such as fragmentation of cellular components and focal accumulation of inflammatory cells, will generally be present. The term necrosis is also used in the nervous system when referencing active destruction of a specific anatomic site or cell type (i.e., necrosis of field CA2 of Ammon's horn after trimethyltin exposure as reported by Chang (11)).

### ***Vacuolation (Figures 28-29)***

Vacuoles or clear spaces are often seen in H&E sections of the brain and spinal cord of laboratory rats. Most often vacuolation is observed in heavily myelinated regions of the CNS. Other terms such as spongiosis or spongy degeneration have been used to describe this process; however, these are not recommended for toxicologic pathology. Interpreting the presence of this lesion may be difficult because artifactual vacuolation is common and must be differentiated from pathologic or chemically induced vacuolation (25). Vacuolization may also occur selectively within the cytoplasm of neurons in response to certain neurotoxins (19), and is occasionally observed in large neurons of aged rats as a background change.

Artifactual vacuolation may occur unilaterally or bilaterally in rats. While there is no absolute method for separating artifactual vacuolation from pathological, some differentiating features may be looked for. Vacuolation of myelinated areas accompanying a pathologic process such as intramyelinic edema or demyelination may be associated with reduced staining of the myelin and phagocytic or inflammatory cells. In contrast, artifactual vacuolation is more typically associated with other evidence of autolysis such as shrunken or darkly staining neurons, and can also be caused by processing problems (37, 56). Mucocytes, which occur artifactualy in the brain of rats on occasion, can undergo dissolution and result in vacuolation of myelinated areas of the brain (51).

Vacuolation in non-myelinated regions of the CNS is less commonly associated with autolysis. Nonetheless, other evidence of a pathologic process should be sought before concluding that vacuolation is a true lesion. Pathologic vacuolation in non-myelinated regions is frequently associated with gliosis, neuronophagia, or mineralization.

### ***Compression (Figure 30)***

Compression of neural tissue in either the CNS or PNS leads to atrophy of the adjacent tissue. In the CNS, compression is most commonly observed in old rats due to the growth of pituitary tumors. This may be associated with hydrocephalus. In the PNS, segmental demyelination and Wallerian degeneration have been reported to occur in the plantar nerves of the hind foot of guinea pigs housed in wire-mesh cages (21). Similar lesions have been observed in rats housed in wire-mesh cages for several months (O'Donoghue, unpublished data).

### ***Arteritis (Panarteritis Nodosa) (Figure 30)***

For a discussion of the morphology of this lesion, the reader is referred to the nomenclature section dealing with diseases of the vasculature.

The vasculature of the central nervous system of the rat is spared the effects of panarteritis nodosa even when the disease is associated with chronic estrogen stimulation (13). However, involvement of small muscular arteries adjacent to peripheral nerves can result in compression of the peripheral nerve or secondary involvement of the peripheral nerve in the inflammatory process.

### ***Inflammation (Figure 31)***

Spontaneous inflammatory lesions in the nervous system of rats may occur but are uncommon (25, 28). Bacterial, viral, and protozoa agents have been implicated. If inflammation is identified, appropriate modifiers should be used to describe the type of inflammation, its location, and the extent of involvement.

## DISCUSSION

While there are many different systems for classifying non-proliferative lesions of the nervous system, the one used in this guide recognizes that most lesions associated with toxic insults can be differentiated into those which primarily affect the axon (axonopathy), the neuron (neuronopathy), or the myelin sheath (myelinopathy) (29, 41, 43, 47). However, lesions of each of these structures often affect other tissues secondarily, as seen with axonopathies and secondary demyelination.

Many different terms have been used to describe axonal lesions. Some of these terms describe the time frame between dosing and the onset of clinical signs or pathologic effects (e.g., delayed neuropathy). Others describe the location of a lesion along the length of the axon (e.g., proximal axonopathy vs. distal axonopathy) or the fact that many axonopathies begin in the CNS and PNS at approximately the same time (e.g., central-peripheral axonopathy). Still others imply knowledge of the pathogenesis of a lesion (e.g., dying-back or Wallerian degeneration). Terms such as Wallerian degeneration have become so frequently used to indicate nearly any type of disintegration of the axon, that the original meaning of the term has almost become lost. If one cannot establish the primary lesion in a damaged nerve, the more generic term axonal or nerve fiber degeneration should be used. Many of the terms used to describe axonal lesions refer to information which is available only after a complete histopathologic examination has been completed and, therefore, were not used in this guide where the purpose of the terminology is to be able to record observations as they are being made.

The laboratory rat is the preferred test species for neurotoxicity studies using national and international guidelines. Therefore, information about spontaneous lesions and artifacts in the nervous system of standard laboratory rats is important for interpreting of the results of toxicology studies for two reasons. First, the occurrence of spontaneous lesions in rats which are similar to those seen in human disease processes demonstrates that the rat is a sensitive species for providing test results relevant to the risk assessment process. Secondly, recognition of spontaneous lesions and artifacts is necessary so that they can be differentiated from toxicant-induced lesions and their potential impact on the interpretability of test results can be assessed.

Because age-related lesions in the CNS and PNS are so common in laboratory rats, the use of rats for the assessment of neurotoxicity following chronic exposure to toxicants may be impaired or impossible for some sites within the CNS and PNS. At the very least, study

directors should be cognizant of the high frequency of spontaneous lesions when designing toxicity studies.

## RECOMMENDED NOMENCLATURE AND DIAGNOSTIC CRITERIA

### AXON

#### *Axonal Degeneration*

1. Axon and its myelin sheath appear as round to oval fragments arranged in short strings of beads or ovoids undergoing phagocytosis

#### *Axonal Swelling (Axonal Dystrophy)*

1. Eosinophilic fusiform or torpedo-shaped structures in longitudinal section
2. Eosinophilic spheroids in cross section may not be apparent unless they are frequent, large, or hyperchromatic
3. Myelin/axon diameter ratio is decreased
4. Frequently occurs in the gracile and cuneate nuclei and rostral fasciculi of rats older than 6 months (also called axonal dystrophy)

#### *Axonal Atrophy*

1. Average axonal diameter is decreased and interaxonal space is increased in peripheral nerves (best observed in cross section)
2. Myelin/axon ratio is increased
3. Differentiate from endoneurial edema, which has increased interaxonal space but normal average axon diameter

### NERVE CELL BODY

#### *Lipofuscin Accumulation*

1. Yellow-brown granules in the cytoplasm of neurons and glial cells (may also appear to be greenish)
2. Associated with normal aging
3. May also be chemically-induced

#### *Chromatolysis*

1. Pale cytoplasm due to loss (dispersion and/or partial disappearance) of Nissl substance
2. In central chromatolysis, neurons become swollen with pale central cytoplasm and nuclei move toward margin of the cell (may be secondary to axonal damage)
3. In peripheral chromatolysis, degenerating neurons may be smaller than normal and show dissolution of the peripheral Nissl substance (may be a recovery stage of central chromatolysis)

#### *Neuronal Necrosis*

1. Eosinophilic and shrunken nerve cell body (H&E)

staining)

2. May have associated glial cell reaction around necrotic cell bodies
3. Includes cell death arising from apoptosis

### ***Neuronophagia***

1. Degenerating or necrotic neuron surrounded by phagocytic microglial cells
2. Differentiate from satellitosis in which oligodendroglia are adjacent to and surround normal neurons

### ***Nerve Cell Loss***

1. Fewer neurons than expected for the same anatomical site in a normal animal
2. Gliosis may be only evidence for earlier neuronal death
3. May occur in sites other than primary targets as a result of trans-synaptic degeneration

## **Myelin Sheath**

### ***Myelin Folding***

1. Increased invagination of myelin into axoplasm
2. A normal process that is increased in peripheral neuropathies

### ***Intramyelinic Edema***

1. Clear spaces or bubbles within the myelin sheath
2. Heavily myelinated regions stain less intensely

### ***Demyelination (and Remyelination)***

1. Myelin ovoids are present in early stages, but are not prominent in later stages
2. Lipid-laden macrophages may be present to clear debris during repair
3. Reduced myelin staining may result from the presence of many demyelinated or hypomyelinated fibers in the chronic phase of primary demyelination
4. Primary demyelination may be differentiated from demyelination secondary to axonal degeneration by the presence of intact denuded axons (both have myelin ovoids)
5. Remyelination or attempts at remyelination may predominate in later stages of demyelination, resulting in variable thickness of myelin segments along the same axon

## **GLIA**

### ***Gliosis***

1. Increased size (hypertrophy) or number (hyperplasia) of glial cells and/or increased eosinophilic background stain due to increased glial

cell processes

2. Usually refers to hypertrophic astrocytes with gemistocytic (enlarged cell bodies with prominent eosinophilic cytoplasm) or pilocytic (swollen cell processes that form a dense, hyalinized network) features
3. Increased microglia may also be a component
4. May need to confirm with special histochemical or immunohistochemical stains for astrocytes and microglia

## **MULTIPLE COMPONENTS**

### ***Epidermoid Cyst***

1. Cyst lined by stratified squamous epithelium and containing keratinaceous debris in the lumen

### ***Hemorrhage***

1. Antemortem escape of blood from the vasculature
2. Pale erythrocytes with blurred cellular outlines, and blood pigments in phagocytic cells in older lesion
3. Not to be confused with artifactual release of blood occurring at necropsy

### ***Hydrocephalus***

1. Expansion of the ventricles with cerebrospinal fluid
2. Can be incidental in young animals, but most often acquired secondary to pituitary tumors

### ***Mineralization***

1. May be primary with no cellular reaction
2. May be secondary to tissue destruction and often associated with a glial reaction
3. Distinguish from bone chips pushed into the brain during necropsy

### ***Infarction***

1. Cell death resulting from obstruction of blood supply; the location of the vascular occlusion is often not identified
2. Early lesion appears as sharply demarcated areas with pyknotic nuclei and decreased eosinophilic staining due to cytoplasmic vacuolation
3. After several days macrophages invade and replace the normal parenchyma
4. Old infarcts often contain many cystic spaces and are surrounded by glial and vascular reactions

### ***Necrosis***

1. Fragmentation and death of various cellular tissue components
2. Focal accumulation of inflammatory cells may be present

### **Vacuolation**

1. Clear spaces of various size, usually in heavily myelinated regions of the CNS
2. May also be intracytoplasmic in neurons
3. Important to determine if chemically induced or an artifact by careful comparison with controls to include location, group treatment, and tissue processing history

### **Compression of CNS parenchyma**

1. Indentation and often atrophy of neural tissue adjacent to a space occupying lesion such as a pituitary tumor
2. May also occur secondary to hydrocephalus

### **Compression of PNS**

1. Chronic compression of axons can result in Wallerian degeneration of axons distal to the site of compression
2. Degenerative lesions may also be observed in axons proximal to the site of compression

### **Inflammation**

1. Increased number of inflammatory cells in nervous tissue

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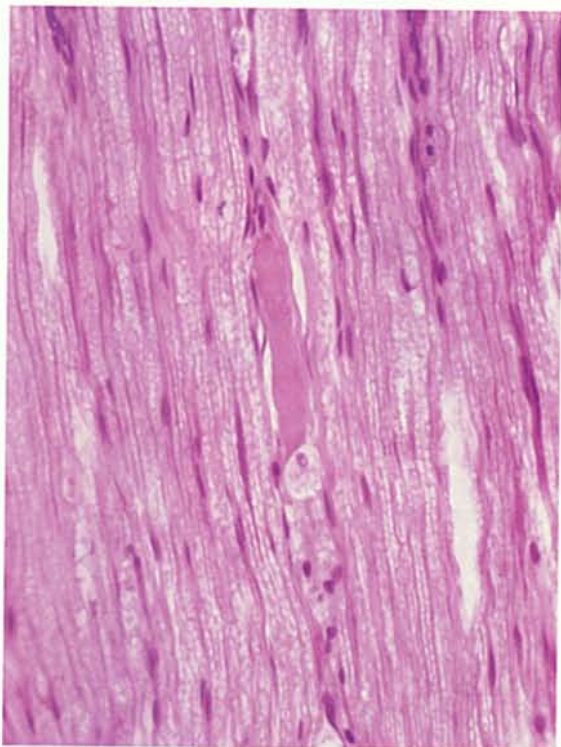
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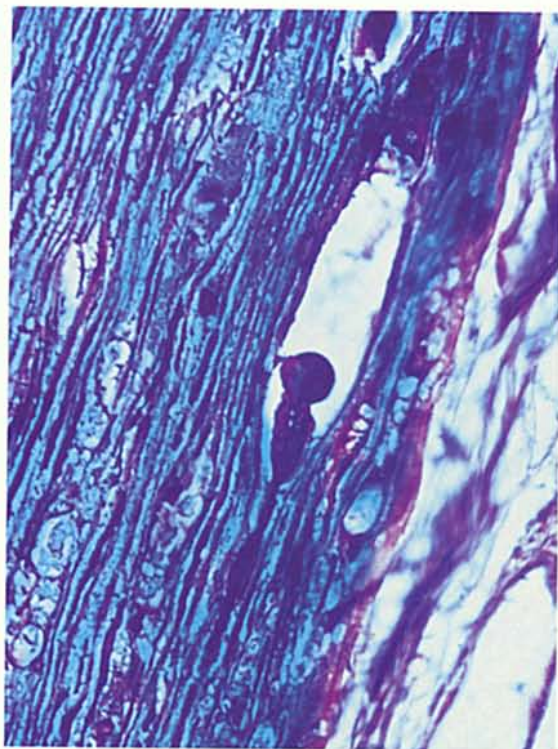
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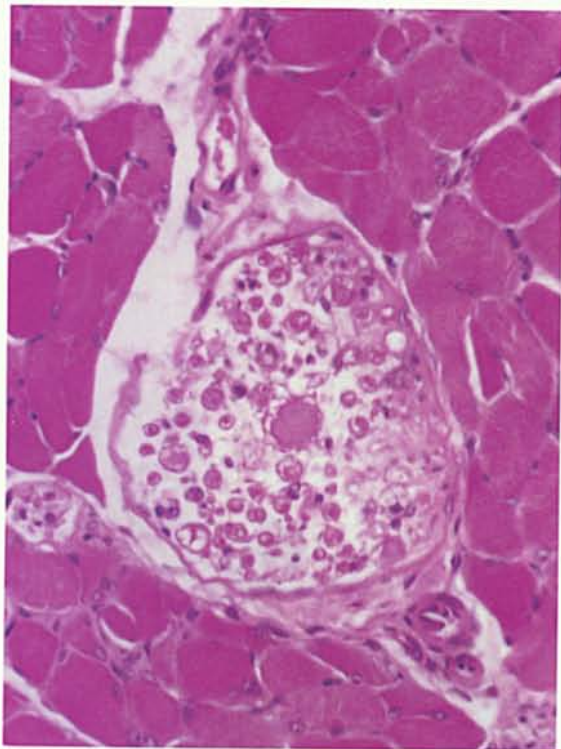
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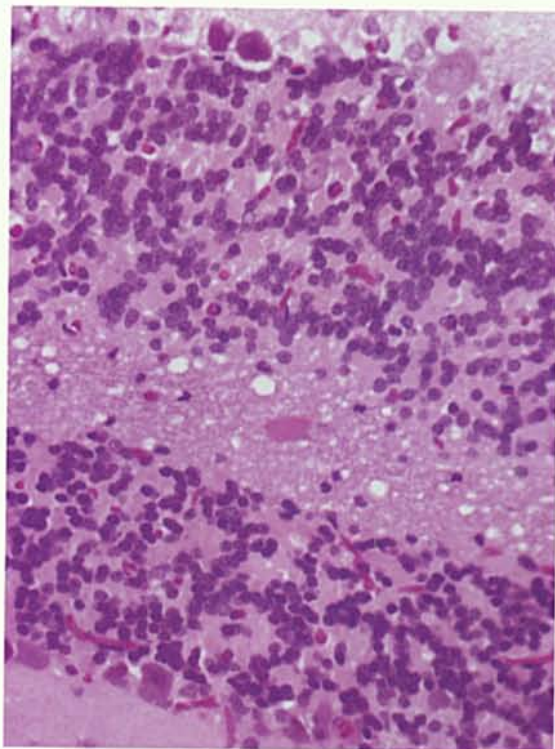
*Fig. 1 - Axonal degeneration, tibial nerve (H&E).*



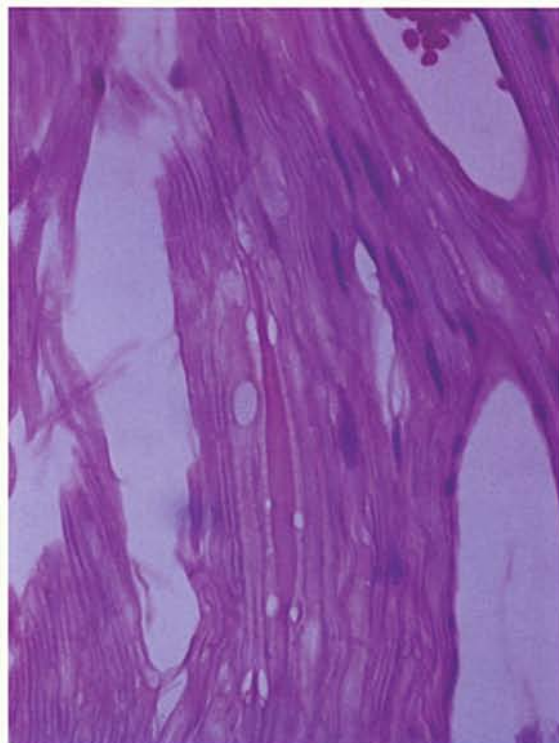
*Fig. 2 - Axonal degeneration, spinal cord (Luxol fast blue - Bodian silver).*



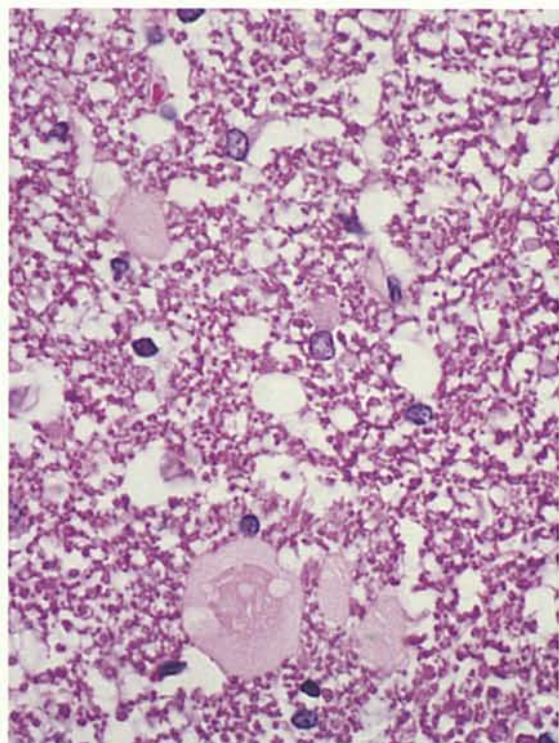
*Fig. 3 - Axonal swelling (giant axon), intramuscular nerve (H&E).*



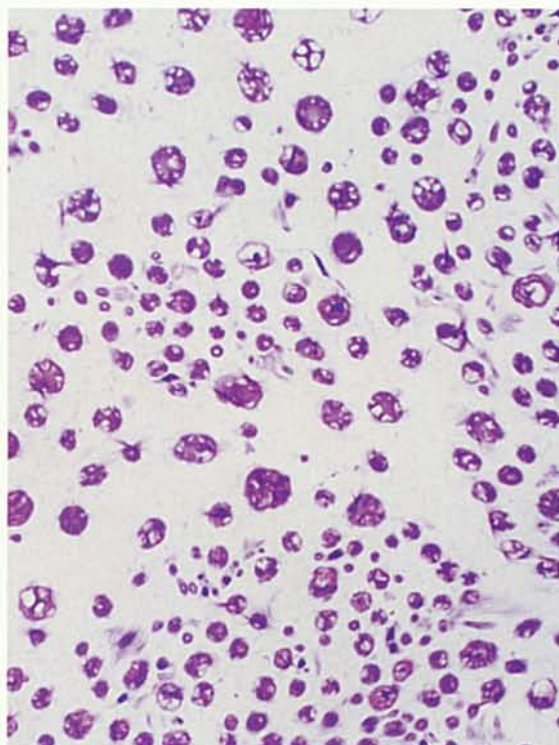
*Fig. 4 - Axonal swelling, cerebellum (H&E).*



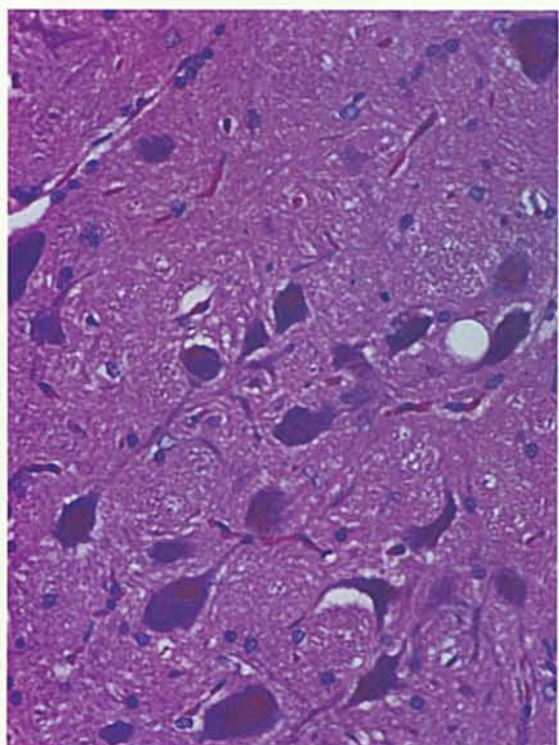
*Fig. 5 - Axonal swelling, sciatic nerve (H&E).*



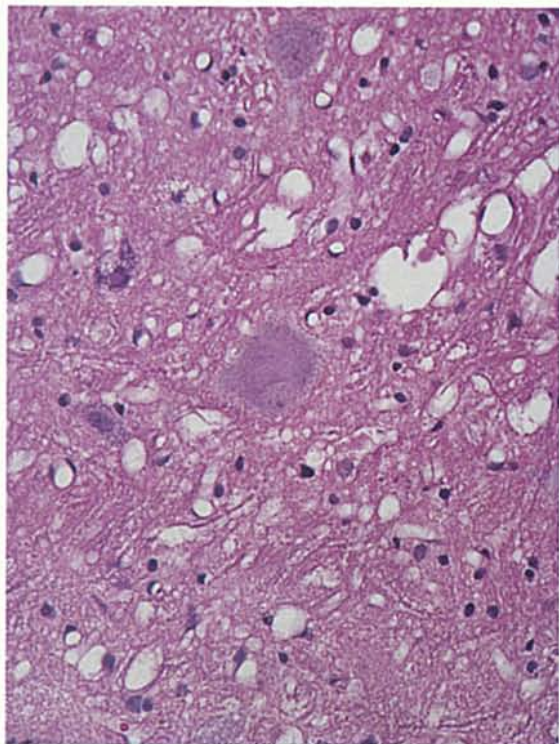
*Fig. 6 - Axonal dystrophy, medulla oblongata (H&E).*



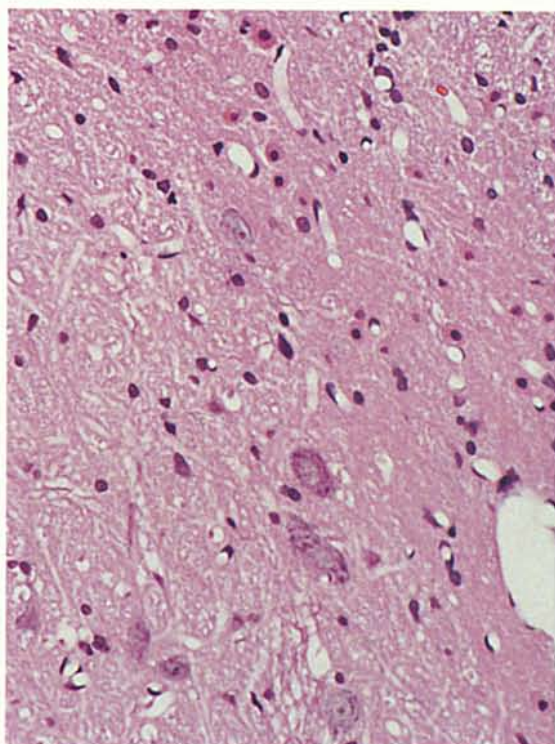
*Fig. 7 - Axonal atrophy, sciatic nerve (H&E).*



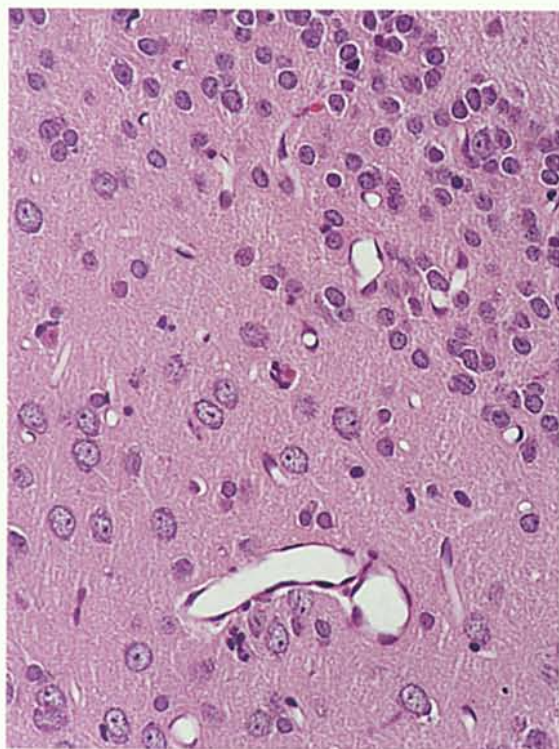
*Fig. 8 - Lipofuscin accumulation, medulla oblongata.*



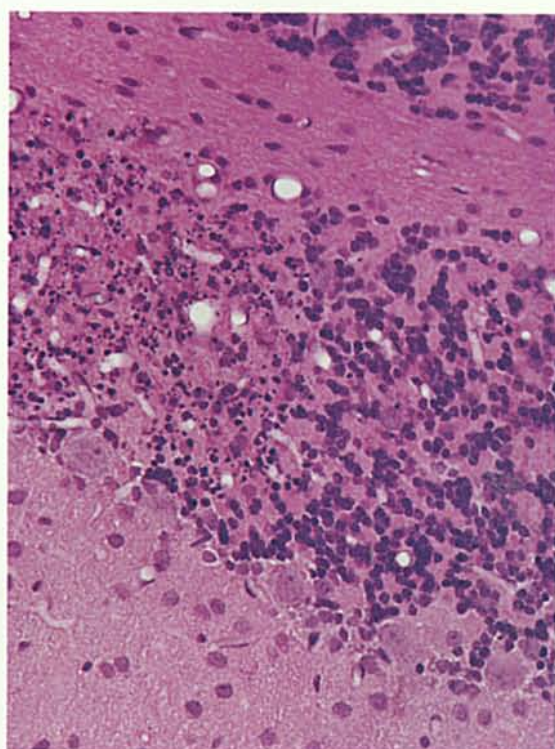
*Fig. 9 - Chromatolysis, pons (H&E).*



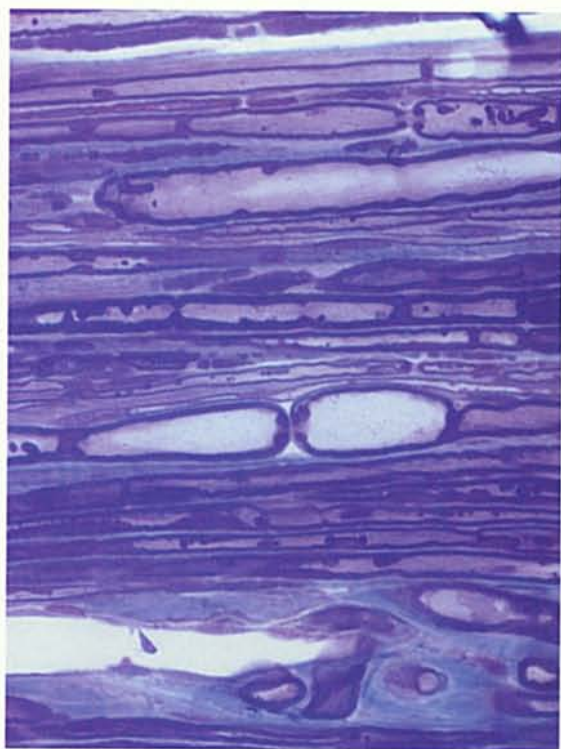
*Fig. 10 - Neuronal necrosis (H&E).*



*Fig. 11 - Neuronophagia (H&E).*



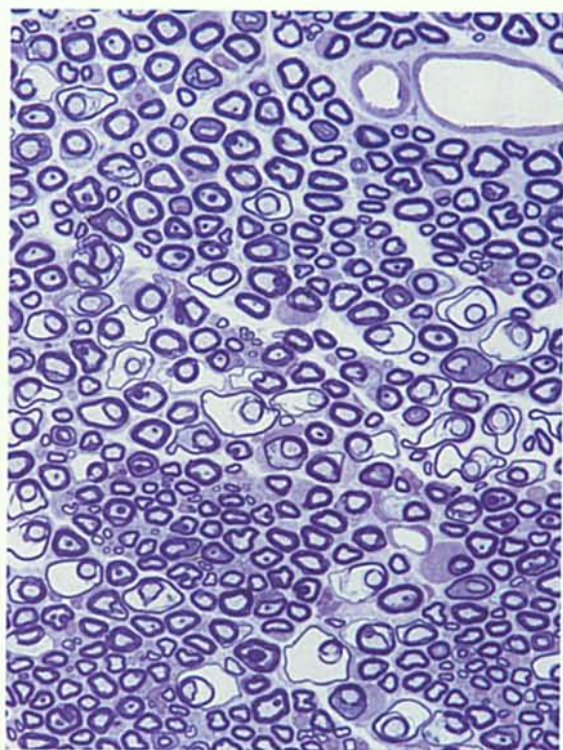
*Fig. 12 - Nerve cell loss and degeneration with gliosis, cerebellum (H&E).*



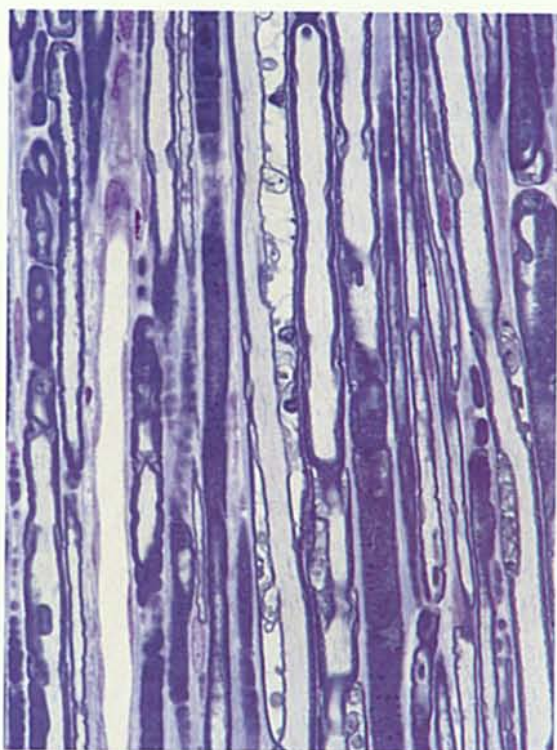
*Fig. 13 - Myelin folding and axonal swelling, tibial nerve (toluidine blue, plastic).*



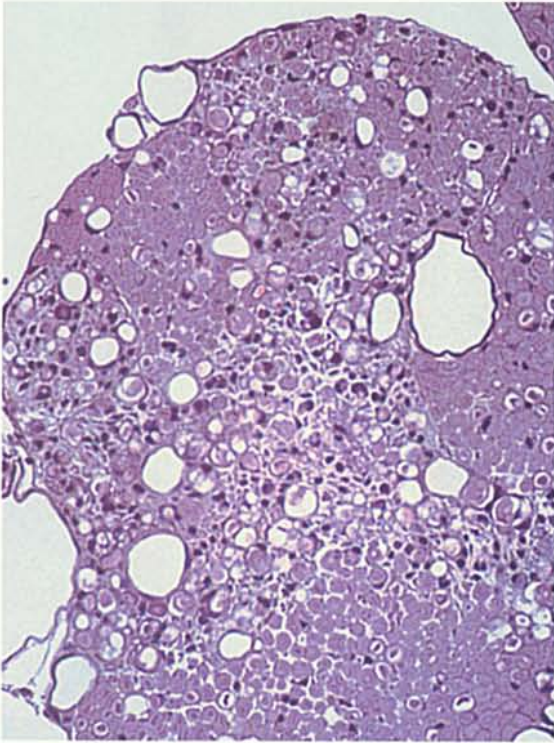
*Fig. 14 - Myelin folding and axonal swelling, tibial nerve (lead citrate-uranyl acetate, electron microscopy).*



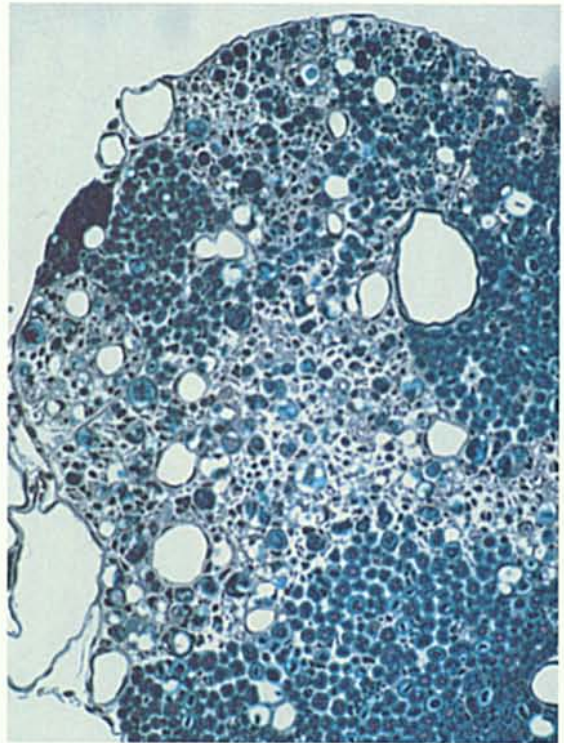
*Fig. 15 - Intramyelinic edema (toluidine blue, plastic).*



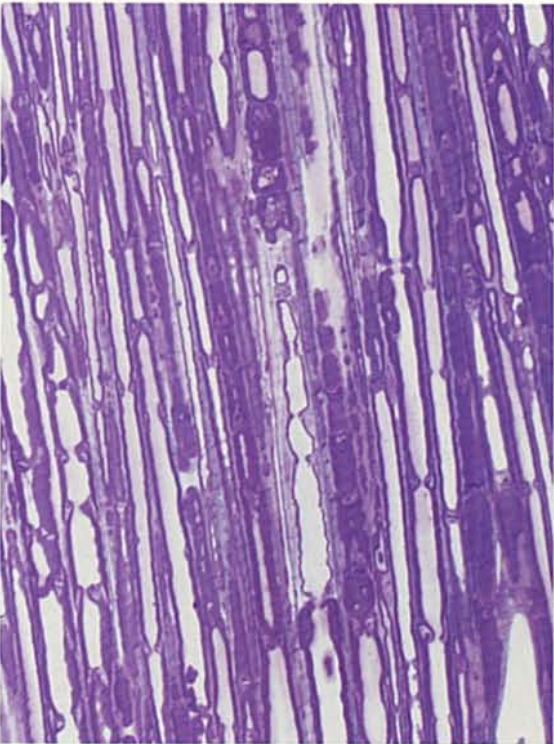
*Fig. 16 - Intramyelinic edema (toluidine blue, plastic).*



*Fig. 17 - Demyelination and axonal degeneration, spinal nerve root (H&E).*



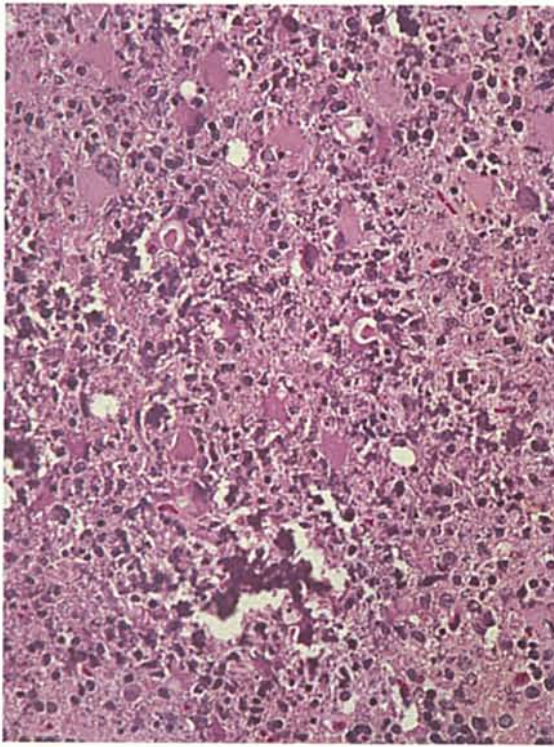
*Fig. 18 - Demyelination and axonal degeneration, spinal nerve root (Luxol fast blue - Holmes silver).*



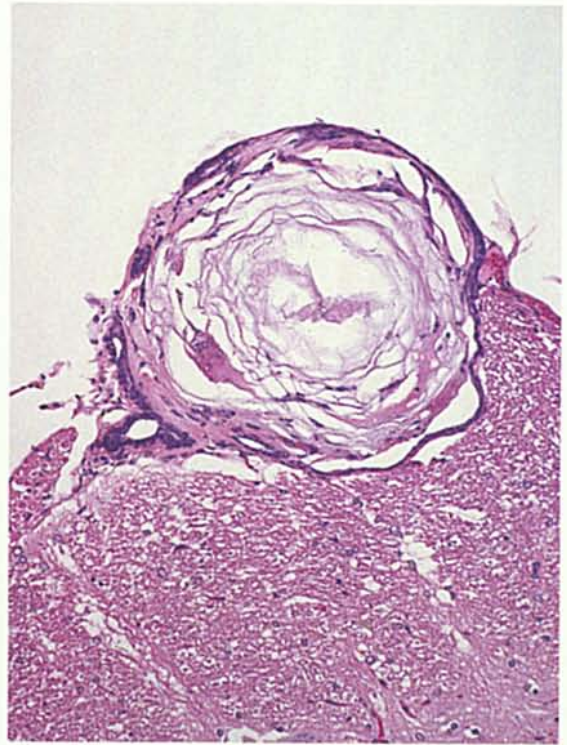
*Fig. 19 - Segmental demyelination and remyelination, tibial nerve (toluidine blue, plastic).*



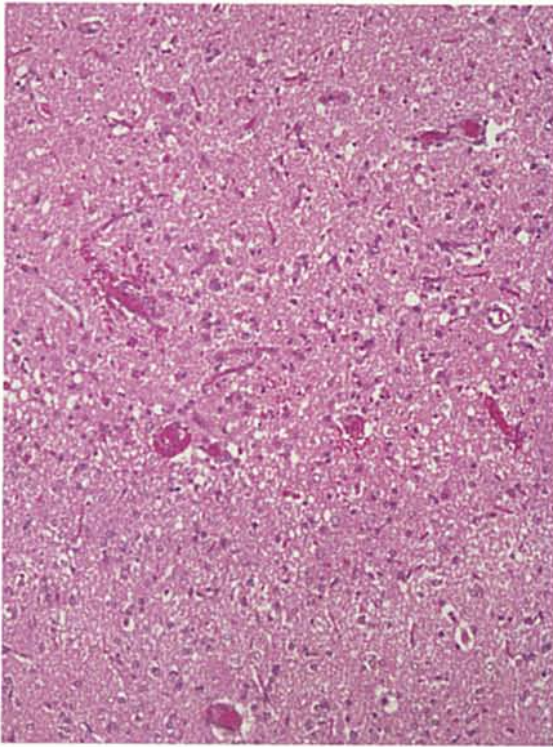
*Fig. 20 - Segmental demyelination, tibial nerve (teased nerve fiber preparation).*



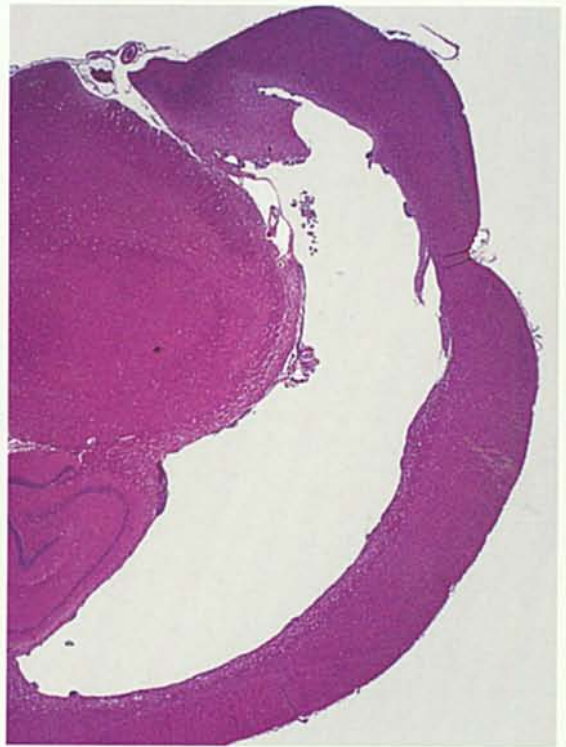
*Fig. 21 - Gliosis and mineralization, cerebellum (H&E).*



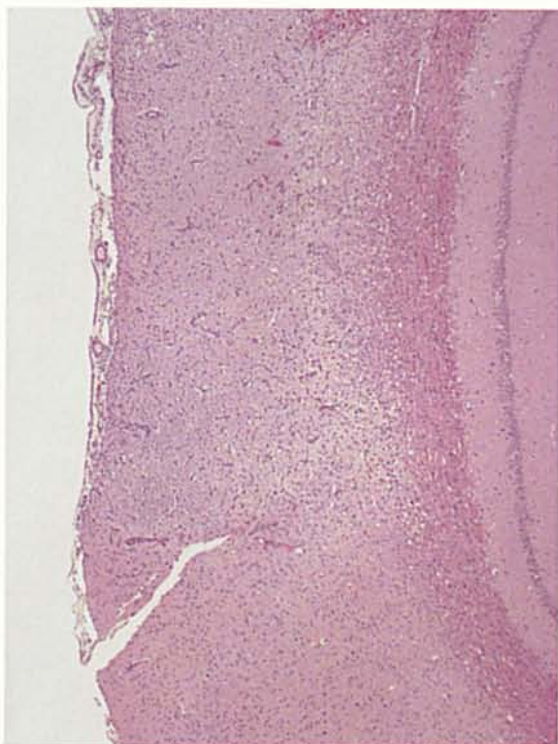
*Fig. 22 - Epidermoid cyst, spinal cord (H&E).*



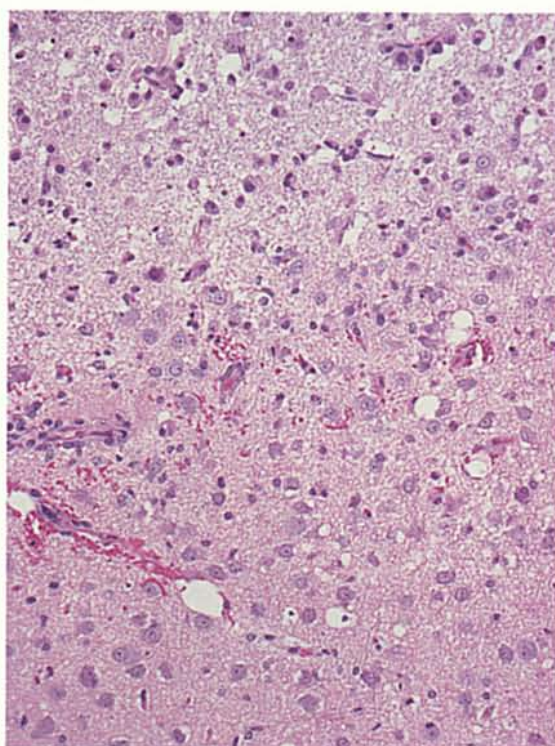
*Fig. 23 - Hemorrhage, pons (H&E).*



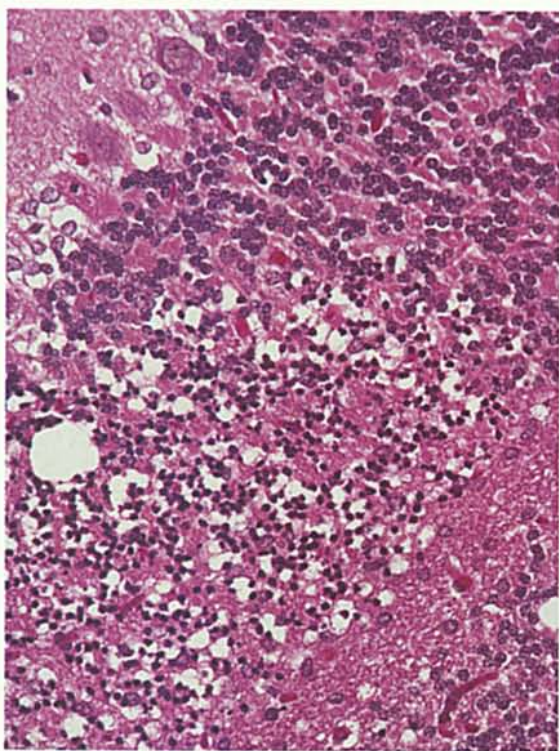
*Fig. 24 - Hydrocephalus (H&E).*



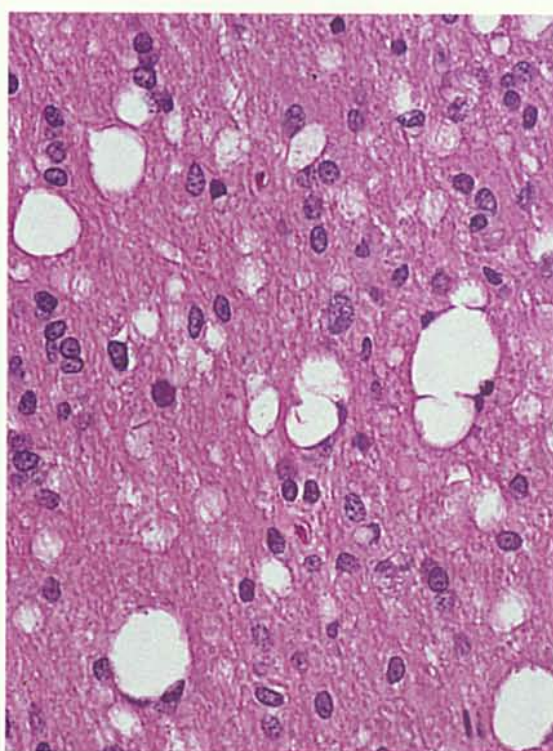
*Fig. 25 - Cerebral infarct (H&E).*



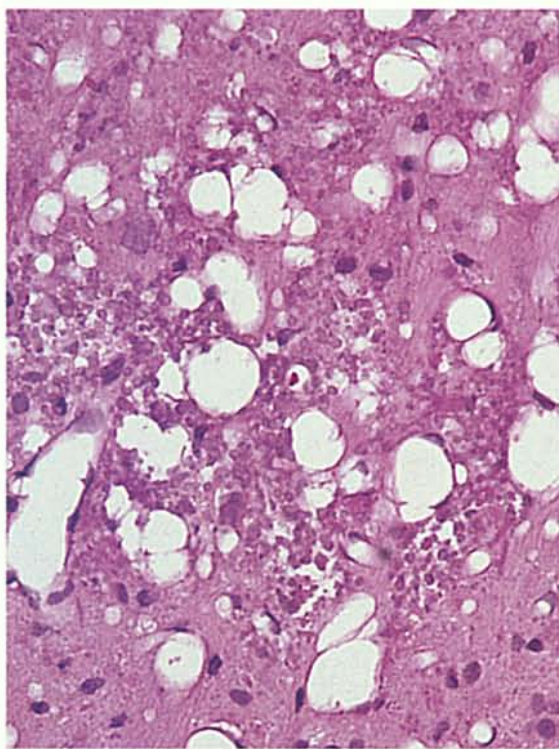
*Fig. 26 - Cerebral infarct (H&E).*



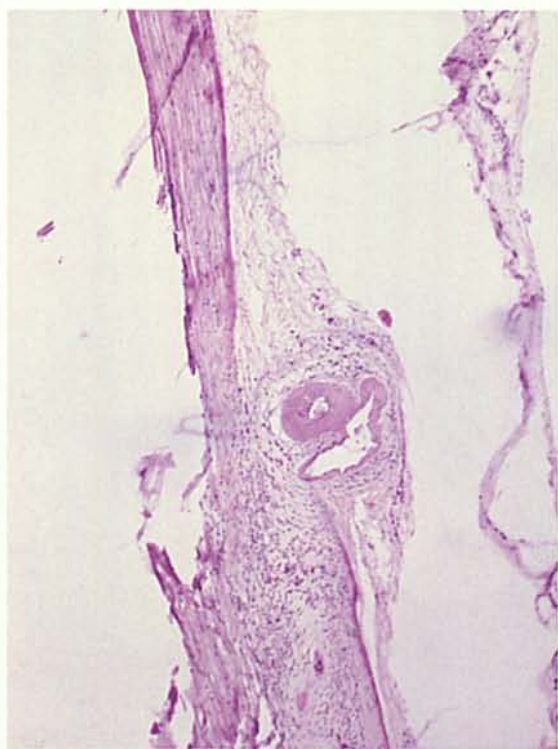
*Fig. 27 - Necrosis, cerebellum (H&E).*



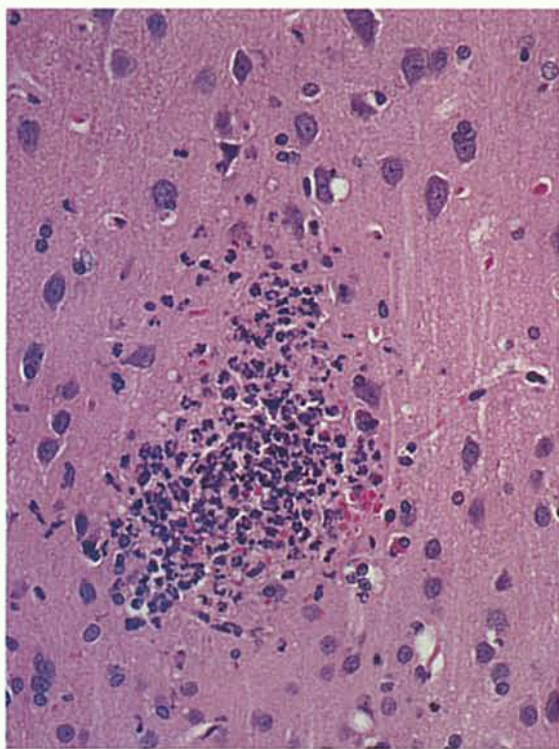
*Fig. 28 - Extracellular vacuoles and mineralization associated with necrotic focus (H&E).*



*Fig. 29 - Vacuolation, artifact (H&E).*



*Fig. 30 - Arteritis, nerve compression and degeneration, plantar nerve (H&E).*



*Fig. 31 - Acute inflammation, cerebral cortex (H&E).*