

Overview of spontaneous and drug induced non-neoplastic lesions of the nervous system.

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Definitions

- **Neurotoxicity is defined as a detrimental effect in the NS caused by biological, chemical or physical agents**
- **Series of events including:**

Entry of agent into organism and contact with site of action

Interaction with specific targets

Initiation of biochemical changes followed by functional and morphological changes

The Issue...

- **Broad range of cellular target sites and mode of action**
- **Different compounds – CNS or/and PNS affection**
- **Development of biomarkers for neurotoxicological injury**
- **e.g.**
 - Caspase**
 - Acetylcholinesterase**
 - Neurofilaments**
 - Tau-Protein**

PNS Injuries - Basis

- **One Schwann cell can form only one myelin sheath segment (nucleus is located in the mid-internodal area)**
- **One oligodendroglia cell can form several myelin segments (nuclei arranged in short rows within white matter)**
- **Oligodendroglia also have supporting cells (astrocytes, microglia).**
- **Largest Schwann cell segments are found in the lumbosacral spinal nerve roots. Long segments with thick myelin sheaths - very susceptible (size!)**

PNS Injuries

- **Easier to access compared to CNS**
- **Different primary sites of expression**
- **However, some axonopathies and neuronopathies affect both CNS and PNS**

PNS Injuries: Distribution

- **Morphological**
 - **Axonopathy (proximal, distal)**
 - **Neuronopathy**
 - **Myelinopathy**
- **Functional**
 - **Sensory**
 - **Motor**
 - **Combined**

PNS Injuries – Specific Conditions/Specific Pattern

Primary sensory, distal, central/peripheral axonopathy:

- **Earliest and most prominent lesion in distal peripheral nerves and endings (e.g. sensory endings in muscle spindles, KRINKE et al., 1978) and distal sensory spinal tracts, fasciculus gracilis and cuneatus**
- **Examine: long and large, susceptible axons contributing to the sciatic nerve:**
 - **spinal cord, dorsal root ganglia and spinal nerve roots, especially at the L4 level (in the rat L4 is the major contributor to the sciatic nerve)**

PNS Injuries – Specific Conditions/Specific Pattern

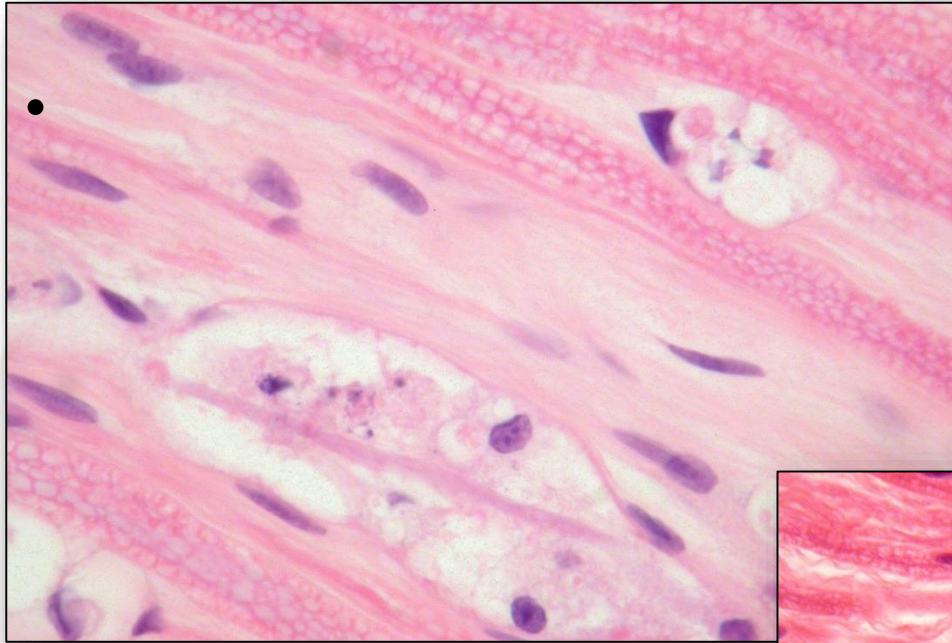
- **Peripheral nerves including the sciatic, tibial and plantar nerve, sensory nerve endings in lumbrical muscle spindles.**
- **Spinocerebellar fibers: mid-longitudinal section of the anterior cerebellar lobules**

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PNS Myelinopathy Characterization

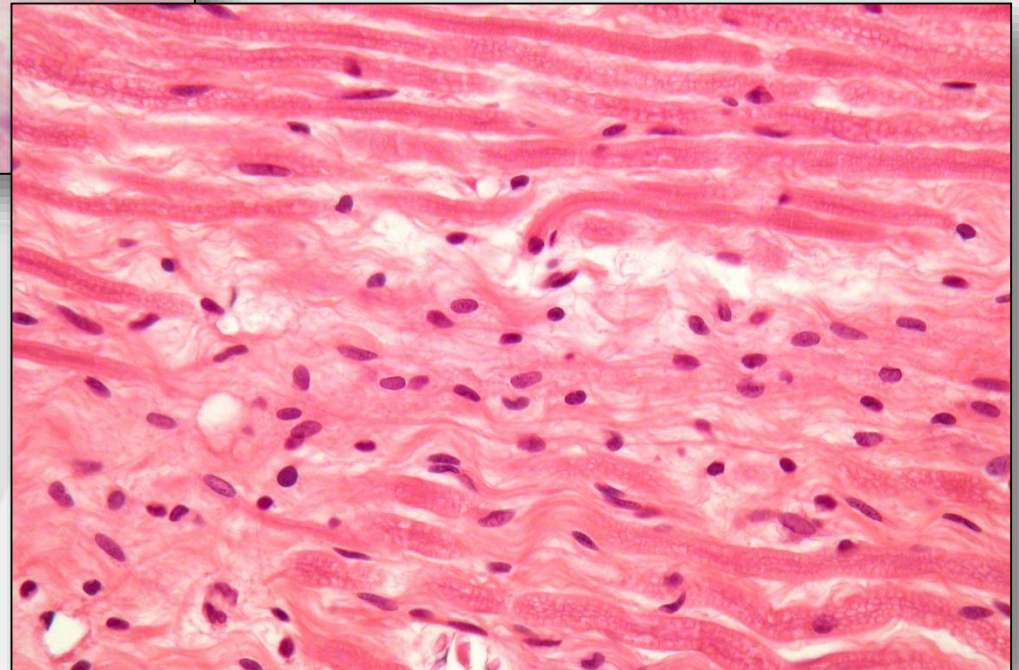
- **formation of ovoidal fragments beginning in the mid-inter-nodal area adjacent to Schwann cell nucleus**
- **ovoids delineated by Schmidt-Lanterman incisures (those become more prominent, but number does not increase (reflects active role of Schwann cells (Krinke et al., 1986)))**
- **fragmentation triggered by signals from axons (local selective damage to myelin sheaths can occur independently of axon damage and also not causing axonal degeneration)**
- **axons can be de-/remyelinated, but the myelin sheaths surrounding damaged axons are unable to persist alone.**

H&E: Spontaneous Sciatic Fiber Degeneration



Myelinopathy

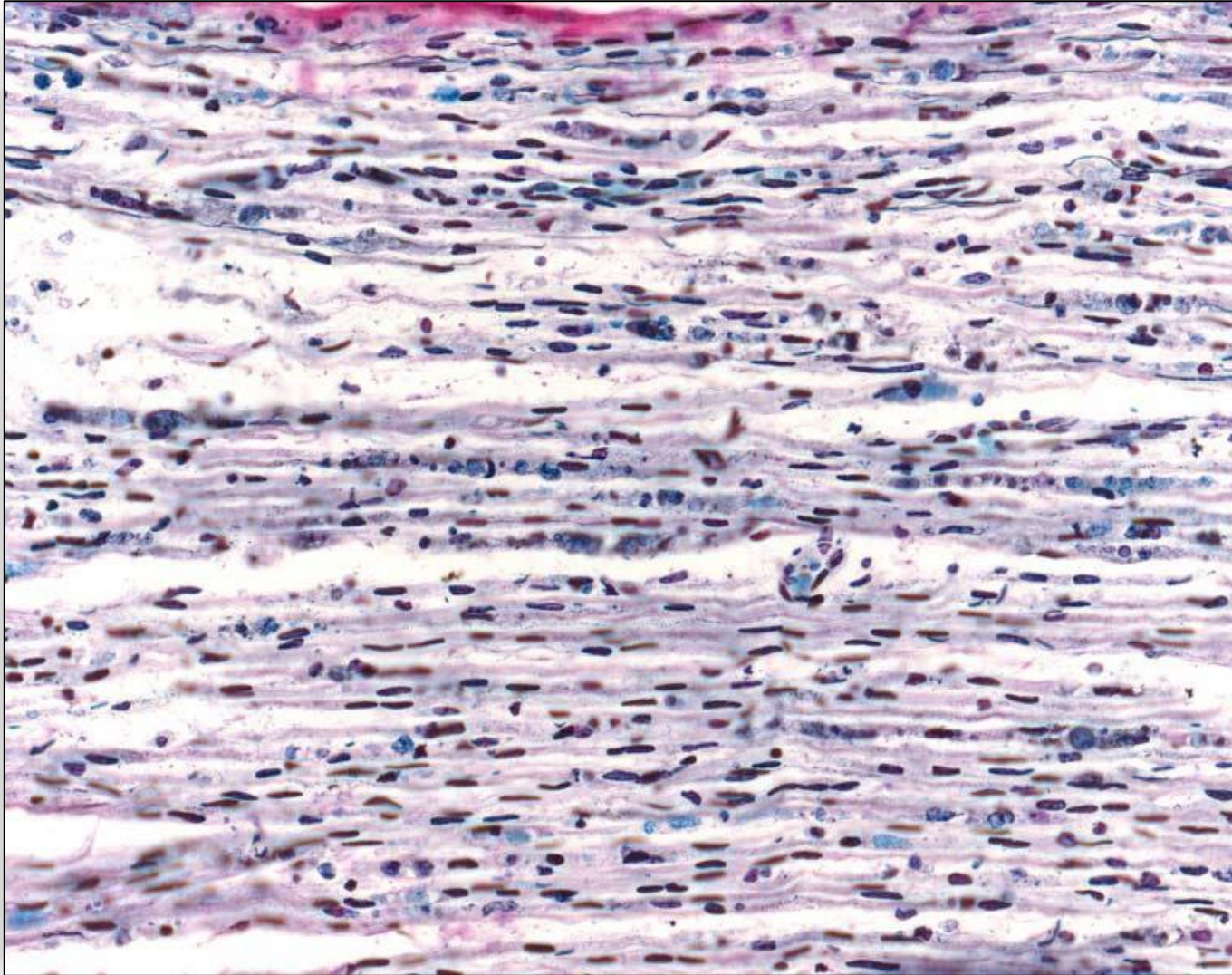
Axonopathy



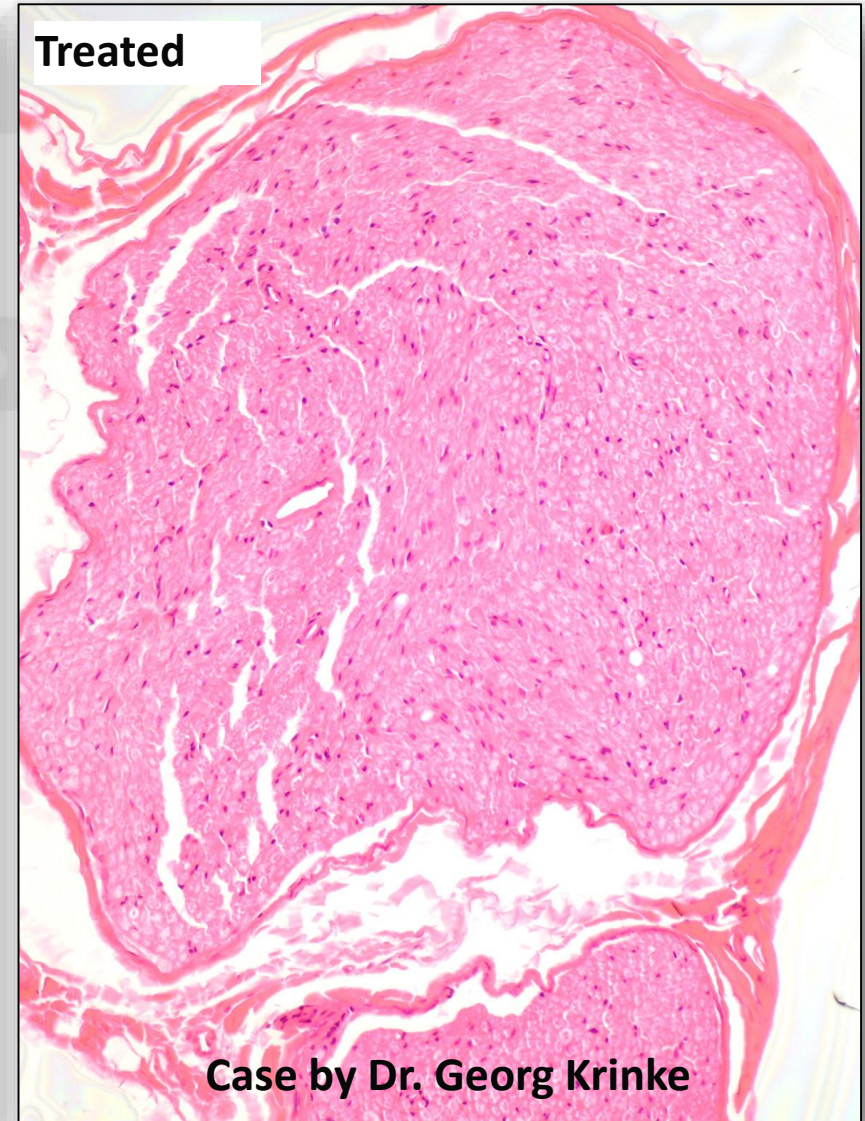
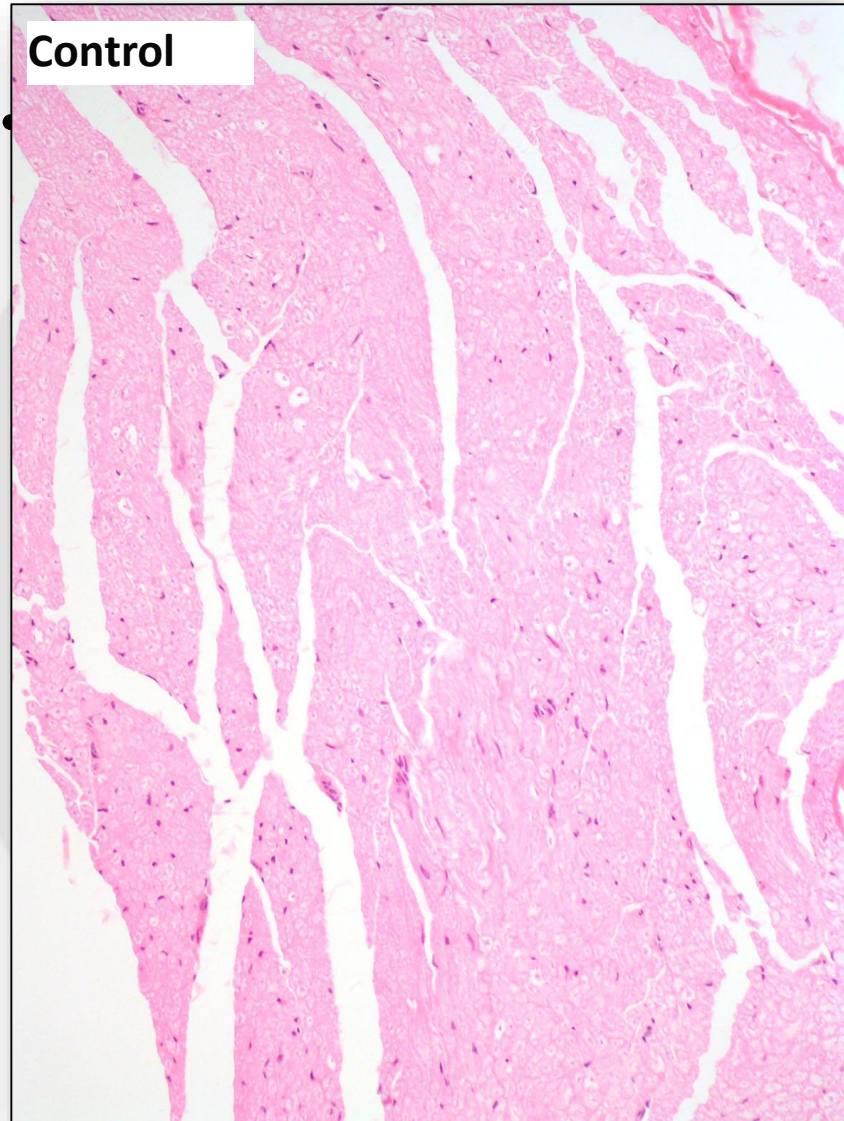
Wallerian Degeneration vs Secondary Degeneration

- **Local damage to nerve fibers:**
fibers distally to injury site with manifestation of axonal fragments and myelin ovoids
- **‘Wallerian degeneration’** when resulting from the mechanical trauma (e.g. transection)
- **‘Secondary degeneration’** by other cause has similar/identical morphological features
- **Difference:**
Wallerian degeneration by total disruption of the nerve fibers
secondary degeneration may be by selective effect on particular organelles/transport mechanisms

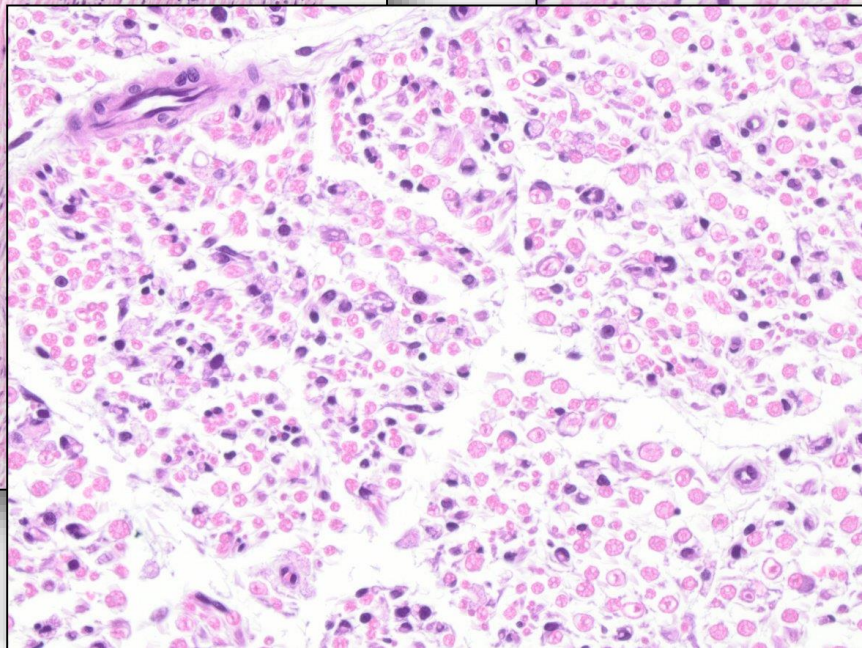
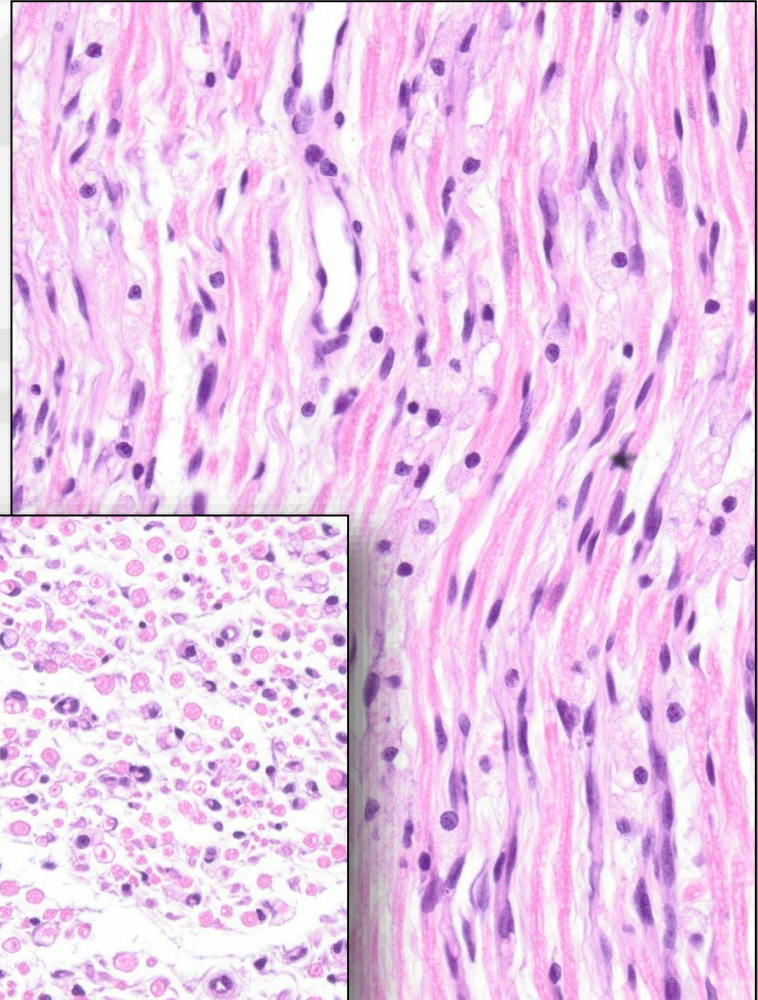
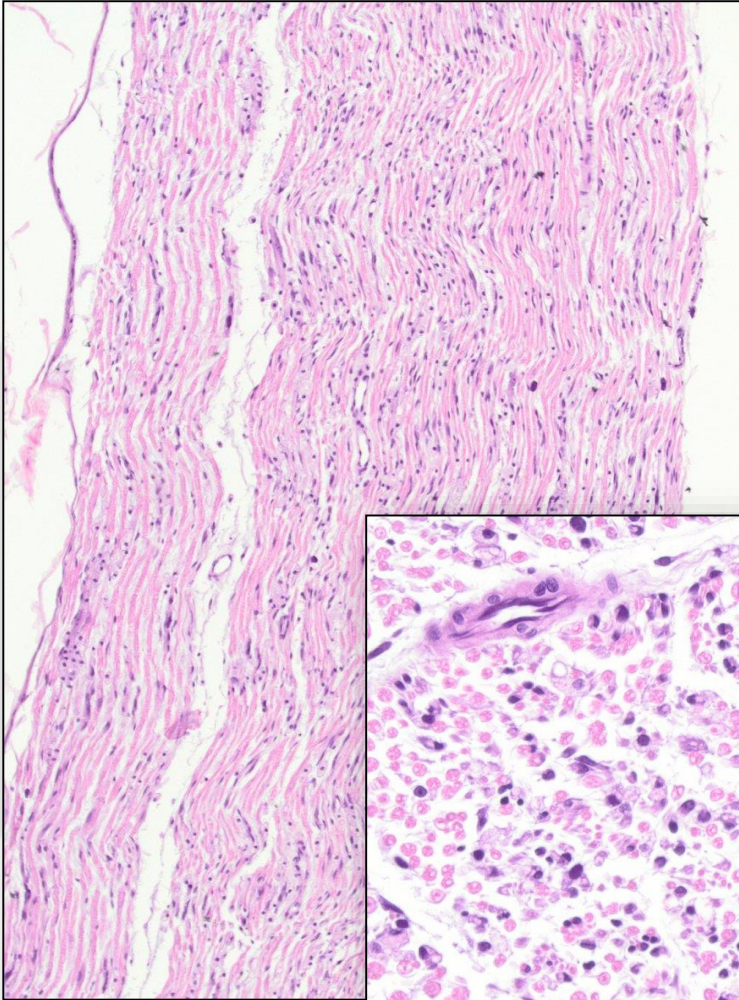
Bodian: Induced Wallerian Degeneration (Bull, Neurectomy)



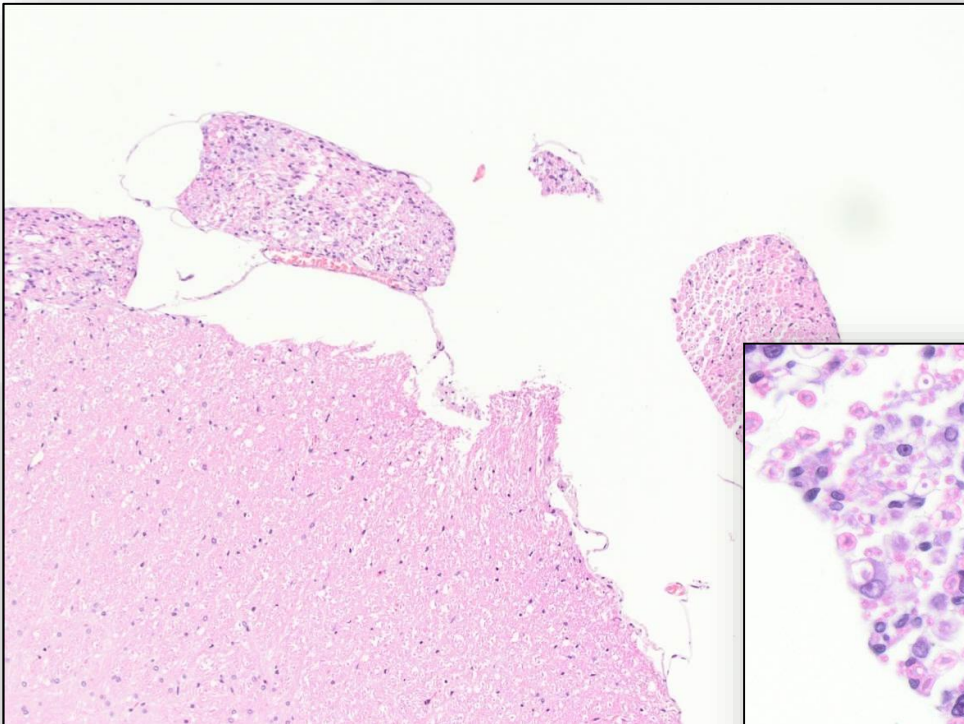
H&E: Increased PNS Cellularity (Demyelination), Rat, Irgacur (photoinitiator)



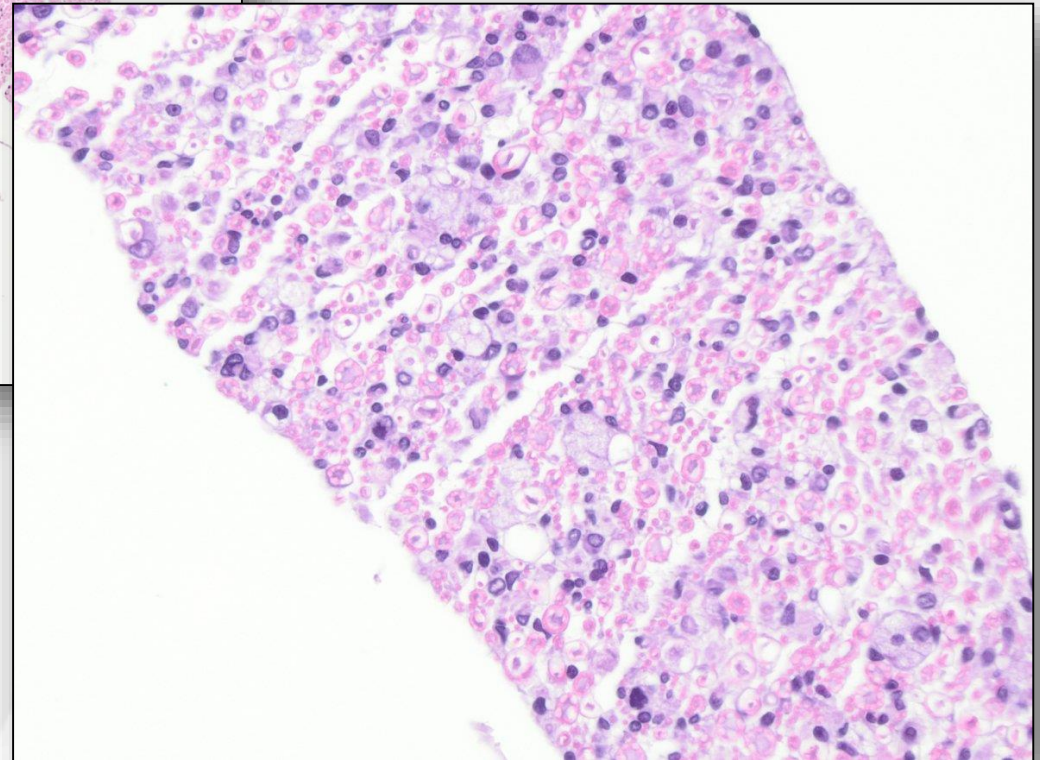
PNS Combination: axonopathy/myelinopathy



PNS Combination: axonopathy/myelinopathy

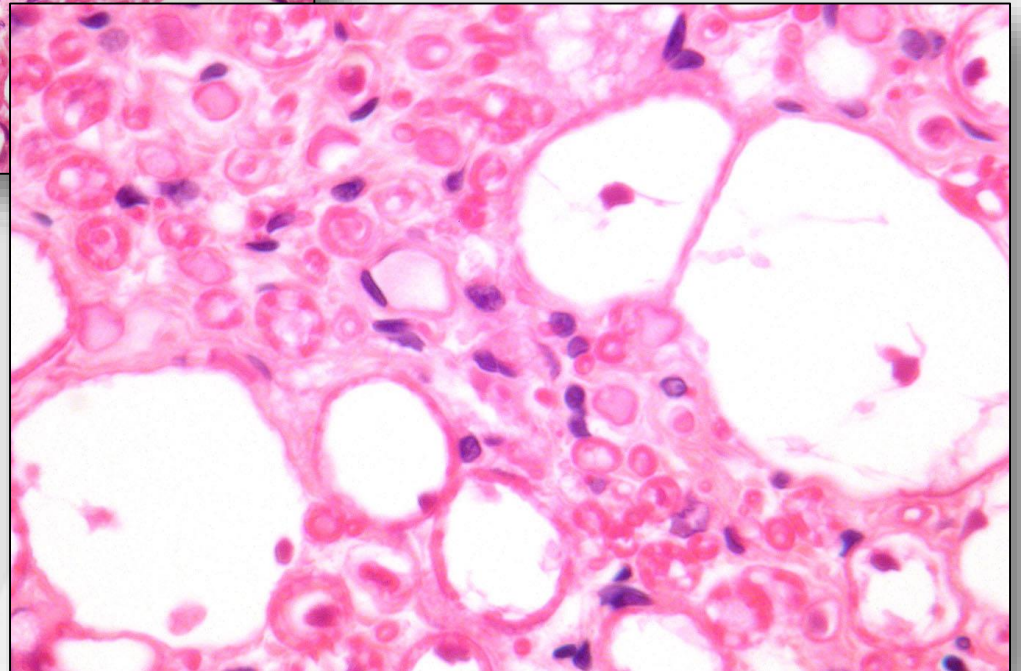
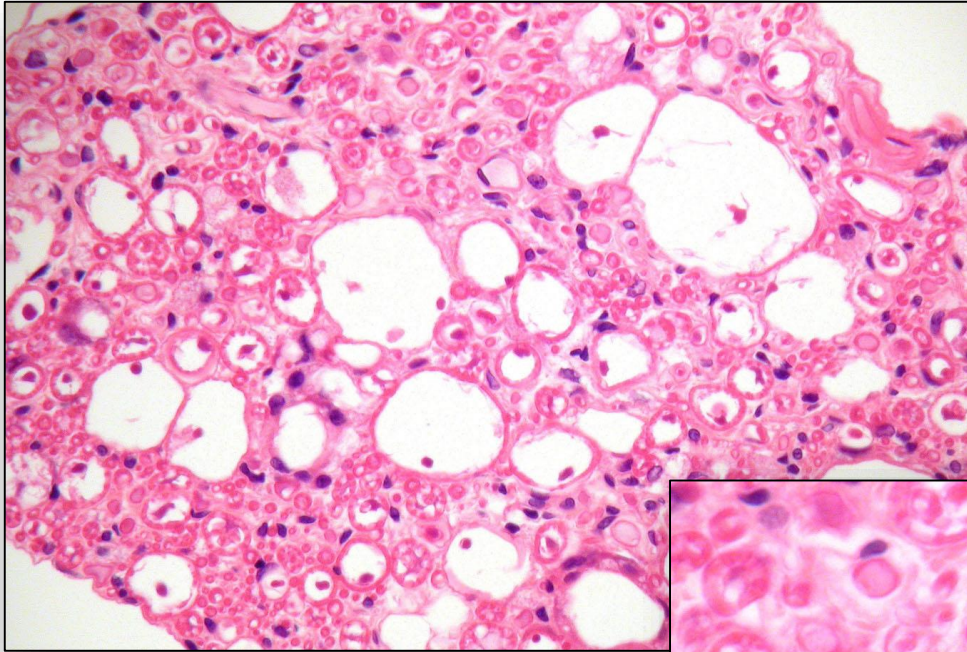


Dorsal root affected



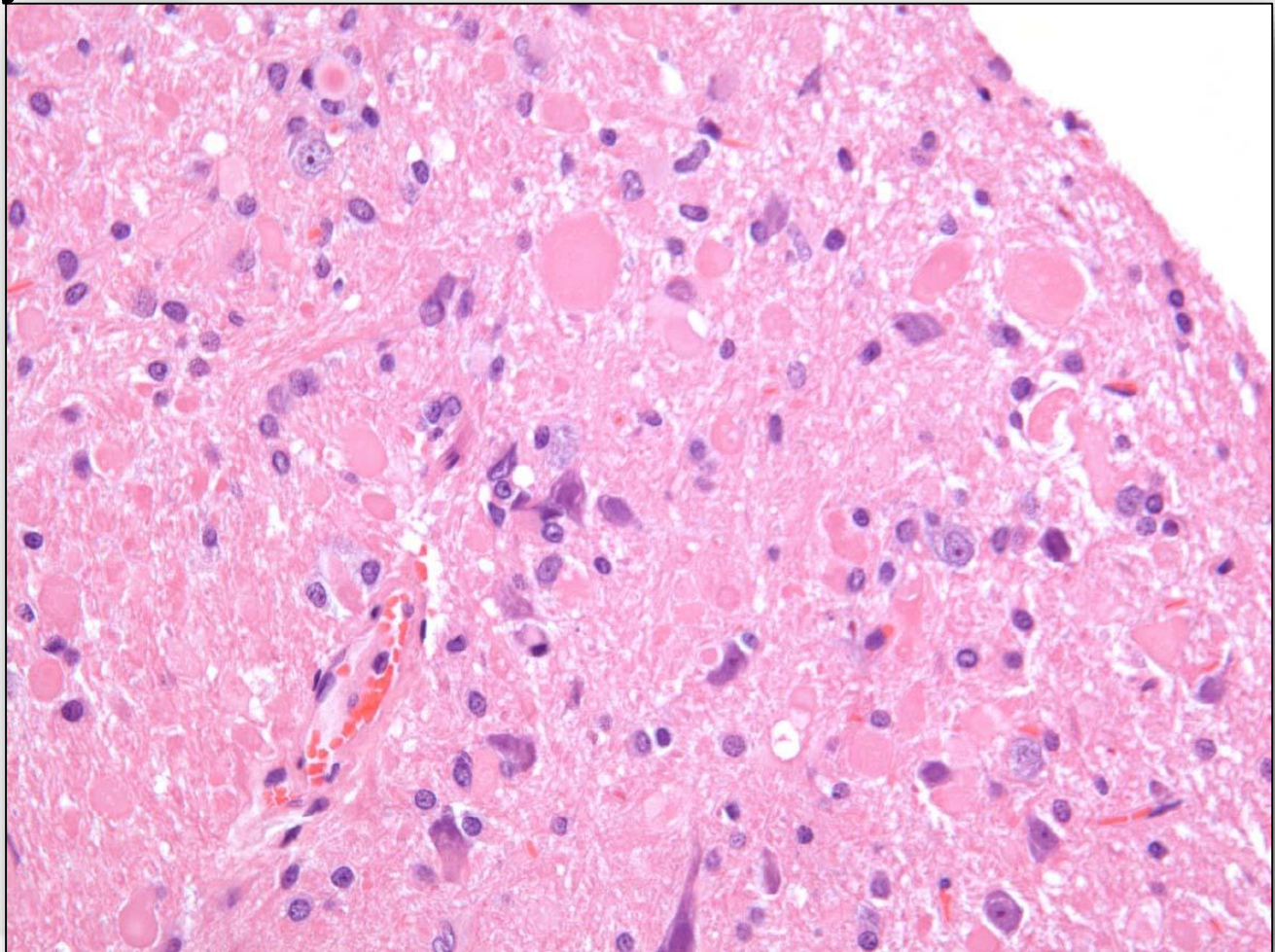
**No finding in spinal cord,
No finding in skeletal.
Muscle
Why?**

H&E: Spontaneous Radiculoneuropathy



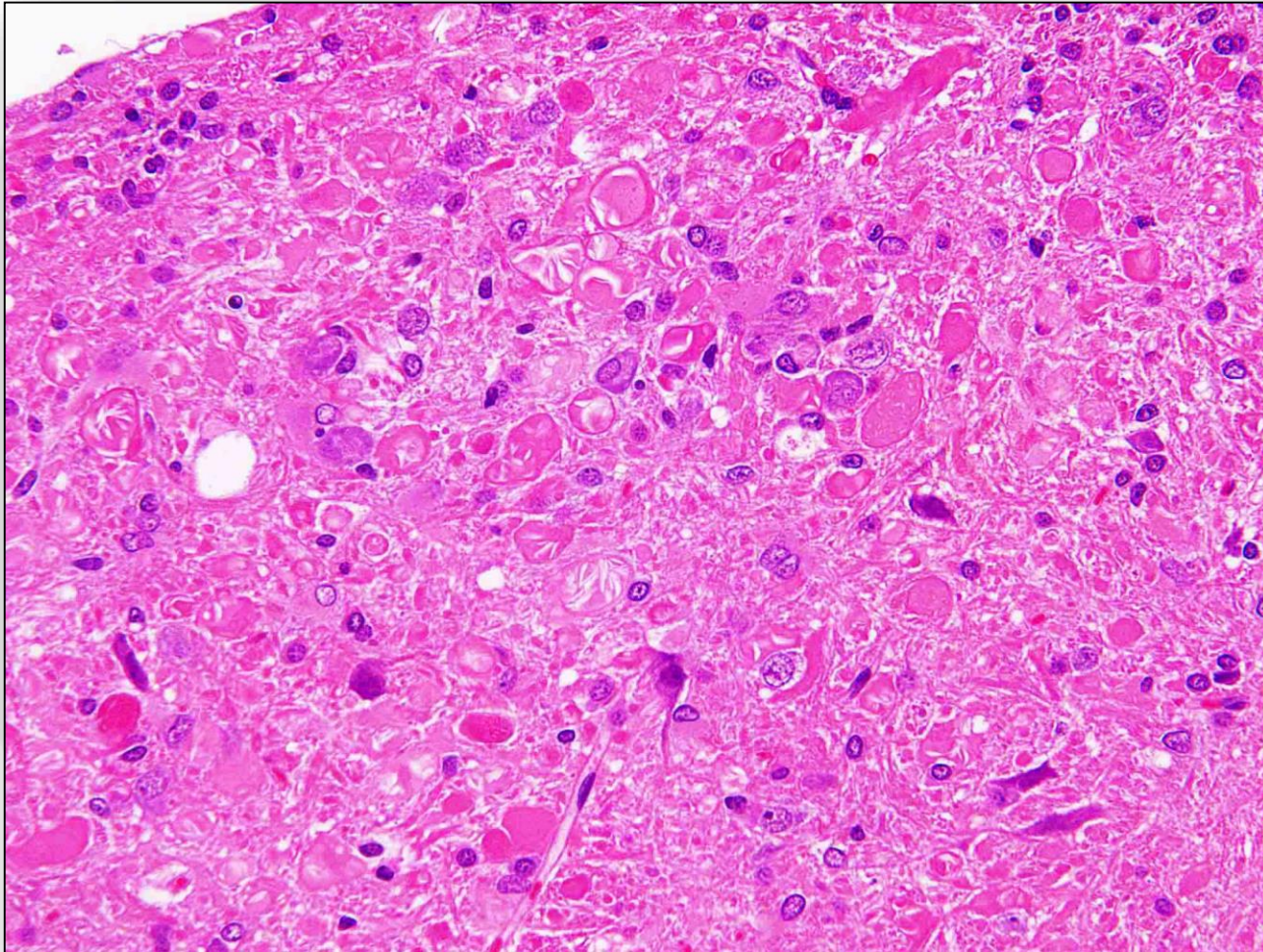
PNS Injuries – Fasciculus cuneatus/gracilis

- Spontaneous, rat
- 13-week studies onwards
- Axonopathy



Medulla, Fasciuli cuneati et gracili, 13-Week, High Dose

- **Induced axonopathy, rat, 13-Week
(distal, central/peripheral axonopathy)**



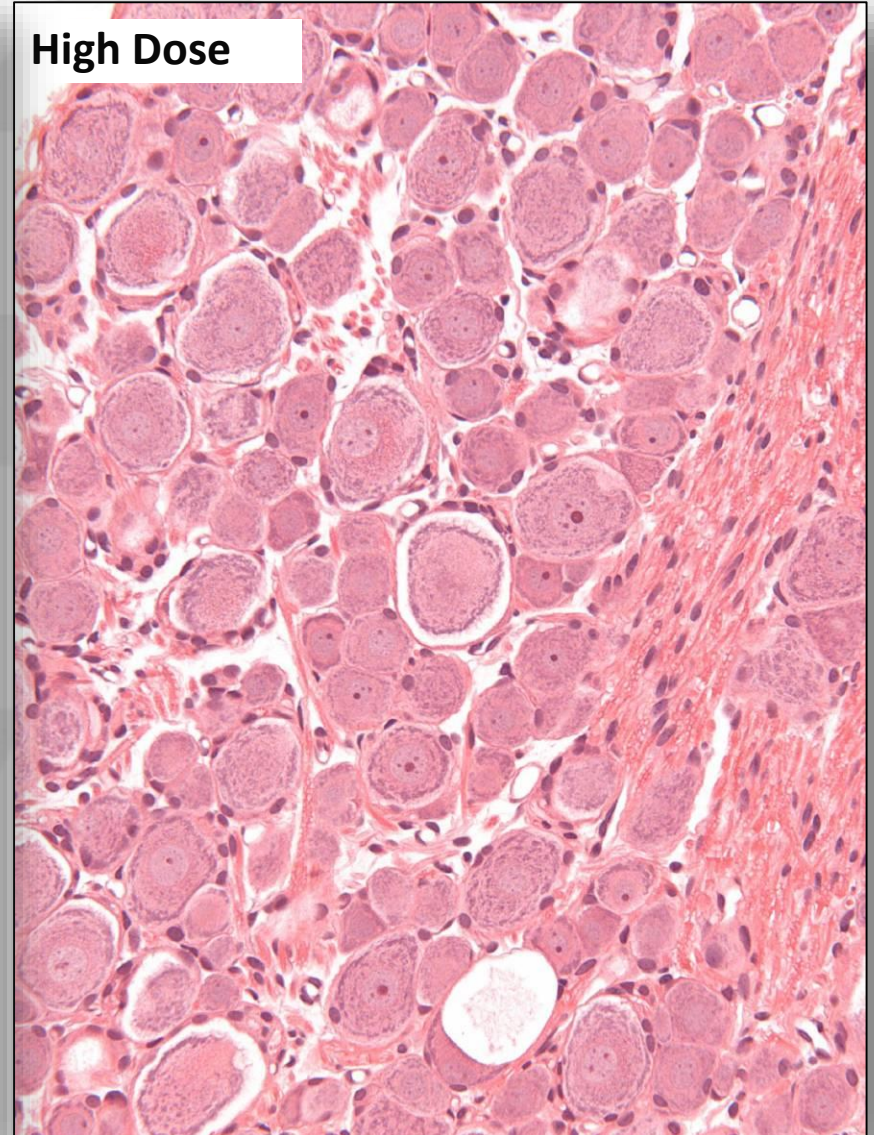
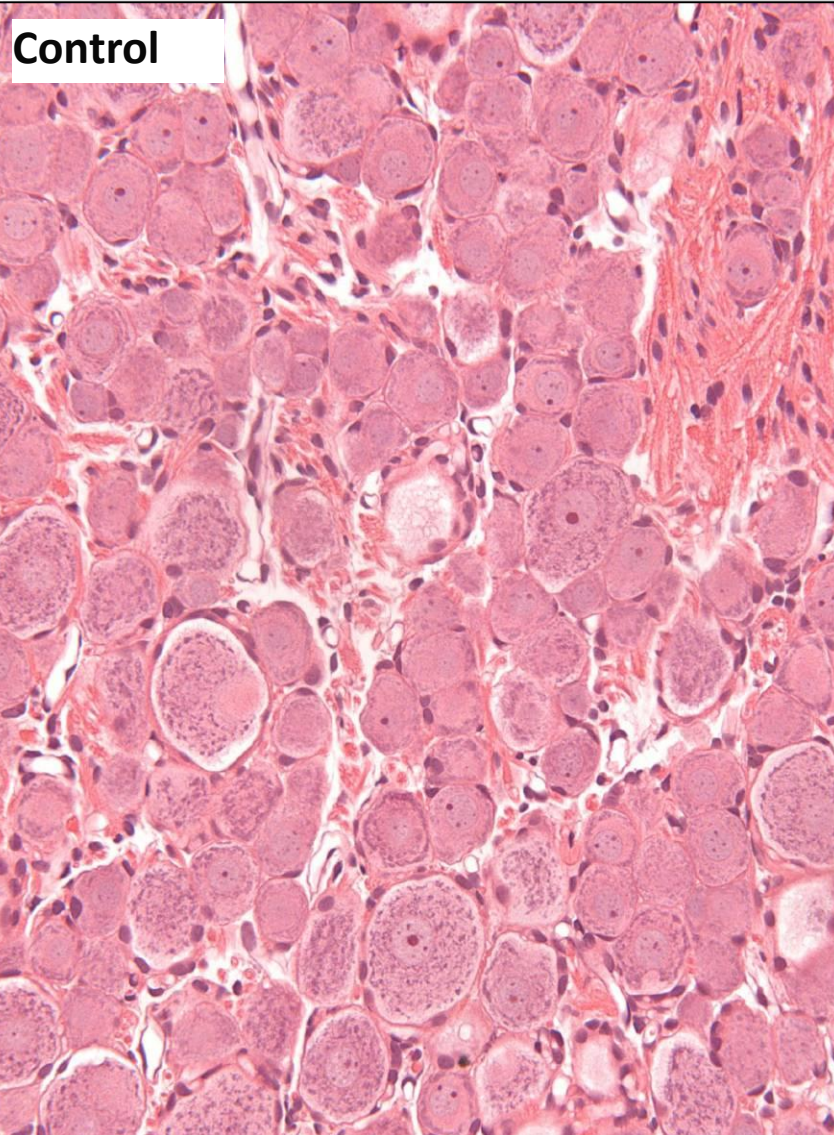
PNS Injuries – Neuropathy

- **early primary changes in the nerve cell bodies**
- **peripheral segmental demyelination may be most prominent in the spinal nerve roots, especially at the lumbosacral level, such as the sciatic nerve roots, which have large myelin segments.**

Acrylamide (3 to 30 mg/kg/day)

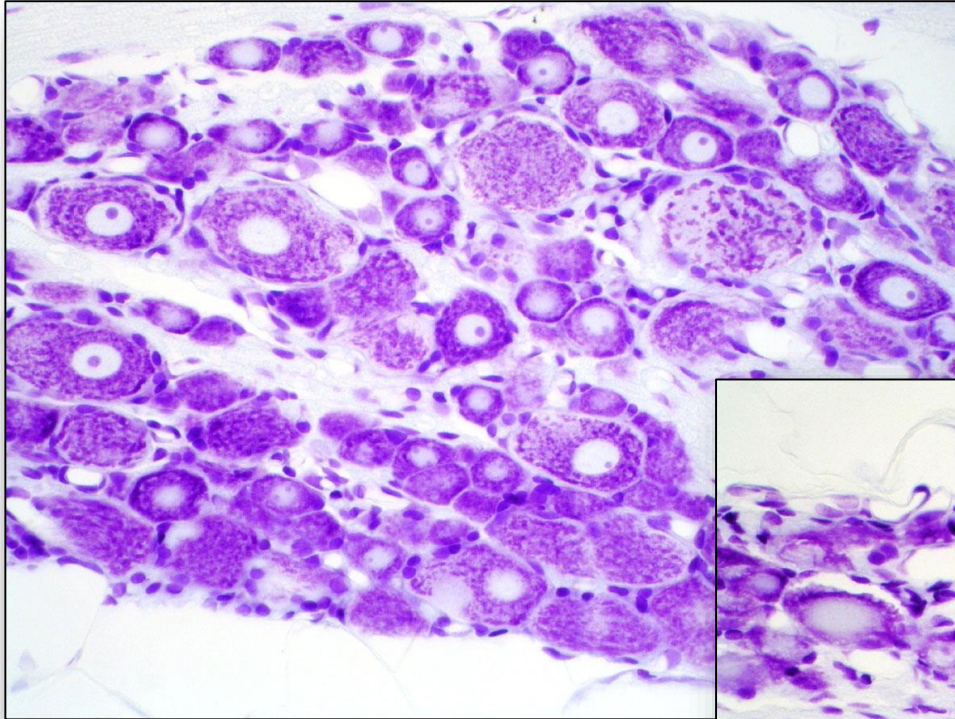
- **28-Day repeated dose neurotoxicity study**
- **FOB testing in week 4 after treatment with 30 mg/kg/day for 17 days revealed e.g.:**
 - **reduced grip strength in the forepaws (about -30%)**
 - **reduced grip strength in the hindpaws (-51% males/-82% females)**
 - **increased landing foot splay (+35% in males/+44% in females)**
- **Motor activity testing revealed:**
dose-dependent reduction in centre time, and motor activity, both distance travelled and rearing activity

Correlate: Chromatolysis

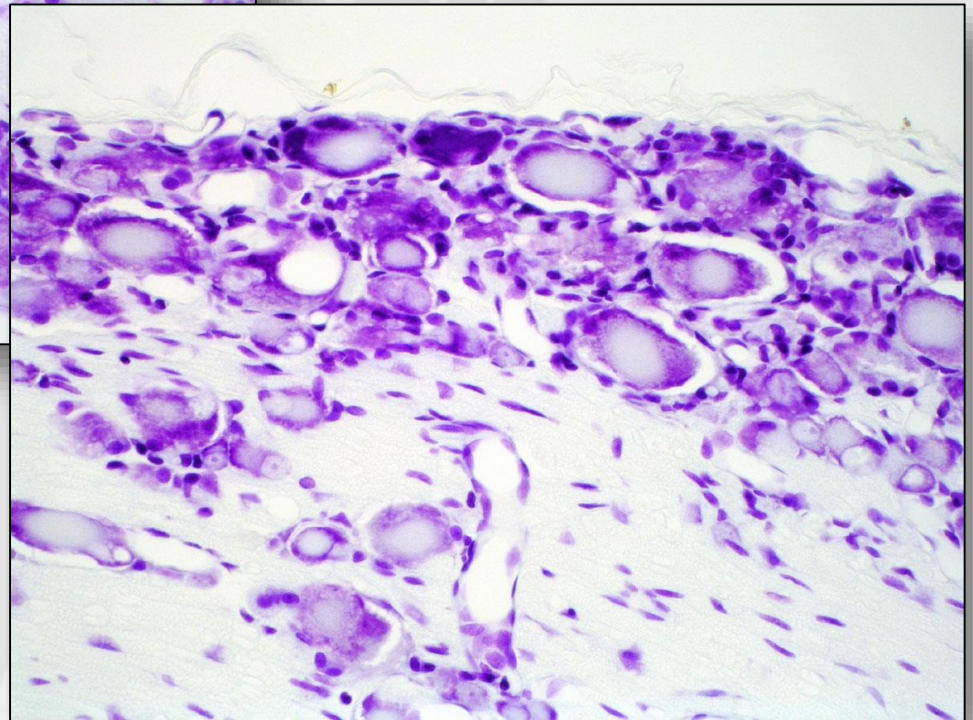


Another case: Dorsal Root Ganglion, Rat, Toluidin Blue

(Dr. G. Krinke)



normal



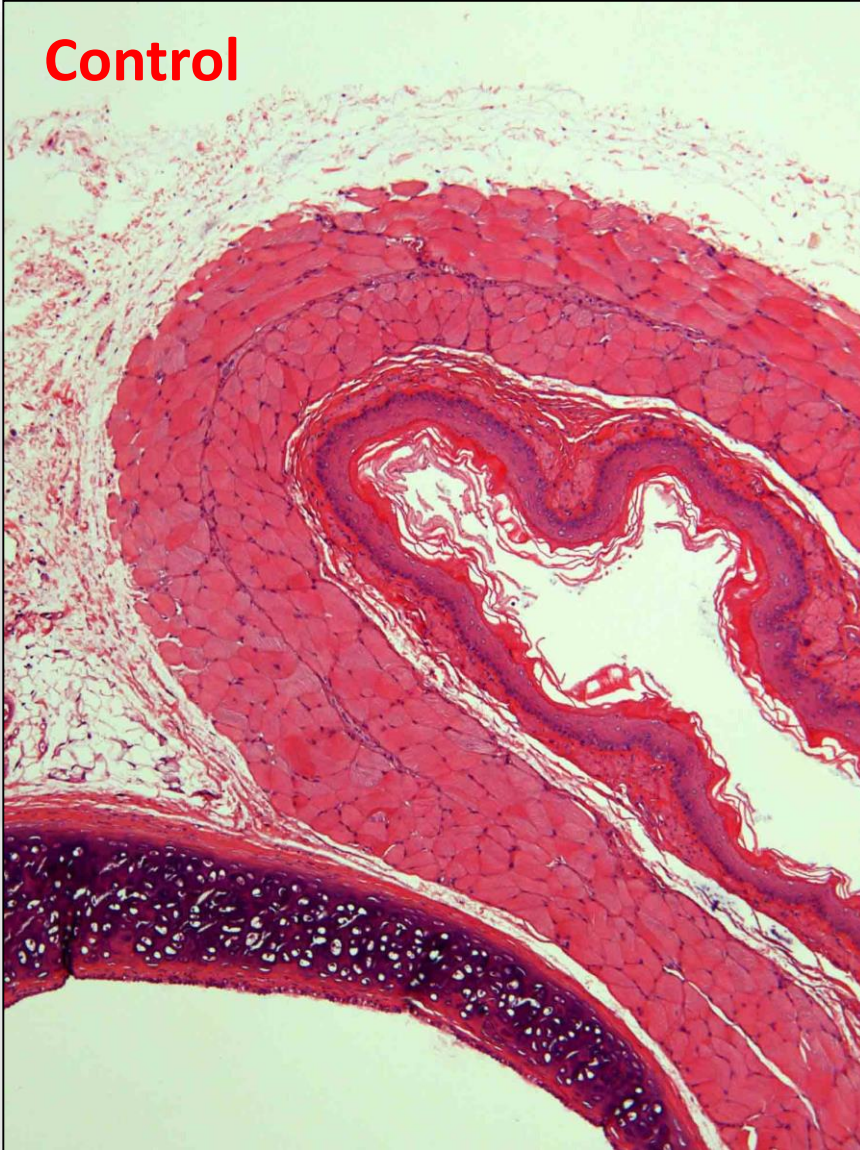
treated

Autonomous NS: Affection of Ganglia

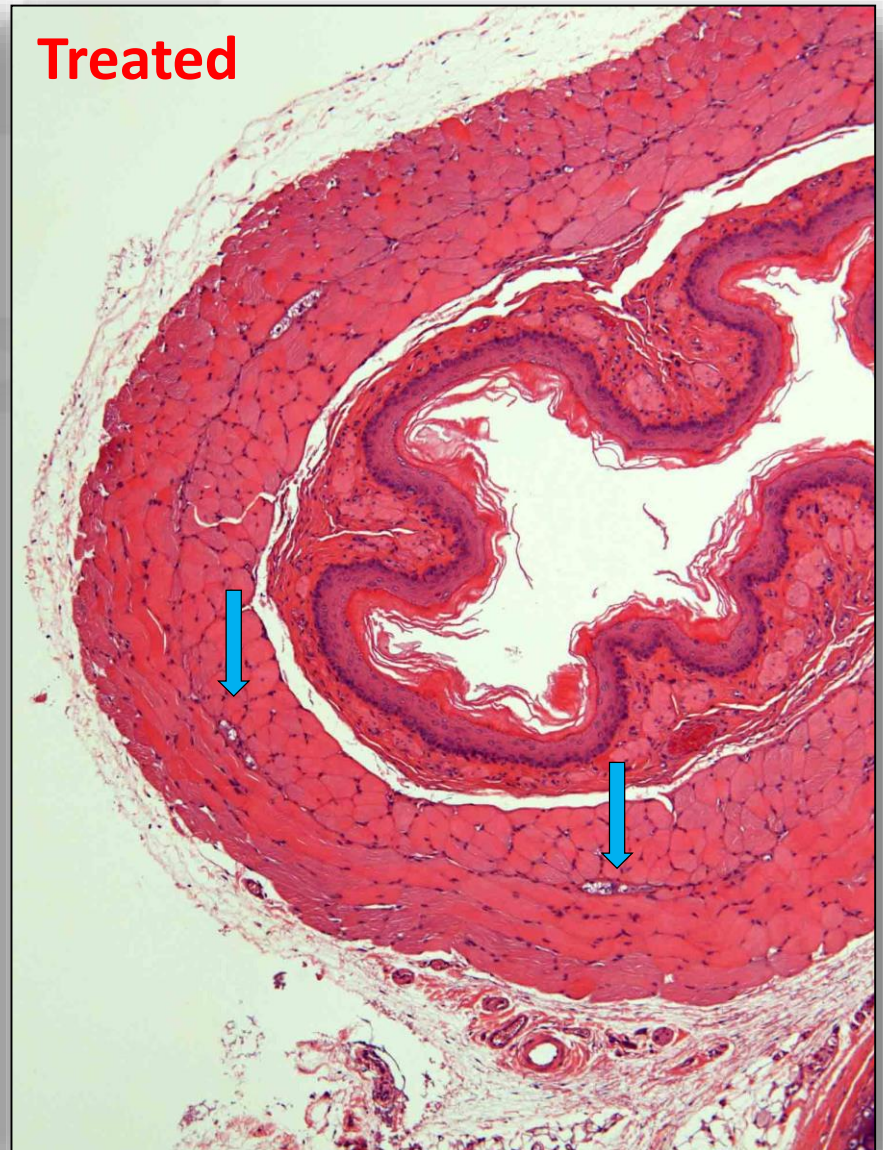
- **Pharmaceutical**
- **4-Week and 13-Week Study**
- **Regular studies**
- **No clinical symptoms**
- **Affected: esophagus, gastrointestinal tract, uterus, kidneys, medullar oblongata, pituitary gland parathyroid glands**

Esophagus: 13-Week

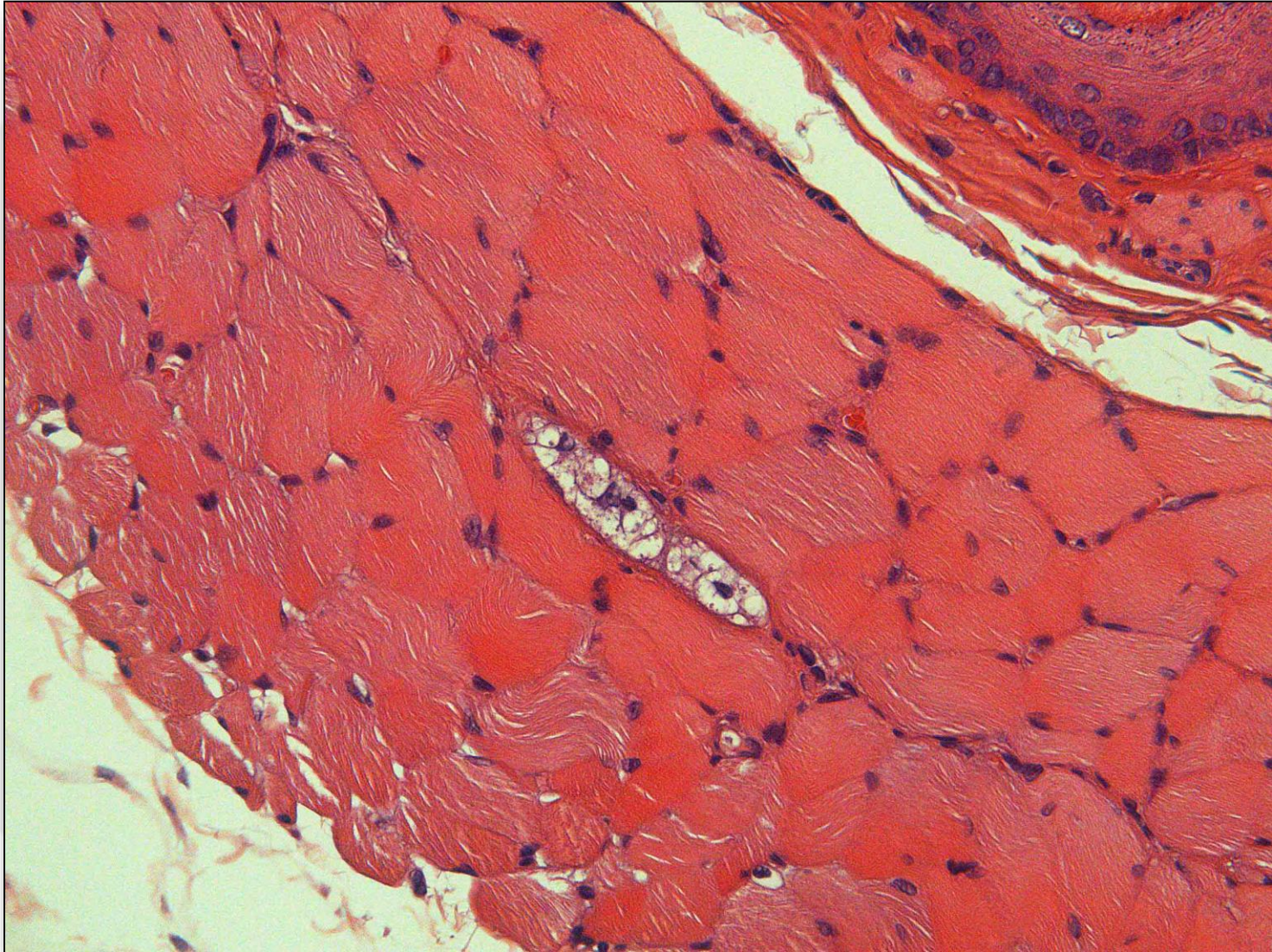
Control



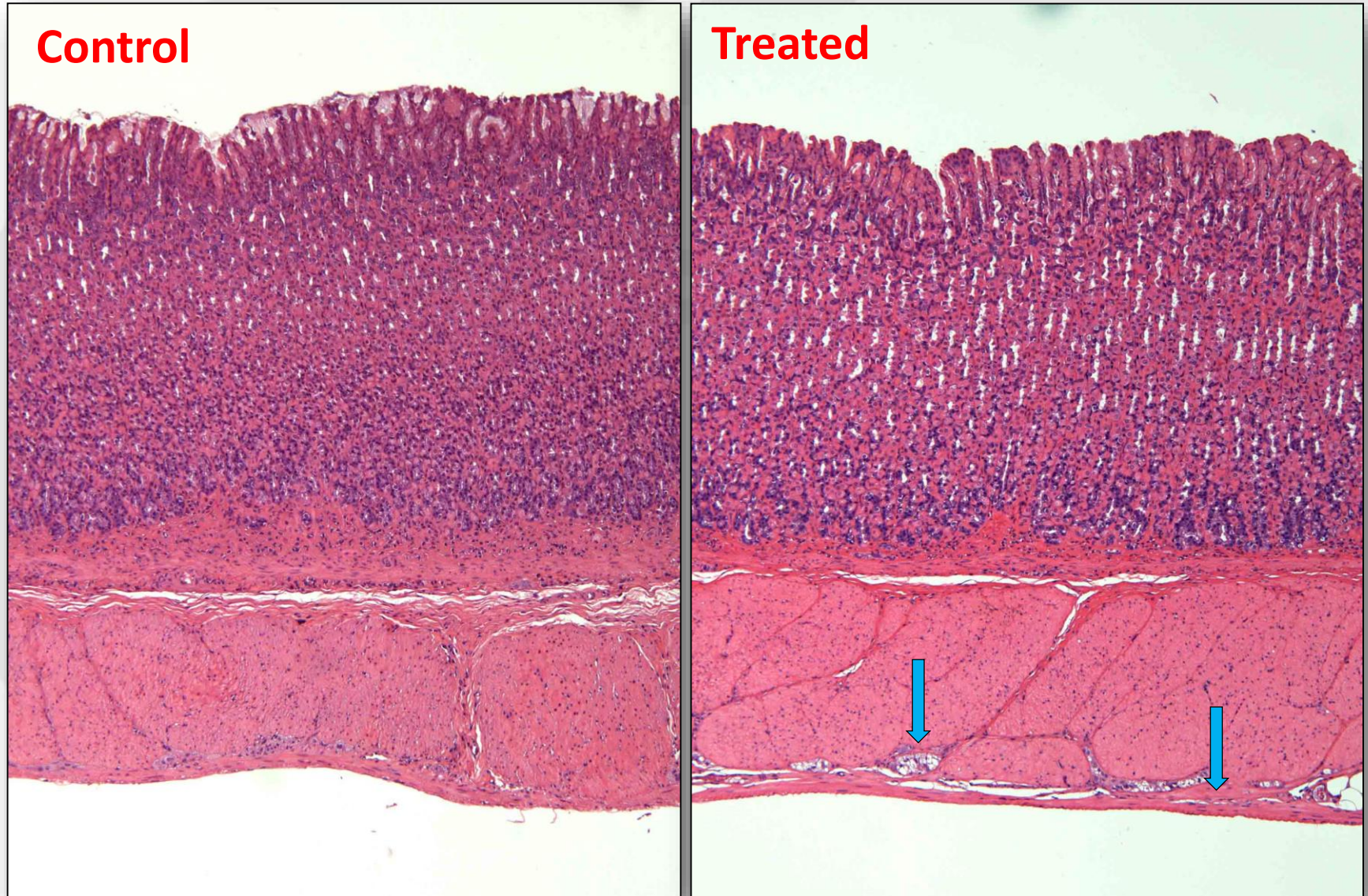
Treated



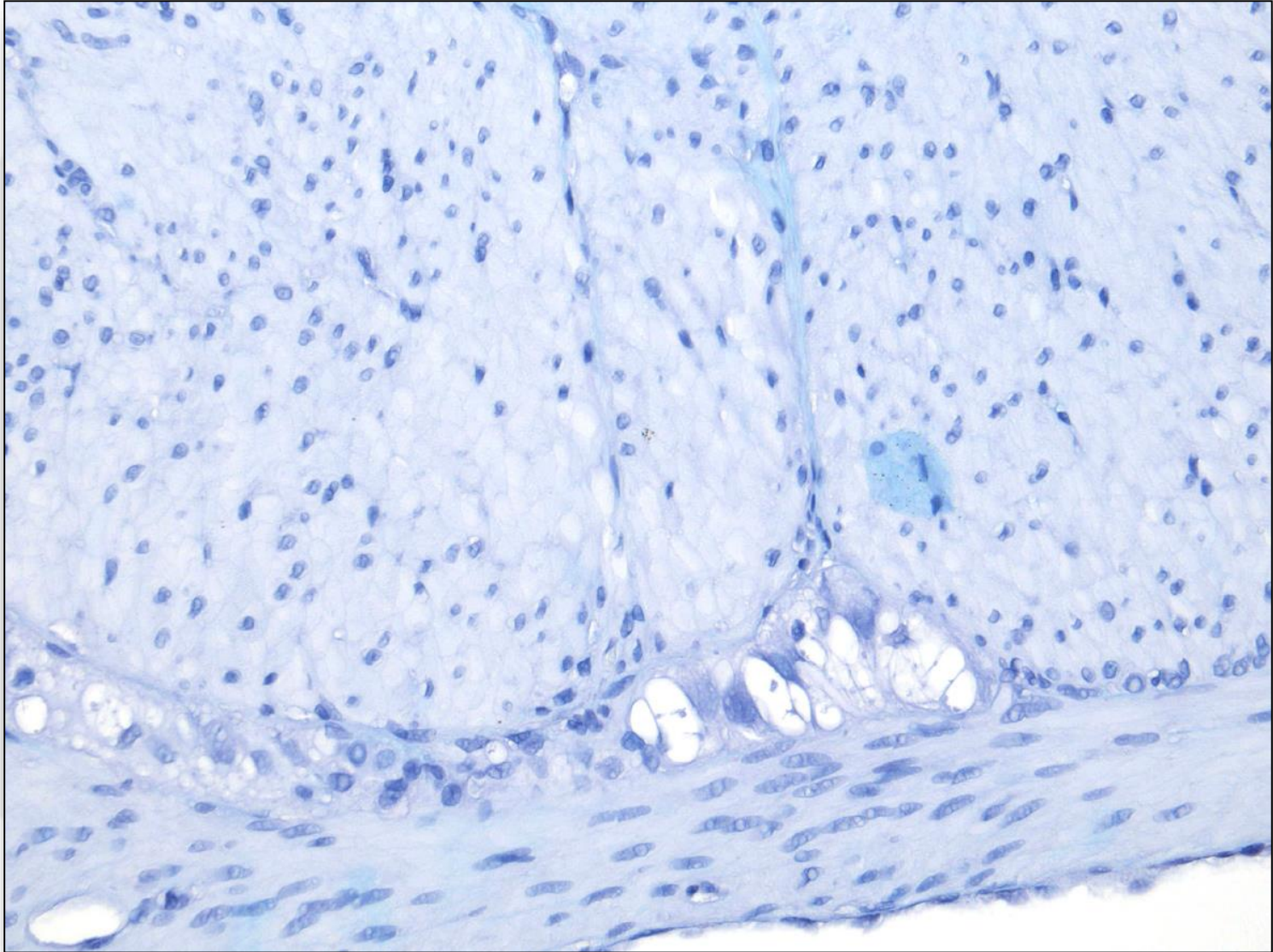
Esophagus: 13-Week



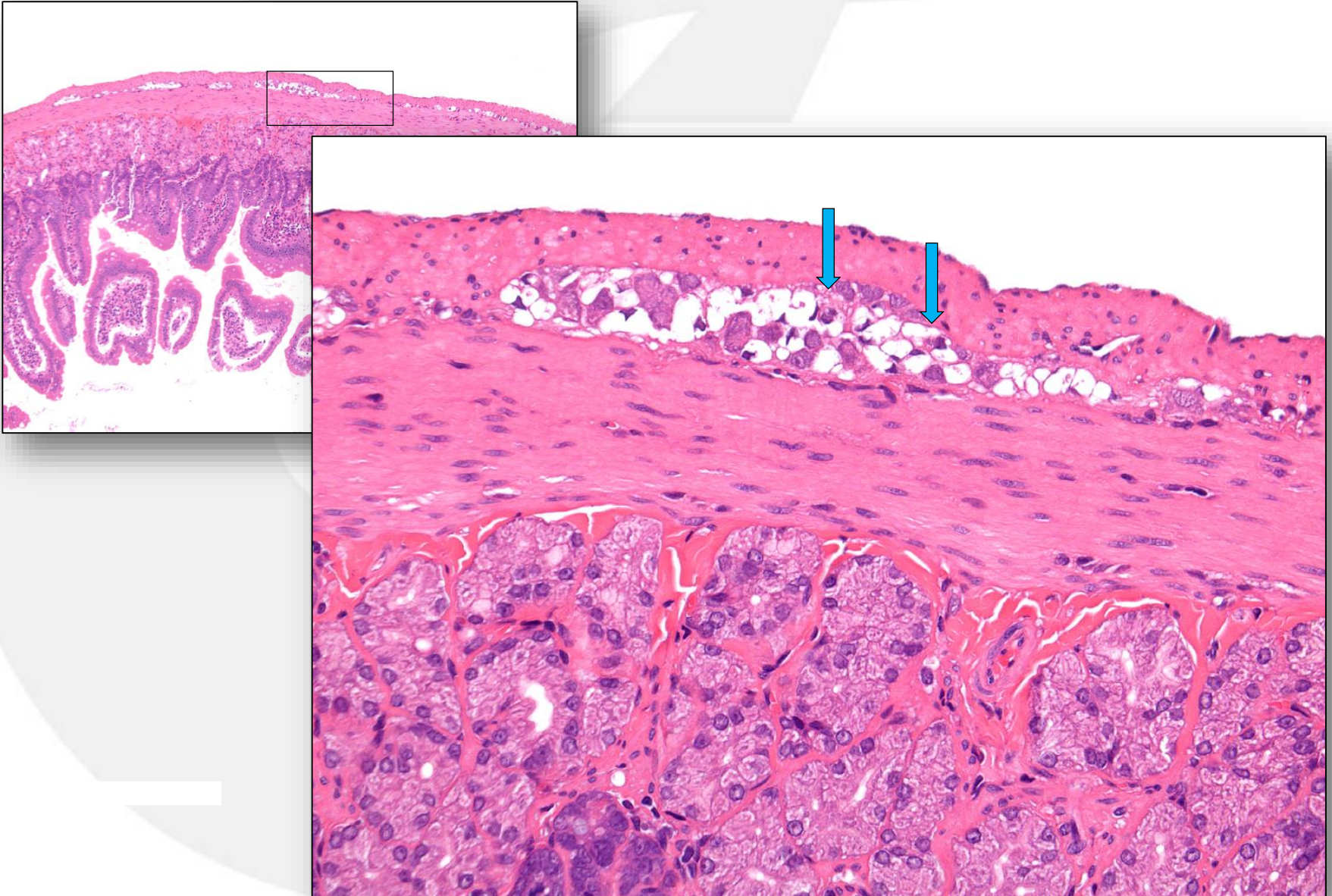
Stomach: 13-Week (Auerbach Plexus only, no Meissner Plexus)



Stomach: 4-Week



Duodenum: 4-Week



Special Technique: Teased-Fibres

- **Specimens of Nerves of approximately 10 mm in length**
- **Removal of fat and connective tissue**
- **Few minutes in proteolytic enzyme (Pronase)**
- **Rinsed in ethanol**
- **24-28 hrs in Sudan-Black**
- **Isolation of fibers on glycerin covered slides in parallel**

(Study in hen with Organophosphates)

Special Technique: Teased-Fibres

Control

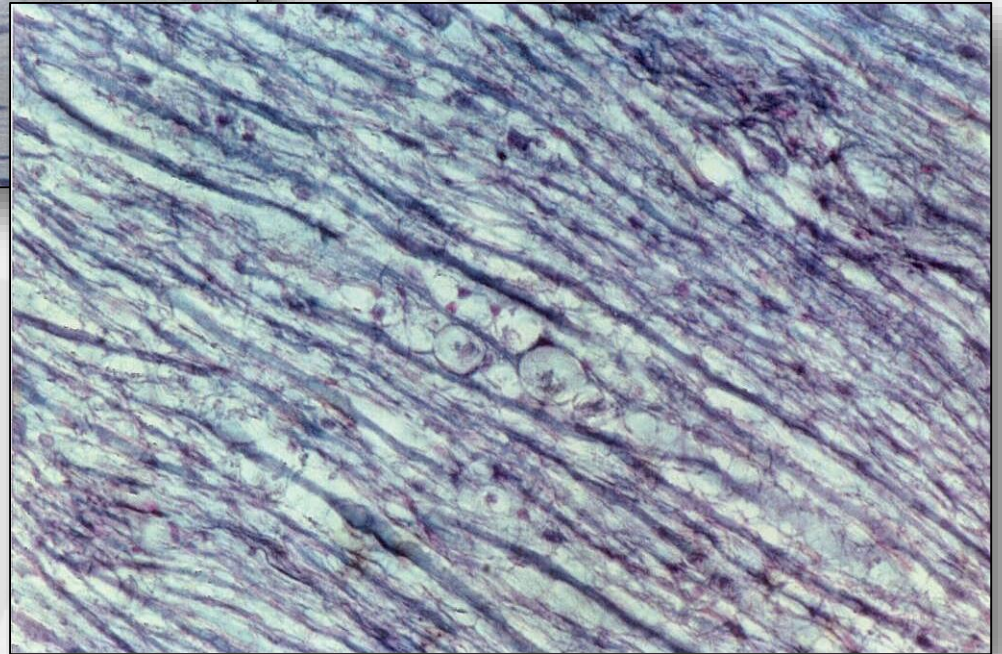
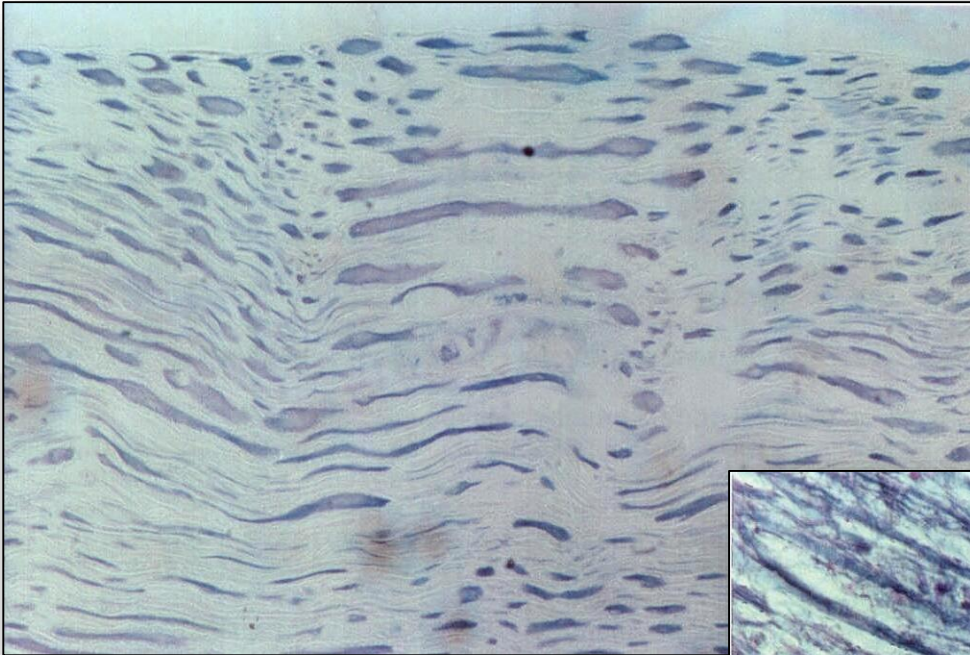


Treated



Case by Dr. Georg Krinke

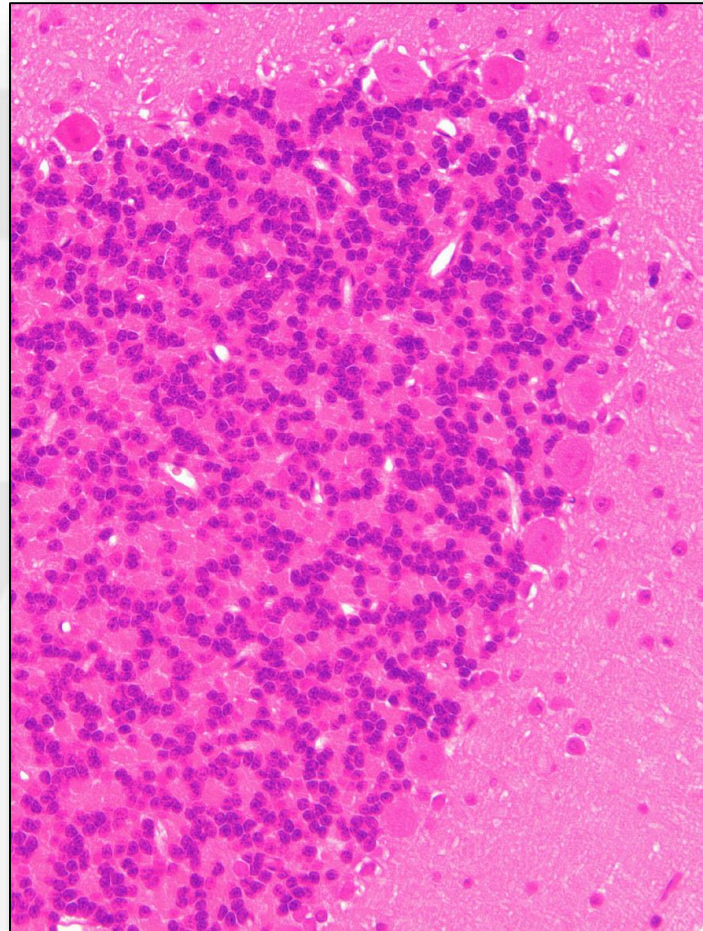
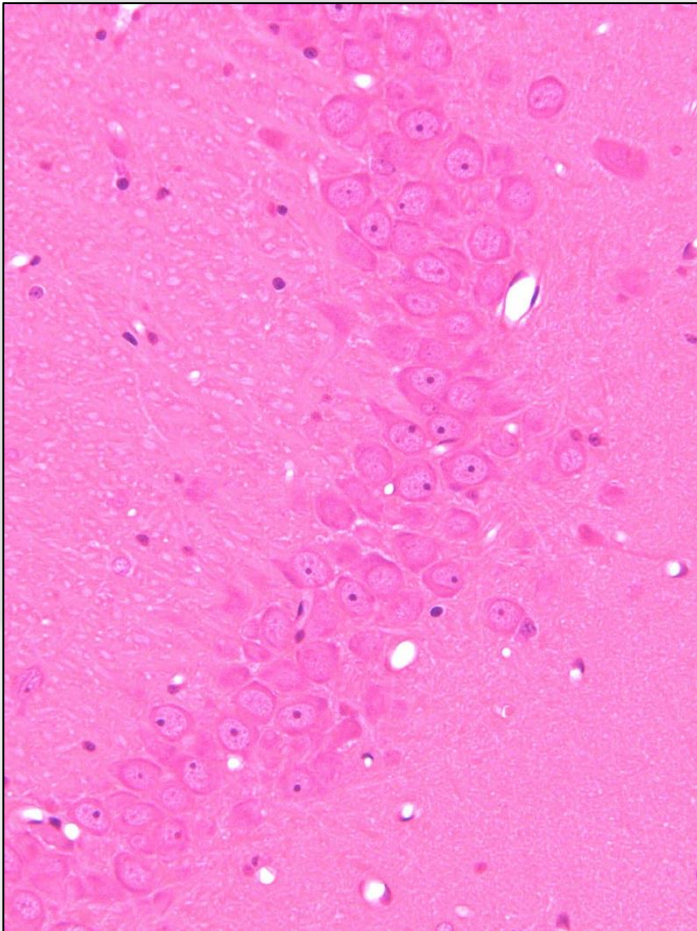
Organophosphate: Hen, peripheral nerve, Example: Bodian



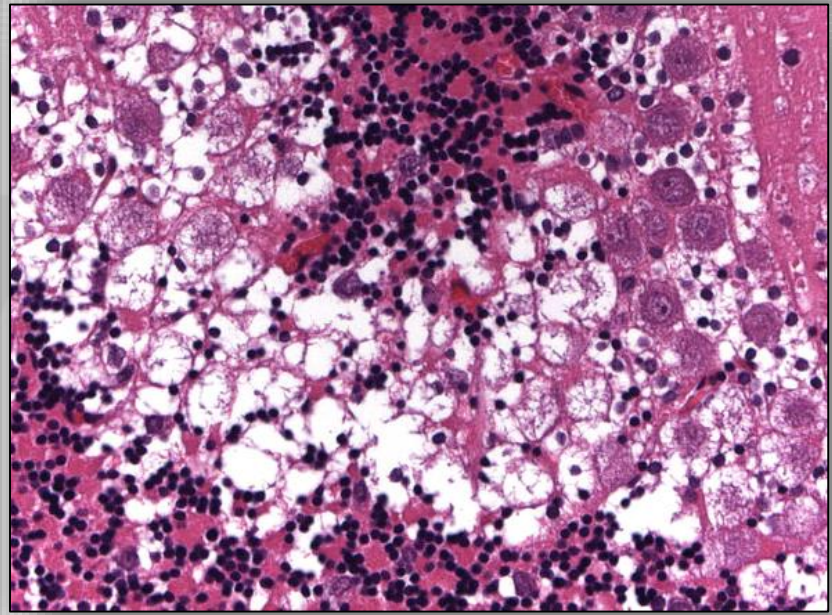
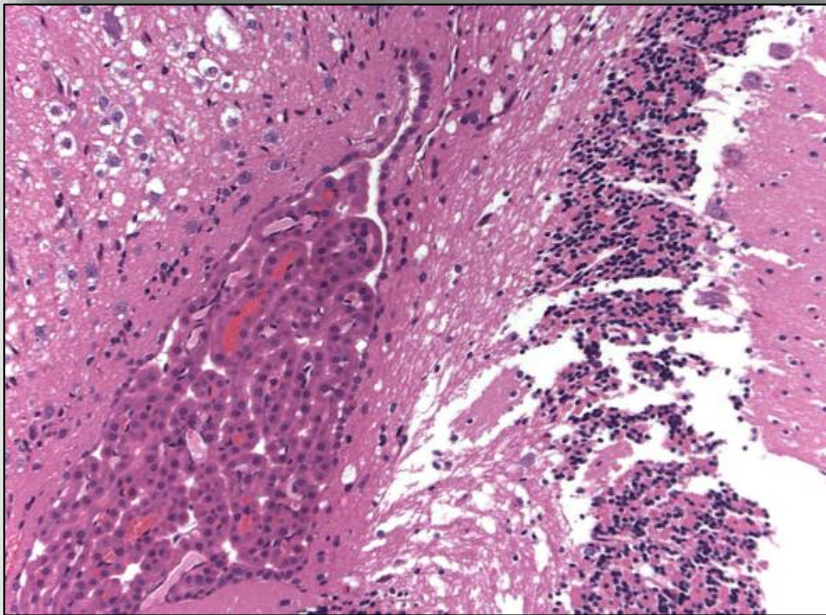
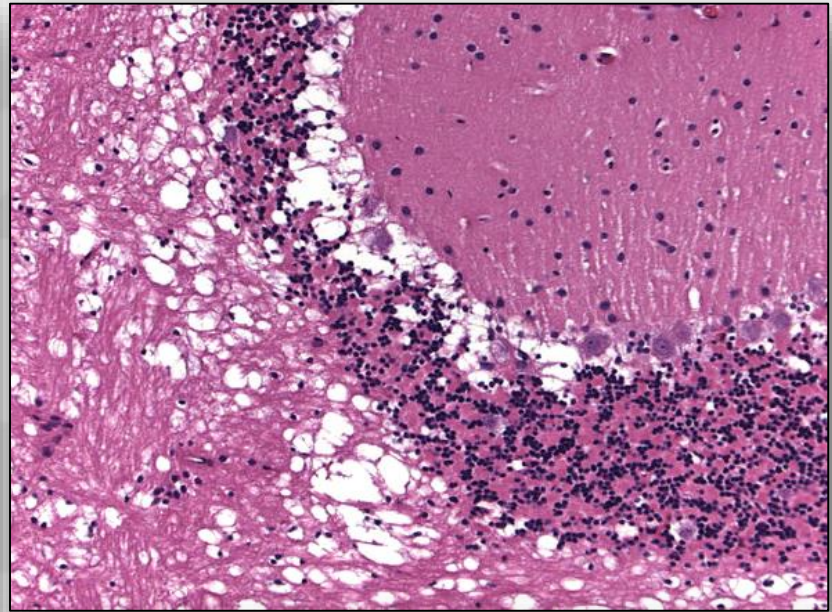
- **“delayed neuropathy”
of organo-phosphorus
compounds (TOTP)**

Technical Considerations

- **Perfusion fixation with previous rinsing (e.g. Ringer`s solution + heparin)**
- **Formaldehyd, glutaraldehyd**



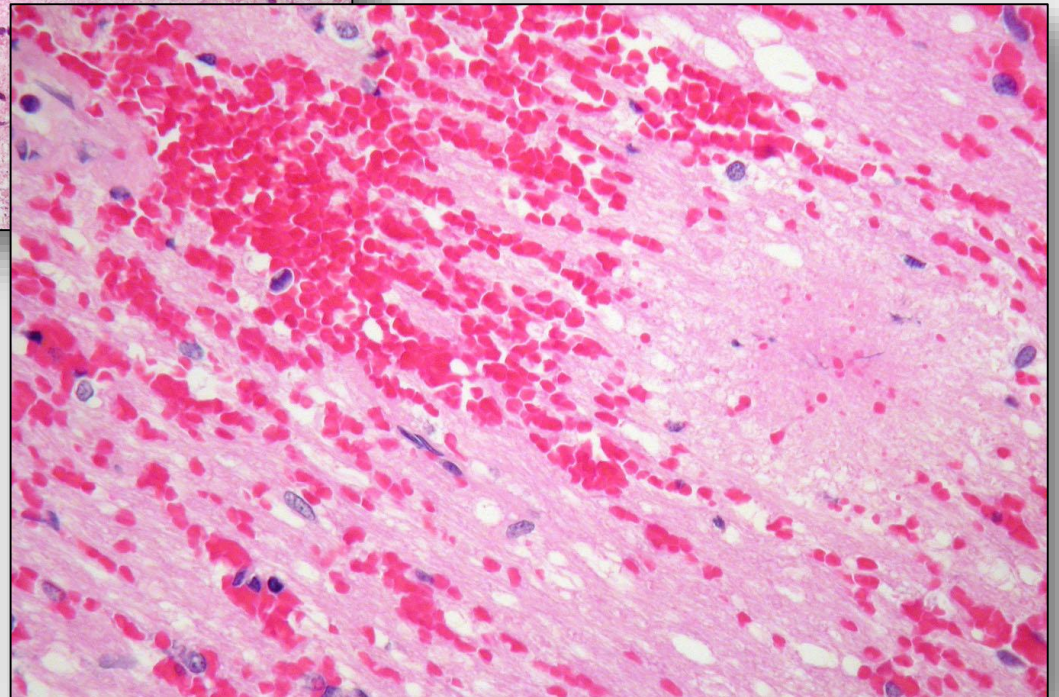
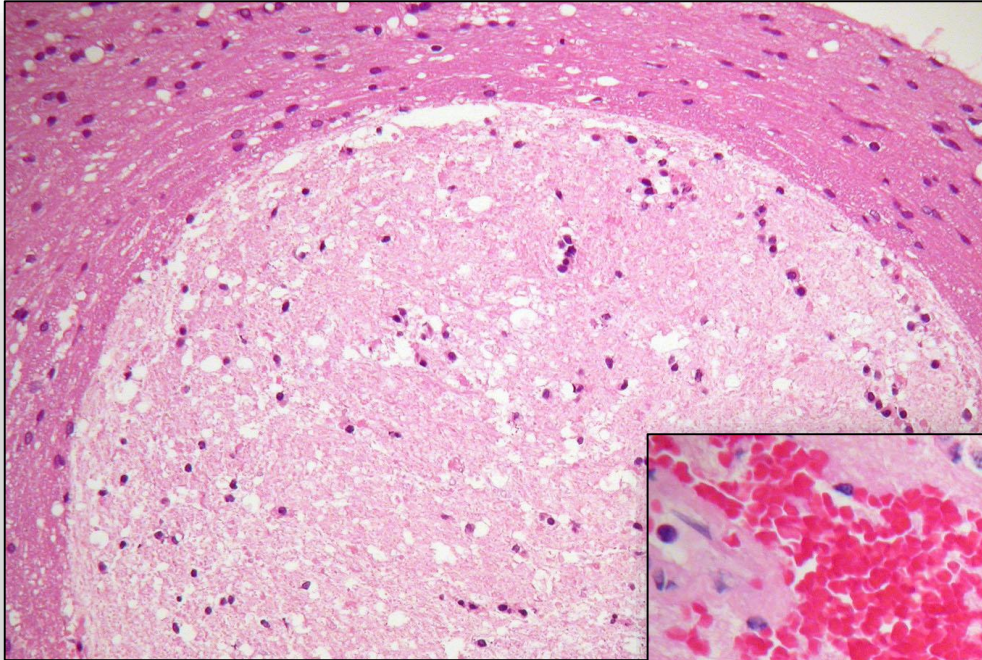
And this is the wrong outcome....



Injury of CNS

- **Targets are similar including axons, myelin sheaths and neurons**
- **Techniques to determine effects are similar, but effects may be difficult to establish because:**
 - **spontaneous lesions may be confusing**
 - **section level**
 - **symmetrical lesion (except raphe)**

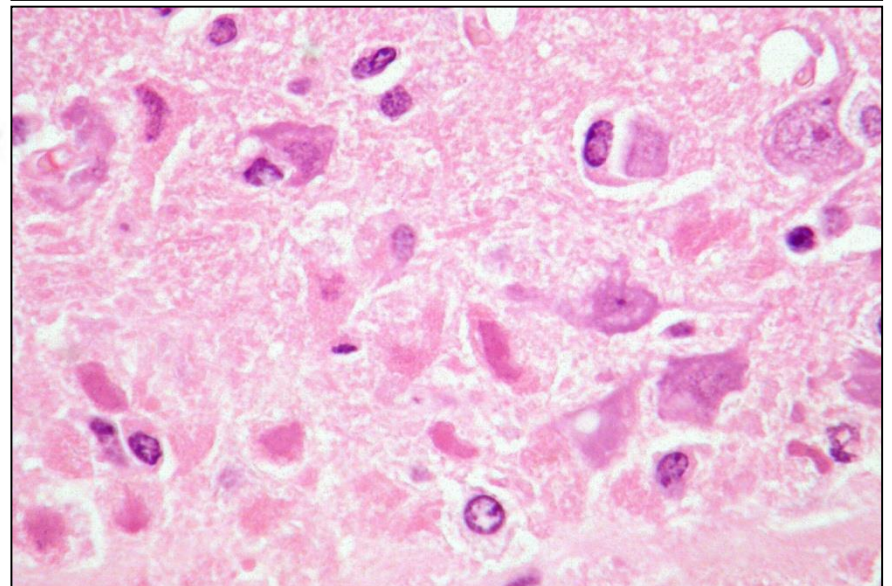
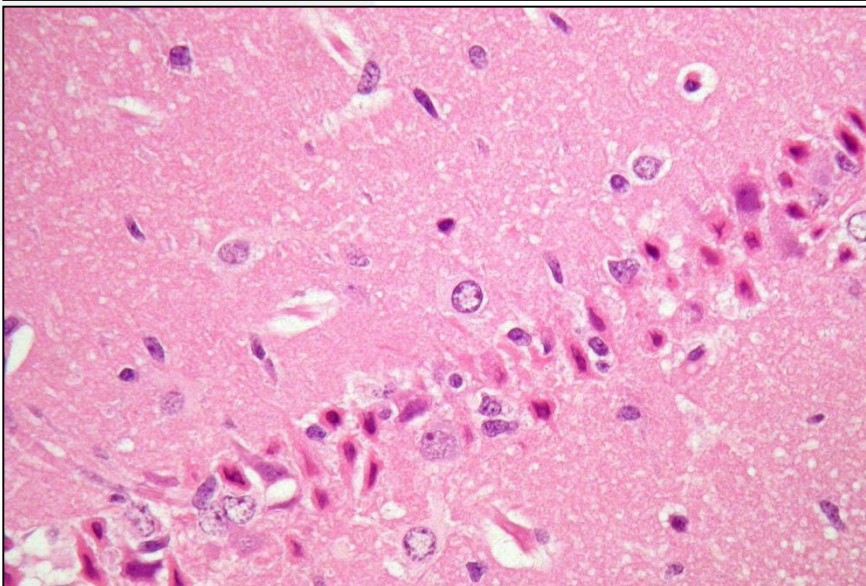
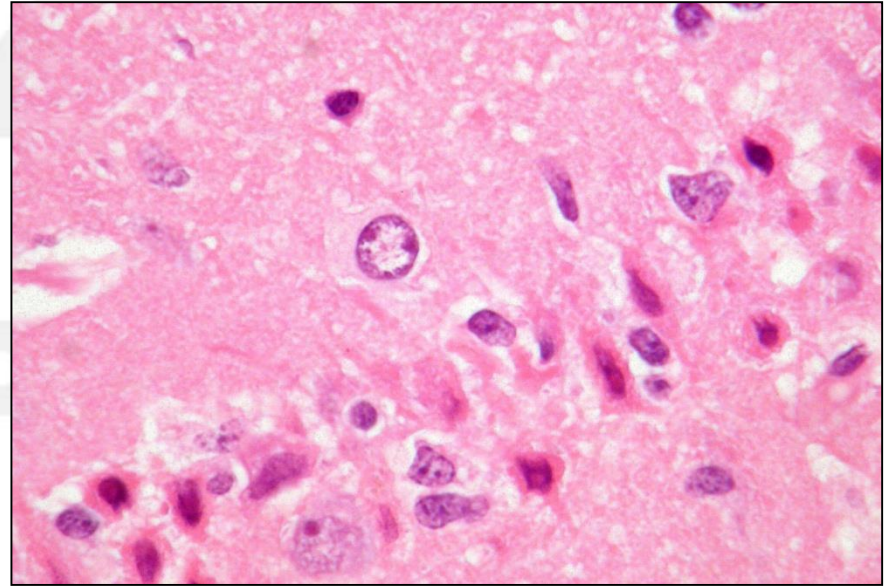
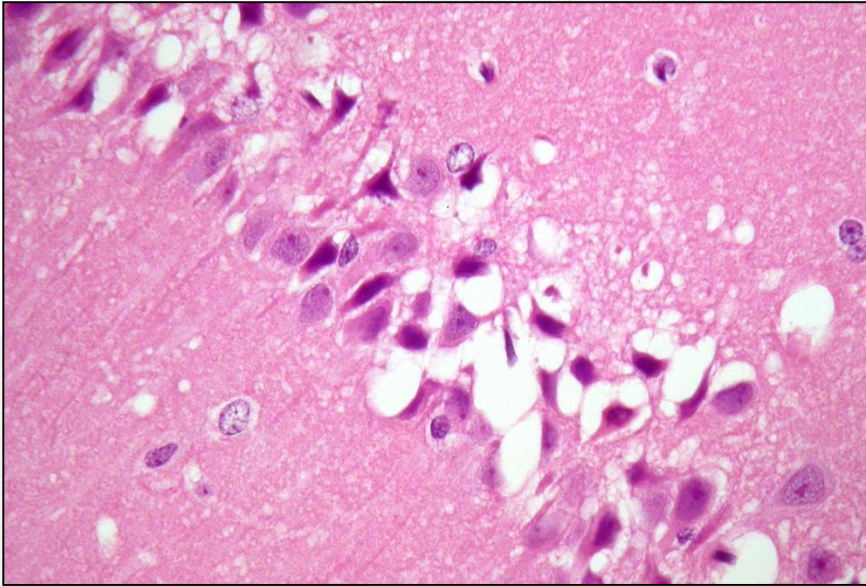
Spontaneous Lesion: Trigeminal Nerve



H&E and Special Stains

- **H&E, Advantages: Routine stain, most lesions detectable**
- **H&E; Disadvantages: some early and subtle changes not visible and non-symmetrical sections may cause misinterpretation**
- **Silver Stains: Frozen section with artefacts**
Complicate, expensive, time-consuming, not easy to reproduce, often not to quantify
- **Flurojade: simple and safe technique, but fading**
- **Plastic embedding: expensive and no additional information in peripheral nerve longitudinal sections**

Opiate: Sommer Sector: Rat, Stroke

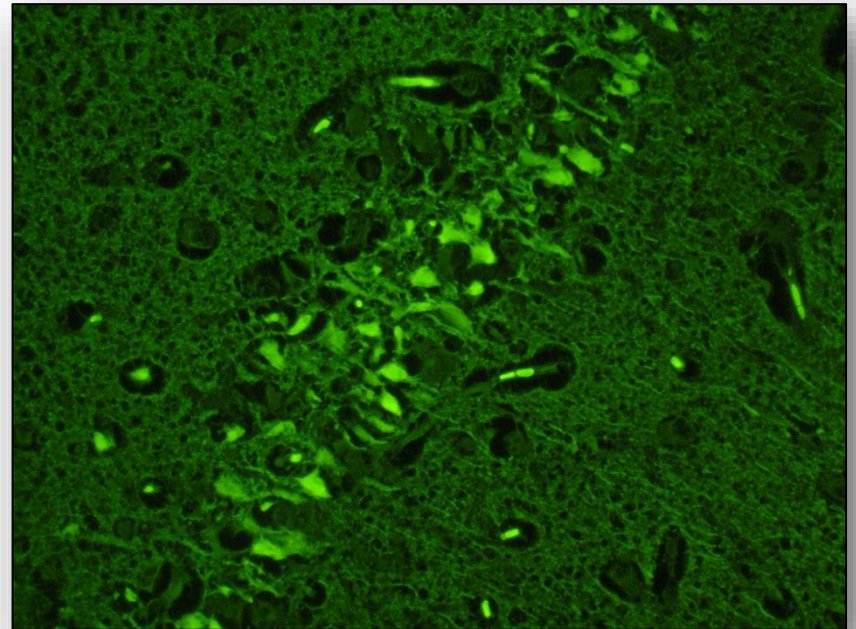
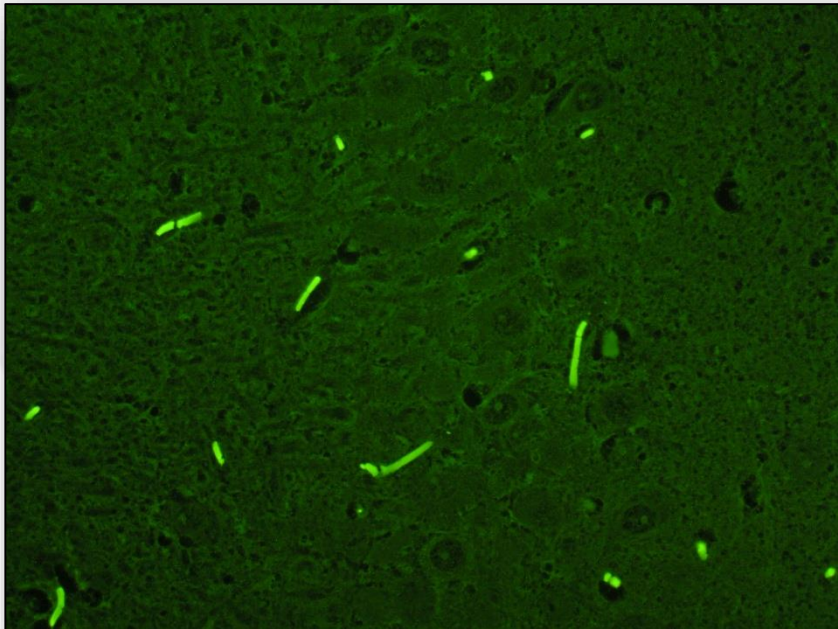


Sommer Sector: Rat, Stroke, Opiate

- **Hippocampal degeneration – unilateral:**
- **Mainly CA1 (Sommer's sector and only partially the CA2 (part of the Resistant Sector) involved**
- **Acute necrosis (dark red staining neurons)**
- **Gliosis of a moderate degree (not a result of primary neurotoxicity but secondary to hypoxia)**
- **Similar lesions may be recorded after seizures**
CA1 and CA2 neurons of the hippocampus are specially sensitive

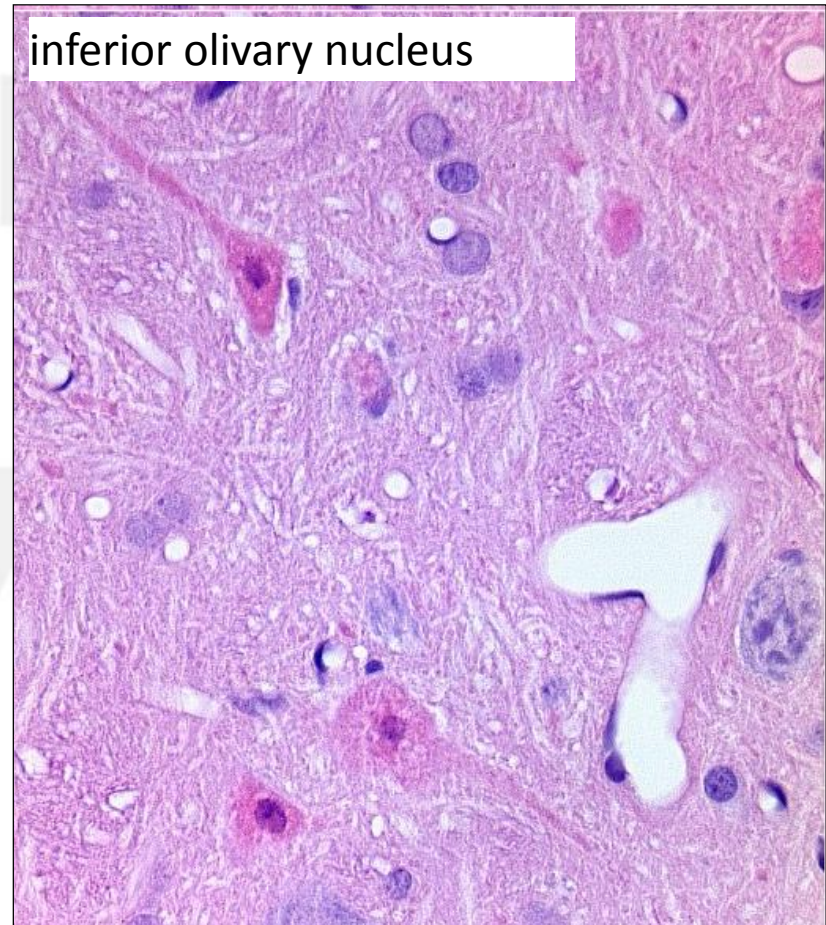
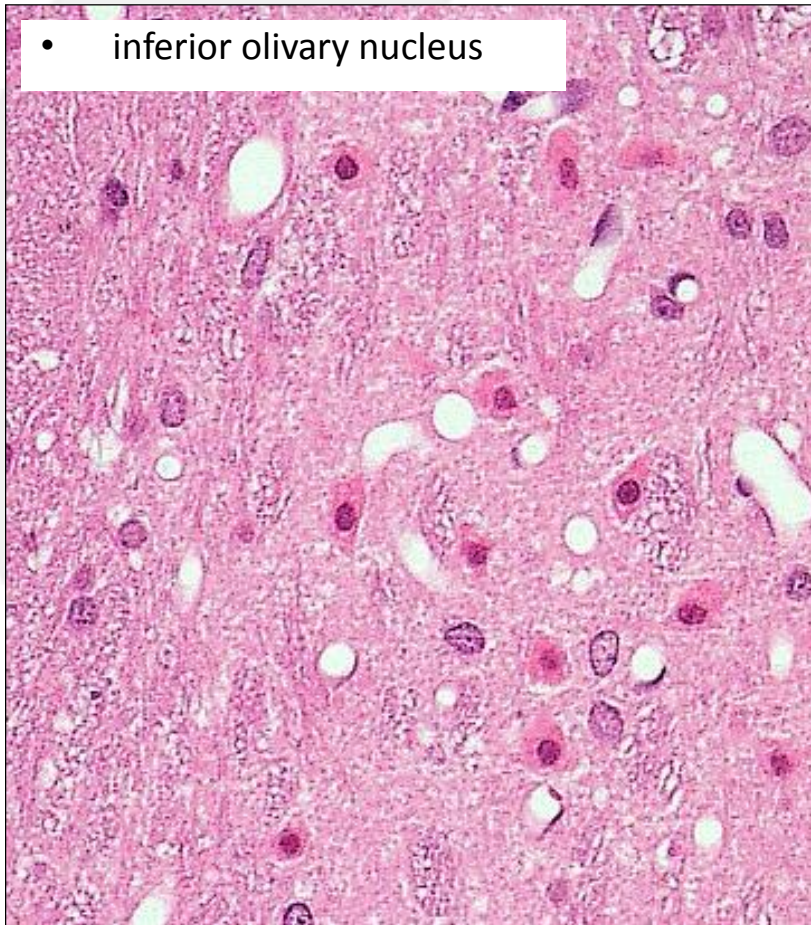
Hippocampal degeneration (Stroke)

- **Control:**
 - healthy pyramidal neurons are dark
 - yellow spots by fluorescence of erythrocytes
- **Treated:**
 - necrotic pyramidal neurons are bright yellow

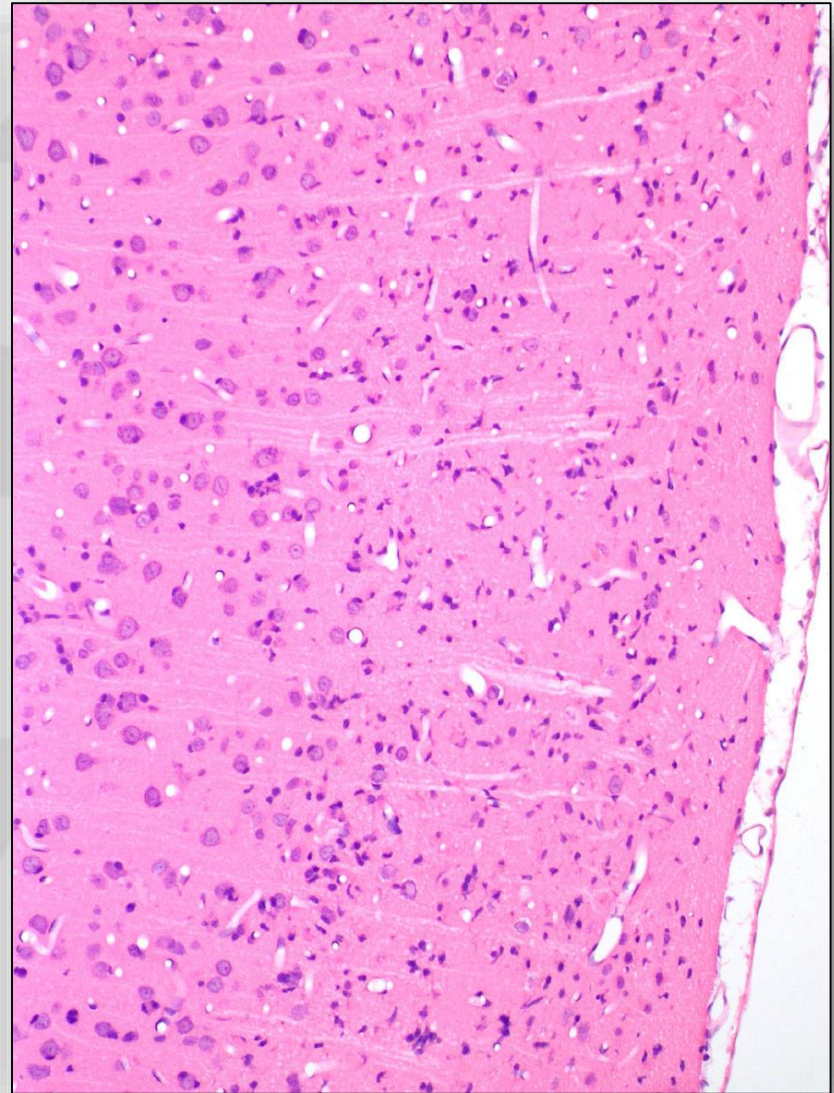
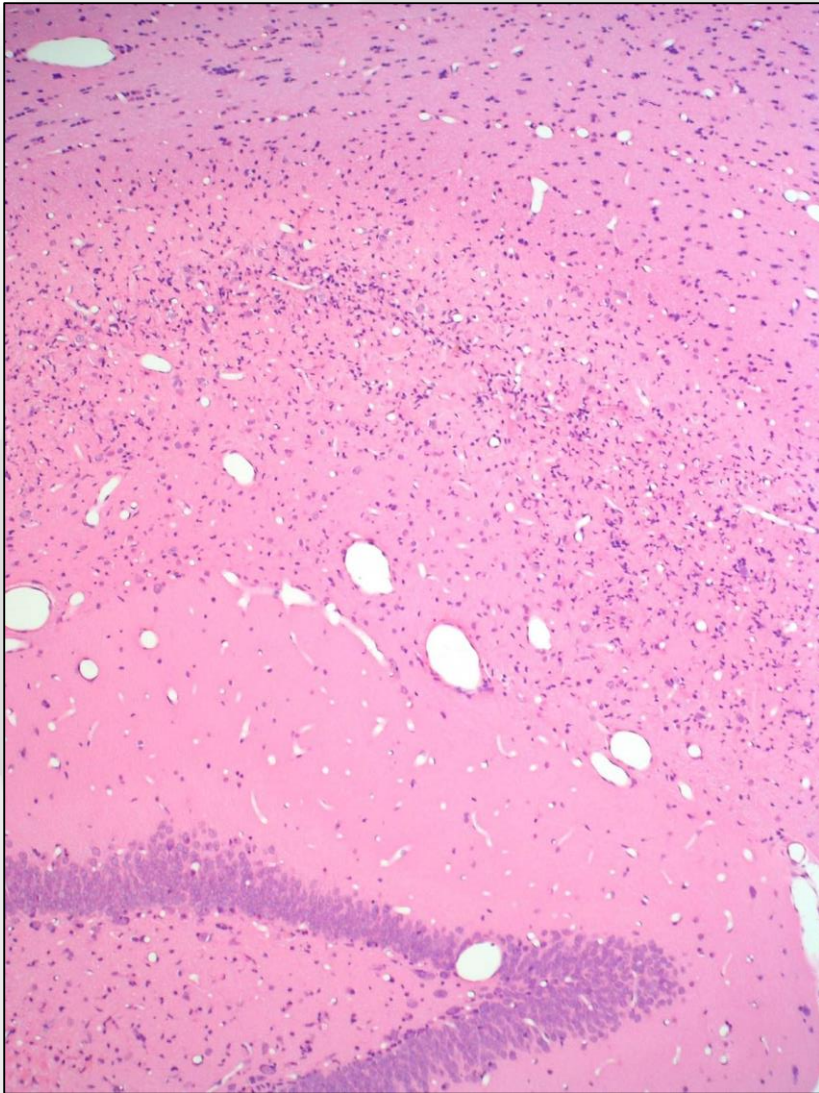


H&E: Acute Neuronal Necrosis. Selective

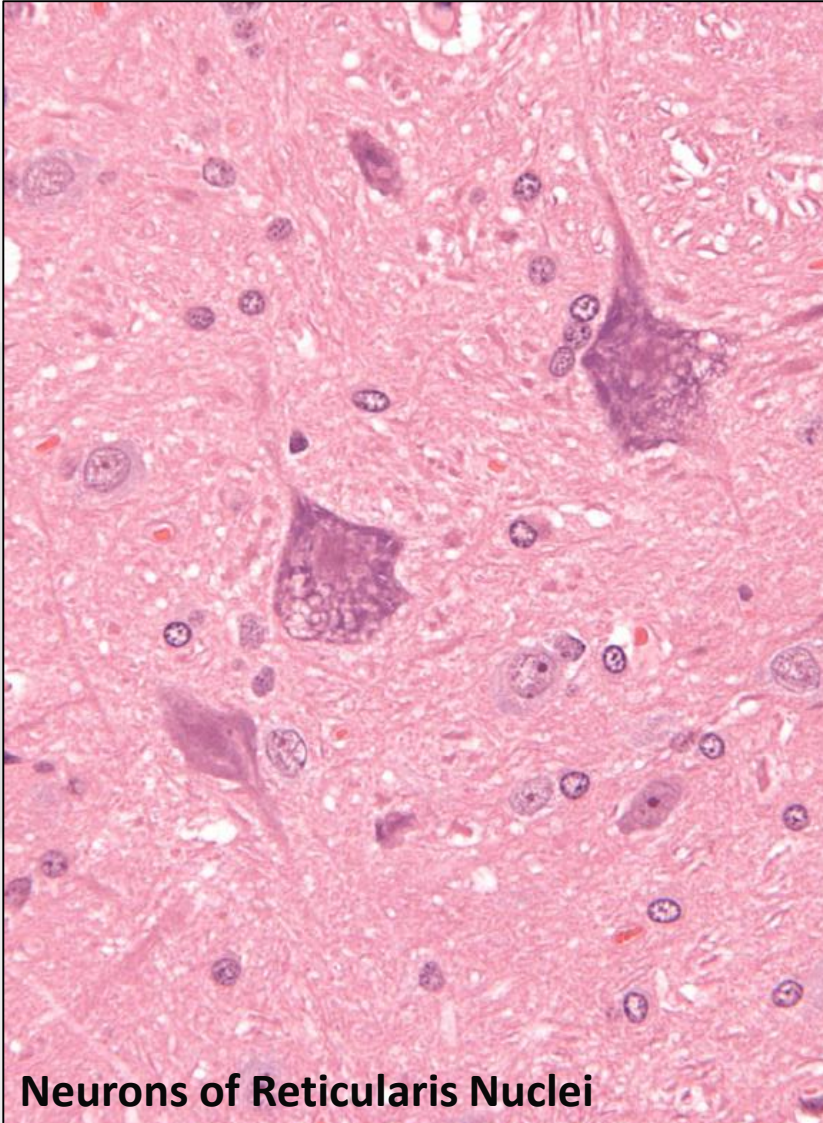
- **3-acetylpyridine: inhibitor of Coenzyme I (3 days)**
- **discrepancy between energy demand of affected**
- **neurons and restricted supply due to energy deprivation**



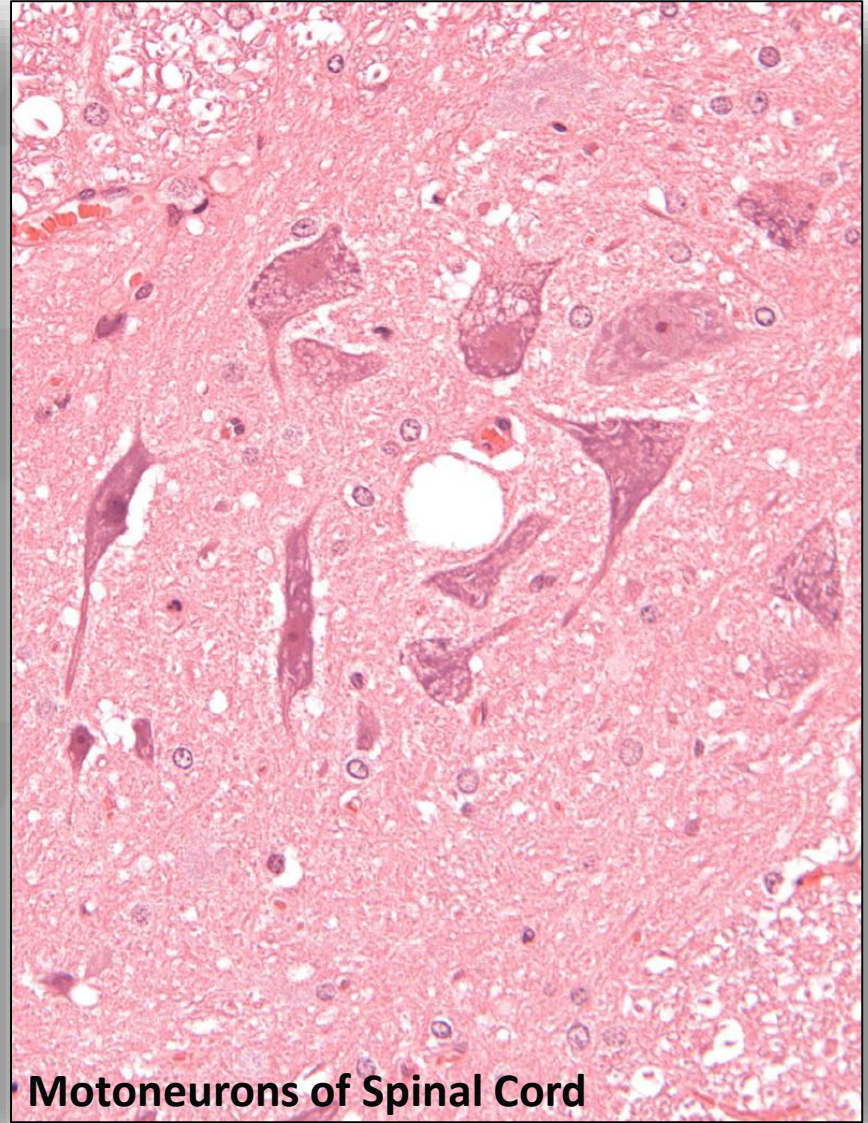
Selective Neuronal Loss and Gliosis, Rat, Entobex



Phospholipidosis: Rat, Antibiotics

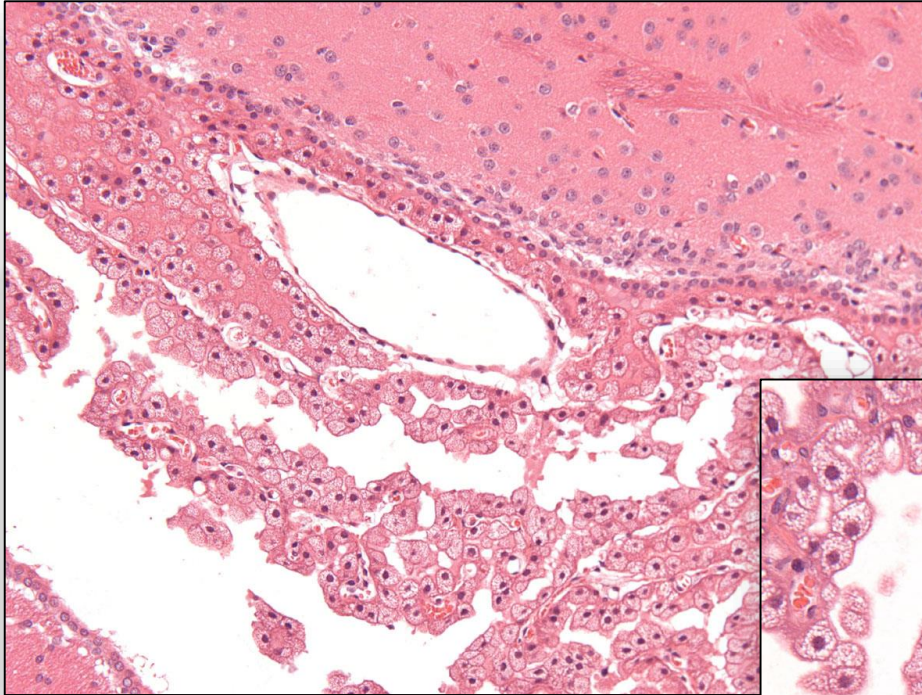


Neurons of Reticularis Nuclei

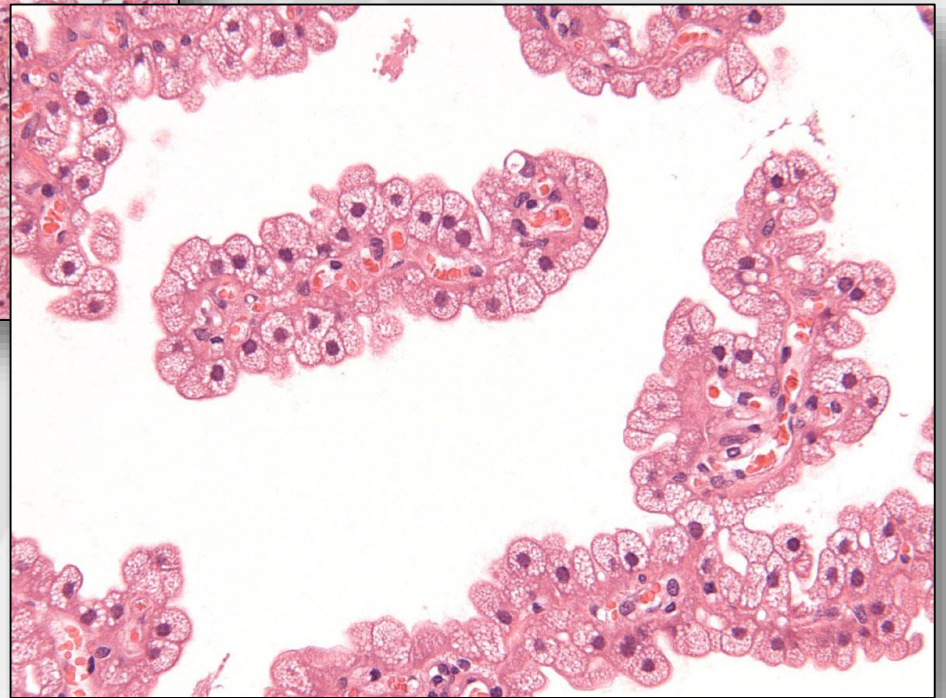


Motoneurons of Spinal Cord

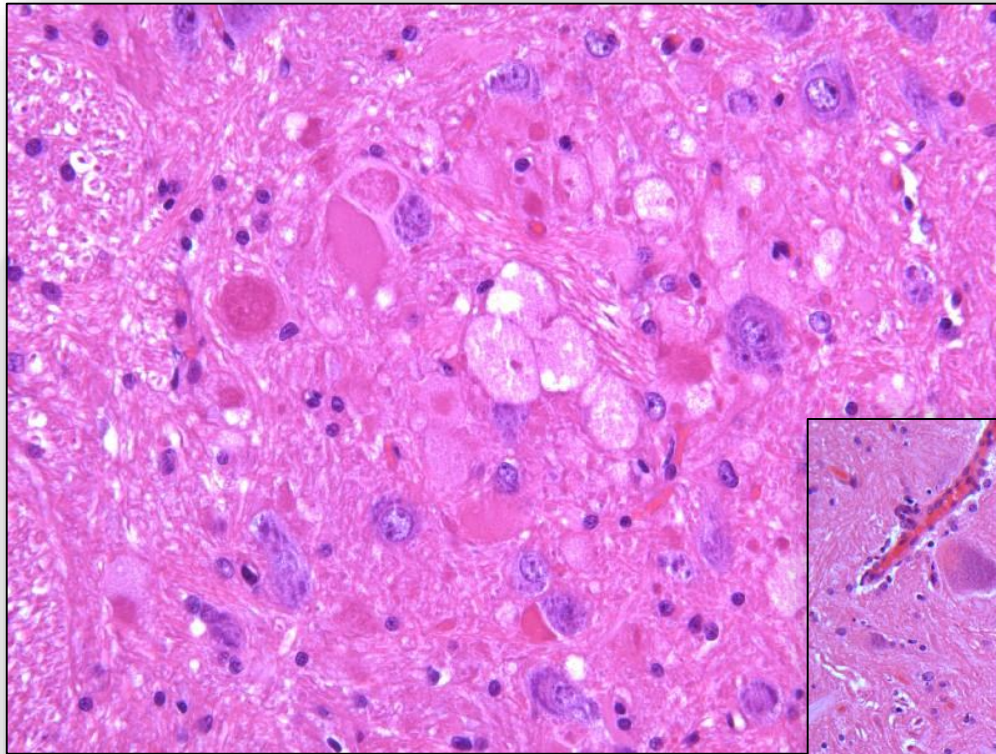
Phospholipidosis: Rat, Antibiotics



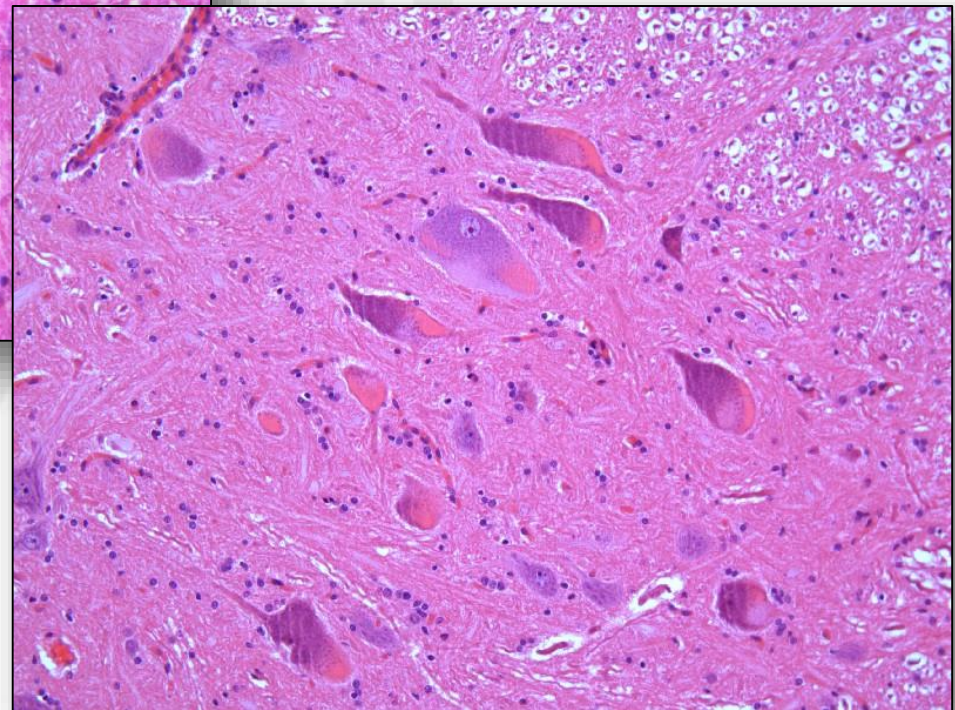
Plexus vacuolation



Phospholipidosis: Dog, Antibiotics

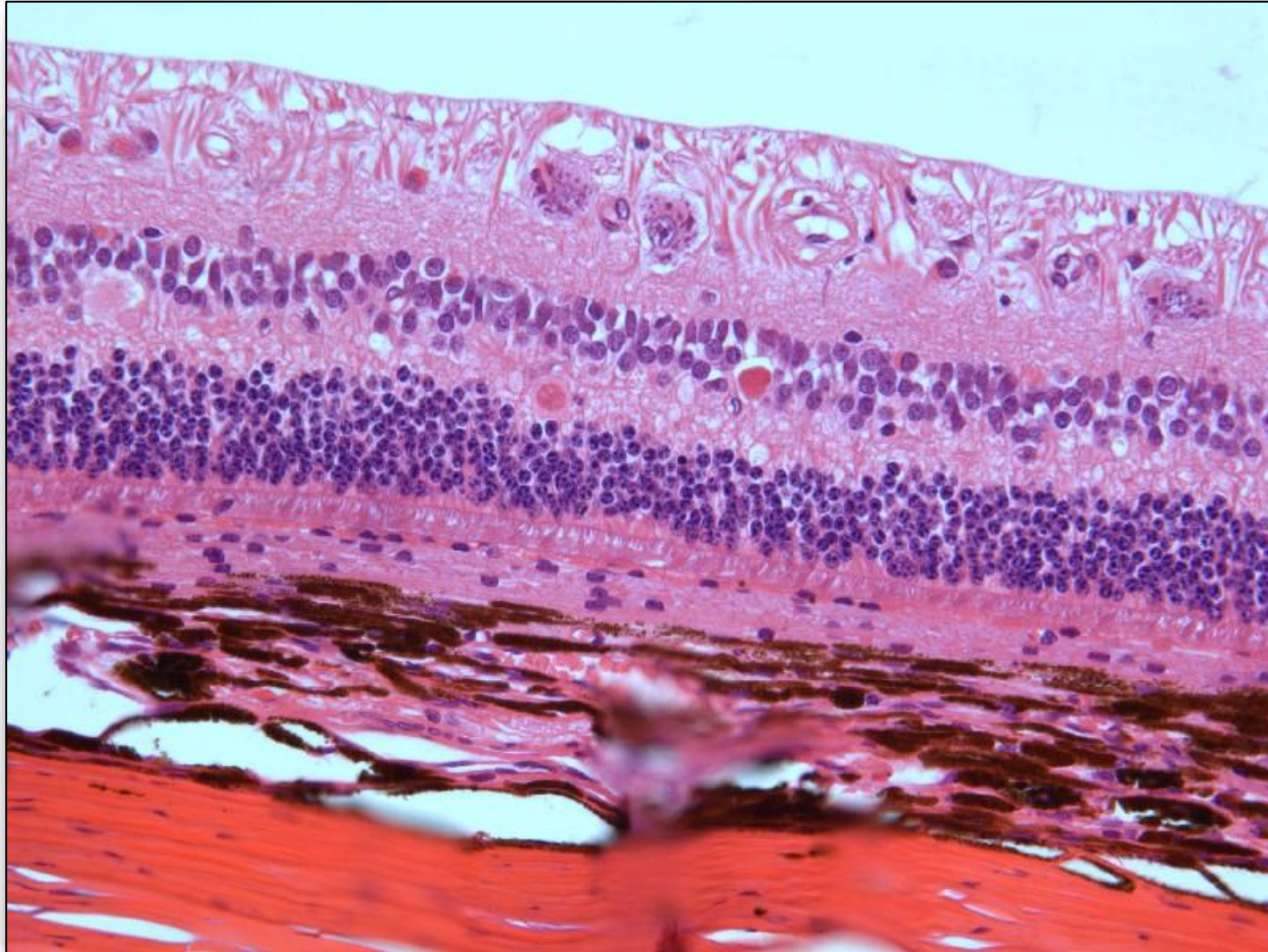


**Neuronal necrosis in
medulla oblongata**

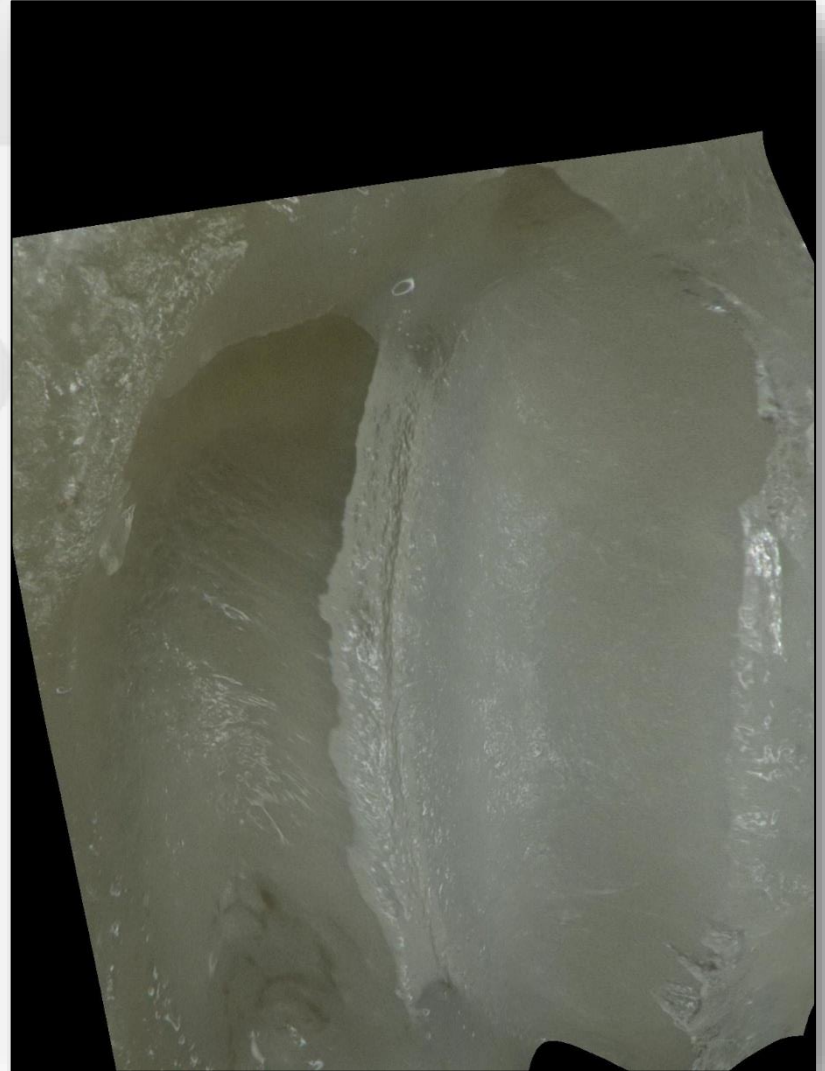


**Chromatolysis in
medulla oblongata**

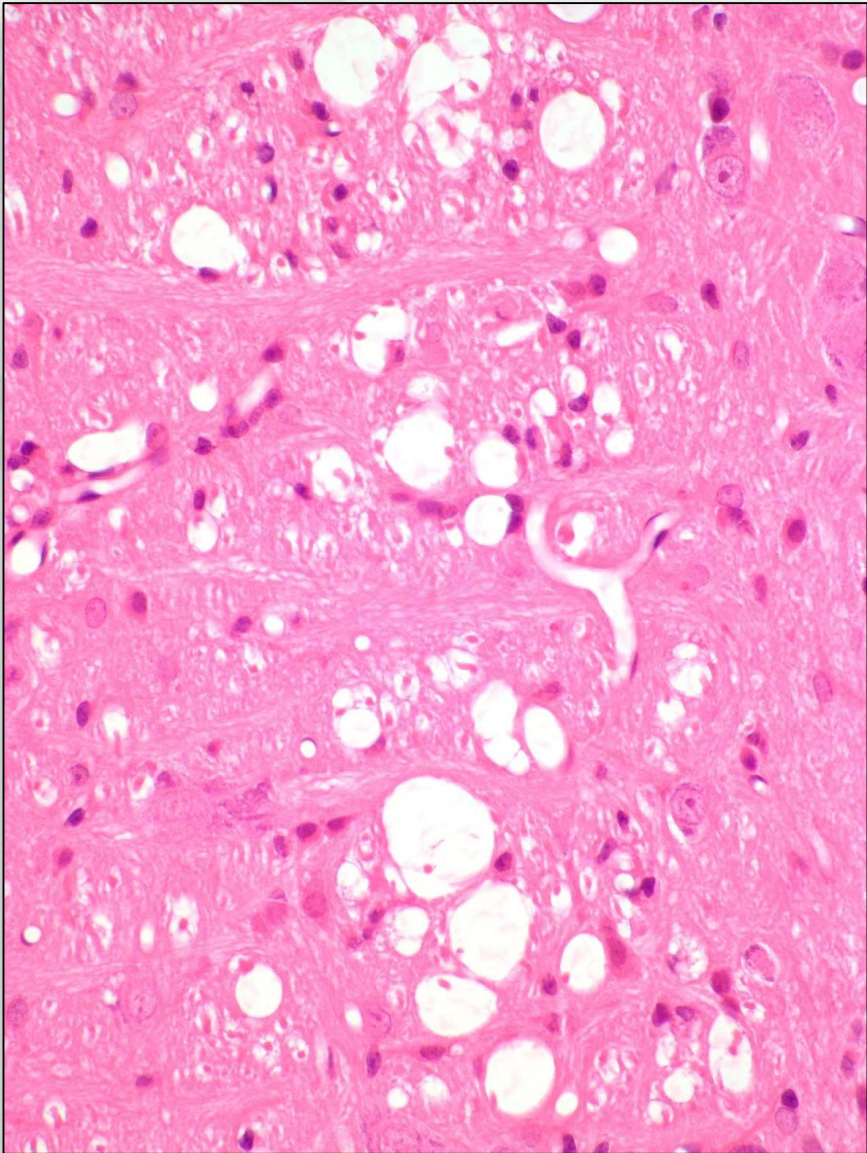
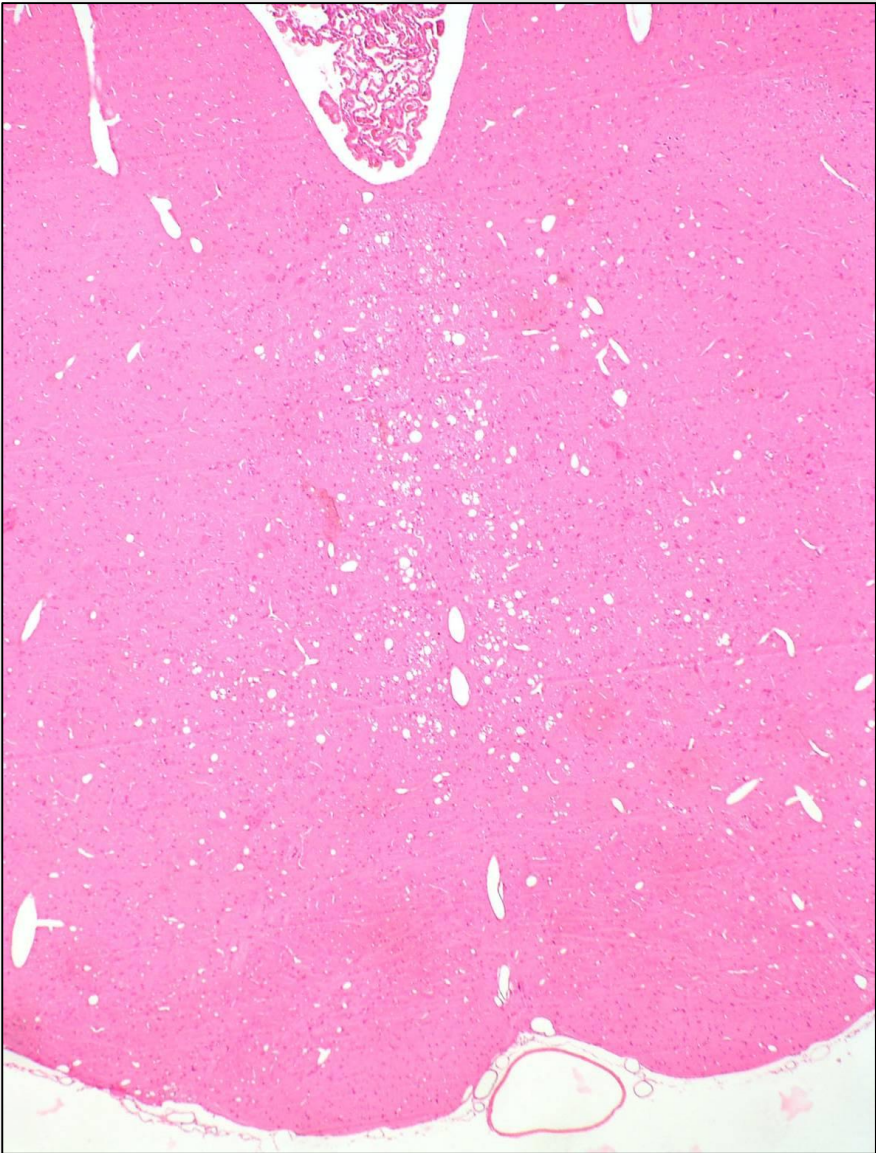
Phospholipidosis: Dog, Antibiotics



Do not forget the Cochlea !!! (Examples, Digital 3D, Laser)



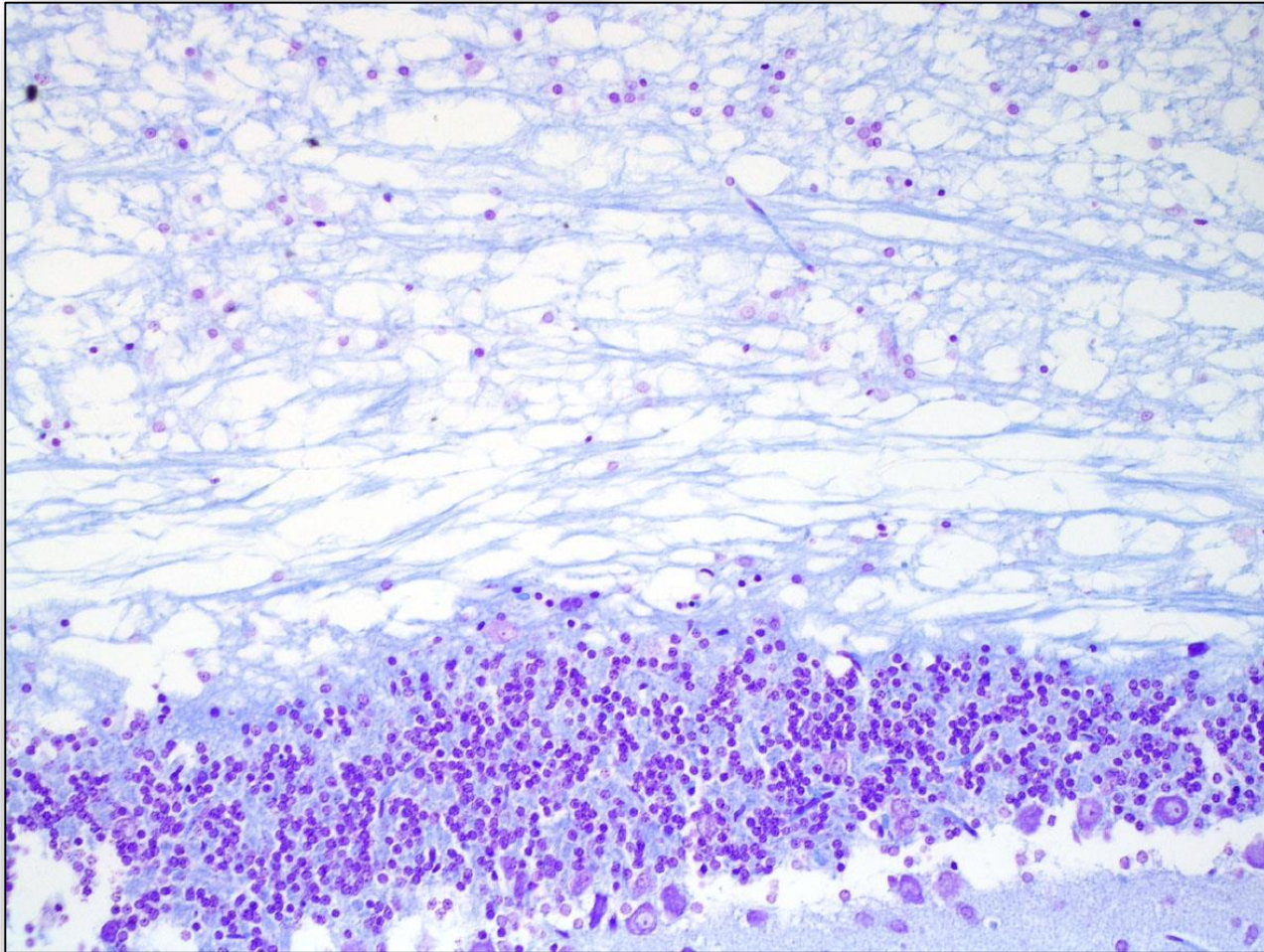
Demyelination, Rat, Diphenylhydantoin-like



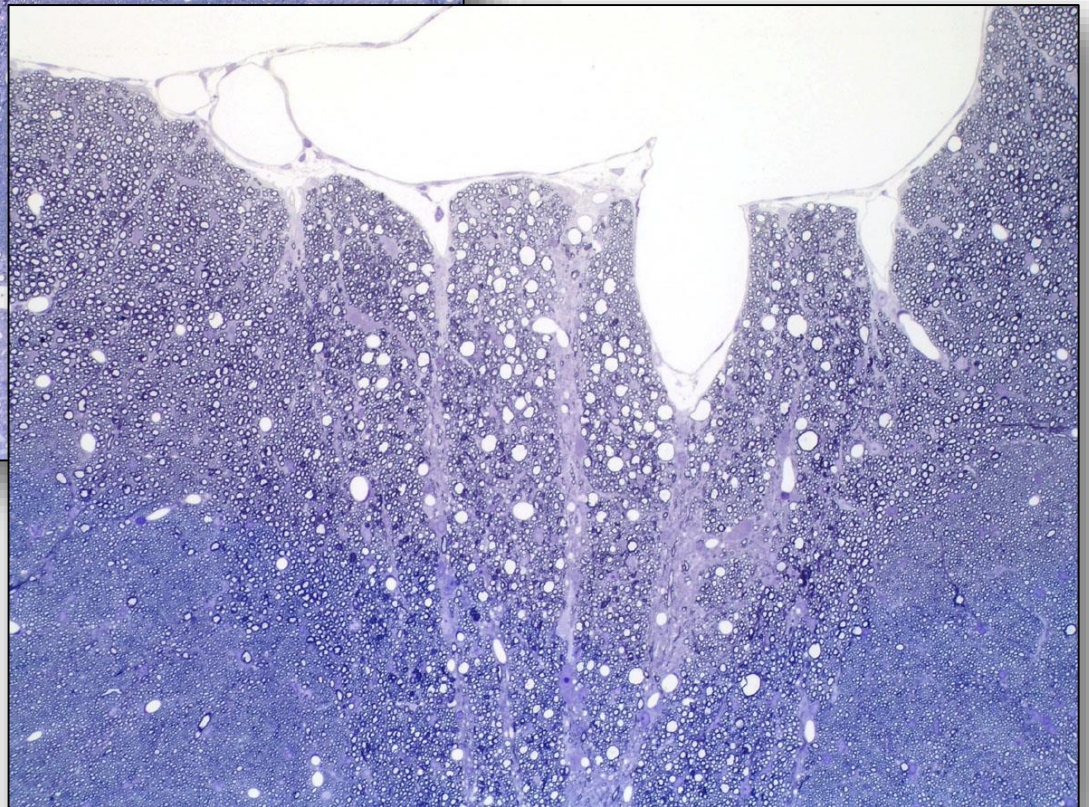
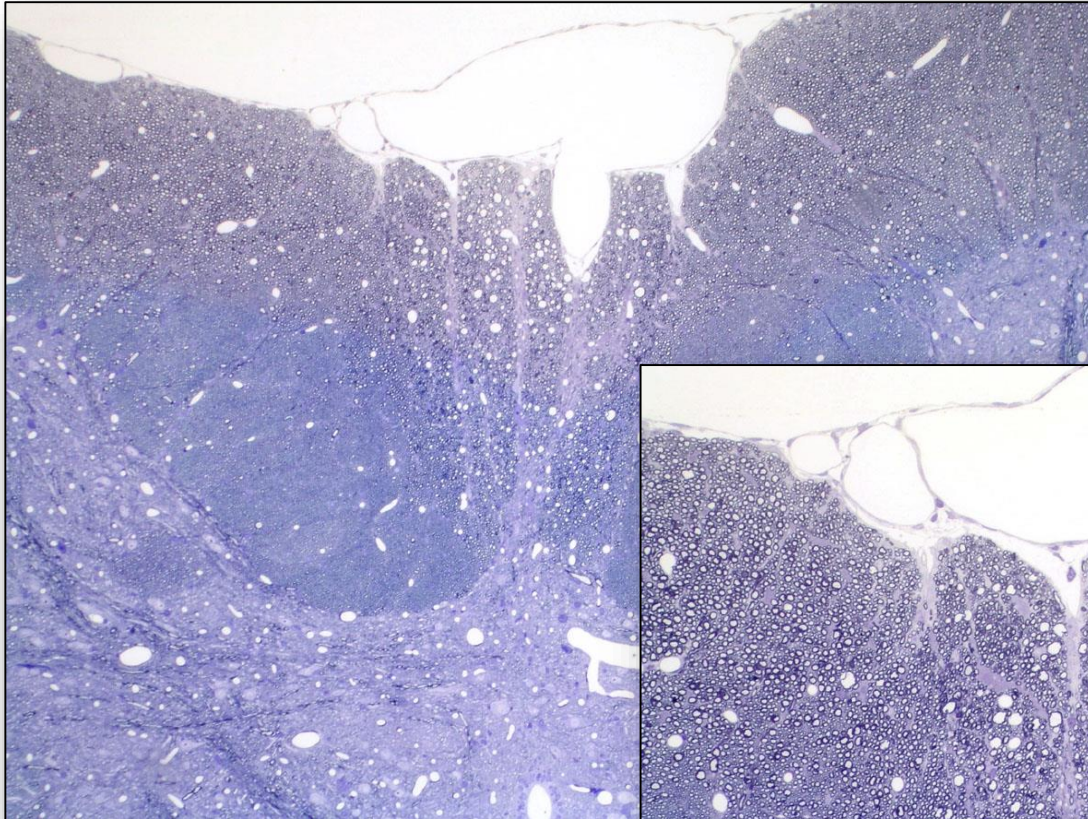
Demyelination: Mouse



Myelin edema, Induced by Hexachlorophen, Rat



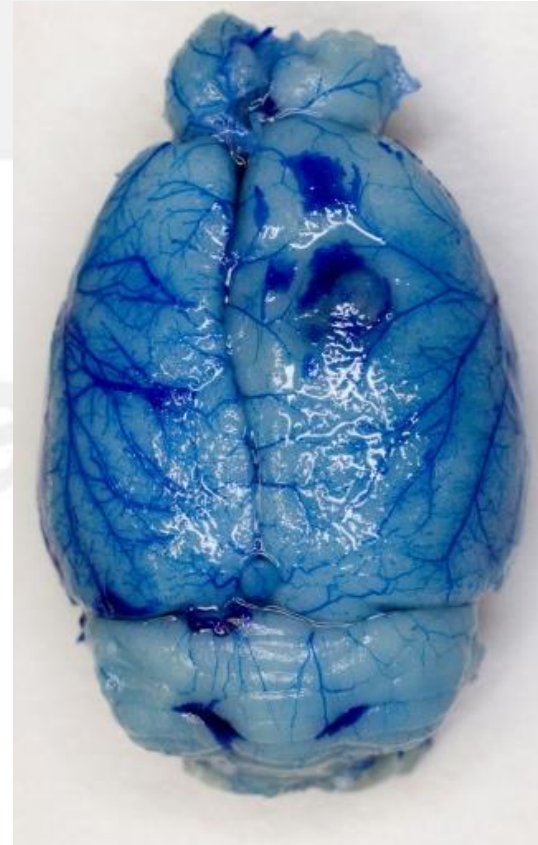
Degeneration of Goll's Tract, Rat, Sensory Fibers, Pyridoxine



Case by Dr. Georg Krinke

Angiofill (by Dept. Anatomy, University Bern)

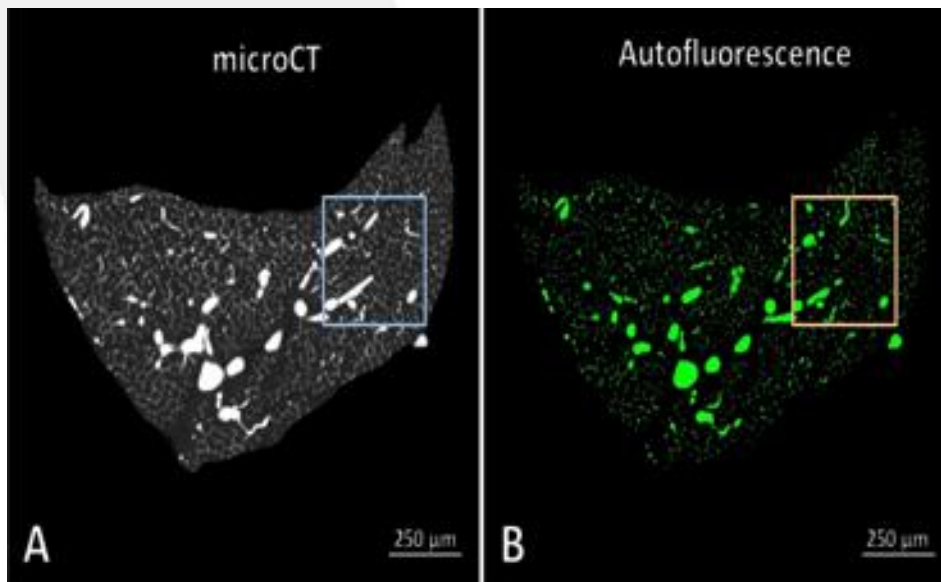
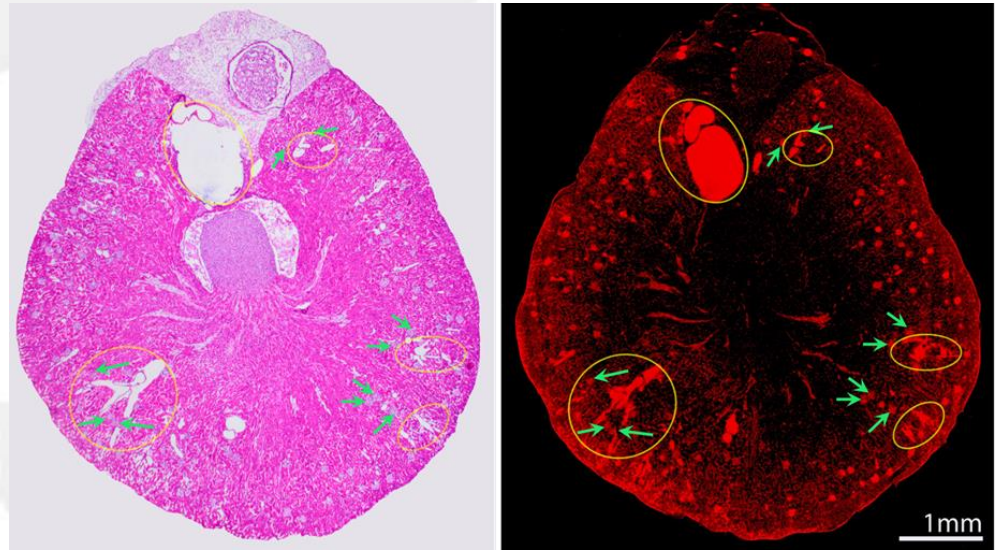
- **Perfusion of Contrast Agent**
- **Harvest Organ of Interest / Fixation**
- **μ CT Scanning**
- **Further Processing for Histology**



Macroscopical image of the perfused and fixed rat brain

Compatibility

- Histological analysis and μ CT within the same kidney
- Dissolvement of polymer
- Correlative morphology can be preformed

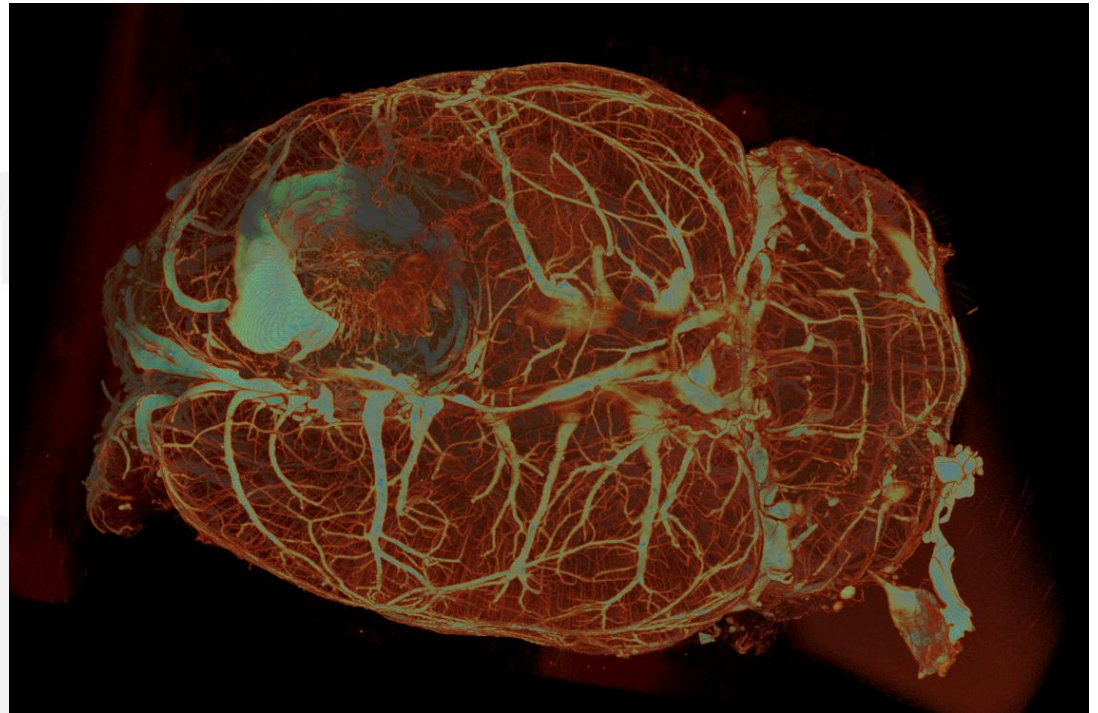


- Fluorescence signal from the contrast agent
- Remains in the vascular lumen even after the processing for histological investigation

Further Technique

Ex-vivo vascular contrast agent for microvasculature detection

- Contrast agent combined with μ CT
- Gives μ CT virtual image
- Down to $0.6\mu\text{m}$ voxel resolution (isotropic)



9L gliosarcoma untreated day 24 post-implantation (rat brain)

Summary

- **Understanding basic morphology and physiology**
- **Check clinical symptoms incl. FOB**
- **H&E provide most information**
- **Symmetrical lesions in CNS**
- **PNS lesions following clear rules**
- **Special stains for better visualization**
- **Mapping may be helpful in recognition**
- **Advanced technology may require help by ,centers‘**

We watch the further development!!

