Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #20 **15 February 2023**

CASE I:

Signalment:
14-week-old, male, Saanen kid

History:
One of five kids from a herd of 60 goats that developed progressive onset hindlimb ataxia and sternal recumbency terminally.

Gross Pathology:
No specific findings. CNS appeared normal.

Laboratory Results:
Liver copper 0.01 mmol/kq, (normal range 0.06 – 2.5 mmol/kg – values for sheep)
Liver molybdenum 9.54 µmol/kq,(normal range 15.6 – 62.0 µmol/kg – values for sheep)

Microscopic Description:
Cerebellum: multifocally, Purkinje cells are lost, and a small number are shrunken/angulated with cytoplasmic eosinophilia and karyopyknosis. Ectopic Purkinje cells noted within an irregularly thinned granular layer. Concurrent prominence of reactive Bergmann glial cells. Occasional light perivascular cuffs of admixed lymphocytes and plasmacytes noted in cerebrum. No significant changes noted elsewhere in brain or in spinal cord.

Contributor's Morphologic Diagnoses:
Cerebellum: degeneration and dysplasia (particularly affecting Purkinje cells), multifocal, moderate

Contributor's Comment:
A diagnosis of delayed enzootic ataxia (swayback) in this goat kid was based on: signalment, clinical signs, the microscopically visible cerebellar lesions and low liver copper concentrations. This neurological disease of sheep and goats is generally accepted to result from a primary deficiency in the intake of copper or a secondary deficiency caused the reduced absorption/availability of copper during pregnancy.[3] In the secondary form dietary molybdenum, zinc, iron and cadmium can interfere with copper absorption.[1,6] Molybdenum is an important antagonist for copper as (in combination with sulfate), it forms thiomolybdates in the rumen which can complex with copper, limiting its absorption.[3] All three affected kids had liver copper concentrations below the normal range. There was no history of swayback in this goat herd and the dams' diet consisted of grass, hay and sheep concentrate (unknown quantity). The

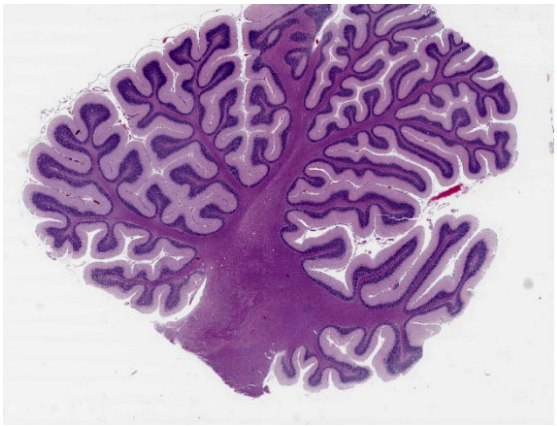

Figure 1-1. Cerebellum, goat kid. A section of cerebellum is submitted for examination. The granular cell layer is diffusely and mildly thinned. (HE, 7X)

goats had access to mineral licks/blocks and were being housed at night.

Clinically, two forms of enzootic ataxia in goat kids and lambs are described: a congenital form, more commonly reported in lambs develops in utero and manifests in the first few days of life; and a delayed form in both lambs and kids up to six months of age.[1,2] Affected animals develop progressive neurologic signs such as ataxia (swaying), hindlimb paralysis, and blindness. Lesions are found in the cerebrum, brainstem, and spinal cord in the congenital form, but are limited to the brainstem and cord in the delayed form. Cerebral lesions may be found in approximately 50% of congenitally infected lambs but rarely in kids: these consist of bilaterally symmetrical cavities in the white matter.[3,6] Microscopically visible lesions in both white and gray matter regions of the brainstem and spinal cord in both congenital and delayed forms are similar in both goats and sheep. Neurons in the medullary reticular, red, and lateral vestibular nuclei in the brainstem and in the lateral and ventral spinal cord exhibit degenerative changes: chromatolysis, cytoplasmic eosinophilia and pyknosis/margination of nuclei. Wallerian-type axonal degeneration is observed in dorsolateral and ventromedial spinal cord funiculi.[3,6] Ultrastructural observations in the goat and the particular involvement of neurons having long processes within the CNS or extending into peripheral nerves, suggest that the neuraxon rather than its myelin sheath is targeted in delayed enzootic ataxia.[14] While myelin degradation in this eventuality would then be expected as a secondary event, myelin in this context is qualitatively abnormal and therefore potentially unstable.[3] Accompanying astrogliosis is mild.[3,6] Cerebellar cortical lesions, particularly targeting Purkinje cells as described here are reported more commonly in goats than in sheep.[2,14]

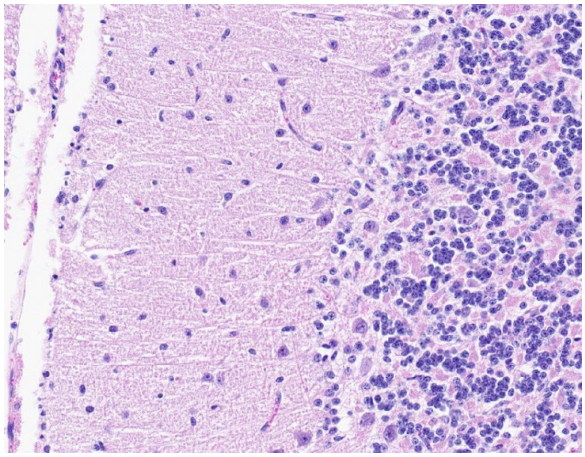

Figure 1-2. Cerebellum, goat kid. There is segmental loss of Purkinje cells and proliferation of Bergmann's glial cells within the molecular layer (HE, 400X)

Copper is required for the activity of several enzymes essential for neural function, including cytochrome oxidase (mitochondrial respiration) and copper-zinc superoxide dismutase (antioxidant activity). Suggested mechanisms contributing to the development of these lesions include altered function of cytochrome oxidase in mitochondria leading to suppression of mitochondrial respiration and reduced phospholipid synthesis. This energy failure is likely to result in axonal/neuronal degeneration. The generation of excessive reactive oxygen species as a consequence of inadequate function of superoxide dismutase ultimately results in oxidative injury within the CNS.[3,6]

Copper deficiency is more commonly encountered in grazing animals because of the poor availability of this element in grass. Foodstuffs from which copper is more readily absorbed are those low in fiber such as cereals. Preservation of grass in the form of hay or silage improves copper availability.[2] The fact that goats have a lower capacity to store copper in the liver than do sheep[10,14] would suggest this species requires a higher daily intake. In previous instances of enzootic ataxia in goats, sheep concentrate, low in copper, had been used as supplementary food. No

further cases occurred following the replacement of sheep concentrate by concentrate appropriate for cattle and horses.[14]

Differential diagnoses in this case would include white muscle disease, spinal trauma/vertebral body abscess, caprine arthritis encephalitis, meningoencephalitis, and possible toxicity (lead, organophosphate). Enzootic ataxia may be prevented through adequate copper supplementation of the dam during the latter half of pregnancy.[2]

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JPC Diagnosis:
Cerebellum: Purkinje and granular cell loss, segmental, moderate.

JPC Comment:
The contributor provides a good overview of the gross lesions, histologic findings, and pathogenic features of this classic entity in small ruminants. Another example of copper deficiency in a young goat was seen in WSC 2021 Conference 2 Case 1. Readers are encouraged to review that case which also demonstrated neuroaxonal degeneration in the spinal cord secondary to copper deficiency. Copper deficiency has also been implicated as a cause of laryngeal neuropathy in adult goats. In a 2017 *Veterinary Pathology* study, researchers described goats with severe copper deficiency that had both ataxia and stridor, while moderately affected goats had ataxia without stridor.[12] The severely affected goats demonstrated atrophy of laryngeal muscles appreciable both grossly and histologically, with Wallerian degeneration of the recurrent laryngeal nerve.[12] Interestingly, both sets of goats also had increased serum iron levels, with the more severely affected goats having higher levels, indicating that high dietary iron may have led to copper deficiency.[12]

As the contributor describes, copper is an essential cofactor in the activity of many enzymes in the body. Copper is an essential component of tyrosinase, which is involved in the rate-limiting step in melanin synthesis, and deficiency results in depigmentation of the hair or wool, causing a rusty brown discoloration and the formation of spectacles in the fur around the eyes (periocular achromotrichia).[5,13] Copper is also important for oxidation of sulfhydryl groups during keratinization, and deficiency causes hair shafts in sheep to be straighter with less

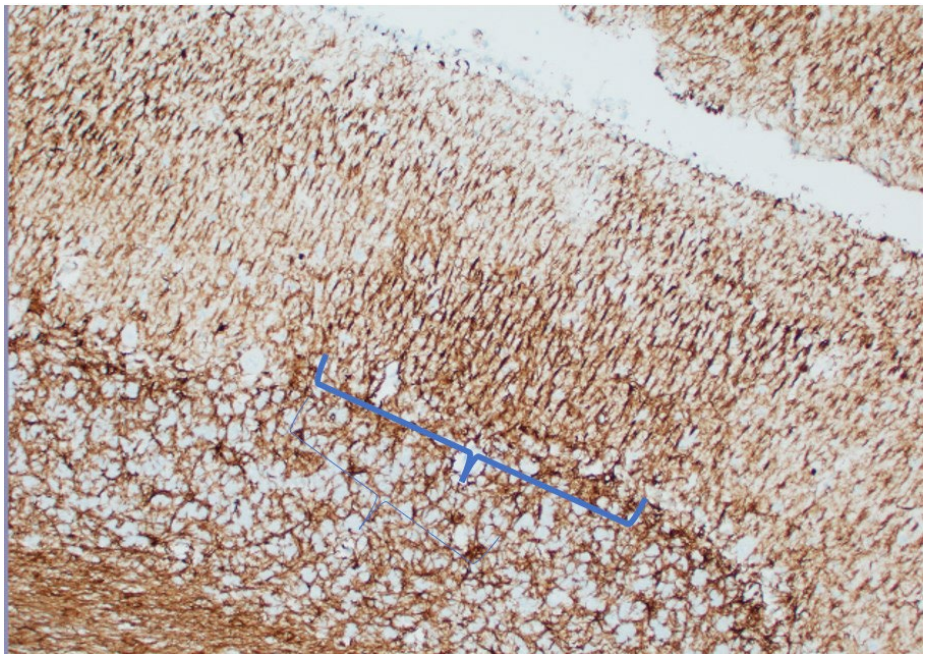

Figure 1-3. Cerebellum, goat kid. A GFAP stain demonstrates the proliferation of Bergmann's astocytic fibers in areas of Purkinje cell loss (bracket). (GFAP, 200X)

crimp ("string" or "steely" wool).[5,13] Alterations in coat color and texture due to copper deficiency have been reported in dogs, cattle, sheep, and moose.[5]

Copper is also a component of lysyl oxidase and is required for the proper crosslinking of collagen and elastin.[4] Copper deficiency can lead to fragile bones and cartilage in dogs, lambs, foals, calves, and pigs.[4] In dogs, copper deficiency is associated with decreased osteoblast activity and can produce metaphyseal lesions similar to the scorbutic lattice of vitamin C deficiency.[4] In foals, copper deficiency may cause the articular epiphyseal cartilage complex to lyse which may resemble osteochondrosis.[9]

Prolonged copper deficiency has been associated with sudden death and myocardial scarring in cattle in Australia and Florida ("falling disease").[11] In a recent study on the effect of prolonged copper deficiency on cardiac function, researchers found histologically apparent fibrosis in the ventricular myocardium, severe decrease in copper-zinc superoxide dismutase activity, and significantly increased levels of lipid peroxidation byproducts.[8]

This week's moderator, LTC Keith Koistinen, Chief of Diagnostic Pathology at the Joint Pathology Center, described the function of Bergmann glial cells (specialized radial glia astrocytes) which are prominent in this case: to provide scaffolding for the migration of Purkinje cells. Bergmann glial cells are difficult to distinguish solely on H&E. In this case, the molecular layer looks slightly denser in the areas of Purkinje cell loss, suggesting proliferation of Bergmann glial cells; however, definitive identification of Bergmann glial cells requires GFAP immunohistochemistry. The moderator and participants also remarked on the fact that Purkinje cells were present in the granular layer, reflective of defective migration referenced in the contributor's morphologic diagnosis of Purkinje cell dysplasia. The moderator also described that the best tissue for submission for copper analysis is brains for neonates and liver for adults.

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CASE II:

Signalment:
6-month-old, Hereford steer (*Bos taurus*)

History:
A 6-month-old Hereford steer presented to the Texas A&M Large Animal Food Animal Medicine and Surgery with a history of acute onset of being unable to rise. At presentation, the steer was recumbent and experiencing seizures. On neurological examination, he was ataxic, head-pressing and appeared blind. His condition was unresponsive to medical intervention, and the decision was made to humanely euthanize and perform a necropsy.

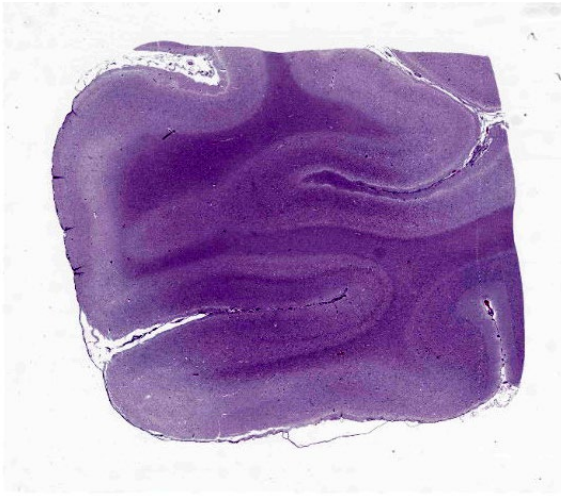

Figure 2-1. Cerebrum, ox. A section of cerebrum is submitted for examination. There are segmental areas of pallor beneath the meninges. (HE 6X)

Gross Pathology:
Flared metaphyses of the ribs with retained calcified cartilage and mild cerebral edema.

Laboratory Results:
Blood lead level= 0.71 ppm (lab normal=0-0.3 ppm); Post-mortem analysis of renal tissue (after formalin fixation) = 6.2 ppm (>3 ppm indicative of toxicity). Radiographs of ribs revealed a flared physis and a radiodense band at the metaphysis.

Microscopic Description:
Cerebrum. The deep cerebrocortical interface has a laminar zone of rarefaction (edema), and many neurons are diffusely hypereosinophilic, shrunken/polygonal with pyknotic nuclei (neuronal necrosis) or are lost. Endothelial cells are swollen. Reactive astrocytes are numerous (gliosis). Occasional vessels have a few surrounding neutrophils. Rarely, the nuclei of neurons have 2-4 µm, round, eosinophilic (lead) inclusions.

Contributor's Morphologic Diagnoses:
Severe, diffuse, laminar cortical neuronal necrosis with gliosis, edema and rare intraneuronal intranuclear eosinophilic inclusions (lead inclusions).

Contributor's Comment:
The clinical signs related to lead poisoning are predominantly neurologic and include depression, inappetence, staggers, muscle tremors, bruxism, head pressing, convulsions, blindness, and death.[1,14] Acute poisoning in cattle often results in death within 12-24 hours.[1] Gross lesions are not striking in cases of lead poisoning but may include brain swelling, hemorrhage, or meningeal and cerebrovascular congestion.[14] Microscopic lesions in acute cases may be confused with early autolysis.[1] In subacute cases, microscopic lesions of laminar cortical necrosis of the cerebrocortical gyri, astrocytosis, perivascular edema or hemorrhage.[14] Other differentials for bovine laminar cerebrocortical neuronal necrosis include, thiamine deficiency and consuming diets or water containing high sulphate (both causing ruminant polioencephalomalacia), or as a residual lesion of cyanide poisoning.[1] This case is remarkable for the number of mitotic figures along with the significant neuronal necrosis. The mitotic population is presumably glial cells responding to the neuronal necrosis, but the population is negative with GFAP.

While multiple species may be affected with lead intoxication, cattle are 10 times more likely to be affected than dogs, cats, or horses.[10] The disease is often acute in cattle and chronic in horses.[1] Sheep are less commonly affected and swine are rarely affected.[1] A retrospective, case-controlled study of cattle at North American veterinary school hospitals revealed a seasonality to lead intoxication cases with the most being reported in the late spring and summer months and an association between age and likelihood of exposure to lead with cattle less than four years of age at an increased risk.[8] The highest risk age group are cattle between 2 and 6 months of age.[8] Calves have higher exposure than adults, which is often from licking painted troughs, walls, etc. while confined. Adult cattle on pasture are often exposed through accidental ingestion of discarded car batteries, old lead paint on farm

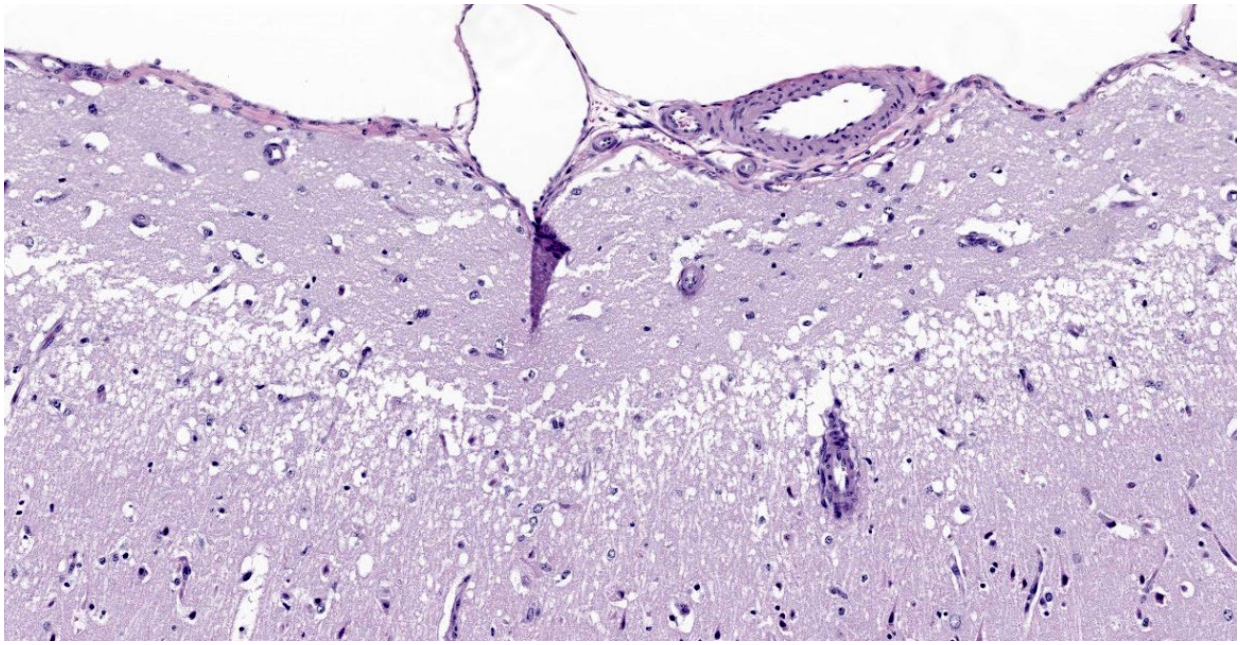

Figure 2-2. Cerebrum, ox. Higher magnification of a laminar area of rarified submeningeal grey matter. (HE 150X)

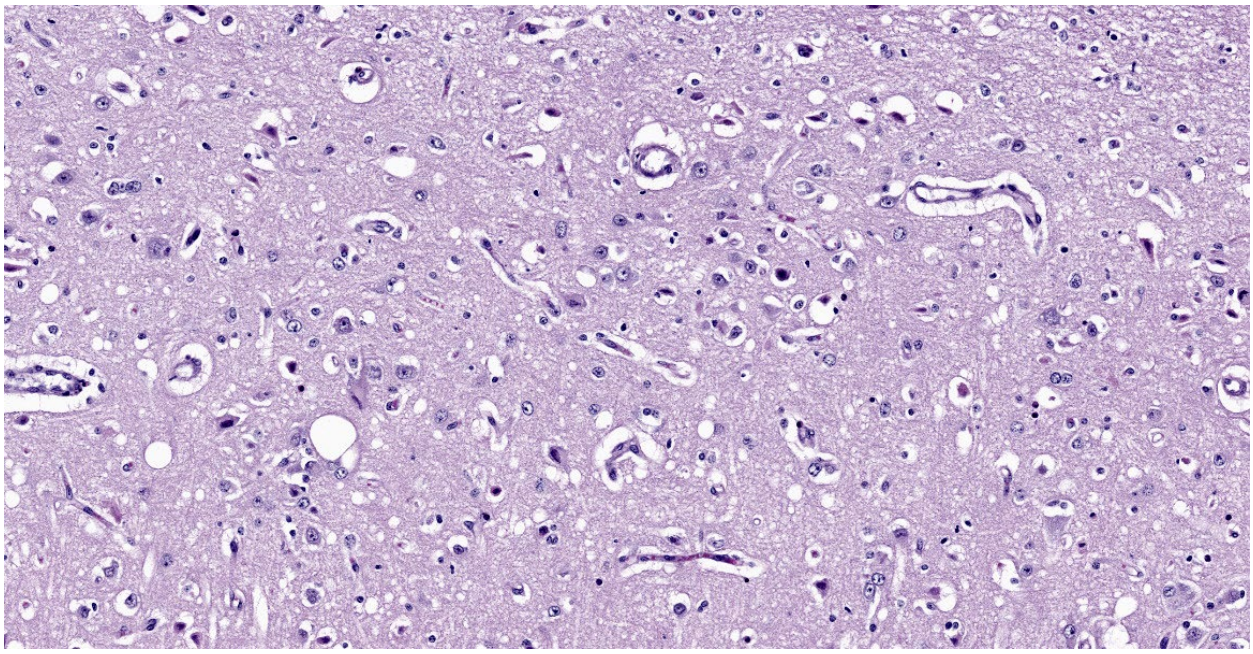

Figure 2-3. Cerebrum, ox. Areas of necrosis are gliotic and contain shrunken neurons and pyknotic glial cells. (HE 170X)

buildings or fences, or old asphalt shingles.[4,14] Experimental models of lead intoxication do not reach the levels of naturally acquired lead intoxication, which may be due to the formation of lead salts that increases ingestion of lead contaminated objects.[1] Other sources of environmental contamination include land near highways with historical lead accumulation in the soil from leaded gasoline exhaust, land near clay pigeon shooting ranges, industrial pollution, accumulation in plants, or fertilizers and pesticides.[5,9,12]

In lead intoxication the laminar necrosis does not autofluoresce under UV-light, suggesting a different pathogenesis from thiamine-related necrosis. The mechanism of action in lead intoxication is not well defined. The two theories of lead toxicosis are ionic mechanism and an oxidative stress mechanism. [2] The ionic mechanism proposes that lead metal ions cross the blood brain barrier using cationic transporters and replace other bivalent ions like Ca^{2+}, Mg^{2+} or Fe^{2+}.[5,14] The direct neuronal toxicity is presumed to be due to altered function of the dopaminergic, cholinergic and glutamatergic neurotransmitter systems.[14] Lead accumulates within neurons and astrocytes by utilizing a calcium transporter channel and disrupts calcium homeostasis, which leads to calcium release from the mitochondria with a subsequent increase in apoptosis. The oxidative stress mechanism proposes that lead toxicosis causes the levels of reactive oxygen species to increase while concurrently decreasing the antioxidant levels of proteins such as glutathione.[5]

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JPC Diagnosis:
Cerebrum: Neuronal necrosis and loss, cortical, laminar, multifocal, with gliosis and edema.

JPC Comment:
This entity was last seen in Wednesday Slide Conference during Conference 10, Case 2 of 2021-2022. That case demonstrated lead toxicosis in the kidney of an African penguin, and readers are encouraged to review that case which details the features of disease in avian species and a brief review of the history of lead toxicity. During that conference, a recent *Vet Pathol* article was discussed which described lead intoxication in bald eagles; in this species, lead toxicosis leads to fibrinoid necrosis in arterioles of the heart, brain, and eyes leading to petechia, hemorrhagic necrosis, and ischemia in these organs.[7]

In the gross findings of this bovine case, the contributor describes flared metaphyses of the ribs and retained calcified cartilage, reflecting the effect of lead toxicity on bone. Lead inhibits the function of osteoclasts, which in growing animals normally resorb mineralized cartilage and woven bone of the primary spongiosa, making way for lamellar bone of the secondary spongiosa during endochondral ossification. [3] In lead toxicosis, osteoclasts fail to resorb bone and, histologically, osteoclasts are detached from the surface of trabeculae and may contain characteristic acid-fast eosinophilic occlusions.[2] Grossly, there is a band of sclerosis and flaring of the metaphysis due to retained mineralized cartilage, and radiographically, this produces a characteristic lead line.[2]

Lead toxicosis can affect other systems as well. During erythropoiesis, lead inhibits heme synthesis and causes a nonregenerative, hypochromic, microcytic anemia with circulating sideroblasts. Lead also causes retention of RNA, leading to basophilic stippling.[11] Lead quickly accumulates in the kidney and concentrates in the tubular epithelium nucleus, where inclusions are readily apparent (the moderator noted that eosinophilic intranuclear inclusions can be normal findings in healthy dogs). Lead inhibits mitochondrial respiration and in acute toxicosis can cause apoptosis of proximal tubular epithelium.[6] In rabbits, lead toxicosis causes outer retinal degeneration secondary to lipofuscin buildup, and in rats, it causes necrosis of the photoreceptor cells and inner nuclear layer of the retina.[13]

Conference participants and the moderator debated on the presence of eosinophilic intranuclear inclusions and could not reach a consensus on whether they were present. Acid-fast staining by JPC did not confirm the presence in this case.

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CASE III:

Signalment:
Male, Sprague-Dawley rat (*Rattus norvegicus)*

History:
This rat was administered a test article to induce seizures in order to evaluate the effectiveness of anticonvulsant therapeutics.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Cerebrum at the level of the thalamus and hippocampus: Multifocally within the cerebral cortex, piriform cortex, amygdala, hippocampus, thalamus, caudate nucleus, and putamen, there is neuronal degeneration and necrosis characterized by shrunken, angular, hypereosinophilic cytoplasm of neuron cell bodies. There is rarefaction of the white matter immediately adjacent to affected neuron cell bodies.

Contributor's Morphologic Diagnoses:
Cerebrum at the level of the thalamus and hippocampus: Neuronal degeneration and necrosis, multifocal, severe, with rarefaction.

Contributor's Comment:
Due to low cost and ease of manufacturing of chemical warfare agents, there is an increased likelihood of use of chemical warfare agents by terrorists, as well as, by various governments. Relatively recent examples of chemical attacks include the use of chlorine gas by Al Qaeda in 2006 and 2007, the use of various chemicals by ISIS in 2015 and 2016, and the poisoning of a former Russian agent in 2018. One of the most notable examples of a chemical warfare agent being deployed is the use of the nerve agent sarin by members of the Aum Shinrikyo religious cult in Japan in 1995 that killed 12 and sickened 5,000 others.

In order to decrease the likelihood of future chemical warfare agent use there is a focus on strategies to research and mitigate the negative physiologic effects of various agents so that their deployment is useless as effective antidotes are readily available.

Mechanism of Action of Nerve Agents. The chemical warfare (CW) nerve agents include the anticholinesterase nerve agents tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), and VX, all of which are, or have been, part of the US domestic munitions inventories.[8] These agents are potent anticholinesterase compounds deliberately formulated to induce debilitating effects or death during wartime hostilities and have been used by military authorities of several nations to develop munitions (e.g., Germany during the Nazi era, the USA, and the former Soviet Union).[8]

All of the listed nerve agents are anticholinesterase compounds and induce accumulation of the neurotransmitter acetylcholine (ACh) at neural synapses and neuromuscular junctions by of nerve agents can result in excessive bronchial, salivary, ocular and intestinal secretions, sweating, miosis, bronchospasm, intestinal hypermotility, bradycardia, muscle fasciculations, twitching, weakness, paralysis, loss of consciousness, convulsions, depression of the central respiratory drive, and death.[8]

Nerve Agent-Associated Injury to the Nervous System. Nerve agents exert their neuropathologic effects through binding and irreversible inactivation of acetylcholinesterase (AChE), the enzyme that hydrolyzes ACh, leading to a toxic accumulation of ACh at nicotinic (skeletal muscle and preganglionic autonomic) receptors, muscarinic (mainly postganglionic parasympathetic) receptors, and central nervous system synapses.[9]

Historically, this has been of concern in organophosphate and carbamate insecticide exposure among farm workers; however, it is likely that use of nerve agents in civilian populations or against military personnel would result in a range of exposures that stem from both the proximity of various groups to the site of deployment and the persistence of residues of some agents in the environment.[9]

Common nerve agents developed for chemical warfare purposes include the G-series (so names because they were first developed by German scientists in the mid-1930s) and V-series (a designation of more ambiguous origin) weapons.[9] As previously mentioned, the G-series includes tabun (GA), sarin (GB), soman (GD), and cyclosarin (GF), while the V-series includes VX, first synthesized by the British in 1954.[9] The G-series compounds are volatile liquids at room temperature, are soluble in both fat and water, and are absorbed readily through the eyes, respiratory tract, and skin.[9] V-series agents are viscous and toxic mainly via skin exposure, and therefore pose a lower inhalation hazard than the G-agents; however, they are notoriously persistent in the environment.[9]

Acute exposure to nerve agents is associated with a range of clinical symptoms, varying from abnormal movements and salivation to limb tremor and muscle fasciculations, to convulsions.[9] In a variety of animal and especially rodent models, animals that survived

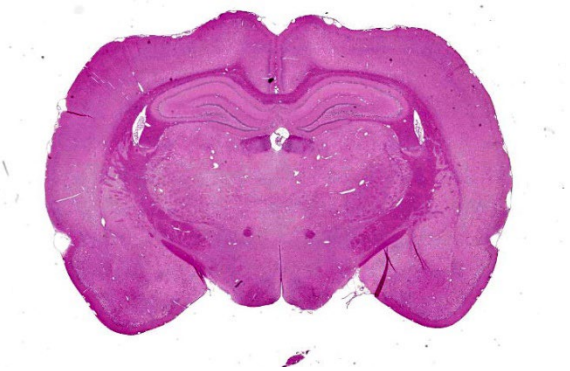

Figure 3-1. Diencephalon, rat. A transverse section of brain at the level of the diencephalon. (HE, 6X)

seizures tended to manifest extensive bilateral brain neuronal necrosis that affected predominantly the forebrain, thalamus, tegmentum, and spinal cord.[9] Acute injury may be attributable to several mechanisms.[9]

Ischemic hypoxia may derive from respiratory insufficiency during prolonged seizures, and evidence of cellular ischemia is present in brains of exposed animals.[9] This consists of shrinkage of the cell soma and proximal dendrites, cytoplasmic microvacuolation due to mitochondrial swelling, dispersion of Nissl substance (cytoplasmic RNA), increased cytoplasmic eosinophilia, nuclear changes including displacement of the nucleus to an eccentric position in the neuron, shrinkage, and darkening.[9] Generally, these nerve agent-associated lesions were described as indistinguishable from those associated with brain ischemia or anoxia.[9]

Within minutes after exposure to nerve agents, there is a marked decrease in AChE activity and associated rise in ACh.[9] The earliest seizure activity begins in the absence of other significant neurotransmitter alterations and is prevented by anticholinergic drugs.[9] These observations suggest that seizure-associated neuropathologic findings that occur upon nerve agent exposure are caused primarily by a mechanism of cholinergic toxicity.[9]

However, if seizures progress untreated, other neurotransmitter systems display secondary alterations, and the involvement of these has been invoked in models of injury to cerebrum that involve mechanism of "excitotoxic" injury.[9] Specifically, these refer to the involvement of the excitatory amino acid transmitter glutamate, which increases intracellular calcium mobilization.[9]

In excitotoxicity, overstimulation of glutamatergic synapses leads to marked neuronal calcium dyshomoeostasis that in turn leads to neuronal injury.[9] Importantly, pretreatment or early post-exposure treatment of experimental animals with anticonvulsants (e.g., benzodiazepines such as diazepam), blocks nerve agent-associated seizures which in turn prevents or diminishes neuropathologic effects.[9] This occurs in the absence of a direct effect on cholinergic processes.[9] These observations, taken together, suggest strongly that excitotoxic mechanisms contribute largely to the structural changes observed in the cerebrum upon nerve agent exposures.[9]

Abrogation of these mechanisms by anticholinergic drugs within 20-40 min of the onset of seizures is sufficient in most cases to significantly diminish neuropathologic lesions.[9] The failure of anticholinergic drugs to prevent nerve agent-associate pathologic changes after this time period has been attributed to the recruitment and dominance of non-cholinergic mechanisms of excitotoxicity and perhaps to secondary loss of the integrity of the blood-brain barrier.[9]

Technical Considerations for Toxicologic Neuropathology. Cost-effective, high-quality neuropathologic evaluations require teamwork involving the recording and assessment of detailed clinical signs and gross postmortem observations, careful and timely collection and processing of tissues, and a working knowledge of both neuroanatomy and neuropathology.[4] The process begins well before the necropsy.[4] It is important for the pathologist to have knowledge of the likely target effects (distribution, biochemistry, secondary changes) of the compound so that tissue collections and evaluations can be optimized.[4]

Evaluation of murine brain tissue requires considerable expertise in the preparation of

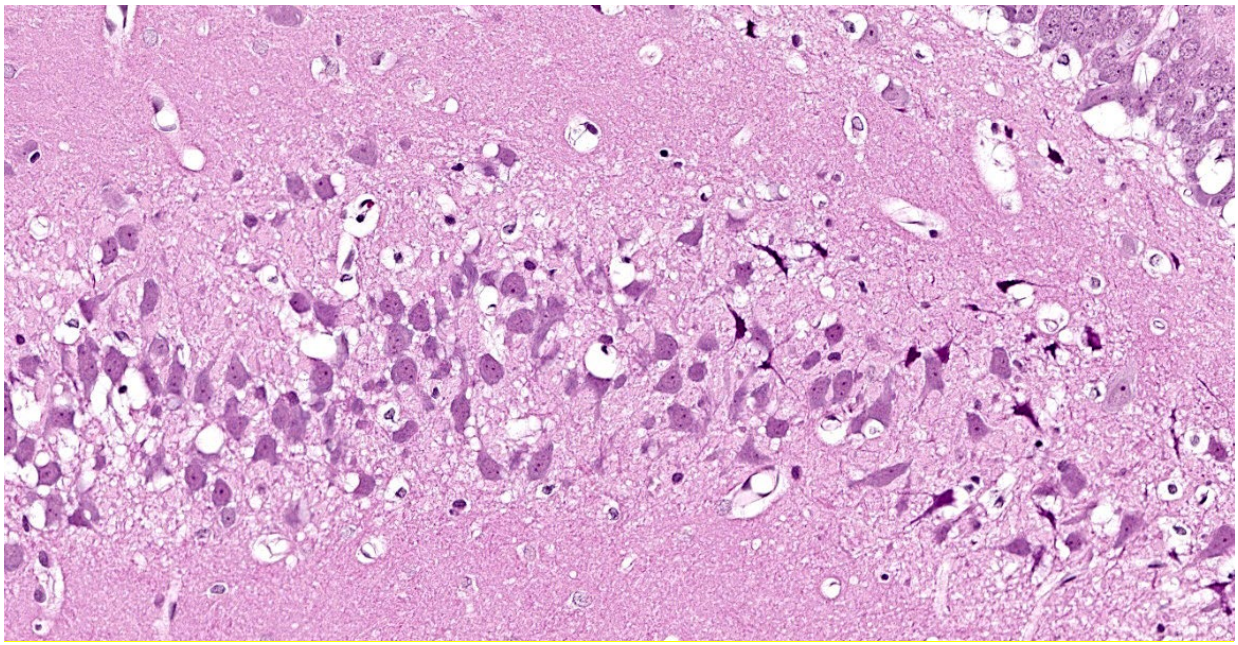

Figure 3-2. Diencephalon, rat. There are numerous degenerating and necrotic neurons within CA3 of the hippocampus. Astrocytes associated with degenerating neurons are markedly swollen by cytosolic edema (HE, 276X)

tissue samples prior to examination by a neuropathologist. The use of whole-animal perfusion techniques and specialized grossing implements (such as brain matrices) are practical and effective tools which can help ensure that virtually identical brain regions are collected and prepared from each animal within a large study.

As with many other organ systems, the most common way for nervous tissue to be fixed is by direct immersion in an aldehyde fixative solution.[3] However, whole body perfusion via the vascular system is preferred, particularly if any degree of special detail is necessary in the study.[3] Once mastered, perfusion yields reproducible, high-quality preparations of nervous tissue.[3] However, this approach requires technical dedication and practice before mastery.[3] The technique cannot simply be written and given to an inexperienced technical specialist for execution without proper instruction and training.[3] An excellent summary of the practical aspects of whole body perfusion is provided in the below referenced article by Fix and Garman.[3]

Investigators seeking to evaluate brain injuries will select a region of interest using a standard brain atlas which localizes the desired coronal section by its relationship to the sutures of the skull. By definition, the Bregma level refers to the junction of the coronal and sagittal sutures on the skull, and a coronal brain section at this level corresponds to a Bregma level of 0.0 mm.[4] Bregma coordinates represent the number of millimeters rostral (positive numbers) or caudal (negative numbers) to this plane.[4]

Rat brain atlases are developed using rats of a particular sex, strain, and weight class (e.g., adult, male, medium-sized [270 to 310 g], Wistar rats were used for Paxinos and Watson's *The Rat Brain in Stereotaxic Coordinates*, 5th edition).[6] For this reason, if rats of a different sex, strain, or weight class are used, stereotaxic accuracy will be affected.[6] In other words, if the rat brain is smaller than the medium-sized rat used for the reference atlas, the distance from the Bregma to the desired coronal section will need to be proportionately scaled down to acquire the region of interest.

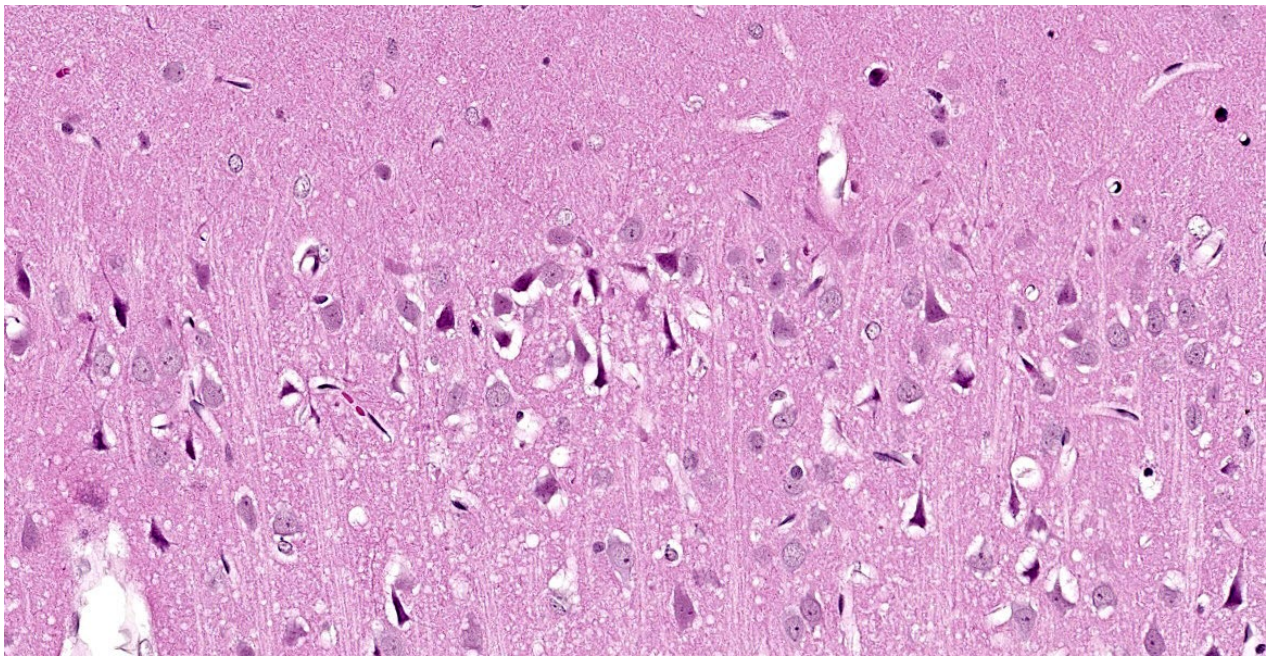

Figure 3-3. Diencephalon, rat. There are scattered degenerating and necrotic neurons within throughout the neocortex, but far fewer than in the hippocampus. (HE, 298X)

Even with a good brain atlas, the process of actually matching slides on the microscope with sites depicted in the atlas can be challenging.[4] Since histologic sections rarely match the exact plane of section depicted in an atlas, the pathologist must have a basic working knowledge of neuroanatomy and the ability to envision how the landmarks will be shifted when sections are tilted or skewed from the true coronal planes typically presented in the atlases.[4] A series of atlases coauthored by Paxinos and Watson provides good neuroanatomic references for rodents, and is available in both hard copy and in electronic format.[4]

Once the target tissue within the brain has been fixed, grossed, and embedded, the technical skill in the histology laboratory is perhaps the most important factor in arriving at a mounted section of the specific region of interest. The target tissue must be embedded with sufficient excess tissue to allow for block facing and sectioning through the block until the desired serial section is mounted on the glass slide.

The skill level of the histology technician cannot be overstated in murine brain research. First, the individual must have a detailed understanding of murine neuroanatomy. Second, he or she must possess the academic interest to study reference brain atlases and understand where the region of interest is located relative to other structures. Third, the technician must be able to recognize detailed brain structures in unstained paraffin-embedded tissue sections. Finally, the technician must be able to make adjustments if the animal's size or gender do not match the reference atlas. Microtomy is, by its nature, a destructive process since the technician must section through the block until the desired section is exposed. Because of this, the technician must know when to stop sectioning so that the region of interest is not inadvertently consumed.

For pathologists with an interest in toxicologic neuropathology, there is an excellent compilation of resources, designed to consolidate a broad range of useful neurobiology, neuropathology, and neurotoxicology resources in a single reference.[1]

Contributing Institution:
https://usamricd.apgea.army.mil/

JPC Diagnosis:
Cerebrum, hippocampus and thalamus: Neuronal degeneration and necrosis, multifocal and segmental, with rarefaction.

JPC Comment:
The moderator remarked on the remarkable preservation of this tissue achieved by perfusion and described the histologic evidence of perfusion: dilated (non-retracted) empty blood vessels and reduced dark neuron artifact.

This case illustrates the classic but nonspecific lesion of acute eosinophilic (acidophilic) neuronal degeneration and necrosis. In this case, neuronal necrosis is due to the effects of a nerve agent, which, as the contributor describes, works in a few ways: by causing direct neuronal acetylcholine toxicity by inhibiting acetylcholinesterase, by causing excitatory nerve injury, and by causing ischemia due to respiratory depression secondary to prolonged seizure activity. Acute eosinophilic neuronal necrosis can occur in other conditions as well, including ischemia or hypoxia, thiamine deficiency, heavy metal toxicosis (including lead and organic mercury), hypoglycemia, and inflammation.[5,7] Neurons are acutely sensitive to ischemic and hypoxic injury because they maintain minimal energy stores.[2] When deprived of oxygen due (i.e. ischemia or cardiac arrest), the most sensitive neurons in the cerebral cortex die within 10 minutes due to decreased ATP generation.[2] Neurons in the cerebral cortex, Purkinje cells, and the hippocampus are the most sensitive to ischemic injury; this phenomenon is referred to as selective neuronal vulnerability.[2,5] Other causes of decreased ATP generation leading to neuronal necrosis include cyanide, which interferes with mitochondrial cytochrome oxidase activity during cellular respiration, and carbon monoxide, which prevents oxygenation of peripheral tissues. [2,5] For the same reason, hypoglycemia (i.e. due to a functional insulinoma) also causes neuronal necrosis, and, in general, neurons display the same sensitivity to hypoglycemia as ischemia (with the exception of Purkinje cells, which are more resistant to hypoglycemic injury).[2]

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CASE IV:

Signalment:
5-month-old, intact male, Labrador, C*anis familiaris,* canine

History:
This dog was presented to the emergency service of a veterinary clinic. Owners reported that he was alert in the morning and ate well. He suddenly became lethargic, and they rapidly brought him to the clinic. On arrival, his temperature was elevated (39,4 ºC), but returned to normal values within an hour. He was in a lethargic state that gradually worsened. Blood parameters revealed only slightly decreased hematocrit (32) and potassium (3.4) values. Blood pressure was normal. He received intravenous fluids and intralipids, but his general state rapidly deteriorated and he was in cardiopulmonary arrest a few hours after his arrival at the clinic. Cardiopulmonary resuscitation was performed unsuccessfully.

Gross Pathology:
The dog weighed 20 kg and was in good body condition. Lungs were diffusely edematous, and the pericardial sac contained a small amount (5-6 ml) of blood-tinged fluid. The heart weighed 174 g (within normal values) and showed no anomaly. Approximately 20 to 25 ml of uncoagulated blood was present in the abdominal cavity. No lesion explaining this blood was found in the abdominal organs. The stomach was full of undigested dry food, amongst which two small blue particles (size of blueberries) were noted. No diarrhea was present. Considering the rapid onset of clinical signs and the fact the dog was healthy and up to date on his vaccination schedule, an intoxication was suspected.

Laboratory Results:
Routine bacteriological culture of the lung, liver and kidney revealed only rare hemolytic *E.coli* and few contaminants. A PCR for canine herpesvirus was negative. Stomach content was sent for toxicological analysis and results showed a large amount of phenethylamine detected by GCMS.

Microscopic Description:
Histological changes are limited to the cerebrum; they are multifocal, present in all lobes, but most severe in the right frontal lobe. There is marked and extensive vasogenic edema involving mainly the white matter, variably extending into the adjacent cerebral cortex, associated with vascular fibrinoid degeneration/necrosis and mostly perivascular hemorrhages. The wall of affected blood vessels is effaced by fibrinoid material, sometimes with pycnotic nuclei, and eryhrocytes and/or fibrin are present in the Virchow-Robin spaces. In affected areas, there are numerous reactive astrocytes and microgliocytes and, multifocally a few to several neutrophils. There are numerous microgli-

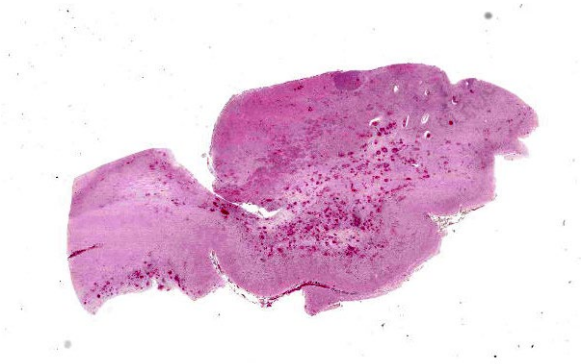

Figure 4-1. Cerebrum, dog. A section of cerebrum is submitted for examination. At subgross magnification, there are numerous areas of perivascular hemorrhage, predominantly within the white matter. (HE, 7X)

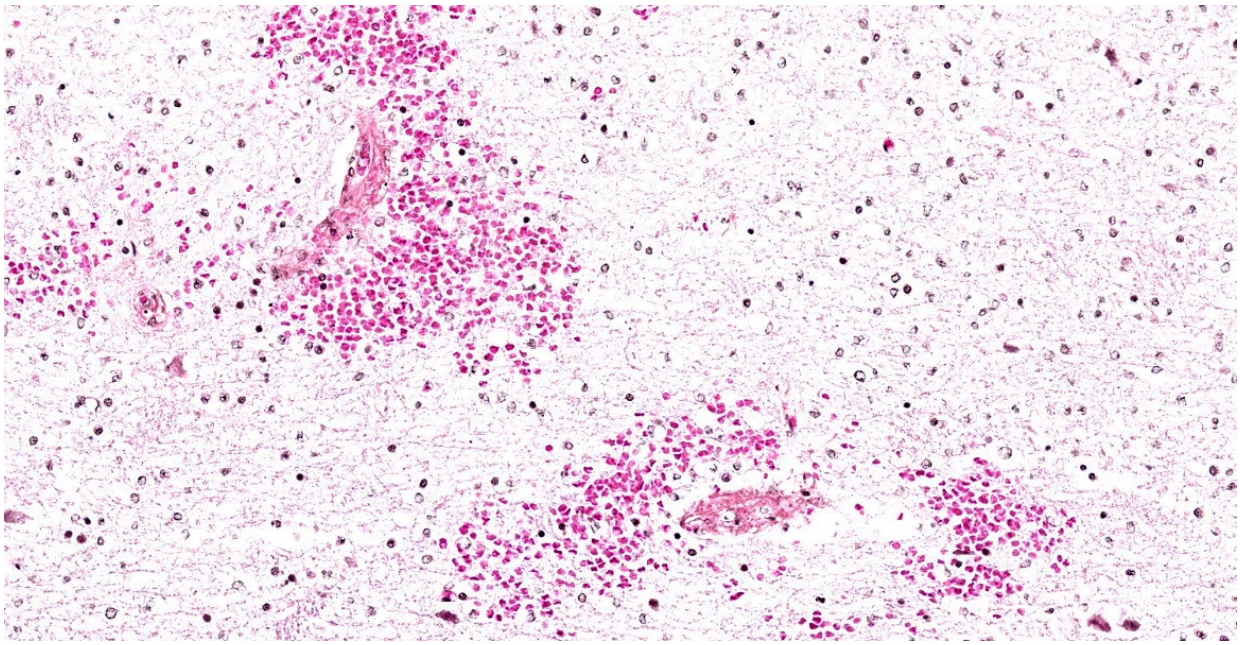

Figure 4-2. Cerebrum, dog. There is fibrinoid necrosis of vessel walls with perivascular hemorrhage. (HE, 248X)

ocytes/macrophages and occasional neutrophils in the perivascular (Virchow-Robin) and subarachnoidal spaces. Neuronal changes are interpreted as artefactual ("dark neurons").

Contributor's Morphologic Diagnoses:
Marked and extensive vasogenic cerebral edema associated with vascular fibrinoid degeneration/necrosis and perivascular hemorrhages (mainly white matter)

Contributor's Comment:
Phenethylamine is an organic compound, natural monoamine alkaloid, and trace amine, which acts as a central nervous system stimulant. It represents the core structure of numerous drugs with stimulant-like properties such as amphetamines and methamphetamines.[8] It is also the major component of stimulant drugs used to treat attention-deficit/hyperactivity disorder (AD/HD) by improving brain levels of serotonin and norepinephrine.[10] In the present case, the exact substance containing phenethylamine that was ingested by the dog could not be identified. The owners suspected that the dog had eaten an illegal substance, possibly from a neighbor's gathering that took place the night before. To the best of their knowledge, the dog did not have access to other possible sources of phenethylamine-containing substances.

Very few reports have documented intoxication of dogs by amphetamines or by drugs prescribed for the management of AD/HD.[2,4,9,11] The principal mechanisms of toxicity in amphetamine poisoning are the release of catecholamine with stimulation of the nervous system and a marked increase in the release of norepinephrine, dopamine, and serotonin. Clinical signs most commonly reported are cardiovascular signs such as hypertension and tachycardia and central nervous system signs such as hyperactivity, agitation, and tremors. Hyperthermia is also often present. Lethargy, depression, and coma have been reported in the latter course of intoxication. Peak plasma concentrations of amphetamine occur 1-3 hours after ingestion.[1] The median lethal dose (LD50) for orally administered amphetamine sulfate in dogs is 20 to 27 mg/kg.[1] Full recovery can be achieved when a rapid diagnosis is made together with an aggressive intervention. In the present

case, lethargy and depression were the principal clinical signs displayed. The very young age of the dog (4 months) and the presence of a large amount of phenethylamine as determined by GCMS analysis could have both contributed to the rapid deterioration of the animal leading to coma and death.

Histological lesions in the present case were present only in the brain and were centered around blood vessels (principally cerebral edema with a marked exudation of fibrin and perivascular hemorrhages). These changes are consistent with a significant increase in vascular permeability. In humans, it is generally assumed that death following a methamphetamine overdose results from heart attack or stroke. Central nervous system alterations are difficult to precisely determine in humans since polysubstance abuse is seen in the majority of cases.[3] Arterial or venous infarcts and subarachnoid or parenchymal hemorrhages have been reported as neuropathological complications.[5] A necrotizing vasculitis, predominantly of small blood vessels, but larger arteries and veins may be involved has also been observed in few cases.[5]

Recent studies using a rat model of acute methamphetamine intoxication showed the development of a temperature-dependent leakage of the blood-brain barrier and the development of vasogenic edema that could finally result in decompensation of vital functions and death.[6,7] More specifically, the authors found the leakage of albumin in the neuropil (reflecting alterations in vascular endothelium), an increased number of glial cells and the presence of several pyknotic neurons in rats that had received 9 mg/kg of methamphetamine.[6,7] These changes were temperature dependent, being more severe in rats that were maintained under warm (29°C) compared to standard (23° C) ambient temperature. Histological changes in the brain of dogs following an intoxication by amphetamines have not been reported. However, the histological lesions observed in the present case are consistent with a severe alteration of the blood-brain barrier following intoxication with a phenethylamine-containing substance.

Contributing Institution:
Département de pathologie et microbiologie
Faculté de médecine vétérinaire
Université de Montréal

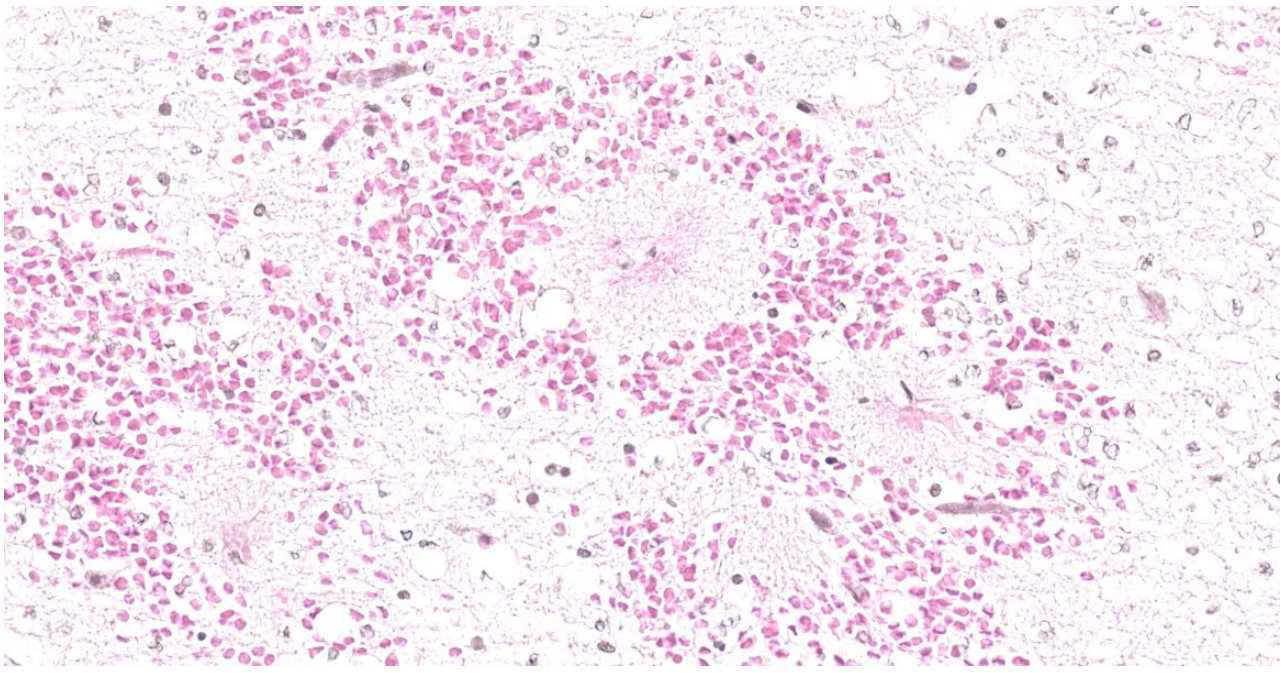

Figure 4-3. Cerebrum, dog. Effete vessels are replaced by polymerized fibrin and surrounded by ring hemorrhage. (HE, 351X)

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JPC Diagnosis:
Cerebrum: Fibrinoid vascular degeneration and necrosis, acute, multifocal to coalescing, with, hemorrhage, edema, and gliosis.

JPC Comment:
Prior to reviewing the clinical history and gross necropsy findings of this case, the moderator and participants discussed possible differentials for fibrinoid vascular degeneration in the cerebrum of a dog, including infectious agents (i.e., endotoxemia, *Rickettsia rickettsia,* and *Ehrlichia canis*), accidental ingestion of illicit substances, and hypertension.

In a nine year retrospective study evaluating 241,261 cases of suspected poisoning events reported to the ASPCA's Animal Poison Control Center in the United States, exposure to human medications accounted for 40% and 32% of canine and feline cases, respectively, and recreational or elicit medications accounted for 1.83% and 0.44% of exposures, respectively.[12] Exposure to prescribed amphetamines and illicit methamphetamine comprised a small portion of these toxicities, accounting for 1.16% and 1.44% of total canine and feline exposures.[12] Human pharmaceuticals more commonly referenced as a source of exposure included analgesics and medications treating CNS, gastrointestinal, and cardiovascular disorders.[12] These results indicate that amphetamine toxicity is an uncommon toxicosis in dogs and cats, but one that clinicians and pathologists should be cognisant of.[12]

Amphetamines are lipid-soluble drugs rapidly absorbed in the gastrointestinal tract and which cross the blood-brain barrier.[1] Clinical signs begin within 20 minutes of ingestion.[1] Peak plasma concentration occurs within 3 hours, and, in dogs, the drug is cleared within 6 hours.[1]

Amphetamines continuously stimulate muscle activity causing energy depletion, calcium overload in the sarcoplasmic reticulum, and constant muscle fasciculations, exacerbating energy demands.[10] Rhabdomyolysis is a common consequence of amphetamine toxicosis in humans and is occasionally reported in dogs.[1,10] Some cases may progress to myoglobinuria and acute kidney injury.[10] Hyperthermia and respiratory failure can lead to disseminated intravascular coagulation, a potential cause of death in intoxicated dogs.[1] Other reported cause of death include cerebral hemorrhage secondary to hypertension and heart failure.[1] Metarubricytosis, hypersegmentation of neutrophil nuclei, and thrombocytopenia presumedly due to hyperthermia have been reported secondary to amphetamine toxicosis in a Boxer.[13]

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