Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #19 — 11 February 2026

CASE I:

Signalment:
Male, 408-gram, age unknown, Fischer 344 rat (*Rattus norvegicus*)

History:
The rat had telemetry placement surgery on 8 June 2015. Post-operative treatments included meloxicam, buprenex along with supplemental feeding. Animal recovered from surgery without complication and was acting normally during the initial post-surgery period. On the 26th of June, the rat appeared pale and lethargic and euthanasia was elected.

Gross Pathology:
This rat has a body score of 3/5 with adequate subcutaneous and visceral fat. The skin incisions over the skull and left thorax from the telemetry surgery have completely healed. The pocket over the left thorax holding the telemetry device contains 5 ml of milky serosanguinous fluid. There is a layer of fibrin coating the telemetry device. The lungs were collapsed and diffusely red. The spleen is enlarged measuring 6.5 x 2 x 1 cm, friable with a homogenous pale red area on one pole (infarct). The liver is diffusely pale (light brown) and friable and the thymus is mottled tan/brown. The pancreas is soft and tan and both kidneys have a brown/green appearance. There is a moderate amount of dry food contents in the stomach, small amount of normal digesta and gas in the small intestine, moderate amount of soft green contents in the cecum and many dry fecal pellets in the colon. There is no urine in the bladder.

Laboratory Results:
N/A.

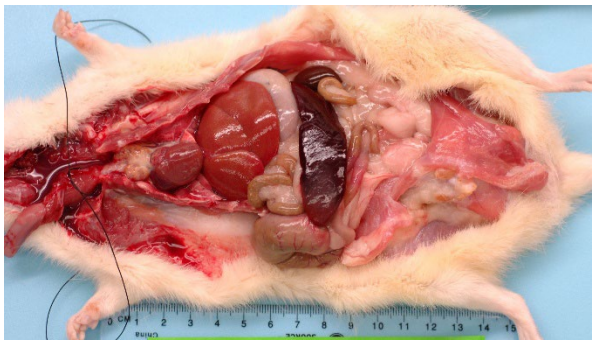

Figure 1-1. Abdominal cavity, rat: The liver and spleen are markedly enlarged. (Photo courtesy of: US Army Institute of Chemical Defense).

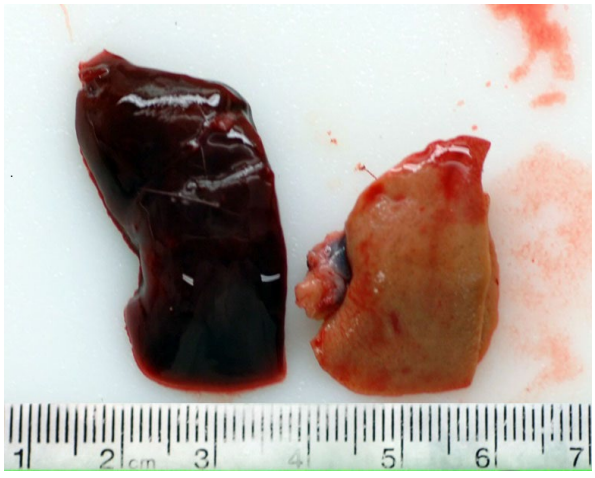

Figure 1-2. Liver, rat: The liver demonstrates marked pallor (normal control on left). (Photo courtesy of: US Army Institute of Chemical Defense).

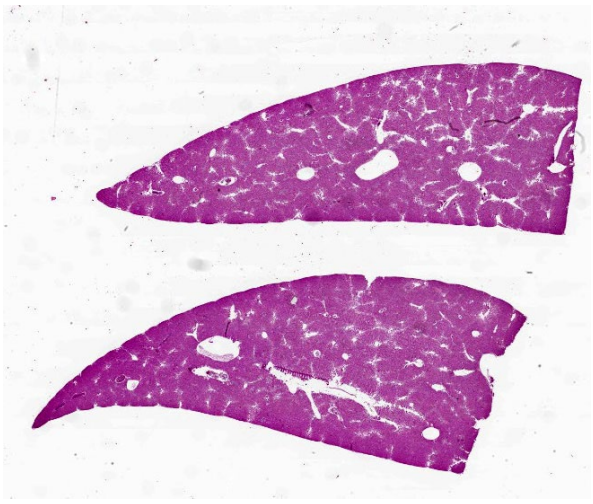

Figure 2-3. Liver, rat: Two sections of liver are submitted for examination. There are no changes at subgross magnification. (HE, 12X)

Microscopic Description:

Liver: Diffusely filling portal veins and expanding sinusoids is a neoplastic population composed of round cells with distinct cell borders, small amount of eosinophilic, granular cytoplasm, round to occasional reniform nucleus, clumped chromatin and indistinct nucleolus. Mitotic rate is <1 per hpf.

Diffusely centrilobular hepatocytes are lost, degenerating or necrotic. Diffusely there is lobular atrophy and sinusoids are expanded by previously described neoplastic cells admixed with eosinophilic cellular debris. Multifocally centrilobular kupffer cells and/or hepatocytes contain hemosiderin pigment. Multifocally within portal regions there is an increase in small bile duct profiles (ductular reaction). Rarely, bile duct epithelial cells are degenerative, necrotic or undergoing regeneration.

Contributor's Morphologic Diagnoses:

Liver: Leukemia, large granular lymphocytic

Contributor's Comment:

Large granular lymphocytic (LGL) leukemia, formally identified as mononuclear cell leukemia (MCL), is the main cause of death among Fischer 344 (F344) rats >20 months of age.[4] Reported frequency ranges from 10 - 35% with 50% of early deaths is some 2-year studies being caused by this disease.[6] LGL leukemia has been reported in other strains of rats.

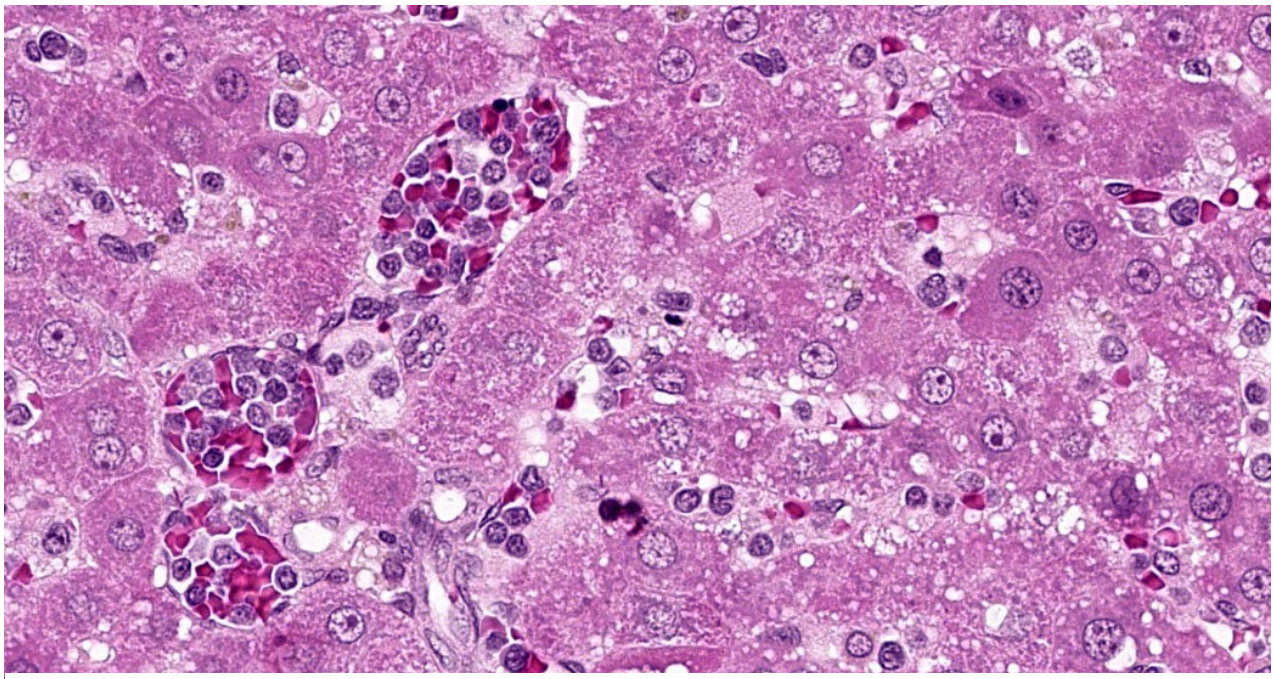

Figure 1-4. Liver, rat: Within portal veins and hepatic sinusoids, there are numerous neoplastic large granular lymphocytes. Few centrilobular hepatocytes are hypereosinophilic, contracted, and without nuclei (necrotic) (HE, 900X)

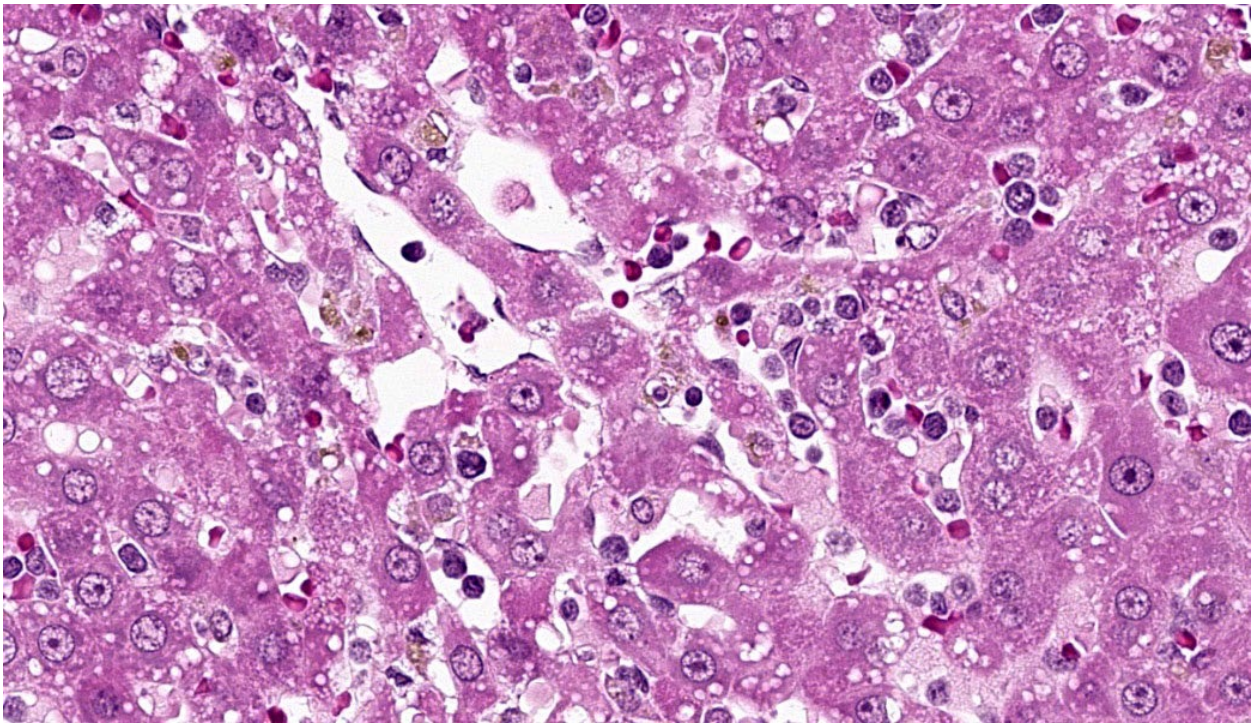

Figure 1-5. Liver, rat: There is loss of centrilobular hepatocytes resulting in enlarged centrilobular veins, and Kupffer cells in this area contain abundant lipofuscin granules. (HE, 900X)

Wistar-Furth rats have the next highest occurrence with an incidence rate of 15-22%, while the disease is rarely seen in Sprague-Dawley rats (0.6%).[7] Naturally occurring LGL leukemia has not been reported in mice.[7]

Clinical signs are characterized by weight loss, anemia, jaundice, and depression.[1] There is usually a concurrent, immune-mediated hemolytic anemia, with thrombocytopenia and clotting abnormalities suggestive of disseminated intravascular coagulation.[1] Splenomegaly is the most common characteristic macroscopic finding in rats afflicted with LGL leukemia.[4]

Histological findings, in general, include diffuse infiltration of the splenic red pulp sinusoids of variable density, infiltration of the hepatic sinusoids with variable centrilobular hepatocellular degeneration, and necrosis and atrophy of hepatic cords and infiltration of the alveolar septa of the lungs.[7] Other tissues affected include lymph nodes, adrenal gland, kidney, and bone marrow.

Contributing Institution:
U.S. Army Medical Research Institute of Chemical Defense
(https://usamricd.health.mil/)

JPC Diagnoses:
Liver: Large granular cell leukemia.

JPC Comment:
Conference 19 was moderated by the esteemed Dr. Kimberly Whitten, a veteran Department of Defense laboratory animal toxicological pathologist who works for USAMRICD. This first case was a classic entity in F344 rats and one that must be at the forefront of any pathologist's mind in this strain. LGL leukemia in F344 rats closely resembles the NK-cell leukemia seen in humans

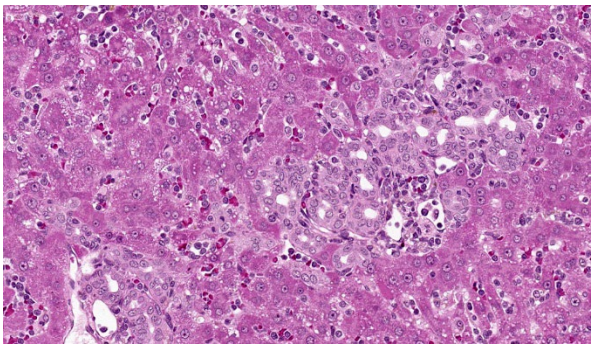

Figure 1-6. Liver, rat: There is moderate biliary hyperplasia. (HE, 750X)

and, as such, they are used as a research model for this aggressive disease.[7] Morphologically, the LGL leukemia cells resemble normal rat LGLs, but there are notable differences in cytotoxicity & surface antigens. Normal rat LGLs usually express surface markers such as CD8, CD2, CD56, and ASGM1, while leukemic LGLs tend to show abnormal or low-level expression of T-cell receptor (TCR) beta chains, may or may not express ASGM1, and can display heterogeneity in markers like CD56.[7,9] This suggests that these leukemias may be of a heterogeneous lymphocytic origin, although NK-cell markers predominate.[7,9]

Natural killer cells are vital, fast-acting lymphocytes of the innate immune system that, unlike most other lymphocytes, identify and destroy virally infected or cancerous cells without prior sensitization. Acting as a first line of defense, they utilize surface receptors to detect absent MHC-I molecules or "stressed" signals on target cells, prompting the NK cells to release cytotoxic granules containing pore-forming enzymes, such as granzyme B.[2]

Dr. Whitten quizzed participants on other common tumors in F344 rats, which included: testicular interstitial cell tumors, which occur in almost 100% of aged male Fischer rats; mesotheliomas of the tunica vaginalis of the testes, which occur in up to 5% of males and may spread to the peritoneal and pleural cavities; pituitary adenomas, which are seen in 30% of rats and may be prolactin producing, which can result in mammary hyperplasia; mammary gland tumors (primarily mammary fibroadenoma), which occur in roughly 40% of female and 20% of male rats; pheochromocytomas, seen in 30% of male rats in a recent study; and thyroid C-cell carcinomas, occurring in up to 10% of aged rats.[3]

References:

1. Barthold SW, Griffey SM, Percy DH. Rat. In: *Pathology of laboratory rodents and rabbits*. 4th ed. Ames, IA: John Wiley & Sons, Inc: 2016;166-167.
2. Mace EM. Human natural killer cells: Form, function, and development. *J Allergy Clin Immunol*. 2023;151(2):371-385.
3. Sass B, Rabstein LS, Madison R, Nims RM, Peters RL, Kelloff GJ. Incidence of spontaneous neoplasms in F344 rats throughout the natural life-span. *J Natl Cancer Inst*. 1975;54(6):1449-1456.
4. Shiga A, Narama I. Hepatic lesions caused by large granular lymphocytic leukemia in Fischer 344 rats: similar morphologic features and morphogenesis to those of nodular regenerative hyperplasia (NRH) in human liver. *Toxicol Pathol*. 2015;43:852-864.
5. Stefanski SA, Elwell MR, Stromberg PC. Spleen, lymph nodes, and thymus. In: Boorman GA, Eustis LS, Elwell MR, Montgomery CA, MacKenzie WF, ed. *Pathology of the Fischer rat: reference and atlas*. San Diego, CA: Academic Press;1990:374-379.

6. Stromberg PC, Vogtsberger LM. Pathology of the mononuclear cell leukemia of Fischer rats. *Vet Pathol*. 1983;20:698-708.
7. Thomas J, Haseman JK, Goodman JI, et al. A review of large granular lymphocytic leukemia in Fischer 344 rats as an initial step toward evaluating the implication of the endpoint to human cancer risk assessment. *Toxicol Sci*. 2007;99:3-19.
8. Ward JM, Reynolds CW. Large granular lymphocyte leukemia: a heterogeneous lymphocytic leukemia in F344 rats. *Am J Pathol*. 1983;111:1-10.
9. Watters RJ, Liu X, Loughran TP Jr. T-cell and natural killer-cell large granular lymphocyte leukemia neoplasias. *Leuk Lymphoma*. 2011;52(12):2217-2225.

CASE II:

Signalment:
7.5-year-old male cynomolgus macaque (*Macaca fasicularis*)

History:
This animal had undergone surgery six days previously to induce acute hepatic failure by ischemic liver injury and had concurrently received a xenographic transplant of swine hepatocytes into the spleen. It had been started on tacrolimus immunosuppressive therapy prior to manipulation. Two days post-operatively, the monkey began progressive clinical deterioration as well as developing diabetes. It was unresponsive to empirical supportive therapy and was euthanized.

Gross Pathology:
Kidneys were reported to be pale and reduced in size without additional specifications.

Laboratory Results:
N/A.

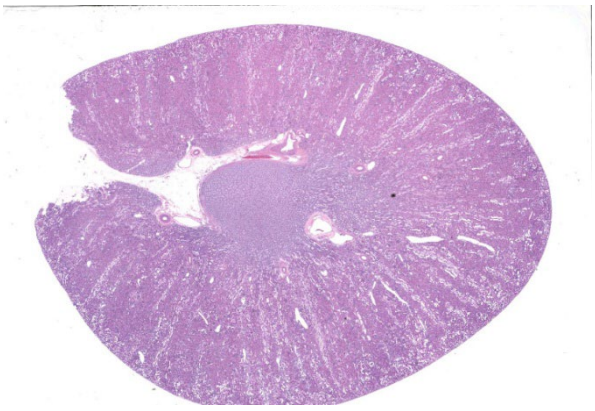

Figure 2-1. Kidney, rhesus macaque: A section of kidney is submitted for examination. At subgross magnification, numerous ectatic tubules are visible. (HE, 9X)

Microscopic Description:
Renal lesions involve primarily the tubular component and are characterized by a variety of morphological features. Swelling and vacuolar change are prominent within proximal renal tubular epithelium, especially in the straight portion of that segment. Many tubules contain prominent cast-structures, typically consisting of granular to globular eosinophilic material, frequently admixed with blueish-gray proteinaceous sheets. Exfoliated epithelial cells are occasionally incorporated into the cast structures with numerous tubules also containing aggregates of mixed, primarily granulocytic inflammatory cells. Individually necrotic renal tubular epithelium is occasional evident. Associated with obstruction created by these casts, there is extensive, widespread tubular dilatation. Although extremely infrequent, fibrin thrombi were noted within occasional glomerular capillary loops. However, other significant glomerular changes were uncommon at the light microscopic level.

Contributor's Morphologic Diagnoses:
1. Renal tubular vacuolization, patchy to focally extensive, primarily proximal tubules, moderate-marked.
2. Coarsely granular eosinophilic tubular casts with occasional proteinaceous sheets consistent with myoglobin and associated

myoglobinuric nephrosis, patchy, moderate, with associated marked tubular dilatation and epithelial cell thinning.
3. Acute tubular injury (ATI), patchy, mild, characterized by renal tubular epithelial cell exfoliation, individual cellular degeneration and patchy, primarily granulocytic inflammatory cell aggregates.
4. Focal glomerular capillary loop fibrin thrombi, minimal.

Contributor's Comment:
Despite administration at standard dosing levels, this animal's tacrolimus levels climbed at one point to 80 ng/ml (target range <16 ng/ml) and the monkey developed clinical signs of toxicity as well as diabetes. Unfortunately, renal function values were not available at the time of necropsy.

Tacrolimus, also known as FK506 is a macrolide immunosuppressive drug derived from the fungus *Streptomyces tsukubaensis*, approved by the FDA in 1994 to lower the risk of allogenic organ transplant rejection. Although not sharing molecular structural properties, it has similar therapeutic mechanisms and toxicity with its older immunosuppressive counterpart, cyclosporine. Both are classified as calcineurin inhibitors (CNI), affecting calcium-dependent events such as nitric oxide synthase activities, apoptosis, and cell degranulation.[13]

Renal toxicity is a well-recognized effect, both to transplanted kidneys as well as the native kidneys of patients receiving other organs. Early nephrotoxicity is primarily associated with acute arteriole vasoconstriction and is thought to be of multifactorial pathogenesis, including increasing vasoconstrictive factors, reducing vasodilator mechanisms and increasing the formation of free radicles.[3,4,6,7,14] Toxicity due to vasospasms can manifest in a so-

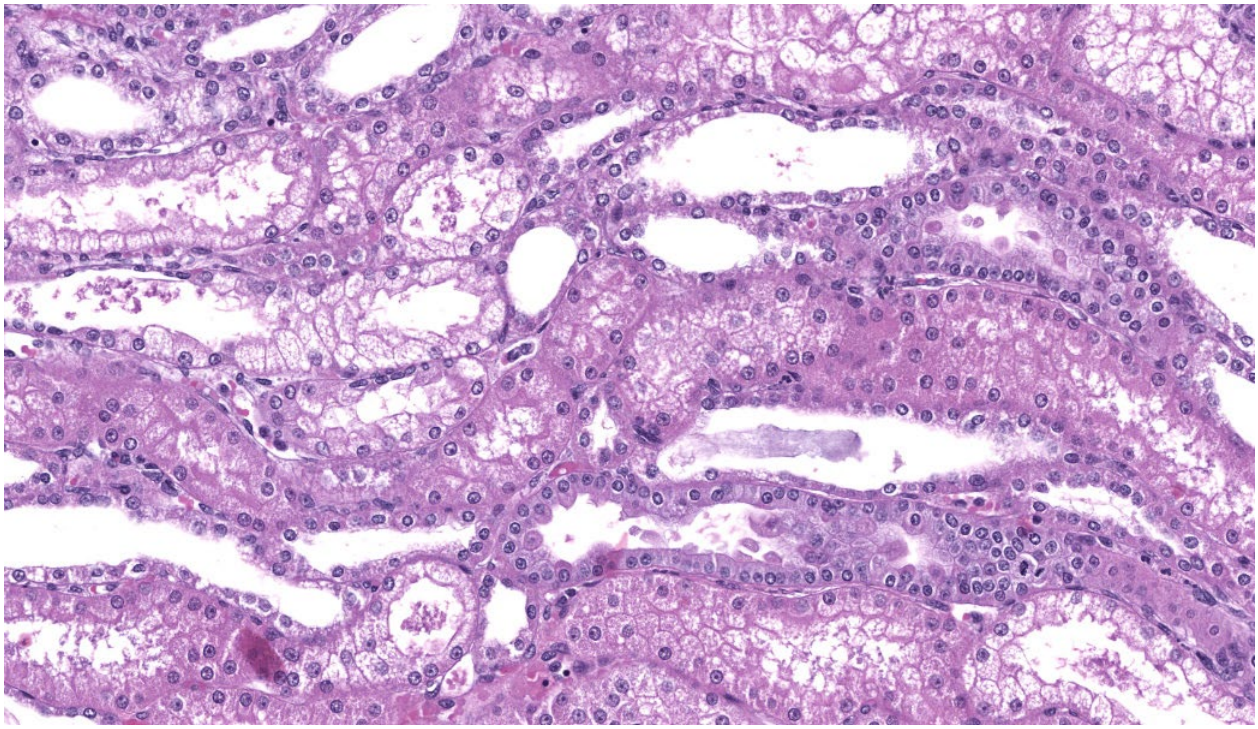

Figure 2-2. Kidney, rhesus macaque: Within the more distal nephron, numerous changes, including ectasia, swelling and cytoplasmic vacuolation, and regeneration are visible. (HE, 380X)

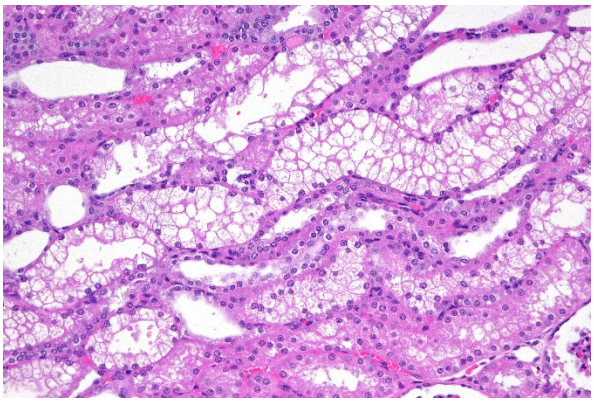

Figure 2-3. Kidney, rhesus macaque: Tubular epithelium is markedly vacuolated. (HE, 200X) (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, www.dlar.pitt.edu).

called "functional form" without any characteristic morphological changes, or with associated structural histological alterations, either acute or chronic.[13] The latter includes effects on both arterioles (especially afferent arterioles) and glomeruli, including smooth muscle cell swelling, endothelial cell injury/necrosis and fibrin thrombi, potentially resulting in thrombotic microangiopathy. Such changes were considered to be relatively modest in this case, although very mild mesangial matrix expansion and hypercellularity of the juxtaglomerular apparatus were occasionally noted. Tubular components of CNI injury can include the development of vacuolization leading to clear, aqueous fluid accumulation (the latter being associated ultrastructurally with dilated smooth endoplasmic reticulum and occur predominantly in the straight portion of the proximal tubules.) This process was prominent in this monkey. As vacuolar change is a common, non-specific lesion often seen with osmotic nephrosis due to agents such as mannitol, inulin, glucose, radiocontrast agents, chemicals that induce vacuolation through phagolysosomes and other causes of tubular ischemia correlation with other factors must be taken in ascribing its pathogenesis.[7]

Another form of renal tubular injury is occurring in this case – likely tacrolimus related as well, but of a more secondary mechanism. Since early after its therapeutic use, tacrolimus has been known to be associated with severe rhabdomyolysis, although the incidence is considered rare.[1,2] The role of calcineurin in skeletal muscles is complex, with both protective and detrimental effects.[14] CNI are potent CYP450 inhibitors and this may be related to an increased incidence of muscle toxicity in transplant recipients, especially associated with other drug interactions.[1]

Renal casts noted in this case (and often admixed with numerous dehisced epithelial cells) were suggestive of myoglobin origin. They are often comprised of round granules that line up in chains or aggregate in clusters. Tinctorially, there is variation between and even within different stains applied with a typical pink to red-brown appearance with H&E, pink to bright magenta with PAS, and often a gray-blue sheet containing fine red granules on Trichrome.[5,8] Immunostaining with antibody to myoglobin was strongly positive in

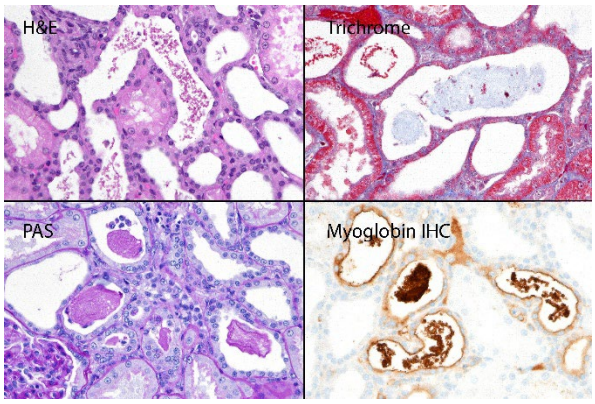

Figure 2-4. Kidney, rhesus macaque: There are eosinophilic tubular casts with occasional crystalline protein consistent with myoglobin and mild associated myoglobinuric nephrosis. (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, www.dlar.pitt.edu).

these casts, often with a prominent rope-like string to beaded appearance. Interestingly, the concept that acute kidney injury is triggered by myoglobin as a direct, sole toxin appears to be oversimplified and there is ample evidence that additional factors such as hypovolemia, renal vasoconstriction or urine acidification are required for nephrotoxicity.[9,12] Of these factors, certainly vasoconstriction directly associated with CNI effects would have likely been present. Again, in this case the myoglobinuric nephrosis present cannot be definitively ascribed to tacrolimus, but this is considered a likely pathogenesis, especially in the absence of other common pathogeneses such as severe trauma, dehydration, and other frequently associated comorbidities.[5]

Although both the direct and indirect effects of tacrolimus toxicity in this case were thought to be substantially responsible for lesions, the impact of the severe ischemic hepatic and biliary injury created prior to the transplant was also considered to have potentially played a role in some of the changes present.

Hepatorenal syndrome (HRS) involves the development of renal failure in patients with severe liver disease and while often seen in chronic cirrhosis, it can also occur in patients with acute liver disease.[10] The pathogenesis is associated with severe renal vasoconstriction resulting from complex changes in splanchnic and general circulations as well as systemic and renal vasoconstrictors and vasodilators.[10] This entity is mentioned only to emphasize the complexity and interconnectedness of the kidneys with other systemic functions.

In addition to renal effects, other common toxic complications associated with tacrolimus use in humans include neurological problems and diabetes, although numerous other clinical problems such as diarrhea, pruritus and alopecia are reported.[16]

Contributing Institution:
Division of Laboratory Animal Resources, University of Pittsburgh; (www.dlar.pitt.edu)

JPC Diagnoses:
Kidney: Tubular vacuolization, necrosis, and regeneration, acute, multifocal, marked, with granular casts and glomerular fibrin thrombi.

JPC Comment:
The contributor of this case provided an exceptional comment and covered much of what was discussed in conference. Hemoglobin and myoglobin were discussed as the two main rule-outs for the granular casts present in the kidney and special attention was drawn to the fact that, with tacrolimus toxicity, the vacuolation of the straight portion of the proximal convoluted tubules (PCT) is the primary process and is associated with dilation of the smooth endoplasmic reticulum of the PCT epithelial cells.[14] This kidney presented a couple of nice examples of tubular regeneration as well. Additional ruleouts in this case that were considered were aminoglycoside toxicity, which tends to appear more like a lysosomal storage disease with build-up of cytoplasmic material in the PCT epithelial cells, mannitol administration, which can cause osmotic nephrosis when given in high volume or over a prolonged period of time, and any other toxins that inhibit CYP450 that may have also resulted in tubular epithelial vacuolation.

CYP450 enzymes in the kidney are critical for maintaining fluid and electrolyte balance, regulating blood pressure, and metabolizing drugs or toxins. They function primarily in the PCTs to catalyze the oxidation of arachidonic acid into vasoactive substances, such as certain HETE molecules (vasoconstrictors) and epoxyeicosatrienoic acids (EETs; vasodilators), which all help to modulate vascular tone

and tubular sodium transport.[11] These potent signaling molecules act as protective, locally acting agents in the kidney to reduce blood pressure, decrease inflammation, and prevent tissue fibrosis.[11] When CYP450 is inhibited, these molecules are not produced effectively, resulting in kidney injury.

References:

1. Dopazo C, Bilbao I, Lazaro JL et al. Severe Rhabdomyolysis and Acute Renal Failure Secondary to Concomitant Use of Simvastatin With Rapamycin Plus Tacrolimus in Liver Transplant Patient. *Transplantation Proceedings*. 2009;41:1021–1024.
2. Hibi S, Misawa A, Tsunamoto K et al. Severe rhabdomyolysis associated with tacrolimus. *Lancet*. 1995;346(8976):70.
3. Issa N, Kukla A and Ibrahim HN. Calcineurin Inhibitor Nephrotoxicity: A Review and Perspective of the Evidence. *Am J Nephrolo*. 2013;37:602-612.
4. Kemper J and Kniska K. Pathophysiology and treatment of calcineurin inhibitor nephrotoxicity. *Kidney Centric*. 2014;1-6.
5. Liapis H, Boils CB, Hennigar R and Silva F. Myoglobin casts in renal biopsies: immunohistochemistry and morphologic spectrum. *Human Pathology*. 2016;54:25-30.
6. Myers BD. Cyclosporine nephrotoxicity. *Kidney International*. 1986;30:964-974.
7. Naesens M, Kuypers DR and Sarwal M. Calcineurin Inhibitor Nephrotoxicity. *American Society of Nephrology*. 2009;4:481-508.
8. Najafian B, Fogo AB, Lusco MA and Alpers CE. AJKD Atlas of Renal Pathology: Myoglobin Cast Nephropathy. Am J Kidney Dis. 2017;69(2):e7–e8.
9. Najafian B, Frankinn DB and Fogo AB. Acute Renal Failure and Myalgia in a Transplant Patient. *J Am Soc Nephrol*. 2007;18:2870–2874.
10. Ng C, Chan M, Morris HI et al. Hepatorenal Syndrome. *Clin Biochem Rev*. 2007;28:11-17.
11. Omata K, Abe K, Sheu HL, et al. Roles of renal cytochrome P450-dependent arachidonic acid metabolites in hypertension. *Tohoku J Exp Med*. 1992;166(1):93-106.
12. Petejova N and Martinek A. Acute kidney injury due to rhabdomyolysis and renal replacement therapy: a critical review. Critical Care. 2014;18:224.
13. Renal Transplantation Pathology. In: Jennette JC, Olson JL, Silva FG and D'Agati VD eds. *Hepinstall's Pathology of the Kidney Vol II*. 7th Ed. Philadelphia US: Wolters Kluwer; 2015:1321-1460.
14. Randhawa PS, Starzl TE and Demetris AJ. Tacrolimus (FK506)-Associated Renal Pathology. *Adv Anal Pathol*. 1997;4(4):265-276.
15. Sathyan S, Baskharoun R and Perlman AS. Prevention of Recurrent Episodes of Rhabdomyolysis With Tacrolimus in a Transplant Recipient With Myopathy. *American Journal of Therapeutics*. 2014;21:e171–e174.
16. Scott LJ, McKeage K, Keam SJ, *et al.* Tacrolimus: A further update of its use in the management of organ transplantation. *Drugs*. 2003;63:1247–1297.

CASE III:

Signalment:

A 2-year-old female spayed Pitbull (*Canis lupus familiaris*)

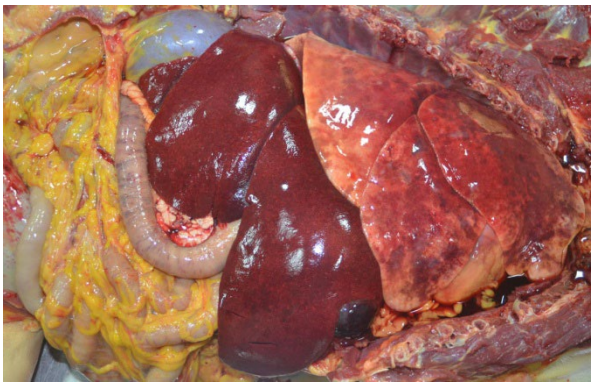

Figure 3-1. Liver, dog: There is icterus of the mesenteric adipose tissue and the liver is enlarged, dark red, and granular. (Photo courtesy of: Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html))

History:
A two-year old pit bull was hospitalized for management of acute liver injury and coagulopathy two days after ingesting three bulbs of cycad palm. Despite aggressive treatment, the patient developed hepatic encephalopathy and shock. Due to a poor prognosis, humane euthanasia was elected.

Gross Pathology:
There was generalized icterus including the skin, mucus membranes, subcutaneous and mesenteric adipose tissue, fascia, and sclera, as well as petechiae and ecchymoses. The liver was enlarged, dark red and granular. There were multiple gastric mucosal ulcers, and melena throughout the gastrointestinal tract.

Laboratory Results:
The dog had hypoalbuminemia, hypocholesteremia, and hypoglycemia as well as unmeasurable PT and PTT, and severe thrombocytopenia. ALT, AST, GGT, ALP, and total bilirubin were all elevated.

Microscopic Description:
Liver: There is massive loss of hepatocytes in all examined sections with approximately 99% of the hepatic parenchyma affected. The portal regions contain scant numbers of remaining hepatocytes with large numbers of macrovesicular and microvesicular lipid vacuoles. Kupffer cells and occasional hepatocytes contain moderate amounts of yellow brown pigment (lipofuscin). There is moderate proliferation of oval cells, which have large open nuclei with a prominent nucleolus. There are increased numbers of circulating neutrophils and monocytes within the sinusoids.

Contributor's Comment:
Cycad palms are primitive palm-like plants that are native to tropical and subtropical regions of the world. All cycads belong to the class Cycadopsida and the order Cycadales. In the United States common cycads palms include *Dycas revoluta*, *Cycas circinalis*, and *Zaminia floridana* and are commonly referred to as sago palms. In addition to being native in some regions, they are also popular ornamental plants in non-native climates.[1,5] All parts of the cycad plant are toxic but the highest concentration of toxin is found in the seeds.[11]

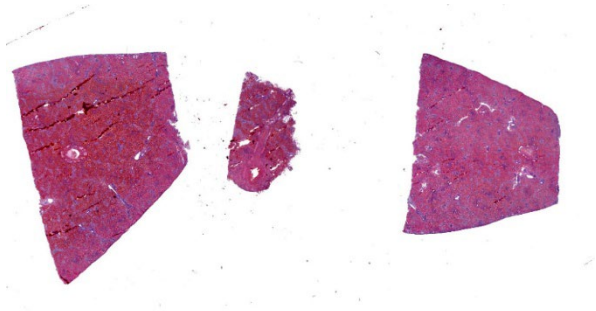

Figure 3-2. Liver, dog: Three sections of liver are submitted for examination; all are similar. At subgross magnification, normal hepatocellular architecture is lost and there is diffuse hemorrhage throughout the hepatic parenchyma (HE, 8X).

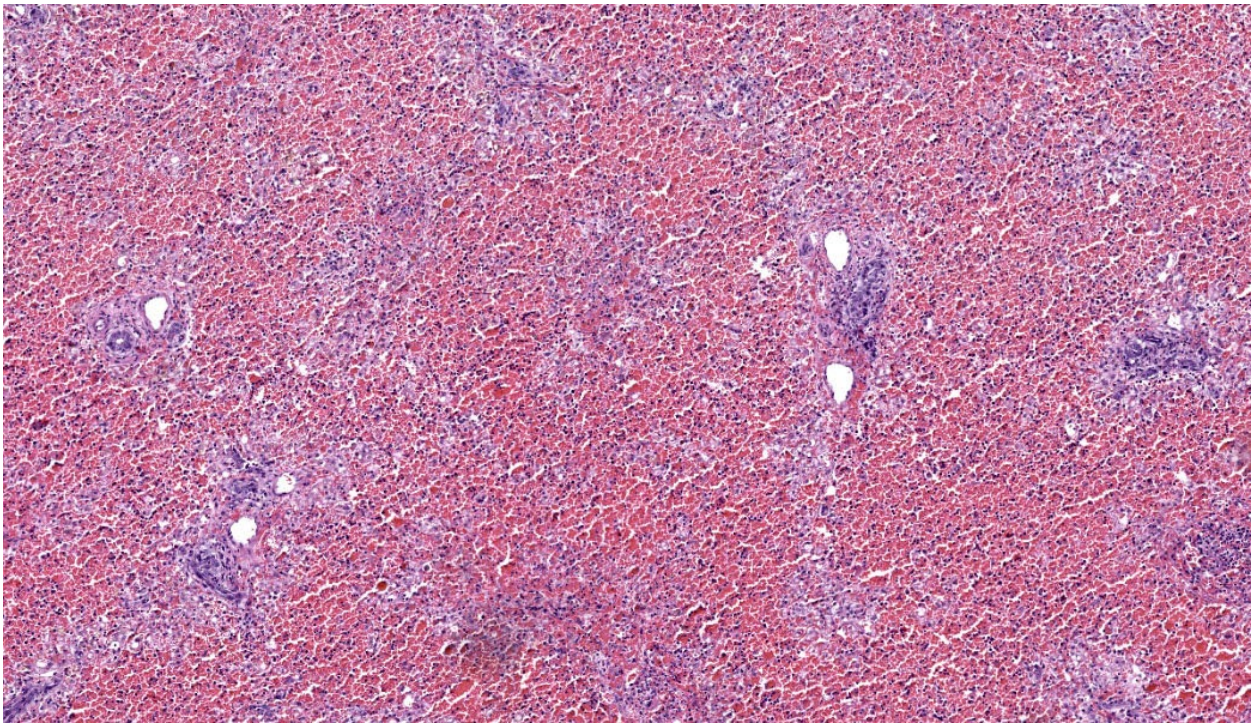

Figure 3-3. Liver, dog: There is massive necrosis of the hepatic lobule with diffuse hemorrhage. Portal areas remain. (HE, 115X)

Cycads contain three types of toxins: azoxyglycosides, of which cycasin is the most prominent member, β-methylamino-L-alanine (BMAA), and an unidentified high molecular weight compound.[1,11] Cycasin, the predominant toxin, is metabolized by intestinal bacteria into the toxic metabolite methylazoxymethanol (MAM). MAM is neurotoxic, carcinogenic, mutagenic, teratogenic, and hepatotoxic.[1,5,11] This carcinogen potential has been documented in rats where chronic exposure leads to cancer development in the liver, kidney, and intestinal tract of laboratory rodents and nonhuman primates.[8,14] Once metabolized in the intestine, MAM undergoes enterohepatic circulation and enters the liver via the portal vein where it is metabolized by hepatic cytochrome P450 (CYPs).

Cycad ingestion in dogs leads to gastrointestinal and hepatic disease caused by methylazoxymethanol (MAM). Common clinical signs in dogs include vomiting, diarrhea occasionally with melena, abdominal pain, anorexia, icterus, hypersalivation, depression, and neurological signs secondary to hepatic encephalopathy. Common clinical pathology findings include hyperbilirubinemia, elevated alanine aminotransferase (ALT), elevated alkaline phosphatase, hypocholesterolemia, hypoalbuminemia, elevated prothrombin time (PT), elevated partial thromboplastin time (PTT), and thrombocytopenia.[1,2,5,11,13]

There are multiple retrospective studies on cycad poisoning in dogs with survival rates ranging from 32 to 64%.[1,3,5] Severe liver injury leads to decreased synthesis of coagulation factors, decreased production of albumin, and disrupted bile acid circulation leading to decreased intestinal absorption of vitamin K.[5,11] All of these lead to the observed clinical abnormalities. Increased vascular permeability also leads to albumin loss through intestinal

and renal losses. Thrombocytopenia may occur due to coagulopathy and gastrointestinal bleeding.

MAM is oxidized by hepatic cytochrome P450 (CYPs) leading to centrilobular necrosis as the most common finding in acute toxicosis. Subacute changes include severe centrilobular coagulative necrosis with some regeneration as well as portal fibrosis and bile duct proliferation. Chronic changes include diffuse hepatocellular necrosis, hepatic degeneration and regeneration with extensive stromal collapse, bridging fibrosis with nodular regeneration, and biliary hyperplasia.[5] There are multiple retrospective studies on cycad poisoning in dogs with survival rates ranging from 32 to 64% and even with recovery chronic hepatic disease can persist.[1,3,5]

Cycad intoxication also occurs in domestic ruminants where it manifests as two distinct syndromes: neurologic and hepato-gastrointestinal.[6,7,8,12] The hepatic-gastrointestinal syndrome causes hepatocellular megalocytosis, centrilobular necrosis, and biliary duct hyperplasia. These may be similar to other hepatic toxicoses such as aflatoxin, pyrrolizidine alkaloids, *Lantana* species, and spordesmin. As in dogs, cycasin and its metabolites have been linked to hepatic-gastrointestinal syndrome in ruminants, and the hepato-gastrointestinal syndrome is more common in sheep compared to the neurologic syndrome which is more common in cattle.[1]

Cycads also contain the neurotoxin beta-methylamino-L-alanine (BMAA) and an unidentified neurotoxin. This unidentified neurotoxin is thought to be the cause of neurologic disease in cattle with chronic cycad poisoning, where progressive proprioception deficits in the hindlegs are attributed to axonal degeneration in the dorsal funiculus and the spinocerebellar and corticospinal tracts.[7,8,12]

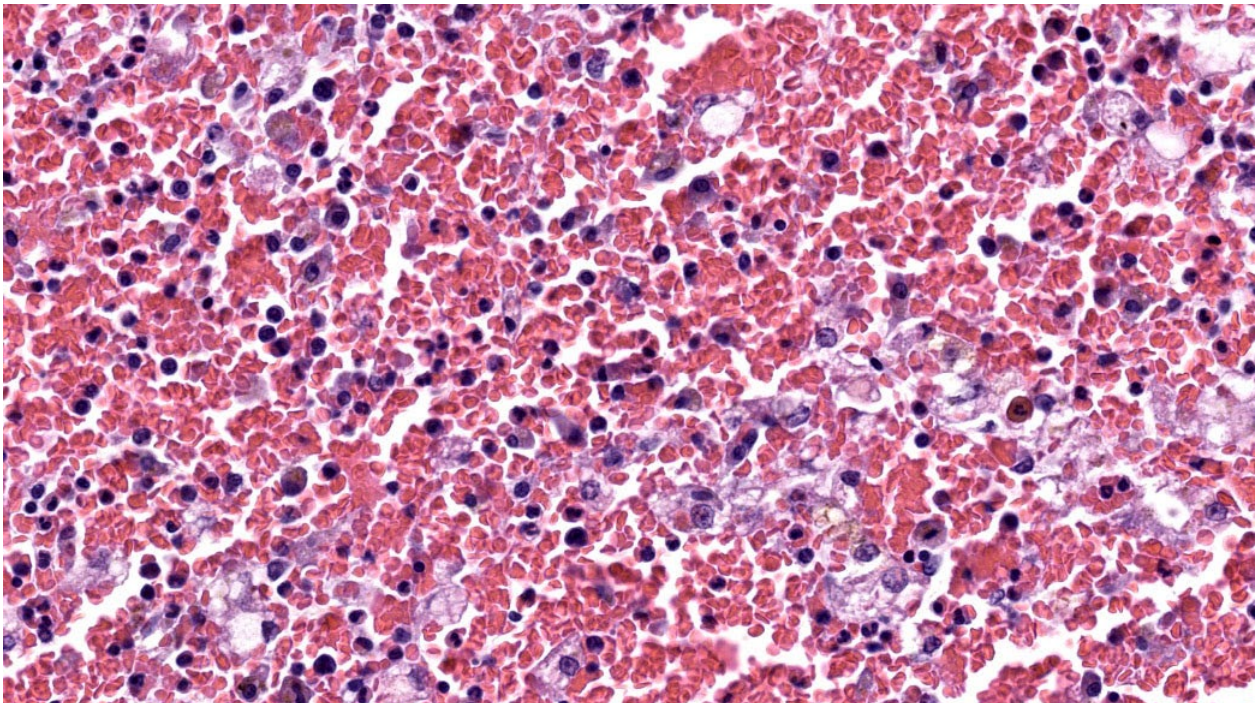

Figure 3-4 Liver, dog: Within the necrotic area of the hepatic lobule, there is disassociation of hepatocytes, and many discernible hepatocytes are pyknotic and fragmented. There is diffuse hemorrhage and infiltrating debris and hemosiderin-laden macrophages. (HE, 731X)

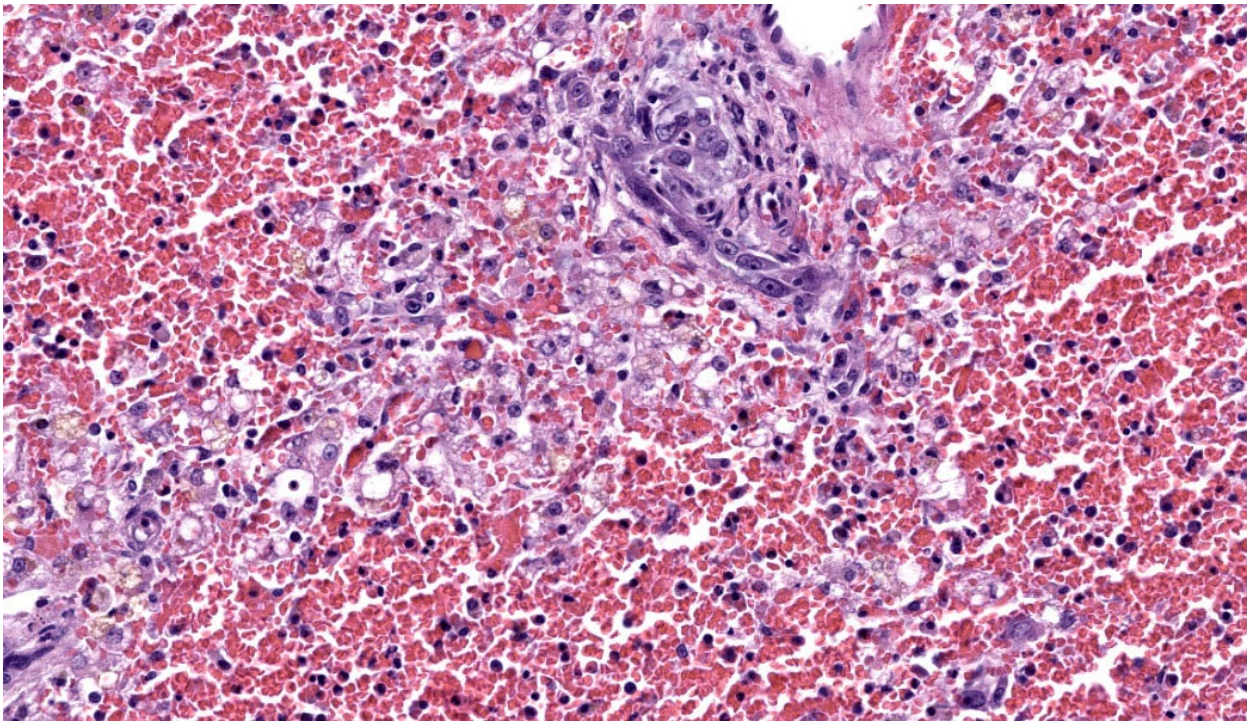

Figure 3-5. Liver, dog: In some lobules, degenerating hepatocytes remain with numerous lipid vacuoles in their cytoplasm. Few fibroblasts are proliferating around/within portal areas. (HE, 481X)

Cycad intoxication is associated with severe disease and fatalities in multiple domestic species caused primarily by hepatotoxicity which leads to secondary effects of hepatic failure including hepatic encephalopathy, coagulopathy, and hypoproteinemia.[1-5,11,13] In the cattle, cycad intoxication can also cause neurologic disease due to axonal degeneration.[7,8,12]

Contributing Institution:
Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html)

JPC Diagnoses:
Liver: Hepatocellular necrosis, acute, massive, diffuse, with hemorrhage and stromal collapse.

JPC Comment:
This contributor also provides an outstanding comment on this entity, which represents a case of acute hepatic necrosis secondary to a toxin in a dog. Much of what was discussed in conference for this case is covered in their write-up. Hepatic damage by cycad palm is progressive and dose-dependent.[9] Dr. Whitten discussed the acute changes seen in toxicity cases such as this, which include centrilobular hemorrhage & massive hepatocellular coagulative necrosis, vacuolar degeneration, hepatic venous congestion, pigmentary bile duct distention, bile duct plugging, and intracanalicular cholestasis.

Participants were also asked about various other potential causes of of massive hepatic necrosis across numerous species. Other toxic and chemical causes discussed included heavy metals, such as copper toxicity in sheep and young cattle, arsenic, and iron; medications/drugs, with specific mention of high-

dose ivermectin, some antibiotics, halothane, and phenobarbital; plants, fungi, and algae, such as *Amanita spp*. toadstools, *Senecio spp.* and *Crotalaria spp.* (pyrrolizidine alkaloids), *Aspergillus spp*. (aflatoxins), *Fusarium spp.* (fumonisins), and blue-green algae (cyanobacteria); and industrial chemicals like carbon tetrachloride, chlorinated hydrocarbons, and pesticides (i.e., paraquat).[4] Infectious causes included canine adenovirus-1 (canine infectious hepatitis), canine herpesvirus-1, rabbit calicivirus (rabbit hemorrhagic disease virus), *Clostridium novyi* (Black Disease), and *Salmonella spp*. A variety of ischemic and metabolic causes were mentioned and, in summary, covered shock/hypoxia, thromboembolisms, heat stroke, and DIC.[4] A brief mention of liver flukes, such as *Fasciola hepatica*, was made due to their contribution to the development of Black Disease via creation of migration tracks that enable a hypoxic environment for *Clostridium novyi* to proliferate. Lastly, there was brief discussion on the massive hepatic necrosis seen with Theiler's disease in horses (caused by equine parvovirus) and the lesions of inherited copper storage disease in Bedlington Terrier and Doberman dogs.

In cases of cycad palm in dogs, cattle, and rodents, lesions of the CNS have been reported in addition to the gastro-hepatic syndrome.[6,7,8,12] The primary lesions seen in the CNS include vacuolation of the neuropil, demyelination, and spongiform change to the brain and spinal cord.[8,12] While sago palm is known to produce BMAA neurotoxin, Dr. Whitten speculated that some of the brain lesions (i.e., astrocyte swelling and cytotoxic edema) may also be secondary to hepatic encephalopathy from severe hepatic necrosis.

References:

1. Albretsen JC, Khan SA, Richardson JA. Cycad palm toxicosis in dogs: 60 cases (1987-1997). *J Am Vet Med Assoc*. 1998;213: 99-101.
2. Botha CJ, Naude TW, Swan GE, Ashton MM, van der Wateren JF. Suspected cycad (*Cycas revoluta*) intoxication in dogs. *J S Afr Vet Assoc*. 1991;62: 189-190.
3. Clarke C, Burney D. Cycad Palm Toxicosis in 14 Dogs from Texas. *J Am Anim Hosp Assoc*. 2017;53: 159-166.
4. Cullen JM, Stalker MJ. Liver and Biliary System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals.* Vol 2. 6th ed. St. Louis, MO: Elsevier; 2016:258-352.
5. Ferguson D, Crowe M, McLaughlin L, Gaschen F. Survival and prognostic indicators for cycad intoxication in dogs. *Journal of veterinary internal medicine*. 2011;25: 831-837.
6. Gabbedy BJ, Meyer EP, Dickson J. Zamia palm (*Macrozamia reidlei*) poisoning of sheep. *Aust Vet J*. 1975;51: 303-305.
7. Hall WT. Cycad (zamia) poisoning in Australia. *Aust Vet J*. 1987;64: 149-151.
8. Hooper PT, Best SM, Campbell A. Axonal dystrophy in the spinal cords of cattle consuming the cycad palm, Cycas media. *Aust Vet J*. 1974;50: 146-149.
9. Lake BB, Edwards T, Atiee G, Stone R, Scott L. The characterization of cycad palm toxicosis and treatment effects in 130 dogs. *Aust Vet J*. 2020;98(11):555-562.
10. Laqueur GL, Matsumoto H. Neoplasms in female Fischer rats following intraperitoneal injection of methylazoxy-methanol. *J Natl Cancer Inst*. 1966;37: 217-232.
11. Milewski LM, Khan SA. An overview of potentially life-threatening poisonous plants in dogs and cats. *Journal of Veterinary Emergency and Critical Care*. 2006;16:25-33.
12. Reams RY, Janovitz EB, Robinson FR, Sullivan JM, Rivera Casanova C, Mas E. Cycad (*Zamia puertoriquensis*) toxicosis

in a group of dairy heifers in Puerto Rico. *J Vet Diagn Invest*. 1993;5:488-494.
13. Senior D, Sundlof S, Buergelt C, Hines S, O'Neil-Foil C, Meyer D. Cycad intoxication in the dog. *The Journal of the American Animal Hospital Association (USA)*. 1985.
14. Sieber SM, Correa P, Dalgard DW, McIntire KR, Adamson RH. Carcinogenicity and hepatotoxicity of cycasin and its aglycone methylazoxymethanol acetate in nonhuman primates. *Journal of the National Cancer Institute*. 1980;65: 177-189.

CASE IV:

Signalment:
Mouse (*Mus musculus*), female, 129S/SvEv background, >6 months of age

History:
These transgenic mice are from a 129S/SvEv genetic background. The colony is showing a high {>50%) incidence of ocular lesions manifesting clinically as crusted eyes with conjunctival swelling and ocular discharge.

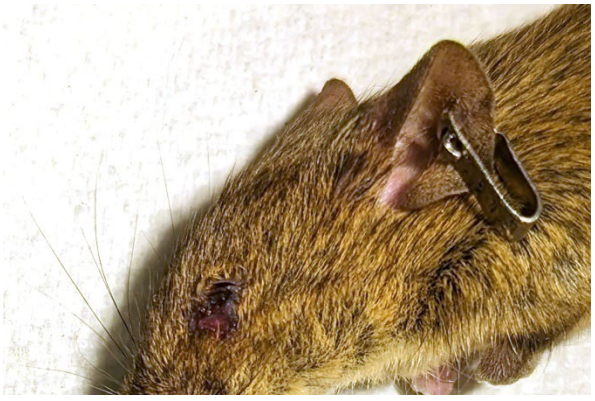

Figure 4-1. Head, 129S/SvEv mouse: Affected animals demonstrated crusted eyes with conjunctival swelling and ocular discharge. (Photo courtesy of: In Vivo Animal Core, Unit for Laboratory Animal Medicine University of Michigan, https://animal-care.umich.edu/ business-services/vivo-animal-core).

Gross Pathology:
Both mice showed serous ocular discharge, porphyrin staining, medial canthus swelling, and blepharospasm bilaterally. No experimental history of eye manipulation was reported. One mouse had been treated with an ocular antibiotic ointment.

Laboratory Results:
N/A.

Microscopic Description:
Bilaterally or unilaterally*, the eyelids contained locally extensive epidermal acanthosis and ortho to parakeratotic hyperkeratosis of varying severity. This was accompanied by epithelial erosion and mild serocellular crusting. There was mild to moderate mixed neutrophilic to lymphocytic inflammation within the conjunctiva and eyelid sebaceous glands (Meibomian glands). Focally there was occasional dilation and mild degeneration of Meibomian gland acini or ductules. The cornea, lens, iris, retina, optic nerve and other interior structures of the eyes were normal.

The Harderian gland was mildly enlarged (section plane-dependent) and glandular lumens contained occasional brown pigmented inspissated material (porphyrin secretions). Glandular epithelium lacked atypia and there was no compression or invasion as might be seen with adenoma or carcinoma.

**Grossly, the lesion was present bilaterally in affected mice but, due to section plane and variable severity, it is microscopically more evident unilaterally in the submitted examples.*

Contributor's Morphologic Diagnoses:
1. Eyelids: Blepharoconjunctivitis, neutrophilic, with epidermal acanthosis, erosions, and ortho to parakeratotic hyperkeratosis, unilateral to bilateral, moderate, chronic.

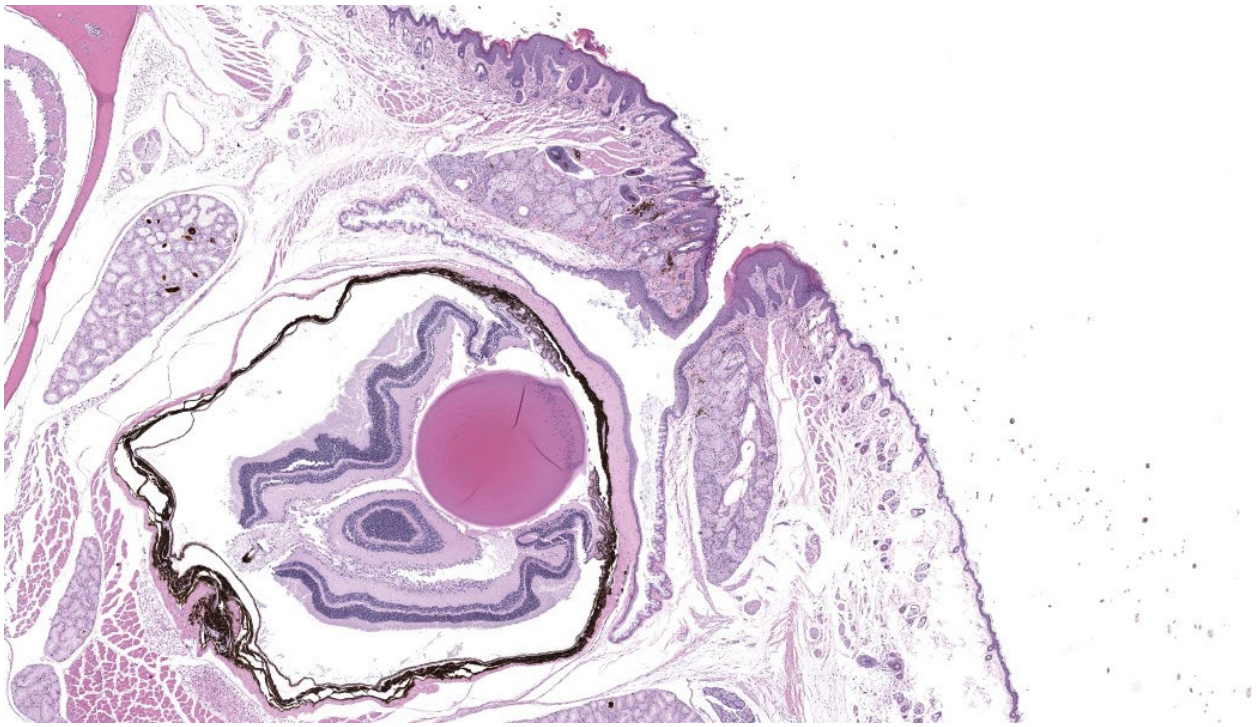

Figure 4-2. Eye, 129S/SvEv mouse: Unilaterally, there is blepharitis and conjunctivitis with epidermal hyperplasia of the eyelid and conjunctiva and overlying serocellular crusting of the eyelid. There is moderate accumulation of porphyrin pigment in the Harderian gland. (HE, 40X)

2. Harderian gland: Hyperplasia with inspissated porphyrin secretions, bilateral, mild, chronic.

Contributor's Comment:

Blepharoconjunctivitis occurs with high frequency in aging 129S/SvEv, 129P, and other 129 origin substrains.[2,3] An incidence of >90% was reported in one lifetime survey of pathology in 129S6/SvEvTac mice (older nomenclature for 129S/SvEv). Findings ranged from suppurative to mononuclear inflammation and epidermal changes included acanthosis and focal erosion or ulceration.[2] In this submission, the epidemiology of the lesion presentation and its similarity to the literature descriptions of blepharoconjunctivitis supported that its occurrence was related to the underlying background strain and not to the knockout gene.

The underlying cause for blepharitis or blepharoconjunctivitis in 129S substrains is unknown. Underlying age-related structural and functional changes in the position and function of lacrimal glands are believed to play a role, possibly by impacting mucocutaneous barrier function. Age-associated anterior migration of the palpebral mucocutaneous junction in mice has been cited as contributing to eyelid laxity and poor Meibomian gland function.[2] *Corynebacterium* species biochemically similar to C. *urealyticum,* C. *pseudodiphtheriticum,* and C. *bovis* have been cultured from affected eyelids. However, these agents were not identified in all affected animals and are also commensally found on the eyelids of normal mice. Additionally, rederivation into SPF housing was not sufficient to prevent occurrence.[2] In the submitted case, the attending clinical veterinarian reported that treatment with topical ocular antibiotic ointment appeared to decrease lesion severity and prolong

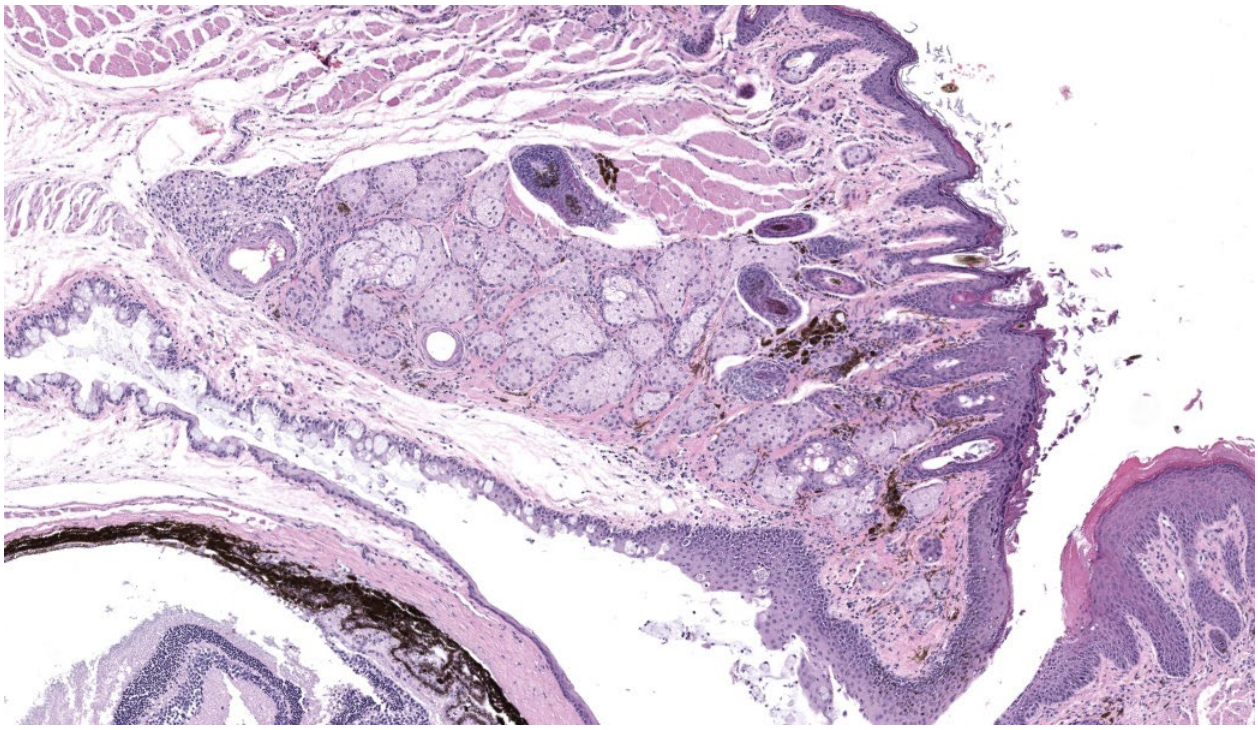

Figure 4-3. Eyelid, 129S/SvEv mouse: The eyelid has hyperplasia of the epidermis extending down into hair follicles, overlying serocellular crust, and mild dermal lymphoplasmacytic inflammation which infiltrates the subjacent Meibomian glands. (HE, 123X)

the time until the animal reached humane endpoints for euthanasia, but did not fully resolve the lesion.

Harderian gland adenomas have been linked to blepharoconjunctivitis in 129S6/SvEvTac mice, but these were believed to be a concurrent aging lesion that played an exacerbating rather than a causative role.[2]

Nomenclature of 129 mice can be confusing. 129 mice are made up of 3 major subfamilies with enough genetic variation between them that they are considered separate strains. As such, these subfamilies have been designated as the parental strain (129P), the Steel strain (129S), and the teratoma strain (129T). There are also several substrains within each of these three. 129S/SvEv mice, the strain in this submission, are widely commercially available and are frequently utilized to generate embryonic stem cells (ESCs) for the production of mice with targeted mutations (knockouts). They were originally bred from 129S mice received from Dr. Martin Evans, giving them their substrain designation.[4]

Contributing Institution:
In Vivo Animal Core, Unit for Laboratory Animal Medicine
University of Michigan
2800 Plymouth Road
Ann Arbor, Ml 48109
(https ://animal care .um ich .edu/ business-services/vivo-animal-core)

JPC Diagnoses:
1. Eyelid: Blepharoconjunctivitis, unilateral, lymphocytic and neutrophilic, chronic, diffuse, mild, with epidermal hyperplasia and erosion.
2. Eyelid, Meibomian gland: Adenitis, lymphoplasmacytic, chronic, focal, mild.

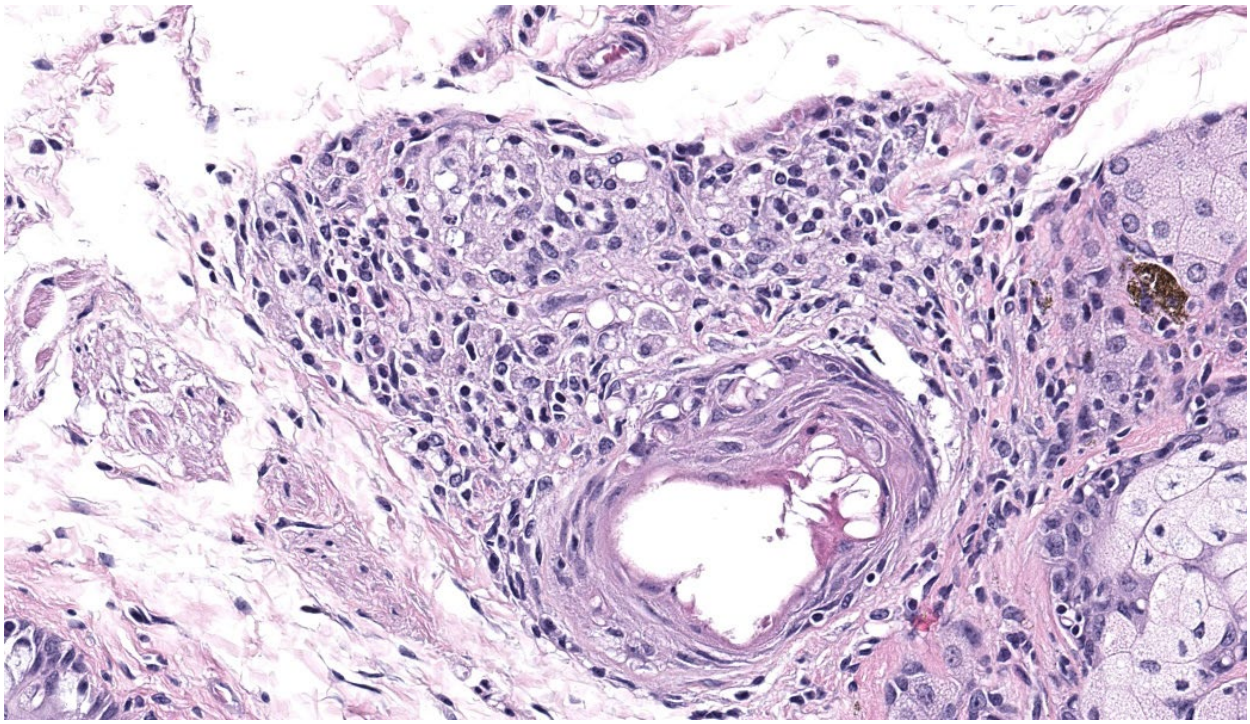

Figure 4-4. Eyelid, 129S/SvEv mouse: There is focal loss of Meibomian gland architecture and infiltration by lymphocytes and plasma cells. (HE, 381X)

JPC Comment:

Although seemingly a not-so-exciting lesion, this is one that needs to be able to be recognized by the pathologist, especially in 129 mice. It is a common finding in 129 substrains! The contributor did a great job discussing this lesion in their comment which, again, covered most of this case's conversation. Some participants were thrown off initially by the size discrepancy between the two lenses, but this is largely due to a function of cut during processing and is not a lesion. The contributor mentioned that this was a grossly bilateral lesion but did acknowledge that this is not seen histologically due to sectioning. In short, don't be thrown off if the lesion isn't obvious on both eyelids despite the gross description.

Although not in the contributor's description, a few participants astutely perceived the presence of a sequestrum characterized by necrotic bone with surrounding osteoclastic activity within the right side of the maxillary bone. This sequestrum is surrounded by moderate inflammation and fibrosis, and there is an underlying neutrophilic and lymphocytic gingivitis. Most participants thought this may have been related to some form of oral trauma that resulted in a penetrating injury to the hard palate. Gingivitis is a common background lesion in laboratory mice, and the sequestrum was a neat secondary lesion to find! The JPC did not include the sequestrum and gingivitis in the morphologic diagnoses, however, to ensure clarity on the primary blepharoconjunctivitis lesion in this case.

Conference attendees were also quizzed on additional common background lesions of 129 mice, which include a reduced or absent corpus callosum (can be up to 80% in some groups of 129 mice), spontaneous testicular teratomas and lung tumors, eosinophilic crystalline pneumonia (also known as acidophilic macrophage pneumonia), hyalinosis of the

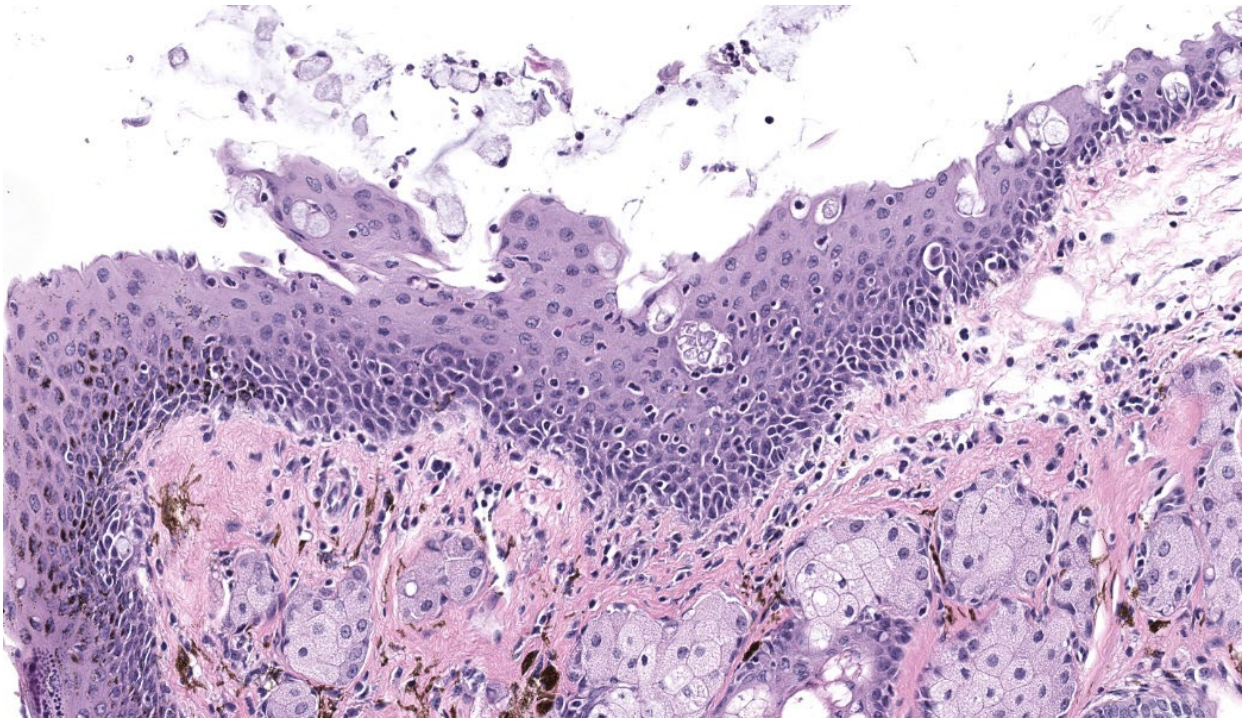

Figure 4-5. Eyelid, 129S/SvEv mouse: The conjunctival epithelium is infiltrated by low numbers of neutrophils and lymphocytes. (HE, 381X)

glandular stomach, respiraoty tract, and gallbladder, urolithiasis, megaesophagus, polyarteritis, and age-related hearing loss in 129P3/J mice. This particular lesion is caused by a Cdh23ahl mutation that results in progressive hearing loss by 3 months of age.[1] In contrast, 129S/SvEv mice are resistant to noise-related hearing loss.[5]

References:

1. Burghard AL, Morel NP, Oliver DL. Mice heterozygous for the Cdh23/Ahl1 mutation show age-related deficits in auditory temporal processing. *Neurobiol Aging*. 2019;81:47-57.
2. Radaelli E, Castiglioni V, Recordati C, et al. The pathology of aging 129S6/SvEv-Tac mice. *Vet Pathol.* 2016;53(2):477-92.
3. Sundberg JP, Brown KS, Bates R, Cunliffe-Beamer TL, Bedigian H. Suppurative conjunctivitis and ulcerative blepharitis in 129/J mice. *Lab Anim Sci.* 1991;41:516-18.
4. Mouse strain 129 substrain nomenclature. Mouse Genome Informatics Database at the Jackson Laboratory, Bar Harbor, ME. Accessed September 13, 2023.
5. Yoshida N, Hequembourg SJ, Atencio CA, Rosowski JJ, Liberman MC. Acoustic injury in mice: 129/SvEv is exceptionally resistant to noise-induced hearing loss. *Hear Res*. 2000;141(1-2):97-106.