Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 22

27 April 2016

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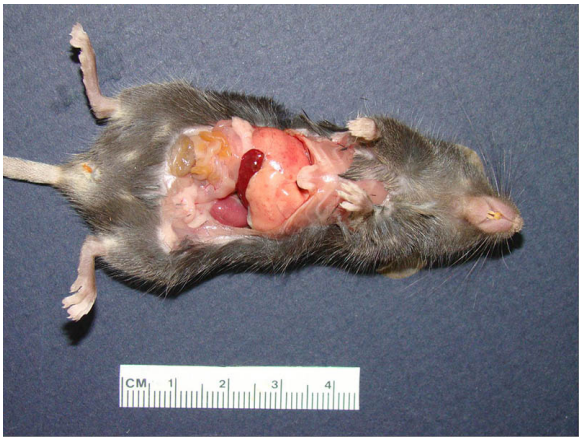
CASE I: NIEHS-087 (JPC 4017222).

Signalment: 11-month-old B6.129S-*Cybb*^{tm1Din}/J mouse (*Mus musculus*)

History: A breeding colony of B6.129S-*Cybb*^{tm1Din}/J mice were housed in an AAALAC International accredited facility. The mice were housed in static micro isolator cases with *ad libitum* autoclaved food (NIH-31) and beta chip bedding. Mice were provided acidified water due to imm-unocompromised state. The mice were housed in the same room as B6 imm-unocompetent mice. Sudden deaths were noted in the colony over a weekend. A total of 87 mice, aged from one to eleven months were affected. Of these, 45 mice were found dead and 19 sick mice were euthanized and necropsied. Twenty males and 38 females were affected.

Gross Pathology: The livers were pale and slightly enlarged. In most of the mice, there



Body as a while, mouse. The liver was slightly enlarged, and there are multiple tan foci in the liver and lung. (Photo courtesy of: National Institute of Environmental Health Sciences, Cellular and Molecular Pathology Branch and Comparative Medicine Branch, P.O. Box 12233, Research Triangle Park, NC 27709, http://www.niehs.nih.gov/research/atniehs/labs/lep/index.cfm)

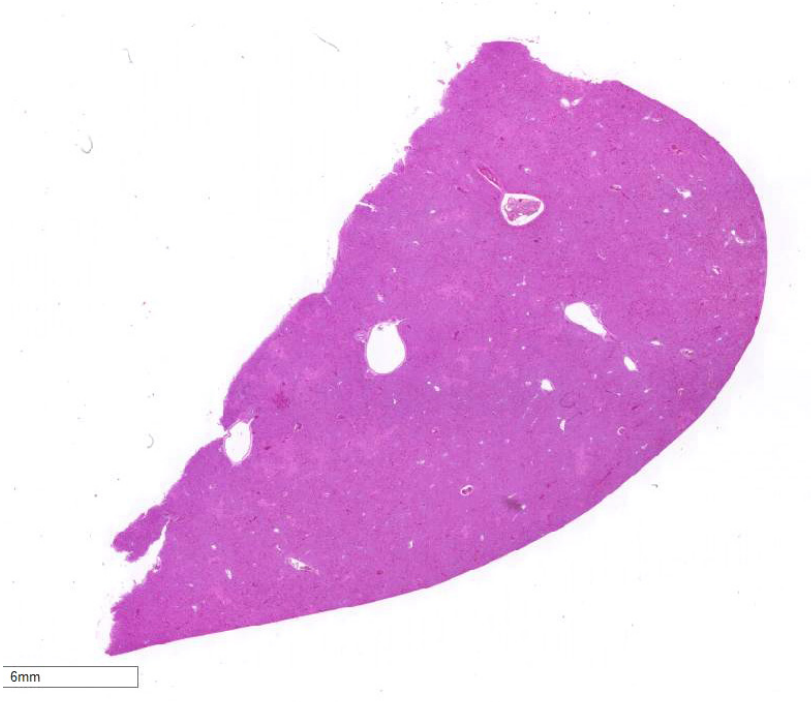
were multifocal tan foci in the liver, spleen and lung.

Laboratory Results: From multiple tissues, a pure culture of *Burkholderia* spp. was isolated. The isolate was further

characterized as *Burkholderia cepacia* with PCR. In the blood smears, monocytes contained bacterial rods.

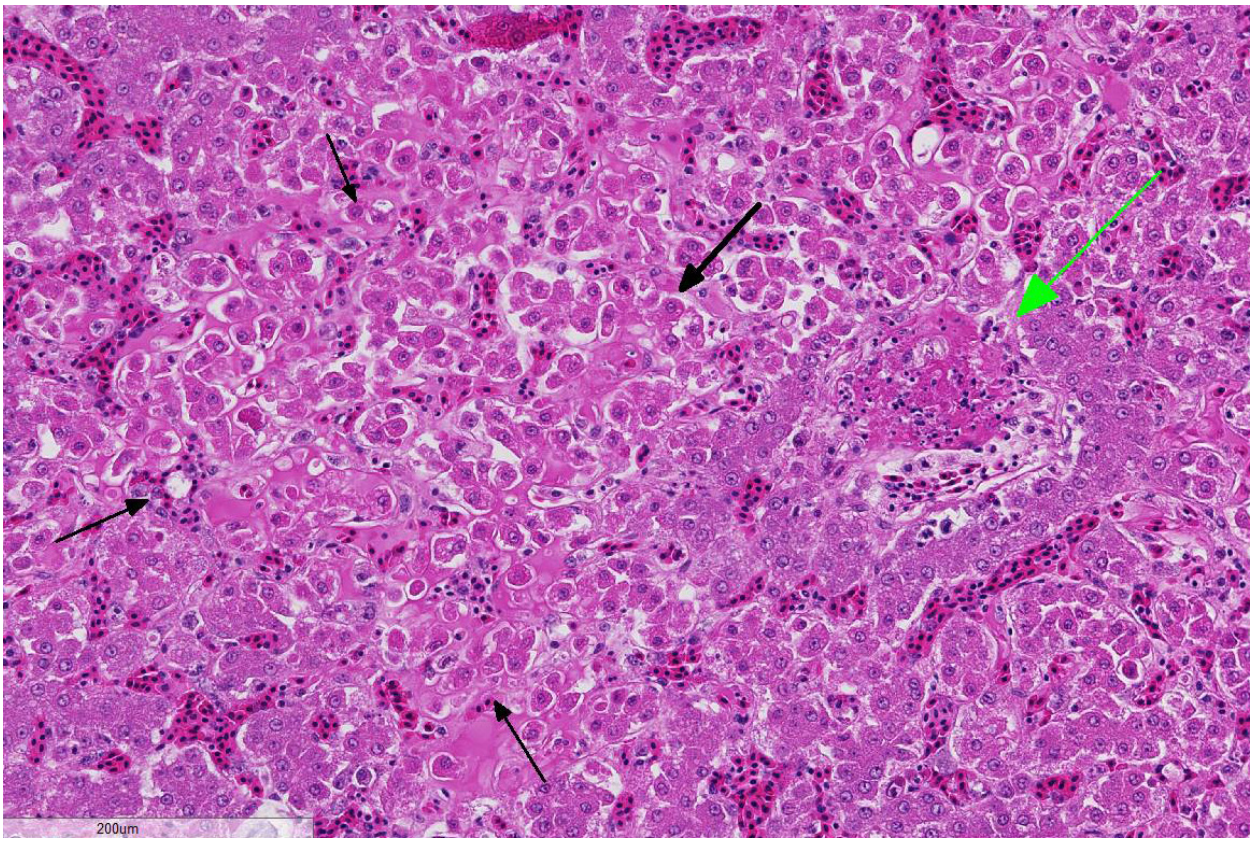
Histopathologic Description: Liver: Multifocally and randomly, there are areas of hepatocyte necrosis containing cellular and karyorrhectic debris and moderate numbers of degenerate neutrophils. Multifocally, many blood vessels contain thrombi. There are large areas of coagulative necrosis surrounding these thrombosed blood vessels. Multifocally, the hepatocyte cytoplasm is vacuolated (glycogen) and, contain clear round vacuoles (lipid). Multifocal portal areas are infiltrated by small numbers of lymphocytes. No bacteria were present in the Gram's stained liver section.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrosuppurative, multifocal, random, moderate with vascular thrombosis.



Liver, mouse. Randomly throughout the section, there are pale foci of necrosis. (HE, 5X).

Contributor's Comment: B6.129S-*Cybb*^{tm1Din}/J mouse is a targeted mutant lacking superoxide production in phagocytes.[9] 129S6 mice is the embryonic stem cell donor in which cytochrome b-245, beta polypeptide(*Cybb*) targeted mutation(*tm*) was created by Dr. Mary C Dinauer(*Din*) (http://jaxmice.jax.org/strain/002365.html; accessed on August 5, 2011). Superoxide production is critical in host defense. Therefore, mice lacking superoxide production are immunocompromised. This mouse is an animal model for X-linked chronic granulomatous disease in humans.[9] These mice are susceptible to various bacterial and fungal infections.[9] Based on the gross lesions in the affected mice differential diagnoses included septicemia, mouse hepatitis viral infection, pox viral infection and Tyzzer's disease. Histologically, the absence of syncytial cells and intracytoplasmic inclusions helped to eliminate the possibility of mouse hepatitis virus and poxvirus infection, respectively. Absence of bacilli in and around the necrotic hepatocytes, eliminated the possibility of Tyzzer's disease. The histologic changes in the current case were consistent with a septicemic process. This was supported by the isolation of bacteria and evidence of bacteremia. *Burkholderia cepacia* complex includes nine members. The bacteria are ubiquitous in nature and can cause pneumonia and septicemia in cystic fibrosis and chronic granulomatous disease patients.[3,7] In the current outbreak the source of



Liver, mouse. There are multiple areas of coagulative necrosis (black arrows), as well as partially occlusive fibrin thrombi within adjacent vessels. (HE, 181X)

infection could not be determined. Because B6.129S-*Cybb*^{tm1Din}/J mouse is a model for X-linked CGD,[9] only male mice should have been affected. However, both males and females were affected in the current case. One possible explanation could be that certain numbers of cells in females can have the mutant X-chromosome resulting in a full expression of an X-linked condition.[5] Also, spontaneous infections with various pathogens in both males and females have been previously reported in this mouse model.[2]

JPC Diagnosis**:** Liver: Hepatitis, necrotizing, multifocal, random, marked with fibrin thrombi.

Conference Comment**:** *Burkholderia cepacia* complex includes a group of motile, aerobic, gram-negative bacilli that are ubiquitous in the environment and can be isolated from soil, vegetables and water.[1] They act as endophytic bacteria on certain types of vegetation and have been used as a potential catalysts, involved in the formation of enzymes, used in the creation of alternative energy sources from perennial grasses.[6] Due in part to the presence of antibiotic resistance mechanisms, they are also important bacterial pathogens in the hospital setting,[1] and are important opportunistic pathogens in cases of septicemia and lung infection in cystic fibrosis patients.[4] The organism has also been isolated from the deep pyoderma in dogs on immunosuppressive therapy (cyclosporine). In those cases the clinical course was acute and infection was widespread. Because *B. cepacia* is not a commensal on canine skin, it is presumably acquired either from the

environment or within hospitals, although reports of environmental *B. cepacia* in veterinary practices are rare. *B. cepacia* has also been reported to cause subclinical mastitis in dairy sheep.[1]

As discussed above, the transgenic mice in this case are deficient in superoxide production in phagocytes. Once phagocytosis of an opsonized particle takes place within a neutrophil, usually via binding of complement or F_c receptors, internal signaling cascades are initiated involving various GTPases and protein and lipid kinases, eventually resulting in release of calcium from the endoplasmic reticulum and production of the oxidative burst within phagolysosomes. The oxidative burst is initiated by formation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which stimulates generation of superoxide free radicals, critical in microbial killing.[8] In this case, a mutation in NAPDH oxidase is involved in superoxide deficiency and absence of a functional respiratory burst.[9] Reactions involving superoxide anion result in the formation of hydrogen peroxide, hydroxyl radical and hypochlorous acid as well as peroxynitrate, all of which are important microbicidal agents.[8]

The conference histologic description was very similar to the contributor's description above. Not all sections have prominent fibrin thrombi but in sections with thrombi areas of necrosis appear spatially associated with thrombosed vessels. The differential diagnosis for this lesion includes *Helicobacter hepaticus*, *Salmonella* spp., *Proteus mirabilus* and *Clostridium piliforme* as well as some viral conditions such as mouse hepatitis virus, as mentioned above. Both Gram stains and silver stains failed to identify infectious organisms. Conference participants discussed *B. cepacia* and its various virulence factors including lipopolysaccharide (LPS), exotoxins, lipases, siderophores and proteases as well as the inherent antibiotic resistance of its cell envelope.[7]

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CASE II: AR07-025012 (JPC 4032917).

Signalment: Adult, male, Fischer 344 rat (*Rattus norvegicus*).

History: This rat was euthanized after presenting for rolling and inability to maintain balance.

Gross Pathology: The spleen was dark red, markedly enlarged (9 x 3 x 1.5 cm) and occupied 2/3 of the abdominal cavity, compressing adjacent organs. The surface was irregular and traversed by prominent white-tan fibrous adhesions. The liver was dark red and moderately enlarged.

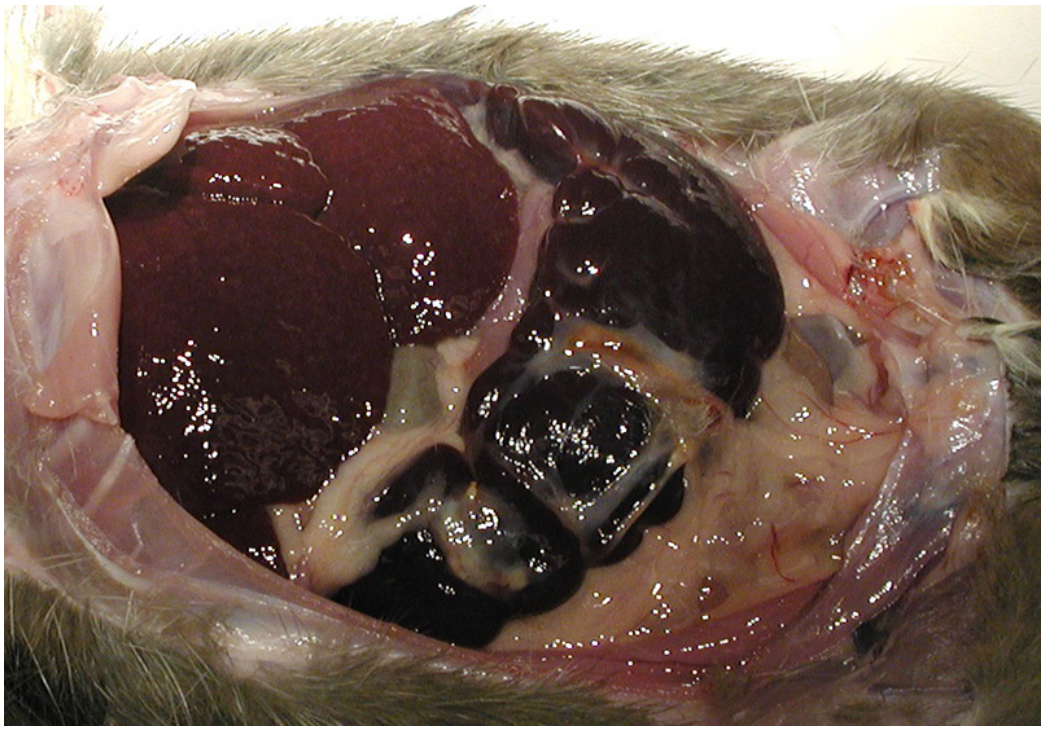
Laboratory Results: None reported.

Histopathologic Description: Testis: Testicular blood vessels are filled with neoplastic round cells resembling lymphocytes, with irregularly round, 6-10 micron diameter hyperchromatic nuclei and scant pale eosinophilic cytoplasm frequently forming thin rims around the nuclei. Mitotic figures are rare. Replacing the testicular parenchyma is another neoplasm, 3 x 4 mm, well demarcated, unencapsulated and multilobular, composed of sheets and packets of round to polygonal cells supported by fine fibrovascular stroma. The neoplastic cells have variably distinct cell borders, abundant eosinophilic finely vacuolated cytoplasm, and oval, central nuclei with finely stippled chromatin and a single, variably distinct basophilic nucleolus. Mitotic figures are rare. The adjacent seminiferous tubules are compressed and shrunken, lined only by Sertoli cells with an absence of germ cells. Some contain rounded germ cells with eosinophilic cytoplasm and nuclear condensation or multinucleated spermatid giant cells. Others are dilated and contain variable amounts of homogeneous eosinophilic material.

Contributor's Morphologic Diagnosis:

1. Testis, vessels: Lymphocytic leukemia (Large Granular Lymphocyte Leukemia)
2. Testis: Interstitial cell tumor
3. Testis, seminiferous tubules: Tubular degeneration and atrophy, multifocal, moderate

Contributor's Comment: Large granular lymphocyte leukemia and interstitial cell tumors are two of the most common neoplasms in aged Fischer 344 (F344) rats. Large granular lymphocyte (LGL) leukemia, also known as mononuclear cell leukemia or Fischer rat leukemia occurs commonly in the F344 rat but is also reported in other strains. It is a rapidly fatal, age-related neoplasia, with a significantly increased risk after 20 months of age.[4] Clinically, rats can exhibit inactivity, weight loss, pale eyes, icterus, and palpable splenomegaly.[6]

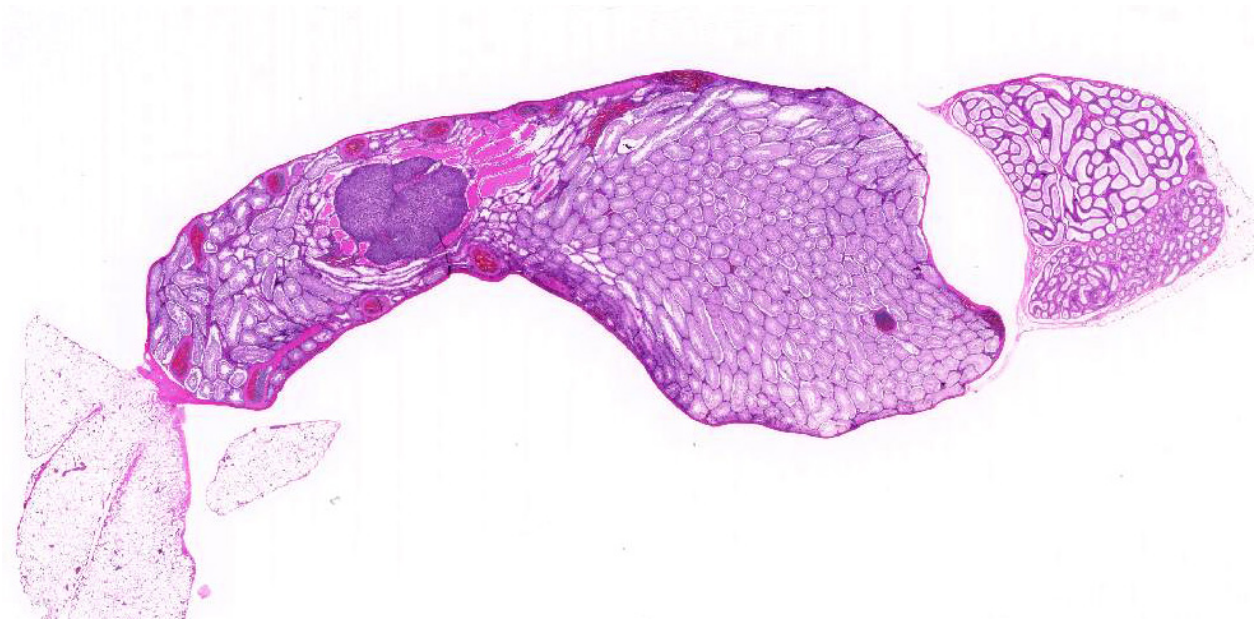


Testis, F344 rat. There is marked splenomegaly with hepatomegaly in an adult Fischer 344 rat. White-tan adhesions multifocally traverse the cobblestoned surface of the spleen. (Photo courtesy of: Wake Forest School of Medicine, Department of Pathology/Comparative Medicine, Medical Center Boulevard, Winston Salem, NC 27157-1040, http://www.wakehealth.edu/School/Comparative-Medicine/Training-Programs/ACVP.htm

Common clinical pathological abnormalities include hemolytic anemia and thrombocytopenia. Gross lesions include a characteristically markedly enlarged, often friable spleen and an enlarged, mottled liver. Enlarged mesenteric lymph nodes and hemorrhage in the lungs, lymph nodes and brain can also be present.[4]

Histologically, LGL leukemia most consistently demonstrates diffuse leukemic infiltration of the red pulp, where the neoplasm is believed to arise, with variable lymphoid depletion.[4,5] The liver and spleen are generally always infiltrated by neoplastic LGLs. In advanced stages, a general leukemia is present, and vessels in lymph nodes, lung, and brain are often infiltrated by neoplastic cells. Bone marrow involvement, if present, occurs late relative to involvement of the spleen.[4] Rats with LGL leukemia can also have focal accumulations of neoplastic lymphocytes,[7] such as were observed in the brain in this case, which led to the neurological clinical signs.

Large granular leukocytes have distinctive azurophilic cytoplasmic granules in blood smears; granules are generally not discernible on histology sections.[5] Well-differentiated LGLs may have reniform nuclei and cytoplasmic granules; poorly differentiated LGLs have few or inapparent granules.[6] LGL cells are generally pleomorphic and 10-15 microns in diameter. Many studies have demonstrated natural killer cell activity of LGLs, but the exact cell of origin is still not fully understood.[6,8]



Testis, rat. Within the body of the testis, there is an interstitial cell tumor.

Interstitial, or Leydig, cell tumors are the most common testicular neoplasms in rats, dogs, cats and bulls.[1,2] Fischer 344 rats have an incidence approaching 100% by 24 months of age.[1] Grossly, interstitial cell tumors tend to be well-circumscribed, tan to yellow-orange, soft masses. There can be multiple tumors and they can be unilateral or bilateral. Histologically, interstitial cell tumors are unencapsulated and composed of uniform round or polyhedral neoplastic cells with abundant eosinophilic finely granular or vacuolated cytoplasm. Peripheral seminiferous tubules are often compressed and show variable degrees of degeneration and atrophy. Nuclei are generally round, centrally-placed and have single nucleoli. Mitotic rates are generally low. Interstitial cell hyperplastic lesions, probably preneoplastic lesions, can be more diffuse, occur between tubules rather than replace them, and have a diameter smaller than three seminiferous tubules.[1] A much less common and more pleomorphic proliferative interstitial cell lesion with cellular atypia and invasion of adjacent tissue, including vessels or the capsule, or with metastases warrants the diagnosis of interstitial cell carcinoma.[1]

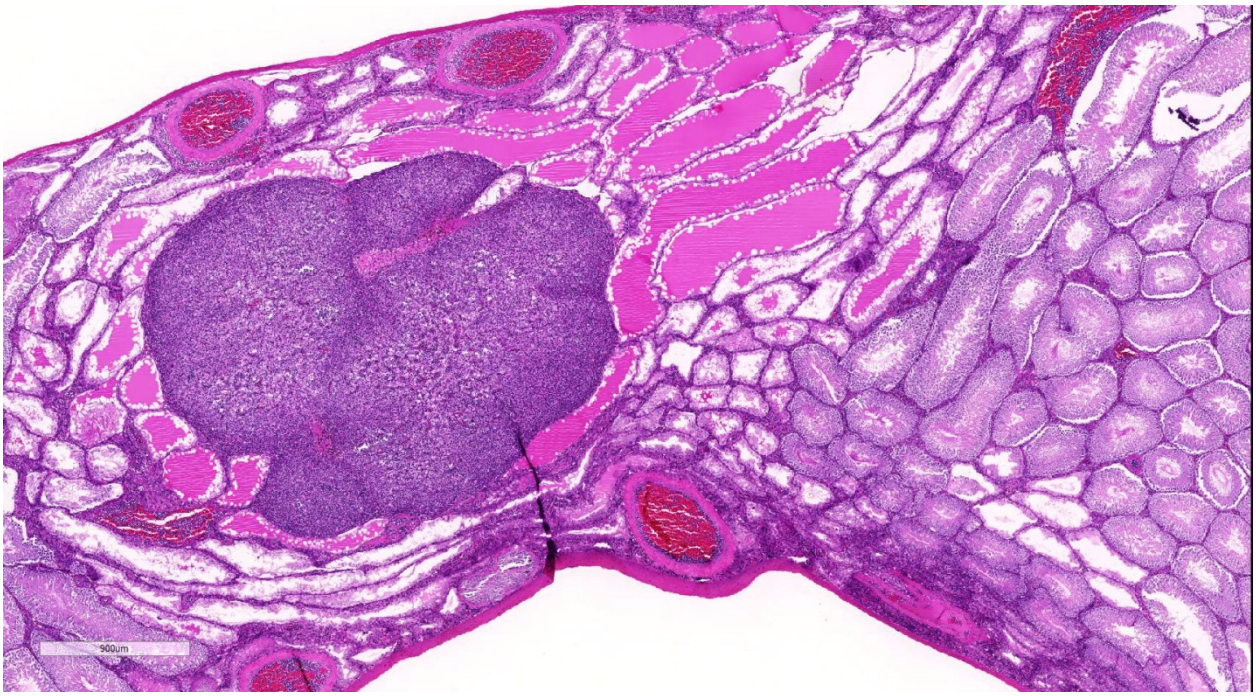
JPC Diagnosis**:** 1. Testis, blood vessels: Mononuclear cell leukemia.
2. Testis: Leydig cell adenoma.
3. Testis, seminiferous tubules: Degeneration, atrophy and loss with marked aspermatogenesis and spermatid giant cells.

Conference Comment**:** Conference participants discussed the nomenclature of large granular lymphocytic (LGL) leukemia. We prefer the nomenclature noted above, in agreement with INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice). The "granular" descriptor refers to the cytologic appearance of neoplastic cells when stained with a Wright-Giemsa stain; the granules cannot be visualized in histologic sections stained with hematoxylin and eosin. The moderator also mentioned that in some severe cases, extravascular infiltrates of neoplastic cells can be seen as well as circulating cells with mitotic figures. The neoplasm is a heterogeneous, non-B,

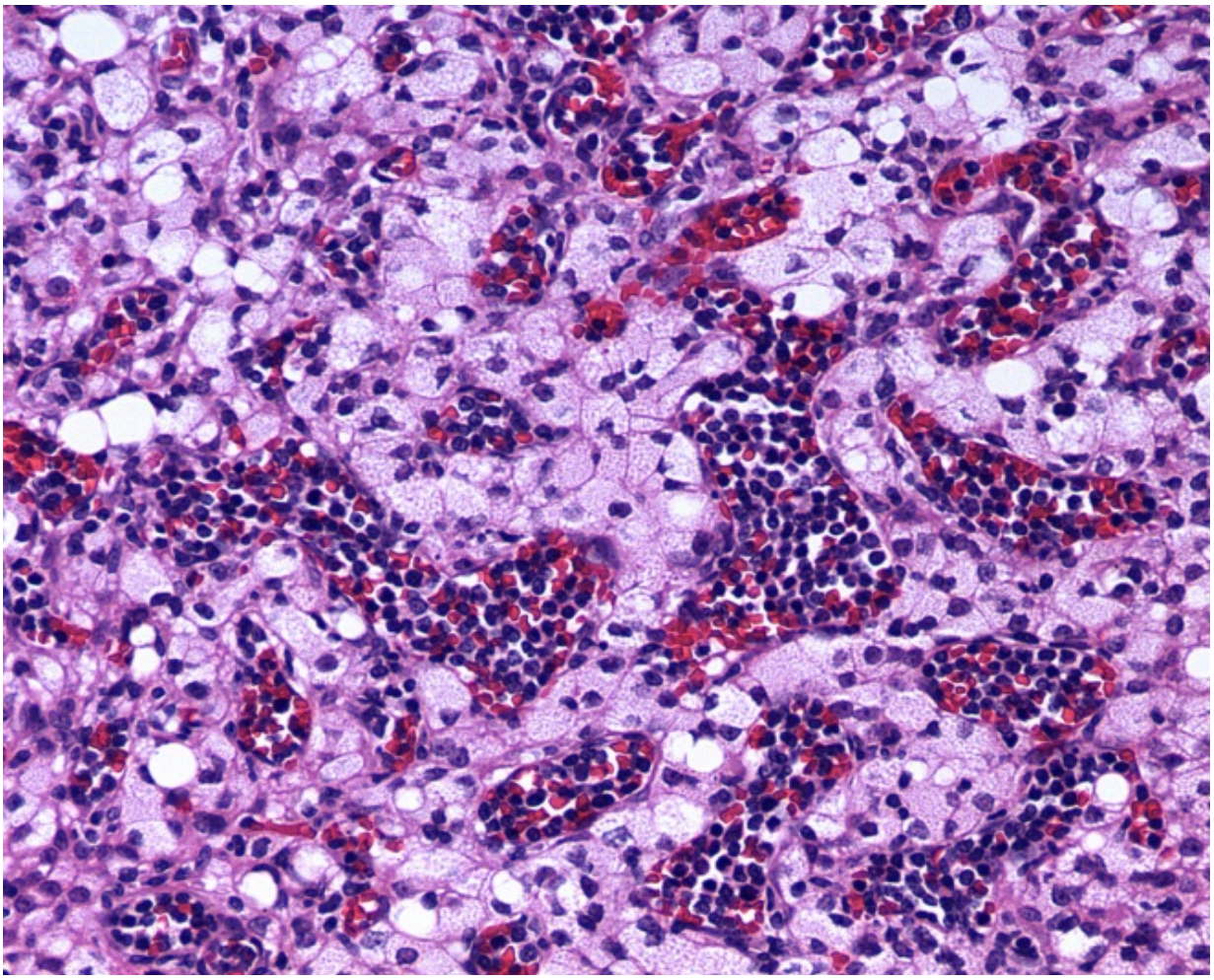
non-T cell leukemia with neoplastic cells having natural killer cell activity; cells are negative for the immunohistochemical stain leukocyte common antigen (LCA, CD45). Icterus is often seen in affected animals secondary to hemolytic anemia; the leukemia cells will also phagocytose erythrocytes and their granules can be erythrolytic. Due to the high incidence of this condition in Fischer 344 rats, Sprague-Dawley and Wistar rats (as opposed to Fischer 344 rats) are used more commonly in toxicology studies.

Early diagnosis of LGL leukemia can be challenging, but the presence of leukemia cells in hepatic sinusoids may aid in the diagnosis. Other hepatic changes include nodular regenerative hyperplasia, characterized by centrilobular atrophy and periportal hypertrophy of hepatocytes secondary to anemia-related ischemic hepatocellular injury; endothelial injury may also play a role in some cases. Splenic congestion, a common and consistent finding, and is postulated to be related to portal hypertension in addition to proliferation of neoplastic LGL leukemia cells in the spleen. The precise cause or pathogenesis of portal hypertension in this condition is unclear.[3]

As noted in the contributor's comments, Leydig cell hyperplasia is a differential diagnosis for Leydig cell tumor. Neoplastic lesions are considered to be a continuum or progression from hyperplastic lesions in most cases. Unlike hyperplasia, however, Leydig cell tumors will generally demonstrate compression of adjacent testicular parenchyma and neoplastic cells will be larger, with larger nuclei and may (or may not) demonstrate atypia. Hyperplastic lesions may be focal, multifocal or diffuse, with diffuse being most common, and cells may demonstrate prominent vacuolation;



Testis, rat. The tumor is surrounded by ectatic, atrophic seminiferous tubules. The loss of spermatocytes within these tubules has resulted in an overall narrowing of the testis at this location, in spite of the presence of the interstitial cell tumor. (HE, 24X)



Testis, F344 rat. Two neoplastic populations are present in the testis. One fills blood vessels (leukemia) scattered amongst the large polygonal cells with finely vacuolated cytoplasm of the other (interstitial cell tumor). (Photo courtesy of: Wake Forest School of Medicine, Department of Pathology/Comparative Medicine, Medical Center Boulevard, Winston Salem, NC 27157-1040, http://www.wakehealth.edu/School/Comparative-Medicine/Training-Programs/ACVP.htm

however, hyperplastic lesions will not compress the surrounding tissue. Another criterion which may aid in the diagnosis of less straightforward cases is the designation of a proliferative Leydig cell lesion with a diameter of greater than three seminiferous tubules as a neoplasm (Leydig cell adenoma).[1]

The pathogenesis of Leydig cell hyperplasia is related to elevated luteinizing hormone levels, paracrine stimulatory factors within the testis or can be secondary to decreased spermatogenesis.[1] Restriction of dietary intake may decrease the incidence of interstitial cell tumor but is not documented to decrease the incidence of LGL leukemia.[7] A diffuse distribution of hyperplastic lesions is more common in the mouse as well as in the rat.

Contributing Institution:

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http://www.wakehealth.edu/School/Comparative-Medicine/Training-Programs/ACVP.htm

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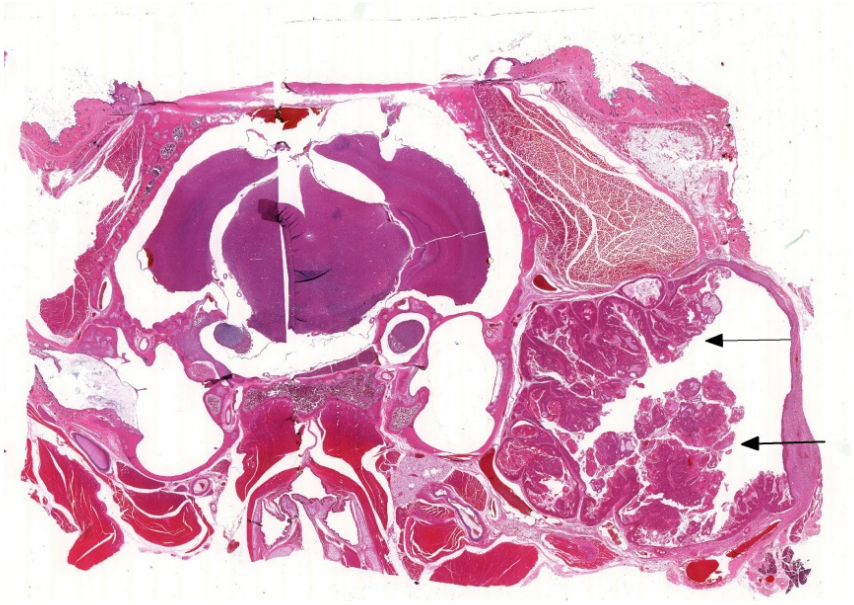
CASE III: 15-V587 (JPC 4067775).

Signalment: >24 month old, male, Fischer F344 rat (*Rattus norvegicus*).

History: An aged male F344 rat enrolled in a behavior study underwent surgery for the placement of head prosthesis. The animal recovered uneventfully, but 4 weeks following the surgery, a small swelling on the right side of the face was noticed. The swelling increased in size over several days. An aspirate of the mass showed eosinophilic acellular linear material (keratin), non-degenerative neutrophils, histiocytes, lymphocytes, and amorphous debris. The rat was anesthetized and the swelling was lanced and flushed, producing copious



Cross section of head, rat. A large, proliferative mass arises from the squamous lining of the deeper aspect of the ear canal. (HE, 6X).

amounts of purulent and sebaceous discharge. The rat was treated with oral antibiotic therapy (enrofloxacin administered in the drinking water); however, facial swelling was still present and animal's condition deteriorated. Humane euthanasia via intraperitoneal barbiturate overdose was elected by the investigative group.

Gross Pathology: There was an approximately 1 cm diameter, firm swelling at the base of the right ear. There was focal ulceration on the caudoventral aspect of the swelling with adhered bedding material. Both kidneys were diffusely, mildly pale, shrunken and irregular in texture, with pinpoint depressions across the surface. The spleen was approximately twice the normal size with an irregular texture. The liver was diffusely moderately enlarged and irregular. Both testicles were approximately 1.5 times larger than normal and yellow to tan.

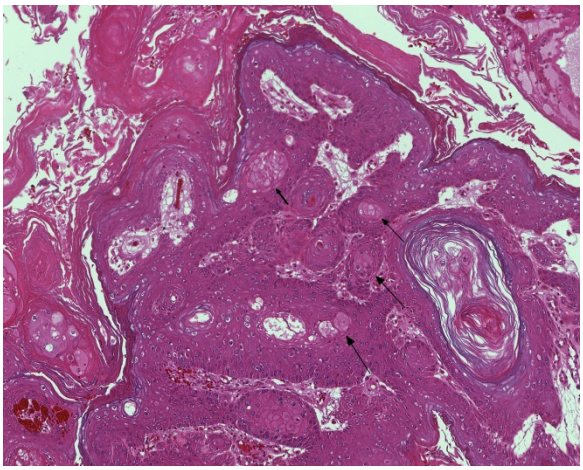
Laboratory Results: None

Histopathologic Description:

Extending through the subcutis from the lateral aspect of the bulla is a large, well demarcated, non-invasive, mul-tilobulated and nonencapsulated neoplasm consisting of papillary projections of stratified sq-uamous epithelium supported by expanded fibrovascular cores. The basement membrane is intact. There is diffuse, marked hyperkeratosis with abundant strands of eosinophilic acellular material (keratin) present within the center of the neoplasm. There are multifocal circular areas of lamellated keratin (keratin pearls). Neoplastic cells are well differentiated squamous epithelial cells with variably well delineated cytoplasmic borders and moderate eosinophilic cytoplasm. An ovoid central nucleus with a single nucleolus is present. There is mild to moderate anisocytosis and anisokaryosis. Mitotic figures are not observed. There is mild, multifocal in-tercellular edema with accentuation of intercellular desmosomes. Multifocal cystic cavities are also present. Sebaceous cells are moderately to markedly enlarged and increased in number, with abundant pale and vacuolated cytoplasm. Extending along the lateral aspect of the neoplasm is a thick layer of mature fibrous connective tissue with mild multifocal extravascular red blood cells (hemorrhage).

Contributor's Morphologic Diagnosis: Zymbal's gland: Squamous cell papilloma with sebaceous hyperplasia and cystic degeneration.

Contributor's Comment: The Zymbal's gland is a multilobulated sebaceous holocrine gland located anterior-ventral to the ear canal in rats and mice, although it is smaller in mice[5,7] This gland consists of 3-4 lobules which each have intralobular ducts that empty into the excretory duct and then ultimately into the ear canal.[5,6] Spontaneous neoplasms are rare in rats with an incidence of 1% or less.[6] Types of neoplasia reported



Ear canal, rat. The mass is composed of papillary projections of squamous epithelium which exhibits normal maturation. There are foci of sebaceous differentiation (arrows) scattered throughout the proliferating epithelium, as well as keratin pearls. (HE, 200X)

include sebaceous cell adenoma, squamous cell papilloma, and carcinoma of sebaceous and/or ductal epithelial origin.[5] The Zymbal's gland is a target organ of many carcinogenic chemicals,[4] with an incidence of Zymbal's gland neoplasms in as many as 67% of experimental animals (versus 0% in controls) in some studies.[3] Chemicals known to induce Zymbal's gland tumors include 2-Acetylaminofluorine, Tris carbonium pamoate, urethan, heterocyclic amines, benzene, 3-3 dichlorobenzidine, 4-aminostilbene, azoxymethane, methychorlanethrene, and cupferron.[6]

In this case, no history of toxin exposure was present. Despite the large size, a benign tumor was diagnosed given the lack of mitoses and cellular atypia as well as the lack of invasion into the surrounding muscle and subcutis. The tumor was characterized as a papilloma given the exophytic multilobulated growth with prominent fibrovascular core. Marked sebaceous hyperplasia was present, which is reported to be common in cases of squamous cell papilloma of the Zymbal's gland.[5]

JPC Diagnosis**:** Zymbal's gland: Squamous papilloma with multifocal sebaceous differentiation.

Conference Comment**:**
Squamous papillomas of the Zymbal's gland originate from the duct epithelium, are composed of stratified squamous epithelium and must be differentiated from squamous cell carcinoma based on absence of invasive growth and cellular atypia. Inflammatory, atrophic and hyperplastic changes are also described in the Zymbal's gland, although their frequency of occurrence varies. The retention of normal tissue architecture aids in differentiating hyperplasia from adenoma. Adenomas will also generally have increased numbers of basaloid cells compared to hyperplasia, are lobulated, and well defined but not encapsulated. Many of the neoplastic cells demonstrate cytoplasmic vacuolation, akin to mature sebaceous gland cells.[5] In this case there was considerable discussion during the conference regarding the presence of vacuolated sebaceous cells in this neoplasm; some participants argued the cells which appear vacuolated may actually represent degenerating squamous epithelium. Regardless, glandular proliferation is often associated with squamous papilloma of the Zymbal's gland and thus, if present in this case, would not be surprising and would not change the diagnosis. Zymbal's gland carcinoma is also described and includes such features as pleomorphism, increased mitotic index, squamous differentiation, ulceration and absence of ducts.[5]
In addition to the mammary gland, the Zymbal's gland is commonly assessed in xenobiotic studies in rodents since it is a target organ of many carcinogens. Aside from neoplastic changes, degenerative, necrotic, regenerative and vascular changes are also described in the gland and many of the changes are similar to what is observed in the mammary gland.[5] Additional tissues which may be sampled in xenobiotic studies include the preputial and clitoral glands, which are also common sites for adenomas in rodents. Degeneration, necrosis and regeneration are often seen in the same gland with repeated toxin exposure. Dilation or cystic degeneration of ducts may also be seen in the Zymbal's gland as a spontaneous aging change in rodents.[5]

Common tumor types in both the F344 and Sprague Dawley rat strains (from the National Toxicology Program historical control database) include mammary gland fibroadenoma, pituitary pars distalis adenoma, thyroid gland C cell adenoma, adrenal medulla benign pheochromocytoma,

and uterine stromal polyp.[1,2] Mammary gland carcinoma[1,2] and histiocytic sarcoma are also common tumors seen in Sprague Dawley rats, and large granular lymphocytic leukemia, interstitial cell tumors in males,[1,2] and mesothelioma originating from the testicular tunic are common in F344 rats.

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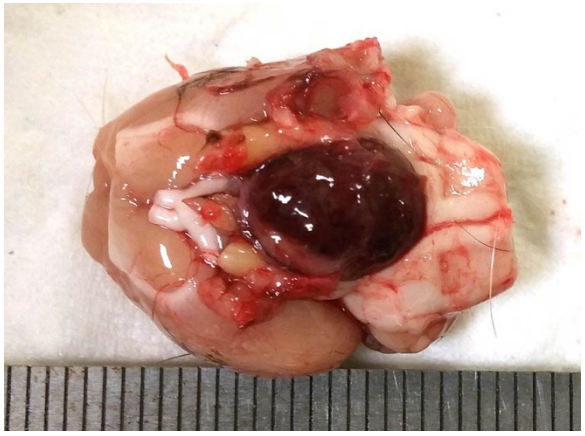
CASE IV: EX 109 (JPC 4069626).

Signalment: An 18-month-old, female, Long–Evans rat (*Rattus norvegicus*).

History: The investigator noticed a large, firm, subcutaneous mass in the right axilla.

Gross Pathology: At necropsy, a firm, circumscribed and lobulated subcutaneous mass approximately 3.5 cm in diameter, was attached to the skin in the axillary area. On removing the brain, a dark red-brown nodular mass, approximately 1 cm in



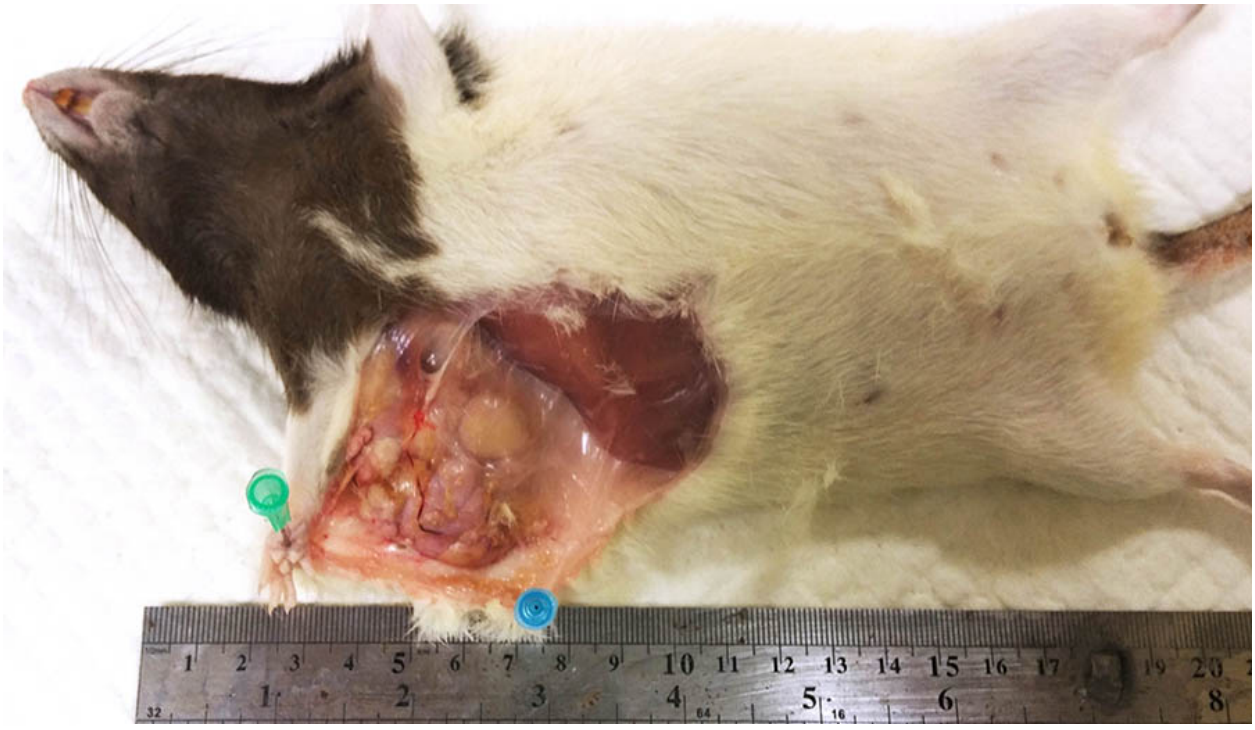
Hypophysis, rat. A dark red-brown nodular mass, approximately 1 cm in diameter, replaces the pituitary gland. (Photo courtesy of: Department of Veterinary Resources, Weizmann Institute, Rehovot 76100, Israel, http://www.weizmann.ac.il/vet/)

diameter, was present in the ventral aspect of the brain, replacing the pituitary gland.

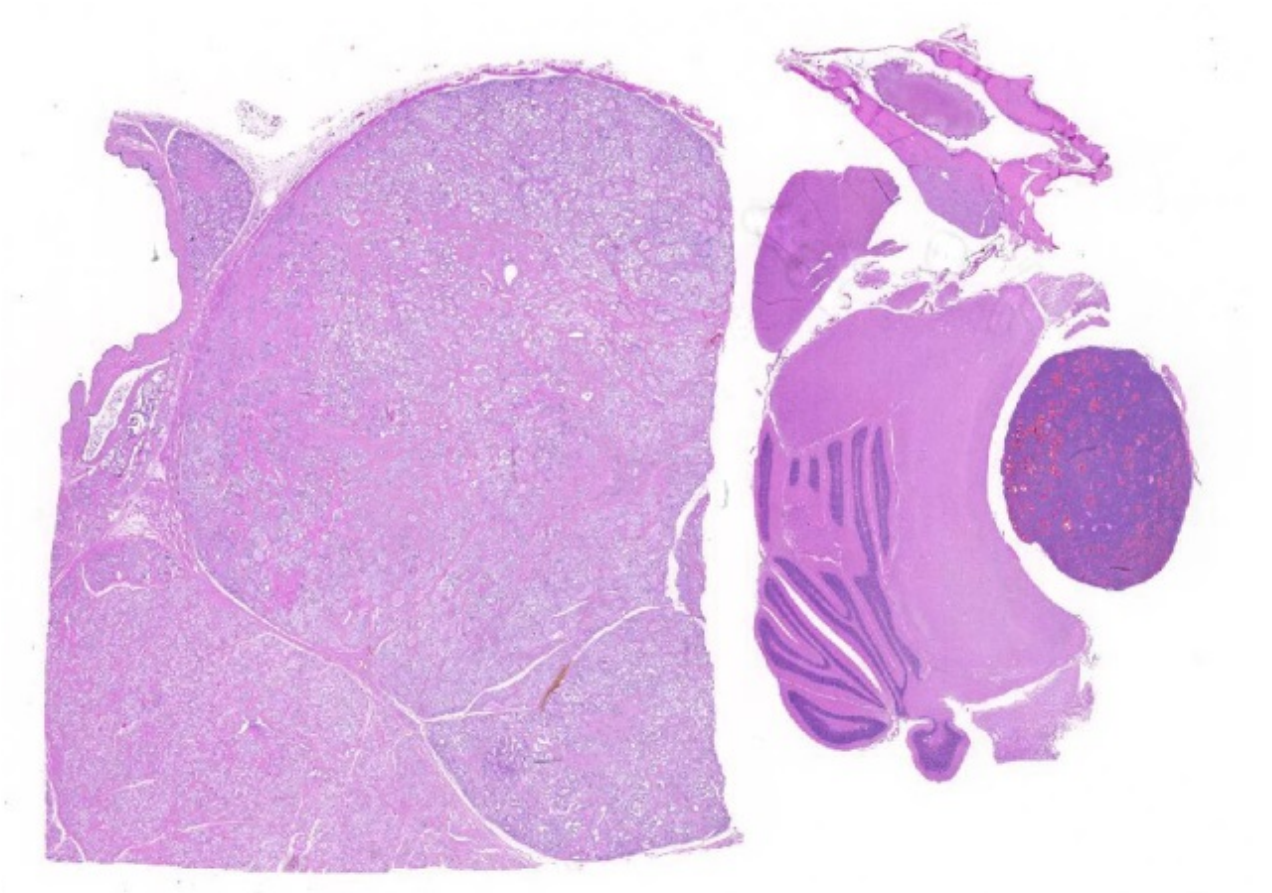
Laboratory Results: None

Histopathologic Description:

Axillary mass - Part of a multilobular mass composed of disorganized epithelial proliferation and connective tissue. The neoplastic epithelium forms variably sized acini and tubuli which are aggregated into lobules dissected and separated by moderate to abundant dense, collagen-rich, connective tissue. The neoplastic cells are cuboidal to irregular and arranged as a monolayer; they have moderate to abundant cytoplasm which is often vacuolated. Vacuoles vary from numerous and small (microvesicular vacuolation) to large, single, lipid vacuoles which lead to significant expansion of the cytoplasm and peripheral displacement of the nucleus. Nuclei are round to slightly irregular, vesicular to finely granular, and have a small nucleolus. There is slight anisocytosis and anisokaryosis. Mitotic figures are not observed. Multifocally, the lumen of acini contains proteinaceous secretory material, which in some cases shows internal layers (corpora amylacea). In other parts of the mass, not submitted, there are large cysts filled with secretory material and lined by epithelial cells as described.



Mammary gland, rat. A firm, circumscribed and lobulated subcutaneous mass approximately 3.5 cm in diameter, was attached to the skin in the axillary area (Photo courtesy of: Department of Veterinary Resources, Weizmann Institute, Rehovot 76100, Israel, http://www.weizmann.ac.il/vet/)



Tissues from a rat. Subgross examination of submitted tissues reveals a large multilobular neoplasm replacing the mammary gland (left), and a large neoplasm replacing the pituitary gland (right) (HE, 6X)

Pituitary mass- Compressing the medulla there is a small, discrete, thinly encapsulated and densely cellular nodular mass composed of a uniform population of polygonal cells arranged into solid islands separated by fine fibrovascular stoma. The neoplastic cells have a moderate amount of eosinophilic to lightly amphophilic cytoplasm, relatively indistinct cytoplasmic margins, and round to slightly irregular, vesicular to finely granular nuclei with a small nucleolus. There is anisocytosis and anisokaryosis. A few mitotic figures are observed. At the edge of the mass there are scattered giant karyomegalic cells. There are numerous large, blood-filled spaces lined by neoplastic pituitary cells. At one edge of the mass there are possible remnants of the pre-existing adeno-hypophsis.

Contributor's Morphologic Diagnosis:
Axillary mass: Mammary gland fibroadenoma
Pituitary gland (adenohypophysis): Adenoma

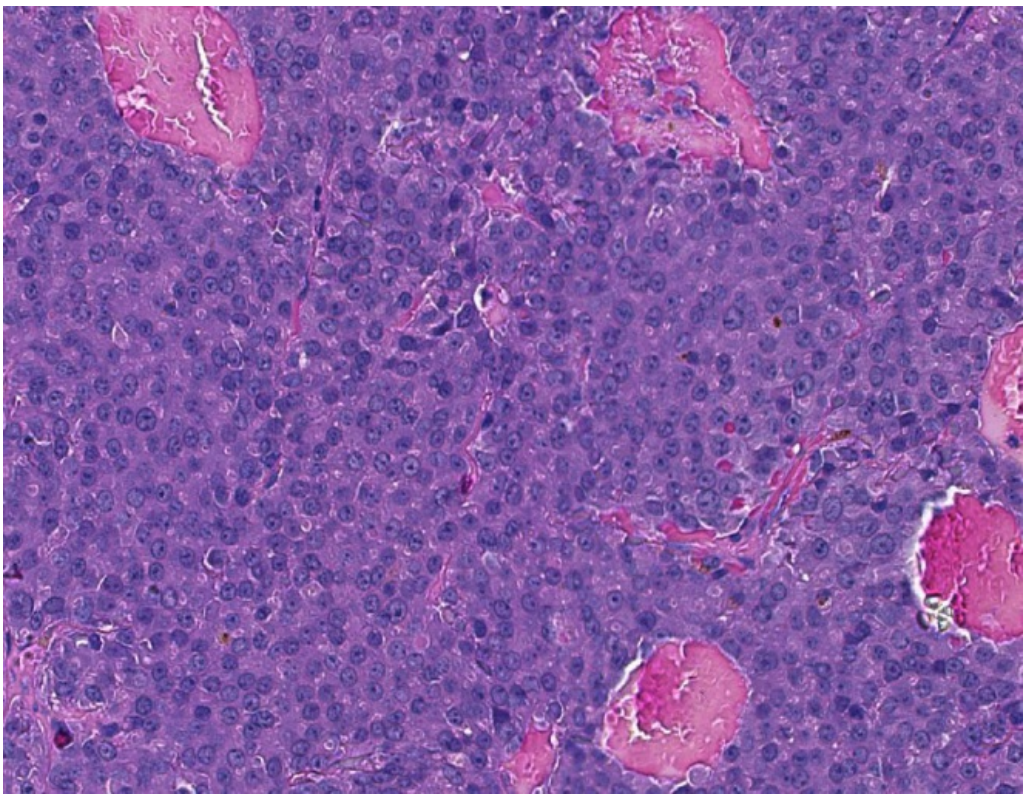
Contributor's Comment: Mammary fibroadenoma: Mammary tumors are one of the most common tumors in old female rats,[1] especially in the SD strain where the incidence of spontaneous tumors often approaches 50% in lifetime studies of control animals.[4] Most are benign fibroadenoma which are composed of mammary epithelial cells and connective tissue.[4] The incidence of these tumors varies considerably between different rat strains,

suggesting that genetic background is an important factor in their development. Other factors which influence their occurrence are diet, environment, and in the case of toxiclogic studies, the degree of differentiation of the mammary glands, and physiologic and hormonal status at the time of chemical exposure.[4] Mammary tumors are rare before 1 year of age, and are generally found after 18 months of age.[1] Mammary fibroadenomas also occur occasionally in male rats.[5]

Histologically, the proportion of glandular and connective tissue in fibroadenomas is variable, and this has led to their subclassification. However, since several subtypes are commonly encountered in a single tumor, this division appears to be of little merit.[1, 4] Exposure to estrogen and prolonged exposure to prolactin increase tumor frequency, whereas parity and ovariectomy decrease the incidence of mammary gland tumors in rats.[1,5] Although increased mammary gland tumors are found in rats with pituitary tumors and high levels of prolactin are considered a contributing factor, a casual effect is difficult to determine.[1,5] Estrogens induce both pituitary and mammary tumors, and the incidence of both types of tumors correlates with body weight.[1]

Mammary fibroadenomas may become very large, but as a rule, they are only locally infiltrative and rarely metastasize. Surgical excision is possible in pet rats or experimentally valuable animals.[5] Spontaneous mammary adenocarcinomas are most common in SD rats and uncommon in other strains. They may develop in existing fibroadenoma, but this is rare. They generally do not metastasize.[1]

<u>Pituitary adenoma</u> is very common in older rats, especially of the Wistar strain. There is conflicting information in standard references regarding their incidence in other strains: according to Boorman and Everitt [1] they are common in F344 and uncommon in SD, while according to Percy and Barthold [5] they are common in the SD strain. Some



Pituitary gland, rat. Neoplastic cells are arranged in nests and packets on a fine fibrovascular stroma and there numerous blood-filled spaces throughout the mass. Nuclei exhibit mild anisokaryosis and a low mitotic rate. (HE, 220X)

studies suggest a slightly higher incidence in females. Other than age, genetic background, diet, and breeding history are thought to play a role in tumor development. Reduction of food intake reduces their incidence and, according to one study, mated females are less prone to these tumors than virgin females.[5] Clinical signs vary from asymptomatic to severe depression, often with incoordination.[5] The neurologic signs are due to compression of the brain.

Histologically, the hallmark of adenoma is compression of the surrounding parenchyma and sharp delineation at the margins of the nodule. The neoplastic cells are generally larger than normal and have more abundant cytoplasm, which is usually pale or faintly basophilic. The mitotic index is usually low. Often, there are prominently dilated vascular channels which may be lined by endothelial cells or neoplastic pituitary cells; this has been referred to as angiomatous or cavernous pattern. Giant cells and areas of necrosis may be present.[3]
Most pituitary tumors are thought to arise from the pars distalis and are diagnosed as chromophobe adenomas based on HE-stained sections.[1,5] Acidophil and basophil tumors have also been described. The diagnosis of chromophobe adenoma provides no information regarding the endocrine status of the tumor.[1] In pituitary tumors studied by immunocytochemistry, prolactin-producing tumors are the most common type,[5] but growth hormone, ACTH, TSH and FSH-secreting tumors have also been described.[1] Lactation in an aging rat is often a sign of a functional pituitary tumor.[1]

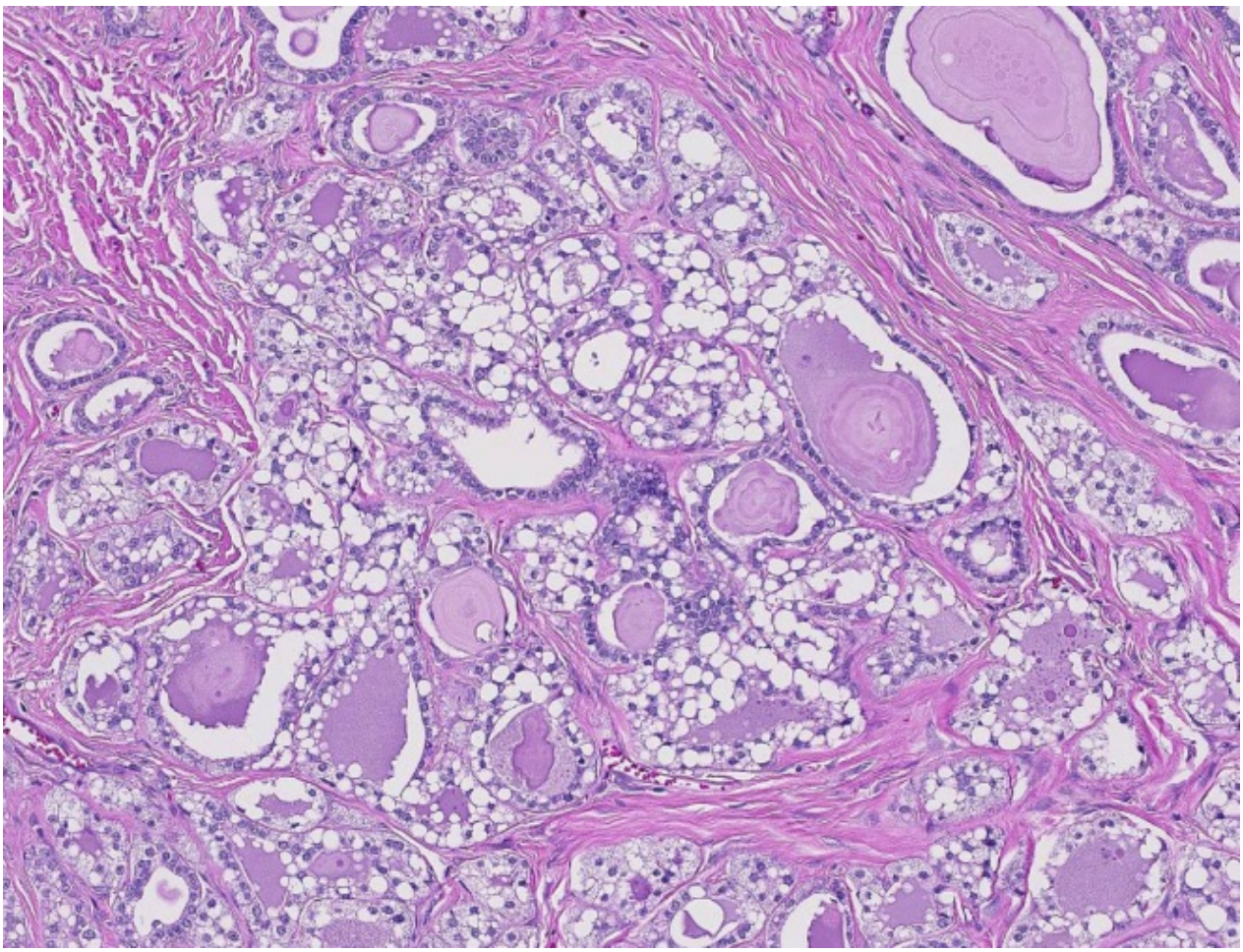
Pituitary adenomas should be differentiated from hyperplastic and hypertrophic lesions. In hyperplastic lesions there is proliferation of cells of normal size, no evidence of pseudocapsule formation, and no significant compression of adjacent pituitary tissue. Nodules of hypertrophic cells form islands of large cells without evidence of encapsulation.[1,3] Pituitary carcinomas are rare and require evidence of invasion or distant metastasis for their diagnosis.

JPC Diagnoses**:** 1. Pituitary gland: Pituitary pars distalis adenoma.

2. Mammary gland: Mammary fibroadenoma.

Conference Comment**:** Mammary gland fibroadenoma is one of the most common rat mammary tumors. It is more commonly seen in female rats and has an especially high incidence in Sprague Dawley rats as mentioned above. It is generally well defined and composed of proliferating glandular tissue surrounded by a proliferation of fibrous tissue. Large sections or an entire mammary gland may be involved. It may have a lobular growth pattern with variation in size and composition of individual lobules. Secretory epithelium is arranged in a single layer, and small foci of pleomorphic cells may be present, but mitoses are uncommon. It is differentiated from adenoma by the conspicuous contribution of a fibrous connective tissue component. Adenocarcinoma may arise from within mammary fibroadenoma.[6] Other less common mammary neoplasms in the rat include ductular carcinoma and cystadenoma.

Another lesion which must be differentiated from benign mammary neoplasia is lobuloalveolar hyperplasia. This condition may be referred to as pseudopregnancy, and is differentiated from neoplasia by maintaining the normal lobular histologic architecture, specifically the relationships among the various mammary tissue components including ducts, glandular epithelium, stroma, and myoepithelium. Cellular pleomorphism is absent; however, focal squamous met-



Mammary gland, rat. Neoplastic cells are arranged in well-defined acini which are filled with variable amounts of purple secretory product. The secretory product is occasionally lamellated (corpora amylaceae). Neoplastic cells are columnar with large cytoplasmic vacuoles; accumulation of secretory product within the lumen results in a cuboidal to attenuated morphology of neoplastic cells in many glands. (HE, 123X)

aplasia can occur and hyperplastic lesions may be focal or diffuse.[6] Diffuse mammary hyperplasia is associated with hormonally-induced physiologic changes during late gestation and lactation. Focal hyperplasia may be accompanied by fibrous proliferation separating acini, but the lobular architecture is maintained and the lesion is not compressive, which aids in differentiating it from mammary fibroadenoma.[6]

Dietary food restriction is known to decrease the incidence of both pituitary and mammary tumors in rats. Lower levels of prolactin are present in rats on a restricted diet, and prolactin is a primary stimulus for the development of mammary neoplasia in rats. Most rat pituitary neoplasms are prolactin-immunopositive and are postulated to be involved in the development of mammary tumors,[2] although a definitive link has not been demonstrated in all cases. Furthermore, not all rat pituitary tumors are prolactin positive. Interestingly, reduction in body weight from decreased caloric intake is paradoxically associated with an increase in uterine neoplasia in rats; this effect is postulated to be related to prolactin's influence on function of the ovary and corpora lutea. In the rat prolactin promotes progesterone production in the

corpus luteum post ovulation, which opposes estrogen's promotion of uterine growth. Therefore, a decrease in prolactin results in elevated levels of estrogen, which stimulates endometrial growth.[2] Long term administration of estrogen to rats also results in prolactin producing pituitary adenomas. These induced tumors may also produce other hormones, such as thyroid stimulating hormone.[7]

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