CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

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COMMON NON-NEOPLASTIC FINDINGS IN CARCINOGENICITY STUDIES

Thanks to John Eighmy at Covance, who graciously shared material for this talk

Non-neoplastic Lesions of Aged Rats

- The diagnosis and documentation of non-neoplastic lesions is an important aspect of the histopathological evaluation of carcinogenicity studies even though the primary purpose of the studies is to identify neoplasms.

Why are non-neoplastic lesions important?

- Masses that arise during the in-life phase of carcinogenicity studies are carefully tracked and documented; some of these masses prove to be non-neoplastic lesions when evaluated microscopically. The diagnoses for these findings must be recorded.
- The cause of death or moribund condition of rats euthanized during the course of a carcinogenicity study must be documented to either establish or exclude a possible relationship to the drug or test article being evaluated. Non-neoplastic changes are often the cause of death or moribund condition.

Why are non-neoplastic lesions important?

- Common background lesions must be differentiated from possible chronic toxic effects of the test article; such findings may not have previously been identified in shorter term studies with the drug or test article.
- Although common non-neoplastic lesions are always present in the background of a carcinogenicity study, the incidence, severity, or time of onset of the non-neoplastic lesions can be exacerbated by the drug or test article.

Chronic Progressive Nephropathy (CPN)

- CPN is the most important age-related disease of the rat kidney; incidence and severity is higher in males than in females.
- CPN is a common cause of death in rats on carcinogenicity studies.
- The disease is spontaneous and has an unknown pathogenesis.

Chronic Progressive Nephropathy (CPN)

- Incidence of CPN varies among different rat strains; Sprague-Dawley rats (a common laboratory strain in North America) often have lesions by 3 months of age. Other rat strains also are affected.
- Calorie and protein restriction in the diet lowers incidence and severity.
- It can be difficult to distinguish age-related kidney changes (components of CPN) from drug-related effects in chronic toxicity and carcinogenicity studies.

Chronic Progressive Nephropathy

- Gross (Macroscopic) Findings:
  - In advanced stages, kidneys may be enlarged, pale, and/or irregularly shaped.
  - Surface may be pitted and contain small cysts.

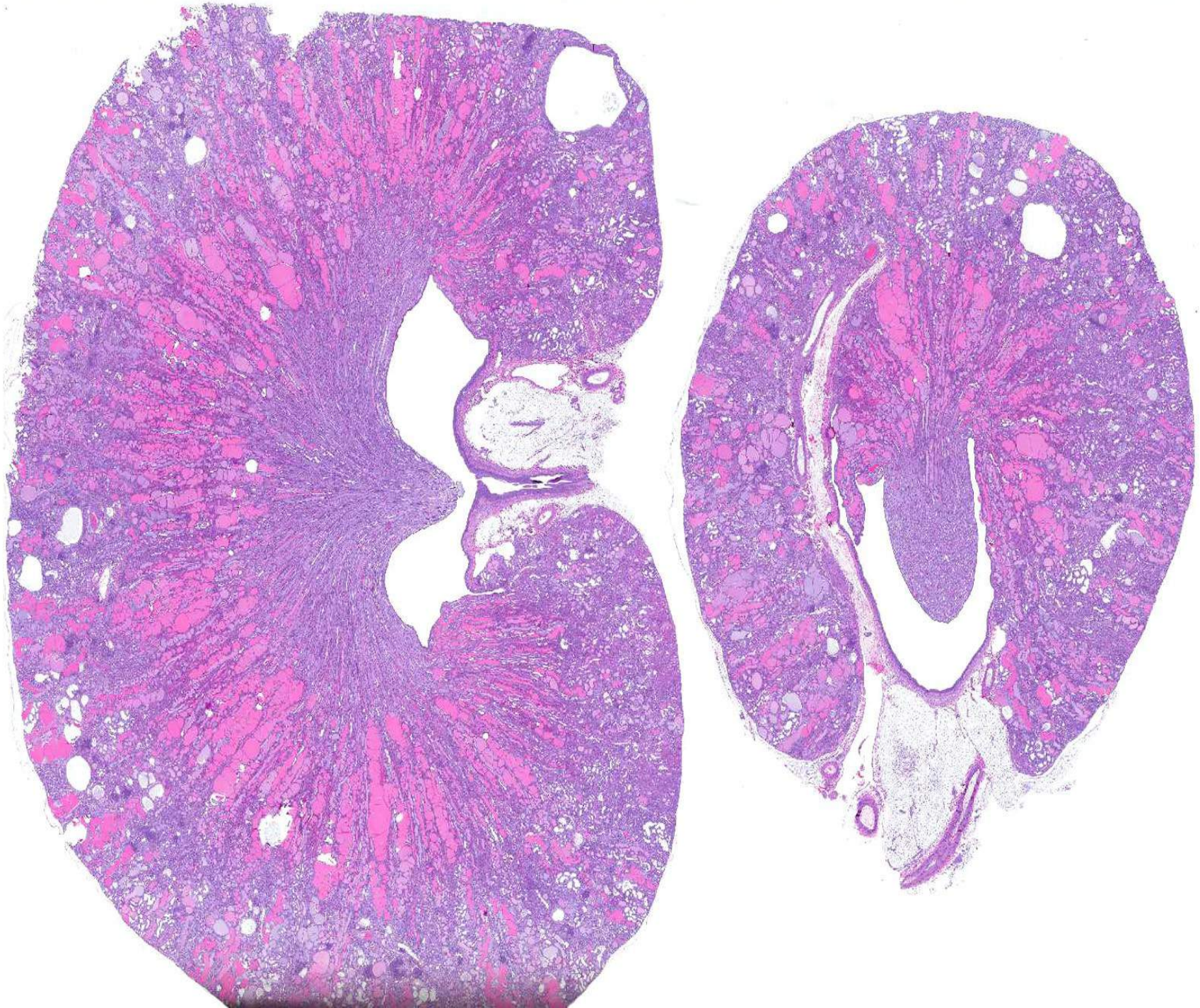

cm

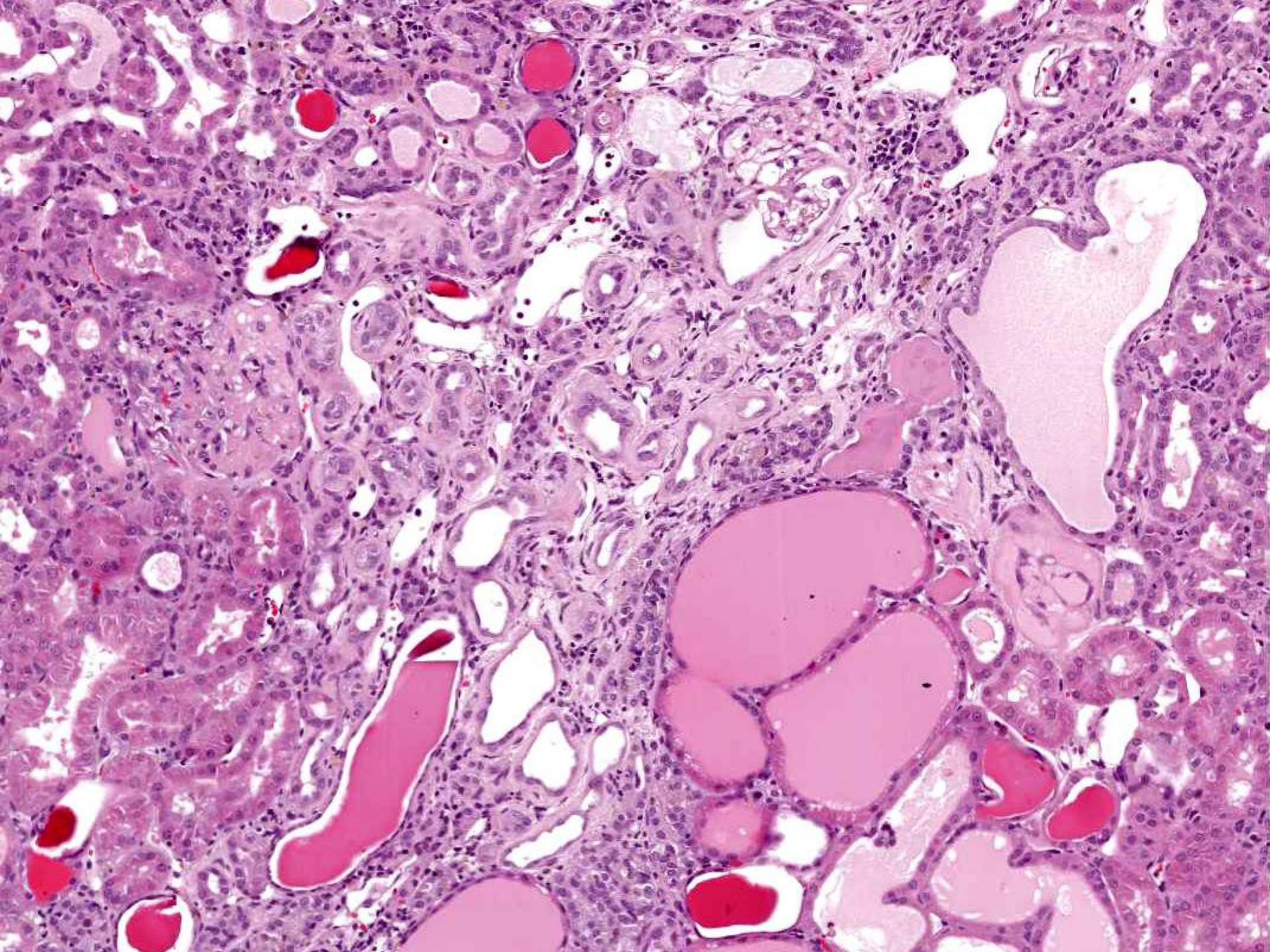
Chronic Progressive Nephropathy

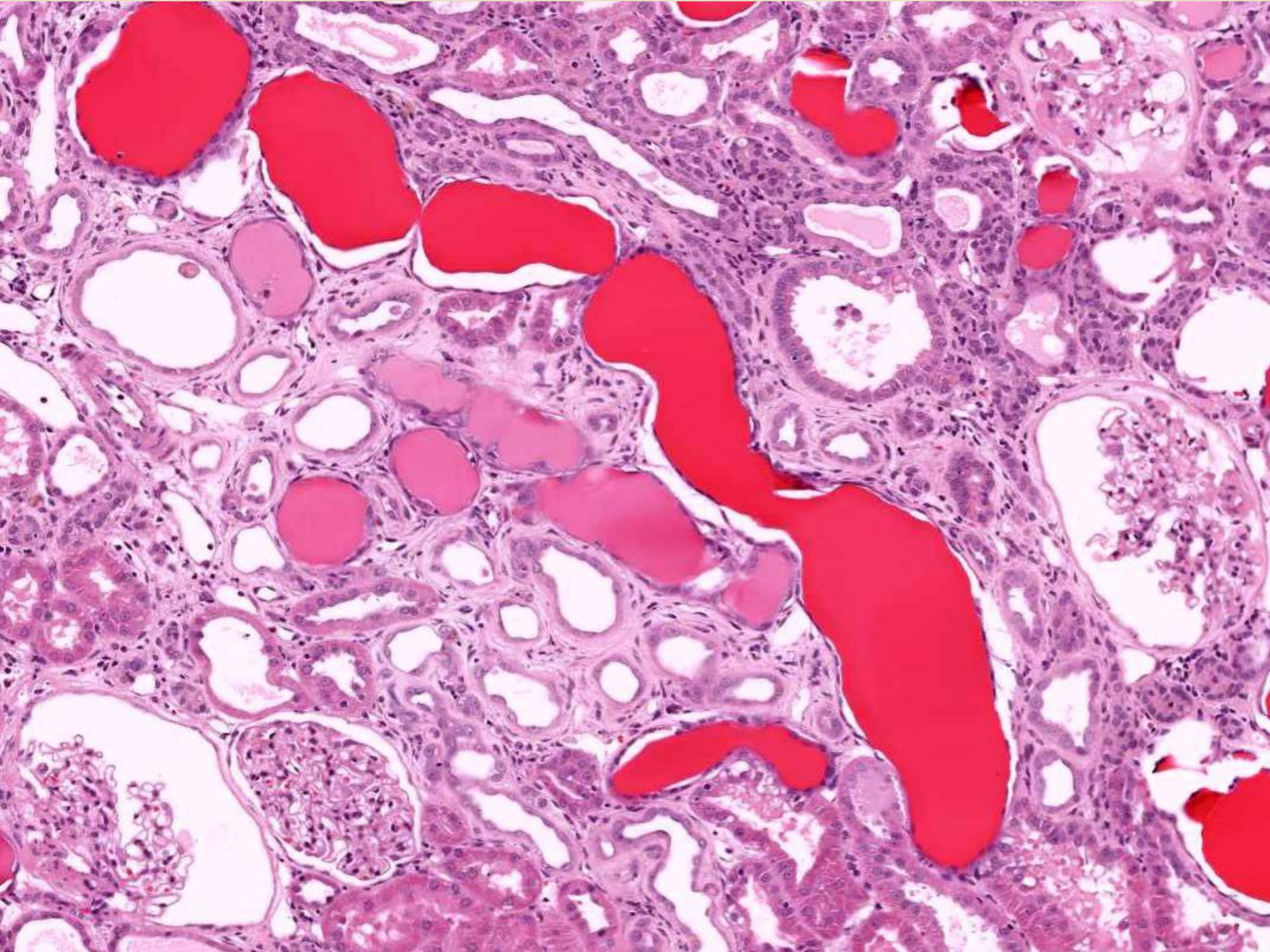
- Microscopic Findings
  - Microscopic changes can be extensive.
  - Glomerular changes include thickened basement membranes, adhesions to Bowman's capsule, segmental glomerular atrophy and sclerosis, and periglomerular fibrosis.
  - Tubules in the cortex and medulla are often dilated, contain eosinophilic proteinaceous casts, and are surrounded by peritubular fibrosis.

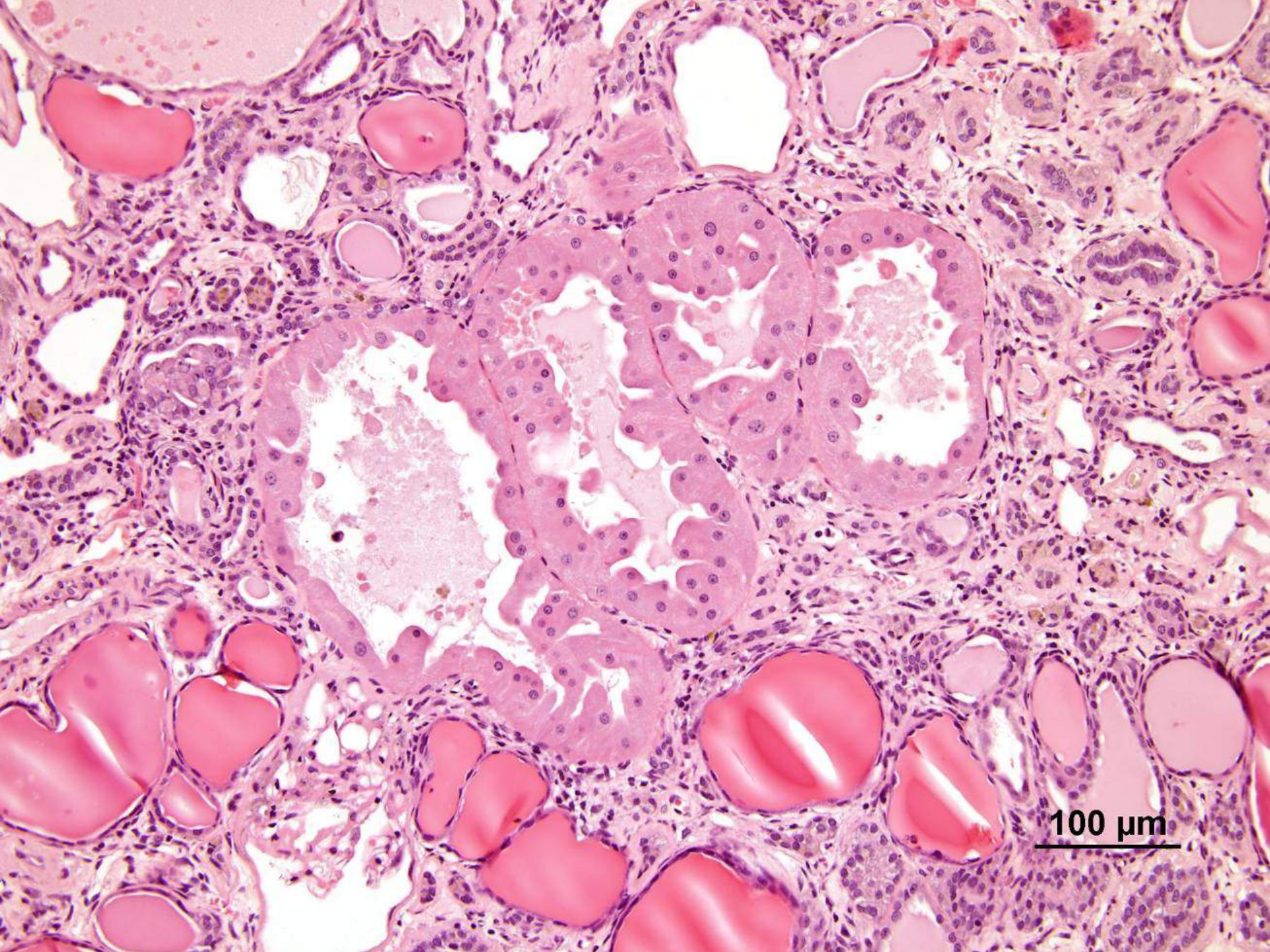
Chronic Progressive Nephropathy

- Other common microscopic findings include:
  - Additional tubular changes such as cystic dilatation, tubular basophilia, atrophy, hyperplasia, or hypertrophy.
  - Nonsuppurative interstitial inflammation and fibrosis.
- Secondary hyperparathyroidism can occur in rats with CPN, resulting in generalized tissue mineralization, including the glandular stomach, lung, and blood vessels in multiple organs.





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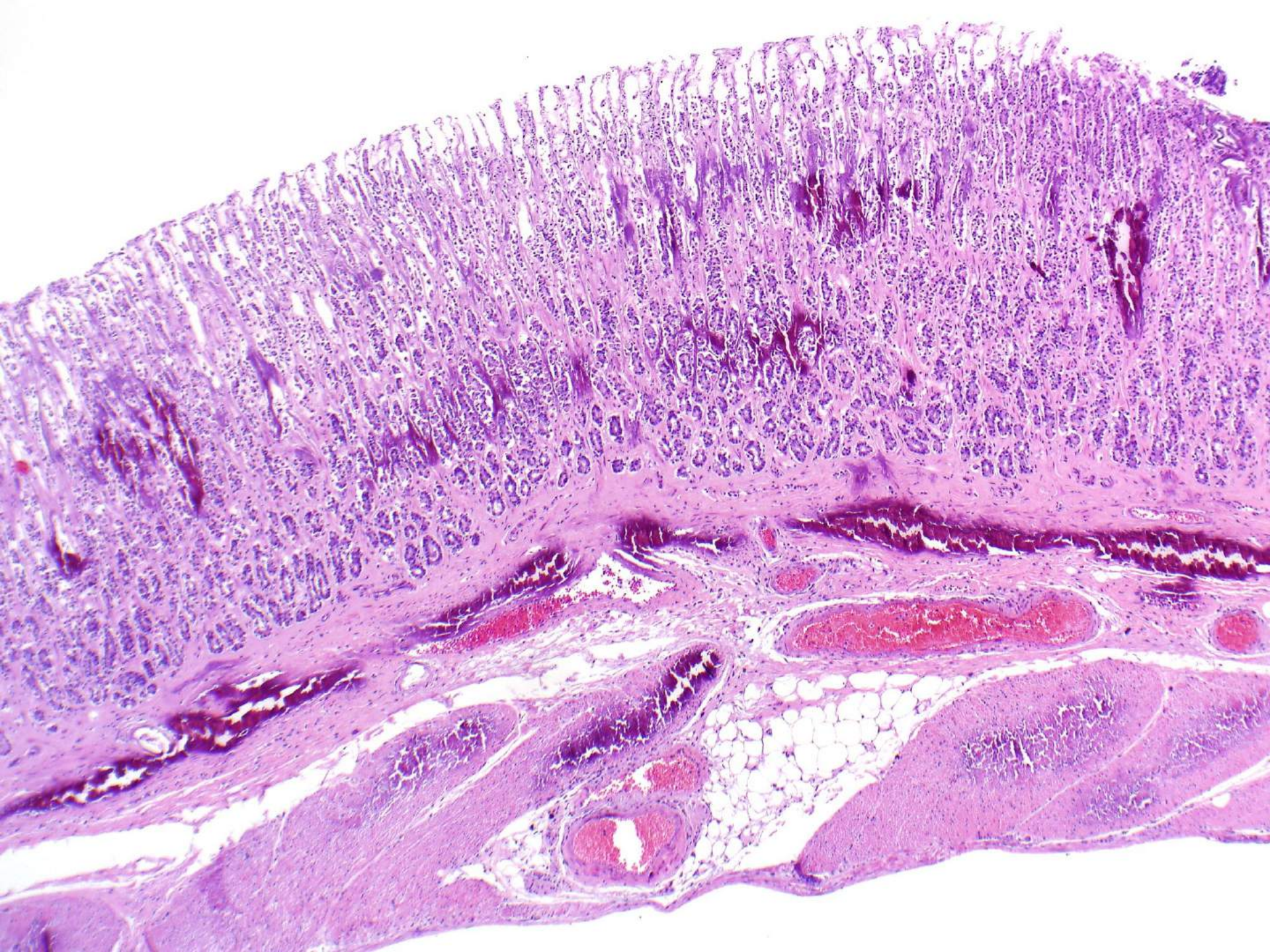

Genitourinary Inflammation

- One or multiple organs in the genital and urinary systems are often inflamed, especially in males. Inflammation can be so severe that it is the cause of death in some rats on carcinogenicity studies.
- In the kidney, pyelonephritis and hydronephrosis may be noted.
- Urinary calculi (mineral) may be present in the kidney, urinary bladder, or lower urinary tract; inflammation and epithelial hyperplasia often accompany the calculi.
- Inflammation may extensively involve male secondary sex glands such as the prostate and seminal vesicles.
- Preputial glands (males) or clitoral glands (females) may be affected alone or in combination with other organs.

Pyelonephritis, Hydronephrosis, Urinary Calculi

- Pyelonephritis, urinary calculi, and hydronephrosis are kidney changes that can occur together or separately.
- Pyelonephritis - inflammation of the kidney and its pelvis; the cause is generally an ascending bacterial infection, but bacteria are not always apparent microscopically.
- Hydronephrosis - enlargement of the renal pelvis due to blockage in the urinary system (such as by calculi) or chronic kidney disease that prevents urine from draining into the bladder. Hydronephrosis can be congenital; it is often unilateral and affects the right kidney more often than left in some rat strains.

Pyelonephritis, Hydronephrosis, Urinary Calculi

- Gross (Macroscopic) Findings:
  - One or both kidneys are enlarged.
  - On cut section, renal pelvis is generally dilated and compresses the renal cortex and medulla.
  - Calculi may or may not be visible in the urinary tract at necropsy, and they are often lost during tissue processing.

Pyelonephritis, Hydronephrosis, Urinary Calculi

- Microscopically, with pyelonephritis, the inflammation is generally chronic but active. The pelvis of the kidney is always involved; inflammation extends variably into the cortex and medulla. Bacterial colonies may or may not be visible in the histologic section.
- Necrosis is a common feature of pyelonephritis; pelvic epithelium may be ulcerated. Hyperplasia of the pelvic epithelium also occurs.
- Hydronephrosis and urinary calculi can accompany pyelonephritis or can occur without inflammation.

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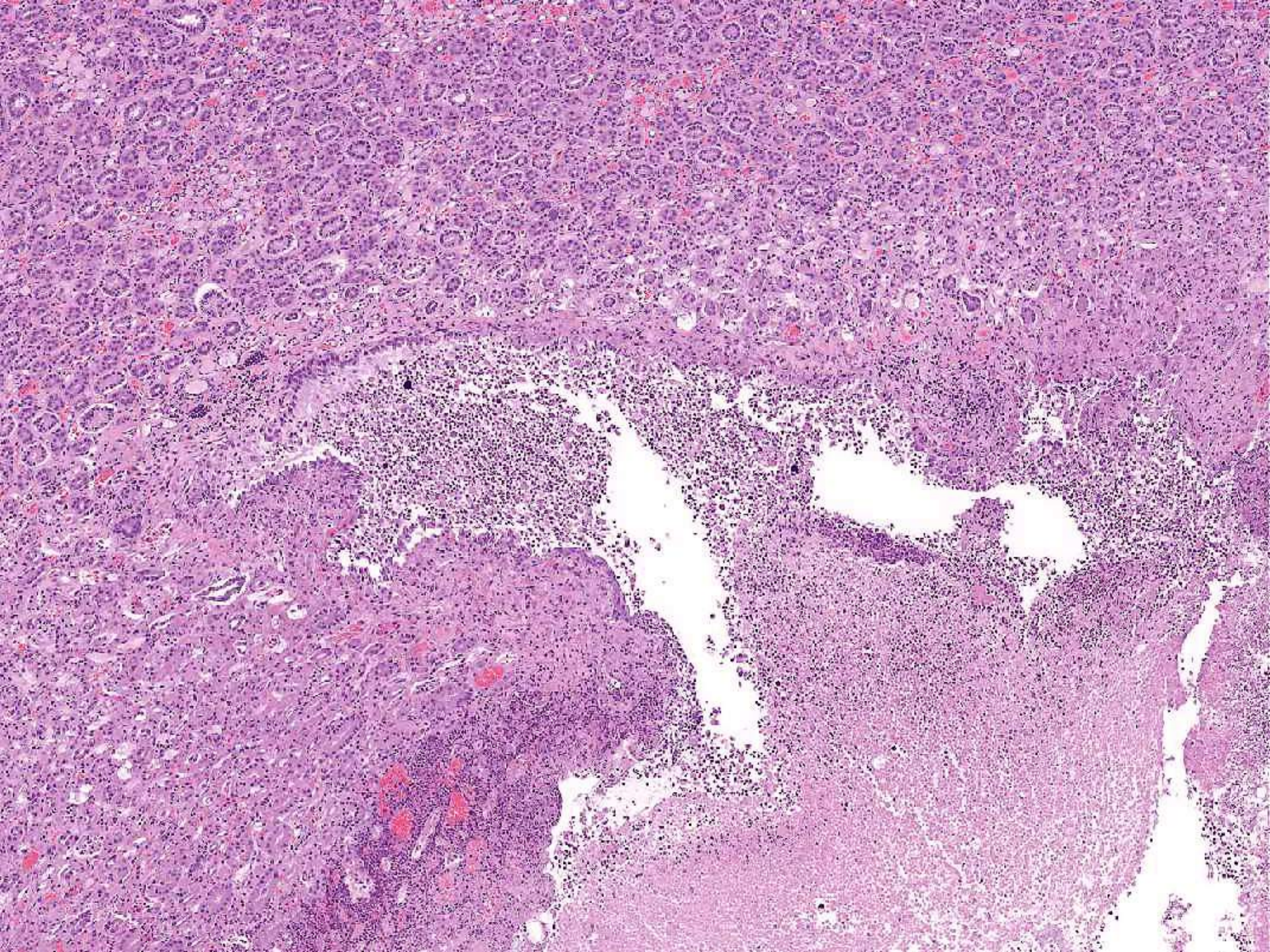
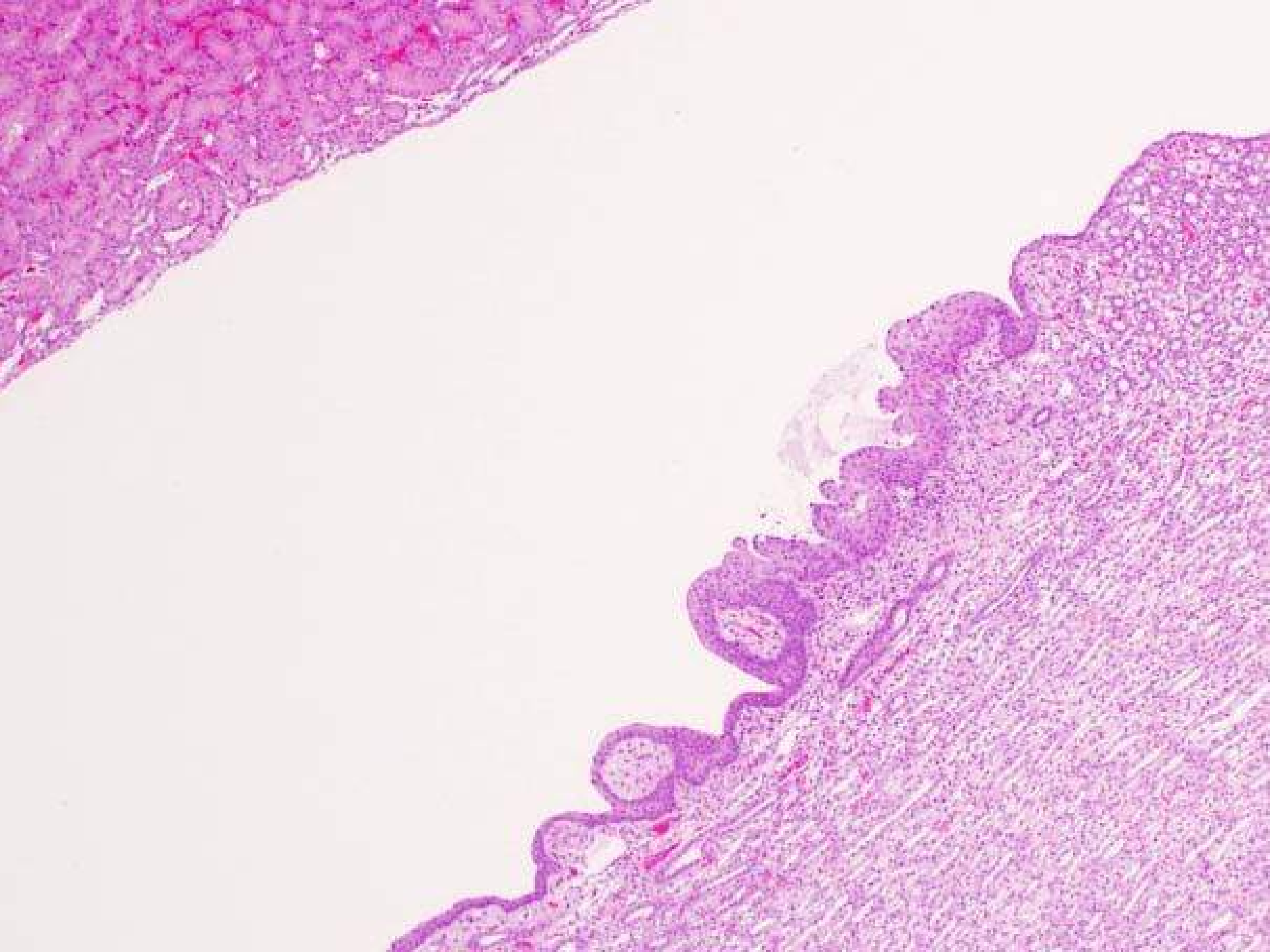
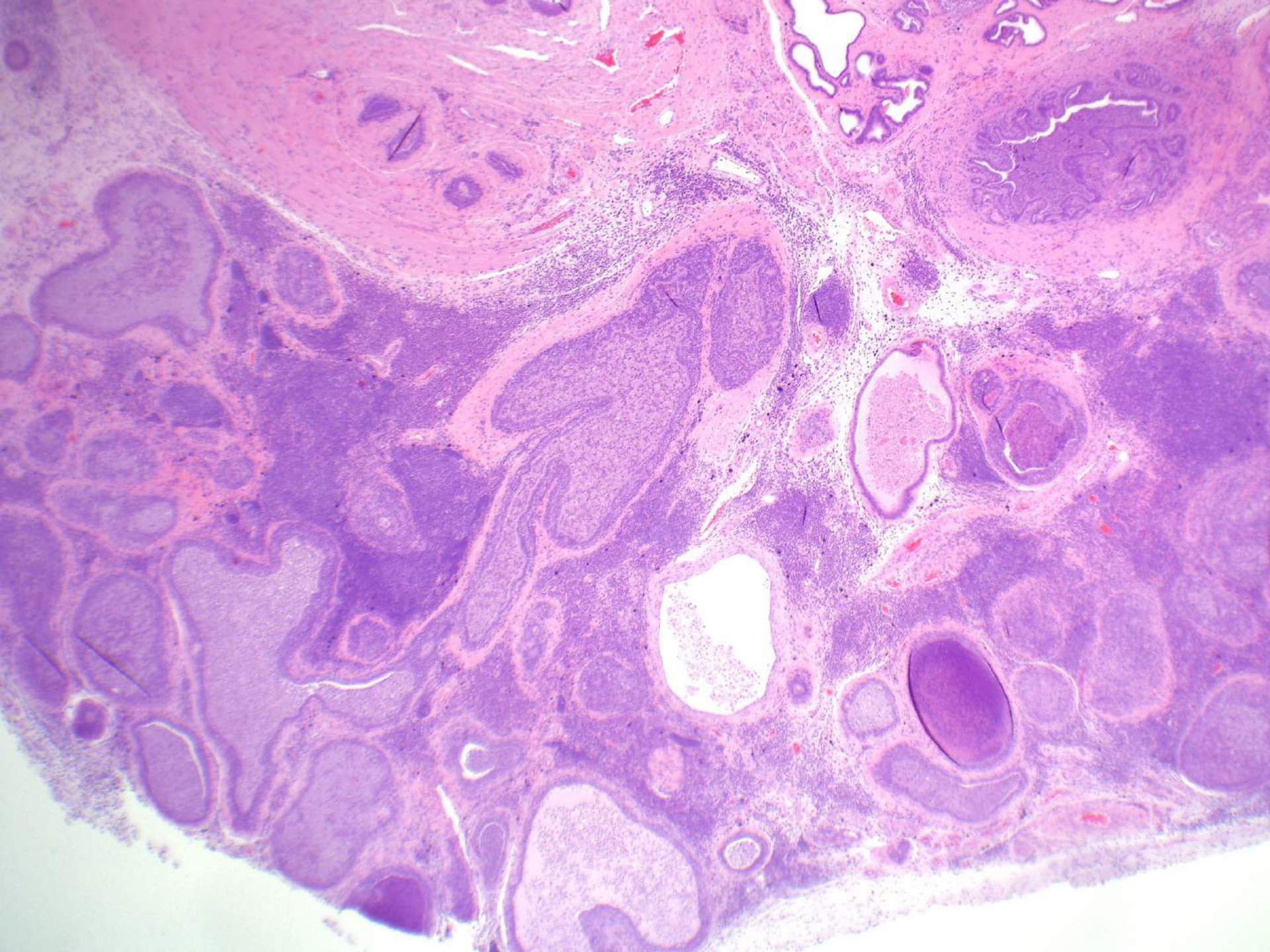
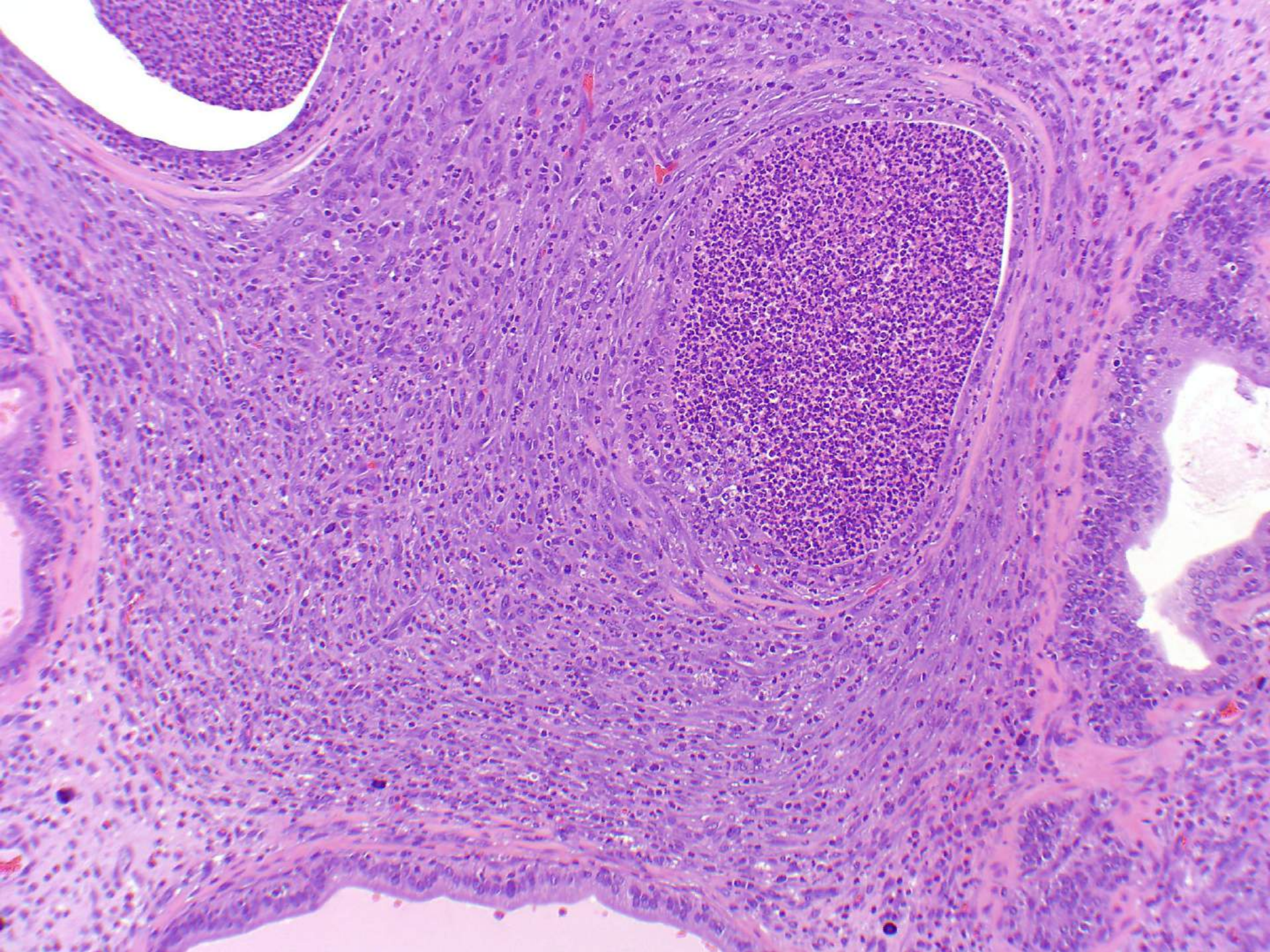




Chronic Prostatitis

- Grossly, the prostate glands may be enlarged and dark red.
- Microscopically, the inflammation is chronic but active; features include dilatation of the glands by inflammatory cells (lymphocytes, macrophages, and neutrophils). Necrosis and obliteration of glands by inflammation may be a feature.
- Small aggregates of mineral may be present.





Preputial / Clitoral Gland Inflammation

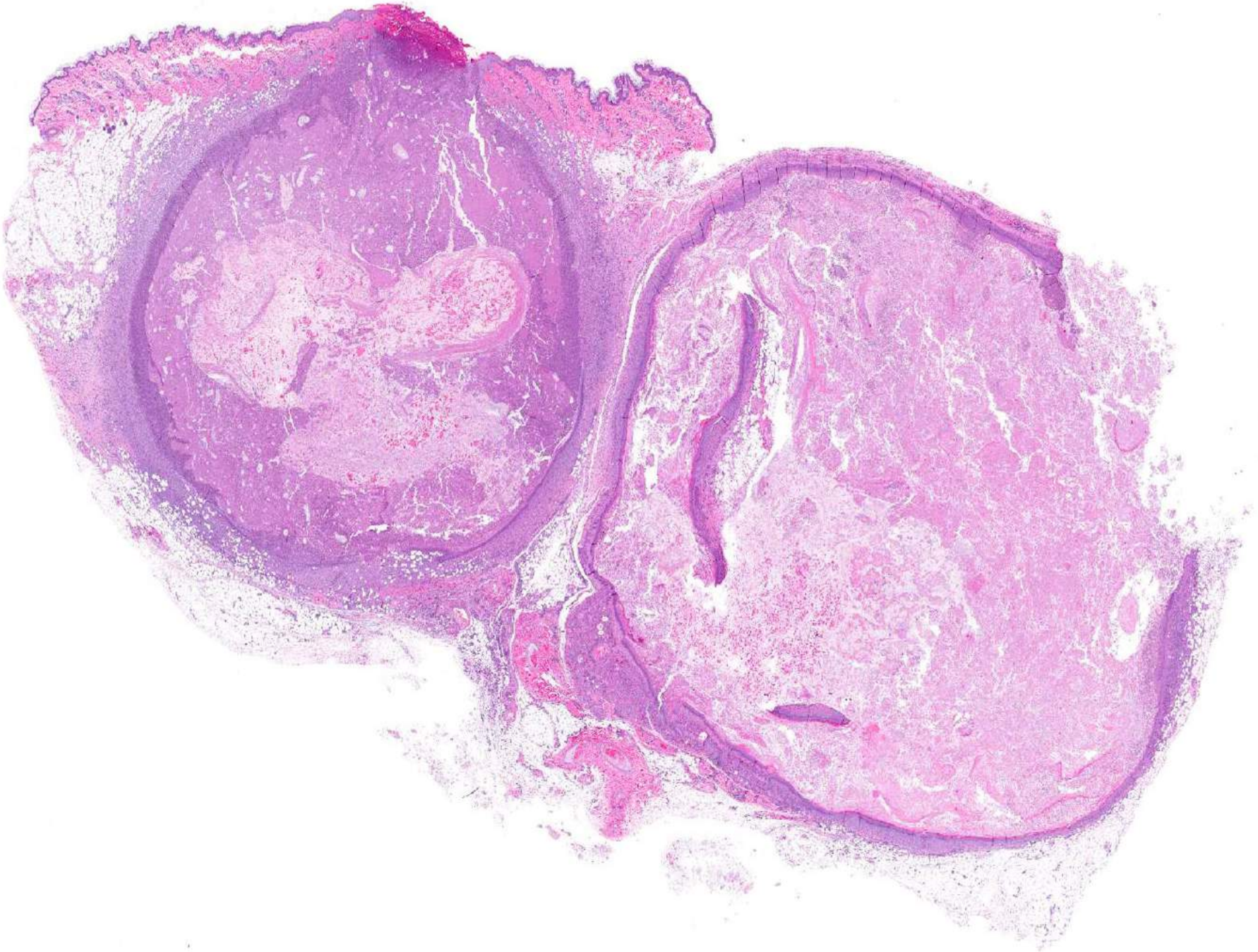
- Preputial glands in males and clitoral glands in females are paired organs in the subcutaneous tissue near the base of the penis or clitoris.
- The glands are considered to be specialized sebaceous glands.
- The glands have large central ducts lined by stratified squamous epithelium that are frequently distended by accumulated secretion.

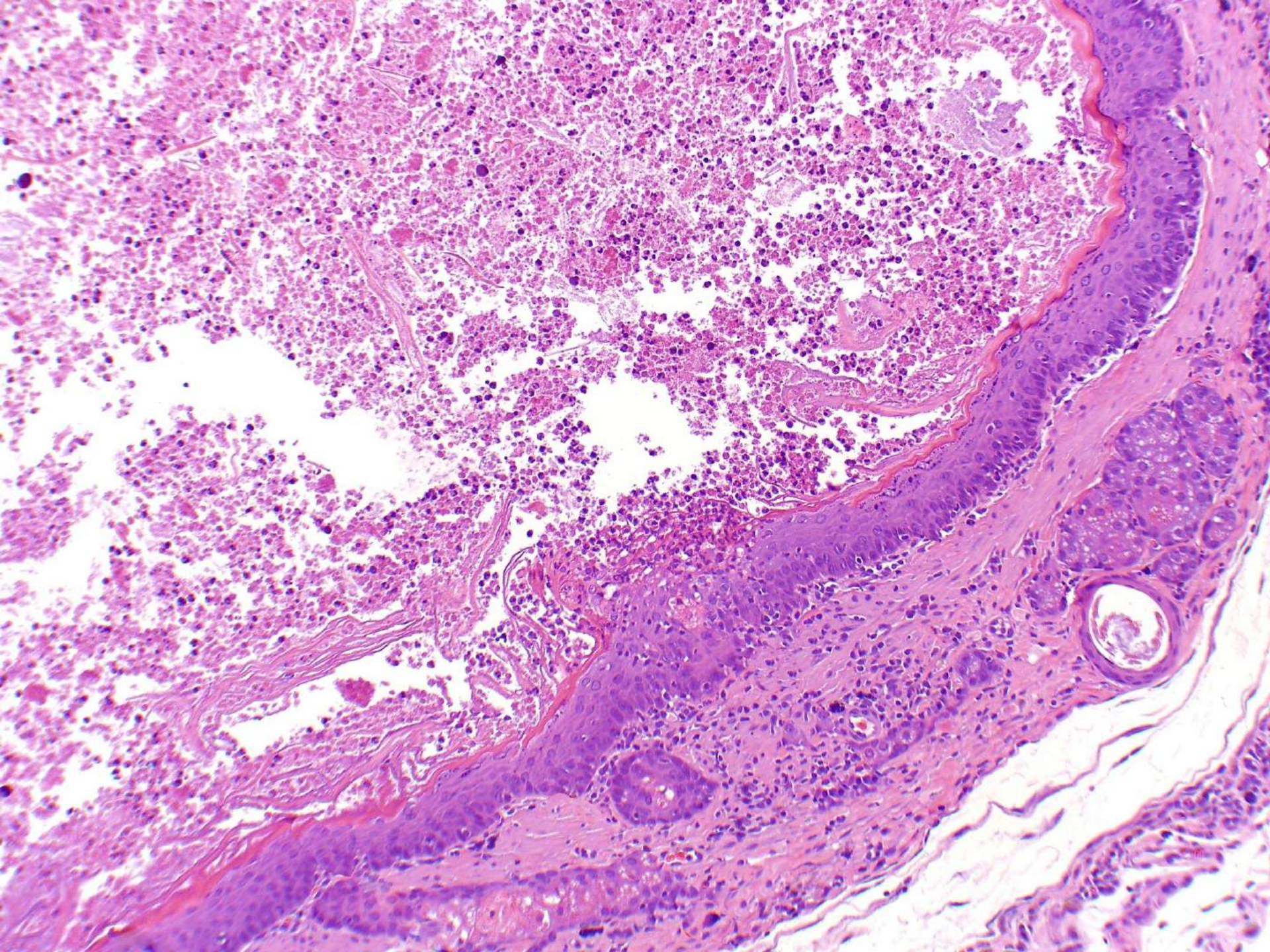
Preputial / Clitoral Gland Inflammation

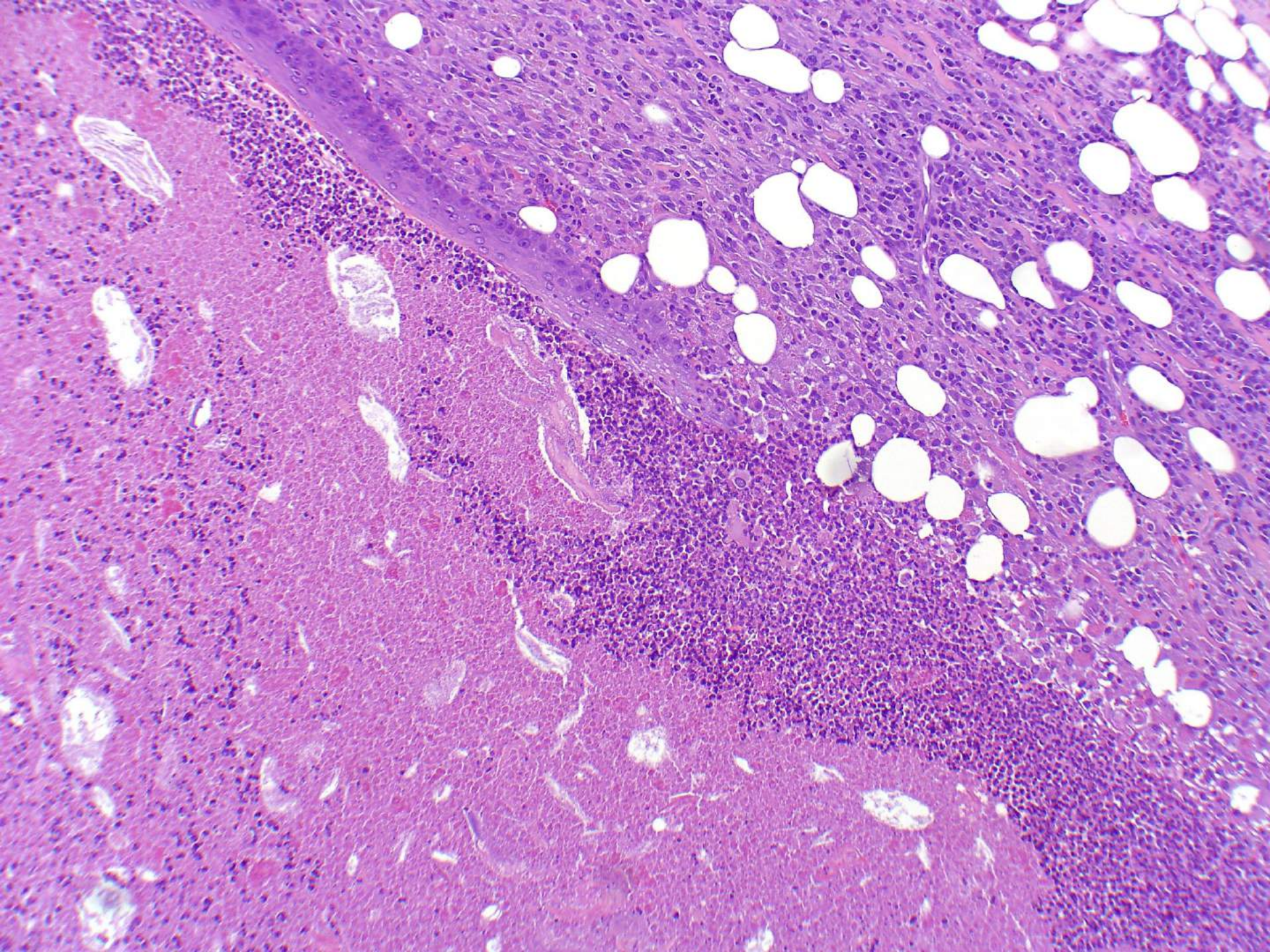
- Grossly, inflamed glands are noted as large swellings in the perineal region.
- Inflamed glands are frequently described as abscesses; pus may be extruded on palpation.
- Enlarged glands may be misidentified at necropsy as skin or mammary neoplasms.
- Some pharmaceutical companies routinely collect the tissue as part of study protocols, but the glands are more often collected only when enlarged.

Preputial / Clitoral Gland Inflammation

- Microscopically, inflammation is generally chronic and suppurative.
- The main ducts of the glands are markedly dilated.
- Necrosis is a common feature; necrosis and inflammation can extend into the adjacent tissues.







Alveolar Histiocytosis

- Prevalence: Very common finding in the lungs of aged rats, moderate incidence in younger animals.
- Grossly:
  - White to pale tan foci, usually about 1 mm in diameter, visible on the pleural surface of the lung.
  - Foci may extend slightly above the pleural surface in uninflated lung and can be numerous.

Alveolar Histiocytosis

- Microscopically, clusters of foamy macrophages are present in alveoli and terminal airways.
- Cause is unknown, but not considered infectious.
- Should not be mistaken for any of the viral pneumonias of rats.
  - Animals are seronegative.
  - Any lymphoid infiltrate is slight and localized.
  - Perivascular lymphoid cuffing is not a prominent feature.

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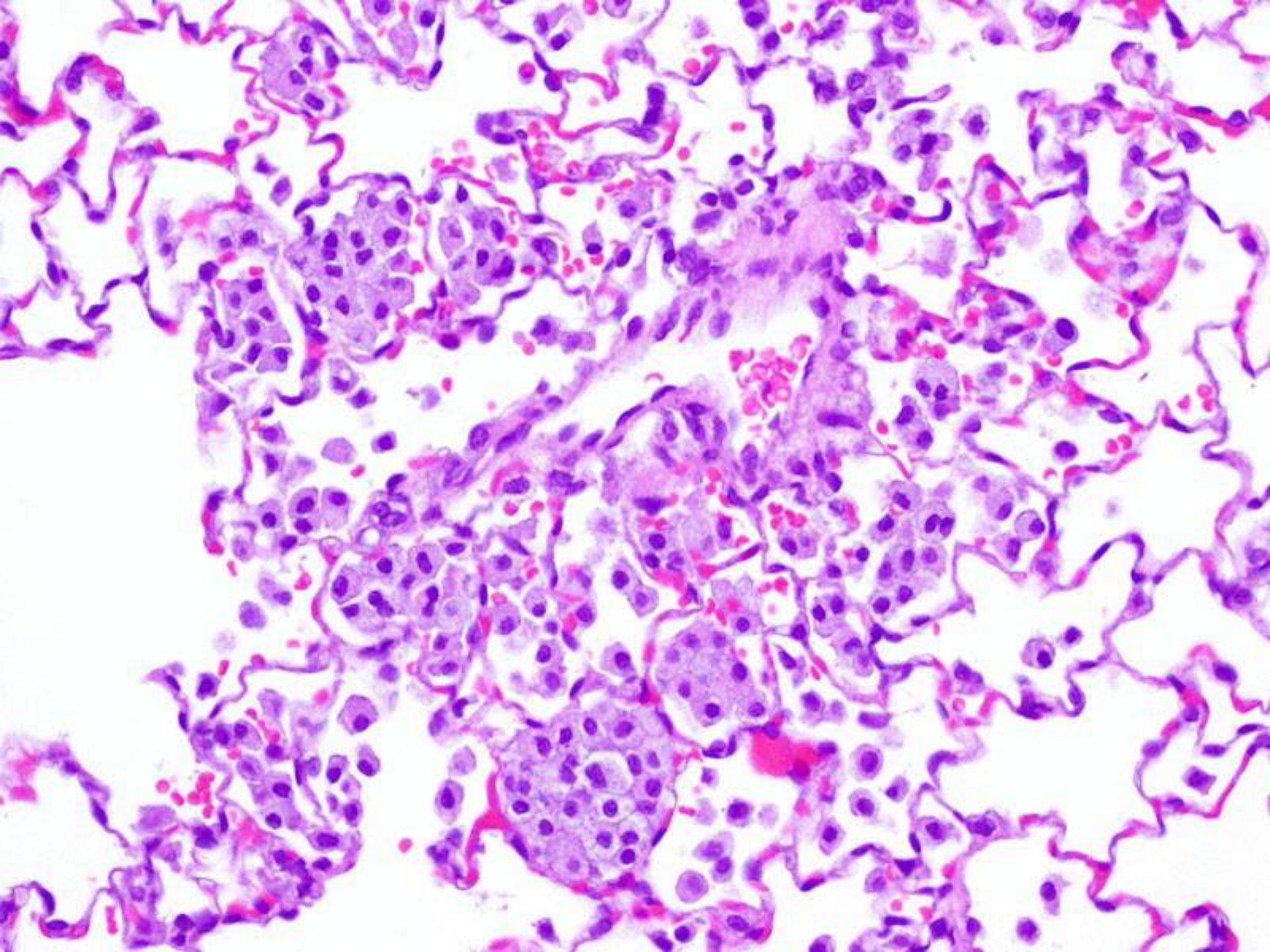
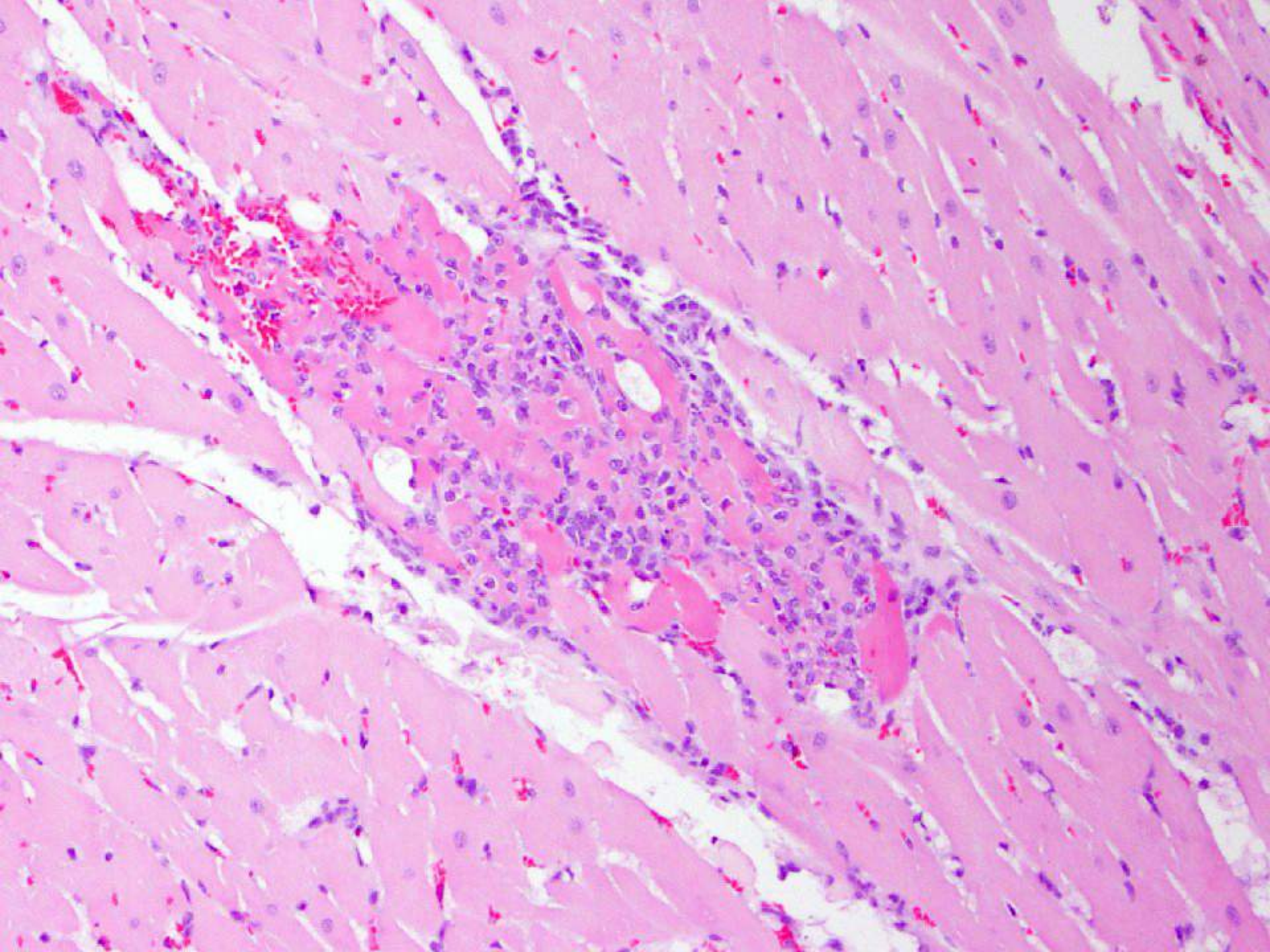


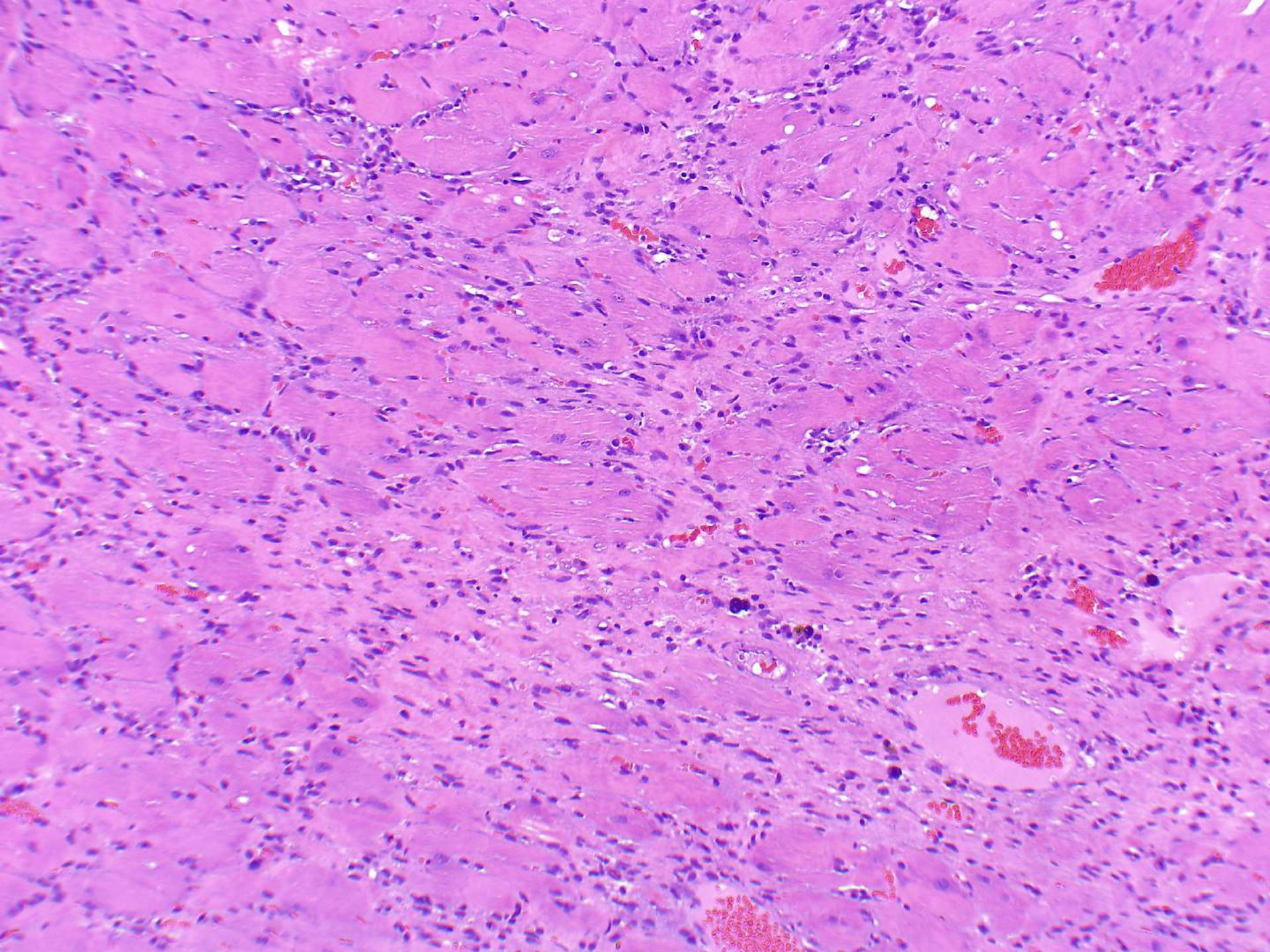
Cardiomyopathy

- A spontaneous, degenerative disease of the rat heart.
- Etiology is unknown, but the incidence, severity, and age of onset are affected by diet and stress.
- Males tend to be more commonly and severely affected than females.
- Some strains (especially Sprague-Dawley) have early lesions by 3 months of age.

Cardiomyopathy

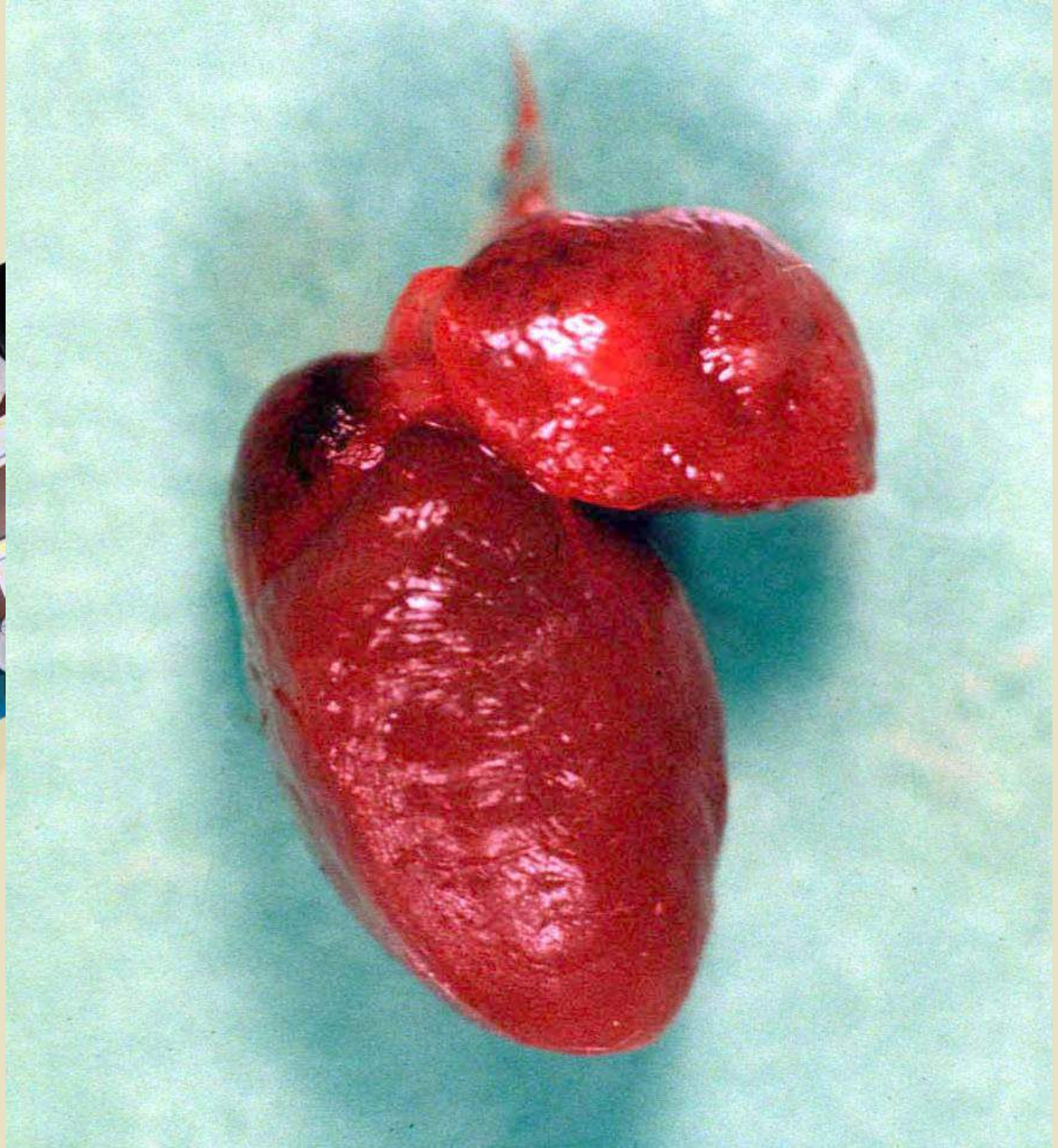
- Gross (Macroscopic) findings:
  - Heart may be pale or tan.
- Microscopic findings include:
  - Myofiber degeneration and necrosis.
  - Interstitial fibrosis.
  - Mononuclear cell infiltrate or chronic inflammation.





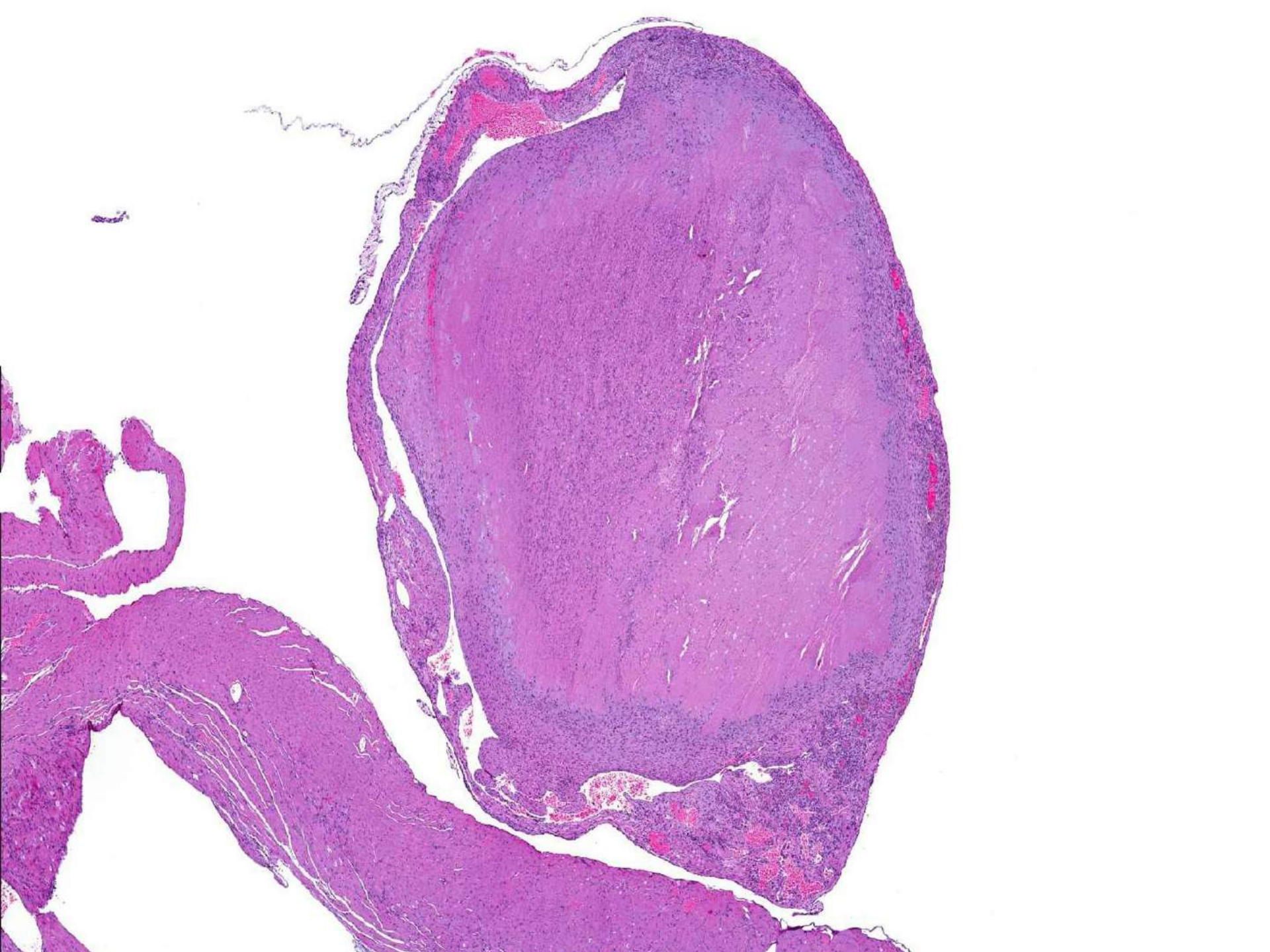
Atrial Thrombosis

- Thrombosis is more common in the heart of males than females.
- It may be the cause of death of animals on carcinogenicity studies.
- Thrombi less commonly affect the ventricles and aorta.
- Gross (Macroscopic) Findings
  - Atrium of the heart is enlarged and has dark red or tan discoloration.



Atrial Thrombosis

- Microscopic Findings:
  - Large, well-organized thrombus (blood clot) occupies the affected atrium.
  - Inflammation associated with the thrombus may extend into the wall of the heart.

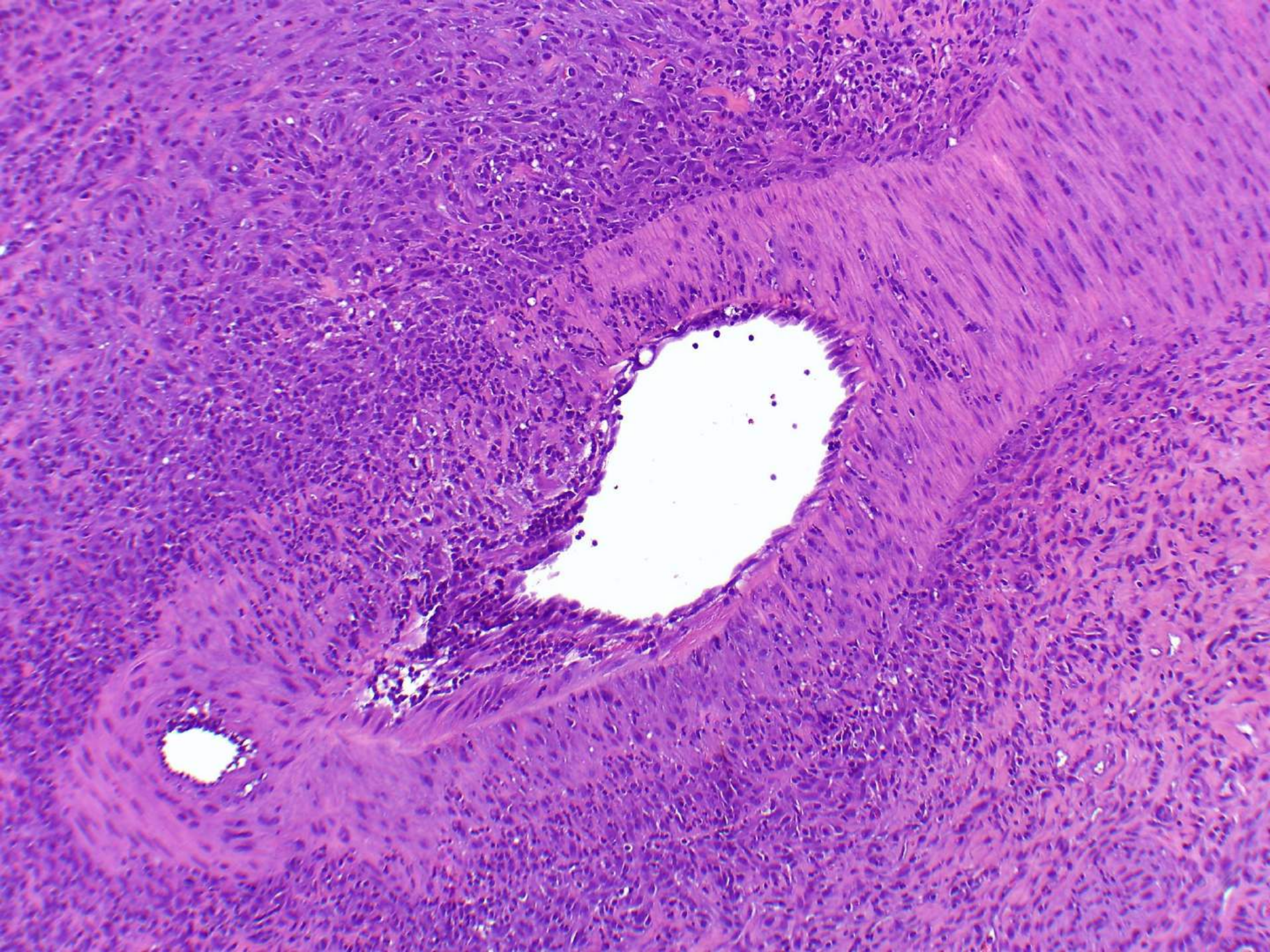


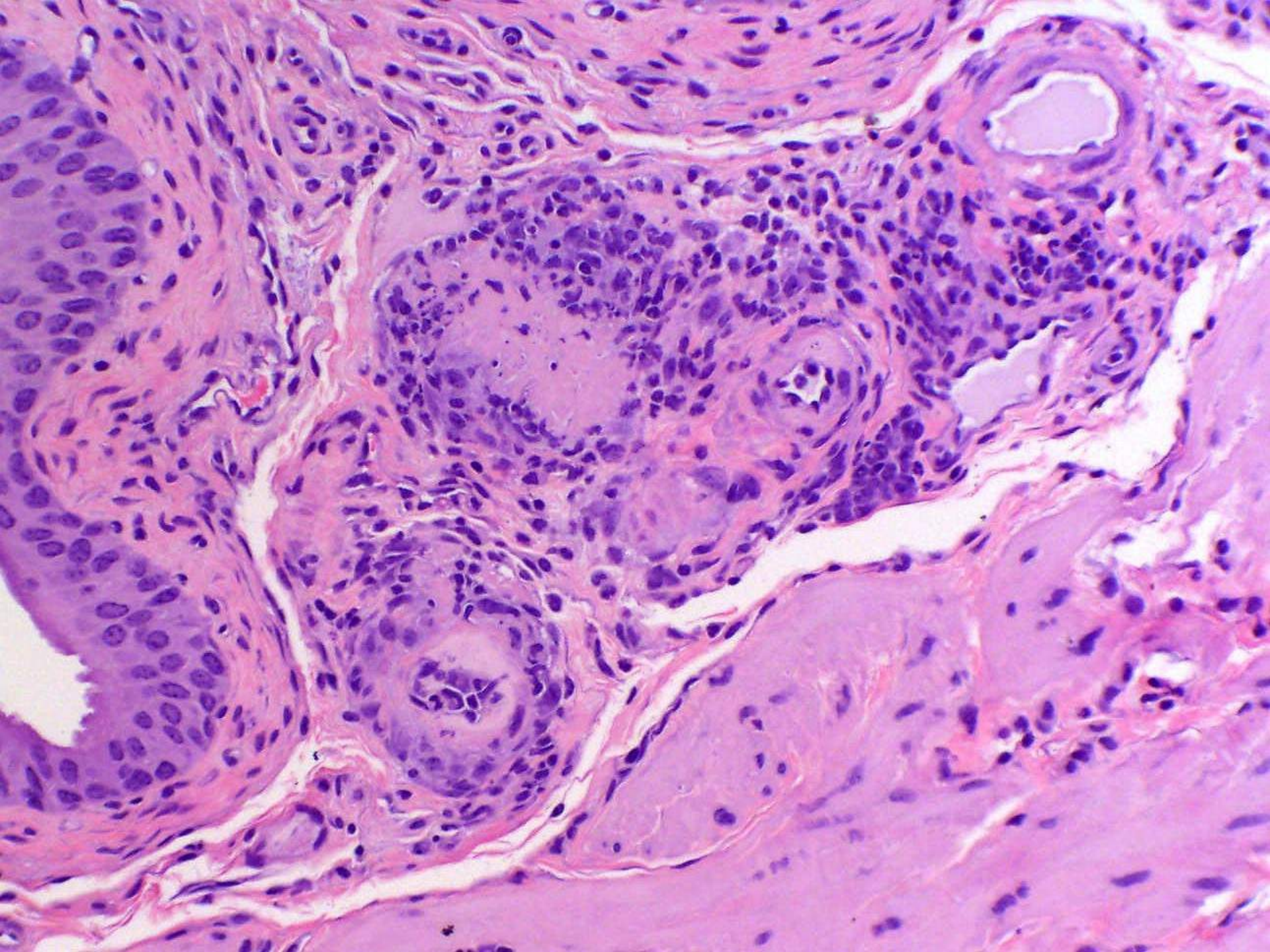
Polyarteritis

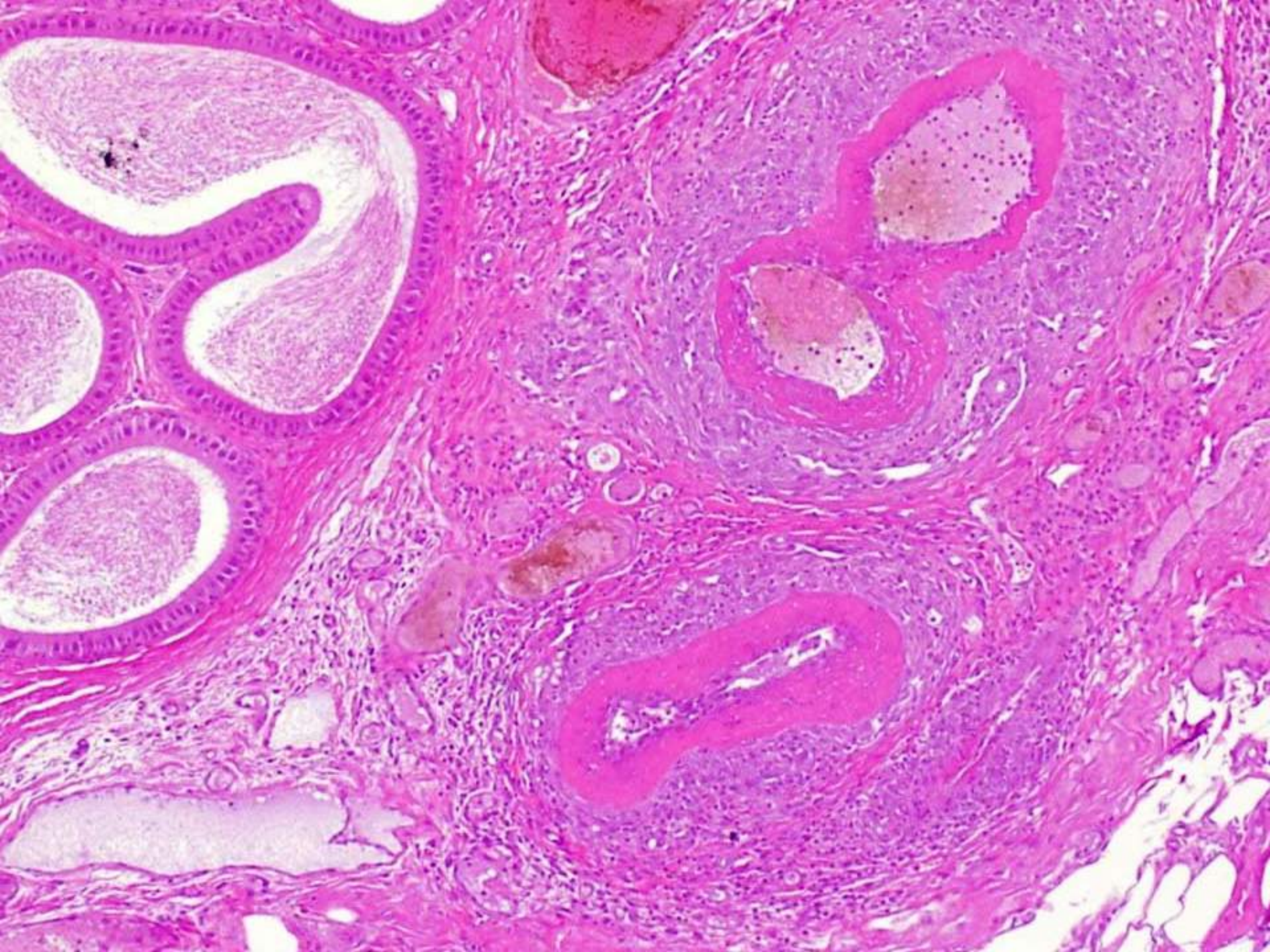
- Polyarteritis is a spontaneous disease that affects arteries and arterioles of rats; the cause is unknown.
- The incidence is higher in males than females and increases with age.
- Gross (Macroscopic) Findings:
  - Nodular, gray to red thickening of arteries or arterioles, especially vessels in the mesentery, pancreas, epididymis, and testis.

Polyarteritis

- Microscopic Findings:
  - Appearance varies from an acute inflammatory reaction to a chronic or chronic-active lesion.
  - Segmental fibrinoid necrosis may accompany the inflammation in acute cases.
  - Vessel lumen may be narrowed and thrombosed.

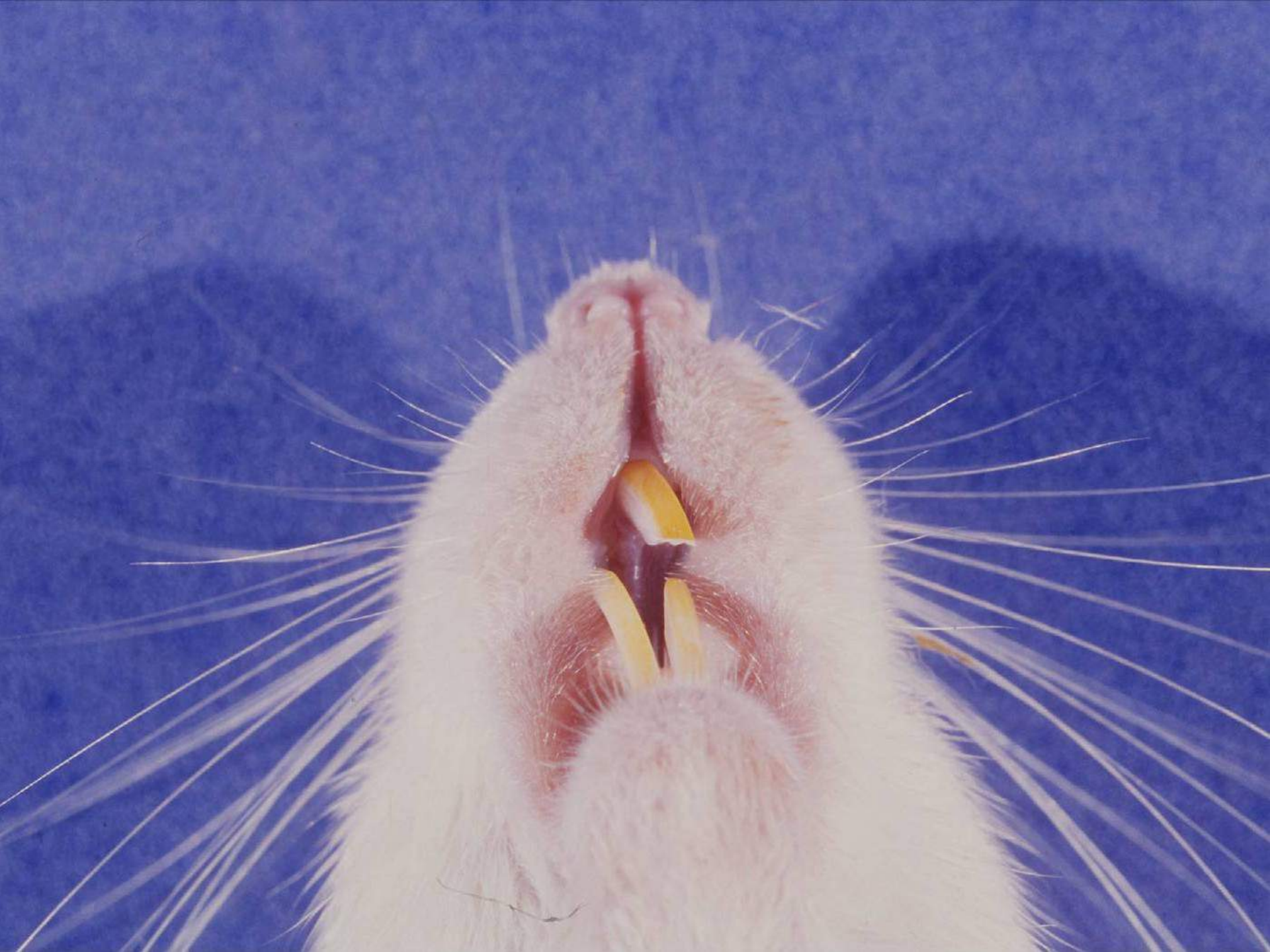


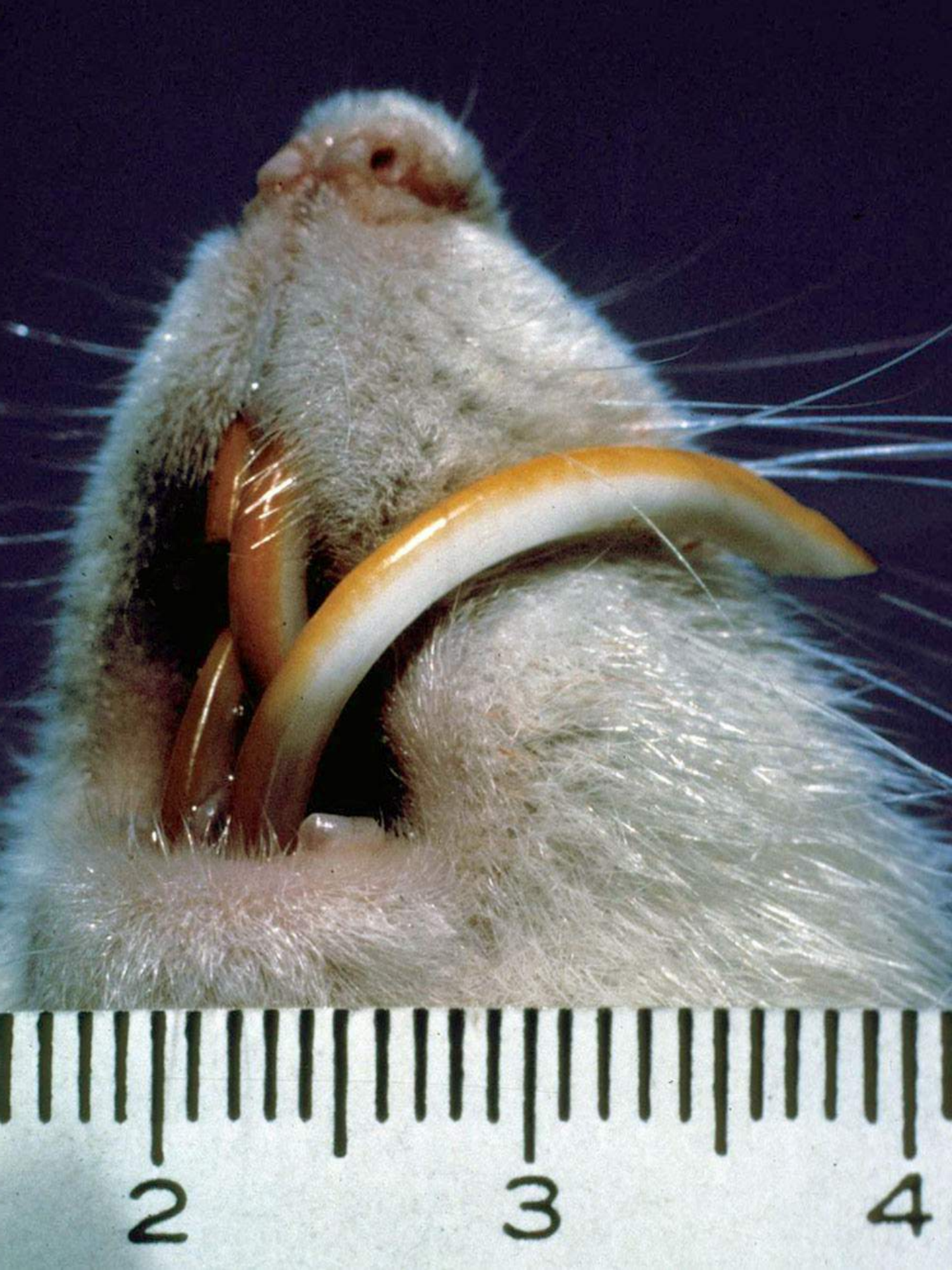




Malocclusion

- The incisor teeth of rats grow throughout life.
- Improper alignment of incisor teeth (malocclusion) due to overgrowth, fracture, or loss prevents normal wear.
- Malocclusion generally occurs at a low incidence, but can be associated with anorexia and weight loss.
- Malocclusion occurs more commonly in studies when rats are fed powdered diets.
- Teeth are not examined microscopically in our laboratory unless they are a known target of the drug being tested.

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Biliary Hyperplasia

- Biliary hyperplasia in the liver is common; it can affect 20-50% of aged rats in a carcinogenicity study. However, bile duct neoplasms are rare in rats, indicating that the hyperplasia has little likelihood of progressing to neoplasia.
- Biliary hyperplasia is not evident grossly in the liver.
- Microscopically, there is proliferation of well-differentiated bile ductules; peribiliary fibrosis may also be present. Not all portal areas are affected.

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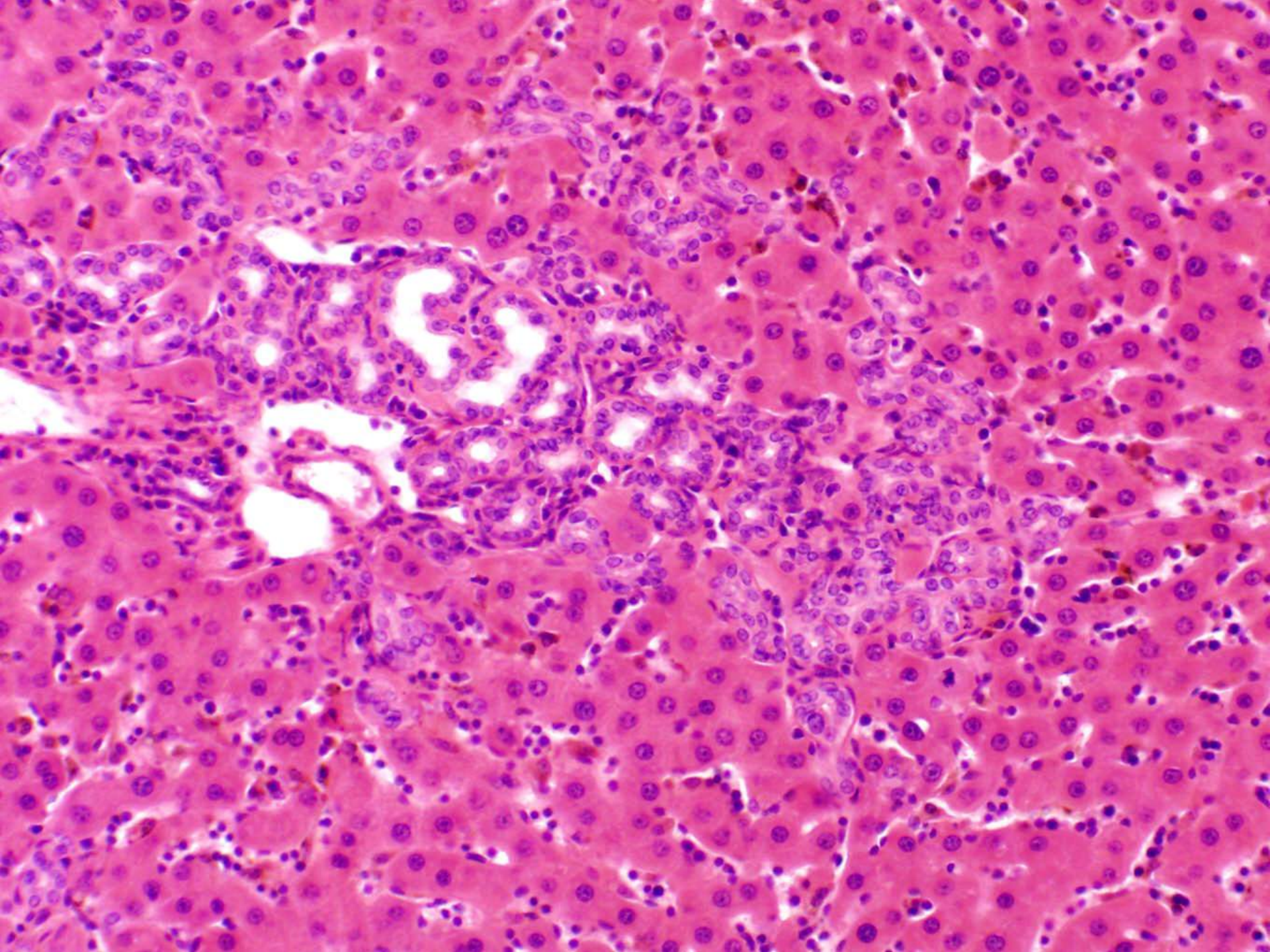
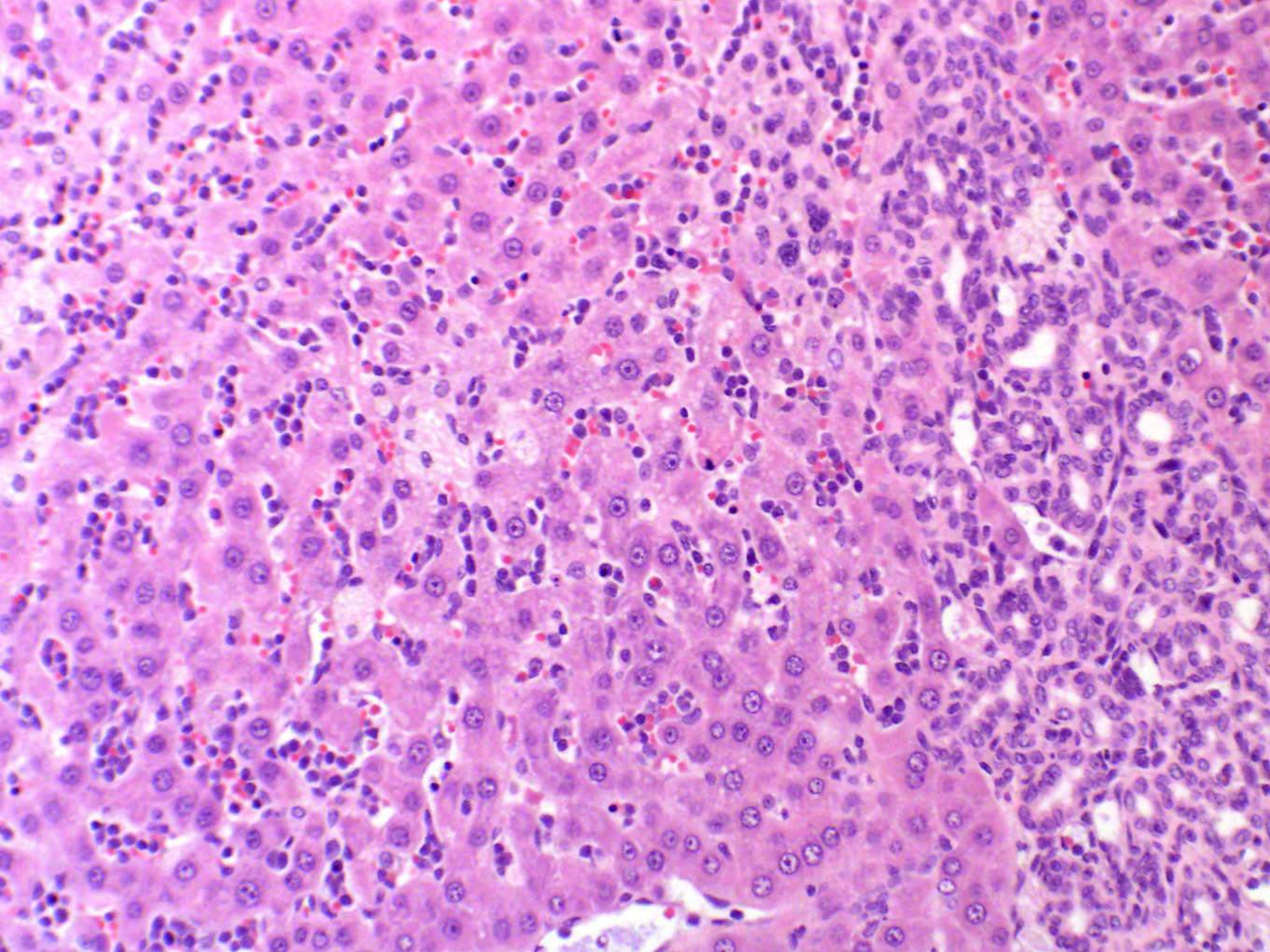


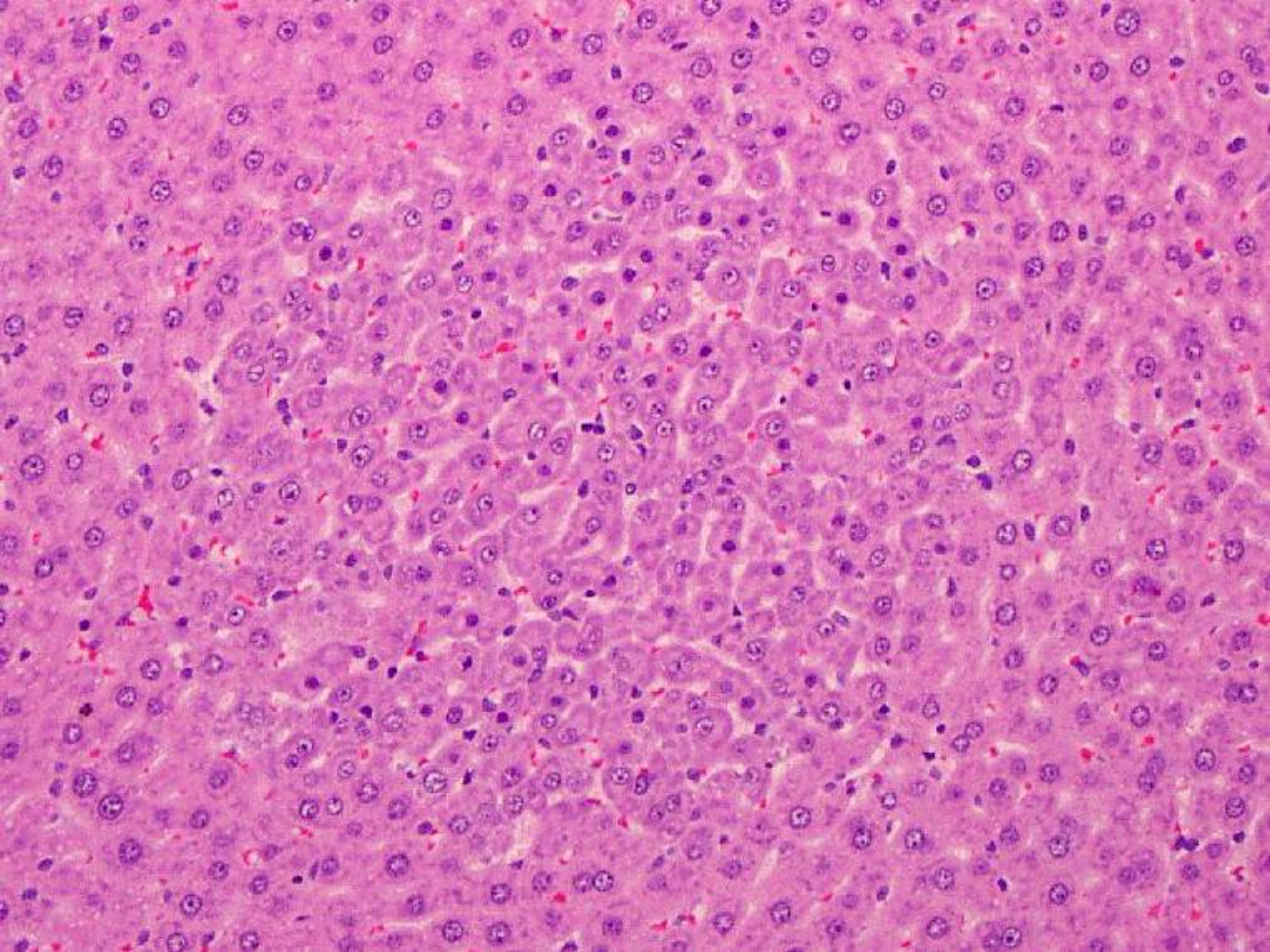


Foci of Cellular Alteration

- Microscopically, these liver lesions must be differentiated from hepatocellular neoplasms.
- Hepatocytes in the foci differ in size and staining quality from the surrounding hepatocytes. Foci of cellular alteration are classified as basophilic, eosinophilic, clear, vacuolated, or mixed.
- Cells at the periphery of the foci can blend with surrounding parenchyma or cause minimal compression; they do not disrupt the normal lobular architecture of the liver.

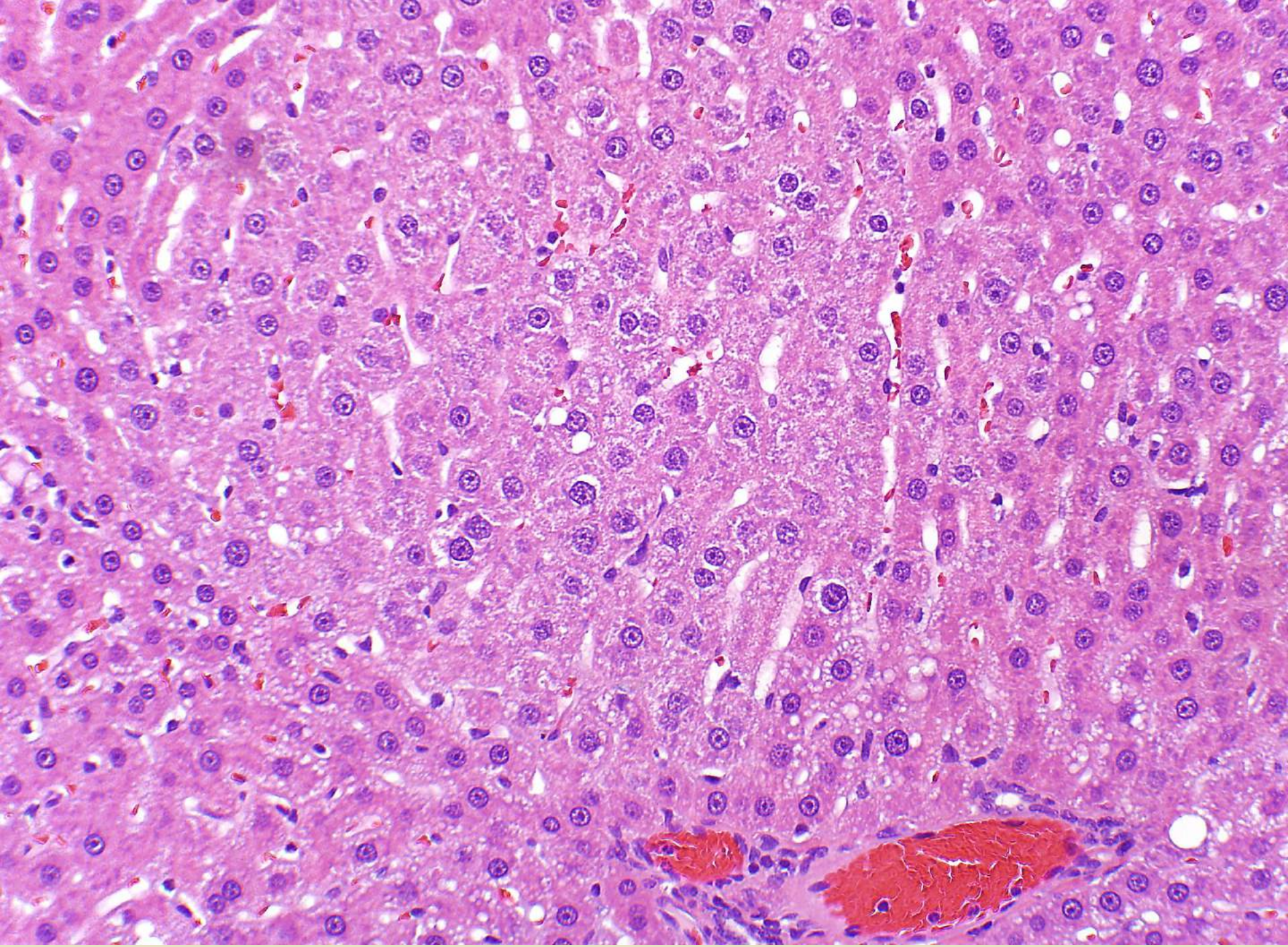
Basophilic Foci of Cellular Alteration

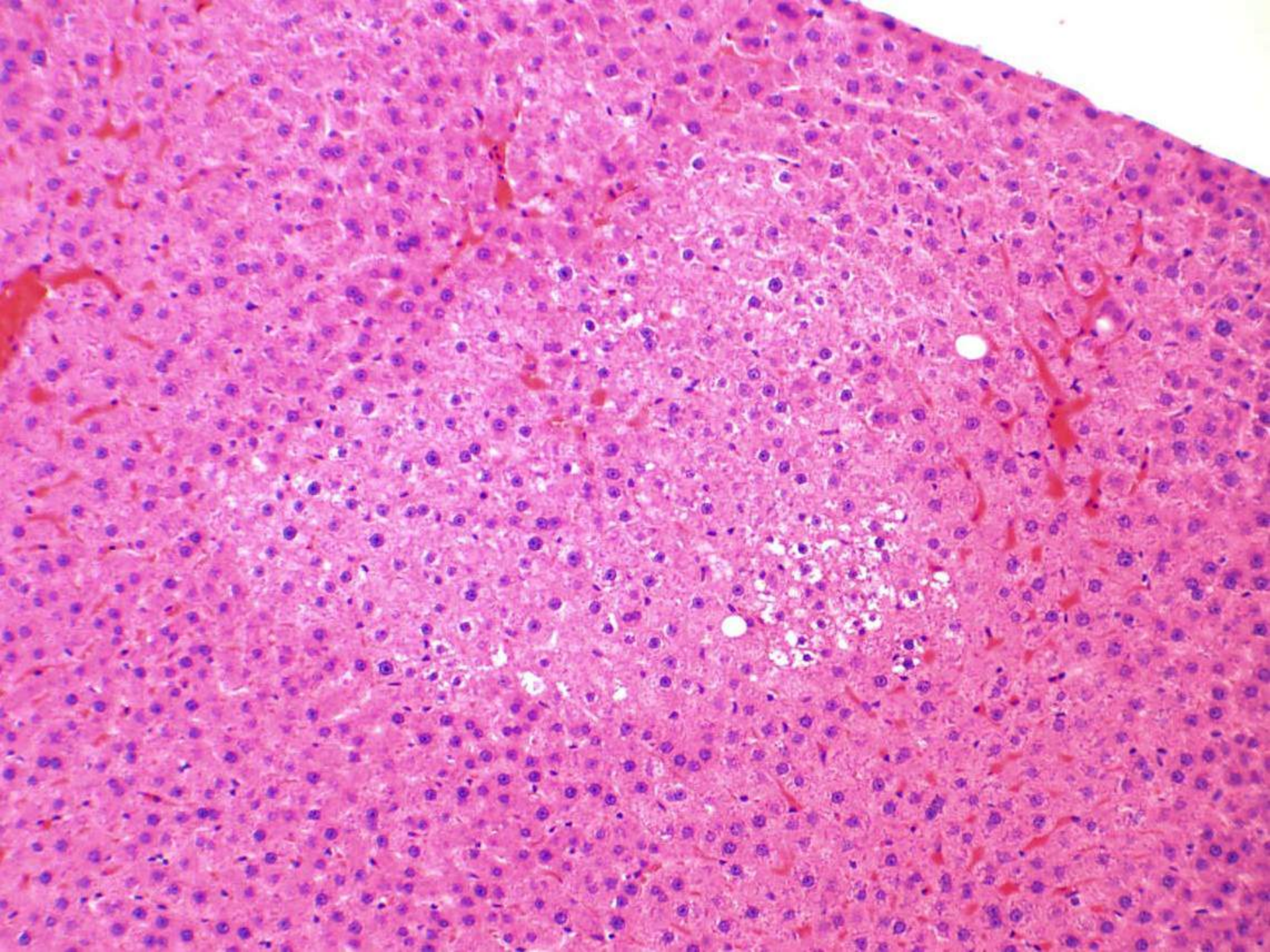
- Basophilic foci generally consist of small, basophilic hepatocytes that contain numerous ribosomes and little glycogen.
- Most spontaneous basophilic foci have cytoplasmic basophilia in dense linear aggregates (tigroid pattern).
- The importance of these foci as a predictor of carcinogenesis is somewhat controversial, but basophilic foci of cellular alteration are considered pre-neoplastic lesions.



Eosinophilic Foci of Cellular Alteration

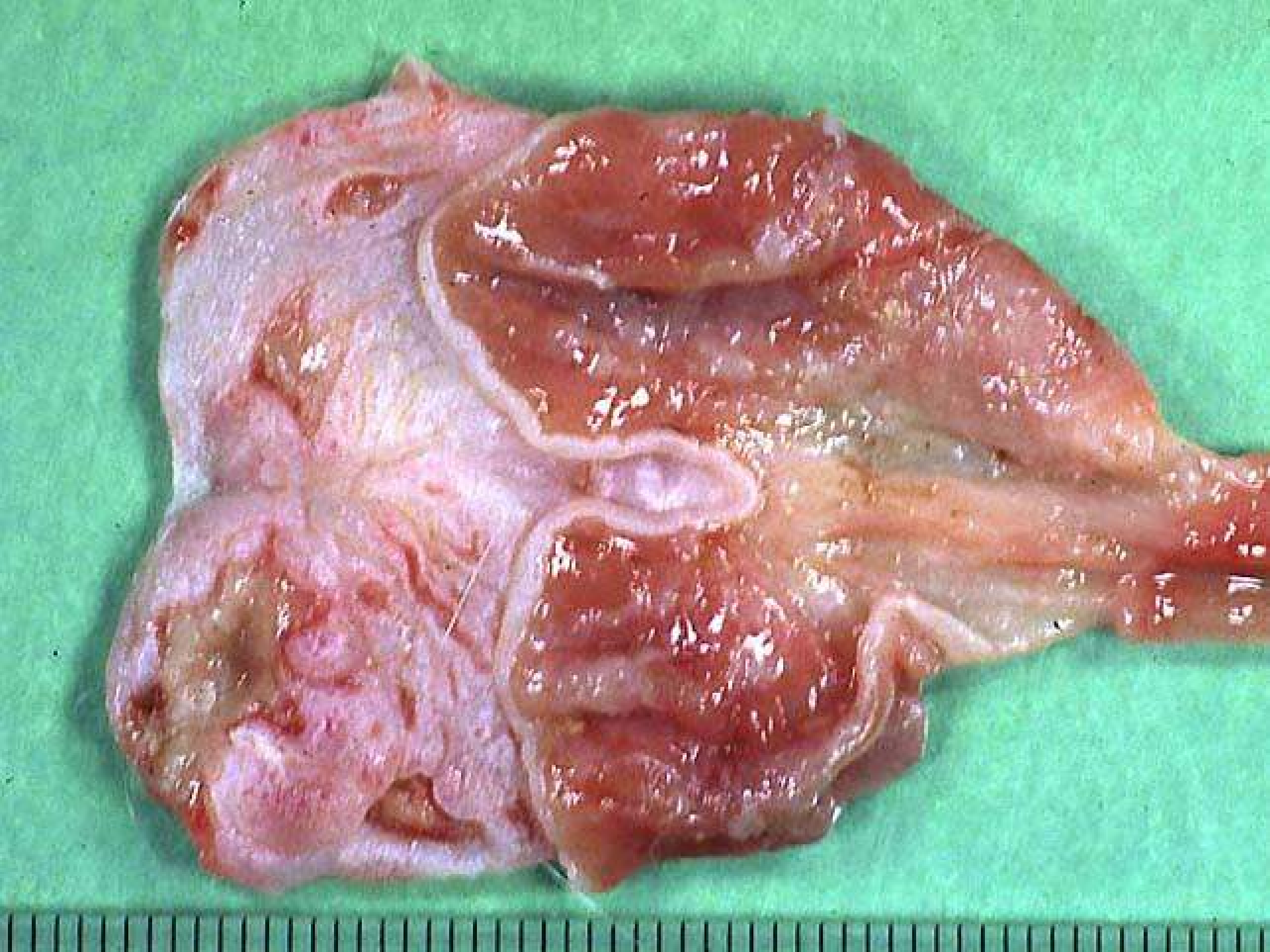
- Eosinophilic foci generally consist of hepatocytes with homogeneous eosinophilic cytoplasm usually resulting from an increase in smooth endoplasmic reticulum.
- Hepatocytes in eosinophilic foci tend to be larger than the surrounding hepatocytes and may cause compression of the adjacent parenchyma.





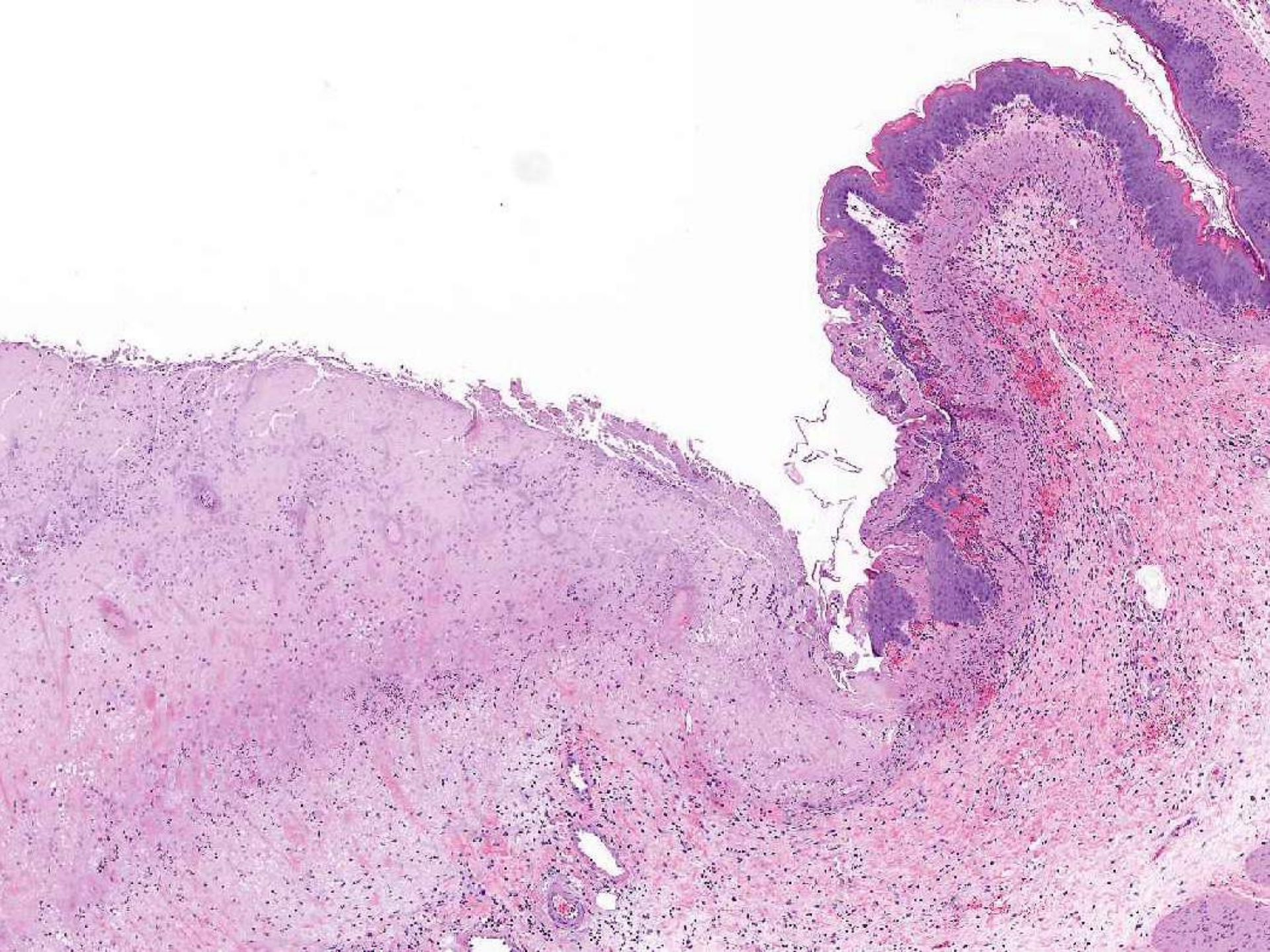
Gastric Erosion / Ulceration

- Grossly, one or more variably-sized, brown or black areas are present on the mucosal surface of the glandular or nonglandular stomach.



Gastric Erosion / Ulceration

- Microscopically, areas of the mucosal epithelium are necrotic. Necrosis may extend through the muscularis mucosa into the underlying submucosa and muscle.
- Gastric erosions and ulcers may be stress-related.

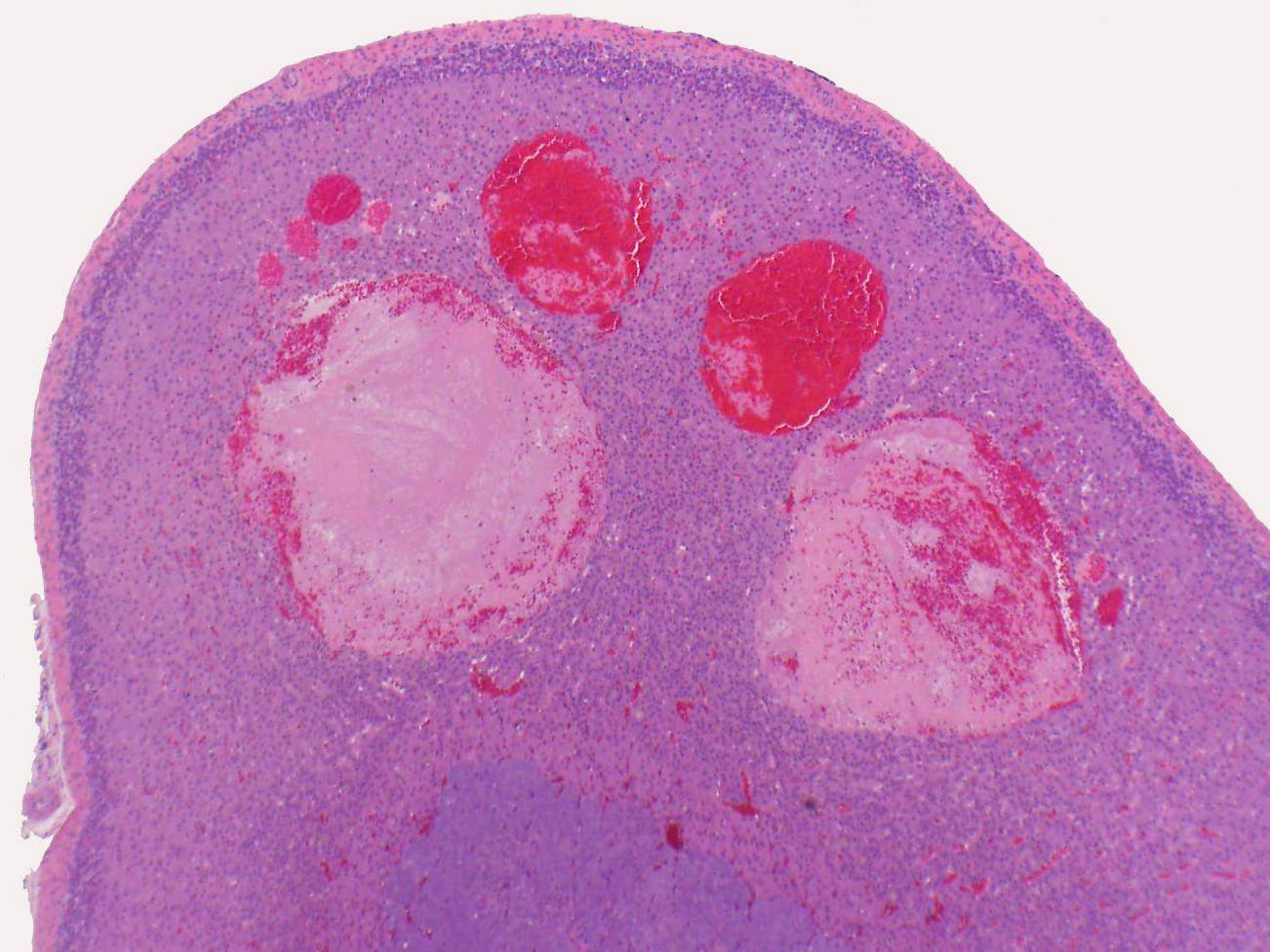


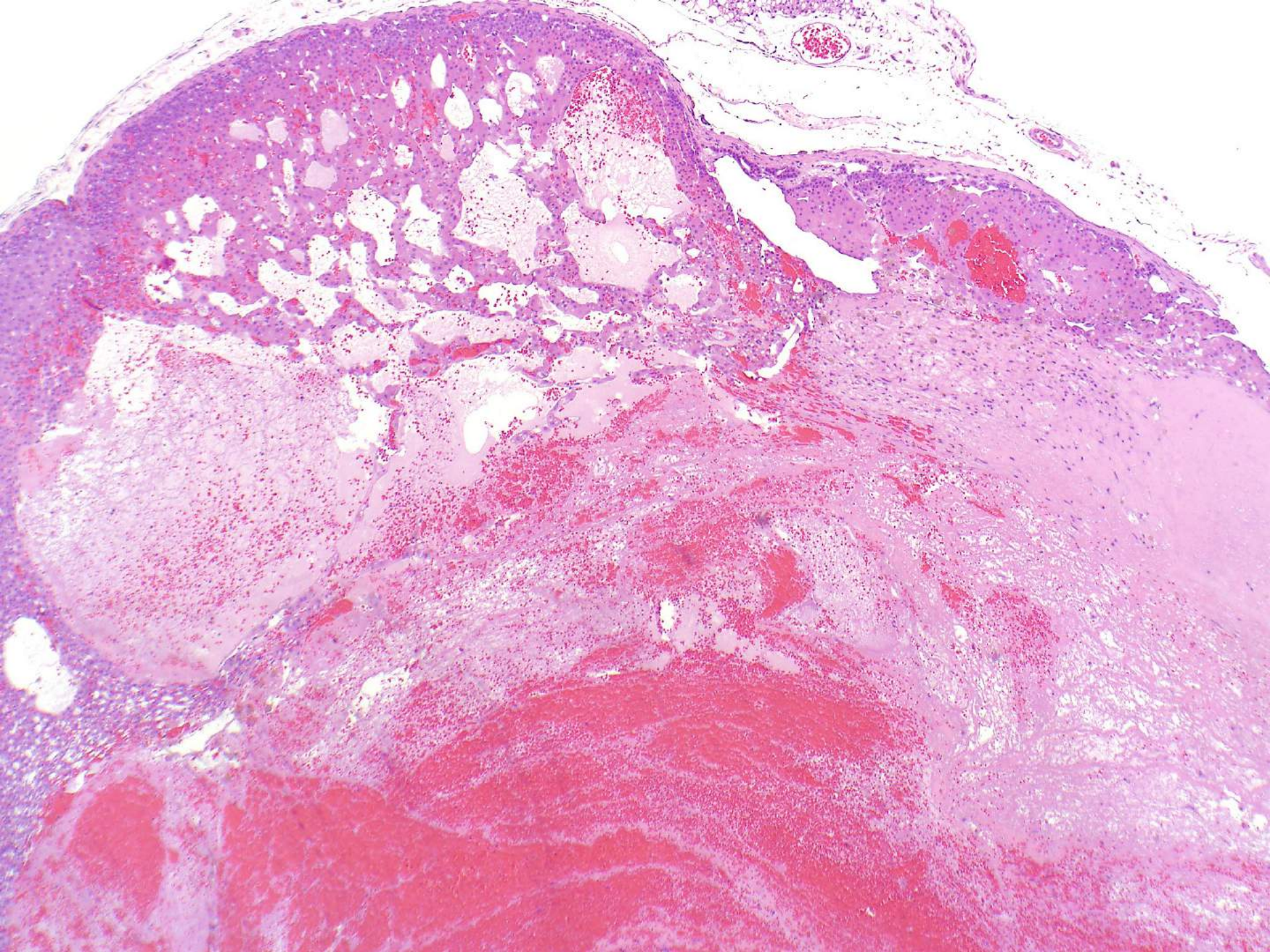
Cystic Degeneration – Adrenal Gland

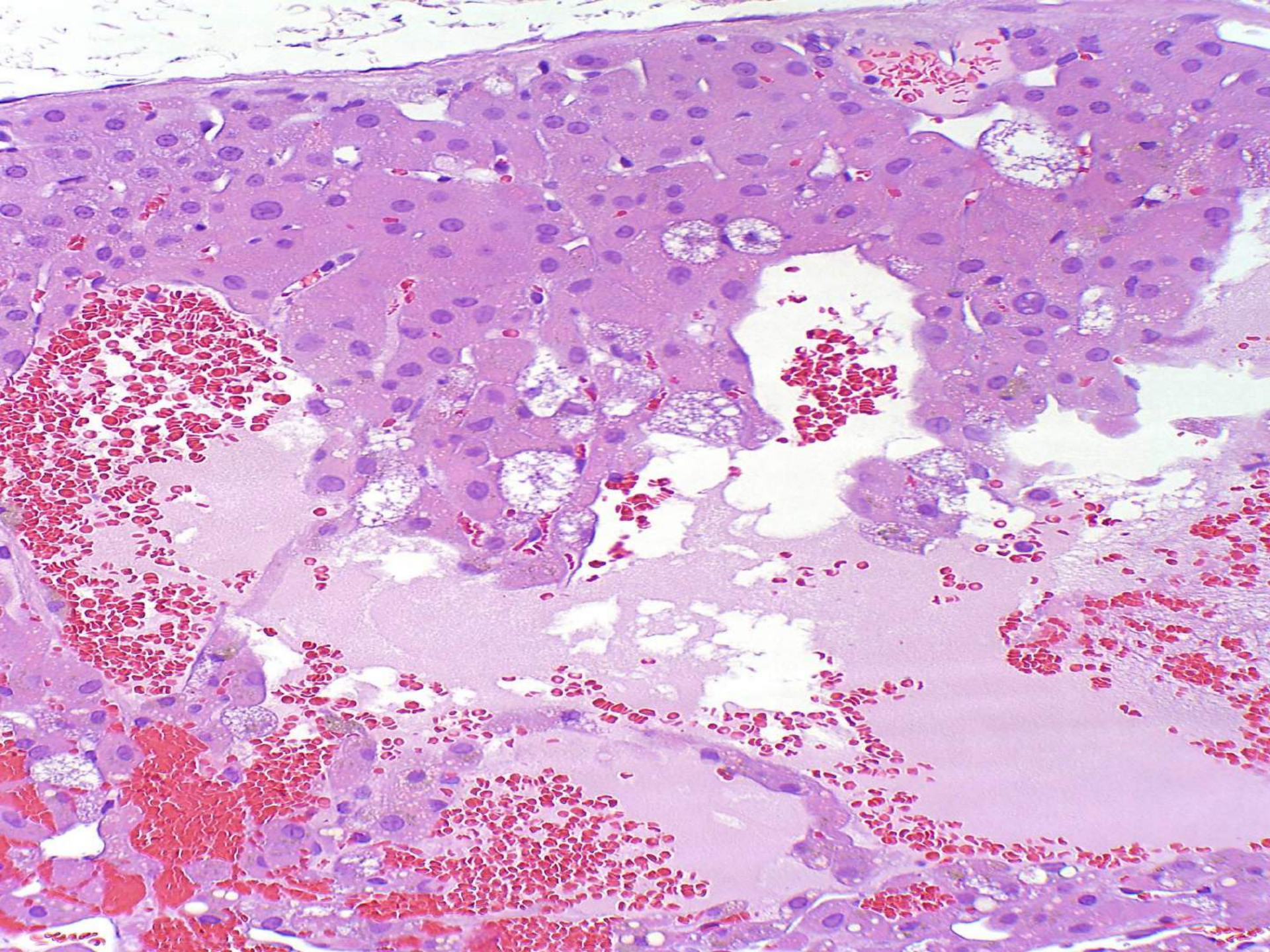
- Cystic degeneration is an aging change in the adrenal cortex of rats.
- The lesion occurs more commonly in females than in males; Sprague-Dawley rats have a particularly high incidence.
- Grossly, the affected adrenal glands are enlarged, soft, dark red, and mass-like.

Cystic Degeneration – Adrenal Gland

- Microscopically, cystic degeneration is characterized by cortical cell vacuolation, cell loss, and the formation of dilated, cystic spaces that contain blood. Thrombi may also be present.
- Lesions are generally focal and well-circumscribed; can be very extensive.
- The areas differ from hyperplasia and neoplasia in that there is a decrease rather than an increase in the total number of cells in the affected areas; mitoses are absent.





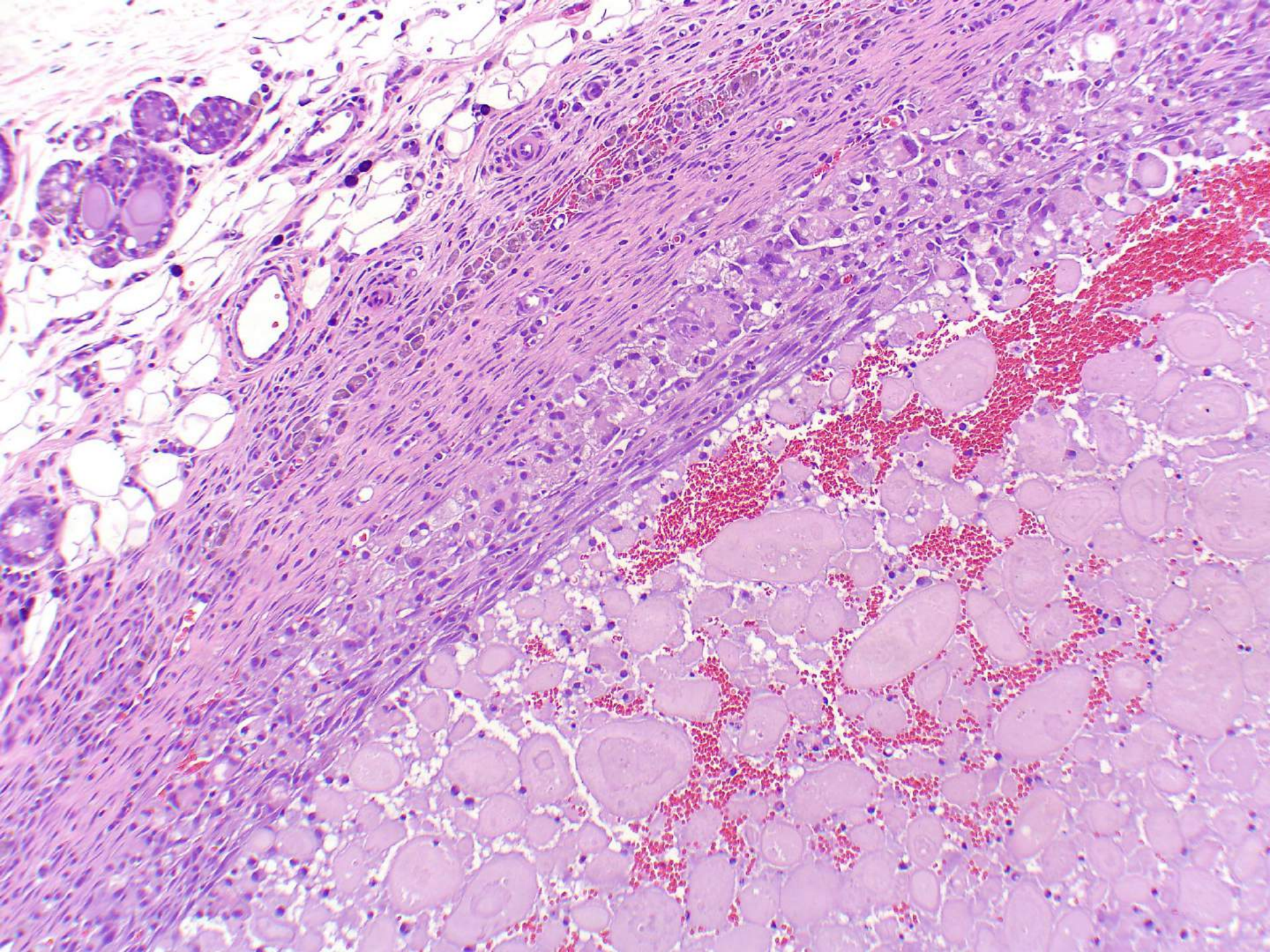


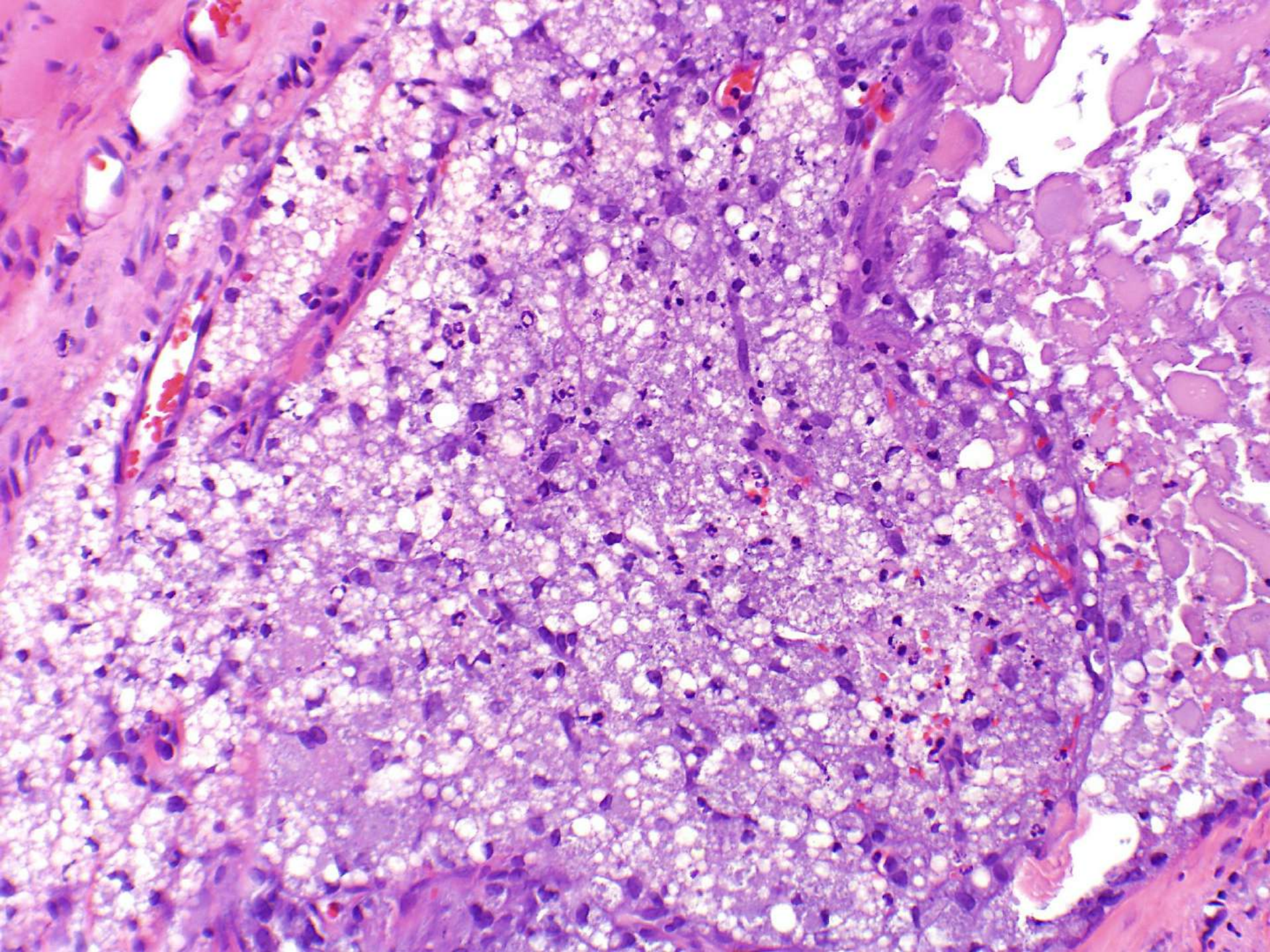
Ectatic Mammary Ducts / Galactoceles

- Ductal and glandular dilatation (ectasia) commonly occurs in the mammary gland of aging rats, especially in females.
- Grossly, the lesions are often described as cysts or masses at necropsy.
- The term galactocele is often used to describe markedly dilated ducts and/or alveoli that are filled with mammary secretion.

Ectatic Mammary Ducts / Galactoceles

- Microscopically, the affected ducts and glands are dilated and filled with secretory material. Cellular debris and inflammatory cells may also be present.
- If the wall of the galactocele ruptures, macrophages with vacuolated, foamy cytoplasm accumulate at the site.



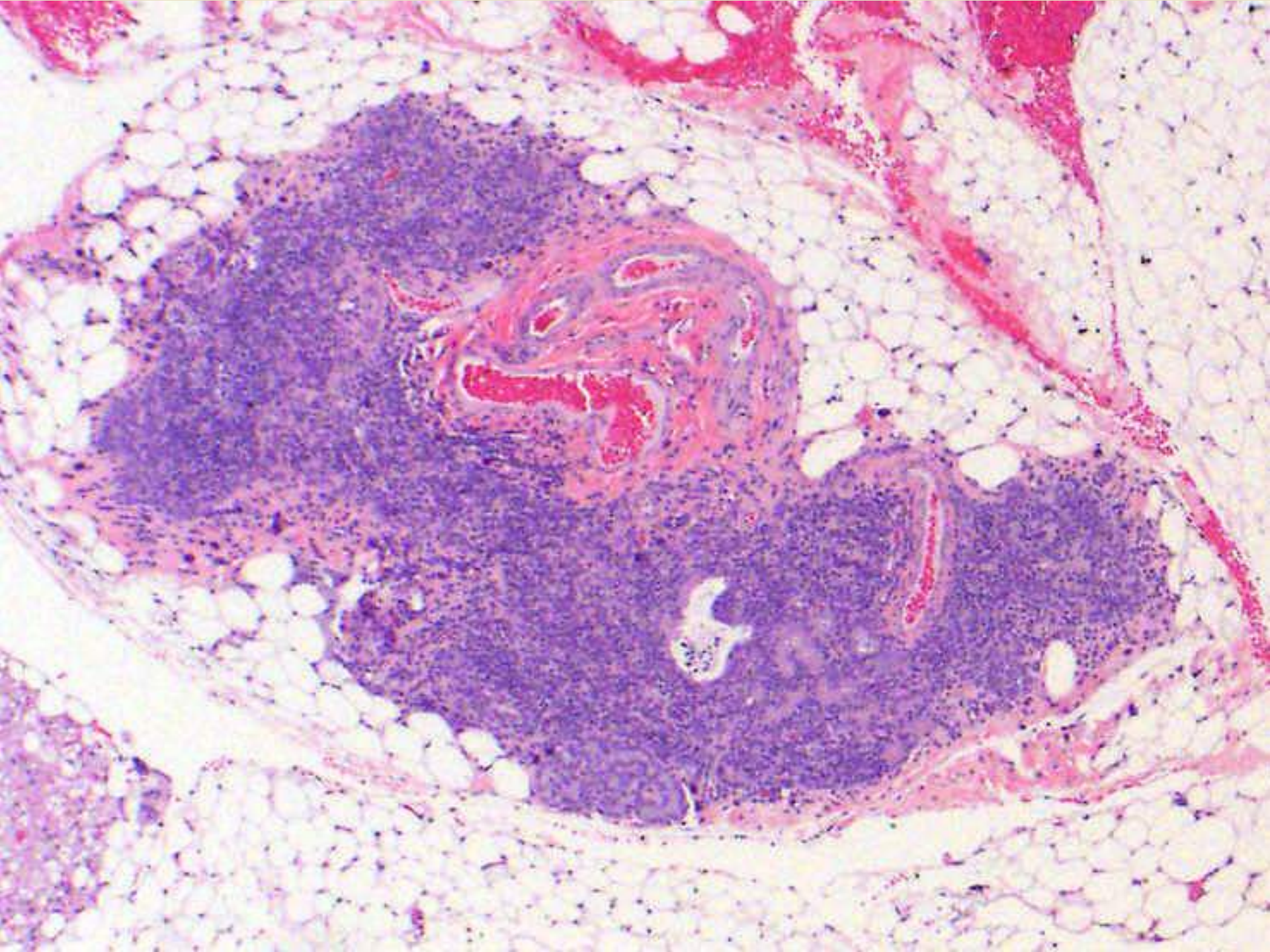


Thymic Atrophy / Involution

- Thymic atrophy or involution is a normal, age-related process.
- The incidence is nearly 100% in old rats.
- Grossly, the thymus is decreased in size; the small size can make identifying and collecting the thymus of aged rats difficult at necropsy.

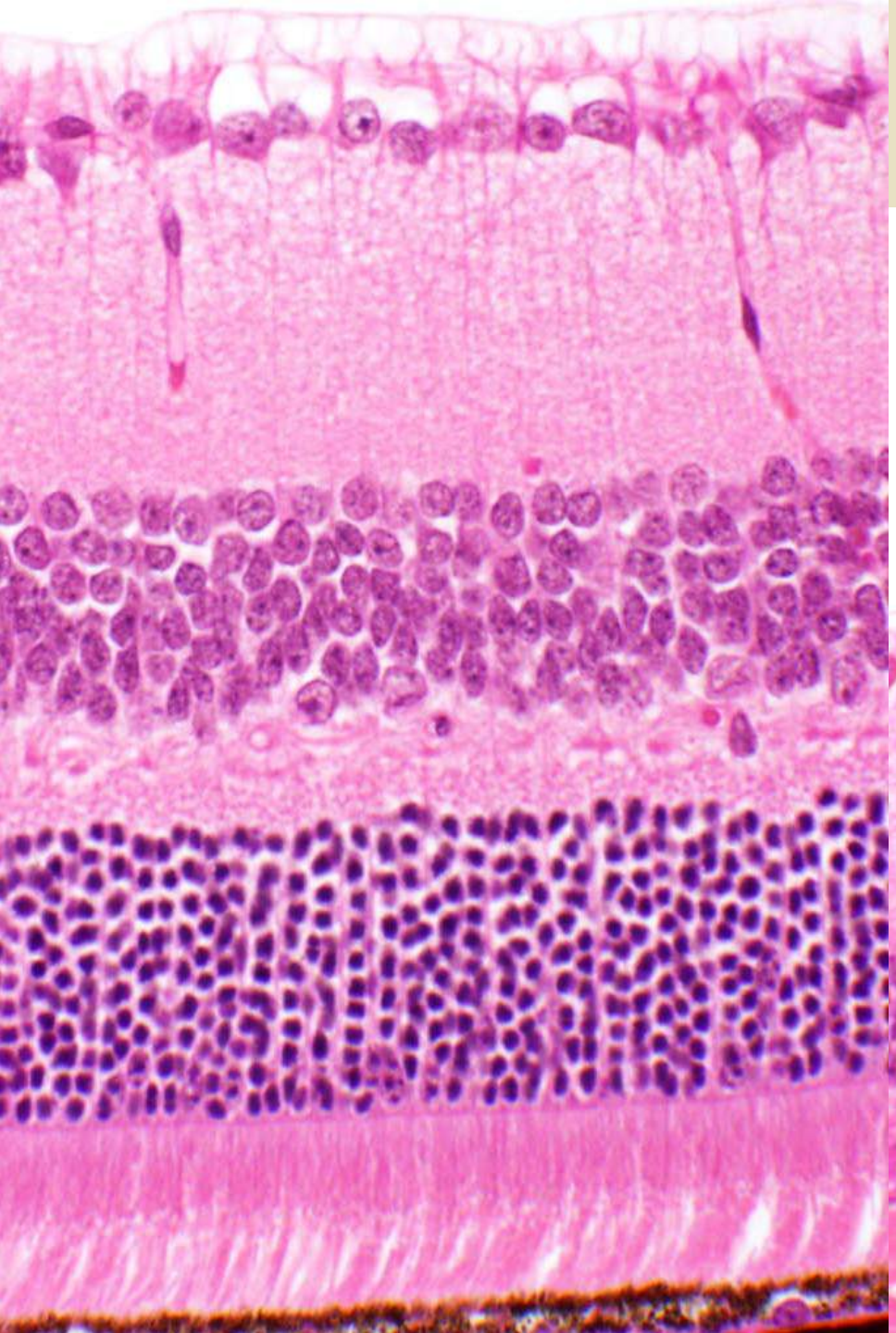
Thymic Atrophy / Involution

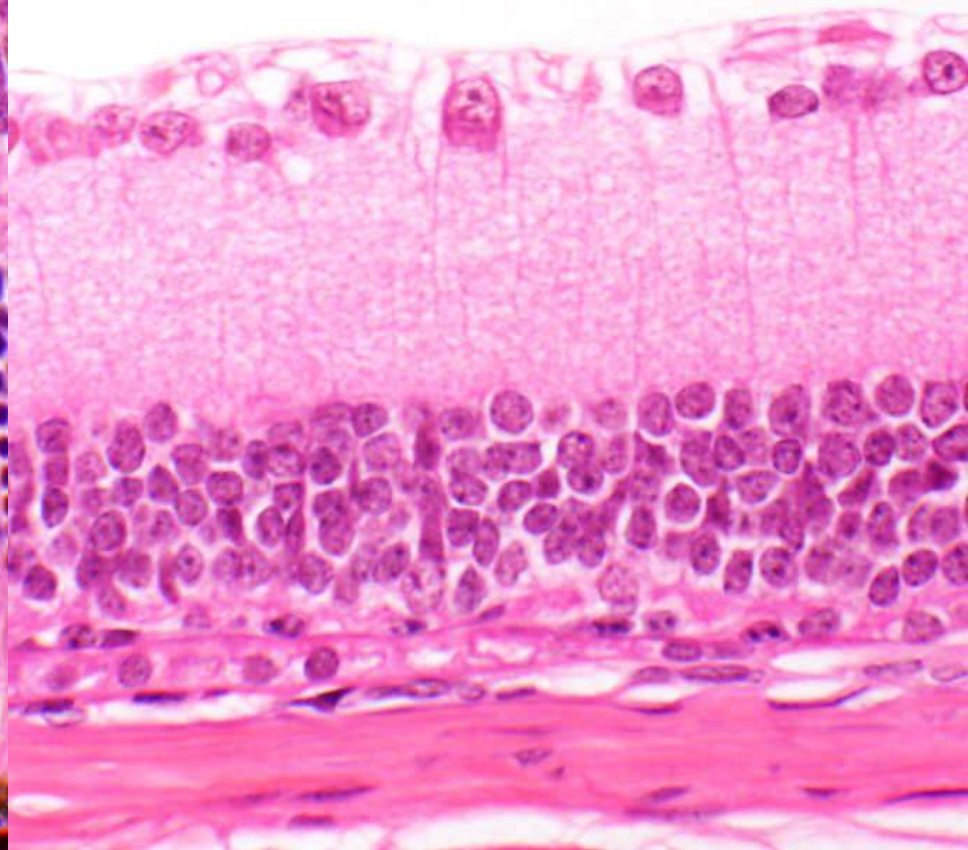
- Microscopically, the lymphocyte population is decreased in the cortex and medulla.
- As the atrophy progresses, epithelial cells of the thymus become more prominent, forming tubular or ductal structures that can become cystic or hyperplastic.
- Fat cells become more prominent and scattered between atrophied thymus lobules.



Retinal Degeneration

- Retinal degeneration causes thinning or loss of the photoreceptor cells of the eye. The outer nuclear layer of the retina is most commonly affected in aging rats, but degeneration can progress to total cell loss.
- Retinal degeneration tends to begin in the peripheral retina if it is age-related; excess light exposure can cause degeneration of the posterior pole of the retina.
- The incidence of light-induced retinal degeneration is minimized by rotating cages in the animal rooms during the study.



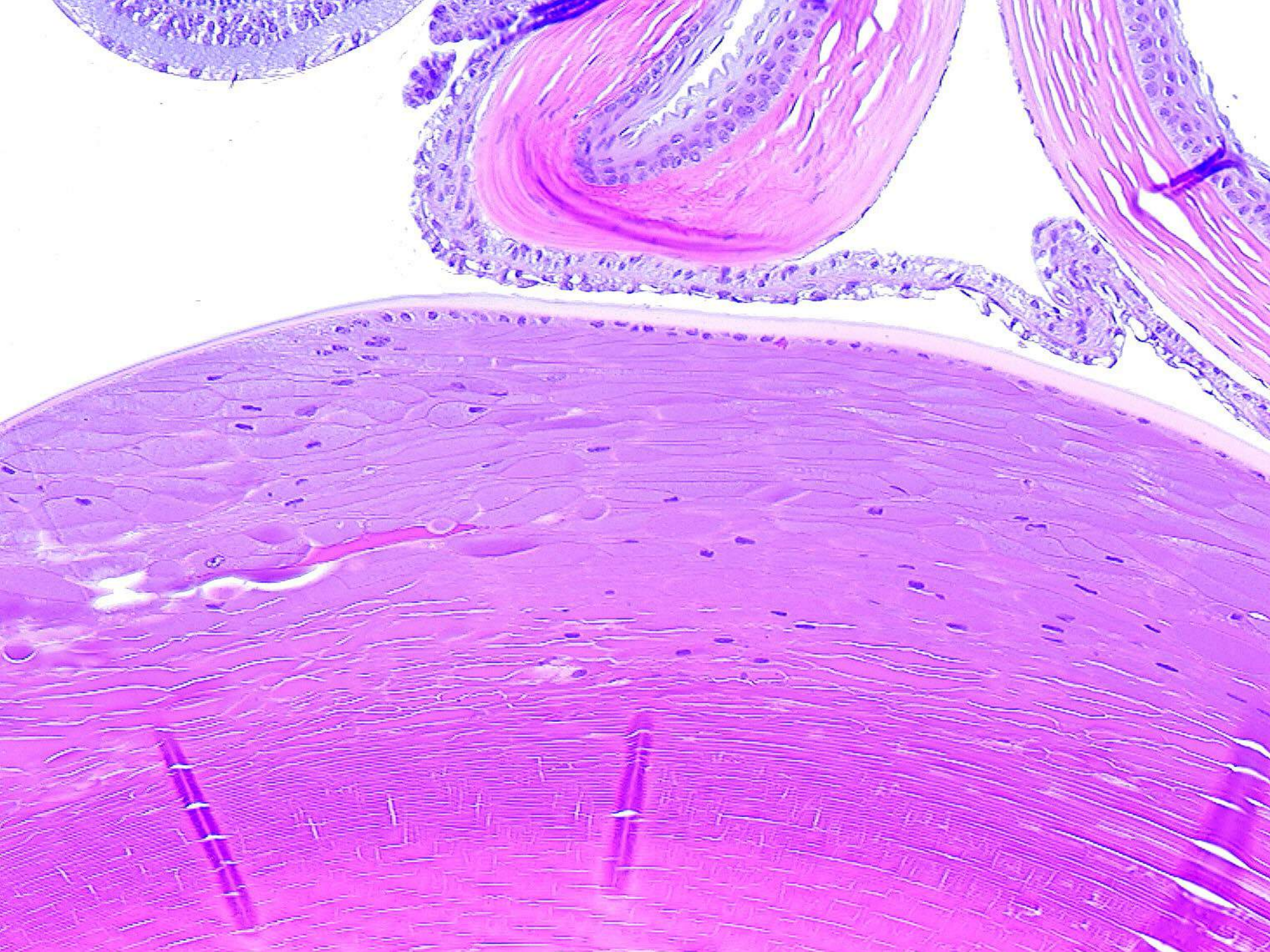


Cataract

- A cataract is an alteration of the eye that causes the lens to become opaque.
- Cataracts can be unilateral or bilateral.
- Grossly, the eyes are opaque or have white discoloration.

Cataract

- Microscopically, the lens fibers are irregularly enlarged and granular.
  - Lens fiber degeneration and mineralization also may be present.
  - The nuclei in the bow area of the lens become more irregularly arranged.

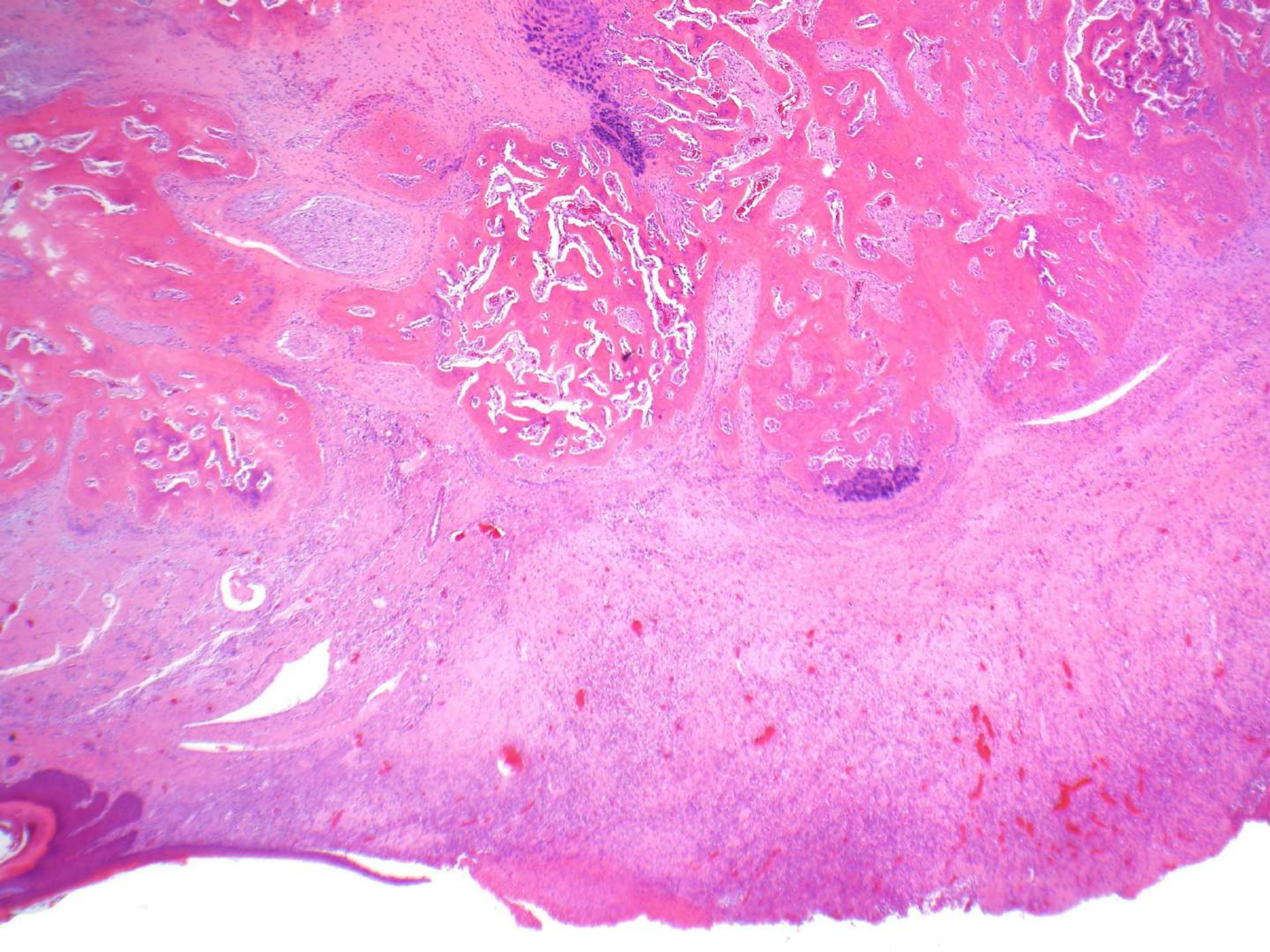


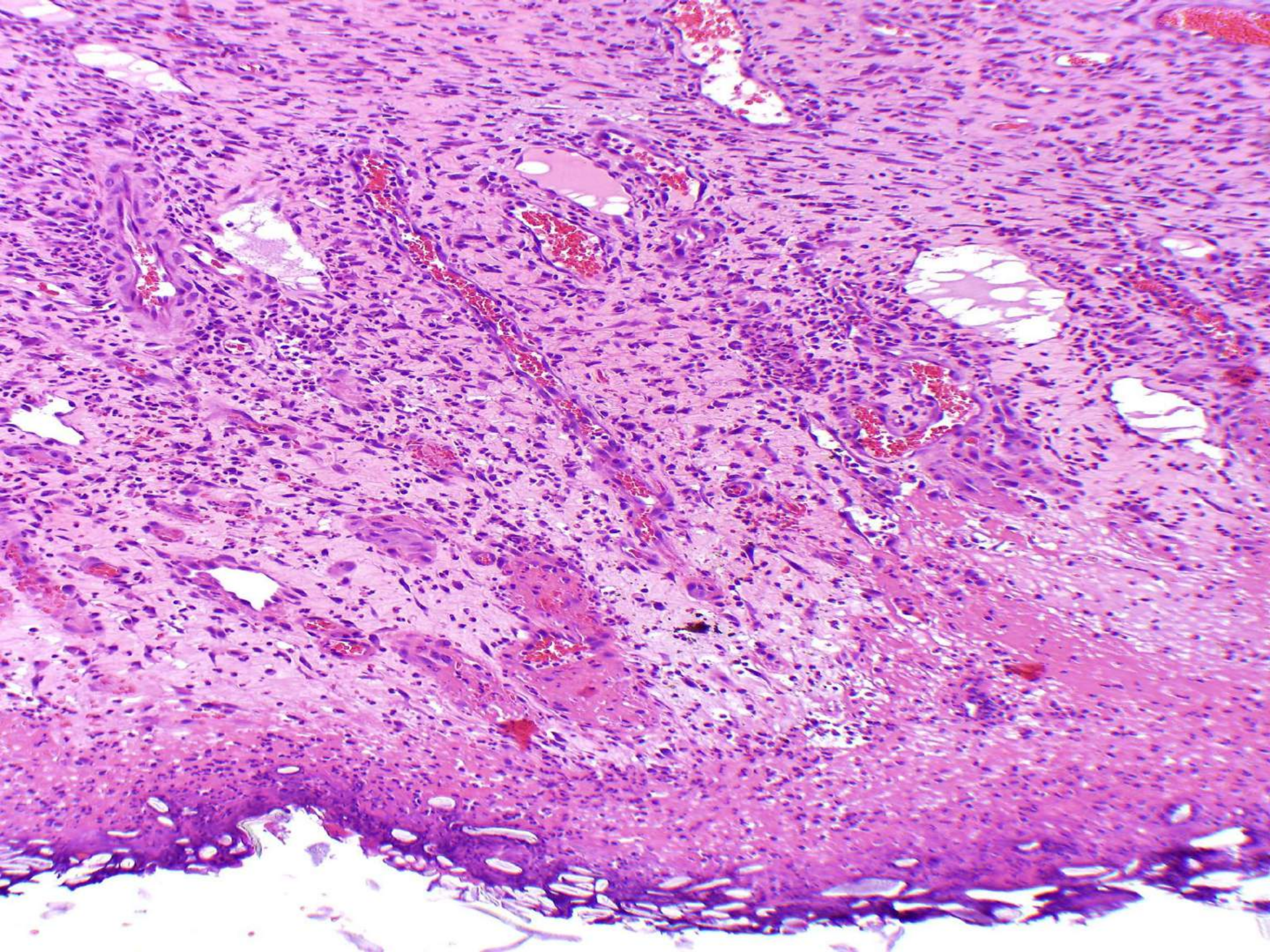
Ulcerative Pododermatitis

- Ulcerative pododermatitis is characterized by ulceration and inflammation of the bottom (plantar surface) of the feet.
- Commonly occurs in rats housed in wire cages, especially involves the rear feet of male rats being fed unrestricted diets.
- Grossly, variably-sized ulcers are present on the plantar surface of the feet.

Ulcerative Pododermatitis

- Microscopically, there is epidermal ulceration, chronic inflammation, and granulation tissue formation.
- Proliferation of the underlying bone (hyperostosis) often occurs in chronic cases.





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