

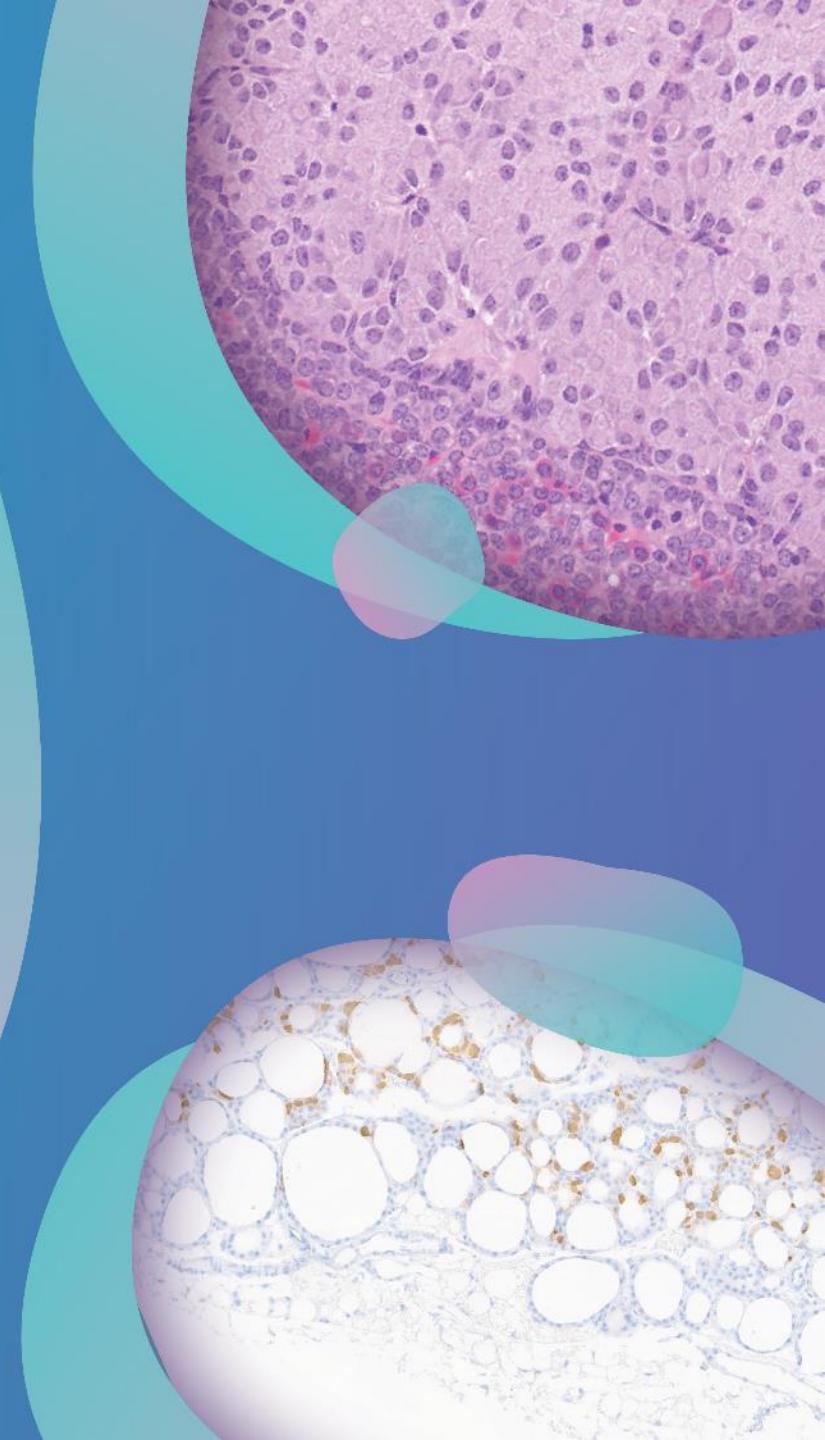


# Adrenal Gland: Histology, Anatomy, Physiology and Incidental Lesions

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## The Adrenal Gland

- Most common endocrine organ associated with chemically induced lesions (Ribelin 1984), while the adrenal cortex, rather than the medulla, is the most frequent site of toxicity (Hinson and Raven 2006) due to:
  - ↑ expression of xenobiotic metabolizing enzymes (e.g., cytochrome P450) → ↑ free radicals and metabolites
  - ↑ unsaturated fatty acids in cell membranes → lipid peroxidation
  - Accumulation of lipophilic compounds due to high lipid/cholesterol/steroid content
  - ↑ possible targets such as receptors, enzymes, and peripheral hormone carrier molecules
  - ↑ vascularization and ↑ blood volume per unit mass
- The adrenal gland contains two distinct endocrine regions (Inomata and Sasano 2015):
  - Medulla
  - Cortex
- The adrenal cortex is essential for life (i.e., aldosterone secretion in the ZG), while the functions of the medulla are not required for life (Rosol et al. 2001)



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## Embryology of the Adrenal Gland

- The adrenal cortex and medulla are formed during embryogenesis by two distinct cell populations with mesodermal and neuroectodermal origins, respectively (Hammer et al. 2005)
- Cortex: derived from SF-1 expressing cells within the urogenital ridge of the coelomic cavity epithelium (i.e., adrenogonadal primordium)
- Medulla: derived from ectodermal tissue of the neural crest
- Precursor medullary cells invade the developing cortical cells forming the embryonic adrenal, which migrates cranial to the kidneys

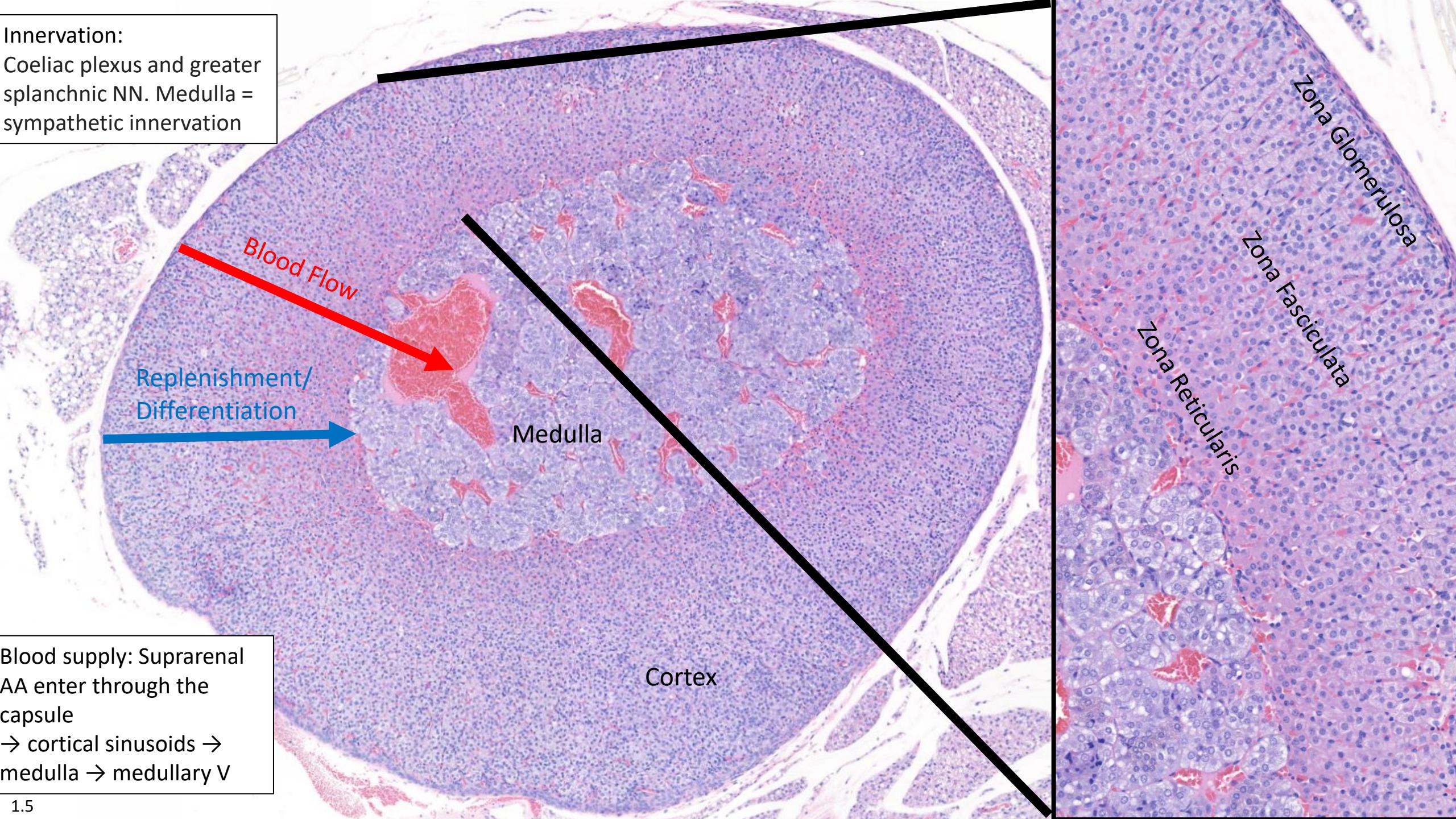


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## Adrenal Cortex

- The adrenal cortex is characterized by three layers:
  - Zona glomerulosa (ZG): aldosterone, outer-most layer of cortex (i.e., sub-capsular)
  - Zona fasciculata (ZF): corticosteroids, middle layer of cortex
  - Zona reticularis (ZR): androgens, inner-most layer of cortex adjacent to medulla
- Species differences in the organization of these zones
  - Rodents: ZR poorly differentiated from ZF in mice; zona intermedia in rats; persistent X-zone (fetal) in mice
  - Others... (e.g., cell arrangement of ZG can vary between species; dog versus rat/human; hence synonym of zona multiformis)
- Zona glomerulosa cells are extremely sensitive to increases in extracellular potassium concentration and maintain K<sup>+</sup> levels by secreting aldosterone
- Zona fasciculata constitutes the bulk of the cortex in laboratory animals/toxicology species
- The zona reticularis is prominent in humans, but it is less distinguishable in rodents (rat > mouse)
- Precursor cells located subcapsular/outer ZG (mitotically active) → centripetal differentiation from ZG to ZR → apoptosis (Vinson 2016)

Innervation:  
Coeliac plexus and greater  
splanchnic NN. Medulla =  
sympathetic innervation



Blood supply: Suprarenal  
AA enter through the  
capsule  
→ cortical sinusoids →  
medulla → medullary V

# Adrenal Cortex: Histology

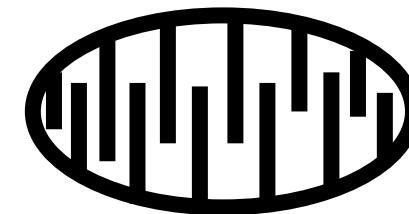
- Light microscopy:

- Zona glomerulosa: cells are polyhedral are arranged in clusters invested with a fine fibrovascular trabecular network that is continuous with the capsule
- Zona fasciculata: cells are polyhedral and arranged in radial cord-like structures (laminae) separated by sinusoids
- Zona reticularis: cells are polyhedral, lack the cord-like arrangement of the ZF and are invested with tortuous sinusoidal structures

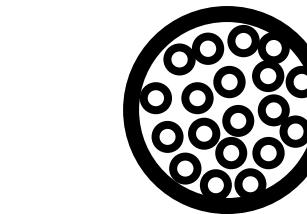
- Ultrastructure:

- Zona glomerulosa:

- Mitochondria = lamellar cristae
- ↓ lysosomes
- ↓ smooth endoplasmic reticulum
- ↓ lipid droplets



Lamellar cristae (ZG)



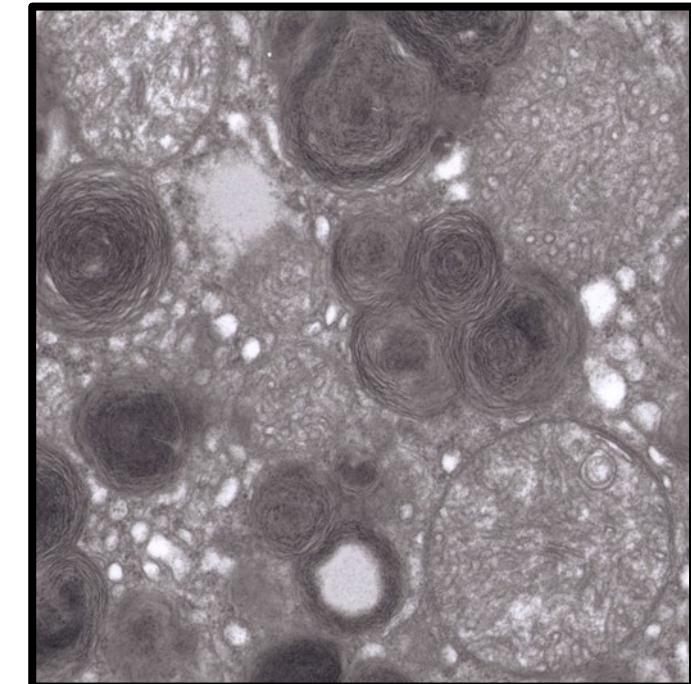
Tubulo-vesicular cristae  
(ZF/ZR)

- Zona fasciculata:

- Mitochondria = short and long tubular/vesicular cristae
- ↑ lipid droplets
- ↑ smooth endoplasmic reticulum and rough endoplasmic reticulum
- ↑ lysosomes

- Zona reticularis:

- Mitochondria = short and long tubular/vesicular cristae
- Lipofuscin granules
- ↑ Lysosomes
- ↓ lipid droplets
- ↑ smooth endoplasmic reticulum and rough endoplasmic reticulum

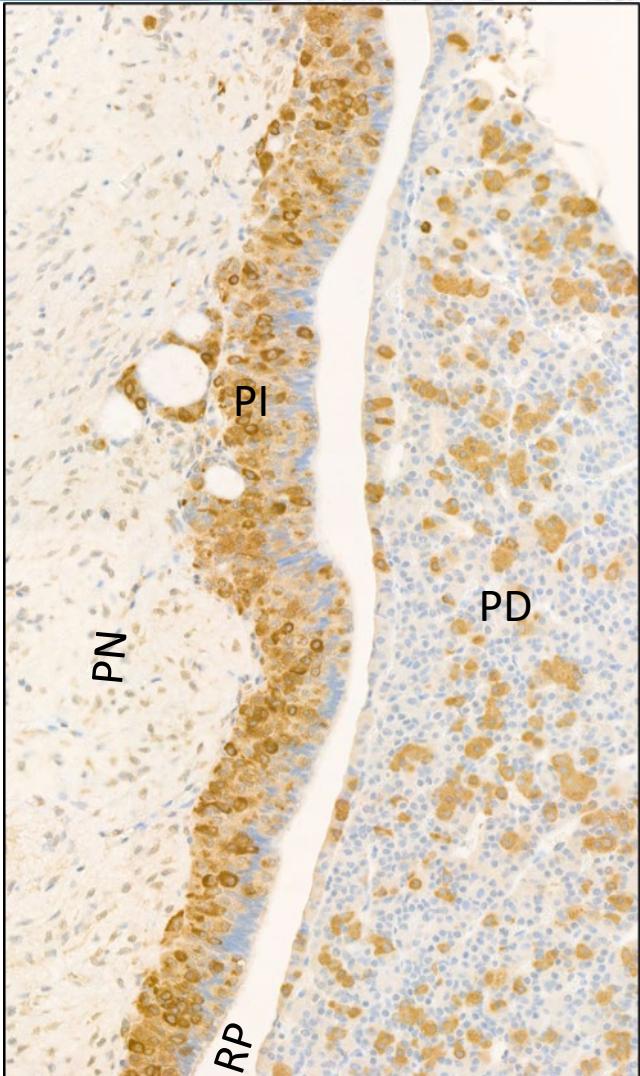




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## Adrenal Cortex: Physiology

- Adrenocorticotropic hormone (ACTH, corticotropin):
  - Synthesized in pituitary corticotrophs (i.e., pars intermedia and pars distalis)
  - Regulates fetal adrenal development and adrenal function in adult animals
  - ACTH works via the ACTH receptor (ACTHR) → stimulates synthesis and secretion of glucocorticoids, mineralocorticoids, and adrenal androgen (Kater et al. 1989)
- ACTH Receptor (ACTHR):
  - Guanine nucleotide binding protein (G protein)-coupled receptor (GPCR)
  - Expressed in the plasma membrane of adrenocortical cells in all three zones
  - ACTHR expression levels vary among species (Xia and Wikberg 1996; Reincke et al. 1998; Müller et al. 2001)
- ACTH signaling via ACTHR induces the proliferation of adrenocortical cells (Imai et al. 1990).
  - Chronic ↑ secretion of ACTH → bilateral, diffuse hyperplasia of the adrenals (Bland et al. 2003)
  - The primary site for mitogenic action of ACTH is the ZG.
  - The outer fasciculata is the primary adrenal zone responsible for compensatory growth, though proliferating cells can be observed in the ZG in the early stages (Engeland et al. 2005).





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## Steroidogenesis in the Adrenal Cortex

- Mineralocorticoid Pathway:
  - Cholesterol → Pregnenolone → Progesterone → 11-Deoxy-corticosterone → Corticosterone → 18-Hydroxy-corticosterone → **Aldosterone** [i.e., major circulating mineralocorticoid; final conversion in ZG mitochondria by Aldosterone Synthase (CYP11B2)]
- Glucocorticoid Pathway:
  - Human, monkey, and dog: Cholesterol → Pregnenolone → 17 $\alpha$ -Hydroxy-Pregnenolone → 17 $\alpha$ -Hydroxy-progesterone → 11-Deoxycortisol → **Cortisol** [i.e., final conversion in ZF/ZR mitochondria by steroid 11 $\beta$ -hydroxylase (CYP11B1)]
  - Rodents and rabbits: Cholesterol → Pregnenolone → Progesterone → 11-Deoxycorticosterone → **Corticosterone**
- Androgen Pathway (not present in rodents):
  - Cholesterol → Pregnenolone → 17 $\alpha$ -Hydroxy-Pregnenolone → Dehydroepiandrosterone → **Dehydroepiandrosterone Sulfate** (i.e., DHEA, major circulating cortical androgen; final conversion in ZR by P450c17 which also possesses 17, 20-lyase activity)

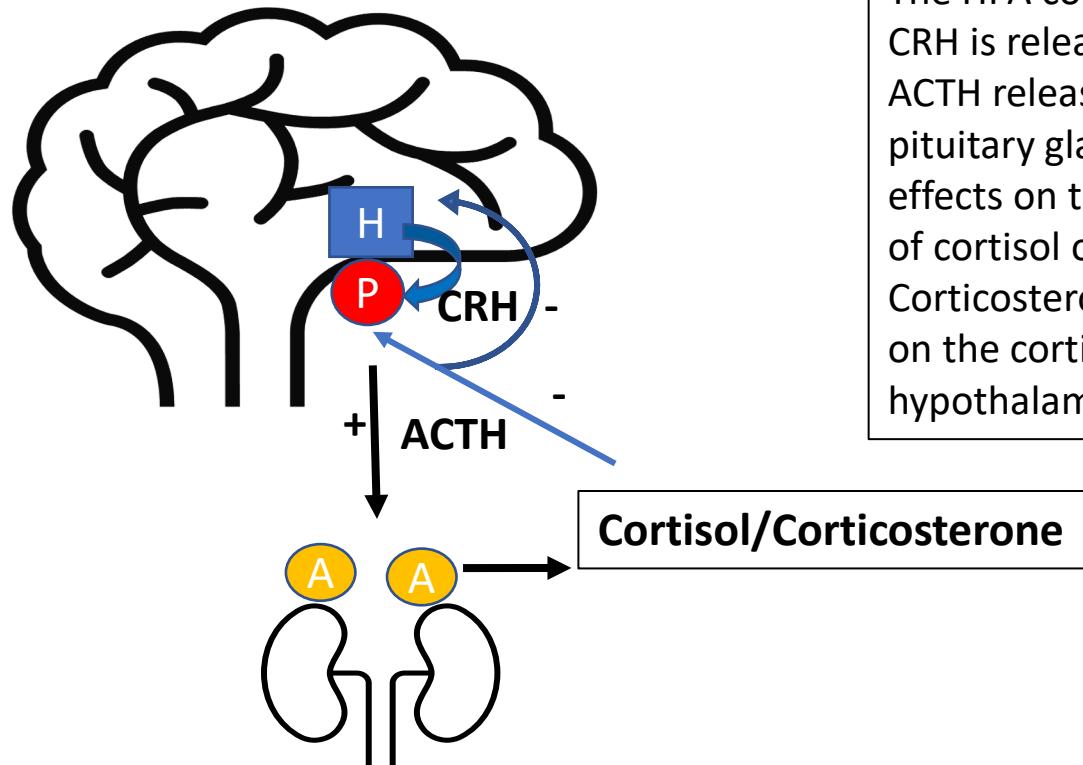


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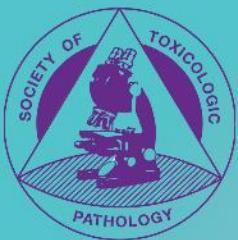
## Adrenal Cortex: Hypothalamus/Pituitary/Adrenal (HPA) Axis

### HPA Disruption:

- ACTH-dependent
  - Pituitary neoplasia ( $\downarrow$  or  $\uparrow$  ACTH)
- Corticosteroid-dependent
  - Adrenal neoplasia ( $\downarrow$  or  $\uparrow$  cortisol)
- Disruption of steroidogenesis
- Exogenous corticosteroids
- Other xenobiotics (e.g., Valproic Acid)
- Stress



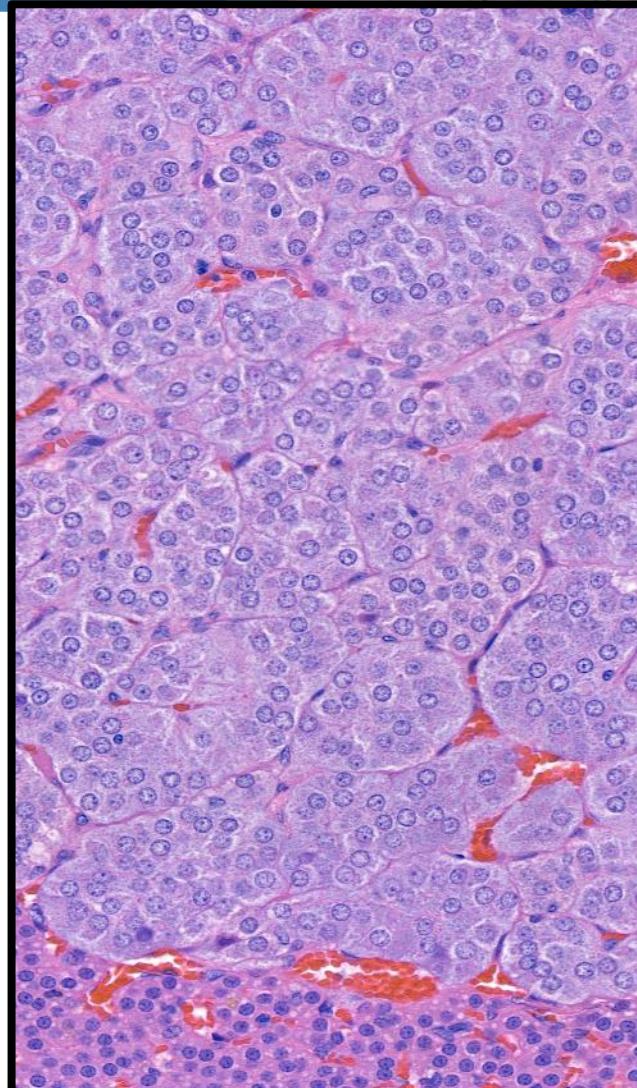
The HPA consists of the hypothalamus (**H**) where CRH is released into the circulation, eliciting ACTH release from corticotrophs in the pituitary gland (**P**). ACTH in circulation has trophic effects on the adrenal cortex (**A**) resulting in release of cortisol or corticosterone from the zona fasciculata. Corticosteroids in circulation have negative feedback on the corticotrophs in the pituitary and neurons in the hypothalamic paraventricular nucleus.



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## Adrenal Medulla

- The medulla comprises ~15% of the volume of the adrenal gland
- Neural crest-derived and consists of three types of cells (Carney 1992; Cormack, 1989; Tischler, 1977):
  - Chromaffin: In the rat: epinephrine (E) cells (the majority), norepinephrine (NE) cells, and small granule-containing cells (Pace et al. 2002; Rosol et al. 2001) The ratios of E/NE-type cells in young adult rats is approximately 4/1 (Tischler 1989)
    - Site of synthesis and storage of catecholamines
    - Derived from a sympathoadrenal neuroblast precursor
    - Express neuronal cytoskeletal proteins
    - Catecholaminergic
  - Neuronal (i.e., ganglion-like cells)
    - Derived from the same sympathoadrenal precursor as chromaffin cells
    - Express neuronal cytoskeletal proteins
    - Catecholaminergic
  - Sustentacular cells (stromal/supportive cells)
    - Stromal or supportive cells that are morphologically and functionally similar to Schwann and satellite cells.





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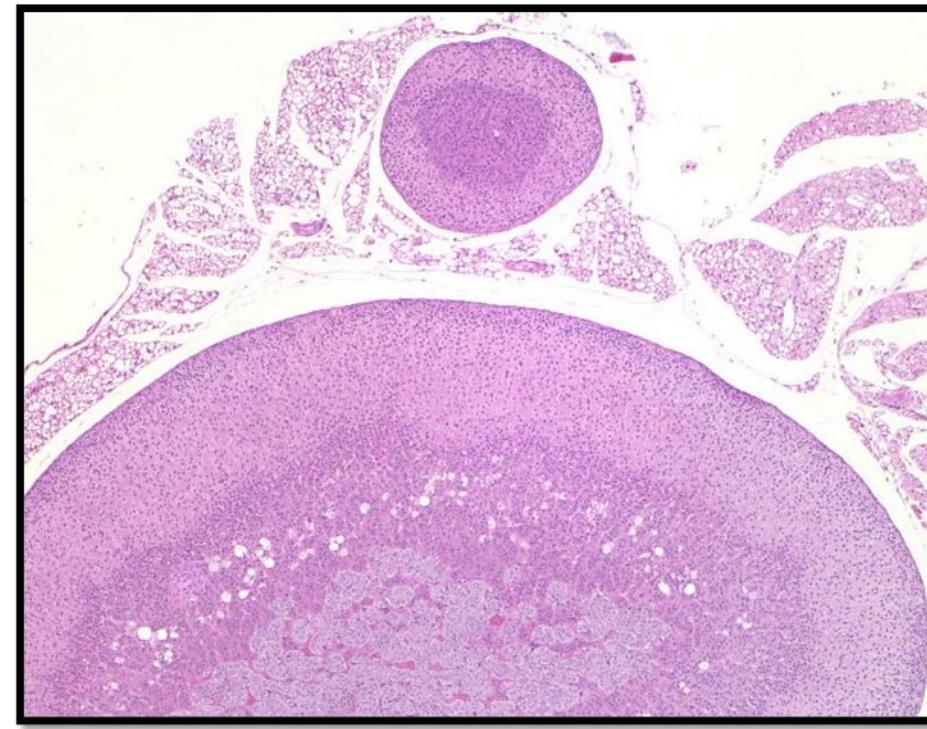
## Adrenal Medulla-Physiology

- Medullary hormones (catecholamines) include:
  - Adrenaline (epinephrine)
  - Noradrenaline (norepinephrine)
  - Dopamine
  - Neurotensin, neuropeptide Y (NPY), enkephalins, serotonin, and histamine are stored in same secretory granules as above (Tischler 1989)
- Catecholamine biosynthesis (in medullary chromaffin cells):
  - Tyrosine → 3,4-dihydroxyphenylalanine (dopa) by tyrosine hydroxylase (TH; rate-limiting step)
  - Dopa is decarboxylated to form dopamine
  - Dopamine is hydroxylated to produce noradrenaline
  - Noradrenaline is methylated to adrenaline by phenylethanolamine N-methyltransferase (PNMT)
- Adrenaline and noradrenaline stored in secretory vesicles in cells of the adrenal medulla and peripheral nerves. The vesicles in the medulla release adrenaline into the blood
- Catecholamines bind to receptors in organs and blood vessels → activate intracellular signal pathways via membrane bound G proteins → ↑ glucose and free fatty acids → ↑ basal metabolic rate, muscular perfusion, cardiac contractility, heart rate, and blood pressure
- Catecholamine secretion controlled by sympathetic innervation and acutely triggered by stress, trauma, and shock, as well as by fasting, hypoxia, hypoglycemia, or pharmacologically active substances such as nicotine, reserpine, or retinoic acid.
- The cardinal symptom of acute and chronic release of catecholamines is ↑ blood pressure



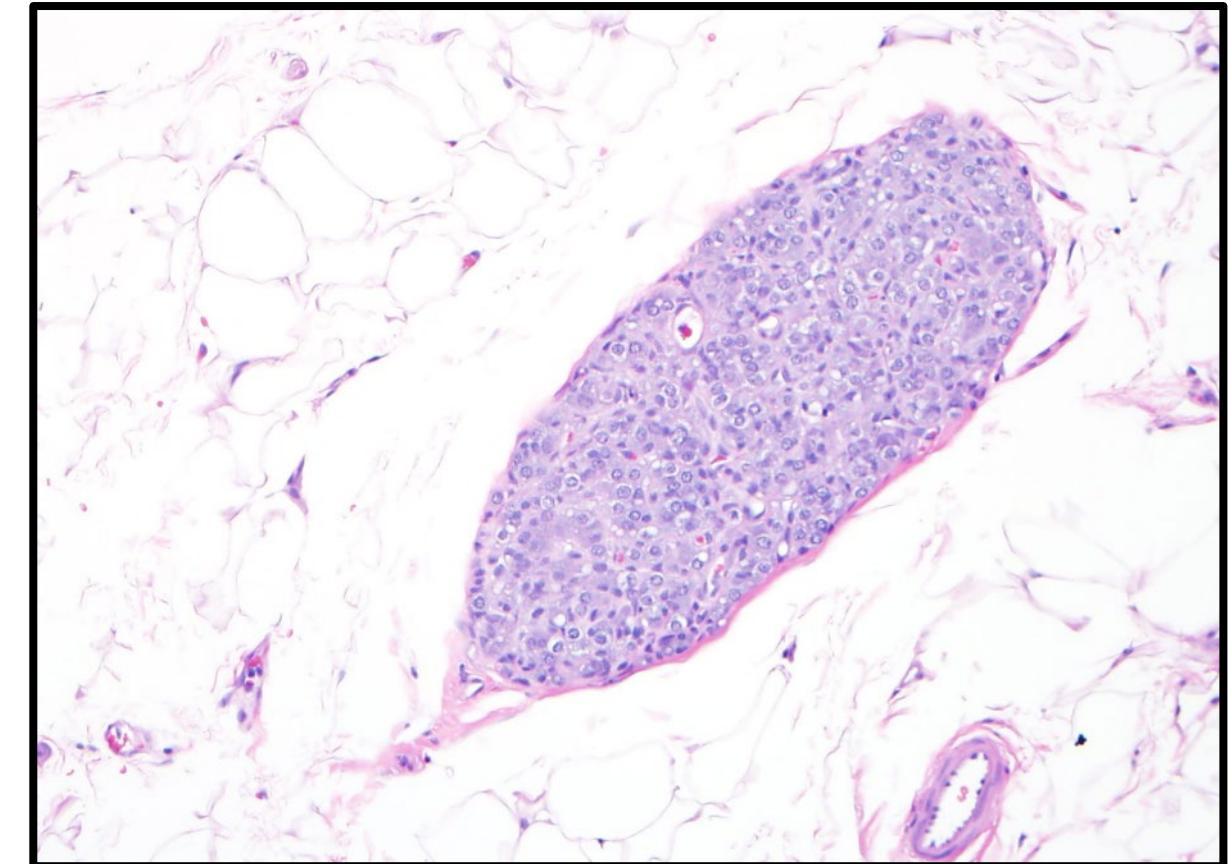
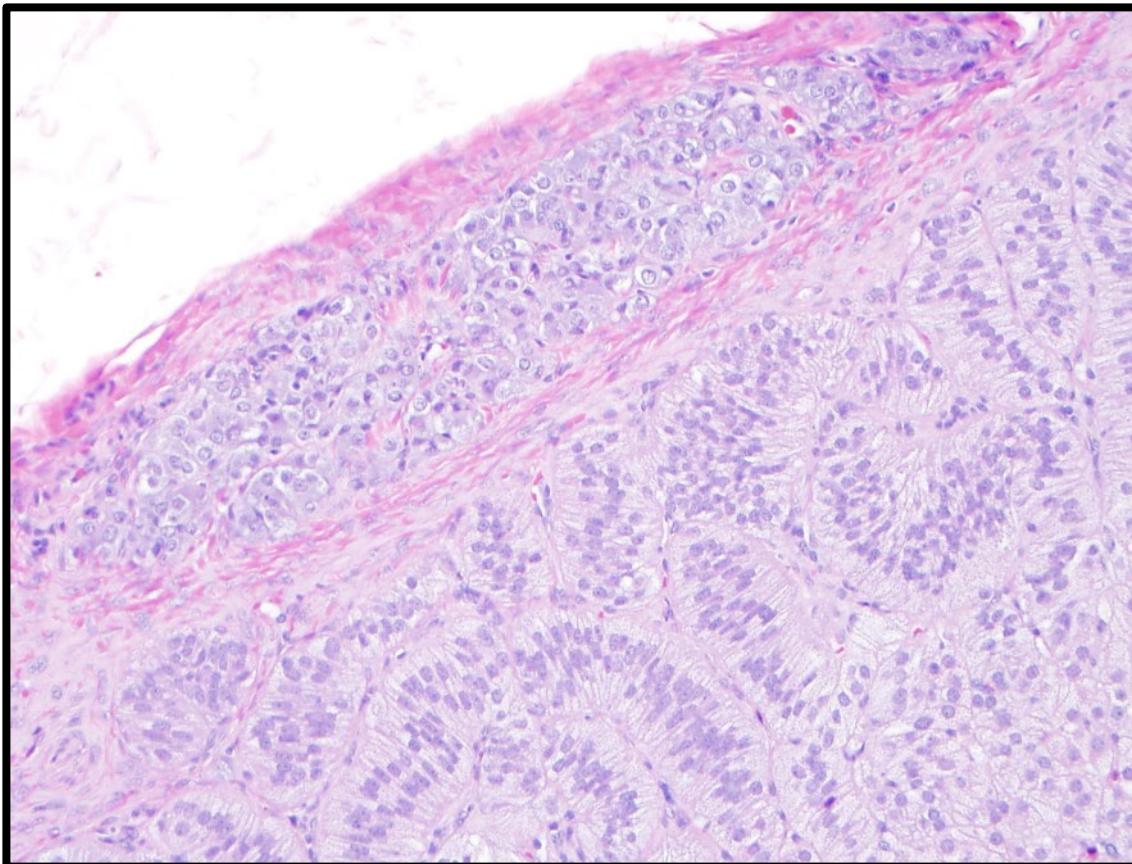
## Incidental Findings in the Adrenal Gland of Laboratory Animal Species

## Accessory (i.e., ectopic) Adrenocortical Tissue



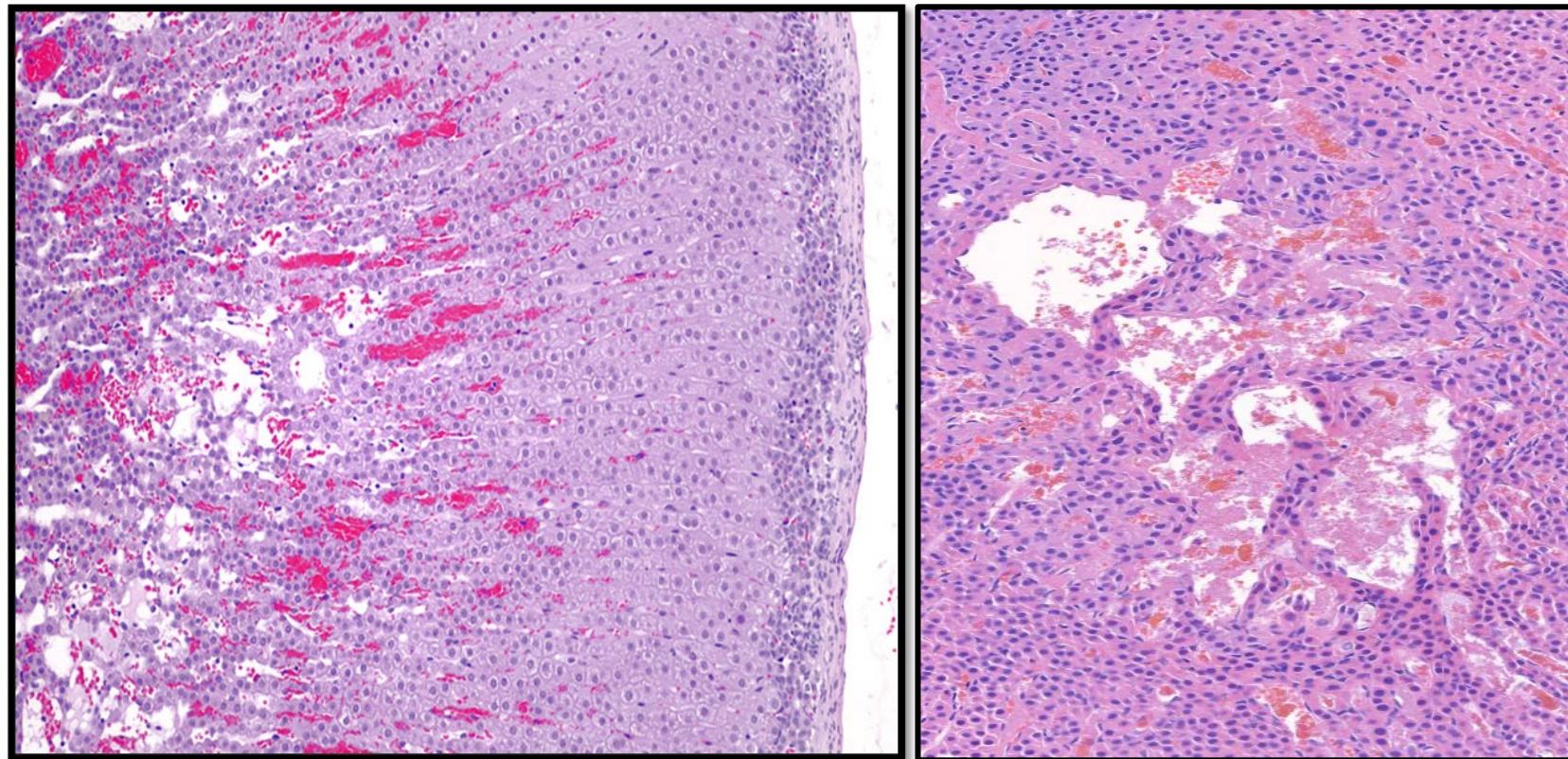
- Presence of adrenocortical tissue outside the adrenal capsule or in the periadrenal tissue (Brandli-Baiocco et al. 2018)
- Composed of normal cortex, either detached or attached to the adrenal gland but with a complete fibrous capsule
- Lack the distinct zonal arrangement of the adrenal cortex and are devoid of medullary tissue

## Accessory (i.e., ectopic) Adrenomedullary Tissue



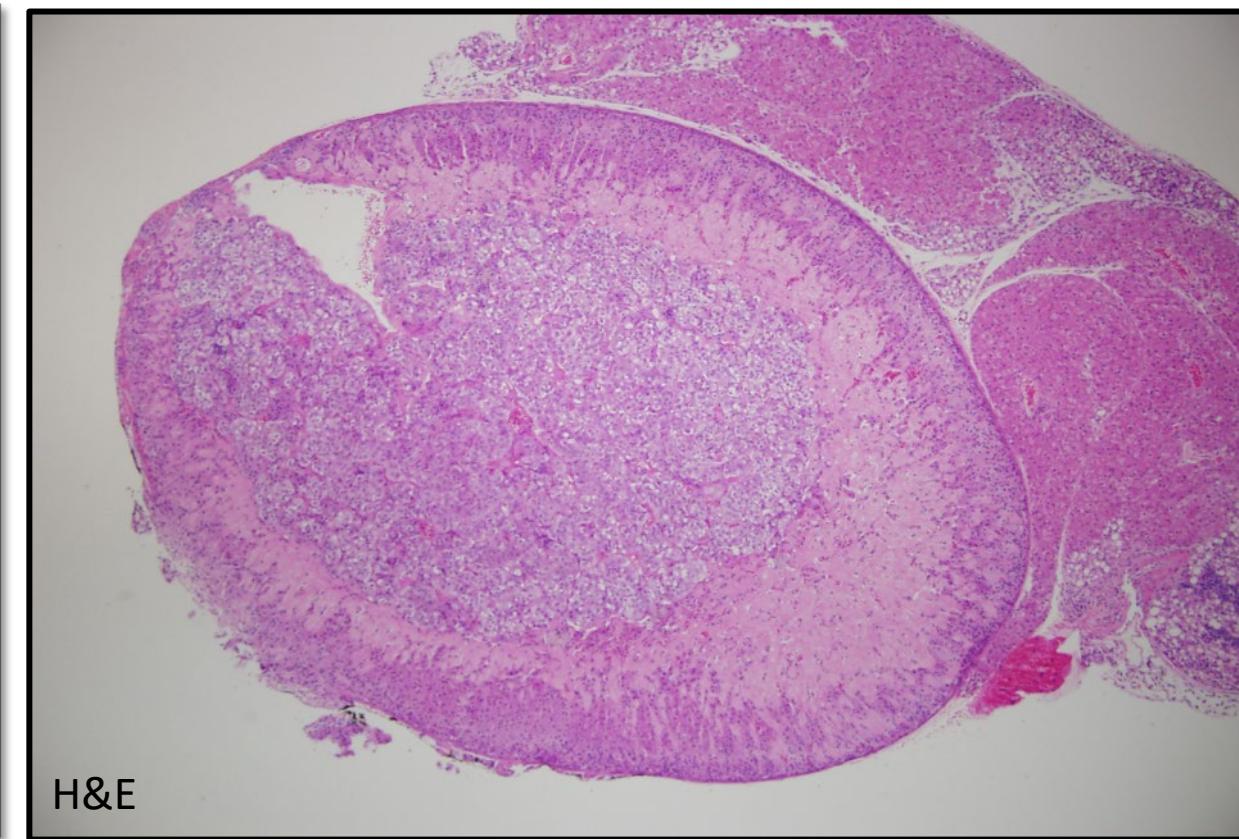
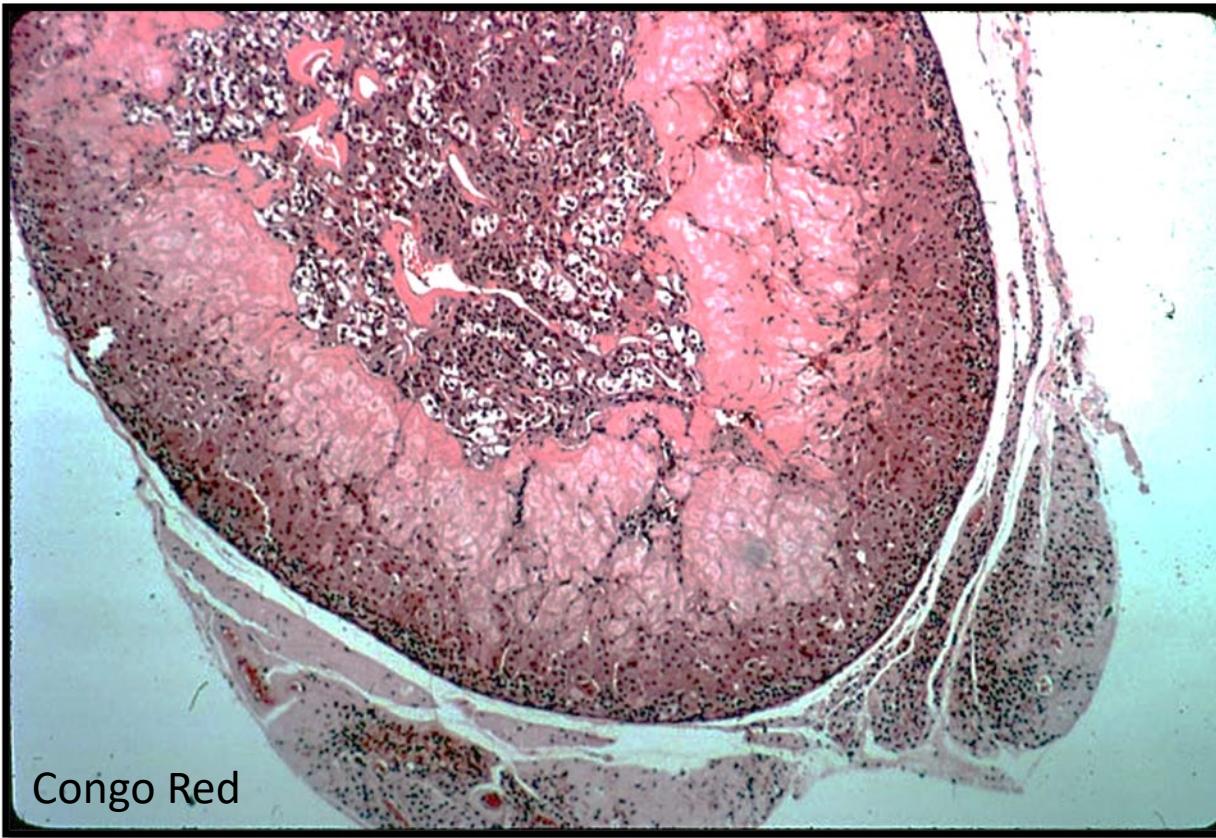
- Presence of adrenomedullary tissue outside the adrenal capsule or in the periadrenal tissue
- Composed of normal medulla, either detached or attached to the adrenal gland but with a complete fibrous capsule

## Angiectasis, cortical



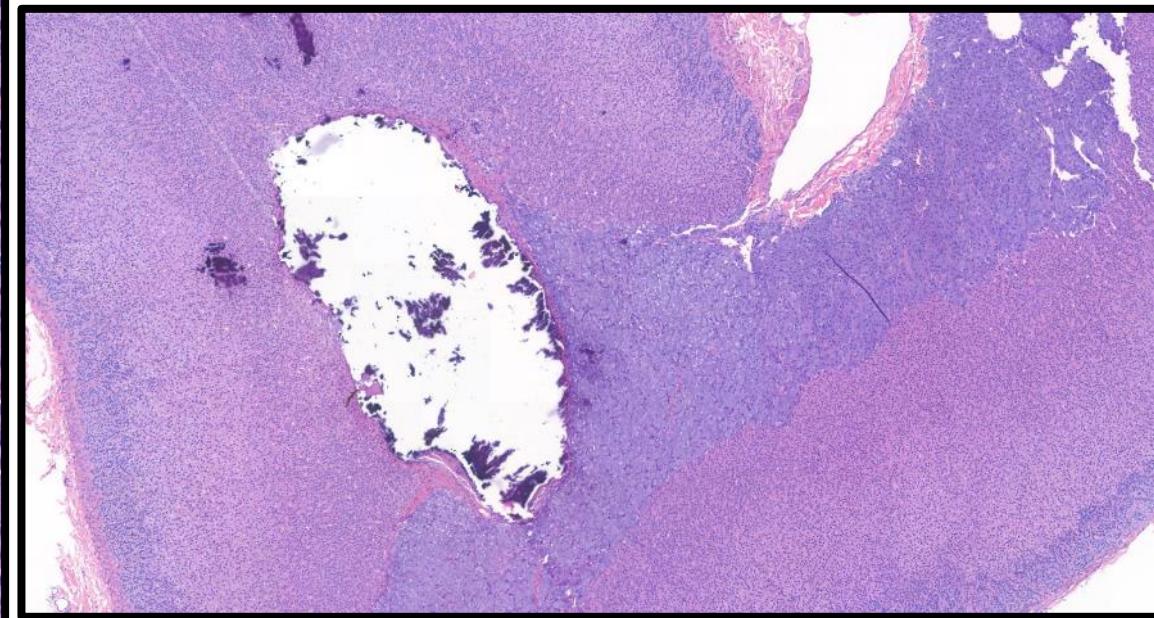
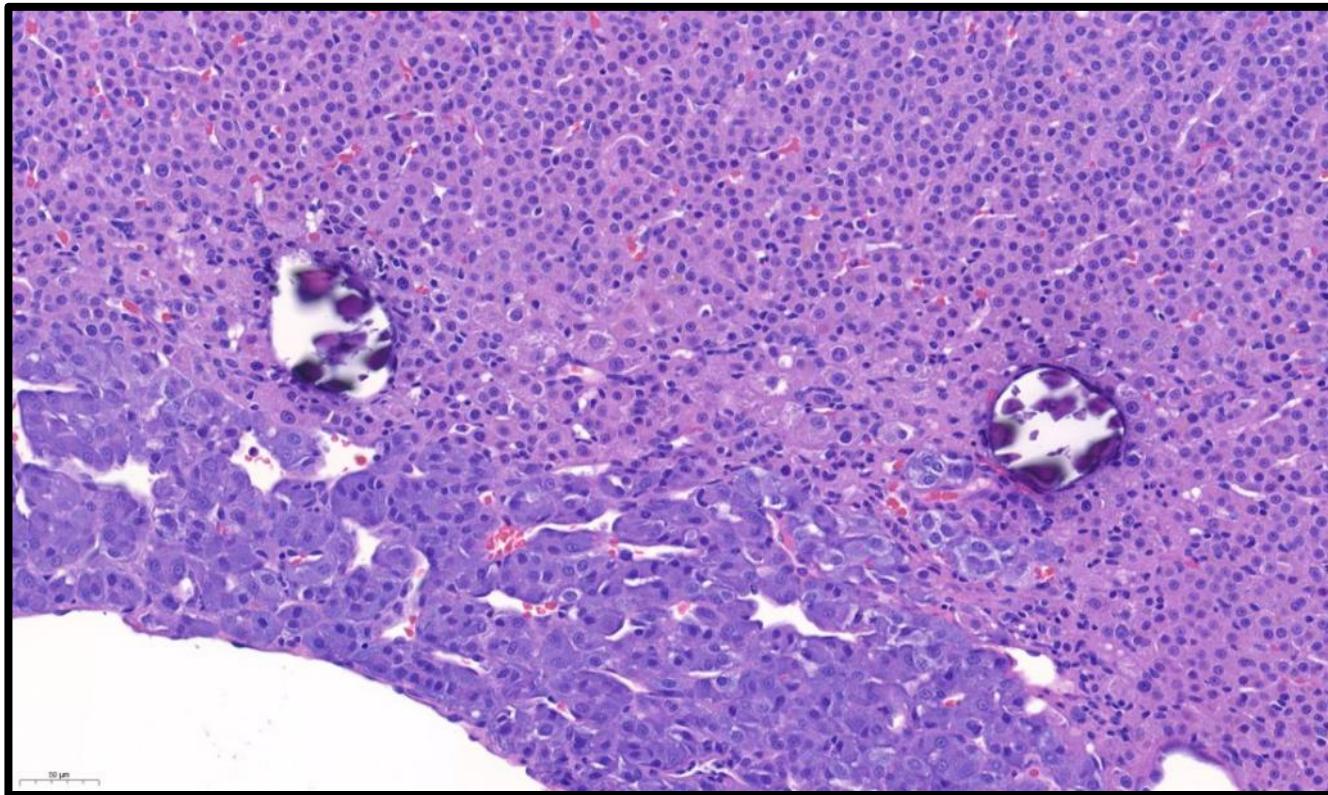
Aged rats and mice develop cortical telangiectasis secondary to parenchymal cell loss resulting in marked dilatation of cortical capillaries (Brandli-Baiocco et al. 2018). This is a common lesion in aging female rats, whereas it is rarely found in mice (Frith et al. 2000).

# Amyloidosis



Systemic amyloidosis is commonly seen in aging mice of several strains (A, L, C3H, C57, and CBA), and frequently involves the adrenal gland. In contrast, adrenal amyloidosis is rare in rats (Brandli-Baiocco et al. 2018). In F344 rats, it has been reported in the absence of generalized amyloidosis. The deposits usually start in the zona reticularis and, in severe cases, may largely replace this zone (Nyska and Maronpot 1999).

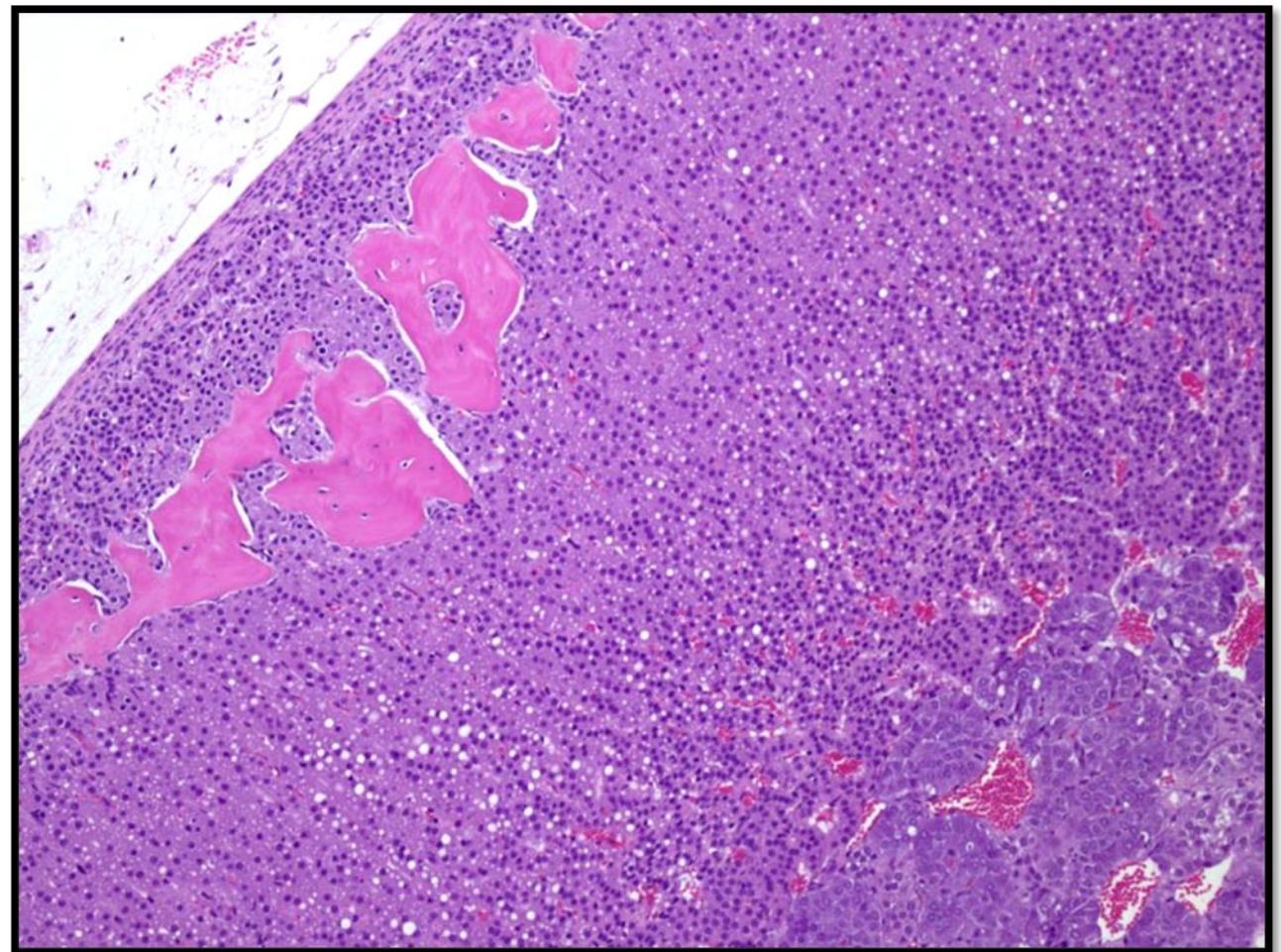
# Mineralization



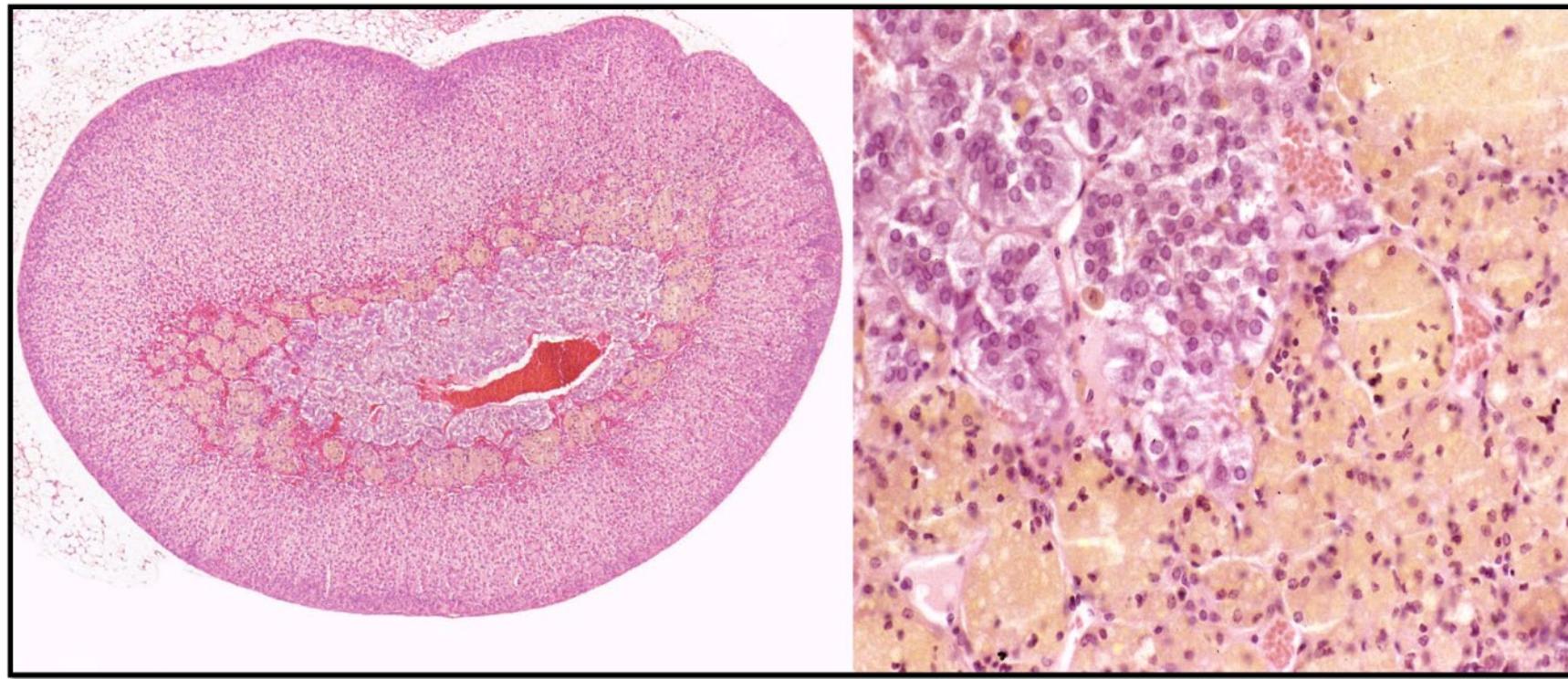
Located at the interface between the cortex and medulla in the cynomolgus monkey which likely represents dystrophic mineralization of remnants of the fetal zone of the adrenal (Brandli-Baiocco et al. 2018).

# Osseous Metaplasia

Focal metaplastic bone formation (i.e., lamellar or woven bone) ± EMH within the adrenal cortex considered a degenerative change  
(Brandli-Baiocco et al. 2018)

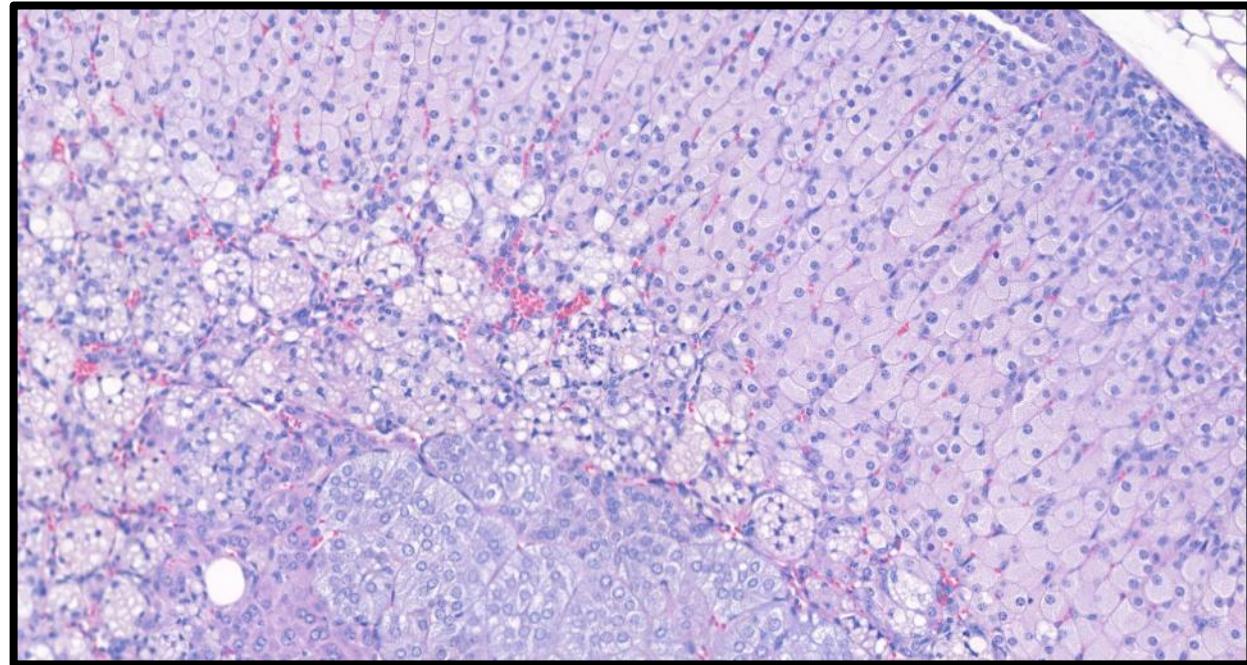
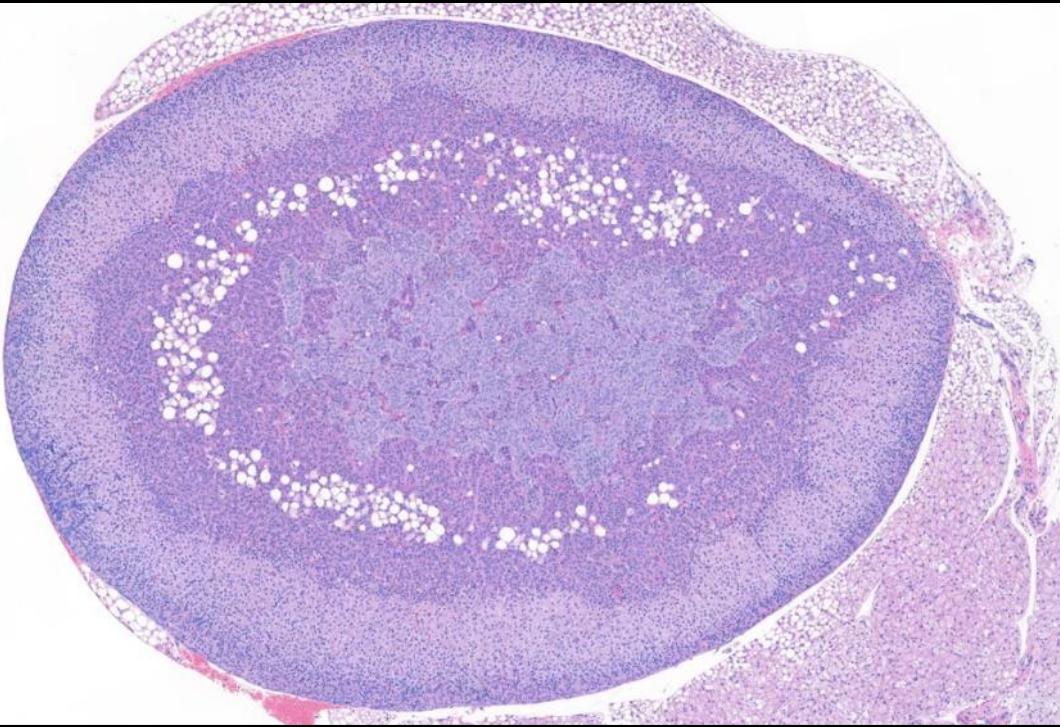


## Pigment



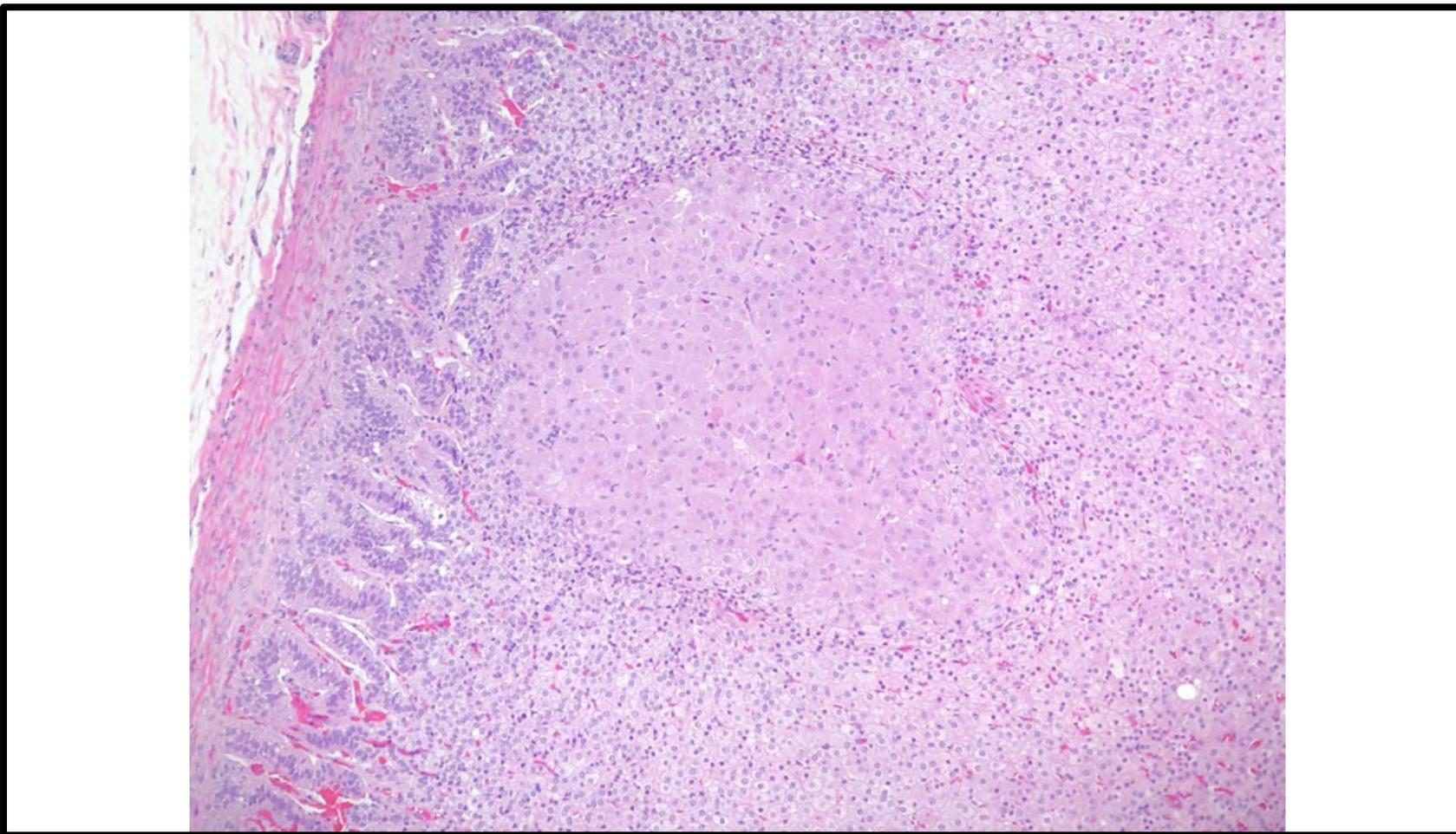
- Lipofuscin pigment deposition in adrenal cortical cells and ZR macrophages is commonly seen in aged mice and rats (Rosol et al. 2001). Lipofuscin deposition can be seen in younger rats and indicate excessive cellular organelle turnover or defective cell metabolism or seen with severe hormone-induced atrophy.
- In mice, ceroid pigment accumulation is prominent in the degenerating X-zone at the corticomedullary interface.
- In aged hamsters, dense aggregates of ceroid pigment may also accumulate at the corticomedullary junction (Nickerson 1979).

## Persistent X-Zone



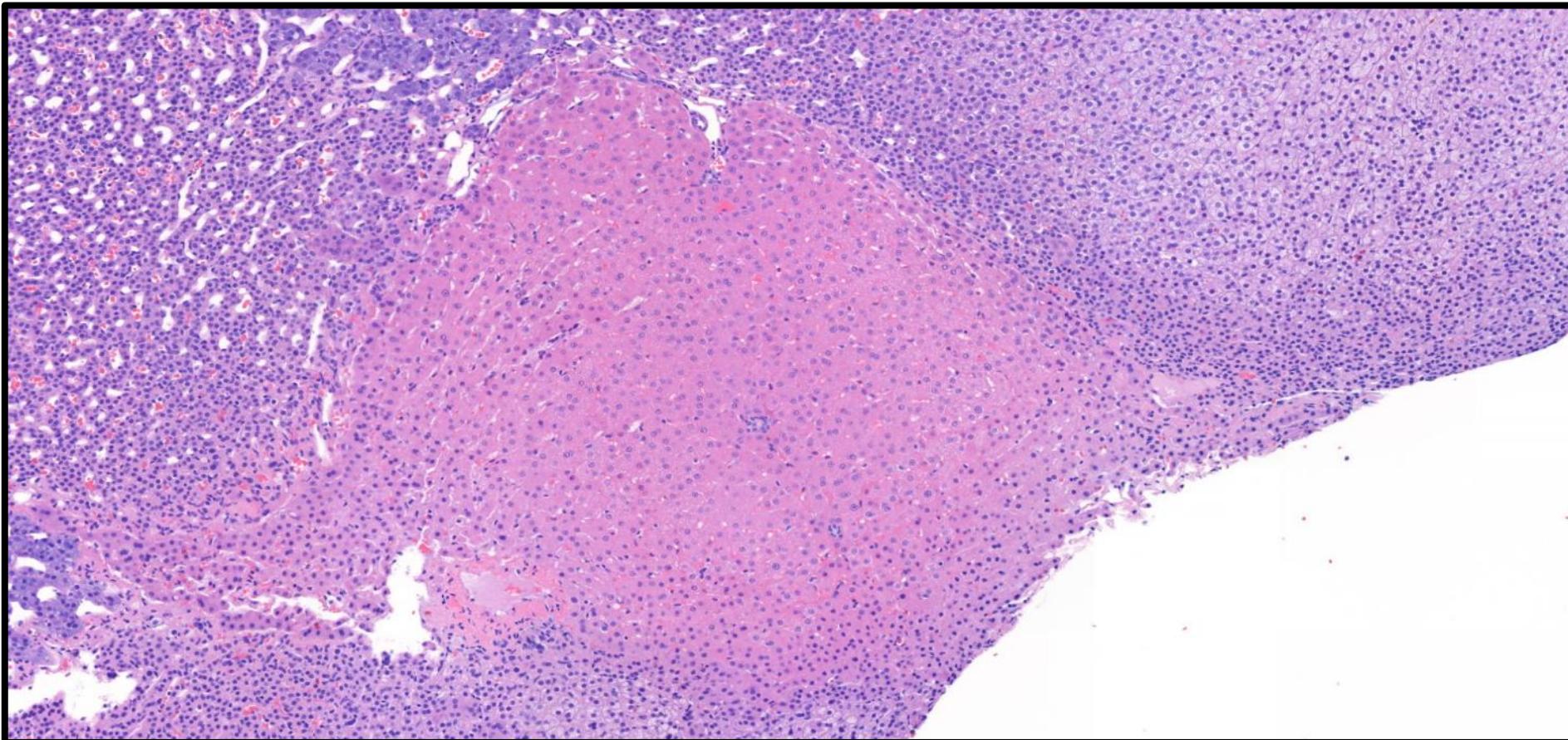
A specific feature of the mouse and rabbit adrenal cortex is the so-called X-zone, a putative post-partal remnant of the fetal adrenal zone located at the junction of the cortex and medulla. In males, this zone disappears rapidly with the approach of puberty (5 weeks), whereas in females, it continues to increase in size to reach a maximum at about 9 weeks and gradually regresses once they reach sexual maturity. There is delayed disappearance of the adrenal X-zone in obese hyperglycemic mice, probably related to hypogonadism (Naeser 1975).

Hypertrophy, cortical, focal

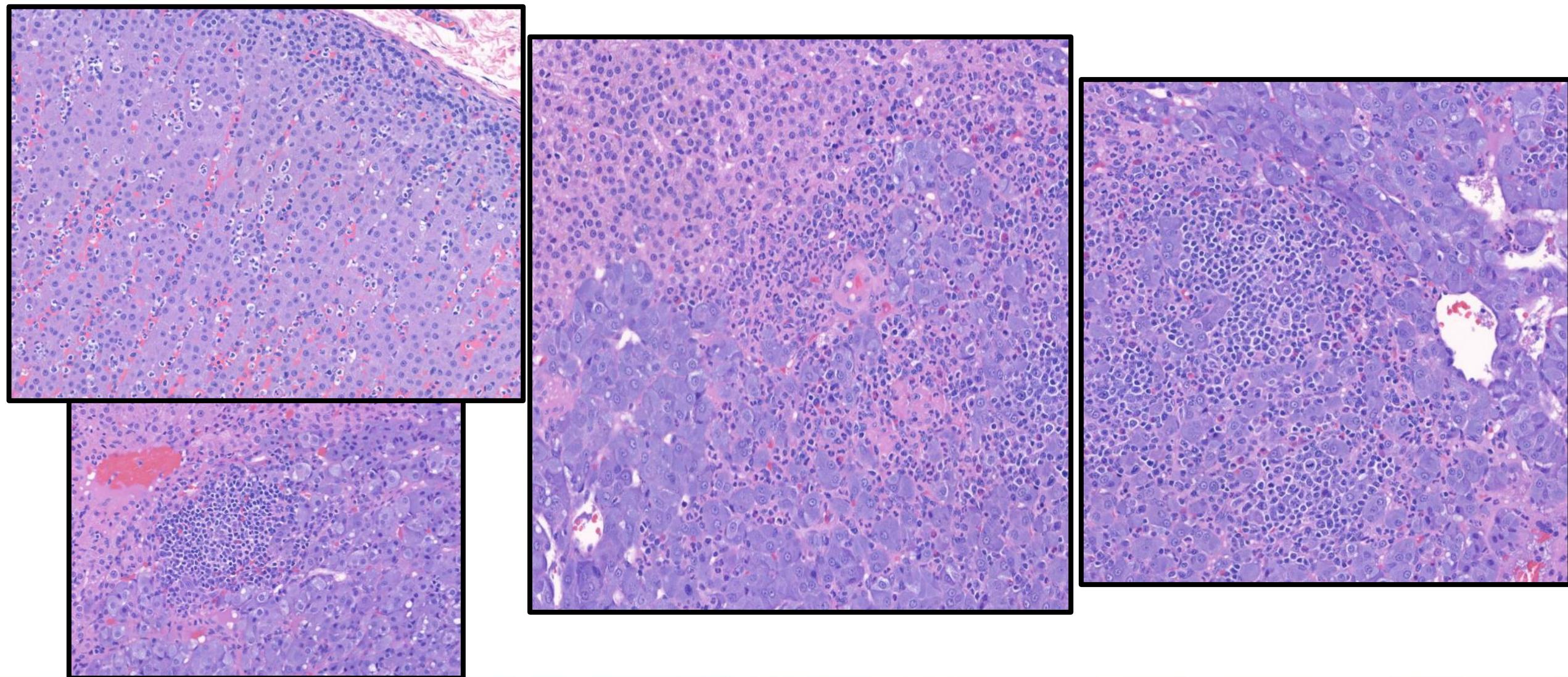


Vacuolation, cortical, decreased, focal

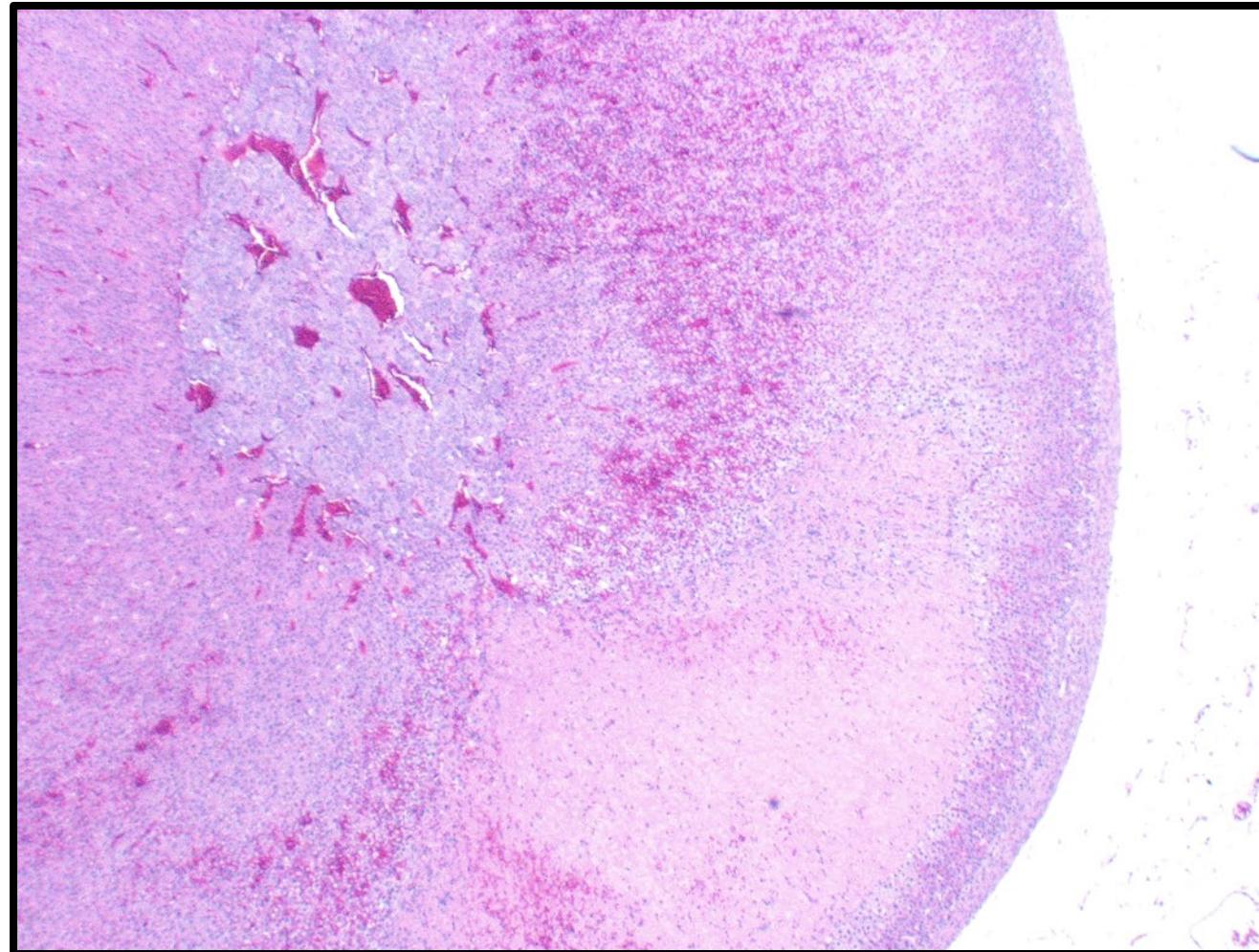
- ZF, ↓cytoplasmic vacuoles, no compression of adjacent tissue



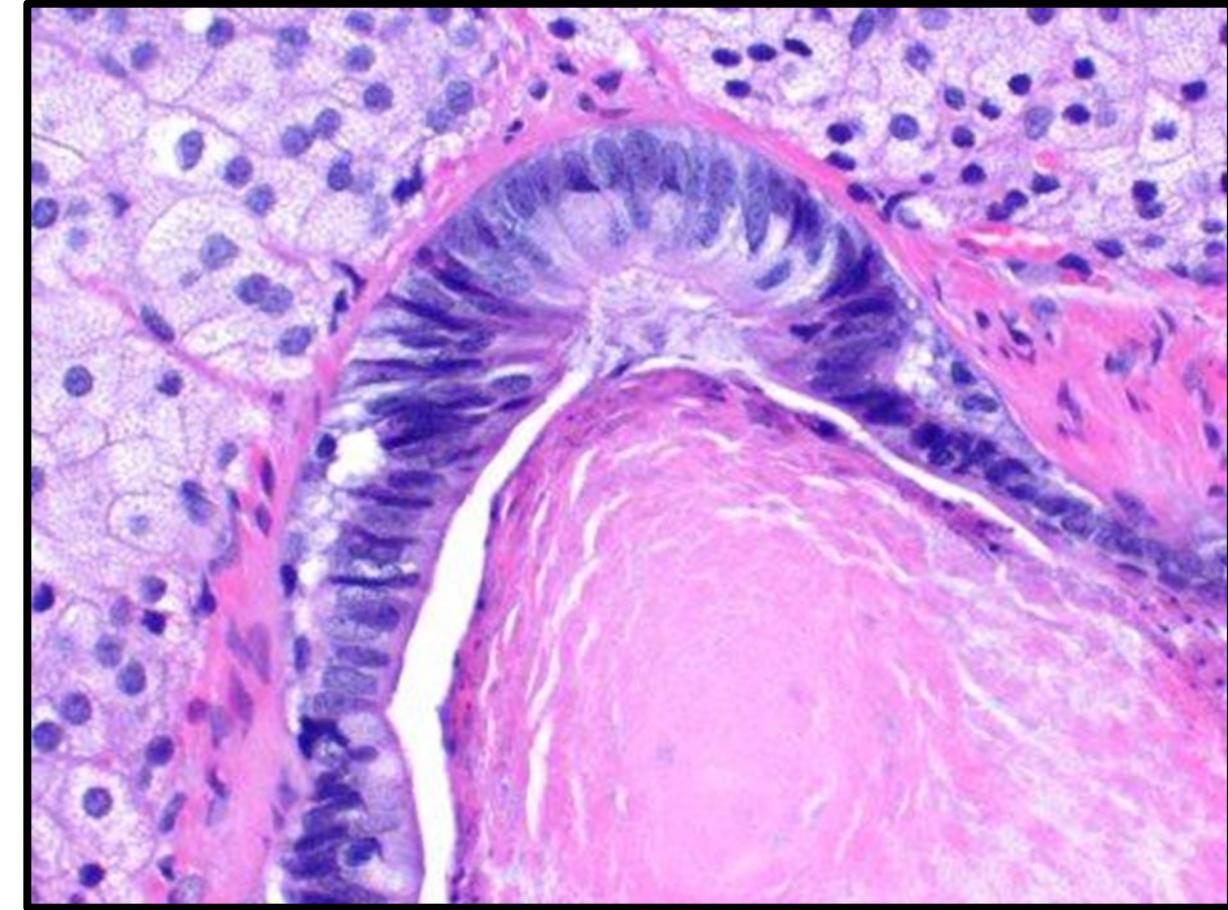
## Infiltrate, inflammatory cell *versus* Inflammation *versus* Extramedullary Hematopoiesis

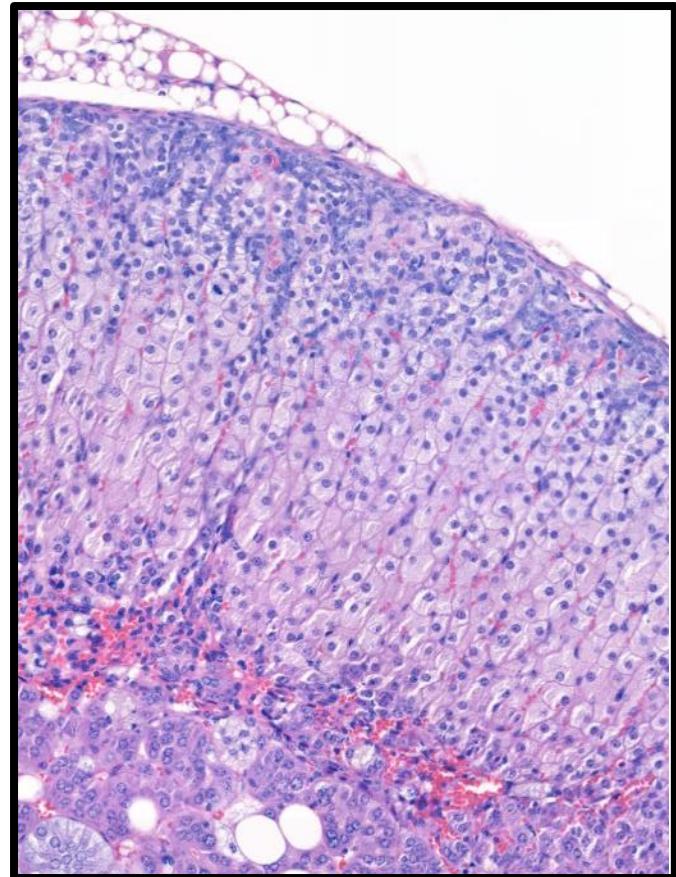
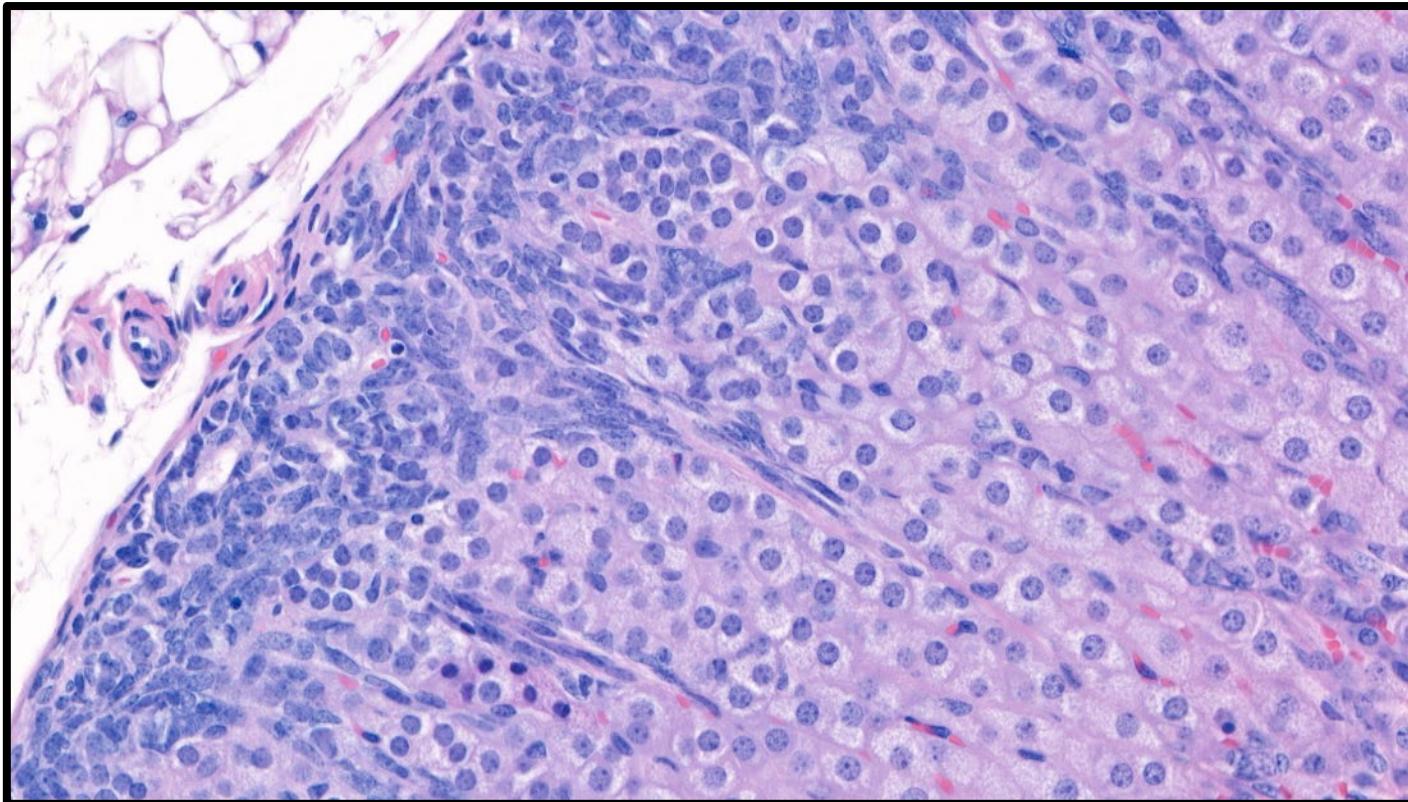


Infarction, cortical: coagulative necrosis, secondary to thrombosis,  
“wedge”-shaped area of necrosis



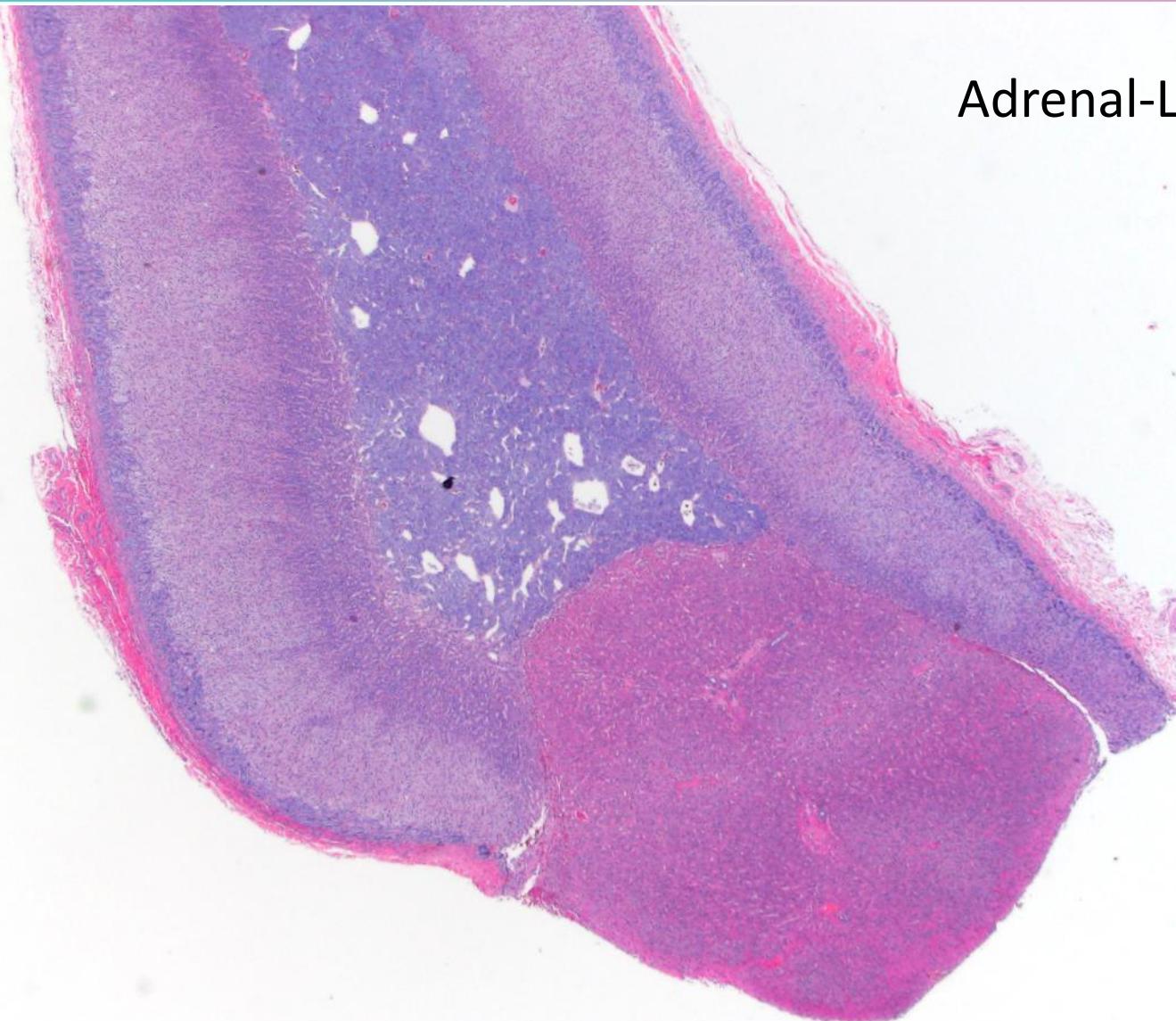
## Adrenal Cyst: developmental anomaly



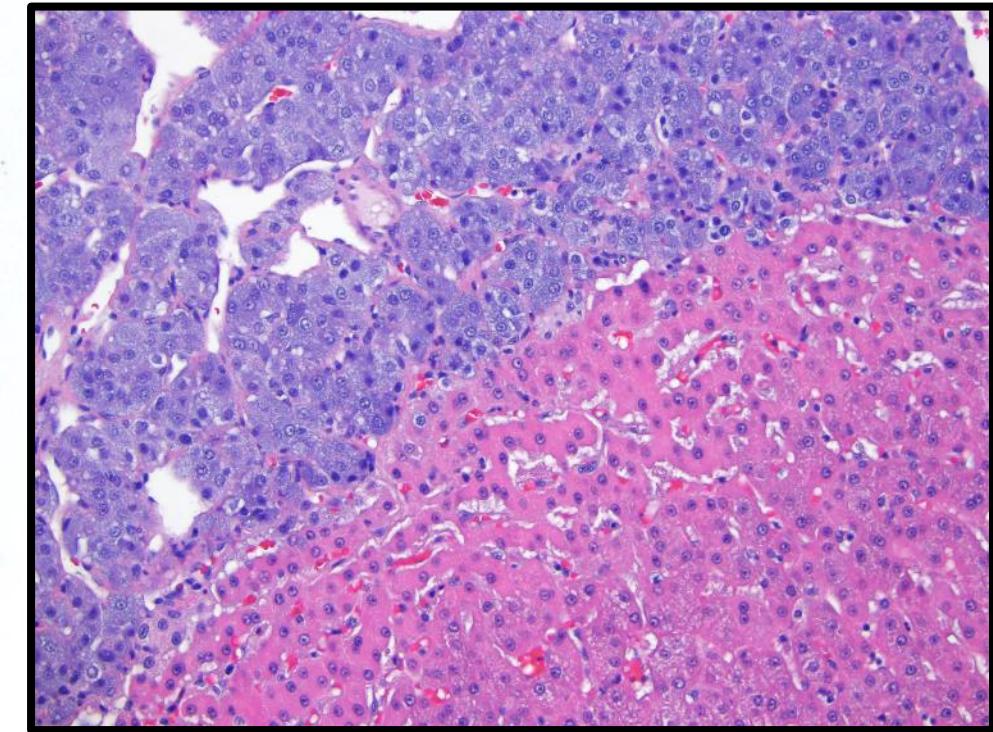


Subcapsular cell hyperplasia (spindle cell hyperplasia)

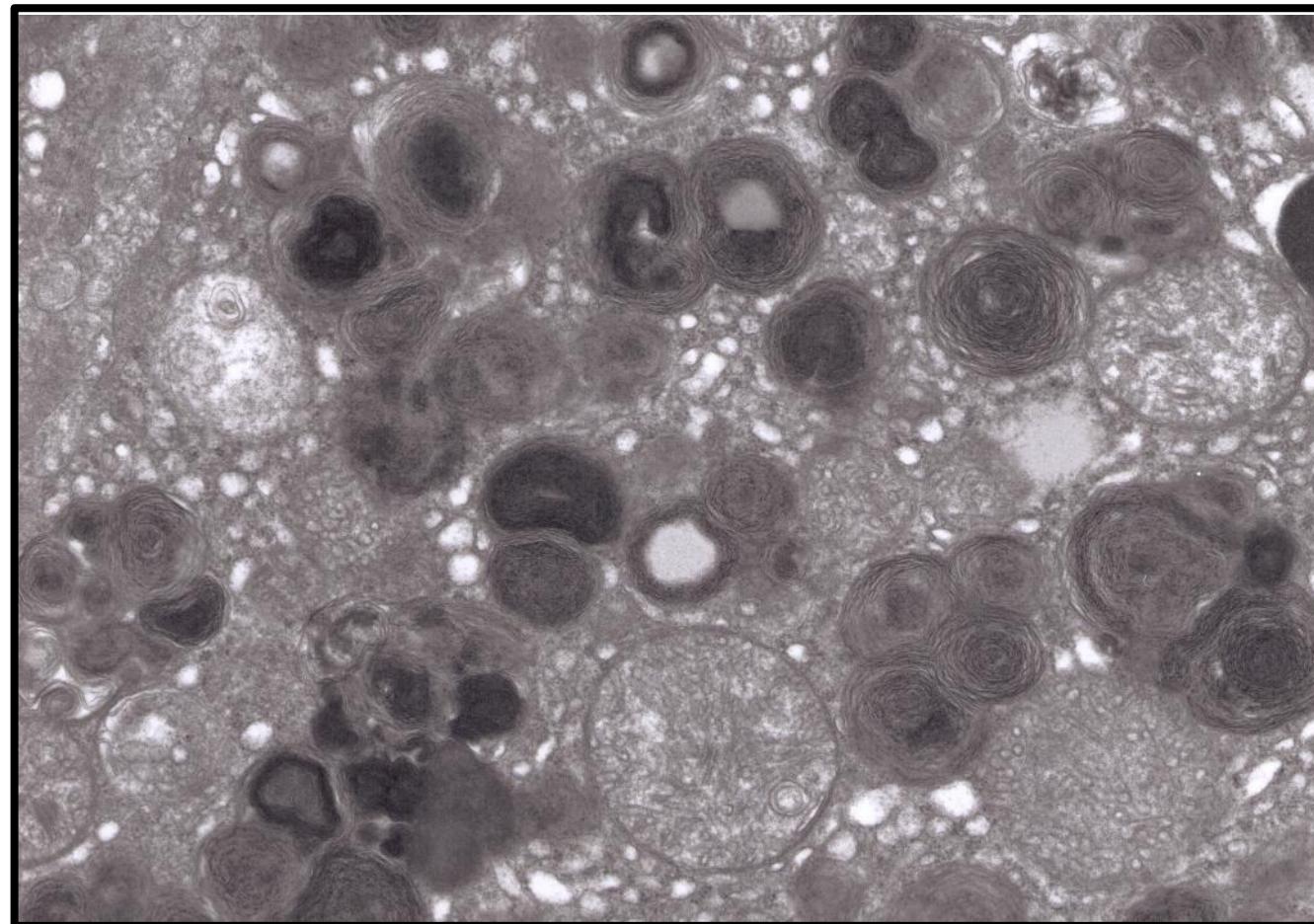
Age-related focal or diffuse change in mice. The function of the spindle cells is unknown. These hyperplastic foci are composed of spindle cells (type A) or polygonal cells (type B), or both. Develop as localized, wedge-shaped proliferations beneath the capsule and can become an extensive mass replacing much of the cortex. Subcapsular adenomas are derived from the same cell population in varying combinations (Nyska and Maronpot 1999).



Adrenal-Liver Fusion: developmental anomaly



Drug-Induced Phospholipidosis (PLD): EM = lysosomal multilamellar whorls; H&E = cytoplasmic vacuolation. Cationic-amphiphilic drugs (CAD) can bind to 1) lysosomal phospholipases and decrease enzyme activity → accumulation of phospholipid from cellular turn-over, 2) bind to membrane phospholipid and inhibiting phospholipase degradation, or in some cases 3) affect lysosomal degradation pathway regulation.





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