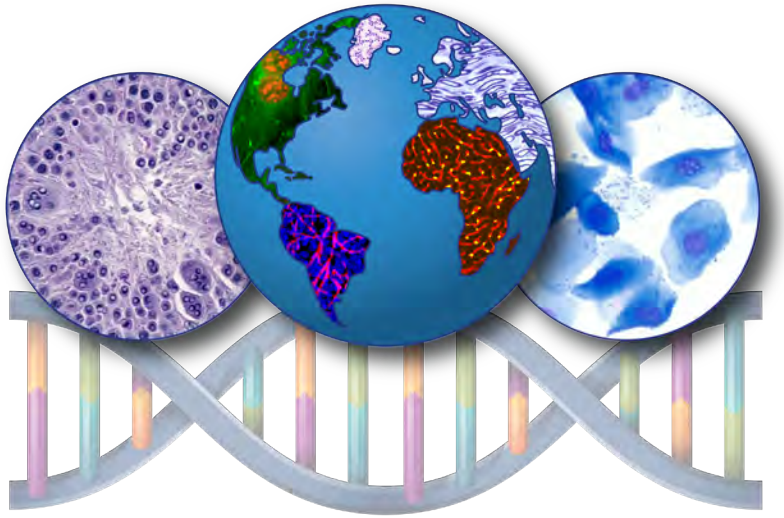
National Institute of
Environmental Health Sciences
Division of Translational Toxicology

Anatomy and Histology of the Normal Rodent Adrenal Gland



Division of Translational Toxicology Global Toxicologic Pathology Training Program

National Institutes of Health • U.S. Department of Health and Human Services

Overview

- Introduction
- Embryology and Development
- Anatomy and Histology
- Cutting and Trimming
- Hormones
- Blood Supply and Innervation
- X-Zone in Female Mice
- Congenital/Developmental Lesions
- Pigment
- Special Stains
- Practical Notes

Adrenal Gland: Introduction

Introduction

- First described in 1563 by Eustatius
- Importance was recognized by Thomas Addison in 1855
- Also referred to as the suprarenal glands
- Composed of outer cortex and inner medulla
- Cortex is essential for life, the medulla is not

Adrenal Gland: Embryology and Development (Rodents)

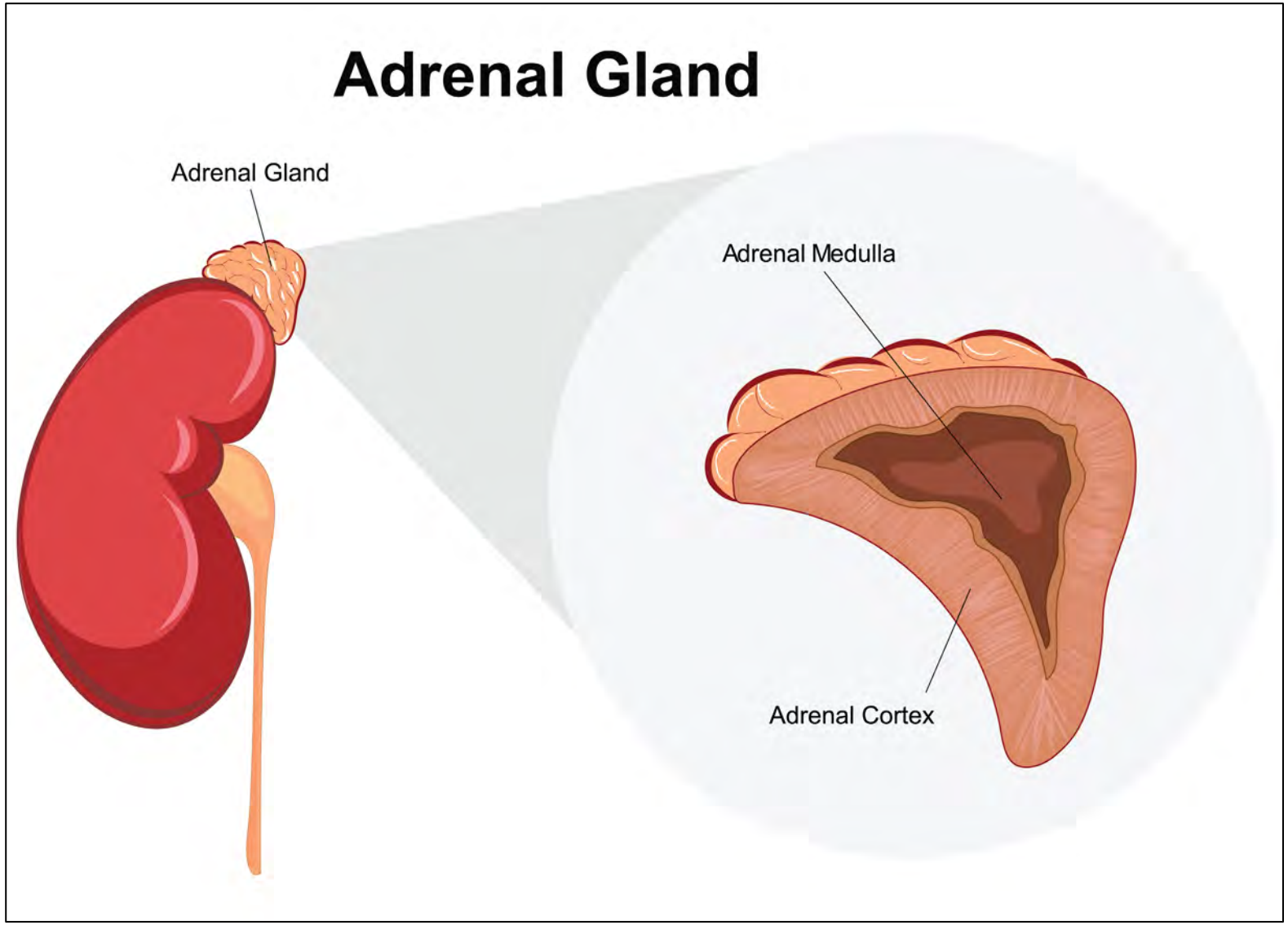
Embryology and Development

- The adrenal gland is composed of two embryologically distinct tissues: the cortex and the medulla
  - Cortex originates from cells within the mesoderm
  - Medulla is derived from ectodermal tissue of the neural crest
  - Both cortex and medulla are formed on gestation day (GD) 11 with development nearly complete at GD 15
  - Both cortex and medulla are fully functional at birth
- Although embryologically distinct, the two portions of the adrenal gland are functionally related

Adrenal Gland: Gross Anatomy

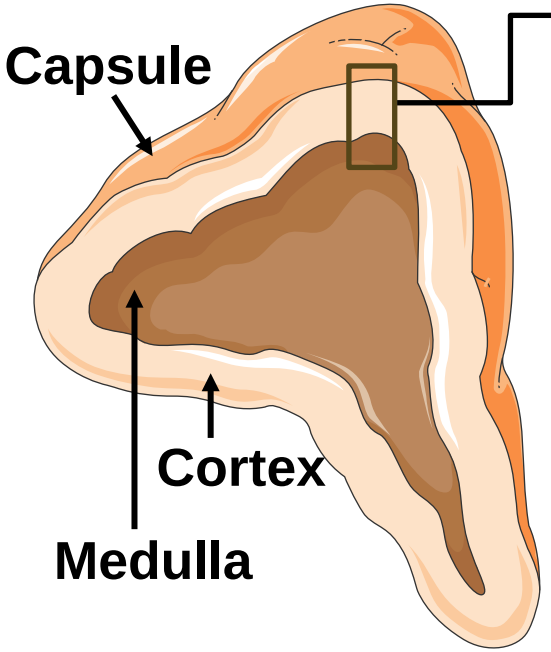
Gross Anatomy

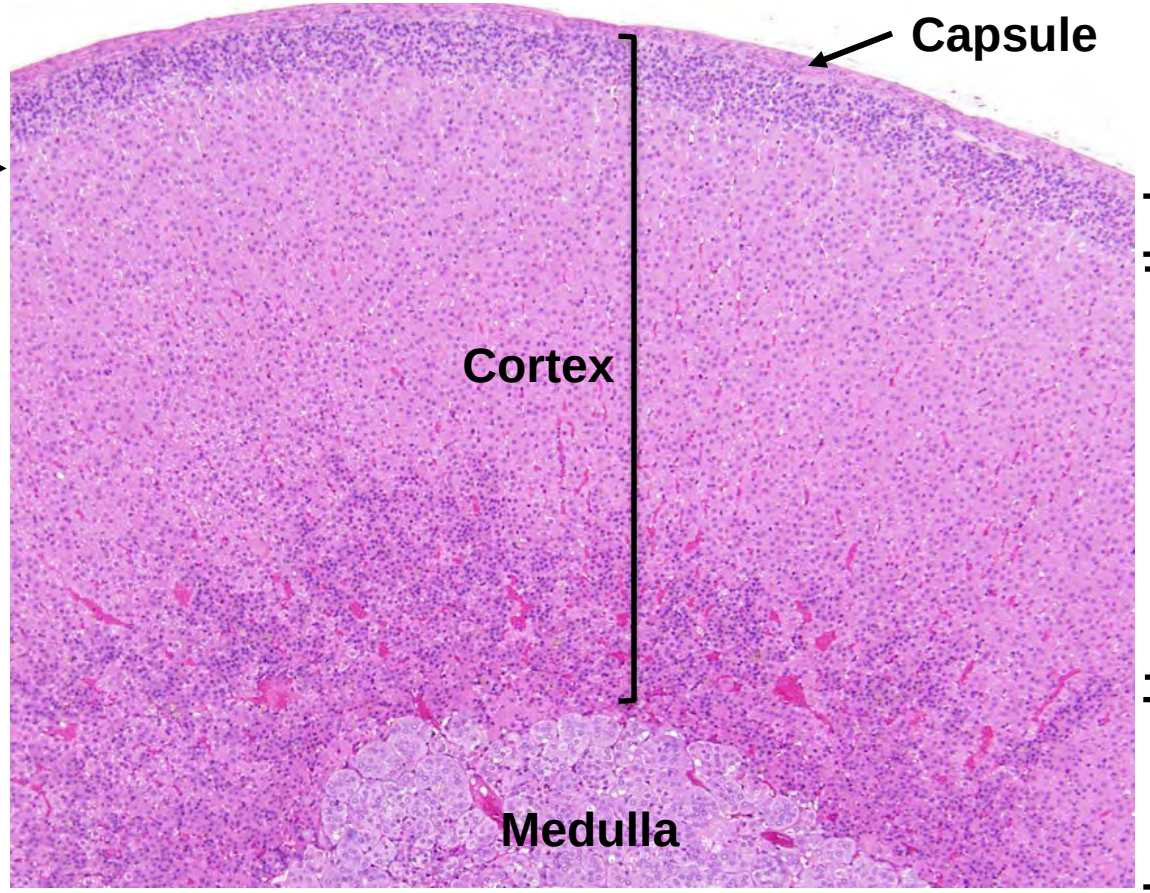
- Small, paired, yellowish organs located in the retroperitoneal space at the anterior pole of the kidneys
- In mice, growth and function of the adrenal glands are markedly influenced by gender and age
- In mice and rats, the adrenal gland of the female is significantly larger than that of the male
  - Relative difference varies among different strains
  - Adult female rats generally demonstrate increased sizes for all zones of the adrenal cortex



Adrenal Gland: Histology

Histology (Rat)



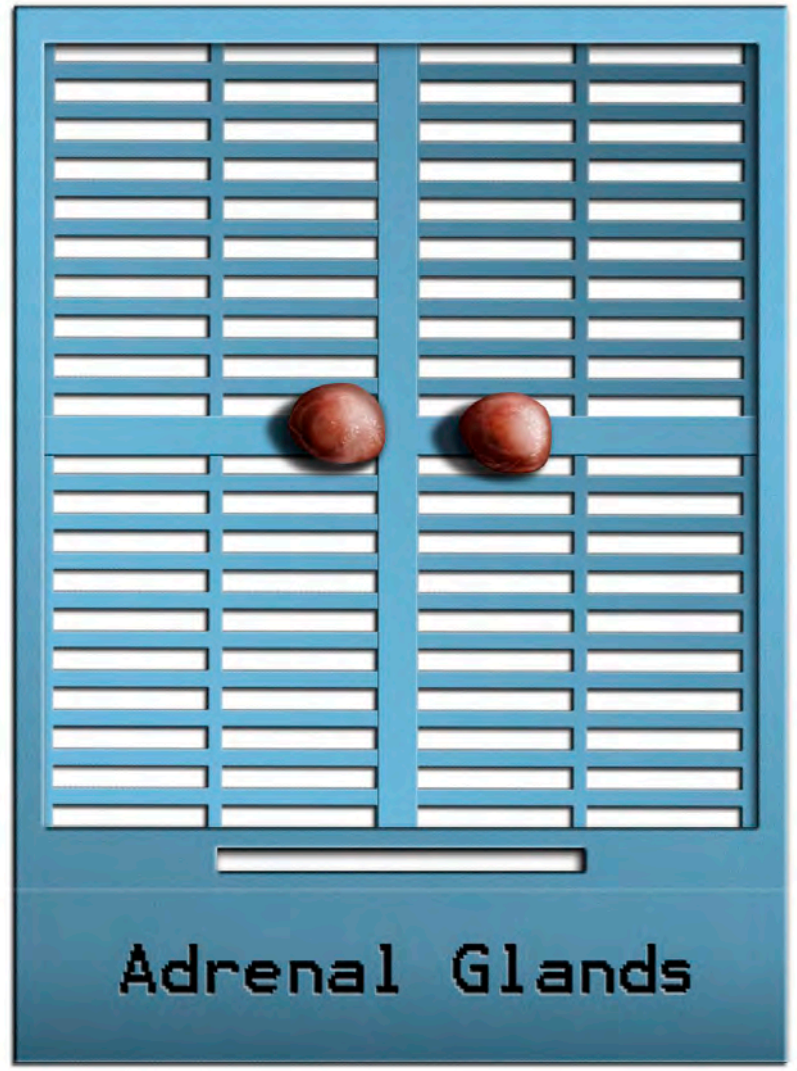


Zona glomerulosa	→	Mineralocorticoids (Aldosterone)
Zona fasciculata	→	Glucocorticoids (Cortisol, corticosterone)
Zona reticularis	→	Androgens (Dehydroepi-androsterone, DHEA)
Adrenal medulla	→	Catecholamines (Epinephrine, Norepinephrine)

Adrenal Gland: Cutting and Trimming

Cutting and Trimming

- The adrenal gland is harvested *in toto* (i.e., in total or altogether) at necropsy
  - Dissection can be challenging
  - Glands are small and can be difficult to locate especially in older animals that may have adjacent retroperitoneal fat
- Adjacent fat tissues should be carefully removed to avoid rupture of the adrenal capsule
- Because of their small size, mouse adrenals are typically embedded without bisecting for histological processing
- Bilateral adrenals are often embedded together



Adrenal Gland: Hormones

Steroid Hormones

- The adrenal cortex is a major site of steroid hormone production (steroidogenesis)
  - Derived from the precursor cholesterol through serial conversions catalyzed by steroid hydroxylases that belong to the cytochrome P450 (CYP) superfamily
- Mediate their actions through binding to nuclear receptors
- Steroid hormone secretion is circadian and immediate
  - Adrenal mineralocorticoid and glucocorticoids are not stored but enter the blood stream directly upon synthesis
  - Blood levels of hormones reflect the rate of synthesis
- Synthesis and secretion of hormones are regulated by the hypothalamic-anterior pituitary-adrenal (HPA) axis and the renin-angiotensin system (RAS)

Adrenal Gland: Hormones (continued)

Hormones

- **Cortex: Zona glomerulosa** cells produce **mineralocorticoids** (aldosterone)
  - Regulate the Na+/K+ balance in extracellular fluids and impact blood pressure homeostasis
  - Mainly regulated by angiotensin II, potassium, and adrenocorticotropic hormone (ACTH)
- **Cortex: Zona fasciculata** cells produce **glucocorticoids** (cortisol in dog, pig, nonhuman primate, and humans and corticosterone in rat, mouse, rabbit)
  - Mobilize fats, carbohydrates, and proteins; enhance the activity of other hormones, including glucagon and catecholamines
  - Regulated by ACTH from the pituitary and controlled by a feed-back mechanism
  - Secretion is controlled by sympathetic innervation and acutely triggered by stress, trauma, and shock

Adrenal Gland: Hormones (continued)

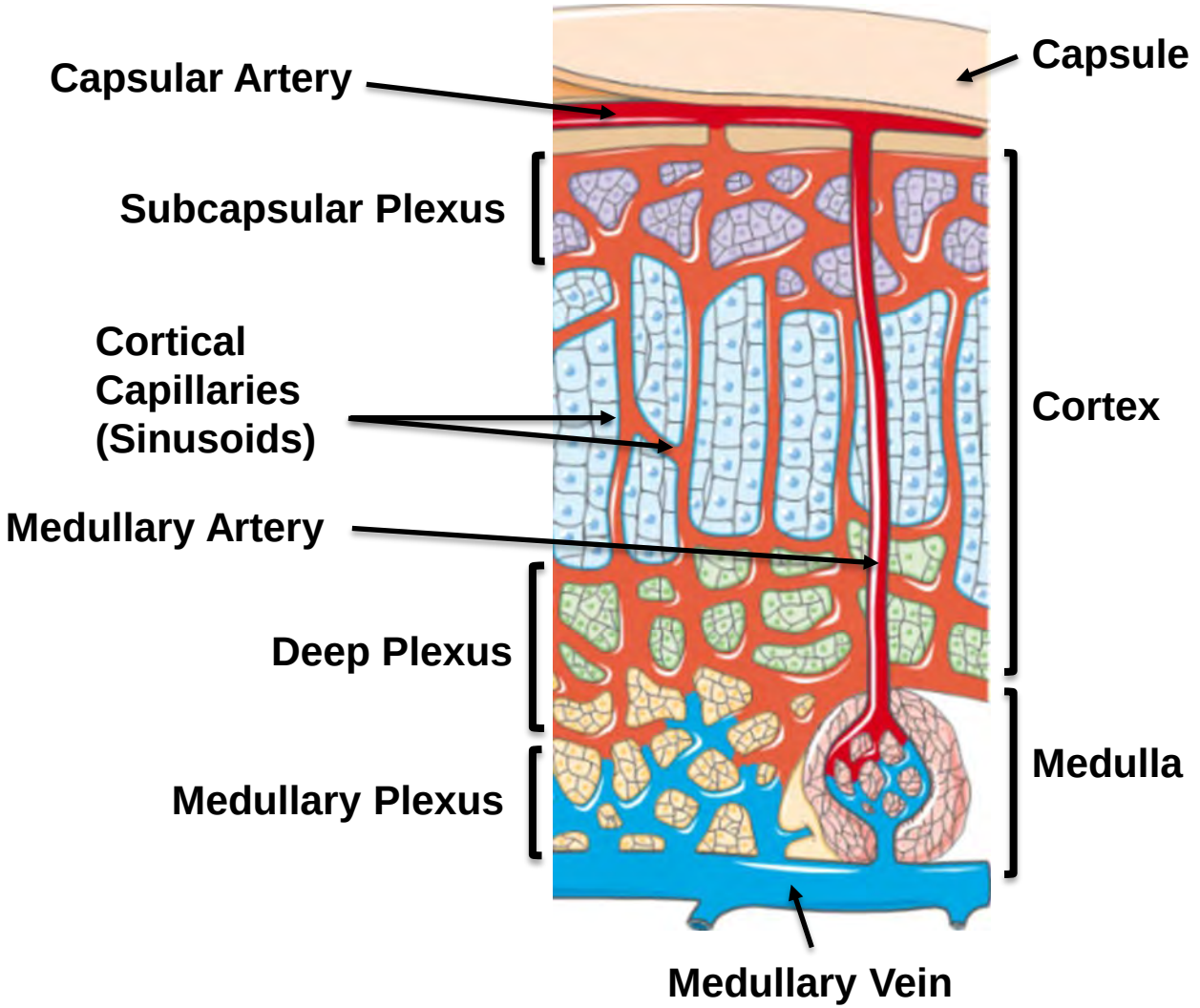
Hormones

- **Cortex: Zona reticularis** cells produce adrenal **androgens in humans** including dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), and androstenedione
  - **Rodent** adrenals lack the enzyme Cytochrome P450 17 alpha-hydroxylase (CYP17) necessary to produce adrenal androgens
  - **Rodent** zona reticularis cells produce little to no sex steroids and produce mainly corticosterone
- **Medulla: Adrenal medulla** chromaffin cells produce the **catecholamines** norepinephrine and epinephrine, which are stored in secretory vesicles
  - In **rodents**, norepinephrine and epinephrine are stored in separate chromaffin cell types, which can be distinguished ultrastructurally by the morphology of their secretory granules

Adrenal Gland: Blood Supply

Blood Supply

- Adrenals receive blood from the aorta and/or the phrenic, renal and lumbar arteries, forming a vascular plexus perfusing the capsule, cortex and medulla
  - Additionally, arteries travel within the cortex without branching and supply blood directly to the medulla
- Allows for transport of glucocorticoids directly from the cortex to the medulla
  - Glucocorticoids are required for the activation of phenylethanolamine-N-methyltransferase (PNMT)
  - PNMT allows for the conversion of norepinephrine to epinephrine by medullary cells



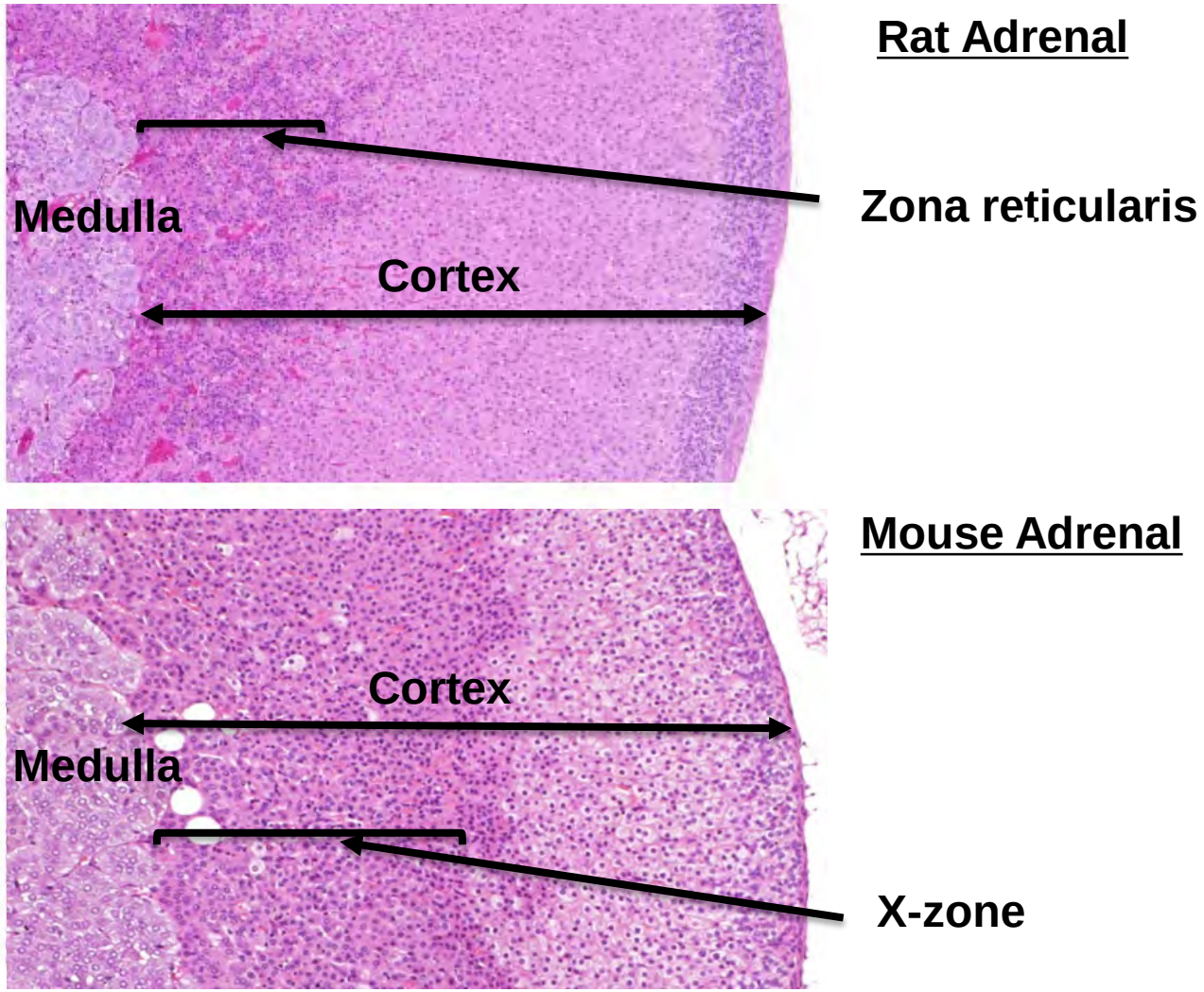
Adrenal Gland: Innervation

Innervation

- Cells of the adrenal gland receive both intrinsic and extrinsic innervation
- Intrinsic innervation arises from ganglion cells sparsely distributed throughout the gland in subcapsular, cortical, and medullary regions
- Most external fibers that project to the adrenal gland travel via the splanchnic nerves
  - The majority of these fibers are cholinergic preganglionic sympathetic fibers arising predominantly from the spinal cord
  - After penetrating the capsule, nerve fibers branch to form an extensive subcapsular network

Adrenal Gland: Species-Specific Characteristics

- The border between the zona fasciculata and zona reticularis (upper image) is not clearly delineated, especially in mice (bottom image)
- Mice have a prominent X-zone at the cortico-medullary junction (bottom image) that regresses
- Mice have deposition of cortical lipogenic pigment resulting from X-zone regression
- Mice commonly have subcapsular proliferations of fusiform cells



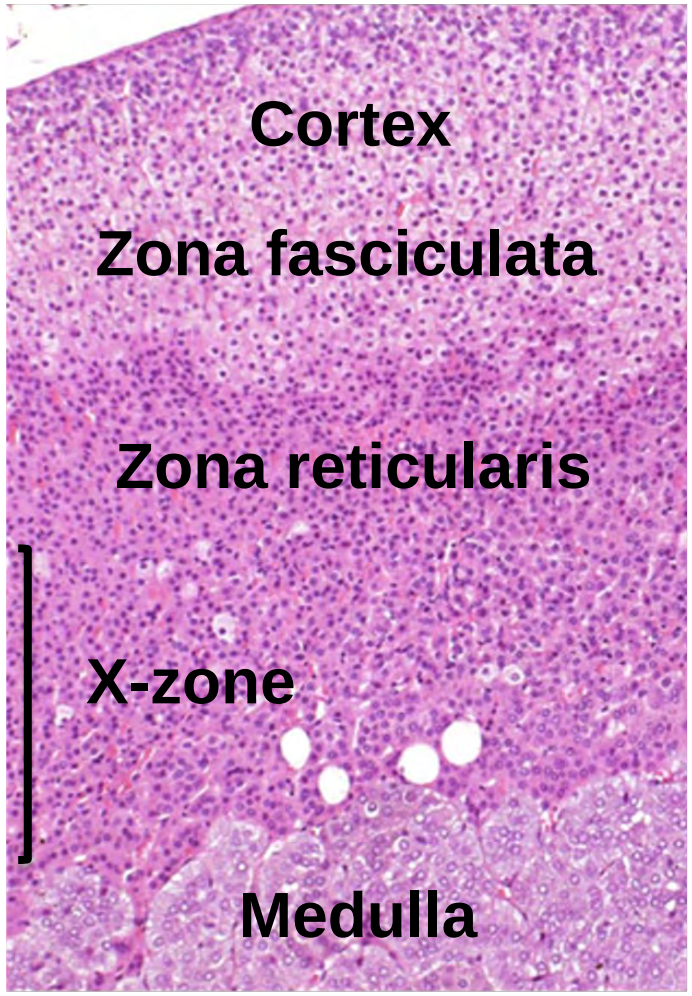
X-Zone in Mice

- Transient cortical region located at the cortico-medullary junction that is unique to the mouse
  - In males and females, the x-zone appears a few days after birth and is fully developed at weaning
  - Gonadectomy preserves the x-zone in both males and females
- In females, the X-zone increases in size reaching a maximum at around 9 weeks of age before undergoing regression/involution (lipid vacuolization)
  - Regression is gradual in virgins and is accelerated by pregnancy
  - After regression, a few fibrous strands may remain
- In males, the X-zone disappears at puberty (5 weeks) without undergoing vacuolization
- Premature or delayed adrenal X-zone involution is a potential treatment-related effect

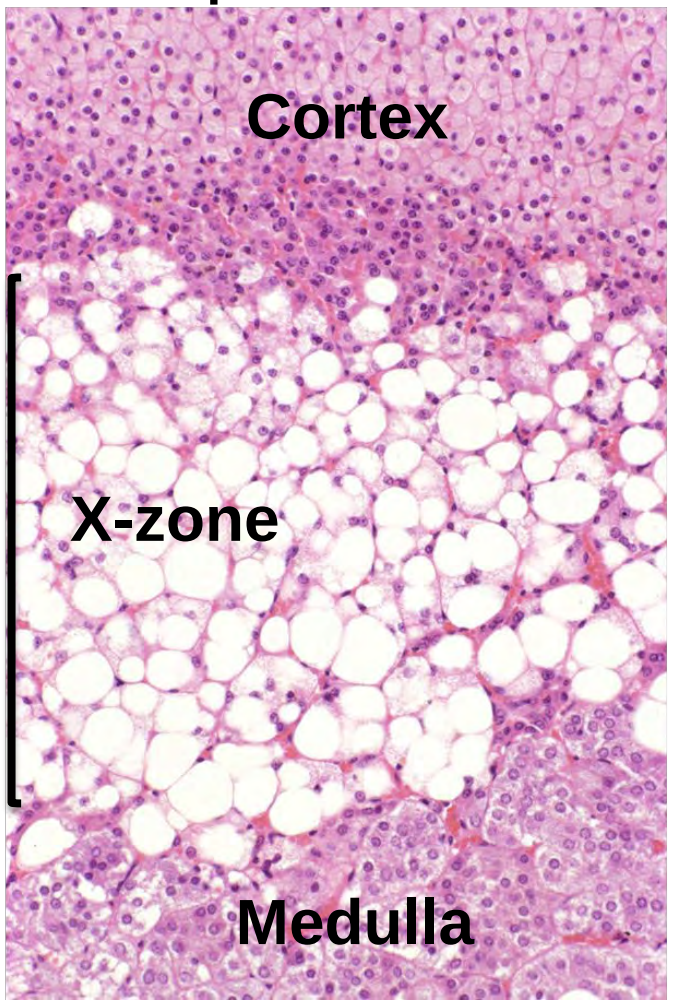
National Institute of Environmental Health Sciences
Division of Translational Toxicology

Adrenal Gland: X-Zone Regression in Female Mice

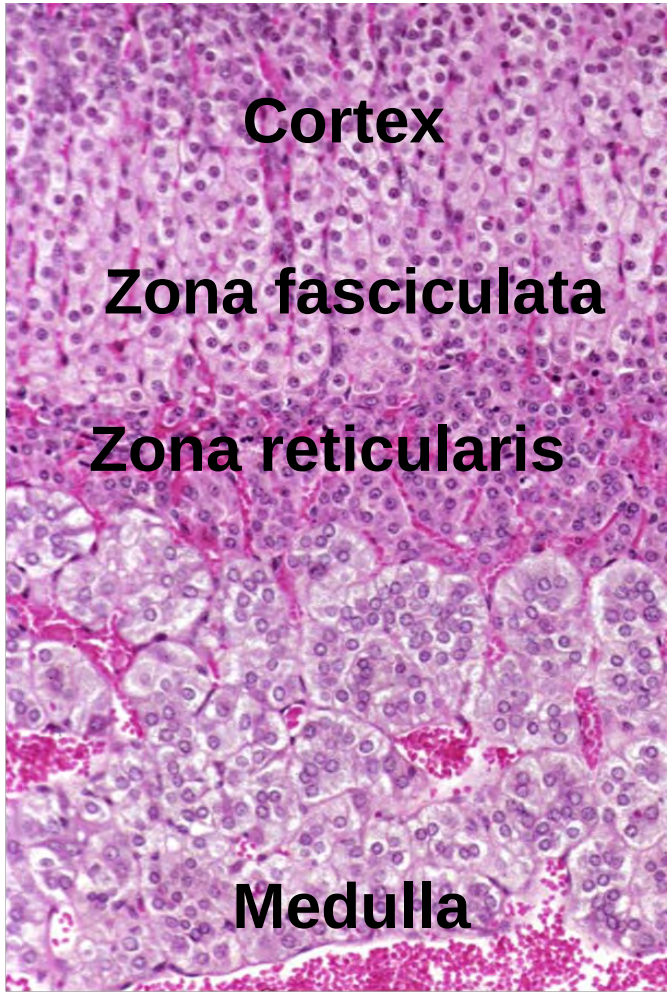
9 weeks of age



Advanced regression with lipid vacuolation



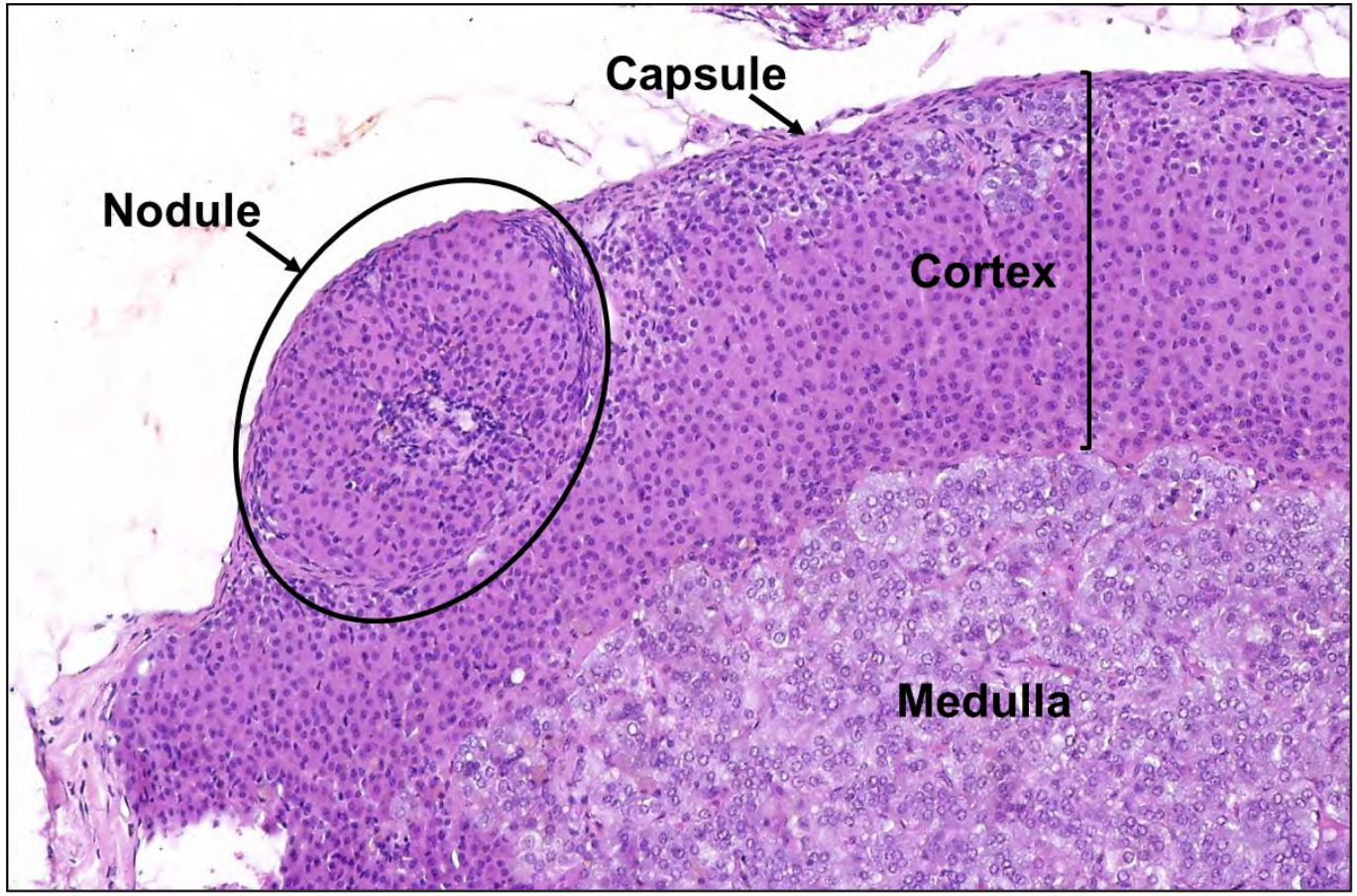
Complete regression



Adrenal Gland: Congenital/Developmental Lesions

Congenital/Developmental Lesions

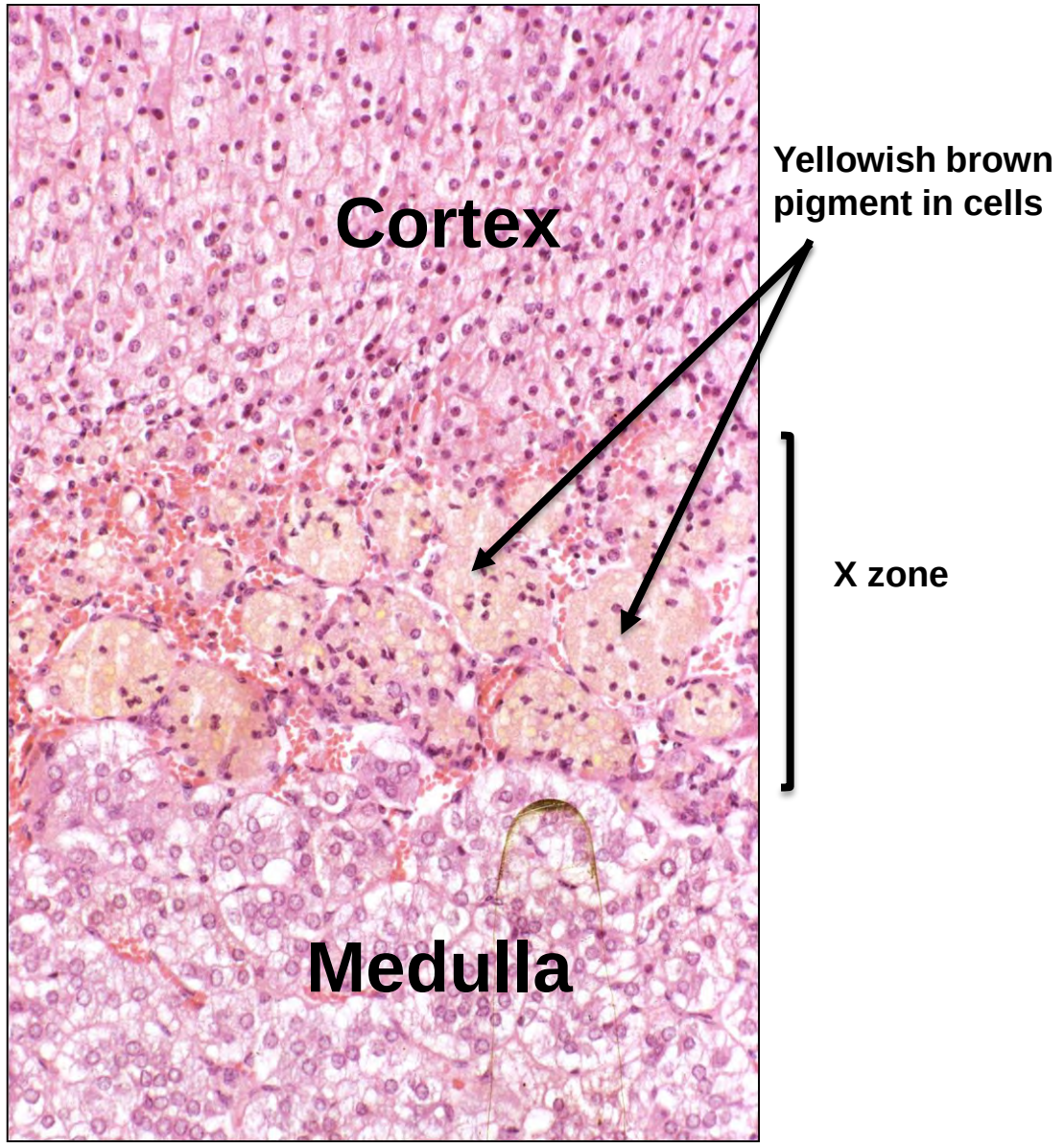
- **Accessory adrenal cortical nodules** are the primary congenital lesion in rodents
  - More prevalent in females
  - Relatively common with up to 50% incidence in some strains
  - Can develop the same degenerative and proliferative changes as the adrenal cortex proper
  - Believed to have an embryological origin, forming during the development of the adrenal cortex
- Other congenital lesions include **adrenal cortical cysts** and **ectopic tissue**



Adrenal Gland: Pigment

Pigment

- Common incidental age-related finding in rats and mice
- Occurs due to the large amount of lipid in the adrenal gland
- Deposition may be exacerbated by treatment
- Origin is from peroxidation of intracytoplasmic fat which becomes insoluble
- Appears as yellowish-brown granular pigment in cortical epithelial cells (H&E)
  - Affected cells become enlarged and lateral displacement of the nucleus gives cells the appearance of macrophages
  - Adjacent affected cells may coalesce into multinucleated clusters sometimes forming a complete band of cells
- May be associated with X-zone regression in mice



Adrenal Gland: Special Stains

Special Stains

- Stains for biogenic amines (epinephrine and norepinephrine) in chromaffin cells
  - Stains for chromaffin cells in the adrenal medulla include Modified Giemsa, Schmorl's, and Wiesel's
  - The ability of these cells to reduce ammoniac silver nitrate to metallic silver results in a black deposit in tissue sections
  - Chromaffin cells have cytoplasmic granules that appear brown when fixed with a dichromate solution
- Stains for lipogenic pigments (lipochrome)
  - These are the breakdown products within cells from oxidation of lipids and lipoproteins commonly found in adrenal cortex (zona reticularis)
  - Can be stained by Sudan black B, long Ziehl-Neelson acid fast, and Schmorl's methods
    - Prussian Blue, which stains iron, can be used to differentiate from hemosiderin
  - May also exhibit a strong orange autofluorescence in formalin-fixed, unstained paraffin sections

Adrenal Gland: Practical Notes

Practical Notes

- Among endocrine tissues, the adrenal gland is most associated with toxicant-induced lesions
  - Highly vascular gland with high rate of blood flow
  - Free radical generation during steroid biosynthesis
  - Potential for bioactivation of toxicants by cytochrome P450 enzymes
- Because of close interplay among endocrine tissues, changes in the adrenal gland may be associated with effects in other endocrine organs
- Age-related changes in the adrenal glands can be induced prematurely and/or be exacerbated by treatment or stress

References

- goRENI (https://www.goreni.org/gr3_index.php)
- INHAND – Nonproliferative and Proliferative Lesions of the Rat and Mouse Endocrine System (https://www.toxpath.org/docs/WED_Endocrine.pdf)
- Nonneoplastic Lesion Atlas (https://ntp.niehs.nih.gov/atlas/nnl)
- Nyska A, Maronpot RR. 1990. Adrenal gland. In: Pathology of the Mouse: Reference and Atlas (Maronpot RR, Boorman GA, Gaul BW, eds). Cache River Press, Vienna, IL, 509-536.

National Institute of Environmental Health Sciences
Division of Translational Toxicology

Author

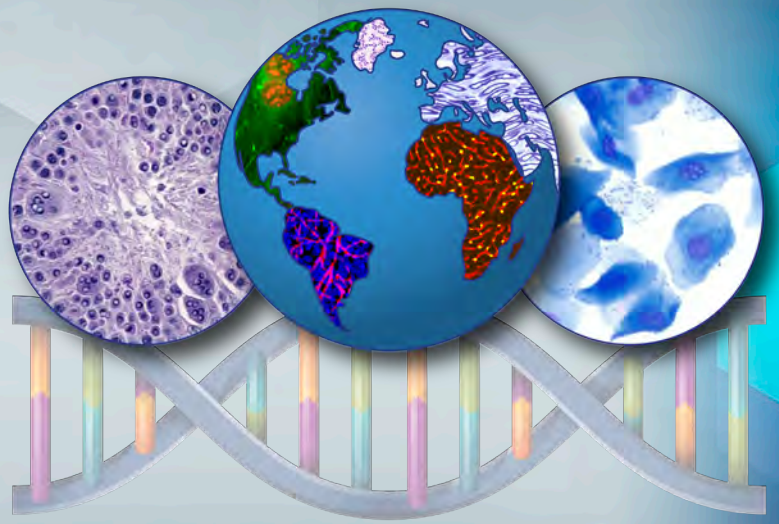
- Marlene Orandle, DVM, PhD – Inotiv-RTP

Reviewers

- Beth Lubeck, PhD, MBA – Division of Translational Toxicology (DTT), NIEHS
- Cynthia Willson, MS, PhD, DVM, DACVP, DABT – Inotiv-RTP
- Karen Cimon, DVM, MS – Experimental Pathology Laboratories

Acknowledgements

- Ron Herbert, DVM, PhD – DTT, NIEHS
- Robert Maronpot, DVM, PhD – DTT, NIEHS (Retired)



Division of Translational Toxicology
Global Toxicologic Pathology Training Program