Endocrine

Breast

Adipose Tissue

Special Stains

2018 Spring

ENDOCRINE SYSTEM

-Endocrinology is the study of hormones, their receptors and the intracellular signaling pathways they invoke.

-The endocrine system is composed of ductless glands that produce hormones.

-Hormones are regulatory chemicals that are secreted into the bloodstream. Hormones induce a change in cellular metabolism.

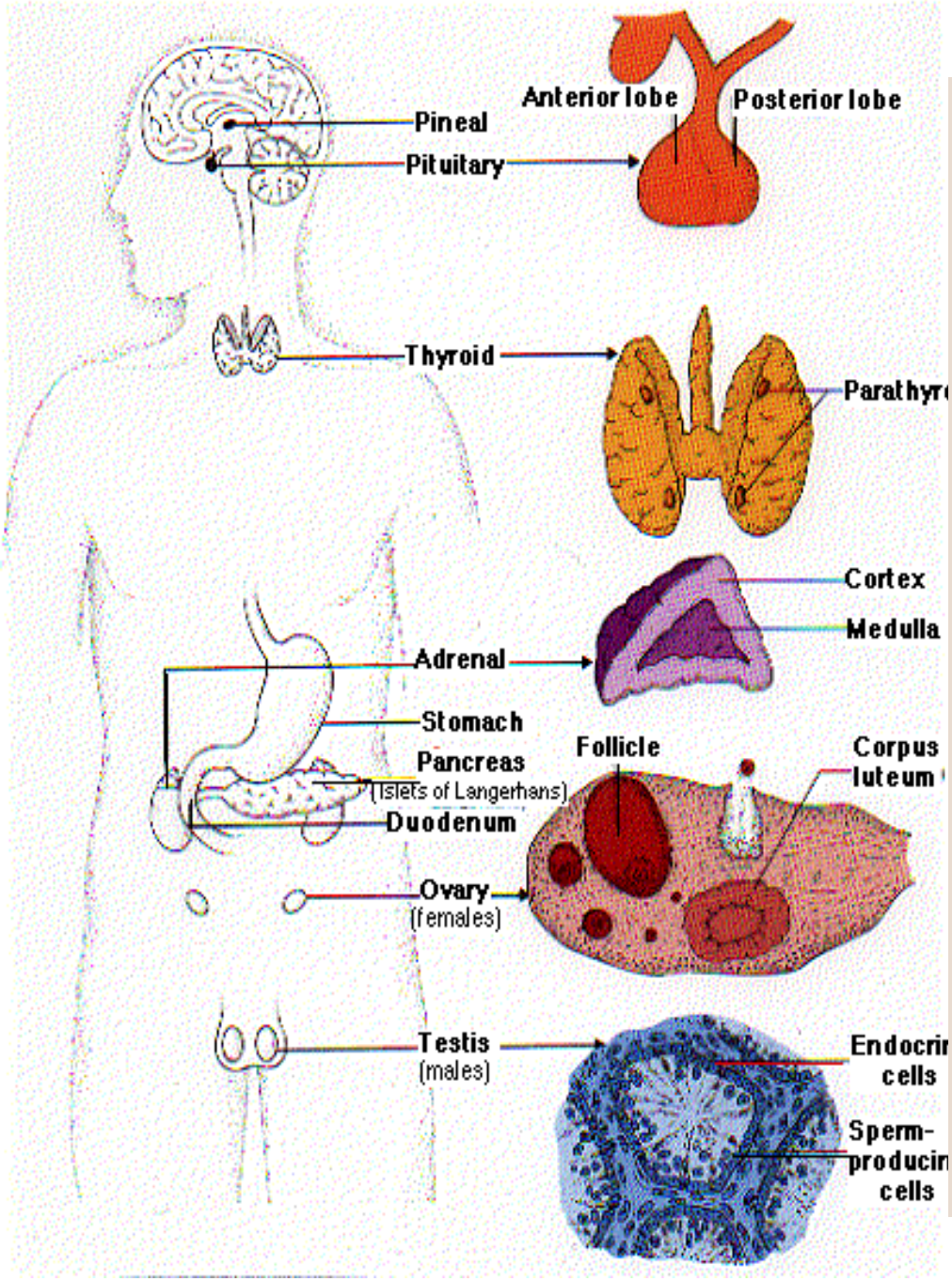
-Distinct endocrine organs are scattered throughout the body.

-In addition to the classical endocrine organs, many other cells in the body secrete hormones. Myocytes in the atria of the heart and scattered epithelial cells in the stomach and intestine are examples of what is sometimes called the "diffuse" endocrine system.

http://www.westmont.edu/~tanowitz/Lectures/EndocrinePhys.html

http://arbl.cvmbs.colostate.edu/hbooks/pathphys/endocrine/basics/overview.html

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/



MAJOR ENDOCRINE ORGANS:

HYPOTHALAMUS

PITUITARY

THYROID

PARATHYROID

ADRENALS

ISLETS IN THE PANCREAS

OVARY **Breast**

TESTIS **Placenta**

ADIPOSE TISSUE

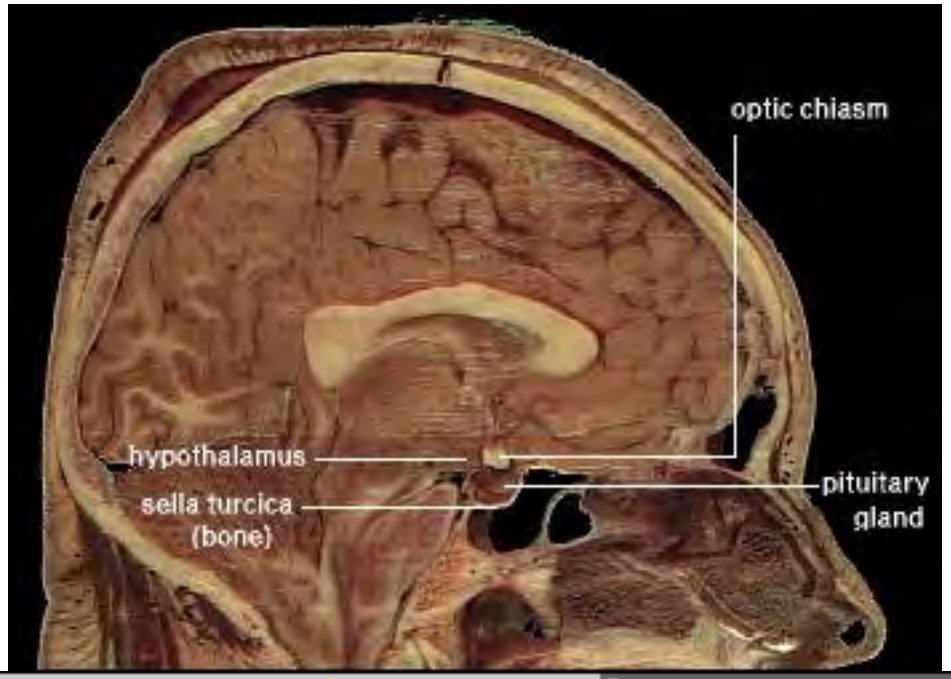
GASTROINTESTINAL HORMONES

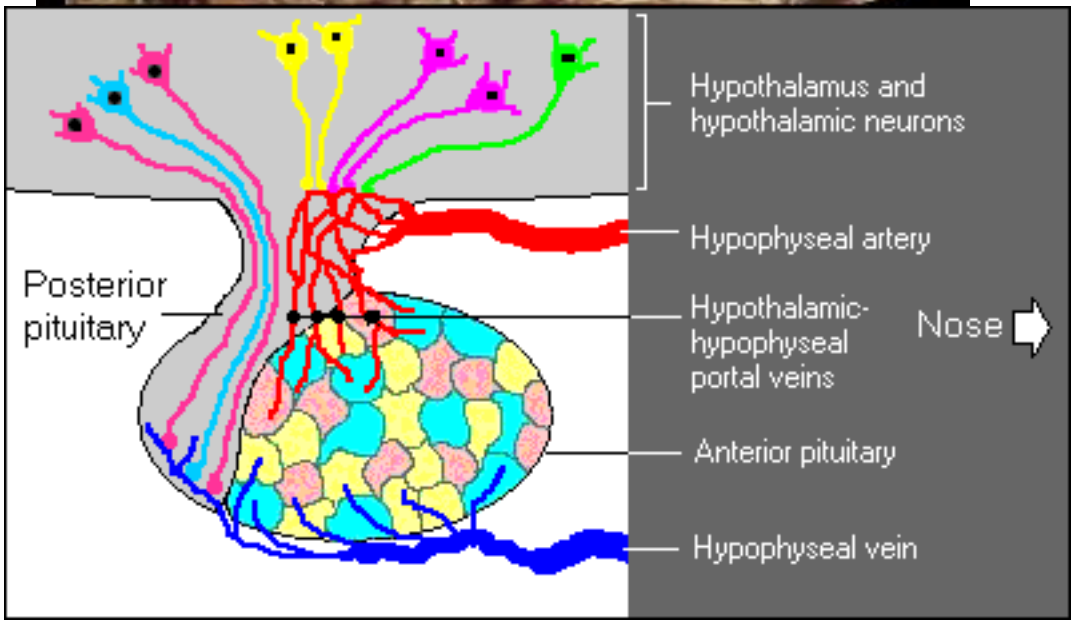
PINEAL GLAND AND MELATONIN

RENIN-ANGIOTENSIN SYSTEM

ETC. ETC.

http://arbl.cvmbs.colostate.edu/hbooks/pathphys/endocrine/basics/overview.html





Hypothalamus : the neurosecretory neurons secrete hormones that strictly control the hormones from the anterior pituitary. There are releasing hormones and inhibitory hormones

One of the most important functions of the hypothalamus is to link the nervous system to the endocrine system via the pituitary gland (hypophysis).

Pituitary

-adenohypophysis
-neurohypophysis

The Hypothalamus is responsible for certain metabolic processes and other activities of the Autonomic Nervous System.

It synthesizes and secretes neurohormones, often called hypothalamic-releasing hormones, and these in turn stimulate or inhibit the secretion of pituitary hormones.

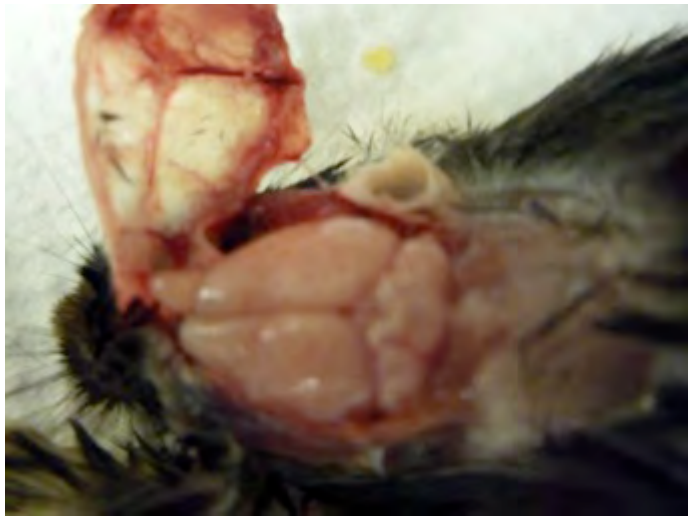
The Hypothalamus controls Blood pressure, Body temperature, hunger, thirst, fatigue, anger, and circadian cycles.

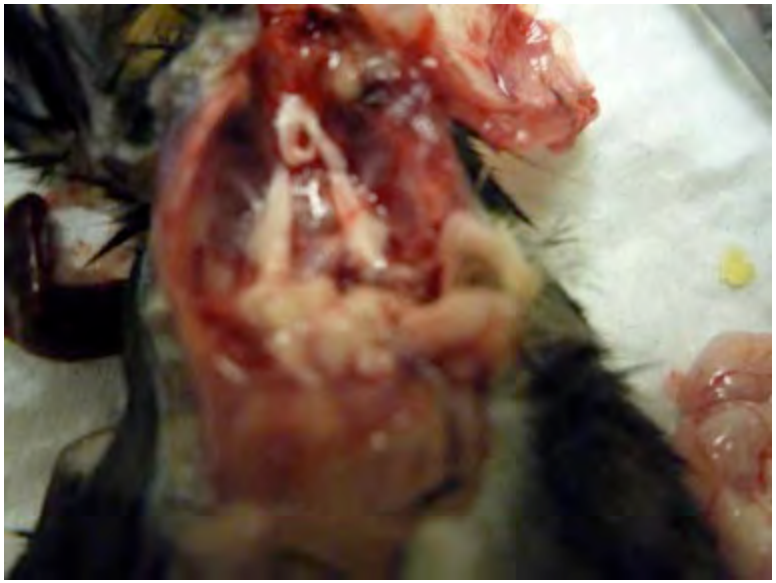
The hypothalamus controls body weight and appetite, but it is not entirely clear how. Sensory inputs, including taste, smell, and gut distension, all tell the hypothalamus if we are hungry, full, or smelling a steak. Yet it is mysterious how we are able to vary our eating habits day to day and yet maintain about the same weight (sometimes despite all efforts to the contrary!) -- leptin gene

The Pituitary

In the mouse, after fixation perfusion of the animal, Remove the top of the mouse skull and expose the Brain

Remove the brain to expose the Sella turcica which holds the Pituitary



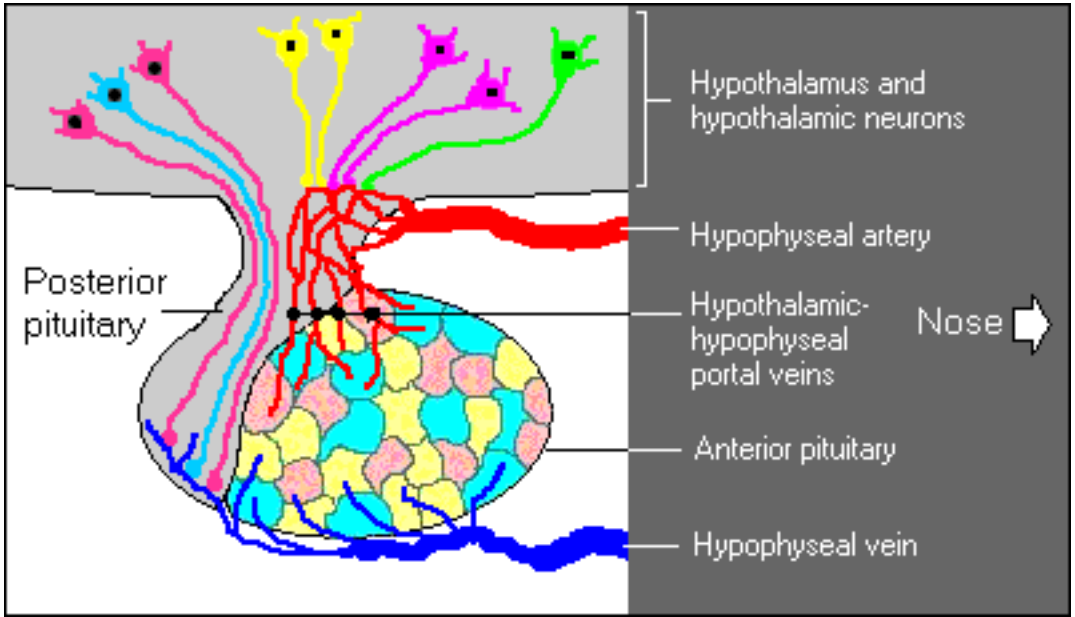


The **pituitary gland** , or **hypophysis** , is an endocrine gland about the size of a pea that sits in the small, bony cavity (sella turcica) at the base of the brain .

The pituitary gland secretes hormones regulating a wide variety of bodily activities, including trophic hormones that stimulate other endocrine glands.

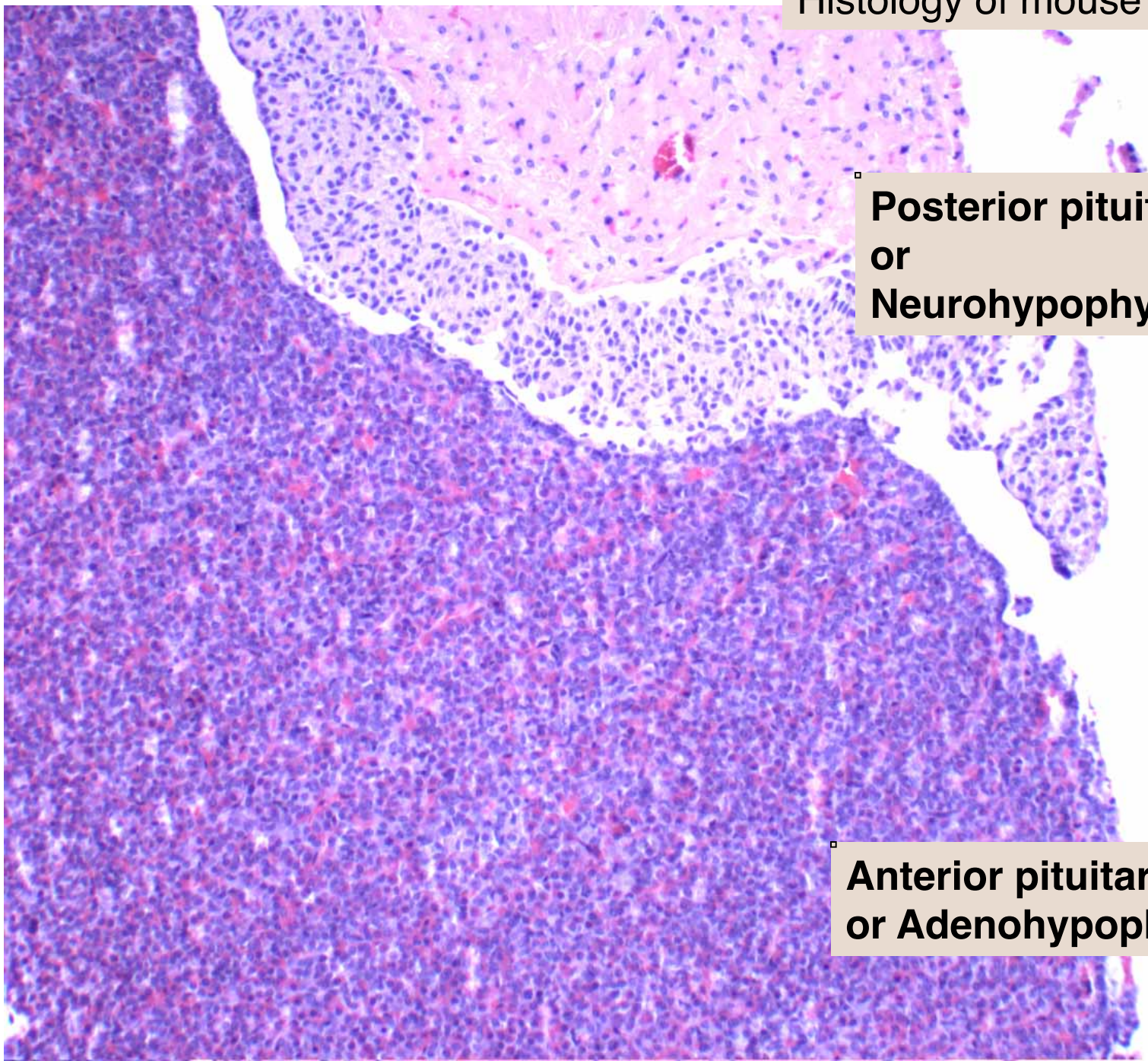
For a while, this led scientists to call it the *master gland* , but now we know that it is in fact regulated by hormones released from the hypothalamus .

The pituitary gland is physically attached to the brain by the **pituitary** , or **hypophyseal stalk** connected with the median eminence .

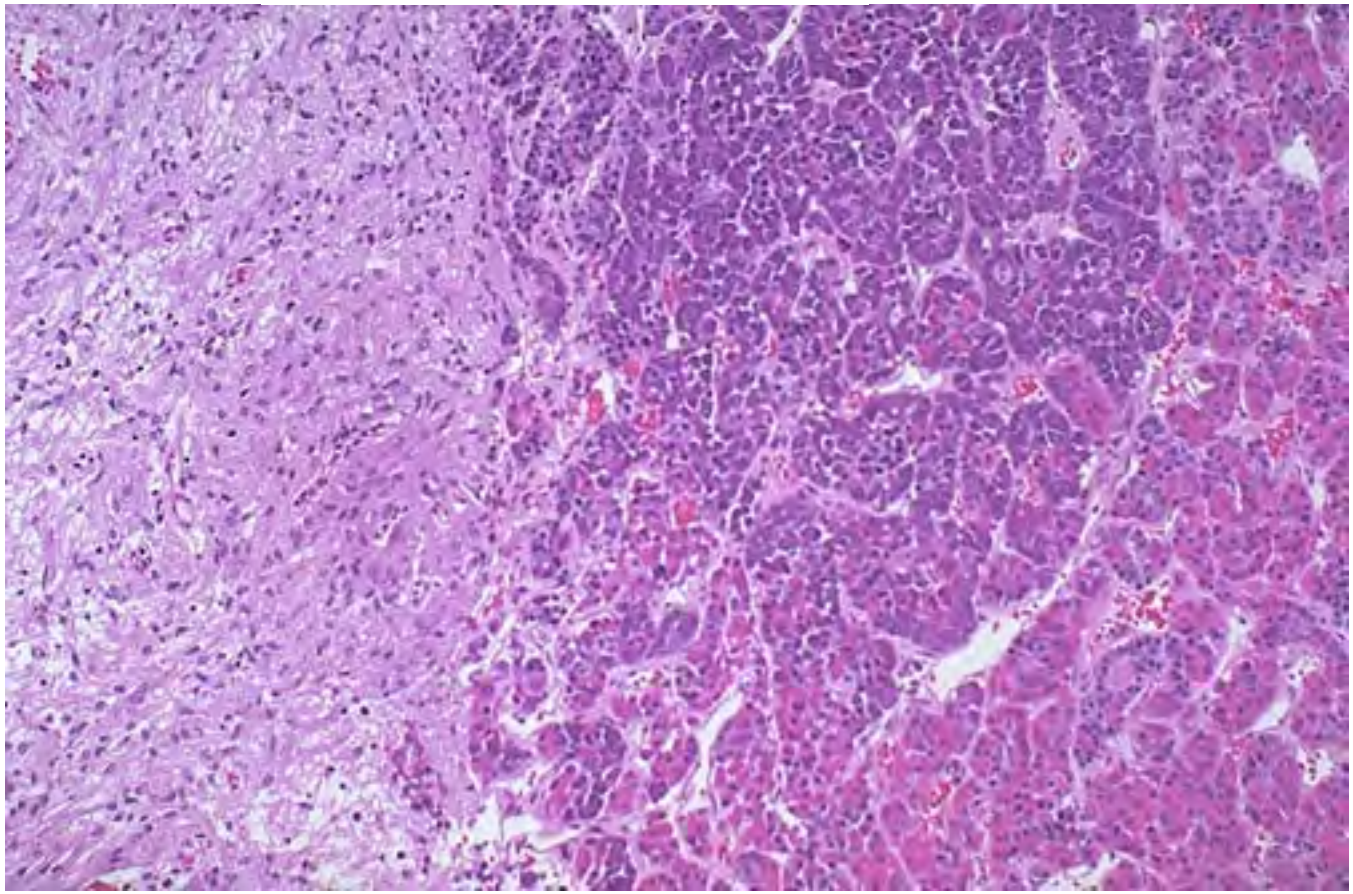


Human Pituitary

Histology of mouse Pituitary



Human Pituitary



Neurohypophysis: - is made up of unmyelinated axons from hypothalamic neurosecretory neurons. About 100,000 axons participate in this process to form the posterior pituitary. In addition to axons, the neurohypophysis contains glial cells and other poorly-defined cells called called pituicytes.

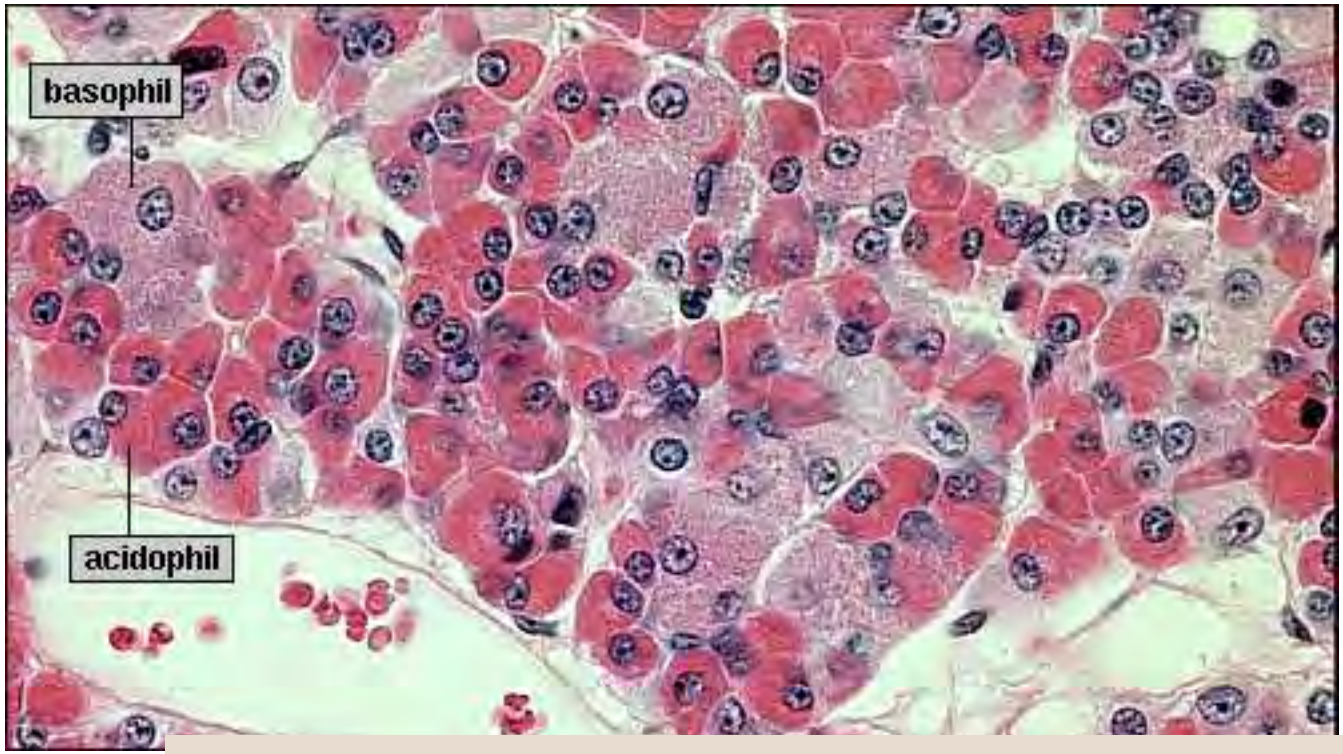
-secretes

-oxytocin : principal actions :

--stimulating contractions of the uterus at the time of birth

--stimulating release of milk when the baby begins to suckle

- antidiuretic hormone: also known as arginine vasopressin. ADH acts on the collecting ducts of the kidney to facilitate the reabsorption of water into the blood. This it acts to reduce the volume of urine formed (giving it its name of antidiuretic hormone)



Adenohypophysis of the pituitary has three cell types:

Acidophils, basophils and chromophobes

Acidophils: - growth hormone

- prolactin

Basophils: -thyroid stimulating hormone, (TSH)

-luteinizing or follicle stimulating hormone (LH or FSH)

-adrenocorticotrophic hormone (ACTH)

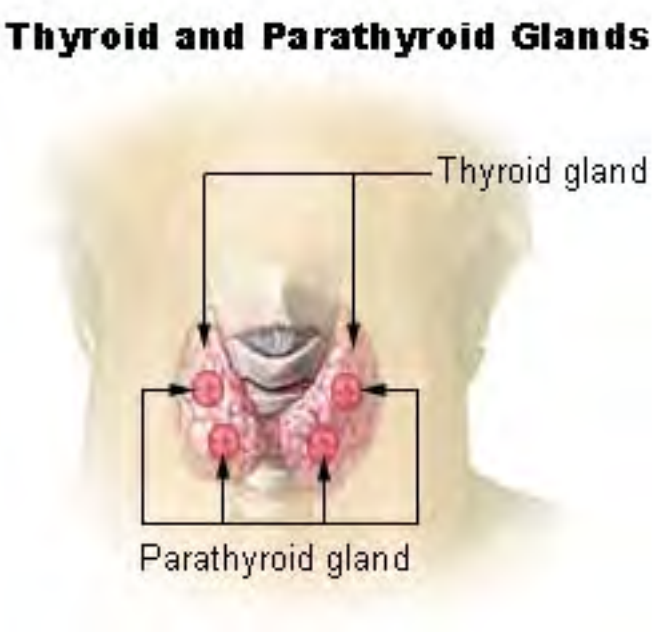
Chromophobes: Stem cells ? Degenerated cells

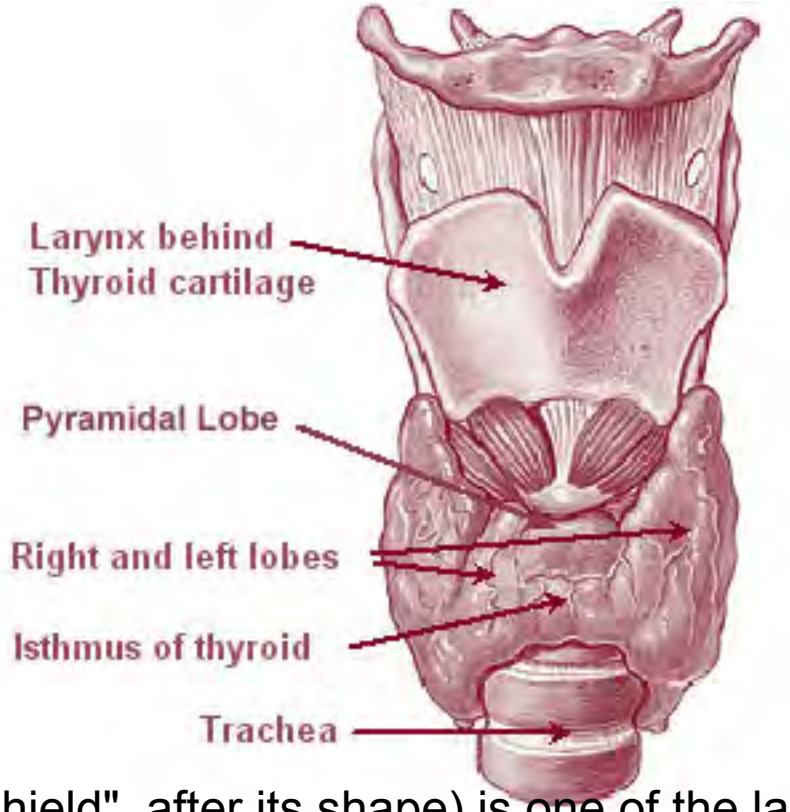
Pituitary adenomas are tumors that occur in the pituitary gland , and account for about 10% of intracranial neoplasms . May or may not be hormone producing

Thyroid : adenomas: About one in 10 people are found to have solitary thyroid nodules . Investigation is required because a small percentage of these are malignant. Biopsy usually confirms the growth to be an adenoma, but sometimes, excision at surgery is required

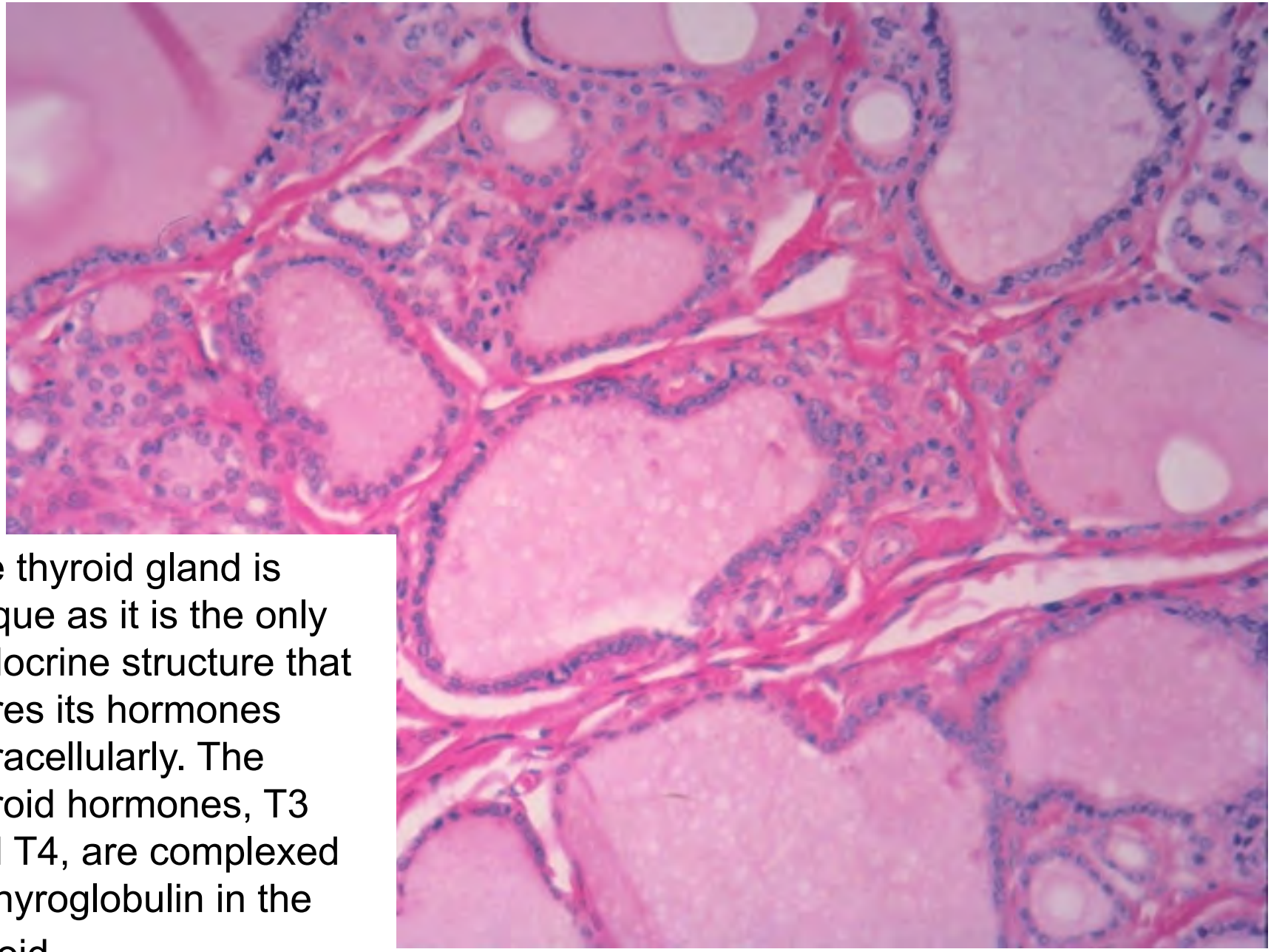
Adrenal: adenomas too

Malignancies



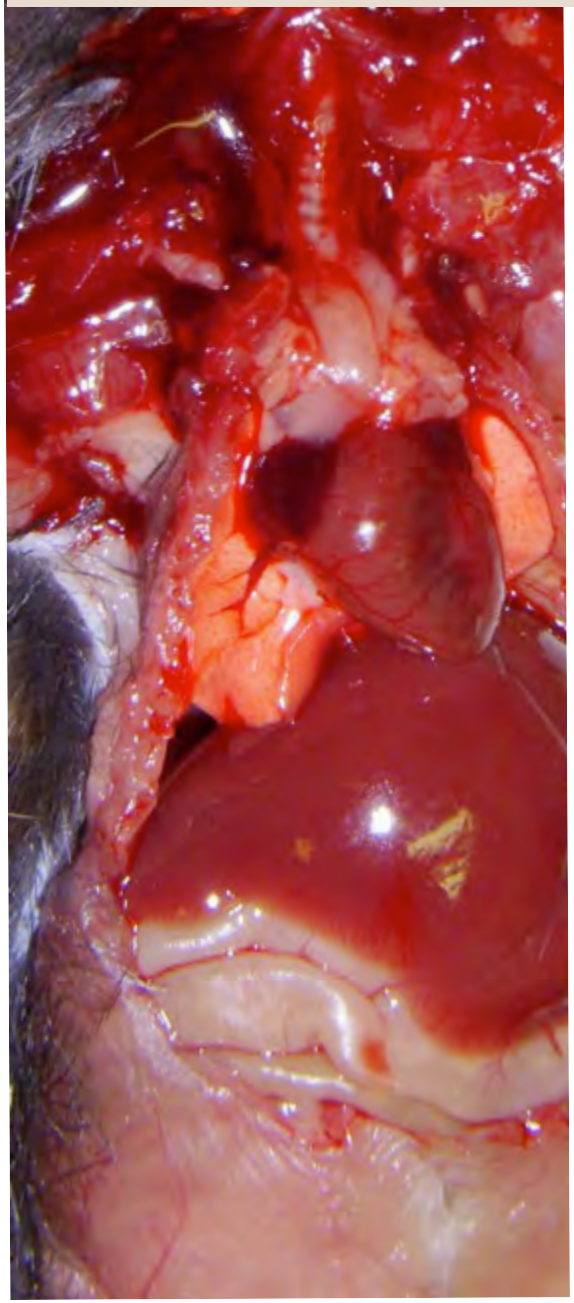


The **thyroid** (from the Greek word for "shield", after its shape) is one of the larger endocrine glands in the body. It is a double-lobed structure located in the neck and produces hormones , principally thyroxine (T 4) and triiodothyronine (T 3), that regulate the rate of metabolism and affect the growth and rate of function of many other systems in the body. The hormone calcitonin is also produced and controls calcium blood levels. Iodine is necessary for the production of both hormones. Hyperthyroidism (overactive thyroid) and hypothyroidism (underactive thyroid) are the most common problems of the thyroid gland.

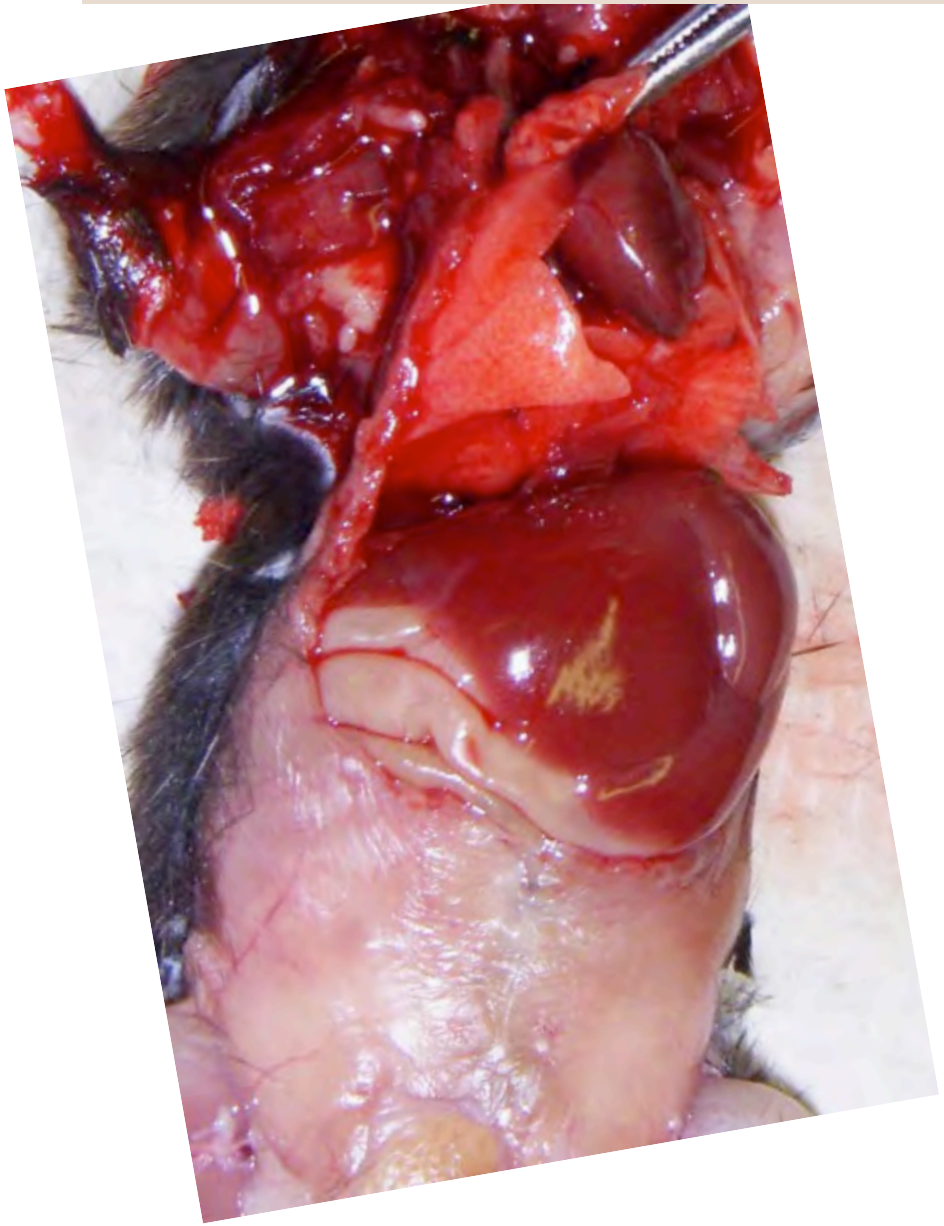


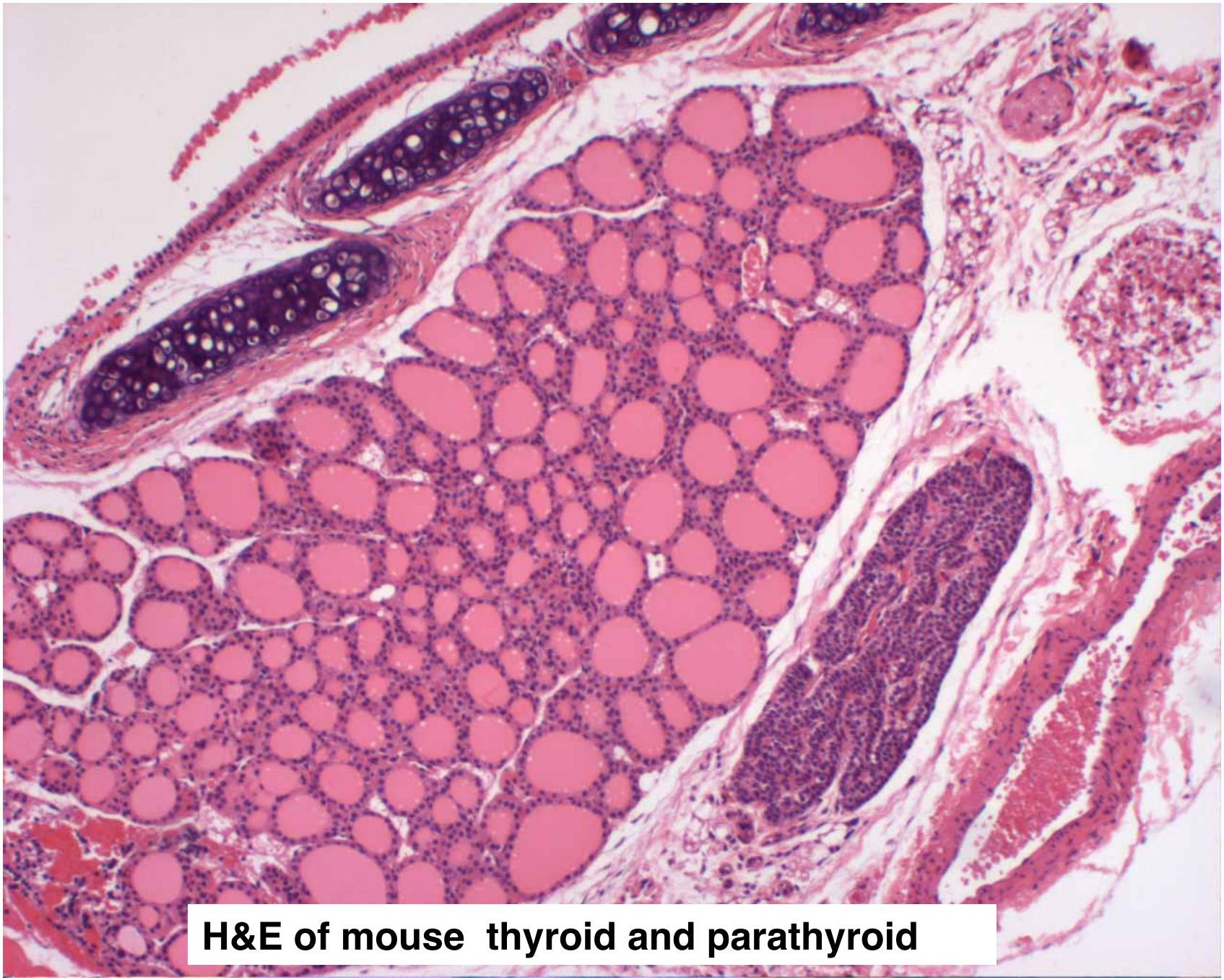
The thyroid gland is unique as it is the only endocrine structure that stores its hormones extracellularly. The thyroid hormones, T3 and T4, are complexed to thyroglobulin in the colloid.

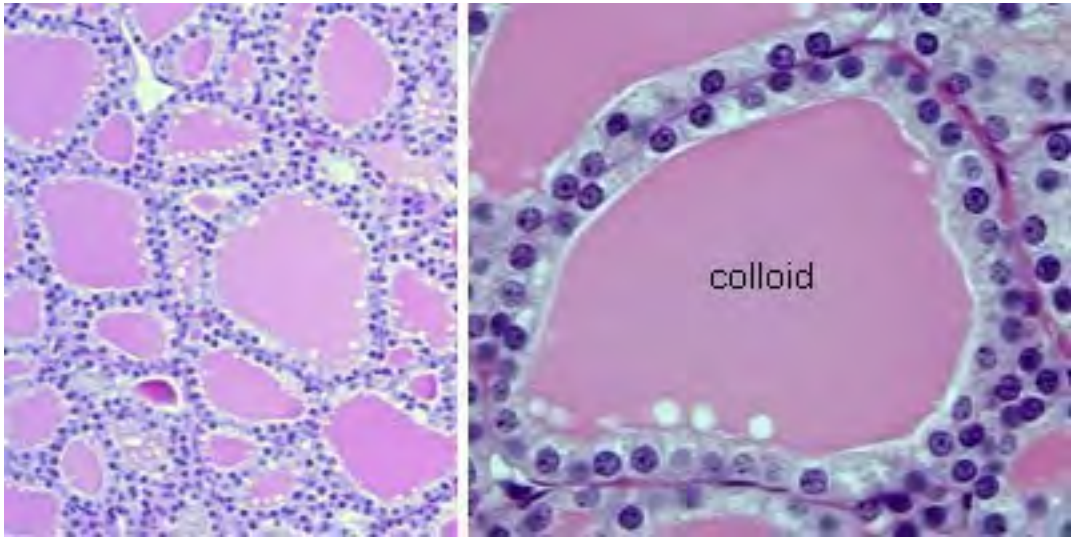
Thyroid and Parathyroid



These are found in sections of the mouse trachea




H&E of mouse thyroid and parathyroid

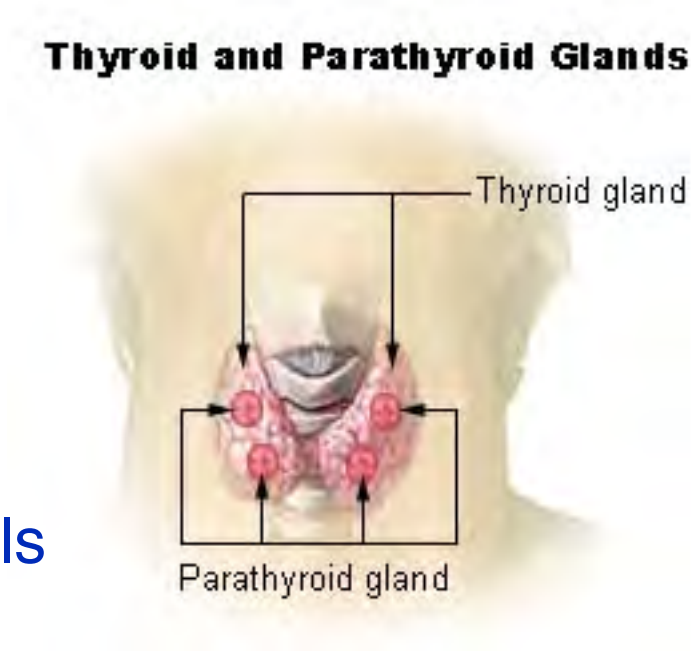


THYROID epithelial cells - the cells responsible for synthesis of thyroid hormones - are arranged in spheres called *thyroid follicles*. Follicles are filled with *colloid*, a proteinaceous depot of thyroid hormone precursor. In the low (left) and high-magnification (right) images of thyroid, follicles are cut in cross section at different levels, appearing as roughly circular forms of varying size. In standard histologic preparations such as these, colloid stains pink.

In addition to thyroid epithelial cells, the thyroid gland houses one other important endocrine cell. Nestled in spaces between thyroid follicles are *parafollicular or C cells*, which secrete the hormone calcitonin.

The parathyroid glands were discovered by Ivar Sandstrom, a Swedish medical student, in 1880. [1] It was the last major organ to be be recognized in humans.

Human parathyroid glands The parathyroid glands are four small glands located on the posterior (closer to the back) surface of the thyroid gland. They distinguish themselves from the thyroid gland histologically as they contain two types of cells- parathyroid chief cells and oxyphil cells .

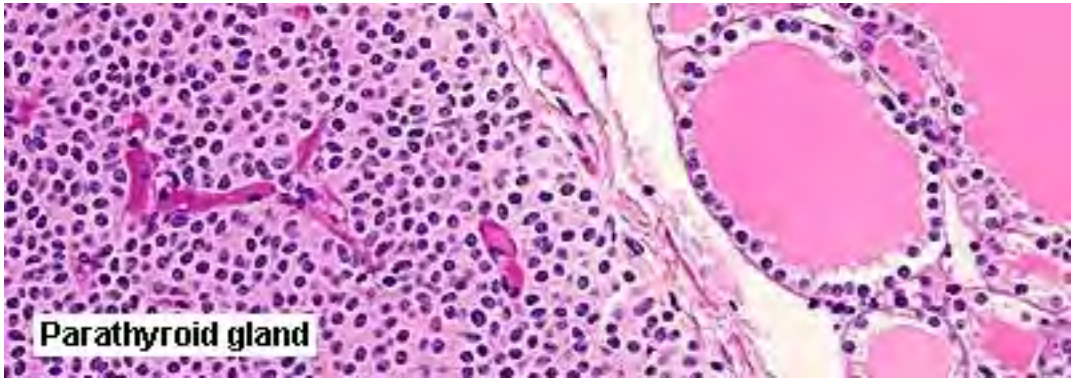


The sole purpose of the parathyroid glands is to regulate the calcium level in our bodies within a very narrow range so that the nervous and muscular systems can function properly.

Parathyroid hormone is a small protein that takes part in the control of calcium and phosphorus homeostasis , as well as bone physiology.

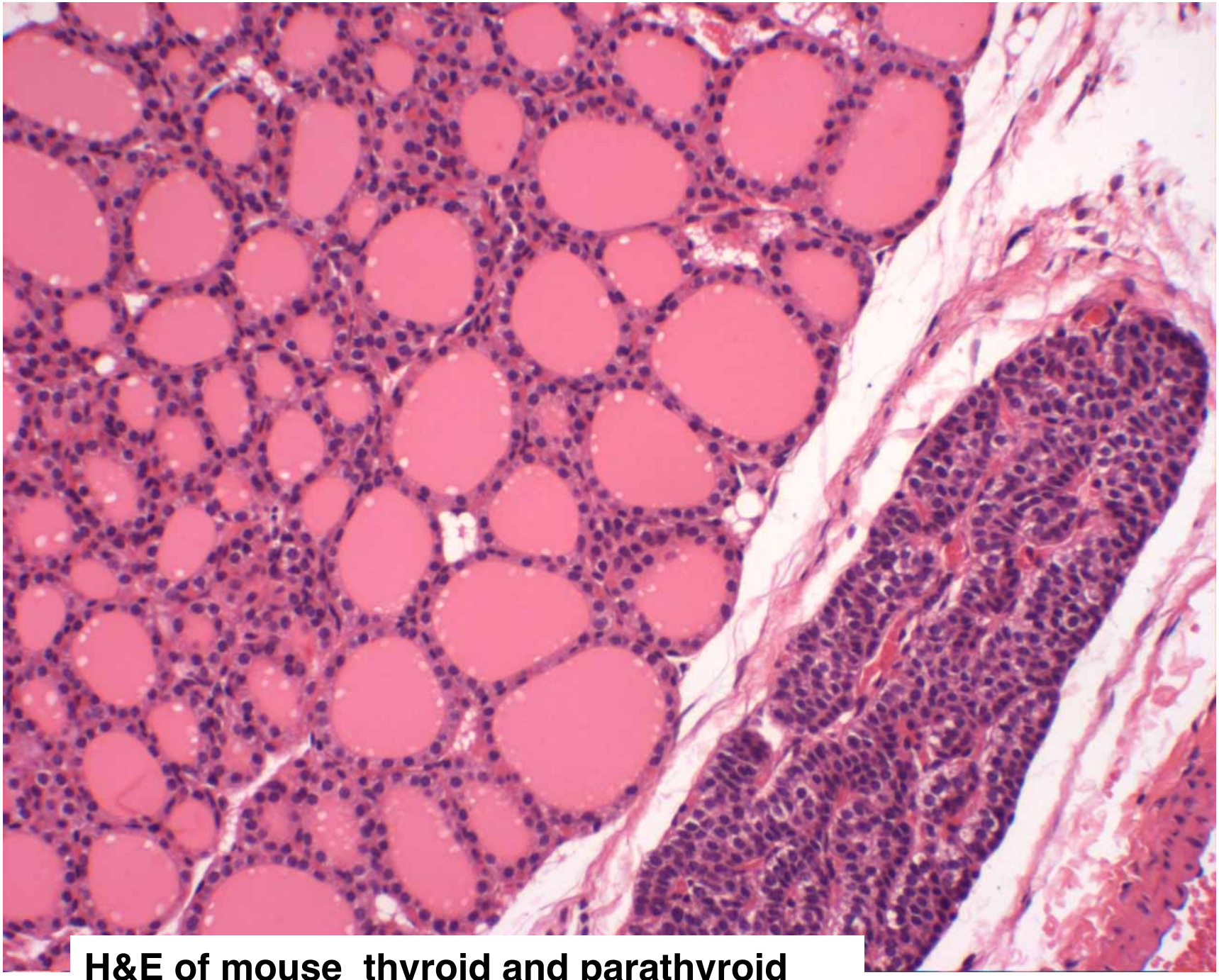
When blood calcium levels drop below a certain point, calcium-sensing receptors in the parathyroid gland are activated to release hormone into the blood.

It then stimulates osteoclasts to break down bone and release calcium into the blood.

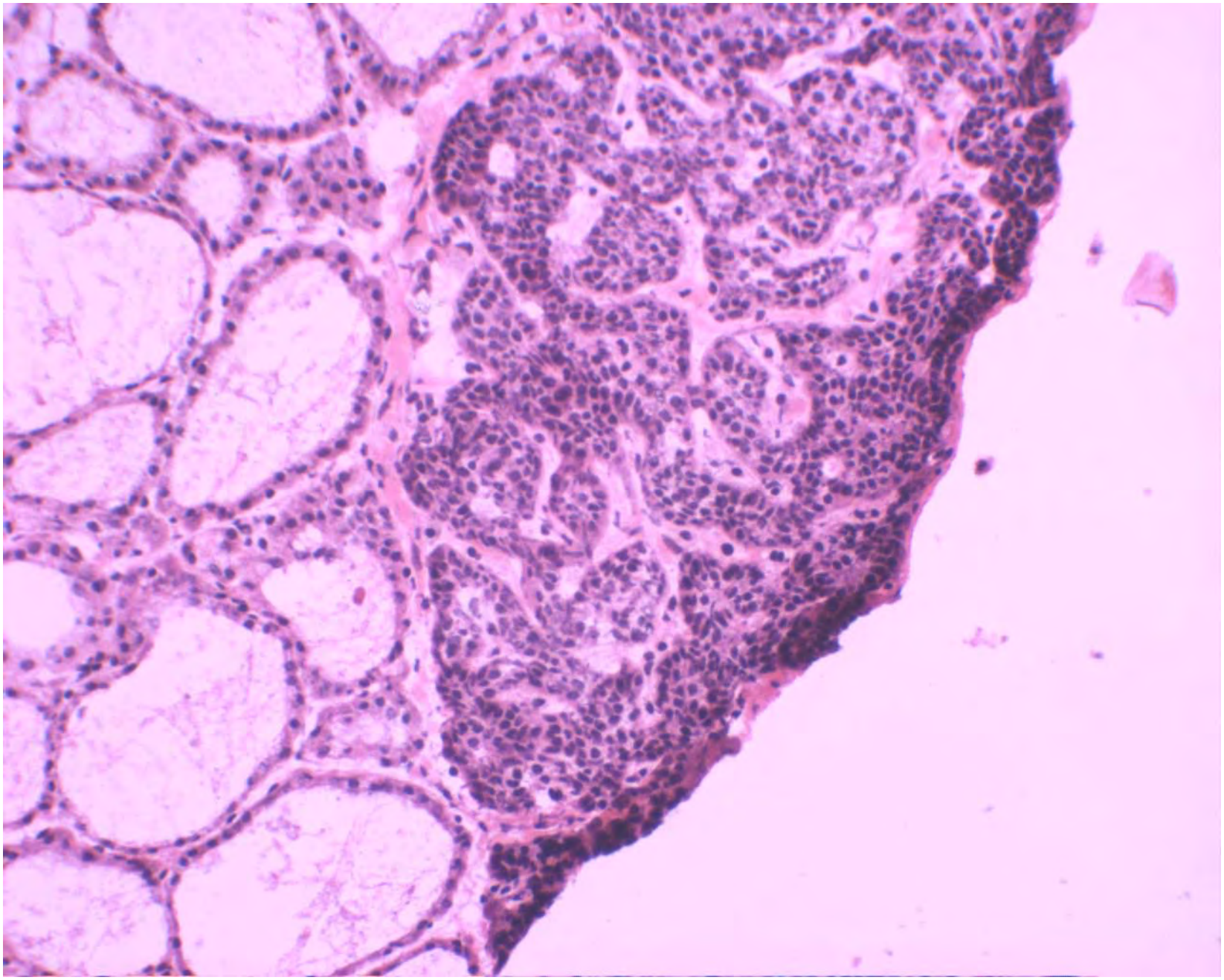


The structure of a parathyroid gland is distinctly different from a thyroid gland. The cells that synthesize and secrete parathyroid hormone are arranged in dense cords or nests around abundant capillaries. *The image shows a section of a parathyroid gland on the left, associated with thyroid gland (note the follicles) on the right.*

Parathyroid hormone and calcitonin participate in control of calcium and phosphorus homeostasis and have significant effects on bone physiology.



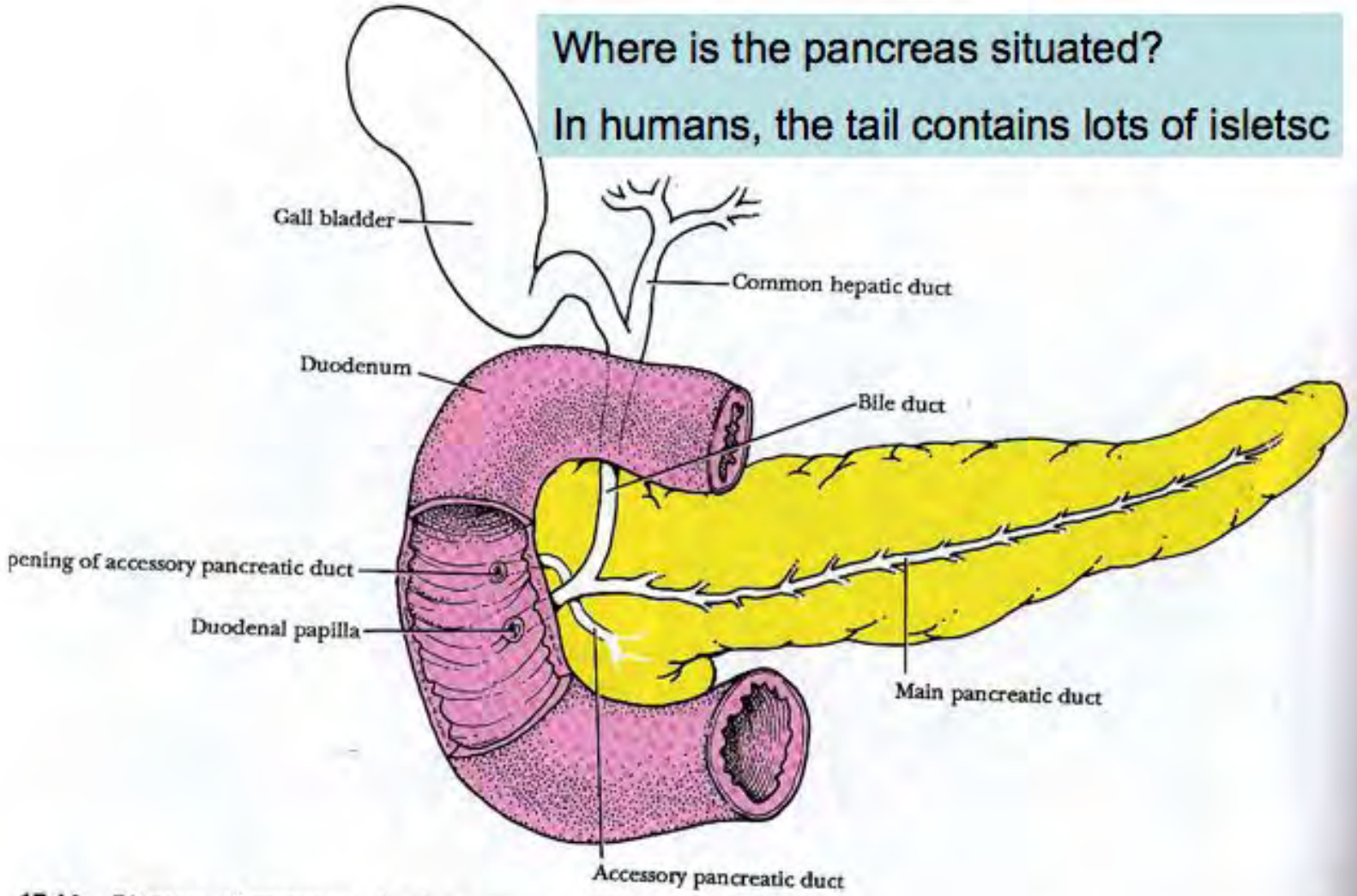
H&E of mouse thyroid and parathyroid



H&E of mouse thyroid and parathyroid

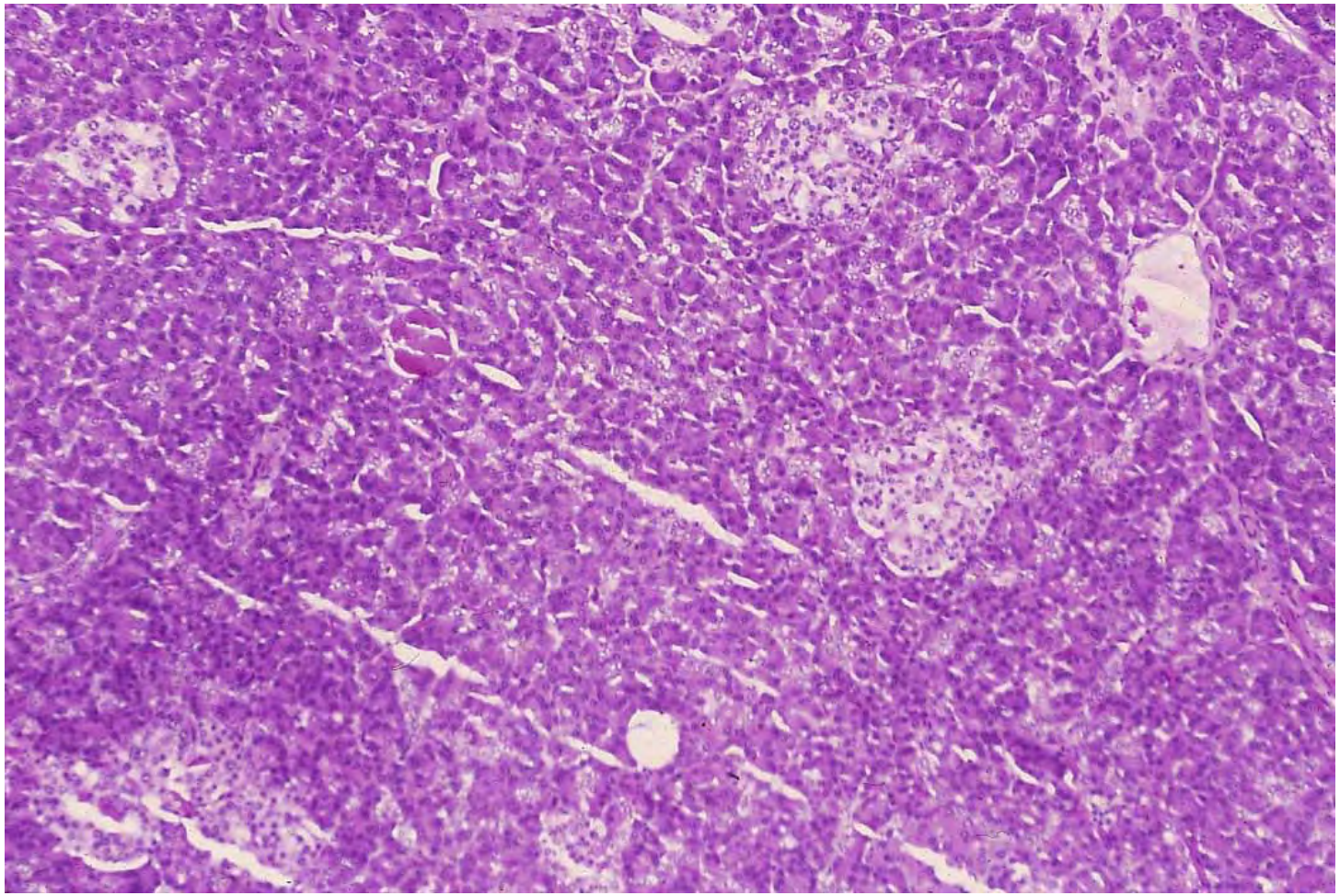
Where is the pancreas situated?

In humans, the tail contains lots of isletsc

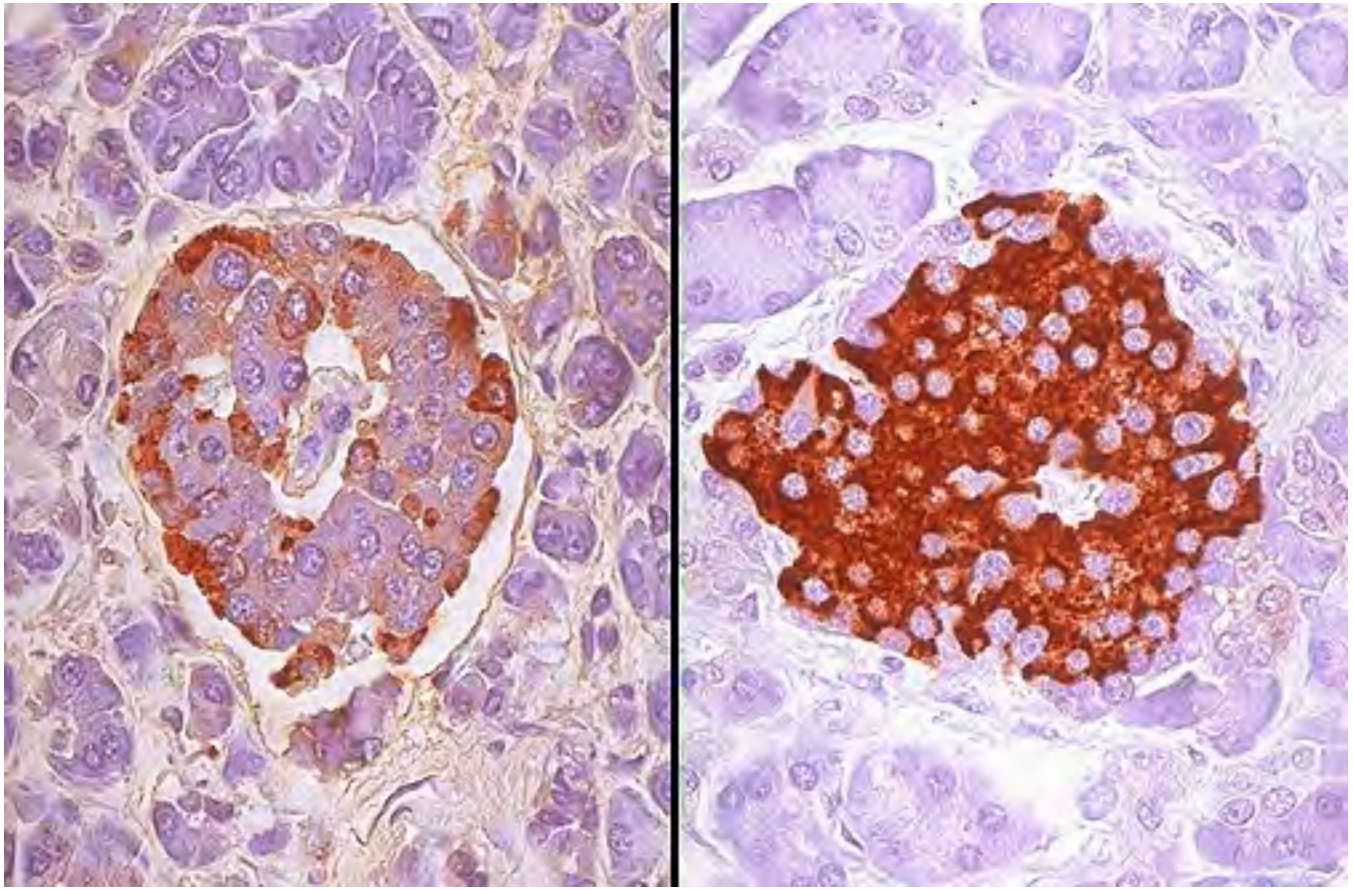


17.14. Diagram of pancreas, duodenum

Microscopic view of Human pancreas



Immunohistochemistry showing insulin and glucagon producing cells in the Human pancreas

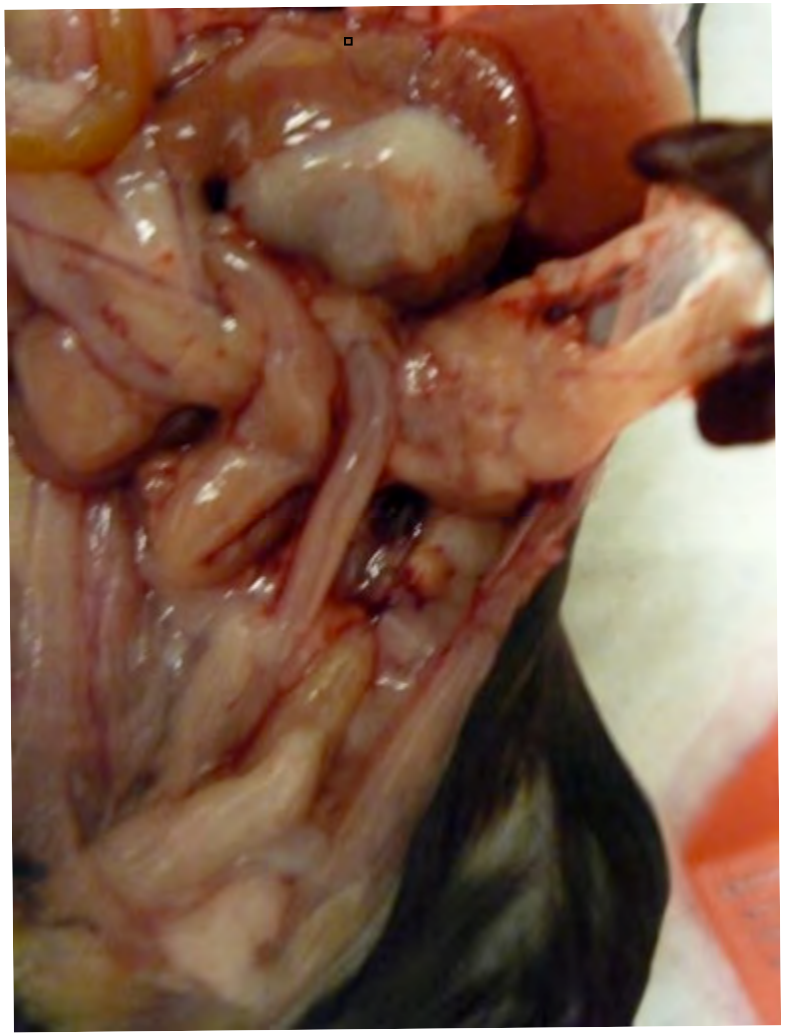


Pancreatic islets of Langerhans house three major cell types, each of which produces a different endocrine product:

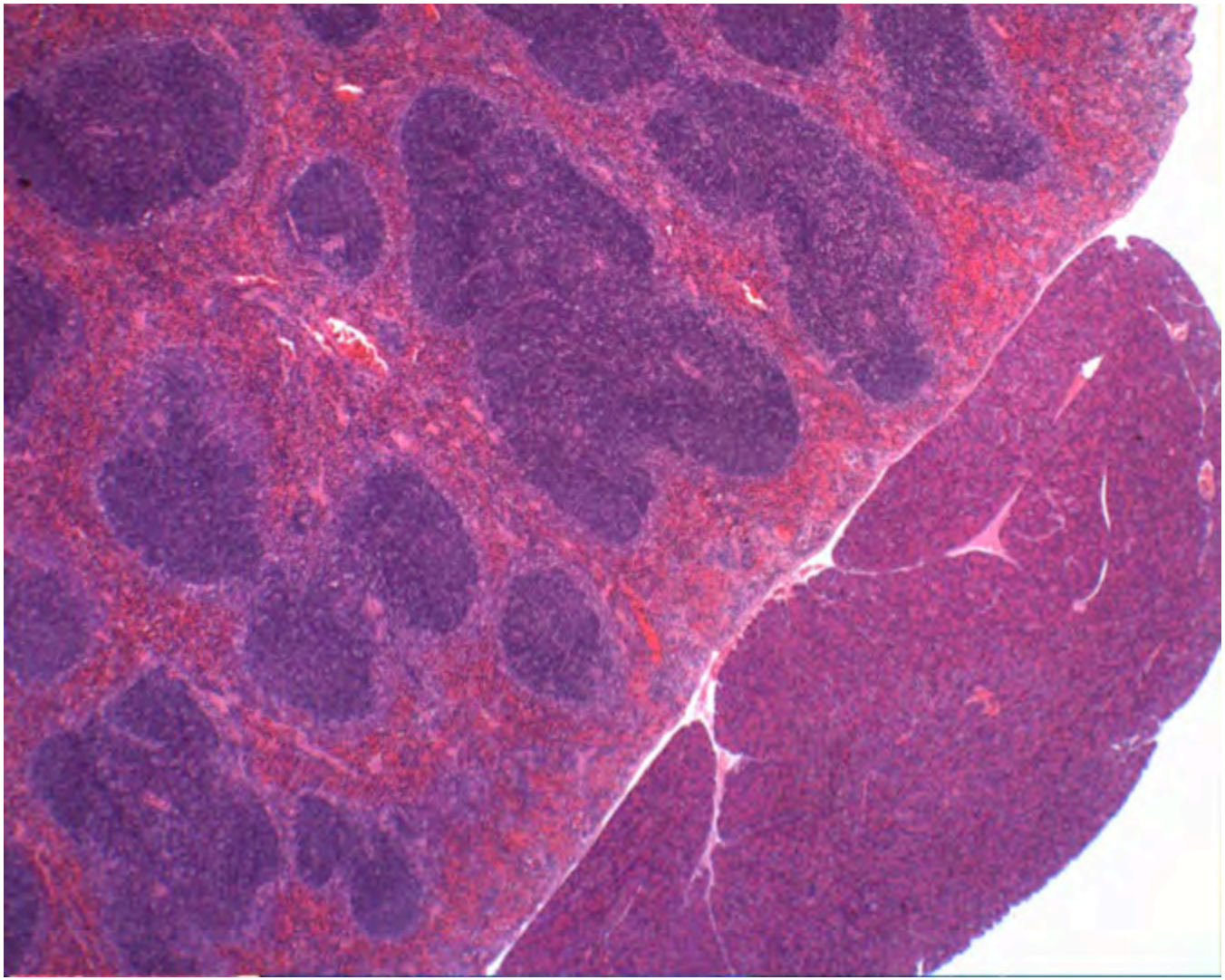
***Alpha cells (A cells) secrete the hormone glucagon.**
•Beta cells (B cells) produce insulin and are the most abundant of the islet cells.
•Delta cells (D cells) secrete the hormone somatostatin, which is also produced by a number of other endocrine cells in the body

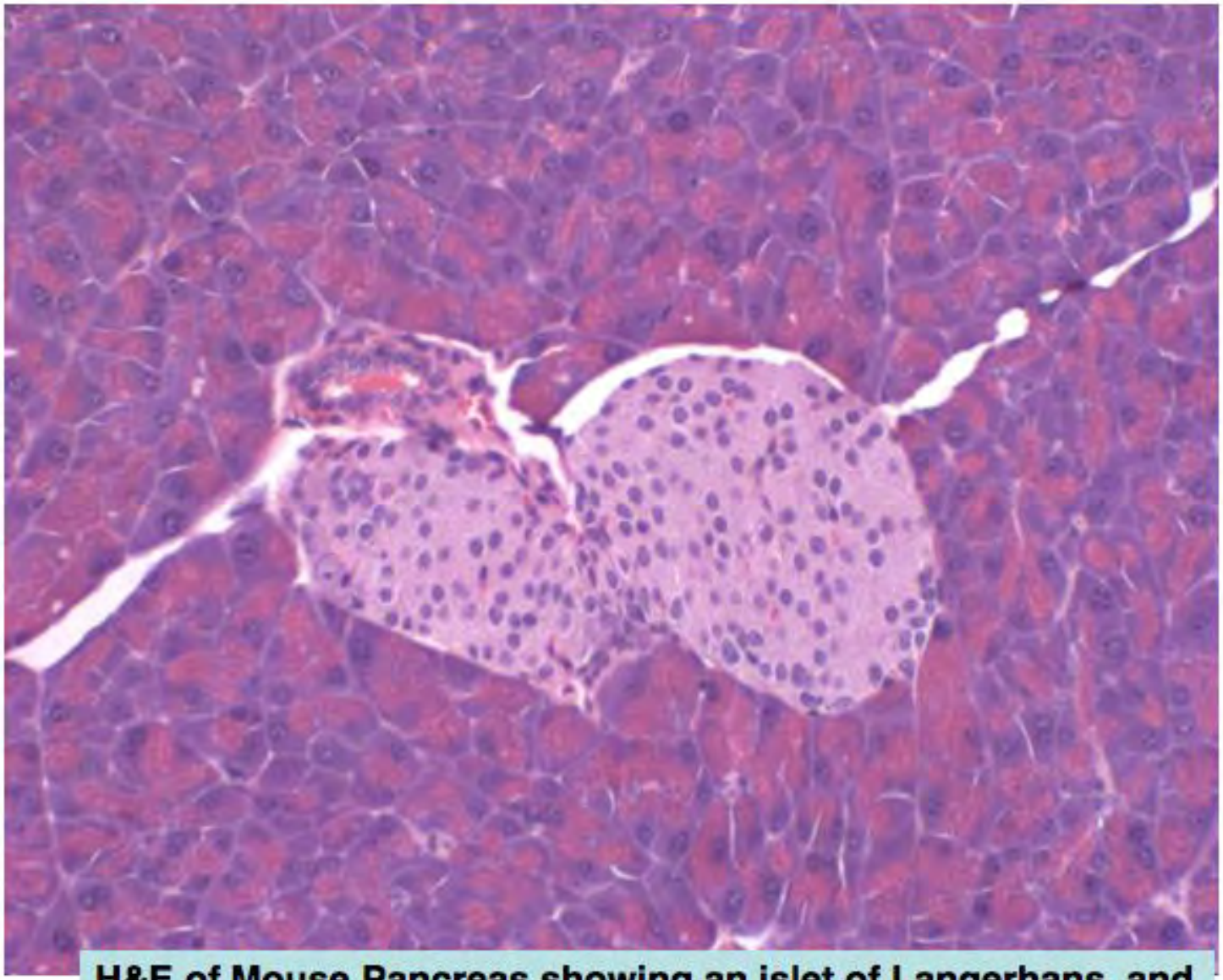
Aside from the insulin, glucagon and somatostatin, a number of other "minor" hormones have been identified as products of pancreatic islets cells (pancreatic polypeptide)

Islets are richly vascularized, allowing their secreted hormones ready access to the circulation. Although islets comprise only 1-2% of the mass of the pancreas, they receive about 10 to 15% of the pancreatic blood flow. Additionally, they are innervated by parasympathetic and sympathetic neurons, and nervous signals clearly modulate secretion of insulin and glucagon.



The Pancreas is close to the spleen



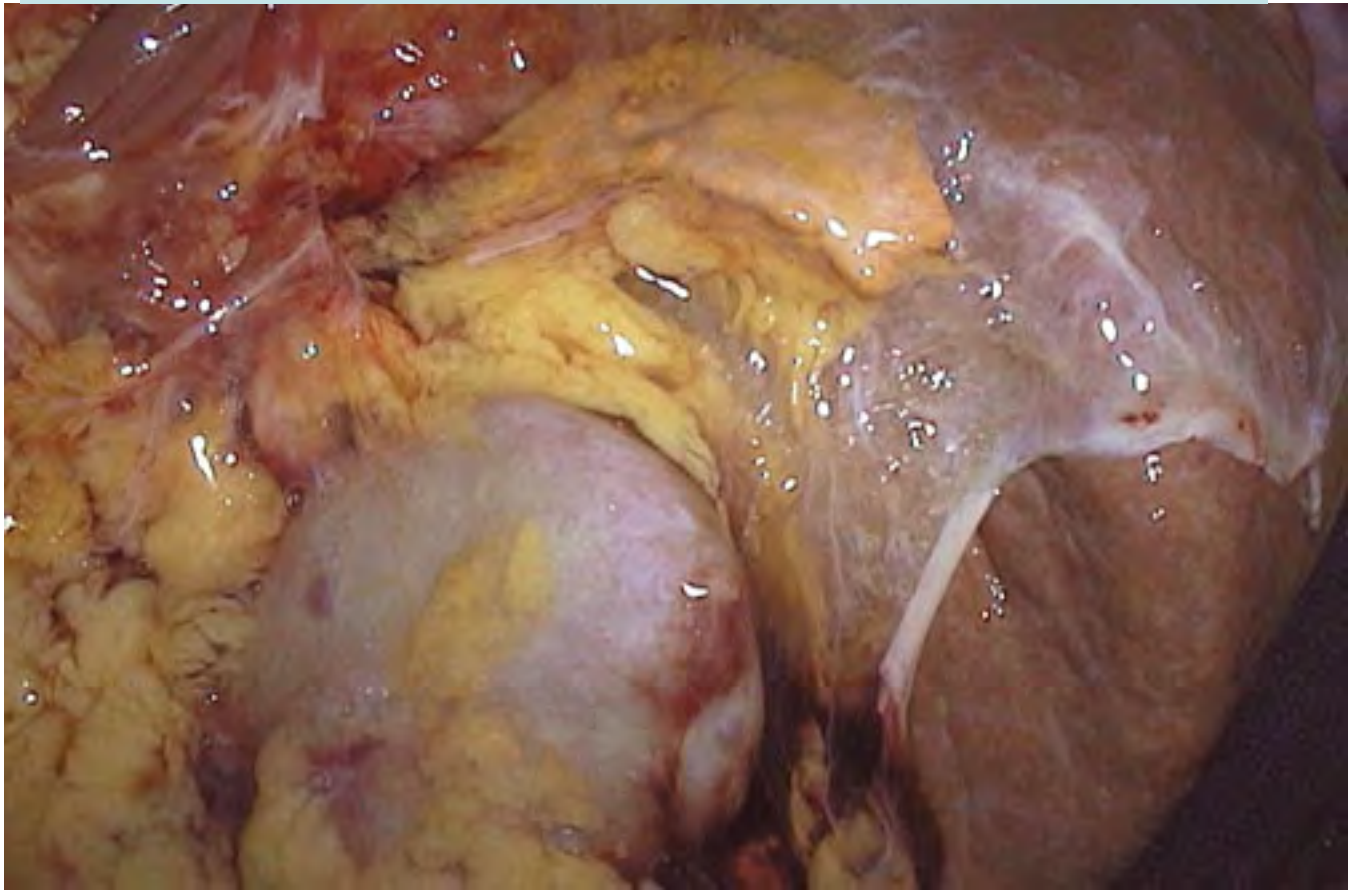


H&E of Mouse Pancreas showing an islet of Langerhans, and a pancreatic duct, surrounded by pancreatic acini

Adrenal glands

Adrenal glands are chiefly responsible for regulating the stress response through the synthesis of corticosteroids and catecholamines , including cortisol and adrenaline .

Human Adrenal gland, right



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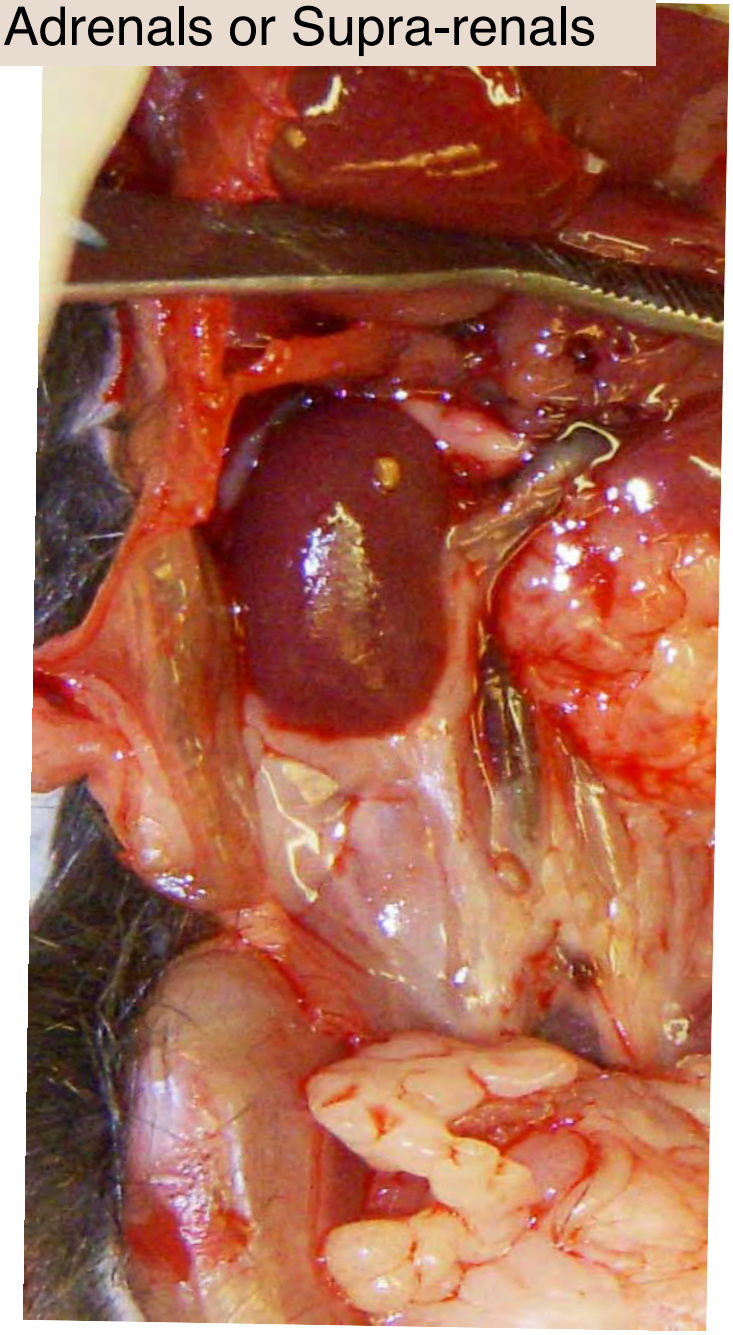
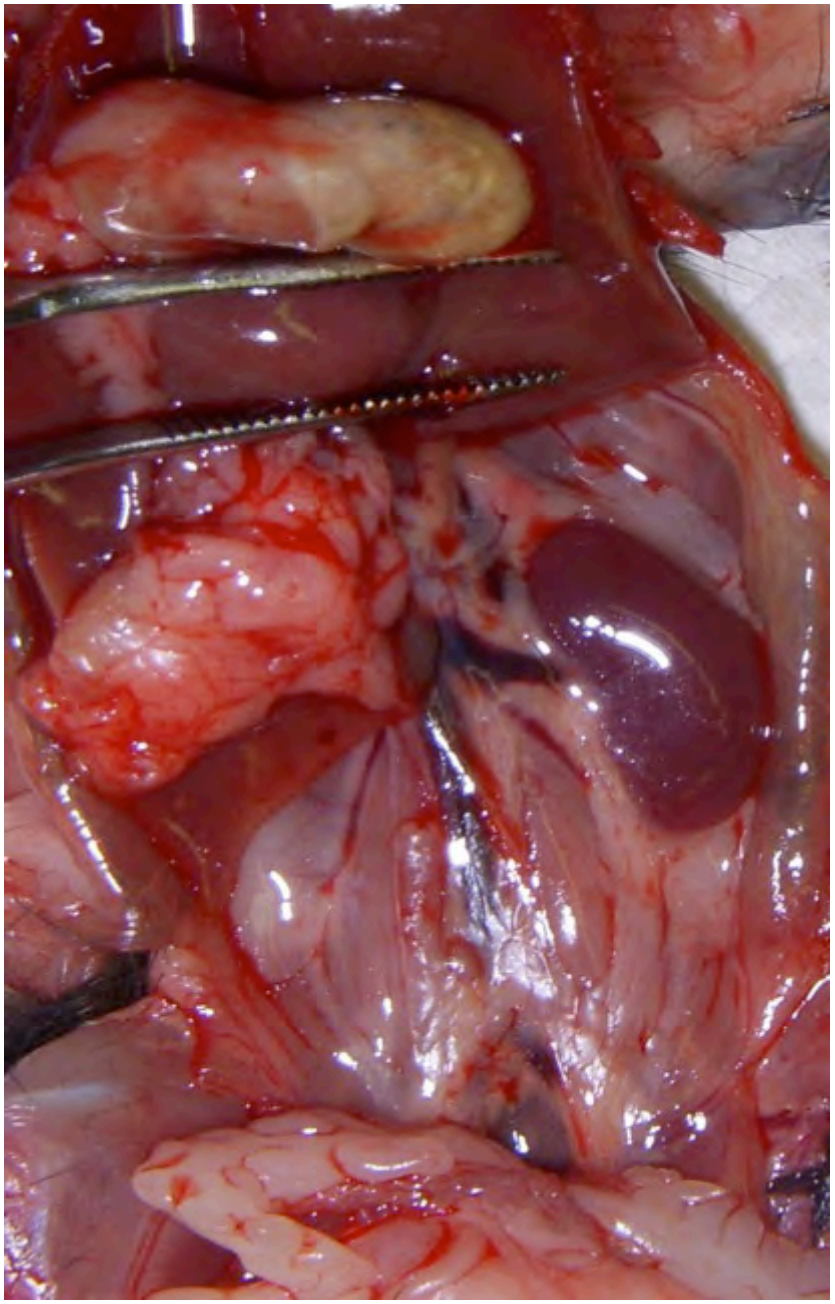
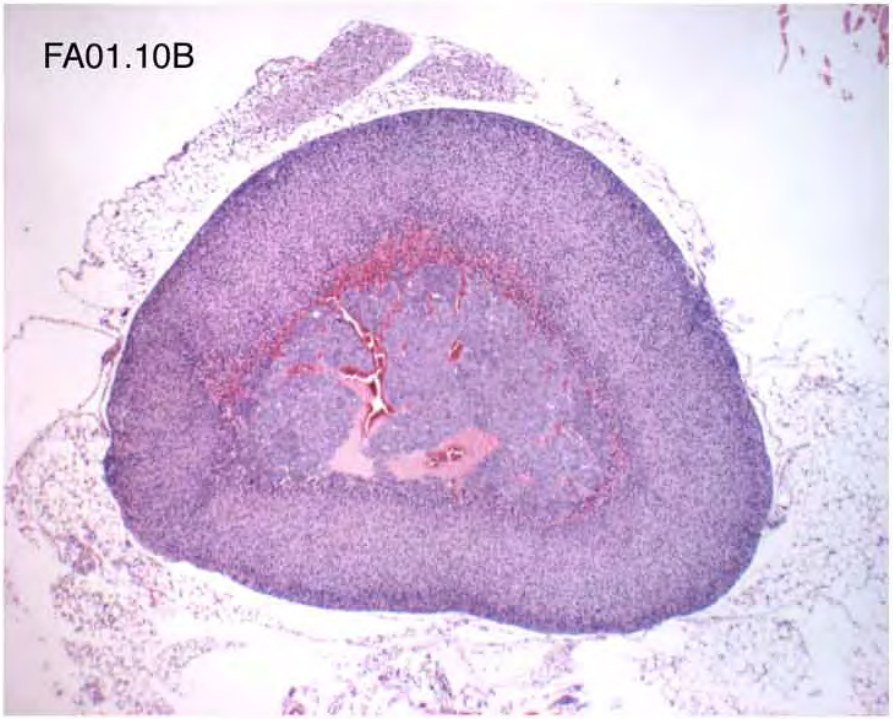
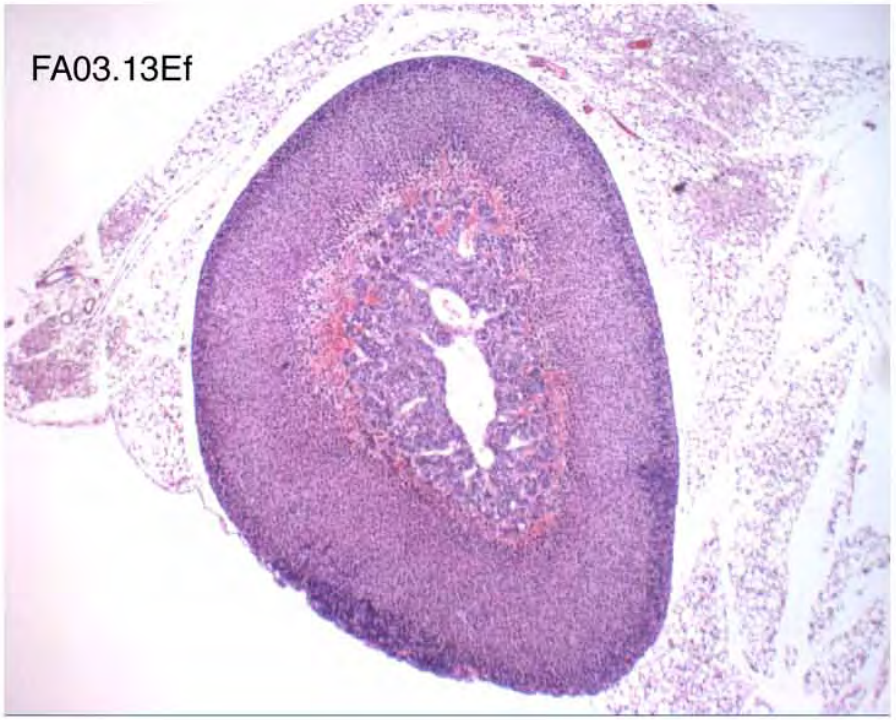
Adrenals or Supra-renals







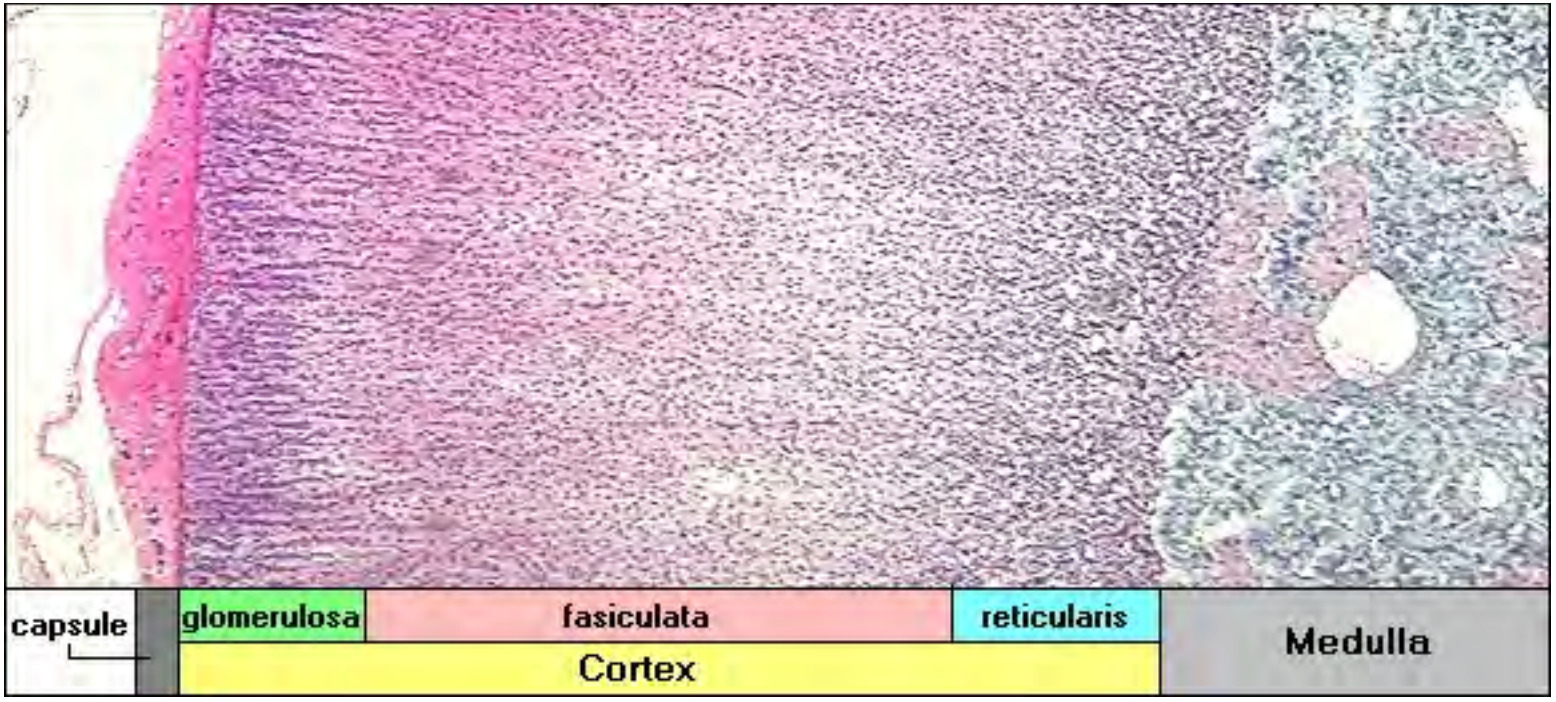


Adrenal from a Male mouse and from a Female mouse

ADRENAL GLANDS:

Despite their organization into a single gland, the medulla and cortex are functionally different endocrine organs, and have different embryological origins.

The medulla derives from ectoderm (neural crest), while the cortex develops from mesoderm

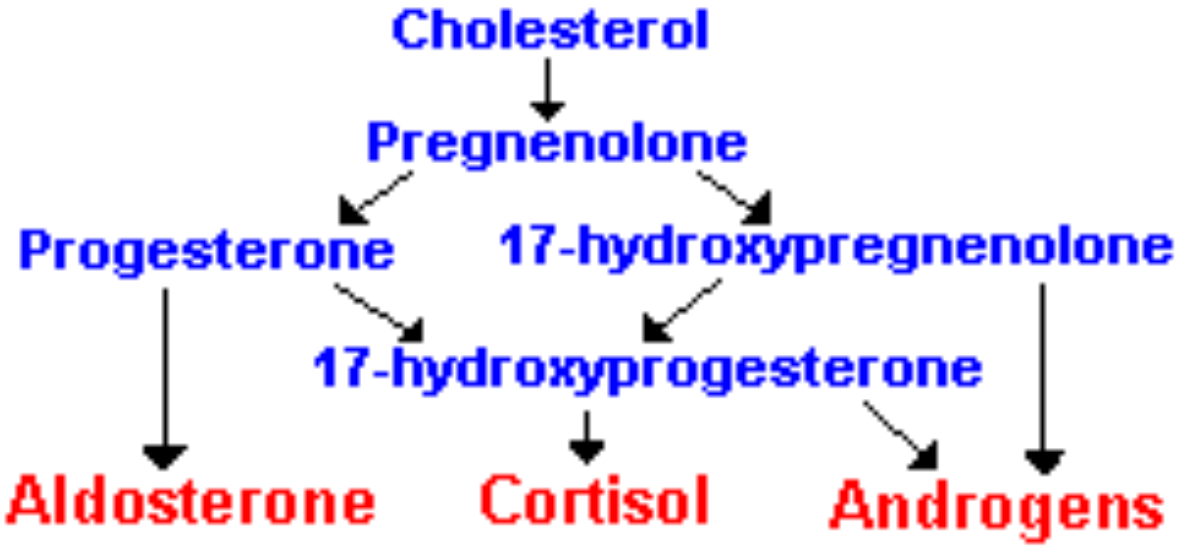


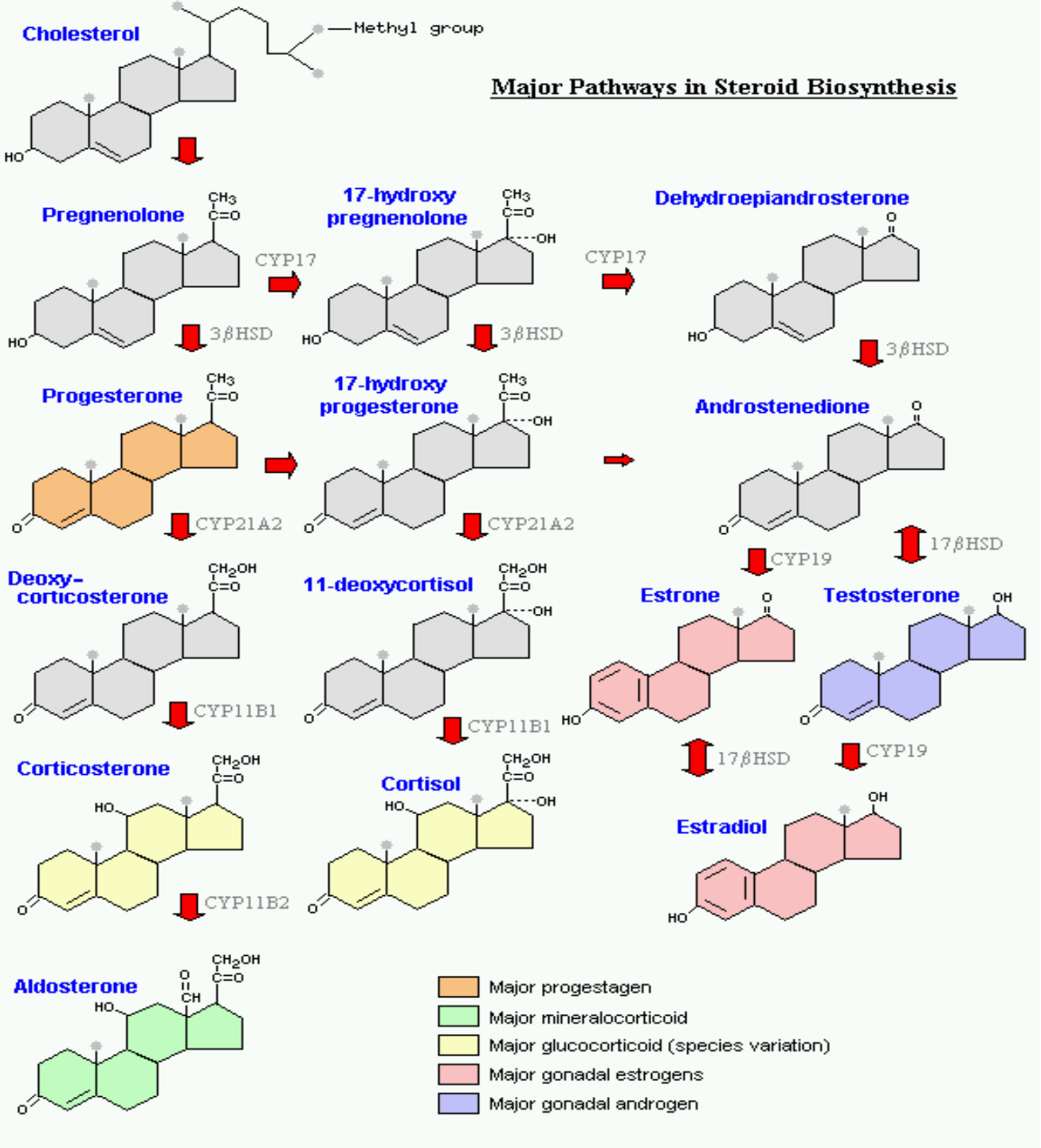
mineralocorticoids (aldosterone) **glucocorticoids , (cortisol),** **sex steroids (androgens)** **catecholamines epinephrine and nor- epinephrine**

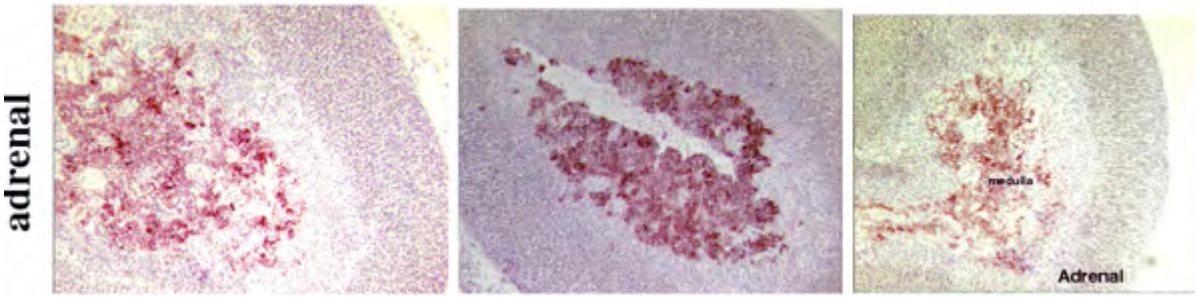
The adrenal cortex is a factory for steroid hormones.

In total, at least two to three dozen different steroids are synthesized and secreted from this tissue,

Class of Steroid	Major Representative	Physiologic Effects
Mineralocorticoids	Aldosterone	Na+, K+, water homeostasis
Glucocorticoids	Cortisol	Glucose homeostasis etc.
Sex steroids	Androgens	




Cholesterol
Methyl group
Major Pathways in Steroid Biosynthesis
Pregnenolone
17-hydroxy pregnenolone
Dehydroepiandrosterone
CYP17
CYP17
3βHSD
3βHSD
3βHSD
Progesterone
17-hydroxy progesterone
Androstenedione
CYP21A2
CYP21A2
CYP19
17βHSD
Deoxy-corticosterone
11-deoxycortisol
Estrone
Testosterone
CYP11B1
CYP11B1
17βHSD
CYP19
Corticosterone
Cortisol
Estradiol
CYP11B2
Aldosterone
Major progestagen
Major mineralocorticoid
Major glucocorticoid (species variation)
Major gonadal estrogens
Major gonadal androgen



The Adrenal Medulla is made up of chromaffin cells which secrete:

Epinephrine and Nor-epinephrine

Epinephrine is also known as adrenaline is a hormone and neurotransmitter [1] that participates in the "fight or flight" response of the sympathetic nervous system.

It is a catecholamine, a sympathomimetic monoamine produced by the adrenal glands from the amino acids phenylalanine and tyrosine.

Other Endocrine organs

Heart produces Atrial-natriuretic peptide (ANP)

Stomach and intestines produce

Cholecystokinin (CCK)

Gastrin, Ghrelin, Neuropeptide Y (NPY), Secretin

Somatostatin

Liver produces Insulin-like growth factor (IGF)

Angiotensinogen Thrombopoietin

In males only: Testes Androgens (chiefly testosterone)

In females only

Ovarian follicle Estrogens (mainly estradiol)

Corpus luteum Progesterone Estrogens (mainly estradiol)

Placenta (when pregnant)

Progesterone Estrogens (mainly estriol)

Human chorionic gonadotropin (HCG)

Human placental lactogen (HPL)

Kidney:

Renin

Erythropoietin (EPO)

Calcitriol (the active form of vitamin D 3

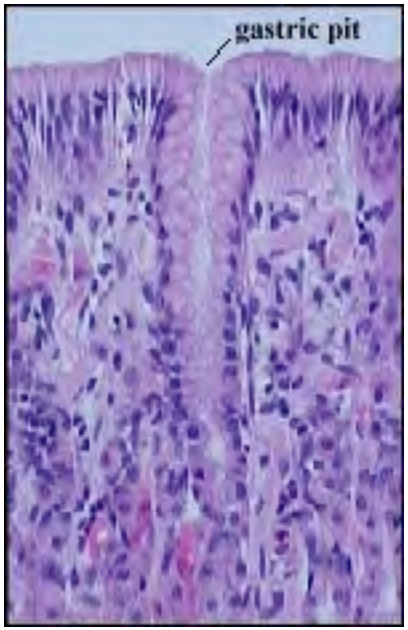
Skin produces Vitamin D 3(calciferol)

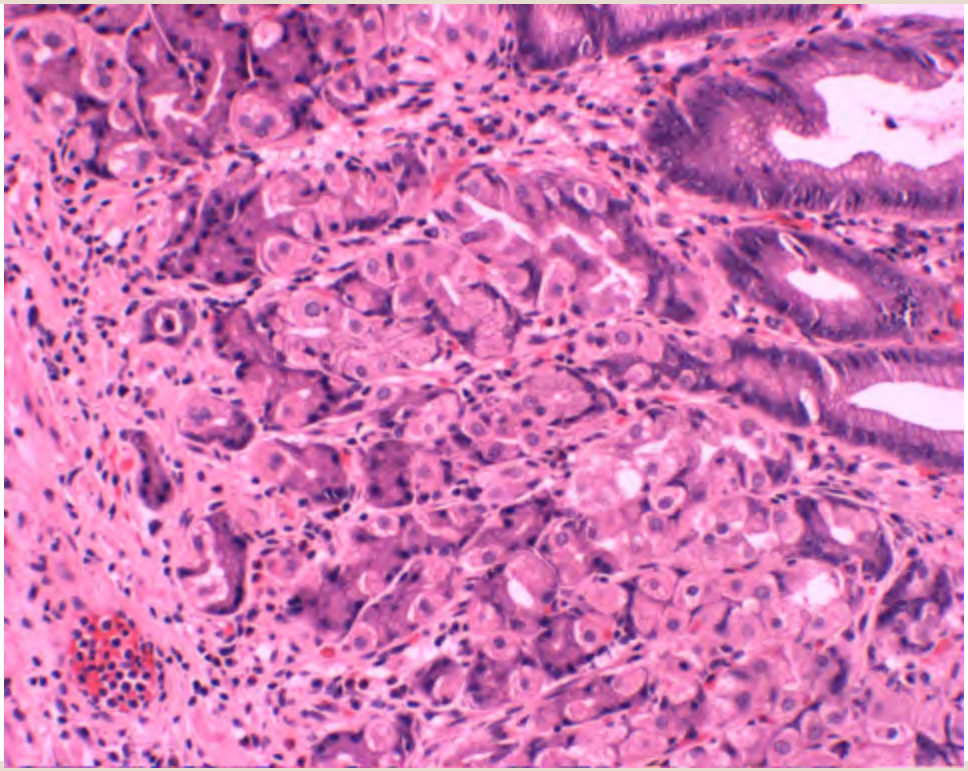
Adipose tissue

Leptin

Estrogens (mainly estrone)

Endocrine cells situated in other organs include Gastrin producing or G cells in the stomach





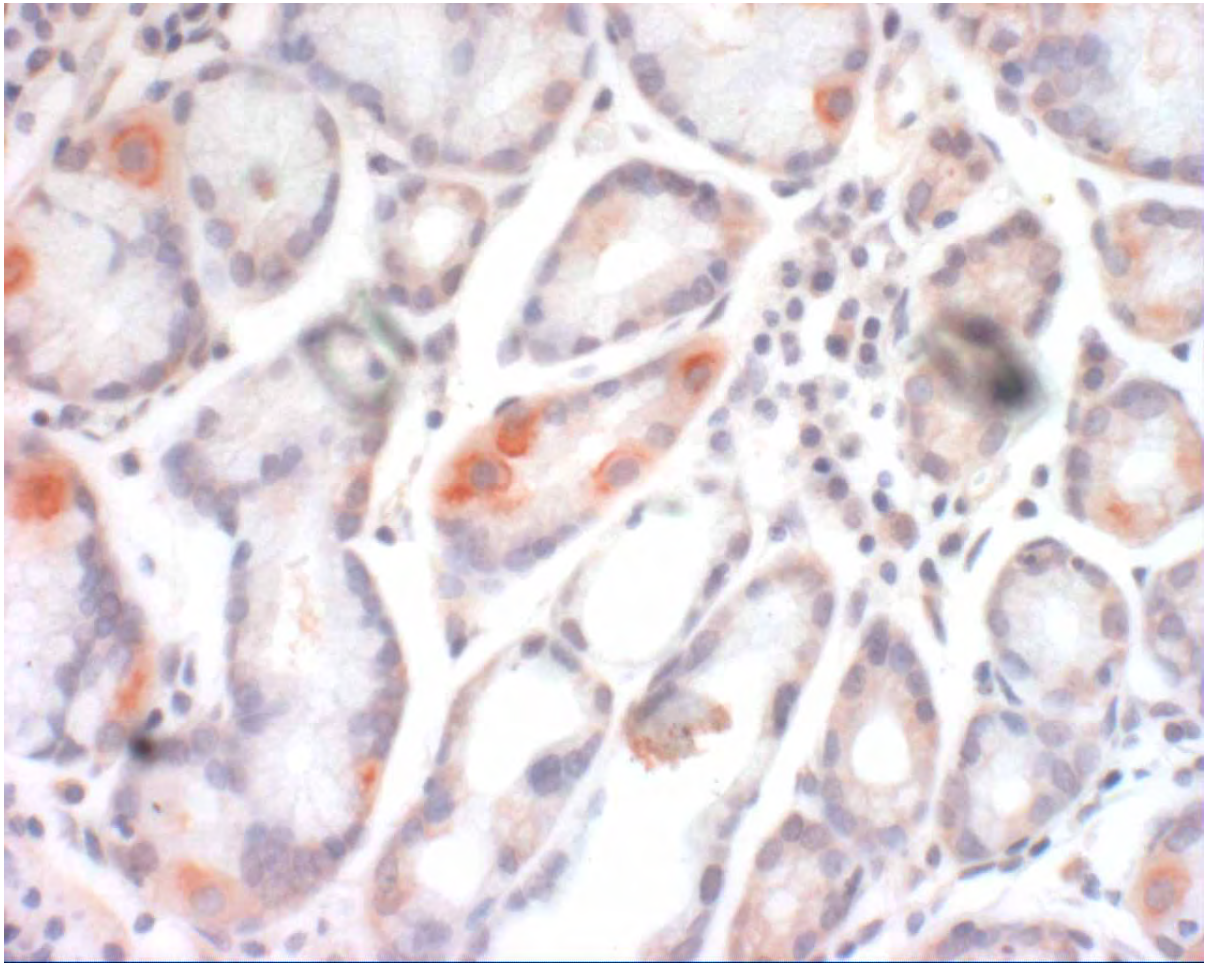
Four major types of secretory epithelial cells cover the surface of the stomach and extend down into gastric pits and glands:

*** Mucous cells: secrete an alkaline mucus that protects the epithelium against shear stress and acid**

*** Parietal cells: secrete hydrochloric acid and Intrinsic factor for Vit B12**

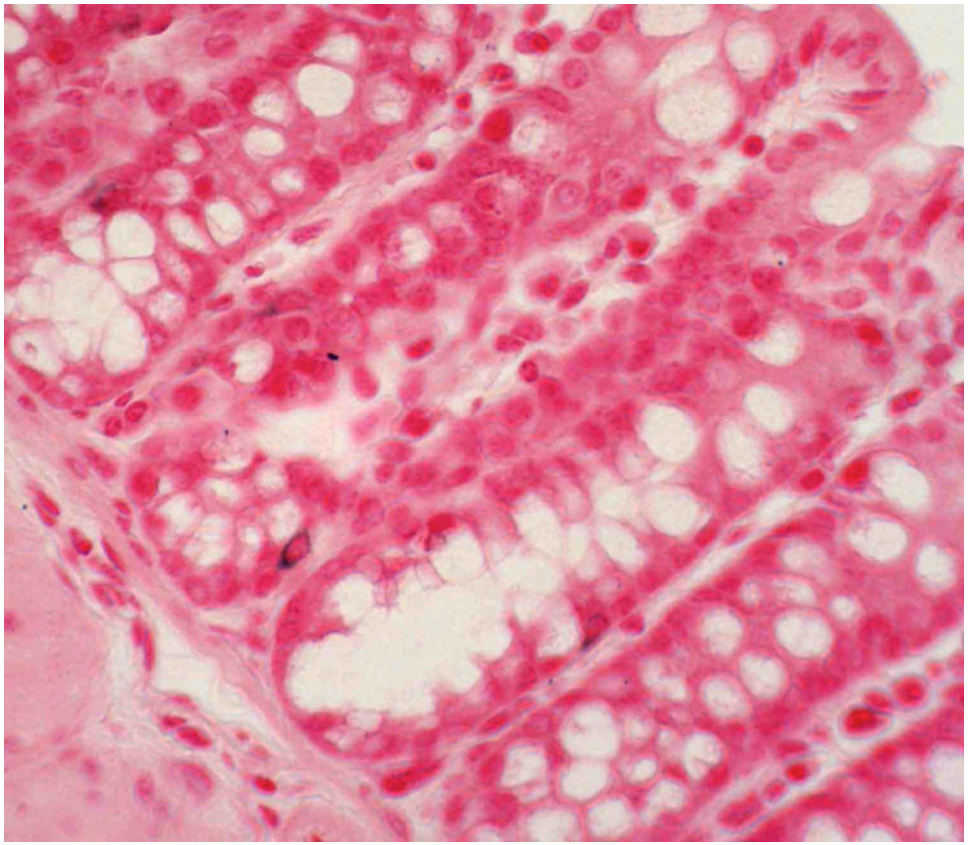
*** Chief cells: secrete pepsin, renin and lipase to start the digestive process**

*** G cells: secrete the hormone gastrin**



G cells in human stomach identified using IHC

Endocrine cells situated in other organs include those in the intestine



Changes that occur during Ovarian Follicle Maturation

FOLLICULAR PHASE

Primary follicle

oocyte

zona pellucida

granulosa cells

theca folliculi

Secondary or antral follicle

cumulus oophorus

corona radiata

Mature or Graafian follicle

ovulation

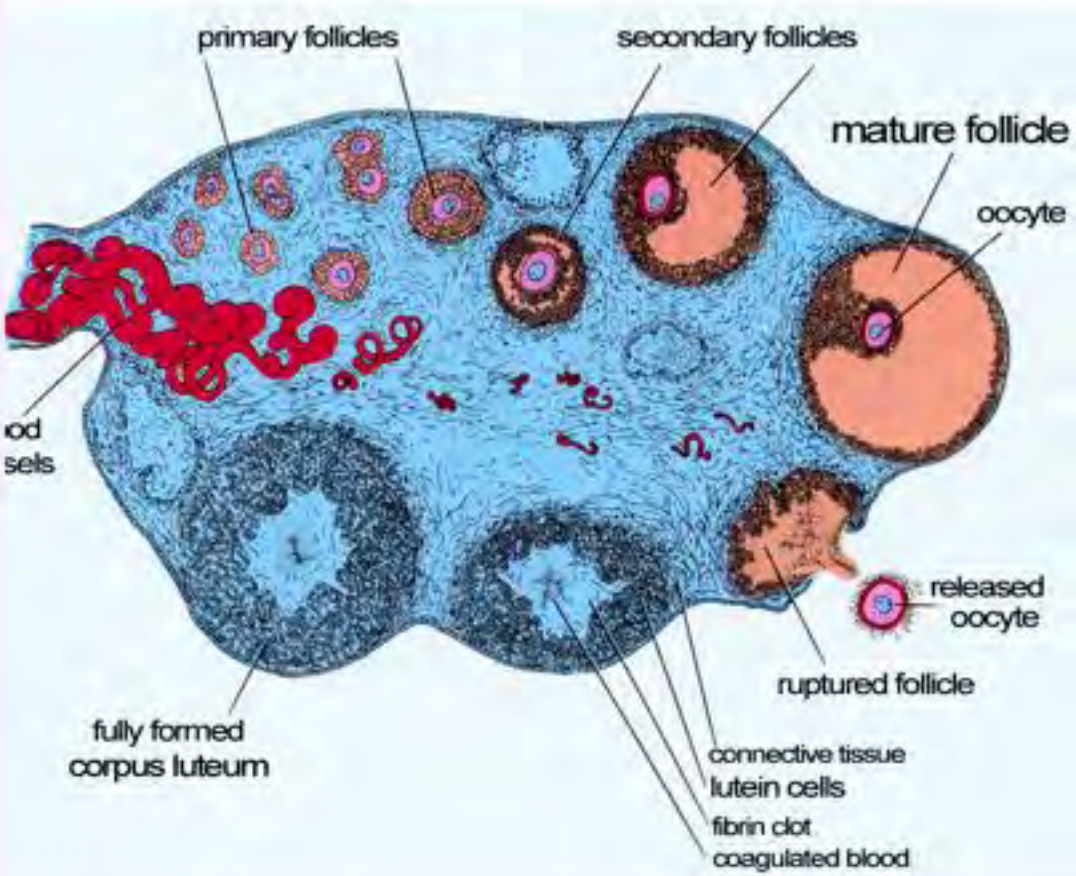
LUTEAL PHASE

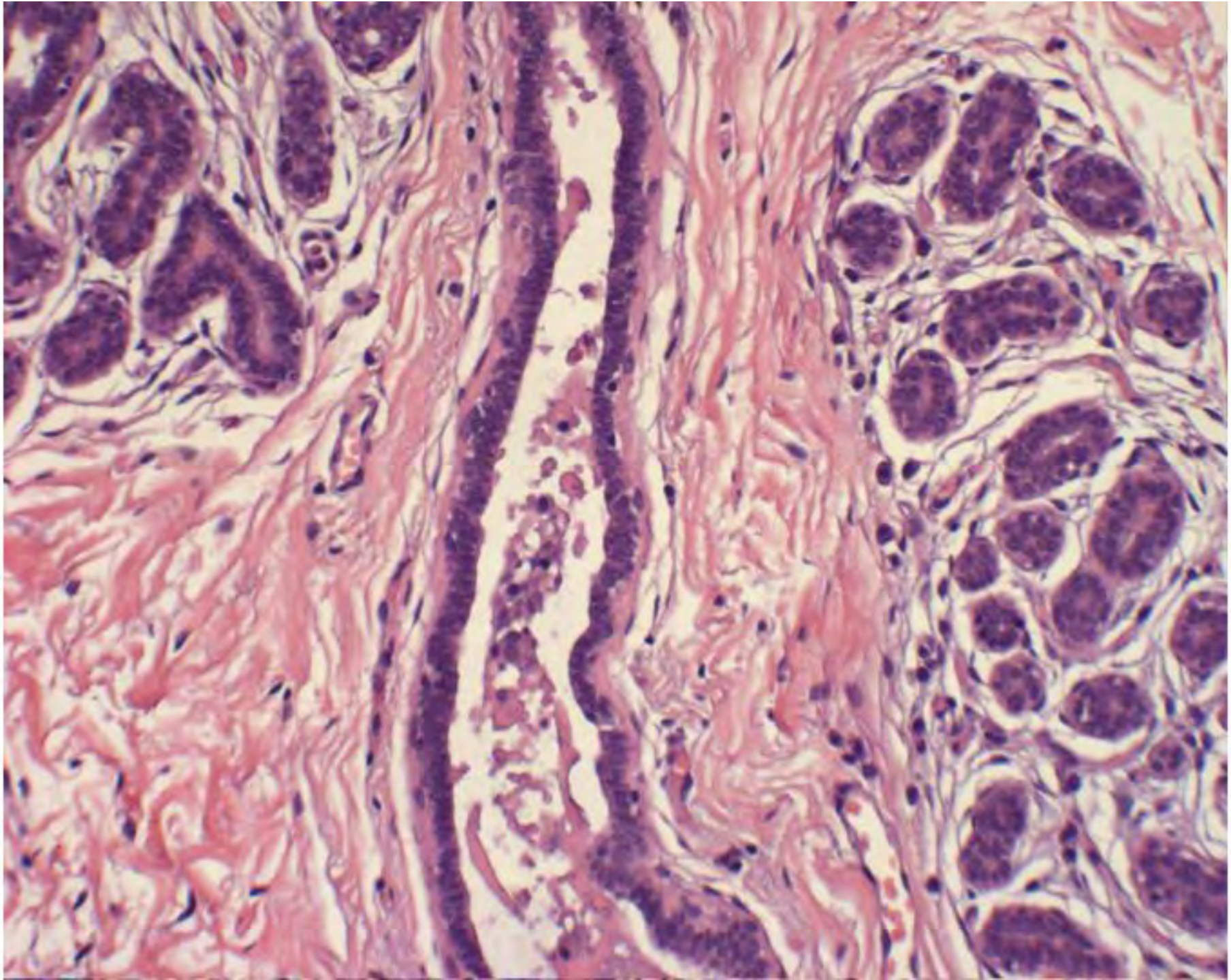
Corpus luteum

granulosa lutein cells

theca lutein cells

(Corpus albicans)





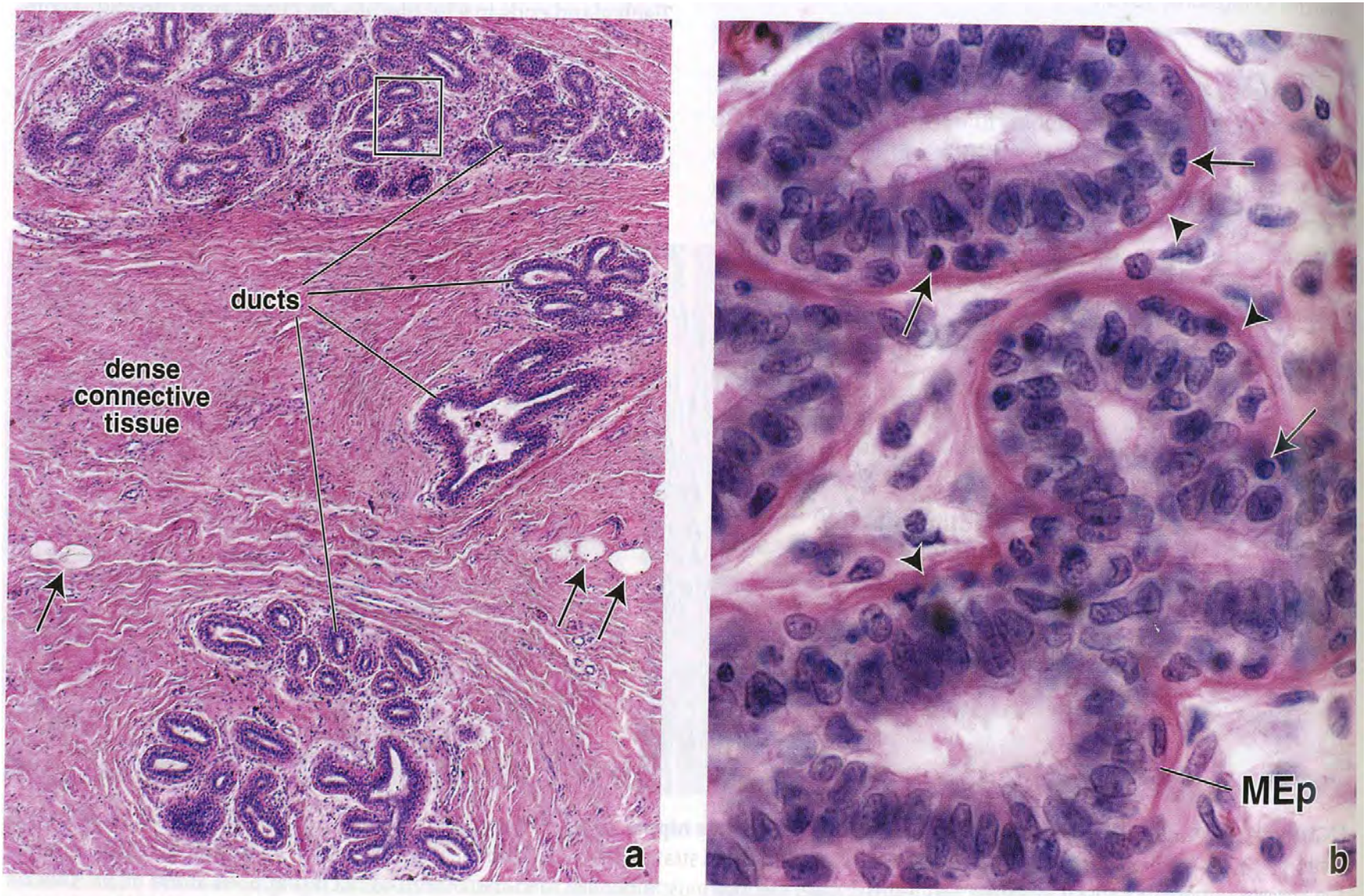
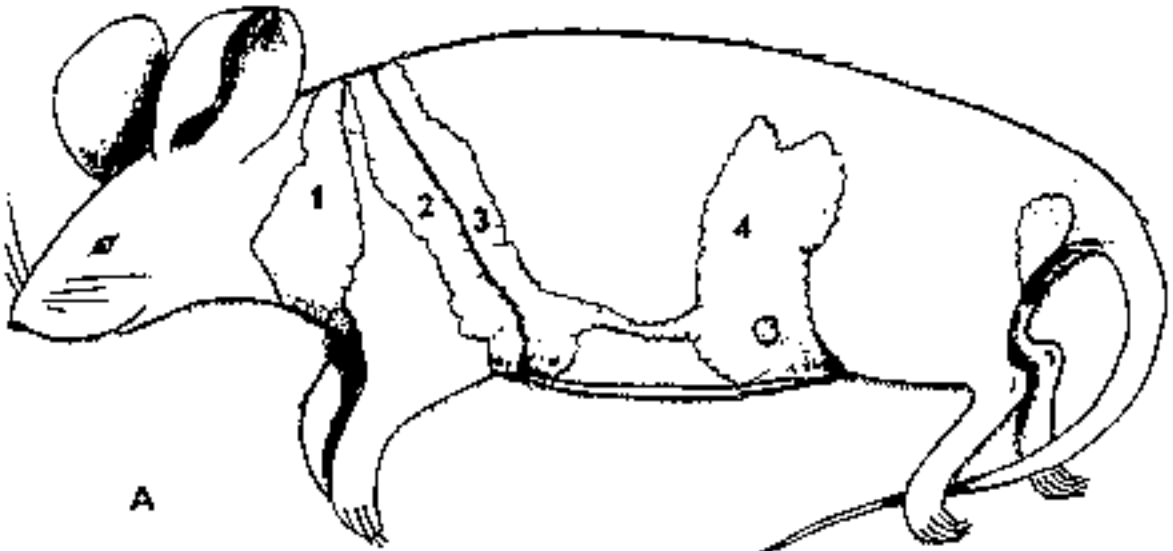
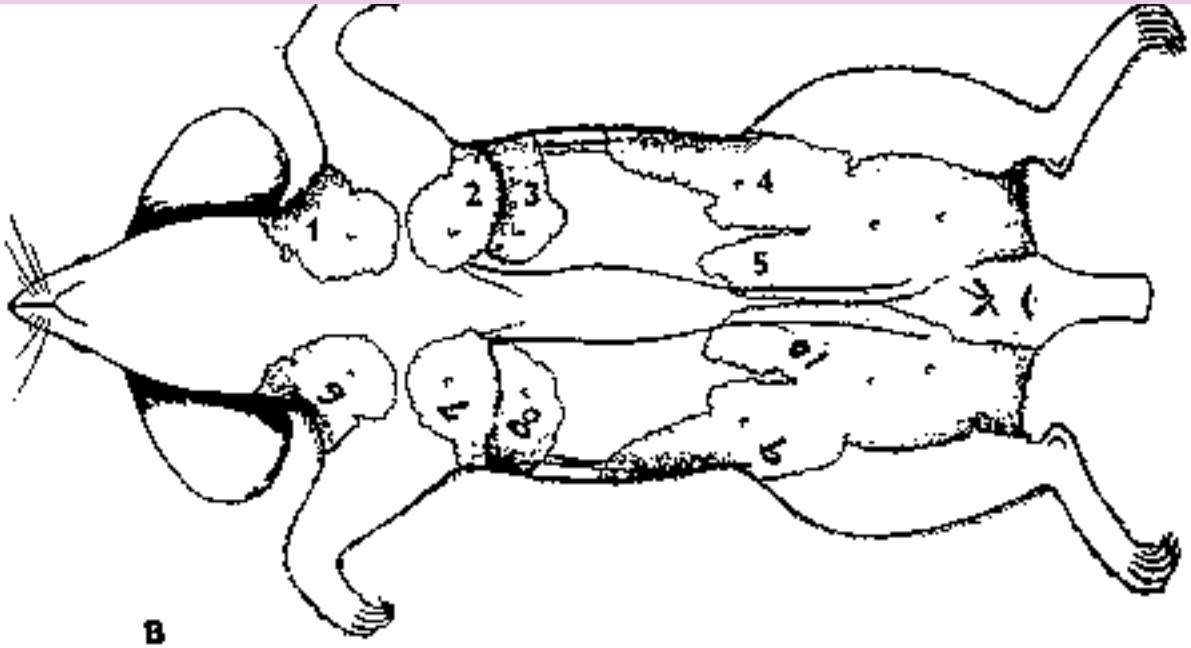


FIGURE 23.34 Photomicrograph of an inactive mammary gland. a. This low-magnification H&E–stained specimen shows several lobules



Location of the mammary glands in the mouse--#4 has adjacent lymph node to help with identification



Adipose tissues

White Adipose Tissue (WAT): Adult adipose tissue contains lipids that dissolve during processing into paraffin.

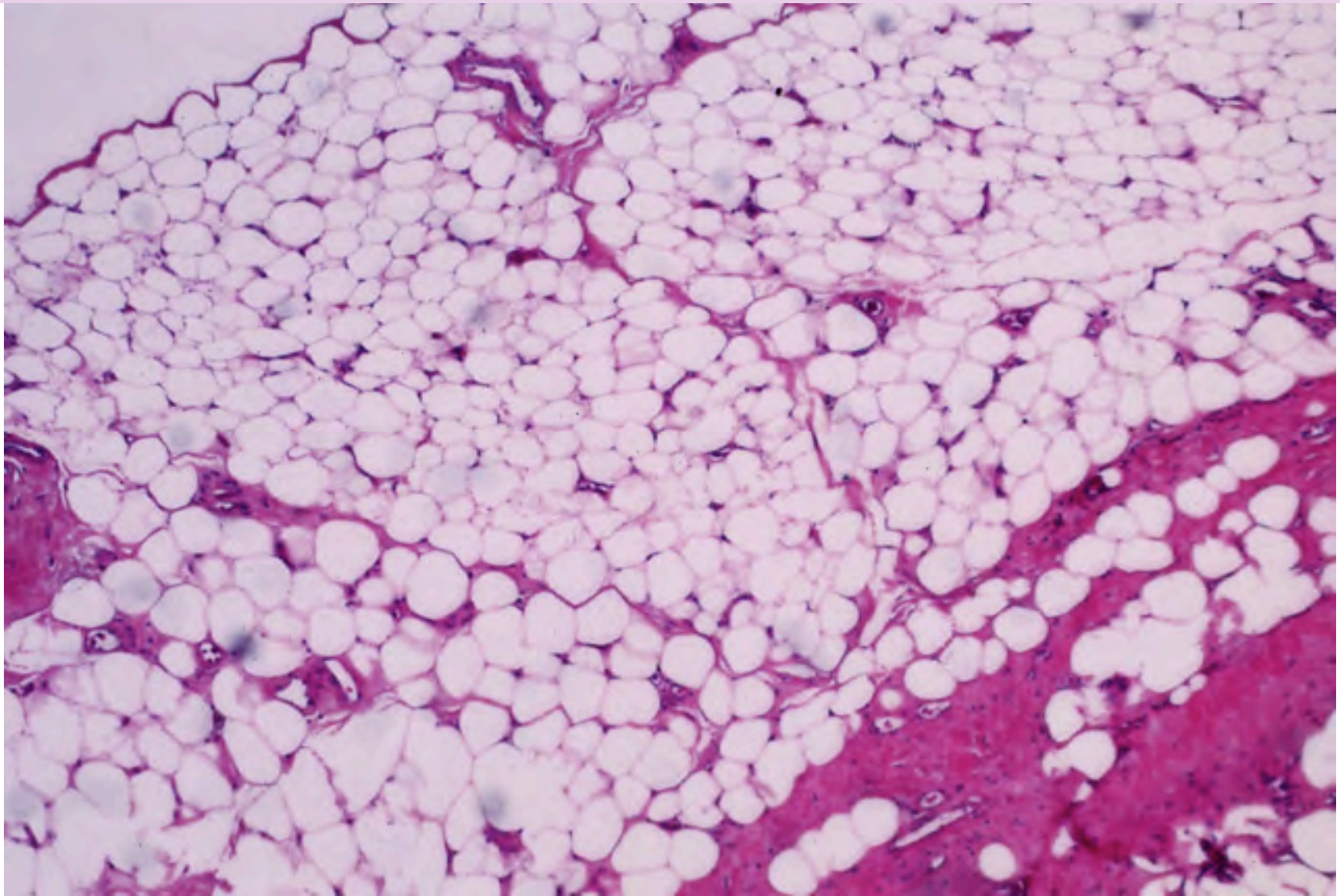
WAT is unilocular and appears like empty vacuoles on H&E

Brown Adipose Tissue (BAT): abundant in the embryo, in the interscapular area.

BAT is multilocular and serves as a source of heat in hybernating animals. The cells contain abundant mitochondria and thus appear pink on H&E.

FROZEN sections of fat: DIFFICULT

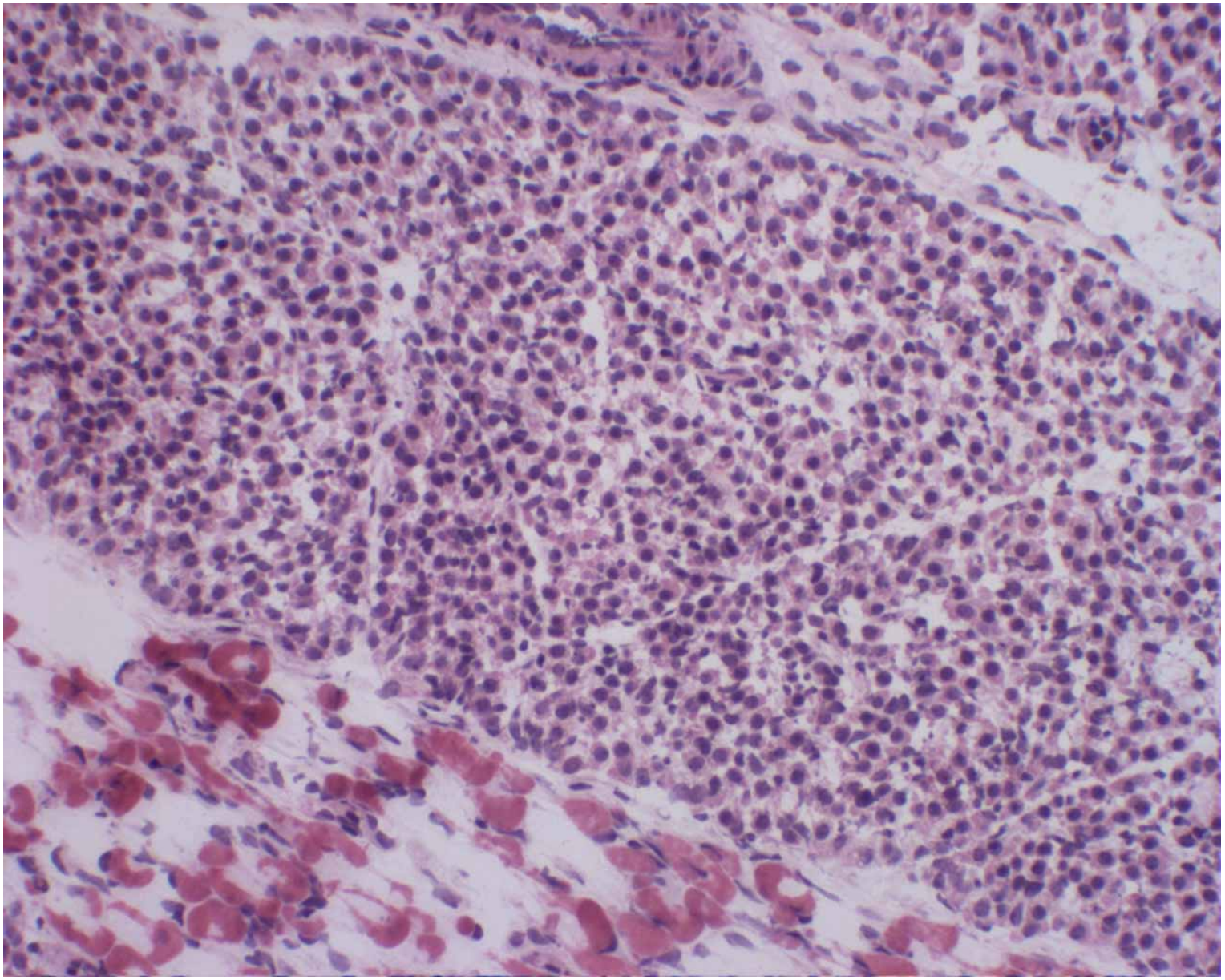
Adipose tissue has to be fixed FLAT and processed into paraffin blocks. Thus fat cells look empty on paraffin sections, material dissolved during processing



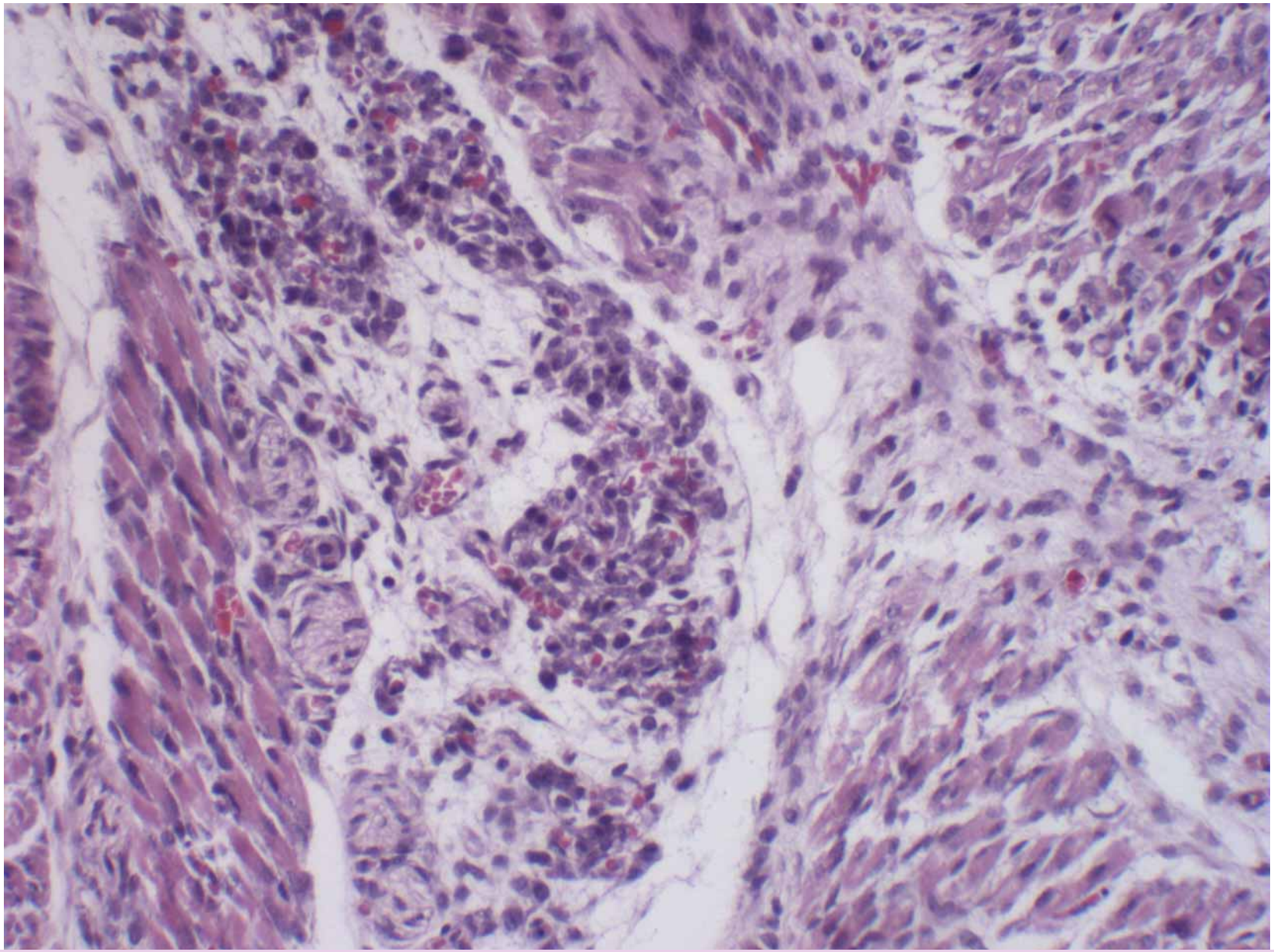
Brown adipose tissue

Frozen sections (Sagittal) of embryo d 14.5)

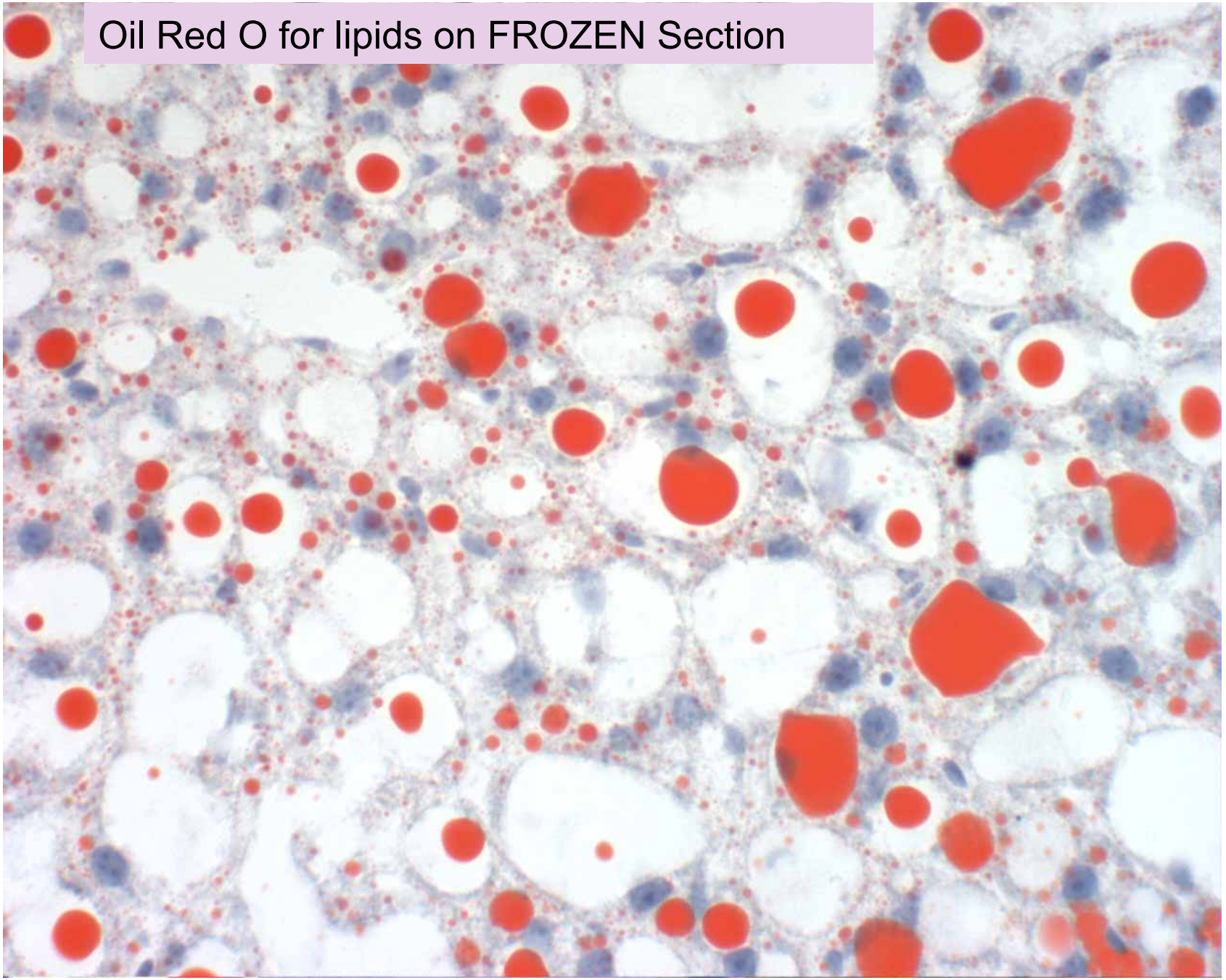
at the midline (thymus is visible)



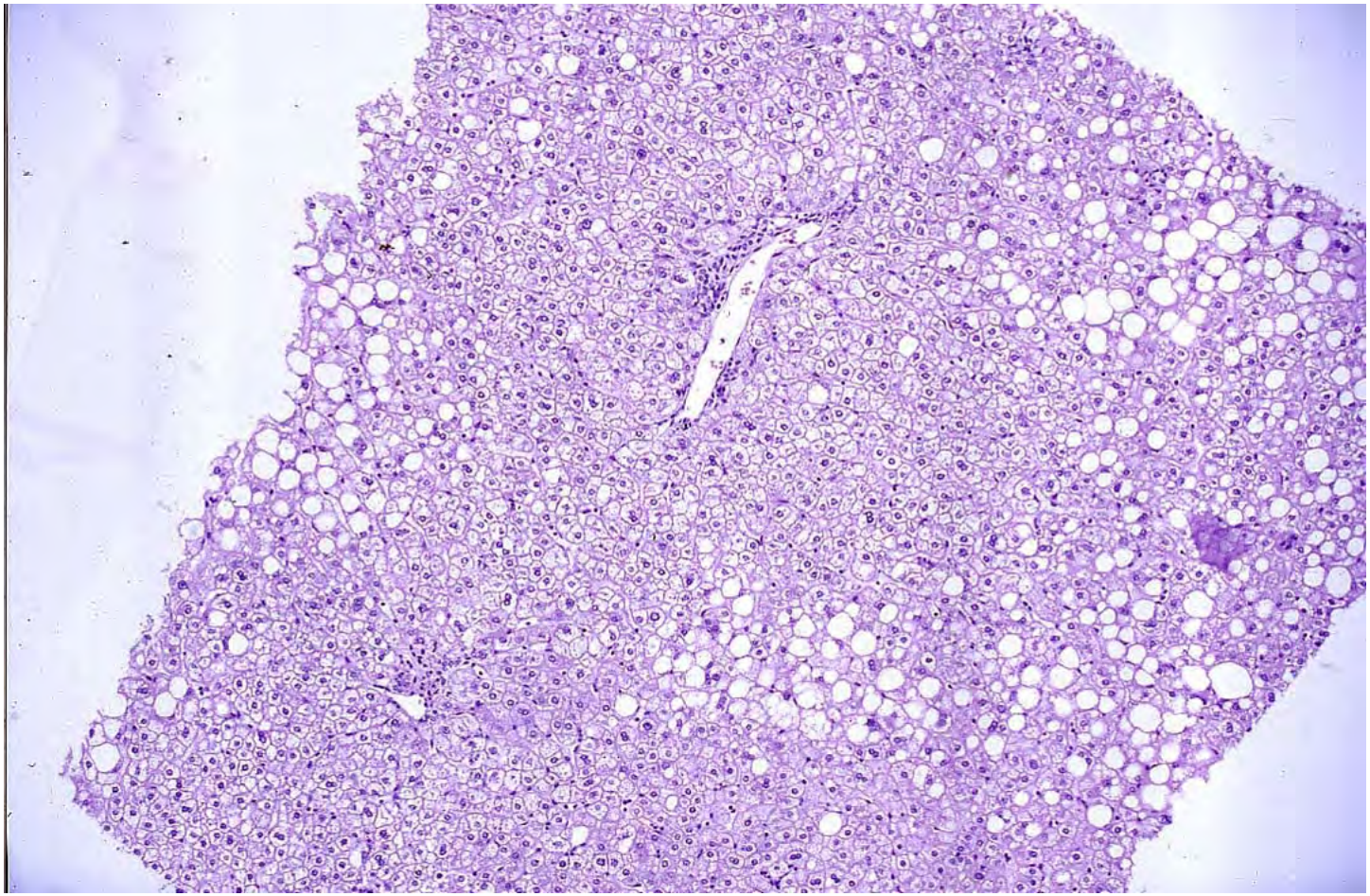
H&E of brown fat--confirm by doing an oil red O stain



H&E of brown fat in a mutant mouse, show that is deficient--confirm by doing an oil red O stain

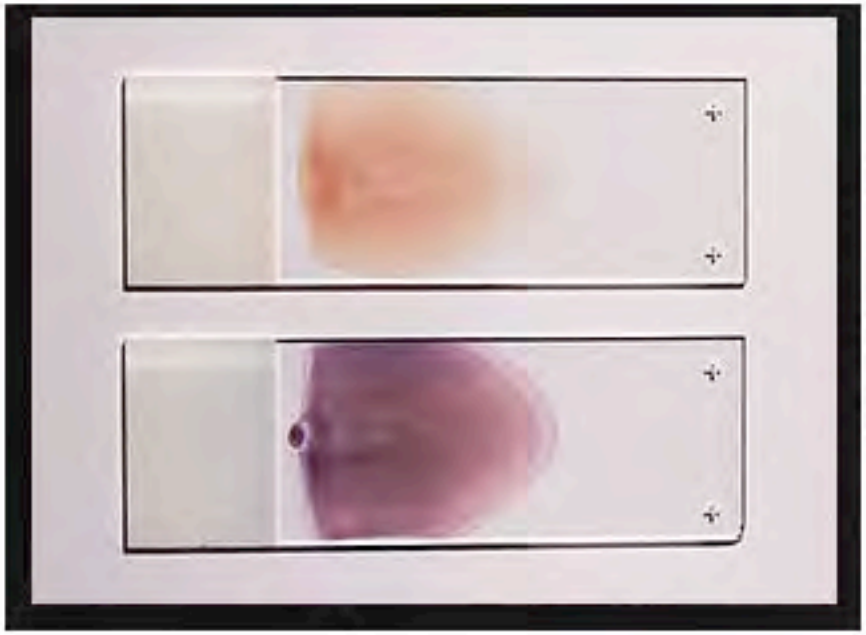

Oil Red O for lipids on FROZEN Section

Cannot do Oil Red O on paraffin sections



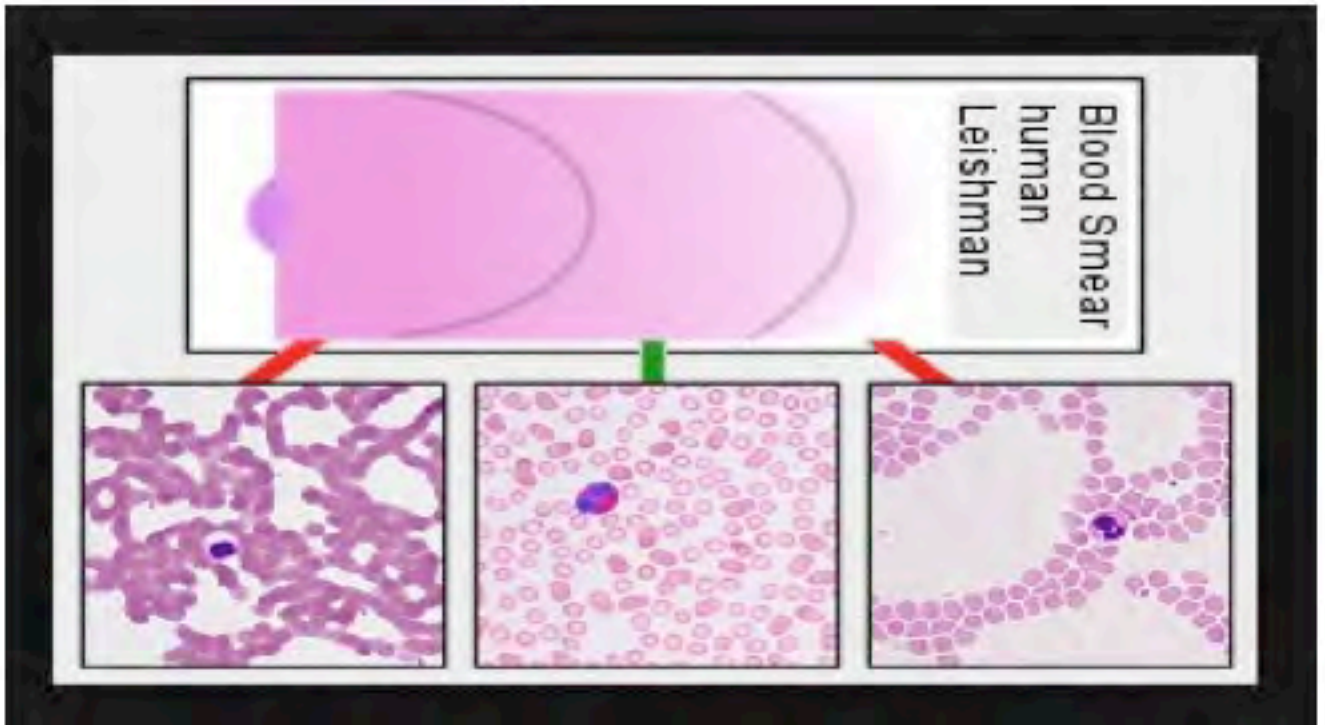
Special Stains in histology

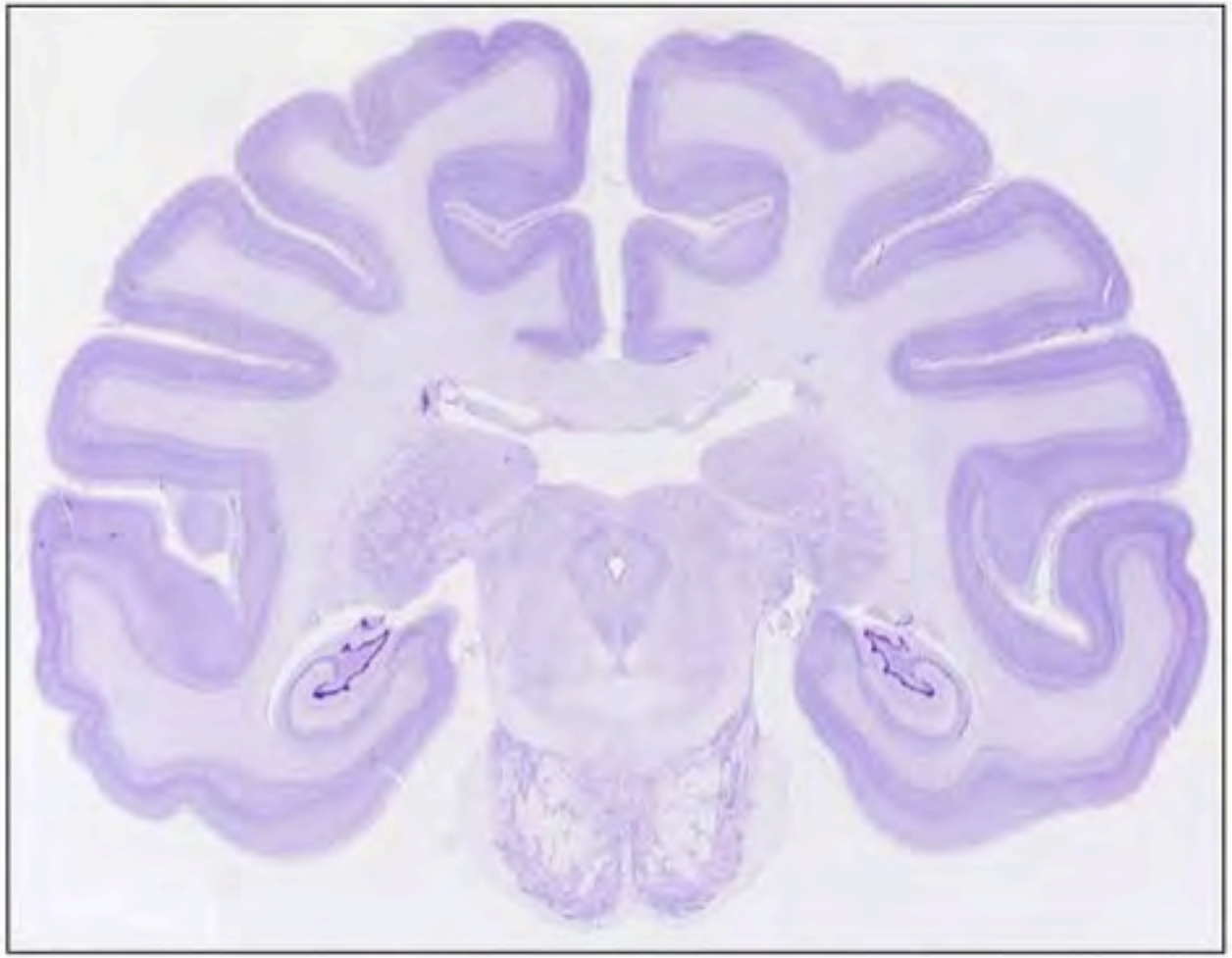
Hematoxylin and Eosin is a routine standard stain that is done to check morphology and to help with diagnosis



Unstained blood smear

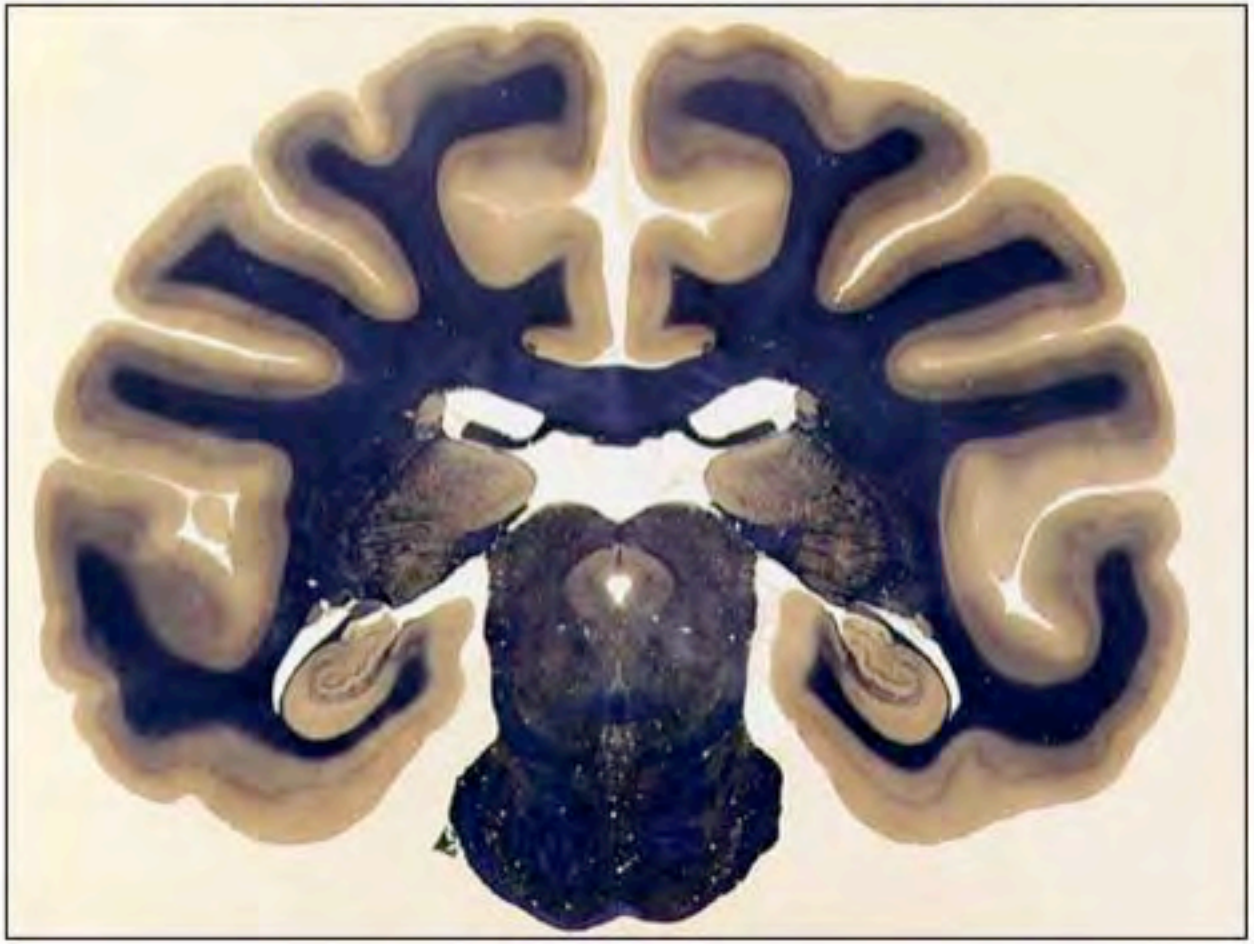
Giemsa-Wright stained blood smear





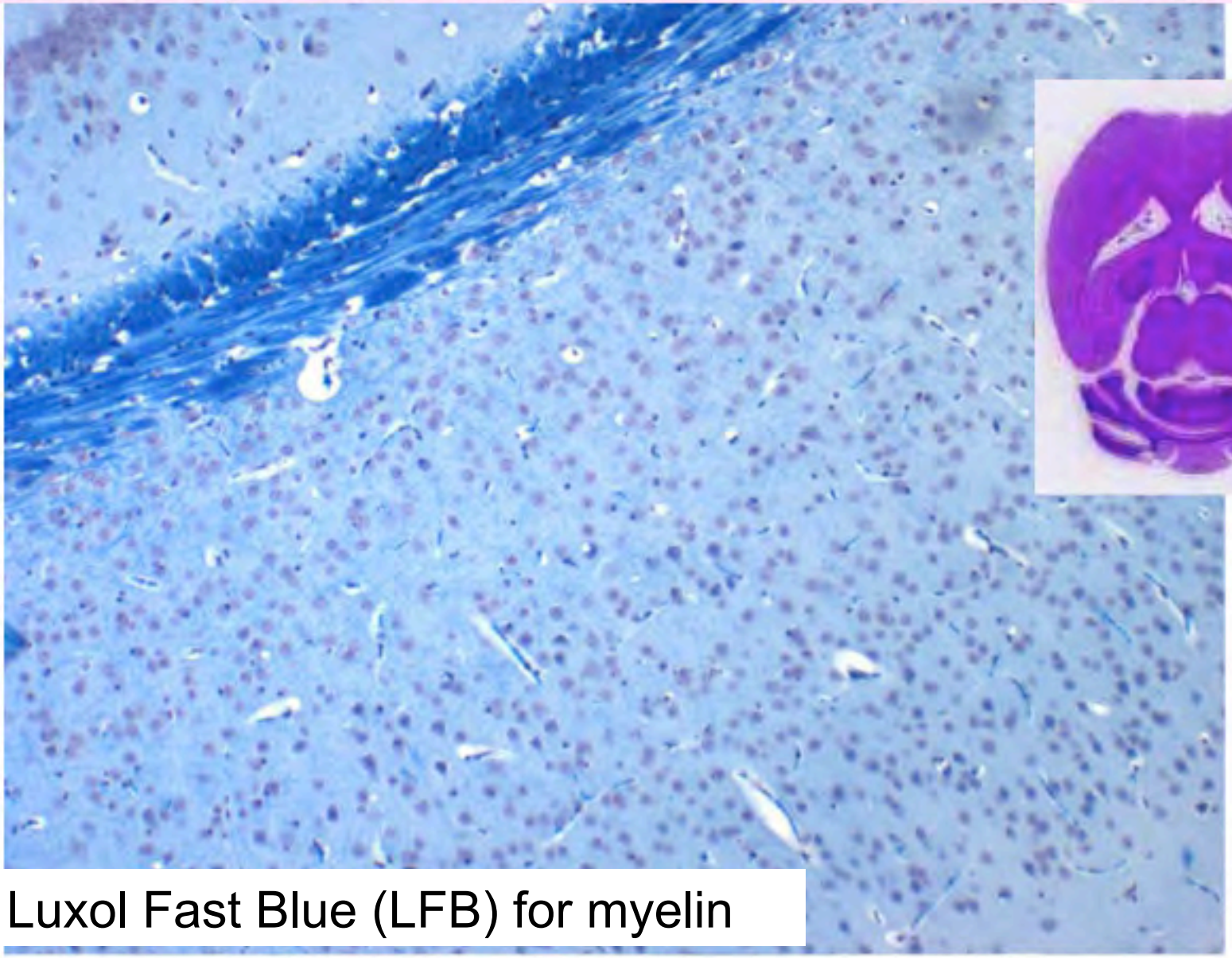
Nissl stain (e.g., cresyl violet, thionin, azure) stains nuclei acids (DNA and RNA). This stain is useful for viewing cell sizes and numbers. **Note the position of the hippocampus**

http://www.neuroscienceassociates.com/Stains/weil_myelin.htm

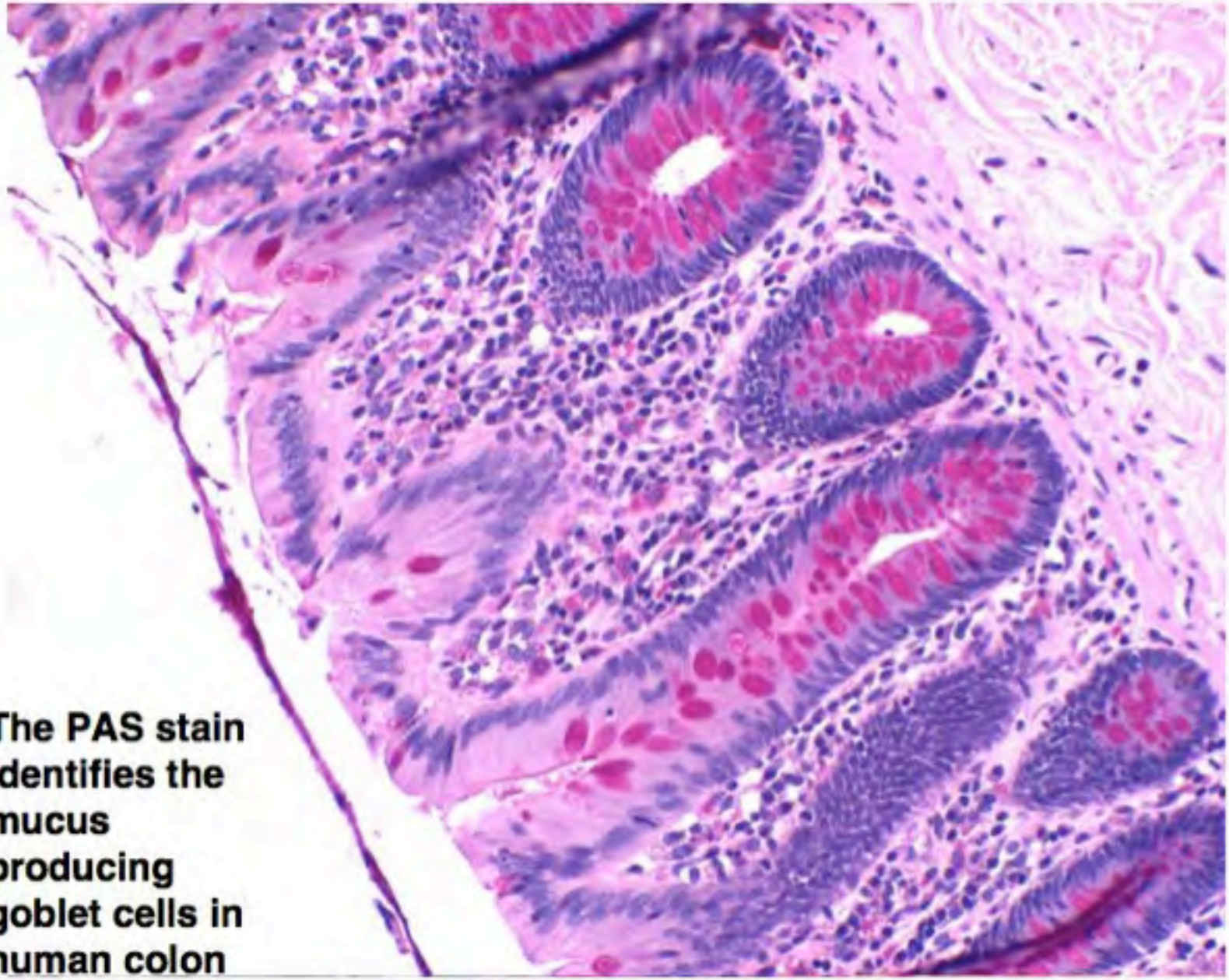


Myelin stain of Human brain

White Matter paraffin sections of Mouse Brain: Myelin stain

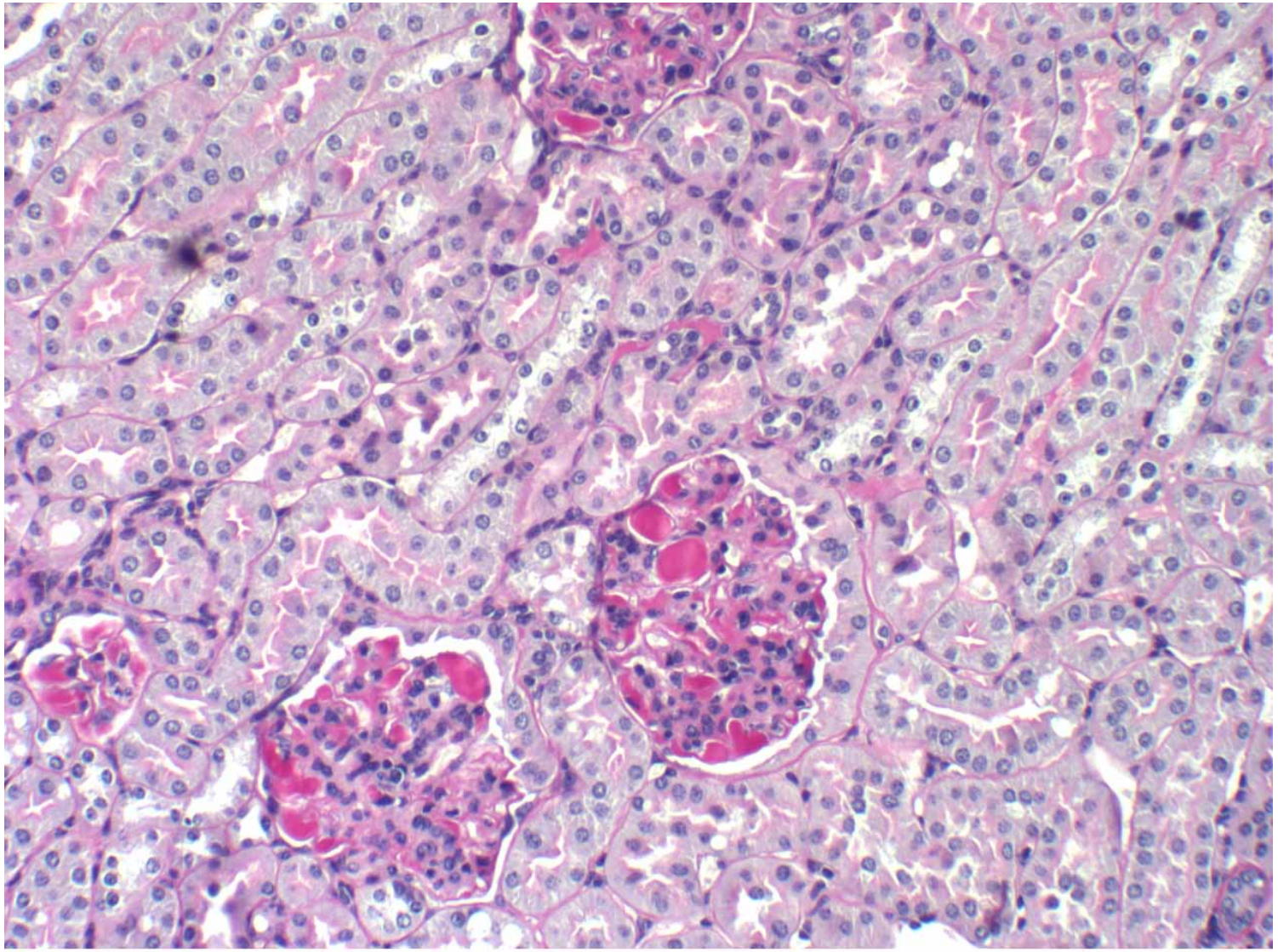


Luxol Fast Blue (LFB) for myelin



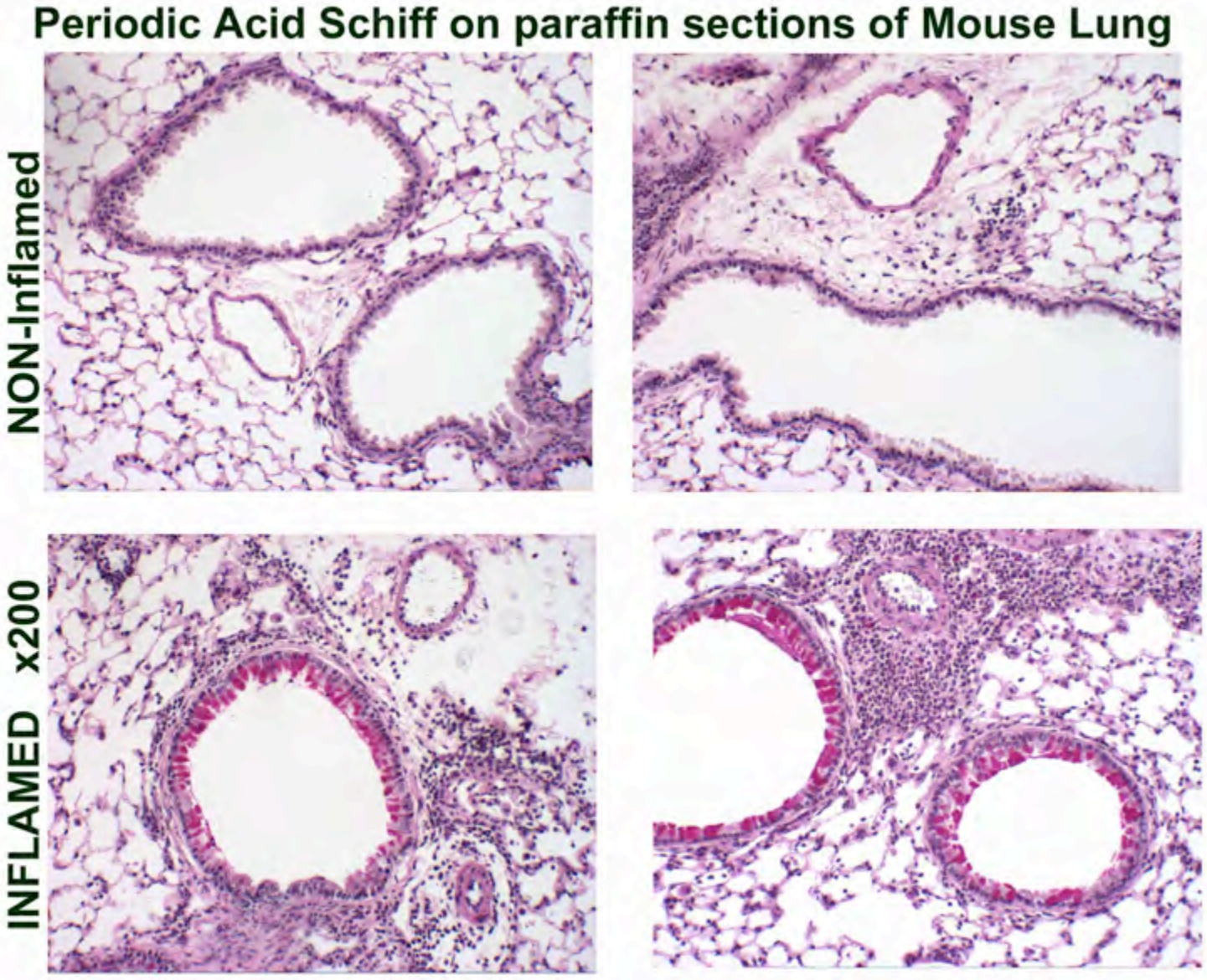
The PAS stain identifies the mucus producing goblet cells in human colon

PAS of kidney to demonstrate carbohydrate-rich accumulations in abnormal glomeruli

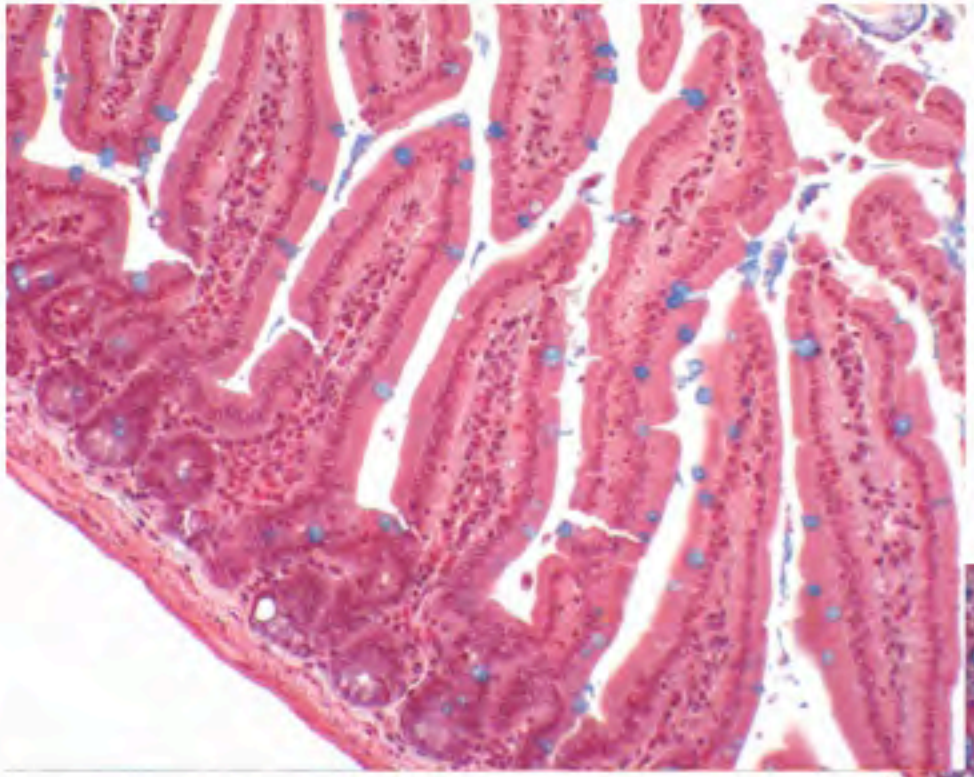


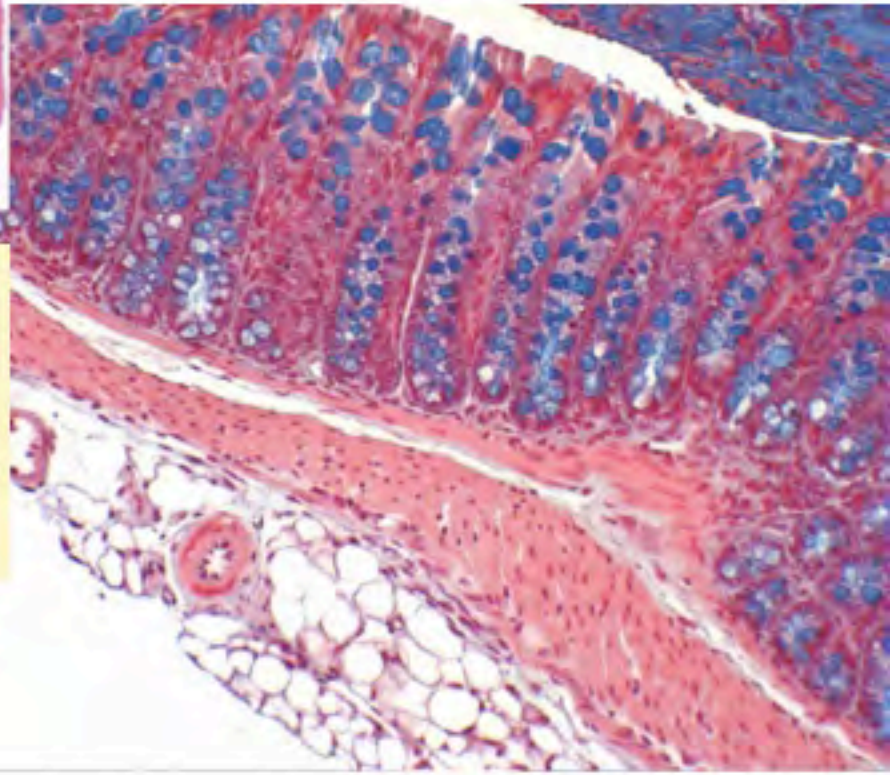
Mouse models of asthma induces inflammation

Normal mouse bronchioles have no mucus secreting cells.



Inflammation induces the bronchial epithelial cells to make mucus, demonstrated using the PAS stain





Mucin identified with Alcian Blue in

Small intestine and in

Colon

Nuclei are stained with nuclear fast red

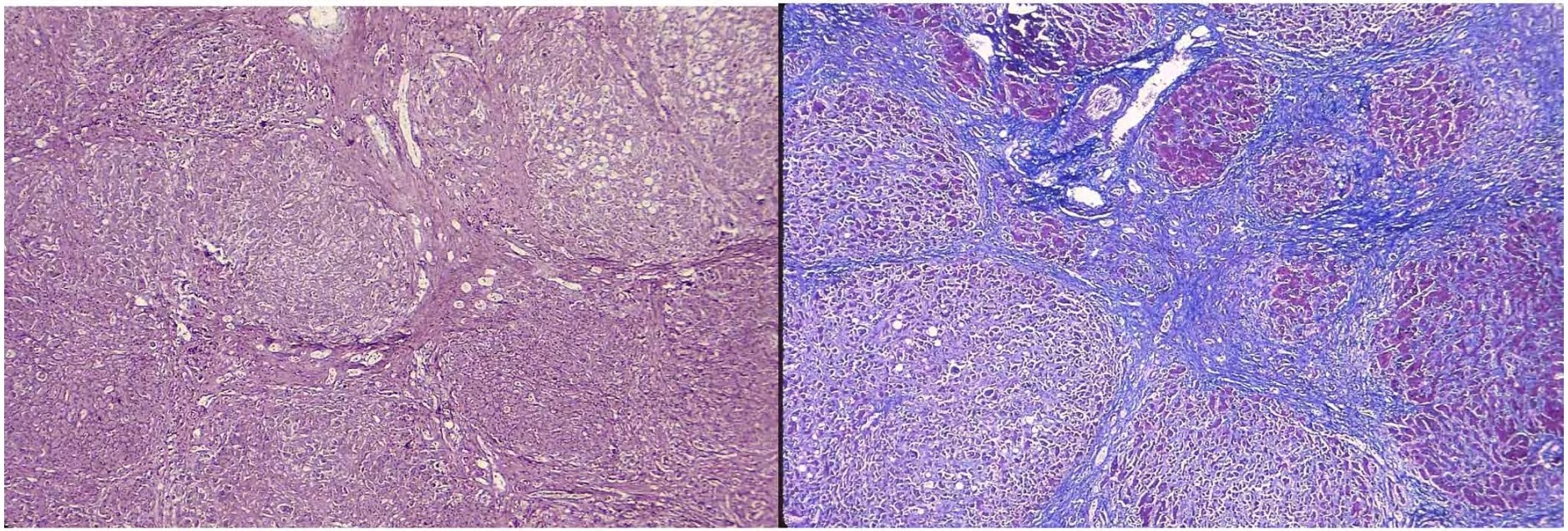
The Mucin and the PAS stain identifies the mucus producing goblet cells in colon and small intestine

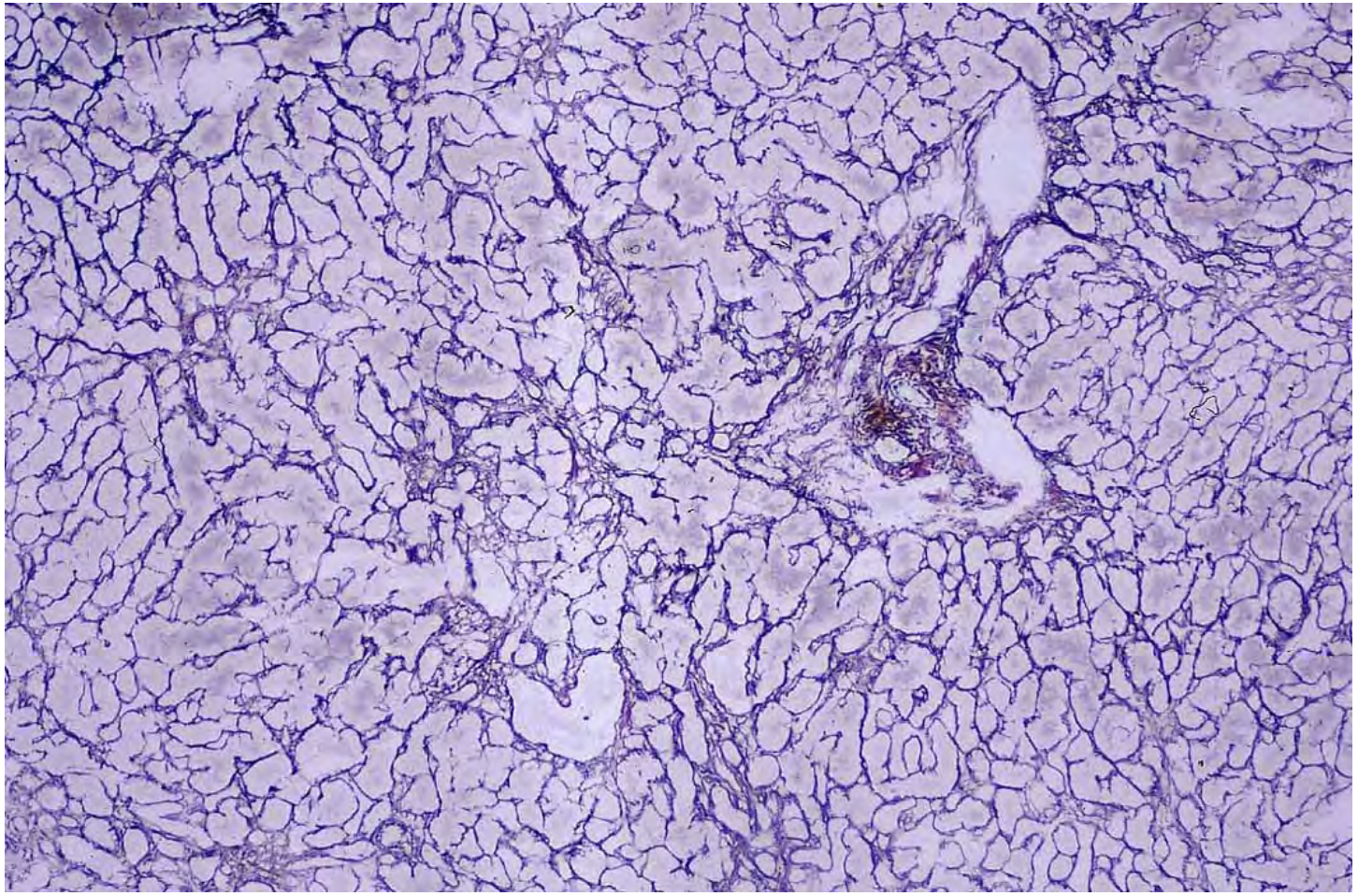
Trichrome stains are used to identify scarring and repair in tissue that occurs after any injury

Liver fibrosis paraffin sections

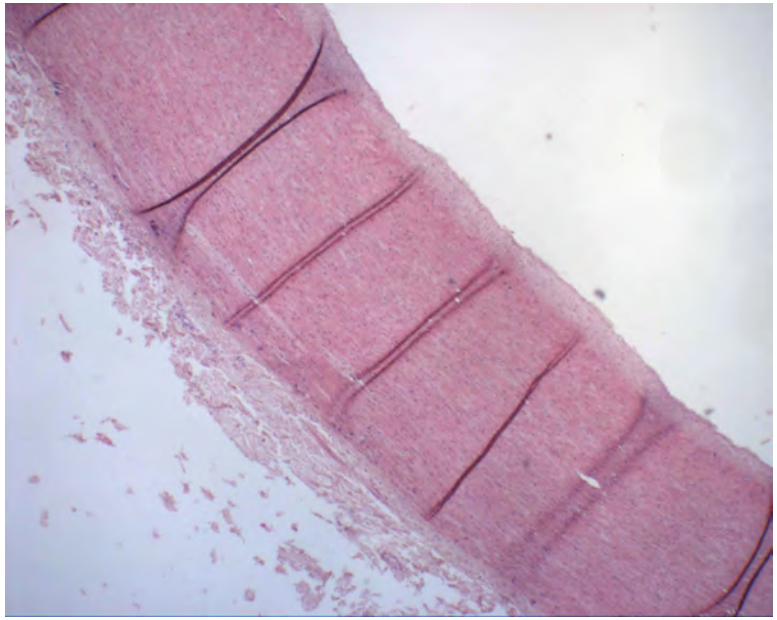
H&E

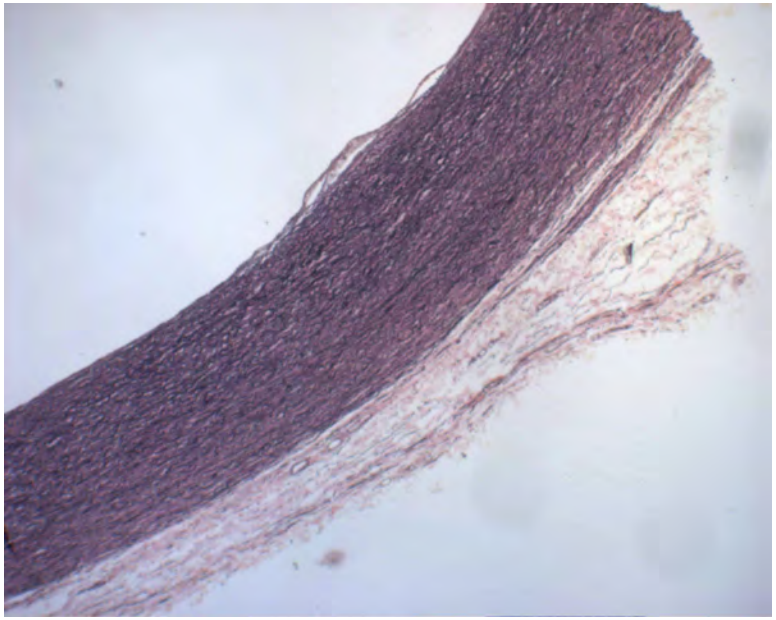
Trichrome



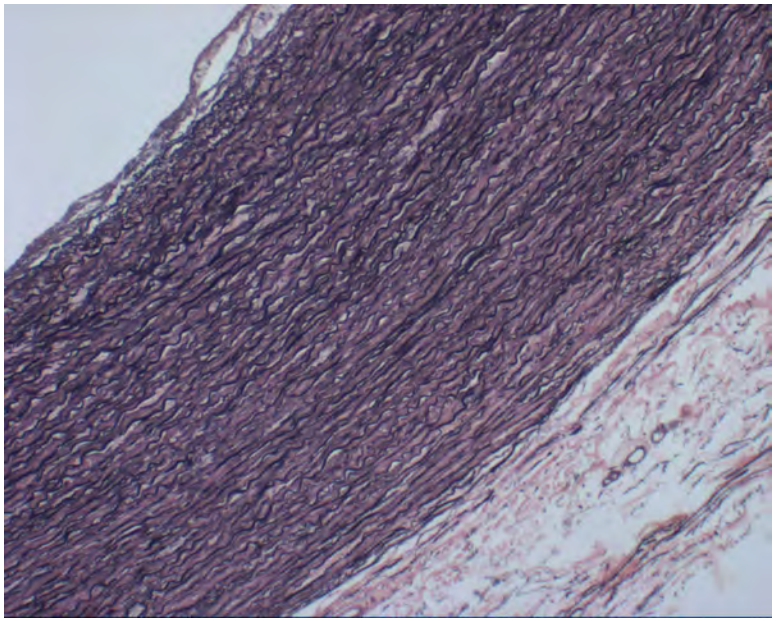


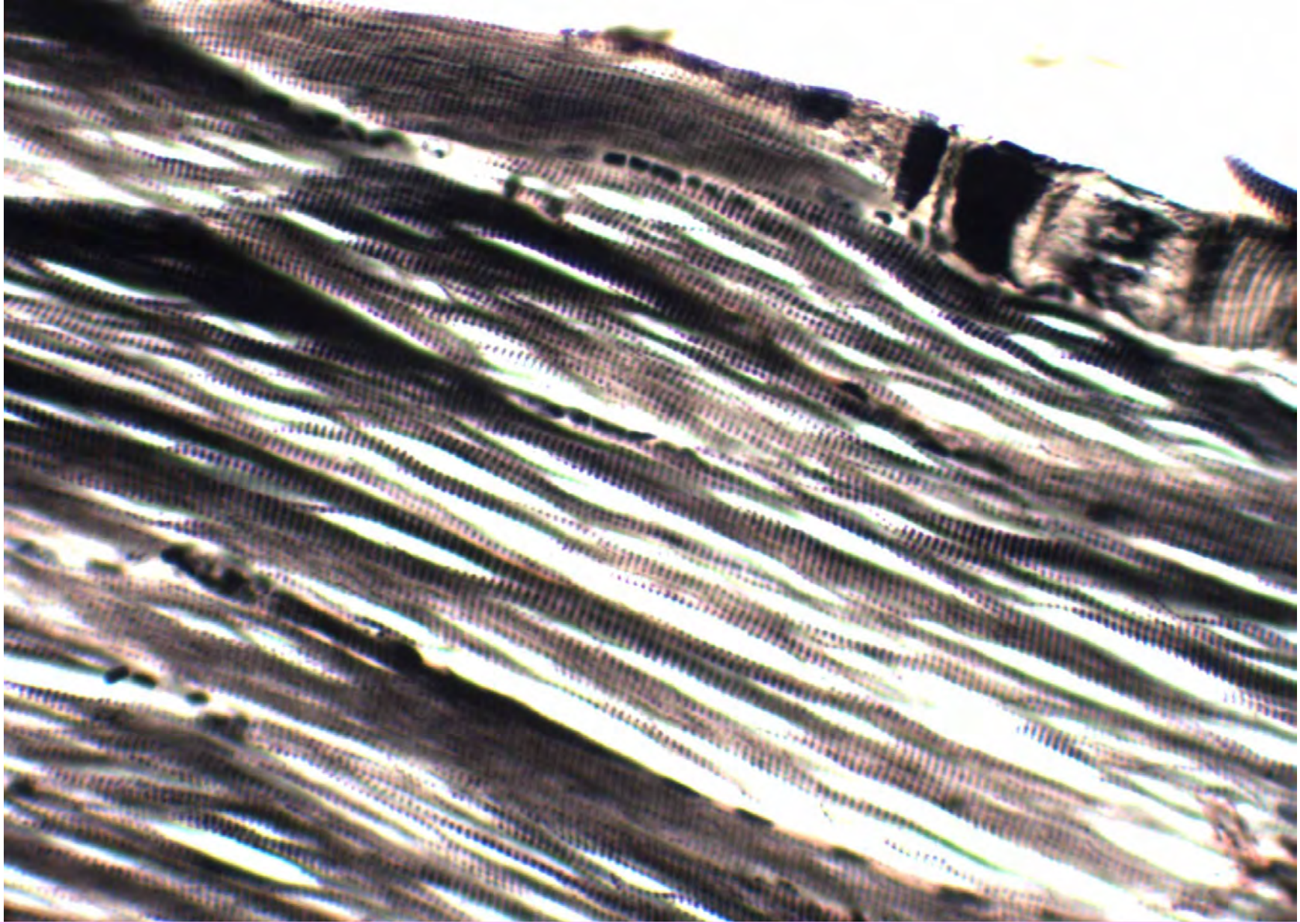
Silver stain to demonstrate reticulin supporting tissue



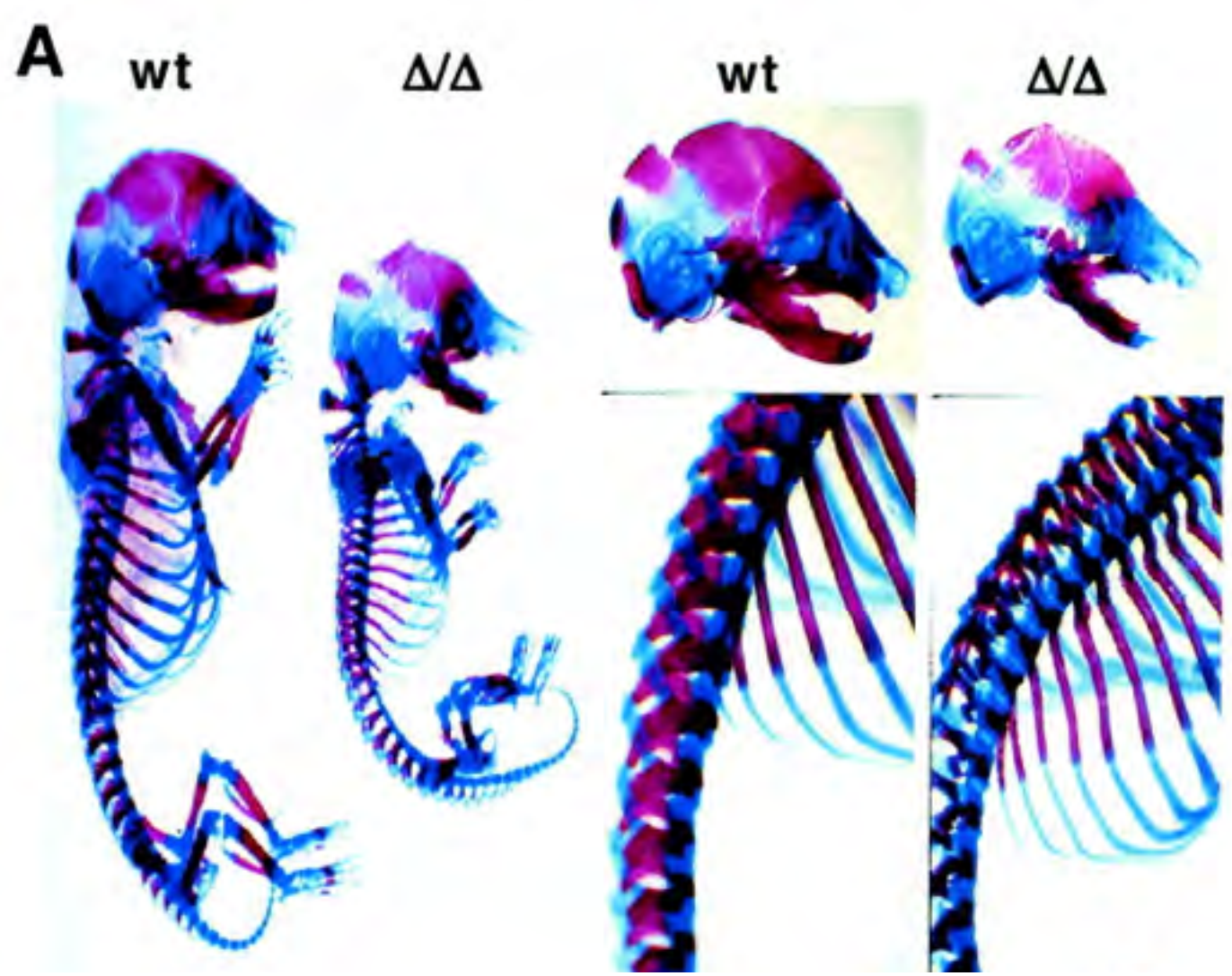


Human aorta: H&E and Elastic stain
This is a large vessel with abundant elastic fibers to contribute strength





Human Skeletal muscle with PhosphoTuncsticAcidHematoxylin PTAH stain to demonstrate striations



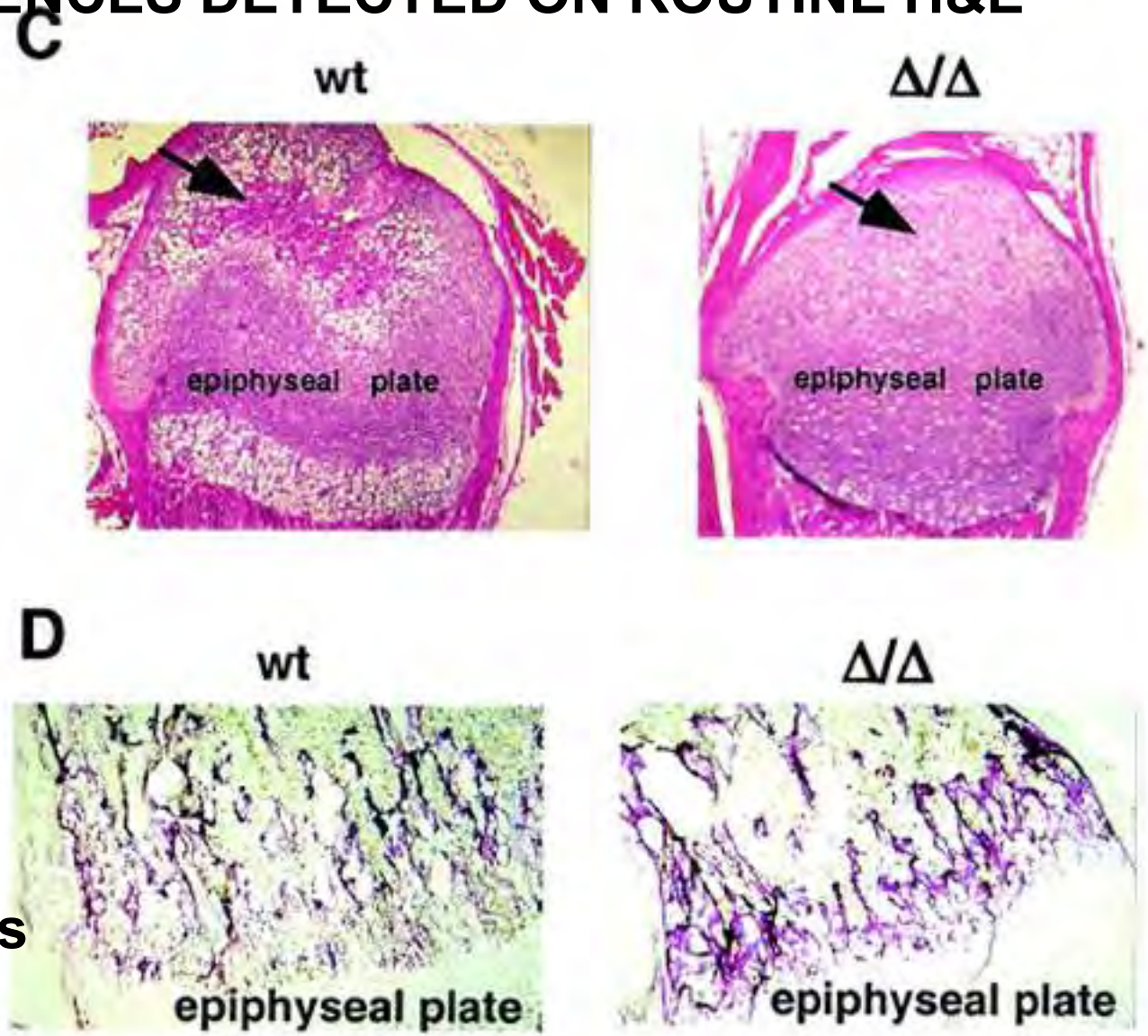
AlizarinRed to identify Bone

Alcian Blue cartilage –Safranin O is a red-orange stain for cartilage

USE OF HISTOCHEMISTRY TO DETECT DIFFERENCES DETECTED ON ROUTINE H&E STAINS

H&E

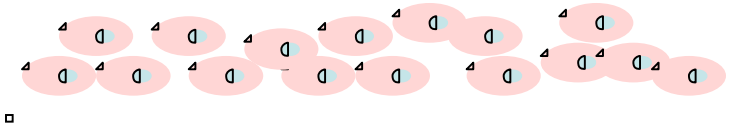
TRAP stain for osteoclasts



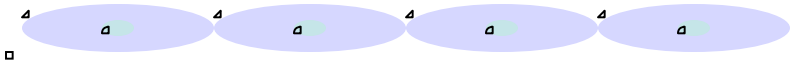
Immunohistochemistry to identify specific cell types, using antibodies

Immunohistochemistry assays may use

cells on slides

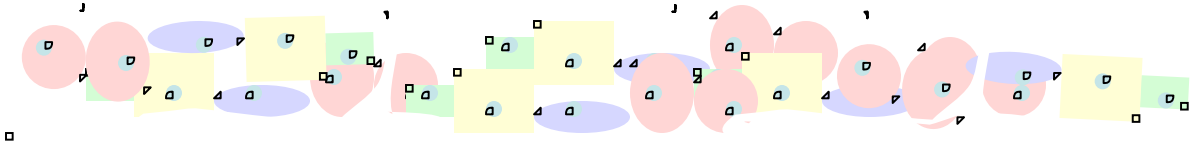


Cells grown, spun into a pellet, frozen or paraffin embedded and sectioned

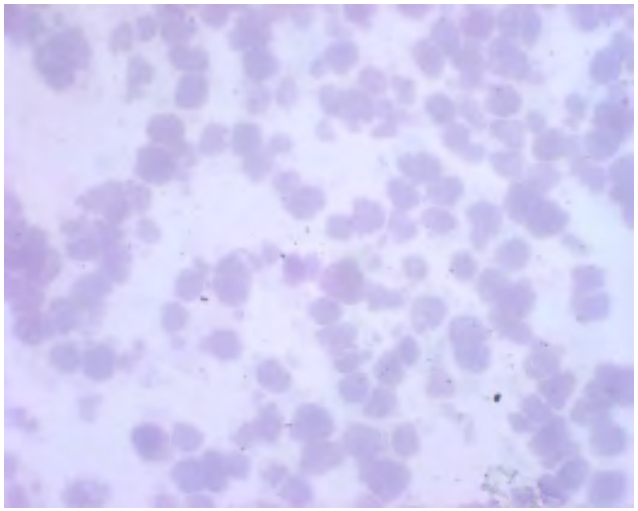


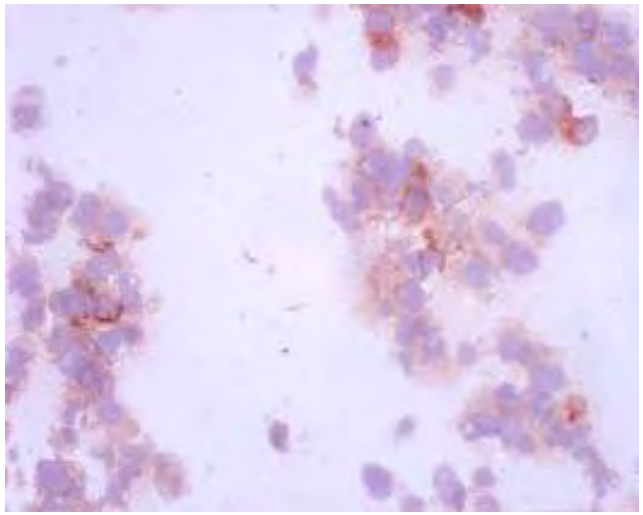
Cells grown as a monolayer

OR use tissue sections that are frozen or paraffin embedded



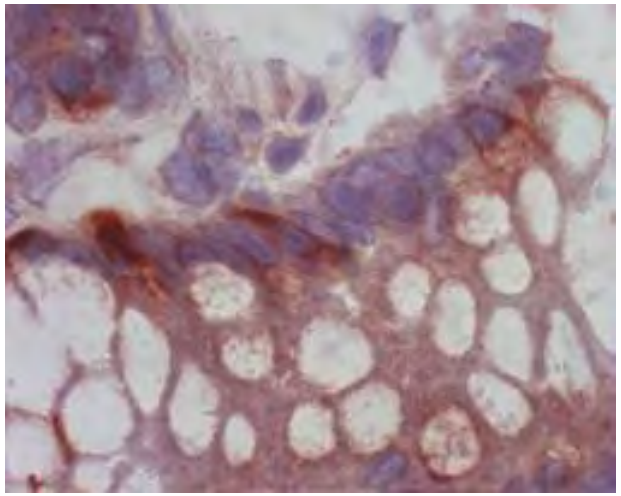
Sections from tissues contain many different kinds of cells as well as extra-cellular matrix components





Negative control and Positive control:
293 cells untransfected or transfected with (-----) plasmid, immunostained with the same antibody

- **Tissue section immunostained on the same slide with the same antibody**



DAB, AEC, red , SG, VIP

Blue, Red (also fluoresces)

HRP

Alk.Phos

AMCA

CY2 , FITC

PE, CY3

Tertiary

fluoresceinated compounds

or with an enzyme

Tertiary reagent is used usually labeled with :

Secondary

Primary

Remove endogenous binding sites in tissue, (biotin, HRP, collagen)

Tissue section: Frozen or deParaffinized

What are the cell / tissue types you will see in breast tissue?

Cuboidal cells--keratin positive

Myofibroblast cells--Smooth muscle actin

Adipocytes

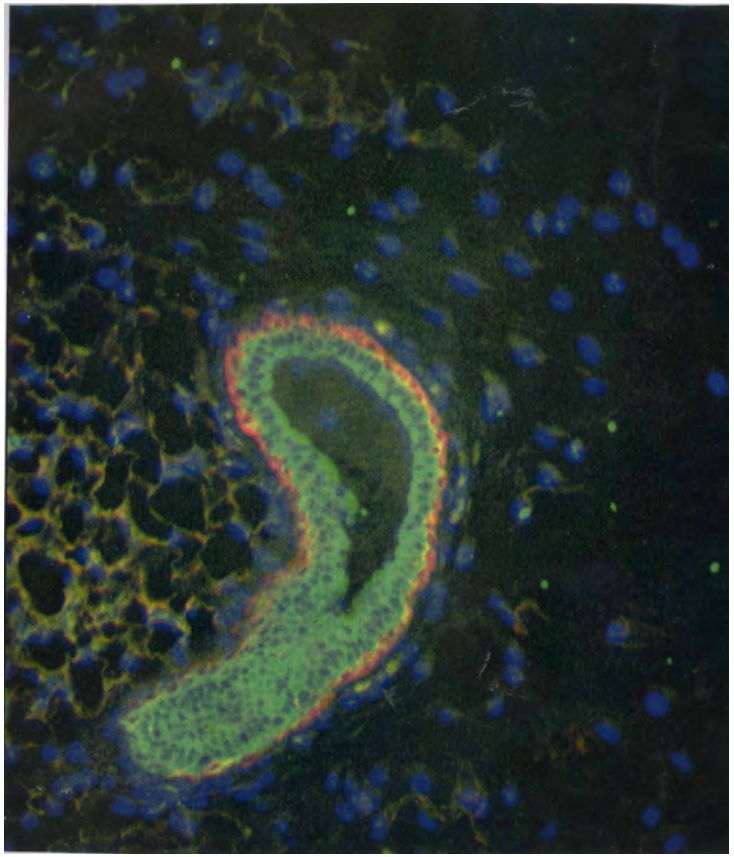
Blood vessels-CD31

Fibroblasts--vimentin

Nerve fibers—neuron specific enolase

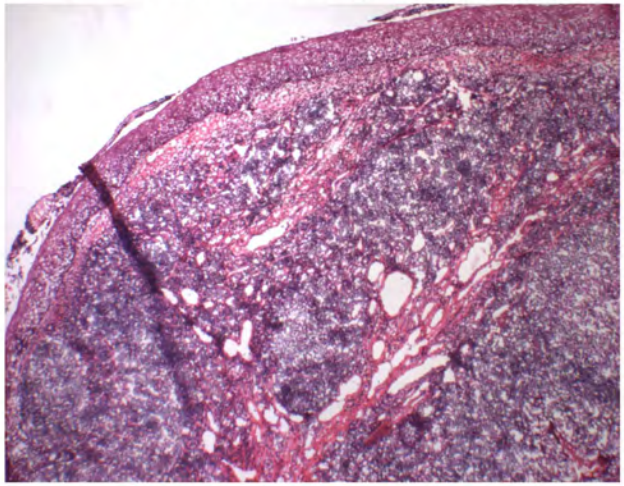
Innate immune cells—CD45

Normal breast ducts and alveoli have an Inner layer of cuboidal epithelial cells (keratin+) and an Outer layer of myoepithelial cells (smooth muscle actin)

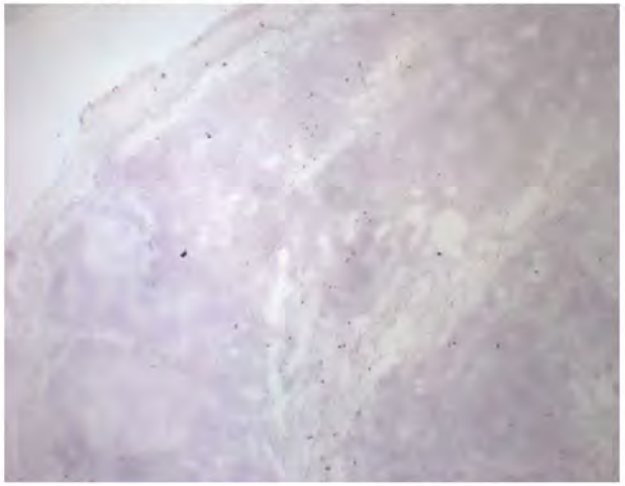


Frozen sections of Human Tonsil

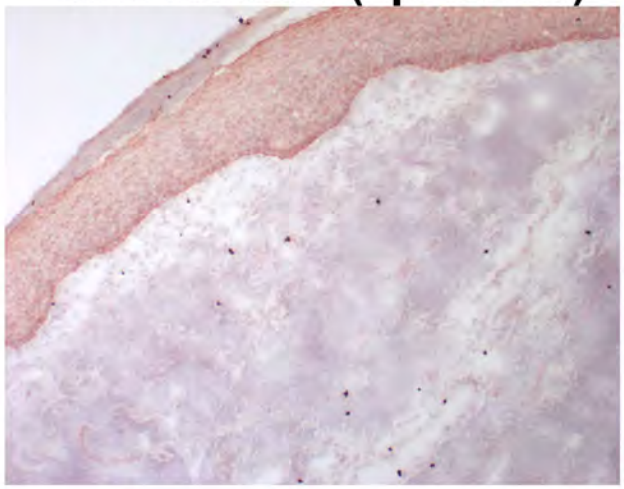
H&E for morphology



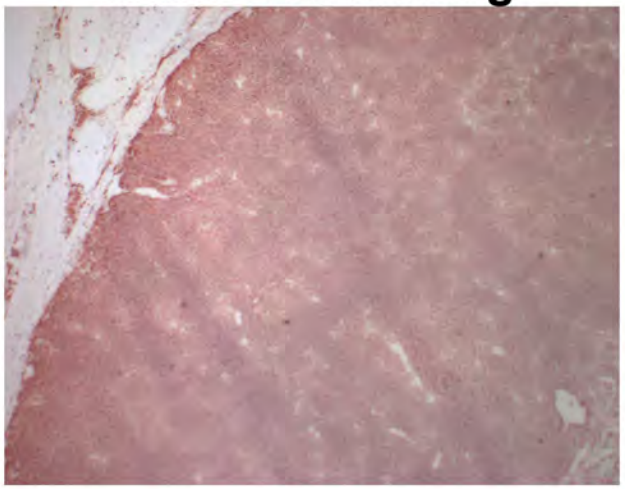
IgG negative control



squamous epithelium
anti-Keratin (epithelial)



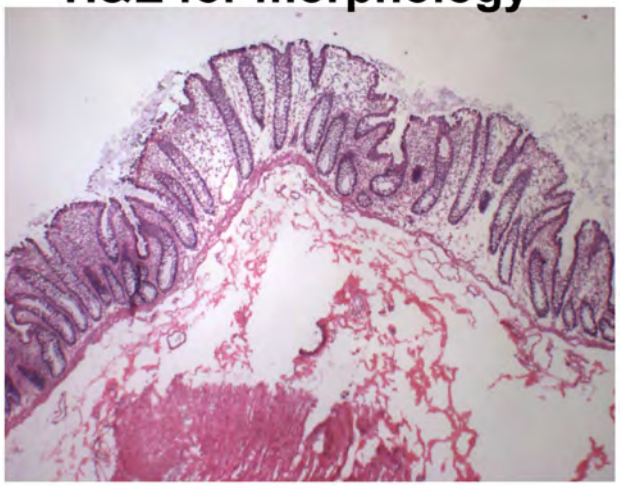
lymphoid cells demonstrate
anti-CD45 binding



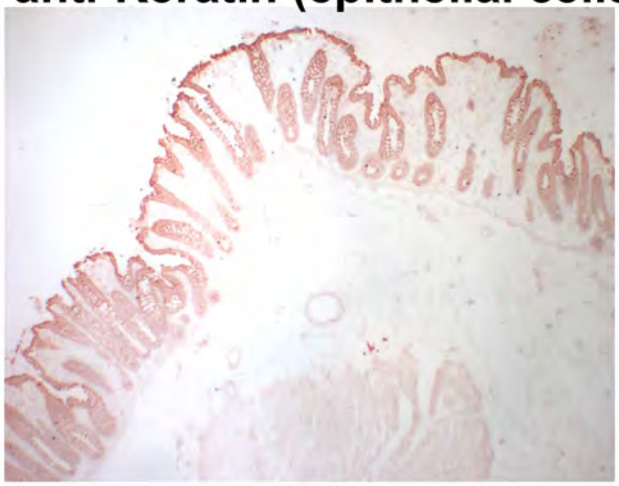
scale bar = 500 microns

Frozen sections of Human Colon

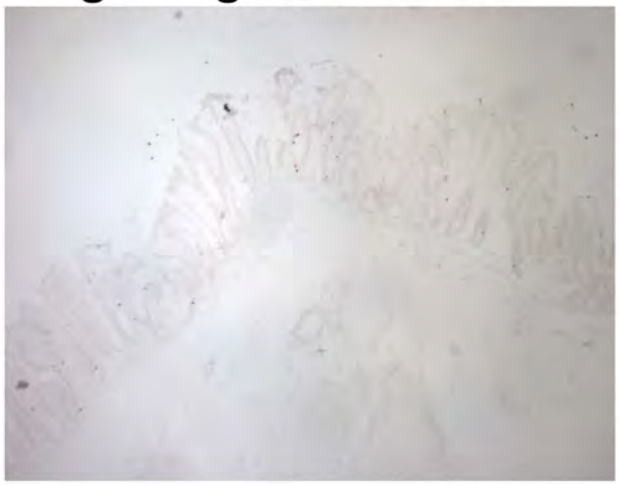
H&E for morphology



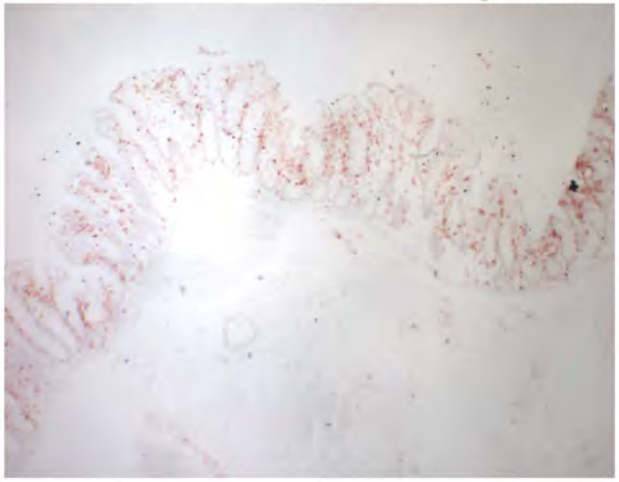
anti-Keratin (epithelial cells)



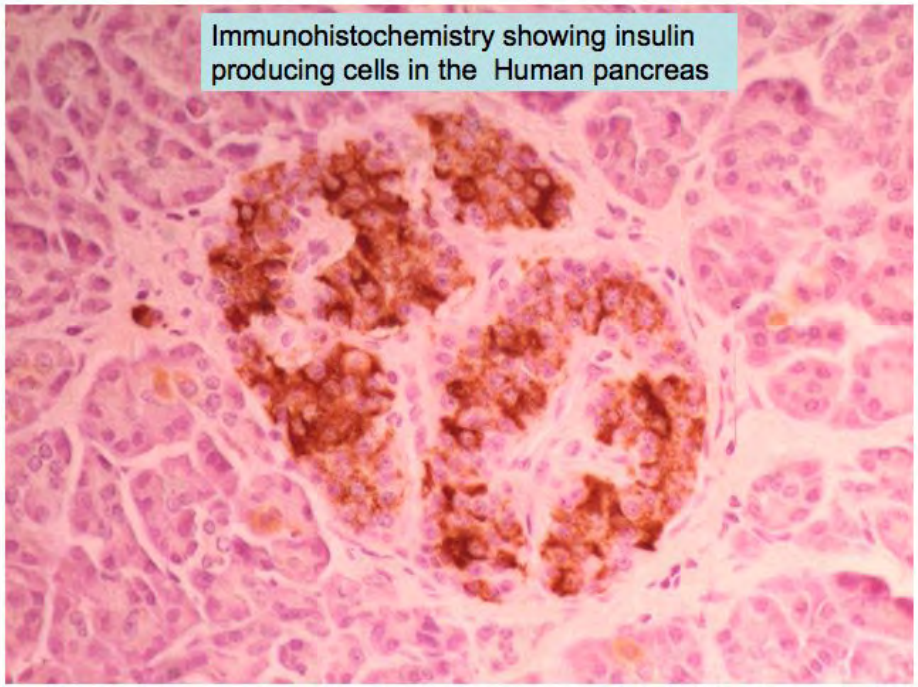
IgG negative control



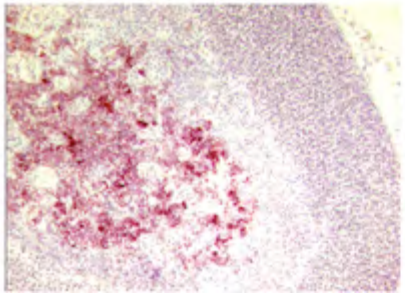
anti-CD45 (leukocytes)

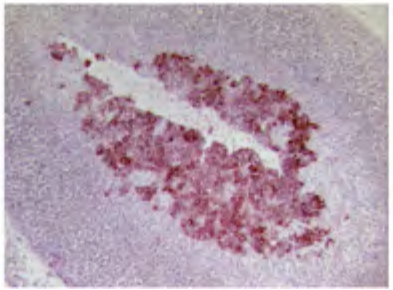


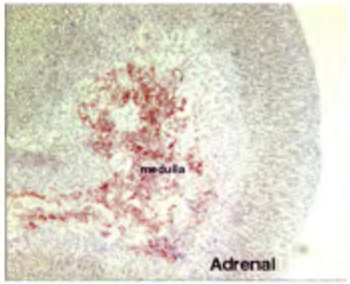
scale bar = 500 microns Magnification x40


Immunohistochemistry showing insulin
producing cells in the Human pancreas

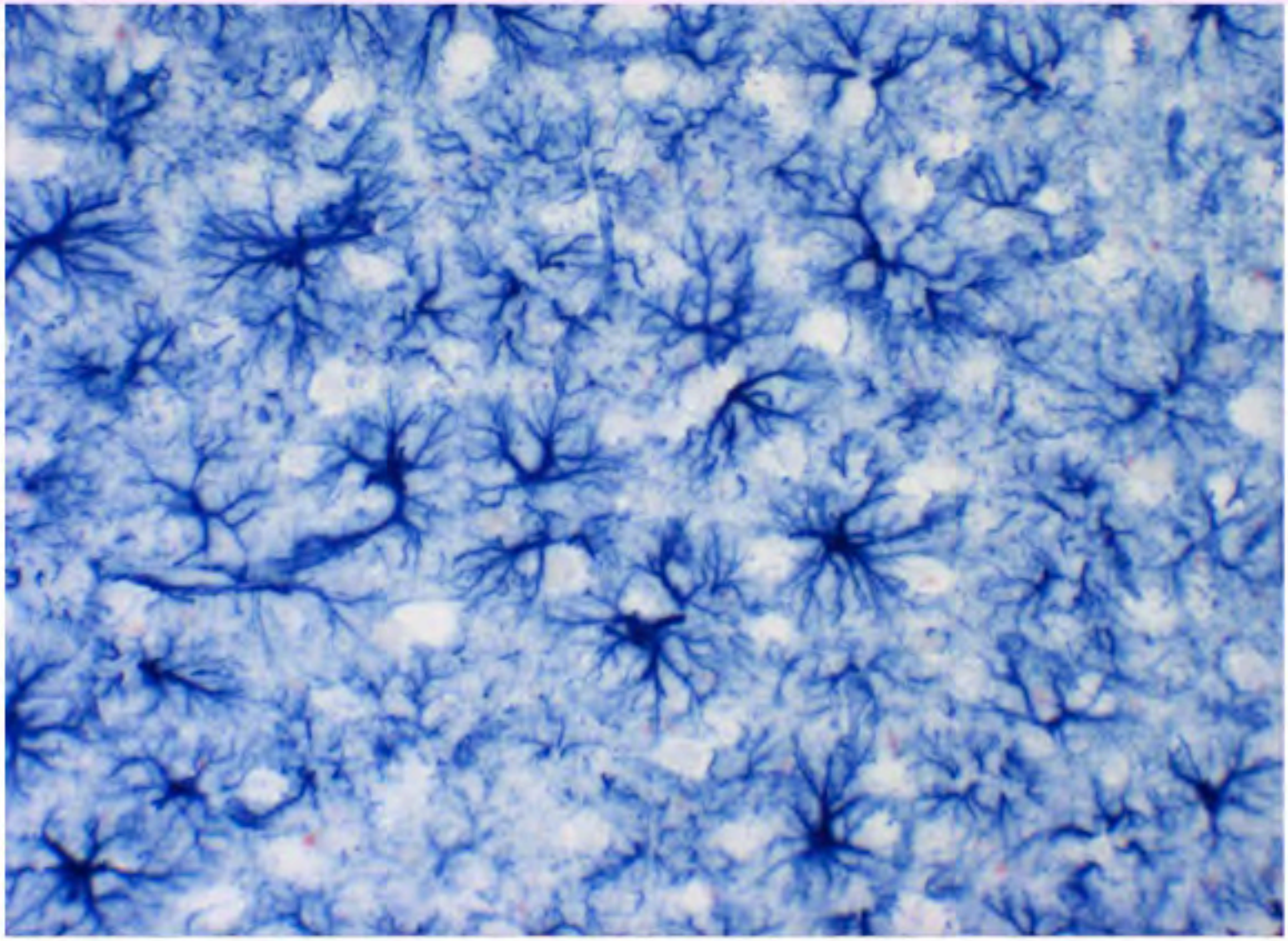
adrenal





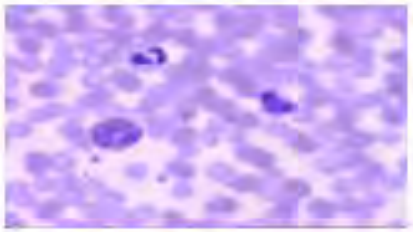

medulla
Adrenal

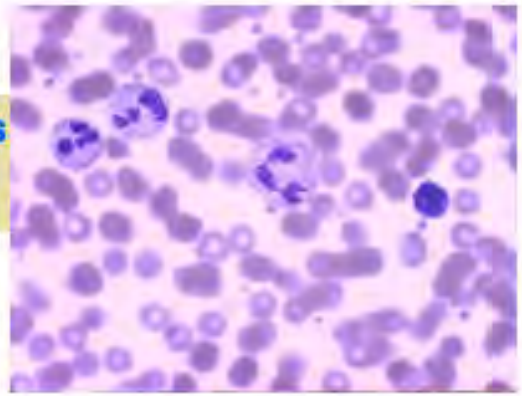
Astroglia/astrocytes in paraffin sections of Mouse Brain with anti-Glial-Fibrillary Acidic Protein antibody



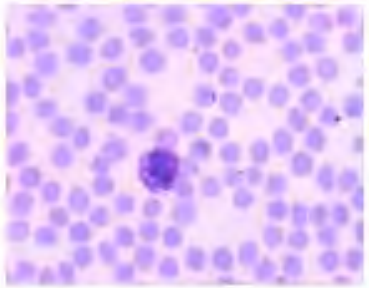
Hematopoietic cells are positive controls for commonly used markers in immunostains of tissues

Erythroid
TER119
Megakaryocytes
Leukocytes
Red blood cells (rbcs)
platelets
CD41
Myelo-monocytic
Lymphoid
Monocytes
Mac-1, CD14
B cells
B220
CD20
T cells
CD3
NK cells
Myeloid
Plasma cells
dendritic cells
macrophages
F480
CD11c
CD4
CD8
Granulocytes
Gr-1
CCR3
Neutrophils Eosinophils Basophils
(polymorphonuclear PMNs)

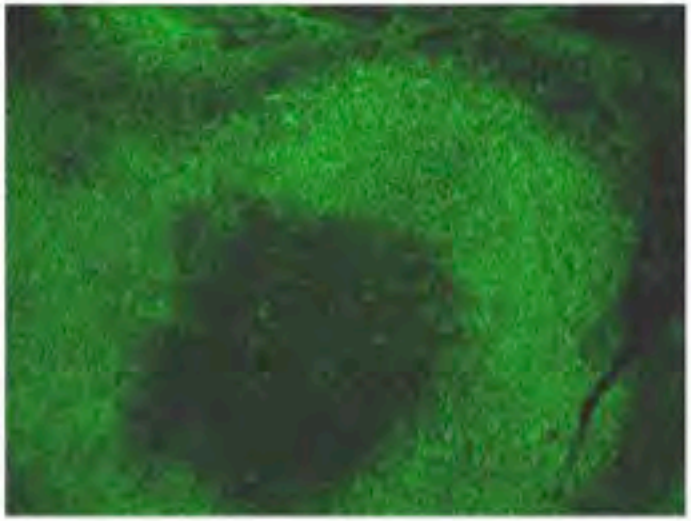




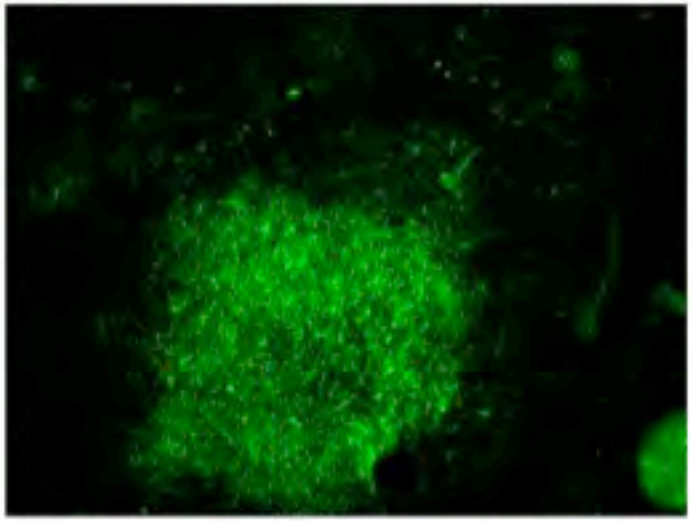
mast cells
Toluidine blue



EXAMPLES OF IMMUNOfluorescence STAINS ON MOUSE SPLEEN SECTIONS

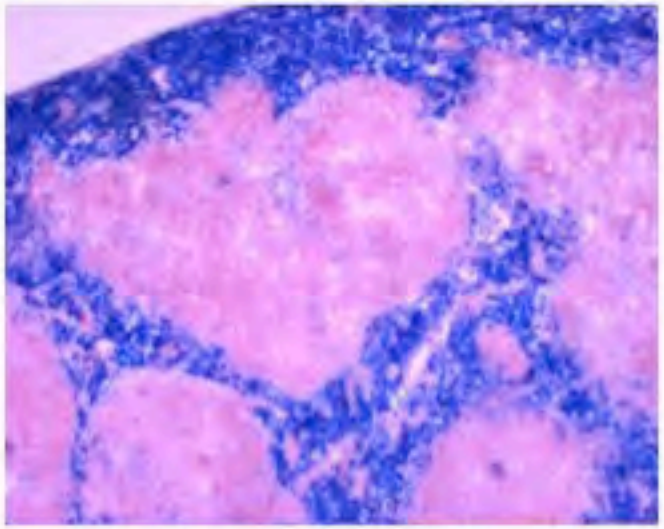


B cell marker B220 on frozen section of mouse spleen, marking the outer aspect of lymphoid follicle

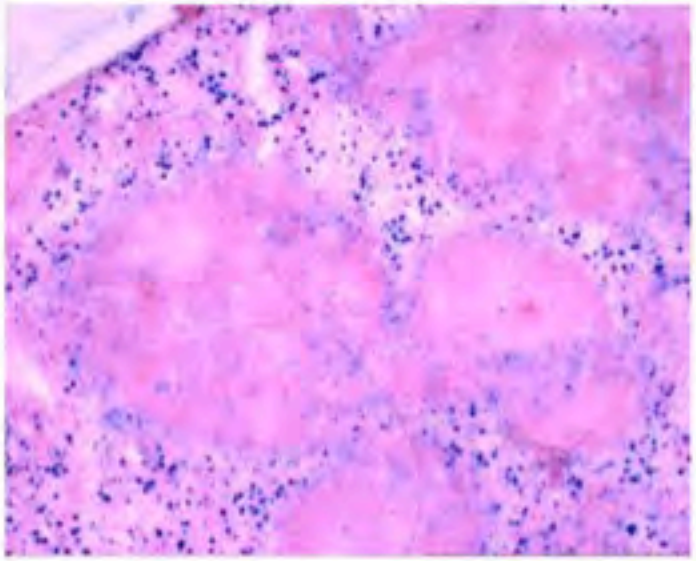


FITC-anti **CD4** on frozen sections of wild type mouse spleen

EXAMPLE OF IMMUNOenzyme STAINS FOR MACROPHAGES IN MOUSE SPLEEN



Biotinylated anti F480 on frozen section of spleen, detected with alkaline phosphatase conjugated streptavidin, Vector Blue substrate and nuclear fast red counterstain



Biotinylated anti Mac 1 on frozen section of spleen, detected with alkaline phosphatase conjugated streptavidin, Vector Blue substrate and nuclear fast red counterstain

IMMUNOHISTOCHEMISTRY
is an important adjunct to histopathologic evaluatio

Epithelium: Keratins

--pan-keratin

and antibodies to keratins of different molecular weights

Supporting connective tissues:

--Vimentin--fibroblasts, blood vessels

--vWF, CD31 (PECAM)-- endothelial cells of blood vessels

Hematopoeitic tissues: CD45, B220, CD3, F Gr-1, CD41

Muscle: desmin, smooth muscle actin

Neural: GFAP, NeuN, F480/Mac-1, MBP, NSE,