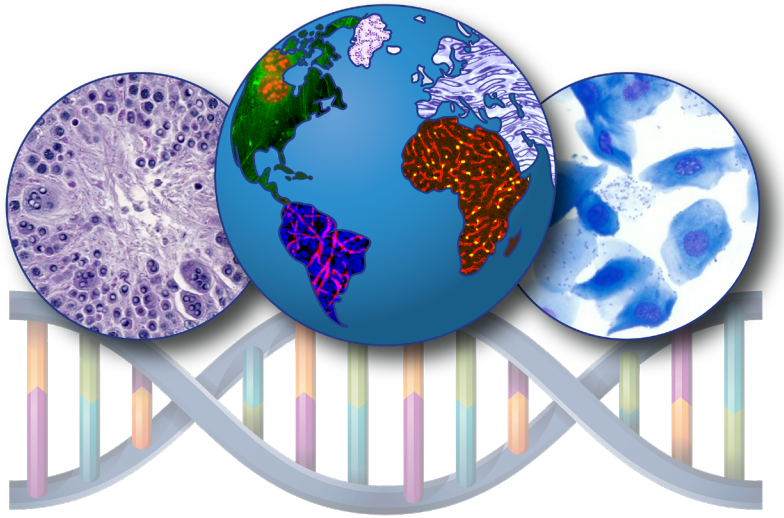
Basic Cellular and Tissue Microscopic Responses to Toxicity



Division of Translational Toxicology Global Toxicologic Pathology Training Program

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

Overview

- “Lesions” and tissue staining used for microscopic diagnosis
- General pathologic processes in response to toxicity
  - Adaptation
  - Cell injury: reversible and irreversible
  - Accumulations
    - Mineralization
  - Vascular changes
  - Inflammation
  - Erosion and ulcer
  - Regeneration and repair
  - Neoplasia

“Lesions” in pathology and tissue staining used for microscopic diagnosis

What is Pathology?

Pathology is the study of Disease

- Disease means any deviation from, or interruption of, the normal function or structure of cells, tissues, or organs
  - *NOT* just illness, and pathology may not be clinically apparent
- Encompasses the study of functional, biochemical, and **morphological (structural) alterations** of cells, tissues, and organs that underlie disease and processes
  - These morphological alterations of cells and tissues can be called **“lesions”**
  - The basic categories of lesions seen in response to toxicity are covered in this module

Basic Tool for Histopathologic Evaluation

Tissue staining with hematoxylin and eosin (H&E)

- H&E staining produces a useful gradient of color changes to evaluate tissues
- **Hematoxylin**
  - A positively charged stain that binds acids (e.g., DNA, RNA) and negatively charged sites on basophilic compounds
    - Stains the nucleus, ribosomes
    - Ribosomes consist of RNA and protein, and can be found free in cytoplasm or attached to endoplasmic reticulum (ER) as rough ER in cytoplasm
  - Basic stain that imparts deep blue to purple color
- **Eosin**
  - A negatively charged stain that binds bases and positively charged sites on acidophilic / eosinophilic compounds
    - Stains the cytoplasm, fluids, proteins (e.g., collagen), extracellular matrix
  - Acidic stain that imparts pink to reddish pink color

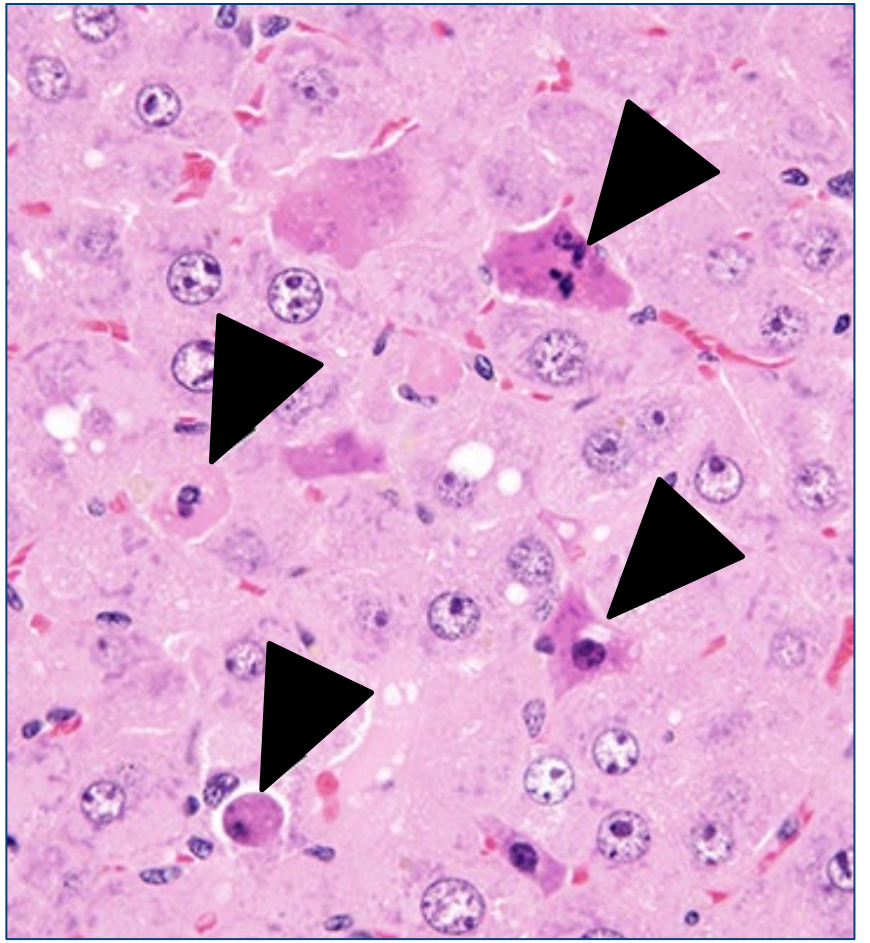
General pathologic processes in response to toxicity

- Adaptation
- Cell injury
- Accumulations
  - Mineralization
- Vascular changes
- Inflammation
- Erosion and ulcer
- Regeneration and repair
- Neoplasia

General Pathologic Processes

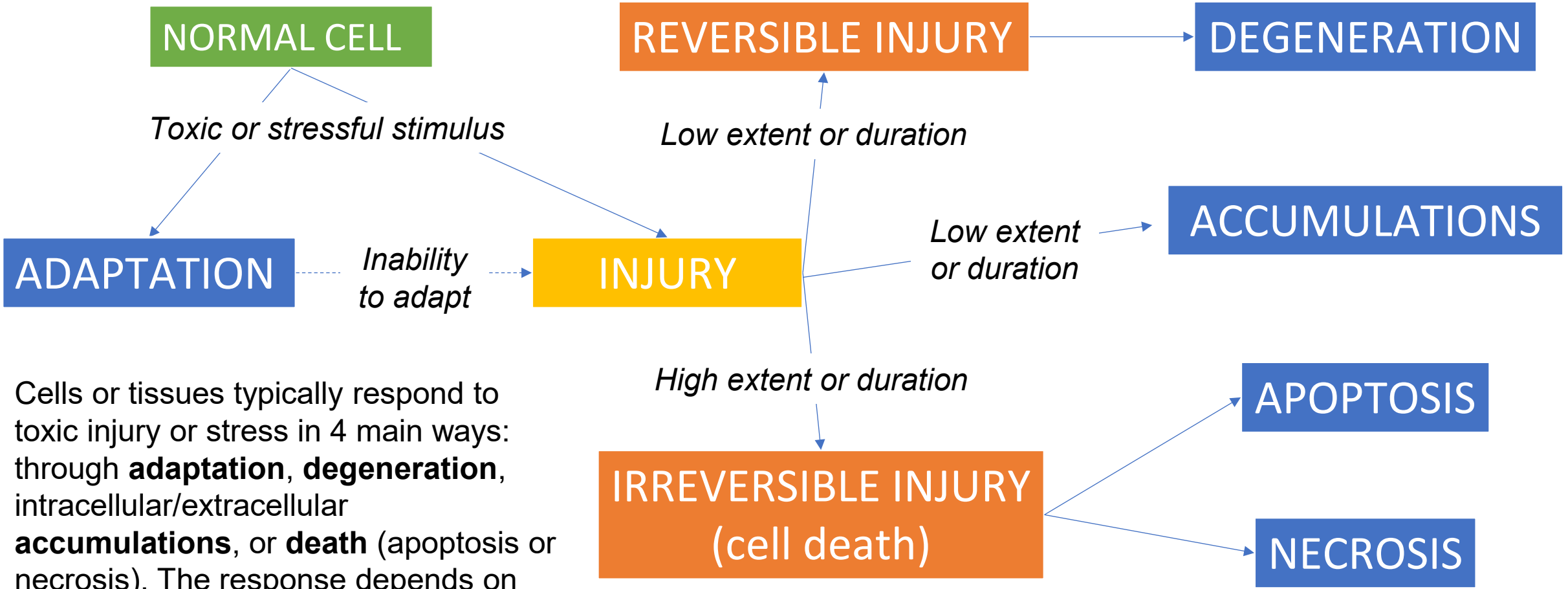
What determines which process will occur?

- Available repair mechanisms for the cell/tissue
- Stage of development
  - Fetus, neonate, juvenile, adult
- Metabolic rate of cell
  - Cells with high metabolic activity are generally more sensitive to injury
  - For example, neurons, hepatocytes, cardiac myocytes, and renal tubule cells are sensitive to hypoxia (decreased oxygen supply), whereas fibroblasts are resistant
- Nature of the injury (intensity/dose and duration)
  - Duration: length of exposure to injurious stimulus
  - For example, acute, high-grade injury may be lethal, whereas chronic low-grade injury may cause degeneration



In this section of liver from a mouse, dead/dying hepatocytes are reduced in size and have brightly eosinophilic cytoplasm and nuclear condensation (arrowheads). (Liver: Hepatocyte – Apoptosis).

General Cell Responses to Injury



Cells or tissues typically respond to toxic injury or stress in 4 main ways: through **adaptation**, **degeneration**, intracellular/extracellular **accumulations**, or **death** (apoptosis or necrosis). The response depends on their ability to adapt and the intensity of the toxic insult.

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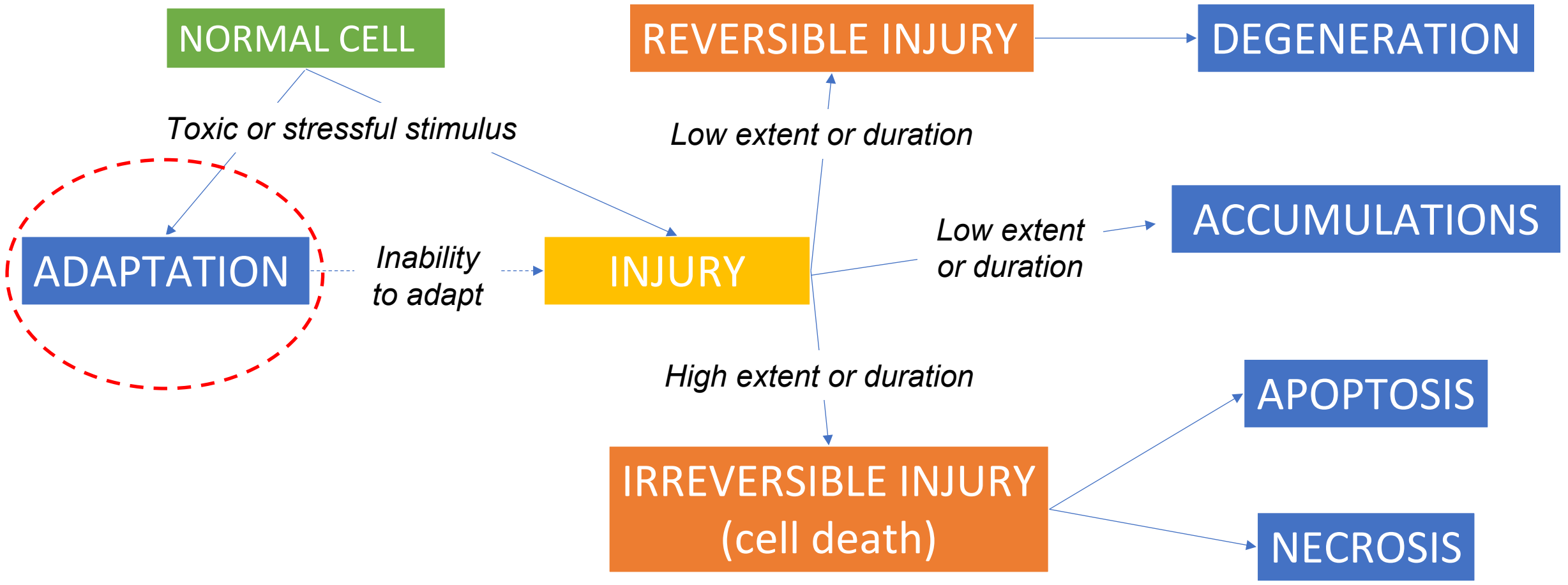
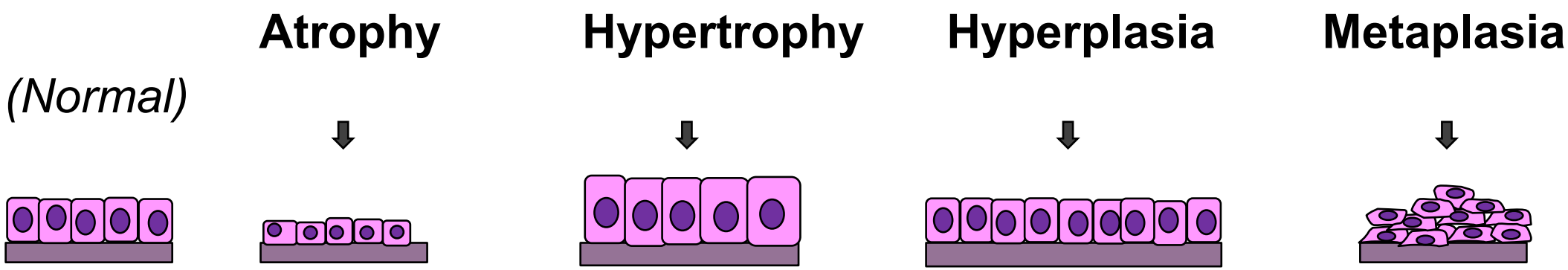
General Cell Responses to Injury



General Pathologic Processes: Adaptation

ADAPTATION

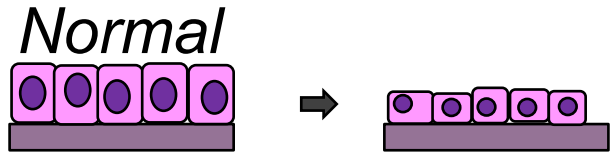
- Adaptation is a change in cell size, number, or phenotype
  - Establishes a new state that preserves viability and function
  - May be harmless or deleterious
  - Can be a normal, compensatory mechanism to combat environmental stressors
- There are four types of adaptations:

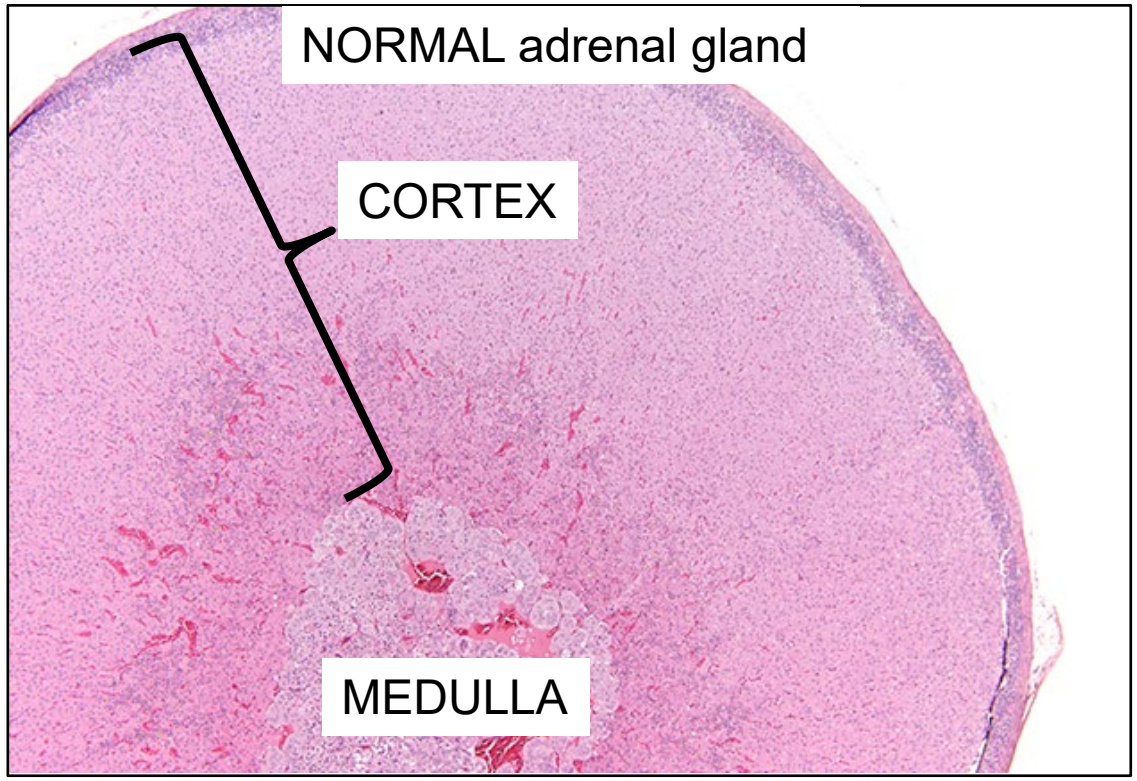


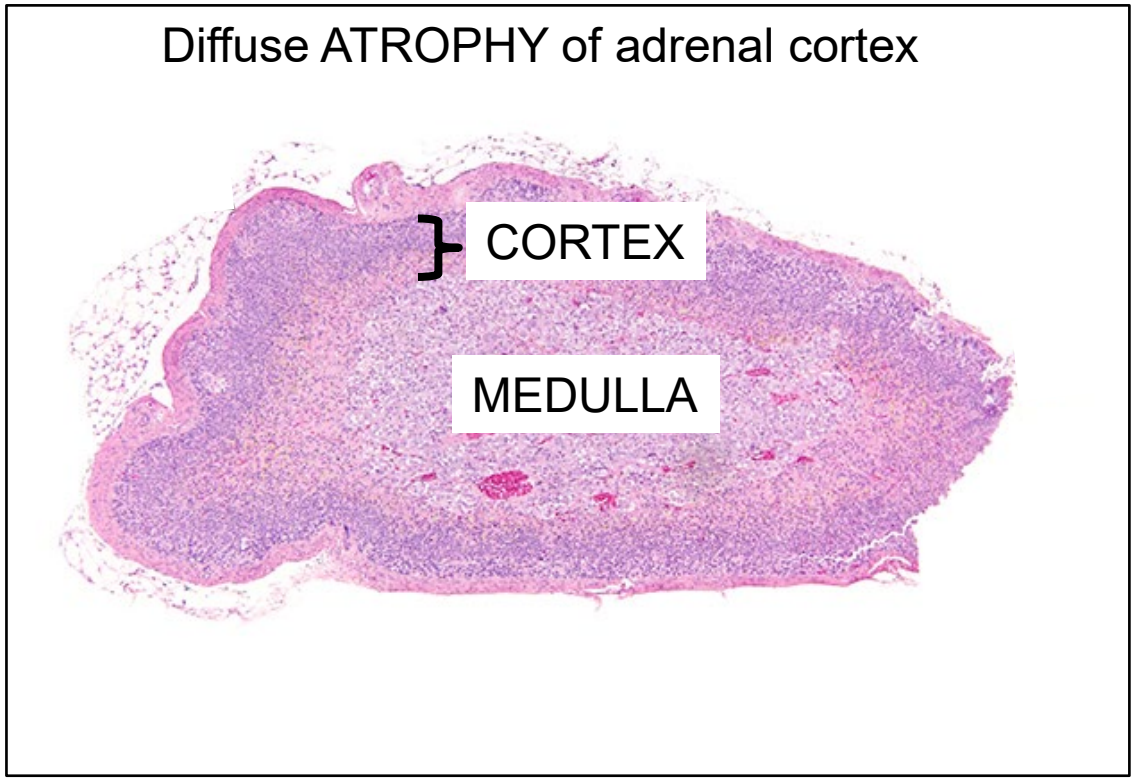
General Pathologic Processes: Atrophy

ADAPTATION: Atrophy

- **Atrophy** is cause by a decrease in the number and/or size of cells
  - Atrophy can affect all or part of an organ/tissue
  - Cells shrink and survive, but usually with reduced function
  - Caused by: Disuse, decreased workload, deficient hormones or nutrients, or pressure





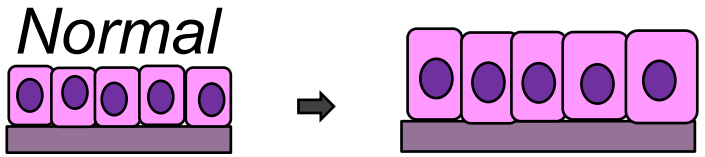


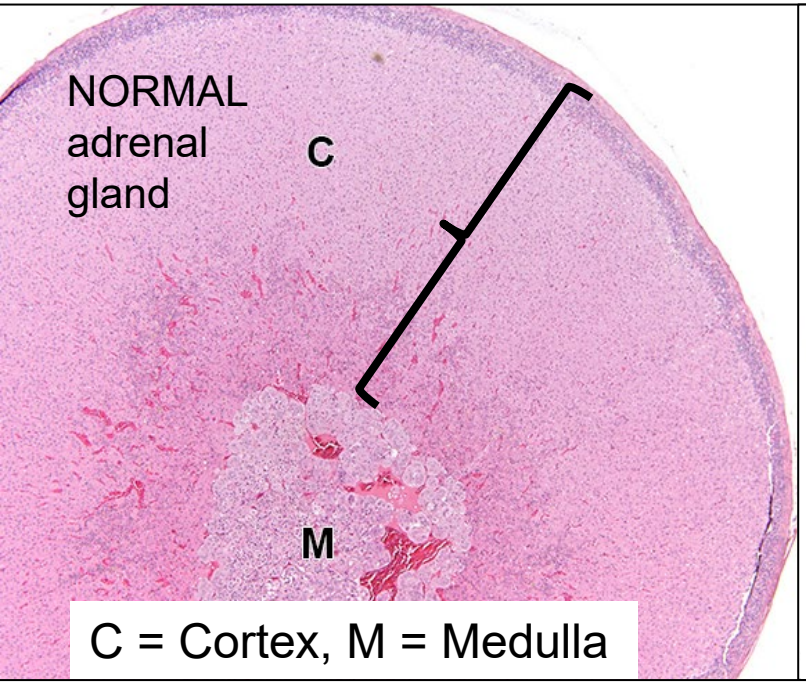
ADAPTATION: Hypertrophy

- **Hypertrophy** is an increase in cell size due to synthesis of more organelles
  - The tissue architecture is generally retained, but the cells are bigger
  - May be focal (in a portion of a tissue) or diffuse (throughout a tissue or region)
- Hypertrophy can occur in most organs and tissues
  - Tends to occur in those that have low potential for replication (e.g., striated muscle)
- Usually caused by demand for increased function
- Cells that are capable of dividing may respond to stress by undergoing BOTH **hypertrophy** (increase in cell size) and **hyperplasia** (increase in cell number)
  - *Permanent* cells are terminally differentiated, generally do not divide after birth, and have limited capacity to regenerate, so they tend to undergo **hypertrophy** without hyperplasia

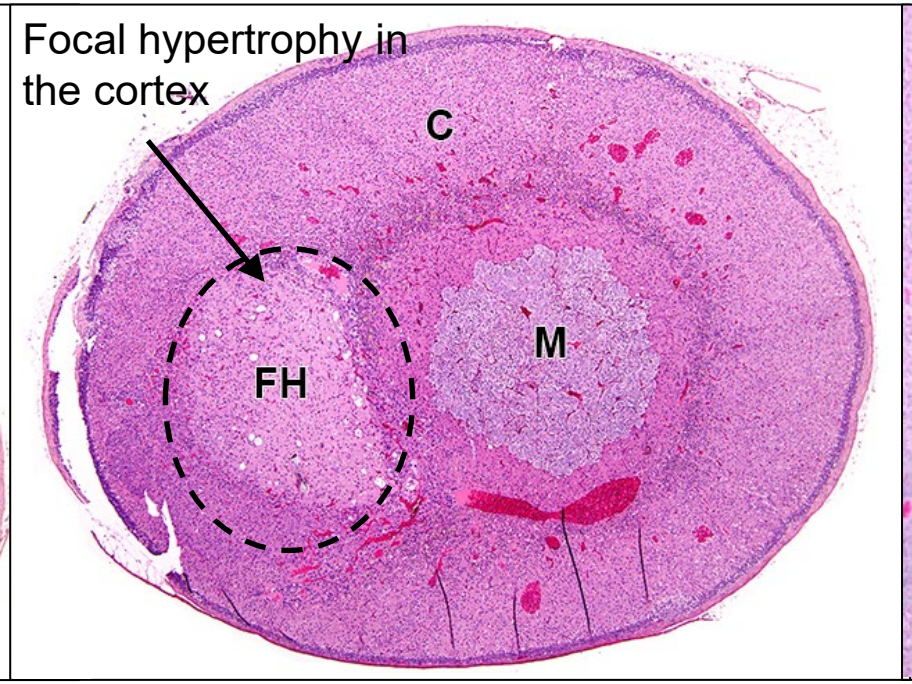
General Pathologic Processes: Hypertrophy

ADAPTATION: Hypertrophy

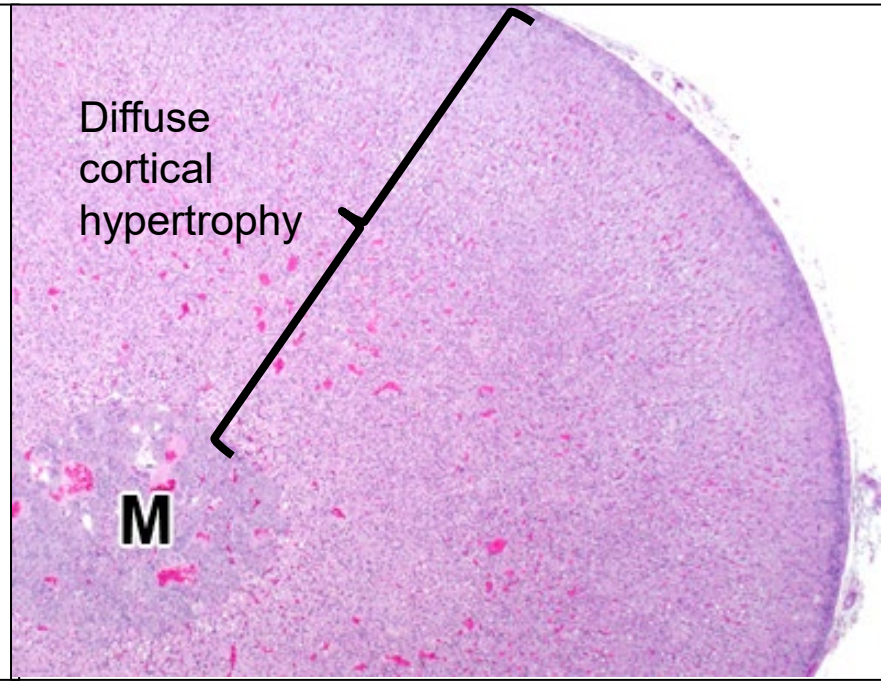




Normal adrenal gland with cortex (C) and medulla (M) regions.



Focal hypertrophy (FH): There is a limited region (focus) within the adrenal cortex with hypertrophied (enlarged) cells.



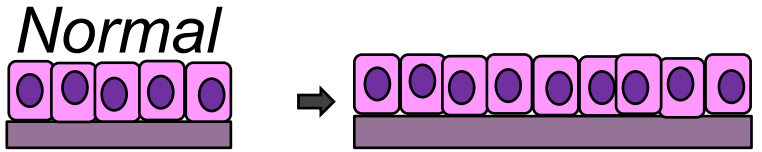
Diffuse hypertrophy: The entire adrenal cortex has enlarged cells. The adrenal cortex is wider than that of a normal adrenal gland.

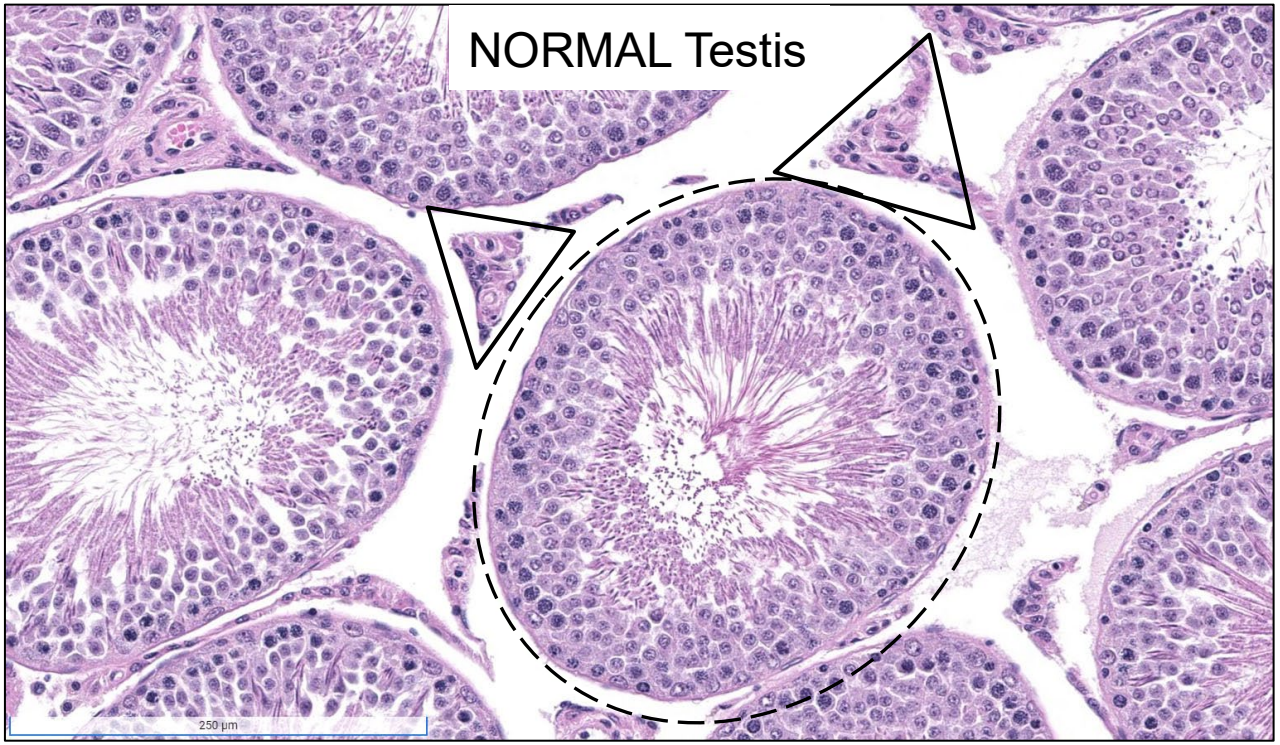
ADAPTATION: Hyperplasia

- **Hyperplasia** is the increase in the number of cells via mitotic division
  - Hyperplasia increases the size of a tissue, organ, or part of an organ
  - May be focal (in a portion of a tissue) or diffuse (throughout a tissue or region)
- Microscopically, hyperplasia can be seen as normal appearing cells that are increased in number
  - However, many cells undergo both **hypertrophy** (increased size) and **hyperplasia**
- Hyperplasia can be caused by a stimulus, such as hormonal stimulation, chronic irritation, compensation (such as after removal of part of a liver) or after an injury
- Cells vary in the ability to adapt via hyperplasia
  - *Labile* cells (continuously divide throughout life), such as of the epidermis, intestinal epithelium, and bone marrow, readily undergo **hyperplasia**
  - *Stable* cells (typically do not divide or divide only after injury), such as bone, cartilage, and smooth muscle, are intermediate in the ability to become **hyperplastic**
  - *Permanent* cells (terminally differentiated), such as neurons, cardiac myocytes, and skeletal muscle, do not undergo **hyperplasia**

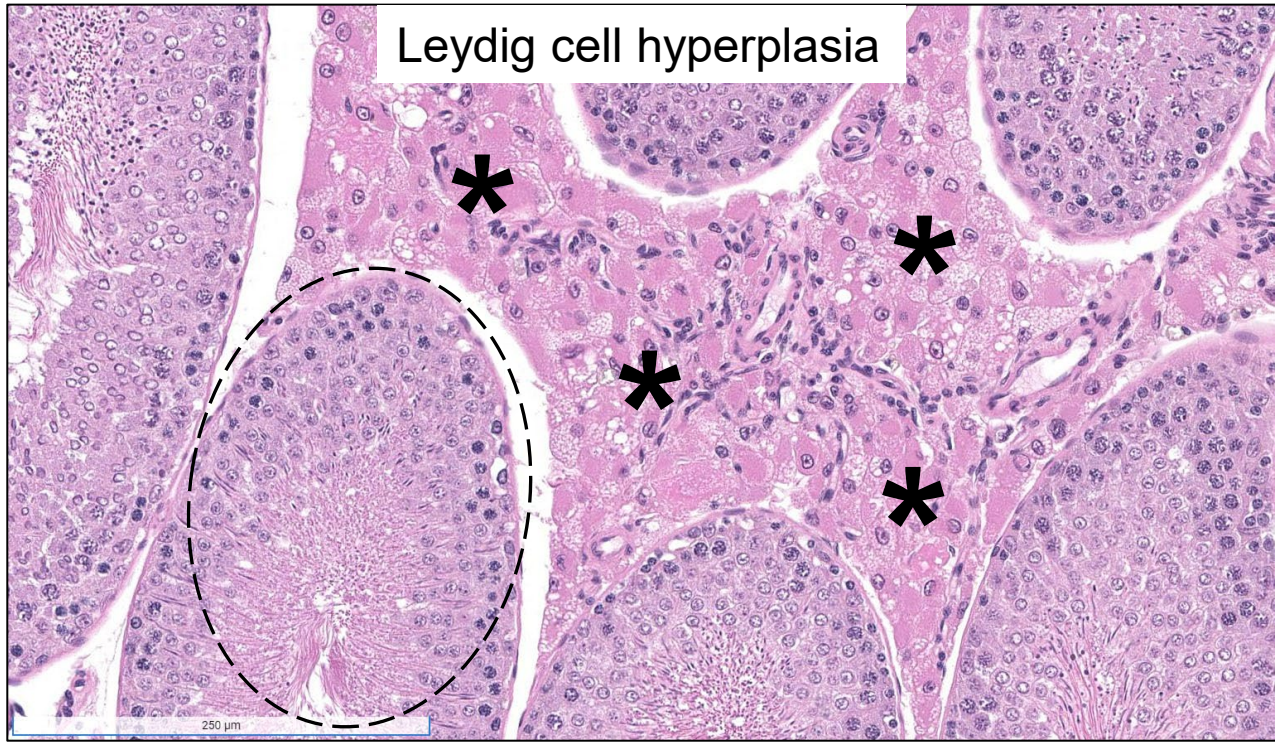
General Pathologic Processes: Hyperplasia

ADAPTATION: Hyperplasia





Normal Leydig cells (within triangles) in the interstitium of the testis, between seminiferous tubule cross-sections (dashed circle).



The Leydig cells (*) have increased in number (hyperplasia). The cells are also increased in size (hypertrophy).

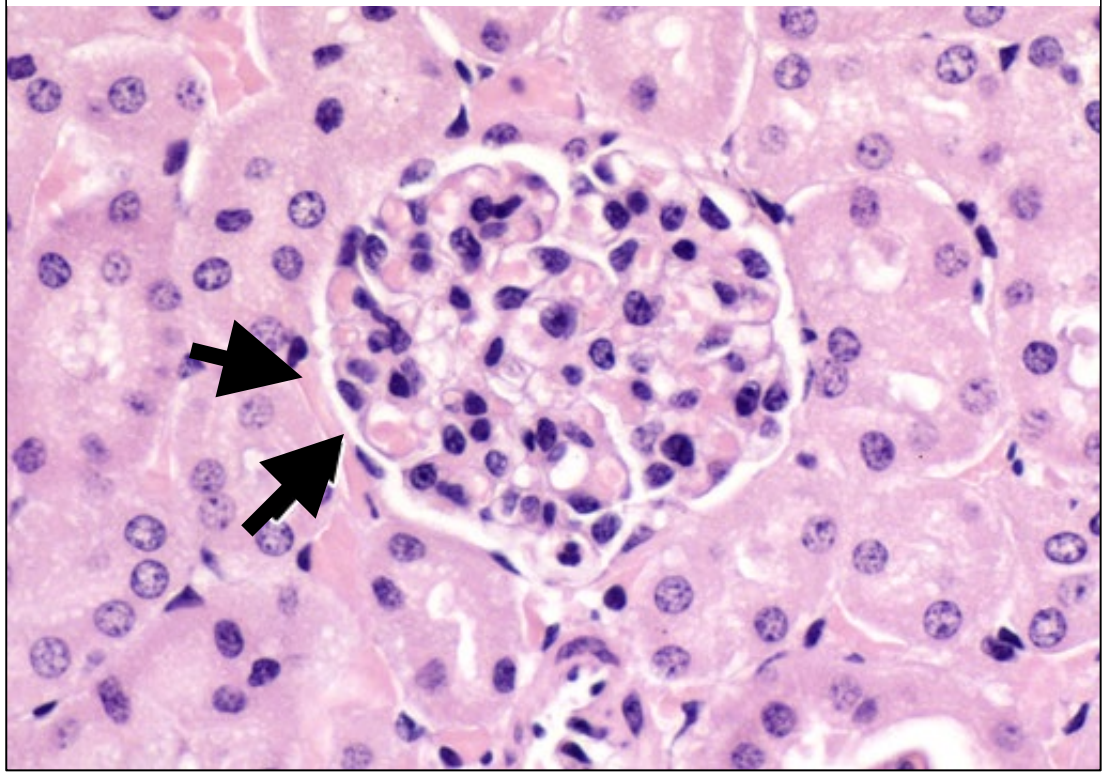
ADAPTATION: Metaplasia

- **Metaplasia** is a potentially reversible adaptation in which one adult cell type is replaced by another adult cell type
  - Usually, but not always, specialized epithelium is replaced by less-specialized epithelium.
- One ADULT cell type does NOT transform into a different ADULT cell type
  - Instead, it is due to reprogramming of stem cells to differentiate into something else
- Some examples:
  - Smoking causes chronic irritation of the normal columnar ciliated epithelium of the trachea and bronchi, resulting in replacement with stratified squamous epithelium (squamous metaplasia), which is more resistant to injury
  - Vitamin A deficiency or estrogen toxicity can cause squamous metaplasia in various tissues

General Pathologic Processes: Metaplasia

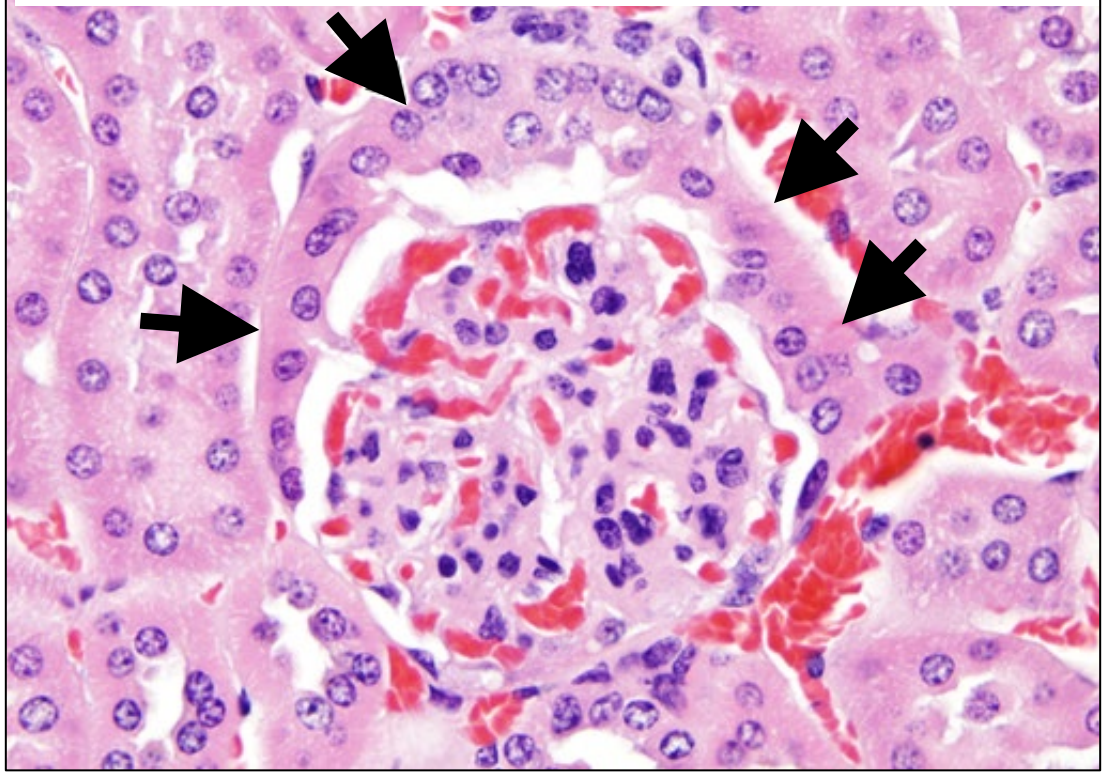
ADAPTATION: Metaplasia

NORMAL glomerulus in a female mouse kidney



In normal female mice, the parietal epithelium lining Bowman's capsule is flattened (arrows). In normal male mice, the epithelium of the Bowman's capsule is normally cuboidal (not shown).

Glomerulus metaplasia in a female mouse kidney



'Male-like' cuboidal epithelium (arrows) in a female mouse exposed to an androgenic test article.

General Pathologic Processes: Adaptation

Core Concept:

- There are 4 types of cellular adaptations to toxic injury or stress
- Cells get smaller (**atrophy**), bigger (**hypertrophy**), more numerous (**hyperplasia**), or change to different type (**metaplasia**)

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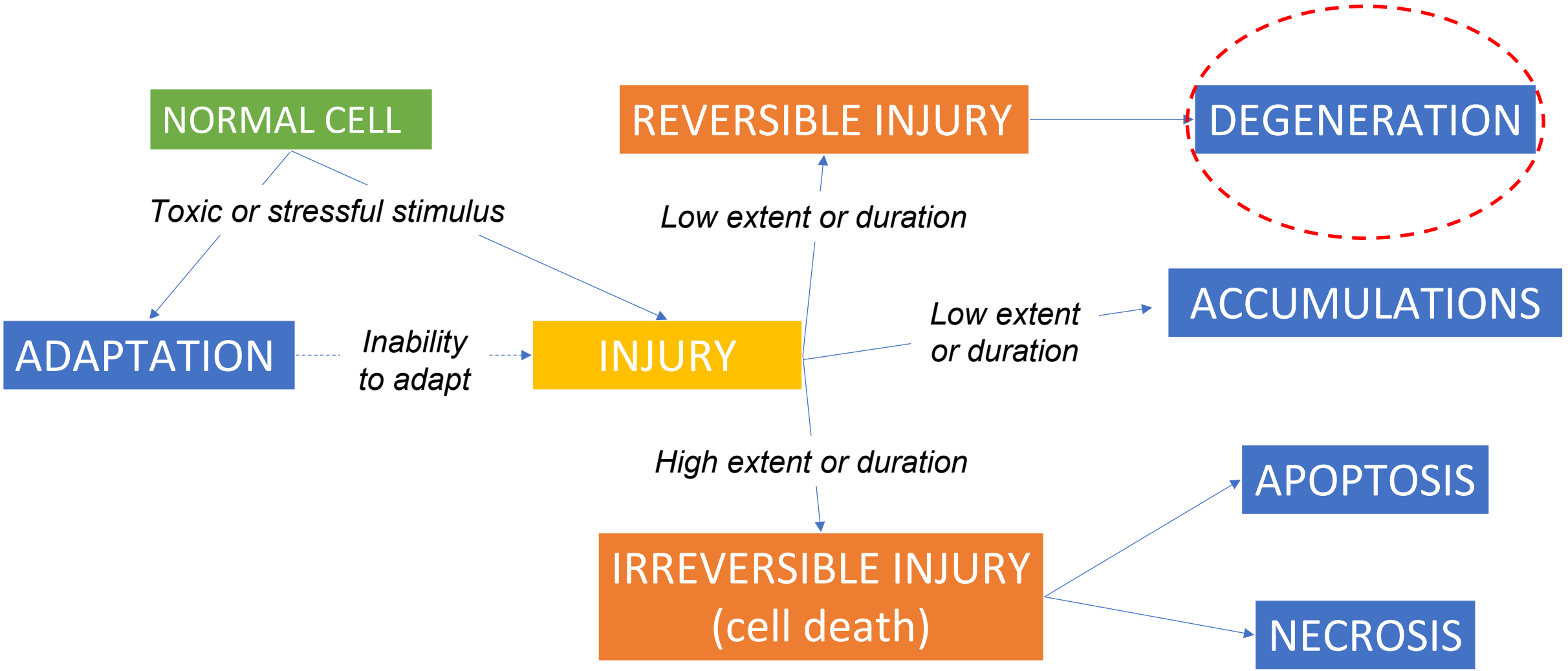
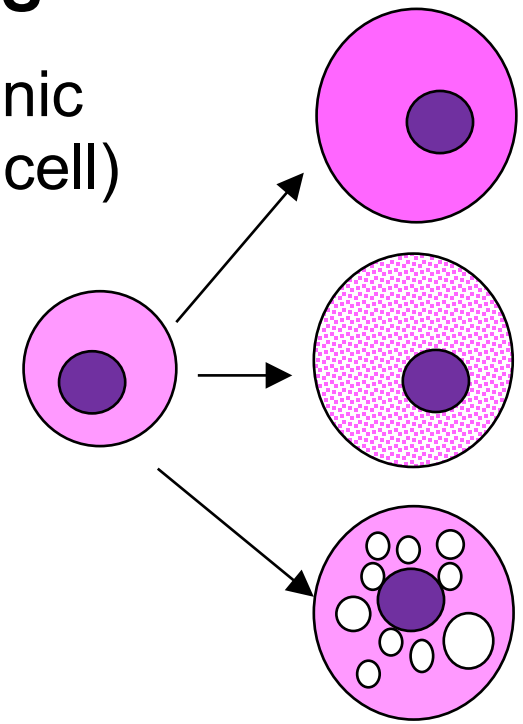
General Cell Responses to Injury



General Pathologic Processes: Degeneration

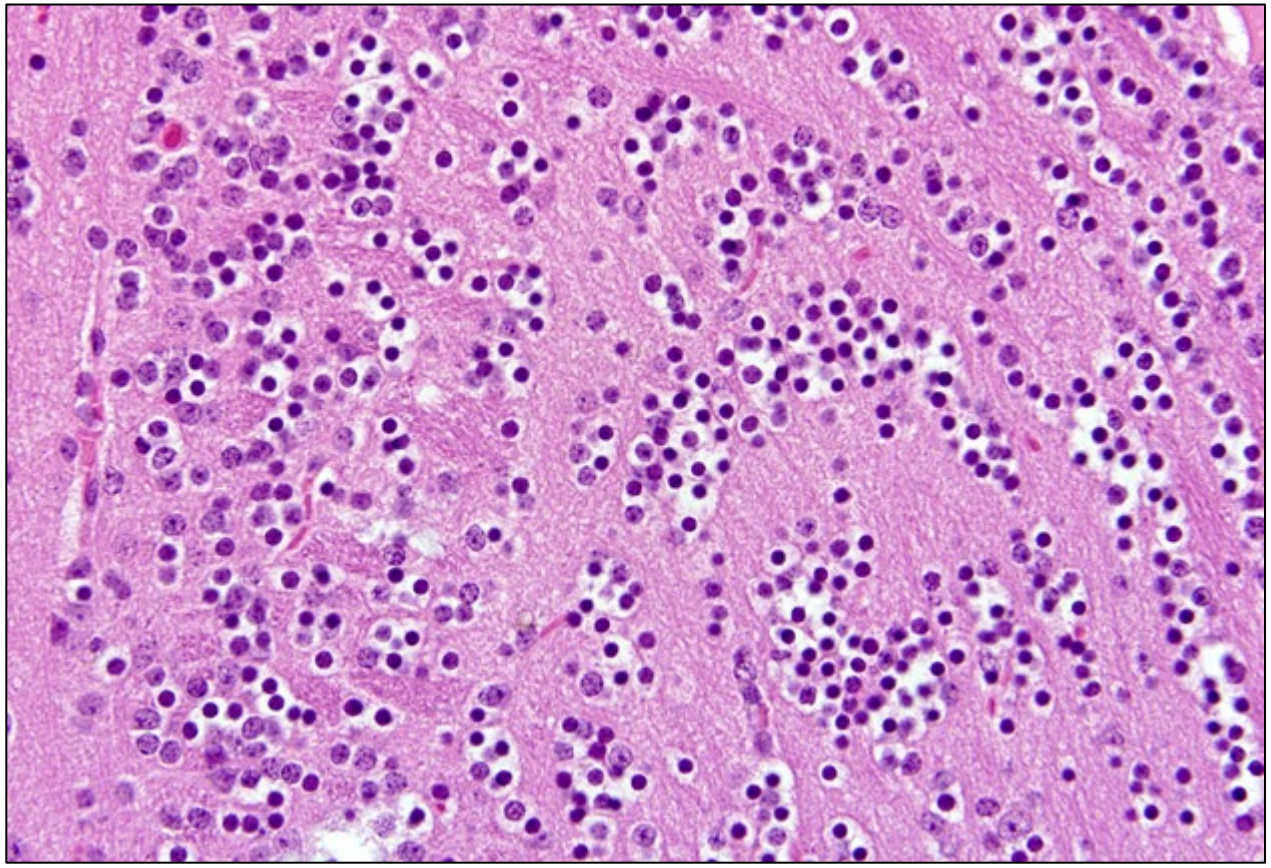
REVERSIBLE INJURY: Degeneration

- **Degeneration hallmarks: CELL SWELLING** and **VACUOLES**
- **SWELLING:** due to failure of the Na^+/K^+ pumps to maintain ionic and fluid homeostasis (therefore, sodium and water enter the cell)
  - The most common manifestation of cell injury
  - Cytoplasm may be **PINKER** or **PALER**
    - **PINKER**: less basophilic cytoplasm due to decreased cytoplasmic RNA
    - **PALER** due to finely vacuolated cytoplasm from water influx diluting the cytoplasm, dilating organelles
- **VACUOLES: WHITE** (clear) spaces
  - Common manifestation of reversible injury (especially in the liver, heart)
  - Vacuoles may be swollen organelles (mitochondria, endoplasmic reticulum) or lipid

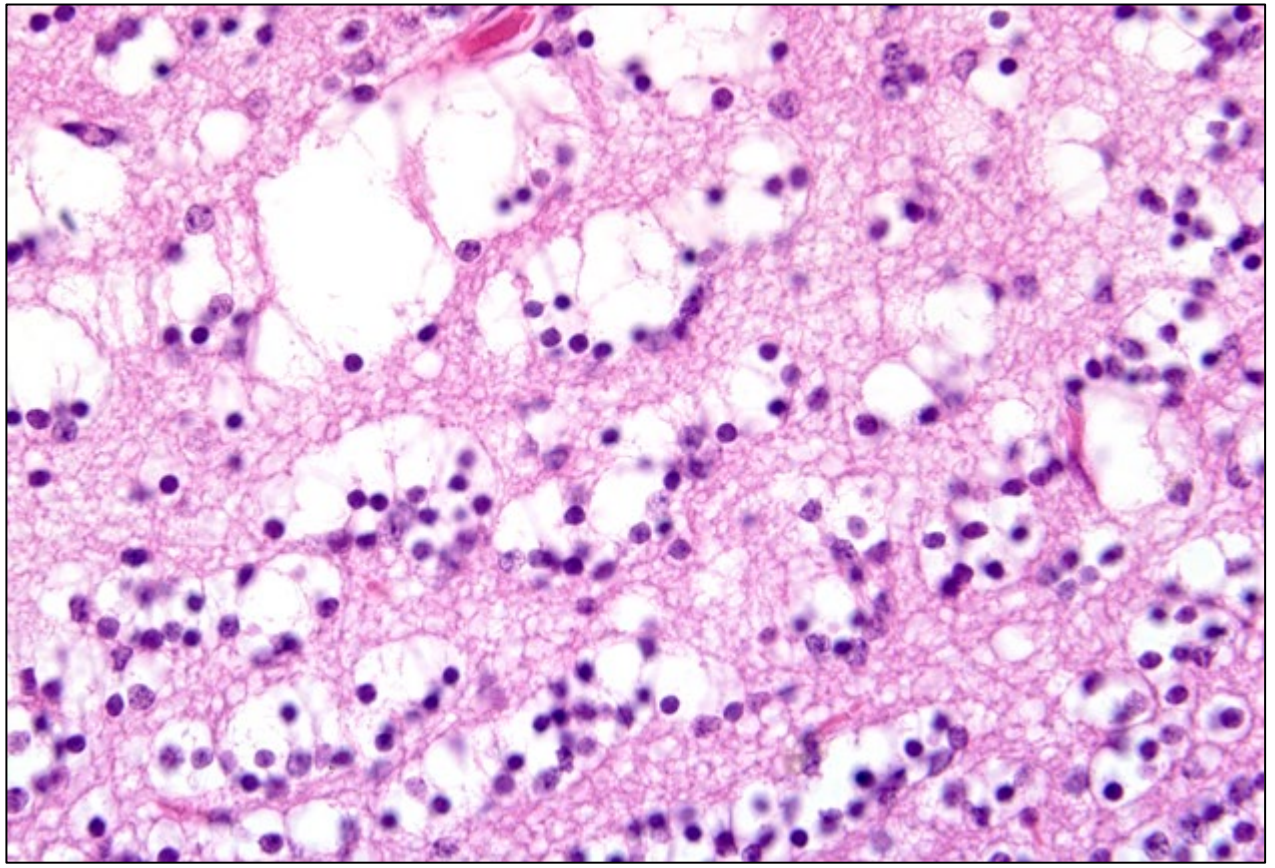


General Pathologic Processes: Degeneration

REVERSIBLE INJURY: Degeneration



NORMAL neurons of the internal granule cell layer of the olfactory bulb of the brain of a mouse.

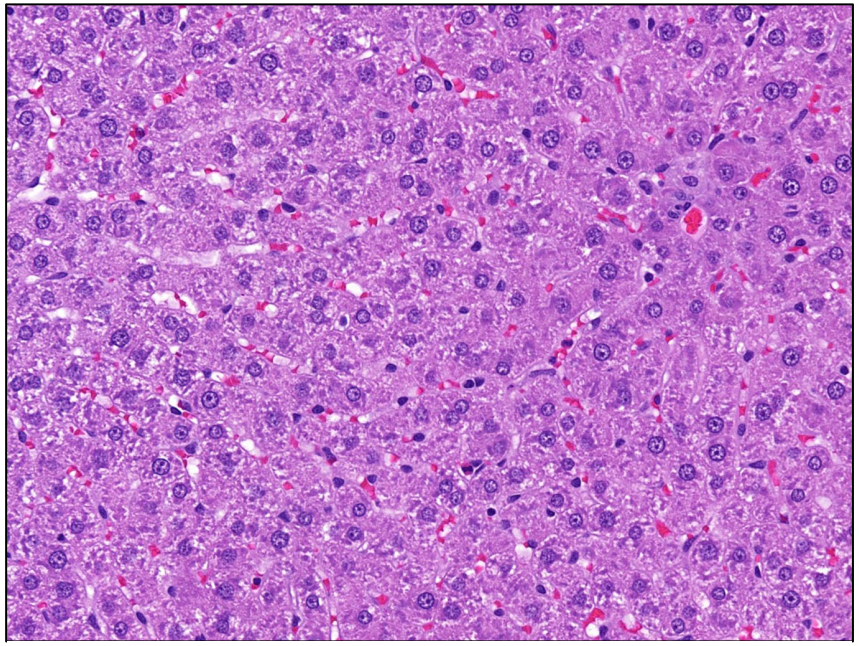


Brain, Neuron – **Degeneration** of the olfactory bulb of the brain of a mouse, seen as swollen neuronal cells with large, prominent vacuoles (large, clear spaces).

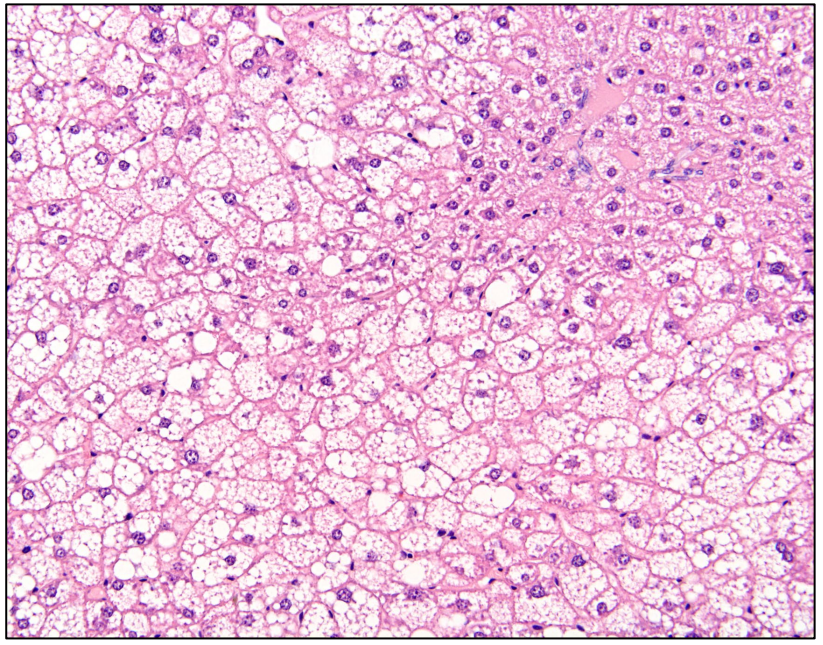
General Pathologic Processes: Degeneration

REVERSIBLE INJURY: Degeneration

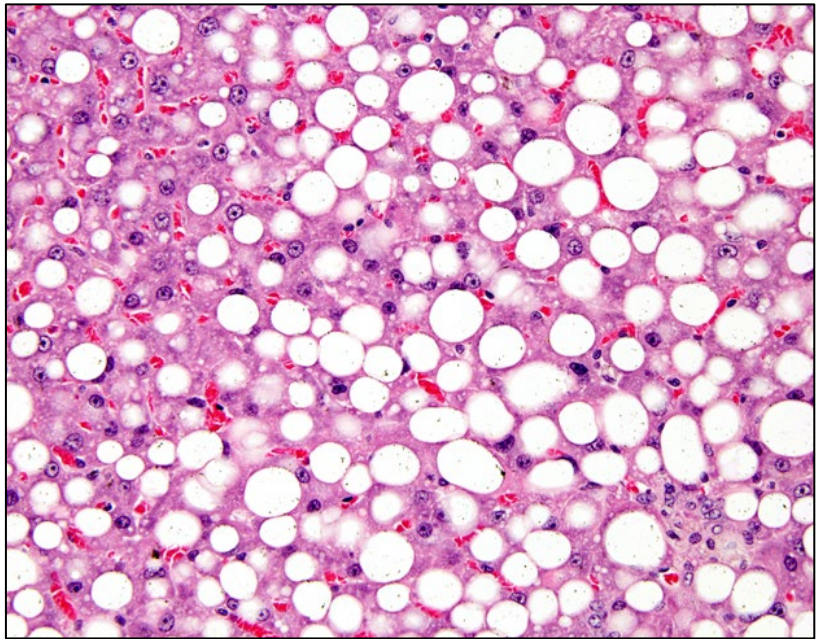
Fatty change is a type of degeneration of that is most common in the liver, heart, muscle, and kidney and results from the abnormal accumulation of triglycerides.



Normal liver from a control mouse. Note that in control animals, fatty change can be a spontaneous finding (not shown here).



Liver – **Fatty change** *(microvesicular vacuolation*, mouse*).* Microvesicular fatty change (multiple small vacuoles without displacement of nuclei) is likely a reflection of toxicity, possibly involving mitochondrial disturbances.

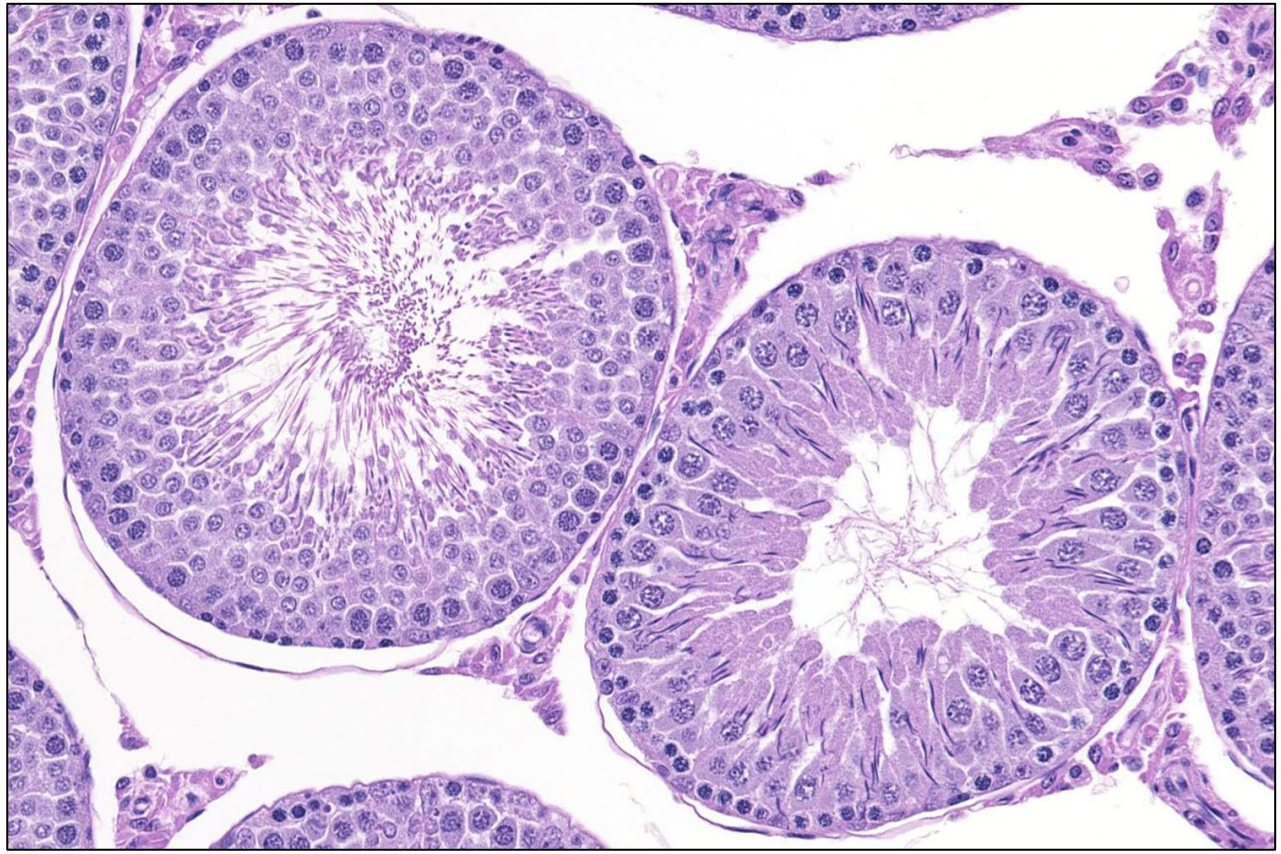


Liver – **Fatty change** *(macrovesicular vacuolation*, rat*).* Macrovesicular fatty change (large vacuoles that displace the nuclei) is often associated with metabolic disturbances.

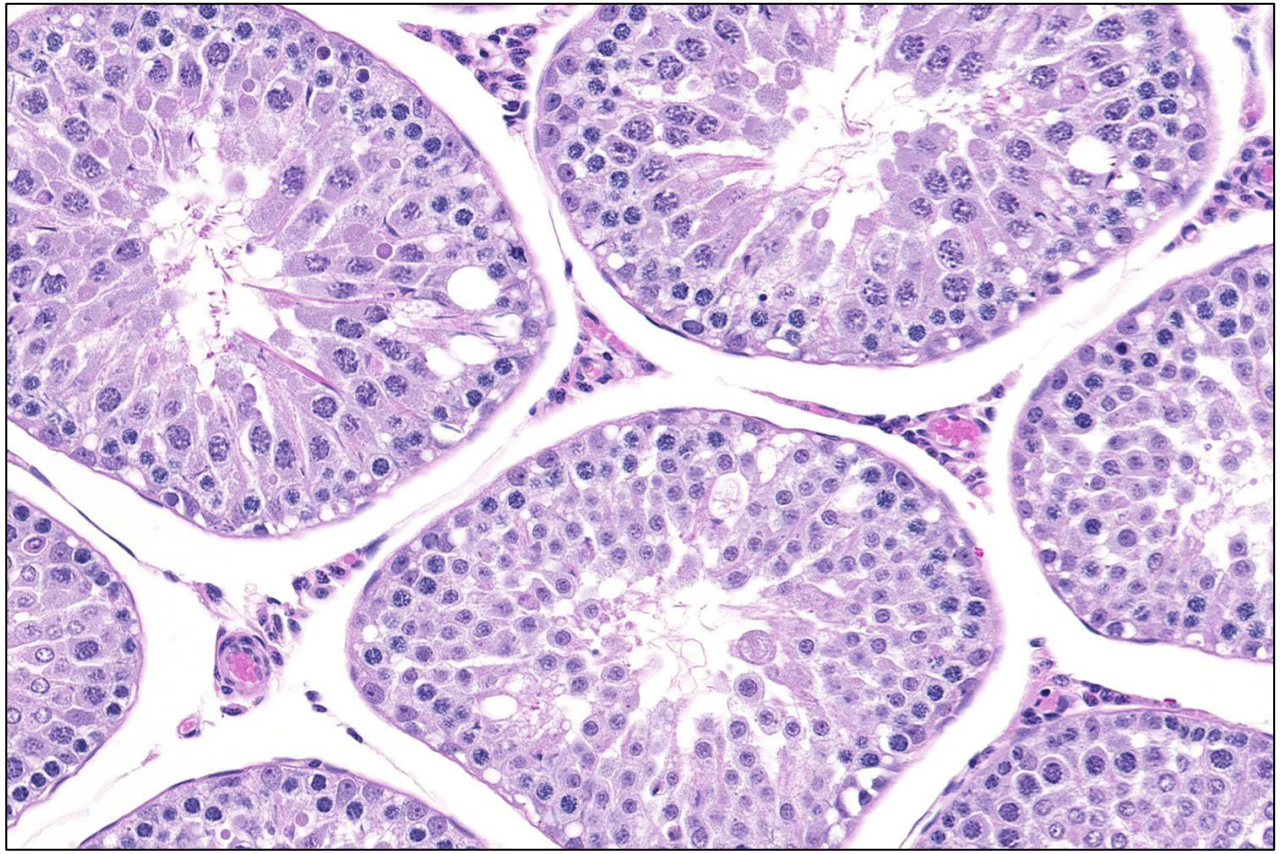
General Pathologic Processes: Degeneration

REVERSIBLE INJURY: Degeneration

At the tissue level, degeneration may appear as swollen cells, clear spaces (vacuolation), cell loss, and disorganization of the normally ordered tissue architecture.



Normal testis in a control rat.



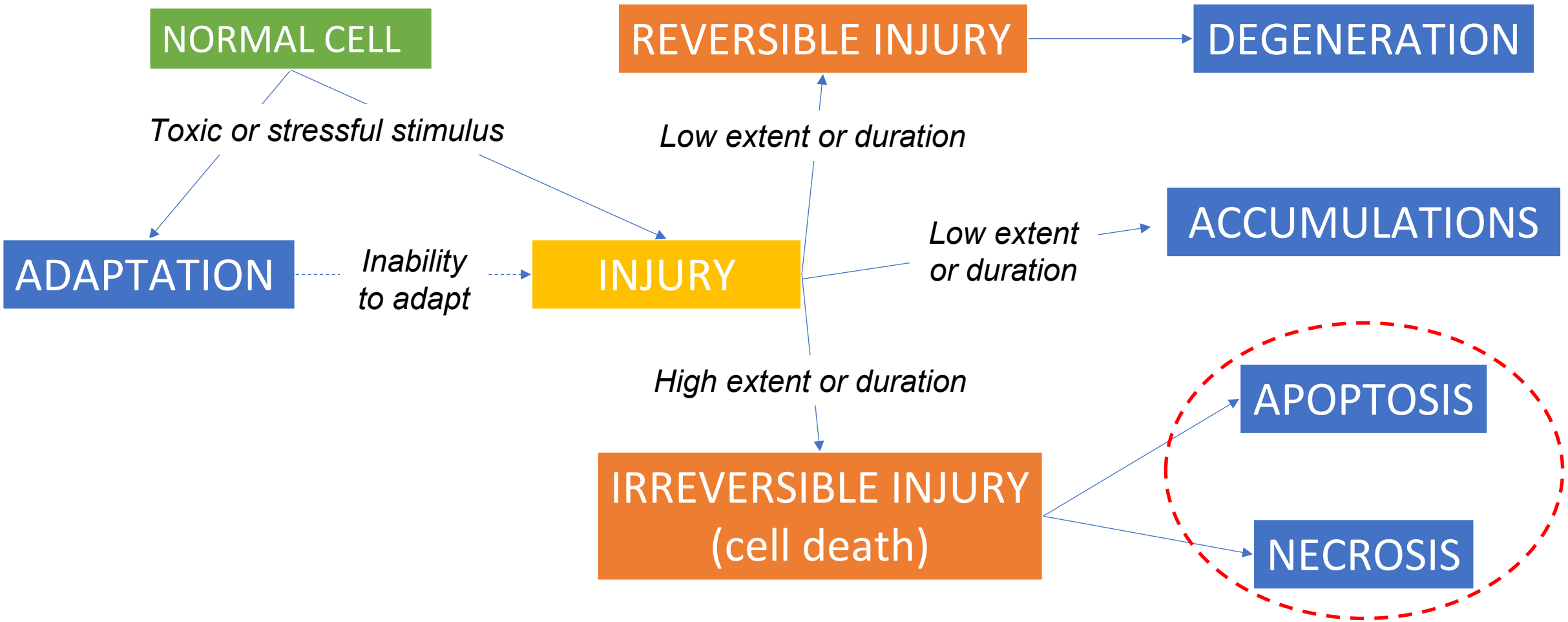
Testis, Germinal Epithelium – **Degeneration**, rat.

General Pathologic Processes: Reversible Cell Injury

Core Concept:

- Reversible cell injury (degeneration) is often seen as swollen cells with clear spaces (vacuoles or lipid) in the cytoplasm
- Degeneration may be called various morphologies (degeneration, fatty change, vacuolation, etc.), depending on the tissue and underlying process

General Cell Responses to Injury

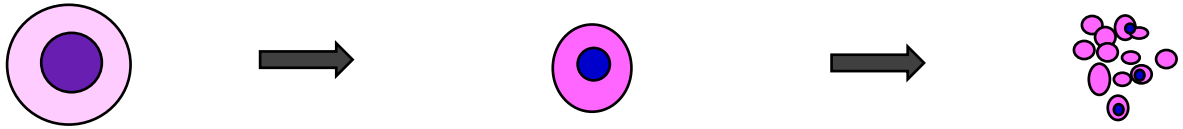


IRREVERSIBLE CELL/TISSUE INJURY: Apoptosis and Necrosis

- Once the damage is so great that injury becomes irreversible, the cell cannot recover and dies
  - There are 2 principal types of cell death: **apoptosis** and **necrosis**
- **Apoptosis** and **necrosis** differ in their morphologic appearance, mechanisms, and roles in normal physiology or disease
  - **Apoptosis**, or "regulated" cell death, is mediated by defined molecular pathways, and can occur as part of normal processes (e.g., to remove cells that are old or no longer needed)
  - **Necrosis**, or "accidental" cell death, is always pathologic
  - In some cases, apoptosis and necrosis can co-occur, or apoptosis can proceed to necrosis

IRREVERSIBLE CELL INJURY: Apoptosis

- Apoptosis is cell death induced by tightly regulated suicide pathways
  - Apoptosis is programmed cell death and requires energy (adenosine triphosphate, ATP); necrosis is unregulated cell death and does not require energy
  - Apoptosis has a distinct morphologic appearance in cells and tissues
  - ATP-dependent cascade of molecular events → **CELL SHRINKAGE** with **deeply eosinophilic, condensed cytoplasm** and **deeply basophilic, condensed nuclear chromatin** (pyknosis) → cytoplasmic **MEMBRANE-BOUND FRAGMENTATION** (apoptotic bodies) → macrophage engulfment (**PHAGOCYTOSIS**)



Normal cell

Apoptotic cell is shrunken, with condensed, deeply eosinophilic cytoplasm and condensation of nuclear chromatin (pyknosis)

Fragmentation of apoptotic cell into membrane-bound fragments (apoptotic bodies), which will be engulfed by macrophages (phagocytosis)

IRREVERSIBLE CELL INJURY: Apoptosis

- Apoptotic cells **shrink** before breaking up into small, membrane-bound fragments of condensed cytoplasm and chromatin called “apoptotic bodies”
  - Unlike necrosis, where cells **swell** before dying due to influx of water
  - Unlike necrosis, plasma membrane integrity persists until late in the process → therefore cell contents are not released, so there is **NO INFLAMMATION with apoptosis**
- Apoptosis can be physiologic (normal) or pathologic (part of disease); necrosis is always pathologic
  - Apoptosis is a normal process in some tissues or at some stages of development
  - Hormone reduction (either physiological or toxicological) can cause apoptosis
- Sometimes, apoptosis and necrosis can co-occur with some agents
  - For example, dexamethasone in the thymus can cause both processes
  - Alternatively, apoptosis may precede necrosis, which then follows after ATP depletion

IRREVERSIBLE CELL/TISSUE INJURY: Necrosis

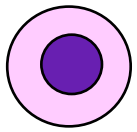
- Necrosis is a pathologic process resulting from severe injury
  - Necrosis can occur on its own, or following or concurrent with apoptosis
- With necrosis, there is depletion of ATP, denaturation of cellular proteins, metabolic failure, and leakage of cellular contents
  - Loss of cell plasma membrane integrity → fluid influx → **swelling** (as in degeneration) → cell death and release of cell contents → incites **inflammation** in surrounding tissue

General Pathologic Processes: Necrosis

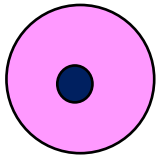
IRREVERSIBLE CELL/TISSUE INJURY: Necrosis

- Possible cytoplasmic changes with necrosis:
  - Early: homogeneously **eosinophilic** due to loss of cytoplasmic ribosomal RNA (which is normally basophilic because RNA binds hematoxylin), or due to consolidation of cytoplasmic components (what starts as degeneration can become necrosis)
  - Later: **pale**, "ghost-like" due to degradation of cytoplasmic proteins
- Three possible nuclear changes with necrosis, all due to DNA breakdown:

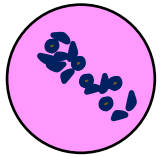
Normal



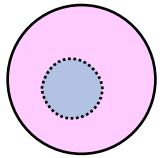
Pyknosis
(shrunken, darker, more basophilic nucleus; as also seen with apoptosis)



Karyorrhexis
(fragmentation of pyknotic nucleus)



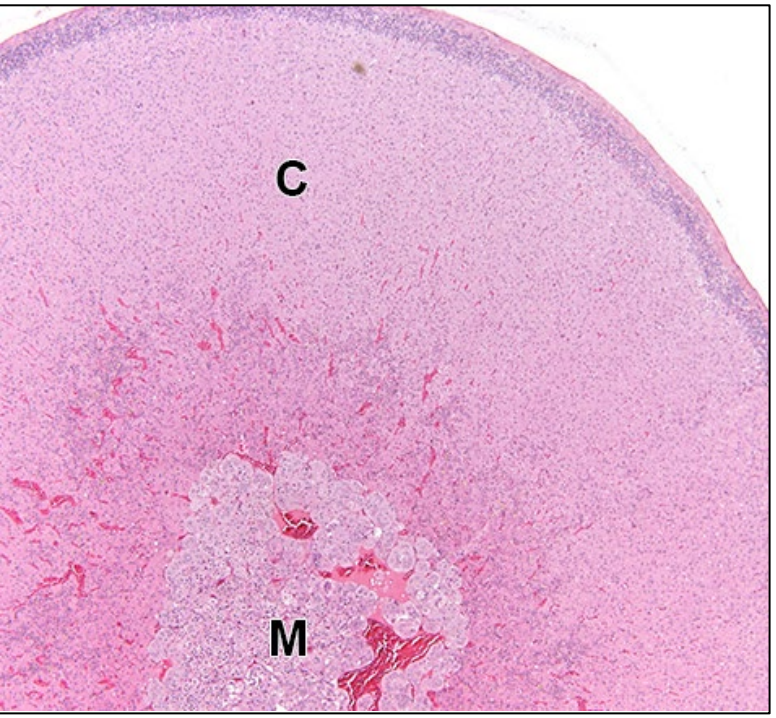
Karyolysis
(extremely pale to absent due to chromatin degradation)



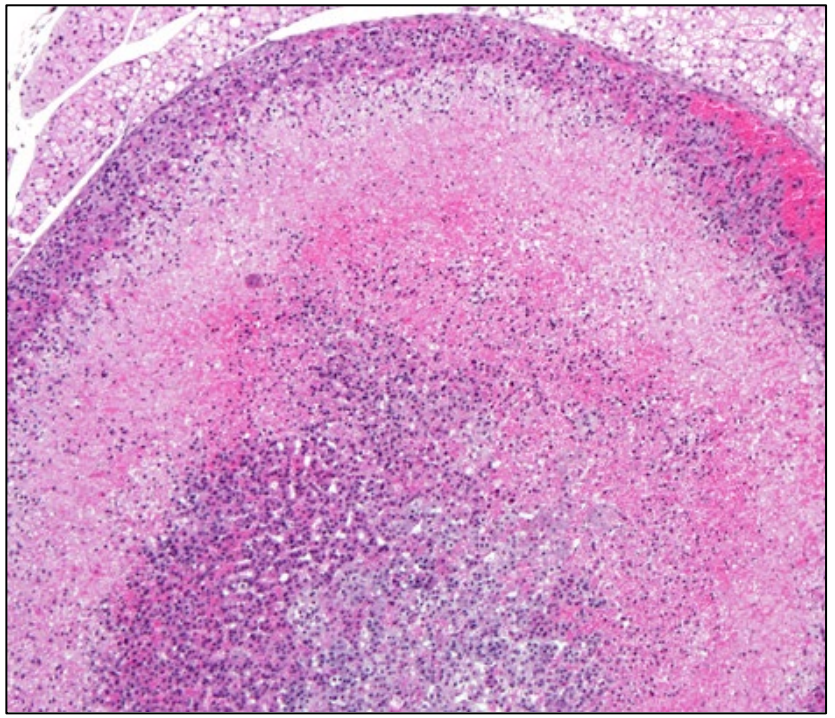
- At the tissue level, the karyolytic cells lend to tissue pallor (paleness due to degradation of nuclear chromatin and cytoplasmic proteins) and loss of cell detail
  - The necrotic cells lose their adherence to the basement membrane, and cells may become individualized

General Pathologic Processes: Necrosis

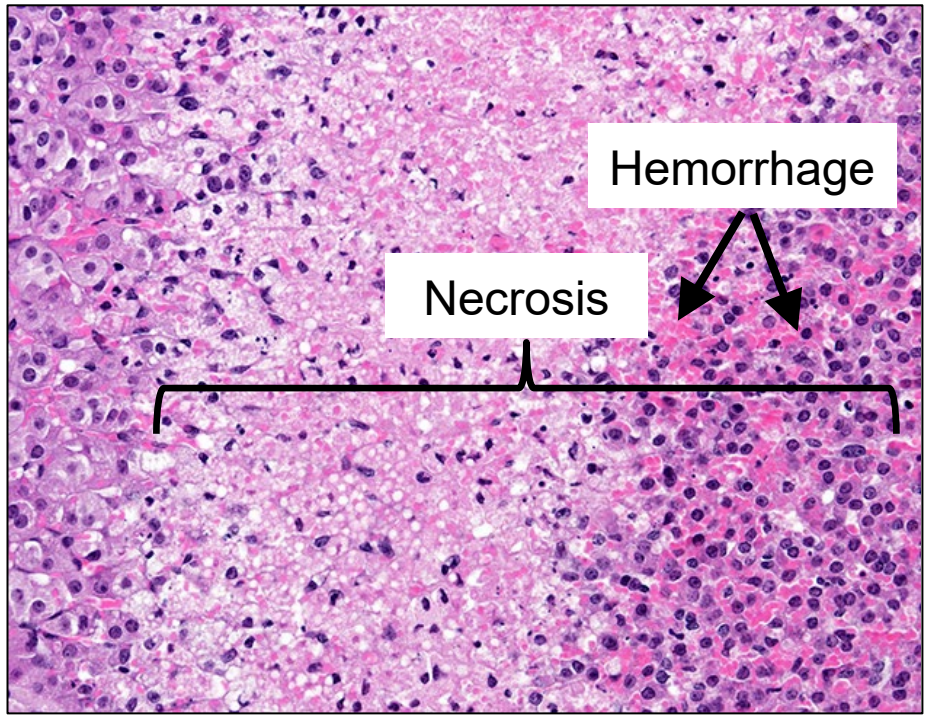
IRREVERSIBLE CELL/TISSUE INJURY: Necrosis



Normal adrenal gland cortex, mouse. C = Cortex; M = Medulla.



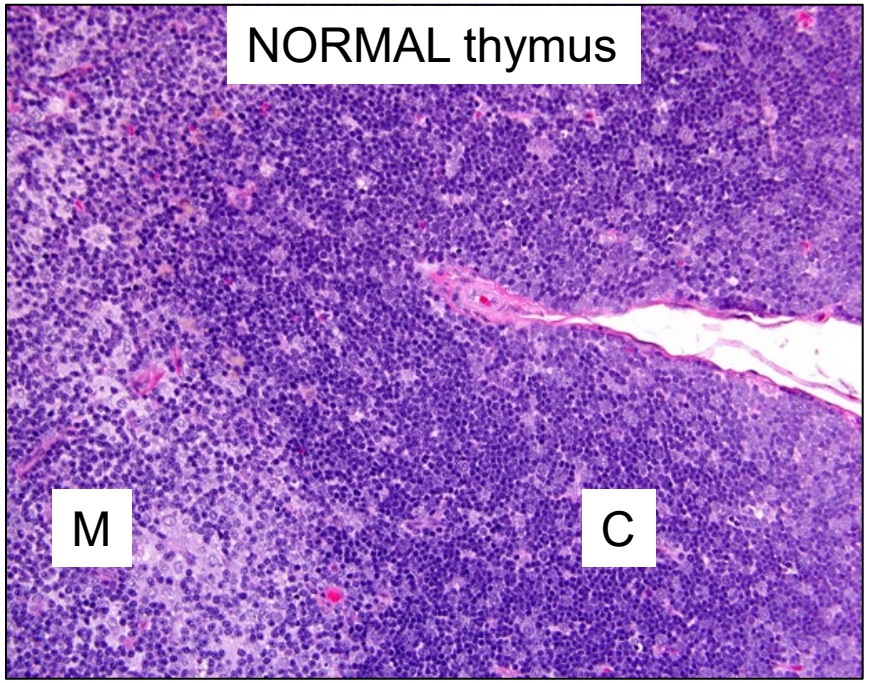
Adrenal gland necrosis, mouse. The tissue architecture is disrupted and there is pallor (pale areas), loss of detail, and hemorrhage (red blood cells outside of vessels).



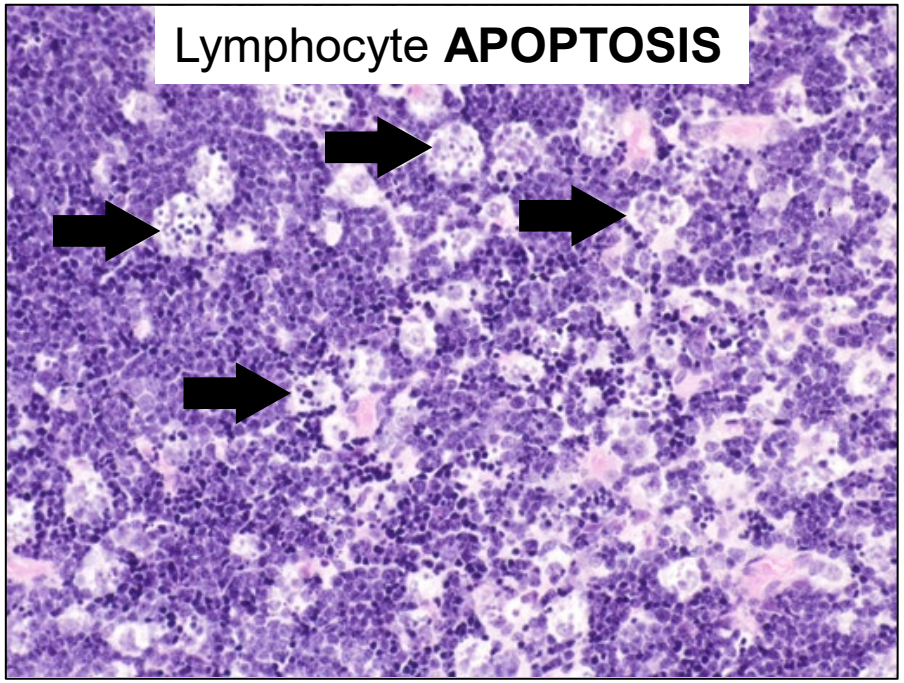
At higher magnification, there is pallor and loss of cellular outlines and detail, cellular debris, hemorrhage, and inflammation.

General Pathologic Processes: Cell Death

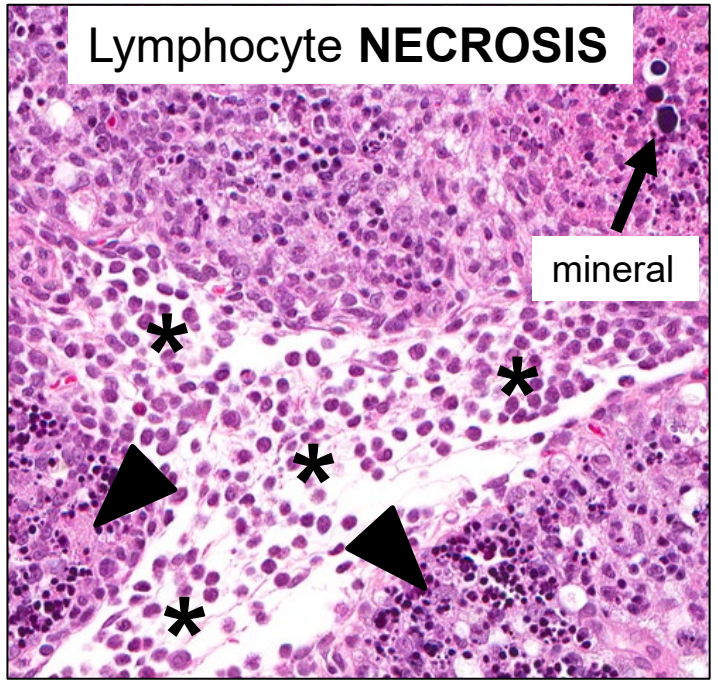
IRREVERSIBLE CELL INJURY: Apoptosis and Necrosis



Normal thymus, mouse. M = Medulla; C = Cortex.



Lymphocyte **apoptosis** in the thymus, mouse. Tingible body macrophages (arrows) contain the apoptotic bodies, giving a “starry sky” appearance to the thymic cortex. Unlike necrosis, apoptosis is not usually associated with inflammation, tissue destruction, mineralization, and/or hemorrhage.



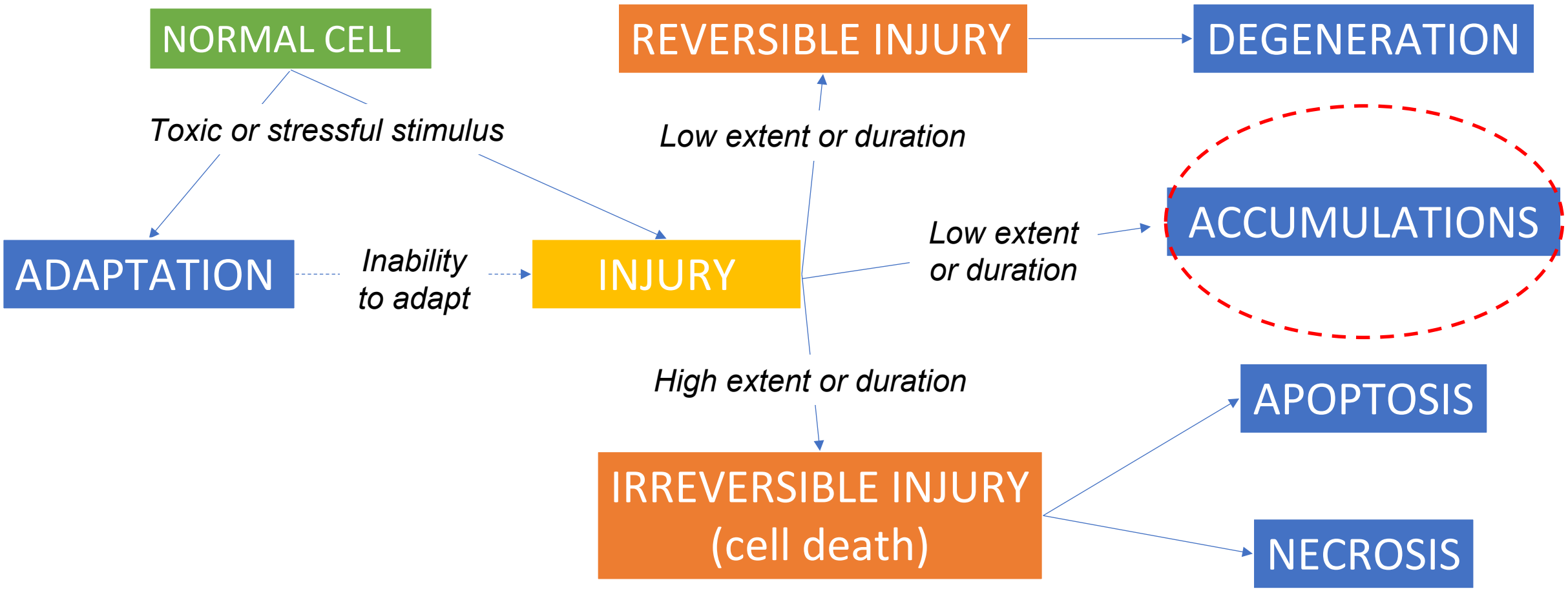
Lymphocyte **necrosis** in the thymus, rat. Tissue architecture is disrupted and there is pallor (pale areas); eosinophilic cytoplasmic and basophilic nuclear cell debris (arrowheads); inflammatory cells (*); and mineral (arrow).

General Pathologic Processes: Cell Death

Core concept:

- Irreversible cell injury typically occurs by apoptosis or necrosis
- **Apoptosis**: cells shrink and there is no tissue inflammation
- **Necrosis**: cells enlarge (at first) and there is tissue inflammation

General Cell Responses to Injury

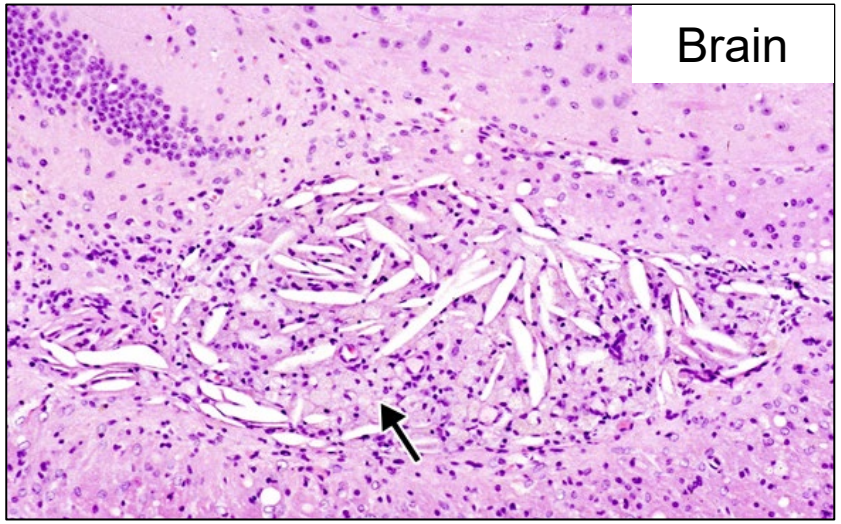


General Pathologic Processes: Accumulations

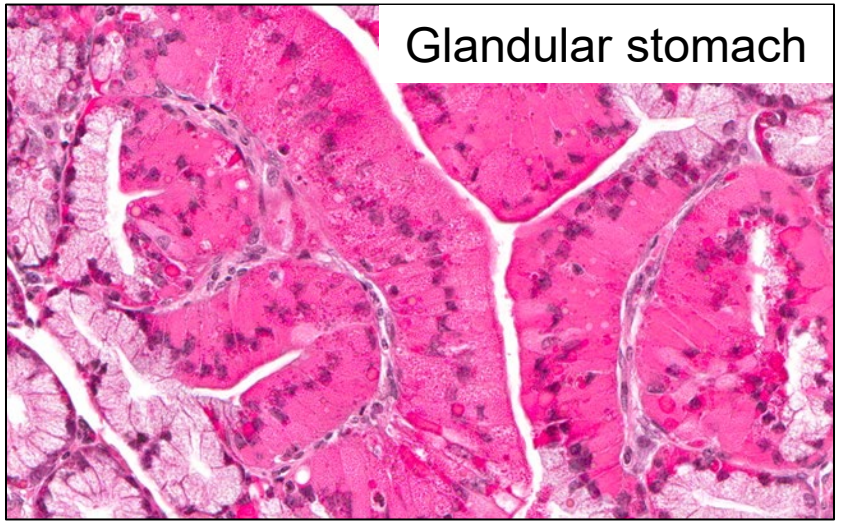
- Instead of the responses of adaptations or cell injury, cells and tissues may respond with metabolic derangements that result in the **accumulation** of abnormal amounts of either normal substances or abnormal substances
- Accumulations may be present in tissue spaces, between cells, or within the cell cytoplasm
- Normal cellular components that accumulate may include water, lipid, glycogen, cholesterol, carbohydrates, protein, or pigment
  - May be seen as **vacuolation** (can also be considered an early form of degeneration) and represent accumulation of **lipids** (triglycerides, phospholipids) or **glycogen**
  - **Protein** often appears as a homogeneous, glassy, eosinophilic substance; may be referred to as “hyaline”
  - Endogenous (inside the body) substances from abnormal synthesis or metabolism (for example, **hemosiderin pigment** from breakdown of red blood cells or **lipofuscin pigment** from lipid peroxidation of cell membranes)
- Alternatively, accumulations may be abnormal components:
  - Exogenous (outside the body) substances like **pigments** (for example, from carbon, tattoos, or dust)

General Pathologic Processes: Accumulations

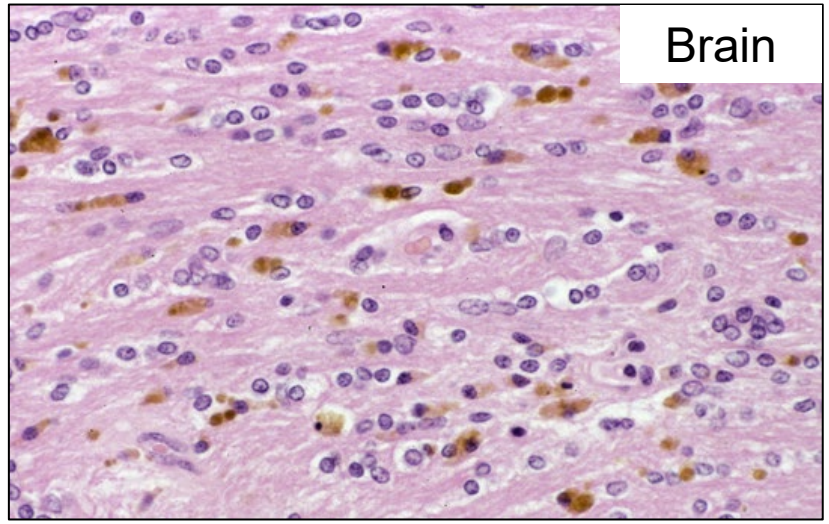
ACCUMULATIONS: Cholesterol, protein, pigment



Accumulation of **cholesterol** crystals ("clefts") in the brain, mouse. The empty, elongated, spicular (needle-like) spaces are surrounded by inflammatory cells (mixed glial cells and macrophages; arrow). The spaces are actually empty, since tissue processing results in removal of the cholesterol. This type of accumulation is usually the result of necrosis in the brain or other tissues.



Hyaline droplet accumulation in gastric glands of the glandular stomach, mouse. Homogeneous, globular, brightly eosinophilic material fills the cytoplasm of gastric mucosal epithelial cells. These types of accumulations are generally comprised of **proteins** and can occur in the lung, kidney, nose, gallbladder, and pancreas.



Pigment accumulation in the brain, rat. The brown pigment has been phagocytized by macrophages ("hemosiderin-laden macrophages") at a site of former hemorrhage in the corpus callosum. Hemosiderin results from the breakdown of red blood cells at the site of hemorrhage. If warranted, special stains are used to definitively determine the type of pigment present.

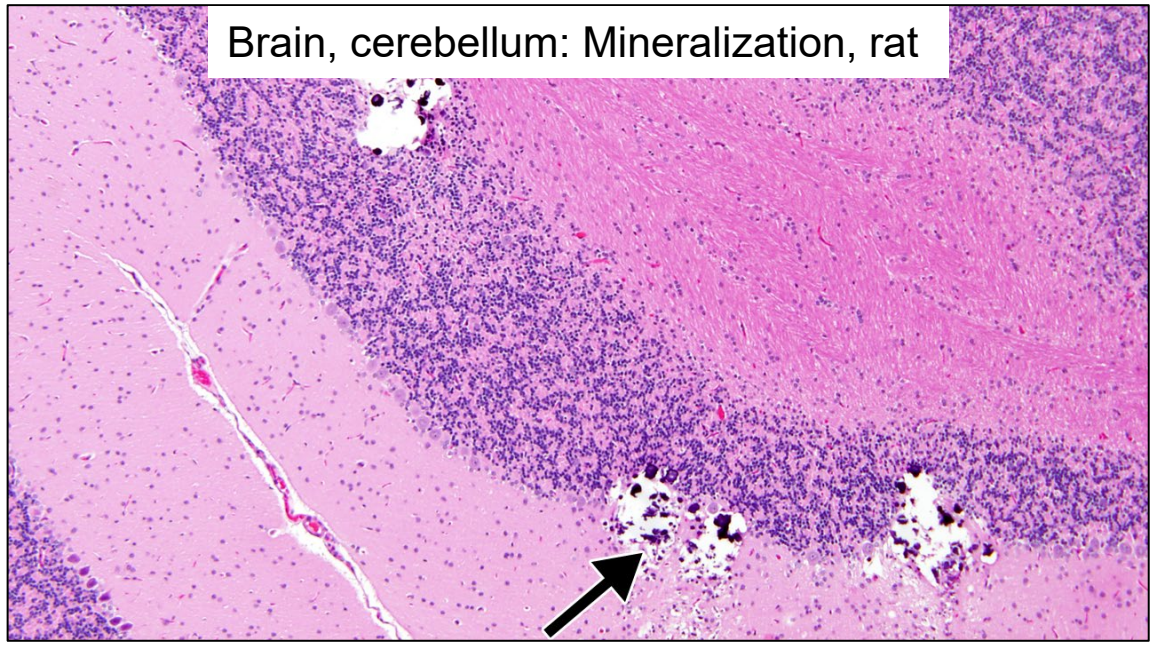
ACCUMULATIONS: Mineralization

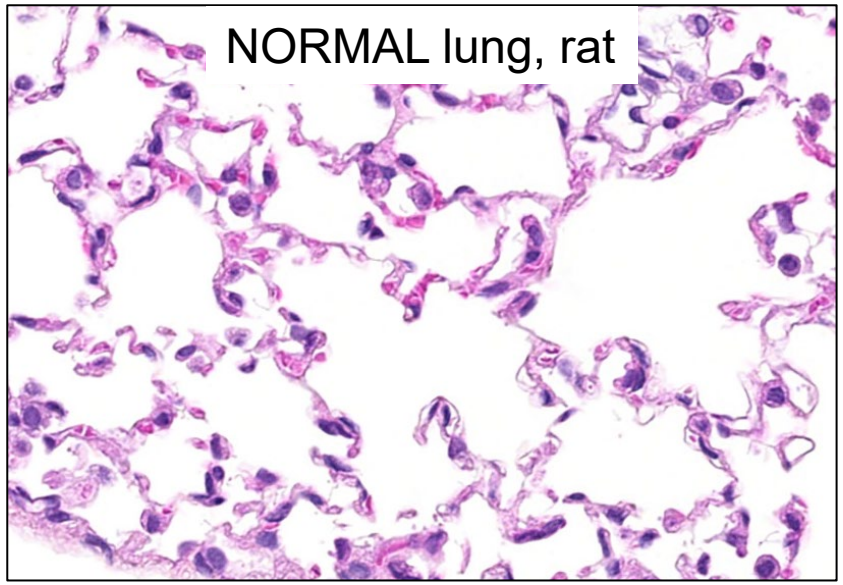
- **Mineralization** is due to pathological calcification, which is the deposition/accumulation of calcium salts in either dead/dying tissue or normal tissue
- Macroscopically (grossly), mineral appears as hard, white, gritty granules
- Microscopically, mineral appears as angular, deeply basophilic intra- or extra-cellular depositions
  - Because mineral is hard, it can lead to artifactual spaces in the surrounding tissue during microtomy
- Mineralization is due to two different categories of calcification: dystrophic or metastatic
  - Dystrophic mineralization occurs in areas of tissue necrosis
  - Metastatic calcification is due to metabolic derangements of calcium and phosphorus
    - Results from hypercalcemia (too much calcium in the blood), increased parathyroid hormone, vitamin D-related disorders or toxicosis, or renal disease/failure resulting in secondary hyperparathyroidism
- Special stains to identify mineral: von Kossa and alizarin-red-S

General Pathologic Processes: Mineralization

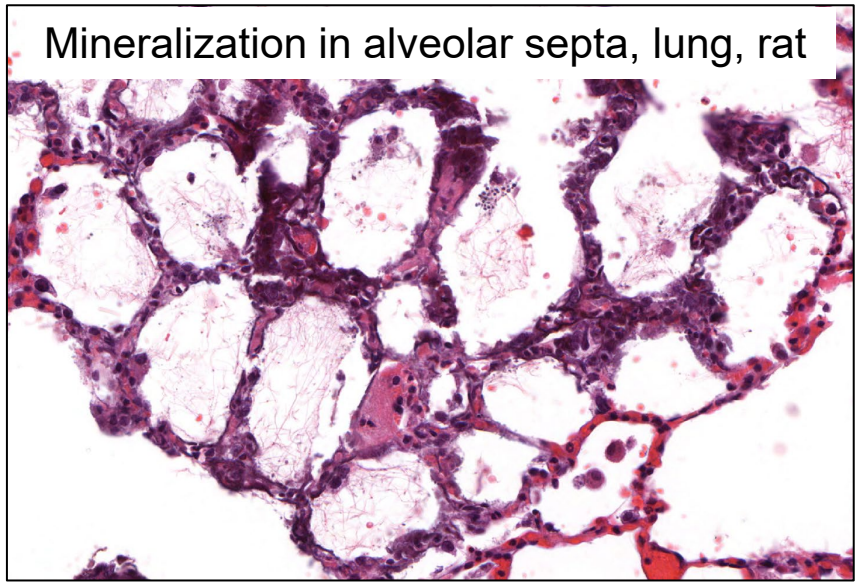
ACCUMULATIONS: Mineralization

Right: Dystrophic **mineralization** at former sites of necrosis in the cerebellum, rat. These mineral deposits of deeply basophilic, angular material (arrow) are present at sites of former necrosis. The clear spaces around the material are artifacts from the microtomy knife cutting the hard material. Mineral in the brain should not be confused with the artifactual presence of bony skull spicules that get pushed into the brain during dissection/trimming or with corpora amylacea, which are normal structures in some species.


Brain, cerebellum: Mineralization, rat


NORMAL lung, rat

In contrast to the normal alveolar septa in the lung (left), the lung on the right shows metastatic **mineralization** throughout the alveolar septa (interstitium) of the lung, rat. The rat also had chronic progressive nephropathy in the kidney. Calcification from metabolic derangements of calcium and phosphorus could occur anywhere, but tends to affect the lungs, gastric mucosa, kidneys, systemic arteries, and pulmonary veins.


Mineralization in alveolar septa, lung, rat

General Pathologic Processes: Accumulations

Core concept:

- Cholesterol accumulations are white, needle-like spaces; often surrounded by macrophages
- Pigment is colored (often **brown**) material that may be endogenous or exogenous
- Proteins are often **deeply eosinophilic (PINK)**
- Mineralization is **deeply basophilic (BLUE)** and angular

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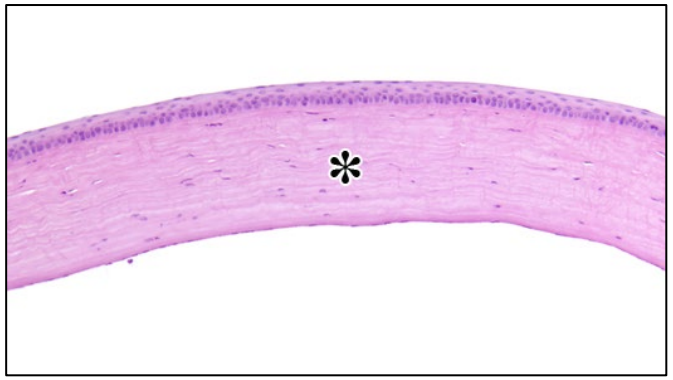
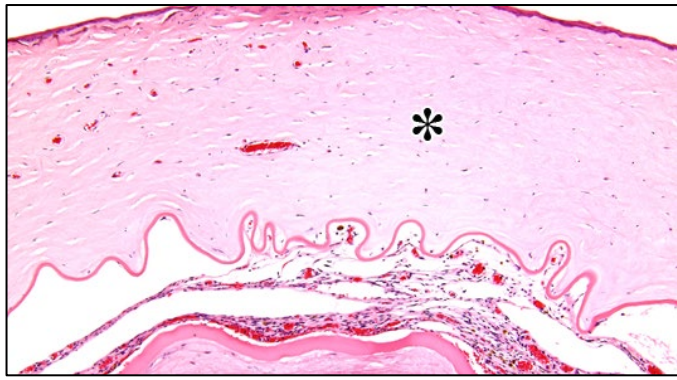
General Pathologic Processes: Vascular Changes

- **Edema**: accumulation of fluid in the extracellular space; seen as expansion by pale pink (upper right image)
- **Congestion**: accumulation of blood within the vascular system; seen as dilated vessels
- **Hemorrhage**: escape of blood from the vascular system; seen as red blood cells in interstitium of tissues
- **Thrombosis**: intravascular coagulation; Seen as layers of pink fibrin / red blood cells / inflammatory cells within blood vessels (thrombus; image on lower left)
- **Ischemia**: reduced blood flow; if stops → infarction (infarct; wedge-shaped necrosis [triangle in image on lower right] ± shrunken tissue)

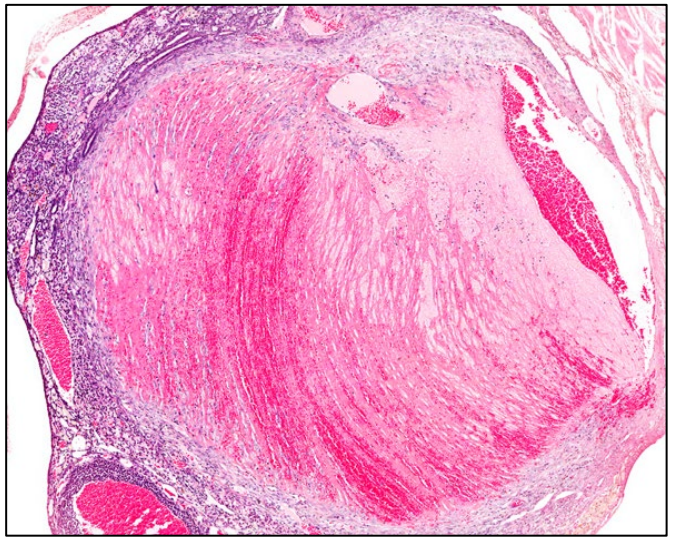
Normal cornea (*) of the eye, rat



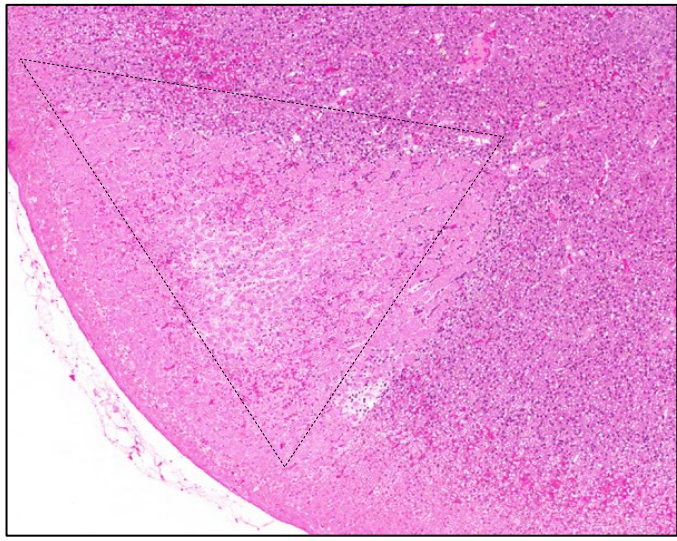
Eye, Cornea (*) – **Edema**, rat



Ovary – **Thrombus** in a vessel, mouse

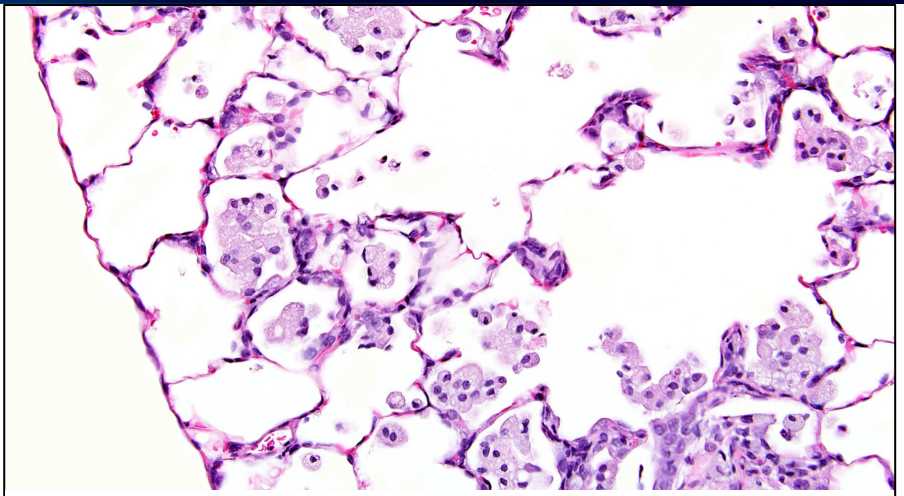


Adrenal gland – **Infarct**, rat

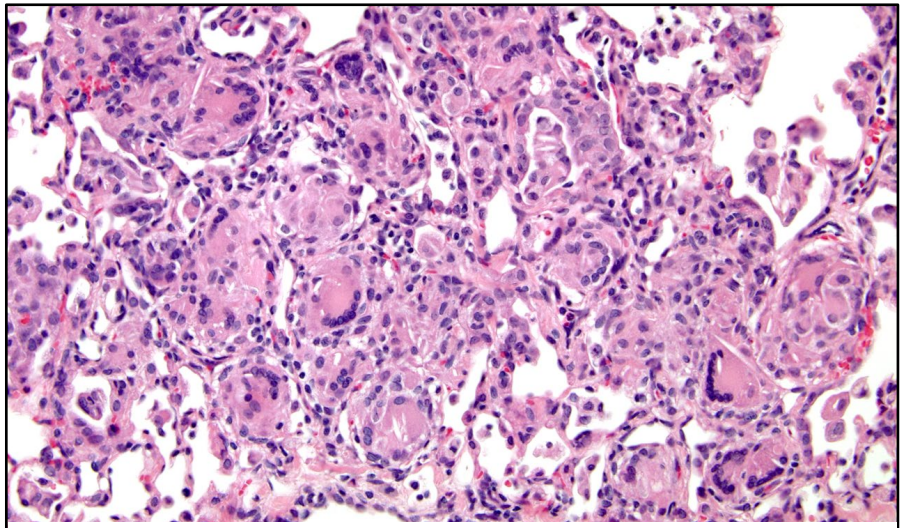


General Pathologic Processes: Inflammation

- **Inflammation** is the reaction of vascularized living tissue to a local injury
- Seen as the presence of inflammatory cells **+ other tissue morphologic features of inflammation**
  - One or more other features are necessary to diagnose inflammation: congestion, edema, hemorrhage, necrosis, fibrosis, tissue disruption/destruction
  - If these other features are absent, consider a diagnosis of **infiltrate** (upper) rather than **inflammation** (lower)
    - Or, no diagnosis if spontaneous, low numbers of inflammatory cells (within normal limits)
- In general, descriptive terms are used in toxicologic pathology
  - Rather than diagnostic terms that end in "-itis"
    - For example, "Kidney: inflammation, mixed cell, mild" rather than "acute nephritis"
    - There are a few exceptions (e.g., polyarteritis nodosa)



Lung, alveoli – Macrophages, increased, rat
DTT terminology: Lung – Infiltration cellular, histiocyte, rat



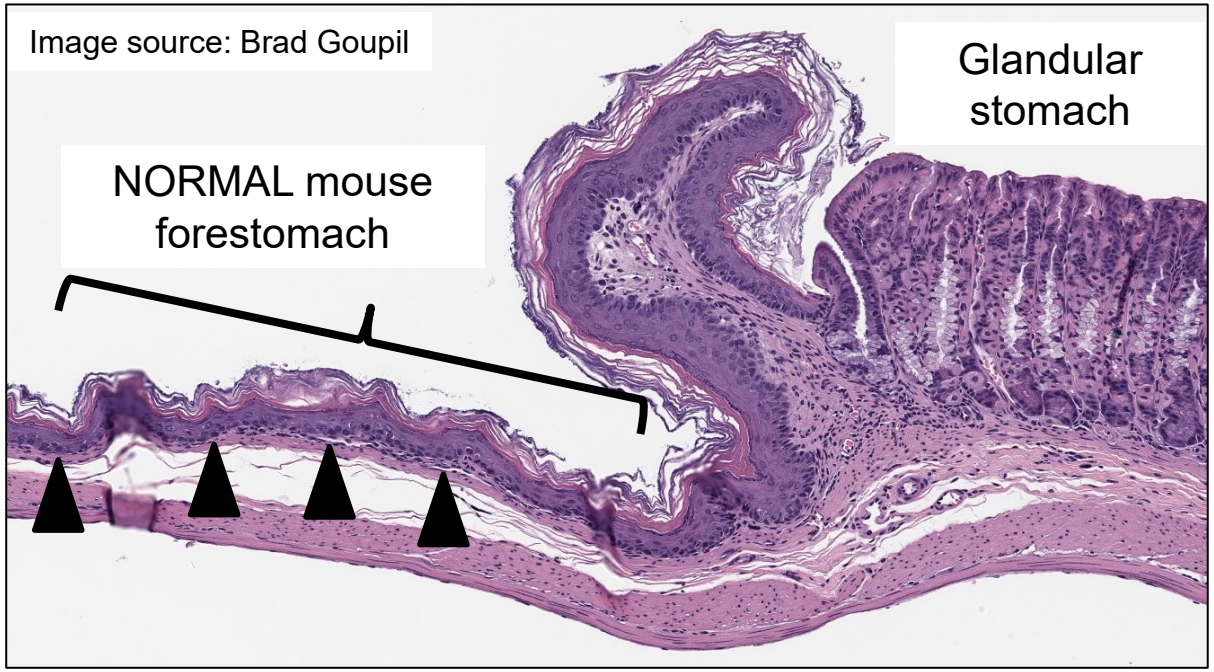
Lung – Inflammation, granulomatous, rat

General Pathologic Processes: Erosion and Ulcer

Basement membrane (arrowheads)

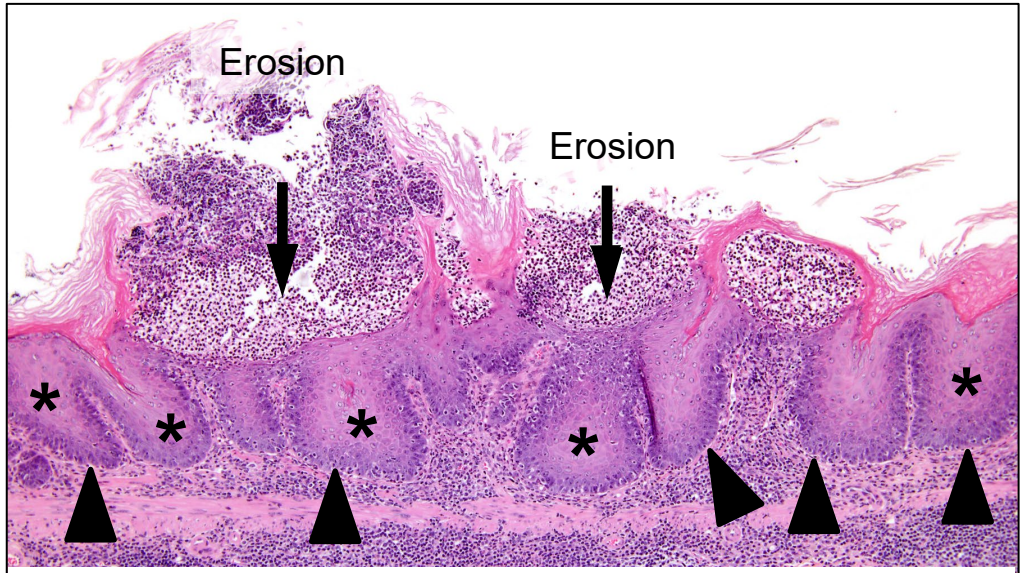
INTACT

- **Erosion**: loss of the superficial epithelial layers of the mucosa (upper right image), basement membrane remains intact; can be associated with inflammation

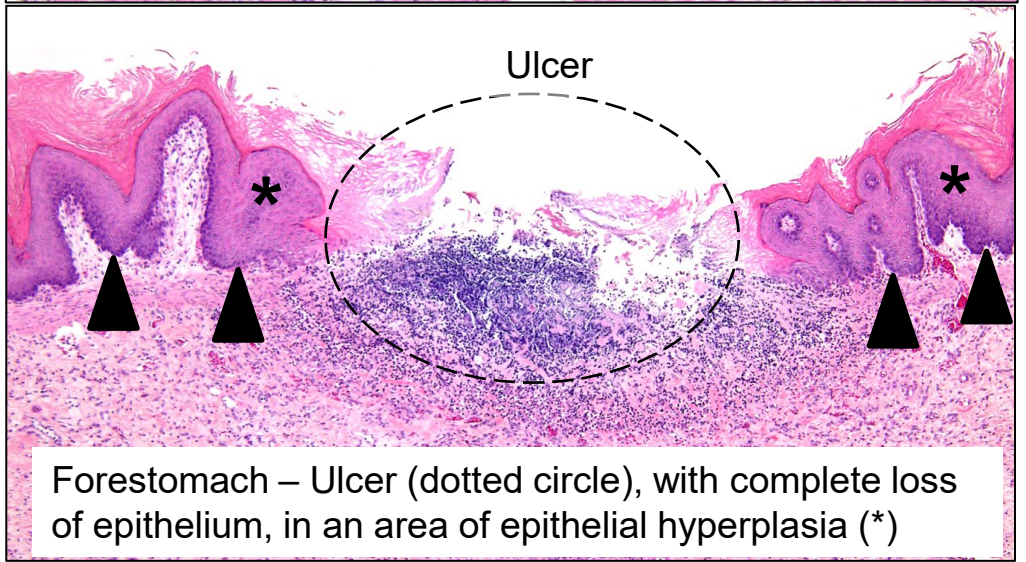


DISRUPTED

- **Ulcer**: loss of **ALL** epithelial cell layers (dotted circle, lower right), extending through the submucosa; basement membrane (region indicated by arrowheads, above and images on right) is breached
- Often associated with inflammation, ± edema, hemorrhage



Forestomach – Erosion (arrows) with partial loss of epithelium within a hyperplastic epithelium (*)



Forestomach – Ulcer (dotted circle), with complete loss of epithelium, in an area of epithelial hyperplasia (*)

General Pathologic Processes: Erosion and Ulcer

Core concept:

- Erosion is tissue damage limited to a portion of an epithelium; the basement membrane remains intact
- Ulcer is tissue damage to the full thickness of an epithelium; the basement membrane is disrupted and is frequently associated with inflammation, hemorrhage, and/or edema

General Pathologic Processes: Regeneration and Repair

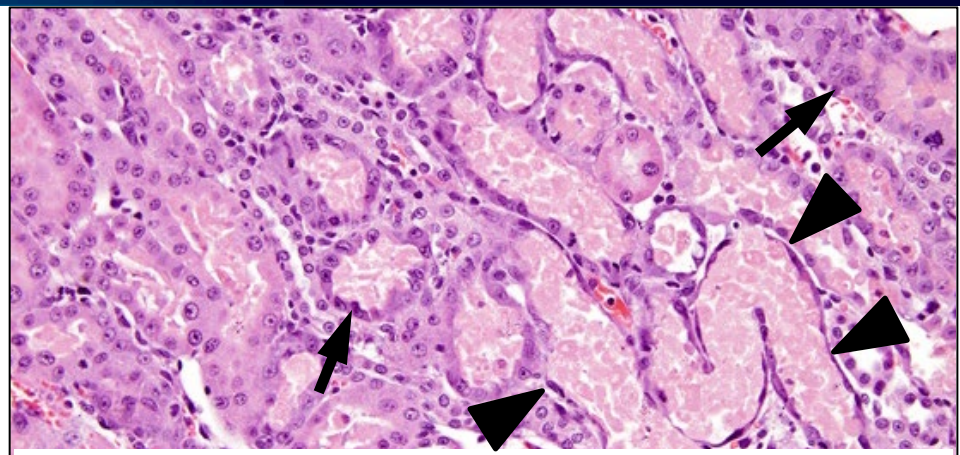
Connective tissue framework

INTACT

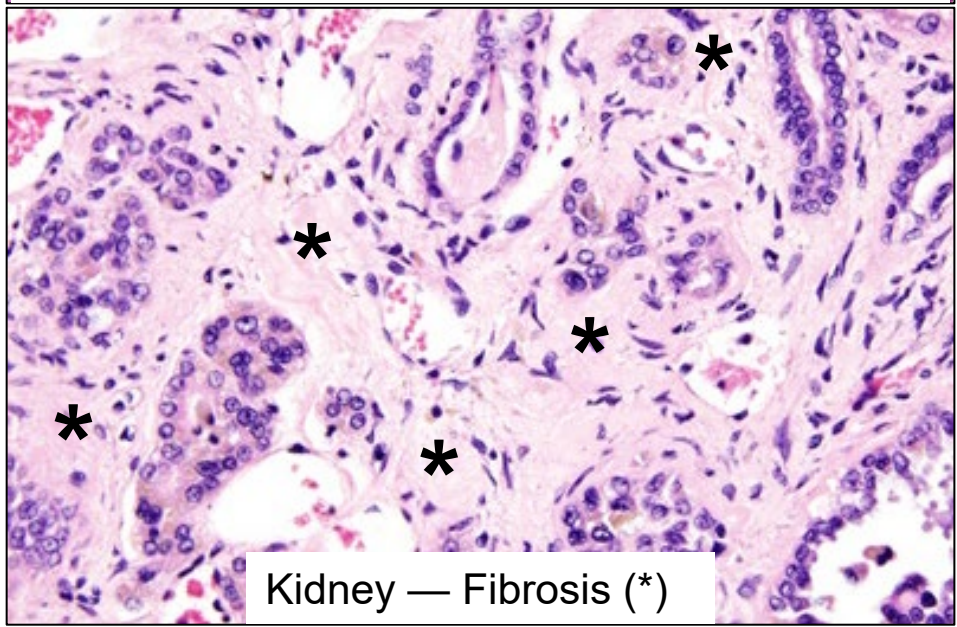
- **Regeneration**: Replacement of damaged cells by cells of the same tissue type
  - Often **darker blue cytoplasm** (arrows, upper right image) because of increased cytoplasmic ribosomes and rough endoplasmic reticulum producing proteins for repair
  - ± Nuclear crowding, mitotic figures, flattened cells

LOST

- **Fibrosis** (scarring): Replacement of injured tissue by fibrous connective tissue (collagen)
  - Pale pink, amorphous material (collagen; *, lower right image)
  - Usually, loss of function and/or strength



Kidney — Regeneration, Tubule. Tubule cell basophilia with nuclear crowding (arrows) and flattening (arrowheads).



Kidney — Fibrosis (*)

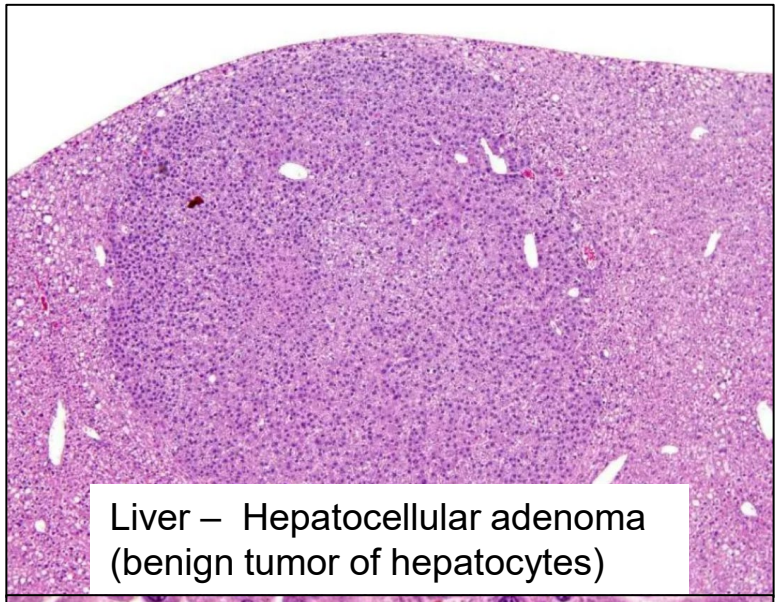
General Pathologic Processes: Regeneration and Repair

Core concept:

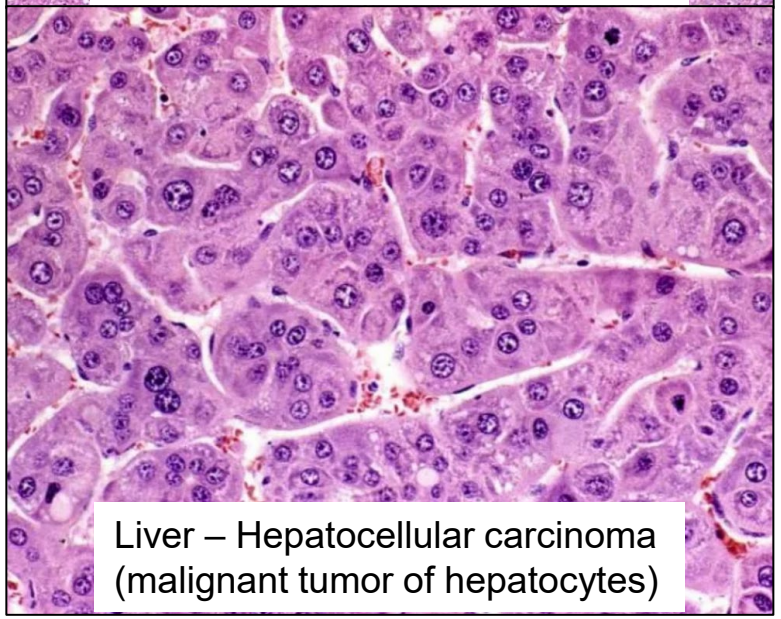
- Cell regeneration is a response to cell injury seen as cells with **more basophilic** than normal cytoplasm due to high protein synthesis; occurs when the connective tissue framework is intact
- Fibrosis is a repair mechanism after tissue injury seen as irregular, very **pale eosinophilic** areas; occurs when the connective tissue framework has been injured

General pathologic processes: Neoplasia

- Proliferation of cells not responsive to normal control mechanisms
  - Benign tumor: *not* cancer
  - Malignant tumor: cancer
    - Often ends with “sarcoma” (mesenchymal cells) or “carcinoma” (epithelial cells)
    - Capable of metastasis
- Potential pre-neoplastic adaptations:
  - Hyperplasia, metaplasia, “atypical hyperplasia” (dysplasia)
- Nomenclature for neoplasms:
  - Tissue/organ
  - Process (reflects cell of origin)
  - ± Biological behavior (benign or malignant)



Liver – Hepatocellular adenoma
(benign tumor of hepatocytes)



Liver – Hepatocellular carcinoma
(malignant tumor of hepatocytes)

General pathologic processes: Neoplasia

Comparison between Benign and Malignant Tumors

Feature	Benign	Malignant
General appearance	Tend to have a regular shape and well-defined borders	May have an irregular shape and poorly-demarcated borders
Differentiation	Well-differentiated (cells resemble the normal cells of the tissue)	Usually some lack of differentiation (variable degree of loss of normal features)
Local invasion	Does not invade surrounding tissue	Invades locally into surrounding tissue
Metastasis	Does not spread to other sites in the body	Frequently spreads to other sites of the body (through blood or lymphatic system)
General growth rate	Slow Mitotic figures rare, normal	Slow to rapid Mitotic figures often numerous ± abnormal

Summary

- There are numerous potential histopathologic diagnoses in toxicologic pathology
- However, most lesions will fall into one of these categories of basic pathological processes:
  - Adaptation: atrophy, hypertrophy, hyperplasia, metaplasia
  - Cell injury: reversible (degeneration) and irreversible (cell death)
  - Accumulations: lipid, cholesterol, proteins, pigment
    - Mineralization
  - Vascular changes: edema, congestion, hemorrhage, thrombus, infarct
  - Inflammation or infiltrate
  - Erosion and ulcer: epithelial tissue injury
  - Regeneration and repair: tissue response to injury
  - Neoplasia: benign or malignant

References

- Cesta MF, Herbert RA, Brix A, Malarkey DE, Sills RC (Eds.), National Toxicology Program Nonneoplastic Lesion Atlas. Available: https://ntp.niehs.nih.gov/atlas/nnl
- goRENI https://www.goreni.org/gr3_index.php
- GENERAL PATHOLOGY and VETERINARY PATHOLOGY TEXTS
  - Kumar V., et al. **Robbins, Cotran & Kumar Pathologic Basis of Disease**, 11th ed, Elsevier, 2025.
  - Zachary JF and McGavin MD. **Pathologic Basis of Veterinary Disease**, 7th ed, Elsevier, 2022.
- GENERAL TOXICOLOGIC PATHOLOGY TEXTS
  - Haschek-Hock, W. et al. **Haschek & Rousseaux's Handbook of Toxicologic Pathology**. 4th ed, Elsevier, 2022.
  - Sahota, P. et al. **Toxicologic Pathology: Nonclinical Safety Assessment**. 3rd ed, CRC Press, 2025.

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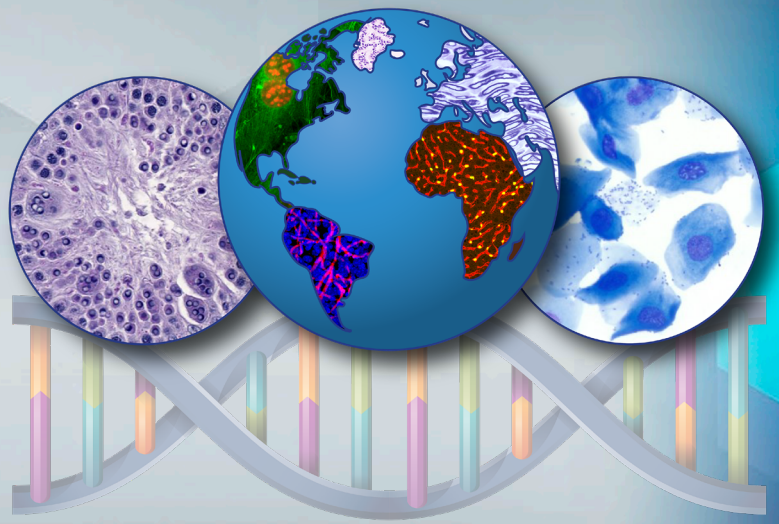

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