

Fish Histology and Histopathology

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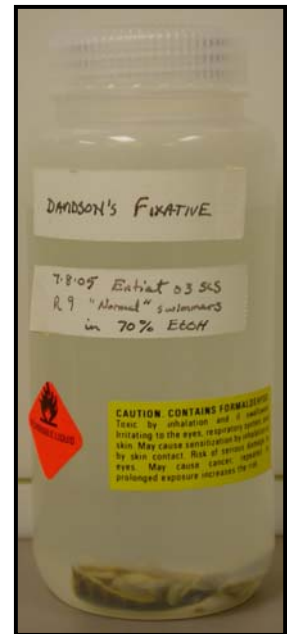
8. Glossary

Processing Tissues For Histology

Turning the Gross Tissues into Beautiful Sections

Sample collection

- Samples should be collected on freshly dead fish, not frozen or “Extremely Dead”
- Sample volume should not exceed 1/10th of the volume of fixative
- Samples should be placed in an appropriate fixative.
- Fry can be fixed whole
 - preferably in Davidson's
- Fingerlings and smaller
 - Gill opercula cut off
 - Cut along midline
 - Viscera pulled out
- Larger Fish
 - Take samples in field
 - Samples should be no thicker than 3mm



Fixation

- Singularly the most important step in producing good histologic slides (Sheehan)

Fixation with Formalin

- Most widely used fixative
 - Must be buffered (pH is as low as 3 without buffering), prevents formalin pigment
 - Penetrates 2 mm in 4 hrs, 10 mm in 24 hrs
 - Tissue Thickness- <3 mm thick for good fixation
 - Does not produce “overfixation”
 - Tissues do not harden unpredictably

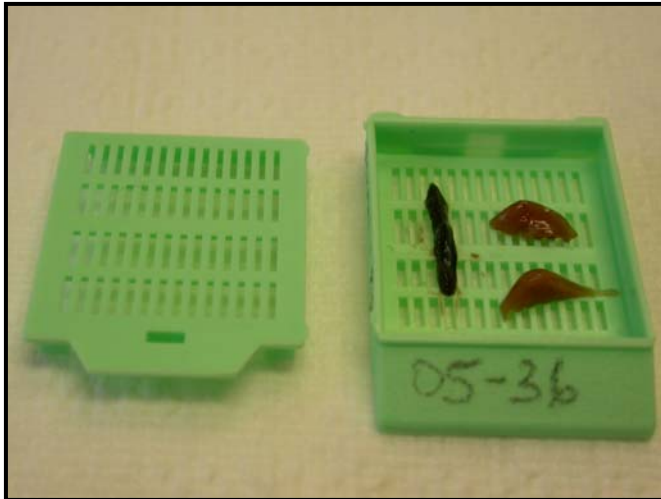


Fixation with Davidson's

- Contains acetic acid
 - Can cause tissue swelling
 - Results in some decalcification
 - Should not be used if red blood cell morphology is needed (destroys cells)
- Tissues should be transferred to 70% EtOH after 12-24 hrs to:
 - Prevent tissues from over-hardening
 - Easier to store/transport

Cutting in of Tissue - Placing tissues in cassettes

- Handle Tissues Gently
- Do not over-stuff Cassettes



Tissue Processing

- Dehydration and infiltration of tissue
 - Pieces must be taken through a series of water/alcohol mixtures to full alcohol and then through a clearant, xylene (HistoClear) or substitute and finally melted paraffin.



Chapter 1 – Tissue Processing

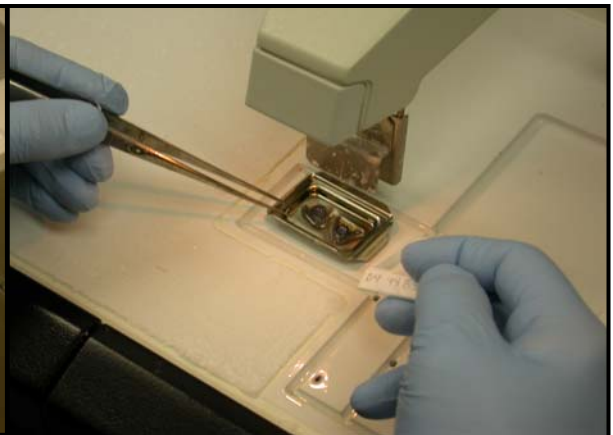
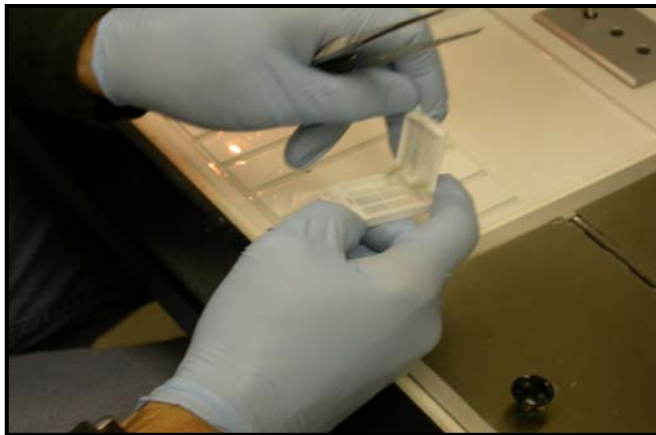
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Purpose of Processing

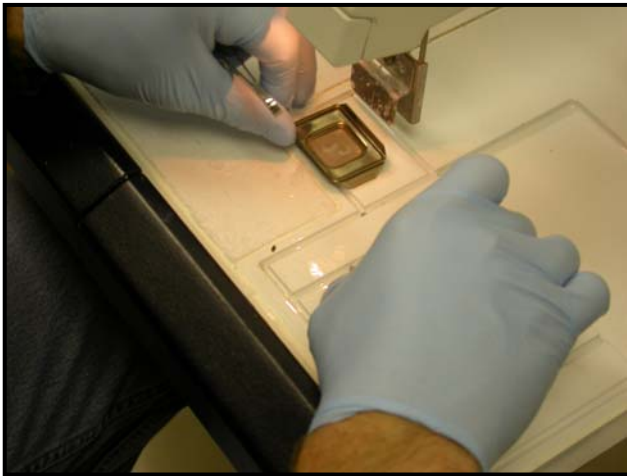
- To provide a matrix that can support the tissues during sectioning
- Wax incompatible with water
 - Dehydrate tissues with alcohols
 - Alcohols incompatible with wax
 - Clearant compatible with wax and alcohol
- Clearant – so named because tissues become translucent: Xylene and alternatives

Embedding

- Embedding tissue into paraffin blocks
 - Pieces must be oriented in paraffin block and allowed to harden



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Sectioning tissue



Floating Tissue Ribbons



Placing sections on slides



Chapter 1 – Tissue Processing

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Staining Slides

- De-paraffinize
- Re-hydrate
- Staining protocol
 - H & E
 - Giemsa
- Coverslip



Staining



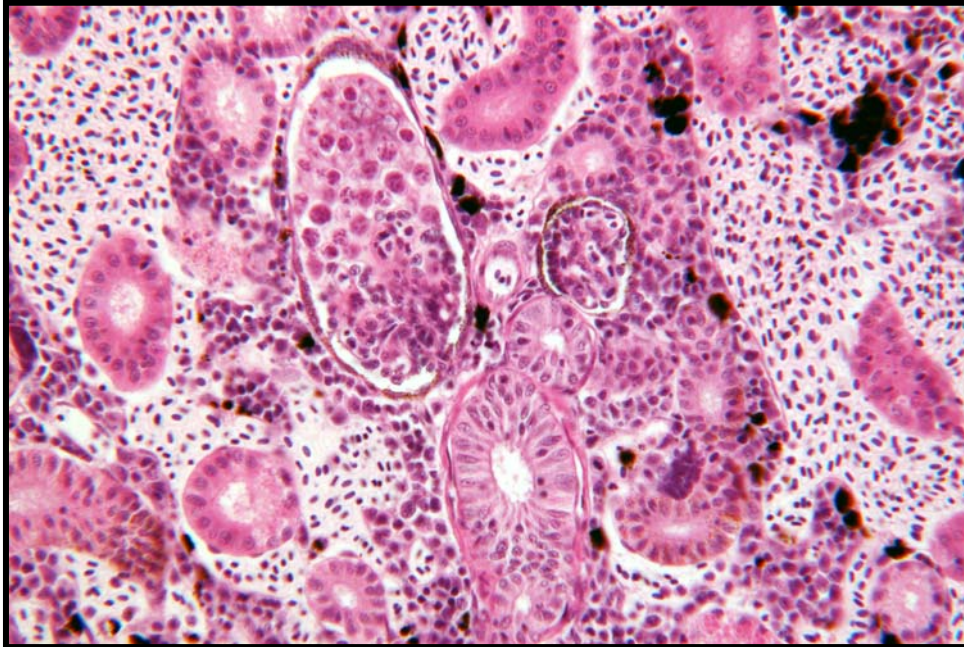
- Hematoxylin
 - Most Commonly Used Natural Dye in Histology
 - Extracted from heartwood of logwood trees
 - 300 years ago Scientist Robert Hook described using it to color fluids.
 - Not extensively used in histology until the 1860's

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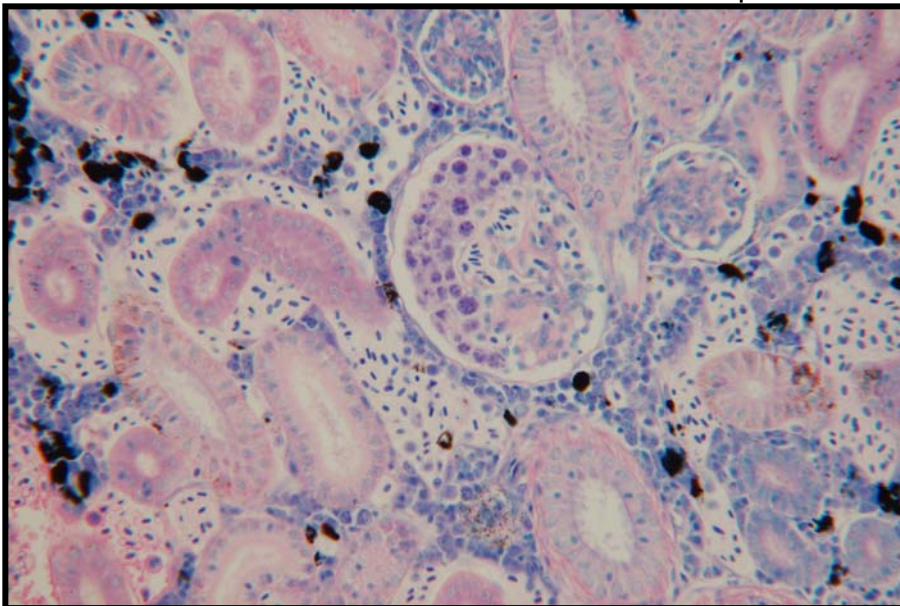
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- Stains Commonly Used in Fish Histology

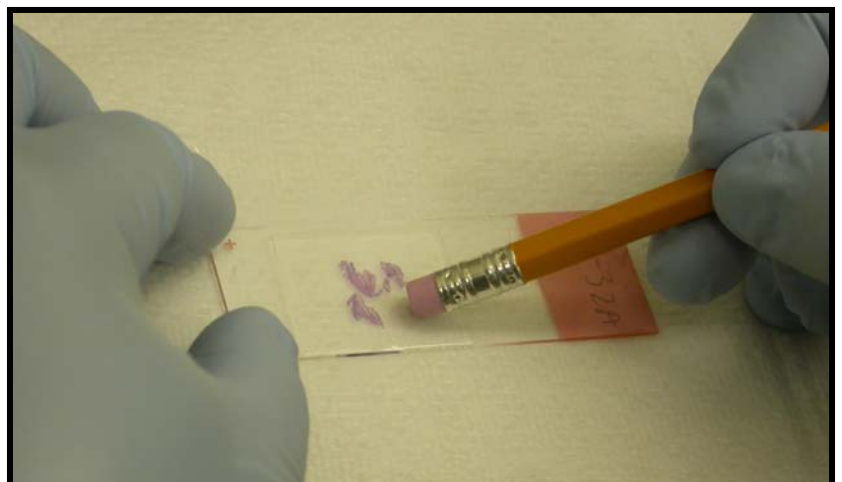
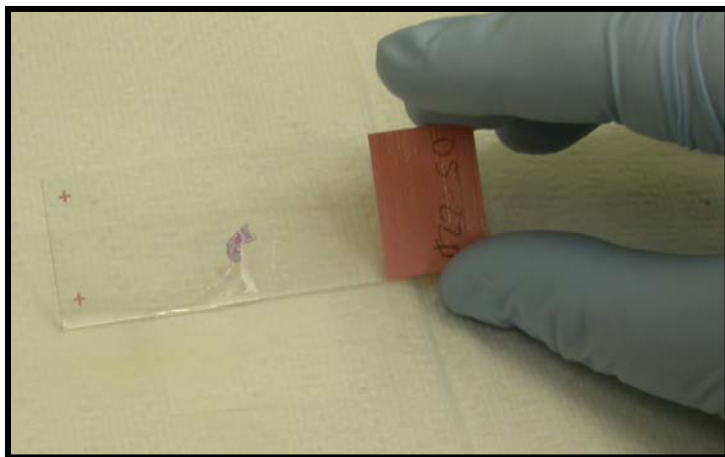
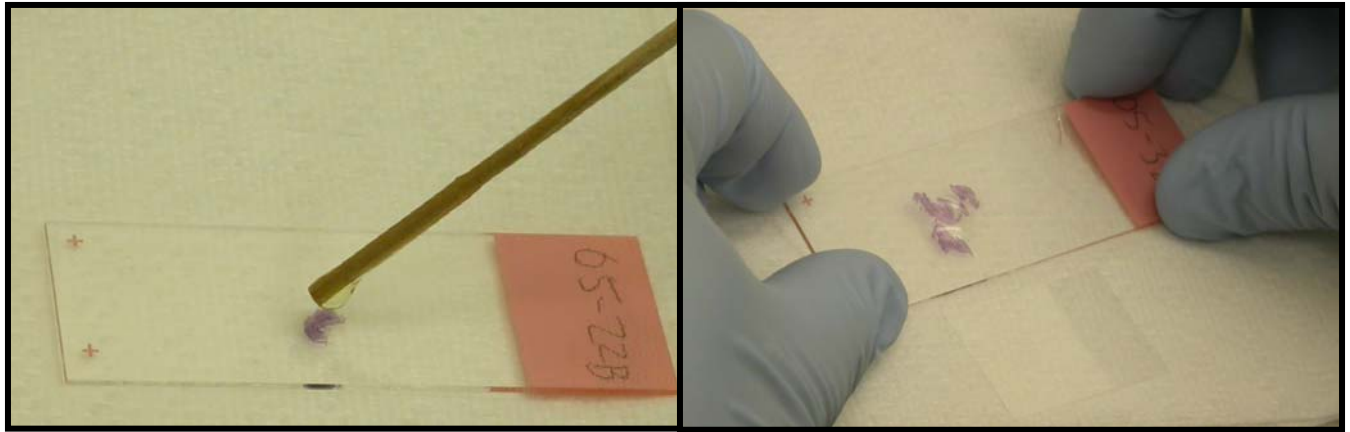
-H&E - Demonstrates a variety of tissue components including nuclei, mitotic structures, mitochondria, etc.



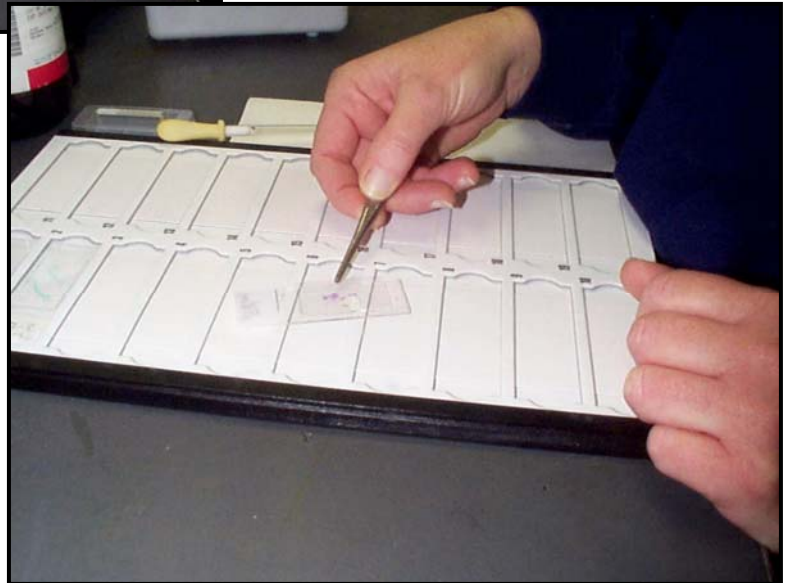
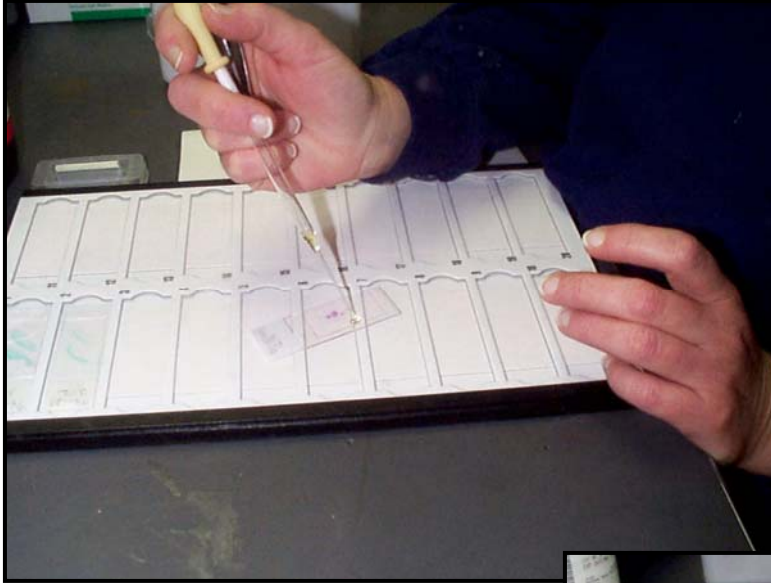
-Geimsa – demonstrates bacteria and parasites



Coverslipping



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Epithelium

Epithelium is one of the four basic tissues which form the organs. The remaining 3 basic tissues are connective tissue, muscular tissue and nervous tissue. Epithelia generally act as boundaries for transport, protection, segregation, sensation, and secretion.

Epithelia are classified first by thickness:

Simple: one cell layer thick, or one cell separates the free (luminal, apical) surface from the underlying basement membrane.

Stratified: many layers of cells which grow from the basal lamina upward and eventually shed into the lumen or free surface.

Epithelia are classified secondly by shape of the cells:

Squamous- very thin and flat cells

Cuboidal

Square columnar- rectangular (taller than it is wide)

Thirdly, epithelia are classified by its apical surface specializations:

Microvilli- or brush border, increases surface area, fuzzy edge, short, hard to see microscopically.

Cilia- very similar to flagella, function in transport and sensation, longer, easier to see.

Gills and Pseudobranchs

The gill epithelium is thin with a large surface area in order for a high level of exposure of gill capillaries to water. This allows for efficient gas exchange of oxygen absorption and carbon dioxide release but also results in vulnerability of the gill to pathogen invasion or irritation. Gills are also responsible for regulating the exchange of salt and water and play a major role in the excretion of the nitrogenous waste products, primarily ammonia. Even slight structural damage can render a fish very vulnerable to osmoregulatory as well as respiratory difficulties.

THE GILL ARCH & PRIMARY LAMELLAE

The gill arch is a curved osseous, or bony, structure from which radiate double rows of paired primary lamellae or filaments. Each of these primary lamellae has a series of secondary lamellae located perpendicular to the primary lamellae. The gill arch is covered by typical teleost epidermal tissue but at the origin of the primary lamellae the epidermis is much thicker and usually contains numerous mucous cells. Below this epidermis there is usually an array of lymphoid tissue. The primary lamella is covered by a mucoid epidermis which may have within it pale-staining saline, or salt secreting chloride cells. These chloride cells are most numerous at the basal (proximal) part of the lamellae. They function in ionic transport with a possible role in detoxification.

THE SECONDARY LAMELLAE

Gaseous exchange takes place across the surface of the secondary lamellae primarily through countercurrent exchange of blood flowing in the opposite direction from the external water. This surface consists of overlapping or interdigitating squamous epithelial cells, usually one layer thick, supported and separated by pillar cells, which are arranged in rows 9-10 μm apart. The pillar cells have primarily a support function. Where the pillar cells impinge on the basement membrane they spread to form flanges which coalesce with those of neighboring pillar cells to complete the lining of the lamellar blood channels. The pillar cells have been shown to contain columns of contractile protein similar to that found in amoebae. Since the blood entering the lamellar blood spaces comes directly from the ventral aorta at high pressure, the presence of contractile elements in the supports of these spaces serve to resist their distension under normal circumstances. The surface of the lamellar epithelium gives rise to microvilli. These serve to aid in attachment of the cuticular (epidermal) mucus, which, in addition to its role in reducing infection and abrasion, has a significant role in regulating the exchange of gas, water and ions. The combined thickness of the cuticle, respiratory epithelium and flanges of the pillar cells ranges from 0.5 to 4 μm . This represents the total diffusion distance for respiratory exchange. Goblet Cells are found scattered among the squamous epithelial cells of the gill lamellae, as well as in the basal region of the lamellae.

THE PSEUDOBANCH AND CHOROID BODY

The pseudobranch is not present in all fish but where present it is a red, gill-like tissue attached to the internal surface of the operculum. It consists of parallel blood capillaries supported by cartilage rods. The pseudobranch has a direct vascular connection with the choroid of the eye, which is composed of similar arrays of capillaries alternating with rows of slender fibroblast-like cells. It is also believed to have a function related to the filling of the air bladder.

Some type of cells seen in gills:

Red blood cells (rbc)

Epithelial cells

Chloride cell—Rounder than epithelial cell, large nuclei, very pink cytoplasm, often at base of lamellae. Increase in numbers with smolting.

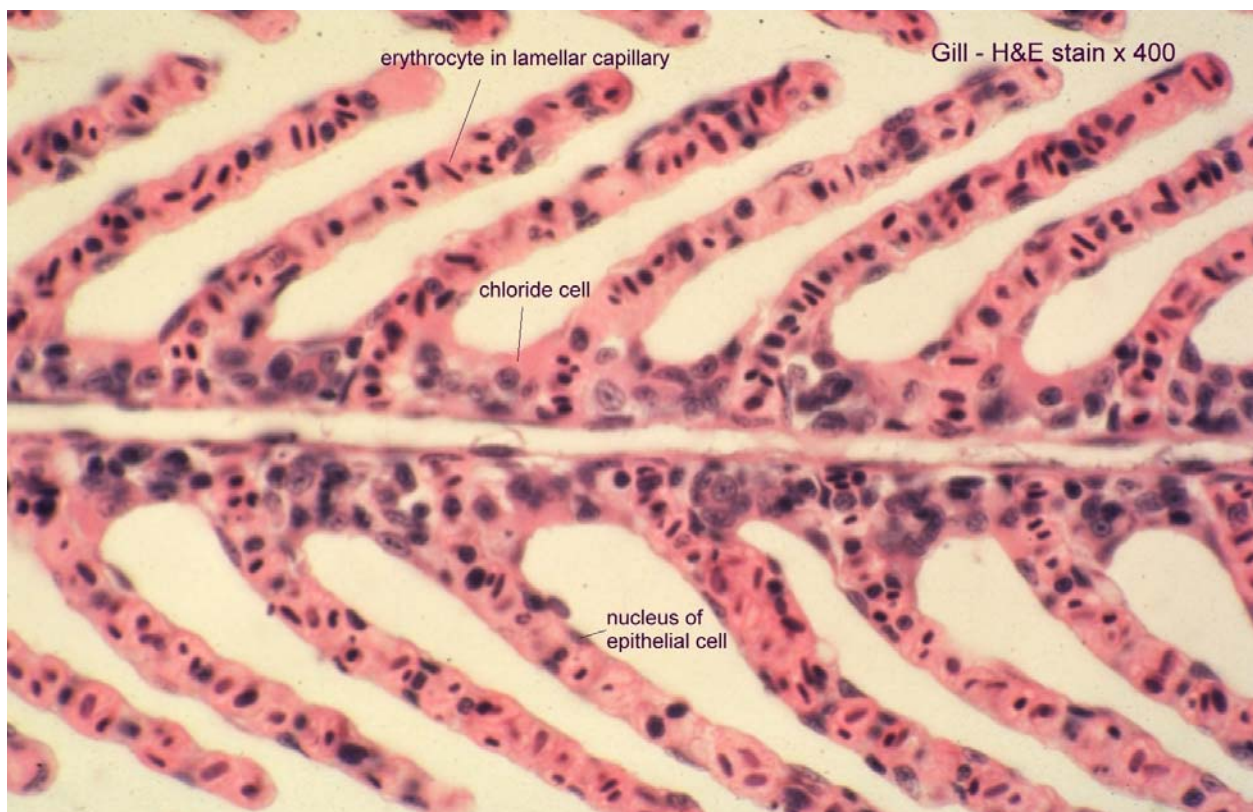
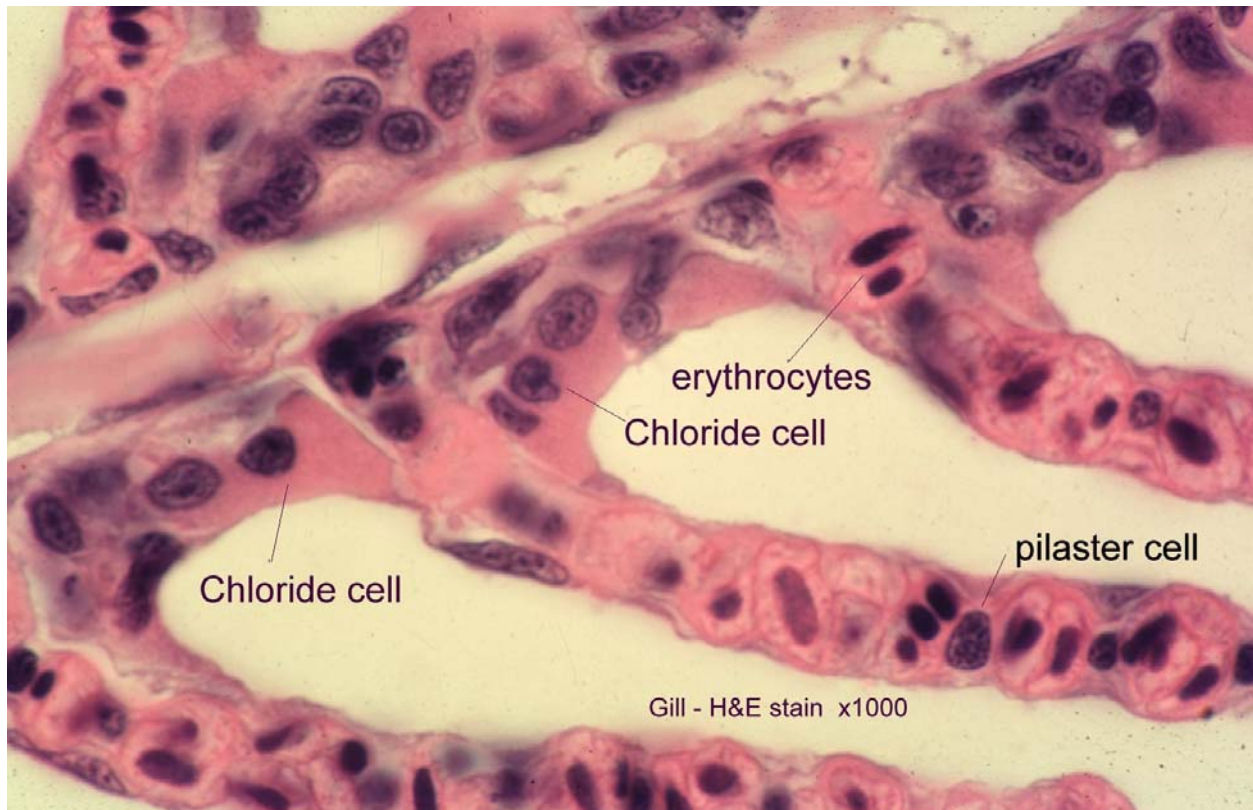
Pilaster cell—(Pillar cell) Structural, supporting, epithelial cell. Very dark staining.

Mucous cells—Granule filled domes or vacuolated cells.

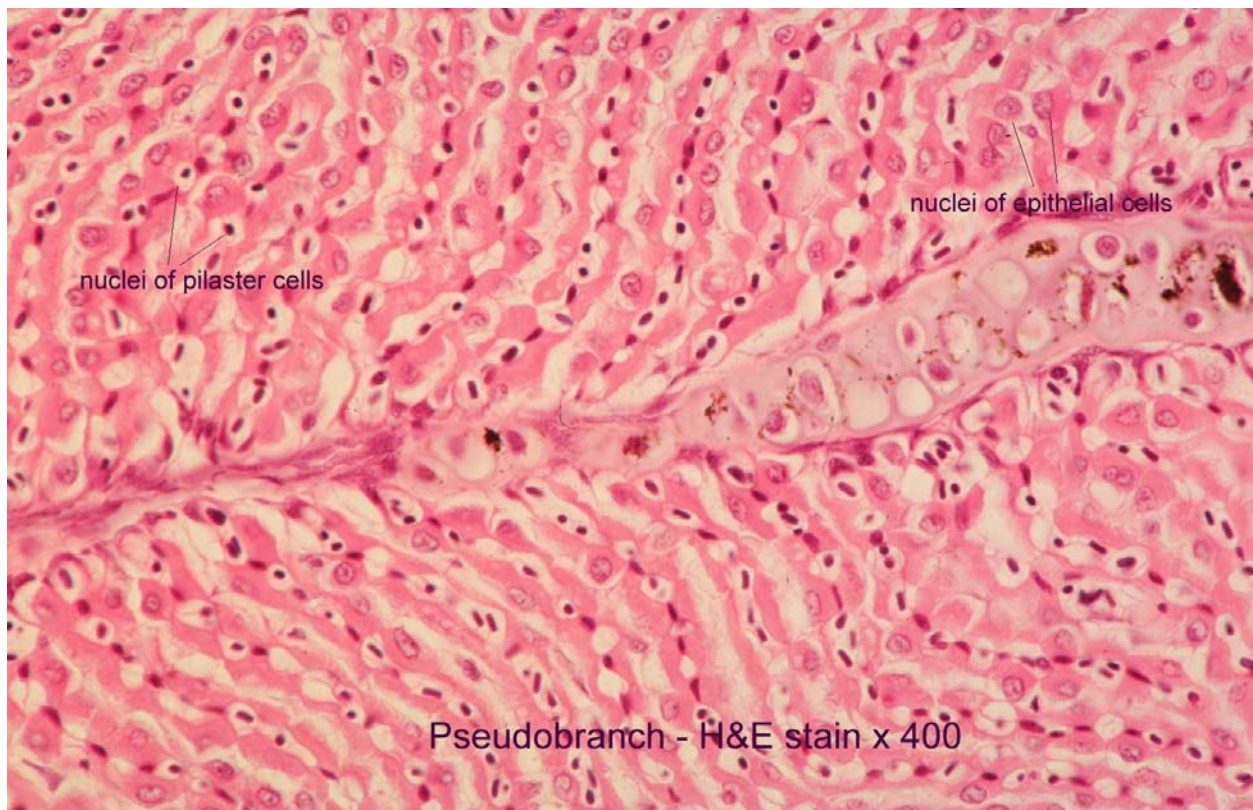


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Integument (Skin and Scales)

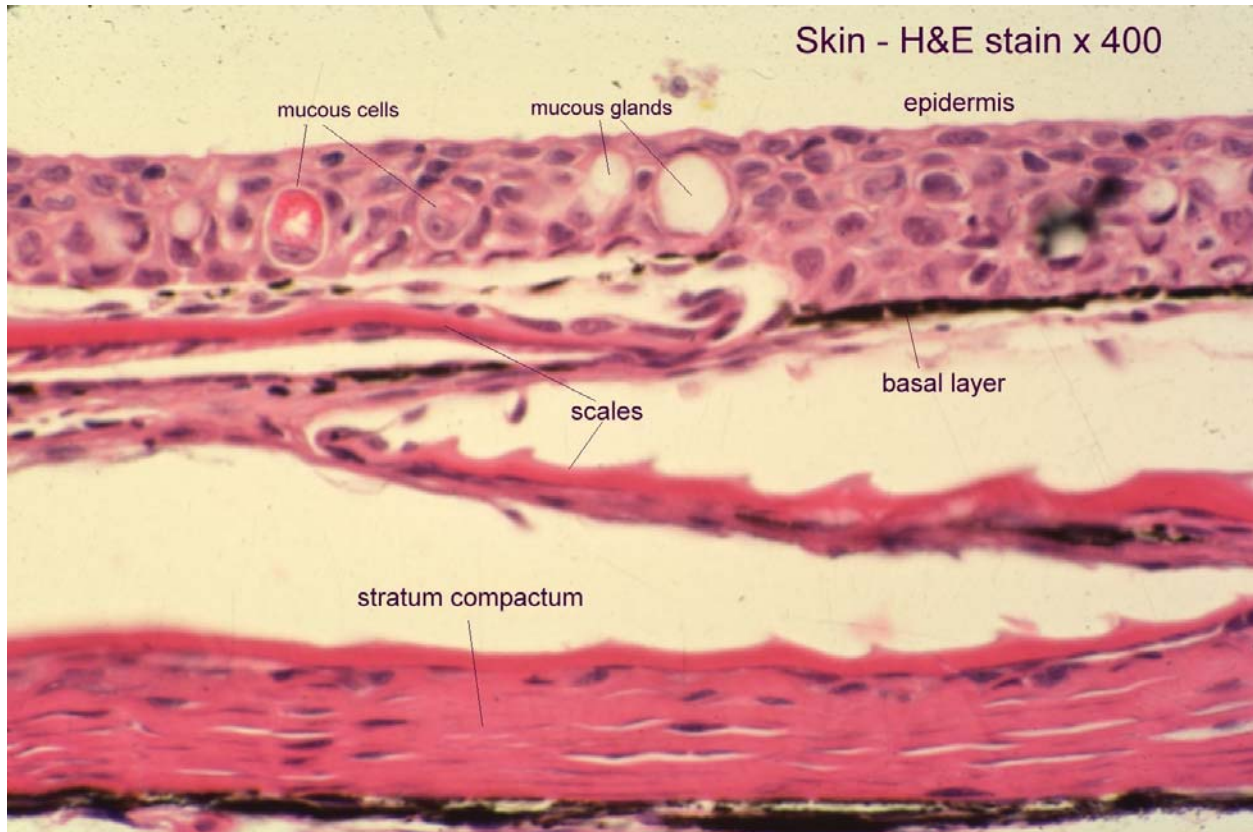
EPIDERMIS

The skin of fishes is composed of two layers, the epidermis and the dermis. The thickness of the epidermis varies greatly depending on the part of the body, age, sex, stage of reproductive cycle, and environmental stresses. The epidermis of a yearling rainbow trout is 5 to 10 cells thick; it consists of outer squamous and cuboidal cells and a basal germinal layer, that gives rise to the differentiated cells outside it. A filament-containing cell (Malpighian cell) is the primary parenchymal cell of fish skin. It contains aggregates of 75'Å diameter filaments. In most species of fish, these cells do not keratinize; consequently the outermost epidermal layers consist of living cells in contrast to the keratinized cell ghosts that make up the surface layers of the skin of other vertebrates. Mucus-secreting cells are found in the epidermis of all fish, but the numbers vary greatly with site and species. These goblet cells usually originate in the middle layer of the epidermis. They increase in size and refine secretions (mainly glycoproteins) as they approach the surface. Other cells found in the epidermis include lymphocytes, macrophages and some species specific cell types.

DERMIS

The dermis, the skin zone between the epidermis and underlying muscle, is composed of two layers of elaborate arrays of connective tissue. An upper (outer) layer is the stratum spongiosum, composed of a network of collagen, fibroblasts, and pigment cells, phagocytic cells, cells of the scale bed and the scales. Directly beneath is the stratum compactum, primarily a non-cellular layer, where a few fibroblasts intersperse between orthogonal (at right angles) bands of collagen. The "plywood" structure of the stratum compactum provides structural rigidity and flexibility from stresses impinging on the skin. Melanophores, the dark, pigment-containing cells in the dermis contain large numbers of membrane-bound electron-dense granules of melanin pigment which can be moved within the cytoplasm of the cell to give a desired coloring and protective effect.

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Kidney

The kidney of the teleost is a mixed organ comprising hematopoietic, reticuloendothelial, endocrine and excretory elements. The kidney of fish is usually located in a retroperitoneal position up against the ventral aspect of the vertebral column. It is a light or dark brown or black organ normally extending the length of the body cavity. It is usually divided into anterior or head kidney, which is largely composed of hematopoietic elements, and posterior or excretory kidney. The ureters, which conduct urine from the collecting ducts to the urinary papilla, may fuse at any level and may be dilated, after fusion, to form a bladder. The urinary ducts open to the outside posterior to the anus.

The primary function of the kidney in fish is the osmotic regulation of water and salts rather than the excretion of nitrogenous wastes as in mammals. In fish, the majority of nitrogenous wastes are excreted by the gills. In freshwater, the kidney must conserve salt and eliminate excess water. This is accomplished by a high glomerular filtration rate, reabsorption of salts in the proximal tubules, and dilution of urine in the distal convoluted tubule.

NEPHRON

The component structure of the fish nephron varies considerably between marine, euryhaline and fresh-water forms mirroring the significant differences between their respective function. Even though this is true, the basic cellular architecture is similar. Each nephron consists of several segments with specific structure and function.

Renal corpuscle (glomerulus within Bowman's capsule) - here an ultrafiltrate of plasma is formed from the blood. This filtrate then passes into the renal tubule where it is altered to form urine.

Proximal convoluted tubule: first segment (PCT)- resorbs 85 % + of the water and sodium & chloride. Also glucose, amino acids, proteins, vitamin C, and inorganic ions. Proximal convoluted tubule – Tall columnar cells with brush border, large spherical, pale-staining, basally located nuclei.

Proximal convoluted tubule: second segment - regulates the ability to produce a hyper or hypotonic urine. Taller columnar cells than first segment of PCT with oval to rounded, centrally located nuclei; a dense apical brush border; and intensely eosinophilic cytoplasm.

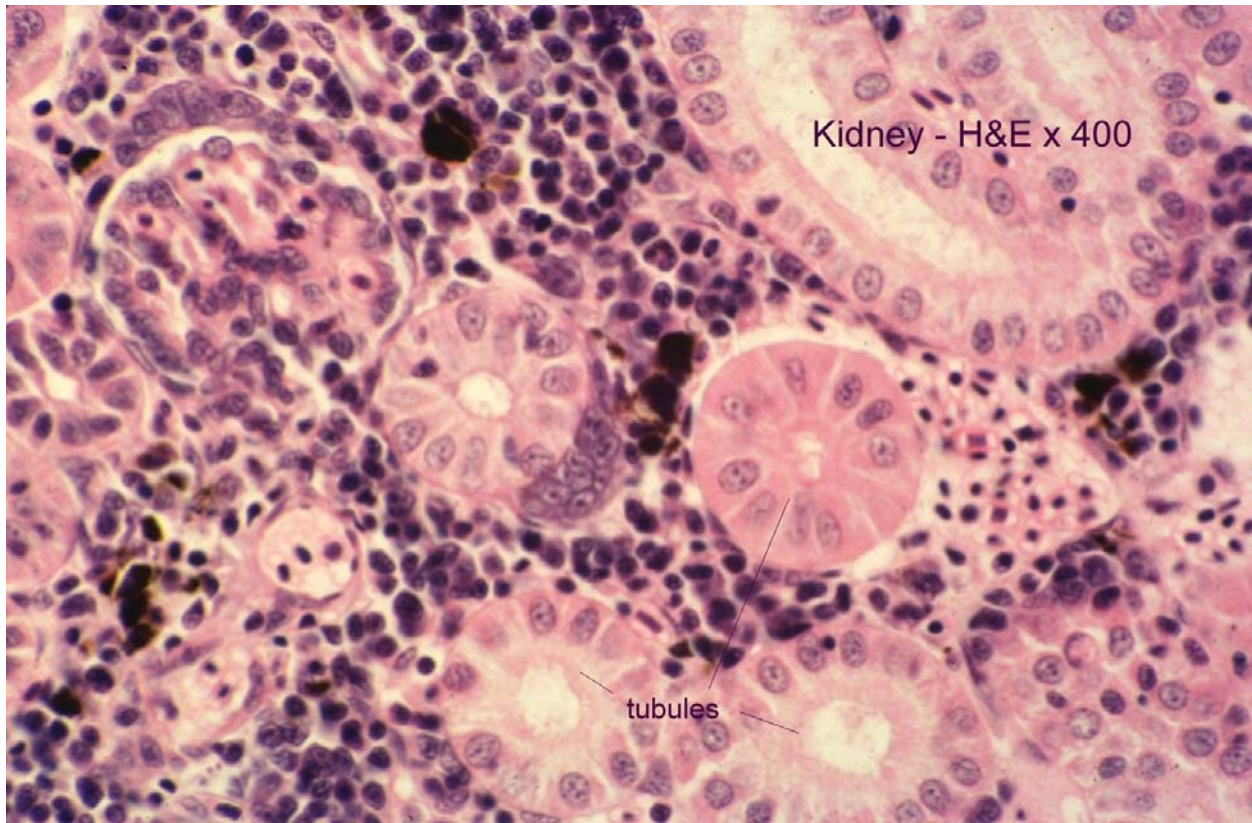
Distal convoluted tubule (DCT)-- more water resorbed, urine concentrated or diluted. Distal convoluted tubule, with generating tubule - Low columnar cells with oval, basally located nuclei and no brush border, stain less intense than PCT.

Collecting tubules -- collection of concentrate for excretion, more water resorption. Collecting tubules, with rodlet cell - Tall columnar cells, basally located nuclei, no brush border, thin layer of smooth muscle and connective tissue. Rodlet cells have been observed, studied, and reported but their nature and function are still unclear and remain controversial. Some believe that they are secretory cells. They have been found

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in many species of fish, and are located in several tissues of the body, such as the heart, kidney, intestine, and gills. Under a light microscope the identifying features of these cells are the electron-dense, light refractory rods or spicules that extend from the interior (basal) end of the cell to the opening at the apex or exterior end of the cell. The cells appear to extrude (burst) their contents to the surface, whether it be the gill surface or the surfaces of internal organs. They have also been reported to be parasites.





Hematopoietic Tissues

Since fish have no lymph nodes and their bones usually have no medullary cavity, hematopoietic tissue is located in the stroma of the spleen and the interstitium of the kidney. To a lesser extent it is also found in the periportal areas of the liver, the intestinal submucosa and the specialized lymphoid organ, the thymus.

Renal Hematopoietic Tissue

In the kidney, the primary site for hematopoiesis in the fish, the hematopoietic tissue forms a support matrix for the nephrons of the posterior kidney but the anterior or head kidney is almost exclusively hematopoietic. The blast, or undifferentiated stem cells, are situated within a stroma of reticuloendothelial tissue similar to that of the bone marrow of the mammal. Another cellular structure, found throughout teleost hematopoietic tissue but not in higher vertebrates, is the melanomacrophage center, which may not be as discrete in salmonids as in other fishes.

Spleen

Functions as an accessory hematopoietic organ, a site for blood filtration, and cell destruction, and for erythrocyte storage. A bag of reserve, immediate use, blood cells. The spleen is involved in all systemic inflammations, generalized hematopoietic disorders, and metabolic disturbances. It is rarely the primary site of disease. The spleen is the only lymph-node like organ to be found in fish. It is dark red or black in color and in health, usually has sharply defined edges. It is situated near the greater curvature of the stomach or the flexure of the intestine. Although usually single, it may in some species be divided into two or more smaller spleens. The most common problem which confronts the fish health biologists as they examine the spleen has to do with its size, color and texture. There appears to be two sizes, too large and too small, many color variations, bright red to very dark purple and lumpy bumpy to smooth. The great majority of spleens examined at necropsy are essentially normal. Always keep in mind that the spleen can hold a large but varying amount of blood.

In some species the pancreas is located as a subcapsular layer to the spleen. The splenic pulp consists of sinusoidal phagocytic tissues in which large numbers of red blood cells may be held and hematopoietic tissue is supported. This is mainly lymphopoietic but not exclusively so. The teleost spleen is different than the mammalian spleen in that red and white pulp areas are diffuse and not very discrete, and the connective tissue framework is not prominent. When the red pulp area is filled with red blood cells, the white pulp nodules, primarily lymphatic tissue, can be seen easier.

Thymus

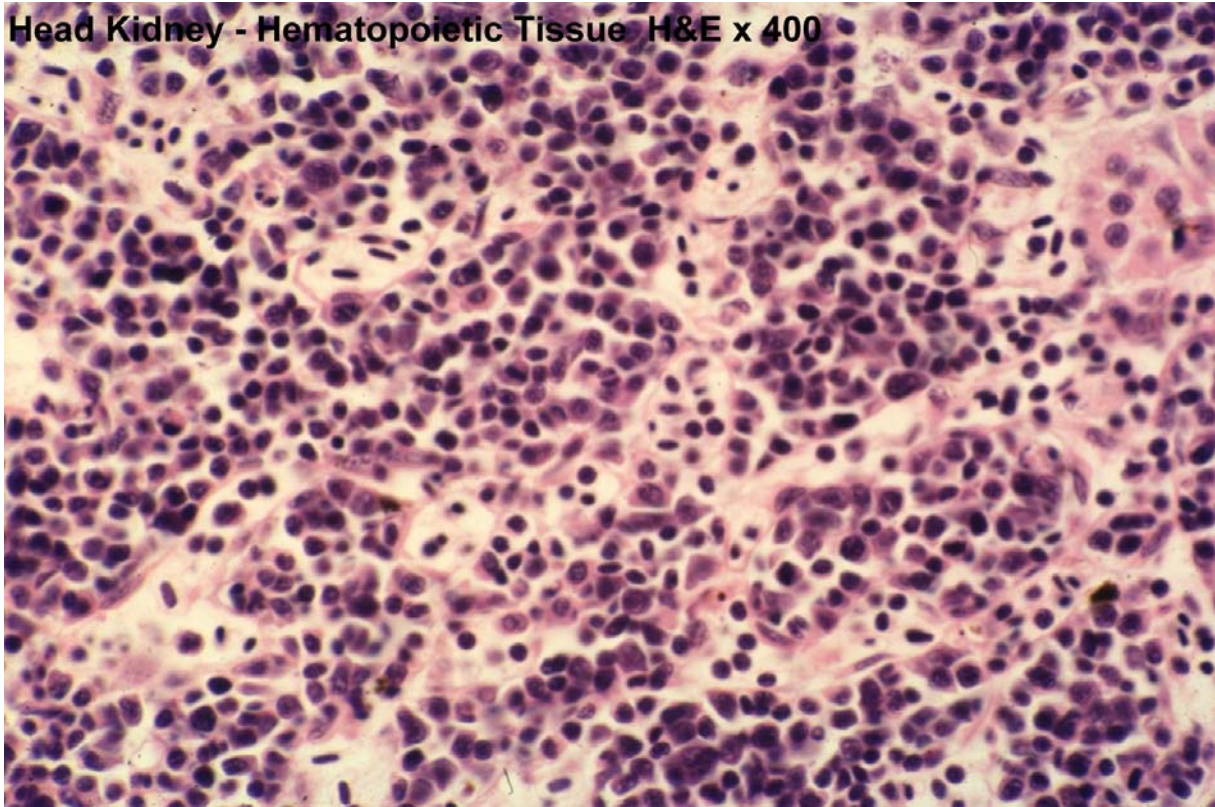
The thymus is a paired organ, an ovoid pad of lymphoid tissue situated subcutaneously in the dorsal commissure of the operculum. It arises from primordia associated with the epithelium of the pharyngeal pouches. Comparative studies of thymic morphology in fish have been made and have found that its life-span was very different in different species, involuting in lower teleosts before sexual maturity but surviving, and even growing, for several years after maturity in higher teleosts. In histological section, the thymus is an aggregate of small lymphocytes with a fibrous capsule and supporting cells. Occasional

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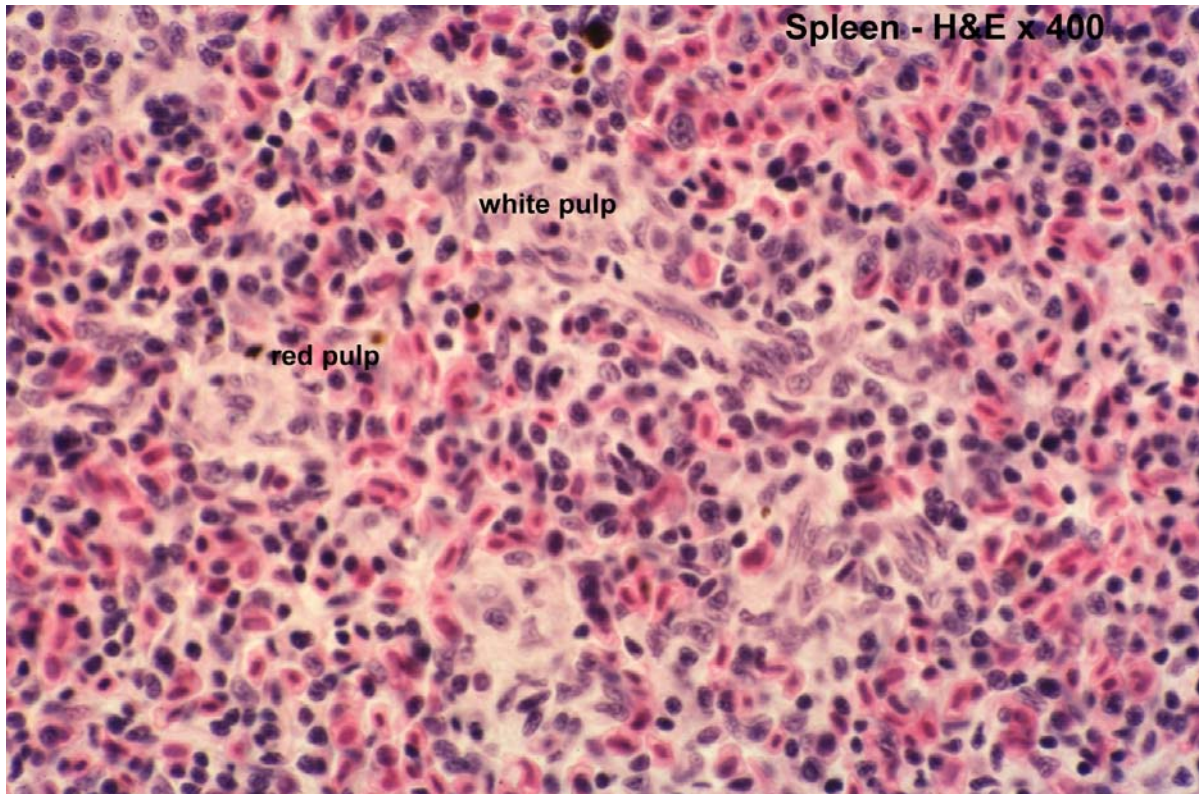
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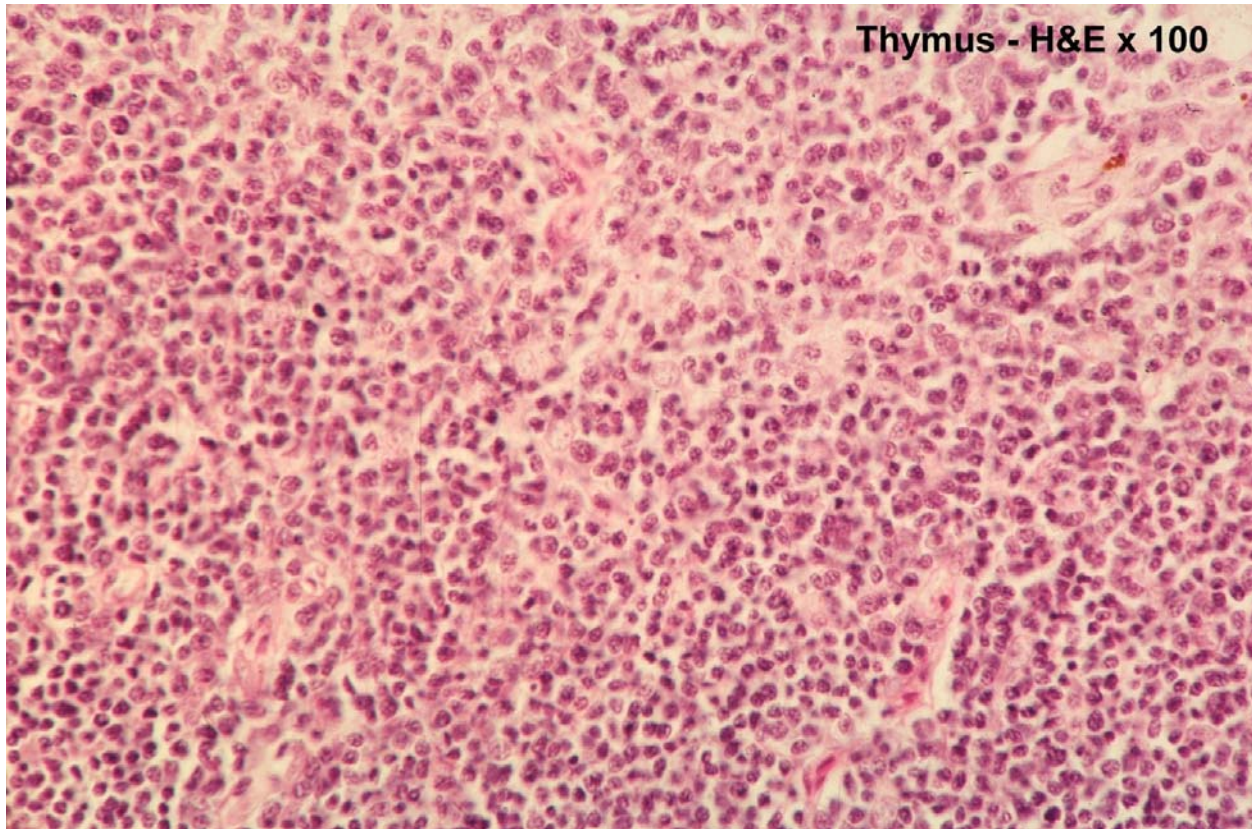
epithelial cords are seen and rarely, focal epithelial nests of Hassall's corpuscles. These are spherical or oval eosinophilic bodies present in the medulla of the thymus gland which increase in size and number with age.

Head Kidney - Hematopoietic Tissue H&E x 400



Spleen - H&E x 400





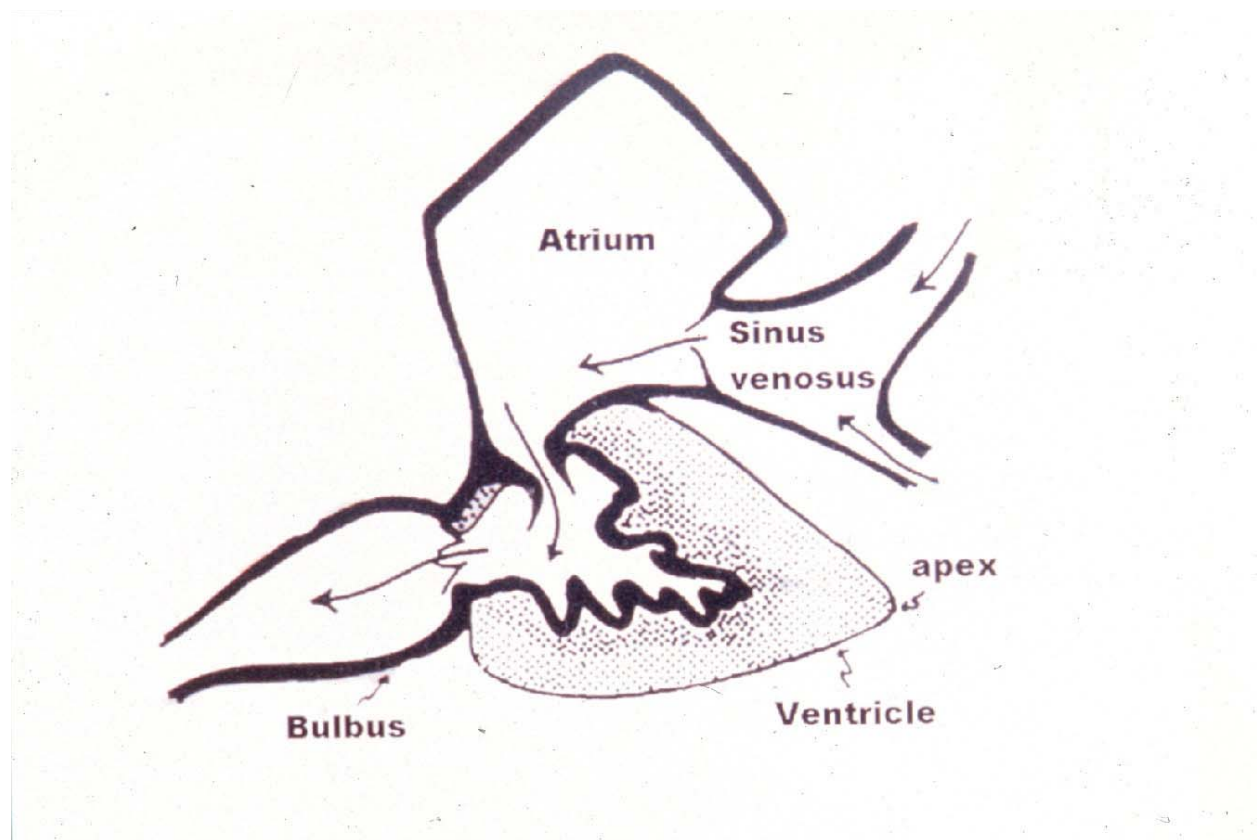
Cardiovascular (Circulatory) System

HEART

Blood circulates from the heart to the gill arches to be oxygenated by diffusion through the epithelium of the gill lamellae. It is then pumped from the dorsal aorta to the arteries, down to the peripheral capillaries before it is returned via the venous system. The heart in teleosts has four chambers through which blood flows in simple succession: sinus venosus, atrium, ventricle, and bulbous arteriosus. Walls of the heart are made of 3 layers: inner endocardium, myocardium (muscle), and the outer pericardium.

Deoxygenated venous blood enters the sinus venosus from the ductus cuvieri, or common cardinal veins, and main veins. There are no inlet valves and the sinus is so small that it can hardly be recognized as a discrete cardiac chamber. The wall is thin, composed mainly of collagenous connective tissue, although in some species it is muscular and contractile.

Through two sino-atrial valves the blood passes into the atrium which lies dorsal to the ventricle. The atrium has a thin wall, and muscular trabeculae traverse the lumen in a loose meshwork. The endothelial lining is therefore large in area and has a phagocytic activity as part of the reticuloendothelial system. Contraction of the atrium forces the blood through valves into the ventricle.

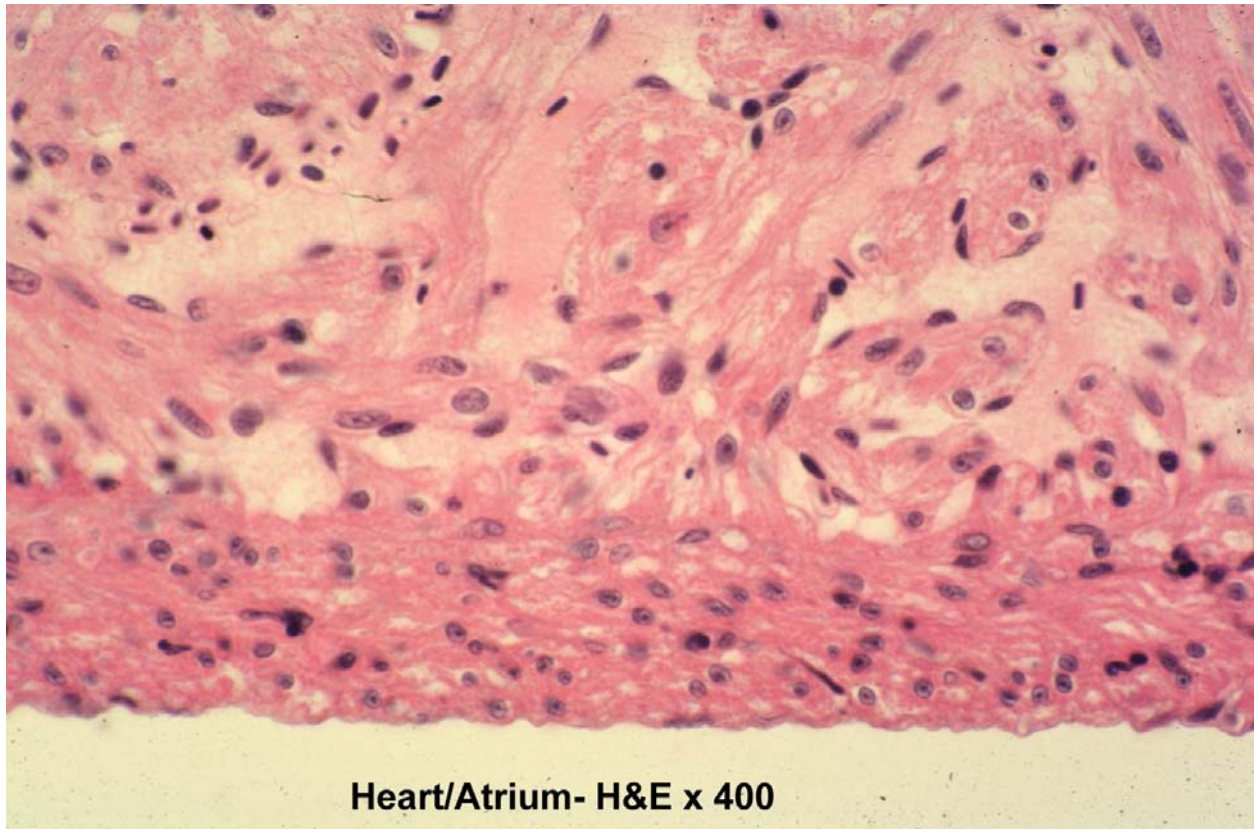


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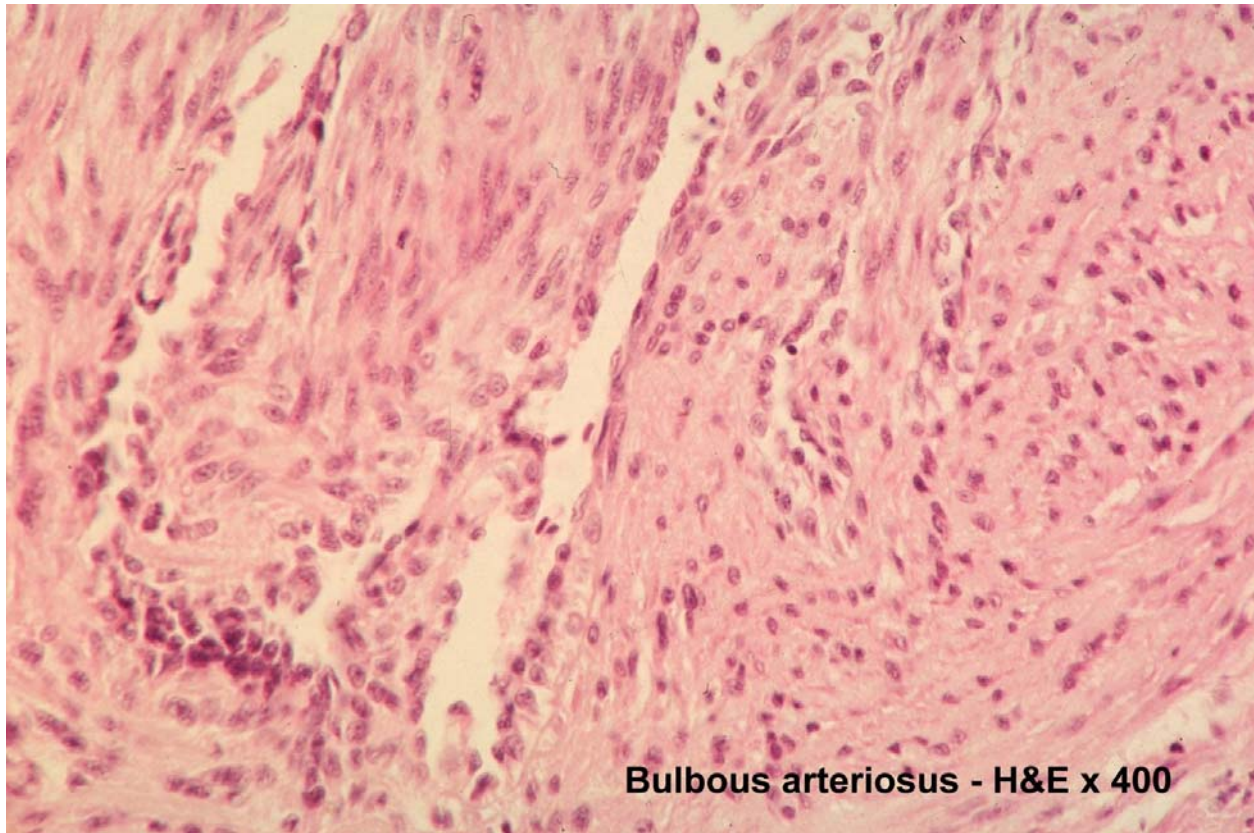
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The ventricle has a much thicker wall than the atrium and in normal histological sections only a small lumen is apparent. Here there are two layers of muscle including a distinct outer compact layer of muscle and an inner spongy layer with numerous trabeculae. The thickness of the compact layer is variable, being almost absent in less active species such as pleuronectides (such as flounders). Coronary vessels run over the outside of the ventricle, supplying the compact muscle while the spongy muscle obtains most of its oxygen supply from the venous blood in the lumen. Individual muscle fibers are approximately 6 μm in diameter, about half that of mammalian muscle. The fibers are similar to mammalian ones with intercalated discs between individual cells. From the ventricle the blood is passed into the bulbous arteriosus through a pair of valves. The bulbous has a thick wall composed of elastic tissue and smooth muscle. It has a complex structure but acts basically as a passive elastic reservoir which smooths the pressure pulse from the ventricle and maintains blood flow during ventricular diastole. The elastic tissue of the bulbous is very different in structure from that of the elastica of arteries.

The outer wall layer, the pericardium, consists of 2 layers. The visceral epicardium encloses the heart in the pericardial space while the second layer called the parietal or outer pericardial sac lines the pericardial cavity. This pericardial space is filled with serous fluid, separating the two membranes.



Heart/Atrium- H&E x 400



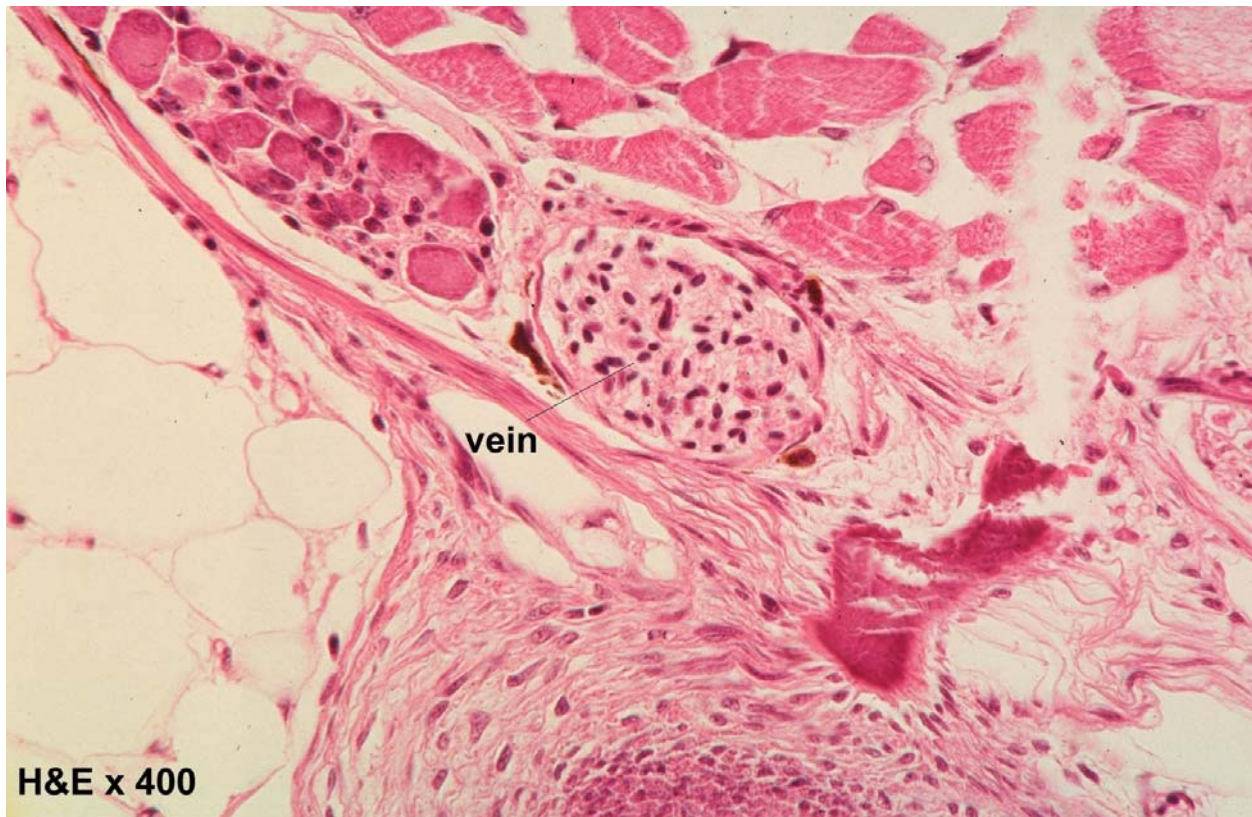
ARTERIES

The ventral aorta runs forward from the heart and distributes blood to the gills via the afferent bronchial arteries. The arteries afferent to the gills have a normal vertebrate arterial structure made up of three wall layers: adventitia on the outside, media and intima. These layers differ in development depending on the vessel and its function. The endothelium of the intima is comprised of flattened cells which can usually be distinguished only by their dark-staining nuclei which bulge into the lumen. Contiguous cells interdigitate so that the endothelium forms a continuous surface. There is a fine basement membrane beneath the endothelium, but this is visible only with the electron microscope. The intima is largely elastic tissue and the media is composed of elastic tissue laminae, or fibers, with smooth muscle cells in between. Adventitia is made up of fibroblasts and collagenous fibers.



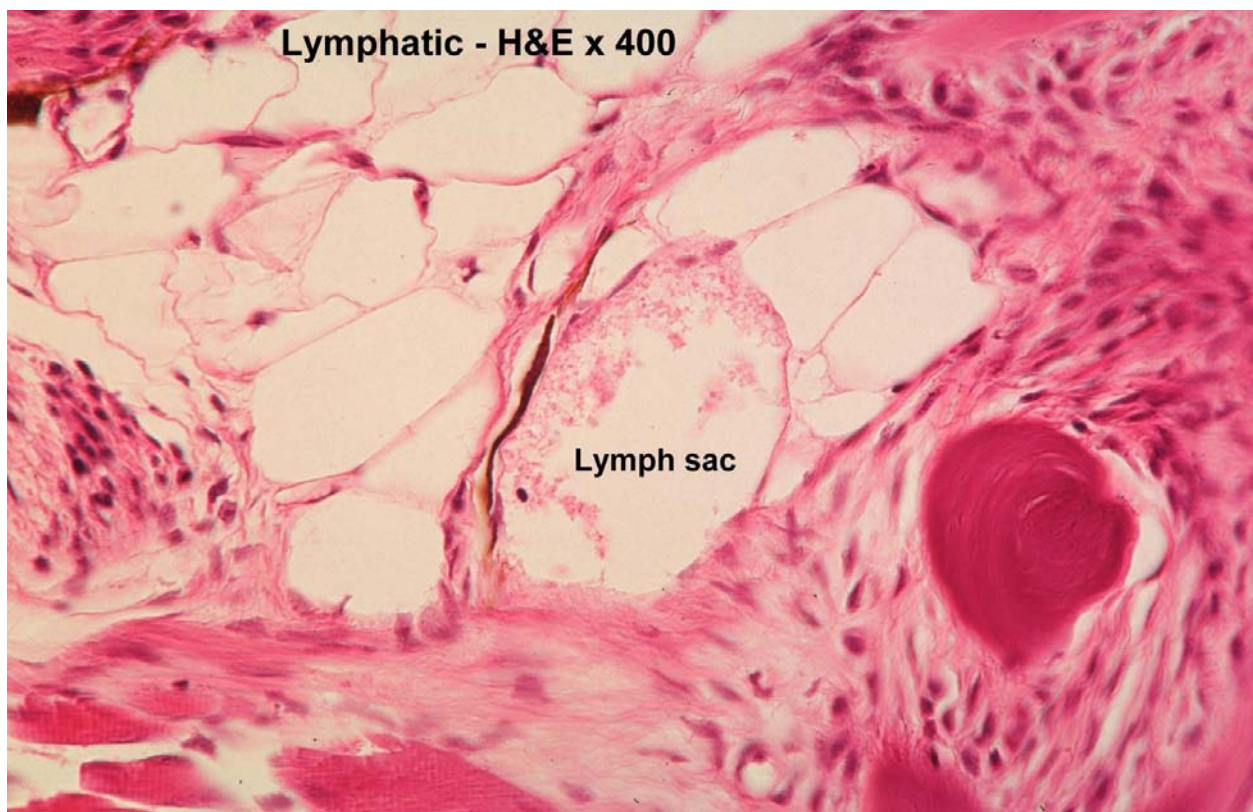
Veins and Capillaries

The veins of fish, as with other vertebrates, are relatively indistensible and have walls composed mainly of collagen. The major veins are large in diameter and pressures are low, being less than 10 mm Hg. Valves are not common in the teleost venous system. Capillaries consist of a single layer of endothelium in order for exchanges of oxygen, nutrients, and waste products to occur.



Lymph Vessels

The lymph drainage system of fish is very extensive, probably because of the high capillary permeability. The lymph volume is about four times the blood volume and its composition is almost identical to that of blood plasma. In the main bulk of the myomeres, or segments of muscle, the lymphatic circulation is the only circulation available since there are no significant blood vessels in the white muscles. There are various lymph propulsors or lymph hearts along the length of the major lymphatic vessels which aid lymph return during breathing movements. Lymph is collected from all areas through a system of vessels, sinuses, and ducts which can appear as empty spaces, or often indiscernible if collapsed, in sections.



Gastrointestinal Tract and Pancreas

Each tubular portion of the digestive tract (stomach, pyloric caeca, intestine (anterior, posterior)) has four layers: Mucosa, Submucosa, Muscularis, and Adventia. These layers are present in different amounts throughout the gastrointestinal tract.

Mucosa (RED) consists of:

- Epithelium
- Lamina propria
- Muscularis mucosa

Surface epithelial membrane on a basal lamina, supported by connective tissue (lamina propria) and a thin muscle layer.

Submucosa (GREEN)

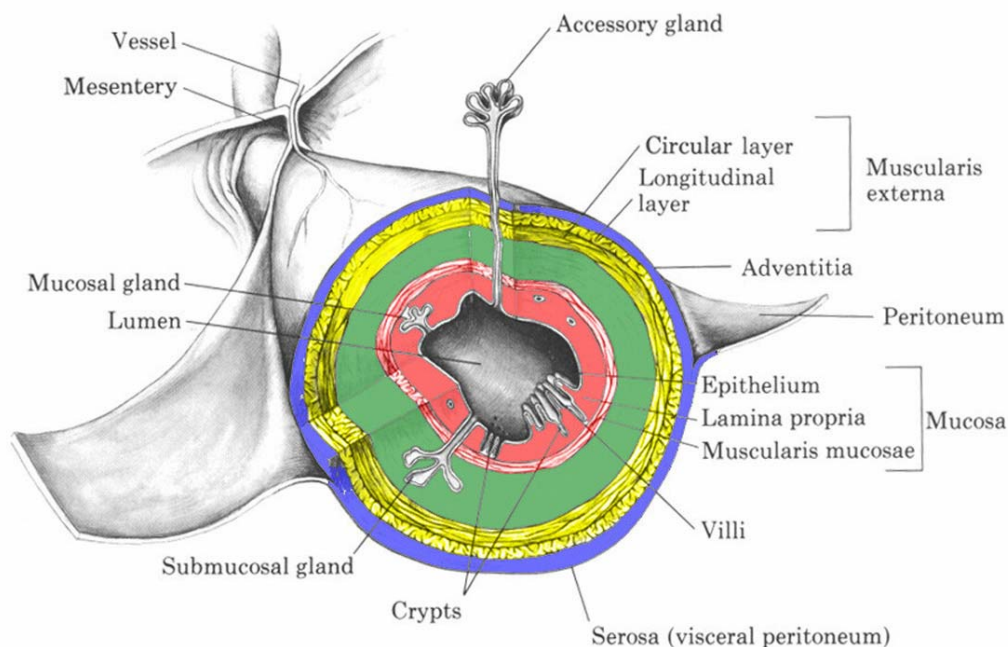
Coarse areolar connective tissue and elastic fibers, containing blood vessels and nerves. It permits mobility of the mucosa.

Muscularis (YELLOW)

Inner layer of circularly oriented and outer layer of longitudinally oriented smooth muscle. Functions to propel food through (peristalsis). Also aids in mixing of food with digestive enzymes.

Adventitia (BLUE)

Relatively dense layer of areolar connective tissue often blending with connective tissue of surrounding structures. When covered by the peritoneum it's called a "serosa". Contains blood vessels, nerves, and lymphatics.

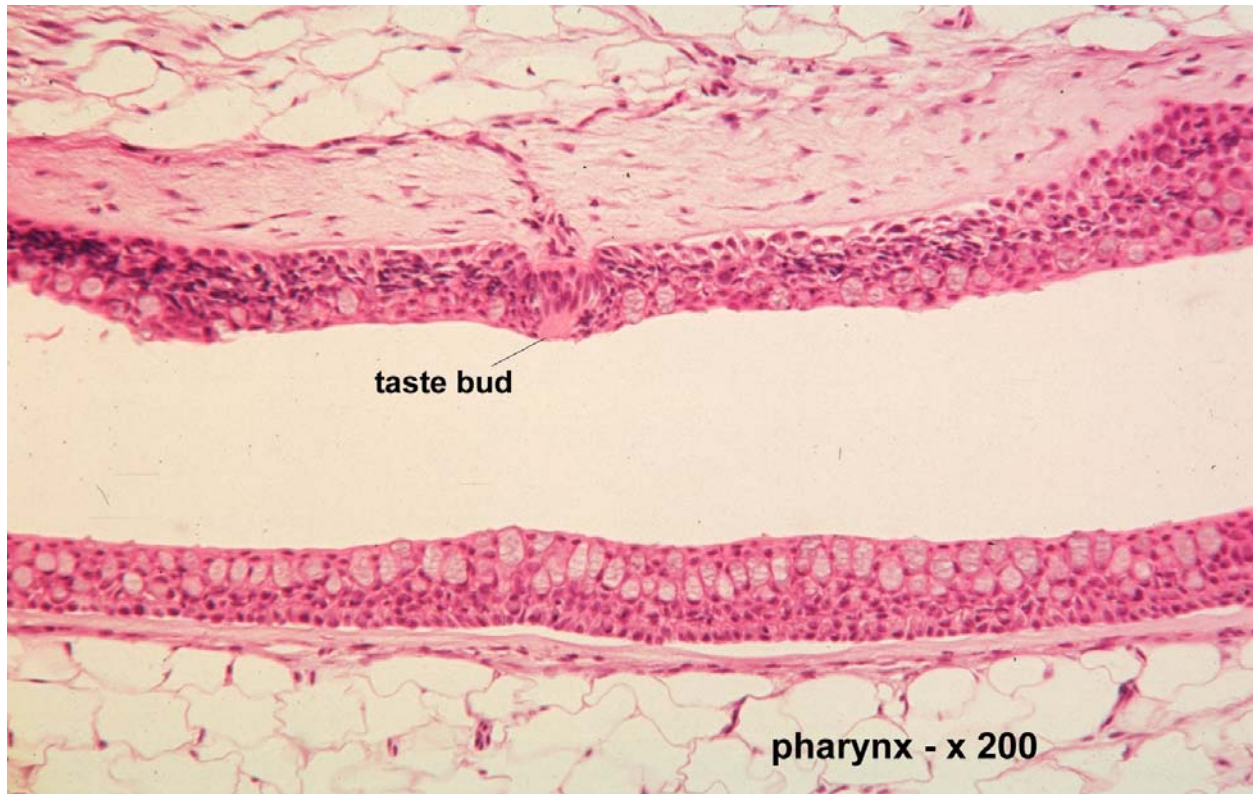


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Mouth

The mouth and buccal, or cheek, cavity are shared by the respiratory and digestive systems. Their digestive function is confined to selection, seizure and orientation of food for transfer to the stomach. Chewing and pre-digestion, found in mammals, are not usually a function of the mouth of the teleost. The mouth and perioral regions contain many sensory nerve endings and teeth. The lining of the buccal cavity consists of a stratified mucoid epithelium on a thick basement membrane with a very condensed dermis binding it to bone or muscle.

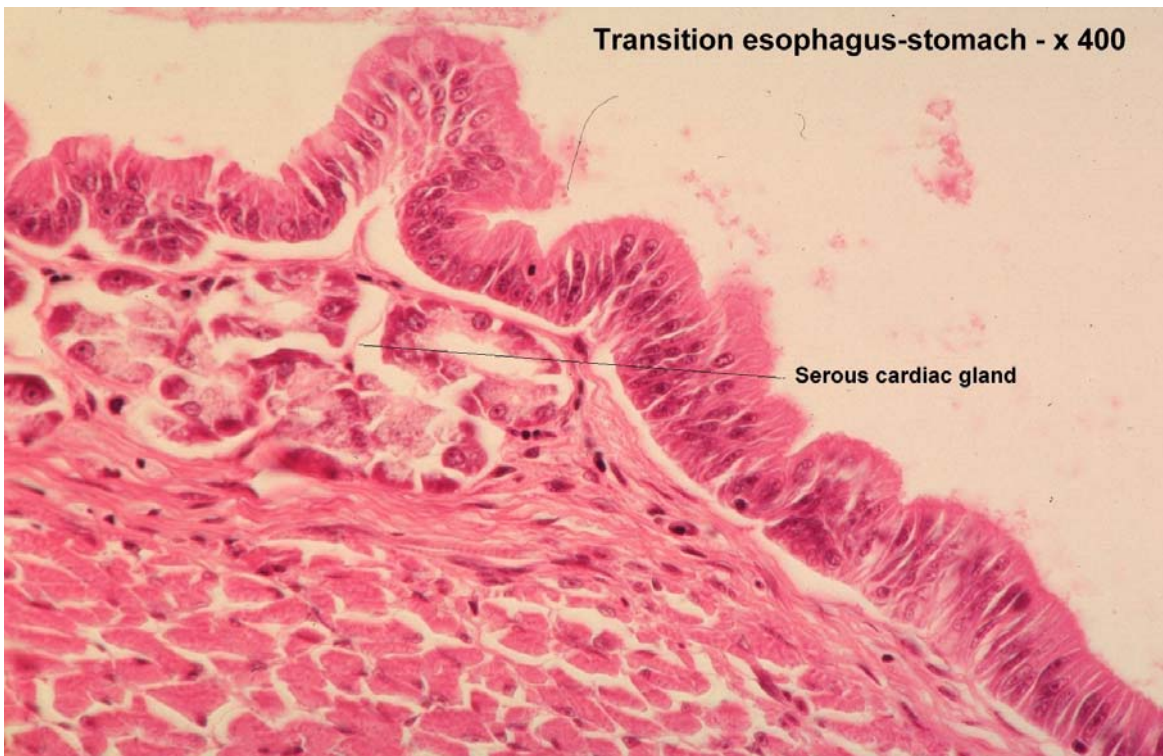
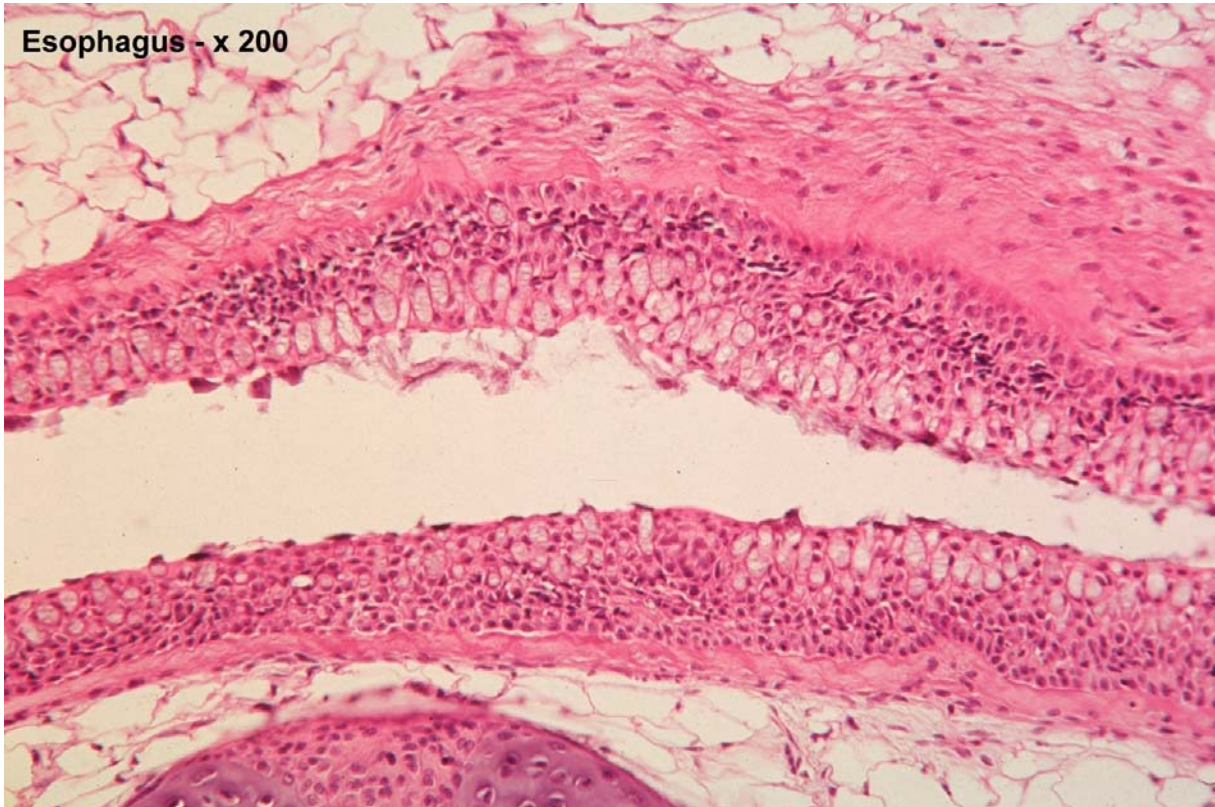


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Esophagus

The esophagus is usually a short, straight and very muscular tube passing from the mouth to the cardia of the stomach. Its combination of an epithelial lining containing abundant mucous cells which provide for more lubrication and the extensive longitudinal folds of the inner surface, allows for easy swallowing of awkward food particles.

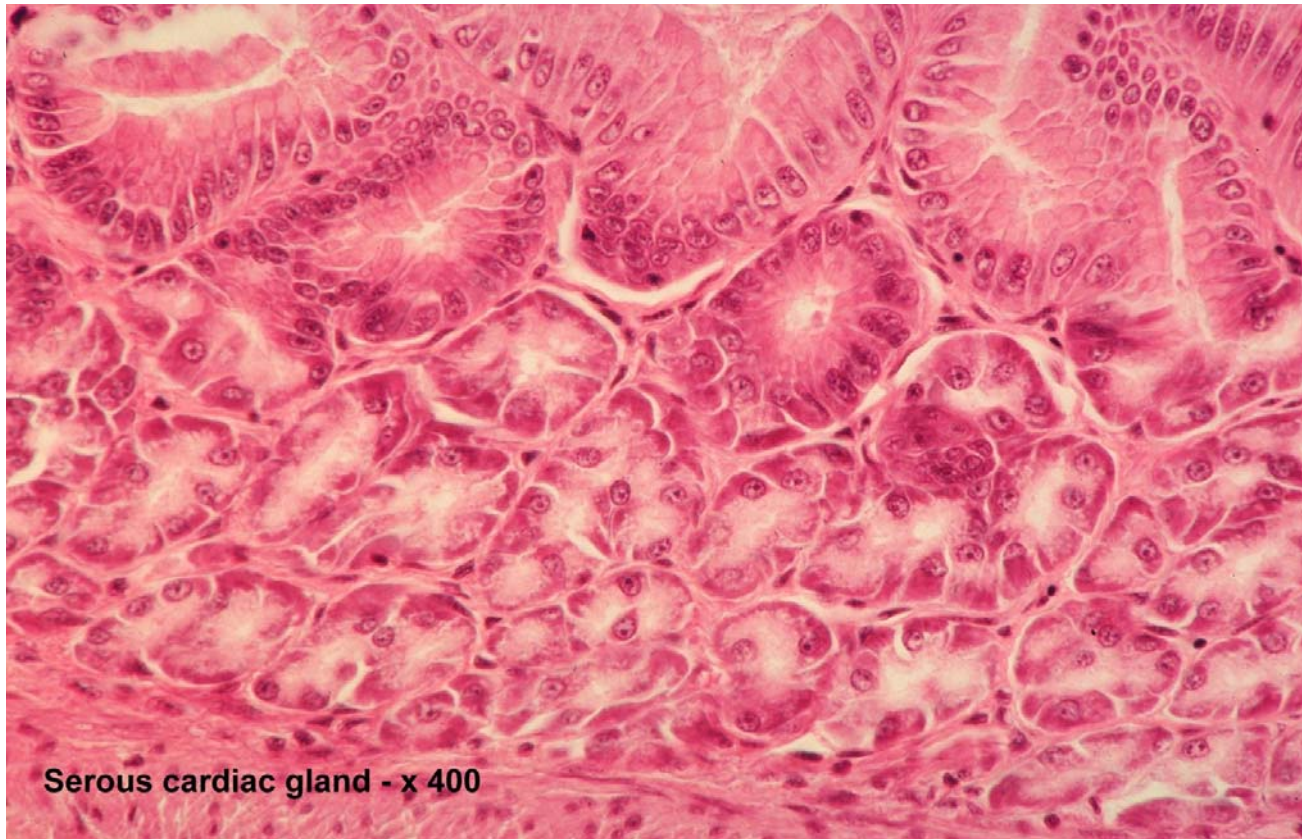


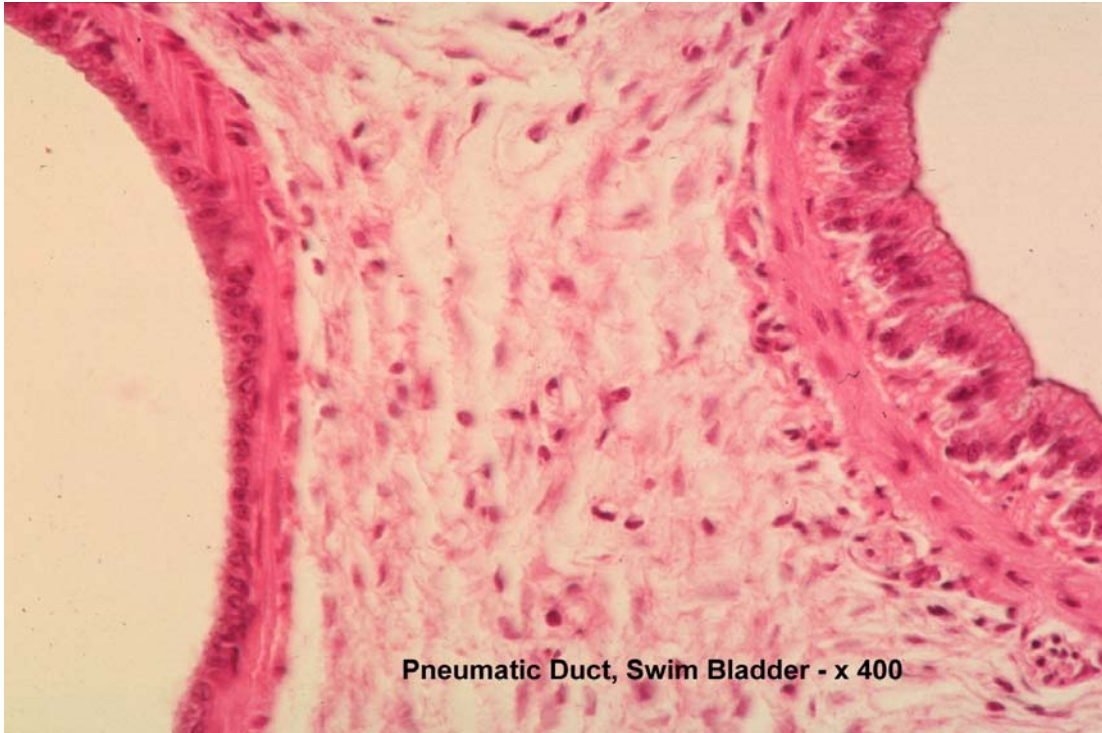
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Stomach

The stomach varies in size. It functions to churn contained material, mixing it thoroughly with the digestive juices that it secretes. Some absorption occurs on a limited basis. Typically it is a sigmoid, highly distensible, sac with numerous folds in its lining. The stomach can be divided into 3 sections: cardiac (anterior), transitional (mid), and pyloric (posterior). All sections are highly muscular with the cardia demarcating the change from the striated muscle of the anterior digestive tract to the smooth muscle occurring distally. There are a number of layers of muscle, including a muscularis mucosa with adjacent layers of connective tissue often containing large numbers of eosinophilic granule cells. The function of these granule cells is uncertain, but they appear to have a role in body defense mechanisms, especially with Infectious Hematopoietic Necrosis Virus (IHNV). The gastric mucosa itself is very mucoid, with numerous glands at the bases of the folds.





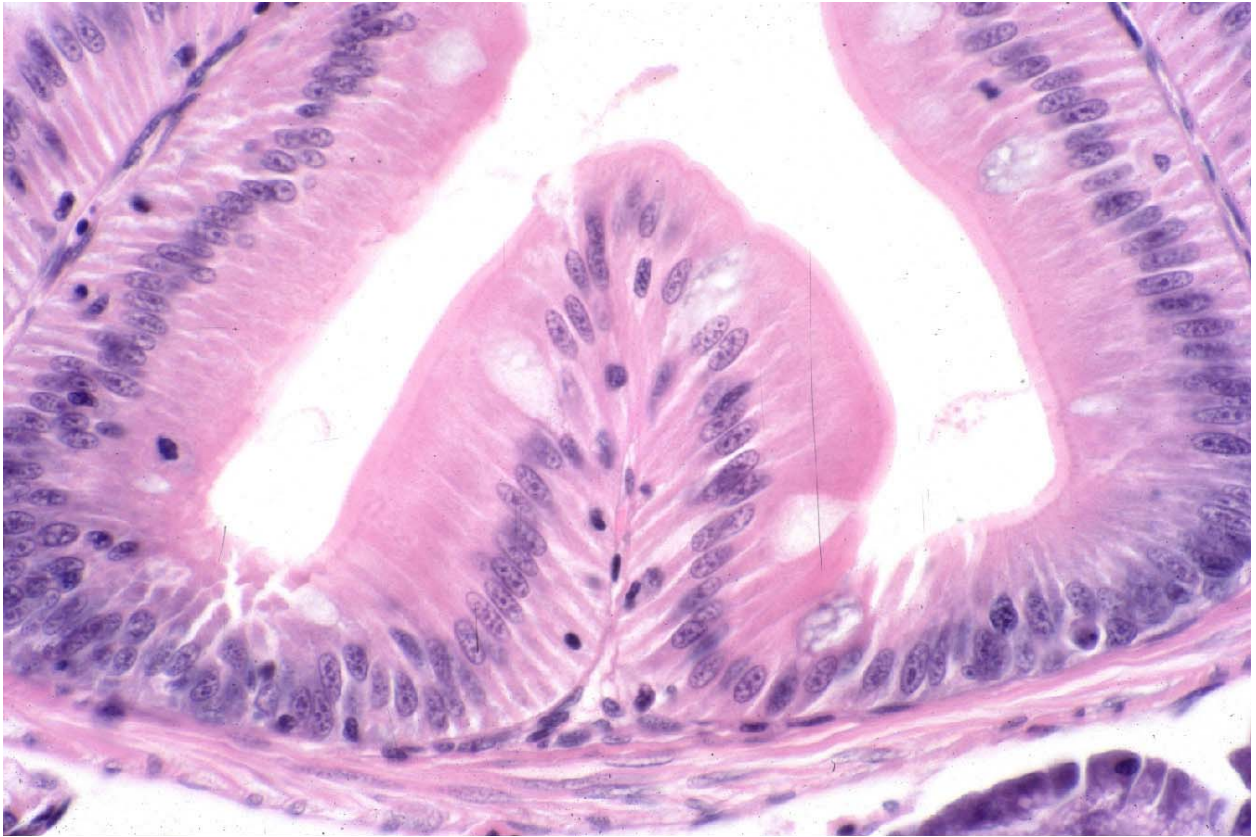
Pneumatic Duct, Swim Bladder - x 400

Pyloric Caeca

Consists of blind-ending diverticula from the distal pyloric valve region of the stomach and the anterior intestine. Found in many species, but notably in the salmonids where they may number 70 or more. Their histological and histochemical features resemble those of the intestine rather than the stomach. The pyloric caeca has a digestive and absorptive function.



Pyloric Caeca - H&E x 160

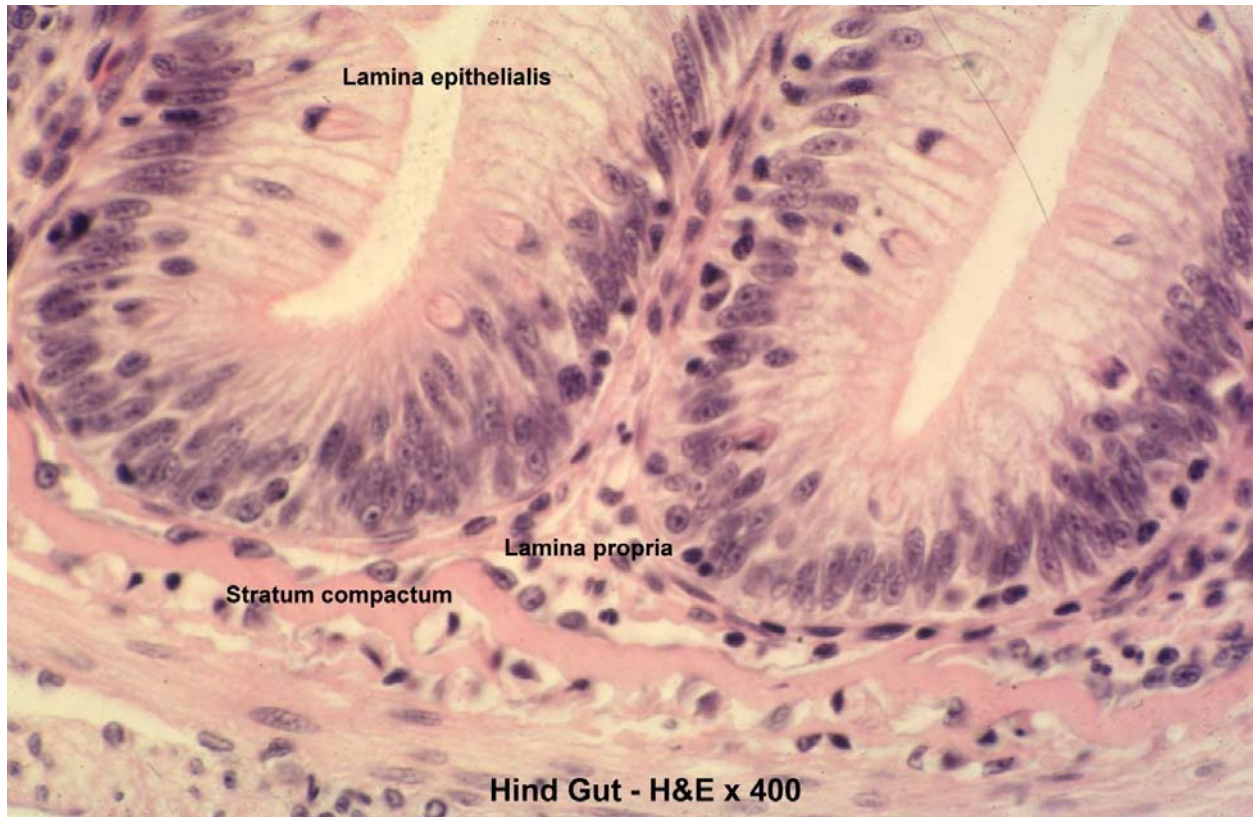


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Intestine

Although its relative length may vary according to diet, the intestine of most fish is a simple tube which does not increase in diameter to form a colon posteriorly. It may be straight, sigmoid or coiled, depending on the shape of the abdominal cavity. It has a simple, mucoid, columnar epithelium, overlying a submucosa often with abundant eosinophilic granule cells and limited by a dense muscularis mucosa and fibroelastic layer. The anterior portion of the intestine functions to 1) transport food material from the stomach to the posterior intestine, 2) to complete digestion by the secretion of enzymes from its walls and from accessory glands, 3) to absorb the final products of digestion into blood and lymph vessels in its wall, and 4) to secrete certain hormones (i.e. Secretin, stimulates pancreatic secretion). The posterior intestine functions include fluid absorption, mucous secretion (more goblet cells), and some digestion which is accomplished by enzymes present in food material, and excretion.



Rectum

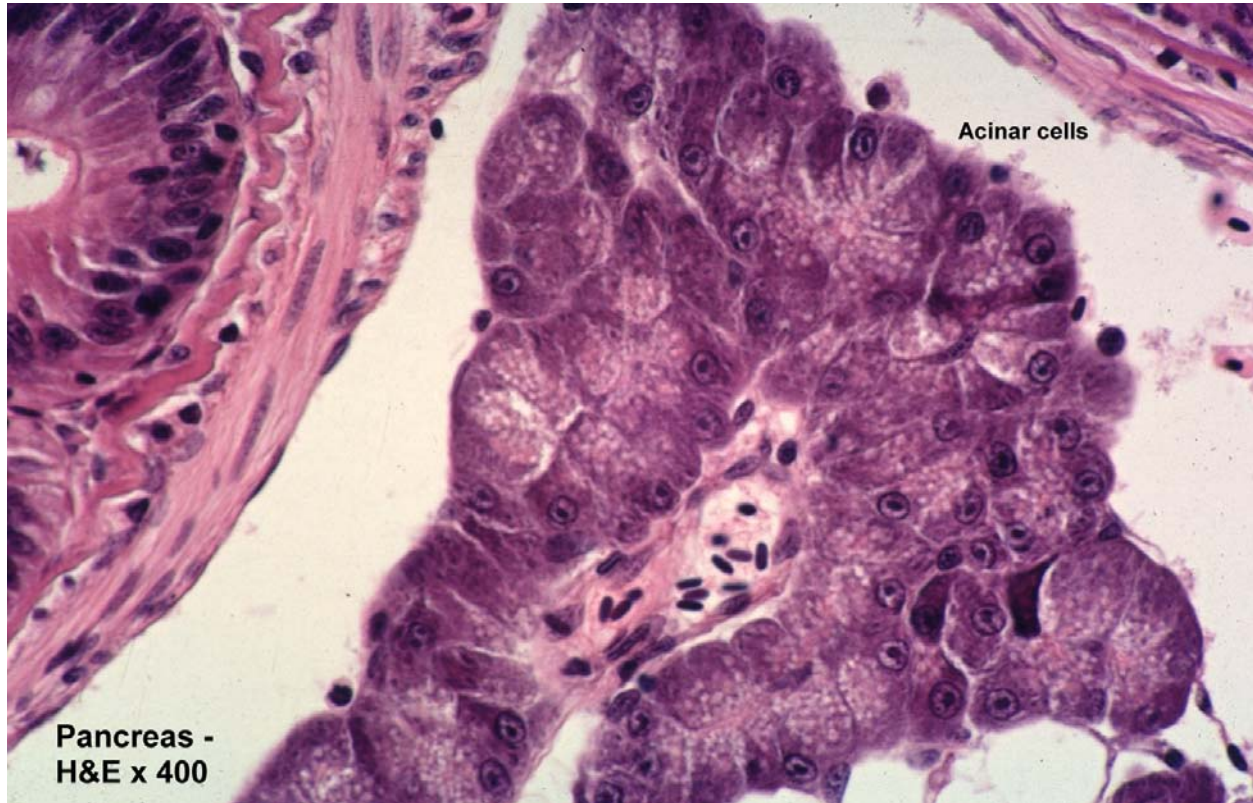
The rectum has a thicker muscle wall than that of the intestine and its lining is highly mucigenic. It is capable of considerable distension.

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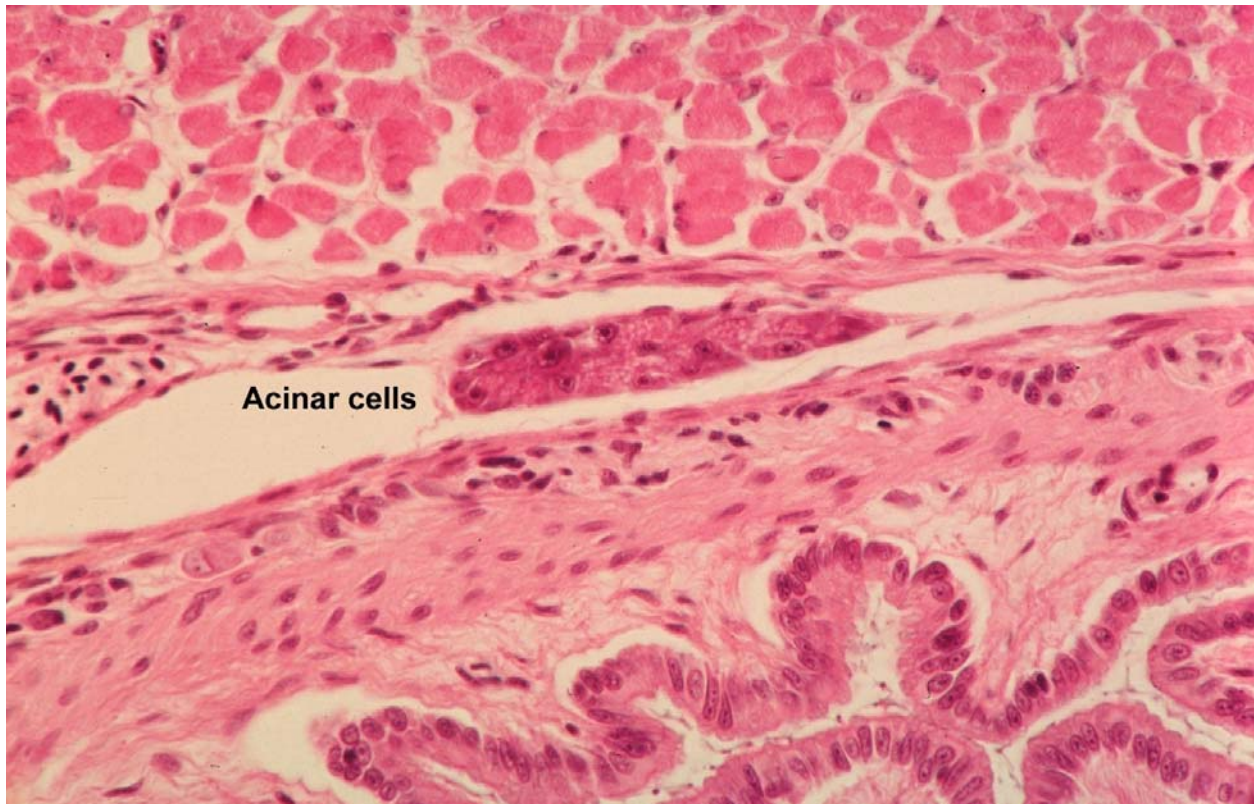
Pancreas

The pancreatic tissue is more variable in location, even within a single species, than the other abdominal viscera. The most common sites for it are as scattered islands of secretory tissue interspersed among the fat cells in the mesentery of the pyloric caeca, as a subcapsular investment, or part, of the spleen and as an external layer around the hepatic portal vein. In salmonids, it is diffuse throughout the tissue (adipose) that surrounds the pyloric caeca. In catfish and bass, it surrounds the portal vessels entering the liver to form a hepatopancreas. The pancreas consists of two types of tissue: exocrine and endocrine.



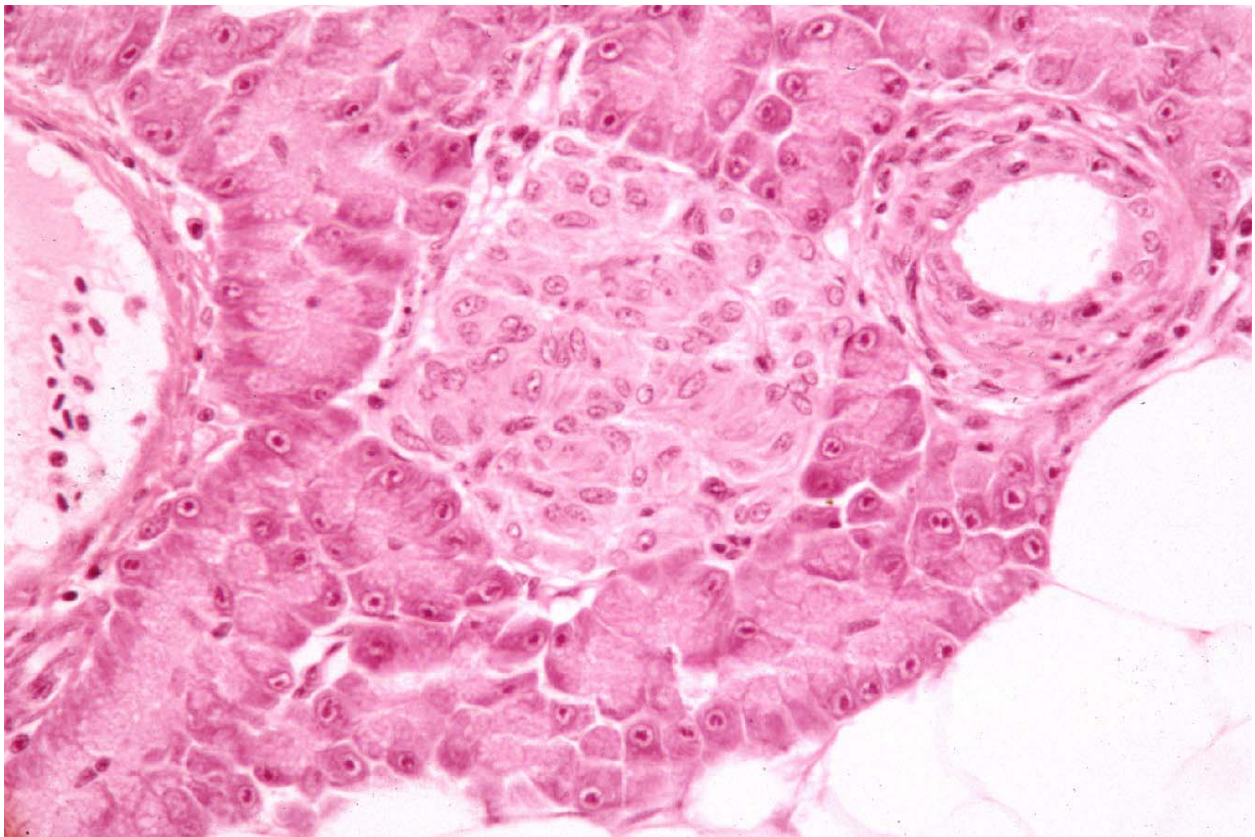
Exocrine Pancreas

The acinar structure of the exocrine pancreatic tissue is very similar to that of the mammal and is comprised of acinar cells that have a large spherical nucleus with 1-3 nucleoli and a very dark basophilic cytoplasm. In actively feeding fish these contain large numbers of bright, eosinophilic, secretory zymogen granules. Digestive enzymes are secreted from these acinar cells into the anterior intestine to break down proteins, fats, and carbohydrates.



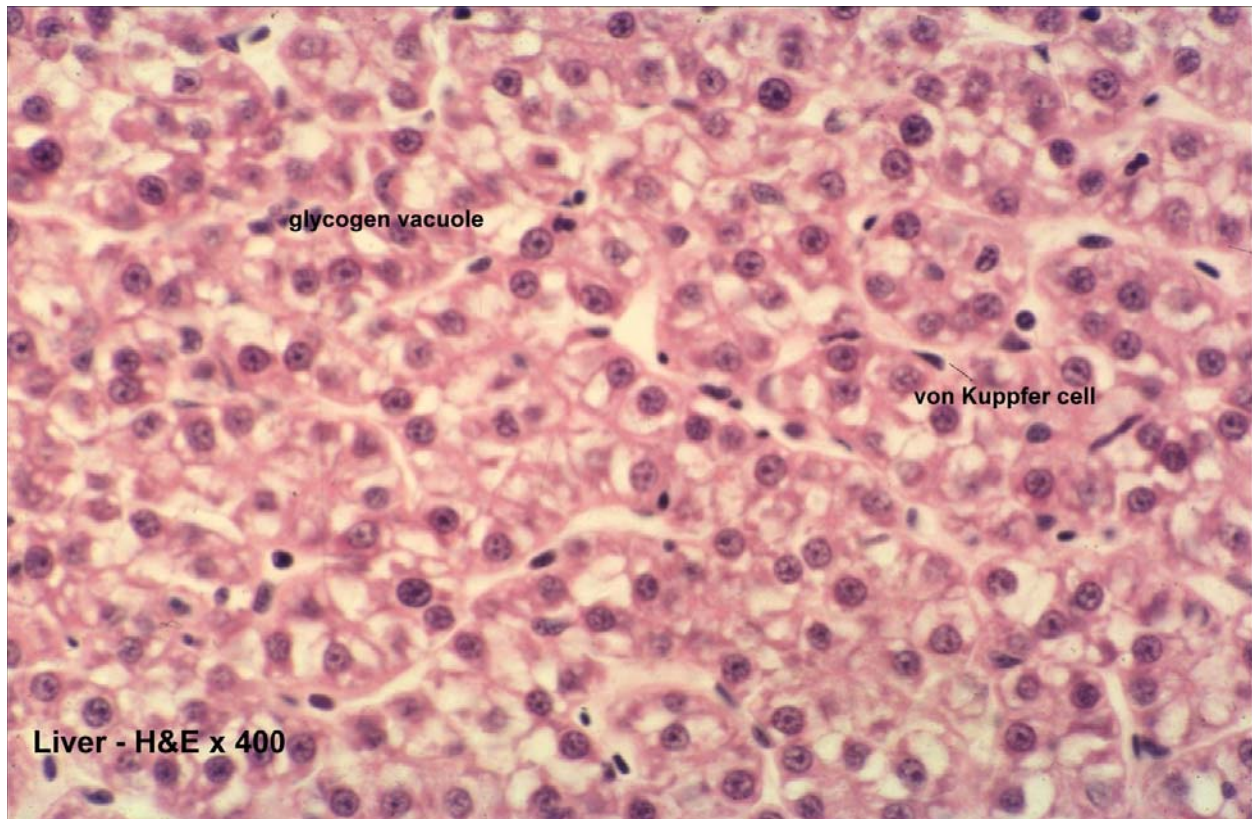
Endocrine Pancreas

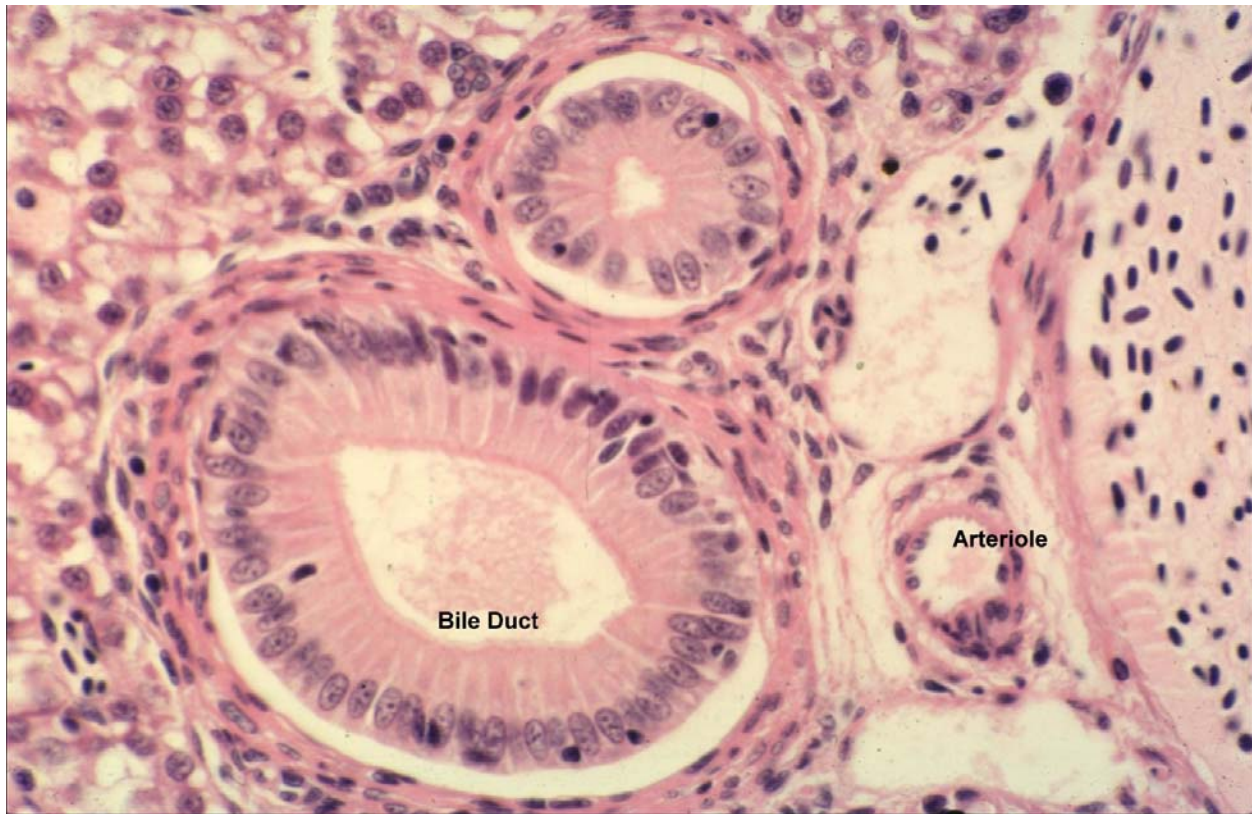
The distribution of the pancreatic tissue varies considerably with species, and the endocrine component is also varied. The endocrine components of the pancreas, the islets of Langerhans, consist of a number of lightly capsulated, spherical masses or clusters of pale staining glandular cells. In salmonids, these clusters are found scattered throughout the pancreas. The size of islet cells may vary with season, and in some species, there is one major islet, known as the Brockmann body. Insulin producing B cells, Beta cells, promote the transfer of glucose across cell membranes which lowers the blood sugar. Glucagon producing A cells, Alpha cells, promote release of stored glycogen which raises the blood sugar. Also, hormone producing D cells and X cells may be present. There is usually considerable change in islet size at spawning, with senility, and with dietary changes. Additionally, there are reported seasonal differences in the proportions of the different cell types.



Liver

The fish liver is a relatively large organ. In wild fish, it is usually reddish brown in carnivores and lighter brown in herbivores, but at certain times of year it may be yellow or even off white. In farmed fish, it can be lighter in color than in an equivalent wild specimen but this is diet dependent. The liver may be a localized organ in the anterior abdomen or may, in some species, have processes which extend the length of the abdomen or are closely applied to the other viscera. In some species it is a compound organ in the form of a hepatopancreas. The histology of fish liver differs from the mammalian in that there is a far less tendency of the hepatocytes to form distinct cords or lobules, and the typical portal triads are not obvious. It is composed of branching and anastomosing, two cell thick laminae or cords of hepatocytes. Distinct endothelial cells line sinusoids, which are irregularly distributed between the polygonal hepatocytes, with very prominent nuclei. Functional phagocytic cells are occasionally observed in the sinusoids. The sinusoidal lining cells are fenestrated and overlie the Space of Disse which is the zone between sinusoid cells and hepatocytes. Hepatocytes are polygonal and have a distinctive central nucleus with densely staining chromatin margins and a prominent nucleolus. In cultured fish, hepatocytes are often swollen with glycogen (extensive irregular vacuolations) or neutral fat. When diet is less than ideal or during cyclical starvation phases, the cells may be shrunken and contain varying amounts of yellow ceroid pigments. The fish liver does contain drug metabolizing enzymes and is one of the most frequently damaged organs, but it has been shown (in mammals) that only 10% of hepatic parenchyma is required to maintain normal liver function.





Gall Bladder

The gall bladder possesses the basic four-layer structure found in the intestine. The mucosa is composed of columnar epithelium with basally located nuclei. These cells appear cuboidal when the gall bladder is distended.



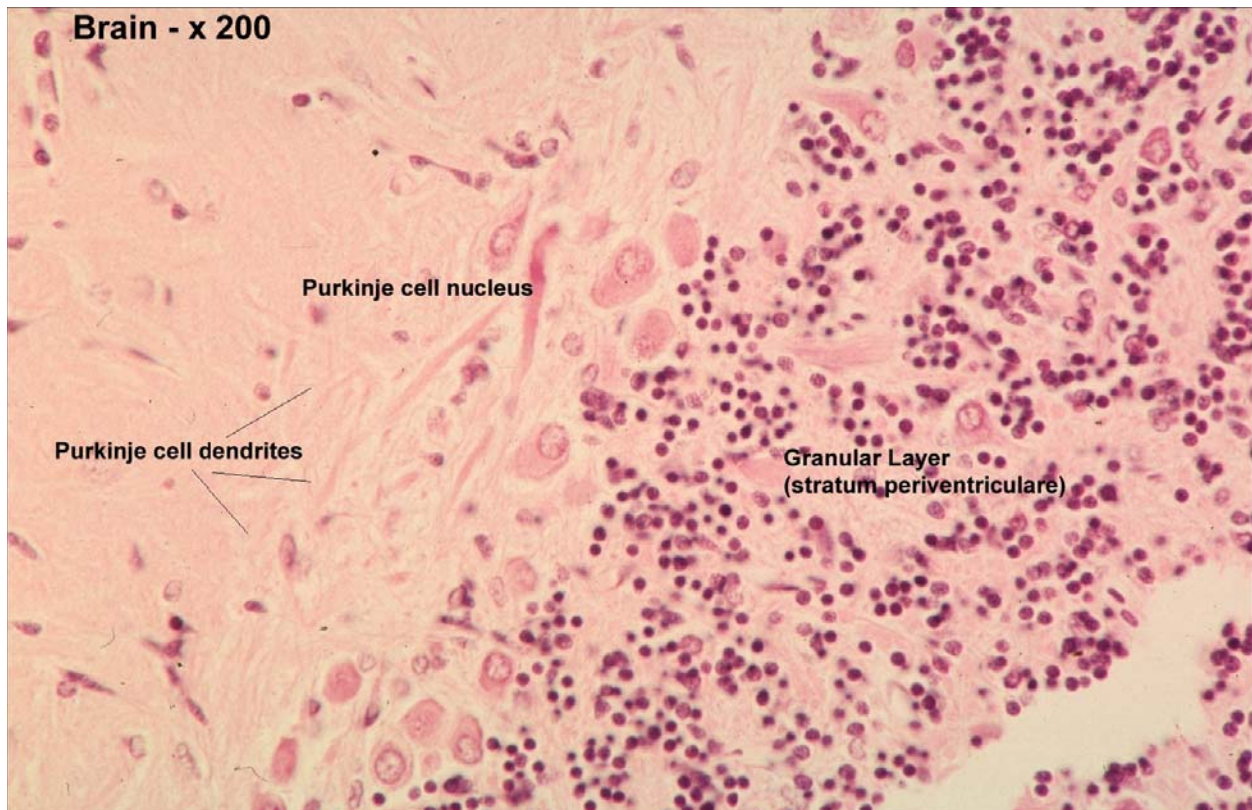
Nervous System

The nervous system is composed of two basic cell types, the neurons, and the neuroglial cells. The neurons conduct the nerve impulses and the neuroglial cells perform a supportive role. Neurons are made up of a cell body with projecting dendrites, and the axon. Usually several dendrites act to conduct nervous impulses to the cell body of the neuron and the single axon enclosed in a myelinated sheath, conducts the impulse away from the cell body. Contacts between neurons are called synapses. Central Nervous System tissue (brain and spinal cord) is divided into the classical gray and white matter consisting of nuclei of neurons, neuroglia, and myelinated axonal processes. The roots of the spinal nerves, especially in the region of the dorsal root ganglia, are usually overlaid by clusters of eosinophilic granular cells which are morphologically similar to those frequently observed in the teleost intestinal submucosa and other loose connective tissues.

Brain

The teleost brain is similar in its basic components to the brain of higher animals, but with differences in form and complexity. For ease of description it is usually divided into five divisions comprised of, from the anterior: the telencephalon, the diencephalon, the mesencephalon, the metencephalon (cerebellum) and the medulla oblongata.

Cells in photo: Giant nerve cell body, Neuroglial nuclei.



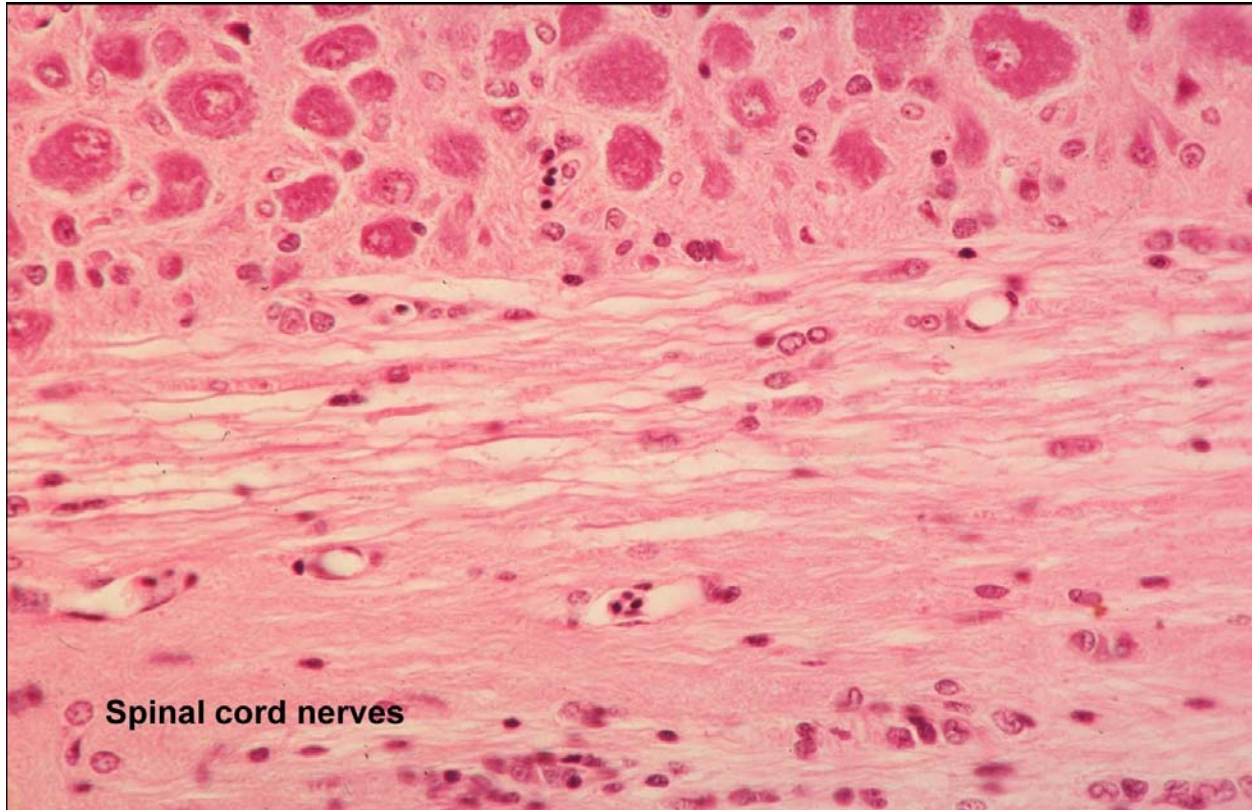
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Spinal Cord

In the spinal cord the most prominent features are the cell bodies of the neurons, and the nuclei of the neuroglial cells, which stain deeply with hematoxylin.

Cells in slides: Giant nerve cell body, Neuroglial nuclei in gray matter and myelinated nerve fiber tracts in white matter.



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Lateral Line Nerve

The lateral line contains high levels of sensory tissue. It is basically a groove on either side of the trunk of the fish overlaid with skin. Pores, alternating with mechanoreceptors which are very sensitive to water particle displacement, are found along its length.

Photo: Cross section of myelinated nerve fibers bundled.



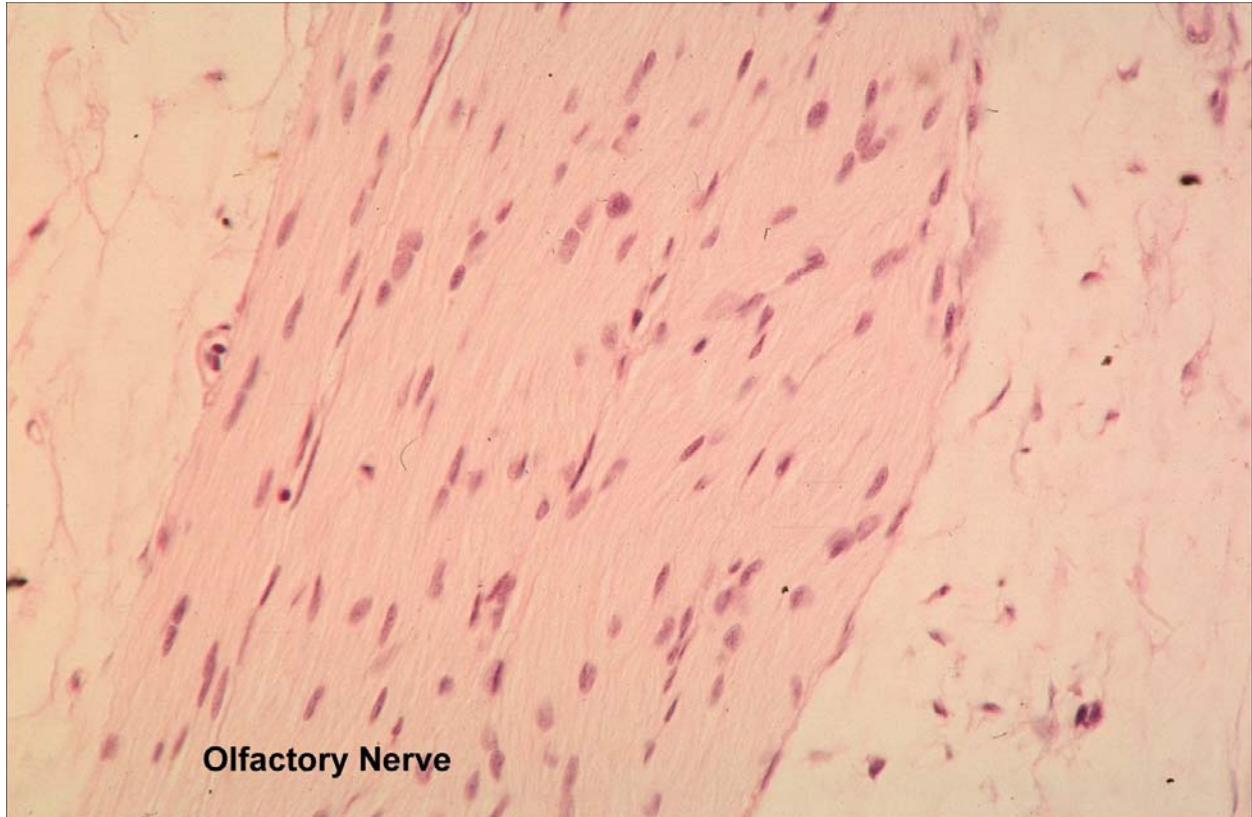
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Olfactory Nerve

Olfactory epithelium is raised in a series of very vascular folds or fingers increasing the surface area of sensory tissue. The olfactory organs are paired areas on the snout. The actual olfactory tissue consists of focal groups of receptor cells surrounded by mucoid and ciliated columnar epithelium.

Longitudinal section: nerve fibers and nuclei (less myelin than lateral line).



Reproductive System

Testes

The testes are paired organs, suspended by mesenteries from the dorsal abdominal wall, alongside or below the swim bladder. The testis itself is comprised of a series of tubules or blind sacs, the seminiferous tubules, which are lined with spermatogenic epithelium. The process of maturation of the male gamete involves the multiplication of spermatogonia which develop from the spermatogenic epithelium (primordial germ cell) to form spermatocytes. Many of these eventually undergo a meiotic division to become the haploid spermatozoa. Spermatozoa attach to the surface of the pyriform (pear shaped), nourishing, cells of the seminiferous epithelium known as Sertoli cells until ready for release.



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Ovary

The ovarian follicle is an aggregate of ova and epithelial cells. These follicles start as oogonia, or primitive egg mother cells, which are periodically generated in the germinal epithelium. The oogonia are made up of cells which are beginning to mature to form and produce oocytes. Small epithelial cells form a single layer surrounding the Oogonia. The epithelial cells grow as the ovum grows and are separated from it by a gradually thickening hyaline capsule. These are responsible for nourishing the ovum and secreting its yolk. In many species, several generations of ova may be found in different stages of development at one time.



Musculoskeletal and Supportive Tissues

Muscle

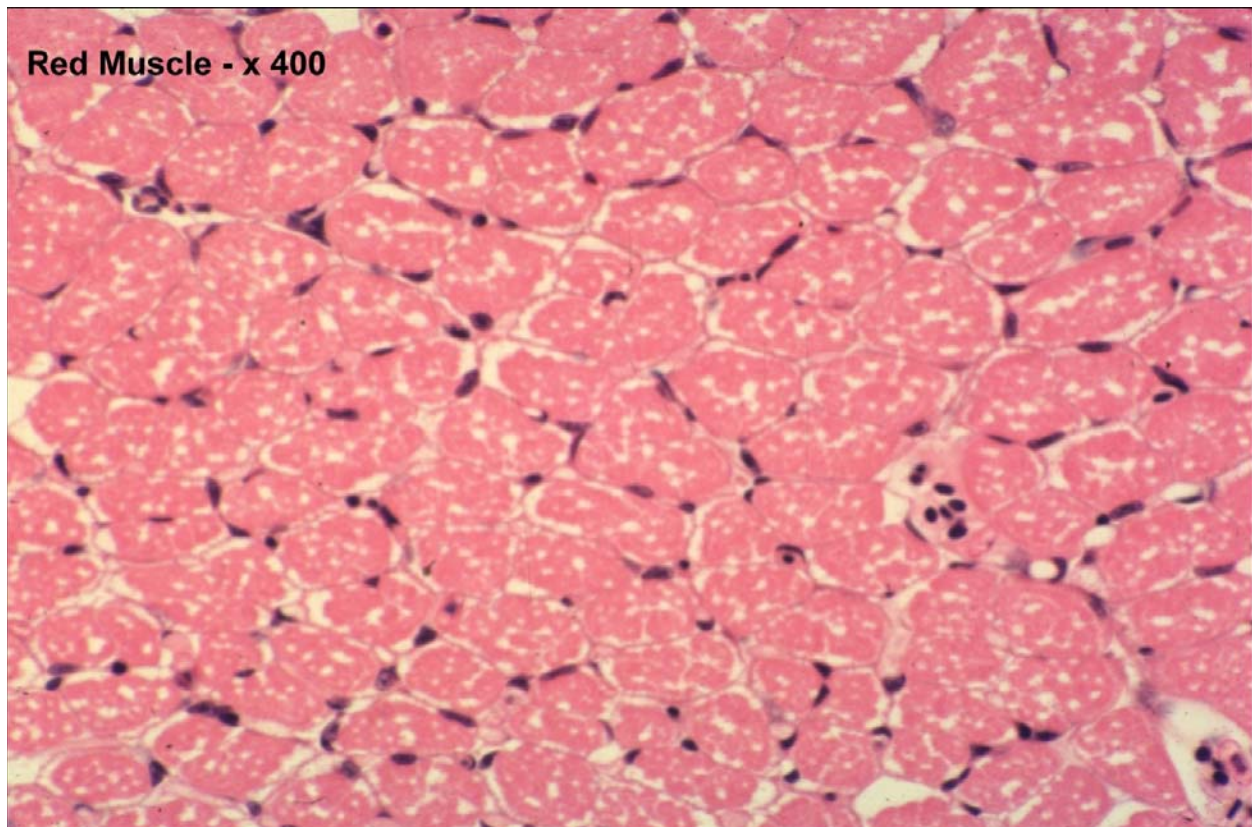
Fish and other higher vertebrates have three types of muscle cells: (1) the striated, unbranched, "voluntary" musculature; (2) the cardiac musculature, consisting of striated, branching "involuntary" fibers; and (3) the smooth unstriated, "involuntary" musculature.

Striated Voluntary

The striated muscle cell of the body musculature, or the skeletal muscles, is multinucleated. The nuclei lie just beneath the membranous sarcolemma that sheathes the cell. Each cell contains several longitudinal myofibrils, each of which are comprised of several myofilaments. The myofibrils can be seen by light microscopy, but myofilaments are visible only by electronmicroscopy. There are "red" and "white" muscle fibers. These two kinds of muscles are involved in two kinds of swimming activity: the red fibers are related to sustained activity, while the white fibers to short, strong bursts of motion.

Red Muscle

The layer of "red" muscle lying as a wedge along the lateral line, just beneath the skin, has a higher lipid content than the white tissue, and a larger number of mitochondria per cell and higher respiratory activity. The red fibers are aerobic, slow-contracting, fibers. The red muscle is also generously supplied with blood, potentially providing a good site for the injection of drugs, anticoagulants and anesthetics.

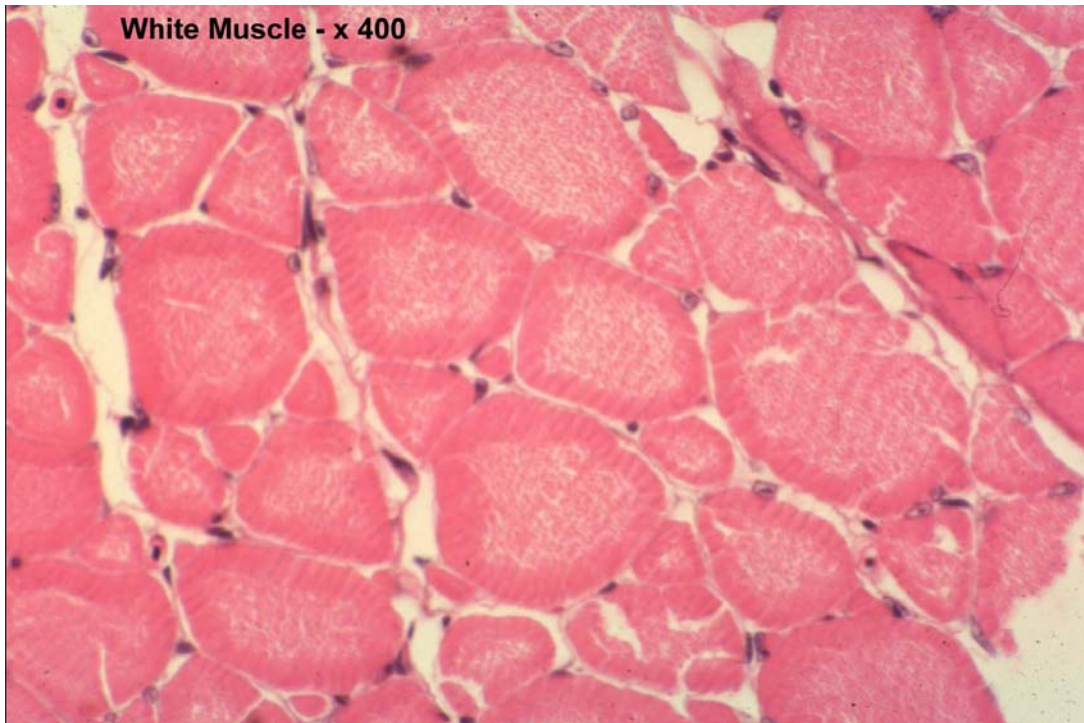


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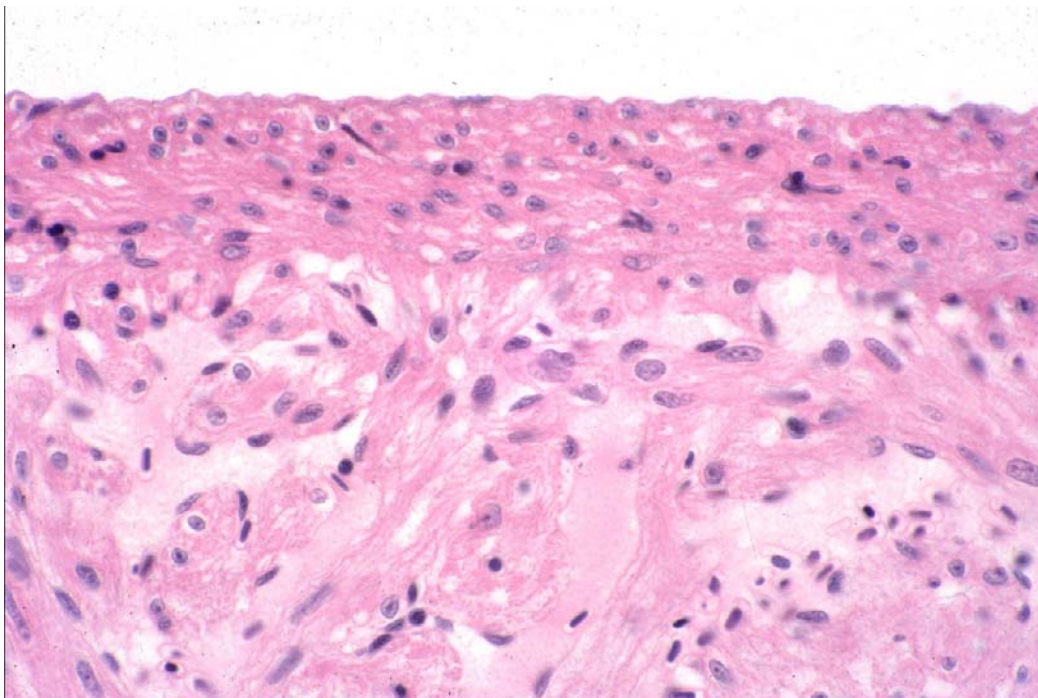
White muscle

White muscle forms the greatest volume of body tissue. Generally low numbers of mitochondria and low respiratory activity. The white fibers are anaerobic, fast-contracting and fast-fatiguing fibers.



Striated Involuntary

The striated muscle fibers of the heart are unlike those of the body musculature in that they are branching and anastomosing. They do not lie parallel to one another, and several planes may be seen when the heart muscle is sectioned for microscopical study. Another important difference is that the nuclei are located at regular intervals near the center of the cell, rather than just beneath the sarcolemma (the plasma membrane of a muscle fiber). Cardiac muscle cells lie end-to-end, rather than as the single, long slender units seen in the body musculature.

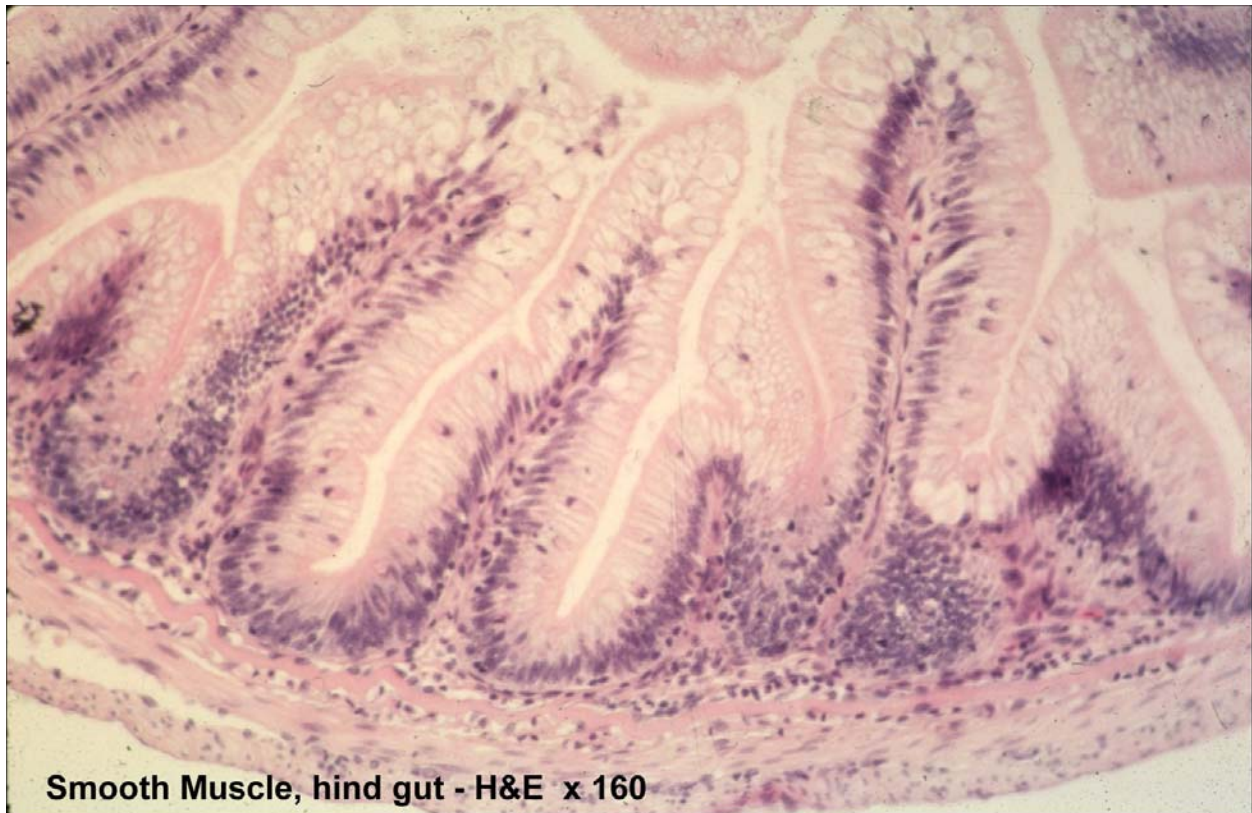


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Smooth Involuntary

Mainly visceral in distribution, no striations, central nuclei. Smooth muscle cells are long and tapering and are not attached to one another end-to-end. They are without striations and thus are said to be "smooth." The nuclei are also long and tapering. If they have contracted, they may appear "wavy." Within the bundles, the cells of smooth muscles are parallel, but in adjacent bundles the cells may lie at right angles. Not only is smooth muscle found in the walls of all parts of the gastrointestinal tract, but also in the walls of blood vessels, especially arteries, and to a lesser extent in glands.



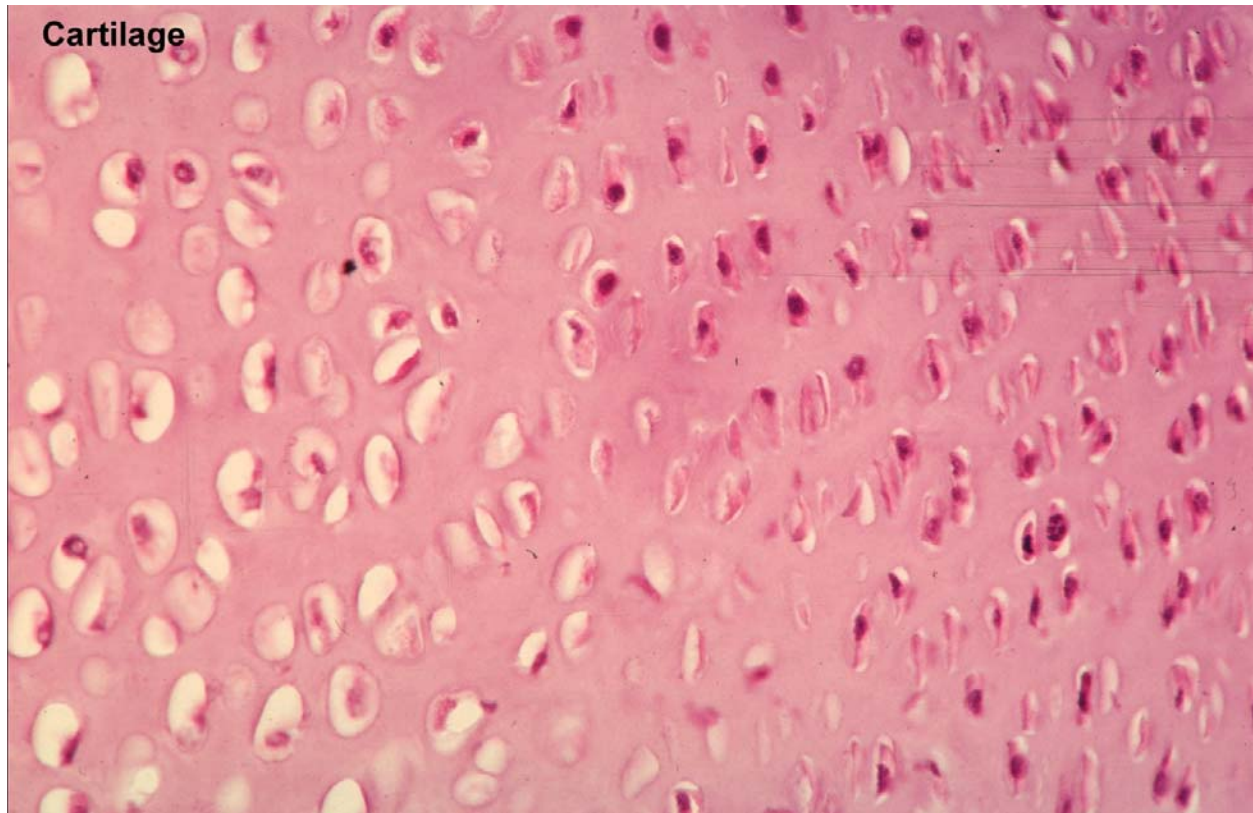
Smooth Muscle, hind gut - H&E x 160

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Cartilage

Cartilage forms the structural elements of the skeleton in embryonic and very young trout, and can almost disappear in older fish. It is a specialized form of connective tissue, consisting of cells (chondrocytes) surrounded by a matrix of collagenous fibers and a ground substance known as chondroitin sulfate. Initially an aggregation of mesenchyme cells become specialized as chondrocytes. These early chondroblasts multiply to form a compact mass of cartilaginous tissue. Eventually, as the basophilic matrix accumulates around the chondrocytes, these cells become isolated in lacunae, which appear in sections as clear, more or less spherical spaces. Surrounding the cartilaginous elements is a perichondrial sheath composed of fibroblasts and collagenous fibers. Cartilage can be roughly divided into three major types: hyaline, elastic, and fibrous. Of the three, hyaline is the most common.

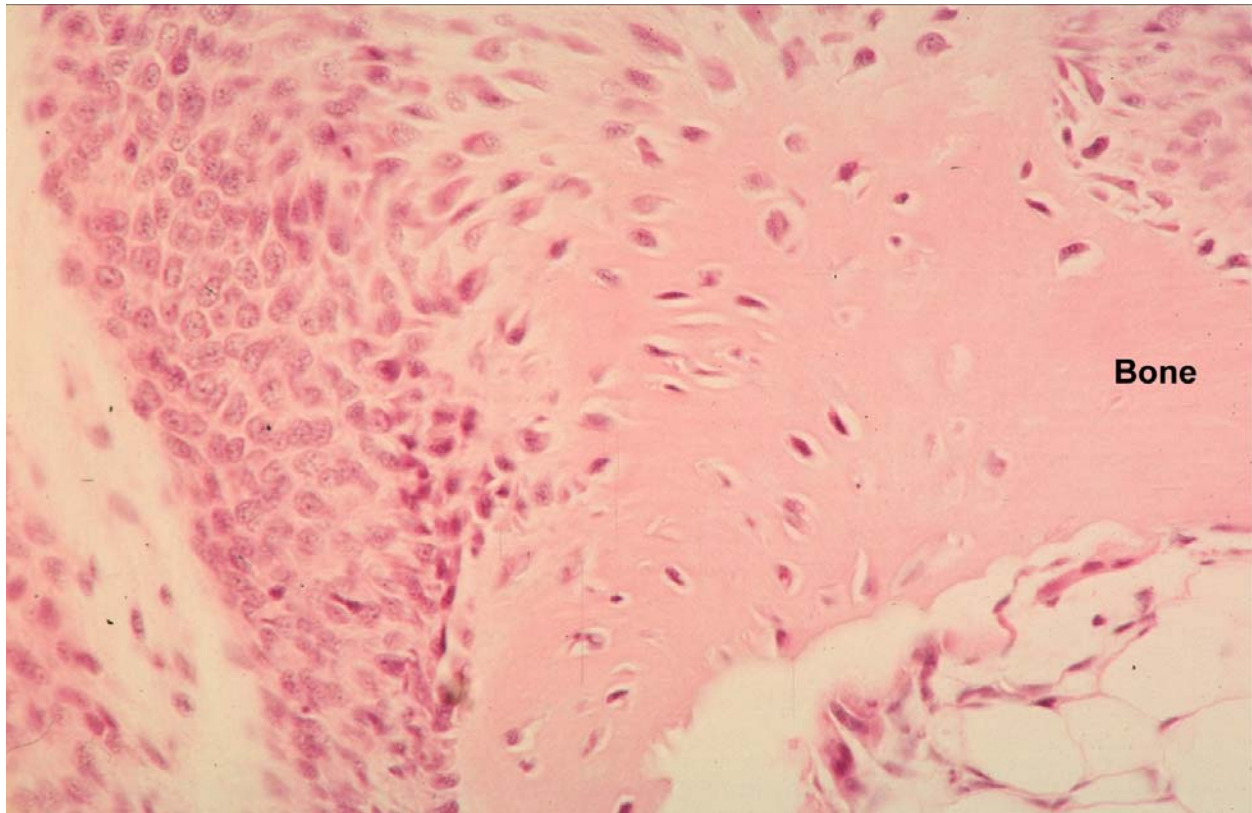


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Bone

Histologically, fish bones are similar to those of higher vertebrates. Unlike mammals, however, fishes do not have any hematopoietic elements within the bone. In general, there are two types of fish bones, cellular and acellular. Cellular bone contains osteocytes and is found in lower orders, such as the Salmoniformes. Fish of higher orders, such as the Perciformes (perch-like) usually have acellular bones, which are characterized by a lack of osteocytes. Bone histogenesis takes place in two forms, direct and indirect. In direct bone formation, the bone is formed in association with the dermis; in indirect bone formation, it is formed by the perichondral ossification of the hyaline cartilage. The osteoblasts of the periosteum do not leave cavities as they become enclosed; the spaces originally occupied by these cells are filled with a "bony" substance, largely calcium phosphate.

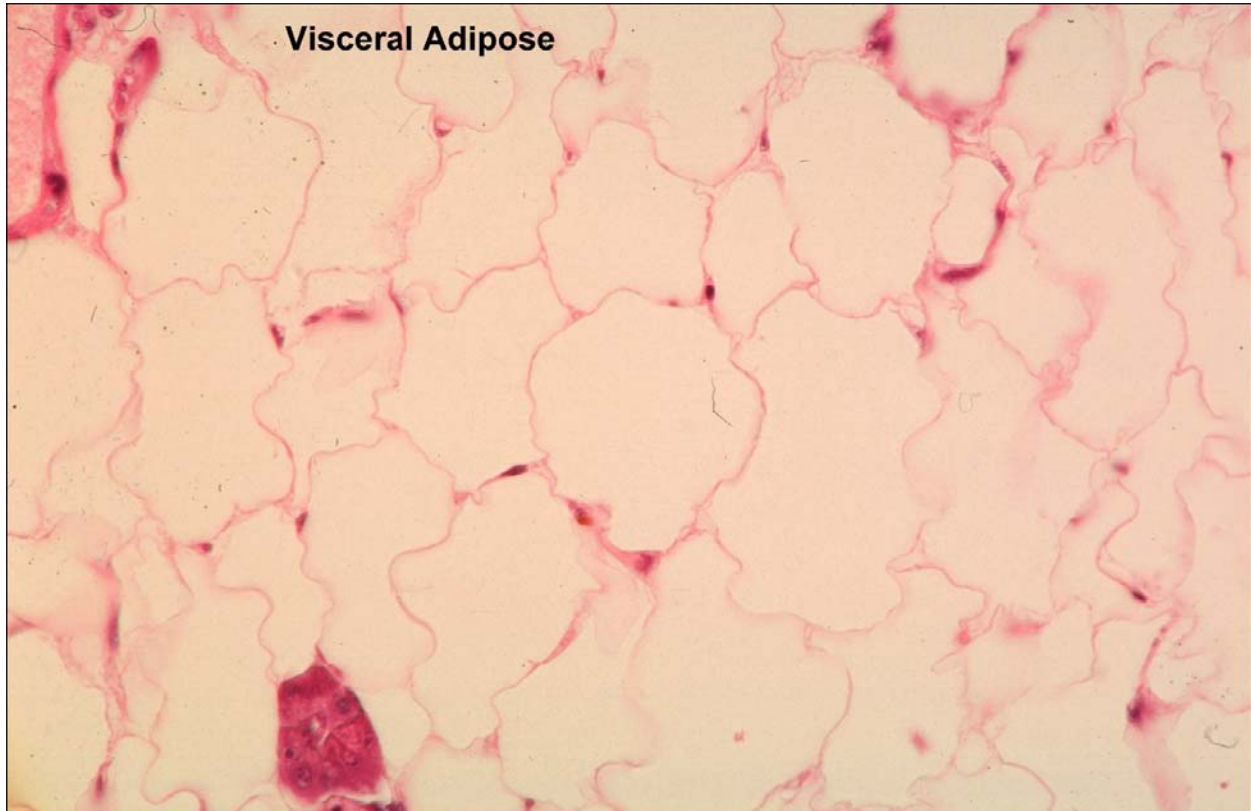


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Adipose

Fat cells are fully differentiated cells and are incapable of mitotic division. New fat cells therefore, which may develop at any time within connective tissue, arise as a result of differentiation of more primitive cells. Although fat cells, before they store fat, resemble fibroblasts, it is likely that they arise directly from undifferentiated mesenchyme cells present within the body. Initially small droplets of fat make their appearance within the cytoplasm. The droplets increase in size and finally coalesce to form a single large droplet, and the cytoplasm is reduced to a thin encompassing layer. The nucleus is compressed and flattened. When fat is utilized it leaves the cell as soluble components (the same form in which it enters), and the cell takes on a wrinkled appearance.

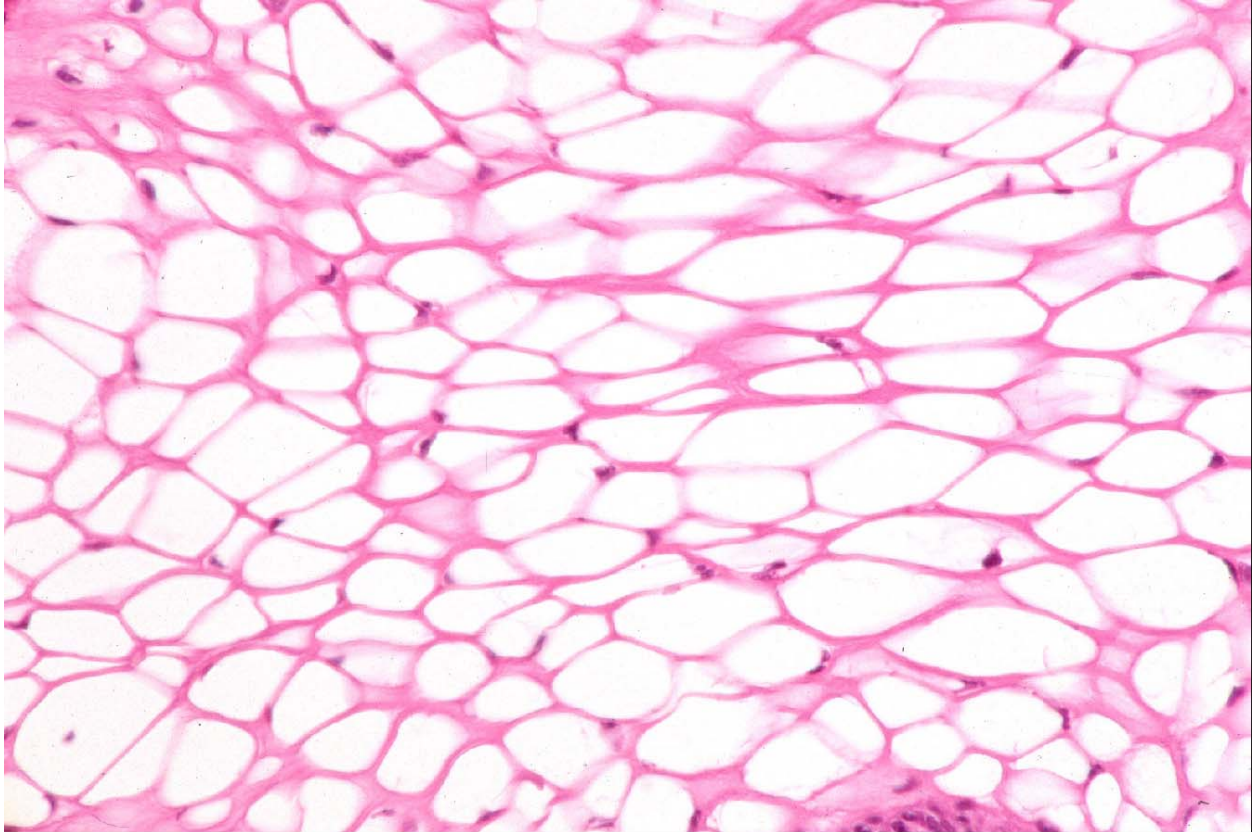


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Notochord

The superficial appearance of notochordal tissue (foundational tissue of the axial skeleton) is similar to that of adipose tissue. Cells that form the notochord resemble fat cells because they result from the accumulation of intracytoplasmic fluid in the cells. This fluid in turn causes the vacuole to occupy almost the entire cell, thus forcing the nucleus and cytoplasm to the periphery of the cell. Cells resembling epithelial cells usually line the inner surface of the notochord. An elastic membrane layer made up of fibroblasts and fibrous tissue surrounds this cell layer, which are associated with formation of the vertebral column.



Endocrine System

An endocrine gland is one without a duct which provides an internal secretion, often a hormone, most commonly into the systemic circulation.

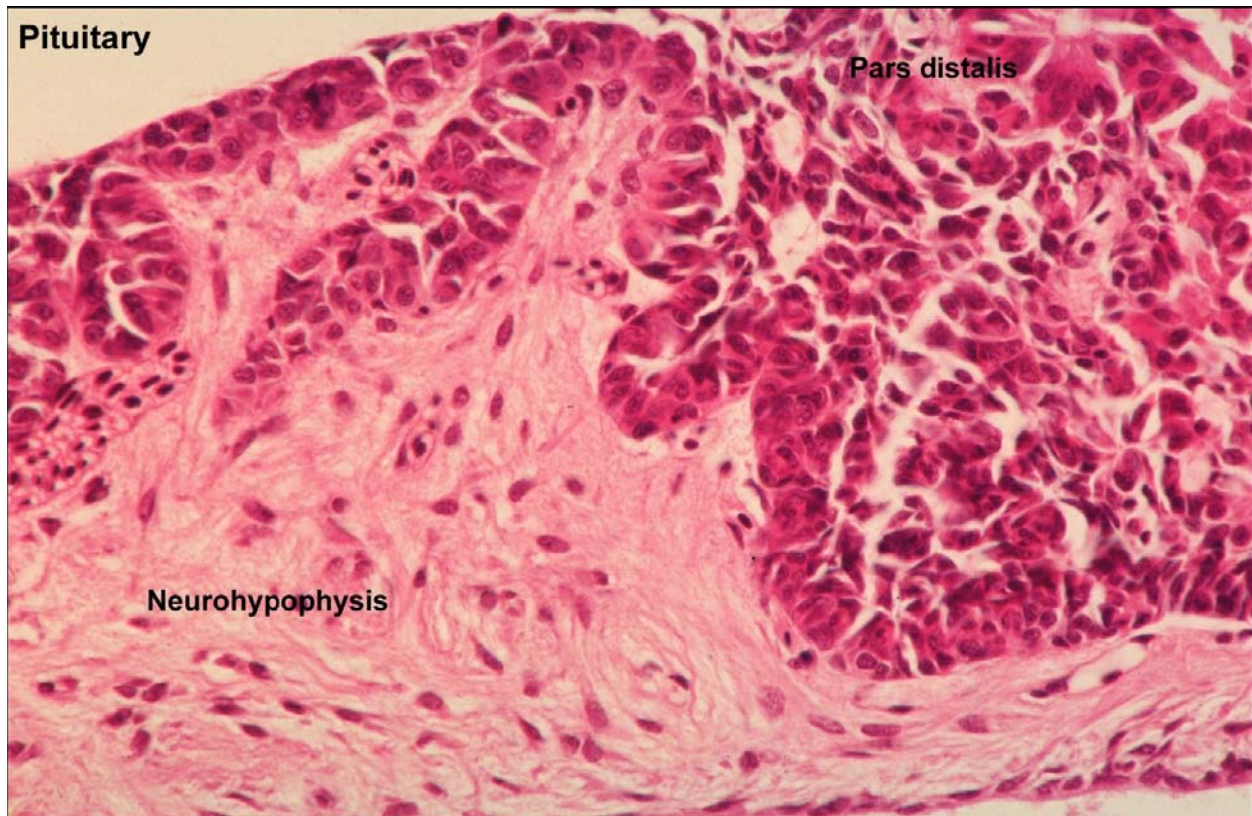
Pituitary

The pituitary gland or hypophysis is ventral to the mid brain and connected to it by a short infundibulum (funnel shaped passageway). The two major components are the neurohypophysis and the adenohypophysis. The adenohypophysis synthesizes, stores, and passes several hormones into general circulation. The neurohypophysis is believed to be responsible for controlling the adenohypophysis, osmoregulation, and some aspects of reproduction. The functions of hormones produced by the hypophysis are numerous including: promoting survival in fresh water, releasing hormones during stress, stimulating growth, stimulating gonad maturation, stimulating thyroid production, ionic regulation and water balance.

In section, the neurohypophysis appears fibrous since it consists of glial cells and three types of neurosecretory fibers originating in the hypothalamus.

The adenohypophysis consists of three areas histologically: rostral pars distalis, proximal pars distalis, and pars intermedia. The rostral pars distalis is composed primarily of prolactin cells which are elongated and often form follicles. The hormone prolactin is believed to play an important role in osmoregulation. The proximal pars distalis contains three cell types: somatotropin (growth hormone), gonadotropin, and thyrotropin (thyroid stimulating hormone). The pars intermedia has only one type of cell, the melanophore stimulating hormone cell.

Photo: Prolactin cells forming follicles (dark purple). Also neurohypophysis (pale cells).

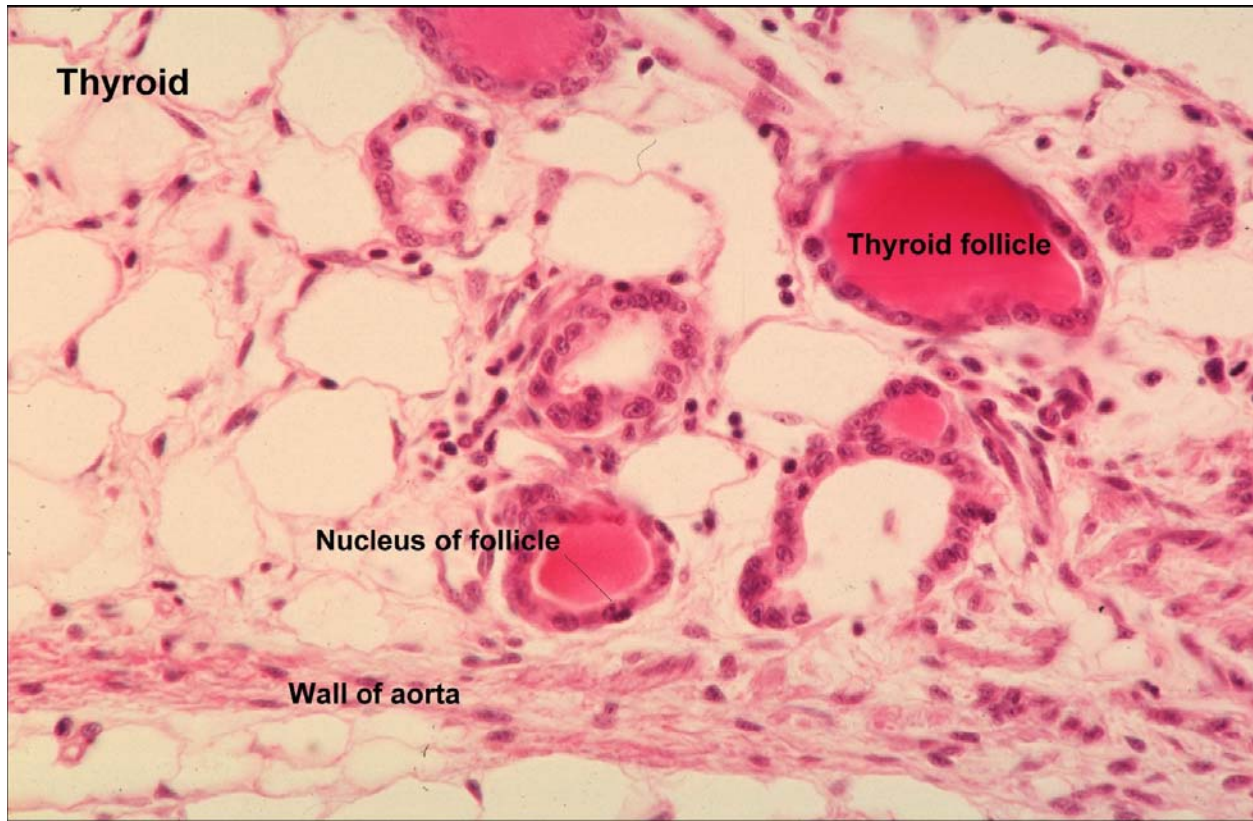


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Thyroid Gland

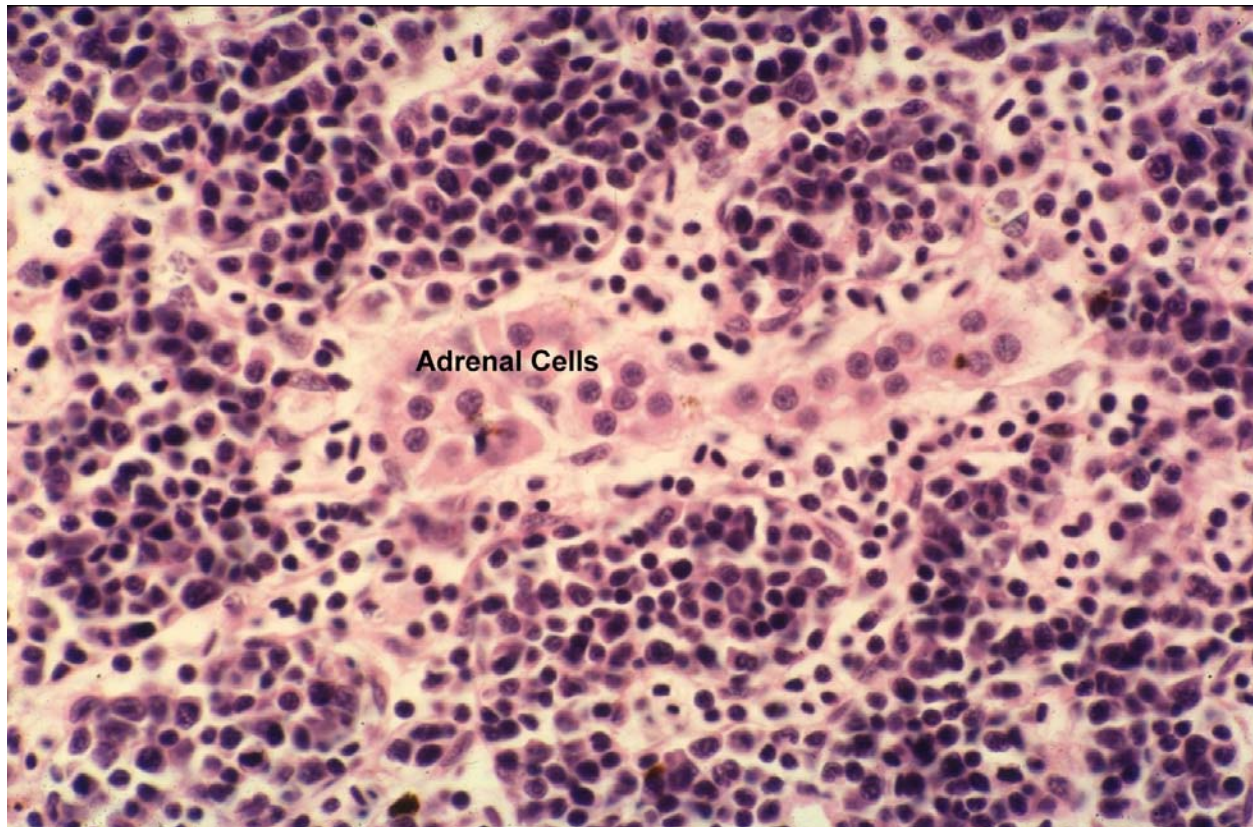
The thyroid gland is similar in basic structure to that of mammals and the thyroid hormone which has a stimulatory effect on many metabolic processes is a similar iodinated thyroxine to that of higher animals. The thyroid follicles are usually round to oval with low cuboidal epithelium and PAS-positive colloid secretion. The colloid is the protein bound form of the thyroid hormone, thyroxine. Instead of being located within a discrete capsule they are distributed throughout the connective tissue of the pharyngeal area or even, in some species, around the eye, ventral aorta, hepatic veins and renal hematopoietic tissue.



Adrenal Gland

Interrenal Tissue

The interrenal tissue is located in the head (hematopoietic) kidney in close association with the cardinal veins. It is homologous to the adrenal cortex of mammals and serves primarily as the cortical steroid producing tissue. The morphology of the interrenal follicles and of the cells themselves is very distinctive. They are embedded in the hematopoietic parenchyma and usually assume a rounded or oval shape. The follicles may occur along the minor blood vessels of the head kidney, where they assume a long, tubular form. The nuclei of the interrenal cells are noticeably uniform, small, nearly spherical and have a well-defined nucleolus.



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Chromaffin Tissue

Chromaffin cells are also found in the head (hematopoietic) portion of the trout kidney, where they are closely associated with the interrenal tissue. The chromaffin cells appear either colorless or have a faint basic reaction. The chromaffin nuclei are oval or irregular in shape and are larger than the nuclei of the interrenal cells. The chromaffin cells lie along the major blood vessels of the head kidney; the interrenal cells are usually scattered throughout the hematopoietic tissue. The chromaffin cells produce both epinephrine and norepinephrine. These are known to function in the stress response-defensive "fight or flight" reactions.

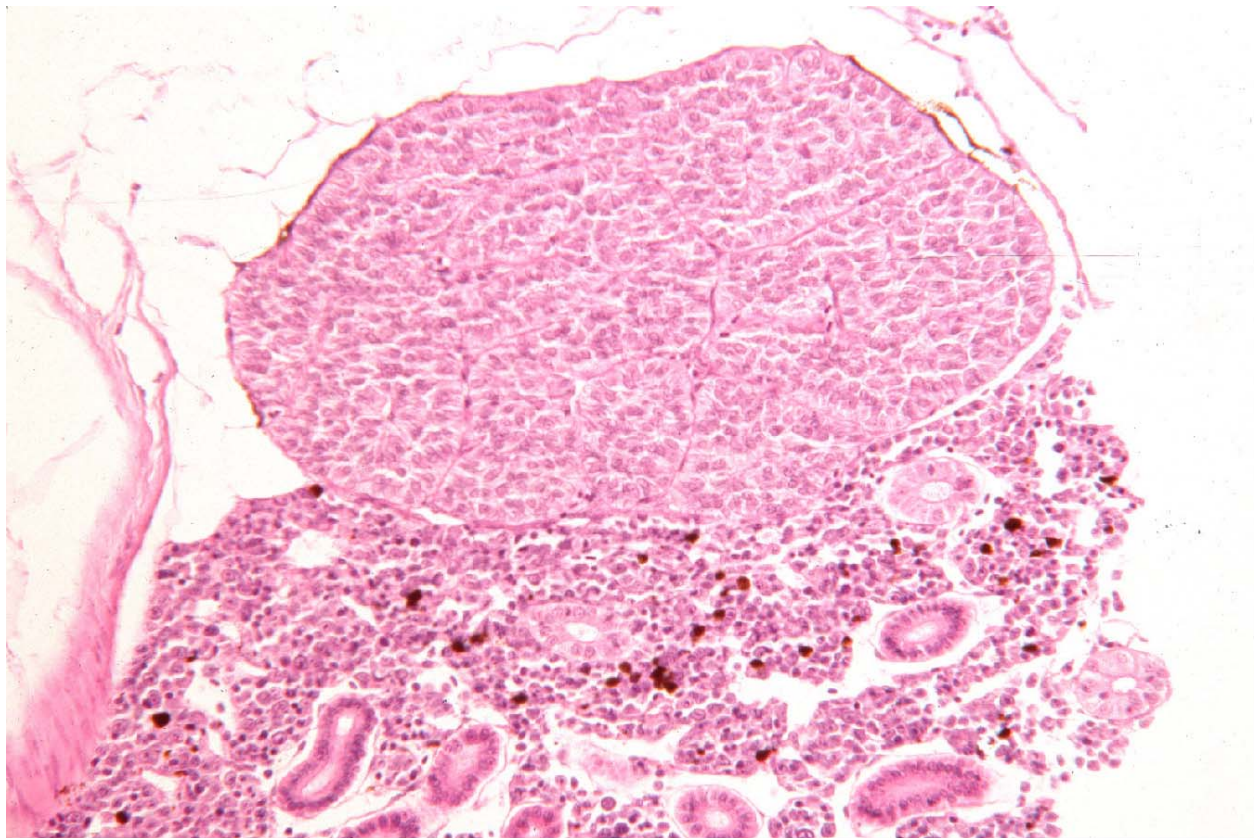


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Corpuscles of Stannius

The glandular bodies or corpuscles described by Stannius in 1829 are usually found on the ventral surface of the kidney. In salmonids, they are light-colored, oval clusters of cells, differing in number and location. Each corpuscle is divided into a variable number of lobules by walls of connective tissue projecting inward from the encapsulating membrane. The lobules are composed of convoluted "cords" of parenchyma. These parenchymal cells are of two types "granular" and "agranular." The granular cells are periodic acid-Schiff positive; the agranular cells are negative. The granules appear to be secretory, but the function of the secretion is not well understood (calcium regulatory function, electrolyte homeostasis, active in smolts not in adults?).

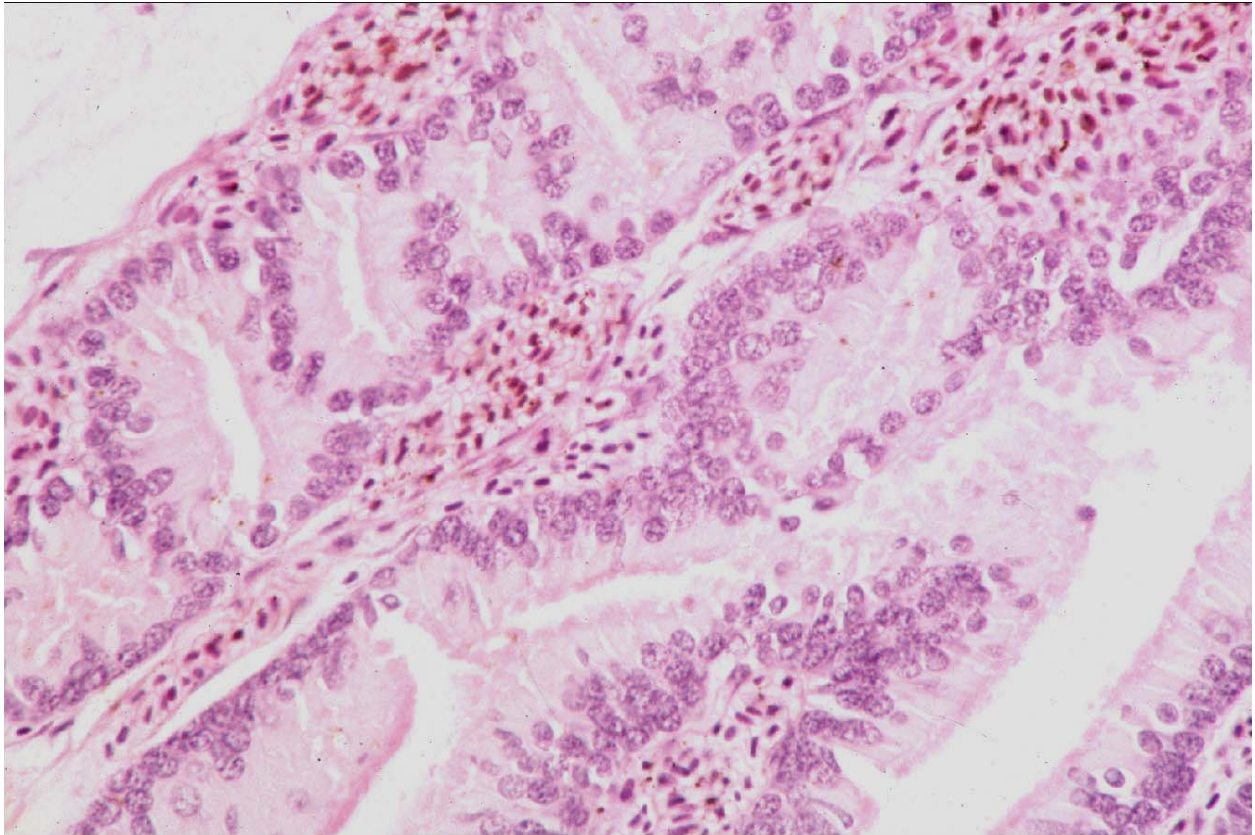


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Saccus vasculosus

The saccus vasculosus is a sac-like protrusion of the mid brains caudal infundibular wall, located directly posterior to the pituitary. The saccus is an organ whose walls are infolded at many points. The lumen of the sac appears empty in paraffin embedded preparations, and blood lies within the folds. Three general functions have been attributed to the saccus vasculosus: sensory, secretory, and absorptive.



Special Sensory Organs and Tissues

Eye



(A) retina; (b) optic nerve; (c) lens; (d) iris; (e) cornea; (f) corneal epithelium; (g) choroid gland

Cornea

The cornea of the fish eye is similar in its refractive index to water. Its layers are comprised of an epidermal conjunctiva with a basement membrane, a dermally derived substantia propria with an internal basement membrane, and an endothelial layer called Descemet's membrane and endothelium.

Lens

The lens is a spherical ball consisting of three tissues. First, an encapsulating sheath of non-cellular transparent material, which is secreted by the second tissue, an underlying layer of cells which are nucleated and capable of both division and secretion. The third tissue, immediately beneath these cells consists of the lens fibers, which constitute most of the lens volume. These fibers are long, slender, transparent, non-nucleated cells lying in layers of long parallel rows. When the lens is dissected, these layers of cells resemble the layers of tissue in an onion. Each layer is one cell thick and is loosely cemented to the layer above and below it. Whereas the mammalian lens is elastic, and can be modified

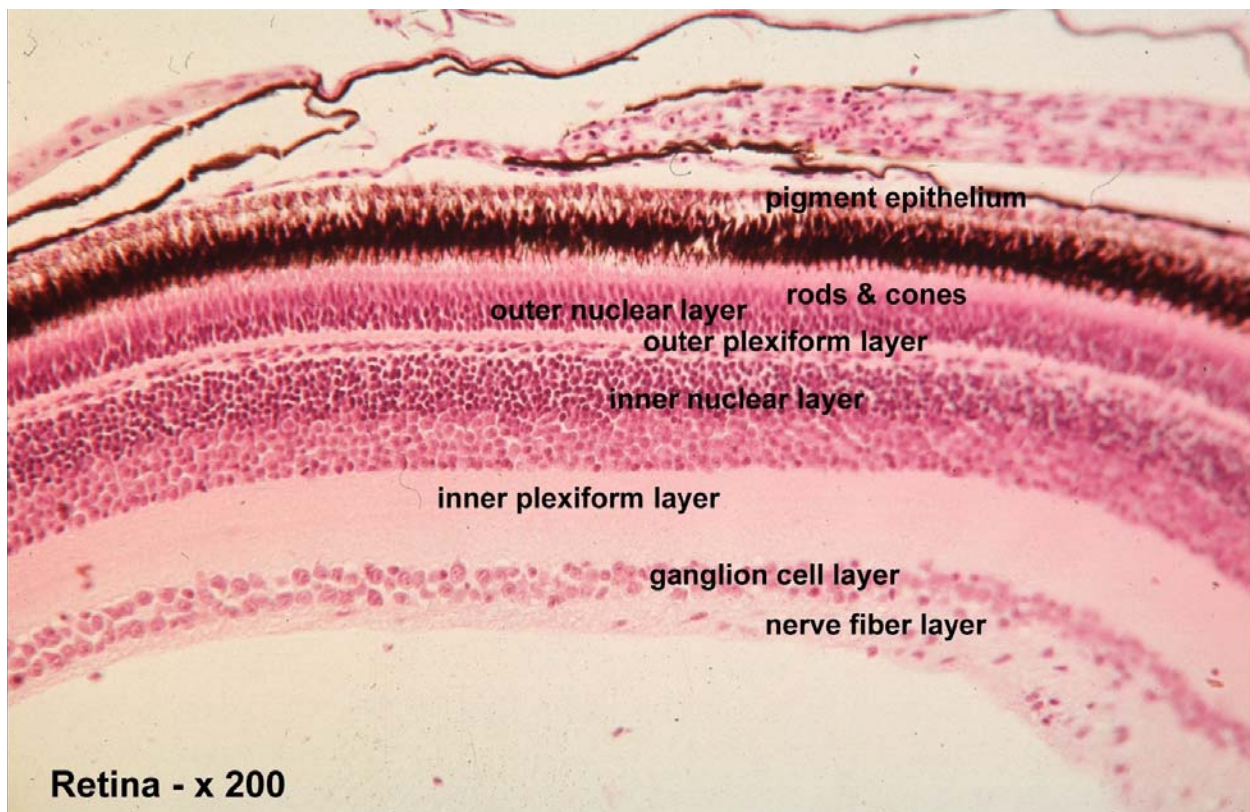
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in shape by contraction of the eye muscles, the fish lens is inelastic and must be drawn inward toward the retina by the retractor lentis muscle to accommodate changing sight requirements. The small degree of accommodation possible in the teleost is achieved by this action of the retractor lentis muscle.

Retina

The retina, the light sensitive tissue, is generally organized as in other vertebrates with internal nervous tissue layers, overlying rod and cone receptor cells, and a black pigmented layer found peripherally. These make up a total of eight specific layers in the retina. The pigmented epithelial layer controls the amount of light which reaches the visual elements beneath it including the ability of needle-like pigment granules to migrate and form fingerlike processes which extend downward into the visual layer. The visual layer of rods and cones consists of three types of receptors: twin cones, single cones, and rods. The nuclei of the cones are large and spherical, whereas those of the rods tend to be small and oval. The ganglion cell layer is composed of a narrow chain of granular, spherical cells surrounded by a fine connective tissue network.



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Choroid

The choroid vessels of the eye form a subscleral network of capillaries for nourishment of the retina. They are associated with the large choroid gland, a network of capillaries which is active in oxygen secretion and whose function is considered to be related to ensuring a high level of oxygen for the retina although it also has blood-monitoring functions.

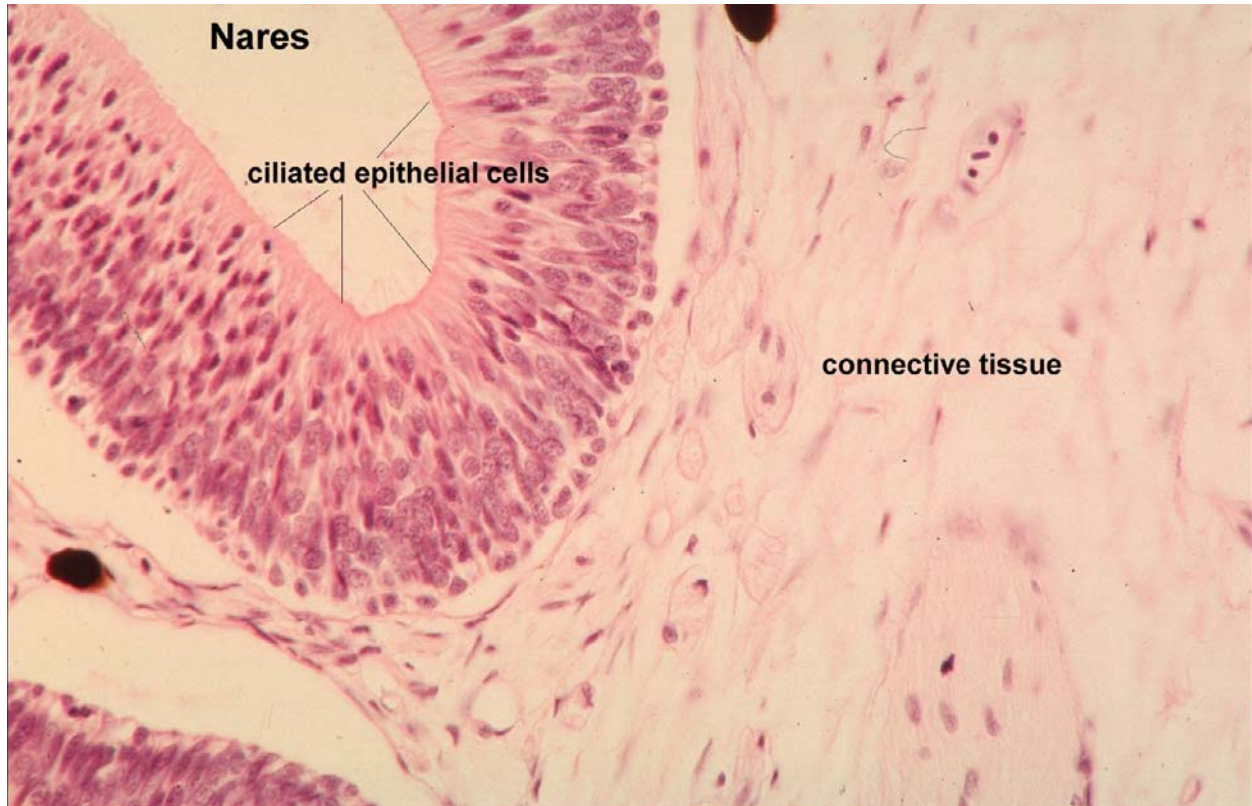


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Olfactory

The neurosensory cells, used in odor detection, are bipolar neurons. The cell body is located within the olfactory epithelium. The dendrite extends toward the surface, where it expands into a ciliated vesicle. The axon proceeds downward to penetrate the basement membrane. The typical ciliated columnar cell has its enlarged ciliated end reaching the surface and the opposite end tapering to a fine process. The mucous cells are less abundant and irregularly distributed. Wandering lymphocytes and macrophages are frequently seen in various areas of the epithelium.

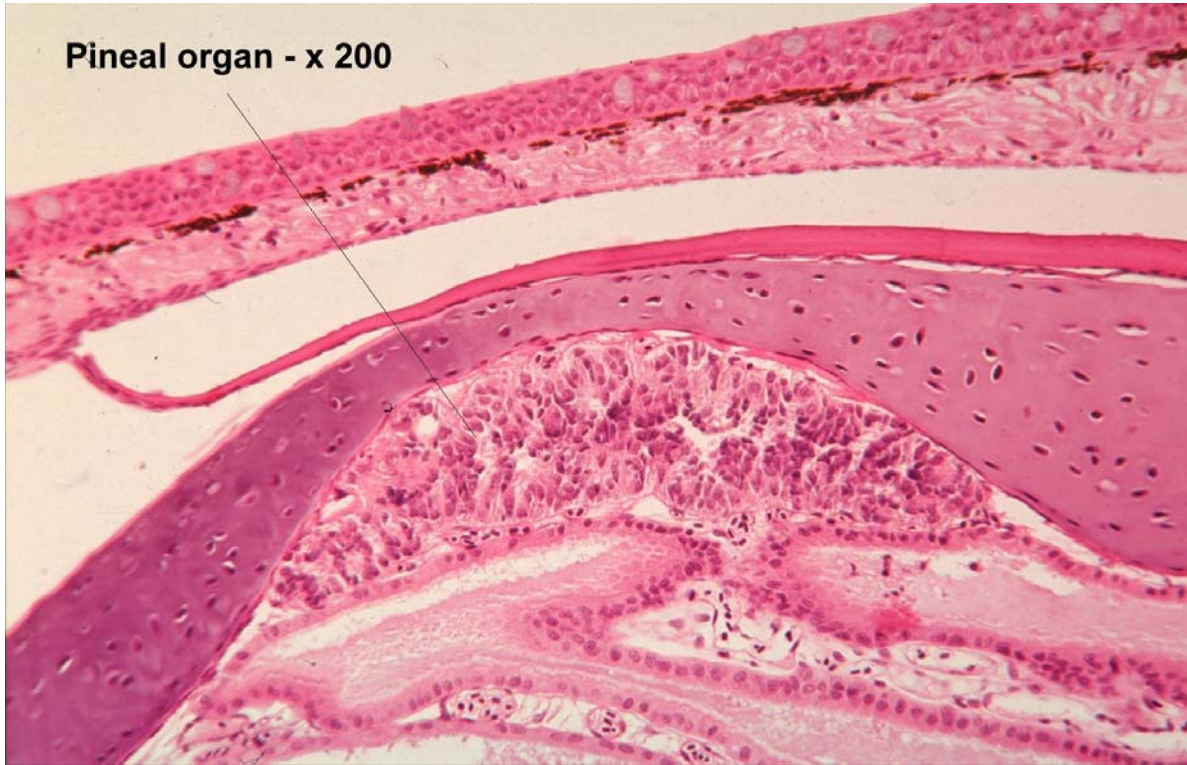


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Pineal Organ

The pineal organ is anterior to the midbrain and dorsomedial to the forebrain. In most species of fish the organ is tubular or sac-shaped. In young salmonids it is visible through the translucent cartilage of the cranium. It is generally believed that the organ is photosensitive, and presumably of greater importance to the younger trout. It has been shown that the pineal organ has a secretory function in some other fish species.



References

Ferguson, Hugh. Systemic Pathology of Fish, A Text and Atlas of Comparative Tissue Responses in Diseases of Teleosts. Iowa State Univ. Press. Ames, Iowa. 1989.

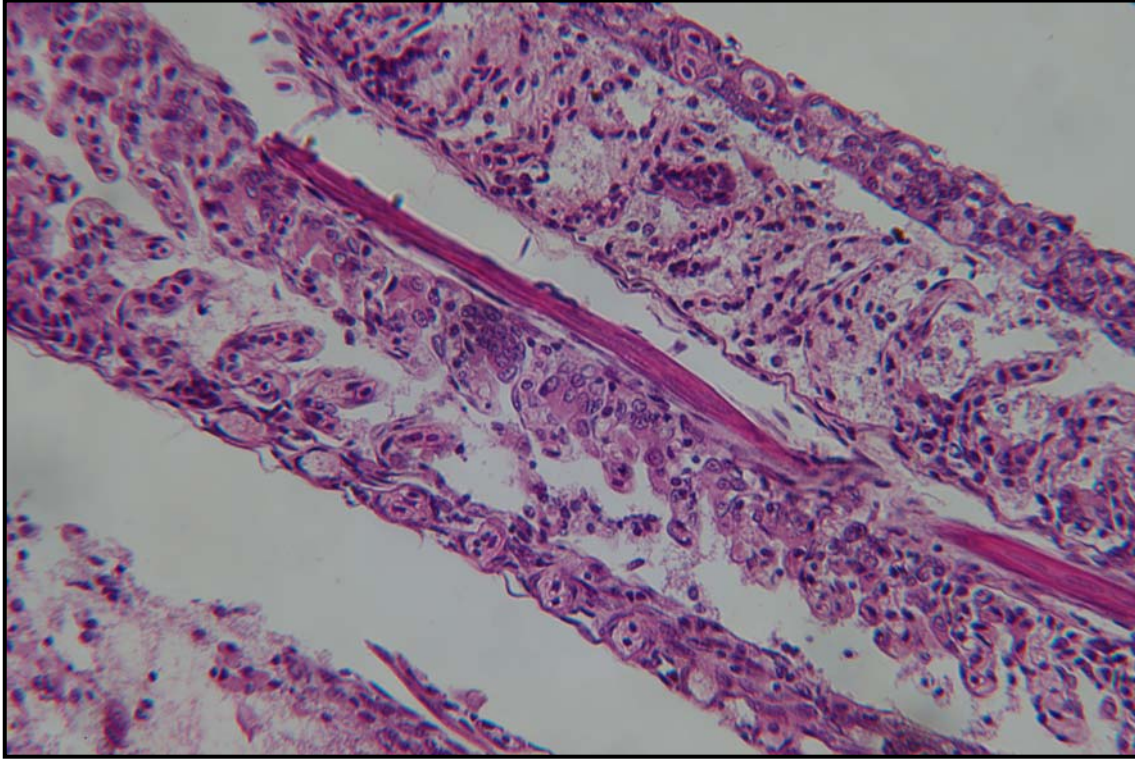
Roberts, Ronald, ed. Fish Pathology. 2nd ed. Bailliere Tindall, London, England. 1989.

Yasutake, William and Wales, Joseph. Microscopic Anatomy of Salmonids: An Atlas. USFWS. Washington, D.C. 1983.

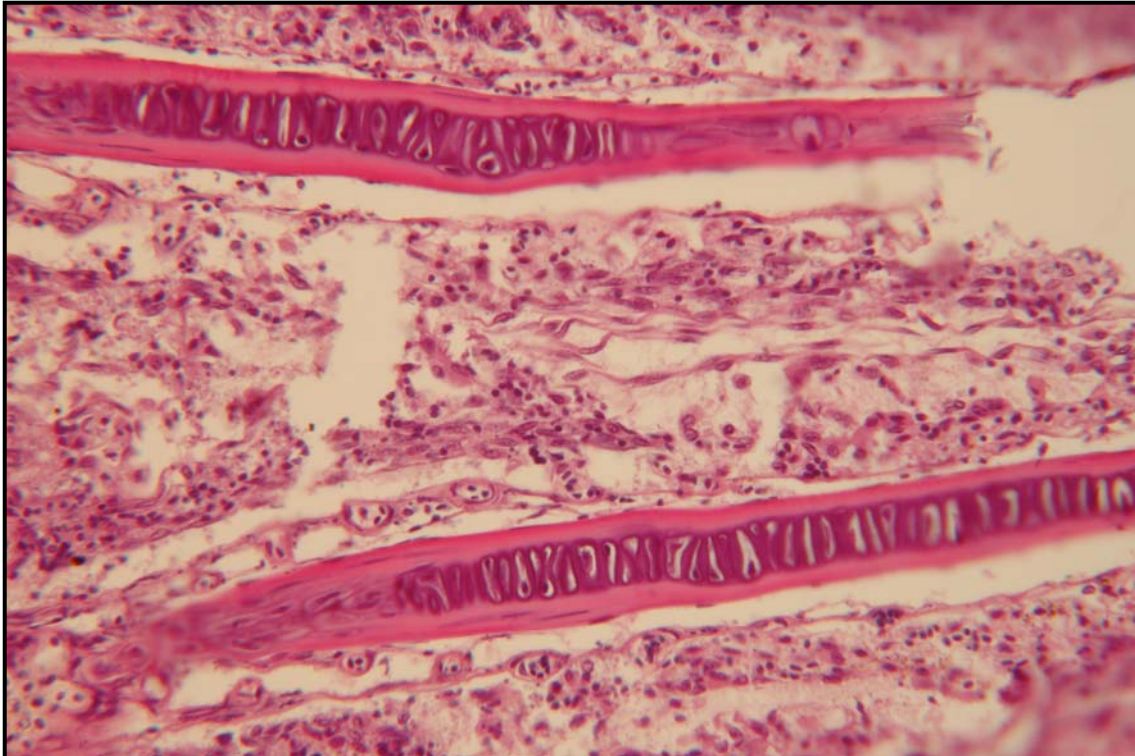
Artifact

1. Prefixation artifacts

Tissues autolyzed prior to fixation

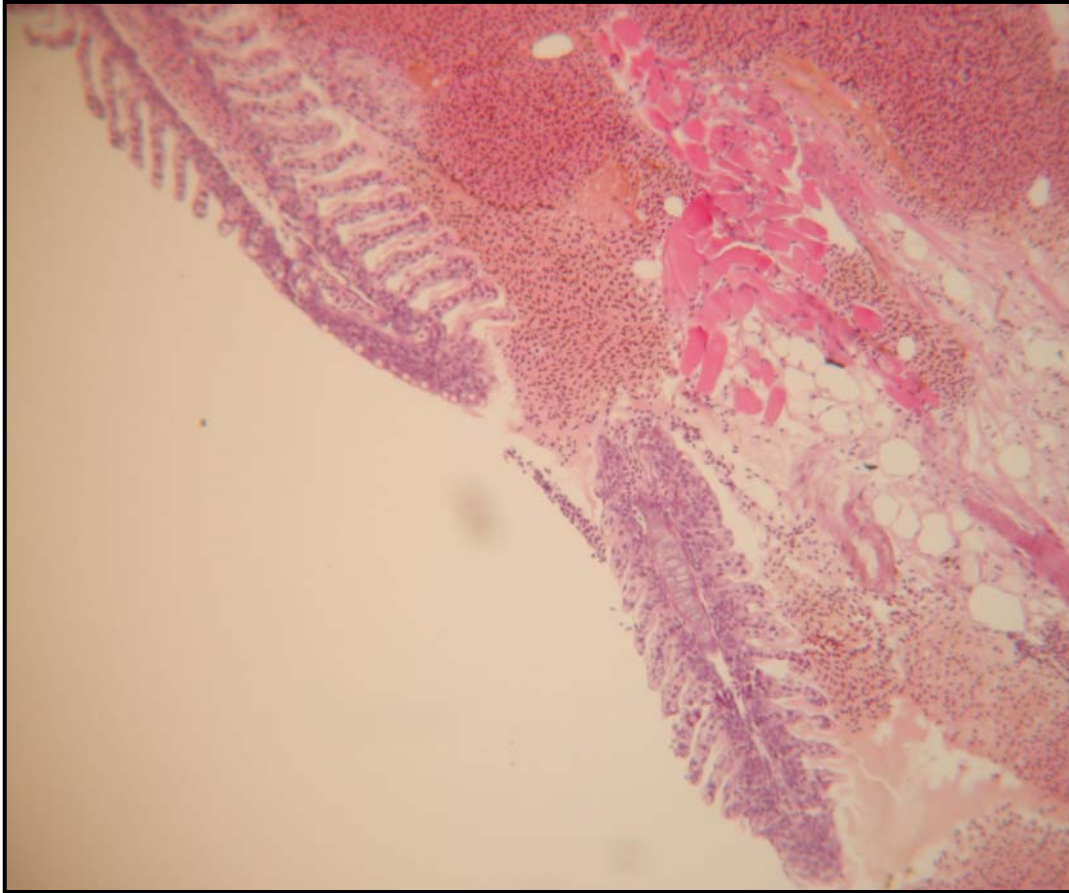


Tissues frozen prior to fixation

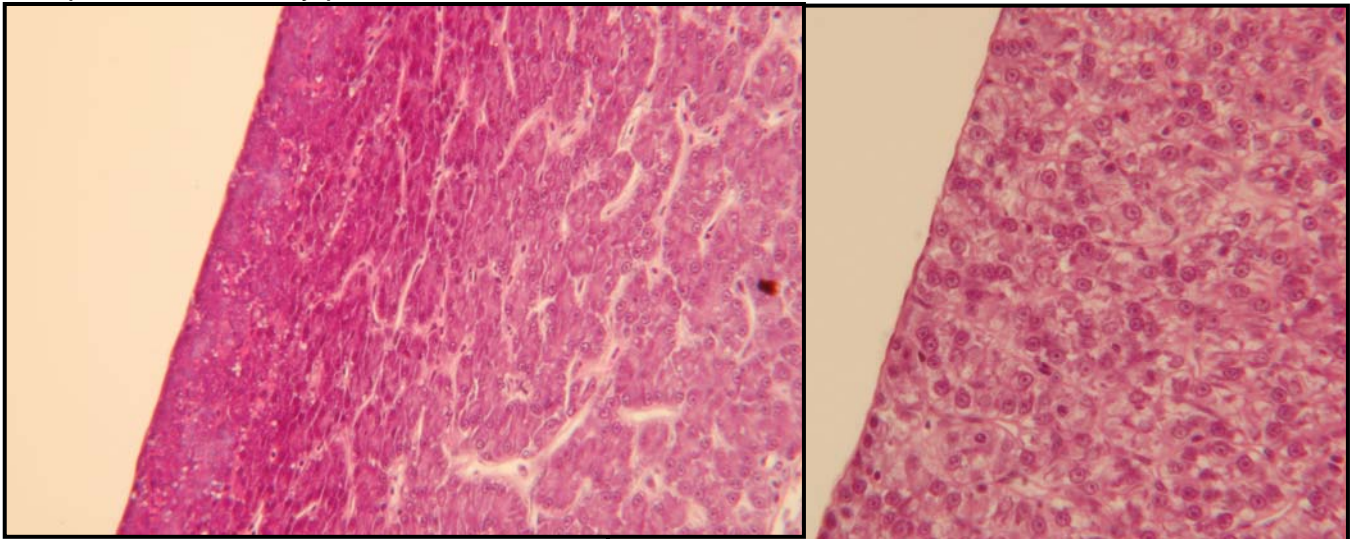


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Fish Histology and Histopathology

Traumatic Sample Collection



Sample allowed to dry prior to fix

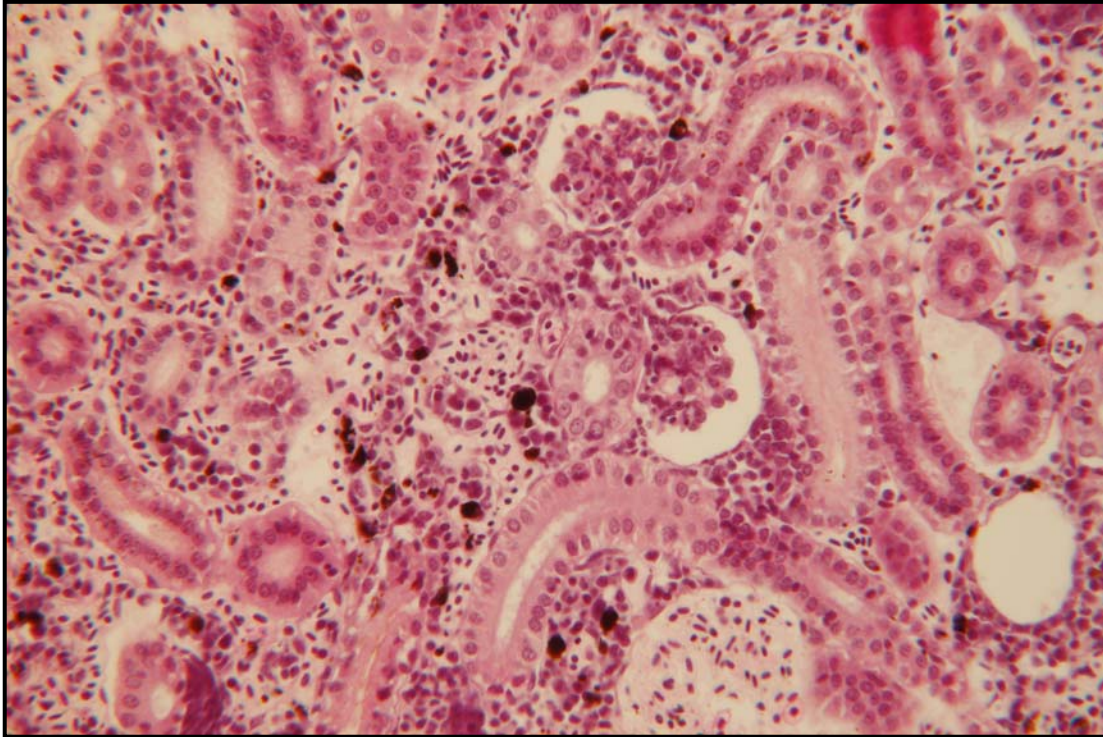


Dry

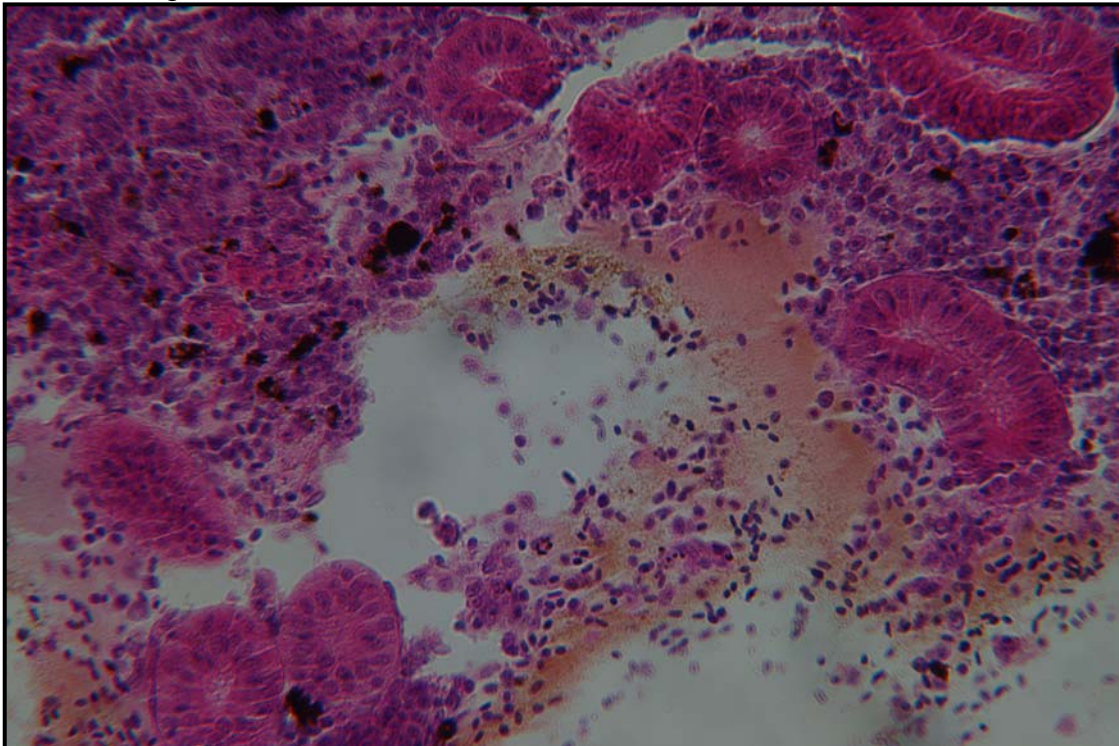
Control

2. Fixation artifacts

Inadequate fixation (tissues too thick)

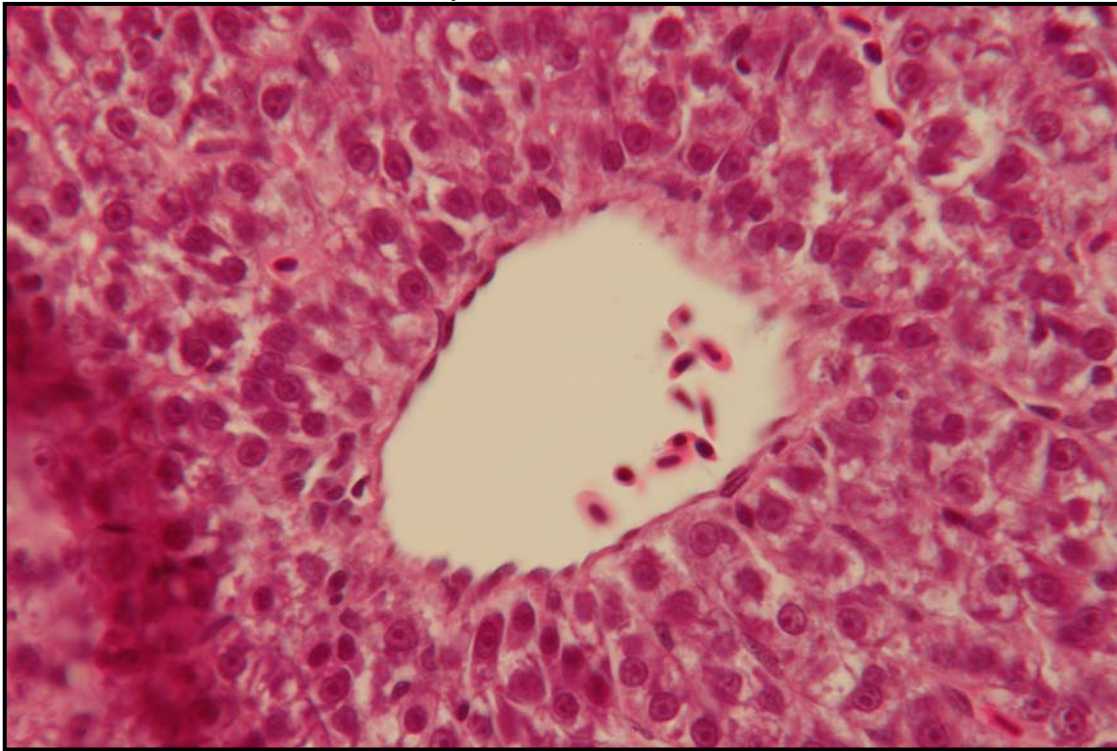


Formalin Pigment

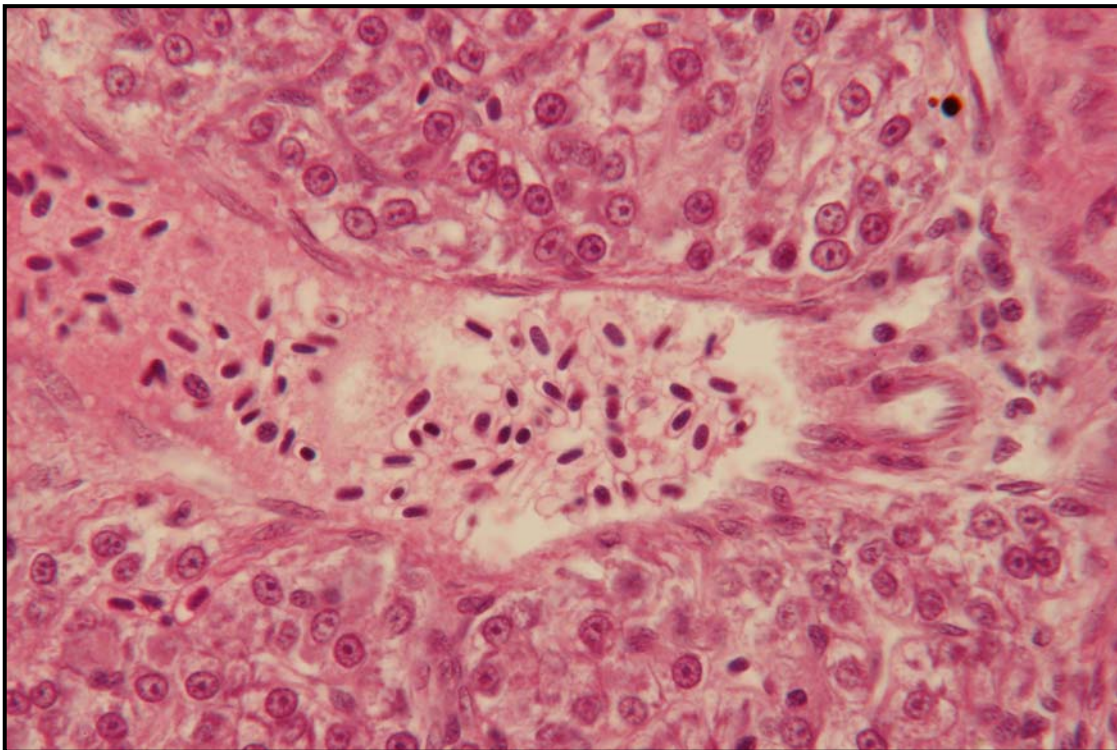


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Acetic Acid in Davidsons destroys RBC's



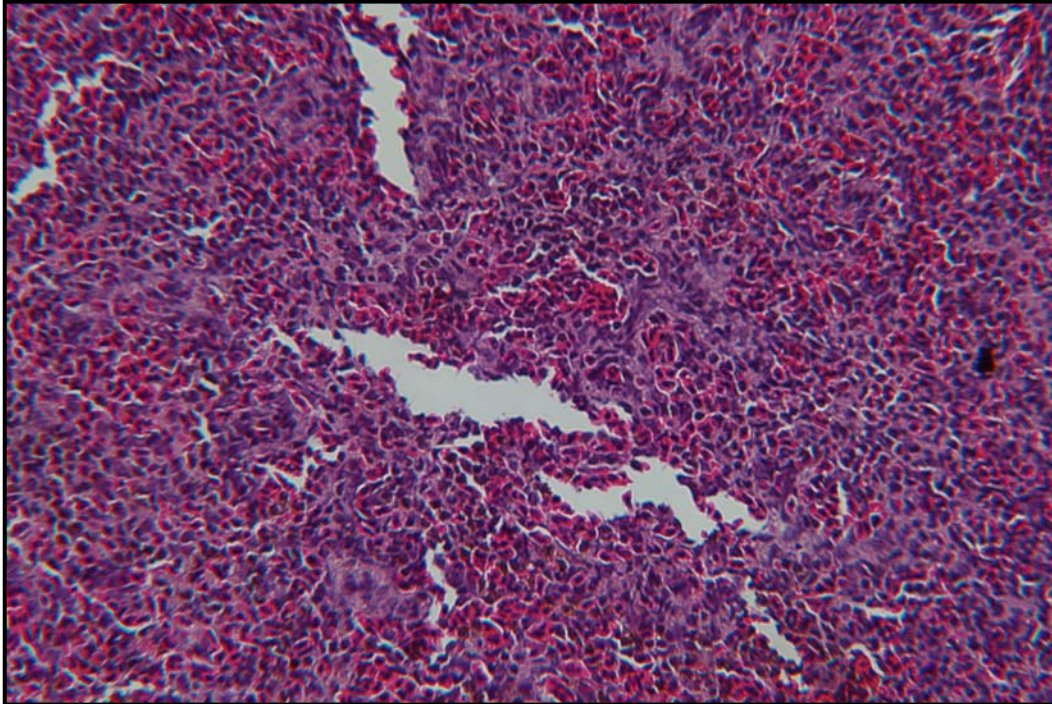
Fixed in Davidsons



Fixed in formalin

3.Processing Artifacts

Parched Earth Effect - Improper processing; Flootation bath too hot

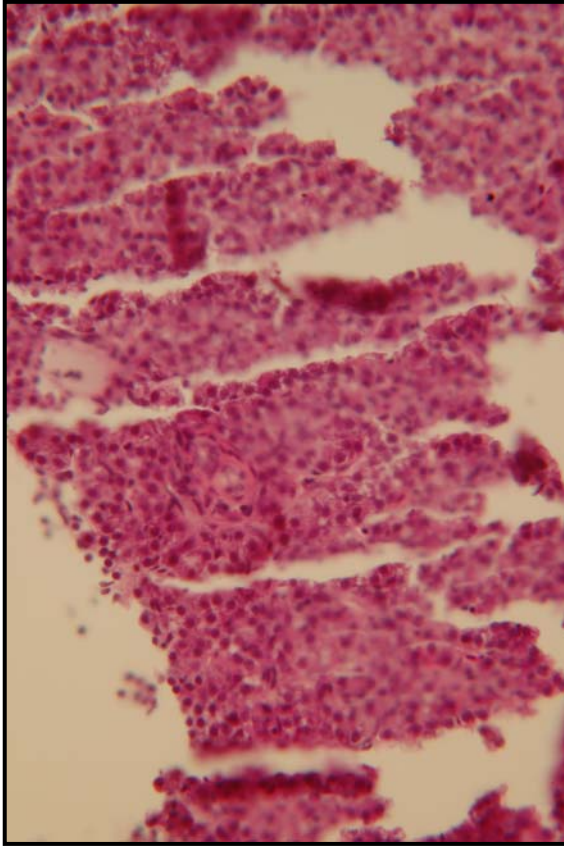


Wrinkling in various directions - From inadequate paraffin impregnation

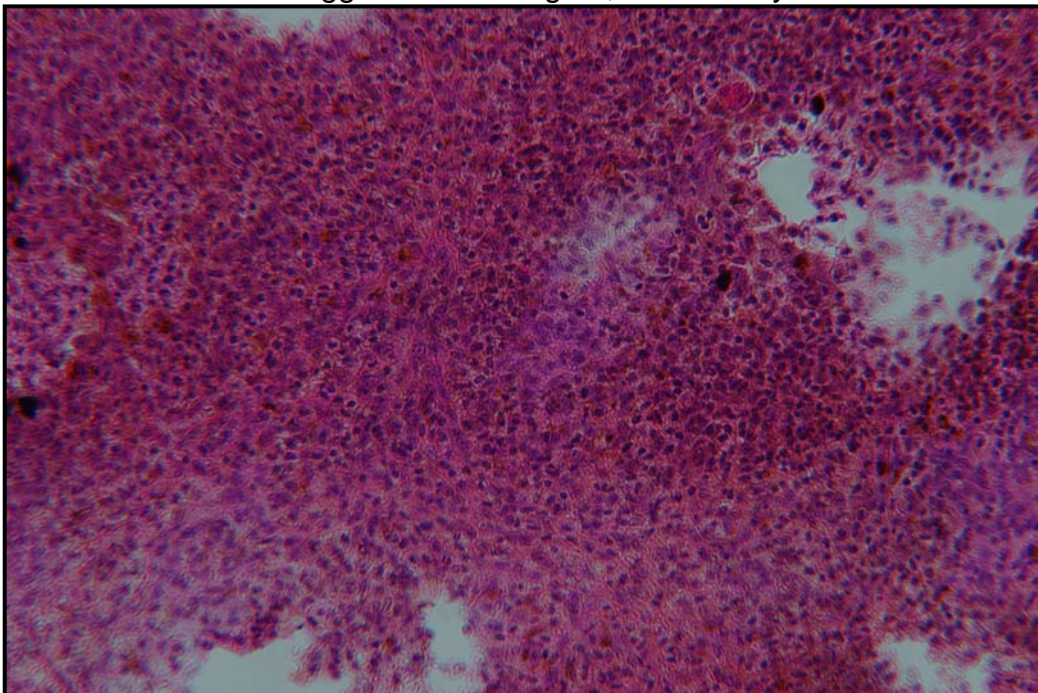


4. Microtomy Artifacts

Microvibrations (lack of rehydration)

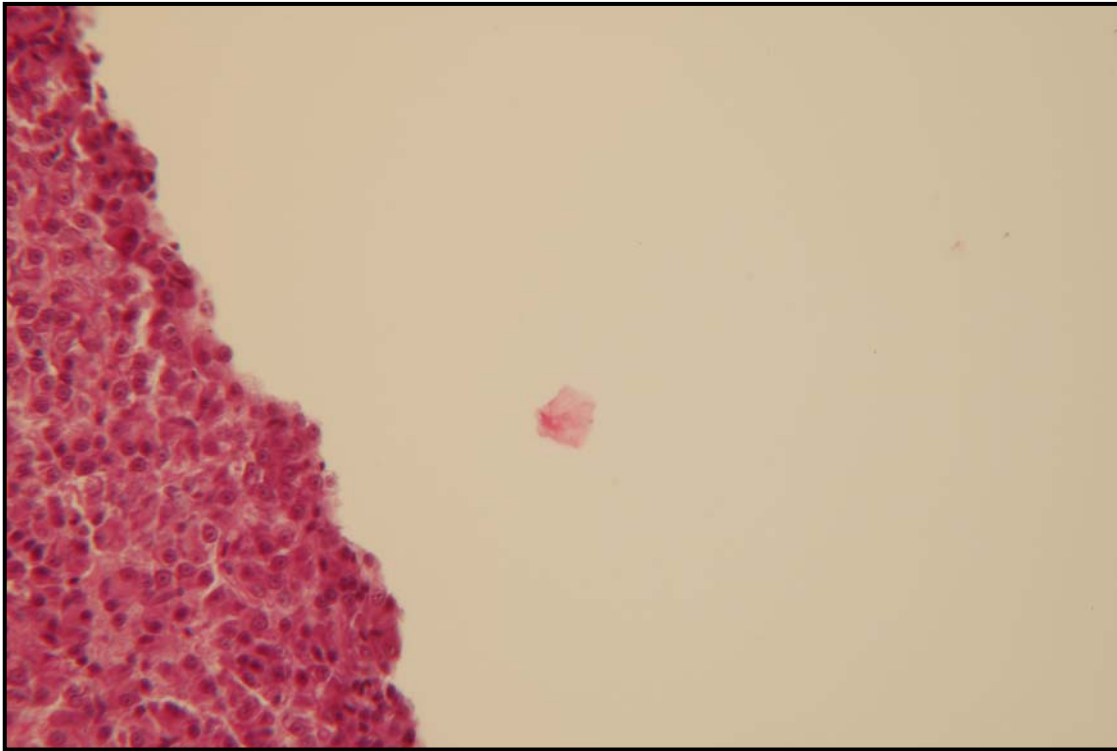


“Moth eaten effect” - Aggressive Facing off; lack of rehydration

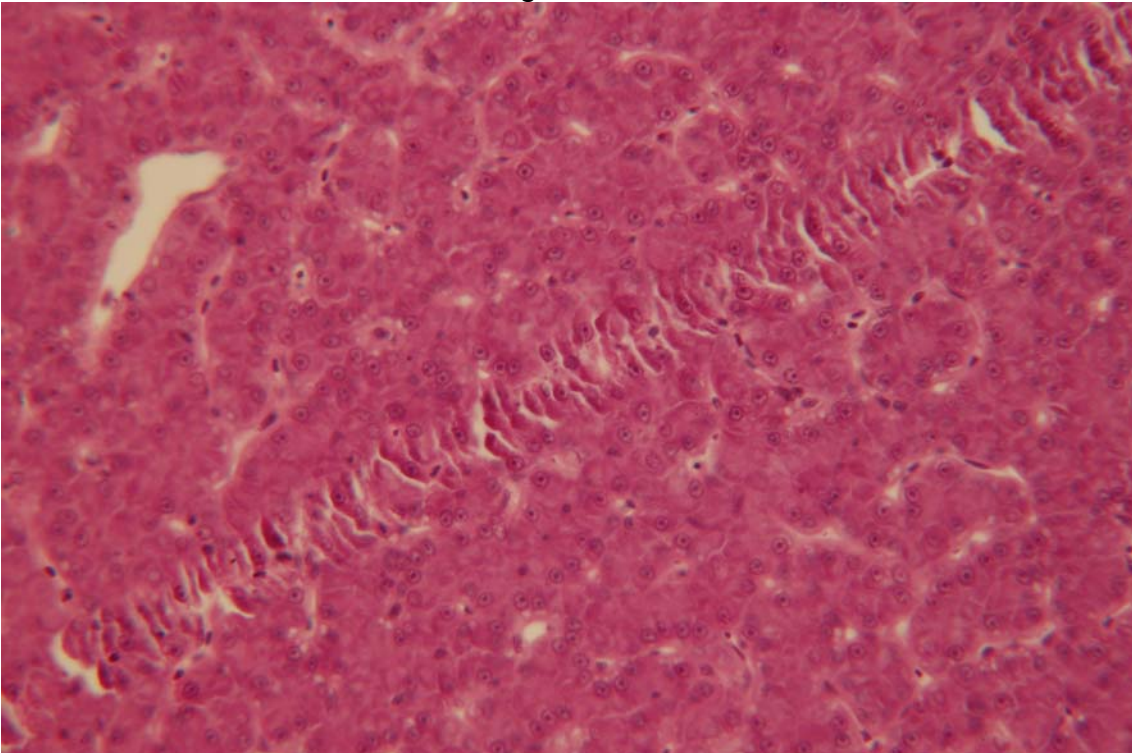


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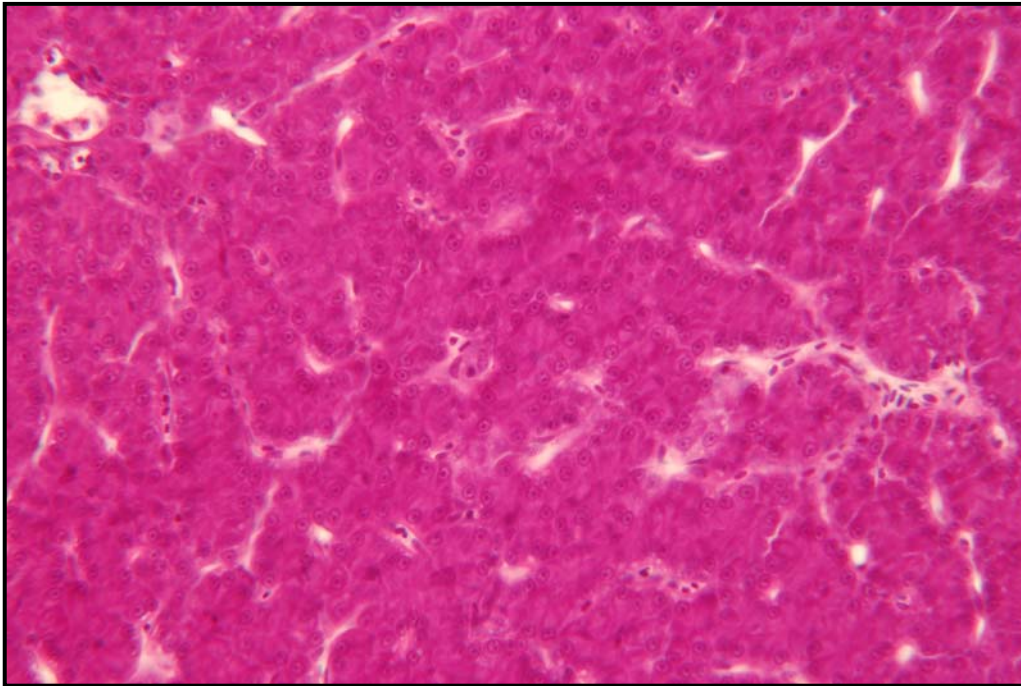
Dandruff



Nick in Microtome - Blade, Something Hard in Tissue

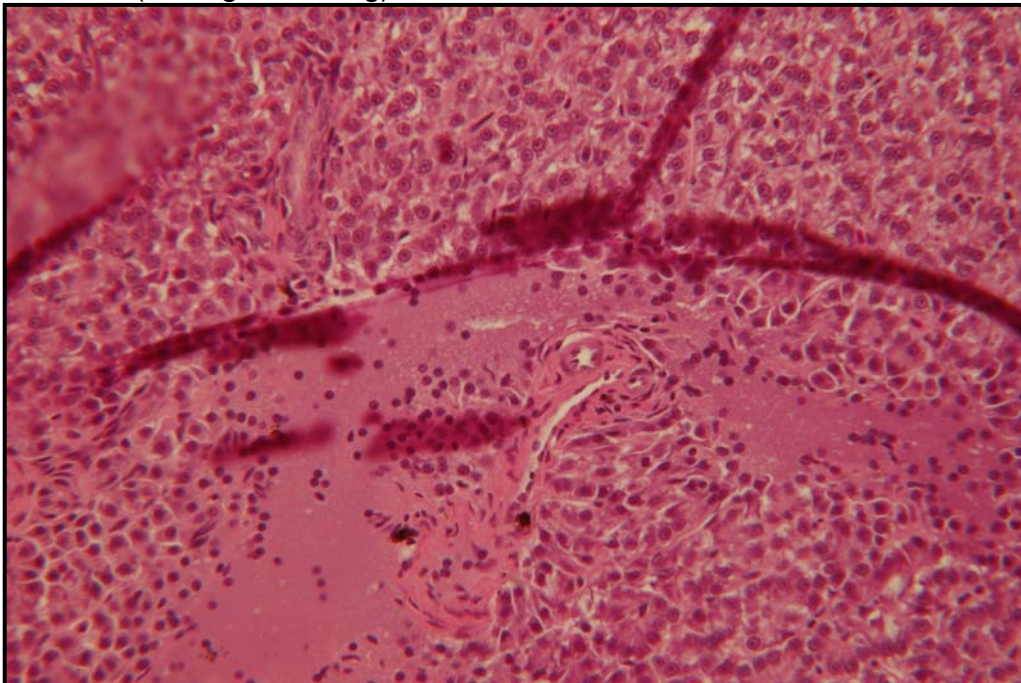


Section Too Thick



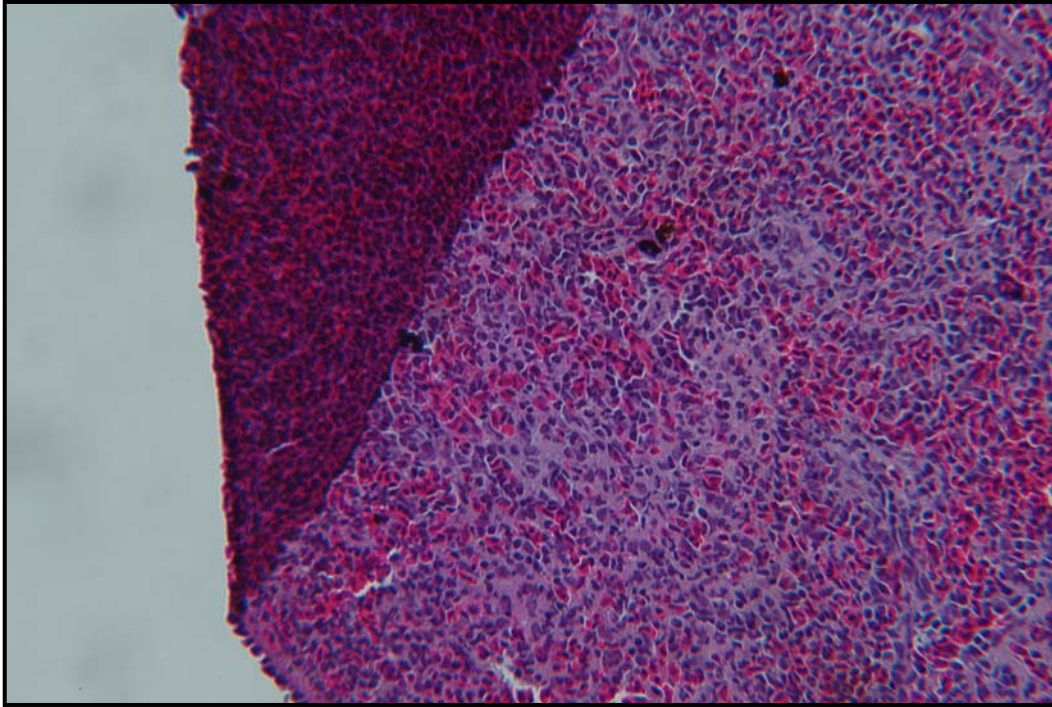
5. Flootation Bath Artifacts

Too cold (folding, wrinkling)

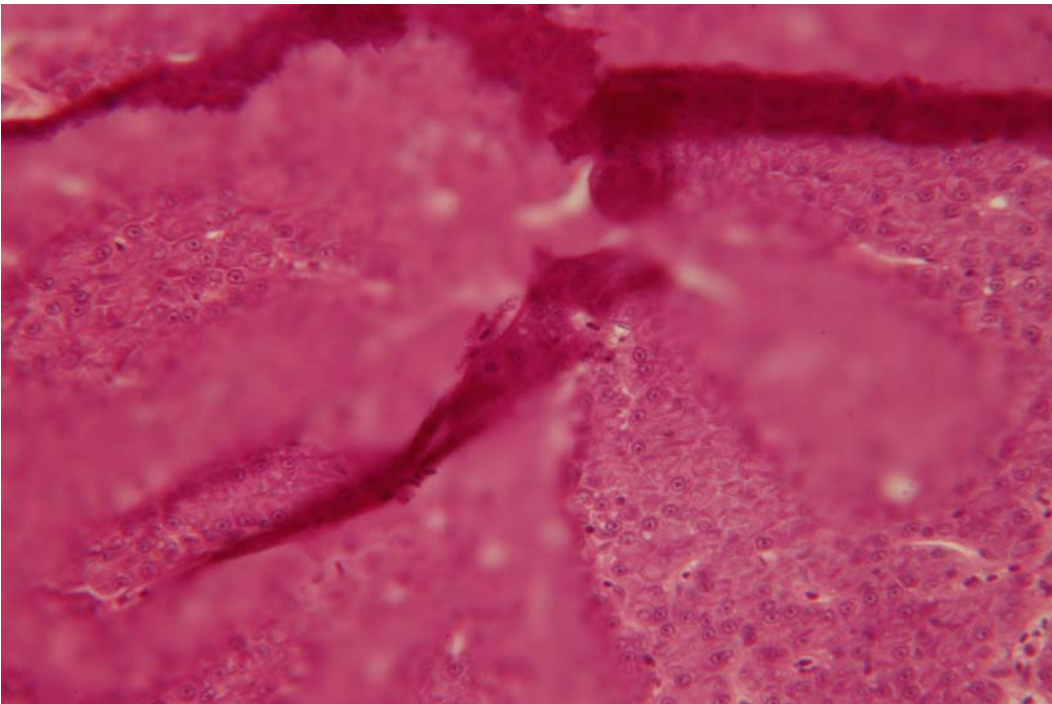


Chapter 3 - Artifact
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5. Floatation Bath Artifacts Continued – too cold (folding)

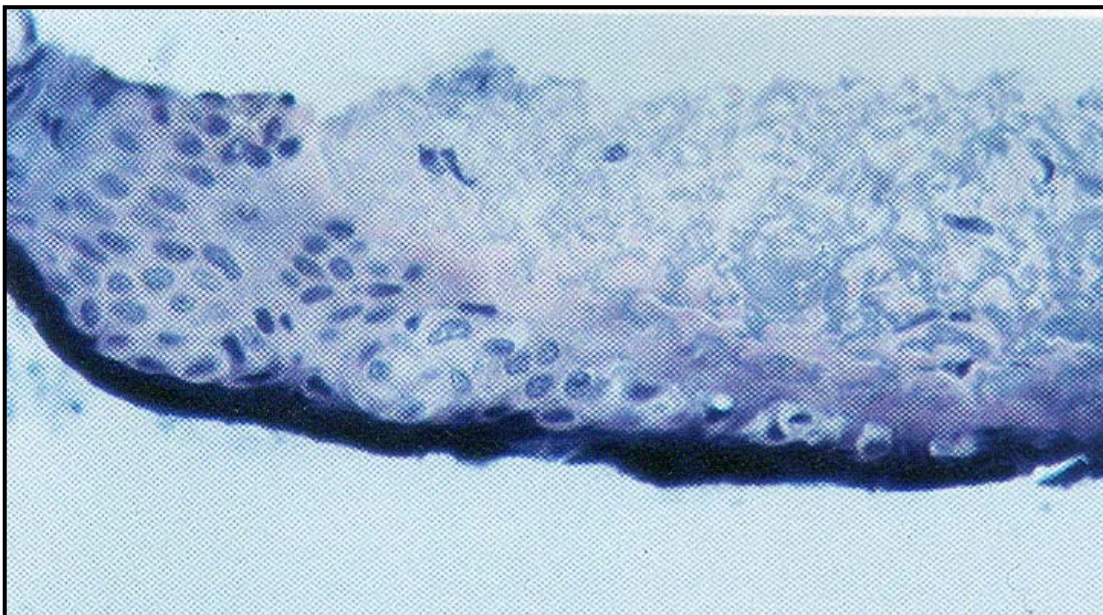
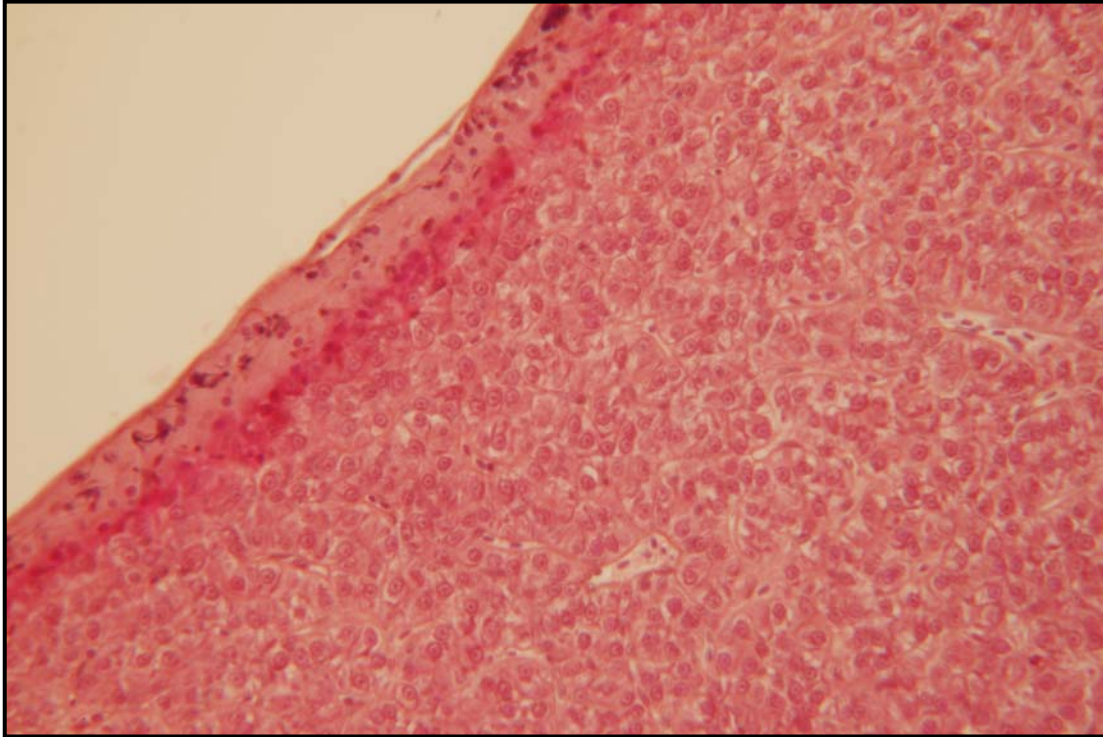


Bubble under section



6.H&E Artifacts

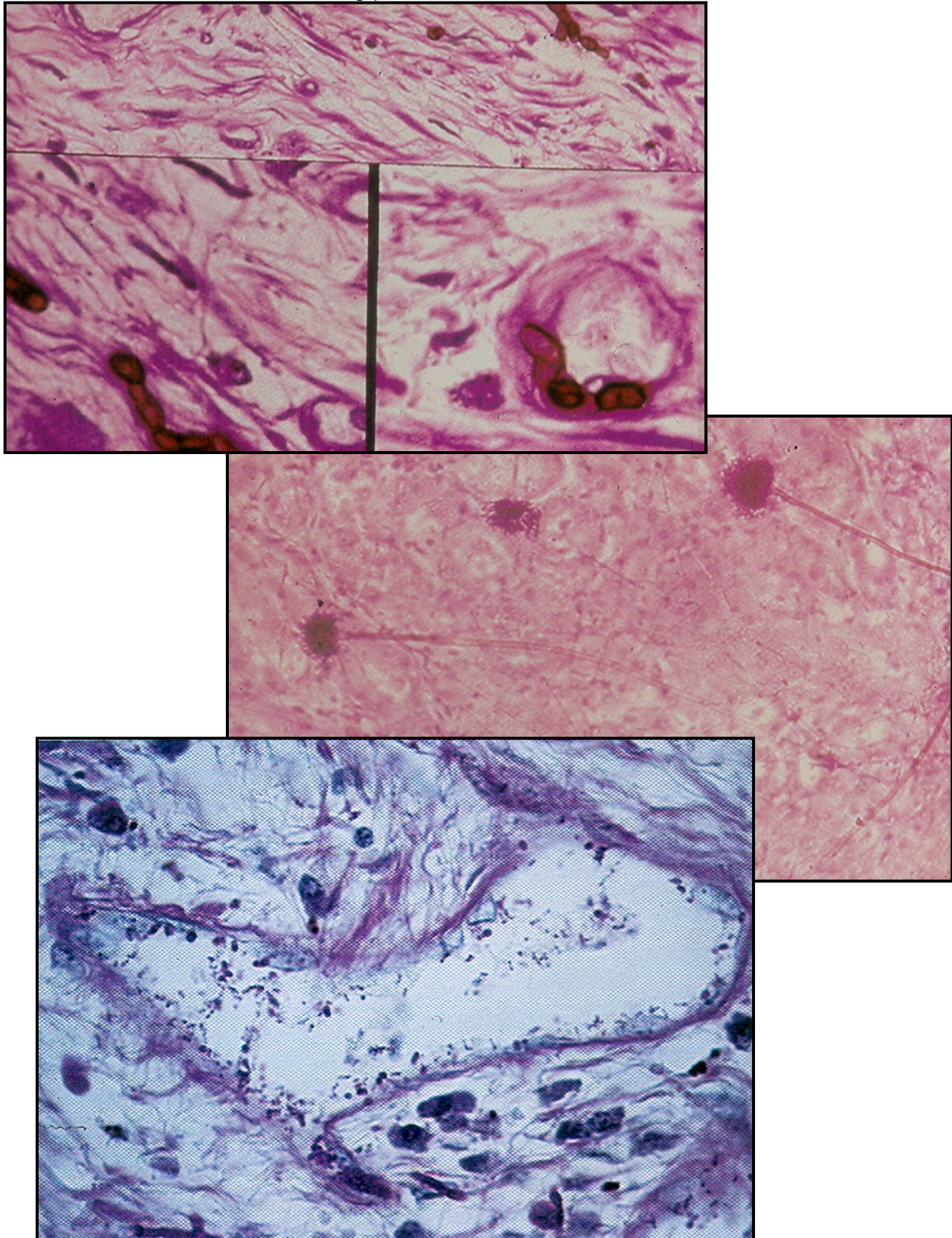
Overoxidized hematoxylin is used



From Luna

7. General Staining Artifacts

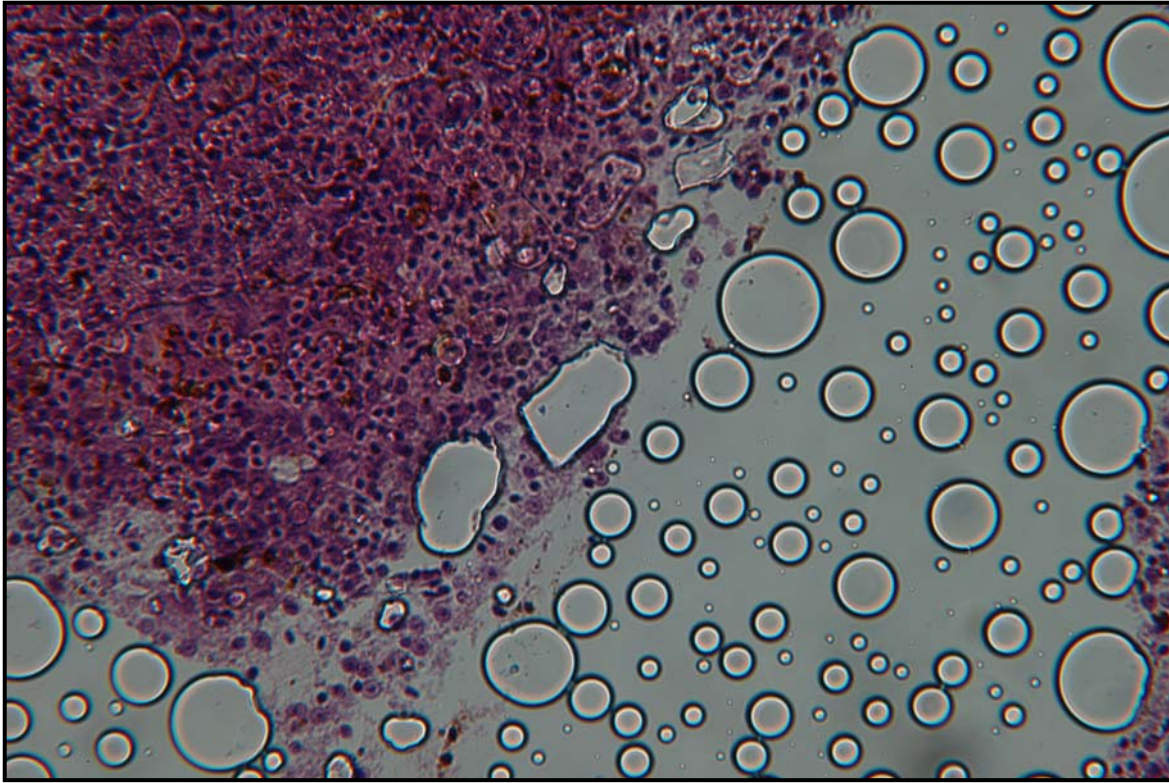
Microbial contamination in staining process



Chapter 3 - Artifact

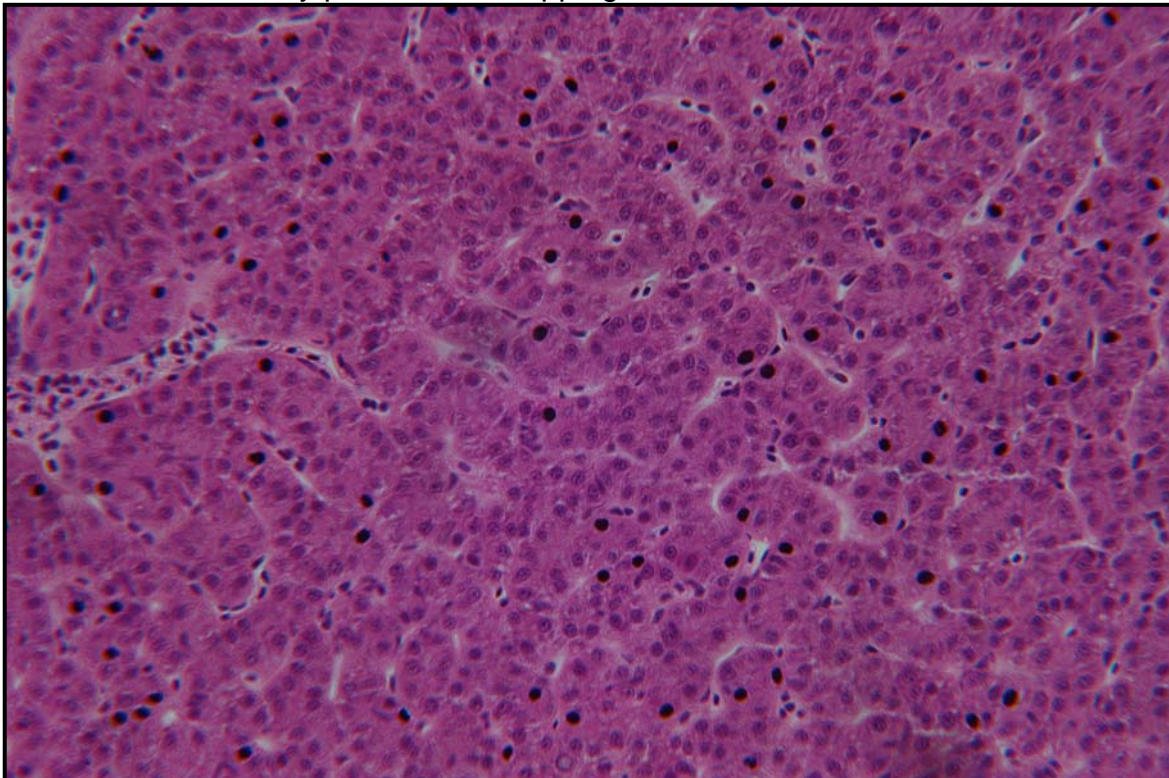
Fish Histology and Histopathology

Poor dehydration and clearing of stained sections, incompatible mounting media and clearant



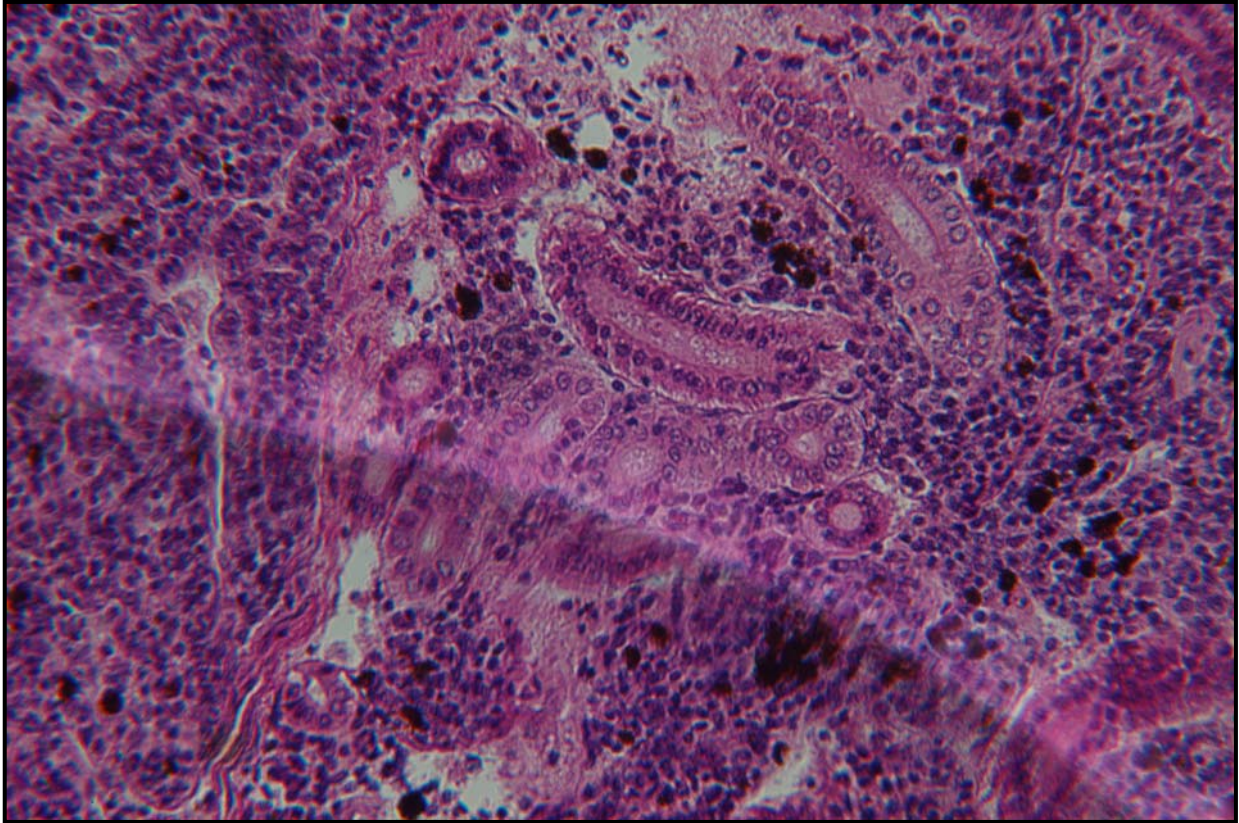
8.Coverslipping Artifacts

Section allowed to dry prior to coverslipping

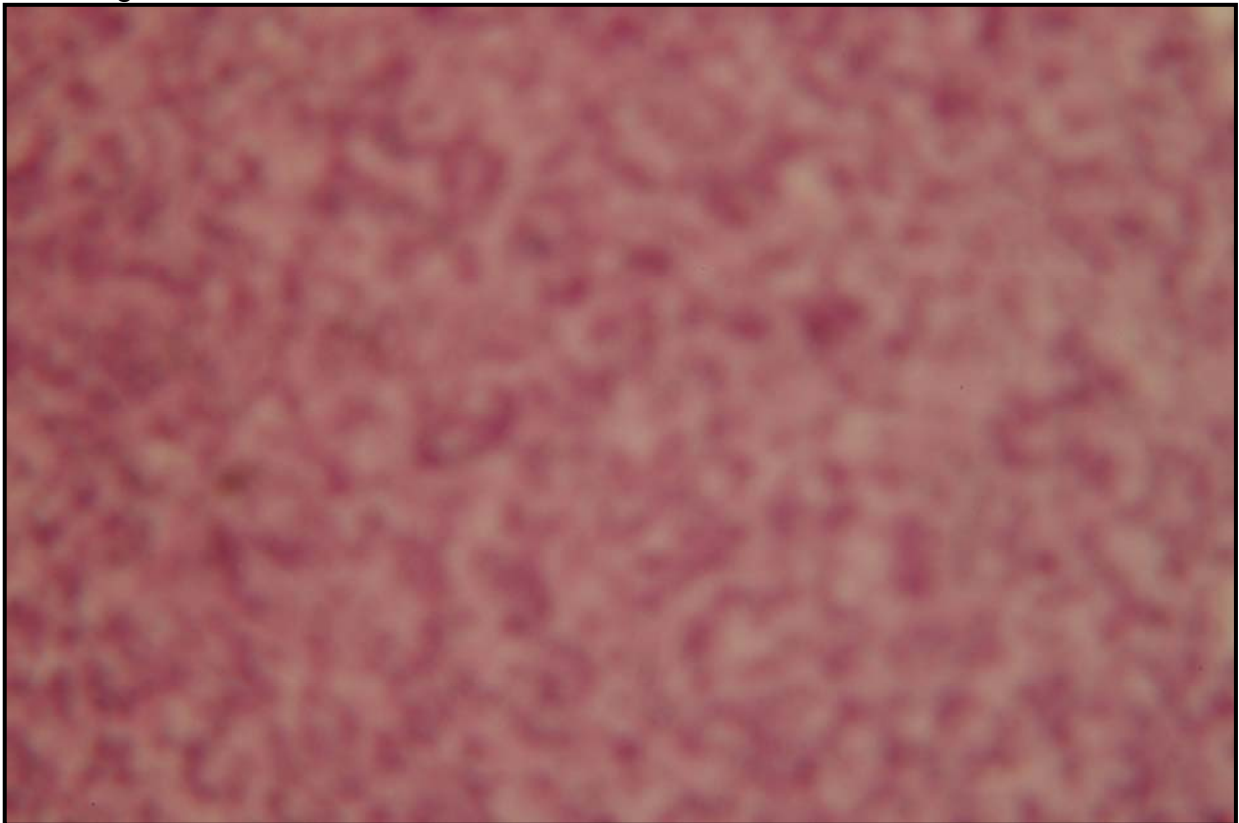


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Mounting media on top of coverslip



Mounting media too thick



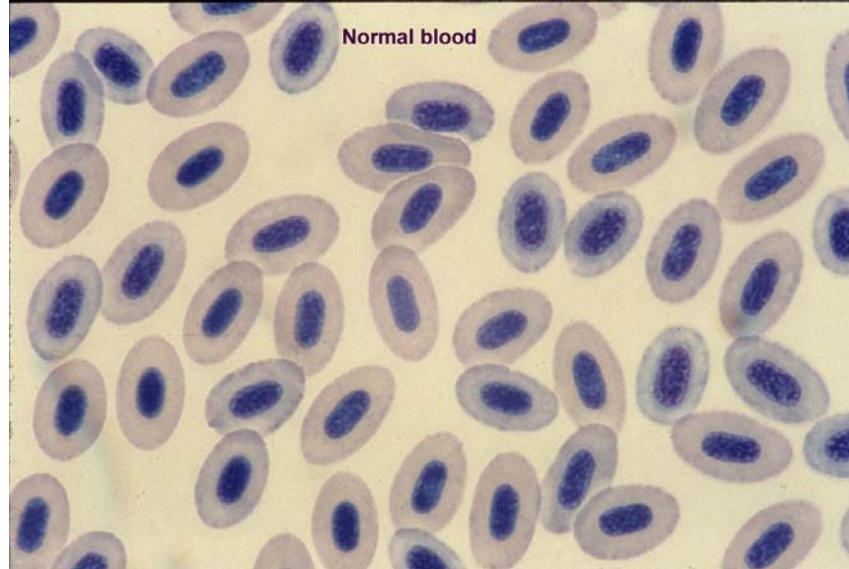
9. Tips on Artifact

If something does not fit with the “global picture” consider artifact as one of the possibilities

Blood and Blood Cell Development

A. Peripheral Blood

1. ERYTHROCYTES



a. The teleost erythrocyte is similar in size, tinctorial properties and ultrastructure to that of the other vertebrates but, like the avian and reptilian erythrocyte, it is nucleated. Hemoglobin is, as in other genera, the main vehicle for transport of oxygen and to a lesser extent carbon dioxide, but unlike mammalian erythrocytes, where anaerobic metabolism predominates, in the teleost erythrocyte, cell metabolism is primarily oxidative phosphorylation, resulting in the production of ATP.

2. NEUTROPHILS

a. The term neutrophil, or polymorphonuclear leucocyte (PMN), is drawn from human histology. Since the granules are not necessarily neutral staining, and the nucleus may not be multi-lobed, in other species of animal the terms heterophil or, in fish type I leucocyte have been suggested but in view of its wide usage the term neutrophil is usually still in general usage.

b. Neutrophils have been identified in teleosts on ultrastructural and histochemical grounds. Good evidence of phagocytic activity such as is found in mammalian neutrophils is not available although they are commonly found at sites of inflammation. Neutrophils in fish are present in about the same numbers as in mammals but they comprise a much smaller proportion of the blood leucocyte population (about 6-8% in fish while they comprise 60-70% in mammals).

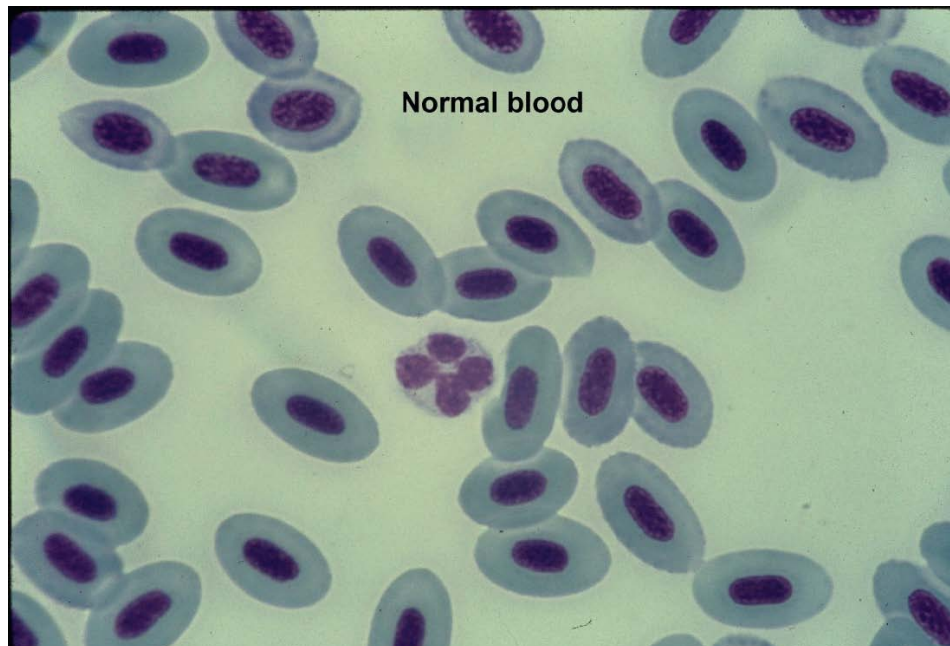
c. Morphologically fish neutrophils closely resemble their mammalian counterparts though the degree of nuclear polymorphism in teleosts varies considerably. The histochemical characters of plaice neutrophils have

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been extensively studied and in most respects they bear close resemblance to mammalian neutrophils, being positive with PAS stain, Sudan Black B and benzidine-peroxidase, acid and alkaline phosphatase tests. Release of neutrophils into blood causing a neutrophilia, is known to occur as a non-specific response to a variety of stress stimuli in mammals and fishes. This is probably mediated through the pituitary and renal axis.

d. The origin of teleost neutrophils is most probably the hematopoietic tissue of the kidney, though the spleen may play a minor role. In smears of teleost kidney, granuloblasts are seen in large numbers and may be characterized by their histochemical properties. These granuloblasts are similar in morphology and staining properties to their counterparts in mammalian bone marrow, the myeloblasts; and myelocytes. There is little information on the life span of teleost neutrophils but they probably have a rapid turnover time of about five days, as in mammals.



3. MONOCYTES

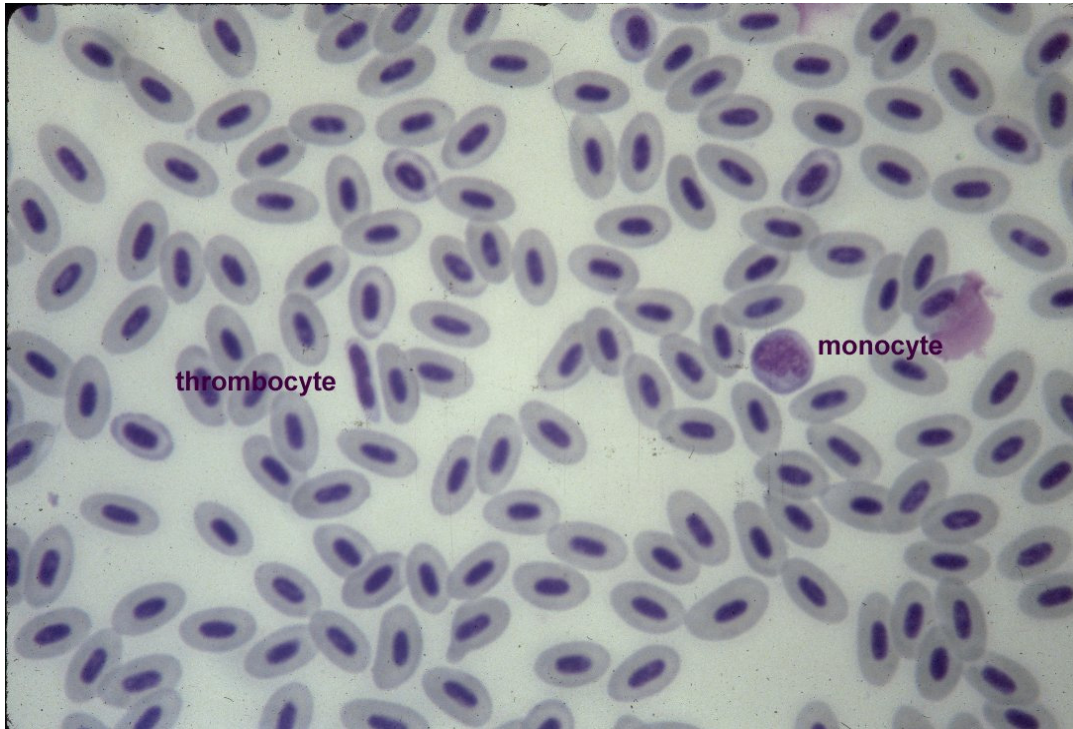
a. Monocytes are partially differentiated cells, which under appropriate circumstances will develop into mature cells of the mononuclear phagocyte system but are not capable of further division. In teleost fishes this system is organized as in other vertebrates, with circulating monocytes arising from renal hematopoietic tissue and being readily able to take up a functional tissue role.

b. Monocytes of fishes form about 0.1% of the circulating leucocyte population, though they increase in number for a short time after injection of foreign particulate matter like colloidal carbon. Morphologically they are very similar to mammalian monocytes which they also resemble histochemically, possessing a few fine scattered granules which stain positively with PAS and acid phosphatase.

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Fish Histology and Histopathology

c. Monocytes in fishes have been observed to take up foreign particulate material such as carbon, though their powers of phagocytosis are limited.

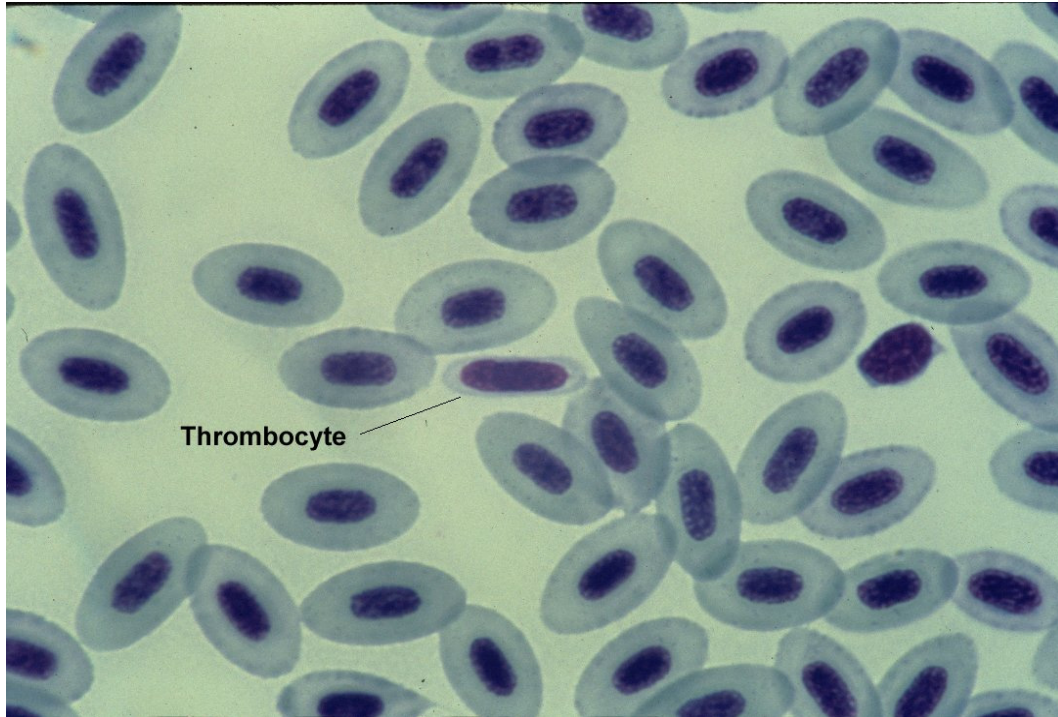


4. THROMBOCYTES

a. Thrombocytes are responsible for blood clotting and are important in preventing the loss of tissue fluids from a surface injury. Thrombocytes are found in all non-mammalian vertebrates. Typically they are elongated cells, often termed spindle cells, though most usually one pole of the cell is drawn out into a point. They clot readily and if care is not taken in the preparation of a blood smear the thrombocytes may cast off most of their cytoplasm and appear as small, densely staining nuclei, surrounded by a minute amount of cytoplasm. It is this spent thrombocyte which has been frequently confused with the lymphocyte. When observed in the living state by phase-contrast microscopy, a retractile vacuole can be seen at the base of the pointed end of the thrombocyte, just anterior to the nucleus. Acid phosphatase and PAS-positive material is associated with that same region.

b. The ultrastructure of the cytoplasm of the teleost thrombocyte has a remarkable similarity to that of mammalian platelets.

c. The difficulty in distinguishing spent thrombocytes from lymphocytes has led to much confusion regarding counts of these cells. Unless the thrombocytes are preserved in their mature, intact, pointed or spindle forms, then differential counts of these cells will not be reliable.



5. EOSINOPHILS

- a. Eosinophils are putatively considered to play a role in defense mechanisms in mammals by phagocytosing antibody/antigen complexes. They may therefore have an important role in maintaining homeostasis during infection and are particularly numerous when antigens are continually being released, as in parasitic diseases. In mammals, eosinophils comprise only 1-3% of blood leucocytes, though their numbers are subject to certain factors like hormone levels.
- b. Eosinophils are characteristically packed with large retractile granules which have a high isoelectric point that is to say they stain with Acid dyes like eosin in alkaline medium.
- c. The literature concerning the presence and nature of eosinophils in fishes is notoriously confused, with many claims, both of their presence and absence, often in the same species. They are normally reported to be rare in fish blood. Most of the descriptions of eosinophils in teleosts refer to the eosinophilic granular cells found in the skin, hematopoietic and digestive tissues, which are almost certainly distinct from the true blood eosinophil. The only criterion for identifying the eosinophil of fishes has been the presence of fairly large eosinophilic cytoplasmic granules.
- d. Fish eosinophils have been implicated in inflammation and some reports of phagocytic activity exist. For example, phagocytosis of bacteria by eosinophils in goldfish and guppies has been reported, while phagocytosis of carbon particles has been claimed and denied.

6. BASOPHILS AND MAST CELLS

- a. The basophils of vertebrates are uncommon granular leucocytes, characteristically containing large basophilic metachromatic granules similar to those of mast cells. The function of basophils is not clear and though they contain histamine in resemblance to mast cells, their relationship to tissue mast cells is not established.
- b. Like eosinophils they are affected by hormones from the adrenal gland and also seem to be involved, in an as yet undetermined way, in allergic and stress phenomena.
- c. The presence of basophils in fishes is, like that of eosinophils, claimed by some workers and disputed by others. Affirmative reports of their presence liken them to the basophils of mammals in their morphology and staining reactions. This cells has not, as yet, been implicated in any recognized defense mechanism in the fish.
- d. The cells designated as mast cells in fishes have been identified solely on the grounds that they have, in common with mammalian mast cells, a connective tissue habitat and cytoplasmic granules which are basophilic and metachromatic, though recent work has shown that the metachromatic granular cells present in the dermis of plaice skin contain histamine.
- e. A property of fish mast cells observed by many workers is the liability of the cytoplasmic granules. It is generally considered that mast cell granules of fish were extremely soluble structures and that it is difficult to preserve them property as well as stain them property.
- f. In mammals the mast cells are mediators of anaphylaxis, causing the contraction of smooth muscle, dilation of blood vessels and increased vascular permeability. It is not at all clear that this phenomenon exists in fish, since attempts to produce anaphylactic-type reactions in fish have not been successful.
- g. In summary, though the presence of eosinophils and mast cells in fishes is disputed, they appear to be present in some species and probably are present in all species. In mammals the defensive role of these cells is coming to light only slowly and at the present time the functional role of so-called eosinophils and mast cells in fish can only be inferred.

7. LYMPHOCYTES

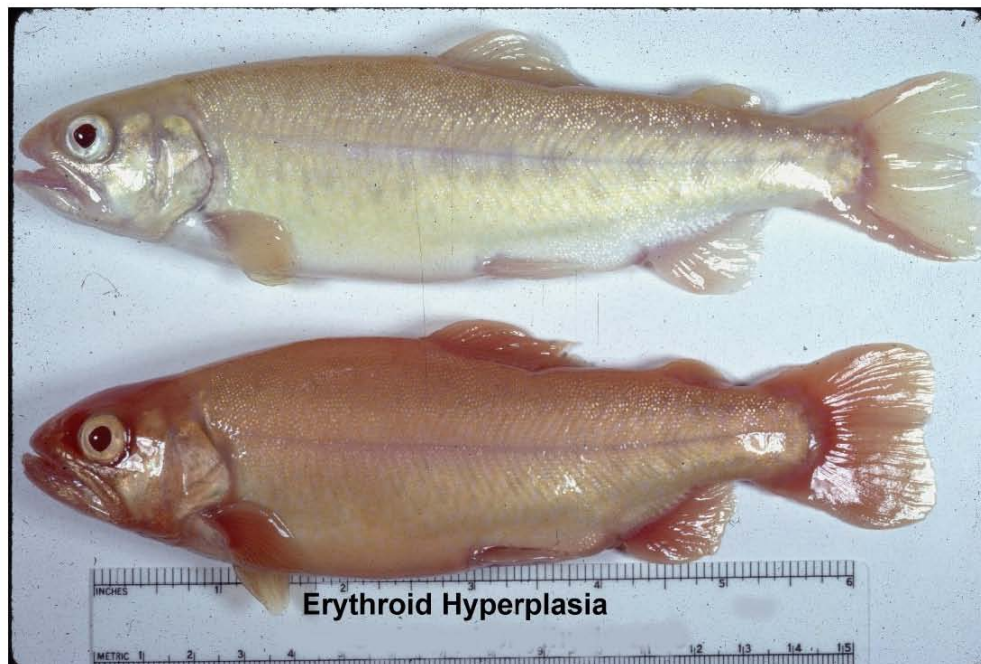
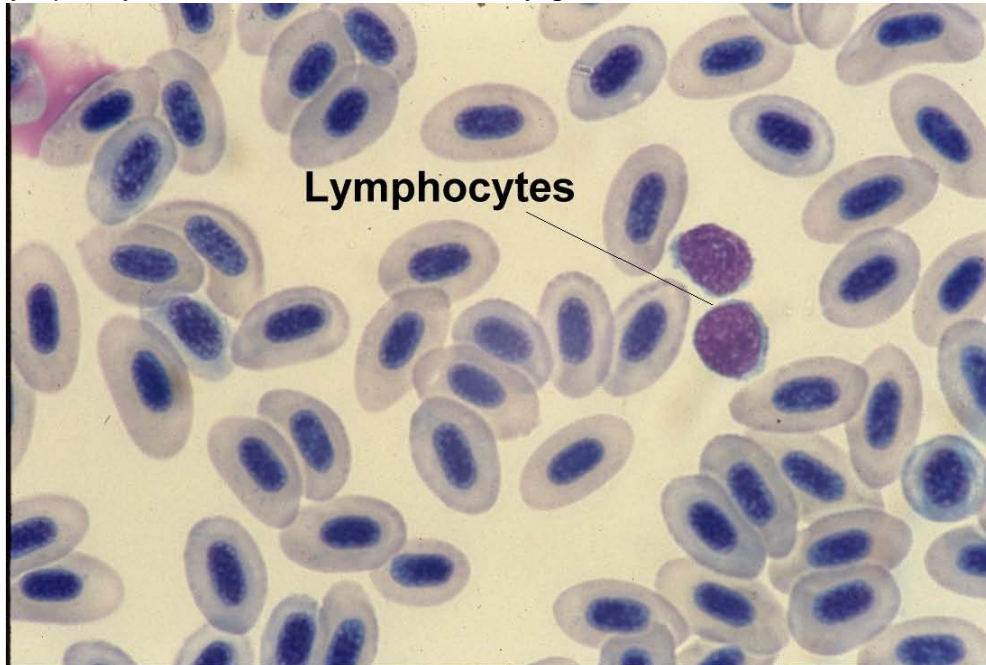
- a. The lymphocyte is the cells responsible for the immune response. The morphology of the lymphocyte is remarkably similar throughout the phylum Vertebrata.
- b. They are usually and arbitrarily separated into large and small categories, for reference, though they probably represent different functional stages of cells within populations of cells rather than a difference in functional capacity. The average size of small lymphocytes may differ between species, for example their diameters average 4.5 μm in plaice, 8.2 μm in goldfish and about 6 μm in man. The nucleus

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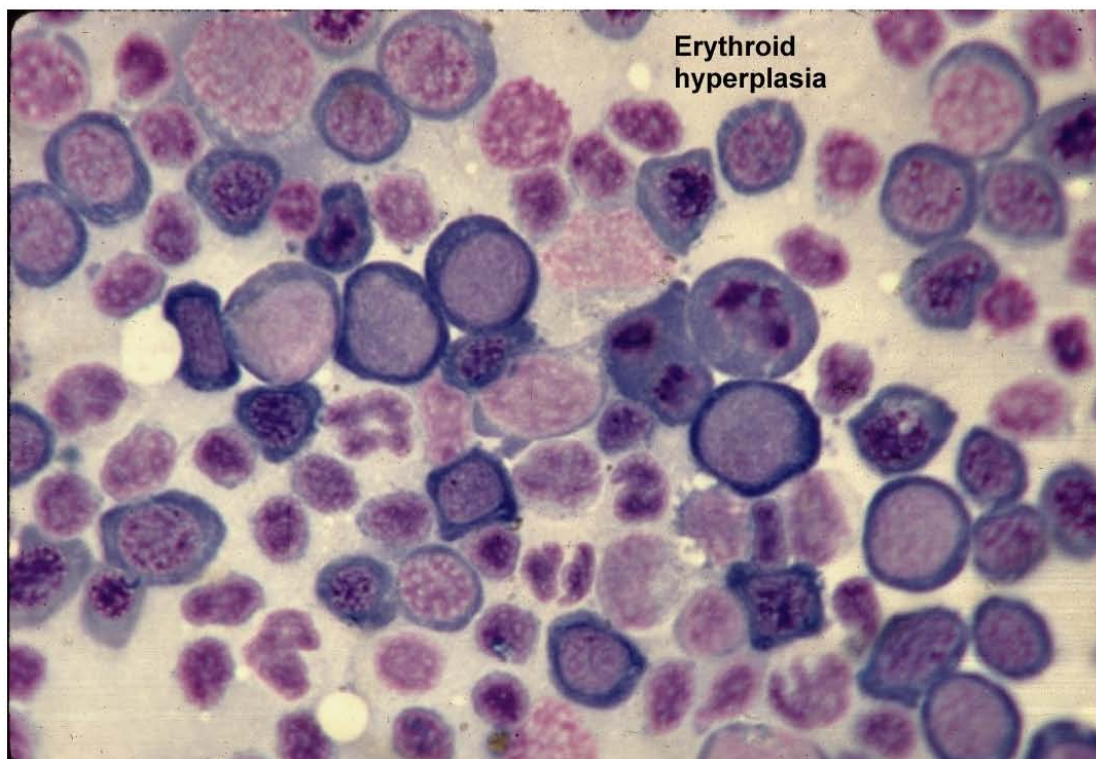
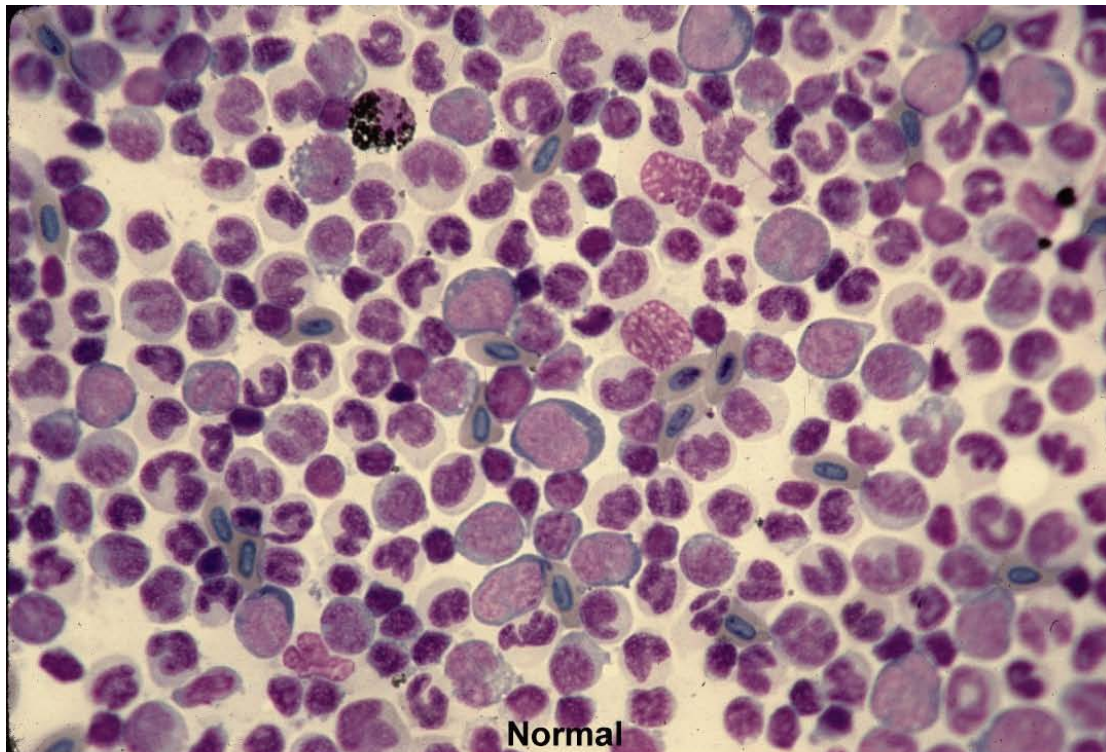
occupies virtually the whole of the cell, leaving only a narrow rim of basophilic cytoplasm in which there are a few mitochondria and isolated ribosomes. In morphology the majority of circulating small lymphocytes appear as inactive undifferentiated cells. They circulate in this form until stimulated into action by their specific antigens. A lymphocyte is said to be mature when it is competent to respond to its antigen.

c. Lymphocytes circulate throughout the blood and lymph of the vertebrate body and congregate in organs which filter body fluids. The number of lymphocytes in the blood is noticeably greater in fishes than in mammals.



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Fish Histology and Histopathology

B. Hematopoiesis: Blood Cell Development



ERYTHROBLAST - I



Cell - Circular.

Nucleus - Circular, centrally located, 80 % of cell.

Cytoplasm - Basophilic, variable width.

Location - More abundant in hemopoietic tissue than in peripheral blood.

ERYTHROBLAST - II



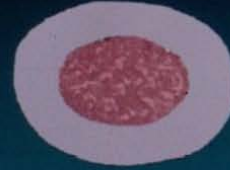
Cell - Circular, but tending toward an oval shape.

Nucleus - Ovalish, centrally located, 60-70 % of cell.

Cytoplasm - Lightly basophilic, variable width.

Location - Can be equally abundant in peripheral blood and in hemopoietic tissue.

PROERYTHROCYTE



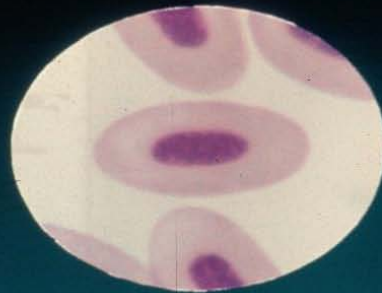
Cell - Becoming oval in shape.

Nucleus - Ovalish, centrally located, 40 % of cell.

Cytoplasm - Grayish, lightly basophilic.

Location - Primarily in peripheral blood.

MATURE ERYTHROCYTE



Cell - Distinctly oval in shape.

Nucleus - Oval, centrally located, 35 -40 % of cell.

Cytoplasm - Orangish-pinkish according to hemoglobin level.

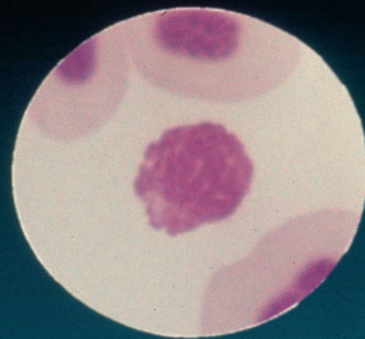
Location - Peripheral blood.

SMUDGE CELL

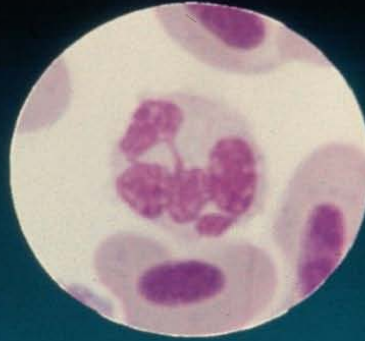
Cell - Shape no longer recognizable.

Nucleus - Pinkish smudge with little definition.

Cytoplasm - No longer recognizable.



NEUTROPHILS



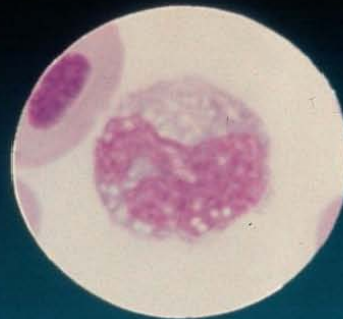
Cell - Spherical.

Nucleus - Multilobed (2 - 5 lobes).

Cytoplasm - Pale to lightly basophilic.

< 10 % of the total WBC's in peripheral blood.

MONOCYTES (MACROPHAGES)



Cell - Spherical.

Nucleus - Large, eccentrically located,
spherical to kidney shaped.

Cytoplasm - Moderately basophilic,
“foamy” if activated.

< 1 -2 % of the total WBC's in normal blood

THROMBOCYTES



Cell - Elongate, spiked, fusiform, spheroid.

Nucleus - Spheroid or elongate (cleft).

Cytoplasm - Very pale basophilic.

< 5 % of the total WBC's in normal blood

LYMPHOCYTES



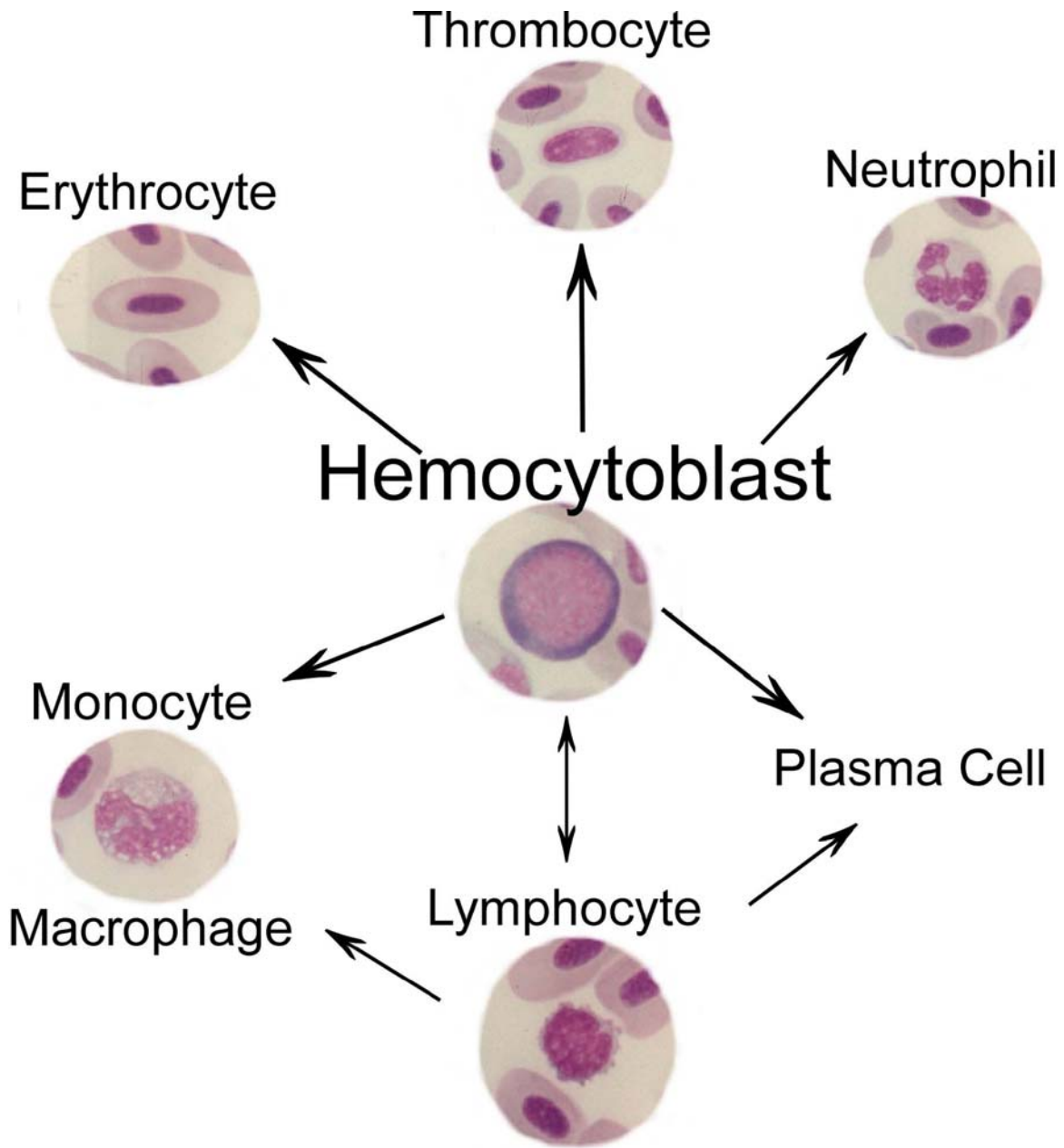
Cell - Spherical.

Nucleus - Spherical, eccentrically located, sometimes cleft.

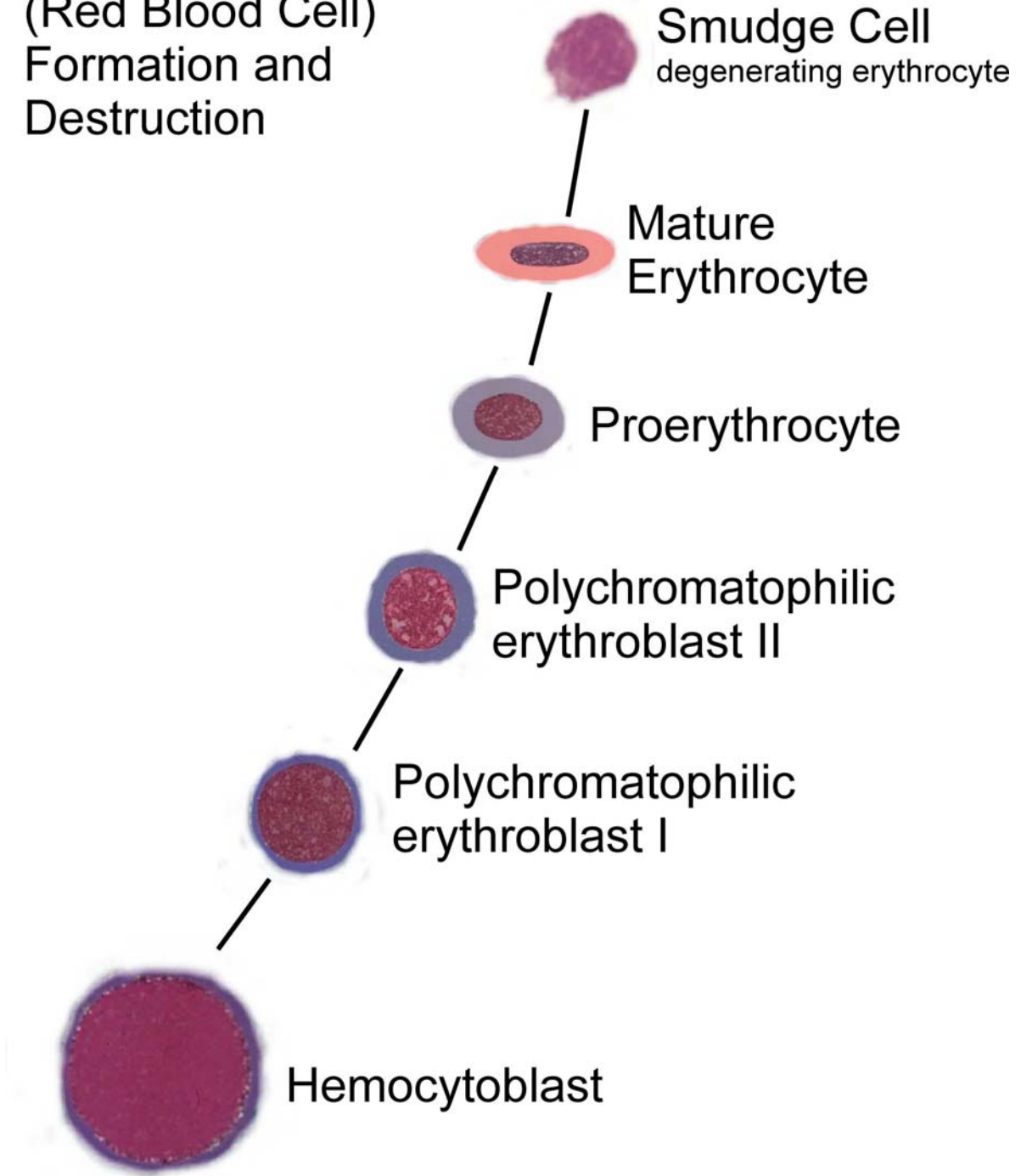
Cytoplasm - Lightly basophilic, narrow, pseudopodial projections.

> 80 % of the total WBC's in normal blood.

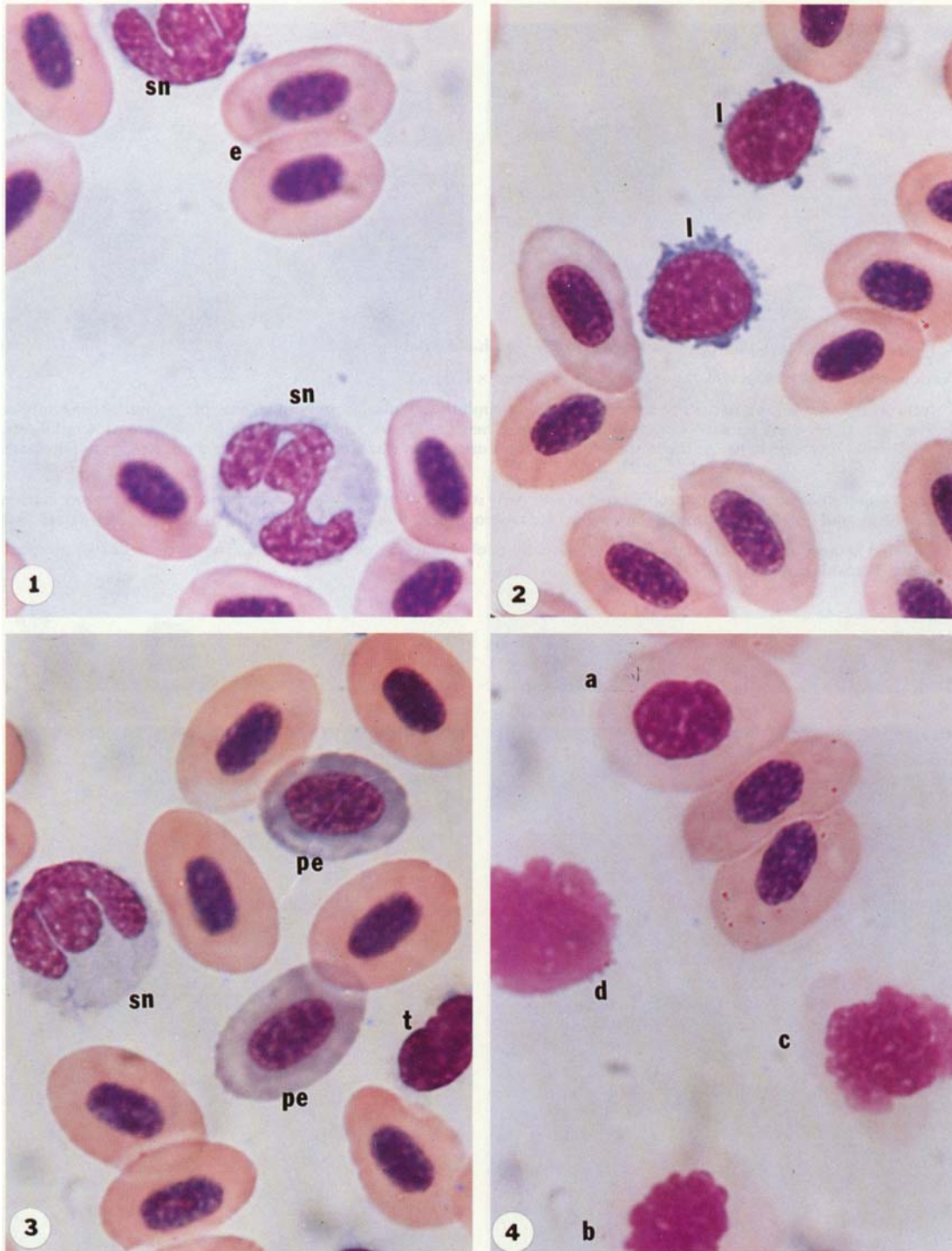
Blood Cell Development



Erythrocyte
(Red Blood Cell)
Formation and
Destruction



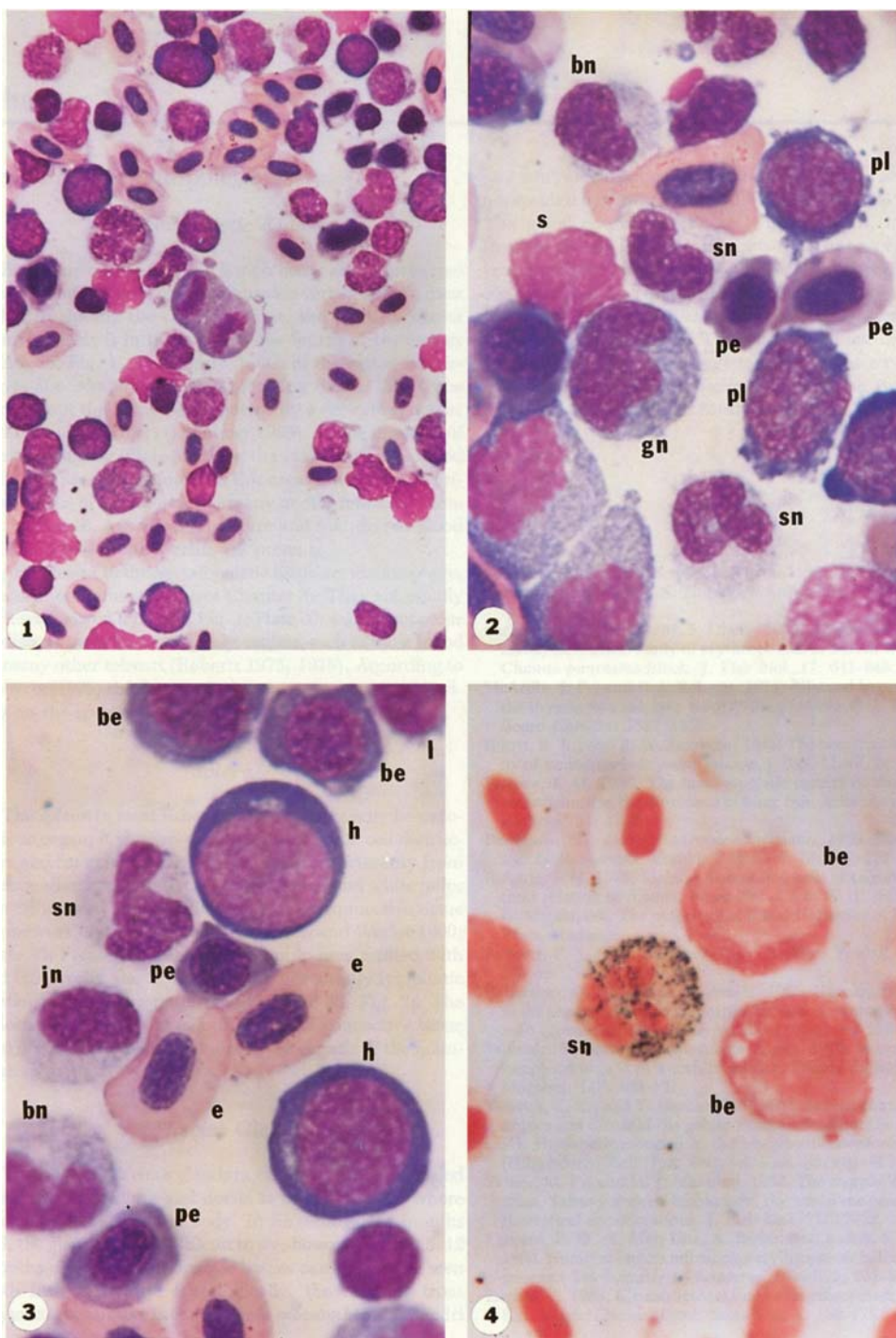
Chapter 4 – Blood and Blood Cell Development
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Peripheral blood smears from 4-month old rainbow trout, stained with Leishman-Giemsa stain.

1. Segmented neutrophil (sn) surrounded by mature erythrocytes (e). X 3600
2. Two mature lymphocytes (l) with pseudopodia, surrounded by erythrocytes. X 3400
3. Segmented neutrophils (sn), polychromatocyte or immature erythrocyte (pe), and thrombocyte (t). X 3600
4. Successive stages (a-d) of erythrocyte "degeneration", ending as a smudge cell (d). X 3600

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Fish Histology and Histopathology



Imprints made from head kidneys of 4-month-old rainbow trout

1. Various states of hematopoiesis. Leishman-Giemsa stain. X 1700
2. Polychromatocyte (pe), an immature erythrocyte with basophilic cytoplasm; prolymphocyte (pl); granulocytes (gn); segmented neutrophil; band neutrophil (bn); smudge cell (s). X 3400
3. Hemocytoblast (h); erythroblast (be); juvenile neutrophil (jn). X 3710
4. Peroxidase stain. X 3700

Chapter 4 – Blood and Blood Cell Development

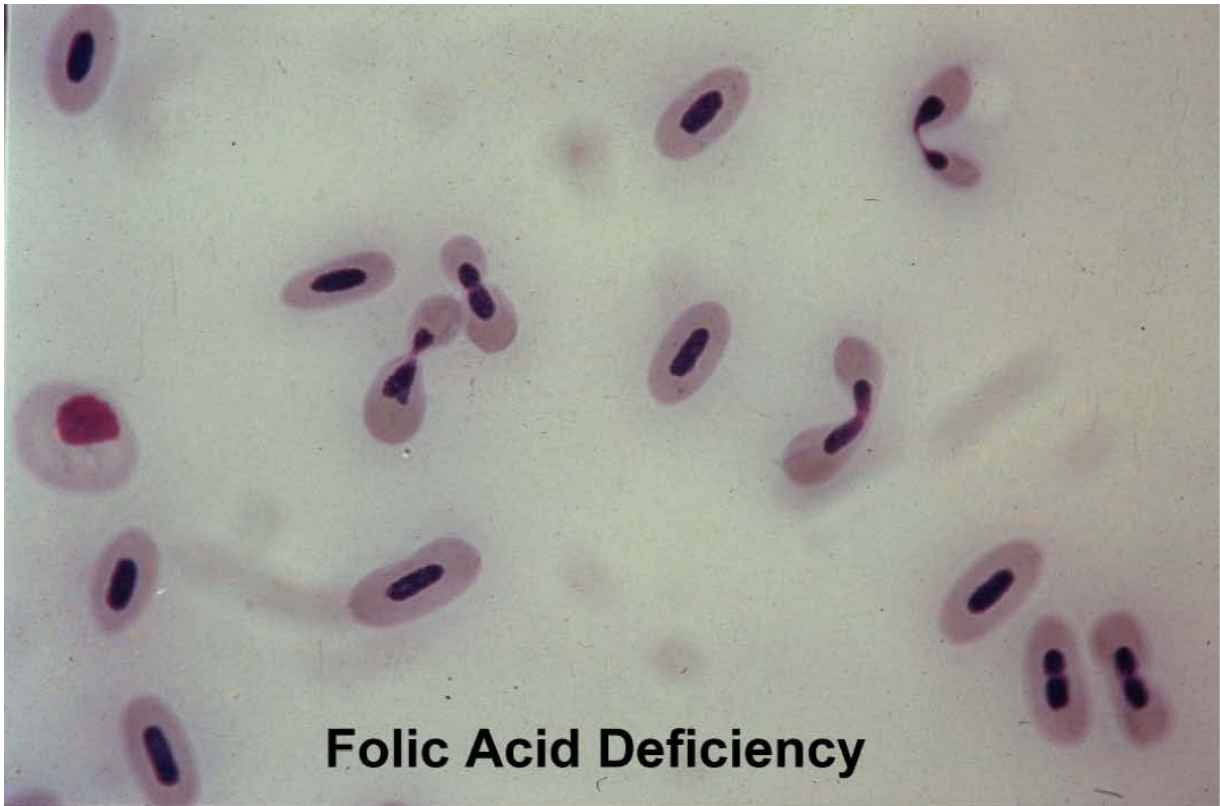
Fish Histology and Histopathology

Blood Disorders

Folic Acid Deficiency

- Folic Acid important in the incorporation of nucleotides into DNA
- Extremely important for red blood cell (RBC) formation
- Clinical signs are severe anemia (based on pale gills), poor growth, exophthalmia, and abdominal distention with ascites
- Deficiency results in a macrocytic anemia- reduced RBC counts but mostly normal hematocrits due to larger size of RBCs
- The anemia is characterized by anisocytosis and poikilocytosis. Immature erythroblasts are megaloblastic
- Nuclear fragmentation and atypia are very common and bilobate RBCs are present but less common



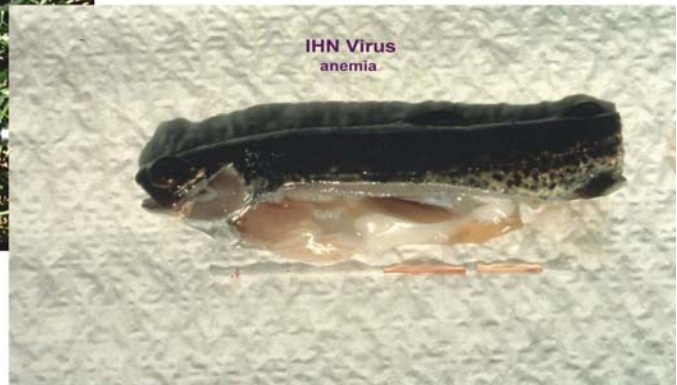


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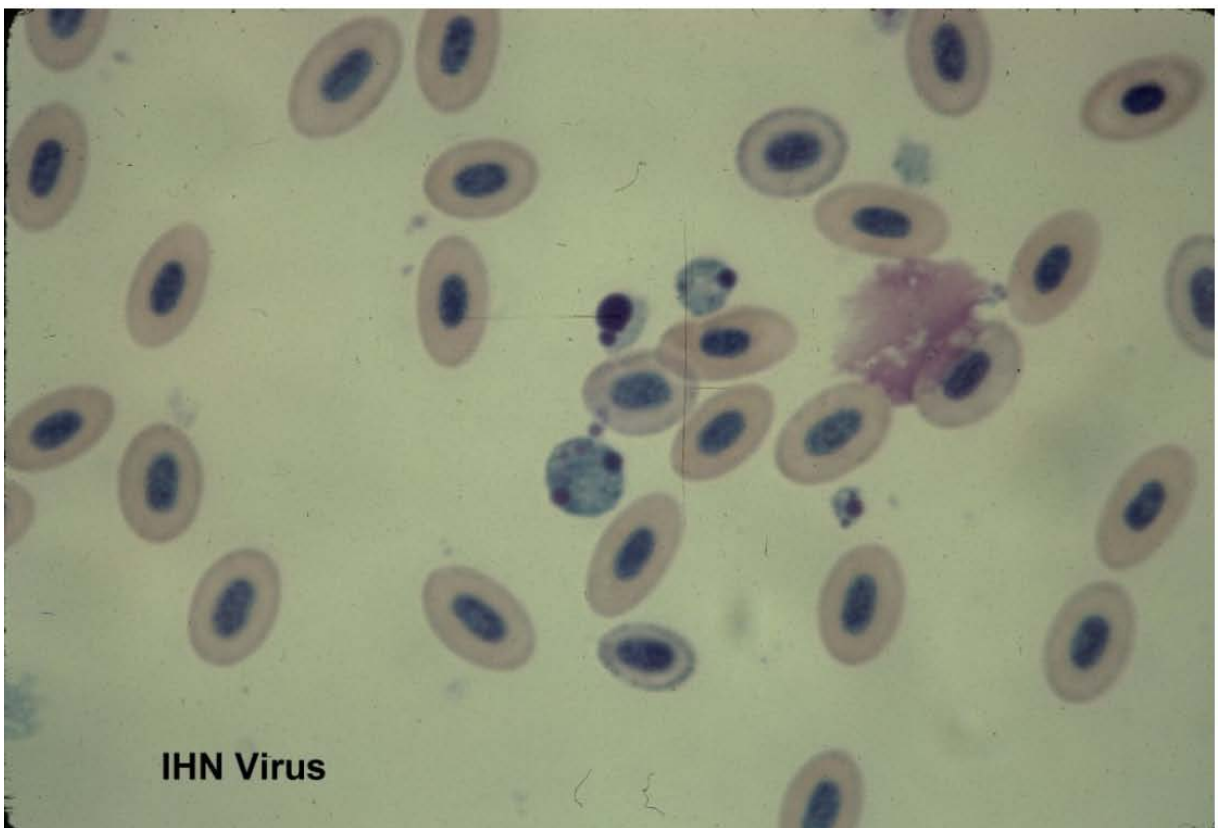
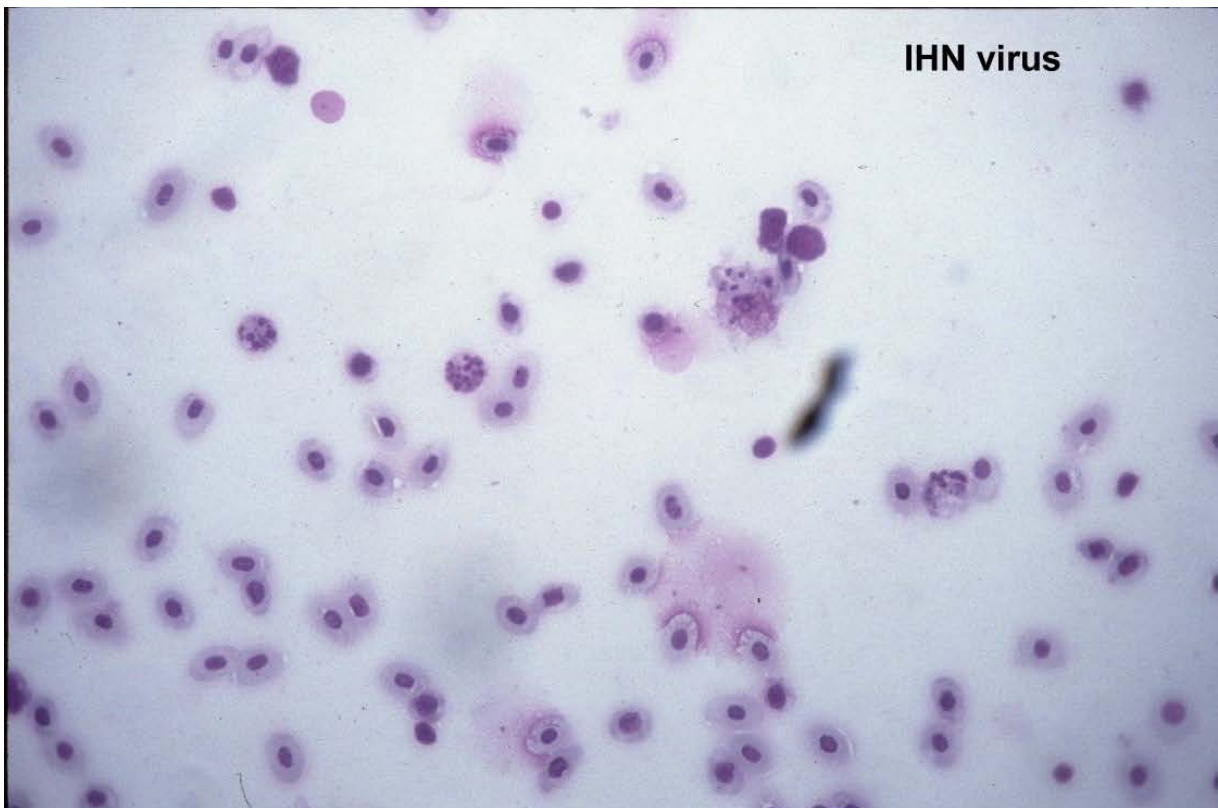
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Infectious Hematopoietic Necrosis Virus (IHNV)

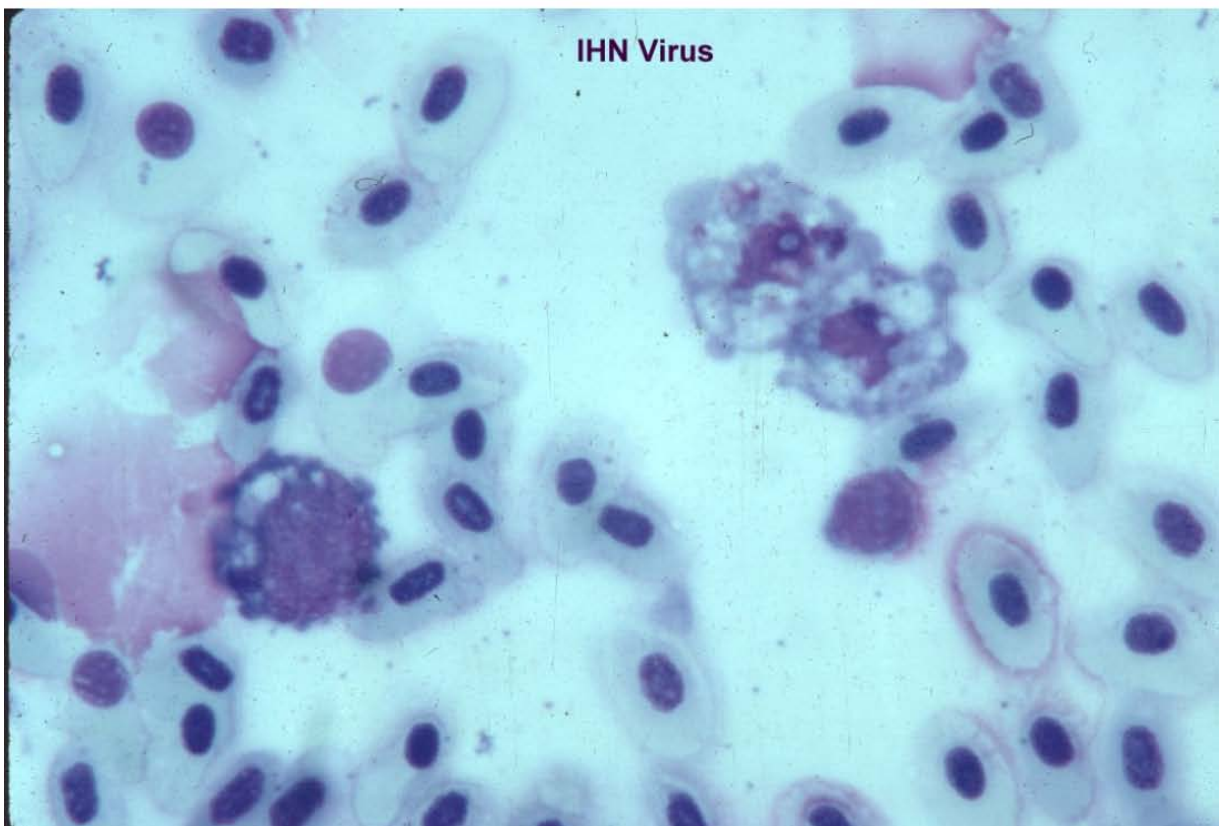
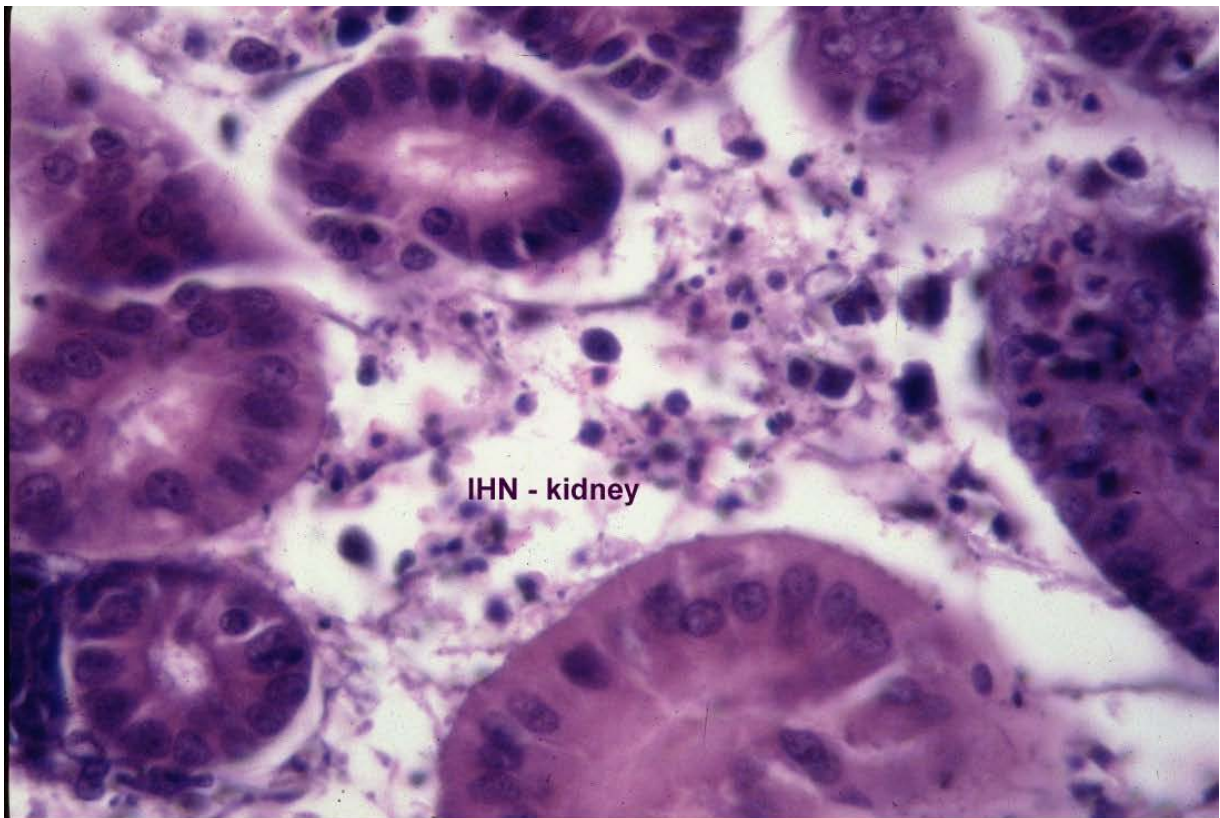
- Virus disease of salmonids that results in a severe anemia
- Clinical signs are usually severe mortality, exophthalmia, darkened color, extremely pale gills, petechial hemorrhages, and abdominal distention from ascites
- Blood may be straw colored with few cells present- hematocrits may be as low as 5 - 10%
- Along with the clinical signs that accompany the disease the use of blood smears and kidney imprints aid in the presumptive diagnosis of the disease
- Characteristics of the blood smears are: Sfew cells are present because of severe anemia Sbi-lobed erythrocytes are usually present in small numbers Snecrobiotic bodies (degenerate cellular debris) are often present Sfoamy macrophages often with engulfed cellular debris are sometimes present
- Characteristics of blood imprints are: Sfoamy macrophages with and without engulfed cellular debris are often present Snecrobiotic bodies and other cellular debris such as cytoplasmic material are often present



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Fish Histology and Histopathology



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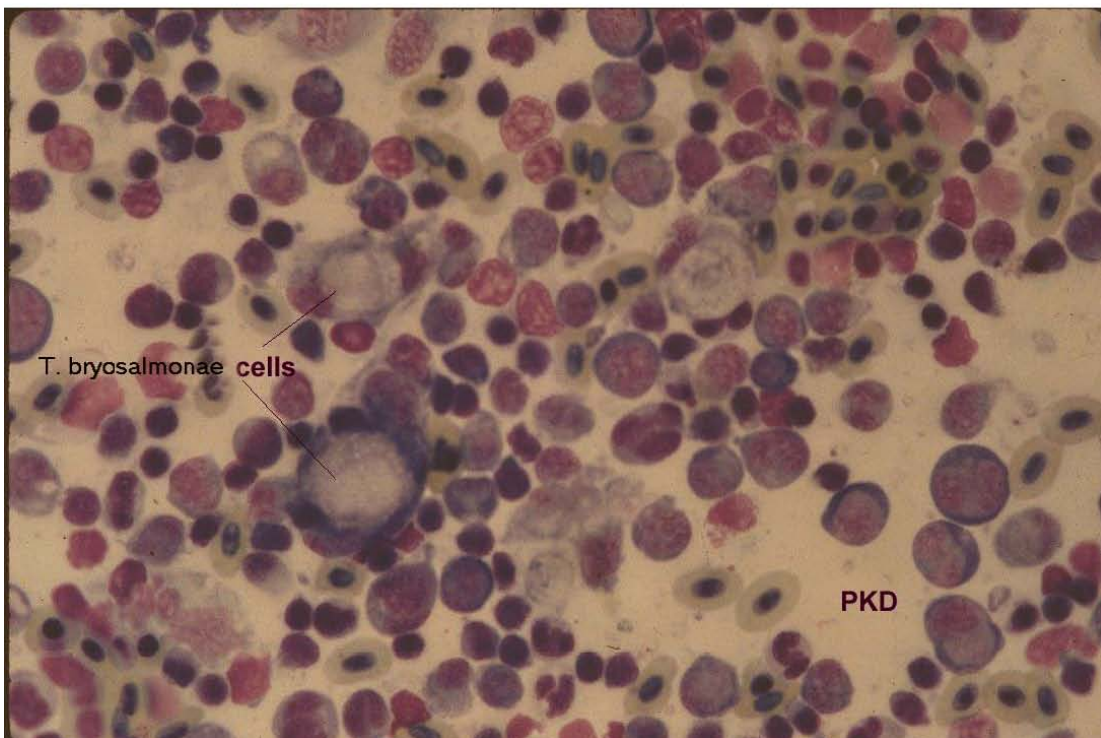
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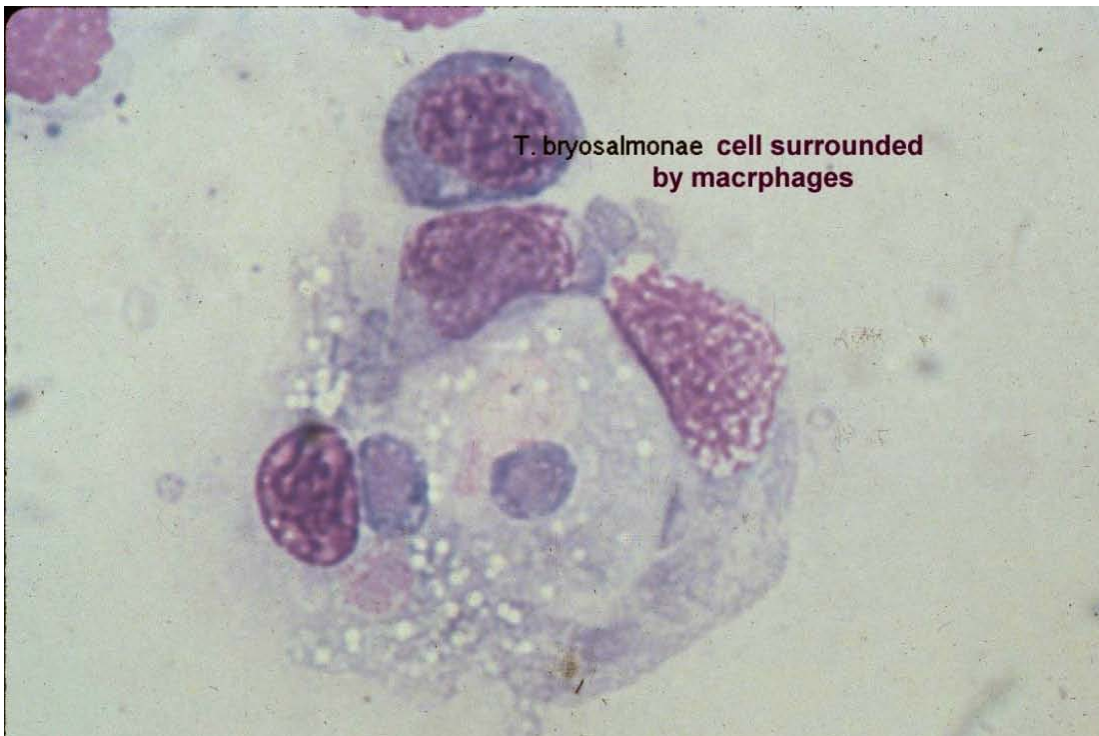
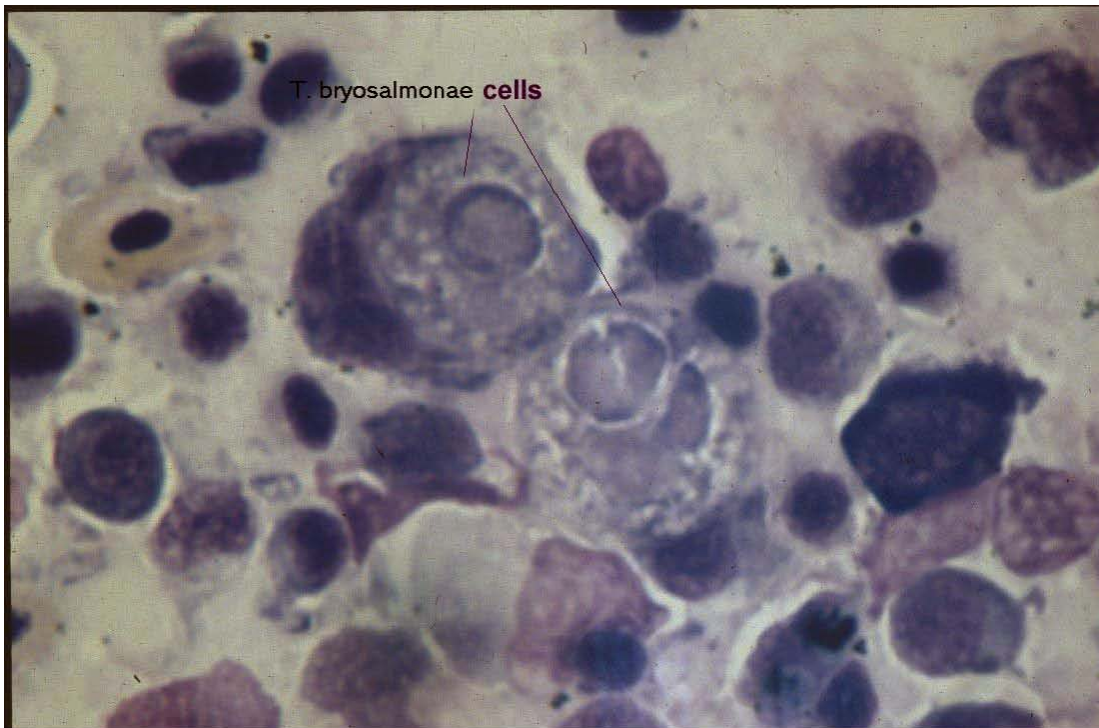
Proliferative Kidney Disease

- Caused by *Tetracapsula bryosalmonae*
- Clinical signs include darkening body color, distended abdomen due to ascites, pale gills indicating anemia, pronounced lateral body swelling, and bilateral exophthalmia
- Enlargement of the kidney and spleen are the most notable internal signs
- The kidney may be grayish throughout or mottled, and is markedly swollen (posteriorly most pronounced)
- In severe cases the capsule may have a folded or corrugated appearance
- Ascites may be present or absent and is usually clear
- Imprints of affected kidney stained with Leishman-Giemsa provide a useful tool for detection of the parasite
- Wet mounts made from affected kidney tissues and examined by bright field or phase microscopy can be used for diagnosis. However, adequate numbers of parasite cells as well as experience in identifying the forms are required to enable one to distinguish trophozoites of the parasite from host macrophages, particularly when the macrophages are laden with cellular debris
- Attachment of macrophages to the parasite aids in distinguishing the parasite from other cells present in the interstitium of the kidney
- The parasite is large (greater than 20 microns) and often contain internal daughter cells or secondary cells within the primary cell. These in turn may contain internal cells or tertiary cells (Endogeny). This is variable between cells

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Fish Histology and Histopathology



Chapter 4 – Blood and Blood Cell Development

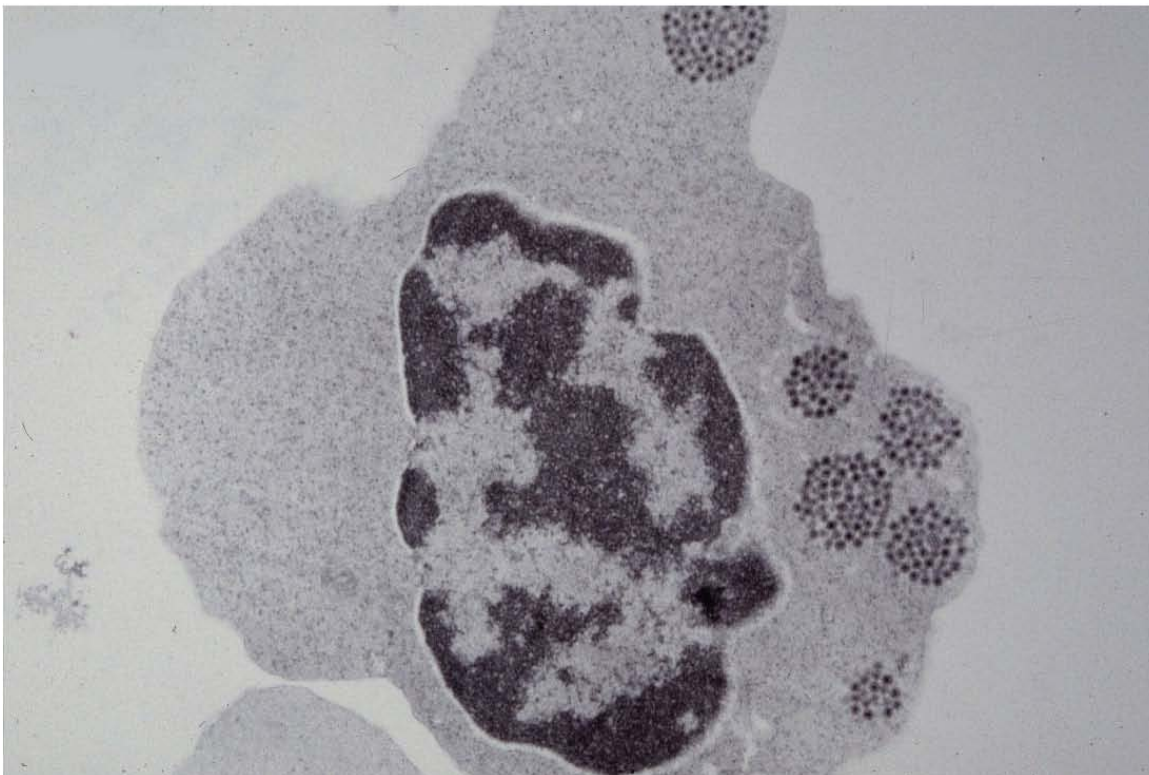
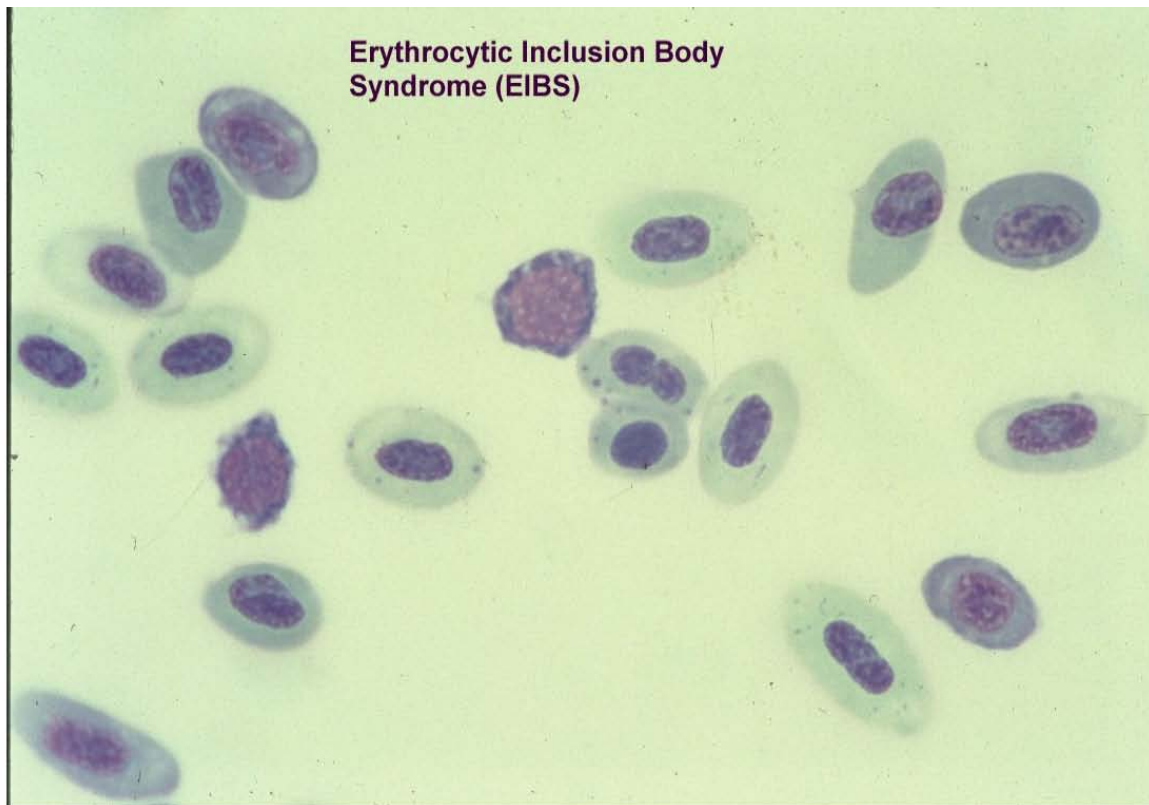
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Erythrocytic Inclusion Body Syndrome (EIBS)

- EIBS is a viral disease of salmonids in which erythrocytes are infected with a small icosahedral virus
- Similar to Viral Erythrocytic Necrosis (VEN) except inclusions in VEN are larger as are the viral particles and they stain much better than do EIBS inclusions
- The most common clinical sign of the disease is anemia which may vary from mild to severe
- Often EIBS is associated with other diseases such as Bacterial Kidney Disease (BKD), coldwater disease, and fungal infections
- The disease is clinically diagnosed by microscopic examination of stained blood smears for the presence of typical intracytoplasmic inclusion bodies in erythrocytes
- Inclusions may be single or several scattered throughout the cytoplasm. They stain pale blue with Leishman-Giemsa
- Blood picture may show many immature cells in blood smear and probably represent an attempt to return to normal
- The staining method of choice is pinacyanol chloride, although inclusions can also be observed when blood smears are stained with Leishman-Giemsa or Giemsa
- Wet mounts of blood can also be used and examined by phase contrast microscopy



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EIBS

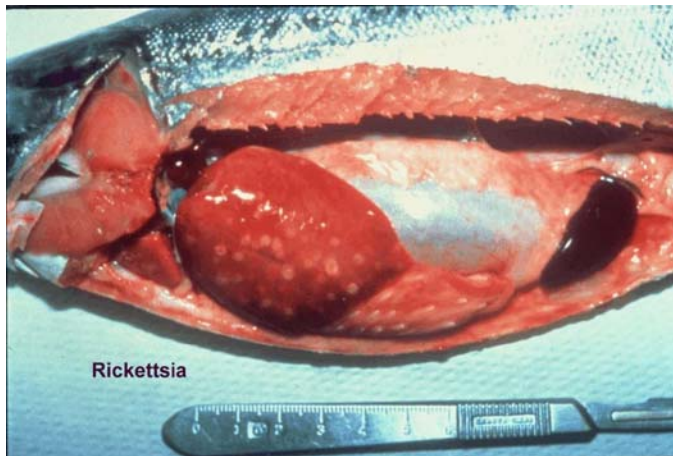
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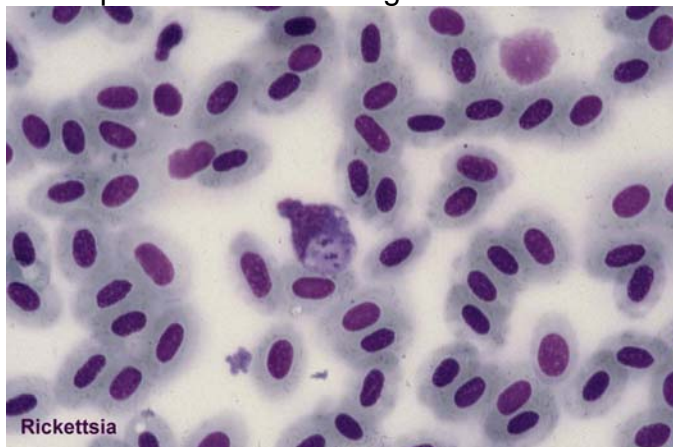
Salmonid Rickettsial Septicemia

(also known as Piscirickettsiosis, coho salmon septicemia, and Huito disease)

Piscirickettsia salmonis is an intercellular rickettsial-like pathogen of fish that replicates within membrane-bound cytoplasmic vacuoles of infected cells. The bacterium is fastidious and does not grow on any known artificial media. The organism has been isolated from salmonid fishes in Chile, Ireland, Norway, and both the east and west coasts of Canada. The organism has been observed in or isolated from coho, Chinook and pink salmon, rainbow trout, and Atlantic salmon. Coho salmon appear most susceptible. Other species are also susceptible to Rickettsia.



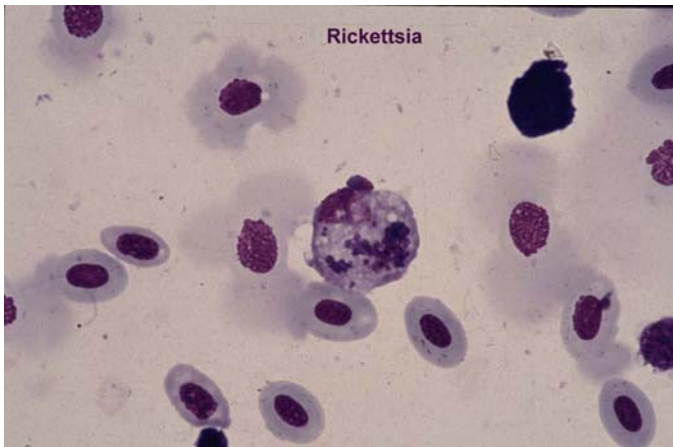
Gross photo of fish showing nodules



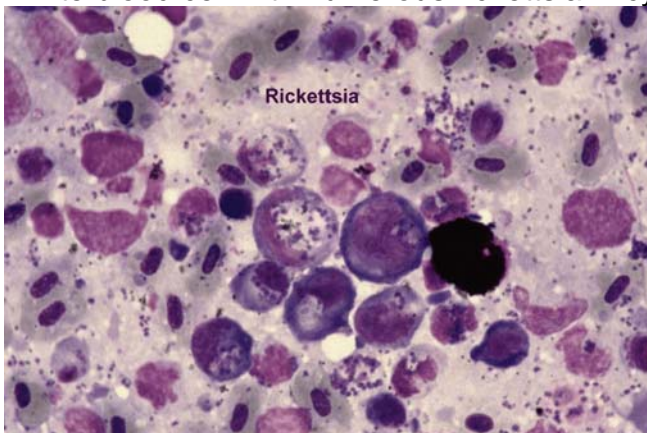
White blood cell with few rickettsia in cytoplasm

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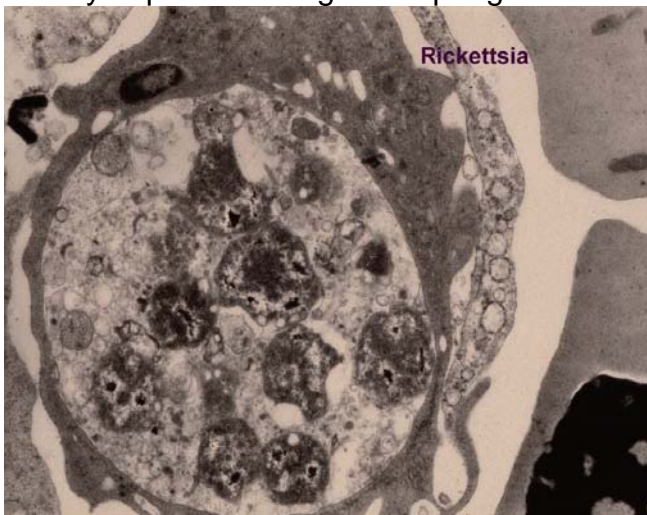
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White blood cell with numerous rickettsia in cytoplasm



Kidney Imprint showing macrophages with rickettsia in their cytoplasm



EM photo showing a cell containing rickettsia

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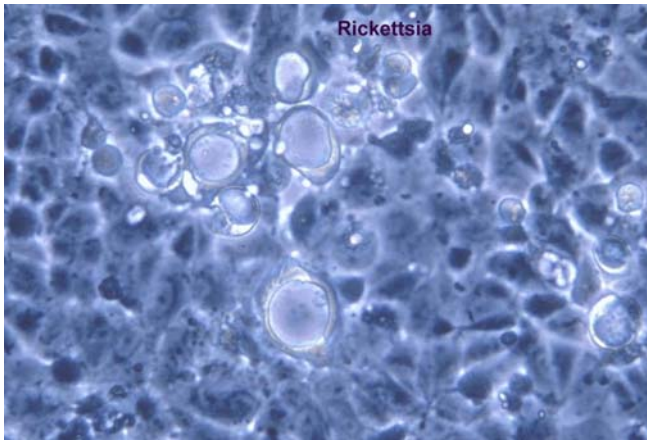


Photo showing infected cells in cell culture

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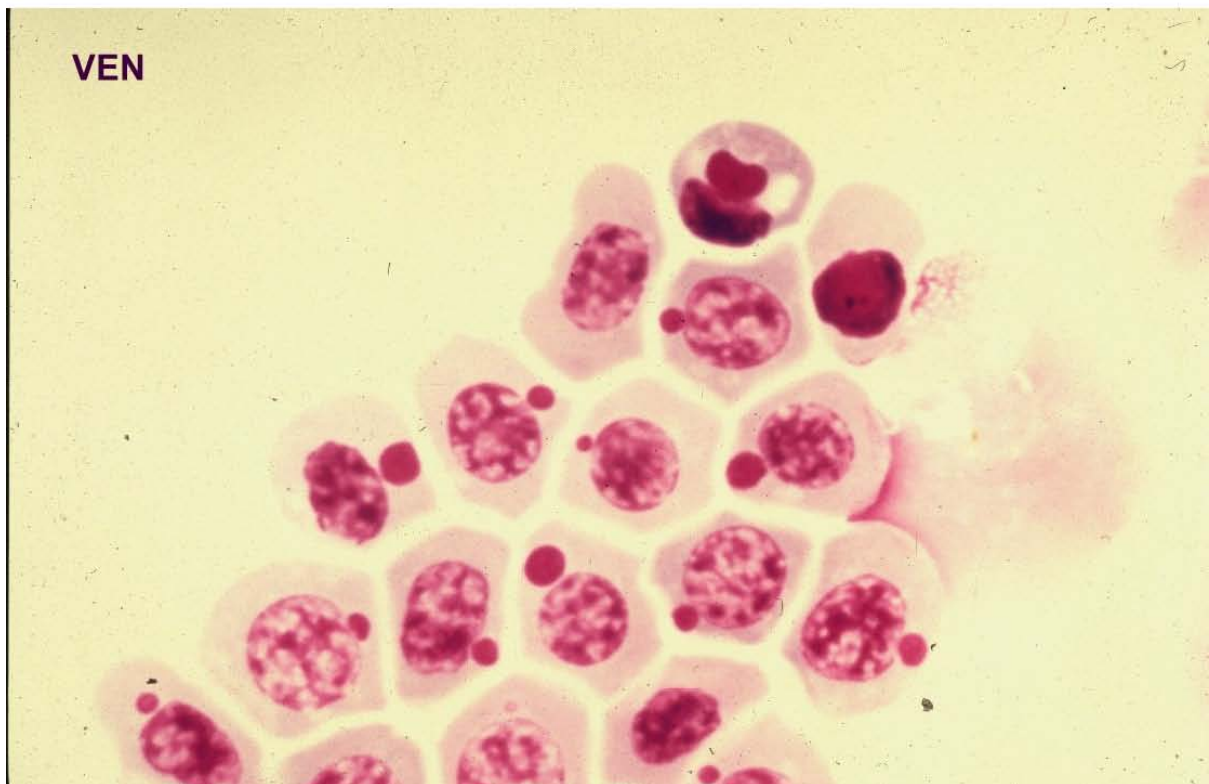
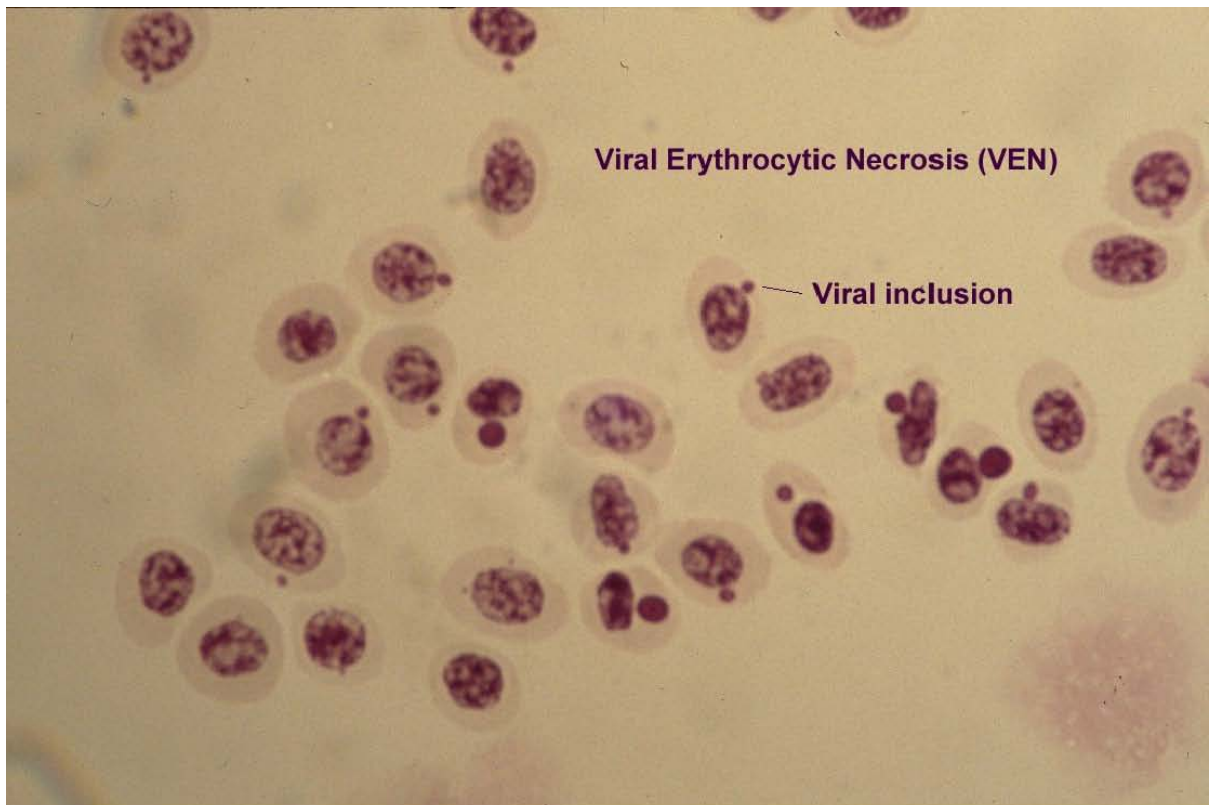
VEN – is caused by erythrocytic necrosis virus (ENV). The disease was originally named Piscine Erythrocytic Necrosis (PEN). The viruses are tentatively placed in the Iridoviridae.

Agents of this disease have not been isolated or fully characterized. There are several morphologically distinct viral particles that cause the disease. Some of these viruses probably cause different signs and have distinct host specificities.

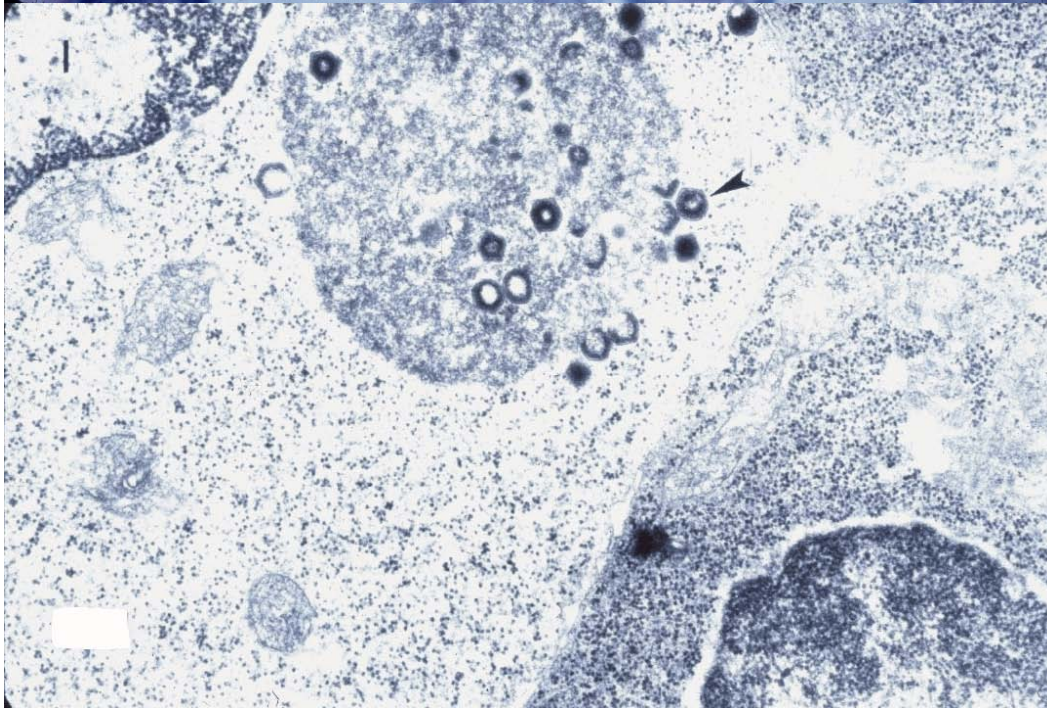
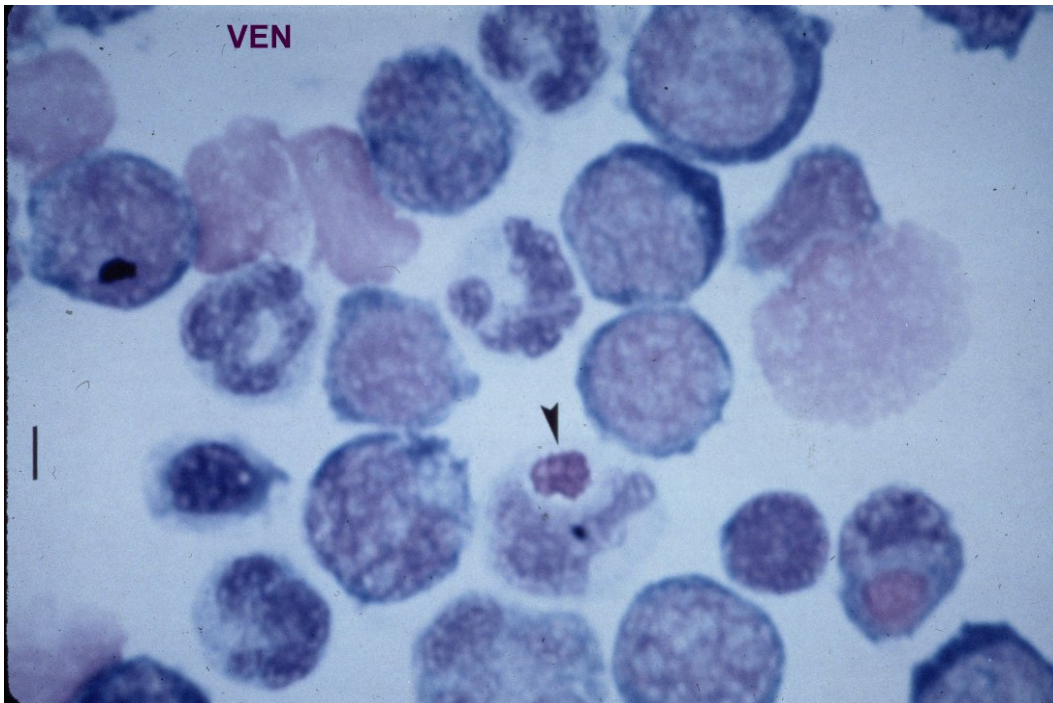
Little is known about the epizootiology of VEN. The numerous agents that cause the disease are manifested as chronic or subacute infections that may not cause overt mortality, but debilitate fish so that other pathogens (e.g. *Vibrio anguillarum*) or adverse environmental conditions cause death of the fish.

The viruses have been demonstrated in many anadromous and marine species of fish. The following photos are from experimentally infected coho salmon.

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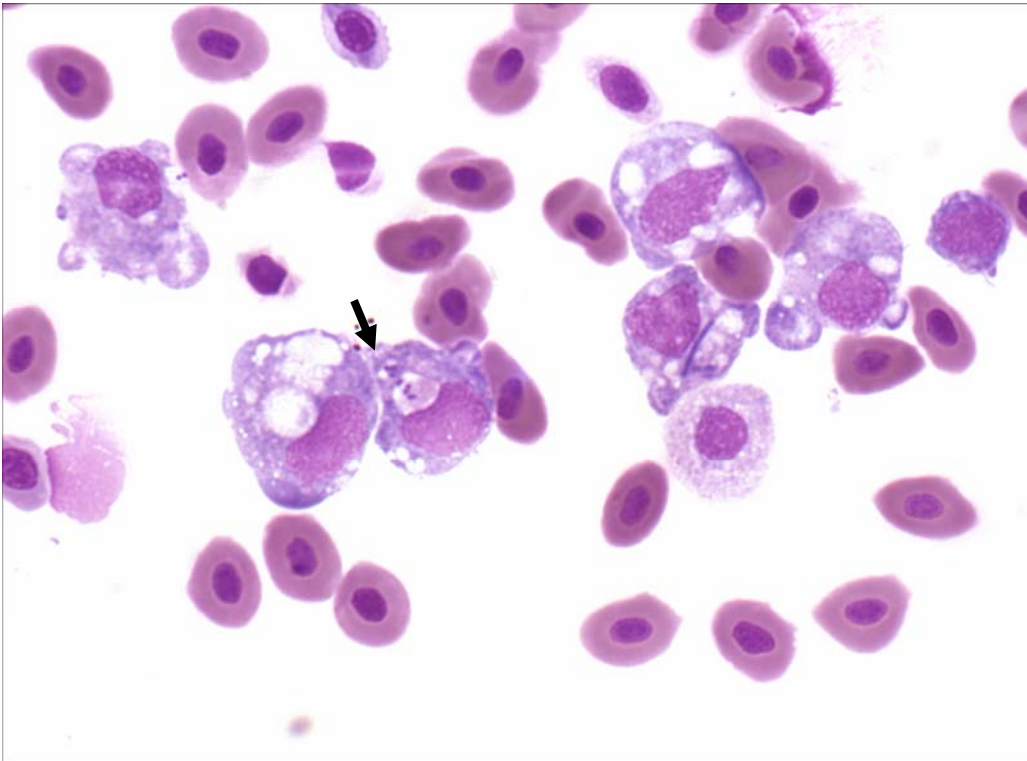
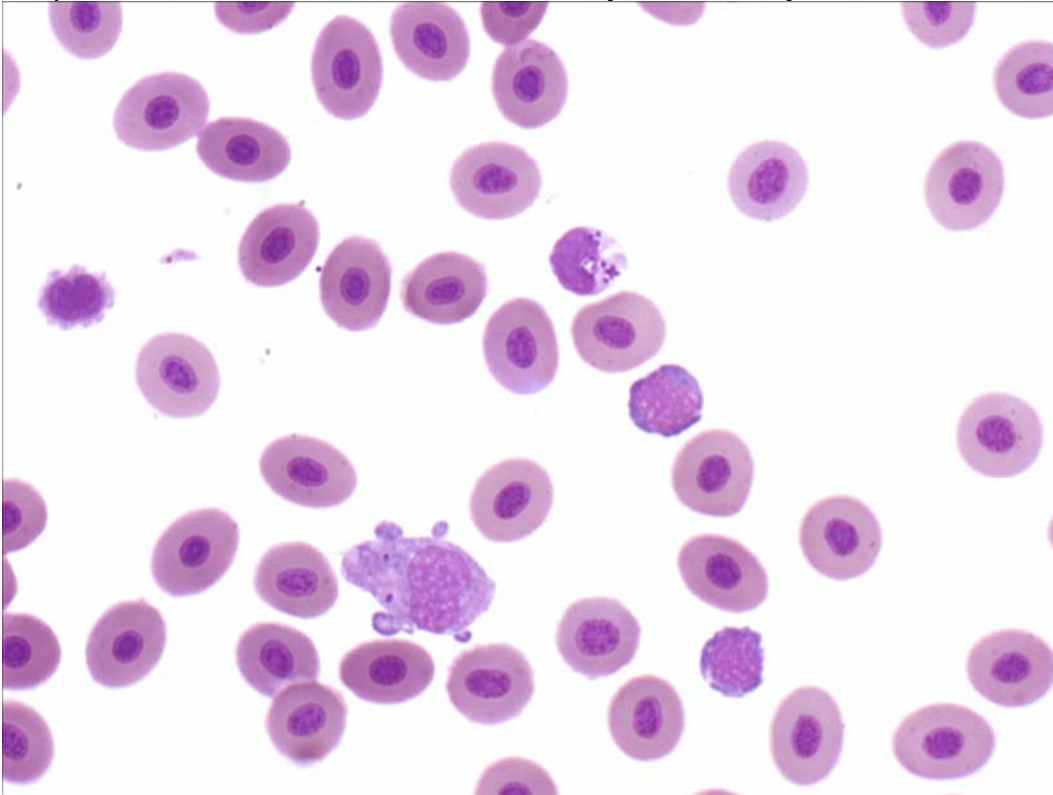


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Tilapia blood smear shows infected monocyte with ***Streptococci***



Numerous macrophages in Tilapia blood smear one of which contains *Streptococci* (arrow).

General Pathology and Histopathology

Organs and tissues respond to stimuli, either physiological or pathological, in various ways, many of which can be identified and studied by histology. These morphologic changes can provide the clues necessary to establish a diagnosis. Hence histology is an important tool for diagnosticians. While sometimes histology is sufficient by itself to make a diagnosis, it can become all the more powerful if complimented by other diagnostic methods such as bacteriology, virology, serology, and toxicology.

In order to understand the histological appearance of injured tissues, it is important to be familiar with the normal histology of the organs and tissues of the type of animal under examination. Look at lots of normal fish as well as sick fish. Be aware that there are ranges of “normal” in any given species, and only by looking at many normal animals will you become familiar with this variation. Considering the diversity of fishes, it is very beneficial to examine numerous specimens from different species as well, in order to better appreciate how variable “normal” can be between species. A solid familiarity with what is considered normal is essential in order to be able to readily identify lesions, particularly those that are subtle. This will also help you sort out those changes that are truly associated with a disease process from those that are part of normal physiologic processes for the animal being examined.

Familiarity with pathologic changes at the gross and histological level will often aid in identification of the etiology, or cause, of a disease. Certain morphologic changes in diseased tissues often provide clues as to what type of injurious agent may have been responsible. A good example is the presence of granulomatous inflammation in various organs of a fish. By recognizing this morphologic pattern, you will immediately consider certain groups of pathogens as possibly being responsible for the lesion: *Renibacterium*, *Mycobacterium*, various fungi. Even without microbiology support, you have narrowed the list of possible etiologies to a few very likely candidates. Don't just hunt for microbes and parasites; use the clues provided by the tissue changes to help you localize where the pathogens may be and to help narrow the field of possible suspects.

Do not be afraid to stray outside the realm of fish. The pathogenesis of disease and the development of lesions are similar across species lines. Inflammation in a fish is similar to inflammation in a dog or a monkey. If you understand histopathology of fish, you can use that knowledge to appreciate similar changes associated with disease in other types of animals.

Causes and Patterns of Cell Injury & Necrosis

Reversible Cellular Changes

A variety of changes in cell morphology and function in response to injury are adaptive and are compatible with cell survival. Injuries often induce changes in cellular structure which are not lethal and are reversible. These changes include acute cellular swelling, hydropic change, and fatty change. These changes may be distinct, or can blend or progress from one to another, or occur simultaneously within a tissue.

Reversible injuries result in structural and functional changes, but adaptation by the affected cells can maintain cell viability. Without adaptation, the changes caused by the injury can progress and may lead to cell death (necrosis).

Examples of reversible cell injury:

1. Acute cellular swelling (Figures 1 through 6)

Cells swell due to increased water uptake following alterations in membrane permeability. Cytoplasm develops a “ground glass” appearance primarily due to fine, watery vacuoles in the cytosol and mitochondria. Mitochondria are very vulnerable to noxious agents; if damaged, cellular metabolism (ionic pump) fails yielding osmotic swelling of the organelle. Acute cellular swelling represents an early and completely reversible manifestation of injury. Seen typically in epithelial cells.

2. Hydropic change (Figures 7 and 8)

Large distinct water vacuoles form within the cell cytoplasm. It is a more pronounced form of swelling than acute cellular swelling. Small vacuoles coalesce, giving a clear vacuolated appearance to the cytoplasm. Water is located in mitochondria and within cisterna of endoplasmic reticulum. Again, epithelium is a frequent target.

3. Fatty Change (Figures 9 through 12)

Fatty change (fatty metamorphosis; lipidosis) is an abnormal and excessive accumulation of intracellular fat. This is an injury which, though reversible, can be severe and can cause severe disruption of cell function. Distinct non-staining vacuoles of fat lie in the cell cytoplasm, displacing and compressing the nucleus. It is most commonly seen in the liver, but also seen in kidney and heart.

There are several mechanisms that can result in excess fat accumulation in a cell. These include: 1) dietary excesses of carbohydrates and/or triglycerides; 2) decreased oxidation of fatty acids leading to increased esterification of fatty acids to triglycerides; 3) decreased lipid acceptor protein (hypoxia, deficient dietary protein); 4) decreased transport from the cell; 5) dietary protein - fat imbalance.

The fat content of the liver is quite variable in normal fish; diet and physiologic events have a bearing on this, as well as the species of the fish; some are always laden with fat (eg. sharks). Therefore be careful when calling a fat-laden liver a lesion. With experience, you will develop an appreciation for the range of normal in various species.

4. Hyaline change

“Hyaline” is a commonly used adjective that does not imply any particular disease. It simply refers to a particular histologic appearance of cells or tissues when stained with H & E stain. Hyaline material is dense, amorphous, homogeneous, and

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eosinophilic. It can be found under normal or pathologic conditions, and may or may not be reversible. It can represent an accumulation of material within the cell, or occur as a result of cell degeneration.

Necrosis (Figures 13 through 20)

Cell injury can progress to a point of no return, where the cell is unable to adapt and homeostasis is no longer possible. Irreversible injury occurs and cell death, or “necrosis,” ensues. Major disruption of the cell membrane occurs during necrosis, accompanied by massive influx of calcium into the cell.

Nuclear and cytoplasmic features are used to identify necrotic cells. The nuclear changes include:

1. pyknosis: shrinkage and condensation of nucleus and chromatin;
2. karyorrhexis: fragmentation of the nucleus;
3. karyolysis: complete dissolution of chromatin and/or nucleus.

The cytoplasmic features of necrosis include intense eosinophilia, loss of basophilia, and fragmentation or hyalinization of the cytoplasmic component. In addition to these cytological features, necrosis will induce localized inflammation (assuming death does not occur too quickly).

There are different types of necrosis that can be recognized histologically.

Coagulative necrosis is characterized by retention of cellular/tissue architecture; cellular detail is retained in the face of cell necrosis. This is associated with diverse causes, including many infectious diseases, ischemia, burns, trauma, and toxic damage.

Caseous necrosis is more easily recognized grossly; they have a dry, cheese-like consistency. Histologically, there is no cellular detail. Mineralization is common. This is seen with some bacterial infections.

Liquefactive necrosis features complete disintegration of the tissue into a liquid of varying consistencies. All histologic features are lost. The liquefaction is caused by enzymes released from host cells, such as neutrophils or other inflammatory cells, or by toxins released from bacteria. Tissues with high fat content, such as the central nervous system, also may liquefy when necrotic.

Necrotic cells are dead and they will not recover. The tissue however may be able to regenerate and heal (see section on Healing and Repair).

Programmed cell death and apoptosis

Programmed cell death and apoptosis are similar processes, but have different triggers. Programmed cell death is a biological function. It is a mechanism for elimination of selected cells during physiological processes of development and growth. The mechanism of cell death is complex, and results in cells with condensed chromatin and cytoplasm that fragment into membrane-bound particles, those fragments being engulfed by phagocytic cells. Apoptosis involves similar mechanisms and morphology, but its onset is triggered by injury, such as viral infection or exposure to a toxin; i.e. it is a pathologic cell death. In both cases, single cells are dead, so gross changes are not obvious. As cell fragments are bound by membranes, inflammation typically seen with necrosis is not present.

Figure 1. Normal liver

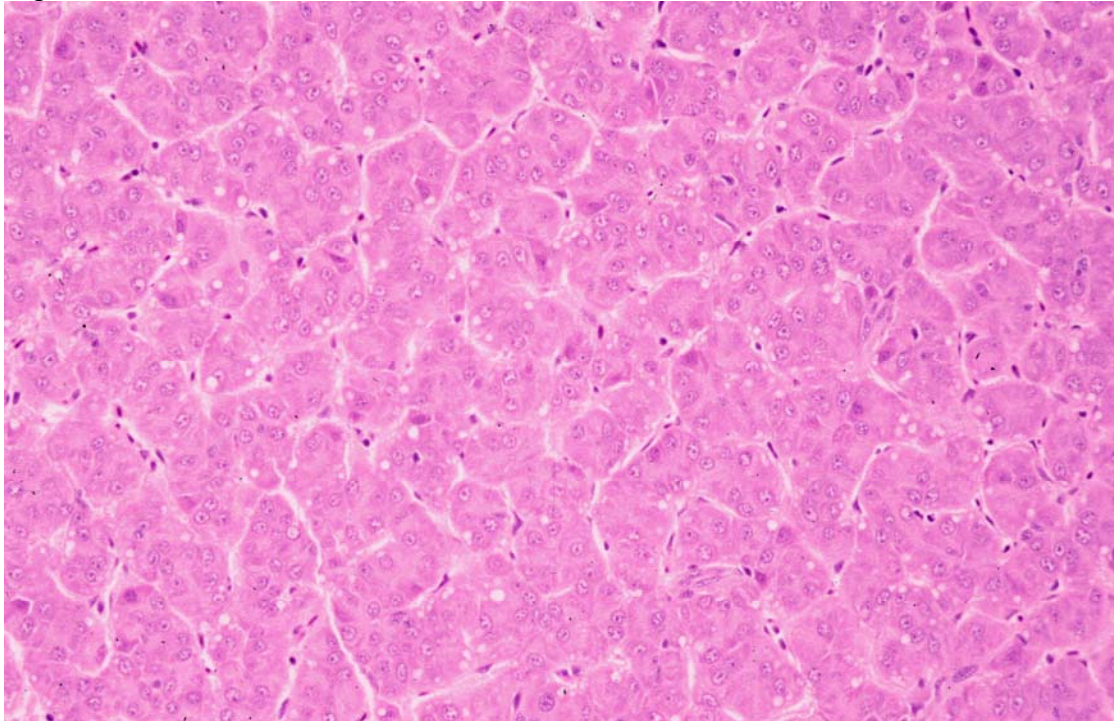


Figure 2. Liver - acute cellular swelling

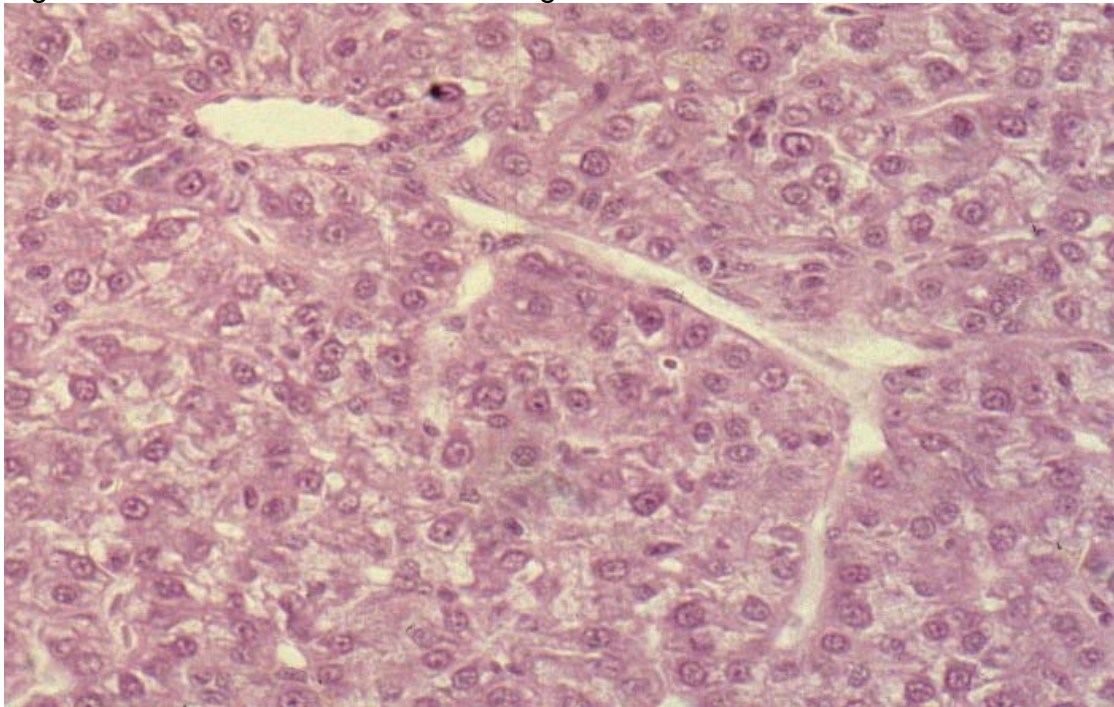


Figure 3. Liver - acute cellular swelling

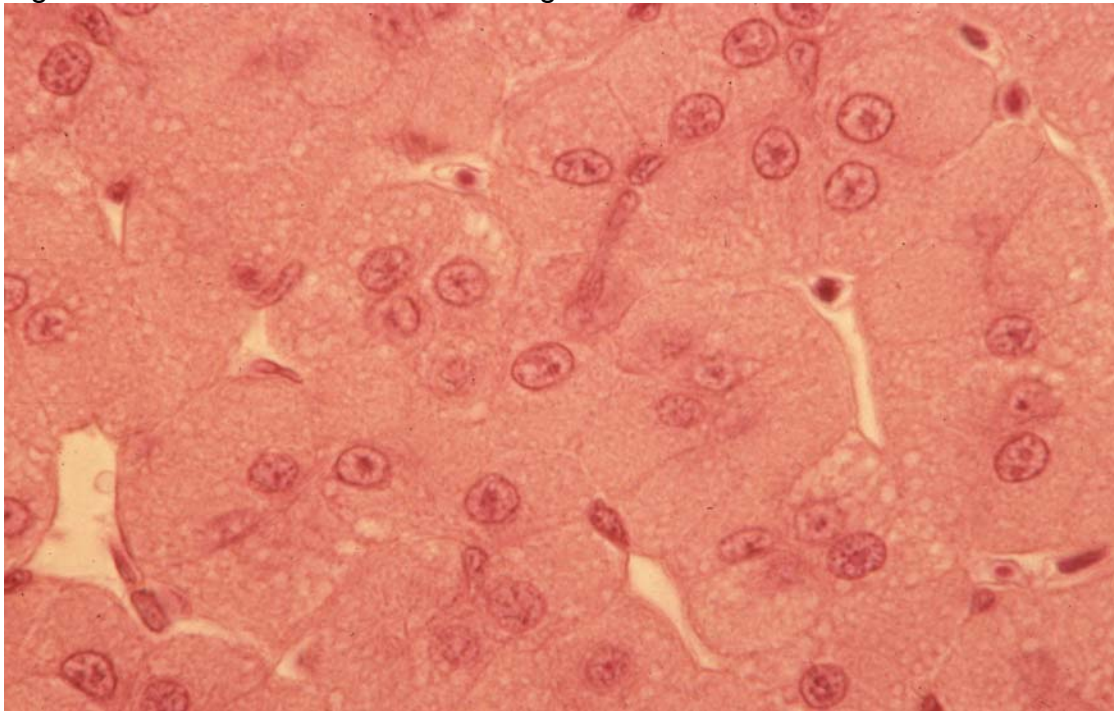


Figure 4. Gill - normal

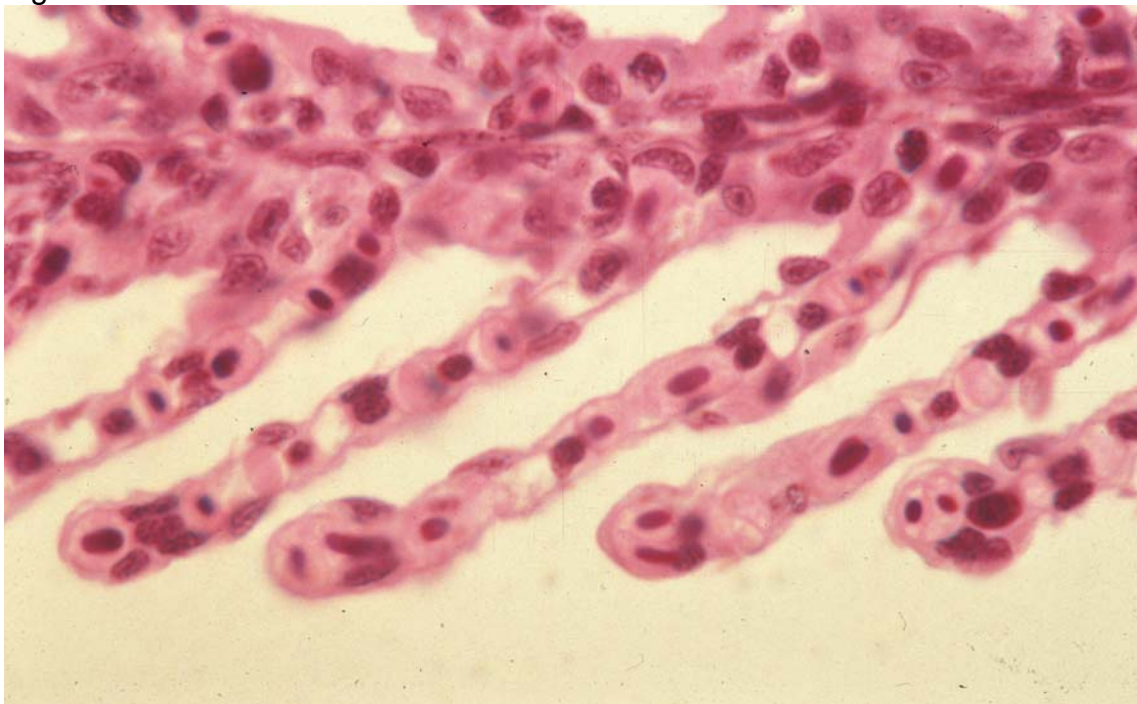


Figure 5. Gill - acute cellular swelling

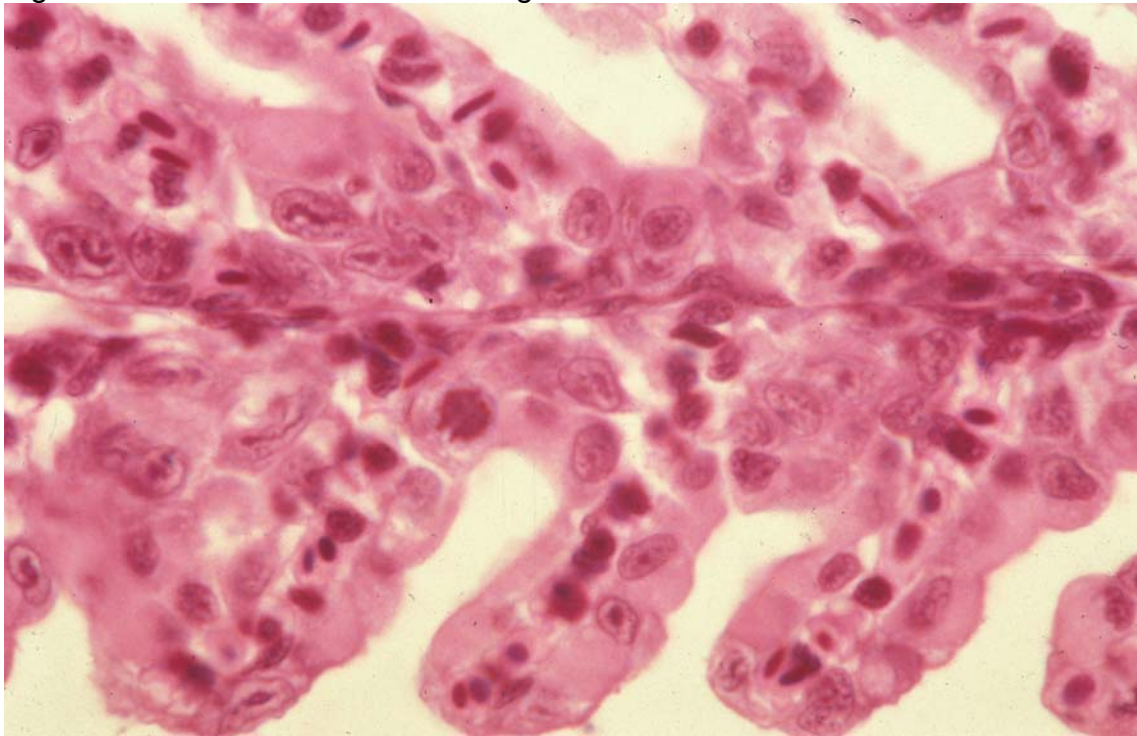


Figure 6. Skin - normal

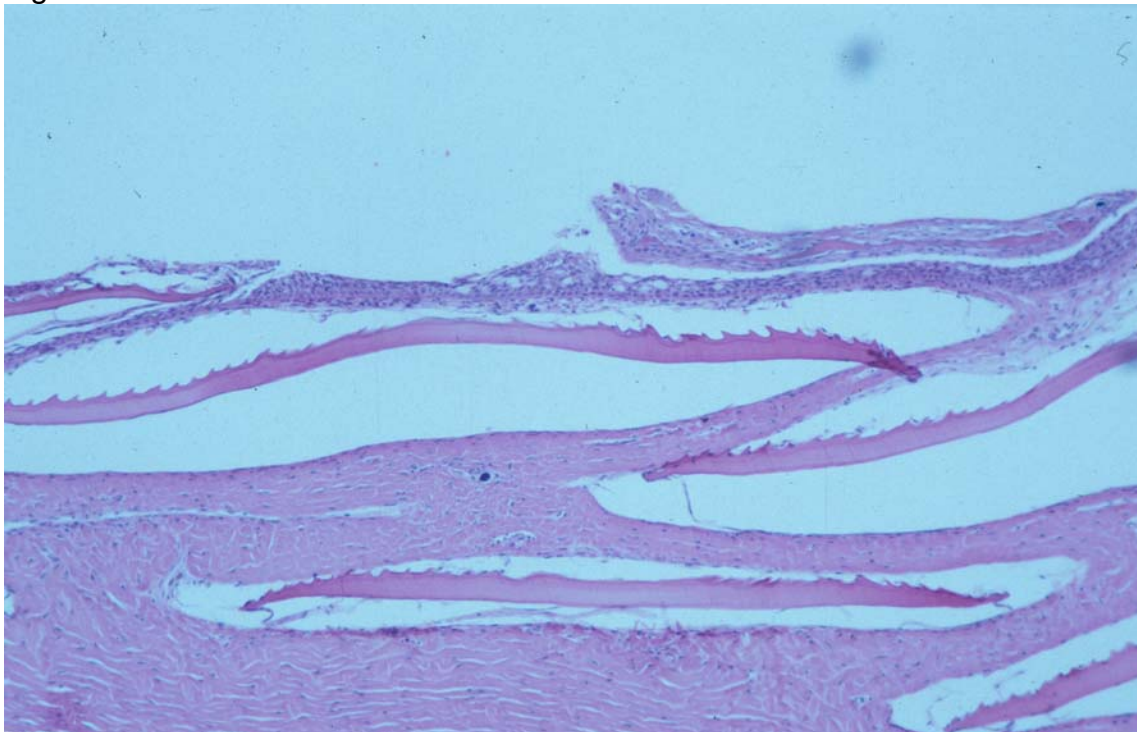


Figure 7. Skin - acute cellular swelling and hydropic change

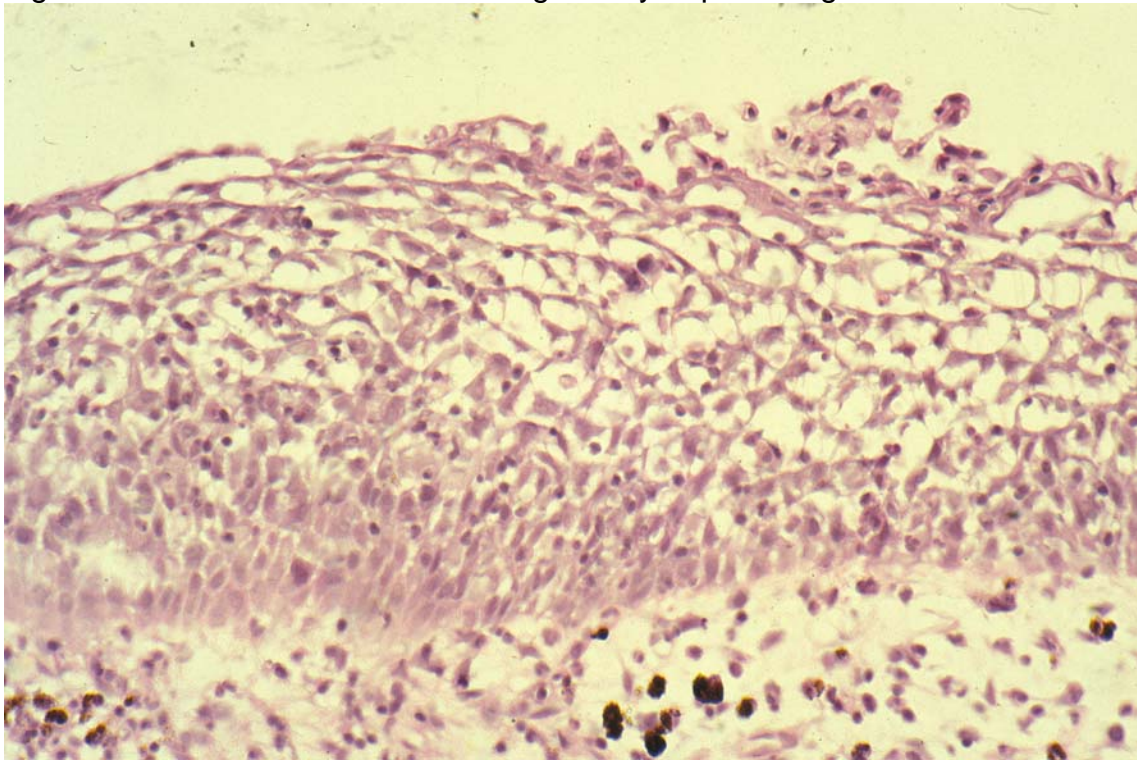


Figure 8. Skin - ulcerative skin lesion is a likely site of cellular swelling



Figure 9. Liver - moderate fatty change

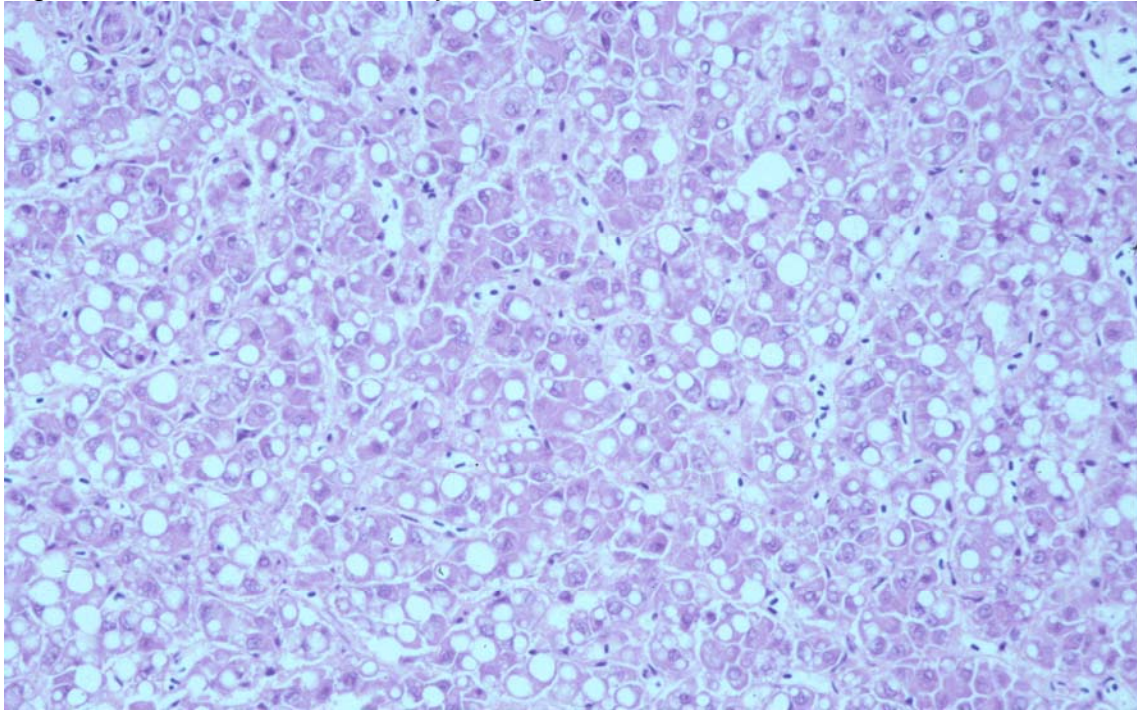


Figure 10. Liver - severe fatty change

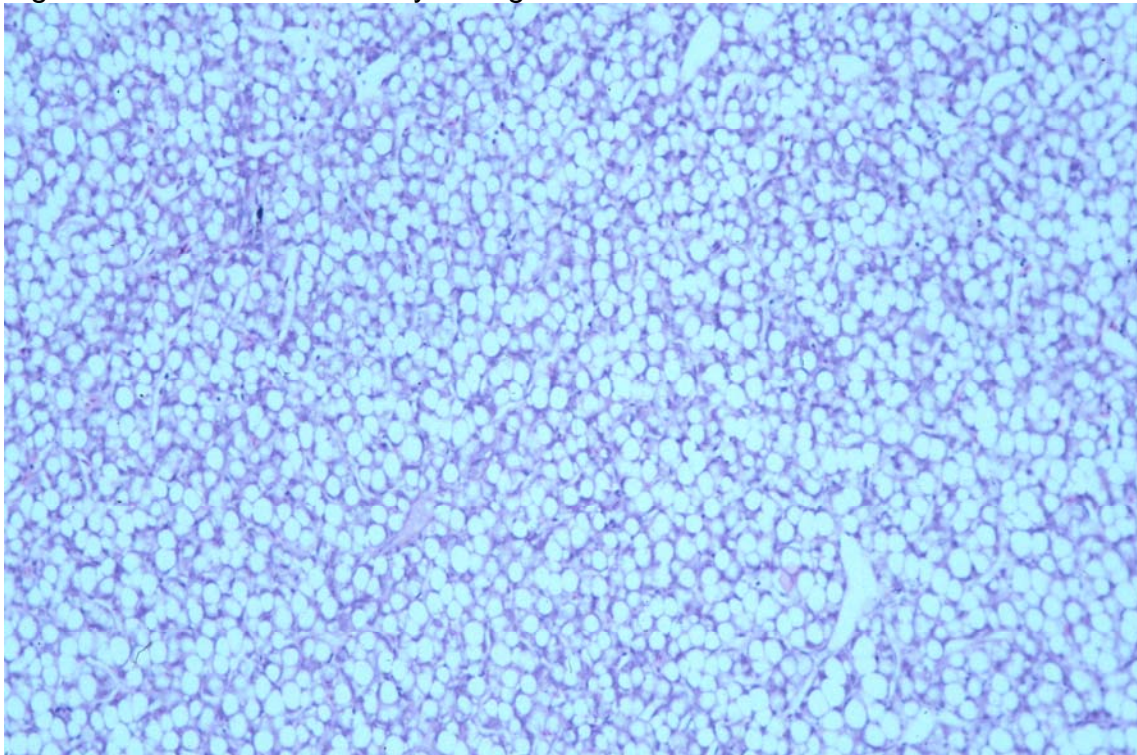


Figure 11. Liver - severe fatty change

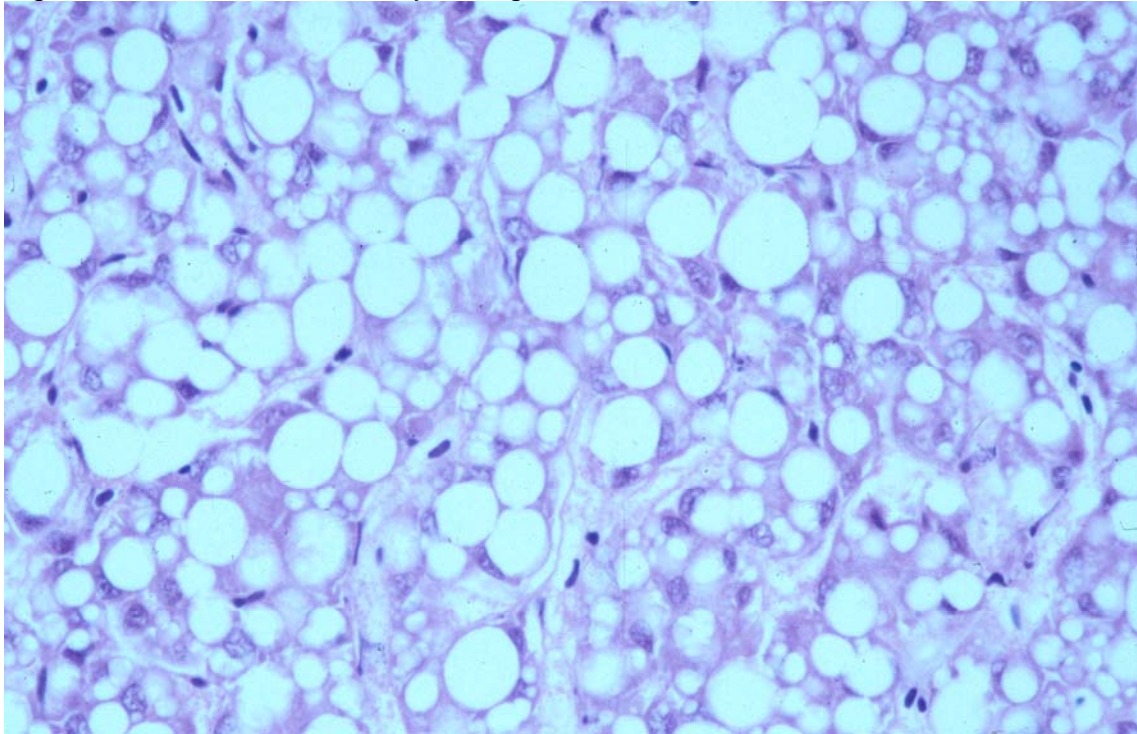


Figure 12. Liver - pale due to fat content

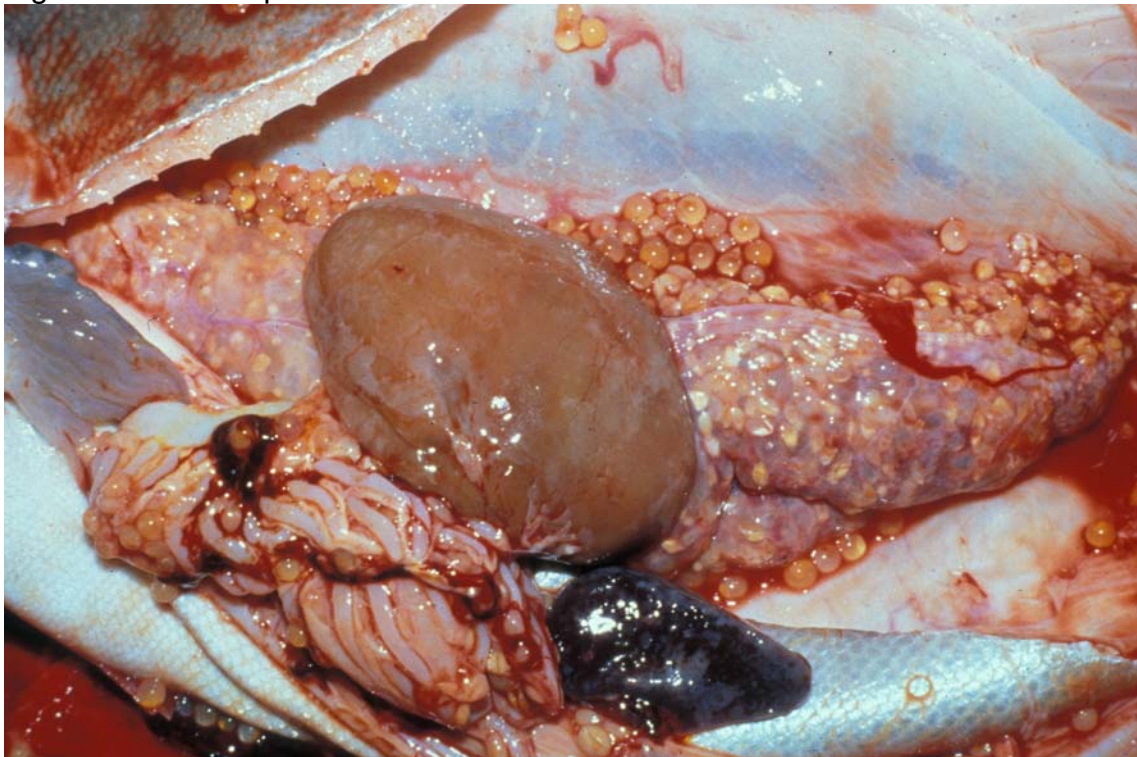


Figure 13. Kidney - necrosis

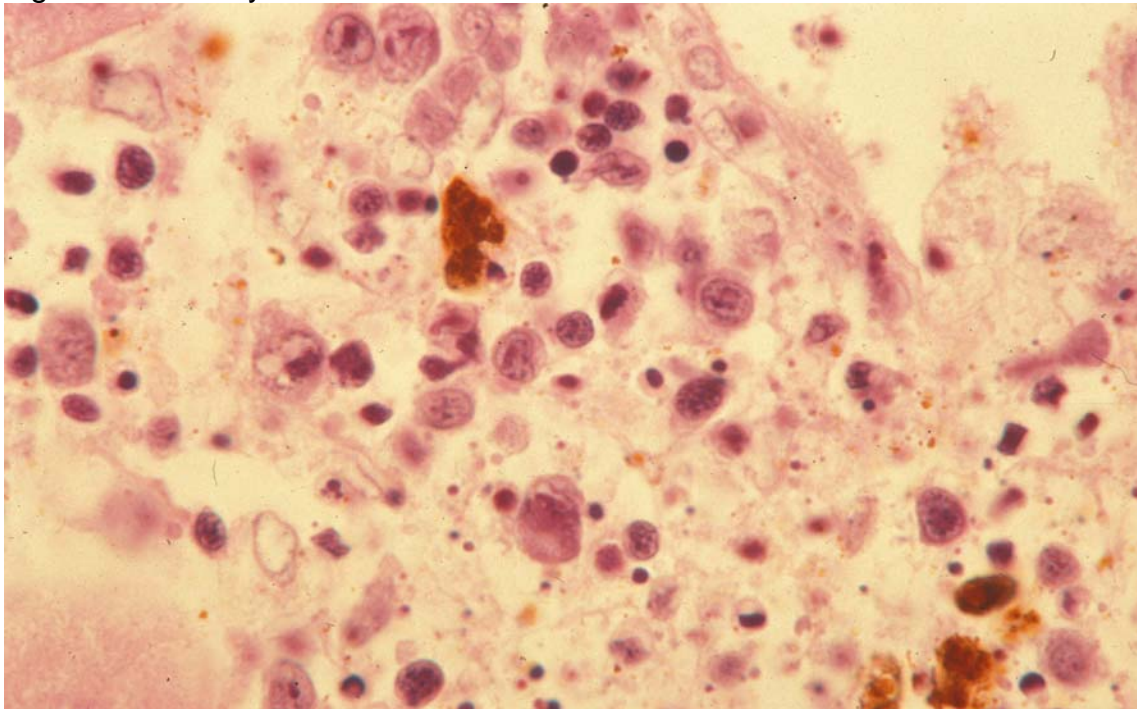


Figure 14. Kidney - necrosis

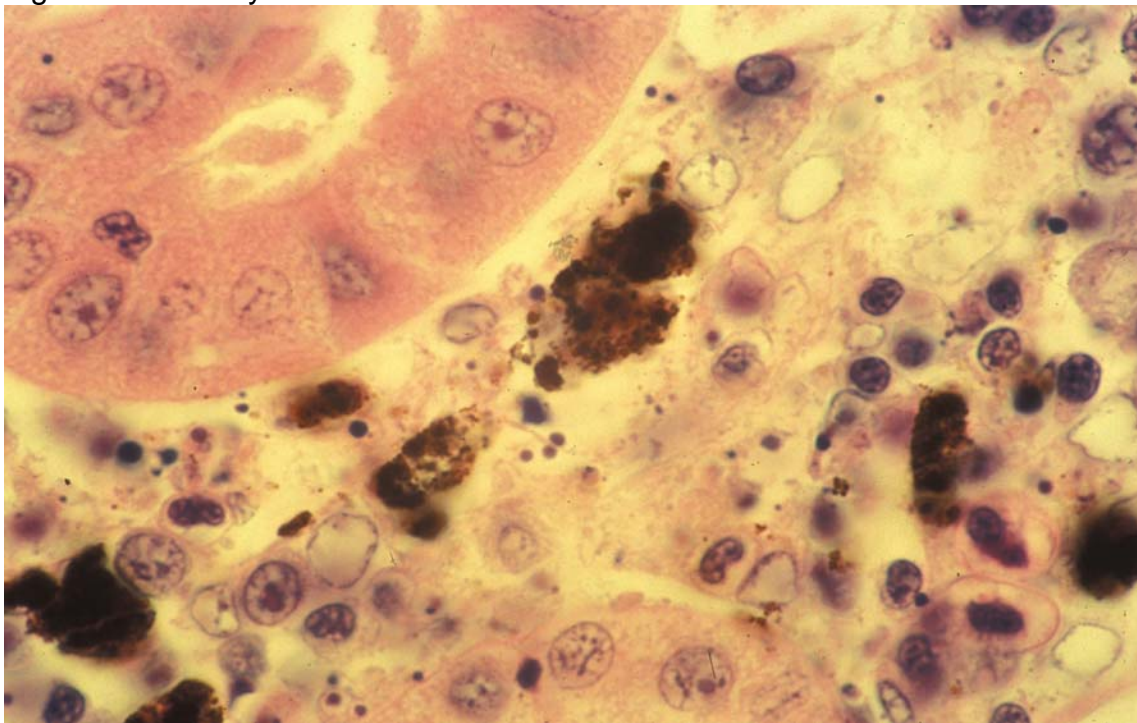


Figure 15. Muscle - necrosis

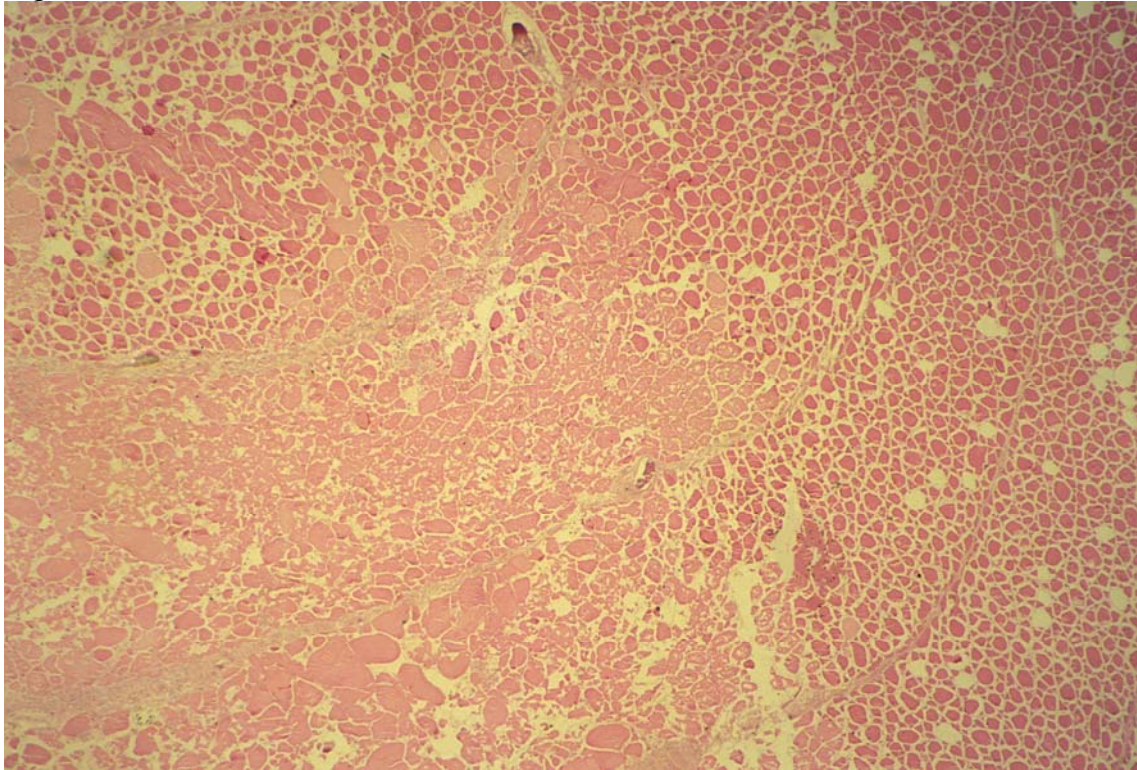


Figure 16. Muscle - necrosis

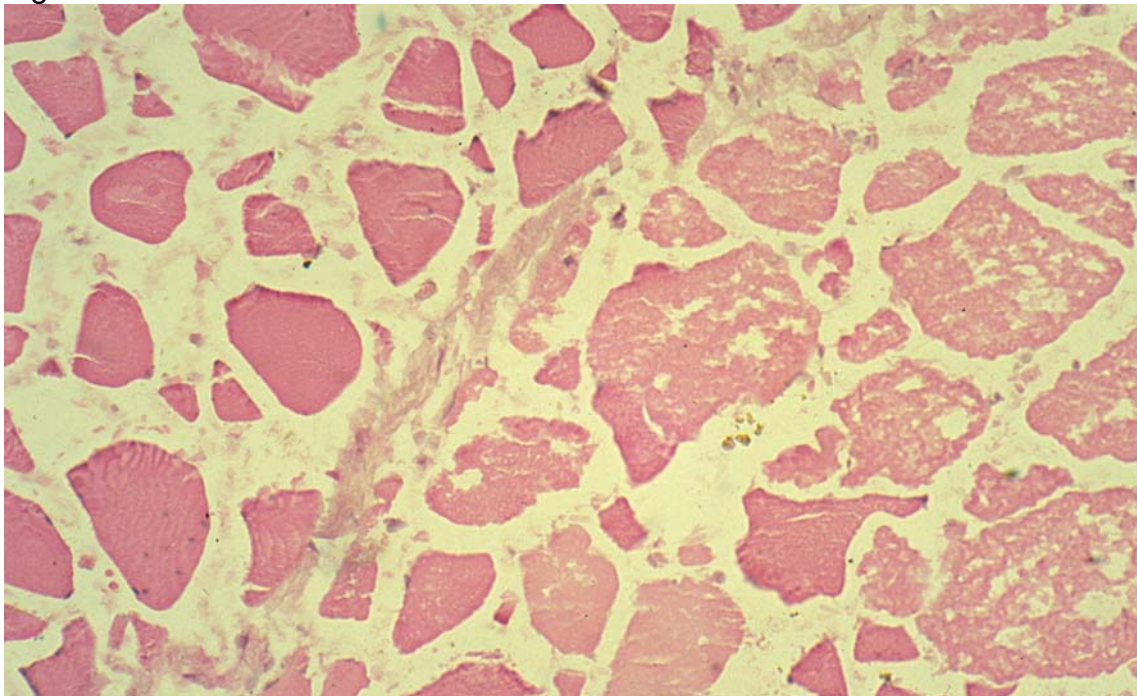


Figure 17. Liver - necrosis

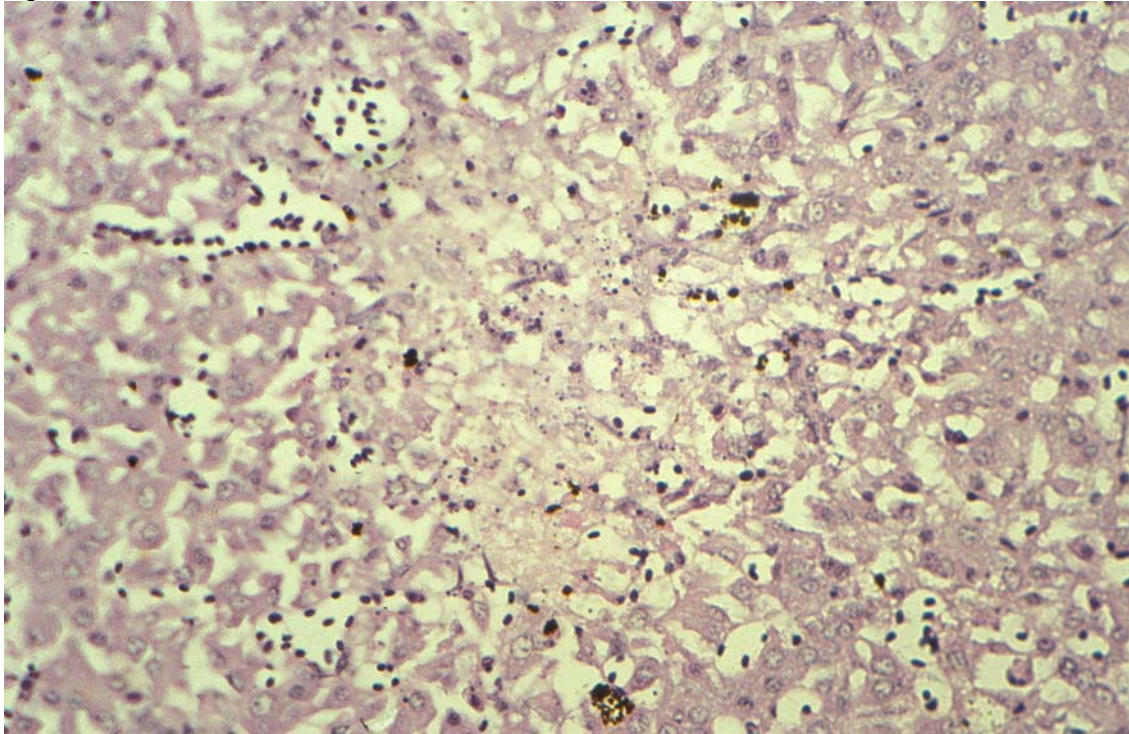


Figure 18. Pancreas - necrosis

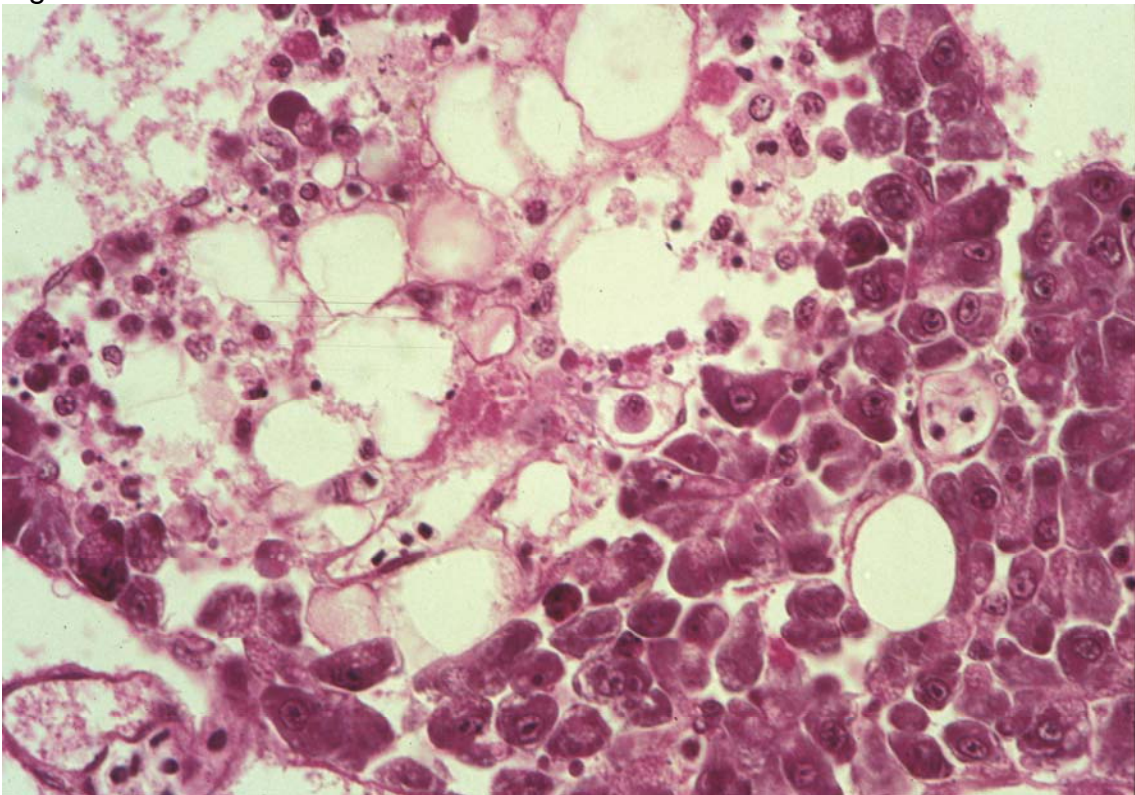


Figure 19. Liver - necrosis

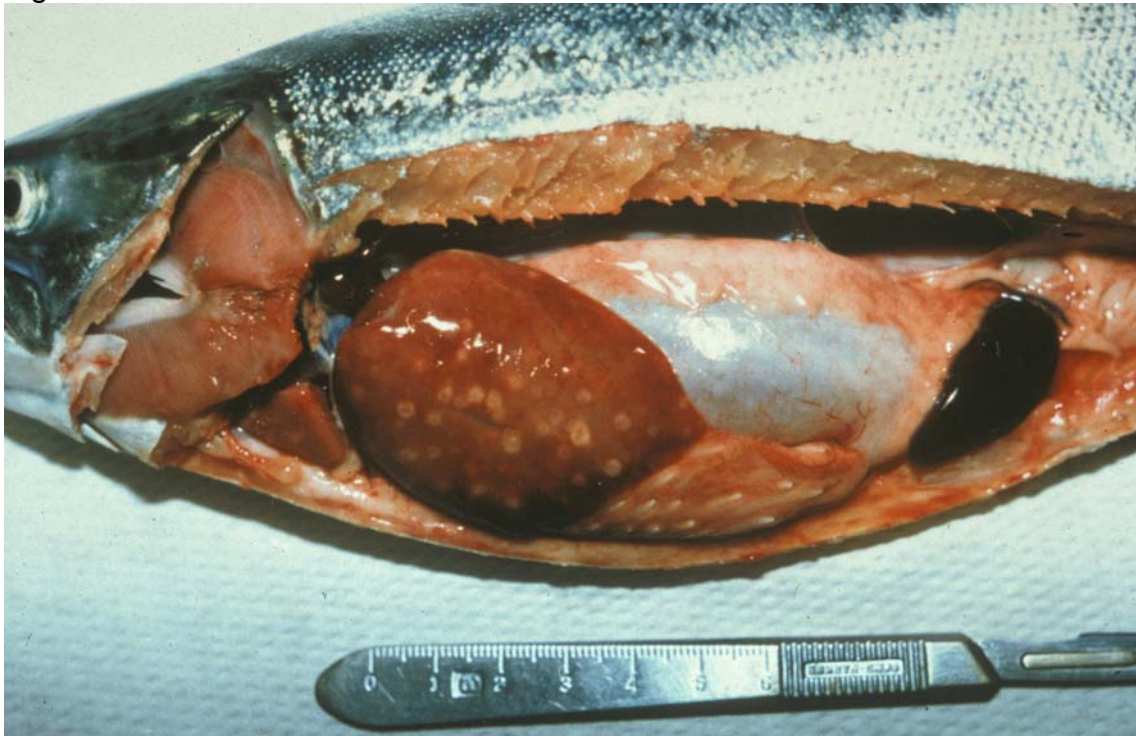
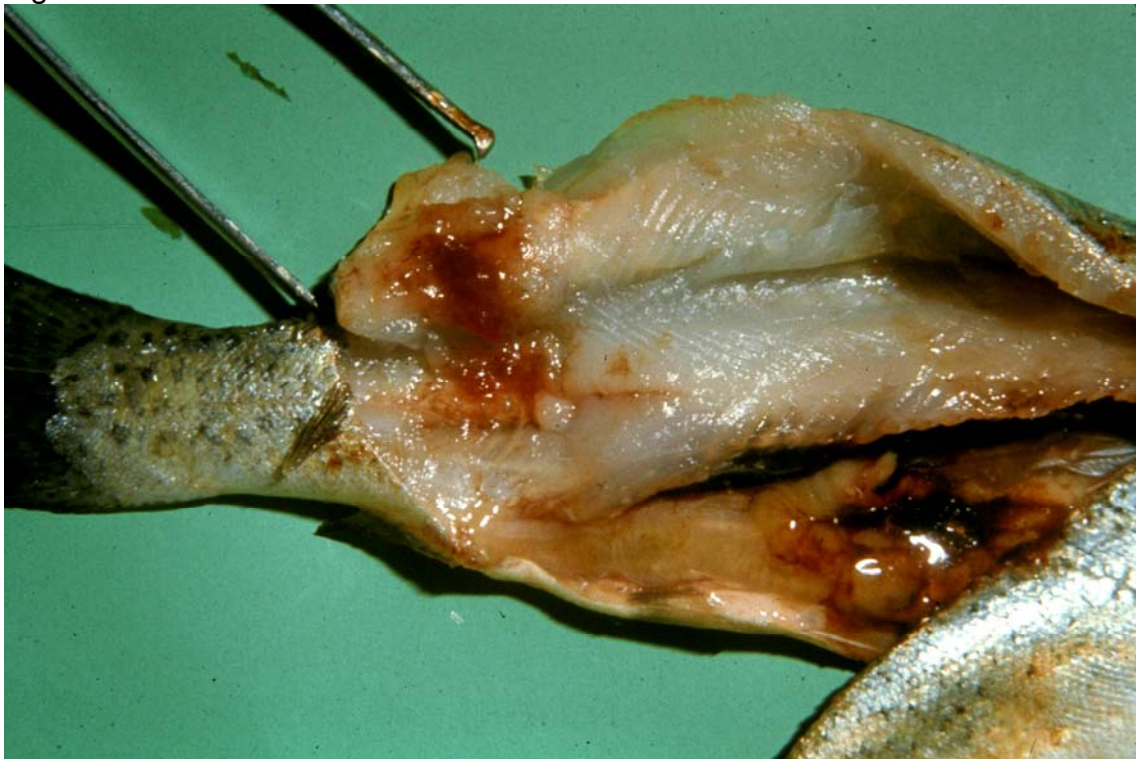


Figure 20. Muscle - necrosis



Disturbances of Circulation

Congestion and Hyperemia (Figures 21 through 24)

Hyperemia (too much blood being delivered to a site) implies an active, arterial-side engorgement of the vascular bed, while congestion (too little blood removed from a site) indicates a passive, venous engorgement. Hyperemia is usually accompanied by evidence of inflammation, and is associated with vascular dilation due to localized release of inflammatory mediators. Passive congestion is associated with reduction in venous outflow due to non-inflammatory events such as cardiac failure, or constriction or obstruction of vascular outflow due to tissue torsions, tumors, or other compressive events. It is often difficult to distinguish hyperemia from congestion histologically; the distinction is usually more obvious at the gross level.

Hemorrhage (Figures 25 through 28)

Hemorrhage is the escape of blood from the vascular system. It is caused by injury to vascular endothelium; this can be due to infection, inflammation, necrosis, neoplasia, or trauma.

Thrombosis (Figure 29)

Thrombosis is the result of activation of the coagulation cascade within the vasculature or heart of a living animal. The resultant mass is a thrombus (thrombi). Thrombi obstruct blood flow, depriving tissues of blood. Ischemia (deprivation of oxygenated blood), results in necrosis of the dependent tissue.

Thrombi can fragment, releasing emboli into the circulation. These in turn can lodge in small vessels, obstruct blood flow, and cause ischemic necrosis.

Infarction

An infarct is a localized area of ischemic necrosis, i.e. necrosis due to loss of blood supply to the tissue. This can be due to thrombosis, embolism, or other vascular obstruction. The necrosis observed in infarcts is most often coagulative, i.e. cell and tissue structure is preserved despite being populated by necrotic cells. The affected area, or infarct, is usually well demarcated from adjacent viable tissue.

Edema (Figure 30)

The accumulation of excessive amounts of extracellular fluid in the interstitial spaces or body cavities is edema. It is often generalized and evident at the gross level. Causes include changes in hydrostatic or osmotic pressure (cardiac failure, vascular obstructions, hypoproteinemia) and increases in vascular permeability that accompanies inflammation.

Figure 21. Blood vessel - normal

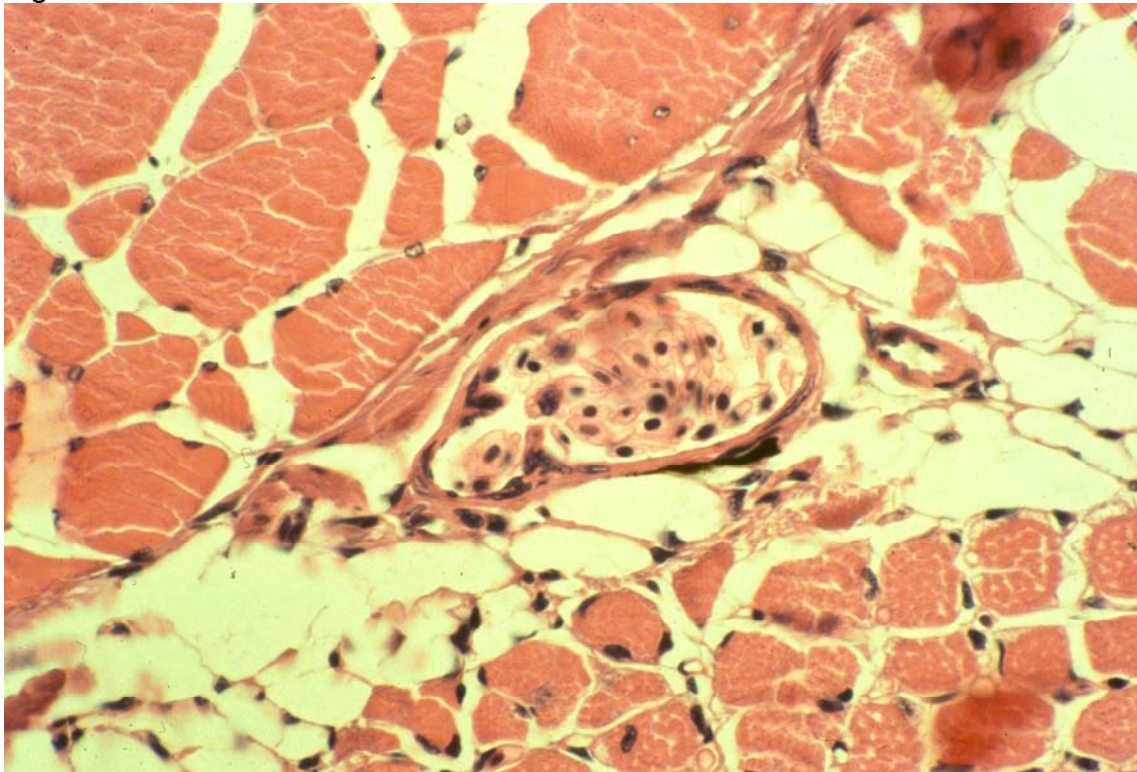


Figure 22. Blood vessel - hyperemia/congestion

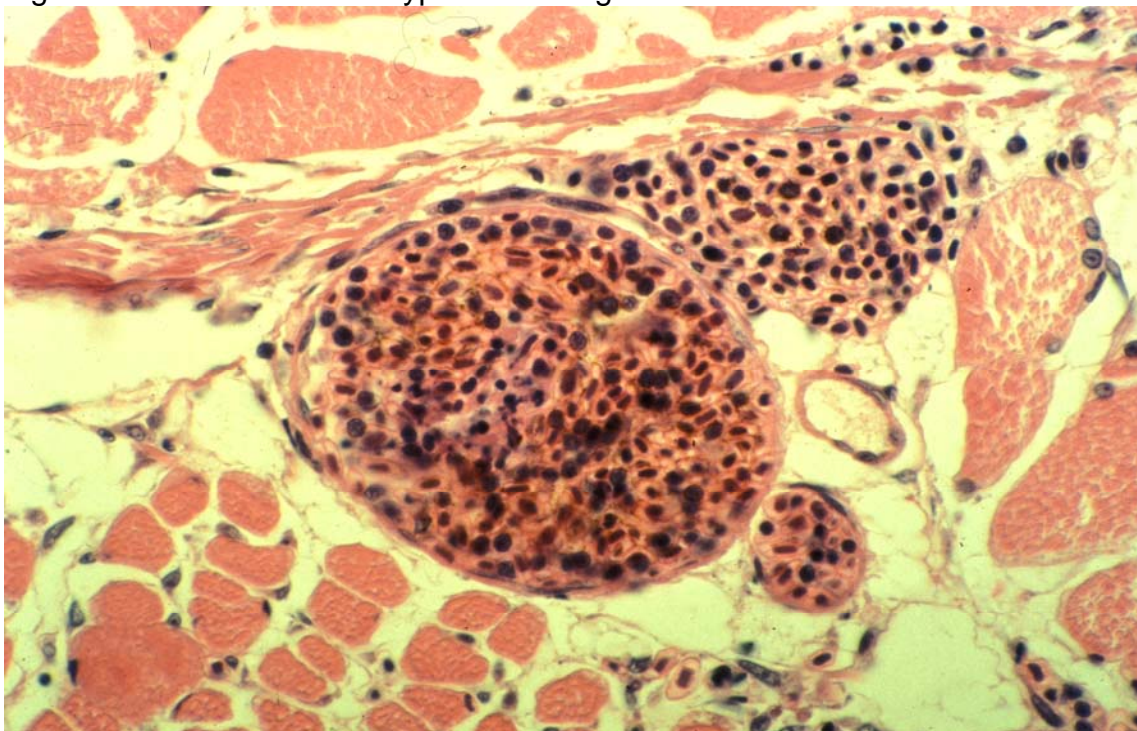


Figure 23. Spleen - congestion

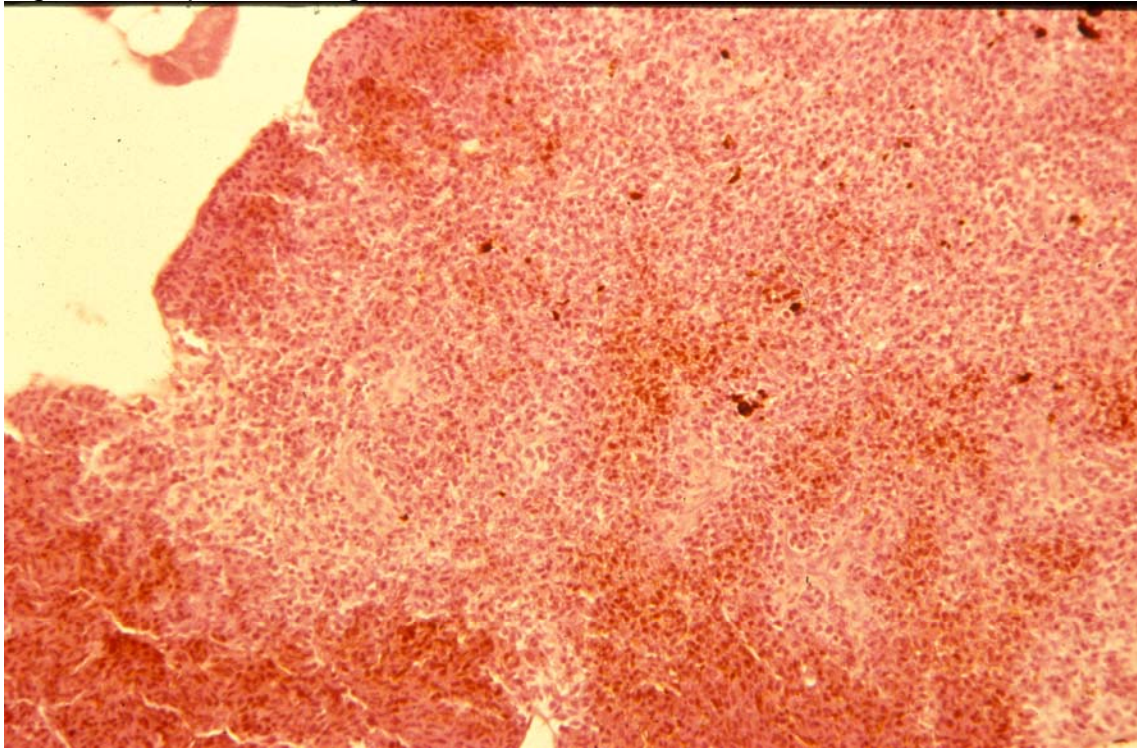


Figure 24. Spleen - congestion

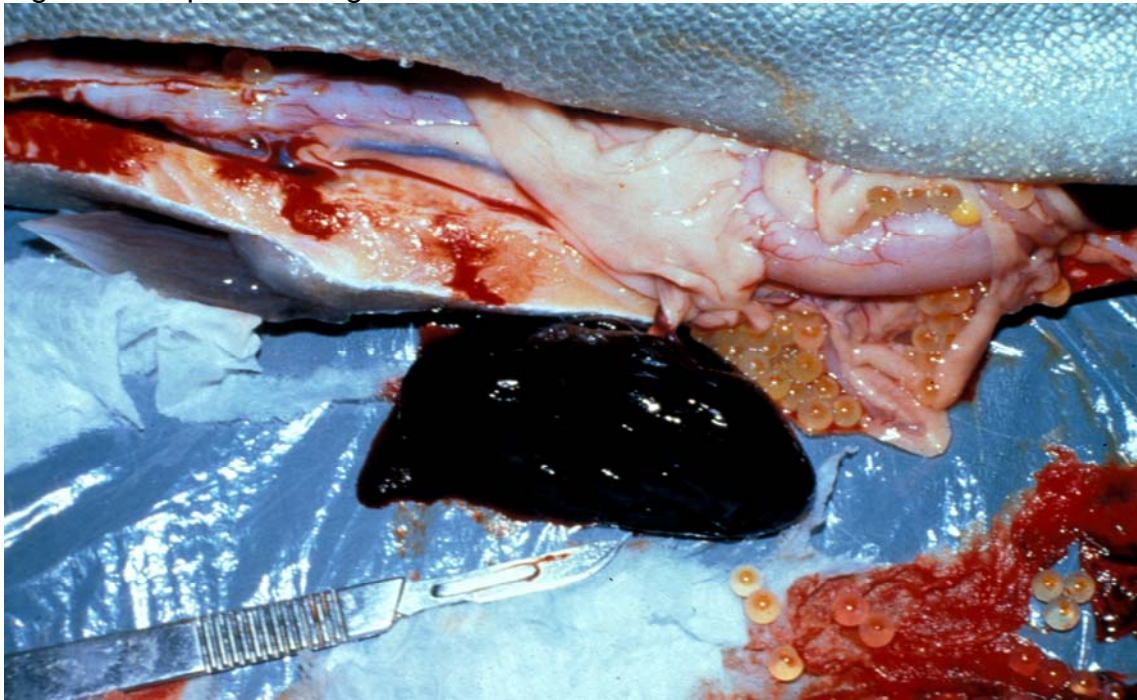


Figure 25. Hemorrhage

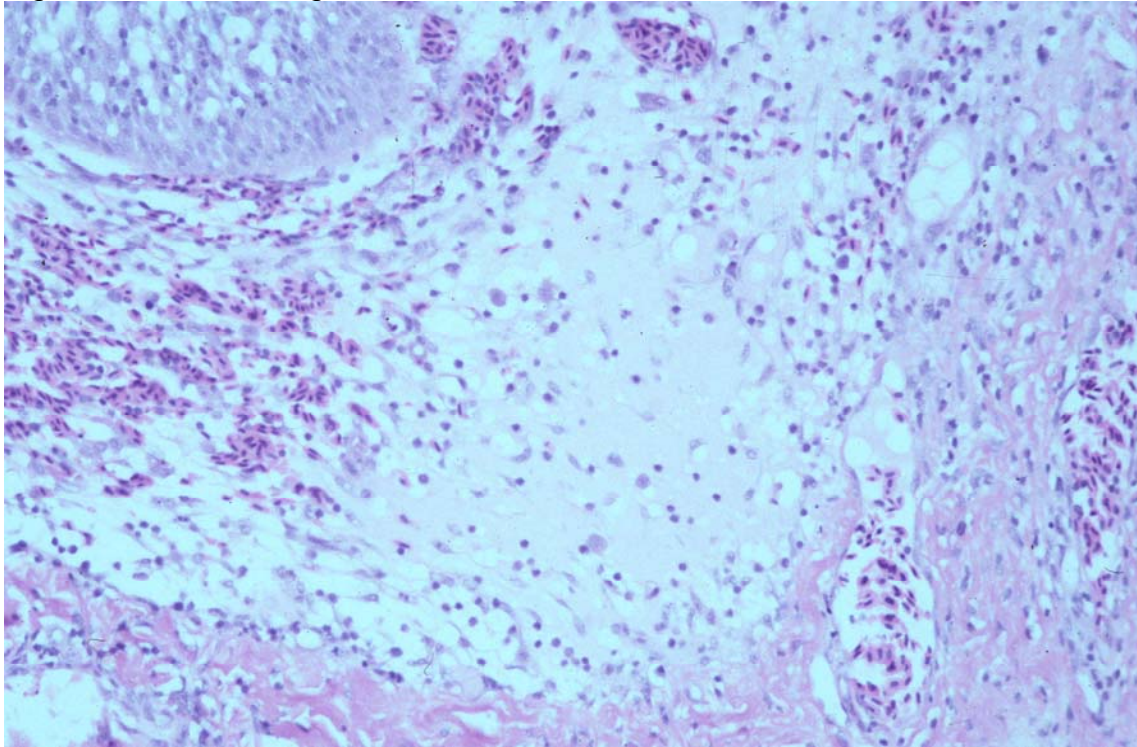


Figure 26. Hemorrhage (operculum, body wall)

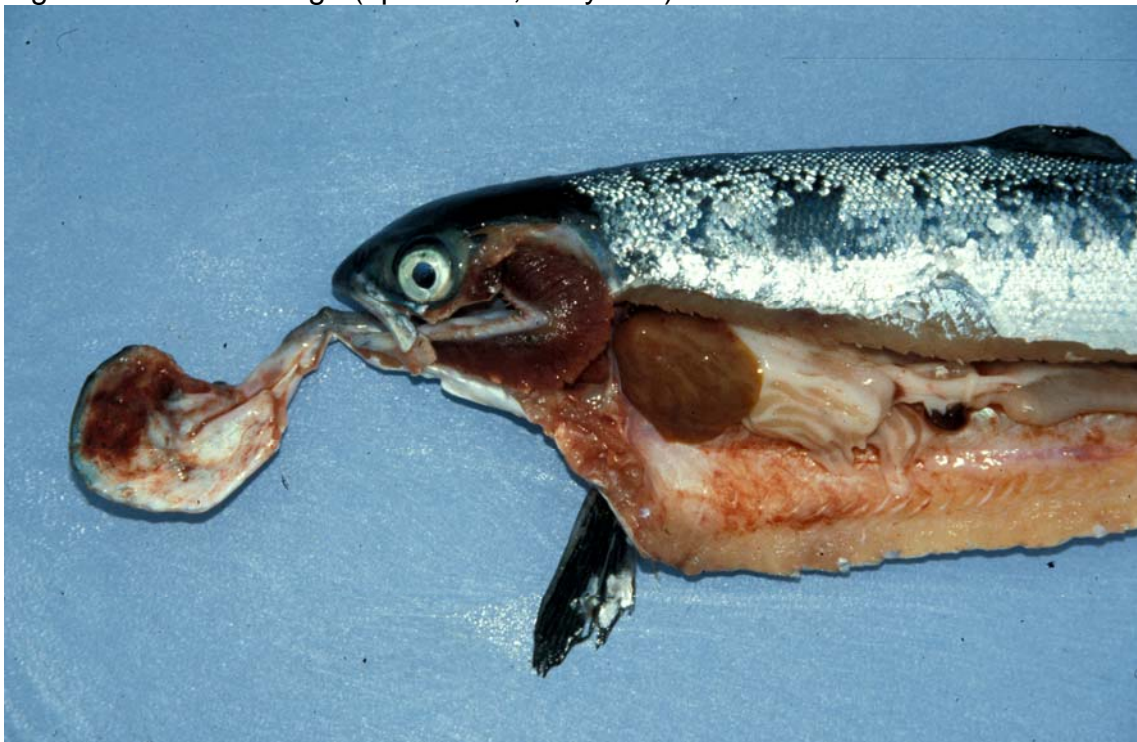


Figure 27. Hemorrhage



Figure 28. Hemorrhage



Figure 29. Thrombus

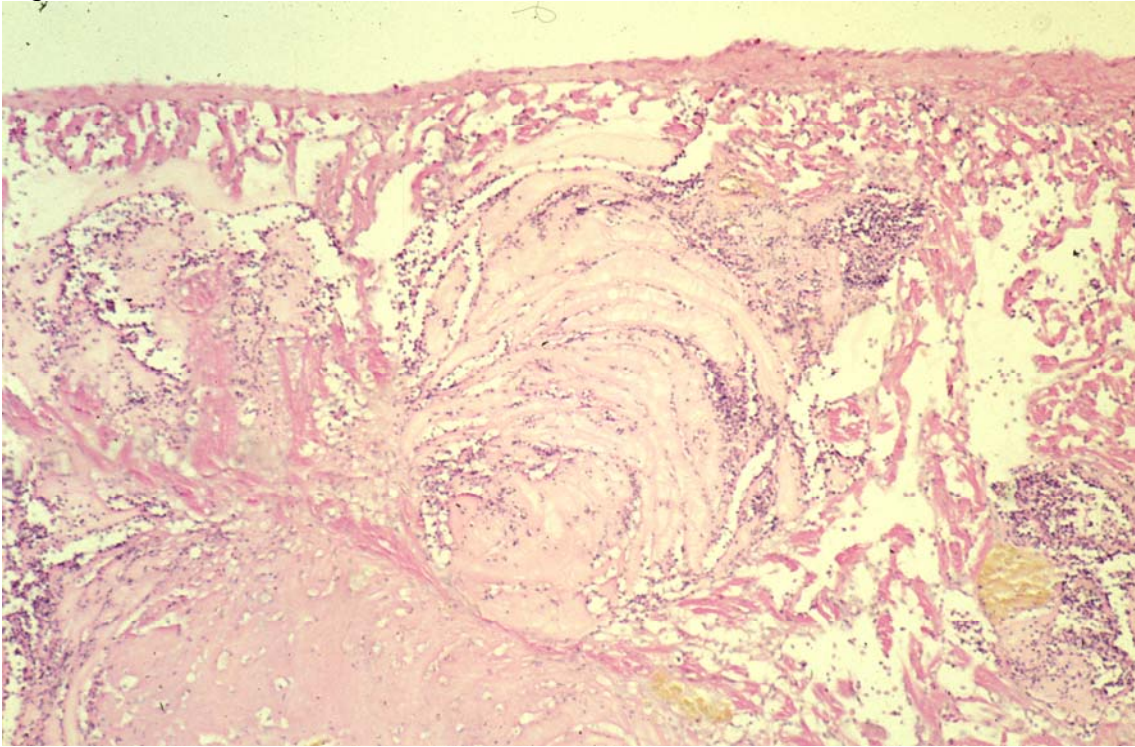
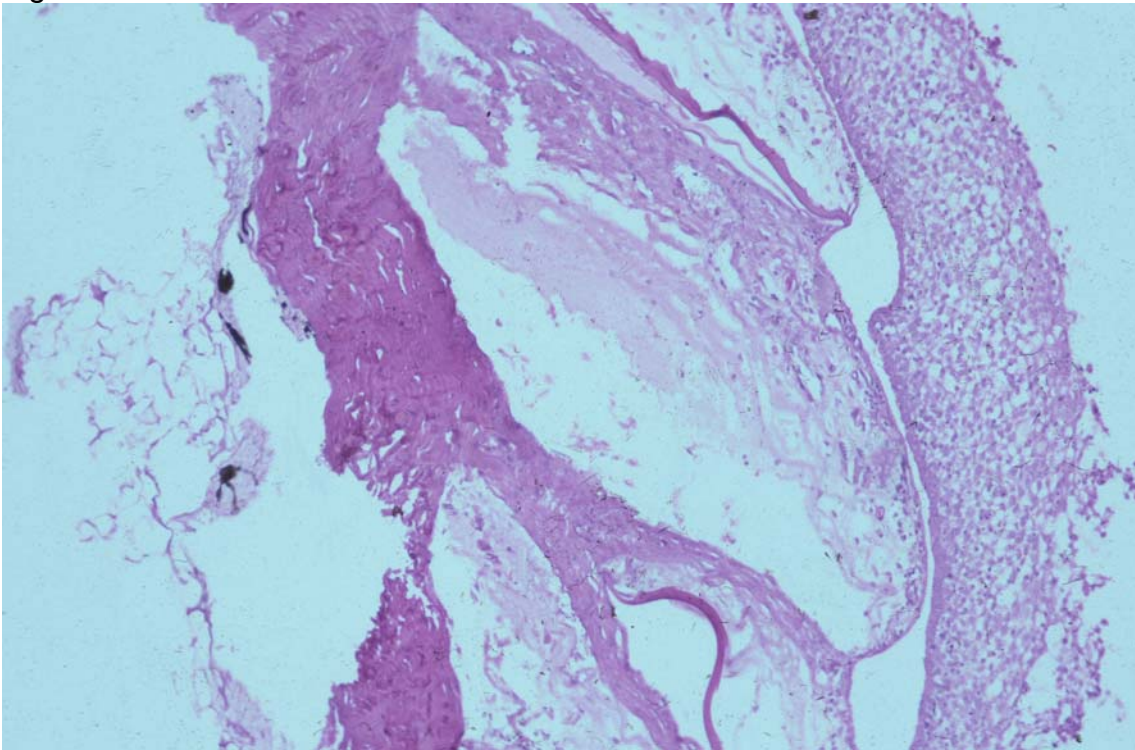


Figure 30. Skin - edema



Inflammation and Healing

Inflammation is a stereotyped response in a living animal to a variety of injuries that involves the vasculature, various inflammatory cells, and potent chemical mediators. The purpose of the inflammatory response is to dilute, isolate, and destroy the injurious agent, and to facilitate healing. The body only has a limited number of ways to respond to an injury, hence the pathogenesis of an inflammatory lesion and the histological appearance of that lesion can be similar whether the injury was caused by a bacterial cell, a foreign body, ionizing radiation, a toxin, or trauma.

Inflammation can be divided into acute and chronic forms which differ histologically as well as in duration. Acute inflammatory episodes, characterized by vascular events and exudation, usually progress over a period of 3 to 10 days, then resolve as the injurious agent is eliminated. Chronic inflammation, characterized by cellular proliferation, can extend from weeks to months to the lifetime of the host, continuing as long as the injurious agent persists.

Inflammation is an important protective process for the host. Defects in the inflammatory response can lead to chronic illness as well as death. In addition to its protective function, inflammation also sets the stage for healing and repair.

Acute inflammation (Figures 31 through 40)

Acute inflammation is a complex interplay of a functioning vascular system, circulating and tissue-based inflammatory cells, and chemical mediators. It is characterized by exudation, the release of fluid and cells from the vasculature into the injured tissue. Following an injury, chemical signals from host cells result in rapid vasodilation and increased vascular permeability in capillaries and post-capillary venules. Immediately, protein rich fluid leaks from the vessels, bathing the site of injury in inflammatory. This fluid contains antibodies, complement, fibrin, and other host defense chemicals. The composition of this inflammatory edema fluid varies with the nature, severity, and duration of the injury, with some being simply watery with smaller proteins to others harboring larger proteins such as fibrin. Grossly, the inflamed tissues will be watery (“serous exudate”). If there is a fibrin component (“fibrinous exudates”), you will find web-like strands or sheets of polymerized fibrin adherent to the tissues. Histologically, these exudates consist of eosinophilic staining in the intercellular space; fibrin will have the appearance of eosinophilic strands.

More severe injuries, particularly bacterial infections, will elicit a cellular component to the exudate. As the vessels dilate and become leaky, neutrophils will move from the bloodstream, marginate to the vessel wall, stick to the endothelium, and migrate between endothelial cells into the extravascular space and proceed to undergo directed migration (chemotaxis) towards the site of injury. These inflammatory cells will then phagocytize and destroy injurious agents such as bacteria. Neutrophilic exudates are liquids of varying consistencies containing varying numbers of neutrophils, and tend to be yellow to tan; pus formation and the classic abscess are typical of a neutrophilic exudate. While most animals readily form neutrophilic (also known as suppurative or purulent) exudates or form abscesses in response to bacterial infections, fish are far less responsive; neutrophilic inflammation will be found, but true pus formation is not seen. Neutrophils are considered to be less phagocytic in fish as opposed to other animals, and don’t form the massive clusters seen in other species. Histologically, neutrophilic responses are characterized by the presence of neutrophils in and around

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the focus of injury. Hint: look for hypercellularity in a low power field as a clue to the presence of inflammation.

Other inflammatory cells can contribute to an acute inflammatory response. It is not unusual to find a few lymphocytes or macrophages in the area; these will increase in number if the injury is not resolved and the process moves toward chronic inflammation.

As the acute inflammatory response resolves, the exudates and any necrotic tissue is removed by macrophages, and the stage is set for healing and repair.

Inflammatory exudates:

- Serous exudate: protein-rich, watery fluid; contains protective proteins such as antibodies and complement; eosinophilic material on histo; e.g. blister
- Mucous exudate: from mucous membrane surfaces, due to proliferation of goblet cells; amphophilic on histo; e.g. mucoid enteritis
- Fibrinous exudate: polymerized fibrin forms a major component of the exudate; strands or sheets adherent to affected tissue; often associated with bacterial infections; eosinophilic strands on histo; e.g. fibrinous peritonitis
- Neutrophilic exudate: neutrophils are the prominent cellular component; common with bacterial infections in most animal species, but this type of exudate is not as prominent in fish as in other species; e.g. abscess

Chronic inflammation (Figures 41 through 56)

If the stimulus persists, the inflammatory response becomes chronic in duration, and the morphology of the lesion is different. Here, macrophages, lymphocytes, and plasma cells predominate, and fibrosis can be significant as well. There is not clear line of demarcation between acute and chronic inflammation; they blend over time. Chronic inflammation usually progresses from an acute inflammatory episode, but primary chronic inflammatory conditions will occur with some injuries. The duration of the chronic inflammatory response can range from weeks to months to the life of the patient.

Chronic inflammatory lesions are proliferative and not exudative. There is no flood of fluid and cells from the small blood vessels. Rather, resident or migrating chronic inflammatory cells accumulate and proliferate at the site of injury, and a mass type lesion is formed. In fact, chronic inflammatory lesions and neoplasms can sometimes be difficult to differentiate grossly.

Chronic inflammatory lesions can be composed purely of macrophages, lymphocytes, or plasma cells. Macrophages provide phagocytic and killing activity, whereas the other cell types provide antibody and cell-mediated immune activity.

Granulomatous inflammation is the more commonly observed form of chronic inflammation in fish as well as other animals. It consists of a mixture of macrophages, lymphocytes, plasma cells, fibroblasts, and sometimes neutrophils, all oriented in and around the site of injury. The lesions of BKD and mycobacteria are examples of this type of inflammatory response. Multinucleated giant cells or epithelioid macrophages are often found in these sites as well. NOTE: Do not confuse granulomatous inflammation with granulation tissue. The latter is a fibrovascular proliferation associated with the healing response.

Granulomas may also be observed in foci of granulomatous inflammation. These are defined nodular collections of macrophages and debris, encircled by a layer of

lymphocytes, in turn encircled by a layer of fibroblasts. These are not uncommon in mycobacteriosis.

As with acute inflammation, if the cause of the injury is neutralized by the chronic response, healing will progress. However, some chronic inflammatory responses persist for the life of the patient, due to the tenacious nature of the offending agent.

Healing (Figure 57)

The inflammatory response sets the stage for healing. Healing can occur by regeneration of the damaged tissue or scar formation. The form of healing that occurs is determined by the nature of the injured tissue and its ability to regenerate as well as the severity and duration of injury. Tissues composed of cells that can readily divide (e.g. epithelium) can easily regenerate, replacing cells lost to inflammation and necrosis. However, for this to occur the infrastructure of the tissue, i.e. reticular fibers, basement membranes, etc., must remain intact and provide a scaffold for cell replacement. If that infrastructure is lost, fibrosis (scar formation) will likely occur. Fibrosis also occurs in tissues composed of cells that cannot regenerate, such as myocardial cells. Fibrosis is typical of the healing process of gaping wounds as well, particularly in the skin. In some cases of extensive tissue loss, a cavity may simply remain at the site of injury (cavitation). This is most often seen in the brain.

Figure 31. Skin - acute inflammation

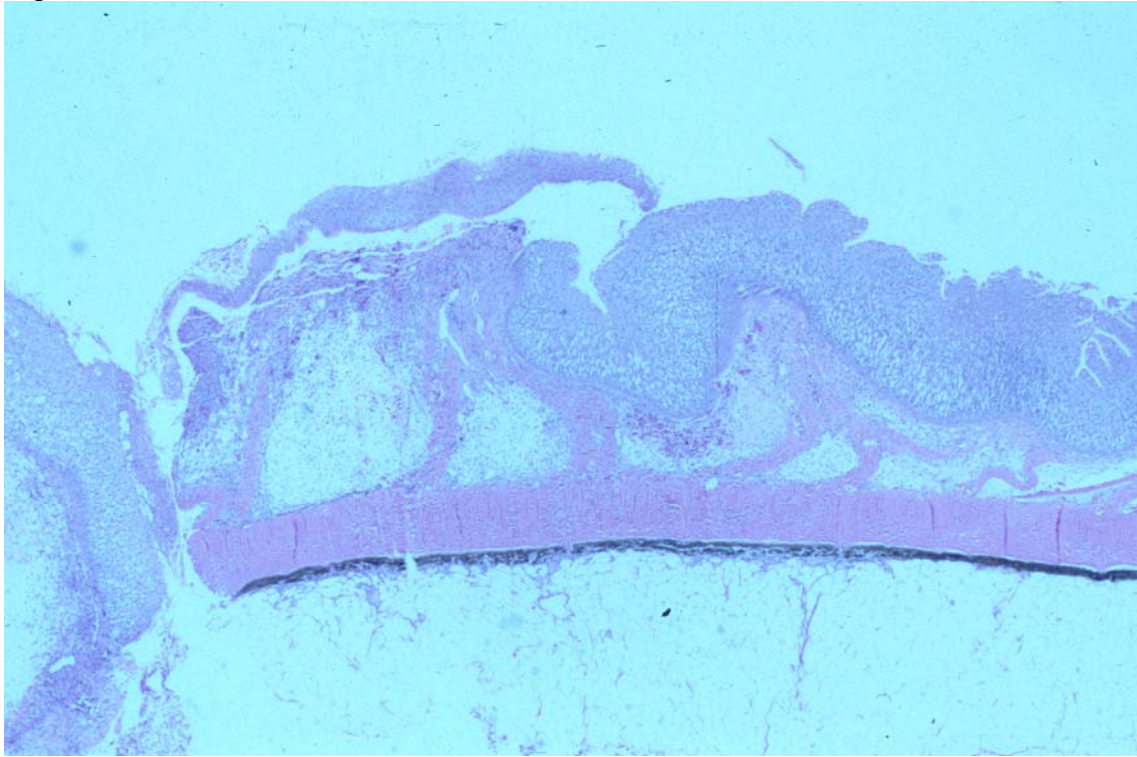


Figure 32. Skin - acute inflammation

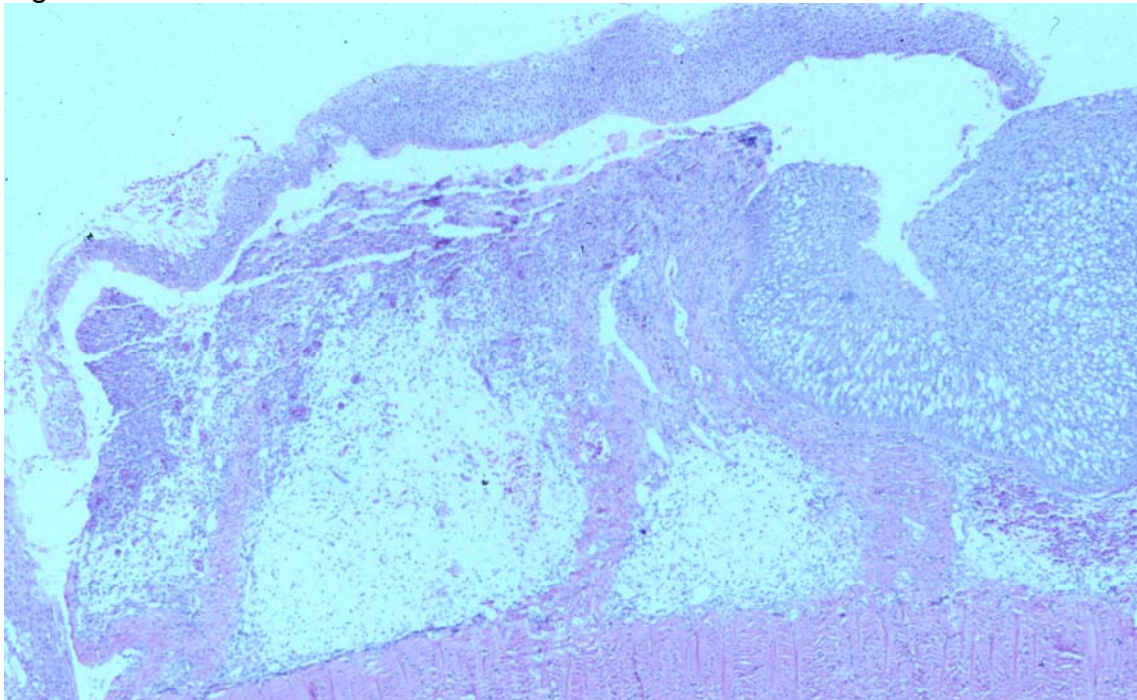


Figure 33. Skin - acute inflammation

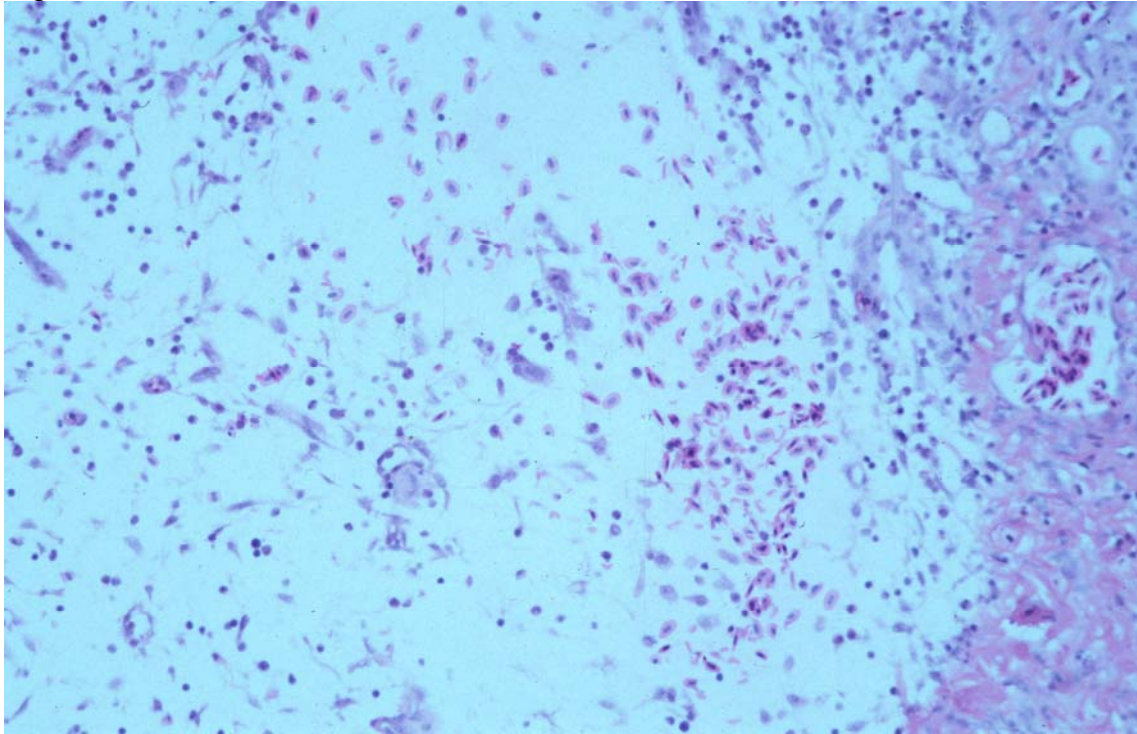


Figure 34. Skin - fibrin

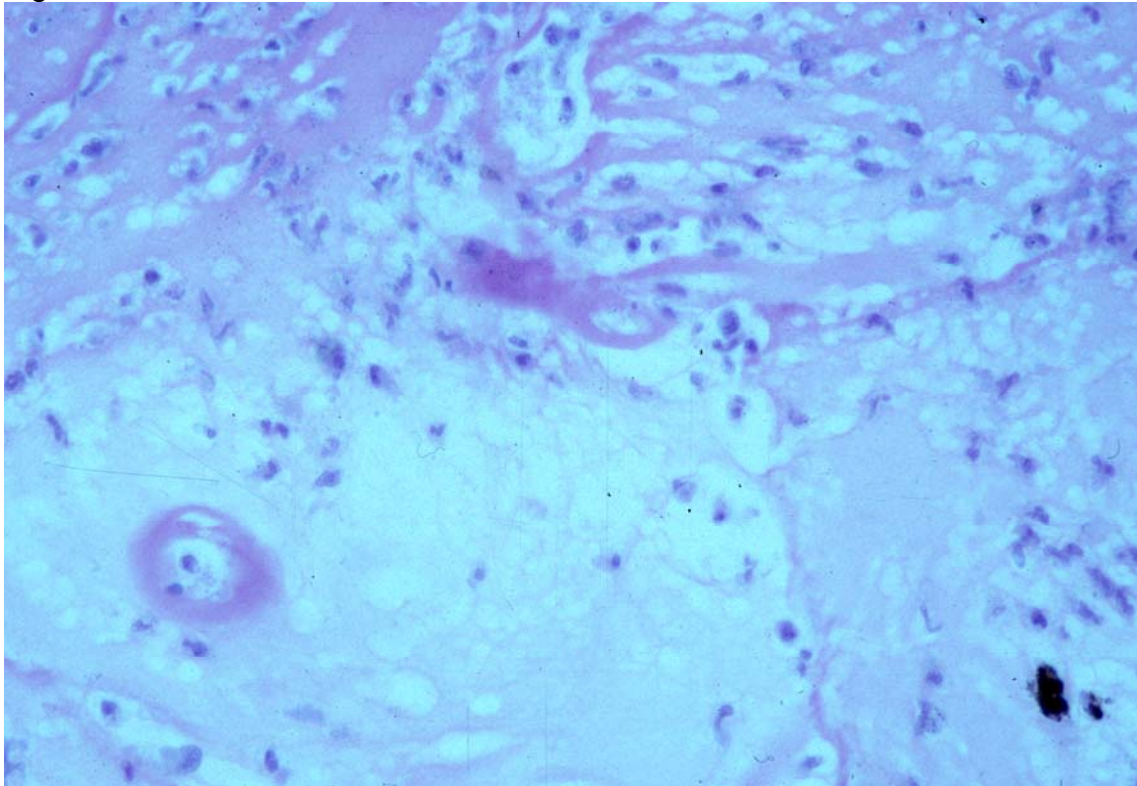


Figure 35. Spleen - fibrinous exudate (peritonitis)

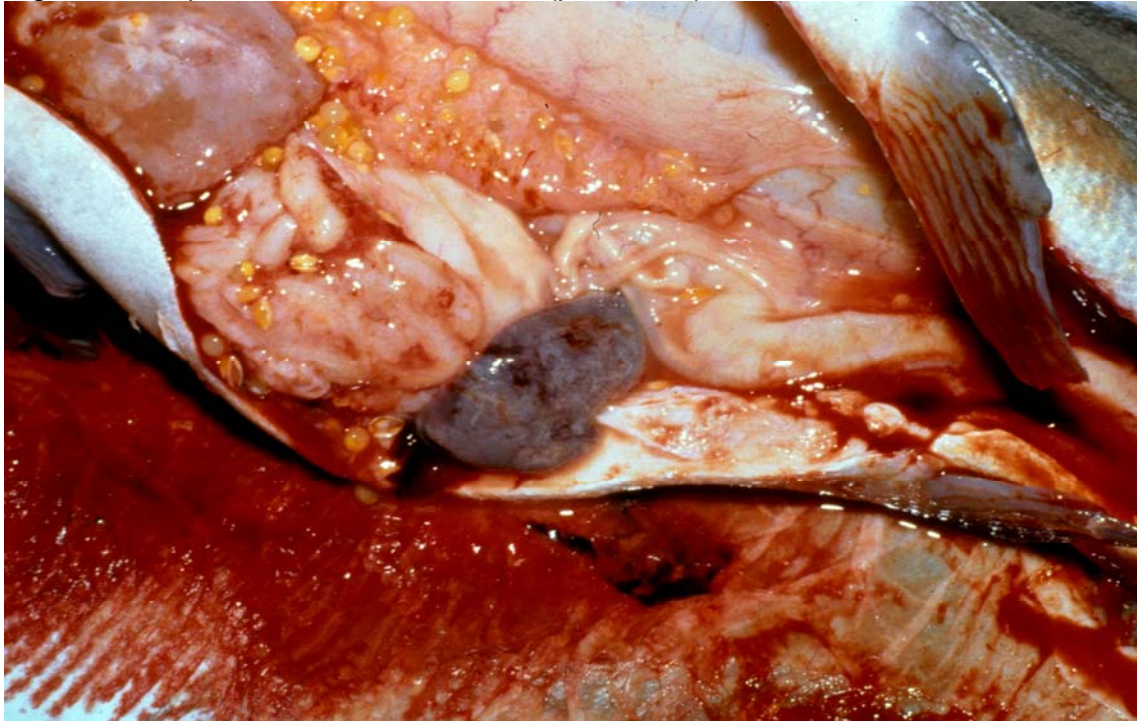


Figure 36. Heart - fibrinous epicarditis



Figure 37. Skin and subcutis - neutrophilic cellulitis

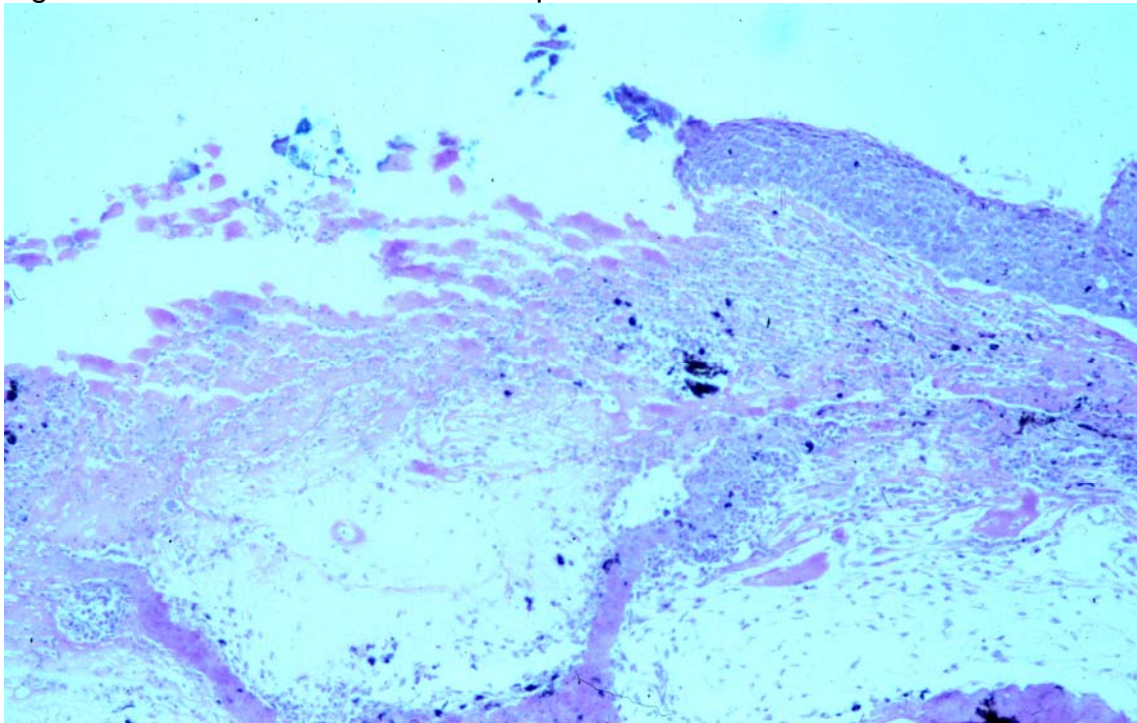


Figure 38. Neutrophilic cellulitis

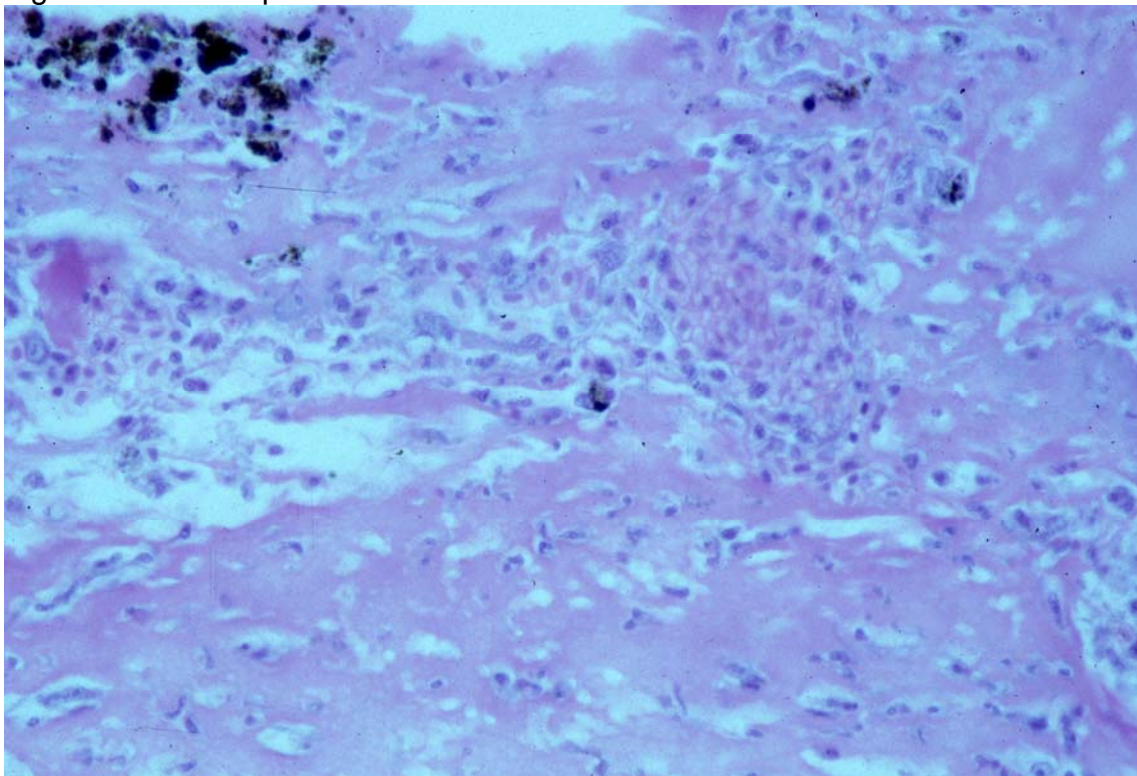


Figure 39. Kidney – Furunculosis; bacteria with no inflammatory exudate

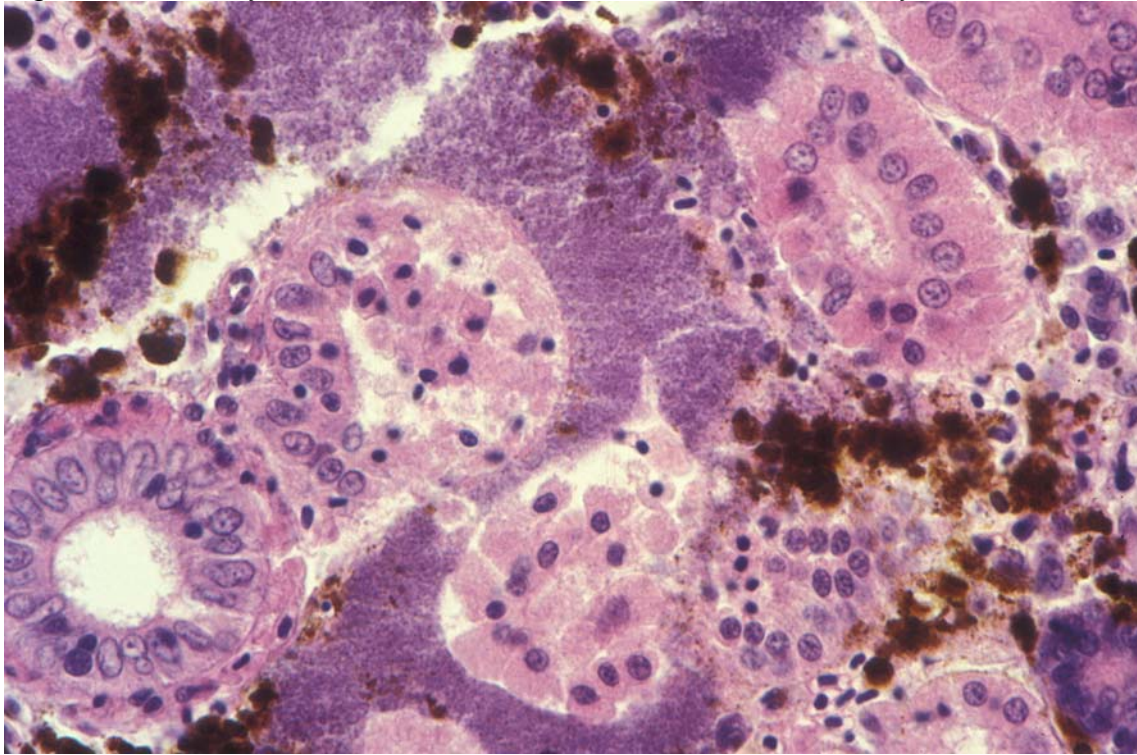


Figure 40. Hypercellularity associated with inflammation

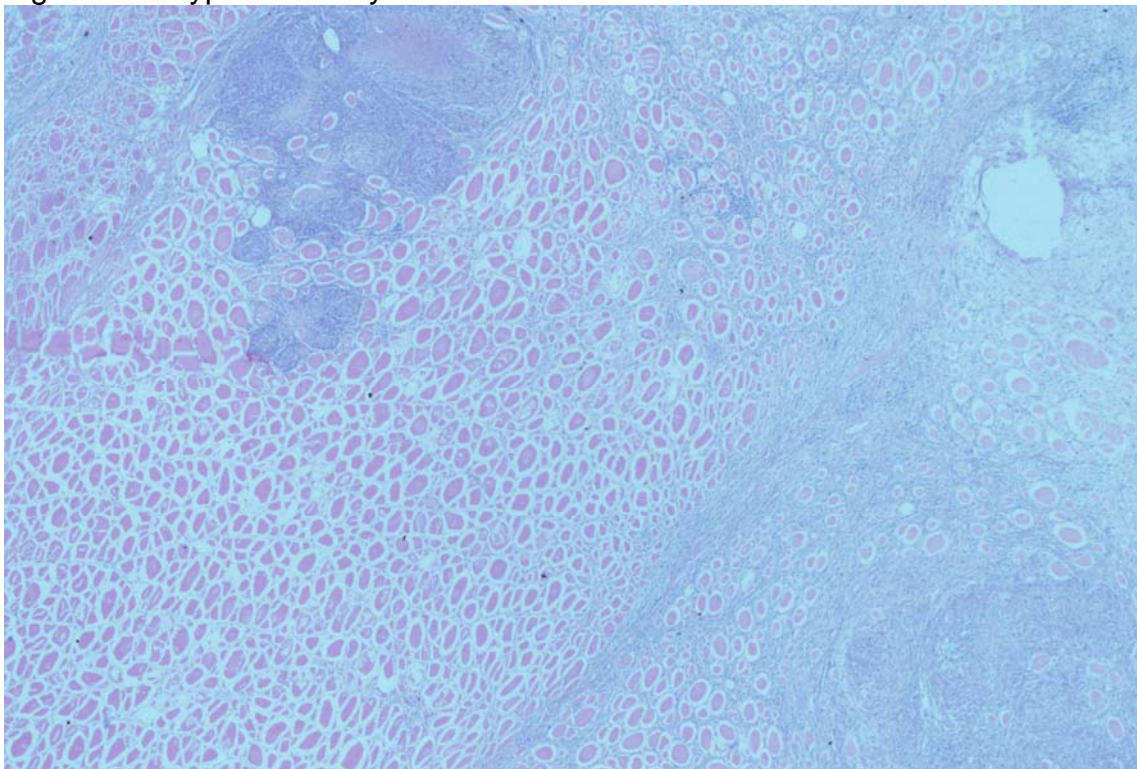


Figure 41. Kidney - chronic inflammation (nephritis); BKD

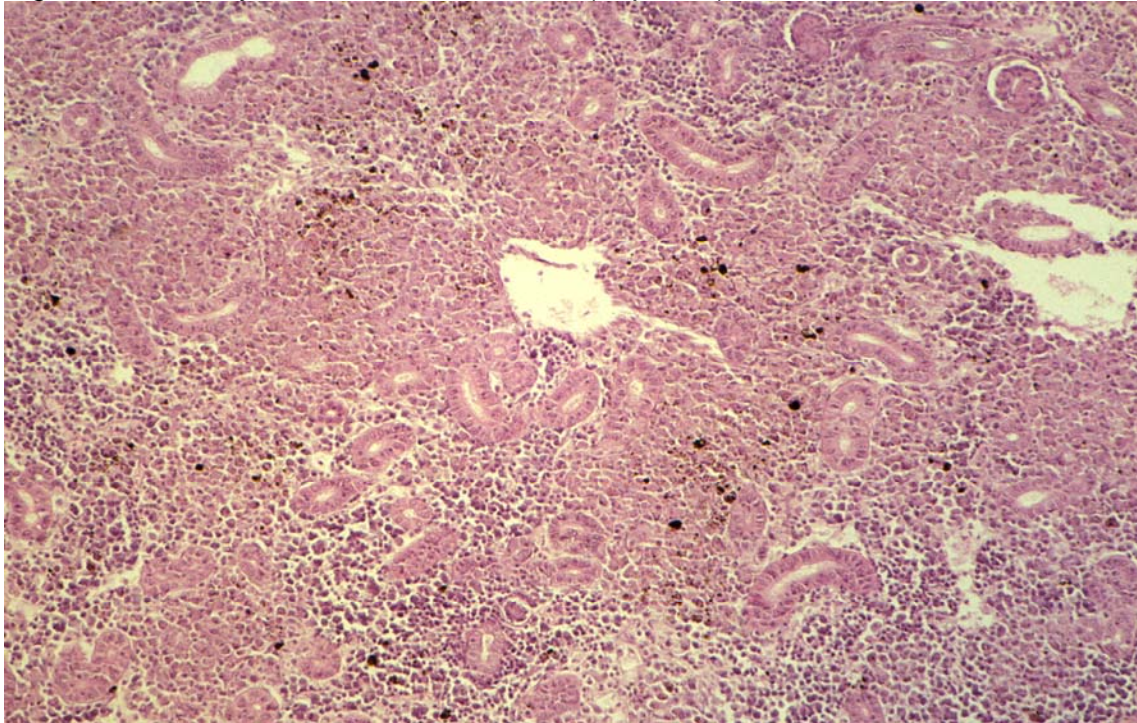


Figure 42. Kidney - chronic inflammation (nephritis); BKD

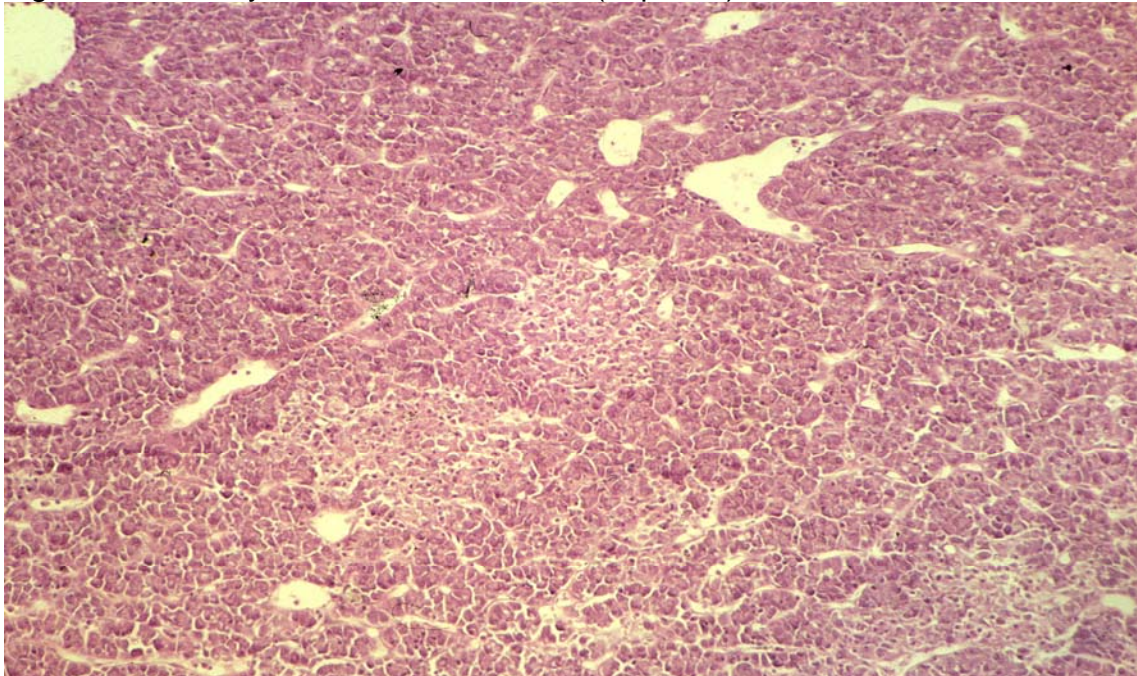


Figure 43. Kidney - granulomatous nephritis; BKD

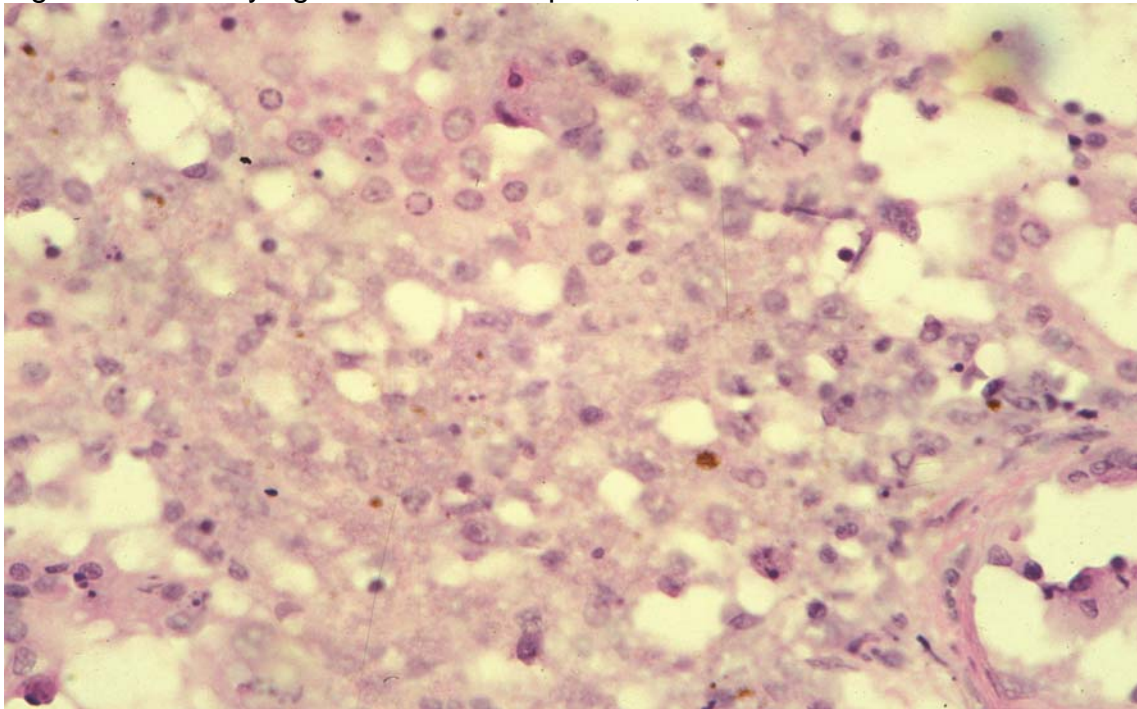


Figure 44. Connective tissue with granulomatous inflammation with giant cells

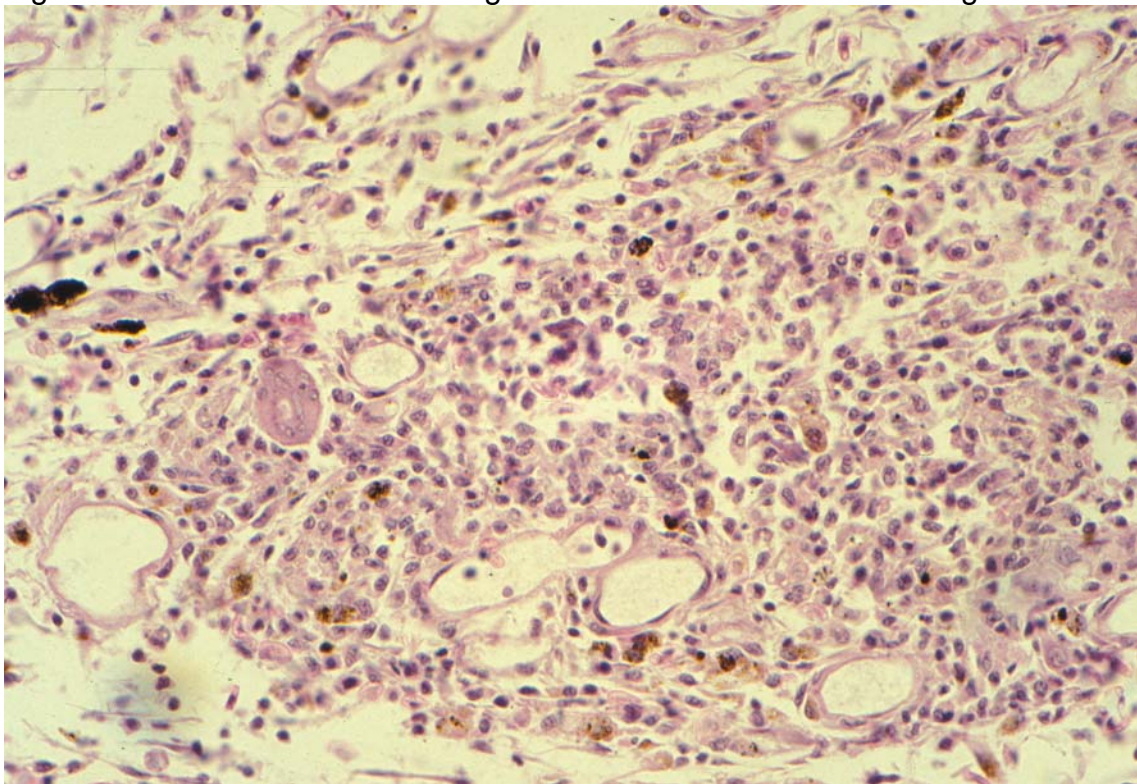


Figure 45. Connective tissue - granuloma

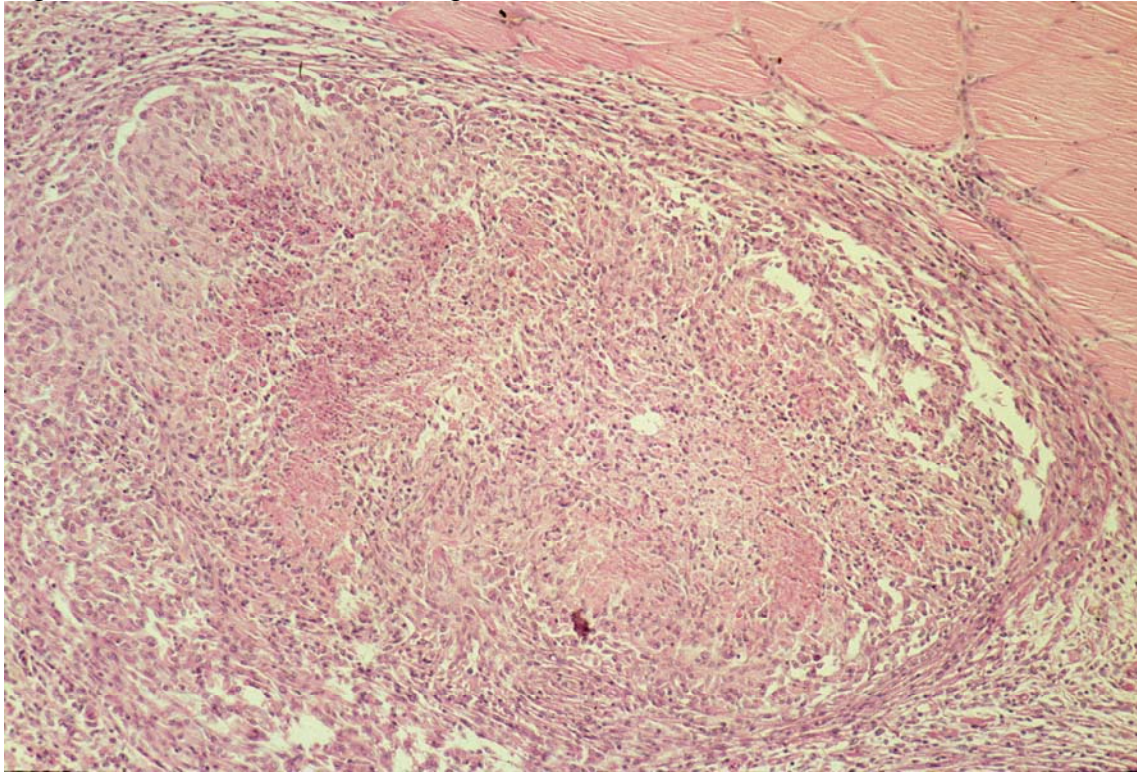


Figure 46. Connective tissue - granuloma

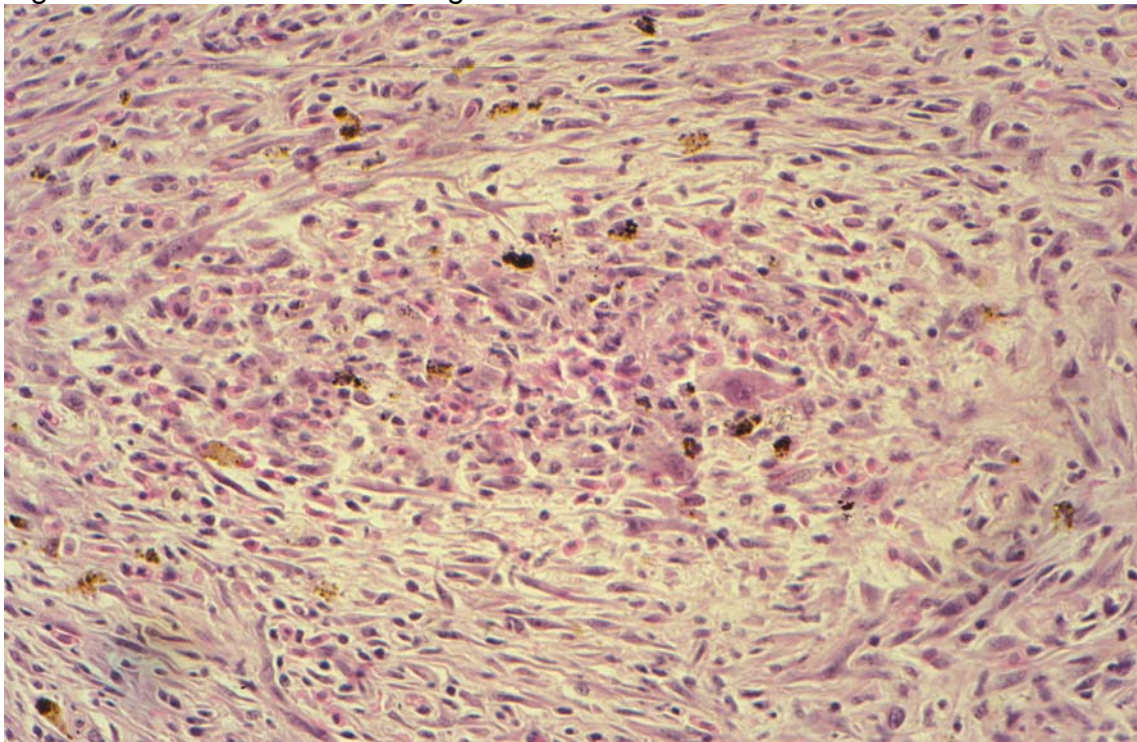


Figure 47. Kidney - granulomas

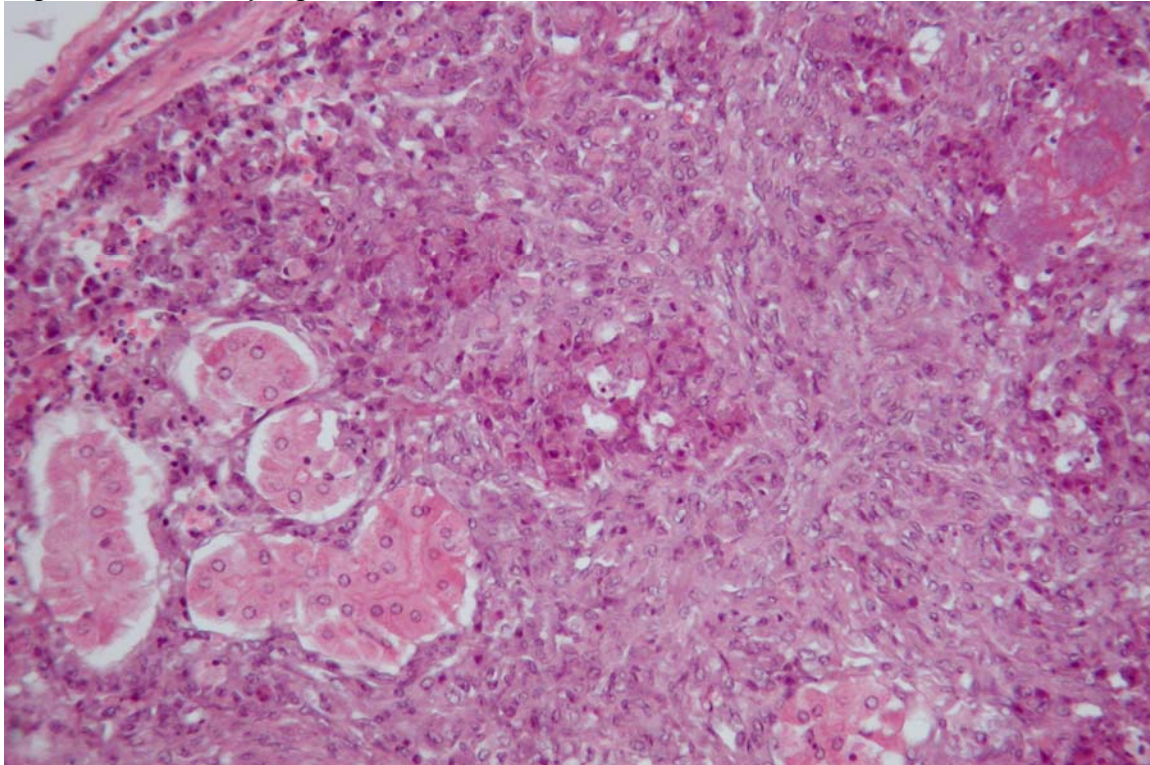


Figure 48. Liver with granuloma

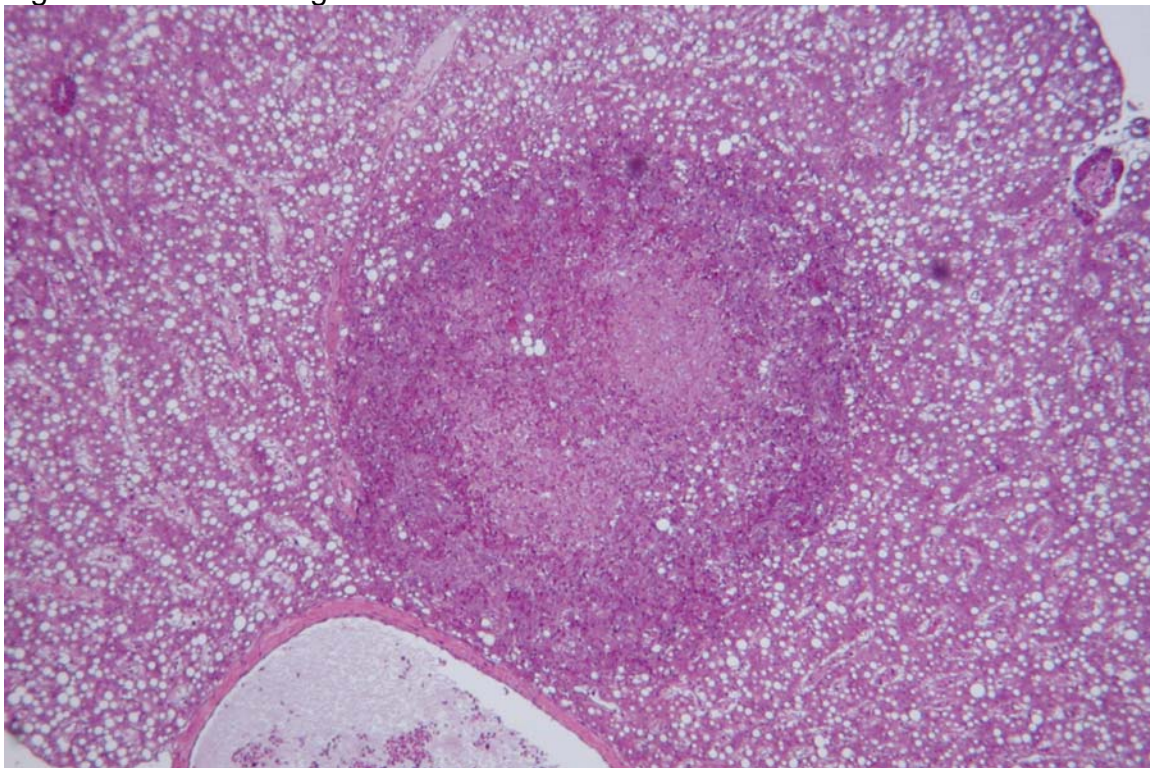


Figure 49. Granuloma with acid fast bacteria

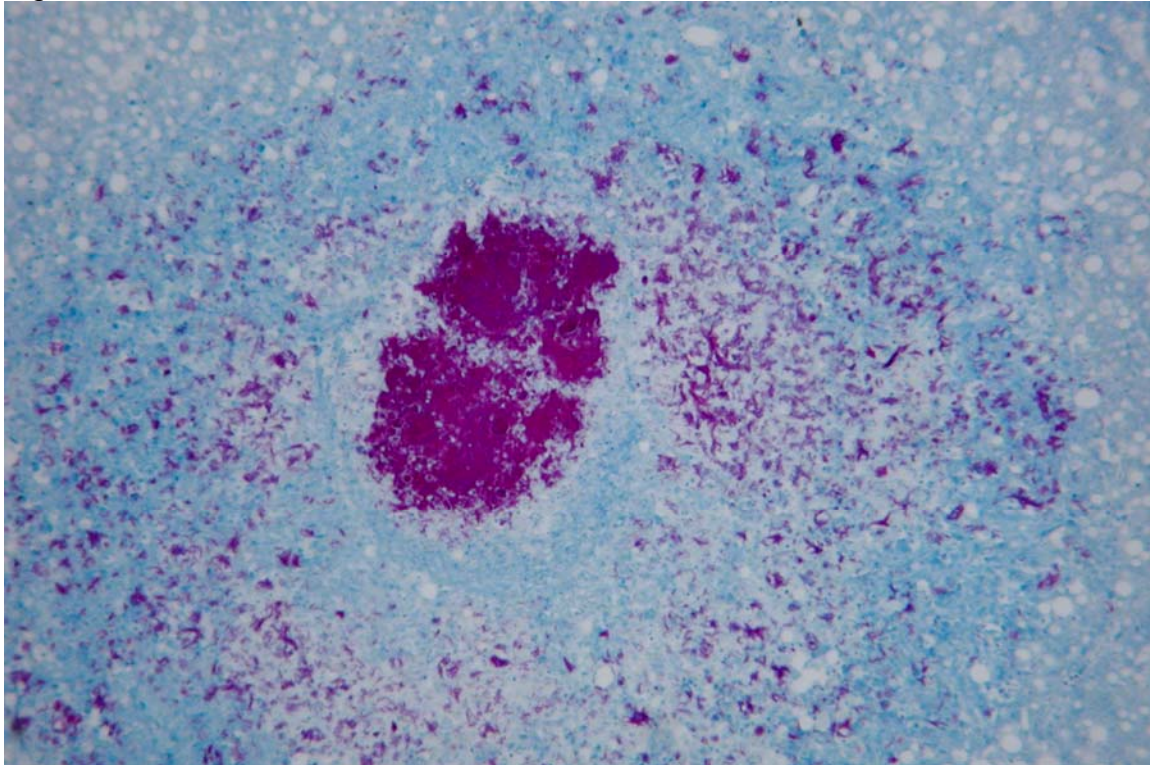


Figure 50. BKD – granulomatous nephritis, splenitis, hepatitis



Figure 51. Liver - granulomatous hepatitis



Figure 52. Muscle - granulomatous myositis



Figure 53. Vertebra - normal



Figure 54. Vertebra - chronic inflammation; bone lysis and new bone formation

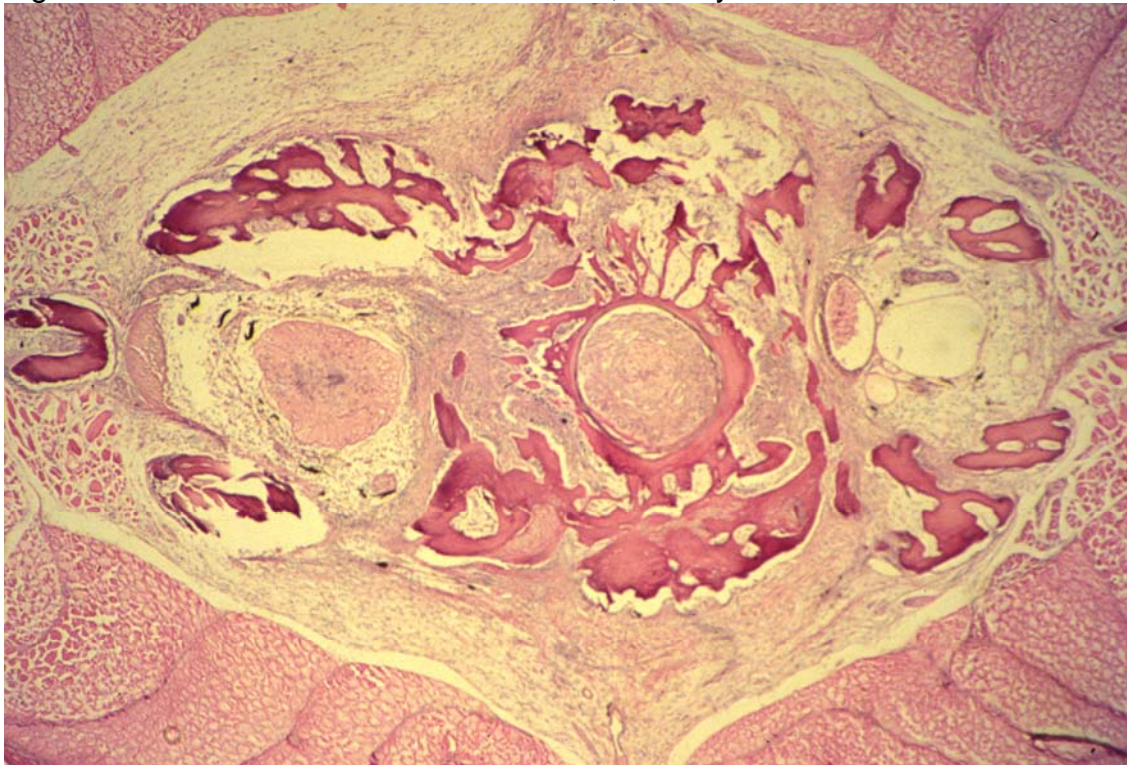


Figure 55. Bone - chronic inflammation; bone lysis and reactive bone formation

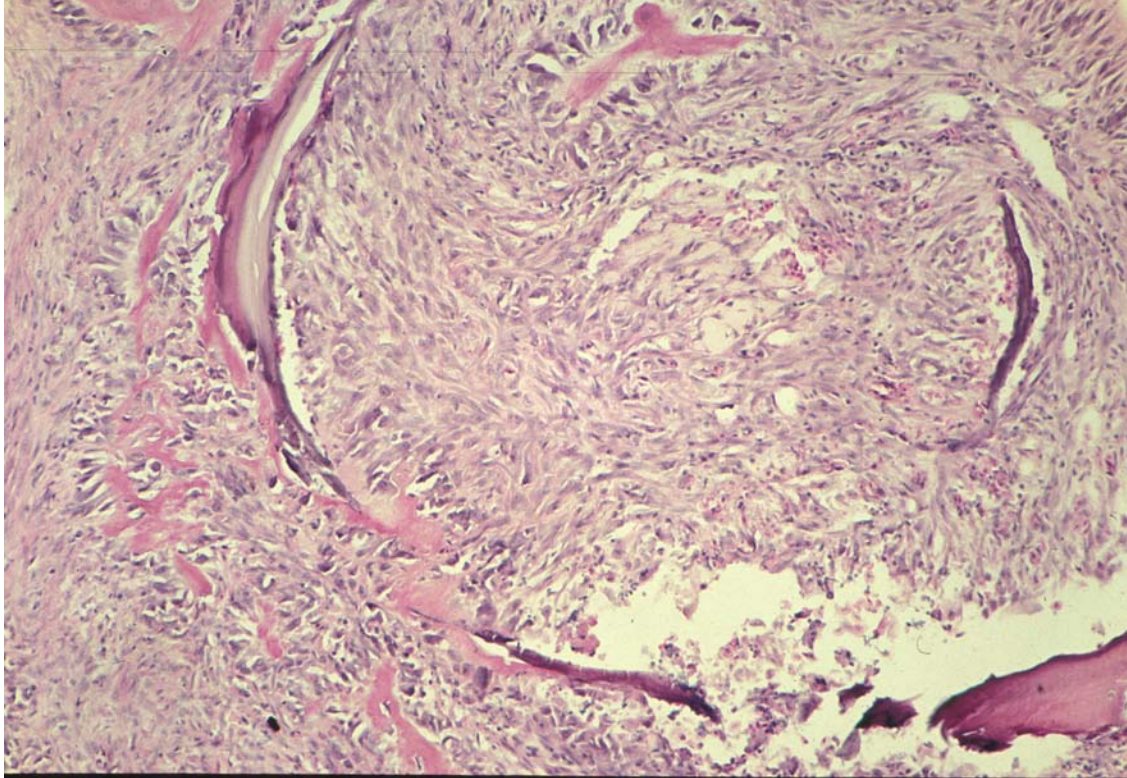


Figure 56. Bone - chronic inflammation with reactive bone formation

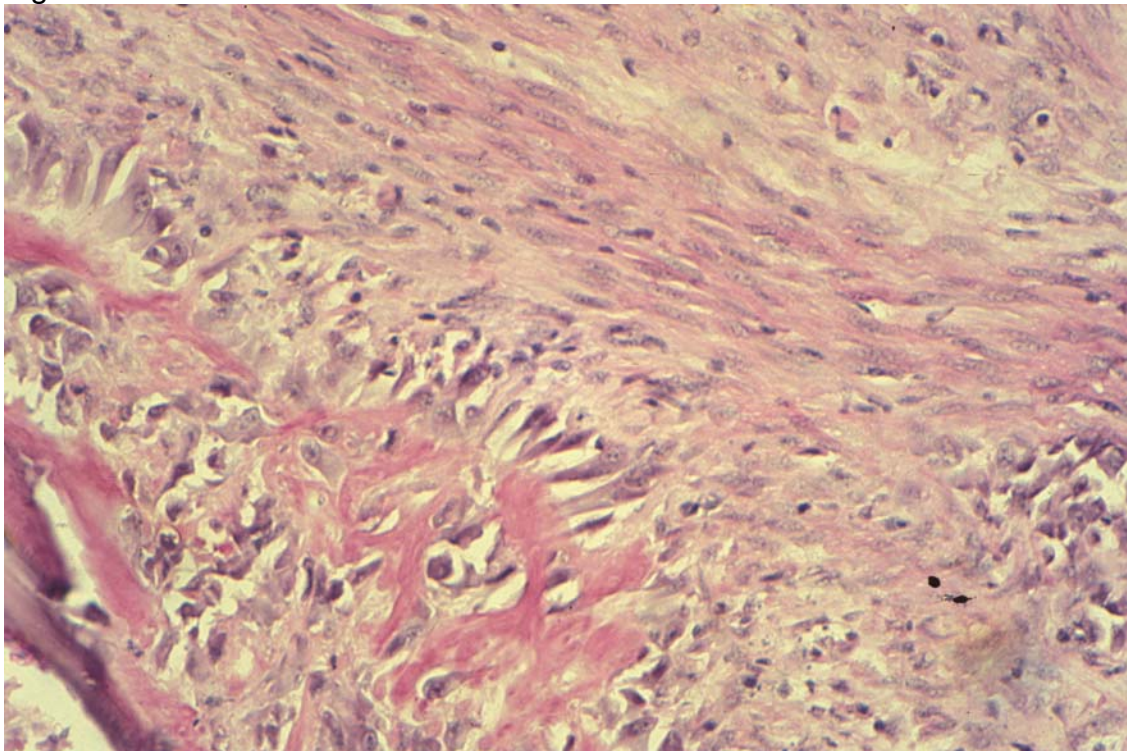
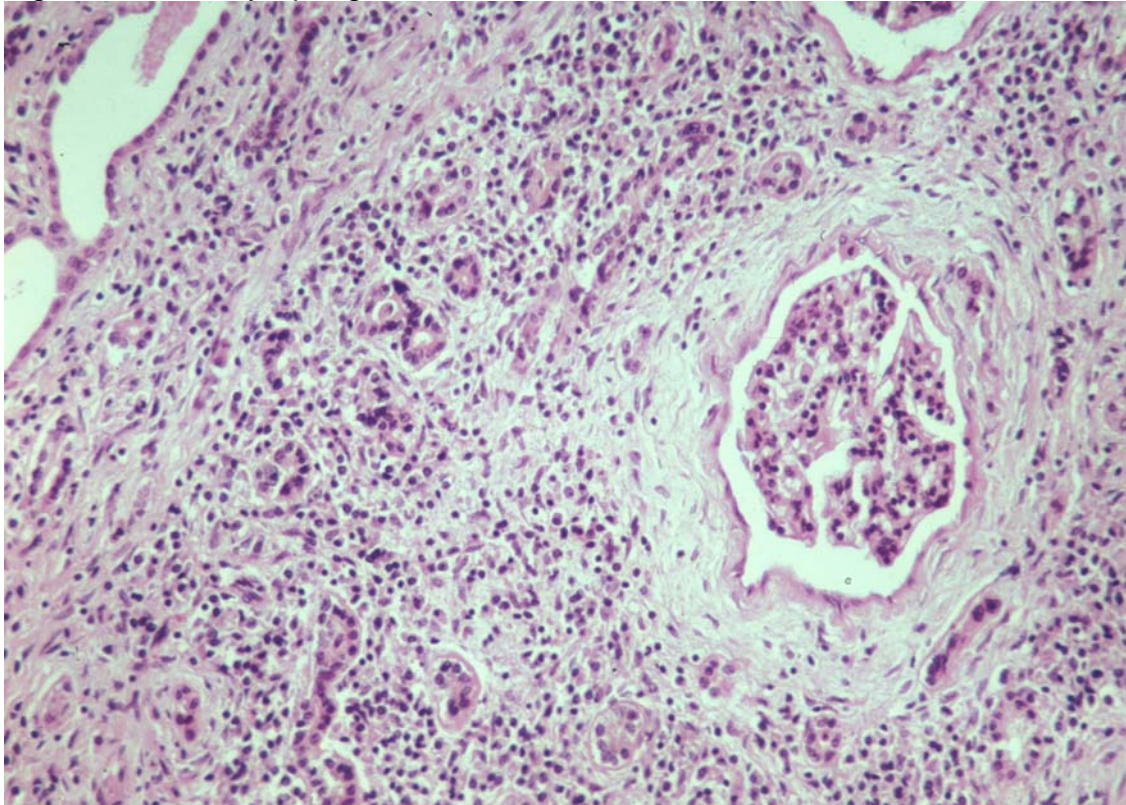


Figure 57. Kidney - periglomerular fibrosis



Mineralization

Mineralization is the deposition of calcium salts in tissues. It can occur by two mechanisms. Dystrophic mineralization is the deposition of mineral at sites of necrosis. The ghosts of necrotic cells are filled with an amphophilic to basophilic crystalline material. This is an irreversible deposition.

Dystrophic mineralization occurs during an upset in calcium and phosphorus metabolism, leading to an excess of calcium in the blood. This may occur due to damage to endocrine control of blood calcium concentration, retention of phosphorus due to kidney disease, or vitamin D toxicity. Calcium is deposited in a variety of tissues, and may or may not have clinical significance. Again this is an irreversible change.

See figures 58 through 62.

Figure 58. Kidney - mineralization

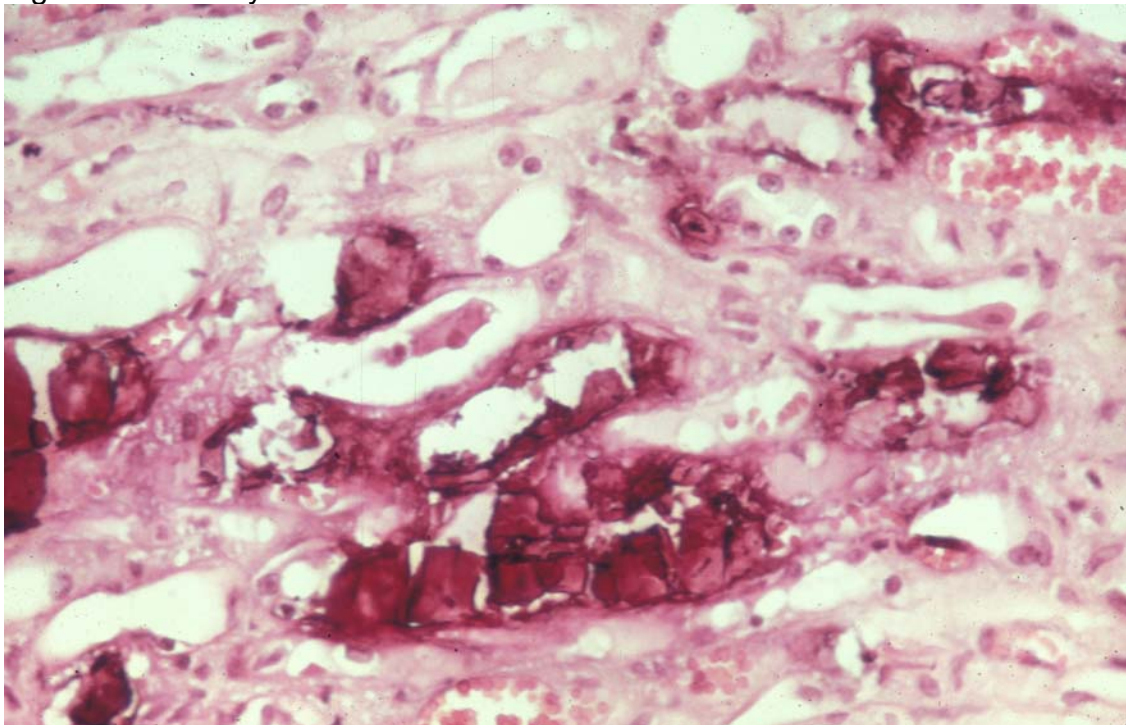


Figure 59. Kidney - nephrocalcinosis

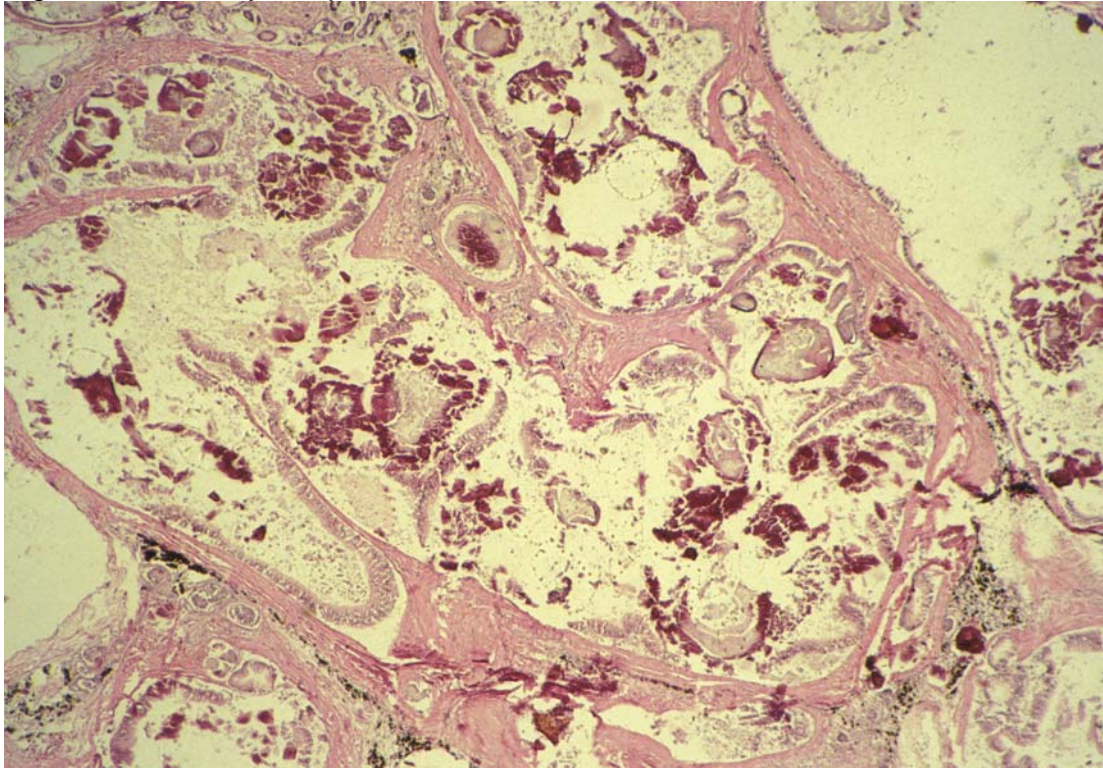


Figure 60. Kidney - nephrocalcinosis

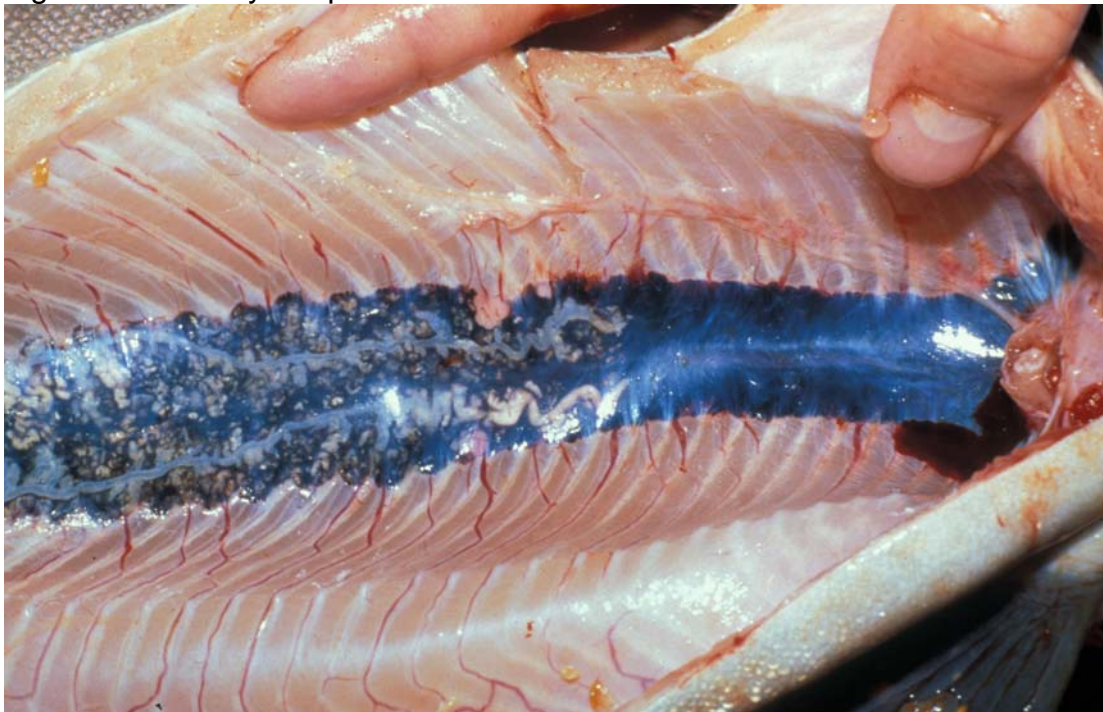


Figure 61. Bulbus arteriosus - mineralization

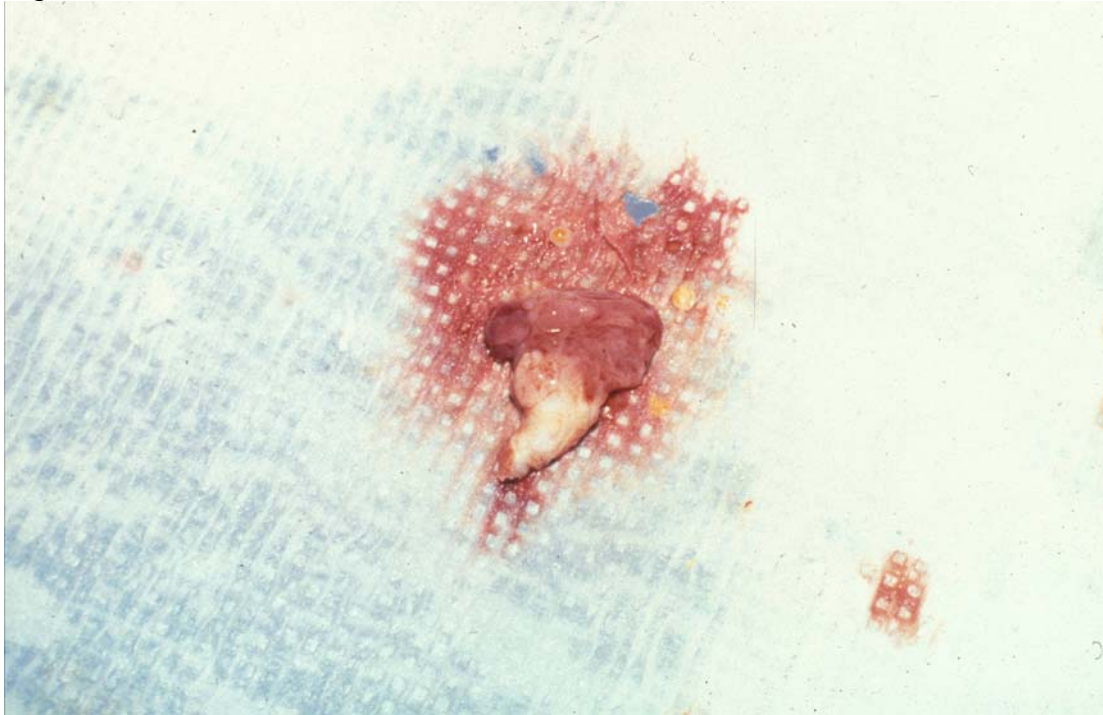
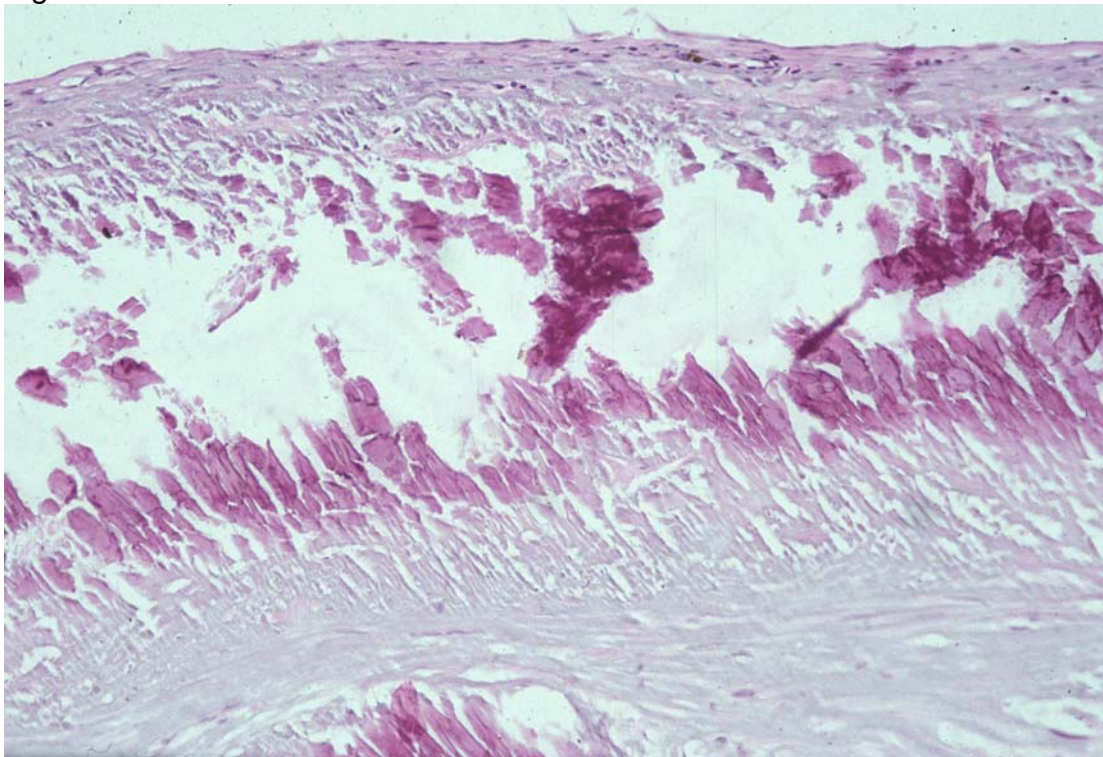


Figure 62. Bulbus arteriosus - mineralization



Pigments

Substances in cells and tissues that have innate color are pigments. They are a diverse group of substances, and may or may not have health significance. Most are varying degrees of tan to brown in histological preparations.

Melanin is a pigment responsible for color of skin, eye, and other tissues. In fish, accumulations of melanin are common at sites of tissue injury, often being visible grossly. Melanin is also present in melanomacrophage centers where it acts as a scavenger for free radicals. These centers increase in size and number in kidney, spleen, liver, and other organs after various types of injury. Melanin is a dark brown granular pigment.

Hemosiderin is an iron containing yellow-brown pigment derived from the breakdown of hemoglobin molecules during red cell destruction or recycling. It is prominent at sites of red cell turnover; during hemolytic conditions in fish, it accumulates in melanomacrophage centers. This pigment can be verified by use of Perl's Prussian Blue stain.

Lipofuscin and ceroid are “wear and tear” pigments found in a variety of cell types. Liver is often the site of accumulation. They are derived from cell membrane breakdown and disruptions of lipid metabolism, and increase with age. Nutritional problems, such as rancid fats in diets, will lead to deposition of these pigments. They are usually finely granular and light tan, but they may not show well in routine H&E preparations. Autofluorescence, and acid-fast, PAS, and fat stains can help identify this material.

Acid hematin is a brown granular pigment formed by the action of acid on hemoglobin. Gastric hemorrhage will lead to acid hematin deposition in the stomach due to stomach acid. The use of unbuffered formalin can lead to artifactual deposition of this pigment.

See Figures 63 through 68.

Figure 63. Kidney - melanin and melanomacrophage centers

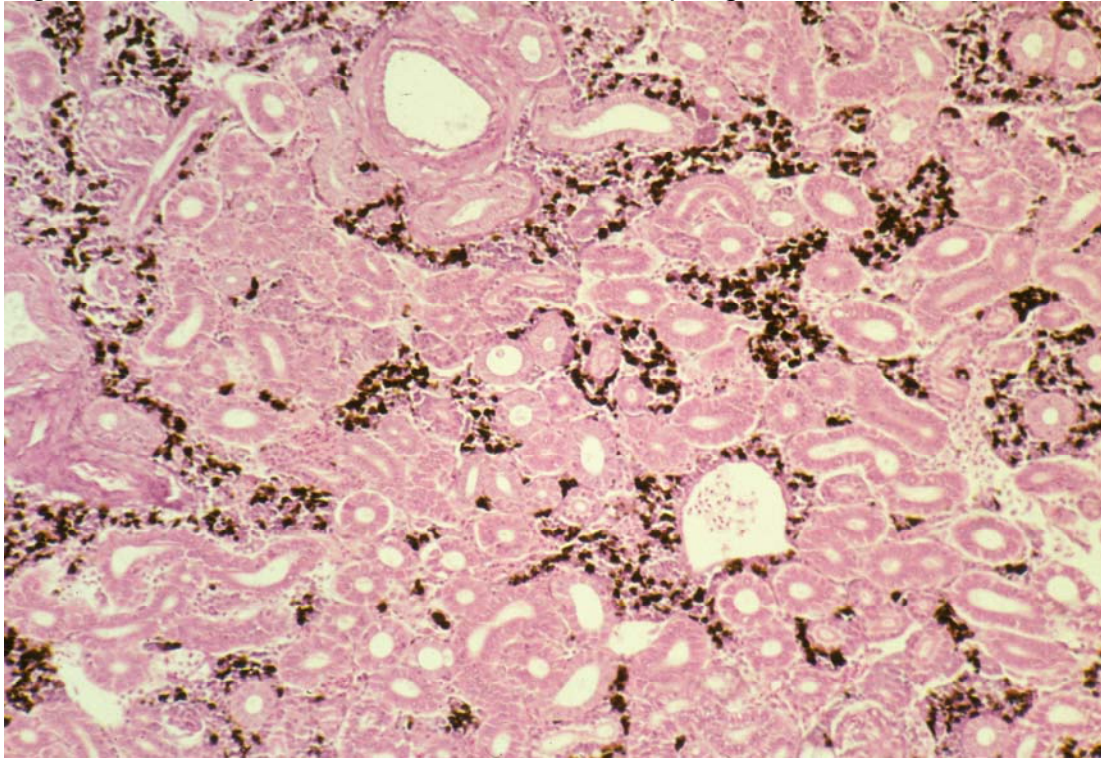


Figure 64. Melanin

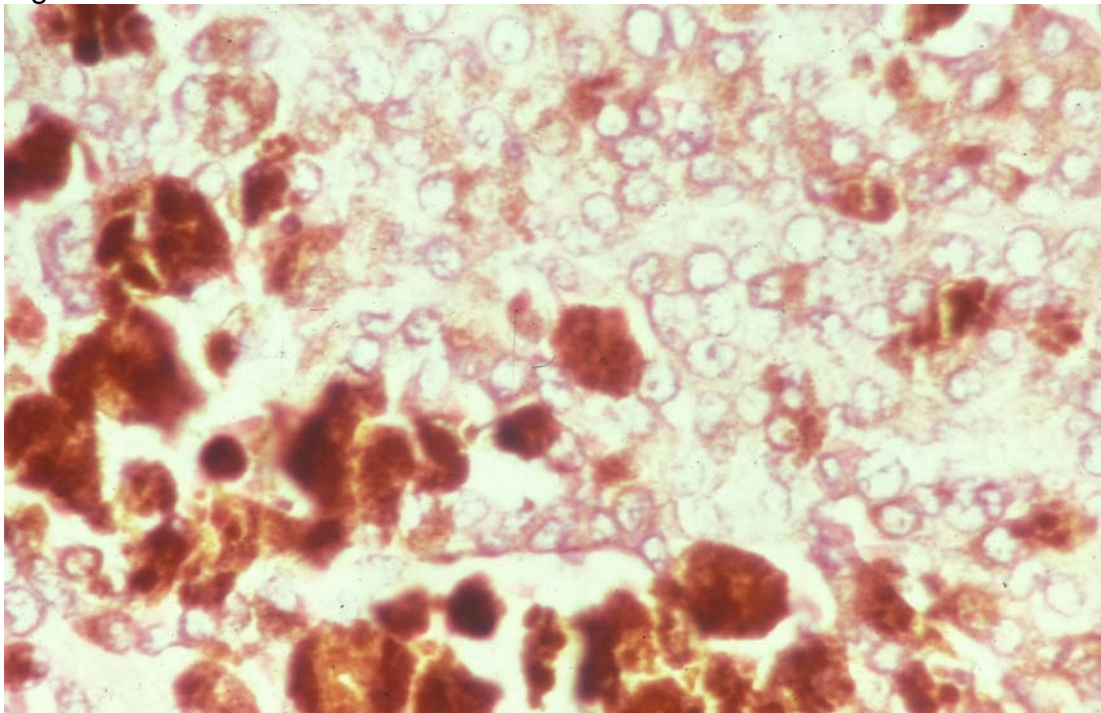


Figure 65. Muscle - melanin

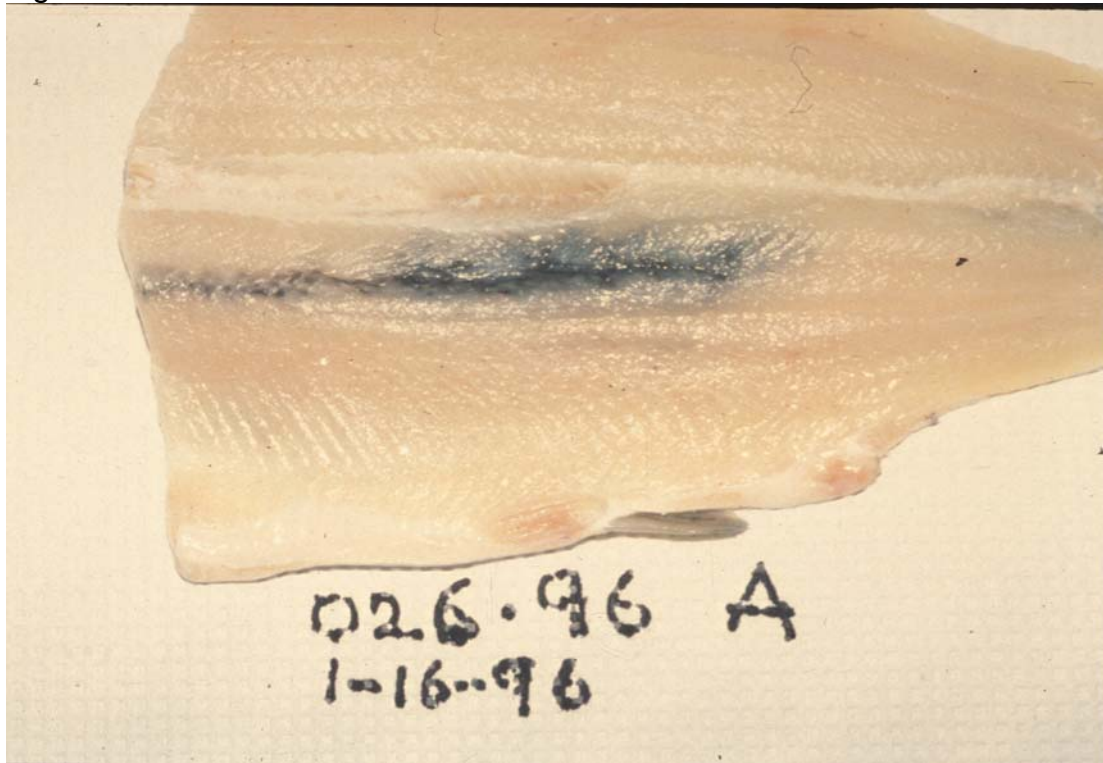


Figure 66. Spleen - hemosiderin

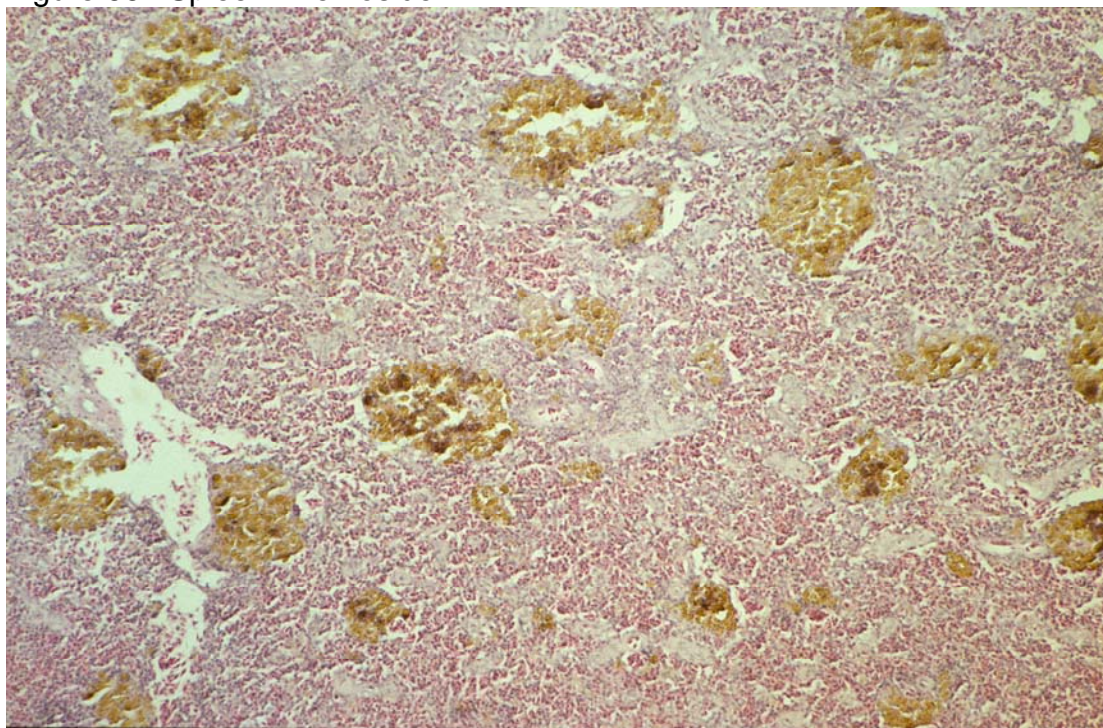


Figure 67. Spleen - hemosiderin (Perls' Prussian Blue stain)

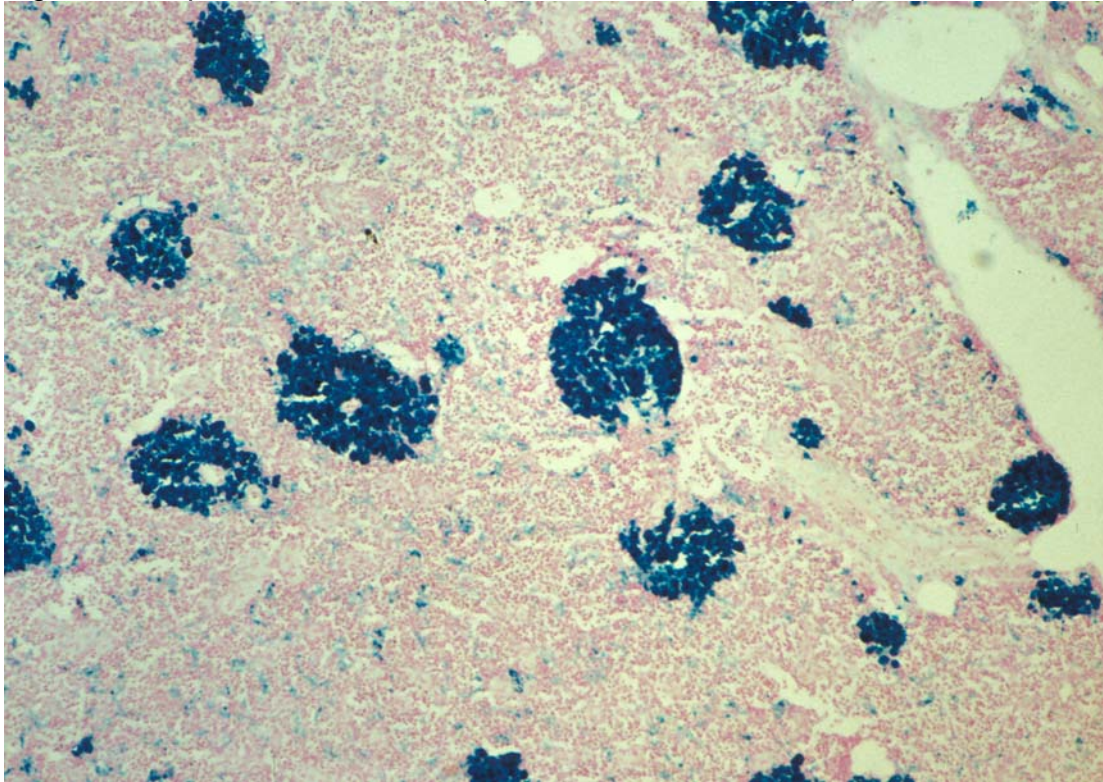
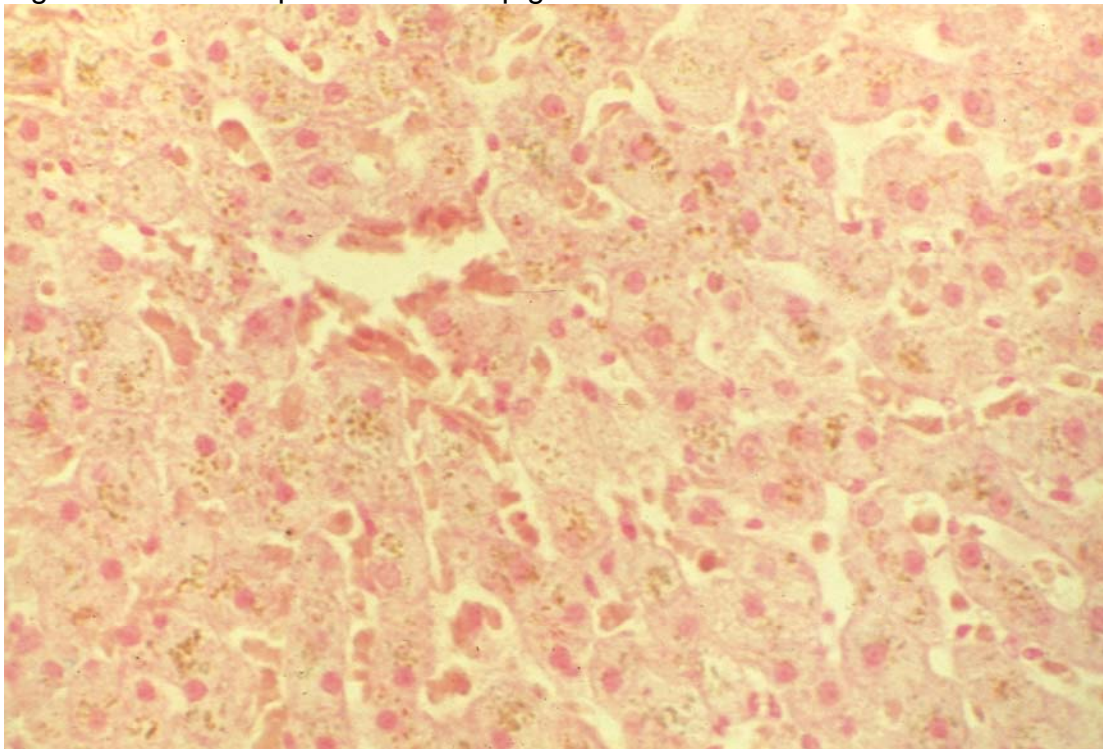


Figure 68. Liver - lipofuscin/ceroid pigment

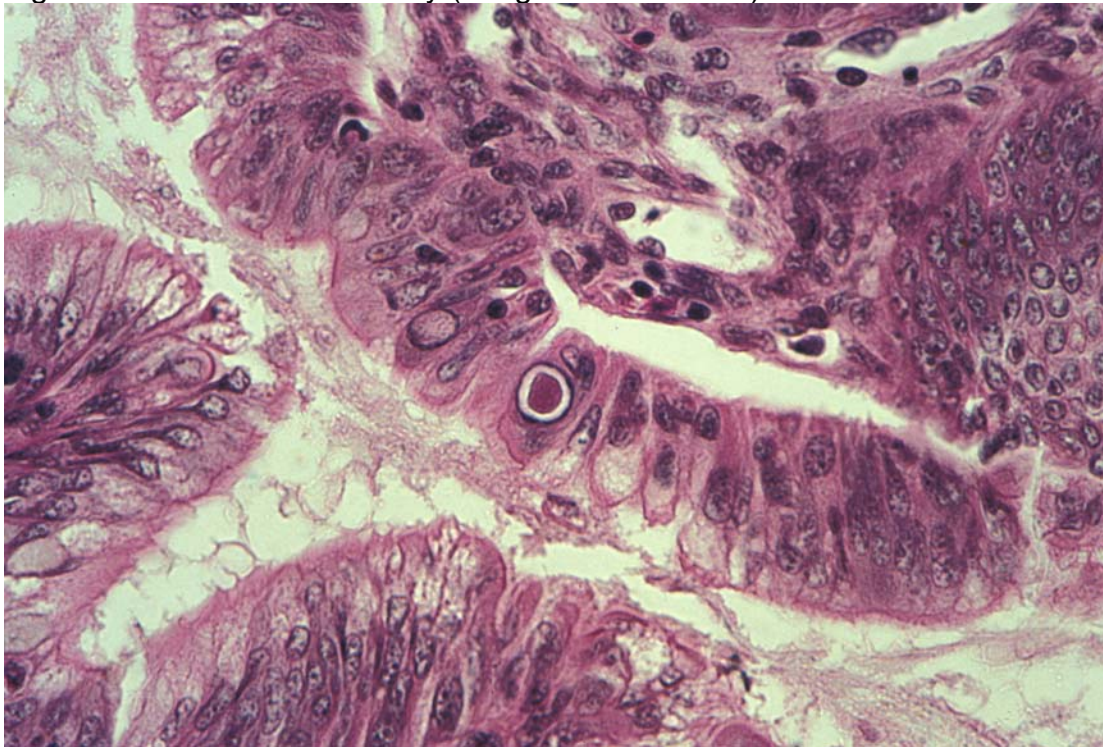


Inclusions

Inclusion bodies are found in a variety of tissues, either associated with viral infections or toxicities. Viral inclusions are tightly packed arrays of viral particles that can become visible with light microscopy. They may be located in the nucleus or cytoplasm, may be eosinophilic to basophilic, and are often very characteristic in size, shape, or location for certain viruses. Inclusions are also found in cases of lead intoxication.

See Figure 69.

Figure 69. Viral inclusion body (sturgeon adenovirus)



Disturbances of Growth

Developmental

- Agnesis - failure of a tissue or organ to develop
- Aplasia - failure of a tissue or organ to grow; a rudimentary version is present
- Hypoplasia - failure of an tissue or organ to grow to normal size

Adaptive (Figures 70 through 82)

- Atrophy: This is a reduction in size of a cell, tissue, or organ. The reduction can be due to a number of causes which include a decreased workload, loss of hormonal stimulation, a diminished blood supply, inadequate nutrition, physical pressure, or denervation.
- Hyperplasia: This is an increase in size of a tissue or organ due to an increased number of individual cells. It is commonly induced by an increased functional demand (renal interstitial hyperplasia), physical or chemical irritation (gill epithelium), or excessive hormonal stimulation (thyroid, goiter).
- Hypertrophy: This is the increase in size of a cell due to synthesis of additional structural components. This increase in size can increase the size of the tissue or organ. Hypertrophy is commonly induced by either increased work load, or physical or chemical irritation.
- Metaplasia: This is a change in which a mature differentiated cell type is replaced by another differentiated type. It comes about due to nutritional abnormalities, constant irritation, or no apparent reason.
- Dysplasia: This is a disorderly cell growth accompanied by cellular atypia (pleomorphism, hyperchromicity, loss of normal maturation progression). It may be pre-neoplastic.

Figure 70. Intestine - normal

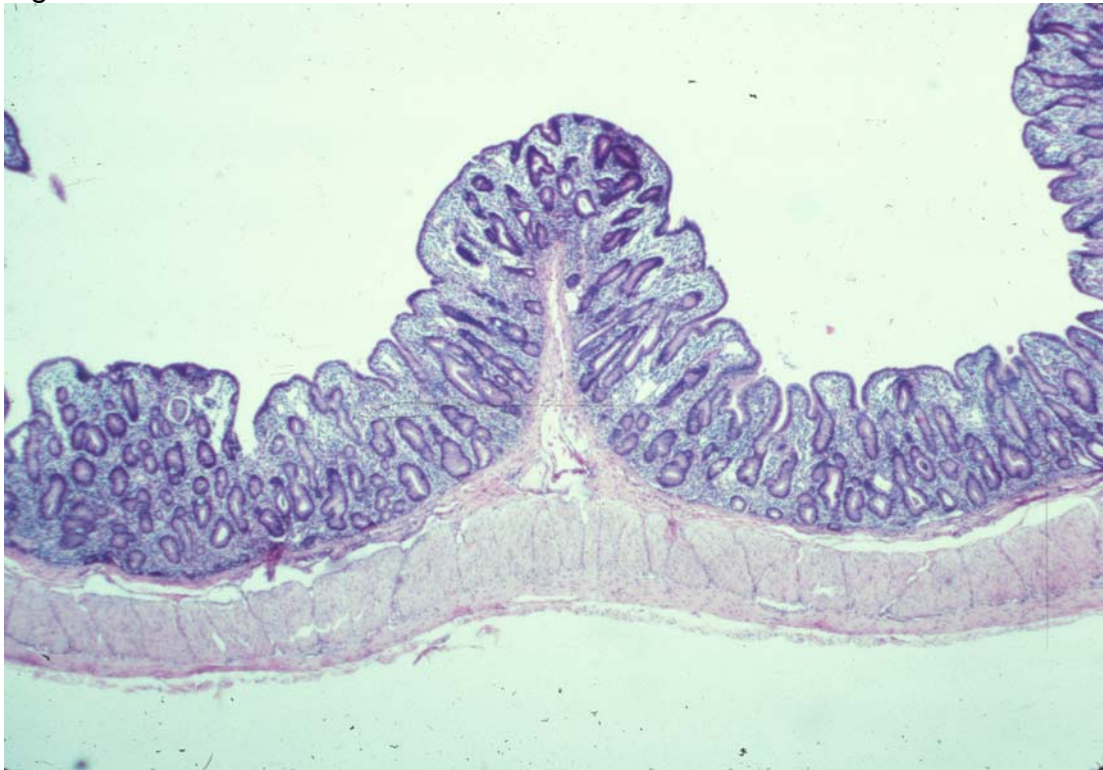


Figure 71. Intestine - hyperplasia of mucosa

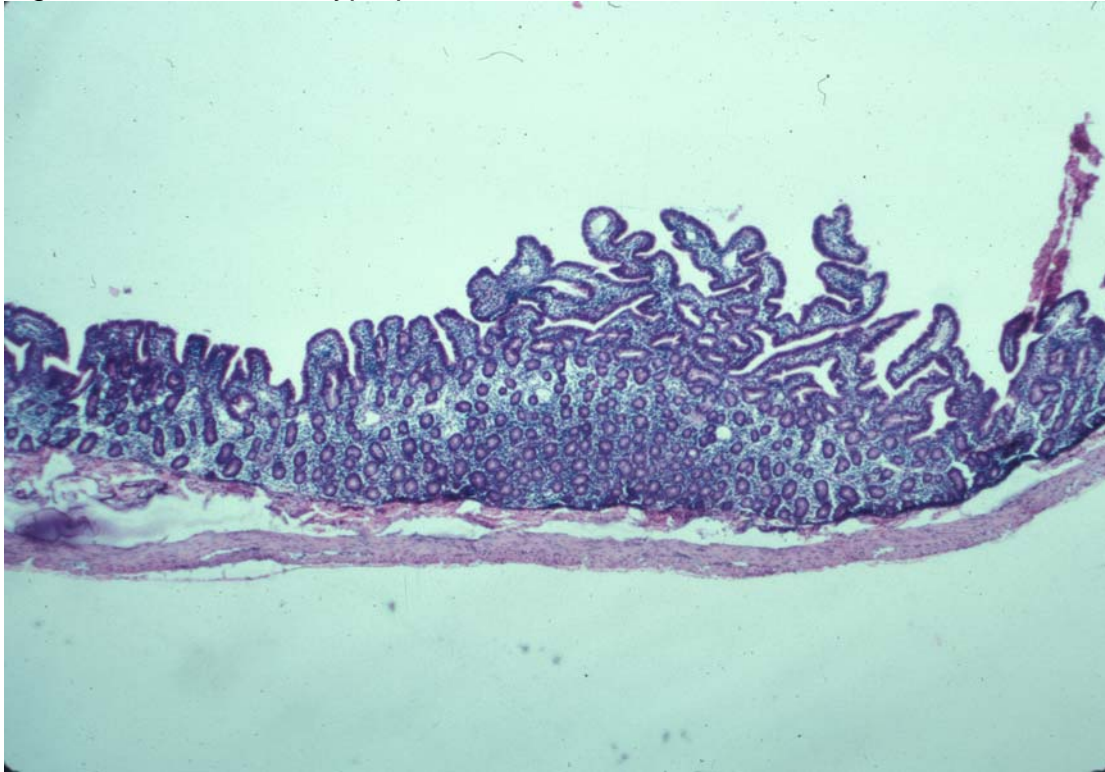


Figure 72. Stomach - hyperplasia of gastric mucosa

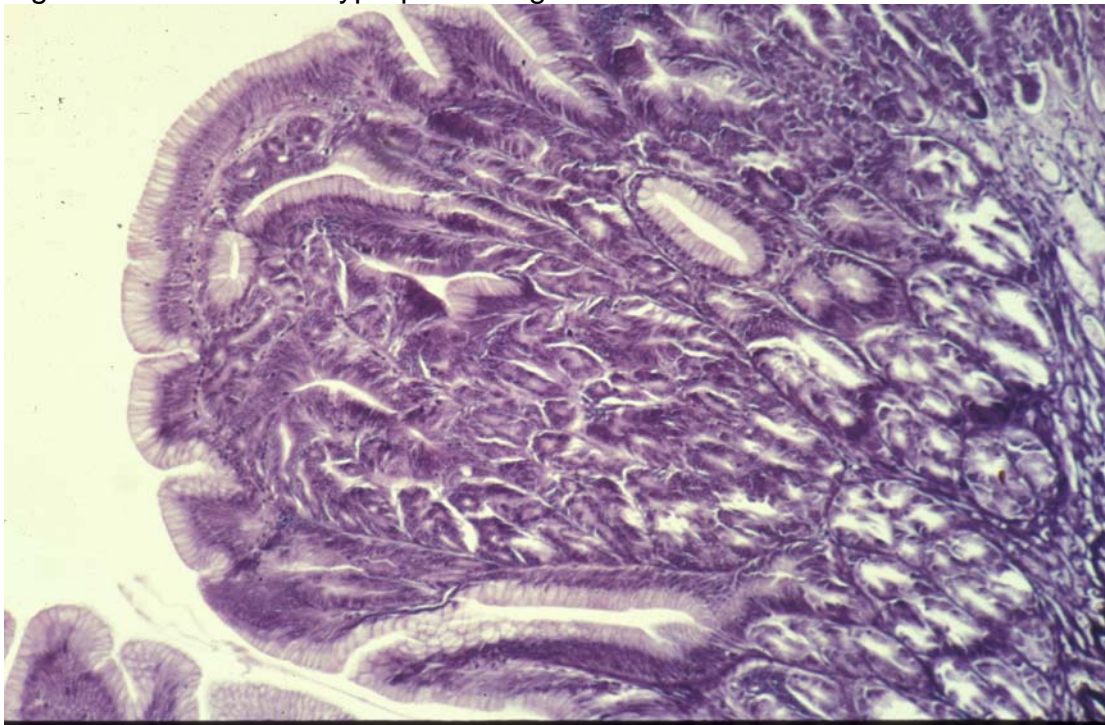


Figure 73. Gill - normal



Figure 74. Gill - hyperplasia of epithelium

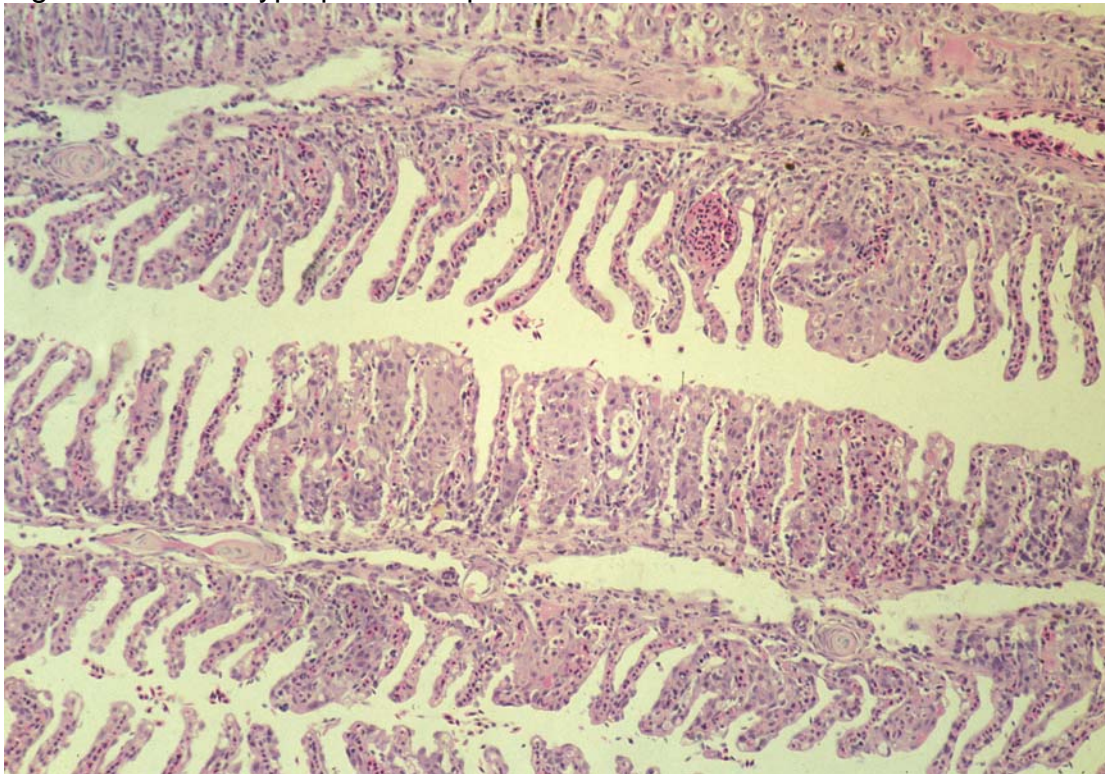


Figure 75. Gill - hyperplasia of gill epithelium (Ichthyobodo)

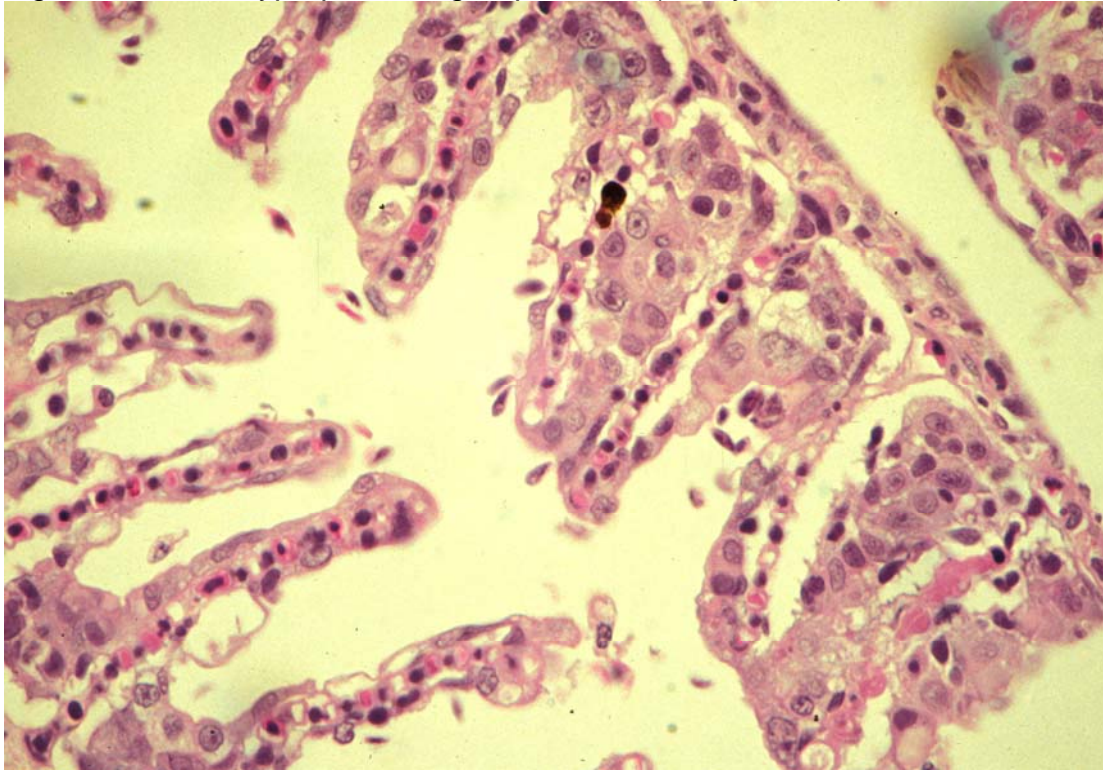


Figure 76. Gill - hyperplasia of gill epithelium

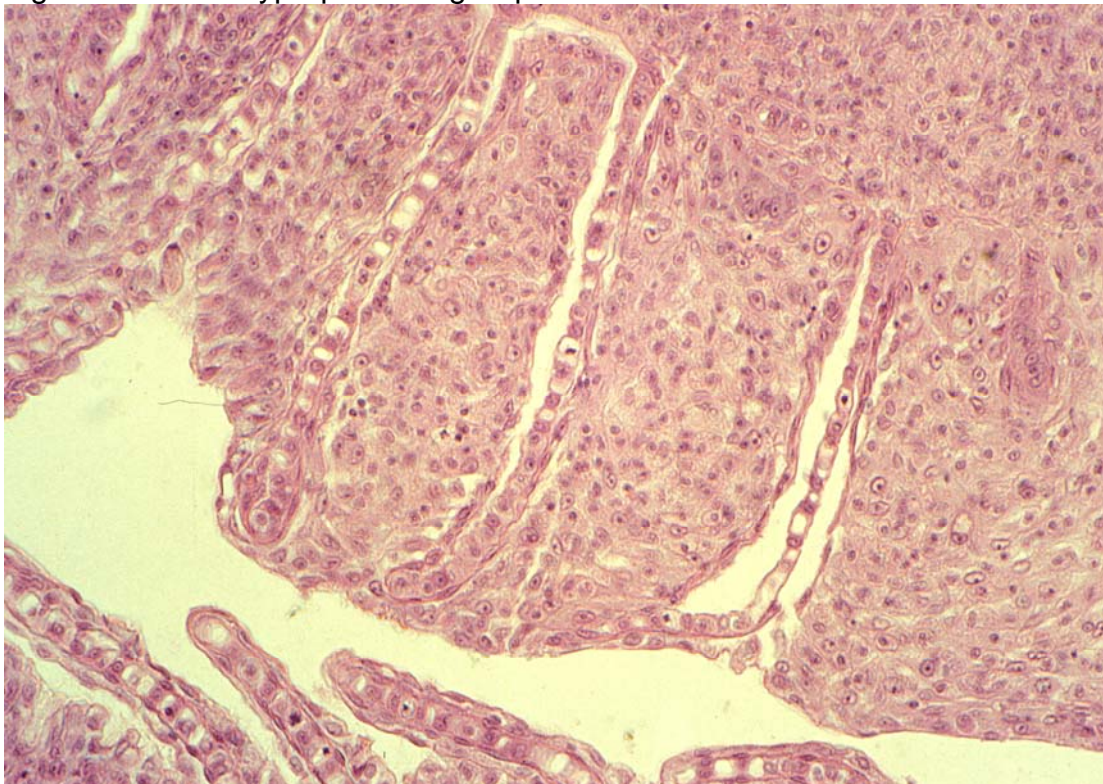


Figure 77. Head kidney - hyperplasia of hematopoietic cells

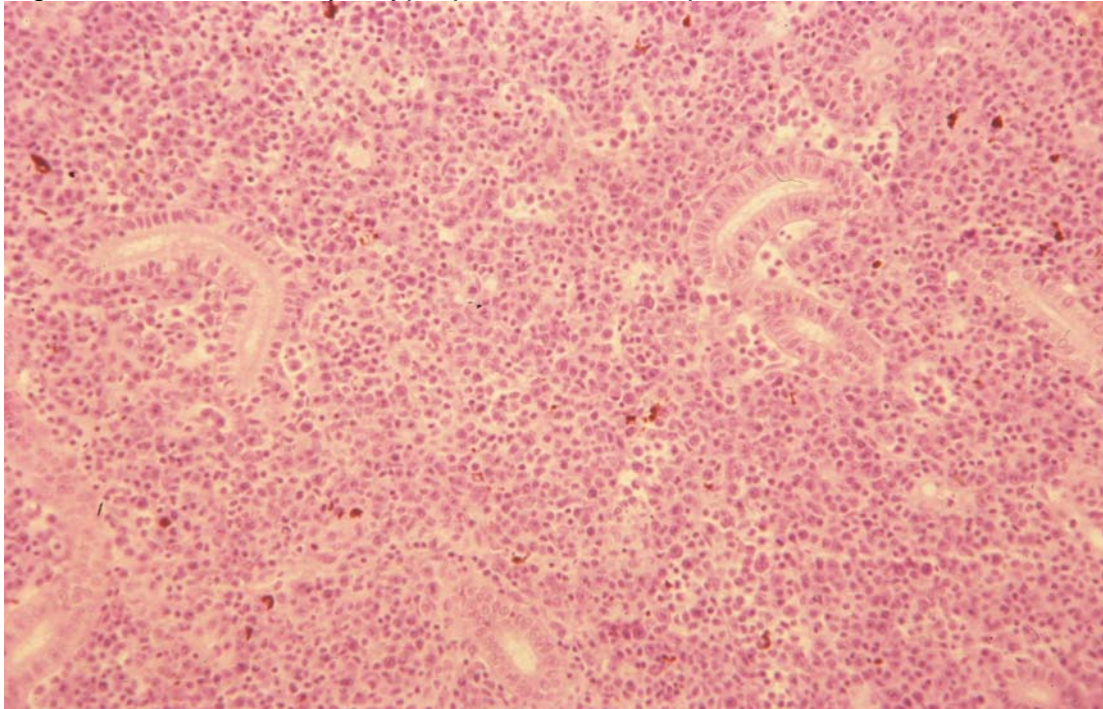


Figure 78. Testes - hyperplasia



Figure 79. Intestine - hypertrophy of smooth muscle



Figure 80. Lung normal epithelium

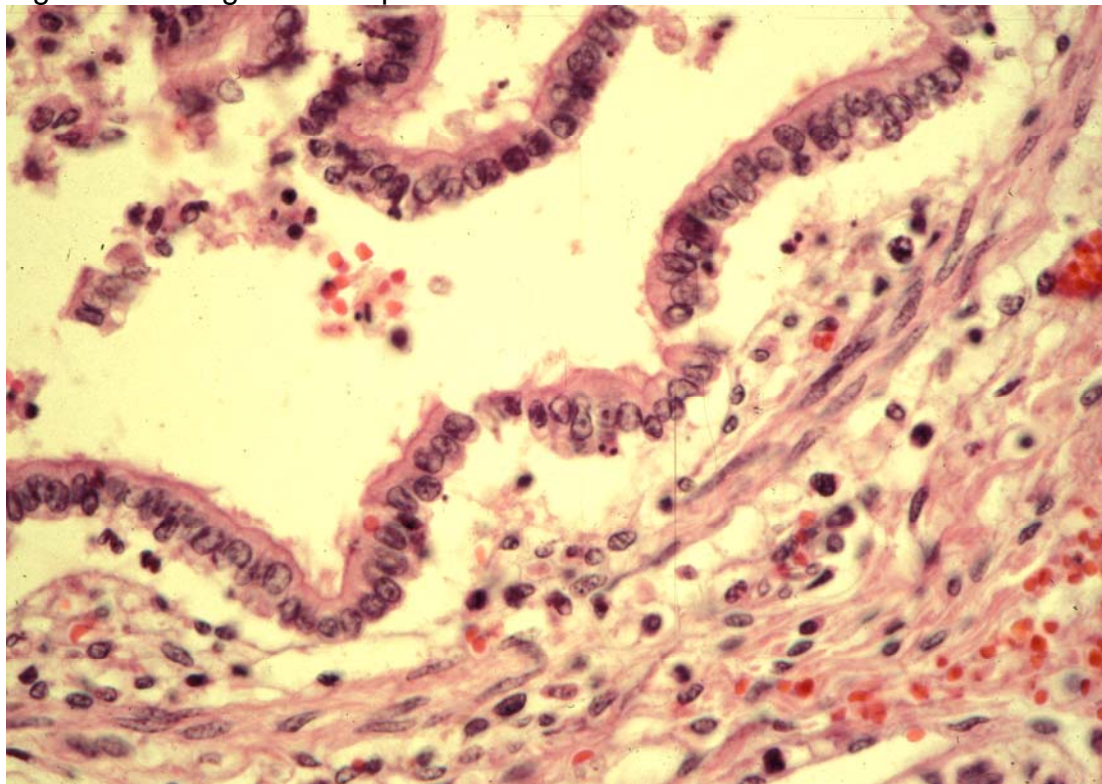


Figure 81. Lung - squamous metaplasia of respiratory epithelium

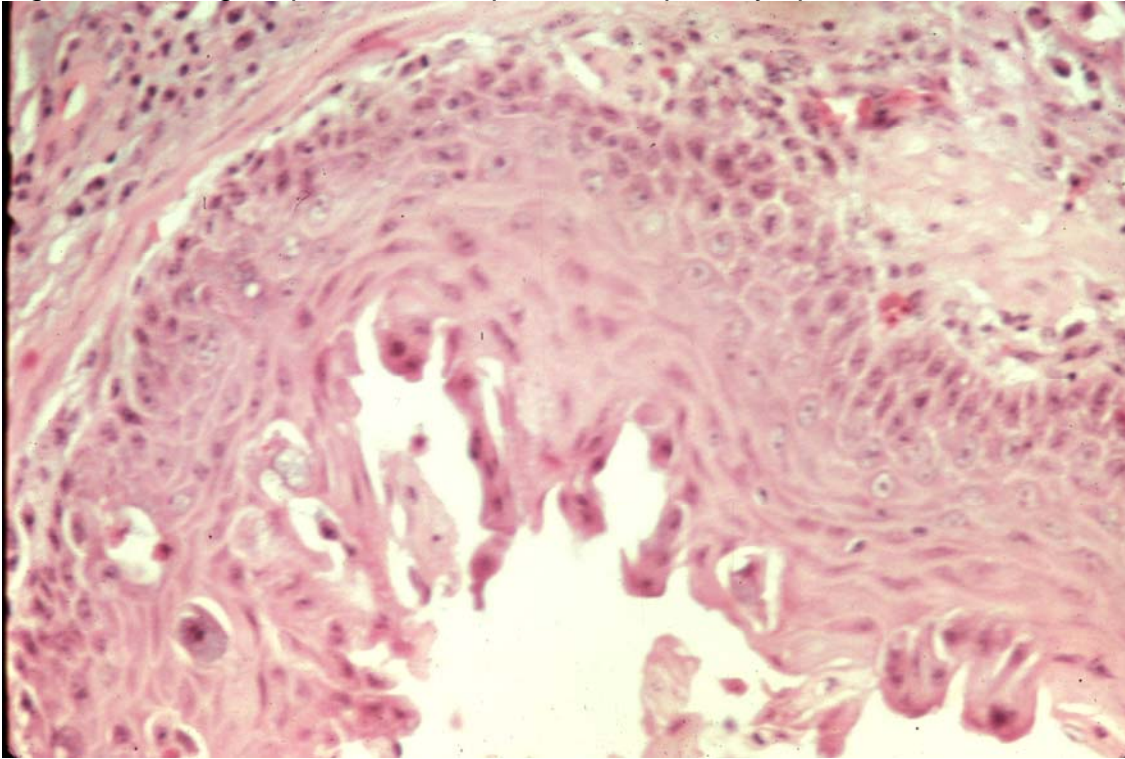
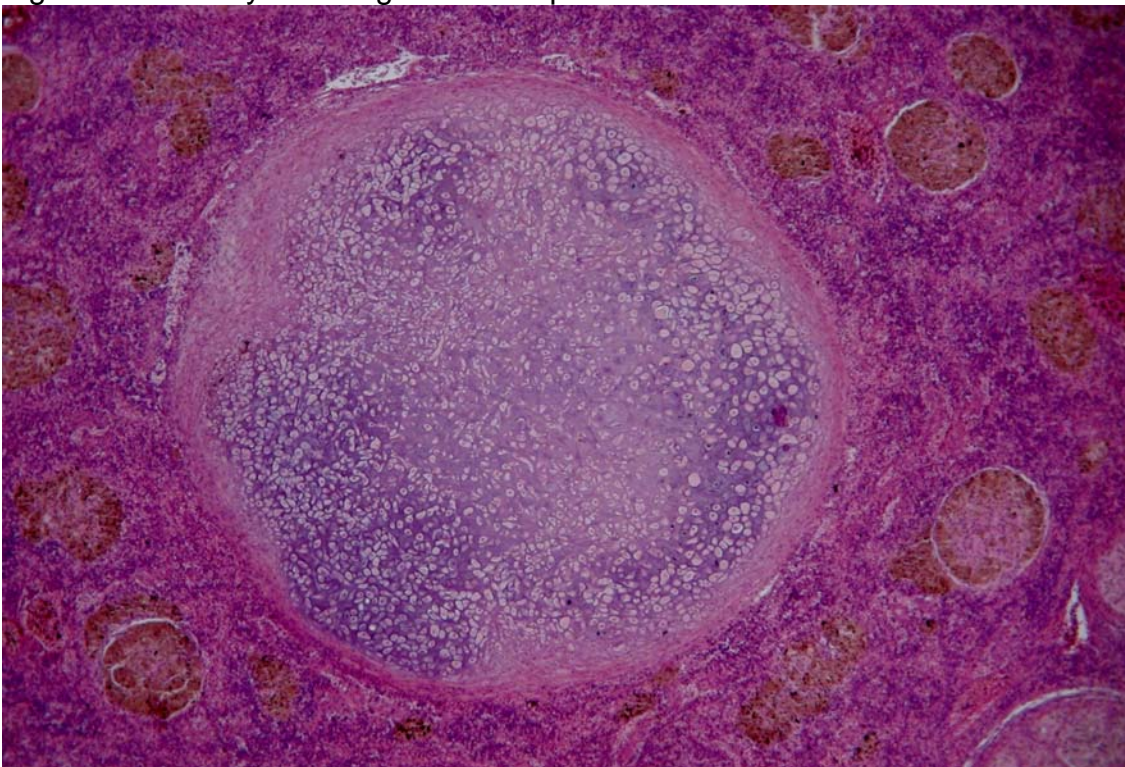


Figure 82. Kidney - cartilaginous metaplasia



Neoplasia

Neoplasia is a disturbance of cell growth characterized by the loss of responsiveness to normal cell control mechanisms. It usually results in the formation of a tissue mass, or tumor. Tumors are incited by a number of mechanisms, from genetic events to toxin exposures to some types of infections. And as control mechanisms are no longer in play, a tumor will persist even if the inciting cause is eliminated.

Tumors may or may not be life threatening. They are allocated to two broad categories, “benign” tumors that are usually (but not always) less deleterious to the host, and “malignant” tumors, those that are more likely (but again not always) to cause the host some degree of harm if not death.

Histologic features of tumors are extremely useful in determining whether the lesion is benign or malignant, what its biological activity (i.e. what will it do over time) is likely to be, whether it is spreading (local invasion or metastasis), whether or not it has been completely removed during a surgical biopsy, and what the overall prognosis is for the host.

Benign tumors tend to be less serious, although there are some benign tumors which can cause the death of the patient. The gross and histologic features that characterize a benign tumor include:

- Histological similarity to the cells/tissue of origin: the tumor cells and their arrangement matches the tissue of origin;
- Well differentiated: the tumor cells show ordered maturation and well defined differentiation; nuclear to cytoplasmic ratio is appropriate; few, if any, mitotic figures; cell products are formed at proper location;
- Encapsulated: these tumors frequently, but not always, are bordered by a discrete fibrous capsule;
- Expansile: these tumors enlarge, compressing adjacent normal tissue;
- Non-invasive: the tumor remains confined with no penetration into adjacent normal tissue;
- No metastasis: the tumor does not spread by way of the circulatory system or by implantation across body cavities.

Malignant tumors tend to be more serious in terms of biological consequence to the host. Many malignant tumors will eventually cause the death of the patient by various means, although some can be less aggressive and may not have a serious impact on long term survival. Histologic characteristics of malignant tumors are:

- Differentiation varies: some malignant tumors are very well differentiated, looking very similar to the tissue of origin; others may show some similarity, while others have no resemblance to the tissue of origin;
- Capsule varies: while some may be weakly encapsulated, most lack this feature;
- Invasive: these tumors frequently invade into adjacent normal tissue;
- Metastasis: the tumor spreads by invasion of the vasculature and release of tumor emboli that spread to distant sites, or tumor cells spread across body cavities and implant on adjacent organs or surfaces; by definition, a metastatic tumor is malignant; metastasis is not commonly reported in fish;
- Necrosis: malignant tumors often grow so rapidly that they outgrow their blood supply;
- Cellular anaplasia: this is the failure to differentiate; the cells may be pleomorphic, may have larger than normal or misshapen nuclei with

Chapter 5 – General Pathology

Fish Histology and Histopathology

hyperchromatic or vesicular chromatin and prominent single or multiple nucleoli; bizarre or giant cells; cell products formed or released in abnormal location;

- Mitotic activity can be high: this can be variable; higher mitotic activity is usually associated with a more aggressive tumor;
- Disorderly cellular arrangements: normal maturation sequences may be disrupted; lack of any organization typical of the tissue of origin.

These histological characteristics of benign and malignant tumors are variable for any given tumor, i.e. not all of them will be present. When examining a tumor in a histologic preparation, keep track of all observed features and when finished, make your diagnosis based upon this overall picture. Be aware that the distinction between benign and malignant is not always clear cut (except in the presence of metastasis, which by definition means malignant), and that for a given tumor, there may be disagreement between pathologists as to its nature and likely biological activity.

Diagnosis of a particular tumor may be very straightforward, particularly if it is a well differentiated neoplasm. But many are not differentiated, and it can be extremely difficult to determine the cell of origin. Special stains can be useful in some cases; for example, poorly differentiated mast cell tumors can sometimes be recognized by the presence of even a few granules that stain with toluidine blue. Electron microscopy can be utilized in order to visualize organelles and cytoplasmic structures that may provide clues to a tumor cell's origin.

Immunohistochemistry is an extremely useful technique for the identification of neoplasms. This technology relies upon antibody-based stains specific for intermediate filaments or surface markers. Basic cell types harbor certain filament types, eg. epithelium contains cytokeratin, connective tissue contains vimentin, muscle contains desmin. Thus, these can be used as markers to provide evidence that a tumor is of epithelial or connective tissue origin. Surface molecules can also be used as markers to identify cell types. For example, CD3 and CD79 can be used to differentiate T-lymphocytes from B-lymphocytes in cases of lymphosarcoma.

See Figures 83 through 110, and appended figures.

Figure 83. Swim bladder - adenoma



Figure 84. Swim bladder - adenoma

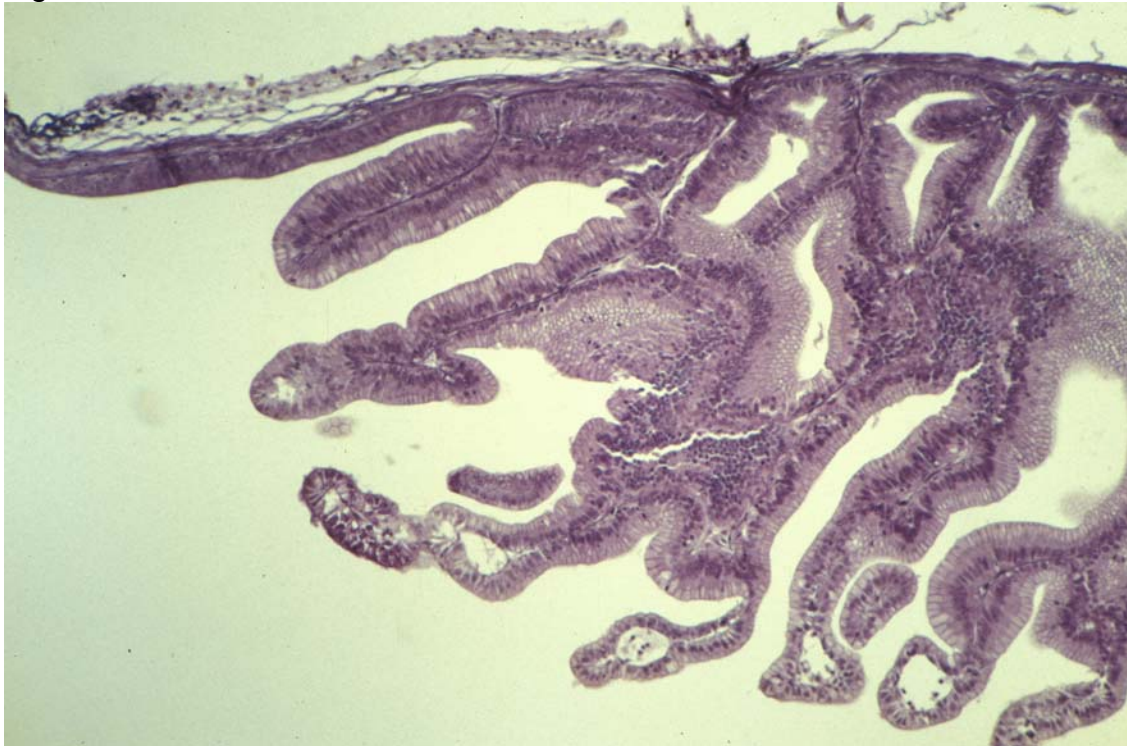


Figure 85. Fibroma

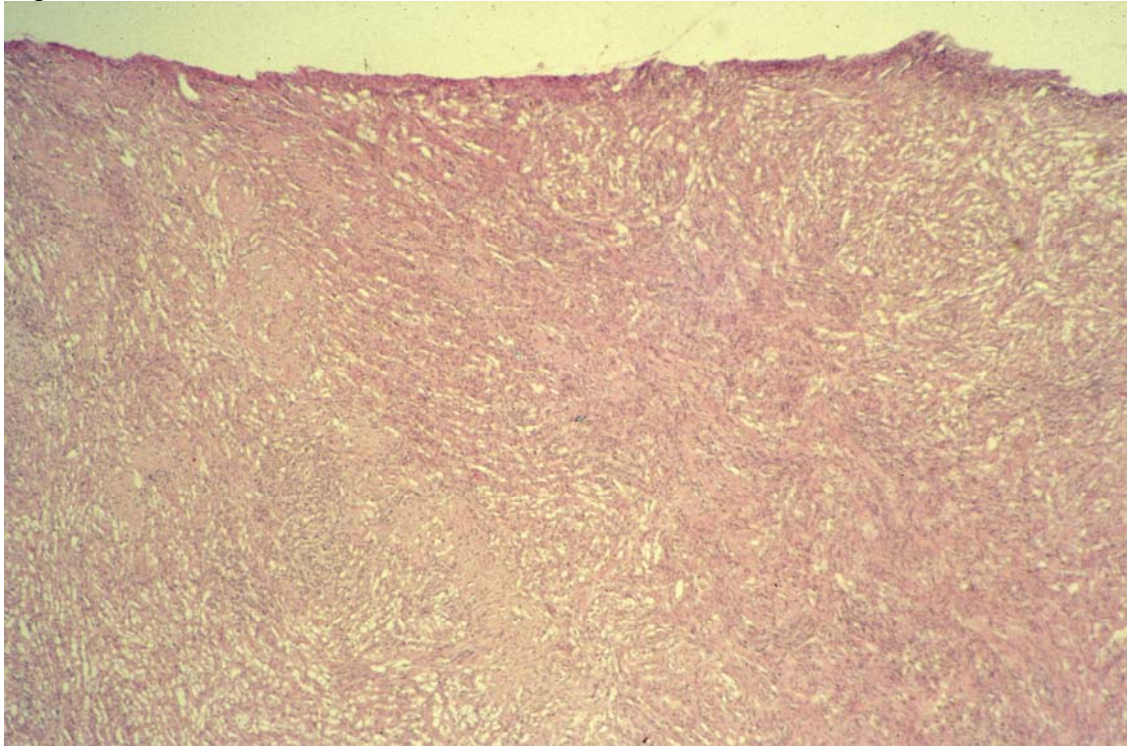


Figure 86. Fibroma

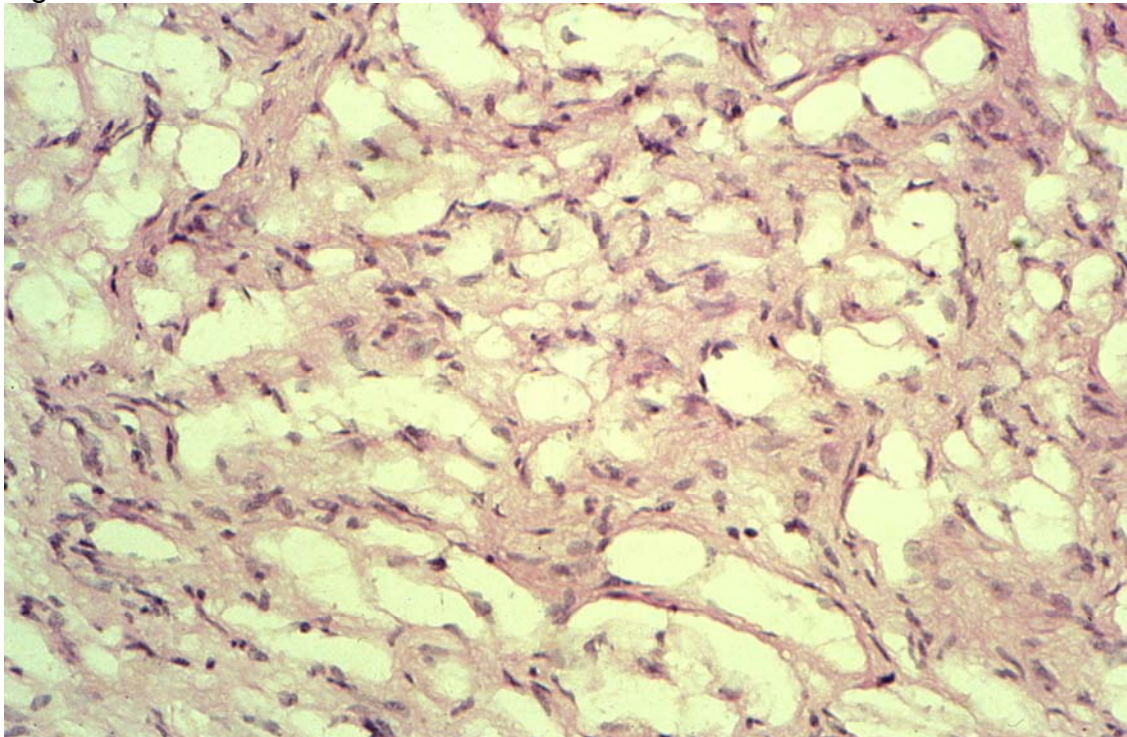


Figure 87. Hemangioma

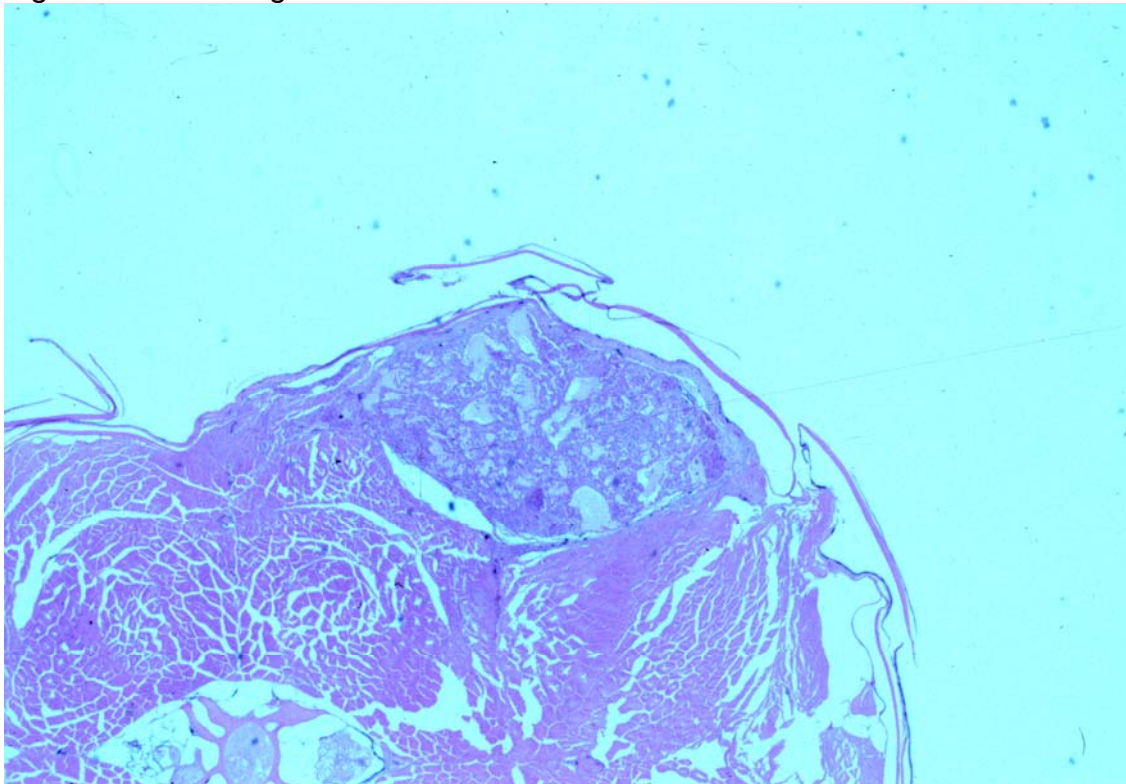


Figure 88. Hemangioma

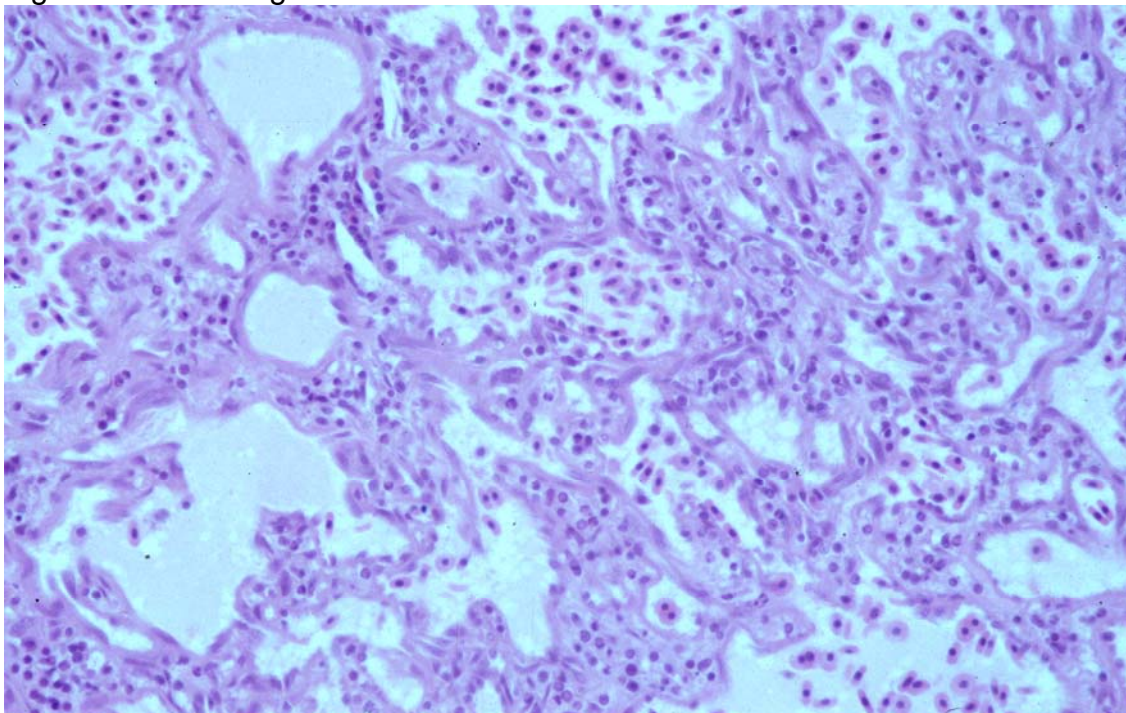


Figure 89. Thyroid adenoma

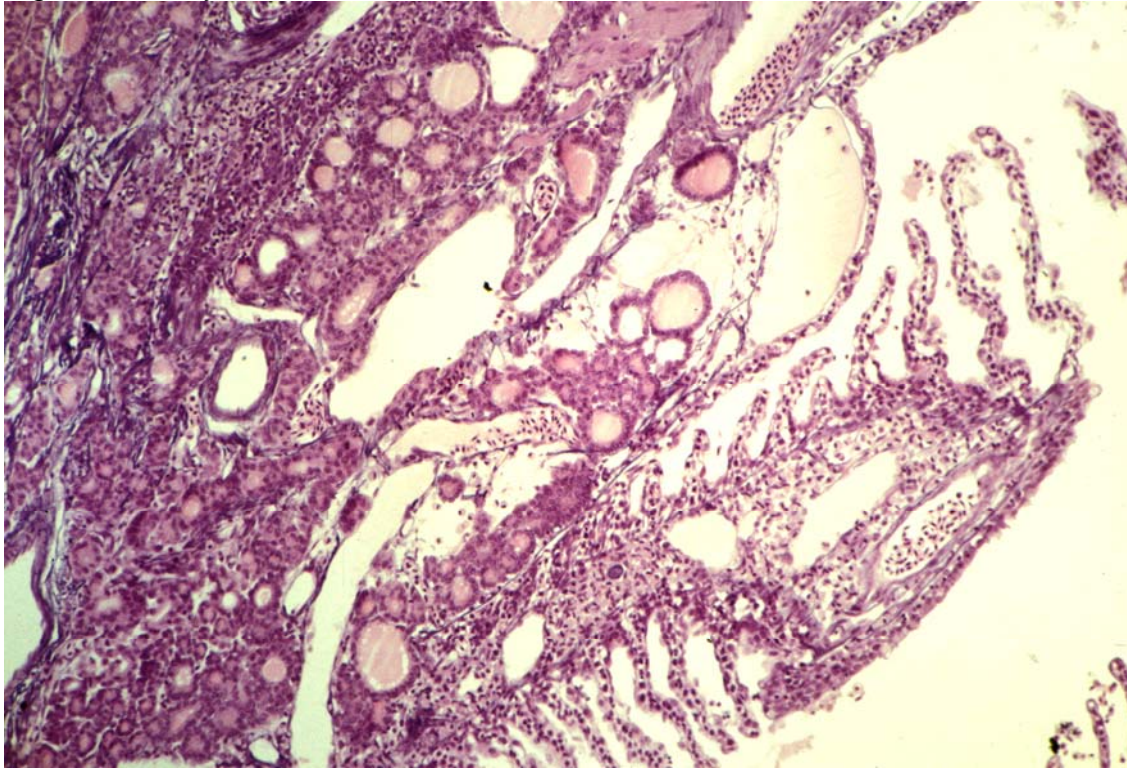


Figure 90. Thyroid adenoma

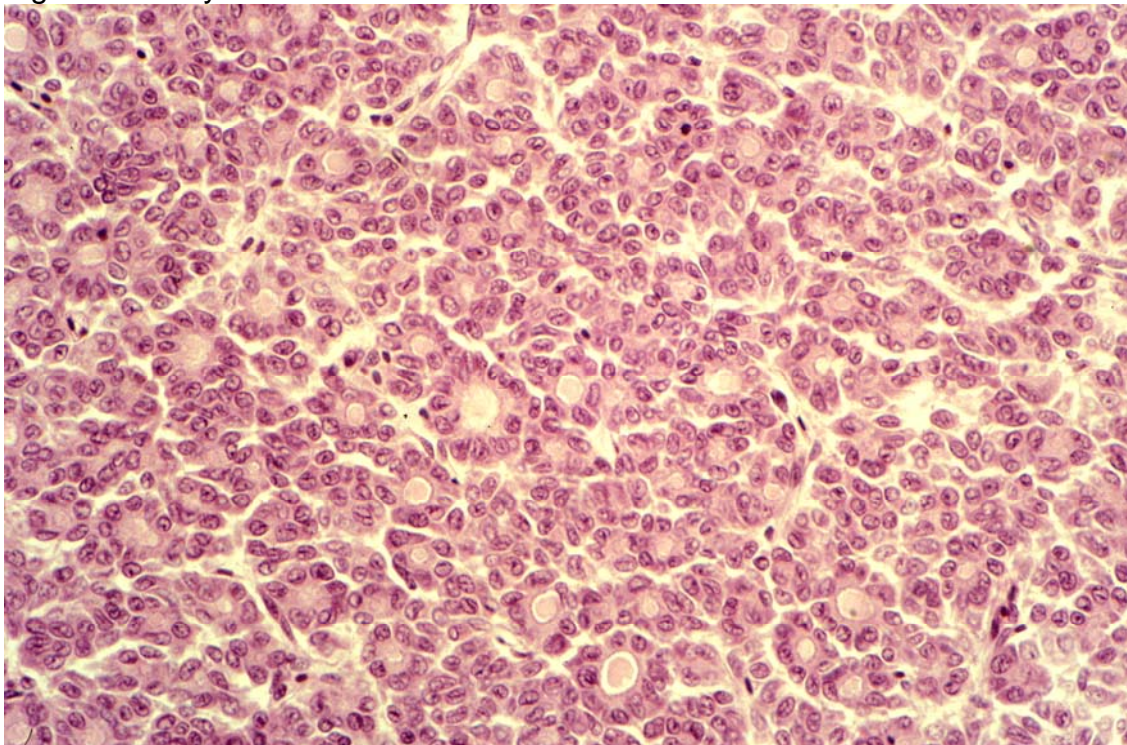


Figure 91. Pancreatic adenocarcinoma

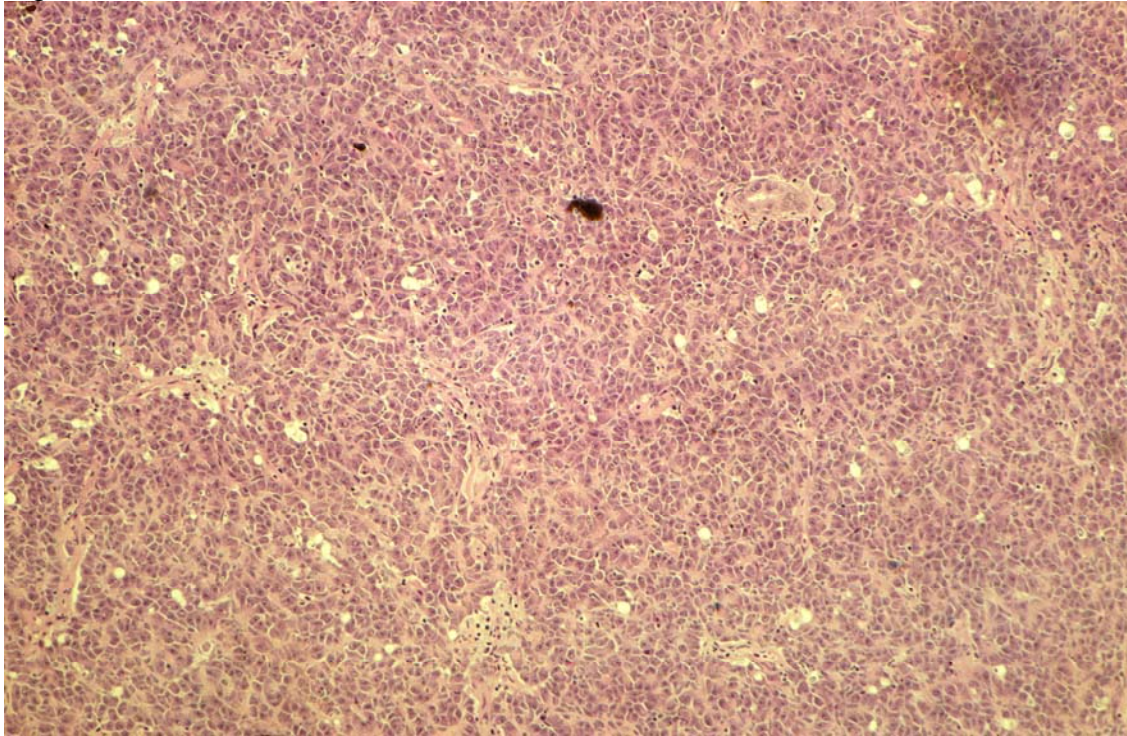


Figure 92. Pancreatic adenocarcinoma

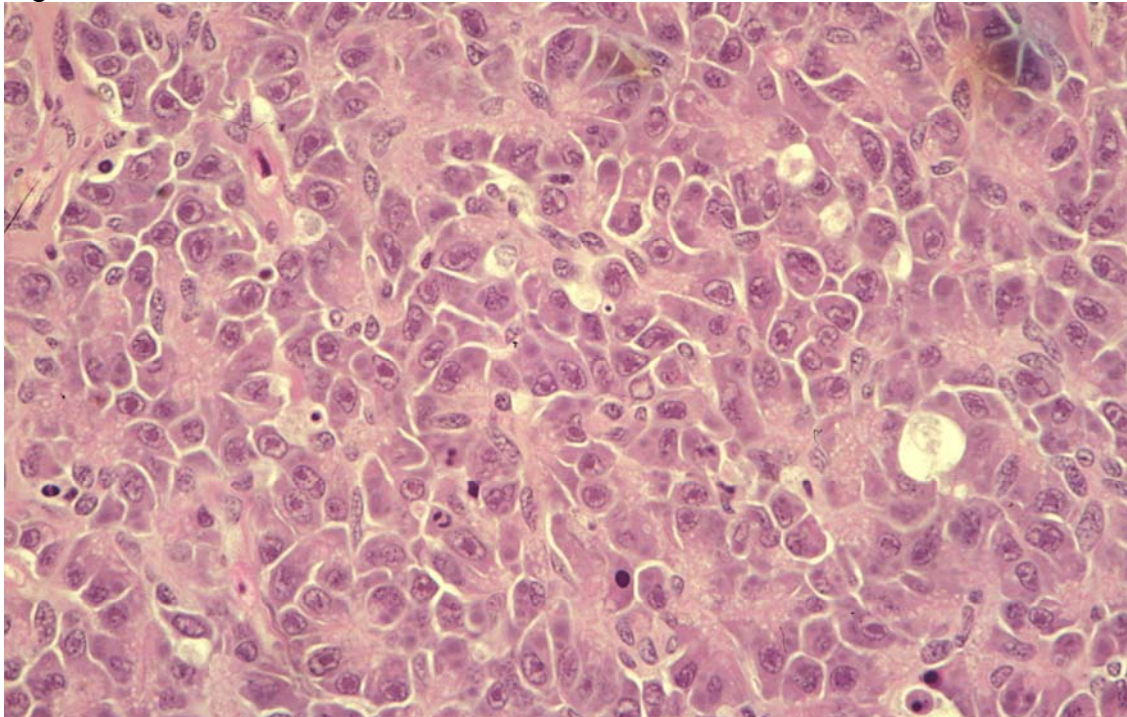


Figure 93. Pancreatic adenocarcinoma

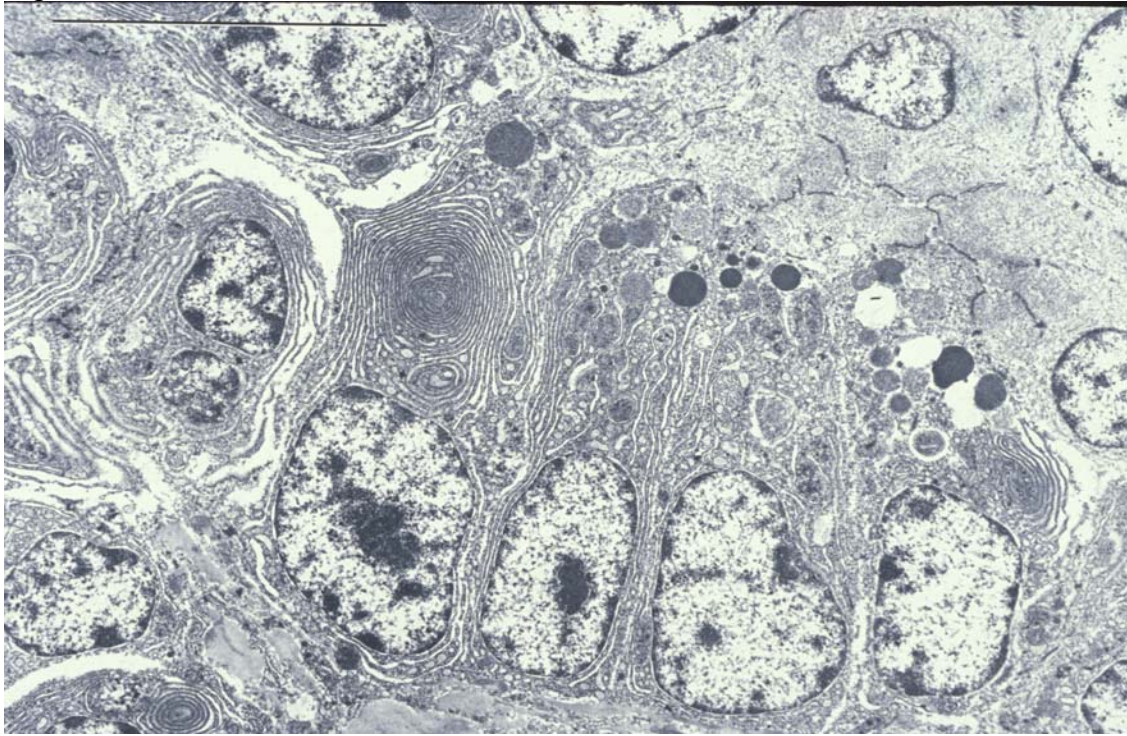


Figure 94. Pancreatic adenocarcinoma

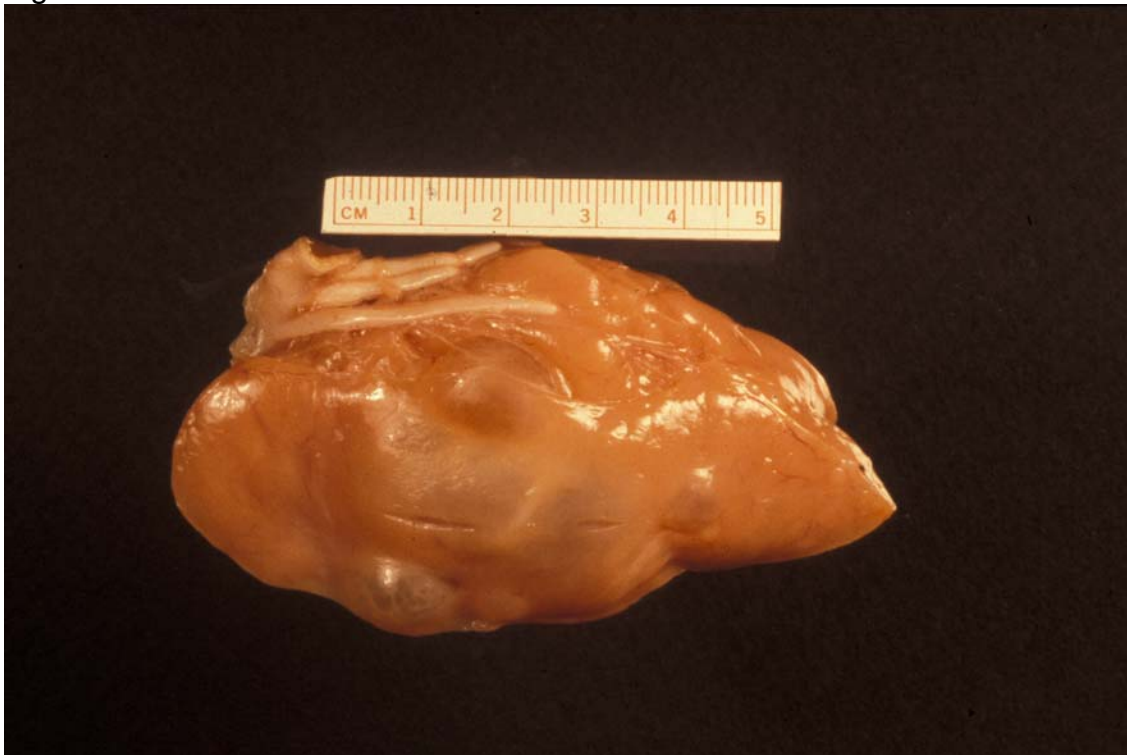


Figure 95. Nephroblastoma

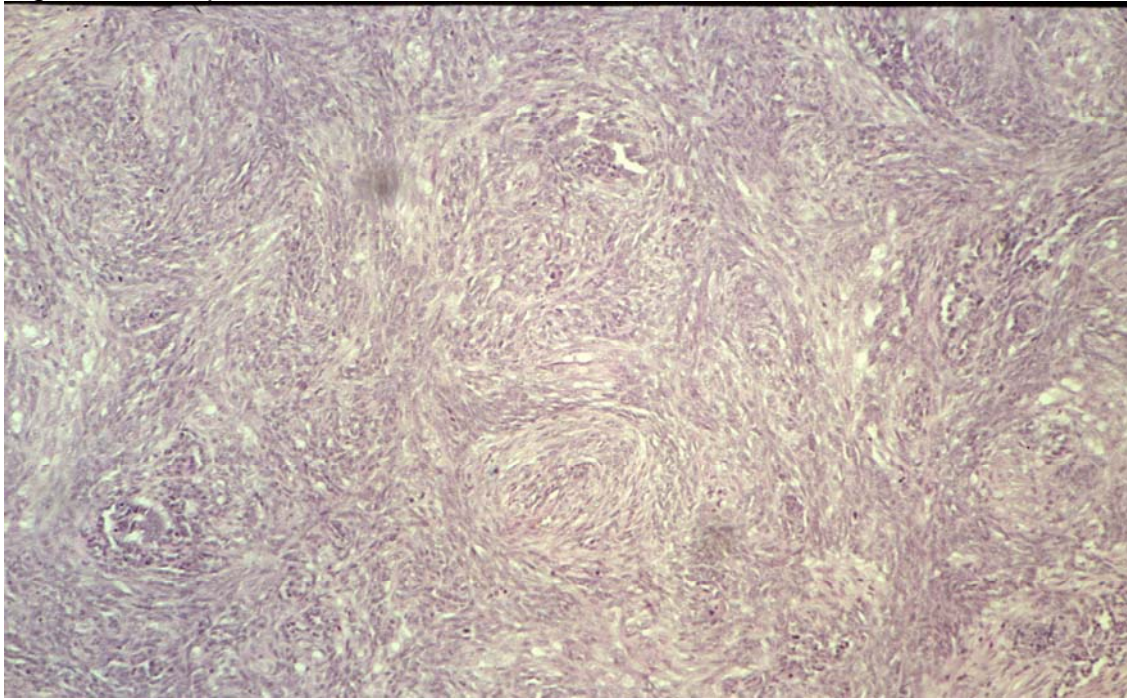


Figure 96. Nephroblastoma

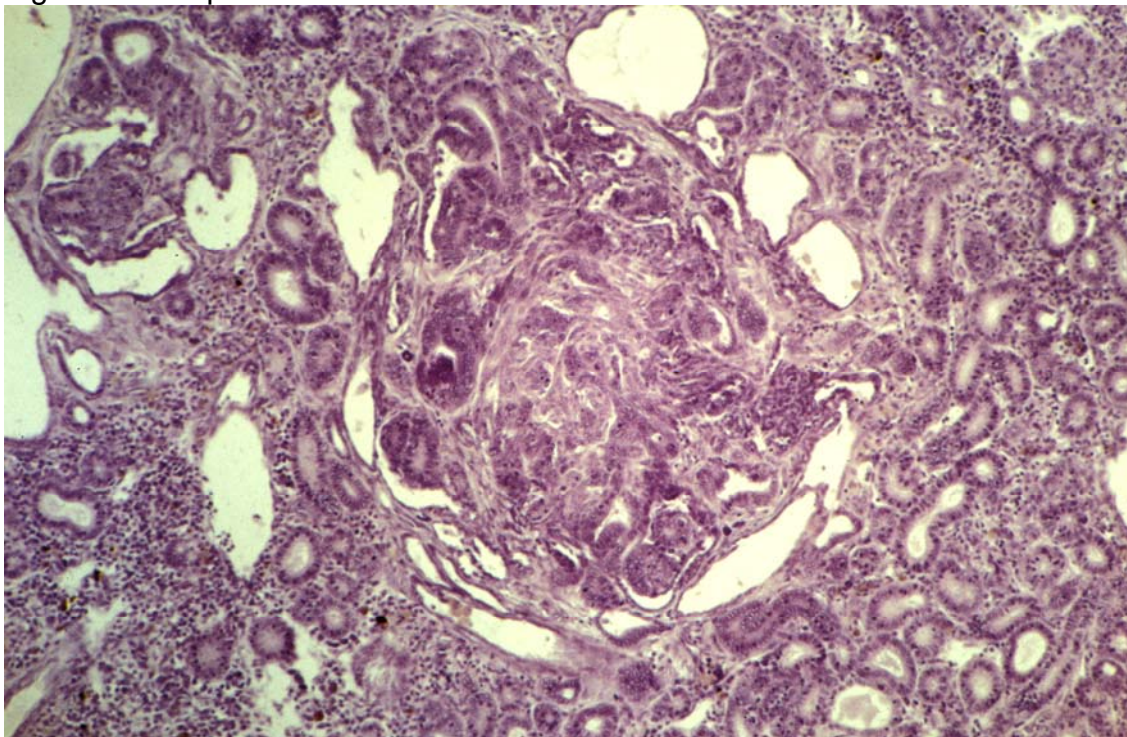


Figure 97. Nephroblastoma

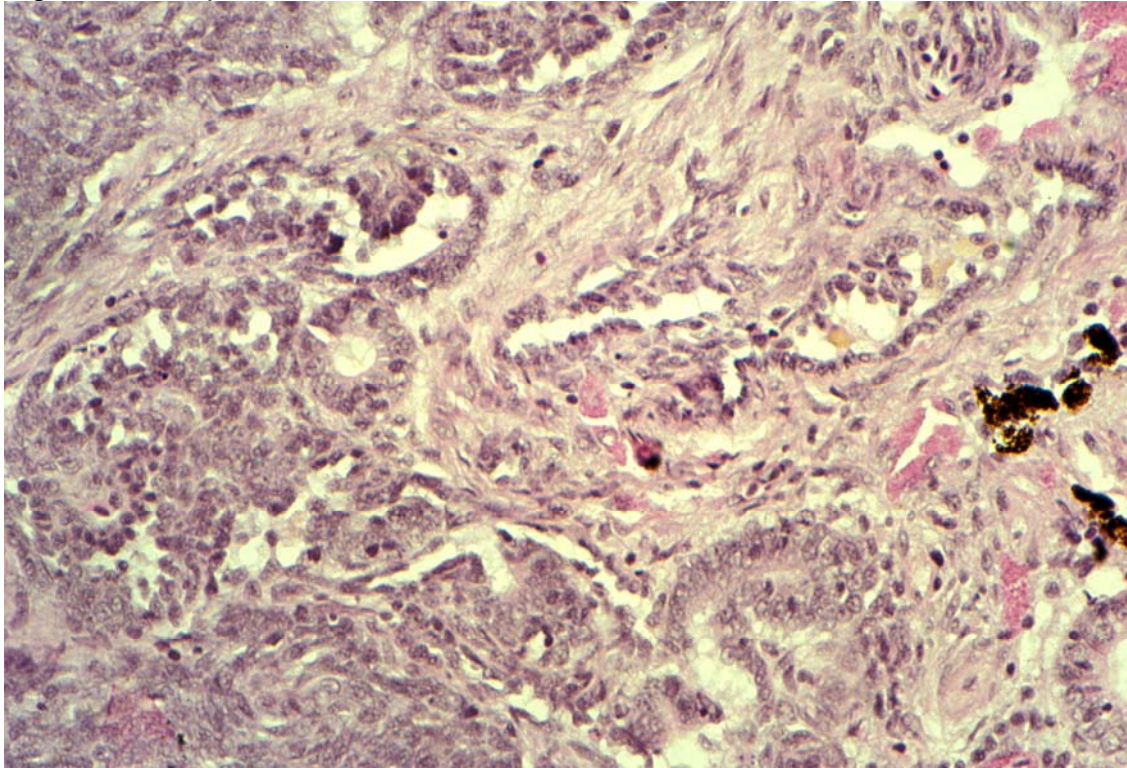


Figure 98. Nephroblastoma

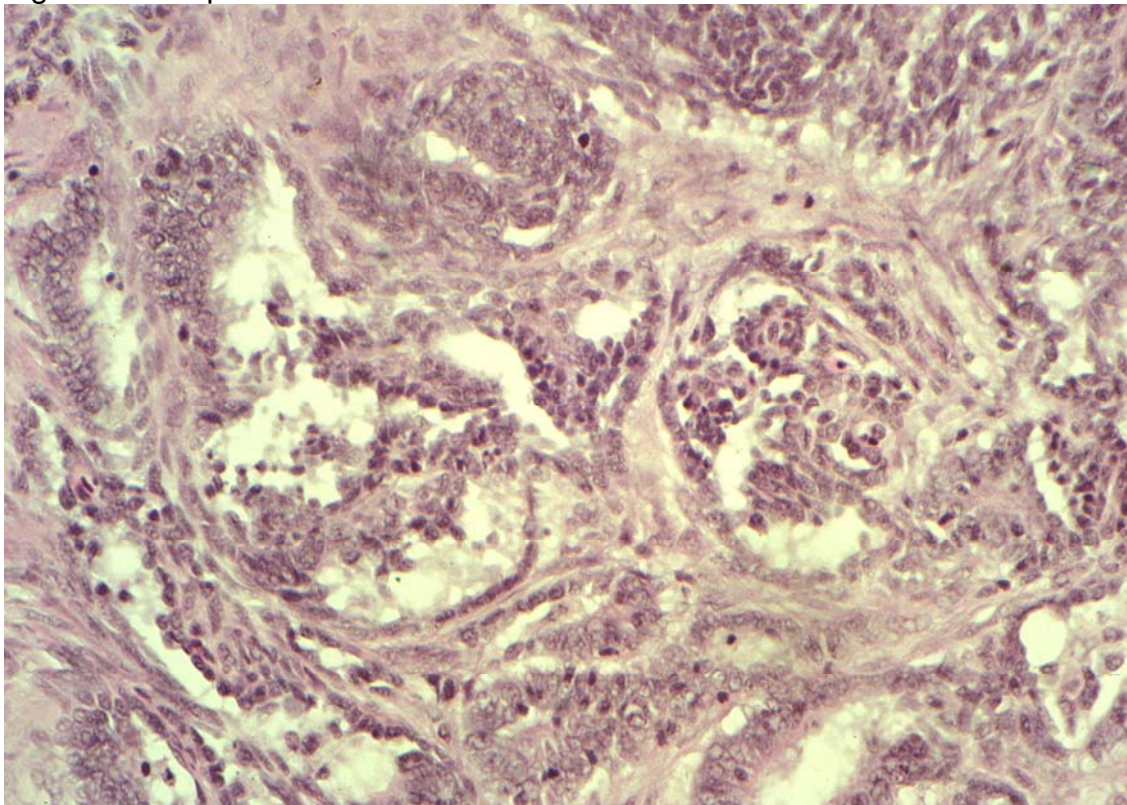


Figure 99. Nephroblastoma

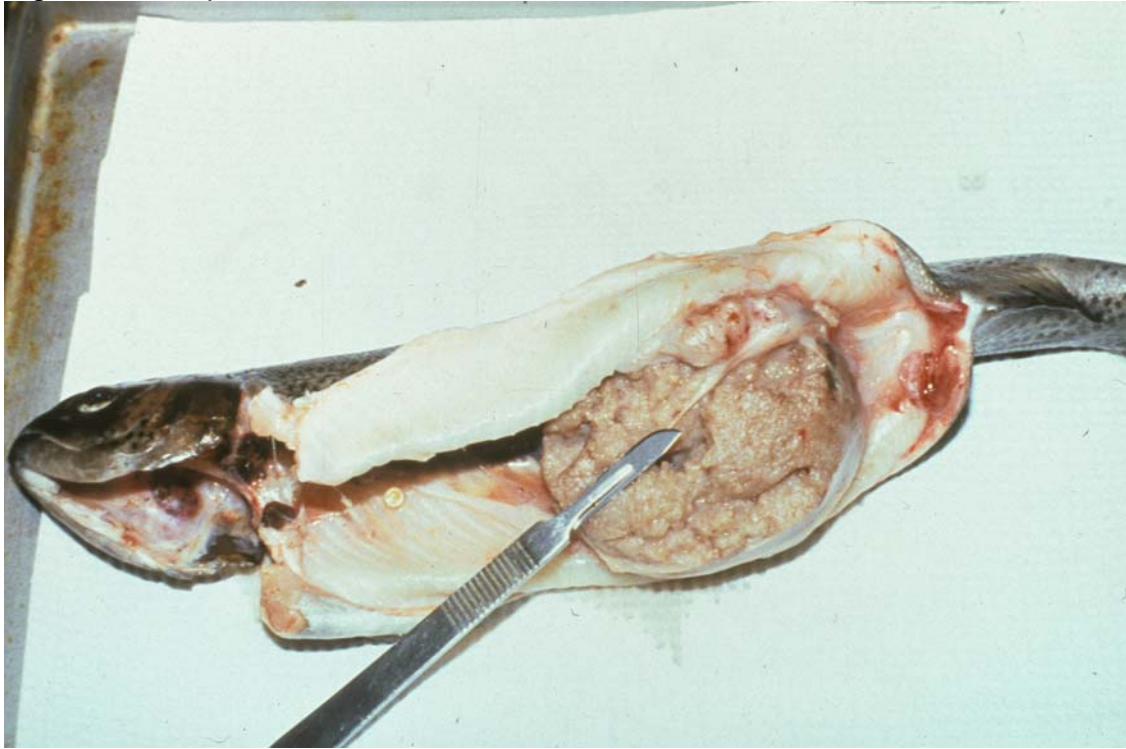


Figure 100. Lymphosarcoma (ovarian)

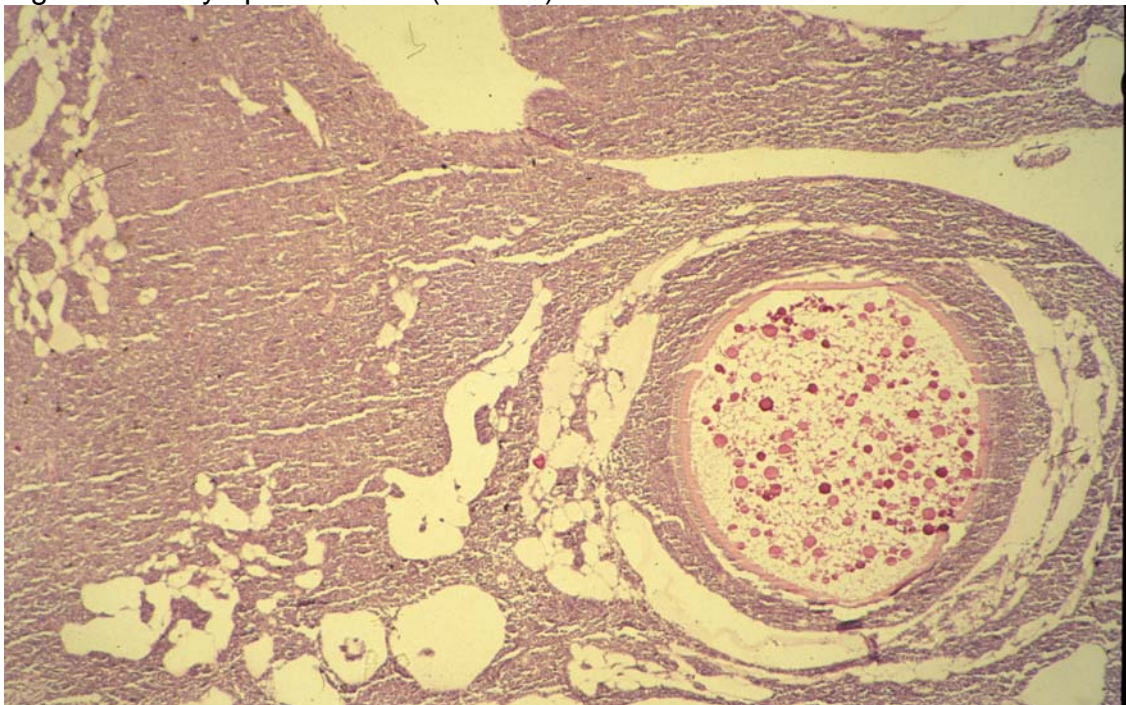


Figure 101. Lymphosarcoma

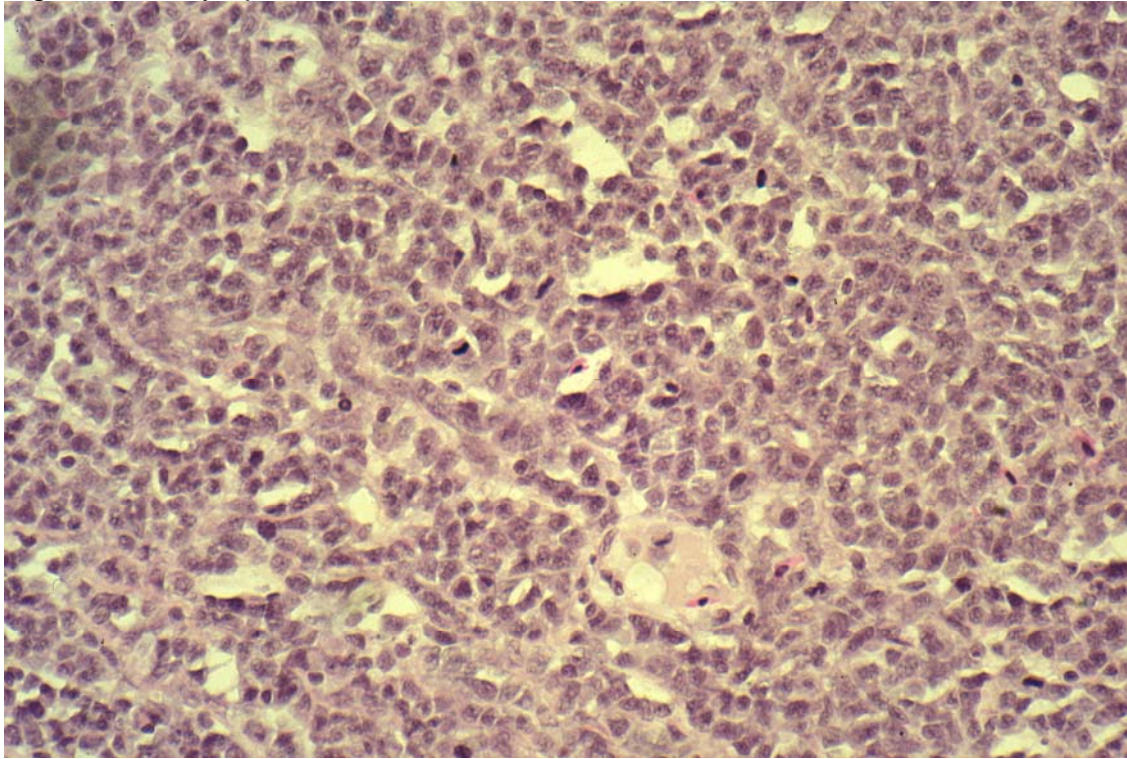


Figure 102. Lymphosarcoma (ovarian)



Figure 103. Fibrosarcoma

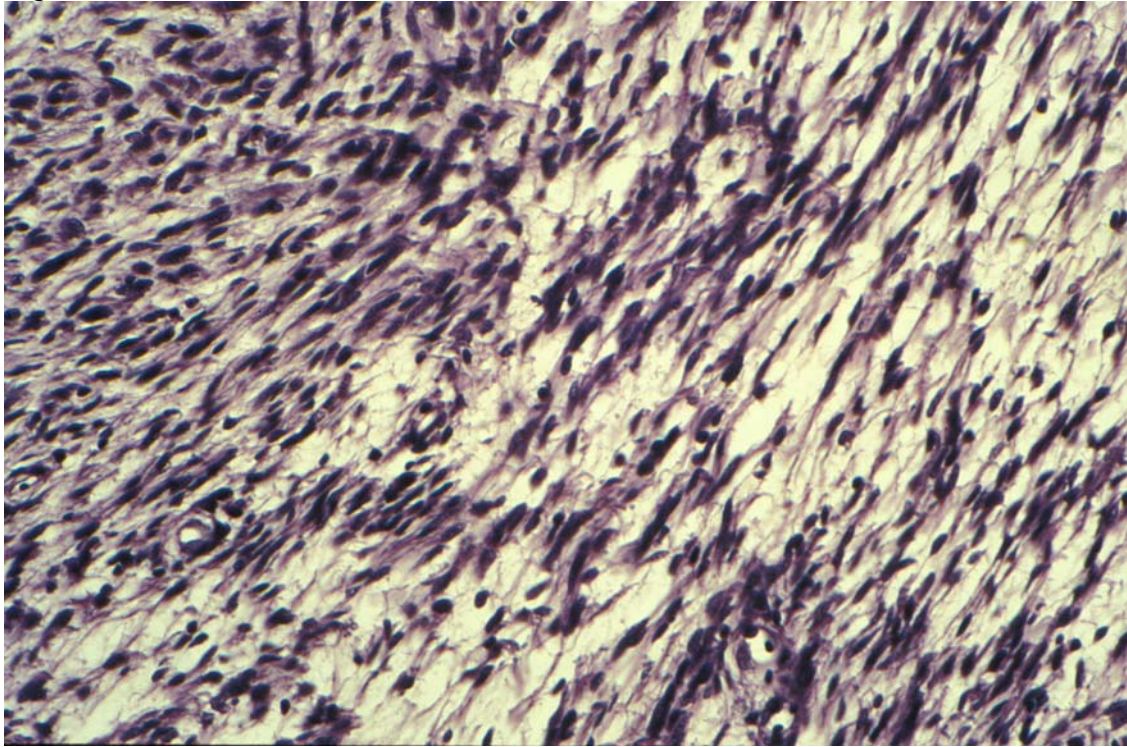


Figure 104. Hepatic adenocarcinoma

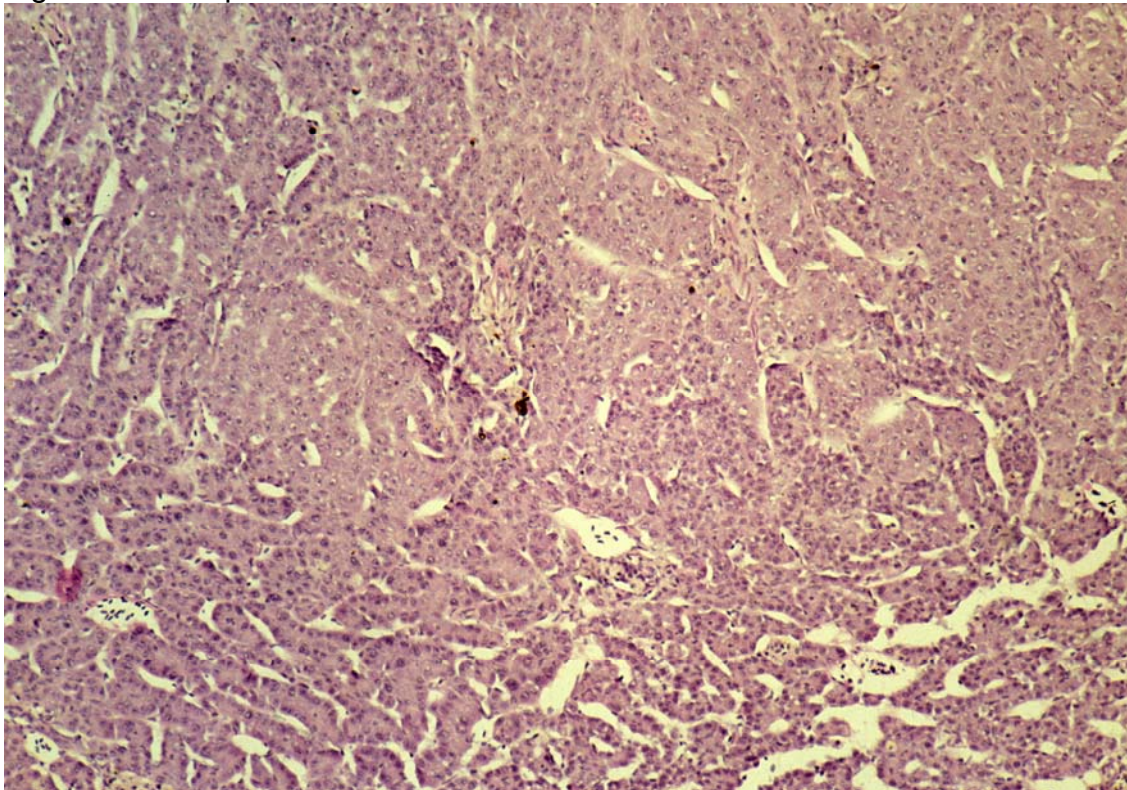


Figure 105. Hepatic adenocarcinoma

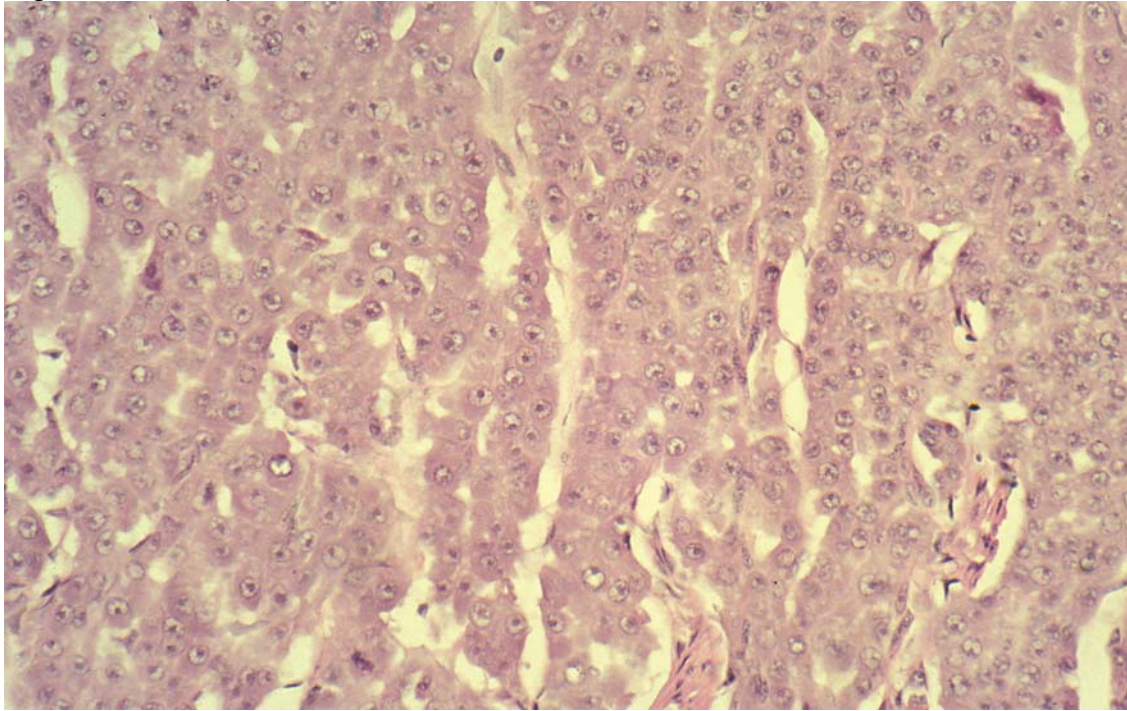


Figure 106. Hepatic adenocarcinoma

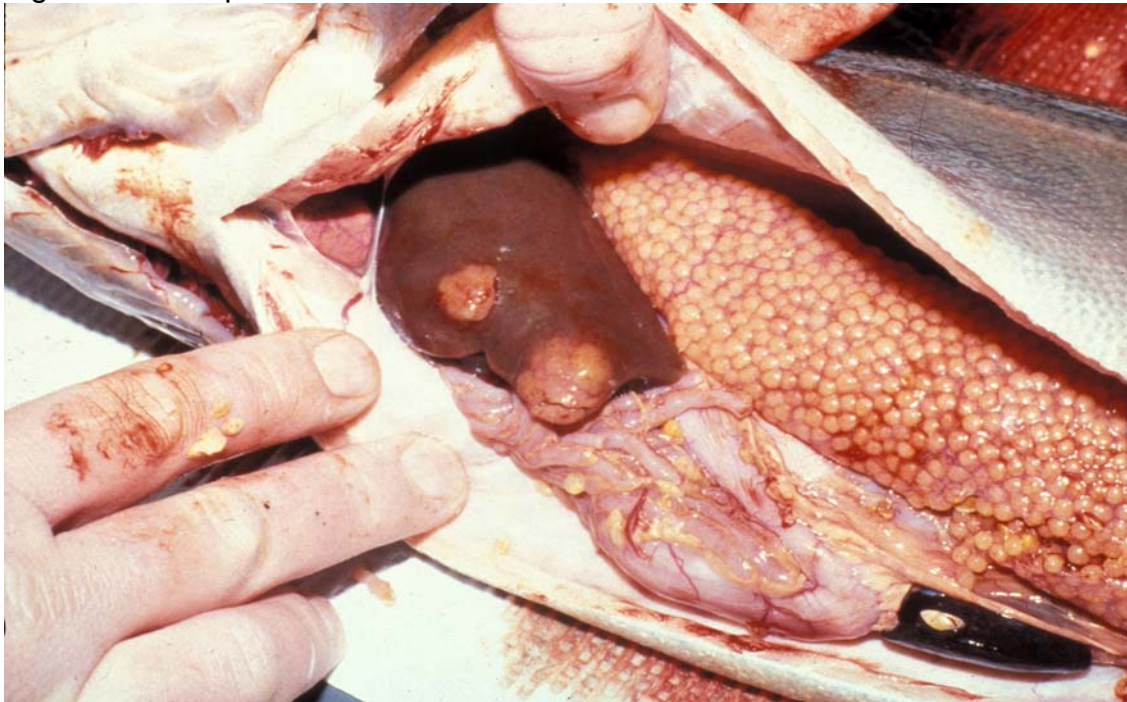


Figure 107. Fin - melanoma



Figure 108. Melanoma

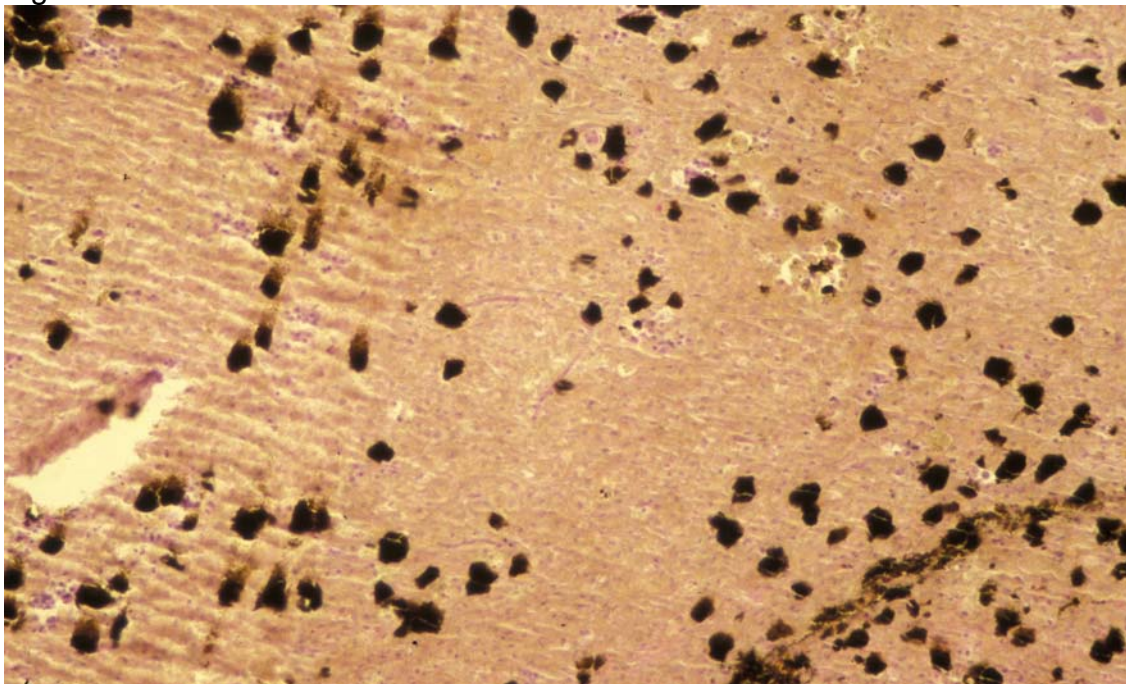


Figure 109. Liver - Monocytic leukemia

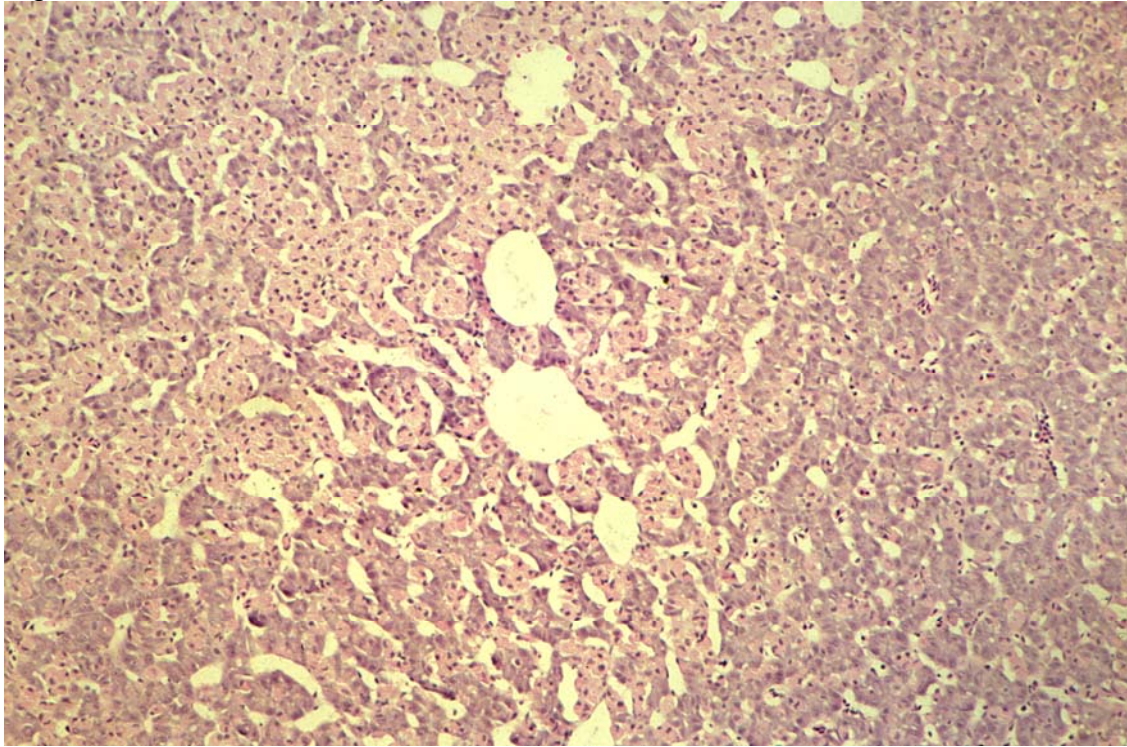
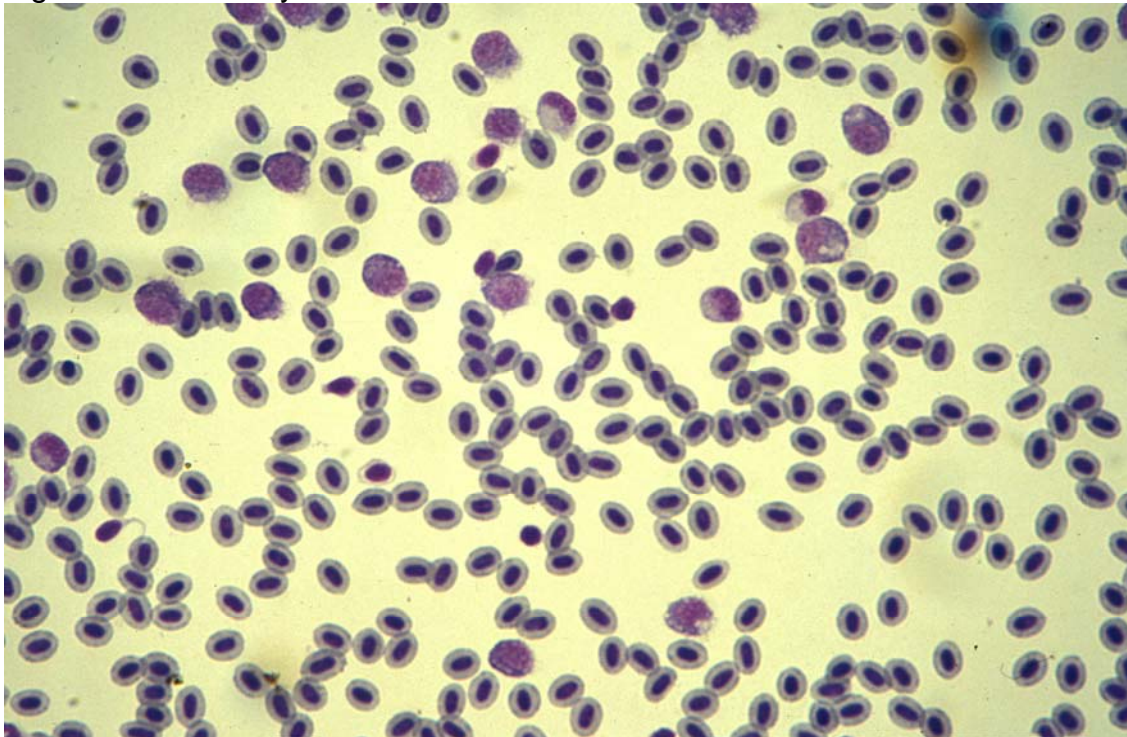


Figure 110. Monocytic leukemia



Post mortem autolysis

Post mortem autolysis is the natural decomposition of tissues after death. It will show up to varying degrees in histological preparations, depending upon how quickly sample collection and fixation occurred after death. Autolyzed tissues are obviously not the best samples to use for histological assessments, and care should be taken to minimize their inclusion in your samples. Sometimes it cannot be avoided. It is important to be able to separate tissue changes due to autolysis from those that are lesions. A few general rules will help:

- Post mortem autolysis can look very much like coagulative necrosis. Cell structure is maintained, and nuclei are faded or absent, similar to karyolysis during necrosis. However, necrosis will usually have a patchy distribution with variability in intensity from severe to absent in a given tissue. Autolysis on the other hand is a diffuse change; the entire tissue is similarly affected.
- Necrotic tissue induces inflammation; you will be able to find evidence of an inflammatory response surrounding necrotic tissue in most cases. Autolysis occurs after death of an animal, so inflammation is not possible.
- Autolysis progresses as a wave of degeneration in some tissues, particularly the intestine. Here, the autolytic change starts on the surface of the mucosa and gradually over time progresses deeper into the intestinal wall. This progression is uniform along the length of the intestine. Intestinal necrosis will have a patchy distribution, with some areas exhibiting prominent necrosis lying adjacent to less severely affected tissue, or even normal tissue.
- Gas bubbles produced by overgrowth of bacteria are common in autolyzed tissue.
- Large bacilli (“cadaver bacilli”) or other bacteria will be found in autolyzed tissue, without any evidence of an inflammatory response. Bacteria in viable tissue will usually cause some degree of inflammation.

See Figures 111 and 112.

Figure 111. Liver - autolysis

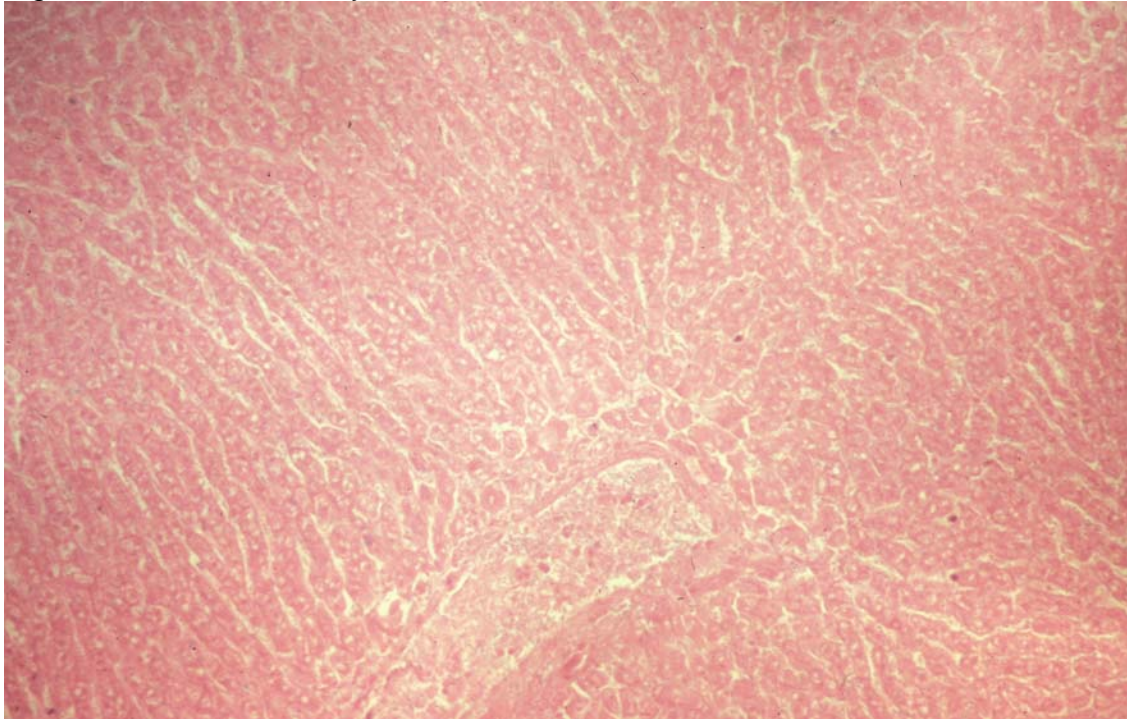
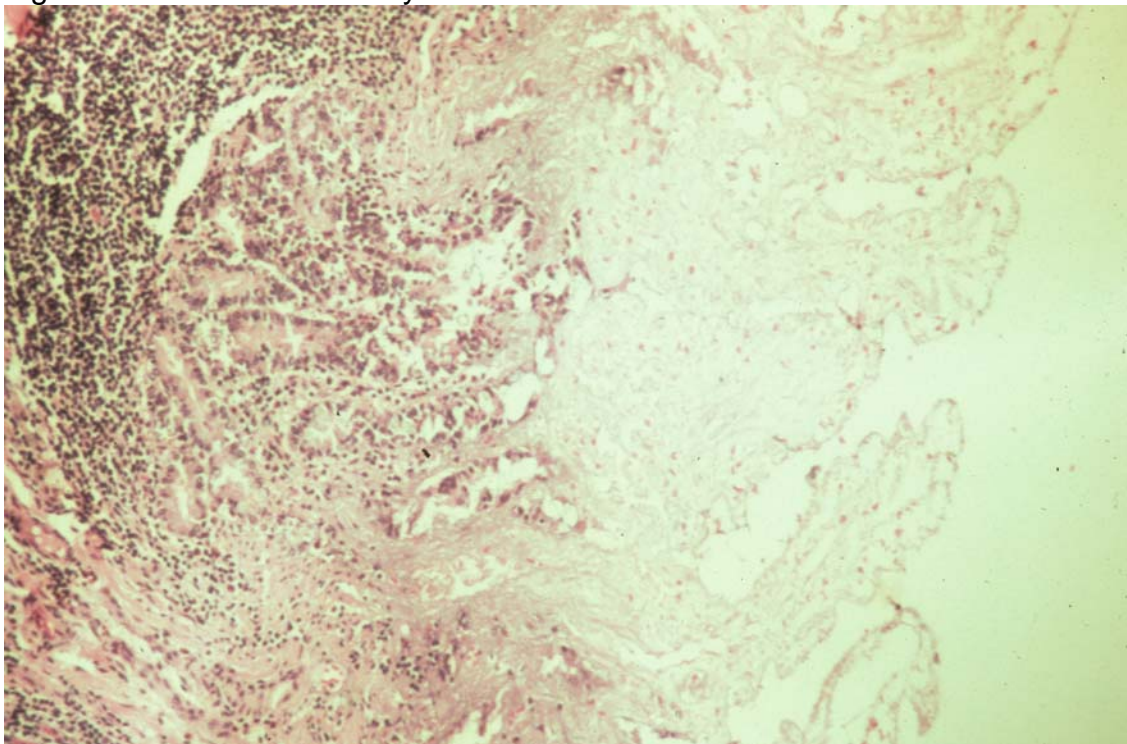


Figure 112. Intestine - autolysis



Freezing artifacts

If a tissue is frozen, ice crystals will form within and between cells. These crystals will lacerate and distort the tissue, and after the tissue is thawed, fixed, and processed, the sections will have freezing-induced artifacts that will obscure histological detail. Be aware of this potential confounding factor when choosing to examine frozen tissues.

Formalin will freeze as well, and any tissues within the frozen formalin will have freezing artifacts. If freezing temperatures cannot be avoided due to working conditions, the freezing point of formalin can be reduced by adding 10% by volume of 95% ethyl alcohol to the formalin; this may help protect the samples.

See Figures 113 and 114.

Figure 113. Freeze artifact

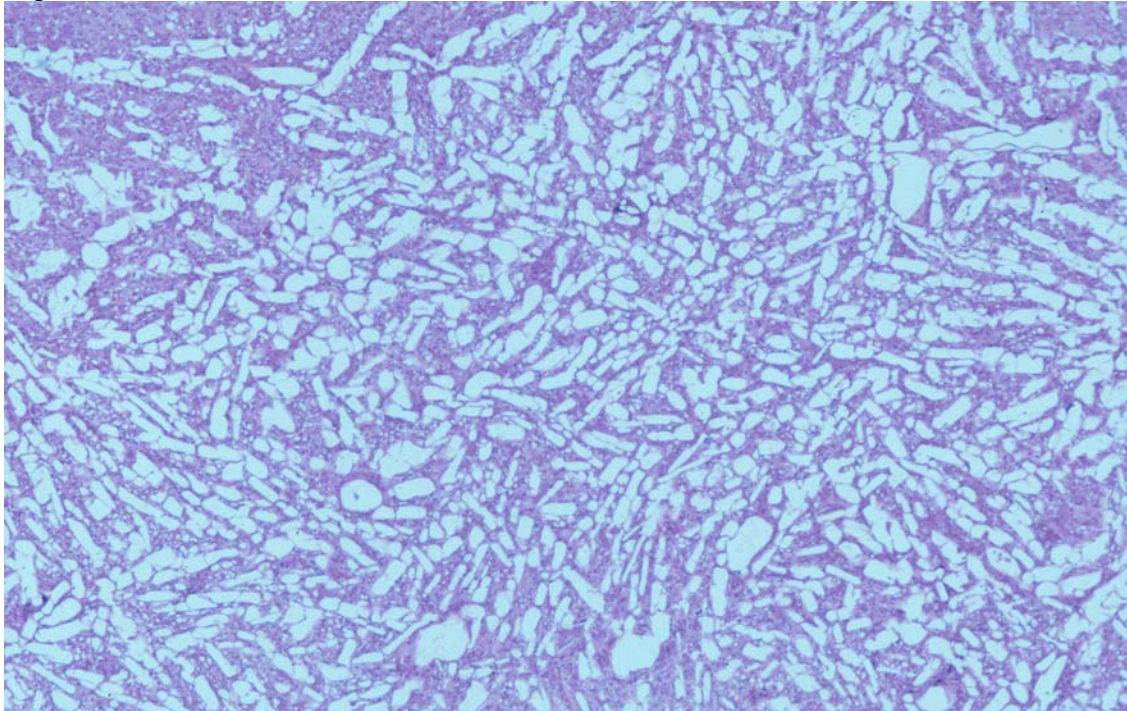
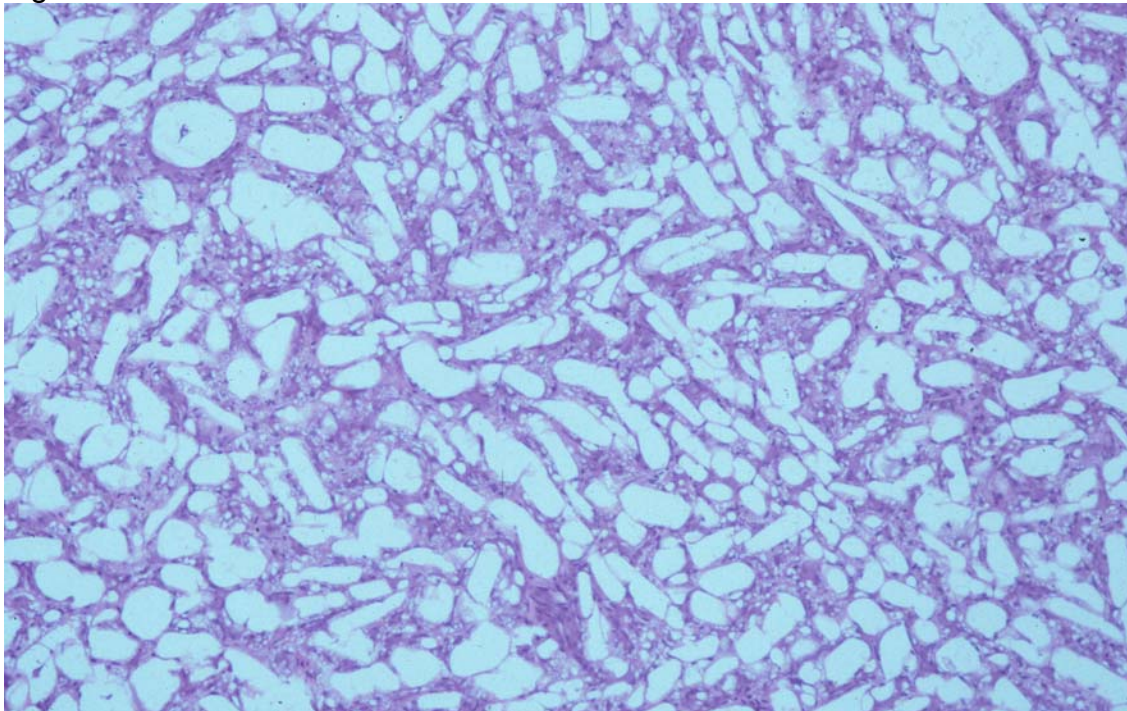


Figure 114. Freeze artifact



Additional Neoplasia Slides

(Legends to follow pictures)

A. Hemangioendotheliosarcoma

Figure 1



Figure 2

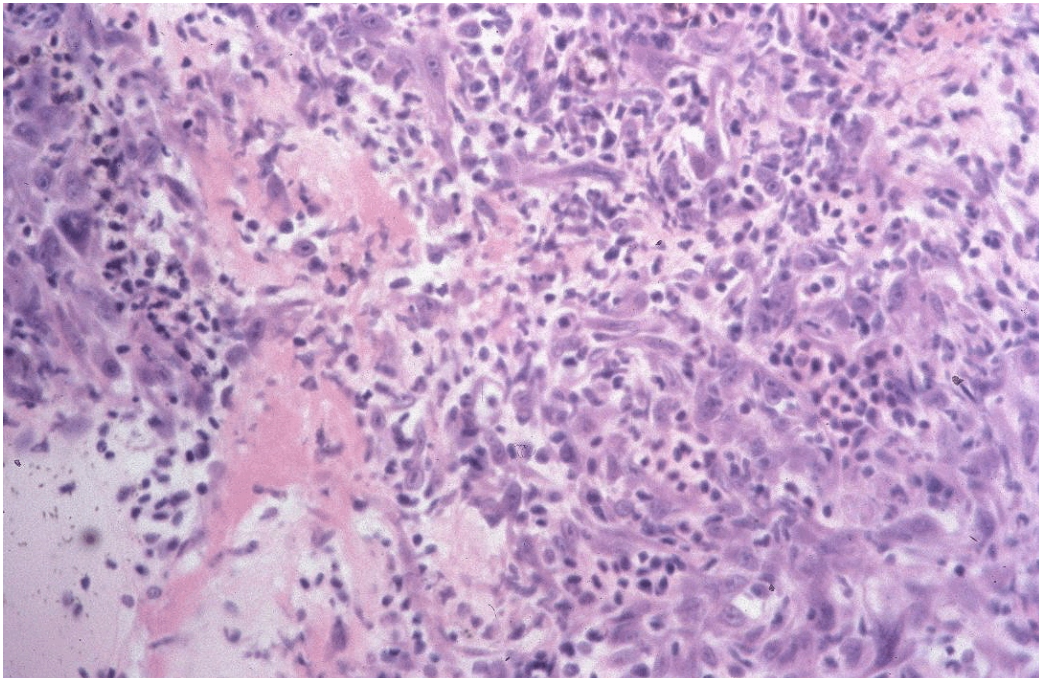


Figure 3

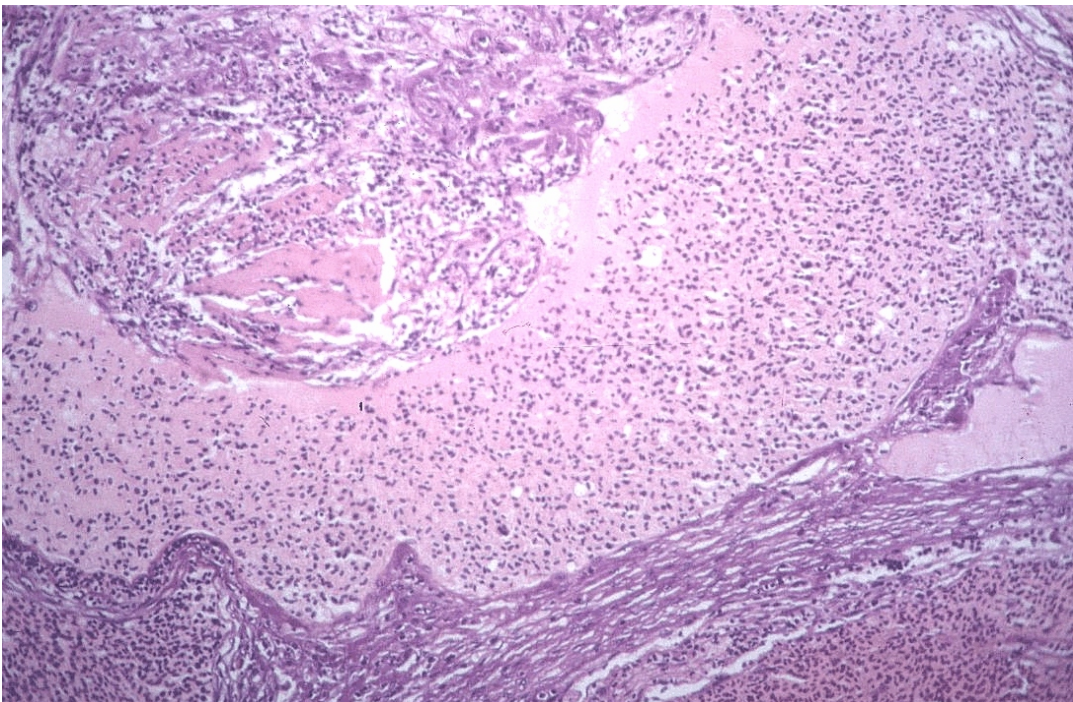
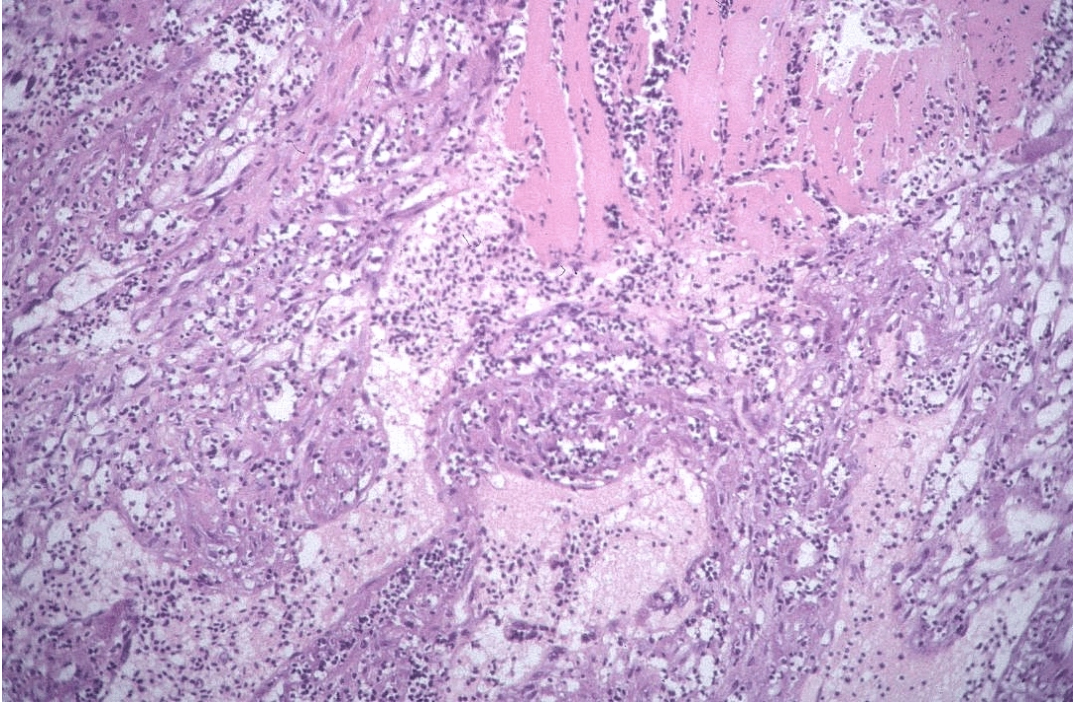


Figure 4

B. Melanoma

Figure 5

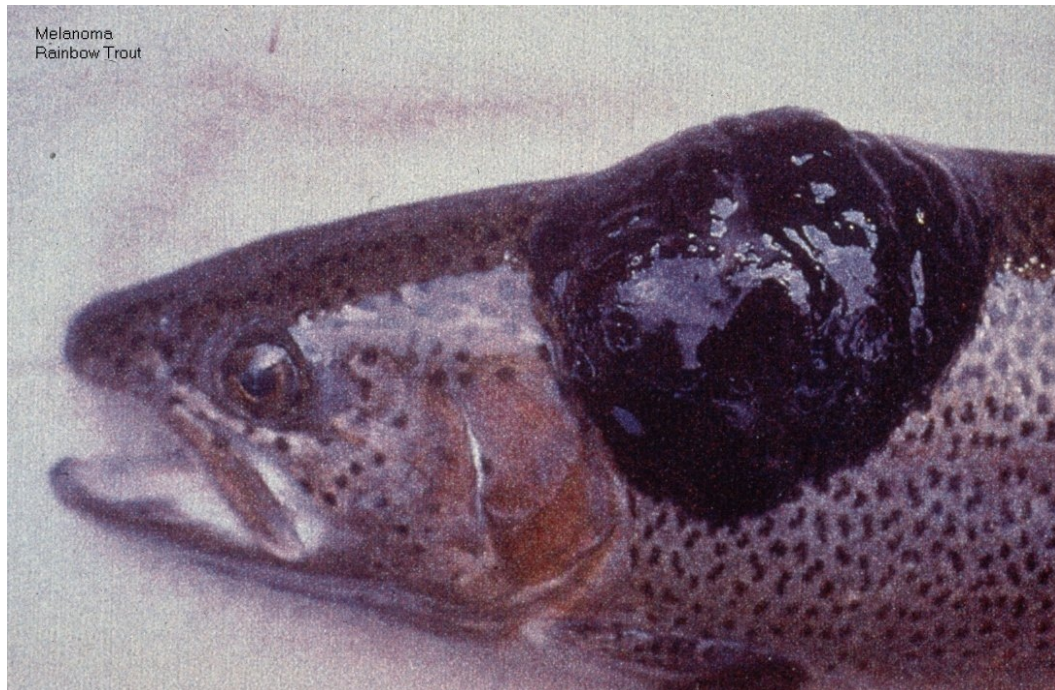


Figure 6



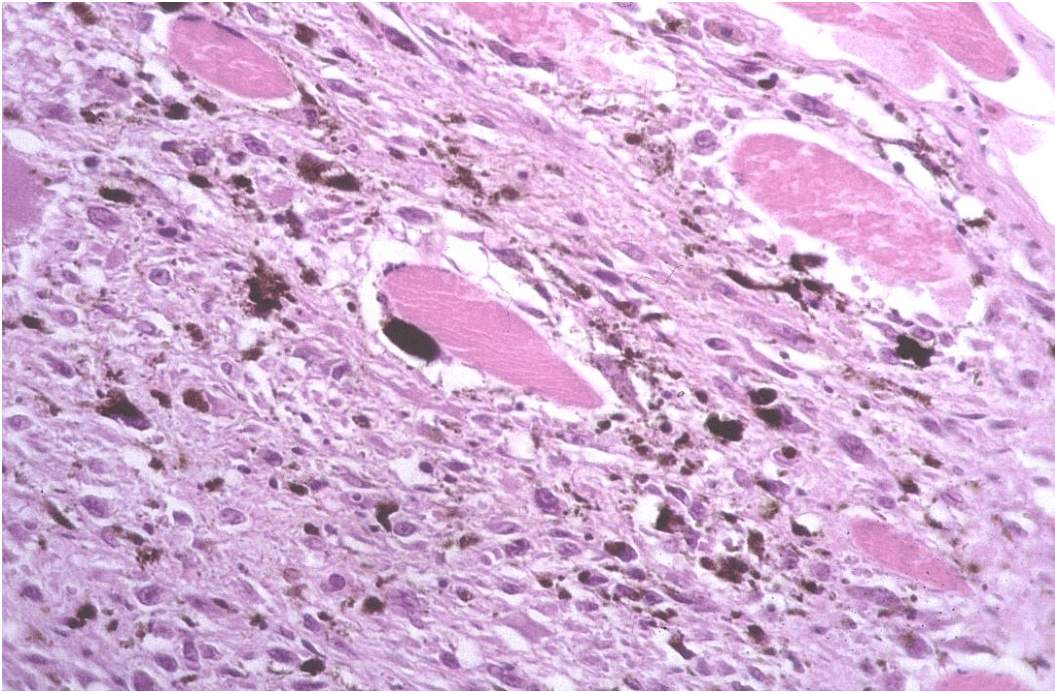


Figure 7



Figure 8

Figure 9

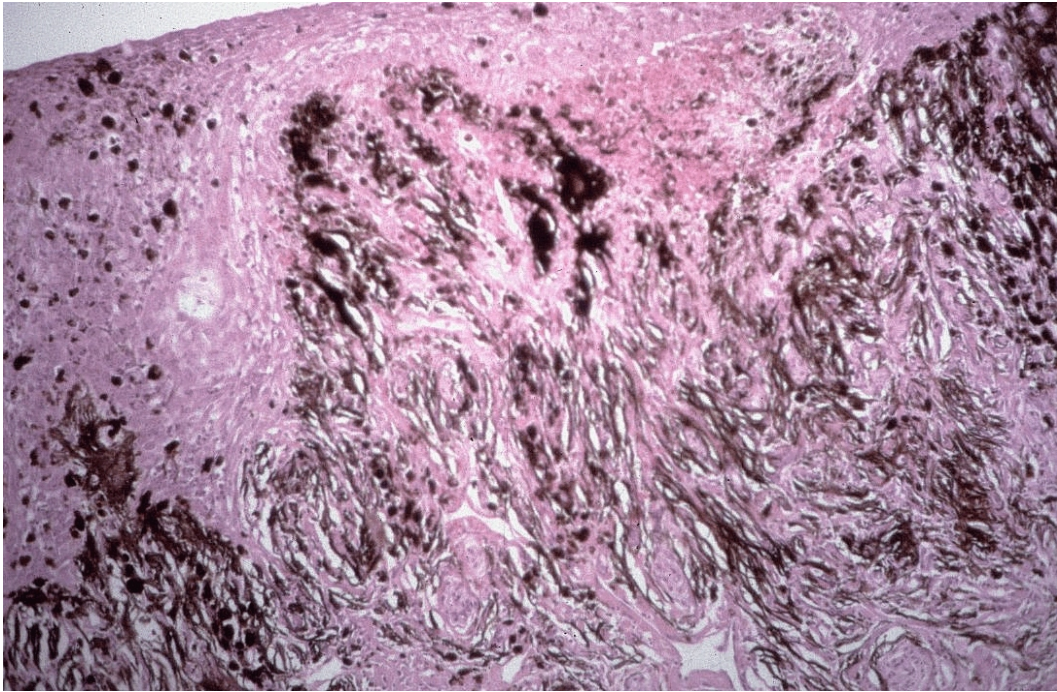
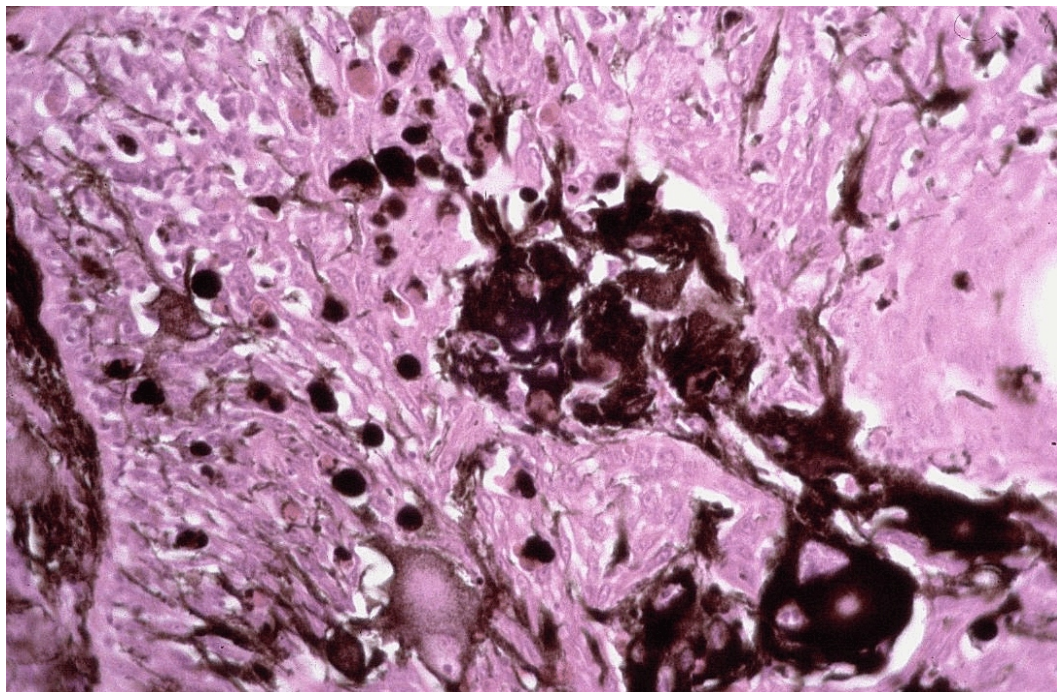


Figure 10



C. Thymoma

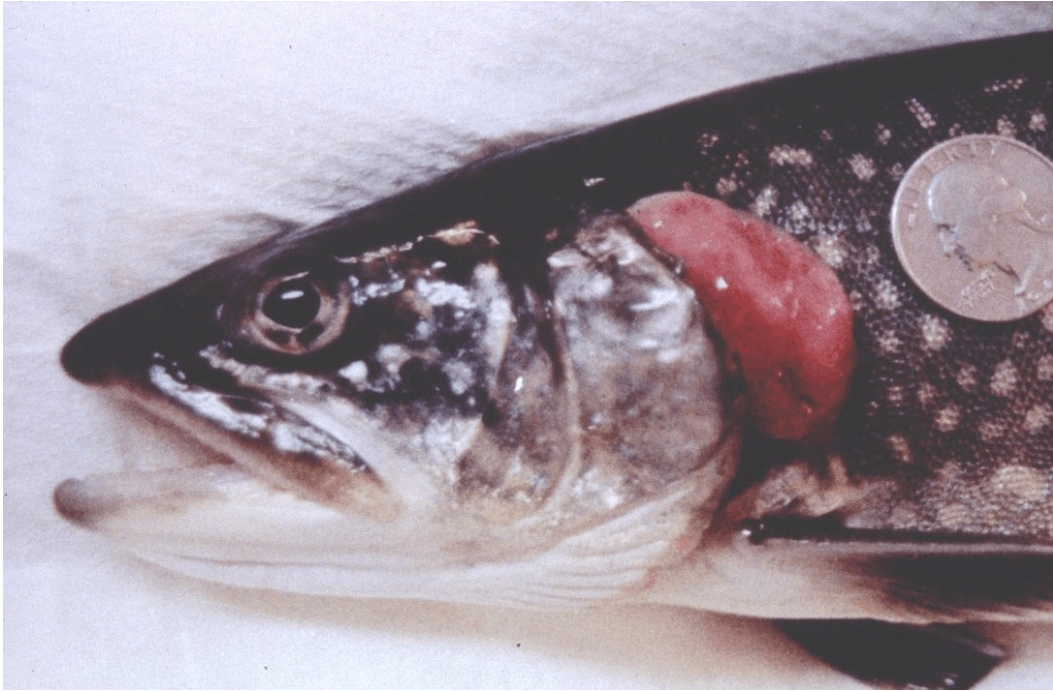
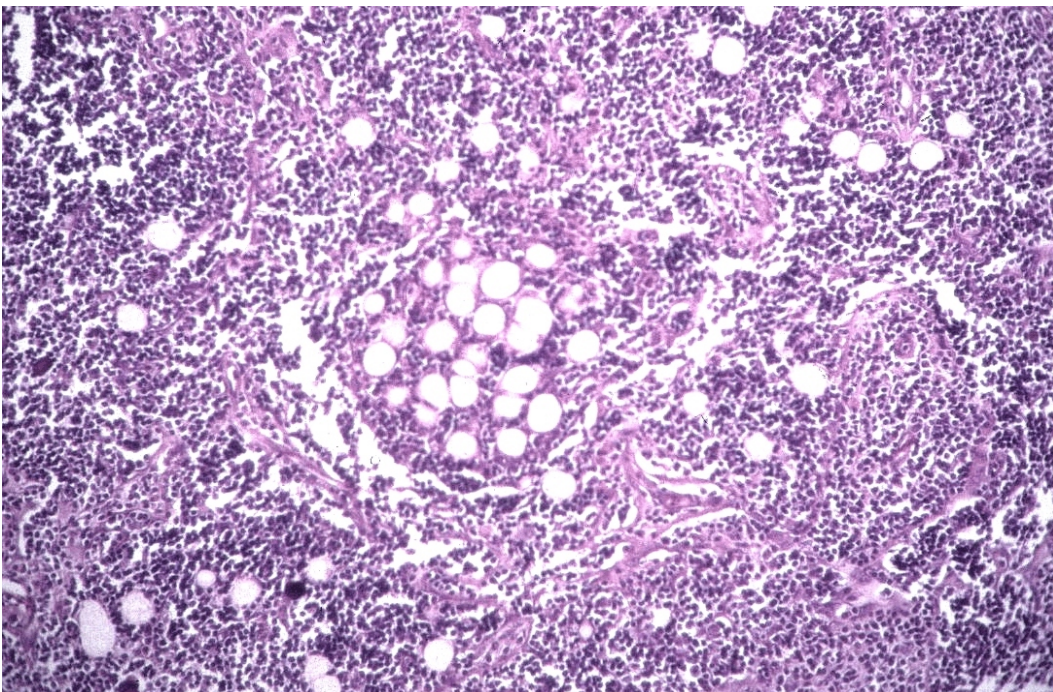


Figure 11

Figure 12



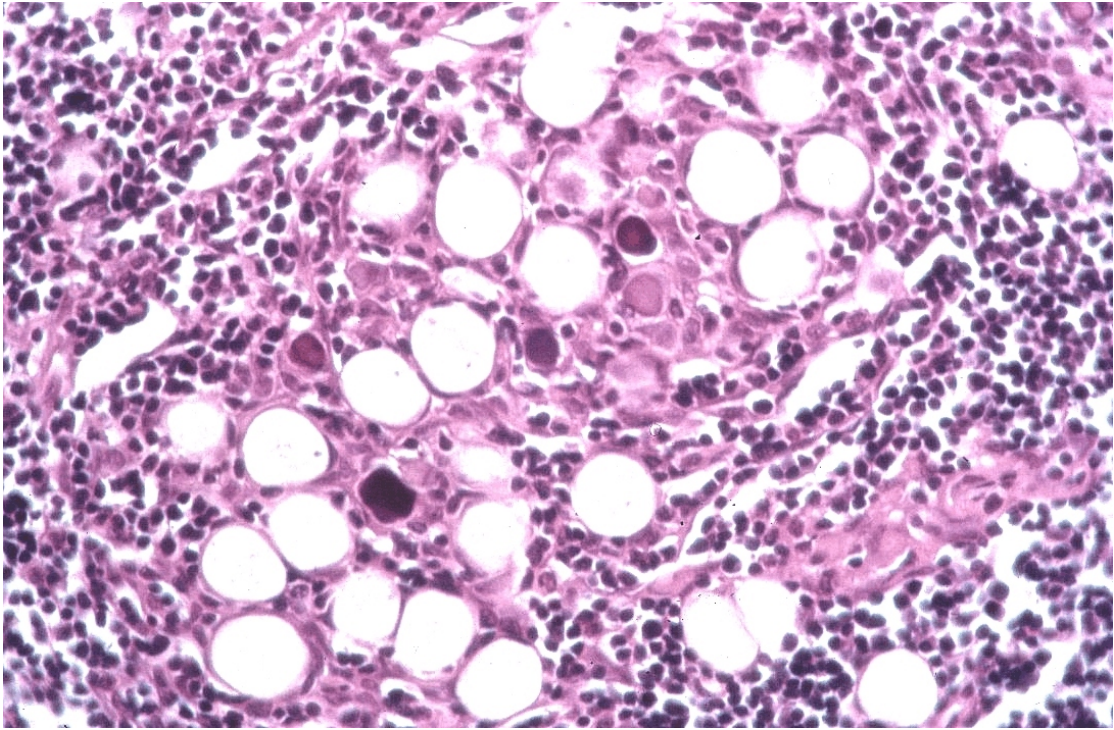


Figure 13

D. Adenocarcinoma

Figure 14

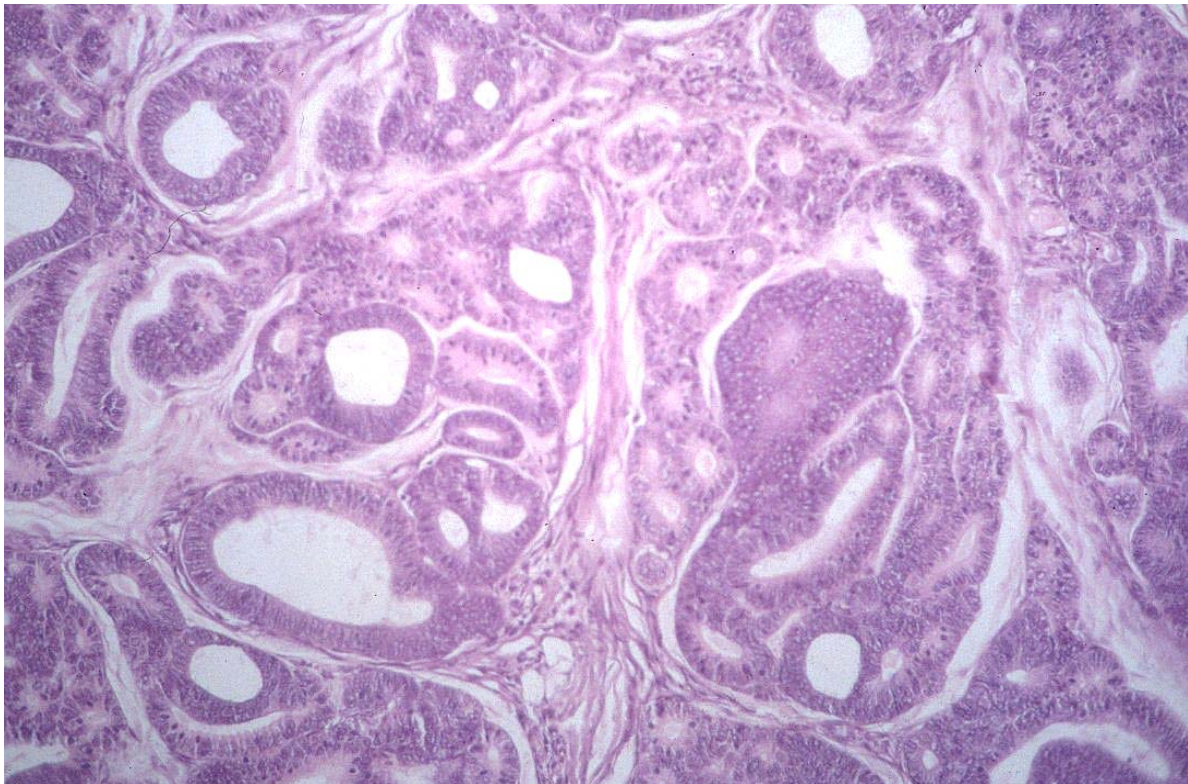
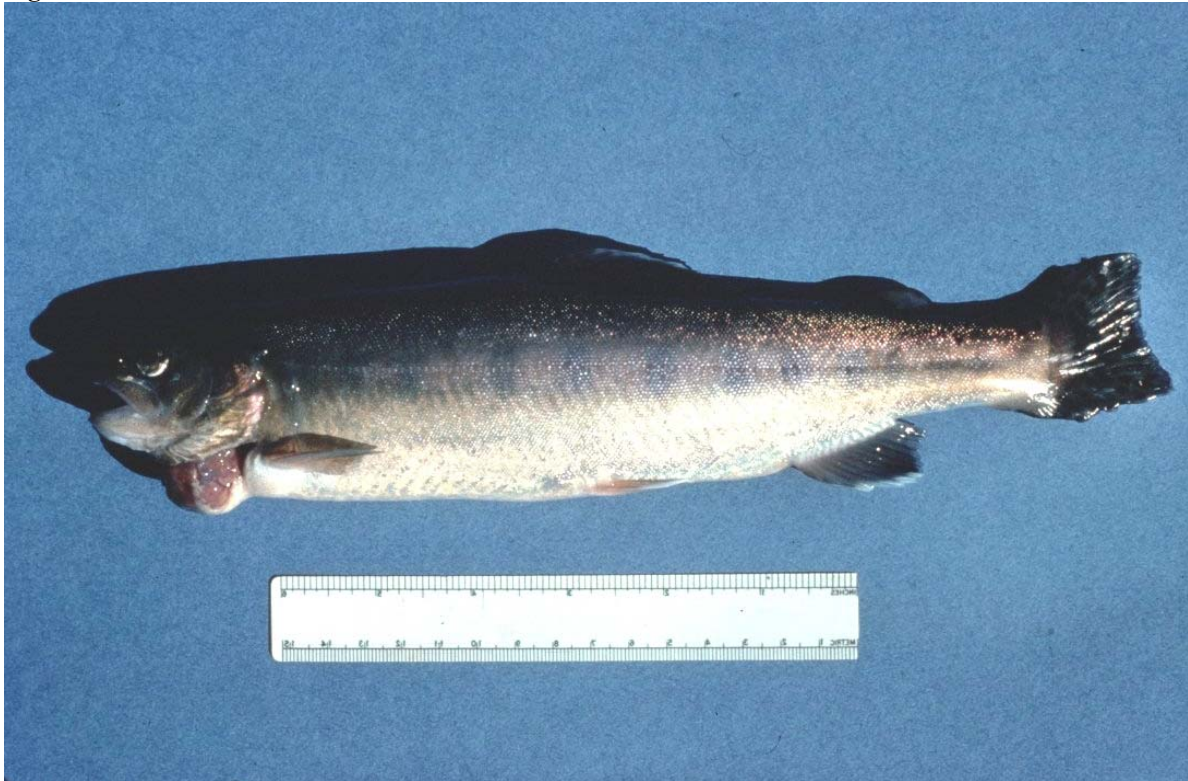


Figure 15

Figure 16

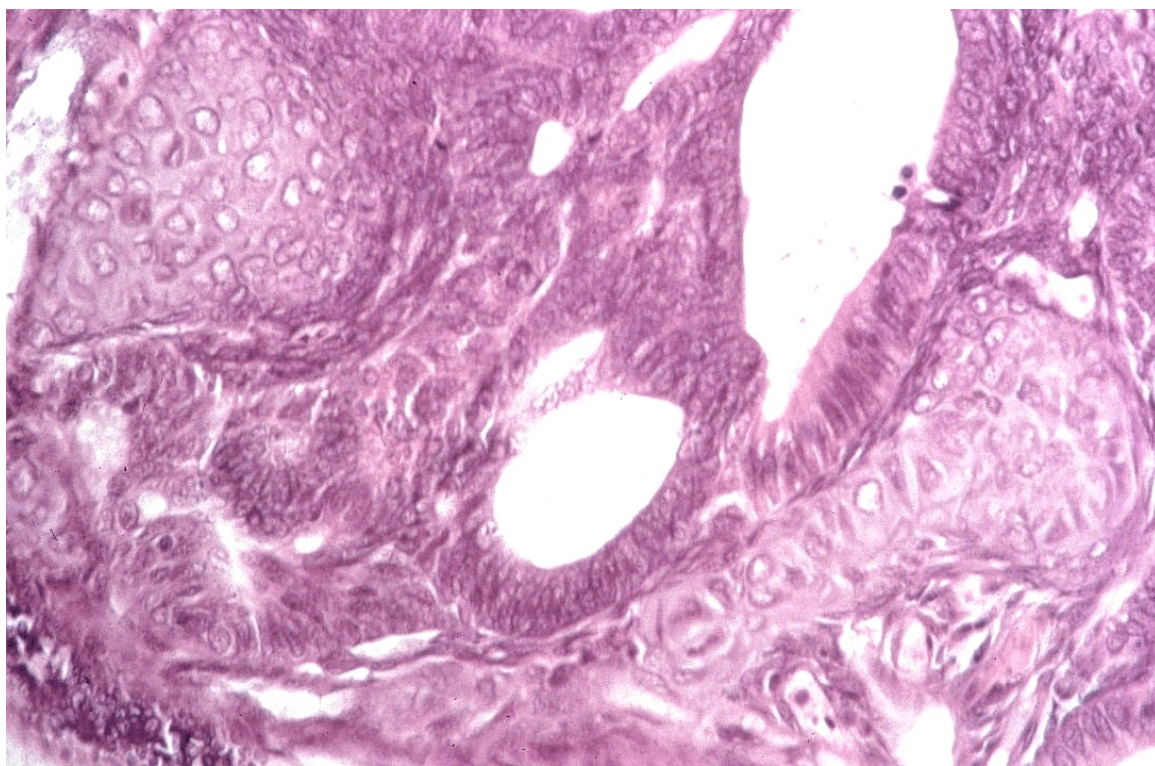
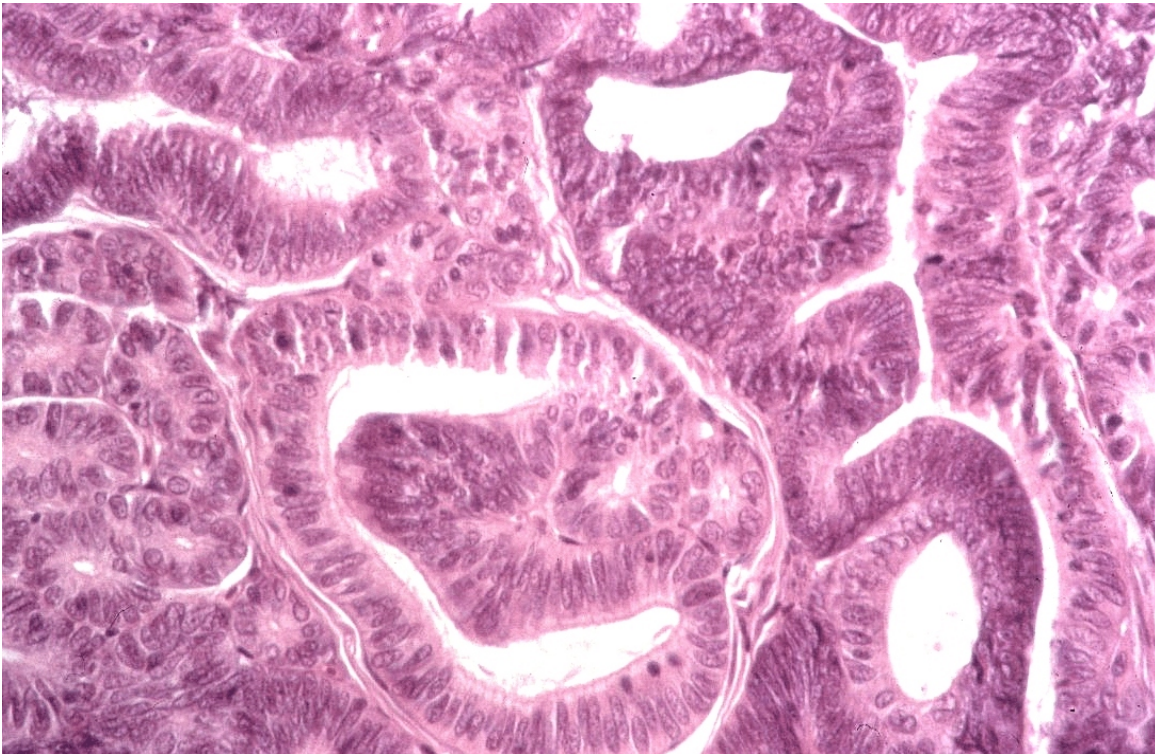


Figure 17

E. Nephroblastoma

Figure 18

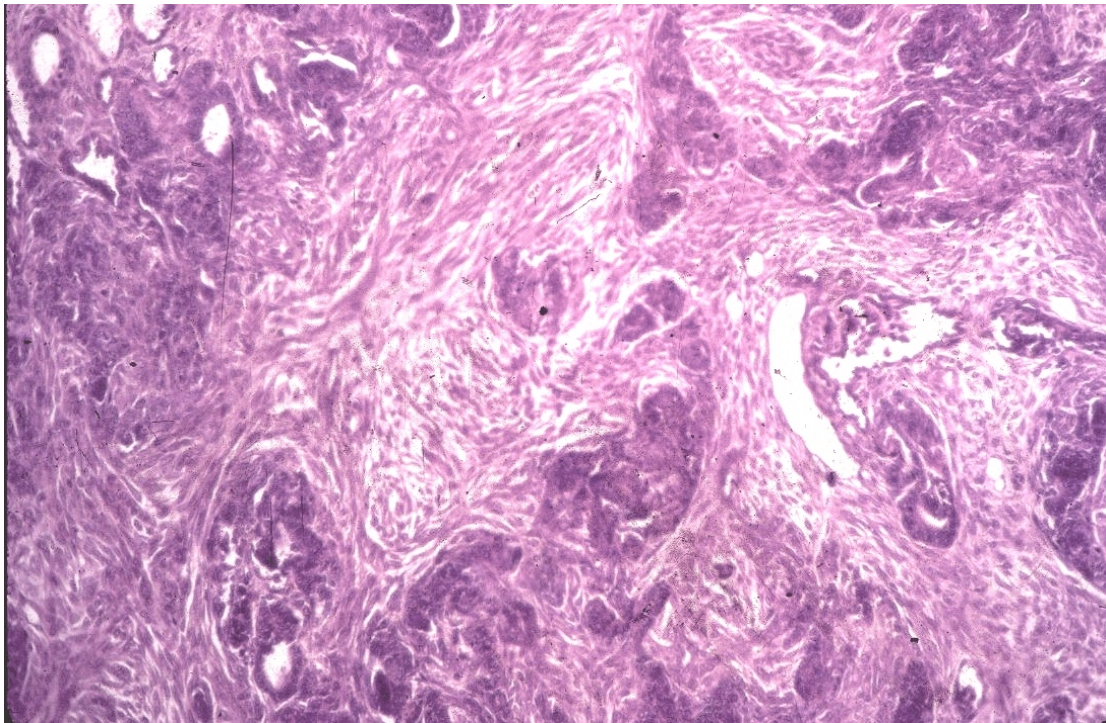


Figure 19

F. Fibrolipoma

Figure 20

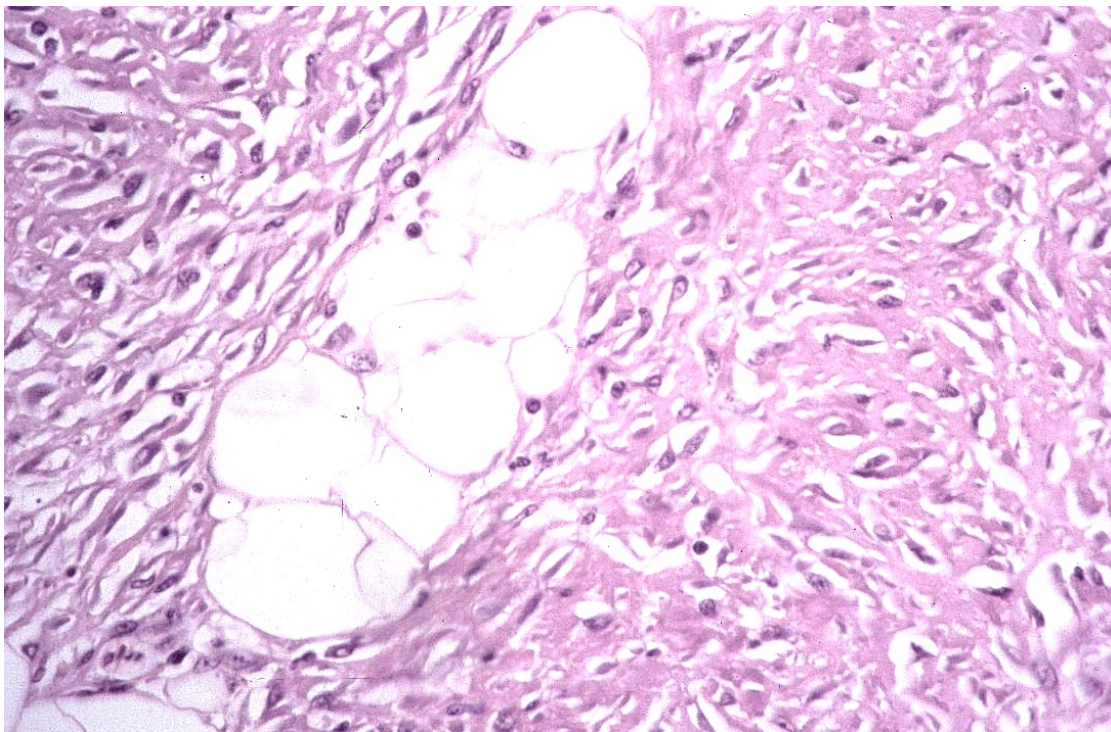
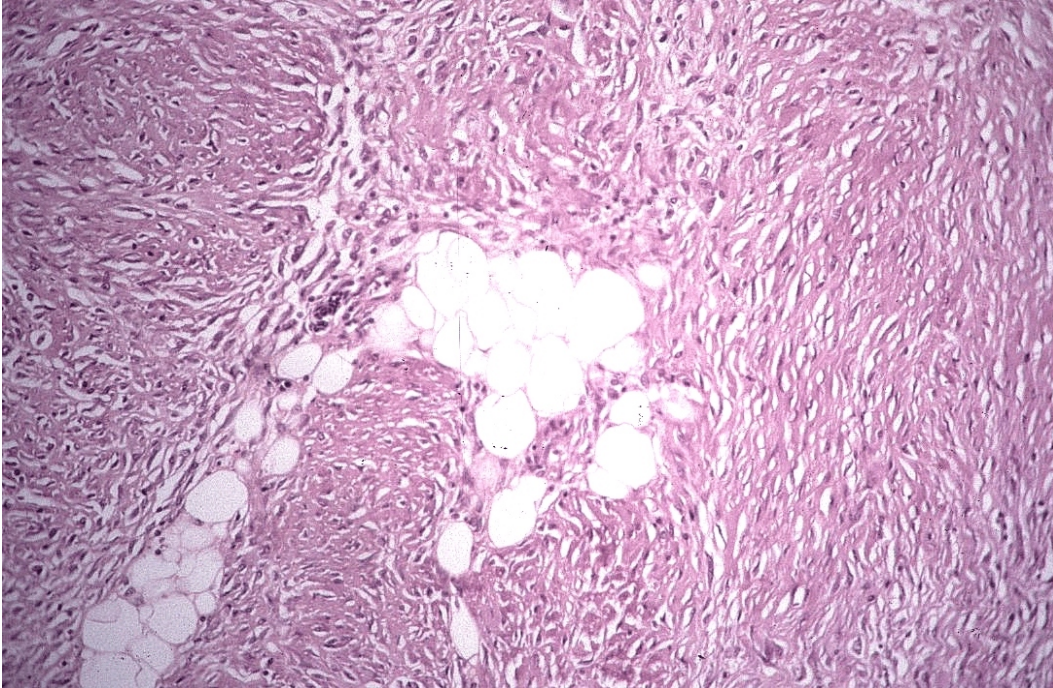


Figure 21

Figure 22



G. Malignant Lymphoma

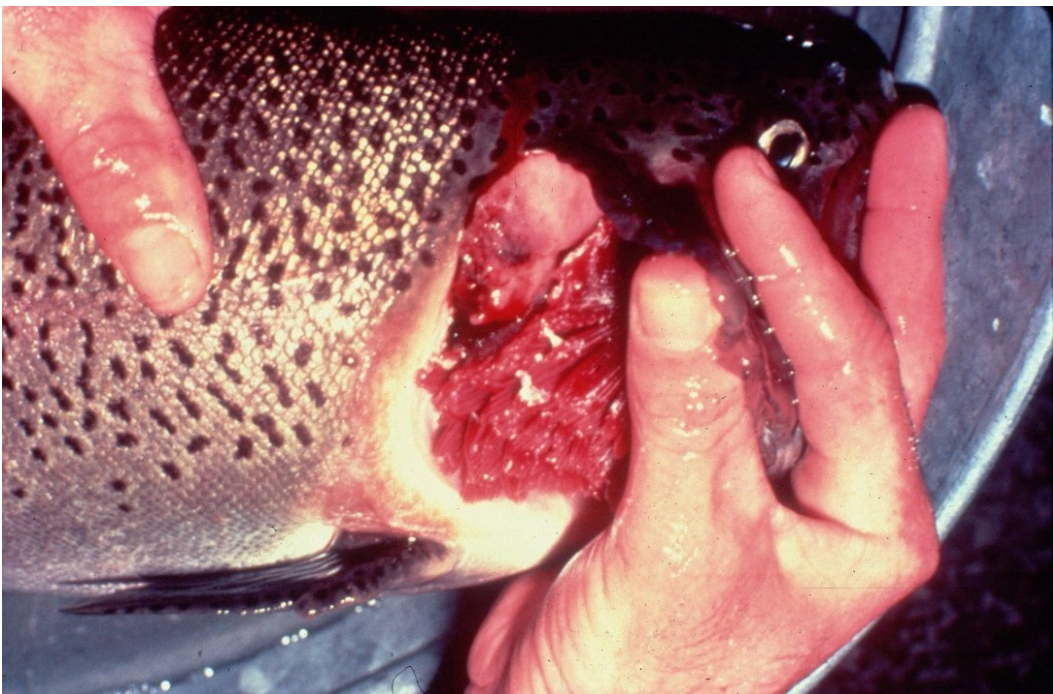


Figure 23

Figure 24

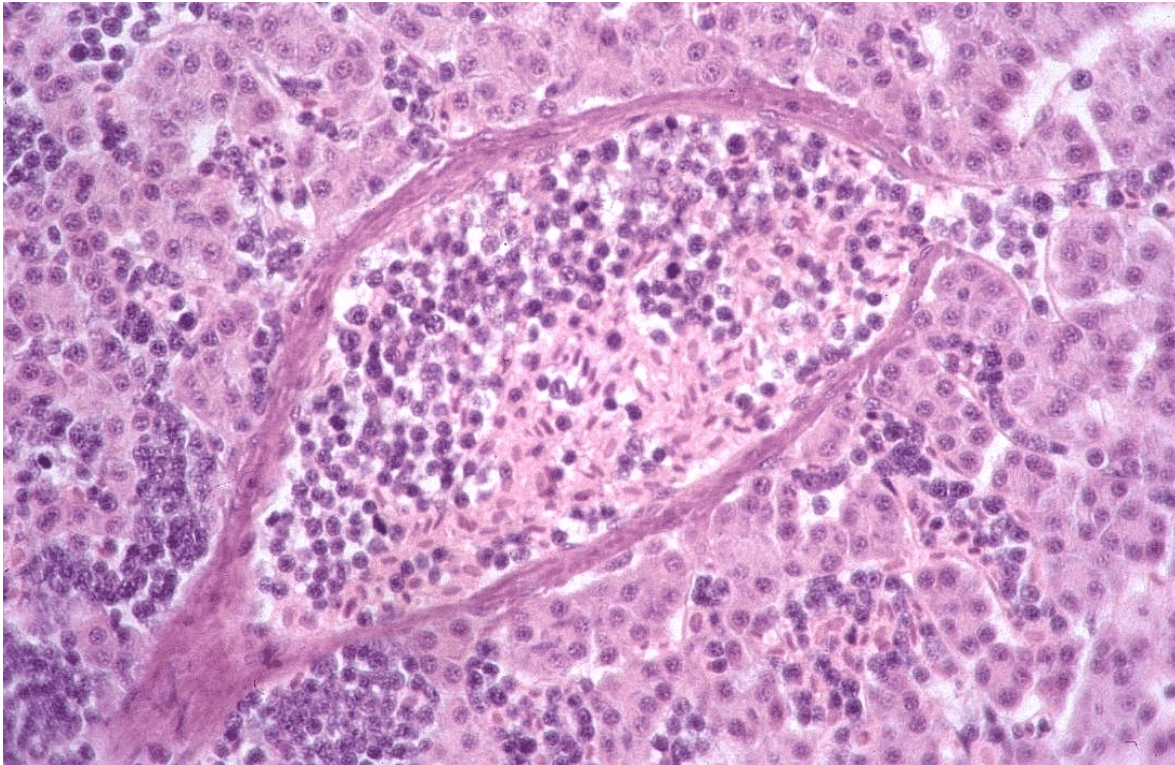


Figure 25

Figure 26

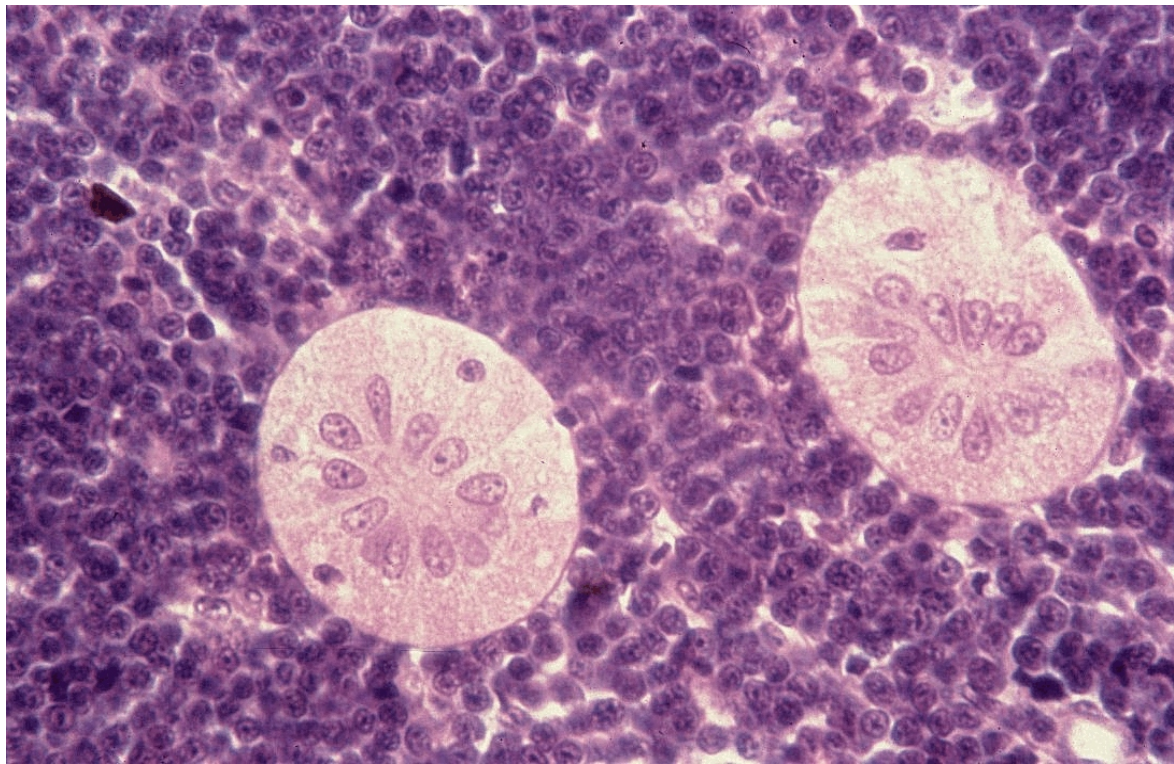
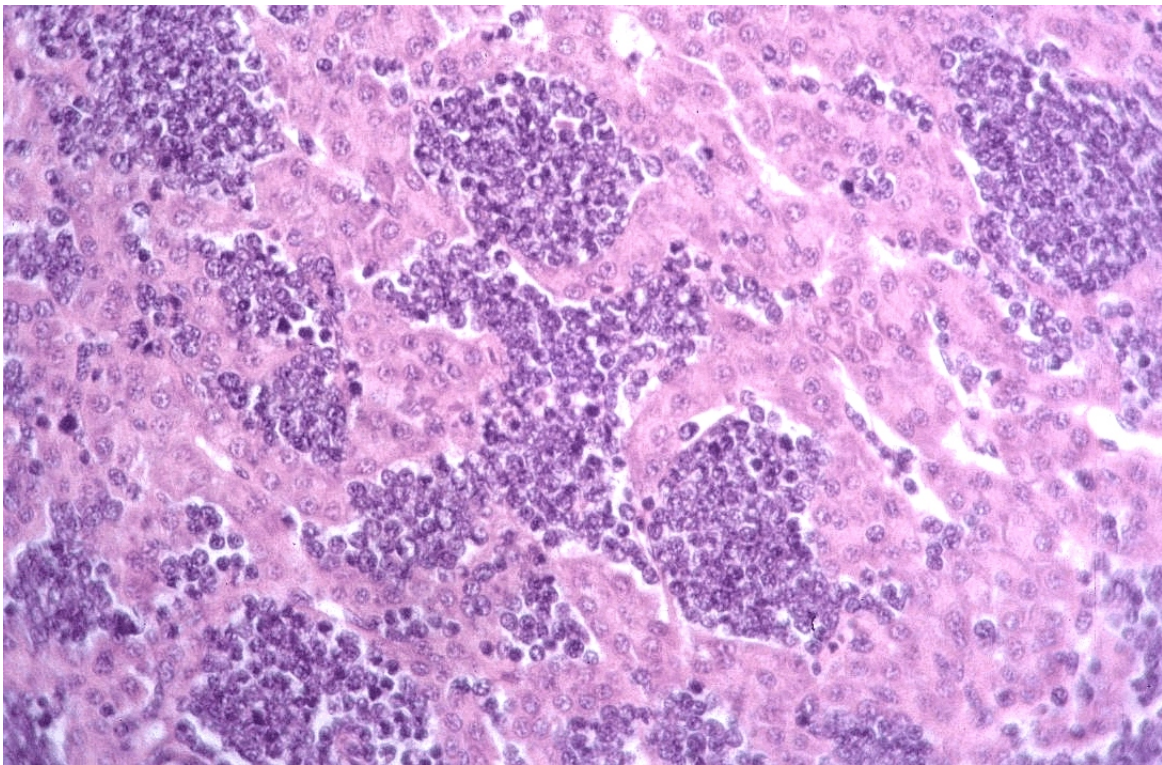


Figure 27

H. Hepatocellular Carcinoma

Figure 28

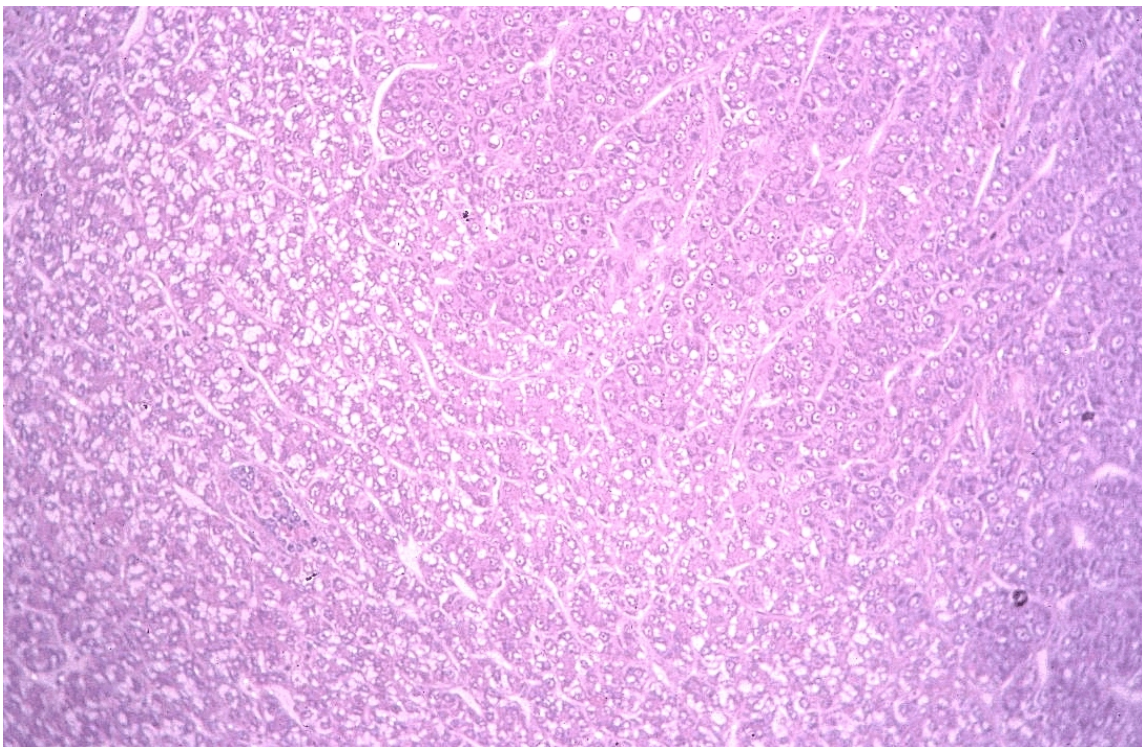
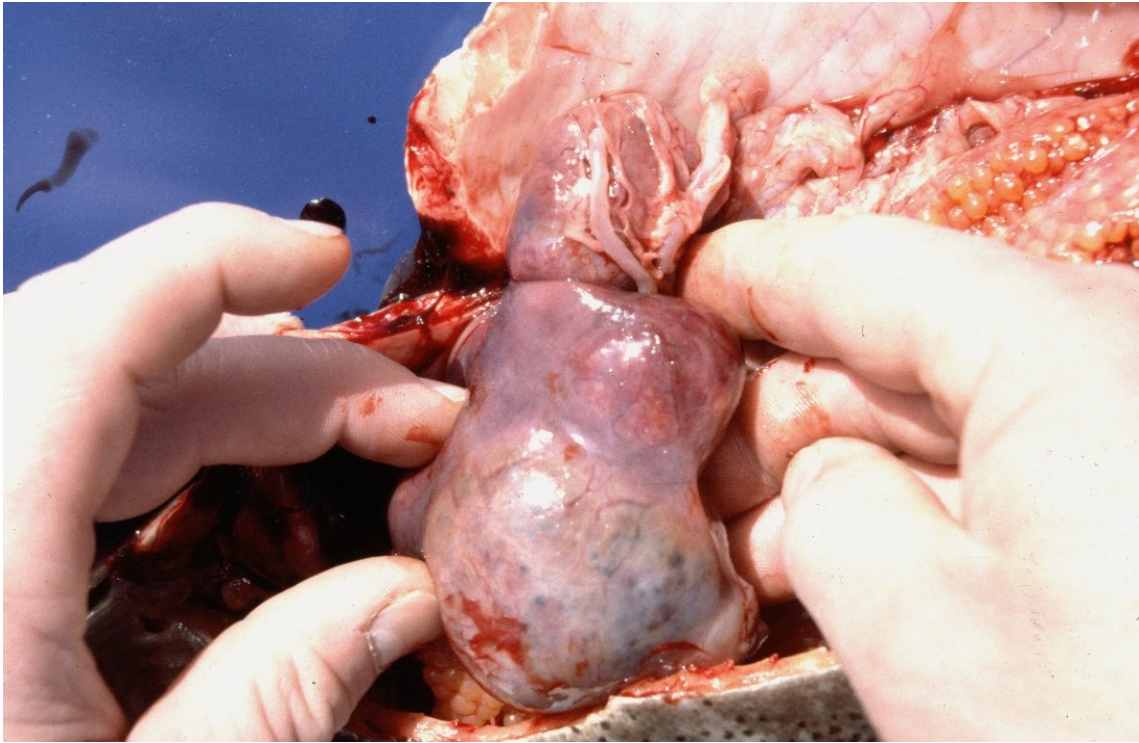


Figure 29

Figure 30

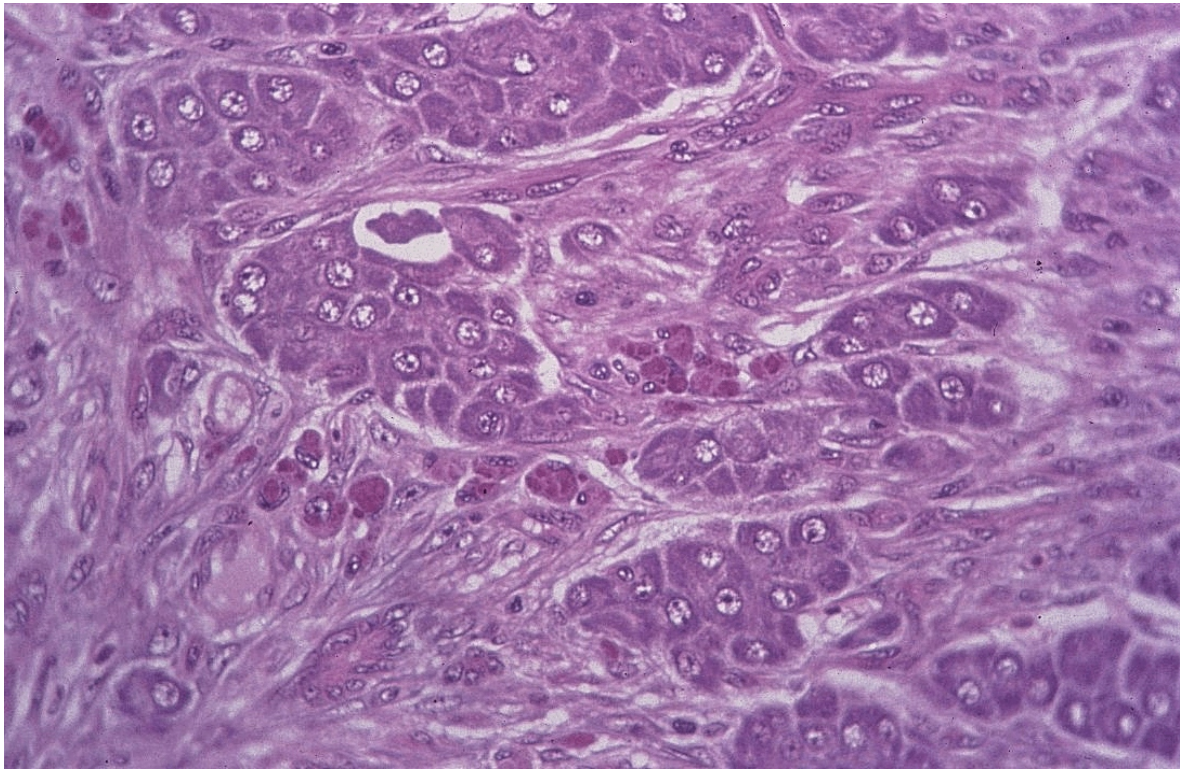


Figure 31

Figure 32

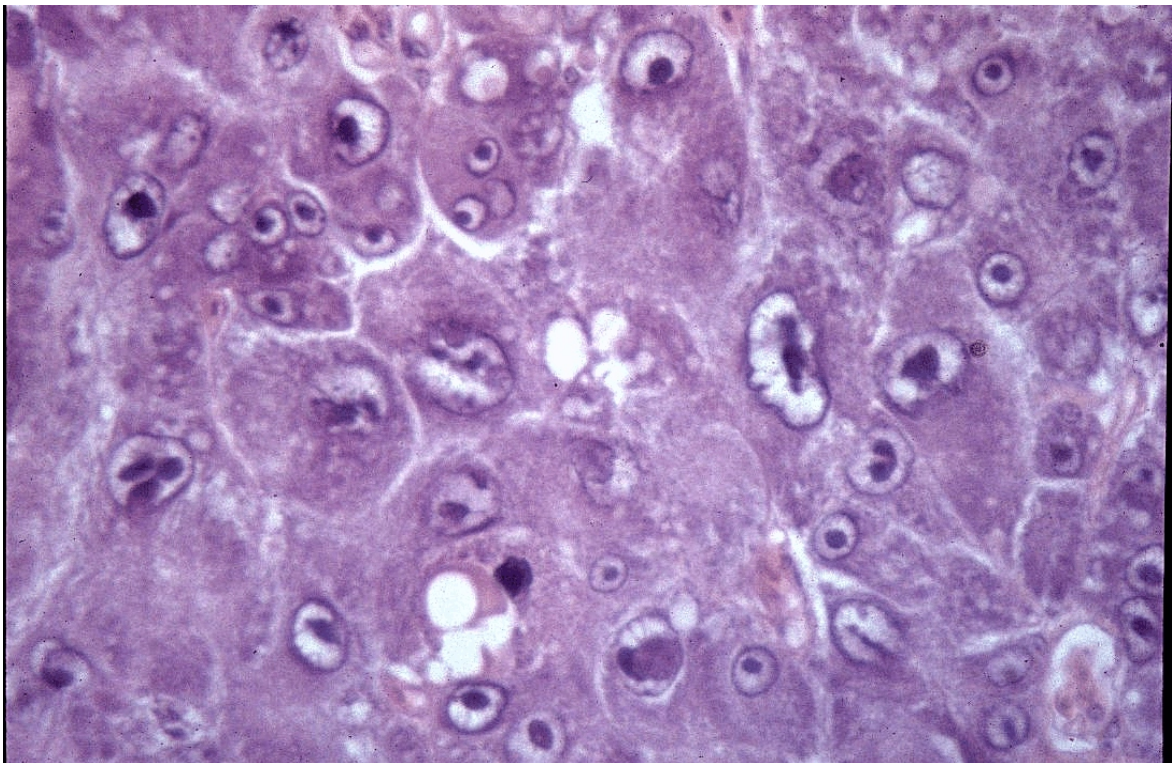
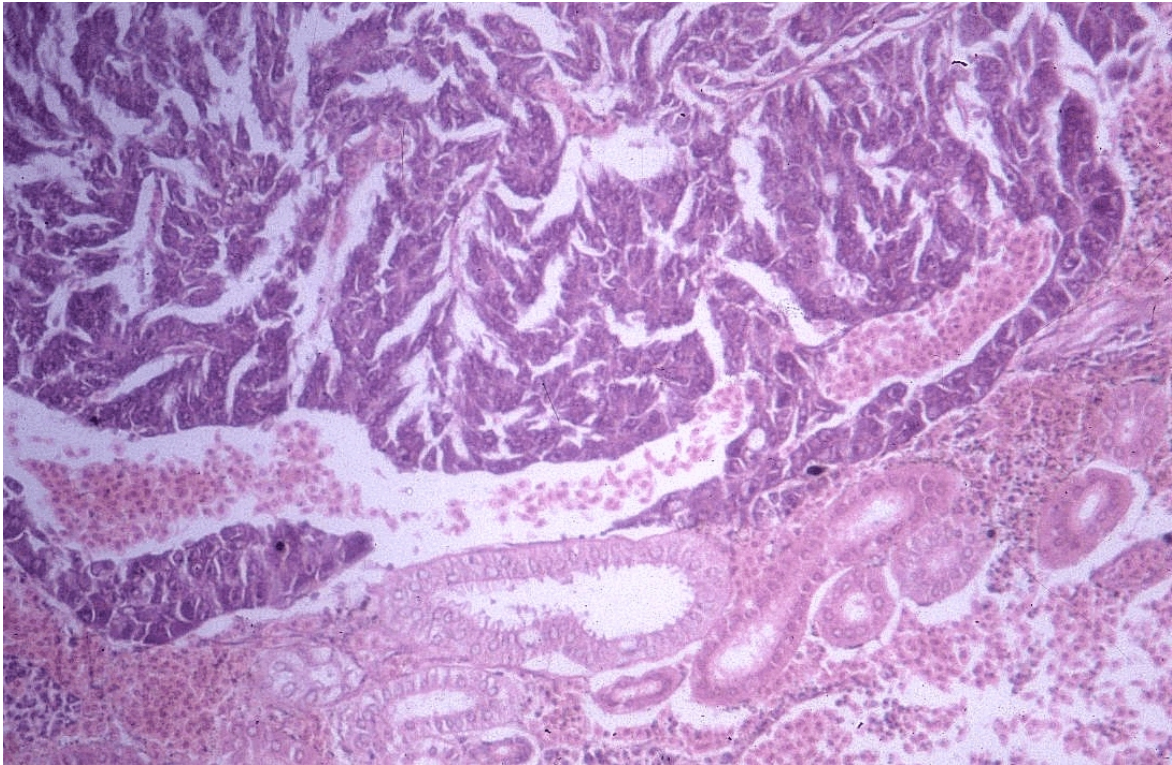


Figure 33

Legends to figures (1-33) of tumor slides

1. Highly vascular hemangioendothelial sarcoma in an adult RBT is invading operculum and underlying muscle tissue.
2. Hemangioendothelial sarcoma composed of spindle-shaped endothelial cells and numerous blood capillaries is invading and destroying muscle tissue.
3. Hemangioendothelial sarcoma composed of spindle-shaped endothelial cells forming blood-filled vascular channels ranging from small to cavernous. Epidermis is present in upper left.
4. Hemangioendothelial sarcoma composed of spindle-shaped endothelial cells forming a large cavernous vascular channel.
5. Large melanoma is shown on surface of adult rainbow trout.
6. Malignant melanoma appears as solid black mass in transverse segment through posterior trunk of adult chinook salmon.
7. Malignant melanoma with melanin-laden melanocytes invading and destroying adjacent muscle fibers.
8. Melanoma of barbels of catfish.
9. Melanophores are shown invading dermis and underlying muscle of catfish with melanoma.
10. Catfish barbel shows invading melanophores in epidermis and dermis.
11. Thymoma protruding from operculum of an adult lake trout.
12. Thymoma from lake trout with nest of epidermal and goblet cells (center) interspersed amongst lymphoid cells. Goblet cells are scattered throughout the section.
13. Higher power view of Thymoma from lake trout shows a nest of epidermal and goblet cells (center) interspersed amongst lymphoid cells.
14. Renal adenocarcinoma of golden trout is located in isthmus area. Note tumor is not capsulated on one surface.
15. Renal adenocarcinoma shows embryonic kidney tubules interspersed among more normal appearing tubules.
16. Renal adenocarcinoma shows embryonic renal tubules.

17. Renal adenocarcinoma shows embryonal renal tubules and ectopic cartilage tissue.
18. Large multi-nodular nephroblastoma in kidney of adult rainbow trout. Liver is on the right.
19. Nephroblastoma with large swirling masses of basophilic nephrogenic blastema interspersed amongst poorly-differentiated, spindle-shaped tubular epithelium. Normal kidney tubules are present in upper left.
20. Adult wild lake trout with large encapsulated fibrolipoma on body surface.
21. Fibrolipoma from lake trout with a mixture of dense fibrous connective tissue and adipose tissue.
22. Fibrolipoma from lake trout with a mixture of dense fibrous connective tissue and adipose tissue.
23. Large thymus tumor in rainbow trout broodfish with malignant lymphoma having hemorrhagic and necrotic areas.
24. Liver of trout with malignant lymphoma shows whitish tumor nodules scattered throughout.
25. Liver of trout in Figure 24 with malignant lymphoma. Note leukemic condition in blood vessel and nests of invading of tumor cells amongst hepatic parenchyma.
26. Liver of trout with malignant lymphoma shows nests of lymphoblasts scattered throughout mostly normal liver cells.
27. Kidney from trout with malignant lymphoma showing compression of degenerate tubules by lymphoblasts.
28. Massive hepatocellular carcinoma in a rainbow trout broodstock.
29. A well-differentiated hepatocellular carcinoma with widened cords and basophilic hepatocytes. Some normal hepatocytes are on left of center.
30. Highly anaplastic hepatocellular carcinoma from a rainbow trout. Hepatocytes are irregular in size and shape, and are not organized into well-defined trabeculae.
31. Massive hepatocellular carcinoma in a Atlantic tomcod.
32. Metastasis of hepatocellular carcinoma to kidney of Atlantic Tomcod.

33. Atlantic tomcod hepatocellular carcinoma shows anaplastic hepatocytes with extreme nuclear pleomorphism and some multinuclear cells.

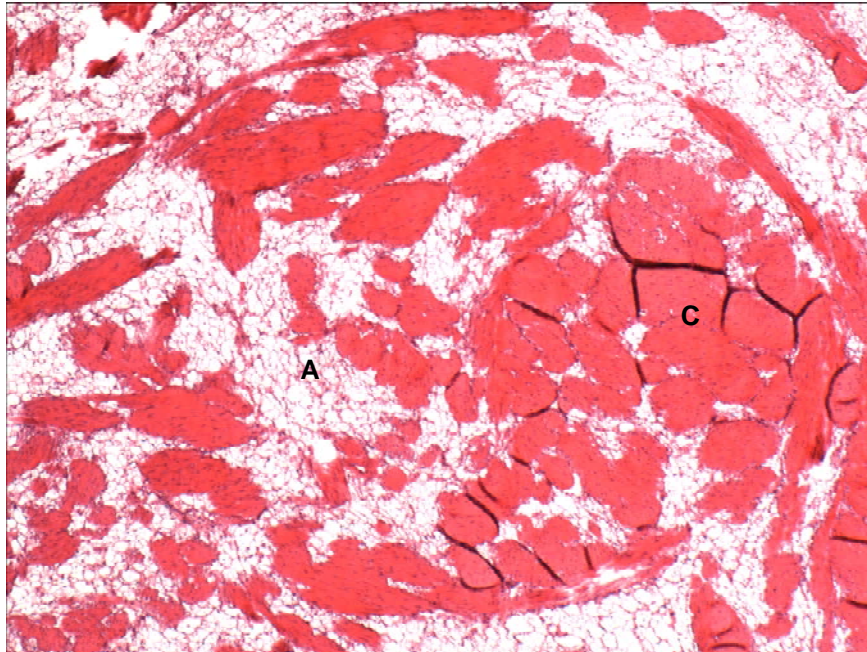
Fibrolipoma – Rainbow Trout



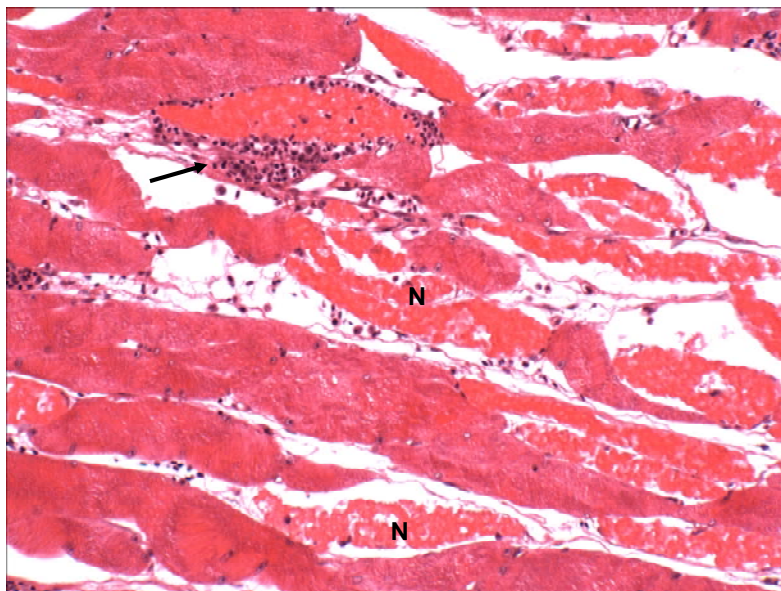
Fibrolipoma rainbow trout



Fibrolipoma originated in kidney and has destroyed muscle tissue



Fibrolipoma rainbow trout – dense fibrous connective tissue (**C**) is interspersed amongst adipose tissue (**A**)



Hemorrhage (arrow) and pressure necrosis (N) in muscle of trout with fibrolipoma

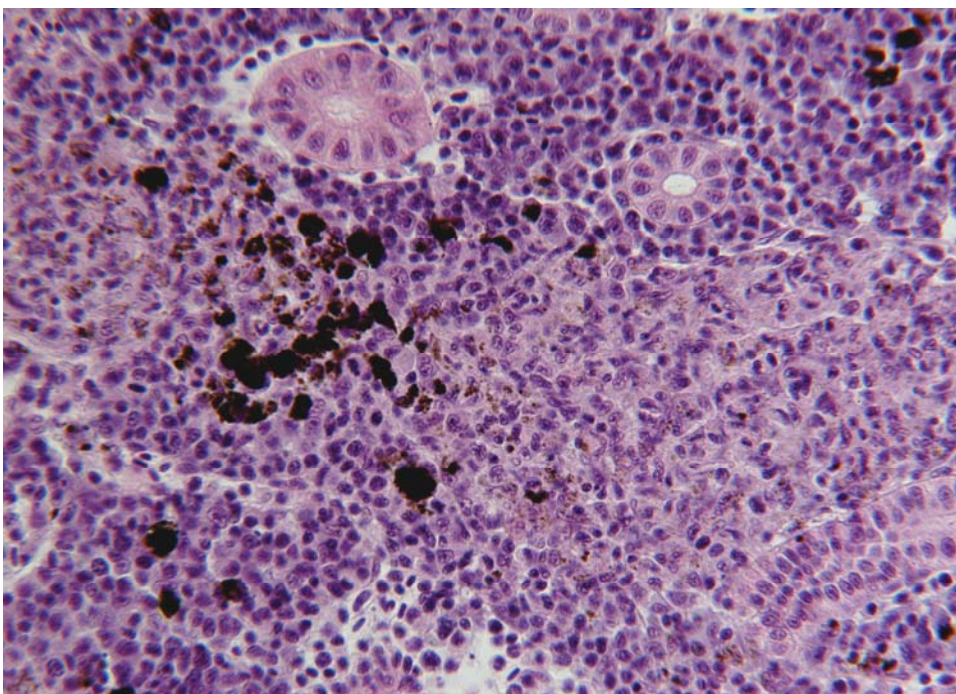
Infectious Diseases

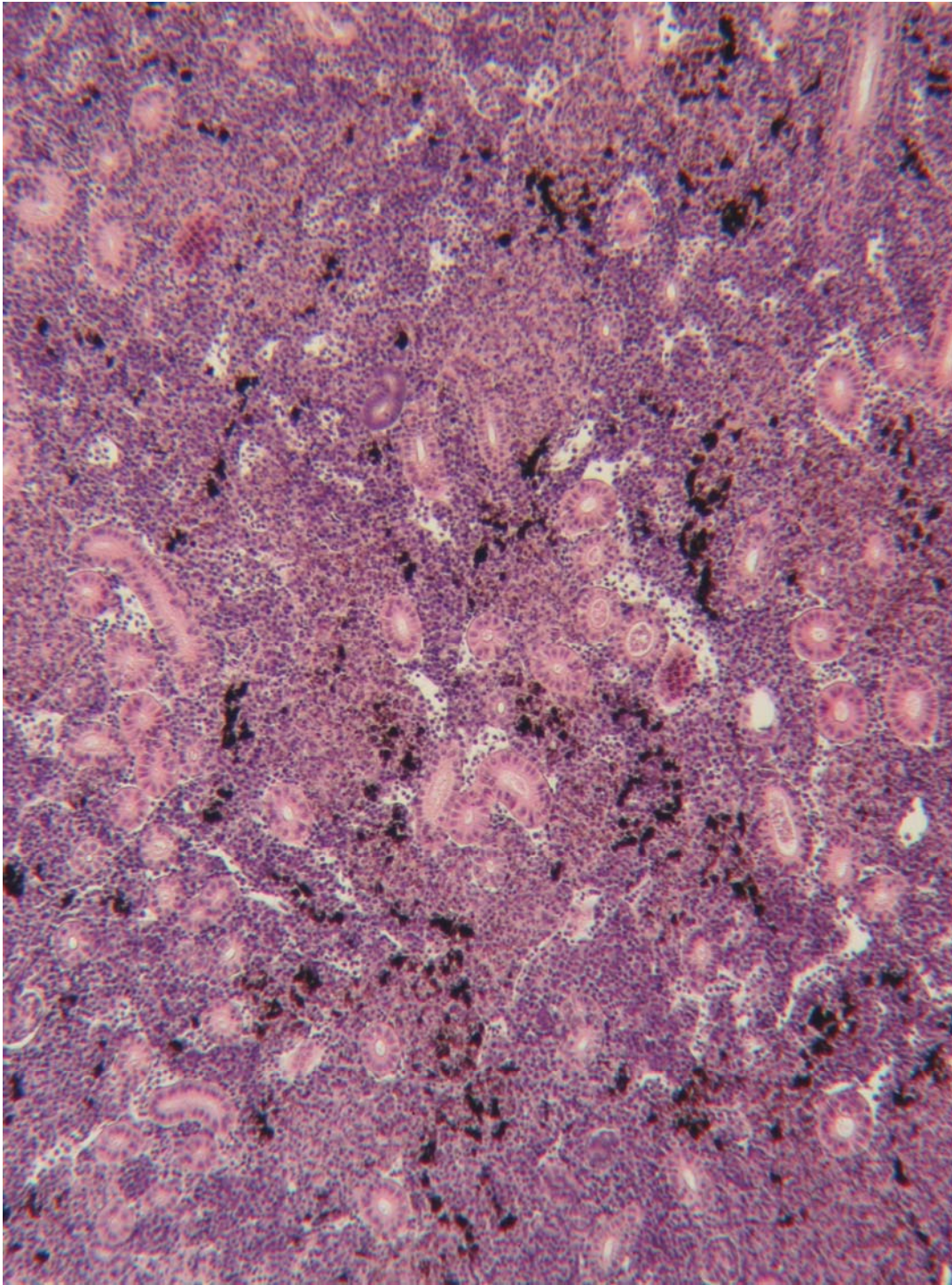
1. General

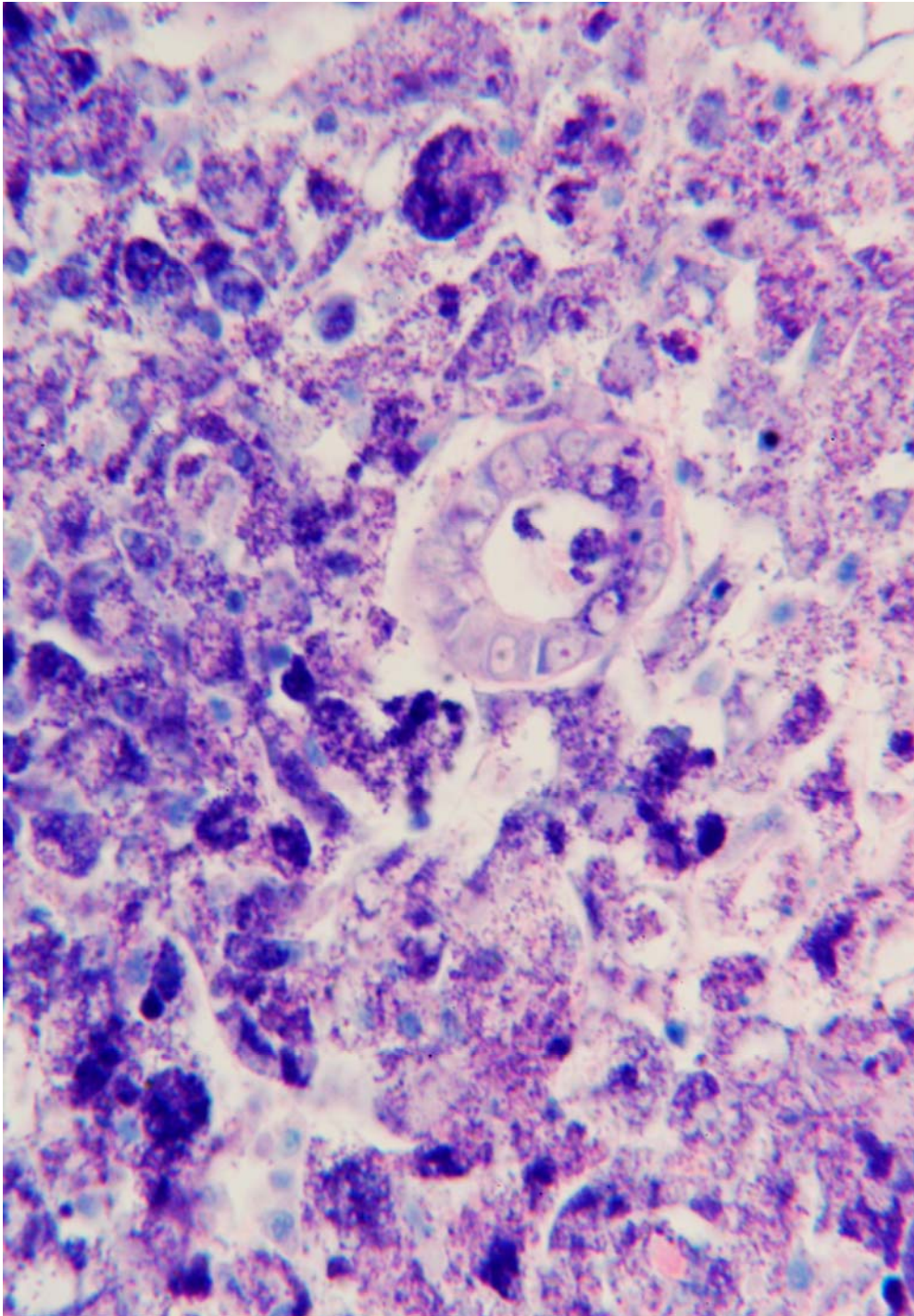
- Infection – state or condition in which the body or a part of it is invaded by a pathogenic agent which under favorable conditions, multiplies and produces effects which are injurious.
- Localized to widely disseminated
- Primary or secondary
- Progressive – early acute, late chronic
- Principle causes - viruses, bacteria, rickettsia, fungi and parasites.

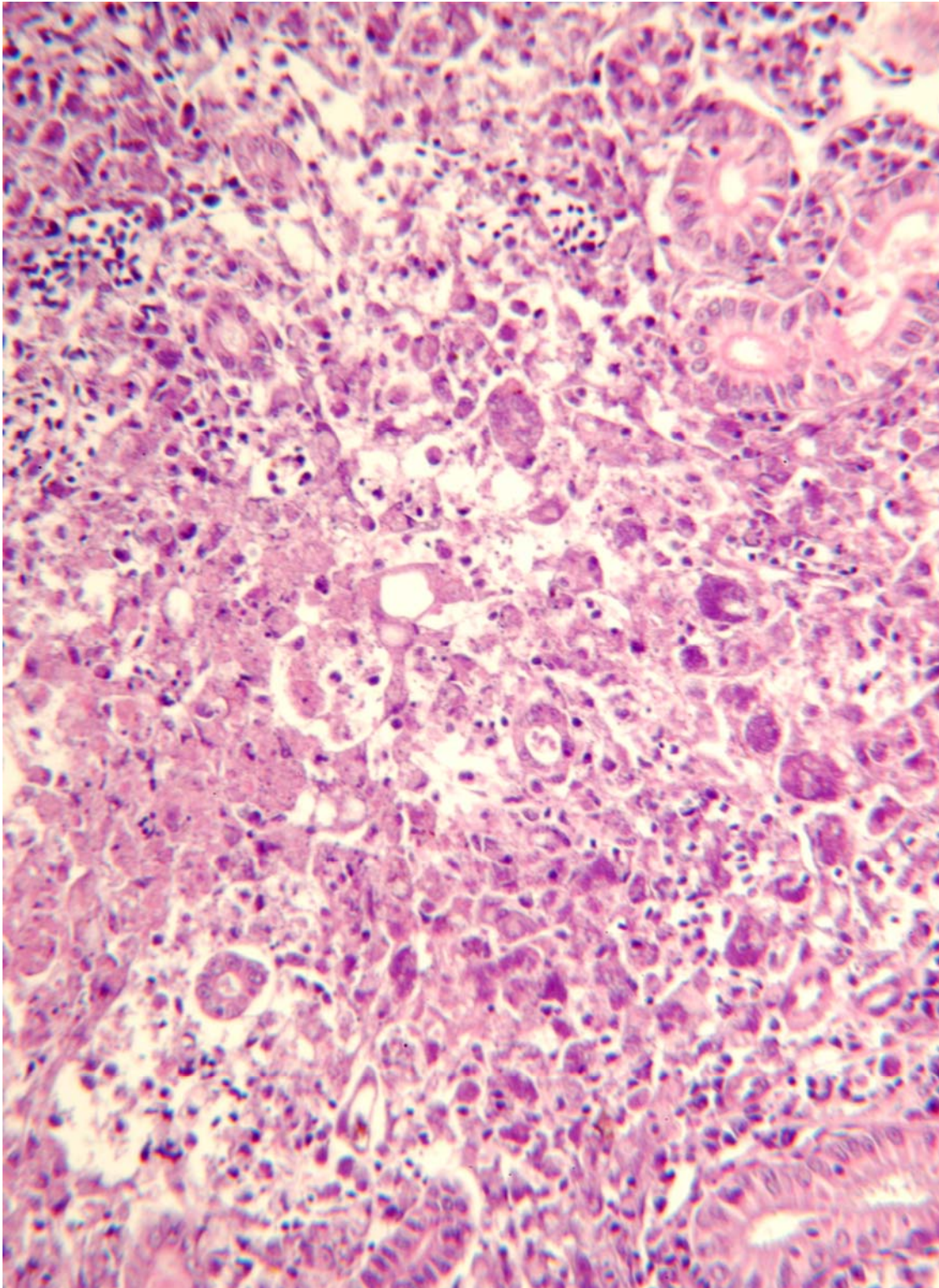
2. Bacteria

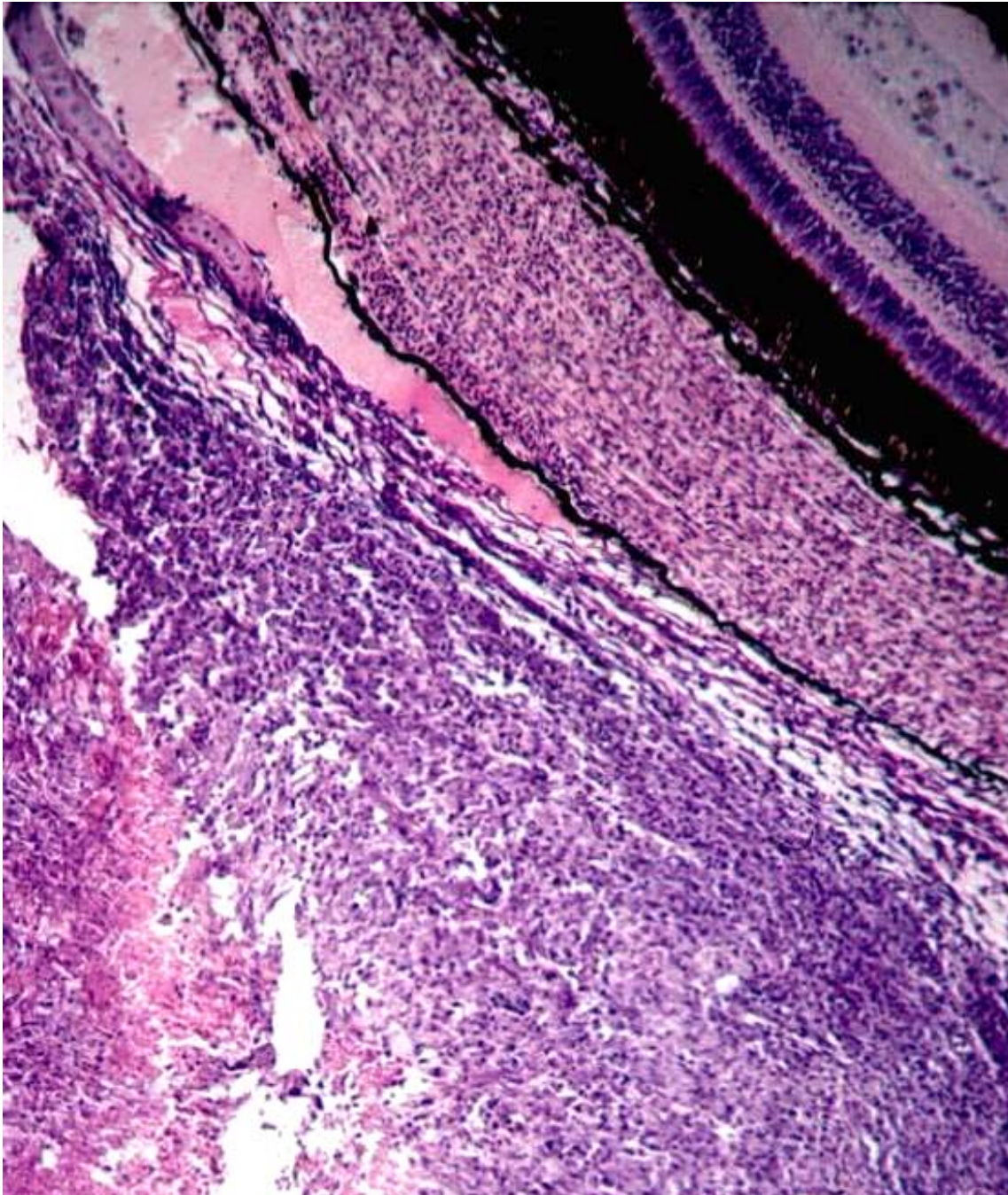
a. Bacterial Kidney Disease (BKD) *Rennibacterium salmoninarum*

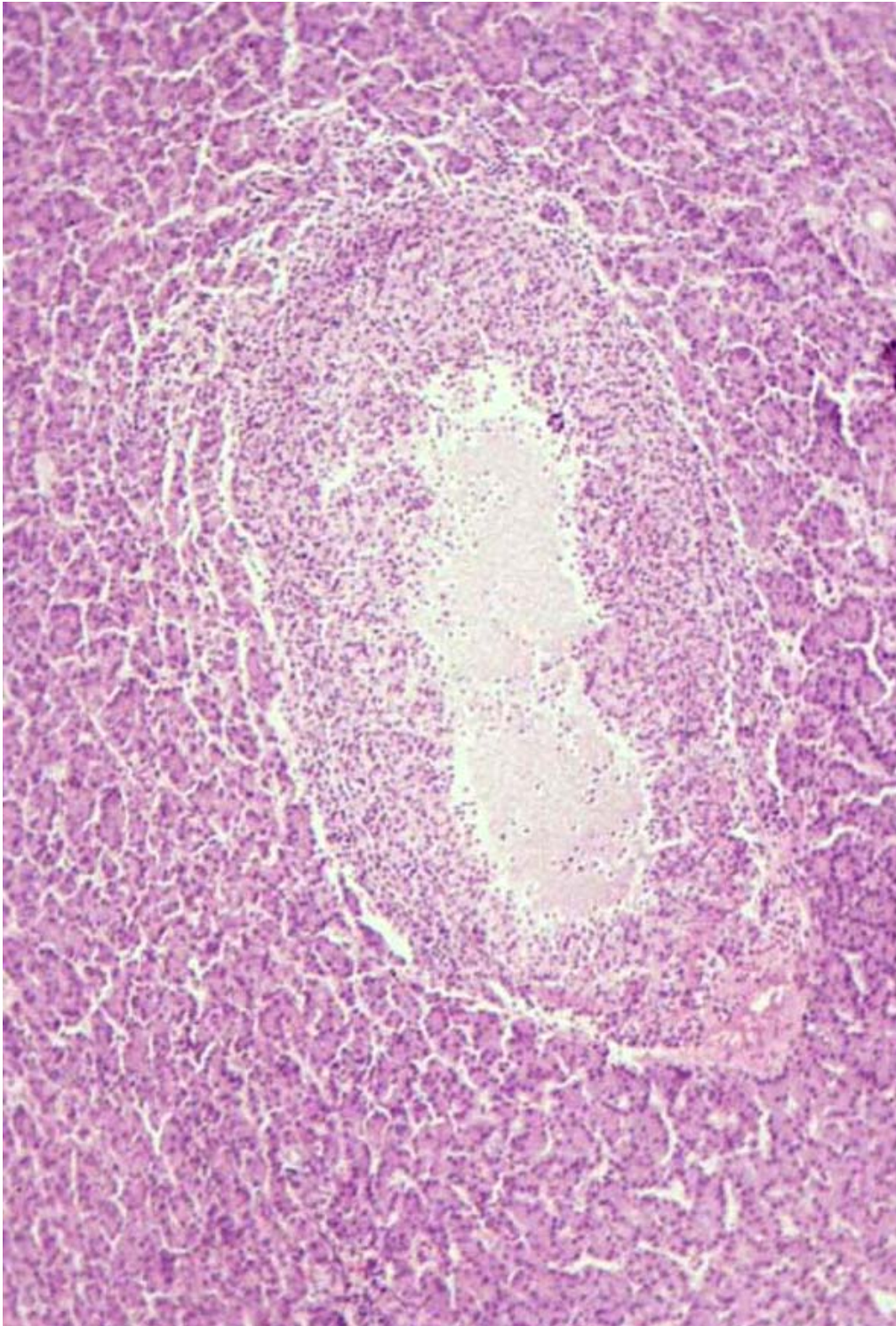






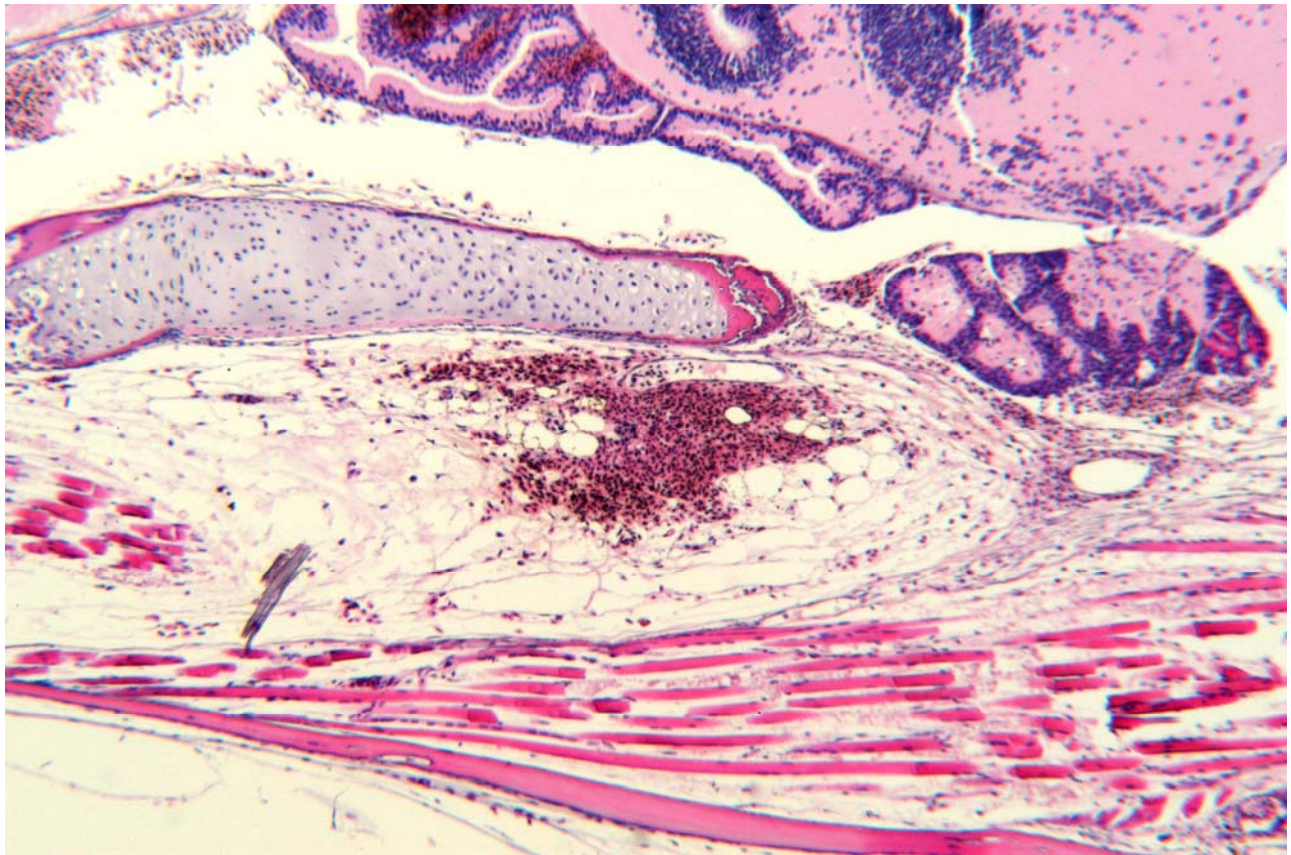


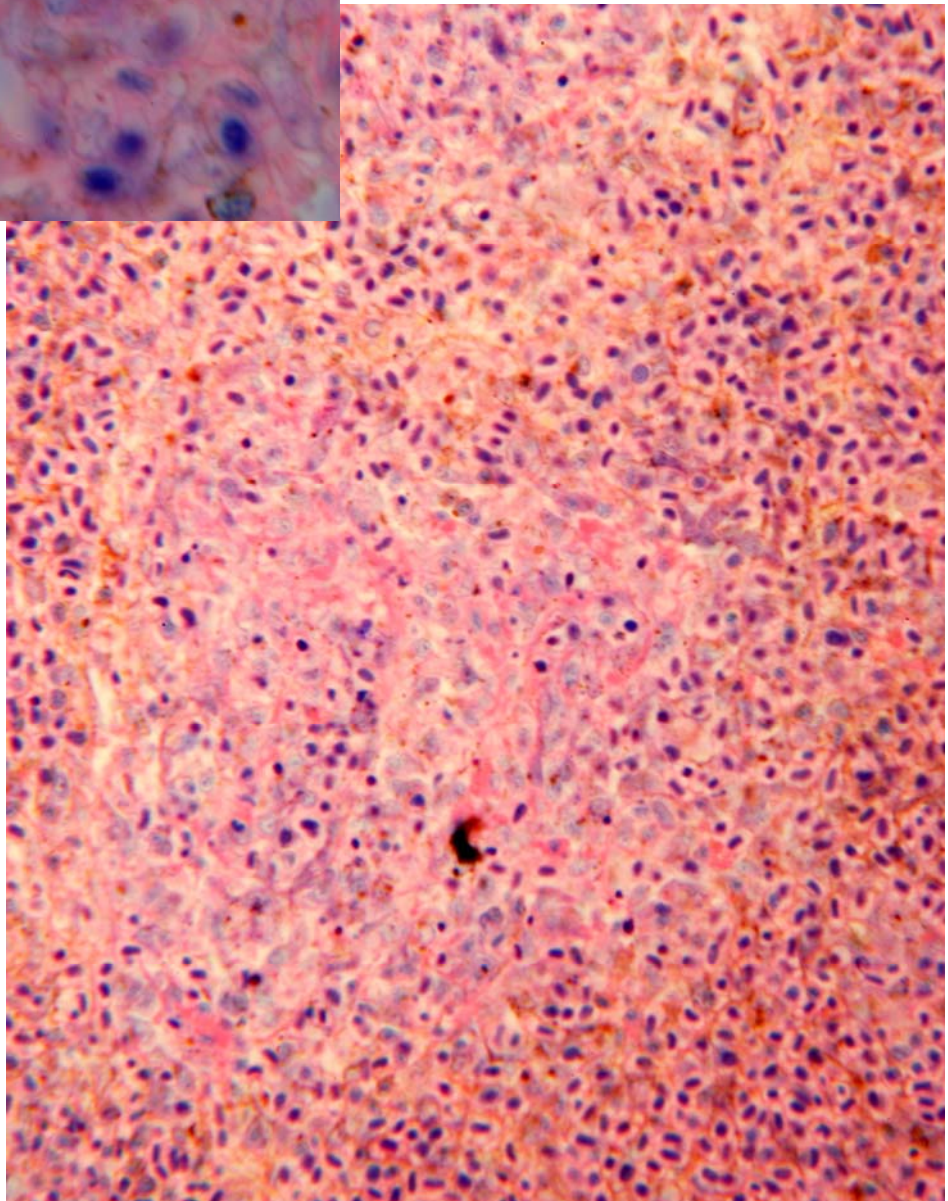
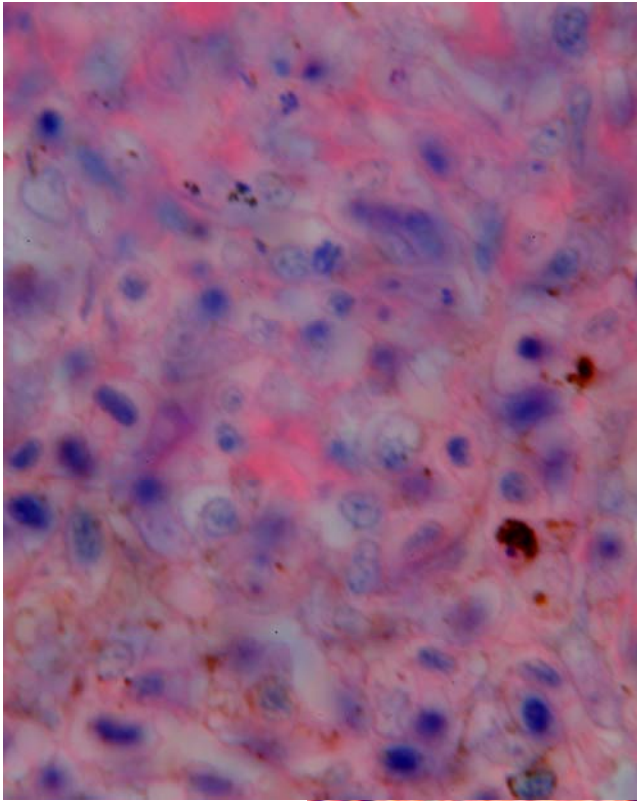




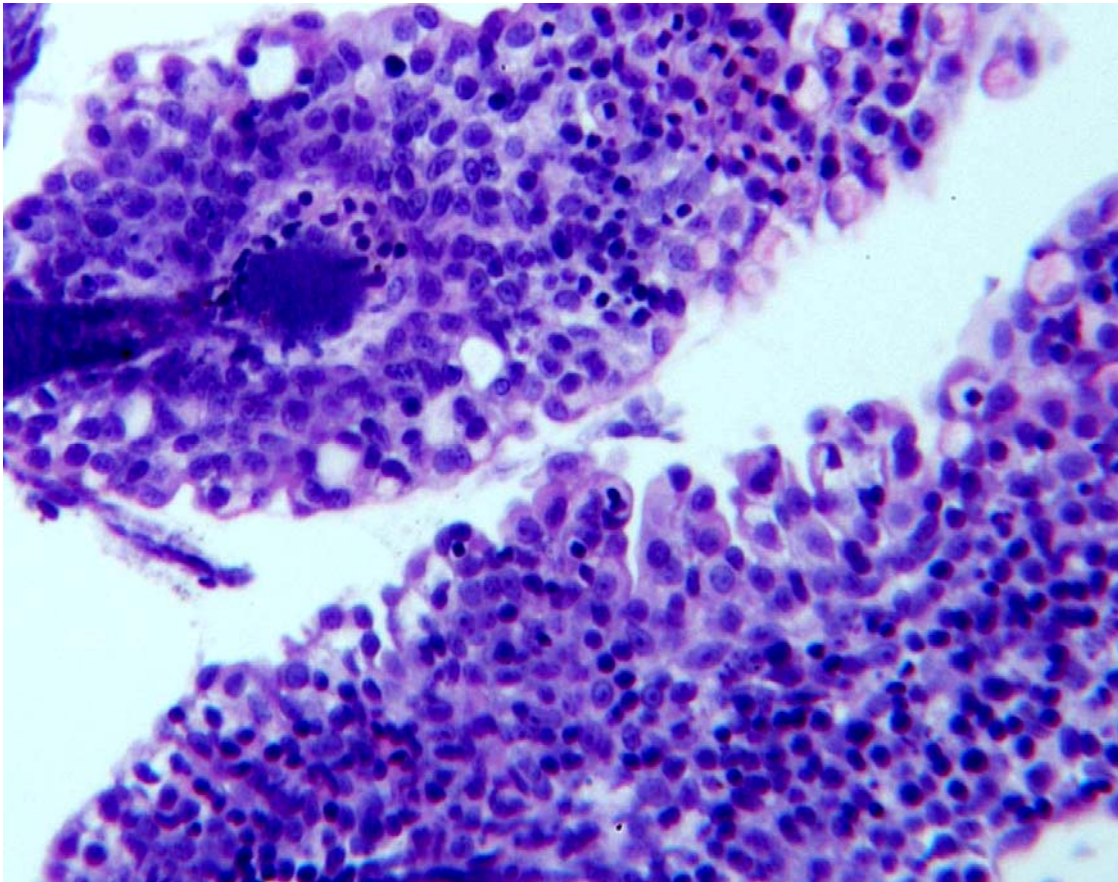
Chapter 6 – Infectious Disease
Fish Histology and Histopathology

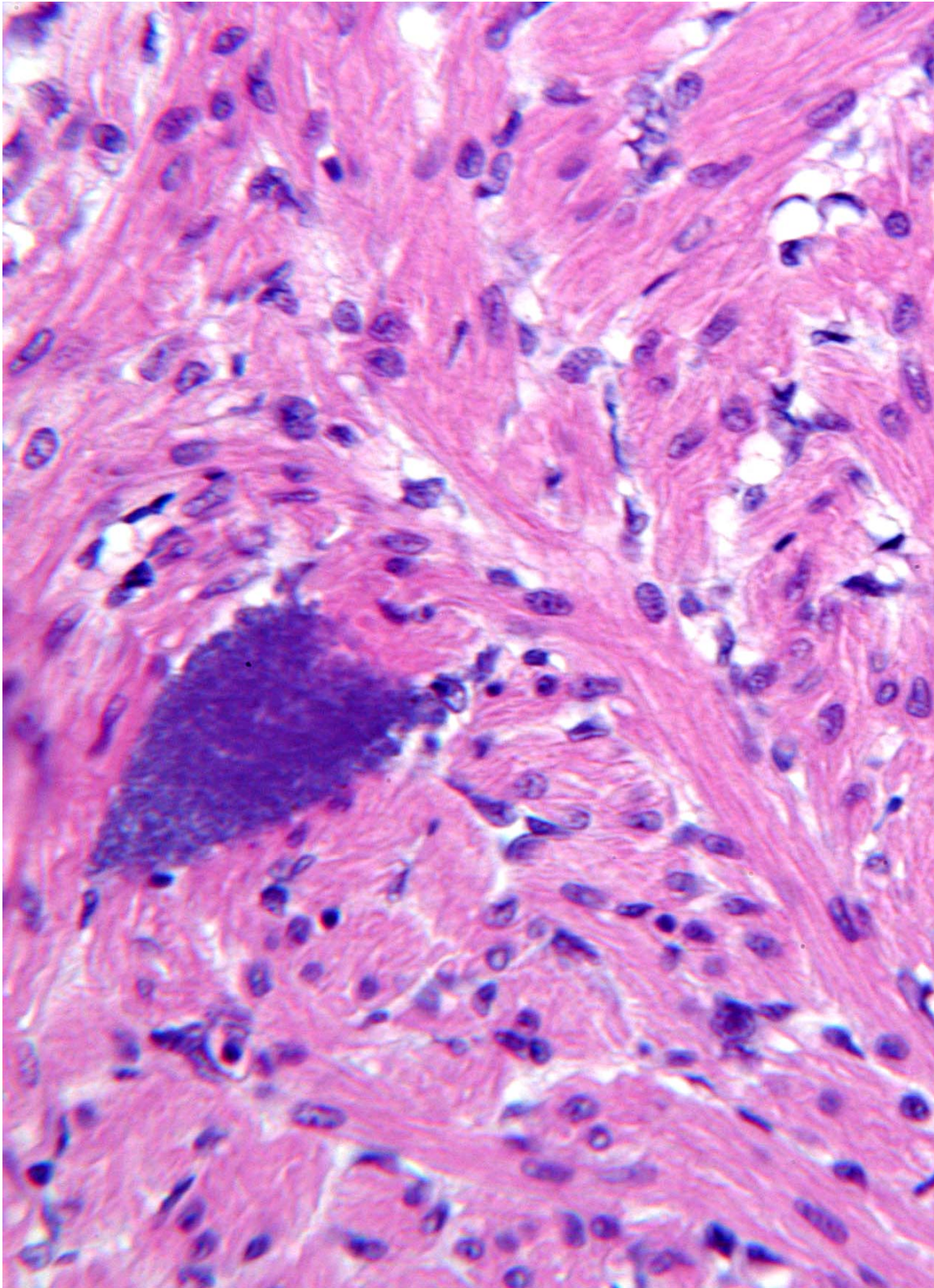
b. Enteric Redmouth (ERM) *Yersinia ruckeri*

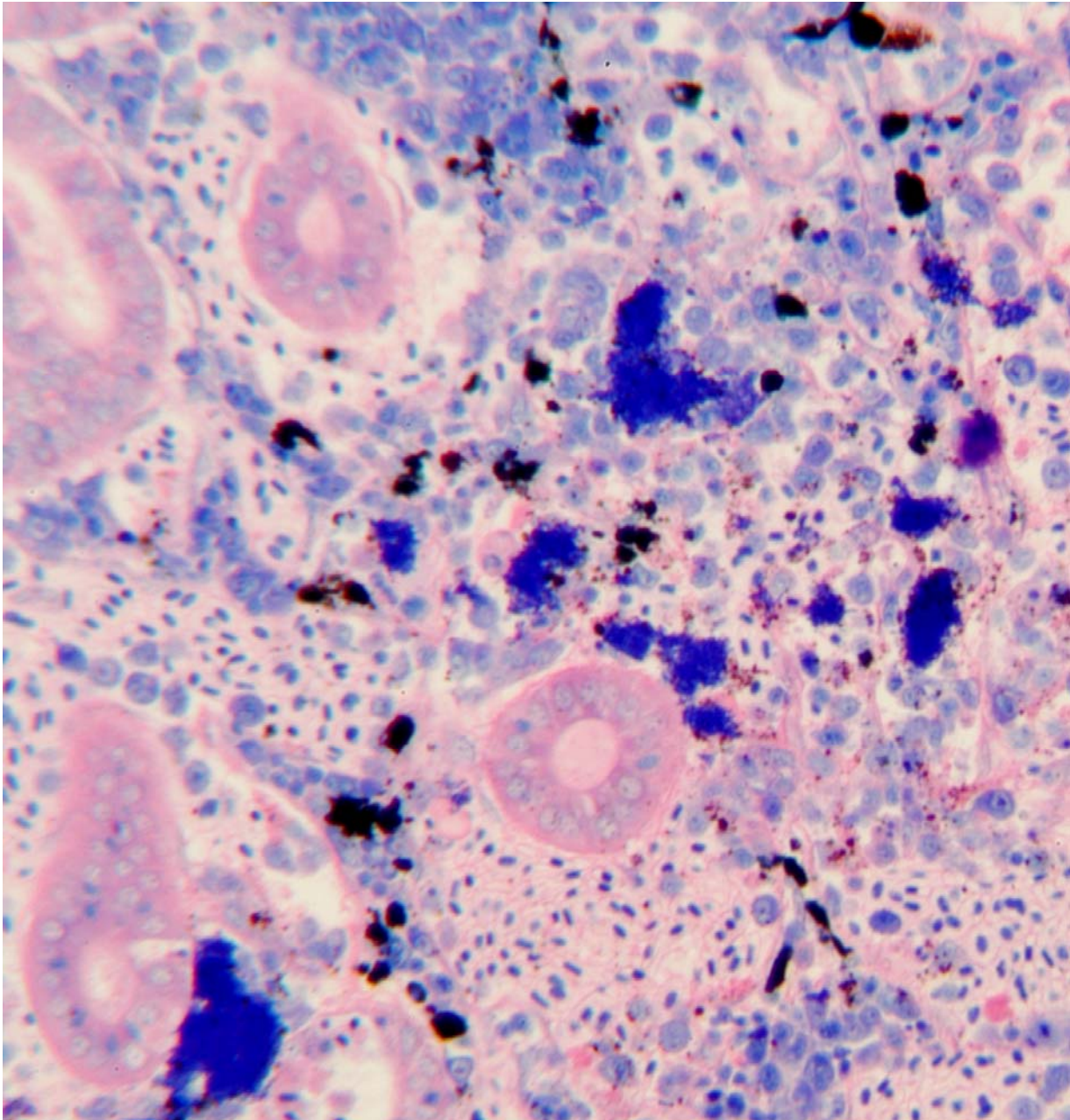


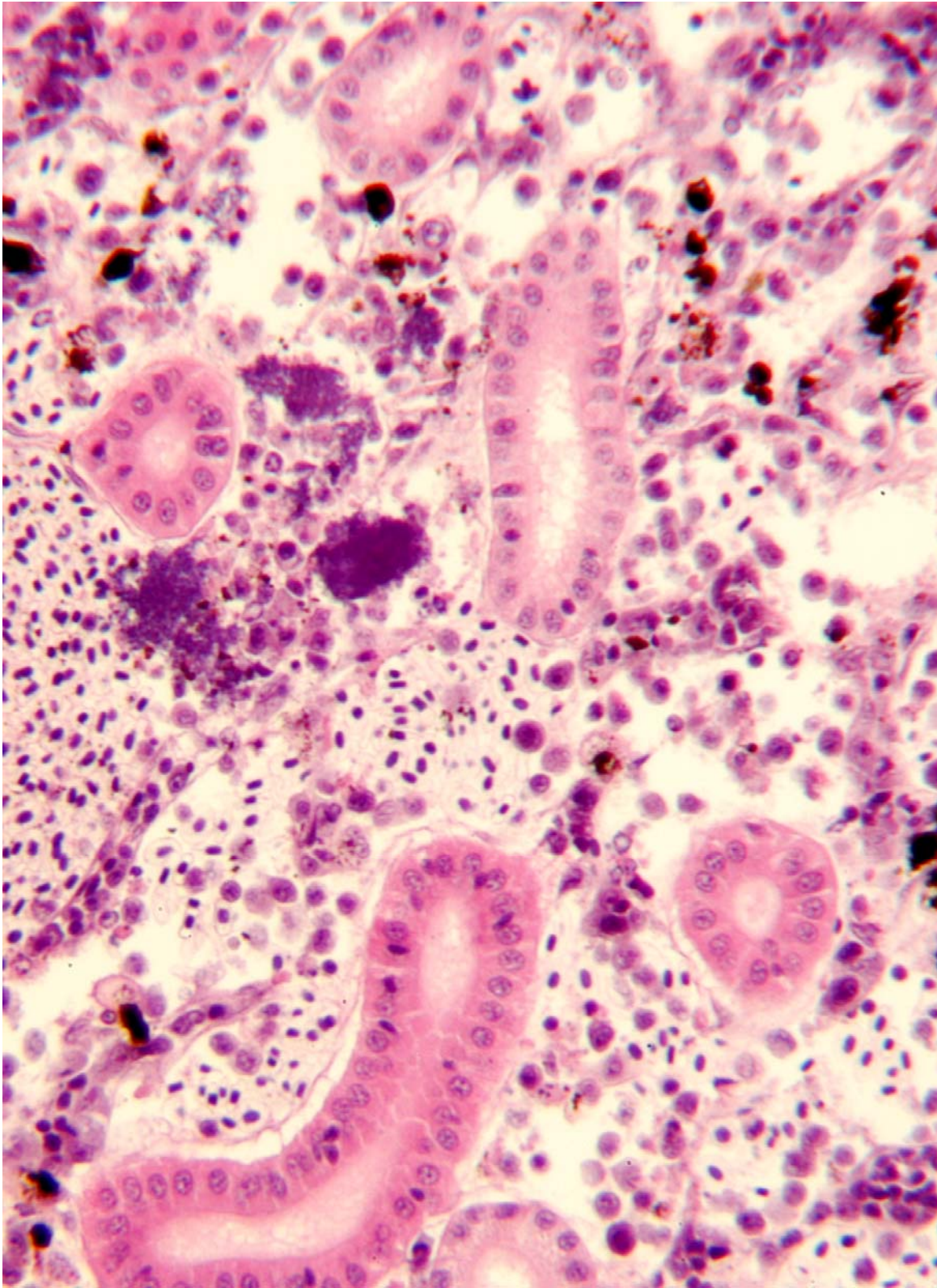


c. Furunculosis – *Aeromonas salmonicida*

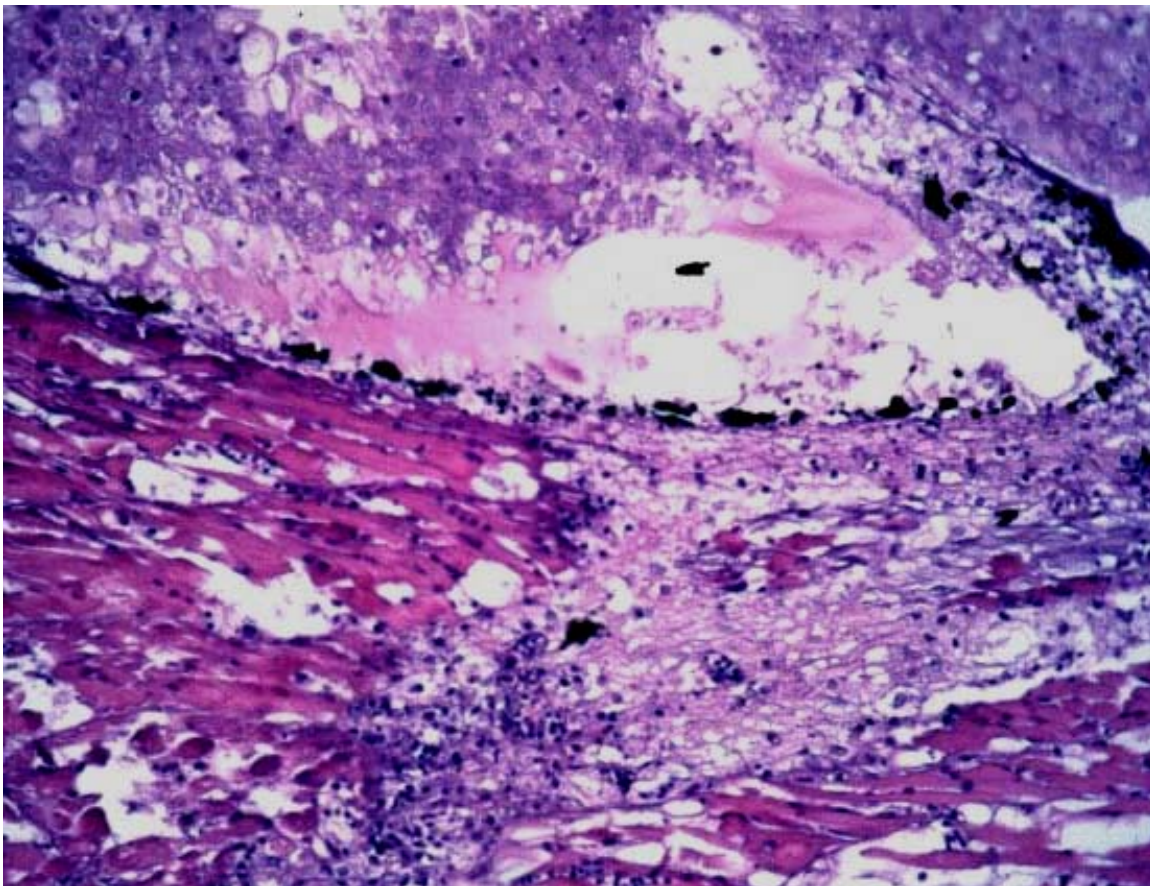
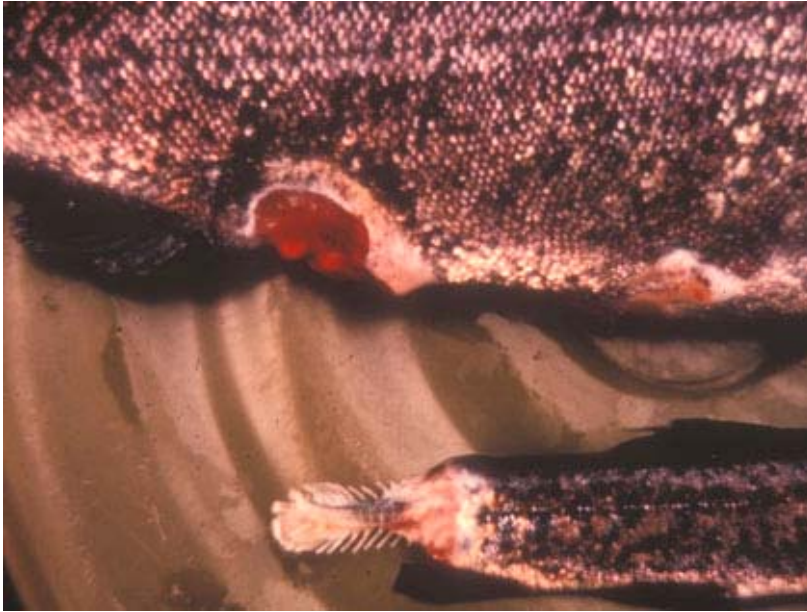


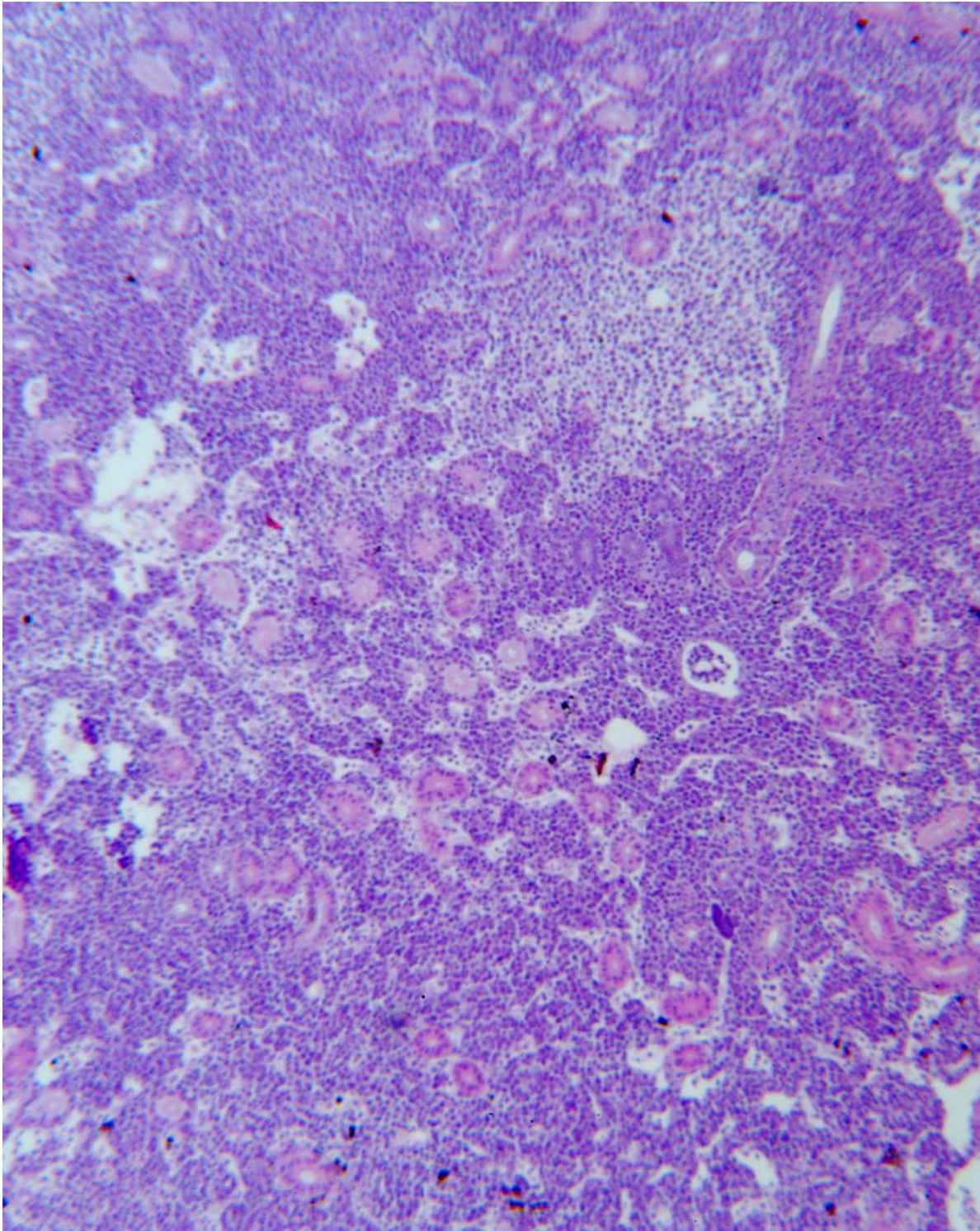


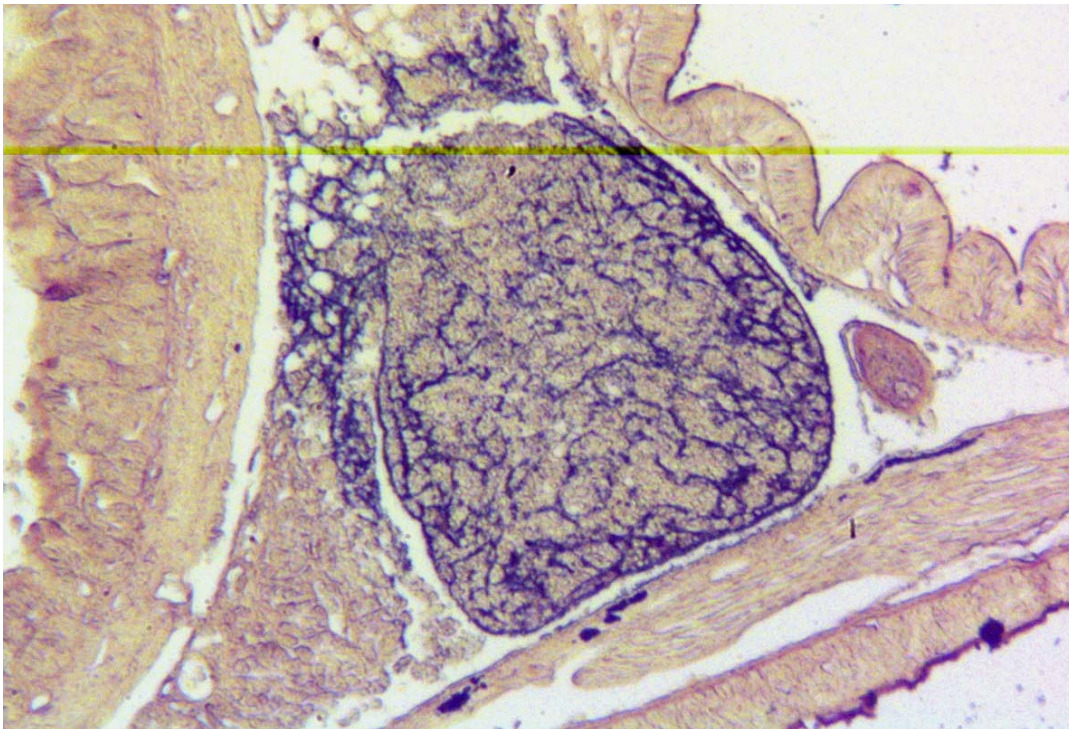
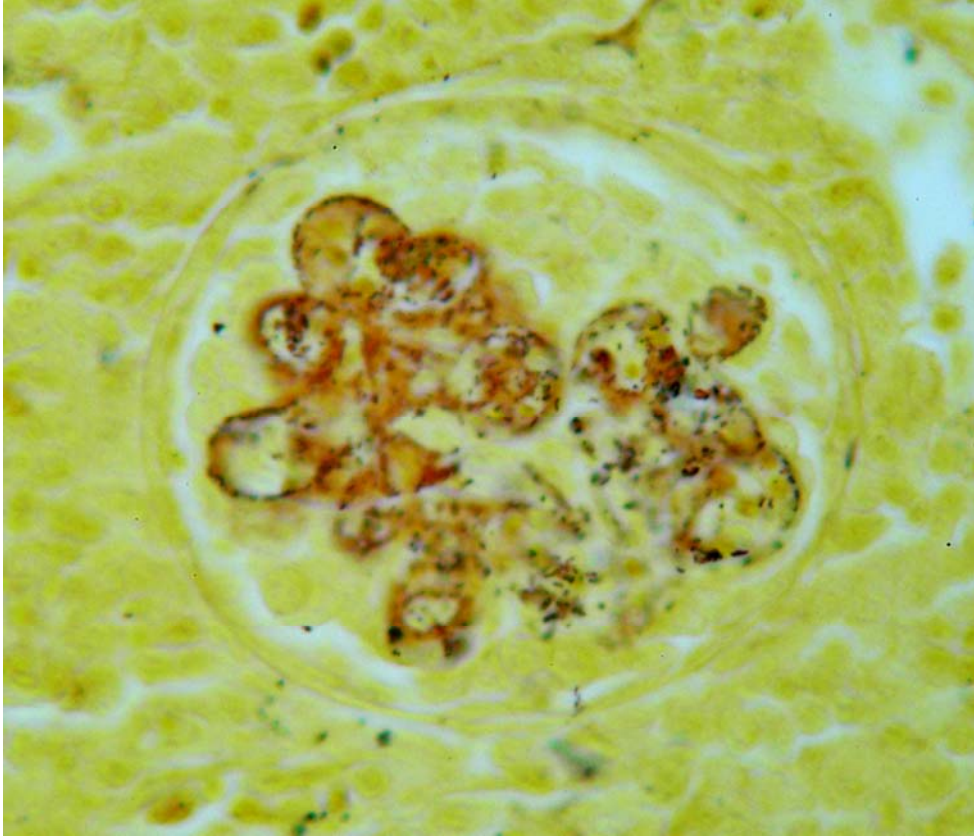


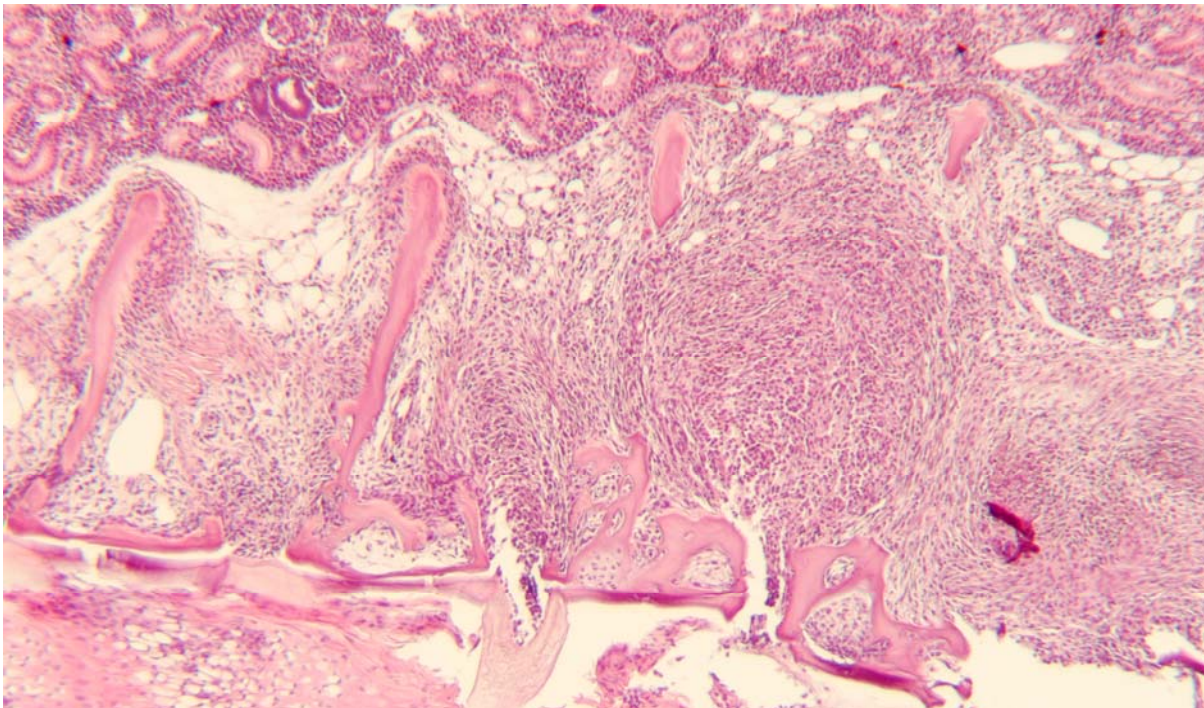
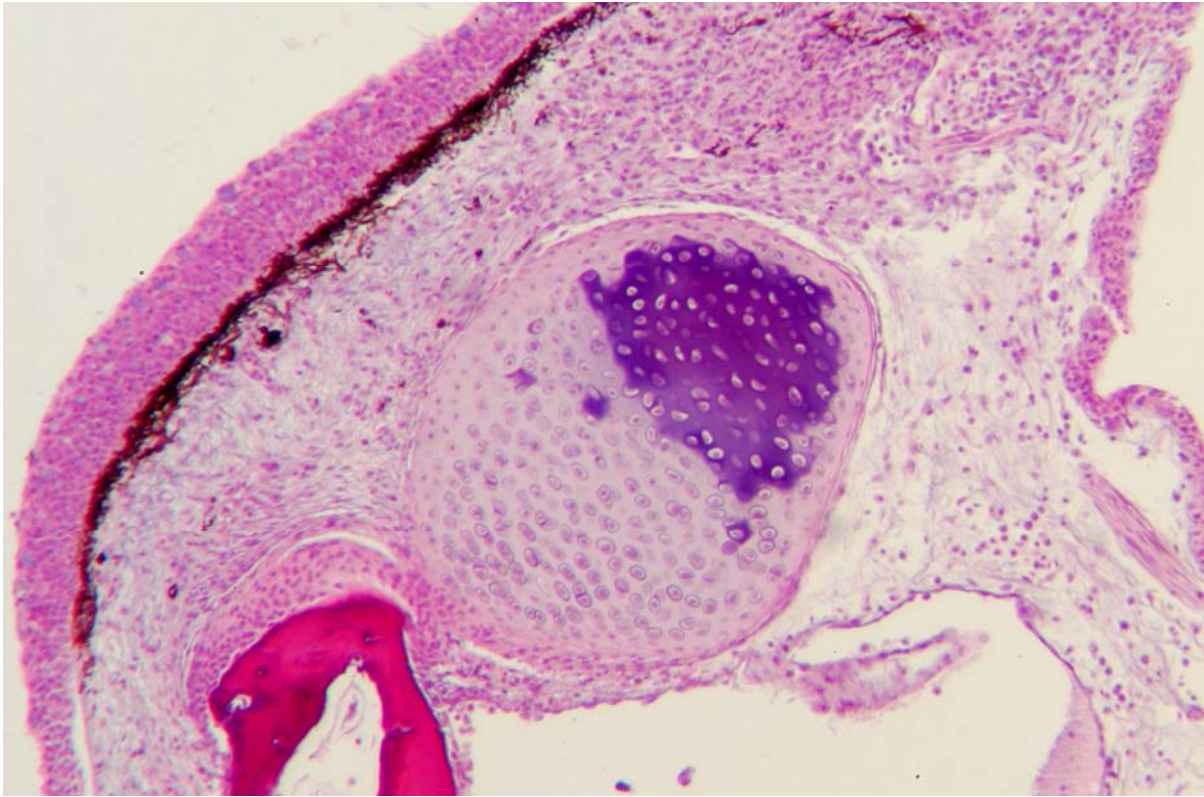


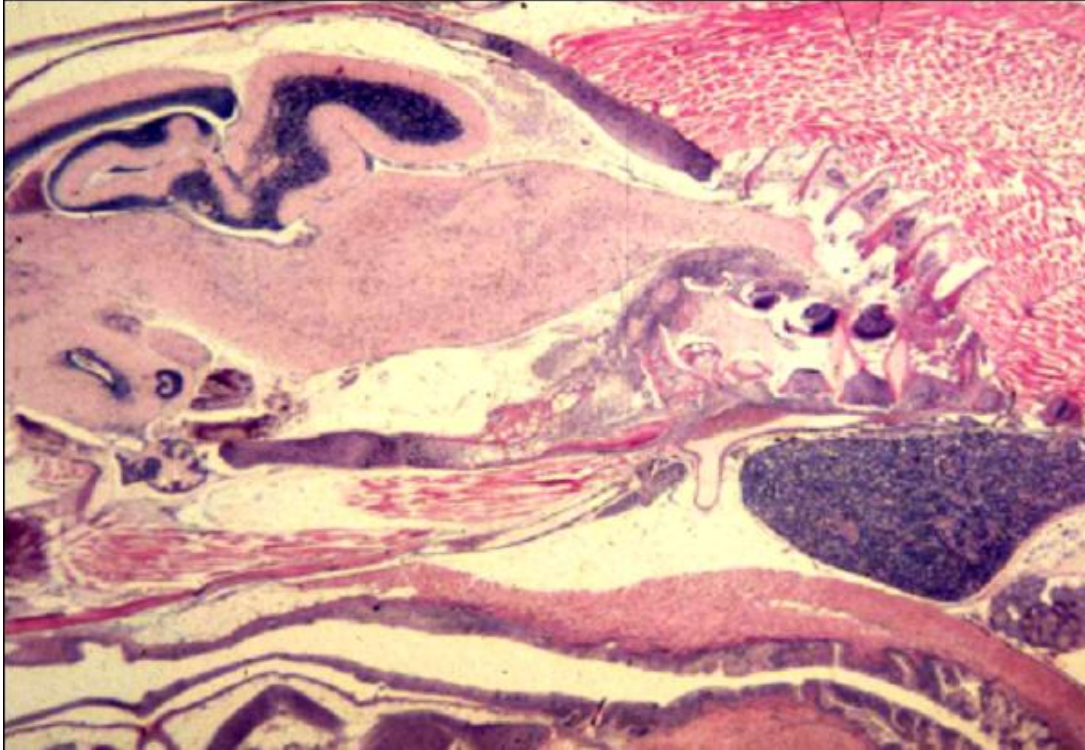
d. Coldwater Disease (CWD) *Flavobacterium psychrophilia*







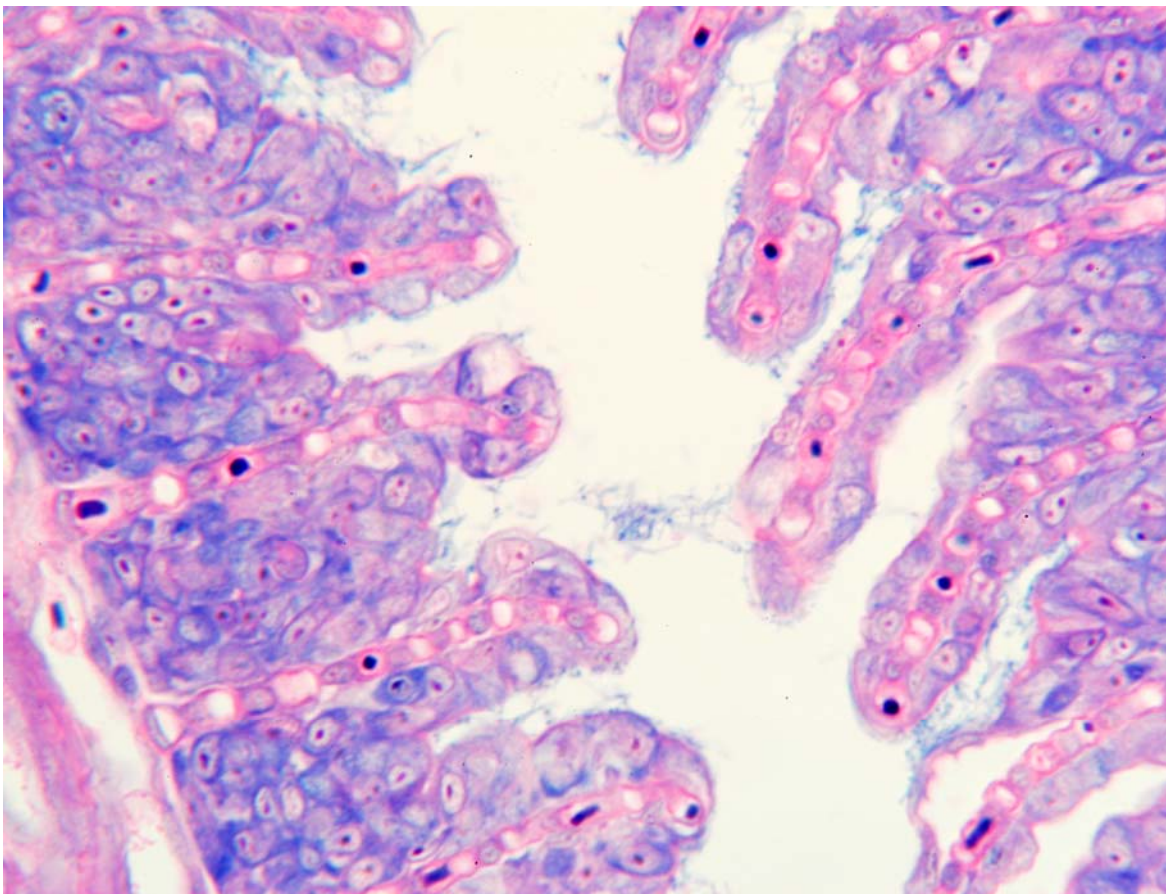
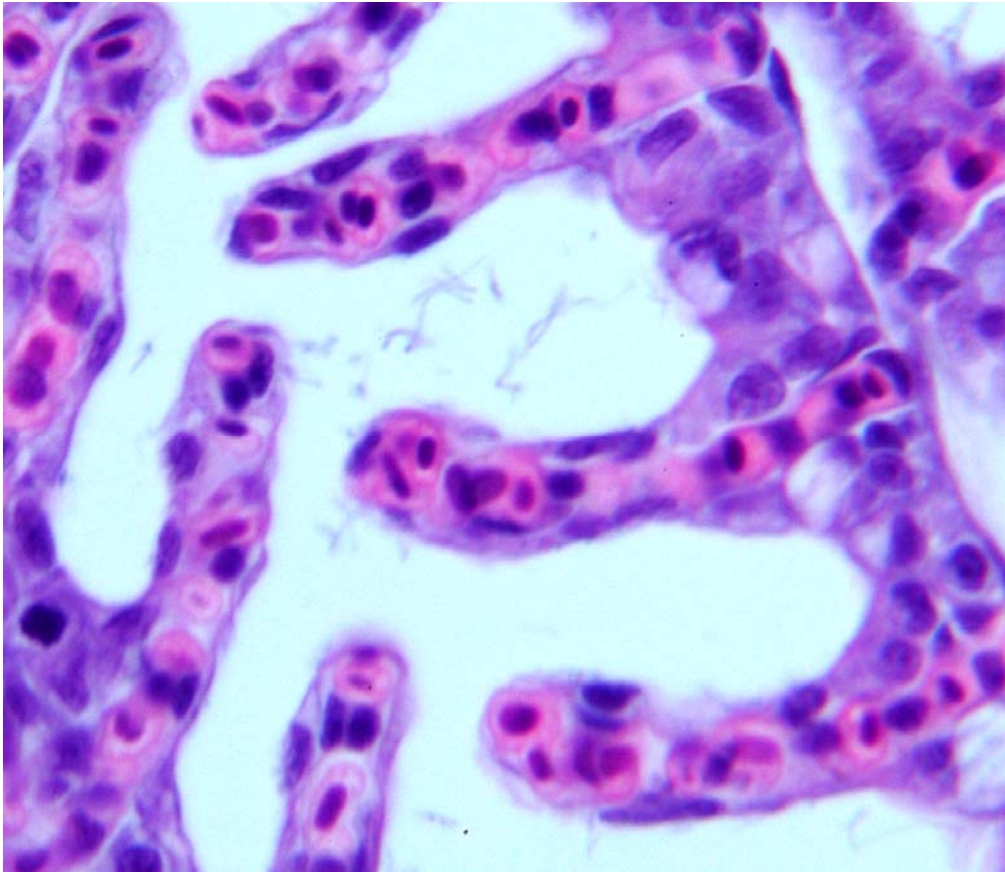


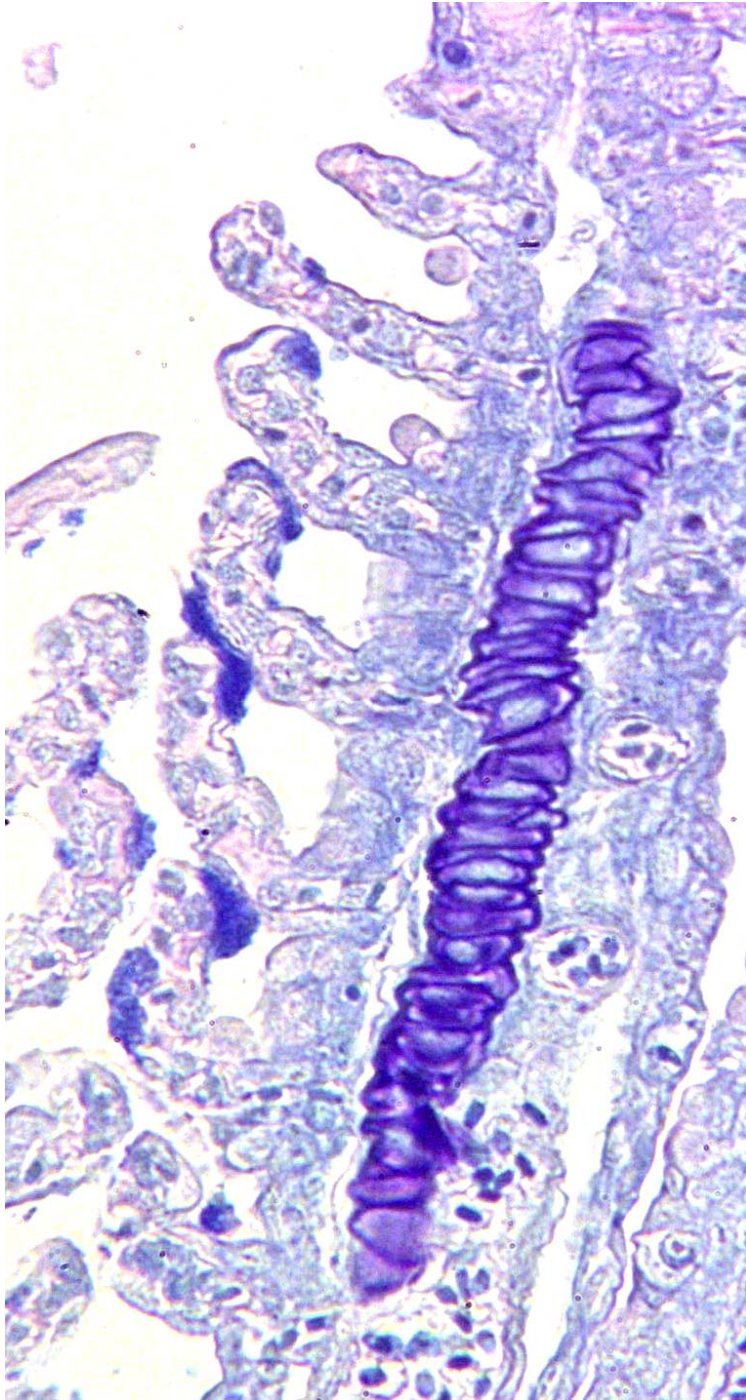


e. Bacterial Gill Disease (BGD) *Flavobacterium branchiophila*

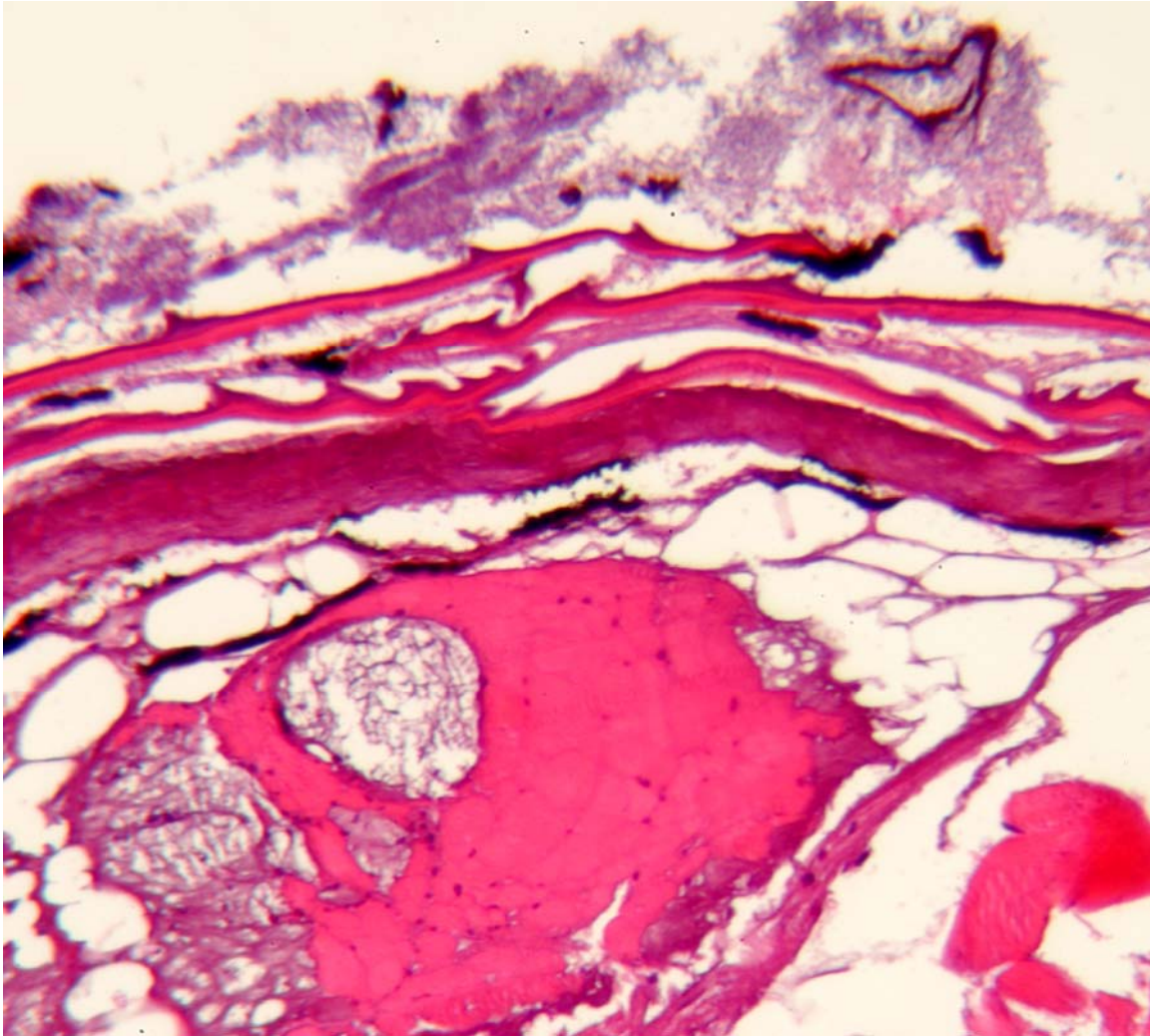


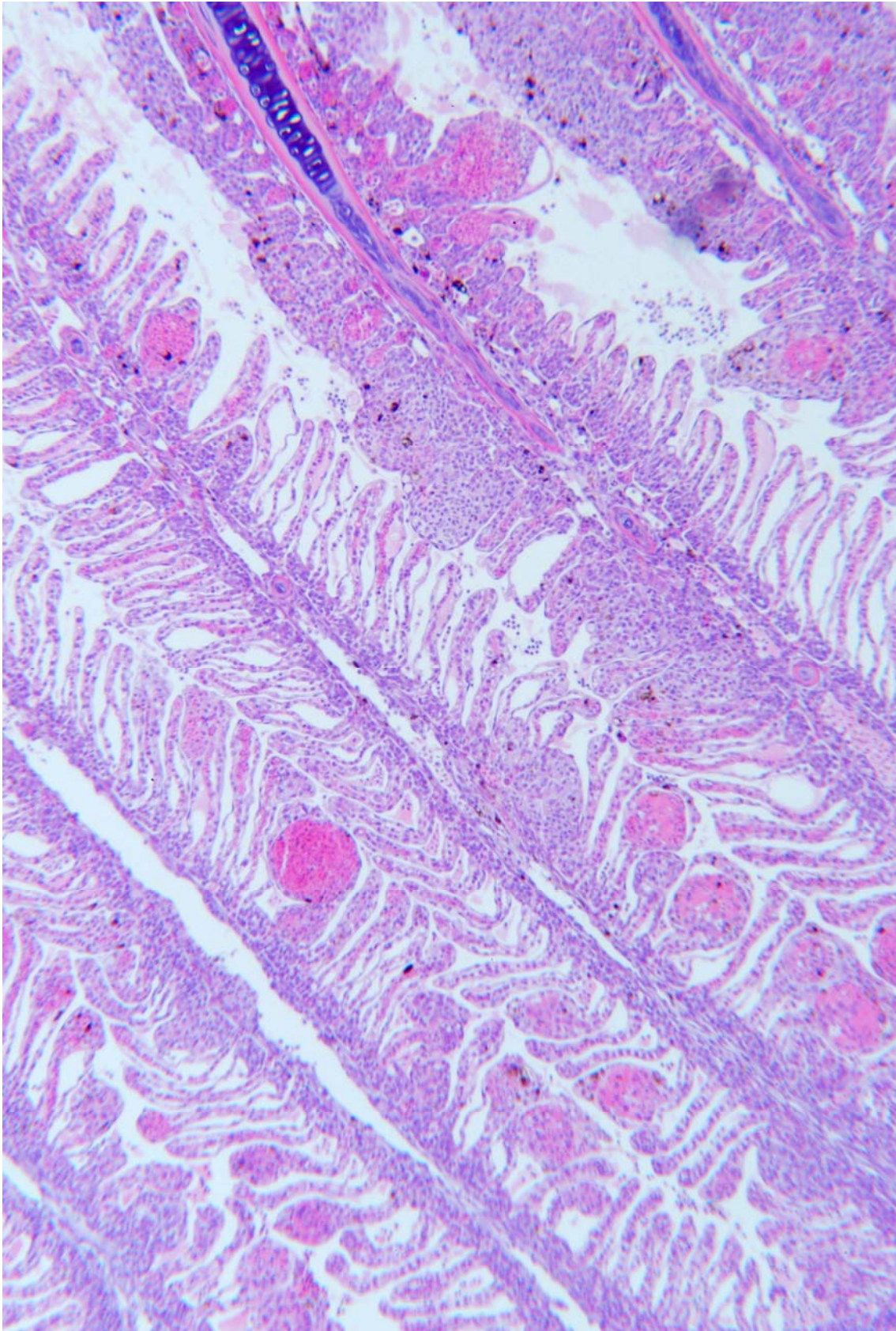
Chapter 6 – Infectious Disease
Fish Histology and Histopathology



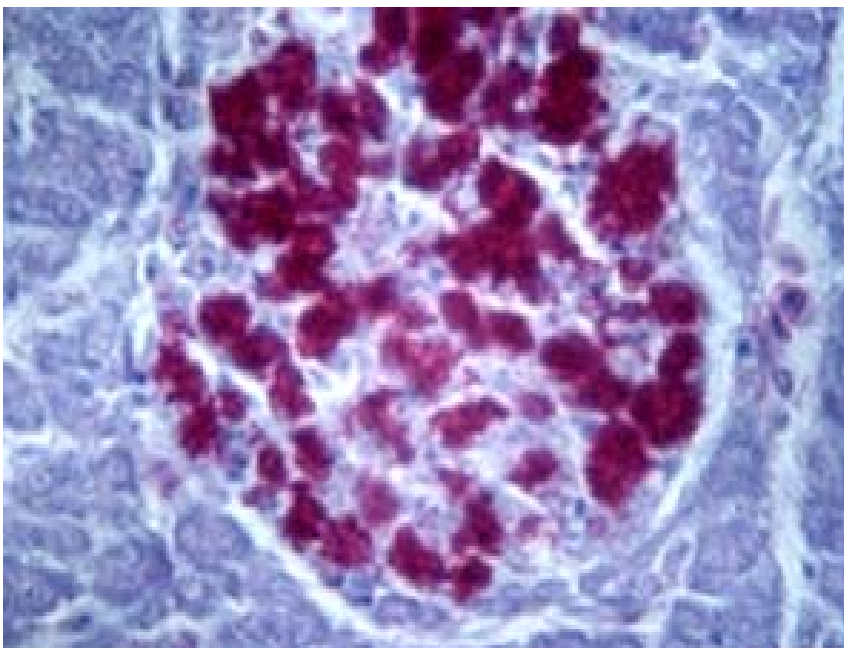
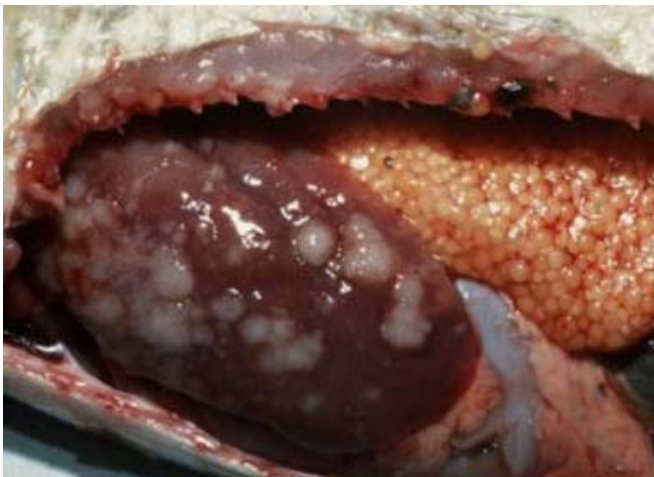


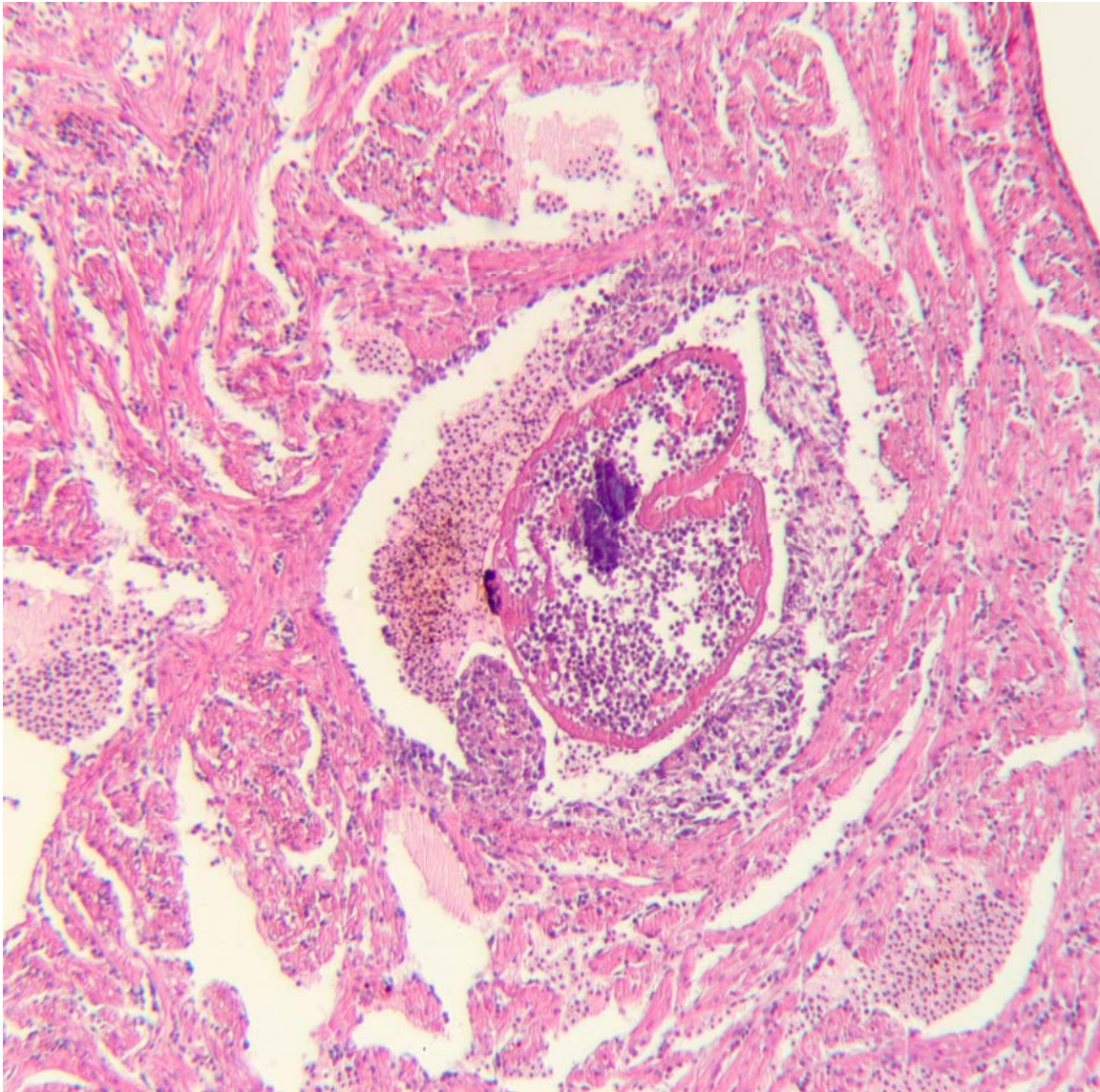
f. Columnaris *Flavobacterium columnaris*



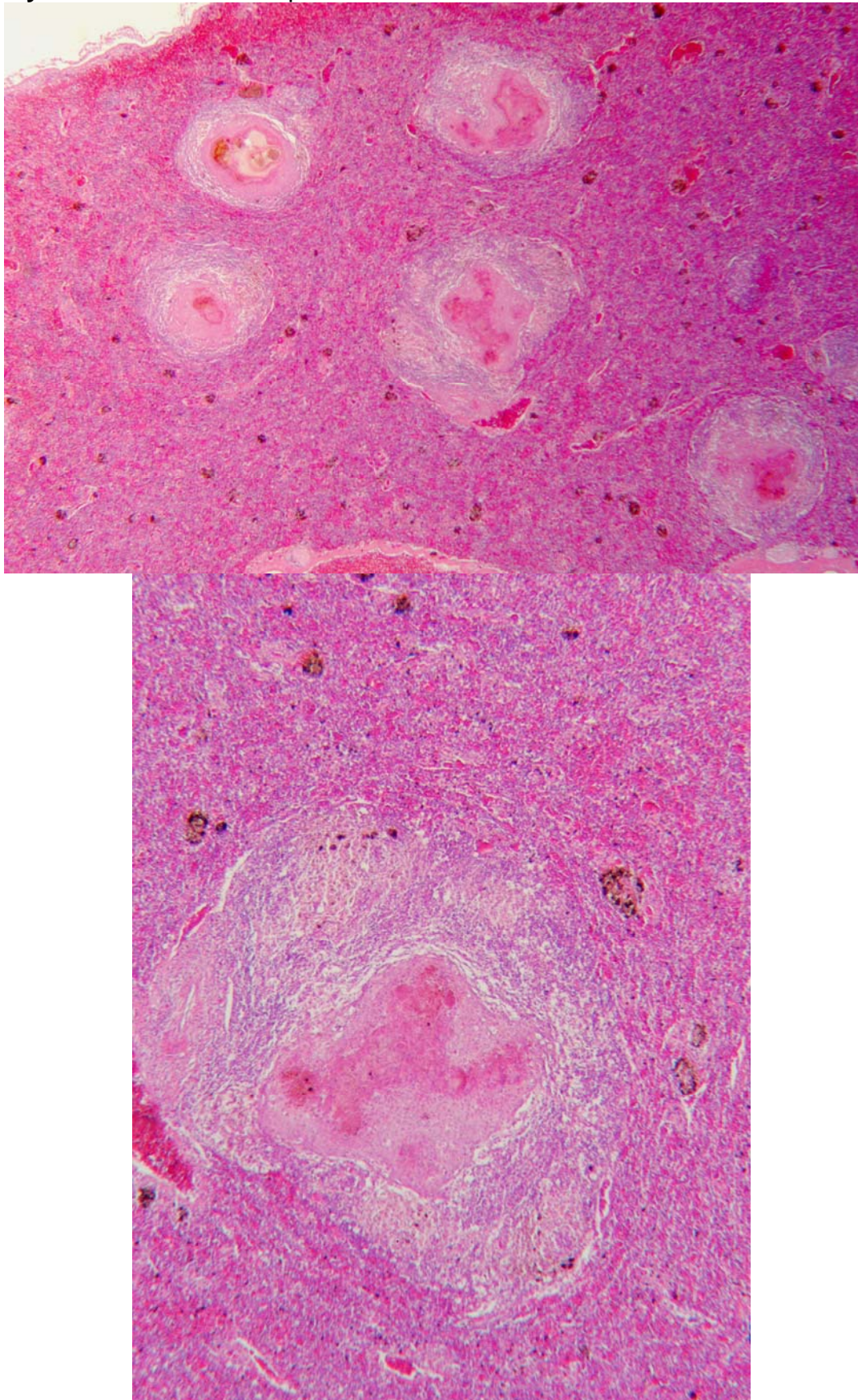


g. Mycobacteriosis *Mycobacteria chelonis*

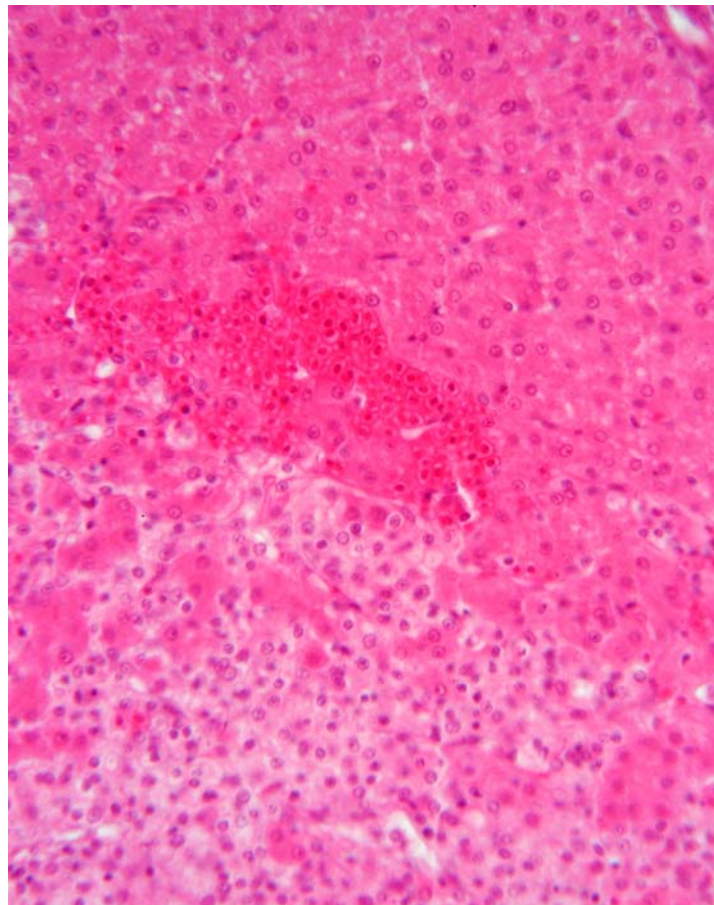
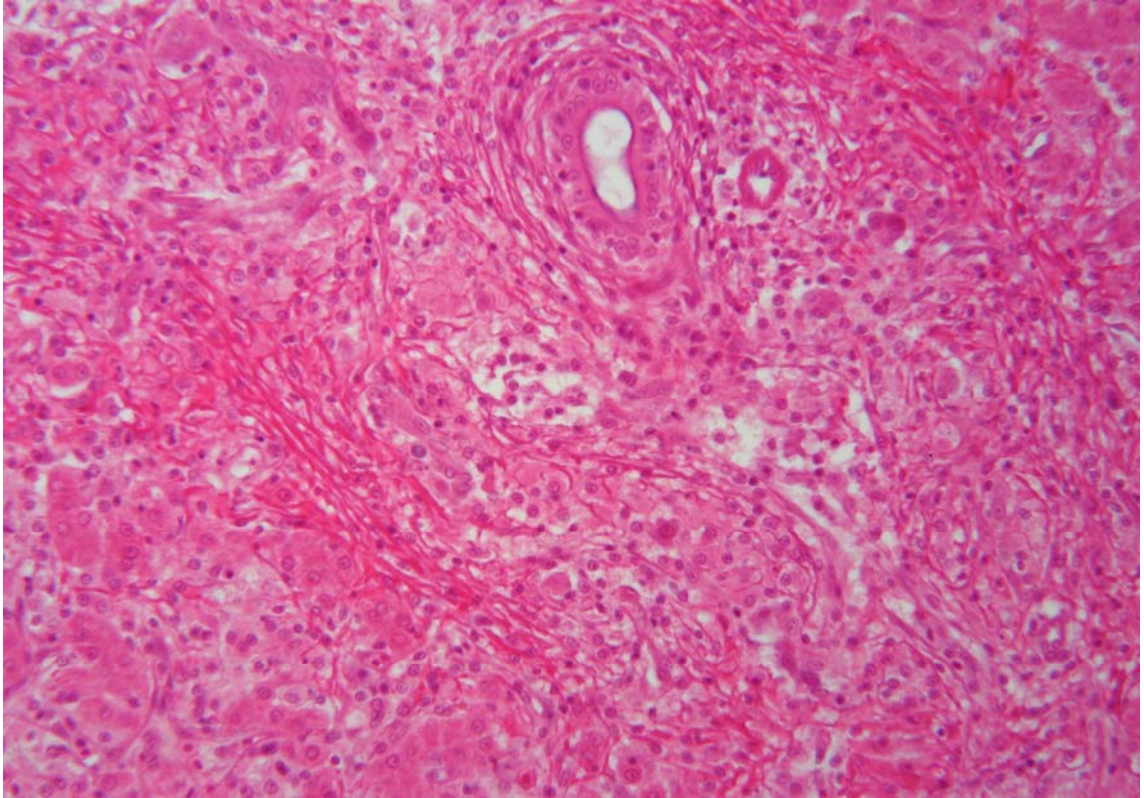




Mycobacteriosis in Striped Bass

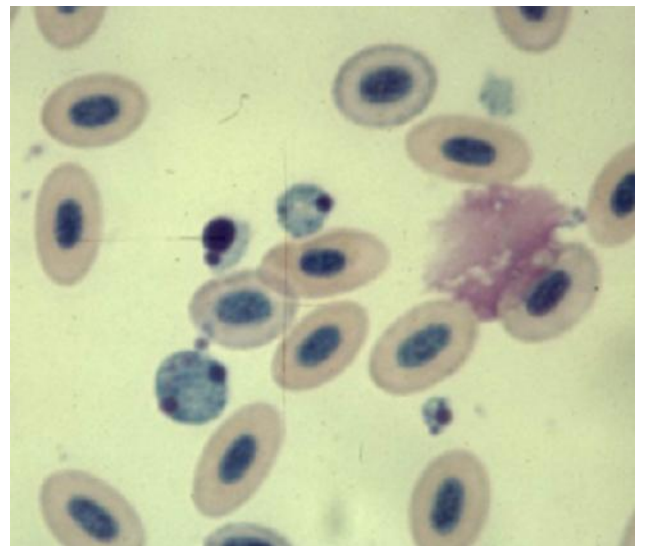
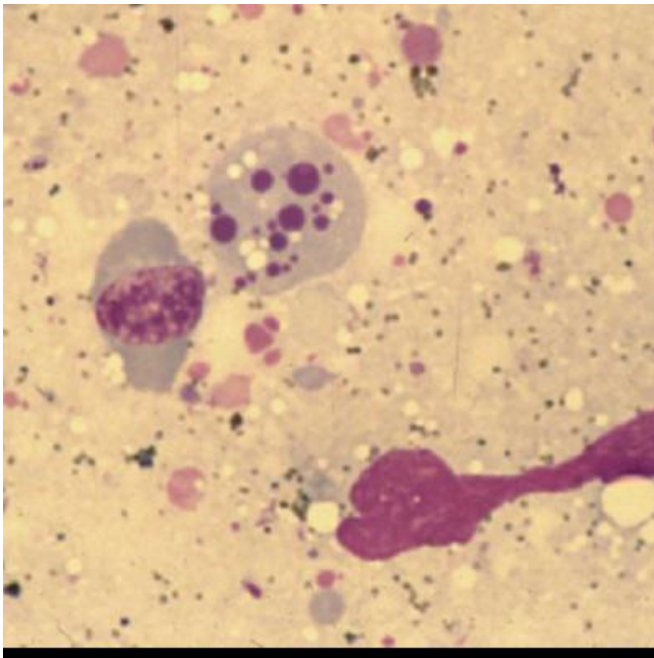


h. *Edwardsiella ictaluri*

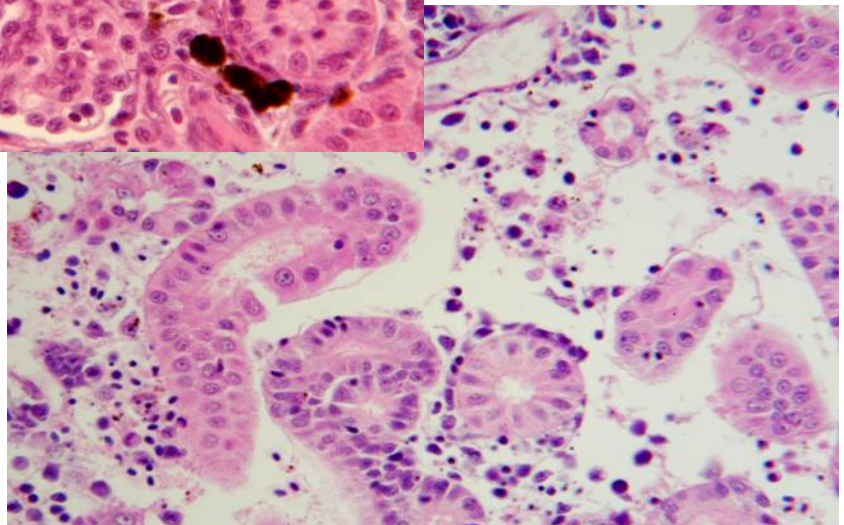
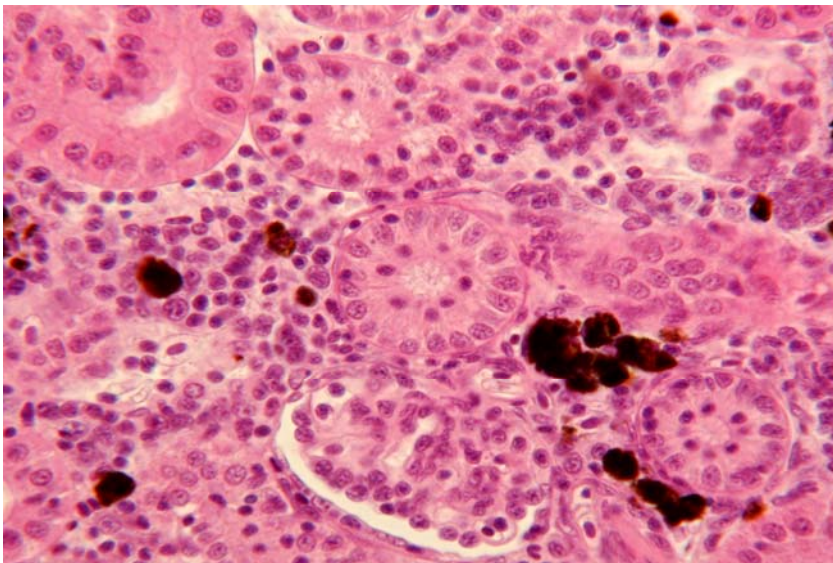
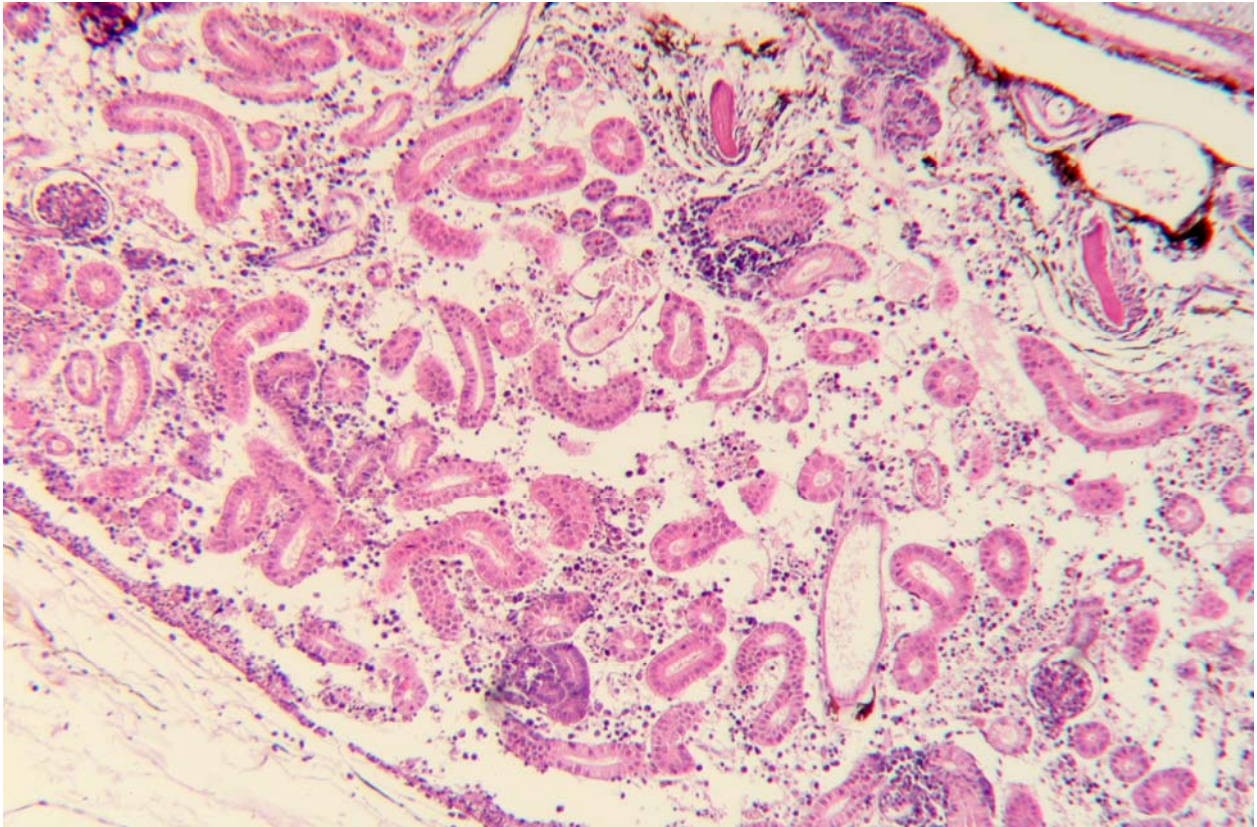


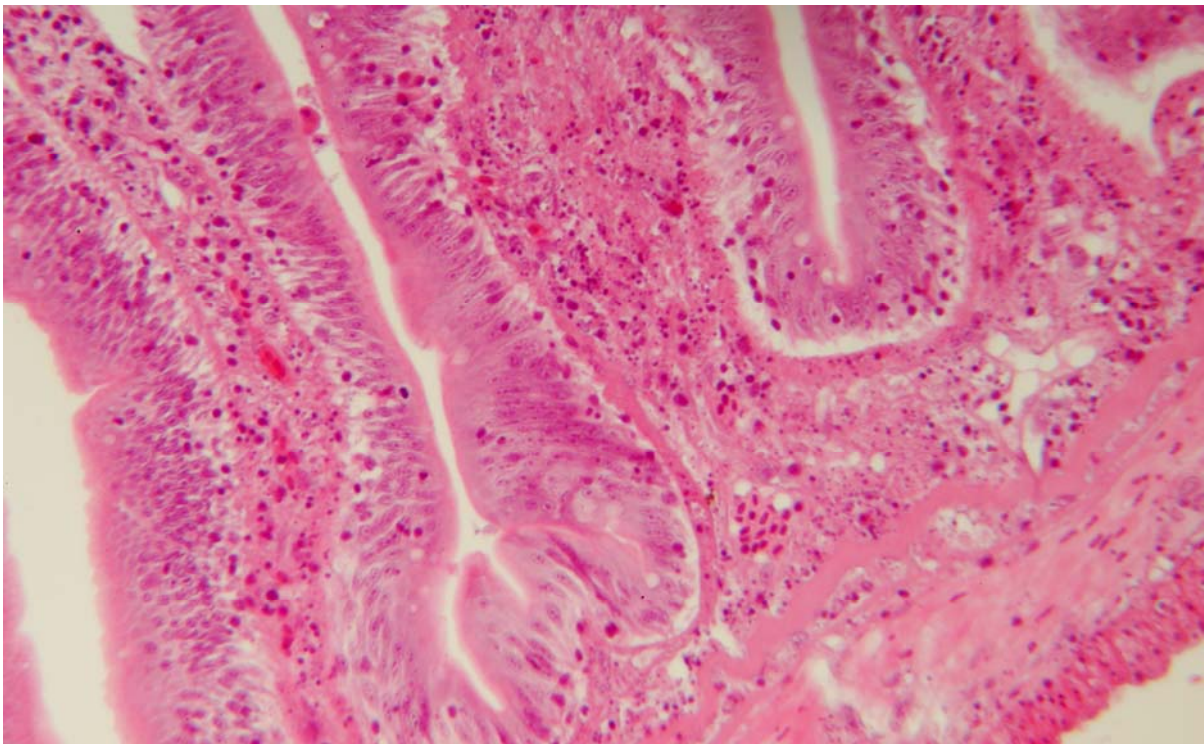
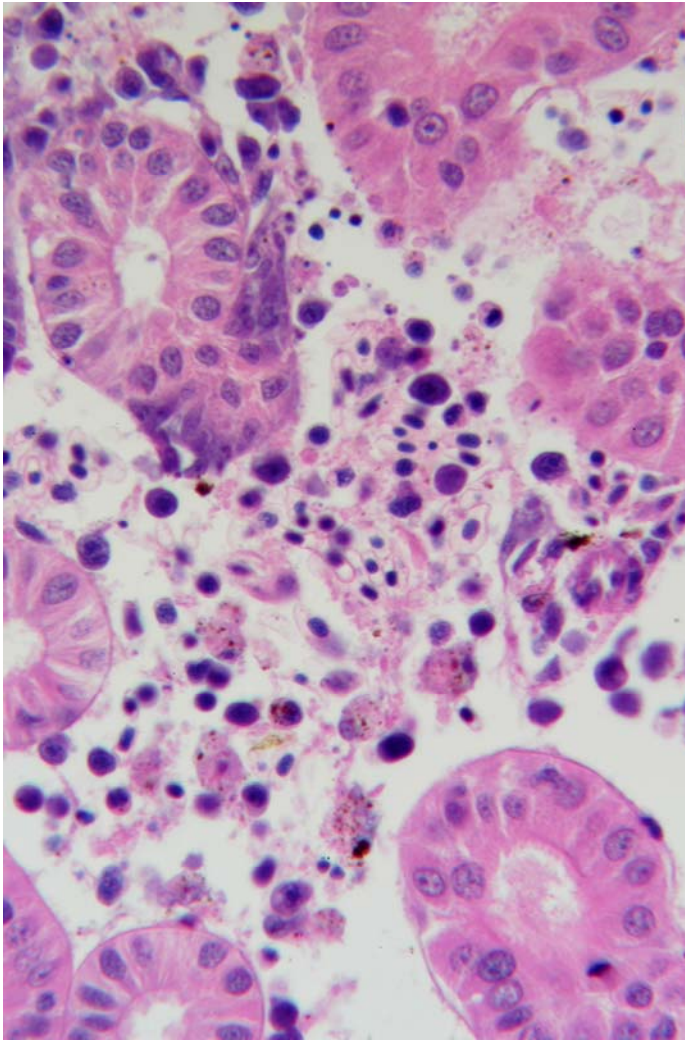
3. Viruses

a. Infectious Hematopoietic Necrosis (IHN)

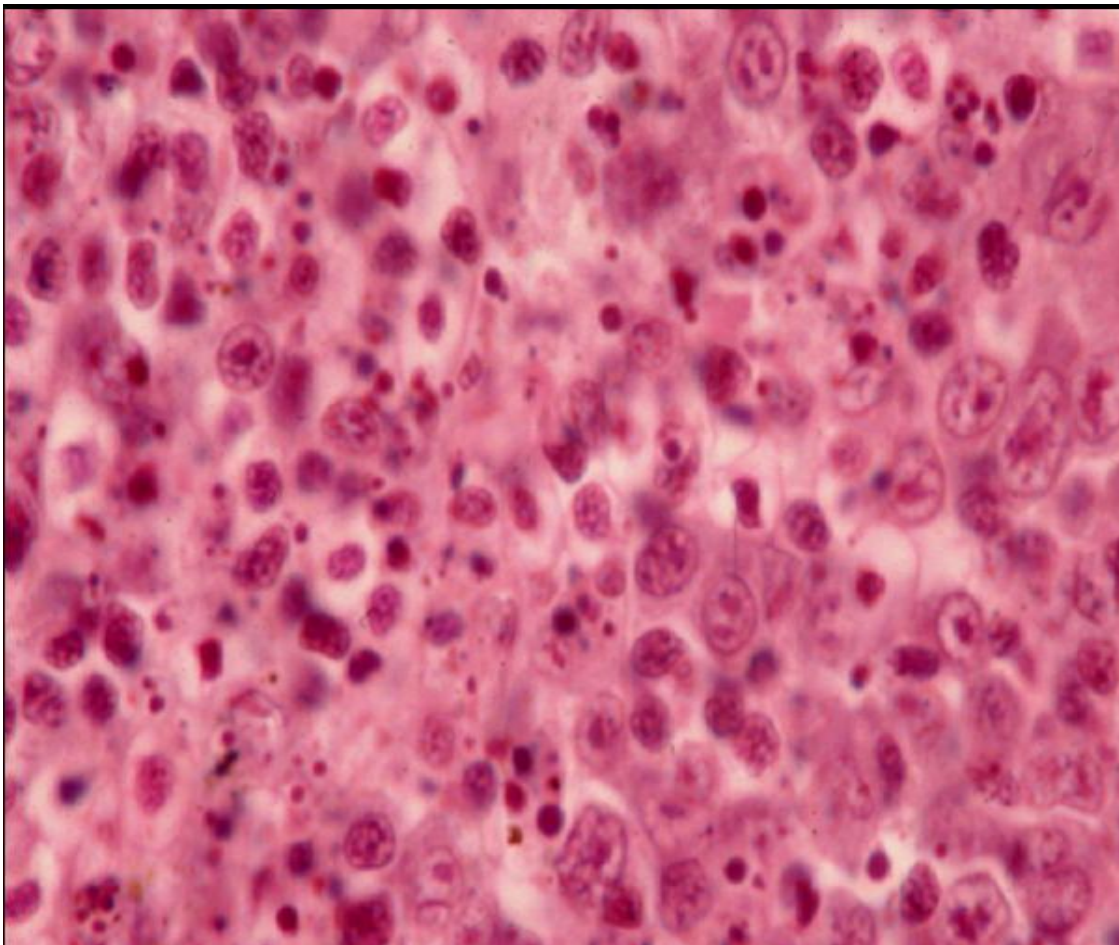
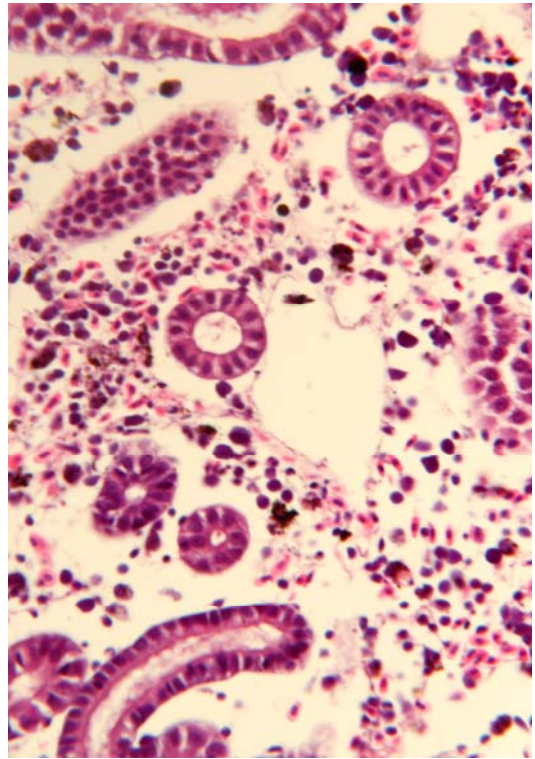
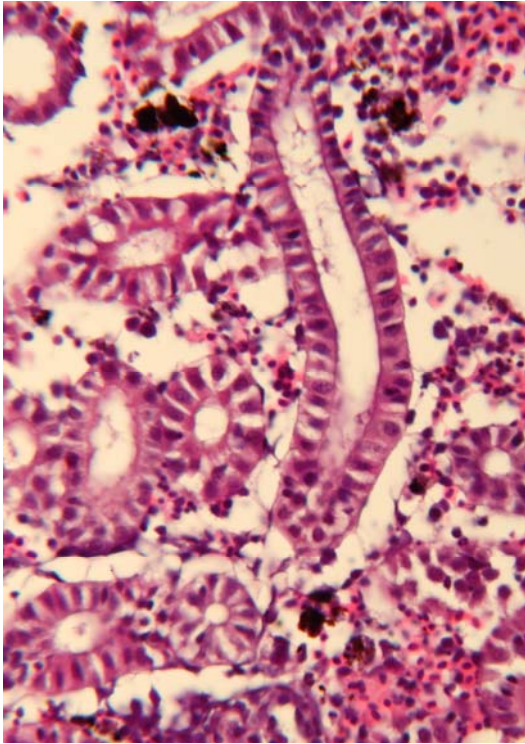


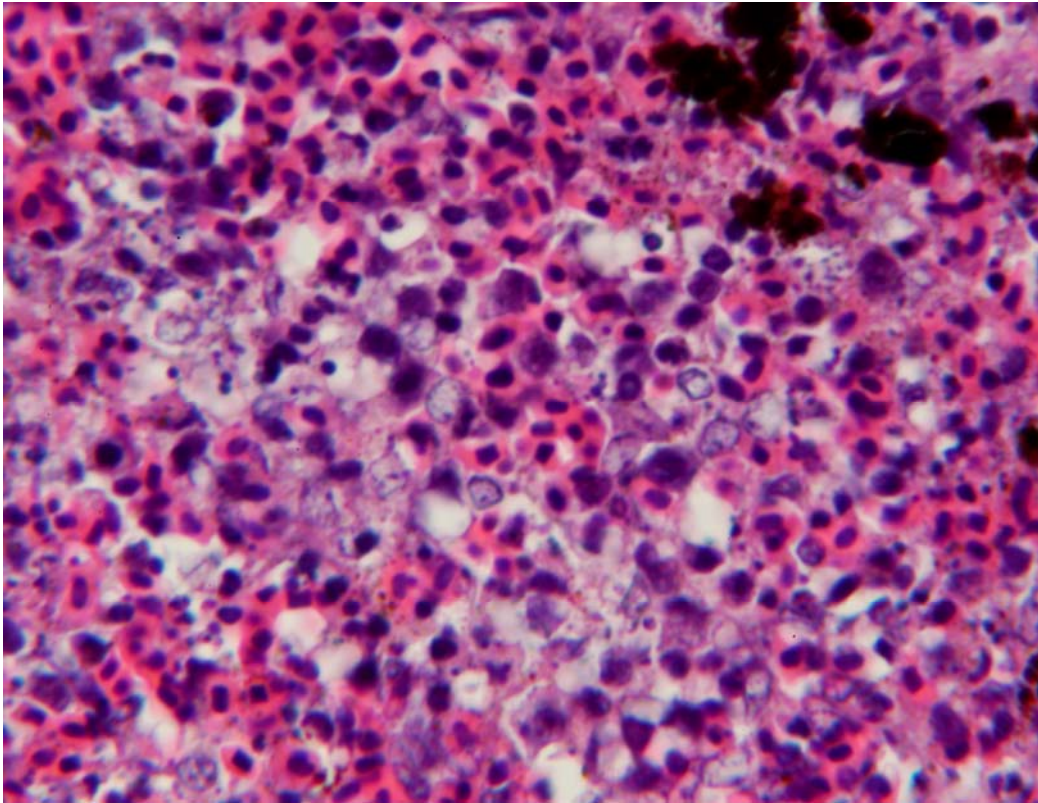
Chapter 6 – Infectious Disease
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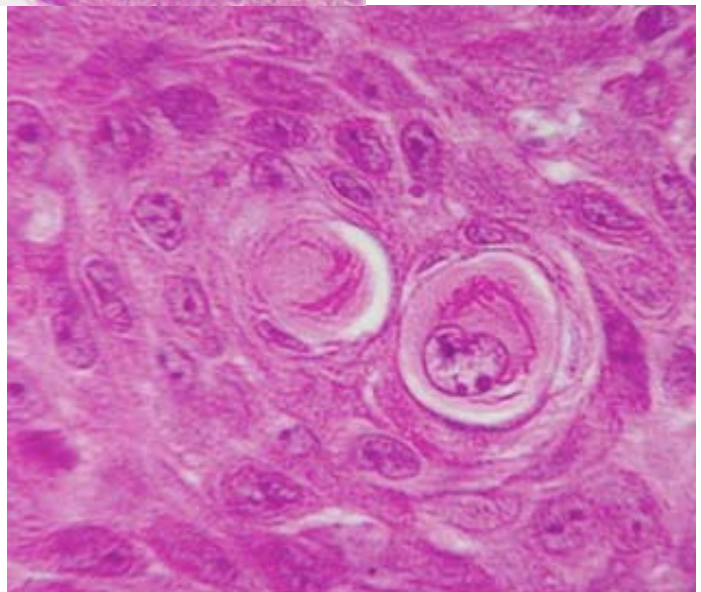
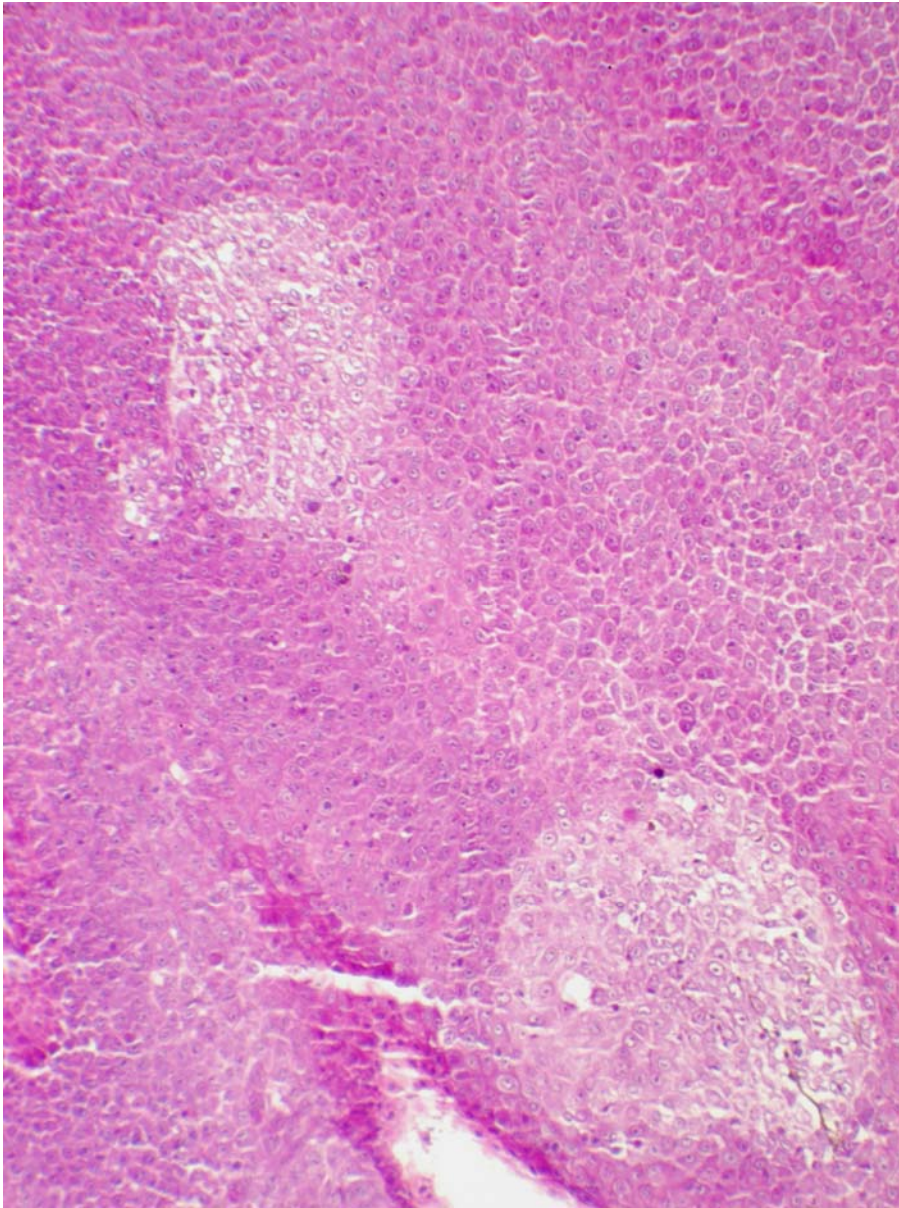
b. Viral Hemorrhagic Septicemia (VHS)

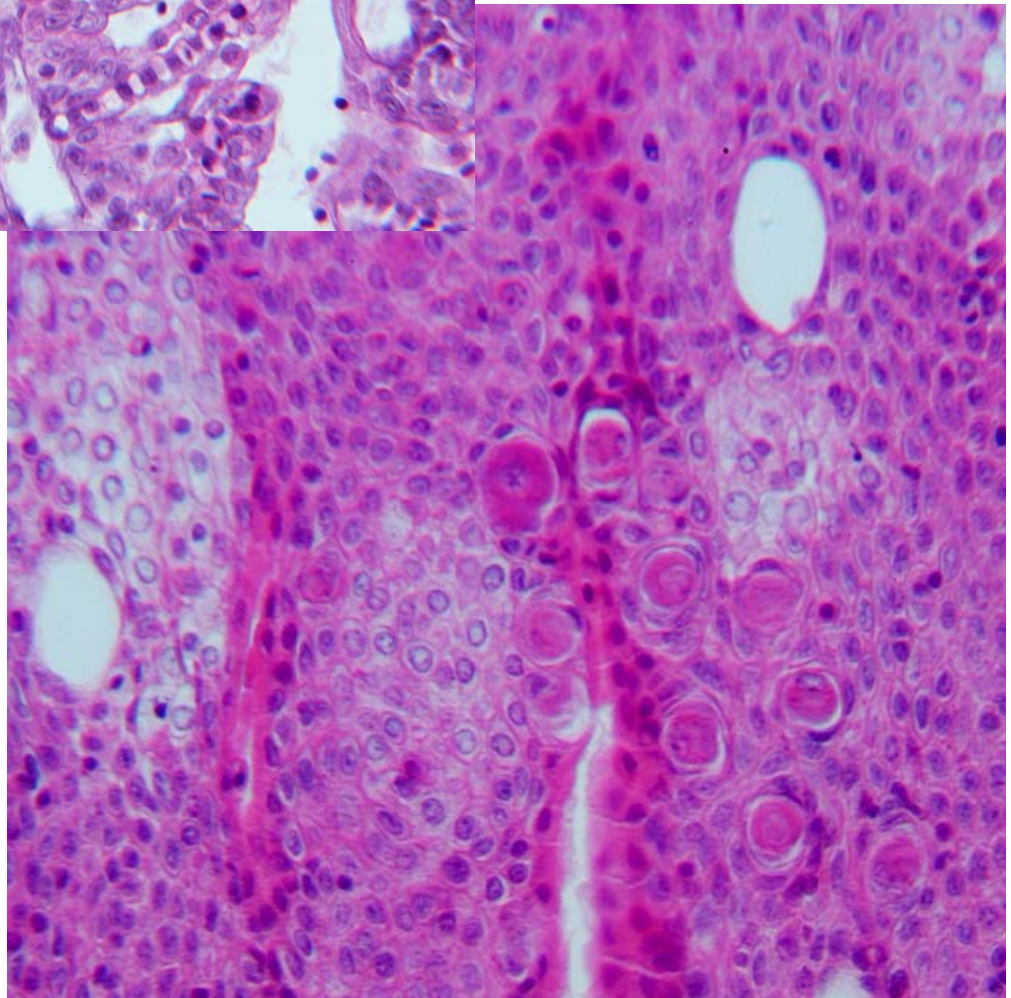
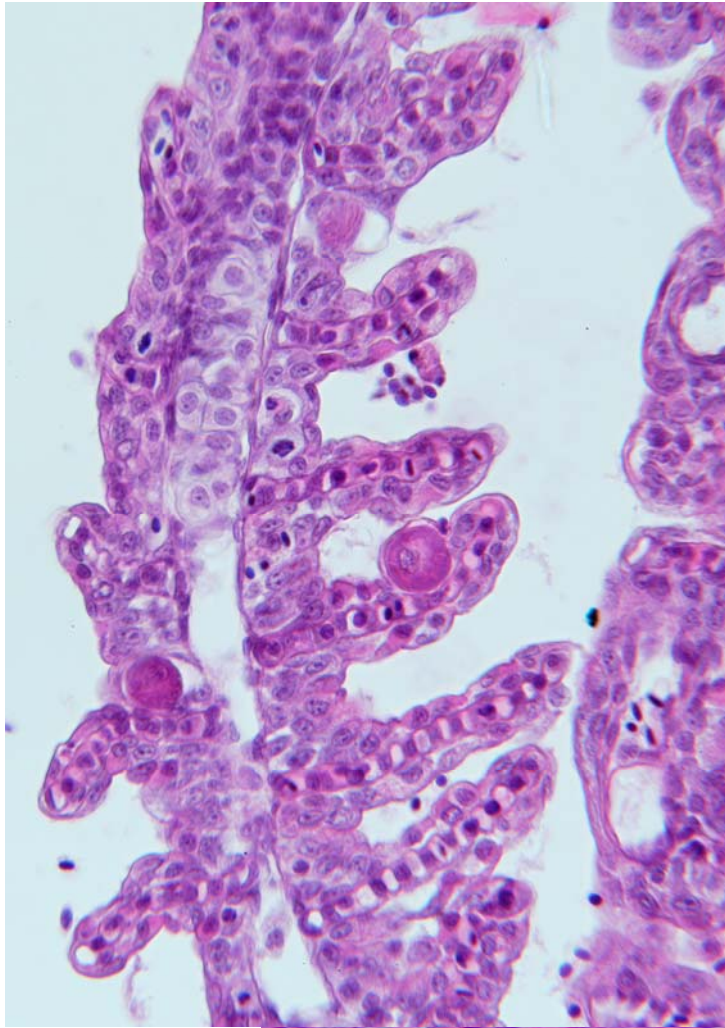




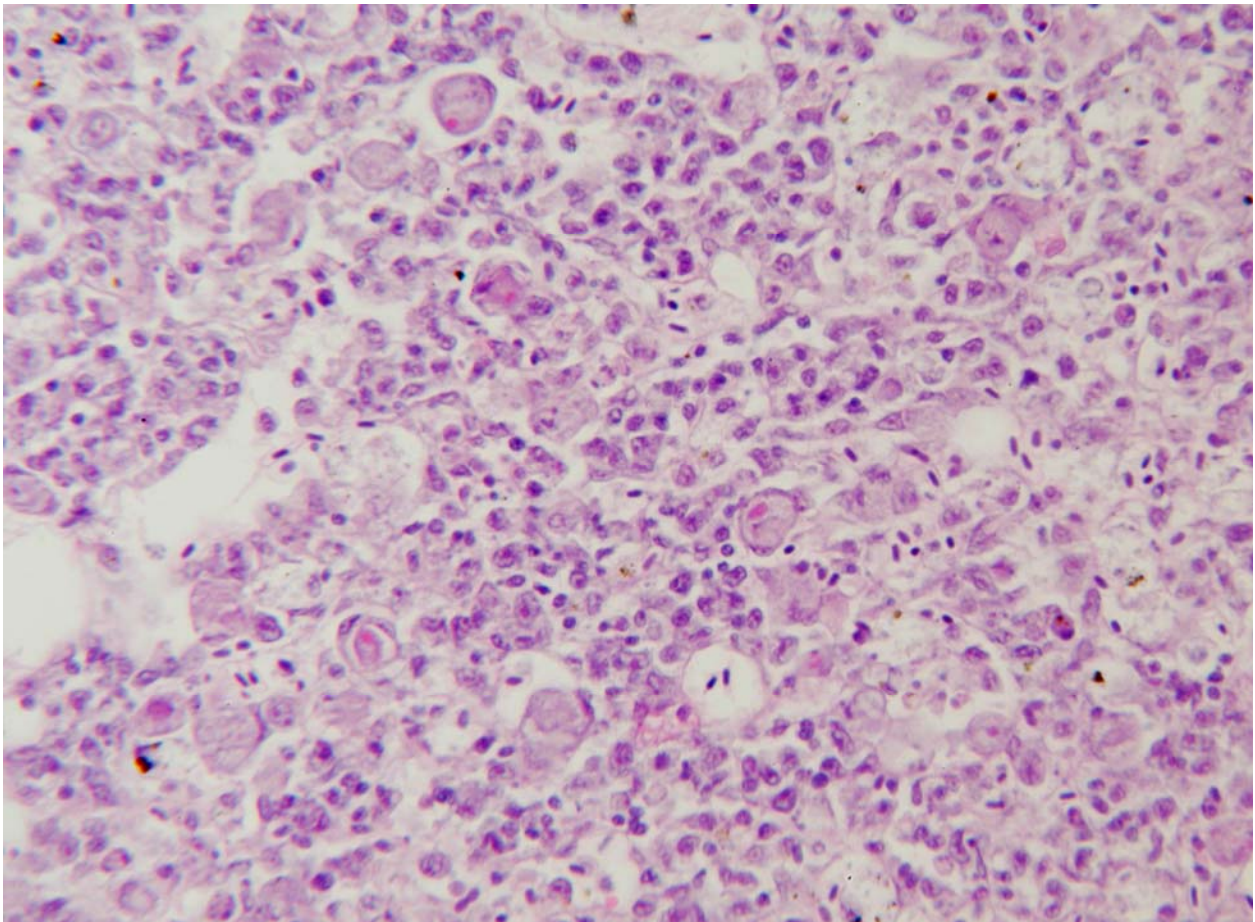
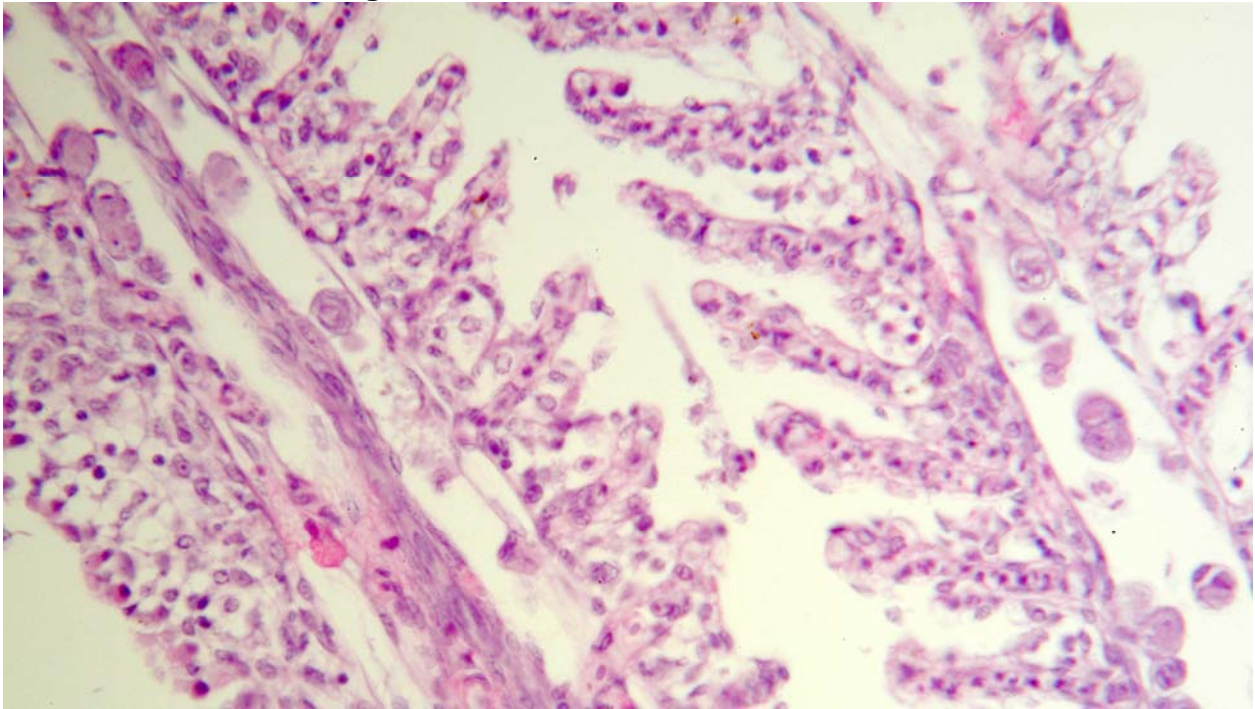
c. Iridovirus – Sturgeon



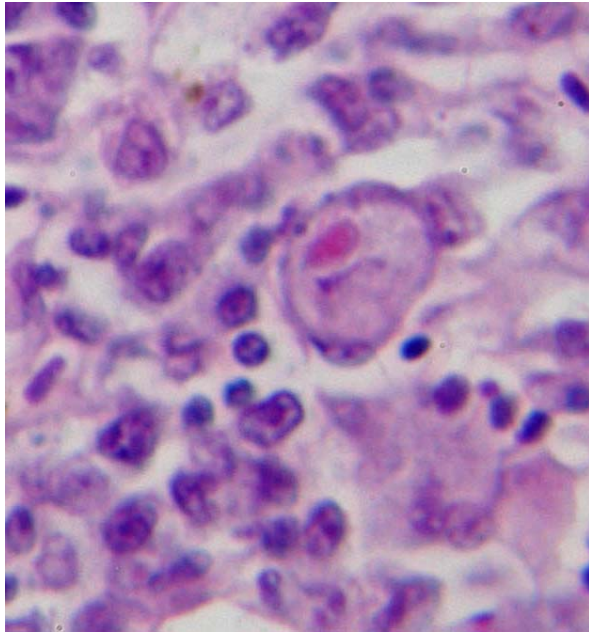




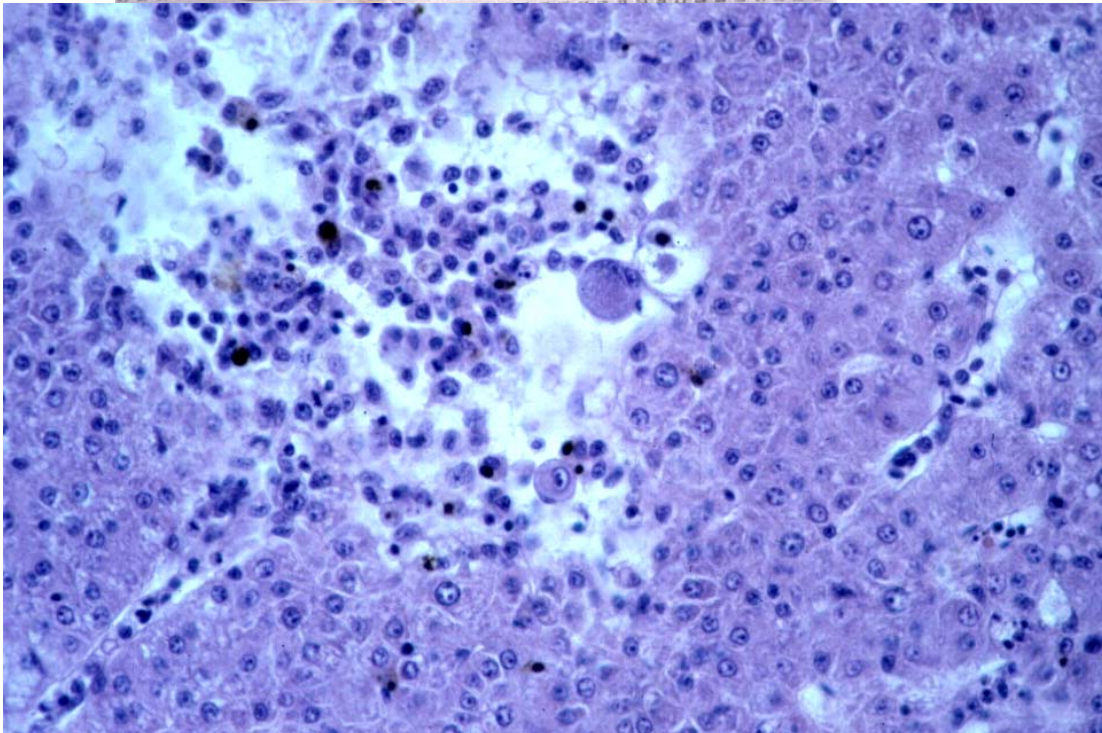
d. Iridovirus - Angelfish

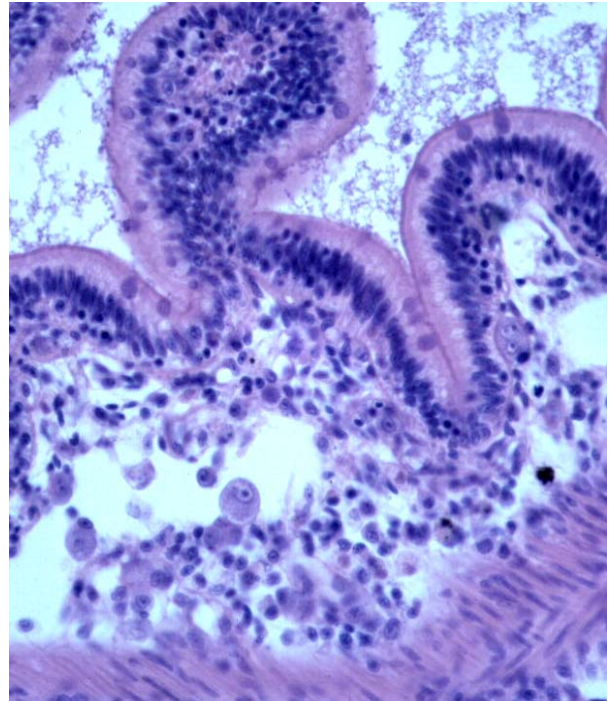
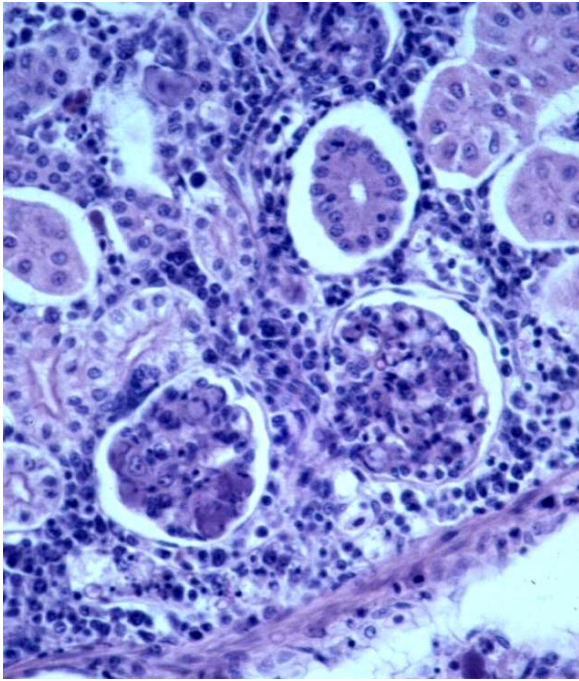


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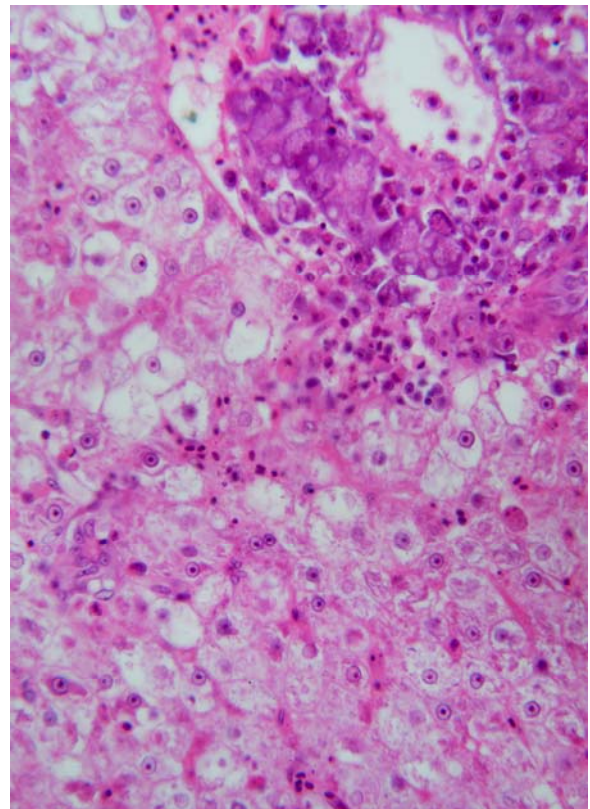
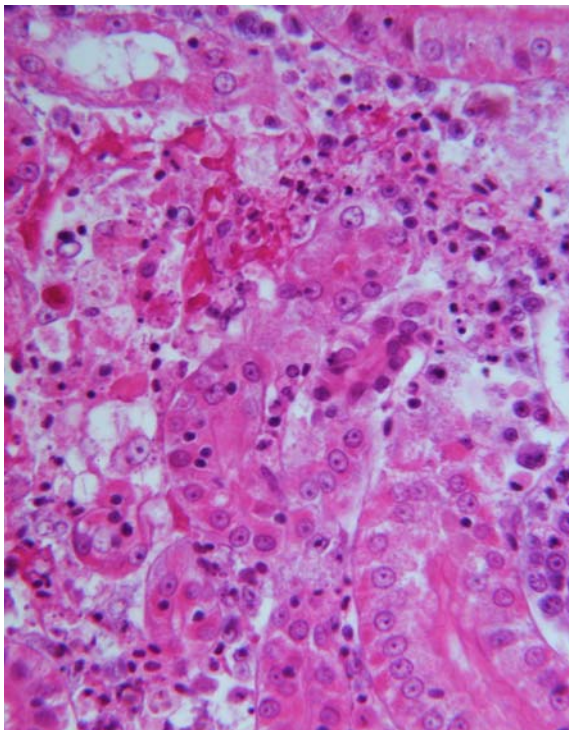


e. Iridovirus – Tilapia





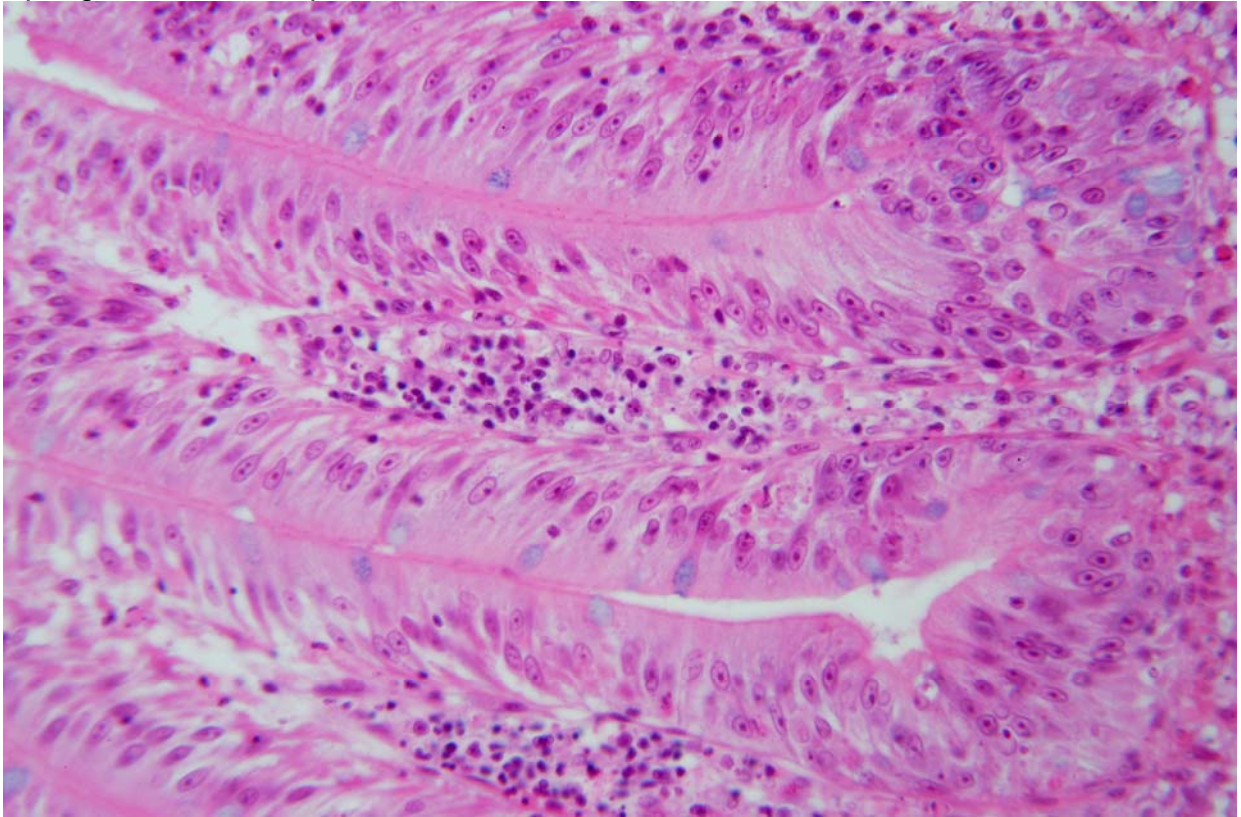
f. Spring Viremia of Carp – SVC



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Spring Viremia of Carp – svc

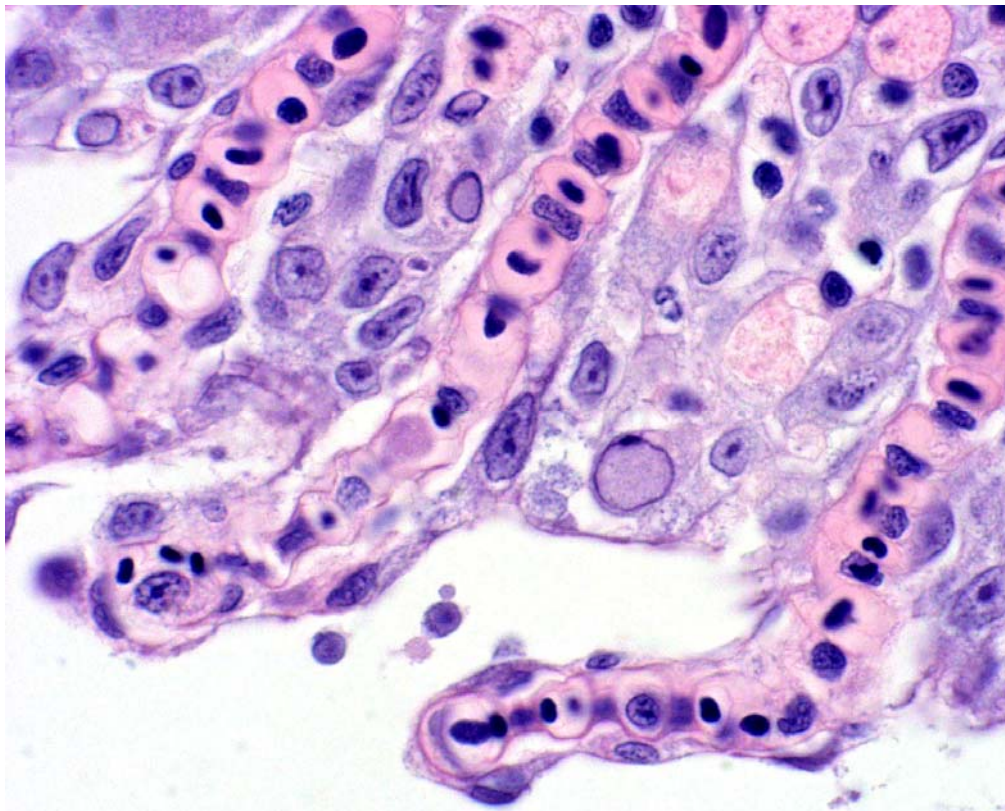
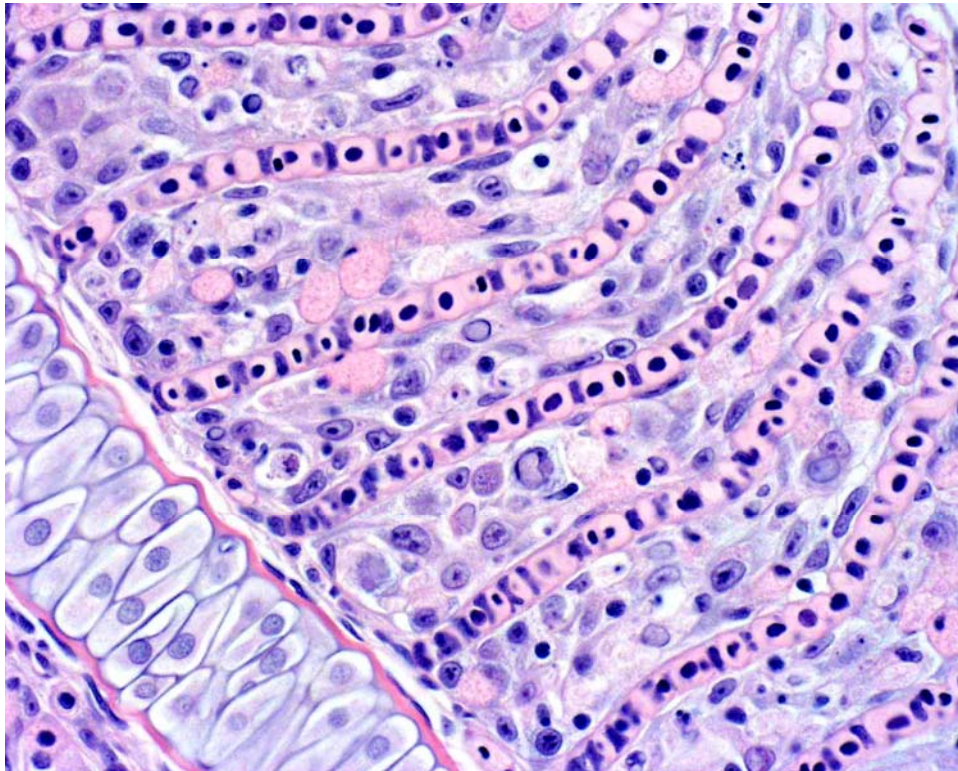


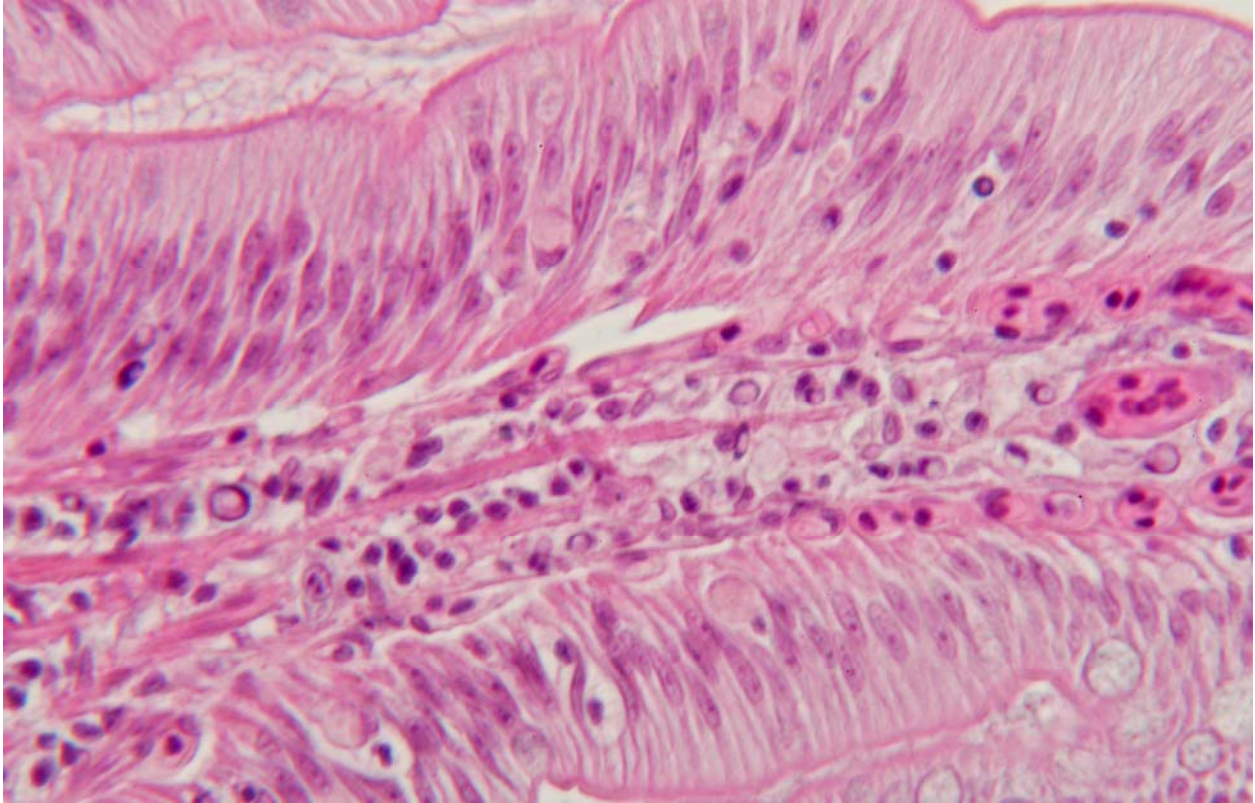
g. Koi Herpesvirus

KHV - Species affected are koi and common carp



Koi Herpesvirus

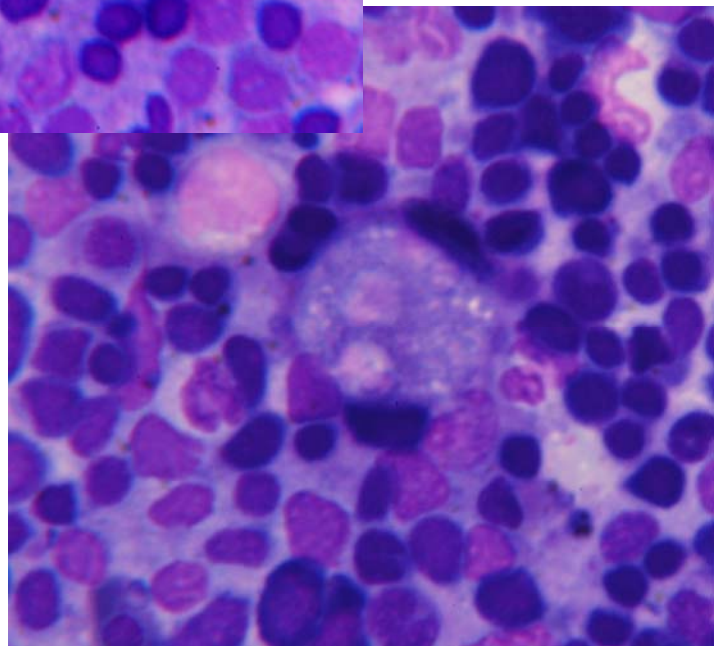
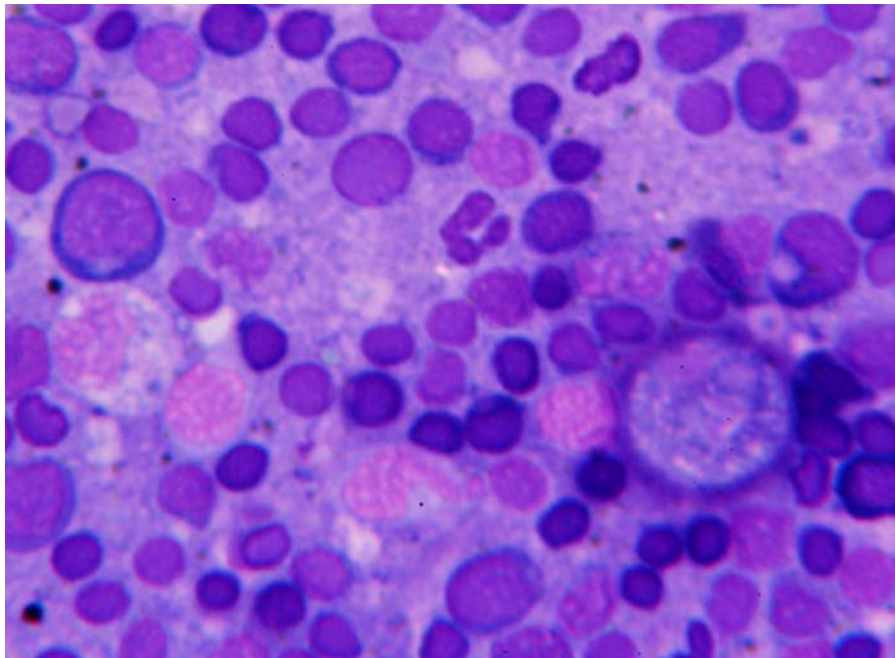




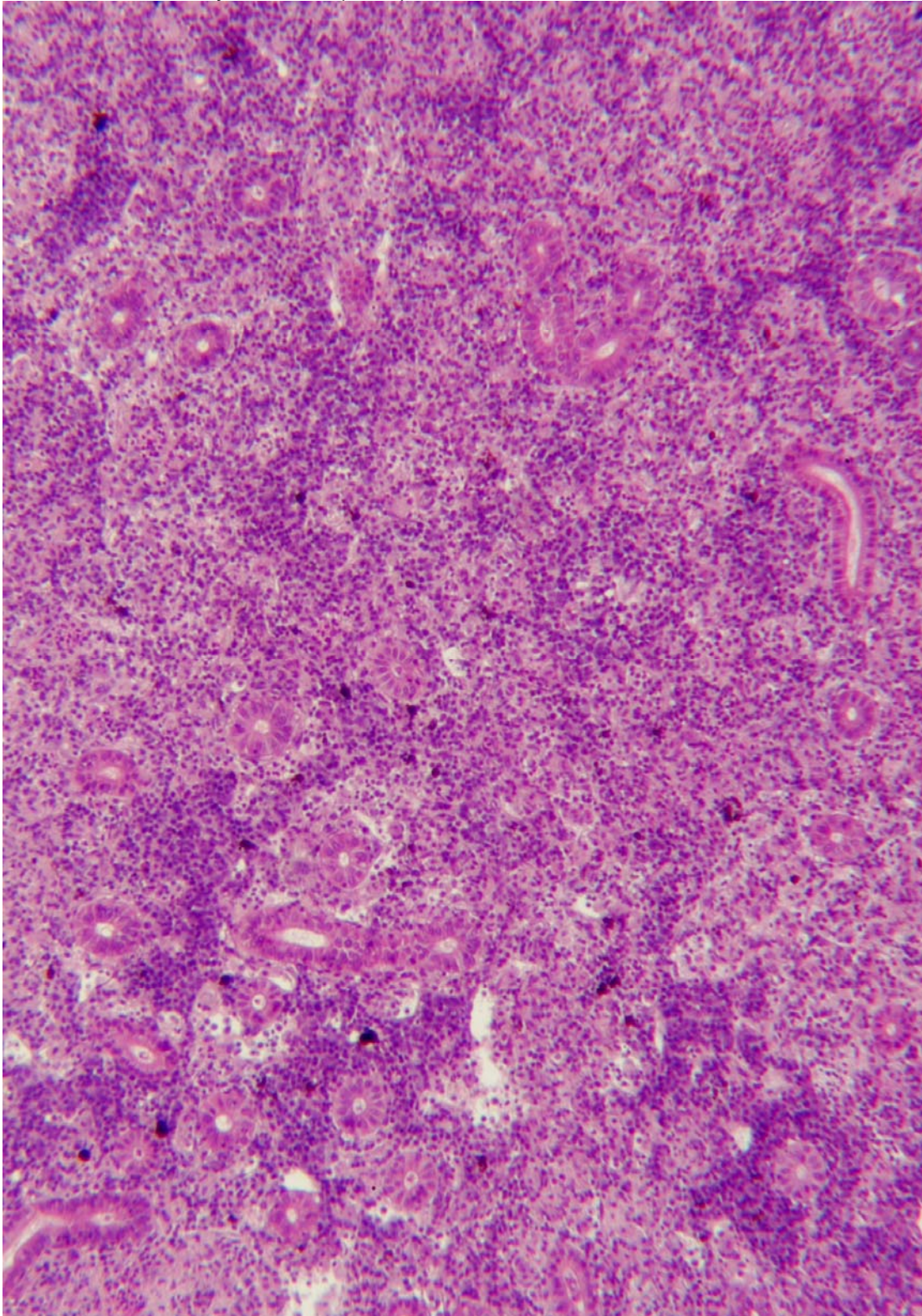
4. Parasites

a. Myxosporean

(1) Proliferative kidney disease (PKD) - *Tetracapsula bryosalmonae*

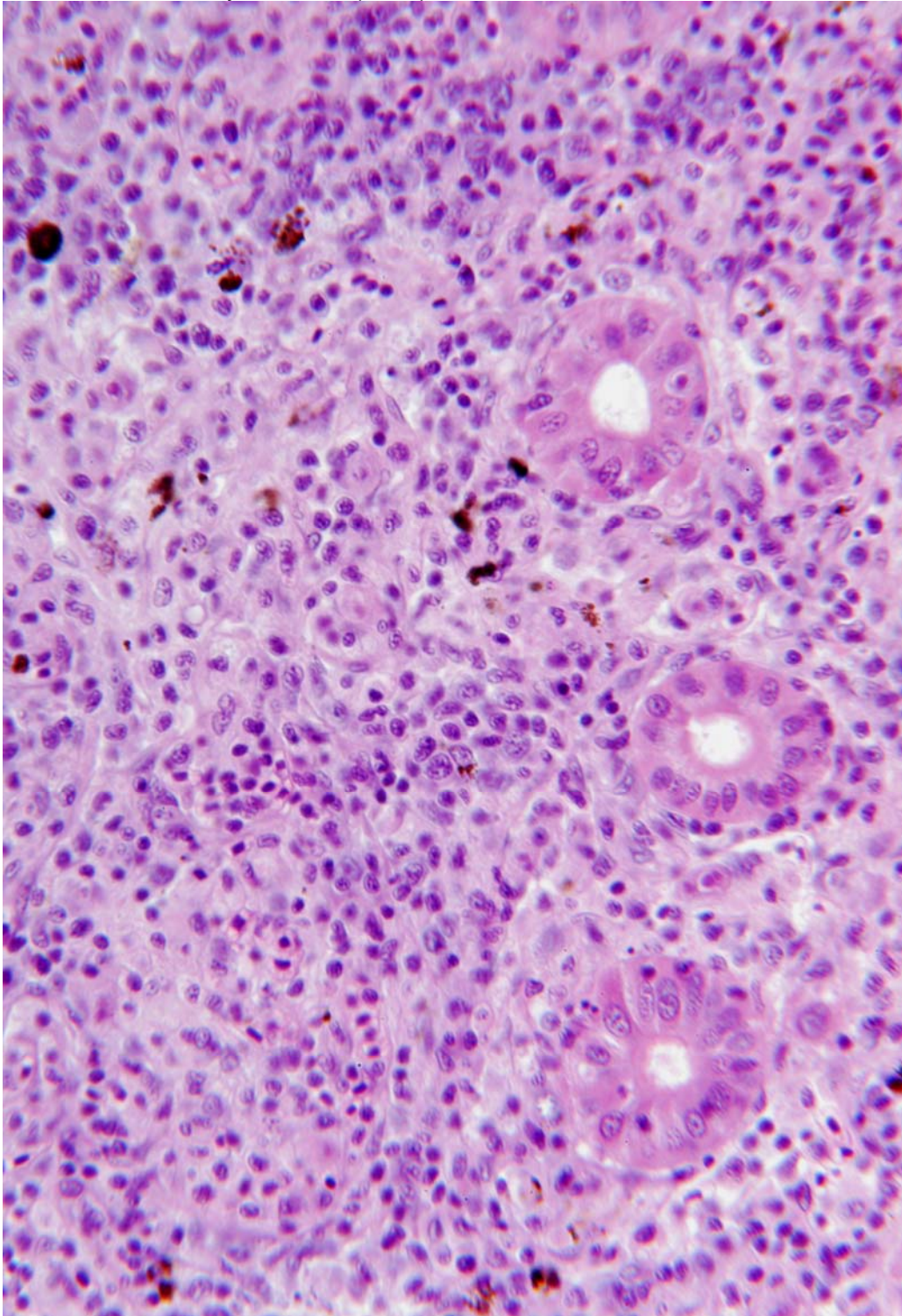


Proliferative kidney disease (PKD)



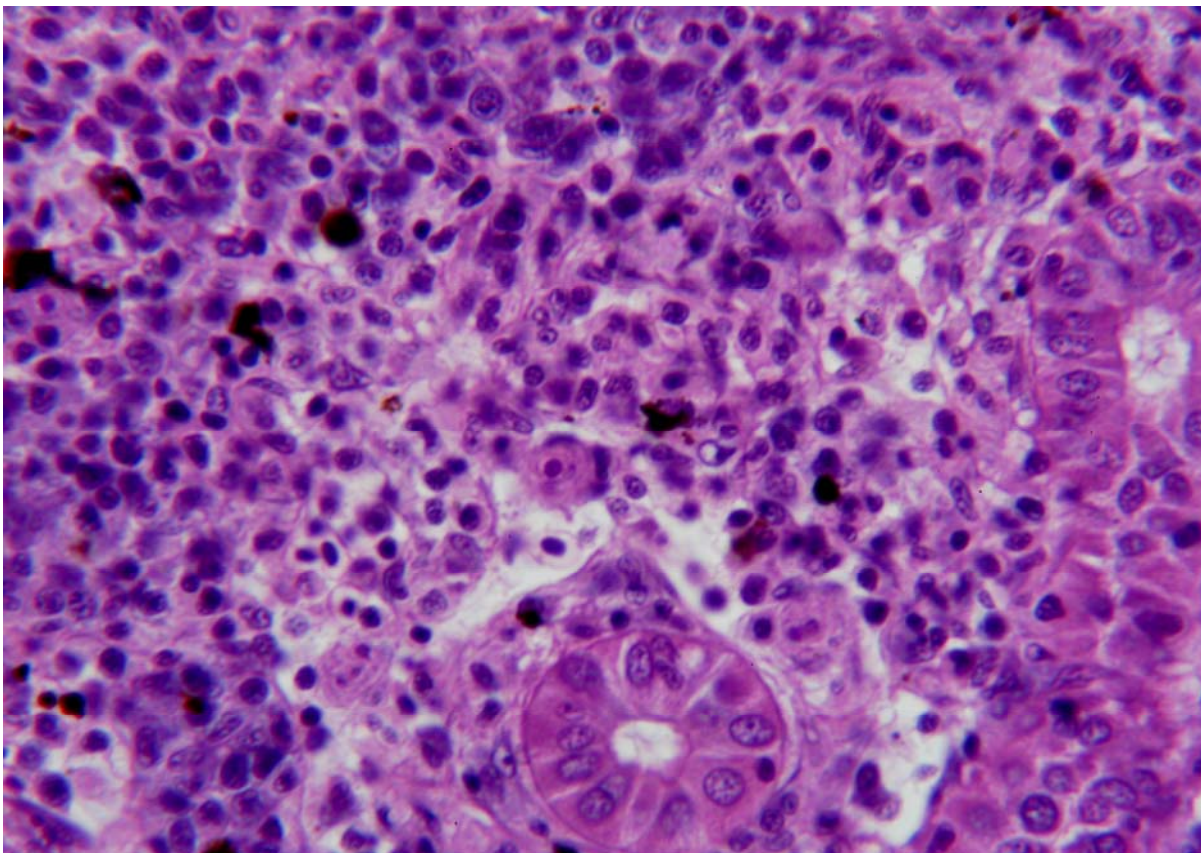
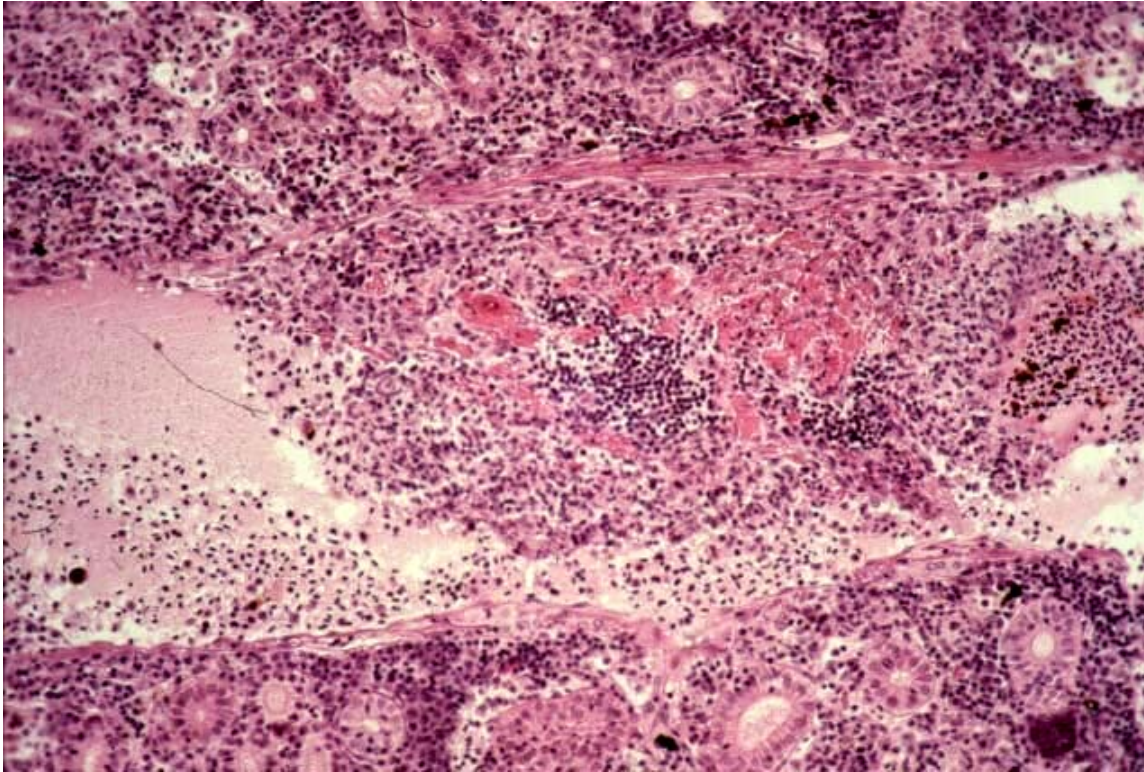
Chapter 6 – Infectious Disease
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Proliferative kidney disease (PKD)



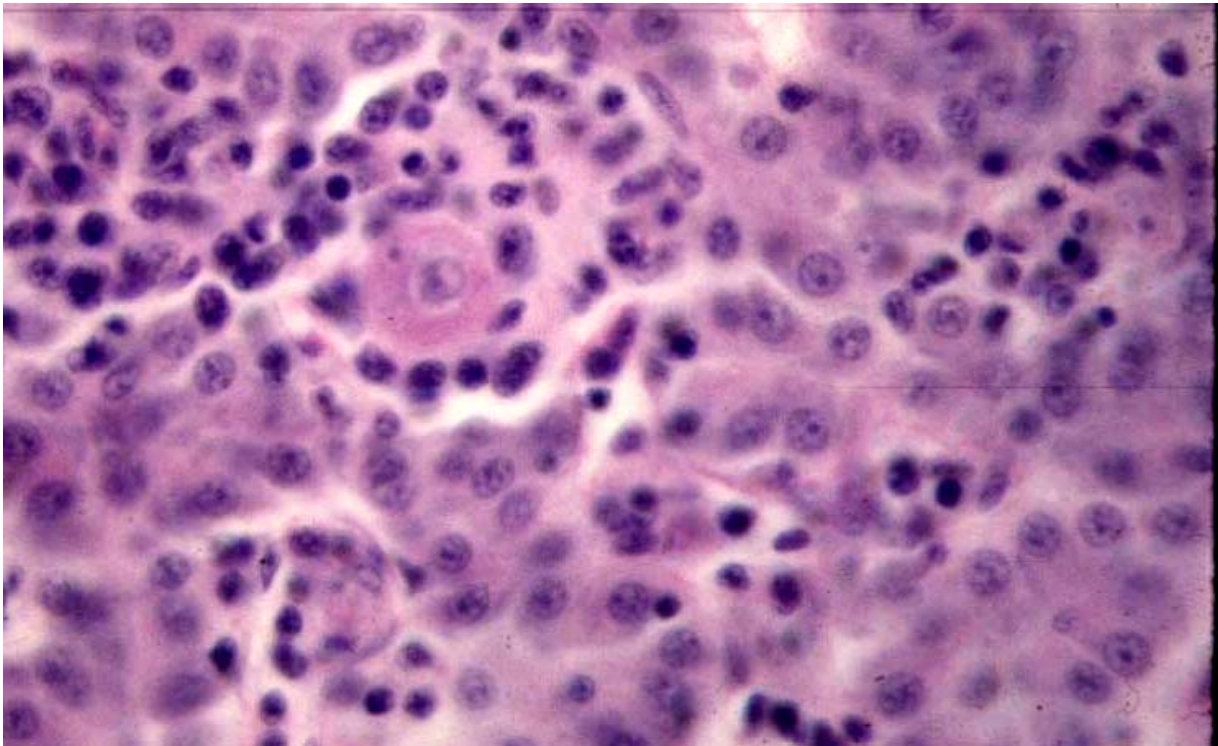
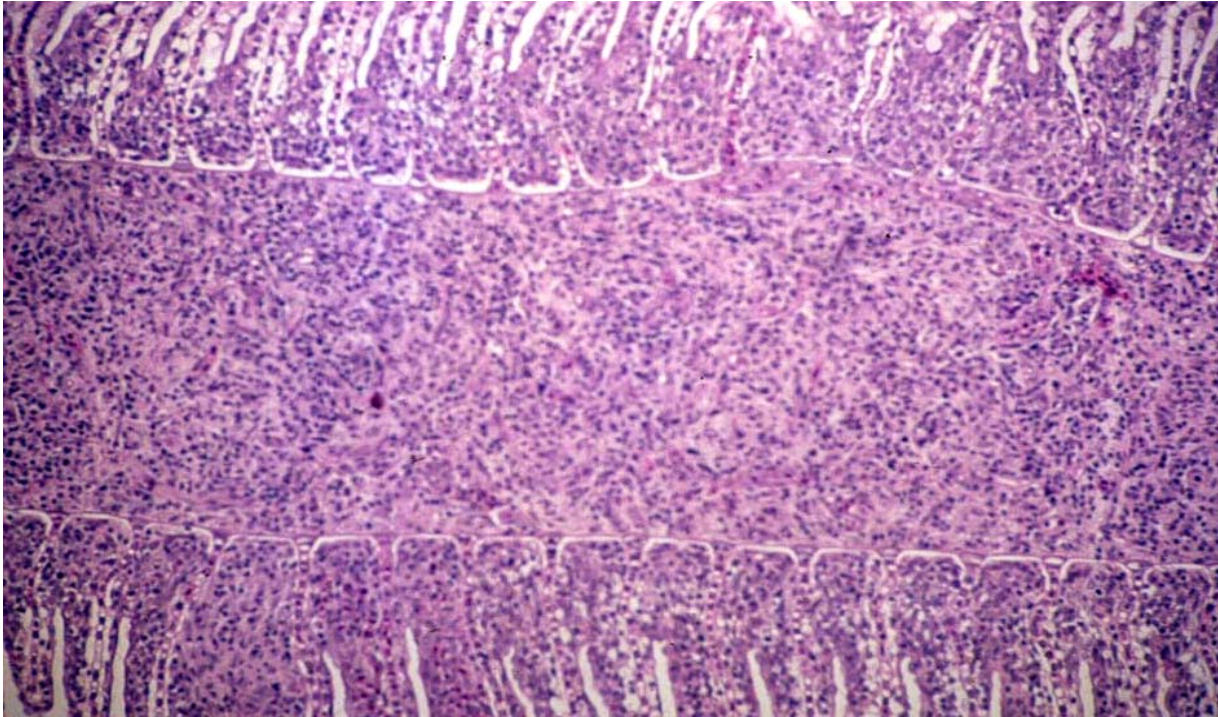
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Proliferative kidney disease (PKD)

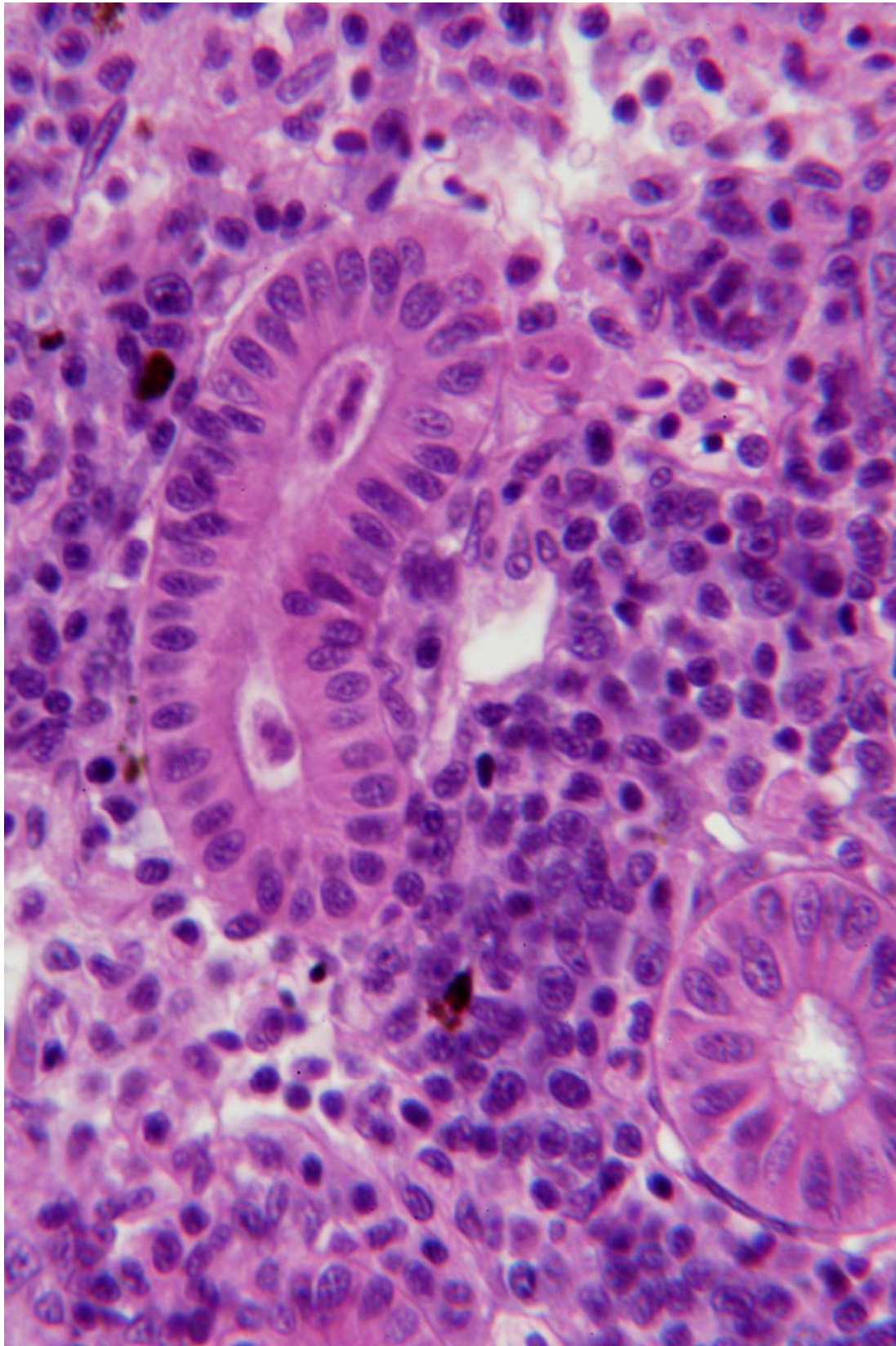


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PKD

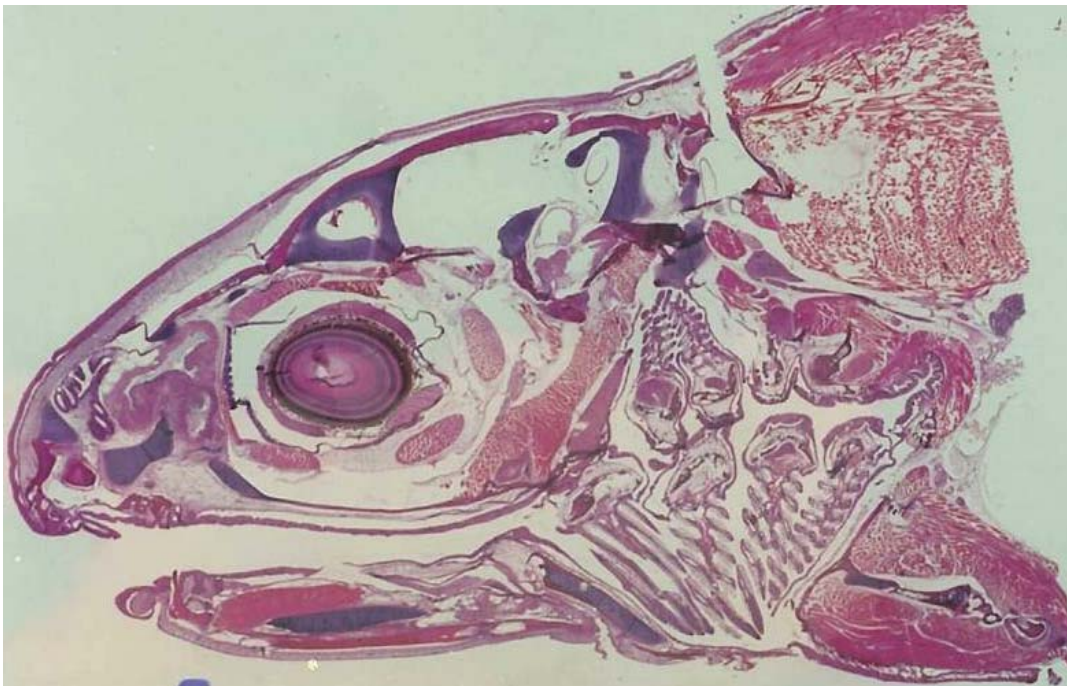


PKD



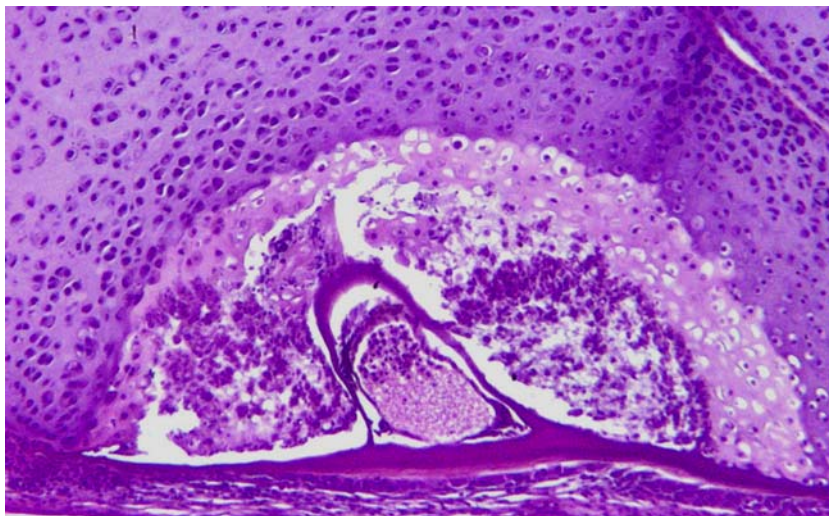
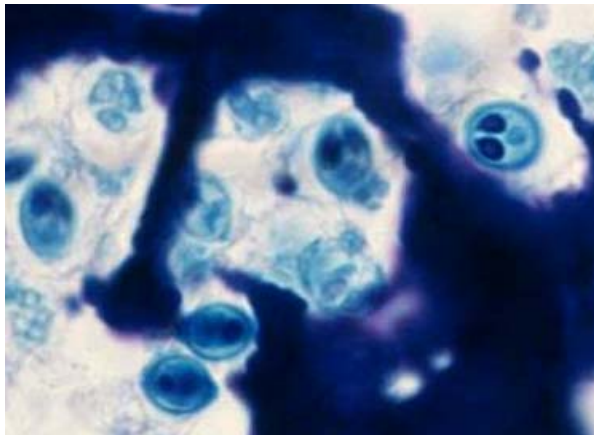
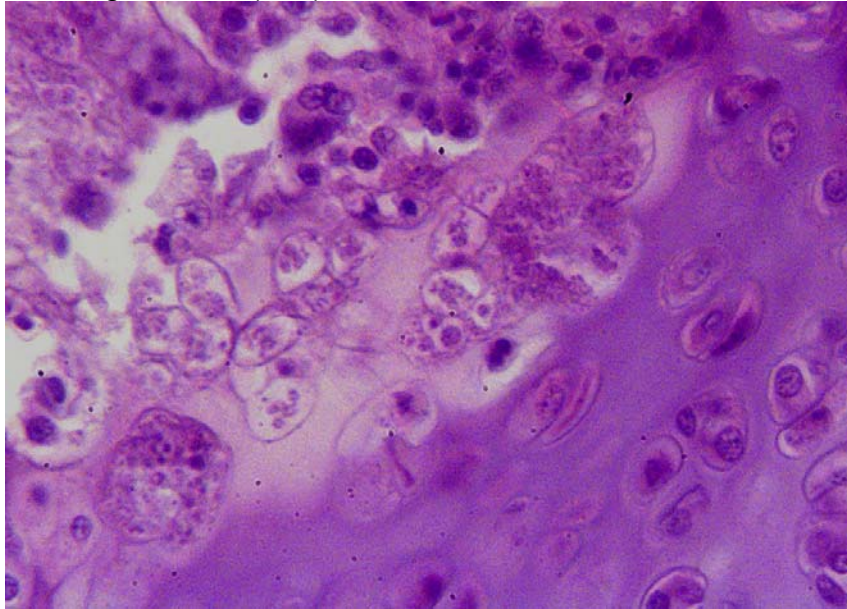
Chapter 6 – Infectious Disease
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(2) Whirling disease (WD) *Myxobolus cerebralis*



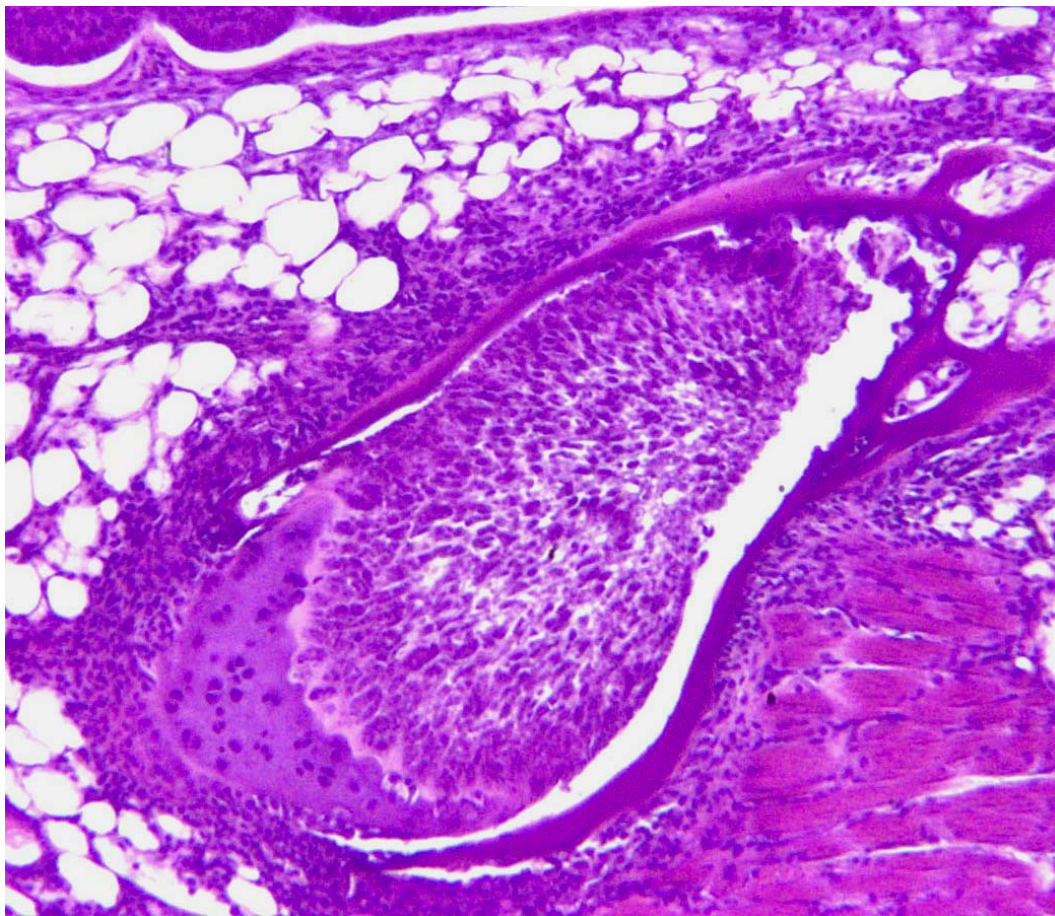
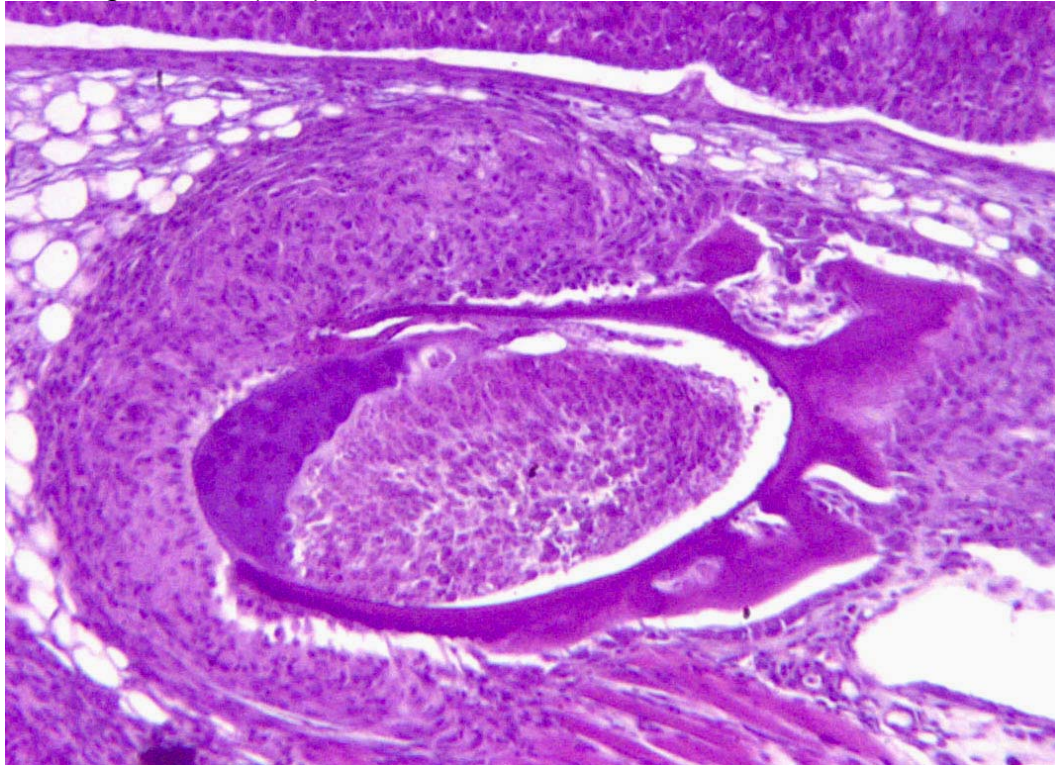
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Fish Histology and Histopathology

Whirling disease (WD)



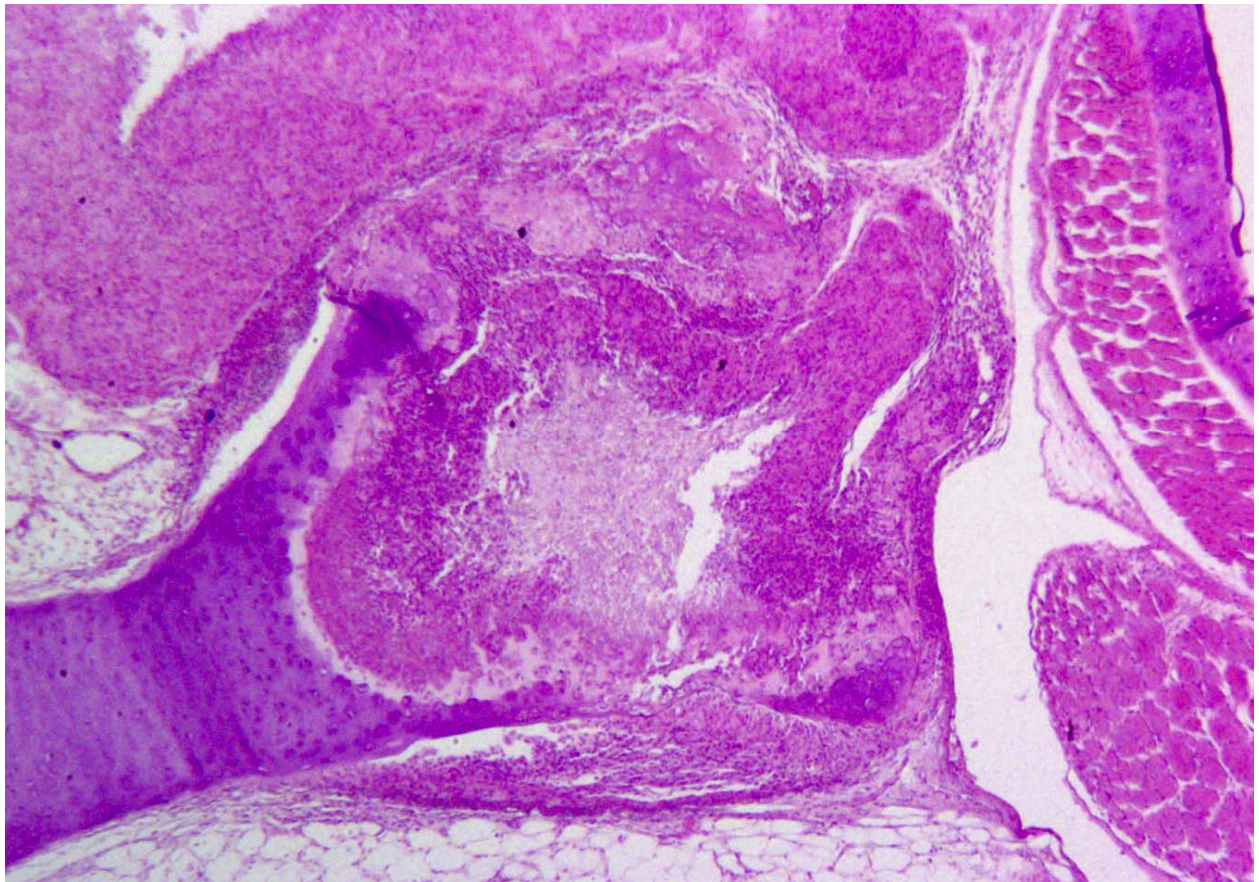
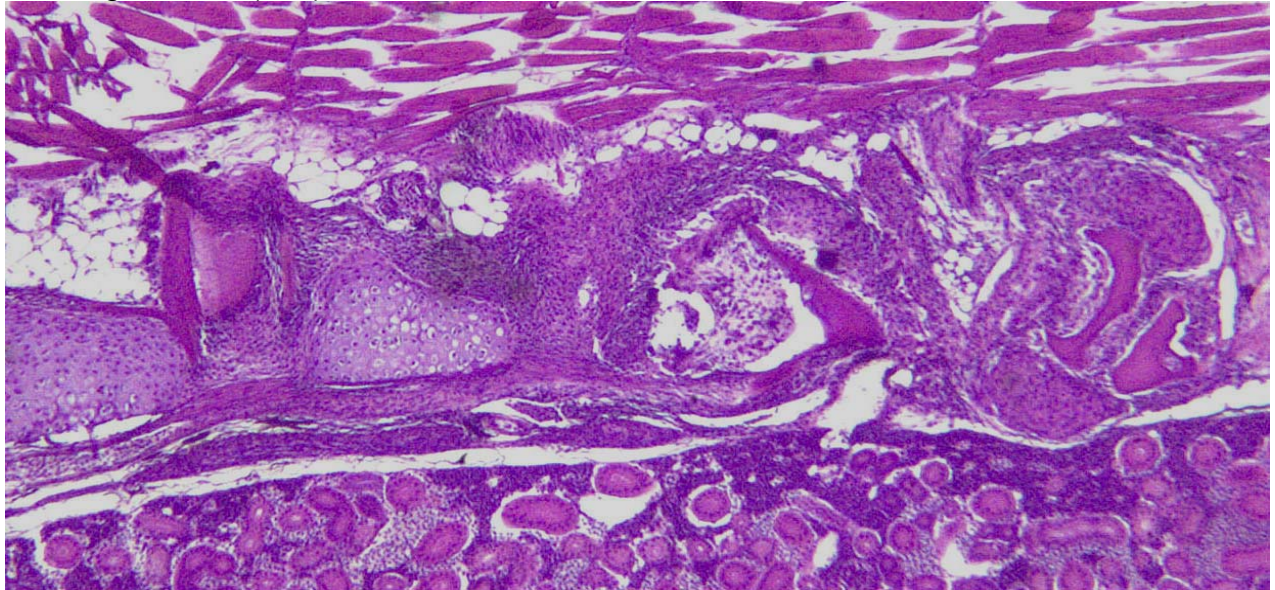
Chapter 6 – Infectious Disease
Fish Histology and Histopathology

Whirling disease (WD)



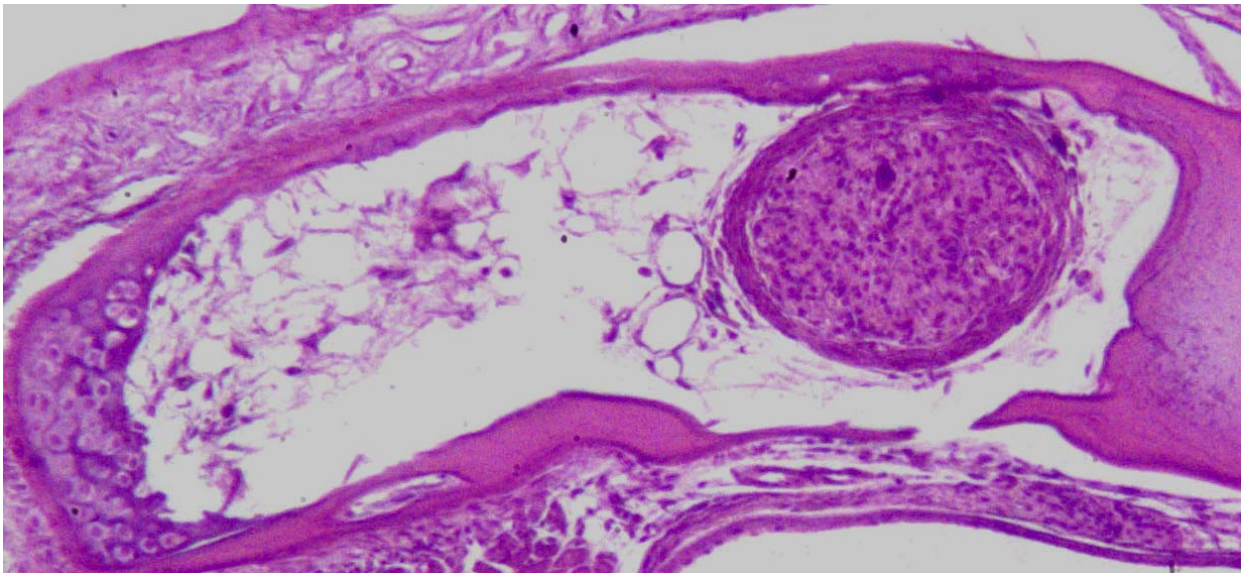
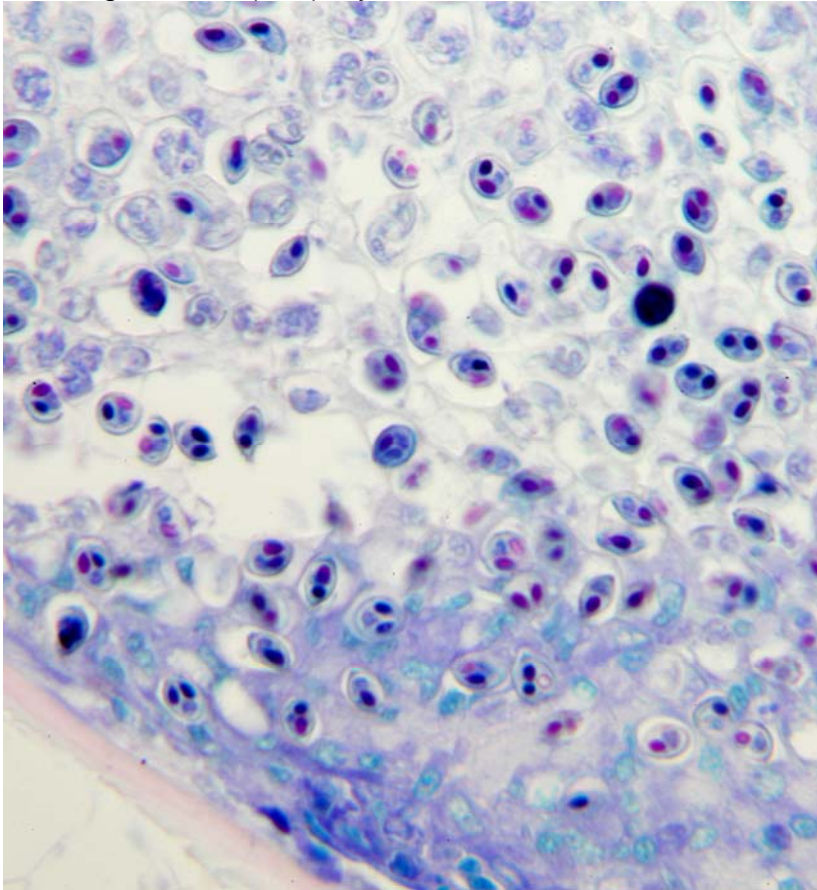
Chapter 6 – Infectious Disease
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Whirling disease (WD)

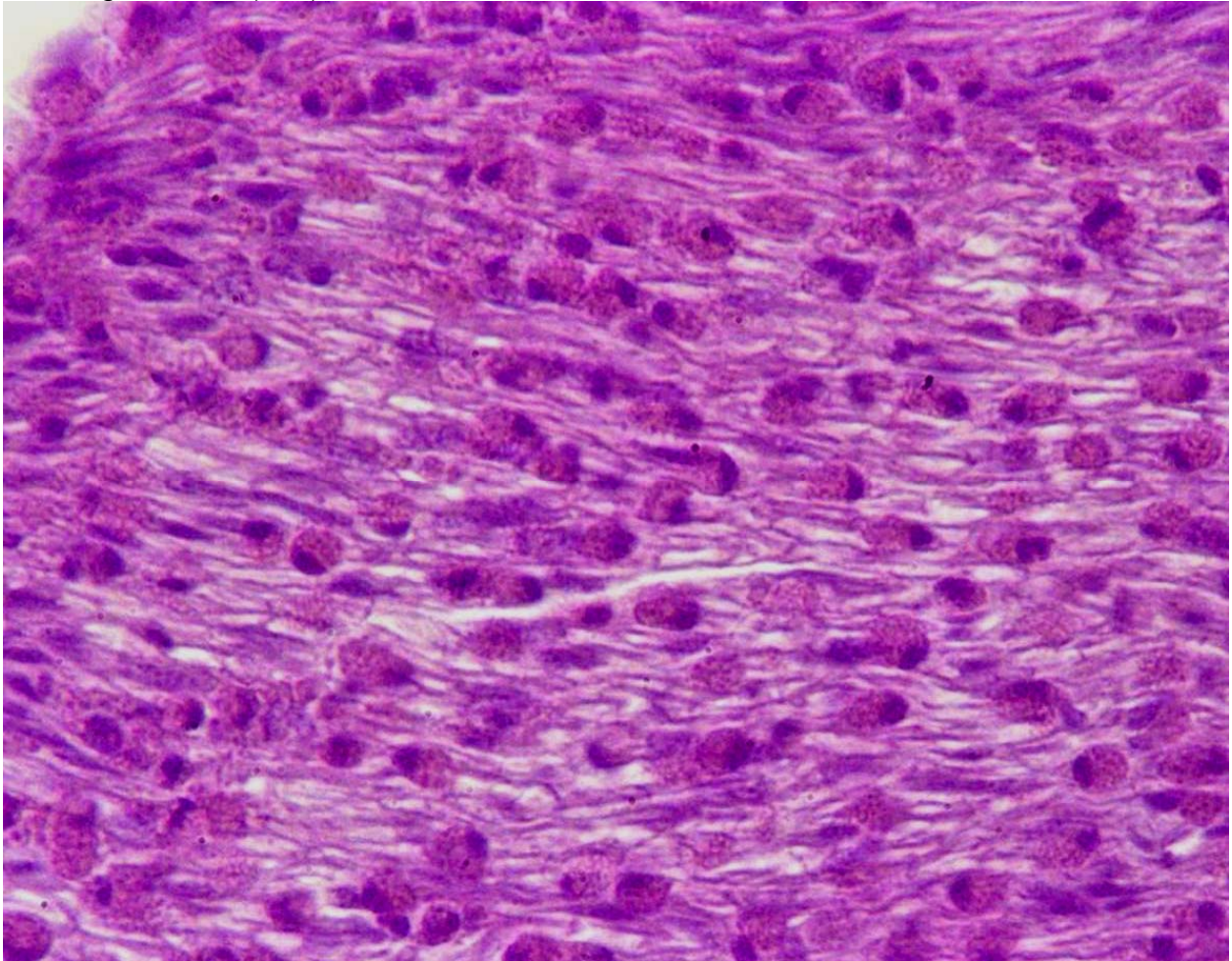


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Whirling disease (WD) *Myxobolus cerebralis*



Whirling Disease (WD)

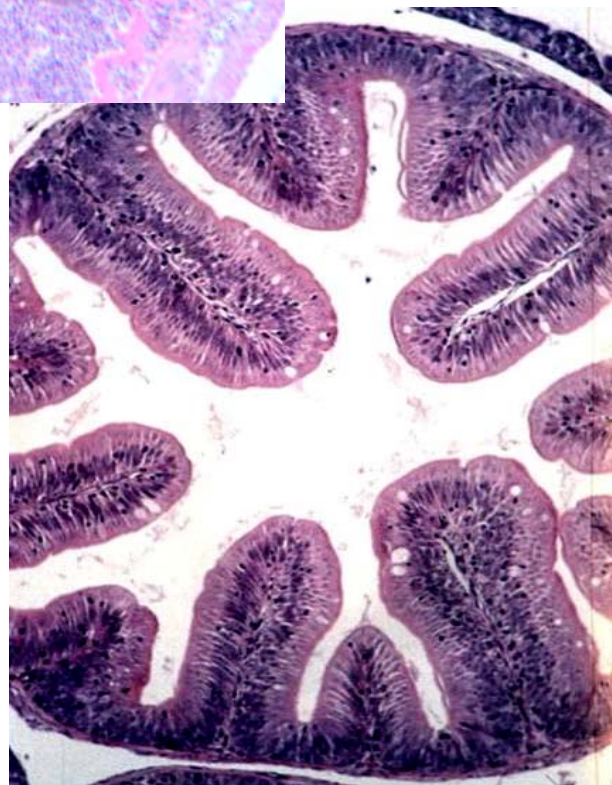
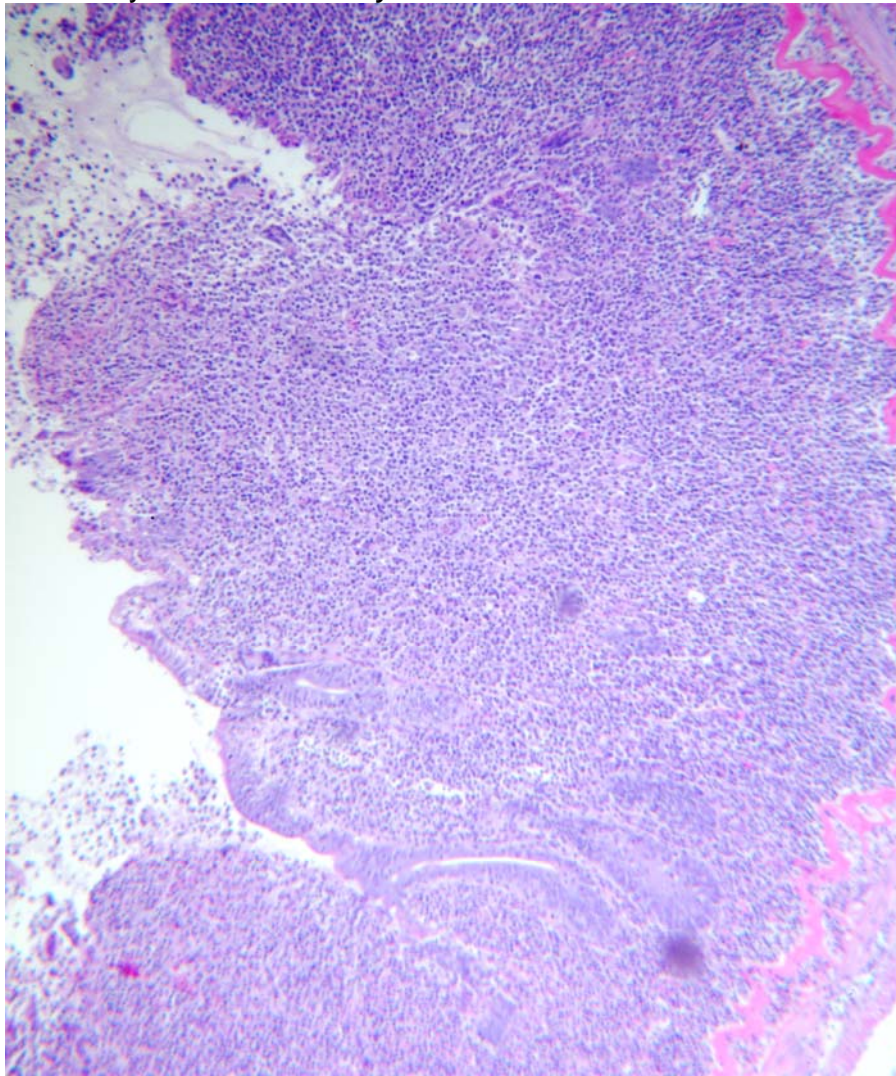


(3) Ceratomyxosis – *Ceratomyxa shasta*



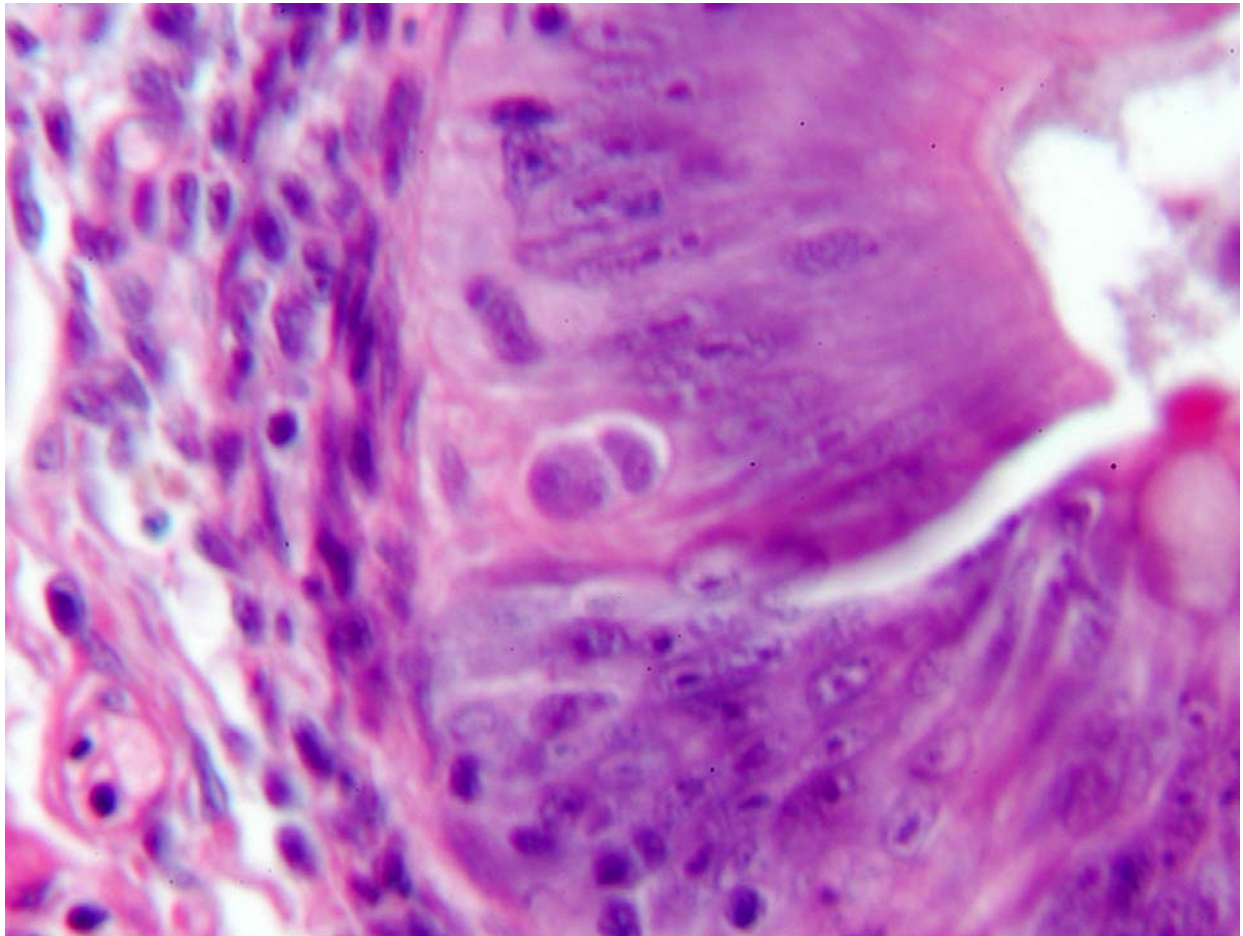
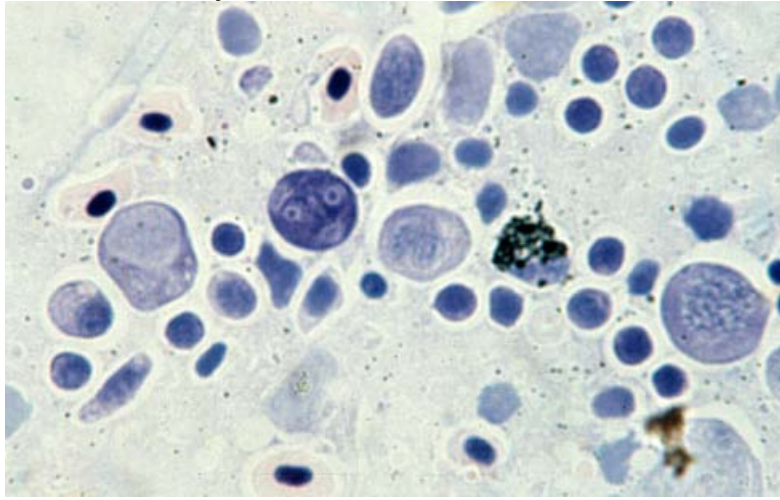
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Ceratomyxosis – *Ceratomyxa Shasta*

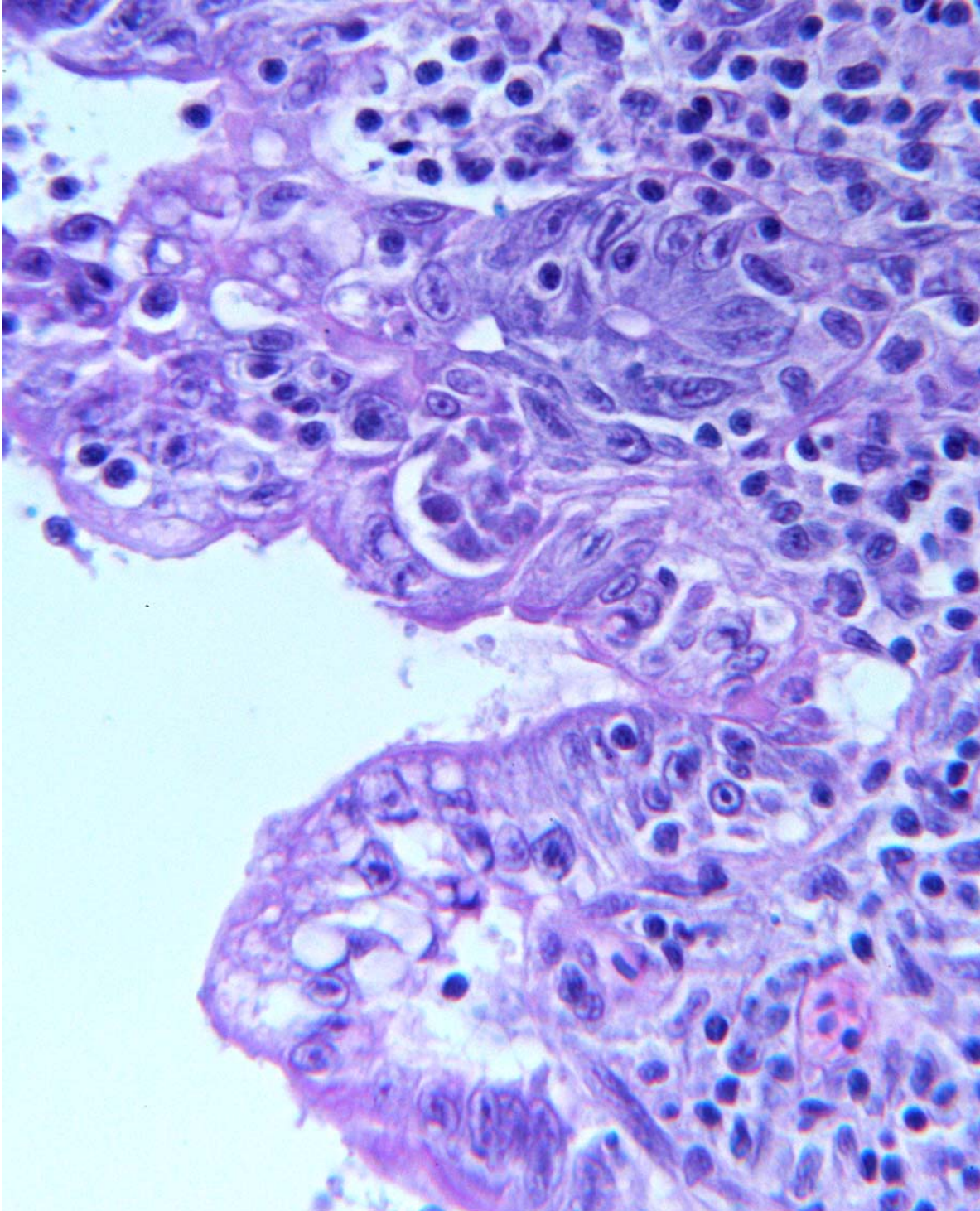


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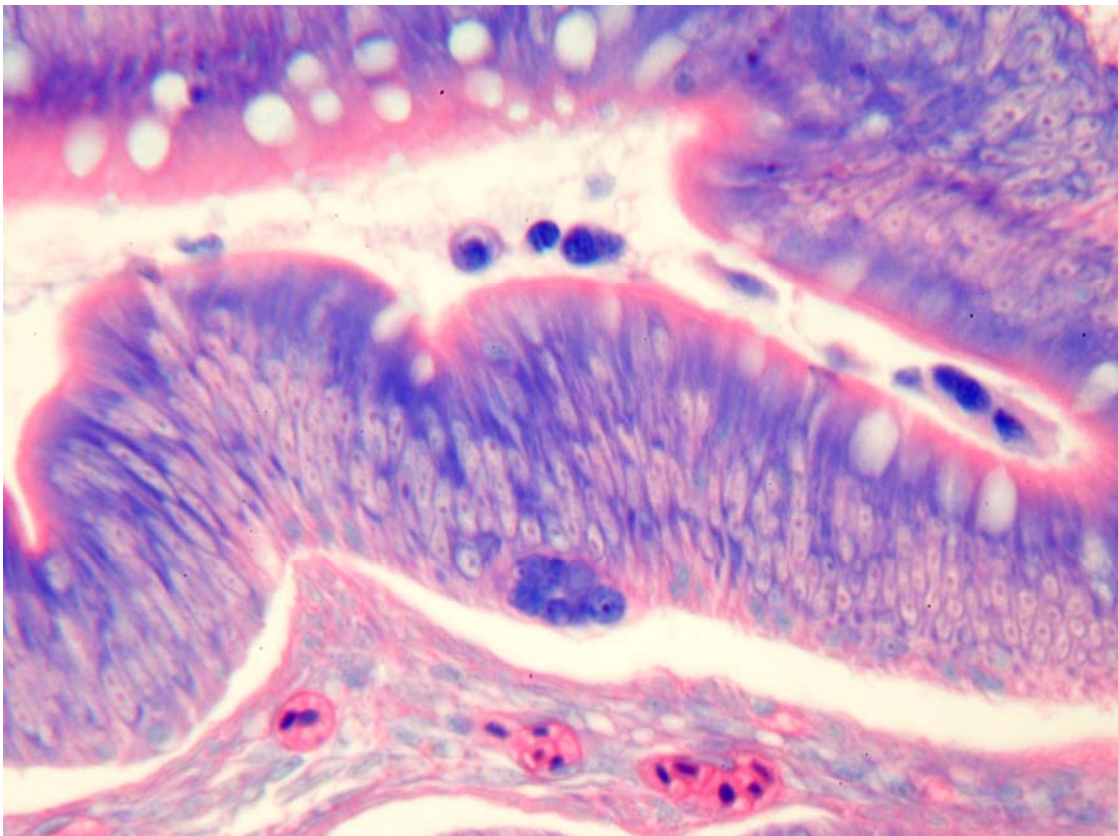
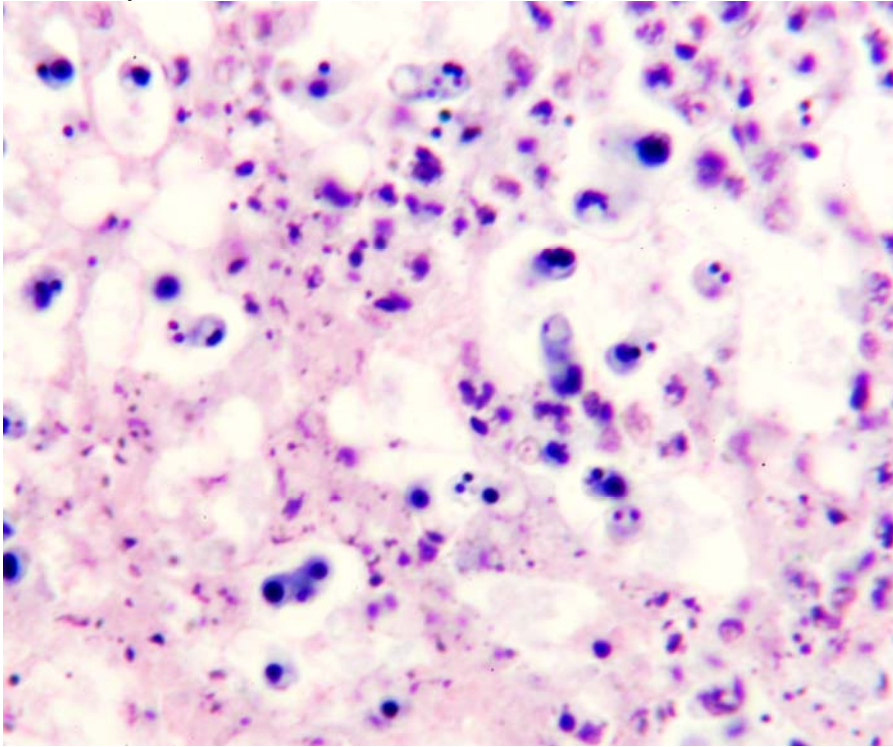
Ceratomyxosis – *Ceratomyxa shasta*



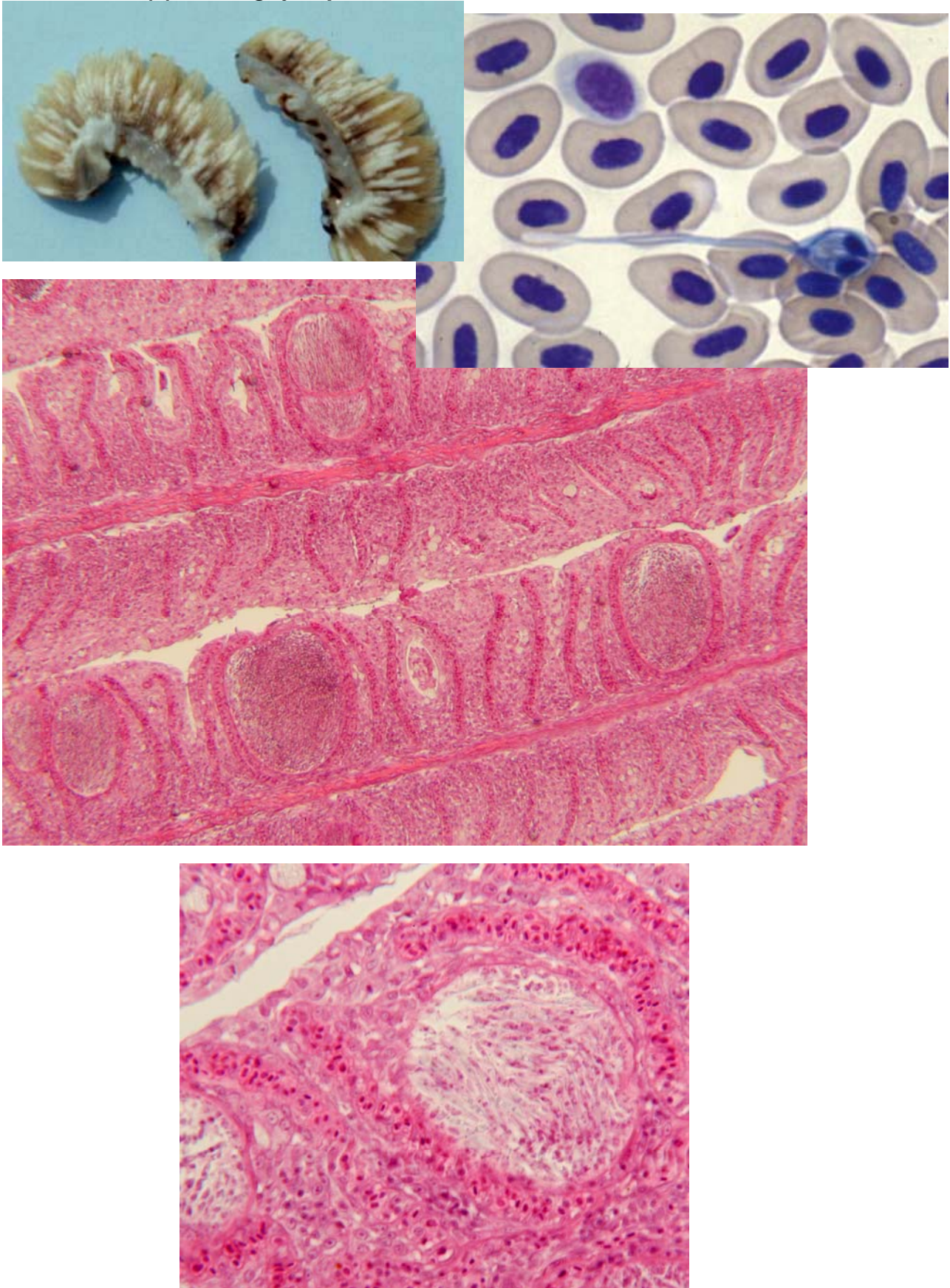
Ceratomyxosis



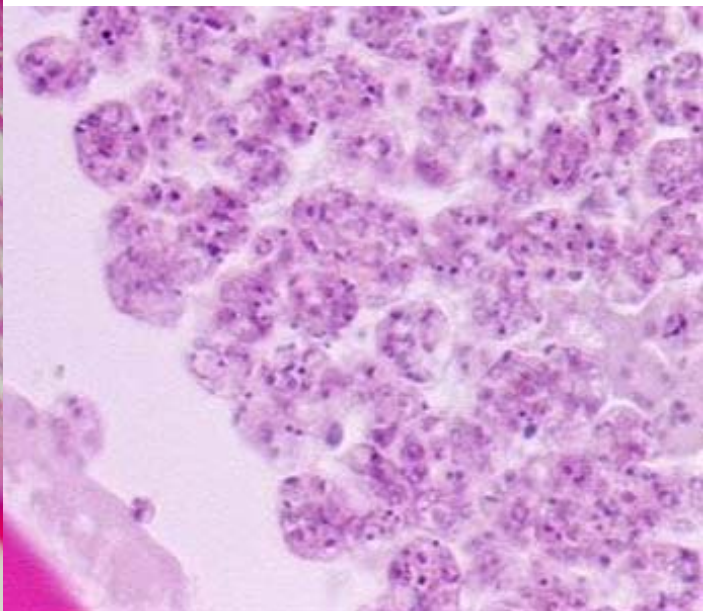
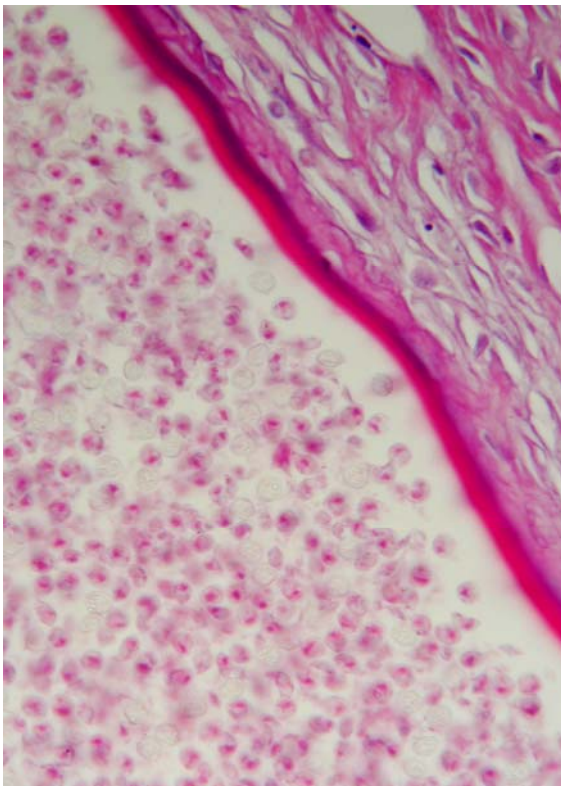
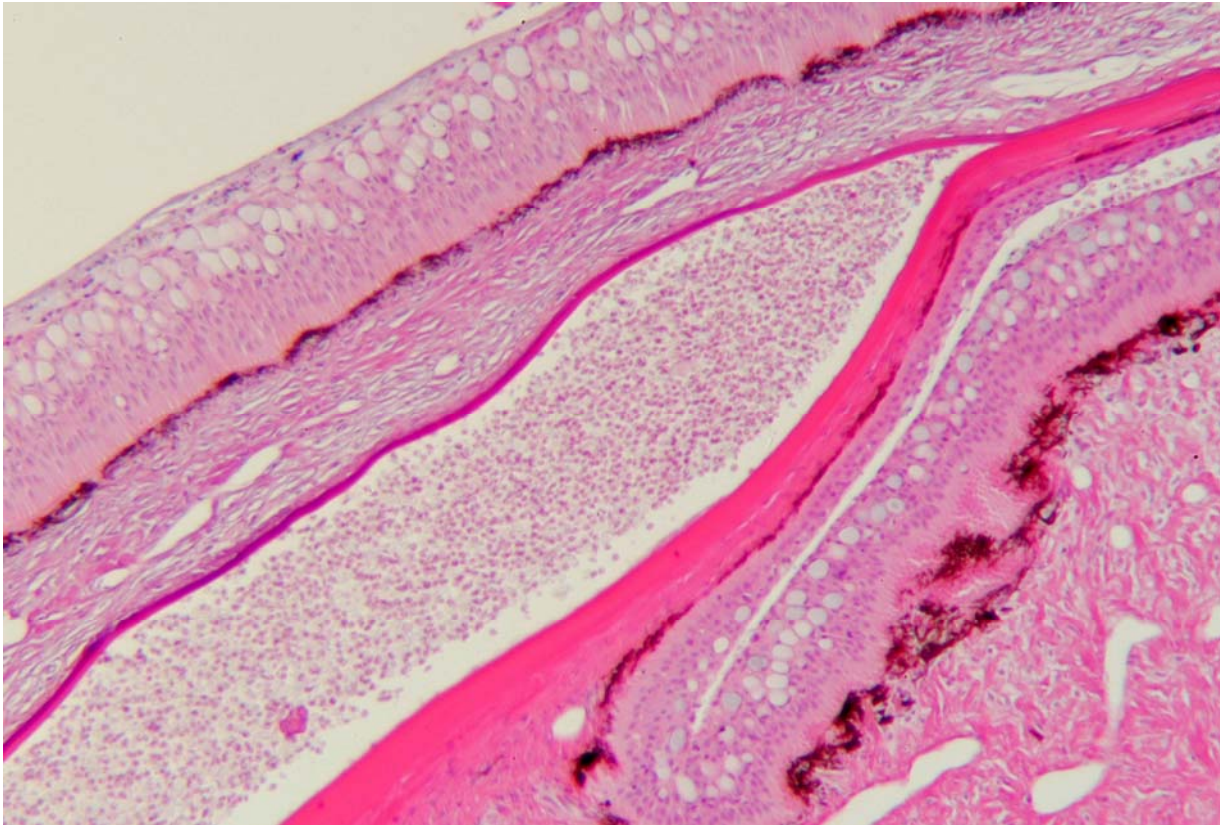
Ceratomyxosis



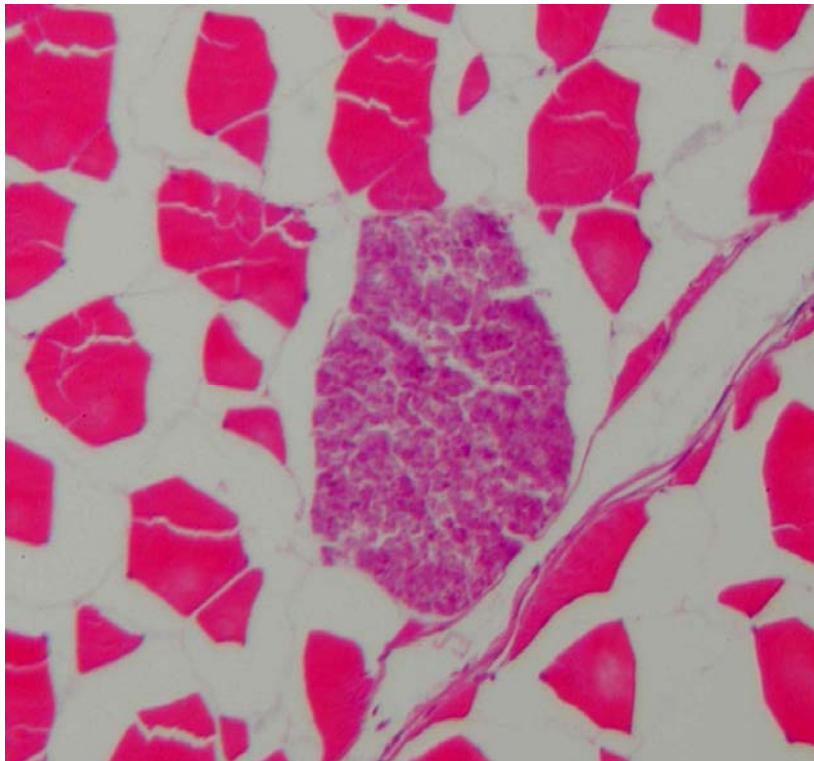
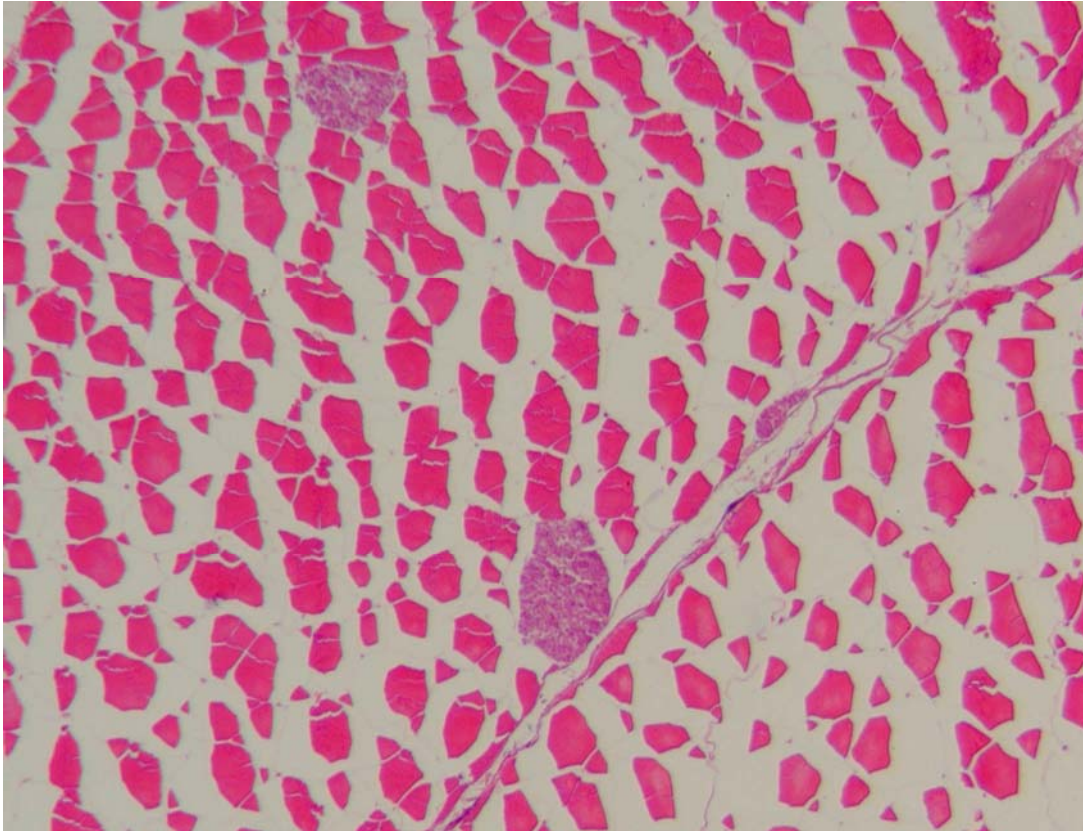
(4) *Henneguya* sp.



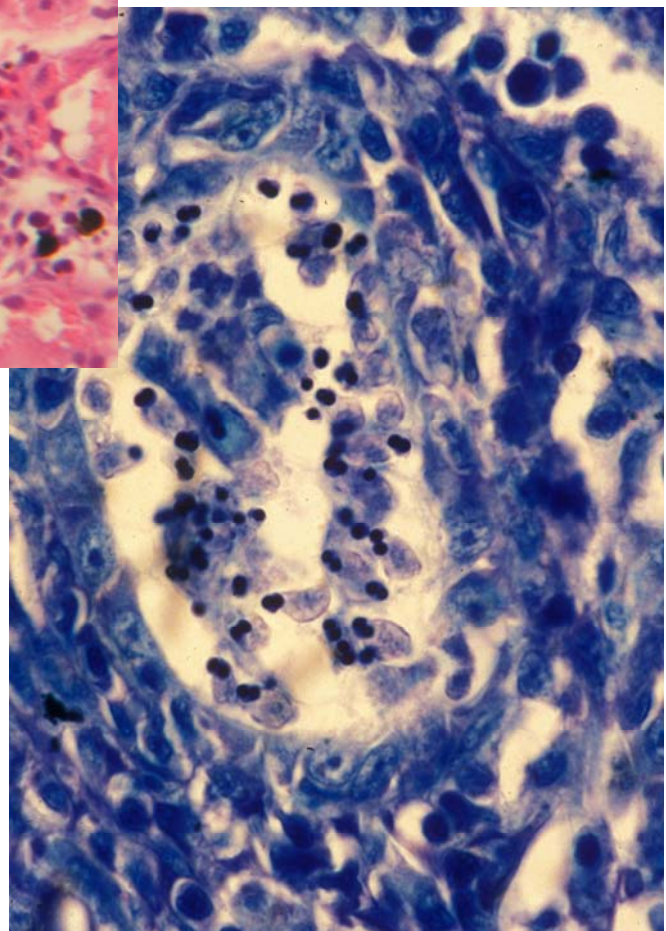
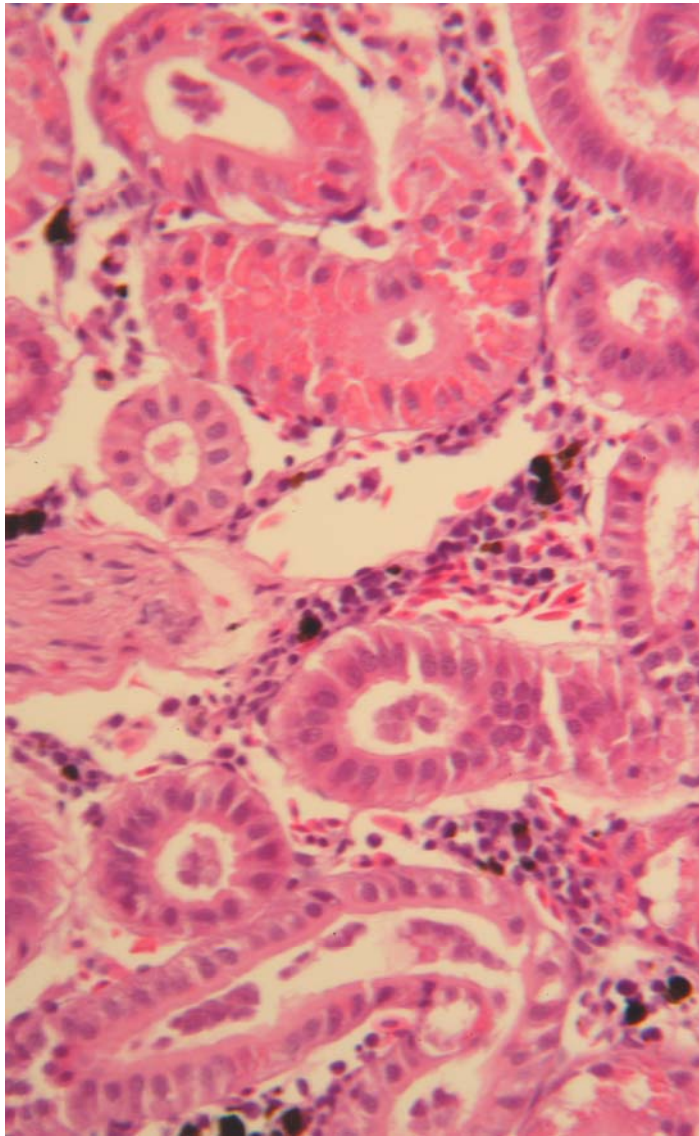
(5) *Myxobolus squamalis*



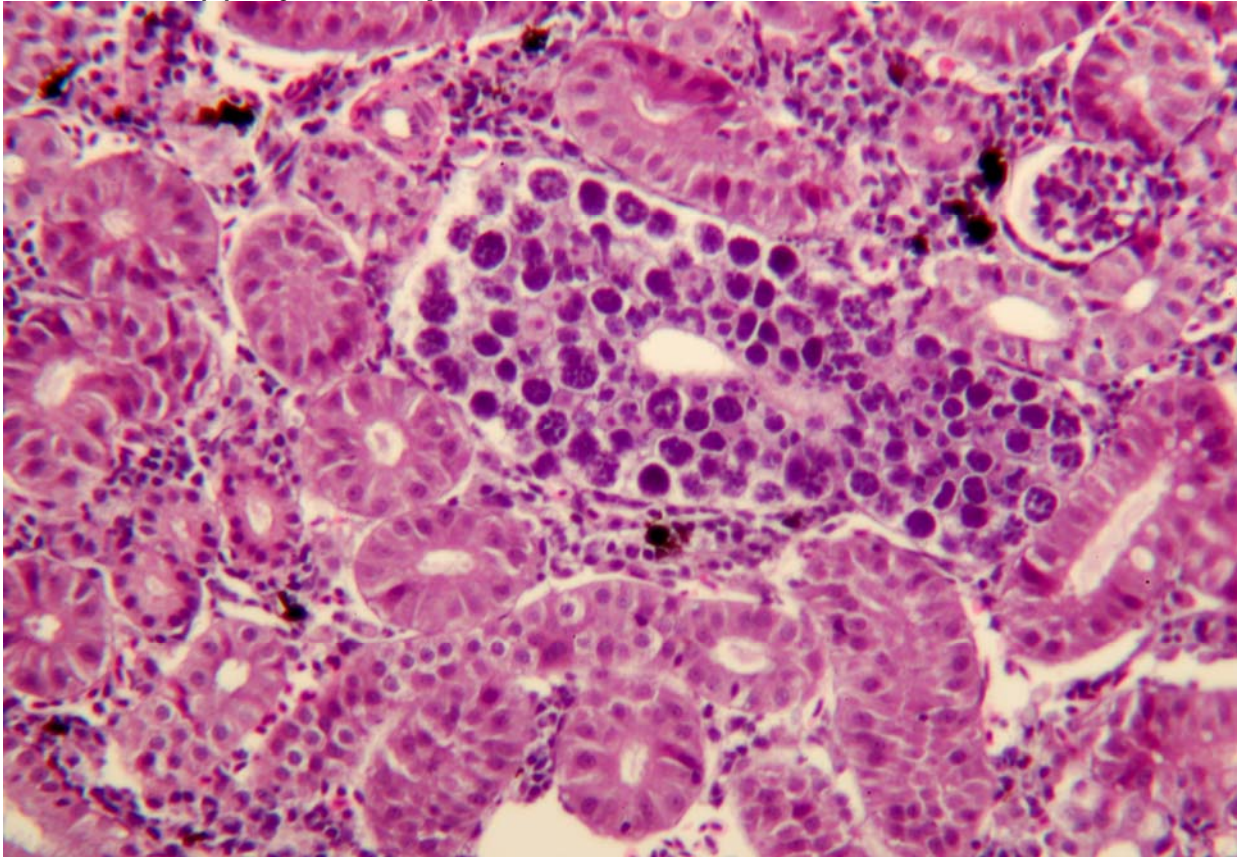
(6) *Kudoa* sp.



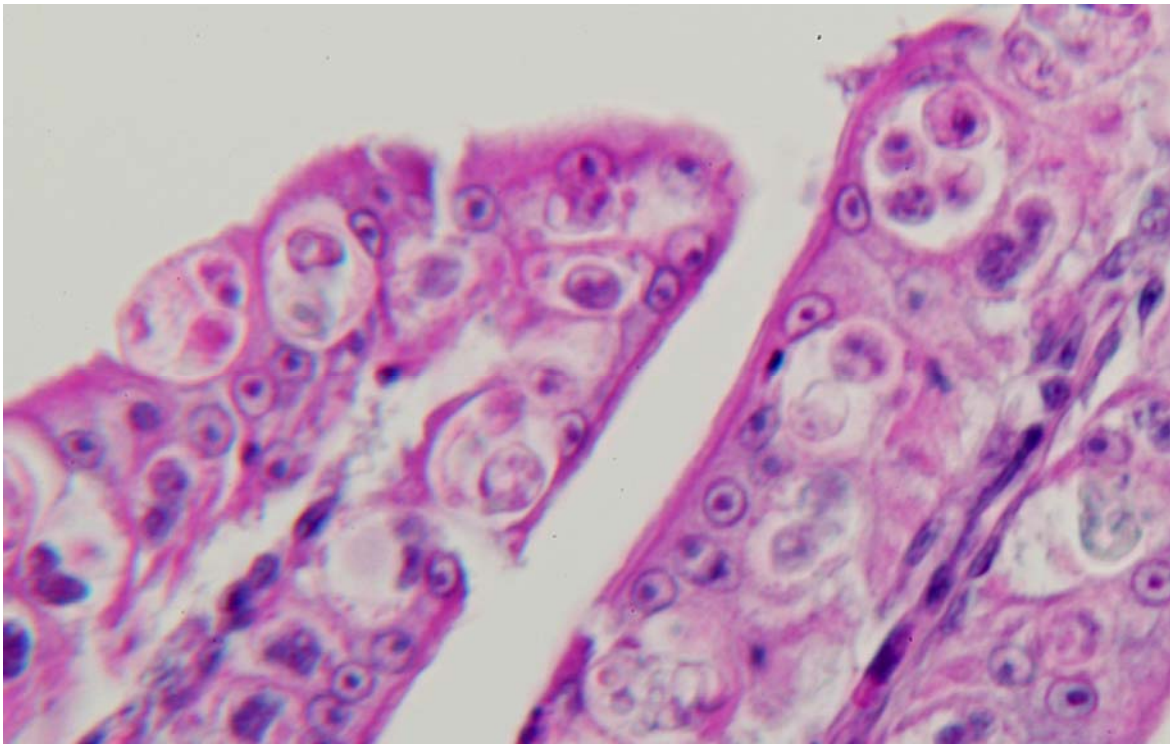
(7) *Parvicapsula* sp.



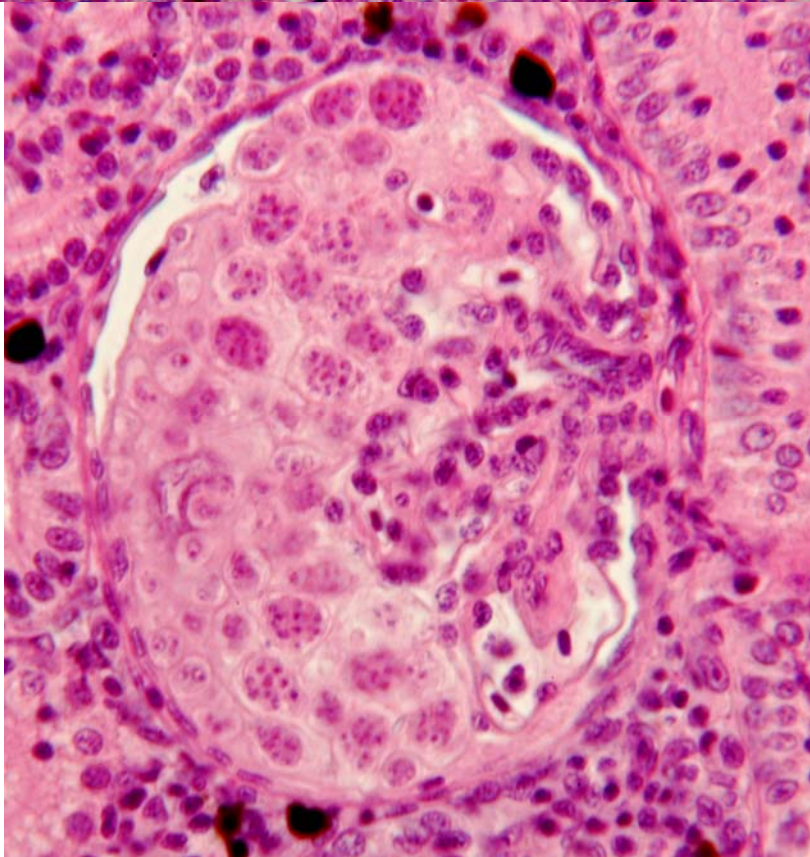
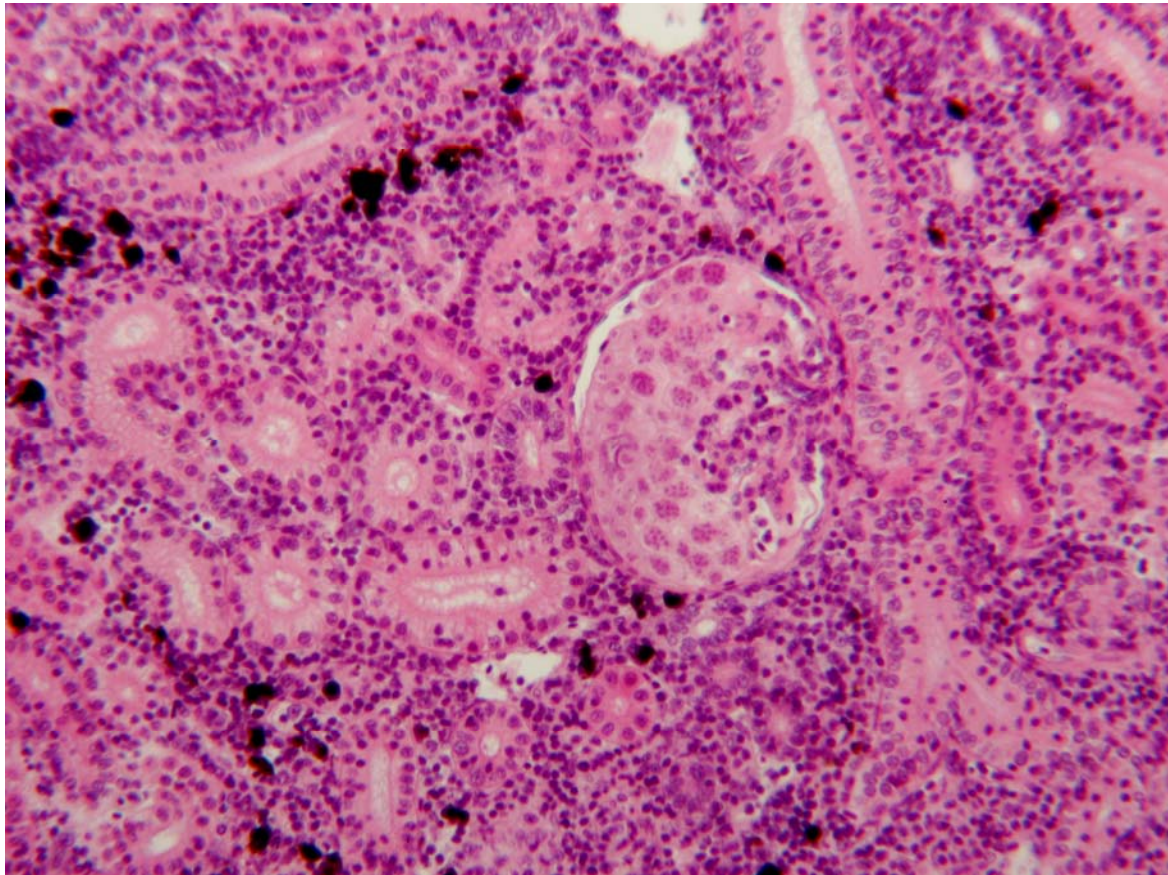
(8) *Myxidium* sp.



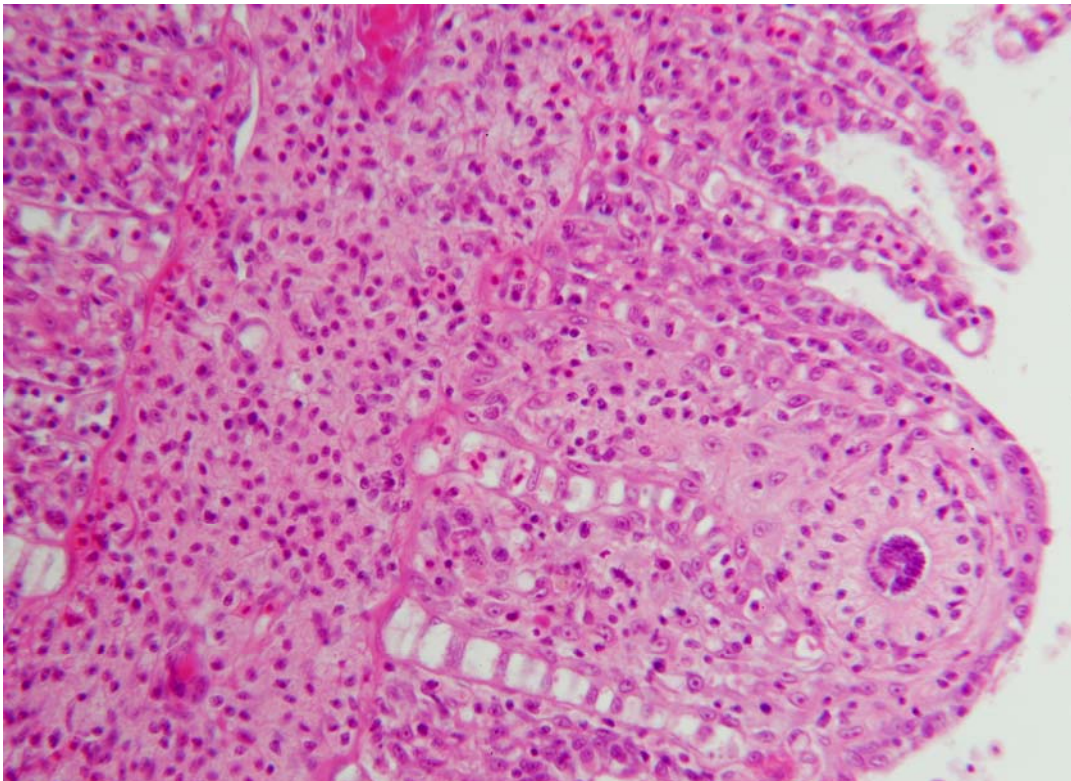
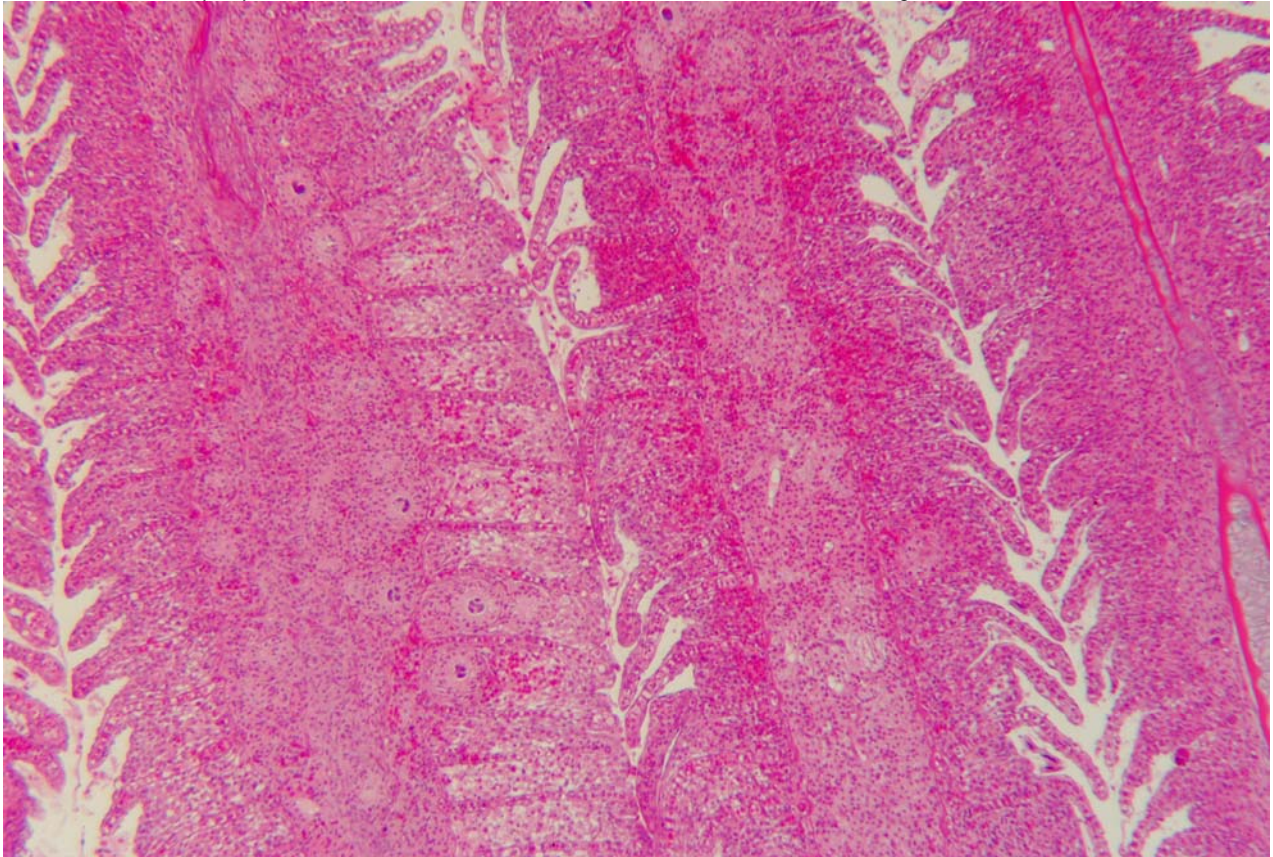
Myxidium lei



(9) *Chloromyxum* sp.

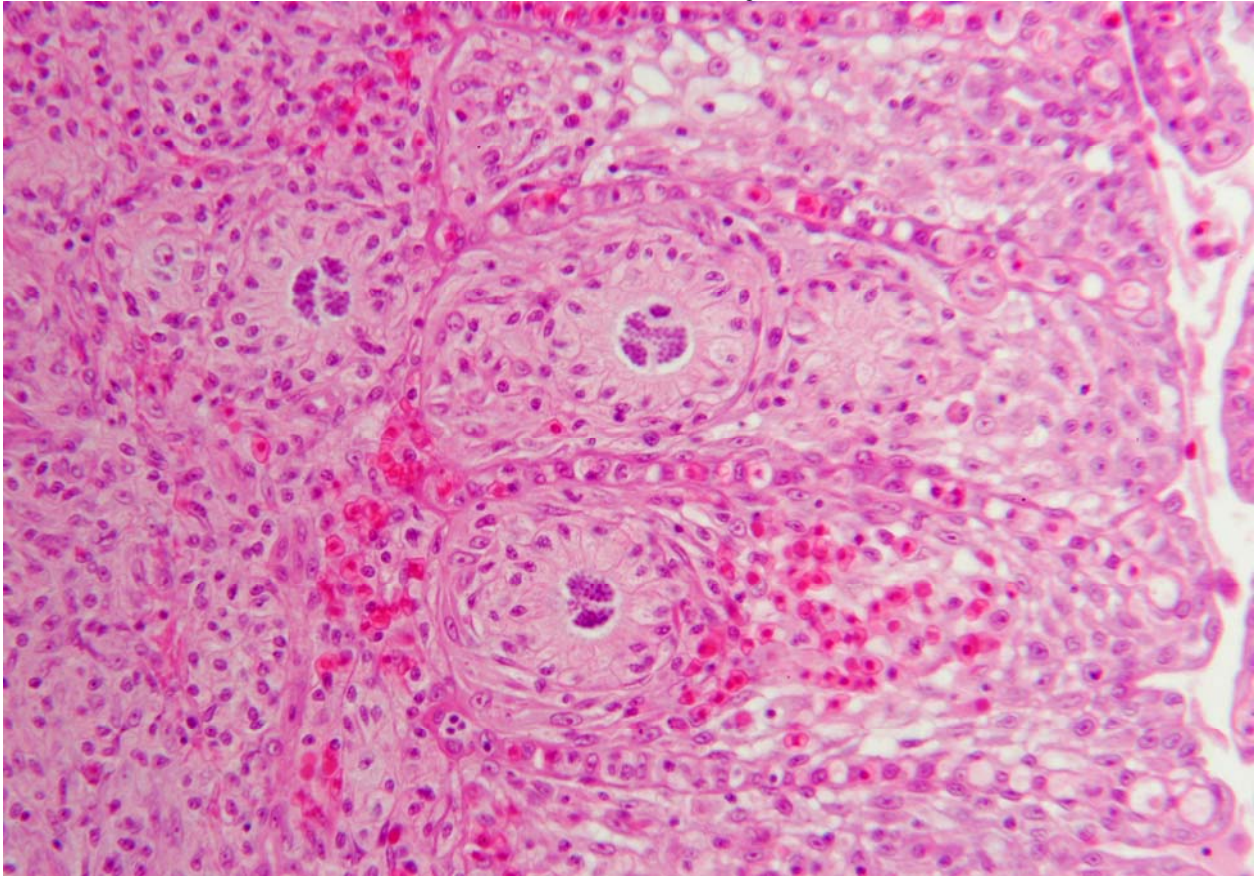


(10) Proliferative Gill Disease - *Aurantiactinomyxon ictaluri*



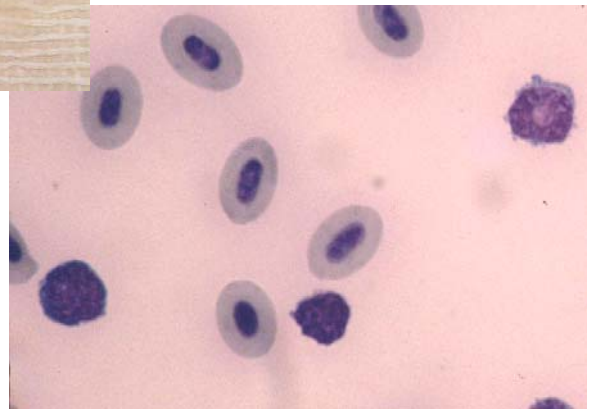
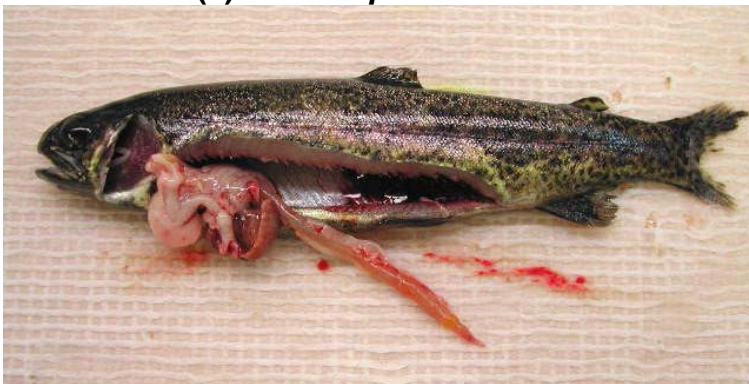
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▪ Proliferative Gill Disease – *Aurantiactinomyxon ictaluri*



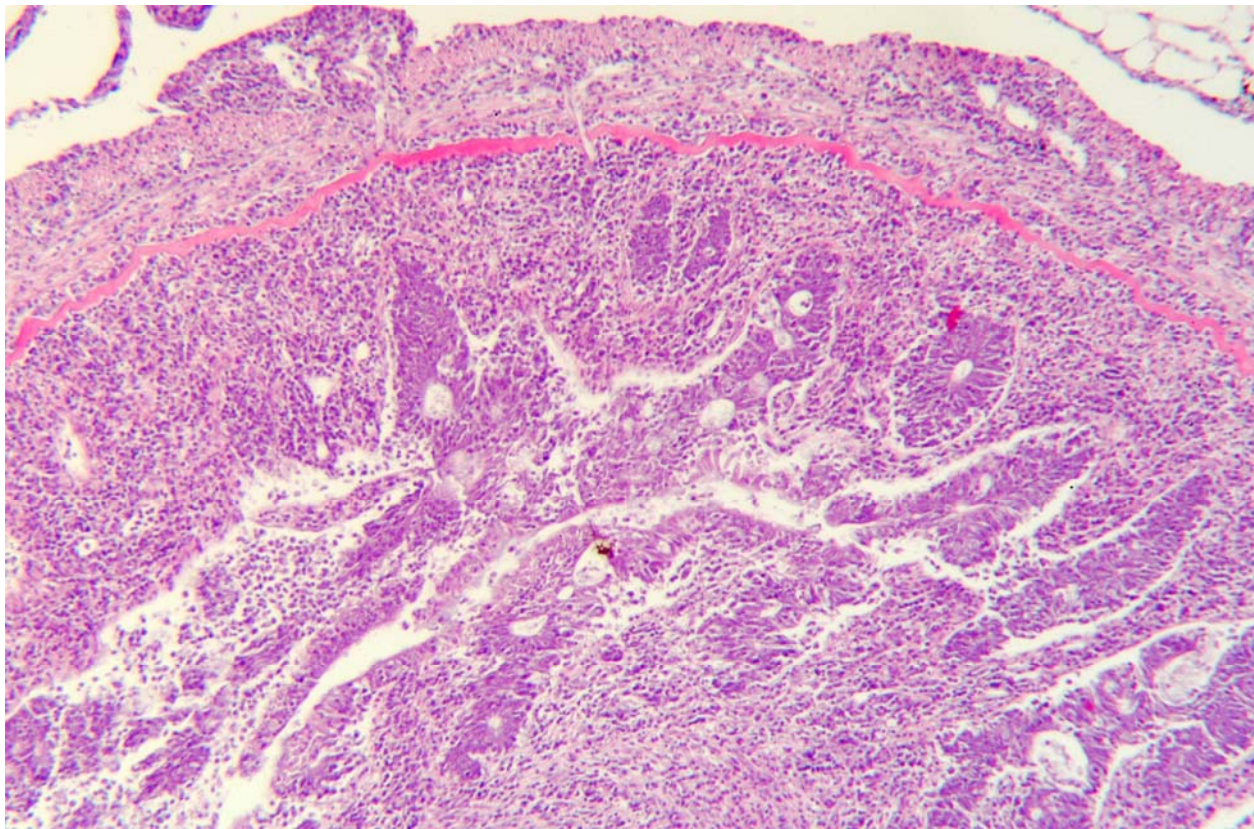
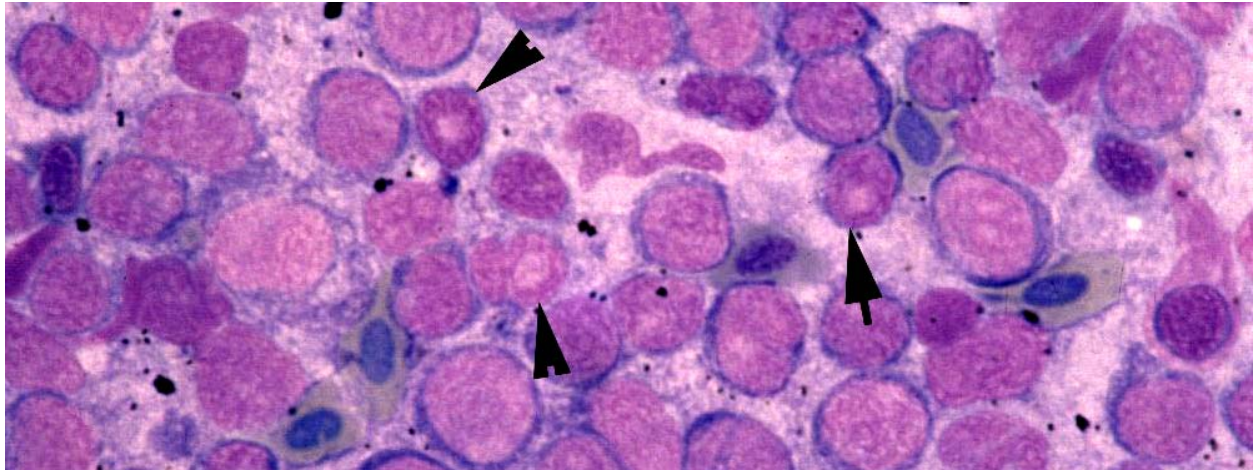
b. Microsporidian

(1) *Nucleospora salmonis*



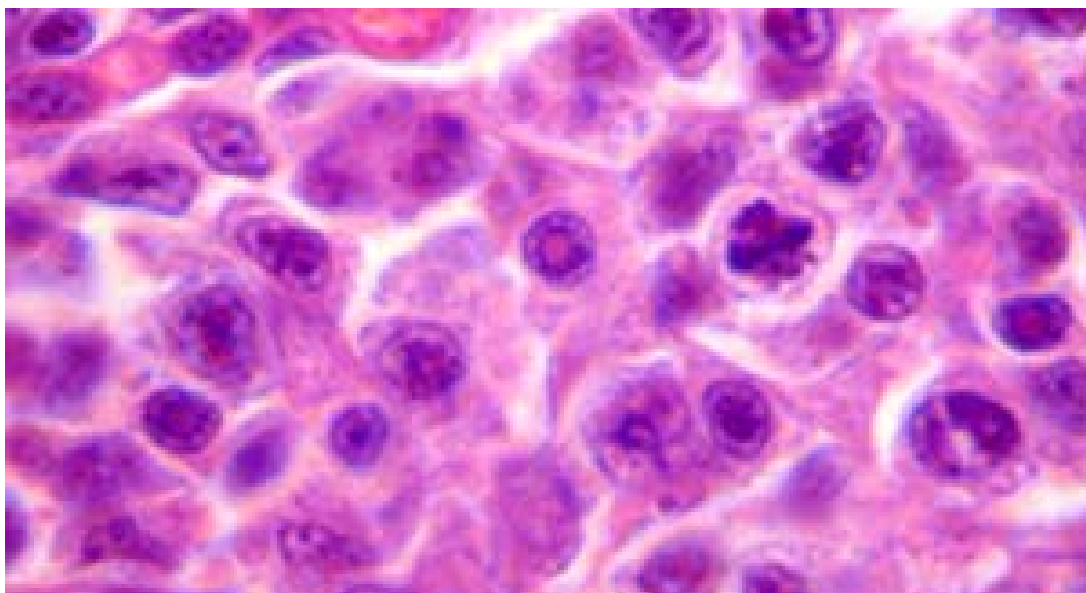
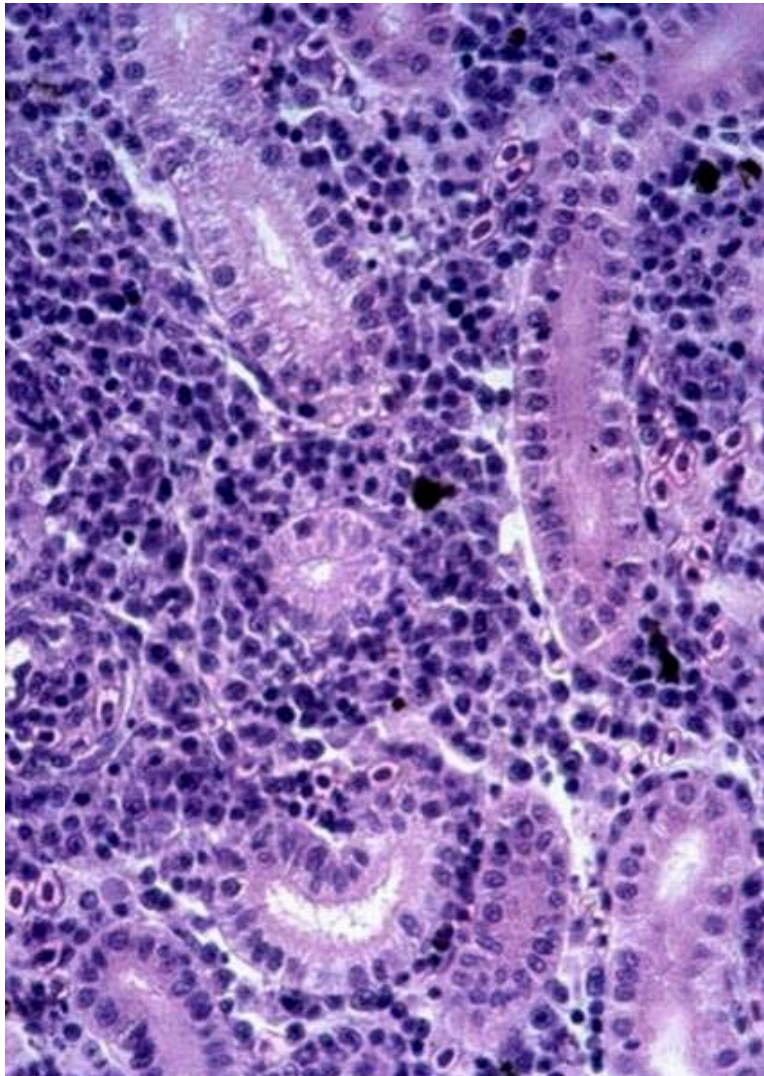
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Nucleospora salmonis

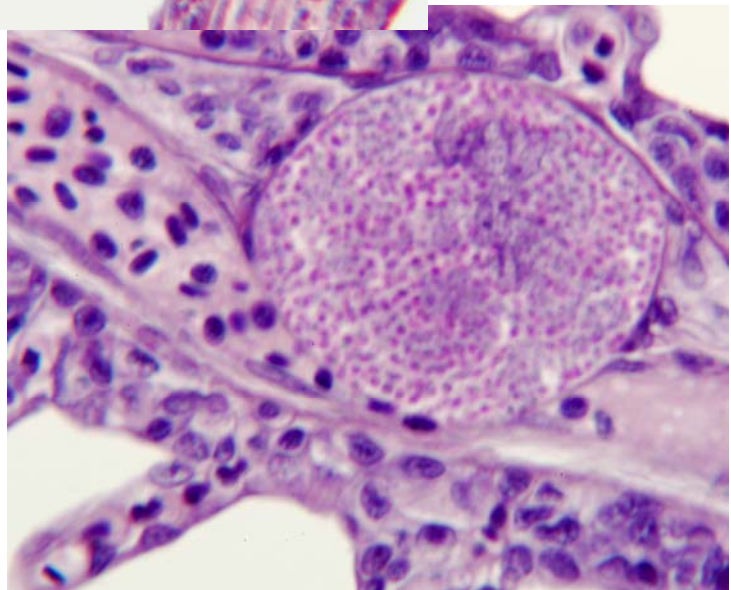
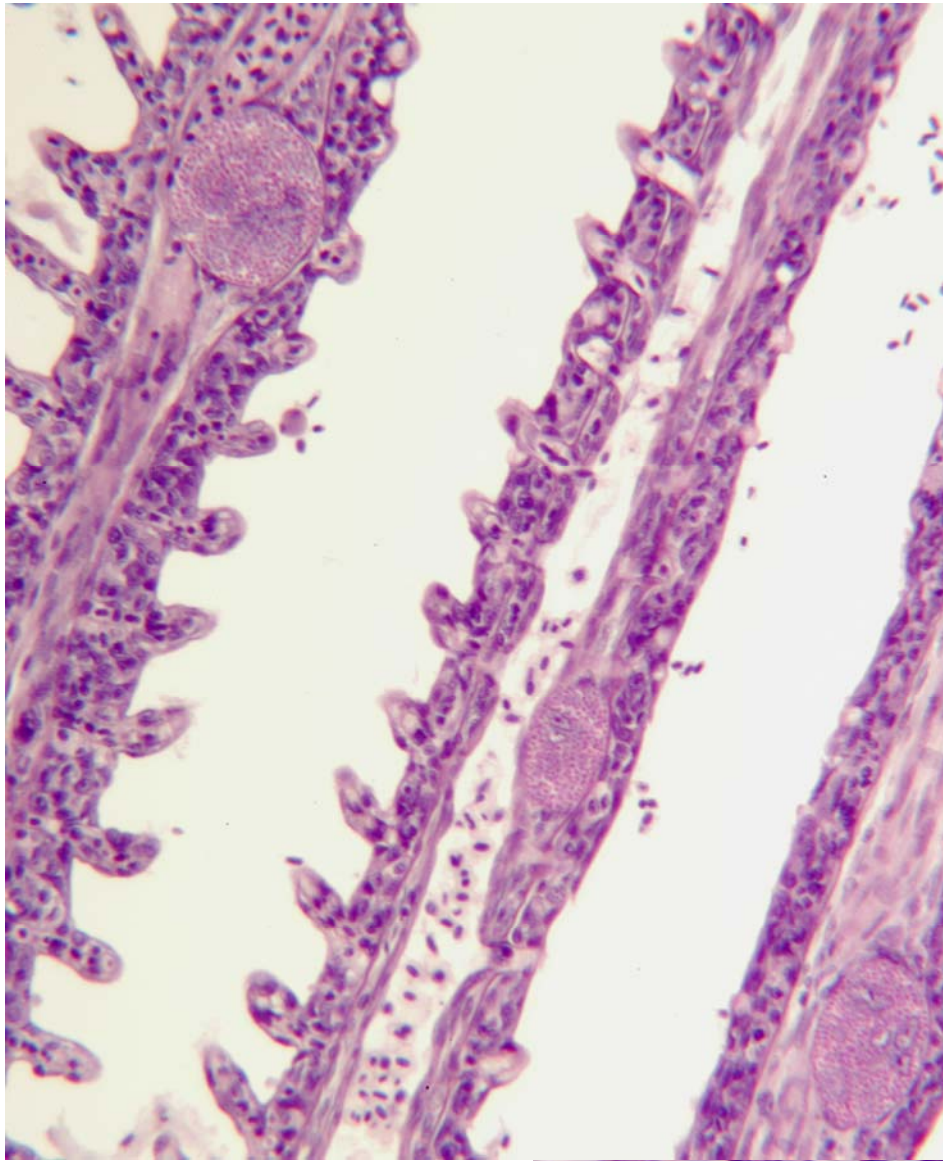


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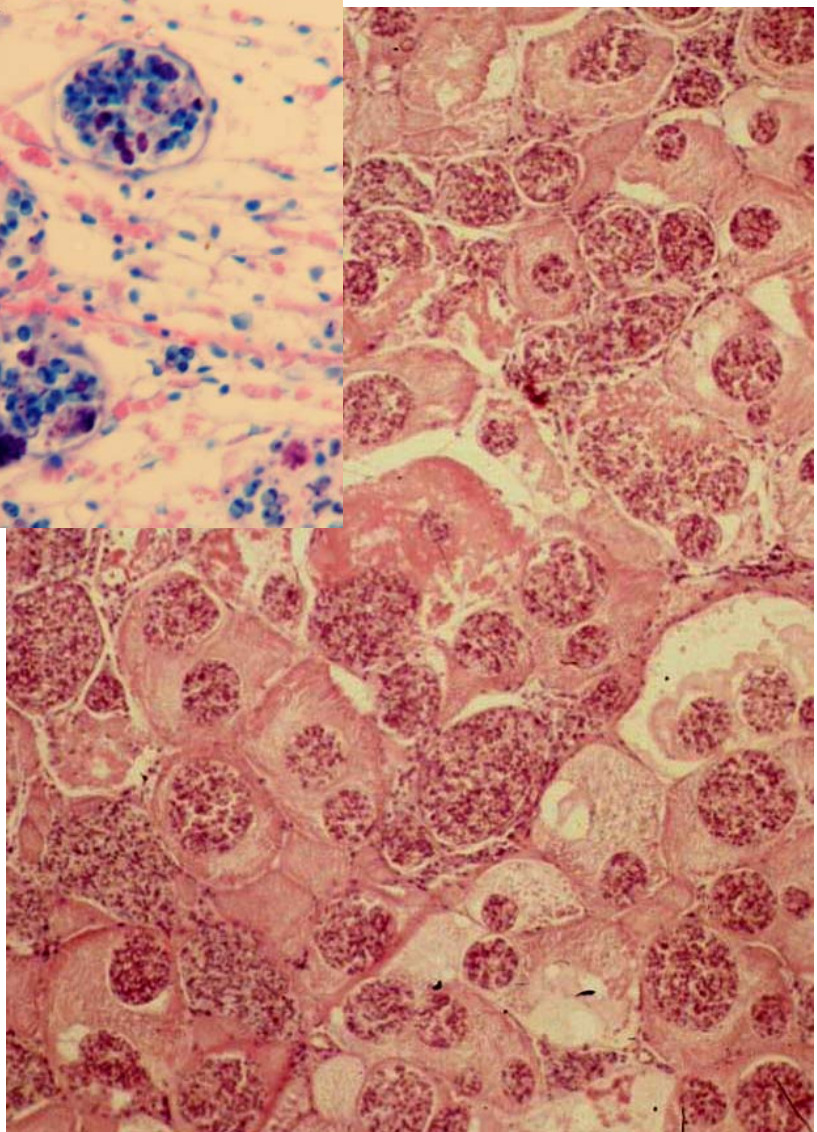
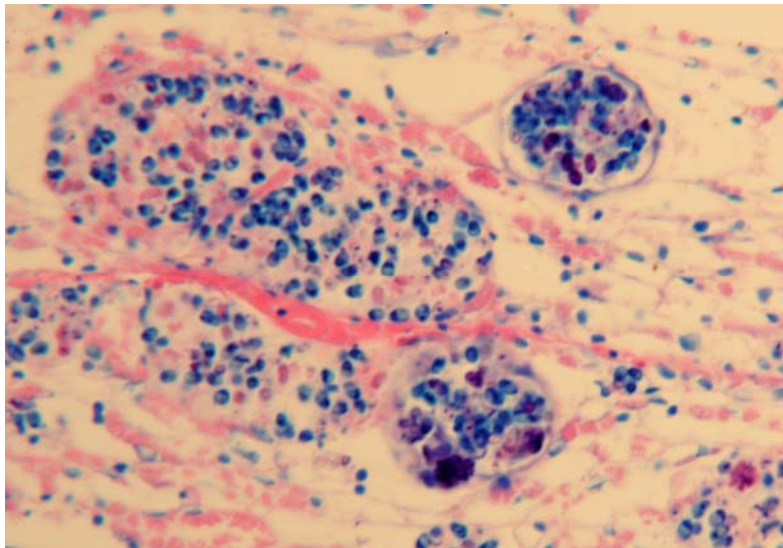
Nucleospora salmonis



(2) *Loma salmonae* - (Pleistophora)

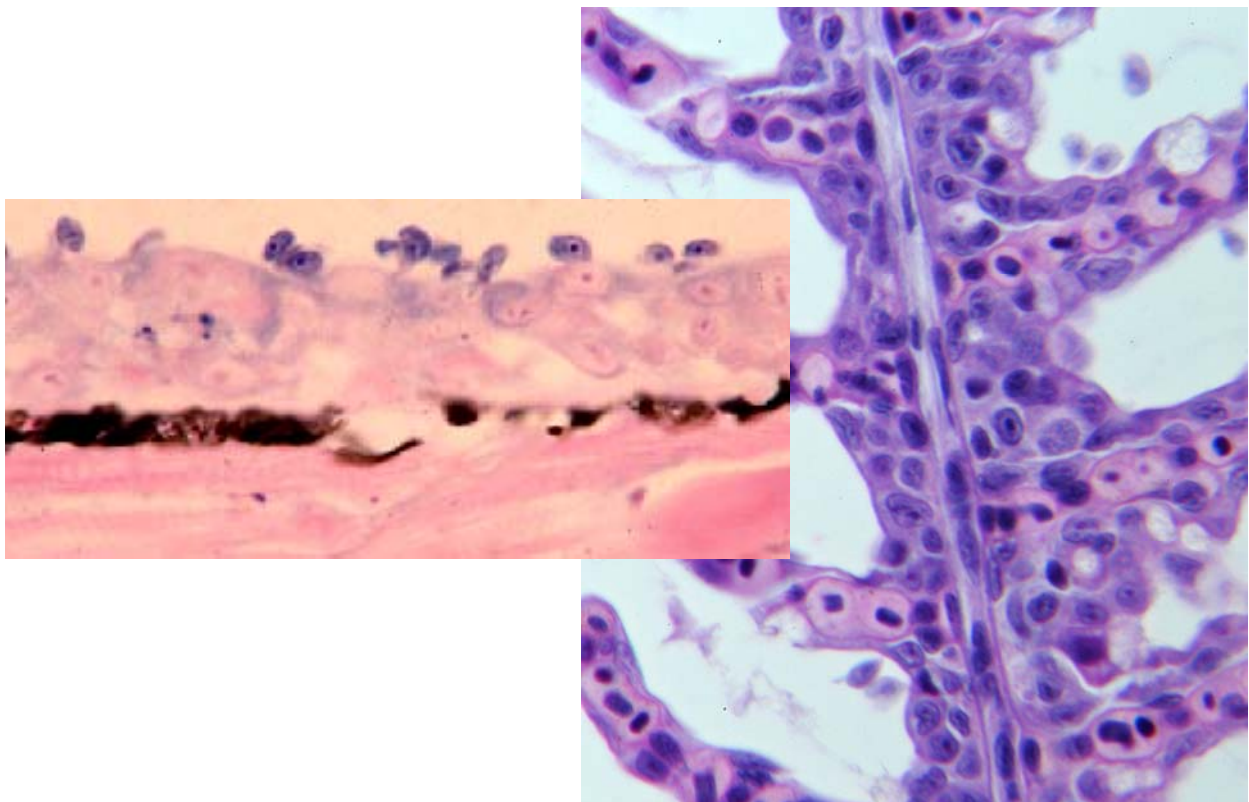
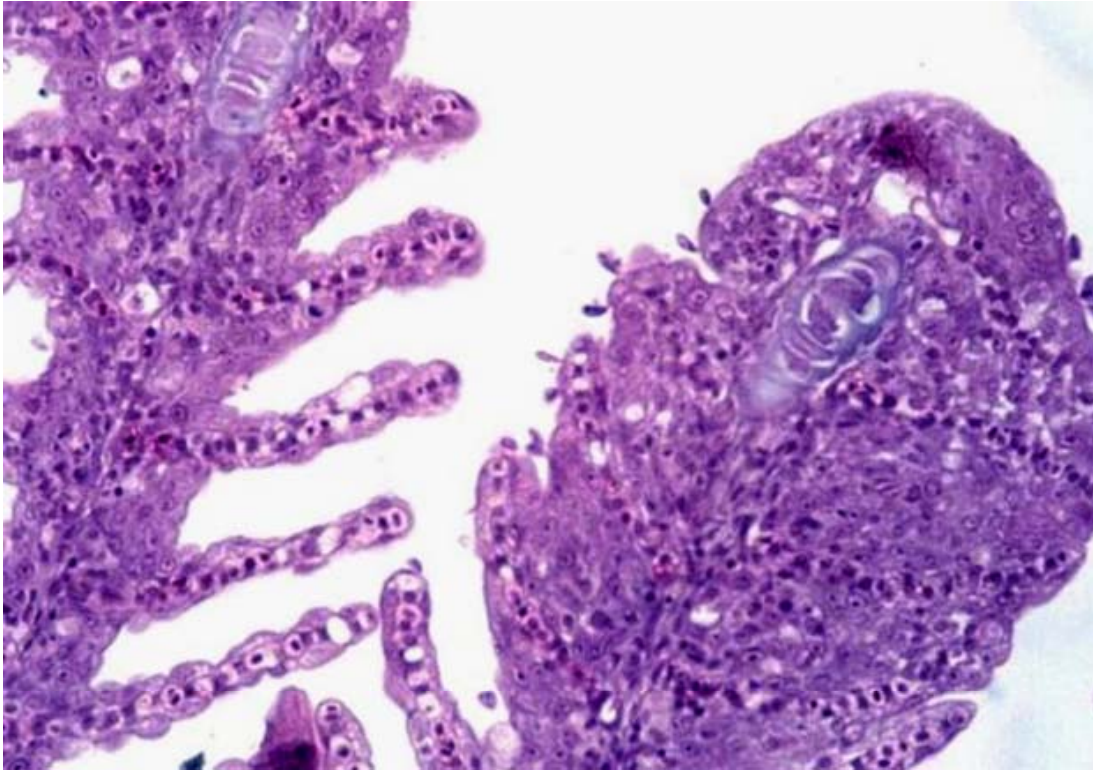


(3) *Heterosporis*

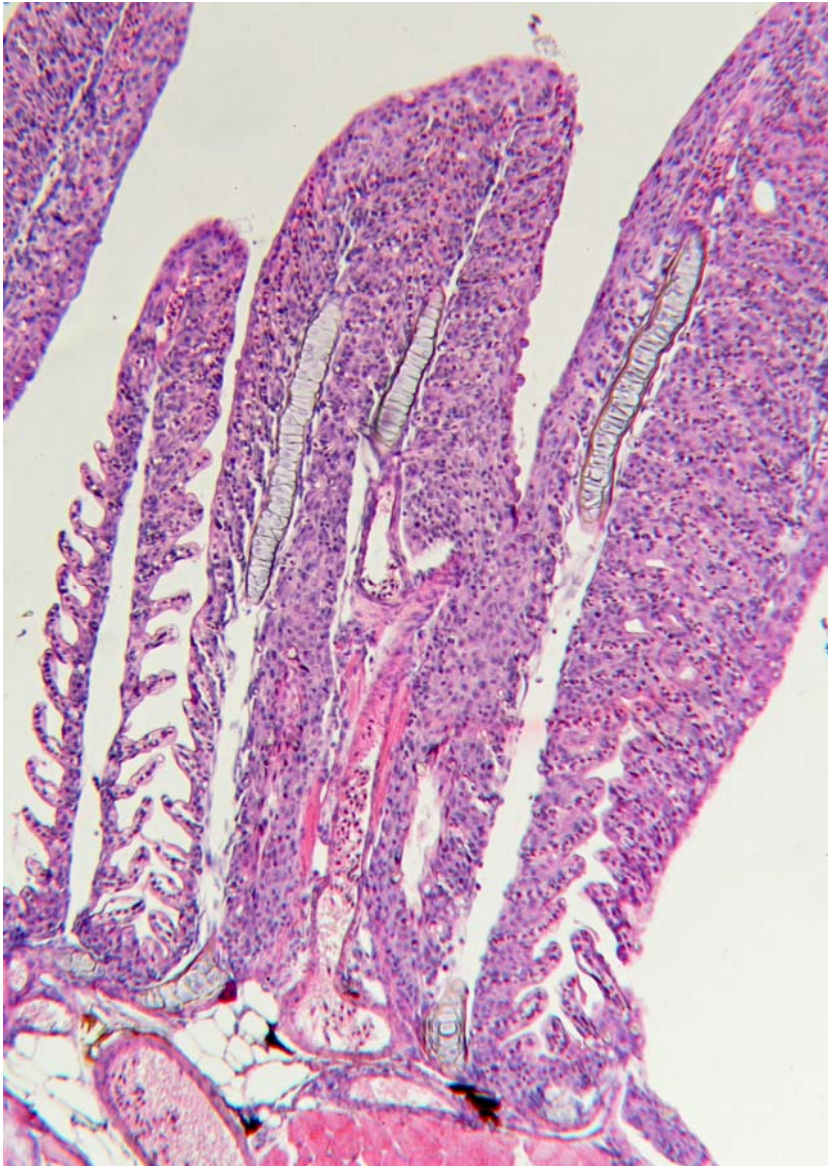


c. Protozoan

(1) *Costia* – *Ichthyobodo necator*

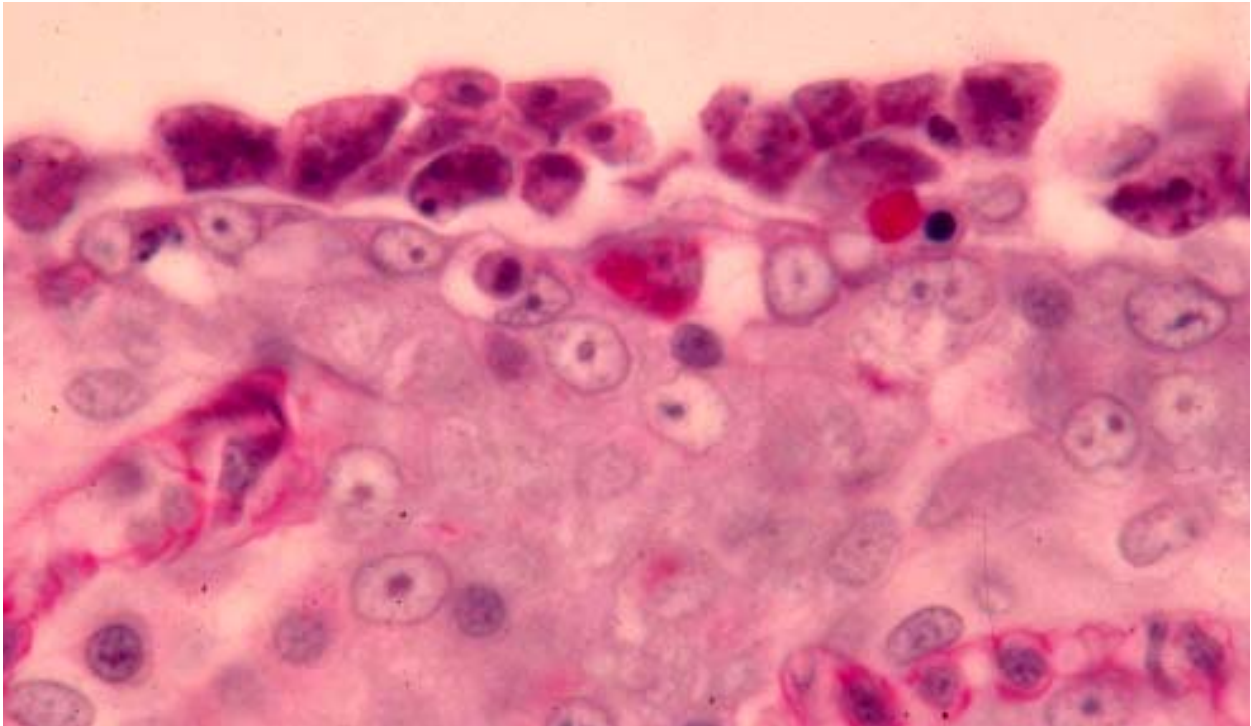


(2) Nodular gill disease - amoeba

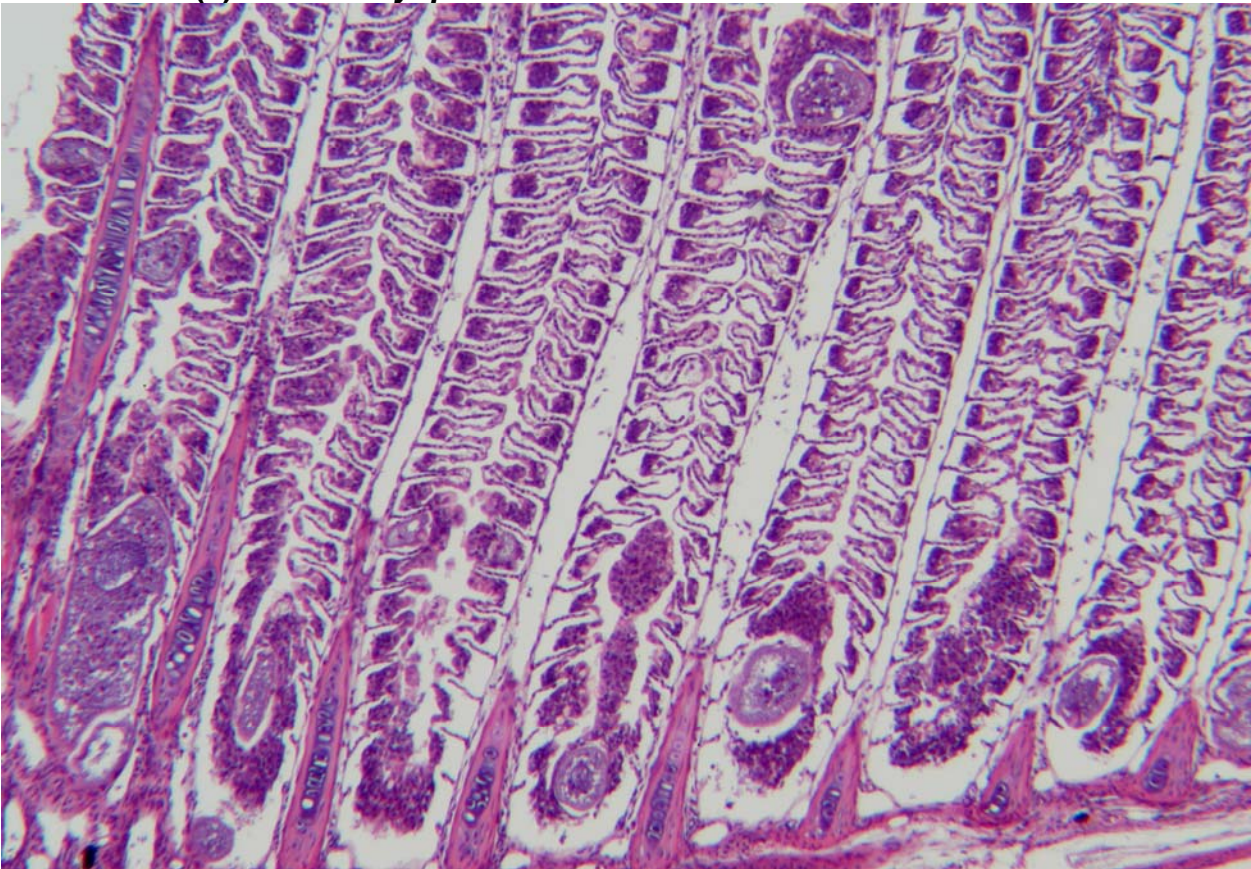


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Nodular Gill Disease

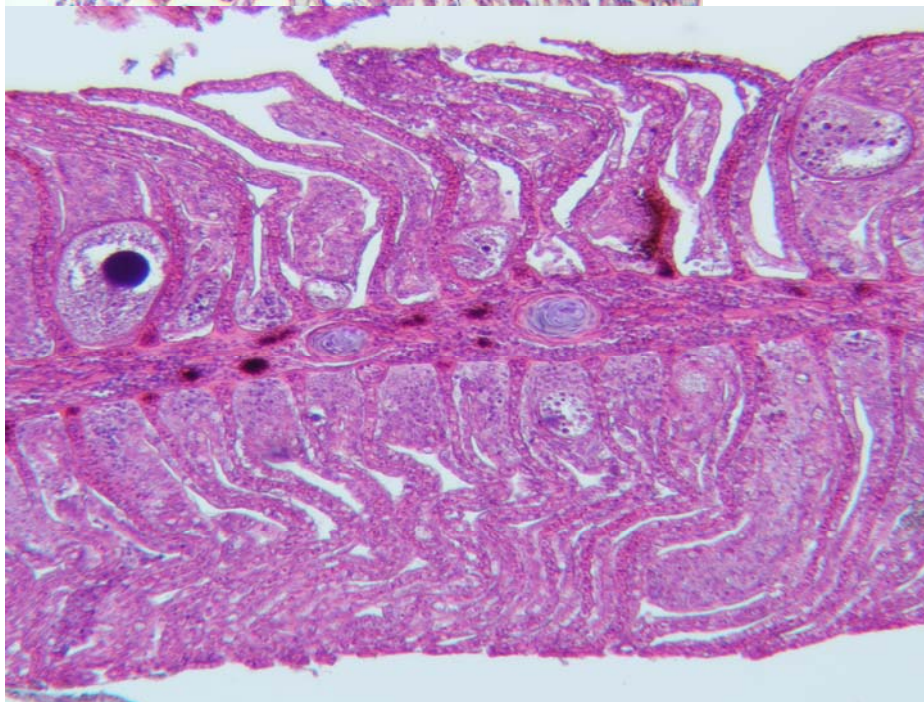
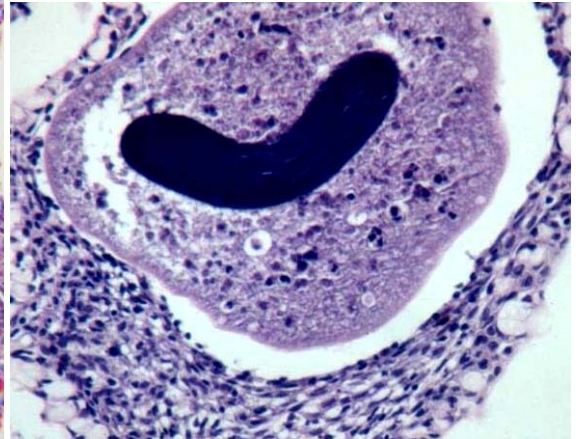
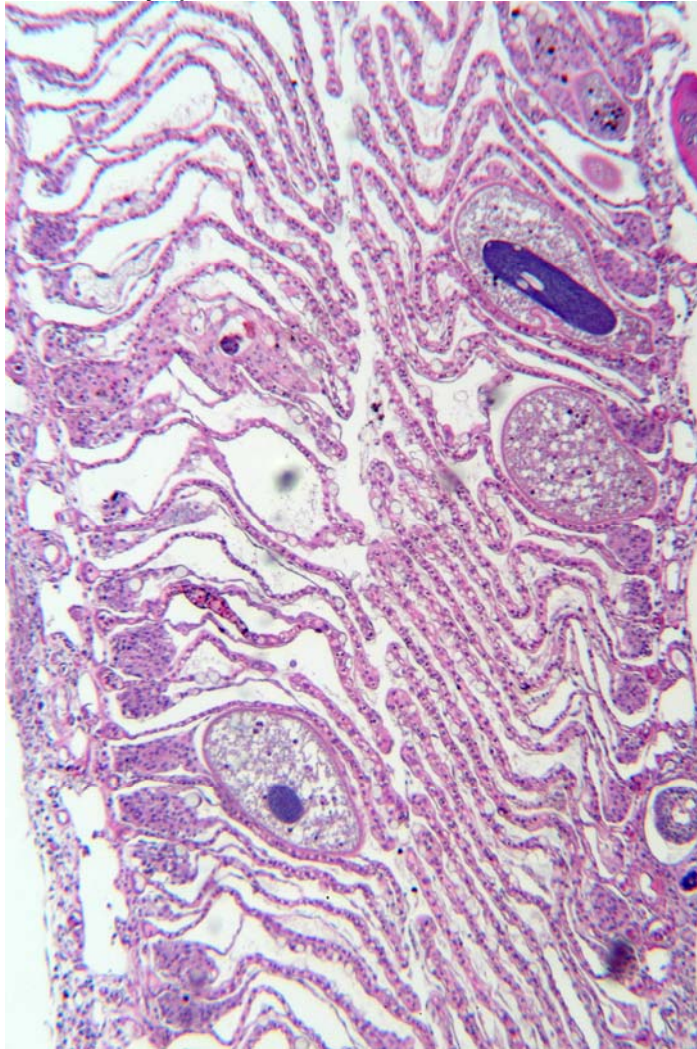


(3) Ich - *Ichthyophthirius multifiliis*

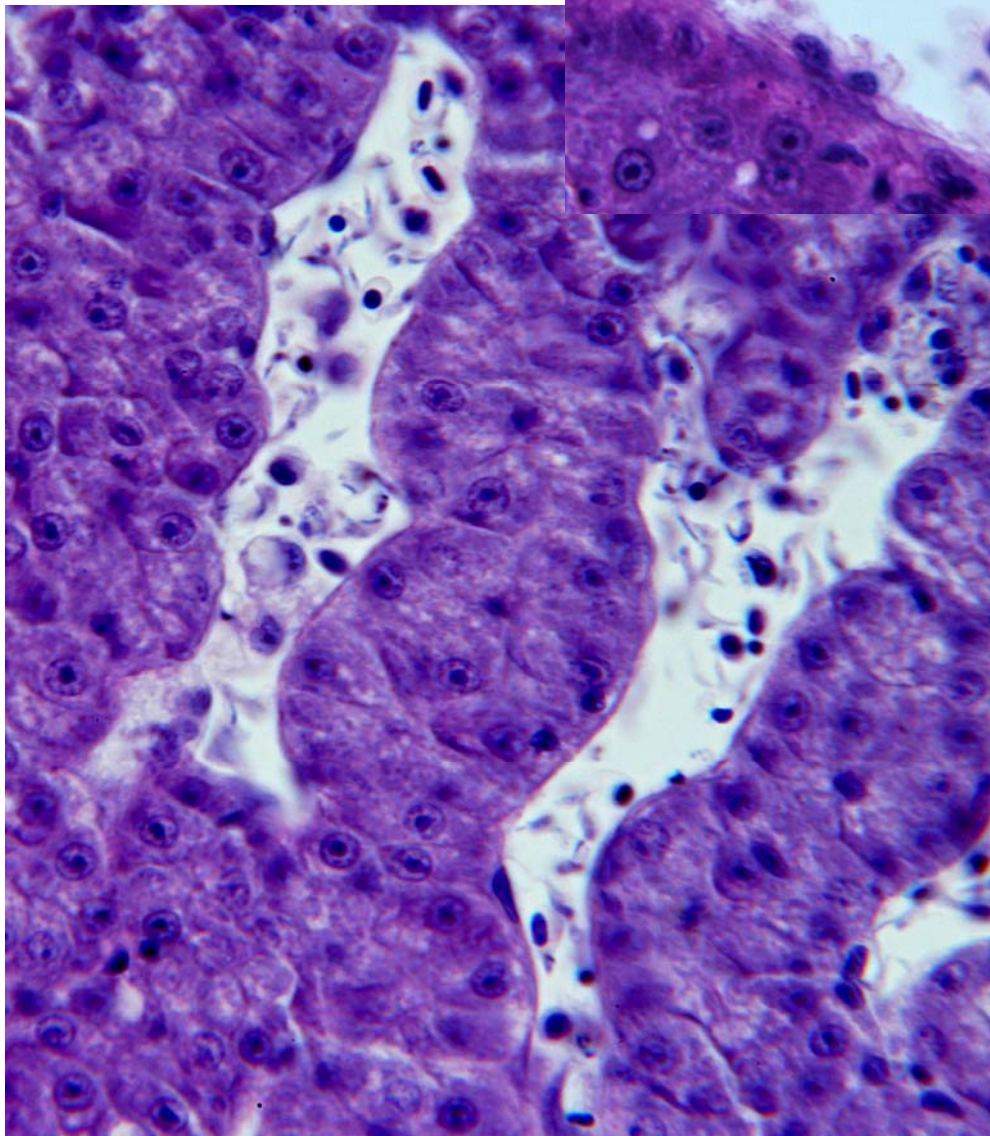
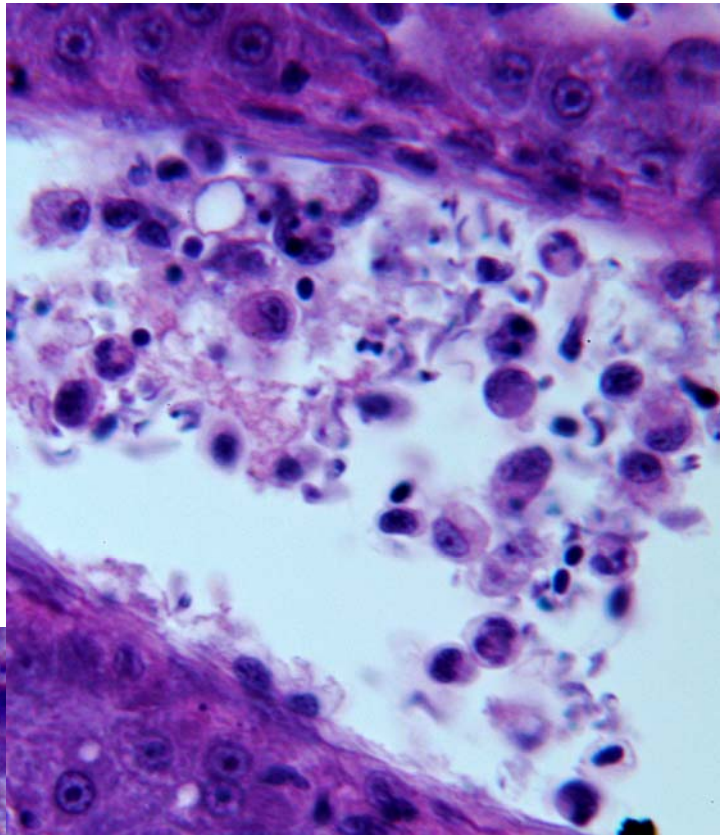
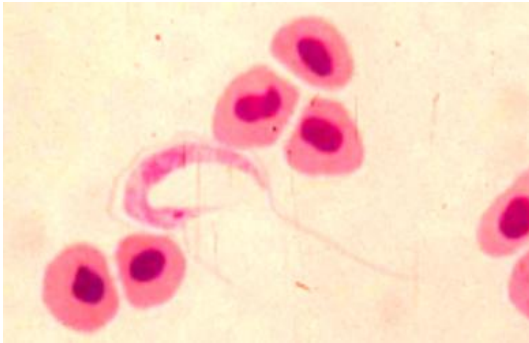


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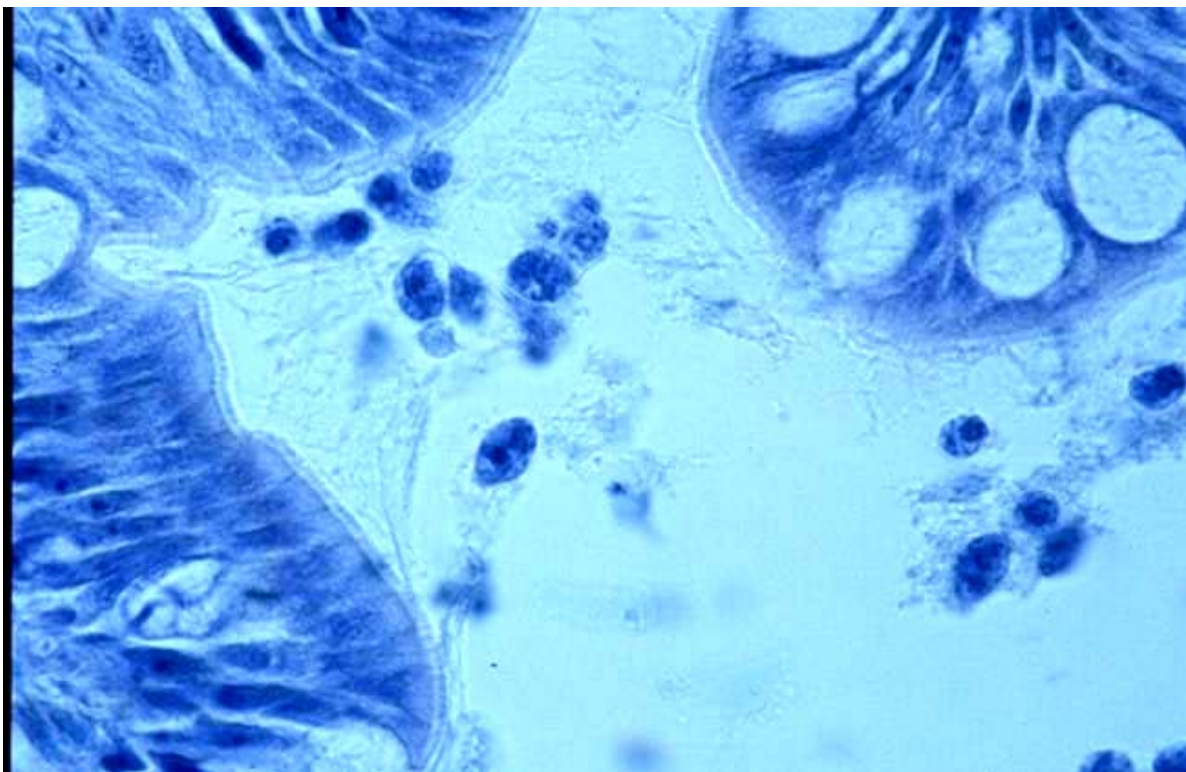
Ich - *Ichthyophthirius multifiliis*



(4) *Cryptobia*

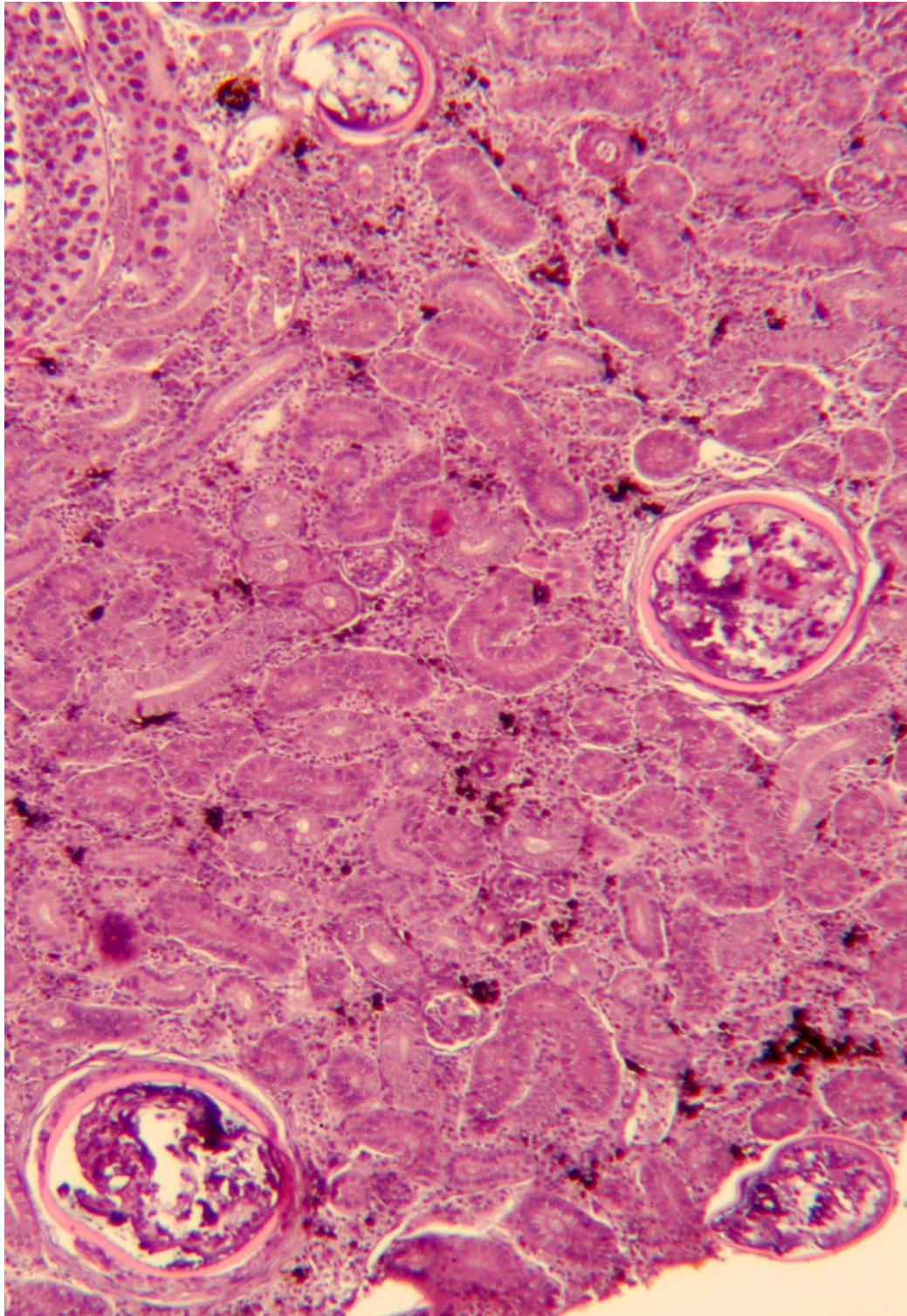


(5) *Hexamita*

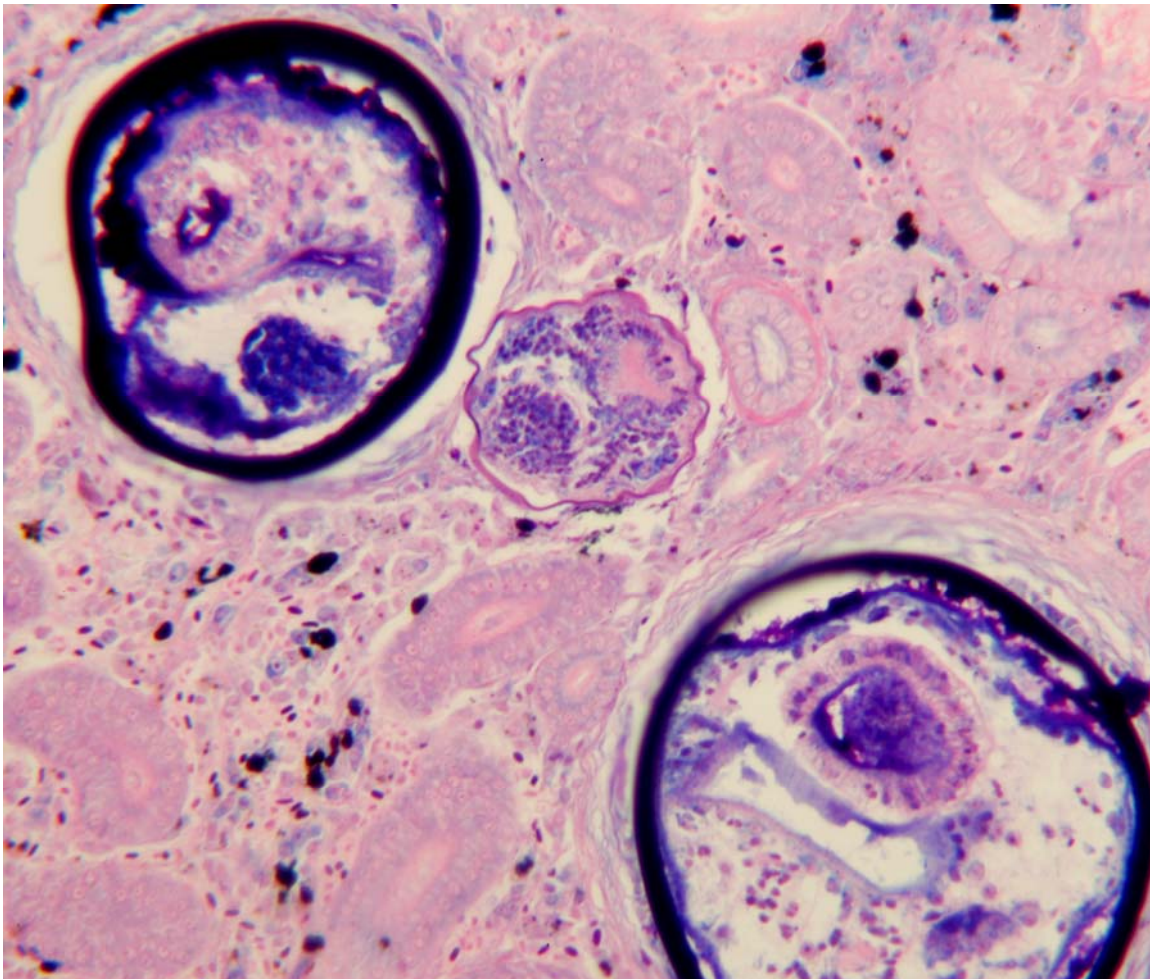
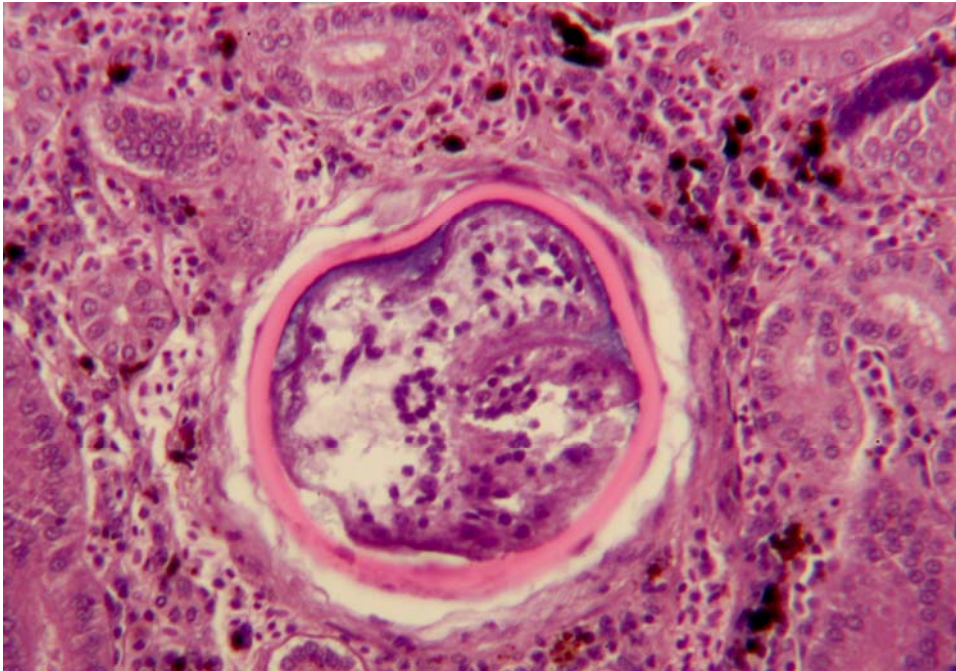


d. Digenetic trematode

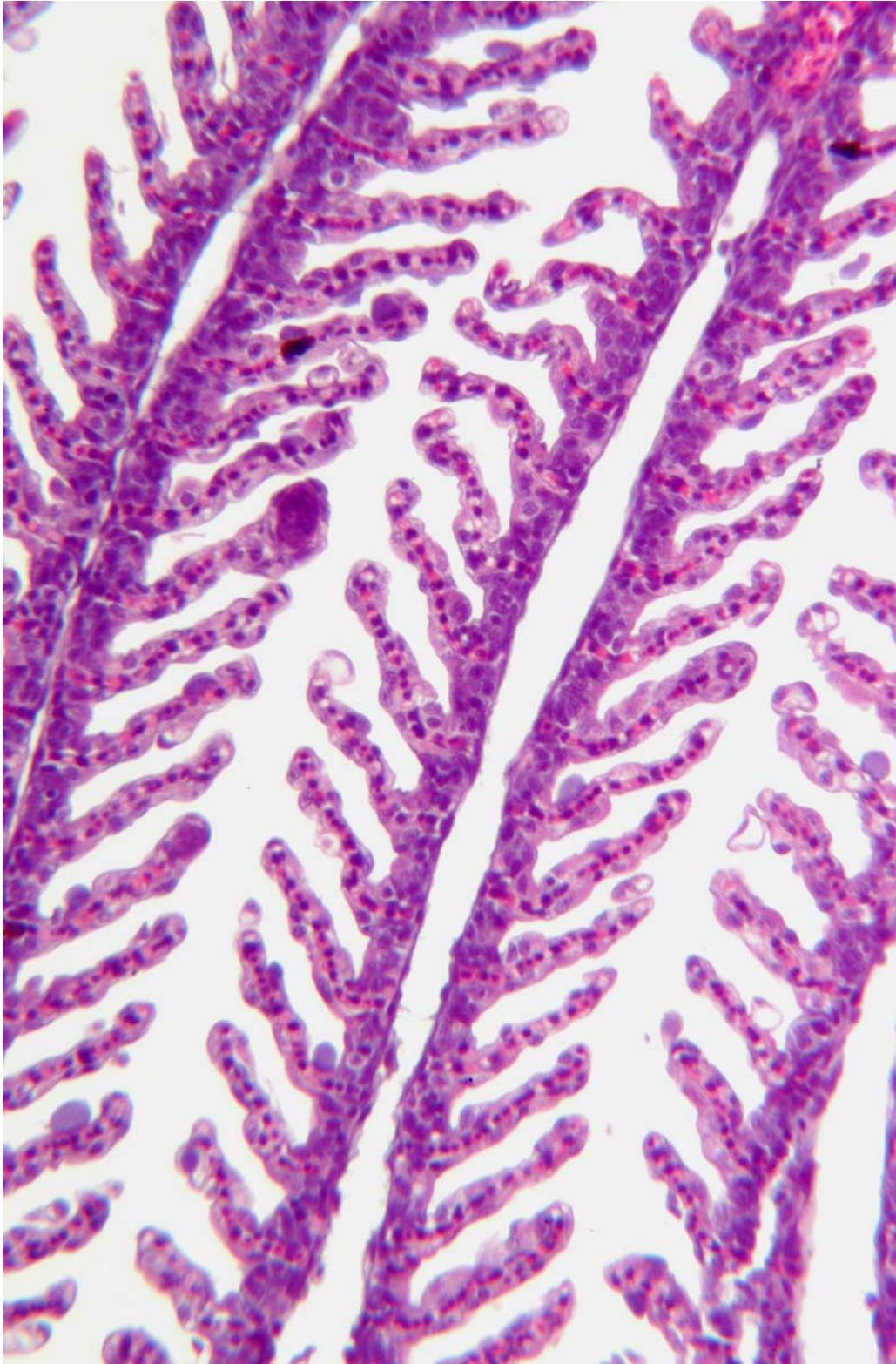
(1) *Nanophyetus salmincola*



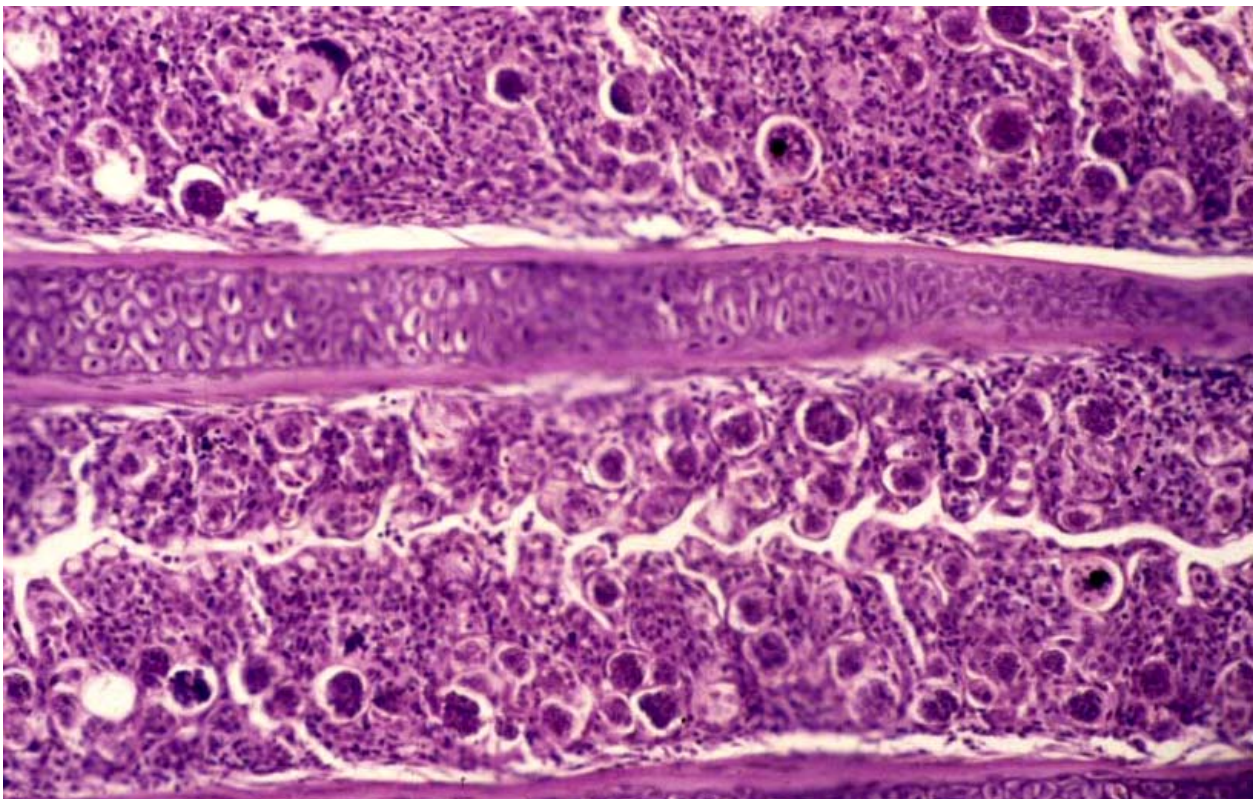
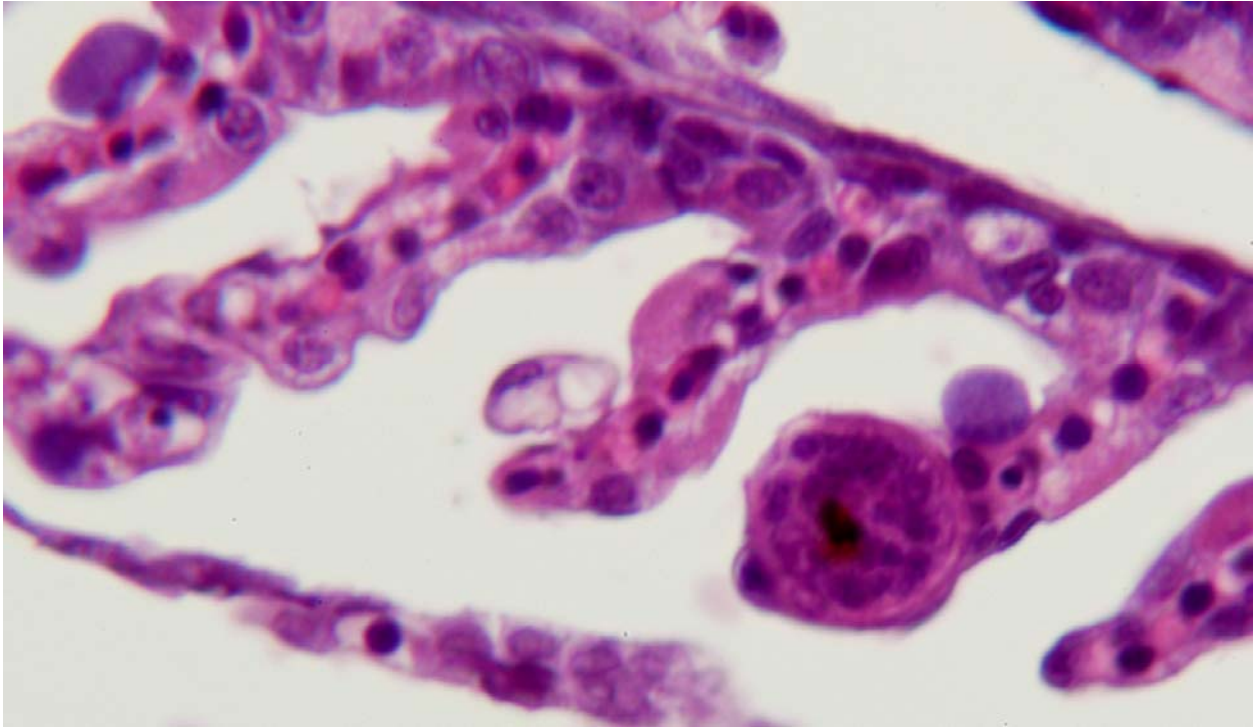
Nanophyetus salmincola



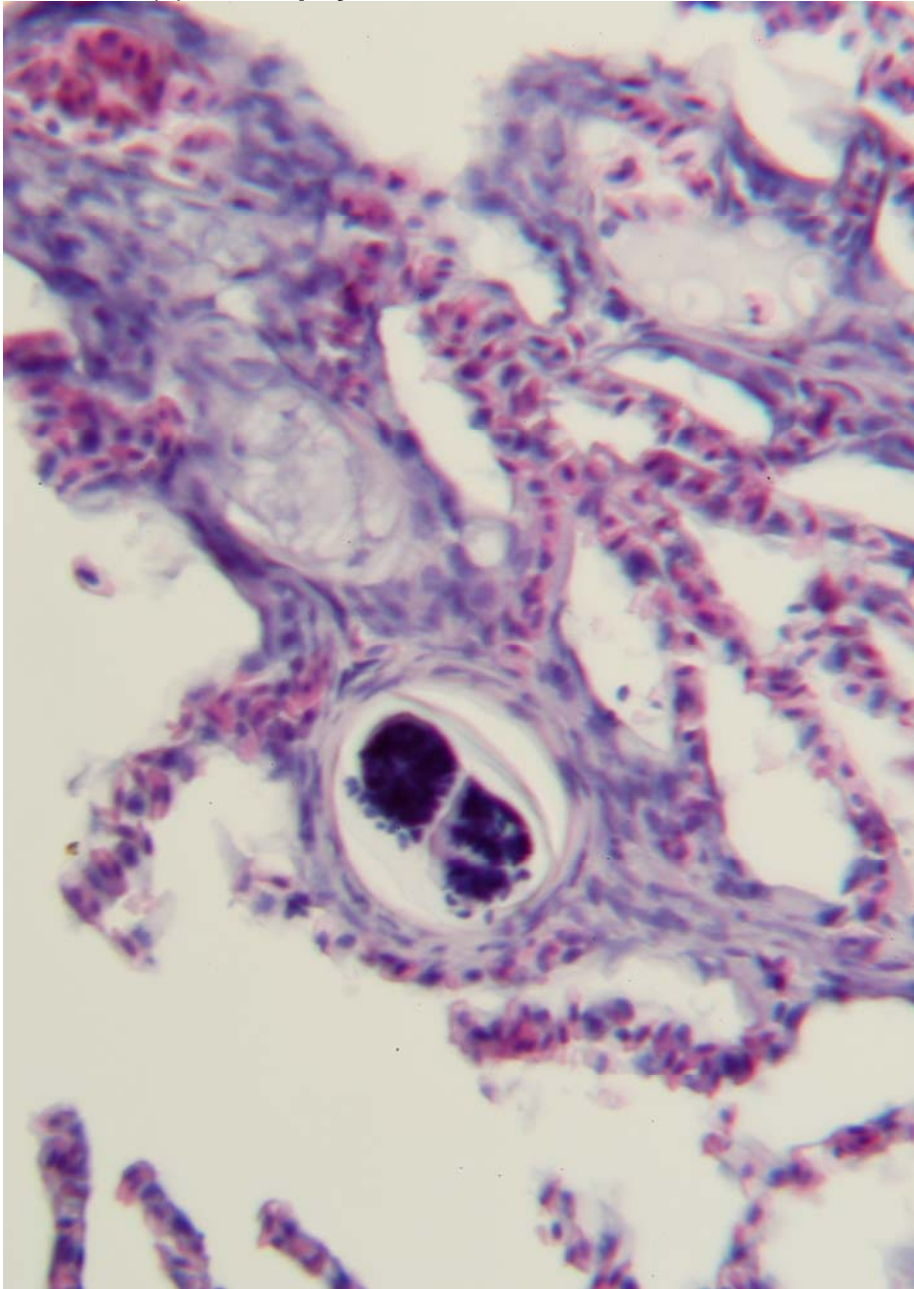
(2) *Sanguinicola* sp.



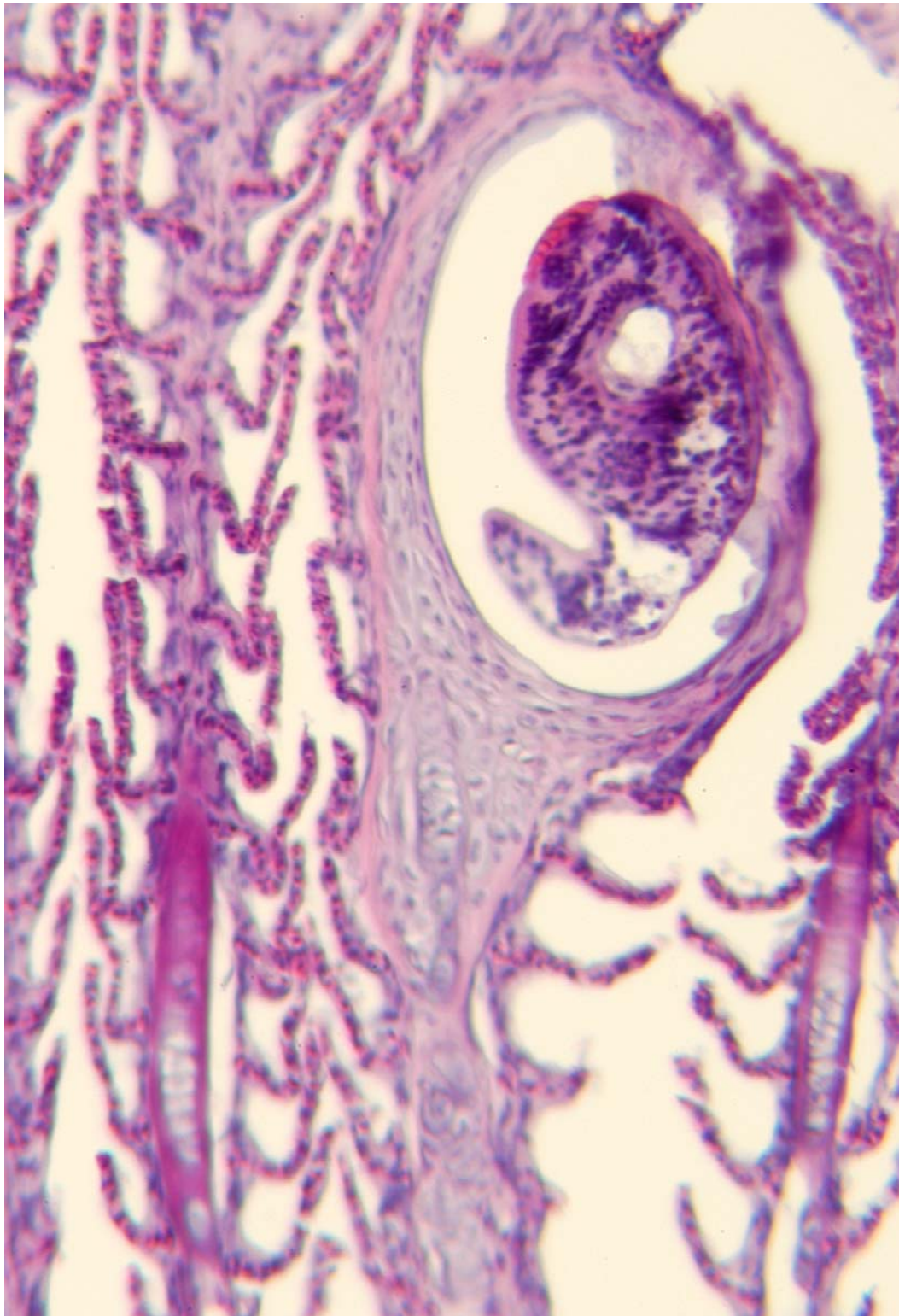
Sanguinicola sp.



(3) Heterophyidae - *Centrocestus formosanus*



Centrocestus formosanus



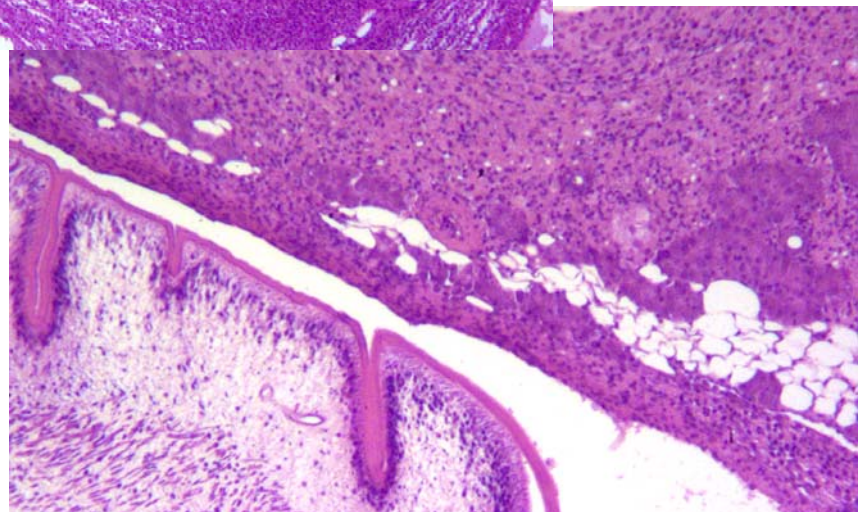
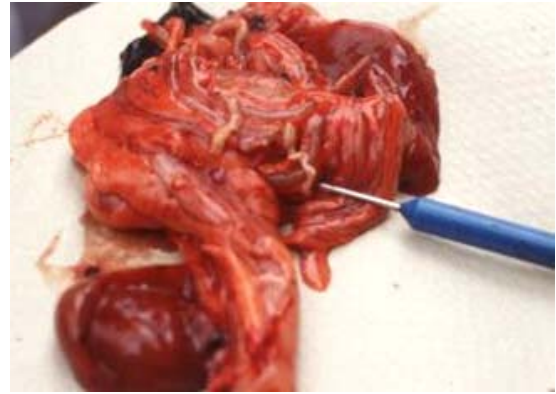
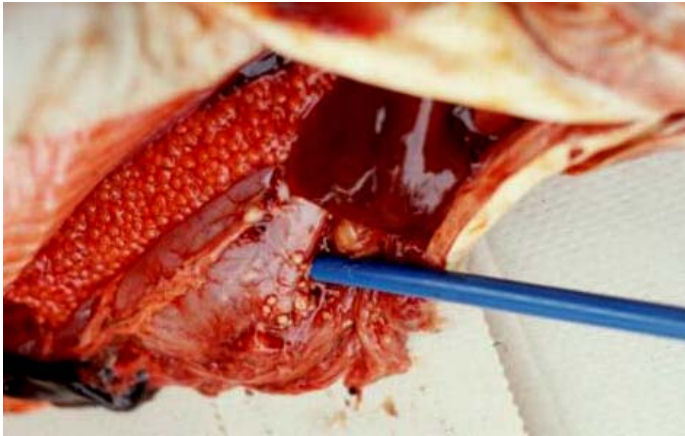
Chapter 6 – Infectious Disease
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(4) *Diplostomulum spathaceum*



e. Cestode

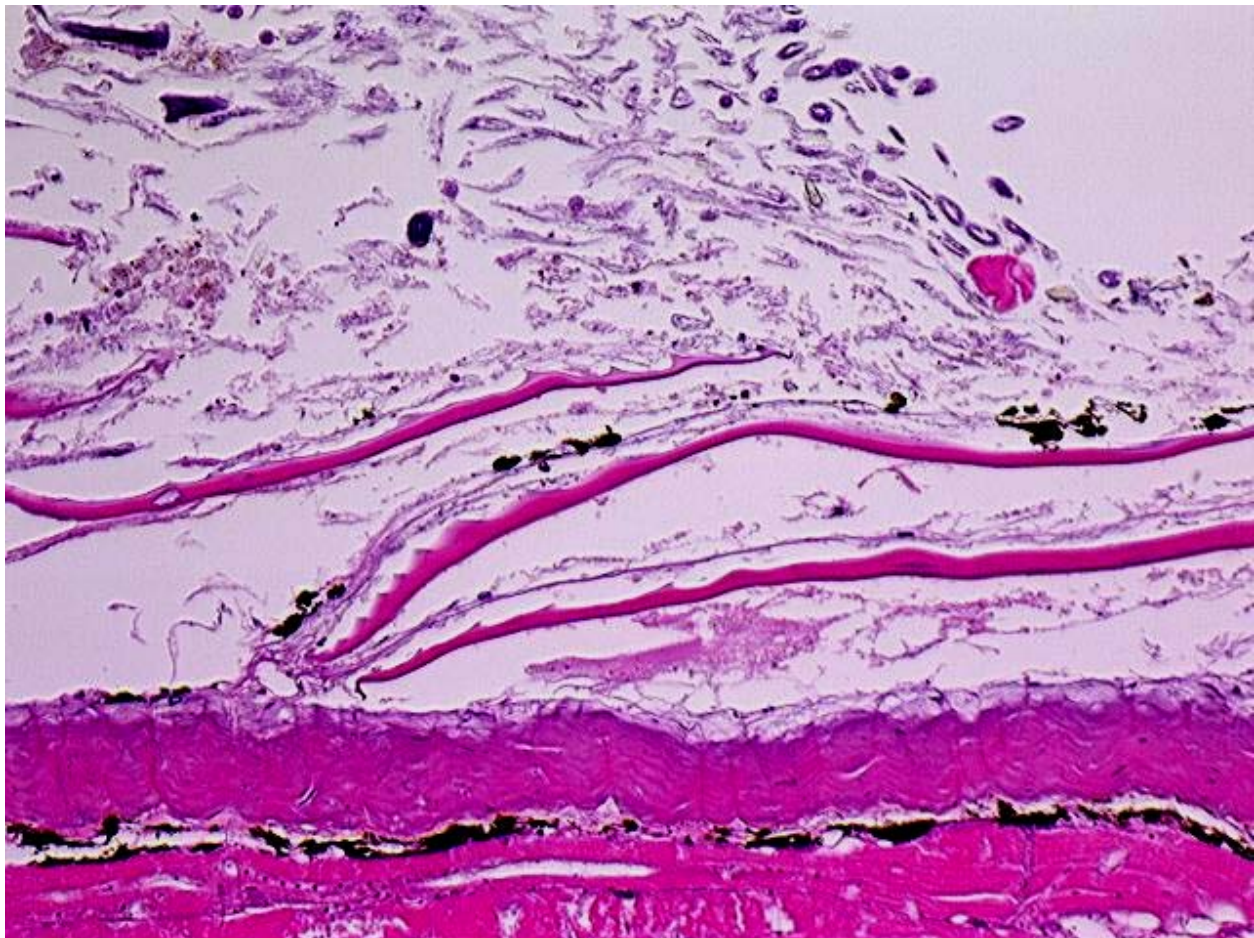
(1) *Diphyllobothrium dendriticum*



5. Fungi

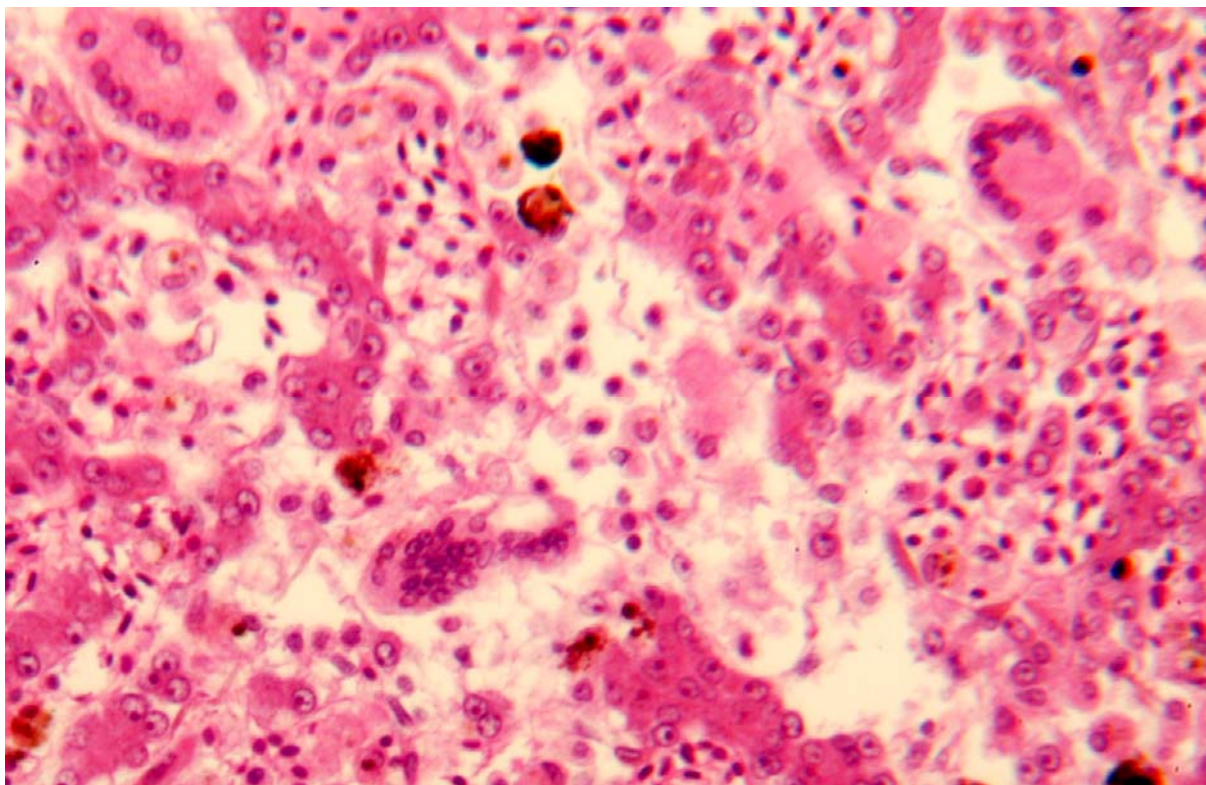
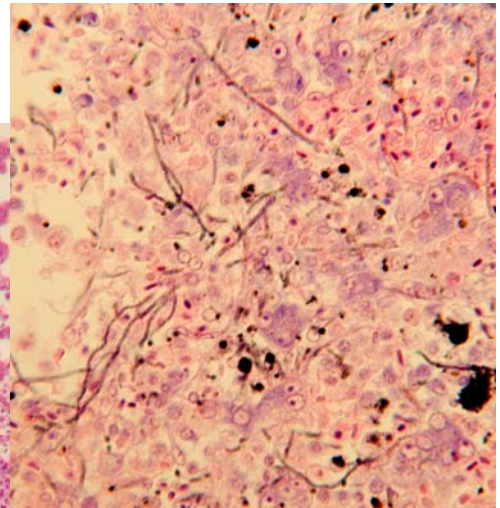
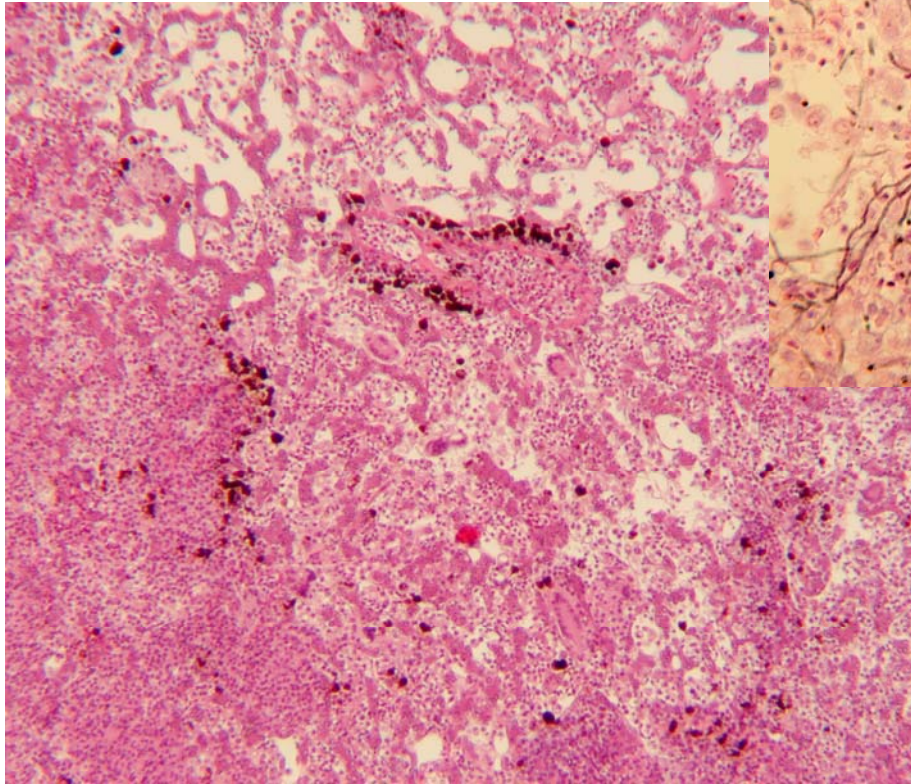
a. Saprolegniasis –

- Superficial fungal infections caused a variety of primarily saprophytic, secondary fungal agents
- Also a problem on salmonid eggs
- Affects a variety of fish species - cold and warmwater, fresh to brackish



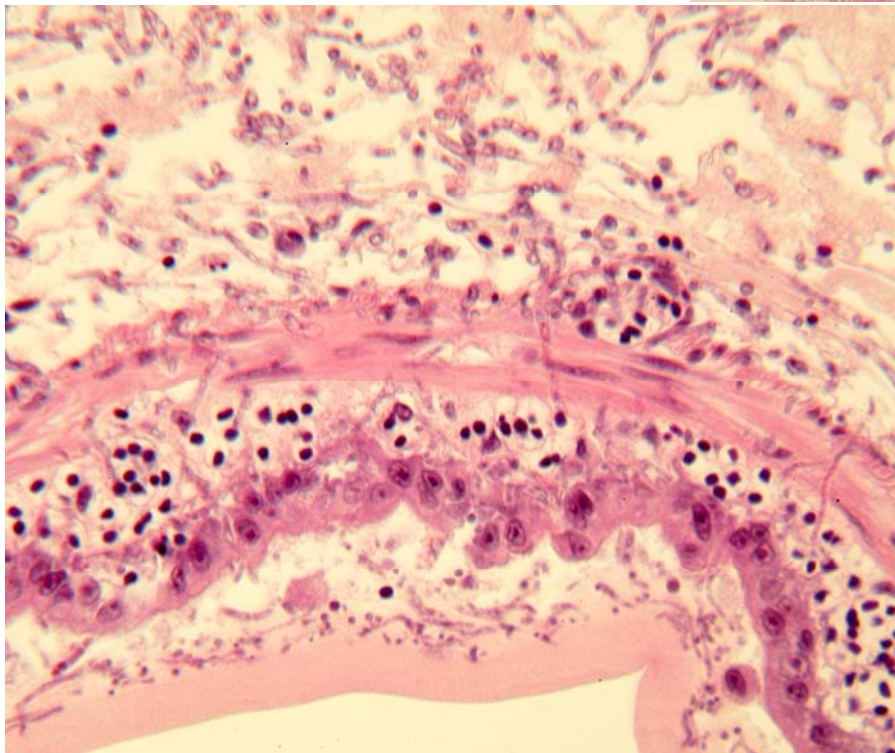
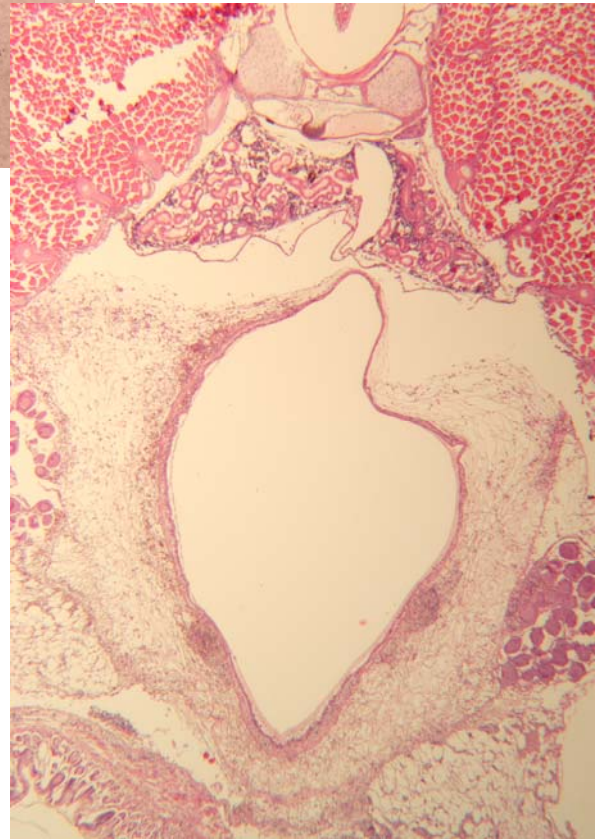
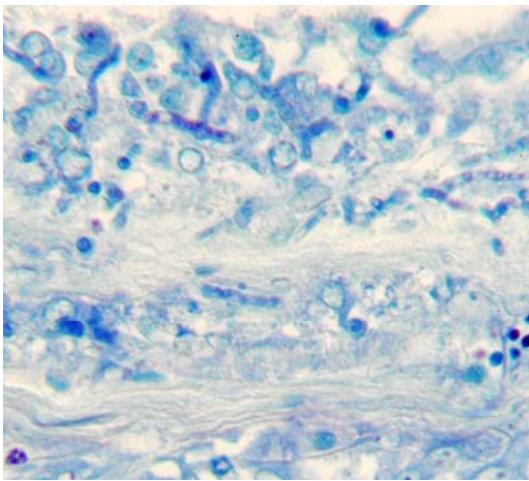
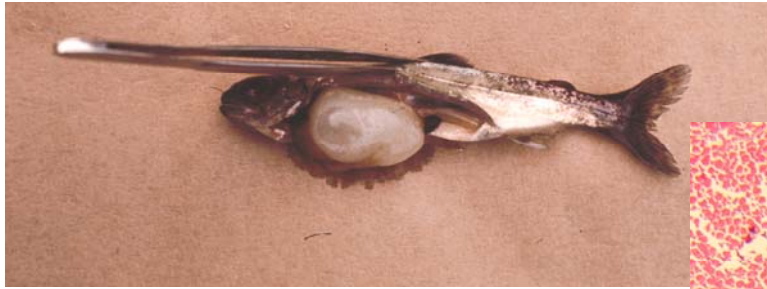
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Saprolegnia

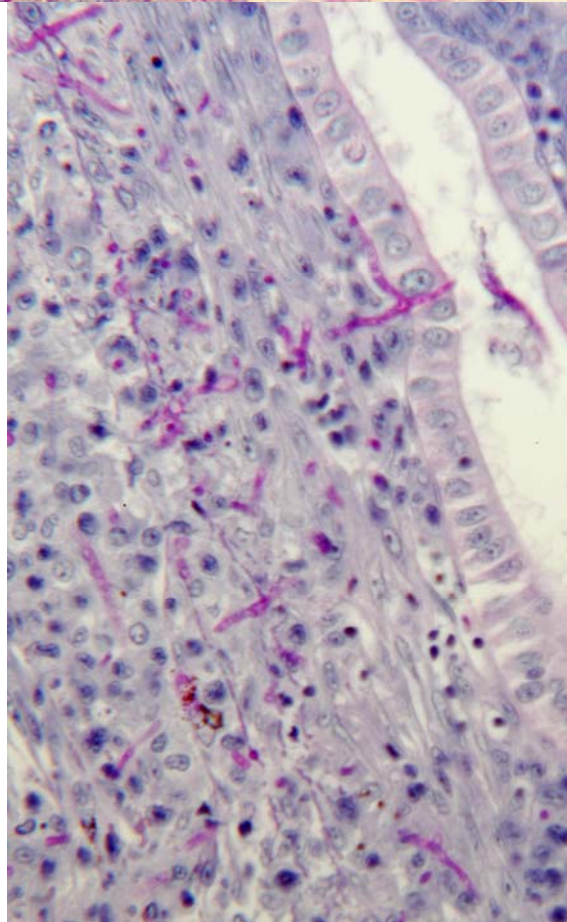
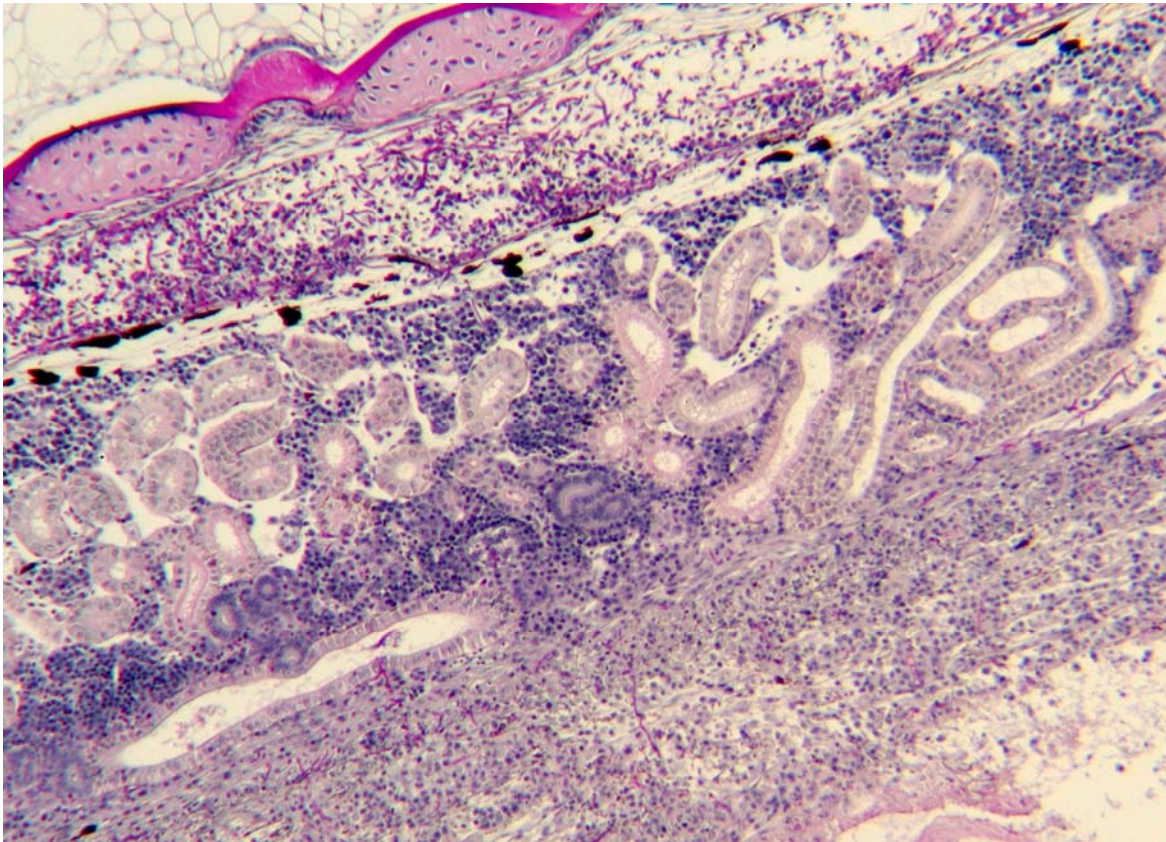


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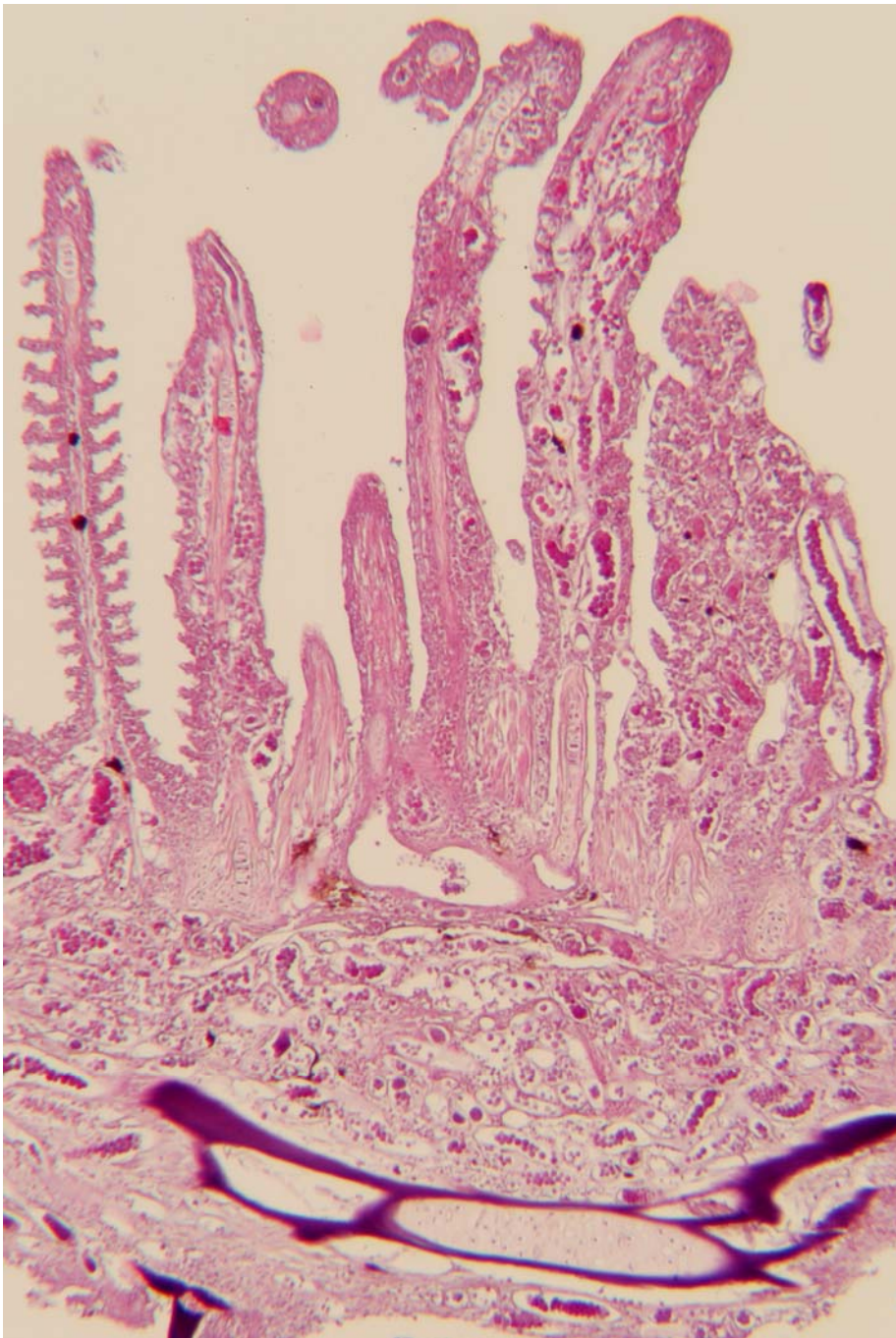
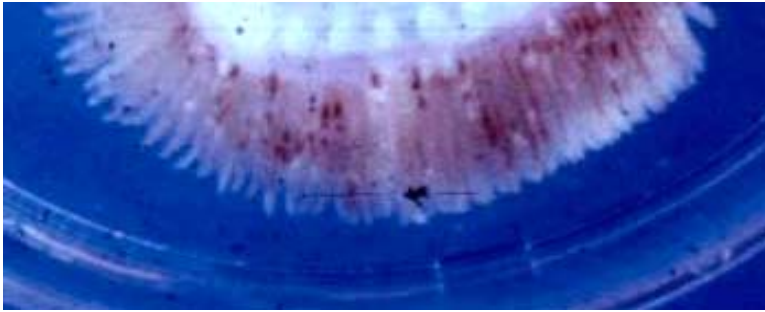
b. Phoma herbarum



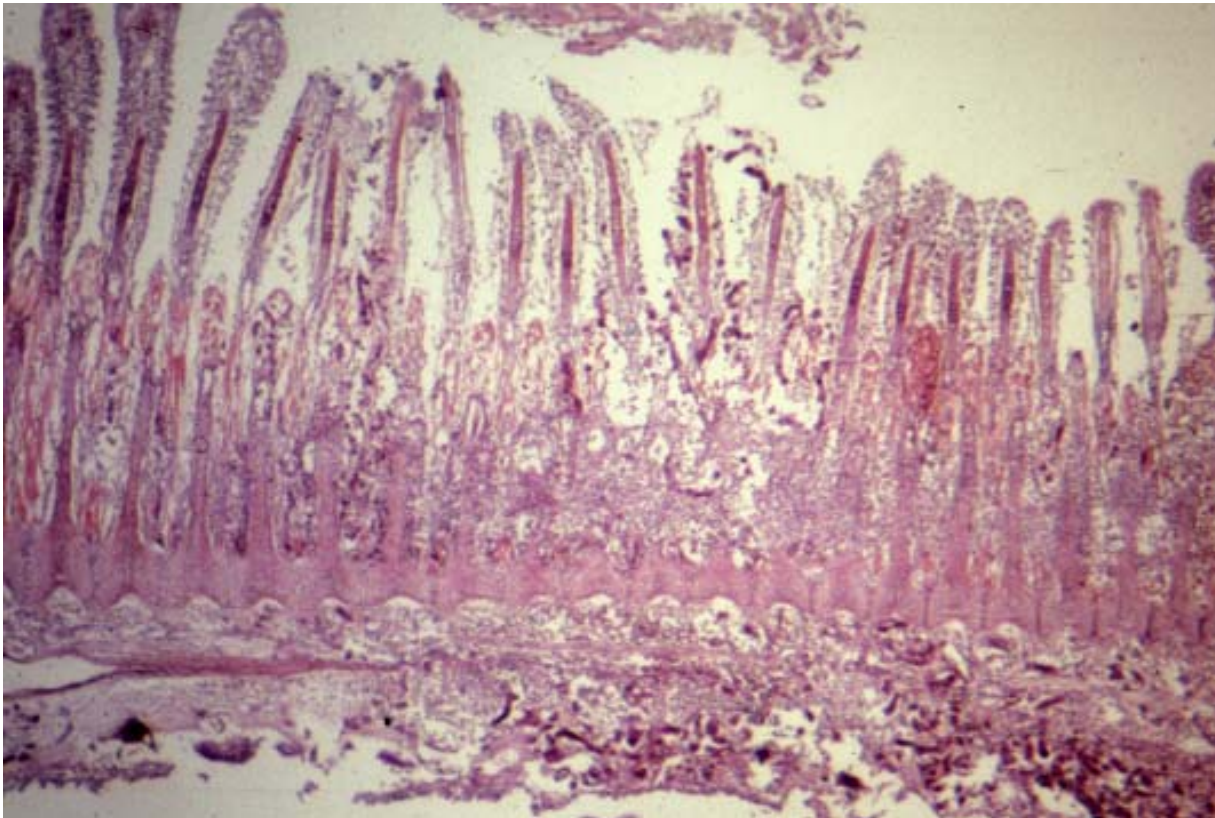
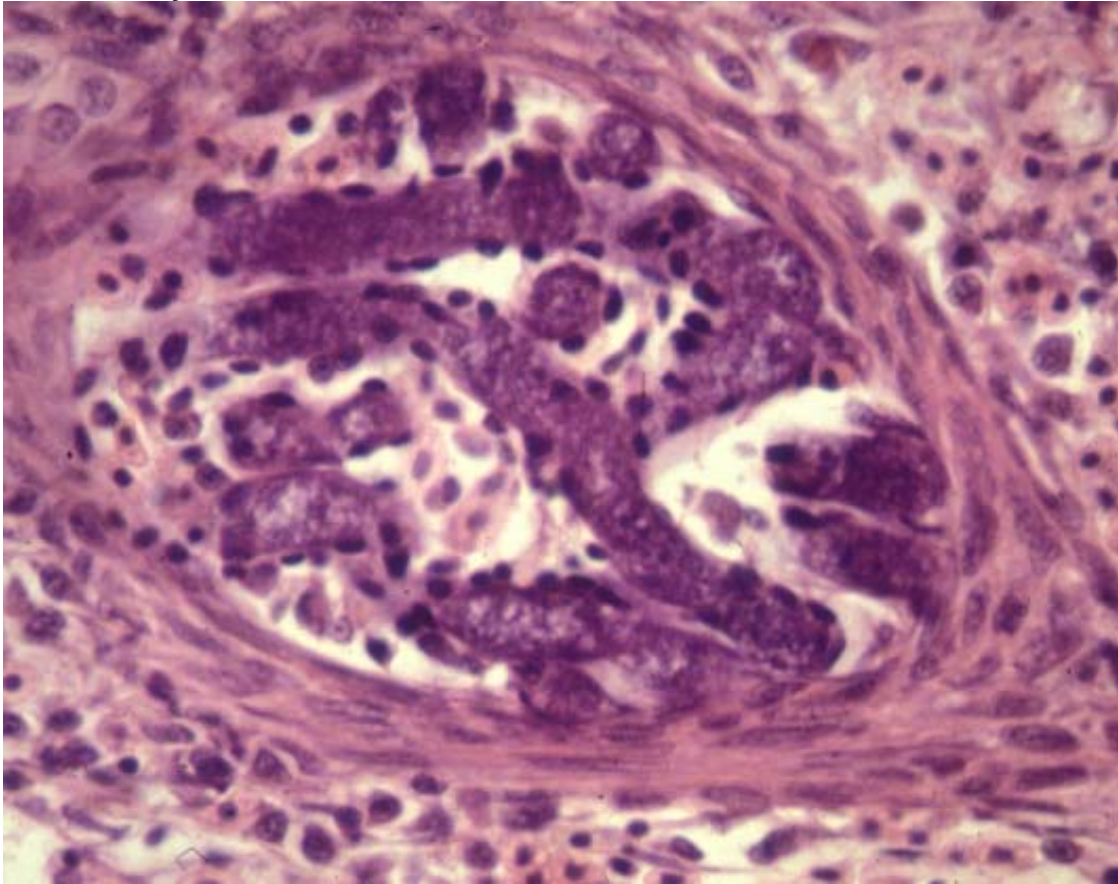
c. Mortiella



d. *Branchiomyces*

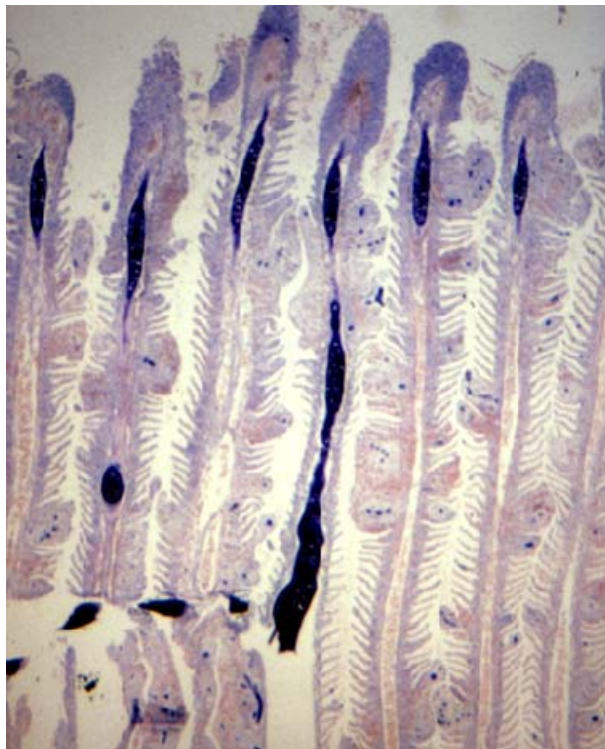


Branchiomyces



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Branchiomyces

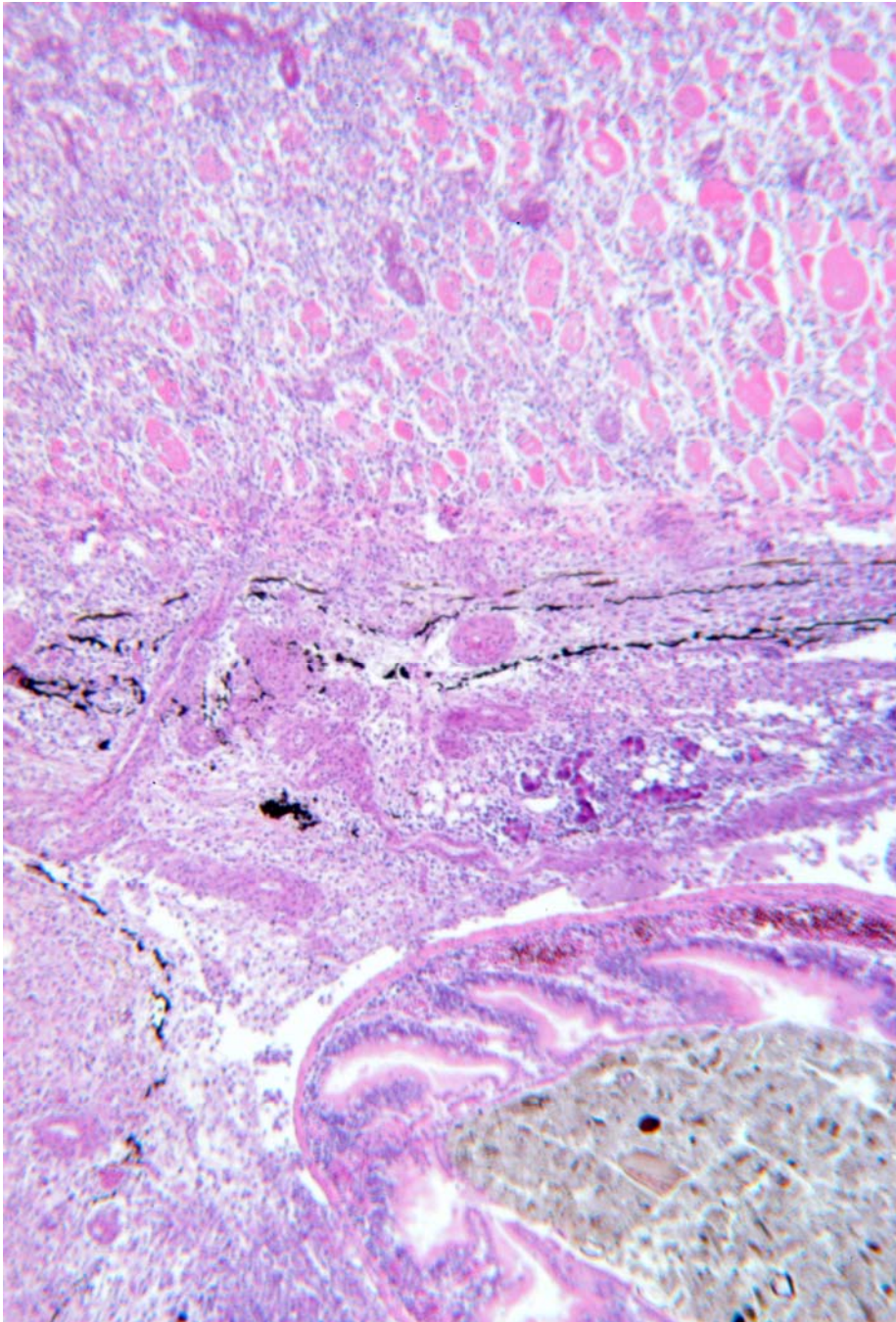


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e. *Aphanomyces invadans*

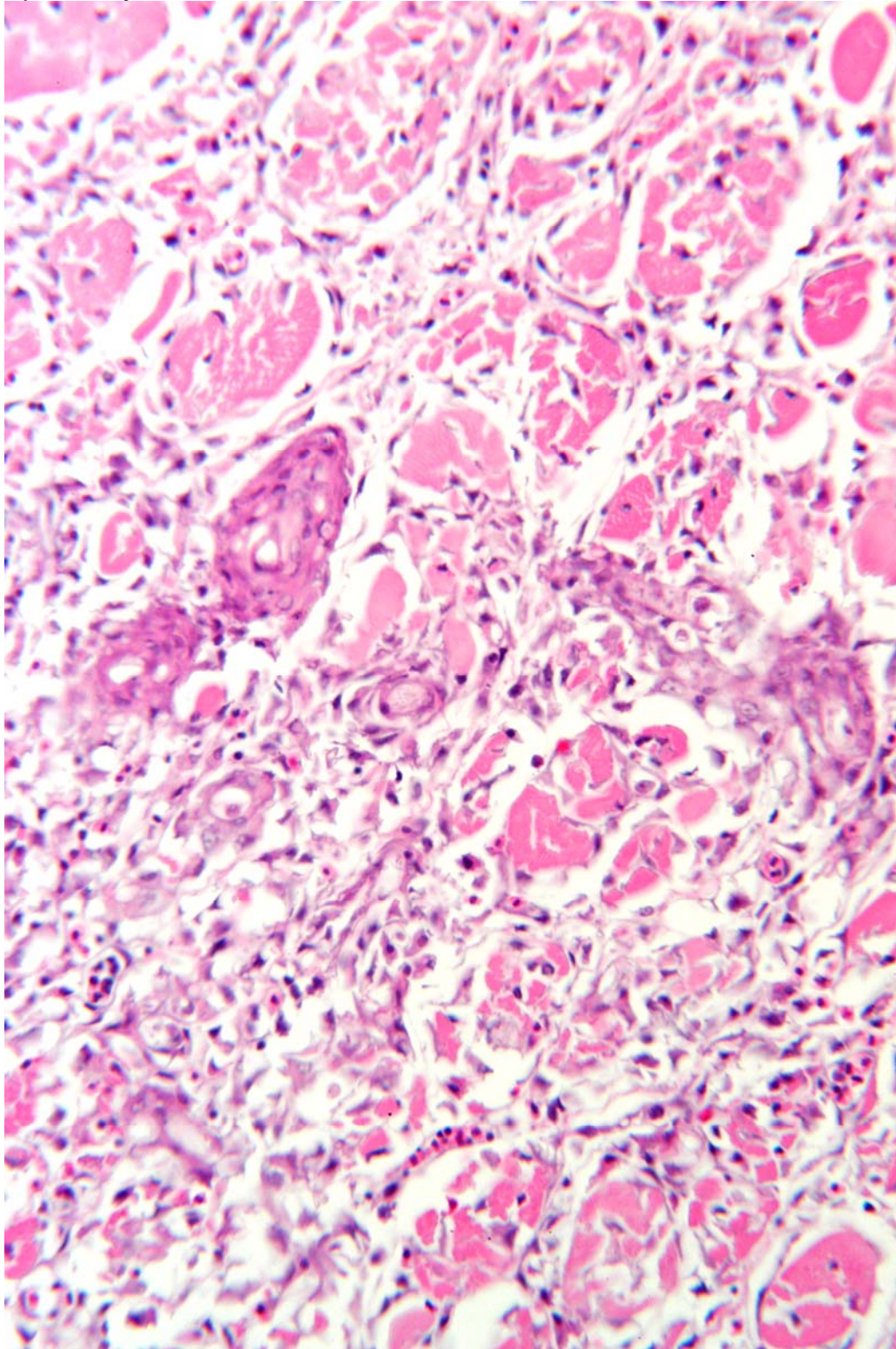
- Ulcerative lesions
- mid to late summer
- 8 - 12 cm Menhaden
- Other species
 - Mullet
 - Bluegill
 - Catfish



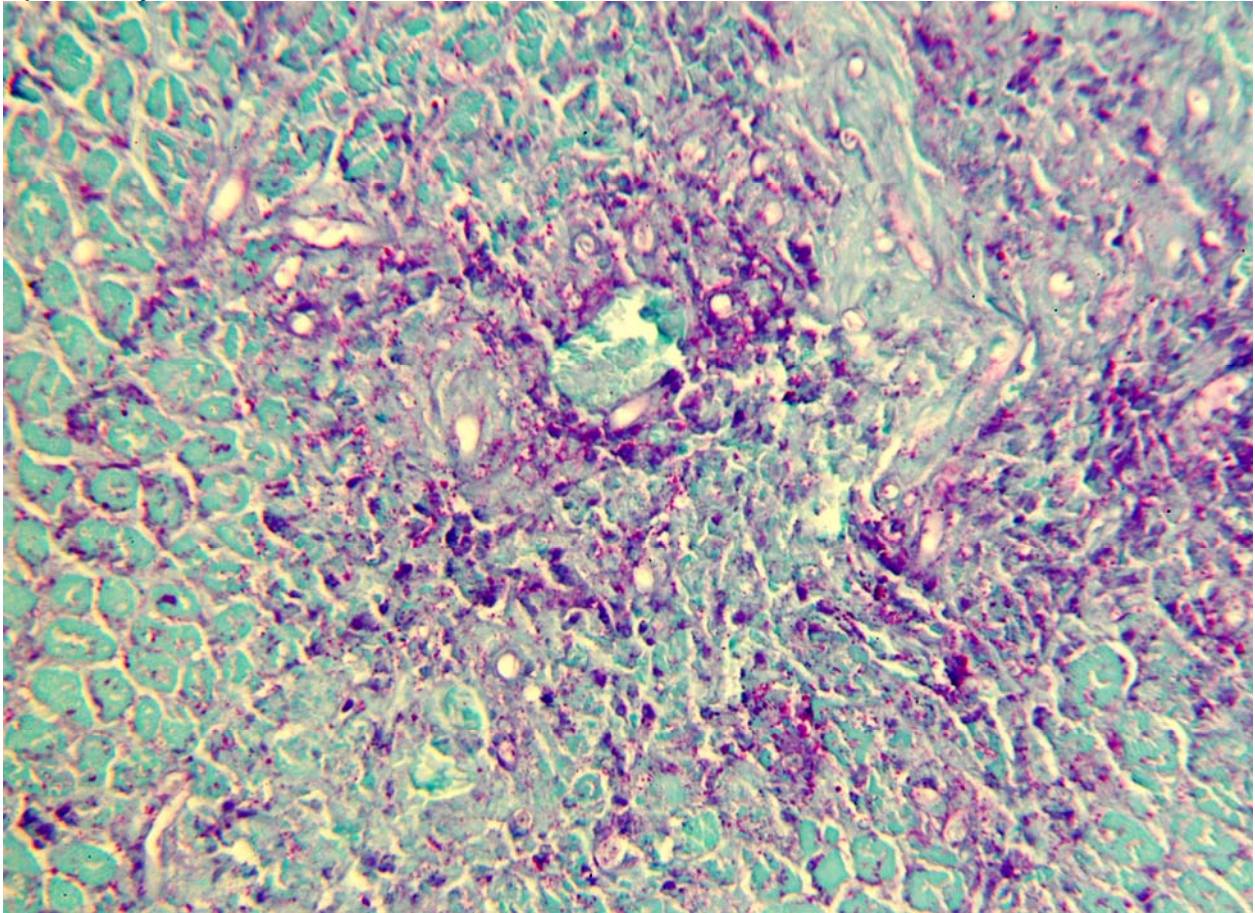


Aphanomyces invadans

Aphanomyces invadans

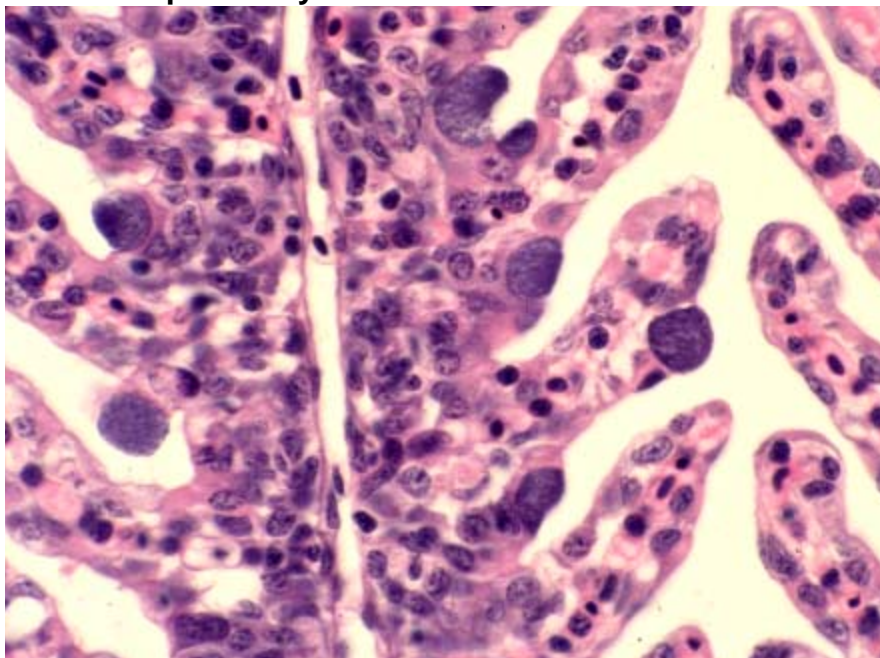


Aphanomyces invadans

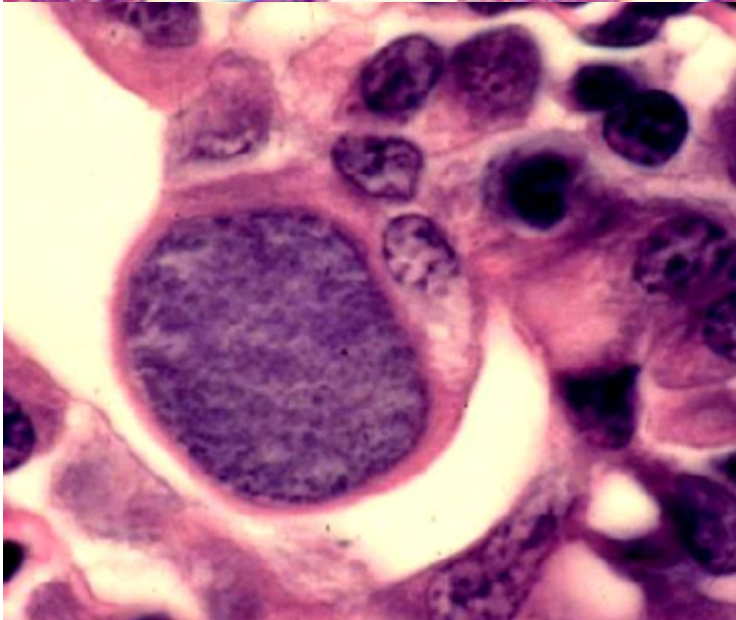
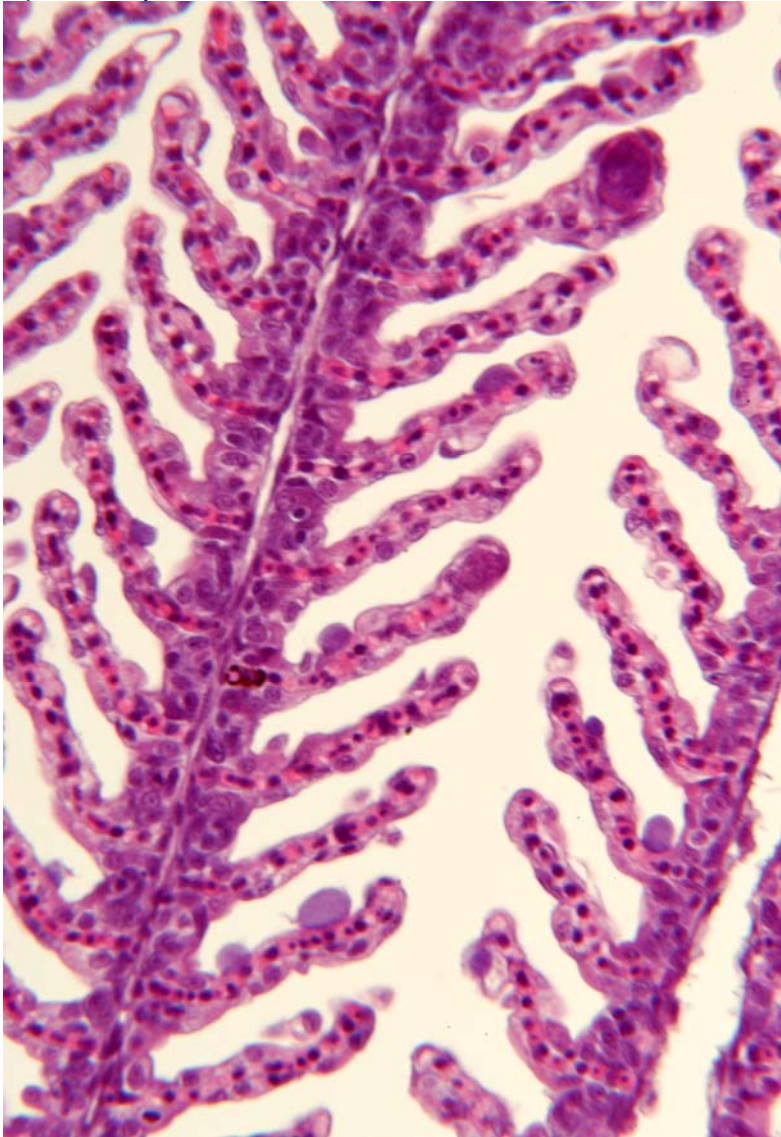


6. Chlamydia and Rickettsia

a. Epitheliocystis

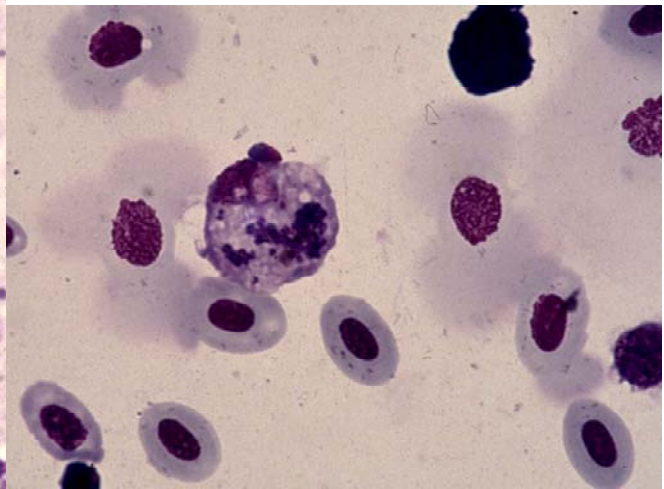
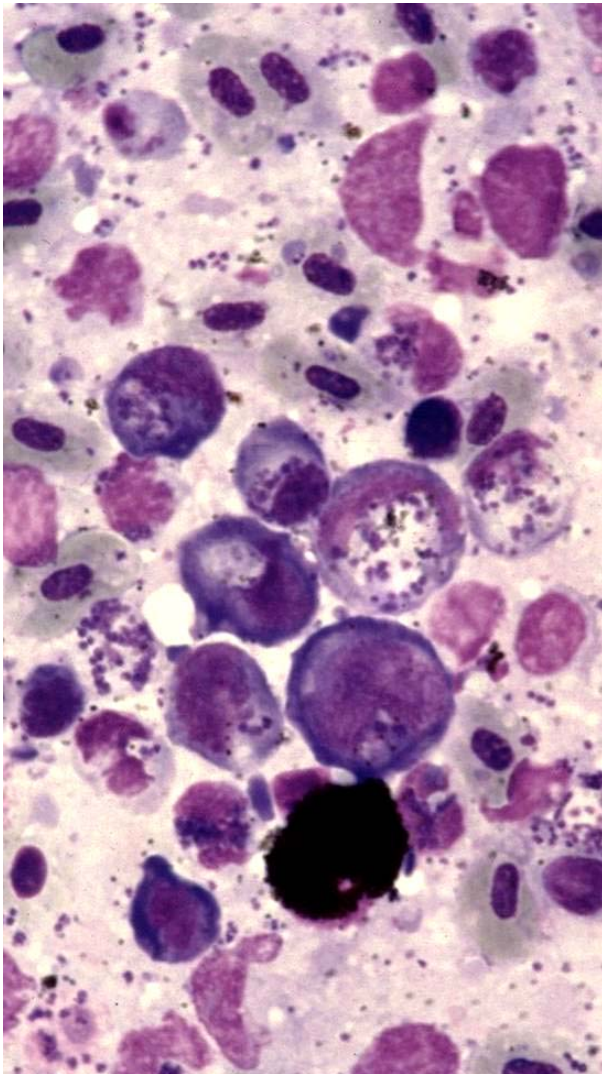
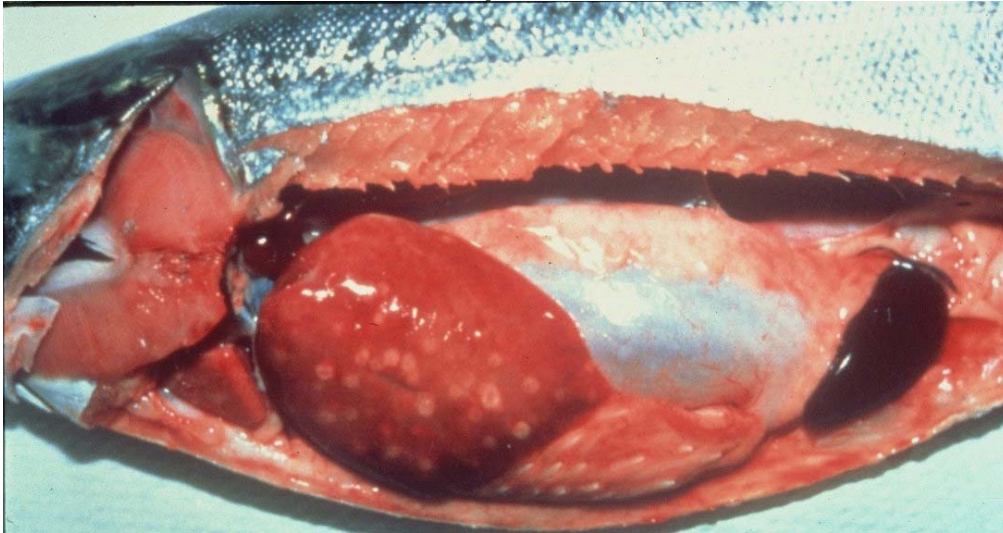


Epitheliocystis



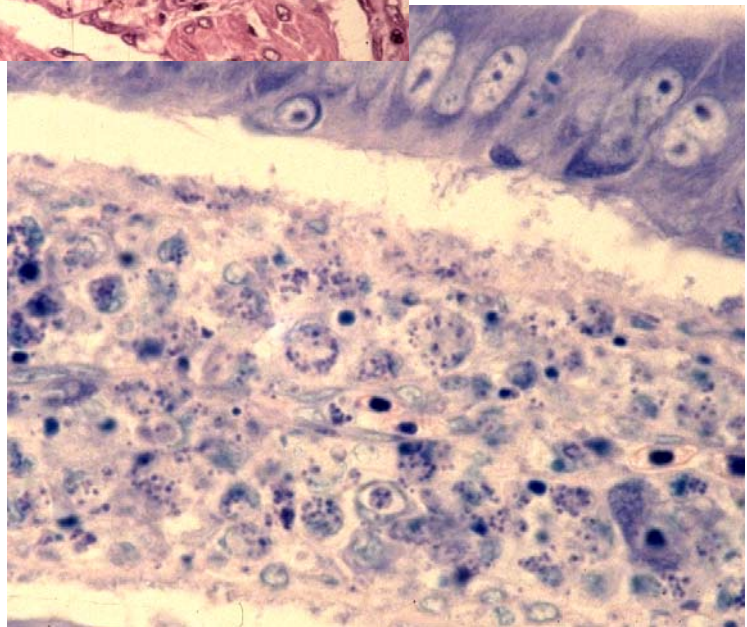
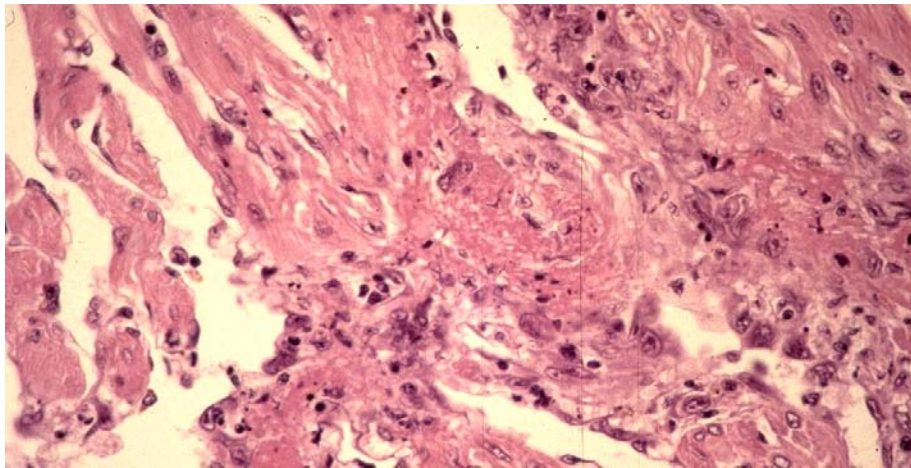
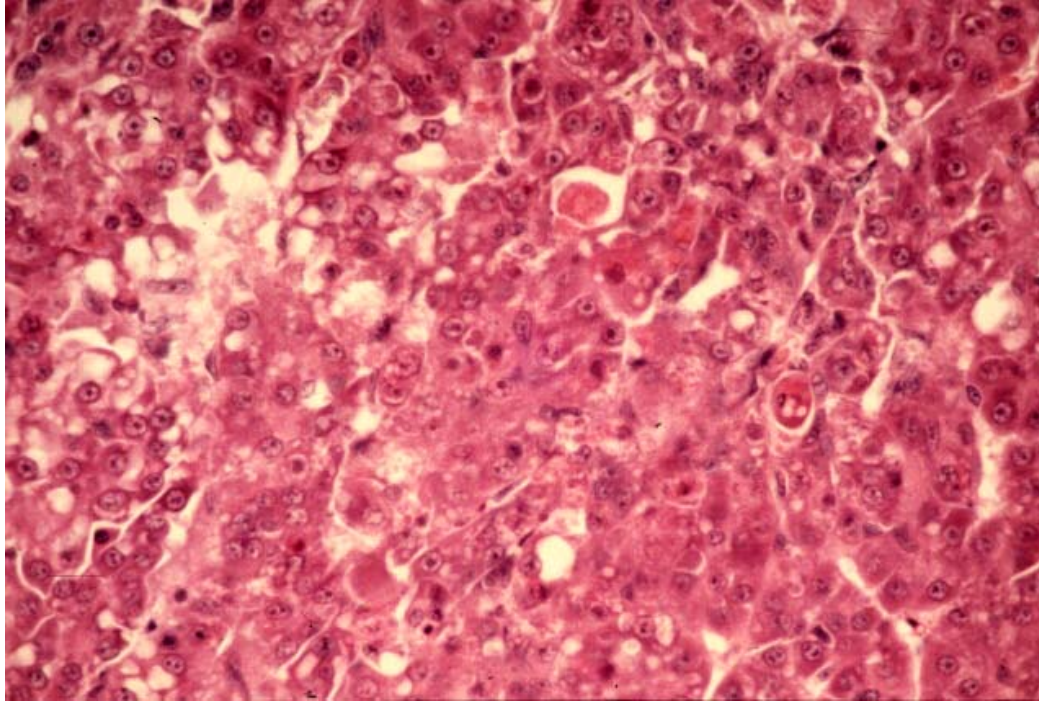
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b. Salmonid rickettsia septicemia - *Piscirickettsia salmonis*



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Fish Histology and Histopathology

Piscirickettsia salmonis



7. DRIPs

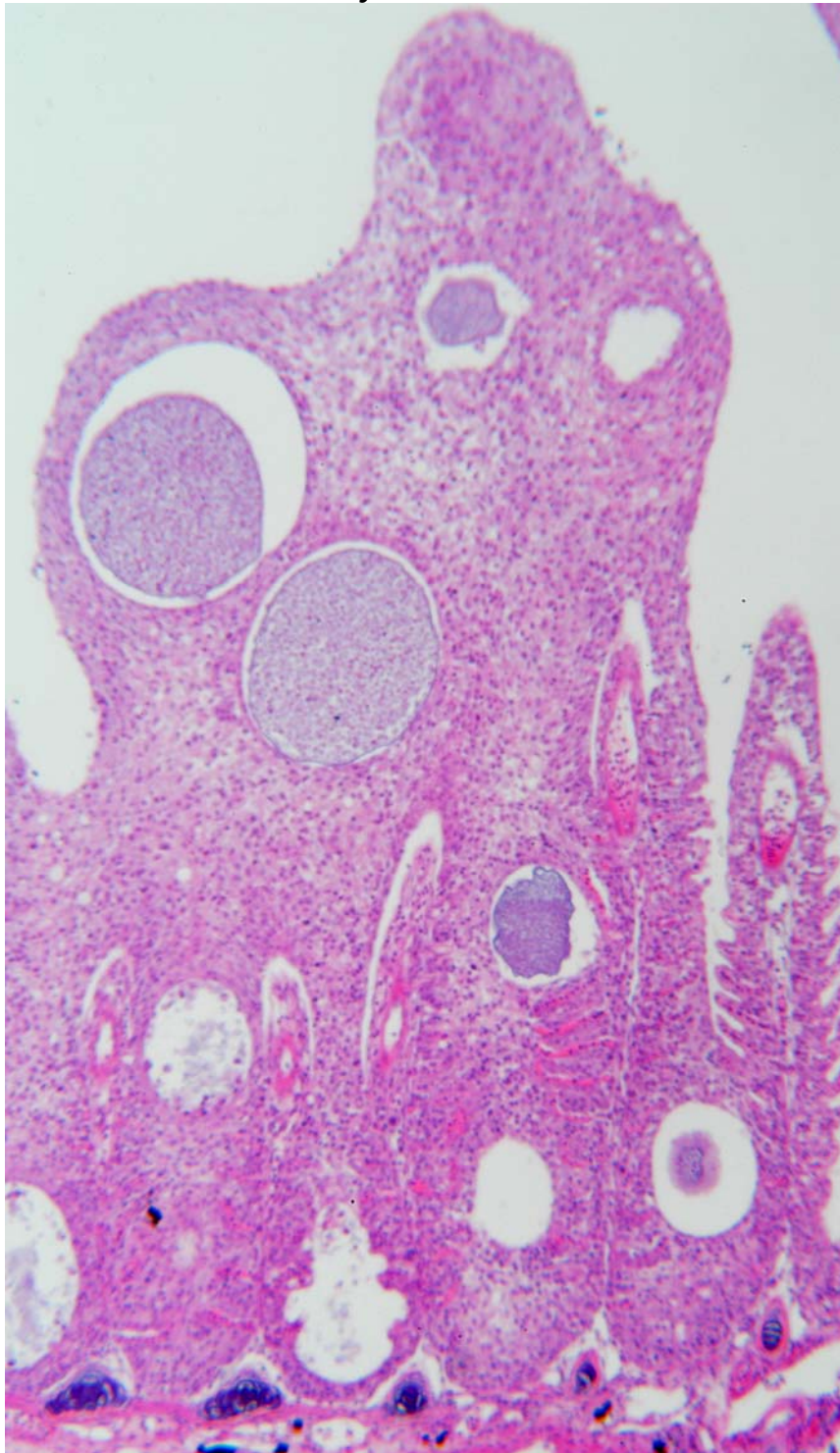
Dermocystidium salmonis

Rosette agent - *Sphaerothecum destruens*

Ichthyophonus hoferi

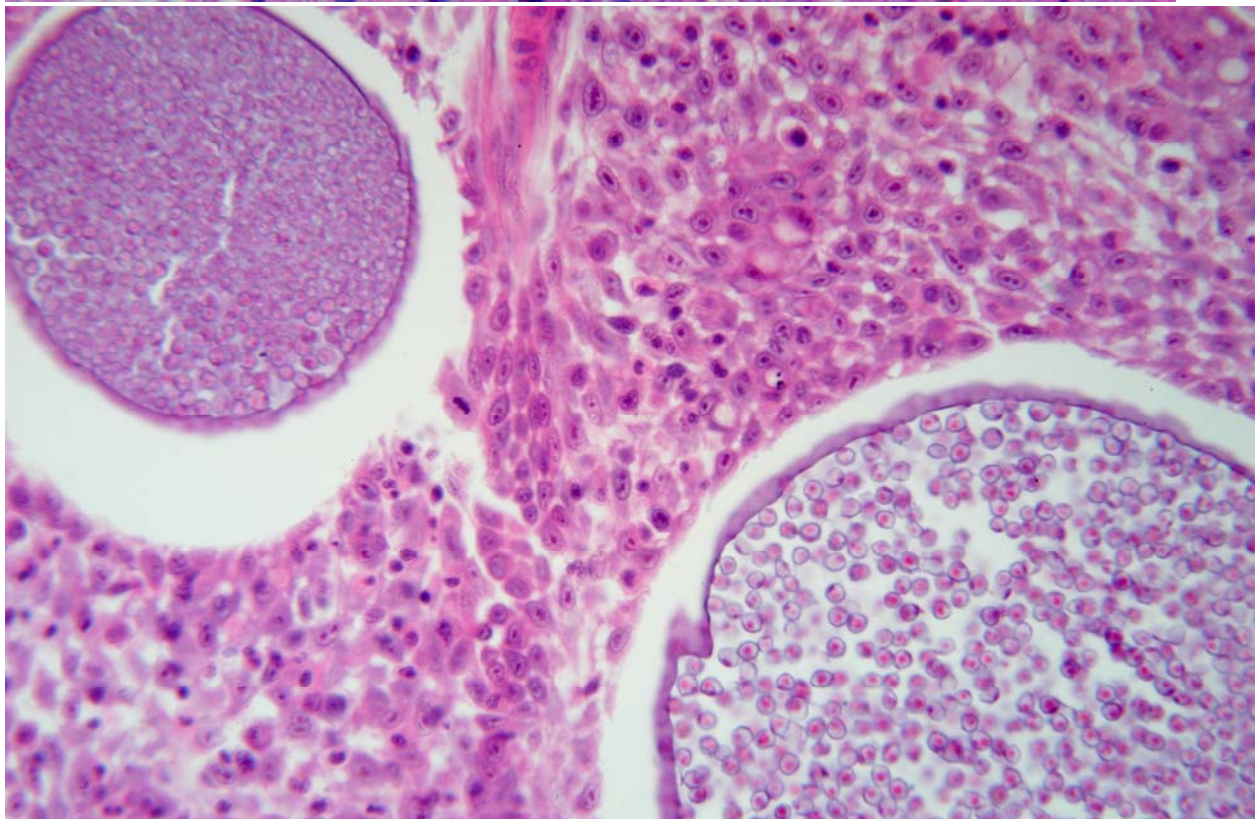
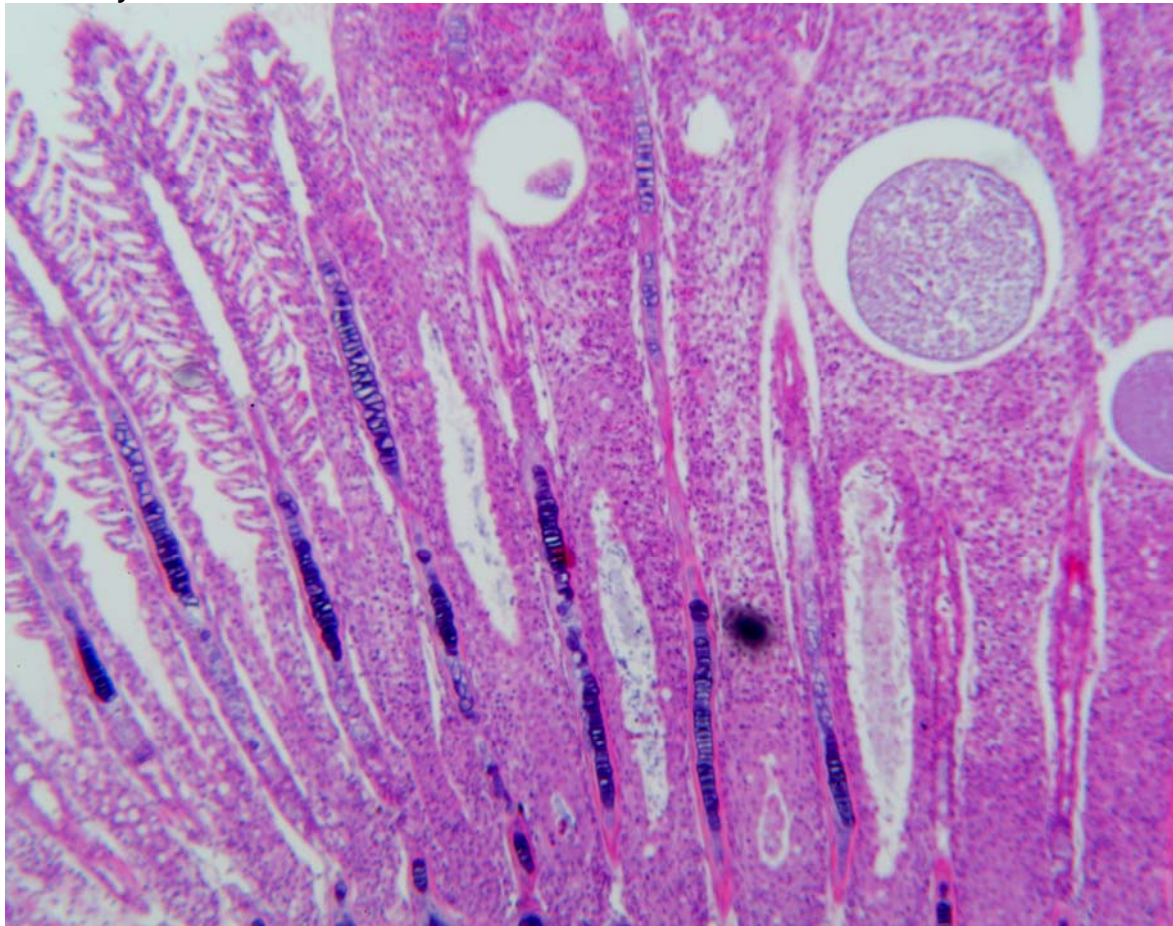
Psorospermium haeckelii

a. *Dermocystidium*



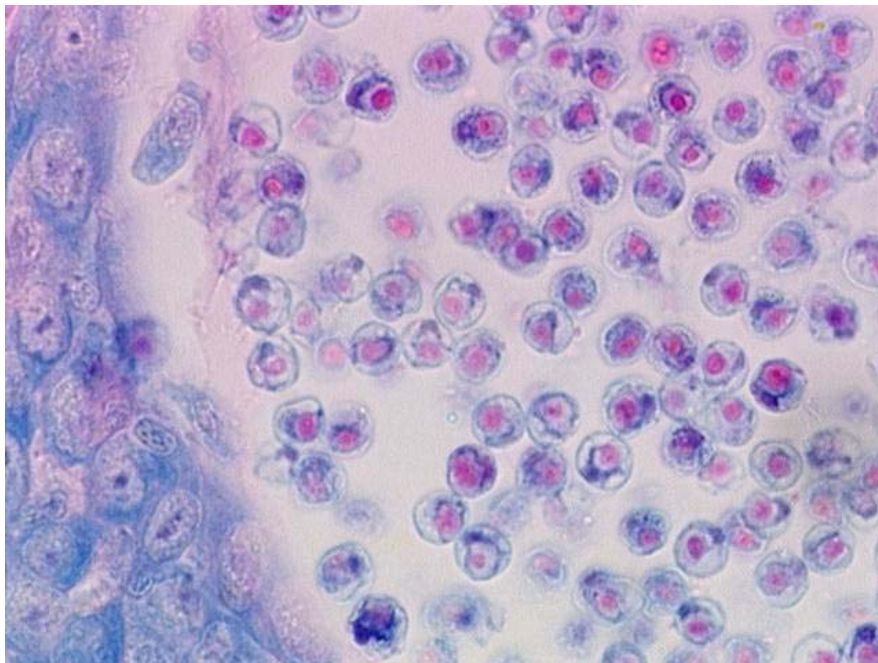
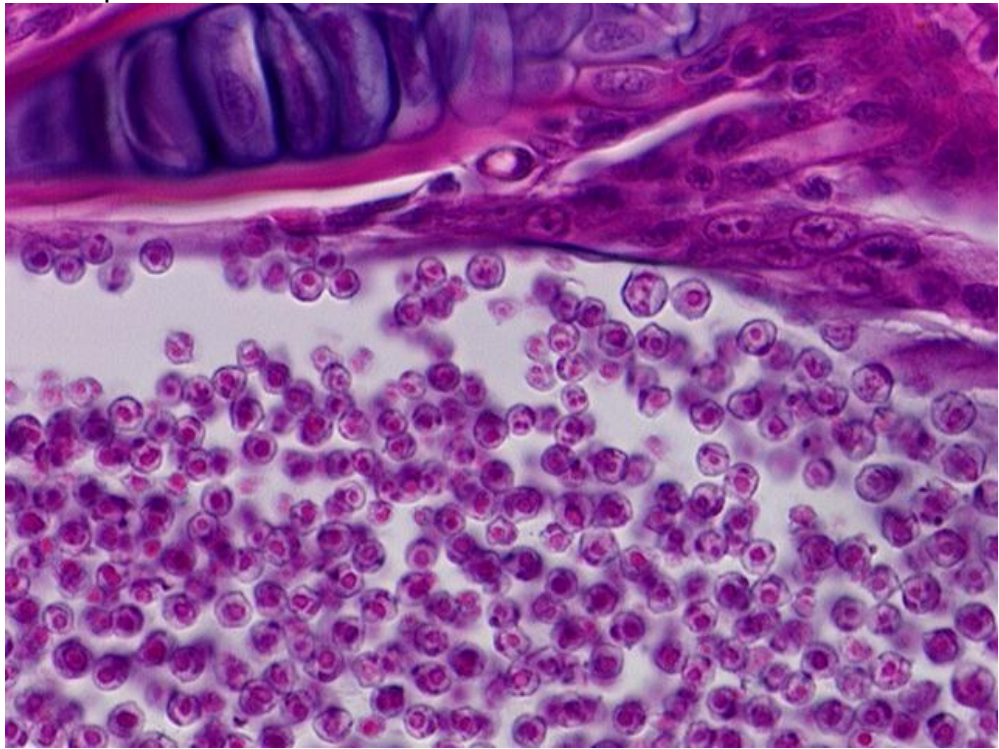
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Dermocystidium



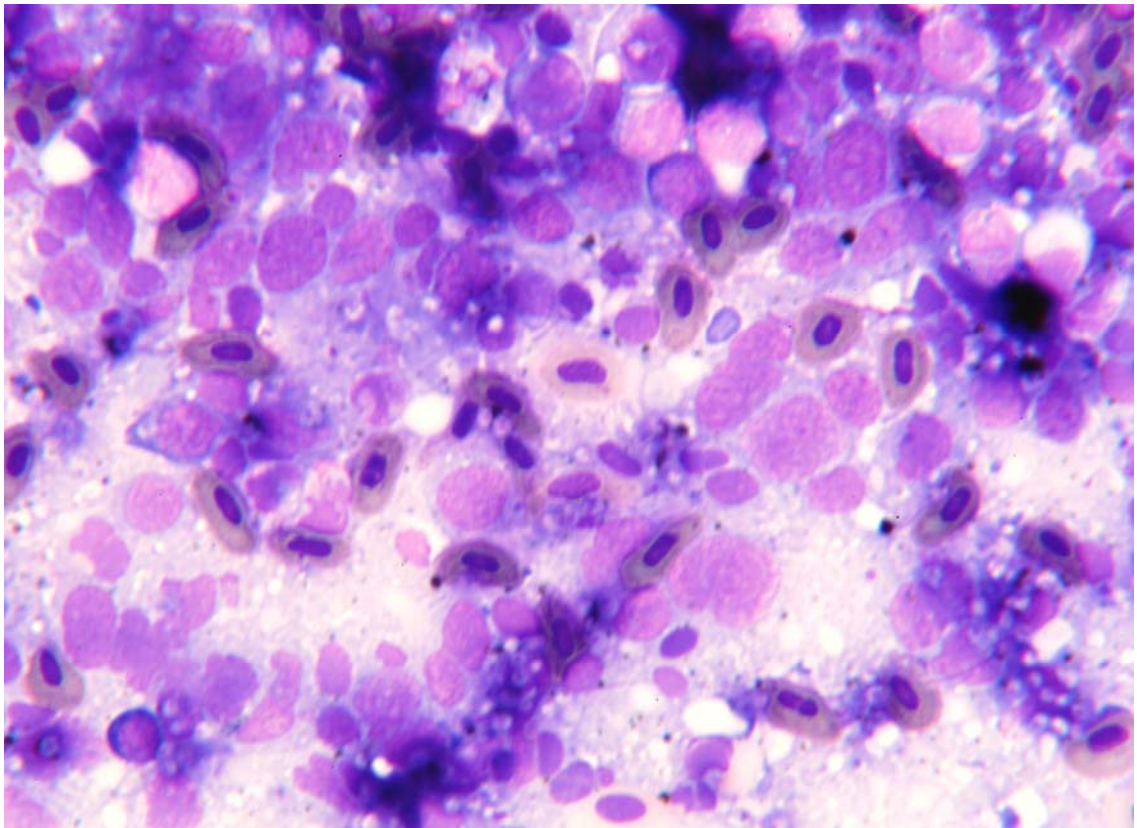
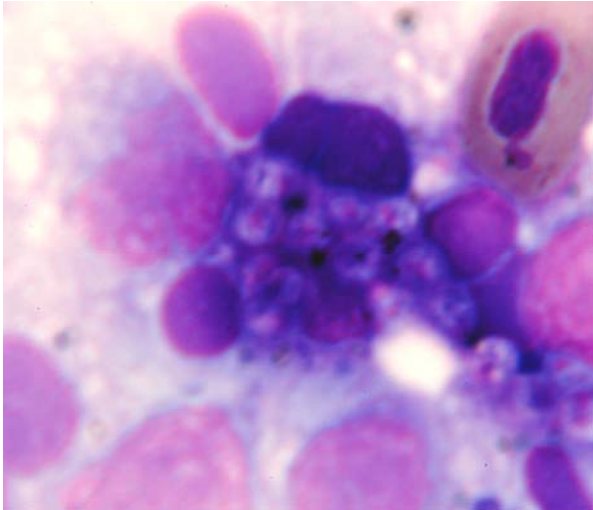
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Dermocystidium – vacuoplast

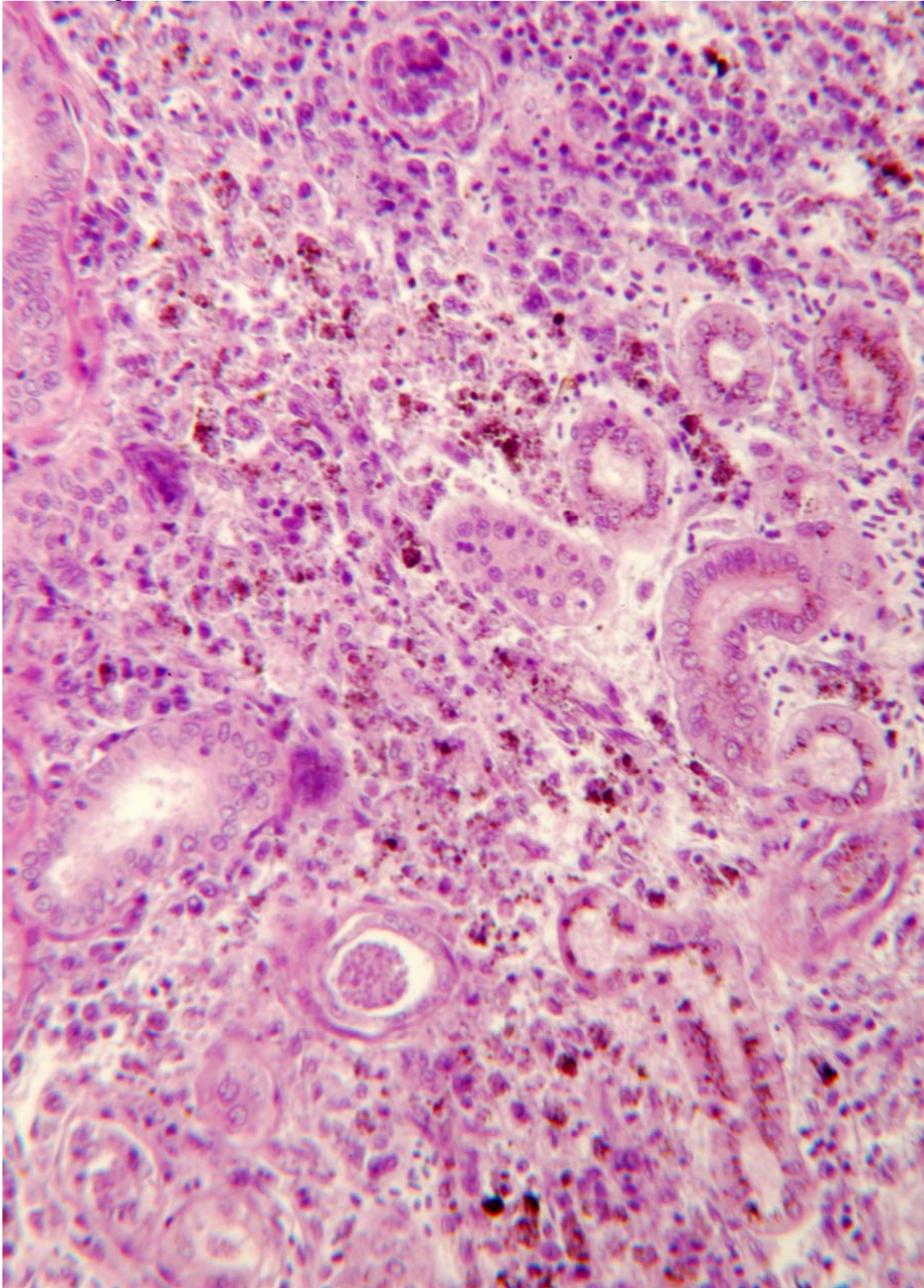


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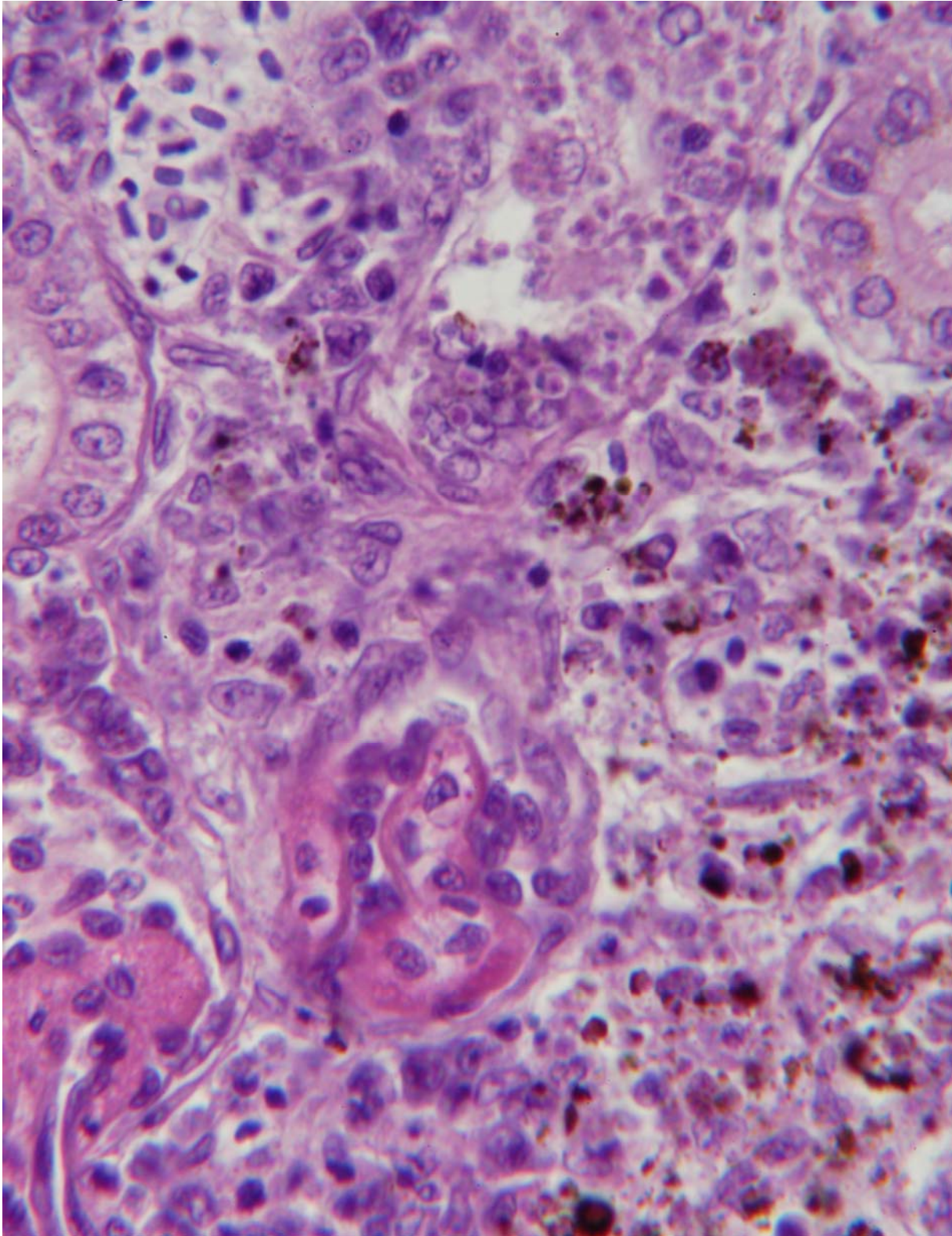
b. Rosette agent - *Sphaerothecum destruens*



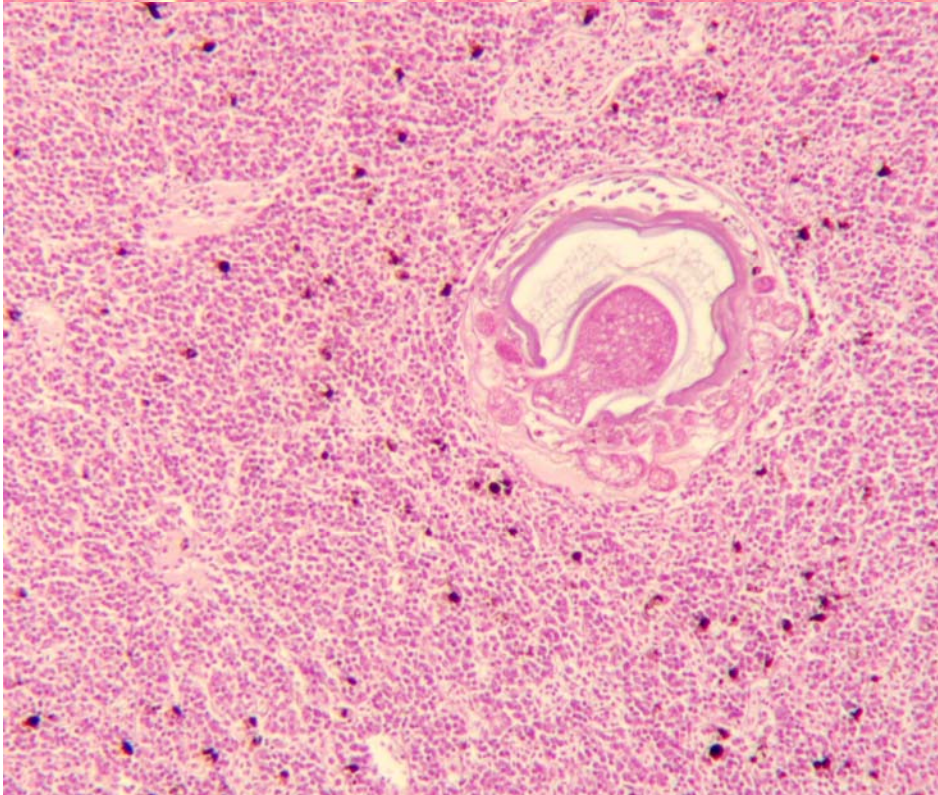
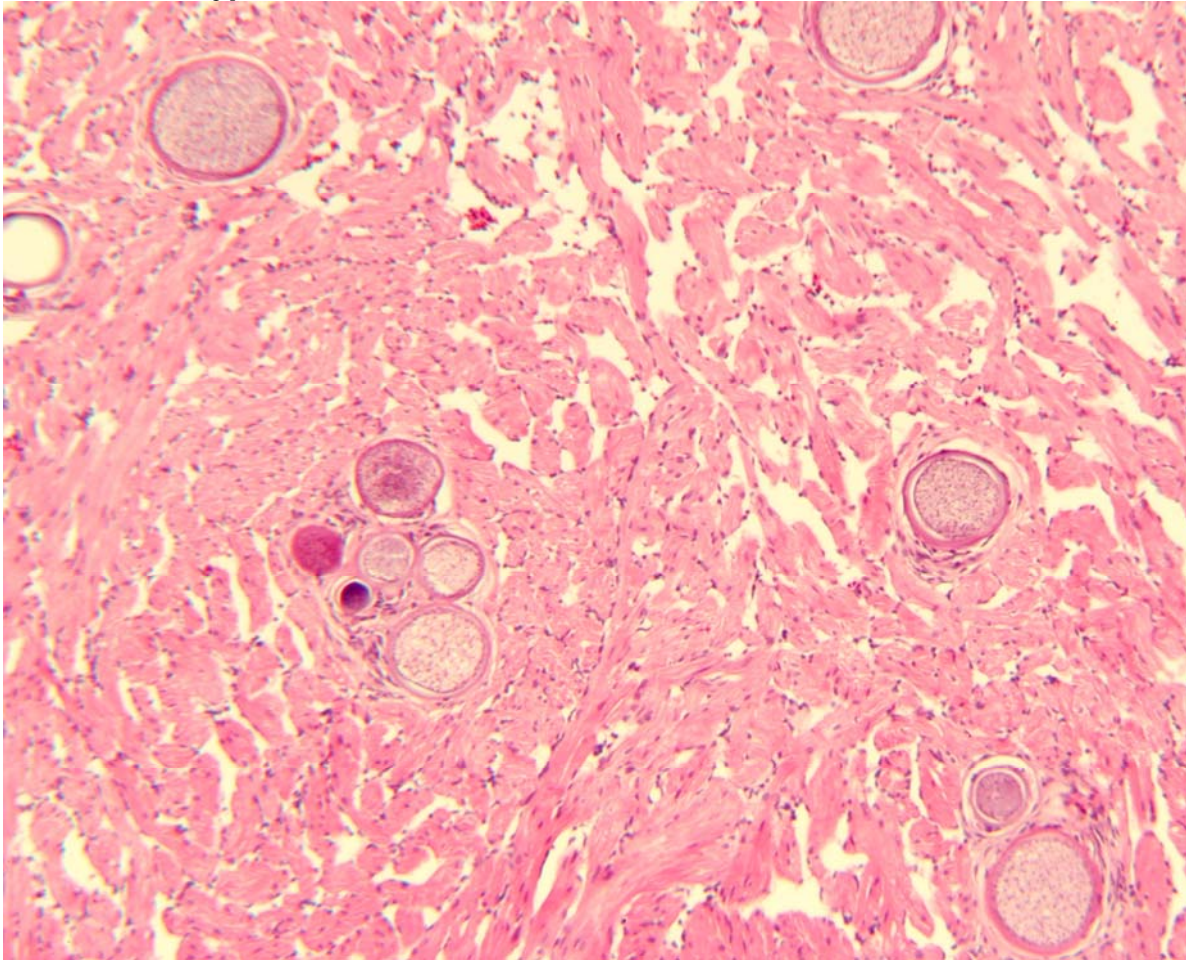
Rosette agent



Rosette Agent

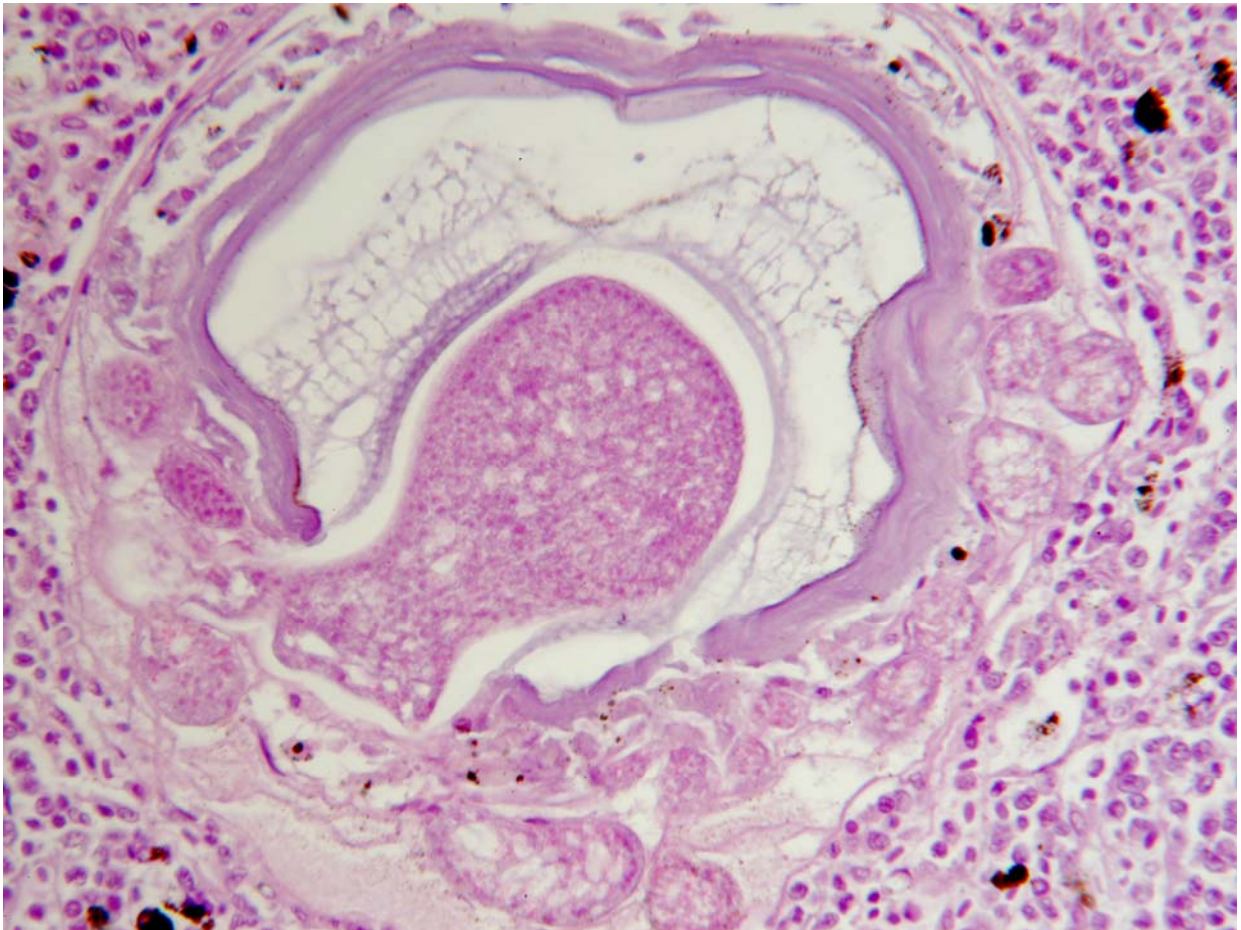
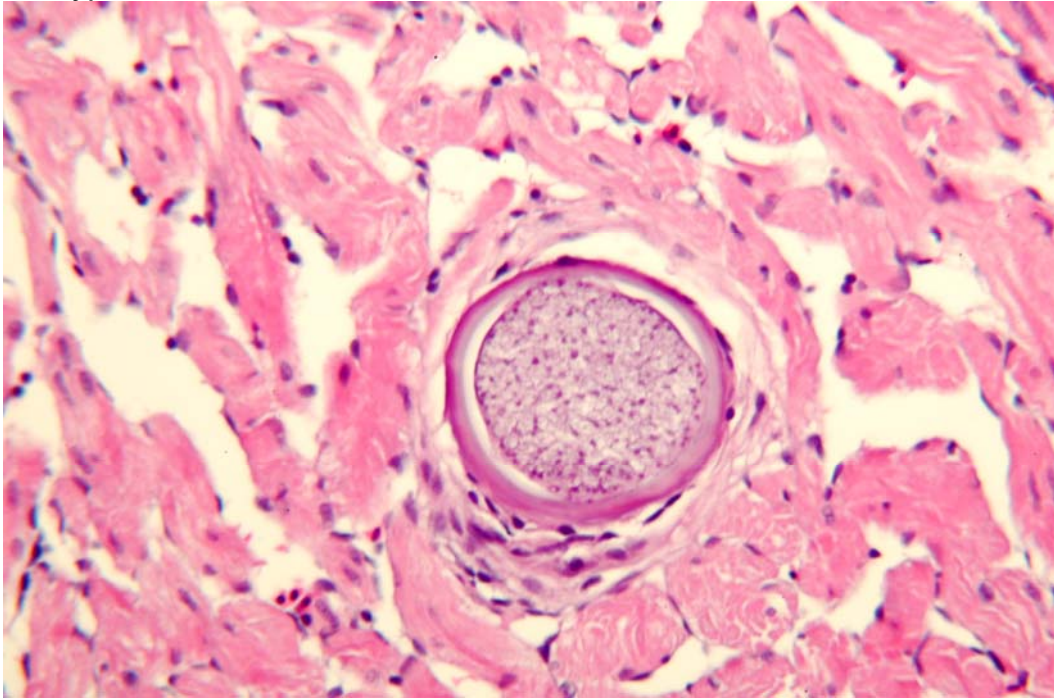


c. Ichthyophonus hoferi

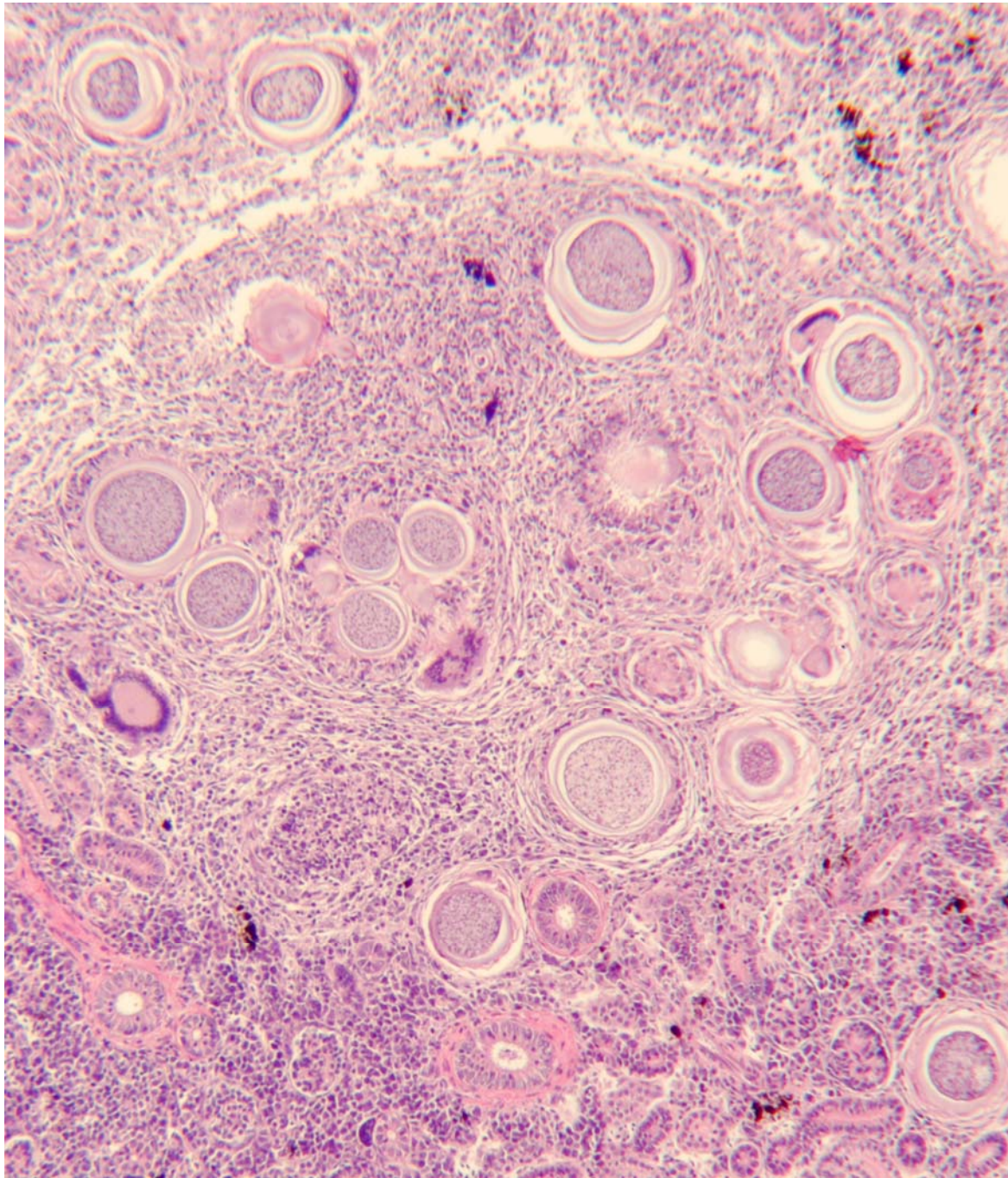


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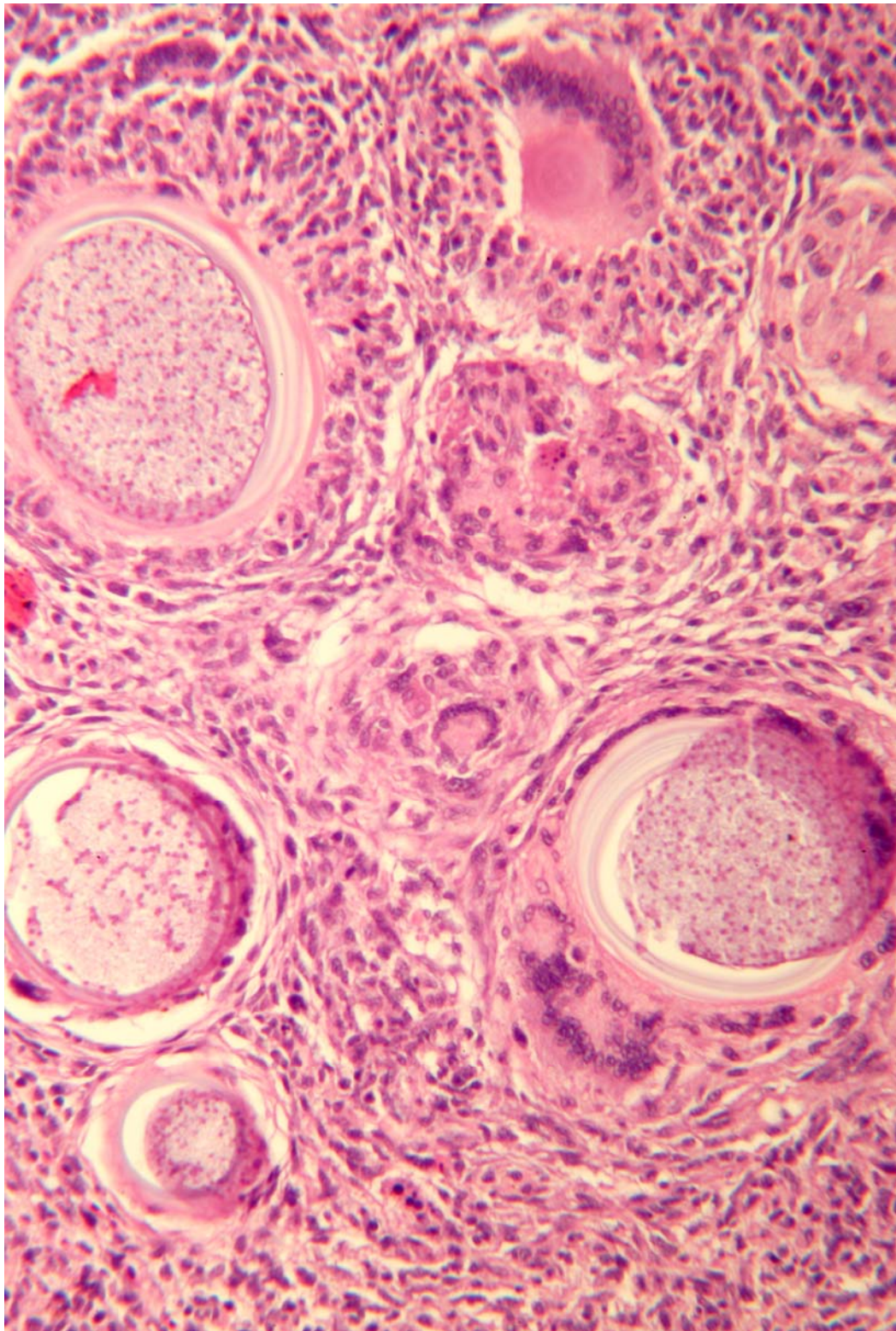
Ichthyophonus hoferi



Ichthyophonus hoferi

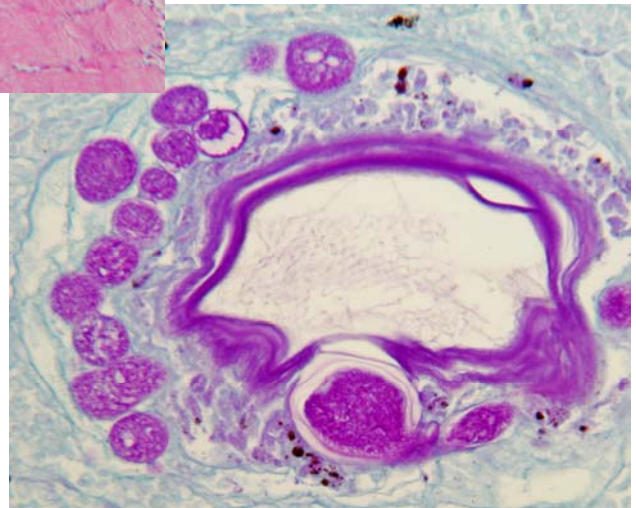
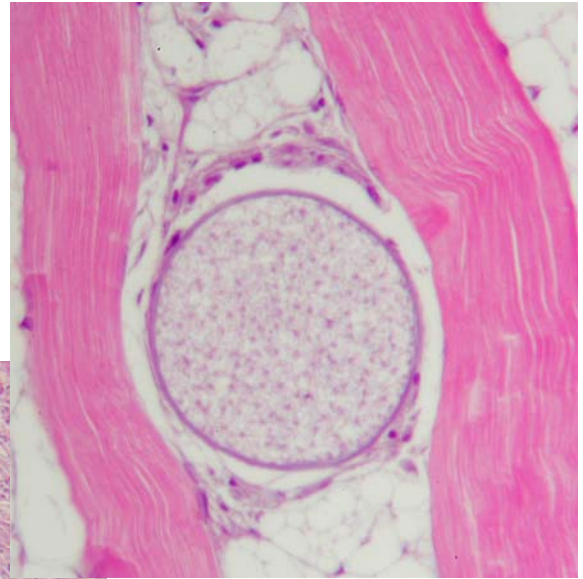
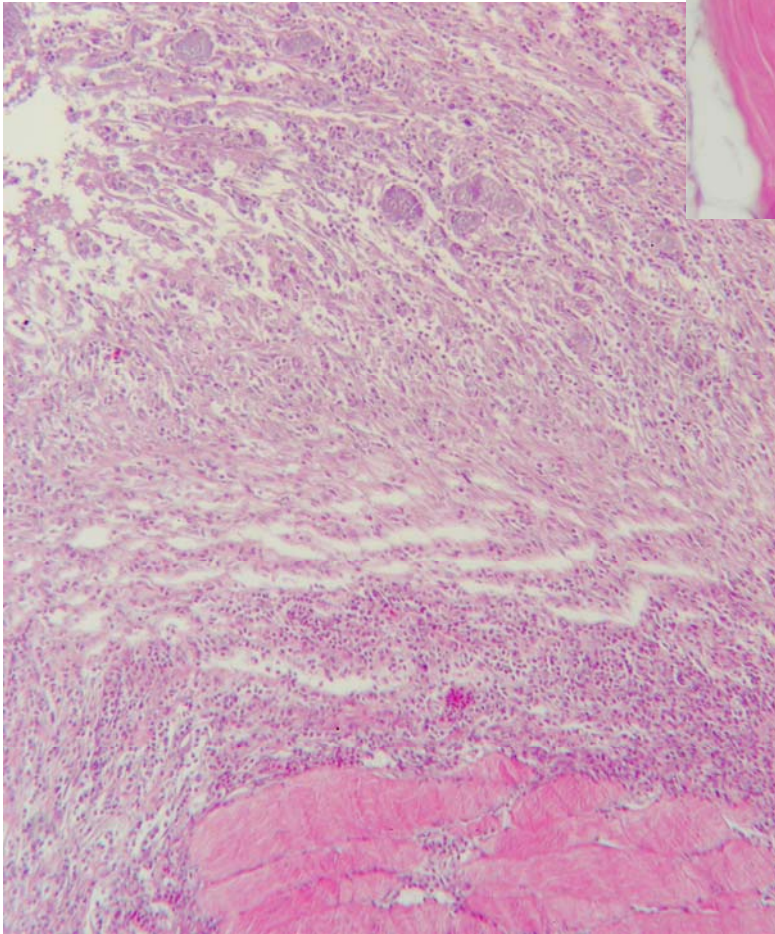


Ichthyophonus hoferi



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Ichthyophonus hoferi



Non- Infectious Diseases

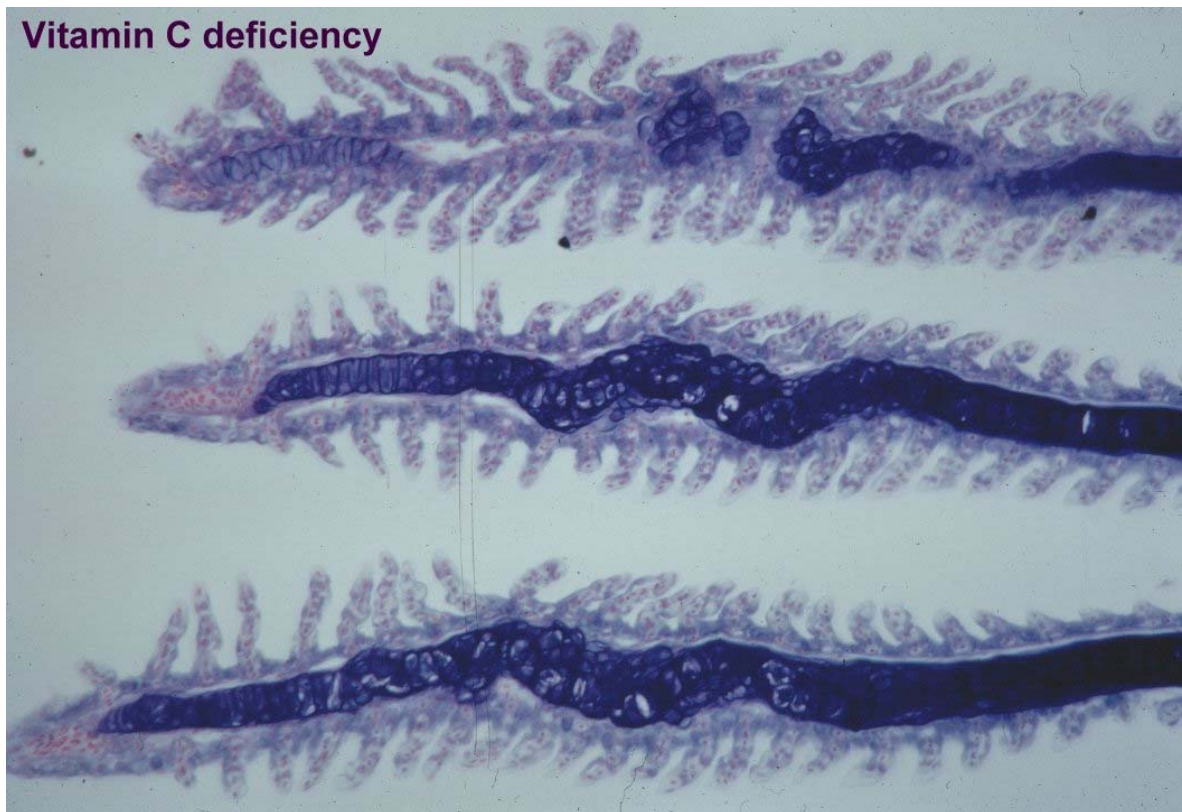
I. Nutritional

- A. Vitamin C deficiency- skeletal deformations
- B. Pantothenic acid deficiency- nutritional gill disease
- C. Folic acid deficiency- anemia/blood disorder
- D. Pyridoxine Deficiency
- E. Lipoid Liver Disease
- F. Vitamin E Deficiency
- G. Magnesium Deficiency
- H. Phosphorus Deficiency
- I. Zinc Deficiency - Cataract

II. Other

- A. Dermatitis/Steatitis
- B. Goiter
- C. Nephrocalcinosis
- D. Visceral Granuloma

A. Vitamin C Deficiency

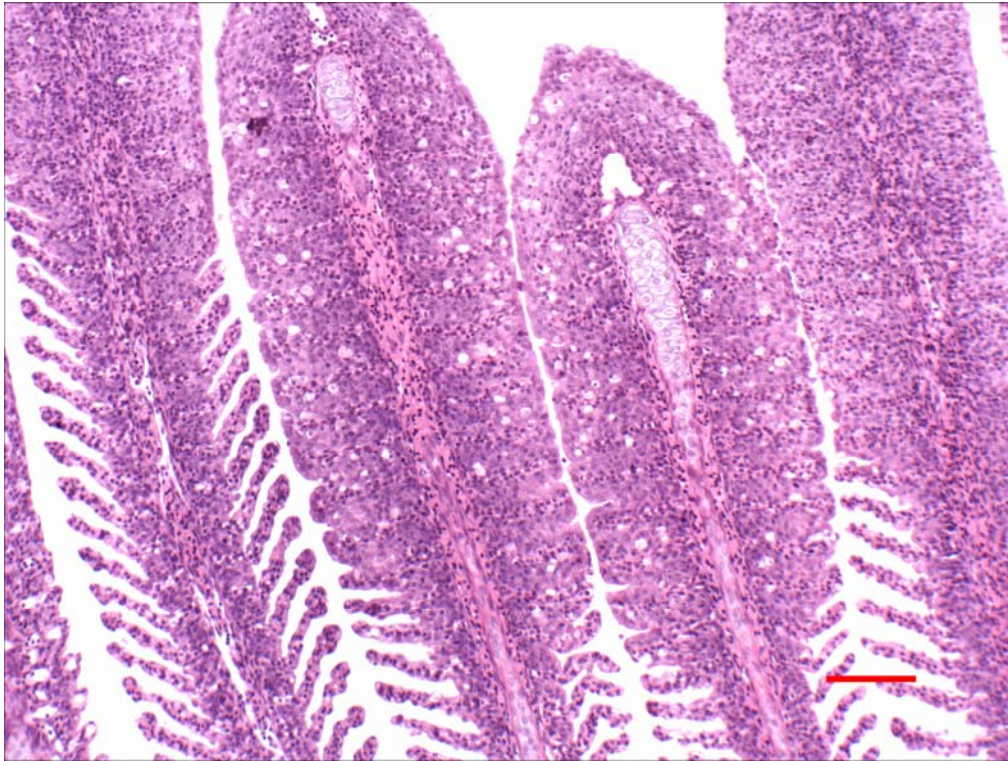




B. Pantothenic Acid Deficiency

Rainbow trout with classic nutritional gill disease due to pantothenic acid deficiency.
Note fusion of filaments

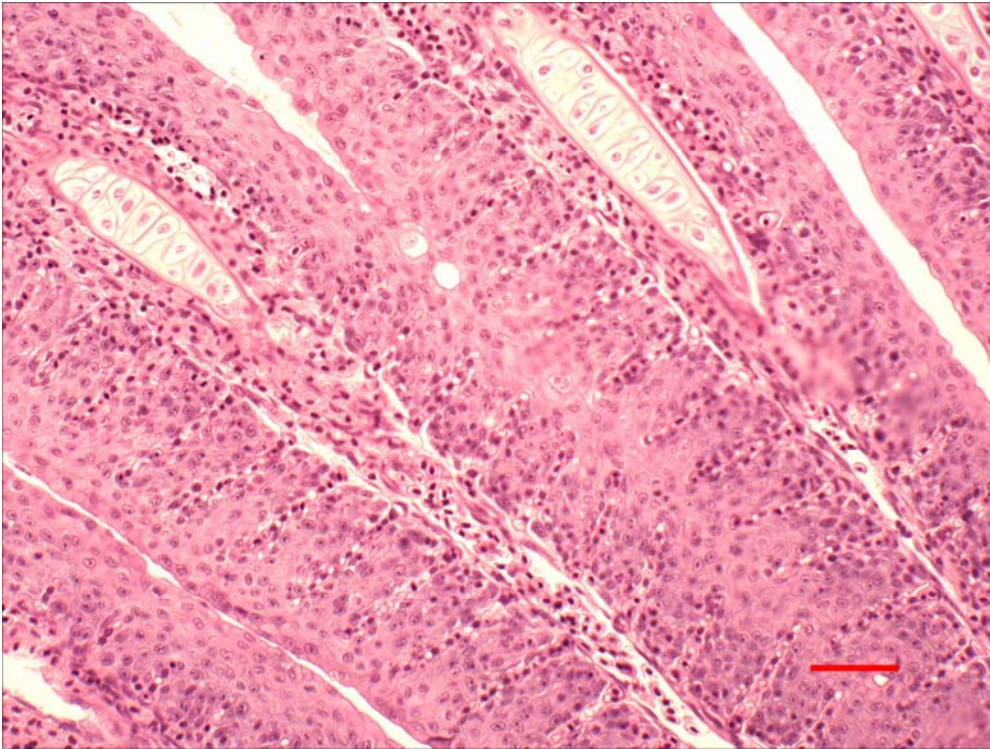




Pantothenic Acid deficiency-early stage of nutritional gill disease. Note fusion of lamellae at tips of filaments; more normal lamellae towards bases (bar = 100µm).



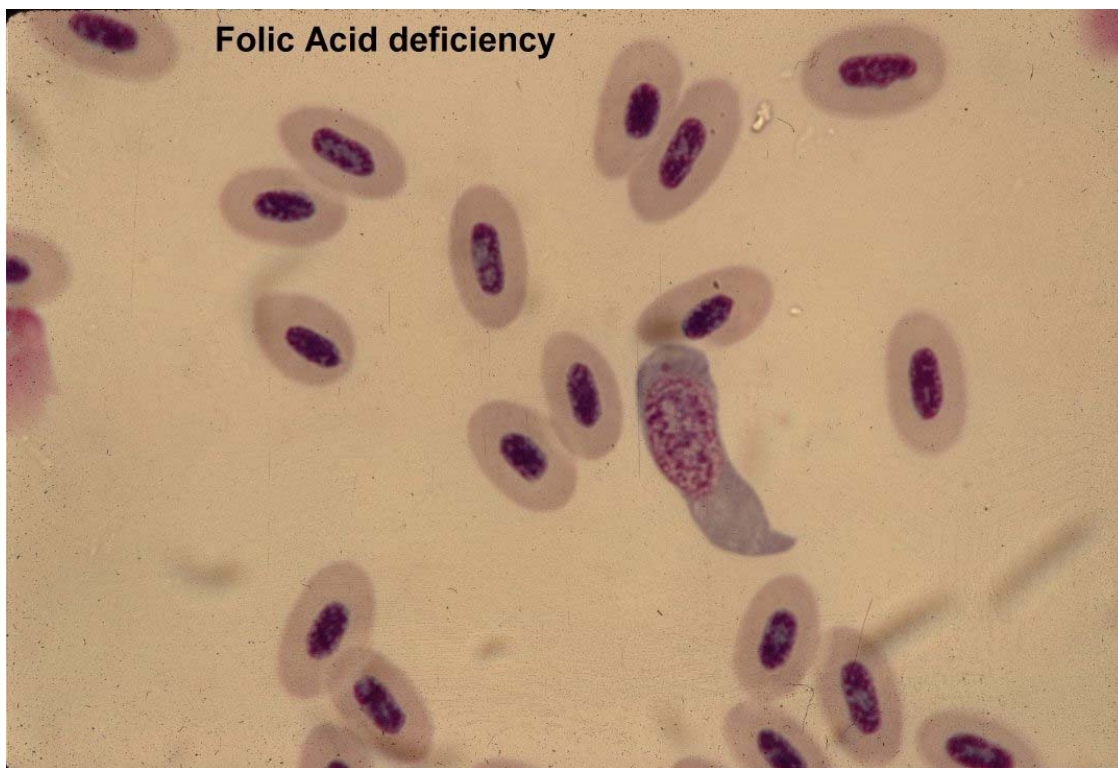
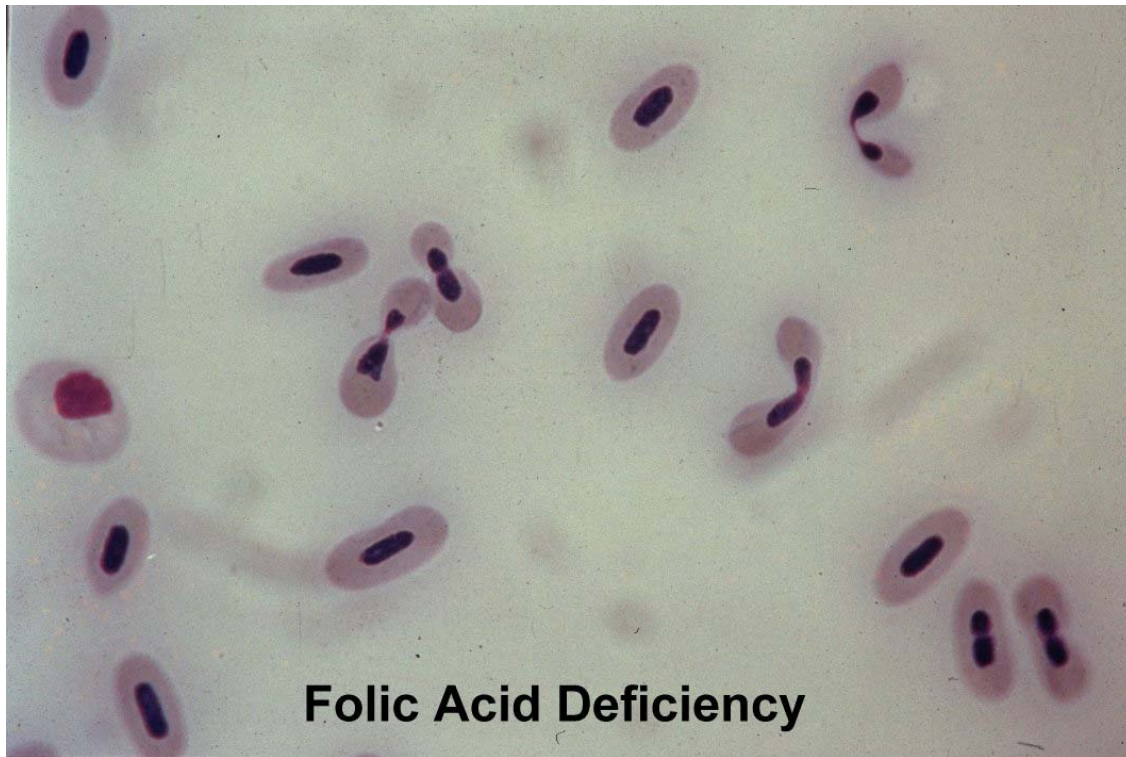
Pantothenic acid deficiency, severe nutritional gill disease. Gill filaments are severely fused (bar = 100µm).



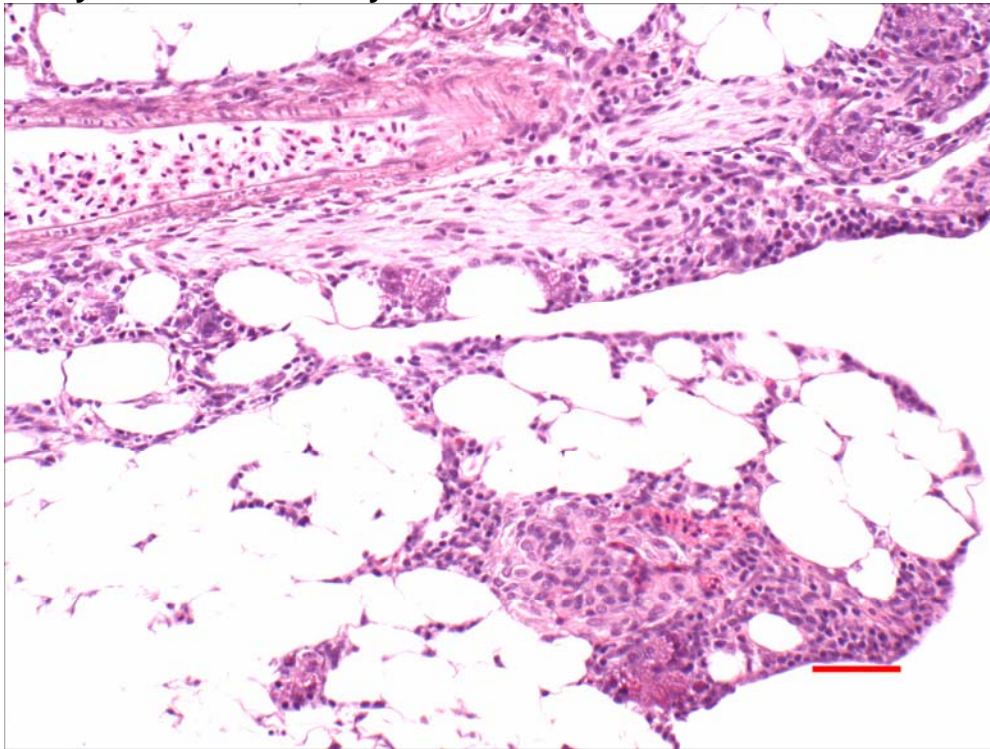
Pantothenic acid deficiency, severe fusion of lamellae and filaments (nutritional gill disease; bar = 50 μ m).

C. Folic Acid Deficiency

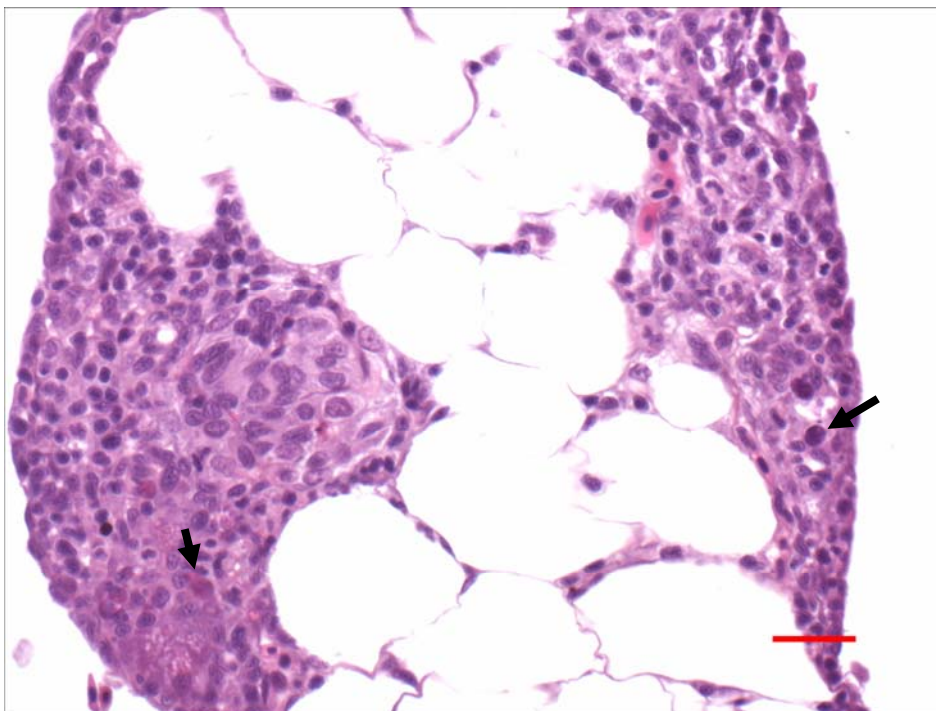




D. Pyridoxine Deficiency



Pyridoxine deficient – pancreatitis and mild degeneration of pancreatic acinar cells. (bar = 50 μ m).

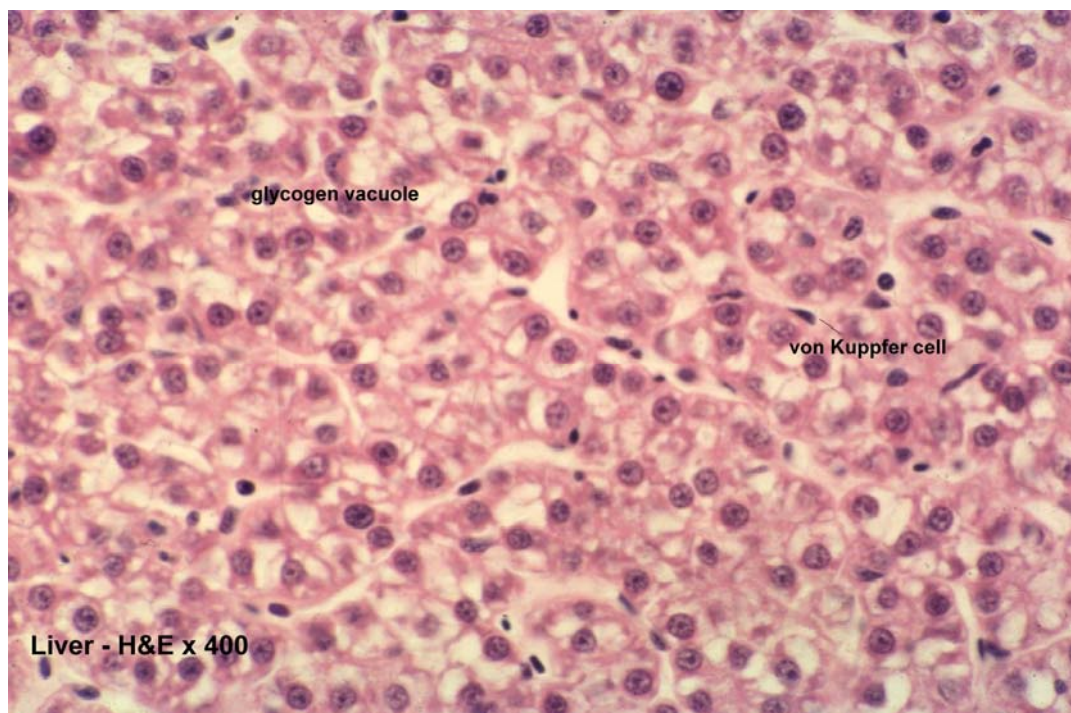


Pyridoxine deficient – pancreatitis and degeneration of pancreatic cells (arrow). (bar = 50 μ m).

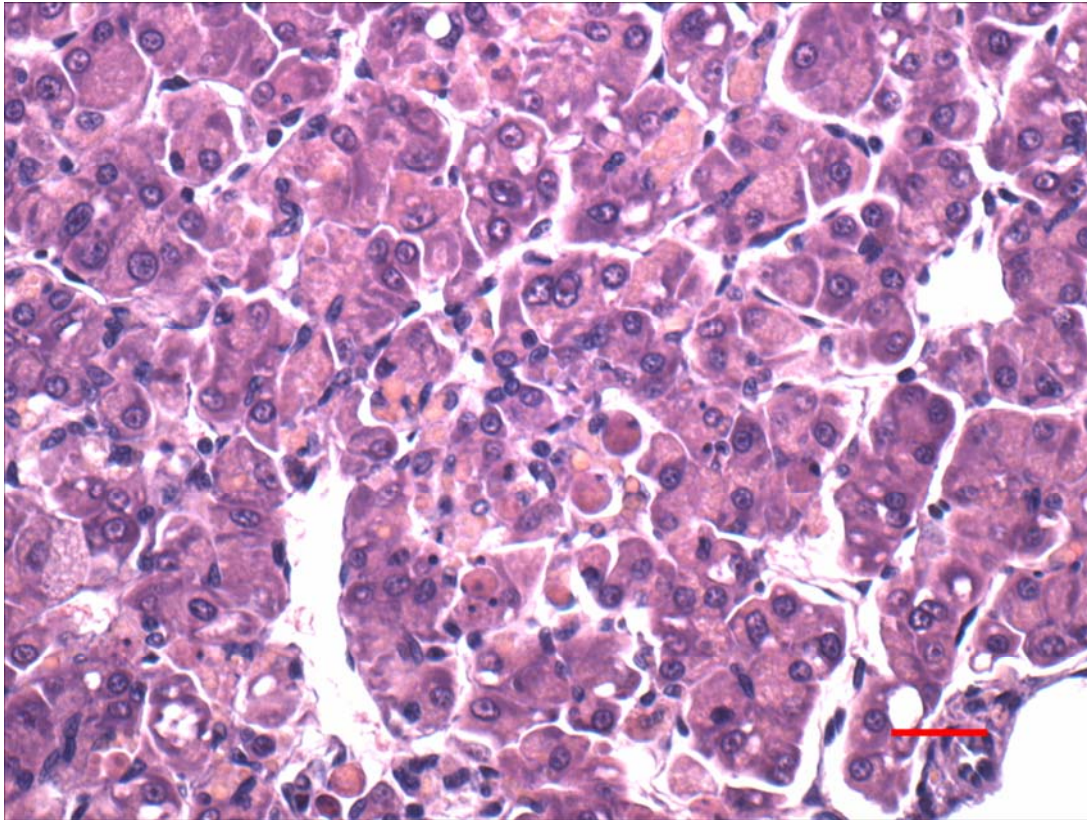
E. Lipoid Liver Disease



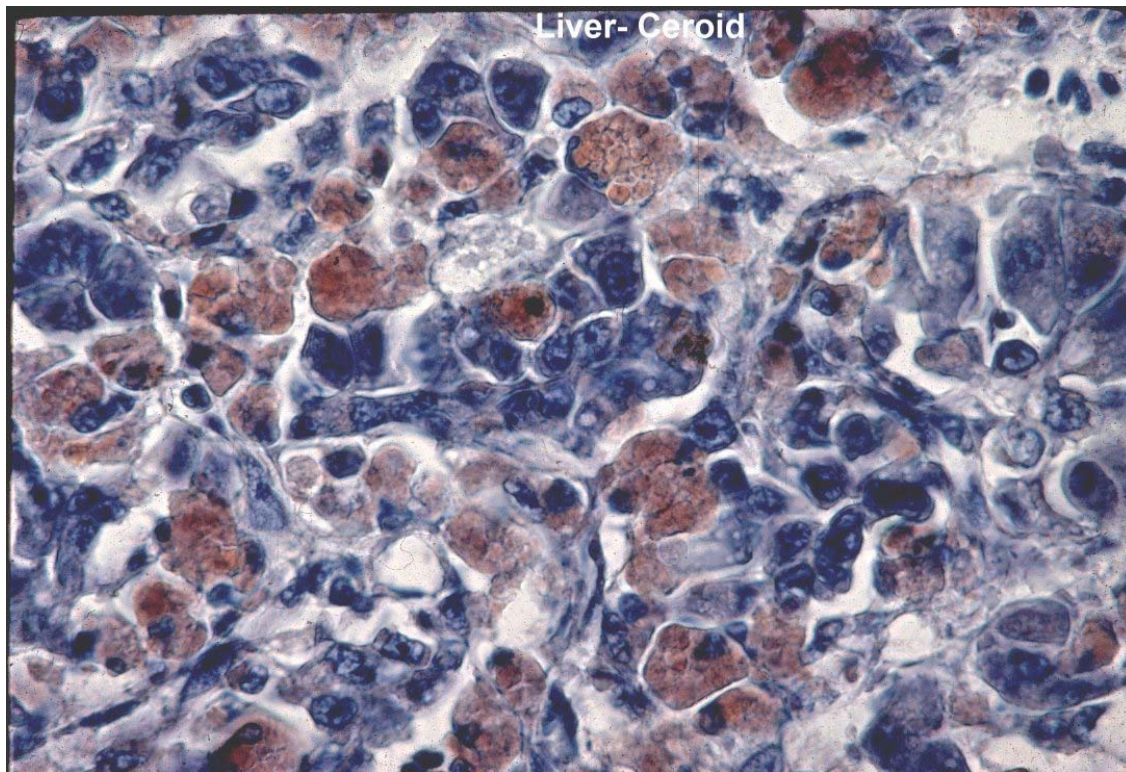
LLD note anemia, yellowish liver and swollen spleen. Due to rancid feed.



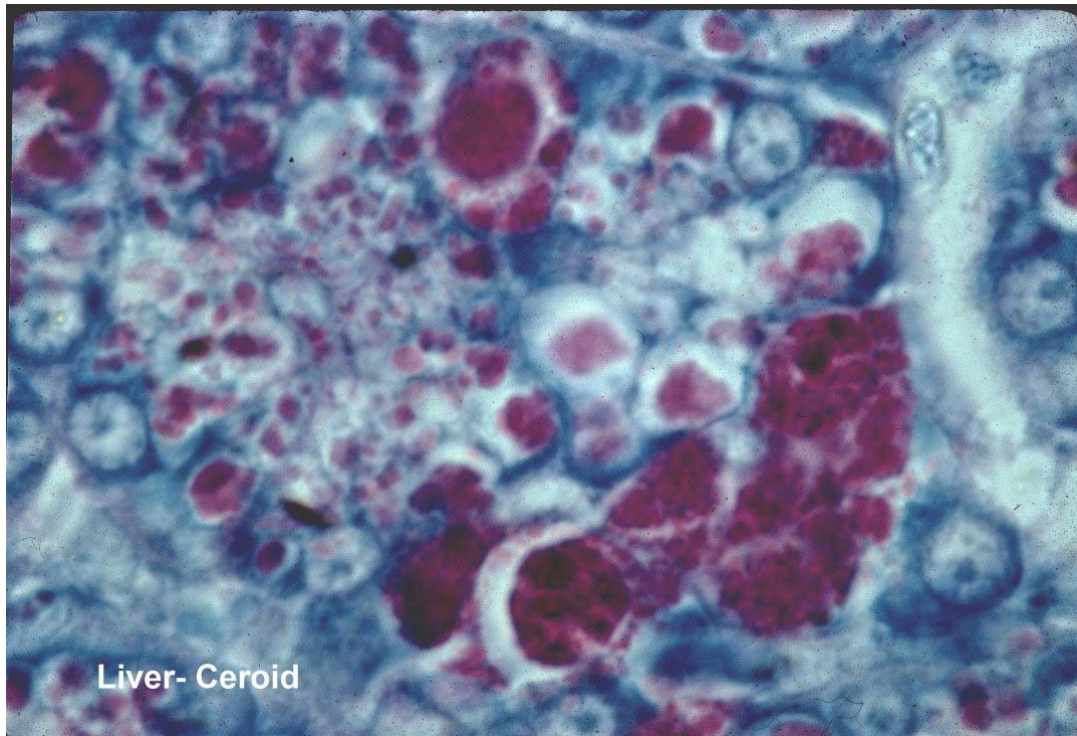
Normal liver which contains mostly glycogen vacuoles.



Ceroidosis in liver of trout fed fish meal diet lacking vitamin premix.(bar = 25µm).

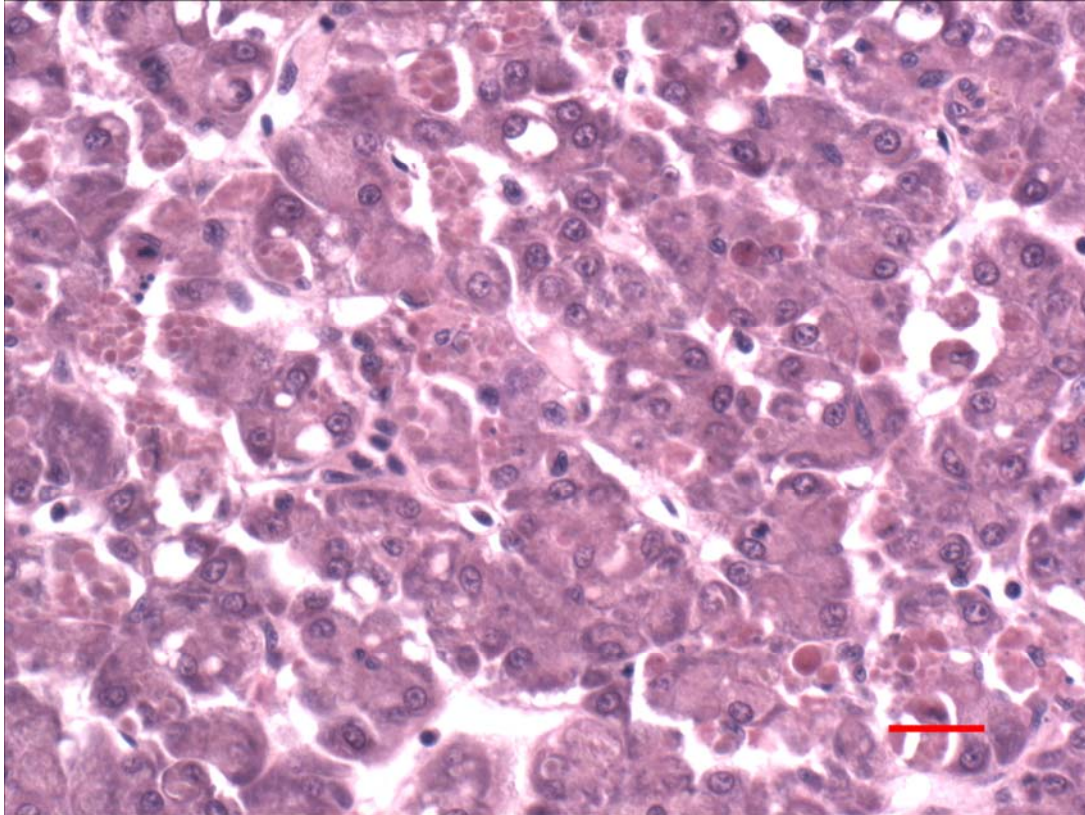


Ceroid in hepatocytes of fish fed rancid feed. Sudan III staining for lipid.

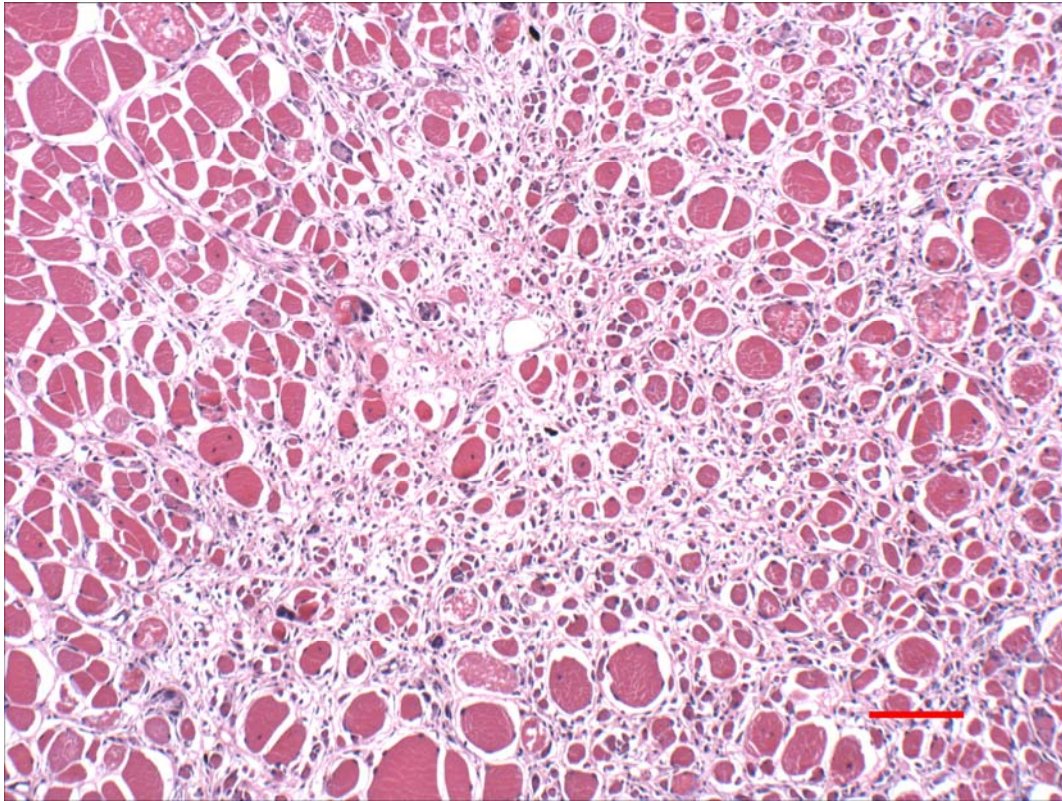


Ceroid in hepatocytes of fish fed rancid feed. Oil Red O staining for lipid.

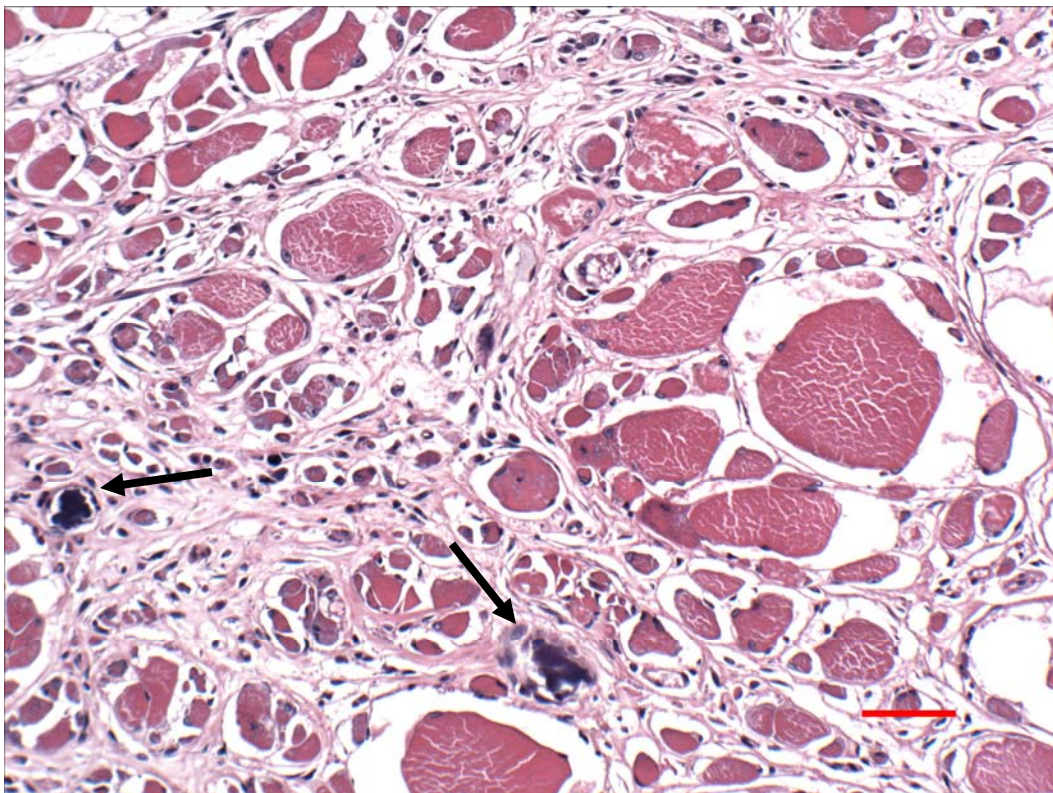
F. Vitamin E Deficiency



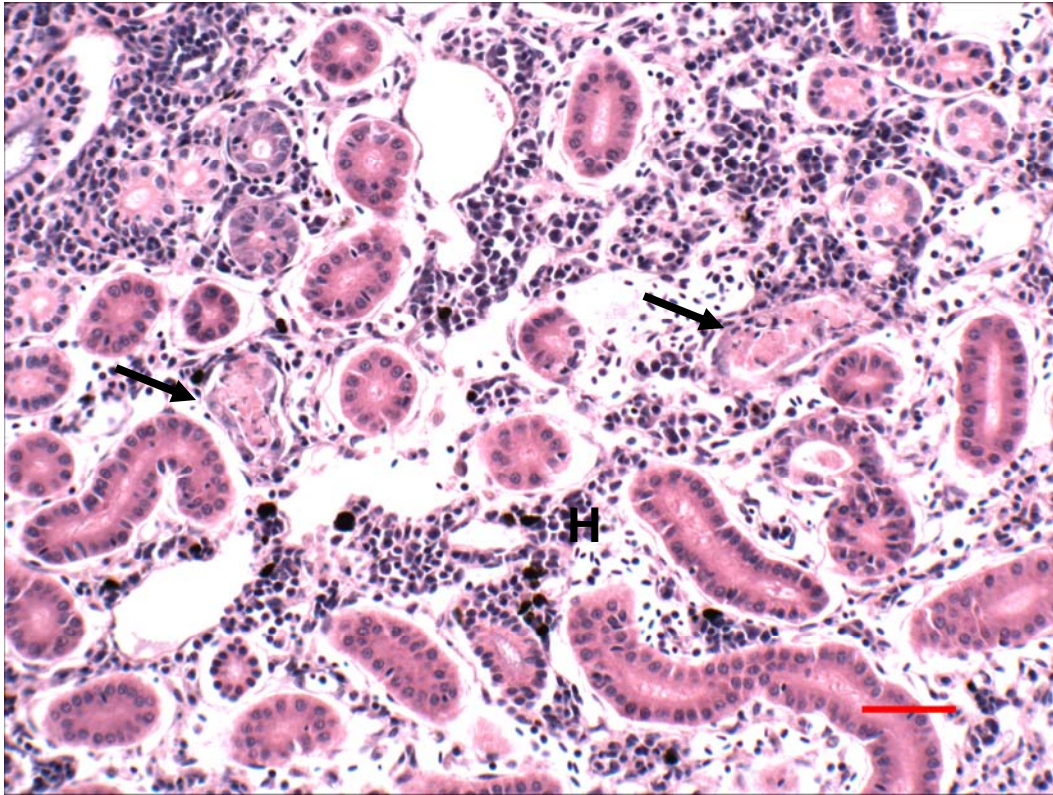
Vitamin E deficiency. Note ceroid scattered throughout.(bar = 25µm).



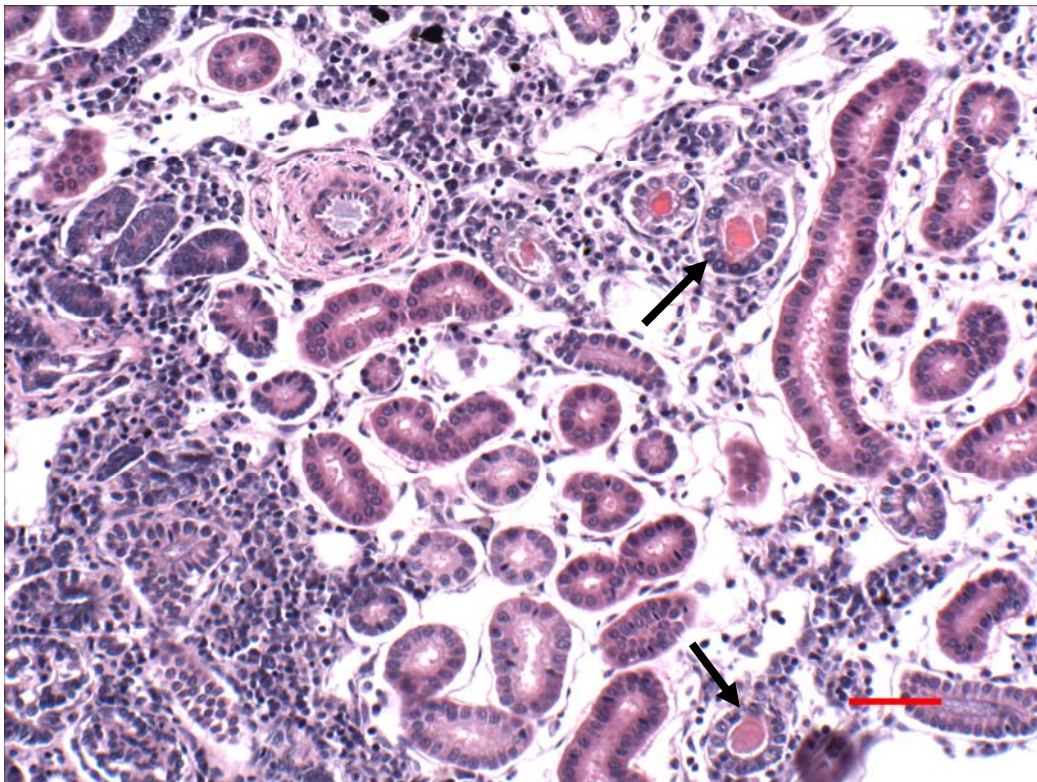
Vitamin E deficiency – severe muscle degeneration, atrophy and fibrosis of degenerate muscle tissue (bar = 100 μ m).



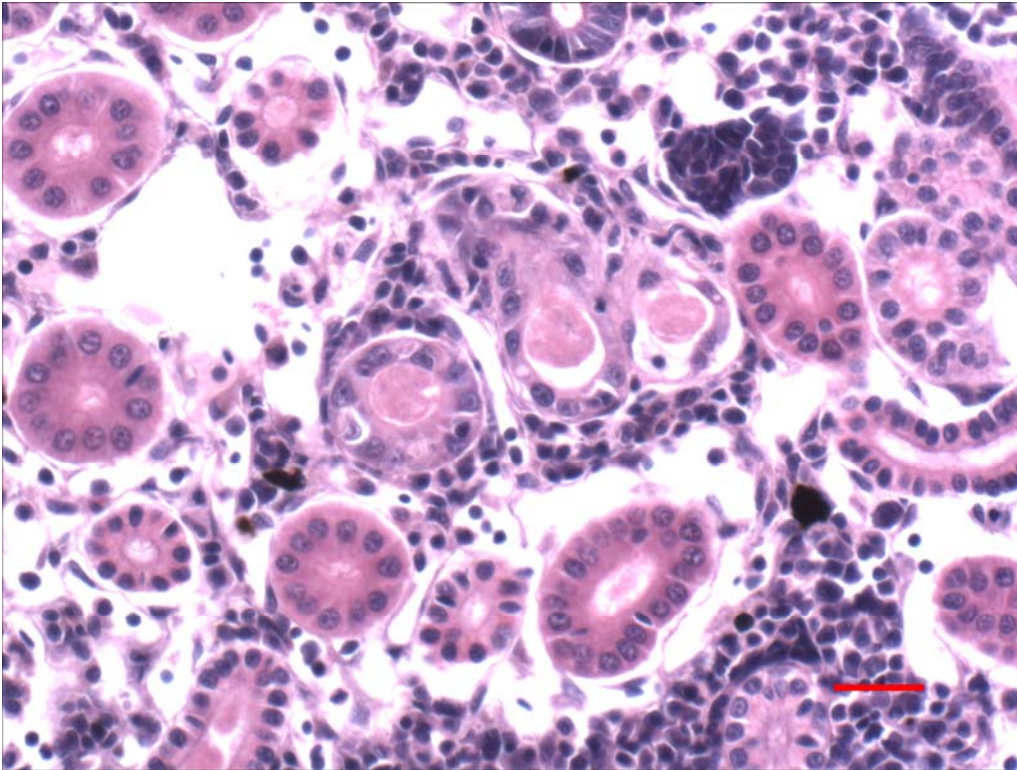
Vitamin E deficiency - severe necrosis, fibrosis and atrophy of muscle fibers in trout. Note calcified foci (arrows; bar = 50 μ m).



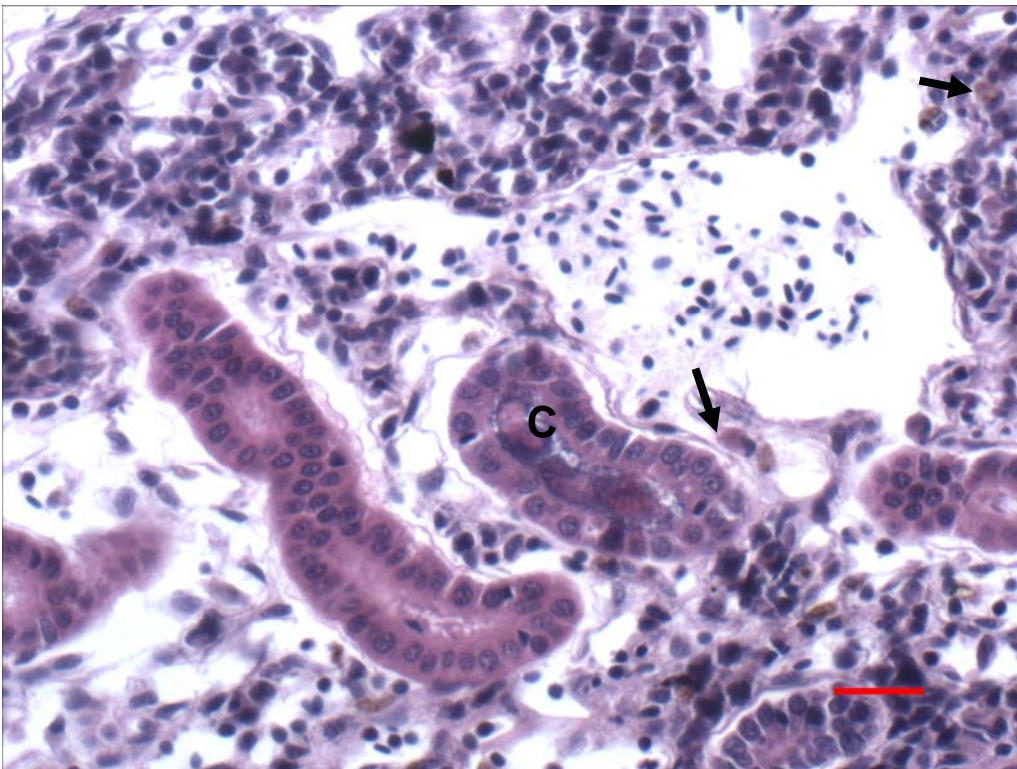
Vitamin E deficiency. Note necrotic kidney tubules (arrows) and hypoplastic hematopoietic tissue (H). (bar = 50µm).



Vitamin E deficiency – eosinophilic casts (arrows) are present in kidney tubules. Note also hypoplastic hematopoietic tissue. (bar = 50µm).



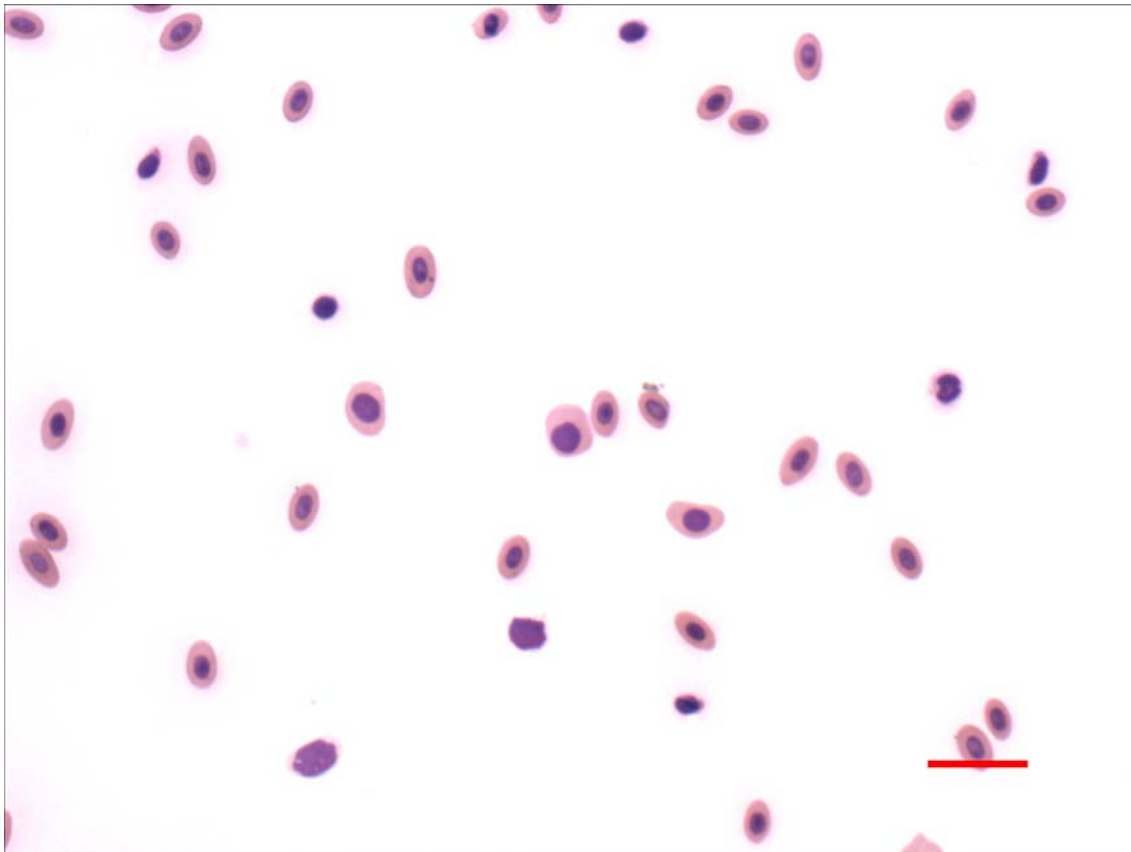
Vitamin E deficiency - necrotic kidney tubules with casts in lumens are seen in center of photo (bar = 25µm).



Vitamin E deficiency, early calcification (C) in kidney tubule of trout. Note reduction of hematopoietic tissue surrounding kidney tubules. Ceroid is scattered throughout hematopoietic tissue (arrows; bar =25µm).

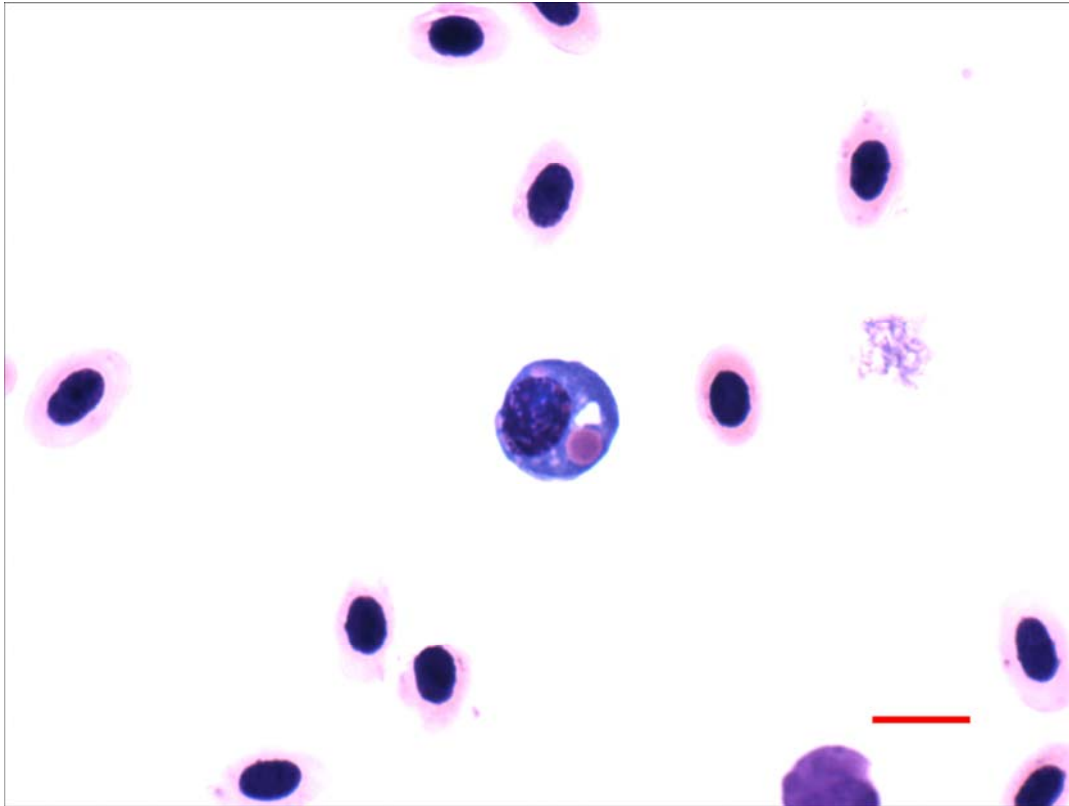


Severe anemia in trout fed fish meal diet with rancid oil and without vitamin premix. Fish showed typical vitamin E deficiency anemia.

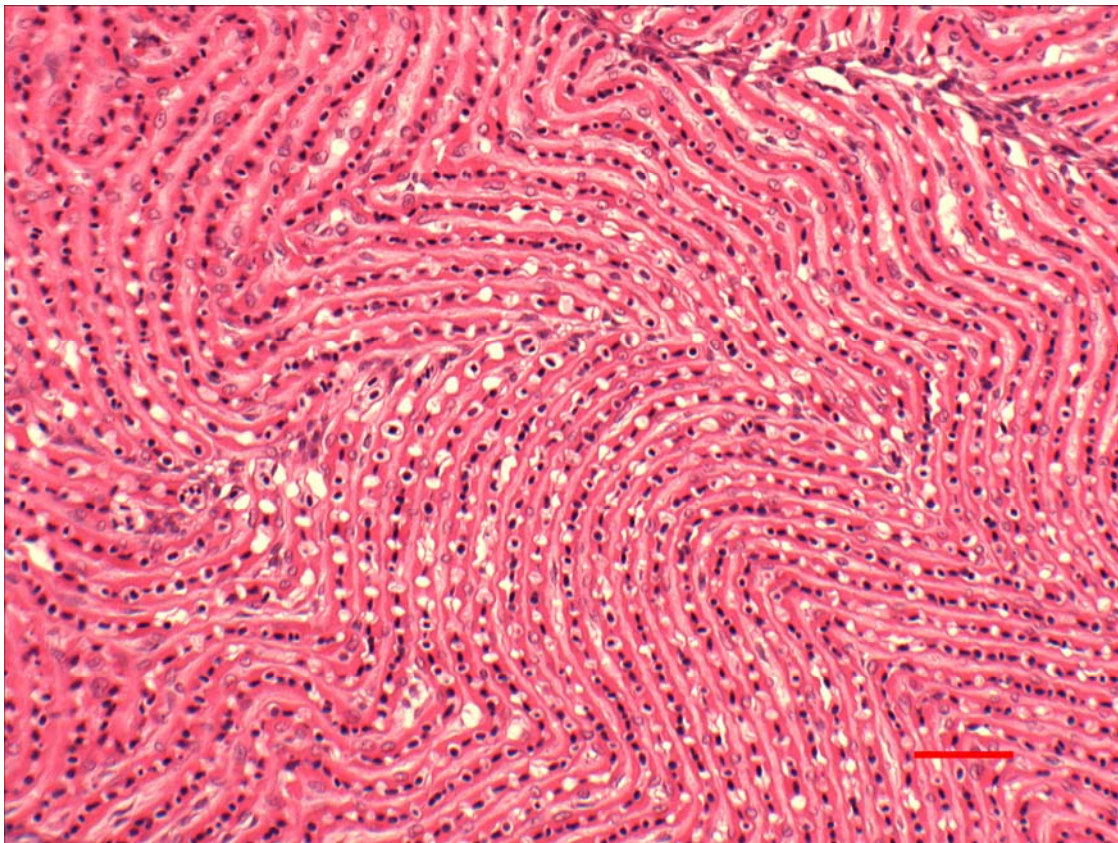


Vitamin E deficient, anemic trout. Note atypical and microcytic rbc's. (bar = 25 μ m)

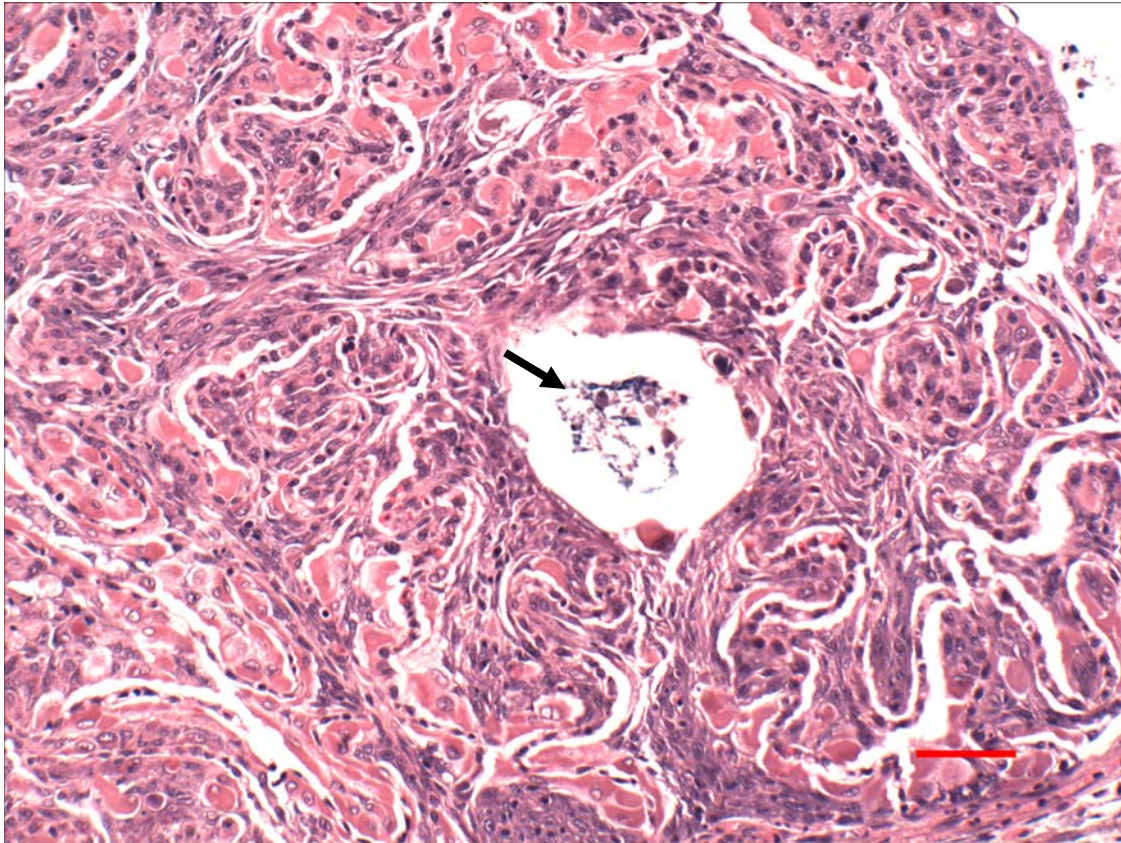
Chapter 7 – Non-Infectious Diseases
Fish Histology and Histopathology



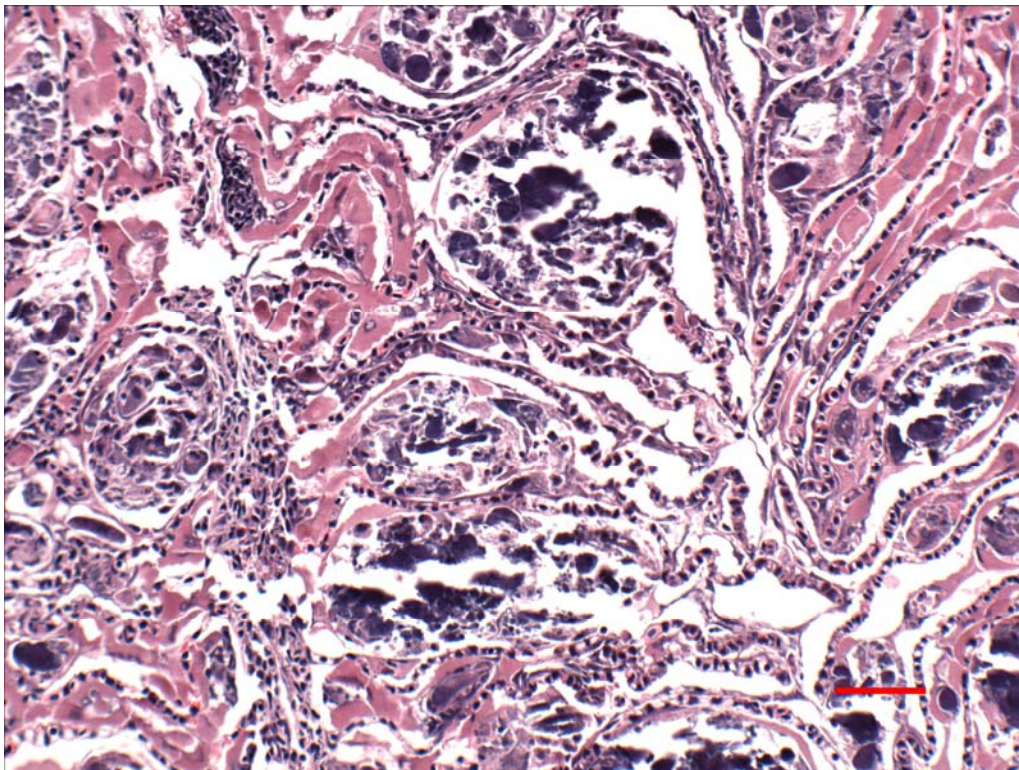
Vitamin E deficient, anemic trout. Note atypical immature rbc with attempt to produce hemoglobin. (bar = 10 μ m)



Normal pseudobranch from rainbow trout (bar = 50 μ m).

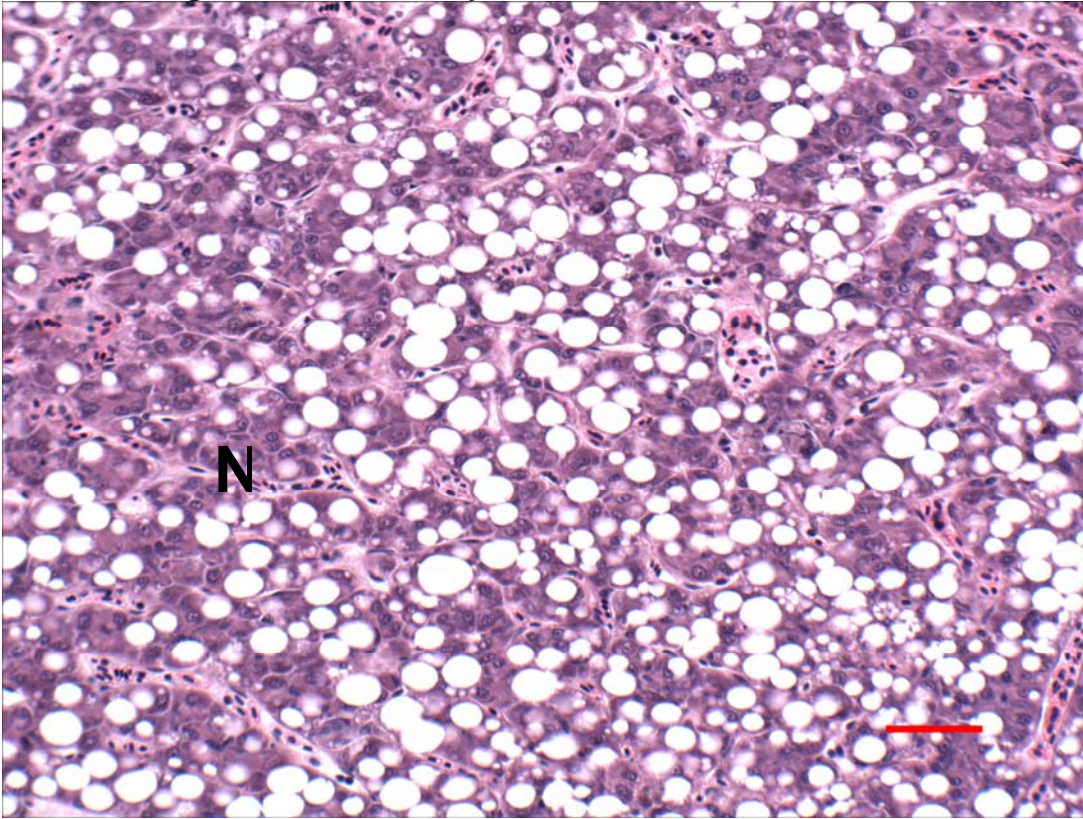


Vitamin E deficiency - hyperplasia and fusion of lamellae of pseudobranch of trout. Note focus of calcification in cystic space (arrow; bar = 100 μ m).

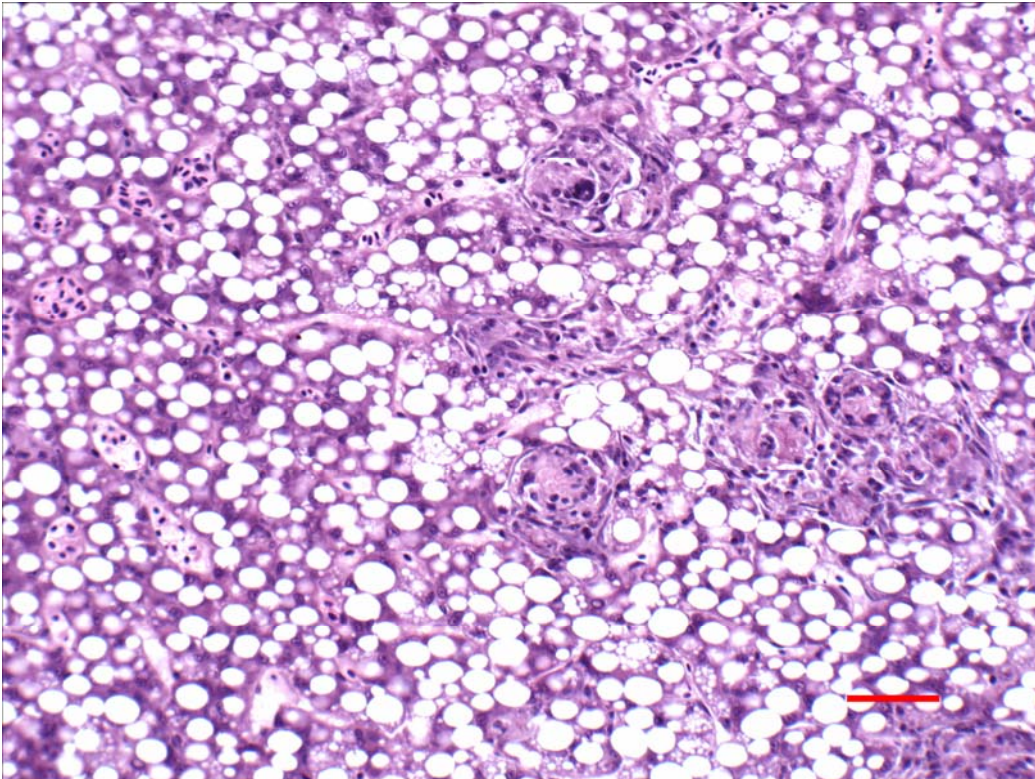


Vitamin E deficiency - severe calcification of pseudobranch of trout. Note hypertrophy and degeneration of glandular cells (bar = 100 μ m).

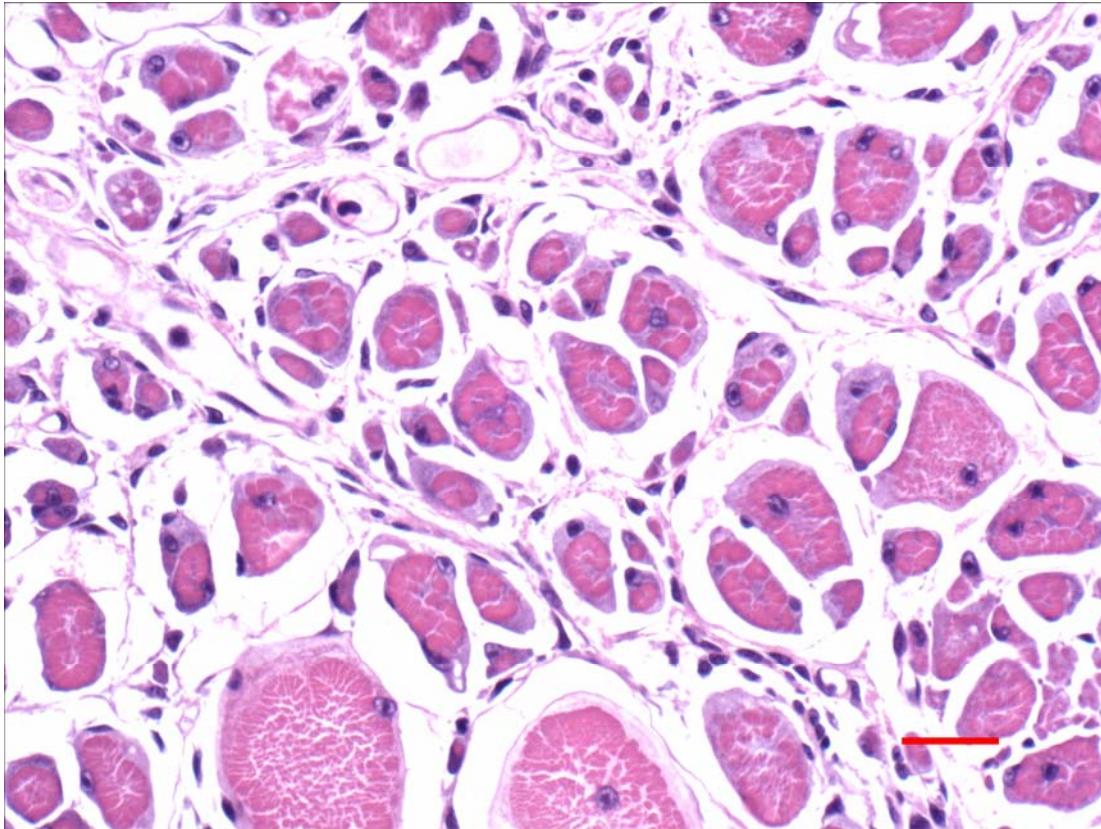
G. Magnesium Deficiency



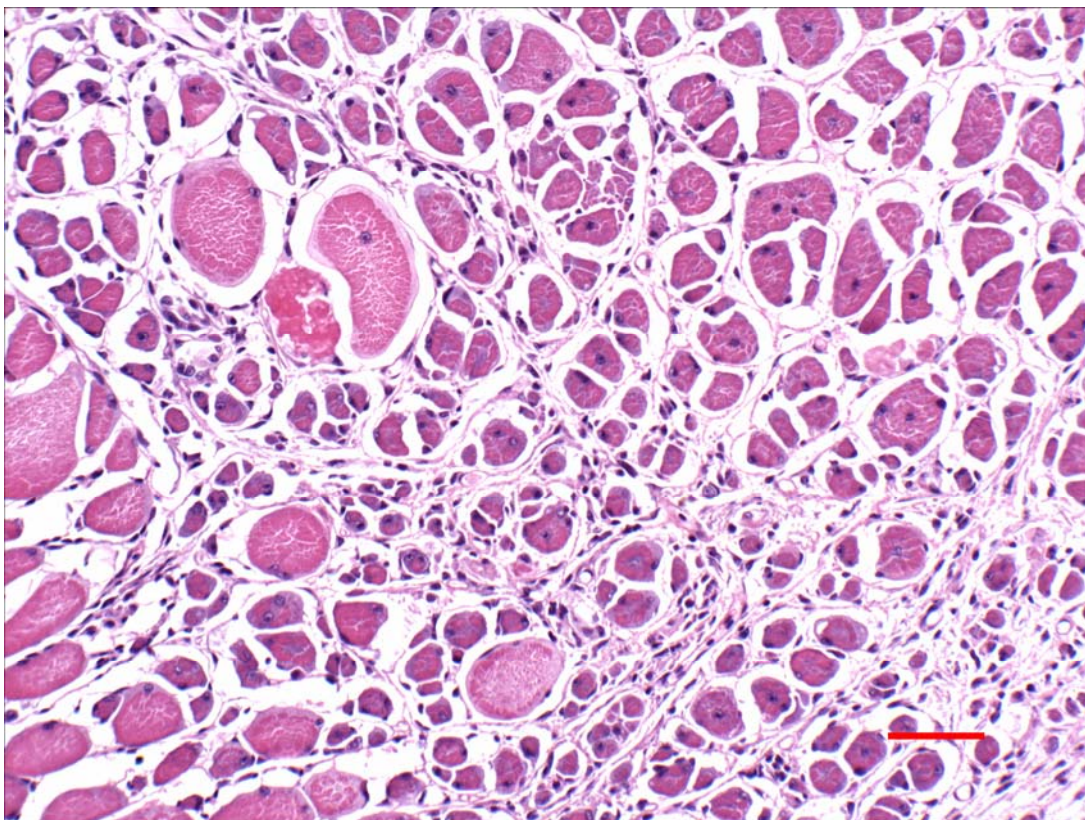
Magnesium deficiency, liver of trout shows fatty change. Note normal hepatocytes (N; bar = 50 μ m).



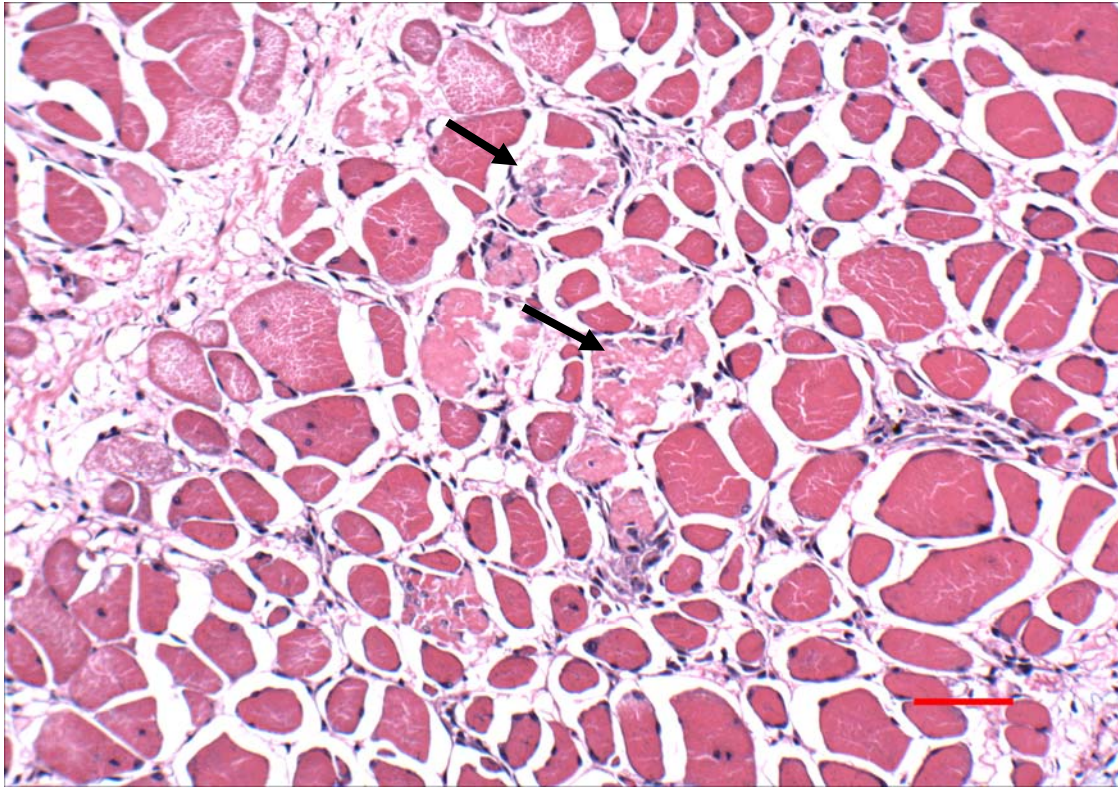
Magnesium deficiency – severe fatty change and granulomas (arrow). (bar = 50 μ m).



Magnesium deficiency – atrophy, necrosis and fibrosis of muscle. (bar = 25 μ m)

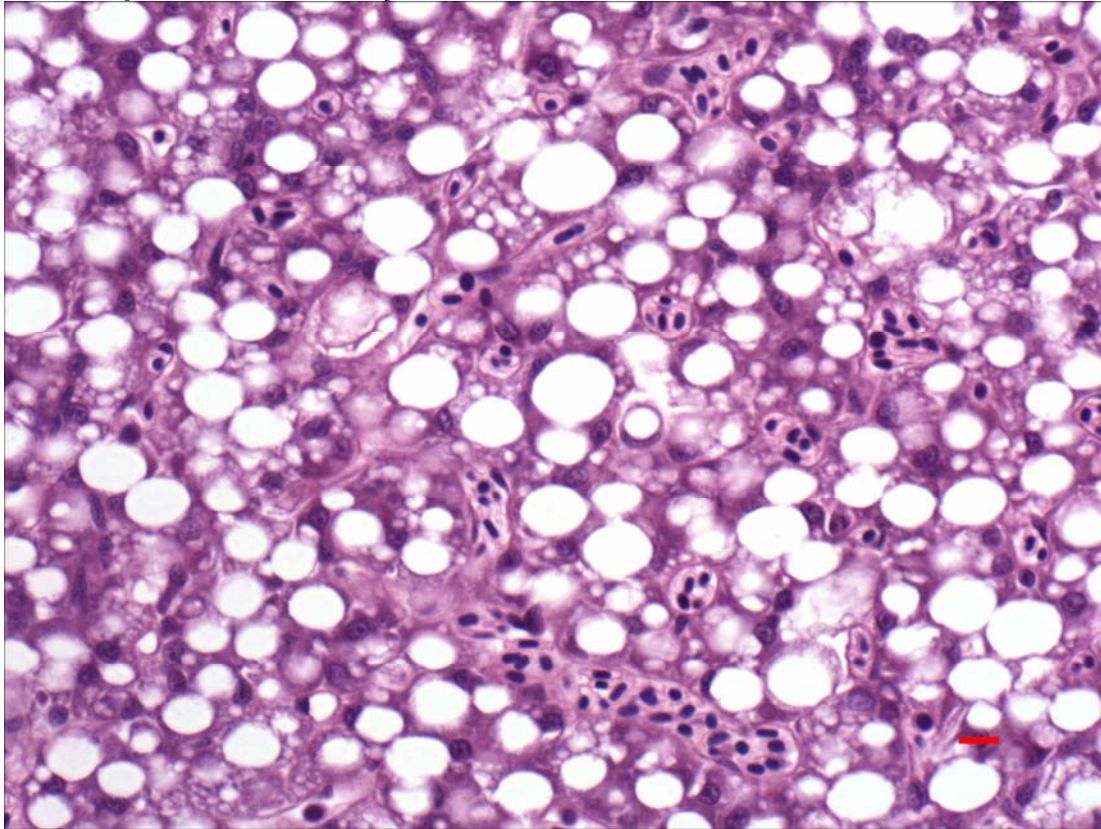


Magnesium deficiency – atrophy, myositis, necrosis and fibrosis of muscle. (bar= 50 μ m).

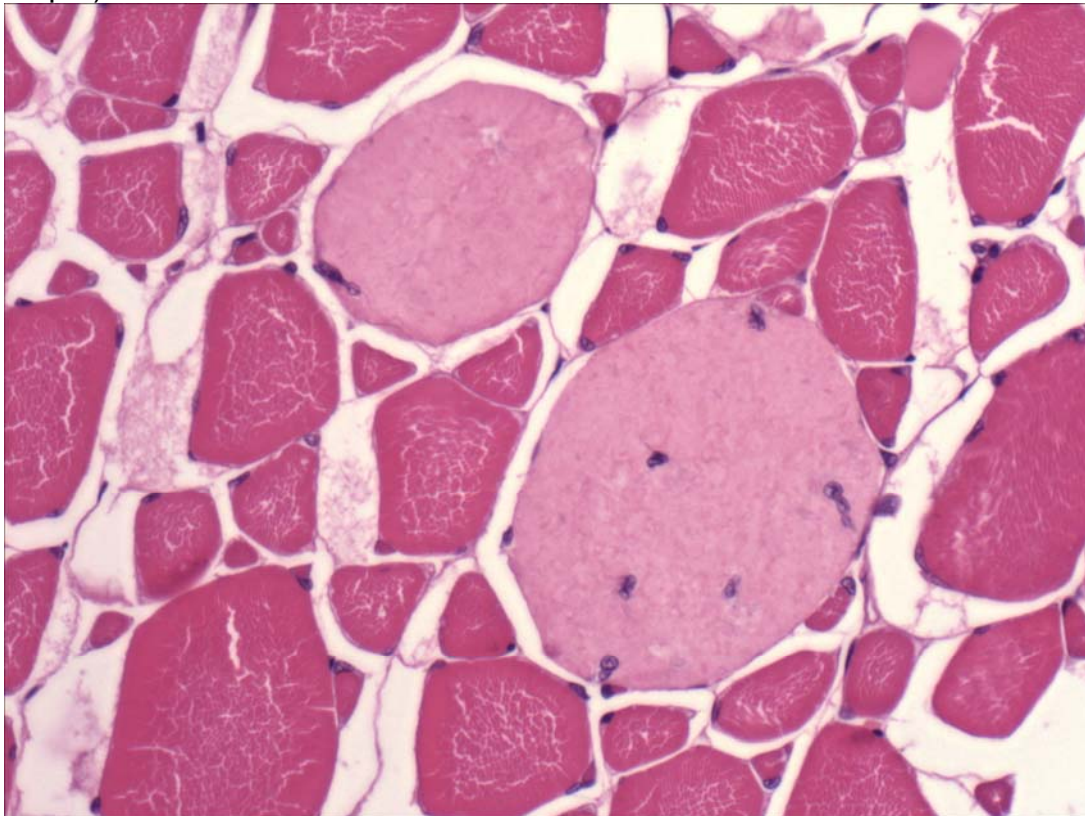


Magnesium deficiency, severe necrosis of muscle fibers is seen in center of photo (arrows). Note atrophy of some fibers (bar = 50µm).

H. Phosphorus Deficiency

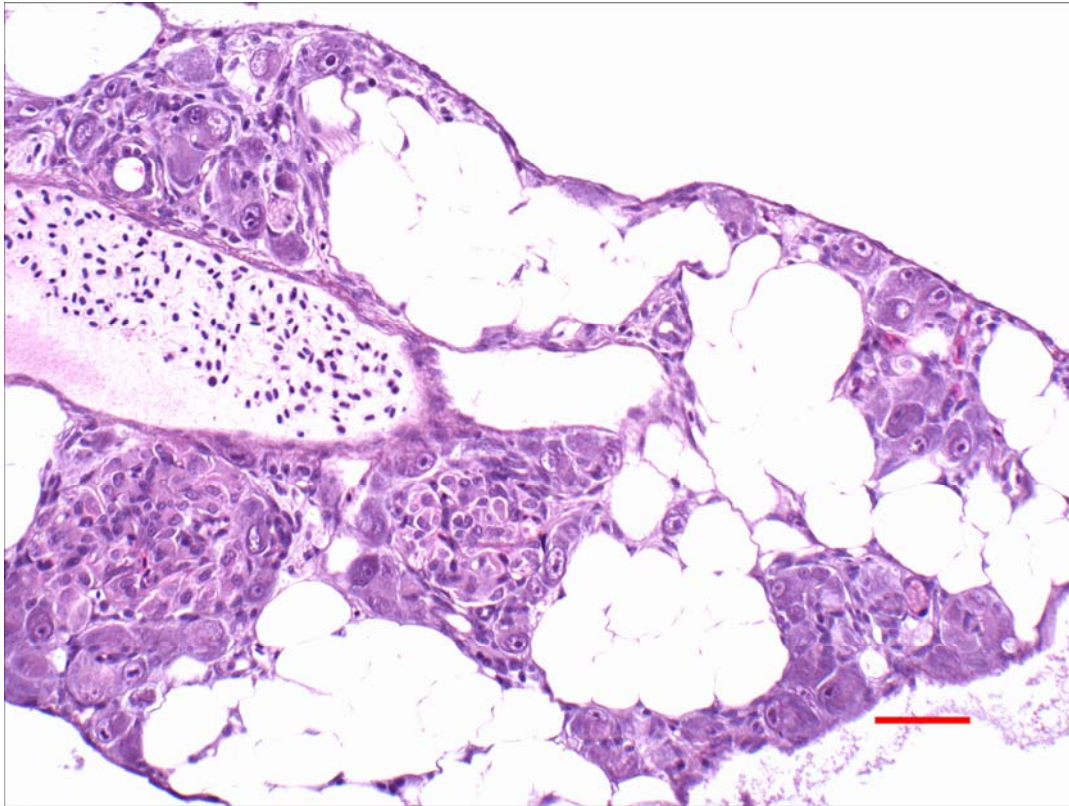


Phosphorus deficiency – severe fatty change in liver. Note congested sinusoids. (bar = 25 μ m)

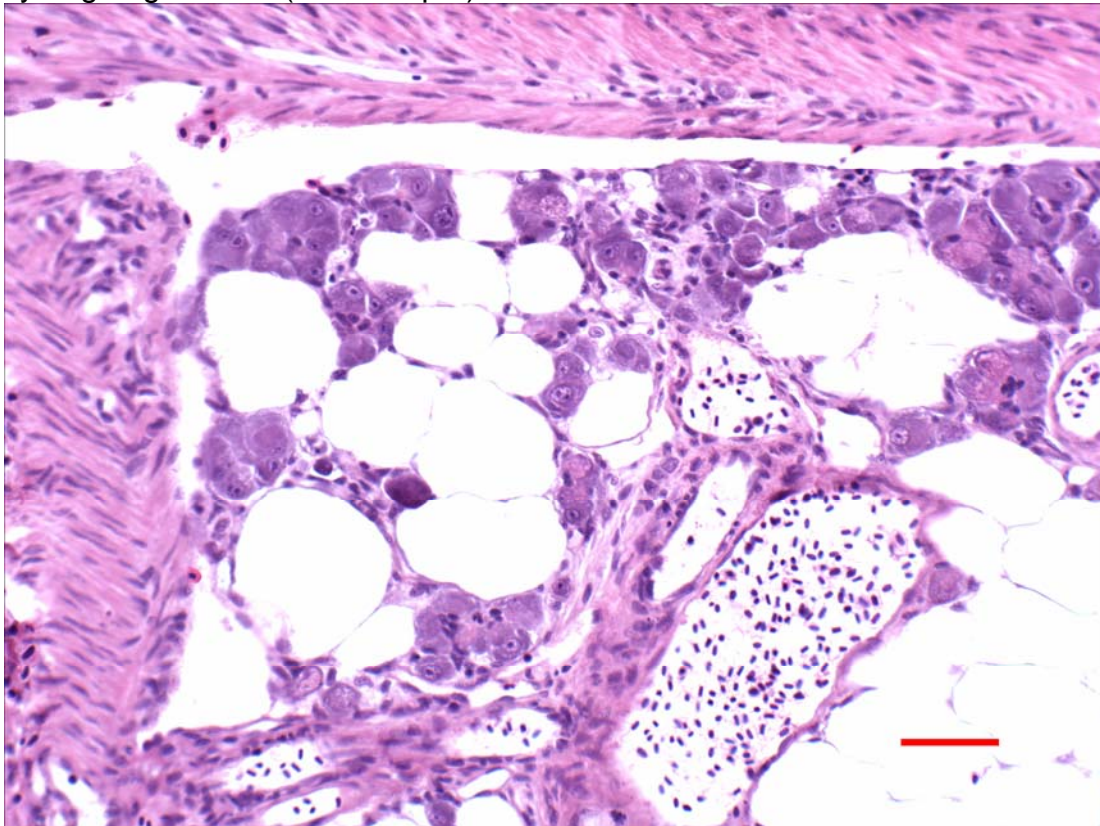


Phosphorus deficiency – necrosis of muscle fibers. (bar = 25 μ m)

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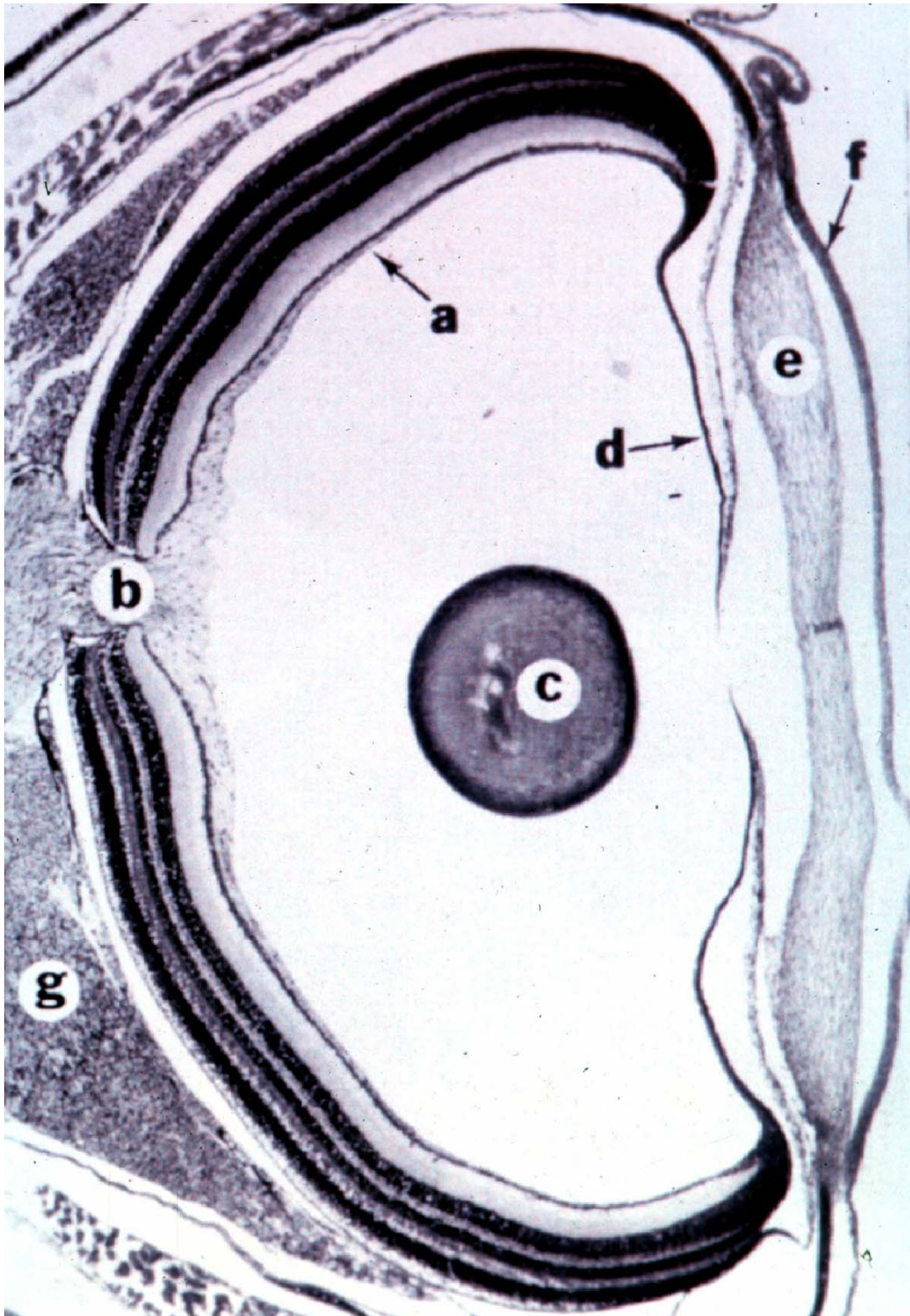


Phosphorus deficiency – note swollen degenerate acinar cells most of which lack zymogen granules. (bar = 50 μ m).

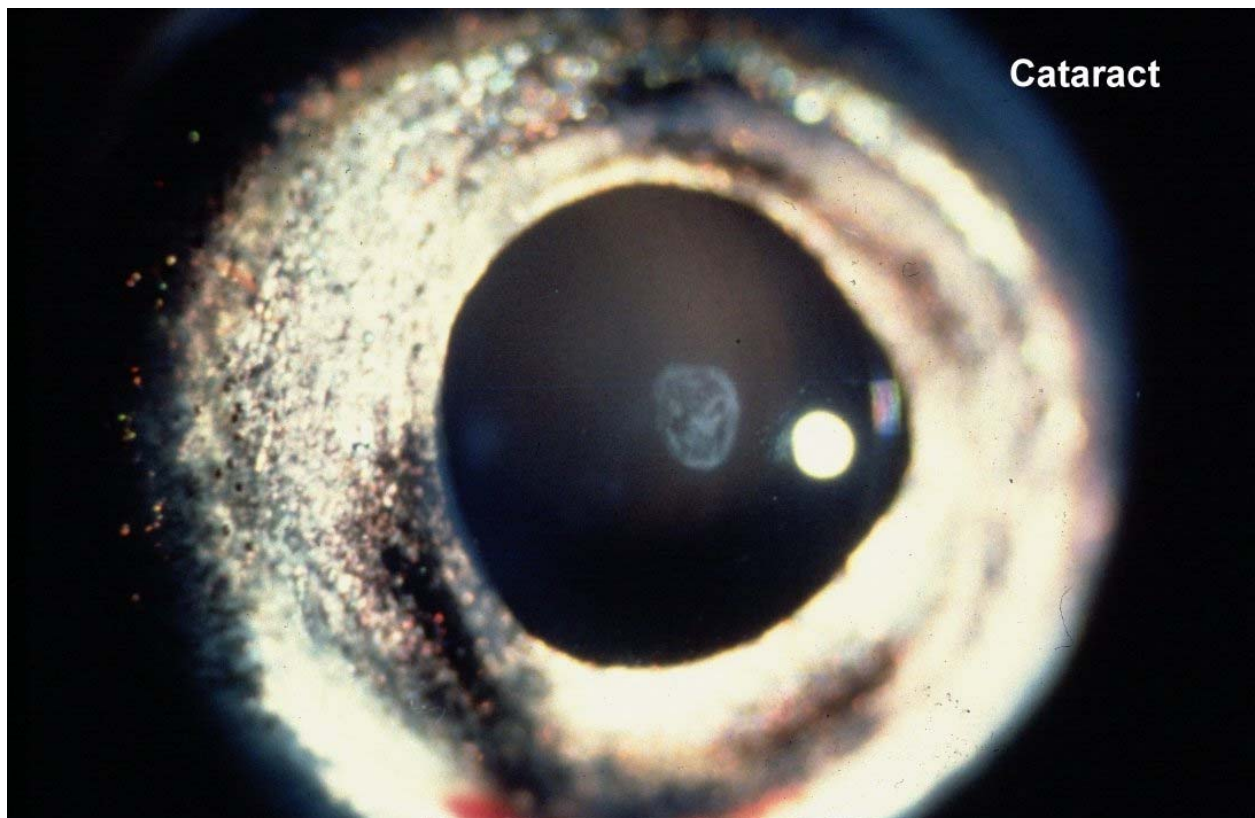
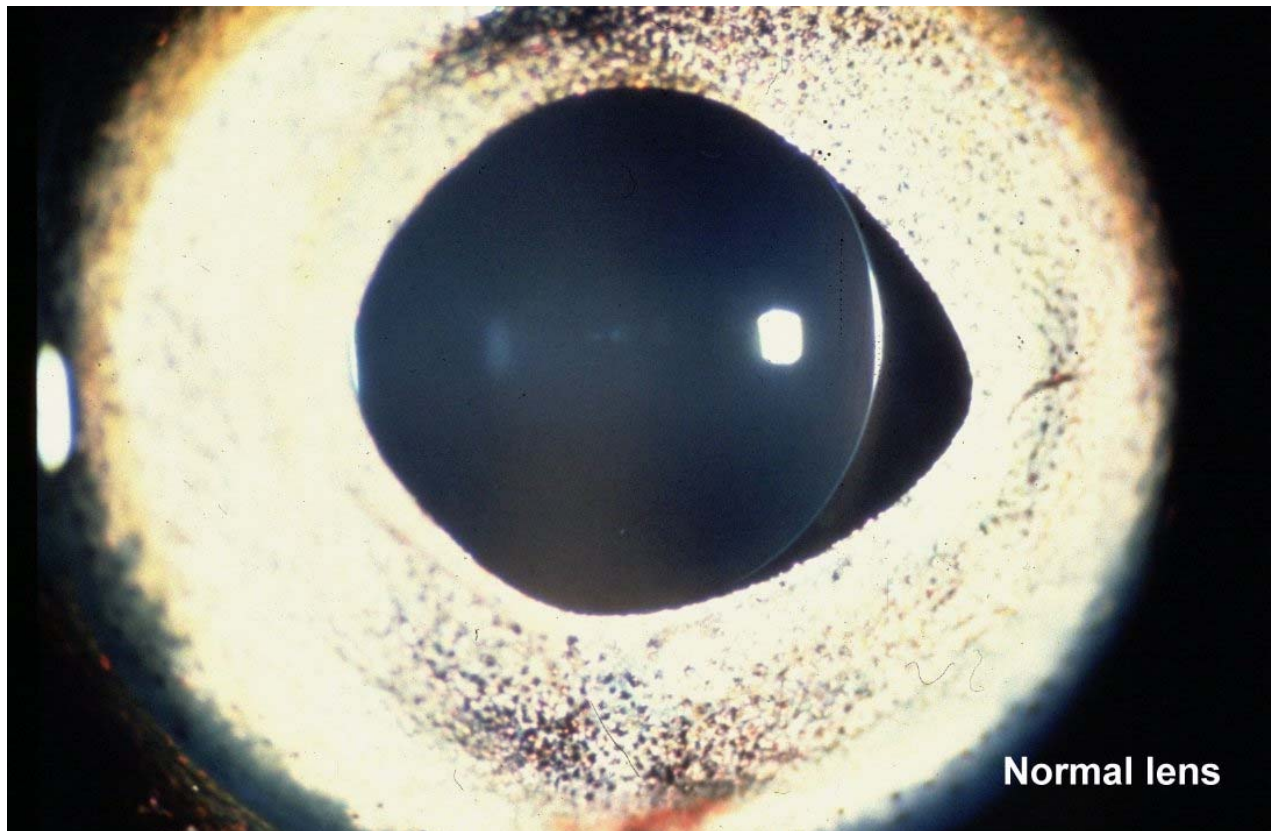


Phosphorus deficiency – pancreatic acinar cells are swollen, lack zymogen & show mild degeneration. (bar = 50 μ m)

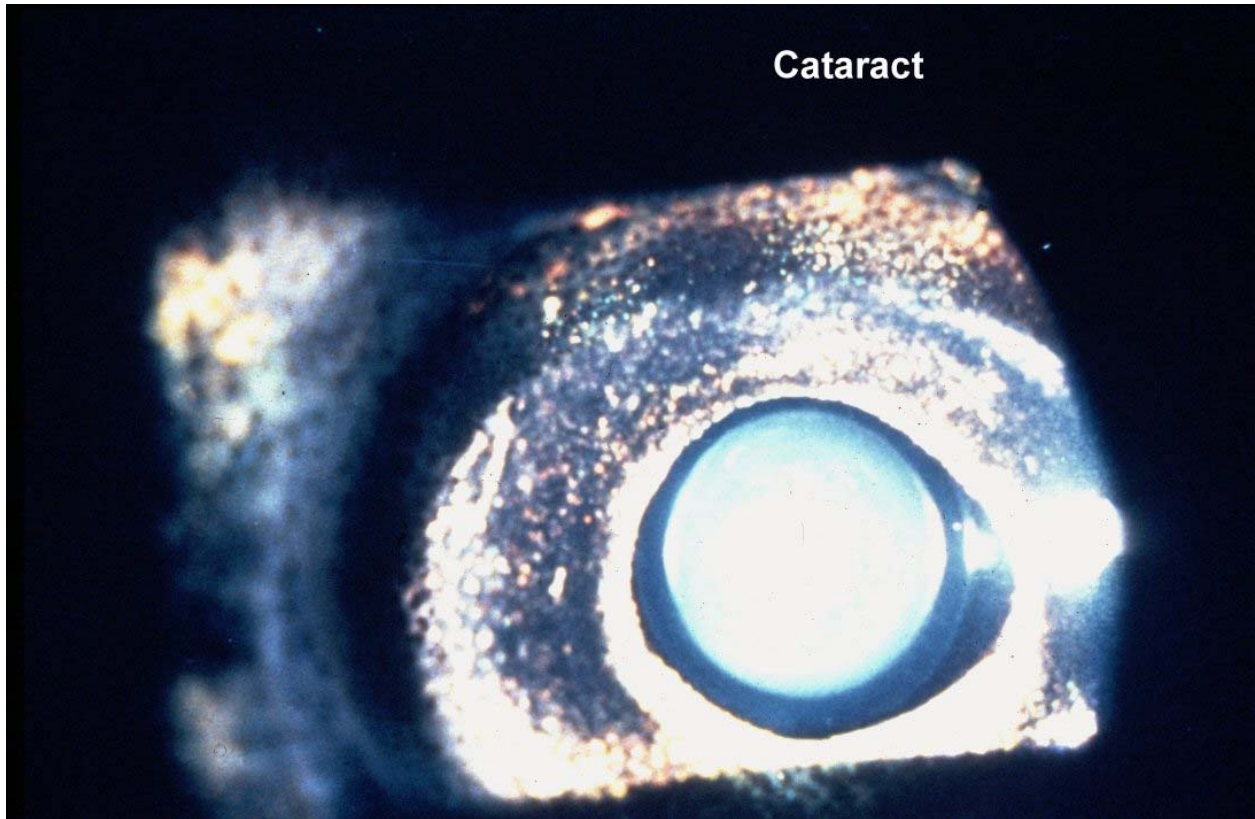
I. Cataract



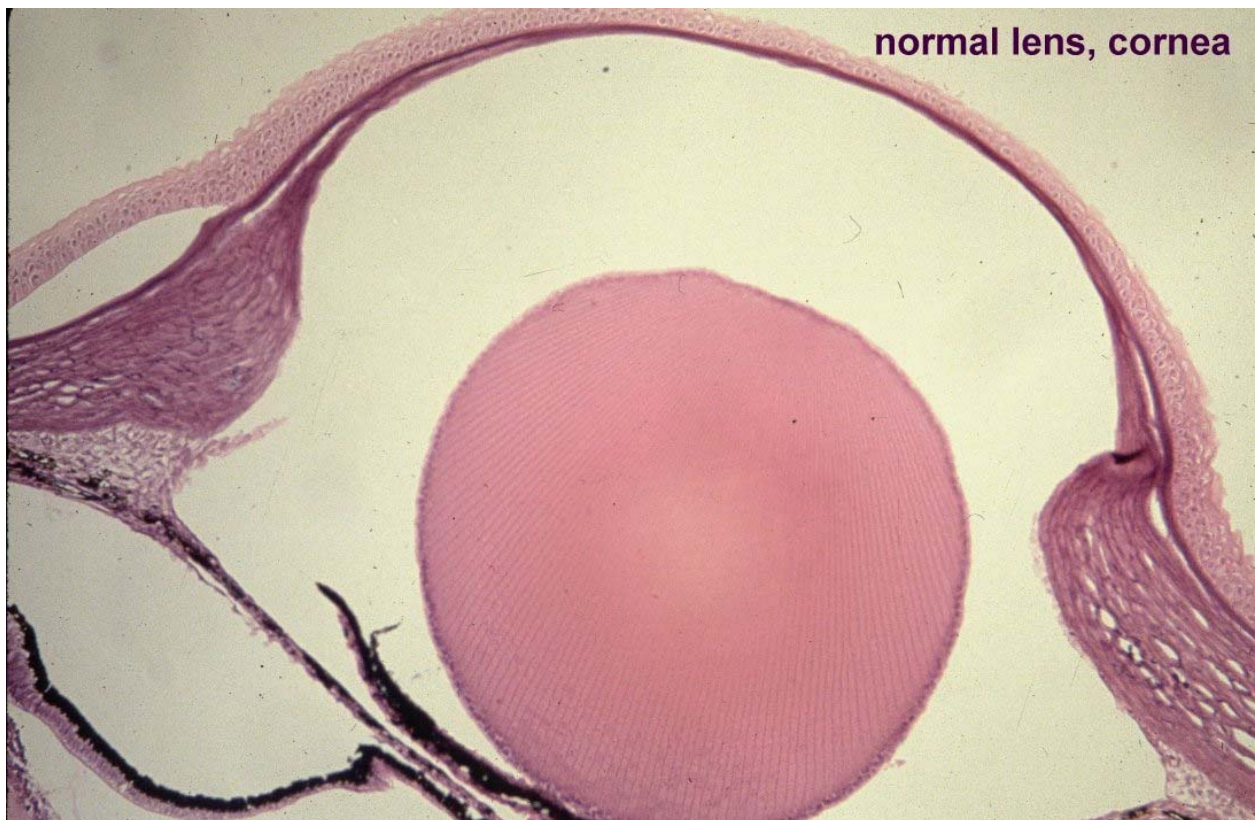
Histological section through eye of trout.



Incipient cataract formation in lens of trout



Advanced cataract in eye of trout



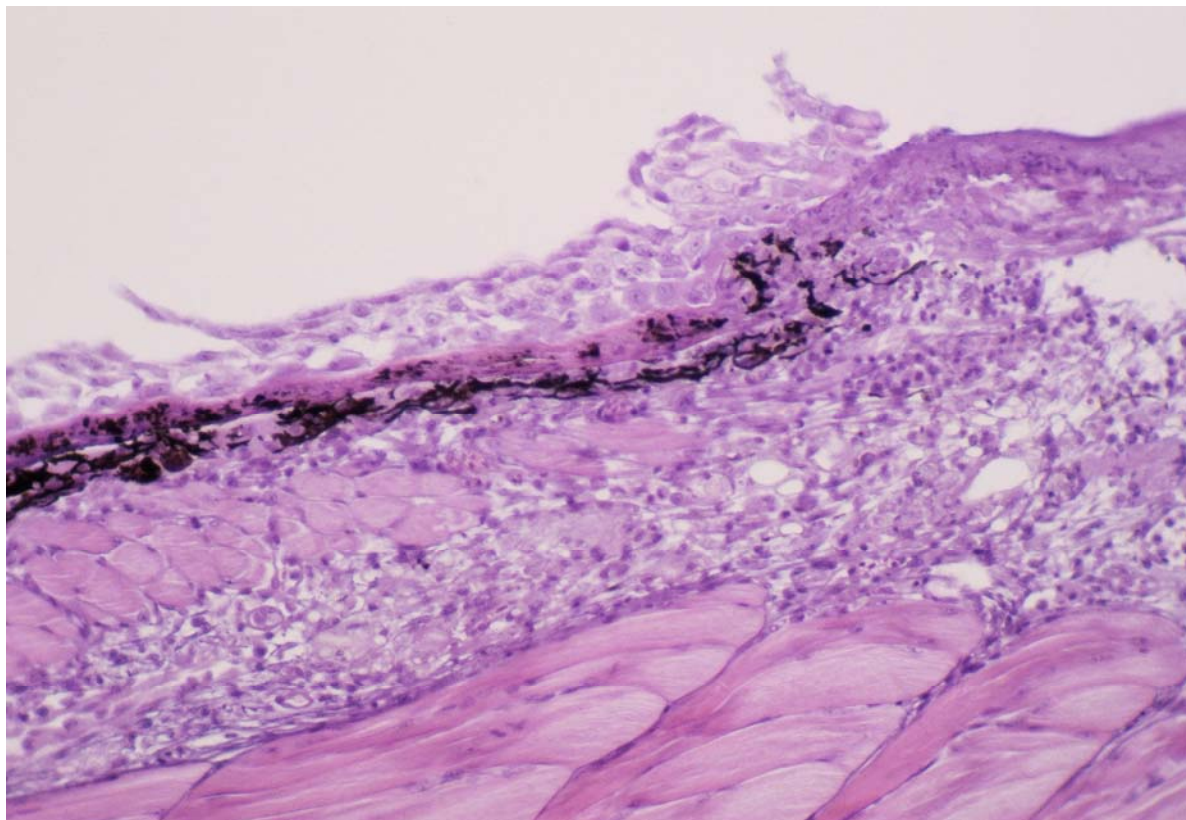






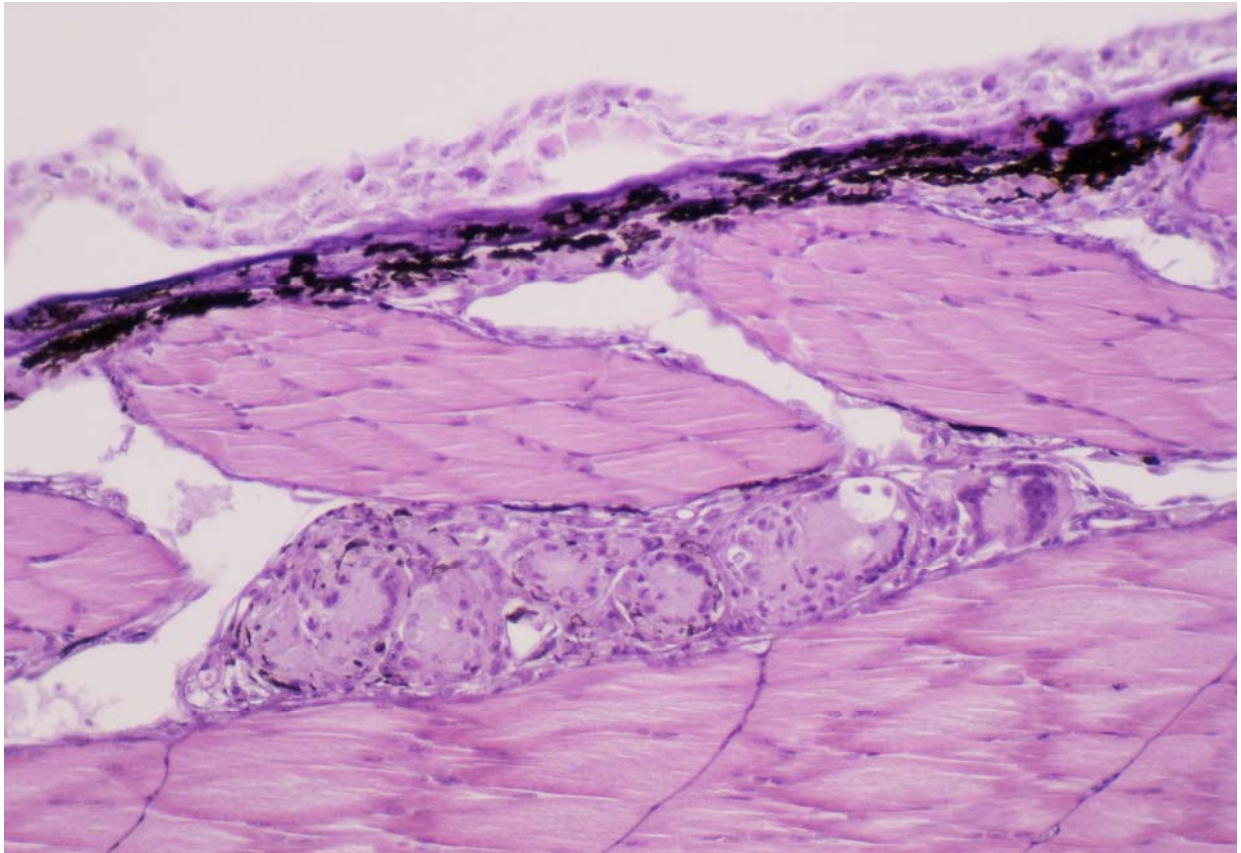
II. Other Diseases

A. Dermatitis/Steatitis

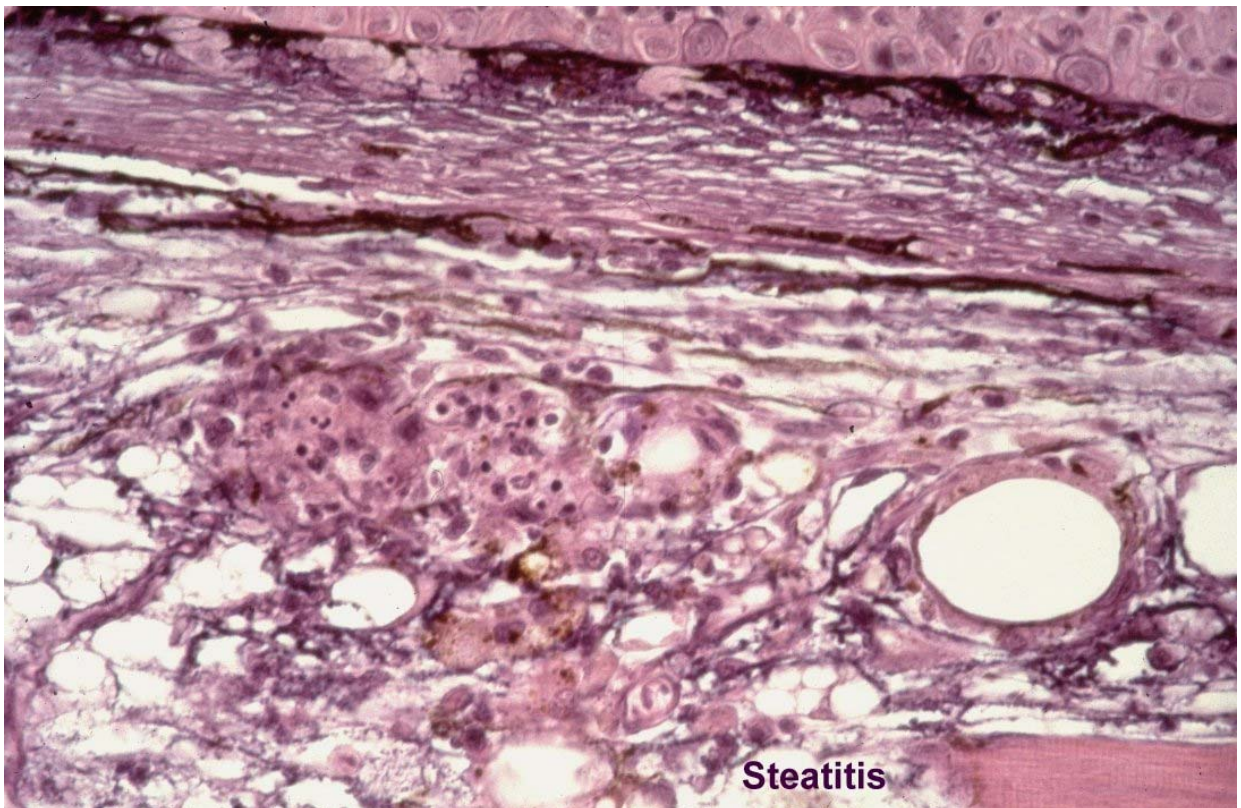


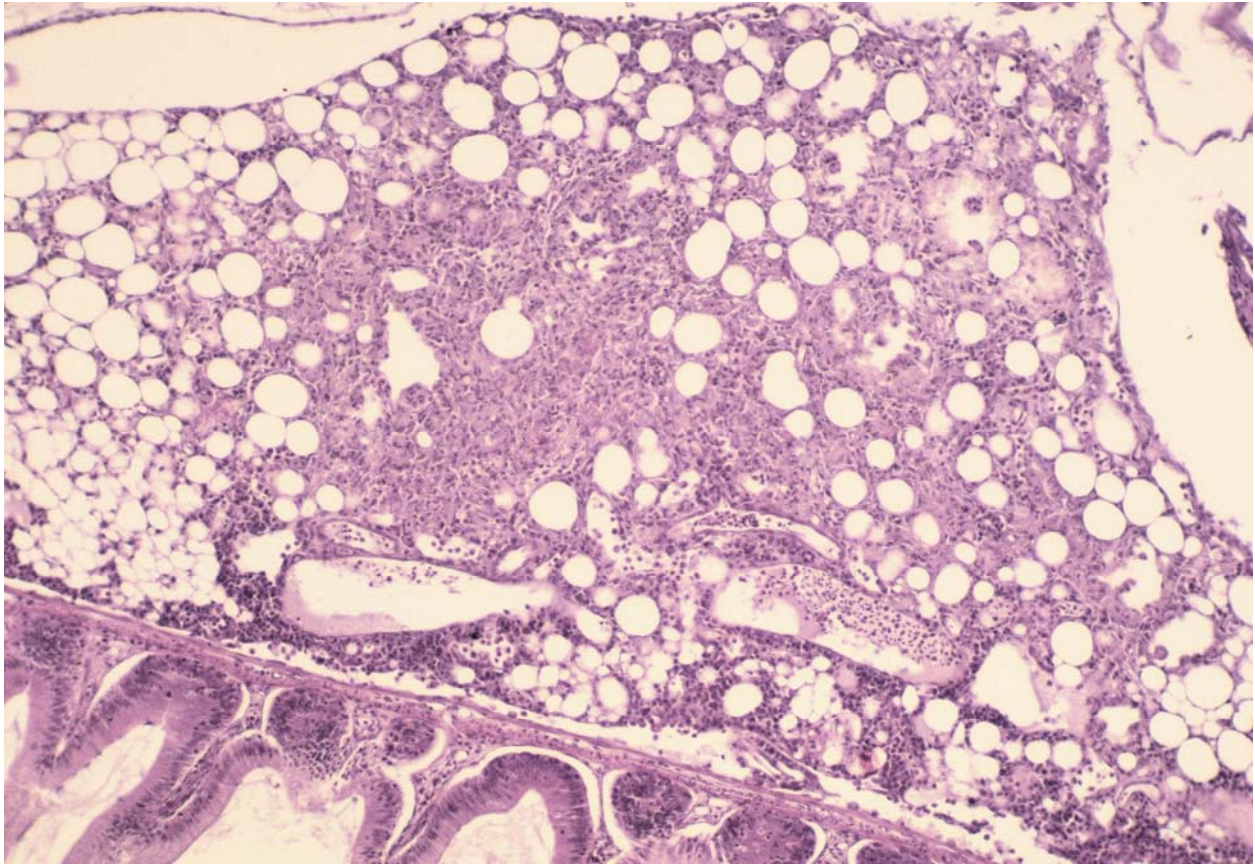


Normal epidermis and dermis of trout.



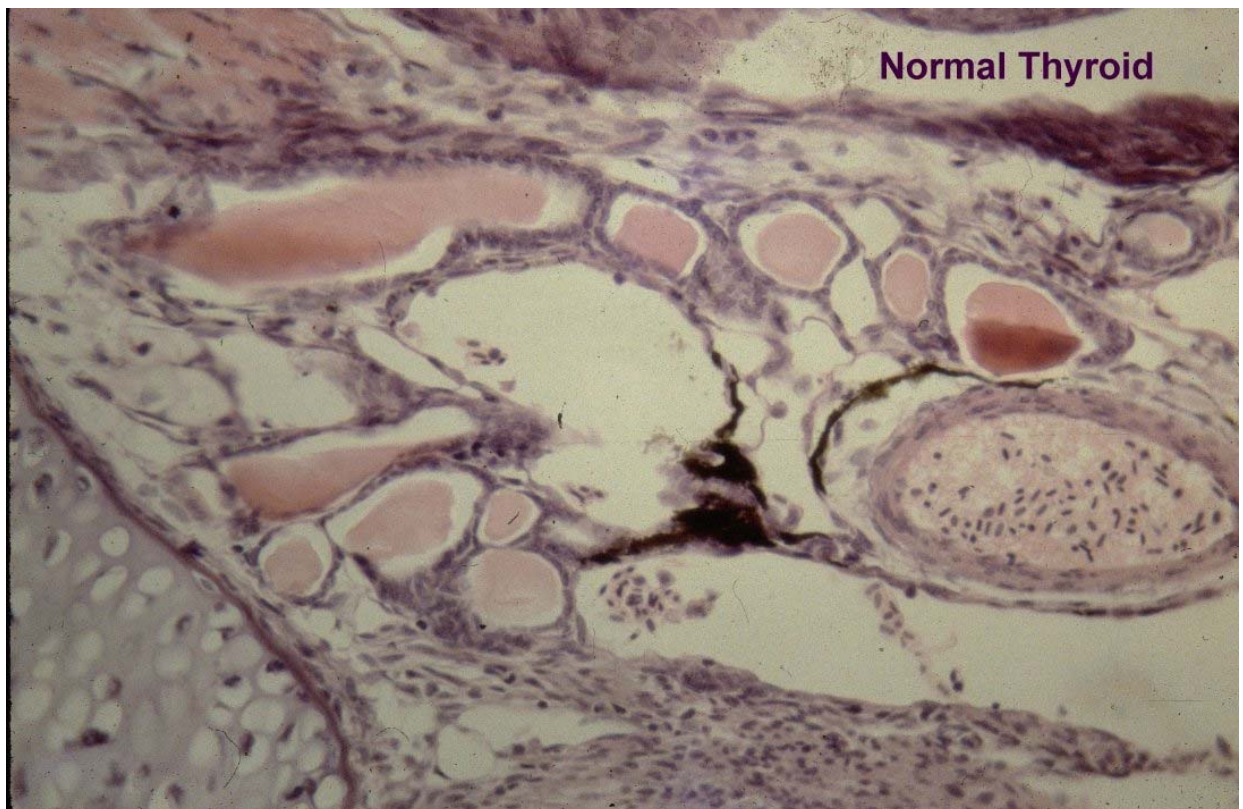
Note granulomatous lesion with giant cells.

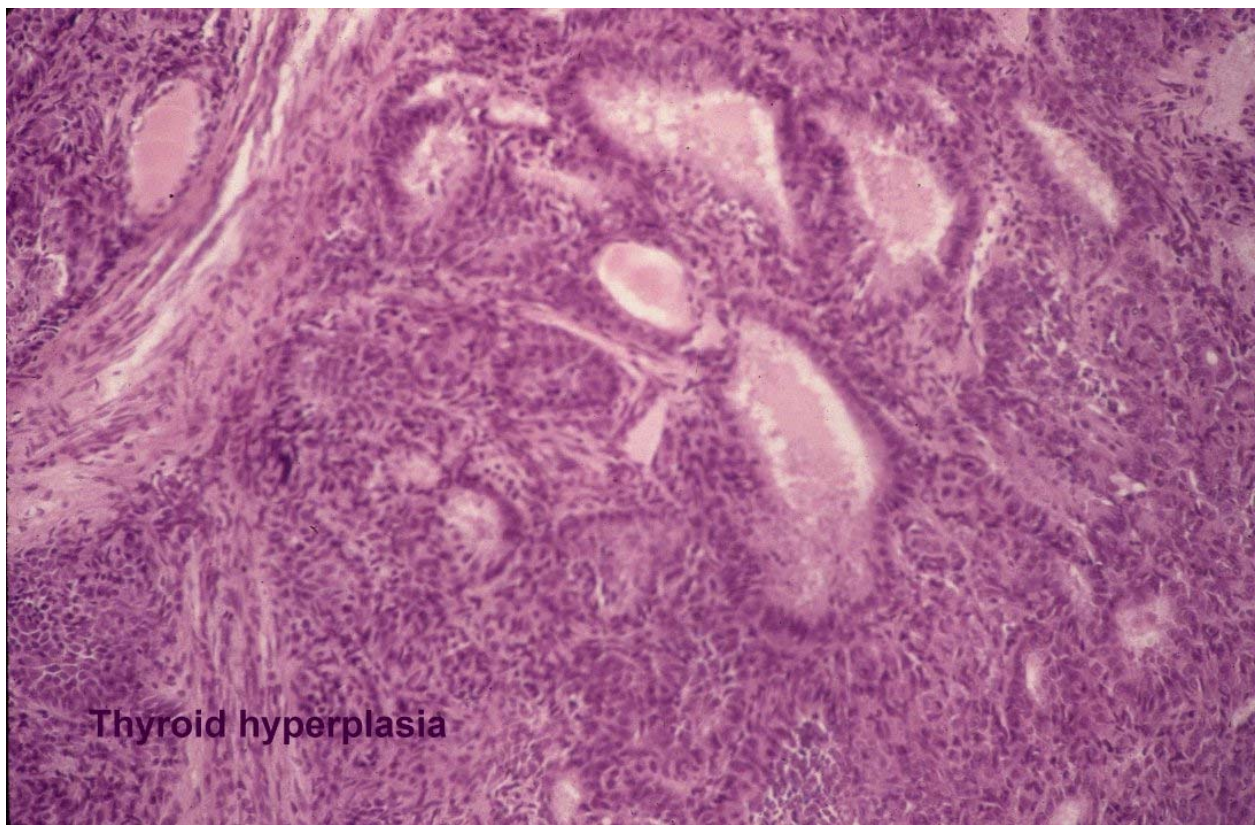
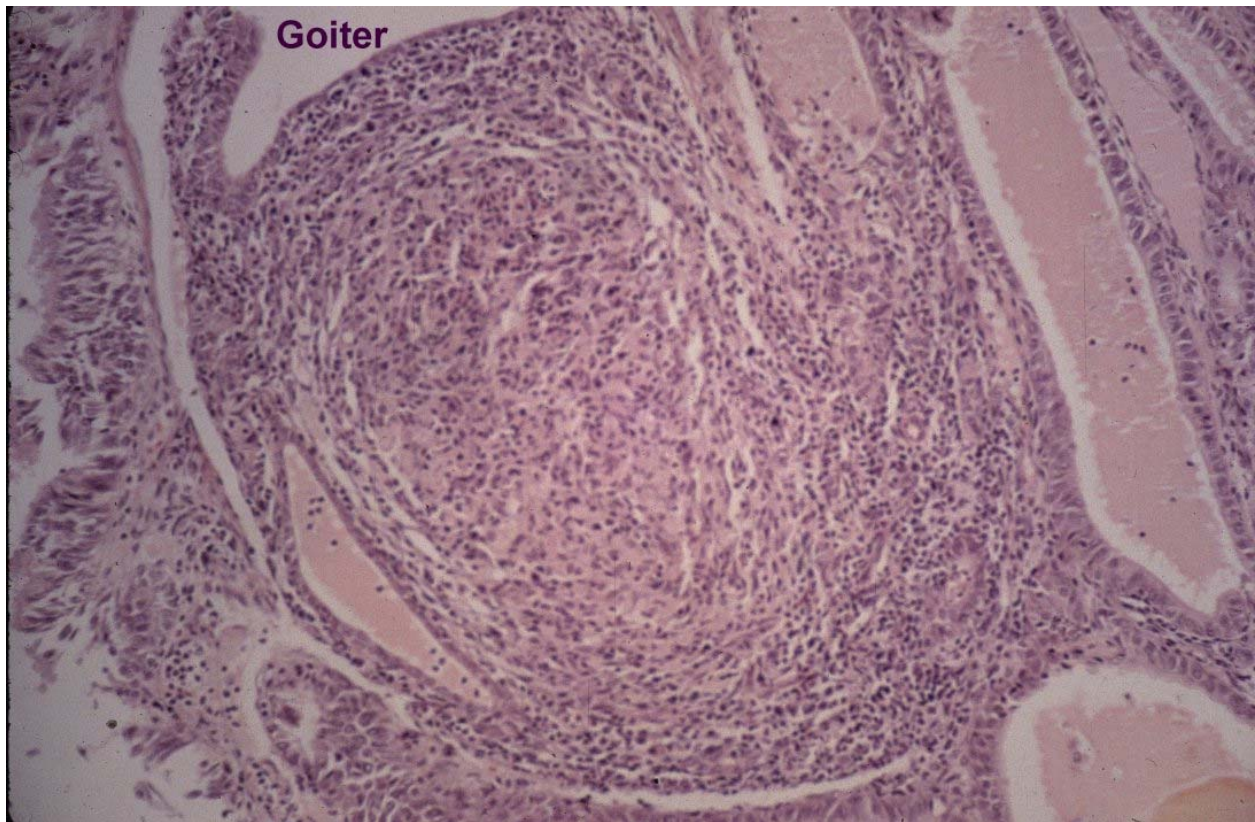


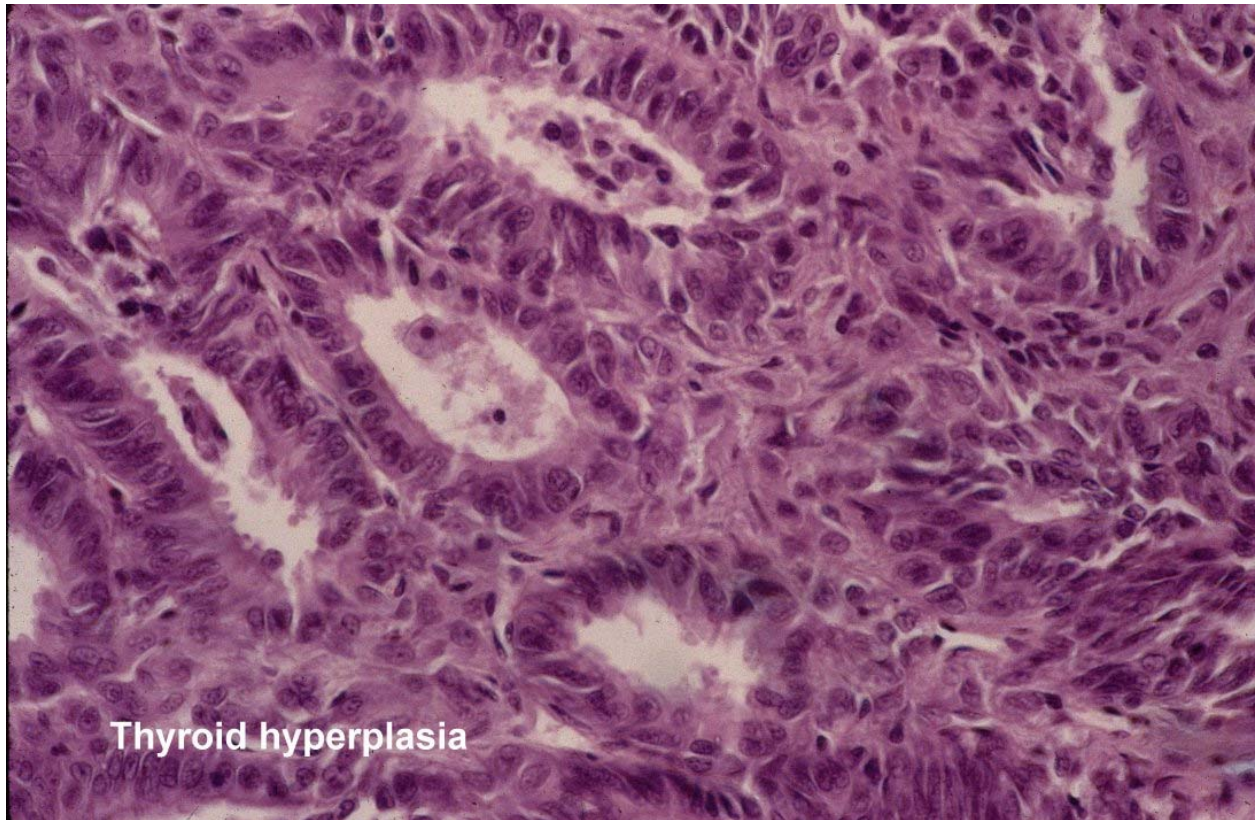


Note steatitis of adipose in visceral cavity.

B. Goiter

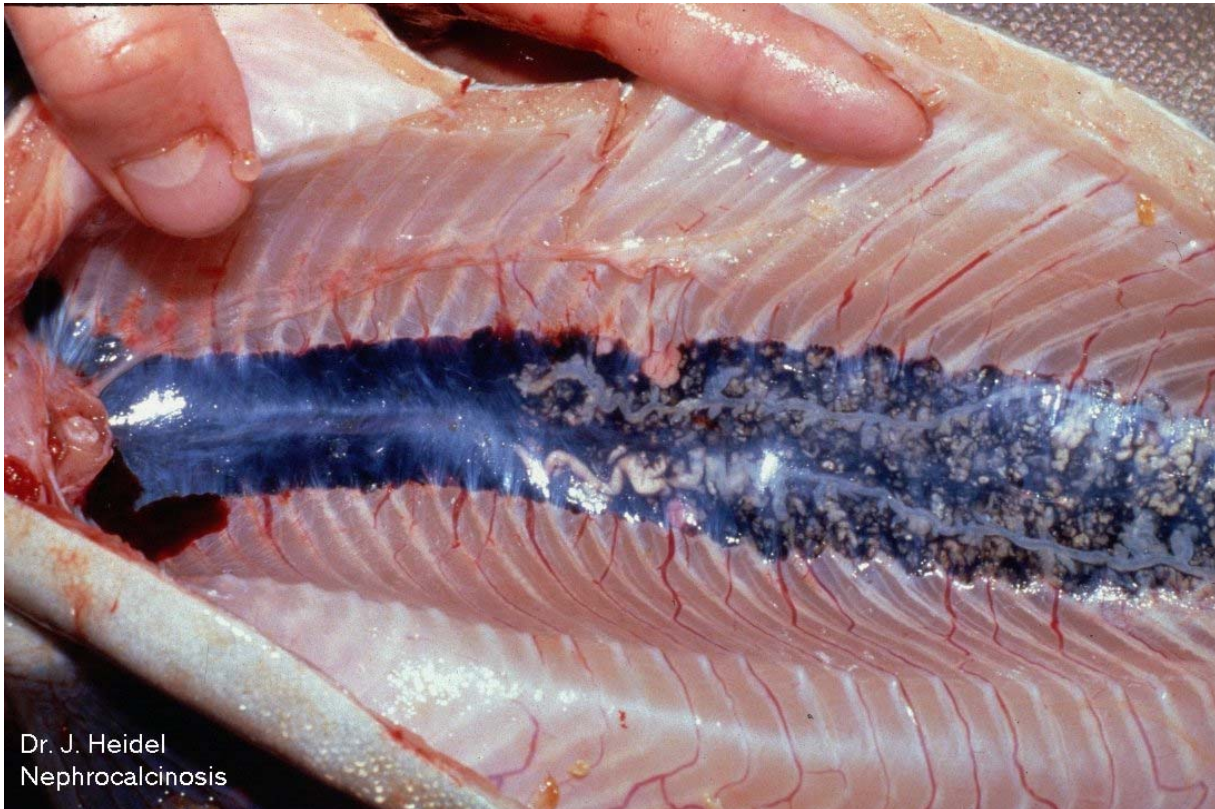


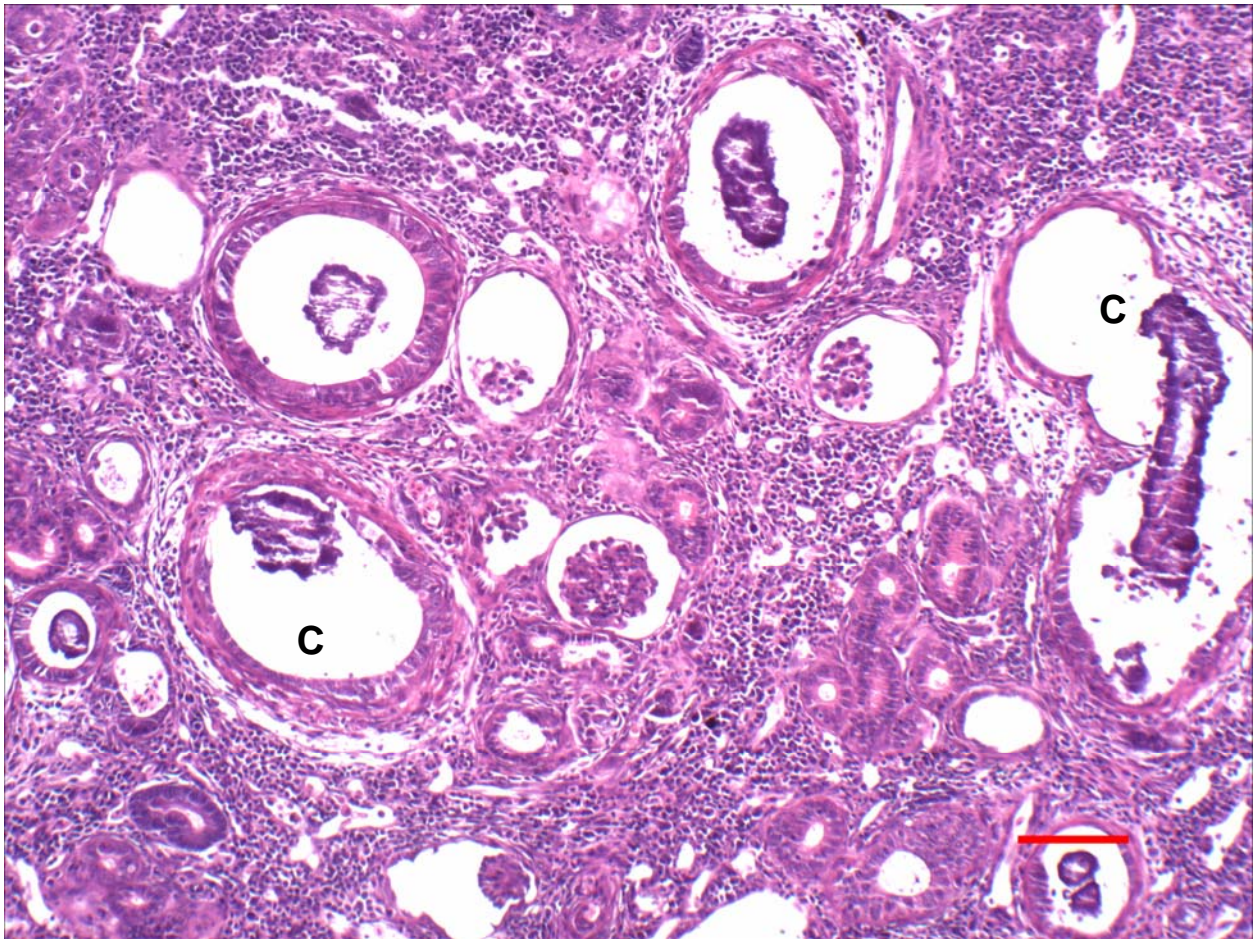




C. Nephrocalcinosis

- Can be nutrition related - due to magnesium deficiency or selenium excess.
- Can also be caused by excess CO₂ in the water
- Other??

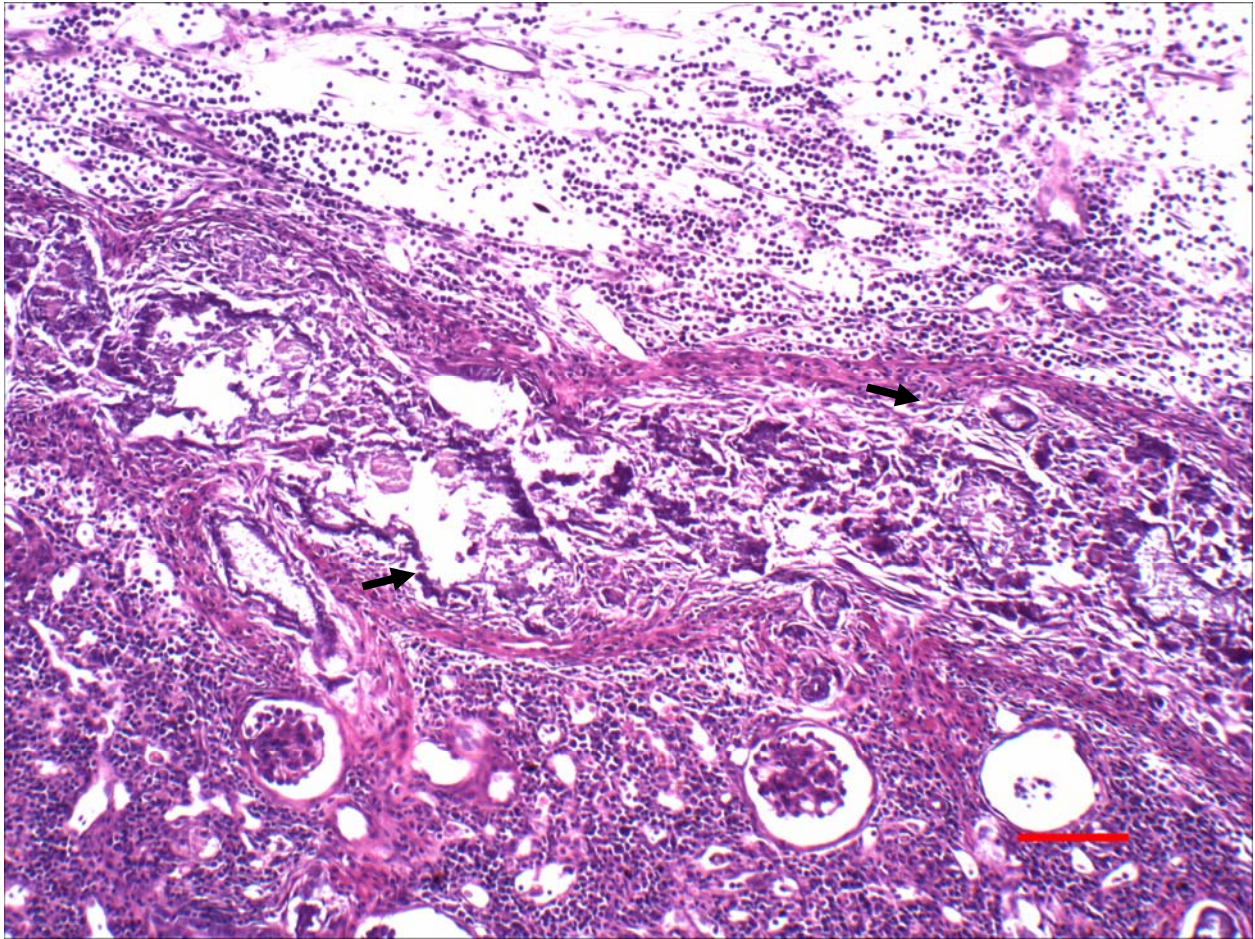




Calcium deposits (C) are present in fluid filled collecting tubules of cutthroat trout with nephrocalcinosis. (bar = 100 μ m)



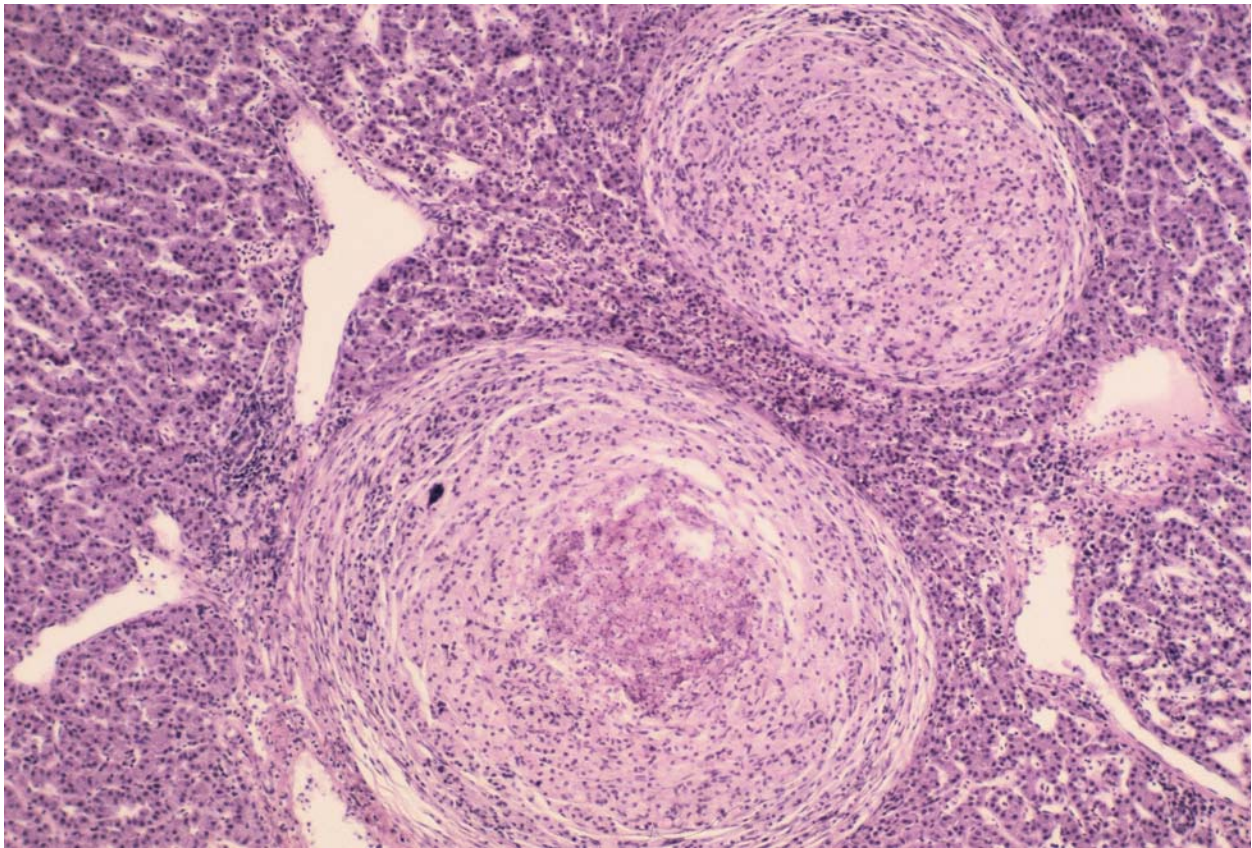
Cutthroat trout on bottom shows severe calcinosis of ureters.



Severe nephrocalcinosis cutthroat trout - Ureter is filled with calcium deposits (arrows). Note edematous change in upper portion of photo (bar = 200 μ m)

D. Visceral Granuloma

- Thought to be nutrition related and perhaps related to nephrocalcinosis in other salmonids.
- Found primarily Salmon and Brook Trout.
- in Atlantic



Large granulomas are present in liver of Atlantic salmon with visceral granuloma.

Glossary — Histology and Histopathology

- Abscess - circumscribed area filled with liquefied cell debris and leukocytes
- Adenocarcinoma - malignant neoplasm of epithelial cells forming glandular structures
- Adenoma - benign neoplasm of epithelial cells forming glandular structures
- Anaplasia - loss of structural differentiation of cell (reversion to primitive cell)
- Aneurysm - circumscribed dilation of an artery
- Anorexia - diminished appetite
- Antemortem - before death
- Ascites - accumulation of serous fluid in body cavity
- Atrophy - wasting of tissue, organs, etc
- Autolysis - self-digestion by own enzymes
- Benign - mild illness; non-malignant neoplasm
- Calculi - concretion formed by salts of inorganic or organic acids
- Carcinogen - cancer-producing substance (e.g. aflatoxin)
- Carcinoma - malignant neoplasm of epithelial tissue
- Cataract - loss of transparency of eye lens or lens capsule
- Ceroid - see lipofuscin
- Cholangioma - neoplasm of bile ducts
- Chondroma - benign neoplasm of cartilage-forming cells
- Chromaffin Tissue - interrenal cells having affinity for chromic salts
- Cirrhosis - fibrosis of liver with loss of architecture and function
- Concretions - aggregation or formation of solid materials, usually in layers
- Congestion - abnormal amount of blood in vessels
- Cyst - abnormal sac filled with fluid or foreign material

Chapter 8 - Glossary
Fish Histology and Histopathology

Edema - excessive fluid in cells, tissues, serous cavities

Embolism - obstruction of vessel by transported blood clot or foreign material (gas)

Emphysema - presence of air (gas) in interstices of connective tissue

Encephalitis - inflammation of brain

Endocarditis - inflammation of the lining of the heart

Enteritis - inflammation of the intestine

Eosinophilic - having an affinity for eosin stain

Epithelioma - neoplasm of epithelial cells

Exophthalmus - protrusion of the eyeballs due to fluid, gas, tissue reaction

Exudate – fluid and/or blood cells passed into tissue due to increased vascular permeability

Fatty (metamorphosis) change - abnormal accumulation of fat within parenchyma cells

Fibroblast - elongate cell capable of forming collagen fibers

Fibroma - benign neoplasm of fibrous connective tissue

Fibrosis - formation of fibrous tissue in reaction to irritant

Fixative - material used to preserve tissue by denaturing and precipitating proteins

Furuncle (boil) - pyogenic infection in hair follicle

Gastritis - inflammation of the stomach

Gastroenteritis - inflammation of the lining of the stomach and intestine

Giant Cell - multi-nucleated cell formed by fusion of macrophages

Glomerulonephritis - inflammation of glomeruli

Glomerulosclerosis - hyaline deposits (scarring) in glomeruli (degenerative process)

Granuloma - nodular inflammatory lesion (wide variation in appearance)

Granulomatosis - disease characterized by multiple granulomas

Hemangioma - proliferation of vascular endothelium which may resemble neoplasm

Chapter 8 - Glossary
Fish Histology and Histopathology

Hemorrhage - bleeding

Hemosiderin - yellow-brown pigment derived from hemoglobin breakdown

Hepatitis - inflammation of the liver

Hepatoma - liver cell carcinoma (malignant)

Histology - science dealing with microscopic structure of cells, tissues, and organs

Histopathology - science dealing with microscopic structure of diseased cells, etc

Hyaline - clear eosinophilic homogenous substance found in some forms of degeneration

Hydropic Degeneration - accumulation of fluid in cytoplasmic vacuoles of cells

Hyperemia - increased blood in part of body (also congestion)

Hyperplasia - increase in number of cells in tissue or organ

Hypertrophy - increase in bulk of organ or part of body

Hypoplasia - decrease in number of cells (or loss of elements of tissue)

Hypotrophy - decrease in bulk of organ or part of body (atrophy)

Infarct - necrosis due to localized insufficiency of blood supply

Infiltration - the diffusion or accumulation in a tissue or cells of substances abnormal to it

Inflammation - response of body to injury

Ischemia - deficiency of blood in a part, due to functional constriction or actual obstruction of a blood vessel

-itis - suffix indicating inflammation

Karyolysis - destruction of nucleus marked by swelling and dissolution

Karyorrhexis - fragmentation of the nucleus (stage of necrosis)

Leiomyoma - benign neoplasm of smooth muscle

Lesion - wound, injury, pathologic change in tissue

Lipofuscin - brown pigment produced by autoxidation of unsaturated fats (wear and tear)

Chapter 8 - Glossary

Fish Histology and Histopathology

Lipoma - benign neoplasm of adipose tissue

Lithiasis - formation of calculi, especially biliary or urinary stones

Lordosis - dorso-ventral curvature of the body (spine)

Lymphosarcoma - neoplastic proliferation of abnormal lymphocytes

Macrophage - mononuclear phagocytic cell

Malignant - (neoplasm) having uncontrolled growth and dissemination through the body

Melanin - brown to black pigment derived from tyrosine by melanocytes

Melanophore - melanin-containing cell contributing to color changes in fish

Melanoma - malignant neoplasm derived from melanocytes

Meningitis - inflammation of membranes covering the brain and/or spinal cord

Metastasis - occurrence of neoplasm remote from the primary site

Micrometer - one-millionth of a meter

Morbid - diseased, pathological

Moribund - dying

Mycosis - disease caused by fungi

Myositis - inflammation of muscle

Necrosis - death of one or more cells

 Liquefaction – produces liquefied material, cells dissolved

 Coagulative – protein denatured, cell outline preserved

 Caseous – combination of liquefaction and coagulative, often in granuloma

Neoplasm - abnormal mass of tissue with uncoordinated growth

Nephritis - Inflammation of kidney tissue except tubules (nephrosis)

Nephroblastoma - mixed renal neoplasm of metanephric blastema

Nephrocalcinosis - presence of calcification in the kidney (form of lithiasis)

Nephrolithiasis - presence of calculi in kidney

Nephron - functional unit of kidney (glomerulus, proximal and distal tubules)

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Fish Histology and Histopathology

Nephrosis - diseases of the kidney tubules

Neurilemmoma (neurofibroma) - benign neoplasm of Schwann cells of nerve tissue

-oma - suffix denoting tumor or neoplasm

Oncology - science dealing with neoplasms

Osteoma - benign neoplasm of bone tissue

Papilloma - benign epithelial neoplasm

Parenchyma - distinguishing cells of an organ (e.g. hepatocytes)

Pathognomonic - characteristic of a disease

Pathology - science dealing with all aspects of disease

Pericarditis - inflammation of pericardium of heart

Peritonitis - inflammation of peritoneal lining of body cavity

Petechia - pinpoint hemorrhages

Post Mortem - after death

Prolapse - falling down of an organ, especially at an orifice

Proteinaceous - resembling protein

Pyknosis - condensed abnormally dark staining of the nucleus

Rhabdomyoma - benign neoplasm of striated muscle

Sarcoma - malignant connective tissue neoplasm

Scoliosis - lateral curvature of the body (spine)

Splenomegaly - enlargement of the spleen

Stroma - connective tissue framework of an organ

Telangiectasia - dilation of terminal blood vessels (spaces)

Teratoma - neoplasm of multiple tissues (embryonic)

Thrombus - blood clot attached to the blood vessel or heart wall

Trauma - injury caused by physical means

Chapter 8 - Glossary
Fish Histology and Histopathology

Tubercle - granulomatous lesion caused by mycobacteria

Tumor - a swelling

Ulcer - open lesion on skin or mucous lining

Vacuoles - clear space in a cell