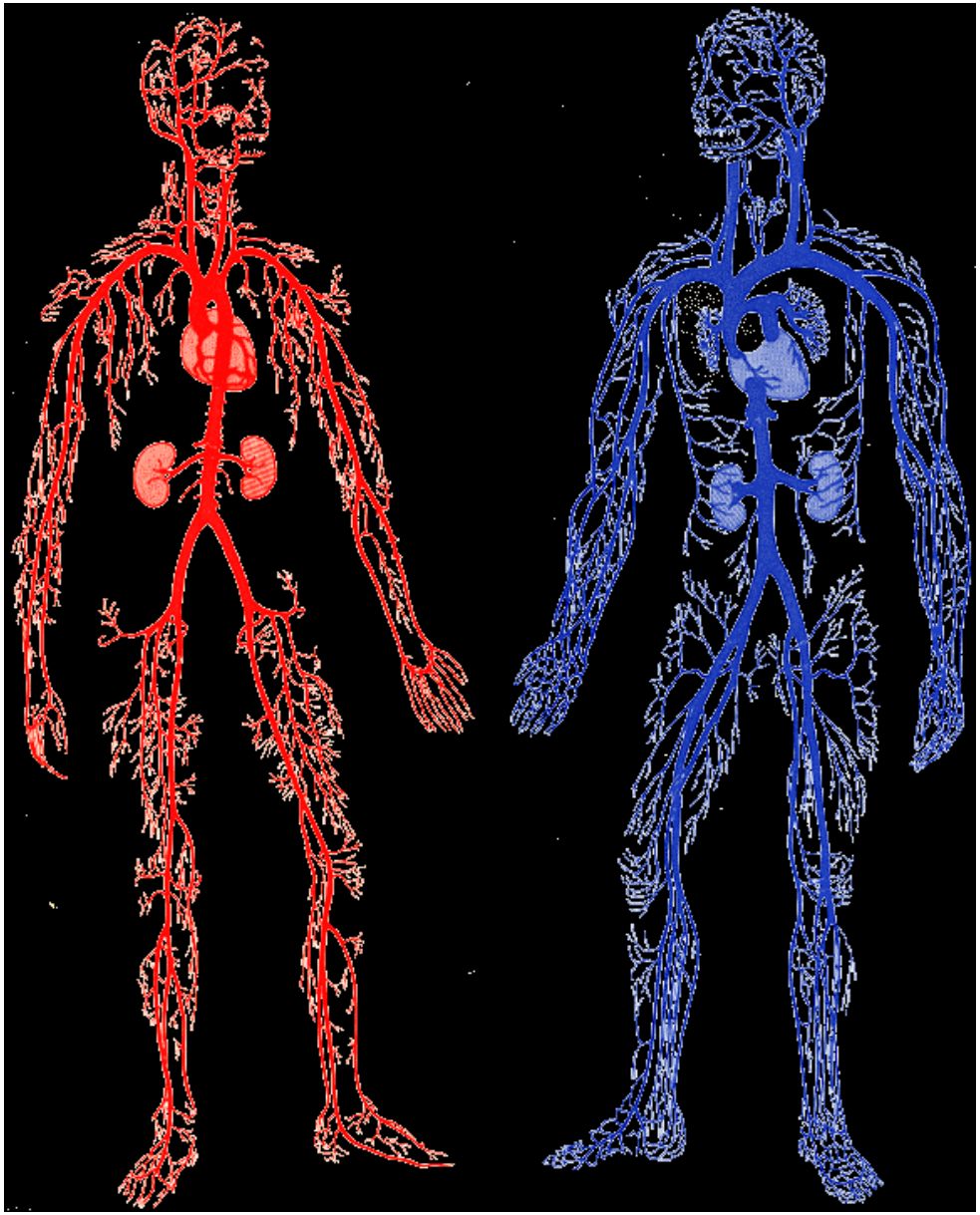


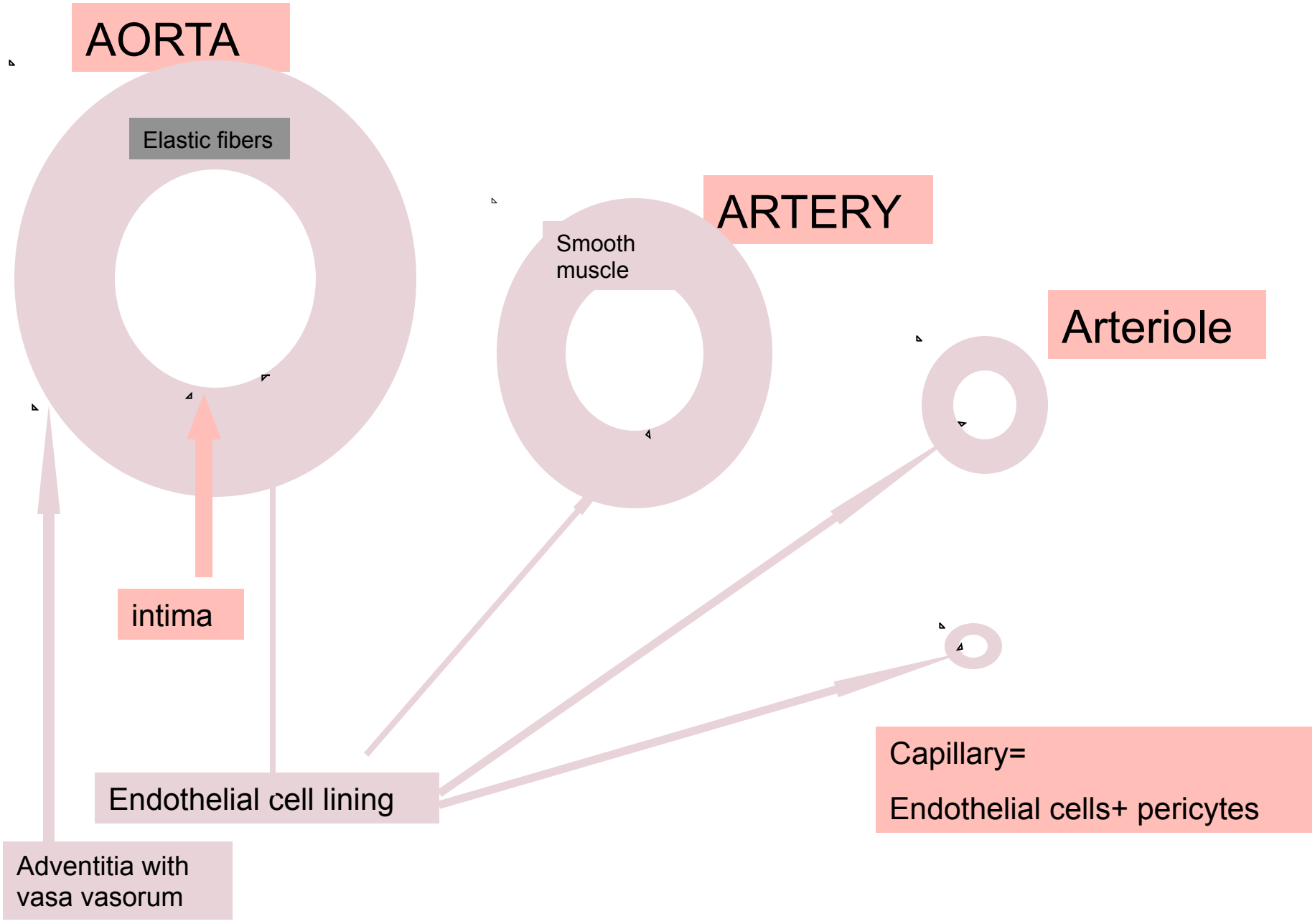
Arterial

Venous systems

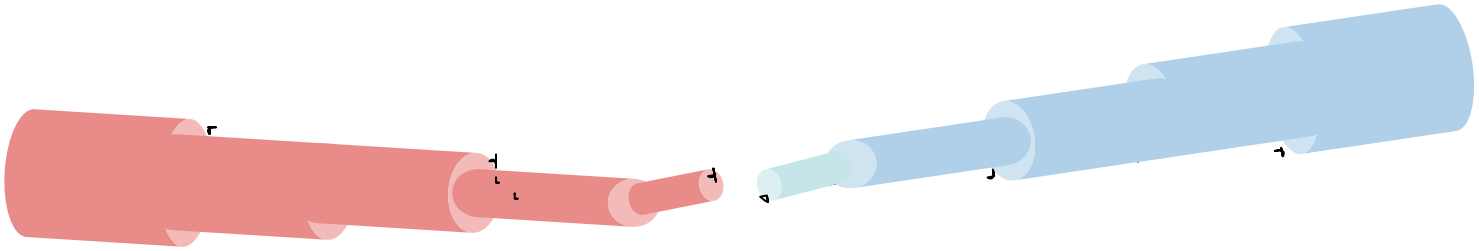
Capillaries

Lymphatics

http://www.accessexcellence.org/AE/AEC/CC/heart_anatomy.html


AORTA
Elastic fibers
ARTERY
Smooth muscle
Arteriole
intima
Endothelial cell lining
Adventitia with vasa vasorum
Capillary=
Endothelial cells+ pericytes

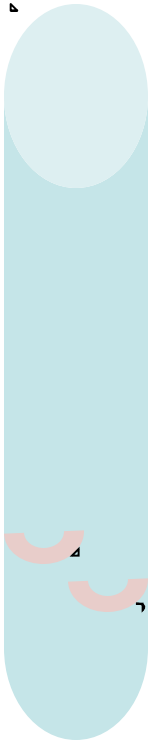
Mechanics of the circulatory system



Aorta to Artery to arteriole to capillary to arteriovenous capillaries to venule to vein

Elastic tissue in wall to smooth muscle to pericyte

Lymphatic system is separate from the blood vessels and is difficult to see without special stains

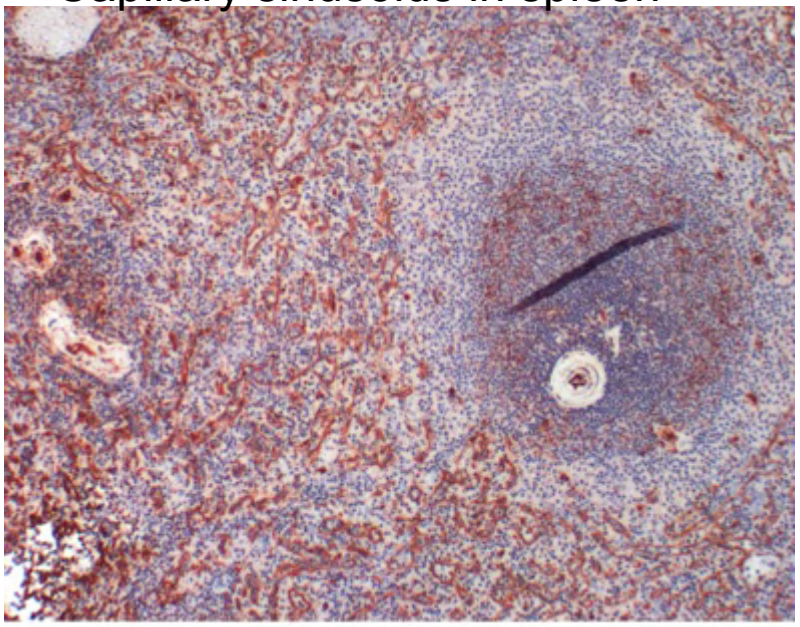


No elastic tissue in walls of veins and venules

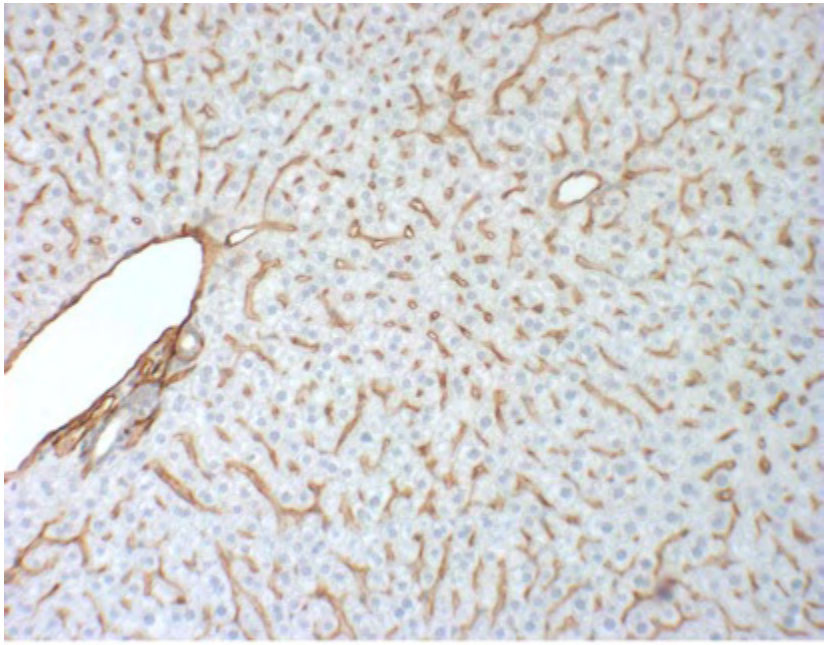
Larger veins have valves in the walls to allow blood keep flowing

Examples of discontinuous capillaries

Capillary sinusoids in spleen

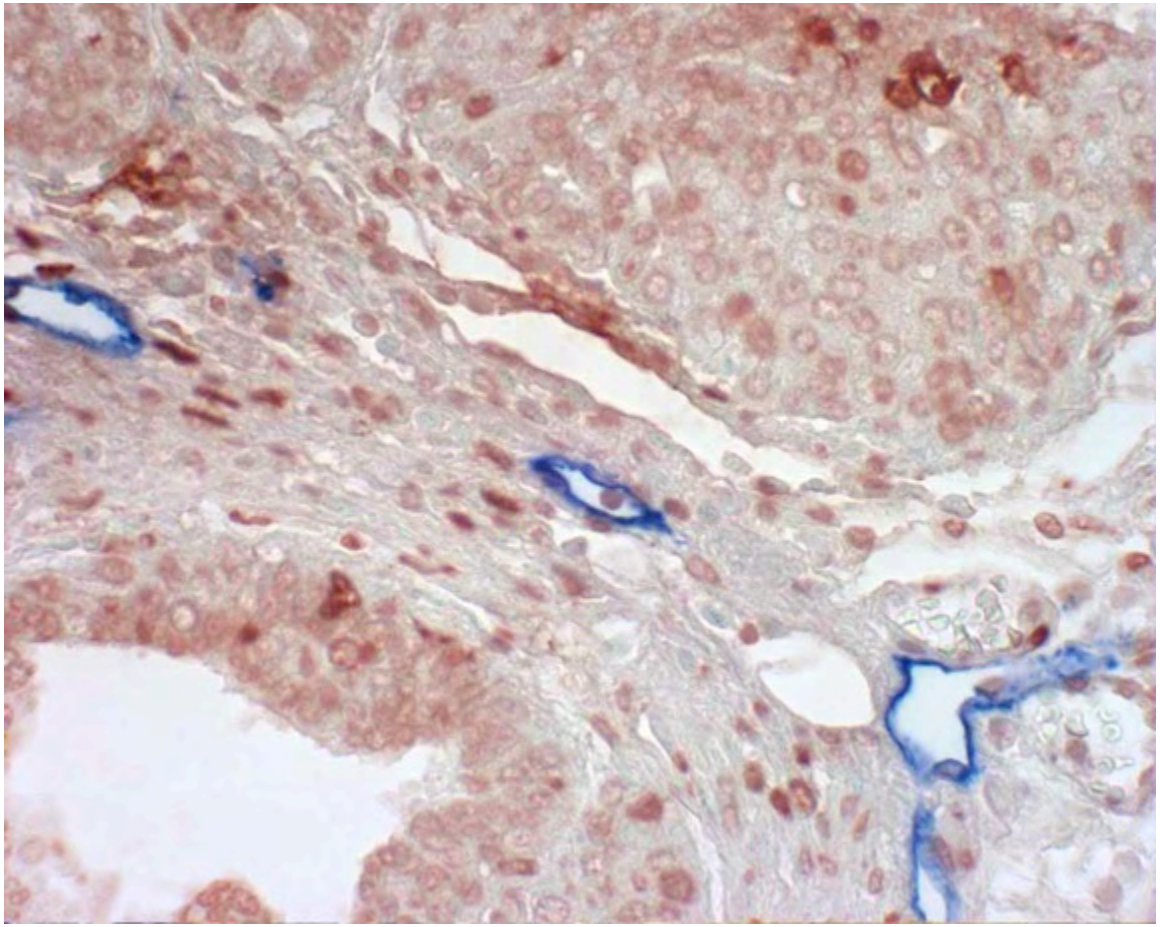


Capillary sinusoids in liver

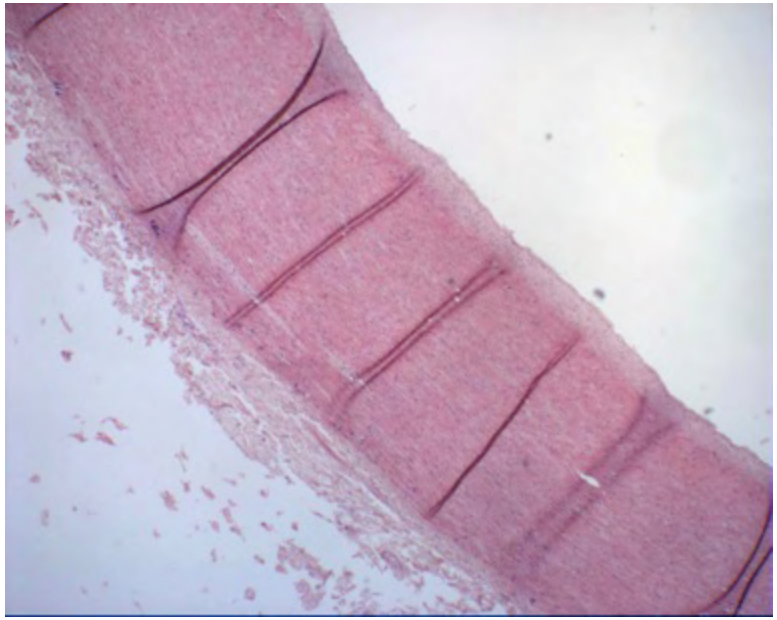


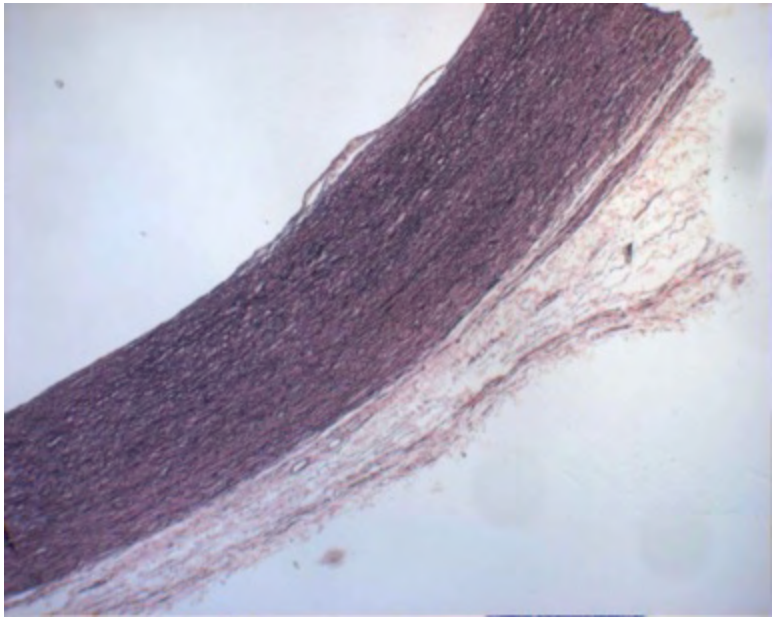
Markers for endothelial cells:

Rabbit anti-von Willebrand's factor (vWF): for large vessels
Anti-CD31 : for all endothelia (not lymphatics)

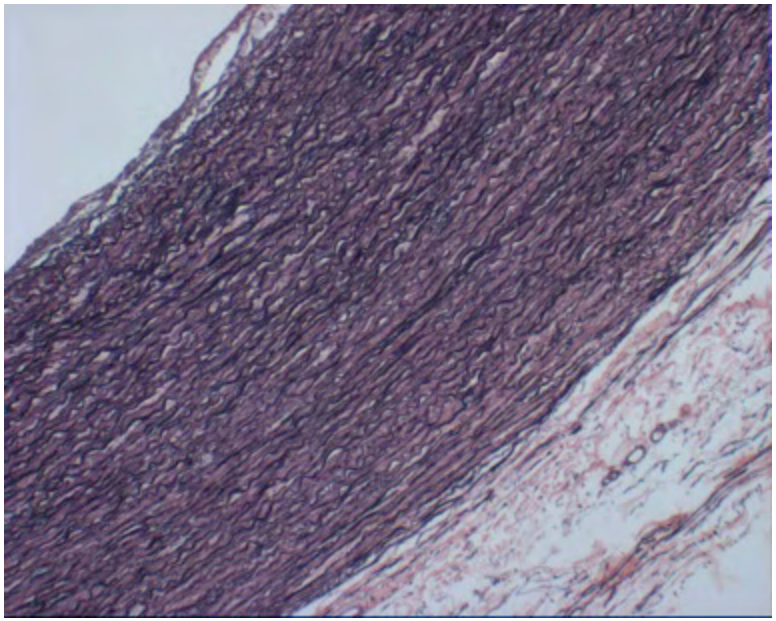


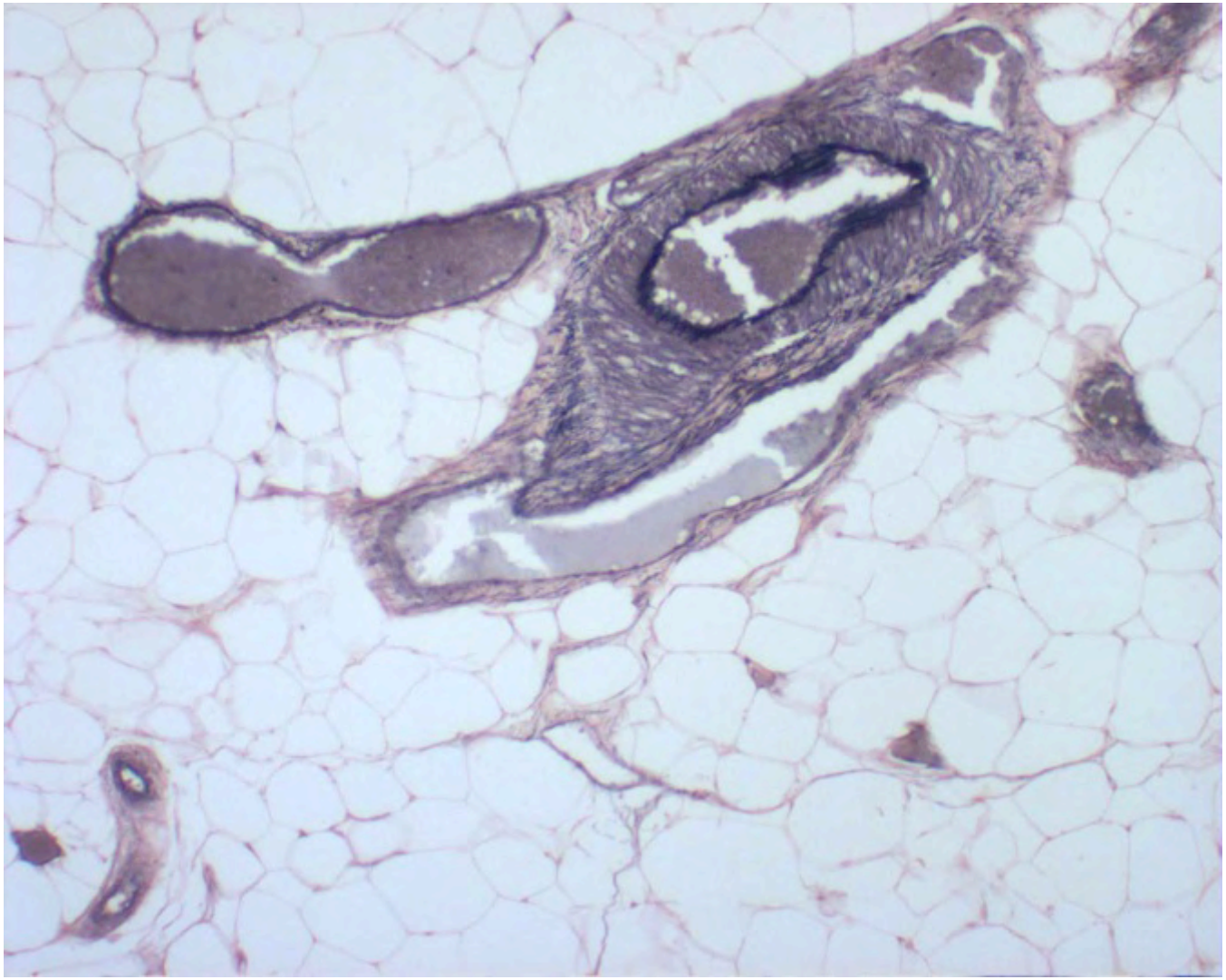
Lymphatics identified with anti-LYVE (blue), surrounding carcinoma cells



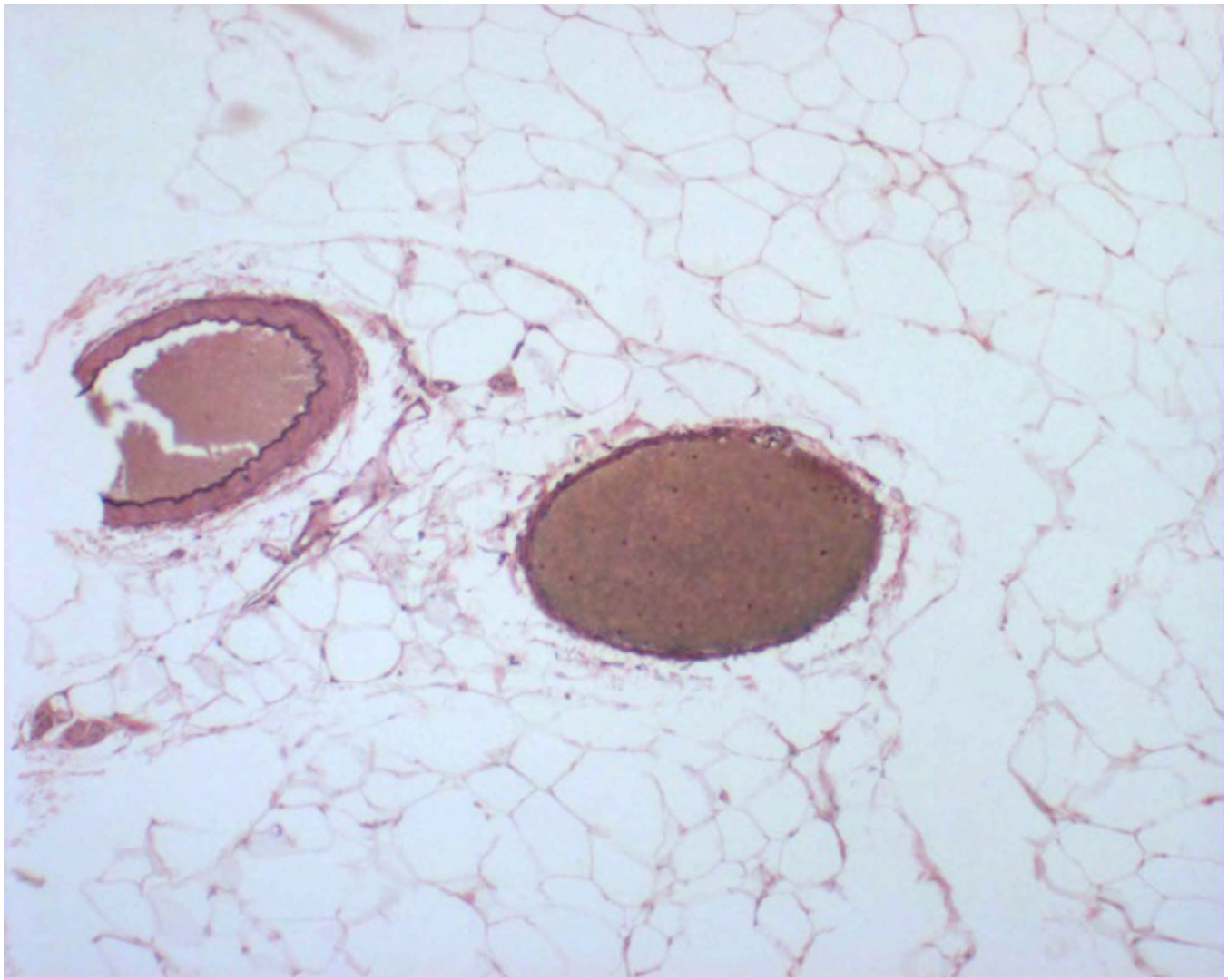


Human aorta: H&E and Elastic stain
This is a large vessel with abundant elastic fibers to contribute strength



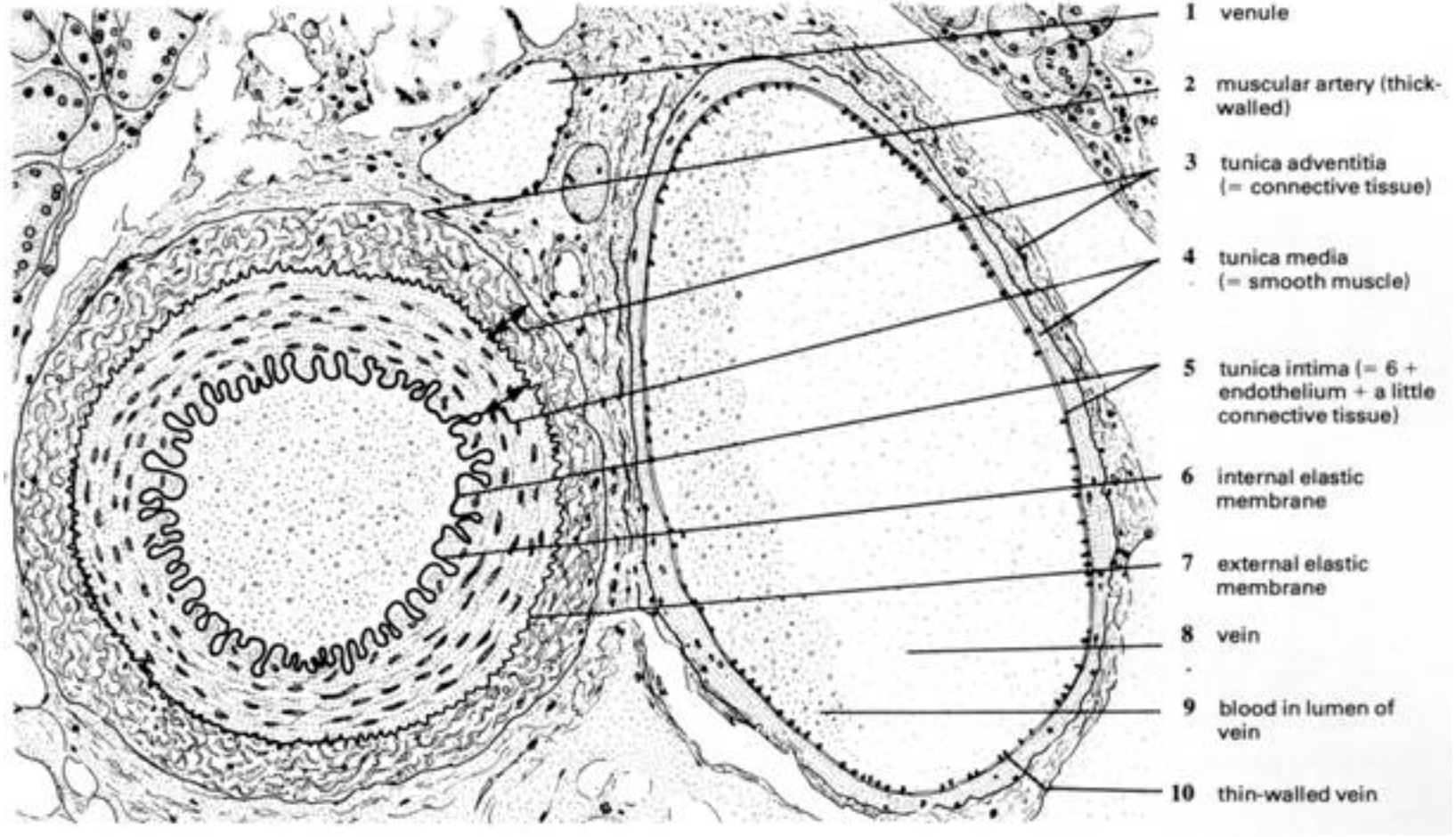


Medium sized muscular artery next to a vein, Elastic stain



Smaller arteriole and vein , elastic stain

http://www.complab.nymc.edu/Histology/CardiovascularSystem/Cardiovascular.htm



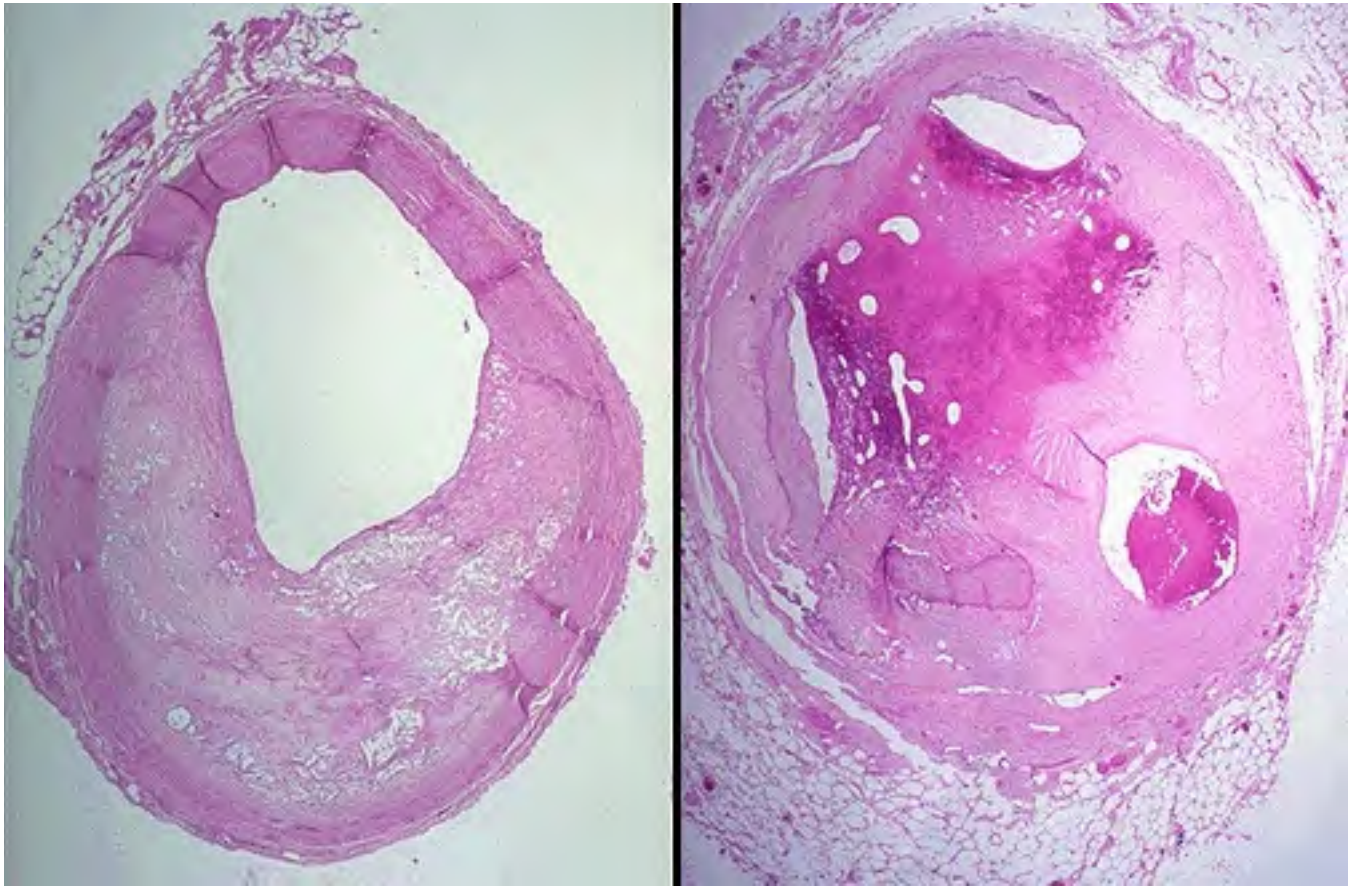
Diseases of Blood vessels include:

Atherosclerosis

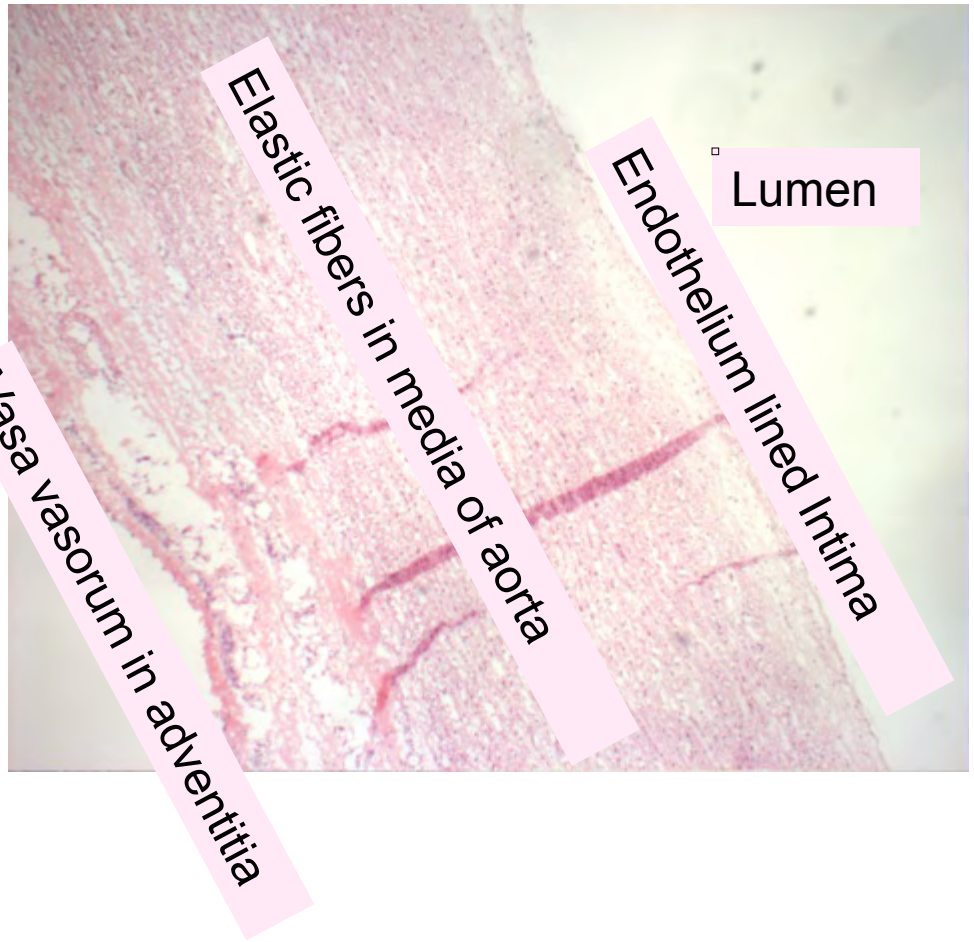
Aneurysms (dilatation)

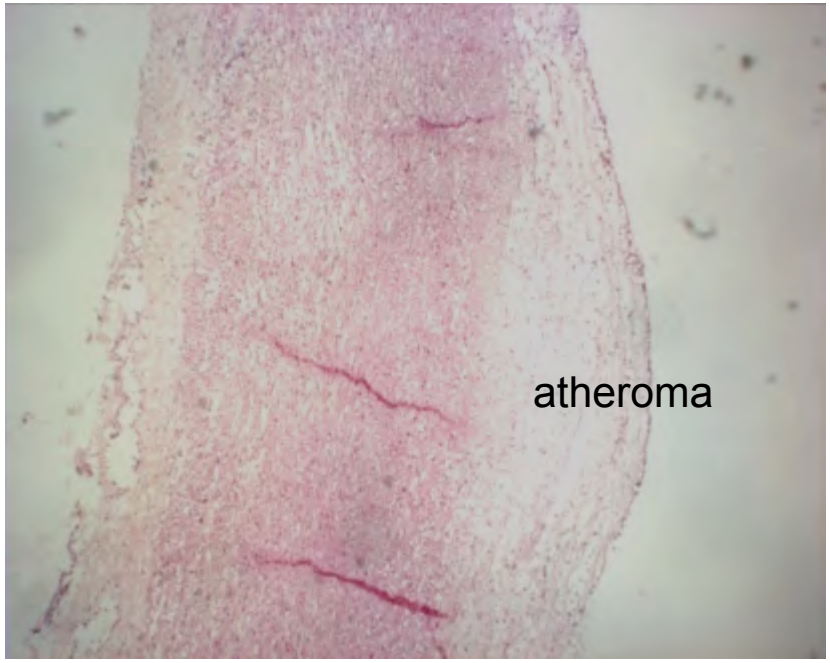
Abnormal collagen support

Atherosclerosis of coronary arteries of human heart

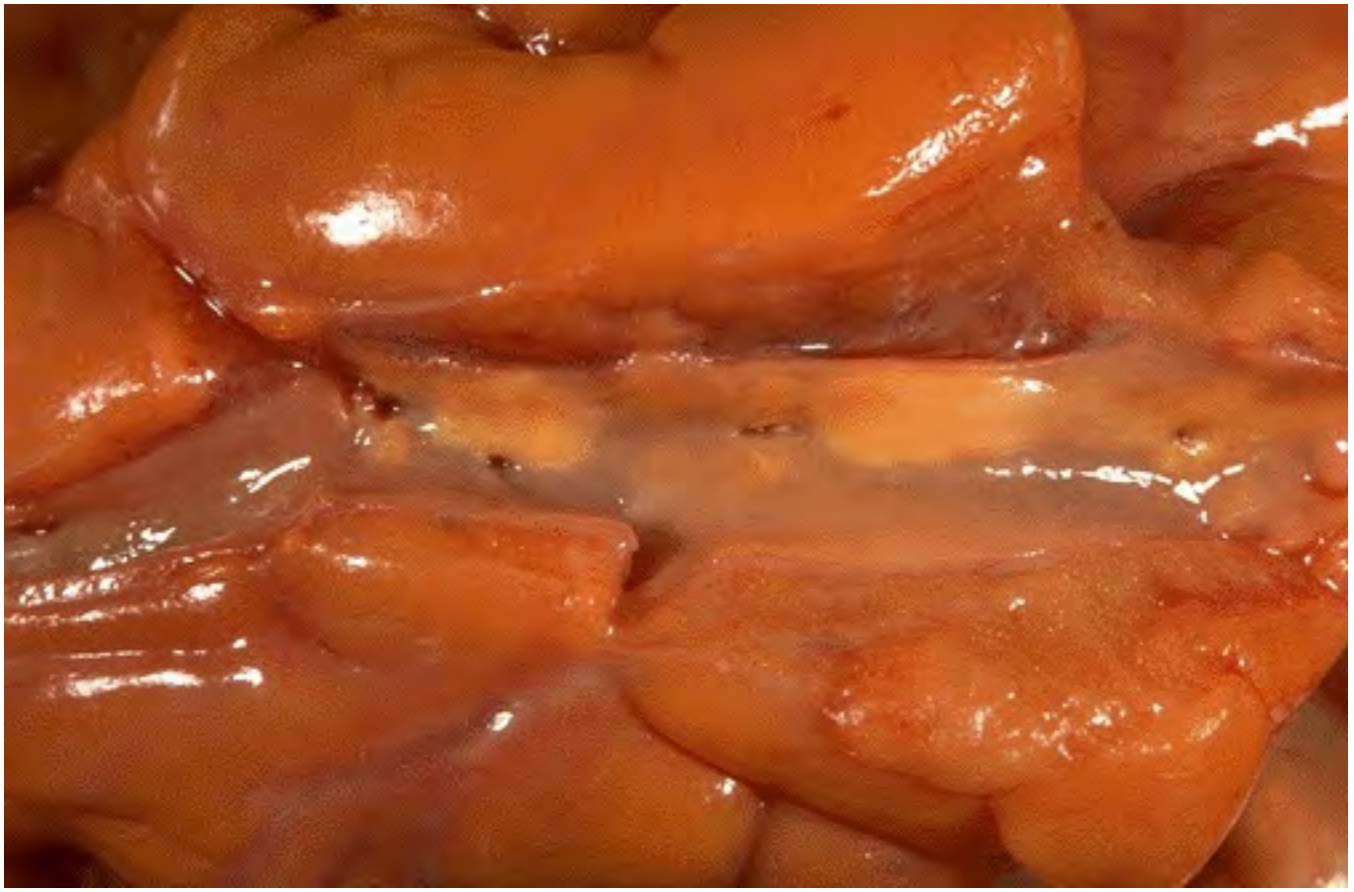


Medlib.med.utah.edu/WebPath


Lumen
Endothelium lined Intima
Elastic fibers in media of aorta
Vasa vasorum in adventitia

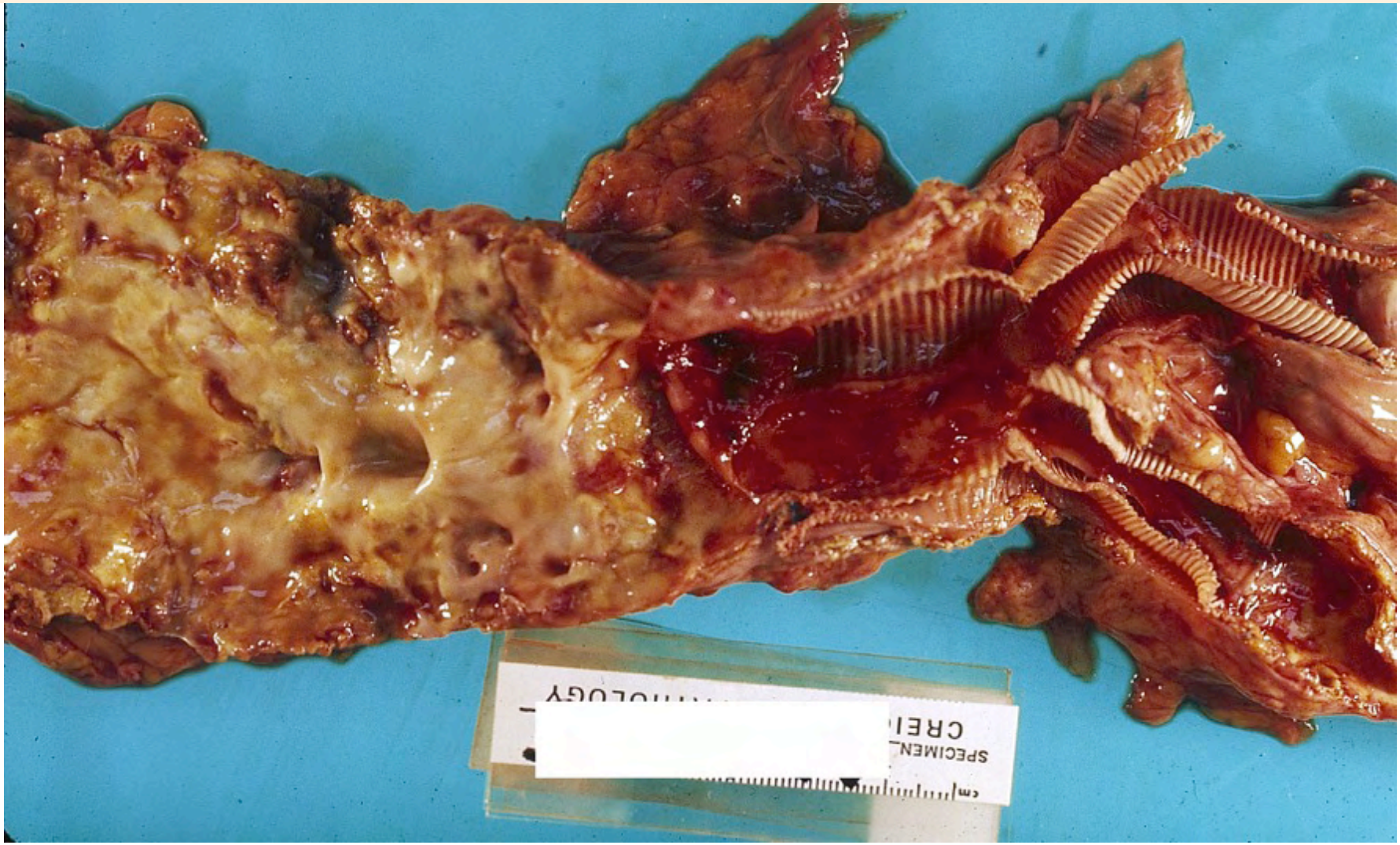

atheroma

Atherosclerosis of coronary artery of human heart

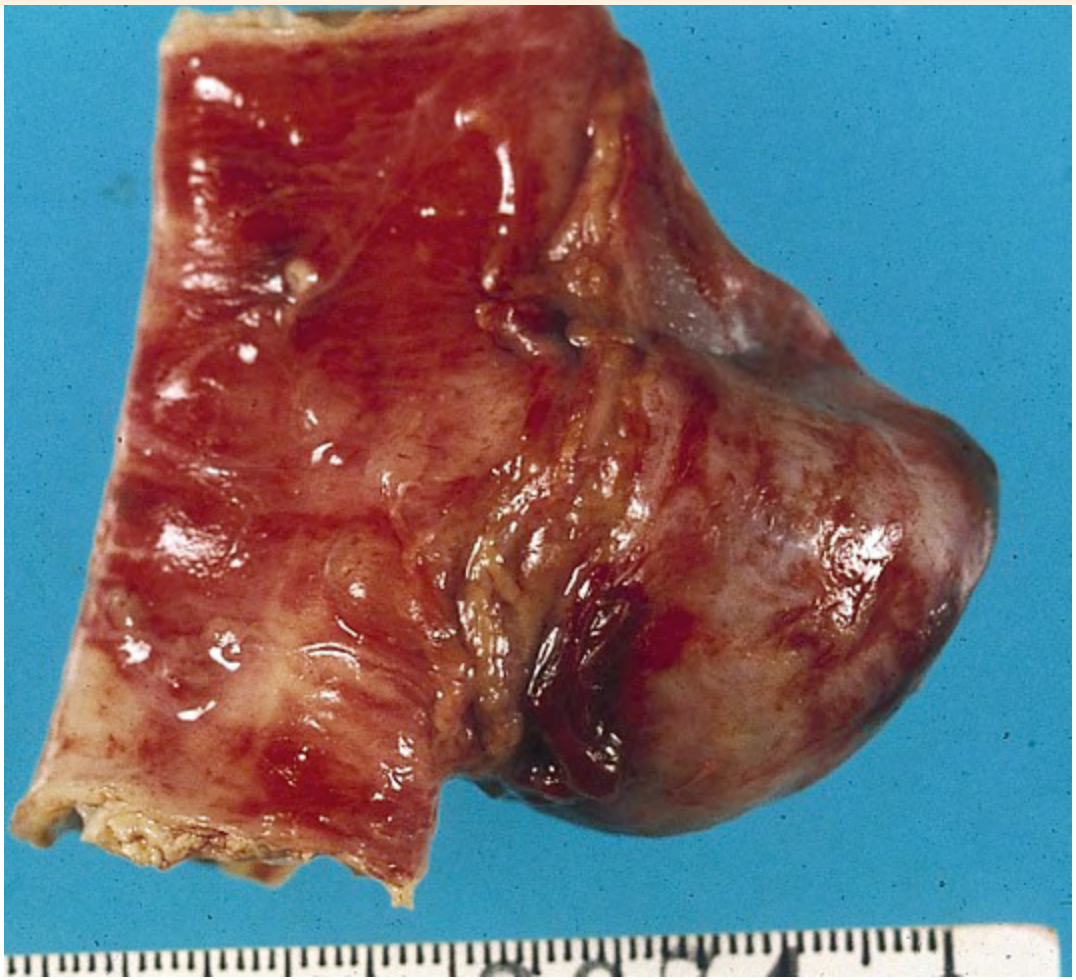


Medlib.med.utah.edu/WebPath

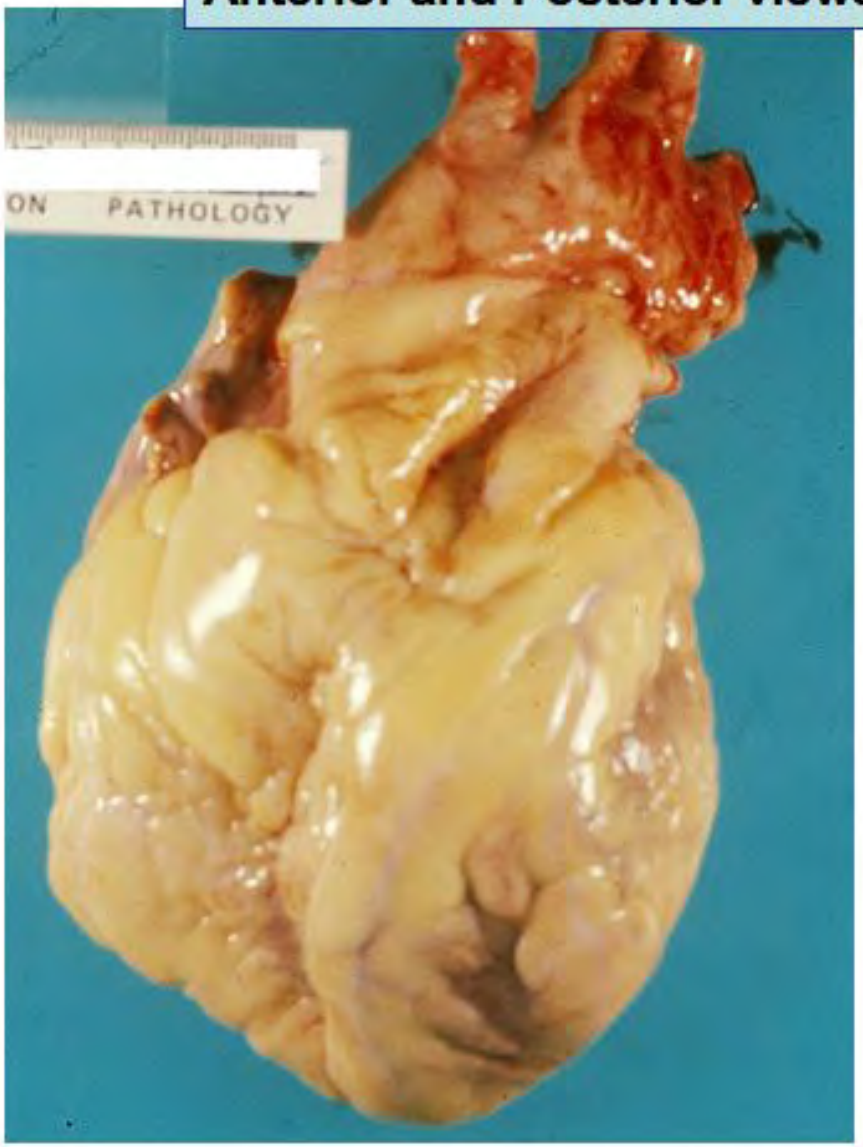
The wall of the aorta shows severe atherosclerosis, and an attempt at surgical repair of narrowed areas at the bifurcations

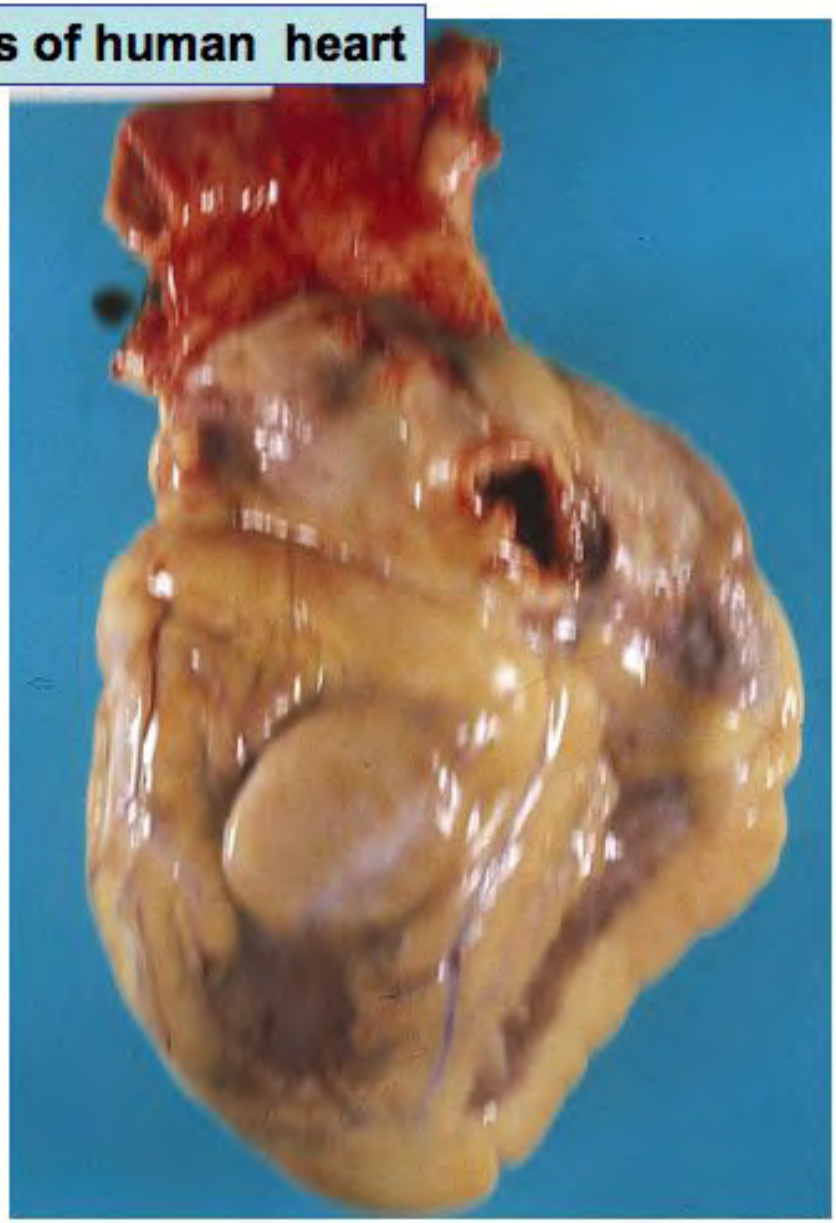


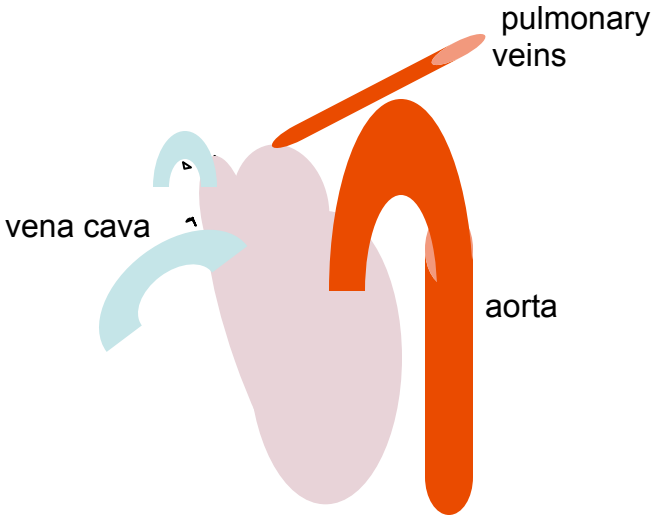
The wall of the aorta is weakened due to pathologic processes such as atherosclerosis, and because of the constant pressure, develops a bulge--aneurysm, complications of which are: thrombosis, rupture, dissection



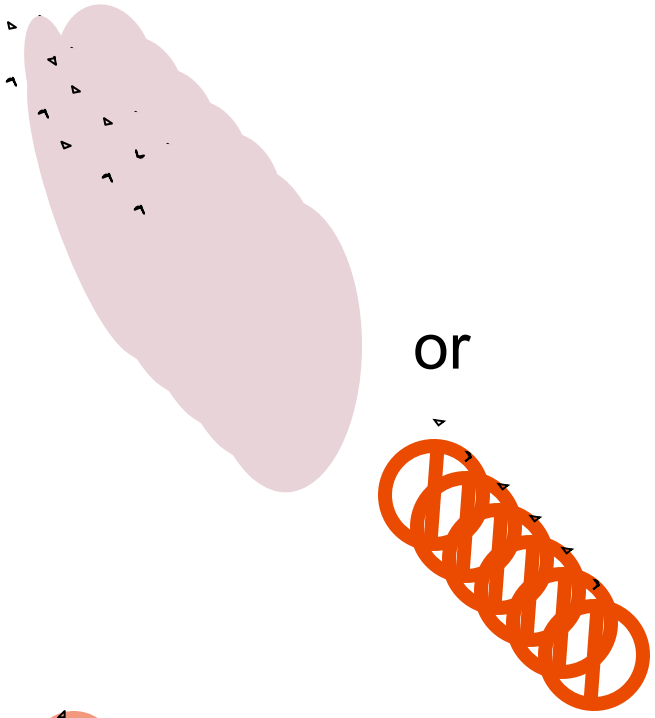
Anterior and Posterior views of human heart

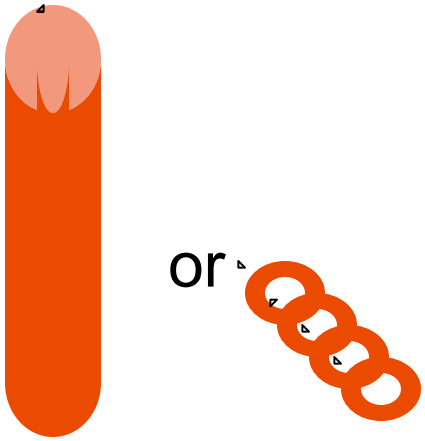






How would one proceed to orient and section to view morphology and relationship of the different structures to each other?





https://instagram.com/candyanatomy/

Mike McCormick - 27yr -Glasgow University Medical Student

candyanatomy 5w

19 likes

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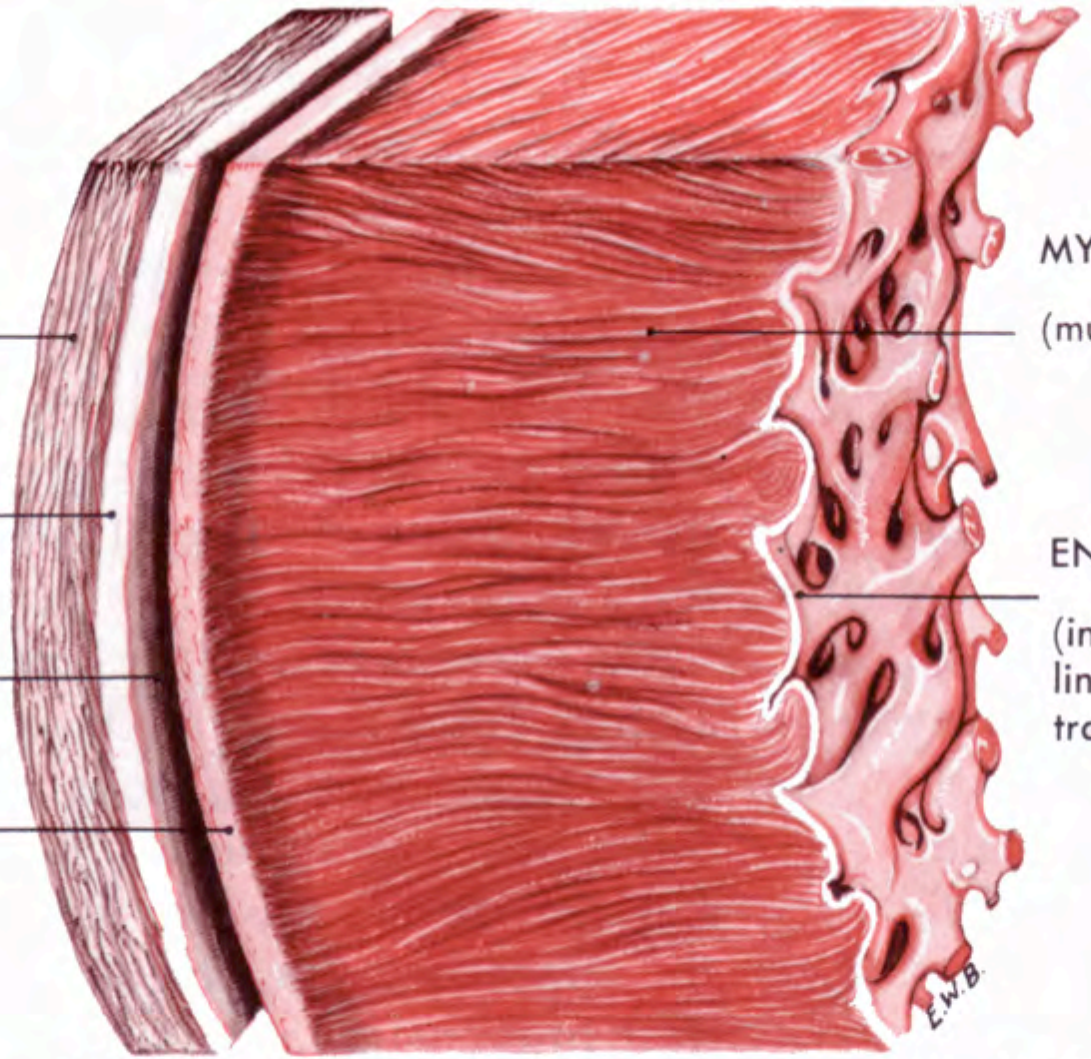
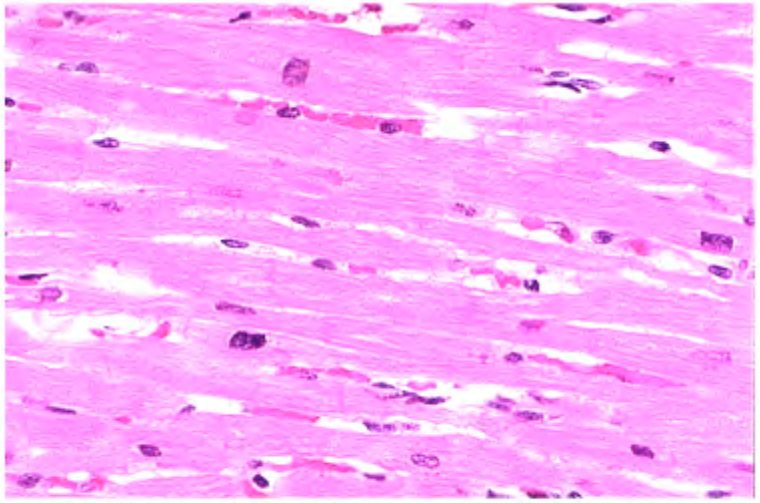
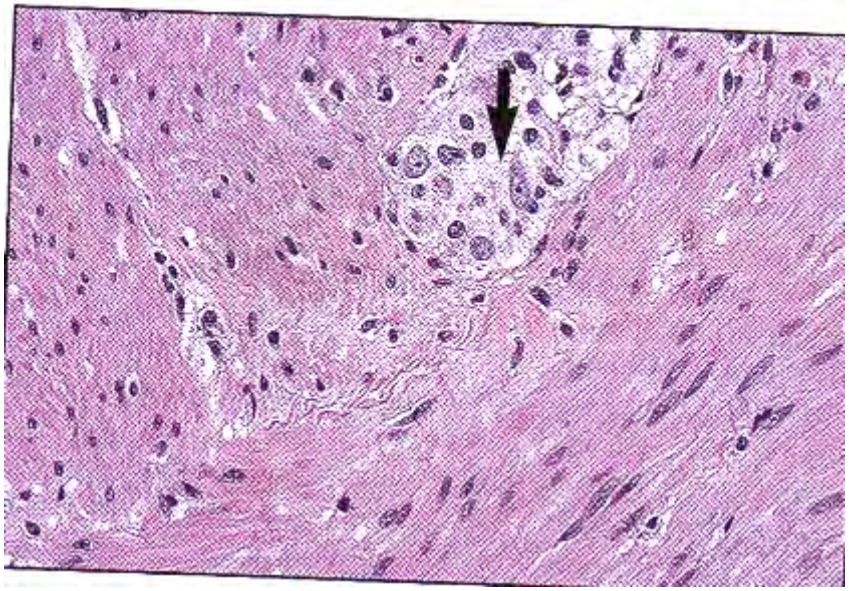


Section of the heart wall showing the components of the outer pericardium (heart sac), muscle layer (myocardium), and inner lining (endocardium).

TYPES OF MUSCLE: Cardiac, Smooth, Skeletal



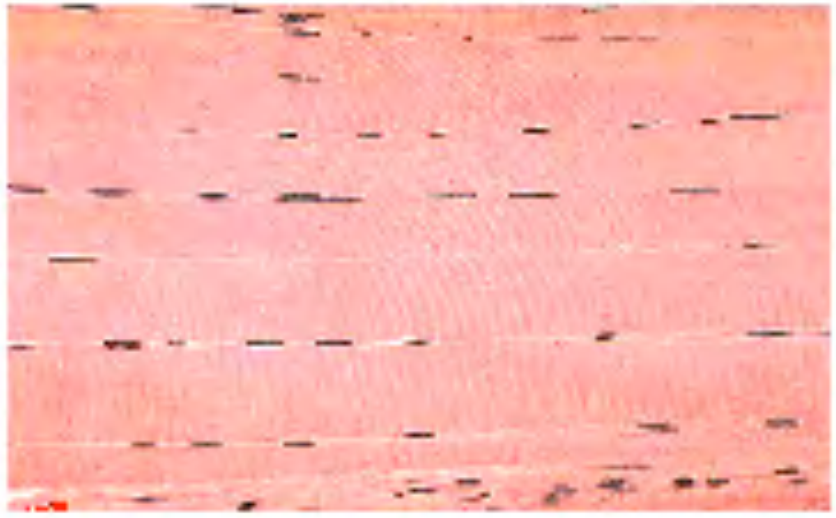
http://medlib.med.utah.edu/WebPath/TUTORIAL/MYOCARD/MI022.html

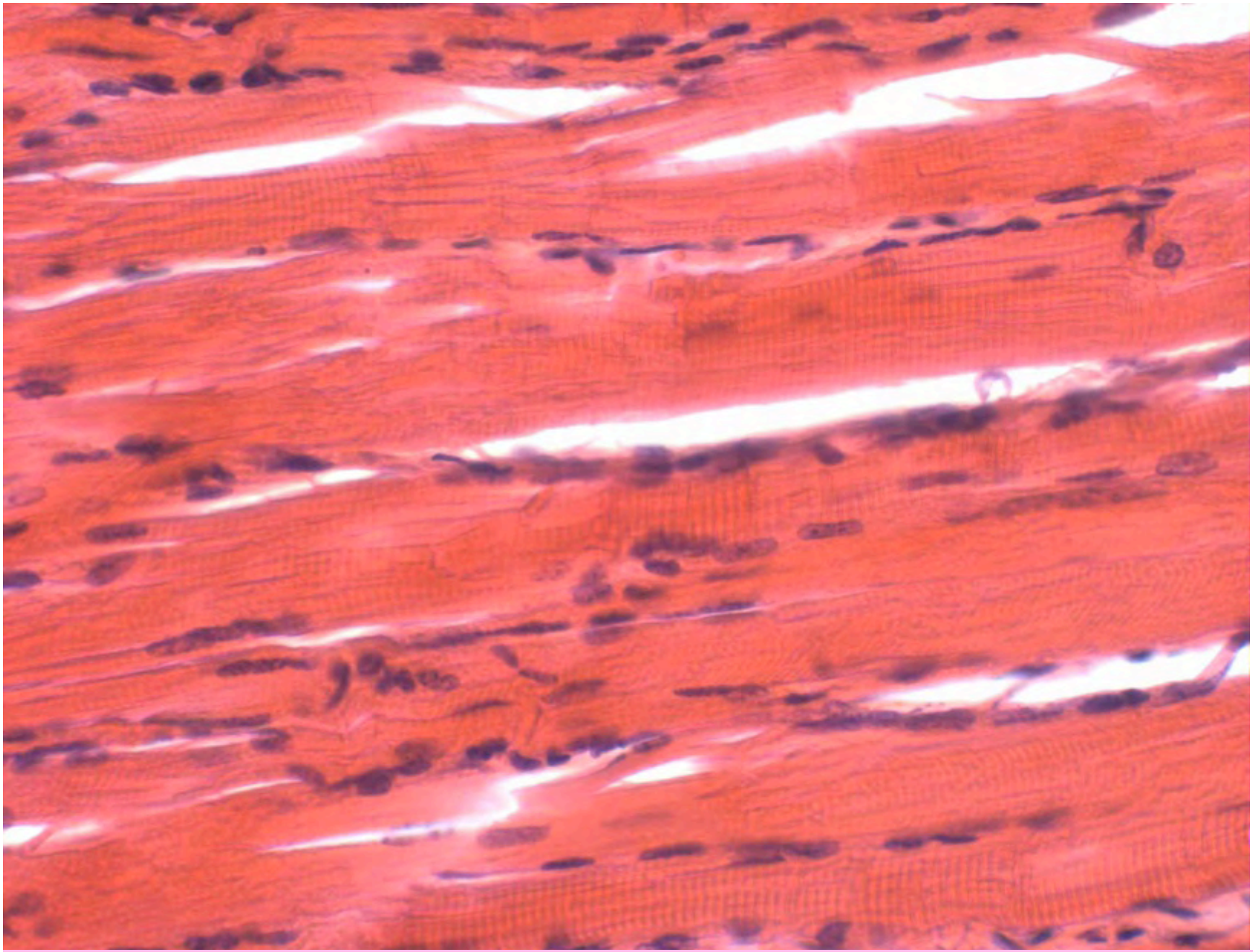


Cardiac: striations + central nuclei

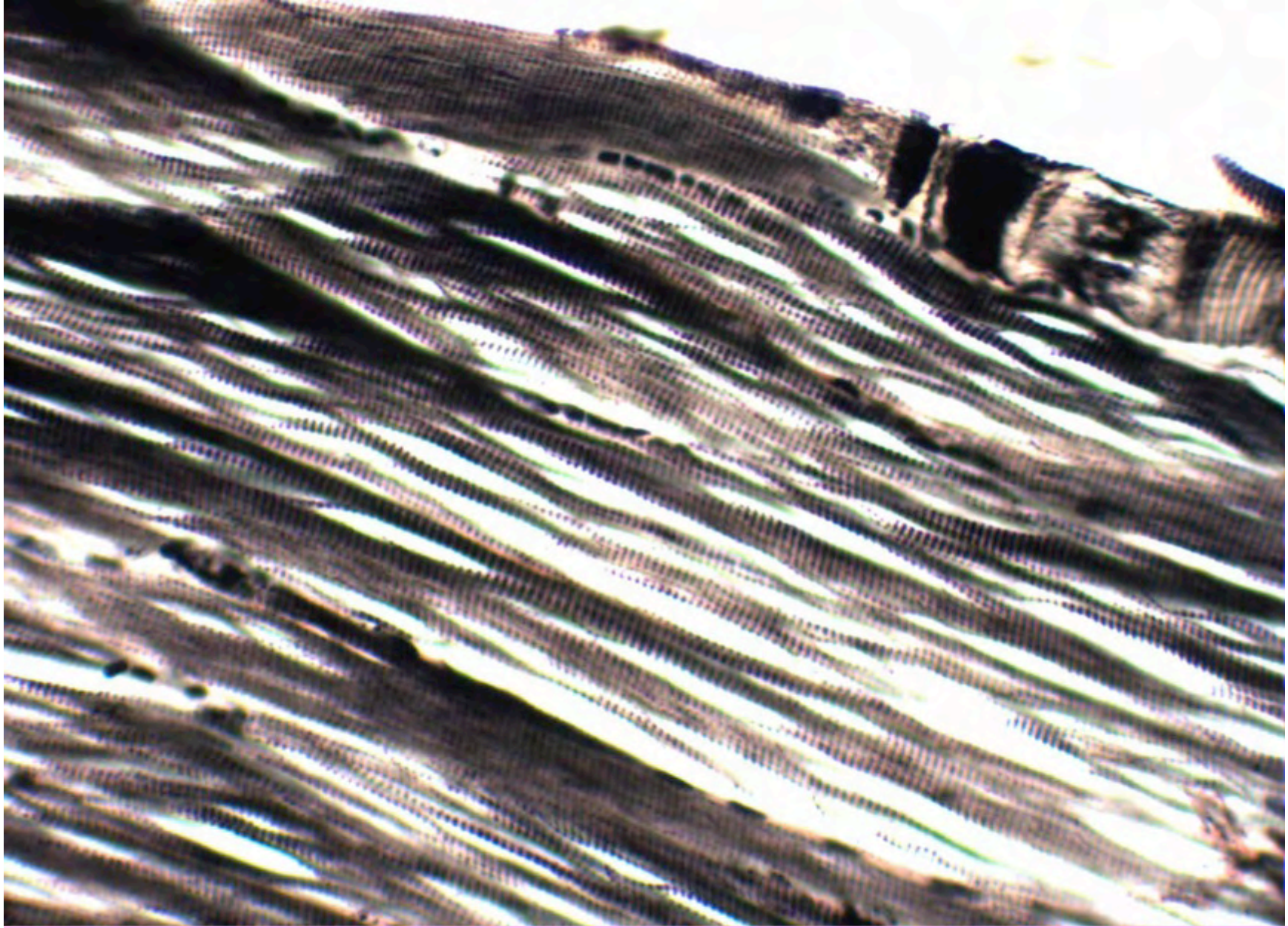
Skeletal: striations + eccentric nuclei

Smooth: central nuclei

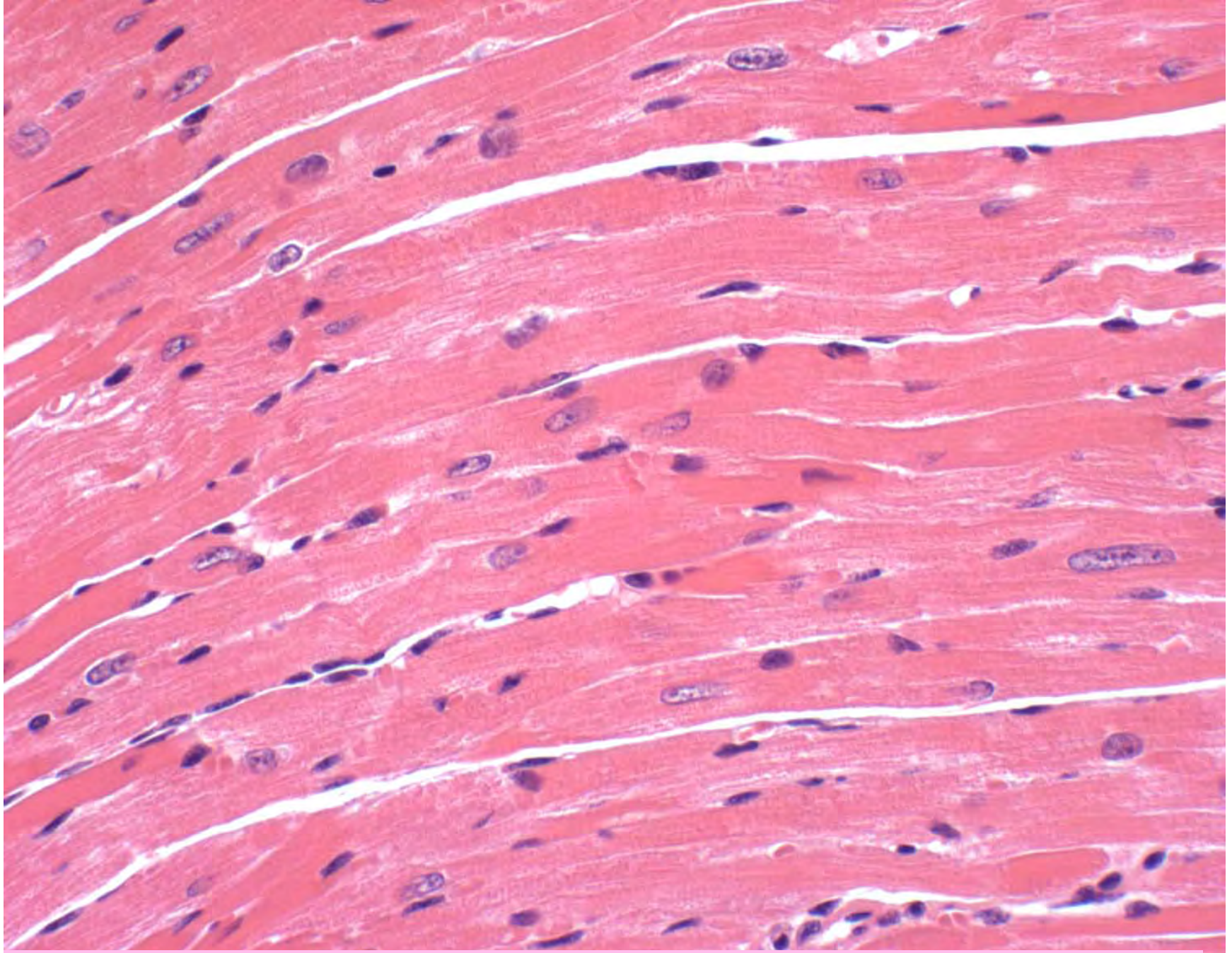




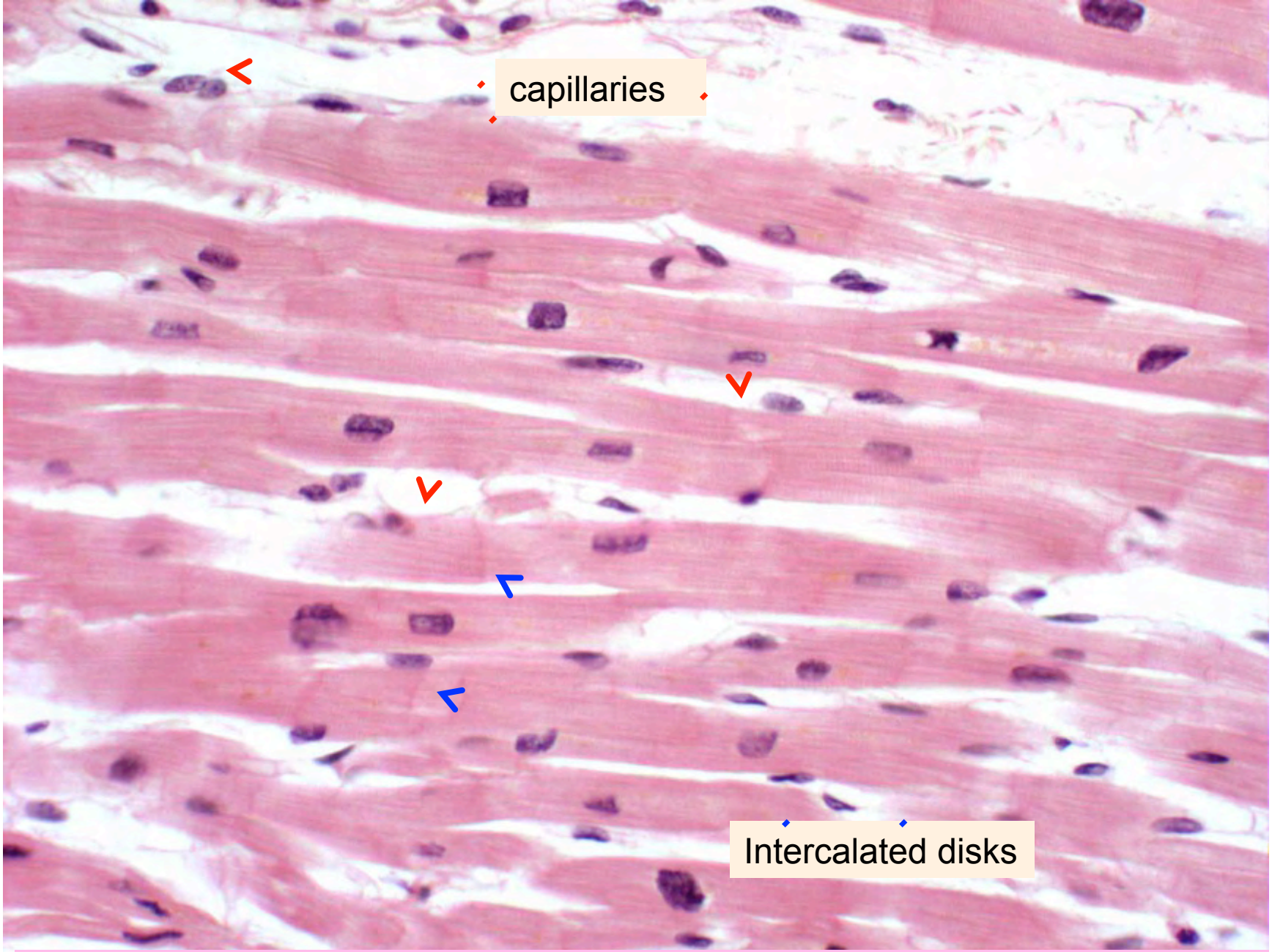
Human Skeletal muscle: nuclei at the edges and striations



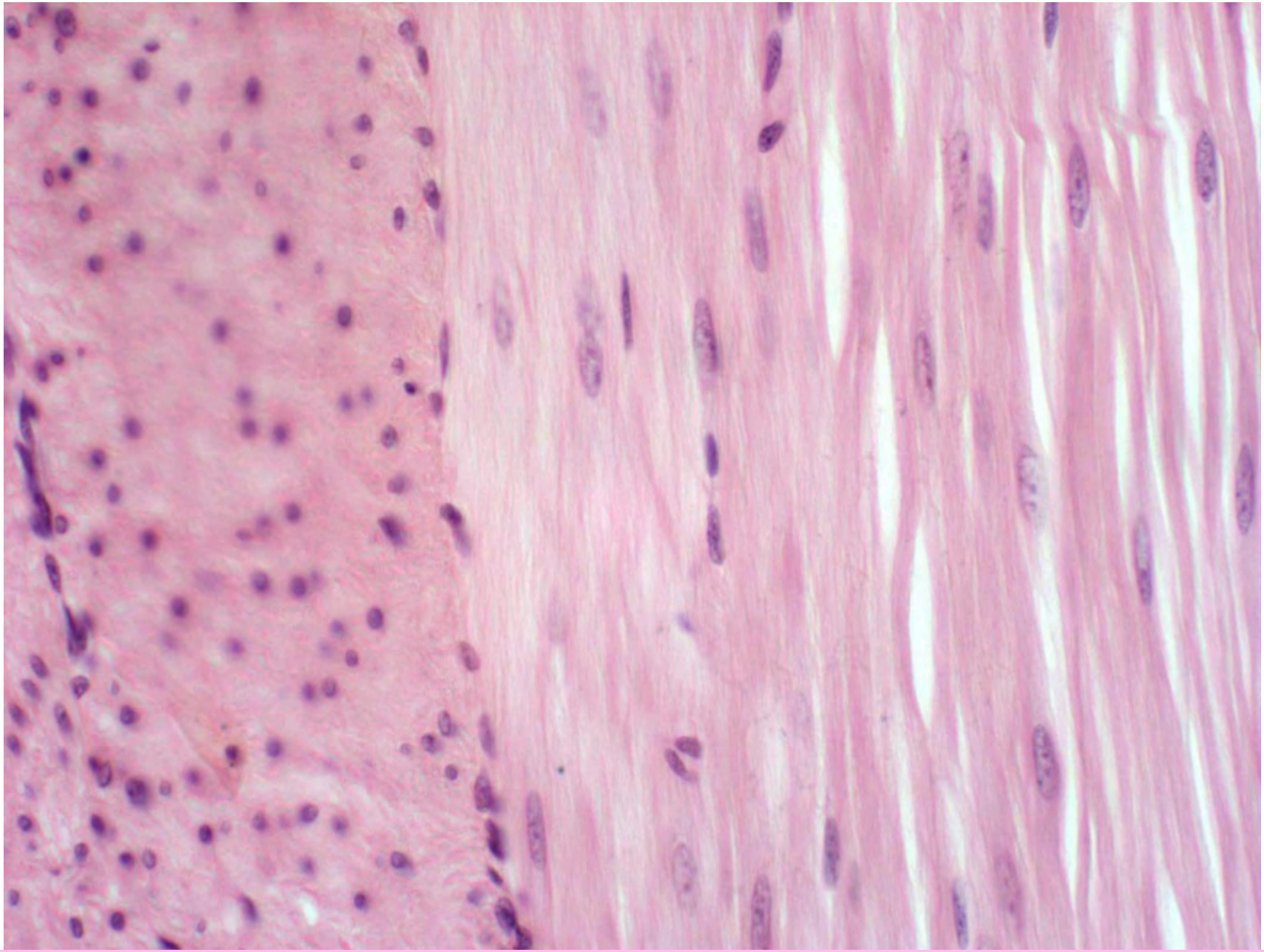
Human Skeletal muscle with PhosphoTuncsticAcidHematoxylin PTAH stain to demonstrate striations



Human Heart cardiomyocytes: central nuclei and striations

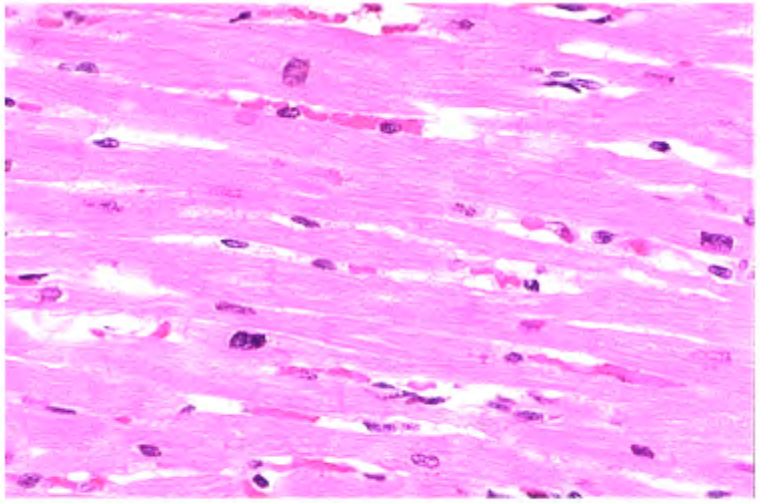


Human Heart cardiomyocytes: central nuclei and striations



Human smooth muscle: central nuclei and No striations

TYPES OF MUSCLE: Cardiac, Smooth, Skeletal



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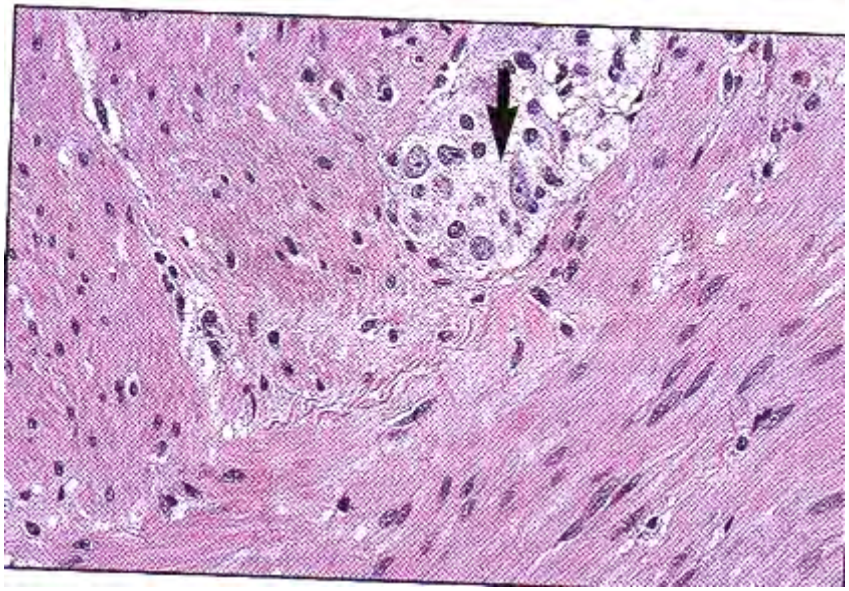
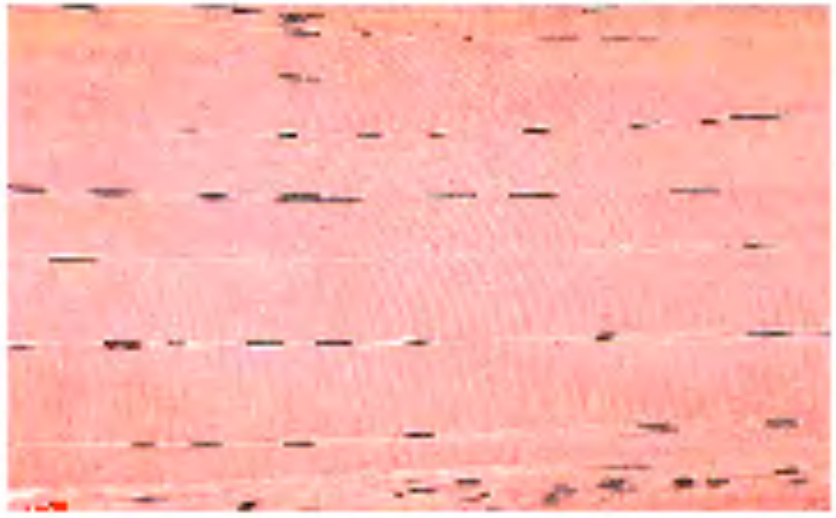


Cardiac: striations + central nuclei

Skeletal: striations + eccentric nuclei

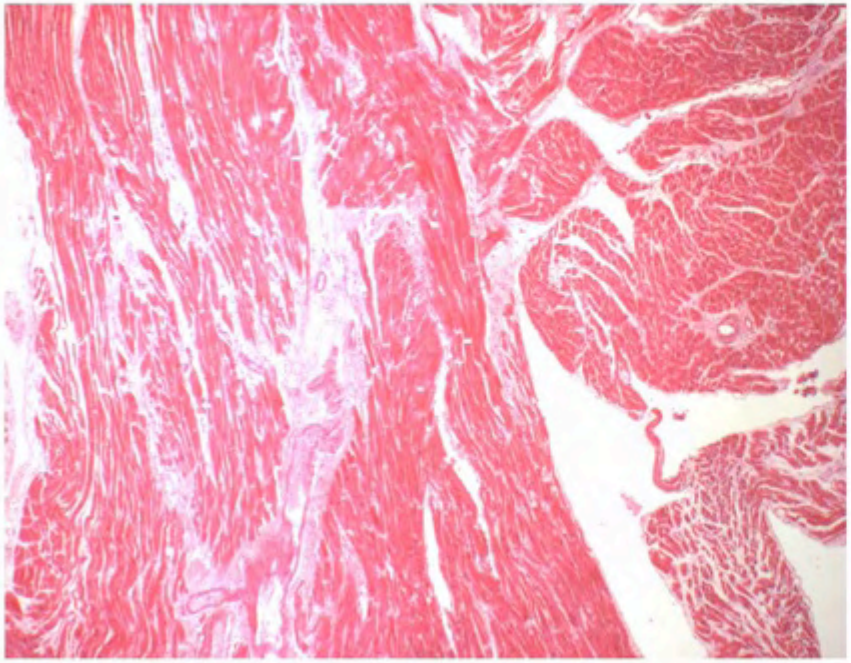
Smooth: central nuclei



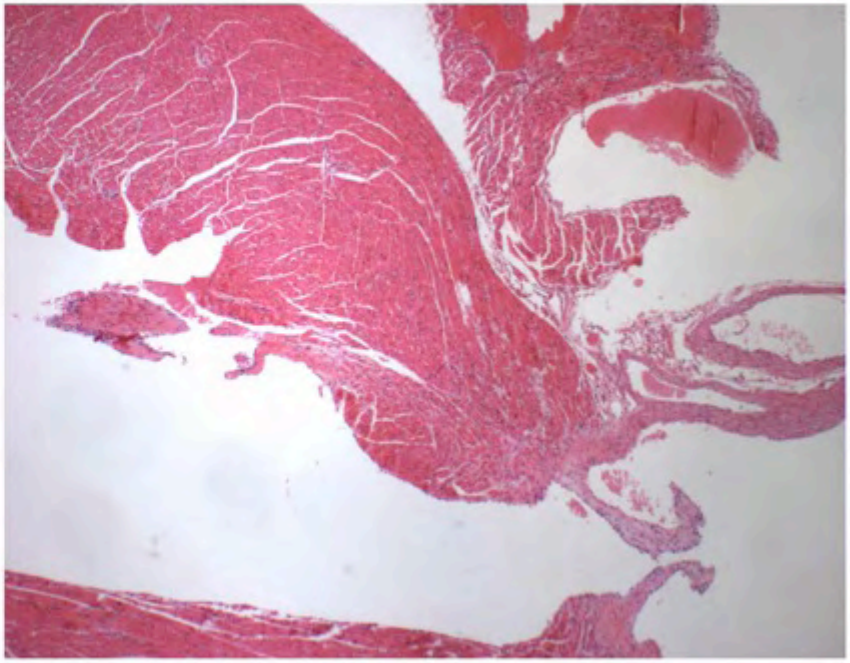
HEART

magnification x40

Human



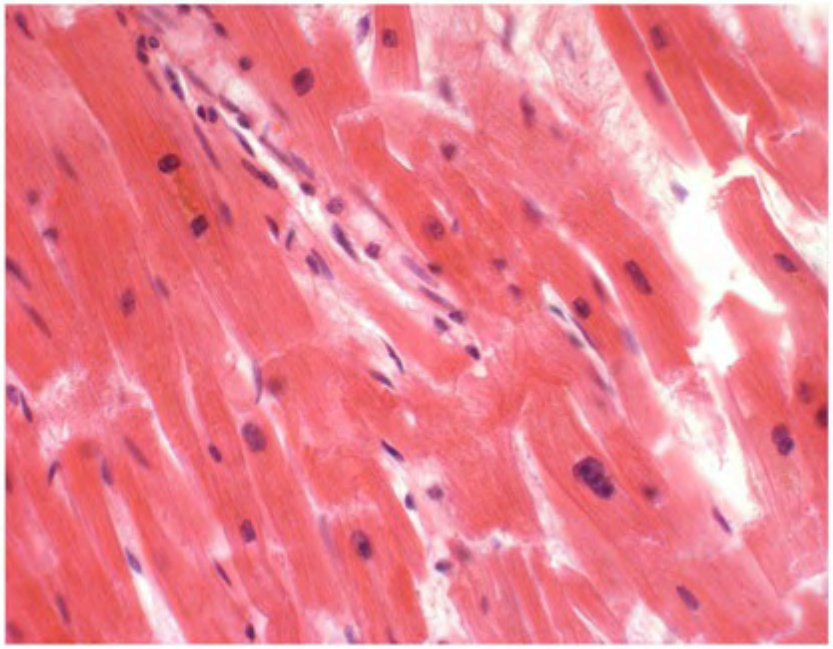
Mouse



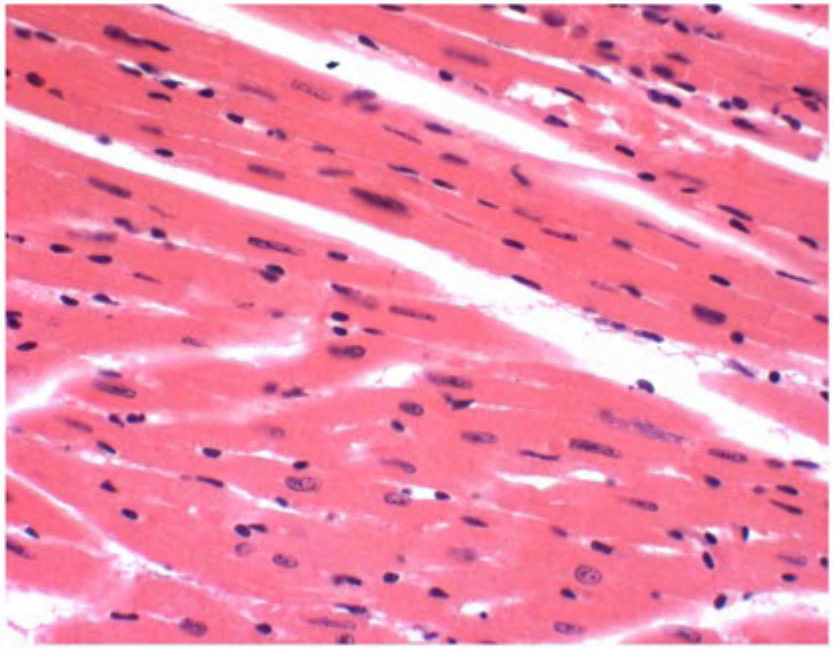
scale bar = 500 microns

HEART

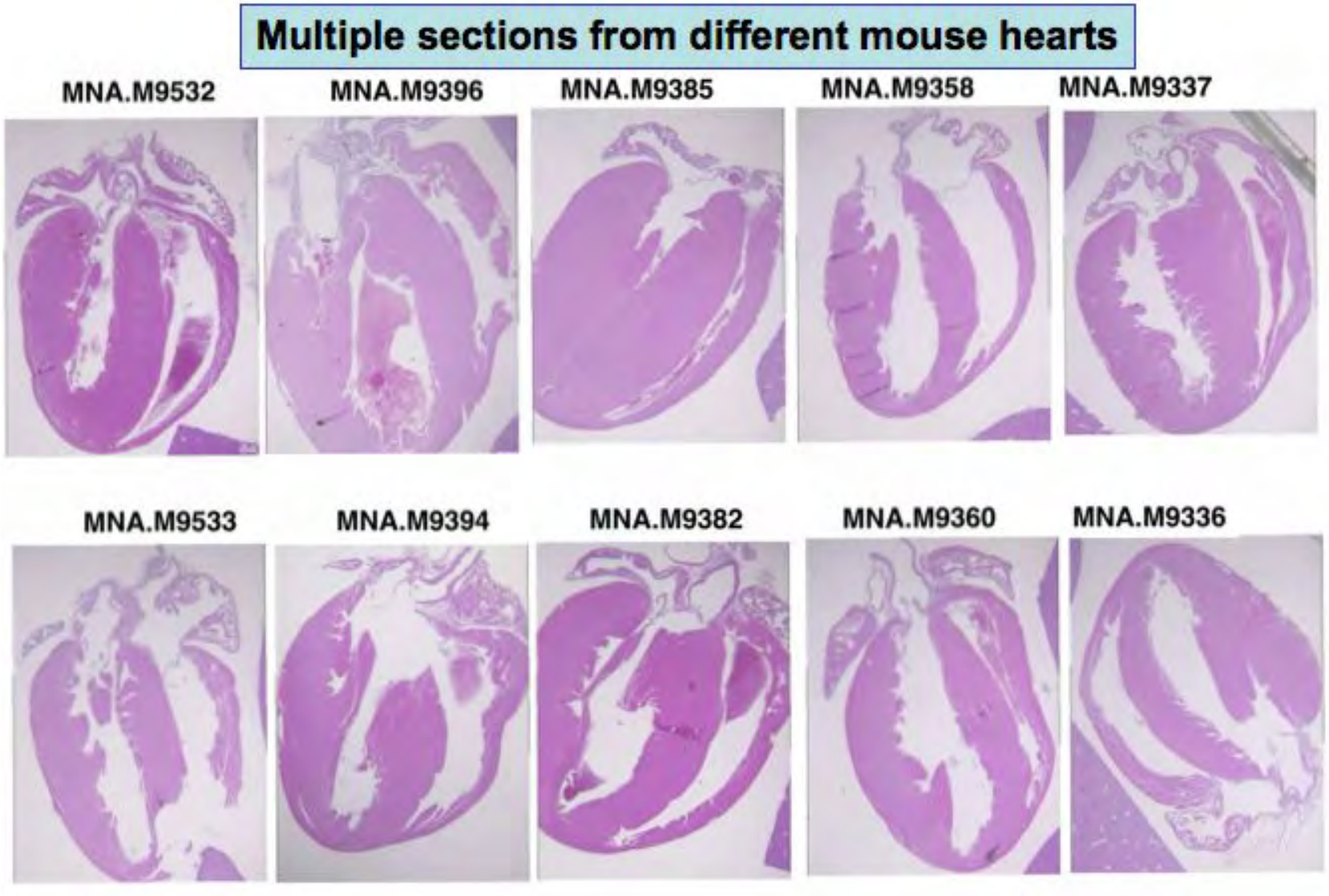
Human



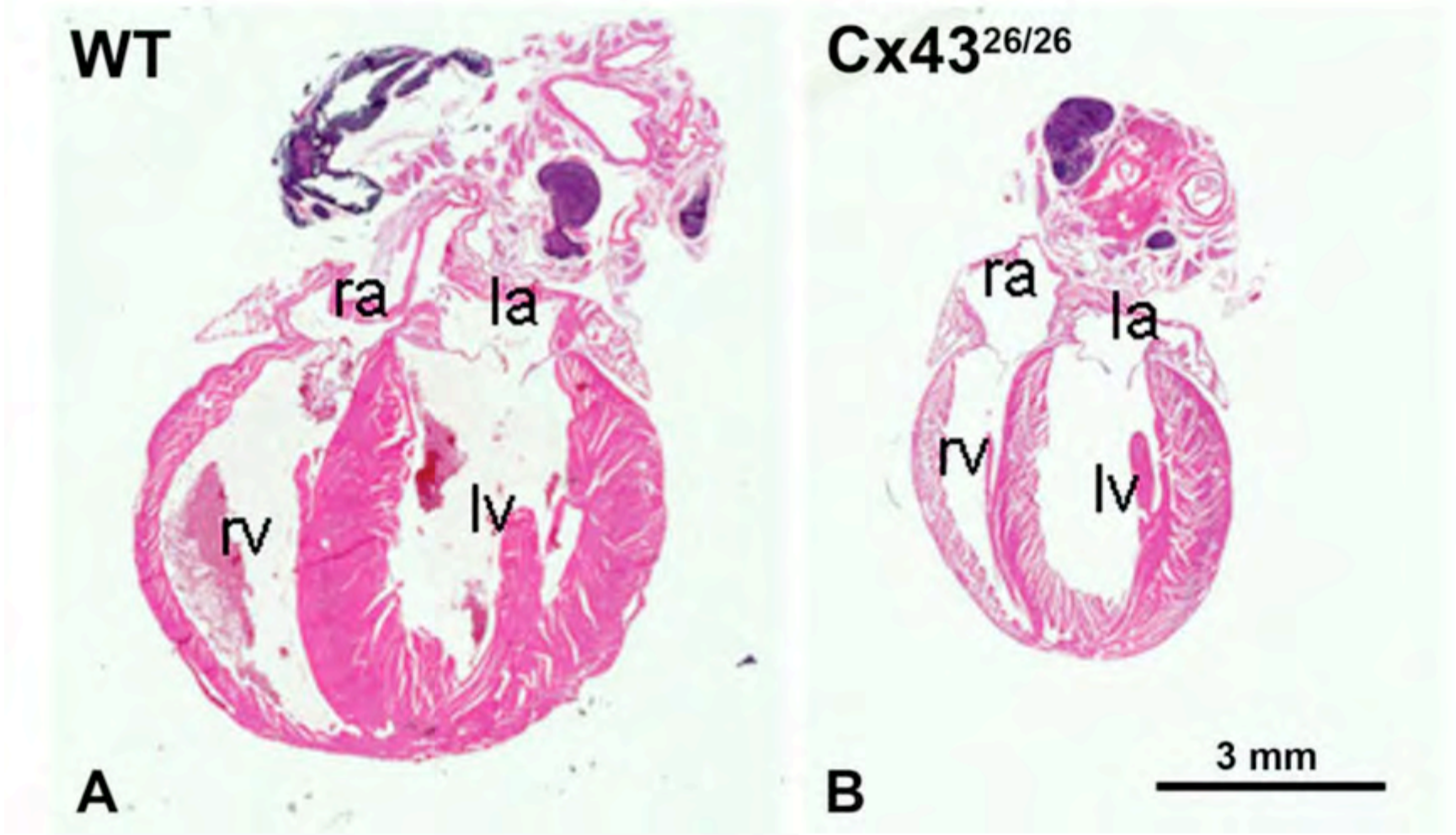
Mouse



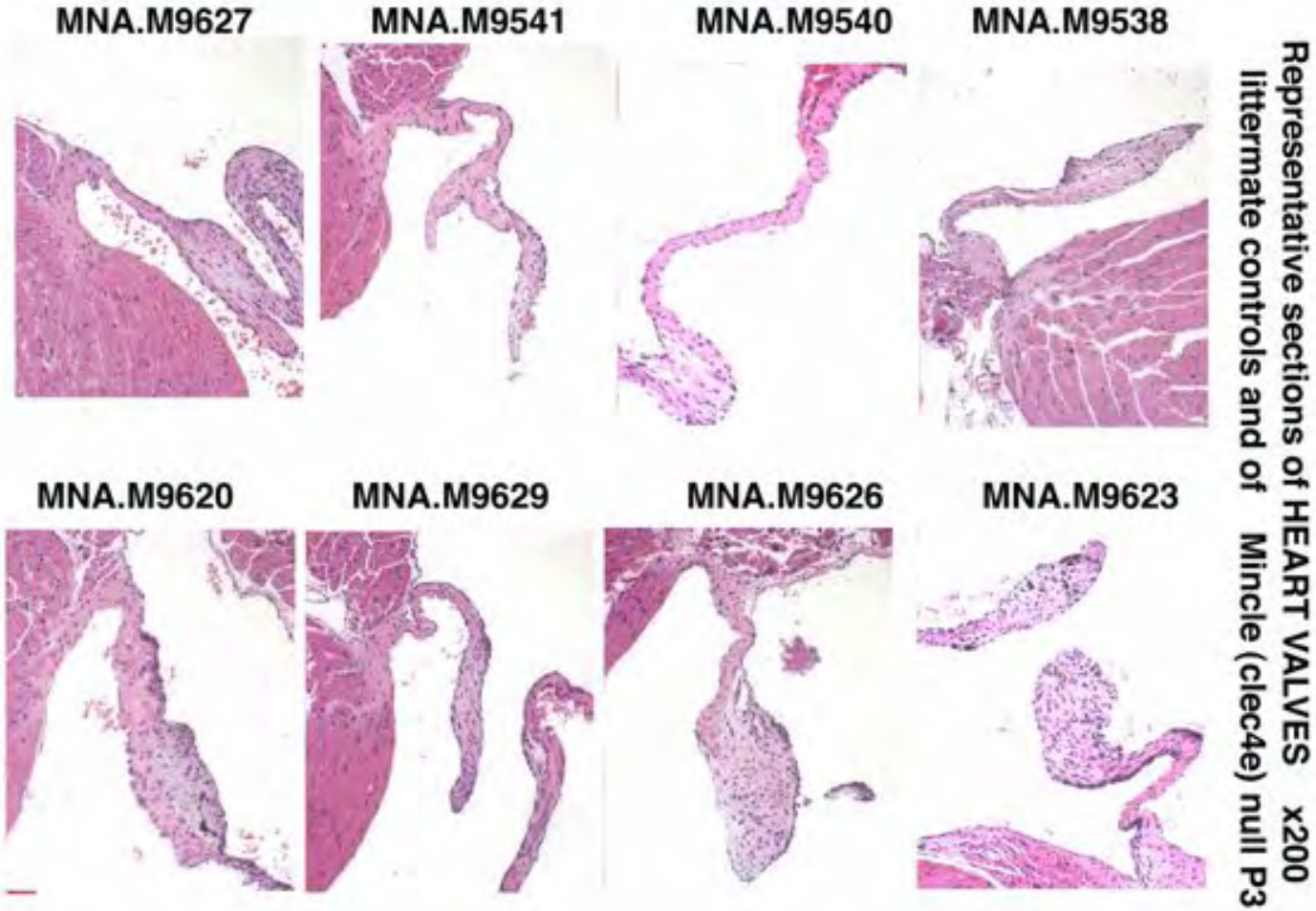
magnification x400 scale bar = 100 microns



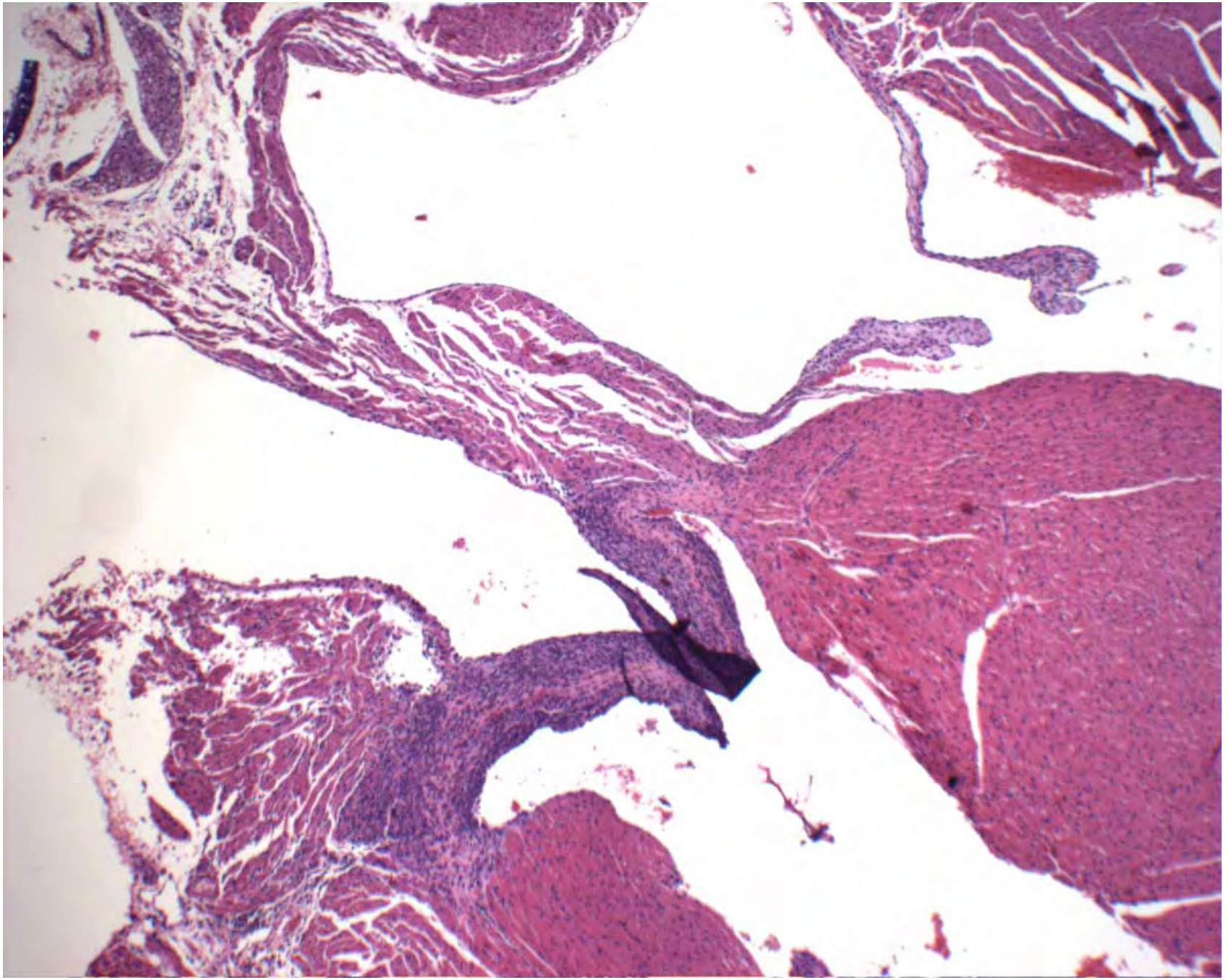
Different orientation of the hearts can help visualize different abnormalities

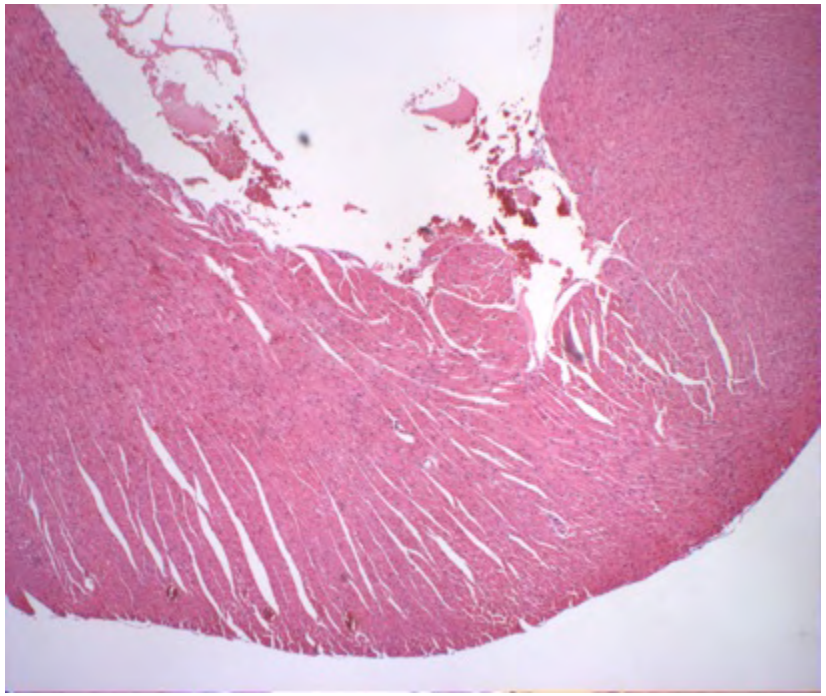

WT
ra
la
rv
lv
A
Cx43 26/26
ra
la
rv
lv
3 mm
B

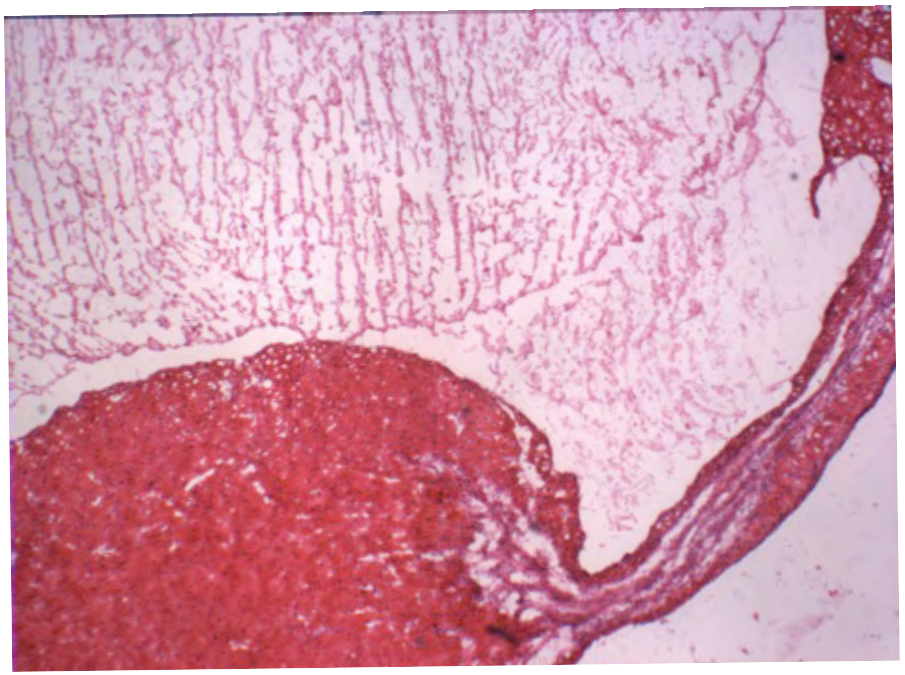
Example of pathology in mouse Heart valves



Representative sections of HEART VALVES x200
littermate controls and of Mincle (clec4e) null P3





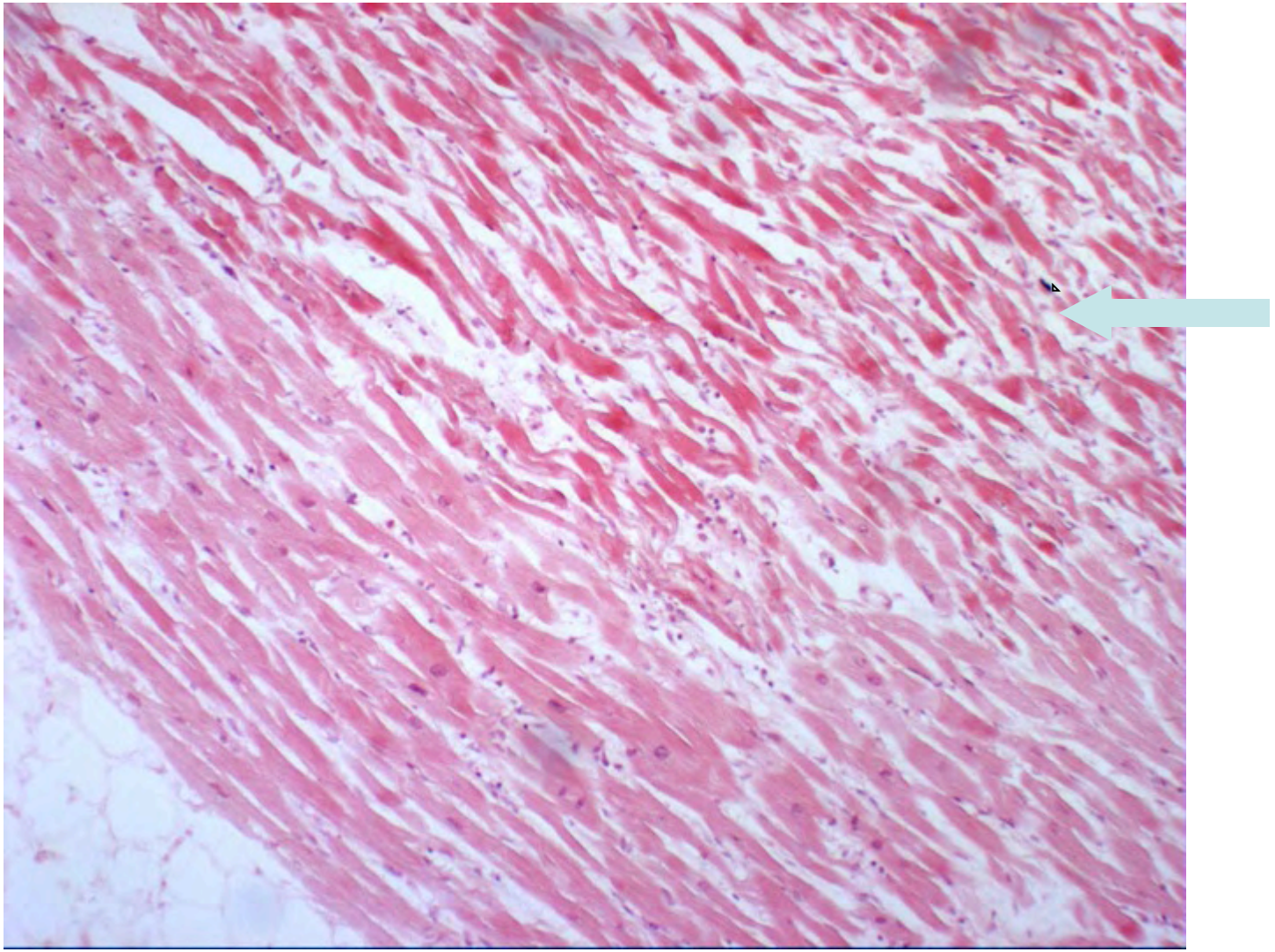


Photos of coronal sections of mouse heart: before and after injury/repair

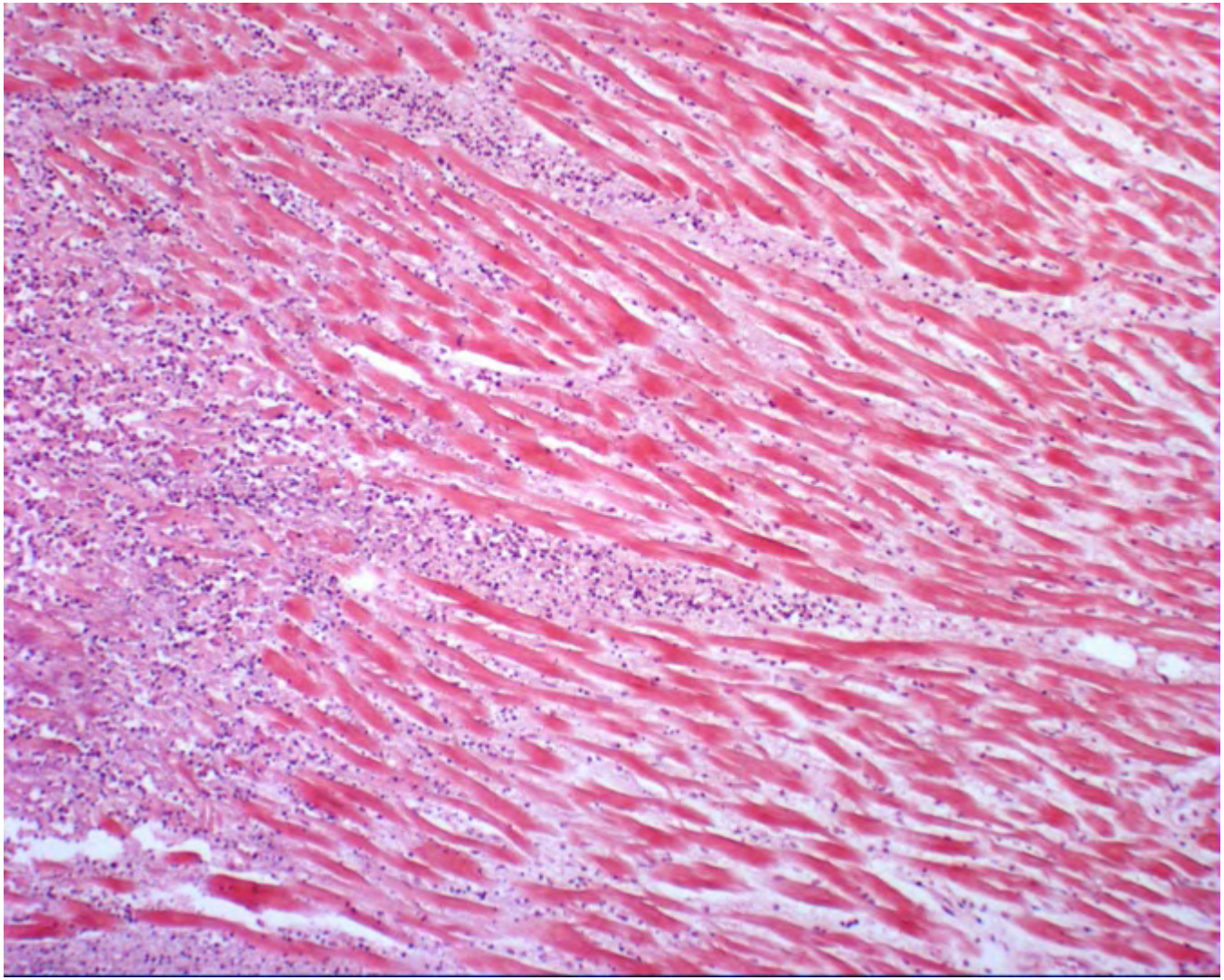
After injury, there is loss of myocardial cells, which do not regenerate

Besides the obvious gross difference in morphology, how would one assess if collagen and thus scar tissue is present?

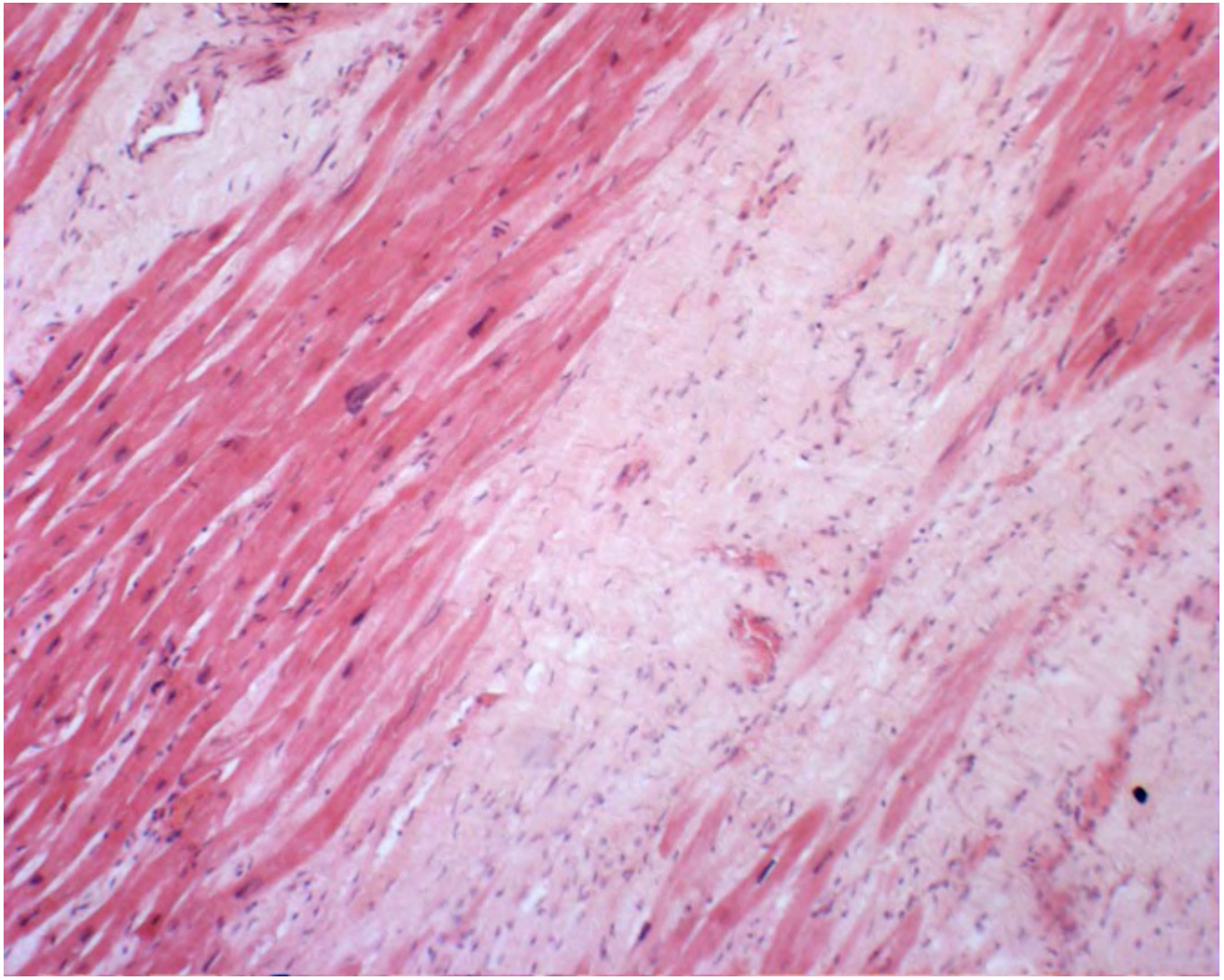
Commonly seen pathology in the heart



Human Heart: early signs of necrosis --loss of nuclei and eosinophilia

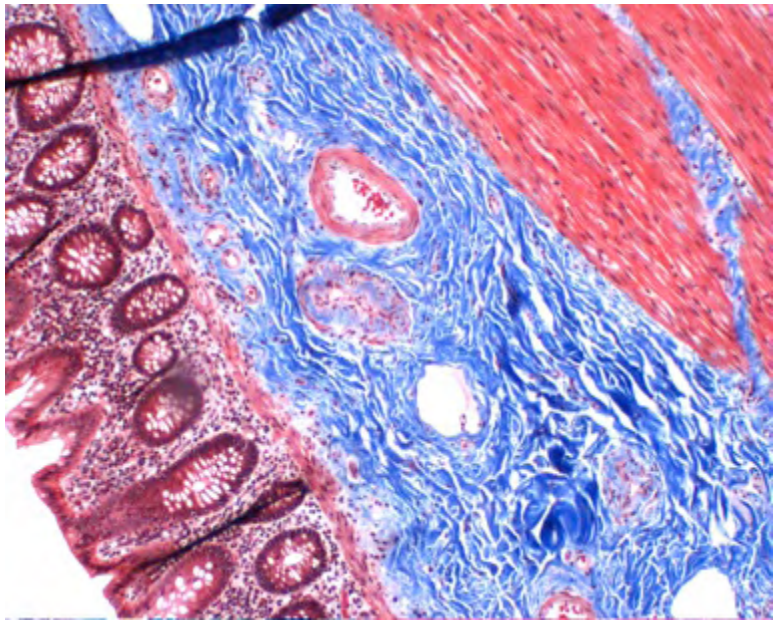


Human Heart: after myocardial infarction-infiltration with leukocytes into dying areas



Human Heart scar with fibrosis and hypertrophied adjacent cardiac myocytes

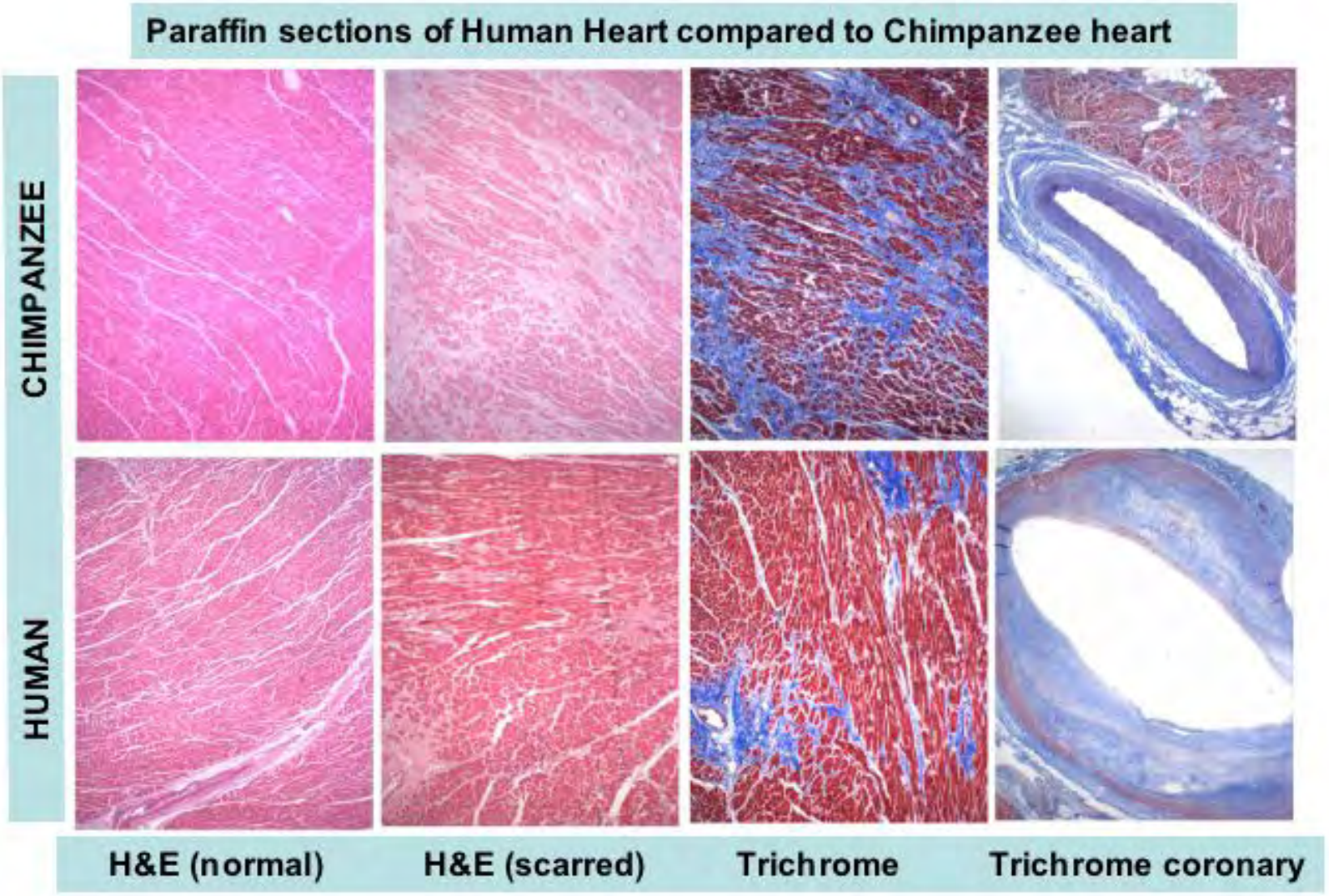
What special stain is done to confirm presence of fibrosis?



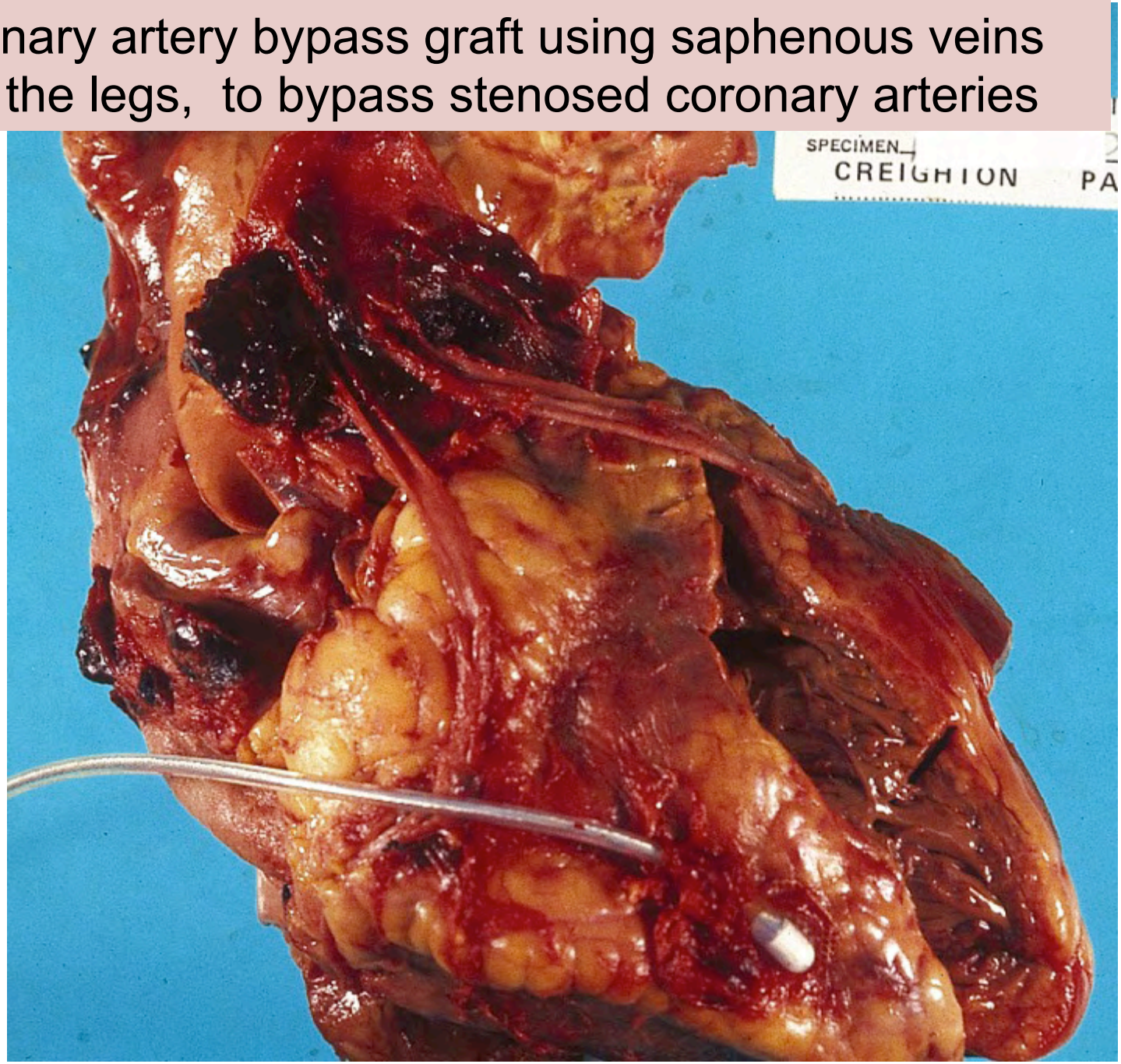
Of several histochemical stains available, the trichrome stain shown here was used is a section of colon, (positive control) showing blue color wherever there is collagen matrix

Increased amounts of collagen are present in healed scar tissue

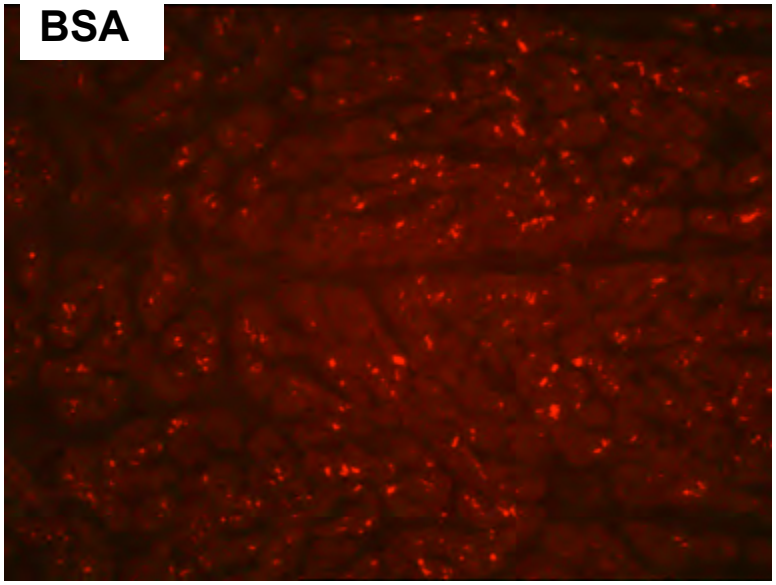
H&E and trichrome to show collagen in heart with scarring after injury and in coronary artery

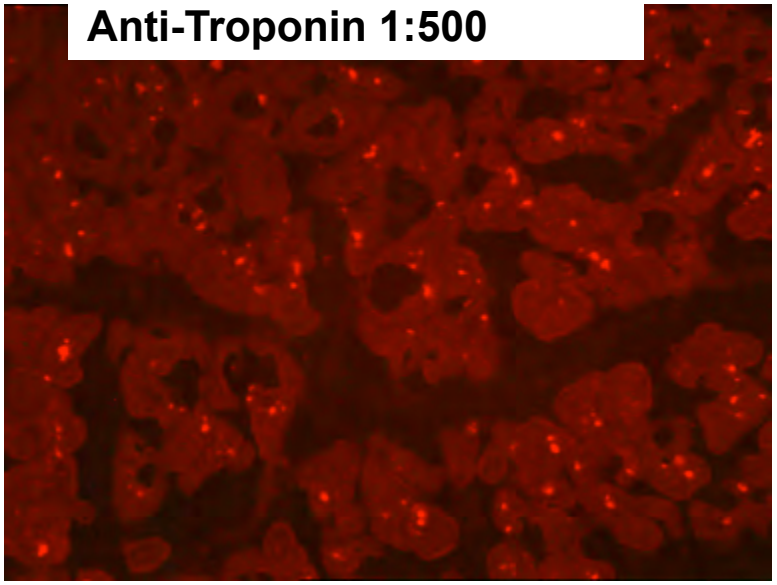


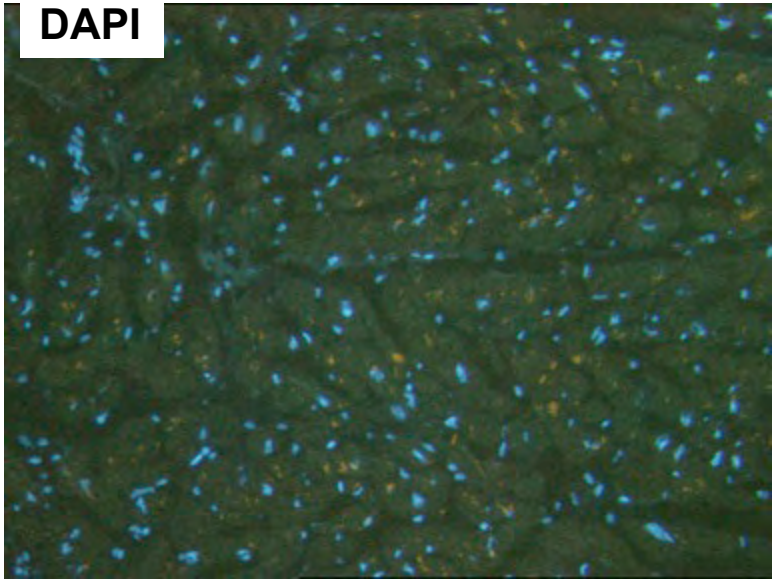
Coronary artery bypass graft using saphenous veins from the legs, to bypass stenosed coronary arteries

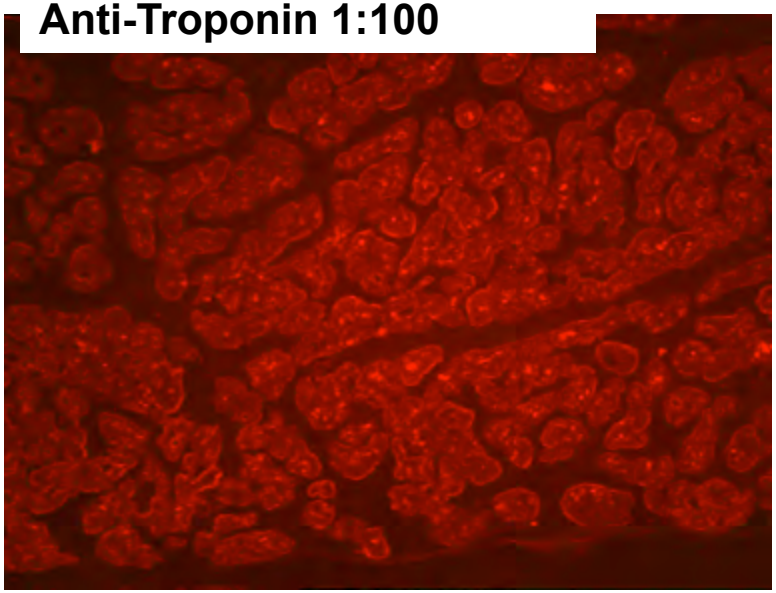


Frozen sections of Human Heart immunostained with anti-troponin

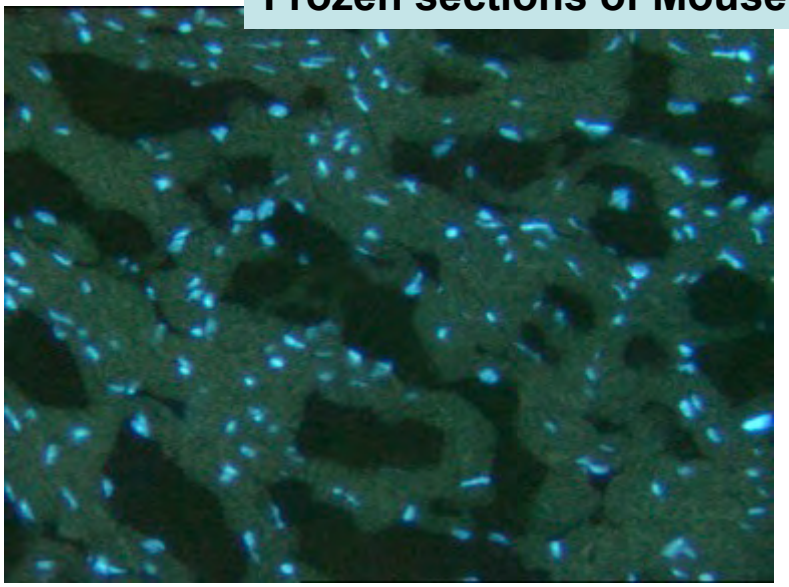


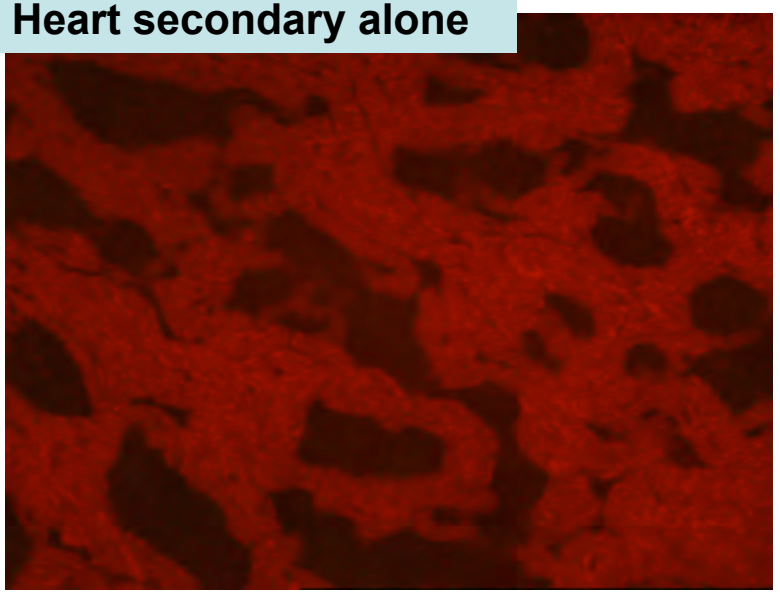




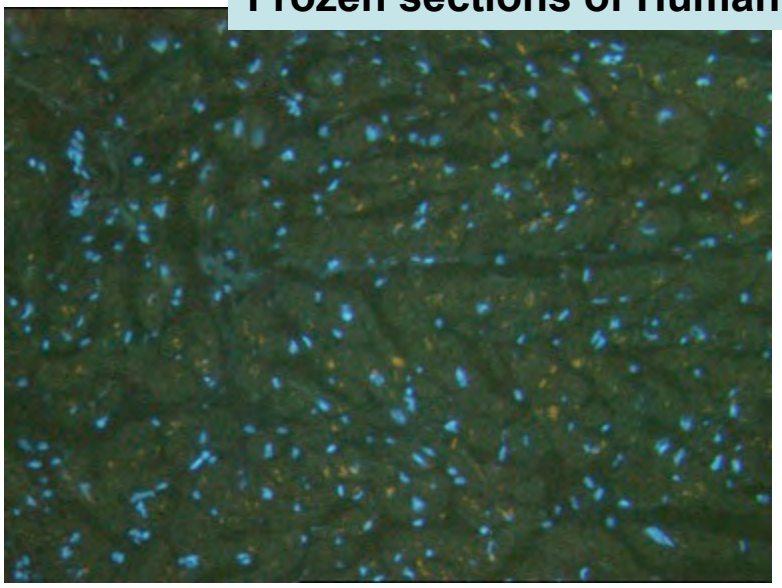


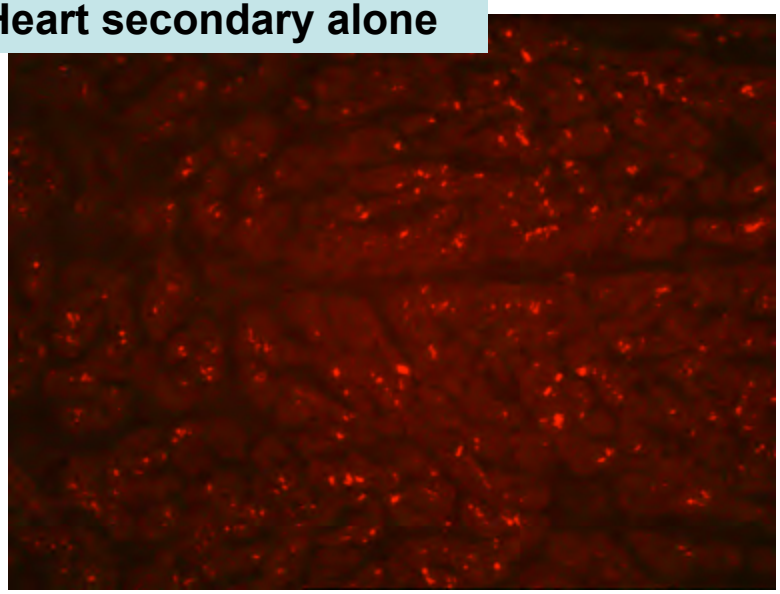
Frozen sections of Mouse Heart secondary alone





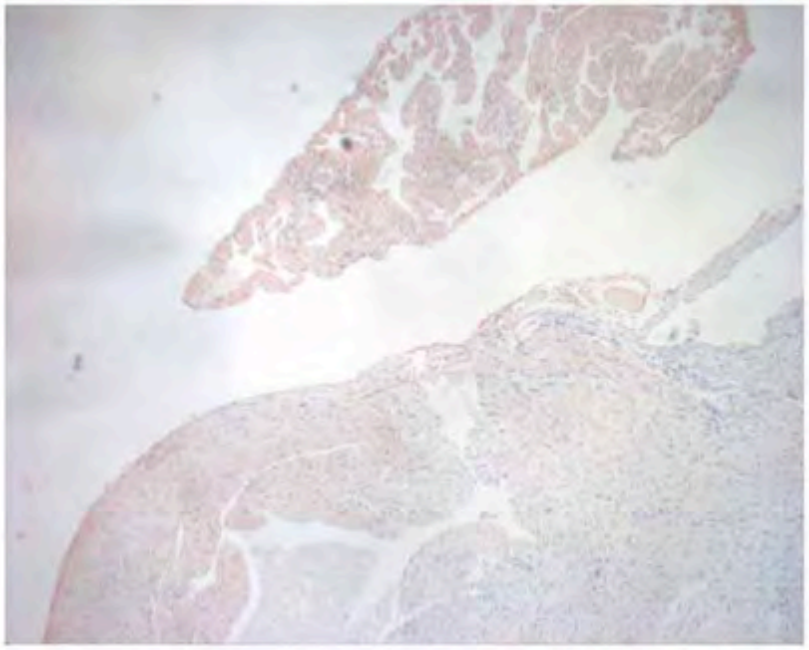
Frozen sections of Human Heart secondary alone

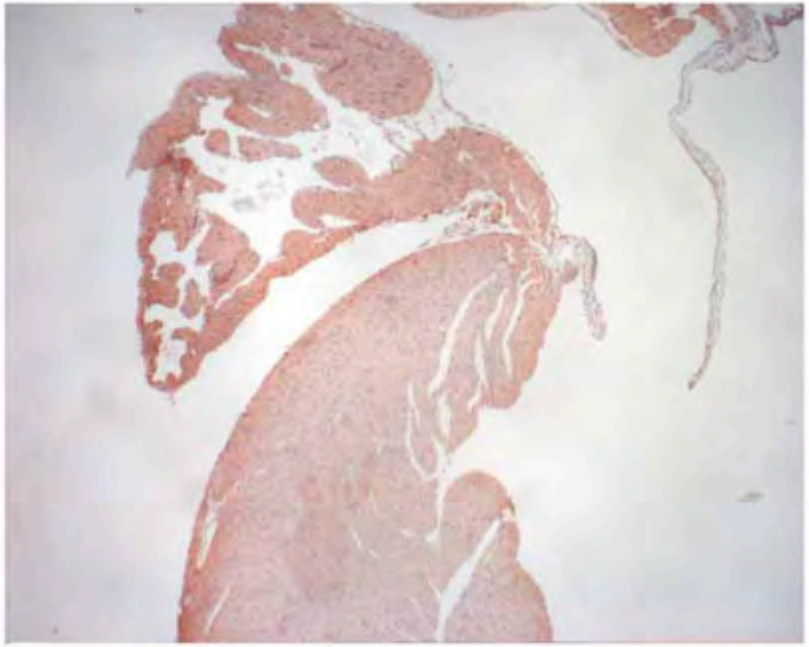




Mouse Heart has high endogenous fluoresence compared to Human Heart

But using enzyme labeled detection systems work well, with no background staining with Ig control





Review of histochemical /immunohistochemical stains so far:

--hematoxylin and eosin: H&E for nuclei and cytoplasm

--Trichrome for collagen in normal and in scars

--PTAH for striations in muscle--only paraffin sections

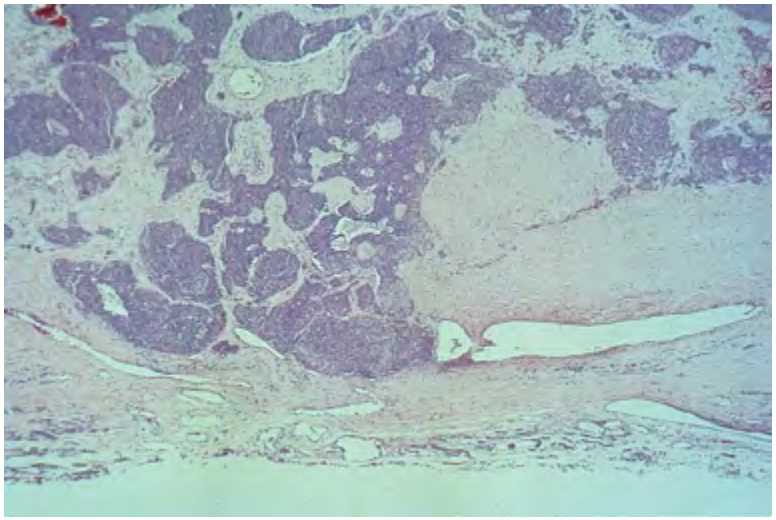
--Elastic stain for elastic in vessel wall --only paraffin sections

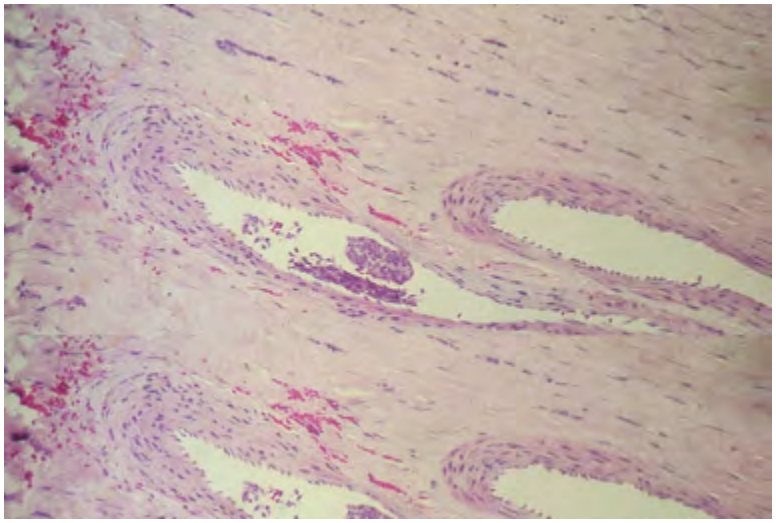
--immunostain for CD31 on endothelial cells

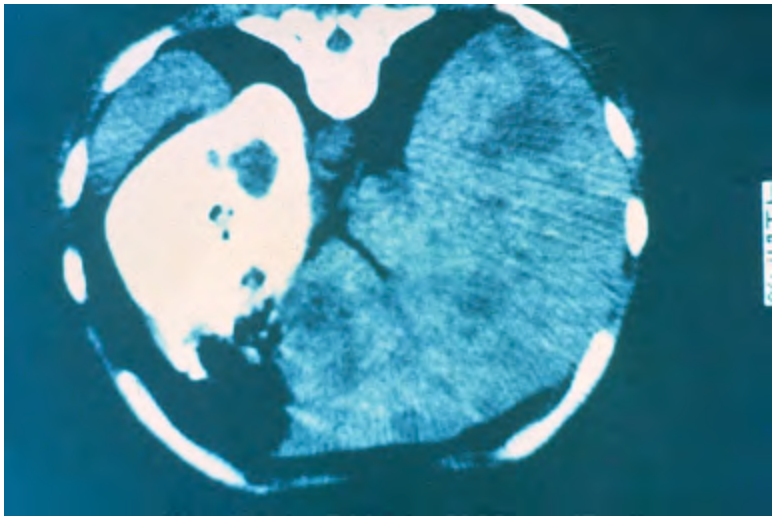
--immunostain for LYVE-1 on lymphatic vessels

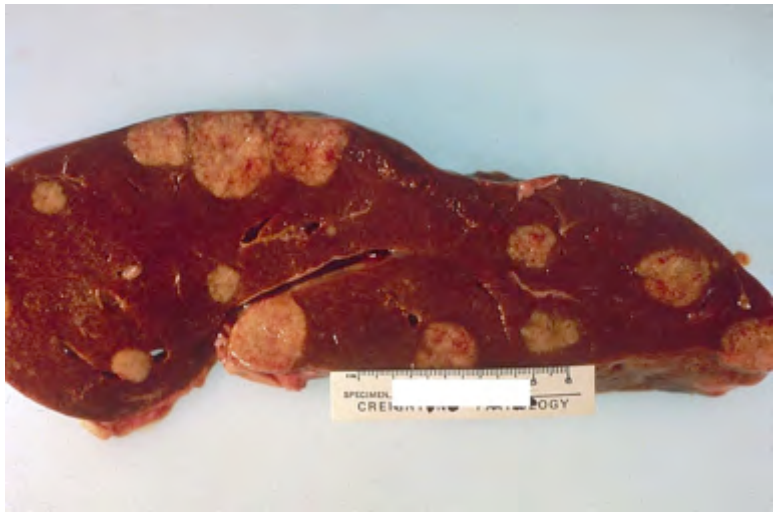
--(UEA lectin for blood vessels in human Not mouse)

Invasion into blood vessels or lymphatics surrounding a malignant tumor, allows hematogenous seeding, and if the distant soil is permissive, metastases seed and grow

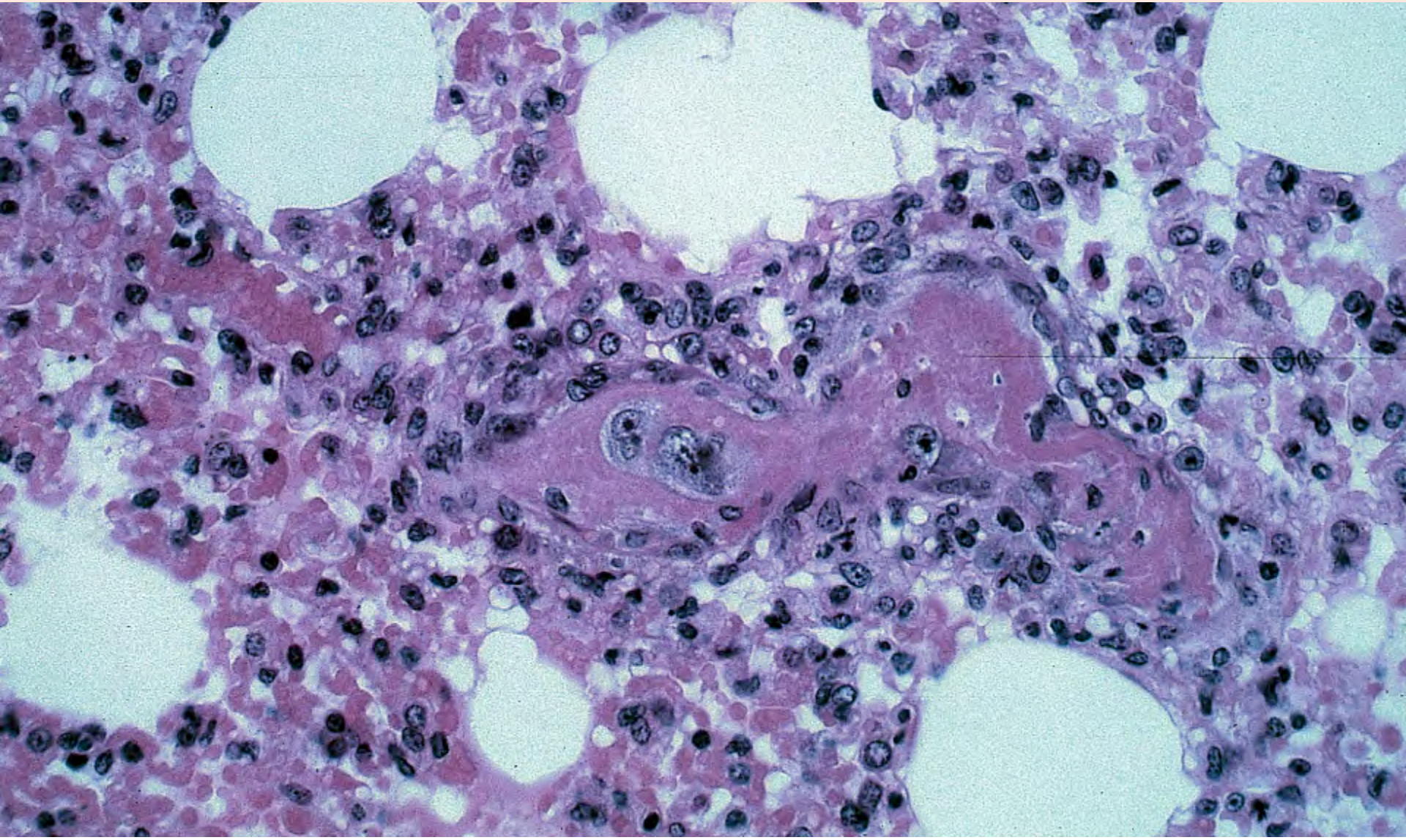




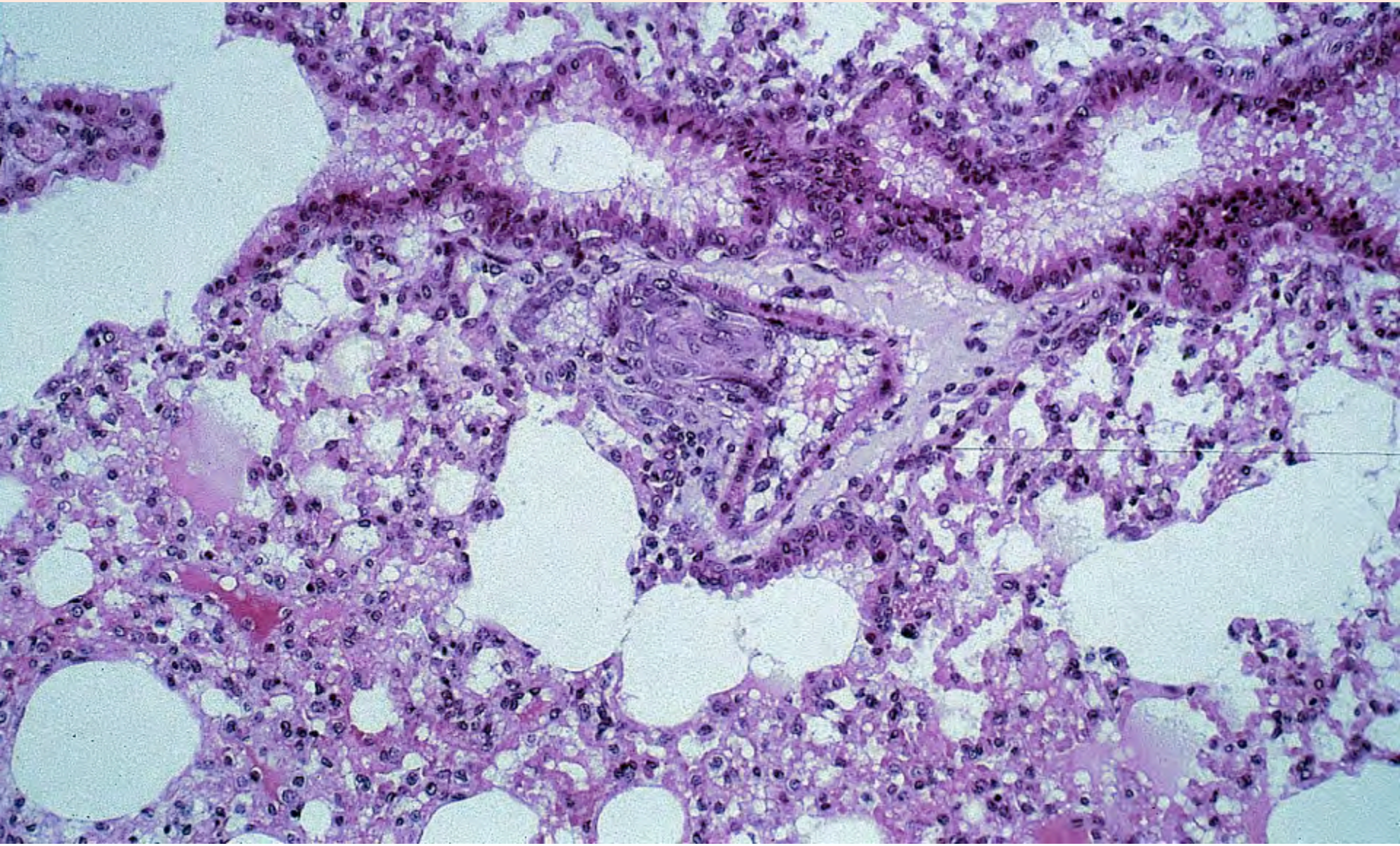




Invasion into blood vessels surrounding a malignant tumor, allows hematogenous seeding----can you identify the malignant cells?

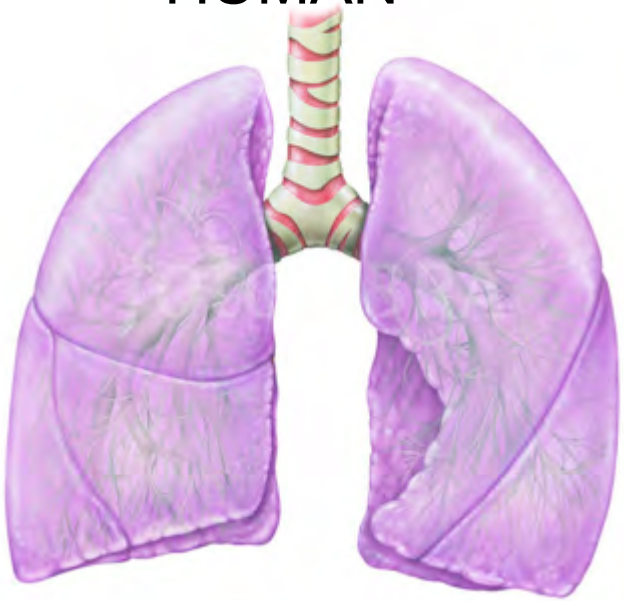


Invasion into blood vessels surrounding a malignant tumor, allows hematogenous seeding----can you identify the malignant cells?

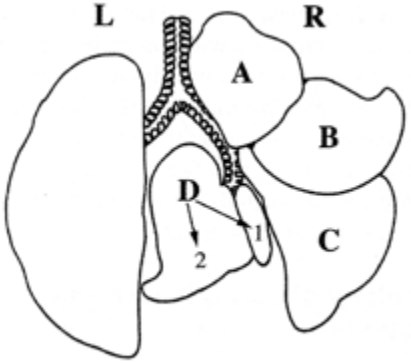


THE LUNGS

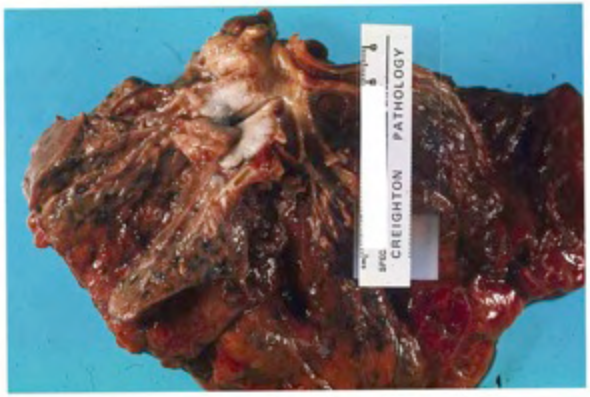
HUMAN



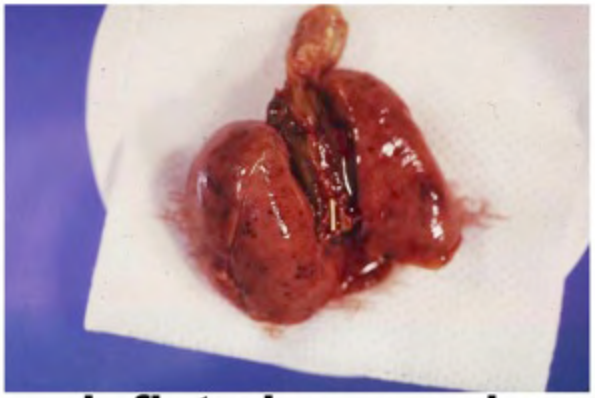
MOUSE



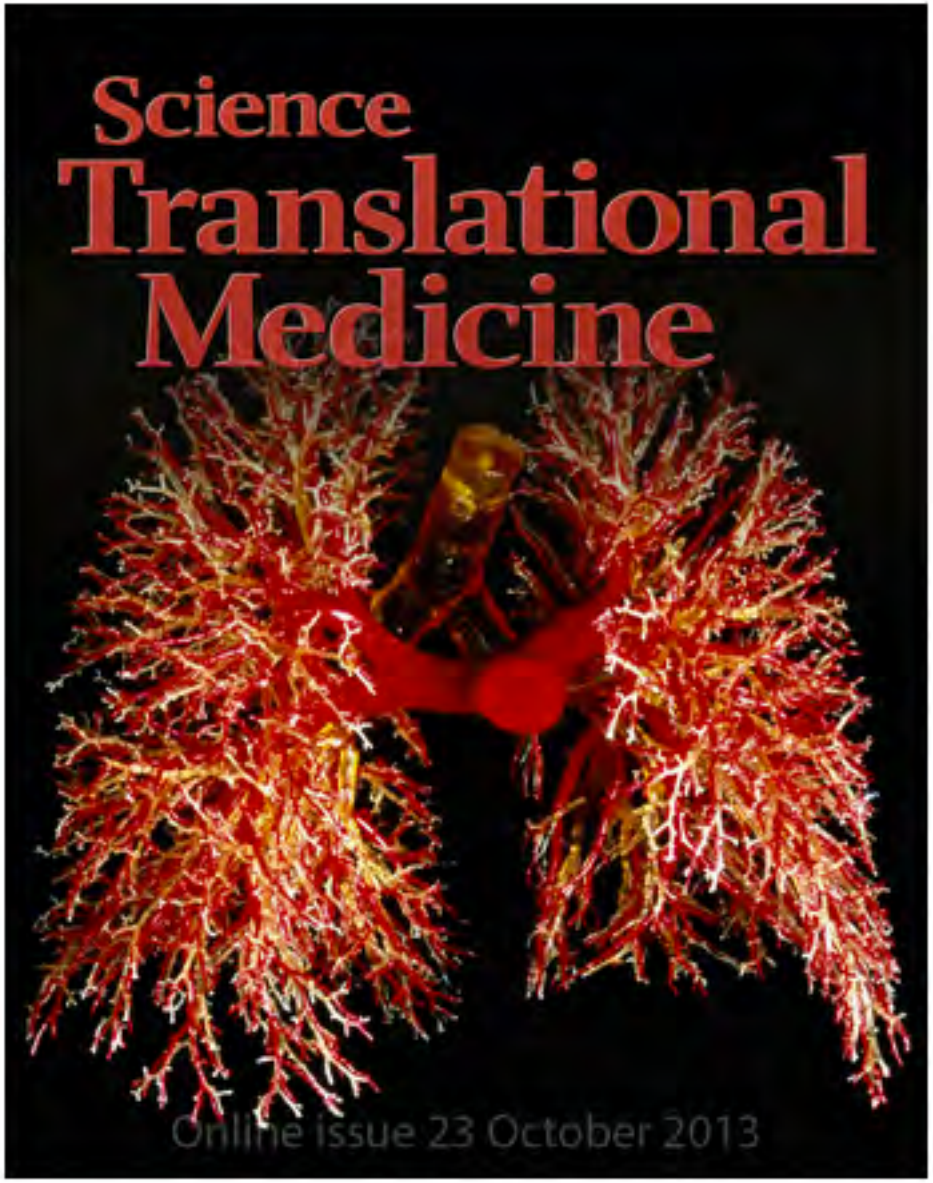
(L) Left Lung (R) Right Lung



Lobe of human lung with primary carcinoma

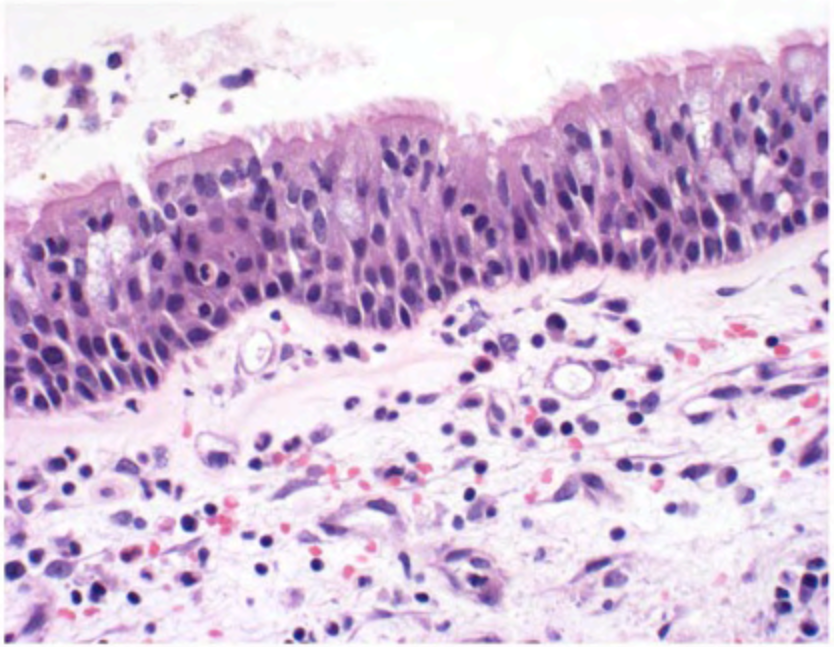


inflated mouse lung with metastases

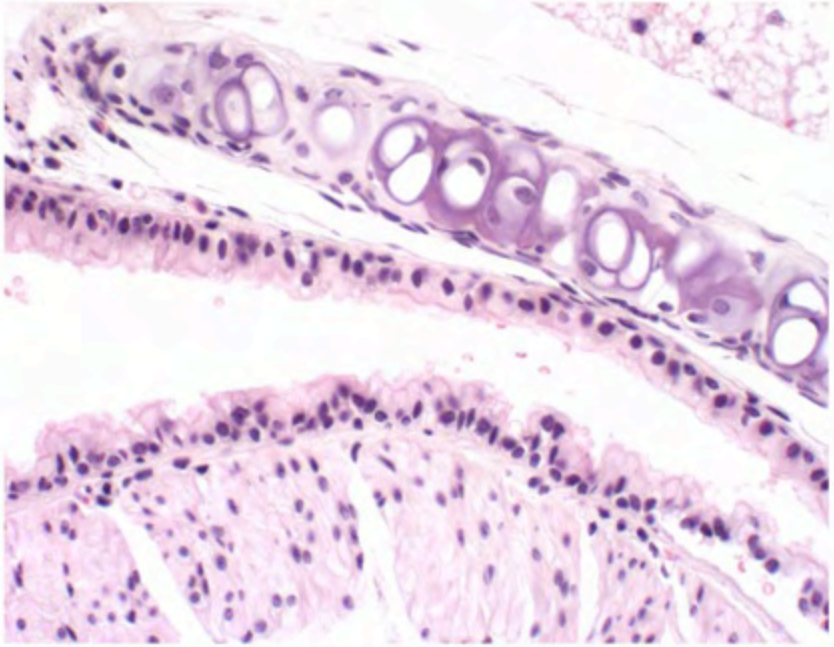

Science
Translational
Medicine
Online issue 23 October 2013

TRACHEA magnification x400

Human

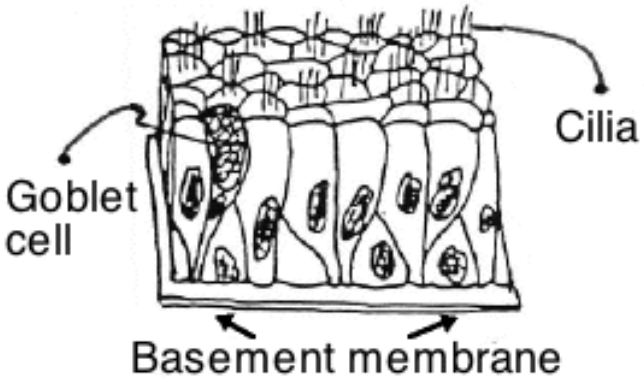


Mouse

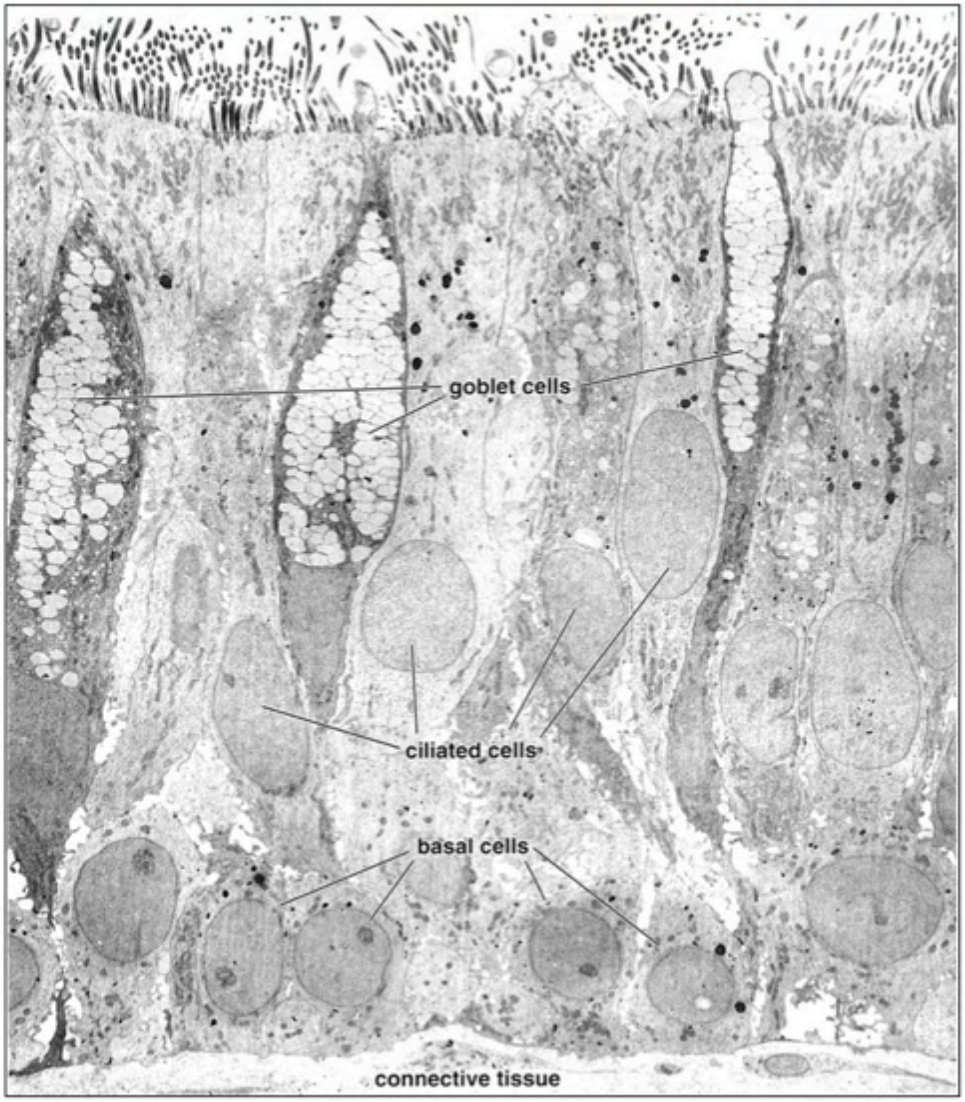


scale bar = 100 microns

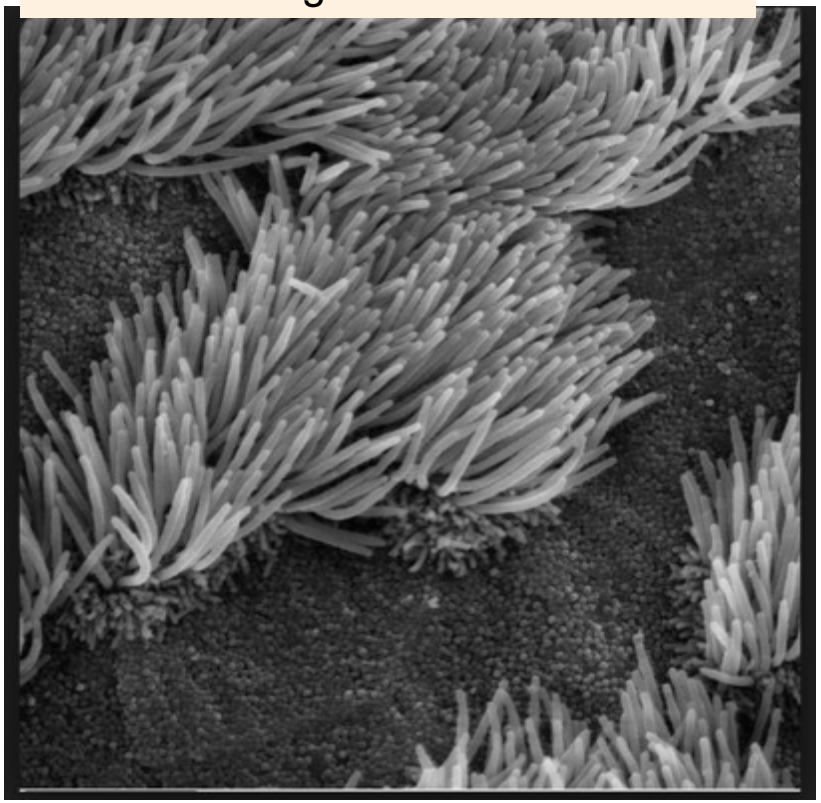
Pseudostratified (ciliated) columnar

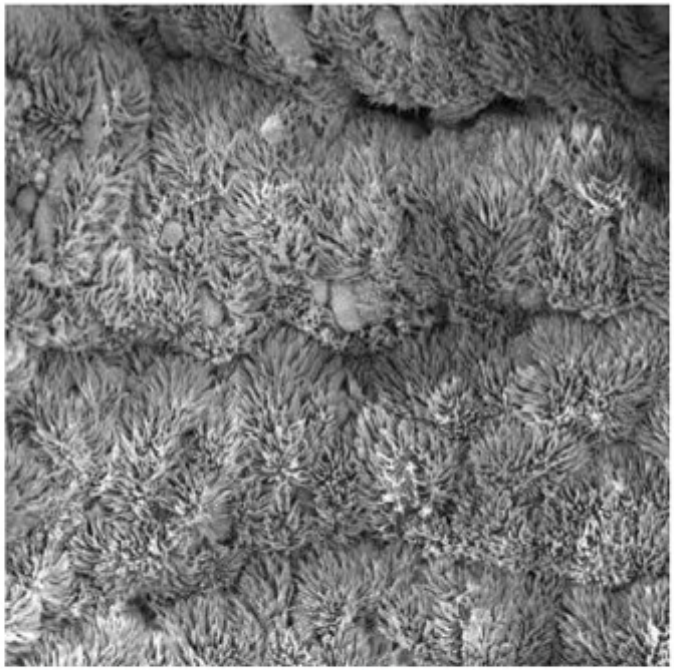


www.columbia.edu/itc/hs/medical/sbpm_histology_old/lab/micro_popup13.html



Human Ciliated tracheal epithelial cell -- scanning EM



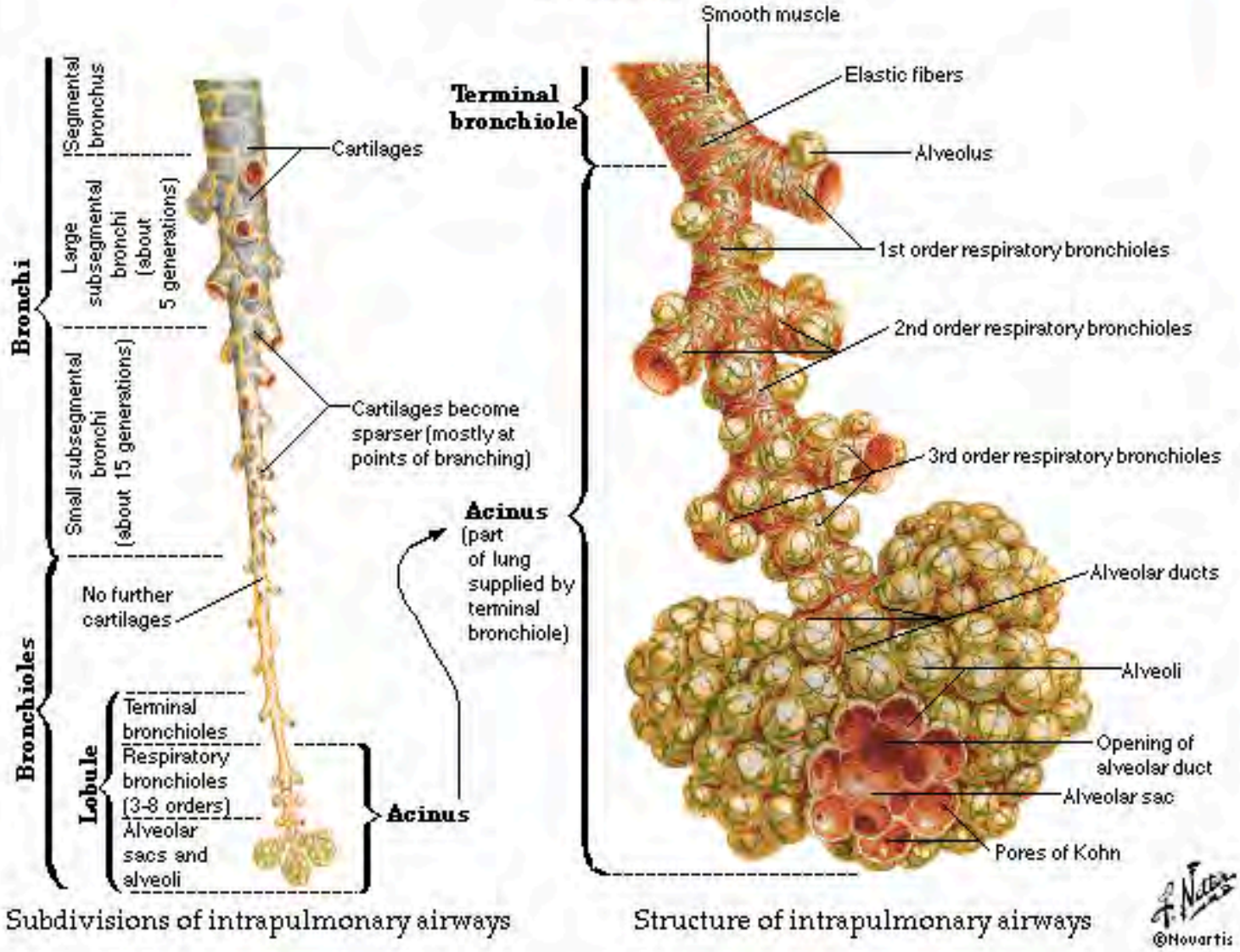


Ciliated tracheal epithelium in a wild-type mouse bending in similar direction

Scanning EM of ciliated tracheal epithelium in a wild-type mouse. The cells are covered with cilia, all bending in a similar direction.

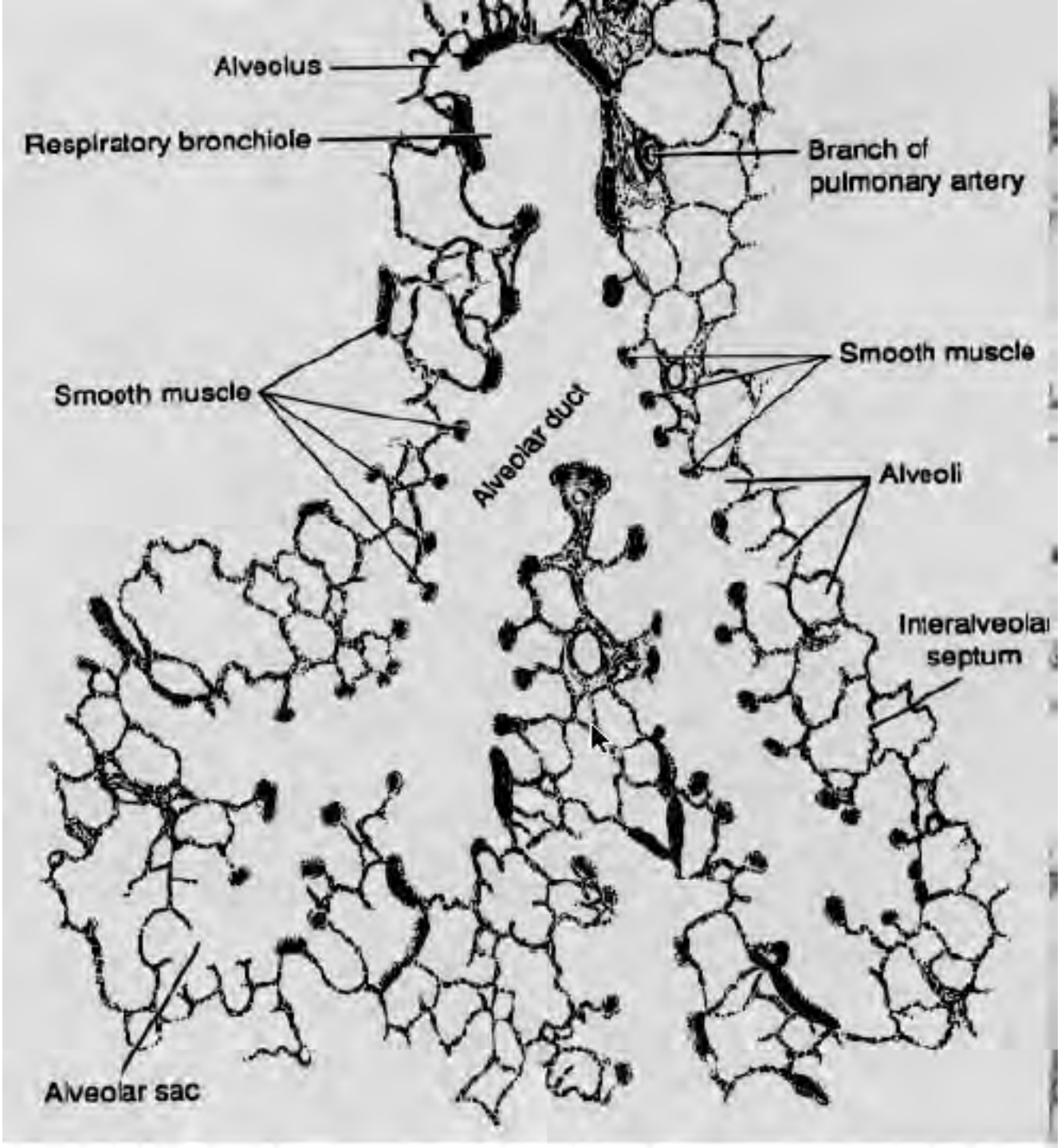
Intrapulmonary Airways

Schema



Subdivisions of intrapulmonary airways

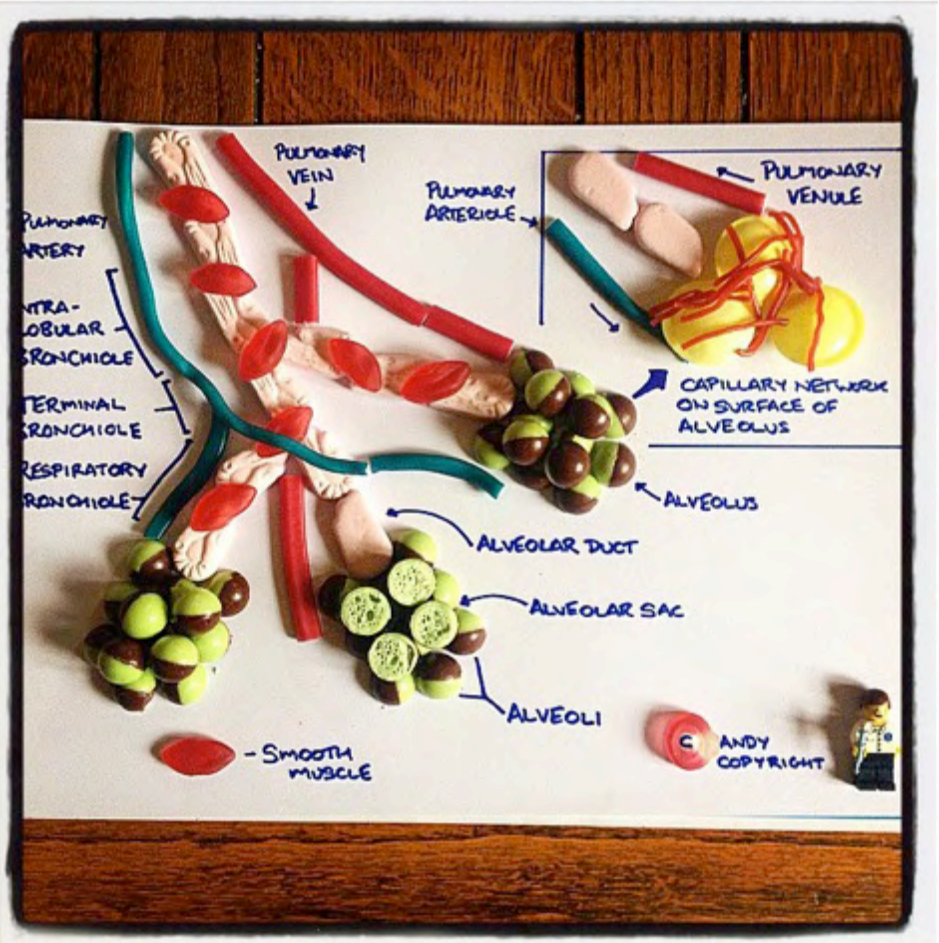
Structure of intrapulmonary airways


Alveolus
Respiratory bronchiole
Branch of pulmonary artery
Smooth muscle
Smooth muscle
Alveolar duct
Alveoli
Interalveolar septum
Alveolar sac

PHOTO

https://instagram.com/candyanatomy/

Mike McCormick - 27yr -Glasgow University Medical Student



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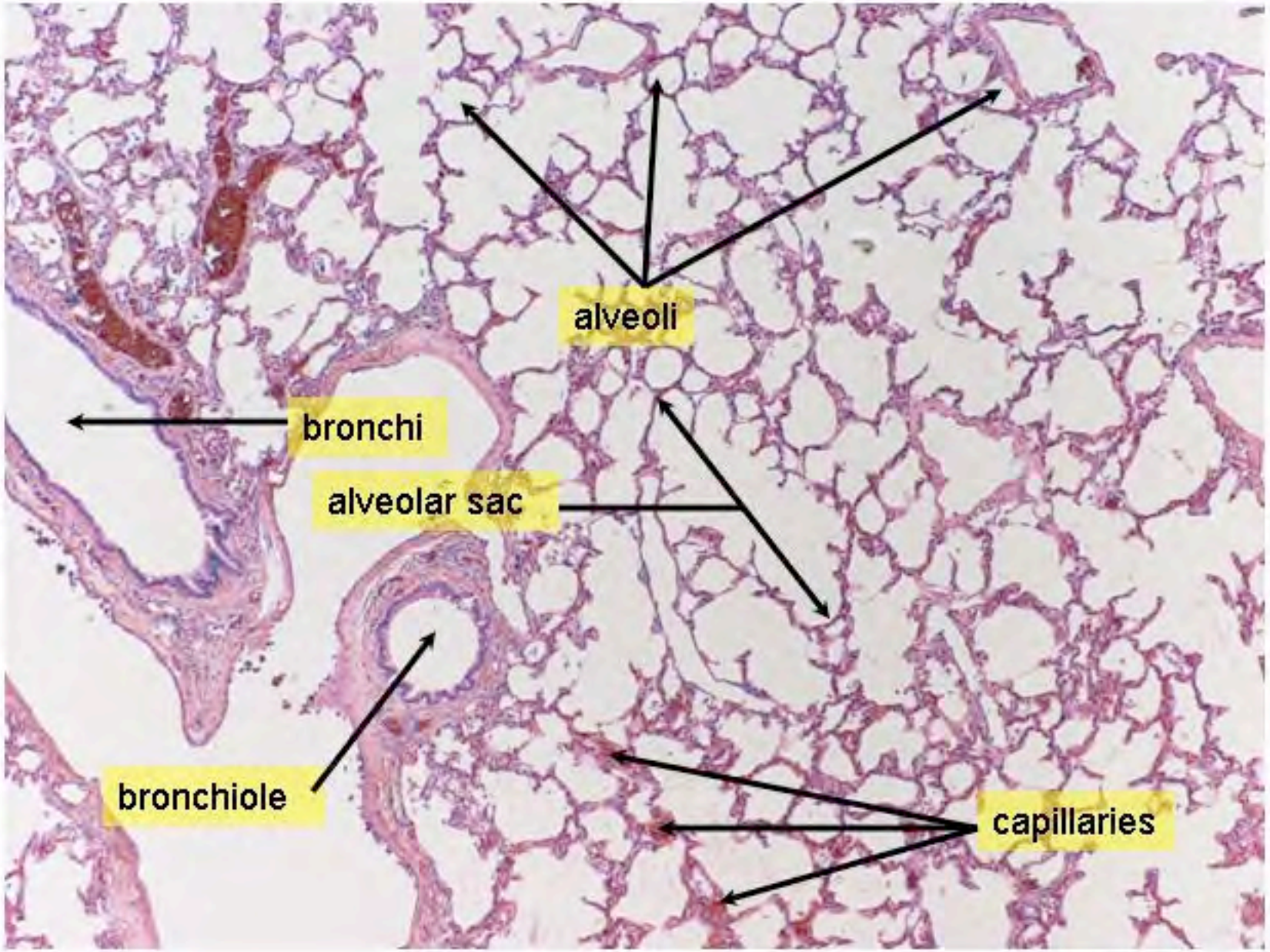
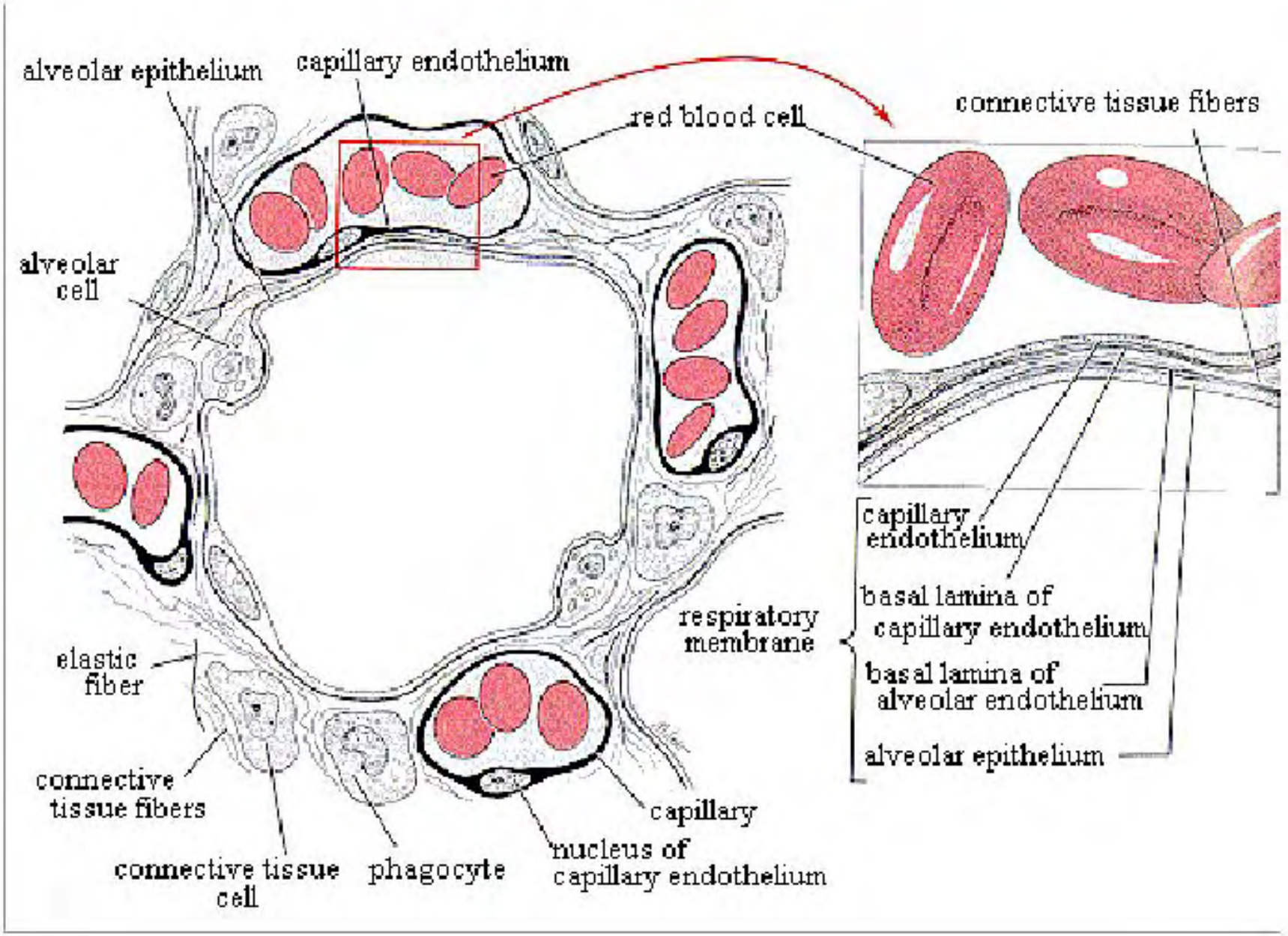
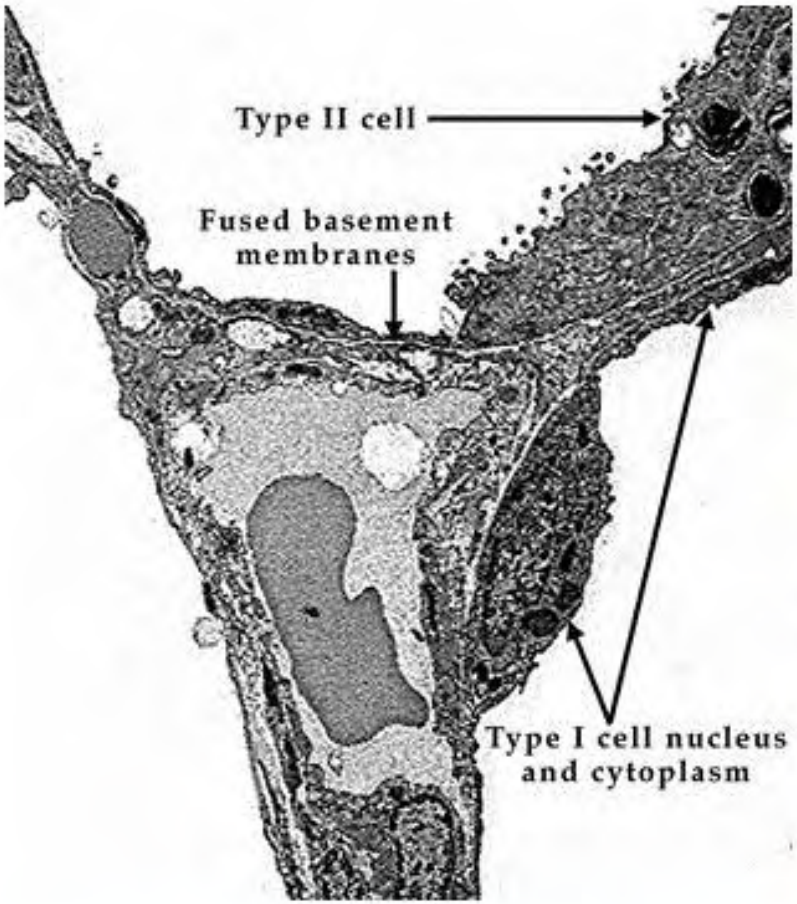

alveoli
bronchi
alveolar sac
bronchiole
capillaries


alveolar epithelium
capillary endothelium
red blood cell
connective tissue fibers
alveolar cell
capillary endothelium
basal lamina of capillary endothelium
basal lamina of alveolar endothelium
alveolar epithelium
respiratory membrane
elastic fiber
connective tissue fibers
capillary
connective tissue cell
phagocyte
nucleus of capillary endothelium



Alveoli are lined by flat type I cells, which cannot be seen with the light microscope, and plump type II cells, which can be seen.

--Normally, each alveolus has one type I cell and 2 type II cells.

--The type II cell secretes surfactant and is the cell that undergoes proliferation after injury, having the capacity to differentiate into a type I cell.

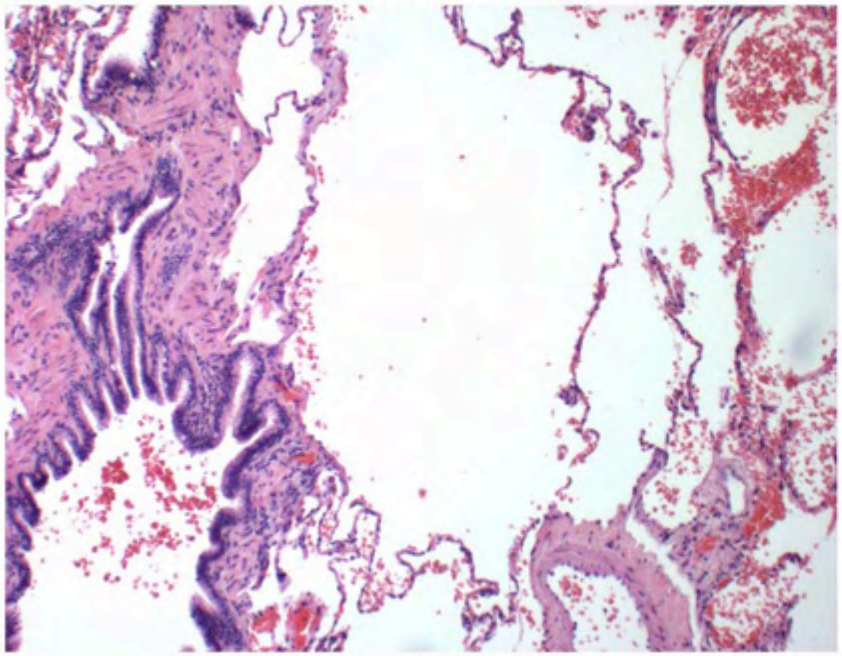
--The alveolar wall has capillaries and connective tissue, including elastic fibers. The capillary basement membrane is focally fused with that of the epithelial cell to facilitate gas exchange. The alveoli contain a few alveolar macrophages, which represent the first line of defense against foreign particles.

The photo shows an alveolar wall with a capillary containing a red blood cell.

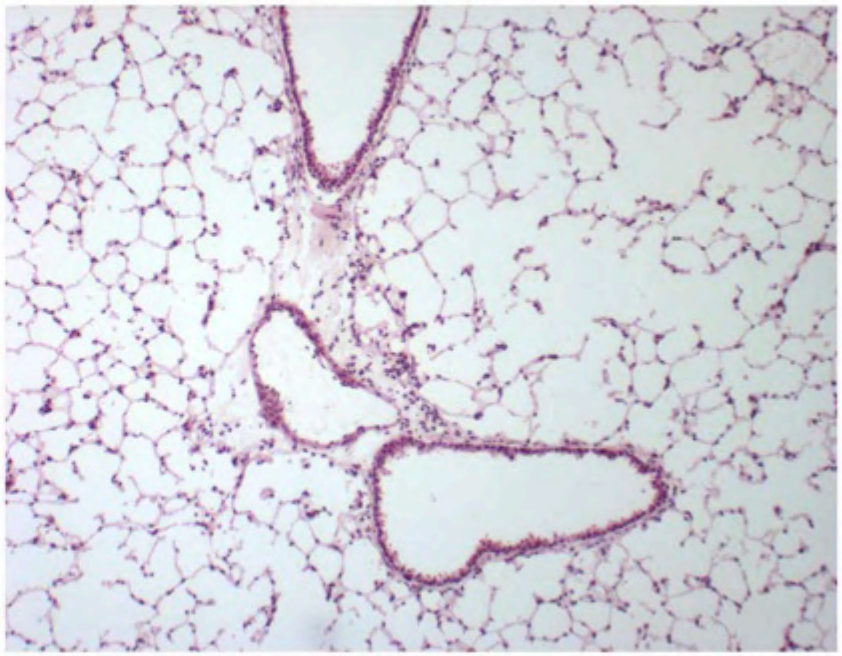
http://pathhsw5m54.ucsf.edu/introduction.html

LUNG magnification x100

Human



Mouse

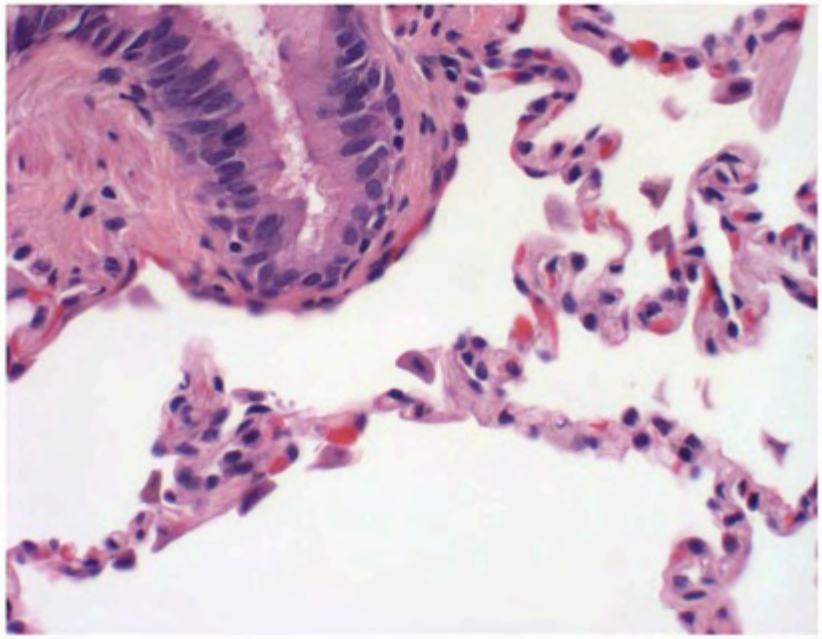


scale bar = 100 microns

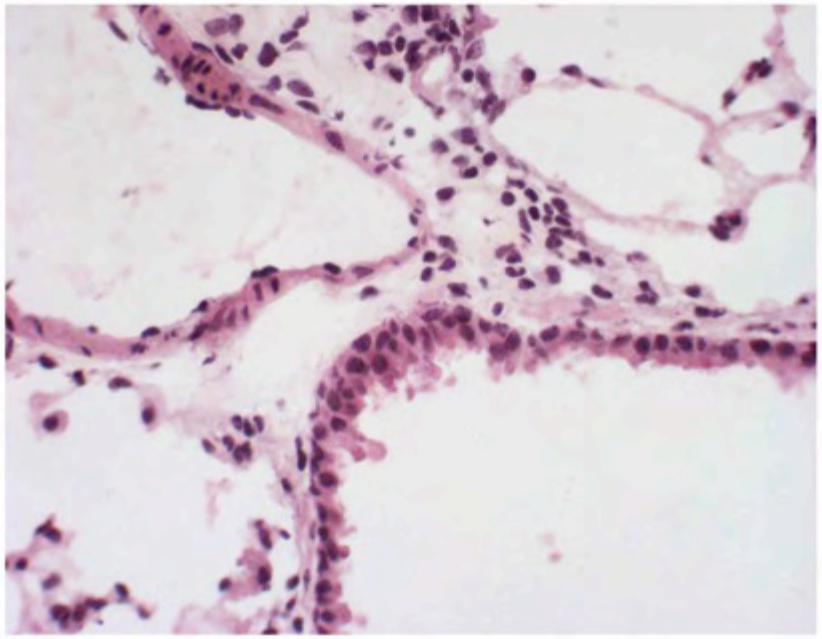
LUNG

magnification x400

Human



Mouse



scale bar = 100 microns

Examples of a few lung diseases include

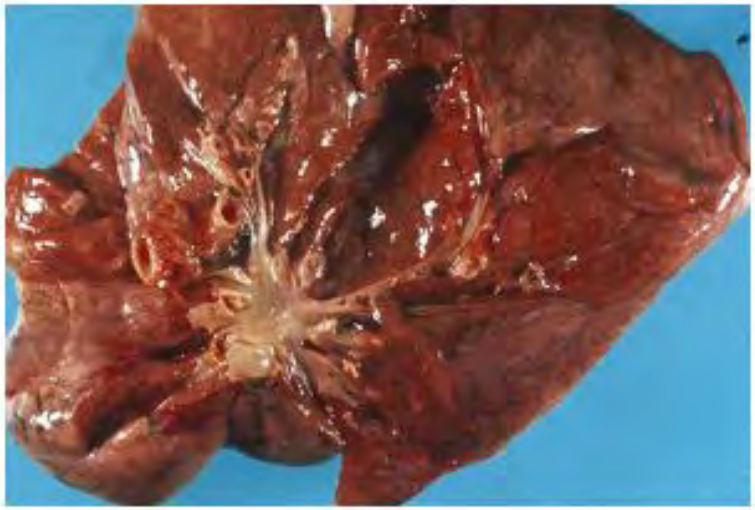
- Pneumonia
- Restrictive lung disease—Asthma
- Chronic obstructive pulmonary disease (COPD)
- Lung cancer—usually of epithelial origin and thus

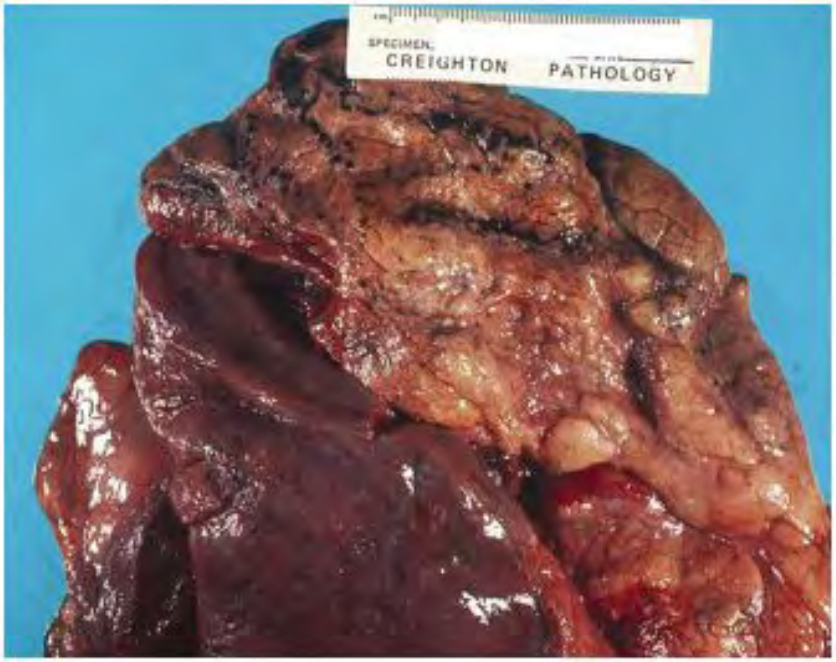
    Lung carcinomas:

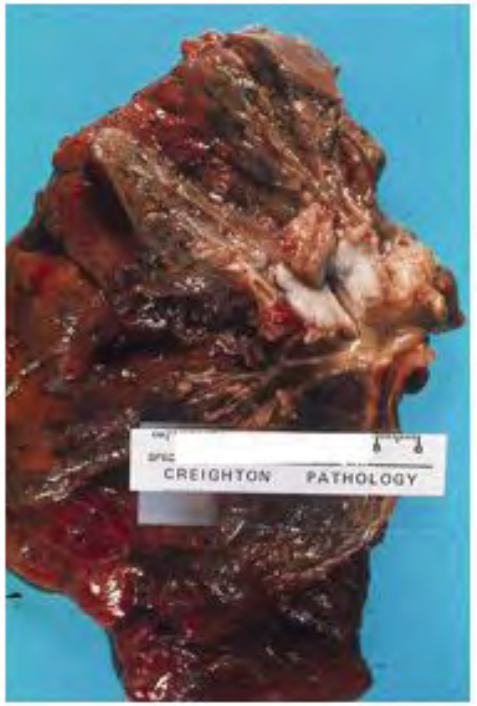
        Squamous carcinomas

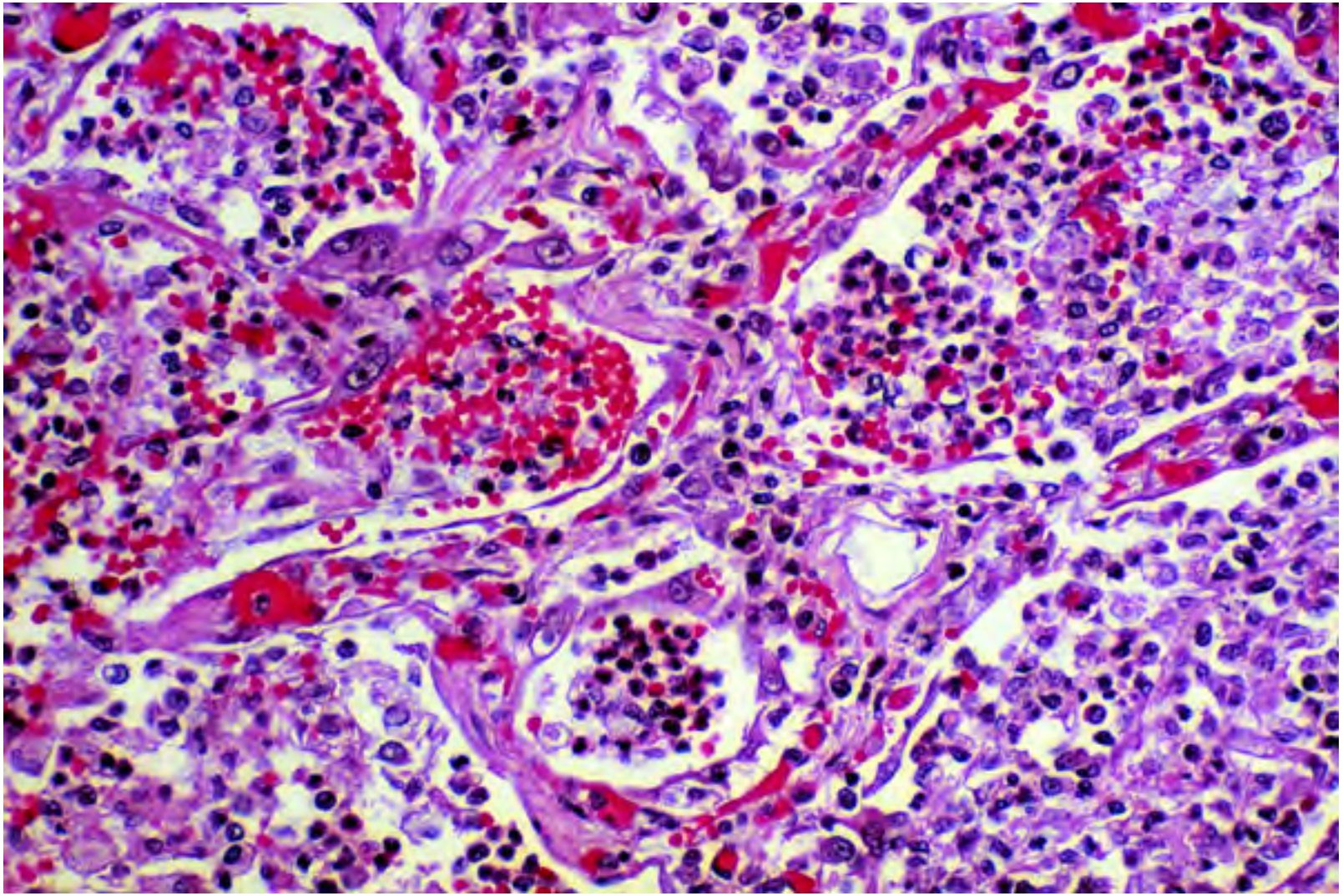
        Adenocarcinoma

        small cell carcinomas

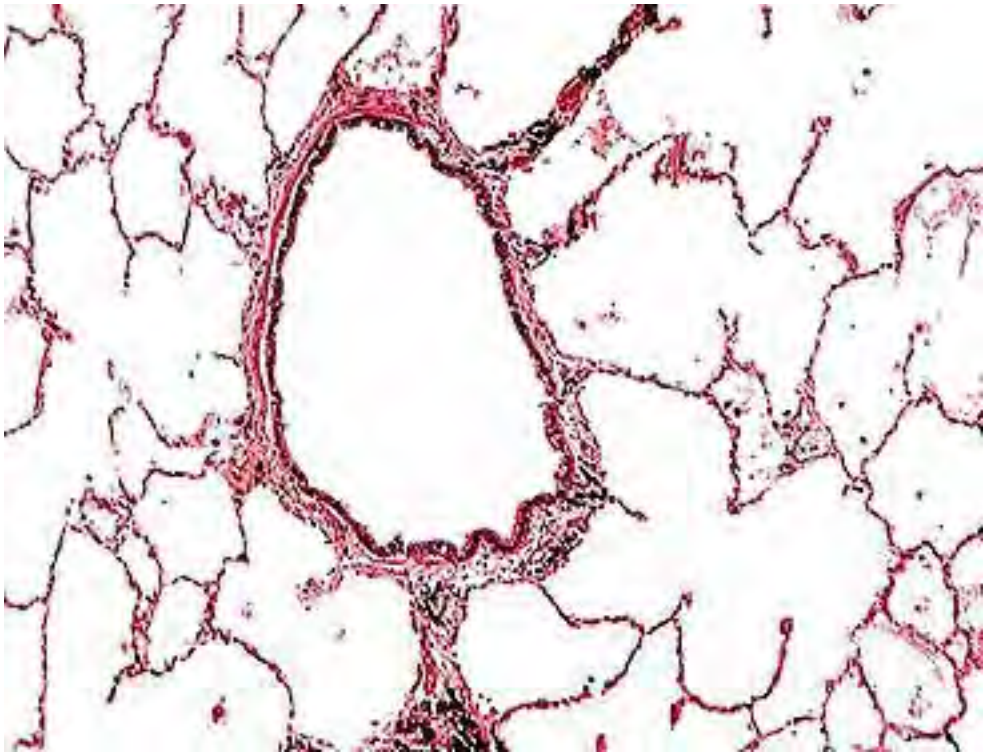


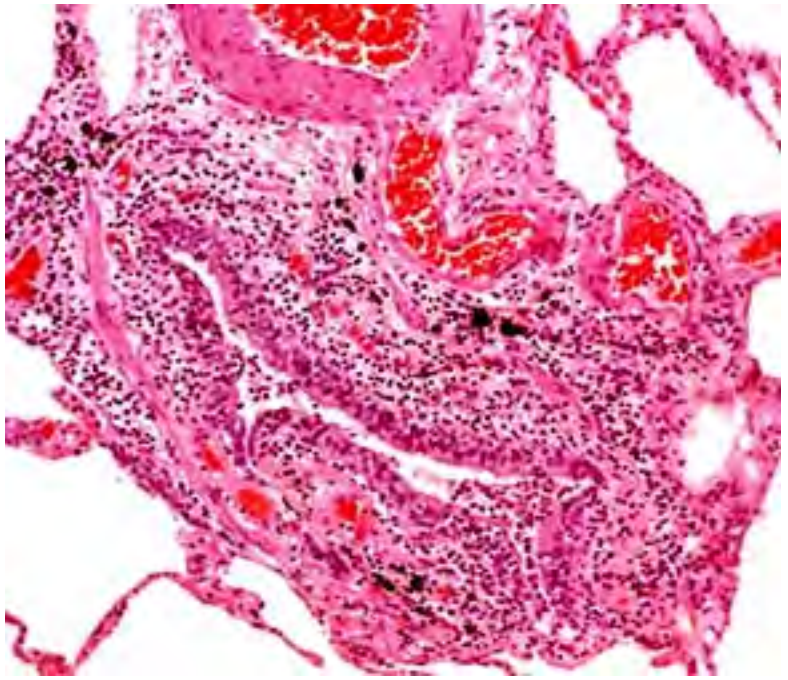

SPECIMEN
CREIGHTON PATHOLOGY


CREIGHTON PATHOLOGY



Histology of pneumonia



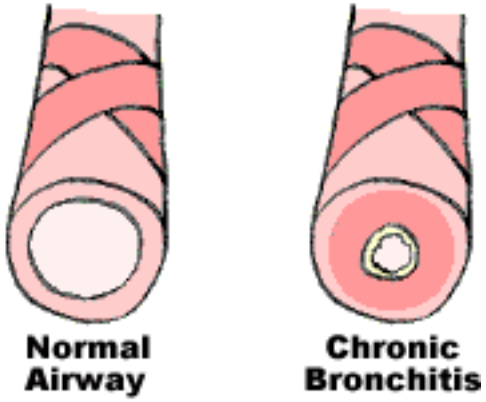


Histology of normal lung parenchyma as compared to a section from a patient who died from long standing chronic Asthma

http://pathhsw5m54.ucsf.edu/introduction.html

COPD: chronic obstructive pulmonary disease

--chronic bronchitis
---emphysems



Chronic Bronchitis

In chronic bronchitis:
***the cells lining the inside of the bronchi are continuously inflamed**
***the airways in your lungs have become narrow and partly clogged with mucus**

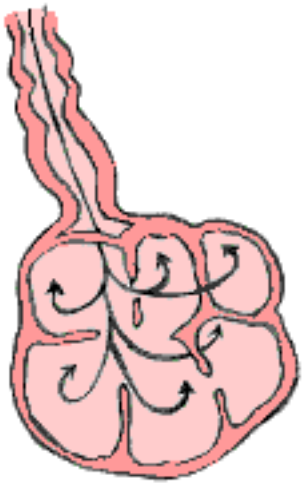
The bronchi are air passages connecting the windpipe (trachea) with the sacs of the lung (alveoli), where oxygen is taken up by the blood. Bronchitis is an inflammation of the bronchi. This inflammation causes excessive production of mucus and swelling of the bronchial walls. Airflow into and out of the lungs is obstructed.

With chronic bronchitis, the mucus cannot be cleared. Instead of helping to clean the lungs, it causes obstruction in the airways. The mucus is thicker and more difficult to cough up. This provides a means for bacteria to settle in the lower airways and increases the risk of infection.

Chronic bronchitis is caused mainly by cigarette smoke. It is characterized by:
***persistent cough**
***production of mucus**
The degree of breathlessness experienced depends on the degree of congestion of the airways and inflammation of the bronchial mucus membranes.

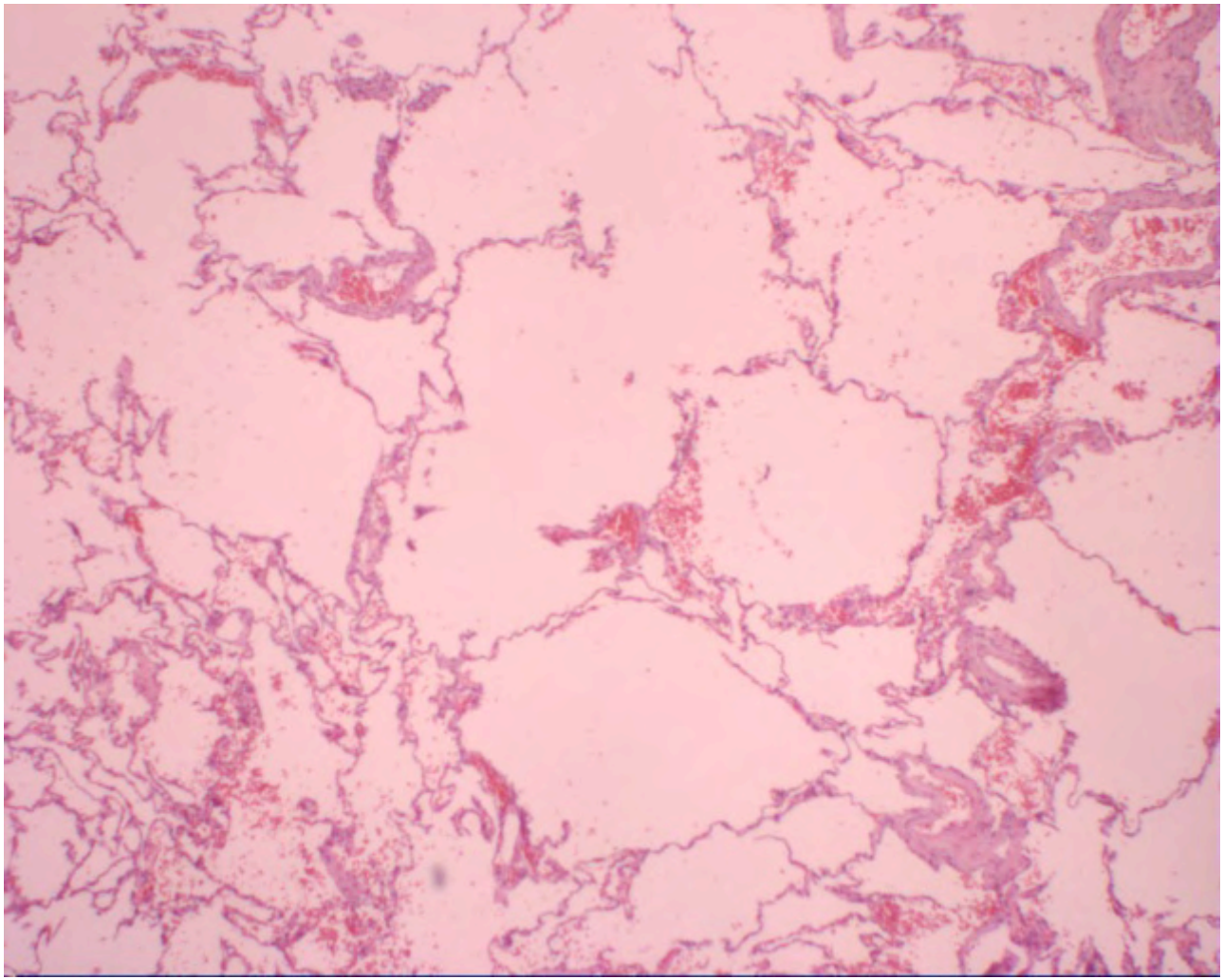
In Emphysema, some of the air sacs deep in the lungs have been damaged.

Healthy Alveolus

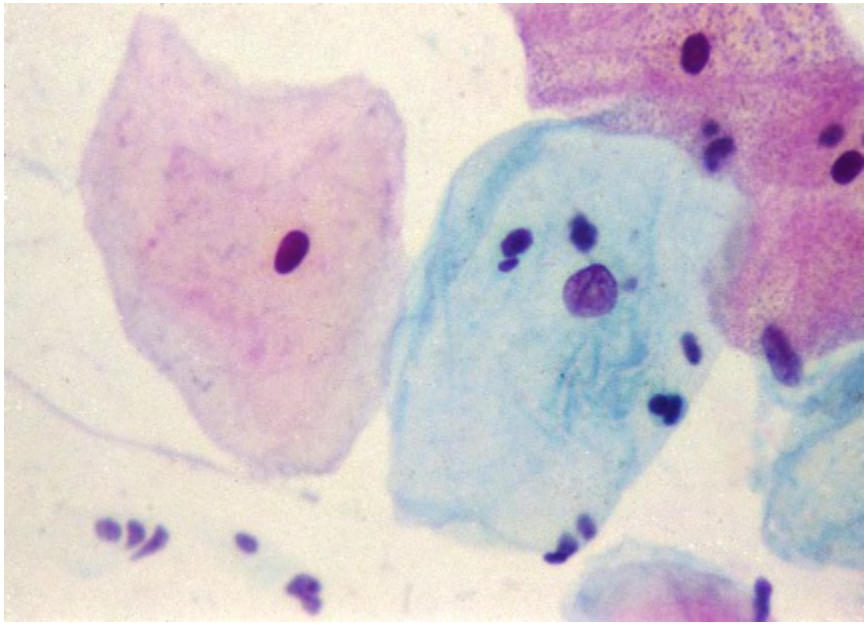


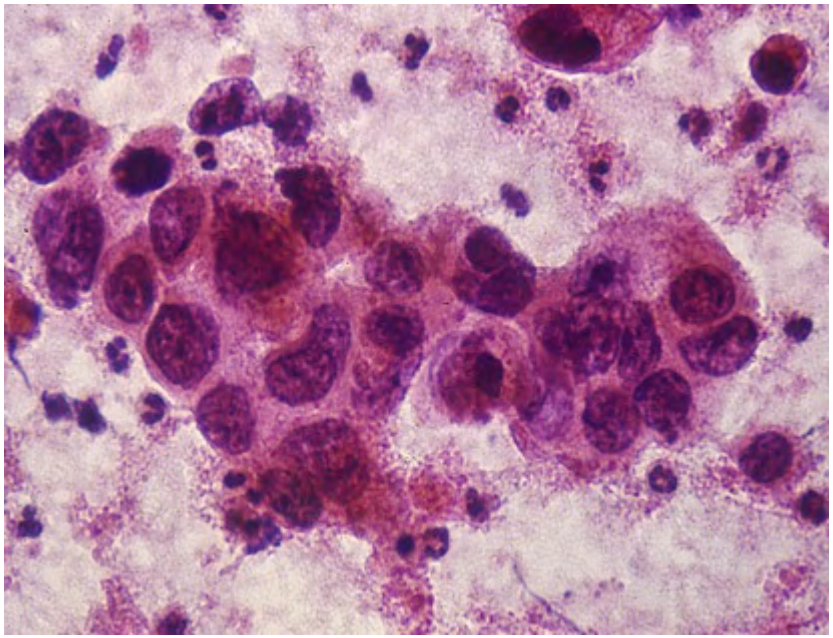
Emphysema

The normal elasticity of the air sacs and the walls of the airways are destroyed. People with emphysema need to forcefully blow the air out in order to empty the lungs. Forcing the air out in this way puts pressure on the airways from the outside, compresses them and causes them to collapse. The walls of the tiny air sacs may even tear. Excessive coughing may cause the airways to collapse as well. As the stretching and tearing of the walls of the air sacs continues, the lungs may become enlarged and less efficient at moving air into the lungs and contaminants out of the lungs



Histology of emphysema



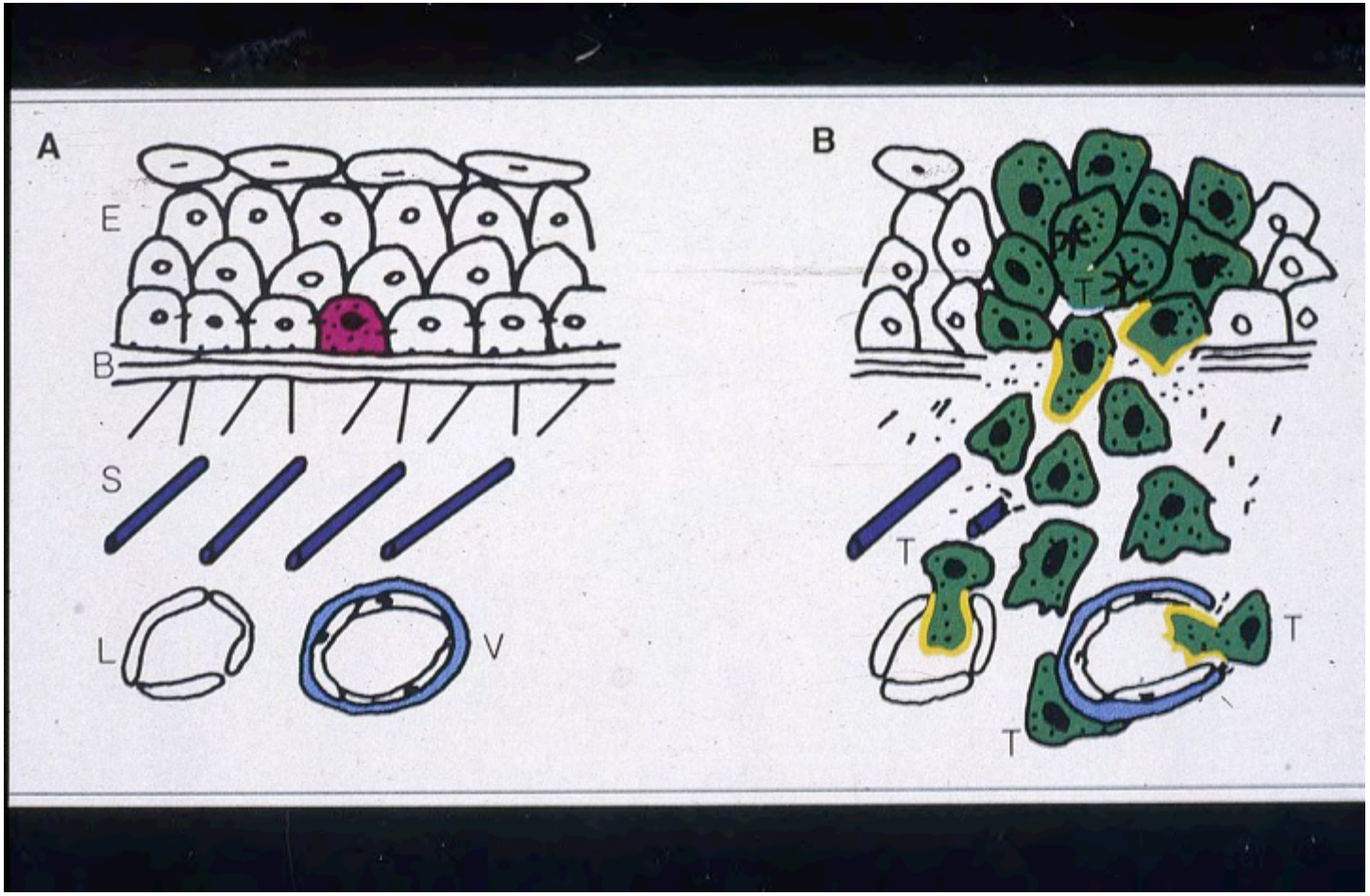


Normal cells

from smear of cervix

Carcinoma cells with

altered nuclear: cytoplasmic ratio


A
E
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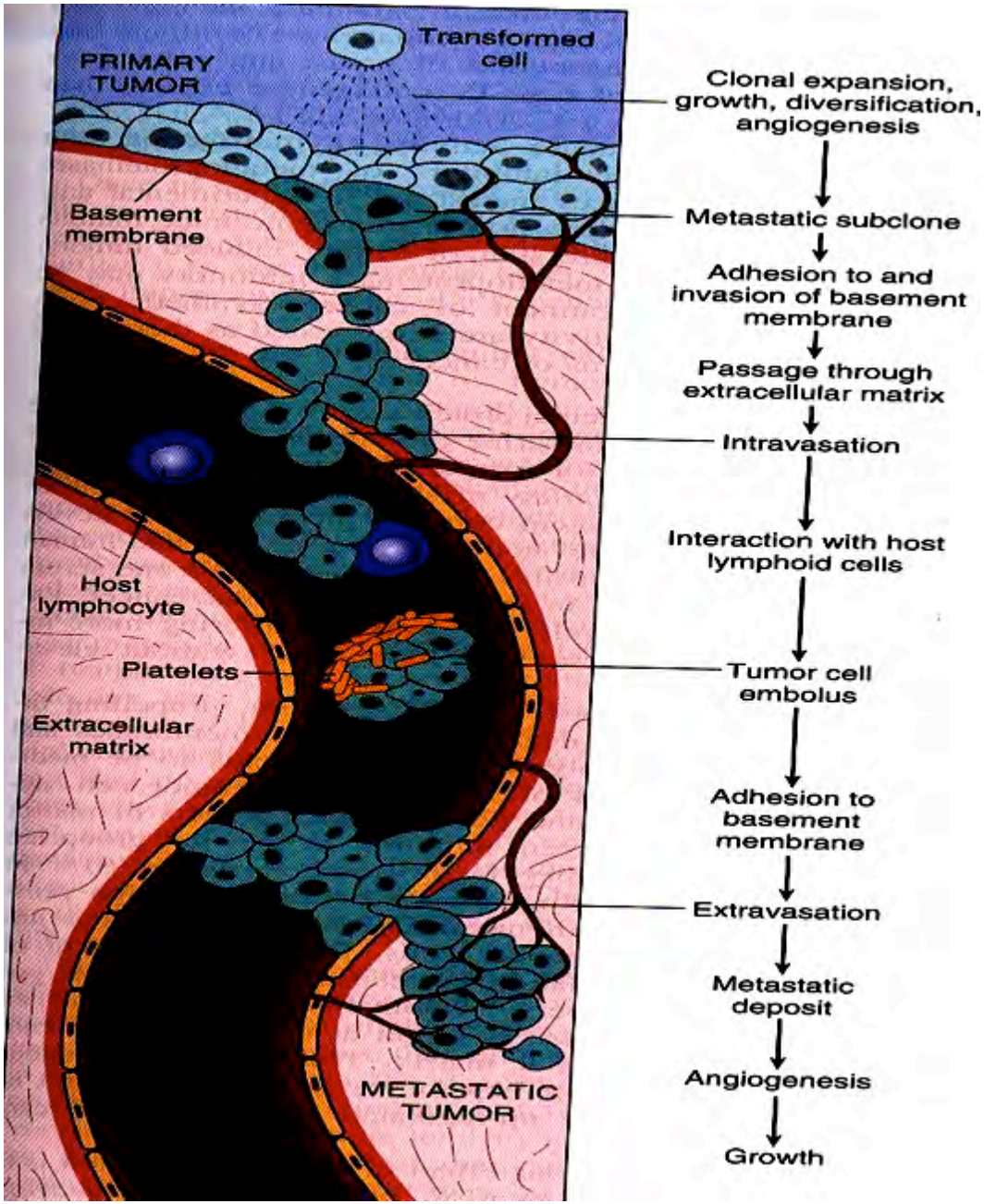
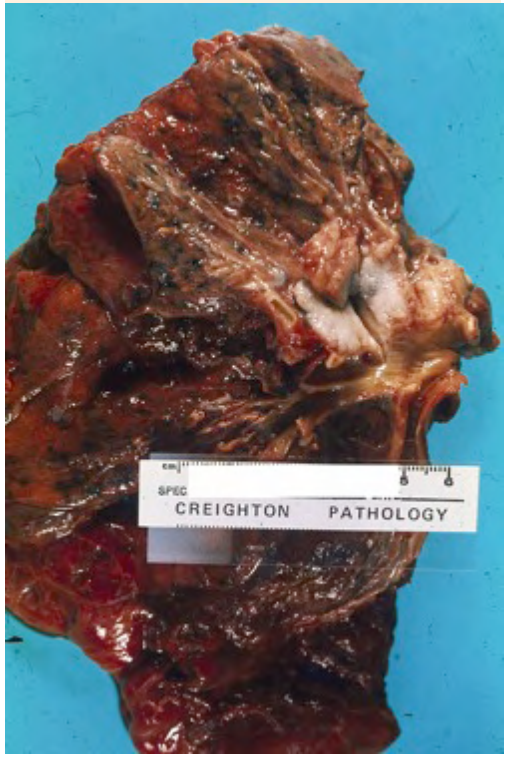
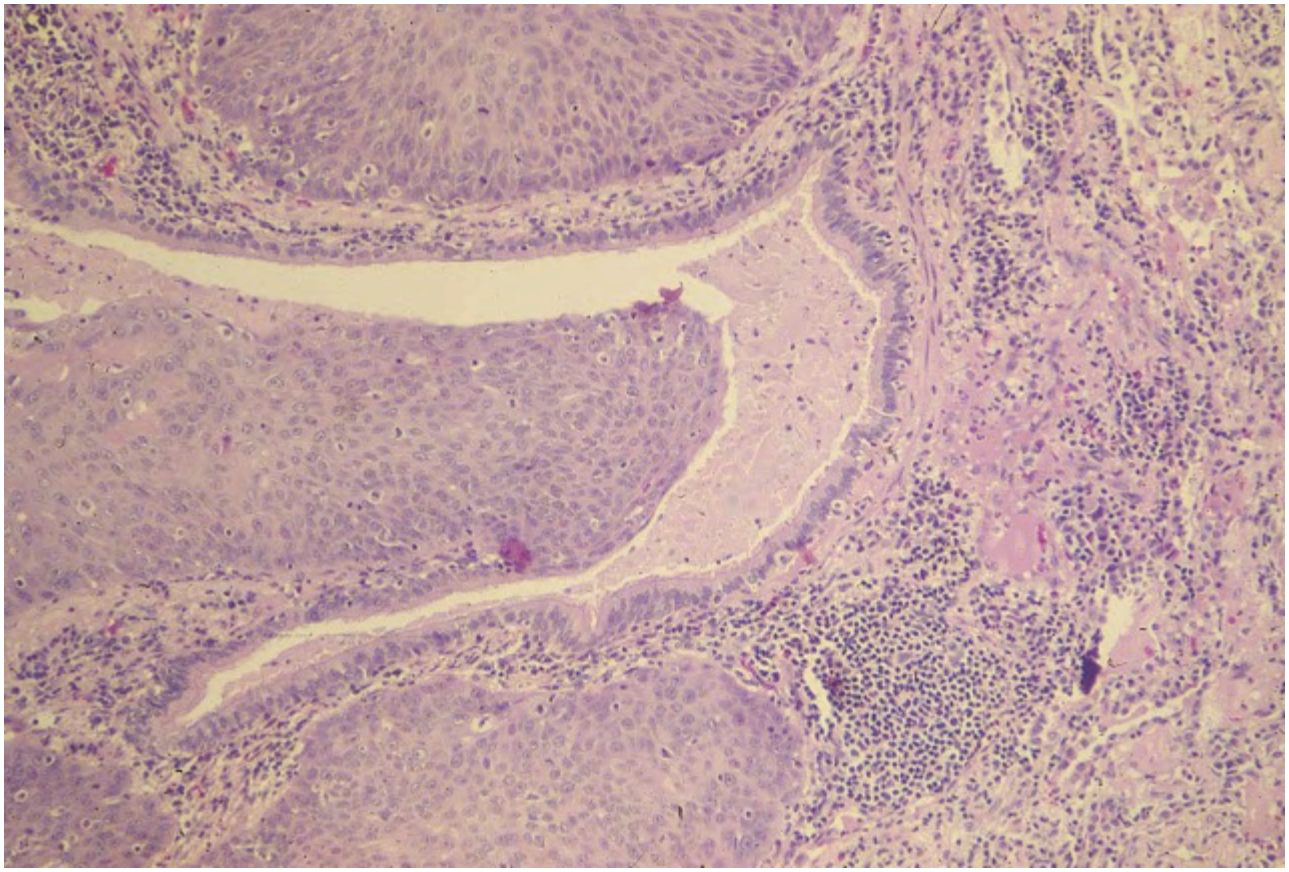
Robbins and Kumar textbook of Pathology description of the process of malignant progression and metastasis



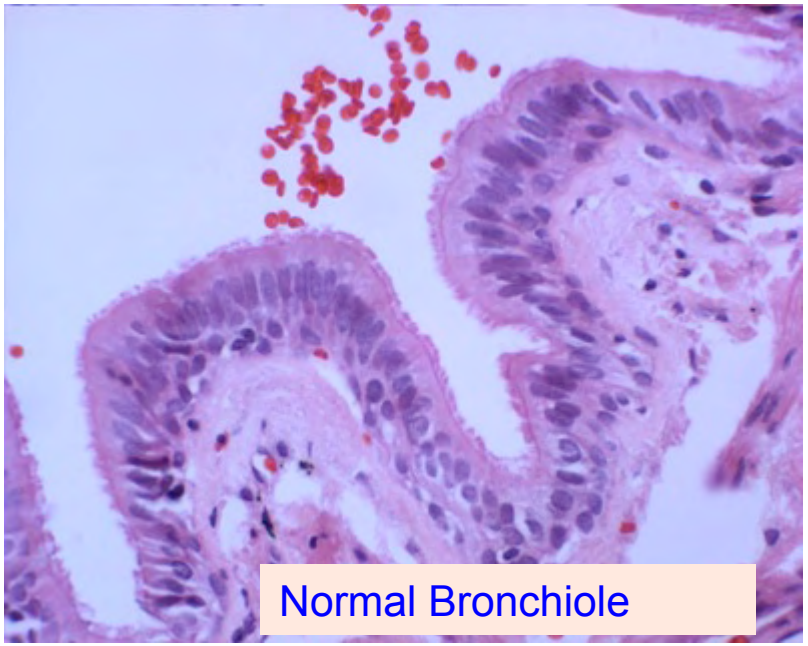
Macroscopic appearance of lung carcinoma

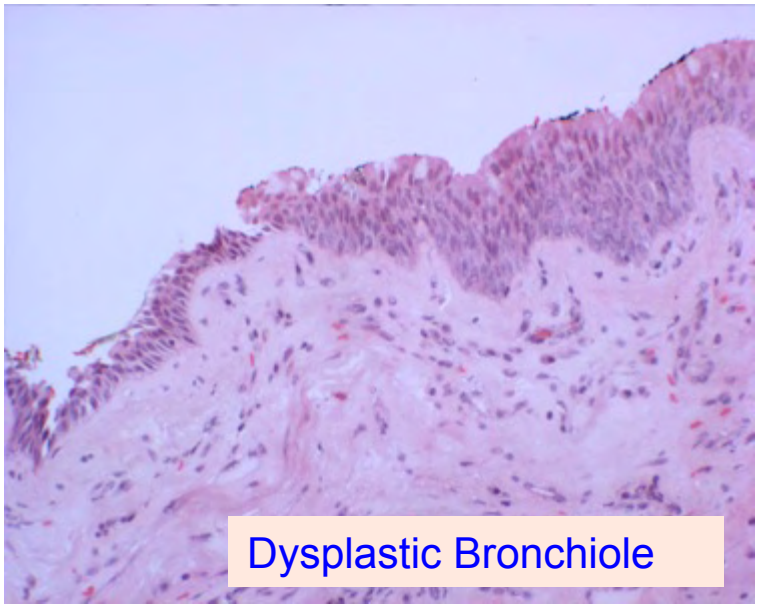


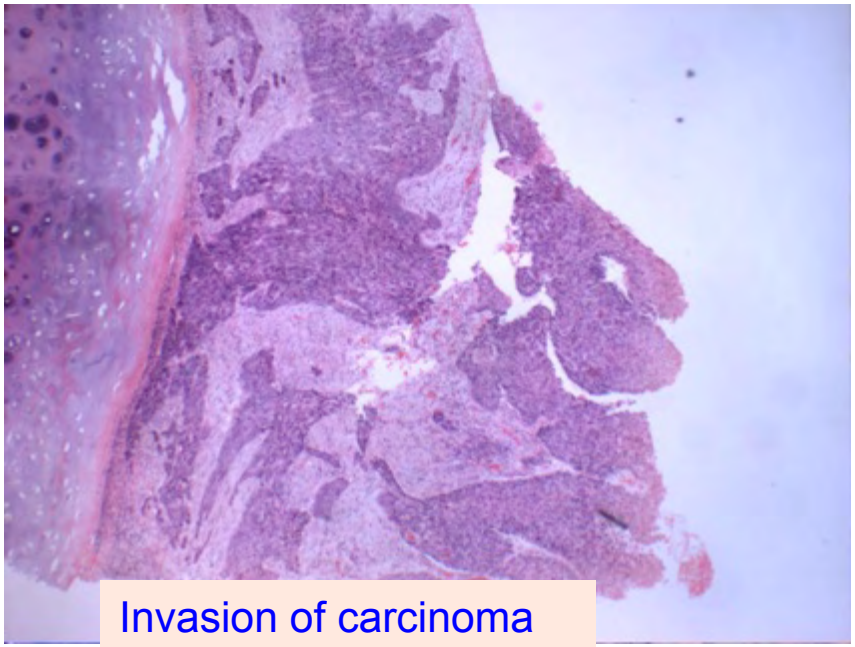
Microscopic appearance of squamous carcinoma of lung arising from bronchiole



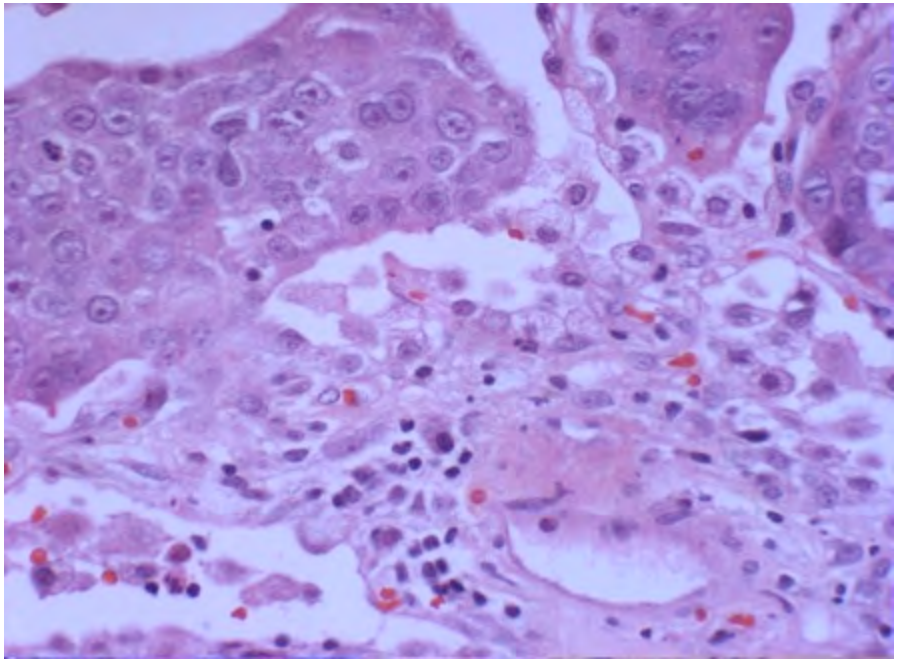

Normal Bronchiole


Normal Bronchiole

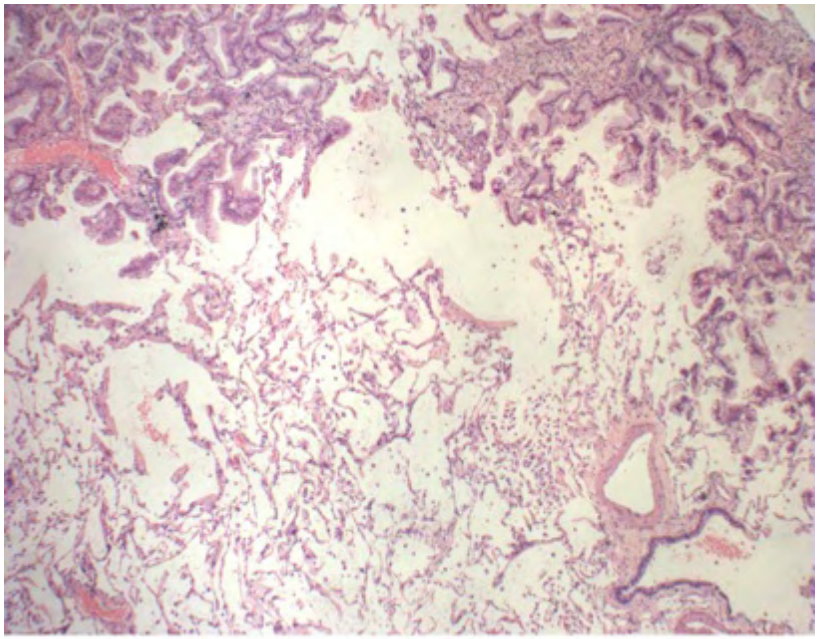

Dysplastic Bronchiole


Invasion of carcinoma

Squamous carcinoma of lung

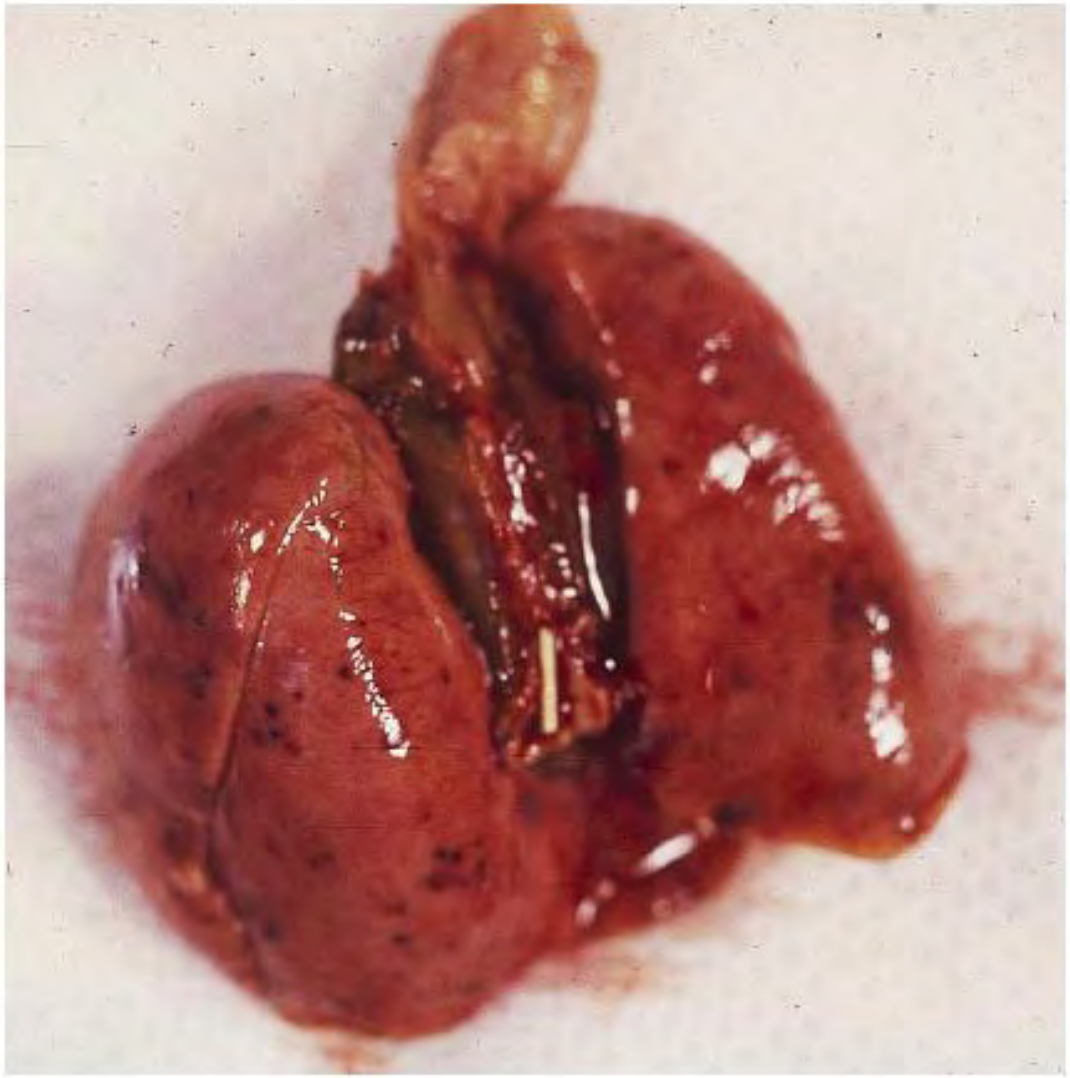


Adeno-carcinoma of lung

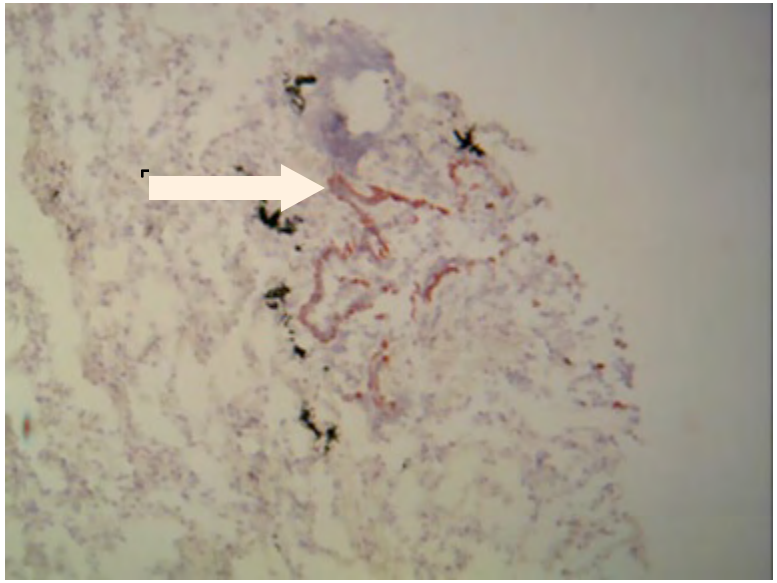


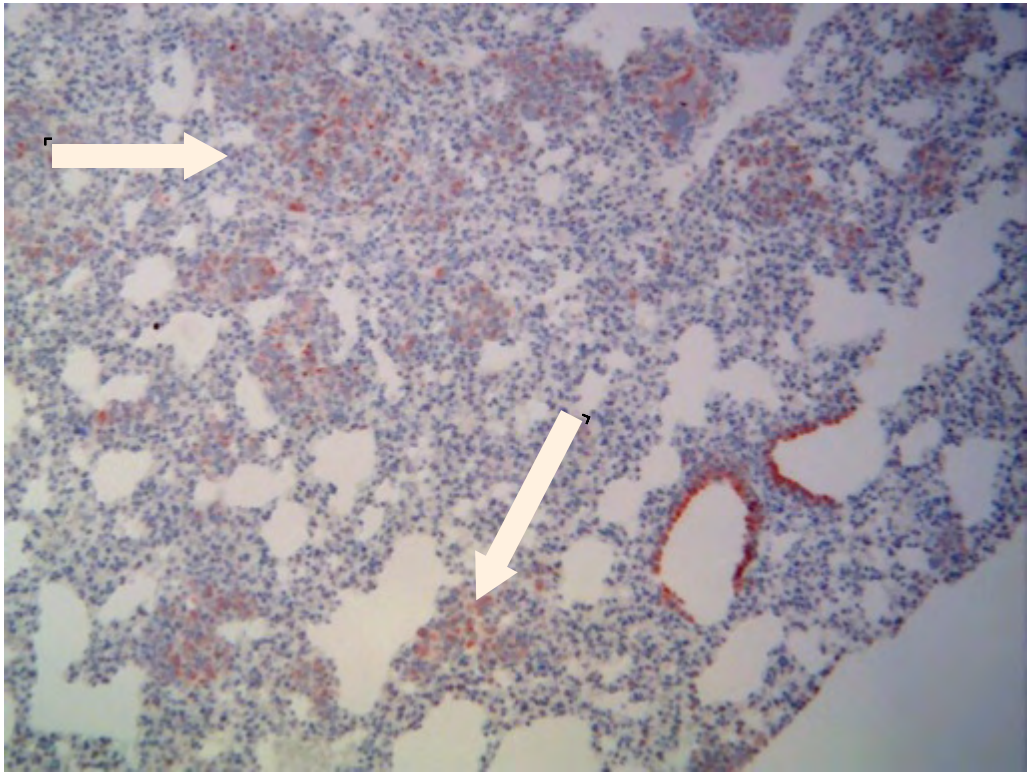
Small cell carcinoma of lung

Large cell carcinoma of lung

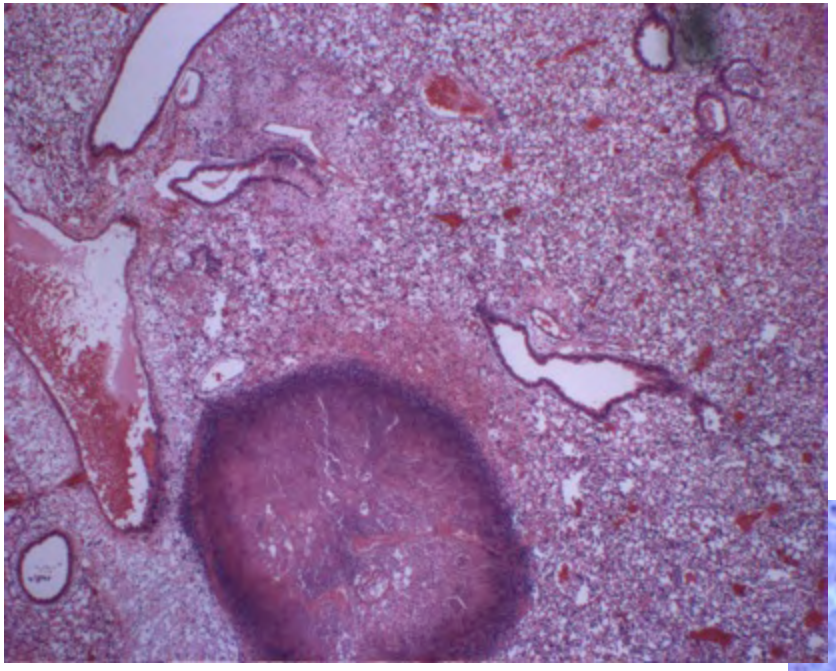


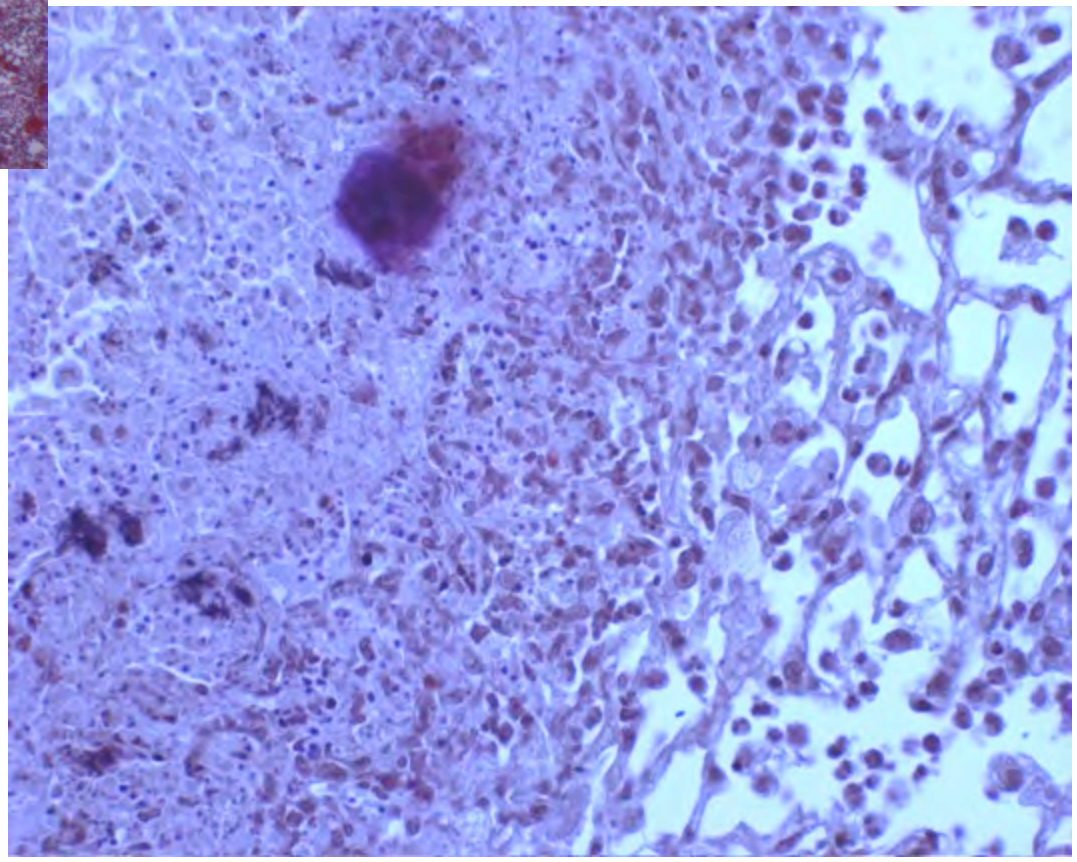
What are these nodules visible on the surface?





Low magnification of anti-keratin on mouse lung
showing endogenousmpositive control of bronchioles and
The metastatic carcinoma is identified as the keratin positive cells

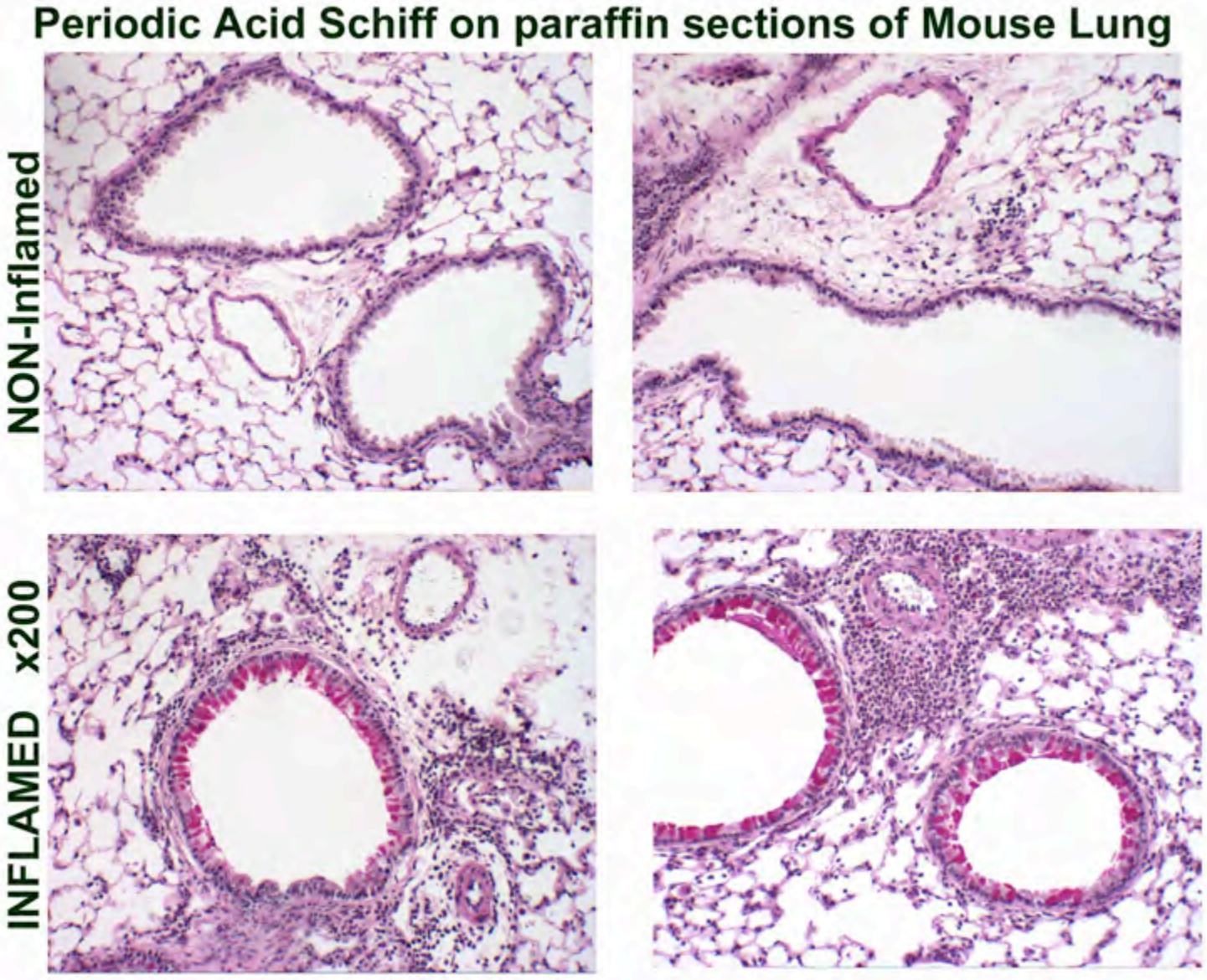




Lesion: Lung Abscess

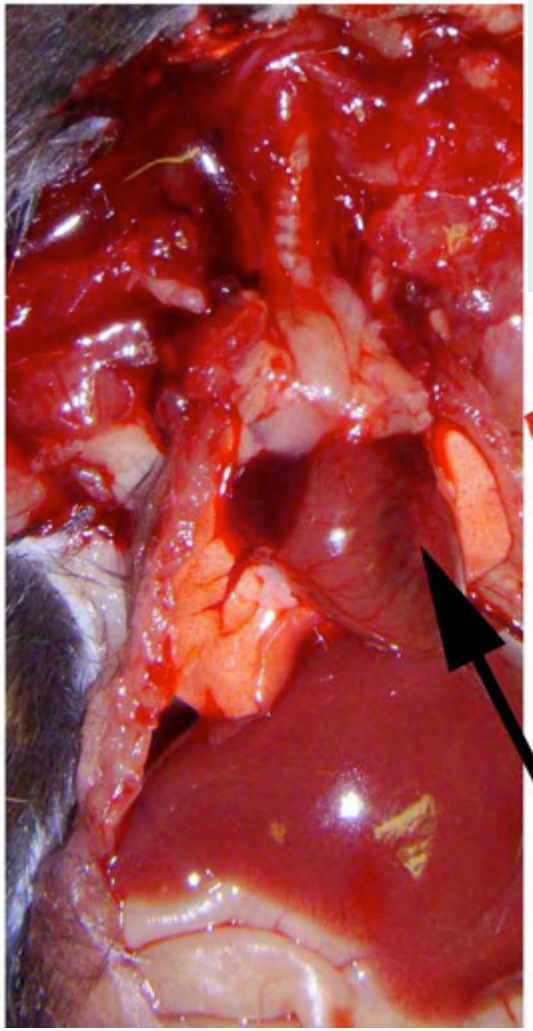
With necrotic center (dead cells,no nuclei with debris and inflammatory cell infiltrates

Mouse models of asthma induces inflammation This induces the bronchial epithelial cells to make mucus

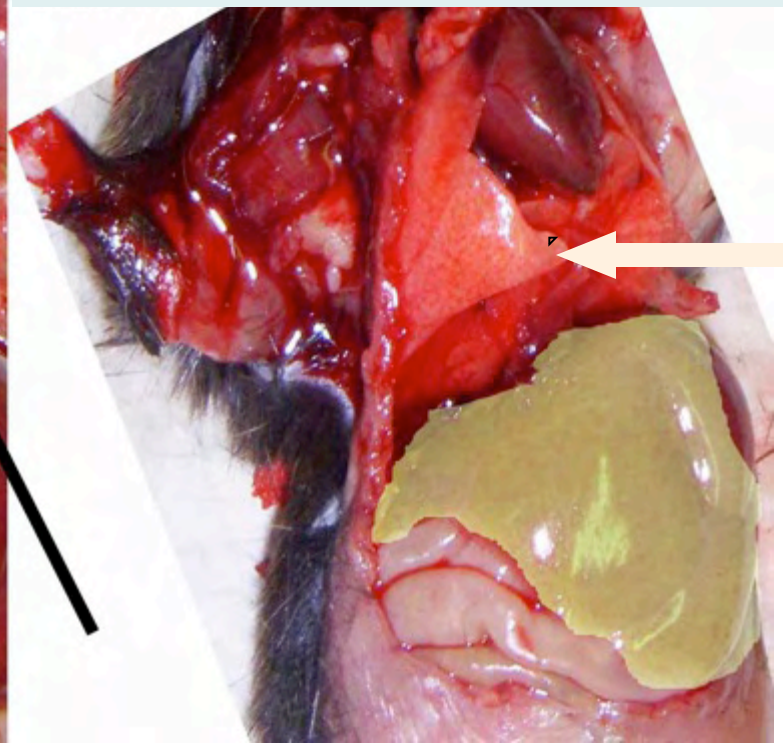


Unlike human lungs, there are no mucin secreting cells in mouse lung, unless they are inflamed.

Inflation of mouse lungs via trachea

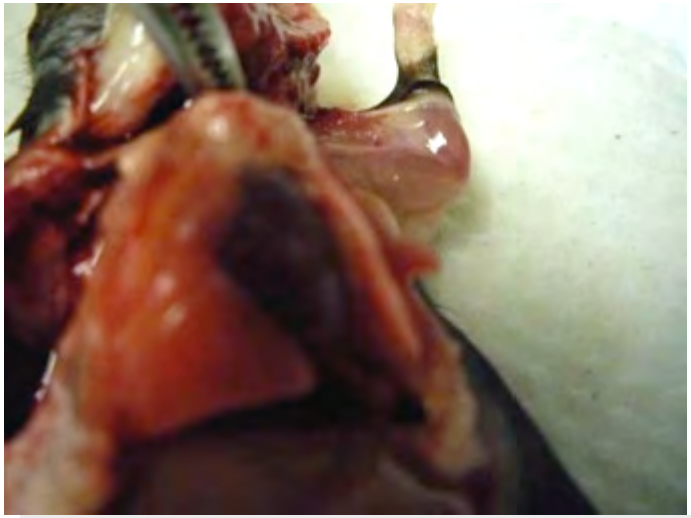


Insert needle of syringe into trachea, hold with clamps and inflate with PBS:OCT to Freeze
or inflate with fixative to fix and then process into paraffin blocks

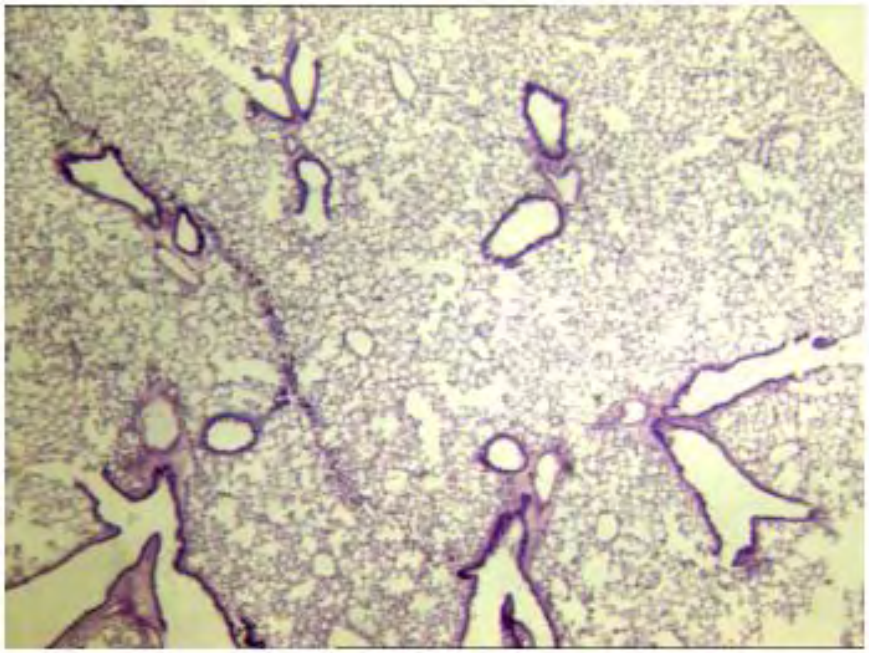


To inflate mouse lungs, Pass needle with filled syringe into trachea, hold on with a clamp, and slowly push fluid and watch lungs inflate. Hold on for about 30 seconds before releasing clamp, dissecting lungs away from thorax and proceed

Mouse lungs before inflation are collapsed



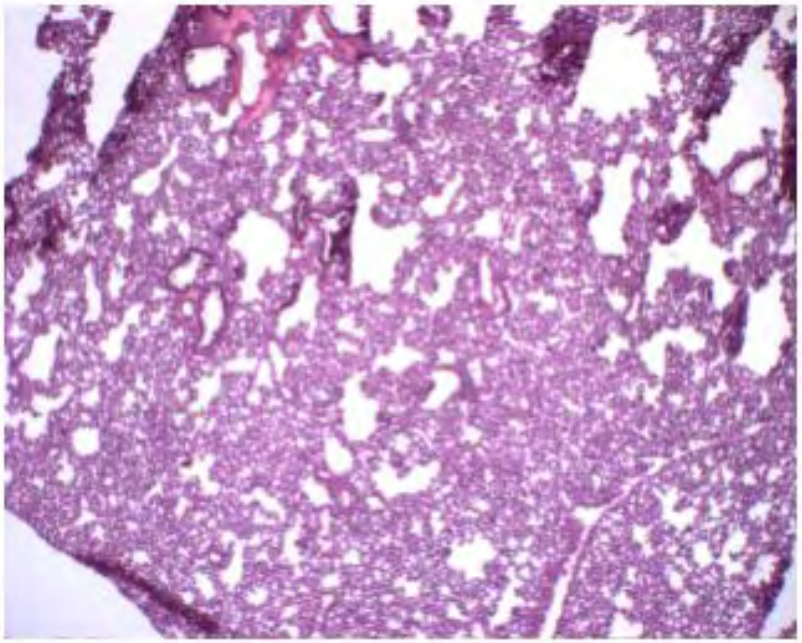
Mouse lungs after inflation



OCT infiltrated lung prior to freezing

Frozen section

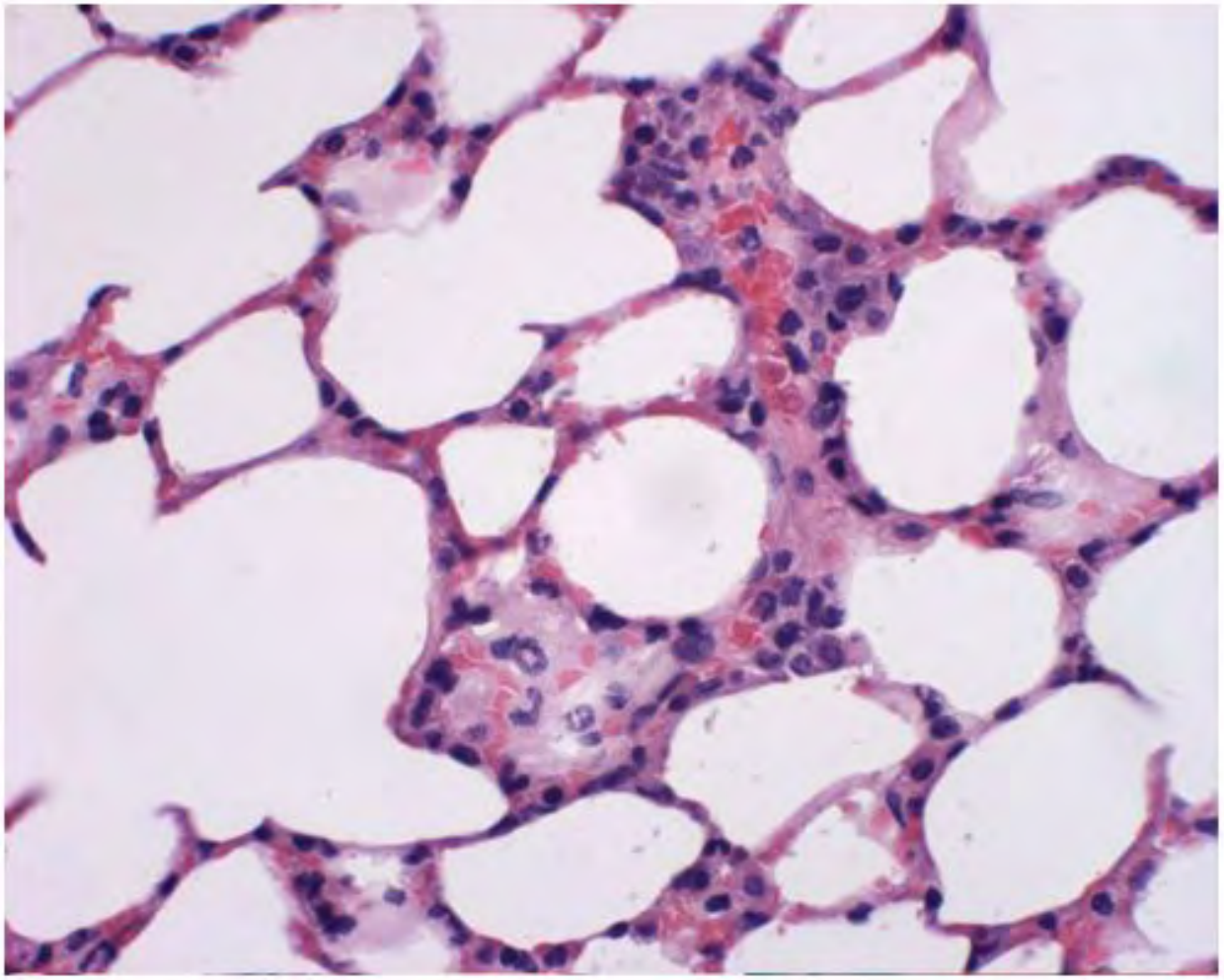
Good morphology



non-OCT infiltrated lung,

Frozen section,

poor morphology



Histology of early pneumonia

Processing of tissue :

Isolate cells for culture

Process into paraffin blocks

Freeze for protein, lipid, sugar, DNA/RNA etc.extracts

Process for EM

Freeze for histology/histochemistry/ & use for immunohistochemistry

-Fix
-Dehydrate
-Infiltrate with xylene
-Infiltrate with hot paraffin wax
-Make blocks for sections
-Store at room temperature
-Deparaffinize sections by
-reversing treatment in xylene,
-alcohol and water

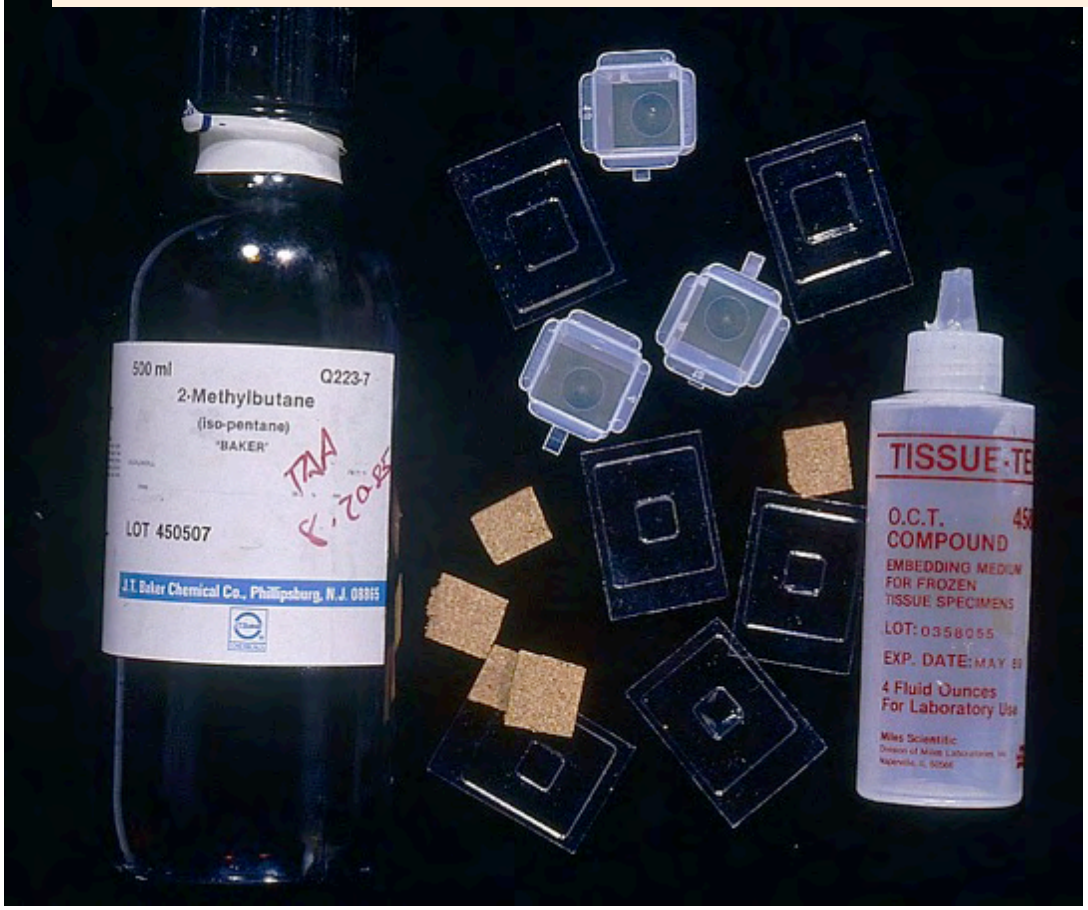
Dry ice in 2-methyl butane

OCT in plastic mold

Frozen or paraffin tissue can then be sectioned for histology

3--30 micron sections

Materials that are needed to freeze tissue for histology



Fixatives

10% Neutral buffered formalin

Zinc formalin fixation requires special processing

Bouin's fixative is quick but it hardens tissues, if fixed for too long, so move specimens to 70% alcohol in 6 hours.

FIXATIVES

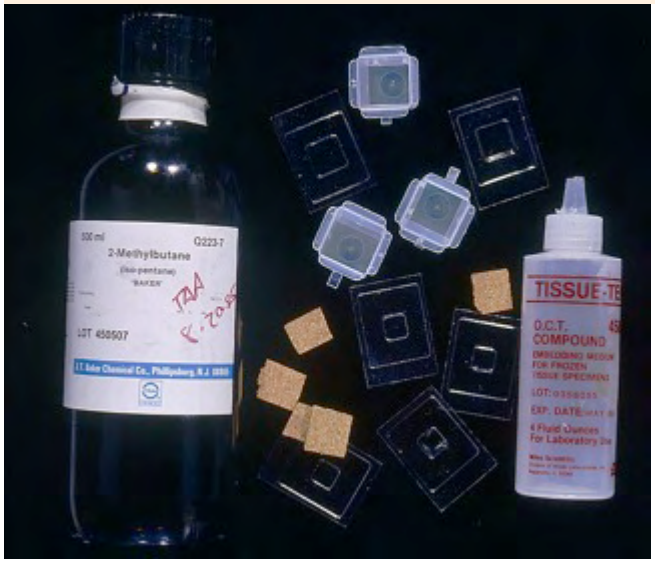
- Fix Thin slices of tissue, or inflated lungs, or tissue in sponges
- In 4% freshly made paraformaldehyde for 24 hours before immersion in 70% alcohol to submit to histotech
- In 10% buffered formalin for 24 hours before immersion in 70% alcohol to submit to histotech
- In Bouin's solution--has picric acid (yellow), acetic acid and formalin--fixes fast, makes tissues hard if left in it for more than 6 hours, many antibodies do not detect epitopes after Bouin's fixation
- Zinc containing fixatives, preserve epitopes for immunostaining

What do you need to do to freeze FIXED tissue for histology?

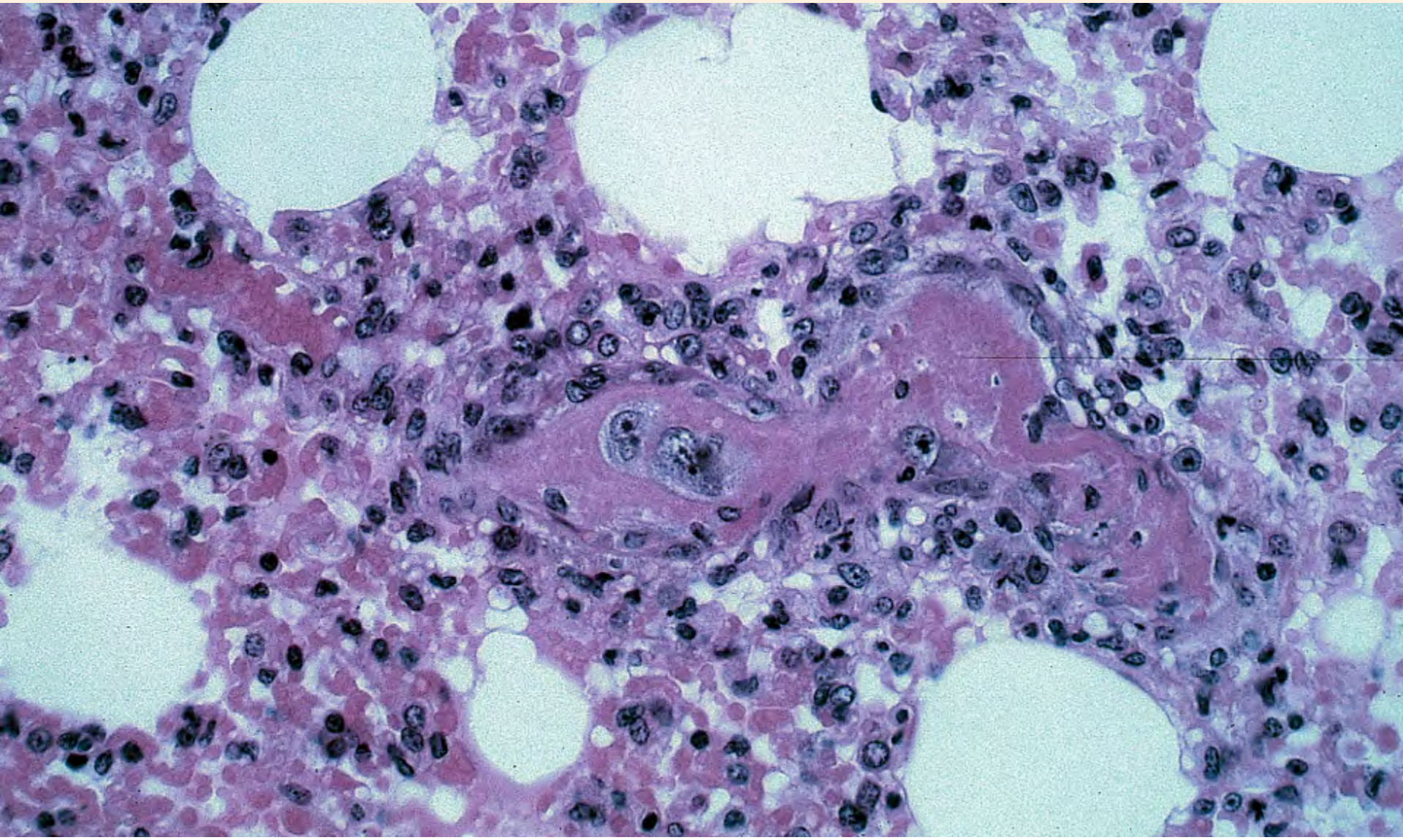
If the animal has been perfusion fixed --the organs have to SINK (Descend to bottom of tube) in 30% sucrose

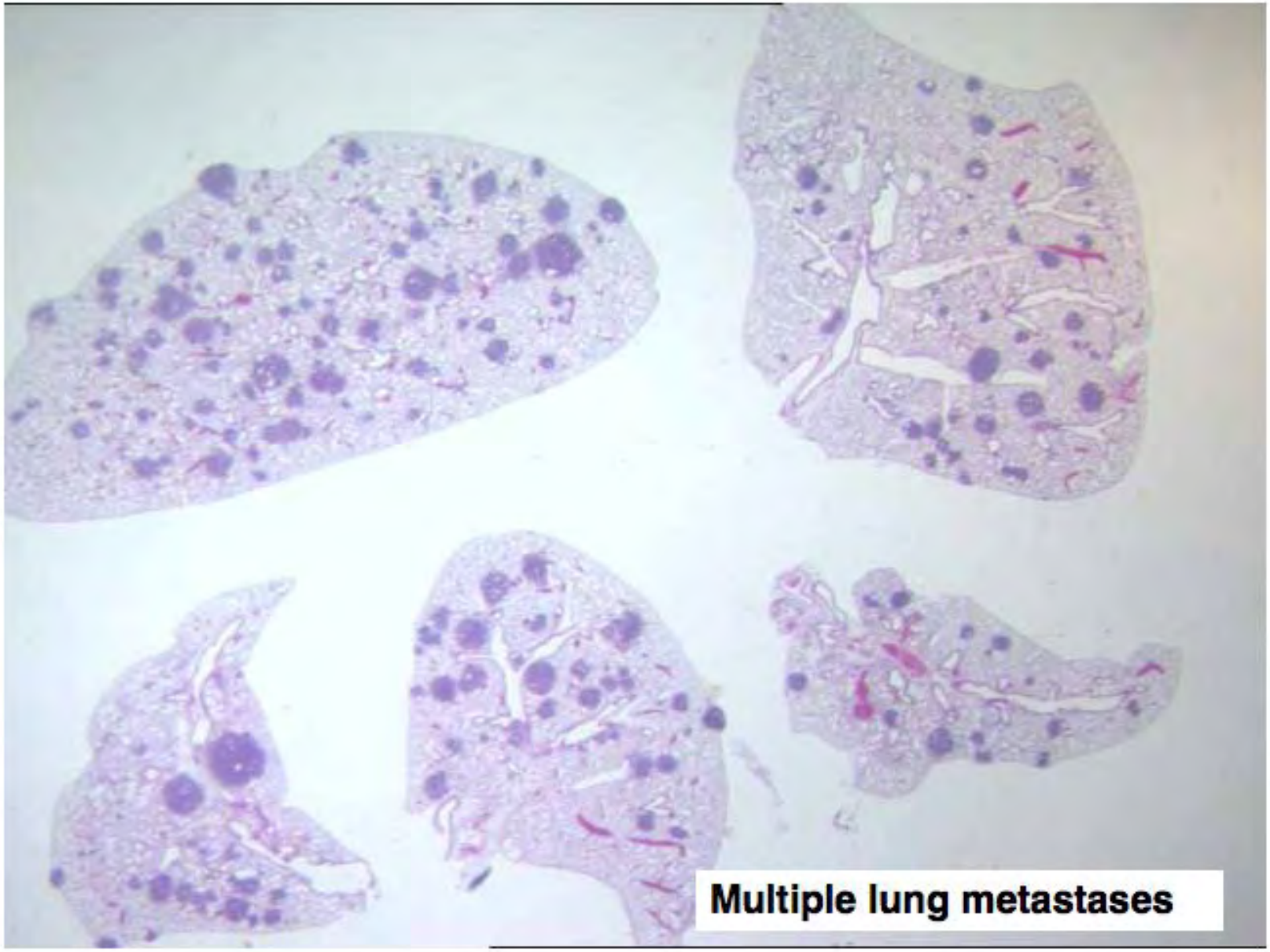
Before blotting well to remove extra sucrose, to freeze in OCT for histology examination

Materials that are needed to use to freeze tissue for histology



Invasion into blood vessels surrounding a malignant tumor, allows hematogenous seeding----can you identify the malignant cells?




Multiple lung metastases

To detect the presence of metastatic malignant cells in the mouse lung:

-----IF GFP is the label used to tag the malignant cells, remove the lungs and extract using methods on the mouse pheno website and detect the fluoresence using a fluorescence plate reader.

To detect the presence of metastatic malignant cells of Human origin in the mouse lung:

Extract the lung and check for Human specific Alu sequences

To detect the presence of metastatic malignant cells in the mouse lung:-----If GFP is the label used to tag the malignant cells, remove the lungs and extract using methods on the mousepheno website and detect the fluorescence using a fluorescence plate reader.

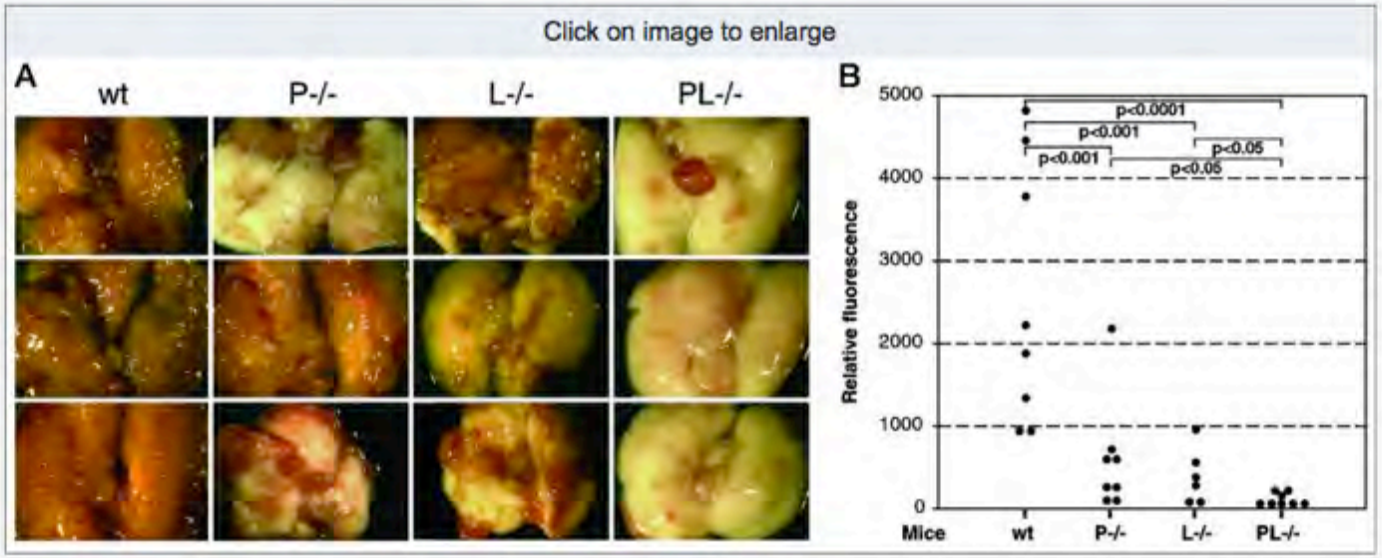


Figure 4

Effect of selectin deficiencies on the metastatic progression of MC-38 mouse adenocarcinoma cells. Mice were injected i.v. with $2–3 \times 10^5$ MC-38-GFP cells and examined 22 days later. Lungs were dissected, photographed, and homogenized, and the homogenate was diluted for a fluorescence read-out. (*A*) Examples of dissected lungs from each mouse genotype. (*B*) Quantitation of metastasis by GFP fluorescence. All mice were in a syngeneic C57BL6 background. The number of animals studied were six to eight per group. Statistical significance was determined by the Bonferonni multiple compare test.

Borsig, L., Wong, R., Feramisco, J., Nadeau. D.R., Varki, N.M., Varki, A.: Heparin and Cancer Revisited: Novel Mechanistic Connections involving Platelets, P-Selectin, Carcinoma Mucins and Tumor Metastasis. *Proc. Natl Acad. Sci. U.S.A.,* 98:3352-3357, 2001

Borsig, L., Wong, R., Hynes, R.O., Varki, N.M., and Varki, A.: Synergistic Effects of L- and P-selectin in Facilitating Tumor Metastasis Can Involve Non-Mucin Ligands And Implicates Leukocytes as Enhancers of Metastasis. *Proc. Natl Acad. Sci. U.S.A.*, 99:2193-2198, 2002.

To detect the presence of metastatic malignant cells of Human origin the Mouse lung:

Extract the lung and check for Human specific Alu sequences

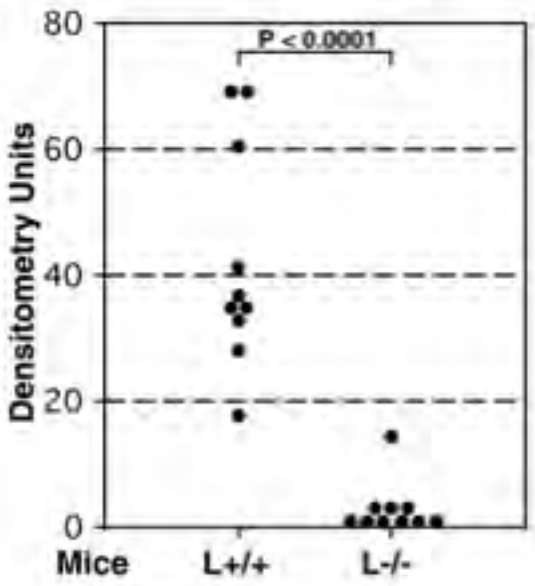


Fig. 1. L-selectin deficiency attenuates metastasis of human adenocarcinoma cells in immunodeficient mice. Mice were injected i.v. with 3–4 × 10^5 LS180 cells and studied 6 weeks later. Human-specific Alu-PCR was conducted on genomic DNA isolated from dissected lungs and densitometrically quantified as described in *Materials and Methods*. The number of animals studied were 9–10 in each group. Statistical significance was determined by the Student's *t* test.

Lungs : Important points to remember

1. Must Inflate mouse lungs before freezing or fixing for histo-pathological examination in order to examine the different cell types in the lung.
2. Keratin positive epithelial cells line bronchi and bronchioles and alveoli
3. Endothelial cells line the abundant capillaries in the alveolar walls (CD31 small vessels, or vWF --large)
4. Lymphatics that travel adjacent to the vessels--LyVe1
5. Plenty of Alveolar macrophages--F480 (CD68)
6. There are wandering lymphocytes and monocytes in the capillaries--CD45

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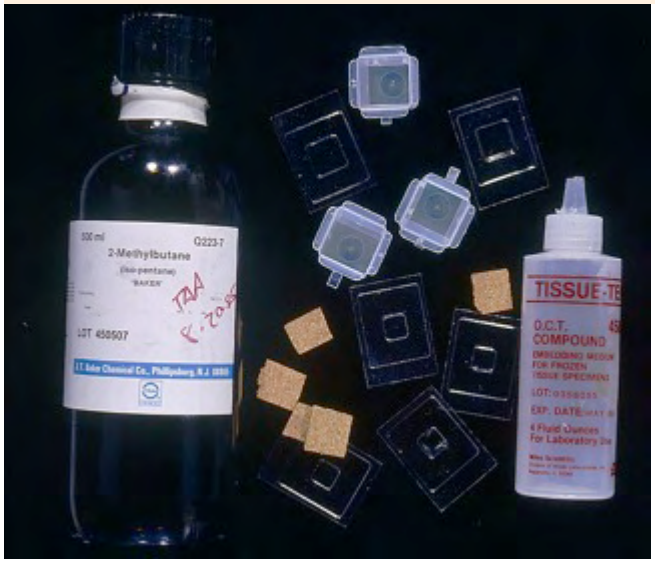
Before blotting well to remove extra sucrose, to freeze in OCT for histology examination

Materials that are needed to use to freeze tissue for histology



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-alcohol and water

Dry ice in 2-methyl butane

OCT in plastic mold

Frozen or paraffin tissue can then be sectioned for histology

3--30 micron sections

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- Zinc containing fixatives, preserve epitopes for immunostaining

Frozen sections and zinc fixed paraffin sections for IHC

Table 2. *Antigen survival*

	Antigens				
Fixative	CD1	CD4	CD7	CD8	CD19
Frozen sections	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
ZnCl	+ + +	+ + + +	+ + +	+ + +	+ + +
Zn acetate	+ + +	+ + + +	+ + +	+ + +	+ + +
ZnCl and Zn acetate	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
NBF	0	0	0	0	0
Z-fix	0	0	0	0	0
1% paraform	0	0	0	0	0
4% paraform	0	0	0	0	0
40% ethanol	0	0	0	0	0
Optimal fix	0	0	0	0	0
Prefer fix	0	0	0	0	0
Quanta fix	0	0	0	0	0
STF	0	0	0	0	0
B*5 without formalin	+ + +	+ + +	+ + +	+ + +	+ + / + + +

Beckstead,J.H. J.Histochem Cytochem 1994 42: 1127

http://www.jove.com/video/2966/diagnostic-necropsy-selected-tissue-sample-collection-rats