Arterial
Venous systems
Capillaries
Lymphatics

http://
www.accessexcellence.org/
AE/AEC/CC/
heart_anatomy.html
Adventitia with vasa vasorum

AORTA

Elastic fibers

intima

Endothelial cell lining

Smoothe muscle

Arteriole

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

No elastic tissue in walls of veins and venules.
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
Diseases of Blood vessels include:

Atherosclerosis
Aneurysms (dilatation)
Abnormal collagen support
Atherosclerosis of coronary arteries of human heart

Medlib.med.utah.edu/WebPath
Elastic fibers in media of aorta
Endothelium lined Intima
Vasa vasorum in adventitia
Lumen
atheroma
Atherosclerosis of coronary artery of human heart
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?
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candyanatomy "Cardio Candy"
#CandyAnatomy #candycopyright #medschool
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

Cardiac: striations + central nuclei
Skeletal: striations + eccentric nuclei
Smooth: central nuclei
Human Skeletal muscle: nuclei at the edges and striations
Human Skeletal muscle with PhosphoTungstic Acid Hematoxylin 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

Cardiac: striations + central nuclei
Skeletal: striations + eccentric nuclei
Smooth: central nuclei
HEART

magnification x40

Human

Mouse

scale bar = 500 microns
Different orientation of the hearts can help visualize different abnormalities.
Example of pathology in mouse Heart valves

Representative sections of HEART VALVES ×200

Intermediate controls and of MUCIE (cleft) 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 fluorescence 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
TRACHEA  magnification x400

Human

Mouse

scale bar = 100 microns
Pseudostratified (ciliated) columnar

- Goblet cell
- Cilia
- Basement membrane
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
Mike McCormick - 27 yr - Glasgow University Medical Student

PHOTO
https://instagram.com/candyanatomy/

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- medschool #anatomy #aero

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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.
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
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. 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
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
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 endogenous positive 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)
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.
OCT infiltrated lung prior to freezing
Frozen section
Good morphology

non-OCT infiltrated lung,
Frozen section,
poor morphology
Processing of tissue:

Isolate cells for culture

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

Freeze for histology/histochemistry & use for immunohistochemistry

Process into paraffin blocks

Dry ice in 2-methyl butane

OCT in plastic mold

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

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?
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 fluorescence 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.

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Figure 4

Effect of selectin deficiencies on the metastatic progression of MC-38 mouse adenocarcinoma cells. Mice were injected i.v. with $2 \times 10^5$ 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 Bonferroni multiple compare test.

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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 $3 - 4 \times 10^5$ 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 histopathological 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.
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**
Isolate cells for culture

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

Freeze for histology/histochemistry/ & use for immunohistochemistry

Process into paraffin blocks

- 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
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• Zinc containing fixatives, preserve epitopes for immunostaining
Frozen sections and zinc fixed paraffin sections for IHC

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

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