PRACTICAL HISTOPATHOLOGY IN MOUSE MODELS OF HUMAN DISEASE:

GUIDES TO PHENOTYPING THE GENETICALLY ALTERED MOUSE

http://mousepheno.ucsd.edu/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3693904/

1. Approval to conduct experiments on animals, following ethical guidelines
2. Use of necropsy facilities in designated scientific buildings
3. Transport of cages appropriately, using covered boxes
4. Transport of and disposal of carcasses, using approved methods
5. Consultation with veterinary personnel for non-routine procedures
6. Consultation with personnel in ACP's BSB laboratory for evaluation of blood and chemistry parameters
7. Consultation with Histology core personnel prior to mouse necropsy

Finish serum chemistry analyses before proceeding to histology

1	C57BL/6 blood chemistry normal ranges		n ~= 400
2	age ~ 2-5 months		
3		mean	sd
4			
5	Glucose (mg/dL)	196.7	91.2
6	Urea nitrogen (mg/dL)	21.4	4.4
7	Creatinine (mg/dL)	0.2	0.2
8	Bicarbonate (mEq/L)	15.9	3.2
9	Chloride (mEq/L)	107.5	4.2
10	Sodium (mEq/L)	150.5	3.9
11	Potassium (mEq/L)	5.0	1.1
12	Calcium (mg/dL)	9.0	0.4
13	Direct bilirubin (mg/dL)	0.1	0.1
14	Total bilirubin (mg/dL)	0.4	0.2
15	Albumin (g/dL)	1.4	0.2
16	Total protein (g/dL)	4.2	0.3
17	Phosphorus (mg/dL)	8.0	1.6
18	AST (SGOT) (IU/L)	72.7	36.3
19	ALT (SGPT) (IU/L)	38.7	25.9
20	Alkaline phos, total (IU/L)	101.5	32.2
21			
22	LIPID PANEL (n~=50)		
23	Lipase (U/L)	53.0	12.9
24	Cholesterol (mg/dL)	98.6	17.9
25	HDL-chol. (mg/dL)	78.6	21.9
26	LDL-chol. (mg/dL)	12.2	8.6
27	Triglycerides (mg/dL)	67.8	26.1
28			

Tests for Kidney function

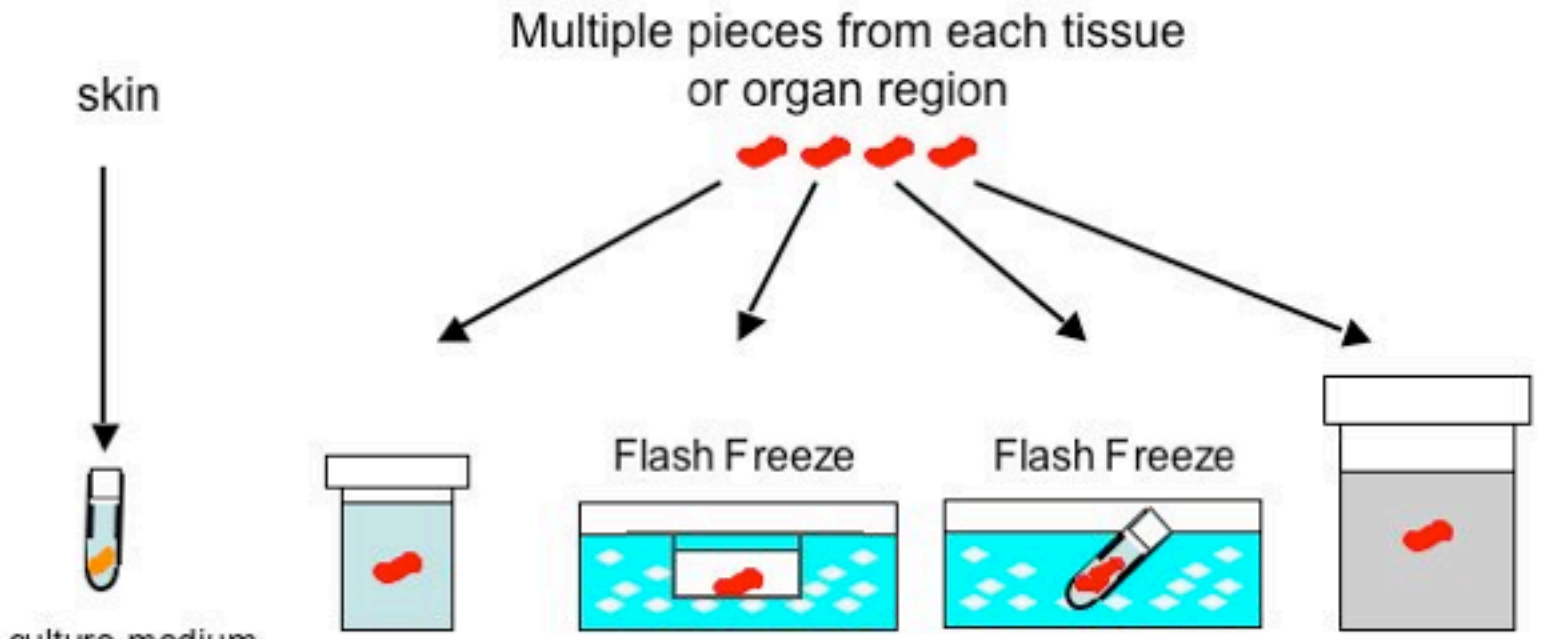
Tests for Liver function

Finish hematology analyses before proceeding to histology

HEMATOLOGY	n = 7		n = 4		
WBC (K/µL)	6.37	3.03	3.82	2.06	
Neutrophils (%)	23.90	10.72	41.34	27.53	
Neutrophils (K/µL)	1.35	0.52	1.84	1.83	
Lymphocytes (%)	71.04	12.19	54.64	28.29	
Lymphocytes (K/µL)	4.74	2.79	1.82	1.13	61.6 % decrease
Monocytes (%)	4.47	1.29	3.51	0.59	
Monocytes (K/µL)	0.26	0.07	0.14	0.10	44.3 % decrease
Eosinophils (%)	0.47	0.59	0.39	0.27	
Eosinophils (K/µL)	0.02	0.03	0.02	0.02	
Basophils (%)	0.12	0.22	0.12	0.08	
Basophils (K/µL)	0.01	0.01	0.00	0.00	
RBC (M/µL)	9.00	0.50	8.13	0.27	9.7 % decrease
HGB (g/dL)	12.0	0.8	10.9	0.9	9.7 % decrease
HCT (%)	40.0	2.1	34.8	1.2	13.0 % decrease
MCV (fL)	44.5	1.8	42.8	1.1	
MCH (pg)	13.4	0.8	13.4	0.7	
MCHC (g/dL)	30.1	1.7	31.3	2.4	
RDW (%)	20.7	1.3	21.2	3.8	
PLT (K/µL)	765	142	942	279	
MPV (fL)	5.16	0.27	5.41	0.47	

When tissues are removed from the body, different preservation methods will help ensure optimal evaluation in order to determine the significance of the pathologic changes induced by disease

An example of different ways to process tissues



	culture medium (alpha MEM + antibiotics)	RNA later	In OCT compound plastic mold	Cryotube	Buffered Formalin
	For primary Fibroblast culture	For RNA Gene expression	For Frozen Histology	For DNA Biochemistry	For Paraffin Histology
Storage:	10 ml screw top tubes	Tight screw top plastic container -20/-80	Frozen sample in mold into labeled plastic bag -20/-80	Frozen sample in cryotube into labeled plastic bag -20/-80	Tight screw top labeled plastic container Room Temp
Shipping temperature:	RT	On dry ice	On dry ice	On dry ice	RT

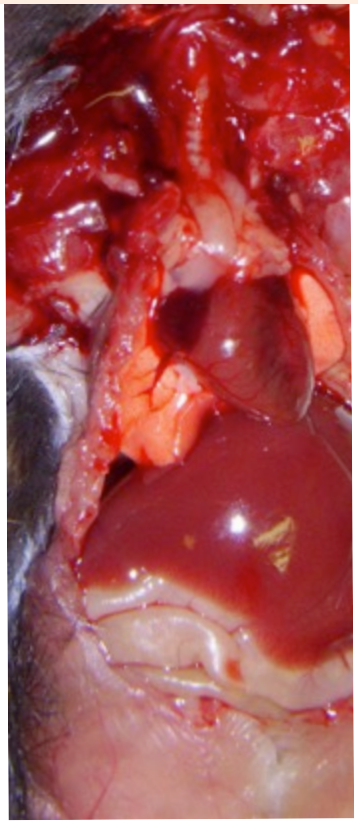
The various tissues and organs that are examined using microscopy

1. NEURAL

2. HEART
/Blood vessels
3. LUNGS

4. LIVER
5. PANCREAS
6. SALIVARY GLAND
7. STOMACH
8. SMALL INTESTINE
9. COLON

10. SPLEEN
11. TONSIL
12. THYMUS
13. LYMPH NODES
14. BONE MARROW



TUMORS

Assess for metastasis

15. KIDNEY
16. BLADDER
17. TESTIS
18. PROSTATE
19. UTERUS
20. OVARY
21. BREAST
22. PLACENTA

29. THYROID/ Parathyroid
30. ADRENAL
31. PITUITARY
---Eyes
---Sinuses

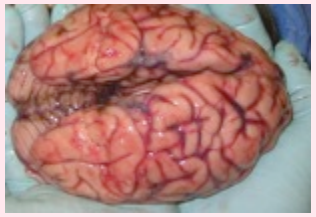
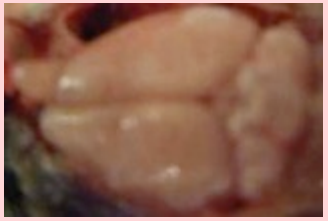
24. SKIN
25. SKELETAL MUSCLE
26. SMOOTH MUSCLE, ADIPOSE

27. CARTILAGE

28. BONE

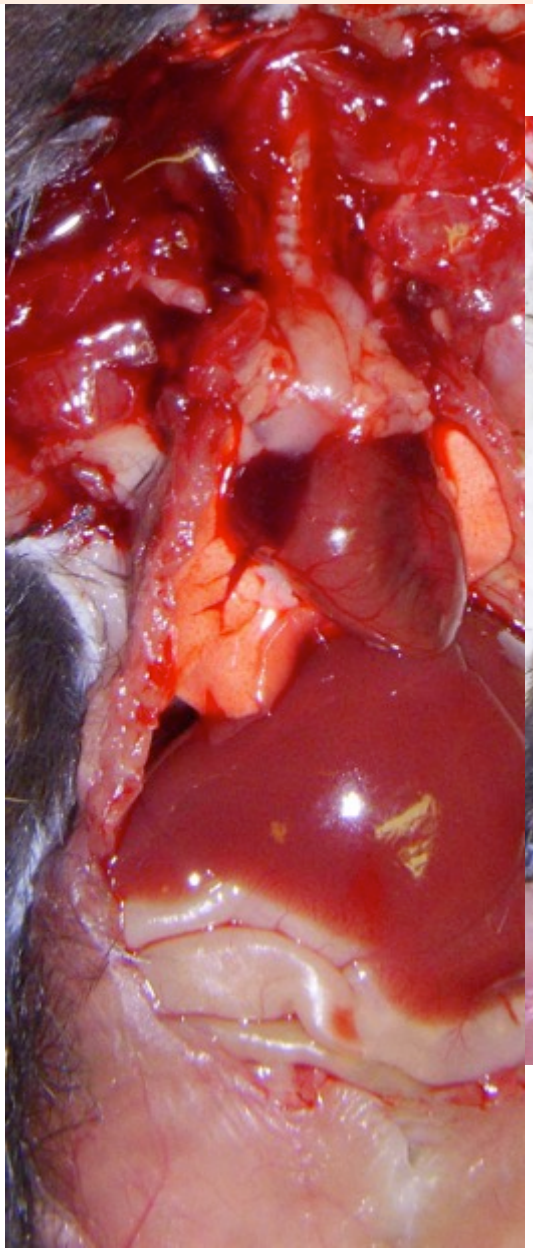
Examples of Human Mouse Differences: in blood counts

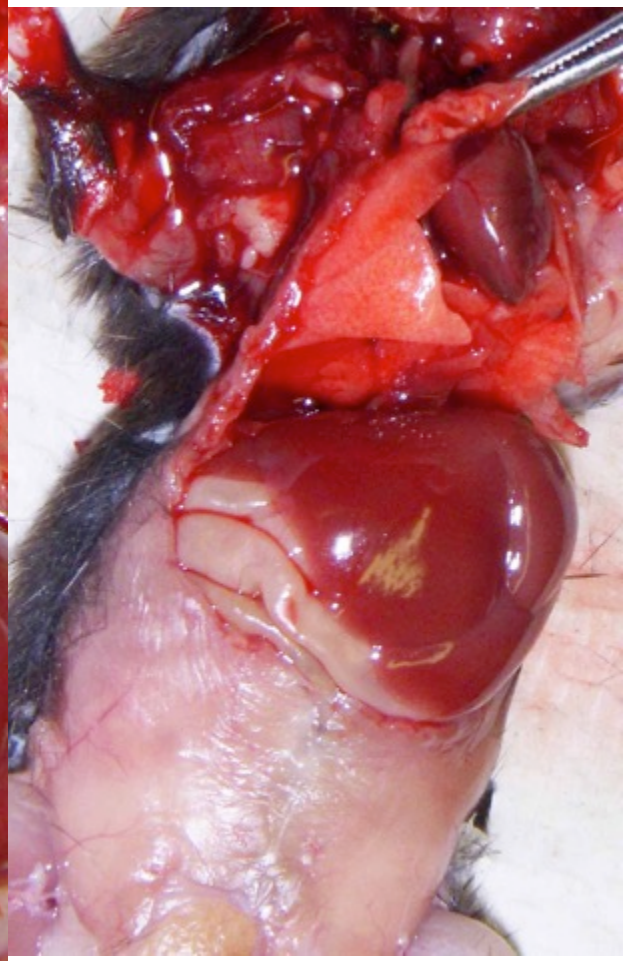
	Human	Mouse
Red blood cell life span	120 days	43 days
White blood cells	Mostly neutrophils	Mostly lymphocytes
Spleen		Abundant megakaryocytes
Markers	CD markers	Different names

A few of many differences	Human	Mouse
Brain	Gyri/sulci 	Lissencephalic brain 
Tonsil	Yes	No
Lungs	3 right lobes 2 left lobes	Many right lobes 1 left lobe
Stomach	Glandular	Squamous + glandular
Colon		Proximal/distal difference
Cecum	Merges	large
Appendix	Yes	No

A few of many differences	Human	Mouse
Liver		Many lobes
Kidney glomeruli		Gender difference
Seminal vesicles		Prominent
Uterus		Bi-cornuate
Ovary		Several follicles develop
Placenta	Distinct	Different
Brown fat	Not prominent	Prominent
Adrenals		Gender difference
Salivary glands	3 separate sites	3 grouped together, with gender difference

Mouse Lungs collapse on opening the thorax--Un-inflated lungs cannot be examined accurately by microscopy





Identify the trachea (shiny cartilagenous rings)

And insert a blunt needle

And INFLATE the lungs with

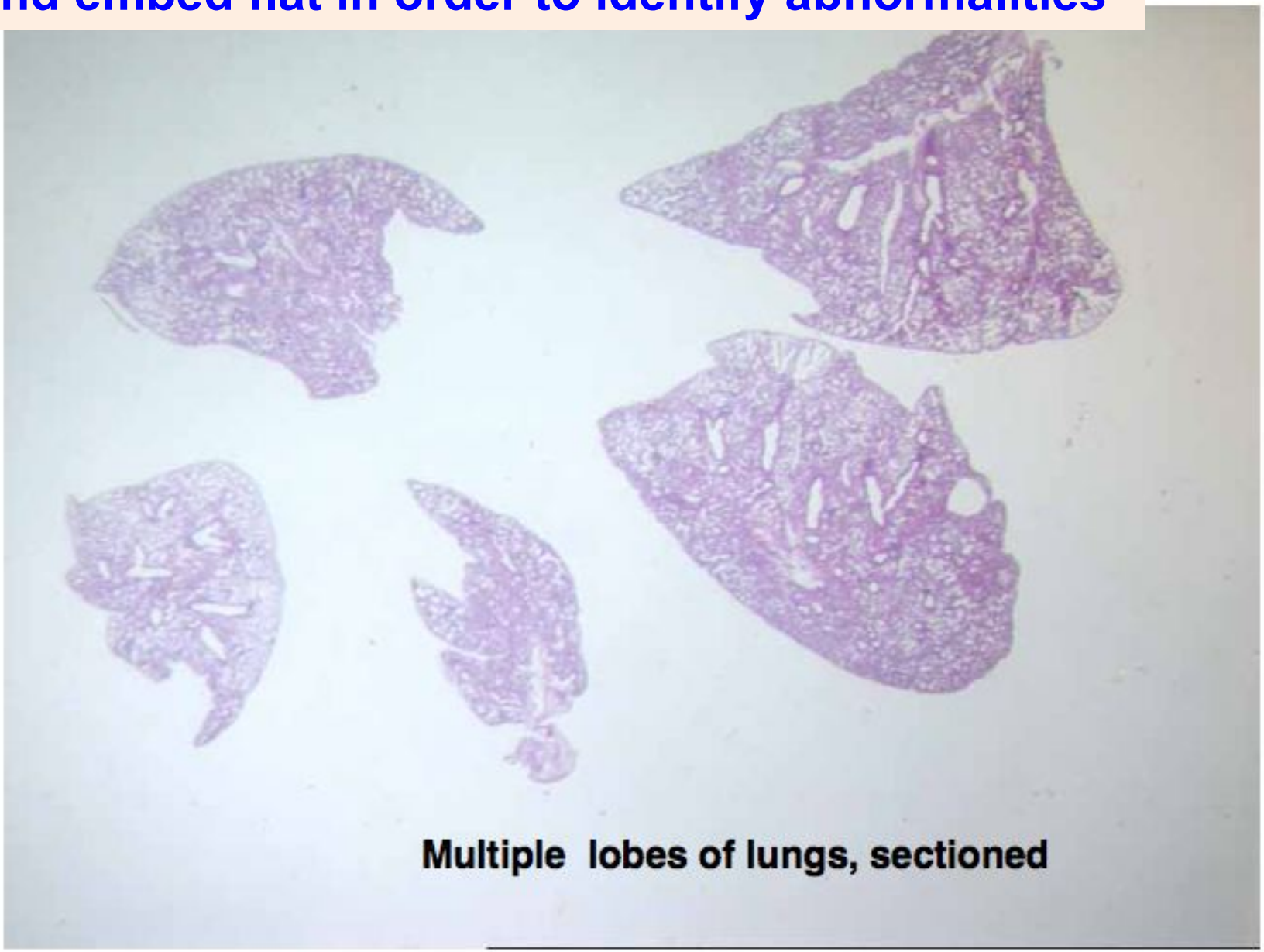
OCT:PBS 1:1 to

FREEZE for use as frozen sections in immunohistochemistry

Or

Inflate with fixative and and transfer to 70% alcohol for processing, embedding and paraffin sectioning

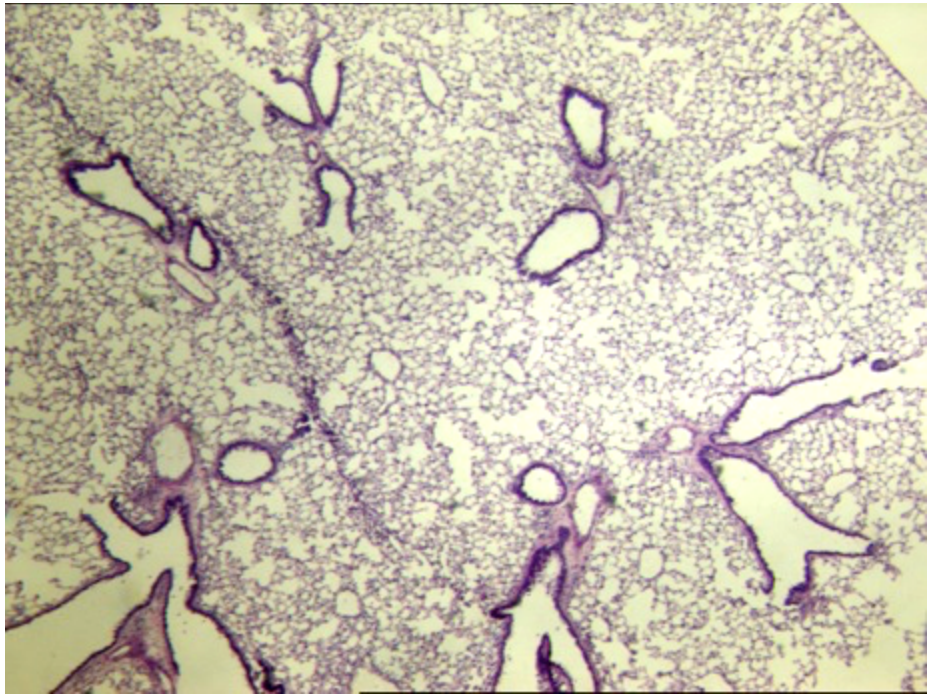
Separate out each of the mouse lung lobes and embed flat in order to identify abnormalities



Multiple lobes of lungs, sectioned

Examples of mouse lung sections

Well inflated

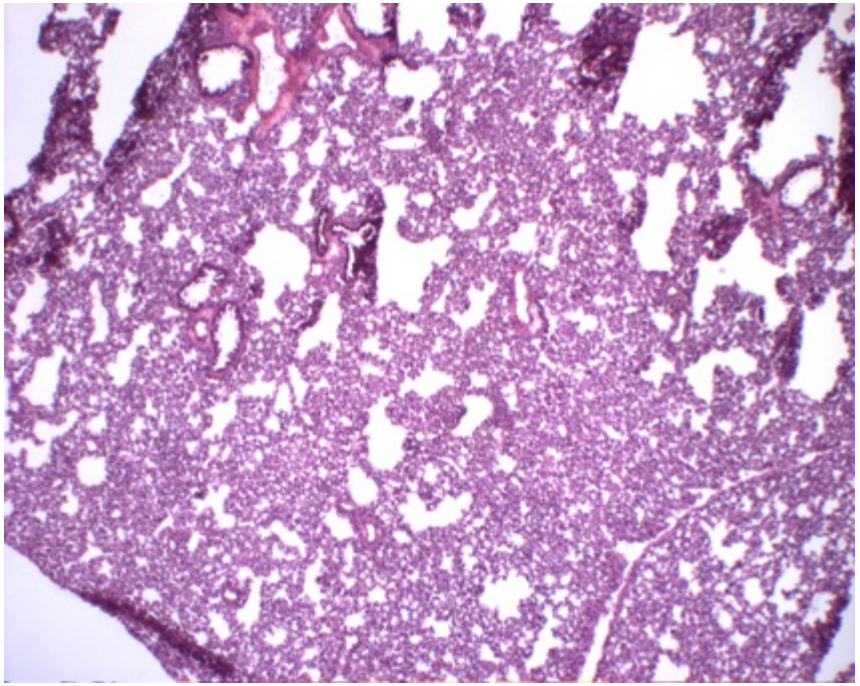


OCT infiltrated lung prior to freezing

Frozen section

Good morphology

not inflated

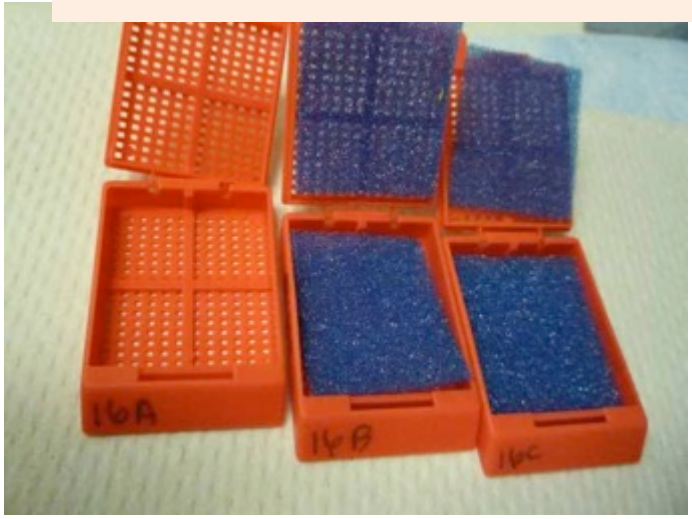


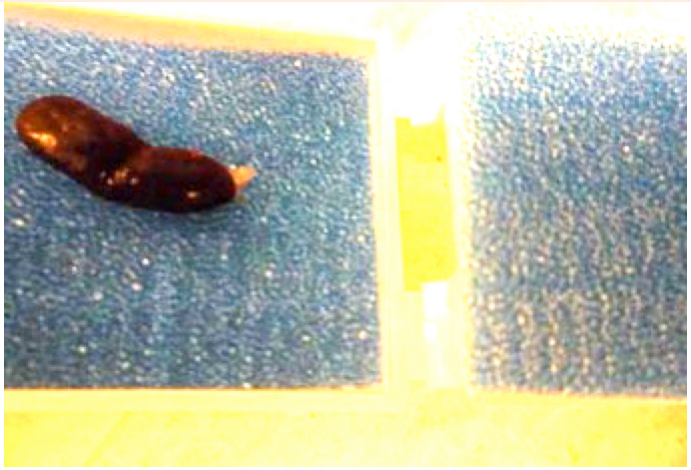
non-OCT infiltrated lung,

Frozen section,

poor morphology

Mouse organs especially spleen are small and delicate and have to be handled carefully





It is important to determine whether the spleen undergoes Fixation (flat between sponges)

Or

Whether it is cryo-protected for correct freezing for immunohistochemistry

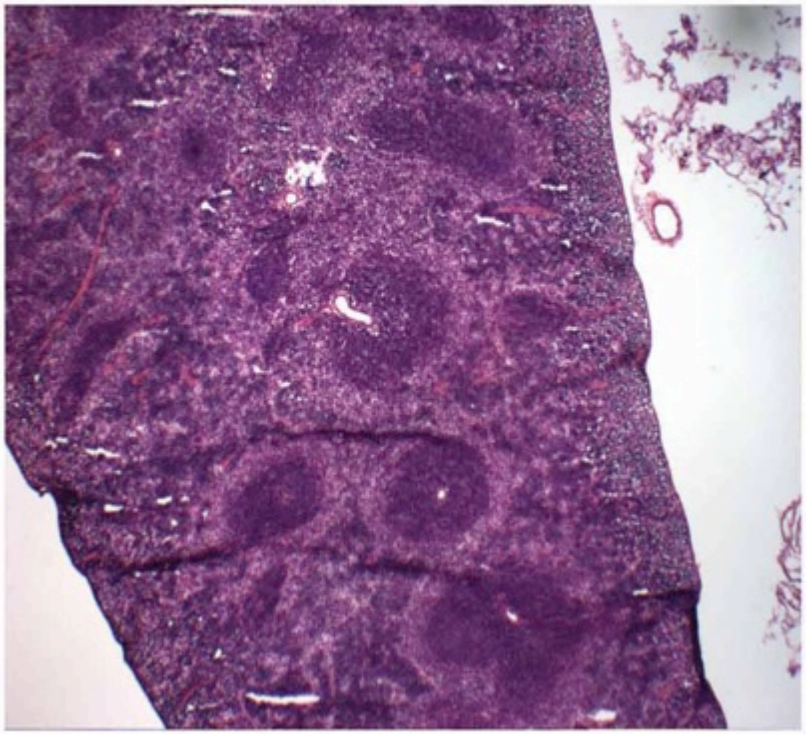
Or

just placed in the freezer for extracts

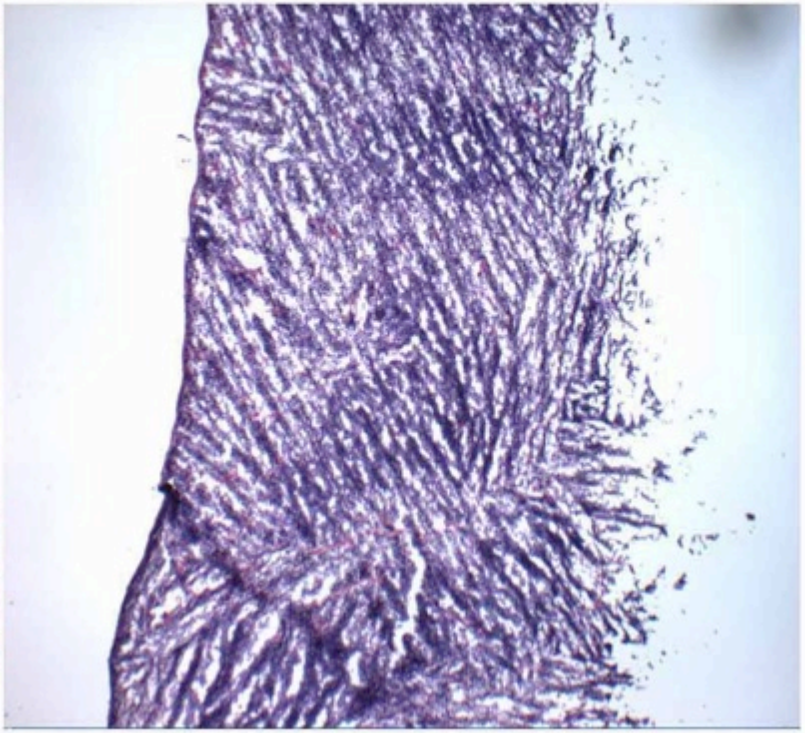
All organs need cryoprotection before freezing, for microscopic examination by frozen sections, to prevent freeze artefact

Frozen sections of mouse spleen with H&E to review morphology

OK to use



freeze artefact, do not use



Tissues that are removed from the body have to be processed correctly for histology

Frozen tissue: Using specific freezing protocols
Snap-freeze tissue which is then stored at minus 80
Use the cryo microtome or cryostat
To do frozen sections

Fixed Tissue: in 10 volumes of fixative for 24 hours and then transfer to 70% alcohol
For processing and embedding into paraffin wax for storage at room temperature
To cut paraffin sections

Processing of tissue :

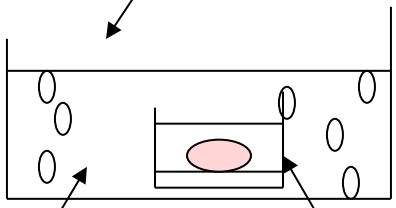
Isolate cells for culture

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

Process for EM

Immerse this sections in appropriate fixative to Process into paraffin blocks

Freeze for use in immunohistochemistry

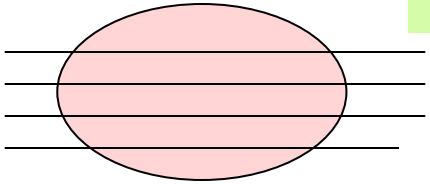


Dry ice in 2-methyl butane

-Fix thin slices in correct fixative
-Dehydrate in graded alcohols
-Infiltrate with xylene
-Infiltrate with hot paraffin wax
-Make blocks for sections
-Store at room temperature
-Deparaffinize sections by
-removing wax in xylene,
- rehydrate in decreasing concentrations of alcohol to water

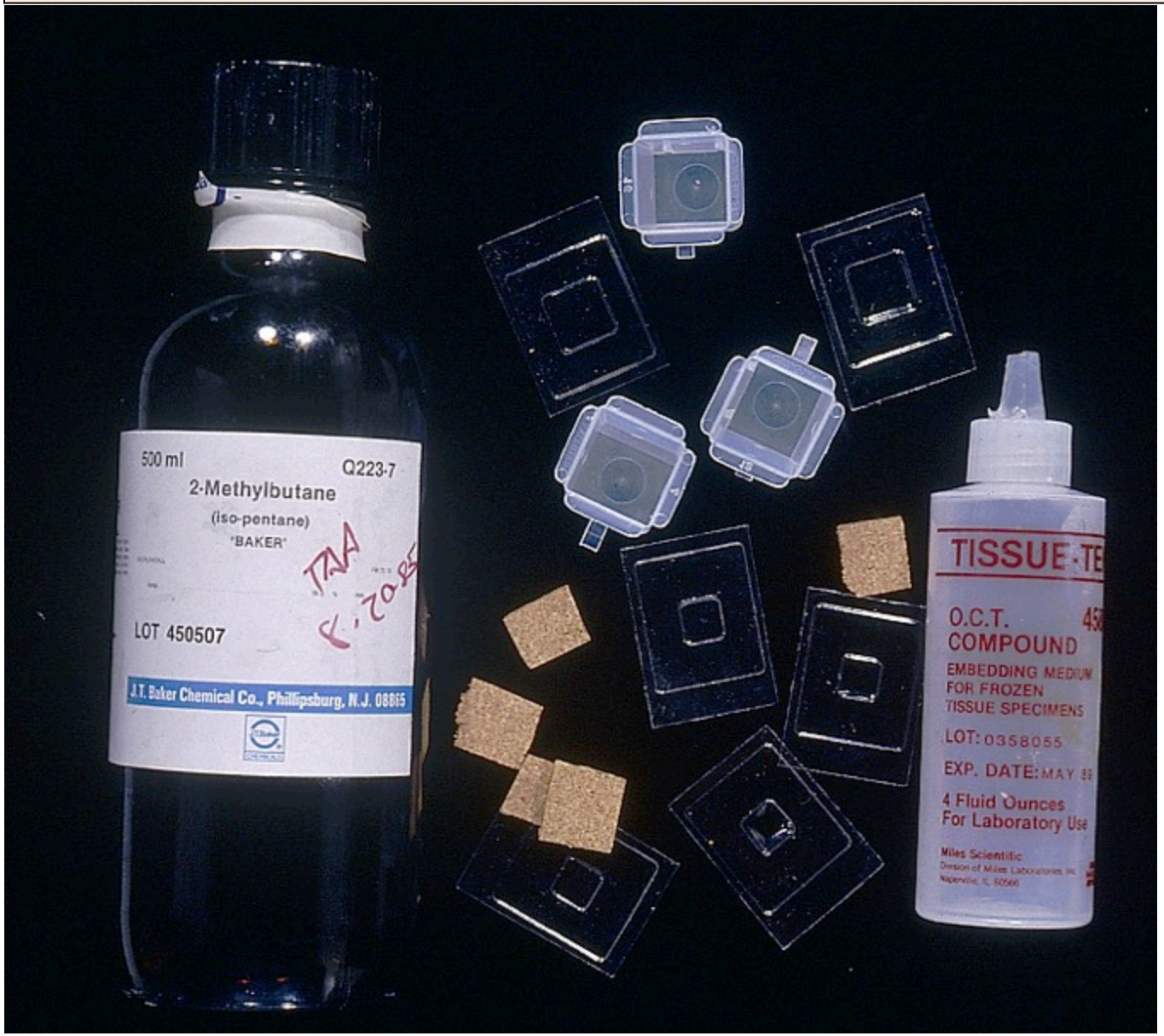
OCT surrounds fresh tissue in plastic mold

Frozen for histology



Frozen tissue or Fixed Paraffin-embedded tissue can then be sectioned for histology into 3--30 micron sections

Materials needed for flash freezing tissue for histology

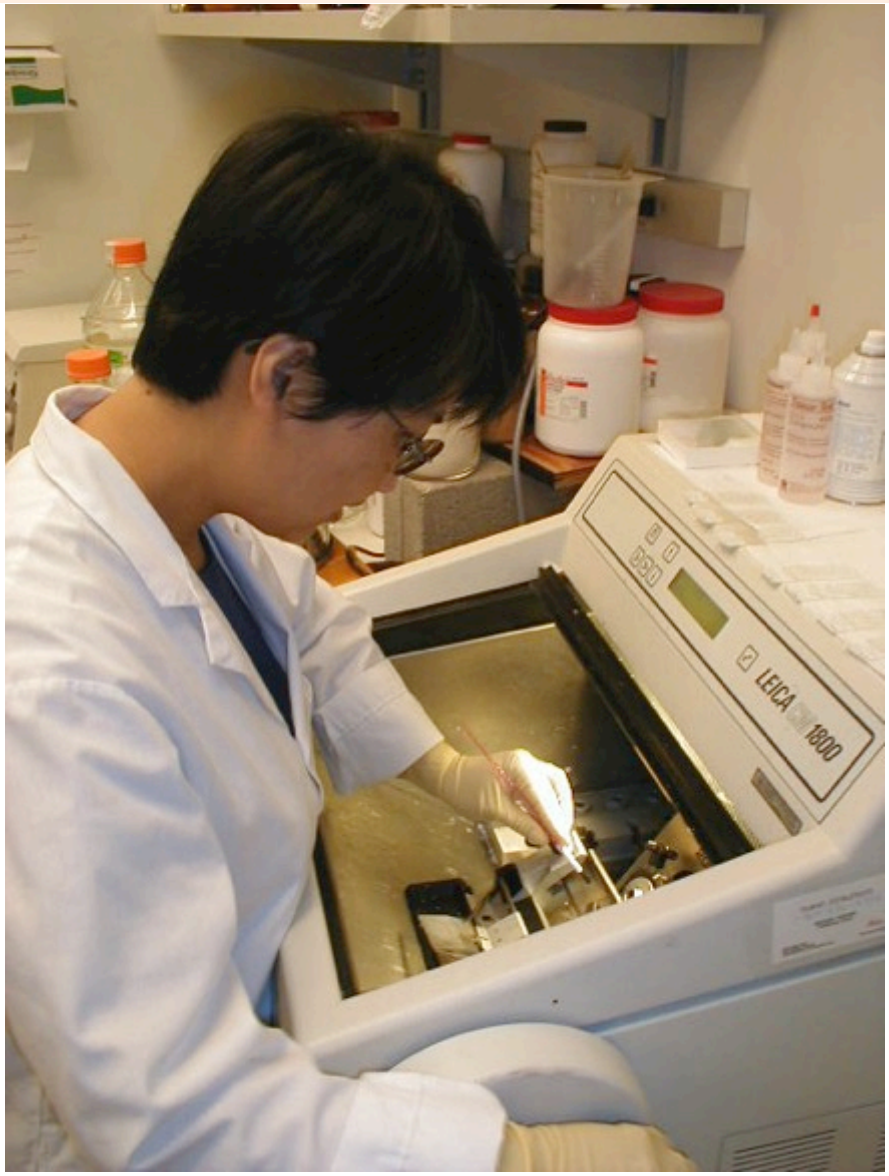


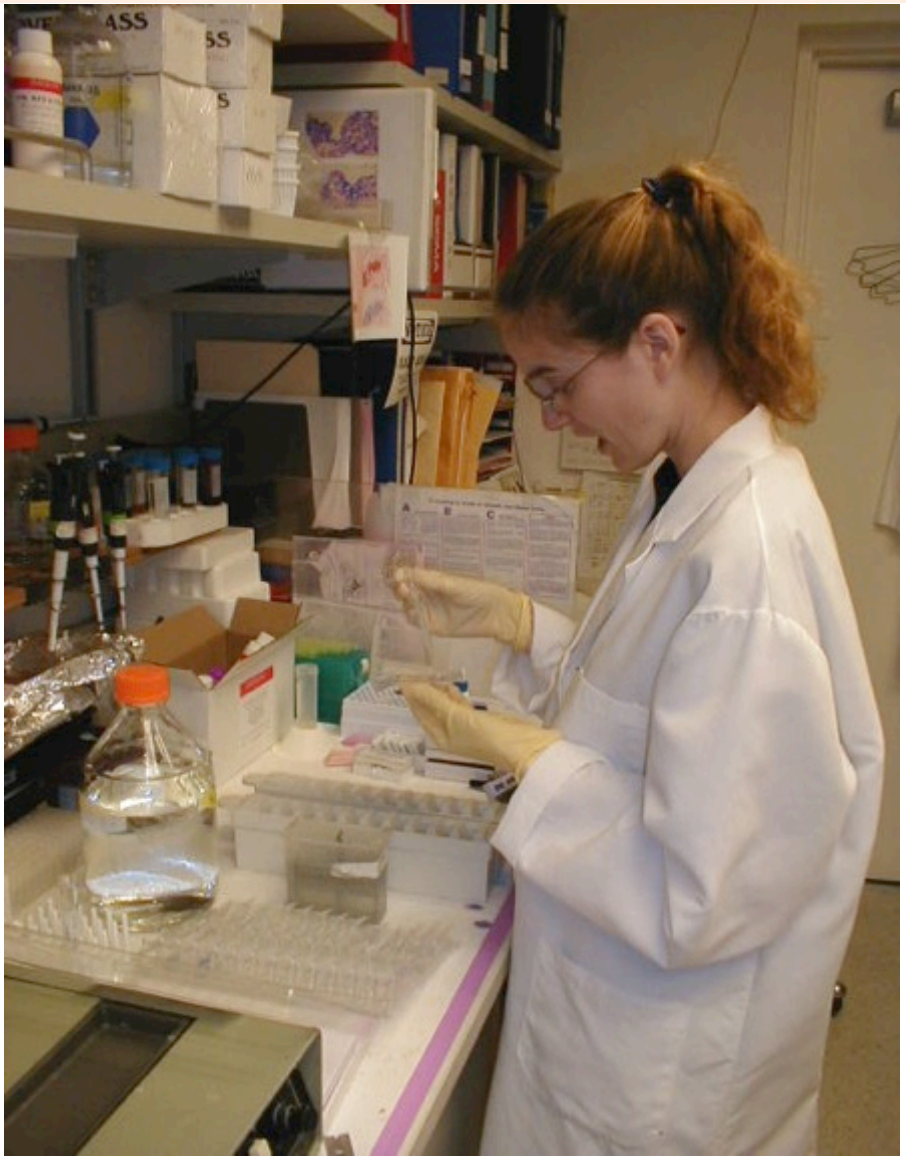
Video of freezing technique

www.mousepheno.ucsd.edu

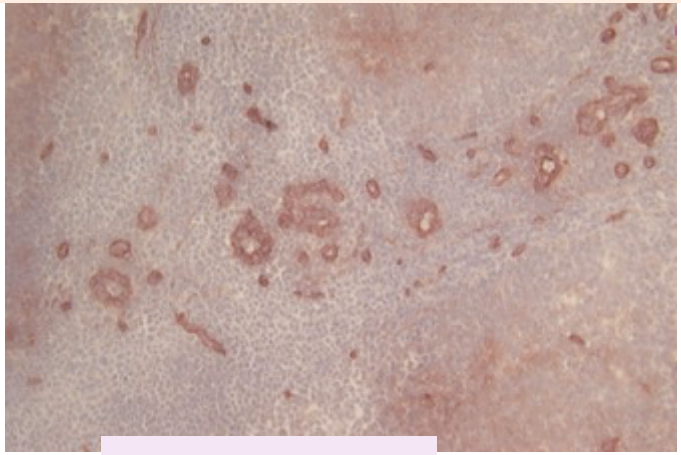
http://mousepheno.ucsd.edu/movies/freezing.MOV

IMMUNOHISTOCHEMISTRY ASSAYS best on frozen sections but paraffin sections may also be used

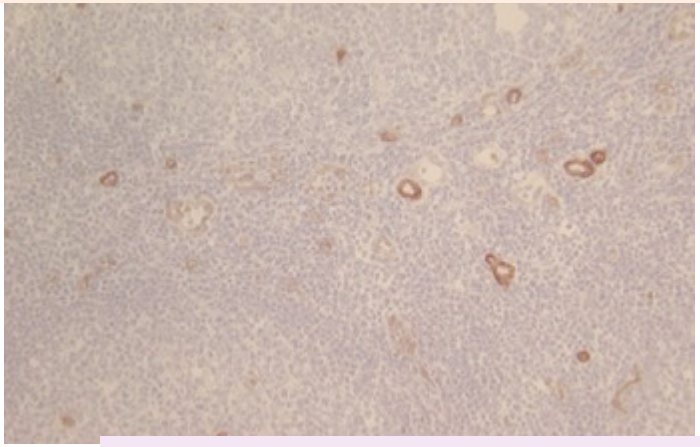




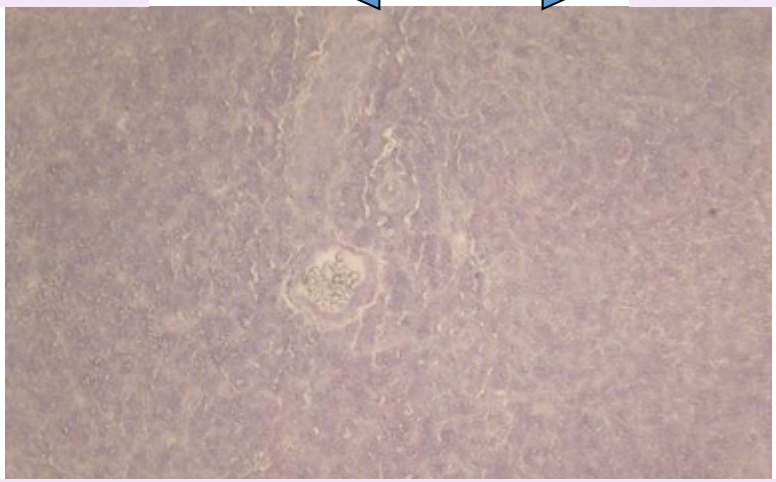
Caveat: Effect of different fixatives on preserving epitopes in frozen sections



Acetone fixed



Paraformaldehyde fixed



If the tissue is paraffin embedded, some mouse monoclonals do not recognize the epitope, in spite of using retrieval techniques

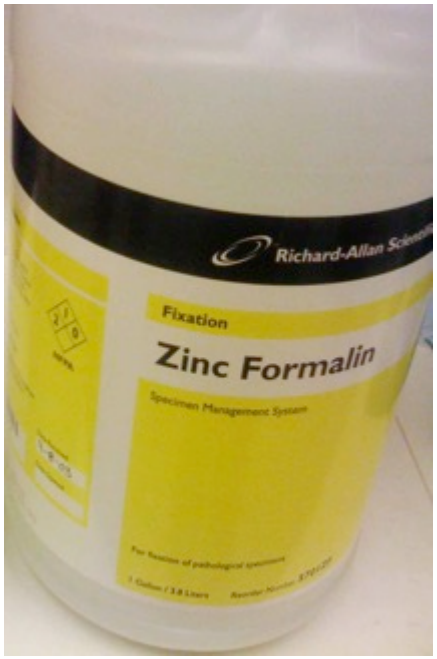
FIXATIVES

- Fix Thin slices of tissue, or inflated lungs, or tissue in sponges
- use 4% freshly made paraformaldehyde for 24 hours before immersion in 70% alcohol to submit to histotech
- Or 10% buffered formalin for 24 hours before immersion in 70% alcohol to submit to histotech
- Or 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
- Or Zinc containing fixatives, preserve epitopes for immunostaining

Four commonly used Fixatives for tissue processing in histology

10% Neutral buffered formalin

4% paraformaldehyde is made fresh in the fume hood before use



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.

Use 10 volumes of fixative for each samples, overnight in labeled cassettes, before transfer to 70% alcohol for processing embedding and sectioning, staining for microscopic analysis

Make 4% paraformaldehyde in the chemical hood with heating and with NaOH and PBS, cool and freeze in aliquots

Add 4 g paraformaldehyde

Wear a mask and be careful while weighing it out, it disperses easily

To 50 ml of water

Heat to 65 degrees

Add 6 ul of 10M NaOH

Solution will clear

Filter via Whatman paper

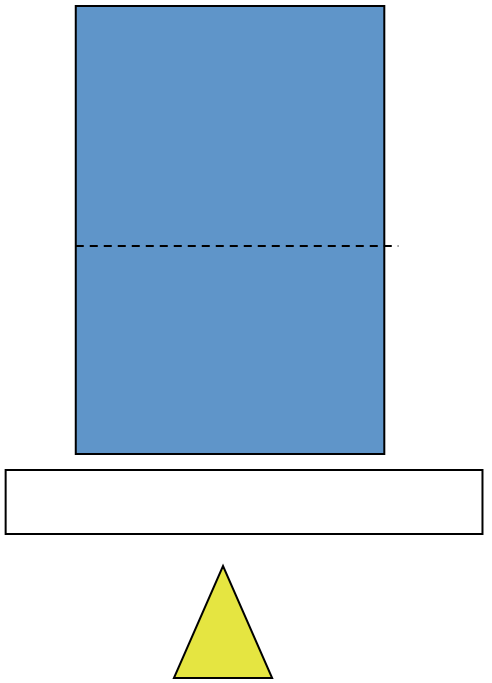
Add 10 ml of 10X PBS

Make up volume to 100 ml

Store at 4 degrees for upto 24 hours

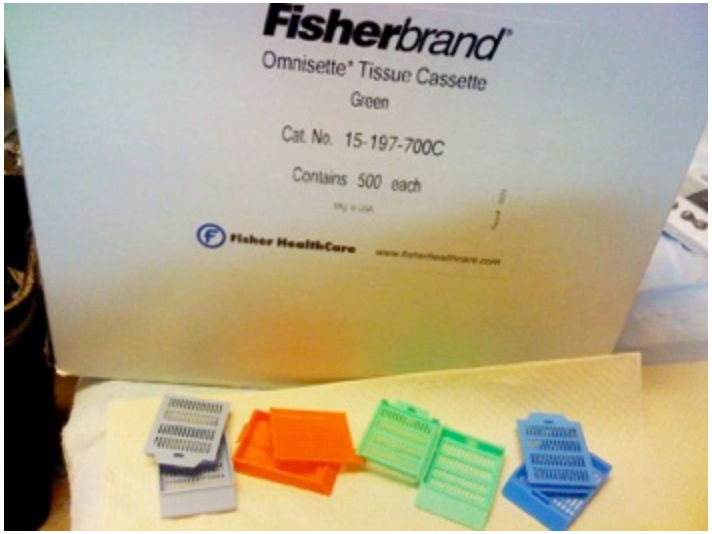
Or freeze in aliquots

In Chemical Fume hood



Use simply labeled cassettes, using indelible pencil, to fix thin slices of organs or rolls of intestine, in 10 volumes of fixative, for less than 24 hours, before transferring to 70% alcohol, for processing into paraffin blocks.

Do not use a “Sharpie “ to label cassettes.



Use Sponges in casettes for to flatten certain organs such as: Spleen,Thymus, Pancreas, adipose tissue, skin, small organs such as adrenals, ovaries, lymph nodes

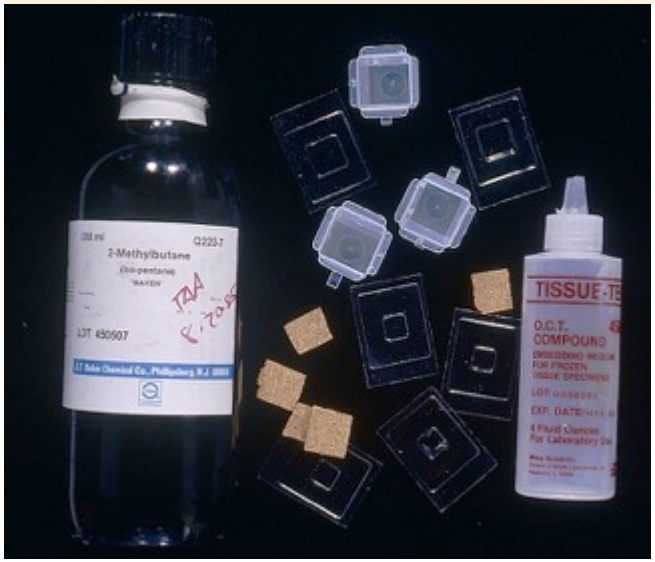
to orient them FLAT for good sections

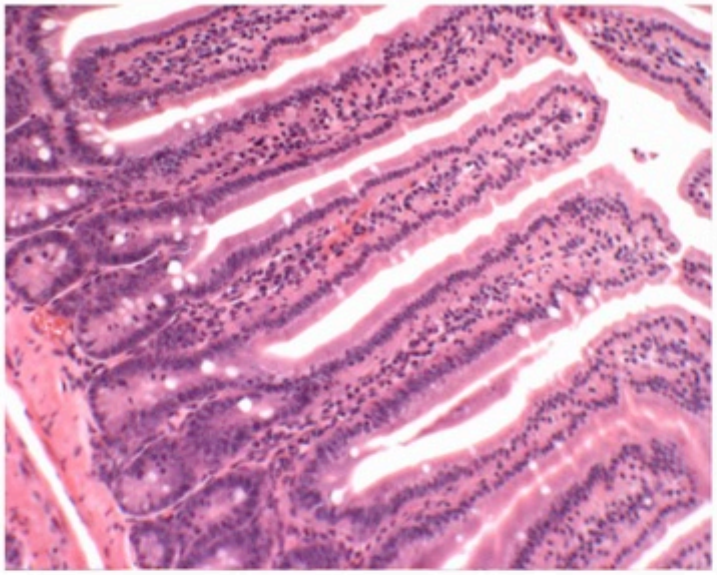
If you need to FREEZE FIXED tissue for histology:

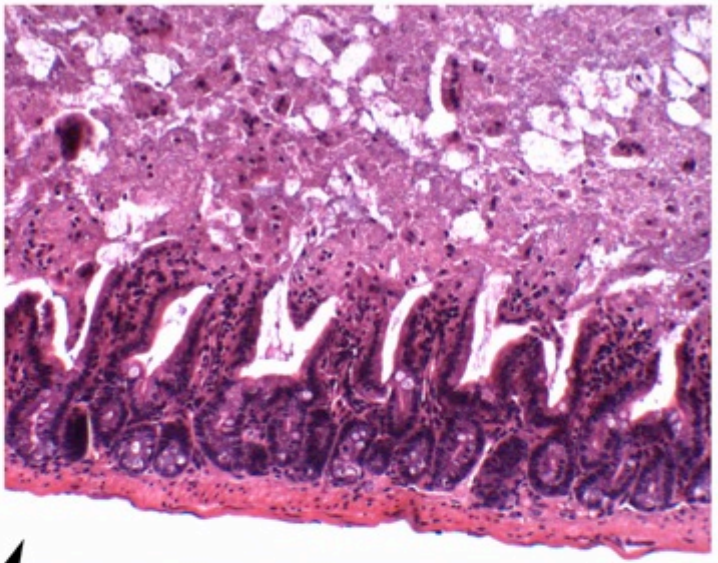
If the animal has been perfusion fixed --the organs have to SINK (Descend to bottom of tube) in 30% sucrose/PBS

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

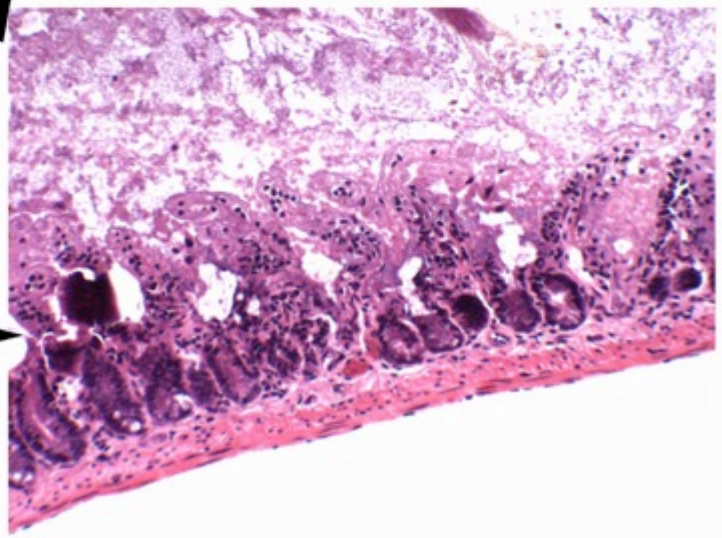






Mouse Small Intestine
Well-fixed, processed, sectioned and stained

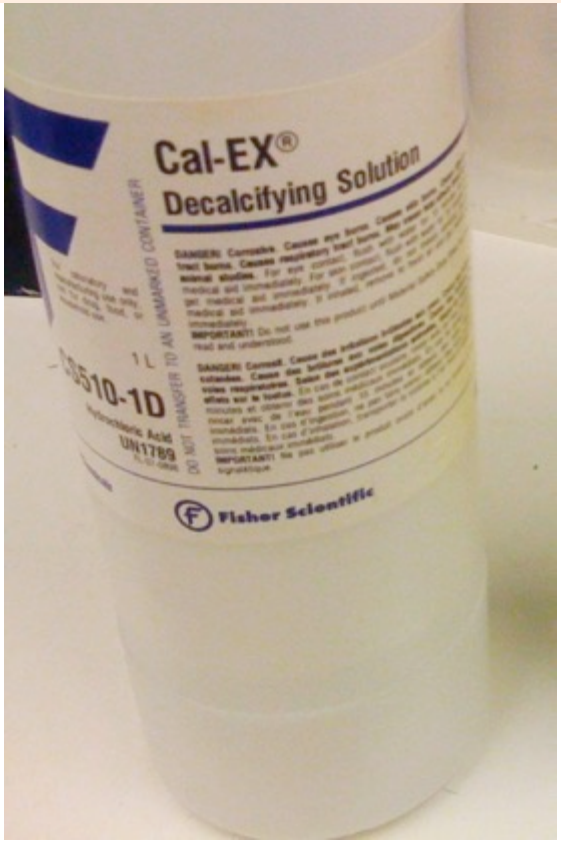
Effects of poor fixation

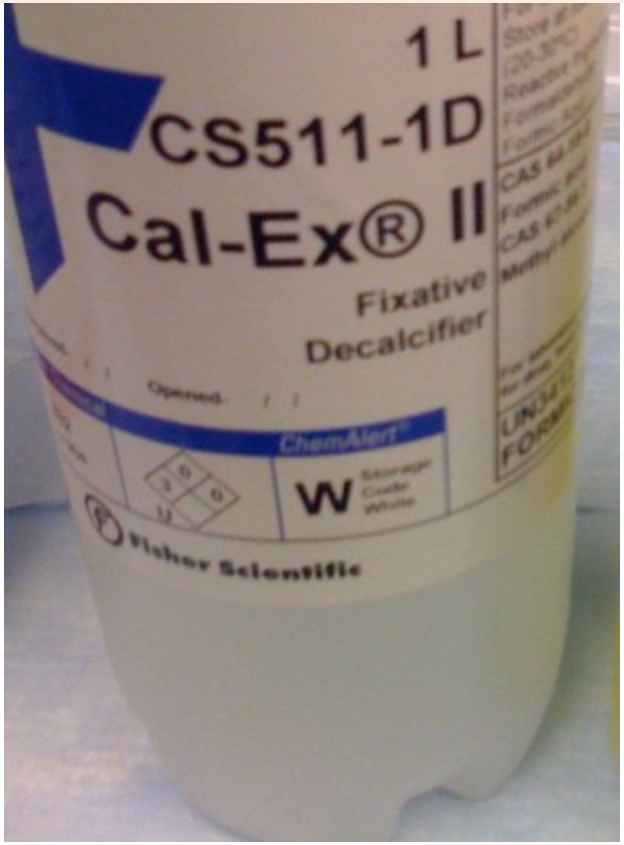


Bones have to be de-calcified after fixation

Decalcification solutions:

HCl; Formalin+ HCl; EDTA only- for slow decalcification for IHC





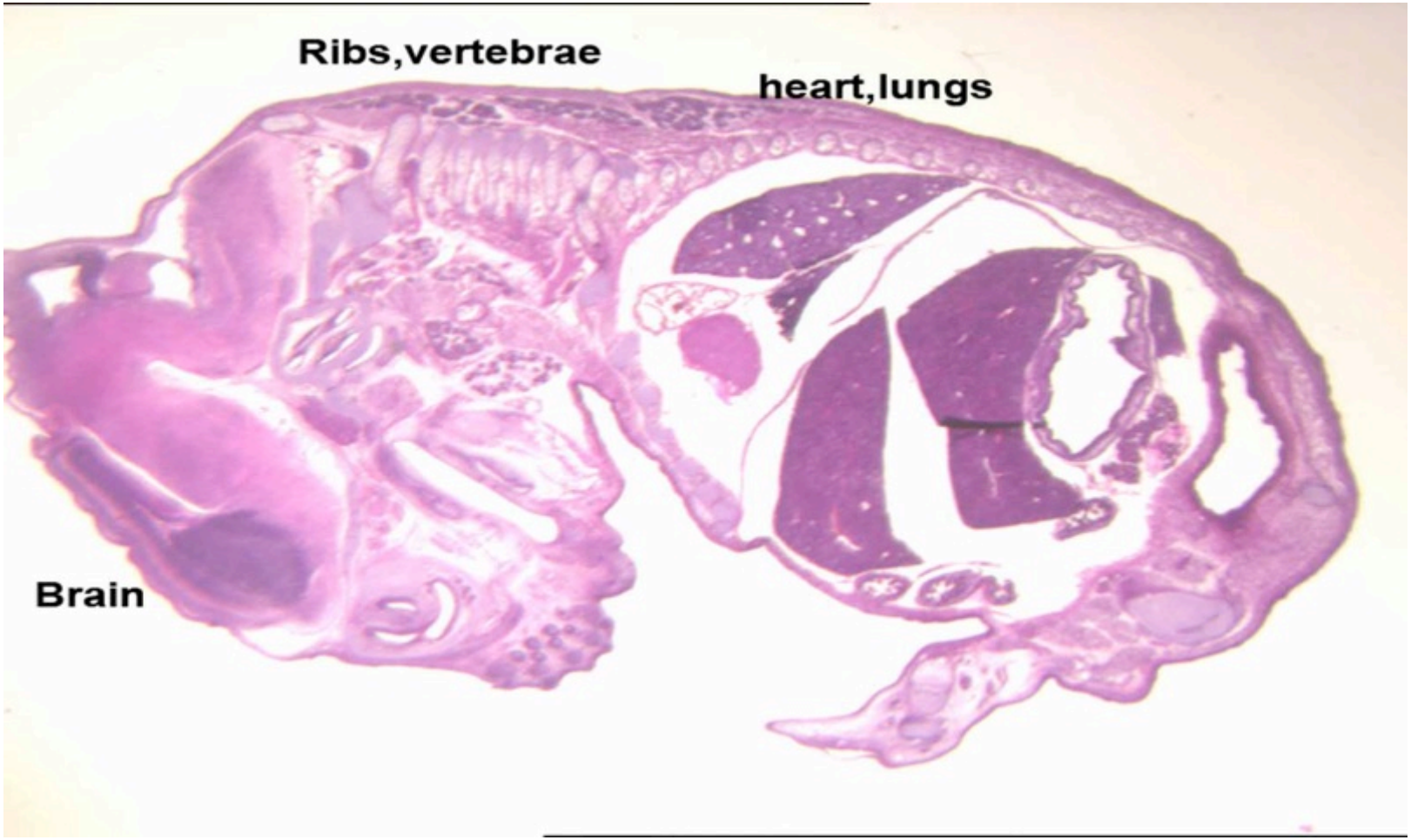
Importance of Orientation of tissues :

Coronal sections

Sagittal sections

Transverse sections

Correct orientation to gain the most information during histopathologic examination, an example of a section of mouse embryo



HEMATOXYLIN AND EOSIN STAINS

H&E= hematoxylin and eosin.

Hematoxylin colors nuclei blue

Eosin colors the cytoplasm pink

HISTO: HISTOLOGY SECTIONS FOR VIEWING UNDER THE MICROSCOPE, using BRIGHTFIELD illumination

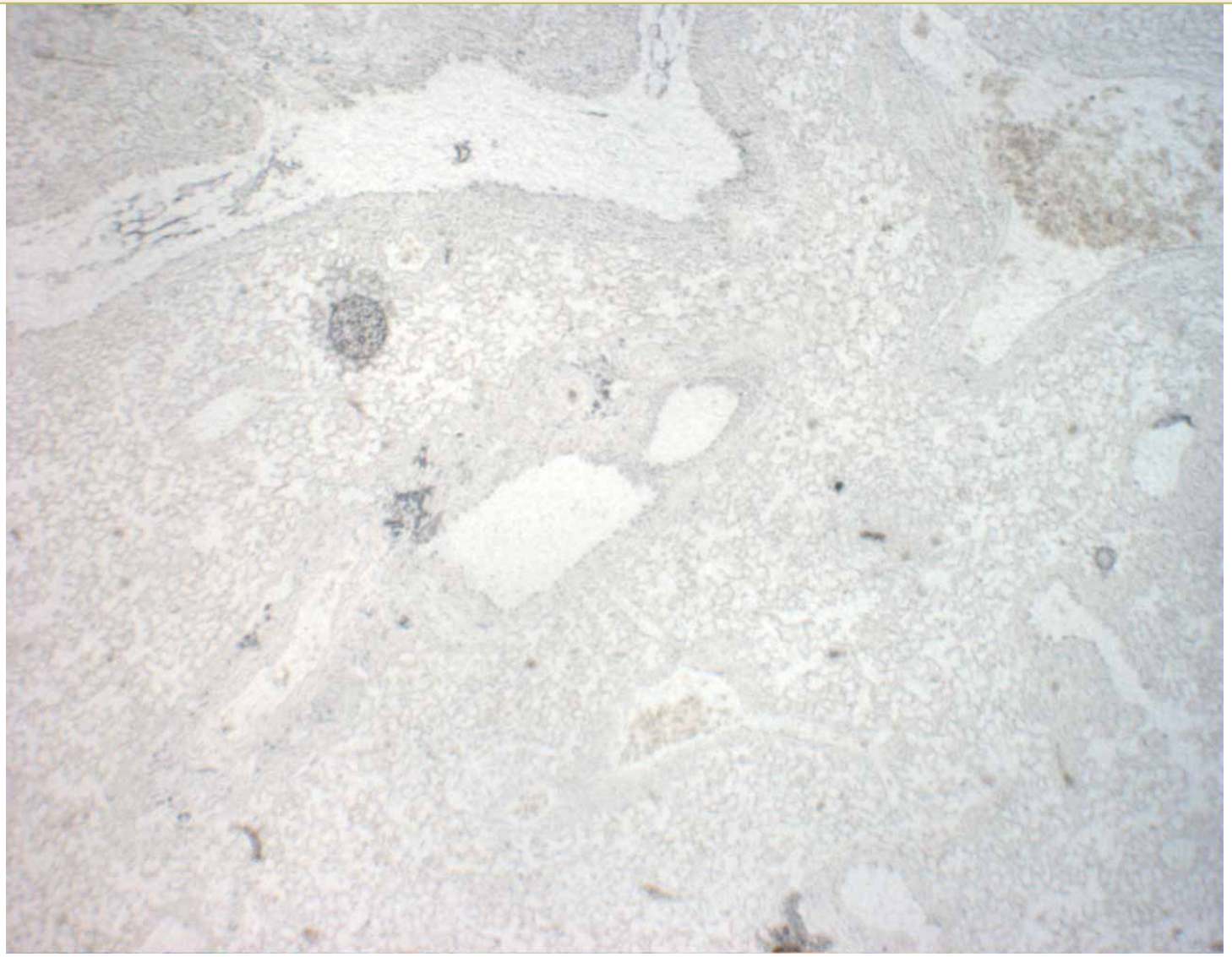
Always review sections using the basic hematoxylin and eosin (H&E) stain before proceeding to perform an immunohistochemical assay

in order to check out the morphology of the tissue and to determine

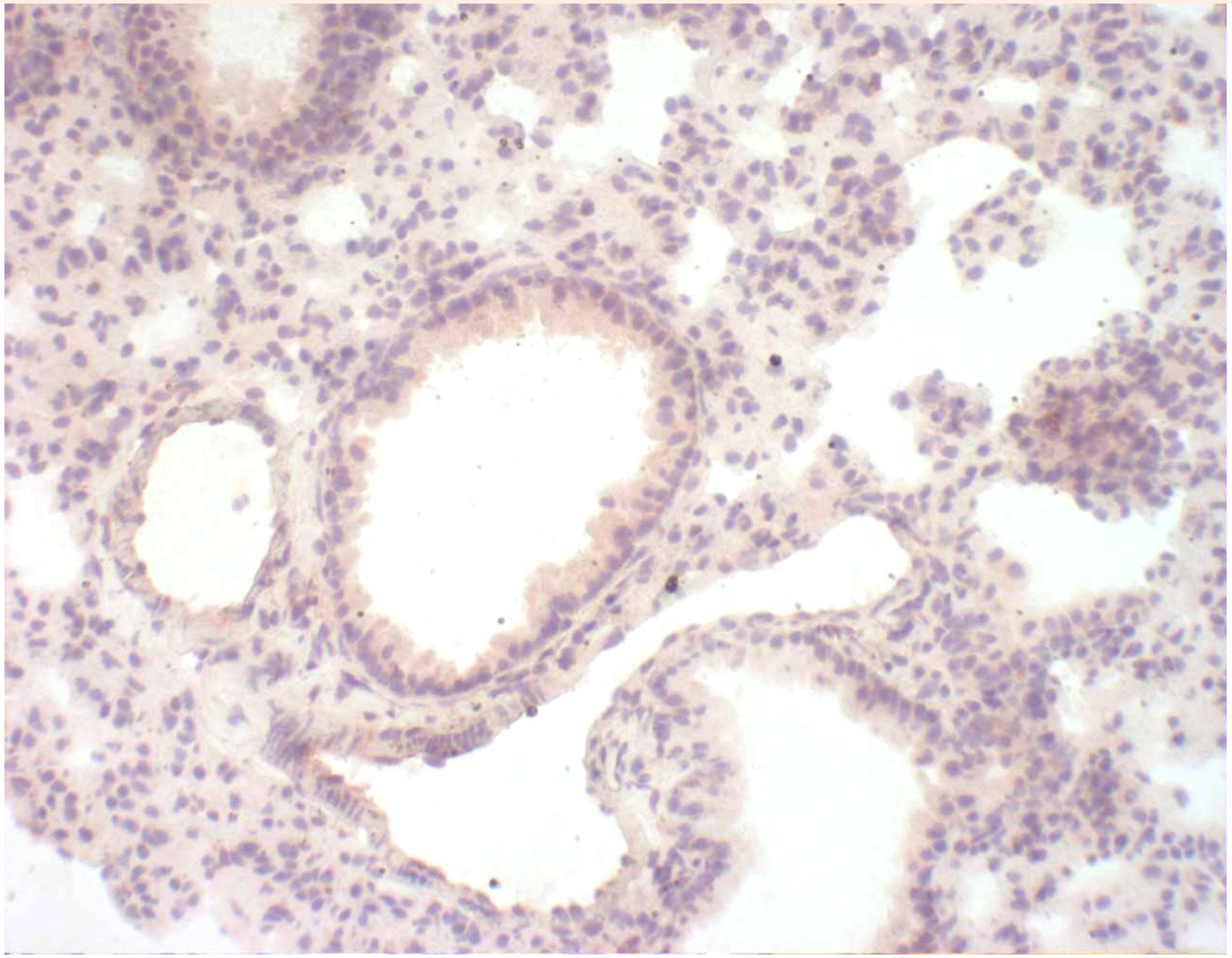
that what you are looking for is present in the section to be immunostained

and that the section has no other abnormalities

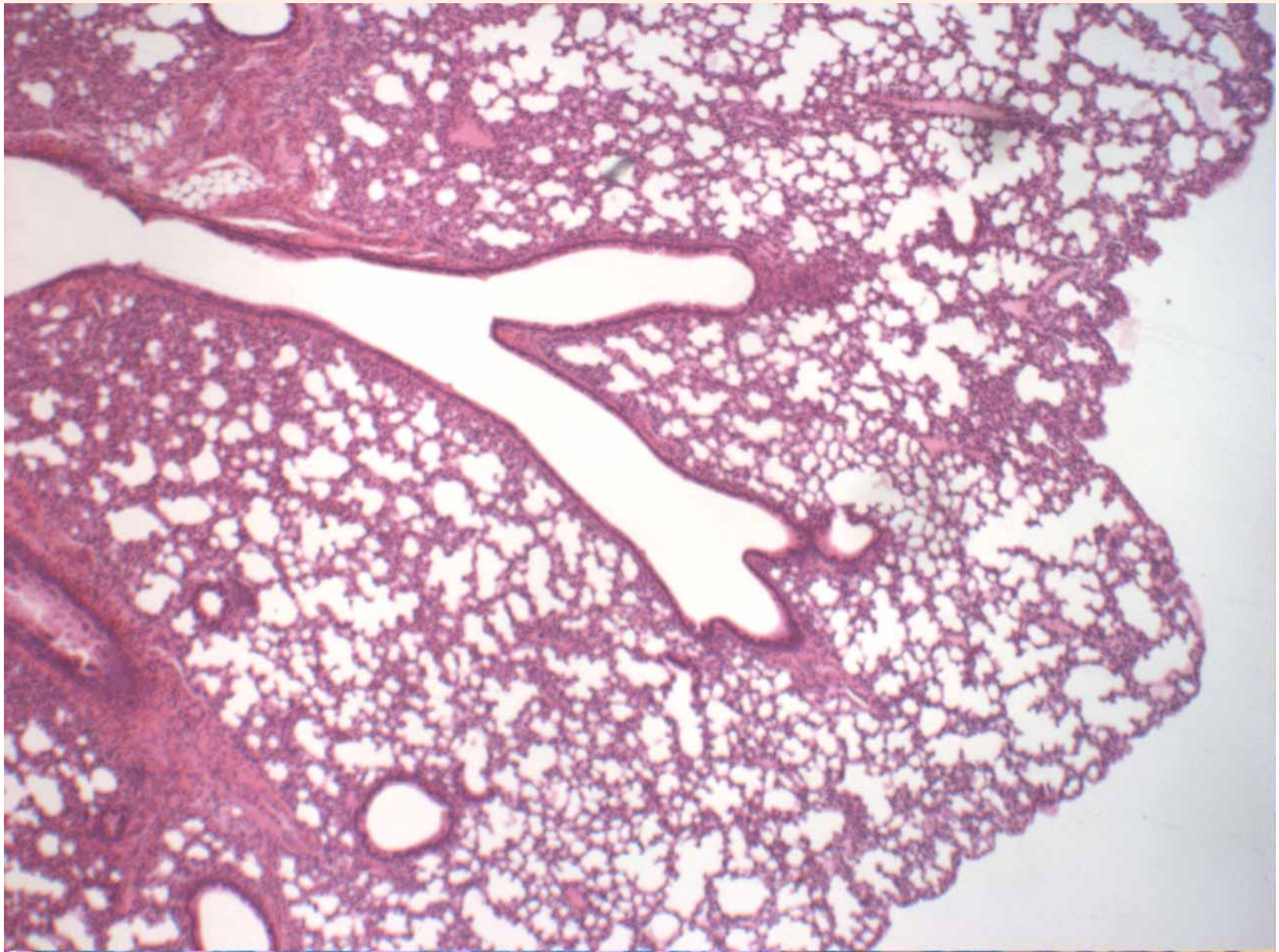
This is a photo of an unstained section on a slide, which needs histochemical stains to help with identification of the tissue



An example of a section of Mouse lung frozen section stained only with hematoxylin

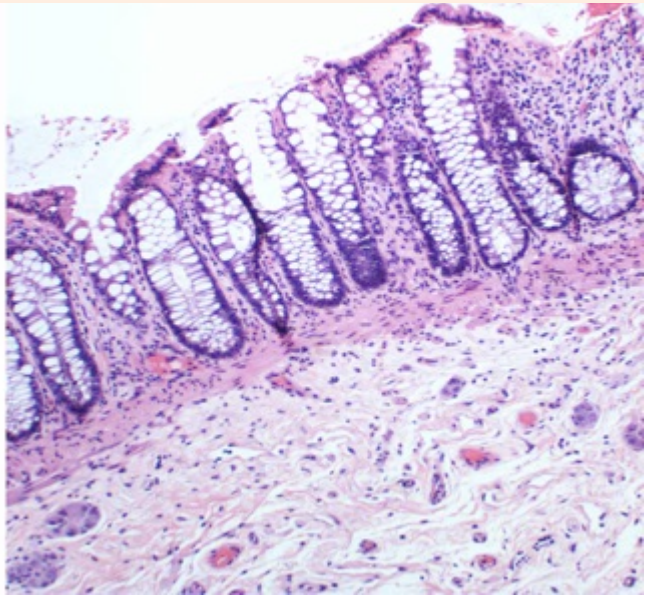


An example of a section of Mouse lung stained with Hematoxylin and Eosin to demonstrate morphology

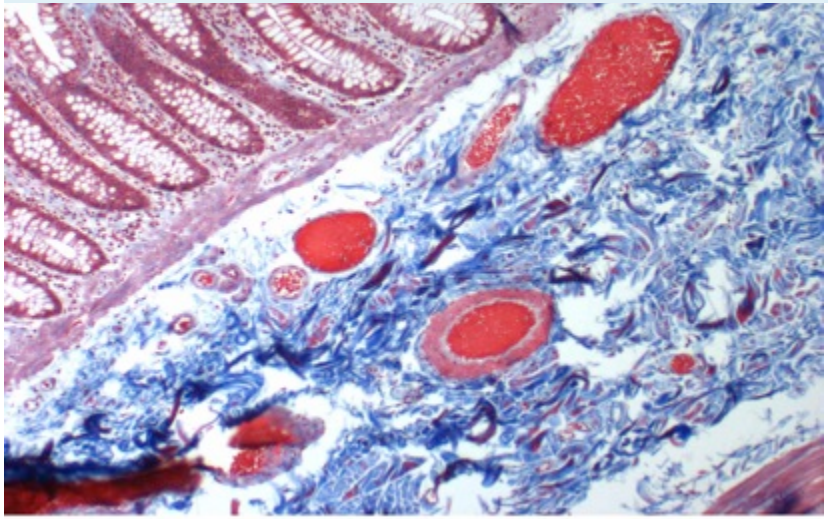


Examples of Different Histochemical stains to demonstrate different components in a section of Human Colon

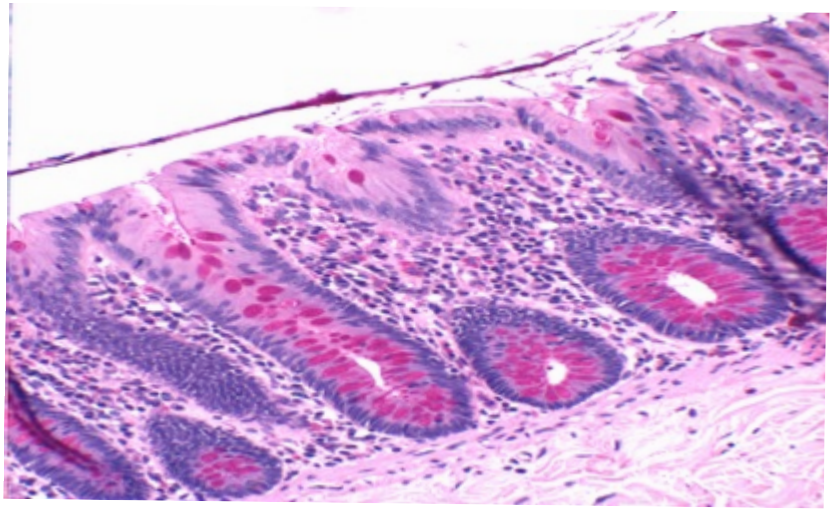
H&E is standard to assess morphology



A Trichrome stain to demosntrate collagen (blue)

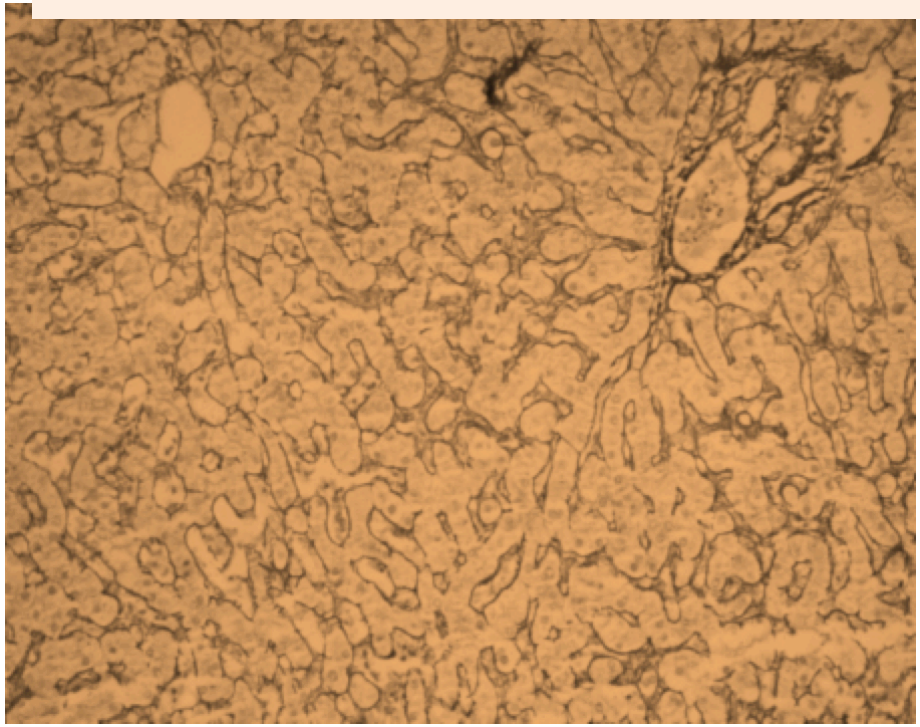


PAS (periodic Acid Schiff) for carbohydrates—here demonstrated fuschia colored mucin in goblet cells of colon epithelium

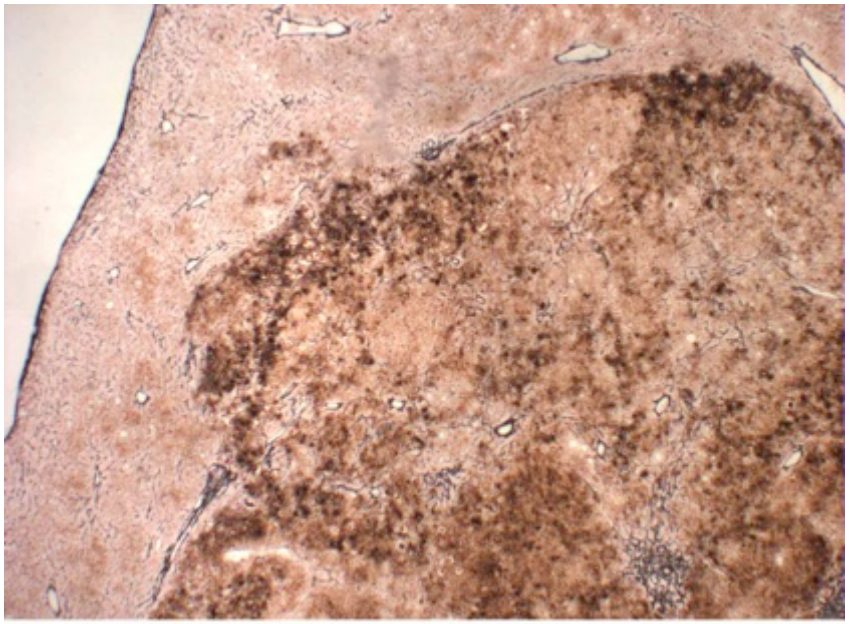


Reticulin stain to highlight supporting support

Normal mouse liver



Liver with invading cancer

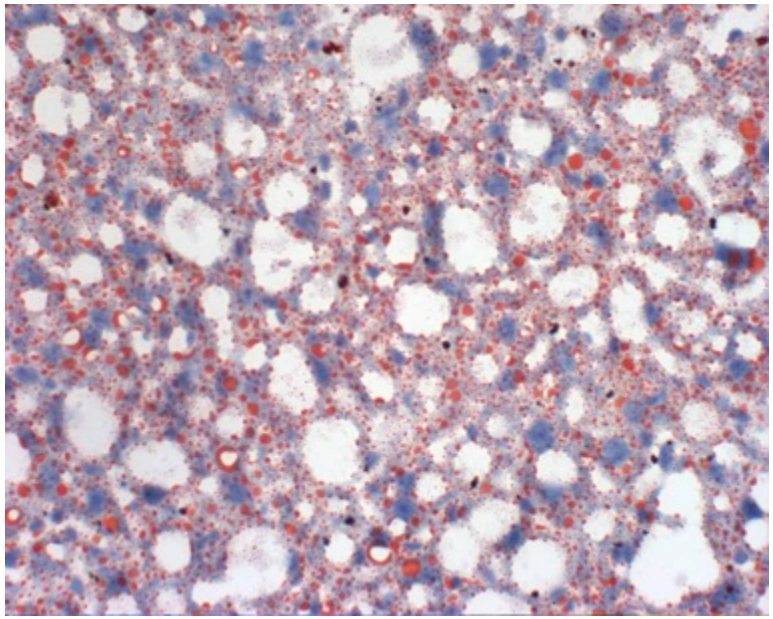


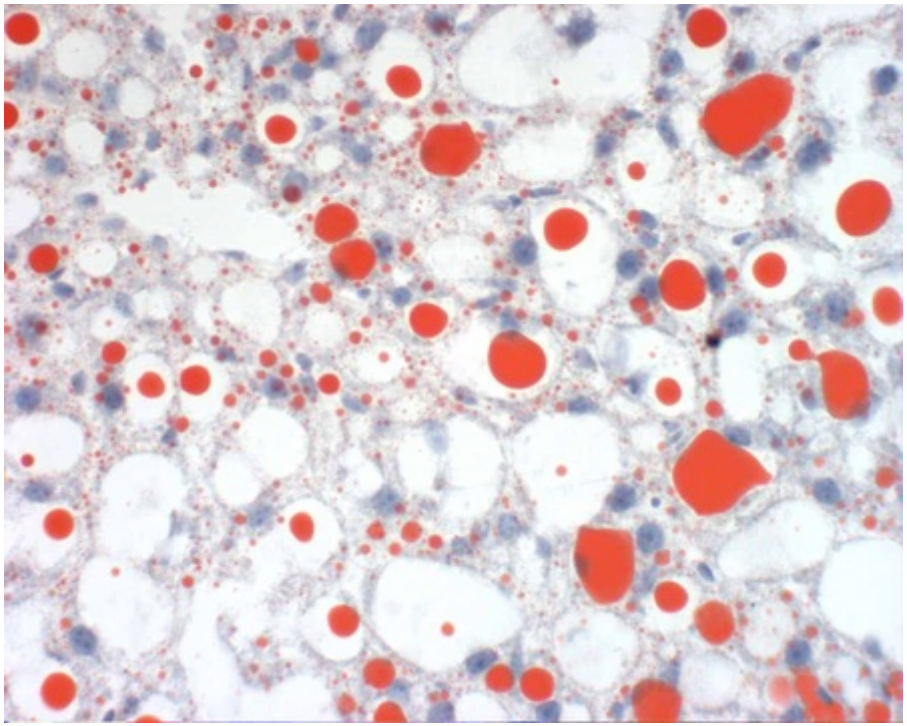
Oil Red O stain of FROZEN section of mouse liver showing

moderate amounts and large amounts

of fatty accumulation in hepatocytes

Control: adipose tissue





Commonly used "Blue" stains in histochemistry:

--hematoxylin: for nuclei

--Trichrome: for collagen and for scarring/fibrosis

--Alcian Blue: for mucin and for cartilage

--Nissl: for nuclei in neurons

--Luxol Fast Blue (LFB): for myelin

Commonly used "Red" stains in histochemistry:

--Eosin: stains cytoplasm and depending in the tissue type, can vary in intensity

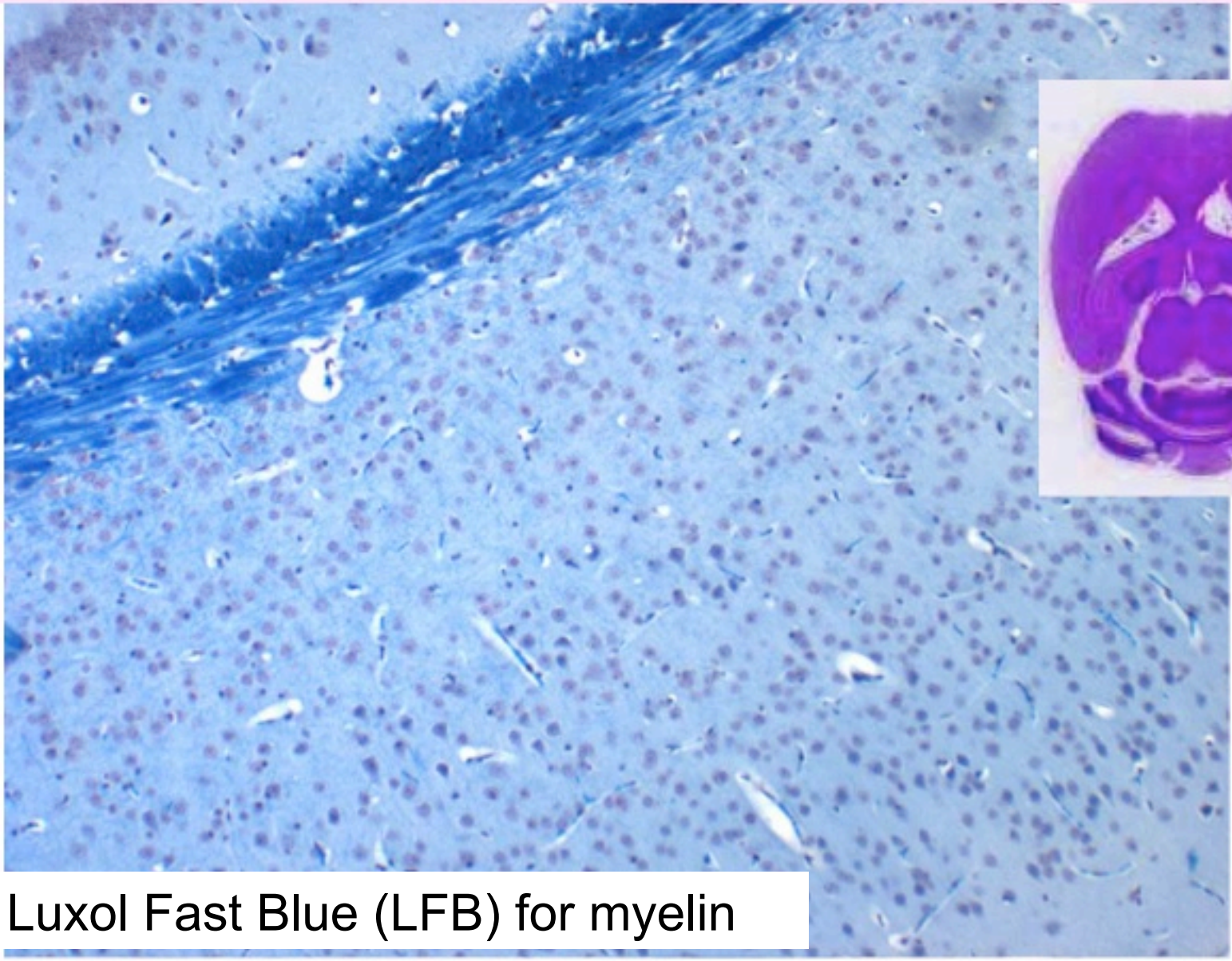
--PAS: Periodic Acid Schiff—for carbohydrate containing material, in mucin and basement membranes

--Alizarin Red: stains bone

--Safranin O: stains cartilage

--Oil Red O—to identify lipid containing cells , has to be done on frozen sections

White Matter paraffin sections of Mouse Brain: Myelin stain



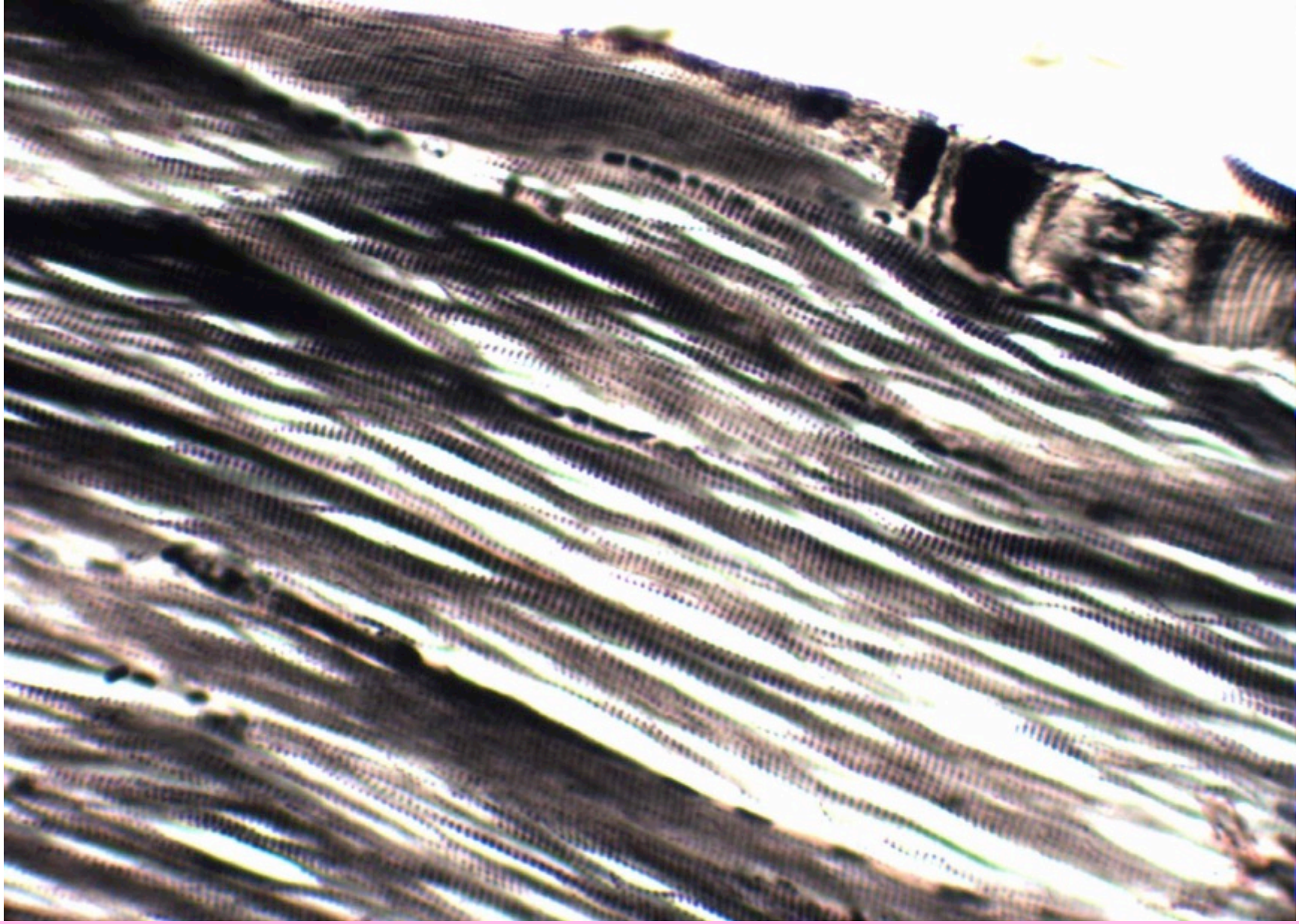
Luxol Fast Blue (LFB) for myelin

Commonly used “Black “ stains in histochemistry:

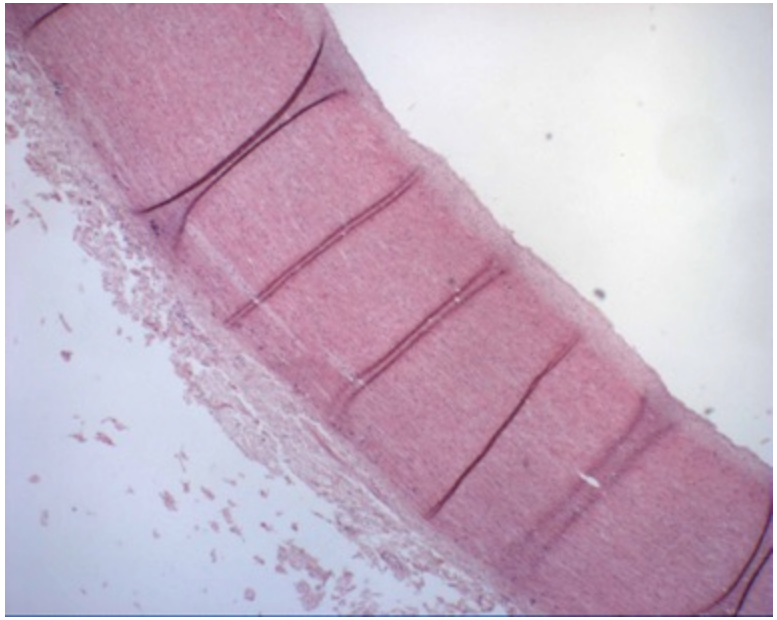
--PTAH: Phosphotungstic Acid Hematoxylin, to identify striations in skeletal muscle and also for collections of abnormal fibrin in clotting disorders

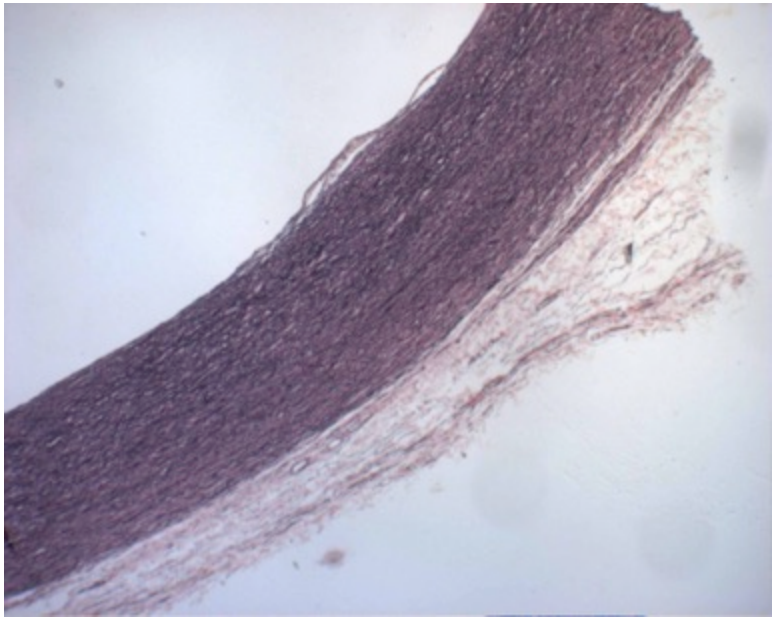
Elastic: to identify elastic fibers

Reticulin: to identify reticulin supporting fibers

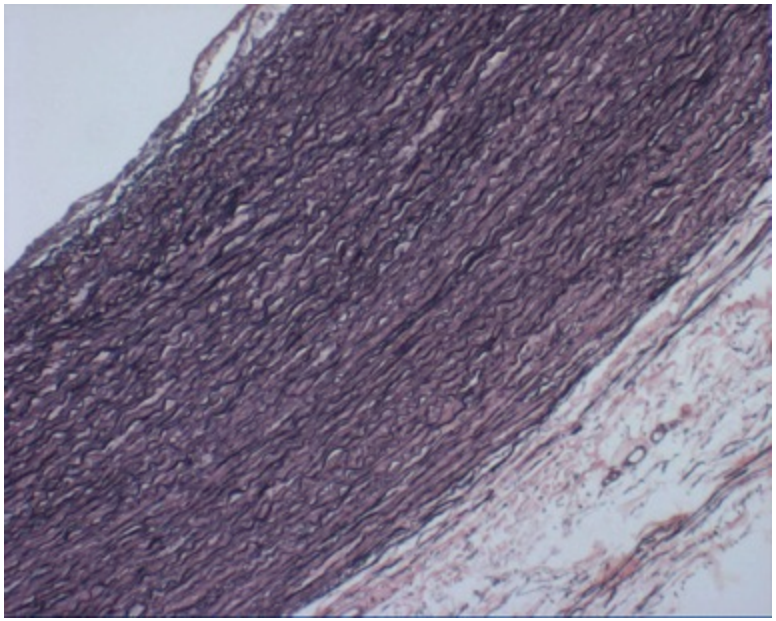


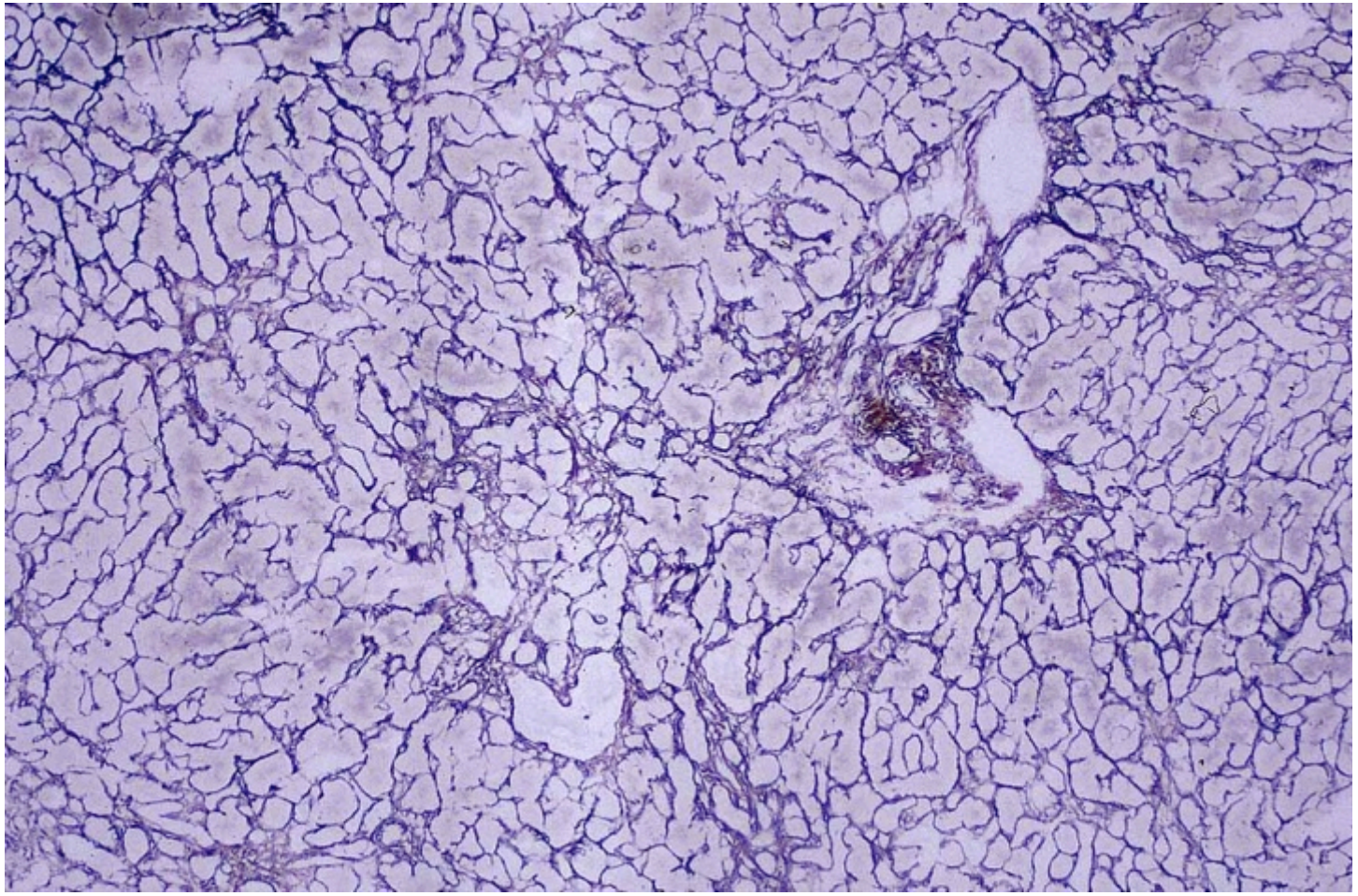
Human Skeletal muscle with PhosphoTuncsticAcidHematoxylin PTAH stain to demonstrate striations





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





Silver stain to demonstrate reticulin supporting tissue

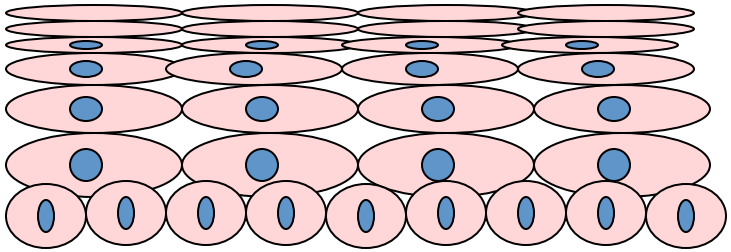
EPITHELIUM is the term given to the cells that

- -cover the exterior surface of the body,
- -lines both the internal closed cavities of the body,
- -lines body tubes that communicate with the exterior (alimentary, respiratory, genitourinary)
- -comprise the various organs (liver)

Epithelium can be

- -impervious (epidermis or bladder) ,
- -secretory (stomach),
- -absorptive (intestines),
- -be a transport system(trachea),
- -receive sensory stimuli (taste buds of the tongue)

Epithelium can be impervious (epidermis or bladder)
Stratified Squamous epithelium—stacked up like plates



Squamous epithelium function helps with shear forces that are encountered as in

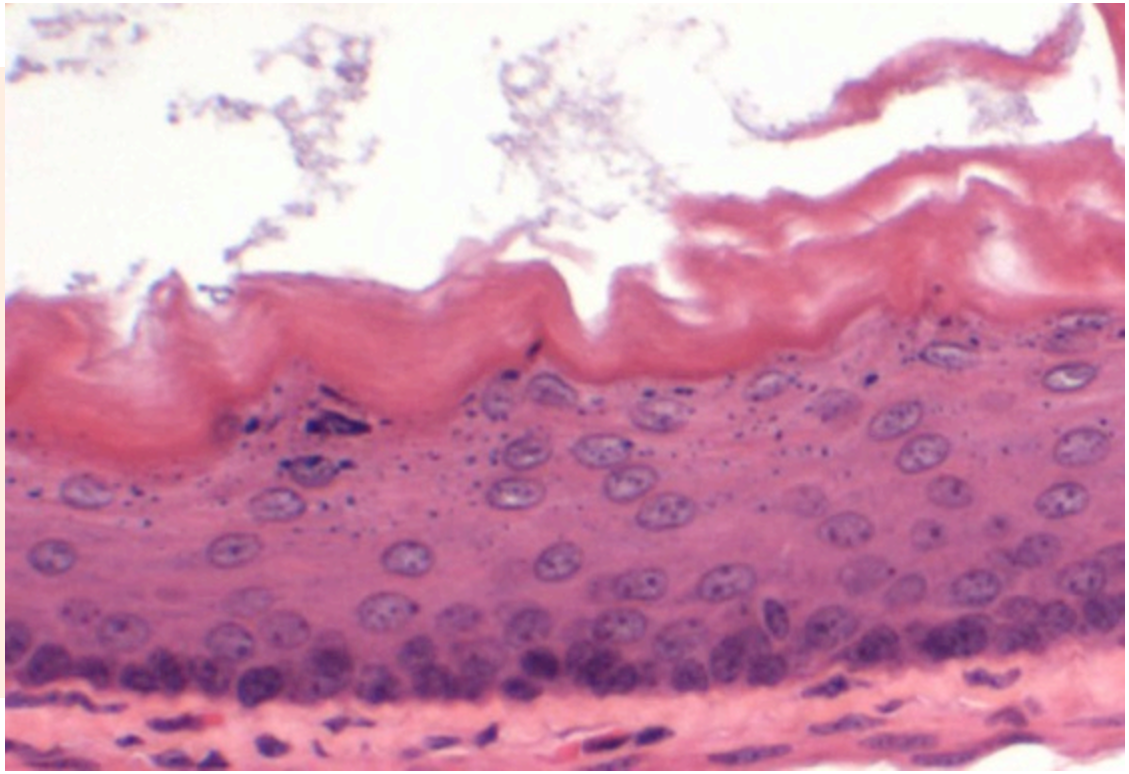
-Skin, with anuclear keratin layer

-Esophagus(No keratin layer)

-Cervix (no keratin layer)

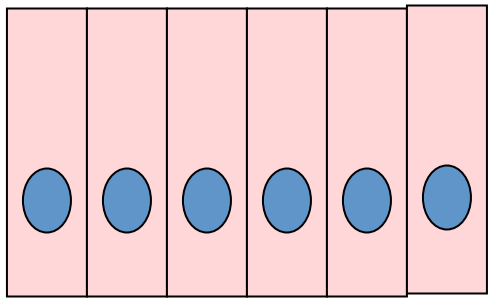
-External ear canal

-Anus

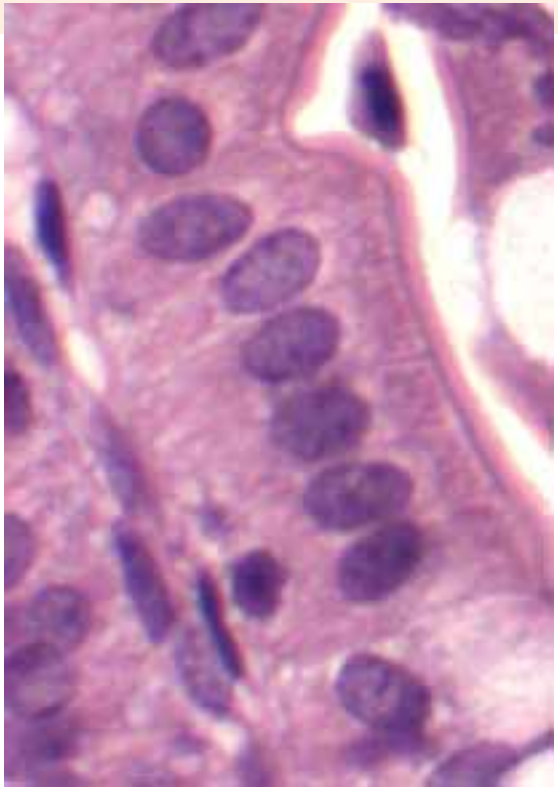


Epithelium can be secretory (stomach), absorptive (intestines),

Columnar epithelium—is so termed because the cells are arranged like columns



The height of the cell is greater than the width

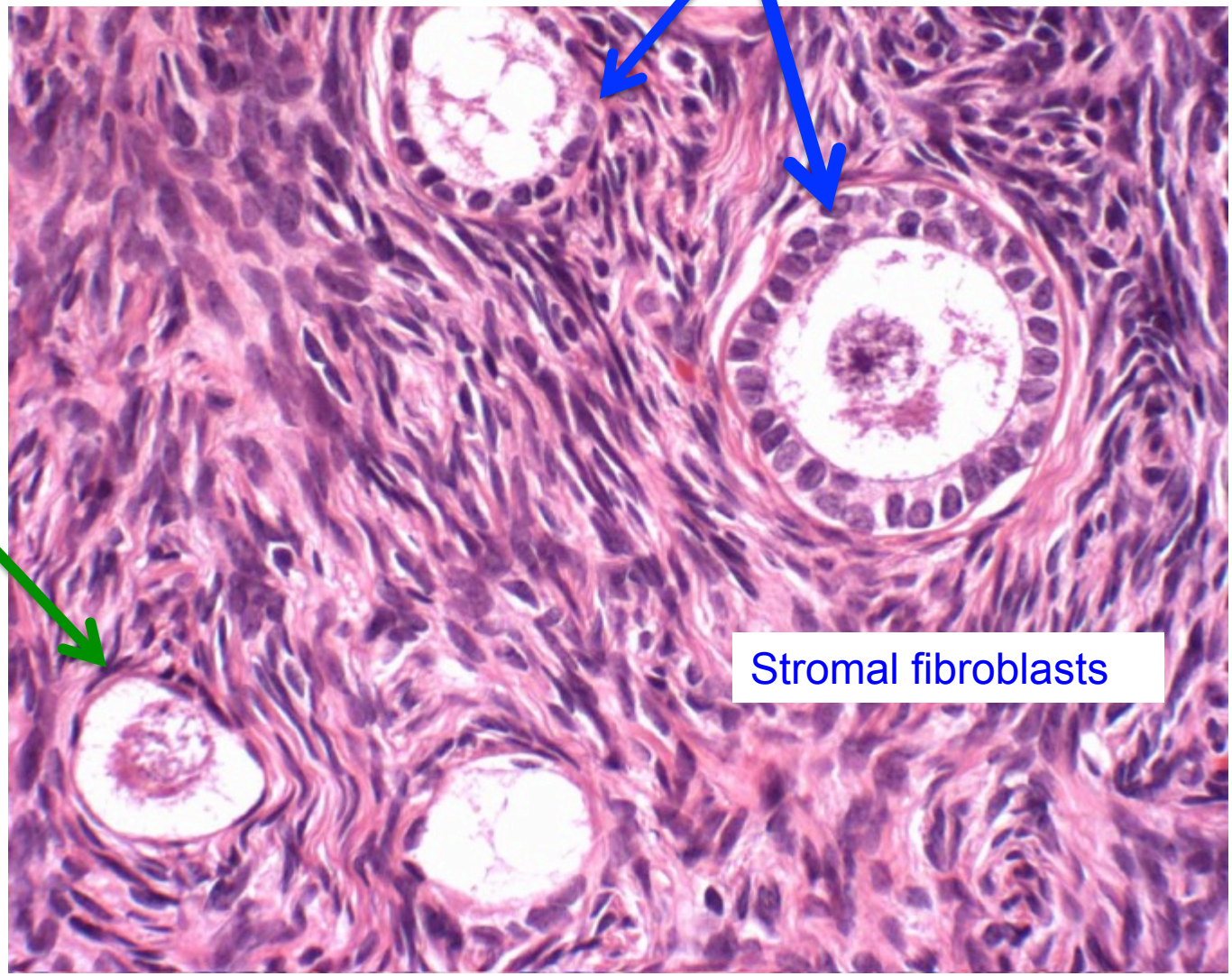


Epithelial lining of intestine

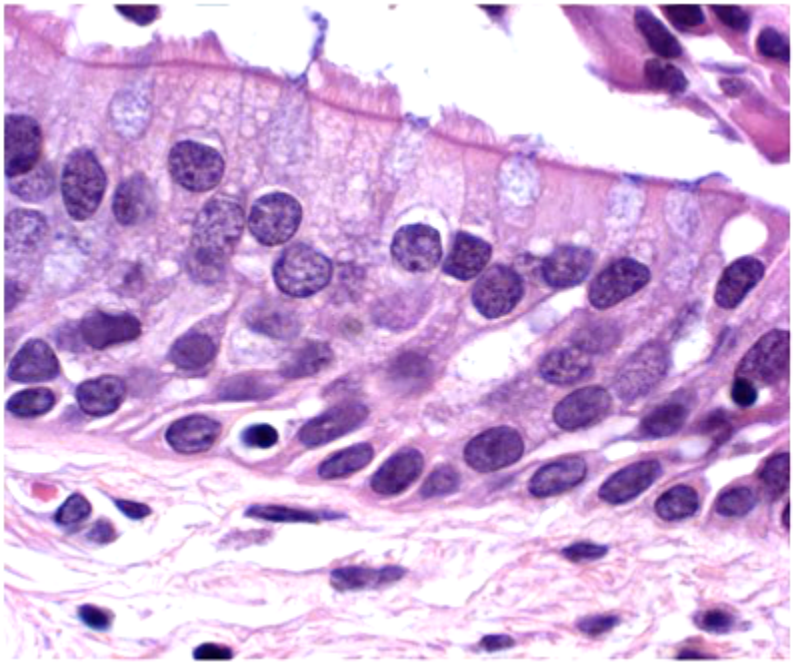
Ovary with developing follicles

Primordial follicles lined by flat squamous epithelium

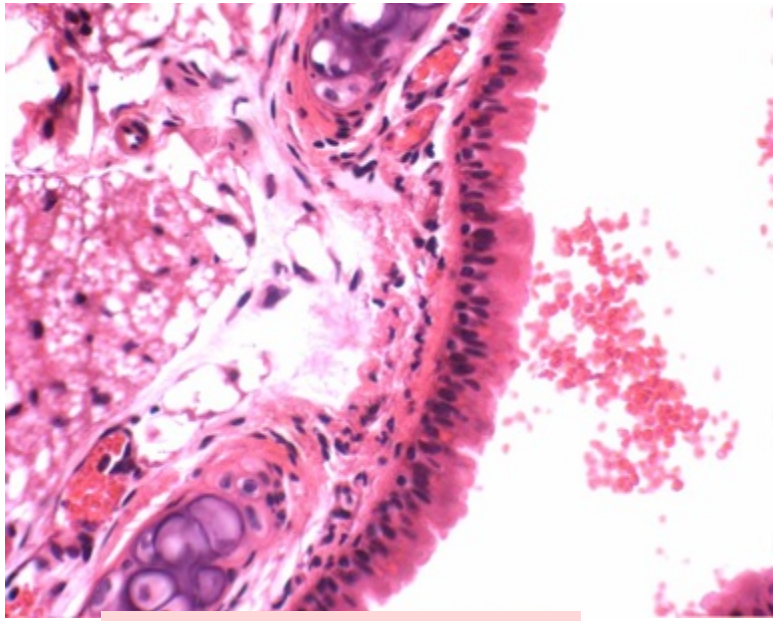
Primary follicles lined by cuboidal epithelium



Pseudo-stratified Columnar epithelium

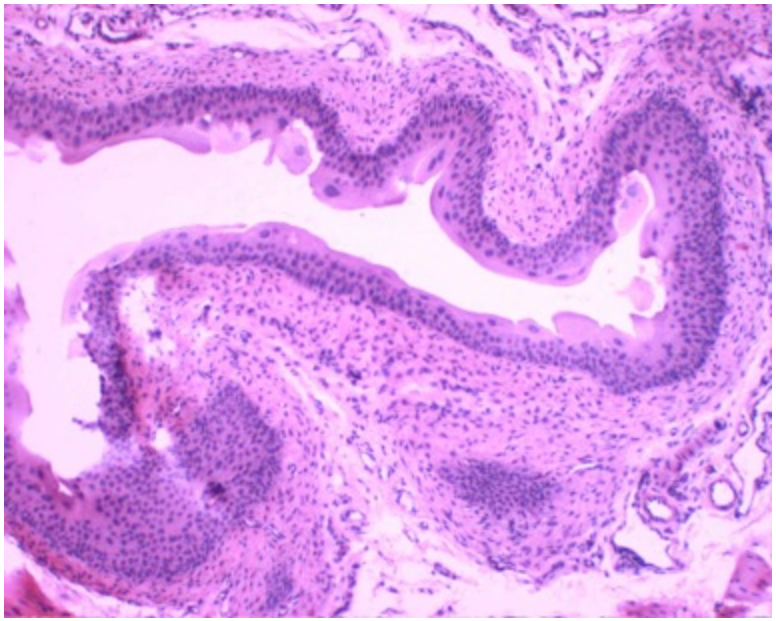


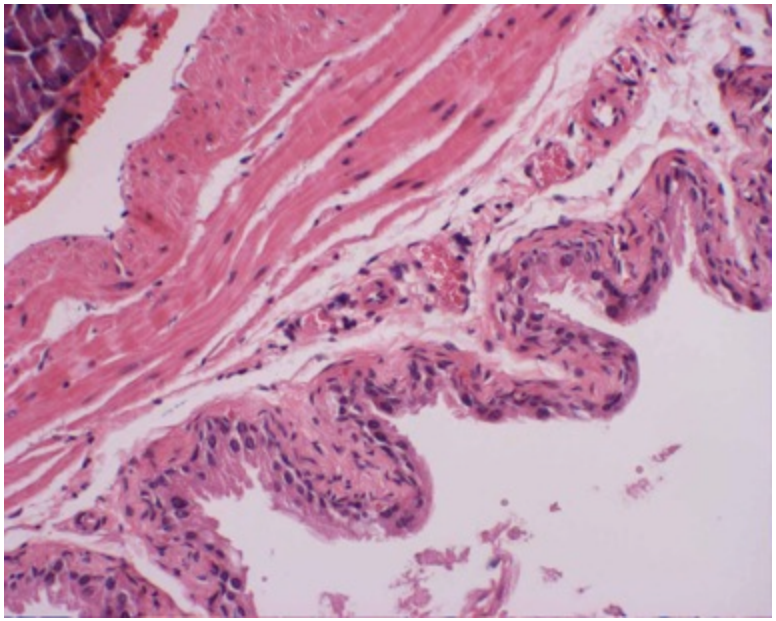
Human trachea



Mouse trachea

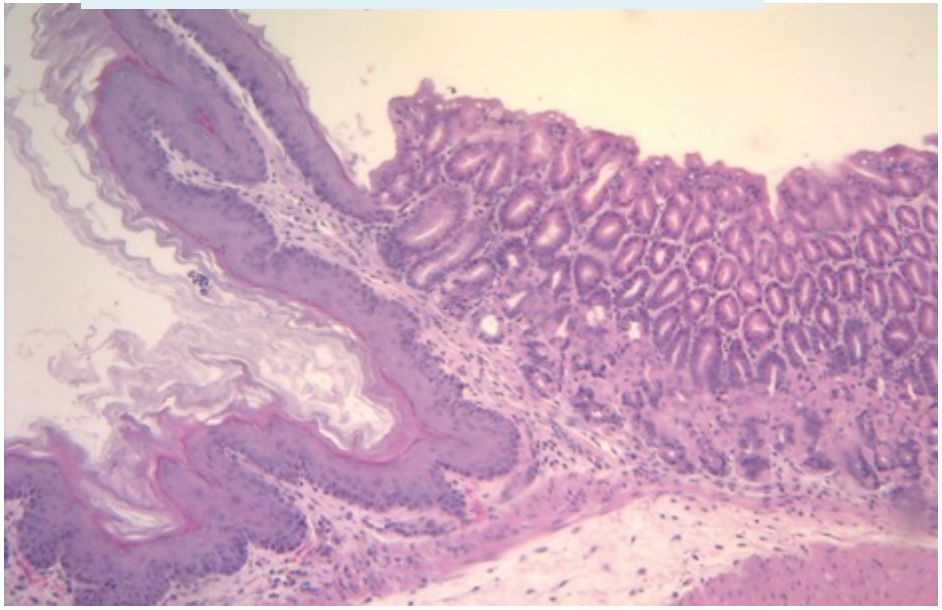
Transitional epithelium of bladder (mouse)



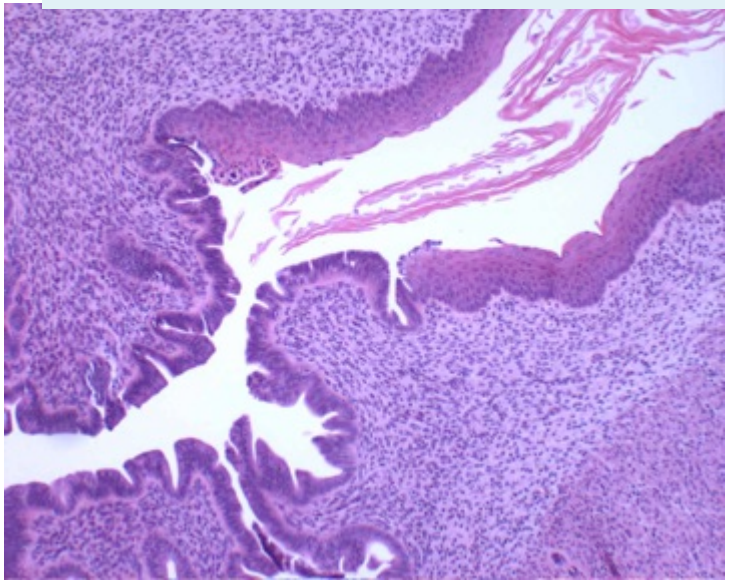


Junctional zone epithelium: Where epithelium of one kind changes naturally to another

Mouse Stomach: half is squamous epithelium

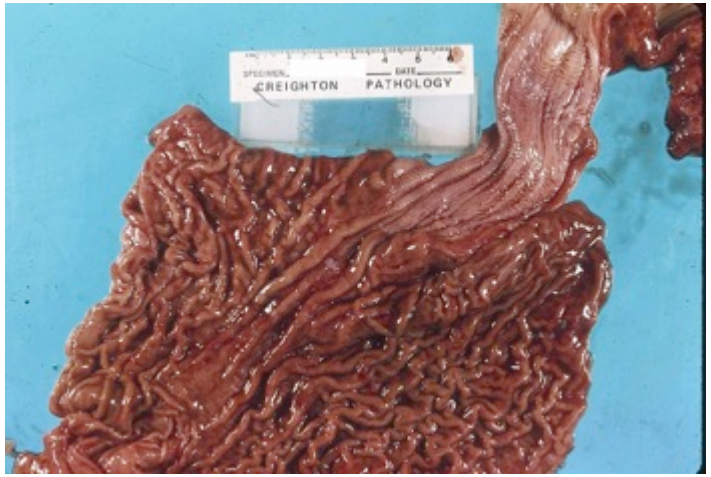


Mouse cervix: external is squamous epithelium



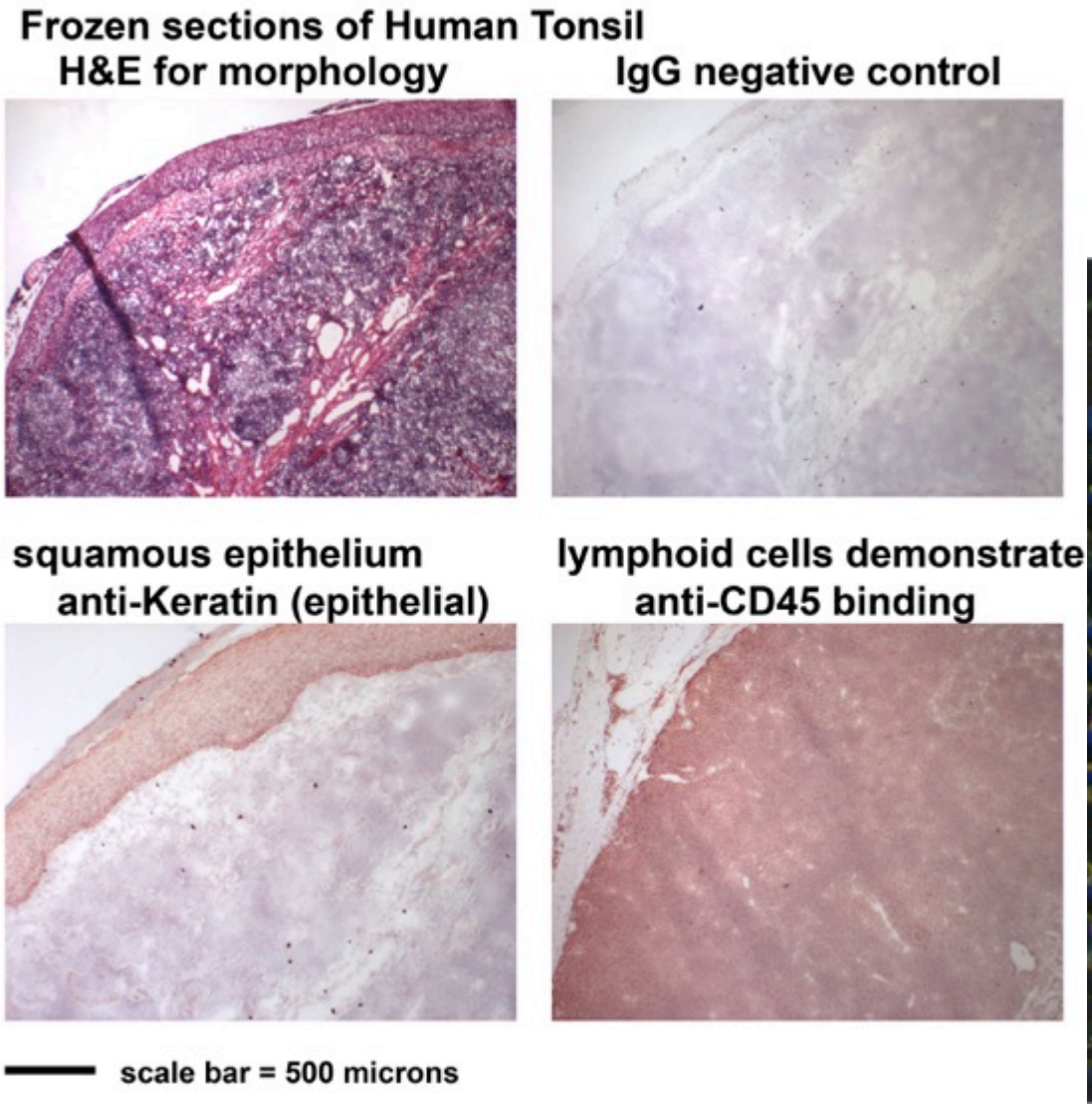
Junctional zone epithelium: Where epithelium of one kind changes naturally to another

HUMAN STOMACH

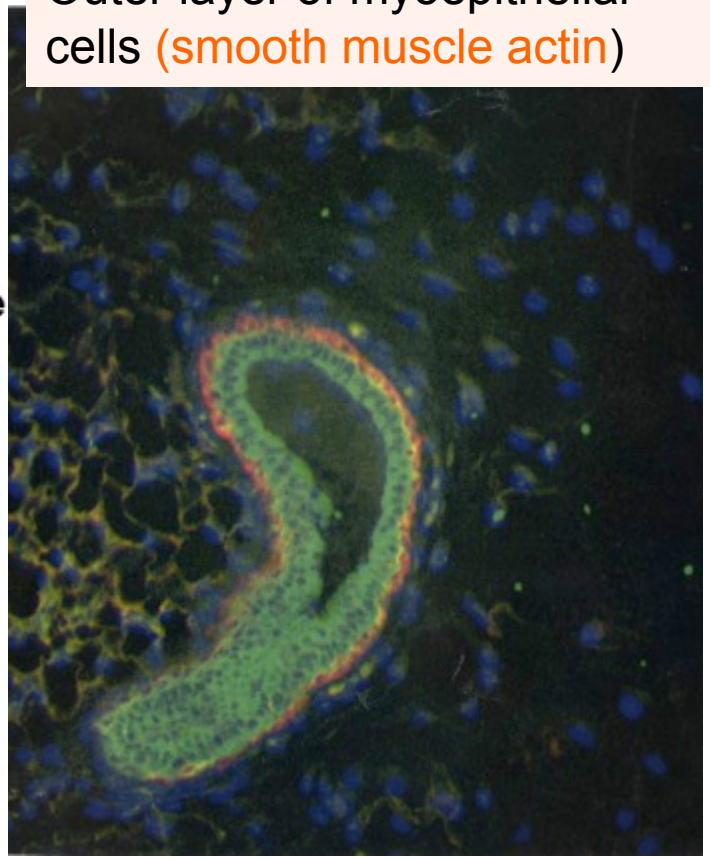


Mouse Stomach: half is squamous epithelium

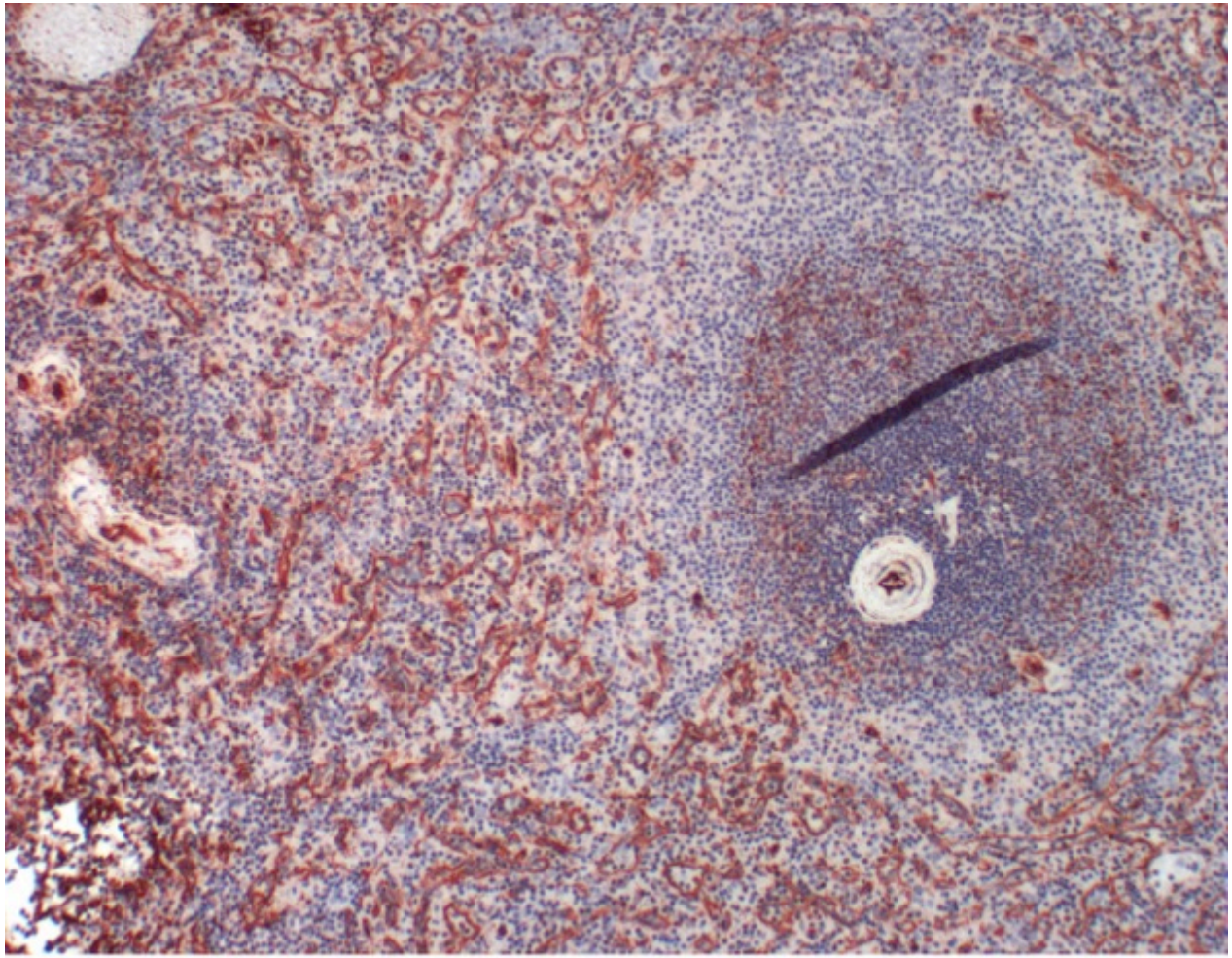
Keratin is a marker for most epithelial cells



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



Vimentin is a marker for stromal fibroblasts and blood vessels



1. Frozen sections are useful for Immunohistochemistry. T/F

2. Frozen sections are useful for Morphologic examination. T/F

3. Paraffin sections are made after fixation. T/F

4. Paraffin sections can be used for immunohistochemistry T/F

5. Length of fixation affect the ability to detect antigens in paraffin sections. T/F

6. Bone has to be fixed and decalcified for two-three days before processing into paraffin blocks, for sectioning and staining. T/F

1. Your genetically altered mouse died last night.

You want to know the cause of death.

Should you fix and look paraffin sections

or plan to sacrifice littermate controls and gene altered animals

at specified time points, harvest organs,

fix and examine paraffin sections?

2. The animal has been perfused with PBS and then with fixative.

Can the tissue from various organs be now frozen

and sectioned for immunohistochemistry?