Peer Review, Related Issues and Findings that Might Cause Differences between Original and Reviewing Pathologists

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with contribution of Dr. J.F.Hardisty, EPL Inc.

Any Section is an artifact because……

- 3-4 um in thickness.
- One moment in agonal stage of cells.
- Shrinking is always there.
- Some structures are stained, others not.
- Covered.

- Can we still do something wrong?

- We can: tresholds, opinion, recognition, substraction of artifacts…..!

But there is something what we should use……

'…Can Pathology rise to the challenges inherent in the new world of One Medicine? For over a century and a half of modern pathology, we have possessed a unique technology, the microscope. We possess a unique skill, microscopic interpretation…'

Cardiff RD, Ward JM, Barthold SW (2008): 'One medicine - one pathology': are veterinary and human pathology prepared? Laboratory Investigation, 88: 18–26

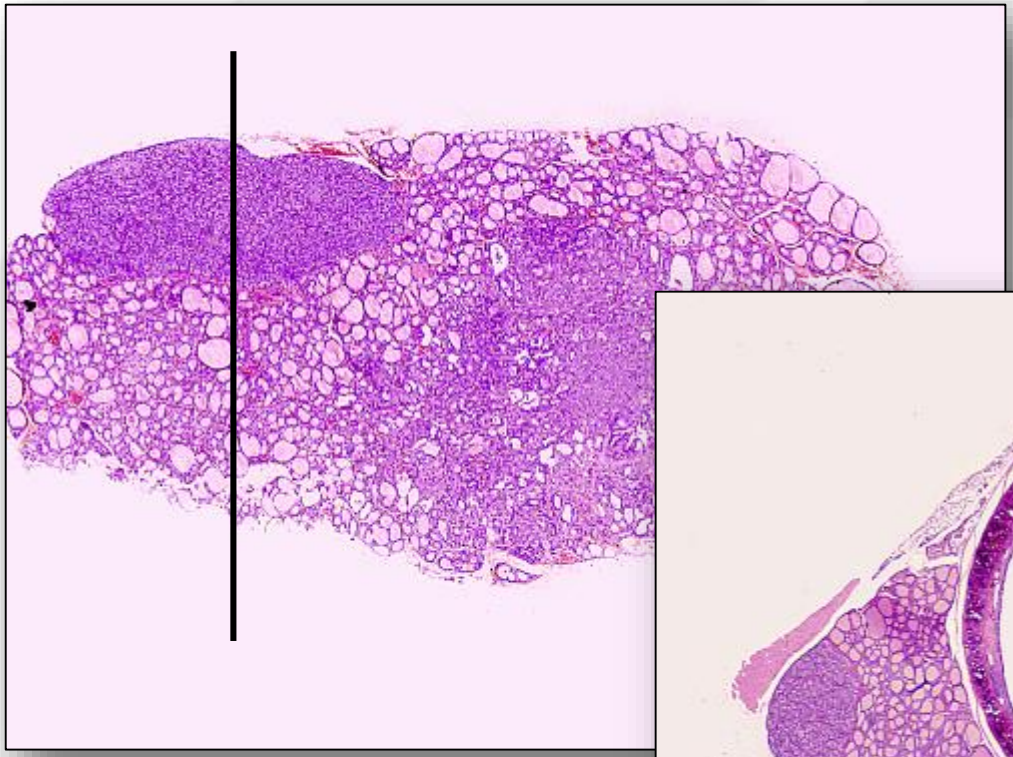
And High Qualified Technicians, and discussion, discussion, discussion……!

What can we do wrong?

Sampling procedures may be insufficient:

- **little tissue**
- **wrong trimming procedures**

Artifacts induced during tissue trimming



Transversal

Longitudinal

What can we do wrong?

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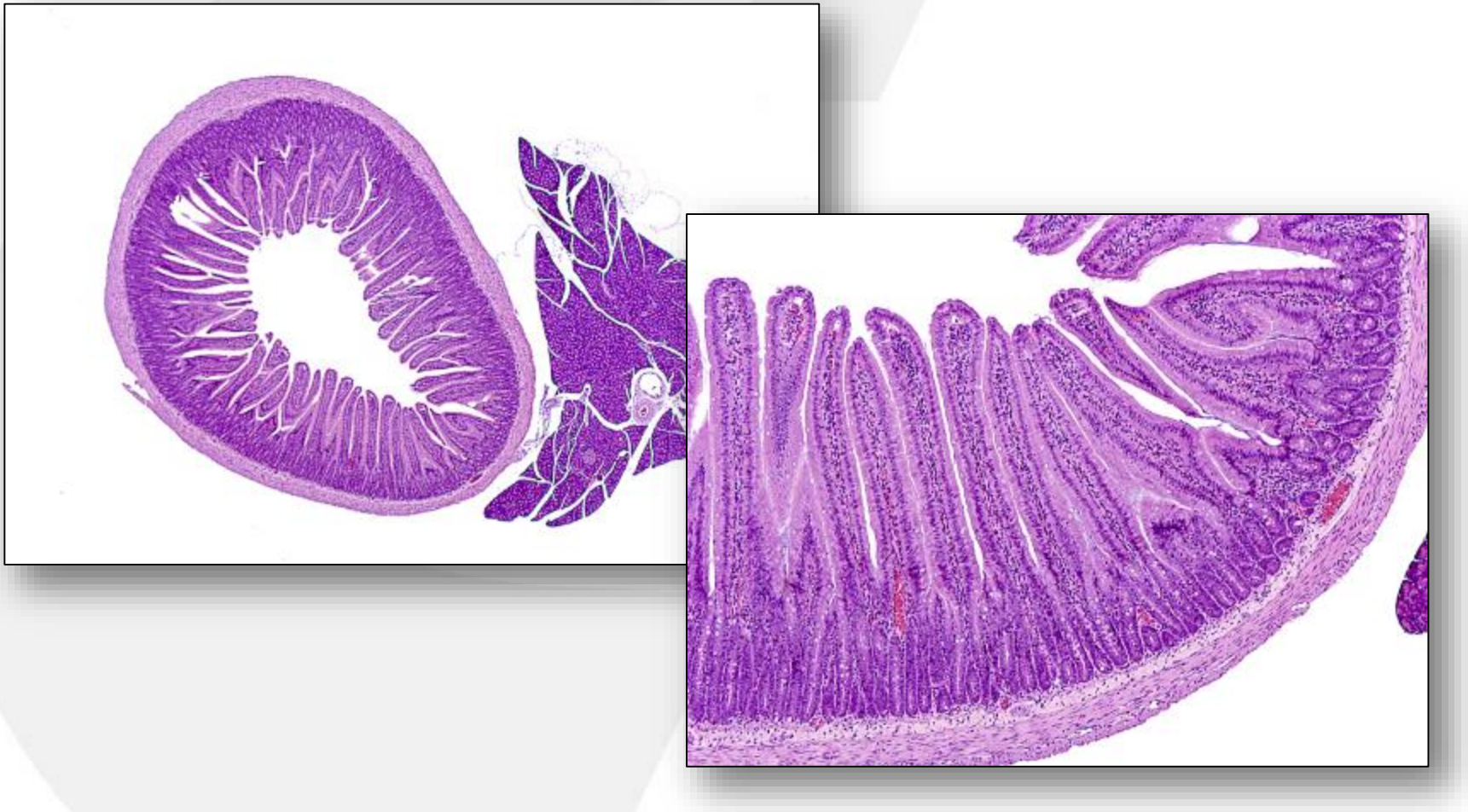
how to avoid the enhancement or unnecessary tissue artifacts

- **pressure**
- **inadequate fixatives**
- **misinterpretation**

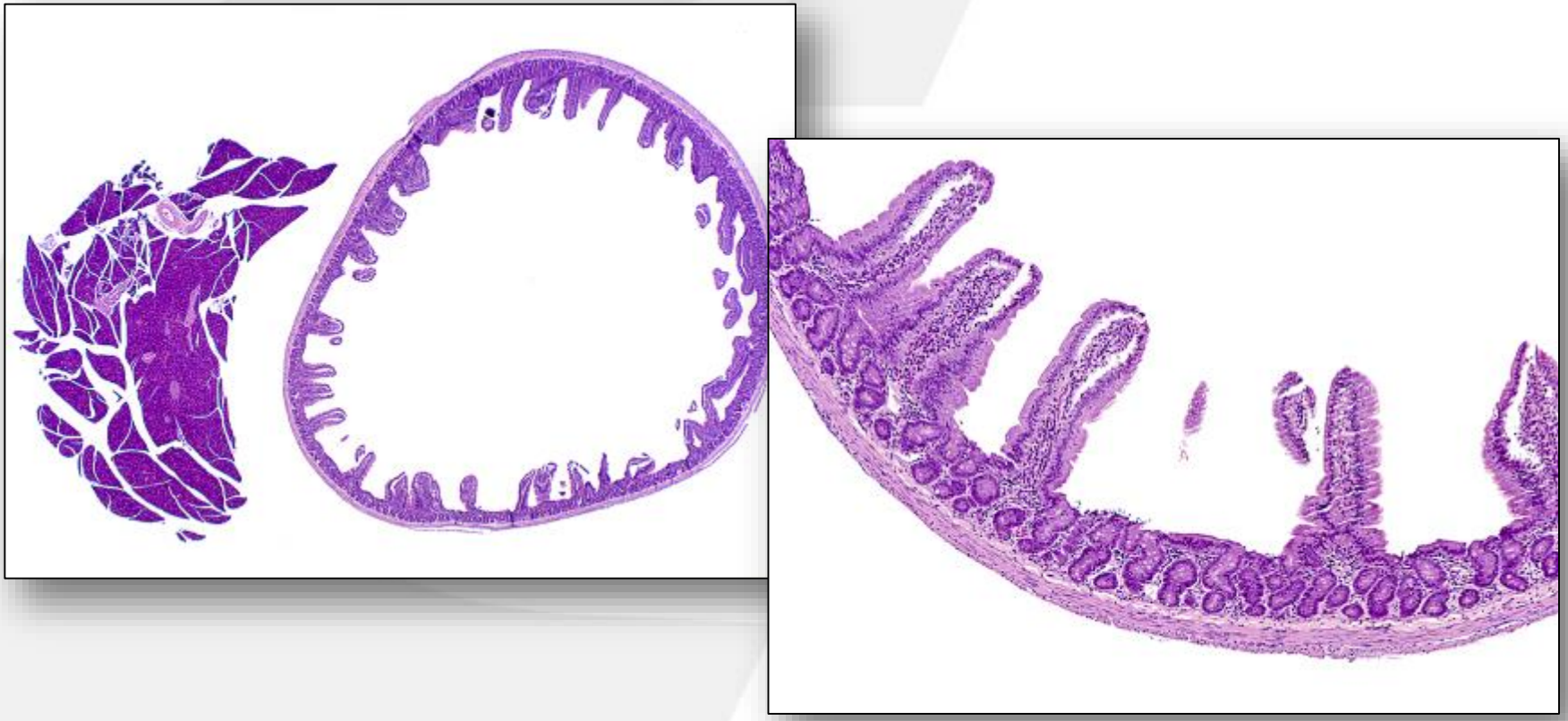
Tissue Artifacts Induced by Fixation Pressure

Tissue Artifacts Induced by Fixation



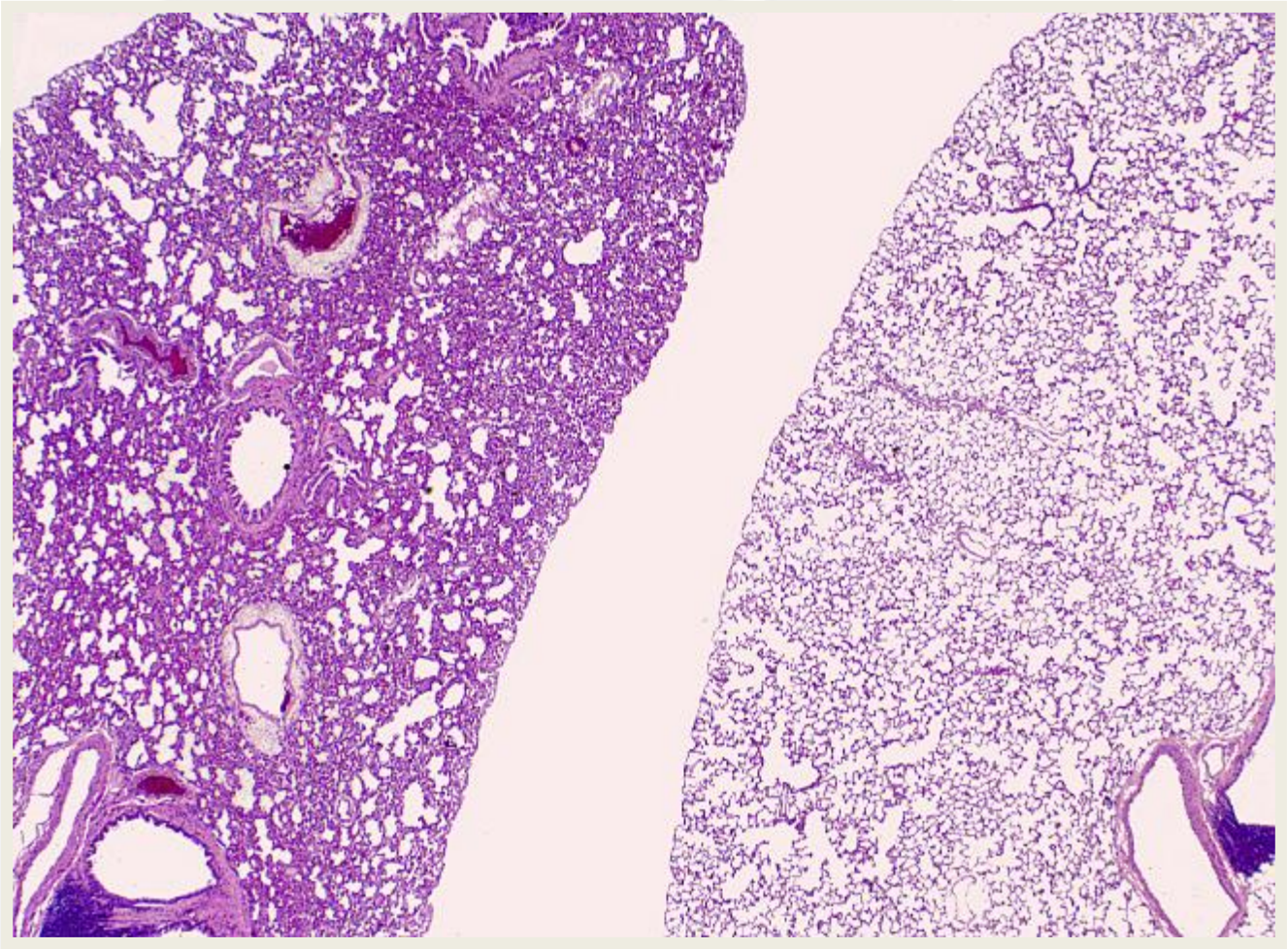
Normal Duodenum and Pancreas. Properly Inflated with Formalin at Necropsy.

Tissue Artifacts Induced by Fixation Pressure



Normal duodenum and pancreas overinflated with formalin at necropsy

Pulmonary Infusion: Why? Influence on Diagnose!

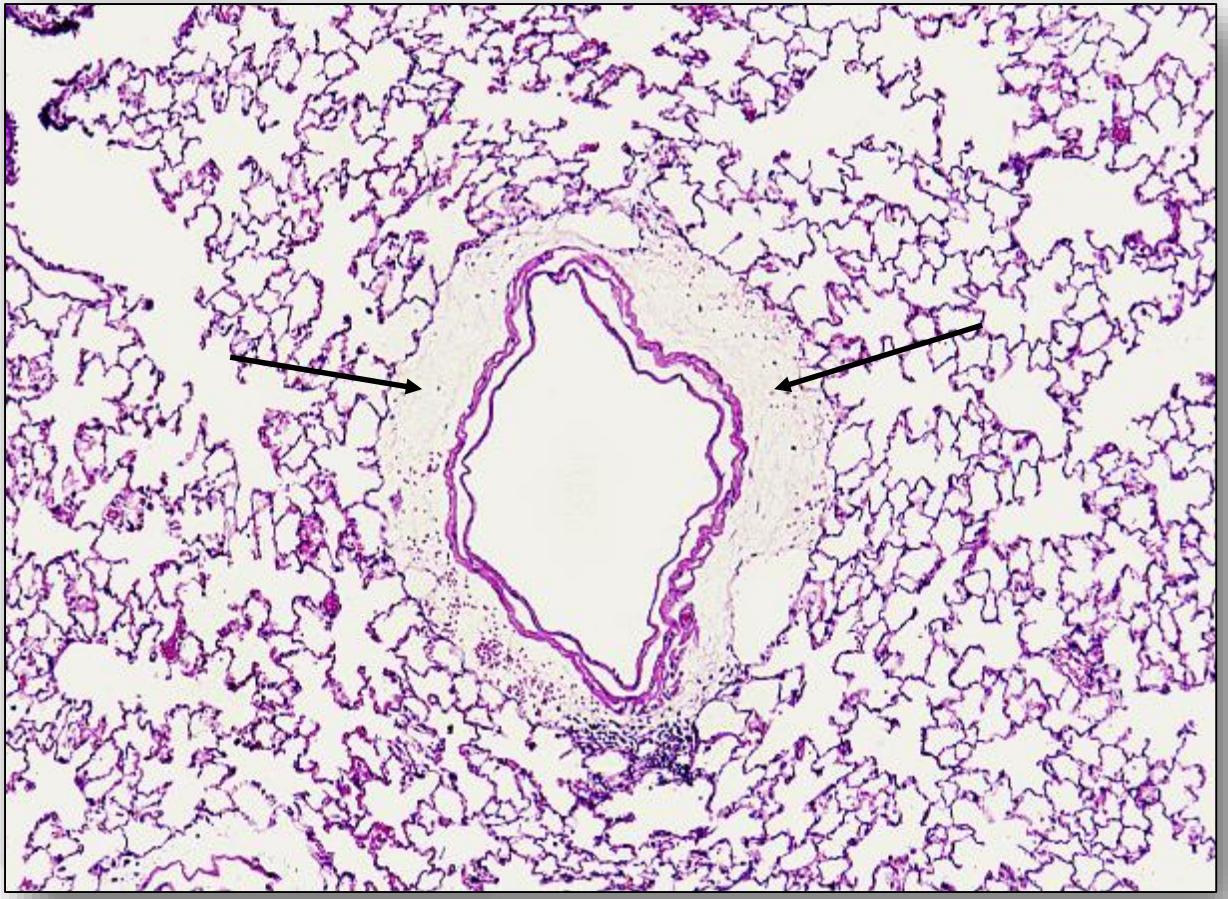


Noninflated lung **Inflated lung**

Pulmonary infusion (mouse)

Pulmonary Infusion may cause artifacts

Separation of the perivascular interstitial tissue sometimes confused with perivascular edema



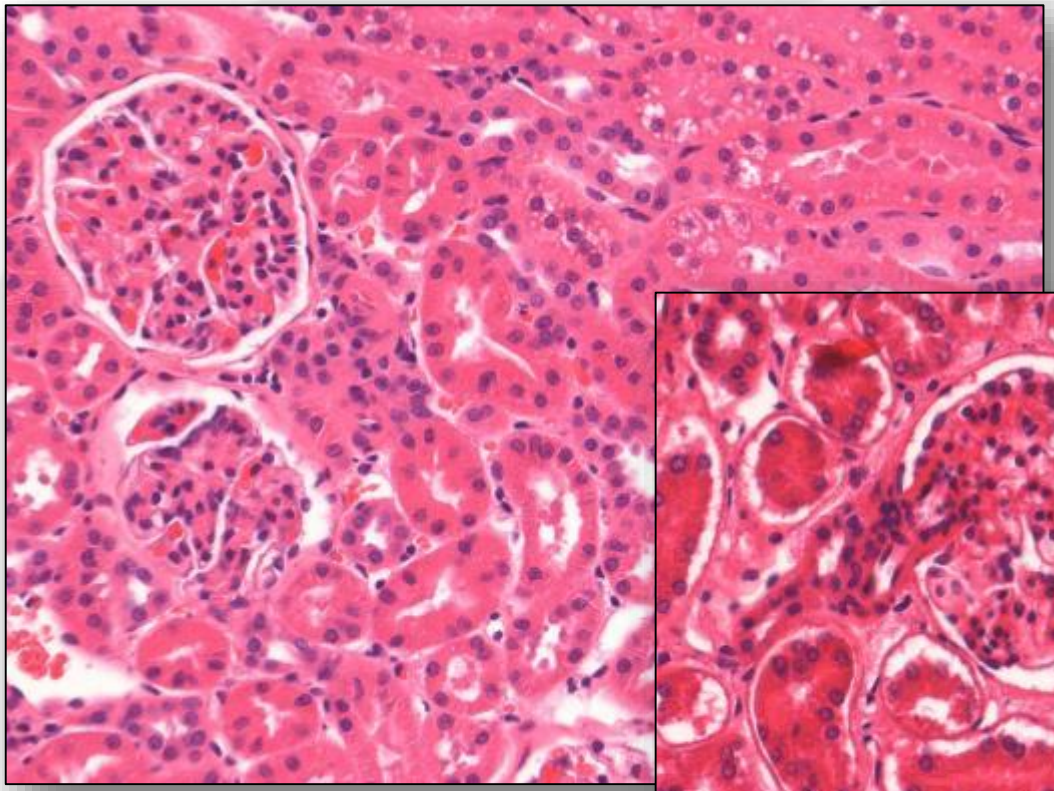
Inappropriate fixative may cause inadequate diagnose

- Study on induced artifacts performed at Harlan incl.

- use of different fixatives, fixation time, drying out of organs, cooling, freezing, autolyis etc.

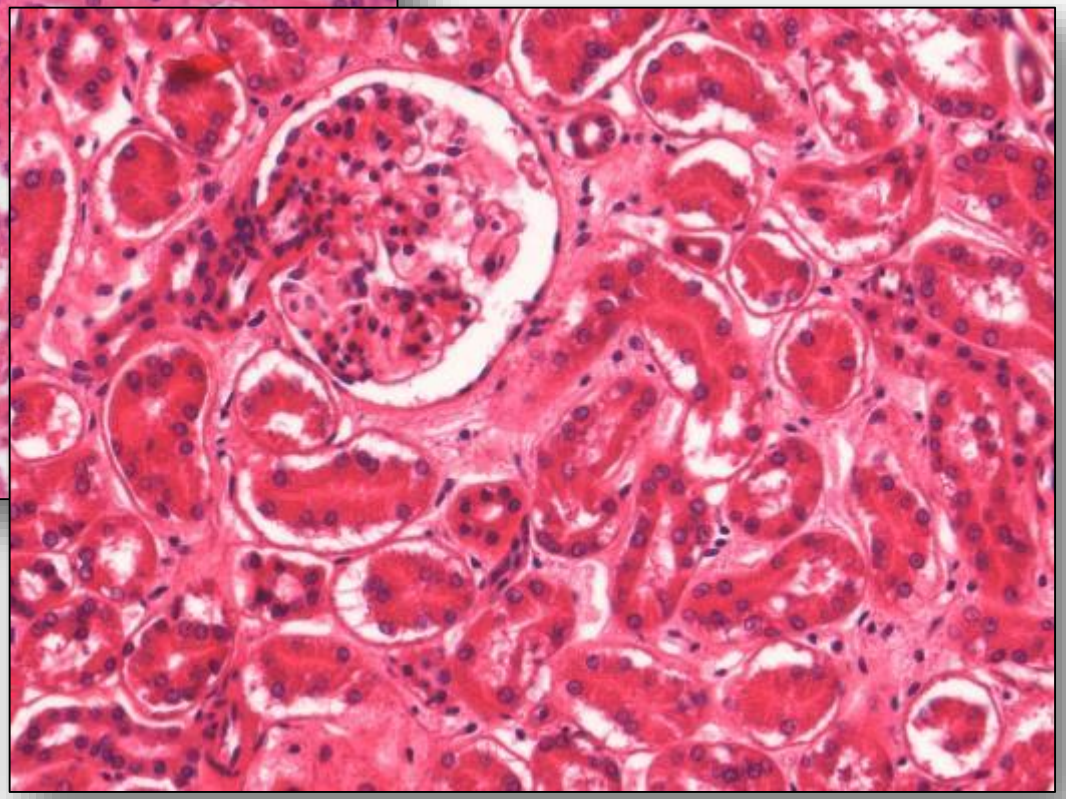
- Inappropiate fixatives may cause alterations confused with induced lesions e.g. degeneration, necrosis etc.

Artifacts by Fixation: Formaldehyde vs Ethanole

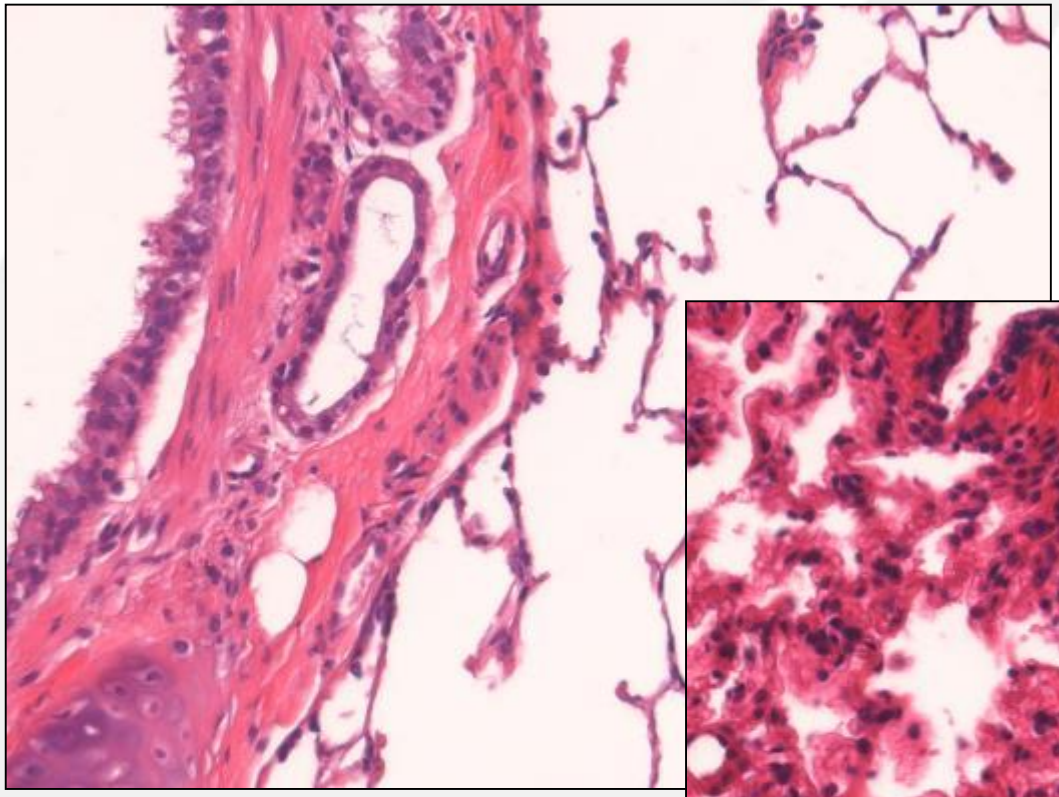


Kidney: block 5x5 mm, F4%,1 day Dog.

Kidney: block 5x5 mm, ET70%,1 day Dog.

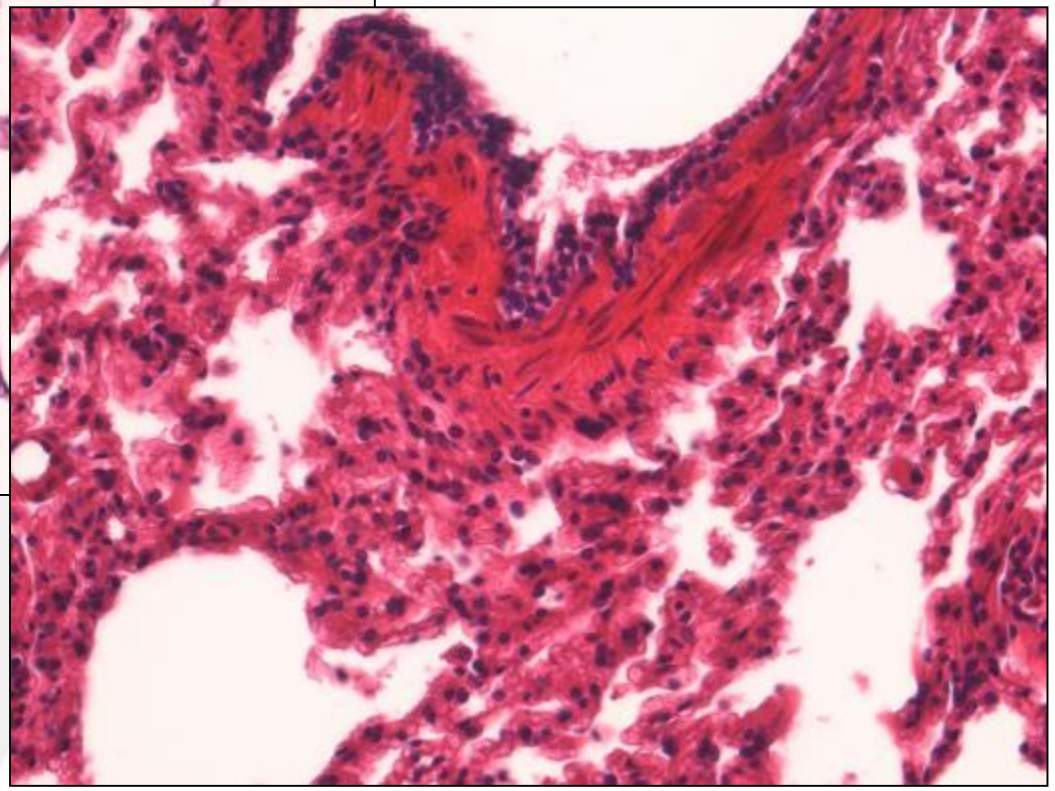


Artifacts by Fixation: Formaldehyde vs Ethanole

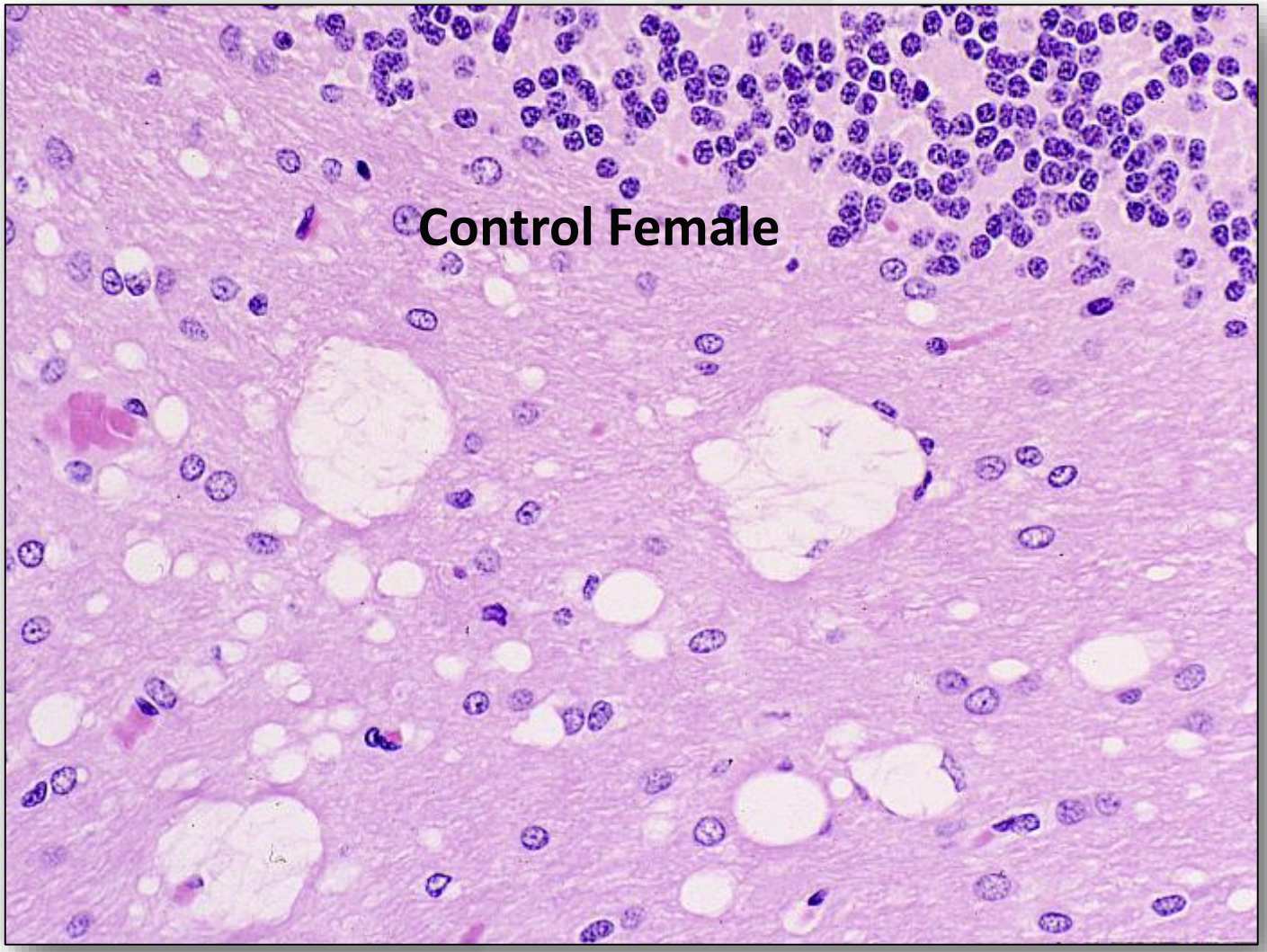


Lung: block 5x5 mm,
F4%,1 day
Dog.

Lung: block 5x5 mm,
ET70%,1 day
Dog.



Cytoplasmic vacuolation in neural tissue produced by ethanol fixatives

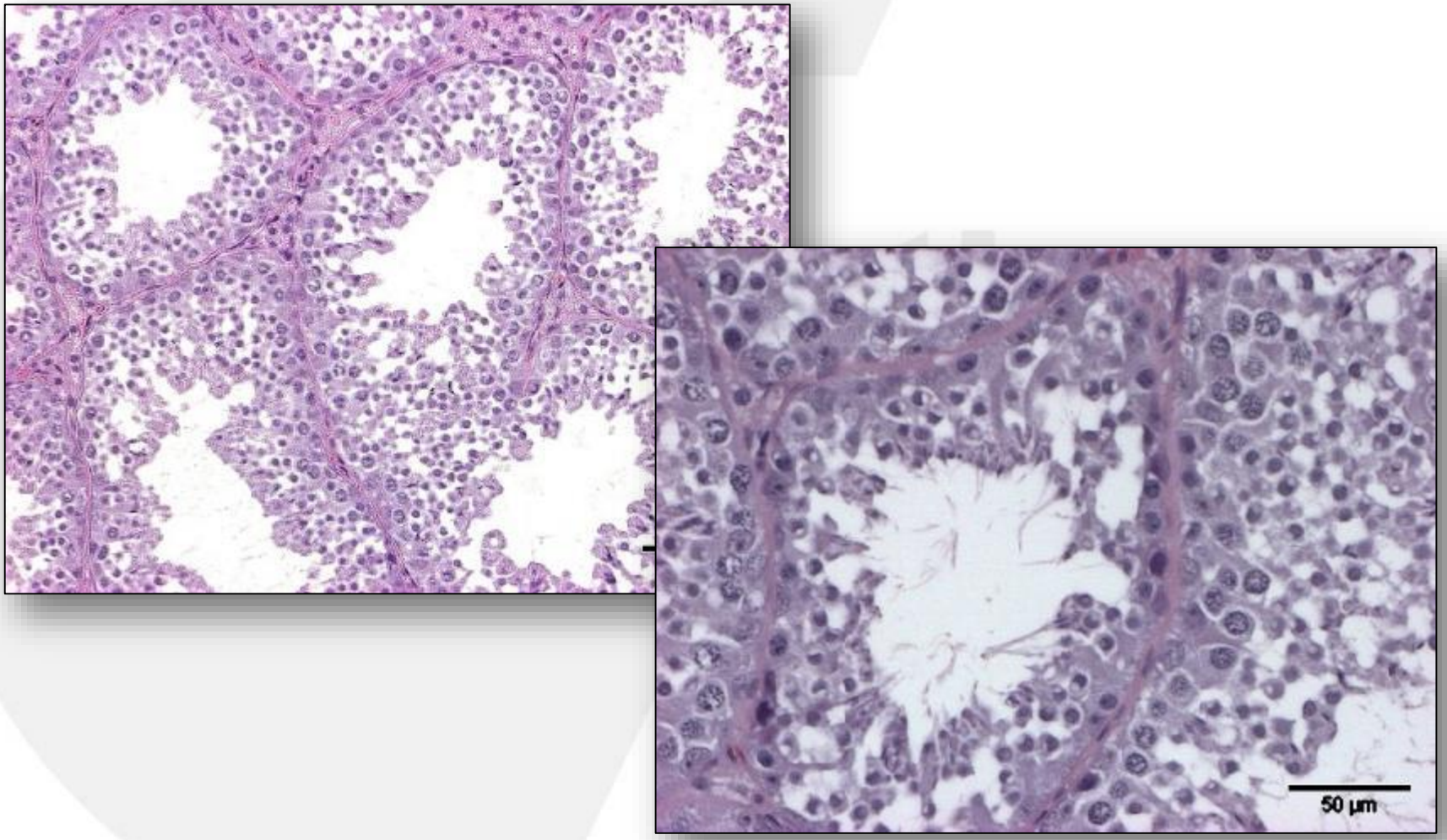


Cytoplasmic vacuolation in neural tissue produced by ethanole fixatives

Cytoplasmic vacuolation in neural tissue produced by ethanol fixatives

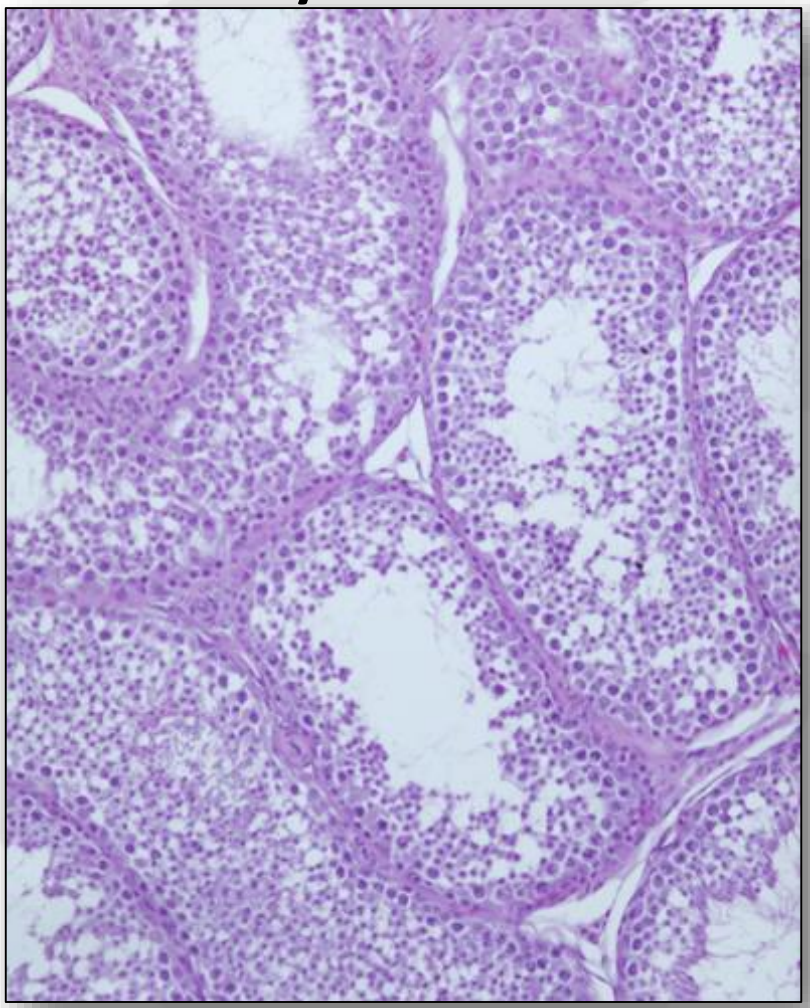
Control Males	Trimmed on Thursday
High Dose Males	Trimmed on Friday
Control Females	Trimmed on Friday
High Dose Females	Trimmed on Monday

Artifacts vs testicular lesions with formalin

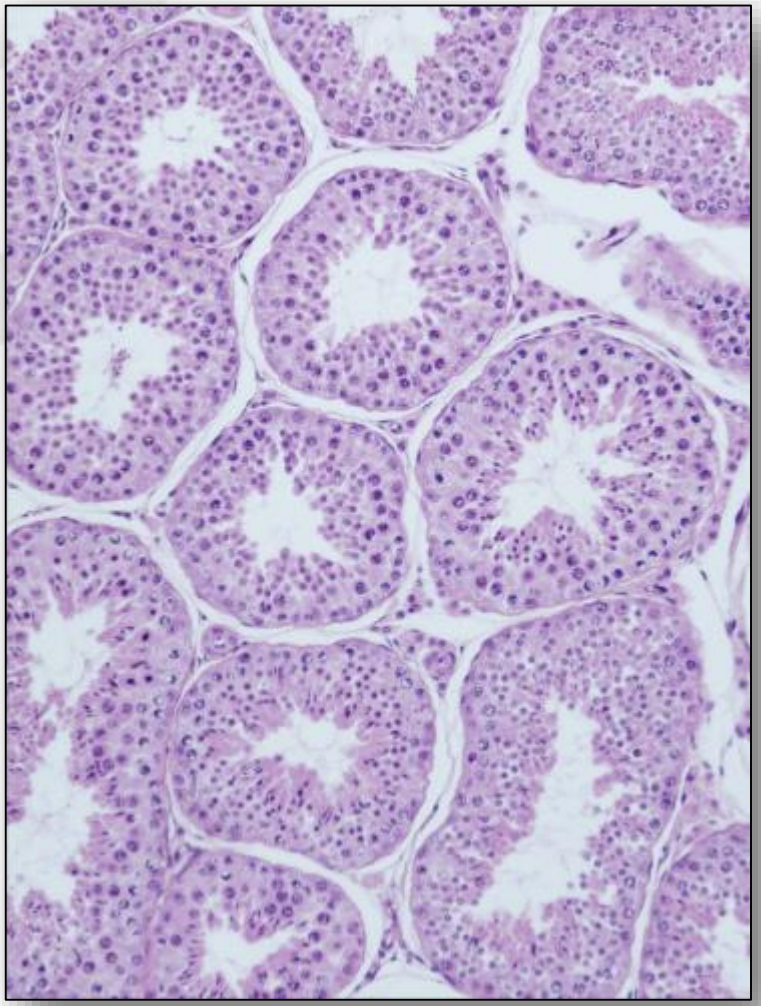


Fixation artifacts involving the testes (control dogs)

Formaldehyde

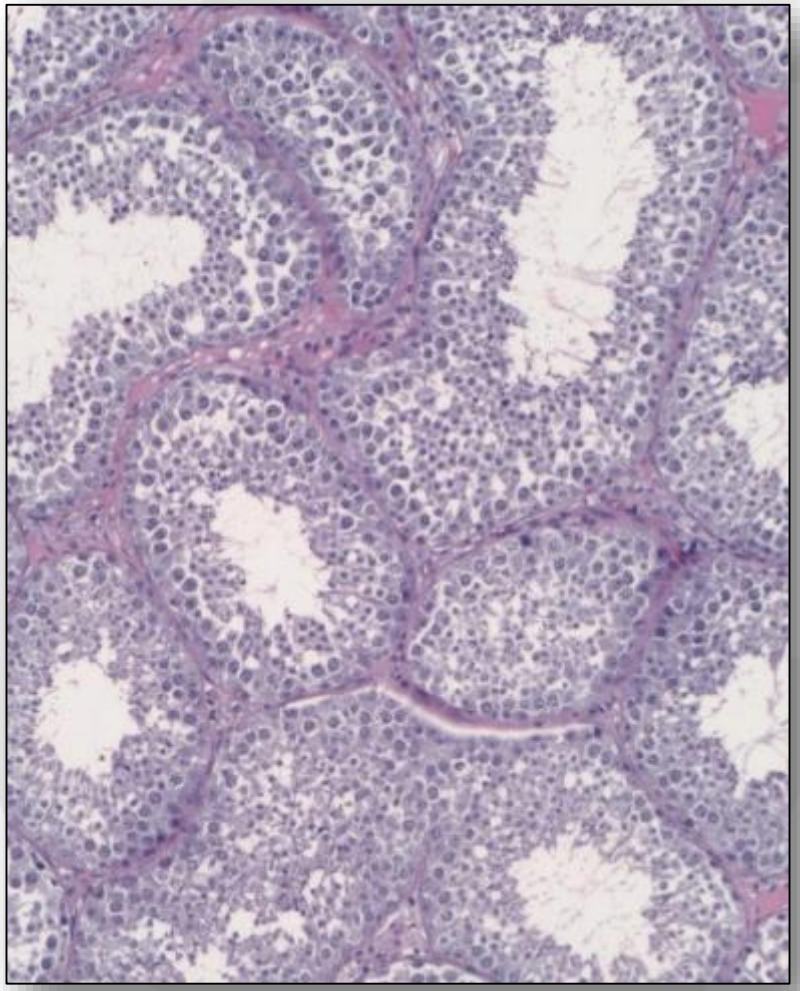


Bouin

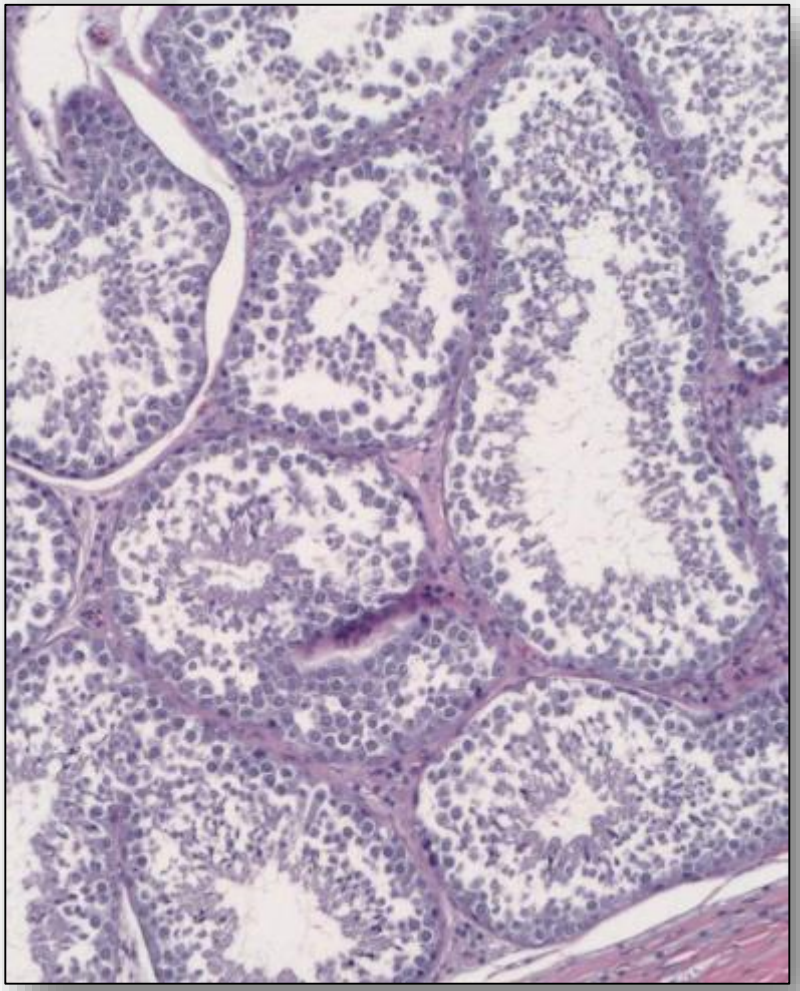


Range of fixation artifact

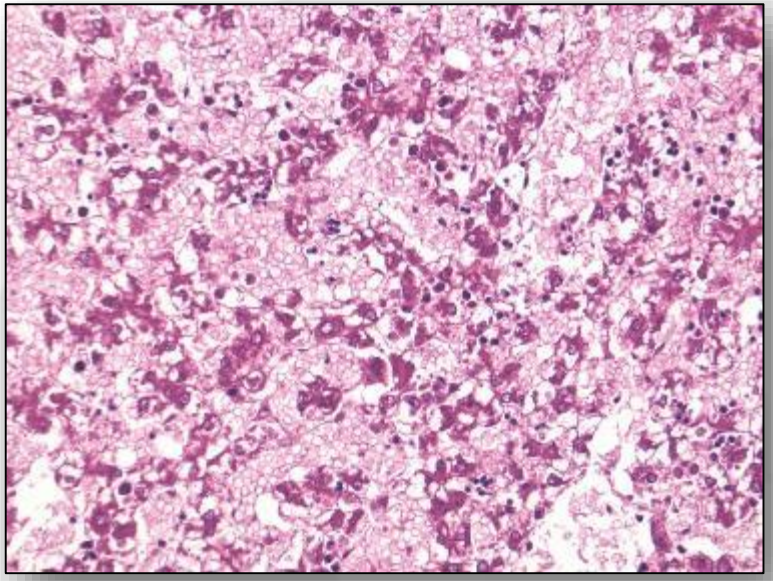
Minimal



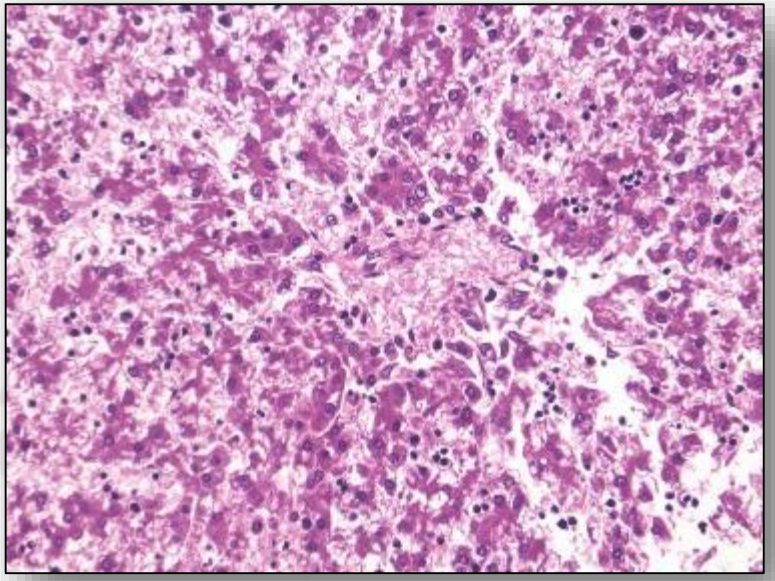
Moderate



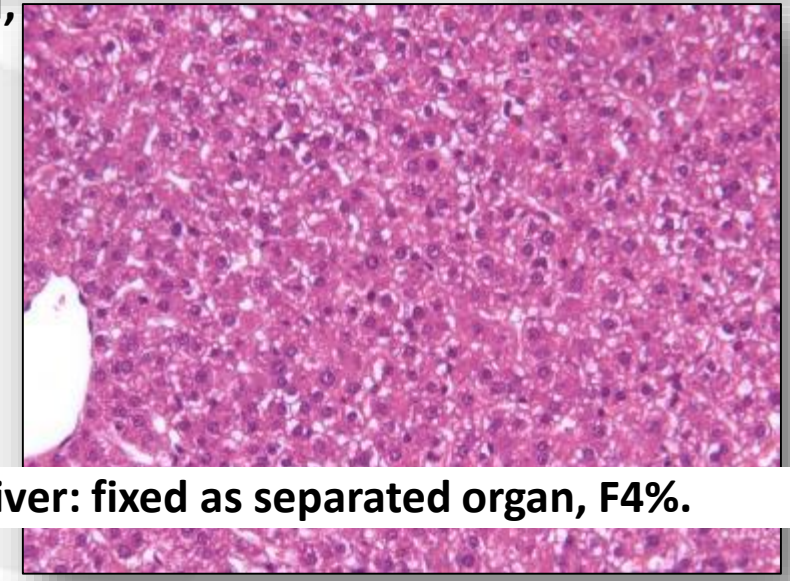
Artifacts by Fixation: Formaldehyde vs Ethanole



Fetal liver: in situ fixed, ET70%.

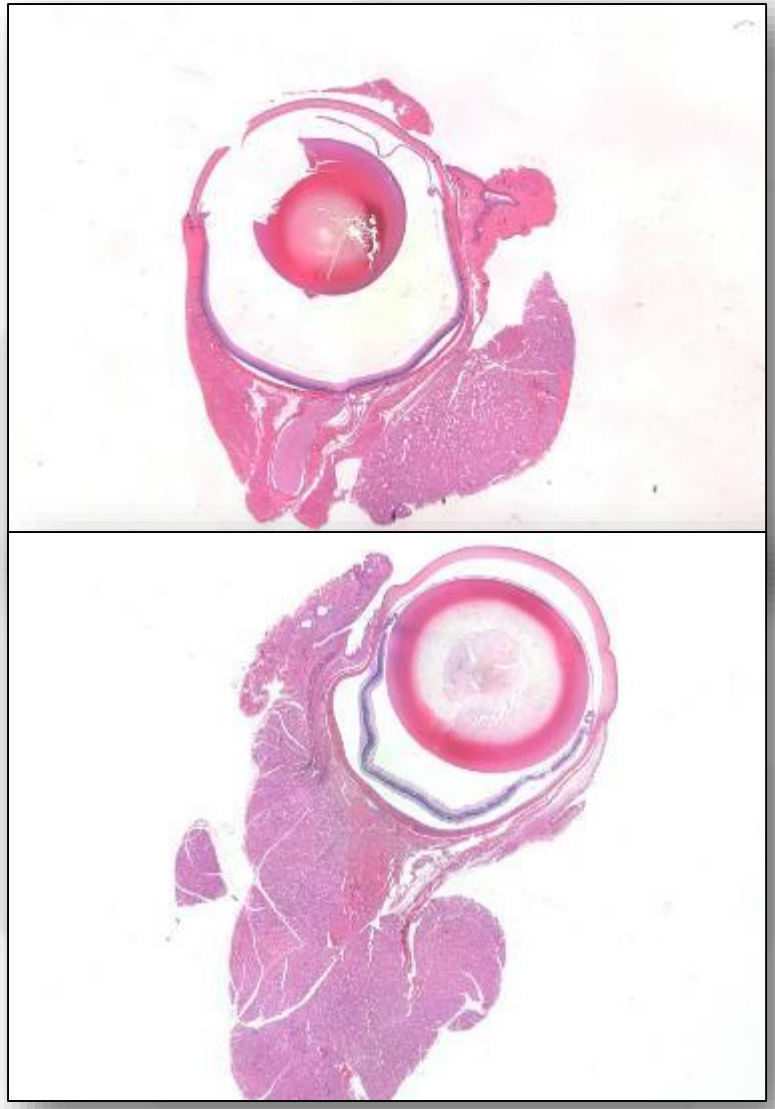


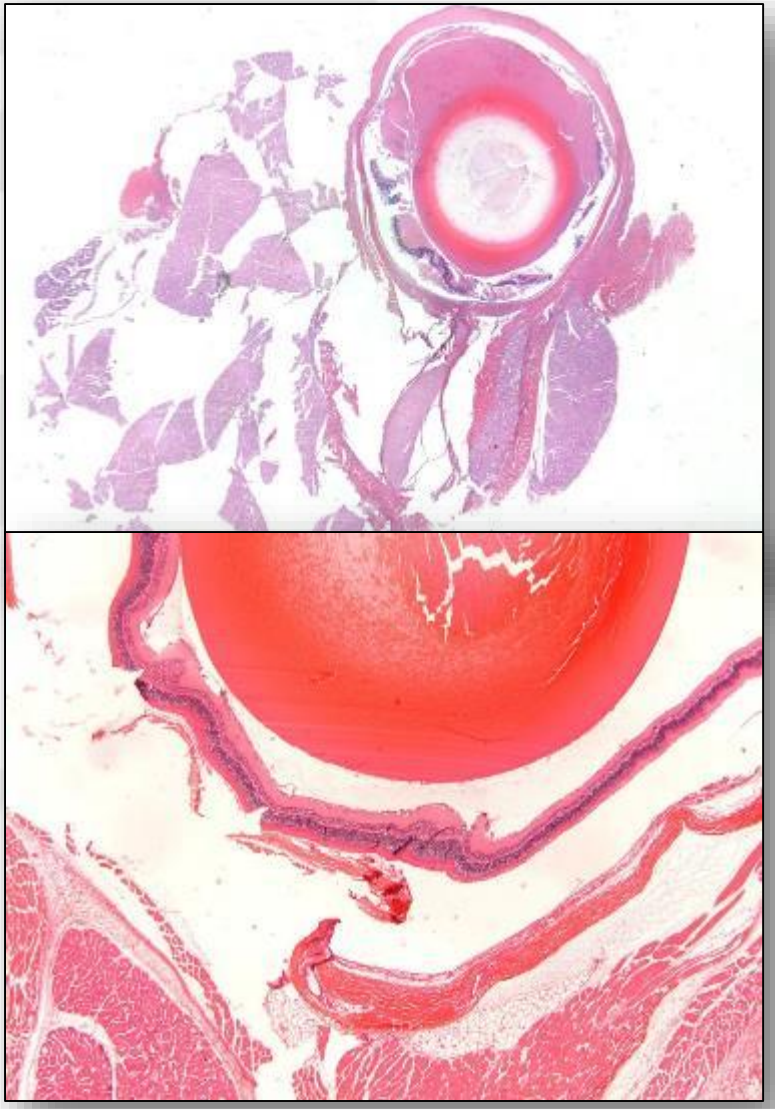
Fetal liver: in situ fixed, F4%.



Fetal liver: fixed as separated organ, F4%.

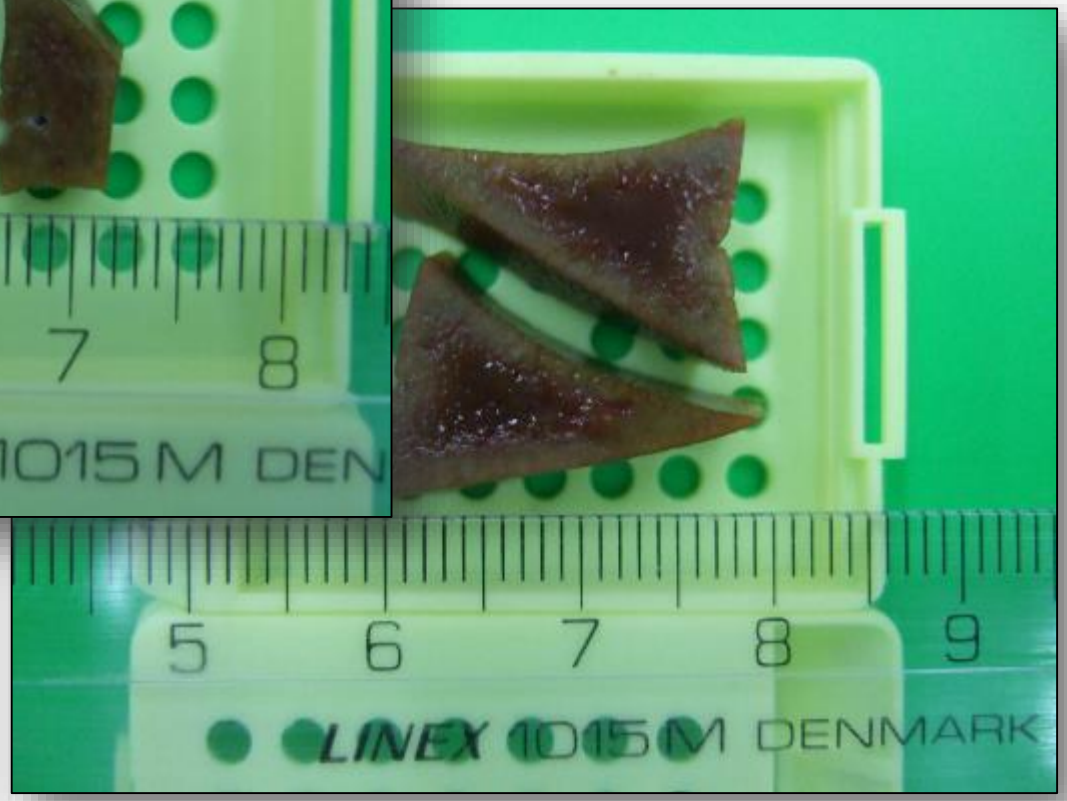
Artifacts by Fixation: Davidson‘s vs Formaldehyde





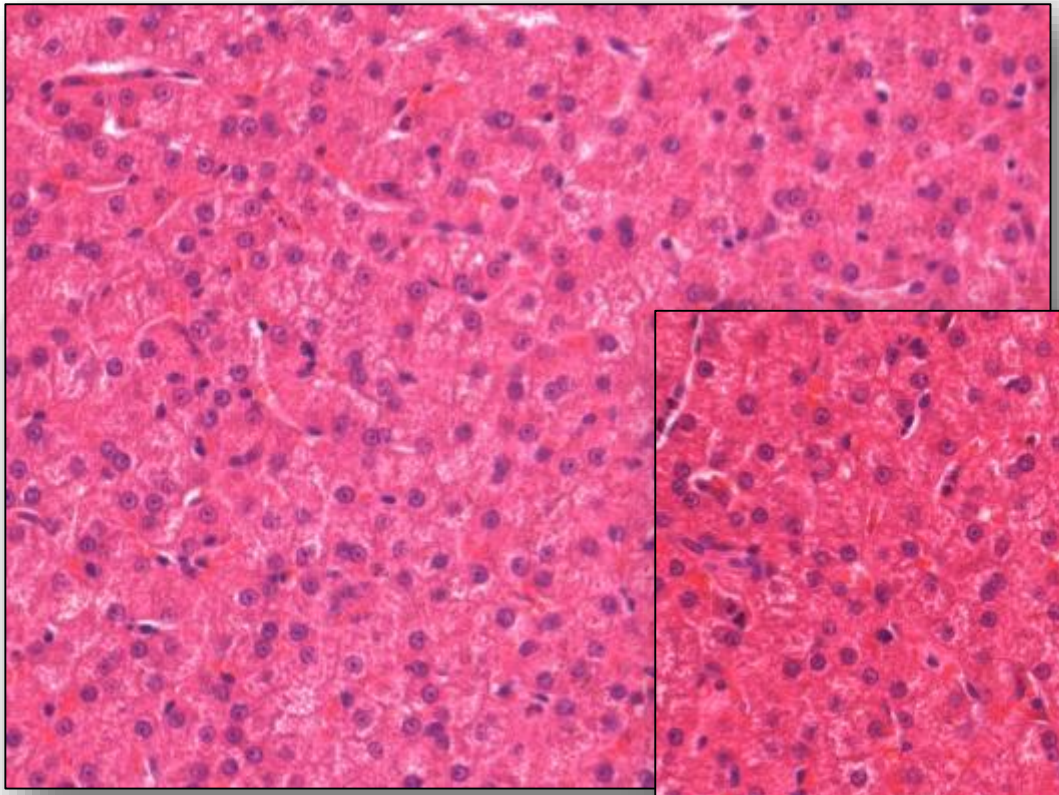
Thickness of Wet Tissues for Fixation

Liver: block 10x10 mm, F4%,1 day Dog.

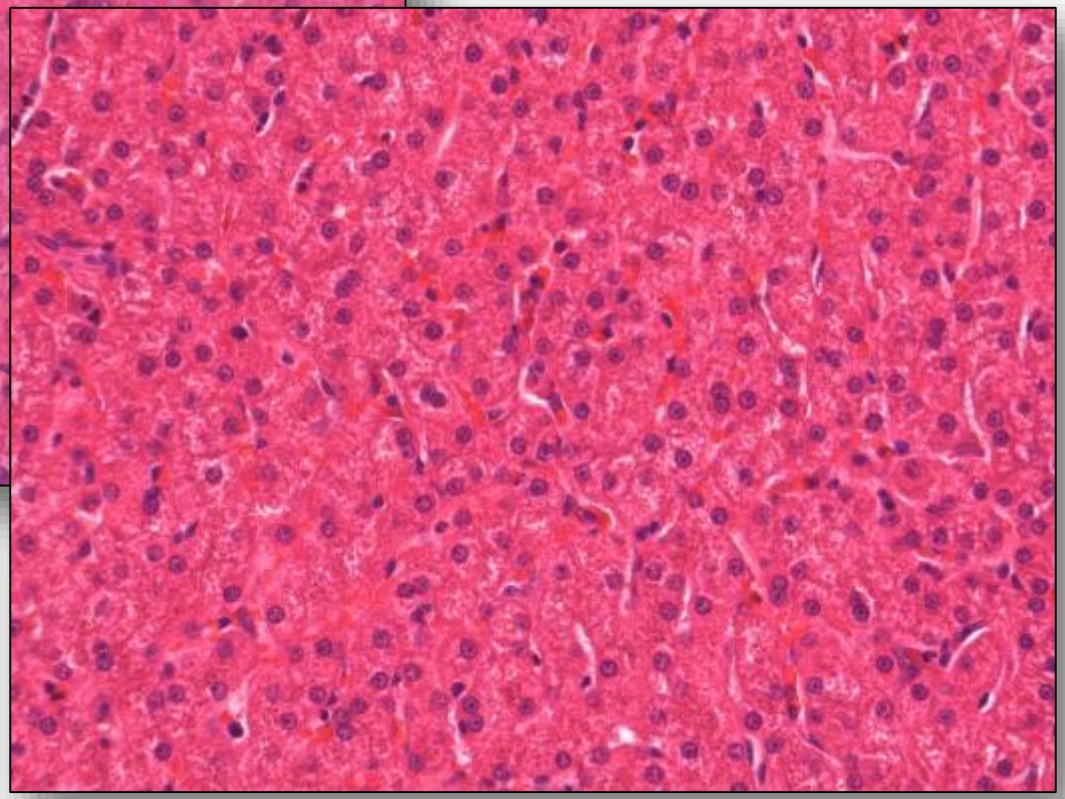


Liver: block 5x5 mm, F4%,1 day Dog.

Thickness of Wet Tissues for Fixation



Liver: block 10x10 mm, F4%,1 day Dog.



Liver: block 5x5 mm, F4%,1 day Dog.

Fixation techniques may cause misinterpretation

- **Perfusion vs immersion fixation causes different appearance of morphological structures in tissues.**

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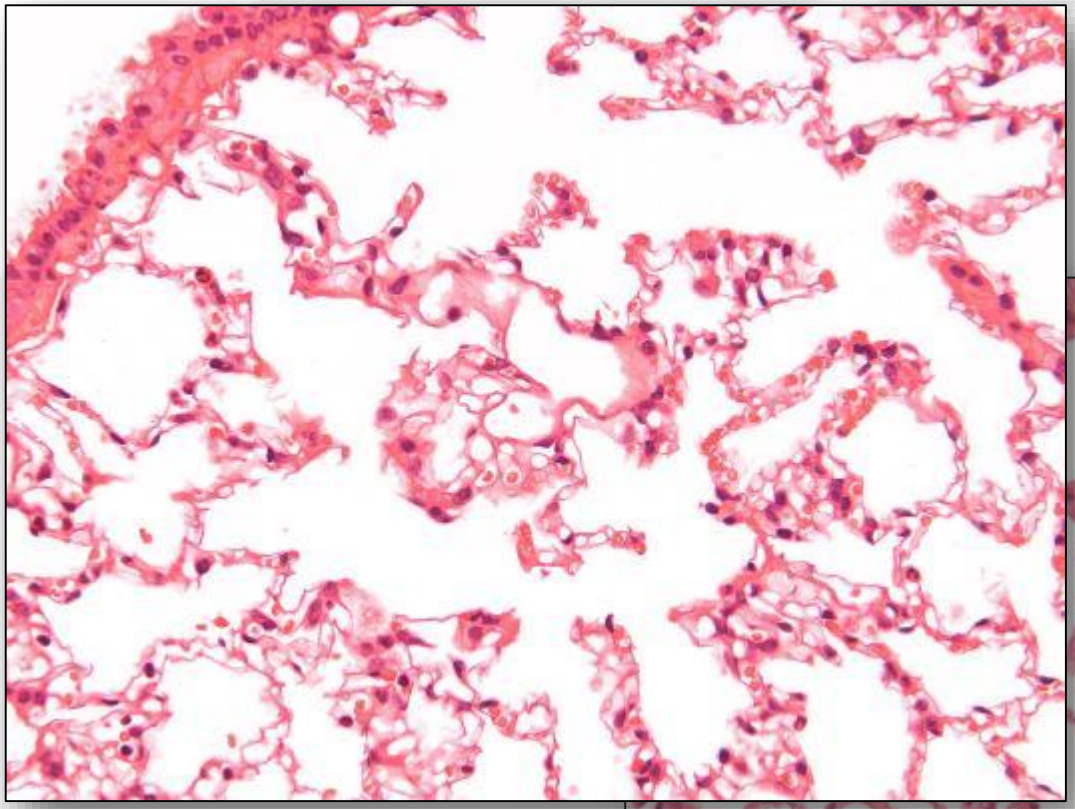
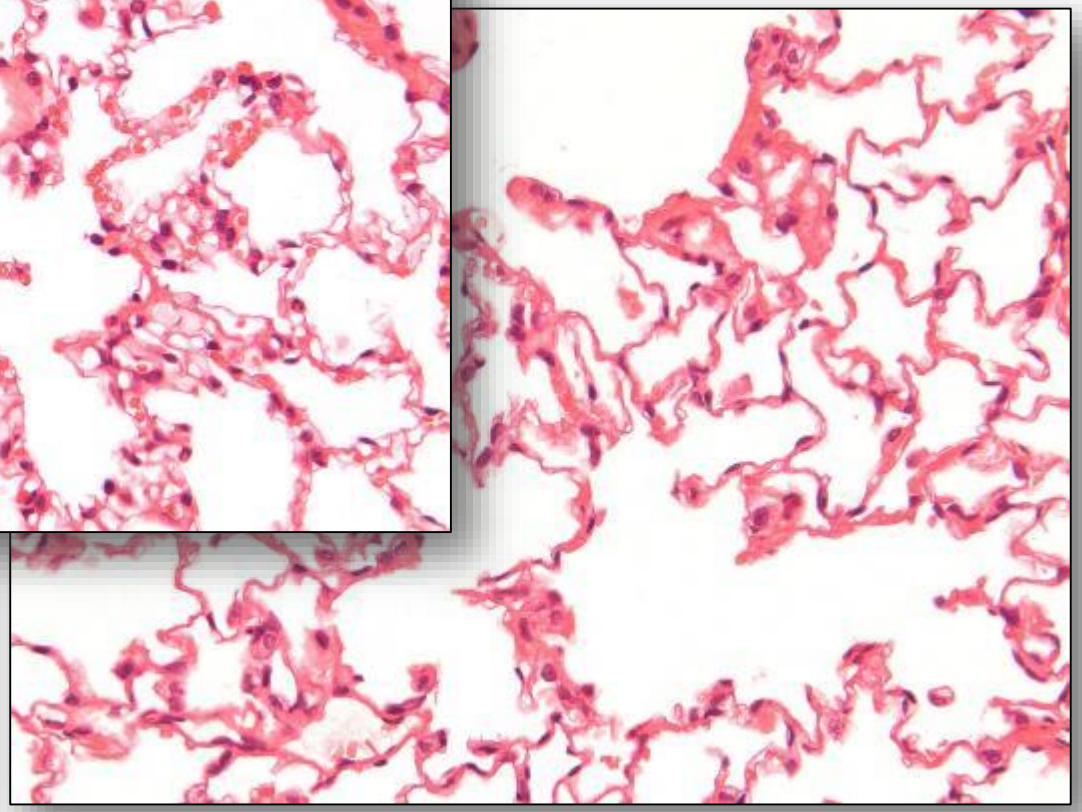
Perfusion with Formaldehyde

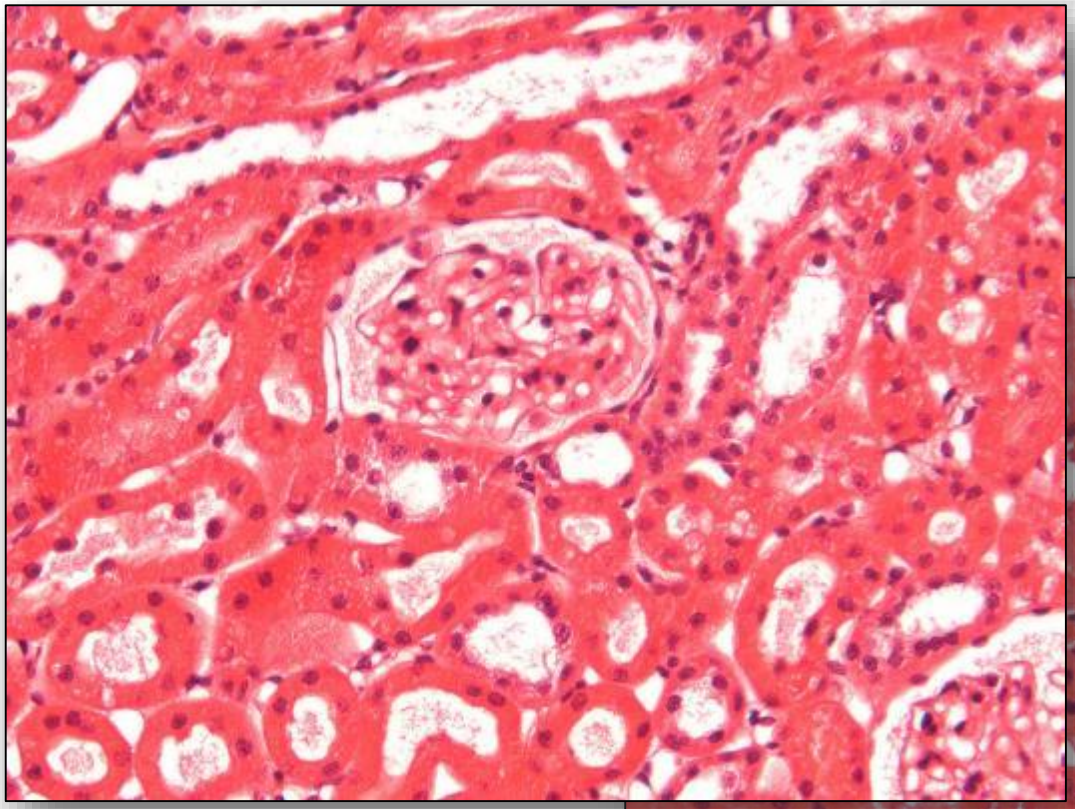


Lung: perfusion, F4%.

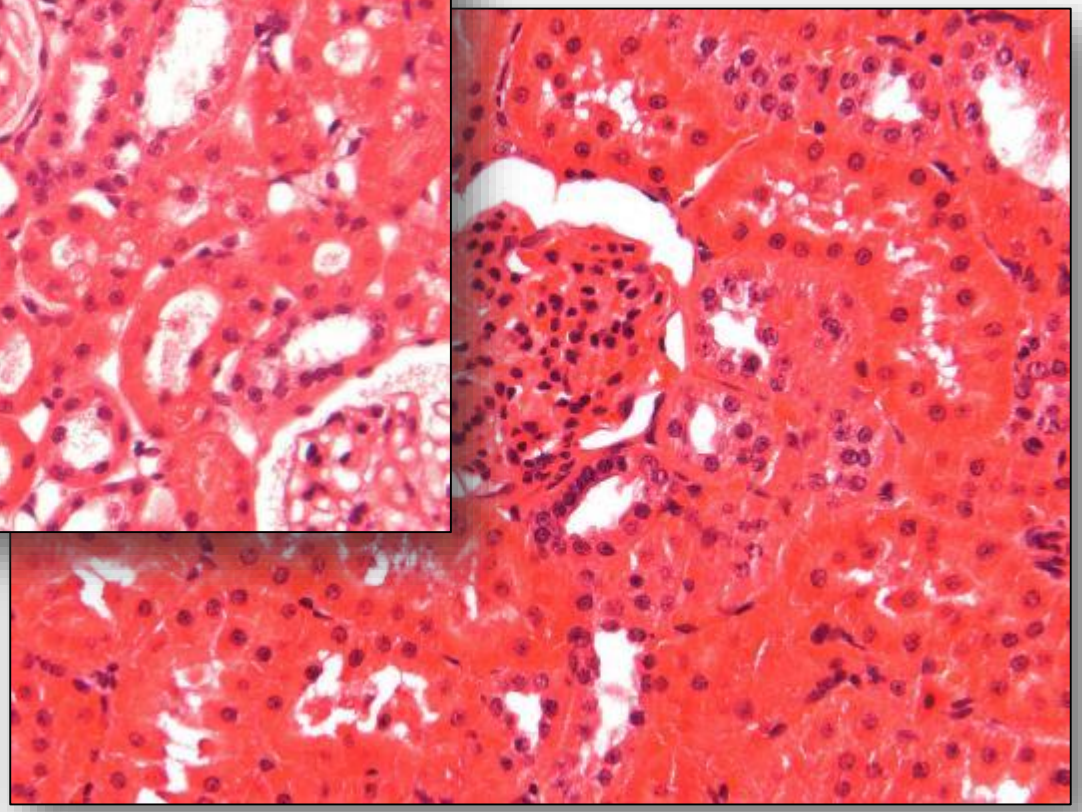


Lung: inflation, F4%.

Perfusion with Formaldehyde

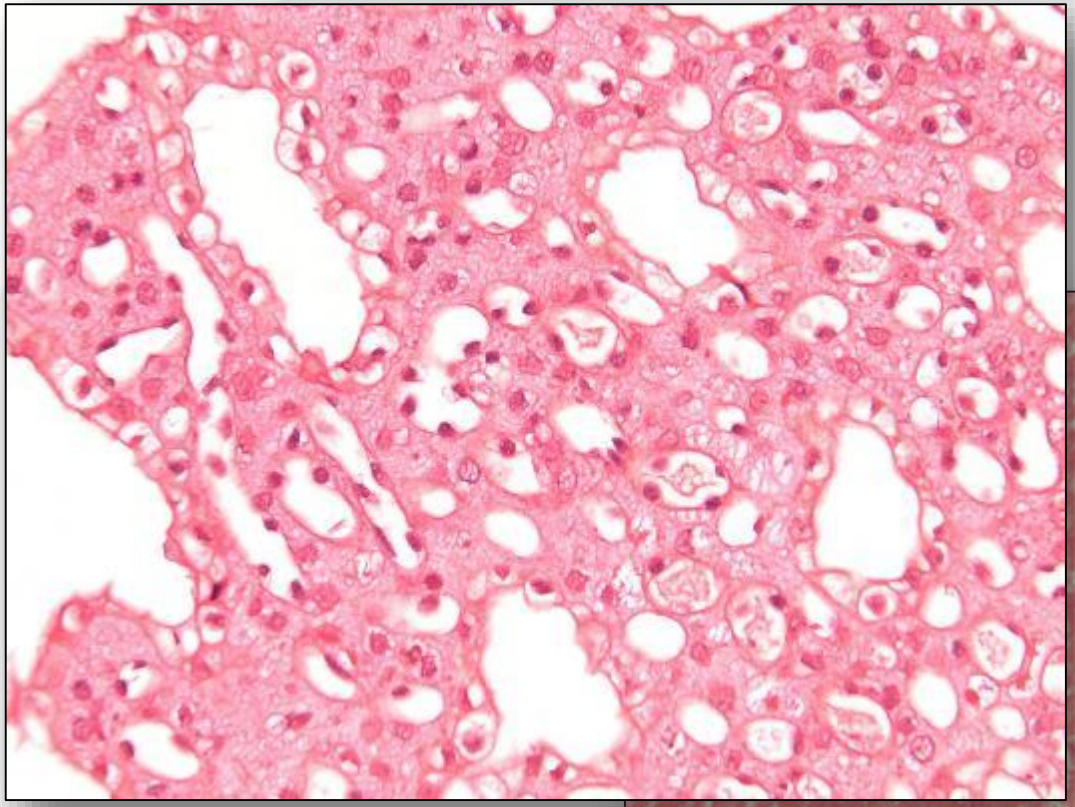


Kidney: perfusion, F4%.

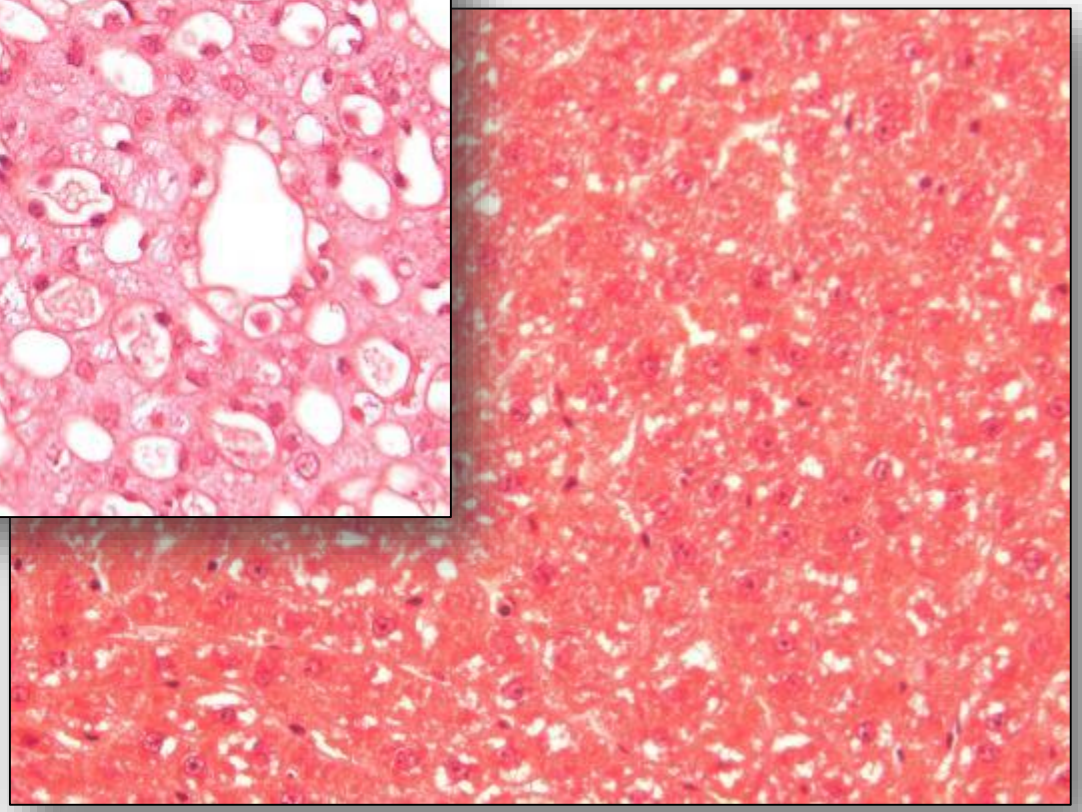


Kidney: immersion, F4%.

Perfusion with Formaldehyde



Liver: perfusion, F4%.



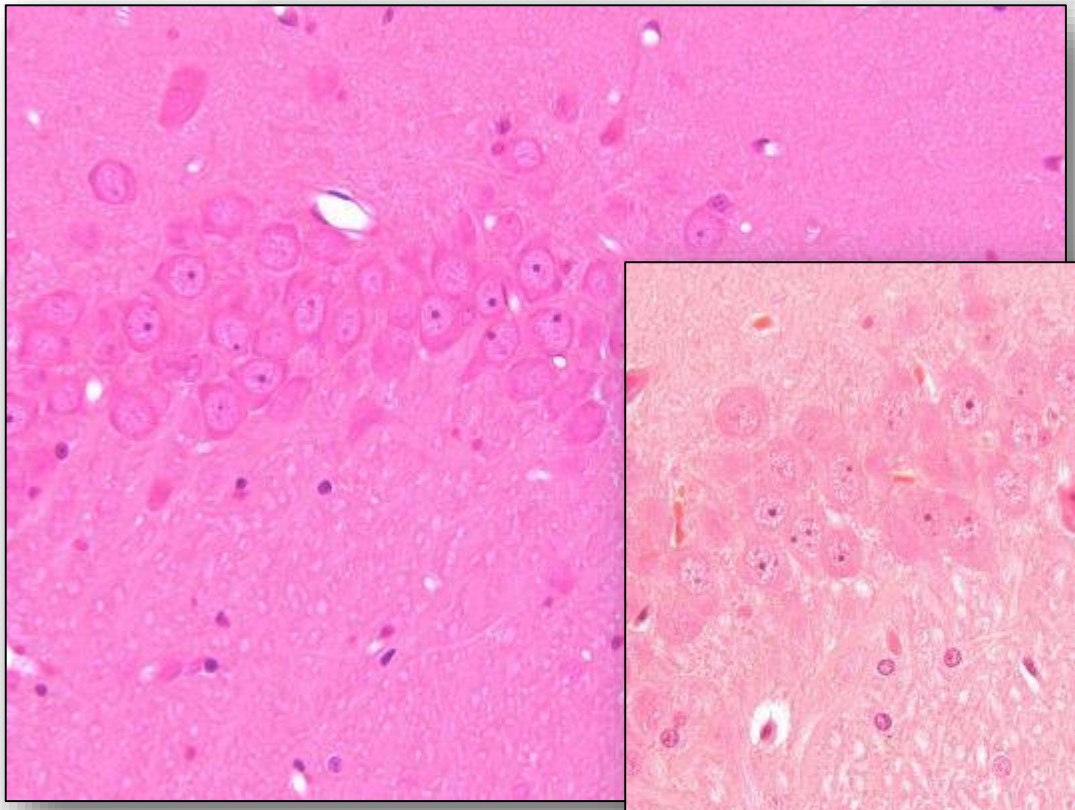
Liver: immersion, F4%.

Fixation techniques may cause misinterpretation

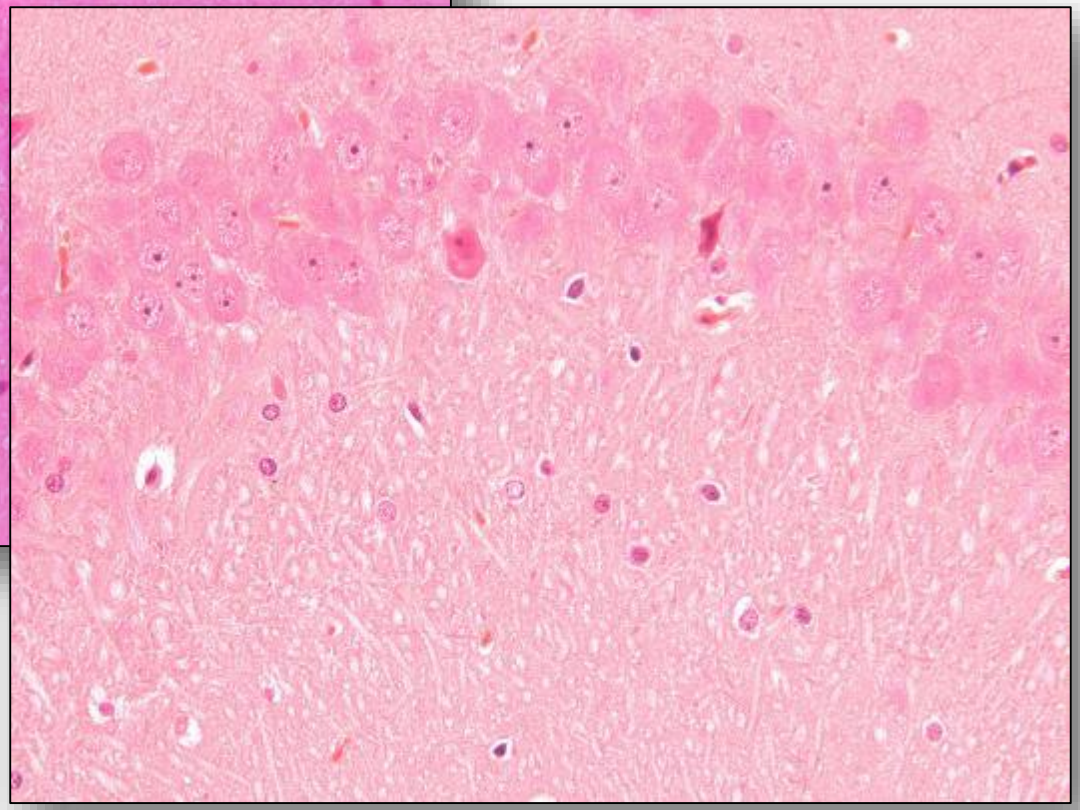
- **Perfusion technique is an absolute MUST in neuro-toxicity studies.**

- **Dark stained neurons in immersion fixed brains may cause overestimations and wrong settings of NOEL/NOAEL!**

Perfusion with Formaldehyde

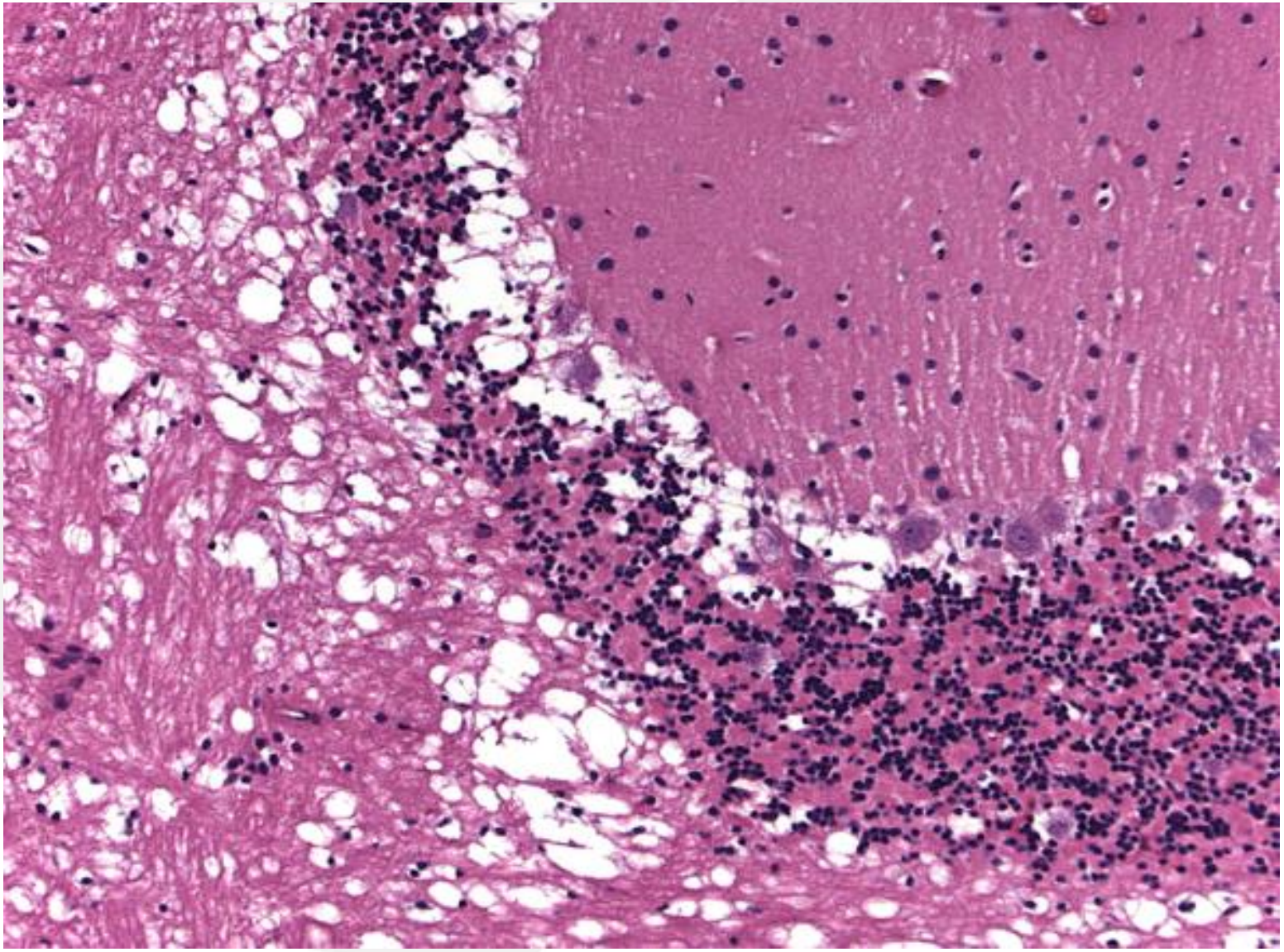


Brain: immersion, F4%.

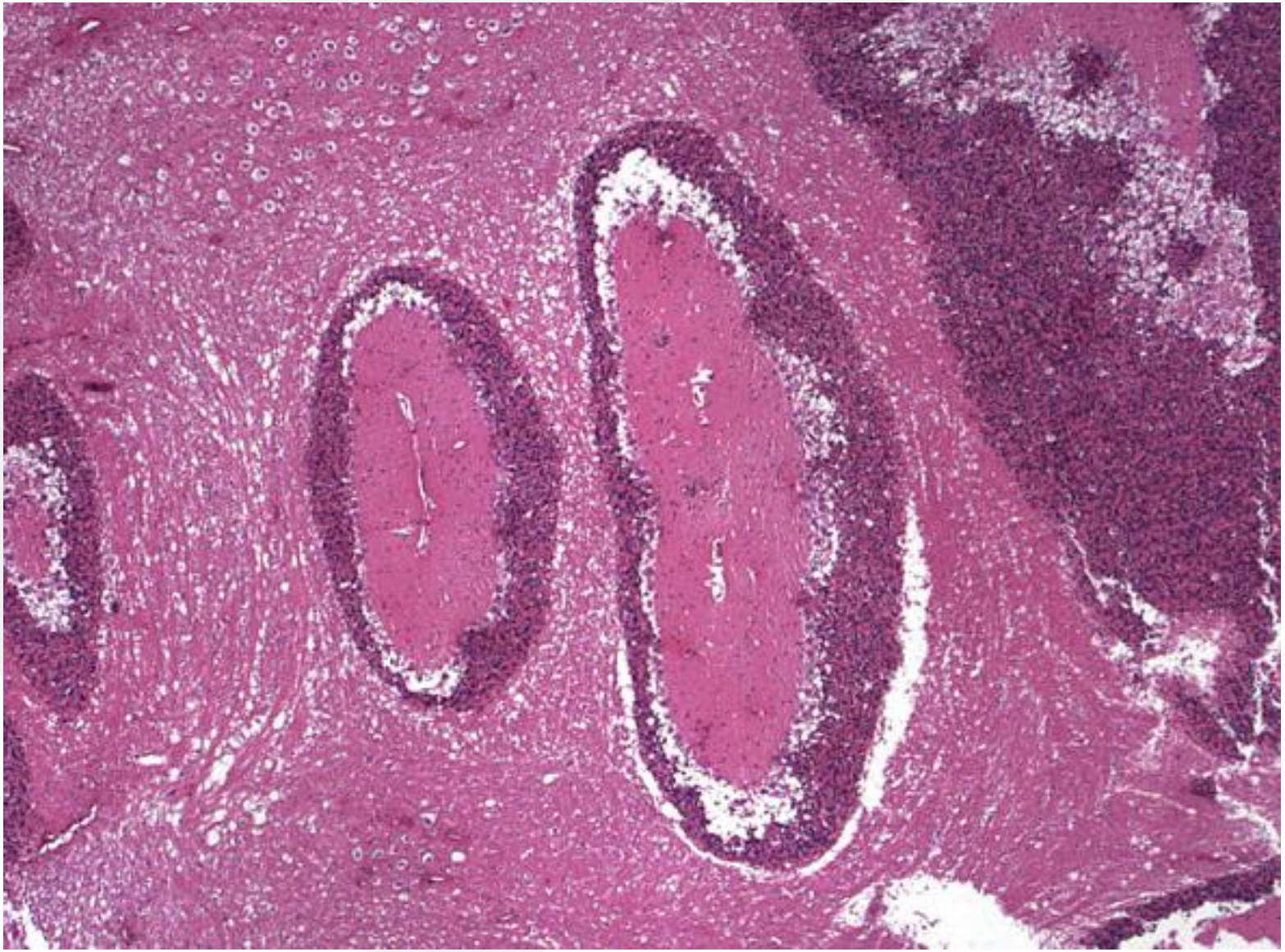


Brain: perfusion, F4%.

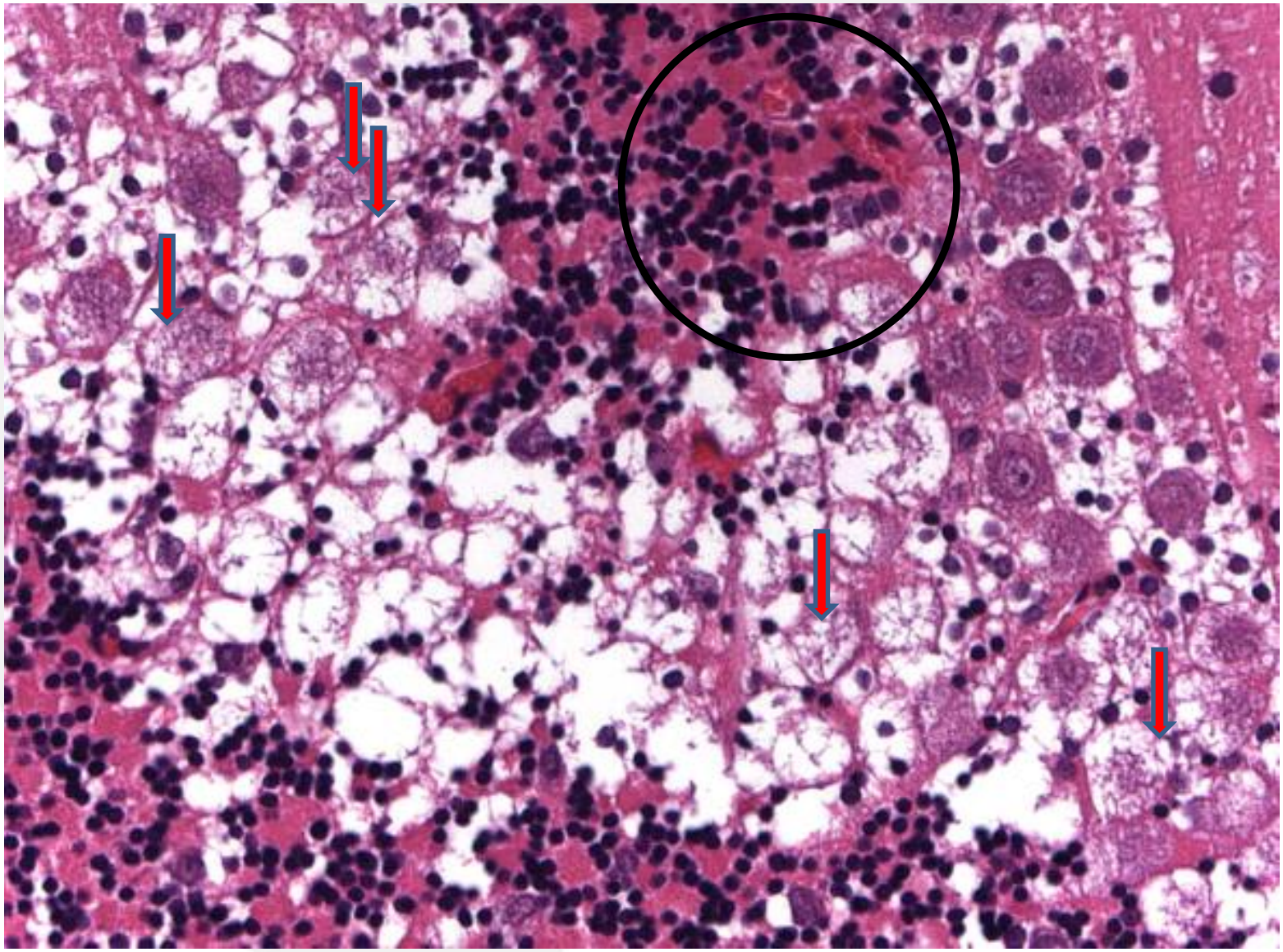
Artefacts by overpression in brain



Artefacts by overpression in brain



Artefacts by overpression in brain



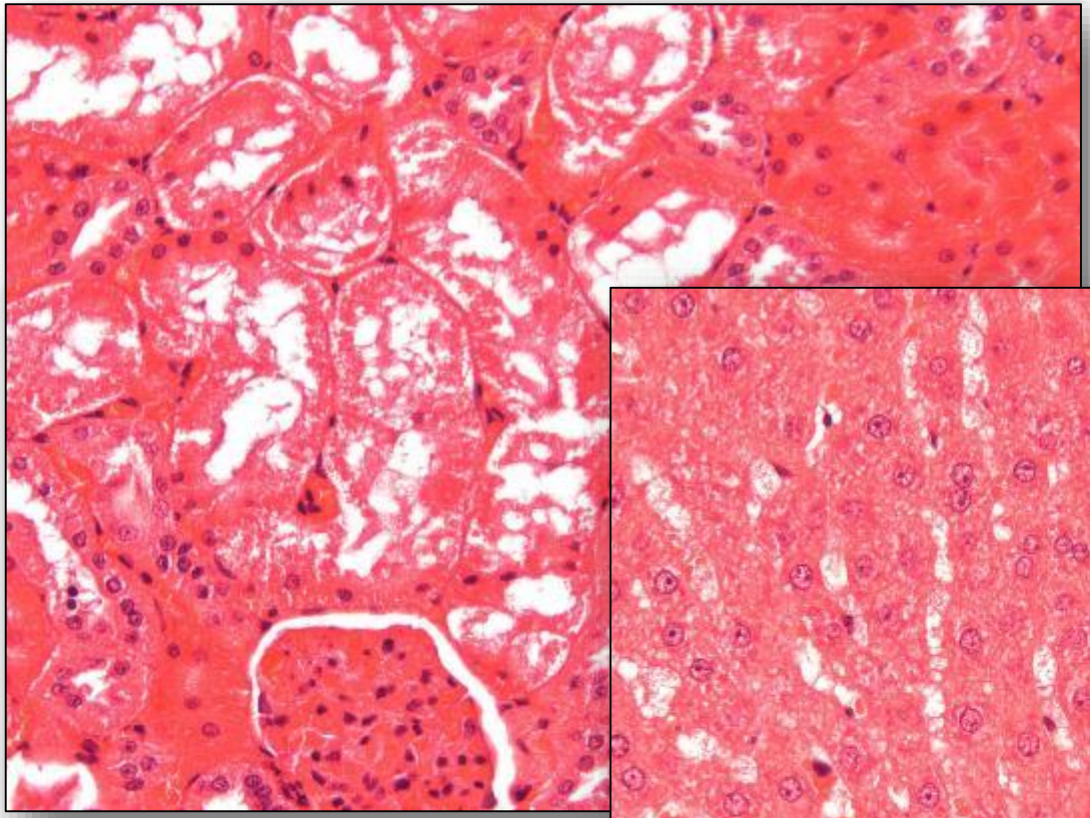
Storage of decedent animals prior to necropsy

- issue mainly in oncogenicity studies

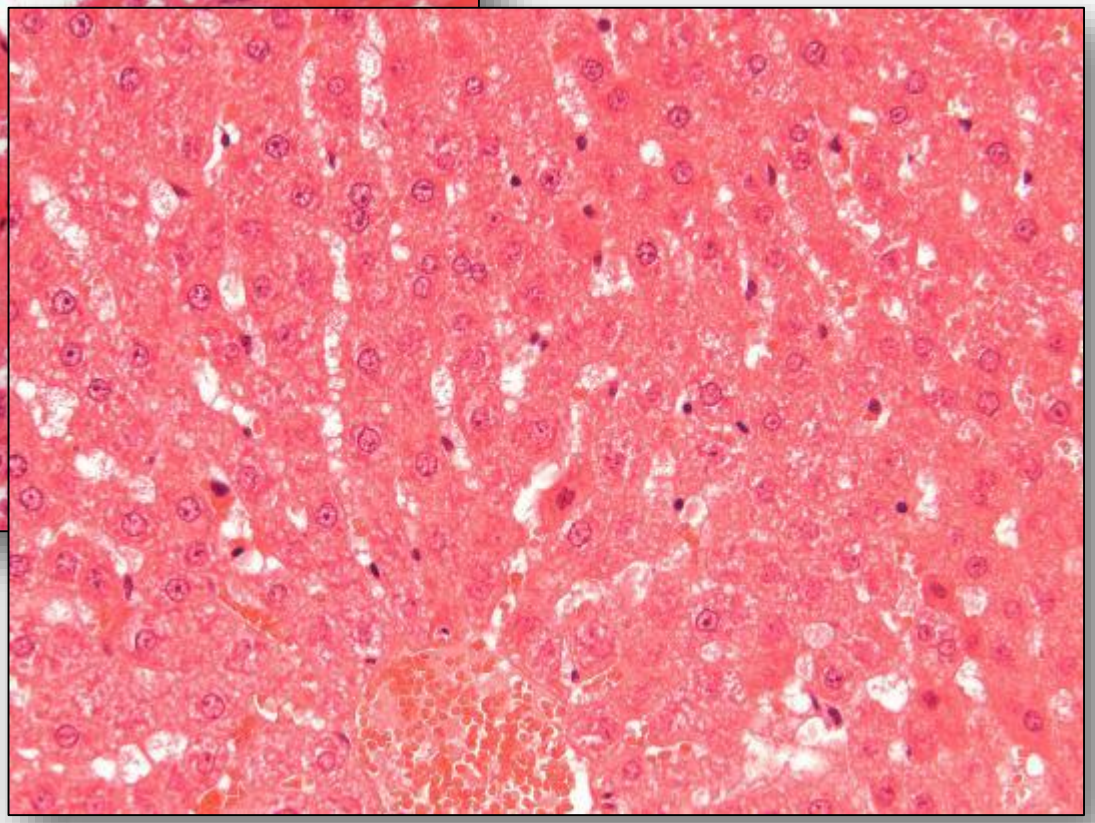
- trained available personell
- weekend programs

- fridgerator is a possibilty but freezers are obsolete

Cooled in fridgerator 5-8 ºC

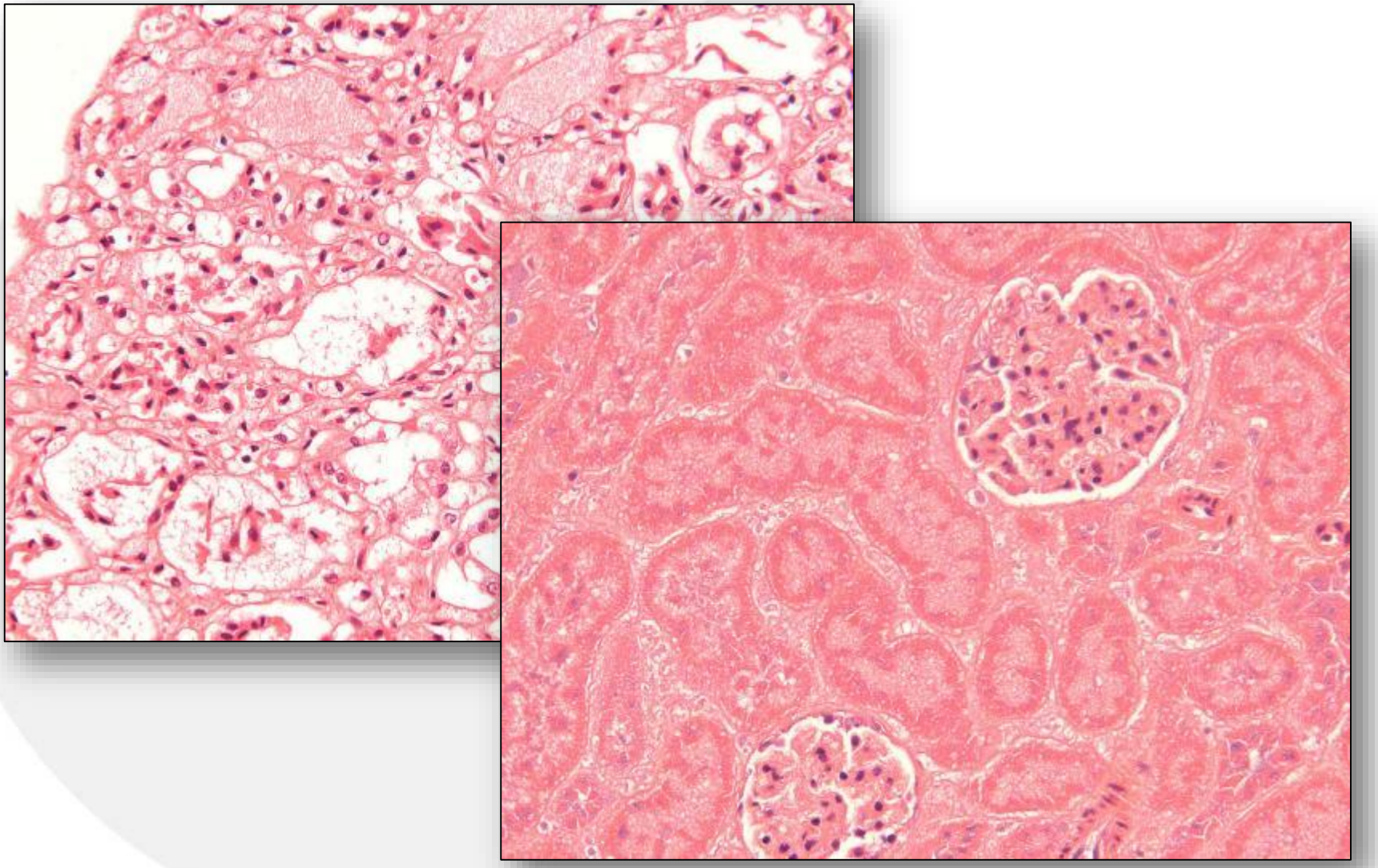


Liver: 24hr, fridgerator, F4%.

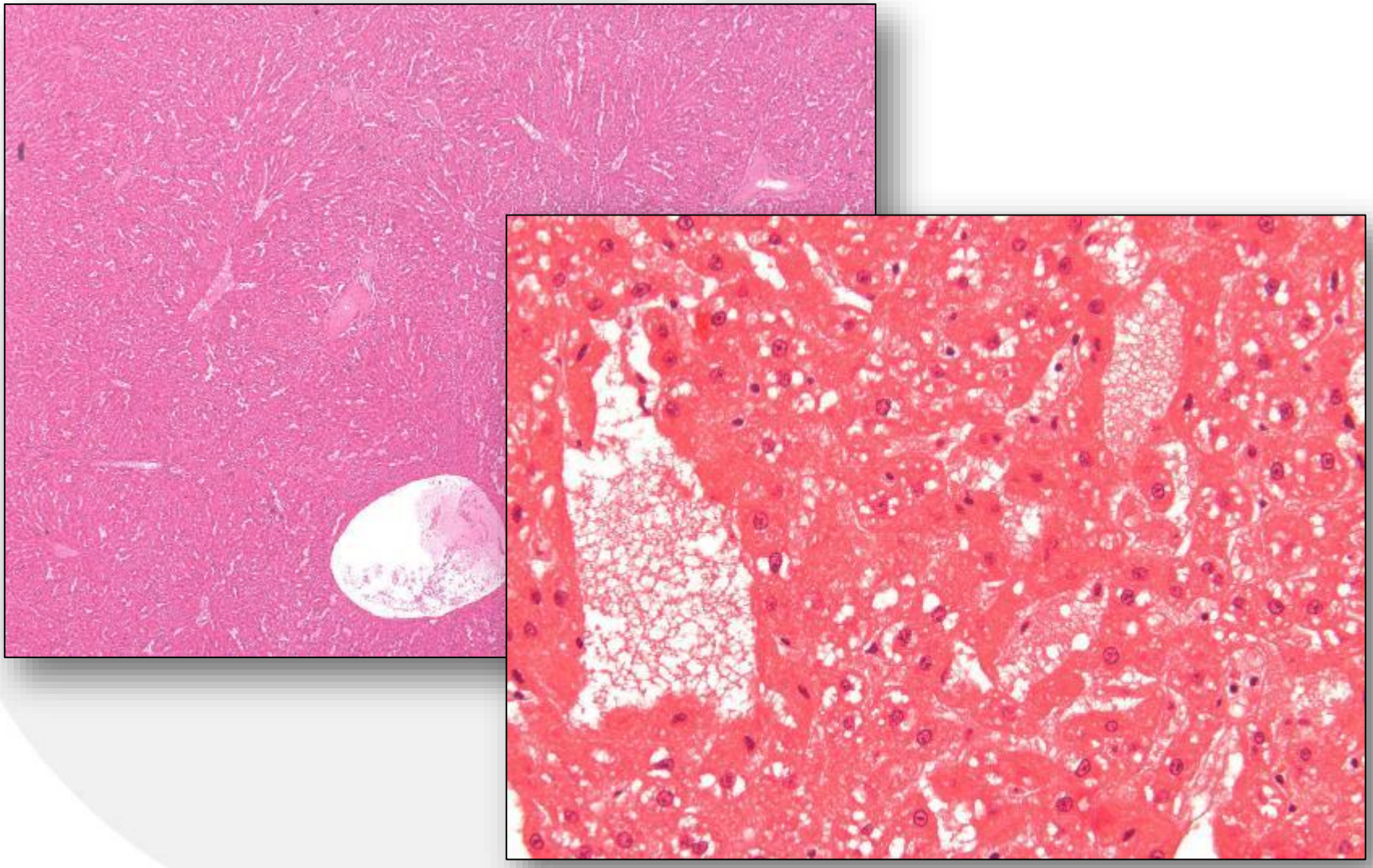


Kidney: 24hr, fridgerator, F4%.

Froozen Tissues: Kidney



Frozen Tissues: Liver



Postmortal changes may cause in misinterpretation

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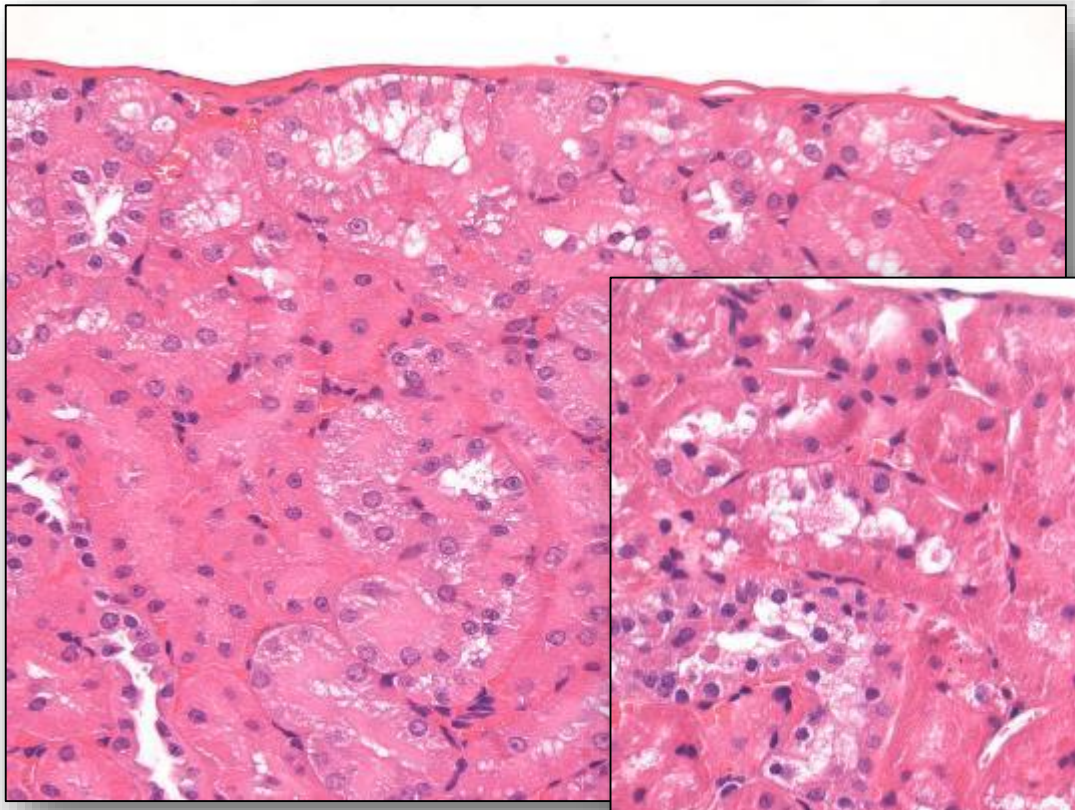
- Study on induced artifacts performed at Harlan incl.

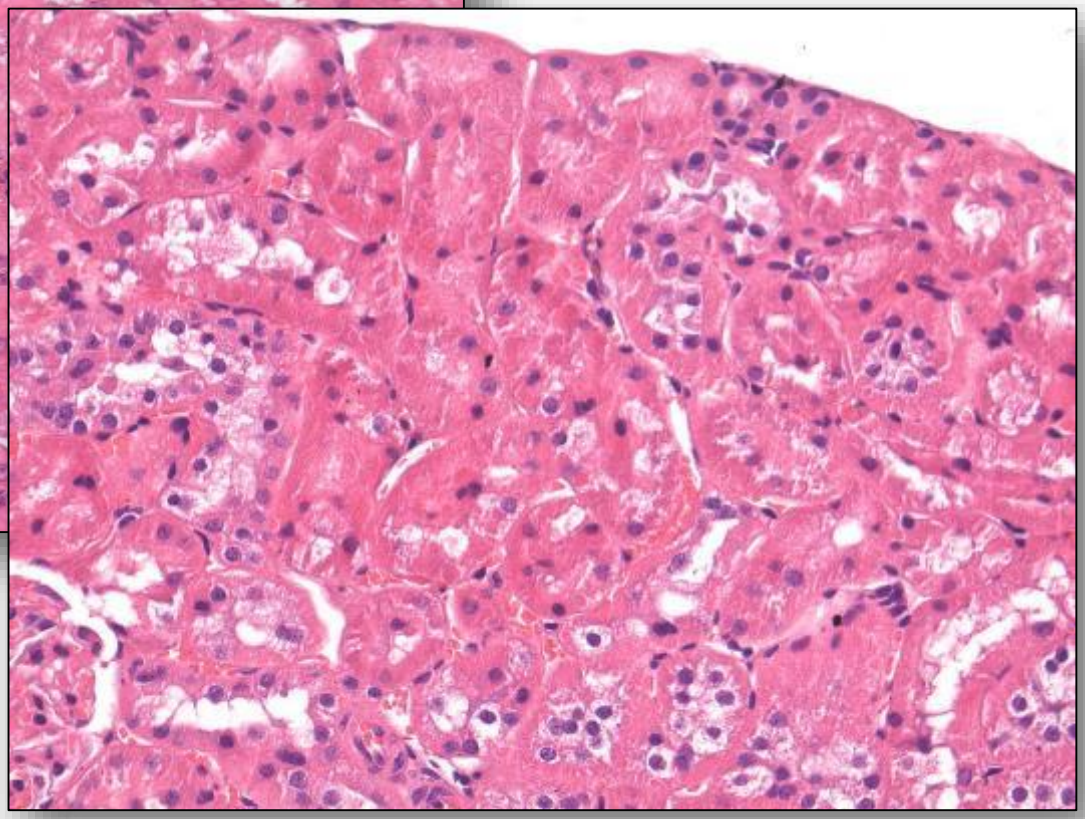
- use of different fixatives, fixation time, drying out of organs, cooling, freezing, autolyis etc.

- Inappropiate fixatives may cause alterations confused with induced lesions e.g. degeneration, necrosis etc.

Drying Artifacts

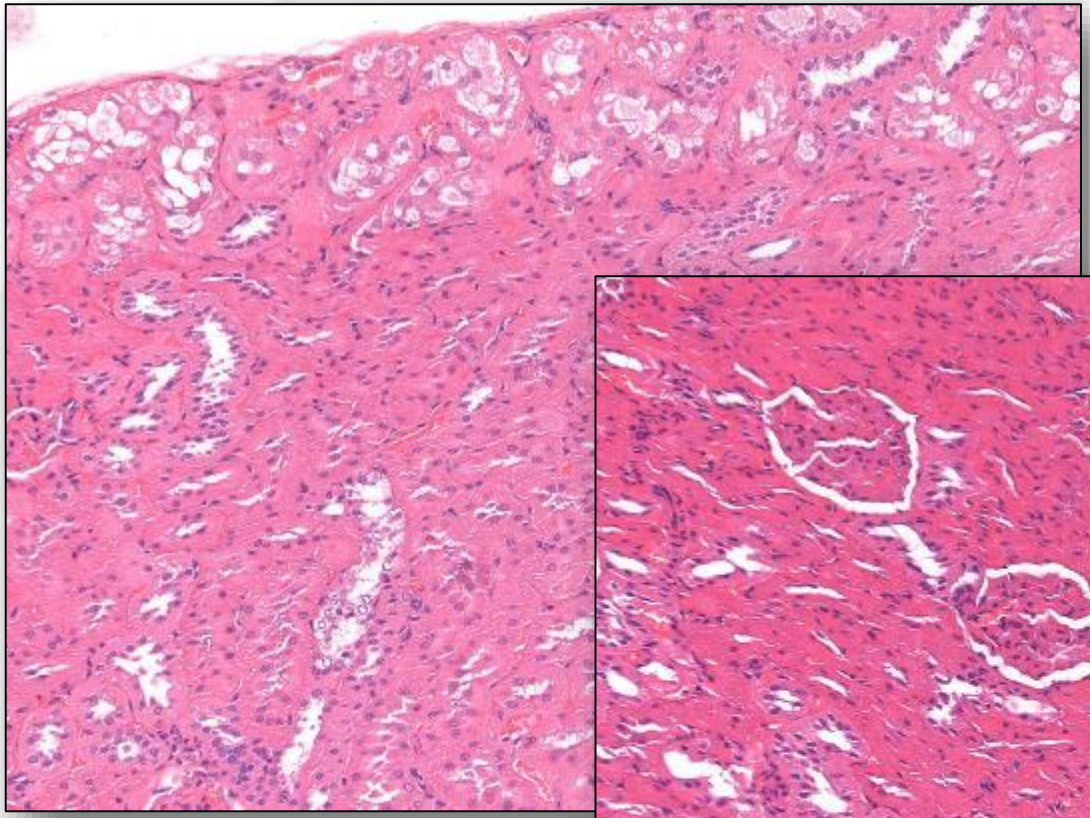


Kidney: 60‘, room temperature, F4%.

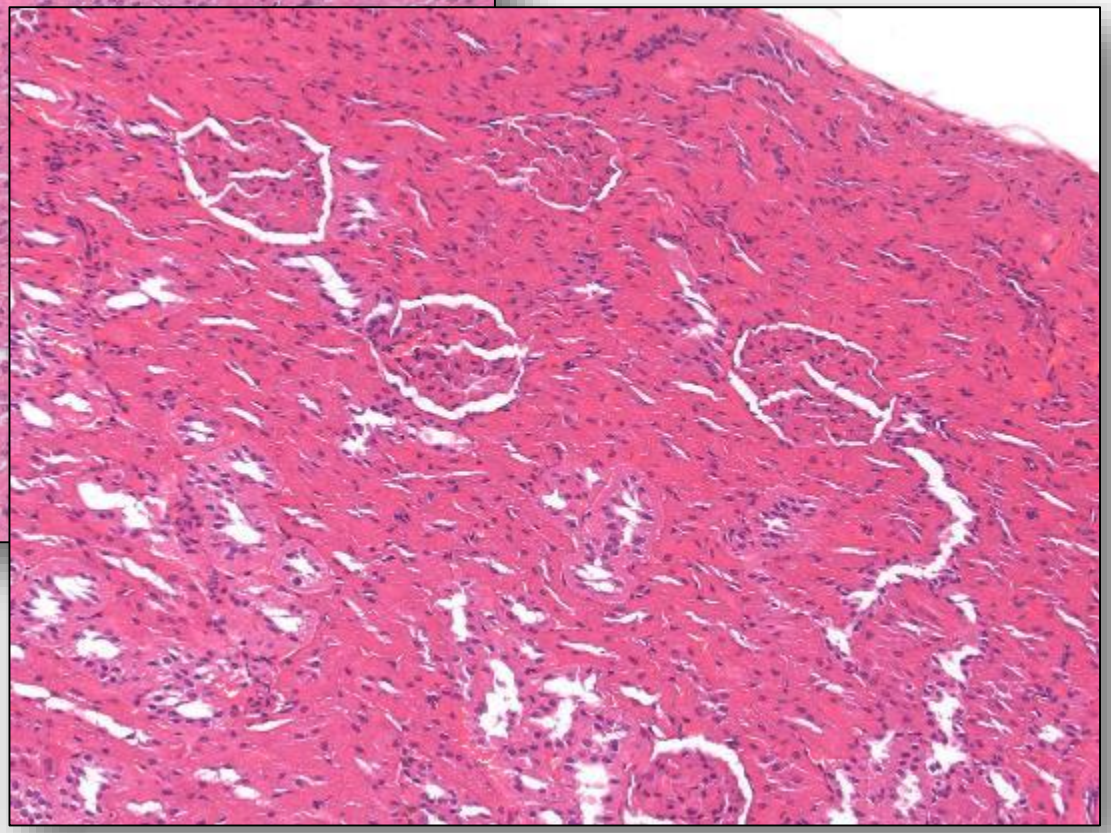


Kidney: 15‘, room temperature, F4%.

Autolysis

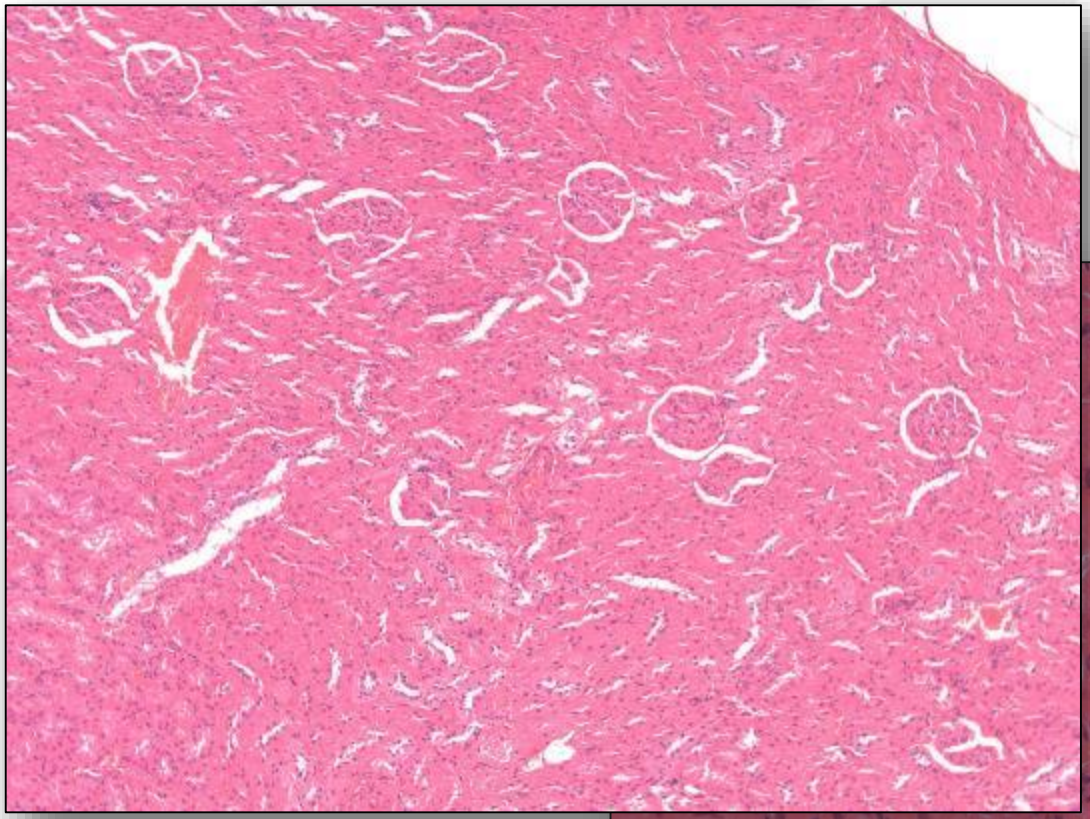


Kidney: 8hr, room temperature, F4%.

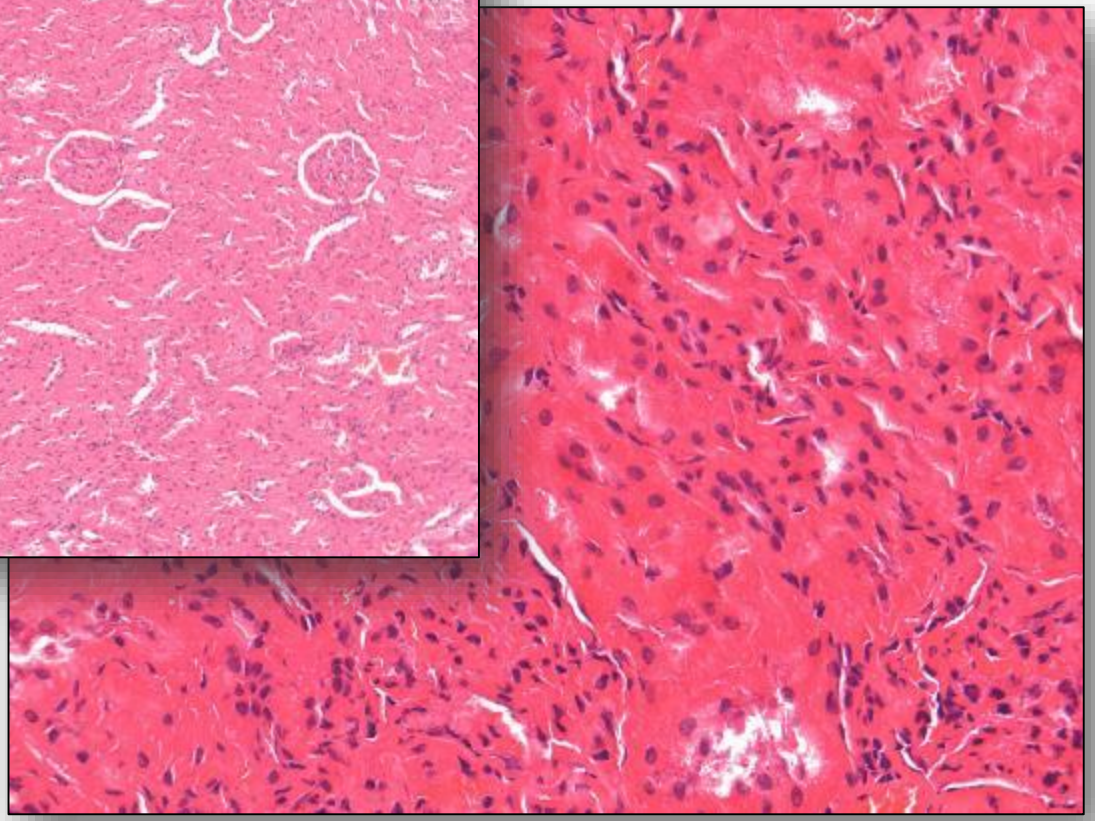


Kidney: 4hr, room temperature, F4%.

Autolysis

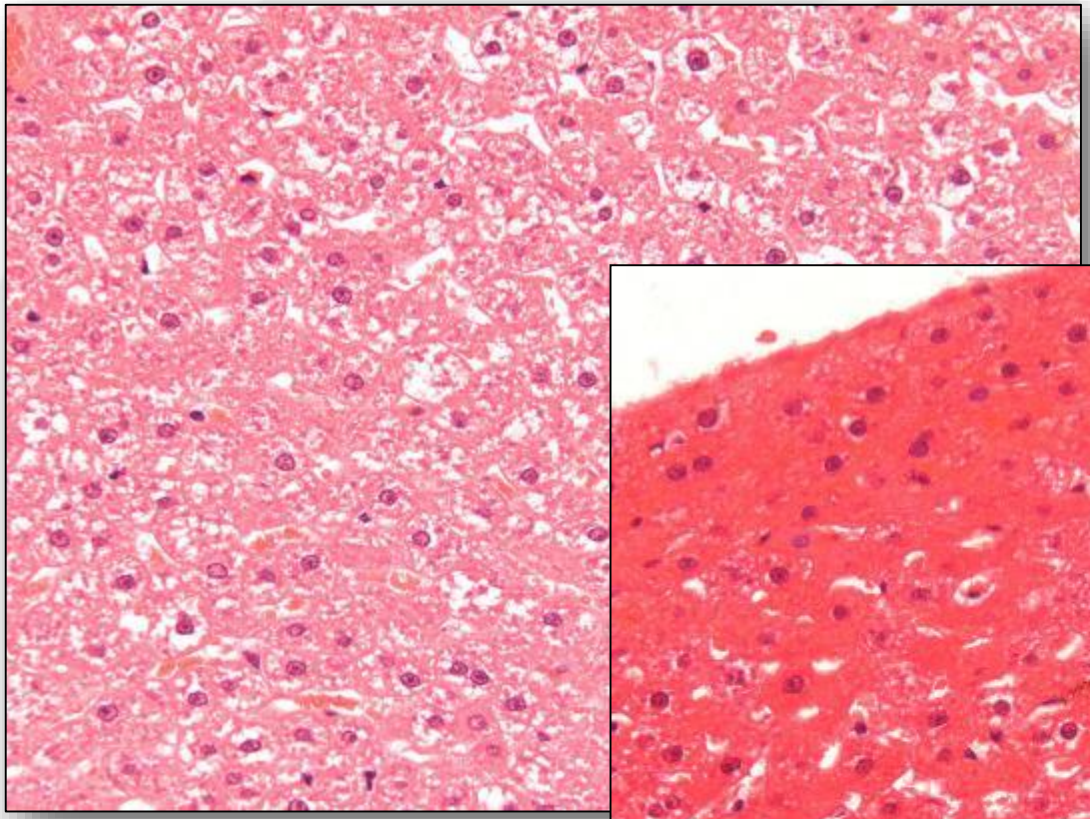


Kidney: 24hr, room temperature, F4%.

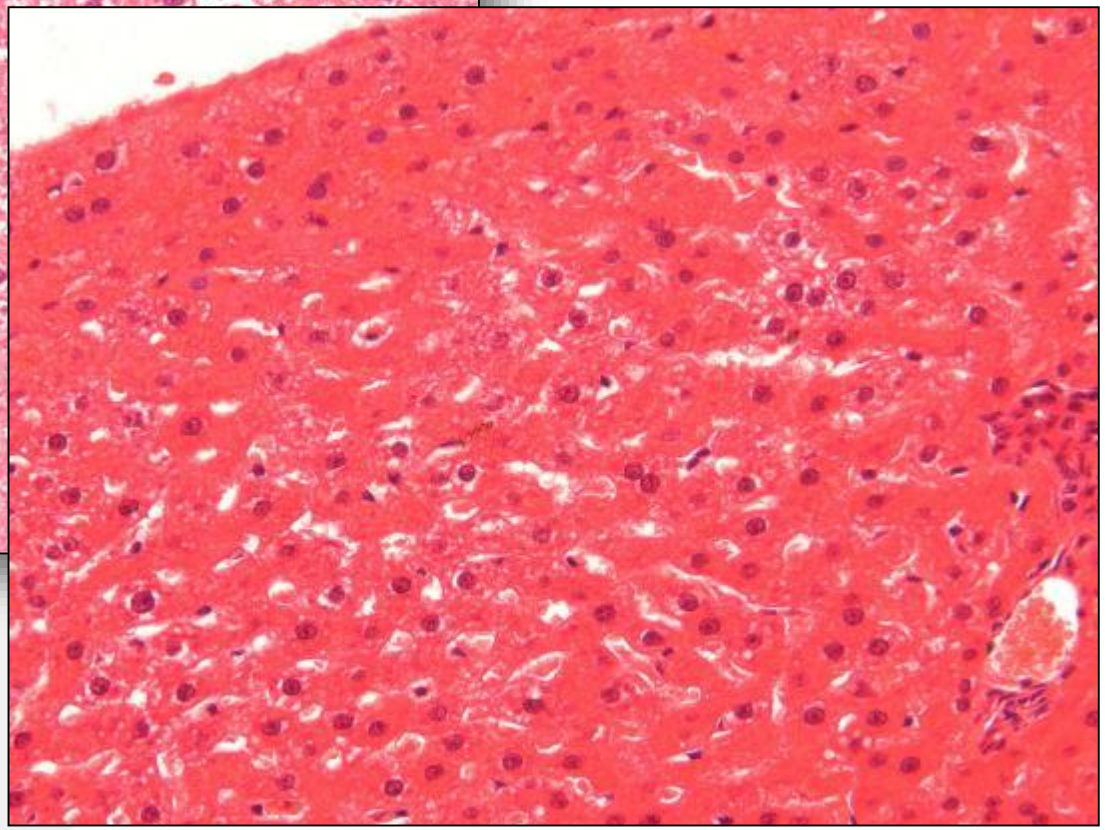


Kidney: 12hr, room temperature, F4%.

Autolysis

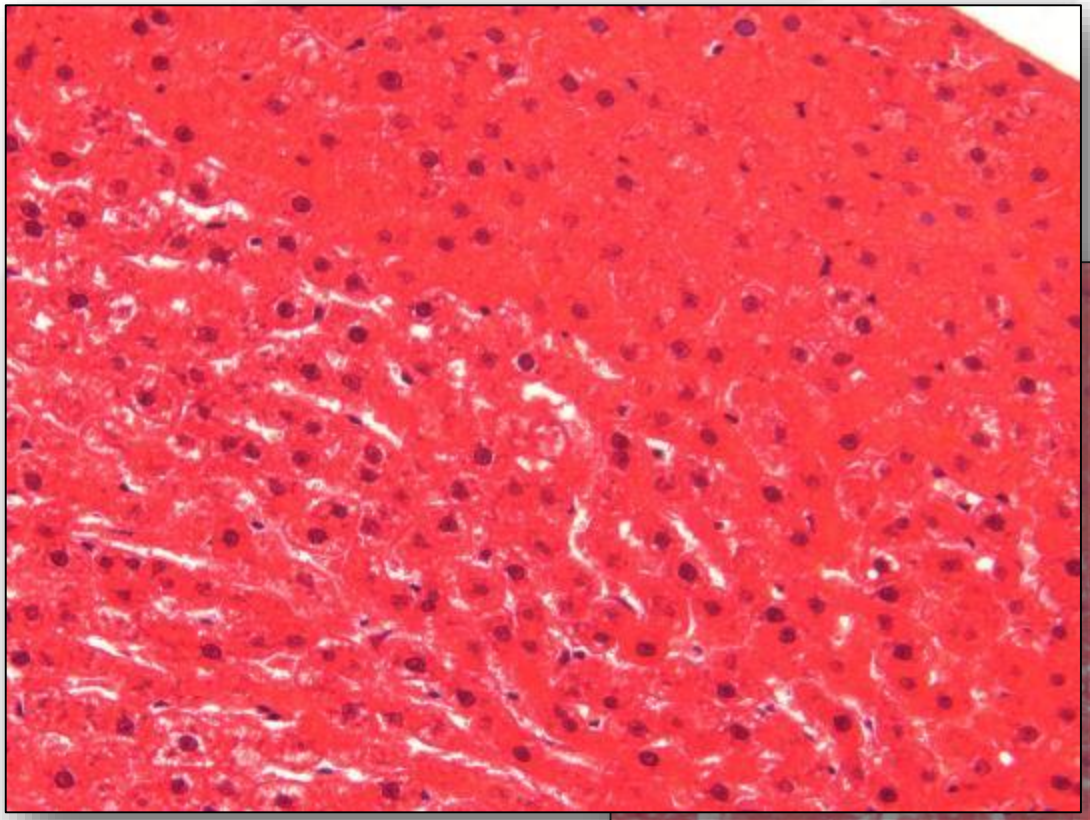


Liver: 8hr, room temperature, F4%.

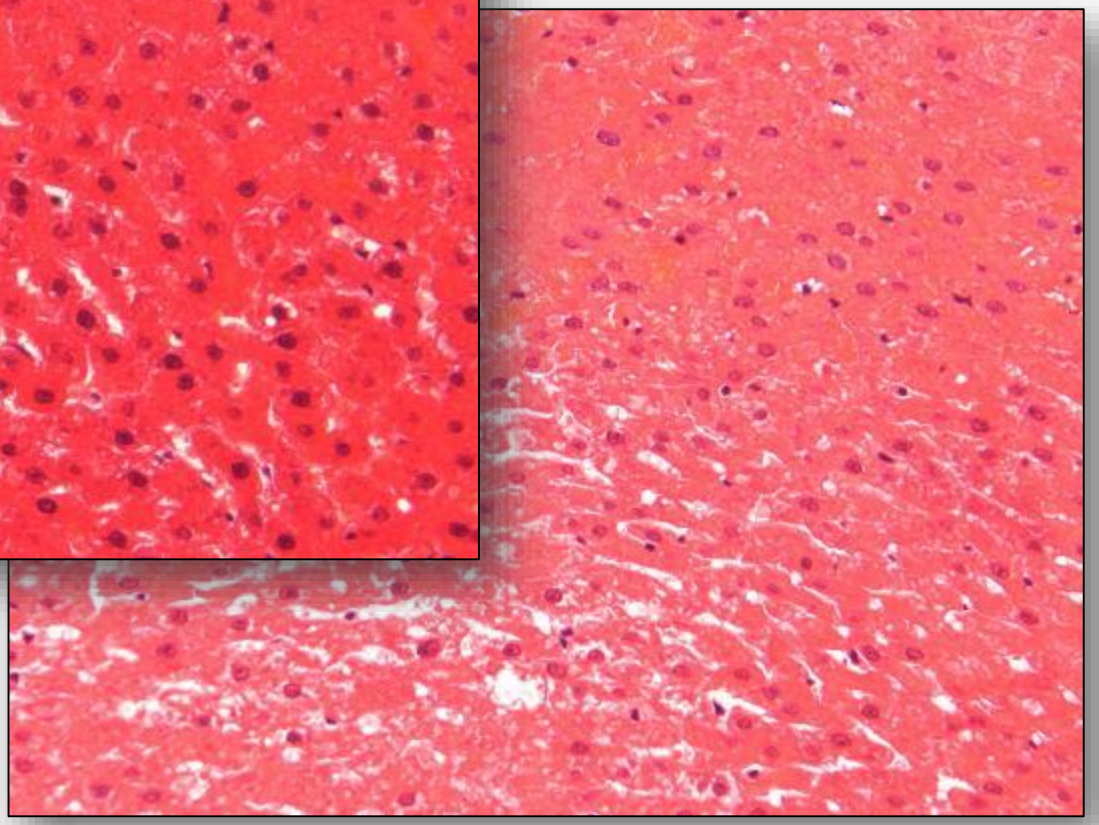


Liver: 4hr, room temperature, F4%.

Autolysis

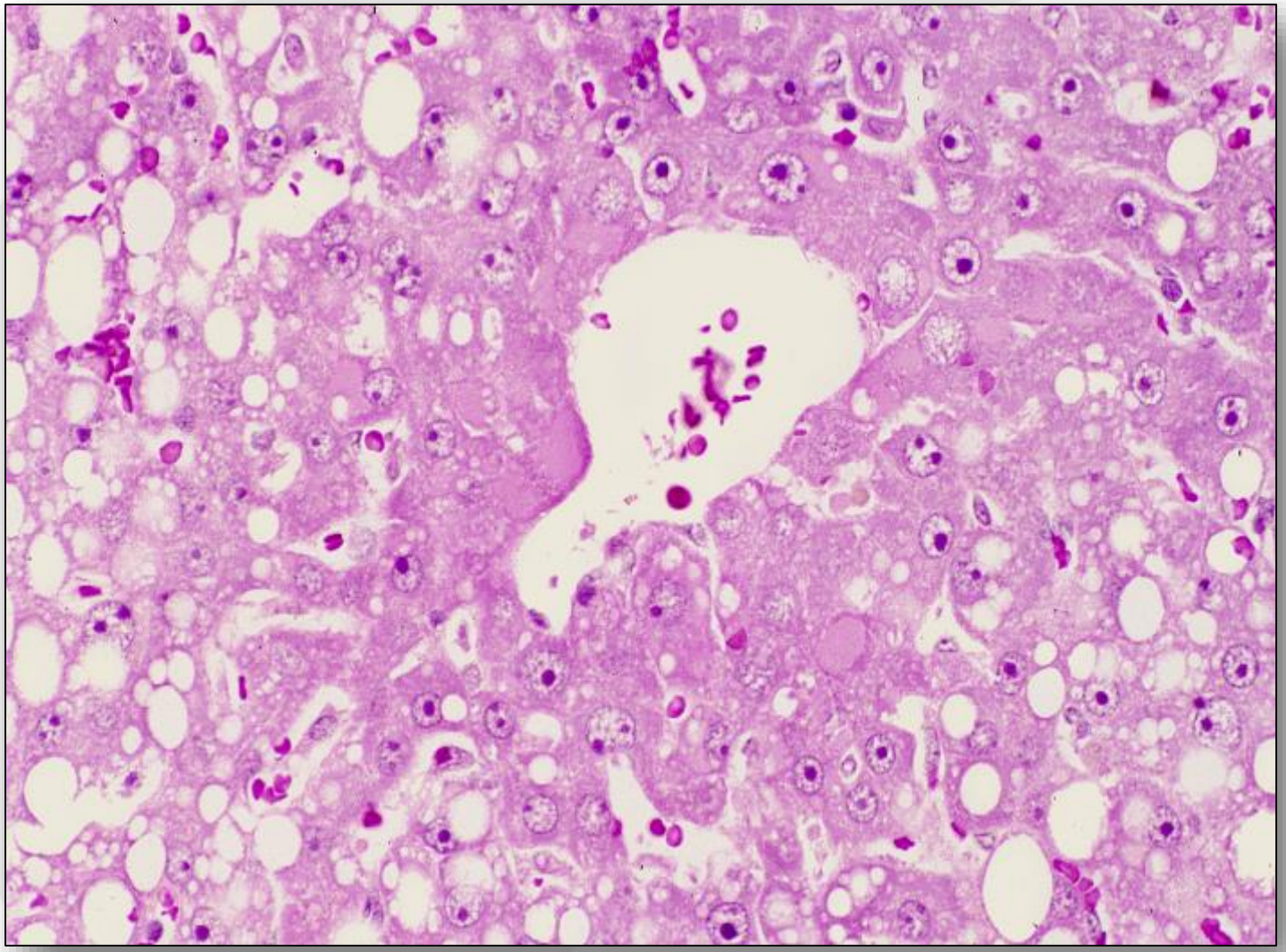


Liver: 24hr, room temperature, F4%.



Liver: 12hr, room temperature, F4%.

Postmortem hepatocellular cytoplasmic vacuolation



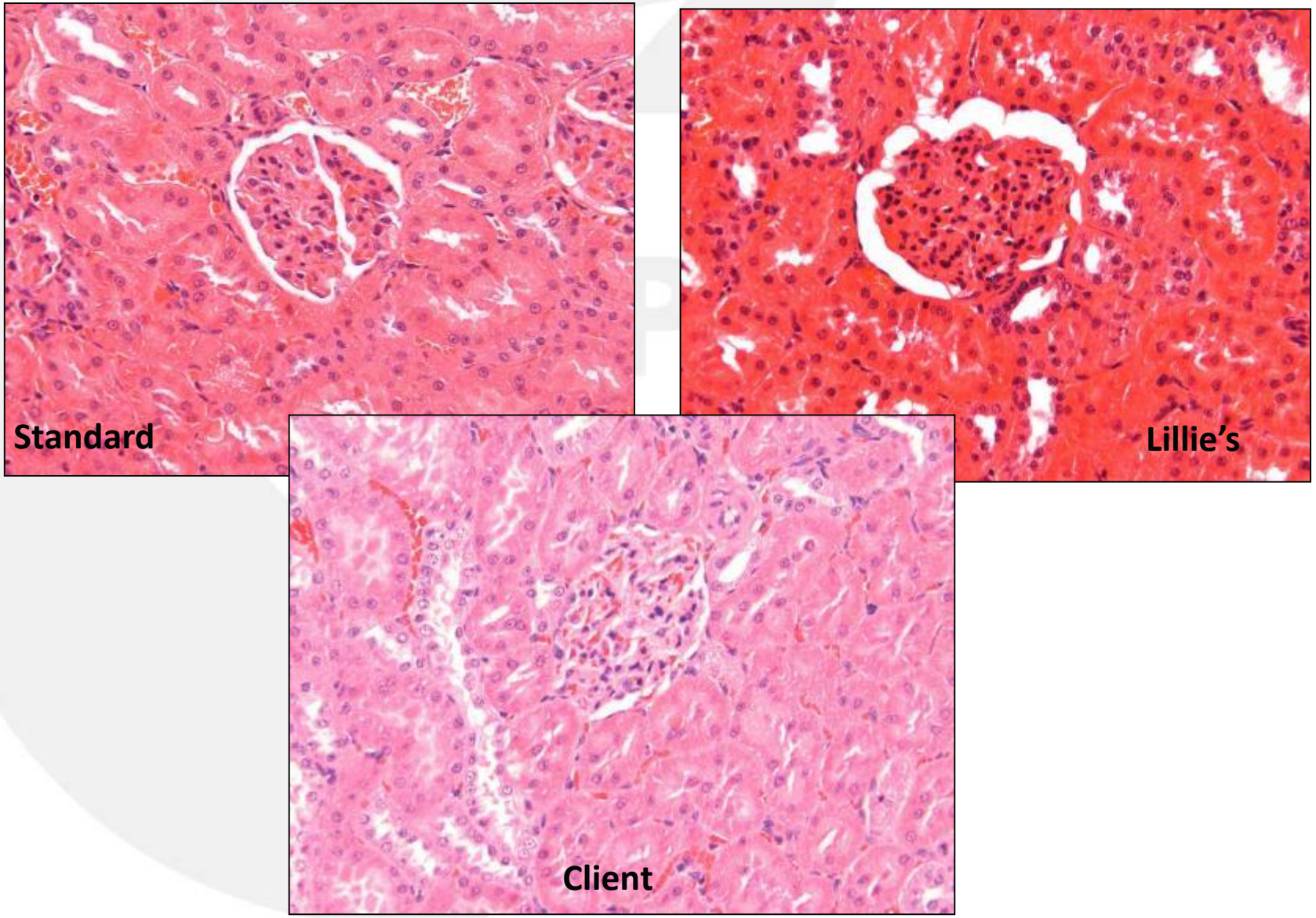
Staining

- routine staining may reveal different appearance of structures EVEN by hematoxylin&eosin

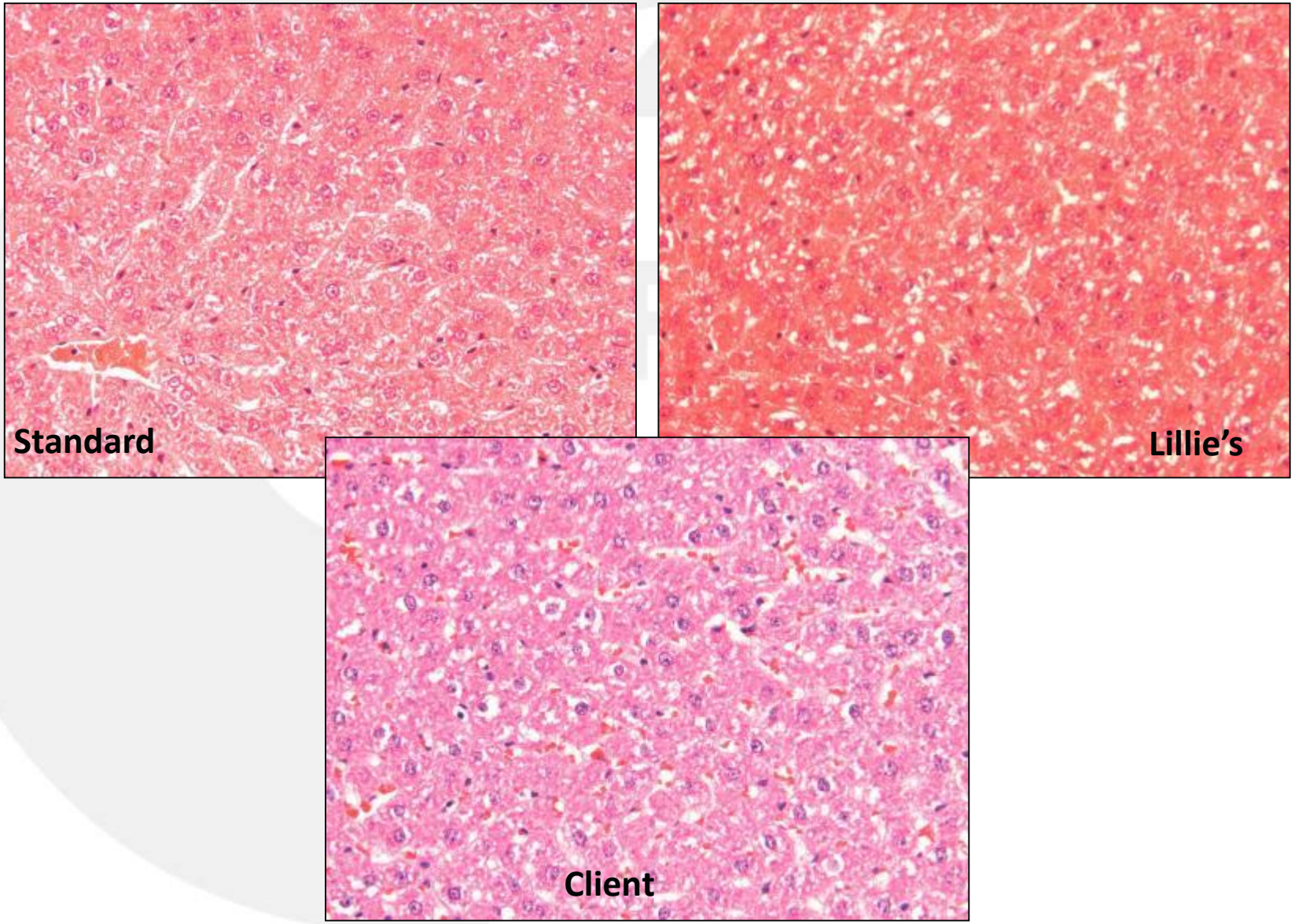
- personal experience and training

- special stains and immunohistochemistry may cause confusion if:
  - tissues under examination that are rarely being evaluated
  - false negative or positive immune reactivity

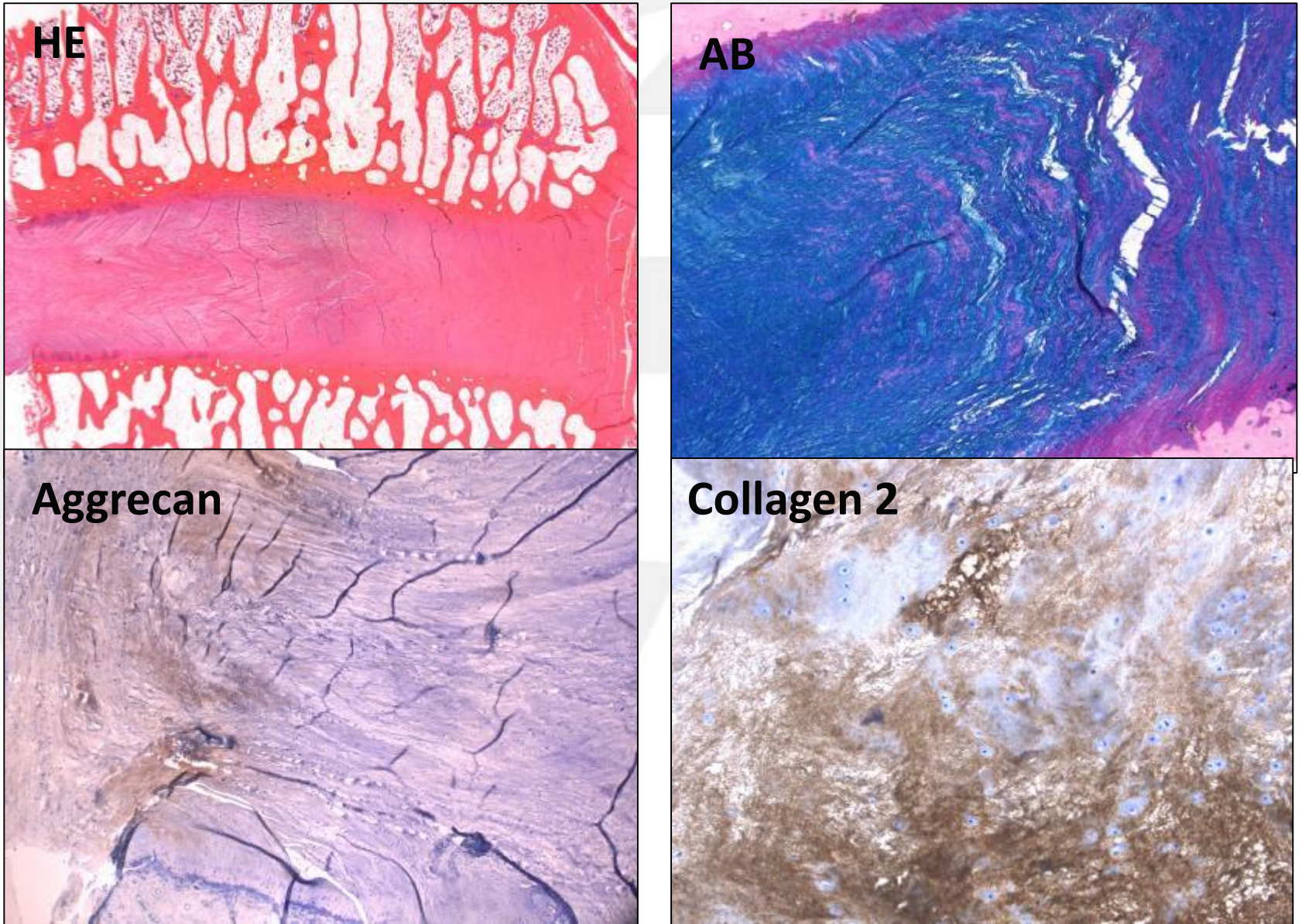
Different H&E Staining: Kidney



Different H&E Staining: Liver



Rarely evaluated tisues: e.g. Sheep-intervetrebral discs



What about experience?

- rare or unusual lesions may be unknown to the pathologist

- over many years there was a lack in case publications

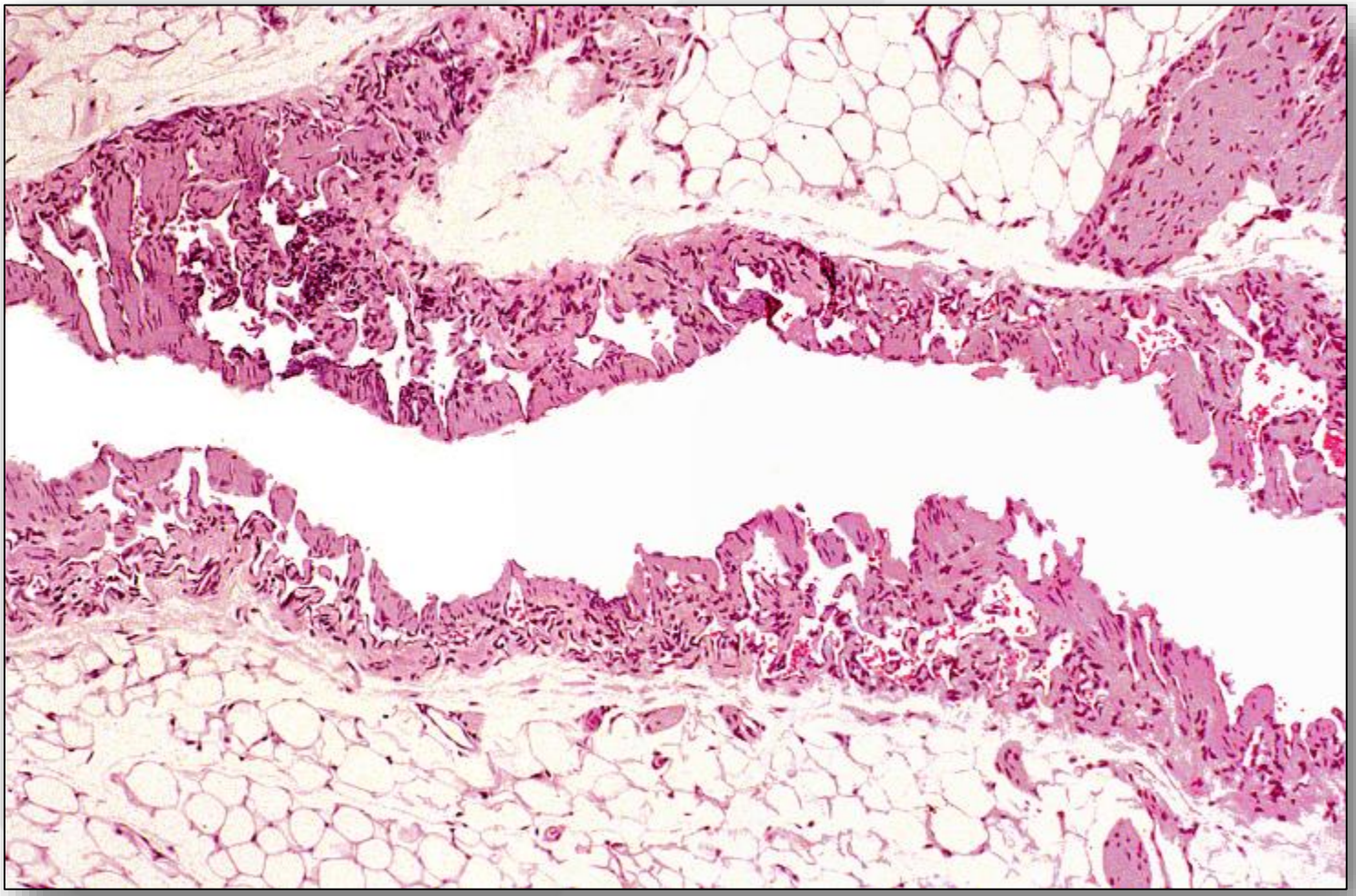
- PR should be applied to each study

‚Reading an oncogenicity study is sooo difficult....!
Is this true?

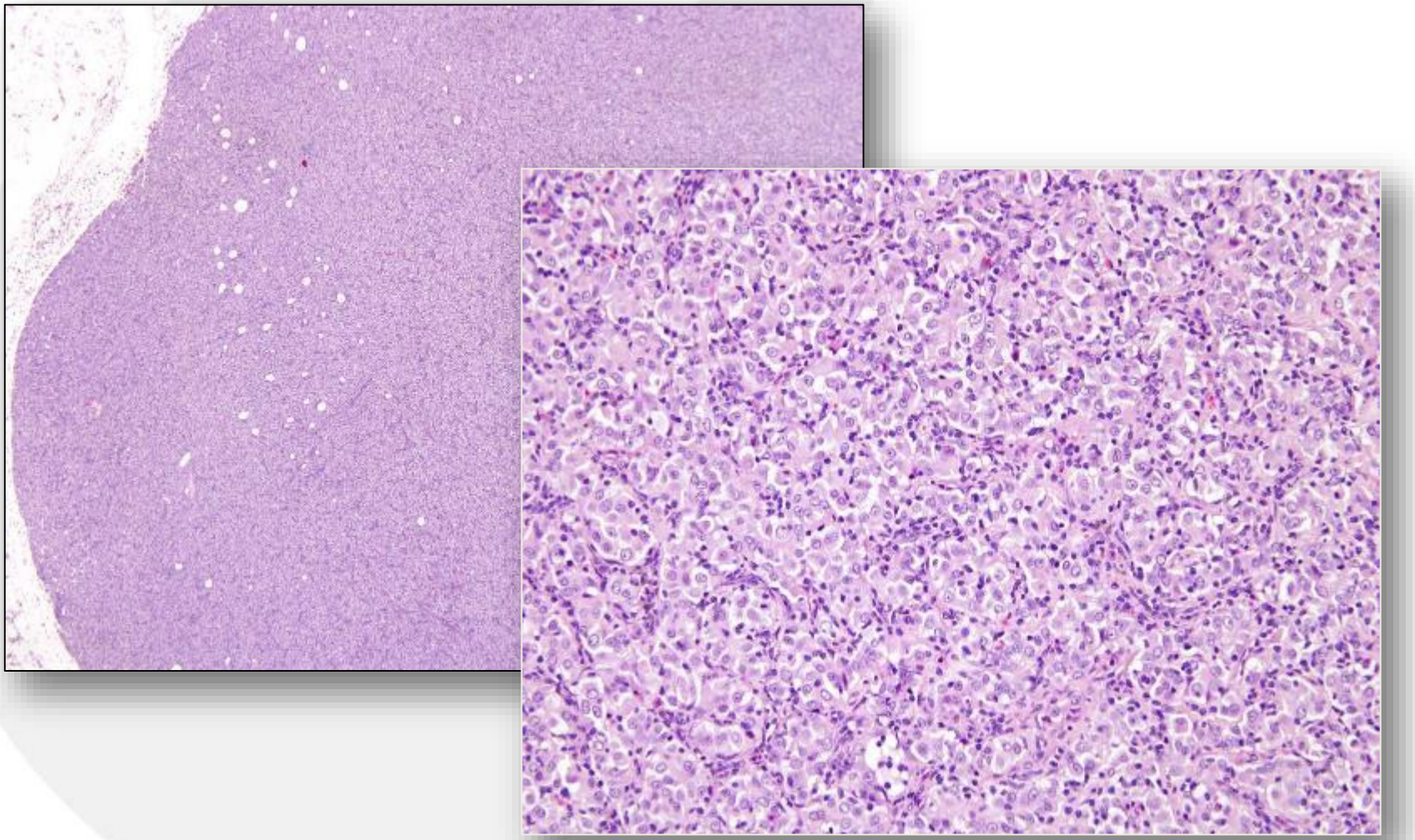
- Short term vs long term studies

Rare or unusual findings

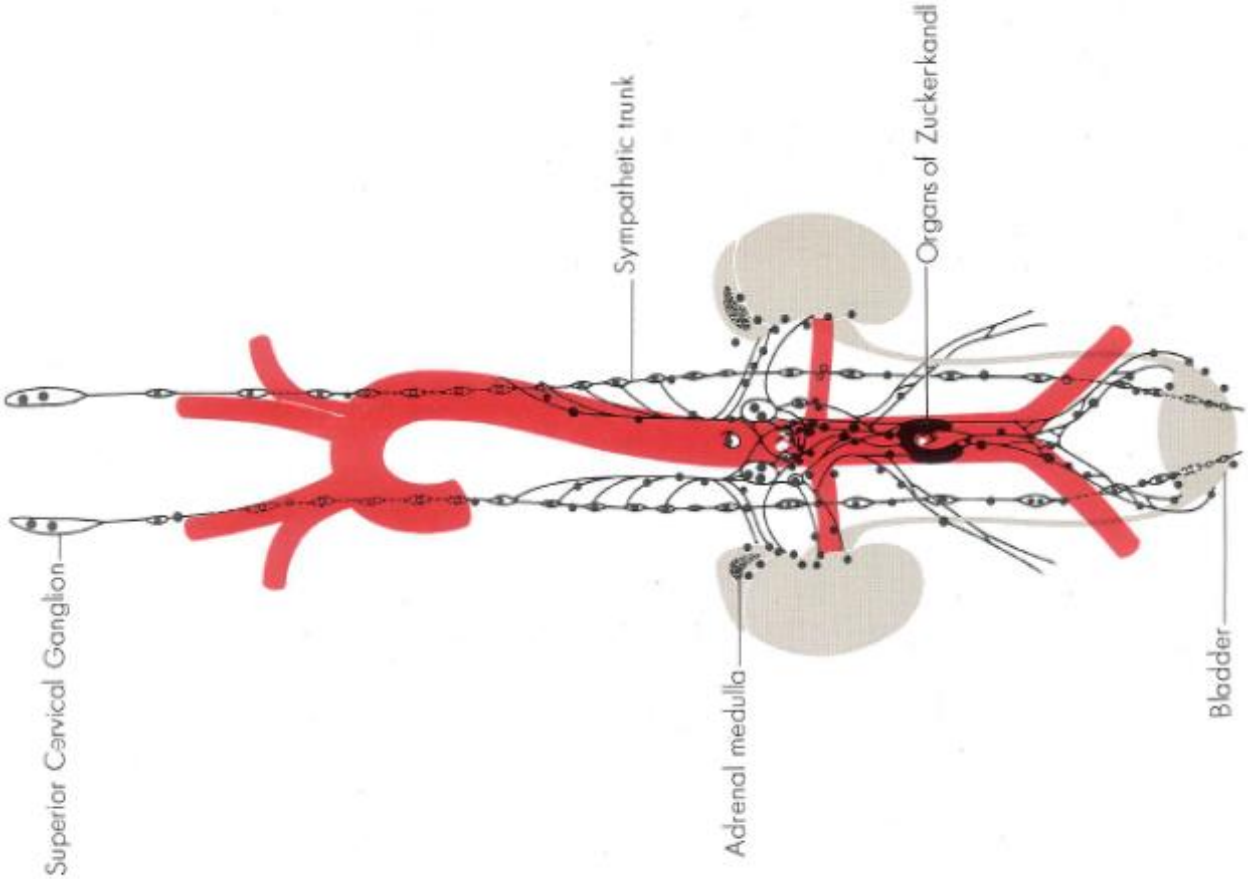
Mesenteric Vein - Plexiform Vasculopathy



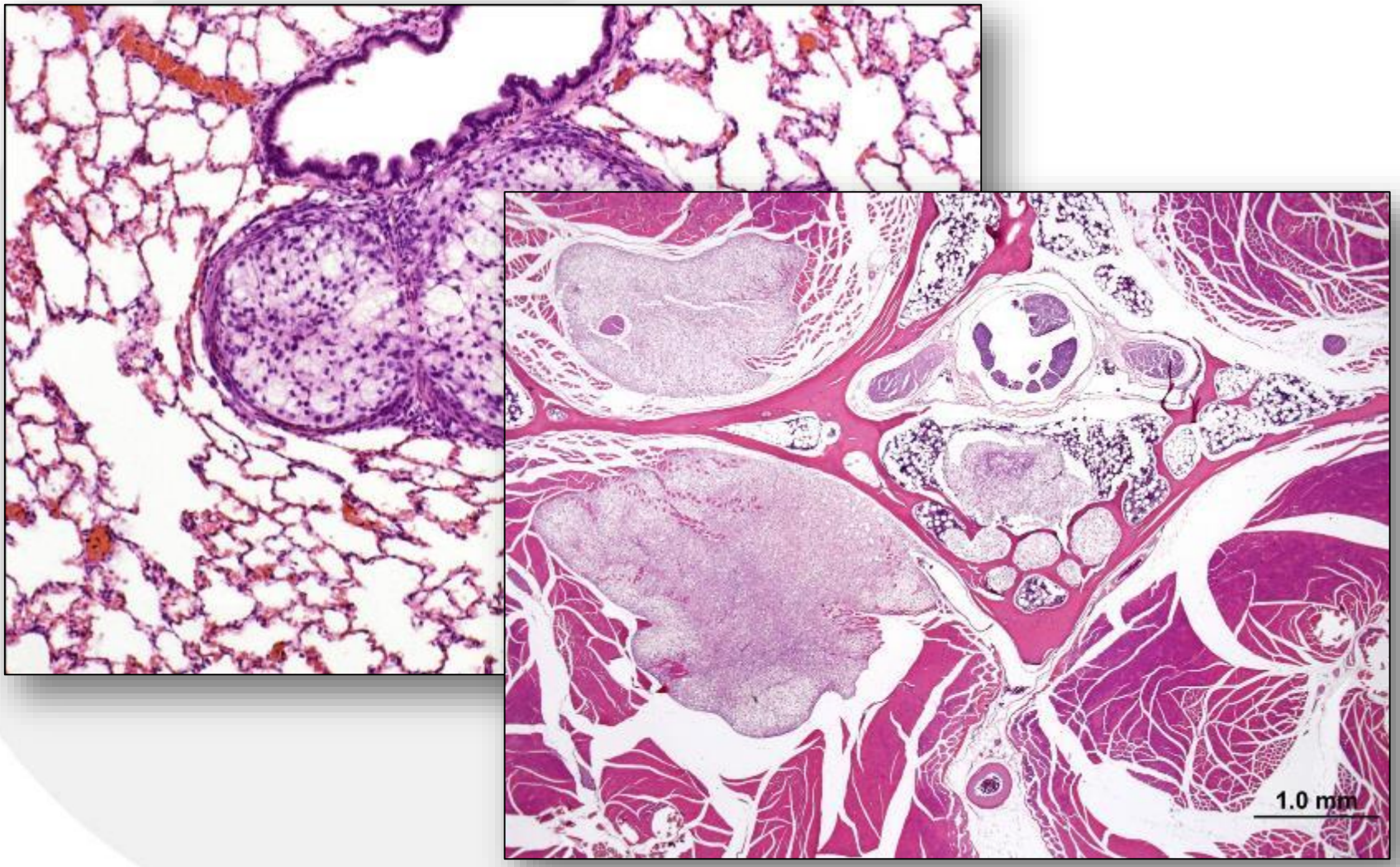
Organ of Zuckerkandl (periaortic paraganglioma)



Organ of Zuckerkandl (periaortic paraganglioma)

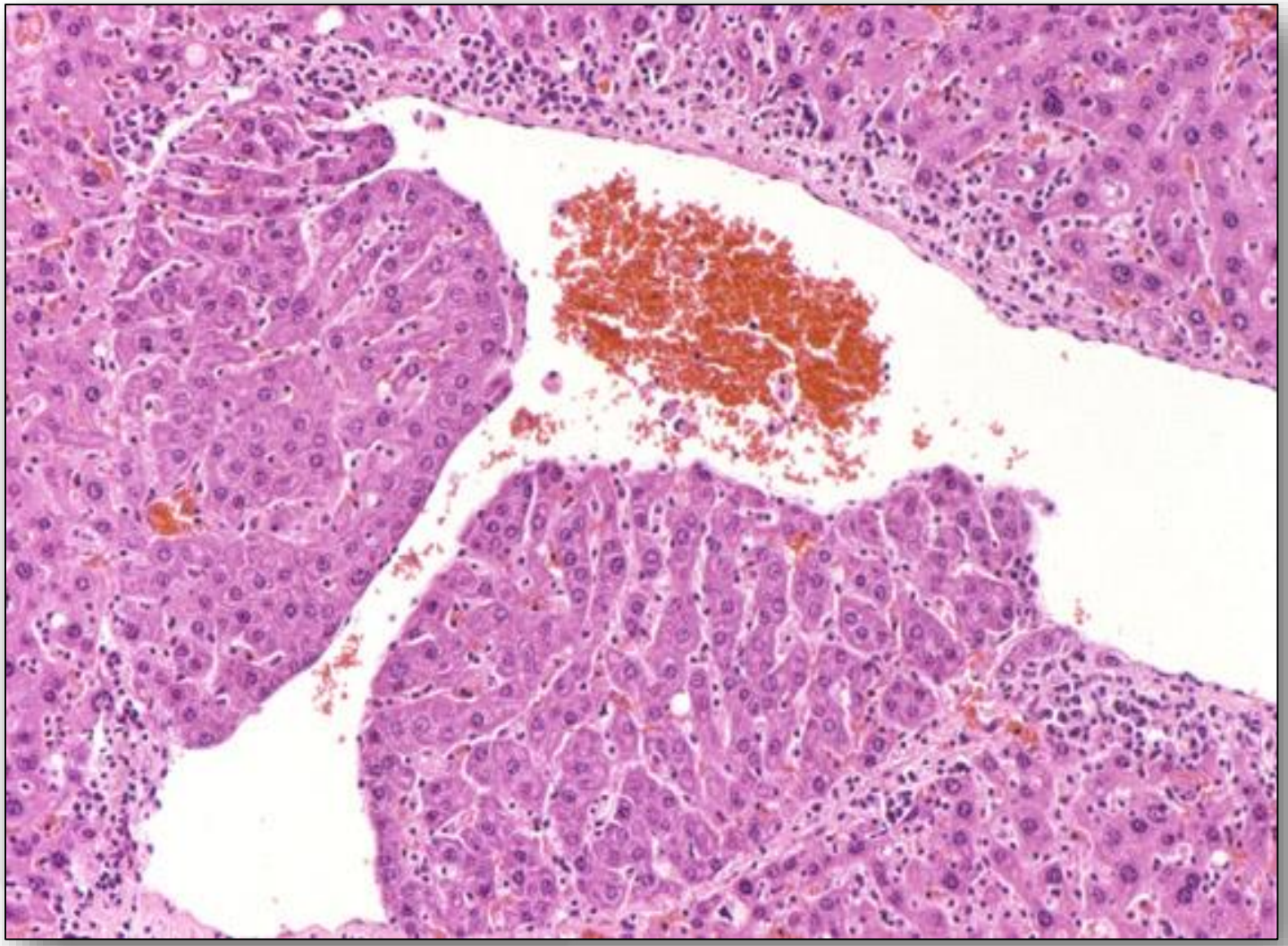


Perivertebral chordoma metastatic to lung

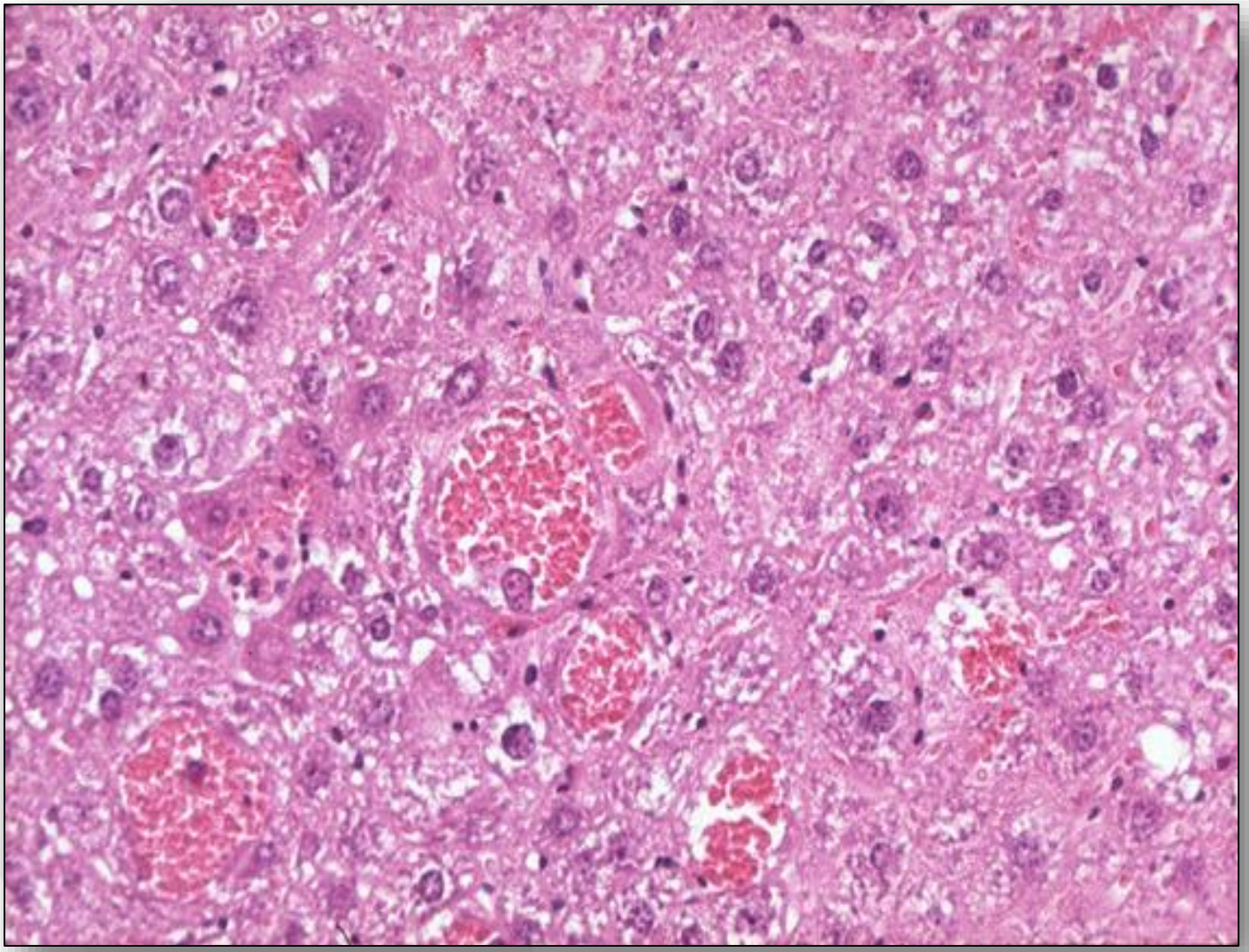


Ito-tumor in mouse liver

Mouse liver – Intravascular hepatocytes



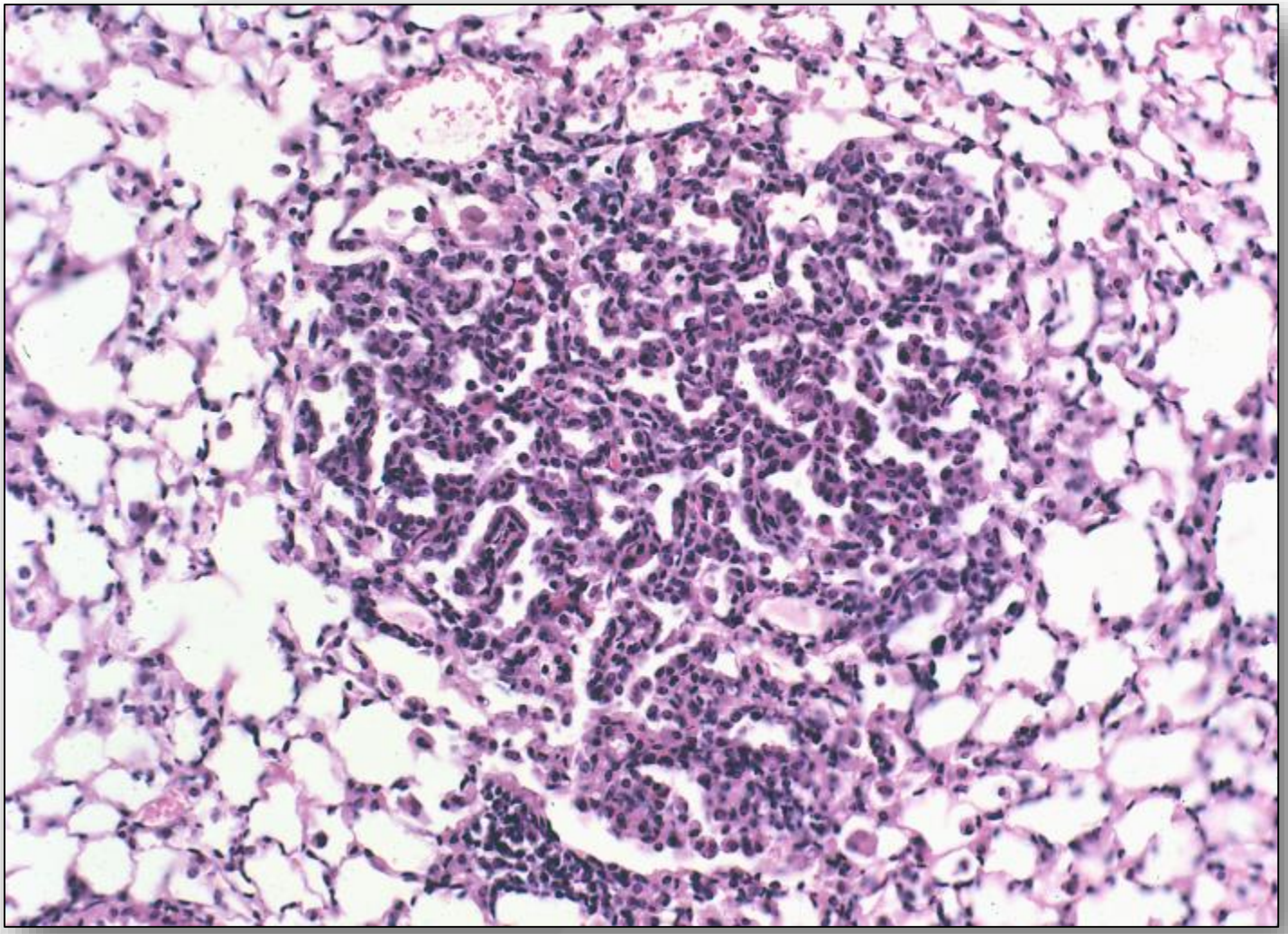
Mouse liver – Intrahepatocytic erythrocytes



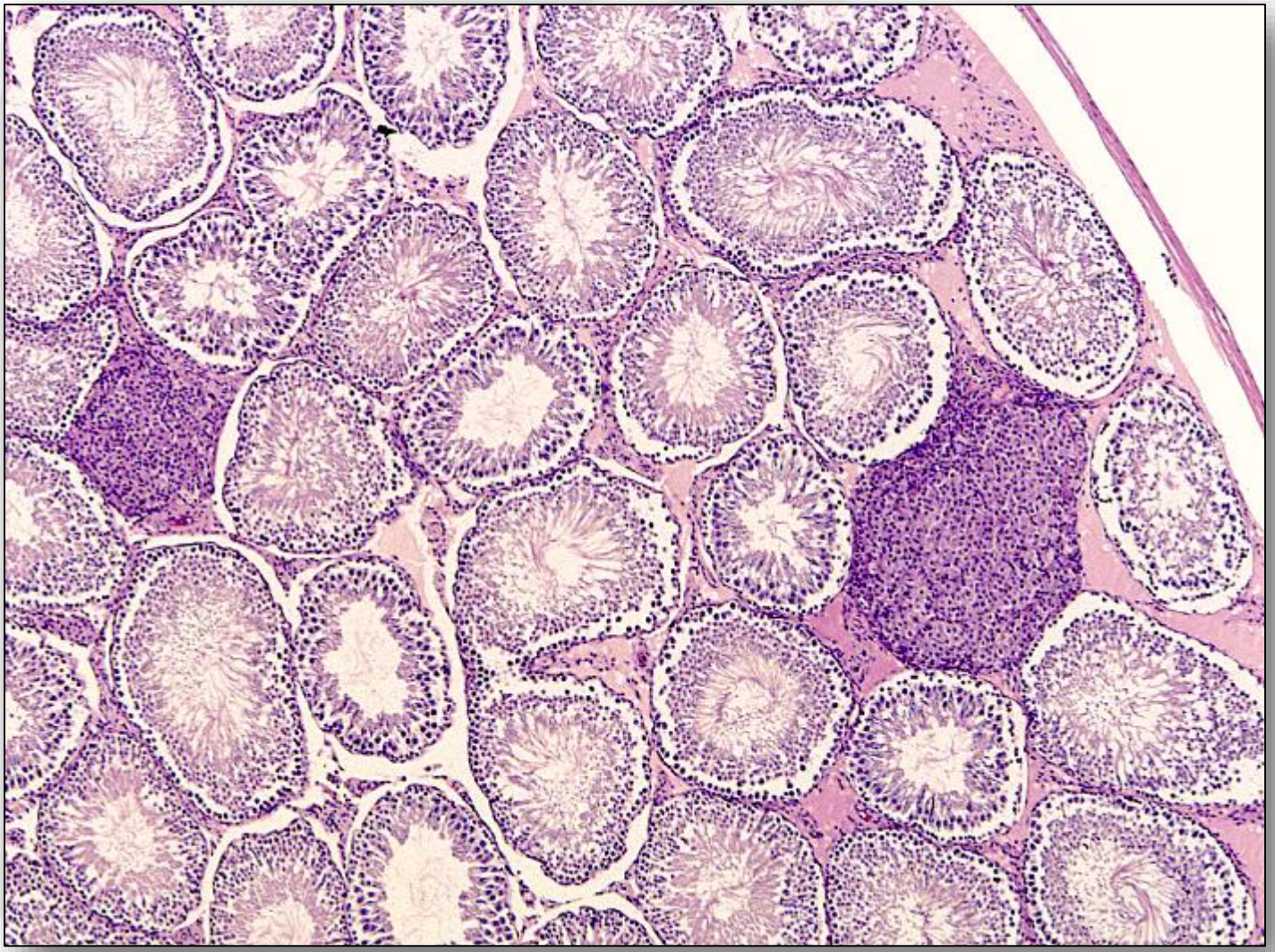
Misdiagnosis

- Threshold for diagnosis of lesion (especially non-neoplastic aging lesions)
- Use of different terminology for same lesions within one study
- Wrongly used terminology

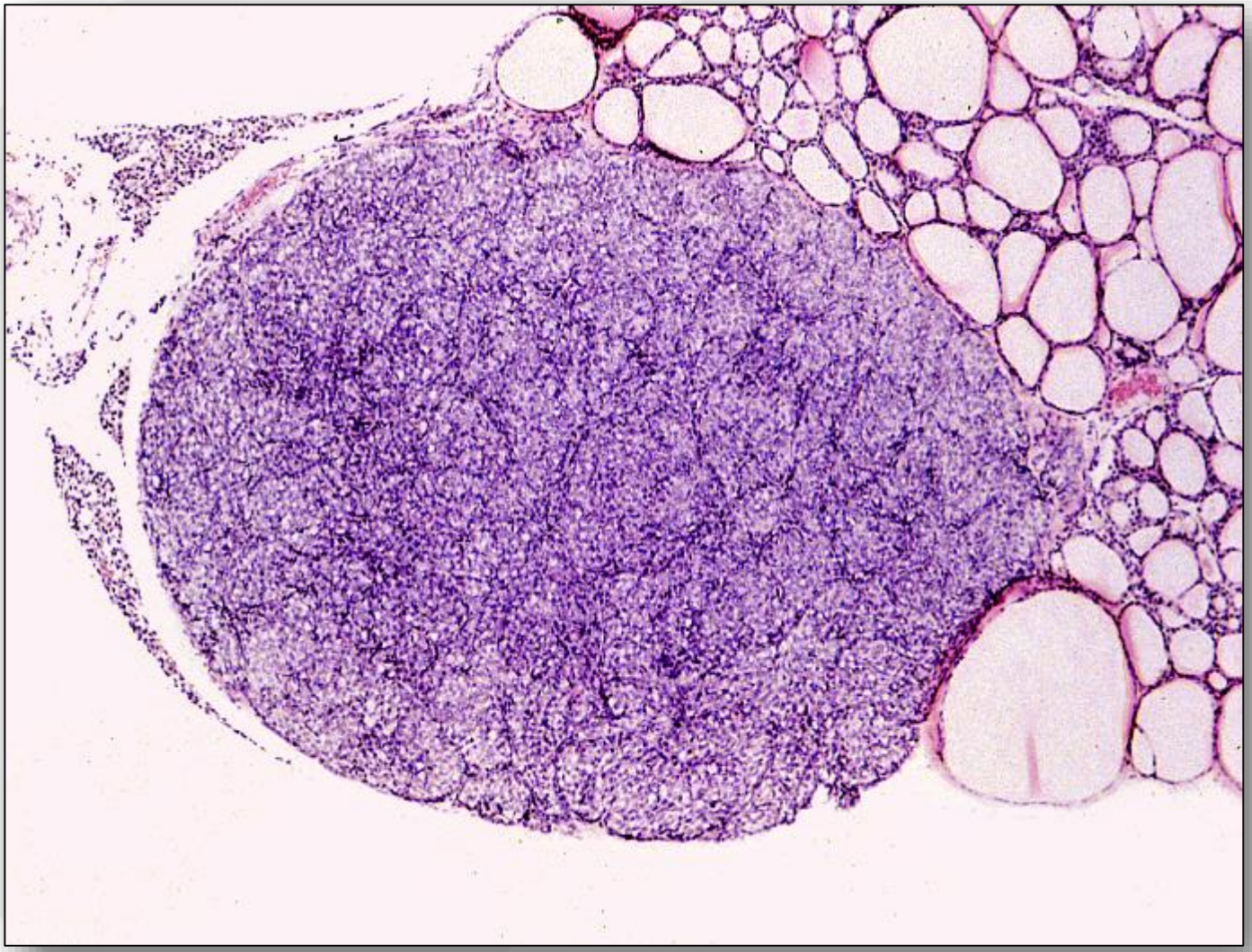
Alveolo-bronchiolar hyperplasia vs Aadenoma



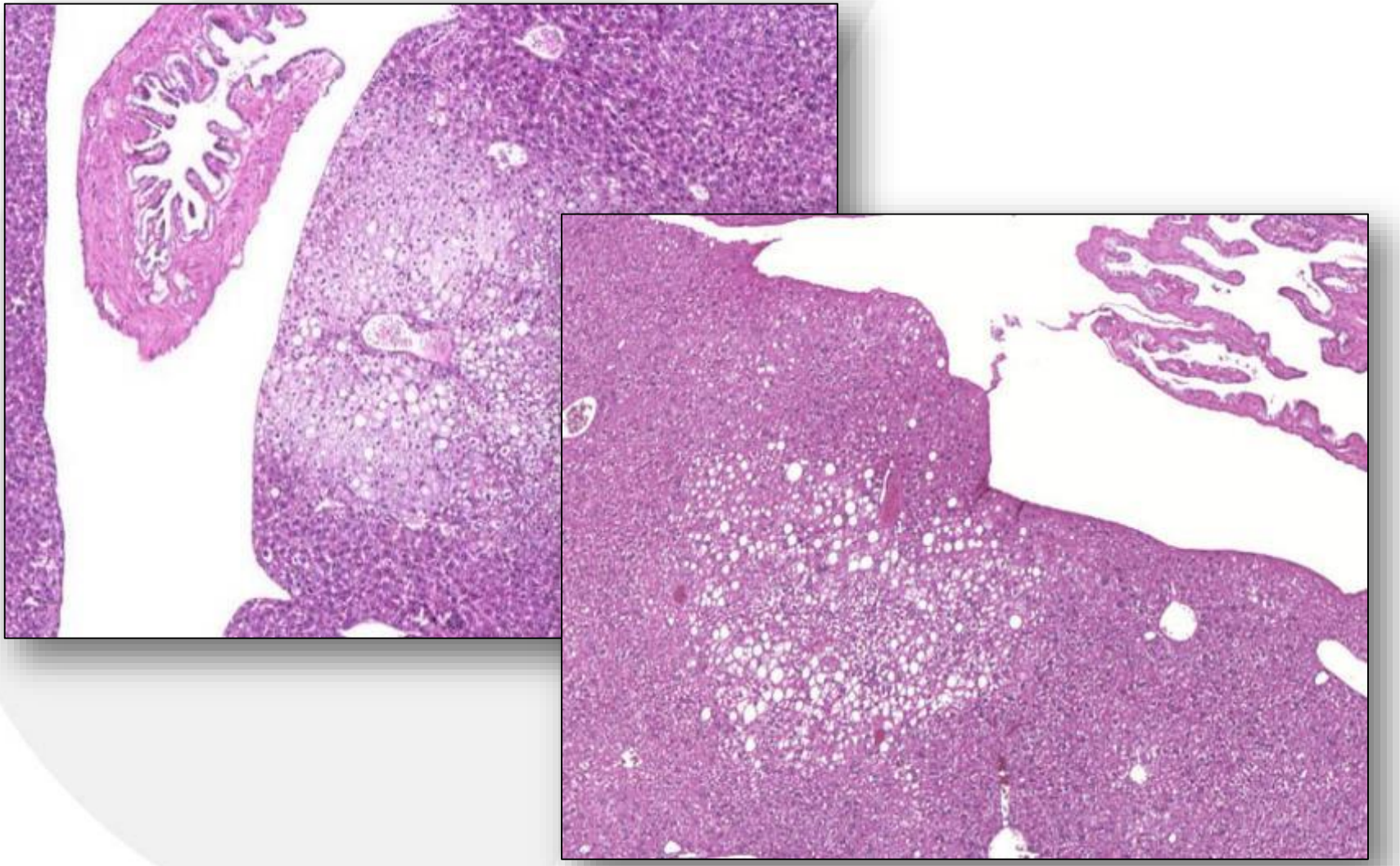
Leydig cell hyperplasia vs adenoma



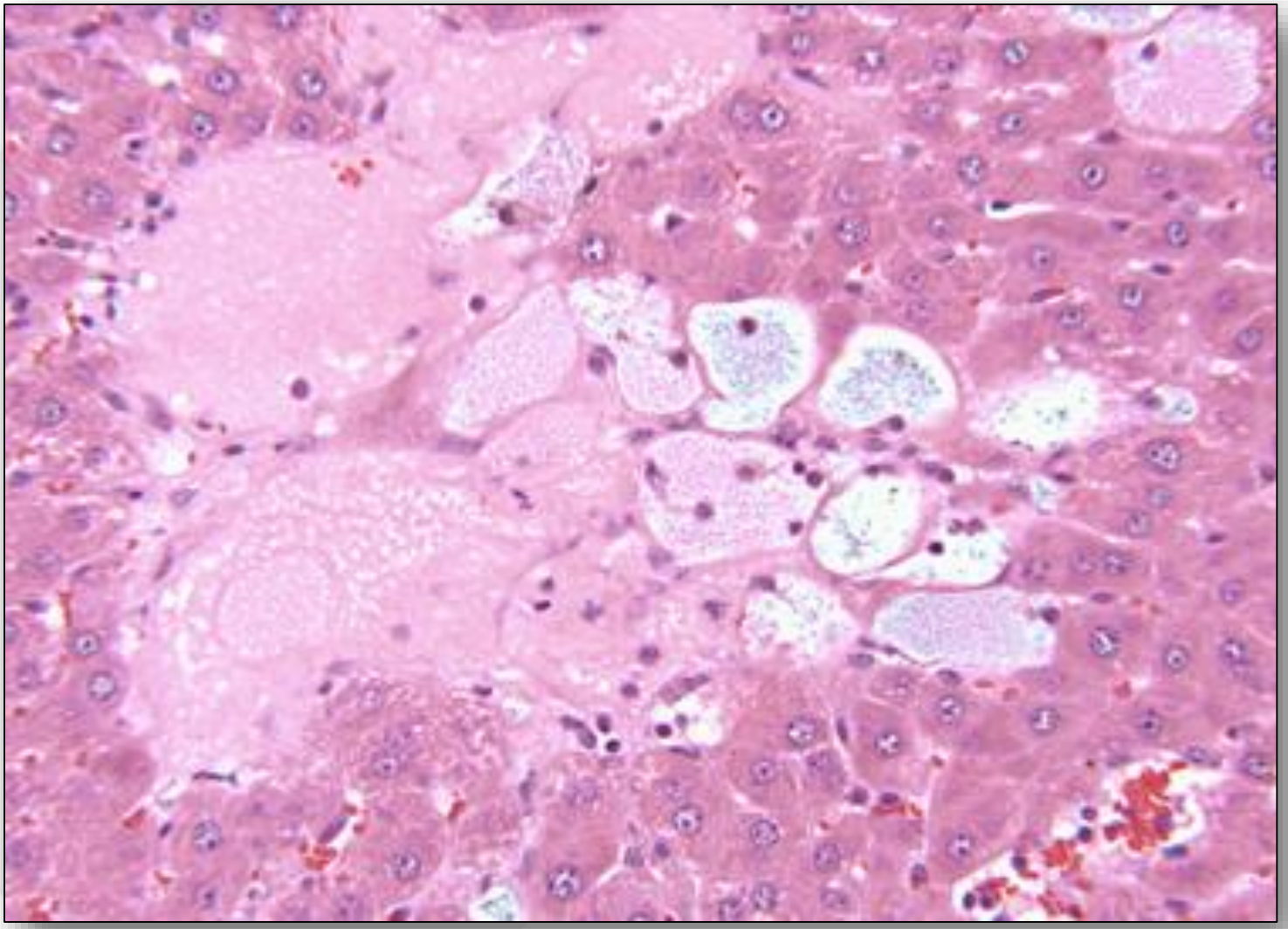
Parathyroid gland hyperplasia vs adenoma



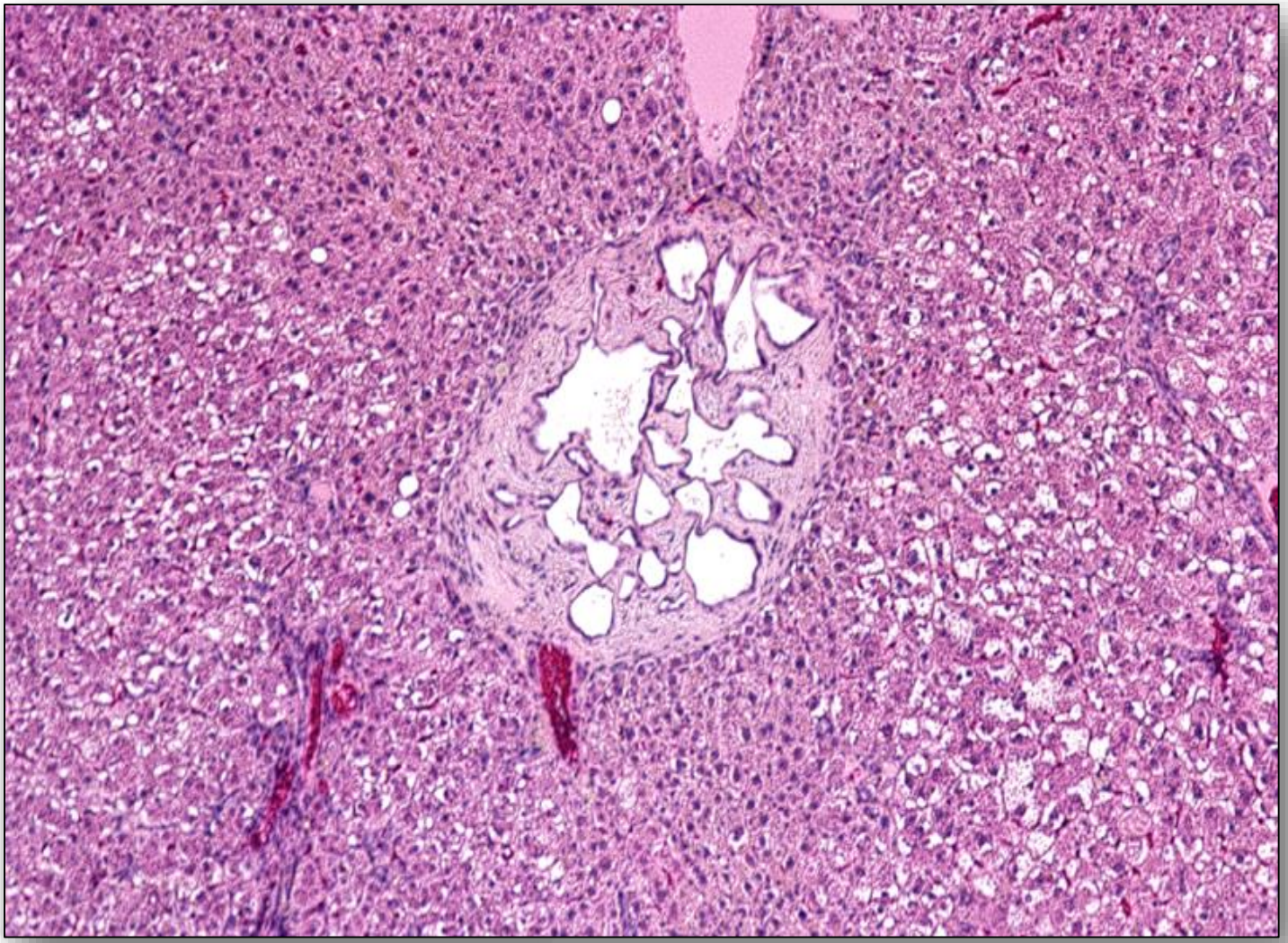
Tension lipidosis



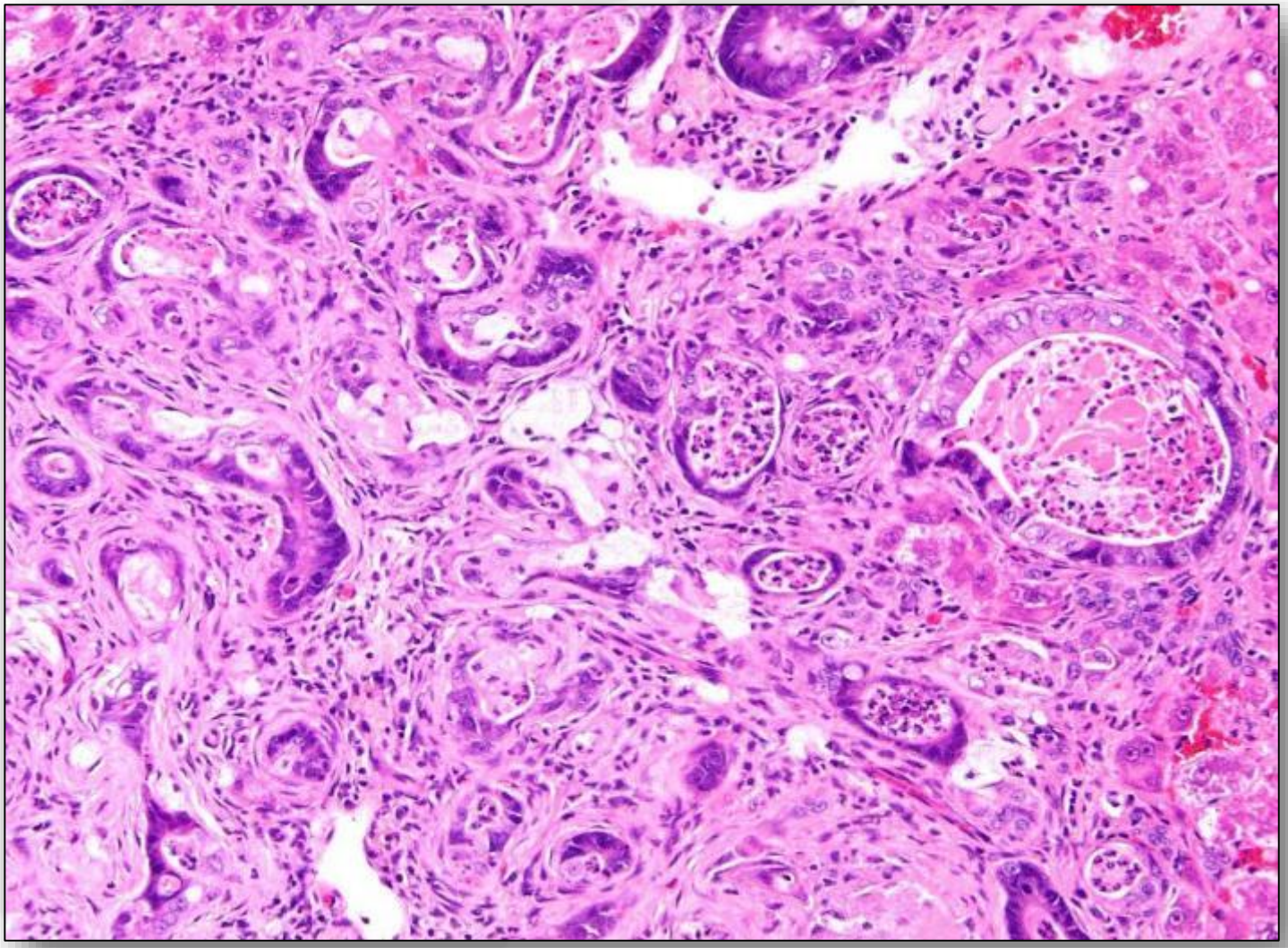
Spongiosis hepatis or Cystic degeneration



Peribiliar fibrosis vs cholangiofibrosis



Cholangiofibrosis



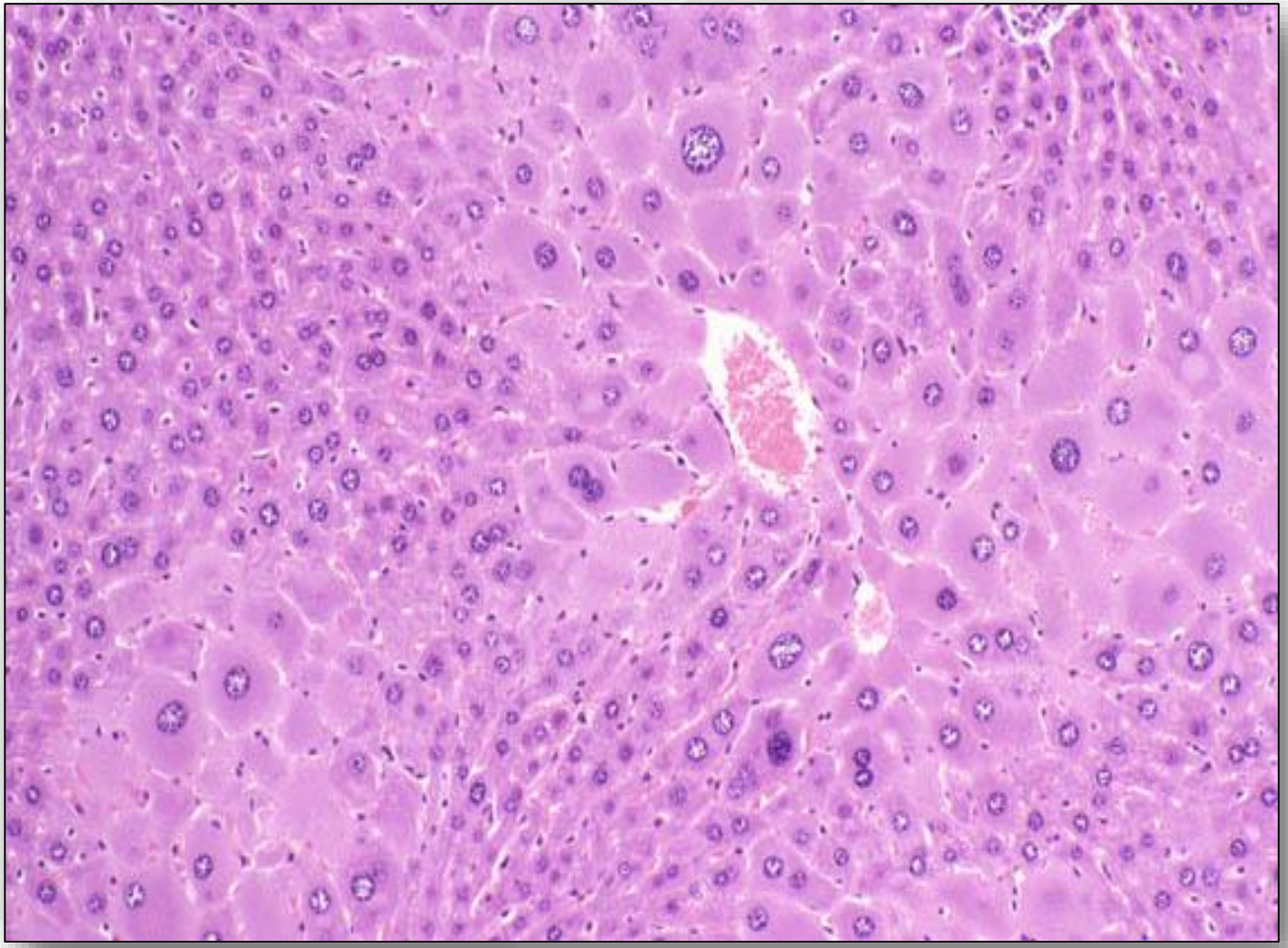
Pharmacologic effects rather than Pathological lesions

- The inappropriate interpretation of pharmacological effects may cause in wrong settings of the NOAEL

- Adverse:
changes that are irreversible and not foreseen, i.e. irreversible changes on cells, tissues, organs or individuals

- Adverse effects include:
degeneration, necrosis, apoptosis, fibrosis, architectural changes etc.

Pharmacological effects:
Examples - Hepatocellular hypertrophy, mouse



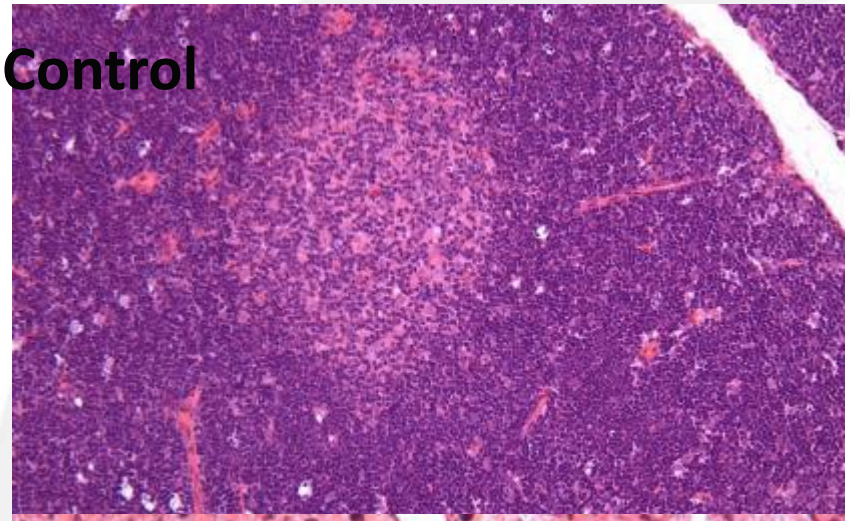
Pharmacologic effects may be proven by mechanistics

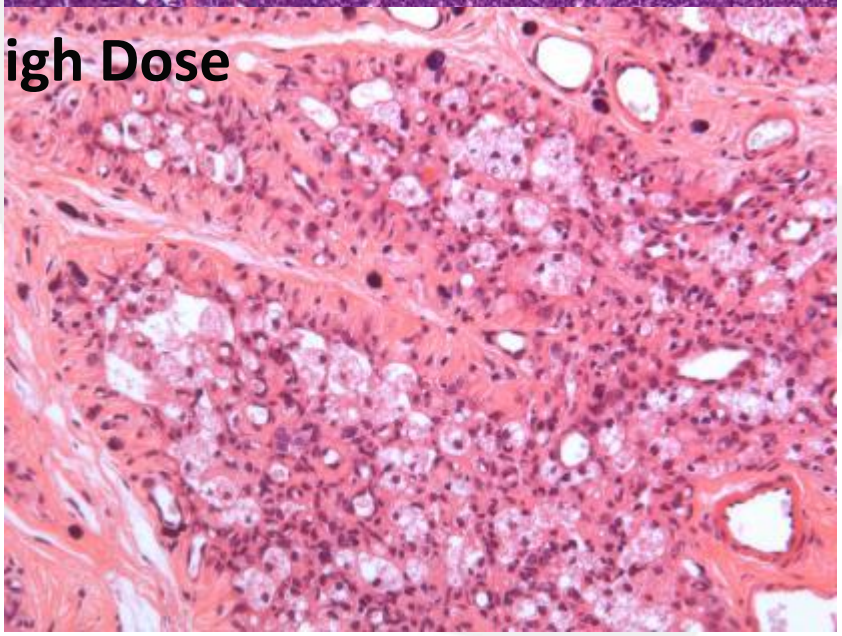
- **Non-adverse effects are reversible**

- **Architectural changes may be confusing but need to be reversible**

- **Human relevance need to be investigated for several lesions especially when considered to be due to endocrine affections**

Pharmacologic effects: Example - low dose corticosteroids, rats

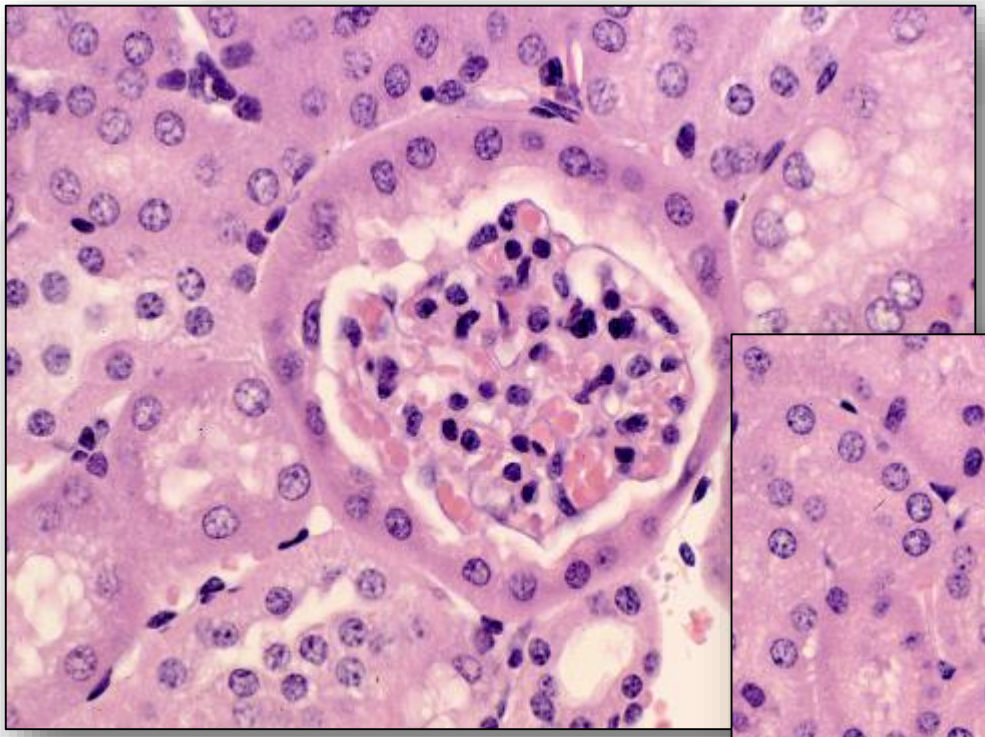




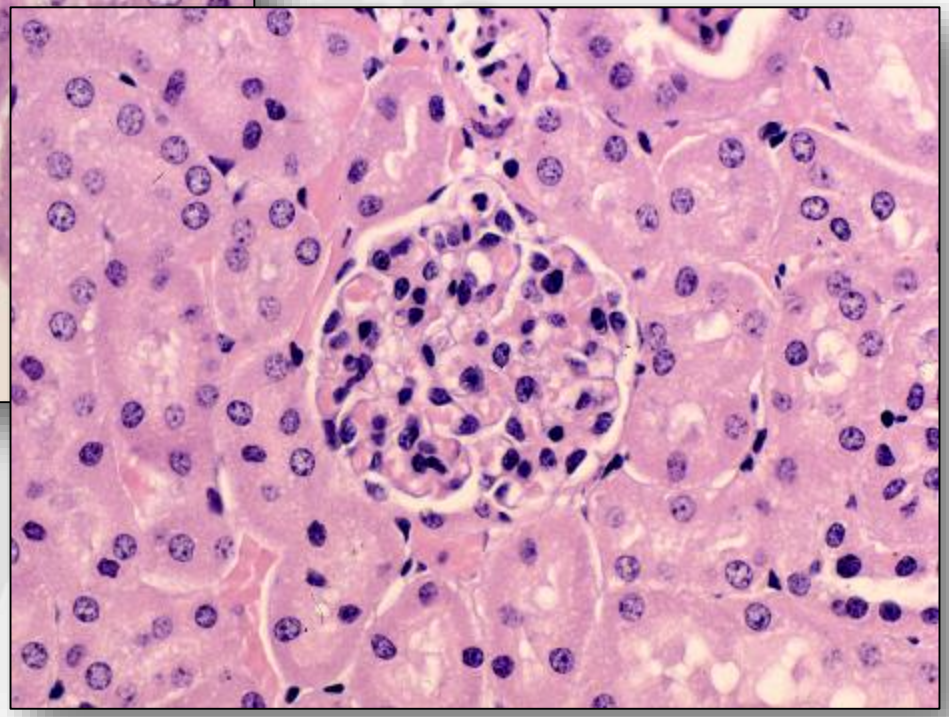
Cortical and medullary atrophy
Increased histiocytosis
Lymphocytolysis
Lymphcytophagocytosis

Full Recovery after 14 days!

Sexual dimorphism

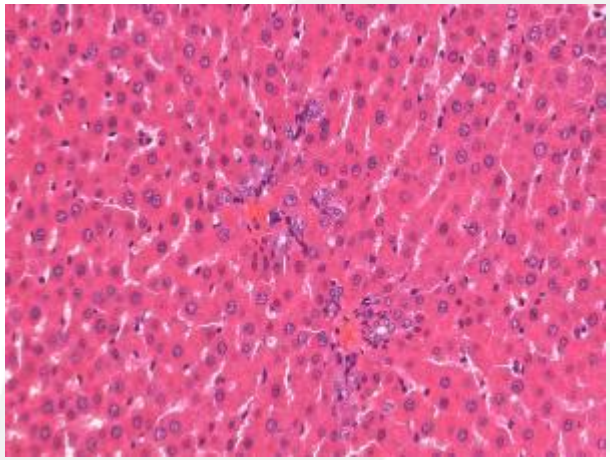


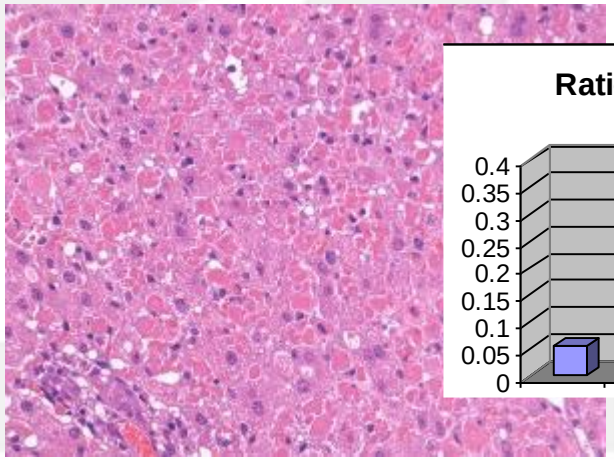
Control Female
Mouse
Renal Glomerulus

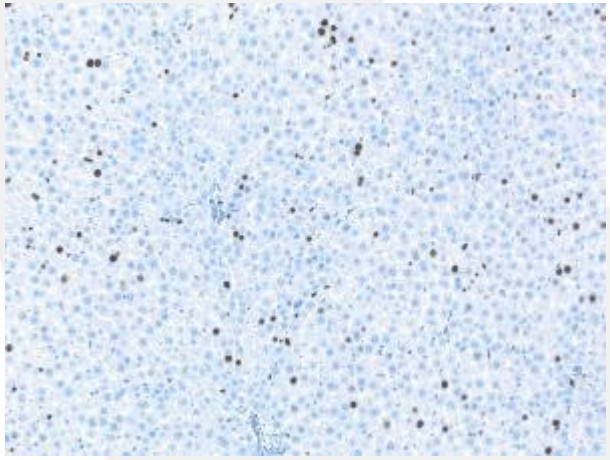


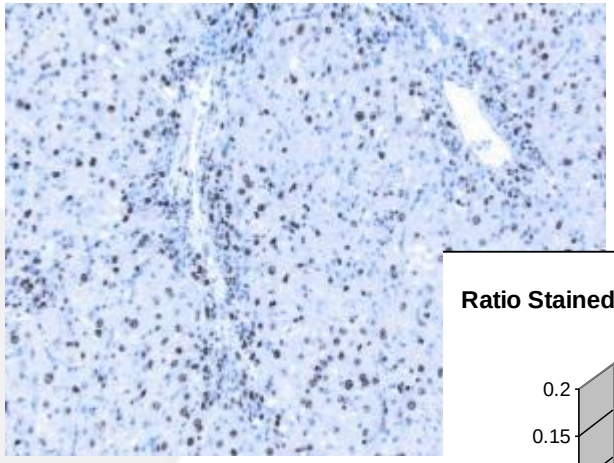
Control Male
Mouse
Renal Glomerulus

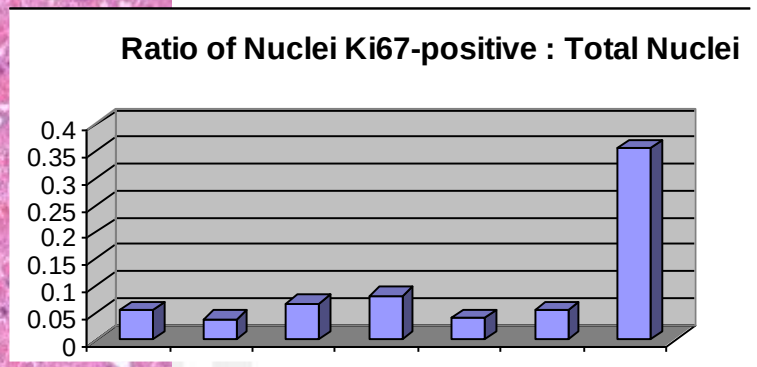
Pharmacologic effects may be proven by mechanistics
Example: FXR agonist rat

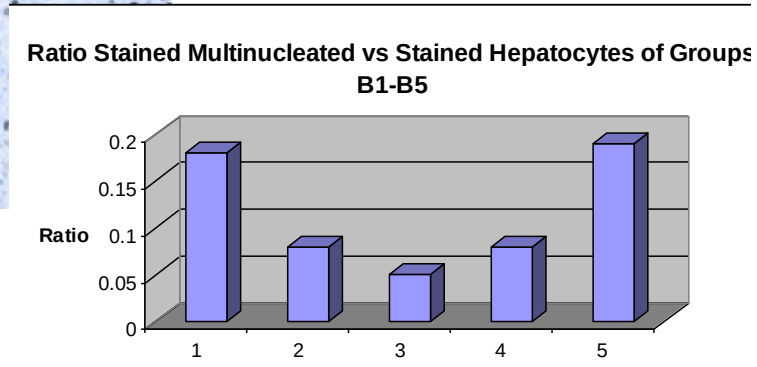












Summary

- All sections are artifacts
- All sections have additionally introduced artifacts
- Some artifacts are avoidable
- Considering correct fixation prior to necropsy
- Clear-cut techniques
- Quality check on sections

- Interpretation and Errors
- PR and PWG
- Adverse vs Non-adverse
- Relevance for Human