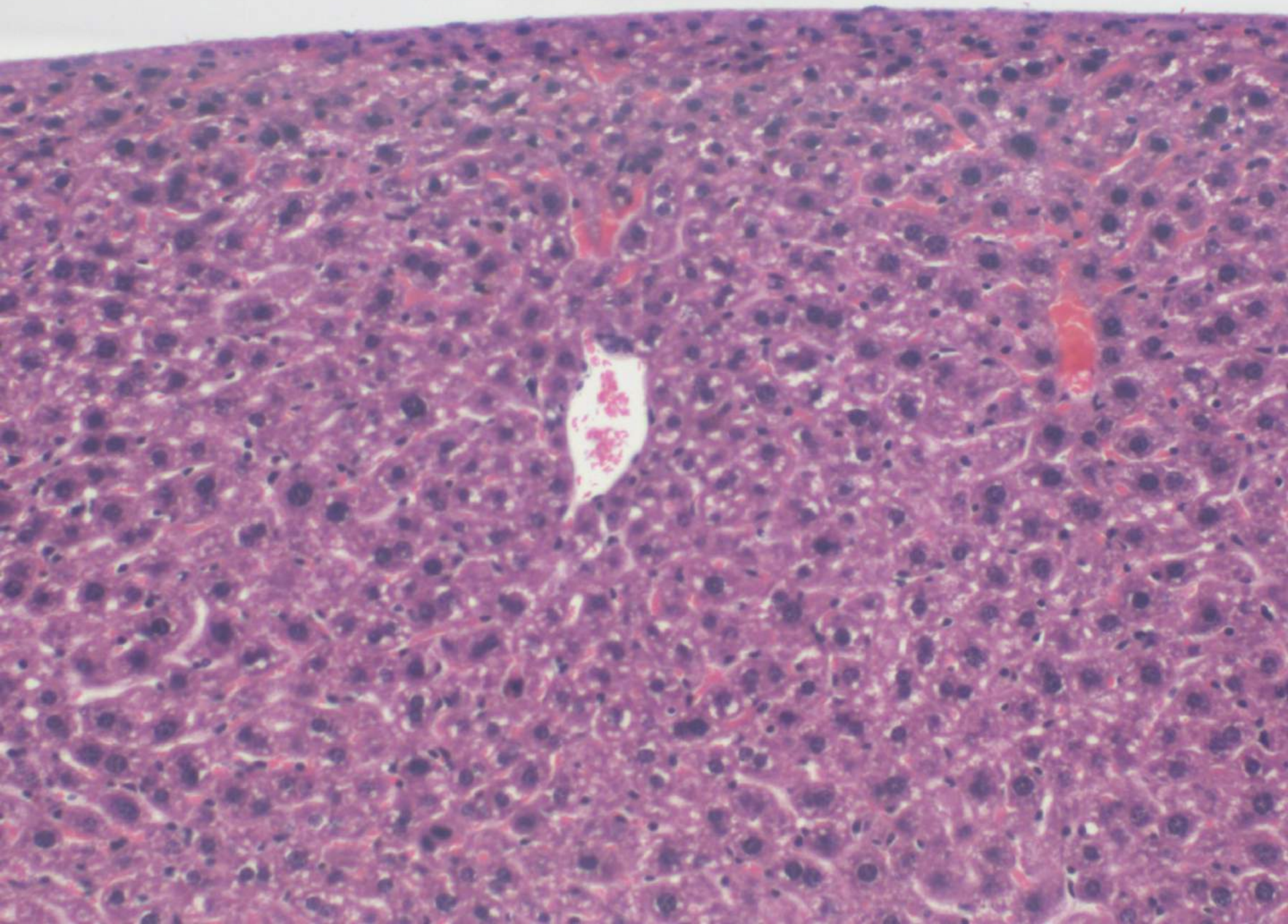


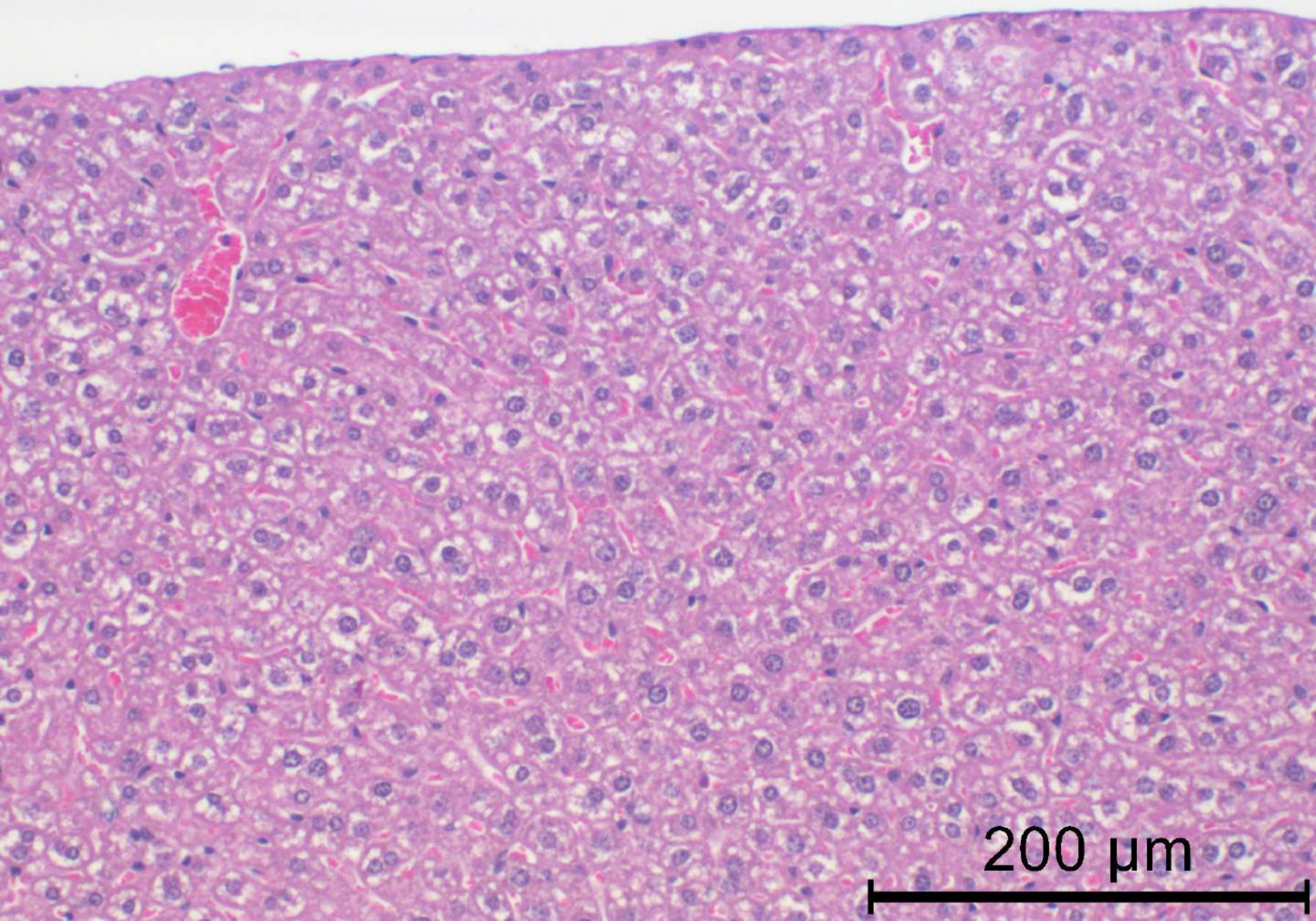
Pathology of Selected Conditions in Laboratory Mice

Ralph M. Bunte DVM, DACVP
Consultant
Laboratory Animal Pathology
Singapore
rbunte@outlook.com
65 96447733

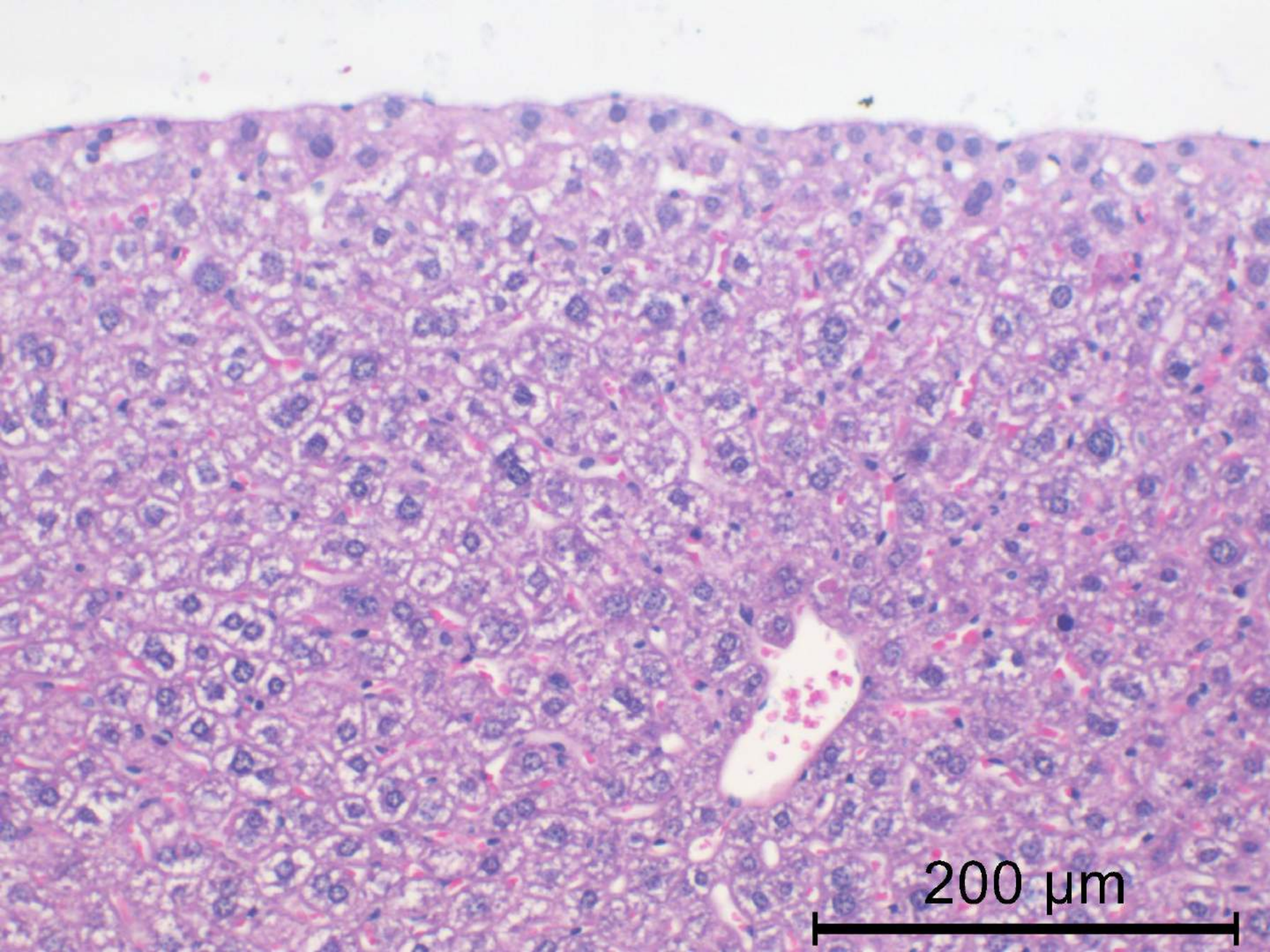
City University of Hong Kong

15 DEC 17

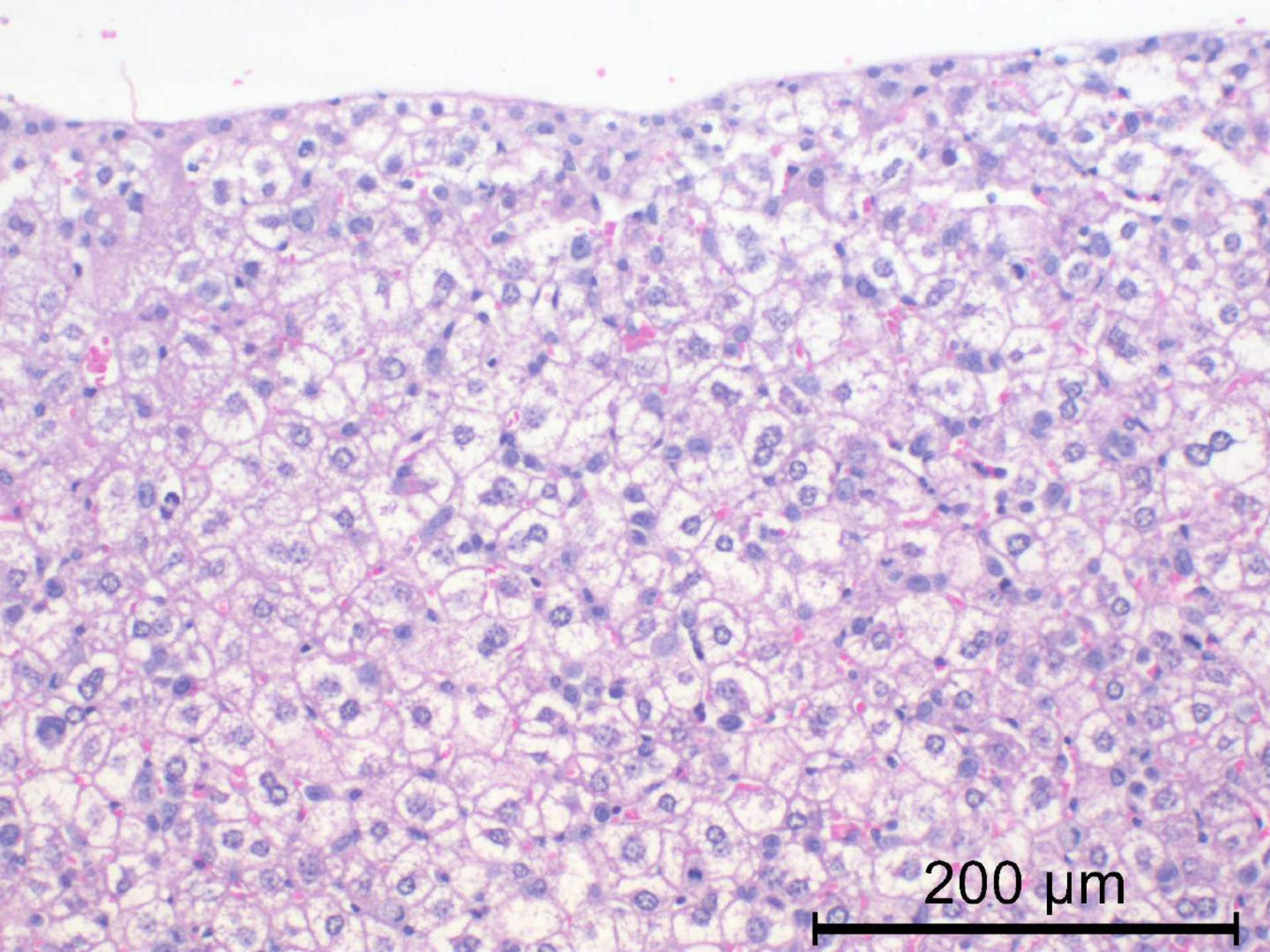




200 μm



200 μm



200 μm

Outline of Presentation

1. Kinds of mice – immunologically
2. Some important unique biological features
 - Spleen
 - Liver
 - Reproductive tract
3. Diagnostic workup - considerations/procedures
4. Selected diseases and conditions
5. Highly infectious diseases that threaten mouse colonies

1. Kinds of Mice - Immunologically

transforming
medicine,
improving lives



Kinds of mice - immunologically

- **Immunocompetent** (immuno-efficient)
 - Are the most robust and healthy
 - Can fend off or recover from all but most pathogenic organisms
- **Immunodeficient**
(immunoincompetent)(immunocompromised)
 - Not as robust and healthy
 - Vulnerable to more microbial agents, especially opportunistic and commensal bacteria
- “Immunoweird” (“immunowedon’tknow”)

2. Some important unique biological features

transforming
medicine,
improving lives



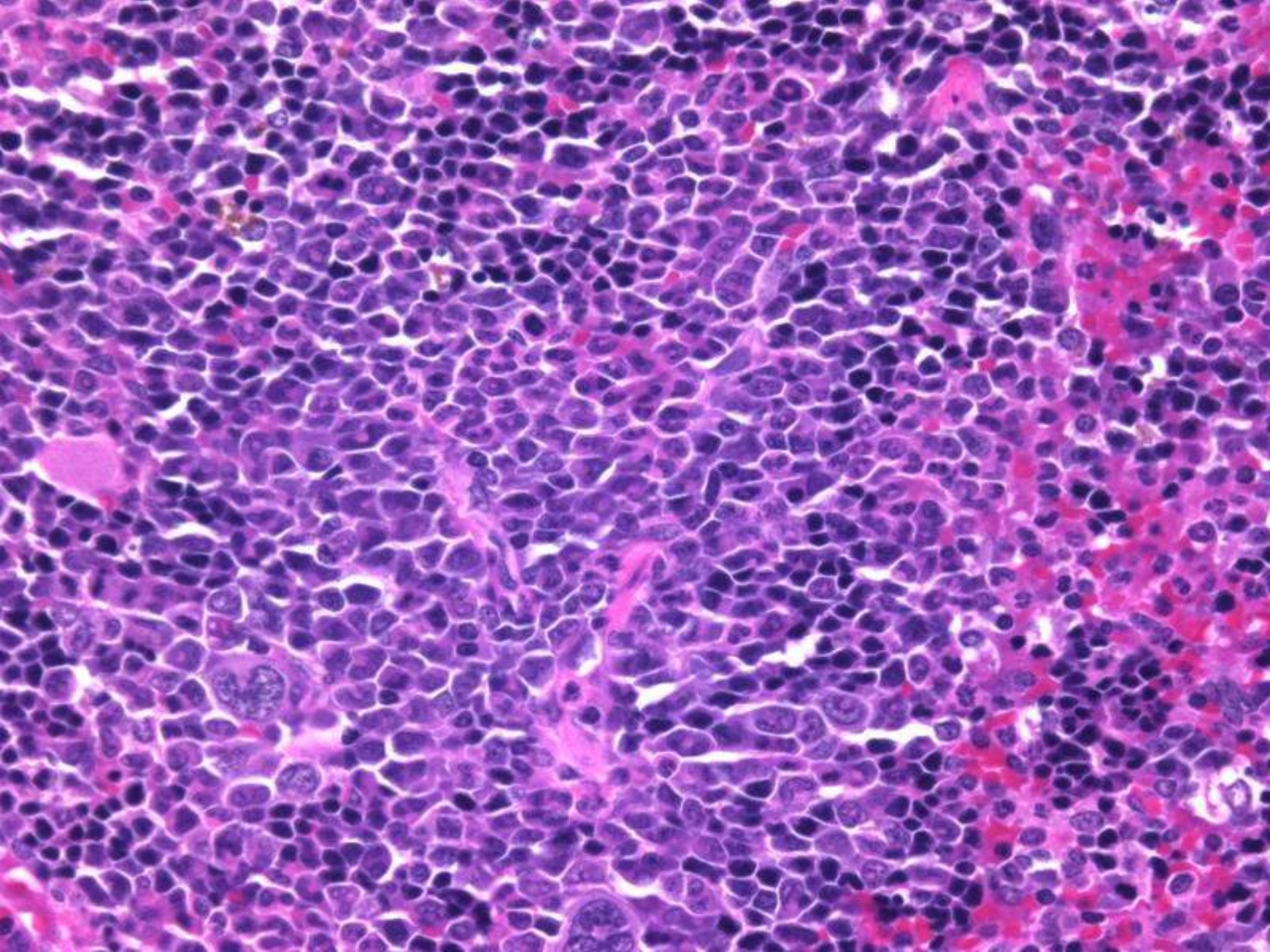
Some important unique biological features- spleen

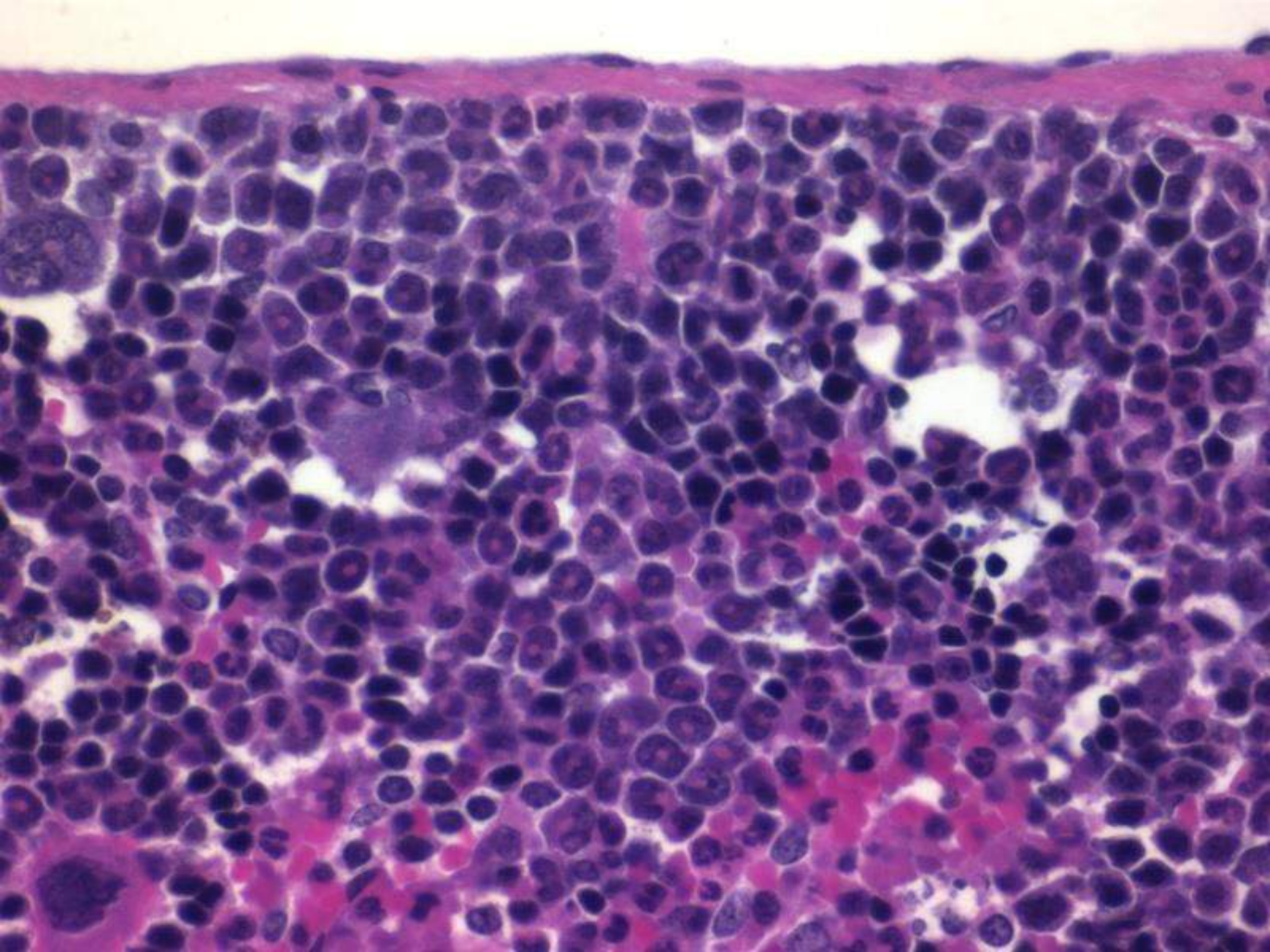
- Spleen is a multifaceted organ
 - White pulp contains lymphoid cells (like most species)
 - Therefore functions like a lymph node
 - Therefore involved in acquired immunity
 - Red pulp contains “bone marrow” cells
 - Therefore functions like bone marrow
 - Therefore involved in innate immunity
 - This is unique to rodents
 - Includes erythropoiesis – the making of erythrocytes
 - Includes granulopoiesis – the making of neutrophils, eosinophils, and basophils
 - Includes thrombopoiesis– the making of platelets
 - Almost all mice, including almost all immunodeficient mice, have intact innate immunity

Some important unique biological features – spleen (cont)

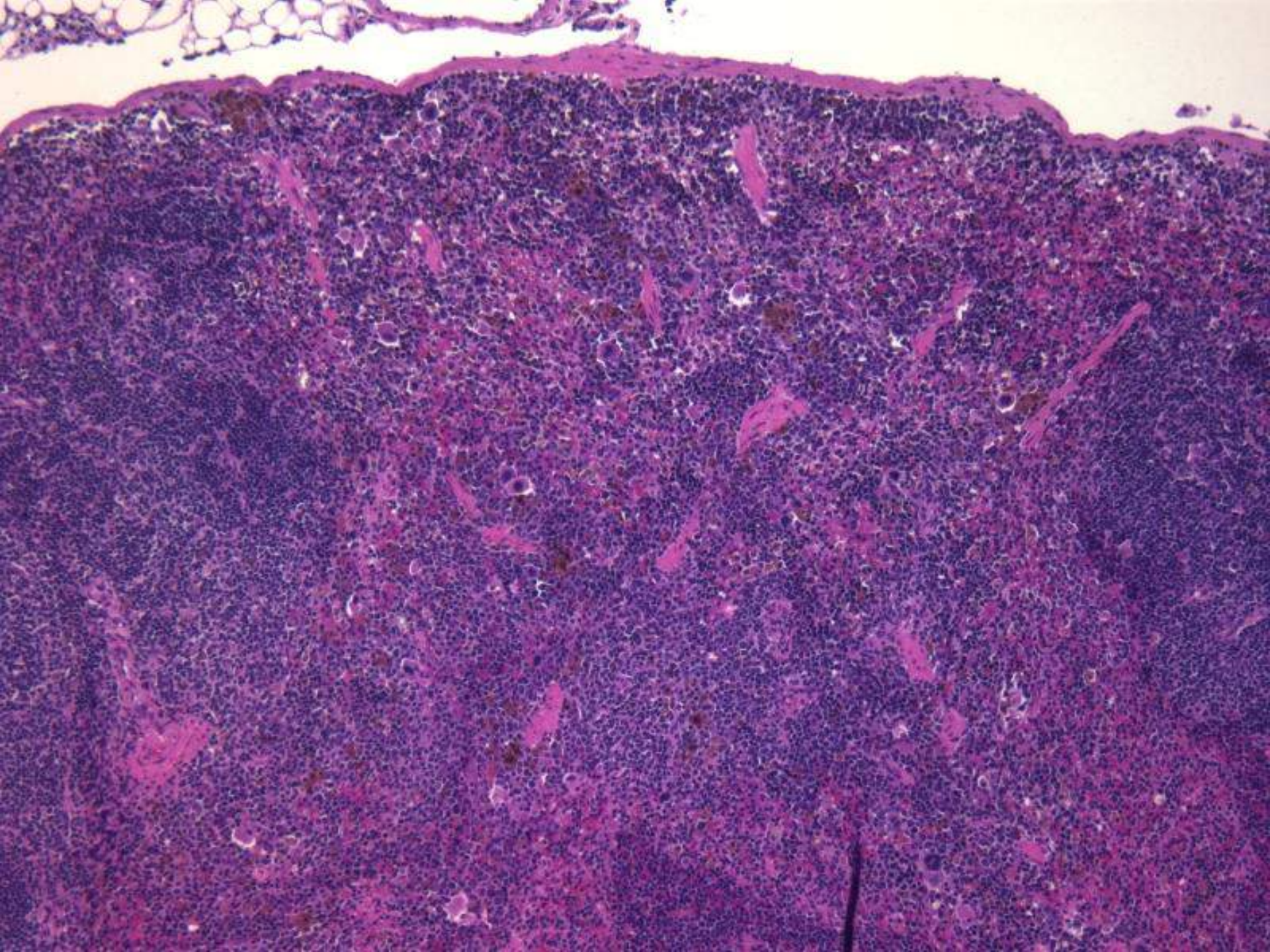
- Almost all immunodeficient strains have:
 - Deficient acquired immunity (spleen therefore small because of decreased white pulp)
 - But intact innate immunity – so can still make neutrophils and macrophages
- Spleen responds quickly to bacterial infections
 - Spleen therefore gets big
 - Should weigh spleen (and mouse) so can use organ weight/mouse weight ratio (mg spleen/gram of mouse)
 - Is an important organ in diagnosing diseases

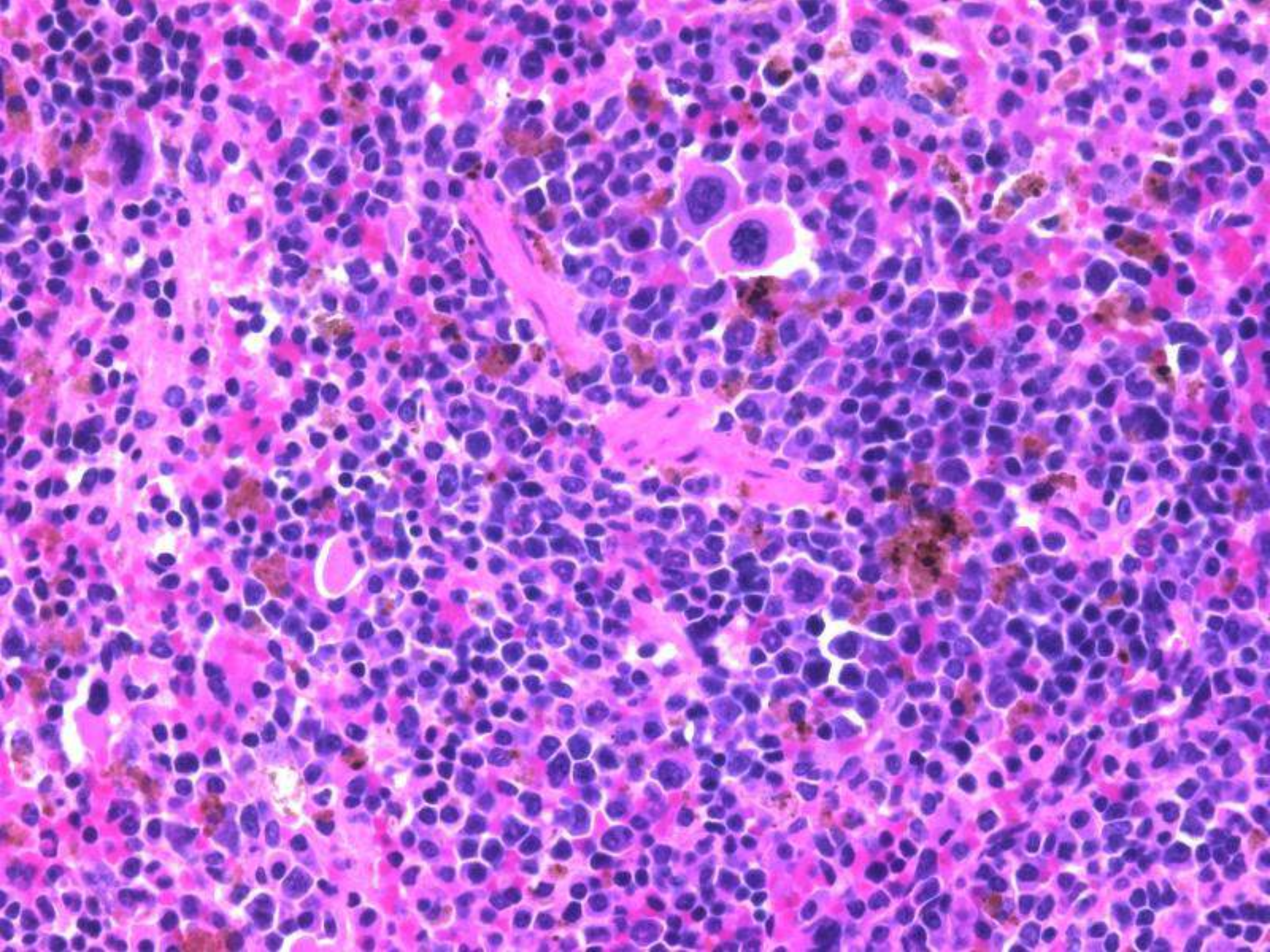


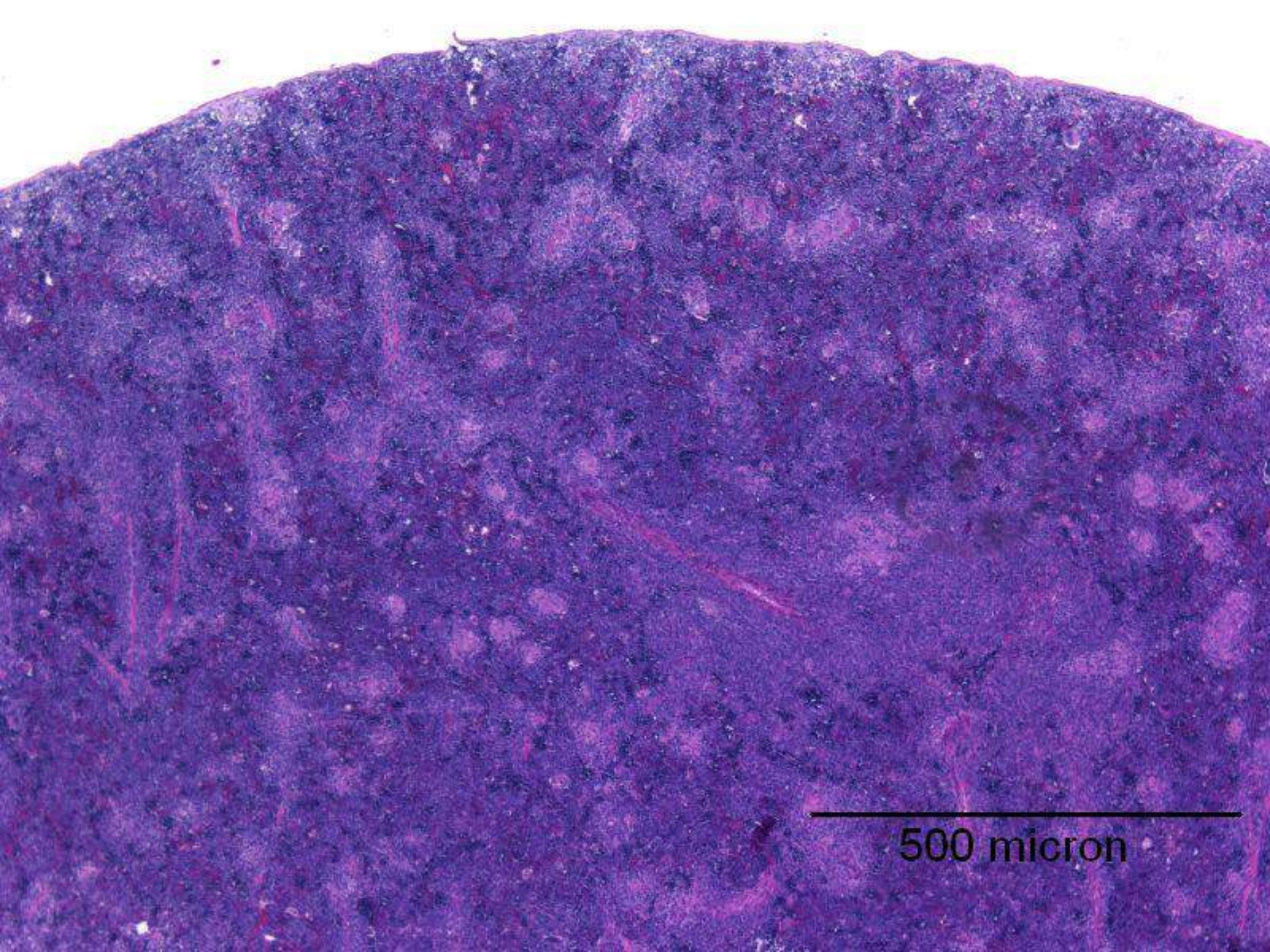




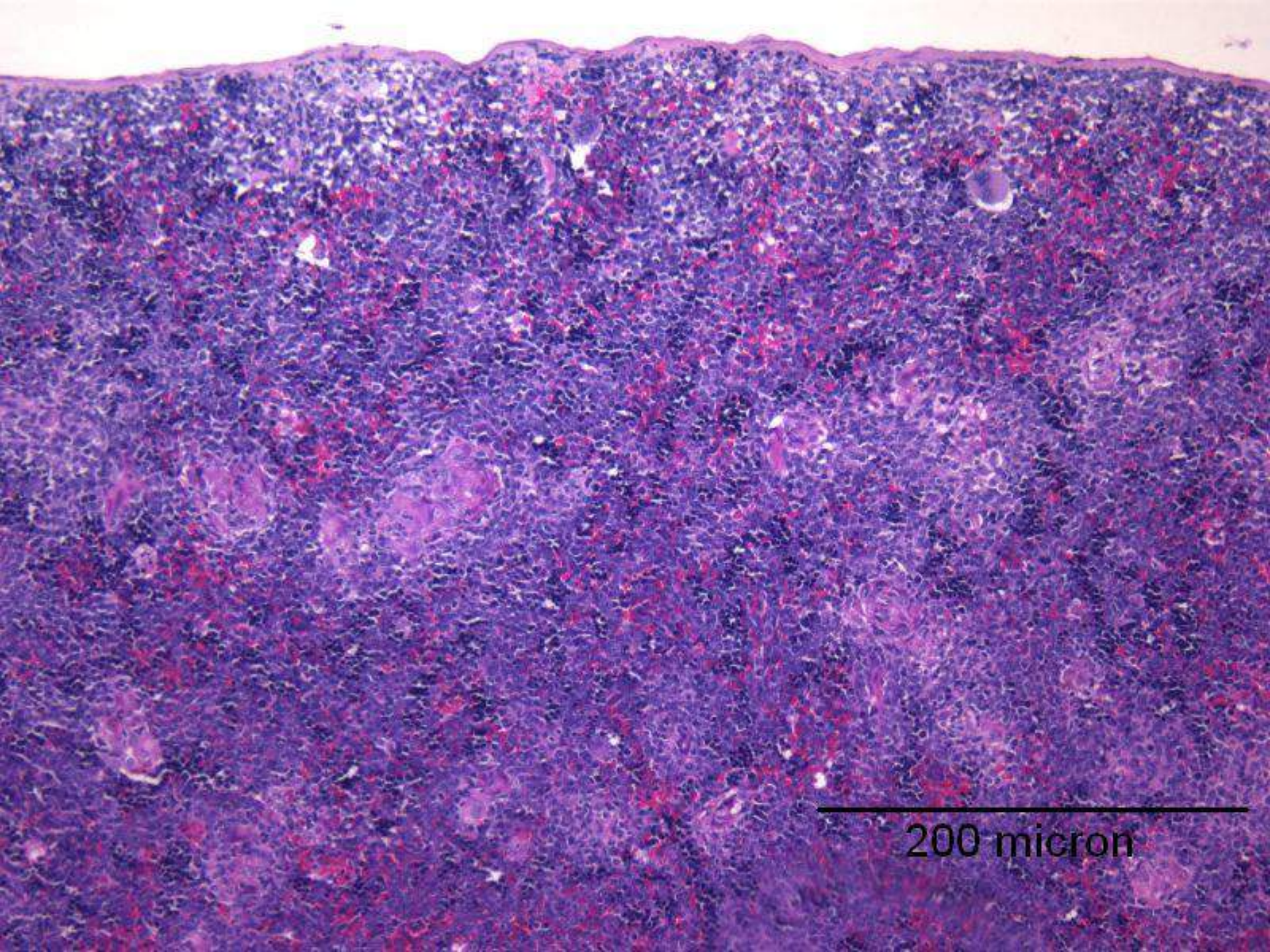




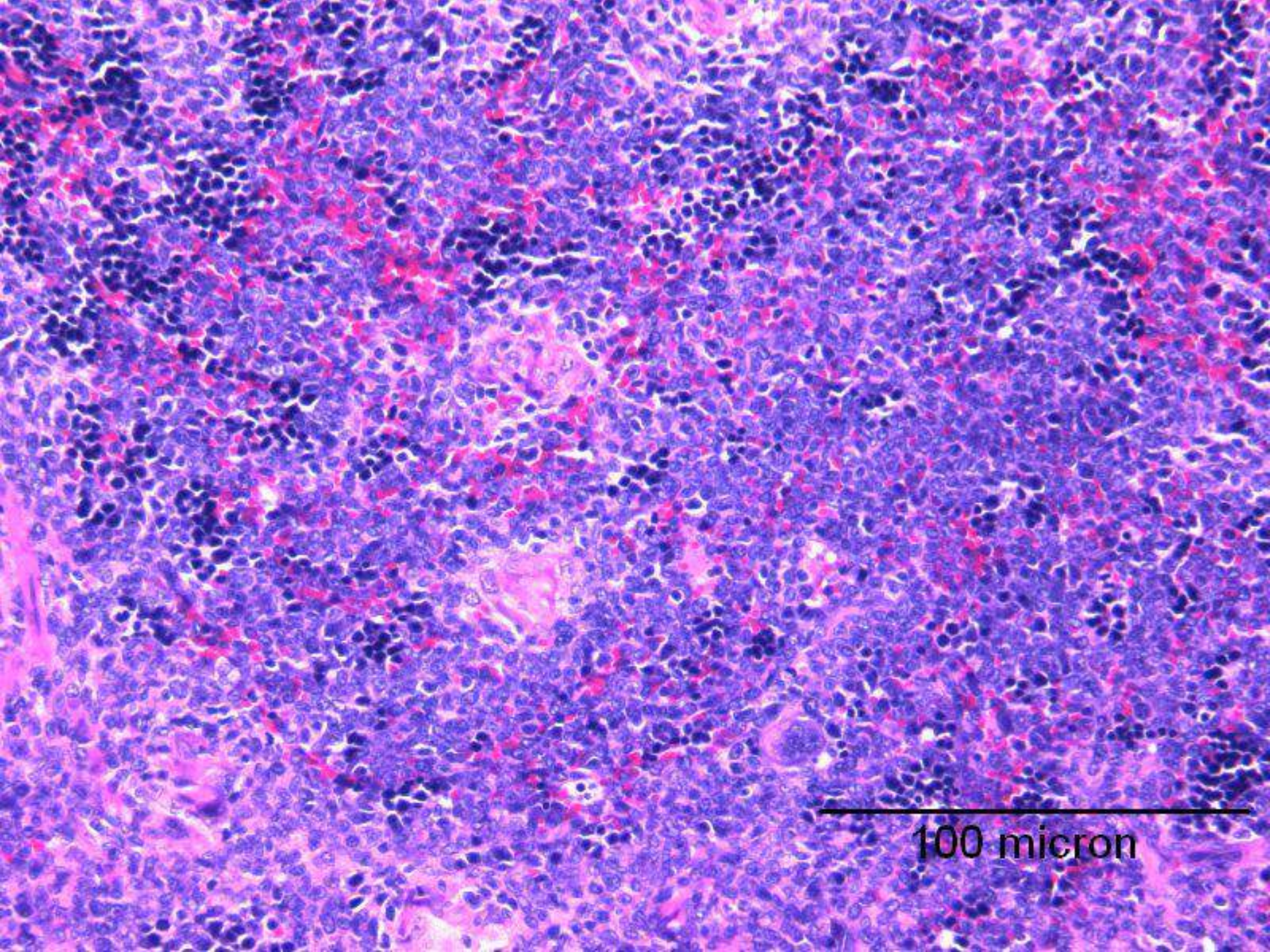




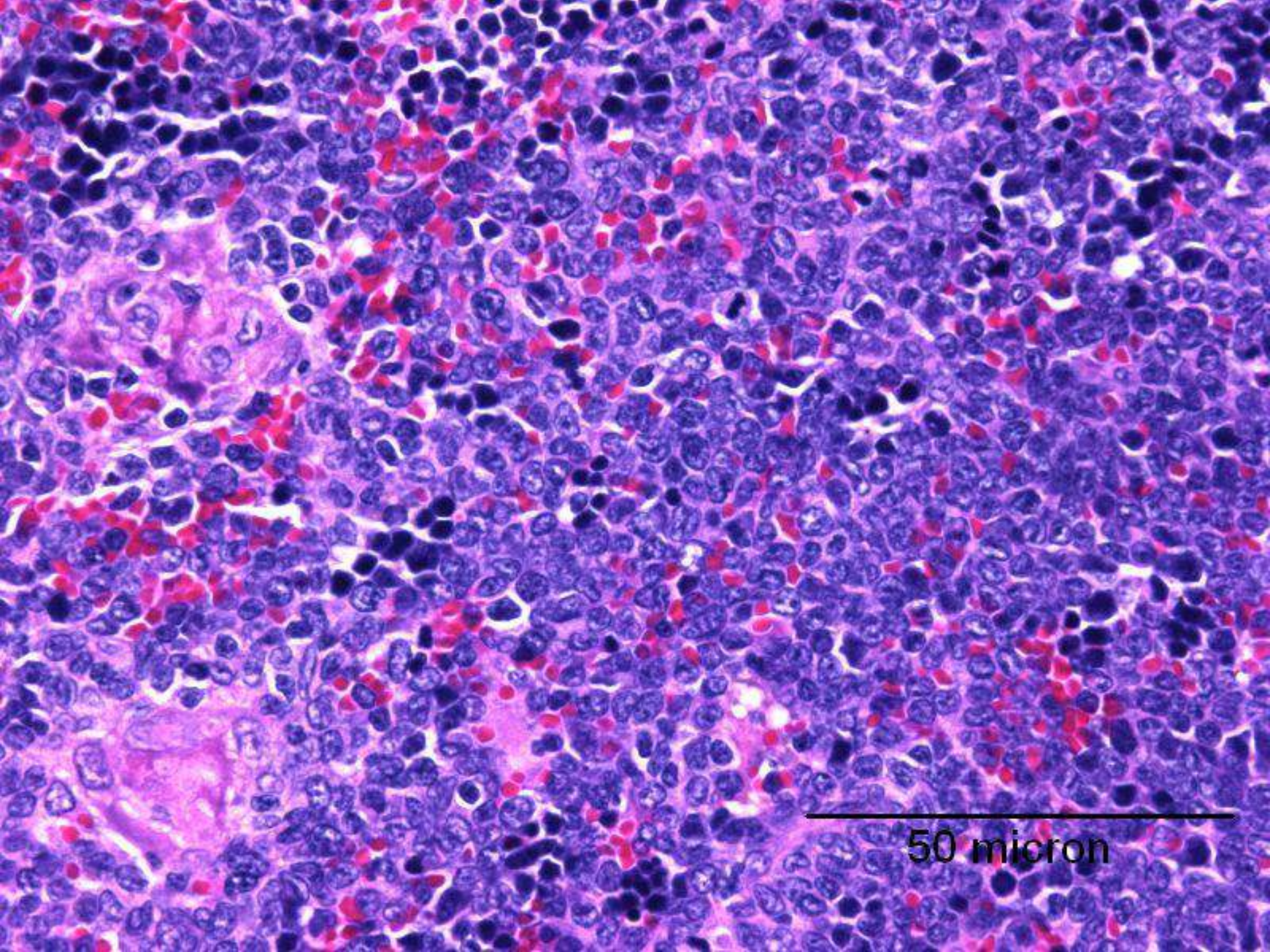
500 micron



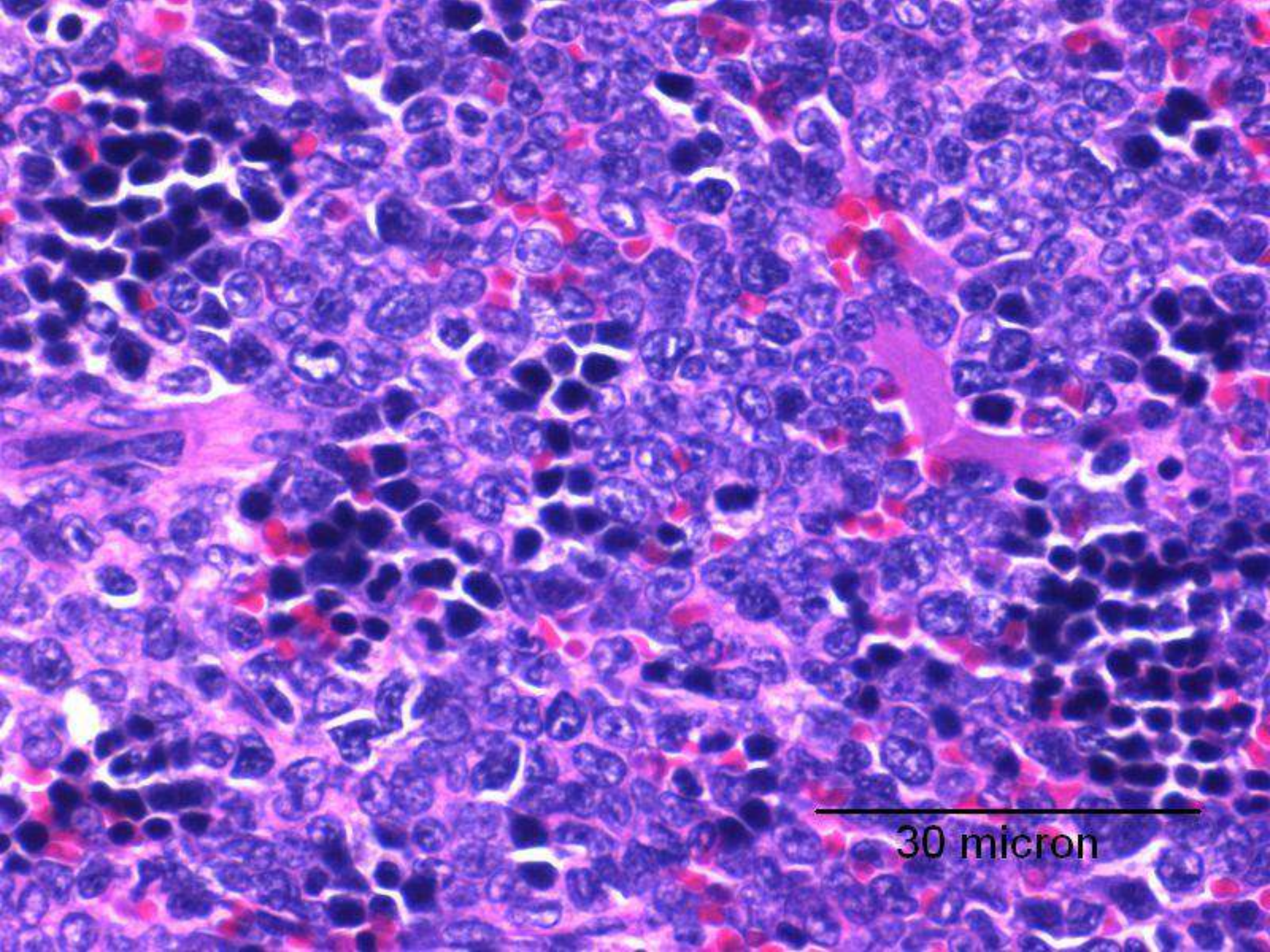
200 micron



100 micron



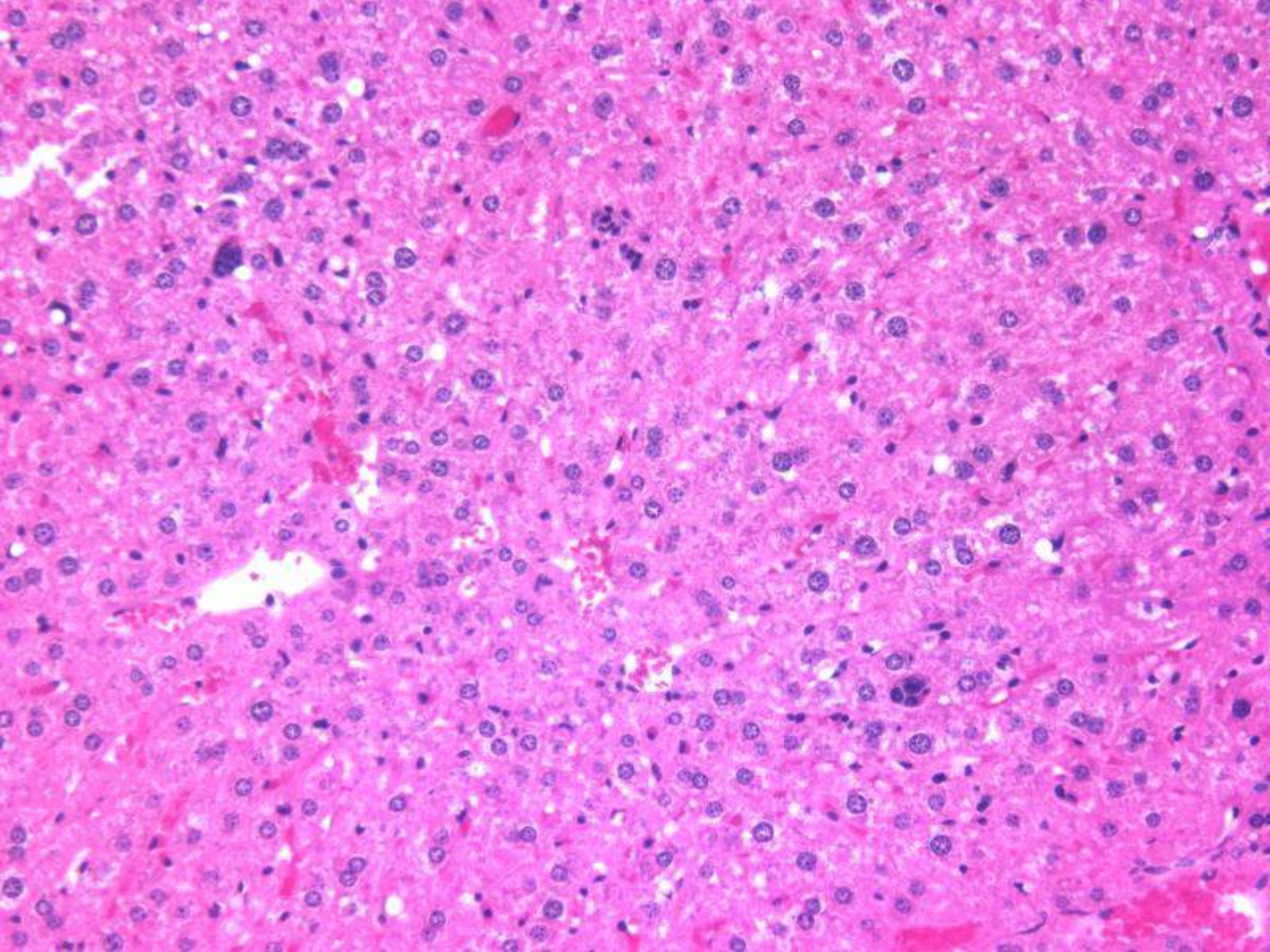
50 micron

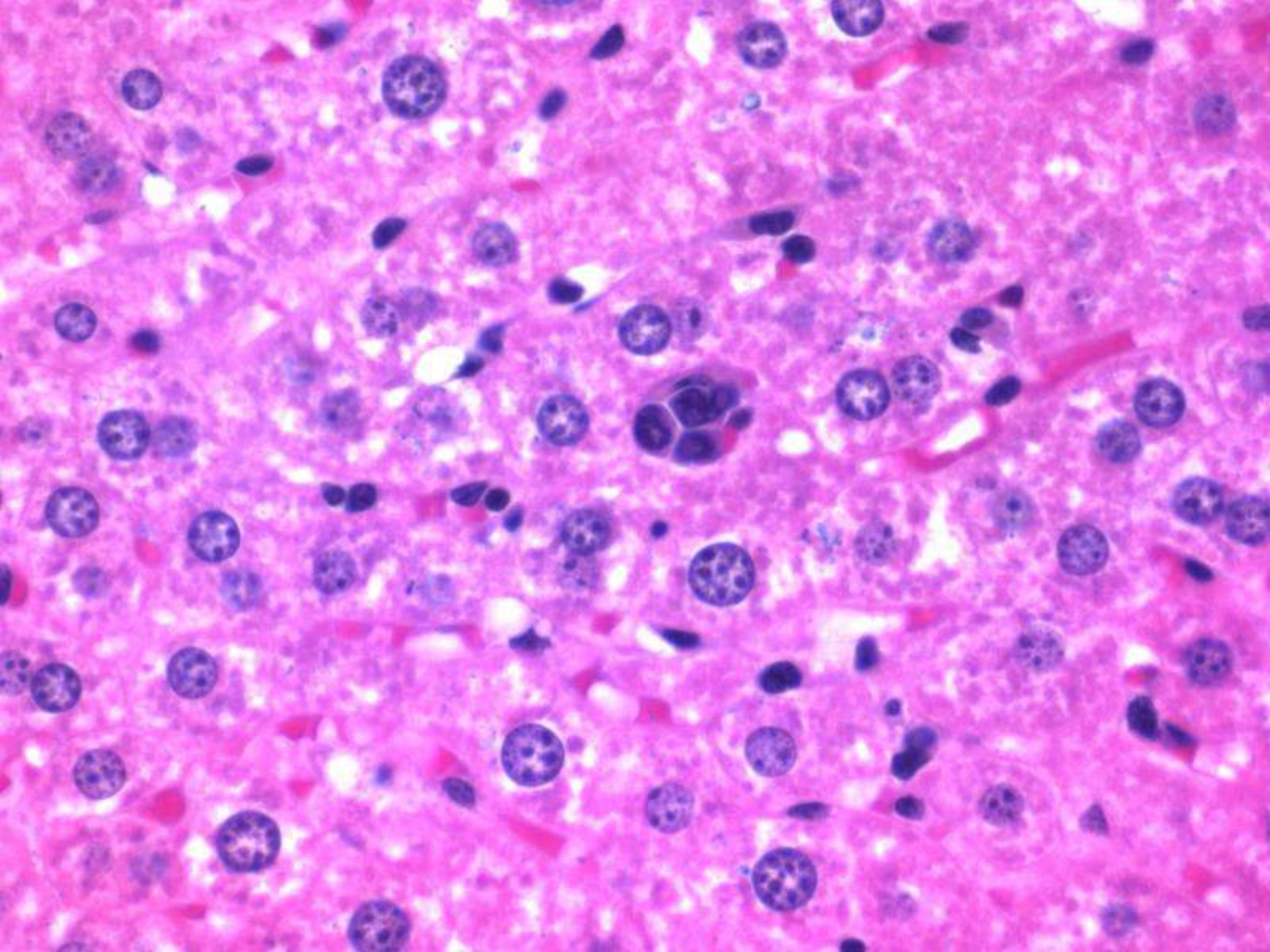


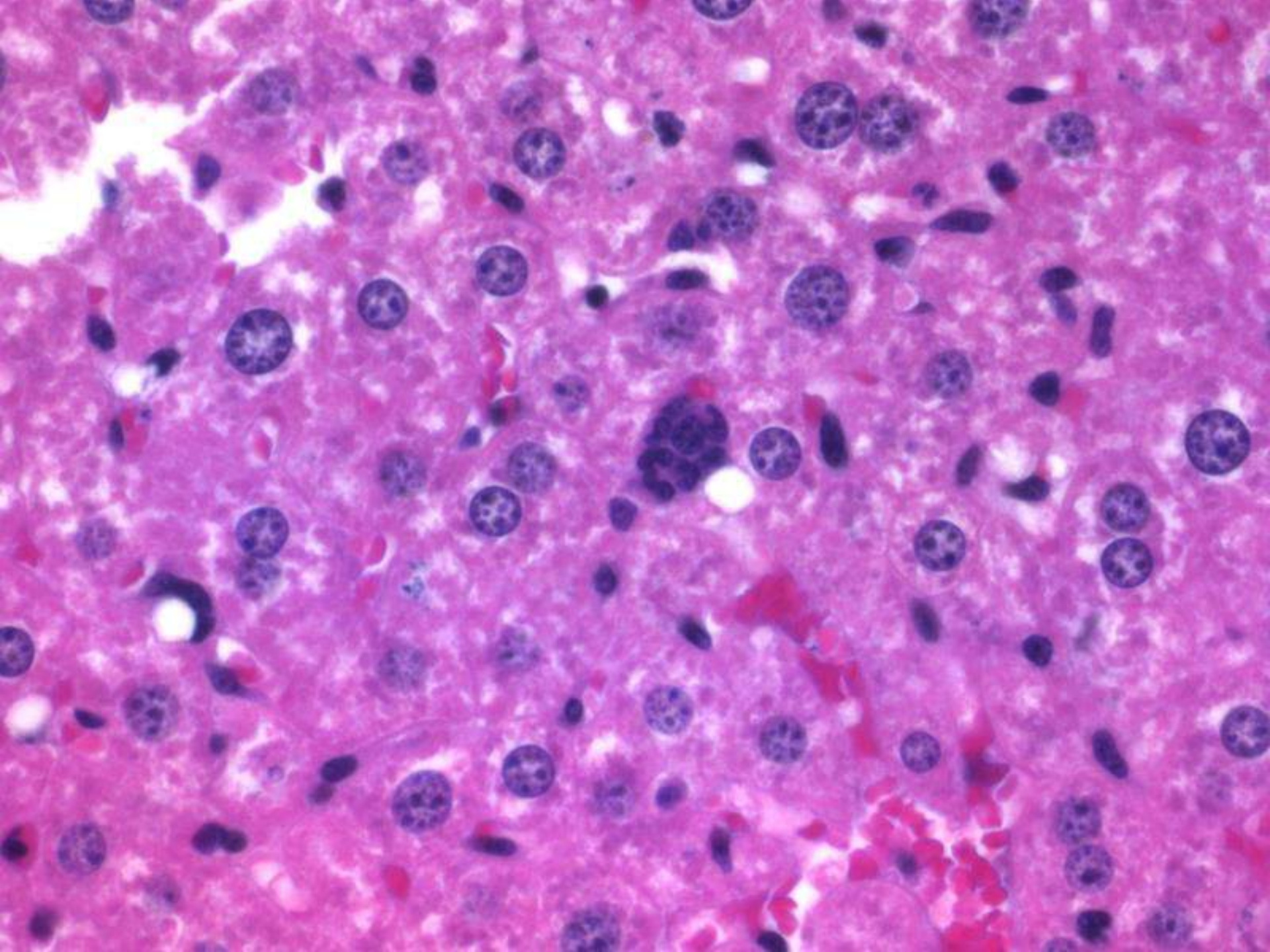
30 micron

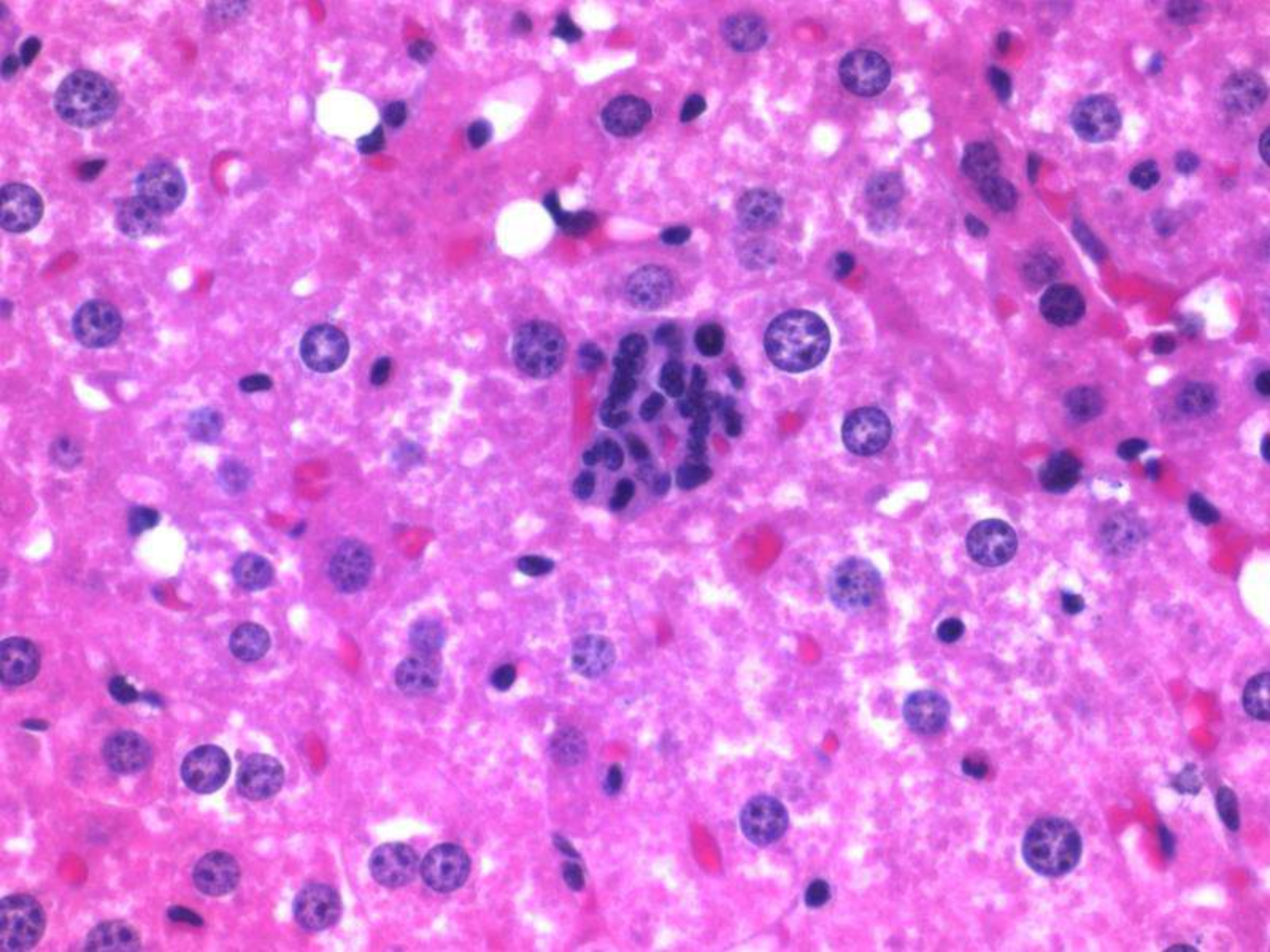
Some important unique biological features– liver

- Liver also a “bone marrow” organ
 - In embryo, and until 1 month of age, there are bone marrow cells in the liver
 - Thereafter, these cells are absent unless and until there is a demand for more blood cells (almost always neutrophils) than the spleen (and bone marrow) can make
 - This is almost always when there are bacterial infections in the mouse and more neutrophils are needed
 - Seldom, if ever, see increased erythropoiesis or thrombopoiesis
 - See erythropoiesis in **Apc** mice and GADD 34 KOs









Some important unique biological features— reproductive tract

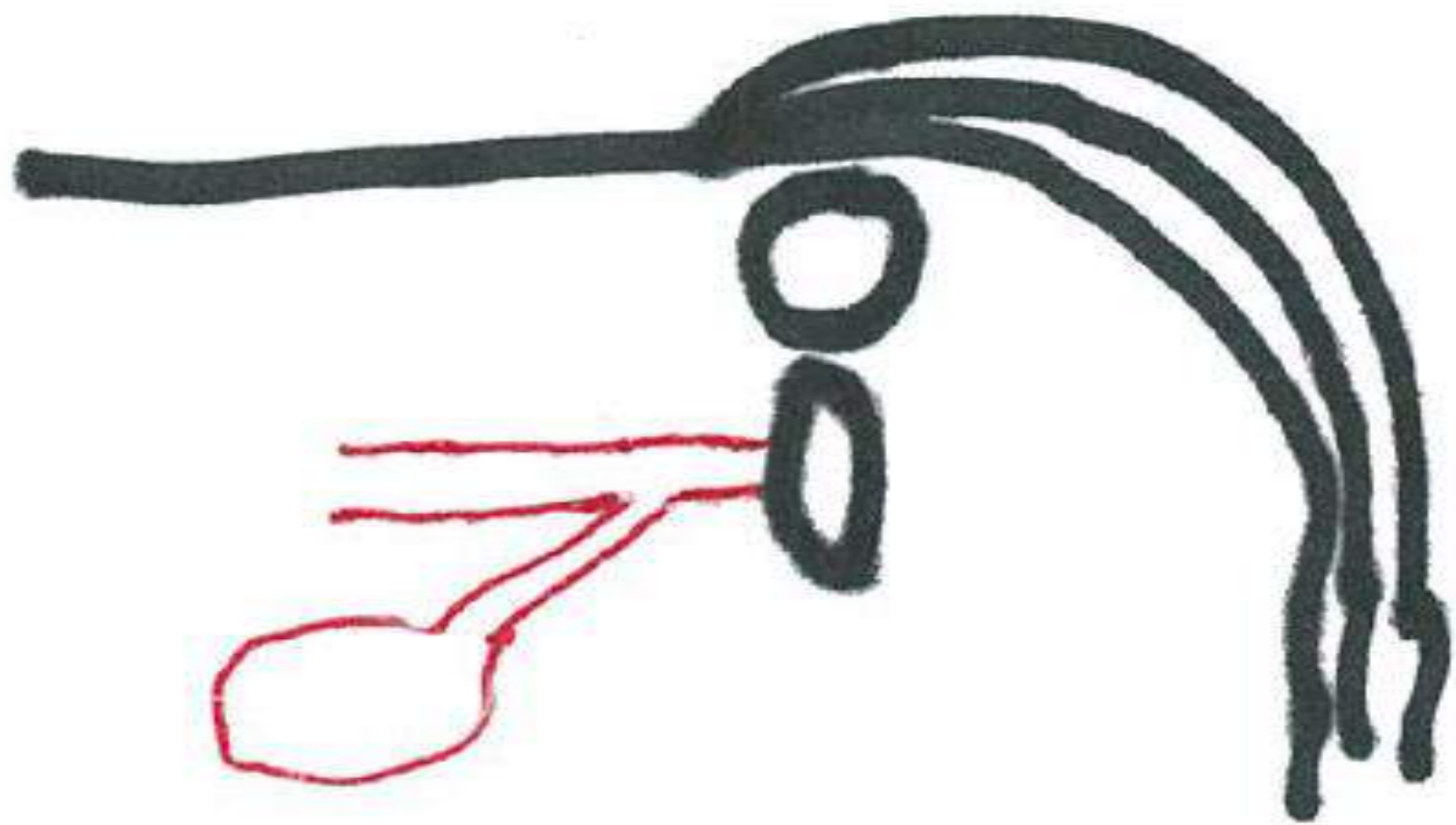
- Males
 - Have many accessory sex glands (most help make very sticky semen and the copulatory (vaginal) plugs
 - Preputial glands (bilateral)
 - Seminal vesicles (bilateral)
 - Coagulating glands (anterior prostates) (bilateral)
 - Ampullary gland (midline)
 - Dorsal prostate (midline)
 - Ventral prostate (midline)
 - Urethral glands
 - Bulbourethral glands (bilateral)

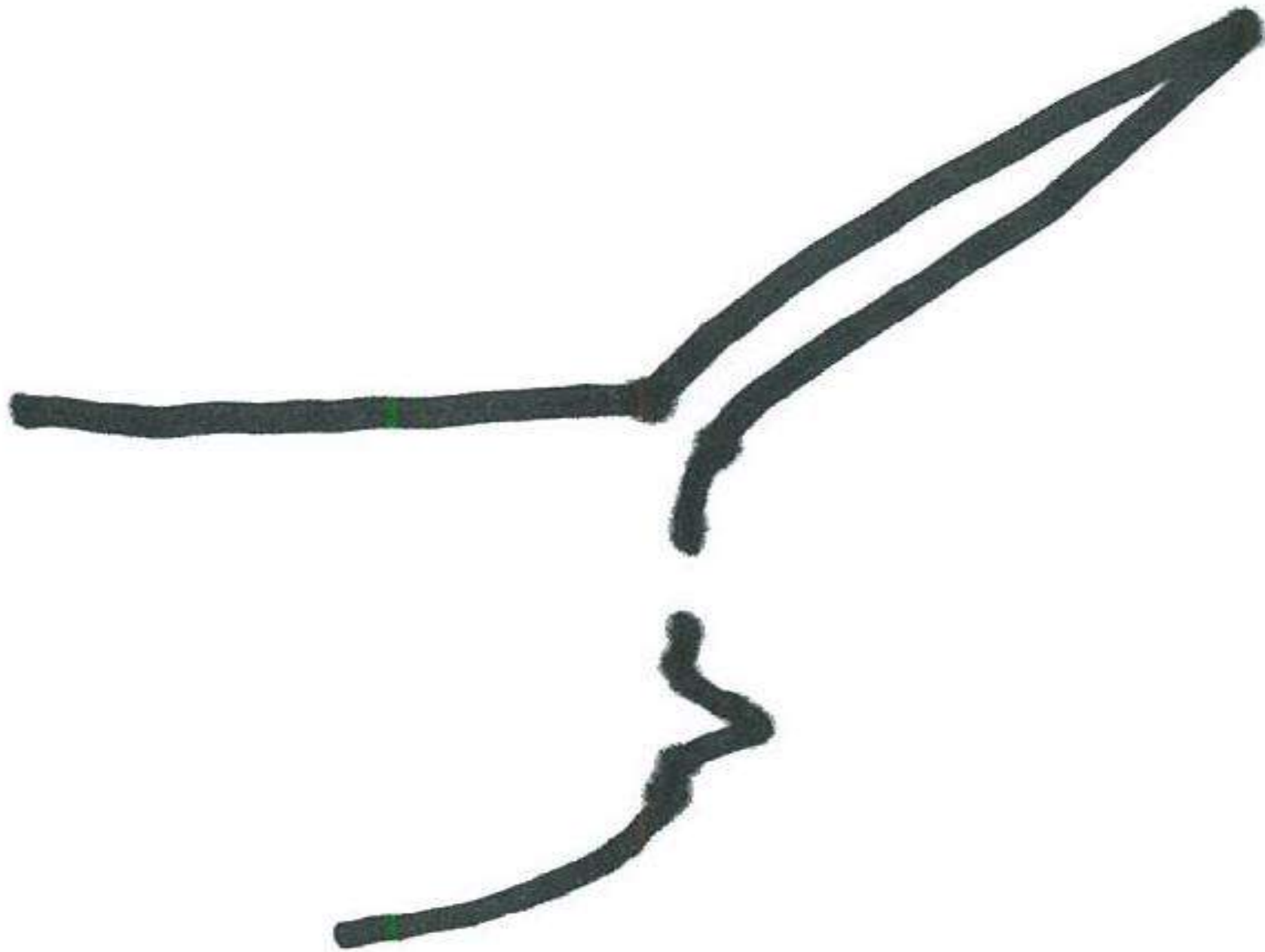


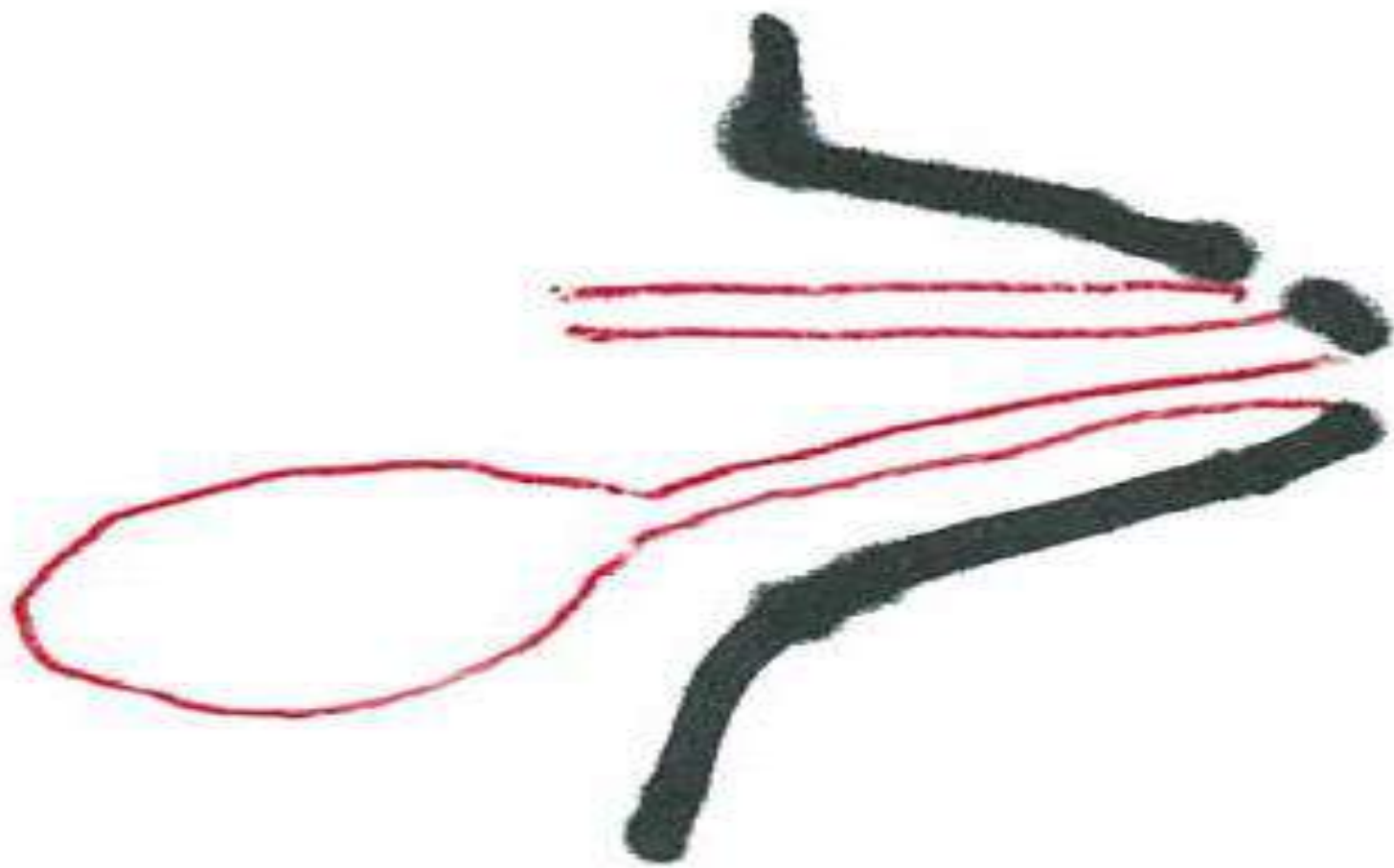


Some important unique biological features - reproductive tract (cont)

- Have seen “semen plugs” on the skin on the head and on the rump of mice
- These glands often infected by bacteria, so need to pay attention to them to explain neutrophilia or increased granulopoiesis
- Females
 - Have urogenital papilla
 - Have separate openings for reproductive tract and urinary tract
 - Have vaginal plugs (semen from male after mating)
 - These can be identified microscopically (see sperm)







3. Diagnostic Workups – Considerations/Procedures

transforming
medicine,
improving lives



When mice die unexpectedly, when they are sick (clinical signs), or when they have gross abnormalities

- Need to find out if any of the above is (are) due to:
 - Natural disease
 - Infections, infestations, neoplasms (tumors), reproductive problems, metabolic diseases
 - Husbandry problems
 - Water deprivation, food deprivation, fighting, other injuries
 - Experimental manipulation
 - Irradiation, anesthesia, surgery, injections, skin applications
 - Any combination of the above

What are options for workup of cases?

- Gross necropsy – stop here if dead too long
- Histopathology
- Ancillary testing
 - Aerobic bacterial culture w/wo sensitivity testing
 - Anaerobic bacterial culture w/wo sensitivity testing
 - Serology for antibodies against a variety of agents
 - PCR for selected agents
 - Toxin assays (e.g. toxin A testing - *Clostridium difficile*)
 - Serum chemistries
 - CBCs (RBC and WBC)

Types of situations typically encountered

- Found dead
 - Could be many things
 - Could be bacteremia/septicemia
 - Could be neoplasm in chest or another critical place
 - Could be water deprivation, especially if other animals also dead (see cannibalism with this)
 - Could be dystocia (difficulty birthing)
 - Could be retained fetuses
 - Could be blocked urinary tract if male
 - Could be enlarged cecum if a germ-free mouse

Types of situations typically encountered (cont)

- Abnormal appearance
 - Alopecia (loss of hair)
 - Ruffled fur
 - Dermatitis
 - Head tilt
- Abnormal behavior
 - Circling (“NASCAR” mice)
 - Scratching
 - Slow moving

Types of situations typically encountered (cont)

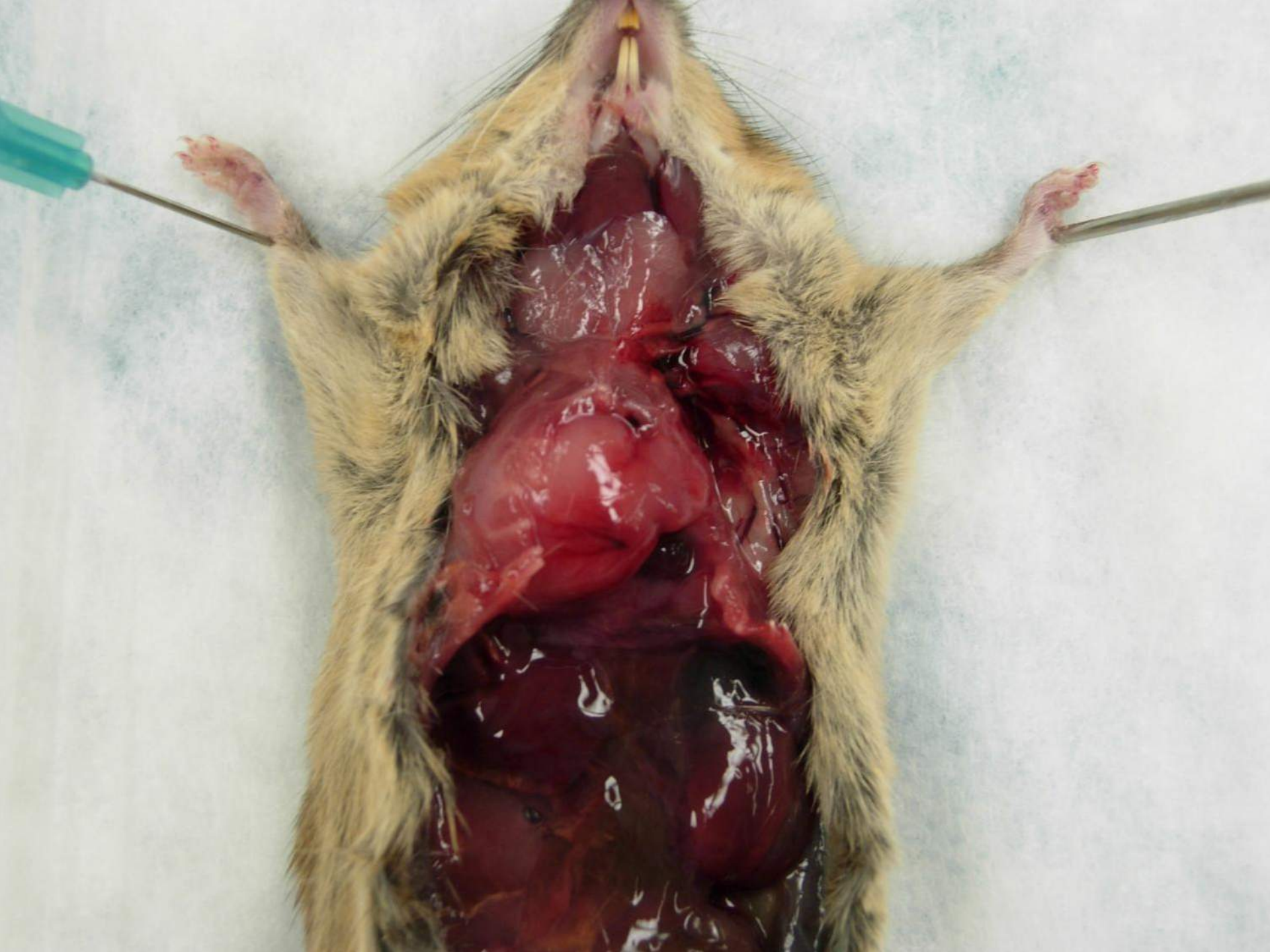
- Lumps and bumps
 - Abscesses
 - Neoplasms (tumors)
 - Benign
 - Malignant (cancer)
 - Granulomas (macrophages primarily present)
 - Hematocyst/hematoma (“blood blister”) e.g. facial vein venipuncture
 - Prolapsed organs through pelvic canal into peri-anal area

How investigate dead animals?

- Be specific about the death
 - Don't write "died on 10 AUG 12" unless you saw it alive on that date and later found it dead on that date, or...
 - Write "found dead at 8 am, 10 AUG 12" or...
 - Write "last seen alive at 5 pm, 9 AUG 12"
- Refrigerate or freeze the animal if necessary
 - No refrigeration needed if do immediate necropsy
 - Refrigerate if necropsy will not be done immediately but within a few hours to a day or so
 - Freeze animal if necropsy can't be done same day
 - Freezing causes "freeze artifact" (ice crystal formation)

How investigate dead animals?(cont)

- Always do gross necropsy on dead mice no matter how decomposed (postmortem change)
 - Good chance may find gross lesions that caused the death, such as neoplasms, heart thrombi, macerated retained fetus(es) with uterine rupture and peritonitis
 - Can harvest heart blood or bone marrow for bacterial culture (these organs last to be contaminated by bacterial overgrowth)



How investigate lumps/bumps?

- Try to aspirate contents, culture, and/or put on glass slide (smear) and stain and examine with a microscope
 - Use sterile 20 gauge needle on end of 3 - 5 cc syringe
 - If pus comes out, it is an abscess
 - If blood comes out, it is a hematocyst/hematoma (“blood blister”) or a neoplasm of endothelial cells (hemangioma/hemangiosarcoma)
 - If clear fluid comes out, it is a seroma or edema fluid
 - If nothing comes out, then it could be a neoplasm
 - It also could still be an abscess or a granuloma

How investigate lumps/bumps? (cont)

- After aspiration open mass using sterile technique
- If looks like pus, culture inside wall of abscess (center might be sterile)
- If is solid mass, make impression smear

4. Selected Diseases and Conditions

transforming
medicine,
improving lives



Some major conditions commonly found in mice

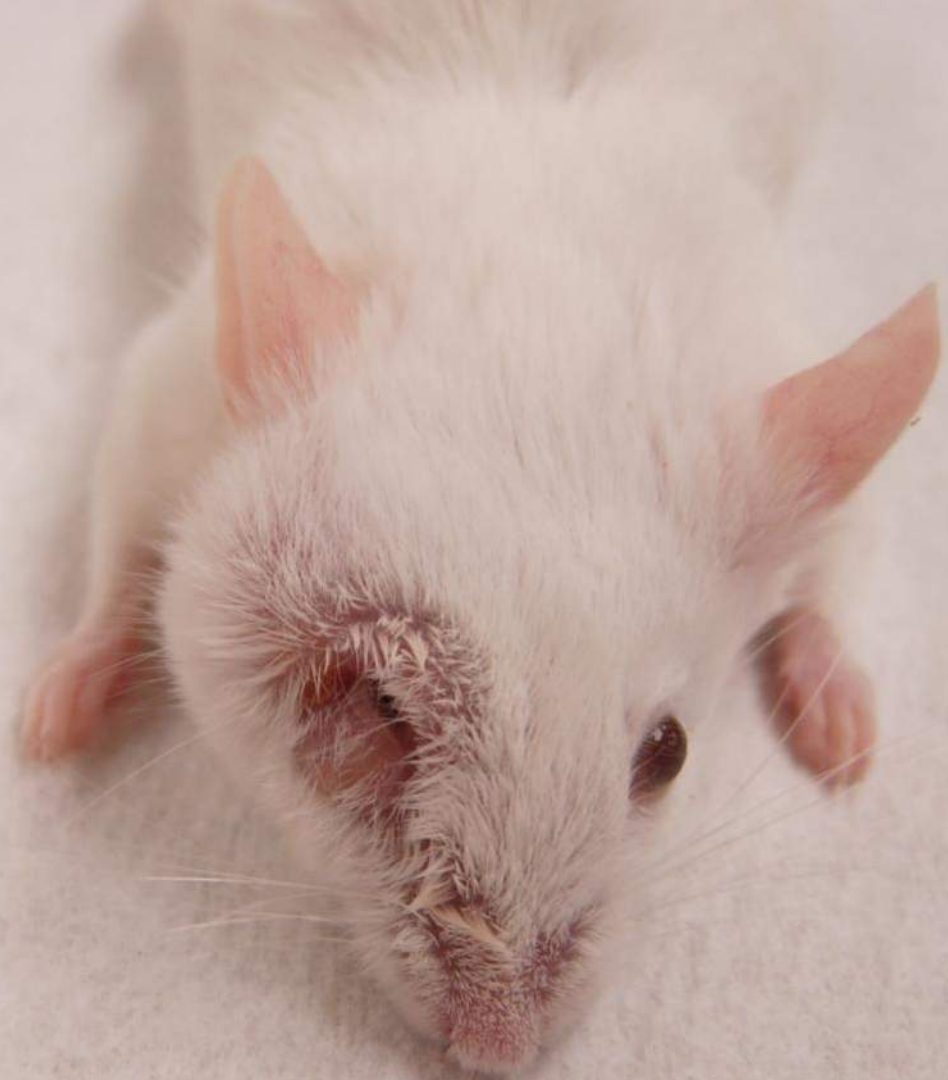
- Abscesses
- Bacteremia/septicemia
- Neoplasms (tumors)
- Rectal prolapse
- Hernias
- Ulcerative dermatitis
- Penile inflammation & necrosis
- Urinary obstruction

Some major conditions commonly found in mice (cont)

- Dystocias (difficult birthing)
- Retained pups after partuition (giving birth)
- Vaginal bleeding

Abscesses – periorbital and other areas on head and around neck

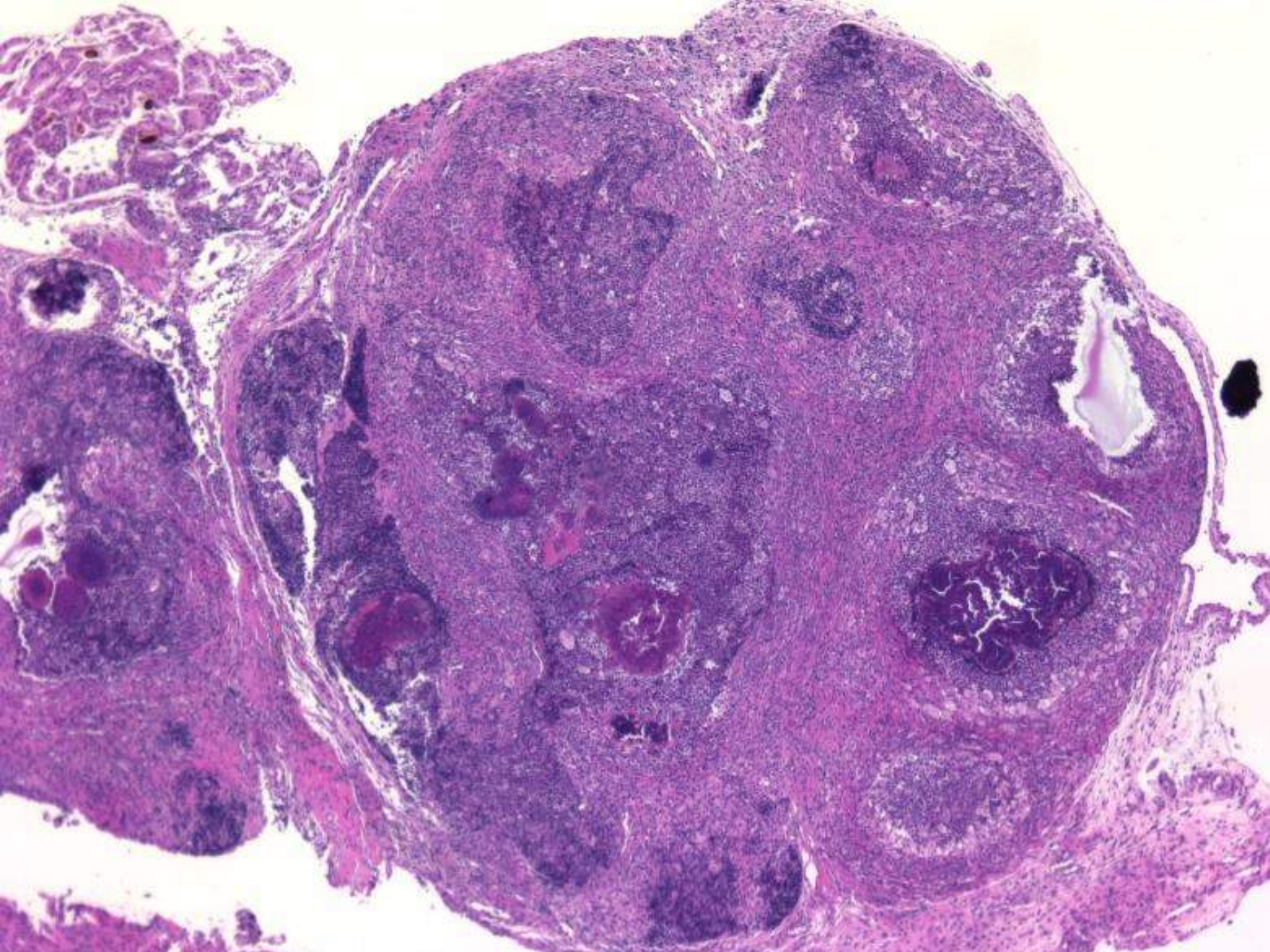
- Common area
- Sometimes behind globe of eye inside the orbit and get bulging of eye outward
- Be sure to culture this for bacteria
- Classic disease if it is “botryomycosis” caused by *Staphylococcus aureus* . If it is this condition, it is a misnomer because it is not a “mycosis” (fungus) but instead a bacterial species
- With botryomycosis see “sulfur granules”

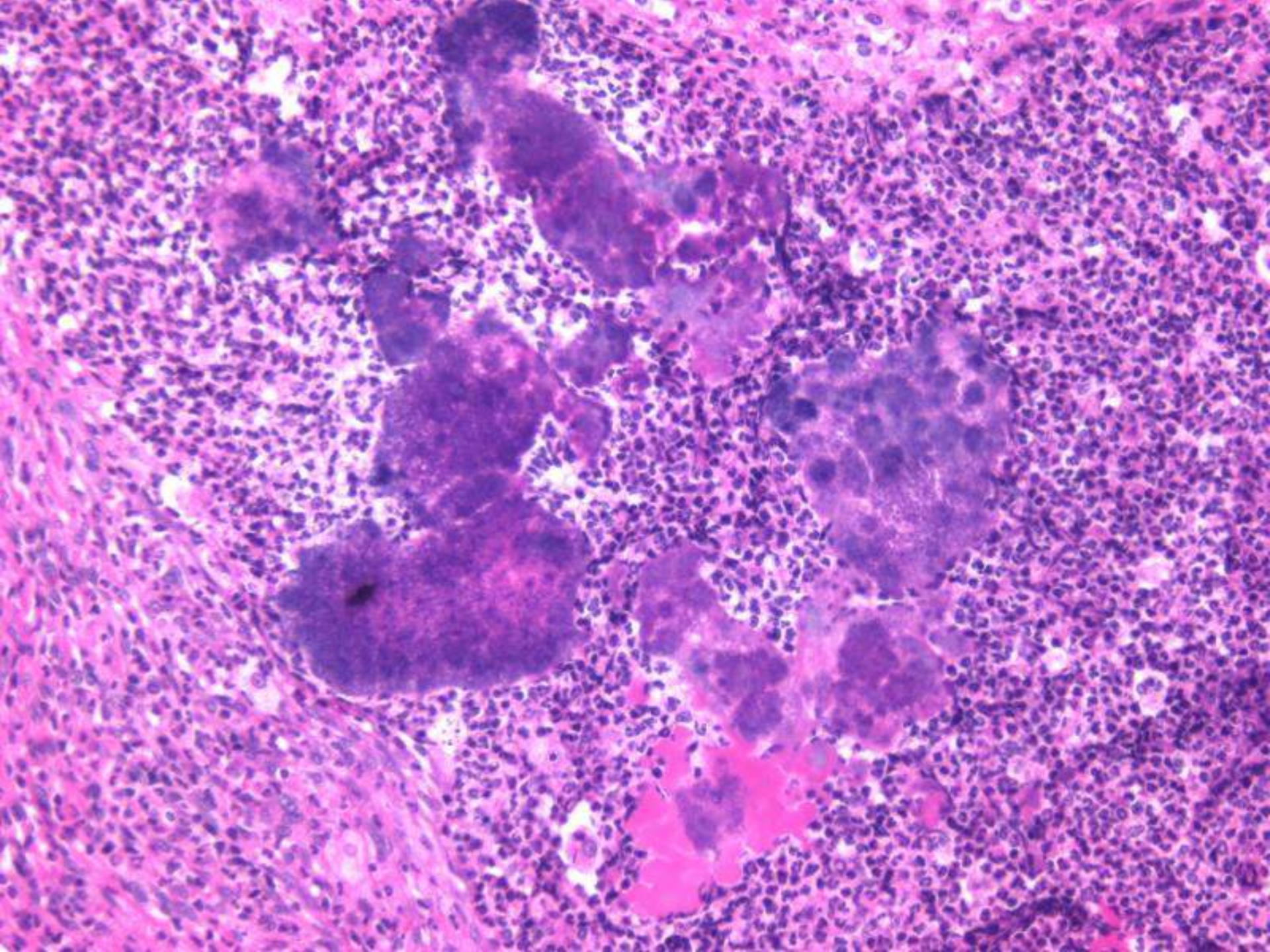


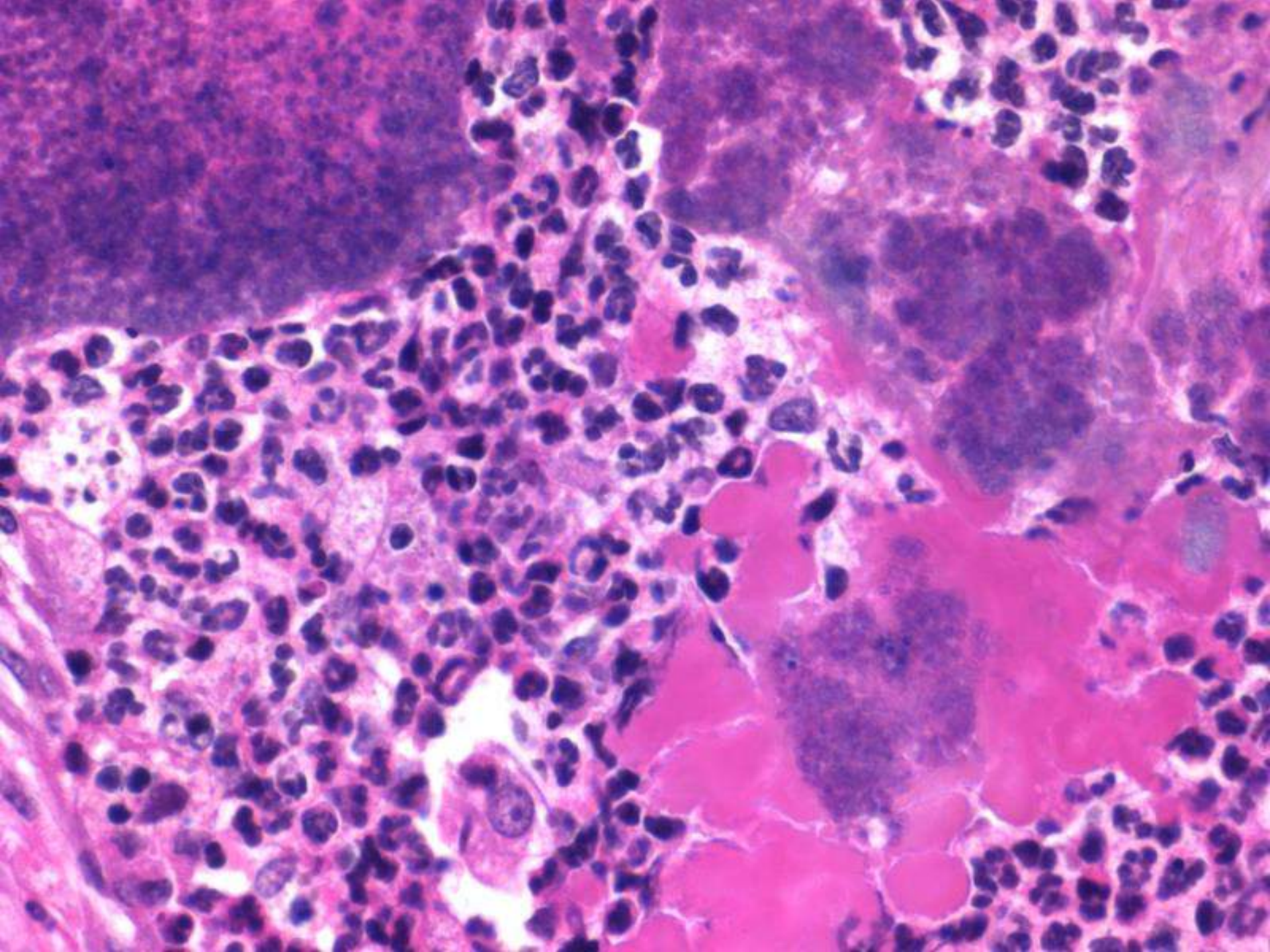


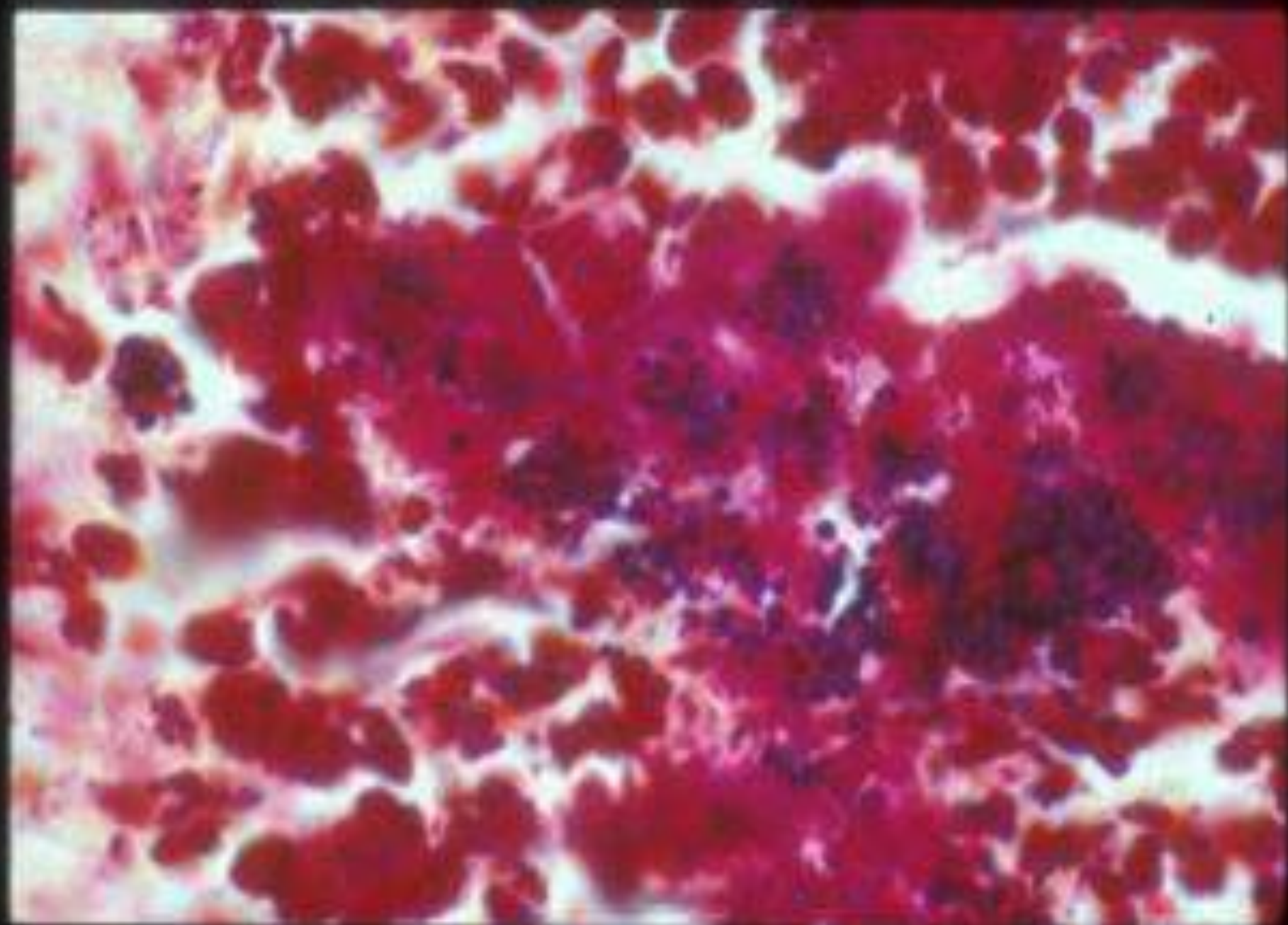












With abscesses – get enlarged spleens

- Get enlarged spleens even with apparently small abscesses
- If have enlarged spleen with a big abscess there likely will be granulopoiesis in the liver as well
- Happens regardless where the abscess develops



Abscesses on tails of NOG and other severely immunodeficient mice

- They are very vulnerable to infection with bacterial species that are considered not pathogenic in immunocompetent animals (e.g. *Klebsiella oxytoca*)
- These mice form abscesses, but the abscesses have very thin walls This indicates that the host has little time to form the protective fibrous connective tissue wall (FCT) around the “pus” during the “battle” between the bacteria and the host



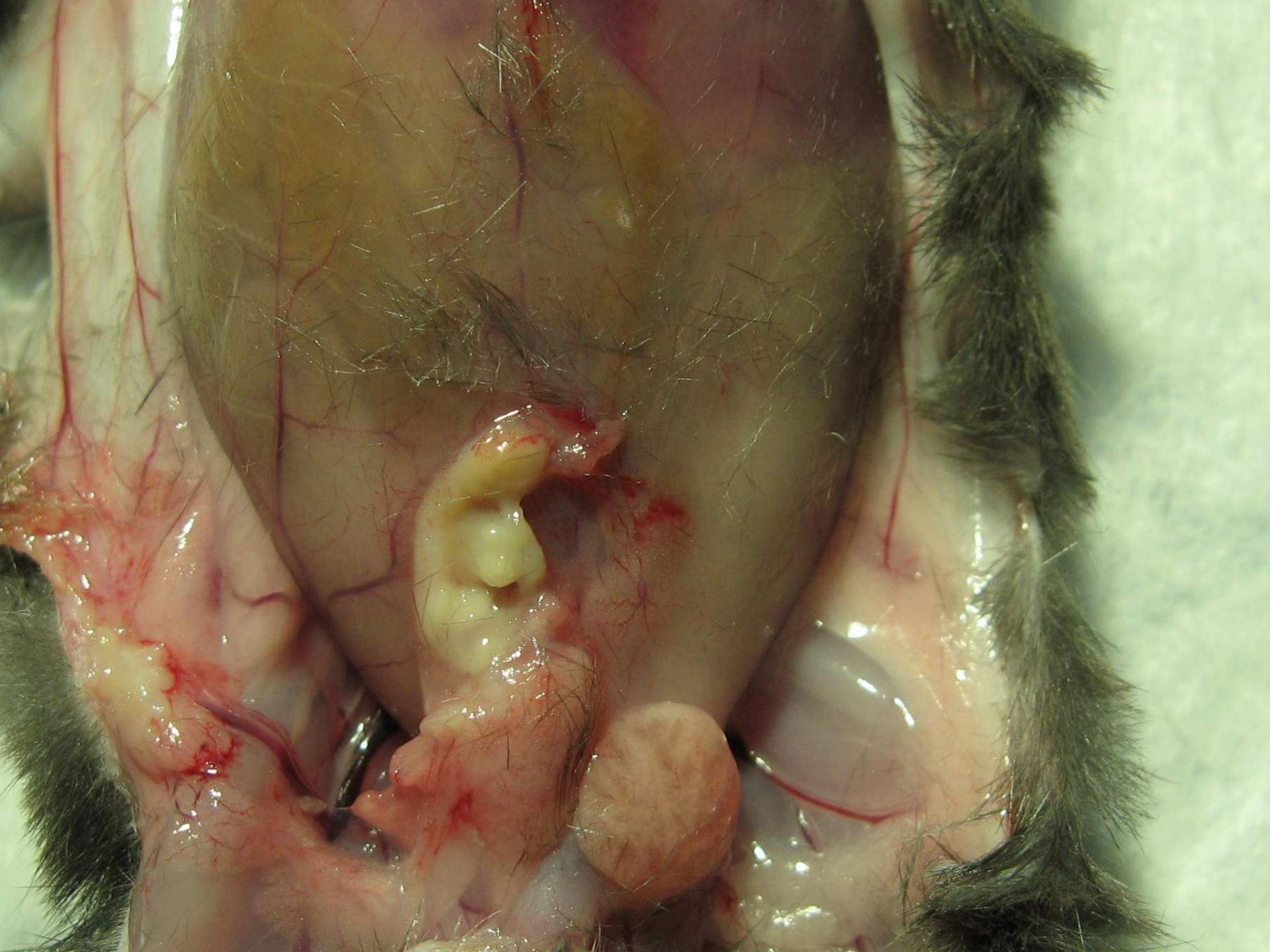




Preputial gland abscesses

- Seem to occur in male cagemates when housed together
- Will produce abnormal WBC profiles - neutrophilia (elevated neutrophil count)

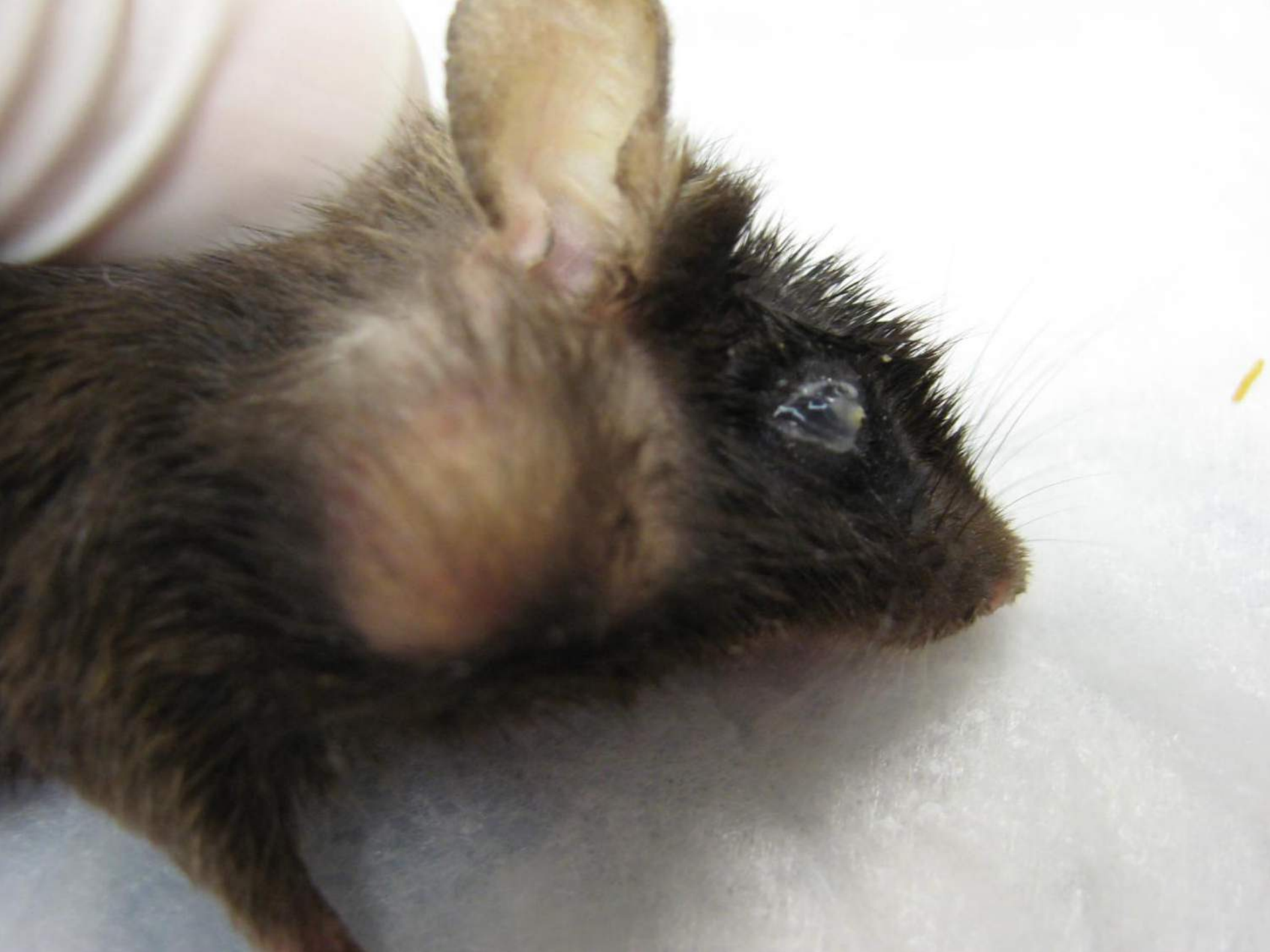




Abscesses elsewhere

- Seem to occur mostly in immunodeficient mice
- Usually have very thin fibrous connective tissue (FCT) walls and will readily rupture when manipulated





Bacteremia/septicemia (sepsis)

- Need to culture to identify bacterial species
- Culture the blood, not organs or tissues
- Take blood from heart or femur using sterile technique
- These sites are best protected from bacterial overgrowth
- Can use sterile saline when withdrawing blood from the marrow cavity in the femur

Neoplasms

- Most common is lymphosarcoma (cancer of lymphocytes)
- Usually affects spleen and lymph nodes
- Can be leukemic or non-leukemic







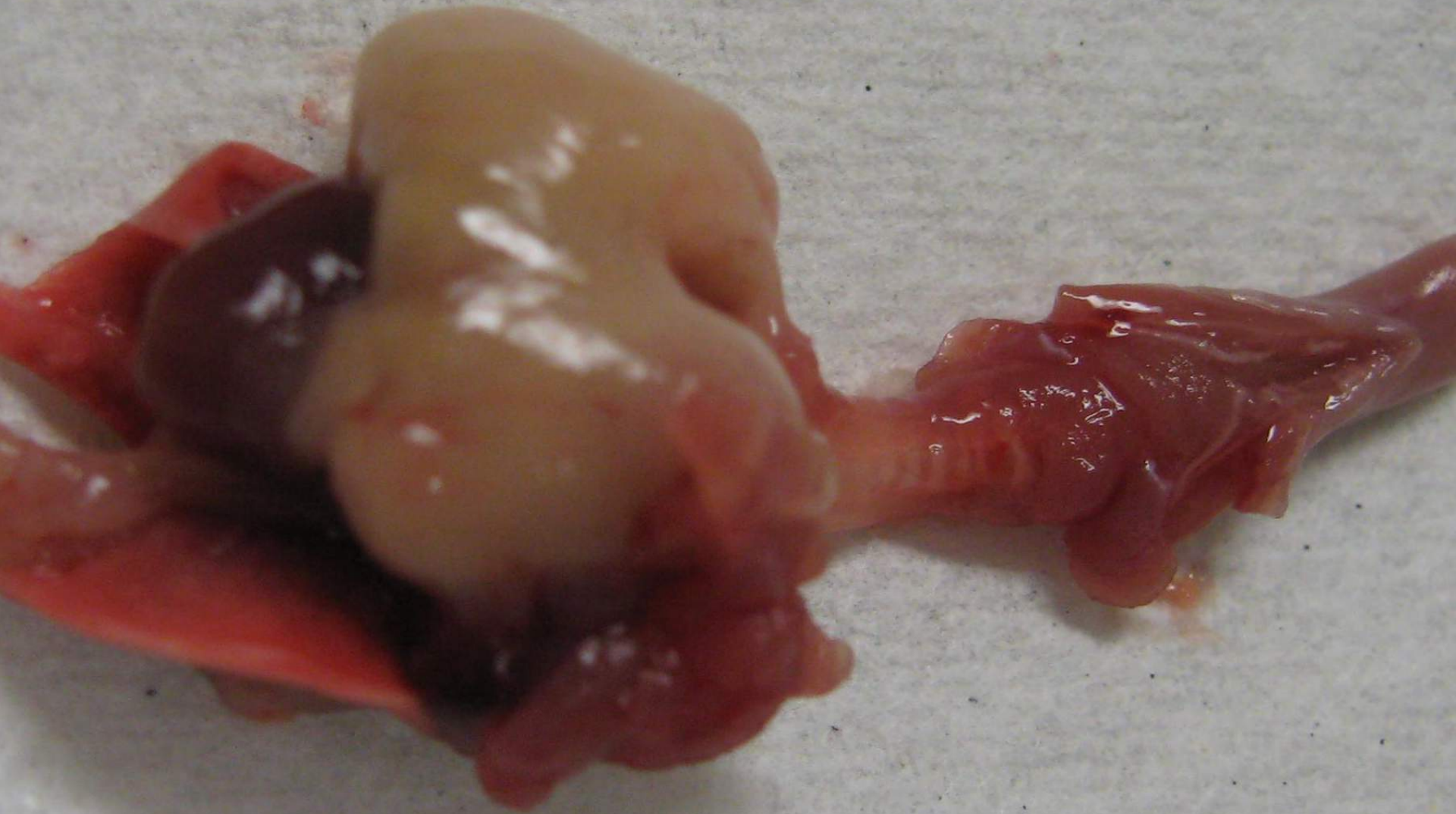


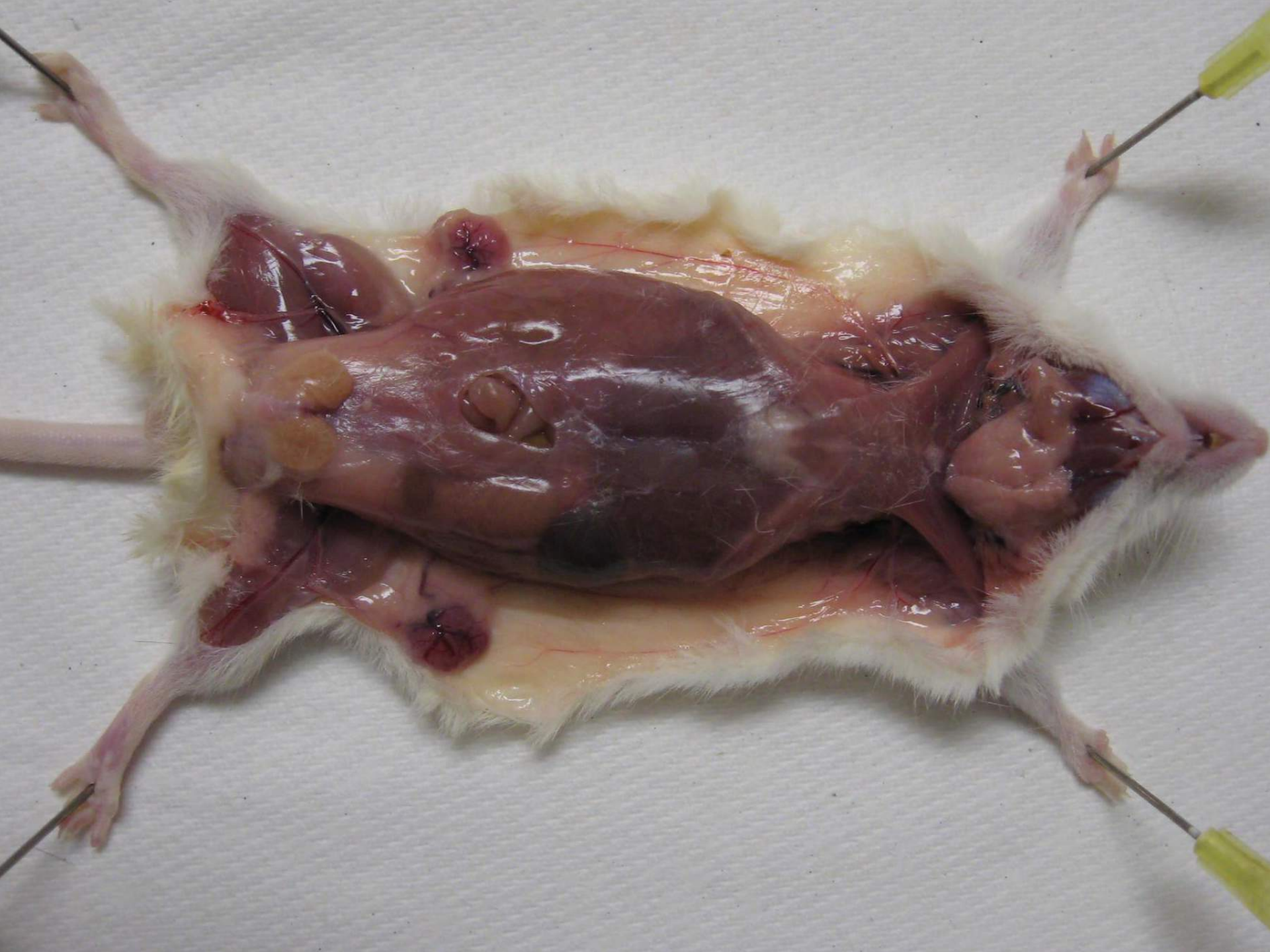










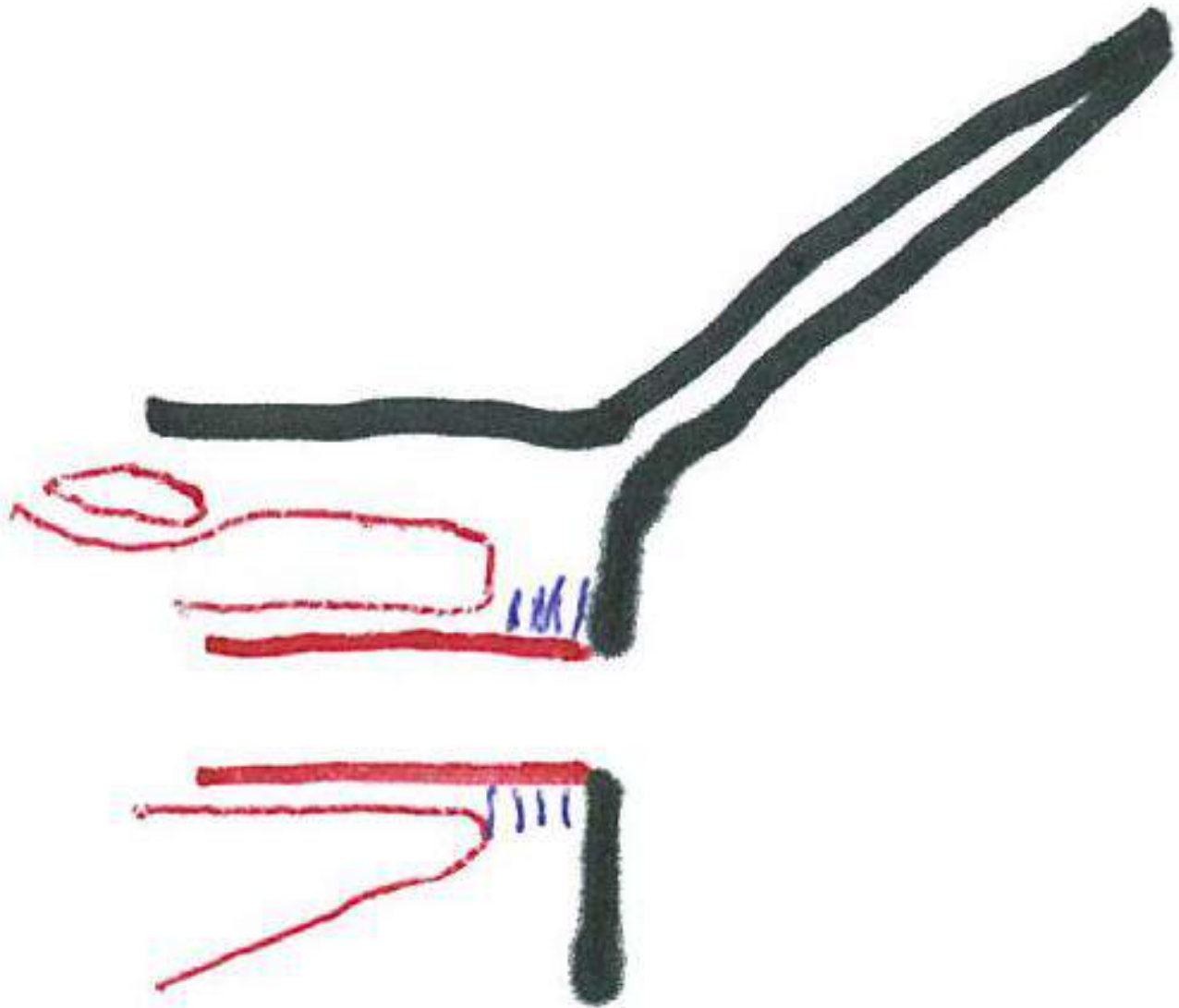


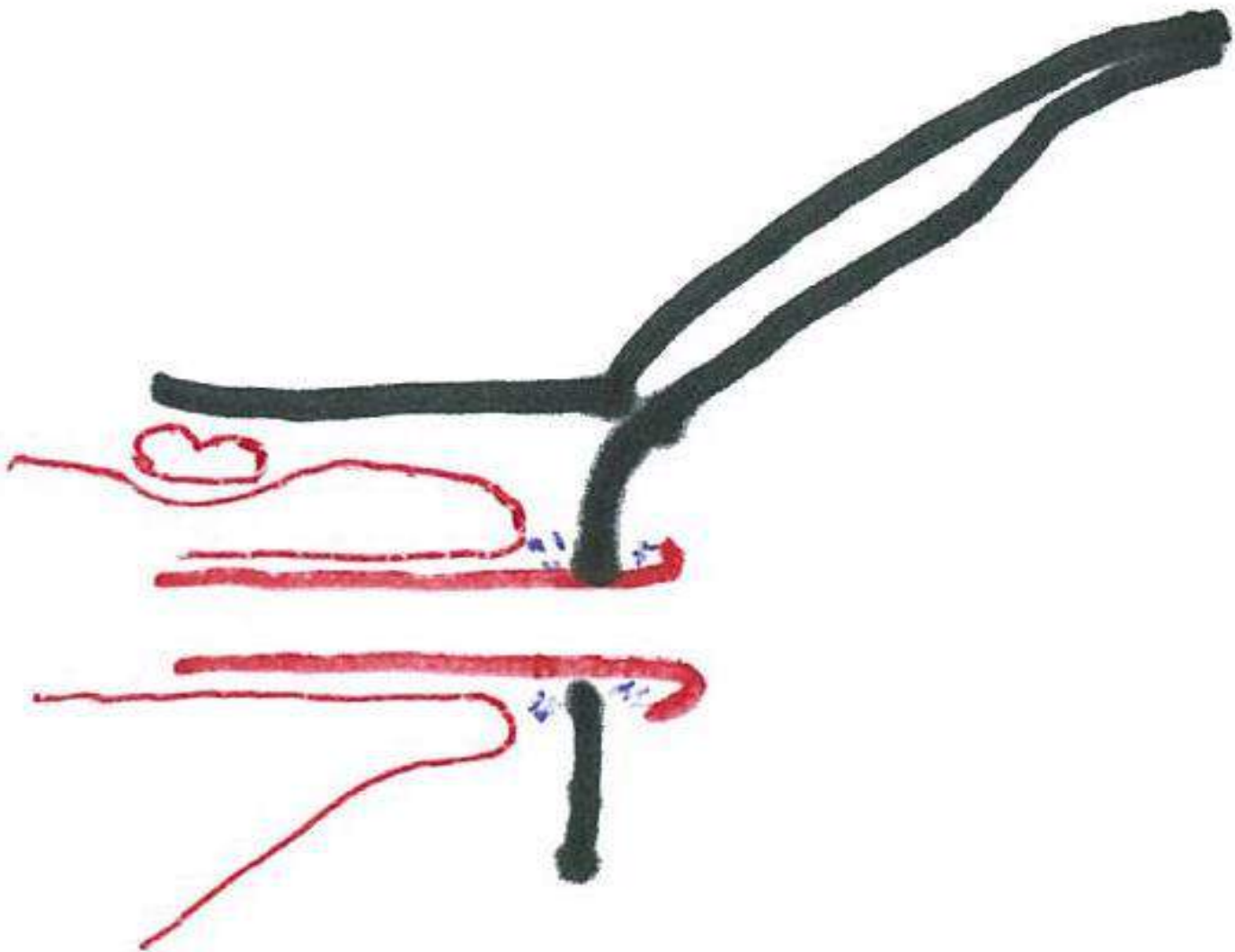
Rectal prolapse

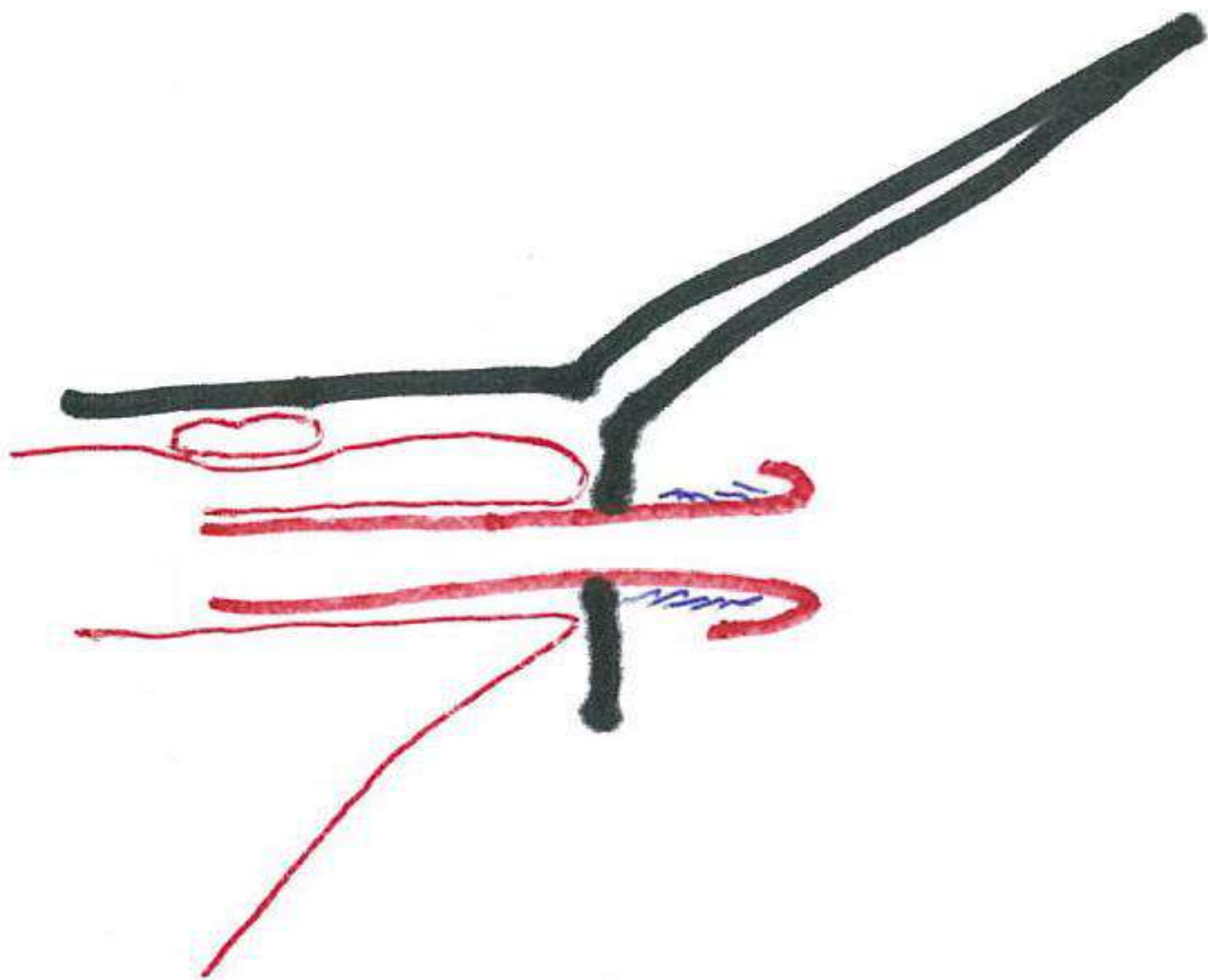
- Occurs occasionally
- Reportedly more common in nude mice

From RADIL Website; Univ. of Missouri









From RADIL Website; Univ. of Missouri



Hernias

- I saw a lot of this in a Pennsylvania institution
- Usually was herniation of the accessory sex glands, especially the seminal vesicles, through the pelvic canal
- Also saw herniation of the cecum through the pelvic canal
- On occasion saw both structures in the hernia



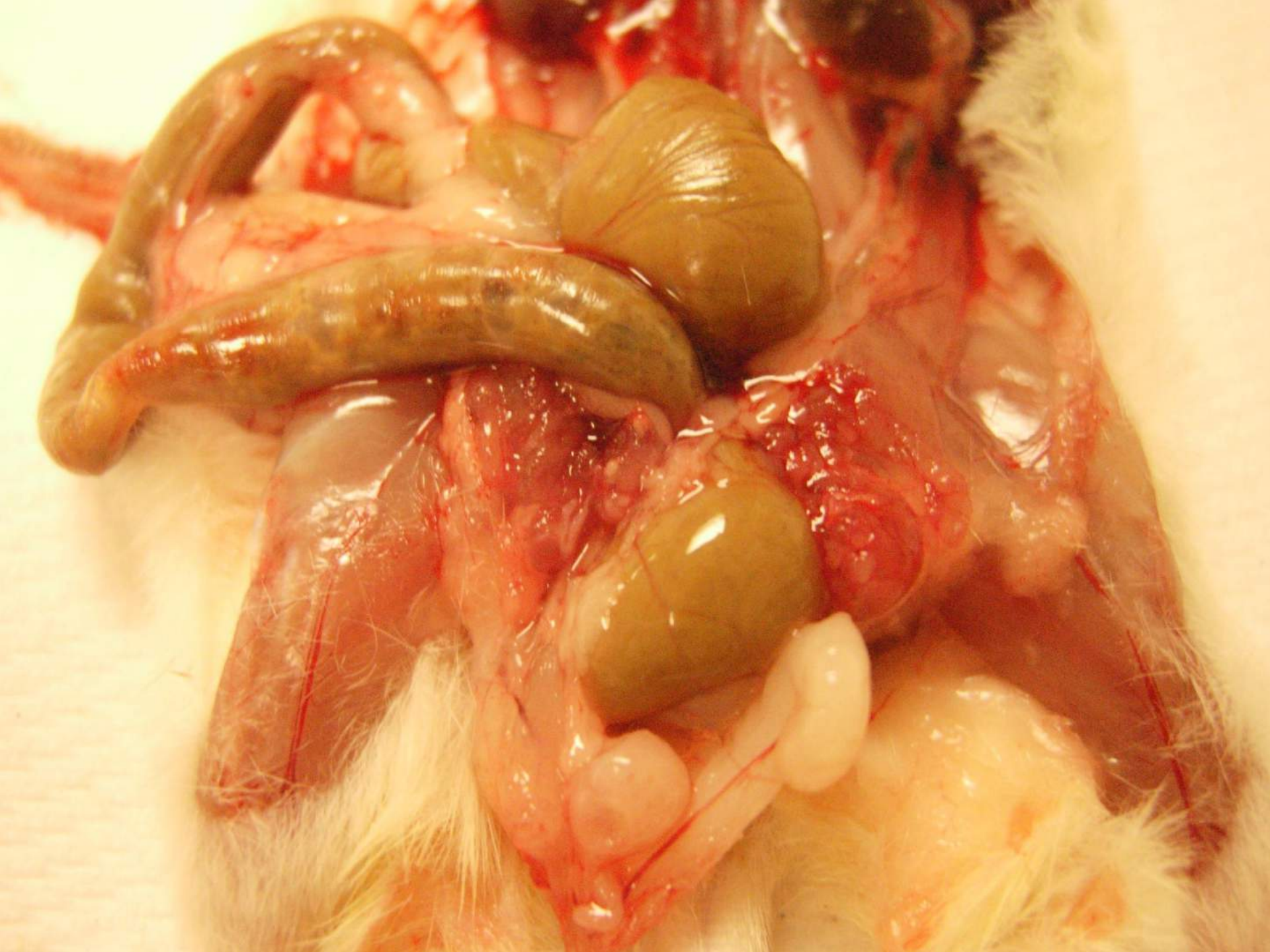












Ulcerative Dermatitis

- Most important non-infectious disease in mice
- Cause still unknown
- Most common in B6 mice

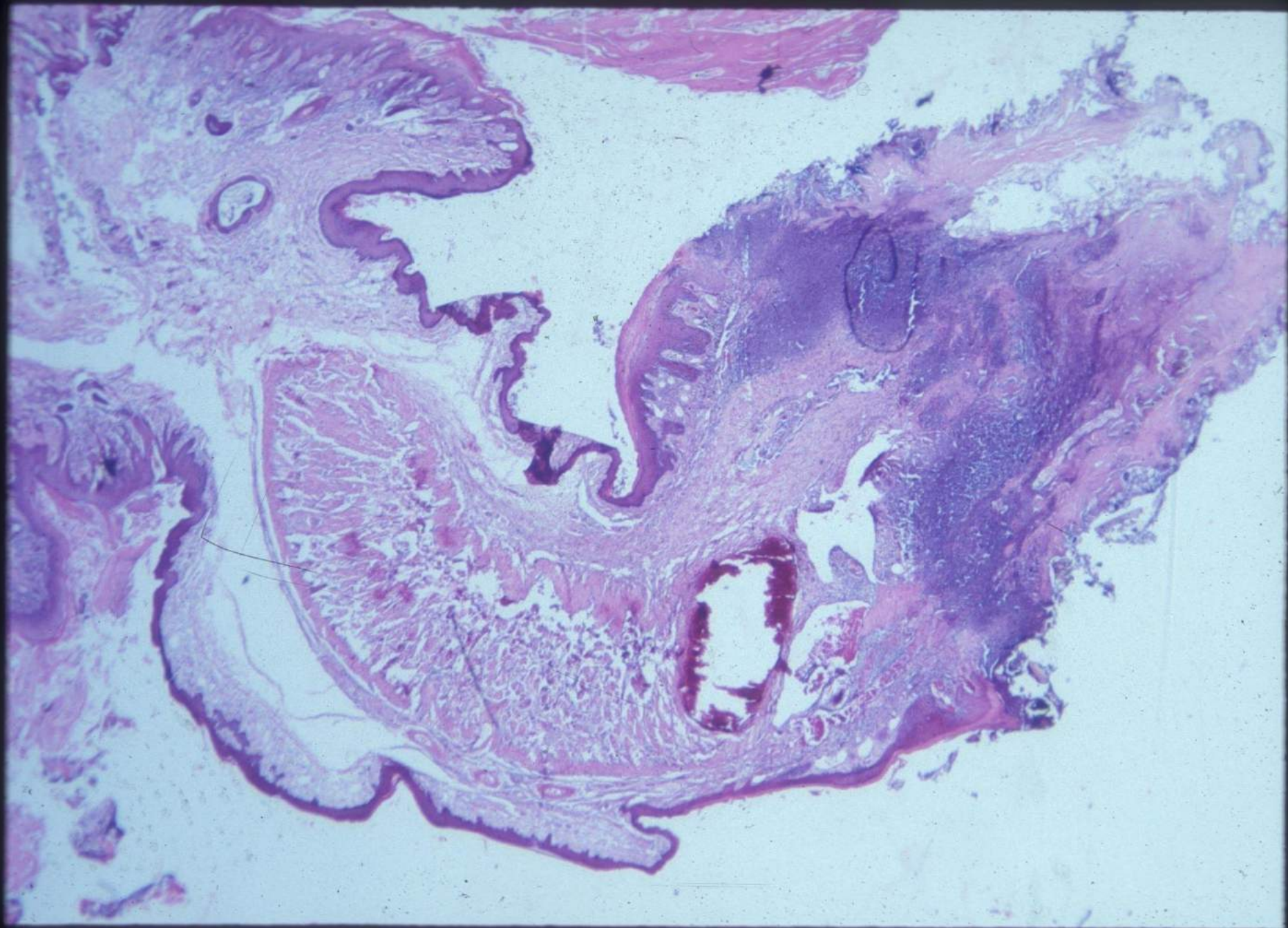




Penile inflammation & necrosis (& sometimes urinary obstruction)

- One report says B6 mice self-mutilate themselves
- Most often due to fighting when “unfamiliar” males are housed together
- Males housed together when young (before weaning or shortly thereafter) generally will not fight - thereafter they do fight
- When they fight they are nasty and will bite each other’s genitalia
- Can result in inflammation producing blocked urethra’s with urinary obstruction





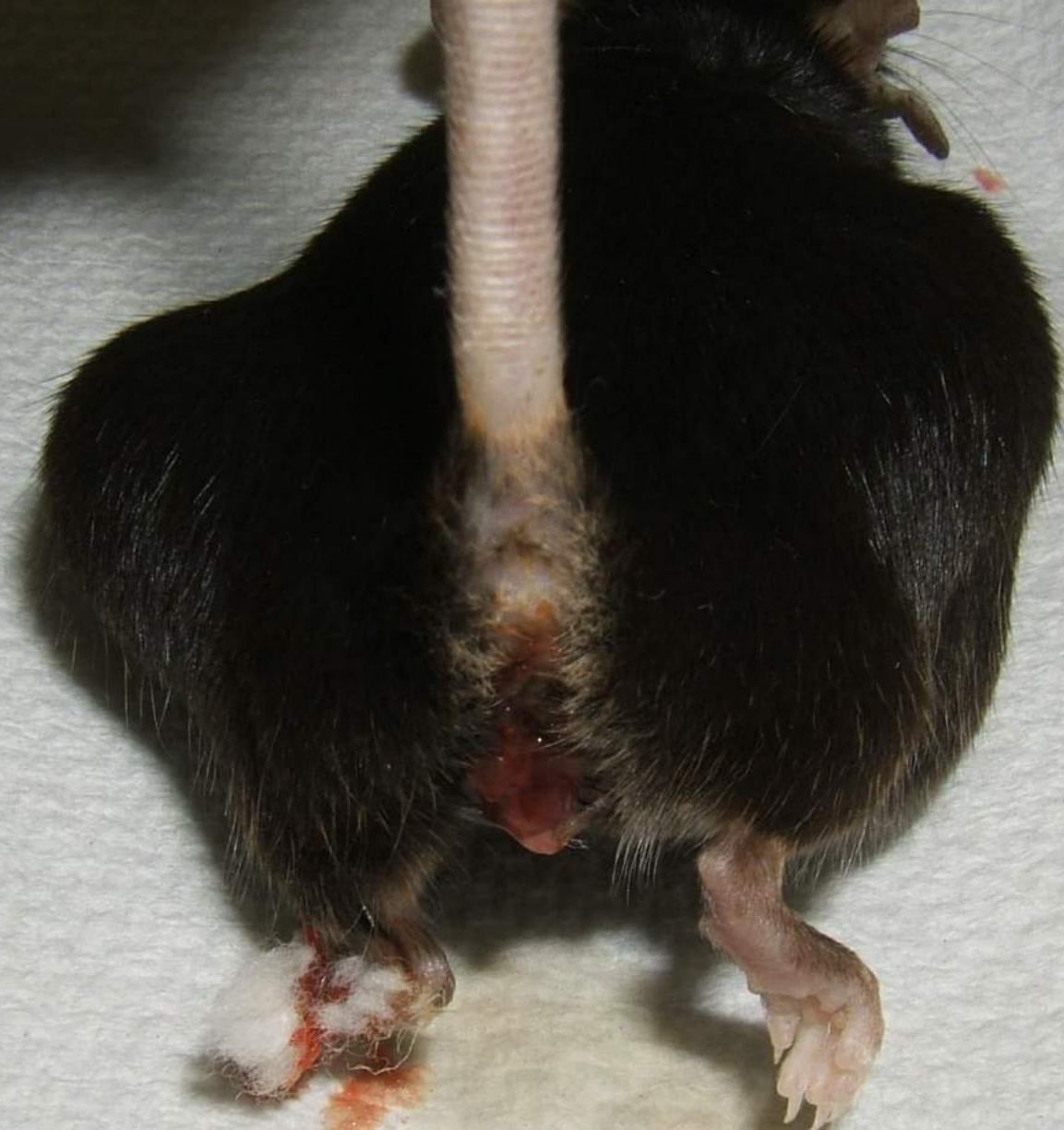
Urinary obstruction caused by cystic dilation of the bulbourethral gland

- The bulbourethral gland is difficult to find if you are inexperienced and know little about mouse anatomy and necropsy techniques
- The gland often becomes cystic
- Can block the urinary tract and therefore, if conditions are just right, produce an enlarged urinary bladder



Dystocias (difficult birthing)

- Occurs frequently
- Usually due to fetuses presenting abnormally
- If mother is valuable, and she will not survive the dystocia, need to consider saving her ovaries and doing ovarian transplantation

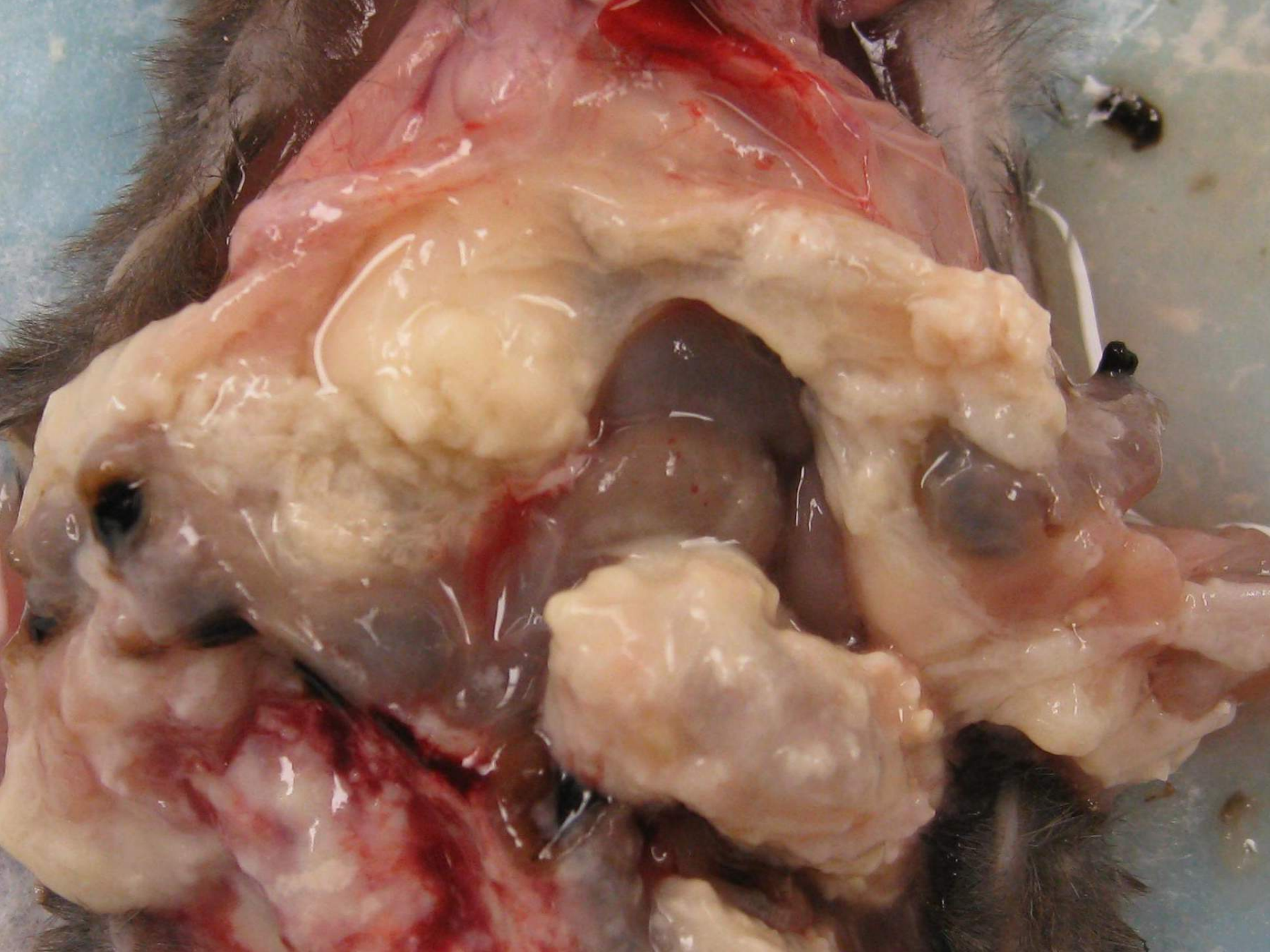




Retained pups after partuition

- Difficult to determine before animal is dying
- Usual cause of death is peritonitis due to bacterial infection in the uterus
- Will often see macerated pups (the bones are the significant finding microscopically)

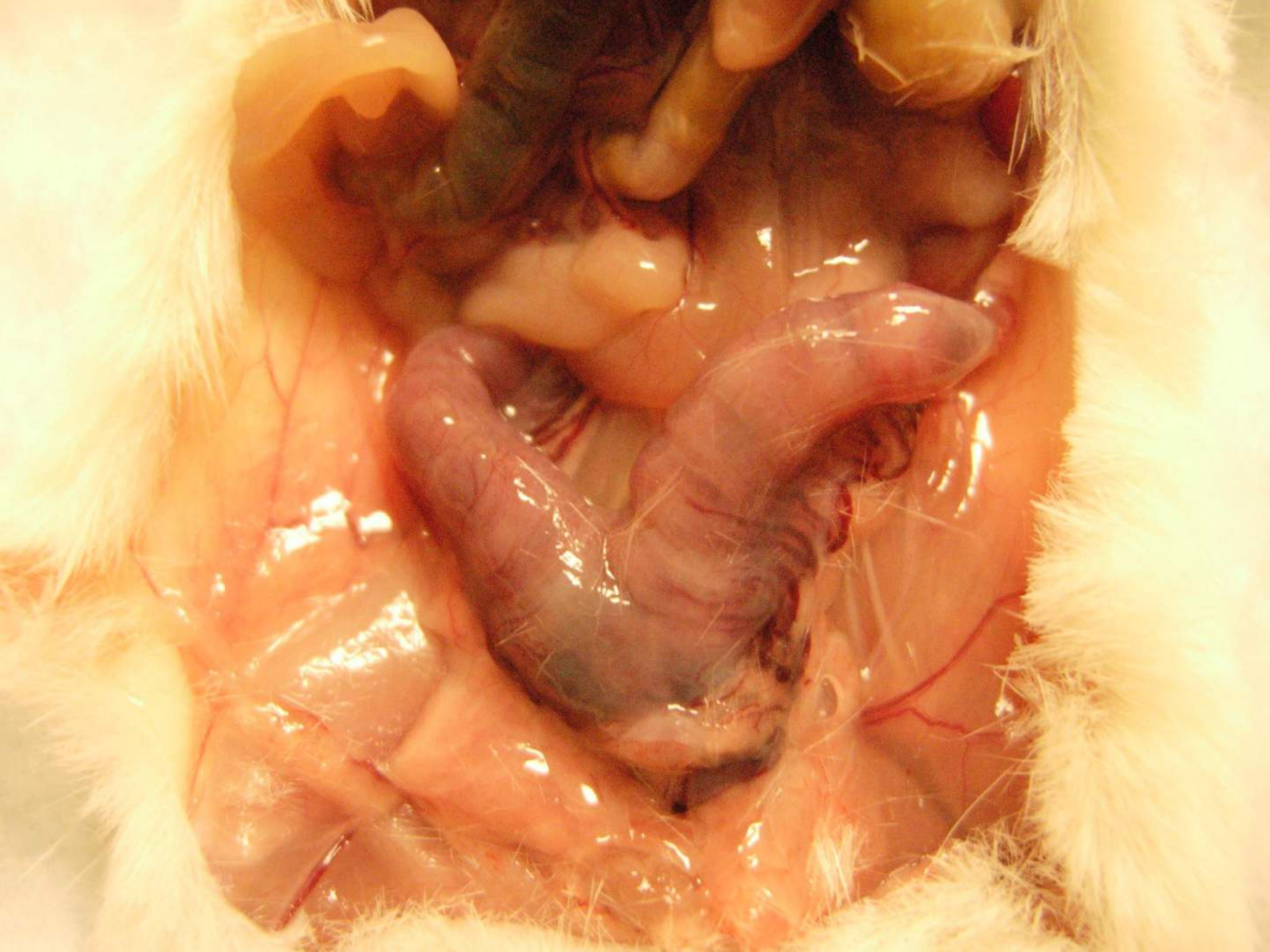




Vaginal bleeding

- Usually occurs during pregnancy
- Cause unknown







5. Highly Infectious Diseases That Threaten Mouse Colonies

transforming
medicine,
improving lives



Highly Infectious Diseases

- Viruses – are 20 viral diseases of mice
 - Most not important
 - Important ones don't kill – they alter immune system (e.g. Mouse Hepatitis Virus, Mouse Parvoviruses)
- Bacteria – are many species
 - Some are pathogens for all mice (e.g. *Pseudomonas aeruginosa*)
 - Some are pathogens in immunocompromised mice
 - Cause localized lesions or systemic disease
 - Need to culture so know identity, can treat, and monitor
 - Culture lesions (e.g. abscesses) or blood (systemic diseases)

Highly Infectious Diseases - continued

- Fungi – most important is *Pneumocystis carinii*
 - Commonly found in all mice (subclinical in immunocompetent mice)
 - But important only in immunocompromised mice
 - Can put mice on antibiotic treatment prophylactically
- Parasites
 - Pinworms (infection) present in many colonies
 - Important because alter the immune system
 - Are difficult to get rid of because of detection problem (*Aspiculuris* sp) or because of the eggs (*Syphacia* spp)
 - Fur mites (infestation) also present in many colonies
 - Important because also alter the immune system

Highly Infectious Diseases – continued

Pinworms (Oxyuriasis) in Mice

- Very common problem worldwide
- Co-infection with 2 species is common
- Very difficult to eradicate
- Eggs are environmentally resistant
- Eggs of *Syphacia* spp tend to drift in air and dust and may be “sticky”
- Expensive to treat

Condition	<i>Aspiculuris tetraptera</i>	<i>Syphacia</i> spp.
Time needed for egg to reach infectivity	5 to 8 days	5 to 20 hours
Prepatent period	21 to 25 days	<i>muris</i> : 7 to 8 days <i>obvelata</i> : 11 to 15 days
Site where eggs are seen	feces	perianal skin (eggs sticky)
Periodicity in egg laying	intermittent egg laying	<i>muris</i> : eggs laid during the day (when host is sleeping) <i>obvelata</i> : continuous laying
Predilection site	large intestine	cecum
Eggs in female (released in 24 hrs?)	17	<i>muris</i> : 450 - 500 <i>obvelata</i> : 350
Hosts	rats and mice	rats, mice, gerbils, hamsters
Eggs found free in the environment?	No	yes



Highly Infectious Diseases – continued

How to detect pinworm infection?

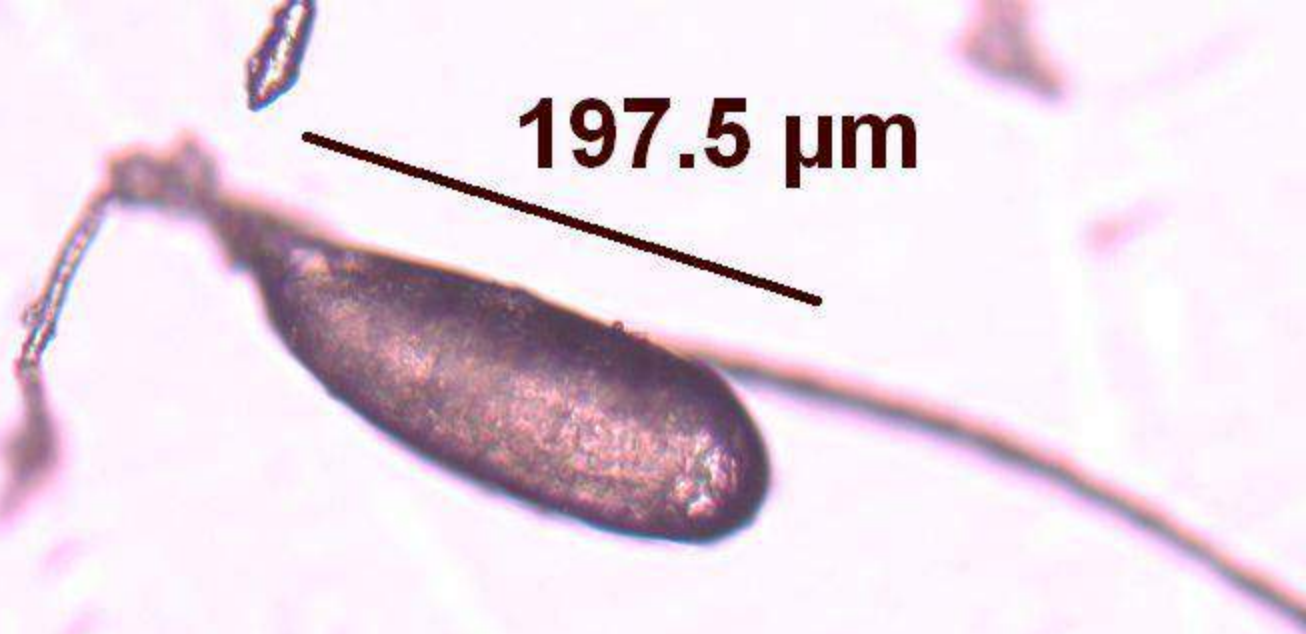
- To detect *Syphacia* sp eggs do tape test and look for eggs (use clear cellophane tape)
- To detect *Aspicularis* sp eggs do fecal float and look for eggs on top of zinc sulfate solution
- With *Aspicularis* sp have about 24% chance of detecting the infection if use 2 fecal pellets
- Have 42% and 78% chance if use 5 or 10 pellets, respectively
- Can examine the cecal and colonic contents and look for larvae or adults

Highly Infectious Diseases-continued

Fur mite infestation with *Myobia musculi*

- Lives on head, neck and shoulder areas
- Not as common as *Myocoptes musculinis*
- But produces most clinically- significant disease
- Adult > egg > larva > nymph
- Adults have 4 pairs of legs
- Larvae have 3 pairs of legs
- Are bulges between their legs
- Second pair of legs have one terminal tarsal claw

197.5 μm

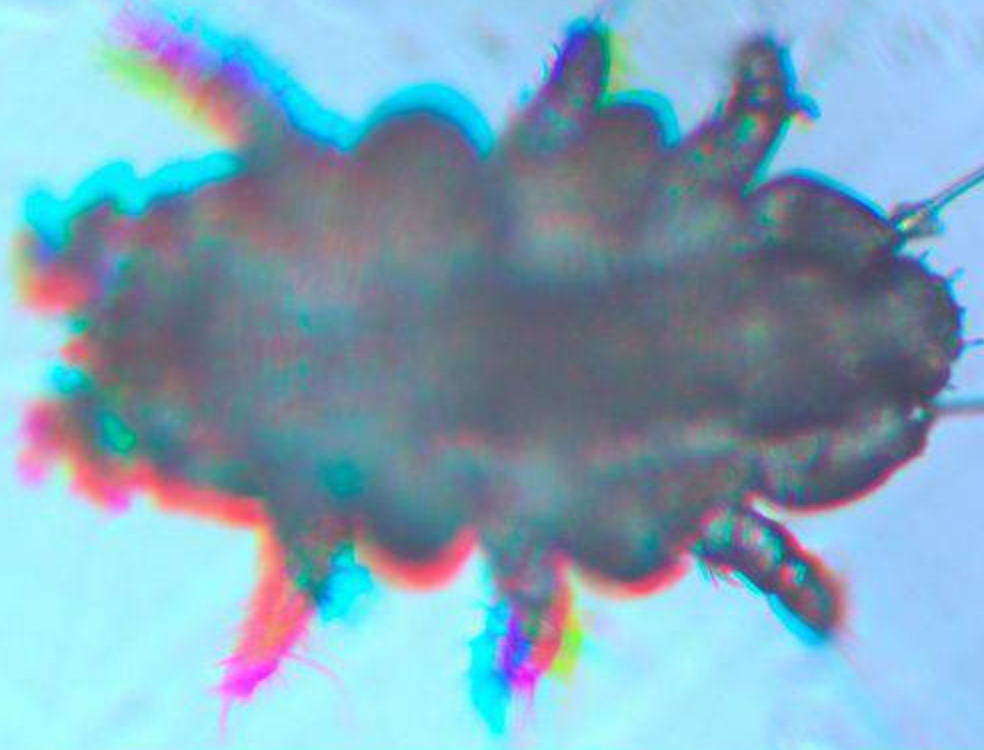


A microscopic image showing a biological specimen, possibly a larva or pupa, attached to a thin, dark, fibrous structure. The specimen is elongated and has a textured, somewhat translucent appearance. A vertical black line is drawn to the right of the specimen, indicating its length. The background is a uniform, light yellowish-brown color.

189.1 μm



293.0 μm



342.9 μm



385.4 μm

Highly Infectious Diseases-continued

Fur mite infestation with *Myocoptes musculinis*

- Lives on head, neck and shoulders, but can be elsewhere with severe infestations
- Usually exists together with *Myobia musculi*
- Adult > egg > larva > nymph
- Adults have 4 pairs of legs
- Larvae have 3 pairs of legs
- Back 2 pairs of legs are big and heavily keratinized and pigmented (“boxing gloves”)



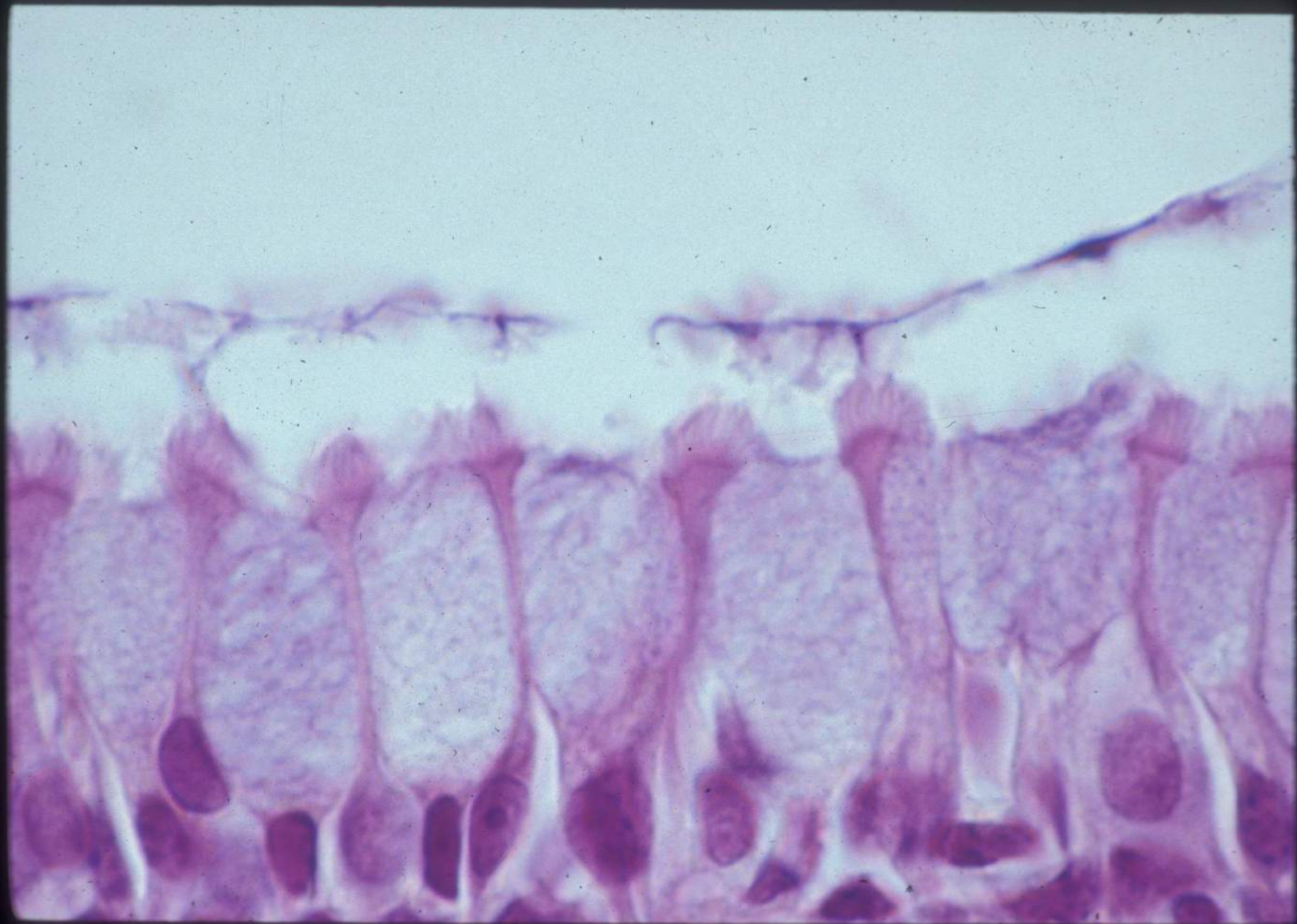
Highly Infectious Diseases-continued

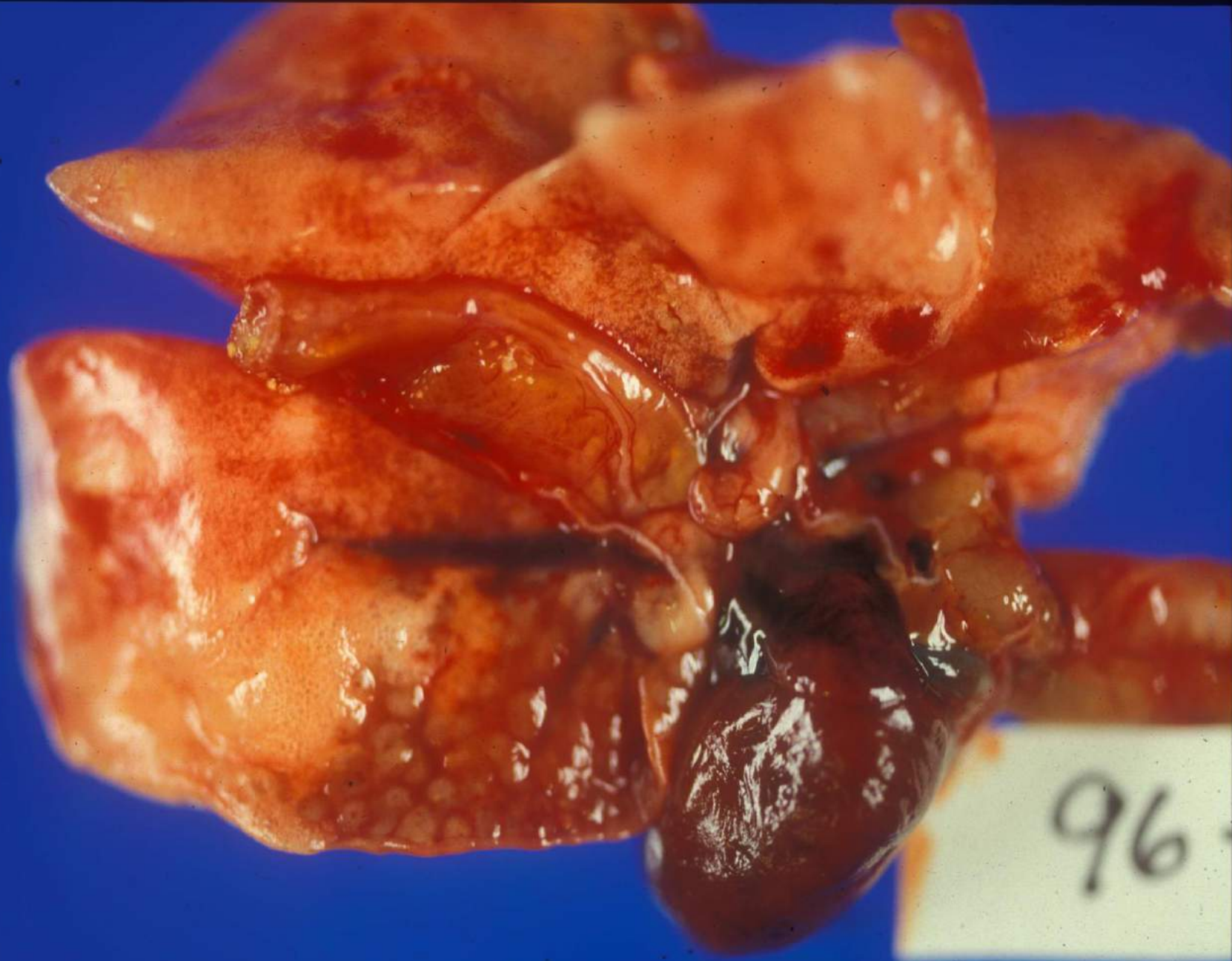
How monitor infectious agents?

- Have a sentinel program (dirty bedding usually)
- For viruses, bacteria, fungi) - do serology and/or PCR and/or histopathology
- For bacteria – serology not good. In addition, culture respiratory tract and intestinal tract, also culture environment
- Parasites – do fur plucks, tape tests and fecal flotations

Selected Rat Diseases

- Mycoplasmosis (*Mycoplasma pulmonis*)
- Cilia Associated Respiratory (CAR) Bacillus
- Parvoviruses
- *Corynebacterium kitcheri*





96



METRIC 1 | LAM 055-82 | 4 | 5

