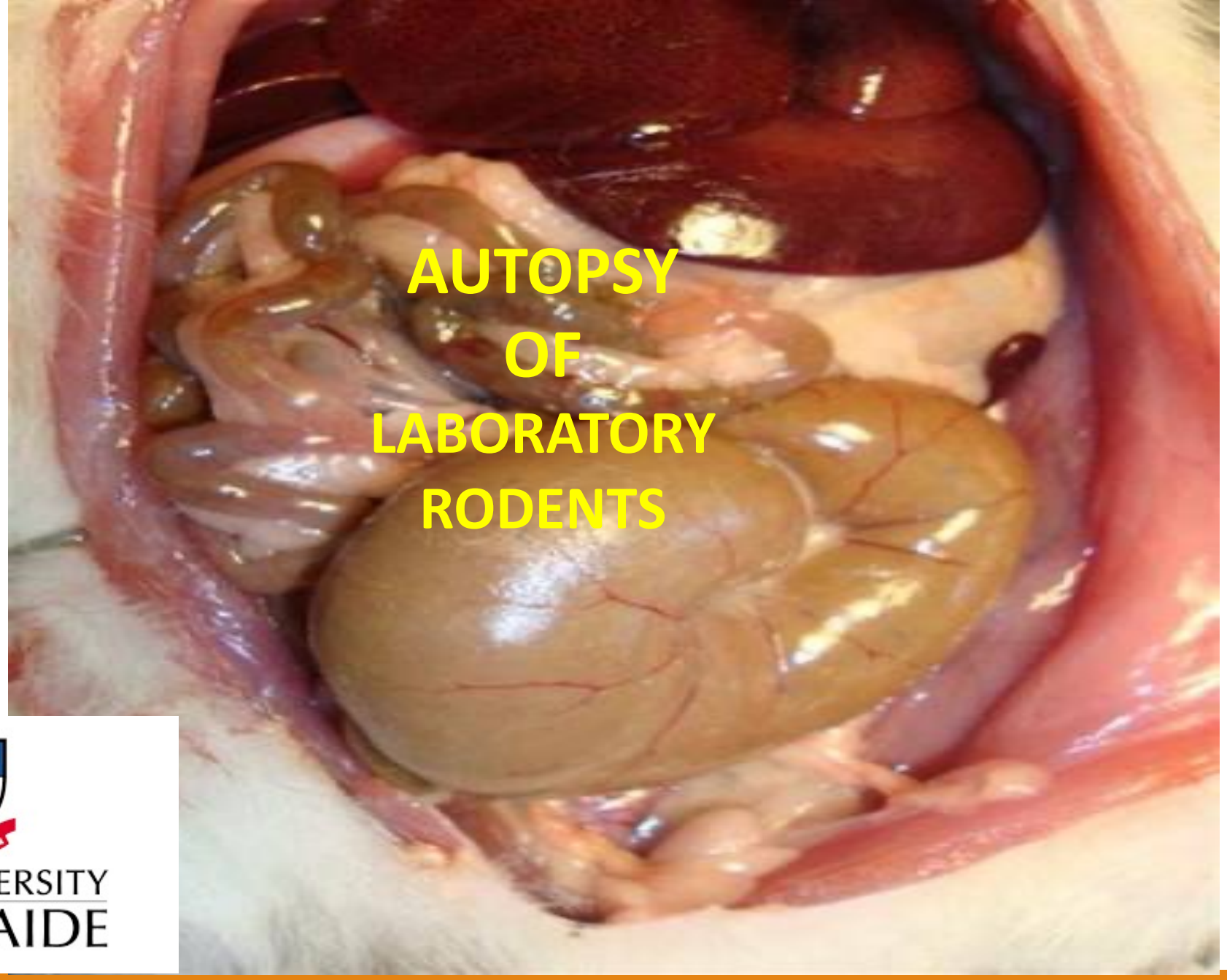

AUTOPSY OF LABORATORY RODENTS

THE UNIVERSITY of ADELAIDE

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Euthanasia

- **The method used must ensure a rapid and relatively painless death**
- **Death is usually indicated by cessation of respiration and heart beat and absence of reflexes**
- **Death must be confirmed by exsanguination or decapitation**
- **Barbiturate overdose, CO_2 (not neonates) asphyxiation, cervical dislocation, stunning, or decapitation can be used**
- **CO_2 has a rapid anaesthetic effect (45-60 sec), leading to respiratory arrest and death with prolonged exposure (5-6 min)**

Blood collection

- Cheek vein bleeding is now common (tail and other large veins can also be used)
- Cardiac puncture by insertion of needle between the ribs into the left ventricle (if unsuccessful, can rapidly open the thorax, incise the heart, and collect exuded blood from the thoracic cavity before it clots)
- For haematology, blood is collected into an anticoagulant (EDTA, Na citrate, heparin) – plasma can be separated by centrifugation
- For serum collection (e.g. for health monitoring serology for viruses/bacteria), blood is allowed to clot and serum separated by centrifugation

Autopsy versus necropsy

- **Traditionally, “autopsy” was the term applied to post-mortem examination of the human body and “necropsy” to a non-human body, but**

- **In the spirit of “One Health”, the term autopsy is now preferred for all post-mortem examinations**

Diagnosis entails integration of

- Clinical history
- Signalment (age, sex, breed/strain)
- Gross (macroscopic) lesions
- Microscopic (histopathological) changes
- Ancillary tests (microbiology, immunology, molecular pathology, toxicology)

The autopsy involves:

- **Prosection** = dissection of a cadaver

- **Lesion description** - size, shape, colour, texture, distribution (random or symmetrical; focal, multifocal, coalescing or diffuse; and severity (mild, moderate or severe)

- Formulation of a **morphological diagnosis**

- Mice have a very high metabolic rate and decompose (autolyse) rapidly after death, so a P/M should be conducted ASAP

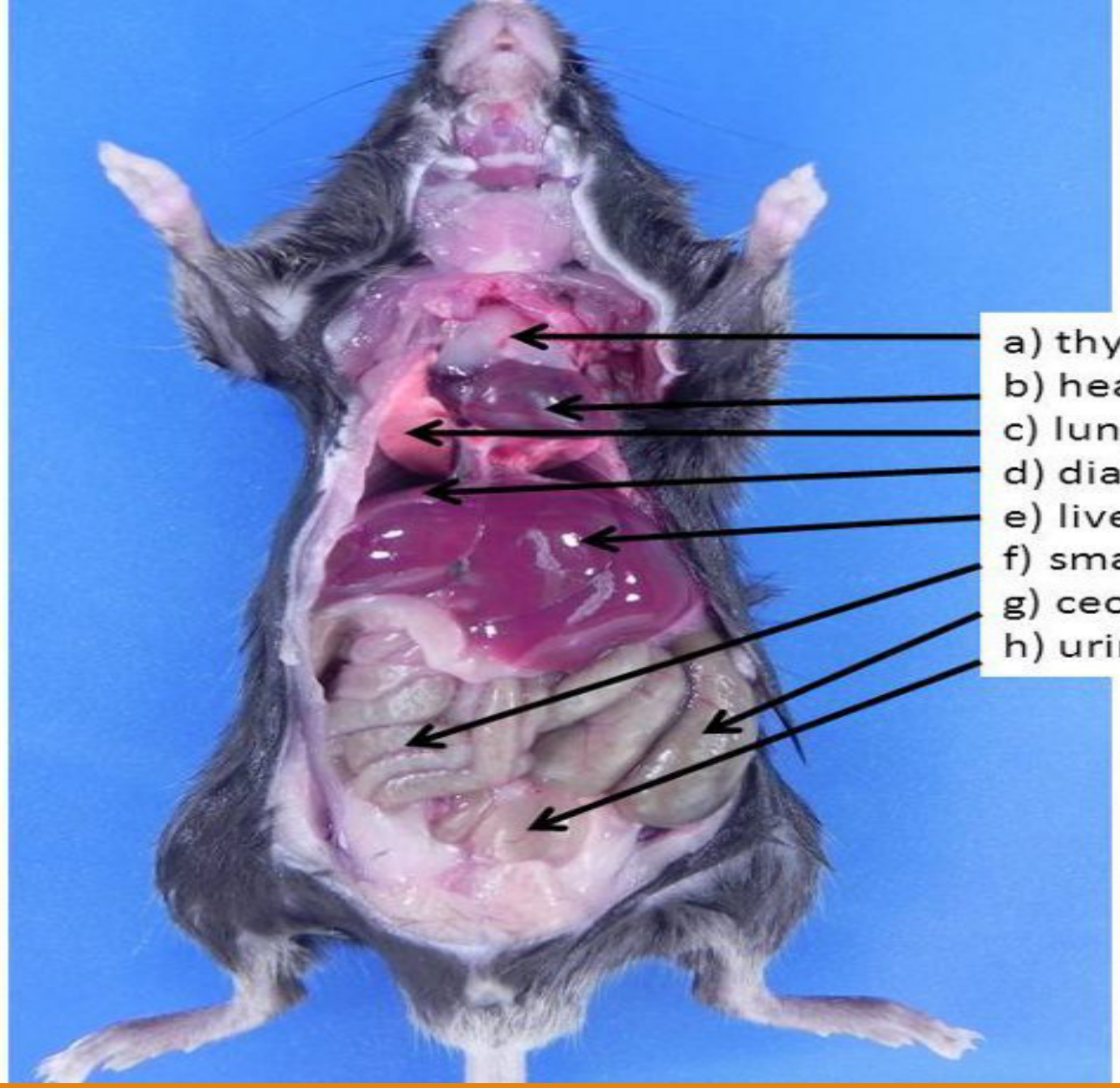
- Cadaver can be kept at 4°C, but not frozen, for thawing produces ice crystal artefacts that markedly disrupt tissues, rendering meaningful histopathology very difficult

- Examine external orifices for any discharge (e.g. faecal staining of the perineum indicative of diarrhoea, nasal discharge) and note the general body condition

- Make an incision in the ventral skin of the neck and expose the trachea – ligate to maintain the lungs inflated when the thorax is opened so lungs can be assessed as they were during life (otherwise they will collapse (termed atelectasis)

- Collect peripheral lymph nodes – cervical beneath mandible (+ large salivary glands), under forelimbs (axillary nodes) and medial aspect of hindlimb (inguinal nodes)

- Open the thorax by cutting up the sternum with scissors and collect the pale-coloured thymus from the anterior mediastinum


a) thymus
b) heart
c) lungs
d) diaphragm
e) liver
f) small intestines
g) cecum
h) urinary bladder

- After cutting the mandible in the midline, grasp the tongue with forceps and gently use scissors to dissect away the trachea/oesophagus, thyroid glands, lungs and heart as one unit

- Lungs should then be inflated with fixative by inserting the needle of a syringe into the tracheal lumen and infusing fixative (do not overinflate as will damage alveoli)

- Remove rib cage (L&R) + sternum to examine bone histology and bone marrow (requires no or only mild decalcification versus prolonged decalcification of long bones)

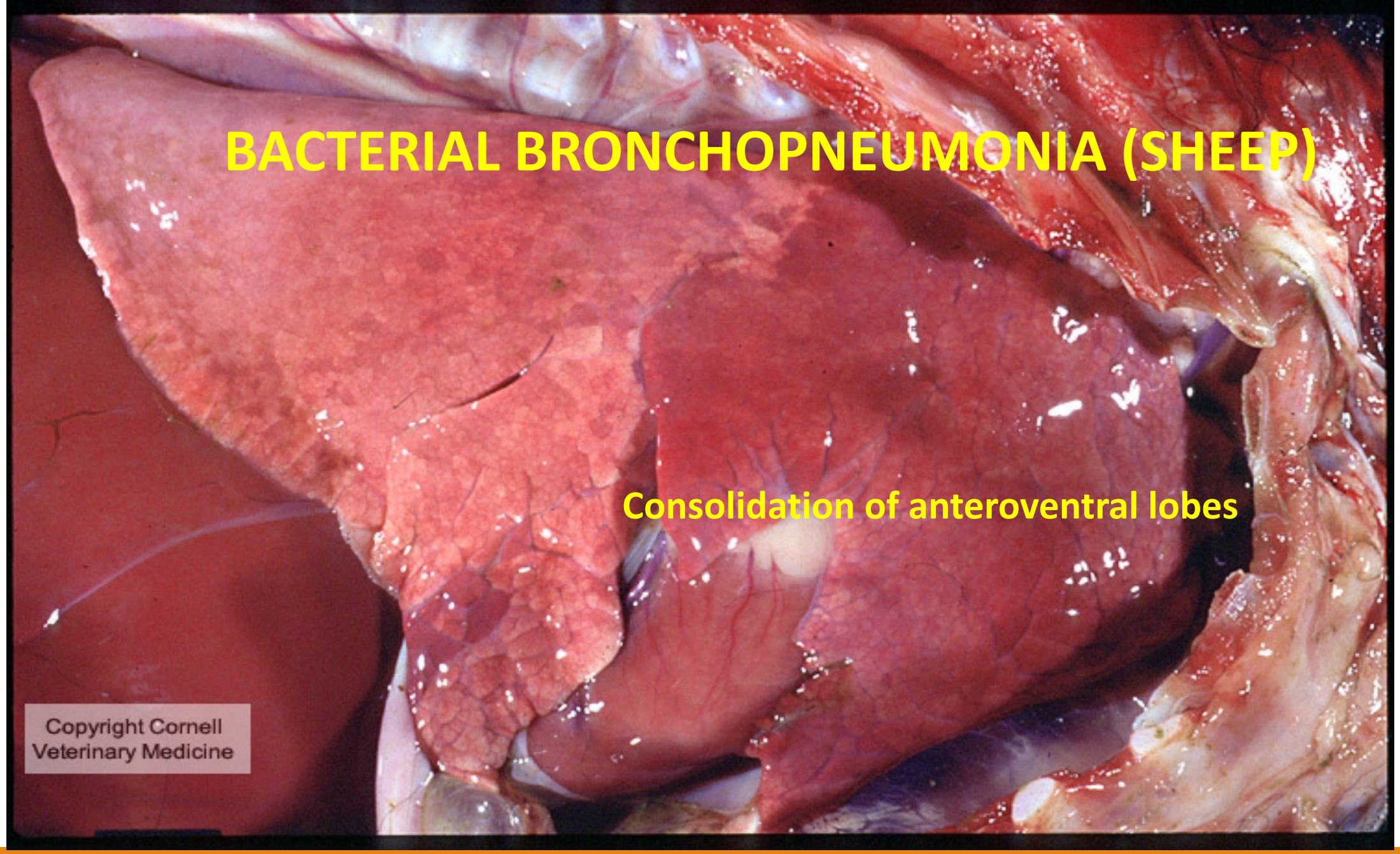
Sectioning of lungs and heart

- In small-sized rodent **hearts**, the fixed specimen is bisected perpendicular to the long axis of the septum to provide a sample for histological study that includes sections of all 4 chambers (atria and ventricles) and the interventricular and interatrial septa. Heart valves should be examined for evidence of bacterial endocarditis.

- Both **lungs** should be collected for histology, including anteroventral and diaphragmatic lobes.

- Note that **bacterial bronchopneumonia favours the anteroventral lobes**, which appear red-grey in colour and are firm to palpate (consolidated with accumulated exudate)

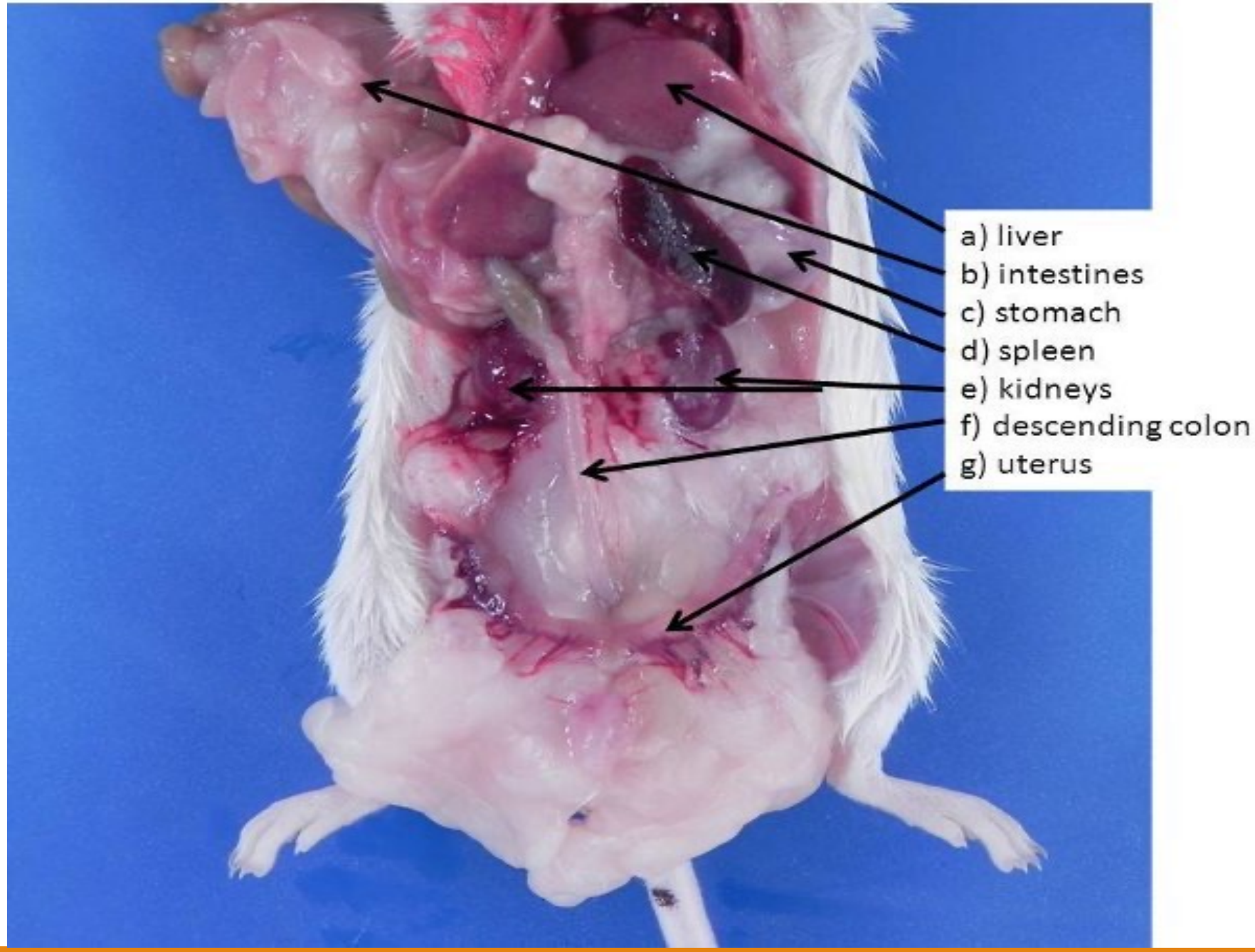
BACTERIAL BRONCHOPNEUMONIA (SHEEP)



- Open the abdominal cavity by a longitudinal incision in the ventral skin and (to prevent blood/faeces obscuring small abdominal tissues), collect mesenteric lymph nodes (form a chain in the mesentery), spleen (adjacent to greater curvature of stomach), adrenal glands (in perirenal fat at anterior pole of the kidney) and pancreas (adjacent to duodenum)

- Separate the stomach from duodenum and remove the caecum – open and flush out contents (ingesta) with saline

- Collect left and right kidneys and bisect by cutting longitudinally from anterior to posterior pole in order to visualise the cortex and medulla from capsule to pelvis. Place cut surface face down in the tissue cassette

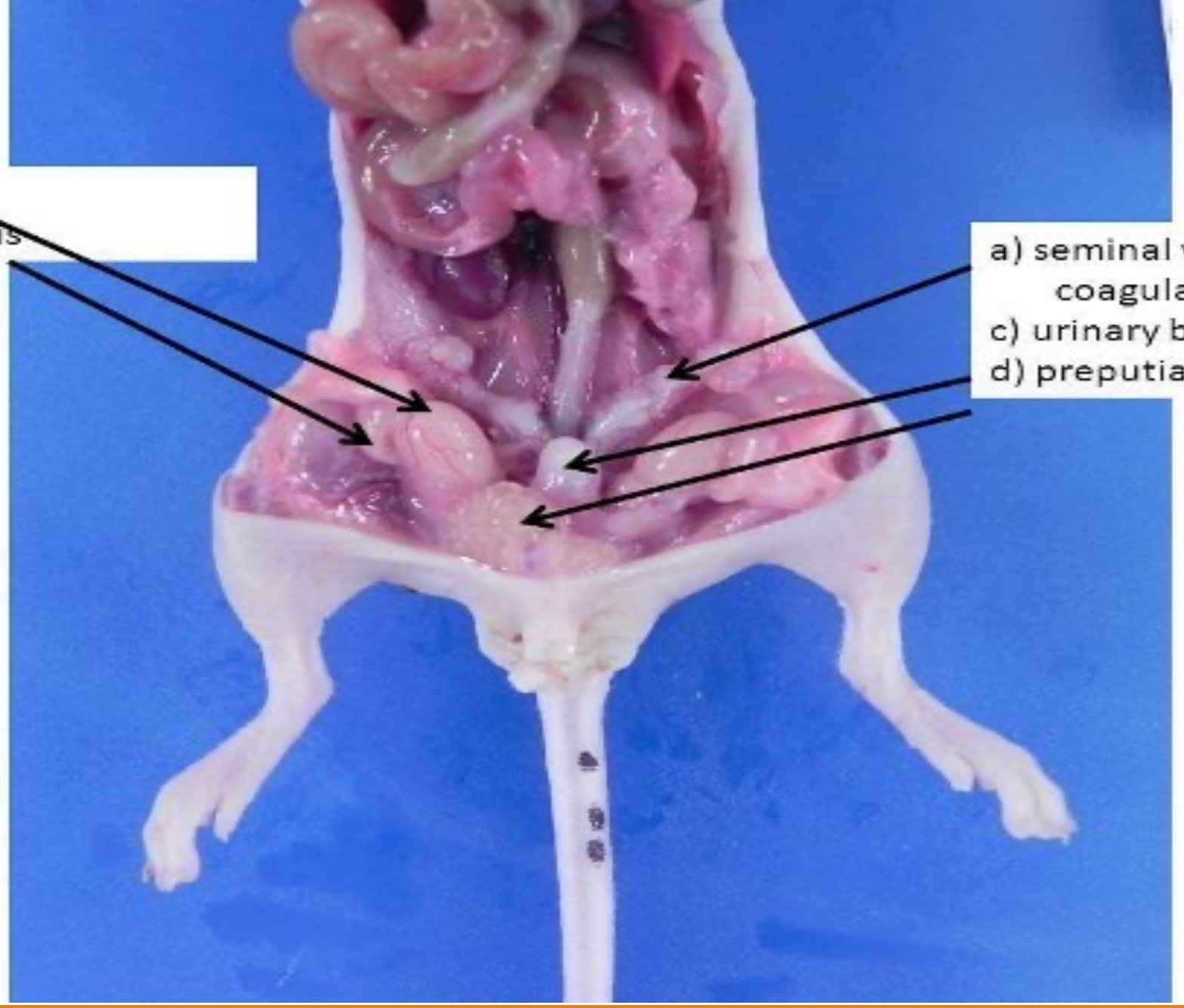

a) liver
b) intestines
c) stomach
d) spleen
e) kidneys
f) descending colon
g) uterus

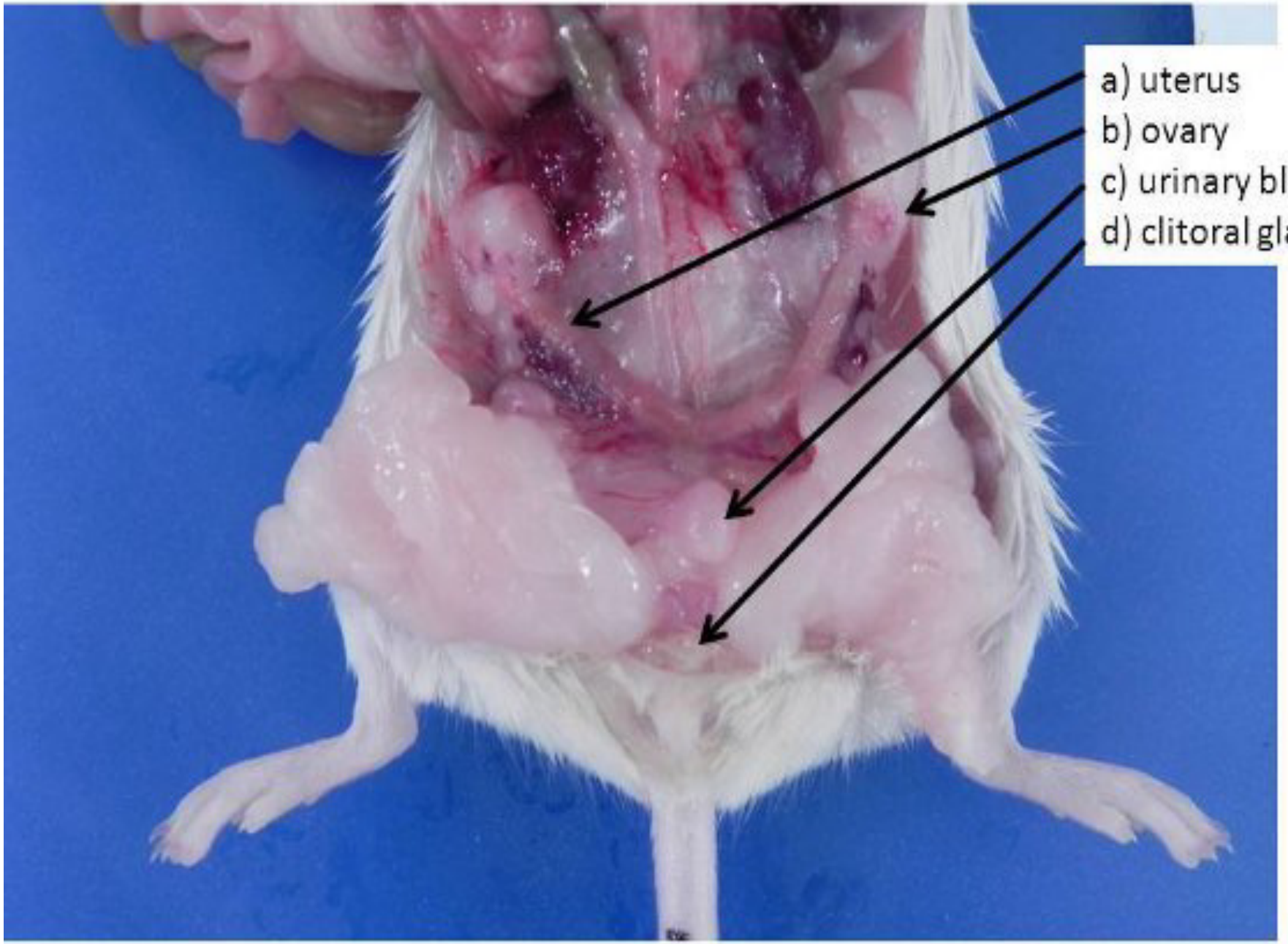
- With forceps, strip the capsule of the kidney away carefully – should be removed without any adherence to the underlying parenchyma – if adherent and kidney tissue is removed could indicate fibrosis (scarring) in kidney

- Intestine can be collected by removing representative short segments (duodenum jejunum, ileum colon, rectum) or the entire intestinal tract can be coiled in concentric, centrifugal circles to create a “Swiss roll” and placed in a tissue cassette

- The intestine should be inflated with fixative to induce rapid fixation as it autolyses rapidly

- Collect representative lobes of liver and the gallbladder (remove bile to facilitate rapid fixation)
- Note that there is NO GALLBLADDER in rats (or horses)
- Inflate the urinary bladder with fixative (~0.5 ml) and remove
- In males, incise the scrotum and remove testes + attached epididymus
- Seminal vesicles (very large in rodents) and prostate gland can be removed as one unit
- In females, collect ovaries, uterus and vagina


b) testis
e) epididymis
a) seminal vesicles and coagulating glands
c) urinary bladder
d) preputial glands


a) uterus
b) ovary
c) urinary bladder
d) clitoral gland

- To collect the brain, separate the skull from spinal cord at atlanto-axial joint with scissors and remove the skin of the scalp

- Insert scissors into the foramen magnum (where the spinal cord exits the skull) and make a circumferential incision in the calvarium, returning to the foramen. Remove the calvarium

- Carefully remove the brain (will need to cut the optic tract at the base of the brain), being careful to prevent manual handling, which will produce mechanical pressure neuronal artefacts

- Both transverse and longitudinal segments of spinal cord should be collected, the former being cut into ~5mm segments to permit entry of fixative into the spinal canal and

- the latter exposed by laminectomy – removing the dorsal vertebral bone to expose the underlying spinal cord

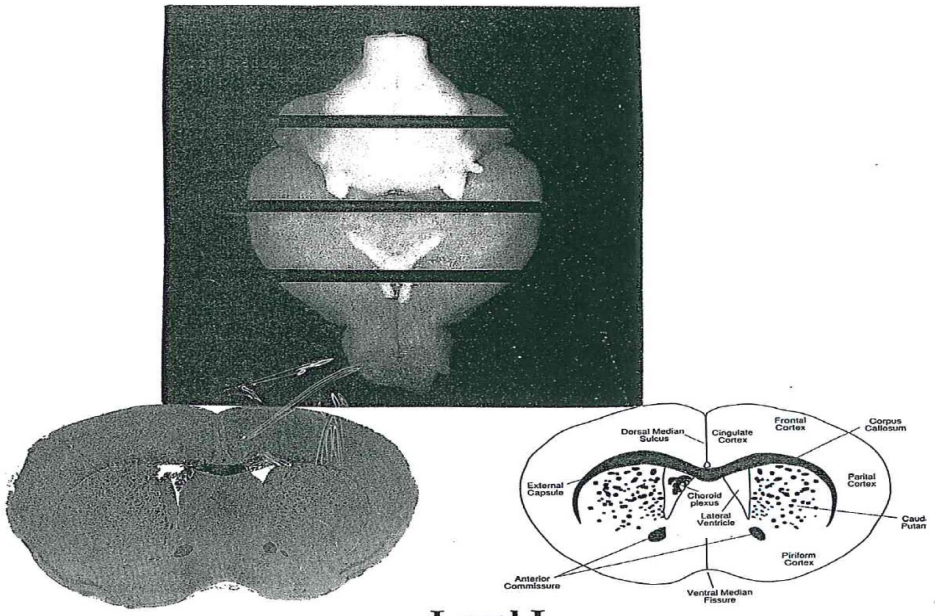
- To collect the eyes, grasp the eyelids with forceps and make a circumferential skin incision around the eye with a scalpel blade. Then, with traction on the eyelids, carefully cut the extraocular muscles with small scissors and remove the eye (with attached optic nerve) from the orbit

- Best to fix the eyes in Davidson's fixative rather than formalin and incise the eyeball to allow penetration of fixative (although small rodent eyes will fix adequately if left intact)

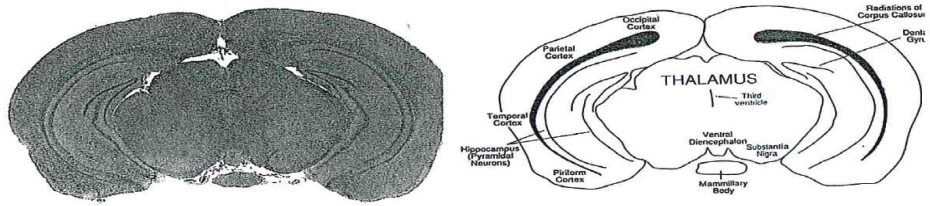
Brain examination

- After fixation, whole **coronal (transverse) sections** of brain are collected at regular intervals from rostral to caudal to permit examination of a wide range of neuroanatomical regions of the cerebral hemispheres, cerebellum and brainstem

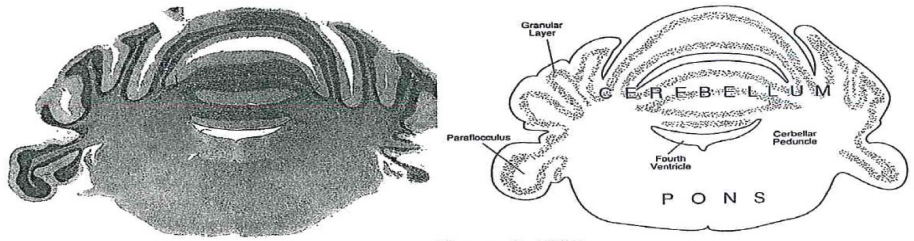
- 3 routine coronal brain sections are commonly examined in mice using landmarks on the ventral surface: just anterior to the optic chiasm (level 1), at caudal borders of mammillary bodies (level 2), and at the widest part of the cerebellum, which includes the pons (level 3)



Level I



Level II



Level III

- **Skeletal muscle is collected with the sciatic nerve by removing a block of quadriceps muscle and incising the muscle to expose the sciatic nerve**

- **Collect a sample of skin (and the underlying mammary glands in females)**

- **To examine nasal passages and the ear canal (e.g. if otitis media is suspected), the whole skull can be decalcified and the skull cut transversely from ear to ear (and at other transverse levels if required)**

Tissue fixation

- **Immersion versus perfusion of fixative**
- **Perfusion gives better tissue preservation**
- **To perfuse the body, anaesthetise the animal, open the thorax, insert the needle of a syringe into the apex of the beating left ventricle, and cut jugular veins to permit escape of the infused fluid**
- **First, perfuse with physiological saline to flush out blood from the vascular system, followed by 10% formalin (or 4% paraformaldehyde)**
- **If successful, the body will become rigid and the organs appear pale due to lack of blood**

Tissue fixation

- Standard tissue fixative is 10% neutral buffered formalin
- Use at least 10 X the volume of fixative to tissue
- Most issues should be trimmed to a thickness of ~2 mm to facilitate penetration of the fixative
- For electron microscopy, collect 2x2mm blocks of tissue and fix in 2.5% glutaraldehyde

Histological stains

- **Haematoxylin & eosin (H&E) is the routine stain**

- **Acidic eosin has an affinity for cytoplasmic proteins, while basic haematoxylin stains nuclear structures**

- **Special histochemical stains can be used to identify different tissue components, e.g. connective tissues, glycogen, mucins, minerals, pigments, amyloid and microorganisms (e.g. Gram stain categorises bacteria as Gram-positive or – negative and silver stains and PAS stain fungi)**

Immunohistochemistry

- Different epitopes can be recognised in histological sections by **combining an antigen-antibody reaction with a chemical reaction**, the latter using a **chromagen** to permit visualisation of the Ag-Ab complex by light microscopy

- Many antibodies can now be used on formalin-fixed, paraffin-embedded tissue sections, but this usually requires **antigen retrieval** to unmask the given antigen and permit antibody binding

- The antibody needs to be tailored to a given species (**include positive and negative controls**) and, when not fully characterised, may need to use a panel (battery) of antibodies. Due to different genetic constitutions, the antibody reaction to a given epitope may also vary between different individuals

- For **bacteriology**, tissues/swabs must be collected aseptically with sterile instruments, being careful not to introduce bacterial contamination, e.g. skin microflora

- For **urinalysis**, urine is collected aseptically for bacterial culture and urine constituents (leucocytes, nitrite, urobilinogen, protein, pH, haemoglobin, specific gravity, ketones, bilirubin, glucose) measured semiquantitatively using dipstick technology. Urine is then centrifuged and the resulting sediment examined microscopically for cells (e.g. epithelial, leucocytes), bacteria, crystals and tubular (e.g. protein) casts

Lesions can be broadly categorised as

- Degenerative/necrotic
- Inflammatory
- Disturbance of growth
- Vascular disturbance

- **Degeneration/necrosis**: cellular swelling imparts pallor to the affected tissue, while necrosis (especially when focal or multifocal) produces a change that is often well-demarcated from surrounding viable tissue

- **Inflammation**: recognition is facilitated when there is an exudate – purulent or suppurative (pus), catarrhal (mucous), fibrinous (fibrin). Inflamed tissues are usually red and swollen

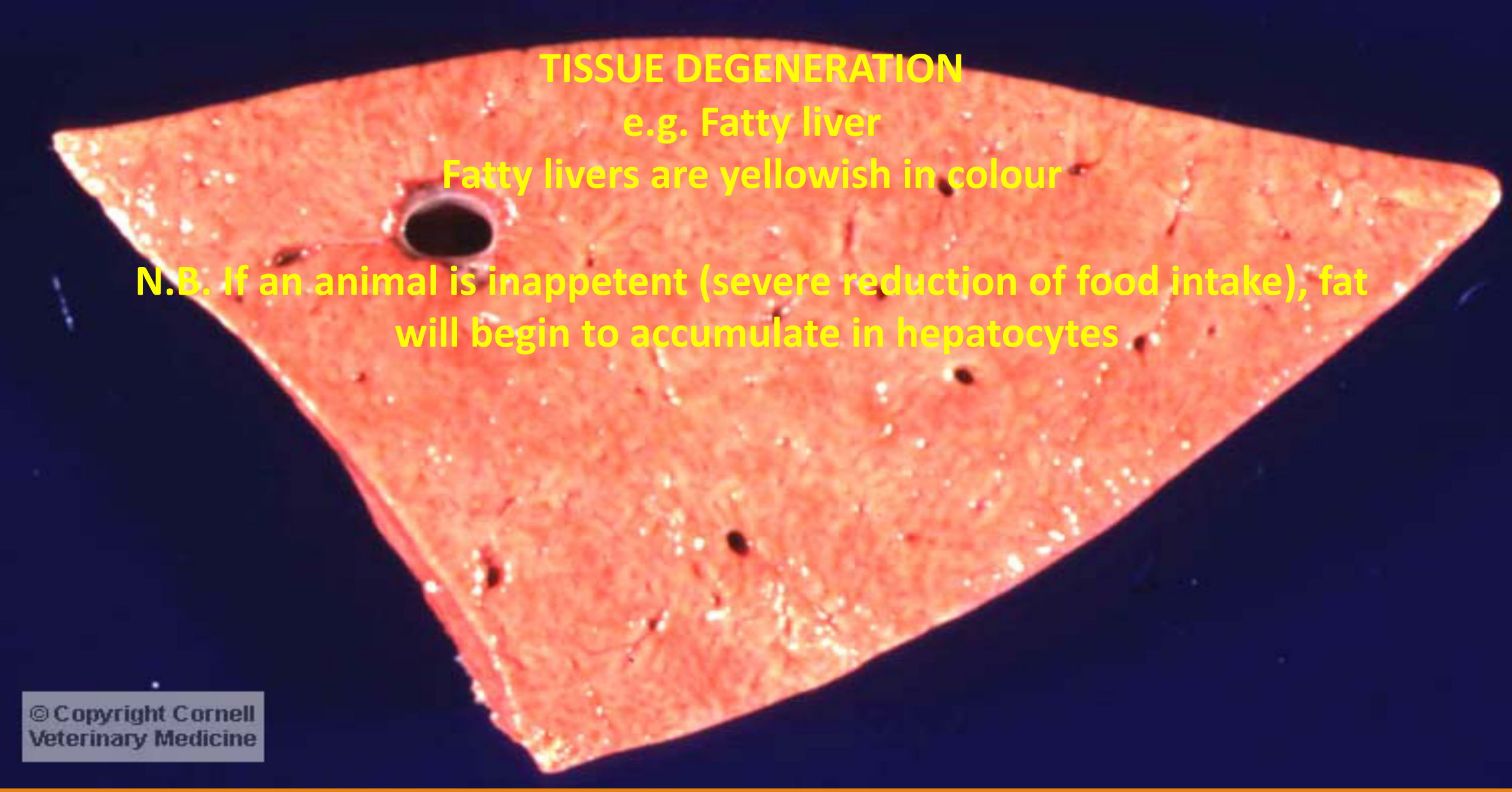
- **Vascular disturbance**: e.g. infarction, thrombosis

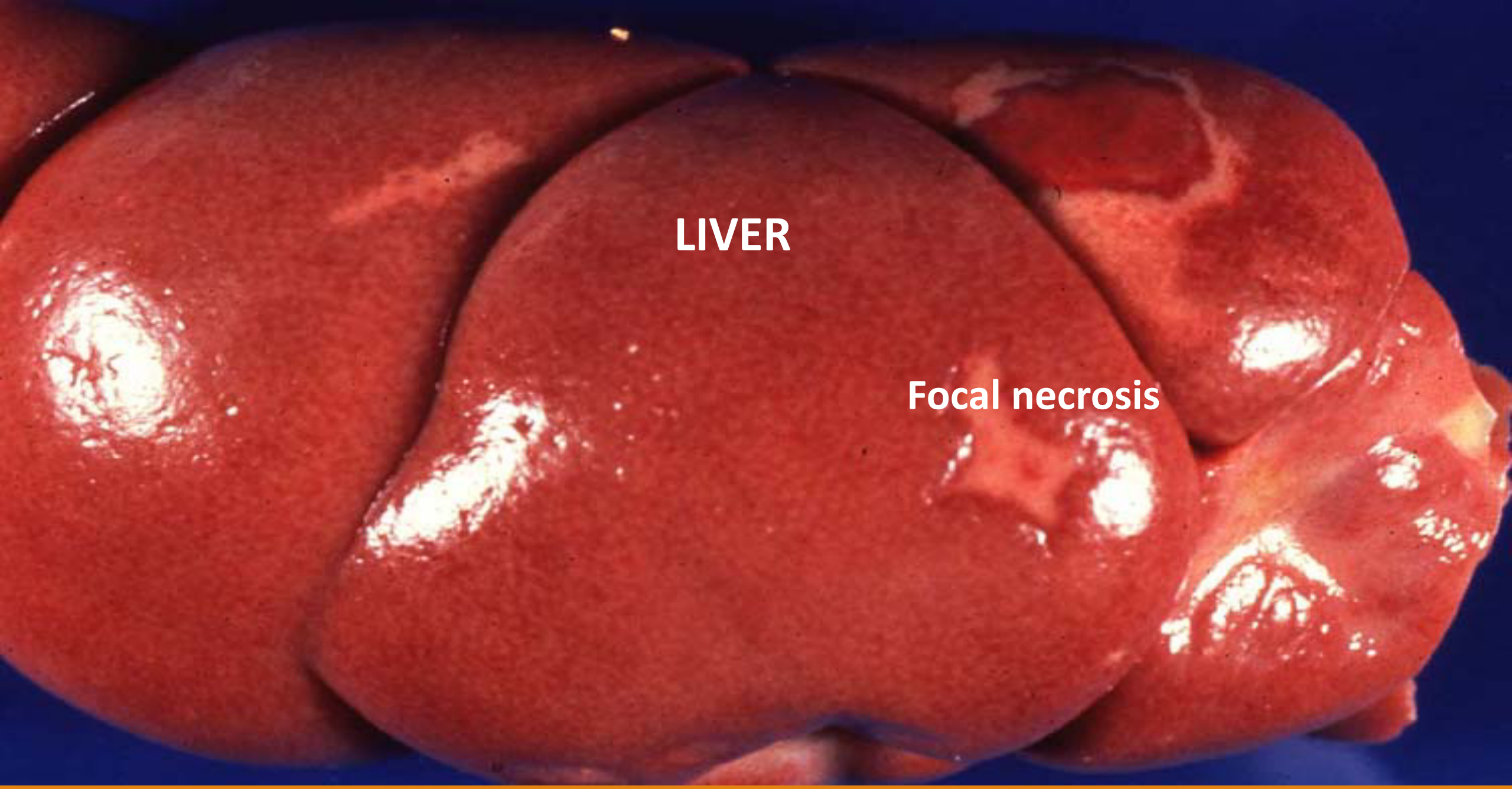
TISSUE DEGENERATION

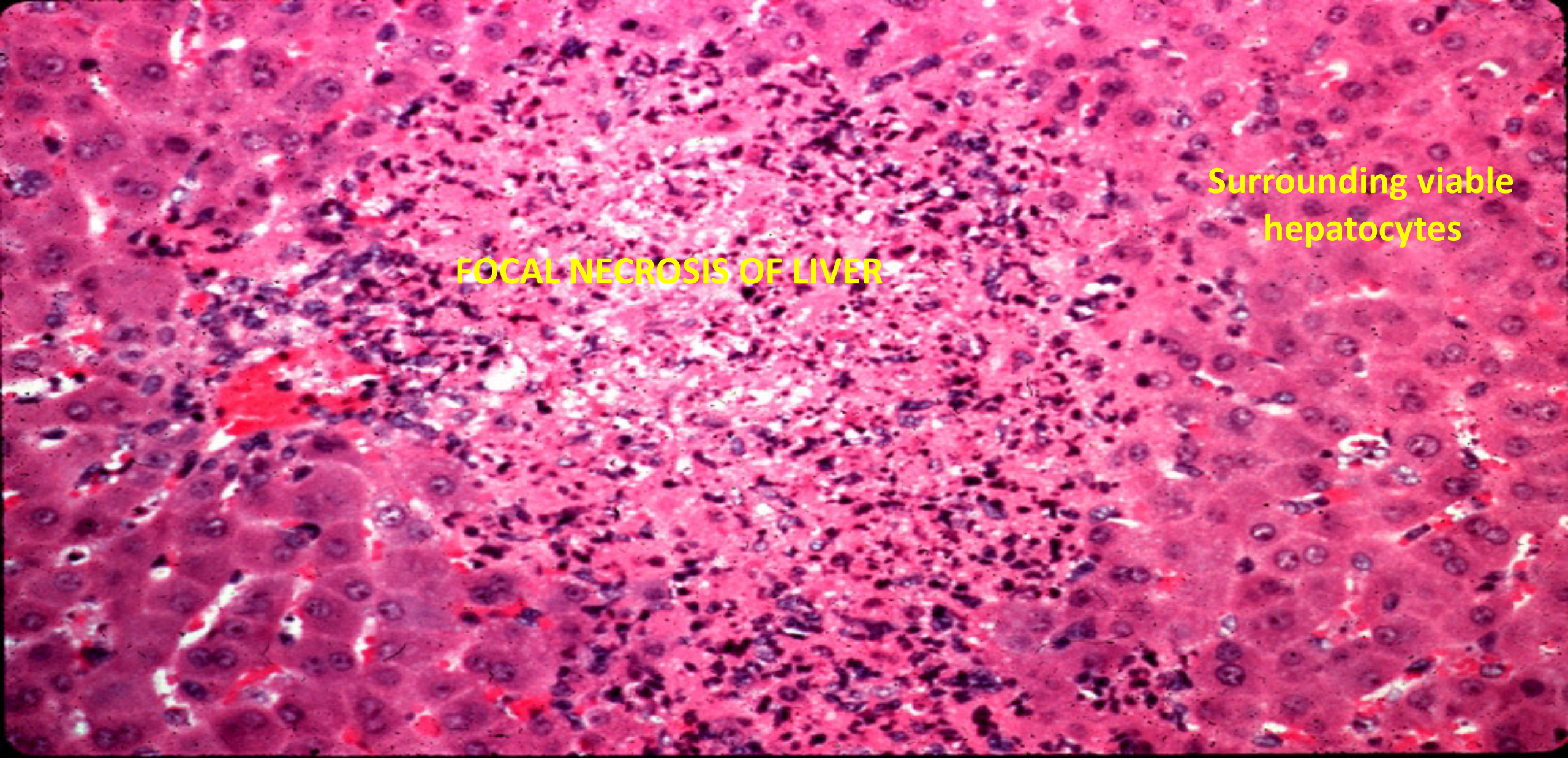
e.g. Fatty liver

Fatty livers are yellowish in colour

N.B. If an animal is inappetent (severe reduction of food intake), fat will begin to accumulate in hepatocytes

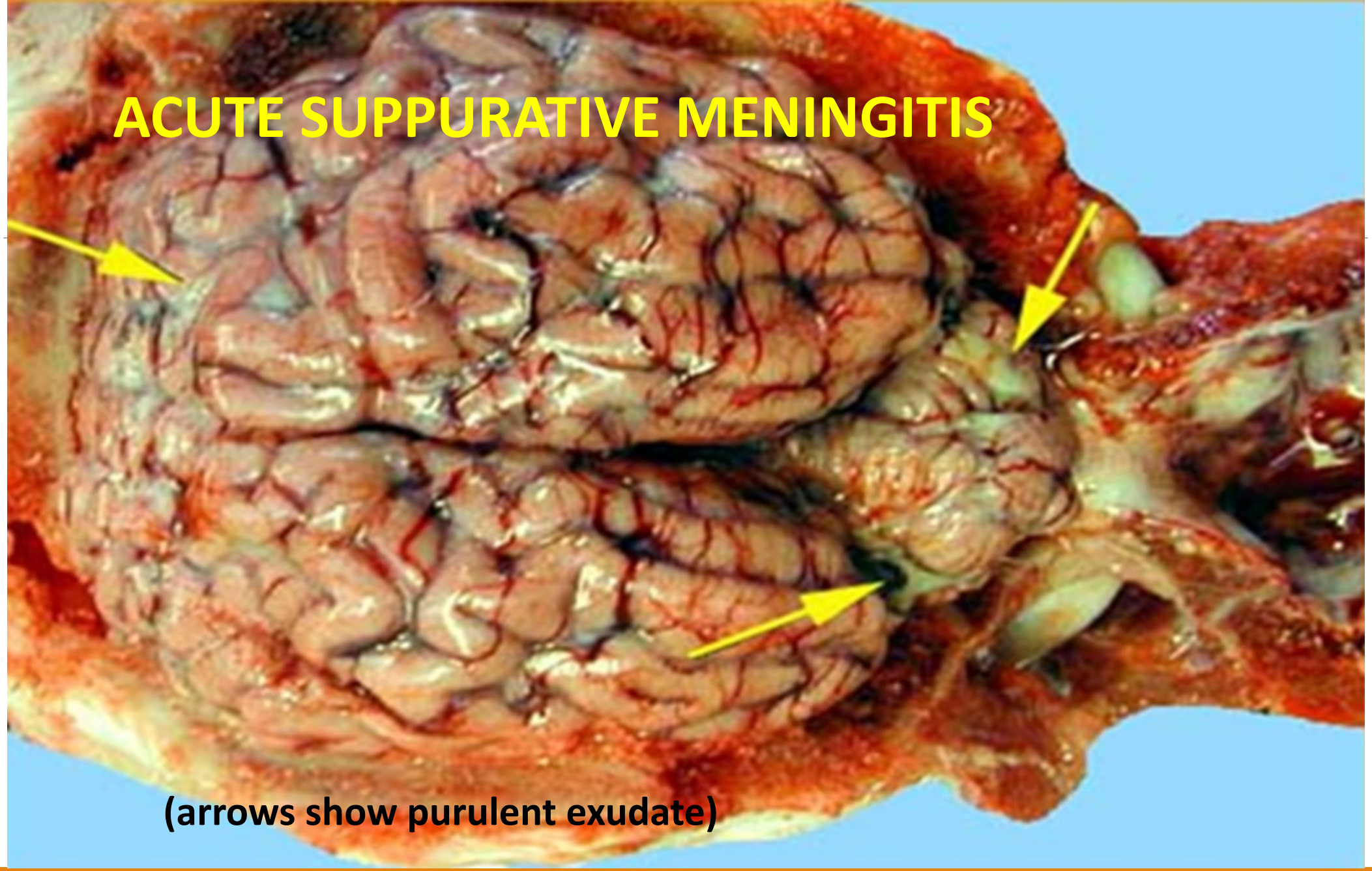



LIVER
Focal necrosis

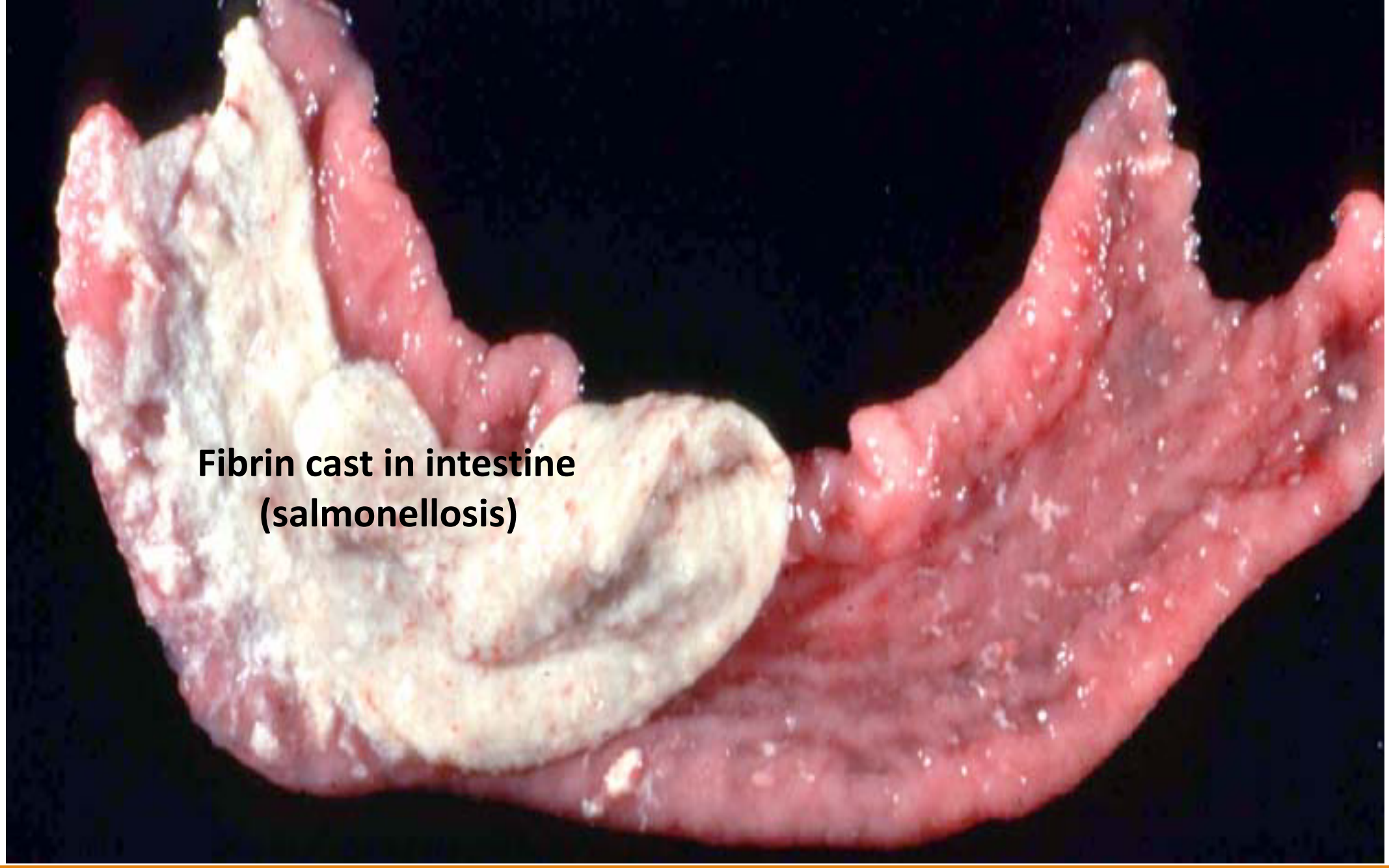


FOCAL HEPATOCELLULAR NECROSIS

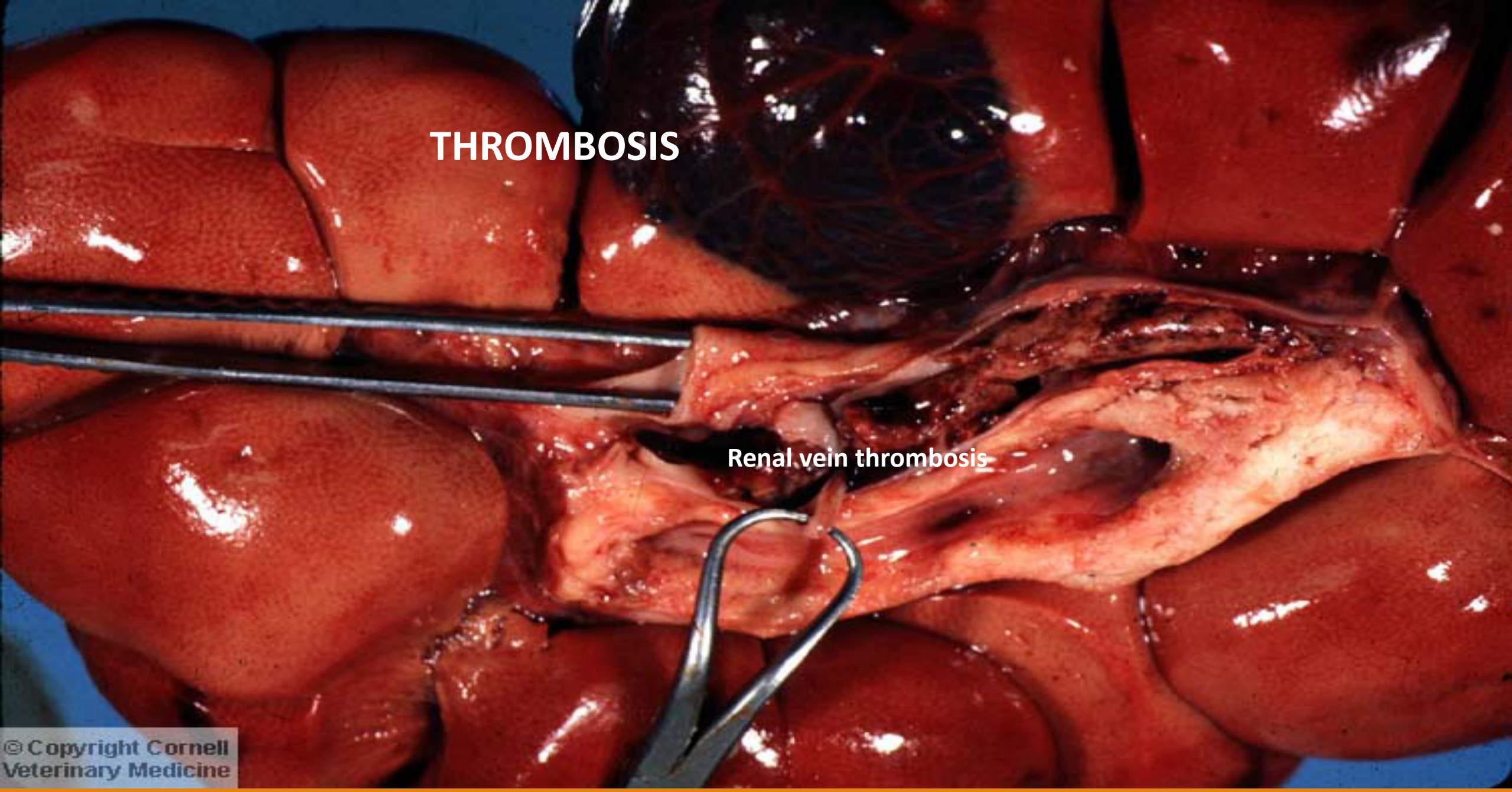
ACUTE SUPPURATIVE MENINGITIS



(arrows show purulent exudate)


Fibrin cast in intestine
(salmonellosis)

THROMBOSIS


Renal vein thrombosis
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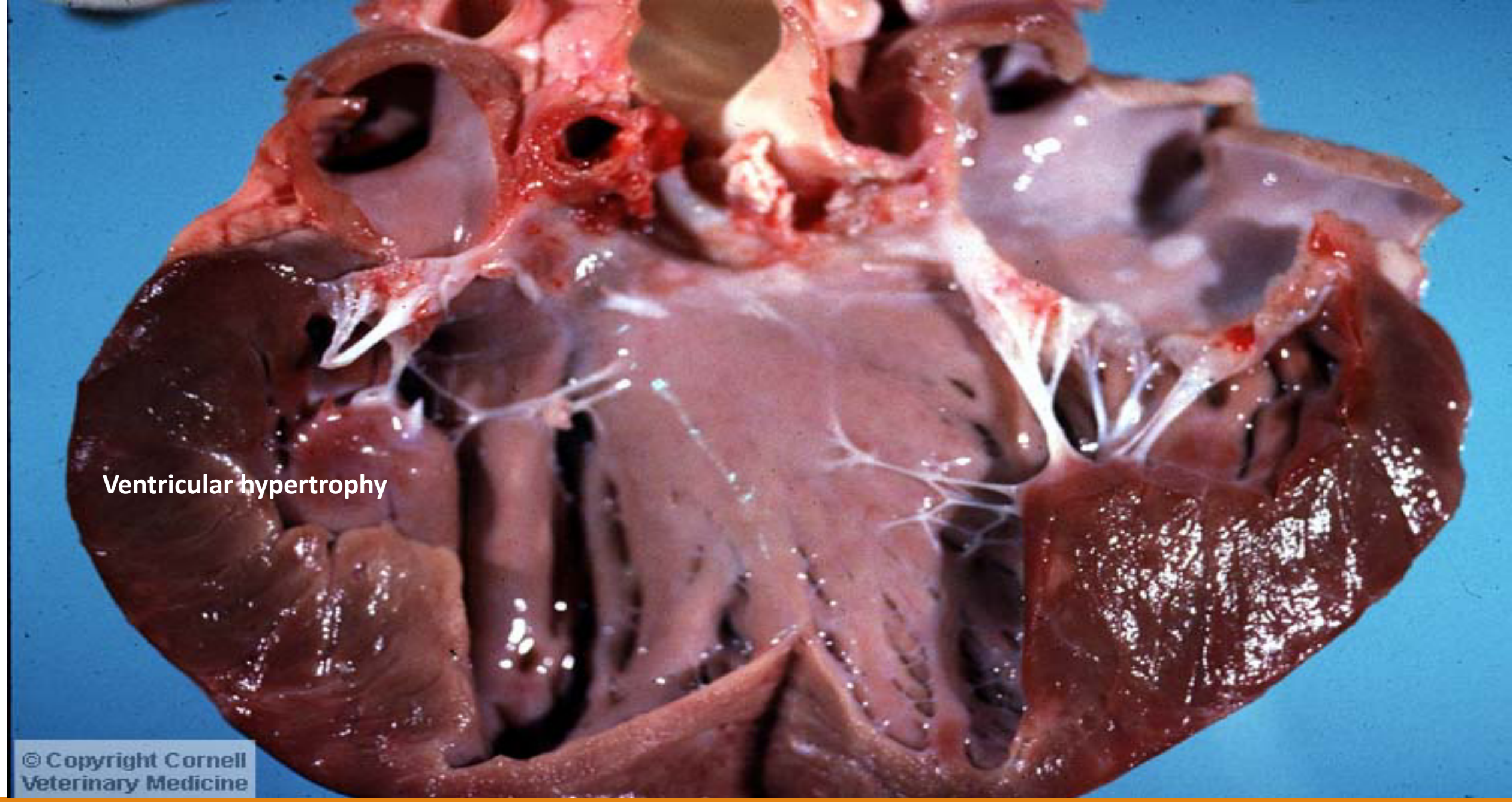
Veterinary Medicine

Disturbances of growth

- **Tissue smaller than normal – hypoplasia (developmental failure to reach full size) or atrophy (shrinkage of a fully formed tissue)**

- **Tissue larger than normal – hypertrophy (increase in individual cell size in post-mitotic cells) and hyperplasia (increase in cell numbers due to mitosis)**

- **Neoplasia (tumour, cancer)**


Ventricular hypertrophy
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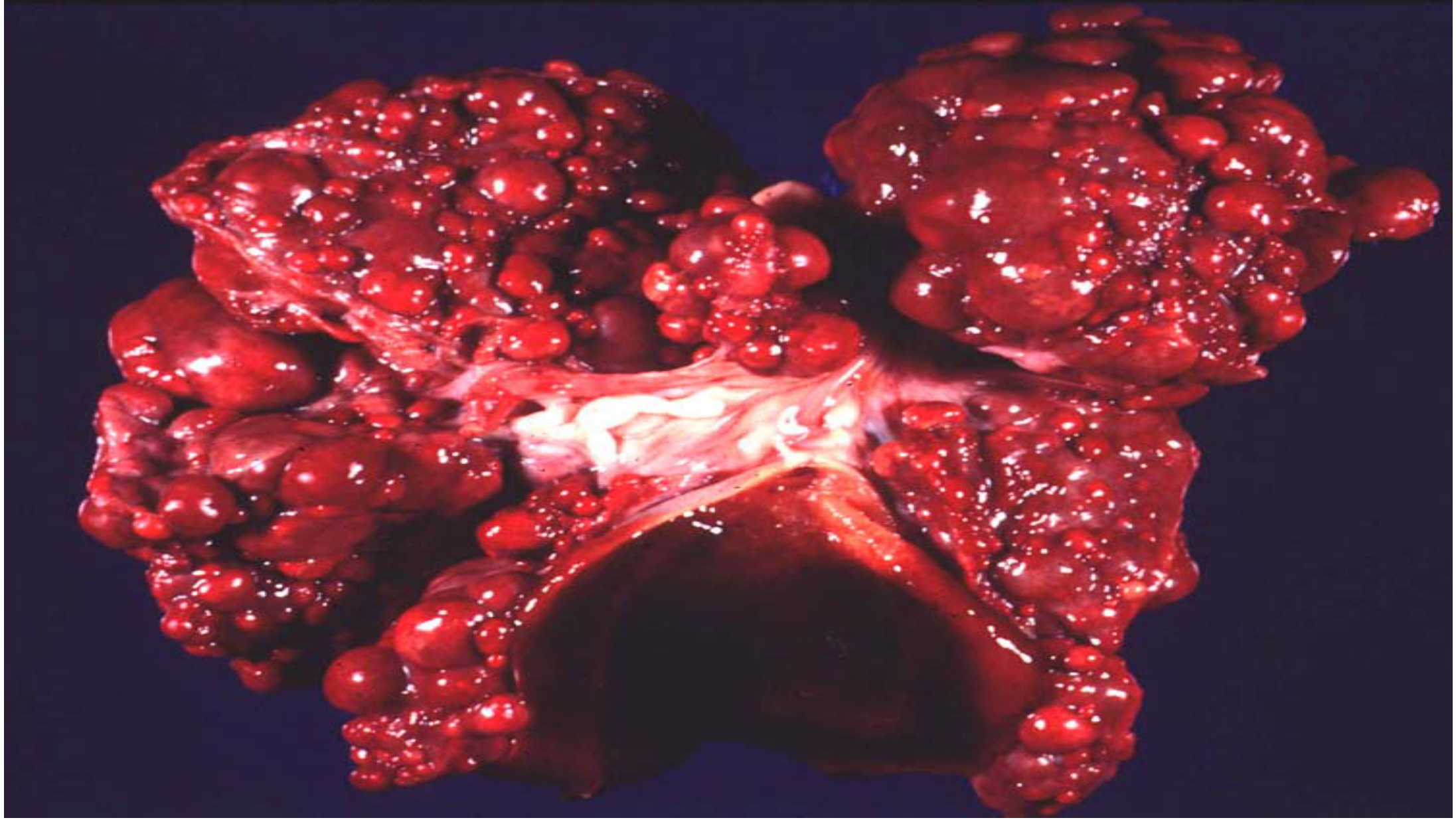
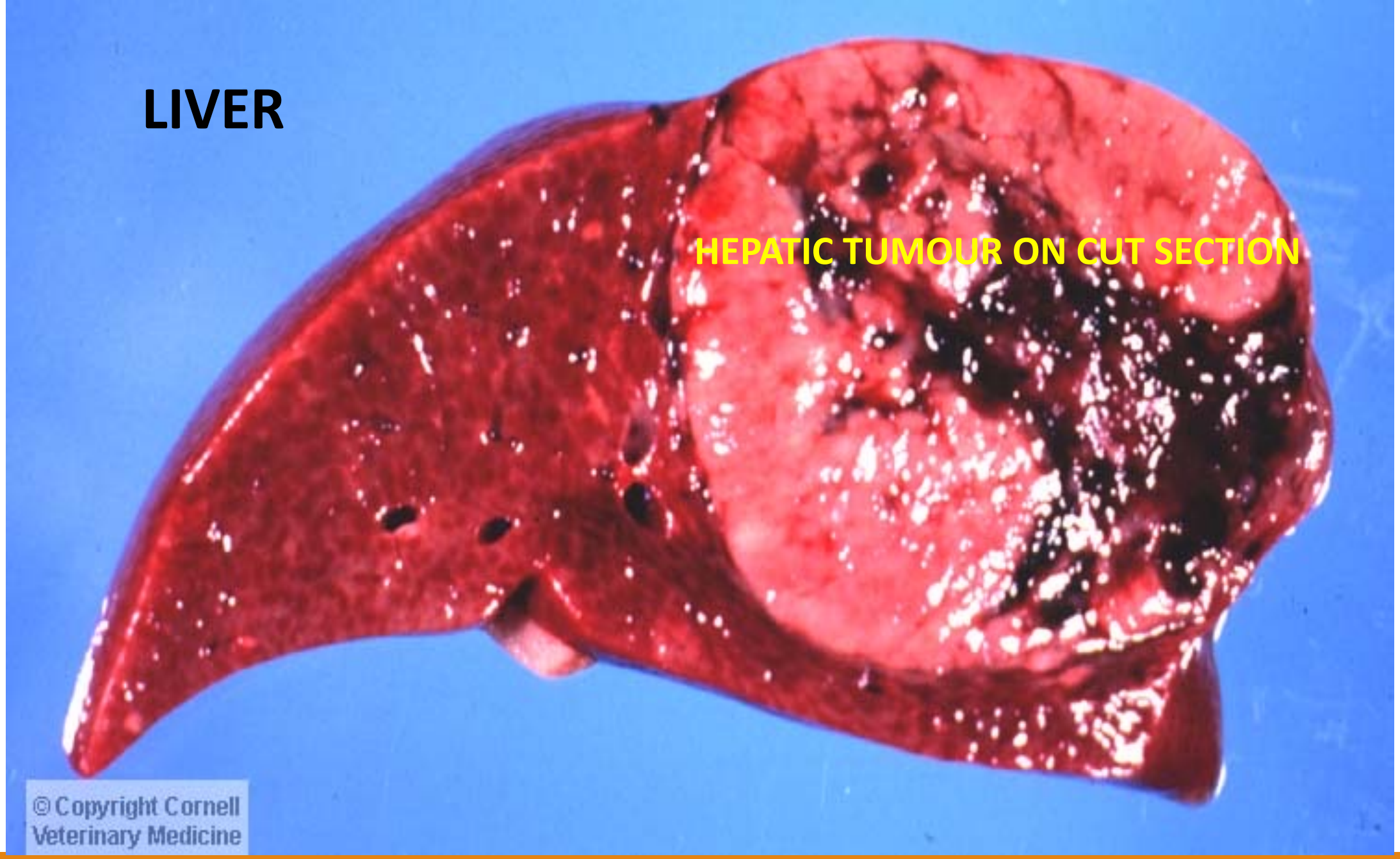


LIVER - NODULAR REGENERATIVE HYPERPLASIA

LIVER


HEPATIC TUMOUR ON CUT SECTION
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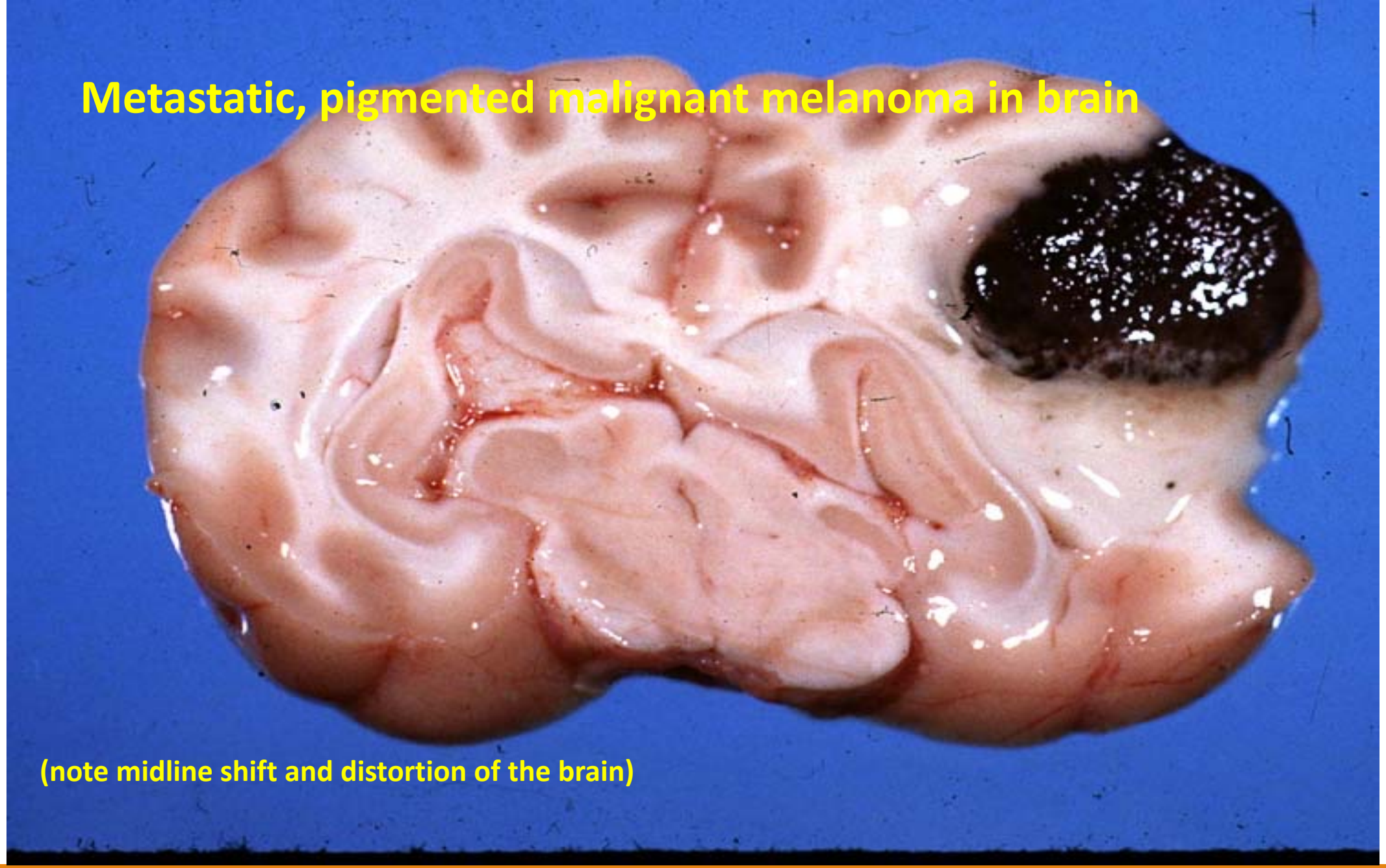
Veterinary Medicine

Metastatic, pigmented malignant melanoma in brain



(note midline shift and distortion of the brain)

Results of an autopsy can lead to

- **Formulation of differential diagnoses (list of possible causes of death)**
  - **Eventually a definitive aetiological diagnosis may be reached**
    - **Results in targeted preventive and therapeutic measures**
    - **Contributes to general disease surveillance information**

Types of disease investigations

- Naturally-occurring (spontaneous) diseases
- Forensic cases
- Anaesthetic deaths (may be no lesions in cases of acute death)
- Experimentally-induced disease

Pattern recognition

- **The thought processes involved in making a definitive morphological diagnosis**

- **Recognition of deviations of tissue architecture from the normal appearance on the basis of prior experience**

- **Where the disease pattern is not immediately recognisable in terms of aetiology, it requires use of background knowledge to ascertain the likely cause (hypothetico-deductive reasoning)**