THE UNIVERSITY OF MELBOURNE

Melbourne Veterinary School

METABOLISM
Laboratory Investigation of the Liver and Biliary System 1

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VETS30017 / VETS90125

Learning outcomes

- Utilise and interpret clinical pathology test results to identify active hepatocellular and biliary disease in the major domestic species
- Discuss the metabolism of bilirubin and the mechanisms behind hyperbilirubinaemia

Lecture Outline

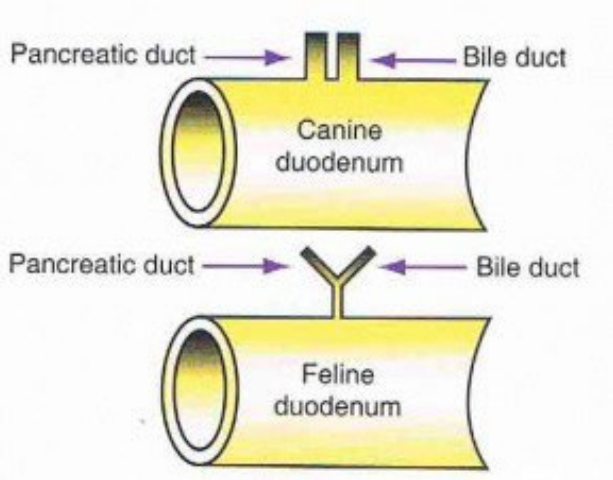
- Introduction
- Assessing for liver disease
- Liver structural damage enzymes
  - ALT
  - AST
  - GLDH
  - SDH
- Cholestasis markers
  - ALP
  - GGT
  - Bilirubin

Introduction

- Liver disease includes any process that results in hepatocyte injury, cholestasis or both:
  - hypoxia, metabolic disease, toxicity, inflammation, neoplasia, trauma, congenital, bile duck blockage
- Liver disease may be primary, or secondary to disease processes in other tissues

  pancreatitis

  inflammatory bowel disease (IBD)



Introduction

- Liver disease frequently presents as a diagnostic challenge, as clinical signs varied and often vague
- In most cases, liver disease can be detected using clinical findings and routine laboratory tests:
  - CBC
  - Biochemistry
  - Urinalysis

Introduction

- Additional diagnostic techniques may then help to provide a more specific diagnosis:
  - liver function tests
  - endocrinology (e.g. TT4, ACTH stim tests)
  - imaging (e.g. radiographs, ultrasound, CT scans)
  - cytology (effusion, FNA) or biopsy for histopathology

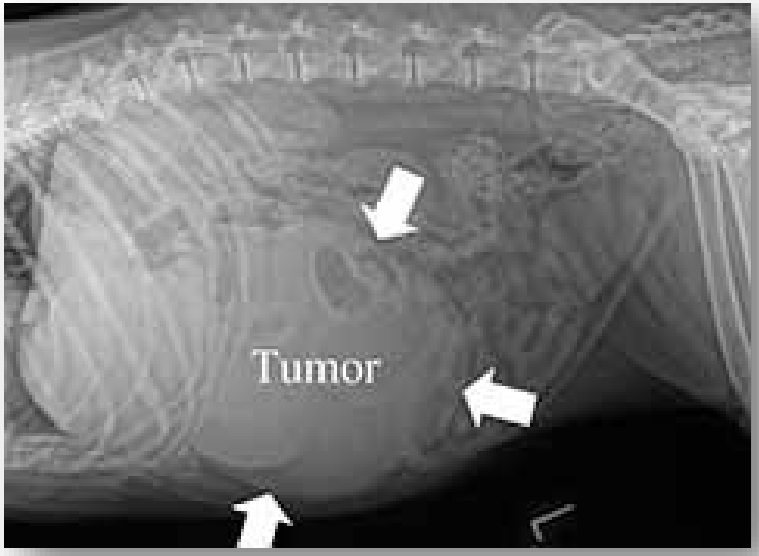
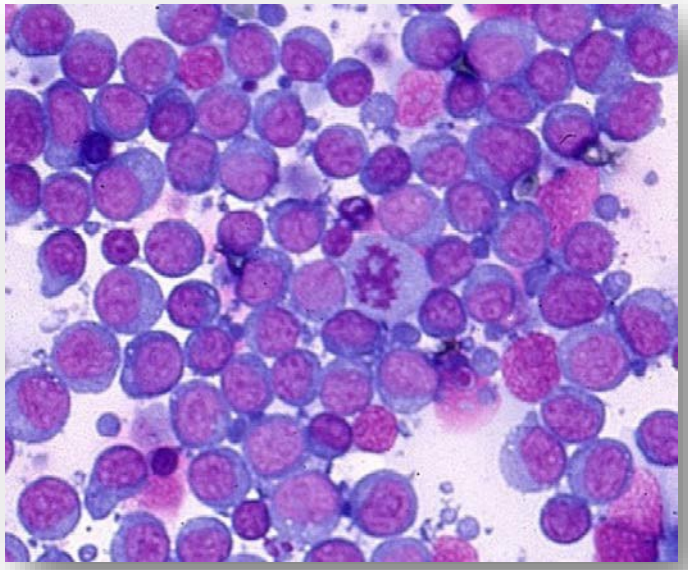
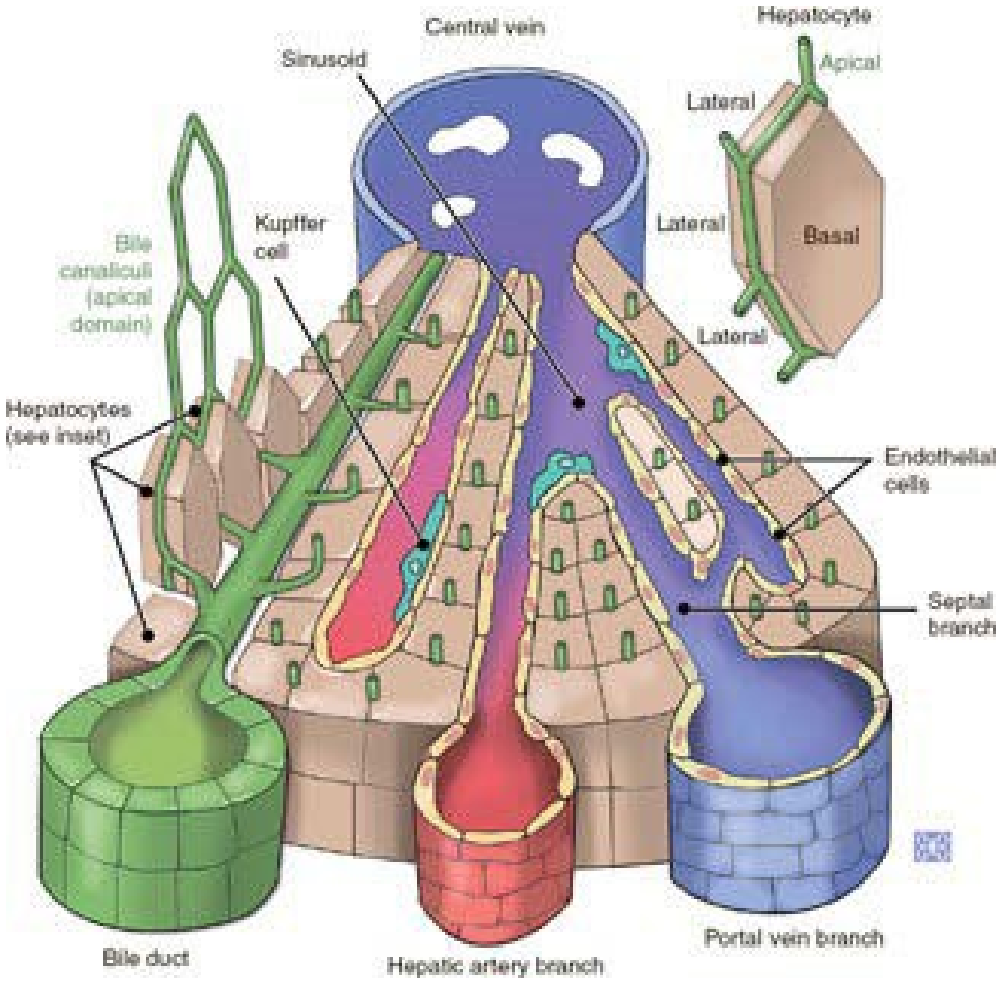


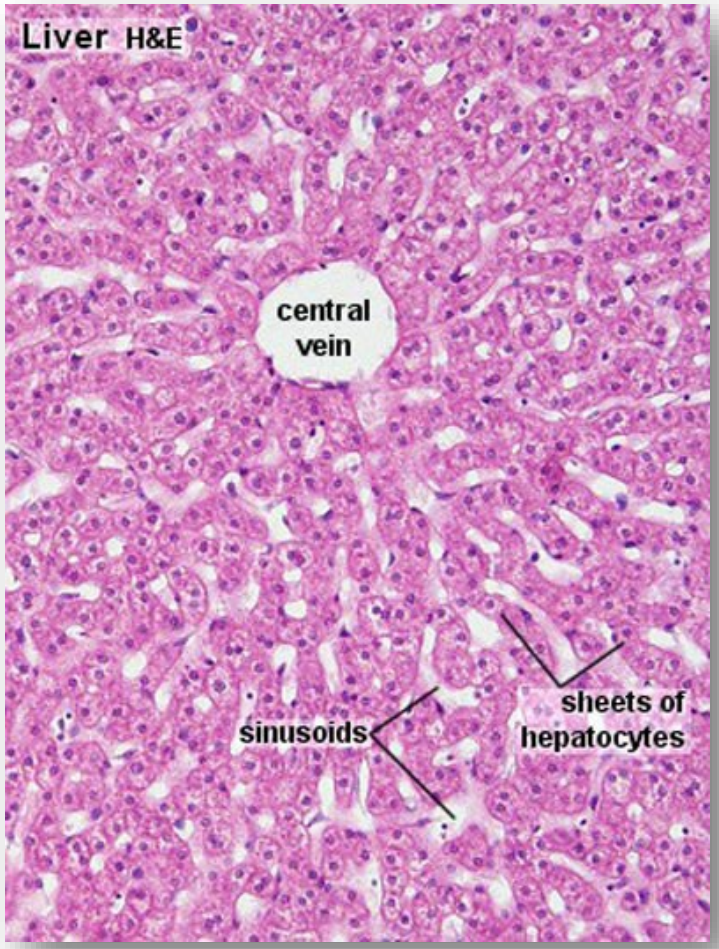
Image: acvs.org



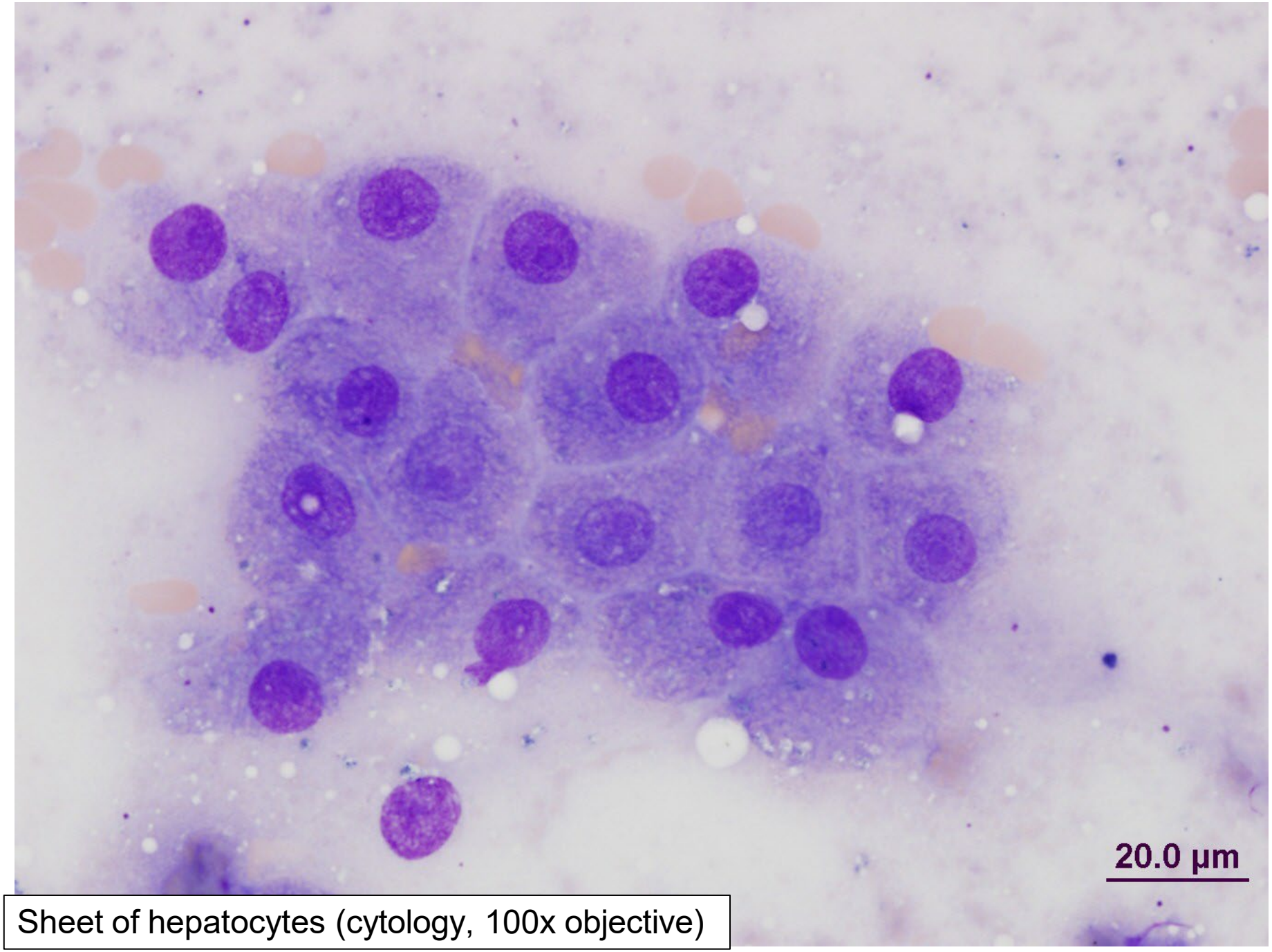
Cytology

Liver Structure





https://www.researchgate.net/publication/236184940_Intracellular_Drug_Concentrations_and_Transporters_Measurement_Modeling_and_Implications_in_the_Liver/figures?lo=1



Sheet of hepatocytes (cytology, 100x objective)

Assessing for liver disease

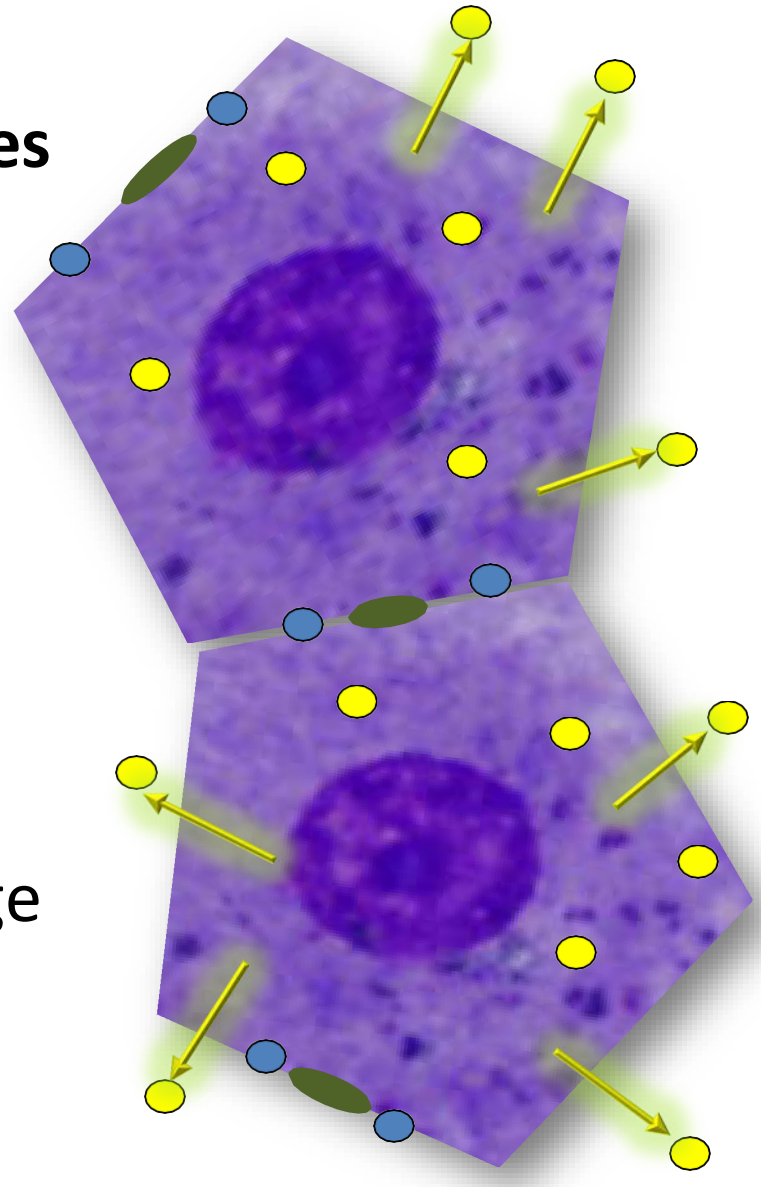
- Is there evidence of cell damage?
  - Increased enzyme leakage
- Is there evidence of cholestasis?
  - Increased bilirubin, induction of enzymes
- Is there evidence of hepatic insufficiency?
  - Decreased urea, cholesterol, albumin, glucose
  - Increased unconjugated bilirubin
  - Functional tests – bile acids, ammonia
- Is the liver disease likely primary or secondary?
  - Clinical findings, other test results

Damage markers

Cytosolic or mitochondrial enzymes

- ALT (muscle)
- GLDH
- AST (muscle, rbc)
- LDH (muscle, rbc)
- SDH

Released due to membrane damage



Hepatocellular injury enzymes

- Elevated enzyme activity alone is not specific as to the cause of the liver injury:
    - hypoxia, trauma, infection, neoplasia, necrosis, toxins, drugs
- Serum enzyme activity depends upon the number of hepatocytes injured, severity of injury and enzyme half life

Acute sublethal injury vs End-stage liver disease

Images: Noahs archive

Which liver will have the highest enzyme activity?

Alanine aminotransferase (ALT)

- Cytosolic enzyme
  - serum half-life ~3 days (dog), ~3 hrs (cat)
- Increased ALT is a major marker of hepatocellular injury in dogs and cats (rats, mice, primates)
  - Less useful in rabbits as rises with other tissue injury
  - Not useful in horses, ruminants and pigs due to low enzyme activity in liver tissue
- Highest activity seen with acute or severe injury:
  - trauma, necrosis, inflammation
- Mild increases seen with:
  - anticonvulsants, corticosteroids, hyperthyroidism

Alanine aminotransferase (ALT)

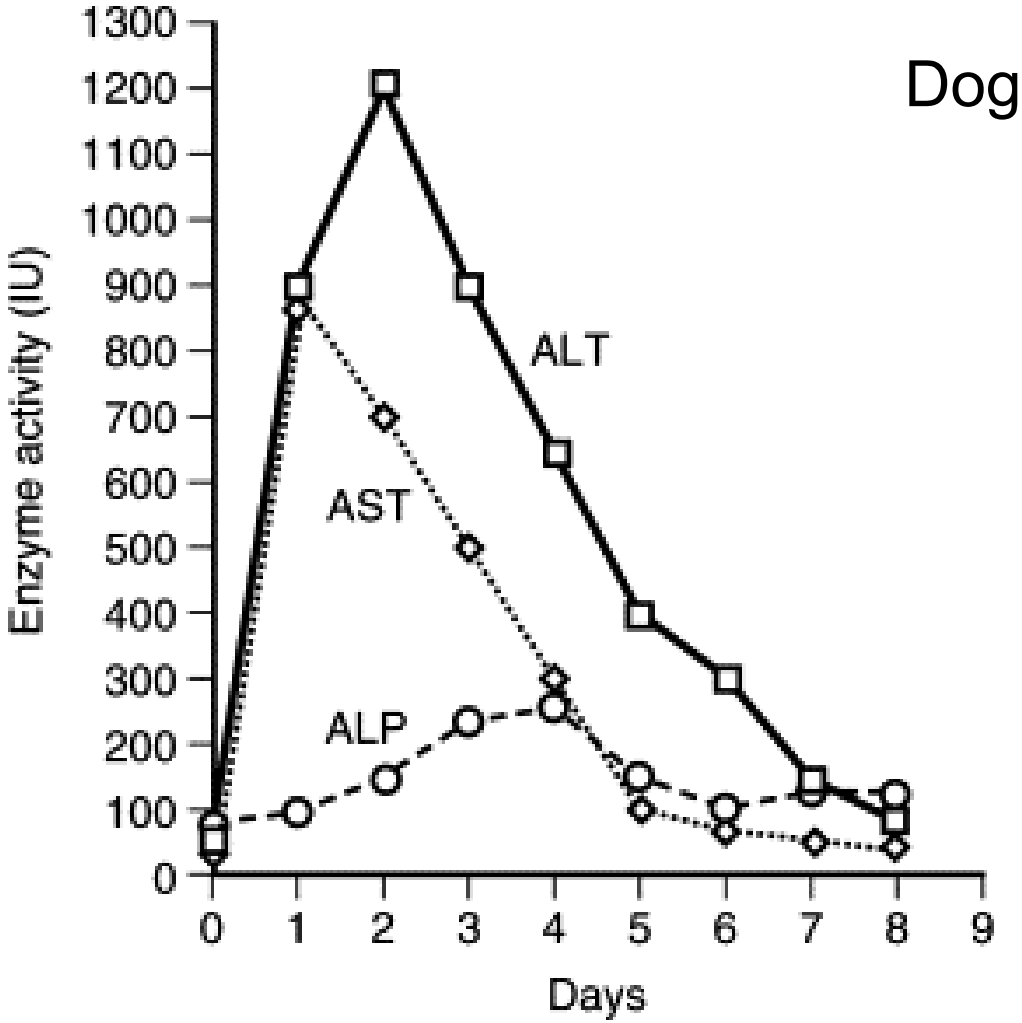
- ALT is also a cytosolic enzyme in skeletal myocytes
- Mild increase in ALT may occur with severe myopathies
    - e.g. ischemic myopathy in cats, muscular dystrophy in dogs, snake envenomation
    - expect creatine kinase (CK) and AST to be high

Aspartate aminotransferase (AST)

- Cytosolic and mitochondrial enzyme
  - serum half-life ~1 week (horse), ~1 day (dog), ~1 hr (cat)
- AST is a useful indicator of hepatocellular injury in most species:
  - horses and ruminants; useful as have minimal ALT
  - cats and dogs; sensitive indicator of liver injury
- AST is not as tissue specific as ALT
  - AST will increase with haemolysis (from erythrocytes)
  - AST will increase with muscle damage (from myocytes)

Enzyme changes after transient insult

Dogs and Cats



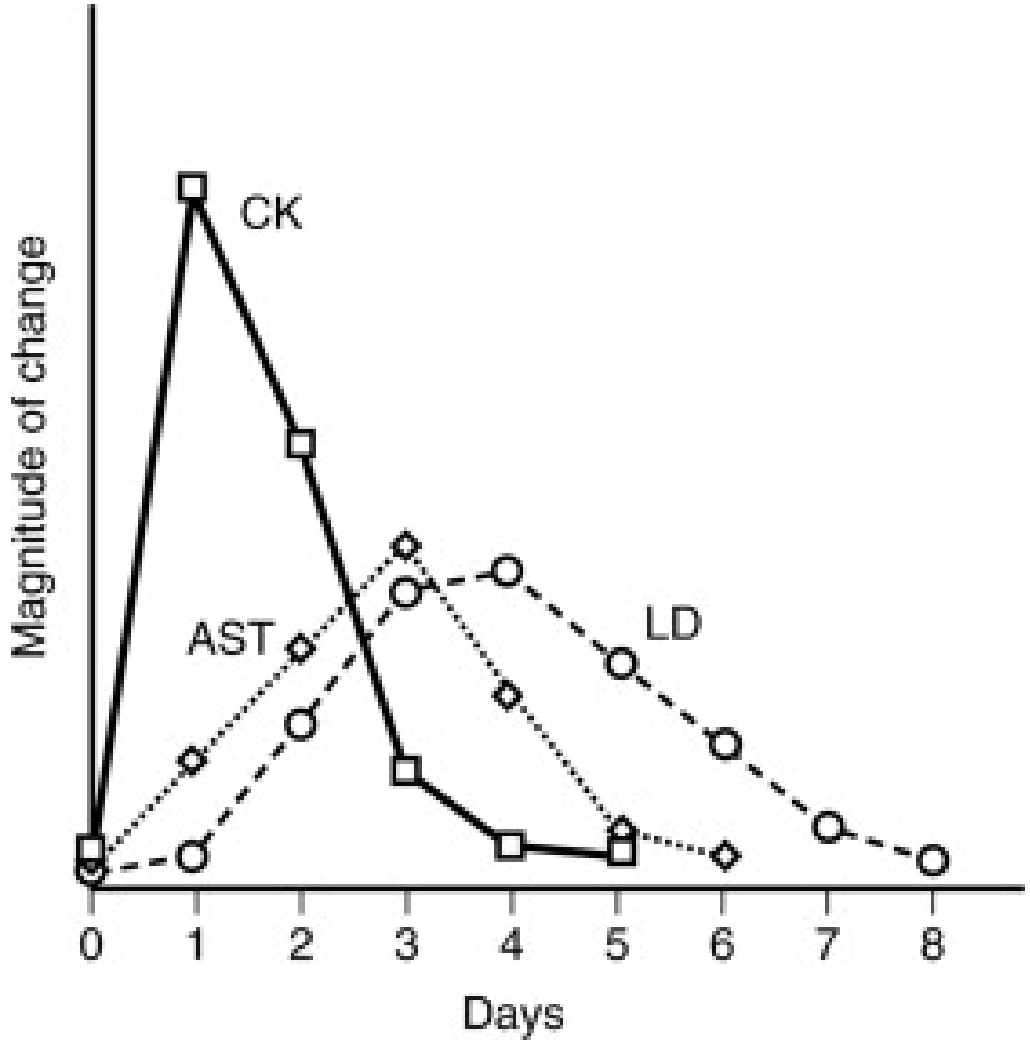
Veterinary Clinical Pathology Secrets, Rick Cowell

AST - muscle vs liver vs haemolysis

1. Check Creatine Kinase (CK)
- Muscle specific enzyme
- Useful to differentiate the source of AST elevation

2. Check for haemolysis
- Haemolytic anaemia
- Invitro haemolysis

Enzyme changes after transient insult



Veterinary Clinical Pathology Secrets, Rick Cowell

Glutamate dehydrogenase (GLDH)

- Mitochondrial enzyme
  - requires greater cell damage to escape into serum
- Half life horses 12-24hrs, cattle 14hrs
- GLDH is a major marker of acute hepatocellular injury in all species
  - useful in large animals, as more specific than AST
  - rarely used in cats and dogs

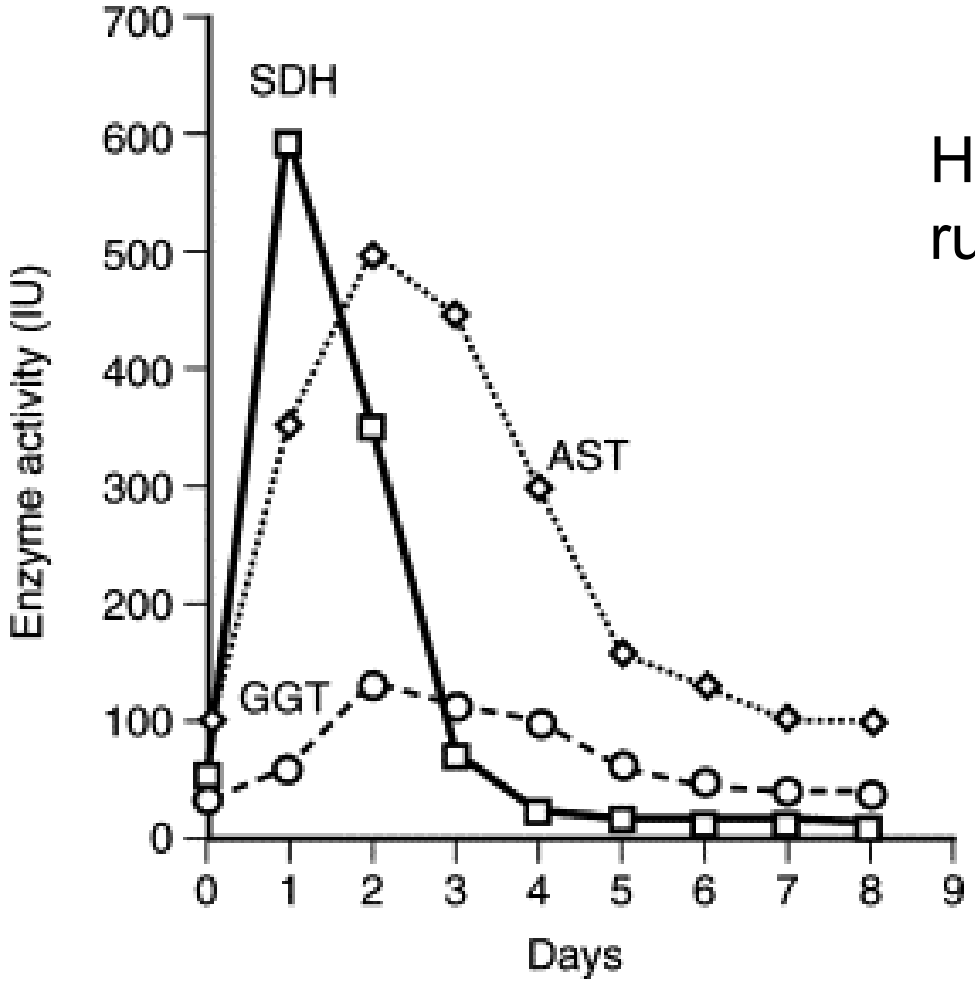
    (ALT considered superior)
  - GLDH useful in birds and reptiles

Sorbitol dehydrogenase (SDH)

- Cytoplasmic enzyme
- SDH is a useful marker of hepatocellular damage in horses and ruminants
  - more commonly used in the USA
  - however enzyme stability is an issue - decreases rapidly in stored samples
- Half life 12-24hrs in horses

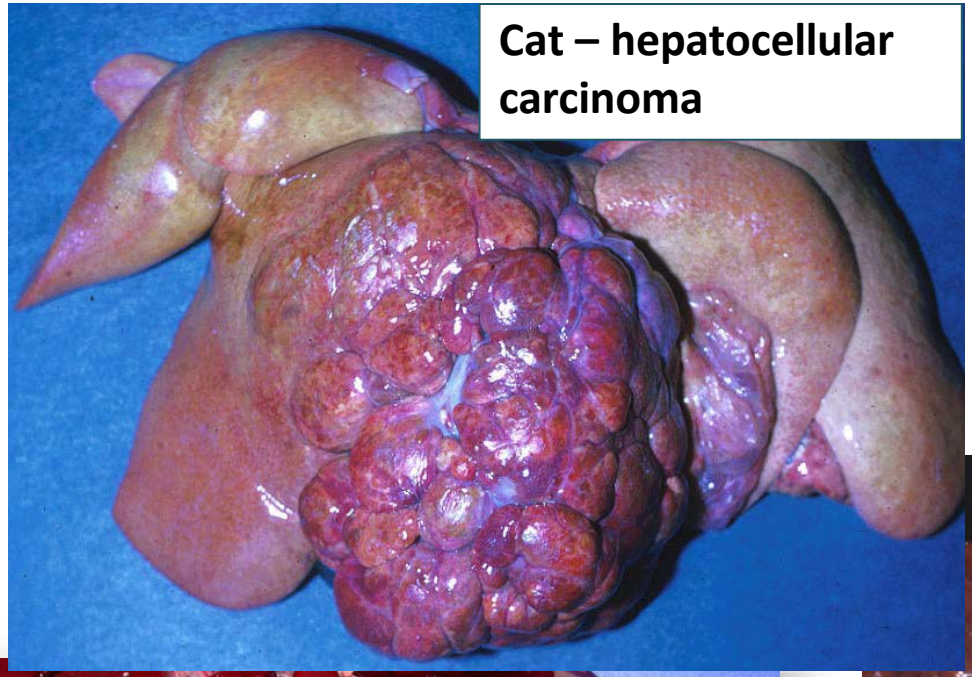
www.thespruce.com

Enzyme changes after transient insult

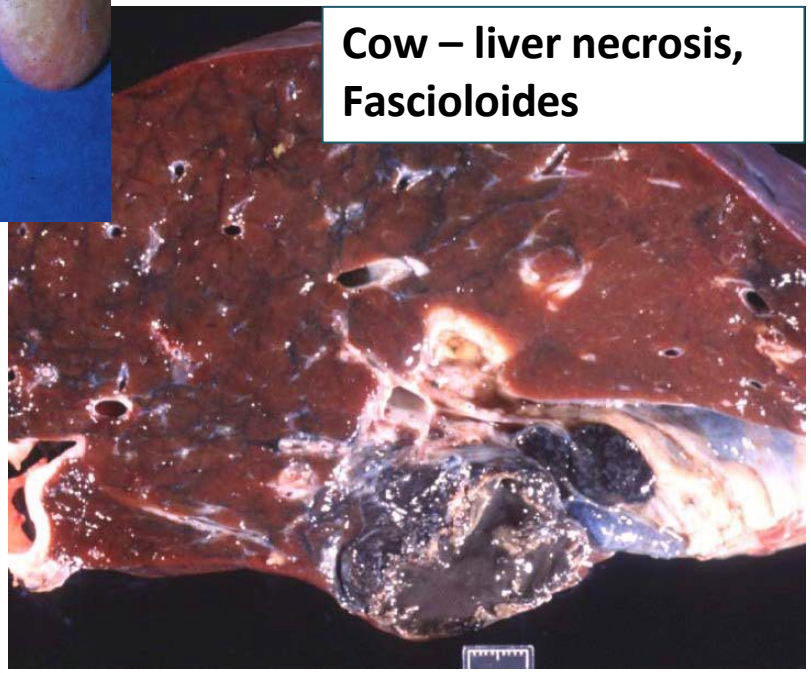


Horses and ruminants

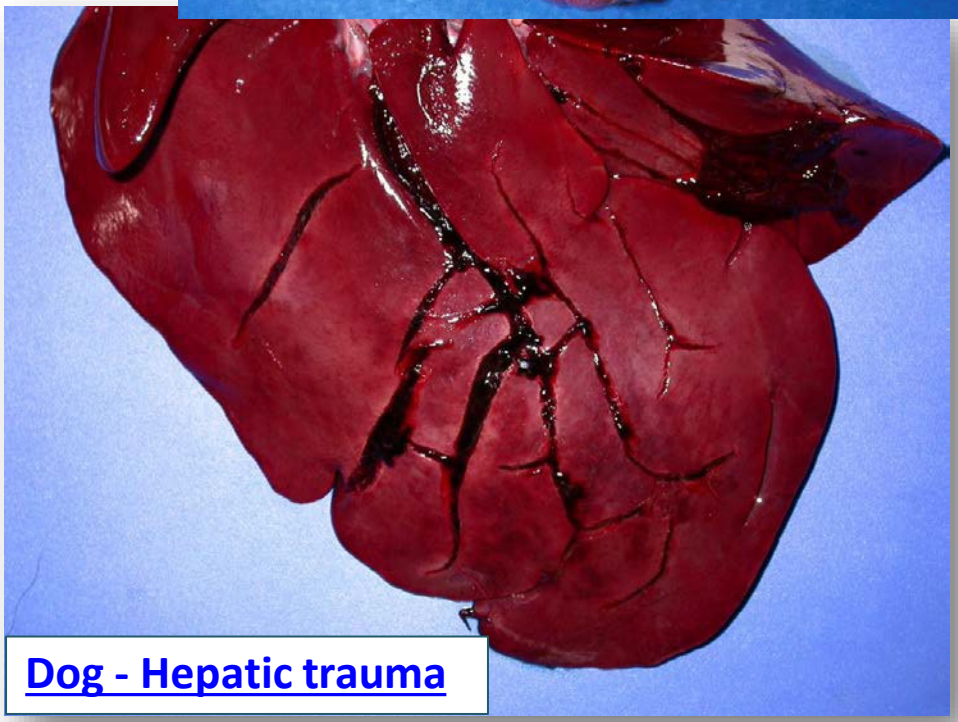
Veterinary Clinical Pathology Secrets, Rick Cowell



Cat – hepatocellular carcinoma



Cow – liver necrosis, Fascioloides



Dog - Hepatic trauma

Noah's Arkive

Diseases causing liver enzyme elevations

Cholestasis

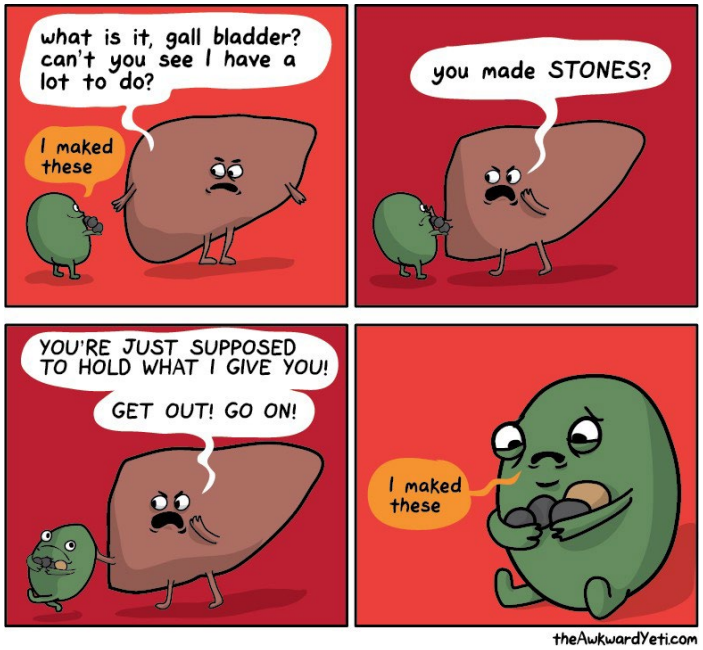
- Reduced outflow of bile - impairment anywhere between hepatocytes and bile duct entry to duodenum
- Functional:
  - Defective or downregulated bile acid transporters

    e.g. endotoxins (sepsis), excess free FA (fasting)

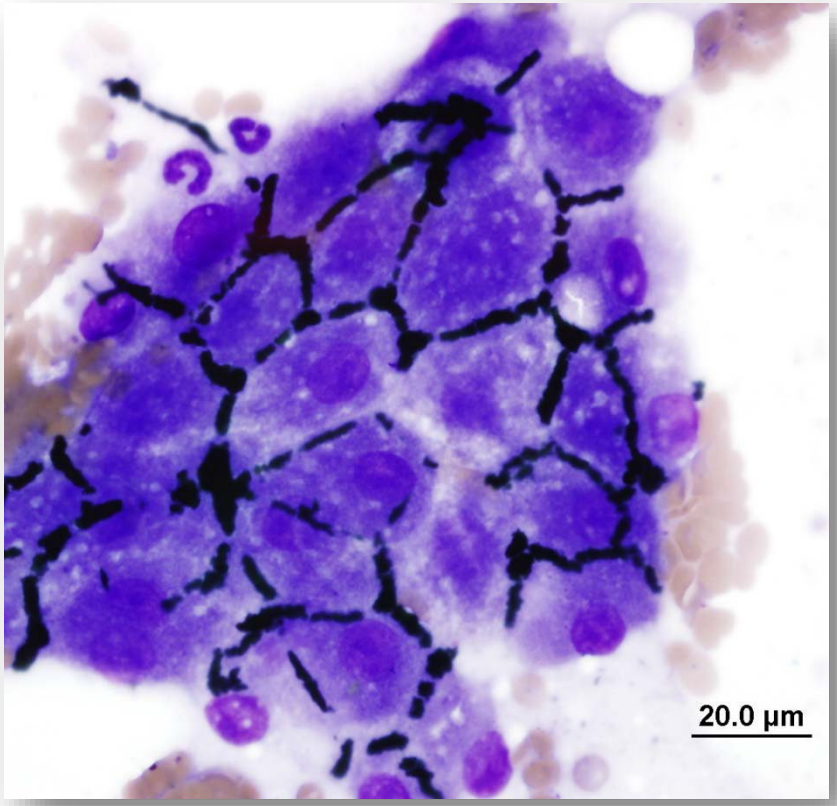
- Structural (obstructive):
  - Hepatic - constriction of canaliculi

    e.g. lipidosis, steroid hepatopathy, neoplasia, fibrosis, bile sludging
  - Post-hepatic – bile duct blockage

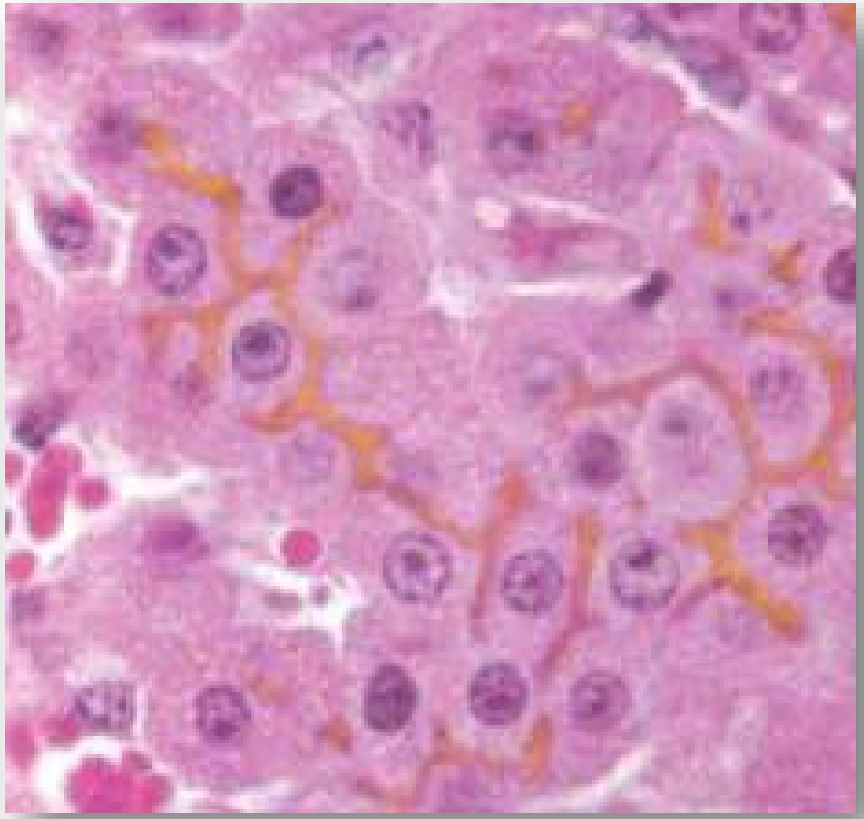
    e.g. cholelithiasis, pancreatitis, neoplasia, mucocoeles, inflammation



“bile plugs or bile casts” = tissue evidence of cholestasis (stoppage or suppression of bile flow)



Cytology



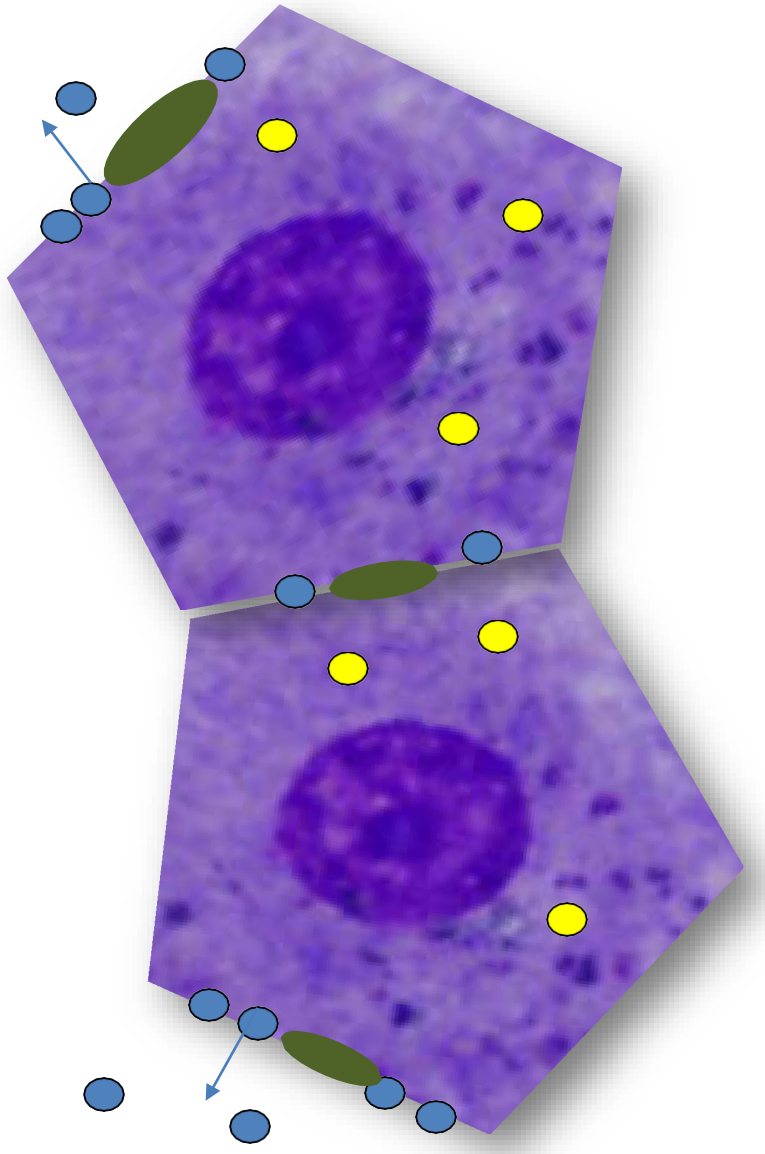
Histopathology

Cholestasis Markers

Membrane enzymes

- ALP
- Corticosteroid ALP in dogs
- GGT

Induced by cholestasis, drugs and hormones

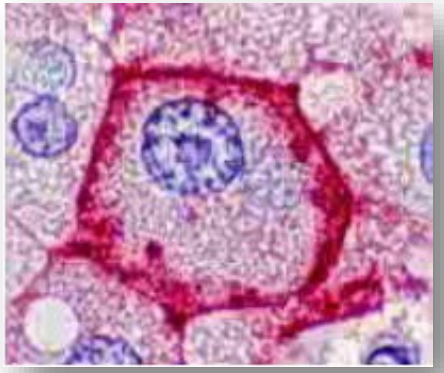


Markers of Cholestasis

- **Hyperbilirubinemia**
  - Bilirubin (normally excreted in bile) may spill over into blood (and urine) with severe cholestasis (hepatic or post-hepatic)
  - Also occurs with haemolysis (RBC lysis)
- **Hypercholesterolemia**
  - Due to decreased clearance
  - Non-specific (increases with endocrine disease, pancreatitis, post prandially)

https://www.cliniciansbrief.com/article/top-5-indications-emergency-corticosteroid-therapy

Alkaline phosphatase (ALP)



- Membrane bound enzyme
- Serum ALP represents total ALP activity, which includes **three major isoforms**:

  1. L-ALP: liver (hepatocytes and biliary epithelium)
  2. B-ALP: bone (osteoblasts)
  3. C-ALP: corticosteroid (hepatocytes) **dog specific**

- Additional ALP isoforms include:
  - intestinal, leukocyte, renal, mammary and placental
  - short half lives and do not typically increase serum ALP
  - may see mild ALP elevations with mammary tumours, and late pregnant queens

Alkaline phosphatase (ALP)

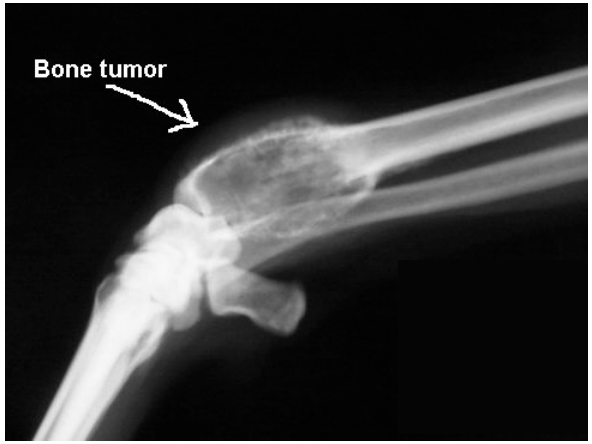
Increased serum ALP: **Cholestasis**

- Bile acid accumulation induces L-ALP synthesis
- Dogs: high sensitivity for detecting cholestasis
  - increased ALP often precedes hyperbilirubinaemia
  - lacks specificity due to C-ALP isoform
- Cats: lower sensitivity but specific for cholestasis
  - produce less ALP and very short half life
  - so any increase is considered significant
- Large animals: poor sensitivity, wide ref interval (GGT preferred)

Alkaline phosphatase (ALP)

Increased serum ALP: **Increased osteoblastic activity**

- Induce B-ALP synthesis
  - young rapidly growing animals
  - osteosarcoma
  - fracture repair
  - active bone resorption
    - hyperparathyroidism
    - hyperthyroidism (also L-ALP)



- Generally only <u>mild</u> increases in serum ALP (< 4 fold)

Alkaline phosphatase (ALP)

Increased serum ALP: Other causes

- **Corticosteroids** (dogs only)
  - induce C-ALP from hepatocytes
  - source:
    - exogenous (therapeutic)
    - endogenous (chronic stress or hyperadrenocorticism)
- Anticonvulsants
  - increased L-ALP production in cats and dogs
    - phenobarbital, phenytoin, primidone (not shown to increase C-ALP)

Gamma glutamyl transferase (GGT)

Membrane bound enzyme

- main source of GGT is the liver
  - especially biliary epithelium but also hepatocytes
  - also in pancreas, kidney, intestine, epididymis (lost into lumens)
- colostrum also contains high levels of GGT
  - dogs, cats, sheep, cattle (but NOT horses)
  - in neonates, decreased GGT may indicate failure of passive transfer
  - measurement of IgG is gold standard

https://kannada.oneindia.com

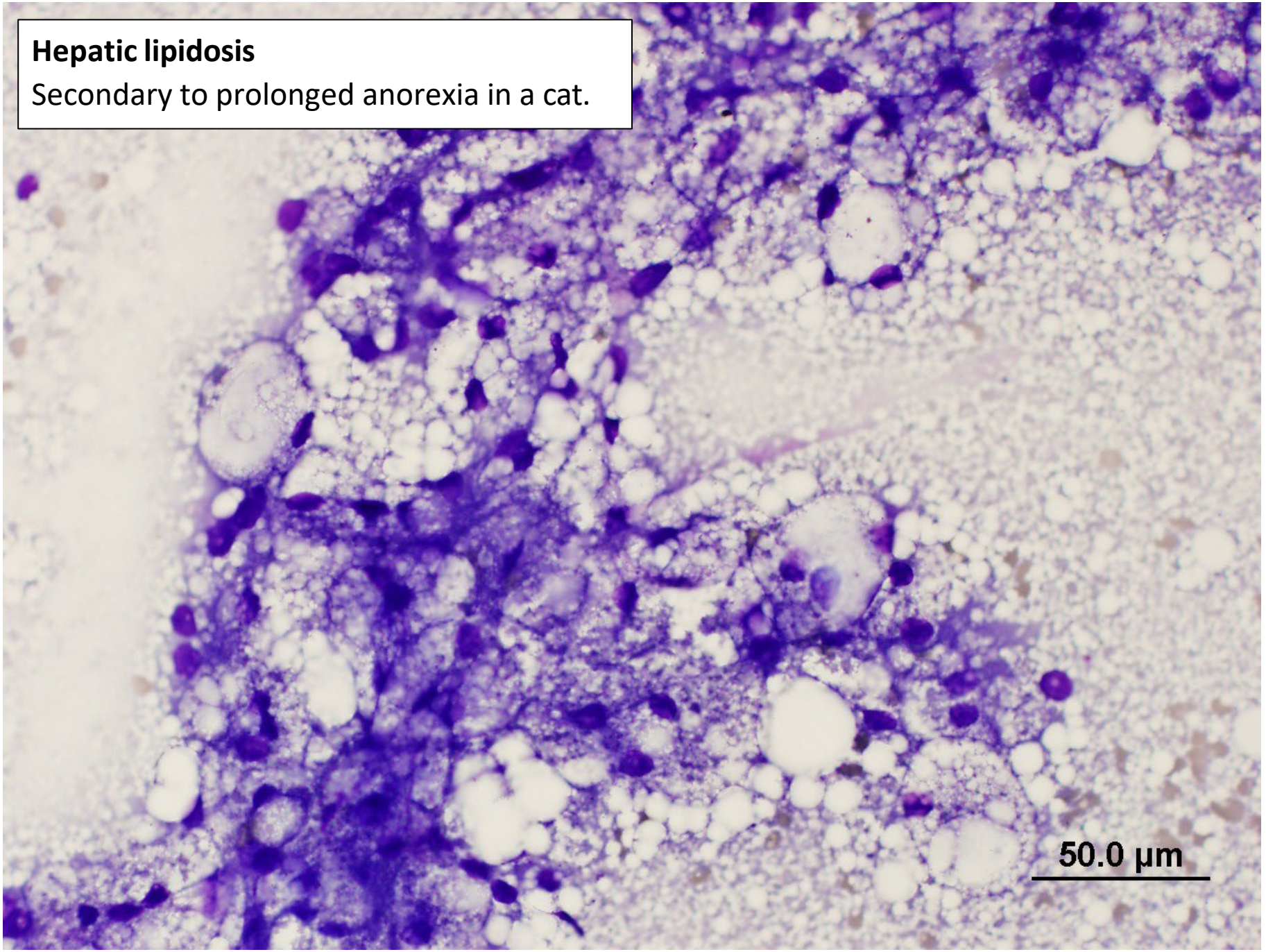
Gamma glutamyl transferase (GGT)

GGT is a sensitive indicator of cholestasis and/or biliary hyperplasia in all domestic species

- GGT more sensitive than ALP in ruminants and horses
  - e.g. pyrrolizidine alkaloid toxicity in cattle
  - e.g. cholelithiasis, cholangiohepatitis in horses
- GGT increases usually precede increases in ALP in cats

  exception = some cases of hepatic lipidosis

  (↑ ALP:GGT ratio is highly suggestive)
- GGT tends to parallel ALP in dogs


Hepatic lipidosis
Secondary to prolonged anorexia in a cat.
50.0 µm

Assessing enzyme abnormalities

1. Identify predominant pathologic process
   - hepatocellular injury vs cholestasis
     - increased enzyme activities cannot identify specific aetiology
     - most diseases are a mix of both processes
2. Identify magnitude of increased activity
   - may relate to the severity of damage (especially with cytoplasmic enzymes)
   - does not differentiate between reversible or irreversible damage or local vs diffuse damage
3. Identify rate of change
   - monitor changes over time to assess if process is active

Serum enzyme interpretation

- Enzyme activities have reference intervals - but are usually described as “fold increases” to interpret their significance/magnitude
  - allows comparison of values between different analysers

    e.g. ALT = 500 U/L (10-50)

    → there is a ‘10 fold’ increase in ALT

- Rule of thumb:

  2-3 fold increase above RI = mildly elevated

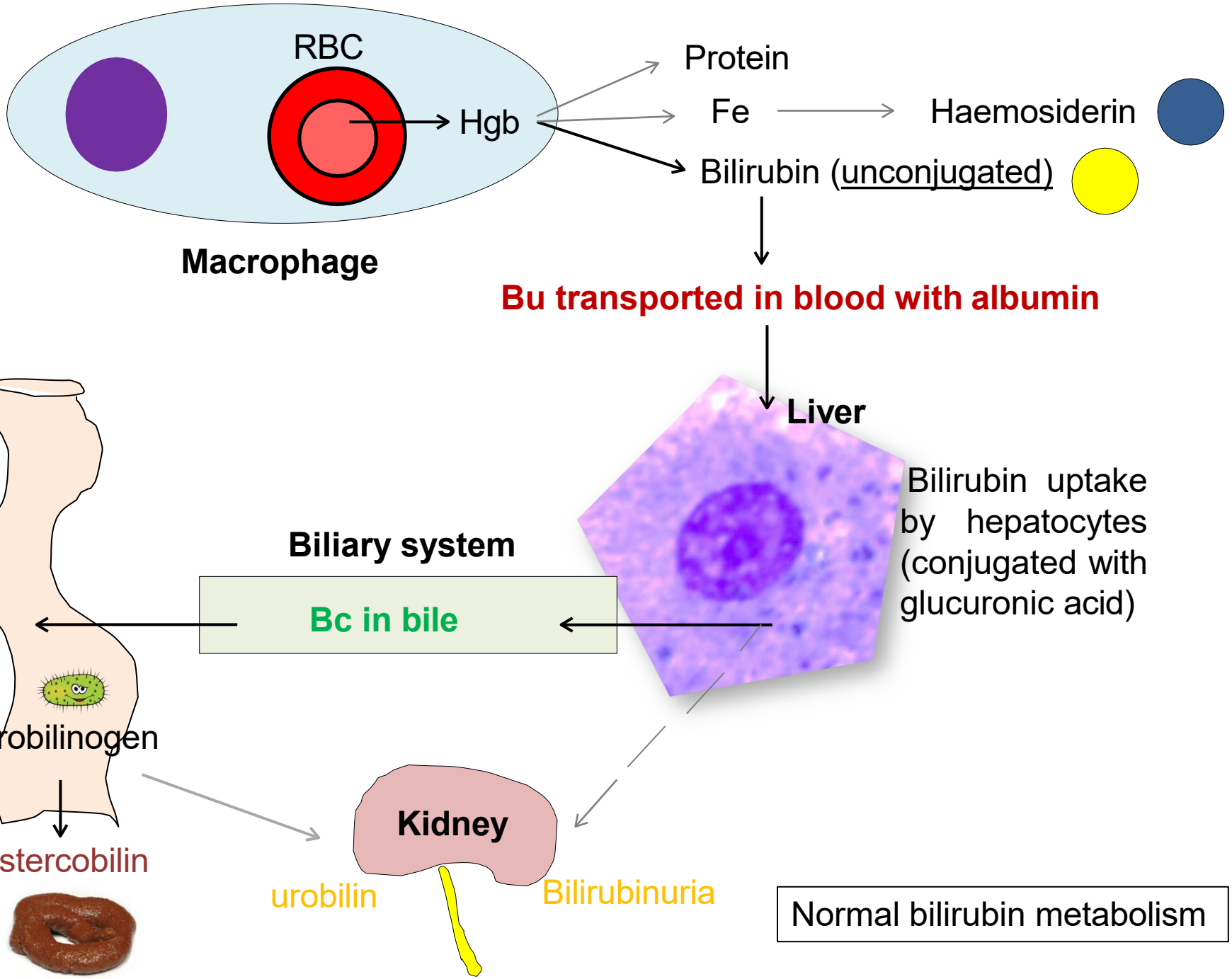
  4-5 fold increase above RI = moderately elevated

  10 fold increase above RI = markedly elevated

Bilirubin

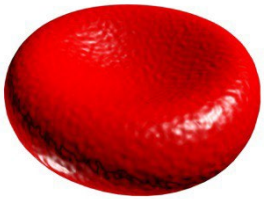
- Yellow pigment
  - derived largely from breakdown of haemoglobin by macrophages (spleen, liver, BM)
- Normally constantly produced and rapidly removed from plasma by the liver
- Elevated bilirubin is used as a marker of liver disease but can indicate haemolysis
- Types of bilirubin
  - Unconjugated (Bu)
  - Conjugated (Bc)
  - Delta bilirubin

} Total Bilirubin (TBIL)


RBC
Hgb
Protein
Fe
Haemosiderin
Bilirubin (unconjugated)
Macrophage
Bu transported in blood with albumin
Liver
Bilirubin uptake by hepatocytes (conjugated with glucuronic acid)
Biliary system
Bc in bile
Intestine
urobilinogen
stercobilin
Kidney
urobilin
Bilirubinuria
Normal bilirubin metabolism

Normal bilirubin metabolism

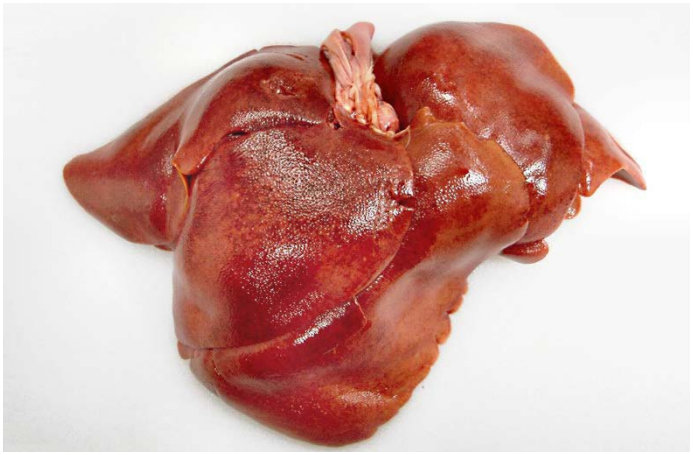
- **Unconjugated** bilirubin (Bu) = indirect Bb/. Produced from degradation of heme from old red blood cells
  = 'indirect' bilirubin
- Bound to albumin in blood and transported to liver
- Uptake by hepatocytes and conjugated to glucuronic acid
- **Conjugated** bilirubin (Bc) = direct Bb. Is water soluble and is excreted as a component of bile, into the intestine
- Converted by intestinal bacteria to urobilinogen
  → stercobilin in faeces (brown colour to faeces)
  → urobilin in kidneys (yellow colour to urine)
- In healthy **dogs**, a small amount of conjugated bilirubin spills into urine – may see (1+) on urine dipstick



Mechanisms of Icterus

Haemoglobin

Bilirubin



Bilirubin conjugation

Bile

Bilirubinuria

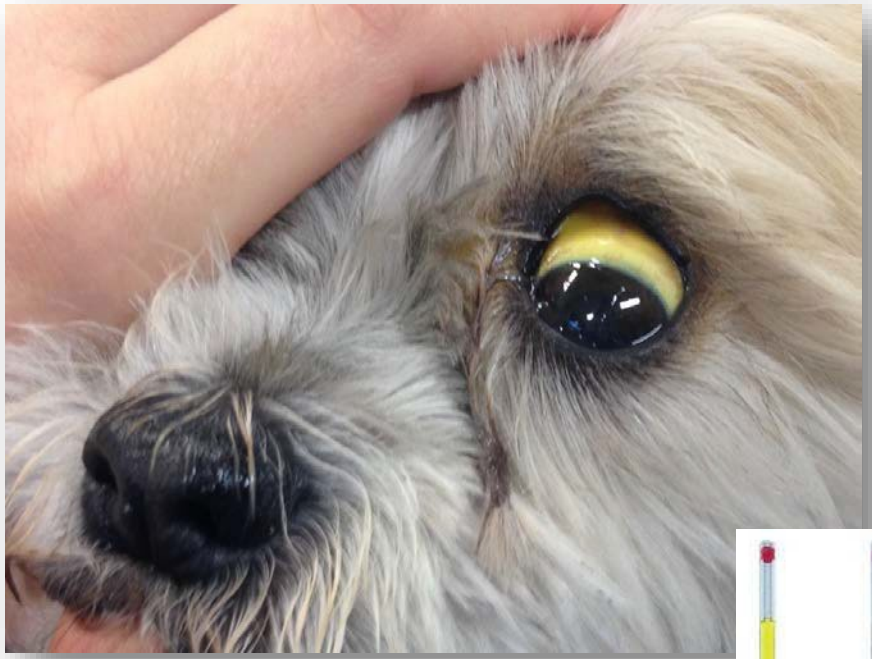
Icterus can be:

- **Pre-hepatic** (haemolysis)
- **Hepatic**
- **Post-hepatic** (bile duct obstruction)

Jaundice = Icterus

Clinical presentation of hyperbilirubinaemia

Hyperbilirubinaemia clinically evident as jaundice/icterus when > 35 - 50 μmol/L



Dog with prehepatic icterus due to IMHA

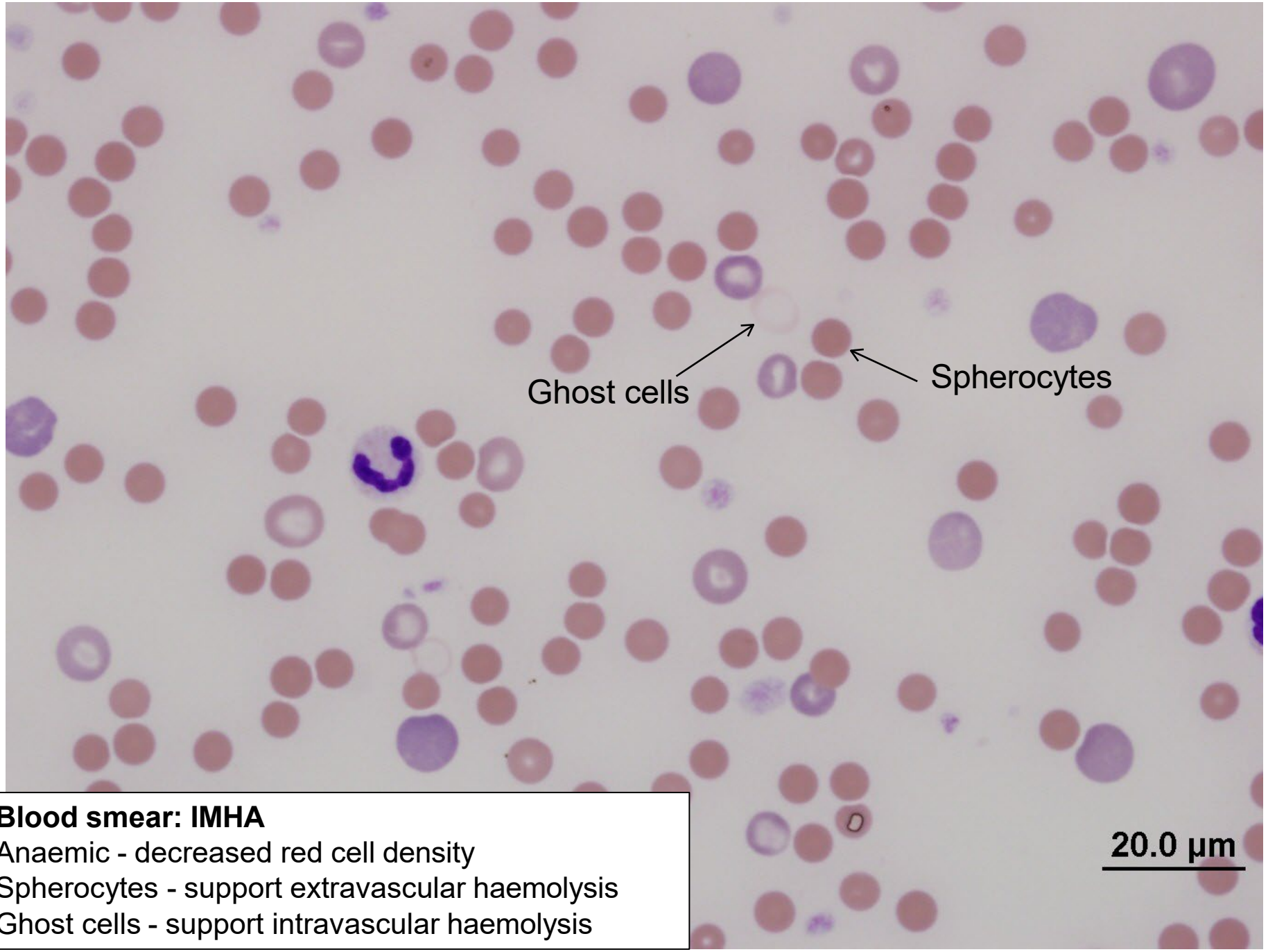
Prehepatic hyperbilirubinaemia

- Increased erythrocyte breakdown
- Rate of Bu production exceeds rate of uptake or excretion by hepatocytes, resulting in elevated TBIL
- Usually due to severe rapid haemolysis

  e.g. immune mediated haemolytic anaemia (IMHA)

  healthy liver can handle substantial haemolysis without an increase in bilirubin
- Typically Bu > Bc, with no increase in liver enzymes

  however, hypoxic injury to hepatocytes or concurrent liver disease may complicate the pattern



Blood smear: IMHA
Anaemic - decreased red cell density
Spherocytes - support extravascular haemolysis
Ghost cells - support intravascular haemolysis

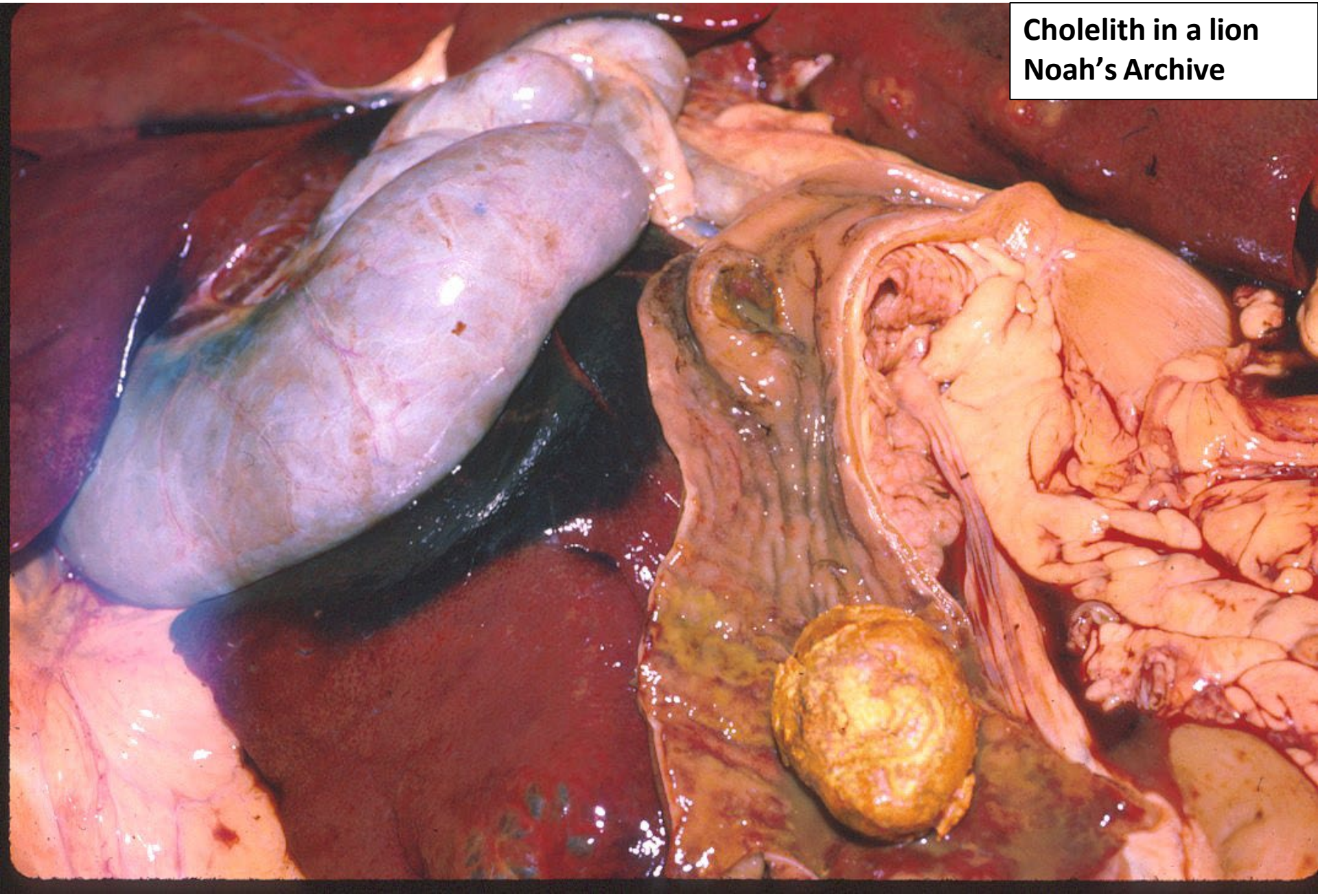
Hepatic hyperbilirubinaemia

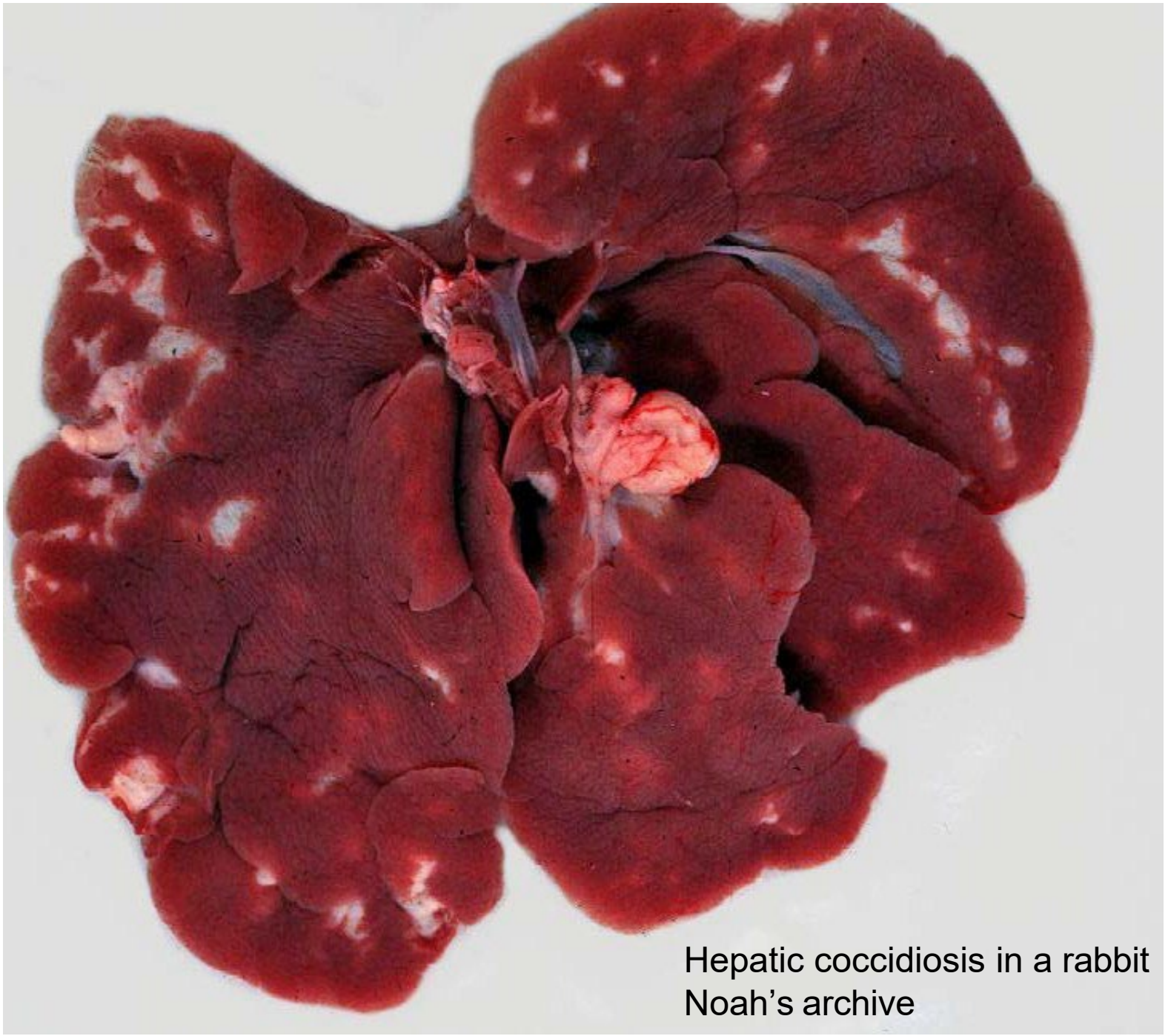
- Elevated TBIL caused by abnormal uptake, conjugation or excretion of bilirubin by hepatocytes:
  - Decreased functional hepatic mass
    - e.g. chronic hepatitis, diffuse necrosis or neoplasia, end-stage liver disease
  - Anorexia/fasting in **horses** (cattle, cats)
    - mobilisation of fatty acids interfere with Bu uptake
  - Sepsis
    - inflammatory cytokines interfere with transporters

- Typically mild elevations in bilirubin
  - Bu may predominate – but dependent on process

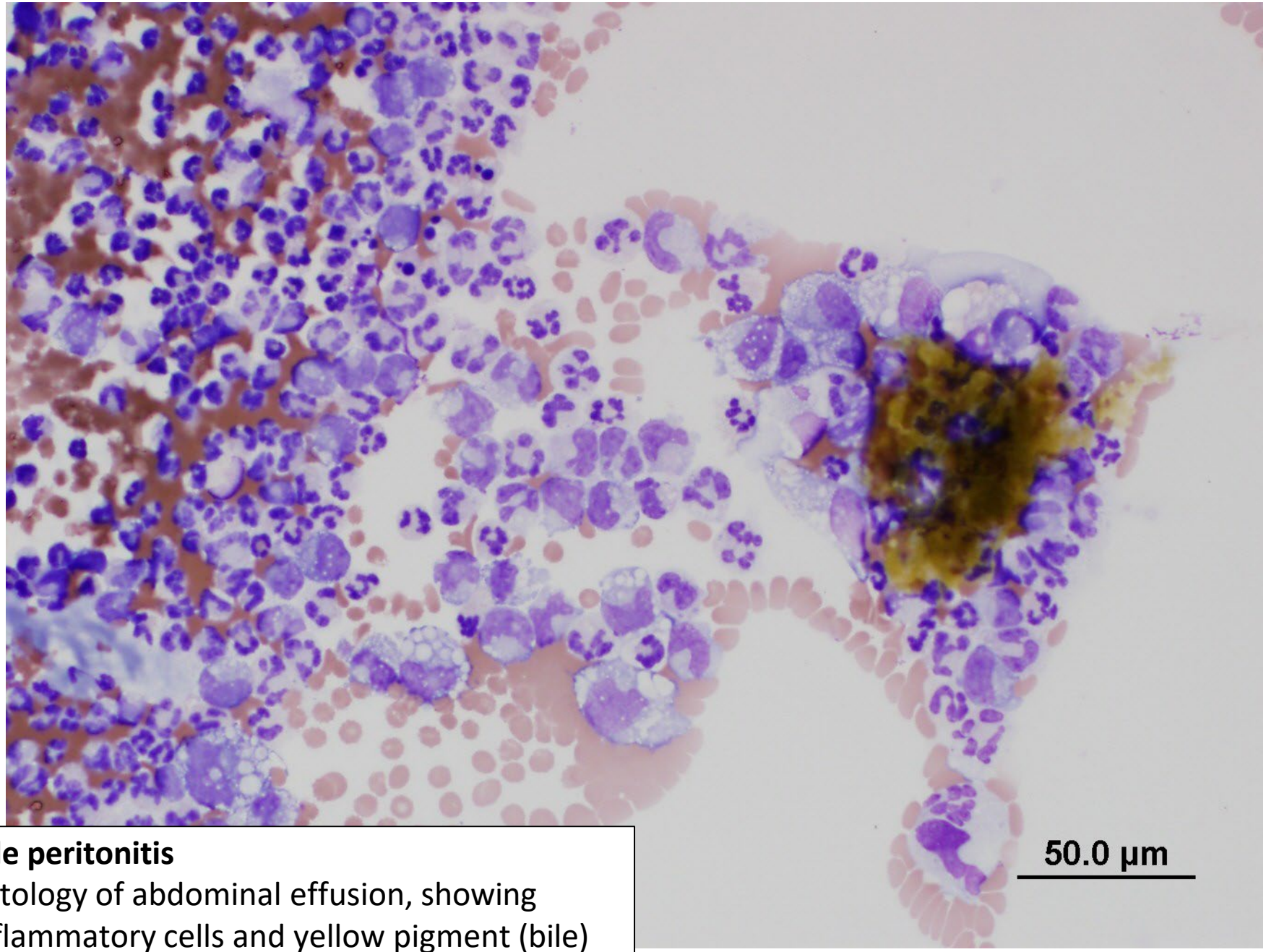
Post hepatic hyperbilirubinaemia

- Increased TBIL caused by obstruction of bile flow
  - Intrahepatic = compression of bile canaliculi
  - Extrahepatic = compression/obstruction of bile ducts
- Typically Bc > Bu (except horses)
  - hyperbilirubinaemia can be marked
  - expect increased ALP/GGT and bilirubinuria


Cholelith in a lion
Noah's Archive



Hepatic coccidiosis in a rabbit
Noah’s archive



Bile peritonitis
Cytology of abdominal effusion, showing inflammatory cells and yellow pigment (bile)

Hyperbilirubinaemia – species variation

- **Dogs**: Elevations in ALP/GGT more sensitive than elevations in bilirubin. Also have low renal threshold for bilirubin, so bilirubinuria precedes hyperbilirubinaemia.
- **Cats**: May see hyperbilirubinaemia before increase in ALP/GGT Bilirubinuria is always significant. Can see slight increase with anorexia
- **Horses**: Have increased bilirubin in health compared to other species (readily become icteric, especially with anorexia). Unconjugated bilirubin dominates, even in cholestatic conditions. Note they often have yellow serum in health (carotene).
- **Cattle**: Icterus commonly due to haemolysis. Rarely due to liver disease or cholestasis in this species.

Thank you!