THE UNIVERSITY OF MELBOURNE

Melbourne Veterinary School

METABOLISM
Laboratory Investigation of the Liver and Biliary System 2

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VETS30017 / VETS90125

Learning outcomes

- Understand the principles of the total serum bile acids assay
- Understand the principles and interpretation of the ammonia test
- List the key lab findings associated with chronic liver disease and liver failure

Lecture Outline

- Introduction
- Assessing hepatic function
- Detecting liver dysfunction
  - Routine biochemistry clues
  - Haematology clues
  - Urinalysis clues
  - Coagulation disorders
  - Special tests
    - Bile acids
    - Ammonia

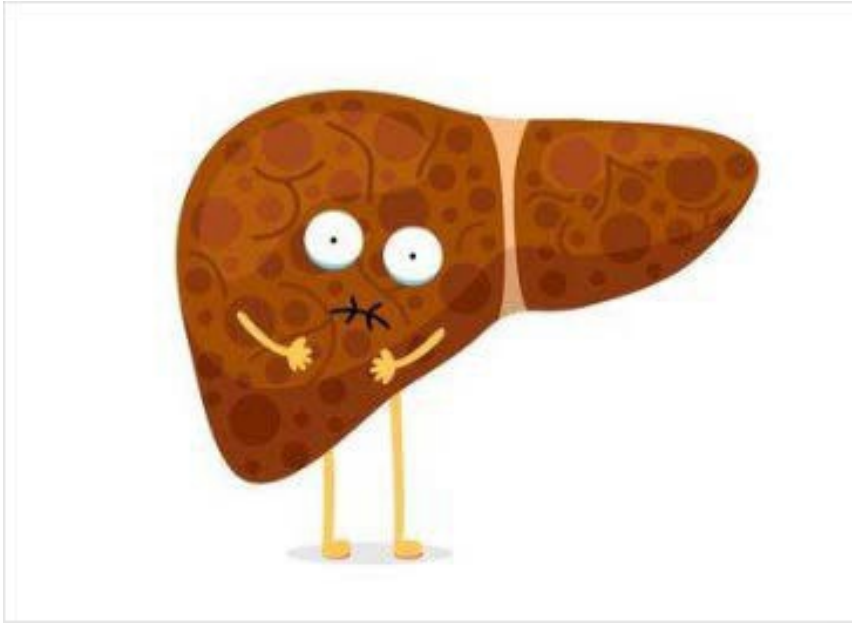
Introduction

- Liver has a marked reserve capacity and ability to regenerate
- Liver failure/insufficiency occurs when more than 70-80% of functional mass is lost:

  - liver fails to synthesise substances normally produced
  - liver fails to clear blood of substances normally eliminated or recycled by the liver

Assessing hepatic function

- Urea synthesis
- Cholesterol synthesis
- Albumin synthesis
- Glucose synthesis
- Bilirubin conjugation
- Bile acids uptake and excretion
- Ammonia uptake and metabolism



Assessing hepatic function

- Enzyme changes suggest the presence of hepatocellular damage and/or cholestasis but don't assess function
- Failure of liver function is identified when more than 70-80% of functional mass is lost :
  - liver fails to synthesise substances normally produced:
    - urea, cholesterol, albumin, glucose, coagulation factors
  - liver fails to clear blood of substances normally eliminated or recycled by the liver:
    - bilirubin, bile acids, ammonia

Assessing hepatic function

Tests that indicate hepatic insufficiency (hepatic failure) **do not** indicate

- Cause of liver dysfunction
- Prognosis for recovery

(i.e. is it reversible or irreversible)

Detecting Liver Dysfunction

Other (non-specific) factors which can help evaluate hepatic insufficiency, especially chronic liver disease:

- Serum biochemistry
- CBC and

erythrocyte morphology

- Urinalysis
- Coagulation proteins

I am sorry, your liver is stuffed

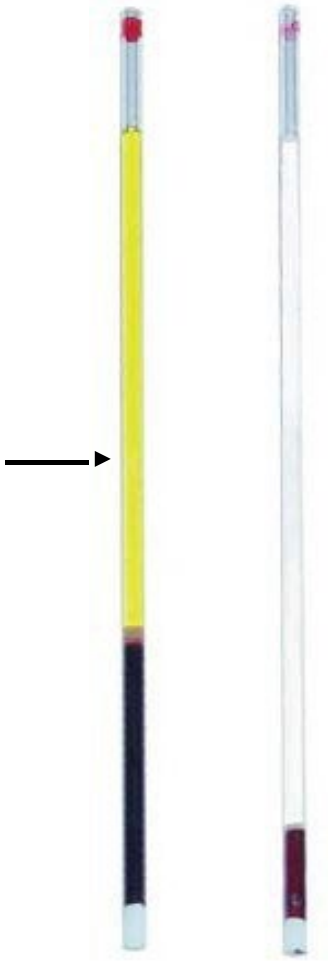
Routine biochemistry clues

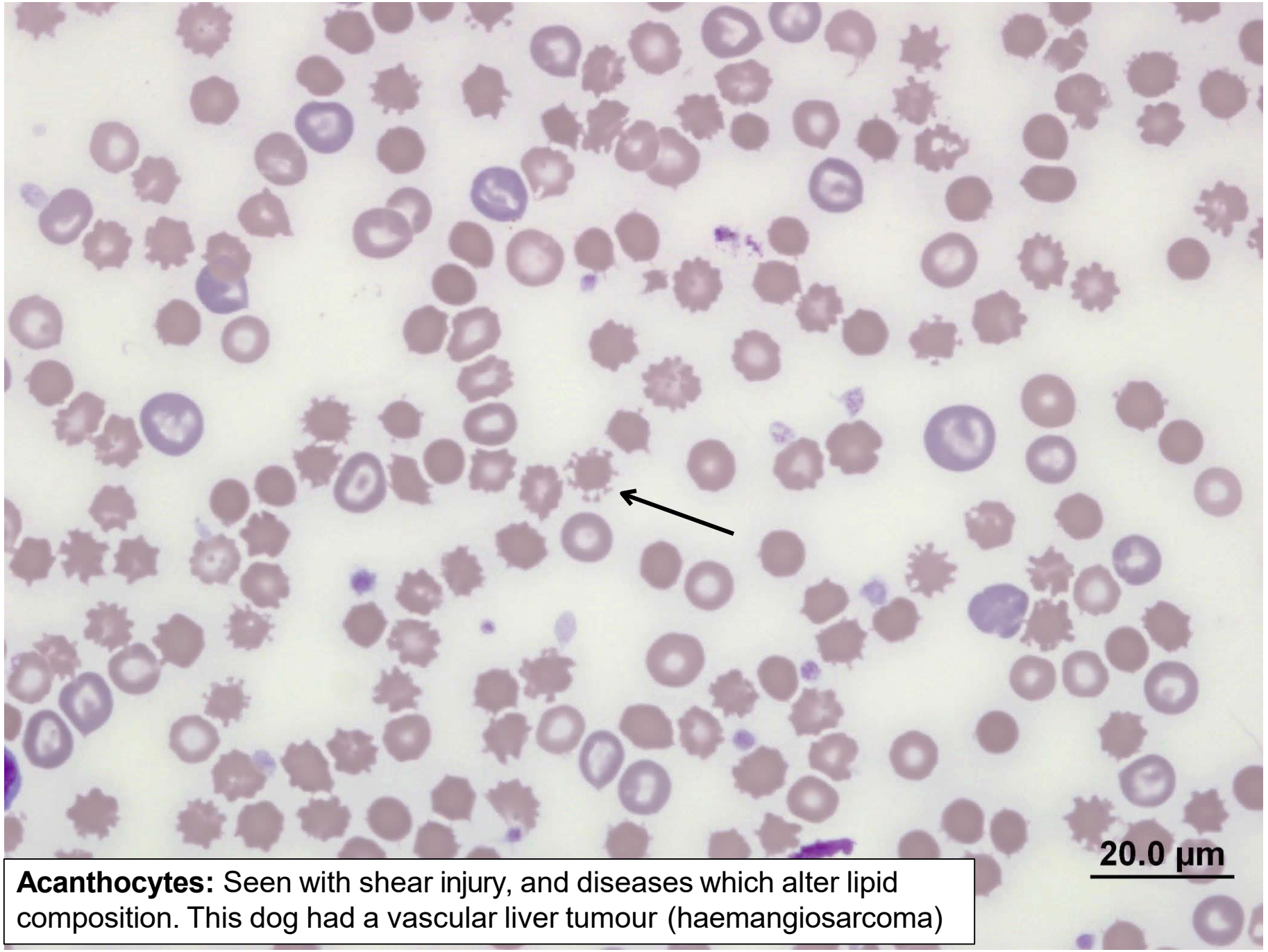
Reduced synthesis of:

- Albumin
  - but long half life (8d dogs, 19d horses)
- Urea
  - ↓ conversion from ammonia
- Cholesterol
  - but may also be normal or increased, as cholestasis can result in hypercholesterolaemia
- Glucose
  - ↓ glycogen stores and ↓ gluconeogenesis

Haematology clues

- Mild non regenerative anaemia
  - e.g. anaemia of chronic disease
- Mild microcytosis
  - may be seen with PSS
- Inflammatory leukogram
  - e.g. cholangiohepatitis, cholecystitis
- Icteric plasma
  - hyperbilirubinaemia in cats and dogs
- Hypoproteinaemia
  - e.g. reduced albumin synthesis





Acanthocytes: Seen with shear injury, and diseases which alter lipid composition. This dog had a vascular liver tumour (haemangiosarcoma)

Urinalysis clues

- Hyposthenuria (and PU/PD)
  - low urea and reduced medullary tonicity
- Bilirubinuria
  - orange urine, bilirubin crystals and positive urine dipstick indicating hyperbilirubinaemia
- Ammonium biurate crystals
  - may be seen with PSS, hepatic dysfunction, Dalmatians and Bulldogs

Coagulation Disorders

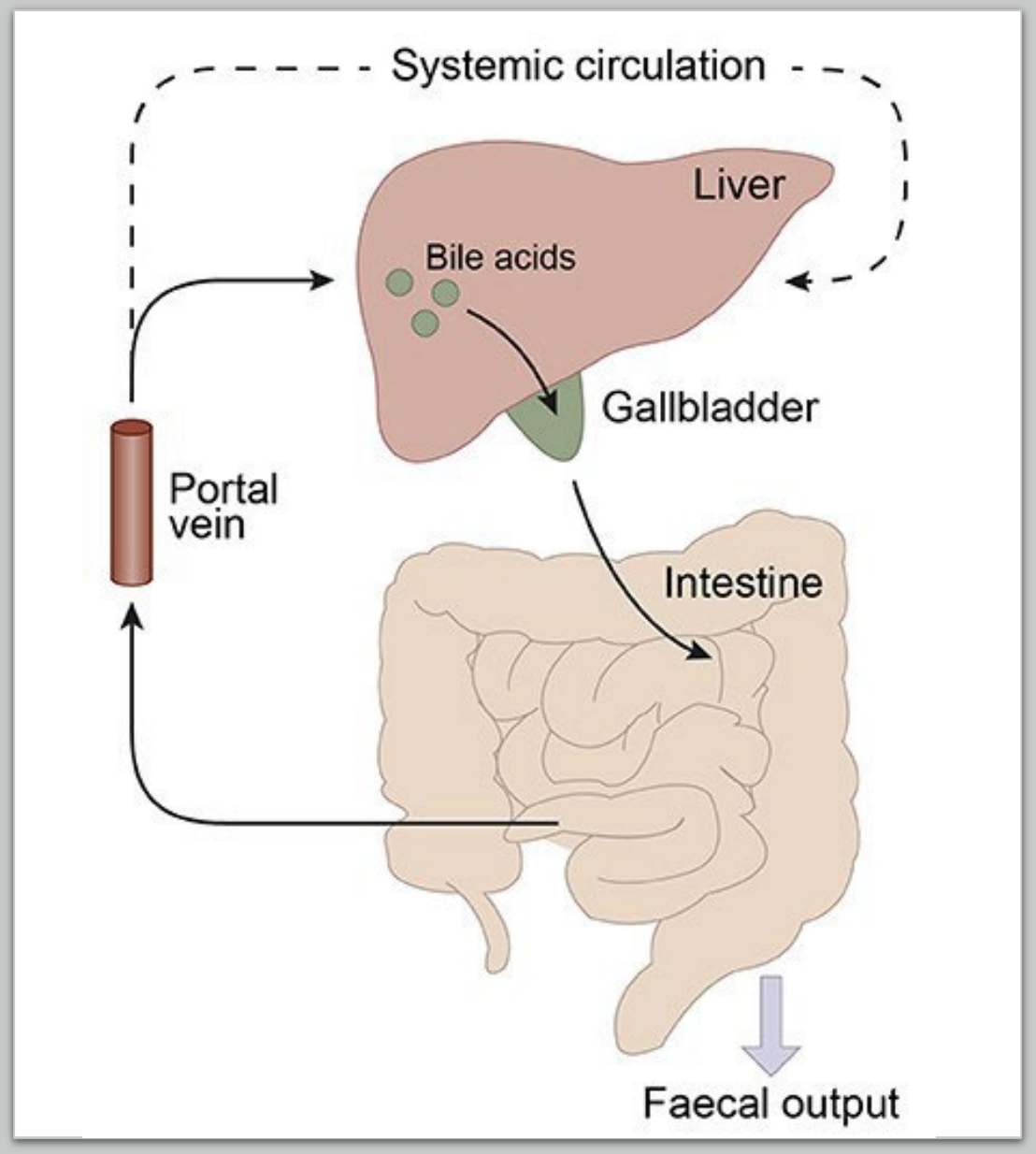
- Coagulation abnormalities are common in liver disease
  - prolonged APTT/PT times
- Causes include:
  - ↓ synthesis of coagulation factors, platelet dysfunction and DIC
  - cholestasis results in reduced absorption of fat soluble Vit K
- Coagulation screening recommended prior to liver FNA/biopsy

Bile acids

- Produced in the liver from cholesterol and stored in the gall bladder (if present)
- Undergo an efficient enterohepatic circulation
  - released into the intestine
  - re-absorbed into portal system
  - extracted by hepatocytes

Enterohepatic circulation of bile acids

- Following food intake, bile acids are released into the duodenum.
- About 95% of the bile acids are reabsorbed in the ileum and are released into the portal vein and redirected to the liver.
- Approximately 5% of the bile acids is lost through fecal output.
- Only a small portion escapes the enterohepatic circulation and reaches the systemic circulation.



Bile acid assay

Causes of increased serum bile acids:

- Decreased functional hepatic mass

(impaired extraction of sBA from the portal blood)

- Portosystemic vascular malformations (MVD, PSVA/PSS)

portal blood by-passes the liver and thus reduces extraction

(esp. post prandially)

- Cholestasis (retention and reflux of sBA back into circulation after bile stasis)

Bile acid assay

- Sensitive and specific test of hepatic function (in the absence of cholestasis)

  e.g. PSS, steroid hepatopathy, neoplasia

- Not useful to assess liver function in animals known to be cholestatic (e.g. hyperbilirubinemia)

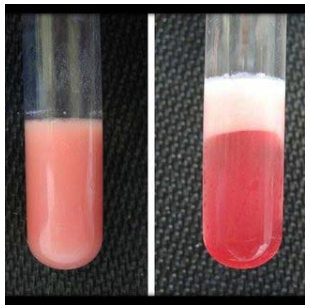
- Abnormal results do not determine aetiology, severity or prognosis

  indicates the need for diagnostic imaging, hepatic biopsy

Bile acid assay

- Single sBA used in horses, camelids
- Dynamic sBA used in dogs and cats:
  - two samples (pre and 2 hours postprandial)
  - increases sensitivity by challenging the liver capacity for BA extraction after gallbladder contraction
- Insensitive test in ruminants

Bile acids test protocol



- Fast the patient for 12 hours
- Collect the first blood sample and label the tube with patient name and “pre” or time zero sample
- Feed a small amount (2-4 tablespoons) of canned maintenance-type diet (high-fat diet is NOT necessary)
- Two hours after feeding, collect the second sample and label with patient name and “2 hr”
    - Post-prandial bile acid concentrations >31 umol/L (dogs) are suggestive of hepatobiliary disease

Microvascular dysplasia

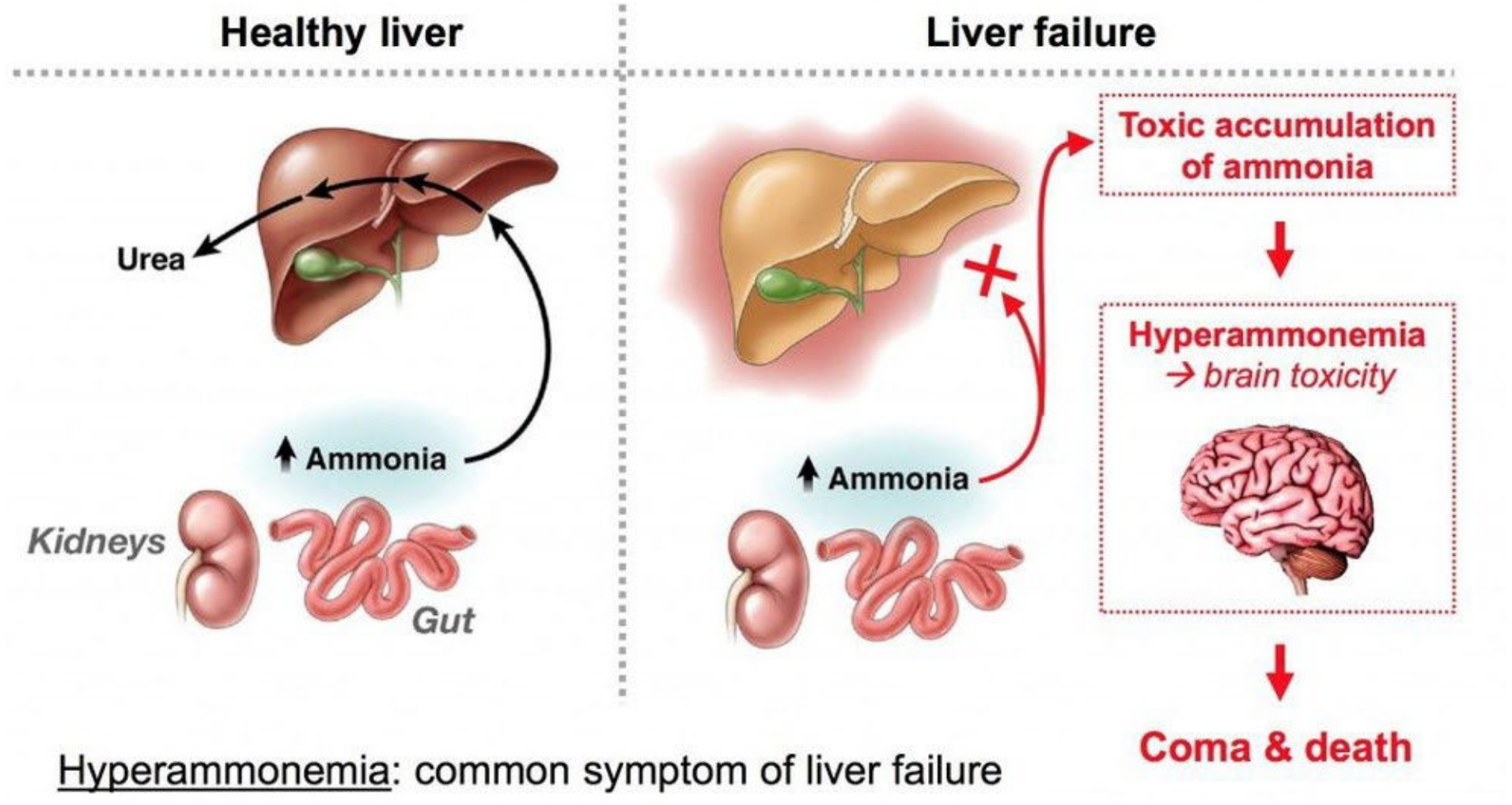
Small breed dogs (Terrier-type)

- high sBA may reflect either portosystemic shunt (PSS) or microvascular dysplasia (MVD)
  - puppies with large shunts often require surgery
  - whereas MVD is not a surgical problem
- ammonia levels may help further evaluate liver function
- **Protein C** values >70% are expected with MVD, whereas a low value would support hepatic insufficiency (e.g. PSS)

Ammonia

- Ammonia concentration can be useful in further evaluating liver function
  - produced from exogenous or endogenous amino acids
  - taken up by the liver where most is converted to urea
  - urea enters circulation and is then excreted into the urine (and GIT system)
- Measurement is problematic as it is very unstable
  - heparin anticoagulant preferred
  - separate plasma and analyse ASAP
  - must be kept chilled

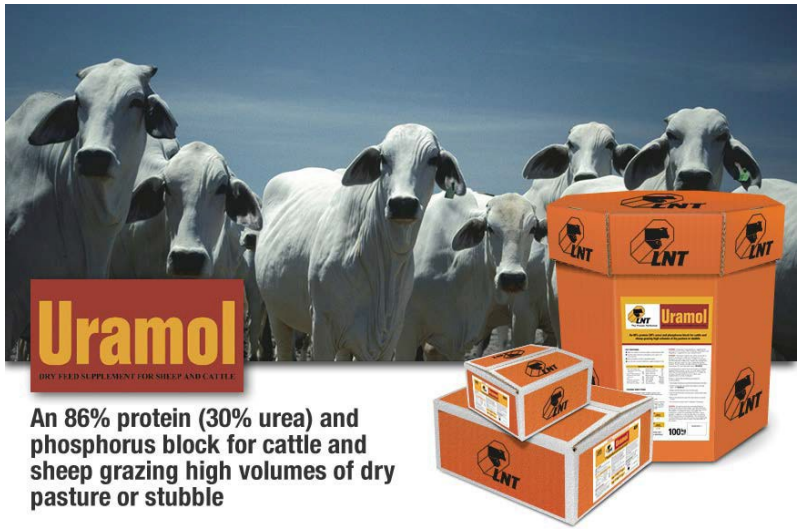
Urea cycle



https://impower.life/blog/2017/6/30/ammonia-choose-the-wrong-protein-and-you-could-lose-85-or-more-to-ammonia

Ammonia

- Elevated blood ammonia levels seen with:
  - Incorrect sample handling
  - Portosystemic shunt
  - Hepatic insufficiency (>70% hepatic function lost)
  - Overgrowth urease-producing bacteria
  - Urea toxicosis in ruminants



Reaching a Final Diagnosis

Lab tests help confirm presence of liver disease, but seldom provide an aetiological diagnosis. For this we need:

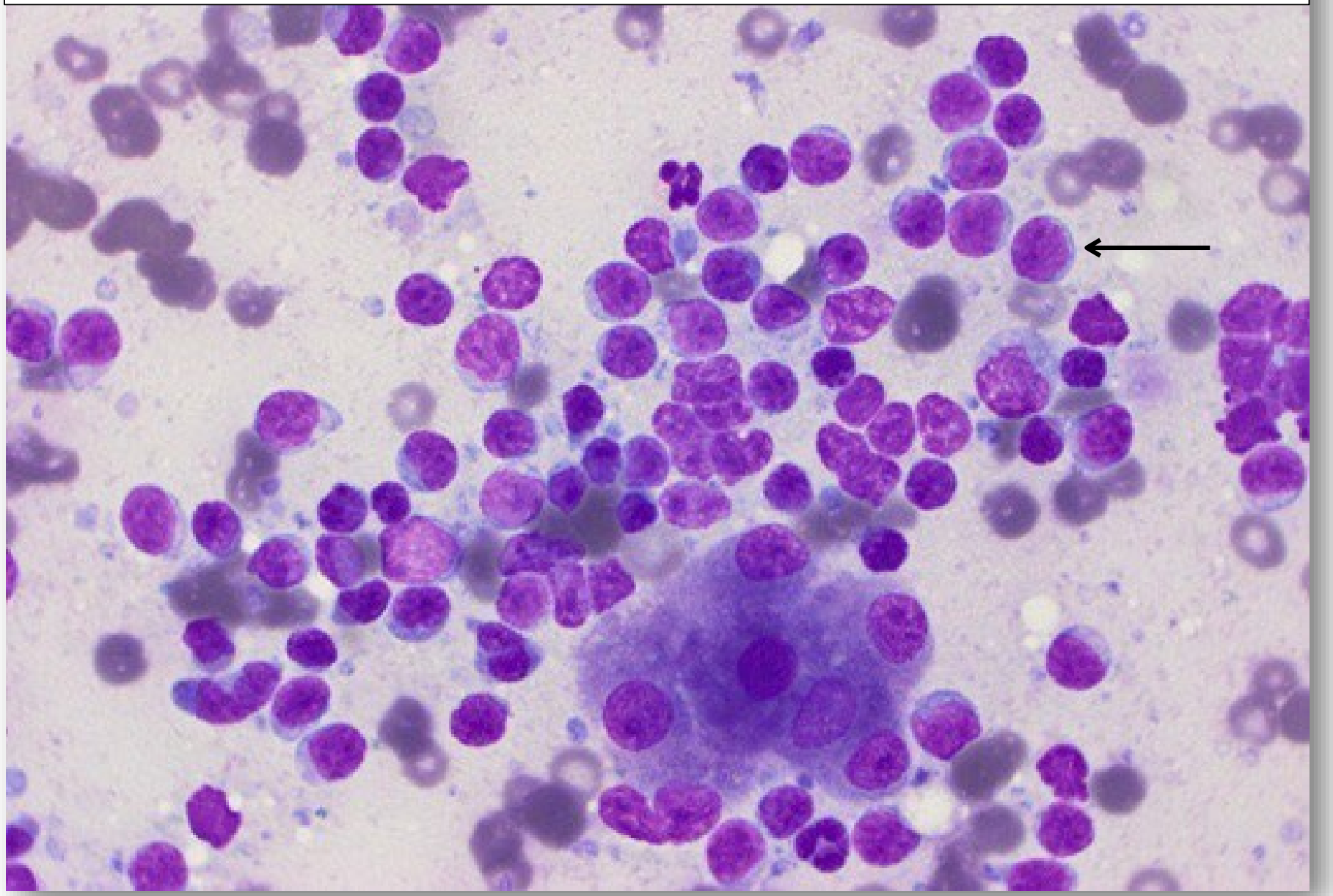
- **Ultrasound**
- **Cytology**

Useful to Dx sepsis, vacuolar change, metastatic neoplasia. Less useful for Dx hepatitis, cirrhosis, hepatocellular tumours

- **Biopsy for histopathology**

Often required for a definitive diagnosis as allows evaluation of architecture

Hepatic lymphoma: Cytology - malignant neoplasia of intermediate to large lymphocytes (arrow). With cluster of normal hepatocytes below.



Thank you!