

CHEMICAL PATHOLOGY

LIVER ENZYMES

ALANINE AMINOTRANSFERASE (ALT)



ALT is a hepatocellular cytoplasmic enzyme which is **liver specific** in dogs and cats. Significant increases in ALT ($> 2-3$ x normal) are indicative of hepatocellular injury, either sub-lethal degeneration or necrosis. Although specific for hepatocellular injury, ALT is not a sensitive indicator of liver disease and there is a poor correlation between the ALT level and hepatic function. In acute **toxic/hypoxic** injury, ALT rises within 12 hours and peaks in 1-2 days. Mild to moderate ALT induction can occur in dogs with many drugs, the most common of which are anticonvulsants (barbiturates, primidone, phenobarbitone), **glucocorticoids** and paracetamol. Large animals do not have significant amounts of ALT in their liver tissue.

ASPARTATE AMINOTRANSFERASE (AST)



AST is not as specific for hepatocellular injury as ALT. The enzyme is found in almost all cells, with high activity in liver and striated muscle. Usually greater hepatocellular injury is required to release AST than ALT. This is due to AST's mitochondrial fraction. AST has a shorter half-life than ALT, therefore, **AST levels are expected to fall before ALT after an acute hepatocellular insult**. If AST levels exceed ALT levels in an individual, at least some of the AST can be assumed to be of non-hepatocellular origin. ALT is considered to have greater liver specificity; however, **AST is regarded as being of greater sensitivity in the detection of hepatic necrosis in cats. AST is used to measure liver necrosis in large animals due to the lack of ALT - bearing in mind it is also elevated in skeletal muscle and myocardial disease.**

ALKALINE PHOSPHATASE (ALP)



ALP is membrane-bound and **found in many tissues**. Some isoenzymes have such short half-lives, they do not influence serum ALP. These include renal, placental and intestinal. Colostrum intake causes a marked rise in **ALP and GGT**, therefore they are of little use in neonates. The isoenzymes of importance are bone, hepatic and steroidal ALP. Increases occur in cholestatic disorders in dogs. **However, the magnitude of ALP increase does not correlate well with the severity of the causal lesion or with the prognosis.** Cholestatic disease in the cat is not always associated with an ALP rise due to its short half-life (6 hours); therefore ALP is not a very sensitive indicator of cholestasis in the cat, but is very specific for cholestasis. **Hence, any ALP elevation in the cat is significant.** The most common causes of ALP increase in the cat are hepatic lipidosis, cholangiohepatitis, hyperthyroidism and diabetes mellitus. In dogs, ALP is a sensitive indicator of cholestasis and elevations due to cholestasis usually precede development of hyperbilirubinaemia, although in most dogs with cholestasis the ALP rise is coincident with bilirubinuria. Hepatic ALP can rise following necrosis due to secondary intrahepatic biliary obstructions and as part of the noidular regeneration process. **ALP elevations in cholestatic disorders in cattle and sheep are less useful due to wide fluctuations in normal values.**

GAMMA-GLUTAMYL TRANSPEPTIDASE (GGT)



Most cells contain GGT but the highest concentrations are found in renal convoluted tubular epithelial cells, biliary epithelium and the canalicular surfaces of hepatocytes. **Serum GGT is chiefly of hepatobiliary origin.** GGT increases are usually attributable to cholestasis although mild increases ($<3-7$ x normal) may occur due to induction by corticosteroids. In cholestatic disease in the dog, GGT increases tend to parallel ALP increases but ALP is regarded as being slightly more sensitive than GGT but is far less specific. However GGT levels do not increase as markedly as those of ALP in hepatic necrosis or bone disorders. **Therefore the combined use of ALP and GGT affords a higher predictive value of hepatobiliary disease and permits distinction of bone ALP isoenzyme increases.** In the cat, GGT is slightly more sensitive than ALP for detection of cholestatic disease. GGT increases may be greater than ALP increases in feline extrahepatic bile duct obstructions, severe cholangiohepatitis and cirrhosis. **Disproportionately low GGT with high ALP can be a useful pointer to hepatic lipidosis. GGT's activity is relatively high in the livers of cattle, horses, sheep, pigs and goats. It is more useful than ALP in these species for detecting cholestatic disorders.**

GLUTAMATE DEHYDROGENASE (GLDH)



A cytosolic enzyme that is fairly liver specific and reasonably sensitive for the diagnosis of acute, but not chronic hepatopathy in most species. It is more stable than SDH, but still less stable than some of the other cytosolic enzymes. **GLDH has been regarded by many as the enzyme of choice as an indicator of the degree of hepatic necrosis in sheep, cattle and goats.** Some authors believe it can be used for this purpose in small animals too but is not superior than ALT. **GLDH can be used as an indicator of liver disease in birds.**

SORBITOL DEHYDROGENASE (SDH)



SDH is a cytosolic enzyme released during hepatic degeneration or necrosis or secondary to altered membrane permeability. The concentration of SDH is greater in the liver than in all other tissues. Although it may be a useful test for recognition of hepatocellular injury in small animals, it offers no advantage over determination of serum ALT activity. SDH is a sensitive liver specific enzyme used to detect hepatocellular damage in large animals including horses, cows, llamas, alpacas, sheep, and goats. SDH activity will increase in < 12 hours and serum activity can return to normal shortly after a single insult (3-5 days). This enzyme is labile *in vitro* and serum must be analyzed within 8-12 hours. Serum stability is best in ruminants, less in camelids and worst in horses. Guidelines for use of this enzyme:

- assay within 5 hours if kept at room temperature
- stable for 48 hours if frozen (72 hours for cattle)
- activity decreases 25% if frozen for one week (horses and cattle)

ARGINASE



Arginase is considered to be liver-specific because it exists in higher concentrations in hepatocytes than in any other tissue. It is a major catalyst in the urea cycle, with large quantities located in mitochondria. With severe hepatic insults, damaged mitochondrial membranes acutely release preformed arginase causing serum elevations. It has been shown to be liver-specific in several species. A simplified method for arginine analysis has made the test feasible for clinical practice, but due to its ability to only detect severe acute hepatic insults, coupled with its short half-life in serum, it has never gained the popularity needed to spread its availability.

LIVER FUNCTION TESTS

BILIRUBIN



Unconjugated (fat-soluble) bilirubin derived from the monocyte-macrophage system is the predominate form circulating in blood bound to albumin. It is conjugated in the hepatocytes to glucuronide to be made water soluble. Secretion of conjugated bilirubin into the canaliculi is the rate-limiting step in bilirubin metabolism.

Bilirubinuria and Hyperbilirubinaemia

Bilirubinuria is anticipated whenever there is a rise in serum conjugated bilirubin (C-BIL). **Bilirubinuria is a very sensitive indicator of conjugated hyperbilirubinaemia (and hence cholestasis) and, especially in the dog which has a very low renal threshold for bilirubin, bilirubinuria commonly precedes hyperbilirubinaemia.** The renal threshold for bilirubinuria is approximately 9x higher in the cat than in the dog and is therefore usually preceded by hyperbilirubinaemia in this species. In health renal tubular epithelial cells in dogs (especially males) can directly take up haemoglobin from the blood, metabolise and conjugate it to C-BIL which will then appear in the urine. This conversion explains why 1-2+ bilirubinuria in an at least moderately-concentrated urine sample from a male dog can be a normal finding. Bilirubinuria in a cat is always of significance and indicative of cholestasis. Detectable icterus of serum/plasma usually occurs when bilirubin exceeds 10-15 $\mu\text{mol/L}$. While yellow discolouration of mucous membranes only occurs when serum bilirubin exceeds 35 $\mu\text{mol/L}$.

Icterus can be classified as prehepatic, intrahepatic or post-hepatic. Prehepatic icterus is most commonly associated with a sudden large influx (haemolysis) of W1conjugated bilirubin (U-BIL) to the liver resulting in a back flow of U-BIL. Intrahepatic icterus due to hepatocellular injury or reduced blood flow to the liver results in inability to take up V-BIL; inability to conjugate U-BIL or inability to excrete C-BIL. Consequently either U-BIL or C-BIL or both would increase. Post-hepatic icterus usually results from a blockage in the biliary tree and consequently a build-up of C-BIL.

Bilirubin assay is based on the diazo reaction. The direct reaction to estimate C-BIL is based on colour development after addition of the reagent; further colour development after addition of alcohol permits measurement of total bilirubin (T-BIL). U-BIL is then calculated as the difference between these two values.

In summary: early haemolytic hyperbilirubinaemias may have < 50% C-BIL; post-hepatic hyperbilirubinaemias may have > 60% C-BIL; hepatocellular hyperbilirubinaemias will have both U-BIL and C-BIL elevated, usually with C-BIL the major component

AMMONIA AND AMMONIA TOLERANCE TEST



NB- The laboratory must be consulted prior to performing this test as the sample requires rapid processing.

Protocol The animals should be fasted 8 hours prior to an EDTA/heparin sample being collected into ice-chilled tubes. Strict anaerobic conditions must be adhered to while collecting and separating the samples. Plasma must be separated immediately and tubes placed on crushed ice. The assay must be run within 30 minutes. If the baseline ammonia level is not abnormally raised and there are no signs of hepatoencephalopathy the ATT should be run. NH_4Cl is administered orally at 100 mg/kg(max. 3g) as a 10% solution in warm water. An EDTA / heparin sample is collected 30 minutes later for ammonia assay.

Interpretation

	Cat	Dog
Normal baseline [NH]	17 - 58 $\mu\text{mol/L}$	26 - 70 $\mu\text{mol/L}$
Post-NH_4Cl	Not greater than double resting value	Not greater than double resting value

TOTAL BILE ACIDS (TBA)



As the TBA assay is easily performed and results are little affected by extrahepatic factors, it has largely replaced dye excretion and clearance tests. Fasting TBA assays yield comparable information to the BSP retention test but are more specific for liver disease. The post-prandial TBA assay yields similar information to the ammonia tolerance test but is far easier to perform and is far less dangerous for patients with hepatic encephalopathy. It is now generally concluded that measurement of TBA adds sensitivity to any liver profile.

Protocol Total serum bile acids may be measured after a 12 hour fast, 2 hours post-prandially or both. The meal provided should contain lipid to provoke a strong cholecystokinin stimulus for gall bladder contraction.

Interpretation

Normal Values

	Dog	Cat
12 hour fast	$< 8 - 15 \mu\text{mol/L}$	$< 5 \mu\text{mol/L}$
2 hour post-prandial	$< 28 - 30 \mu\text{mol/L}$	$< 15 \mu\text{mol/L}$

TBA increases reflect hepatocellular disease, cholestatic disease or portosystemic shunting (either intra- or extrahepatic). Fasting TBA levels have been reported to be of high diagnostic value in portosystemic shunts in dogs

Increased TBA in hepatic disease may reflect:

1. Reduced functional mass with decreased uptake of TBA from portal blood.
2. Cholestasis with regurgitation of TBA back into blood.

However:

- Not all patients with liver disease have elevated TBA.
- The magnitude of increase gives no indication of the reversibility of the change and hence prognosis.
- Some GIT lesions may slow the delivery of bile acids to the ileum and therefore lower the post-prandial level.
- Ileal disease leading to malabsorption will lower the reabsorption of enteric bile acids; while bacterial overgrowth will increase conjugated bile acid levels and thus falsely increase reabsorbed bile acid levels.
- In patients with hyperbilirubinaemia the bile acids will be raised due to cholestasis and the competition between bile acids and bilirubin for the same excretory pathway.

LIVER ENZYMES – FURTHER READING

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