Blood chemistry for the diagnosis of hepatobiliary disease in birds. 
A review.

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SUMMARY  
A review is presented of current knowledge with regard to blood chemistry for the diagnosis of hepatobiliary disease in birds. Straightforward conclusions are difficult, because research on this subject has been limited. 
Jaundice caused by hyperbilirubinaemia occurs infrequently in birds, because the main bile pigment is biliverdin and not bilirubin. A yellow discolouration of avian plasma is often caused by the presence of carotenoids. The diagnostic value of plasma ALAT concentrations is controversial. Although ASAT and LD are not specific for the liver they appear to be sensitive enzymes to detect liver cell damage. 
GLDH is liver specific in a number of animal species (including birds) and might prove to be a useful enzyme for diagnostic purposes. AP and GGT seem less useful. Little information is available on the diagnostic value of plasma bile acids and plasma dye clearance tests.

INTRODUCTION
In canine and feline medicine, jaundice in the absence of anaemia indicates hepatobiliary disease. Furthermore, the enzyme alanine aminotransferase (ALAT) is highly liver specific in these species. The activity of this enzyme in serum or plasma is elevated in 85 per cent of cases of hepatobiliary disease. The combined determination of the activity of heat-labile alkaline phosphatase (AP) and ALAT even permits identification of 98 per cent of cases of hepatic disease (29). 
In pet bird practice identification of hepatobiliary disease is less straightforward. Although the liver is involved in many diseases of pet birds, the correlation of serum parameters with liver pathology remains controversial (23). 
This paper reviews current clinicopathological knowledge relating to the diagnosis of avian hepatobiliary disease.

BILE PIGMENTS
In chickens (Gallus gallus domesticus) the major bile pigment is biliverdin and not bilirubin, because of the absence of the enzyme biliverdin reductase in liver and other tissues, which converts biliverdin to bilirubin (21,22,36). It has been suggested that in birds biliverdin may be converted to bilirubin by bacteria or non-specific reducing enzymes (20). When in chickens both bile ducts are ligated the concentration of plasma bile pigments rises immediately but stabilises after 2 weeks at about 85 μmol/l, which is a much lower level than in mammals with total biliary obstruction (21). 
In sera of healthy ducks low levels of bilirubin may be detected (15,32) and significantly elevated levels have been reported after experimental duck virus hepatitis infection (1). However, the observed levels of 17 ± 3.6 μmol/l (mean ± sd)
were well below the serum concentration of 34-51 μmol/l which has been mentioned as the level above which jaundice becomes apparent in man (19). These observations might explain the infrequent occurrence of jaundice in birds (23). Frequently a yellow colouration of plasma caused by carotenoids can be seen in healthy birds. This is often misinterpreted as being icteric plasma. It has been suggested that in psittacine species liver disease is indicated by biliverdinuria (23). In this condition fecal urates are coloured an intense yellow-green instead of white (14, 35).

ALANINE AMINOTRANSFERASE (ALAT, ALT, SALT, EC.2.6.1.1)

While ALAT (formerly glutamic pyruvic transaminase, GPT) is highly liver specific in dogs, this holds not true for the chicken (Fig. 1).

![Tissue distribution of ALT](image)

**Fig. 1.** Tissue distribution of alanine aminotransferase in the dog and in domestic fowl. H = heart; L = liver; S = skeletal muscle; K = kidney; B = brain. (Drawn from data presented by Trautschold and Werle, 1966).

Furthermore in the chicken the activity of ALAT in plasma or serum is reported to be low or even absent (7, 12, 17, 24, 37). It has been demonstrated that activity of ALAT in chicken erythrocytes is 1.6 times as high as the activity in serum. Therefore hemolysed blood is not suitable for ALAT measurements (37). Experimentally induced poisoning with carbon tetrachloride (CCl4) induced a rise in serum ALAT concentrations in chickens, which was attributed to liver cell damage (17). However, elevated ALAT concentrations might have been caused by damage to other organs. CCl4 is known to be nephrotoxic as well (10) and in chickens highest ALAT activity occurs in this organ (Fig. 1). It should be noted that birds are reported to be largely resistant to the hepatotoxic action of CCl4, because CCl4 is not metabolised in the avian body, and therefore no formation of toxic free radicals occurs (13b).

In experimentally induced virus hepatitis in ducks Ahmed et al. (1) and Bokori and Karsai (7) attributed elevated ALAT concentrations to liver cell damage. However,

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1 EC = Enzyme number from the Enzyme Commission of the International Union of Biochemistry.
it is known that duck virus hepatitis can cause degeneration of the kidneys as well
and in ducks (Anas platyrhynchos) highest ALAT activities have been reported to
occur in these organs (30).

In geese the ALAT activities in liver, heart, skeletal muscle and lung have been
reported to be low (5). Bokori and Karsai (7) reported a considerable rise in plasma
ALAT and aspartate aminotransferase (ASAT) activity in geese experimentally
poisoned with CCl₄ and phosphorus. They concluded that the rise of plasma
ALAT, ASAT, and aldolase (ALD) activities is a reliable indicator for the
severity of liver lesions in waterfowl. However, the animals with the highest enzyme
activities in plasma also had myocardial and renal degeneration, which might have
attributed to these elevations of plasma enzyme activities.

In turkeys the ALAT activity is low in the liver and heart, and the highest activity
occurs in skeletal muscle (6). In this species the effect of experimental infection with
Histomonas meleagridis on plasma ALAT, ASAT and LD concentrations has been
studied (3). Although it was concluded that there is a decrease in plasma concentra-
tions of all three enzymes associated with the acute caecal lesions of histomoniasis
from the 7th day onwards, and an increase associated with severe liver lesions from
about the 11th day onwards, the numerical data presented do not support this
conclusion. While there is a decrease in ASAT and LD concentrations during the
phase of caecal lesions, there is an increase in ALAT concentrations, suggesting
that elevated plasma ALAT concentrations might have been caused by caecal
rather than liver damage. No information is available, however, on ALAT concen-
trations in caecal tissue, to support this explanation.

McDougald and Hansen (25), studying the same disease in chickens and turkeys,
found no significant changes in plasma ALAT activity attributable to liver damage,
even when other enzymes were greatly elevated.

Campbell (9), who studied activities of various plasma enzymes after inducing liver
damage with aflatoxin B₁ reported elevated ALAT activities in 75 per cent of
pigeons (Columba livia), 100 per cent of red tailed hawks (Buteo jamaicensis) and
great horned owls (Bubo virginianus) and 85 per cent of cockatiels (Nymphicus
hollandicus). No mention was made of the integrity of other organs, however.

In conclusion, the literature on the applicability of plasma ALAT concentrations
for the diagnosis of liver disease in birds is controversial. Interspecies variations
and concomitant injury to other organs in experimentally-induced liver disease are
possible explanations for this controversy. Because in a number of birds ALAT
activity is highest in the kidney, increased urinary ALAT levels might be diagnostic
for renal disease in these species.

ASPARTATE AMINOTRANSFERASE (ASAT, AST, SAST, EC 2.6.1.1.)

Aspartate aminotransferase (formerly glutamic oxaloacetic transaminase, GOT) is
widely distributed in avian tissues, especially in heart, liver, skeletal muscle, kidney
and brain. There is considerable species variation regarding the relative distribu-
tion among various tissues, which makes interpretation of elevated plasma ASAT
activities difficult (5, 6, 11, 12; Fig. 2).

Significant elevations of plasma ASAT activities have been reported in chickens
with inherited muscular dystrophy (13). In turkeys with liver cell damage due to
histomoniasis elevated plasma ASAT activities have been demonstrated (2, 3, 16,
25). Elevated plasma ASAT activities have been reported in geese (7) after
experimental poisoning with the hepatotoxic agents such as CCl₄ and phosphorus,
as described above. Two- to four-old increases of plasma ASAT activities have been
associated with 'soft tissue' injury in 'birds of prey'. (18). Elevated serum ASAT
activities were observed by Campbell (9) in 75 per cent of pigeons, 100 per cent of

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red-tailed hawks and 40 per cent of great horned owls and cockatiels due to liver cell damage as a result of experimentally induced aflatoxin B, poisoning.

Although the foregoing data indicate that ASAT is not liver specific, in our experience it is the single highly useful plasma enzyme (when compared to ALAT, AP and 7-GT) for detecting liver disease in Amazon parrots (Amazona spp), African grey parrots (Psittacus erithacus), and budgerigars (Melopsittacus undulatus). Currently we perform a liver biopsy for histological examination in these species when plasma ASAT concentrations are elevated, unless the bird was recently injected intramuscularly.

LACTATE DEHYDROGENASE (LD, LDH, EC 1.1.1.27.)

LD occurs in most avian tissues (11). Electrophoresis of LD isoenzymes in pigeon tissues suggests that the major isoenzymes in the heart are LD 1 and 2, while the liver is rich in LD 2, 3 and 4 but has relatively little LD 1 or 5 (4).

Galvin (14) stated that in the various tissues of ‘psittacine birds’ which he tested, he could only detect one LD type, which was similar to the human LD isoenzyme. Furthermore, he found that LD was negligible in psittacine red cells and hence hemolysis would not elevate plasma LD as it can in mammals. Although soft tissue damage can lead to elevation of plasma LD concentrations, in the psittacine species examined by Galvin, elevated LD concentrations reportedly almost always indicated liver disease.

Elevated LD concentrations associated with liver damage have been reported in turkeys after experimental infections with Histomonas meleagridis (3,25).

Campbell (8) reported elevated serum LD concentrations in 33 per cent of pigeons, 92 per cent of cockatiels and 100 per cent of red-tailed hawks and great horned owls, after experimental poisoning with the hepatoxic agent aflatoxin B,.

GLUTAMATE DEHYDROGENASE (GLDH, GDH,EC 1.4.1.3.)

GLDH is virtually liver specific in man and most of the domestic animals (31). In the cockerel, duck and turkey significant amounts are found only in the liver, kidney and brain (11; Fig. 3).
It is remarkable that this enzyme has not been used by veterinarians for the diagnoses of liver disease in birds, for it appears to be the most liver specific of all enzymes tested.

ALKALINE PHOSPHATASE (AP, EC 3.1.3.1.)

AP is a good example to illustrate the complexity of the use of plasma enzyme concentrations and isoenzymes for the diagnosis of disease in animals. Classically elevations of AP in mammals are observed with skeletal disorders or hepatobiliary obstruction (31).

The increase in AP activities in plasma or serum is not a simple reflection of tissue activities. In hepatobiliary obstruction the increased serum AP activity is due to overproduction of AP in liver cells, not to reduced biliary excretion. In the dog, AP activity in the intestine is about 700 times greater than that in the liver, but AP from the intestine does not contribute significantly to elevated serum AP activities. This is because the half-life of intestinal AP is much shorter than that of hepatic AP (7b).

In chickens little AP activity has been demonstrated in the liver and none has been found in lung, skeletal muscle or heart (5). In turkeys highest AP activity was in the testes, followed by the liver (6). In ducks high AP activity has been demonstrated in the duodenum and kidney (30).

Elevations of AP in avian species have been predominantly associated with increased osteoblastic activity (20, 33, 34), e.g., skeletal growth, nutritional secondary hyperparathyroidism, rickets, fracture repair and osteomyelitis, as well as impending ovulation.

Reports of elevated plasma AP concentrations associated with liver disease in avian species are rare. Ahmed et al. (1) found significantly elevated serum AP concentrations after inducing duck viral hepatitis infections in White Peking ducklings. Campbell (9), studying the effects of aflatoxin B1 on plasma enzyme activities, concluded that determination of AP activity in serum is not a sensitive test for detecting liver disease in pigeons, cockatiels, red-tailed hawks and great horned owls.
GAMMA GLUTAMYL TRANSFERASE (GGT, r GT, EC 2.3.2.2.)

Very little is known about the significance of plasma r GT concentrations for the diagnosis of hepatobiliary disease in birds. Campbell (9) concluded that determination of r GT is not a sensitive test for the detection of liver disease in the pigeon, cockatiel, red-tailed hawk and great horned owl. Galvin (14), however, found this enzyme to be elevated in some avian patients with liver disease and among the avian tissues he tested, the liver had the greatest concentration. The information from Galvin, however, is too casual to permit conclusions. Lewandowski et al. (20) created some confusion by discussing ‘gamma glutamyltranspeptidase (GGPT)’ and ‘gamma glutamyltransferase (GGT)’ as two different enzymes. These two names are synonymous, the former being obsolete.

BILE ACIDS

The determination of the concentration of bile acids in plasma or serum provides a very sensitive test for liver disease in man (19). Many patients with normal results of conventional tests of liver function exhibit elevated bile acid levels in the peripheral blood. So far there seem to be no reports on the use of plasma bile acid concentrations for the detection of liver disease in birds.

PLASMA DYE CLEARANCE TESTS (BSP AND ICG)

The hepatic uptake and excretion of a number of organic dyes after intravenous administration have been used in a variety of animal species as an index of hepatic function. Of these dyes bromsulphthalein (BSP) and indocyanine green (ICG) have been used experimentally in birds (11,28). Both are excreted primarily via the bile. In addition to normal hepatic biochemical integrity, the excretion of these dyes requires a normal hepatic blood flow. Measurement of BSP is the simpler and more economic of the two tests. In chickens BSP clearance is markedly influenced by age and sex (8,26,27). BSP should be injected with care, because perivascular leakage outside the vein causes severe pain and tissue necrosis. One advantage of ICG for diagnostic purposes is that it is non-irritating if accidently injected perivascularly. Preliminary results of the plasma half-life and clearance rate of ICG in three raptor species have been reported (28).

The clinical usefulness of plasma dye clearance tests in birds awaits further investigations.

REFERENCES


