Development of a Biliverdin Detection Assay in Birds

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Birds commonly are presented for clinical illness related to liver disease. Histologic lesions frequently are observed in liver specimens from sick birds. These histologic specimens include hepatic biopsies from live birds as well as liver tissue obtained from dead birds at necropsy.

Biochemical tests, such as serum enzyme activities and bile acid concentration, have been used for the antemortem diagnosis of liver disease in birds, but these tests have clinical limitations. The serum enzymes of interest in avian liver disease are broadly classified as "leakage" enzymes or "induction" enzymes. Leakage enzymes are located in the cytosol and leak from the hepatocyte into the blood following cellular injury. Examples of these enzymes include glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), and aspartate aminotransferase (AST). Induction enzymes exhibit increased serum enzymatic activity with cholestasis or following the administration of certain drugs. Examples of these enzymes include gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP). Although GLDH and SDH are relatively liver-specific, AST activity may originate from liver, muscle, and hemolyzed erythrocytes. Increased serum activity of ALP may be due to liver or bone disease. Serum bile acids are recycled via enterohepatic recirculation. They evaluate the ability of hepatocytes to extract bile acids from portal blood and recycle them for dietary lipid digestion. Bile acid concentrations may be elevated with portosystemic shunts, cholestasis, or loss of hepatic function from widespread hepatic necrosis, fibrosis, or end stage liver disease (cirrhosis). Hepatic disease or dysfunction must be severe to increase serum bile acid concentrations.

In mammals, bilirubin quantitation is used to assess increased erythrocyte destruction and cholestasis. Bilirubin is a waste product of hemoglobin degradation and is produced via monocyte-macrophage destruction of erythrocytes. Senescent or damaged erythrocytes are phagocytosed by macrophages. Hemoglobin from erythrocytes is catabolized to amino acids, iron, and bilirubin. Bilirubin is formed in a stepwise reaction where the heme moiety is enzymatically converted to biliverdin by heme oxygenase. Biliverdin subsequently is reduced to bilirubin by biliverdin reductase. The bilirubin that is produced by macrophages is then secreted in the bloodstream, bound to albumin, and transported to the liver. The albumin-

bound form of bilirubin is unconjugated and hydrophobic. Following hepatocellular uptake of bilirubin, it is conjugated to glucuronic acid and excreted into the bile. The conjugated form of bilirubin is water soluble. If cholestasis is present, conjugated bilirubin may be regurgitated into the blood. Because of its water solubility, conjugated bilirubin is rapidly filtered from the plasma by the glomerulus and excreted in the urine. Hyperbilirubinemia is often observed in liver and hemolytic disease. Therefore, total, conjugated, and unconjugated serum bilirubin concentrations and the presence or absence of bilirubinuria are used routinely in mammals as screening tests for hepatic function or extrahepatic disease. Liver diseases associated with hepatocellular swelling, hepatic fibrosis, hepatic inflammation or extrahepatic diseases resulting in red cell hemolysis, bile duct inflammation or obstruction may cause hyperbilirubinemia and bilirubinuria. The measurement of conjugated bilirubin in urine is of value in mammals because bilirubinuria often precedes the onset of hyperbilirubinemia. Measuring the serum concentrations of conjugated and unconjugated bilirubin can aid in differentiating extrahepatic from intrahepatic disease in mammals.

Birds lack biliverdin reductase; therefore, they do not produce bilirubin. In birds, biliverdin (not bilirubin) is the waste product of hemoglobin destruction. Since bilirubin is not usually present in avian serum and urine, its measurement is not useful in evaluating liver disease or hemolytic disease in birds. Experimentally, mild hyperbilirubinemia has been reported in chickens with ligated bile ducts and in ducks experimentally infected with duck hepatitis virus. The conversion of biliverdin to bilirubin was attributed to nonspecific enzymatic activity or conversion of biliverdin to bilirubin by enteric bacterial enzymes with subsequent absorption of bilirubin into the enterohepatic circulation.

The majority of biliverdin is excreted in an unconjugated form into the bile. Blood biliverdin concentration increases when hepatic “regurgitation” due to decreased hepatic function or biliary obstruction occurs, resulting in the typical green discoloration of plasma. Quantitation of avian serum biliverdin concentration may aid in the diagnosis and classification of extra- and intrahepatic diseases of birds. We have developed a biochemical assay for quantitating biliverdin in avian serum and biological fluids. This test may prove useful as an adjunct to serum enzyme activities and bile acid concentrations in evaluating intra- and extrahepatic diseases in birds.

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