Cooking with Blood Chemistry: Understanding What your Machines are Telling You

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One of the hardest concepts to understand is blood chemistry testing. We all learn the tests' names, but not necessarily what they mean and specifically what they indicate. I believe that it is important to understand both concepts, not only how to accurately run the tests, but what the tests themselves indicate.

Various chemical substances are found in the blood and can be quantitatively analyzed. Results from these tests can provide important information and diagnostic clues to the veterinary health care team in the management of health and disease.

With the expanding technological changes in in-house automated equipment, it is now possible to process large numbers of samples and perform several tests on a single specimen, in a relatively short period of time. These in-house chemistry analyzers are easy to operate and usually require little maintenance, other than routine cleaning and quality control calibration checks.

Important factors related to chemistry analysis

Testing is performed to measure substances in the blood, which can indicate normal or abnormal functioning of various organs. These substances are found in the serum, plasma, urine and other body fluids. It is important to follow the instructions of the specific manufacturers or the various instruments, as to the type of sample which can be used on each analyzer. Some tests use serum, others use plasma usually collected in a lithium heparin (green top) tube or anticoagulated whole blood collected in lithium heparin tube. The sample requirements can be found in the instruction manual for each machine used. EDTA samples are not usable by most machines because it causes binding of calcium and may affect other metabolites. Sodium heparin is not recommended as it adds sodium to the patient sample and can affect electrolyte readings.

Chemistry Testing

Blood urea nitrogen (BUN) or Serum urea nitrogen–Kidney

This is probably the most frequently performed test in a veterinary clinic. It determines the ability of the renal filtration system to remove this waste product of protein metabolism from the blood stream.

This test is used to evaluate renal function. Urea, which is the principle waste product of the body's protein metabolism, is hard to determine in the laboratory. Laboratory methods have been developed to instead determine the nitrogen in urea found in the blood (BUN).

The blood concentration of urea nitrogen is proportional to the protein content of the diet and renal excretory capacity of the animal. Increased values can be seen with dehydration, urinary obstruction, uremia, nephritis, surgical shock and intestinal obstruction. Intestinal bleeding may also increase the BUN. Because the RBCs are digested as part of the diet and contribute additional protein to the animal

Decreased values can occur in acute liver failure and intestinal disease. Because this value is related to protein metabolism, it is very dynamic and can change quickly based on changes in the diet as well as the condition of the kidneys. BUN should not be the sole value used to assess renal function.

Creatinine –Kidney

Creatinine is a non-protein nitrogenous product of muscle metabolism. It is formed from creatine, an amino acid found in muscle. This value is less affected by diet and protein catabolism that BUN and provides a more accurate measure of renal function. Creatinine levels are elevated by conditions that reduce glomerular filtration. Specific gravity ~1.010 suggests a renal cause for the elevation, while a higher specific gravity suggests prerenal or postrenal causes. Remember that ~ 75% of the kidneys must be nonfunctional before elevated values will be seen. Even though neither BUN nor creatinine is produced by the kidneys, they are still seen as renal tests because the kidneys are essential in removal of these by-products from the system.

Glucose - Pancreatic function (Beta cells), liver.

Glucose is the primarily energy source for all the cells within the body. If the hormone, insulin is missing, glucose cannot get into the cells to provide energy and instead builds up in the blood stream. Blood glucose levels can also be increased with corticosteroid use. The cortisone competes with insulin for the same binding sites; this competition causes glucose to remain in the blood stream rather than moving into the cells. Insulin-secreting pancreatic tumors, starvation, hypoadrenocorticism, shock and severe exertion can cause hypoglycemia. The most common cause of hypoglycemia is improper blood handling. Red blood cells are living cells and will continue to metabolize and use glucose when left in contact with plasma after collection. The longer they are left in contact with the plasma the lower the glucose level will become. This can lead to falsely decreased values. When actual glucose levels are too low (<50 mg/dl) seizures can occur because the brain is not receiving enough energy to function properly. This can be seen with neonates, young small breed animals and some diseases.

Total protein –Liver, kidney, intestine

Protein is manufactured in the liver from nutrients in the diet. Total plasma protein includes albumin, globulin and fibrinogen. Total serum protein does not include fibrinogen, and will be slightly lower than plasma protein measurements. Hypoproteinemia can be seen with diseases involving the intestines, kidneys or liver. The kidneys are responsible for recovering the protein and returning it to the system when filtering the blood. If the kidneys are not able to do this properly, protein will be lost in the urine and the serum protein levels can decrease. With intestinal disease, if protein is not absorbed into the blood stream but instead remains in the intestinal tract, low serum levels can be seen. Protein synthesis occuris primarily in the liver, with significant liver dysfunction protein synthesis may become affected causing lower levels in the plasma. Elevations in protein can be seen with dehydration, certain cancers and some viral infections (FIP).

It is easy enough to tell if the liver is function properly and able to make protein, and proteins lost in the urine can be detected on screening tests. Protein absorption problems in the intestines are harder to find, and are usually diagnosed by process of elimination. If the liver is fine, and there is no protein loss in the kidneys, then the loss is usually through lack of absorption through the intestines.

Total albumin

Albumin is one of the proteins measured by the total protein. It makes up 35%-50% of

the total plasma protein in most animals. This value helps to distinguish which element is being affected when either an increase or decrease in total protein levels are found. Albumin is the most “osmotically active” particle in the blood, and is involved in maintaining the osmotic pressure in the blood vessels as well as transporting medications and other metabolites throughout the body. Albumin is absorbed by the intestines, synthesized by the liver and retained by the kidneys. Any disease that affects one of these three organs can cause a decrease in albumin levels. Hypoalbumenia with normal globulin levels suggests decreased albumin production or increased loss. If both albumin and globulin are low, hemorrhage, exudation and dilution are likely causes. Severe dehydration often increases serum albumin levels.

Total globulin - immunological antibody response.

Globulins are another protein measured by the total protein. They are composed of lipoproteins (Alpha globulins) and immunoglobulins (Gamma globulins). The Gamma globulins can be further divided up into IgG, IgM. IgD, IgA and IgE. To determine the quantity of these individual components of globulin the sample would need to be sent to a reference laboratory for protein electrophoresis.

This test involves the passing of an electric current through the serum sample; the changes in current flow correlates with the various globulins. This breakdown is helpful in determining if the increase is due to inflammation or cancer. Inflammation and certain diseases (FIP in cats, Ehrlichia in dogs) can cause a polyclonal gammopathies (many types of globulin are increased). Lymphocyte/plasma-cell disorders (lymphosarcoma and multiple myeloma) can cause monoclonal gammopathies. Marked hyperglobulinemia can cause problems because of increased serum viscosity. Globulin concentrations are normally estimated by determining the difference between the total protein and albumin concentrations-this difference is the globulin fraction.

A/G ratio

A measure of the ratio of albumin to globulin (A/G ratio). This helps to determine which value is elevated or decreased. An alteration in the normal ratio of albumin to globulin ratio can be an indicator of a protein abnormality. This is calculated by dividing the albumin concentration by the globulin concentration.

Total Bilirubin - liver

Bilirubin is an insoluble molecule derived from the breakdown of hemoglobin by macrophages in the spleen, and circulates in conjugated and unconjugated forms. Measurement of the circulating levels of the various types of bilirubin can help pinpoint the cause of jaundice. Differences in the relative solubility of each of these molecules allow them to be individually quantified. Total bilirubin is comprised of both conjugated and unconjugated bilirubin.

Conjugated bilirubin

In most animals, the prehepatic bilirubin comprises ~ 2/3 of the total bilirubin found in the serum; this is called conjugated bilirubin because it is bound to albumin. An increase in this fraction indicates problems with uptake pointing at liver damage, bile duct injury or obstruction. Any bilirubin in the urine is conjugated because it is the only bilirubin that is water soluble.

Conjugated bilirubin is sometimes referred to as direct bilirubin because test methods directly measure the amount of conjugated bilirubin in the sample.

Unconjugated Bilirubin

Unconjugated bilirubin is not water soluble and does not pass in to the urine. Increased levels of unconjugated bilirubin are seen with red blood cell destruction or defects in the transport system that allows bilirubin to enter the hepatocytes for conjugation. Unconjugated bilirubin is sometimes referred to as indirect bilirubin because its concentration is indirectly calculated by subtracting the conjugated bilirubin concentration from the total bilirubin concentration. Conjugated and unconjugated bilirubins are released into the intestines to aid in fat digestion. Failure to release these compounds into the duodenum through bile duct obstruction can affect fat digestion and lead to pale, greasy looking stools.

Cholesterol – Liver, lipid metabolism, intestines.

Cholesterol is one of the lipids found in the blood. It composes only ~ 10-15% of the total fat found in the body. Cholesterol is primarily produced in the liver and excreted in the bile. Increases occur with obstructive biliary disease, hypothyroidism and hyperadrenocorticism. Hepatocellular disease, diabetes mellitus and anorexia decrease cholesterol production and reduce serum cholesterol levels. Hypercholesterolenemia does not cause lipemia in the blood as it is clear. Dogs and cats do not have the same problems with cholesterol as do people.

Triglyceride - Thyroid, liver, lipid metabolism

Triglycerides are the most abundant fat found in the body composing ~85% of the total fat found.

This is the fat fraction most responsible for lipemic blood samples. Elevated triglycerides can indicate recent ingestion of a high fat meal, impaired fat metabolism or intestinal disease. Fasting hypertriglyceridemia, also known as hyperlipidemia can be caused by diabetes mellitus, hypothyroidism, hypercortisolemia, cholestasis and idiopathic conditions in some miniature Schnauzers. Hyperlipidemia is both a cause and a result of acute pancreatitis.

Ammonia (NH^3) –Liver.

Ammonia is a by–product of protein metabolism. Increased baseline blood ammonia levels or persistently high blood ammonia levels after oral administration of ammonium chloride indicate hepatic insufficiency. Congenital or acquired hepatic shunts, bile duct obstruction, cholangiohepatitis and cirrhosis can increase blood ammonia levels.

Enzymes

Enzymes require special attention in preparing samples and carrying out chemistry determinations related to them. The following factors should be adhered to: 1) the age of the sample can affect test results-enzymes deteriorate over time. 2) serum or plasma used for these procedures should be removed from the red blood cells as soon as possible by centrifugation and extraction. Leaving these fluids on the erythrocytes for a long period of time can result in hemolysis and leakage of additional enzymes from the RBCs into the serum or plasma. This could falsely elevate some enzymes. 3) Hemolysis should be avoided because the test results could be falsely elevated. 4) Chemistry analyzers must be checked for proper

maintenance as to time, temperature and proper warm up.

ALT (Alanine Aminotransferase) - Liver

This enzyme was formerly known as **SGPT.** It is found in large quantities in the cytoplasm of canine and feline liver cells. This enzyme enters the blood stream when liver cells are damaged or destroyed, and circulates for a few days. Elevated serum ALT activity indicates recent or on-going liver cell damage. An increase of at least 3X normal indicates significant liver damage within the preceding 2-5 days. Corticosteroids and anticonvulsant medications may also lead to increases in serum ALT levels. There is no correlation between the blood levels of the enzyme and the severity of liver damage.

AST (Asparate Aminotransferase) - Liver

This enzyme was formerly known as the **SGOT.** It is mitochondria-bound and is found in several body tissues, but especially liver and striated muscle. Elevations can be seen with skeletal muscle necrosis, and hepatocellular necrosis. Elevated AST with normal ALT indicates muscle necrosis. In liver damage, AST activity rises more slowly than does ALT and indicates more complete cellular disruption because it leaks from the cell only with necrosis, not membrane instability. Hemolysis and lipemia can falsely elevate serum AST activity. Large animals (equine/bovine) tend to have a higher normal AST compared to small animals (canine/feline).

ALKP (Alkaline Phosphatase) - Liver, Bone

Elevated AlkP activity in the serum indicates increased production by the liver, bile ducts and growing bone or decreased excretion in bile or urine. The enzyme is induced by bile stasis and corticosteroid use. Elevated AlkP does not suggest liver or bone necrosis. The major causes of increased AlkP activity in dogs are cholestatic disease and excessive exogenous corticosteroids. Cats have less AlkP than do dogs, and slight excesses are rapidly excreted by the kidneys. Any increase in AlkP activity in cats is significant and suggests cholestasis. Normal values are higher for puppies and kittens because of active bone growth.

GGT (Gamma Glutamyltranspeptidase) - Liver

GGT is a liver enzyme that indicates disease of the portal biliary system. Increases in GGT activity parallel those of AlkP, but GGT is not found in bone. Corticosteroids and bile stasis can increase GGT production. In cats, GGT activity tends to increase more than AlkP activity with cholestasis. In dogs, increased GGT activity suggests cholestatic liver disease or cortisol excess.

By looking at the liver enzymes both individually and together, you can figure out which part of the liver is affected. With increases in ALT and AST we would look at the hepatocytes. Increases in T. bili and GGT would point towards problems with the cholangeo-hepatic system or bile ducts. An increase only in the Alk phos would indicate a need to look for exogenous steroid exposure. If all the values are increased equally-the entire liver is unhappy!

CK (CPK) - (Creatine kinase, Creatine phosphokinase) - Muscle

CPK is found in high levels in the CNS and skeletal muscle. Muscle trauma, IM injection, myositis, vigorous exercise (or vigorous resistance to restraint!), and occasionally

CNS damage can cause elevated serum CPK activity. Increased CPK activity parallels rise in AST activity with muscle necrosis. Mild elevations can be seen with traumatic venipuncture or resistance to restraint. Bit wounds, hit by car and other traumas can cause higher levels, myositis, as seen with Mastitory myositis or tetanus can cause some of the highest increases.

Amylase- Pancreas

Amylase is the first enzyme involved in carbohydrate digestion. It is found primarily in the pancreas, but in dogs, can also be found in the saliva. Cats do not have salivary amylase. Pancreatic inflammation, necrosis or pancreatic duct occlusion releases amylase into the blood and peritoneal cavity, elevating serum amylase levels 2-3 times normal. Increased absorption from upper intestinal inflammation and decreased renal excretion can also elevate serum amylase activity. EDTA should not be used for sample collection because amylase requires the presence of calcium for activity, and EDTA acts as a calcium binder. The presence of lipemia may reduce amylase activity.

Lipase - Pancreas

Lipase is a pancreatic enzyme normally secreted into the duodenum during digestion of fats. Because lipase is more pancreatic specific than amylase, it may be more sensitive in detecting pancreatitis.

It can be pathologically activated within the pancreas by lipemia or pancreatic trauma. Pancreatic necrosis sometimes elevates serum lipase activity to 2-7 times normal within 48 hours. Lipase activity also rises with increased absorption seen with upper intestinal inflammation and from decreased excretion with renal failure. Serum lipase activity remains elevated longer after pancreatic insult than does serum amylase activity. Corticosteroid administration is correlated with increased lipase activity with no concurrent change in amylase activity.

Suggested Reading

Hendrix, Charles, Sirois, Margi. Laboratory Procedures for Veterinary Technicians 5th ed. Mosby Elsevier 2002