

BIOCHEMICAL PROFILES AND INDIVIDUAL BIOCHEMICAL PARAMETERS

A guide to their Interpretation

Combined with haematology and urinalysis the biochemical profile forms the data base for most diagnostic investigations. Many biochemical parameters tend to have specificity for an organ and/or a limited range of pathological processes. Interpretation of diagnostic biochemical patterns requires an understanding of the pathological implications of each abnormal result. Together with the normal results these form a pattern which reflects one or more underlying disease process. Investigative biochemical profiles are designed to provide all the data necessary for a broad investigation of internal disease. Profiles with limited data are best used for monitoring an established diagnosis for which the results of a more wide ranging profile have already been obtained. Individual biochemical evaluations may be used, for example, for therapeutic drug testing (*phenobarbitol, bromide, digoxin*), assessing vitamin status and monitoring liver function (*bile acids*) and diabetic control (*fructosamine*).

Test for assessing Hepatobiliary Damage and Liver Function

SERUM TRANSAMINASE ACTIVITY

Aspartate aminotransferase (AST) is present in many tissues and is useful in evaluating muscle and liver damage in small and large animals. AST is not liver specific in any domestic animal species and the reference range in horses is rather broad. Skeletal muscle is the second largest source of AST in animals. It is an absolute prerequisite to eliminate extrahepatic tissue damage as a possible source of serum AST when evaluating the enzyme in relation to the liver.

In combinations with the physical examination and history, the evaluation of other serum enzymes should aid in differentiating the source of increased AST levels.

AST is present in both the cytoplasm and mitochondria of hepatocytes (*and many other cells*) and will elevate in states of altered membrane permeability. In such cases, levels are expected to be less than in states of frank necrosis, when both cytoplasmic and mitochondrial enzymes are released.

Alanine aminotransferase (ALT) is considered to be liver specific in small animals. This enzyme is present in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular damage/necrosis, hepatocyte proliferation, or hepatocellular degeneration. ALT is a cytoplasmic enzyme, and is considered to be liver specific in dogs, primates and some other small animal species. There is little hepatic ALT activity in large domestic animals. Thus, further comments regarding ALT will relate only to dogs and cats.

Elevation of serum levels of both AST and ALT can occur with states of altered hepatocellular membrane permeability. Because ALT is located only in the cytoplasm, serum levels tend to be relatively higher than AST, as a result of membrane leakage from the hepatocyte. Mitochondrial enzymes are less likely to be released with most of the conditions which result in increased membrane permeability.

Many causes of altered membrane permeability are potentially reversible but some may progress to hepatocellular necrosis which is essentially an irreversible change. Causes of increased cell membrane permeability include:

- Anoxia/circulatory hypoxia
- Exposure to toxins and toxemia
- Inflammation
- Metabolic disorders
- Hepatocyte proliferation

The magnitude of both AST and ALT elevations in serum is generally related to the number of hepatocytes affected. However, the level cannot be used to predict either the type of lesion, or whether cell damage is reversible (*leakage*) or irreversible (*frank necrosis*). In fact, focal necrosis may yield a lower concentration of both AST and ALT than would severe, transient hypoxia in which all cells may be affected resulting in a potentially reversible alteration in membrane permeability and diffuse enzyme leakage. Equally increases in ALT and AST may be relatively mild in cases of severe cirrhosis/fibrosis of the liver since there is no ongoing hepatocellular damage.

Another factor to be considered when interpreting AST and ALT levels is the rate of clearance from plasma. Both enzymes are molecularly too large to permit glomerular filtration and are primarily stereochemically denatured. The half-life of these enzymes is approximately 2-4 days and some prognostic information may be gleaned with this

knowledge. Thus, if an elevated serum level falls by 50% after 2-4 days, the prognosis is generally more favourable than if the enzymes remain persistently elevated or are only slightly decreased after this time period.

Finally, it must be remembered that ALT is liver specific only in the dog and cat. AST is present in many tissues, but because of organ size and relative enzyme content, it may be used with care to evaluate liver disease in large animals. Elevated AST and ALT in large animals may also reflect muscle damage or degeneration (*in which case CK is also elevated*).

Lactate dehydrogenase (LDH)

LDH is an intracellular enzyme which is widely distributed throughout the body and is found at high levels in tissues that utilise glucose for energy; it is therefore not organ specific. As a result, an increase in LDH can reflect damage to a number of different tissues (*skeletal or cardiac muscle, kidney, liver*).

LDH levels may be increased whenever there is cell necrosis or when neoplastic proliferation of cells causes an increase LDH production. Erythrocytes have high levels of LDH, therefore, even slight haemolysis can alter the serum activity considerably. Also LDH will diffuse out of the RBC's into the serum, if serum is not separated quickly. Nonhaemolysed serum samples must be submitted if valid LDH values are to be obtained.

Although not organ specific, elevated LDH activity indicates tissue damage, and other more specific diagnostic tests may help identify the source. The various isoenzymes of LDH can be identified by electrophoresis and these may also help in identifying the source of tissue damage.

Glutamate dehydrogenase (GLDH)

GLDH rises significantly with hepatic necrosis. This enzyme is highly concentrated in liver tissue and is located in cell mitochondria and, therefore, complete cell disruption is necessary before it is released in large quantities. Hence any significant rise in serum GLDH is indicative of hepatic necrosis.

Alkaline phosphatase (ALP)

The alkaline phosphatases are a group of enzymes which catalyse the hydrolysis of a phosphate group from an organic molecule at an alkaline pH. They are called isoenzymes because they catalyse the same reaction in the same species but have different biochemical properties.

ALP is primarily bound to cell membranes. The physiological function of these isoenzymes is not fully understood although recent information suggests that one of the biological roles of ALP is detoxification of endotoxin.

ALP is found, to some extent, in all tissues and is relatively stable in serum. However, only a few organs actually contribute to the circulating enzyme level.

An elevated alkaline phosphatase concentration is generally due to cholestasis in most adult domestic animals. A mild elevation in immature animals is likely to be the result of normal bone growth. In dogs, when an elevated ALP value is seen, liver disease, Cushings disease, and recent steroid therapy should all be considered. Prolonged steroid therapy resulting in iatrogenic Cushings disease can be diagnosed on the basis of low pre and post ACTH cortisol levels.

The liver isoenzyme will be elevated in any active liver disease. In acute hepatocellular necrosis, ALT, AST and GLDH are markedly elevated while ALP is only minimally elevated. Intrahepatic and extrahepatic biliary obstruction causes more dramatic elevations of ALP, which in some cases can be 10-20 times the normal level. This is due to recycling as well as increased synthesis of the liver isoenzymes. Extrahepatic biliary obstruction can be caused if the hepatic or common bile duct is obstructed either partially or completely. Possible causes include tumour, granulomatous inflammation, abscesses, pancreatitis and duodenitis.

The anticonvulsant drugs phenobarbital, diphenyl hydantoin (*phenytoin*) and primidone can cause minimal to marked elevations of the liver isoenzymes in dogs. The activity of ALT is also usually increased in such situations. In cats, the liver contains much less ALP per gram of tissue than dogs, and it is cleared from serum much more rapidly. This causes the normal value to be lower than in dogs, and mild elevations can be significant.

Elevations of the bone isoenzyme can be seen in young animals as a result of normal bone growth, and occasionally with bone tumours. These elevations are usually minimal and are seldom more than 2-3 times the normal value. The ALP elevation which is frequently present in hyperthyroid cats is due to release of the bone isoenzyme.

The intestinal, renal and placental ALP isoenzymes are cleared so rapidly from the circulation that they are rarely, if ever, detected in the dog and cat. In the horse, however, intestinal ALP (*SIP*) becomes elevated in serum, following

damage to intestinal mucosa.

The corticosteroid-associated isoenzyme (*SIAP*) has been found only in dogs. It may cause serum elevations of ALP that are greater than 10-20 times normal.

When total ALP levels are within normal limits you may consider not running SIAP.

Tests for detecting the presence of the different ALP isoenzymes (*SIP, SIAP and ALP*) are available through Axiom Laboratories.

COMMON CAUSES OF ELEVATED ALP

- Liver disease
- Cushing's disease (*dog*)
- Steroid therapy (*dog*)
- Antiepileptic drugs
- Bone growth in young animals
- Intestinal damage (*horse*)
- Hyperthyroidism (*cats*)

Gamma glutamyl transferase (γ GT)

Gamma glutamyl transferase has been shown to be a sensitive marker of cholestasis. It may be used in conjunction with other tests, to determine the presence and origin of cholestasis. γ GT has been found to be a valuable tool in the diagnosis of hepatobiliary disorders. Most cells have some γ GT activity, especially kidney, liver and pancreas, but most of the serum γ GT is derived from the liver. It is present in cell cytoplasm and also bound to membranes. It is a carboxypeptidase which cleaves glutamyl groups and transfers them to peptides and other appropriate receptors.

The physiological function of γ GT is unknown, but it could be associated with glutathione metabolism. Elevation of serum γ GT appears to be quite specific for intrahepatic or extrahepatic cholestasis. In liver damage γ GT may be used as an indication of chronic change, due to its slower release and metabolism, compared with transaminases. As such it is often associated with cirrhosis. Gamma GT is particularly useful for identifying chronic hepatic disease in horses. It is induced by corticosteroids in dogs and cannot be used to discriminate between steroid-induced elevations of ALP and cholestasis.

Bilirubin

Bilirubin and its components may be helpful when evaluating liver function or haemolysis. These tests may be useful in distinguishing prehepatic from hepatic or posthepatic hyperbilirubinaemia.

Bilirubin is mainly formed from the breakdown of erythrocytes. It is then carried in the plasma loosely bound in albumin. This bound form is not water soluble and is often referred to as INDIRECT reacting, free, prehepatic, or UNCONJUGATED bilirubin.

The hepatocyte conjugates the indirect bilirubin with glucuronic acid and it is then referred to as DIRECT or CONJUGATED bilirubin. Direct bilirubin is water soluble.

Direct bilirubin is excreted into the intestine via the biliary system. Some of the direct bilirubin is reabsorbed back into the circulation from the intestine. The direct bilirubin is not bound to albumin and is freely filtered by the glomerulae. The renal tubular epithelial cells readily reabsorb the filtered bilirubin in most animals. However, the dog is an exception and small amounts of bilirubin are normal in concentrated urine samples while bilirubinuria in cats is generally considered to be abnormal.

An elevation of indirect bilirubin is a rather uncommon finding in small animals, but when it occurs, it is generally the result of acute and severe haemolysis. A healthy liver is capable of conjugating large amounts of bilirubin and that is why many haemolytic anaemias have normal bilirubin values. The haematocrit and red blood cell counts are low when elevated indirect bilirubin is caused by haemolysis.

Direct reacting hyperbilirubinaemia occurs as a result of impaired hepatic secretion of bilirubin and/or obstruction to bile flow. Obstruction to bile flow can be intrahepatic, extrahepatic or both. Most jaundiced animals have elevations in both indirect and direct bilirubin. Haemolytic disease may also result in an increase in direct bilirubin since a large proportion of the free bilirubin is conjugated.

DOGS

Consider haemolysis when direct bilirubin is less than 25% of the total bilirubin concentration and the animal is

anaemic. Intrahepatic disease with associated cholestasis is suggested when direct/total bilirubin is 40-50%. Complete extrahepatic obstruction is suggested when the percentage of conjugated bilirubin is greater than 75%. Remember that alkaline phosphatase (*ALP*) is also elevated with cholestasis and hence normal alkaline phosphatase concentrations all but rule out biliary obstruction. Serum bilirubin tests are not very sensitive and total bilirubin must be 17 mmol/L or greater for correct interpretation of total, direct, indirect and direct bilirubin concentrations in the dog. Haemolysis can artefactually increase bilirubin levels.

CATS

Total serum bilirubin values >10 mmol/L in the cat may be caused by a variety of conditions (*anorexia, liver disease, renal disease, gastrointestinal disease, FIP etc.*). On the other hand, values above 50 mmol/L are generally caused by liver disease (*if the haematocrit is normal*). Bilirubinuria in cats is considered to be abnormal.

Hepatic lipidosis, cholangiohepatitis and FIP are common causes of feline hepatic disease. Liver biopsy is essential for a definitive diagnosis and for determining prognosis.

HORSES

The normal range for bilirubin is considerably higher in the horse than other species due to the lack of gall bladder. Most of the serum bilirubin is indirect and elevations may be observed secondarily to many conditions. Anorexia, haemolytic anaemias, hepatic disease, endotoxaemia and colic are commonly associated with high total and indirect bilirubin values in the horse. Measurement of direct/indirect bilirubin is not justified in this species.

Interpretation of hyperbilirubinaemia should always be performed in conjunction with liver enzymes, haematology, history and reference to species. The diagnostic changes in direct/indirect bilirubin are usually associated with acute disease. In chronic hyperbilirubinaemia, the ratio of direct to indirect bilirubin is usually 50:50.

Cholestasis

Cholestasis can result from a variety of pathophysiological mechanisms including:

- * Interference of sodium and water transport into the bile.
- * Increased intraluminal biliary pressure due to either intrahepatic or extrahepatic obstructions of ducts.
- * Interferences with normal biliary micelle formation by drugs and abnormal bile acids.
- * Altered bile salt concentrations.

Since the bile duct is closely associated with the pancreas and empties into the duodenum, various disorders causing swelling or constriction of the liver, pancreas or duodenum can also cause increased pressure within the biliary tree and thus cholestasis. In cases where there is evidence of cholestasis, it is therefore necessary to determine if the obstruction is primarily intrahepatic (*e.g. periportal abnormalities*) or extrahepatic (*e.g. pancreatitis, duodenitis*). Amylase, lipase and TLI tests are useful to rule out pancreatitis. Radiography/ultrasonography to check for possible tumours or evidence of hepatomegaly can also be helpful.

Markers of cholestasis include ALP, γ GT, total/direct bilirubin, urine bilirubin, bile acids and cholesterol. These markers should be interpreted in the context of other liver-specific enzymes, which if moderately to markedly elevated, can indicate primary liver damage. Once the cause has been determined and treated it can take days to weeks for the enzymes to return to normal, depending on how long the disorder has been present (*enzyme induction*) and the serum half-life of each enzyme (*clearance rate*).

In the cat, the increase in γ GT can be more marked than the increase in ALP. In dogs, moderate to marked ALP elevations can also be induced by exogenous or endogenous steroids. There is some evidence that this is really a reduced clearance of intestinal phosphatase rather than an induction of a separate isoenzyme. In horses, γ GT appears to be more sensitive than ALP as a marker of cholestasis and the elevations tend to be more pronounced. γ GT appears to be the test of choice for the diagnosis of hepatobiliary disorders in cattle and sheep.

A total lack of bile in the stool produces steatorrhea. Prolonged accumulation of bilirubin in the blood produces jaundice. In most cases of cholestasis the obstruction of the bile flow is incomplete and these changes may not be evident. In addition, conjugated (*direct*) bilirubin is water soluble and can be excreted in the urine. Dogs are the most efficient at excreting excess bilirubin in the urine, cats are less efficient and large animals are the least efficient. This is why dogs can have bilirubinuria without bilirubinaemia. In dogs and cats, prolonged anorexia is sufficient to cause bilirubinuria. The presence of bile in the intestine normally suppresses hepatic and intestinal synthesis of cholesterol. With prolonged cholestasis the serum cholesterol concentration can increase markedly.

Cholesterol

Serum cholesterol is a useful ancillary aid in the diagnosis of several metabolic diseases although is not in itself diagnostic of any single disorder. Cholesterol is the precursor of cholesterol ester, bile acids and steroid hormones. It is implicated in vascular disease and is of diagnostic importance in hypothyroidism.

Cholesterol absorption is dependent upon biliary secretion as well as the hydrolytic activity of pancreatic lipase. In pancreatic insufficiency, despite the lack of fat hydrolysis, some cholesterol absorption still occurs due to bile salt emulsification. Cholesterol synthesis is primarily dependent on hepatocyte metabolism but may occur in any tissue.

The amount of cholesterol from dietary sources and hepatic synthesis is under close homeostatic control with the rate of synthesis inversely proportional to absorption. The dietary cholesterol ester is utilised almost completely in the liver, and losses are in the form of bile acids and free cholesterol and its derivatives in bile.

Hypocholesterolaemia is recognised in inherited lipoprotein deficiencies (*beta*lipoproteinaemia and *alpha* lipoprotein deficiency), intestinal malabsorption/maldigestion, and advanced liver disease.

Hypercholesterolaemia may occur independently or concomitantly with lipaemia and hypertriglyceridaemia. Hypercholesterolaemia with lipaemia may occur with hypothyroidism, hyperadrenocorticism, diabetes mellitus, acute pancreatitis, hepatic disease (*especially if extrahepatic biliary obstruction*), *protein-losing* enteropathy and nephritic syndrome (*glomerulonephritis*). It may also occur in the postprandial period and with starvation. It is also a feature of steatitis. The concentration of serum cholesterol is thyroid-dependent, with thyroid hormone enhancing both the rate of cholesterol synthesis and the rate of catabolism. In hypothyroidism cholesterol utilisation is less than cholesterol synthesis. The net result is an increase in cholesterol concentration.

Since many other factors influence cholesterol concentration, hypercholesterolaemia is not diagnostic for hypothyroidism, but it may be used as an ancillary diagnostic aid. The diagnostic accuracy of the serum cholesterol level for hypothyroidism in the dog is about 60%, but a high cholesterol concentration (*greater than 13 mmol/L*), with diabetes mellitus ruled out, increases its diagnostic accuracy.

Conversely, hypocholesterolaemia is not an index of hyperthyroidism. Since cholesterol concentration decreases in response to thyroid replacement therapy, it may be an indication of effective therapy.

In uncontrolled diabetes mellitus, increased serum cholesterol accompanies a general increase in serum lipids. This is due to the absence of insulin which results in decreased mobilisation of serum triglycerides into fat deposits. In diabetes mellitus, hypercholesterolaemia is also due to decreased activity of insulin-dependent lipoprotein lipase and decrease of cholesterol. The resultant hypercholesterolaemia predisposes to atherosclerotic vascular disease.

Hypercholesterolaemia provides supportive evidence for, but is of limited prognostic value in, hepatic disease. Abnormal serum cholesterol levels usually accompany liver disease in animals, but as such, abnormal levels are not diagnostic of hepatic disorders. In obstructive biliary disease, hypercholesterolaemia may be due to retrograde flow through the biliary system and/or bile salt retention. As a result cholesterol remains in a soluble state and there is a reduction in tissue uptake. In severe hepatocellular disease with loss of hepatic synthetic capacity, a progressive decrease in total and esterified cholesterol occurs and represents a poor prognostic sign.

Excessive serum cholesterol levels are also associated with glomerular disease, especially membranous glomerulonephritis and amyloidosis ie the nephrotic syndrome. The pathogenesis of hypercholesterolaemia in renal disease is not understood, but is related to hypoproteinaemia, since serum cholesterol and serum albumin concentrations maintain an inversely proportional relationship.

CAUSES OF HYPERCHOLESTEROLAEMIA

- Hypothyroidism
- Hyperadrenocorticism
- Extra-hepatic biliary obstruction
- Liver disease
- Protein-losing enteropathy
- Nephrotic syndrome
- Diabetes mellitus
- Pancreatitis
- Post-prandial sampling or starvation

Bile Acids

Bile acids have replaced the BSP retention test, fasting ammonia and ammonia tolerance test for assessing hepatic function. Bile acids are synthesized by the liver and secreted in the bile. Most are then resorbed in the ileum and undergo enterohepatic recycling being removed from the plasma by the hepatocytes before being secreted in bile once again. During feeding the gall bladder contracts and bile flows into the small intestine. Most of the bile acids are resorbed into plasma and reassimilated into hepatocytes within 2 hours of a meal leaving a low fasted serum concentration.

If there is impaired hepatic function, portosystemic shunting or cholestasis the removal of bile acids from plasma is

impaired leading to elevated fasting concentrations. Increased sensitivity is achieved by combining fasting bile acids (8-12 hour fast) with post prandial bile acids (2 hours after a meal). This test (dynamic bile acid test) is the most sensitive index of liver function available and should be run in parallel with hepatic enzyme assessments since the hepatic enzymes only reflect the integrity of hepatocytes not the overall impact of a disease process on liver function. Bile acids are usually not markedly altered in hepatopathies associated with hyperadrenocorticism, corticosteroid therapy or anticonvulsants. Dynamic bile acids are the best screening test currently available for portosystemic vascular anomalies.

N.B: In the presence of jaundice due to a post-hepatic or intrahepatic process, the bile acids lend no extra diagnostic information, however, they can be helpful in discriminating between prehepatic jaundice due to haemolysis and jaundice of hepatic or post hepatic origin.

INTERPRETATION OF OTHER BIOCHEMICAL PARAMETERS

Glucose

Blood glucose is an important source of energy for many cells. Blood glucose is normally maintained by the breakdown of dietary carbohydrates and a rather complex system of endogenous production. Endogenous production results from glycogenolysis (*glycogen broken down to glucose in the liver*) and from gluconeogenesis (*formation of glucose from biochemical precursors*). The maintenance of normal plasma glucose requires delicate balance of glucose availability with glucose utilisation.

Glucose is not the only energy source which fuels the energy requirements of the body tissues. Fatty acids, proteins and other substances also provide energy. However, glucose is an obligate fuel for the central nervous system. Consequently maintenance of a normal blood glucose concentration is essential for the survival of brain tissue. Glucose transport from the circulation into the brain can become rate limiting if the blood glucose falls into the hypoglycaemic range. In general clinical signs may appear when the blood glucose levels fall below 3 mmol/L (*often less than 2.5 mmol/L in the dog*).

Many hormones are involved with glucose regulation (*glucagon, epinephrine, cortisol, insulin*). Insulin, secreted from the B cells of the pancreas, is the most noteworthy and dominant glucoregulatory factor. Insulin primarily stimulates glucose utilisation by a variety of insulin-sensitive tissues including muscle, fat and liver. Small changes in insulin result in substantial changes in blood glucose values. An increase in insulin will generally lower plasma glucose levels.

Glucagon, epinephrine and cortisol are all glucose-raising hormones. Glucagon acts on the liver by stimulating both glycogenolysis and gluconeogenesis. Epinephrine both limits glucose utilisation and stimulates its production. Cortisol antagonises the effects of insulin and limits both the stimulation of glucose utilisation and the suppression of glucose production by insulin.

It is the alterations in these glucoregulatory hormones which cause hypoglycaemia and hyperglycaemia.

CAUSES OF HYPOGLYCAEMIA

- Hepatic disorders
- Hyperinsulinism/insulinoma
- Insulin overdose
- Extrapancreatic tumours
- Idiopathic in Toy Breeds
- Sepsis
- Endocrine disorders eg hypothyroidism, hypoadrenocorticism
- Malabsorption
- Prolonged starvation
- Renal glucosuria (*severe cases*)
- Artifact eg blood sample collected into EDTA or heparin
- Neonatal hypoglycaemia

CAUSES OF HYPERGLYCAEMIA

- Post-prandial sampling
- Hyperadrenocorticism
- Administration of corticosteroids
- Diabetes mellitus.
- Pancreatitis
- Drugs eg morphine, IV fluids containing dextrose or glucose

- Stress/excitement (*especially in cats*)
- Dioestrus in the bitch
- Acromegally
- Glucagonoma

Amylase and lipase

Amylase and lipase are useful in diagnosing pancreatitis. Both enzymes are produced by the pancreatic acinar cells but since they are cleared from the blood by the kidneys, anything which decreases glomerular filtration rate will increase amylase and lipase concentrations in the serum.

Lipase is found primarily in the pancreas, and amylase is found in intestinal mucosa and liver as well as in the pancreas. However, the serum amylase level is derived mainly from the pancreas. The other tissue sources contribute very little activity because, the tissue levels are low, the circulation half life is short, or they are inactivated rapidly. The normal serum half-life of amylase is about 5 hours, and the half-life of lipase is about 2 hours.

In the presence of normal renal function, the relatively short half lives of these enzymes means that blood levels in an animal with pancreatitis can vary considerably, depending on the severity of the lesion and the length of time from onset of illness to presentation.

The levels can also change markedly within only a few days, so serial samples are often very helpful.

There are some technical problems which can interfere with both tests.

- Lipaemia, which is often present with pancreatitis can falsely raise or lower the amylase result and often lowers the lipase result.
- Gross haemolysis can also affect results.

The very wide reference range of amylase and lipase can cause problems in interpretation. For example, the amylase can be elevated three times the baseline and still be within the reference range. Also, amylase results between 2000 and 3000 lu/L are sometimes observed in clinically normal animals due to the large standard deviation. Parenteral use of corticosteroids will increase lipase 3-4 fold while decreasing amylase and this can complicate interpretation. High TLI values may also be diagnostically significant but can also be increased in patients with azotaemia. Other tests such as calcium, glucose, urea, creatinine, phosphorus, ALT, ALP, cholesterol, and urine analysis should be used to help interpret changes in amylase and lipase and to arrive at a diagnosis. Currently the most sensitive and specific test for pancreatitis in both dogs and cats is pancreatic lipase immunoreactivity (*PLI*).

Creatine kinase (CK)

Creatine kinase is useful in diagnosing skeletal muscle or cardiac muscle degeneration. The clinical diagnosis of neuromuscular disease can be aided by serum enzyme determinations. Creatinine phosphate is the major form of high energy phosphate required by muscle for contraction.

Increases in CK can be caused by skeletal muscle damage and excessive exercise, muscle anoxia, from prolonged recumbency, myositis, nutritional myopathy, and myocardial infarction. Frequently CK will increase after intramuscular injections due to local areas of muscle necrosis. CK in CSF may be useful in diagnosing disease of the central nervous system.

The half-life of CK is very short and levels decrease rapidly. This is in contrast to the pattern which serum AST follows. AST is also useful in the diagnosis of muscle damage and can act as a prognostic indicator. Elevated CK values indicate that muscle damage is active or has recently occurred. If the CK continues to remain elevated, the muscle damage is continuing. If elevated AST levels are associated with decreasing or normal CK levels, the muscle damage is no longer active.

Common causes of increased CK activity

MYOSITIS

- Clostridial myositis
- Purulent myositis caused by pyogenic bacteria
- Eosinophilic myositis

MUSCLE TRAUMA

- Contusions
- Recumbency
- Intra-muscular injections
- Seizure activity

MISCELLANEOUS

- Azoturia of horses and greyhounds
- Nutritional myopathies
- Degenerative myopathies
- Myocardial infarction

UREA AND CREATININE

Urea is formed in the liver and is mainly excreted by the kidneys. Consequently urea is useful in evaluating kidney function in conjunction with creatinine which originates from the muscle and is filtered by the kidney.

UREA

The majority of the blood urea is synthesized in the liver from ammonia. Once formed, urea diffuses freely throughout all body fluids. The kidney is the most important route of urea excretion and as a result, urea has long been used as a barometer of renal function.

Urea appears in the glomerular filtrate in the same concentration as is found in the blood. This filtration process does not require energy. Decreased glomerular filtration increases urea. Some urea is passively resorbed from the tubules back into the blood. The amount resorbed is inversely related to the rate of urine flow through the tubules the lower the urine flow rate the greater the tubular urea resorption resulting in an increased urea.

- An increase in urea may be considered under three categories:
 1. **Prerenal** - Fever, infection, tissue necrosis and corticosteroid administration and circulatory changes may all result in urea elevation. Increased protein digestion resulting from intestinal bleeding will likewise cause an increase. Anything that decreases glomerular filtration will increase urea. A high protein diet may also affect the urea concentration.
 2. **Renal** - Increased urea values are seen when approximately 75% of the nephrons become non-functional. As such urea may reach much higher levels (greater than 36 mmol/L) than found in pre-renal uraemia but lower values may also be renal in origin.
 3. **Post-renal** - Urea increases as a result of obstruction of the urinary tract and may reach very high values (90 mmol/L). The magnitude of the increase is dependent on the degree of the obstruction. Urinalysis, especially urine specific gravity, is useful in determining whether elevated urea is pre-renal, renal or postrenal. With pre-renal uraemia urine specific gravity generally is greater than 1.030 in the dog and 1.035 in the cat, while renal uraemia has a lower urine specific gravity.

CREATININE

Most creatinine originates from the non-enzymatic conversion of creatine in muscle. This spontaneous degradation of creatine to creatinine occurs at a rather constant and uniform daily rate. Creatinine is freely filtered by the glomerulus and clearance of creatinine from the plasma to the urine can be used to provide an approximation of the glomerular filtration rate. A small amount of creatinine is secreted by proximal tubules in the kidney but, in contrast to urea, none is resorbed by the tubules.

Causes of creatinine increases may generally be placed in the same three categories described for urea. However, creatinine values are not significantly affected by catabolic factors and diet. Diuresis and other factors affecting urine flow rate have less effect on creatinine than urea because creatinine is not resorbed by the renal tubules.

EARLY RENAL DISEASE

Simultaneous elevations of urea and creatinine on a biochemical profile denote azotaemia. The cause of azotaemia can be prerenal i.e. reduced GFR due to hypovolaemia, renal i.e. due to renal damage, or post-renal i.e. due to urinary obstruction. Due to the great functional reserve of the kidney, renal azotaemia and many of the clinical signs of uraemia only develop when between 60-75% of nephrons are non-functional.

The clinical differentiation of renal from prerenal azotaemia is critical since the former immediately implies serious disease and the latter is usually readily reversible on restoration of normovolaemia. Clinical differentiation is usually based on urine specific gravity since most patients with prerenal azotaemia will have highly concentrated urine while patients with renal azotaemia will have isosthenuric urine.

Development of techniques for detecting the presence of renal disease before 60-75% loss of functional nephrons, at a stage when the disease process might be reversible, has long been a major objective of research in nephrology. One approach that has been quite successful in the human field is to measure renal tubule-specific enzymes in urine

as markers of renal tubular damage.

Most of these renal enzymes are unstable in urine but NAG is reasonably robust. To allow for filtration differences urinary creatinine is also measured and it is the NAG/Creatinine ratio that is important. Increased ratios are indicative of renal tubular damage.

Increased NAG clearance can be detected before the development of renal azotaemia and at a stage when renal tubular damage may be reversible. Abnormal clearance of electrolytes and phosphorus can also be early markers of renal tubular dysfunction.

Micro albumen may also be measured in canine urines as an alternative marker of early renal disease.

Glomerular lesions such as amyloidosis and glomerulonephritis often induce the development of more generalised chronic renal pathology and renal failure. Significant proteinuria is an early marker of glomerular damage. This is detected on a single urine sample by measuring the protein/creatinine ratio which has been shown to correlate well with 24 hour urinary protein loss. It is essential to differentiate glomerular proteinuria from non-specific proteinuria due to urinary tract inflammation. The latter must be ruled out by assessment of the urinary sediment before a positive protein/creatinine ratio is considered indicative of glomerular damage.

The early renal profile has been designed to help identify renal disease at an early stage so that appropriate treatment measures can be instituted before irreversible renal damage occurs. The profile addresses the differentiation of prerenal and renal azotaemia by comparing the degree of azotaemia with urine specific gravity. It assesses renal tubular damage by measuring the NAG/Creatinine ratio and the fractional excretion of sodium, and it provides an indicator of early glomerular damage by assessment of protein/creatinine ratio.

Total proteins, albumin, globulins and the acute phase proteins

Plasma proteins represent a heterogeneous group with albumin constituting the major portion. Albumin serves as a regulator of osmotic equilibrium. Globulins are also important plasma proteins and they are primarily associated with antibodies. Acute phase proteins are associated with the acute inflammatory response and are useful markers for acute and chronic active inflammation.

SERUM PROTEINS

Almost all proteins in the serum are produced by the liver. Immunoglobulins are the notable exception and they are produced by lymphoid tissue. Serum proteins are relatively short-lived with most having half-lives of about 10 days. The breakdown of these proteins occurs mostly in the liver with some catabolic activity in the intestine and kidney. Animal plasma normally contains 25-35 gm/L of albumin which constitutes 40 -60% of the total protein concentration. Fluid accumulations in body cavities and tissue usually result when albumin levels drop below 10 gm/L. However, fluid may accumulate with higher albumin concentrations if hypertension, and loss of vessel integrity, etc. are present. Plasma and serum proteins, act as anions in acid-base balance, take part in coagulation reactions, and serve as carriers for many compounds. In addition to albumin, plasma contains globulins, fibrinogen (*removed from serum by the clotting process*), glycoproteins, lipoproteins, acute phase proteins and transport proteins.

The globulin component is subdivided into important subfractions identified by electrophoresis as alpha, beta and gamma globulins. The alpha and beta fractions are important carriers of lipids, lipid soluble hormones and vitamins. Gamma globulins are primarily associated with antibodies.

Conditions causing inflammation usually cause a measurable increase in serum levels of gamma globulins and often alpha-2 globulins (*e.g. α ALT*). Fibrinogen is a plasma acute phase protein which is utilised in the coagulation process. It is therefore absent in serum. Glycoproteins (*carbohydrates bound to protein*) and lipoproteins (*lipids bound to protein*) are the other major plasma proteins. Both of these serve as carriers of the substances bound to them.

Hypoalbuminaemia

- Primary or secondary intestinal malabsorption
- Exocrine pancreatic insufficiency
- Malnutrition, dietary or parasitism
- Chronic liver disease eg atrophy or fibrosis
- Glomerulonephropathy resulting in proteinuria
- Acute inflammation (*negative acute phase response*)
- Severe exudative skin disease or burns

Hypoglobulinaemia

- Immunodeficiency disease, either primary or secondary

Hypoalbuminaemia/hypoglobulinaemia

- External haemorrhage
- Protein-losing enteropathies
- Johne's disease

CAUSES OF HYPERPROTEINAEMIA

Increased albumin

- Dehydration (*relative increase*)
- Lactation (*common in dairy cows*)

Increased fibrinogen +/- other acute phase proteins

- Acute inflammation

Increased globulins

- Monoclonal gammopathy, Polyclonal gammopathies, Multiple myeloma, acute inflammation, infection, neoplasia, Ehrlichiosis FIP (OX), Leishmaniasis chronic liver disease, FIP, SLE.

Measurement of albumin, along with a separation of globulin into its fractions, can be accomplished by serum protein electrophoresis. When placed in an electric field, these proteins migrate at different rates yielding a familiar electrophoretic pattern. Values obtained from measuring serum proteins can provide an accurate reflection of an animal's health status.

PHOSPHORUS AND CALCIUM

Phosphorus and calcium determinations are important in evaluating profiles. These two determinations and the calcium: phosphorus ratio should be related to other enzyme determinations, particularly those relating to kidney, bone, muscle, digestive reactions and neoplastic processes. These evaluations should always be included in profiles evaluating animal health or disease processes.

PHOSPHORUS

Phosphorus is an important ion, but is most physiologically active as the phosphate radical. It is used in the structural proteins of cell wall, bone and other tissues and in active metabolic enzymes and pathways. Serum concentrations of phosphorus are regulated primarily by the renal tubules responding to parathyroid hormone stimulation. Parathyroid hormone accelerates urine loss of phosphorus by decreasing the tubular resorption of phosphorus. Increased tubular resorption occurs when the circulating parathyroid hormone level is decreased. Vitamin D enhances phosphorus absorption from the intestine and resorption from bone.

There are various disease processes which alter phosphorus levels and many inter-relationships with other systems. The renal system is closely involved in the control of phosphorus levels and thus urea and creatinine are important adjunct determinations. Any high phosphorus should be correlated with renal evaluations. The phosphorus concentration is also related to protein intake and should be correlated with nutritional status.

Hyperphosphataemia can result from increased intestinal absorption, decreased phosphate excretion in urine or a shift in phosphate from the intracellular to the extracellular compartment. The extracellular shift of PO_4 mirrors that of potassium occurring in mineral acidosis, insulin deficiency and tumour lysis syndrome.

Hypophosphataemia can result from decreased intestinal absorption, increased urinary excretion or a shift from the extracellular compartment to the intracellular compartment. Intracellular PO_4 shift may occur with acute alkalosis or insulin-mediated glucose uptake by cells.

CONDITIONS CAUSING HYPOPHOSPHATAEMIA

- Inadequate intake (*dietary or malabsorption*)
- Primary hyperparathyroidism Hypercalcaemia of malignancy (*PTH-related peptide*)
- Diabetes mellitus
- Hypovitaminosis D
- Translocation from extracellular to intracellular locations due to administration of glucose, insulin (*treatment of diabetic ketoacidosis*) or development of alkalosis
- Renal tubular defects
- Eclampsia

- Bicarbonate and diuretic therapy

CONDITIONS CAUSING HYPERPHOSPHATAEMIA

- Age of sample (*phosphorus is released from red cells after 12- 24 hours*)
- Young growing animals
- Renal secondary hyperparathyroidism
- High phosphorus intake, poor quality protein (*nutritional secondary hyperparathyroidism*)
- Vitamin D toxicity
- Hypoparathyroidism
- Tissue trauma/necrosis/tumour lysis syndrome
- Haemolysis (*intravascular/in vitro*)
- Occurs idiopathically and transiently in anorexia or vomiting
- Decreased GFR (*pre and post renal azotaemia*)
- Phosphate enemas
- Rhabdomyolysis

CALCIUM

Calcium is one of the most important ions in the body. It is utilised in bone and structural organisation, enzyme function, blood coagulation, in osmotic pressure and maintenance of fluid balances, and is essential in muscle activity. As such, calcium interrelates with any other system and has a close relationship to many enzymes and values measured in a profile.

The majority of calcium in circulation exists as protein-bound and ionised calcium. Calcium in both forms is normally measured and reported as a total calcium value. When evaluating calcium, it is important to relate total calcium to the quantity of albumin in the serum and the acid-base status of the animal. The total calcium concentration can increase in hyperalbuminaemia and decrease in hypoalbuminaemia. Acid-base changes alter the ratio of ionised to protein-bound calcium. Acidosis increases the ionised calcium fraction, whereas alkalosis increases the protein-bound fraction. Therefore total calcium, albumin and bicarbonate levels are important in evaluating calcium concentrations and related diseases.

CONDITIONS CAUSING HYPOCALCAEMIA

- Hypoalbuminaemia
- Renal secondary hyperparathyroidism
- Eclampsia
- Pancreatitis with fat necrosis
- Hypoparathyroidism
- Excessive phosphate intake
- Intestinal malabsorption
- Hypovitaminosis D
- Chelation by EDTA
- Iatrogenic parathyroid damage during thyroid surgery or as a sequel to parathyroidectomy

CONDITIONS CAUSING HYPERCALCAEMIA

- Young growing animals
- Hypercalcaemia of malignancy (*lymphoma, myeloma, apocrine anal gland carcinoma and other carcinomas*)
- Hypoadrenocorticism
- Primary renal failure
- Osteomyelitis and metaphyseal osteopathy (*rare*)
- Hypervitaminosis D
- Primary hyperparathyroidism
- Hyperalbuminaemia
- Lipaemia
- Granulomatous disease (*rare*)

In mammals, calcium concentrations in the serum are primarily regulated by parathyroid hormone and vitamin D. Alterations of the serum concentration of vitamin D3 and/or PTH can result in hypercalcaemia or hypocalcaemia. From the lists of conditions altering calcium levels, it is obvious that many other systems and conditions are involved. Besides protein and bicarbonate levels, other enzymes or values need to be known to evaluate calcium changes.

Hypercalcaemia in the presence of normal or elevated phosphorus may cause renal damage and nephrocalcinosis.

Primary renal failure may itself cause an elevation of total calcium (*so called tertiary hyperparathyroidism*), this being quite a common finding in young animals with congenital renal disease. Thus the presence of persistent hypercalcaemia should always prompt careful examination of renal parameters such as creatinine, urea and also phosphate. The calcium phosphorus product is particularly important in this respect since it determines the risk of calcium and phosphorus precipitating out in tissues and leading to renal damage through nephrocalcinosis.

Persistent hypercalcaemia in the absence of hyperalbuminaemia in the dog (*and cat*) is highly diagnostically significant since it immediately implicates one of a short list of possible underlying aetiologies. These are hypercalcaemia of malignancy (*the most common cause in the dog*), primary hyperparathyroidism, vitamin D toxicity, Addison's disease and tertiary renal hyperparathyroidism. The last is usually obvious due to advanced signs of renal failure. Hypercalcaemia of malignancy (*HCM*) is most frequently caused by lymphosarcoma (*often cranial mediastinal*) but may also be due to apocrine anal gland carcinomas and other diverse carcinomas. It has also been reported in cases of multiple myeloma. Most tumours causing HCM do so by releasing an embryonic growth factor called PTH-related peptide which binds to the PTH receptor and mimics its actions. With these inter-relationships, it is important to evaluate a complete profile to analyse why calcium might be altered.

Clinical note on calcium and phosphorus

Dystrophic mineralisation is most likely to occur when the calcium concentration (*mmol/L*) multiplied by the phosphate concentration exceeds 5 mmol/L. With a high calcium x phosphorus product in the face of dehydration, renal disease, cardiac arrhythmias or neurologic dysfunction, rapid treatment is necessary. Of course, in the face of high calcium levels, adequate hydration is necessary. Even in the face of other alterations, with an increased calcium, a water deprivation test or any form of water deprivation is contraindicated.

SODIUM AND POTASSIUM

Sodium and potassium may fluctuate for many reasons. These changes can be used as an aid in the diagnosis and treatment of many disorders. Often ratios are calculated to add more information and to help determine the cause of the altered electrolyte concentrations.

SODIUM

Sodium is the primary anion in extracellular fluid. Hypernatraemia generally indicates a lack of access to water and dehydration, however, hypernatraemia can occur in animals drinking salt water.

Hyponatraemia may be associated with diarrhoea, hyperglycaemia, Addison's disease, severe congestive heart failure and the administration of sodium-free fluids. Lipaemia may falsely decrease sodium because sodium is only in the aqueous phase and in lipaemic samples a portion of the aqueous phase is displaced by a lipid phase (*pseudohyponatraemia*).

POTASSIUM

Potassium is located primarily in intracellular fluid (*ICF*). The extracellular fluid (*ECF*) potassium concentrations are controlled by renal excretion. Therefore, a decrease in the glomerular filtration rate can cause an increase in the potassium concentration. Acidosis can also increase the ECF potassium concentration by an exchange of potassium ions from the ICF for hydrogen ions in the ECF. This ion exchange is a normal buffering process in the body. Many other conditions such as Addison's disease, ruptured urinary bladder, diabetes etc. can cause an elevated potassium concentration in the ECF. In the dog the clotting mechanism releases potassium, probably from platelets. For accuracy plasma potassium values should be measured in heparinised plasma and preferably, but not essentially, separated from RBCs within 30 mins of sampling. Removal of serum after clotting will reduce the artificial elevation but not remove it. Measuring potassium on serum which has not been separated will always produce an artefactual increase in potassium. Decreased ECF potassium concentrations may be due to vomiting, diarrhoea, administration of diuretics, alkalosis (*ECF potassium ions being exchanged for ICF hydrogen ions*), and many other causes. Fluctuations in the ECF potassium concentration do not necessarily reflect the total body potassium level.

Hypernatraemia

- Pure water deficit
- Diabetes insipidus
- Primary hypodipsia
- Hypotonic fluid loss from the GIT
- Third space fluid loss (*e.g. burns/effusions*)
- Renal dysfunction
- Salt poisoning

Hyponatraemia

- Addison's disease
- Pseudohyponatraemia
- Hyperglycaemia
- Liver disease
- Congestive heart failure
- Nephrotic syndrome
- Renal failure
- Psychogenic polydipsia
- Vomiting/diarrhoea
- Frusemide administration

Hyperkalaemia

- Addison's disease
- Pseudohyperkalaemia (*e.g. thrombocytosis, Akitas, equine RBCs*)
- Acidosis
- Urethral obstruction or ruptured urinary bladder
- Acute renal failure
- Diabetic ketoacidosis
- Acute tumour lysis syndrome
- Reperfusion after saddle thrombus in cats

Hypokalaemia

- Administration of potassium-free fluids
- Alkalosis
- Hypokalaemic myopathy in cats
- Insulin and glucose
- Vomiting/diarrhoea
- Chronic renal failure
- Post-obstructive diuresis
- Frusemide administration

Possible causes of abnormalities in sodium and potassium

Increased Na:K ratio

- Alkalosis
- Diarrhoea

Decreased Na:K ratio

- Addison's disease
- Diarrhoea
- Ruptured urinary bladder
- Akita breed

Abnormalities in sodium:potassium ratio

Sodium/Potassium Ratio

Sodium/Potassium ratios are used primarily as indicators of Addison's disease. However, anything that decreases the serum sodium concentration and/or increases the serum potassium concentration can decrease the sodium/potassium ratio. Ratios below 23:1 are suggestive of decreased mineralocorticoid activity. However, an ACTH stimulation test should be performed to confirm Addison's disease because other diseases (*diarrhoea, ruptured urinary bladder*) may cause sodium/potassium ratio to decrease below 23:1.

Artificial increases in potassium are common especially in clotted/aged samples and should be interpreted with care. The Akita breed has a naturally occurring high RBC potassium level and interpretation in this species is difficult. No diagnostic significance is put on an elevated sodium/potassium ratio, however, any disorder (*metabolic alkalosis, diarrhoea, etc.*) which will cause elevated sodium and/or decreased potassium concentrations can result in an elevated sodium/potassium ratio. Simultaneous elevations (*dehydration*) or depressions (*diarrhoea, etc.*) in both sodium and potassium concentrations generally result in a normal sodium/potassium ratio.