Plasma

The plasma is basically extracellular fluid (ECF). It transports a large number of substances from sites of absorption or production to sites of utilization or excretion and contains many other substances which are essential in precise concentrations for the proper function of the ECF. Therefore, analysis of plasma samples can provide a variety of types of information.

Plasma is the fluid portion of whole blood in which the cells are suspended. It is composed of approximately 90% water and 10% dissolved constituents such as proteins, lipids, salts, waste material, antibodies, and other ion and molecules. If the sample cannot be centrifuged within 1 hour, it must be refrigerated. Most biochemical tests can be performed on either plasma from a *heparinized* sample. Also note that clotted blood must be collected into *glass* vials, or plastic vials which have been specially coated to be suitable for the purpose.

Serum

Serum is plasma from which fibrinogen; a plasma protein has been removed. During the clotting process, the soluble fibrinogen in plasma is converted to an insoluble fibrin clot matrix. Serum can be refrigerated or frozen. Freezing may affect some test results.

<u>Haemolysis causes</u>:

1. Use of wet syringe.

2. Expel directly from syringe through needle.

3. During separation of serum, ringing of the blood clot is to be done gently which otherwise leads to hemolysis.

4. Cleansing the venipuncture site with alcohol and not allowing the site to dry may cause hemolysis.

5. The use of a small-bore needle, resulting in a large vacuum force applied to the blood, may cause shear stress on the red blood cells, causing them to rupture.

6. Bacterial and Chemical contamination.

Hemolysis can interfere with the result of investigation e.g. total protein, albumin, bilirubin, glycerol, potassium or may cause interference in the test method (e.g., spectrophotometric methods).

The Plasma Proteins

Normal total protein around 60 - 80 g/l (a little lower in dogs). Normal albumin around 25- 35 g/l (dogs and cats lower than large animals). Plasma contains a mixture of proteins , albumin, `globulins', enzymes, specific transfer proteins (e.g. transferrin), protein hormones and clotting factors. Most are synthesized in the liver from amino acids. All have different specific functions but as a group they function to maintain the osmotic pressure of the plasma. Total protein is usually measured by the biuret method, but refractometry is useful if an emergency result is required. Separation of protein fractions in the first instance is done by measuring albumin separately, and subtracting this from the total protein result to give the `globulin' concentration.

Increased total protein concentration

Increase in total serum protein (hyperproteinemia) is observed in

1. Shock.
2. Dehydration.
3. neoplasms like lymphosarcoma and plasmacytoma.

Decreased total protein and/or albumin concentration

A decrease in protein content is known as hypoproteinemia which is observed in

1. maldigestion
2. malabsorption
3. starvation
4. burns
5. lactation
6. renal disease
7. liver disease
8. pregnancy
9. parasitic diseases
10. diarrhea and dysentry.

Calcium

Normal plasma concentration about 2- 3 mmol/l (2.5- 3.5 mmol/l in horses) function in the body which includes maintenance of neuromuscular excitability, maintenance of activity of many enzymes, facilitation of blood coagulation. It is also a major component of bone. About half of the plasma calcium is free, and this is the active proportion, while the other half is inactive, bound to albumin. Low serum calcium should result in an increased production of Parathyroid hormone (PTH). PTH increases calcium resorption from bone and absorption of calcium from the GI tract. Calcitonin is a hormone produced in the thyroid gland. If the calcium is increased, calcitonin is produced to decrease calcium absorption from the intestines, decrease calcium reabsorption from the renal tubules, and inhibit resorption of calcium from bones.

The increased serum calcium levels are known as hypercalcemia which is observed in

1. hyperproteinemia
2. hyperthyroidism
3. hyperparathyroidism and after administration of vitamin D.

The hypocalcemia, decreased serum calcium levels, which is observed in

1. hypothyroidism
2. starvation
3. ketosis
4. milk fever
5. pancreatitis, and rickets.

Phosphorus

Phosphorus is an important component of adenosine triphosphate (ATP) and therefore essential for energy production. Phosphorus contributes to the structure of the matrix of bone, phosphoproteins, and phospholipids.

The increased serum phosphorus levels are known as hyperphosphatemia, which is observed in

1. renal failure
2. hypoparathyroidism
3. increased dietary intake
4. tissue necrosis, acidosis.

Hypophosphatemia, decreased serum phosphorus level, which is observed in

1. inadequate intake
2. rickets
3. hyperparathyroidism
4. heavy parasitism
5. rheumatism like syndrome, and Increase renal excretion.

Magnesium

Normal plasma concentration about 1- 2 mmol/l.

Magnesium is the fourth most common cation in the body and the second most common intracellular cation. Magnesium is found in all body tissues. More than 50% of the magnesium in the body is found in bones related to calcium and phosphorus. Magnesium plays an important role in production and destruction of acetylcholine, a compound required for impulse transmission at neuromuscular junctions. Magnesium occurs at a higher concentration in erythrocytes than in plasma in all animal species except the bovine.

Hypermagnesemia

1. Decreased excretion
2. increased intake
3. Cellular necrosis, and adrenocortical hypofunction

Hypomagnesemia

Hypomagnesemia has been associated with

1. hypoparathyroidism
2. diabetes mellitus
3. lactation tetany, and inadequate dietary magnesium.

Urea

Normal plasma concentration around 3- 8 mmol/l (up to 15 mmol/l in cats).

Urea is a nitrogenous waste product which is formed in the liver as the end product of amino acid breakdown. After the urea has been formed in the liver it is transported in the plasma to the kidneys where it is excreted in the urine.

The concentration of urea in serum or blood is increased in

1. chronic or acute nephritis
2. urinary obstruction
3. intestinal obstruction
4. cirrhosis of liver and increased protein intake.

The decreased serum urea levels are observed in

1. acute hepatic insufficiency
2. chronic diseases
3. nephrosis, and decreased protein intake .

KETONES

Ketone bodies' (acetone, acetoacetate and betahydroxybutyrate)

Older methods of measurement of ketones generally were sensitive only to acetone but nowadays assays specific for β-OH butyrate are most commonly used. All values given here are for β-OH butyrate. Ketosis develops due to a deficiency of glucose passing through the glycolytic pathway in the cells. Lack of the products of glycolysis prevents the Krebs' cycle from functioning. At the same time fatty acids are being utilized as an alternative body fuel and the ketone bodies are formed as an abnormal by product of fatty acid catabolism due to the blocked Krebs' cycle.

In ketotic animals ketones are readily detected in the urine by ordinary dipstix. Rothera's powder has been used for many years to test for ketones in milk and appears to be more sensitive. Many people can actually smell the ketones in the animal's breath.

Causes of ketonemia and ketonuria

1. Anorexia
2. starvation
3. diabetes mellitus
4. bovine ketosis, and ovine pregnancy toxemia.

Alanine aminotransferase (ALT)

In dogs, cats, and primates, the major source of ALT is the hepatocyte, where the enzyme is found free in the cytoplasm. ALT is considered a liver specific enzyme in these species. Horses, ruminants, pig, and birds do not have enough ALT in the hepatocytes for this

enzyme to be considered liver specific. Other sources of ALT are renal cells, cardiac muscle, skeletal muscle and the pancreas. Damage to these tissues may also result in increased serum ALT level. ALT is used as a screening test for liver disease because it is not precise enough to identify specific liver diseases.

Aspartate aminotransferase

AST is present in hepatocytes both free in the cytoplasm and bound to the mitochondrial membrane. More severe liver damage is required to release the membrane bound AST. AST is found in significant amounts in many other tissues including erythrocytes, cardiac muscle, skeletal muscle, the kidney and pancreas. The most common causes of increased blood level of AST are hepatic disease, muscle inflammation or necrosis and hemolysis.

Alkaline phosphatase

Alkaline phosphatase (AP) is present as enzymes in many tissues particularly osteoblasts in bone, chondroblasts in cartilage, intestine and placenta and cells of the hepatobiliary system in the liver. AP concentration is most often used to detect cholestasis in adult dogs and cats. Because of wide fluctuations in normal blood AP levels in cattle and sheep this test is not as useful for detecting cholestasis in these species.

Gamma glutamyltranspeptidase

GGT is found in many tissues including renal epithelium, mammary epithelium, and biliary epithelium, but is primary source is the liver. Cattle, horses, sheep, goats, and birds have higher blood GGT activity than dogs and cats. Other sources of GGT including kidney, pancreas, intestine, and muscle cells. The blood GGT elevated with liver disease especially with obstructive liver disease.