



TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

LECTURE NOTES ON
UNIT – 3
VETERINARY ANALYTICAL BIOCHEMISTRY

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VETERINARY ANALYTICAL BIOCHEMISTRY

Disorders of Carbohydrate Metabolism

DIABETES MELLITUS

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood leading to hyperglycemia, polydipsia, polyphagia, polyuria.

There are two principle forms of diabetes:

- In type 1 diabetes mellitus (previously called juvenile-onset or insulin-dependent), insulin production is absent because of autoimmune pancreatic beta-cell destruction possibly triggered by an environmental exposure in genetically susceptible people. This form develops most frequently in children and adolescents, but is being increasingly noted later in life.
- In type 2 diabetes mellitus (previously called adult-onset or non-insulin-dependent), insulin secretion is inadequate because patients have developed resistance to insulin. Hepatic insulin resistance leads to an inability to suppress hepatic glucose production, and peripheral insulin resistance impairs peripheral glucose uptake. This combination gives rise to fasting and postprandial hyperglycemia. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Symptoms and Signs

The most common symptoms of diabetes mellitus are those of hyperglycemia. More significant hyperglycemia causes glycosuria and thus an osmotic diuresis, leading to urinary frequency, polyuria, and polydipsia that may progress to orthostatic hypotension and dehydration. Severe dehydration causes weakness, fatigue, and mental status changes.

Diagnosis:

Diabetes mellitus is characterized by recurrent or persistent high blood sugar and is diagnosed by demonstrating any one of the following.

- Fasting plasma glucose level $\geq 7.0 \text{ mmol/l}$ (126 mg/dl) - A persistent fasting hyperglycemia is the single most important diagnostic criteria of diabetes mellitus. According to the current definition, two fasting glucose measurements above 7.0 mmol/l (126 mg/dl) is considered diagnostic for diabetes mellitus.

- Glucose tolerance test (OGTT): Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 gram oral glucose load.
- Glycated proteins:
 - Glycated hemoglobin (HbA_{1c}) -The glycated hemoglobin, HbA_{1c} , is known to reflect the average blood glucose level over the preceding 60 days. In Diabetes mellitus, $\text{HbA}_{1c} \geq 48$ mmol/mol (≥ 6.5 DCCT %).
 - Fructosamine (FrAm) - Fructosamines reflect the average blood glucose over the preceding 2 weeks in a manner analogous to HbA_{1c}. This means that FrAm could be used to monitor the average blood glucose on a biweekly interval. This has the advantage that changes in blood glucose can be detected more quickly than with HbA_{1c} and allows for timely clinical intervention.

WHO diabetes diagnostic criteria				
Condition	2-hour glucose	Fasting glucose	HbA_{1c}	
Unit	mmol/l(mg/dl)	mmol/l(mg/dl)	mmol/mol	DCCT %
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycaemia	<7.8 (<140)	$\geq 6.1 (\geq 110)$ & $<7.0 (<126)$	42-46	6.0–6.4
Impaired glucose tolerance	$\geq 7.8 (\geq 140)$	$<7.0 (<126)$	42-46	6.0–6.4
Diabetes mellitus	$\geq 11.1 (\geq 200)$	$\geq 7.0 (\geq 126)$	≥ 48	≥ 6.5

DM in animals:

In animals, diabetes is most commonly encountered in dogs and cats. Middle-aged animals are most commonly affected. Female dogs are twice as likely to be affected as males, while according to some sources, male cats are also more prone than females. In both species, all breeds may be affected, but some small dog breeds are particularly likely to develop diabetes, such as Miniature Poodles.

Feline diabetes mellitus is strikingly similar to human type 2 diabetes. The Burmese breed, along with the Russian Blue, Abyssinian, and Norwegian Forest cat breeds, showed an increased risk of DM, while several breeds showed a lower risk. There is an association between overweight and an increased risk of feline diabetes.

The symptoms may relate to fluid loss and polyuria, but the course may also be insidious. Diabetic animals are more prone to infections. The long-term complications recognized in humans are much rarer in animals. The principles of treatment (weight loss, oral antidiabetics, subcutaneous insulin) and management of emergencies (e.g. ketoacidosis) are similar to those in humans.

KETOSIS

Ketosis is a metabolic disorder characterised by increased levels of ketone bodies in blood. The three ketone bodies are acetone, acetoacetate and β -hydroxybutyrate. Acetone is relatively volatile, whereas the other two ketones are not. Ketone bodies are normally produced in the body and utilised by peripheral tissues regularly. In the non ruminant, the liver is the sole source of ketone bodies and In ruminants, the rumen epithelium and mammary gland are also sources of ketone bodies.

The balance between production and utilisation reflects the level of ketone bodies. The ketone bodies appear in the body fluids when production exceeds the capacity for utilization. They are low renal threshold substances as well as being highly volatile so they readily appear in urine, milk, and in the breath. A high concentration of ketones in the plasma results in a metabolic acidosis known as ketoacidosis. The most significant ketoacidoses commonly encountered in domestic animals are in diabetes mellitus and ovine pregnancy toxæmia. Ketosis occurs when there is a deficient carbohydrate metabolism leading to increased utilisation of fatty acid oxidation as seen in starvation, Diabetes mellitus etc.

The most common qualitative test for ketones is the Rothera test or alkaline nitroprusside test, which detects the ketones in whole blood, serum, plasma, urine, and milk. The test is highly sensitive for acetoacetate and acetone to lesser extent.

BOVINE KETOSIS

Bovine ketosis is actually at least three different syndromes that occur in cows during lactation. The syndromes are characterized by anorexia, depression (usually), ketonemia, ketolactia, ketonuria, hypoglycemia, Acetone (pear drop) smell of breath/ or milk and decreased milk production.

The three syndromes are Underfeeding ketosis, alimentary ketosis, and spontaneous ketosis.

- **Starvation / Underfeeding ketosis** occurs when a dairy cow receives insufficient calories to meet lactational demands plus body maintenance. This occurs when the cow has a normal appetite but is given an insufficient quantity of feed or a diet with low metabolic energy density or when a cow has some other disease, such as hypocalcaemia, mastitis, or metritis, which suppresses appetite and causes the cow to consume insufficient nutrients.
- **Alimentary ketosis** occurs when cattle have been fed spoiled silage that contains excessive amounts of butyric acid. The rumen epithelium has a high capacity to activate butyrate to acetoacetate and 3-hydroxybutyrate. Under conditions where excessive butyrate is presented to the rumen epithelium, large amounts of 3-hydroxybutyrate will be produced and released to the circulation with resulting ketosis.
- **Spontaneous ketosis** is the most common form of ketosis which occurs in high-producing dairy cows that are near the peak of lactation. Increased milk production leads to increased requirements of glucose for lactose synthesis. If there is a mismatch between mammary drain

of glucose for lactose synthesis and gluconeogenesis in the liver, hypoglycaemia will result. This leads to hypoglycaemia and ultimately ketonemia.

OVINE PREGNANCY TOXAEMIA

Pregnancy toxæmia in sheep and goats is also known as lambing sickness and twin-lamb/kid disease. It is a common metabolic disorder of ewes that is caused by the increased energy requirements in the late stage of pregnancy being greater than the energy provided by the diet consumed. It occurs in sheep usually carrying multiple foetuses. It is widespread and may affect any age or breed of pregnant ewe. Ewes in over-fat or very poor condition are most at risk.

Pregnancy toxæmia is most likely to occur in late pregnancy because most fetal growth (and hence most glucose demand) occurs in the final weeks of gestation. The growing foetuses continually remove large quantities of glucose and amino acids for their growth and energy requirements. It may be triggered by insufficient feed energy intake (anorexia due to weather conditions, stress or other causes), producing ketone bodies. Among ewes with pregnancy toxæmia, beta-hydroxybutyrate in blood tends to be higher.

The disease is characterized by depression and weakness in the ewes, which is associated with hypoglycaemia, ketonemia, and ketonuria. There is also considerable fatty deposition in the liver to the extent that it may interfere with liver function. Eventually, the ewes are unable to rise, become comatose, and die if untreated.

HYPOGLYCAEMIA IN BABY PIGS

Hypoglycemia of baby pigs occurs during the first few days of life and is characterized by hypoglycaemias of <2.2 mmol/liter (<40 mg/dl), apathy, weakness, convulsions, coma, and finally death. The newborn baby pig is particularly susceptible to hypoglycemia. At birth, the blood glucose level is >6 mmol/liter (>110 mg/dl) and unless the pig is fed, its blood glucose drops rapidly to hypoglycaemic levels within 24-36 hours. In contrast, newborn lambs, calves, and foals are able to resist starvation hypoglycemia for more than a week. If the baby pig suckles, its ability to withstand starvation progressively increases from the day of birth.

Gluconeogenic mechanisms are undeveloped in the newborn pig, which indicates that the gluconeogenic enzymes of the baby pig are inadequate at birth. This also indicates that these enzymes need to be induced by feeding so they can reach their maximal activities within 1 or 2 weeks after birth.

Starvation of the newborn pig under natural conditions can occur due to factors relating to the sow (agalactia, metritis, etc.) or to the health of the baby pig (anemia, infections, etc.), either case resulting in inadequate food intake. The requirement for feeding to induce the hepatic gluconeogenic mechanisms in the newborn baby pig explains its inability to withstand starvation in contrast to the newborn lamb, calf or foal which is born with fully functioning hepatic gluconeogenesis.

HYPERINSULINISM IN DOGS

Hyperinsulinism is known to be due to a persistent hyperactivity of the pancreas as the result of insulin-secreting islet cell tumors. Hyperinsulinism in dogs is characterized by a persistent hypoglycemia with periods of weakness, apathy, fainting and during hypoglycemic crises, convulsions and coma. This is seen in dogs with insulinomas. A history relating the attacks to periods after fasting or exercise provides a clinical basis for further investigations. Establishment of the diagnosis depends on finding a hypoglycemia of 3 mmol/l (55 mg/dl) at the time of symptoms and a hyperinsulinemia, usually >20 µU/ml. The symptoms are also relieved by glucose administration.

HORMONAL REGULATION OF CARBOHYDRATE METABOLISM

INSULIN

- Insulin is a protein hormone secreted by the β-cells of pancreas.
- It is a hypoglycaemic hormone. High blood glucose level is the most important stimulus for the release of insulin.
- Insulin stimulates the glucose transporter, GLUT4, present in skeletal muscles and adipocytes, thereby increasing the transport of glucose into these tissues.
- However tissues like brain, liver, kidney cortex and blood cells do not require insulin for glucose transport; since the glucose transporter (GLUT) molecules in these tissues are different and do not require the presence of insulin for stimulation.
- Insulin indirectly helps in glucose metabolism by stimulating glucokinase and phosphorylating glucose to glucose 6-phosphate, which results in the trapping of glucose inside the cells.
- Insulin stimulates glycogen synthesis in the liver by stimulating glycogen synthase.
- Insulin promotes amino acid uptake by the cells and increases protein synthesis, thereby reducing the amino acid availability for gluconeogenesis.
- Substances like amino acids, free fatty acids, ketone bodies, glucagon, secretin and the sulfonylurea drugs, tolbutamide cause the release of insulin.
- Epinephrine and nor epinephrine inhibit the release of insulin.
- Insulin is the only hormone, which lowers blood glucose level and promotes glucose storage.
- cAMP levels decrease in the presence of insulin.

GLUCAGON

- A protein hormone secreted by α-cells of pancreas in response to low blood glucose level (hypoglycemia). It has its effects via the production of cAMP.
- It is a hyperglycaemic hormone.
- The effect of glucagon is opposite to that of insulin.
- Glucagon has its effect only on liver cells.
- Glucagon restores blood glucose level
 - 1. by stimulating glycogenolysis and
 - 2. by enhancing gluconeogenesis from amino acids, glycerol and lactate.

EPINEPHRINE

- It is an amino acid derived hormone, synthesised from phenyl alanine or tyrosine.
- Epinephrine is secreted from adrenal medulla in response to stressful stimuli (the 3 'F"s - Fight, fright & flight, excitement, hemorrhage, hypoxia, hypoglycemia, etc.,).
- Epinephrine stimulates glycogenolysis both in liver and skeletal muscles, via the production of cAMP.
- Muscle glycogen is not a direct source of glucose due to the absence of glucose- 6- phosphatase. Therefore, glycogenolysis results in the formation of lactate, which is taken to liver for gluconeogenesis.
- Epinephrine decreases the peripheral utilization of glucose.

THYROID HORMONES - THYROXINE

- Thyroxine (T4) and Triiodo thyronine (T3), are amino acid derived hormones, synthesized from tyrosine and secreted by the thyroid gland.
- It increases the serum glucose level, which may be due to the enhanced absorption of glucose from the intestine.
- It is also involved in the depletion of glycogen. The liver, which is depleted of glycogen can be easily damaged. The damaged liver is unable to take glucose resulting in the increased glucose level.

CORTICOSTEROIDS

- Glucocorticoid hormone, cortisol is secreted from adrenal cortex.
- It increases the level of glucose in plasma mainly by gluconeogenesis in liver.
- It increases protein catabolism in extrahepatic tissues and increases amino acid uptake by the liver for gluconeogenesis.
- It also increases hepatic glycogenesis and decreases peripheral glucose utilization.

GROWTH HORMONE

- It is a peptide hormone, secreted by pituitary gland.
- It increases the blood glucose level by decreasing the glucose uptake by extra hepatic tissues and increases glucose uptake in liver.
- Growth hormone mobilizes the free fatty acids from adipose tissues, for energy production,
- sparing glucose, leading to an increased blood glucose level.

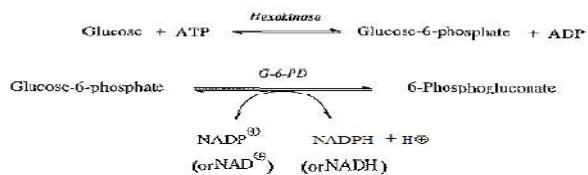
Biochemical tests for the detection of disturbance in carbohydrate metabolism

A) Measurement of Glucose in Blood

Hexokinase or glucose oxidase are widely used in assays to measure the concentration of glucose in blood. Glucose dehydrogenase is used in some methods.

a) Hexokinase Method

In the "hexokinase method," glucose-6-phosphate formed from glucose and ATP by hexokinase (HK) is oxidized by NAD in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PD) to give NADH, which is quantitated spectrophotometrically at 340 nm (alternatively NADP⁺ may be used as the co-enzyme). The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

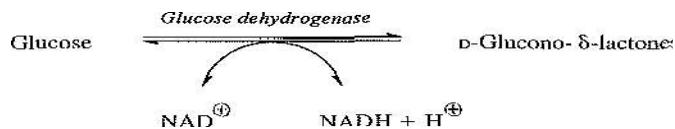


b) Glucose Oxidase Method

Glucose oxidase is highly specific for D-glucose. Glucose oxidase methods are suitable for measurement of glucose in CSF. Glucose is oxidized by glucose-oxidase (GOD) in the presence of atmospheric oxygen to gluconolactone and hydrogen peroxide. The hydrogen peroxide is then oxidized by peroxidase (POD) in the presence of 4-aminophenazone and phenol to form the red dye 4-(*p*-benzochinone-monoimino)-phenazone, which is quantitated spectrophotometrically at 505 nm.

c) Glucose Dehydrogenase Method

The enzyme glucose dehydrogenase catalyzes the oxidation of glucose to gluconolactone. The amount of NADH generated is proportional to the glucose concentration.



B) Tolerance tests

1) Glucose Tolerance Tests

Glucose tolerance (GT) is referred to the amount of glucose that could be ingested by an animal without producing glucosuria.

- **Oral glucose tolerance tests (OGTT):**

Used to measure how well the body can process a larger amount of sugar. If the blood sugar measured in the test is above a certain level, this could be a sign that sugar is not being absorbed enough by the body's cells. The glucose tolerance test can be used to screen for type 2 diabetes and diagnose gestational diabetes.

The oral glucose tolerance test is ineffective in the ruminant because the ingested carbohydrate is almost totally fermented by the rumen micro flora. The OGTT has been used in dogs by feeding of a test meal consisting of 4 g glucose/kg body weight. A fasting blood sample is taken, the test meal given and blood samples are taken at 30-minute intervals for 3 hours.

- **Intravenous Glucose Tolerance Test:**

0.5 g glucose/kg body weight is infused IV as a sterile 50% solution in 30 seconds. The OGTT and the insulin response are of greatest value in the diagnosis of diabetes, particularly those cases with a mild hyperglycemia and without persistent glucosuria.

2) Insulin tolerance test:

An insulin tolerance test (ITT) is a medical diagnostic procedure during which insulin is injected into a patient's vein, after which blood glucose is measured at regular intervals. This procedure is performed to assess pituitary function, adrenal function, and sometimes for other purposes.

About 0.1 unit of crystalline zinc insulin per kilogram body weight is injected intramuscularly or subcutaneously and blood samples are taken every 30 minutes for 3 hours. The test measures (1) the sensitivity of the blood glucose level to a test dose of insulin and (2) the response of the animal to insulin-induced hypoglycaemia.

3) Glucagon Stimulation Test

Glucagon via hepatic glycogenolysis and gluconeogenesis has a hyperglycemic effect that in turn evokes an insulin response. In addition, glucagon is an insulin secretagogue second only to glucose. These are the bases for the glucagon stimulation test (GST) and have been used for the diagnosis of diabetes in cats.

The test is performed by the IV injection of 30 µg glucagon/kg body weight. Samples for blood glucose and insulin are obtained before injection (0 time) and at 5, 10, 15, 30, 45, and 60 minutes after injection.

4) Epinephrine Tolerance Test

Epinephrine also has a post injection hyperglycaemic effect via hepatic glycogenolysis. The blood glucose level rises to a maximum of 50% above the fasting level in 40-60 minutes and returns to the original level in 1.5-2 hours. The test is performed by obtaining a fasting blood sample (0 time), injecting 1 ml of 1: 1000 epinephrine-HCl (in the dog) intramuscularly and obtaining blood samples every 30 minutes for 3 hours.

5) Leucine-Induced Hypoglycemia

The oral administration of L-leucine induces a marked and persistent hypoglycemia in hyperinsulinism due to pancreatic islet cell tumors. The hypoglycaemia is associated with a rise in plasma insulin due to increased release of insulin by the tumorous islet cells. The test is performed by the oral administration of 150 mg L-leucine/kg body weight as an aqueous suspension to the fasting dog. A fasting blood glucose sample is taken before administration (0 time) and every 30 minutes for 6 hours. A hypoglycemic effect is seen quickly at 0.5-1 hour and may persist for as long as 6 hours in hyperinsulinism. The normal dog exhibits no hypoglycemic effect.

6) Tolbutamide Test

The intravenous administration of tolbutamide, an oral hypoglycemic agent, induces the release of insulin from the pancreas and is used as a test of the availability of insulin from the pancreas. The blood glucose curve during the test parallels the insulin tolerance test. This test has not been used in animals.

C) Urinalysis

Tests to detect the presence of sugar in urine sample.

- **Fehling's Test-** In Fehling's test, Fehling's solution-A and Fehling's solution-B are used as the reagents. Fehling's solution-A is an aqueous solution of copper (II) sulphate, having blue colour, while Fehling's solution-B is clear colourless aqueous solution of sodium potassium tartrate.
- **Benedict's Test** - Benedict's reagent is a combination of sodium carbonate, sodium citrate and copper (II) sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). This is both qualitative and semi quantitative test

On boiling the urine sample with the reagents, the copper (II) sulphate (CuSO_4) present in the Benedict's solution and Fehling's solution is reduced by the reducing agent, glucose (sugar), to form a coloured precipitate of cuprous oxide.

Depending upon the concentration of glucose, green, yellow and brick red precipitates of cuprous oxide are formed. Below is the table showing the color sequence depending upon the concentration of glucose level.

Colour of precipitate	Percentage of sugar present
Blue	sugar absent
Green	0.5 to 1%
Yellow	1 to 2 % sugar
Brick Red	2 % or more sugar

- **Methylamine test :** For detection of lactose in urine

Methods for detecting ketones in urine:

- **Rothera's test** (alkaline nitroprusside test) - Acetoacetic acid and acetone react with alkaline solution of sodium nitroprusside to form a purple colored complex. This method can detect above 1-5 mg/dl of acetoacetic acid and 10-20 mg/dl of acetone. Beta-hydroxybutyrate is not detected.
- Nitroprusside test is available as a test tablet (Acetest) and as a coated reagent strip (Ketostix)
ACETEST Tablet Test: The Acetest tablet consists of sodium nitroprusside, glycine, and an alkaline buffer. A purple lavender discoloration of the tablet indicates the presence of acetoacetate or acetone (≥ 5 mg/dl). A rough estimate of the amount of ketone bodies can be obtained by comparison with the color chart provided by the manufacturer. The test is more sensitive than reagent strip test for ketones.
- **Gerhardt's Ferric Chloride Test**
Addition of 10% ferric chloride solution to urine causes solution to become reddish or purplish if acetoacetic acid is present. The test is not specific since certain drugs (salicylate and L-dopa) give similar reaction. Sensitivity of the test is 25-50 mg/dl.
This is an indirect method to detect the β - hydroxybutyrate in the urine.
- β - hydroxybutyrate dehydrogenase enzyme assay: For BHBA estimation, coupled with NBT reduction.

D) Other tests:

- Glycated hemoglobin (HbA1c): Estimated by HMF-TBA (5- hydroxyl methyl furfural – thiobarbituaric acid) colorimetric method, HPLC methods.
- Fructosamine : Measured by a colorimetric method

PLASMA PROTEINS

Plasma proteins are proteins that are synthesized in the hepatocytes or liver cells and release in the blood plasma except immunoglobulins.

Plasma proteins play a vital role in the body metabolism being part of blood, they move from vessels to organs supplying their functions.

Function of plasma proteins

- Maintenance of colloid oncotic pressure (eg : Albumin)
- They help in the transportation of molecules or substances like Lipid transported by apoproteins, cortisol transported by cortisol transporting proteins, Iron by transferrin, drugs and toxic substances by albumin etc.
- They also help in defending the body against foreign organism and particles (Immunoglobulins and complements)
- They are involved in blood coagulation or clotting e.g clotting factors such as fibrinogen
- Buffering the Hydrogen ions of blood.
- Hormones
- Enzymes
- Viscosity (eg: fibrinogen)
- Tumour markers
- Anti- proteases

TABLE 5.2 Common Serum Proteins, Their Function, and Changes in Disease

Protein	M _r (Da)	Function	Change in disease
Prealbumin	54,400	Thyroxine transport	Increase: nephrotic syndrome Decrease: liver disease, protein deficiency
Albumin	69,000	Osmotic pressure regulation, general transport	Increase: dehydration Decrease: liver, kidney, gastrointestinal disease, malnutrition, blood and plasma loss
α_1 -Globulins (α_1 and α_2)			
Thyroxine-binding globulin (TBG)	54,000	Thyroxine transport	Increase: pregnancy
α_1 -Fetoprotein	65,000	Unknown	Increase: hepatoma, pregnancy
α_1 -Antitrypsin	45,000	Trypsin inhibitor	Increase: acute inflammatory disease Decrease: liver disease, chronic pulmonary disease
α_1 -Antichymotrypsin	68,000	Chymotrypsin inhibitor	Increase: acute inflammatory disease
α_1 -Acid glycoprotein (orosomucoid, seromucoid)	44,000	Unknown	Increase: acute inflammatory disease Decrease: liver disease, nephrotic syndrome, malnutrition
α_1 -Antithrombin III	65,000	Thrombin inhibitor	Increase: disseminated intravascular coagulation, liver disease
α_1 -Lipoprotein (HDL, α -lipoprotein)	200,000	Lipid transport	Increase: nephrotic syndrome, diabetes mellitus, hypothyroidism, steroid therapy
α_2 -Lipoprotein (VLDL, pre- β lipoprotein)	1,000,000	Lipid transport	Increase: nephrotic syndrome, chronic active liver disease, acute inflammatory disease
α_2 -Macroglobulin	820,000	Insulin binding, trypsin inhibitor	
α_2 -Globulin	54,000	Thyroxine transport in dogs	
Ceruloplasmin	151,000	Copper transport, ferrioxidase	Increase: acute inflammatory disease
Haptoglobin	100,000	Hemoglobin binding	Increase: acute inflammatory disease
Protein C	62,000	Protease, anticoagulant	Increase: acute inflammatory disease
β -Globulins (β_1 and β_2)			
β_2 -Lipoprotein (LDL, β -lipoprotein)	2,750,000	Lipid transport	Increase: nephrotic syndrome, hypothyroidism, hepatocanicular disease
Transferrin	76,000	Iron transport	Increase: anemias, iron deficiency, pregnancy, acute liver disease, nephrotic syndrome Decrease: iron storage disease, chronic liver disease, acute inflammatory disease
Ferritin	465,000	Iron transport	Increase: iron storage disease, acute inflammatory disease Decrease: iron deficiency
Hemopexin	80,000	Heme transport	Decrease: hemolytic anemia, chronic active liver disease
C3 complement	75,000	Complement C3 factor	Increase: acute inflammatory disease, atopic dermatitis Decrease: autoimmune disease
C-Reactive protein	140,000	Activates complement	Increase: acute inflammatory disease
C4 complement		Complement C4 factor	Increase: acute inflammatory disease Decrease: autoimmune disease
Plasminogen		Proenzyme of plasmin, fibrinolysis	Increase: disseminated intravascular coagulation
Fibrinogen	340,000	Fibrin precursor, coagulation	Increase: acute inflammatory disease Decrease: disseminated intravascular coagulation, afibrinogenemia
γ -Globulins (γ_1 and γ_2)			
Immunoglobulin G (IgG)	150,000	Major antibody formed in response to infectious agents, toxins	Increase: infectious disease, connective tissue disease, liver disease, myelomas and other tumors of the reticuloendothelial system Decrease: fetuses, newborn animals before intake of colostrum, immune deficiency diseases, agammaglobulinemia
Immunoglobulin A (IgA)	150,000	Secretory antibodies in the fluids of the respiratory, gastrointestinal, and the genitourinary tracts	Increase: infectious disease, connective tissue disease, liver disease, myelomas and other tumors of the reticuloendothelial system Decrease: fetuses, newborn animals before intake of colostrum, immune deficiency diseases, agammaglobulinemia

Protein	M_r (Da)	Function	Change in disease
Immunoglobulin E, IgE	200,000	Antibodies in allergy	Increase: allergies, anaphylaxis Decrease: aglobulinemia
Immunoglobulin M, IgM Immunoglobulin D, IgD	900,000 160,000	Cold agglutinin, initiator Unknown, not seen in animals Part of the immunoglobulin molecule	Increase: chronic disease, primary cell reactions macroglobulinemia (Waldenstrom's)
Light chains (Bence-Jones protein)	30,000	molecule	Increase: myeloma

* M_r , Relative molecular mass.

Bence jones proteins:

Bence Jones protein is a monoclonal globulin protein or immunoglobulin light chain found in the urine, with a molecular weight of 22-24 kDa. Bence-Jones proteins are not usually seen in urine. Detection of Bence Jones protein may be suggestive of multiple myeloma or Waldenstrom macroglobulinemia. These proteins are also found in some people with lymphoma.

The proteins are immunoglobulin light chains (paraproteins) and are produced by neoplastic plasma cells. They can be kappa (most of the time) or lambda. The light chains can be immunoglobulin fragments or single homogeneous immunoglobulins. They are found in urine as a result of decreased kidney filtration capabilities due to renal failure. The light chains have historically been detected by heating a urine specimen (which causes the protein to precipitate) and recently by electrophoresis of concentrated urine.

Cryoglobulins:

Cryoglobulins (CRs) are special serum immunoglobulins that precipitate at temperatures <37°C, but mostly at 0 – 4°C, and that dissolve when re-warmed to 37°C . The majority (95%) of CRs are immune complexes (IC) that contain rheumatoid factor (RF). Such CRs are known as “mixed” cryoglobulins to differentiate them from the CRs with monoclonal bands that do not contain RF or antigen-antibody complexes.

Cryoglobulinemia is characterized by the presence of cryoglobulins in the serum. This may result in a clinical syndrome of systemic inflammation (most commonly affecting the kidneys and skin) caused by cryoglobulin-containing immune complexes

Significance of plasma protein concentration in diseases condition

Diseases may occur when there are defects in some plasma protein catabolism and in situation of dehydration and over hydration. Those plasma proteins involved in these disease conditions include

1. Albumin

Albumin has a low molecular weight and it is the most abundant in blood plasma making it the main protein responsible for the regulation of colloid oncotic pressure of blood plasma. Albumin is synthesized in the hepatocytes and has a high capillary permeability.

Decrease in albumin concentration or synthesis occurs in:

- Liver disease (only in chronic liver disease)
- Malnutrition
- Malabsorption
- Catabolic state especially during injury or surgery
- Renal diseases especially nephrotic syndrome

2. Pre-albumin and Retinol binding protein

They are mostly present in small quantities and are synthesized by the hepatocytes. They help in transporting Vitamin A and thyroxine. Fall in their concentration occurs during injury and in both acute and chronic liver disease. They are better markers than albumin to determine nutritional status such as Malnutrition or overnutrition.

3. Transferrin

Involved in transport of iron to the bone marrow. The Total iron binding capacity (TIBC) is a measure of the amount of transferrin. Decrease in the concentration or level of transferrin occurs in protein losing condition, infection and neoplastic disease. Increase transferrin will occur in iron deficiency state.

4. Haptoglobin

Haptoglobin is an Acute phase reactant. It belongs to the Alpha-2 globulin group.

Decrease haptoglobin occurs in Haemolytic anemia, liver disease and rarely in congenital abnormality. Increase level of Haptoglobin occurs in acute infection following trauma being an acute phase reactant.

5. Ceruloplasmin

This is a copper containing protein which binds about 90% of copper found in the blood plasma. Decrease occurs in Wilson's disease, malnutrition and nephrotic syndrome. Raise levels occurs during pregnancy and acute infections.

6. Alpha-1 antitrypsin

It is an alpha globulin protein and another acute phase reactant. Decrease or deficiency occurs during liver diseases while raised level will occur in pregnancy, acute infection that follows trauma.

7. Alpha 2 macroglobulin

This is the major alpha 2 globulin. It has an antiproteinase activity and decrease levels has not been recorded yet.

8. Alpha 1 fetoprotein (AFP)

Present in tissue and plasma of foetus. This plasma protein rapidly falls after birth but minute amount can be detected in adults. Increase or high level occurs in patients with hepatocellular carcinoma and gonadal neoplasm and in women carrying foetuses with open neural tubes.

9. Carcinoembryonic antigen (CEA)

Normally present in fetal intestinal cells but not measurable in healthy adults. This plasma protein reappears in the cells of intestinal neoplasm in adults. The appearance of CEA in the plasma was a diagnostic of intestinal carcinoma in adult like colon cancer. CEA is also released by carcinomas occurring in other sites and from non-carcinomatous lesions of the intestines. Post operatively, plasma CEA provide a means of assessing whether the tumor has been removed that is it should retain to normal within 6 weeks post operation.

10. C-Reactive protein (CRP)

This plasma protein is synthesized by the liver and released into the blood plasma. Small amounts are also made by a subset of peripheral lymphocytes but this remain bound to the surface of the cell. It is the first recognised acute phase reactant. It has a number of immunologic function which include:

- Activation of complements and monocytes
- Initiation of opsonization and phagocytosis
- It is also important in the recognition of necrotic tissues
- Its levels in plasma may increase markedly during acute phase response.

Acute-phase proteins

Acute-phase proteins (APPs) are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the acute-phase reaction (also called acute-phase response).

Positive acute-phase proteins serve (as part of the innate immune system) different physiological functions within the immune system. Some act to destroy or inhibit growth of microbes, e.g., C-reactive protein, mannose-binding protein, complement factors, ferritin, ceruloplasmin, serum amyloid A and haptoglobin. Others give negative feedback on the inflammatory response, e.g. serpins. Alpha 2-macroglobulin and coagulation factors affect coagulation mainly by stimulating it.

"Negative" acute-phase proteins decrease in inflammation. Examples include albumin, transferrin, transthyretin, retinol-binding protein, antithrombin, transcortin. The decrease of such proteins may be used as markers of inflammation. The physiological role of decreased synthesis of such proteins is generally to save amino acids for producing "positive" acute-phase proteins more efficiently.

Classification

Positive Acute Phase Proteins:

- α_1 -Globulins
 - α_1 -Antitrypsin
 - α_1 -Acid glycoprotein (orosomucoid, seromucoid)
- α_2 -Globulins
 - α_2 -Macroglobulin
 - Ceruloplasmin
 - Serum amyloid A
 - Haptoglobin
- β -Globulins
 - Fibrinogen
 - Complement, C3, C4
 - Protein C
 - C-reactive protein
 - Ferritin
 - Amyloid A

Negative Acute Phase Proteins:

- Prealbumin
- Albumin
- Transferrin

Major Responders in various species

Species	Major APP
Cat	TNF- α
Cow	Hp, SAA
Dog	CRP, SAA
Horse	SAA
Mouse	SAA, AGP
Pig	CRP
Rabbit	CRP
Rat	α_2 -Macroglobulin, AGP

Dysproteinemias

Dysproteinemia is a clinical state characterized by abnormal, often excessive, synthesis of immunoglobulin (Ig) molecules or subunits. Dysproteinemia results from clonal proliferation of plasma cells or B lymphocytes.

Classification based A:G ratio and serum protein electrophoresis (SPE) profile

-
- A. Normal A : G—normal SPE profile
 - 1. Hyperproteinemia: dehydration
 - 2. Hypoproteinemia
 - a. Overhydration
 - b. Acute blood loss
 - c. External plasma loss: extravasation from burns, abrasions, exudative lesions, exudative dermatopathies, external parasites; gastrointestinal disease, diarrhea
 - d. Internal plasma loss: gastrointestinal disease, internal parasites
 - B. Decreased A : G—abnormal SPE profile
 - 1. Decreased albumin
 - a. Selective loss of albumin: glomerulonephritis, nephrosis, nephrotic syndrome, gastrointestinal disease, internal parasites
 - b. Decreased synthesis of albumin: chronic liver disease, malnutrition, chronic inflammatory disease
 - 2. Increased globulins
 - a. Increased α_1 -globulin
 - i. Acute inflammatory disease: α_1 -antitrypsin, α_1 -acid glycoprotein (orosomucoid, seromucoid)
 - b. Increased α_2 -globulin
 - i. Acute inflammatory disease: α_2 -macroglobulin, ceruloplasmin, haptoglobin
 - ii. Severe active hepatitis: α_2 -macroglobulin
 - iii. Acute nephritis: α_2 -macroglobulin
 - iv. Nephrotic syndrome: α_2 -macroglobulin, α_2 -lipoprotein (VLDL)
 - c. Increased β -globulin
 - i. Acute hepatitis: transferrin, hemopexin
 - ii. Nephrotic syndrome: β -lipoprotein (LDL), transferrin
 - iii. Suppurative dermatopathies: IgM, C3
 - d. β - γ Bridging
 - i. Chronic active hepatitis: IgA, IgM
 - e. Increased γ -globulin (broad increases)—polyclonal gammopathies: IgG, IgM, IgA
 - i. Chronic inflammatory disease, infectious disease, collagen disease
 - ii. Chronic hepatitis
 - iii. Hepatic abscess
 - iv. Suppurative disease: feline infectious dermatitis, suppurative dermatitis, tuberculosis
 - v. Immune-mediated disease: autoimmune hemolytic anemia, autoimmune thrombocytopenia, Aleutian disease of mink, equine infectious anemia, systemic lupus erythematosus, autoimmune polyarthritis, autoimmune glomerulonephritis, autoimmune dermatitis, allergies
 - vi. Tumors of the reticulendothelial system (RES): lymphosarcoma
 - f. Increased γ -globulin (sharp increases)—monoclonal gammopathies: IgG, IgM, IgA
 - i. Tumors of the reticulendothelial system (RES): lymphosarcoma
 - ii. Plasma cell dyscrasias: multiple myeloma, Aleutian disease of mink
 - iii. Macroglobulinemia
 - iv. Canine ehrlichiosis
 - v. Benign
 - C. Increased A : G—abnormal profile
 - 1. Increased albumin: does not occur except in dehydration
 - 2. Decreased globulins
 - a. Fetal serum
 - b. Precolostral neonate
 - c. Combined immunodeficiency of Arabian foals
 - d. Aglobulinemia

LIPID PROFILE IN DIAGNOSIS

Hyperlipidemia is the increased concentration of triglyceride (hypertriglyceridemia), cholesterol (hypercholesterolemia), or both in the blood. Hyperlipidemia in dogs and cats can be physiological (postprandial) or pathological. Pathological hyperlipidemia can result from increased lipoprotein synthesis or mobilization or decreased lipoprotein clearance. It can be primary (genetic or idiopathic) or secondary to other disease processes.

Hyperlipidemias- Refers to increased plasma levels of cholesterol and triacylglycerols

Lipemias – refers to severe hyperlipidemia in which the plasma looks milky.

Hyperlipoproteinemia is often used synonymously with hyperlipidemia but is best used when plasma lipoprotein analysis is actually conducted. One of the first indications of hyperlipidemia is observation of turbid (i.e., cloudy) or lactescent (i.e., white) plasma, which suggests that lipemia.

The most common form of hyperlipidemia is post prandial hyperlipidemia, which is observed after an animal consumes a meal containing fat and is primarily a result of increased chylomicron levels.

Primary lipid disorders are rare in dogs, and the mechanisms inducing them are often unknown. Idiopathic hyperlipidemia in miniature schnauzers and hypercholesterolemia in briards are the two recognized primary lipid disorders in dogs. Familial hyperlipidemia occurs most often in miniature schnauzers and beagles.

Secondary lipid disorders

Causes include

- high-fat diets
- Hypothyroidism
- Uncontrolled Diabetes mellitus
- Acute pancreatitis
- Hyperadrenocorticism (Cushings syndrome)
- Cholestatic liver disease
- Nephrotic syndrome

Canine fasting hyperlipidemias

Healthy dogs do not develop significant hyperlipidemia on fasting. Fasting hyperlipidemias in dogs is usually an abnormal sign. It is commonly observed in dogs with hypothyroidism (congenital or acquired). The main lipid that is increased is cholesterol, but TAG can be increased too.

Fasting hyperlipidemias in felines appears to be associated with Diabetes mellitus and Nephrotic syndrome as in dogs. A well characterized familial hyperlipidemia is associated with lipoprotein lipase deficiency and hyperchylomicronemia.

Xanthomas (i.e., yellowish raised skin nodules or plaques composed of foamy macrophages laden with lipid) are often seen in cats with hyperlipidemia but are rarely present with hyperlipidemia in dogs

Equine hyperlipidemias may be seen in conditions like maxillary myositis and Equine infectious anemia.

Clinical Enzymology

Clinical enzymology can be described as the branch of science which deals with the application of enzyme activity analysis to the diagnosis and treatment of disease. It also helps us in the prognosis of disease and in the screening of abnormal organ functions.

The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance. This is because the presence of these enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood.

Plasma Enzymes

Enzymes present in plasma can be classified into 2 types, viz. Functional Plasma enzymes and Non-functional plasma enzymes.

Functional plasma enzymes (Plasma-derived enzymes)

- They function in plasma.
- Mostly synthesized by the liver.
- Present in plasma at higher concentration than tissues.
- Usually decreased in disease conditions. Eg. lipoprotein lipase, Clotting enzymes
- Their clinical importance is limited to diseases related to their own synthesis and function. i.e., abnormalities of metabolism of plasma lipoproteins and blood clotting and the organ function of their synthesizing tissues, e.g., thromboplastin as a liver function test.

Non-functional plasma enzymes (Cell-Derived enzymes)

- Present in plasma at lower concentration than tissues. A very low plasma level normally exists due to normal wear and tear and diffusion through undamaged cell membranes.
- Do not have any function in plasma.
- Mostly synthesized by liver, skeletal muscle, heart, brain etc
- Usually increased in disease conditions.
- Gross damage to the cells or abnormal membrane permeability, overproduction of the enzymes or abnormal high cellular proliferation may allow their leakage in abnormally high amount into plasma and other body fluids.

- The amount and nature of the plasma enzyme(s) reflects the extent and nature of the damaged tissue.
- Measurement of these enzymes in plasma can be used in the diagnosis of the disease.
Eg. Creatine kinase, Alanine transaminase etc

They are further subdivided into; secretory and metabolic non-functional plasma enzymes:

- Secretory: They are synthesized and secreted by specialized glands into body lumens mainly for digestion. Their retrograde escape into blood reflects damage in the tissue of their origin, e.g., pancreatic amylase and lipase in pancreatitis.
- Metabolic: They are intracellular metabolic enzymes and their appearance in the plasma is mainly due to cellular damage among other factors.

Estimation of enzymes activities in the serum has many applications

- Diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased),
- Assessing prognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before the occurrence of jaundice).

Isozymes (also known as isoenzymes)

Multiple molecular forms of the same enzyme that differ in amino acid sequence but catalyze the same chemical reaction. They differ in their kinetic parameters (K_m and V_{max} values), electrophoretic mobility and localization. They all have independent action. They can be differentiated from each other and can be clinically quantified. Isozymes are encoded by different genes and expressed in a distinct organelle or at a distinct stage of development. They can be used to identify the specific affected tissues.

Eg. Lactate dehydrogenase has 5 isoenzymes

Isozymes were first described by R. L. Hunter and Clement Markert.

Distribution and application of clinically important enzymes

Enzymes	Tissues	Clinical applications
Alanineamino transferase	Liver	Hepato parenchymal diseases
Alkaline phosphatase	Liver, bone, intestinal mucosa, Placenta	Liver and bone diseases
Amylase	Salivary glands, Pancreas	Pancreatic diseases
Aspartate amino transferase	Liver, Skeletal muscle, Heart, Erythrocytes	Hepatic parenchymal disease, Muscle disease
Cholinesterase	Liver	Organophosphorus insecticide poisoning, Hepatic parenchymal diseases
Creatine kinase	Skeletal muscle,Heart	Muscle diseases
Gamma glutamyl transferase	Liver	Hepatobiliary diseases, Marker of alcohol abuse
Lipase	Pancreas	Pancreatic diseases
Lactate dehydrogenase	Heart, liver, skeletal muscle erythrocytes, lymph nodes, Platelets	Hepatic parenchymal diseases, muscle diseases Hemolysis, tumor marker
5' nucleotidase	Liver	Hepatobiliary diseases
Trypsin	Pancreas	Pancreatic diseases

Some important enzymes of clinical significances are discussed below:

Alanine Aminotransferase (ALT)

- Alanine aminotransferase formerly known as glutamic pyruvate transaminase, catalyzes the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and L-glutamate. ALT, found in the cytoplasm of hepatocytes, is also found in mitochondria but generally at considerably lower concentrations, depending on species and tissue.
- Based on tissue concentrations of ALT, increased serum ALT activity is somewhat specific for hepatic injury in dogs and cats but offers no specificity for detection of liver injury in horses and cattle.
- Increased serum ALT activity occurs with a wide range of other disorders including hypoxia secondary to anemia, metabolic diseases such as lipidosis, nutritional disorders such as copper toxicosis, inflammatory or infectious diseases, neoplastic diseases, and traumatic liver injury. Increased serum ALT activity has also been associated with numerous drugs causing hepatocellular toxicity. Exposure to carbon tetrachloride, mushroom alkaloids, or acetaminophen is clearly a hepatotoxic event.
- Mild to moderate increases in serum ALT activity are also observed in dogs and cats with endocrine diseases such as diabetes mellitus, hyperthyroidism, hyperadrenocorticism and hypothyroidism.

Aspartate Aminotransferase(AST)

- Aspartate aminotransferase (formerly glutamic oxaloacetic transaminase; GOT) catalyzes the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate.
- AST is located in the cytosol but is in higher concentrations in mitochondria. Serum AST has a longer blood half-life than sorbitol dehydrogenase and creatine kinase and is stable for days in serum at room temperature, refrigerated or frozen. AST is routinely used for diagnosis of liver and muscle diseases in horses and food animals.
- Increased serum AST activity is observed with both reversible and irreversible injury to hepatocytes and can be seen following hepatocellular injury and cholestasis. Serum AST is also increased following myocyte injury.
- Markedly increased serum AST and sorbitol dehydrogenase activity suggest acute or active hepatocellular injury, whereas markedly increased serum AST with modest to moderate sorbitol dehydrogenase activity suggests chronic hepatic injury or recovery from acute liver injury.

Sorbitol Dehydrogenase (SDH)

- SDH is located in the cytoplasm of cells. The highest concentration of SDH activity is in liver followed by kidney. The half life of SDH in blood is relatively short in all species. Because of its short half-life and the labile nature of SDH activity in serum, SDH activity is less favored for detection of hepatic disease in dogs than serum ALT activity.
- Serum SDH activity is of greater value than serum AST activity in large animals because of its increased specificity for hepatocellular injury. Serum SDH activity has been used for the detection of hepatic lipidosis, hepatic necrosis, leptospirosis, fascioliasis and hepatic abscesses in cattle and for detection of hepatic necrosis, lipidosis and cirrhosis in horses.

Creatine Kinase (CK)

- CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle.
- CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). The CK-3 isoenzyme is normally responsible for almost all CK enzyme activity in healthy people. Isoenzymes BB predominates in brain, prostate, gut, lungs, bladder, uterus, placenta and thyroid, MM predominates in skeletal muscle and heart muscle and MB isoenzymes is present in varying degree in heart muscle (25%-46% of CK activity) and some in skeletal muscle.
- Serum CK activity is greatly elevated in all types of muscular dystrophy. CK activity in serum invariably increases after myocardial infarction (MI). CK-2 levels rise 3 to 6 hours after a heart attack similar to AST. If there is no further damage to the heart muscle, the level peaks at 12 to 24 hours and returns to pre infarction level in 12 to 48 hours.
- In all species, CK has the advantage over serum aspartate aminotransferase in being specific for muscle injury and not affected by hepatocellular injury.

Lactate dehydrogenase (LDH)

- LDH is widely distributed in most of the tissues as it is one of the glycolytic enzymes active under hypoxic condition. LDH is composed of four subunits of two types i.e. H and M (H for heart and M for muscles).
- There are five isoenzymes with different subunit composition named as LDH1 to LDH5.
 - Isoenzyme and Subunits
 - LDH1 HHHH
 - LDH2 HHHM
 - LDH3 HHMM
 - LDH4 HMMM
 - LDH5 MMMM

LDH1 fraction predominates in cells of cardiac muscle, erythrocytes and kidneys.

LDH 5 is the most abundant form in the liver and in skeletal muscle.

- Predominant elevation of LDH1 and LDH5. (LDH1 greater than LDH5 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction).
- Predominant elevation of LDH2 and LDH3 occurs in acute leukaemia: LDH3 is the main isoenzyme elevated due to malignancy of many tissues.
- Elevation of LDH5 occurs after damage to the liver or skeletal muscle.

Alkaline phosphatase

- Alkaline phosphatases are present in almost all tissues of the body. They are membrane bound. Alkaline phosphatases are a group of true isoenzymes, encoded by at least four different genes: tissue non-specific, intestinal, placental and germ-line ALP. The isoforms derived from the tissue non-specific isoenzyme by post translational modification include the variants of the enzyme found in the liver, bone, kidney and the placenta. Some malignant tumors can produce a placental form of the enzyme called the Regan's isoenzymes.
- Bone diseases with increased osteoblastic activity shows increased ALP level in the serum.
- The corticosteroid-induced isoenzyme of ALP (CALP) has been identified in dogs treated with corticosteroids, dogs with hyperadrenocorticism, or in some older dogs with chronic disease or possibly chronic stress. So Serum CALP activity is a good screening test for hyperadrenocorticism in dogs.
- Cholestasis is the most common cause of significant increases in serum LALP in most species. Increases in serum LALP activity are also observed in steroid hepatopathy in dogs.

Acid Phosphatase

- Acid phosphatase is an enzyme found throughout the body, but primarily in the prostate gland. The highest levels of acid phosphatase are found in metastasized prostate cancer.
- Increased levels are also seen in Prostatitis and is used for diagnosis along with prostate specific antigen (PSA) in dogs and human beings.

γ-Glutamyltransferase (GGT)

- GGT is present in serum which originates primarily from the hepato-biliary system. It is predominantly located in the cell membrane. GGT is located on the luminal surface of the proximal tubular cells of the kidney where it is shed into urine during tubular injury. In liver, GGT activity is primarily associated with the biliary epithelial cells.
- Serum GGT activity was more sensitive than serum ALP activity for detection of extrahepatic cholestasis, cholangiohepatitis, and cirrhosis.
- Serum GGT activity is an especially useful clinical indicator of cholestasis in horses and cattle because of relatively high liver GGT activity compared to dogs and cats. Serum GGT activity in horses and cattle has relatively higher sensitivity for the identification of cholestatic disorders than serum ALP activity.
- The suggested advantage of serum GGT activity determination over serum ALP activity in dogs is increased specificity, as GGT activity is derived solely from liver whereas serum ALP activity is derived from bone and liver as well as the canine corticosteroid induced isoenzyme of ALP.

Amylase

- Amylase is present in many organs and tissues. The highest concentration is found in pancreas. In acute pancreatitis, serum amylase activity increased within 2 to 12 hours of the onset of the disease with maximal levels in 12-72 hours and the level returns to normal by the third or fourth day.
- A significant amount of serum amylase is excreted in the urine, so the rise in serum amylase is reflected in the rise of urine amylase activity.

Lipase

- The main source of lipase is pancreas. Lipase activity in the serum and other body fluid is measured exclusively for pancreatic disorders. Activity assays for serum lipase have been used classically for the diagnosis of acute pancreatitis in dogs, but is of limited use in the diagnosis of exocrine pancreatic insufficiency.
- In acute pancreatitis, increased lipase activity in the serum is seen after 4 to 8 hours of an attack, peaks at about 24 hours, and come to the normal level by 8 to 14 days. Serum lipase activity may also be useful in differentiating severe from mild pancreatitis.
- Serum lipase activity assays have little use in the diagnosis of pancreatitis in cats.

5' nucleotidase (5'NT)

- It is ubiquitously present in all the tissues and is localized in the plasma membrane of the cells in which it is present.
- Serum 5'NT activity is generally elevated in hepatobiliary diseases, especially with intra hepatic obstruction, but, unlike serum alkaline phosphatase, serum 5'NT activity is not increased in infancy, childhood, pregnancy, or osteoblastic disorders.
- It can help confirm the hepatic origin of an elevated ALP.

LIVER FUNCTION TESTS

Biochemical tests are of immense value in diagnosis and monitoring of liver diseases. These tests are usually referred to as “liver function tests” (LFT). LFTs are the most widely performed biochemical tests in the laboratory.

Classification of liver function tests

A. Classification based on laboratory findings

Group I: Tests of hepatic excretory function

a) Bile pigment metabolism

- Serum – Bilirubin estimation (total, conjugated and unconjugated)
- Urine – Bile pigments, bile salts and urobilinogen
- Fecal urobilinogen

b) Excretion of injected substances (foreign dyes)

- Bromosulphalein (BSP) test
- Indocyanine green(ICG) test

Group II: Tests for detoxification function of liver

- Hippuric acid test
- Blood ammonia

Group III: Clinical Enzymology (Liver enzyme panel)

- Alanine amino transferase (ALT)
- Aspartate amino transferase (AST)
- Alkaline phosphatase (ALP)
- Gamma glutamyl transferase (GGT)

Group IV: Tests for synthetic function of liver

- **Plasma proteins** -Total proteins, Serum albumin, globulins, A/G ratio
- Prothrombin time
- Alpha-1-antitrypsin (AAT)
- Alpha-fetoprotein (AFP)
- Ceruloplasmin

Group V: Tests for metabolic function of liver

- Carbohydrate metabolism eg: galactose tolerance test
- Protein metabolism eg: aminoaciduria,Serum proteins
- Lipid metabolism eg: Serum cholesterol estimation, Determination fecal fats

Group VI: Special tests

- Liver biopsy

B. Classification based on Clinical aspects

Group I: Markers of liver dysfunction

- Serum bilirubin, total, conjugated
- Urine: Bile pigments, bile salts and UBG
- Total protein, serum albumin and A/G ratio
- Prothrombin time
- Blood ammonia

Group II: Markers of hepatocellular injury

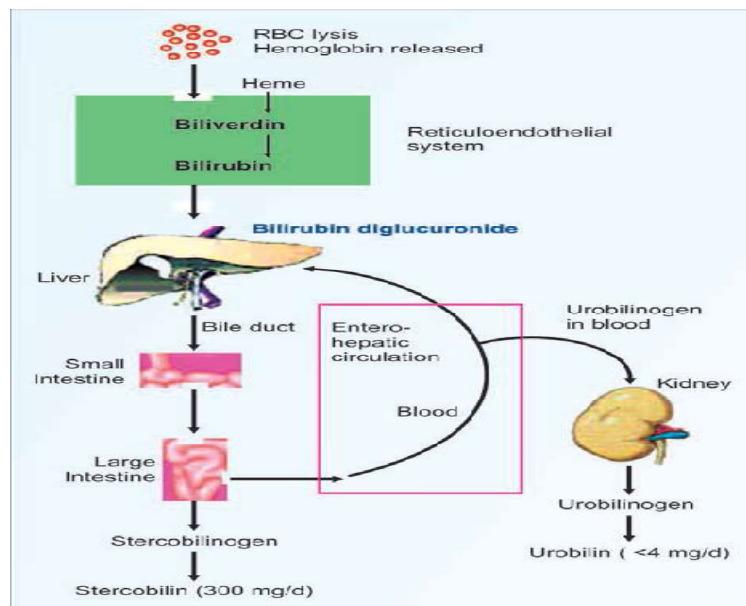
- Alanine amino transferase (ALT)
- Aspartate amino transferase (AST)

Group III: Markers of cholestasis

- Alkaline phosphatase
- Gamma glutamyl transferase

Liver function tests

1) Measurement of Bilirubin



Bilirubin is the excretory product formed by the catabolism of heme. It is conjugated by the liver to form bilirubin diglucuronide and excreted through bile. Measurements of bilirubin as well as detection of bilirubin and urobilinogen in urine are important tests of liver function. Normal plasma bilirubin level

ranges from 0.2– 0.8 mg/dl. The unconjugated bilirubin is about 0.2–0.6 mg/dl, while conjugated bilirubin is only 0 –0.2 mg/dl.

If the plasma bilirubin level exceeds 1 mg/dl, the condition is called hyperbilirubinemia.

Levels between 1 and 2 mg/dl are indicative of latent jaundice. When the bilirubin level exceeds 2 mg/dl, it diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin and mucous membrane resulting in jaundice. (Gk – Icterus).

Van den Bergh Test

Bilirubin reacts with diazo reagent (diazotized sulphanilic acid) to produce colored azo pigment.

At pH 5, the pigment is purple in color. Conjugated bilirubin, being water soluble gives the color immediately; hence called direct reaction. Free bilirubin is water insoluble. It has to be extracted first with alcohol, when the reaction becomes positive; hence called indirect reaction.

- When bilirubin is conjugated, the purple color is produced immediately on mixing with the reagent, the response is said to be van den Bergh direct positive. Eg: obstructive jaundice, (conjugated bilirubin is elevated).
- When the bilirubin is unconjugated, the color is obtained only when alcohol is added, and this response is known as indirect positive. Eg: hemolytic jaundice (unconjugated bilirubin is increased).
- If both conjugated and unconjugated bilirubins are present in increased amounts, a purple color is produced immediately and the color is intensified on adding alcohol. Then the reaction is called biphasic. Eg: hepatocellular jaundice (both conjugated and unconjugated bilirubins are increased).
- Unconjugated hyperbilirubinemia is observed when there is increased production of bilirubin (e.g., hemolytic anemia) or when either hepatic uptake or conjugation of bilirubin is diminished.
- Bilirubinuria is not characteristic in animal patients with unconjugated hyperbilirubinemia.
- Hyperbilirubinemia of the conjugated type is caused either by intrahepatic cholestasis or extrahepatic bile duct obstruction.
- Test for urinary urobilinogen is characteristically negative in complete extrahepatic obstruction.
- The normal horse has a much higher total serum bilirubin than any of the other domestic species and values as high as 4.0 mg/dl or higher have been observed in otherwise healthy individuals. In addition to hepatic and hemolytic diseases, hyperbilirubinemia is observed in horses with intestinal obstruction.

- Food restriction alone causes an abrupt increase in the unconjugated serum bilirubin of the horse and decreased bile flow is a probable factor of the hyperbilirubinemia observed in fasting horses.

Tests useful to distinguish different types of jaundice

Differential Diagnosis of Three types of jaundice							
COMMON LABORATORY TESTS	TYPES OF JAUNDICE						
	Pre-hepatic		Hepatic			Post hepatic	
	HDN*	Hemolytic	Acute Injury	Chronic Injury	Cholestasis	Cirrhosis	Obstruction (Cholestasis)
Bilirubin – Total	↑↑↑	↑	↑↑	N↑	↑↑↑	↑	↑↑↑
Bilirubin – Conjugated	N	N	↑↑	N↑	↑↑↑	↑	↑↑↑
Urine Bilirubin	o	o	↑↑↑	↑	↑↑↑	↑	↑↑↑
Urine Urobilinogen	o	↑	↑	↑	N↑	N↑	↓↓
Fecal Urobilinogen	o	↑↑↑	↓	↓	↓	N↓	↓↓
Prothrombin Time	Normal		Increased			Increased After Vit. K Inj. Becomes normal	
Thymol Turbidity Test	Negative		++			Negative	
ALT/SGPT	Usually Normal		Marked Increase (500-1500 IU/L)			Increased (100 - 300 IU/L)	
Alkaline Phosphatase	Normal		Slightly Increased (<30 KA Units)			Marked Increase (>35 KA Units)	

* Hemolytic disease of the newborn

2) Dye Excretion test

The rate of removal of organic anions can be measured and used to assess the functional capacity of the liver and hepatic blood flow. Sulfobromophthalein (BSP, bromsulphalein) and indocyanine green (ICG) have been used most frequently to assess hepatic function. Following bolus intravenous administration, these dyes are removed rapidly from the plasma primarily by the liver and excreted in the bile. Delayed plasma clearance is taken to be indicative of abnormal hepatocellular or biliary tract function or hepatic circulation.

In the dog and cat, a standard dose of 5mg/kg of BSP is administered as an intravenous bolus. A sample of blood is removed at 30 min and the BSP concentration is determined spectrophotometrically at 540nm.

The clearance rate of ICG provides a useful estimate of hepatic excretory function. ICG clearance has been used to estimate circulation time and hepatic blood flow.

3) Icterus index

The yellow color of serum is due to its bilirubin content and its content is compared with an artificial standard consisting of a 1:10000 solution of potassium dichromate in water. The dilution factor which matches the sample is called as Icterus index. The normal value will be less than 5 units.

4) Turbidity/Flocculation tests

- Thymol turbidity test: The degree of turbidity produced when serum is mixed with a buffered solution of thymol. Sera from patients with liver disease tend to produce more turbidity.
- Zinc Sulphate turbidity test: Addition of normal serum to a solution of zinc sulphate produces turbidity. Sera with higher concentration of gamma globulin produce more turbidity.

BIOCHEMICAL TESTS OF RENAL FUNCTION

URINE ANALYSIS

In clinical biochemistry, urine is tested and report is given on a urine sample. The procedure is called urine analysis or urinalysis. The following parameters are usually checked when reporting on a urine sample.

A. Physical Characteristics of Urine

- **Volume:** The average output of urine is about 1.5 liters per day. Urine volume may be increased in excess water intake, diuretic therapy, diabetes mellitus and in chronic renal diseases. Urine volume may be decreased in excess sweating, dehydration, edema of any etiology, kidney damage.
- **Odor:** Normal urine has a faintly aromatic smell due to presence of volatile organic acids. Urine in diabetic Keto acidosis may have fruity odor due to acetone.
- **Color:** Normal urine is straw-colored (amber yellow) due to the pigment, urochrome. Presence of bilirubin makes urine yellow in jaundiced patients.
- **Specific gravity:** Normal specific gravity of urine is 1.015-1.025. Theoretical extremes are 1.003 to 1.032. The specific gravity will be decreased in excessive water intake, in chronic nephritis, and in diabetes insipidus. It is increased in diabetes mellitus, nephrosis and in excessive perspiration.

B. Chemical Characteristics of Urine

- **Reaction to Litmus**

The pH of urine varies from 5.5 to 7.5. If diet is rich in proteins, sulfuric and phosphoric acids are produced from amino acids, and the urine becomes acidic. If the diet is rich in vegetables, urine is alkaline because the organic acids (citric and tartaric) present in vegetables are converted to bicarbonate in the body.

- **Proteins**

Proteinuria is an important index of renal diseases. In normal urine, protein concentration is very low, which cannot be detected by the usual tests. The proteinuria is commonly assessed by the heat and acetic acid test.

- **Blood**

Hematuria is seen in nephritis and postrenal hemorrhage. Hemoglobinuria is due to abnormal amount of hemolysis.

- **Reducing Sugars (Glycosuria)**

Benedict's test is used as a semiquantitative method for sugar estimation in urine. The approximate concentration of sugar will be 0.5 g/100 ml (green), 1 g% (yellow), 1.5 g% (orange) and 2 g% (red). Dipstick is now replacing the old Benedict's test for detection of glucose in urine.

- **Ketone Bodies**

They are acetoacetic acid, beta hydroxybutyric acid and acetone. Ketonuria is seen in diabetes mellitus, starvation, persistent vomiting, von Gierke's disease, etc. Ketone bodies are analyzed by Rothera's test.

- **Bile Salts**

Bile salts are present in urine during the early phase of obstructive jaundice .Their presence is identified by Hay's test.

- **Bile Pigments**

Bilirubin appears in the urine during obstructive jaundice .It is detected by Fouchet's test.

- **Urobilinogen**

The oxidation of urobilinogen to urobilin is supposed to be the cause of the deepening of color of urine on standing. In hepatocellular jaundice, urobilinogen is absent in urine. It is identified by Ehrlich test or Schlesinger's test.

TESTS OF GLOMERULAR FUNCTION

Measurement of glomerular filtration rate (GFR) provides the most useful general index for the assessment of the severity of renal damage.

CLEARANCE TESTS

Clearance is defined as the volume of blood or plasma completely cleared of a substance per unit time and is expressed as milliliter per minute. It is expressed as milliliter of plasma per minute (ml/minute)

$$U \times V$$

$$C = \frac{U \times V}{P} ,$$

$$P$$

where U = concentration of the substance in urine;

P = concentration of the substance in plasma or serum

V = the ml of urine excreted per minute.

- Exogenous markers are inulin, ^{51}Cr -labelled EDTA, ^{99}Tc -labelled EDTA
- Endogenous markers are urea and creatinine

Inulin clearance

Inulin is a polysaccharide of fructose. It is not appreciably metabolized by the body. It is neither absorbed nor secreted by the tubules. Therefore, inulin clearance is a measure of GFR. The value of GFR as measured by inulin clearance is 125 ml/minute.

Urea Clearance Test

Most commonly used to assess renal failure. Urea is freely filtered by the glomerulus and passively reabsorbed in both PCT and DCT. Hence the urea clearance is less than GFR. Not preferred for estimation of GFR. Normal urea clearance is 75 ml/min.

Creatinine Clearance Test

Creatinine is a waste product, formed from creatine phosphate. This conversion is spontaneous, non-enzymatic, and is dependent on total muscle mass of the body. Creatinine is filtered by glomeruli, and actively excreted by the tubules. Creatinine excretion is constant in a particular person. Since the production is continuous, the blood level will not fluctuate much, making creatinine an ideal substance for clearance test.

A decreased creatinine clearance is a very sensitive indicator of reduced glomerular filtration rate.

Creatinine coefficient

It is the urinary creatinine expressed in mg/kg body weight for 24 hours. The value is elevated in muscular dystrophy.

Others:

- **Cystatin C** is a LMW non-glycosylated protein which is expressed in virtually all organs of the body. Serum level of cystatin is a better test for kidney function (GFR) than serum creatinine levels. Since there is no tubular secretion of Cystatin C, it is extremely sensitive to minor changes in GFR in the earliest stages of chronic kidney diseases.
- **Phenolsulfonaphthalein (PSP)** is a dye that is cleared exclusively by the kidney and used in renal function tests.
- **Para amino hippuric acid (PAH)** clearance is a measure of renal plasma flow.

TESTS FOR TUBULAR FUNCTION

Concentration tests:

1. Water deprivation test
2. Exogenous ADH excess administration
3. Dilution test/Water load test
4. Acid excretion test

Role of BUN, Uric acid and Creatinine in diagnosis

Increased levels of Non protein nitrogenous substances like BUN, Uric acid and Creatinine in blood leads to Azotemia. It is largely related to insufficient or dysfunctional filtering of blood by the kidneys. It can lead to uremia and acute kidney injury (kidney failure) if not controlled.

BLOOD UREA NITROGEN (BUN) / BLOOD UREA

Urea is a small hydro soluble molecule (MW 60) synthesized in the liver from bicarbonate and ammonia in the Krebs-Henseleit cycle. Urea is the main form in which nitrogen is eliminated in mammals. After synthesis, it is distributed into the total body water compartment. It is freely filtered by the kidney glomeruli and reabsorbed from the collecting tubule.

Serum urea is increased in all forms of kidney diseases. In acute glomerulonephritis values may be as high as 300 mg/dl. In early stages of nephrosis, serum urea may be normal, but in late stages serum urea increases along with decreasing renal functions.

Causes for Increased Blood Urea

1. Pre-renal conditions

- Dehydration: Severe vomiting, Intestinal obstruction, diarrhea
- Diabetic coma and severe burns
- Fever and severe infections

2. Renal diseases

- Acute glomerulonephritis
- Nephrosis
- Malignant hypertension
- Chronic pyelonephritis

3. Post-renal causes

- Stones in the urinary tract
- Enlarged prostate
- Tumors of bladder

Decreased Blood Urea

- late pregnancy
- starvation
- diet grossly deficient in proteins
- hepatic failure

CREATININE

Creatinine is a small molecule (MW 113) produced by degradation of creatine and creatine-phosphate, an energy-storing molecule mainly present in skeletal muscles. Creatine is synthesized from the amino acids glycine, arginine, and methionine, the final step occurring in the liver. It is then taken up by the muscles where it is reversibly phosphorylated by creatine-kinase into creatine-phosphate. Skeletal muscles contain about 95% of the total body creatine and creatine-phosphate pool. The estimated turnover of creatine- phosphate (about 2%) is fairly constant in a given individual. Creatinine is freely filtered by the glomerulus and secreted by active transport in the proximal tubule in humans.

P-Creatinine is the most efficient indirect marker of GFR in mammals. It is increased in chronic and acute renal failure and also in some conditions not directly involving the kidney.

- Chronic kidney disease
- Kidney obstruction - eg: enlarged prostate or kidney stone
- Kidney infection
- Dehydration
- Gout
- Rhabdomyolysis
- Muscular dystrophy

URIC ACID

The end product of purine nucleotide catabolism is uric acid. Uric acid is sparingly soluble in water.

The most common abnormality is an elevation of uric acid level in blood, referred to as hyperuricemia.

Accumulation of urate crystals in the synovial fluid leads to Gout. The drug Allopurinol acts as a competitive inhibitor of xanthine oxidase and thereby decreases the formation of uric acid.

Hyperuricemia - Elevation of uric acid levels in blood.

1. Increased production of uric acid

- Rapidly growing malignant tissues, e.g. leukemias, lymphomas, polycythemia.
- Increased tissue breakdown after treatment of large malignant tumors.
- deficiency of HGPRTase enzyme and

2. Reduced excretion rate

- Renal failure.
- Treatment with thiazide diuretics which inhibit tubular secretion of uric acid.
- Lactic acidosis and keto-acidosis due to interference with tubular secretion.

Hypouricemia - decrease in levels of uric acid in blood.

• Adenosine Deaminase (ADA) Deficiency

Hypouricemia is due to defective breakdown of purine nucleotides.

• Xanthine Oxidase Deficiency

It is a genetic defect. Characteristic features are hypouricemia, increased excretion of hypoxanthine and xanthine and liver damage.

BIOTRANSFORMATION

Biotransformation is the process whereby a substance is changed from one chemical form to another by a chemical reaction within the body. Biotransformation also serves as an important defense mechanism in that toxic xenobiotics and metabolites are converted into less harmful substances or as substances that can be excreted from the body.

In general, biotransformation reactions generate more polar metabolites that are readily excreted from the body. The liver plays the most important role in the biotransformation reactions.

The biochemical processes whereby the noxious substances are rendered less harmful and more water soluble, are known as detoxification.

Lipophilic toxicants are hard for the body to eliminate and can accumulate to hazardous levels.

Xenobiotics are compounds which may be accidentally ingested or taken as drugs or compounds produced in the body by bacterial metabolism (Greek, *xenos* = strange).

Bioactivation is a process in which metabolites after undergoing biotransformation becomes more toxic than the parent substance.

Biotransformation reactions are usually classified as Phase one and Phase two reactions.

PHASE I REACTIONS

- Involves the alteration of the foreign molecule, so as to add a functional group, which can be conjugated in phase 2. Phase 1 reactions result in the formation of compounds with decreased toxicity (detoxification).
- Reactions are non-synthetic in nature and in general produce a more water-soluble and less active metabolites.
- The phase 1 reactions include hydroxylation, oxidation, reduction, hydrolysis, dealkylation, epoxidation, etc. In these types of reactions, a polar group is either introduced or unmasked, so the drug molecule becomes more water-soluble and can be excreted.
- The majority of metabolites are generated by a common hydroxylating enzyme system known as Cytochrome P450.

1. Oxidative Reactions

- Aromatic or aliphatic hydroxylation
- N-oxidation or O-oxidation
- Sulfoxidation
- Epoxidation
- N and O- dealkylation
- Deamination

2. Reduction Reactions

- Aldehydes or ketones are reduced to alcohols
- Nitro compounds are reduced to their amines

3. Hydrolysis

- Addition of water splits the toxicant into two fragments or smaller molecules.
- Esters, amines, hydrazines, amides, glycosidic bonds and carbamates are generally biotransformed by hydrolysis,
- e.g. aspirin, procaine, xylocaine

The products of metabolic transformations are either excreted directly or undergo further metabolism by phase two reactions.

PHASE II REACTIONS(CONJUGATIONS)

- A xenobiotic that has undergone a Phase one reaction is now a new metabolite that contains a reactive chemical group, e.g. hydroxyl (-OH), amino (-NH₂), and carboxyl (-COOH). These metabolites must undergo additional biotransformation as a Phase two reaction.
- Phase two reactions are conjugation reactions, i.e a molecule normally present in the body is added to the reactive site of the Phase one metabolite.
- In most cases, the conjugation will make the compounds non toxic and easily excretable.
- The final compounds have a larger molecular weight.
- Phase 2 reactions are sulfation, acetylation, methylation and conjugation with glucuronic acid, glutathione or glycine.

1. Glucuronic acid conjugation

- Glucuronide conjugation is the most common Phase two reactions.
- Glucuronic acid can conjugate with hydroxyls (both phenolic and alcoholic), carbonyl, sulphydryl and amino compounds.
- The activated form UDP-glucuronic acid, is added to xenobiotics by UDP-glucuronyl transferases, present in the endoplasmic reticulum.
- Bilirubin is a good example for a compound conjugated and excreted as Bilirubin glucuronide.
- Phenol, Benzoic acid, Steroids Amines can also conjugate with glucuronic acid.
- Glucuronide conjugation is limited in cats. Pig is more efficient than the human at glucuronidation.

2. Sulfation

- In general, sulfation decreases the toxicity of xenobiotics.
- The highly polar sulfate conjugates are readily excreted through urine. Often glucuronidation or sulfation can conjugate the same xenobiotics.
- The enzyme is sulfo-transferase and the sulfate group is transferred from PAPS (3'-phosphoadenosine-5'-phosphosulfate)
- Sulfation is limited in pigs.
- Phenolic and alcoholic compounds are conjugated with sulfate.
- Eg: steroids and indole compounds

3. Acetylation

- Conjugation with acetic acid is taking place with drugs like sulfanilamide, isoniazid and PAS.
- Enzymes involved are N-Acetyl transferases which uses acetyl CoA as co enzyme.
- Acetylation is limited in dogs.

4. Methylation

- Amino, hydroxy or thiol groups are methylated.
- S-adenosyl methionine (SAM) is the methyl donor and the enzyme is usually O-methyl transferase.
- Eg: Epinephrine, Pyridine, Mercapto ethanol

5. Conjugation with Glycine

- Benzoic acid is conjugated with glycine to form hippuric acid (benzoyl glycine), which is excreted in urine.
- In cats, taurine conjugation is important.

6. Conjugation with Gluthathione

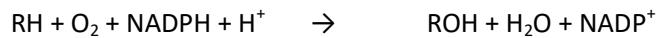
- Takes place in liver, kidneys.
- Alkyl or aryl halides, epoxides and alkenes are detoxified.
- Enzymes involved is glutathione S-transferases.

Phase III – further modification and excretion

- After phase II reactions, the xenobiotic conjugates may be further metabolised.
- A common example is the processing of glutathione conjugates to acetylcysteine (mercapturic acid) conjugates.
- Conjugates and their metabolites can be excreted from cells in phase III of their metabolism.
- Phase III involves drug transporters, which influence the effect, absorption, distribution and elimination of a drug.

Cytochrome P450 System

- Cytochromes P450 (CYPs) are a family of enzymes containing heme as a cofactor that function as monooxygenases. Cytochrome P450 enzymes are the most important enzymes in Phase I metabolism in mammals, and are primarily responsible for the metabolism (degradation and elimination) of drugs.
- Cytochrome P450 enzymes are primarily found in liver cells but are also located in cells throughout the body in mitochondria and microsomes.
- CYPs are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorized as P450-containing systems. The term "P450" is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with carbon monoxide. The active site of cytochrome P450 contains a heme-iron center.
- The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the aliphatic position of an organic substrate (RH) while the other oxygen atom is reduced to water:



- Many hydroxylation reactions (insertion of hydroxyl groups) use CYP enzymes.
- They are inducible enzymes. Eg: Phenobarbital increases the activity of P450.

DISTURBANCE IN ACID BASE BALANCE AND ITS DIAGNOSIS

ACIDOSIS

1. Metabolic Acidosis

- Metabolic Acidosis is also called as Primary HCO₃ deficit and is the commonest acid-base disturbance observed clinically.
- This condition is produced by the addition of an acid or loss of bicarbonate, or dilution of bicarbonate.

Causes:

- GI Loss of Bicarbonate: Diarrhea -Small bowel or pancreatic drainage - Obstructed ileal loop -Anion exchange results.
- Renal loss of Bicarbonate: Carbonic anhydrase inhibitors-Renal Tubular Acidosis (Hyperparathyroidism-Hypoaldosteronism plays role in acid base balance.)
- Increased Acid Production: Lactic acidosis-Starvation ketoacidosis –Alcoholic ketoacidosis -Diabetic ketoacidosis.
- Failure of Acid Excretion: Acute and chronic renal failure.

2. Respiratory Acidosis

- Respiratory Acidosis is a clinical disturbance that is due to alveolar hypoventilation which increases the level of pCO₂.
- Alveolar hypoventilation leads to an increased pCO₂.
- The increase in pCO₂ in turn decreases the HCO₃-/pCO₂ ratio and decreases pH.

Causes

- Depression of central nervous system
- Chronic respiratory acidosis may be secondary to many disorders, including COPD.
- Disease of lung
- Thoracic muscle disorders.
- Neuromuscular disorders, interstitial fibrosis and thoracic deformities.
- Lung diseases that cause abnormality in alveolar gas exchange, cause stimulation of ventilation and hypocapnia secondary to hypoxia.
- Hypercapnia only occurs if severe disease or respiratory muscle fatigue occurs.

ALKALOSIS

1. Metabolic Alkalosis

- Metabolic Alkalosis may result from excessive loss of hydrogen ions, excessive reabsorption of bicarbonate or ingestion of alkalis.
- Excess H⁺ loss: Loss of gastric secretions. e.g. Vomition
- Ingestion of Alkalies eg: Alkaline antacids

2. Respiratory Alkalosis

- Respiratory Alkalosis is also called as primary H₂CO₃ deficit. This results when there is a decrease in [H₂CO₃] fraction with no corresponding change in HCO₃⁻ in plasma.
- Excessive quantities of CO₂ may be washed out of the blood by hyperventilation.

Causes:

- Respiratory alkalosis is an abnormal physiological process in which there is a primary increase in the rate of alveolar ventilation relative to the rate of CO₂ production.
- Hypoxia
- Lung diseases like pneumonia, asthma, atelectasis, fibrosis, pulmonary edema.
- Central nervous system disorders, cerebral diseases: tumor, encephalitis, meningitis.
- Septicemia.
- Cirrhosis of the liver.

Test to measure acid base balance:

1) Anion gap: The anion gap can be calculated as the difference between total plasma anions (HCO₃⁻, Cl⁻) and cation (Na⁺ and K⁺).

$$\text{Anion gap} = (\text{sodium} + \text{potassium}) - (\text{chloride} + \text{bicarbonate})$$

The anion gap for most species of domestic animals appears to be approximately 10 to 20 mEq/l.

Causes of Alterations in Anion Gap

Decreased anion gap:

- Increased cationic protein
- Polyclonal gammopathy (IgG)

- Hypoalbuminemia
- Hyperchloremic acidosis- Altered protein anionic equivalents

Increased anion gap:

Metabolic acidosis

- Organic acids (lactic, keto acids)
- Hypovolemic shock
- Anaerobic exercise
- Diabetes
- Grain overload
- Ketosis

Nonmetabolizable acids

- Inorganic acids (sulfate, phosphate)
- Uremic acidosis
- Intoxication or poisoning
 - ✓ Salicylate
 - ✓ Paraldehyde
 - ✓ Metaldehyde
 - ✓ Methanol
 - ✓ Ethylene glycol

2) Measurement of PCO₂

3) Blood pH

BIOCHEMISTRY OF DIGESTIVE DISORDERS

Digestive Disorders in Ruminants

Acute Rumen Indigestion (Rumen Overload, Lactic Acidosis)

- Acute rumen indigestion occurs in sheep or cattle consuming high-roughage diets when they inadvertently are allowed access to large amounts of readily fermentable carbohydrate (e.g., grain) resulting in the high concentrations of lactic acid accumulate in the rumen and subsequently in the blood.
- *Streptococcus bovis* is the rumen microorganism believed to be chiefly responsible for production of large quantities of lactic acid.
- When lactic acid accumulates more rapidly than it is absorbed, rumen pH falls and rumen atony develops.
- Rumen bacteria produce a racemic mixture of lactic acid, L-lactic acid is absorbed and metabolized but D- lactate cannot be utilized, which contributes to the acid load. This result in metabolic acidosis.
- Lactic acid accumulation in the rumen reduces the pH to 5 or less , which allows the growth of acid producing bacteria.
- Accumulation of lactate increases the osmolality of the rumen, which results in the absorption of water from the systemic circulation. This causes severe dehydration, which in turn may lead to hypovolemic shock.

Acute Rumen Tympany (Bloat)

- The gases (CO_2 , methane) produced during the rumen fermentation are removed by the process called eructation. When the process is blocked gas produced by the rumen microbes cannot escape and the pressure is increased, which causes acute tympany, leading to death.
- Two general types of bloats are simple bloat (Free gas) and frothy bloat.
- Interruption of the normal eructation reflex or mechanical obstruction of the esophagus typically results in free gas bloat.

- Frothy bloat is the most important form of bloat, is seen in cattle consuming large quantities of legumes or in feedlot cattle on high-concentrate diets.
- Pectin methyl esterase, a plant enzyme, acts on pectin to release pectic and galacturonic acids, which greatly increase the viscosity of the rumen fluid, resulting in formation of a highly stable foam.
- Presence of tannins inhibits microbial activity, hence acts as an anti bloat agent.
- Non ionic detergent with surfactant property can be used for treatment eg. Sodium alkyl sulfonate.

Urea Poisoning

- Urea and other NPN substances are used by microbes to synthesize microbial proteins, which are subsequently utilized by the ruminants for the synthesis of body proteins. Urea is hydrolyzed into CO_2 and NH_3 by urease enzyme produced by rumen bacteria.
- Urea poisoning may occur accidentally when animals engorge on large amounts of urea-containing dietary supplement (more than 3% level). The free ammonia can cross the cell membrane thereby producing harmful effects.
- Oral administration of acetic acid has been shown to reduce acute urea toxicity apparently. The proton of acetic acid converts free ammonia to ammonium ion, thereby reducing the absorption of NH_3 from the rumen. (Since NH_3 has no charge, it diffuses freely but ammonium ion is charged and diffusion is prevented).

Digestive Disorders in Non Ruminants

Vomiting

- It is a complex reflex act, which results in the rapid, forceful ejection of gastric contents through the mouth.
- A number of conditions can stimulate vomiting are presence of foreign objects, intussusception, neoplasia, pyloric stenosis, chronic gastritis, presence of parasites, acute nephritis, hepatic disease, presence of poisons.
- Dog and cat vomit easily. In horse it is rare. It is mainly controlled by centres in brain.

- Severe vomiting leads to loss of water and HCl. This results in dehydration and metabolic alkalosis with increased level of bicarbonate ion and decreased level of chloride ion concentration.
- Gastric vomiting may also cause hypokalemia, which may be due to increased urinary excretion during alkalosis. Gastric contents also contains potassium and loss due to vomiting may also contribute to potassium deficiency.
- Potassium deficiency and hypovolemia due to dehydration may cause renal tubular damage and kidney failure.

Diarrhoea

- It is the rapid elimination of watery faecal material with increased frequency and volume or both .
- Diarrhoea results in dehydration associated with H^+ and electrolyte disturbances.
- Dehydration cause haemoconcentration, which leads to hypovolemic shock.
- **Metabolic acidosis** caused by (1) decreased excretion of H^+ resulting from decreased renal perfusion and (2) increased production of organic acids
- **Hyperkalemia** - in such cases is the result of increased movement of cellular K^+ into the extracellular fluid and to decreased renal excretion.
- Hypoglycemia and Disturbance in absorption of all nutrients.

Gastric dilatation - volvulus (GDV)

- It is an acute GI tract disorder, which is due to the accumulation of gas and fluid in the stomach causing mechanical and functional disturbances to pyloric out flow.
- The stomach distends and rotates causing obstruction due to which there is necrosis and perforation of the stomach wall.
- Hyperkalemia and hyperphosphatemia were consistent findings due to reduced renal flow. There is release of intracellular potassium from the damaged tissues.
- Due to the leakage of fluid from the blood vessels into tissues, there is haemoconcentration, which results in increased blood urea nitrogen and creatinine values.
- Due to degeneration of stomach cells and alteration of liver , the transaminases activities are increased
- There is increase lactic acid production, which cause metabolic acidosis.

Tests of Malabsorption

Steatorrhea, the presence of excessive amounts of fat in the feces, is a prominent sign of intestinal malabsorption in dogs. The stools are bulky, gray or tan, and, grossly, may have an oily appearance.

A reliable diagnostic procedure for Steatorrhea qualitatively is by staining the fresh stool with a lipophilic stain, such as Sudan III, and observing increased numbers of oil droplets under the light microscope.

The following methods can be used for testing malabsorption

1. Cobalamin and Folate Absorption

- May give an indication of the site of intestinal dysfunction in dogs and cats.

2. Glucose Absorption

- The absorption of glucose can be evaluated by the oral glucose tolerance test (OGTT) where an oral test dose of glucose is given and the blood glucose levels are measured at half-hour intervals for 3 to 4h.
- In canine malabsorption, the OGTT curve of blood glucose is diminished or flat.
- The test also has been used in the horse for evaluation of small intestinal malabsorption.

3. D-Xylose Absorption

- D-xylose is used clinically to evaluate intestinal absorption.
- The body does not metabolize D-xylose to any significant degree and tissue interferences are avoided.
- The test is also used for differential diagnosis of equine diarrheal diseases.

4. Oleic Acid and Triolein Absorption

- The absorption of ^{131}I -labeled oleic acid and ^{131}I -labeled triolein has been used in dogs with intestinal malabsorption.
- The ^{131}I -labeled oleic acid and ^{131}I -labeled triolein tests are used to differentiate steatorrhea caused by pancreatic enzyme deficiency from that caused by a primary defect in absorption.
- If steatorrhea is caused by a lack of pancreatic lipase, oleic acid absorption will be normal, whereas that of triolein, which requires lipolysis for absorption, will be significantly reduced.
- The absorption of both compounds is reduced in intestinal malabsorption.

5. Vitamin A absorption

- The vitamin A absorption test measures intestinal lipid absorption.
- After oral administration of 200,000 units of vitamin A in normal dogs, serum vitamin A concentrations reach their peak at 6 to 8 h, with values ranging between three and five times fasting serum levels.

BIOCHEMISTRY OF OXIDATIVE STRESS

Oxidative stress is defined as a disturbance in the balance between the production of free radicals and antioxidants in favor of the oxidants. The imbalance between those two fractions may potentially lead to cell damage at molecular level.

A free radical ($R\bullet$) is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital. The uneven number allows them to easily react with other molecules. Free radicals can cause large chain chemical reactions in the body because they react so easily with other molecules.

The following are the **Reactive oxygen species** or **ROS**.

- a. Superoxide anion radical ($O_2--\bullet$)
- b. Hydroperoxyl radical ($HOO\bullet$)
- c. Hydrogen peroxide (H_2O_2)
- d. Hydroxyl radical ($OH\bullet$)
- e. Lipid peroxide radical ($ROO\bullet$)
- f. Singlet oxygen (1O_2)
- g. Nitric oxide ($NO\bullet$)
- h. Peroxy nitrite ($ONOO--\bullet$).

Effects of Reactive Oxygen Species

- Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens.
- Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.
- Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. O_2- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide).
- Polyunsaturated fatty acids, particularly arachidonic acid and linoleic acid, are primary targets for free radical and singlet oxygen oxidations.

- Peroxidation of PUFA (poly unsaturated fatty acids) in plasma membrane leads to loss of membrane functions. Lipid peroxidation and consequent degradation products such as malondialdehyde (-CHO-CH₂-CHO-) are seen in biological fluids.
- Oxidation of sulfhydryl group containing enzymes leads to loss of function and fragmentation of proteins. Polysaccharides undergo degradation.
- DNA is damaged by strand breaks. The DNA damage may directly cause inhibition of protein and enzyme synthesis and indirectly cause cell death or mutation and carcinogenesis
- Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling.

Free Radical Scavenger Systems

1. Superoxide dismutase (SOD)

The mitochondrial SOD is manganese dependent; cytoplasmic enzyme is copper-zinc dependent.

SOD is a non-heme protein. A defect in SOD gene is seen in patients with amyotrophic lateral sclerosis

2. Glutathione peroxidase

The H₂O₂ produced is removed by glutathione peroxidase (POD). It is a selenium dependent enzyme.

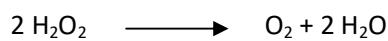
3. Glutathione reductase

The oxidized glutathione, in turn, is reduced by the glutathione reductase (GR), in presence of NADPH. This NADPH is generated with the help of glucose-6-phosphate dehydrogenase (GPD) in HMP shunt pathway. Therefore in GPD deficiency, the RBCs are liable to lysis, especially when oxidizing agents are administered (drug induced hemolytic anemia).

4. Catalase

When H₂O₂ is generated in large quantities, the enzyme catalase is also used for its removal.

Catalase



Anti-oxidants

Antioxidants are molecules that can donate an electron to a free radical without making themselves unstable. This causes the free radical to stabilize and become less reactive.

1. Vitamin E (Alpha tocopherol) is the lipid phase antioxidant. It acts as the most effective naturally occurring chain breaking antioxidant in tissues.
2. Vitamin C is the aqueous phase antioxidant.
3. Ceruloplasmin can act as an antioxidant in extracellular fluid.
4. Caffeine is another effective anti-oxidant.
5. Cysteine, glutathione and vitamin A are minor antioxidants.
6. Beta carotene can act as a chain breaking antioxidant, but is less effective than alpha tocopherol.
7. Polyphenols - contain flavones, isoflavones, flavonols, catechins and phenolic acids.

BIOCHEMICAL BASIS OF FLUID THERAPY

Total body water (TBW) comprises approximately 60% of a patient's body weight. Approximately 67% of TBW is found inside the body's cells and is referred to as intracellular fluid (ICF). The remaining 33% of TBW is the extracellular fluid (ECF), which is further divided as follows:

- Interstitial fluid, which bathes cells and tissues (~24% of TBW)
- Plasma, the liquid portion of blood, which constitutes most of intravascular volume (~8%–10% of TBW)
- Transcellular fluid, which comprises synovial joint fluid, cerebrospinal fluid, bile, and the fluid in the linings of the peritoneal cavity, pericardium, and pleural space (~2% of TBW)

Reasons for Fluid Therapy

Veterinary professionals provide fluid therapy to patients for many reasons, including correction of dehydration, expansion and support of intravascular volume, correction of electrolyte disturbances, and encouragement of appropriate redistribution of fluids that may be in the wrong compartment

GENERAL CONCEPTS OF FLUID AND ELECTROLYTE THERAPY

A. INSTITUTION OF FLUID THERAPY

Fluid therapy should be instituted for the following conditions: dehydration, acid–base disturbances and/or electrolyte imbalances, nutritional problems, and loss of body fluids.

1. BASIS FOR INSTITUTION OF FLUID THERAPY

a. Accurate diagnosis based on clinical examination and laboratory data is important for fluid therapy.

The clinical signs for detection of dehydration include: loss of skin elasticity, dry buccal mucosa and tongue, and sunken eyeballs should be taken into account.

b. Signs of vomiting, diarrhea, abnormal respiratory pattern, and CNS depression or excitation may help with the diagnosis of acid–base disturbances.

c. Blood gas and urine analyses are useful for the precise diagnosis of acid–base and electrolyte disturbances.

2. DEHYDRATION

a. General considerations - Dehydration may be considered in three general categories:

(1) Hypertonic dehydration, which is attributable to loss of pure water or hypotonic fluid.

(2) Isotonic dehydration, which is attributable to loss of isotonic body fluids. However, isotonic dehydration is only seen in acute cases, since with some degree of water replacement, isotonic dehydration will become hypotonic dehydration.

(3) Hypotonic dehydration. The loss of a hypertonic fluid or loss of isotonic fluid with water replacement results in hypotonic dehydration.

b. Causes

(1) Decreases in water intake usually lead to hypertonic dehydration.

- (a) Lack of water source.
- (b) Disorders and pain of the buccal cavity and pharynx.
- (c) CNS disturbances.

(2) Increases in body fluid excretion usually lead to hypotonic dehydration.

- (a) Polyuria - Diabetes, nephrosis, hypoaldosteronism, and diuretics.
Diabetes insipidus will cause hypertonic dehydration.
- (b) Respiratory loss of water during high temperature may lead to hypertonic dehydration.
- (c) Profuse sweating in horses.
- (d) Vomiting/diarrhea.
- (e) Third space loss. Body fluid lost to the body cavities and hollow organs.

c. Role of electrolytes on hydration states and acid-base balance:

(1) $\uparrow [Na^+]$ in ECF \rightarrow water retention

(2) Changes in $[K^+]$ in ECF result in changes in acid-base balance:

- (a) $\uparrow [K^+]$ in plasma $\rightarrow \uparrow [K^+], \downarrow [H^+]$ in urine \rightarrow Acidemia

(b) ↓ $[K^+]$ in plasma → ↓ $[K^+]$, ↑ $[H^+]$ in urine → Alkalemia.

d. Role of carbohydrate metabolism on hydration states and acid–base balance:

(1) ↓ Carbohydrate utilization → Hyperglycemia → Glucosuria → Polyuria → Dehydration

(2) ↓ Carbohydrate utilization → ↑ Gluconeogenesis → Ketoacidosis

(3) ↑ Carbohydrate intake (grain overload) in herbivores → ↑ Lactic acid production → Acidosis.

e. Treatment (amount of fluid to be used) must be based on the body water maintenance plus replacement of the deficit and ongoing loss

(1) Amount of body water maintenance

(a) On the basis of body water turnover

(b) A total of 50–75 mL/kg/day (average 65 mL/kg/day)

(2) Determination of water deficit (dehydration)

Dehydration of 4, 6, 8, and 12% (of body weight), only loss >4% needs a replacement

a) A total of 4% dehydration (mild)

- Animals with 4% dehydration have a history of fluid loss, but without significant signs of dehydration.
- No replacement is needed.

b) A total of 6% dehydration (moderate)

- Animals with 6% dehydration have decreased skin turgor. In dogs and cats, **when the skin over the lateral thorax is picked into a tented fold, it will return to normal slowly**; in species having tight skin, pinch the dorsal eyelid to do the test.
- A decrease in skin elasticity is also seen in cachexia; thus, one cannot conduct this test in cachectic animals.
- Animals with 6% dehydration have dull hair coat and **dry mucous membranes**.

c) About 8–10% dehydration (severe).

The animals with 8–10% dehydration have the following signs:

- The skin lacks pliability. In dogs and cats, when the skin is pinched into a tented fold, it will tent and stay after the pinch is released.
- Dry mucous membranes and tongue.
- Soft eyeballs that are sunken into the orbit.
- Cold extremities.
- Capillary refill time >3 seconds (normal <2 seconds).

e) About 12% dehydration (extremely severe).

The animals with 12% dehydration have following signs:

- All the signs seen with 8–10% dehydration.
- Circulatory collapse (shock).

(3) Estimation of water deficit.

The replacement volume for the initial deficit is estimated according to the following equations:

$$\text{Replacement volume (L)} = \% \text{dehydration} \times \text{body weight (kg)}$$

(4) The composition of replacement fluid should be similar to the volume of fluid lost.

For example, if the deficit is due to loss of the electrolyte-rich GI fluid, then a balanced salt solution containing Na^+ , K^+ , Ca^{2+} , Cl^- , and HCO_3^- (or indirect alkalinizing agents) should be used. In contrast, if the deficit is due to loss of pure water, volume can be replaced with 5% dextrose (glucose in water) over 24–72 hours. An isotonic solution of 2.5% dextrose and 0.45% NaCl can also be used.

(5) The ongoing loss must be taken into account when estimating the fluid therapy volume. The ongoing loss of fluid via vomiting, diarrhea, and polyuria must be estimated and replaced.

(6) Additional factors need to be considered

- (a)** Dehydration affects young animals much faster than adult animals.
- (b)** Old animals with chronic diseases require more water than younger adult animals.
- (c)** Physical and weather conditions may affect the requirement, particularly when it is hot and humid.
- (d)** Drugs will alter requirements, particularly diuretics and mineralocorticoids can affect water and electrolyte balances.

Fluid Therapy Formulas

- **Calculation of Dehydration Deficit¹**

Body weight (kg) × % dehydration as a decimal = liters of fluid required to correct dehydration

- **Calculation of Maintenance Fluid Requirements** (UC Davis School of Veterinary Medicine fluid therapy formula.)

✓ **Dogs:** Body weight (kg)^{0.75} × 132 = 24-hour fluid requirement in milliliters

✓ **Cats:** Body weight (kg)^{0.75} × 80 = 24-hour fluid requirement in milliliters

Ongoing losses (e.g., from diarrhea, vomiting, or polyuria) must be calculated and added to the total maintenance requirement obtained from these formulas.