UNIT-III
VETERINARY ANALYTICAL BIOCHEMISTRY
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Lecture-1

Keto acidosis

Ketoacidosis is a metabolic acidosis due to an excessive blood concentration of ketone bodies (acetone, acetoacetate and beta-hydroxybutyrate). Ketone bodies are released into the blood from the liver when hepatic lipid metabolism has changed to a state of increased ketogenesis. The abnormal accumulation of ketones in the body occurs due to excessive breakdown of fats in deficiency or inadequate use of carbohydrates. It is characterized by ketonuria, loss of potassium in the urine, and a fruity odor of acetone on the breath. Untreated, ketosis may progress to ketoacidosis, coma, and death. This condition is seen in starvation, occasionally in pregnancy if the intake of protein and carbohydrates is inadequate, and most frequently in diabetes mellitus.

Different Types of Ketoacidosis

Diabetes Keto Acidosis (DKA): Due to lack of insulin glucose uptake and metabolism by cells is decrased.Fatty acid catabolism increased in resulting in excess production of ketone bodies.

Starvation Ketosis: Starvation leads to hypoglycemia as there is little or no absorption of glucose from intestine and also due to depletion of liver glycogen. In Fatty acid oxidation leads to excess ketone body.

Bovine Ketosis

Introduction:

- Bovine ketosis occurs in the high producing dairy cows during the early stages of lactation, when the milk production is generally the highest.
- Abnormally high levels of the ketone bodies, acetone, acetoacetic and beta-hydroxy butyric acid and also iso- propanol appear in blood, urine and in milk.
- The alterations are accompanied by loss of appetite , weight loss, decrease in milk production and nervous disturbances.
- Hypoglycemia (starvation) is a common finding in bovine ketosis and in ovine pregnancy toxemia.
- In non-ruminants, liver is the sole source of ketone bodies.
- In ruminants, the rumen epithelium and mammary gland are also sources of ketone bodies production.
- Amoung the ketone bodies acetone does not ionize to the appreciable level, whereas, acetoacetic and b hydroxybutyric acids will ionize readily.
- Acetoacetic and b-hydroxybutyric acid are more powerful acids than the volatile fatty acids.

Lecture-2

Ketosis in Lactation

- During lactation plasma glucose is drained for the synthesis of lactose by the mammary gland.
- The two sources of plasma glucose are absorption from the gut and gluconeogenesis.
- In ruminanats little glucose is absorbed from the gut.
- Most of the glucose is synthesized in the liver and in the kidney.
- The chief substrates are propionate , which is produced in high grain diet.
- When there is a mismatch between mammary drain of glucose for lactose synthesis and gluconeogenesis in liver, hypogycemia will result.
- The condition leads to ketosis.

Underfeeding Ketosis

- This type of ketosis occurs when a dairy cow receives insufficient calories to meet the lactational demands plus body maintenance.
- This type is further divided into Nutritional Underfeeding Ketosis and Secondary Ketosis.
- The Underfeeding Ketosis
- It occurs when the cow is given an insufficient quantity of feed or a diet with low metabolic energy densities.
- The Secondary Ketosis
- It occurs in cows that has other disease like hypocalcemia, mastitis, or metritis, which suppresses appetite and prevents feed consumption.
    - This type of ketosis resembles starvation ketosis and the difference is that there is the additional caloric and glycemic burden of milk production.
    - There are at least three type of syndromes that occur in cows during lactation.
    - The syndromes are characterized by anorexia, depression, ketonemia, ketolactia, ketonuria, hypoglycemia and decreased milk production.
    - The three syndromes are underfeeding ketosis, alimentary ketosis and spontaneous ketosis.

Alimentary Ketosis

- This type of 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 betahydroxybutyrate.
- When there is excess presence of butyrate, large quantity of beta hydroxbutyrate will be formed and released into the circulation with resulting ketosis.

Spontaneous Ketosis

- This is the most common form of ketosis and occurs near peak of lactation, that have access to abundant high quality feed, and that have no other diseases.

- The disease is not accompanied by severe acidosis and spontaneous recovery is common although there is a large decrease in milk production.

Hypoglycemic Theory

- The most widely accepted theory of bovine ketosis is the hypoglycemic theory.
- During lactation mammary gland might withdraw glucose from the plasma more rapidly than the liver can supply it, which leads to hypoglycemia.
- The hypoglycemia will lead to ketonemia as more of the LCFAs will reach the liver and oxidized. The net result of this is an increase in the level of ketone bodies.

Lecture-3

Ovine Pregnancy Toxemia

- This syndrome occurs in pregnant ewes that are carrying more than one fetus and that have been subjected to caloric deprivation or stress.
- Susceptibility increases as ewes approach term because the fetal glucose demands increase with increasing body size.

The Laboratory Test

- Rotheras test is used as a qualitative test for ketone bodies.
  - The test is most sensitive for acetoacetic acid.
  - Acetone gives only a slight response, whereas b-hydroxbutyric acid is insensitive to this test.
  - A number of drugs (substances) having keto, aldehyde or sulfhydril groups can also react with nitroprusside and give false positive result.

Lecture-4

Hormonal control of carbohydrate metabolism

1. **Insulin**: lnsulin is produced by B-cells of the islets of Langerhans in response to hyperglycemia (elevated blood glucose level). Some amino acids, free fatty acids, ketone bodies, drugs such as tolbutamide also cause the secretion of insulin. Insulin is basically a hypoglycemic hormone that lowers in hlood glucose level through various means. lt is an anti-diabetogenic hormone.
2. **Glucagon:** Glucagon is synthesized by alpha-cells of the islets of Langerhans of the pancreas. Hypoglycemia (low blood glucose level) stimulates its production. Glucagon is basically involved in elevating blood glucose concentration. lt enhances gluconeogenesis and glycogenolysis.
3. **Epinephrine:** This hormone is secreted by adrenal medulla. lt acts both on muscle and liver to bring about glycogenolysis by increasing phosphorylase activity. The end product is glucose in liver and lactate in muscle. The net outcome is that epinephrine increases blood glucose level.
4. **Thyroxine:** lt is a hormone of thyroid gland. It elevates blood glucose level by stimulating hepatic glycogenolysis and gluconeogenesis.
5. **Glucocorticoids:** These hormones are produced by adrenal cortex. Clucocorticoids stimulate protein metabolism and increase gluconeogenesis (increase the activities of enzymes-glucose 6-phosphatase and fructose 1,6-bisphosphatase). The glucose utilization by extrahepatic tissues is inhibited by glucocorticoids. The overall effect of glucocorticoids is to elevate blood glucose concentration.
6. **Growth hormone and adrenocorticotropic hormone (ACTH):** The anterior pituitary gland secretes growth hormone and ACTH. The uptake of glucose by certain tissues (muscle, adipose tissue etc.) is decreased by growth hormone. ACTH decreases glucose utilization. The net effect of both these hormones is hyperglycemic.

Lecture-5

Regulation of blood sugar and Clinical Significance of Blood glucose

Conditions in which hyperglycemia may occur:

1. Diabetes mellitus in the dog, cat, cow and sheep. This may arise due to:

a. Pituitary neoplasms.

b. Adrenal hyperplasia.

c. Pregnancy toxaemia.

d. Acute pancreatic necrosis.

2. Convulsions as in:

a. Eclampsia.

b. Intracranial trauma.

c. Epilepsy.

d. Tetany.

3. Transient conditions:

a. Exposure to cold.

b. Following general anaesthesia.

c. Administration of morphine.

d. Injection of epinephrine.

e. Intravenous glucose injection.

f. Feeding large quantities of carbohydrates.

g. Excitement.

4. Hyperthyroidism: There is increased glucose production by the liver in excess of utilization.

5. Chronic nephritis.

Conditions in which hypoglycemia may occur:

1. Starvation.
2. Hypopituitarism.
3. Hypothyroidism.
4. Hyper insulinism - a) Tumour of pancreas, b) Excess of insulin in therapy.
5. Adrenal cortical insufficiency.
6. Severe excretion- Glucose utilization is increased and liver glycogen is depleted.
7. Ketosis in cattle and sheep.
8. Hypoglycemia of baby pig (manifested by convulsions, weakness, coma and death). Blood glucose level is 40 mg%.

Lecture-6

LIVER FUNCTION TESTS (LFTs): Introduction of liver Functions

Liver is a versatile organ which is the central organ of body metabolism and independently involves in many other biochemical functions. Liver perform several diversified functions enumerated below:

1. **Metabolic function:** Liver is the key organ and the principal site where the metabolism of carbohydrate, lipids, protein, minerals and vitamins take place.
2. **Secretory function:** Liver is responsible for the formation and secretion of bile in the intestine. Bile pigment bilirubin formed from heme catabolism is conjugated in liver cells and secreted in the bile. Cholesterol and bile salts are also secreted in the bile into the intestine.
3. **Excretory function:** Exogenous dye BSP (bromosulphthalein) and Rose Bengal dye are excreted through liver cells.
4. **Detoxification and Protective function:** Ammonia is detoxified to urea. Liver cells can detoxified drugs, hormones and convert them into less toxic substances for excretion. Kuffer cells of liver perform phagocytosis to eliminate foreign compounds.
5. **Storage function:** Liver stored glycogen, trace mineral iron and vitamin A, D and B_{12}.
6. **Hematological function:** Liver participates in the formation of blood particularly in the embryo (adults in some abnormal states), synthesis of plasma proteins and blood clotting factors and destruction of erythrocytes.

Lecture-7

LFTs : Standard liver function tests

Numerous laboratory investigations have been proposed in the assessment of liver diseases. **"Standard Liver Function Tests (LFTs)"** will help to detect the abnormilities and extent of liver damage.

Classification of LFTs: Classification is based on the specific functions of the liver involved.

1. Tests based on metabolism:

i. Carbohydrate metabolism:
- Galactose tolerance test
- Fructose tolerance test

ii. Lipid metabolism:
- Serum cholesterol: Free and esterified form of cholesterol estimation and their ratio
- Estimation of fecal fats

iii. Protein metabolism:
- Estimation of total protein, Albumin, Globulin and A/G ratio
- Determination of prothrombin time
- Flocculation tests: Thymol turbidity test, Zinc sulfate test, Colloidal gold test, Cephalin cholesterol flocculation test, Formal gap test.
- Amino acids in urine

2. Tests based on detoxification and protective functions of liver:

i. Conversion of ammonia to urea:
- Estimation of blood urea
- Estimation of blood ammonia

ii. Formation of bilirubin diglucuronate:
- Estimation of serum bilirubin (Direct and Indirect)
- Icteric index
- VD Bergh reaction
- Urinary estimation of bilirubin and uribilinogen

iii. Hippuric test

3. Tests based on excretory functions:
- Bromosulfthalein (BSP) test
- I^{131} Rose Bengal test

4. **Tests based on storage functions of liver:**

i. Glycogen estimation

ii. Lipid estimation

iii. Estimation of vitamin A, D, B_{12}

iv. Estimation of serum iron and serum iron binding capacity

5. **Tests based on serum enzymes derived from liver:** Determination of

- Transaminases
- Alkaline phosphatases
- S- nucleotidase
- ϒ-glutamyl trans peptidase

6. **Cellular structural studies:**

Liver biopsy

The importance of liver function tests are:

1. To assess the severity of liver damage
2. To differentiate different types of jaundice
3. To find out the presence of latent liver diseases.

- The first group of tests includes regarding secretory, excretory and enzymatic functions are: Serum bilirubin test, bilirubin and urobilinogen in urine, BSP excretion test, Serum alkaline phophatase estimation and SGPT.
- Second group of tests meant for assessing the protein synthetic functions are: Total protein estimation, A/G ratio and prothrombin time.
- The final group include that are meant for lipid metabolic functions are: Estimation of serum cholesterol and determination of free and esterified cholesterol ratio.

Tests based on abnormalities of bile pigment metabolism

1. **VD Bergh reaction and Serum Bilirubin:** Bilirubin is estimated by VD Bergh reaction involving Diazo reagent. Van Den Bergh reaction consists of two parts, the direct and indirect reactions.

A. **Direct reacton:** In immediate development of violet colour in 10-30 seconds and delayed direct reaction in which colour appears from 5-30 minutes and then develops slowly to a maximum.

B. **Indirect reaction:** Indirect reaction is a essentially method for the quantitative estimation of serum bilirubin. Serum diluted with distilled water and methanol added in an amount

insufficient to precipitate the proteins, yet sufficient to permit all the bilirubin to react with the diazo-reagent.

Bilirubin as such is insoluble in water while the conjugated bilirubin is soluble and gives direct positive Van den Bergh reaction. Unconjugated bilirubin gives indirect positive Van den Bergh reaction. If the serum contains both unconjugated and conjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensify by the addition of alcohol (indirect positive). This type of reaction is known as "biphasic".

Interpretation: Normal serum bilirubin level is 0.2-0.6 mg/dl.

Response of VD Bergh reaction can differentiate the jaundice as follow:-

- **In hemolytic/pre-hepatic jaundice** un-conjugated bilirubin increased Hense indirect positive reaction obtained. Occasionally it may be a delayed direct reaction.
- **In obstructive/post-hepatic jaundice** conjugated bilirubin is increased hence an immediate direct positive reaction obtained.
- **In hepatic jaundice** either or both may be present (biphasic reaction).

2. **Bilirubin in urine:** The conjugated bilirubin, is excreted in urine due to water soluble. This is in contrast to unconjugated bilirubin, which is not excreted. Bilirubin in urine can be detected by Gemlin's or Fouchet's test.

Tests based excretory function of liver:

- **Bromosulphthalein test (BSP):** A measured amount of dye is injected intravenously. The liver removes the dye rapidly and excretes in the bile. If the liver function is impaired, the excretion is delayed and larger proportion of dye remains in the serum. It is very sensitive test and is most useful in liver cell damage without jaundice, in cirrhosis and chronic hepatitis. In healthy adults not more than 5% dye should be remain in blood but the bulk of dye is removed in 25 minutes.

  In hepatic diseases, cirrhosis, 40-50 % of dye retension takes place. Also abnormal retention of dye in hepato-cellular or obstructive jaundice takes place.

Tests based metabolic capacity of liver:

- **Galactose tolerance test:** LFT can be assessed by measuring the utilization of galactose. This is known as galactose tolerance test. The animal is given intravenously galactose (about 300 mg/kg body weight). Blood is drawn at 10 minutes intervals for the next 2 hrs and galactose estimated. If the normal individuals half life of galactose is about 10-15 minutes. This is markedly elevated in hpatocellular damage (infective hepatitis, cirrhosis).

- **Serum protein, Albumin and A/G ratio:** Serum protein estimation yields most useful information in chronic liver disease. The liver is the site of albumin, fibrinogen and some of α and β-globulin synthesis. The half life of albumin is about 20-25 days. In advanced liver diseases, the albumin is decreased and globulin is increased so that albumin-globulin ratio is reversed in liver cirrhosis. Serum proteins are decreased in malnutrition and liver damage. Low serum albumin is found in severe liver damage due to impairment ability of the liver to form albumin.
- **Estimation of total and esterified cholesterol:** Liver synthesizes esterified cholesterol and excretes into bile. So cholesterol level is the marker for liver disease. Cholesterol level is decreased in hepatitis, cirrhosis, hyper-thyroidism, malabsorption syndrome in severe wasting in acute infections pernicious anemia etc. Cholesterol level is increased in obstructive jaundice, intrahepatic obstruction, myxedema, lipid storage disease, atherosclerosis, nephritic syndrome, diabetes mellitus etc.
- **Prothrombin time:** The time required for clotting to take place in citrated plasma to which optimum amounts of thromboplastin and calcium has been added. Prothrombin is formed by the liver cells, vitamin K. When bile salts are not present in the intestine, the absorption of vitamin K form the intestine is impaired. In jaundice and liver disease the prothrombin time is prolonged.

Normal values for icteric index and prothtombin time:

Species	Icteric index	Prothrombin time
Cattle	8.4-9.8	7 minutes
Buffalo	7.1-9.8	9 minutes
Horse	15.5-18.0	11 minutes
Sheep	6.8-10.7	2-3 minutes
Dog	5.8-6.0	4 minutes

Tests based detoxification:

- **Hippuric acid synthesis:** Liver is the major site for detoxification. This test is ideal test for assessing the detoxification function of liver. Hippuric acid is produced in the liver when benzoic acid combines with glycin. 3 gm of hippuric acid, expressed as Benzoic acid or 3.5 gm of sodium benzoate should be excreted in healthy condition. Smaller amounts are found when there is either acute or chronic liver damage. Amount lower than 1 gm may be excreted by patients with infectious hepatitis.

Value of serum enzymes in liver diseases:

A large number of enzyme estimations are available which are used to ascertain liver function.

Most routinely used laboratory tests are two: Serum transaminase (Amino transferase) and Serum alkaline phosphatase.

A. **Serum Alkaline Phosphatase:** Normal level is 3-13 KA(King-Armstrong) Units/100 ml (23-92 IU/L).

Increased level of alkaline phosphatase is found in post necrotic disease, cirrhosis, carcinoma of liver, obstructive jaundice, hepatocellular jaundice

Liver Biopsy: Histopathological studies of liver biopsy reveal various pathological states of liver cells. Indications for Liver biopsy are fibrosis or neoplasms of liver, in metabolic diseases, arsenic poisoning selenium poisoning and for estimation of Vitamin A, Vitamin E, Cu, Zn, Liver glycogen. **Biopsy is contraindicated in liver abscess.**

Lecture-8

Kidney Function Tests (KFT)/ Biochemical Tests for Renal Function

Kidney plays an important role in the maintenance of water volume, electrolyte and acid-base balance in the body. Kidney serves an important function of excretion of products of metabolism and other harmful substances. Renal/ Kidney function tests are done to assess the functional capacity of kidney (Blood flow to the kidney, glomerular filtration and tubular function). The aim of renal function tests is to detect impairment of renal function as early as possible. The kidney function can be assessed by examination of blood and urine.

The following are commonly used kidney function tests:-

(A) Urine examination: Simple routine examination of urine for Volume, pH, Concentration test / specific gravity test, Osmolality and presence of certain abnormal constituents (Proteins, ketone bodies, blood, glucose etc.).

(B) Blood/serum analysis: Estimation of blood urea nitrogen, serum creatinine, protein and electrolyte.

(C) Glomerular function tests: Clearance test (Urea, inulin, creatinine)

- **Inulin clearance test:** This test is done to find the **glomerular filtration rate (GFR)**. Inulin is filterated by the glomerulus but it is neither secreted nor absorbed by tubules. Inulin is given subcutaneously or by intravenous infusion. The amount of inulin excreted in each minutes is equal to the amount filtered by the glomeruli. Normal rate is 110 to 150 ml per minute.

(D) Tubular function tests: Urine concentration or dilution test, urine acidification test.

Other important renal function tests:

- **Phenol Sulfonaphthalein (PSP) test:** It indicates a general loss of nephron function. A measured amount of phenol Sulfonaphthalein is injected intravenously and then urine is collected at intervals of 40 to 60 minutes. The rate of disappearance of the dye from the plasma can be determined from blood sample taken prior to and then at regular intervals of 30 minutes following its injection. The dye clearance time is prolonged in kidney disease.
- **Methylene blue excretion test:** Methylene blue (2% @0.4 ml/kg) is injected intramuscularly and examined for clearance of dye. In normal condition the excretion of dye reaches its maximum after 1 hour and the clearance is complete within 24 hours. Delay in time indicates kidney dysfunction.

  The choice of kidney function tests starts with routine urine examination, followed by serum creatinine and/or other blood urea estimation and finally the specific tests to measure the tubular and glomerular functions (Clearance tests).

Lecture-9

Estimation and clinical significance of creatinine

Creatinine, in a protein-free filtrate, is determined by its reaction with alkaline picrate to form a yellow- red tautomer of creatinine picrate, the Jaffc's reaction. The intensity of the colour is proportional to the optical density which is measured at 520 nm.

Clinical Significance:

- Clinically insignificant at lower values. It is higher in males since it is related to body size.

Increased values:

- Increased serum levels are seen in renal failure and other renal diseases in a manner similar to urea.
- Creatinine, however, does not increase with age, dehydration and catabolic states (eg fever, sepsis, haemorrhage) to the same extent as urea.
- It is also not affected by diet.
- But the Jaffe's reaction for measuring serum creatinine is not as sensitive and reliable as method for urea. It is interfered with by Ketone bodies and glucose and hence false high values may be obtained in diabetes ketoacidosis.
- serum creatinine is not significant. It is associated with muscle wasting diseases.
- The creatinine production depends on the modification of the muscular mass, and it varies little and the levels usually are very stable.

Lecture-10

ESTIMATION OF BUN (Blood Urea Nitrogen) (DIACETYL MONOXIME PROCEDURE)

The urea reacts with diacetyl in hot acid solution at nearly 100°C, which is released from diacetyl monoxime by an oxidative condensation reaction, to give a coloured product. Diacetyl monoxime is used because of its greater stability. The absorbance colour developed is measured at 480 nm. The intensity of the colour developed is proportional to the concentration of urea present in the sample.

Clinical significance:

The urea concentration varies with the amount of protein in the diet.

Increase of levels: Increases in urea is significant as a measure of renal function. Increase in blood urea occurs in a number of diseases in addition to those in which the kidneys are primarily involved. The causes can be classified as;

Pre Renal: When there is reduced plasma volume it leads to decreased renal blood flow and hence GFR leading to urea retention. Seen in Reduced plasma volume:-

- Acute intestinal obstruction – Severe and prolonged vomiting.
- Severe diarrhoea.
- Pyloric stenosis with severe vomiting.
- Ulcerative colitis with severe chloride loss.
- Diabetic Ketoacidosis.
- Shocks, severe burns and haemorrhage.
- Salt and water depletion
- Hematemesis
- In crisis of Addison's diseases
- Increased protein catabolism:- Fever, Thyrotoxicosis,Cardiac failure -

Renal Disease: Blood urea is increased in all forms of renal diseases like;

- Acute glomerulonephritis.
- Renal failure
- Malignant hypertension
- Malignant nephrosclerosis
- Hydronephrosis
- Chronic pyelonephritis
- Congenital cystic kidneys

Post renal: Due to obstruction to flow of urine there is retention and so reduction in effective filteration pressure at the glomeruli; when prolonged produces irreversible kidney damage. Causes are:

- Enlargement of prostrate.
- Stones in urinary tract.
- Stricture of the urethra
- Tumors of the bladder affecting urinary flow

Decreased levels: It is rare but may be seen: In some cases of severe liver damage. Physiological condition- Blood urea has been seen to be lower in pregnancy than in normal non pregnant.

Lecture-11

Biochemical aspects of digestive disorders

Stomach is major organ of digestion and performs the following functions.

1. Stomach is a reservoir of ingested foodstuffs.
2. It has a great churning ability which promotes digestion.
3. Stomach elaborates HCl and proteases, which are responsible for the initiation of digestive process.
4. The products obtained in the stomach (peptides and amino acids) stimulate the release of pancreatic juice and bile.

Secretion of gastric HCI : The parietal cell of gastric glands produce HCI. A unique enzyme K^+ activated ATPase present in the parietal cells is connected with the mechanism of HCI secretion. The process involves an exchange of H^+ ions (of the parietal cells) for K^+ ions (of the lumen). This is coupled with the consumption of energy, supplied by ATP. The H^+ are continuously generated in the parietal cells by the dissociation of carbonic acid. The bicarbonate ions liberated from the carbonic acid dissociation, enter the blood in exchange for Cl^- ions. The latter diffuse into the gastric lumen to form HCI. Gastric peptide hormone of gastro-intestinal tract stimulates HCl secretion.

Following a meal, there is a slight elevation in the plasma bicarbonate concentration which is linked with gastric HCl secretion. This is referred as alkaline tide.

Tests to assess gastric function

1. Pentagastrin stimulation test:

Pentagastrin" is a Synthetic peptide which stimulates the gastric secretion on in a manner Similar to natural gastrin. The stomach contents are aspirated by Ryle's tube in a fasting condition. This is referred to as residual juice. The gastric juice elaborated for the next one hour is collected and pooled which represents the basal secretion. Pentagastrin (5 pg/kg body weight) is now given to stimulate gastric secretion. The gastric juice is collected at 15 minute intervals for one hour. This represents the maximum secretion. Each sample of gastric secretion collected is measured for acidity by titration the samples with N/10 NaOH to pH 7.4. The end point may be detected by an indicator (phenol red) or a pH meter.

Lecture-12

Pancreatic function tests

The pancreas is a specialized organ with exocrine and endocrine functions. The exocrine functions involve the synthesis of pancreatic juice containing several enzymes and bicarbonate. The major enzymes of pancreatic juice are trypsin, chymotrypsin. Carboxy-peptidase, amylase and lipase.

Pancreatic enzymes in serum

Serum lipase and amylase are commonly employed to assess pancreatic function. Both these enzymes activities are elevated in acute pancreatitis, obstruction in intestine and/or pancreatic duct.

Determination of Serum Amylase Activity by Sterkel and Kirsner method:

Principle: Starch is incubated with serum at a controlled pH for a standard period of time. The serum amylase activity is then destroyed by the addition of tungstic acid and the reducing substances present before and after incubation determined by the method of Folin and Wu.

Procedure: Take two test tubes (20 X 200 mm) and place 5 ml of starch solution and 2ml of sodium chloride solution. In one tube place 1 ml of non-hemolysed serum and mix well. Incubate both the tubes at exactly 37°C for exactly 30 minutes. Place 3 ml of distilled water and 8 ml of 0.085 N Sulphuric acid into each tube. Add 1ml of non-hemolyzed serum to the second tube (control) and mix well. Add 1ml of sodium tungstate to both the tubes, mix and let stand for several minutes until precipitation is complete. Filter and determine the reducing sugar content on 2ml of each filtrate, using Folin-Wu method.

Calculation:

mg% sugar in test - mg% sugar in control X 2

= Serum amylase activity in somogyi units/100 ml serum

Interpretation: The normal range for serum amylase activity varies with the method used for determining reducing sugars. Serum amylase values rise within several hours after the onset of acute pancreatitis, reaching peak values within 24 hrs and returning to normal in 3 to 6 days. Pancreatitis may be extra pancreatic in origin as in acute appendicitis or biliary obstruction or intrapancreatic as in cancer of hepato pancreatic ampulla. Increased values are observed in appendicitis. In chronic pancreatitis, acute attacks are associated with similar elevation but the return to normal is often slower. After repeated acute attacks in chronic pancreatitis, destruction

of tissue may become widespread as to prevent measurable production of the enzyme. In these instances increased serum amylase activity may not be observed.

Determination of Serum Lipase Activity by Cherry and Crandall Method:

Principle : Serum is incubated with an olive oil emulsion and fatty acid produced are titrated with sodium hydroxide.

Procedure : lnto each of two tubes or flasks, place 3ml distilled water and 1ml serum. Place one tube (control) into boiling water for 5 minutes and cool. Add 0.5 buffer solution and 2ml of olive oil emulsion to both tubes, shake well and incubate at 37°C for 24 hrs. Then add 3ml of 95% alcohol and 2 drops of phenolphthalein indicator and mix. Titrate each tube with 0.05N sodium hydroxide until the appearance of a permanent pink color (pH, 10).

Calculation :

ml NaoH for unknown - ml NaoH for control = **units lipase activity/ml serum**

Interpretation: The normal serum lipase activity by this method is upto 1.5 units. The determination of serum lipase activity is complementary to the serum amylase determination, since elevations of serum lipase disappear much more slowly. Greater organ specificity is achieved using olive oil emulsion, thus permitting differentiation between pancreatitis and appendicitis. Elevations of serum lipase often occur in chronic pancreatitis after the condition has reached a state where elevations of serum amylase activity no longer occur.

Basal Acid Output (BAO) : Refers to the acid output (millimoIe/hour). Under the basal conditions i.e. basal secretion. In normal conditions it is 4-10 m mole/hr.

Maximal Acid Output (MAO) : Represents the acid output (millimoIe/hour) after the gastric stimulation by pentagastrin i.e. maximum secretion. In normal conditions it is 20-50 m mole/hr.

Insulin test meal :

This is also known as Hollander's test it is mainly done to assess the completeness of vagotomy (vagal resection). Insulin (0.1 unit/kg body weight) is administered intravenously, which causes hypoglycemia (blood glucose about 40 mg/dl), usually within 30 minutes in normal persons. If the vagotomy operation is successful, insulin administration does not cause any increase in the acid output compared to the basal level.

Lecture-13

Disturbance of rumen function

The digestive process in the rumen involves the interplay between many species of bacteria and other microbes. The proteins and carbohydrates are fermented to short chain fatty acid, which are the energy sources for ruminants.

Acute rumen Indigestion

- When the animals consuming roughages are overloaded with readily fermentable carbohydrates, high concentrations of lactic acid accumulate in the rumen and subsequently in the blood.
- Rumen bacteria produce 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.

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 (Foamy).
- When cattle consumes legumes, which contains soluble plant proteins a stable froth is formed in which gas is trapped as small bubbles which are eliminated by eructation.
- When cattle are with high concentration diet, formation of extracellular dextran slime by amylolytic bacteria in the rumen is the cause of stable foam.
- A plant enzyme pectinand pectin methyl esterase act on pectin to release pectic and galacturonic acids, which increases the viscosity of the rumen fluid resulting in the formation of stable foam.
- Presence of tannins inhibits microbial activity, hence acts as an antibloat agent.
- Non ionic detergent with surfactant property can be used for treatment eg. Sodium alkyl sulfonate.

Urea poisoning

- Ammonia and other NPN substances metabolized to ammonia are used by microbes to synthesize microbial proteins, which are subsequently utilized by the ruminants for the synthesis of body proteins.
- Urea poisoning the develops when urea is fed at more than 3% level as urea is hydrolysed to co_2 and NH_3 by urease enzyme of ruminal bacteria. (excess urea result in release of excess of ammonia in excess of what the liver can tolerate). The free ammonia crosses the cell membrane thereby producing harmful effects.
- When acetic acid is given orally, the proton of acetic acid converts free ammonia to ammonium ion, which reduces the absorption of NH_3. The NH_3, which has no charge, will diffuse freely, whereas ammonium ion is charged, diffusion is prevented.

Lecture-14

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, intussuception, 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 control by centres in brain.

Biochemical changes during vomition

- During vomition loss of water and HCl. These loses result in dehydration and metabolic alkalosis with increased level of bicarbonate ion and decreased level of chloride ion concentration.
- Gastric vomition 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 hypovolemic due to dehydration may cause renal tubular damage and kidney failure.

Diarrhoea

- It is rapid elimination of watery fecal material with increased frequency and volume or both .
- It is due to parasite, infection by bacteria or virus in the intestinal tract, feeding poor quality diet, sudden dietry change, food poison, heavy metal and presence of organophosphorus compound.

Biochemical changes

- Diarrhoea results in dehydration associated with H^+ and electrolyte disturbances.
- Dehydration cause haemoconcentration, which leads to hypovolemic shock, this is characterized by decreased excretion of hydrogen,over production of lactic acid, Hyperkalemia.
- Hypoglycemia

- 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.
- There is hyperkalemia, hyperphosphotemia 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.

Lecture-15

Lactose Intolerance

Definition

Lactose intolerance-the inability to break down the lactose in milk due to defeciency of enzyme lactase secreted by the intestinal cells.

- The ubiquitousness of this condition causes some to feel that it is not really a disease among adults.
- Lactose malabsorption and milk products intolerance symptoms are the most common alimentary tract disorders.
- Especially seen in young ones.

Cause

- Lactase is an intestinal enzyme that helps digest lactose, a sugar that is found in many foods, especially dairy products.
- Diarrhea, gas, and abdominal pain can occur when there is not enough lactase to digest milk products.
- Lactose intolerance was identified as the cause of bovine neonatal diarrhea
- Although lactase deficiency is the most common carbohydrate malabsorption syndrome, other enzymes needed to absorb various sugars (disaccharides).
- The he clinical symptoms of lactose intolerance belongs: nausea, vomiting, abdominal distension, cramps, flatulence, flatus, diarrhea and abdominal pain.

Laboratory Diagnosis

- A lactose tolerance test-the administration of a lactose drink followed by monitoring for gastrointestinal symptoms-confirms the diagnosis.
- During this test, the blood may also be tested for glucose (sugar), which rises in the lactose-tolerant.
- Other confirming tests include stool analysis for a high acid content, which signifies intolerance.

Lecture-16

Regulation of acid-base balance

Balance between acid and based is essential for metabolic processes. Reaction of any solution depends on free hydrogen ions concentration ($[H^+]$). The term used to indicate $[H^+]$ is pH . pH is negative logarithm of hydrogen ions concentration:

$$pH = - \log [H^+]$$

pH depends on balance between $[HCO_3]$ and CO_2. CO_2 concentration is regulated by lungs. Bicarbonate ion $[HCO_3]$ is a base, metabolized mainly in kidneys. CO_2 dissolves in plasma, forming carbonic ion (H_2CO_3), which is main acid component of blood, as it's difficult to determine H_2CO_3 concentration directly, acid component is expressed as carbon dioxide concentration in normal CO_2 to HCO_3 ratio is approximately 1/20. In different cases of acid base disturbances when acid content increases - acidosis will develop, if base content increase – alkalosis will develop.

Regulation of acid - Base Balance

The main buffer systems are following:

1. **Bicarbonate buffer:** The most important extracellular buffer produced by kidneys, has the largest buffering capacity.
2. **Haemoglobin buffer:** Main intracellular buffer of the blood.
3. **Protein buffer:** An extracellular buffer together with bicarbonate buffer, represented by plasma proteins.
4. **Phosphate buffer:** It takes part in hydrogen ions excretion in renal tubules, is not of great importance in blood.

Main blood buffer systems:

Buffer system	Buffering capacity (%)
Bicarbonate	53
Haemoglobin	35
Protein	7_
Phosphate	5

Cellular mechanisms of regulation of acid-base: Change in blood pH causes activation of cellular mechanisms of maintaining constancy of hydrogen ions concentration in extra-cellular fluid:

• If pH increases hydrogen ions move from cells to extracellular fluid in exchange of potassium ions that enter the cells and alkalosis is usually accompanied by hypokalaemia.

- If pH decreases hydrogen ions enter the cells in exchange of potassium ions that leaves the cells and acidosis may cause hyperkalaemia. In such a way electro-neutrality law is maintained by cellular regulation. According to it, the sum of the positive and negative charges (cations and anions) is equal. So, hydrogen to potassium exchange between ECF and ICF should be equal.

Organ mechanisms of regulation of acid-base:

- **Respiratory mechanisms:** Lungs are responsible for volatile acid (carbon dioxide) emanation. CO_2 content in plasma depends on alveolar ventilation. Changes in pH lead to stimulation of chemo-receptor's in the brain stem, causing a compensatory mechanism; therefore changing the respiratory rate. In acidosis alveolar ventilation increases, $PaCO_2$ decreases and pH tends to return to normal. These changes occur rapidly, but it takes 12 to 24 hours to stabilize acid-base status. Alkalosis causes hypoventilation and rise in $PaCO_2$, that leads to fall in pit.

- **Renal mechanisms:** Renal mechanisms are the most complex and effective. Renal compensation occurs by three main mechanisms:
  1. Bicarbonate ions reabsorption in proximal tubules
  2. Bicarbonate ions regeneration in distal tubules
  3. Hydrogen ions excretion.

CO_2 reacts with water to produce carbonic acid into the renal tubular cells. Carbonic acid dissociates to yield H^+ and HCO_3^- reaction is catalyzed by carbonic anhydrase. Bicarbonate ion enter the systemic circulation, is secreted into the lumen. The secretion is coupled to the reabsorption of Na* and electro neutrality preserved. The secreted reacts with filtered bicarbonate to produce carbonic acid that dissociate into carbon dioxide and water. Hydrogen ions excretion begins at the second stage when the whole bicarbonate is reabsorbed. HPO_4 ion can't be reabsorbed from renal tubules because of charge, but it can bind secreted hydrogen ions. Produced H_2PO_4 - is excreted in urine, HCO_3 is reabsorbed into the blood.

After depletion of the latter mechanisms, the kidneys switch to ammonia buffer (NH_3/NH_4^+). The main source of ammonia is glutamine deamination. As NH_3 has no charge, it moves freely across the tubular cell membrane and appears in the urine, where it binds scatted proton to produce ammonium ions (NH_4^+). NH_4^+ can't be reabsorbed because of its charm. This process is termed as ammoniogenesis.

Lecture-17

LABORATORY ACID-BASE (or blood gas) ANALYSIS

When you request an acid-base or blood gas analysis, always remember to send

1. Arterial or capillary blood: To measure arterial pO_2 and pCO_2 values.
2. A heparinised sample: Most 0_2 is carried in red blood cells so we need an anti-coagulated sample.
3. In a sealed syringe: To prevent 0_2 and CO_2 diffusing out of the sample.
4. On ice : To prevent ongoing red cell metabolism from generating a lactic acidosis.

Acid-Base Balance Disturbances

There are several classifications of acid-base balance disturbances. The main ones are shown in table:

Parameter	**Typs of distrurbance**
Blood pH	Acidosis Alkalosis
Primary disturbance	Respiratory Metabolic Mixed Combined
Compensation	Compensated Subcompensated Non- compensated

According to compensatory changes of different types of acid-base balance disturbances

Type of disturbance	[H+]	pH	Primary disturbance	Compensation
Metabolicacidosis	↓	↓	↓ $[HCO_3]$	↓ pCO_2
Metabolicalkalosis	↓	↑	↑	↑
Respiratoryalkalosis	↑	↑	↑	↓
Respiratoryalkalosis	↓	↑	↓	↓

Lecture-18

Respiratory acidosis and alkalosis

Respiratory Alkalosis

Respiratory alkalosis is defined as a pH greater than 7.45 with a pCO_2 less than 35 mm Hg. Respiratory alkalosis appears if removal of CO_2 is greater than production by tissues.

Parameters	**Acute Respiratory Alkalosis**	**Chronic respiratory Alkalosis**
pCO_2	↓	↓
$[HCO_2]$	Normal or ↓	↓
pH	↑	↑Or normal

Any condition that causes hyperventilation can result in respiratory alkalosis. These conditions include:

1. Increased metabolic demands, such as high fever, sepsis, pregnancy, or thyro-toxicosis
2. Psychological responses, such as anxiety or fear.
3. Central nervous system lesions, raised intracranial pressure, which may stimulate the respiratory fever.
4. Hysterical overbreathing.
5. Mountain sickness.
6. Lack of oxygen, hypoxia.
7. CNS injury, neuroinfection, cerebral haemorrage, brain tumor.
8. Salicylate overdosage or other respiratory stimulants (theophyllin, estrogens).
9. Excessive artificial respiration
10. Pulmonary diseases: lobar pneumonia, asthma, pulmonary oedema, pulmonary collapse or fibrosis, pulmonary embolism.

Respiratory Acidosis

Respiratory acidosis is defined as a pH less than 7.35 with a $PaCO_2$ greater than 45 mm Hg. Acidosis is caused by an accumulation of CO_2, lowering the pH of the blood.

Parameteres	Acute Respiratory Acidosis	Chronic Respiratory Acidosis
pCO_2	↑	↑
Hco_2	normral	↑
pH	↓	↓

Any condition that results in hypoventilation can cause respiratory acidosis. These conditions include:

1. Respiratory center depression:

• Central nervous system depression related to head injury, neuroinfection, stroke, brain tumor, increased intracranial pressure.

- Central nervous system depression related to medications such as narcotics, tranquilizes, barbiturates, or anesthetics;

2. Neuromuscular diseases:

• Impaired respiratory muscle function related to spinal cord injury, or neuromuscular blocking drugs, poliomyelitis, Guillian-Barr syndrome, muscular dystrophy, hypokalaemia.

3. **Chest abnormalities:** Hypoventilation due to pain, chest wall injury/deformity (kyphoscoliosis), abdominal distension, pneumothorax, hydrothorax.

4. **Pulmonary disorders:**

• Atelectasis, pneumonia, bronchitis, asthma, pulmonary oedema, emphysema, or bronchial obstruction

• Massive pulmonary embolus.

Lecture-19

Metabolic acidosis and alkalosis

Metabolic Alkalosis

Metabolic alkalosis is defined as a bicarbonate level greater than 26 mEq/liter with a pH greater than 7.45, either an excess of base or a loss of acid within, the body can cause metabolic alkalosis.

Causes of Metabolic Alkalosis

1**. Saline- responsive urinary chloride excretion < 20 mmo1/1 (chloride depletion):**

- **Gastric losses:** vomiting, mechanical drainage, gastric aspiration
- **Diarrhoeal states:** venous adenoma, congenital chloridiarrhoea
- **Diuretic therapy:** e.g. furosemide, chlorothiazide, bumetanide
- **Cystic fibrosis** (high sweat chloride)

-Acute or chronic milk-alkali syndrome (in patients, who drink lots of milk or Calcium –
Containing antacids.

- Exogenous alkali (sodium citrate, lactate, gluconate, acetate).
- Massive blood transfusion.
- Bicarbonate ingestion massive or with renal insufficiency.

2. Saline-unresponsive urinary chloride excretion < 20 mmol/1 (Potassium depletion/ Mineeralocorticoid excess):

- Primary hyperaldosteronism (Conn's syndrome);
- Secondary hyperaldosteronism;
- Cushing' s syndrome;
- Eddie s syndrome (hypermineralocorticism, hypertension and hypokalaemic

Compensatory mechanism: hypoventilation.

Metabolic Acidosis

Metabolic acidosis is defined as a bicarbonate level of less than 22 mEq/1 with a pH of less than 7.35. Metabolic acidosis is caused by either a deficit of base in the bloodstream or an excess of acids, other than CO_2.

Causes of Metabolic Acidosis

1. Kidney dysfunction, that results in retention of nonvolatile acids; impairment of the ability of renal tubules to generate bicarbonate ions (distal renal tubular acidosis); renal losses of bicarbonate (proximal renal tubular acidosis).
2. Increased endogenous organic acids production - ketoacidosis due to insulin deficiency (diabetic ketoacidosis) or due to lack of glycogen (starvation); enzyme defects; lactic acidosis due to tissue hypoxia.
3. Intake of exogenous acids, their precursors or substances, that block certain metabolic pathways, that leads to nonvolatile acids accumulation in the body (poisoning by salicylate, ammonium chloride, methanol, ethanol, ethylene glycol).
4. Gastrointestinal bicarbonate loss: diarrhoea, GIT drainage.

Compensatory mechanism: hyperventilation through stimulation of central chemoreceptors

Lecture-20

Plasma proteins

Blood proteins, also termed **plasma proteins**, are proteins present in blood plasma. They serve many different functions, including transport of lipids, hormones, vitamins and minerals in activity and functioning of the immune system. Other blood proteins act as enzymes, complement components, protease inhibitors or kinin precursors. Contrary to popular belief, haemoglobin is not a blood protein, as it is carried within red blood cells, rather than in the blood serum.

Serum albumin accounts for 55% of blood proteins, is a major contributor to maintaining the oncotic pressure of plasma and assists, as a carrier, in the transport of lipids and steroid hormones. Globulins make up 38% of blood proteins and transport ions, hormones, and lipids assisting in immune function. Fibrinogen comprises 7% of blood proteins; conversion of fibrinogen to insoluble fibrin is essential for blood clotting. The remainder of the plasma proteins (1%) are regulatory proteins, such as enzymes, proenzymes, and hormones. All blood proteins are synthesized in liver except for the gamma globulins.

Separating serum proteins by electrophoresis is a valuable diagnostic tool as well as a way to monitor clinical progress. Current research regarding blood plasma proteins is centered on performing proteomics analyses of serum/plasma in the search for biomarkers.

Families of blood proteins

Blood protein	Normal level	%	Function
Albumins	3.5-5.0 g/dl	55%	create and maintain osmotic pressure; transport insoluble molecules
Globulins	2.0-2.5 g/dl	38%	participate in immune system
Fibrinogen	0.2-0.45 g/dl	7%	Blood coagulation
Regulatory proteins		<1%	Regulation of gene expression
Clotting factors		<1%	Conversion of fibrinogen into fibrin

Lecture-21

Clinical significance of plasma proteins

Abnormal Protein Concentration

- The liver synthesizes of albumin, fibrinogen, prothrombin and most of the globulins particularly alpha and beta globulins.
- The gamma globulins are synthesized in the lymphoid organs.
- The normal range of total protein levels in most of the animals ranges between 5 and 8 g/dL.
- Edema develops when the total protein concentration in plasma falls below 5g/dL.

Hypoproteinemia: (decreased protein concentration)

- Hypoalbuminemia with hypoglobulinemia: It may be due to decreased concentrations of albumin, globulin or both.
- Blood loss
- Due to proportional loss of all blood constituents, interstitial fluid moves into the circulatory system and dilutes the remaining blood causing a decrease in the level of albumin and globulin.
- Protein losing entropy
- During a variety of intestinal lesions both albumin and globulin leak from the intestinal wall into the intestinal lumen and then are digested or excreted.
- Severe exudative skin disease
- This results from vascular permeability that allows both albumin and globulin to escape from the blood.
- Severe burns: These cause increased vascular permeability that can result in loss of both albumin and globulin.
- Effusive disease: This results in the accumulation of body cavity fluids with high protein concentrations that can result in decreased albumin and globulin concentrations.
- The decrease depends on the degree of increased vascular permeability.
- Hypoalbuminemia with normal to increased globulin concentrations.
- The deceased albumin concentration can result from either decreased production or increased loss of albumin.
- If the concentration of globulin is increased the total protein level may be normal.
- Decreased production of Albumin can occur in the following disorders:
    - Hepatic Failure
    - Starvation
    - Gastrointestinal Parasitism
    - Malabsorption
    - Exocrine pancreatic insufficiency (EPI)
    - Inadequate digestion of dietary proteins can result from EPI, in which amino acids are not liberated from the protein by digestion in the intestine, so they are not available for absorption.
    - Decreased albumin production.
    - Increased loss of proteins can occur in the following disorder

- Glomerular Diseases: Albumin are smaller than globulin, they leak more readily through damaged glomerular membrane.

Hyperproteinemia: (Increased Protein Concentration)

Hyperalbuminemia and Hyperglobulinemia

Causes:

- Loss of water from the blood causes an increased concentrations of albumin and globulin.
- The albumin : globulin ratio is not altered because both fractions are concentrated equally.
- Hyperglobulinemia: It depends on the type of globulin that is increased: Increased gamma globulin concentration.
- Acute inflammation is the most common cause.
- Concentrations of several proteins in the globulin fraction (e.g., Ceruloplasmin, haptoglobin, and alpha 2 macroglobulin) are increased.
- These proteins are collectively called as acute phase proteins.
- Increased beta globulin concentrations can occur with acute inflammation, nephrotic syndrome, liver disease and immune response.
- Concentrations of several acute phase proteins in this fraction (e.g., C-reactive proteins, complement, ferritin) increase during acute inflammation.
- Increased gamma globulin concentration: This fraction includes most of the immunoglobulins.
- Increases in gamma globulin concentration are termed as gammopathies and they are divided in to polyclonal (have broad based peak in the beta and gamma regions) and monoglonal gammopathies (have a narrow based electrophoretic peak in the beta and gamma regions), which suggests chronic inflammatory diseases (e.g., chronic bacterial, viral, fungal or rickttsial disordrer, parasitism (cutaneous parasites), cancer and immune mediated diseases.
- Multiple myeloma is due to the proliferation of single clone of B lymphocytes.This clone produces a homogenous type (monoclonal immunoglobulin) of protein called as paraprotein or M-component.

A/G ratio: A/G ratio provides a systematic approach to the interpretation of protein values.

- Normal A/G ratio: Dehydration with water loss results in hyperproteinemia with out a change in the A/G ratio.
- Albumin and globulin fractions are increased proportionately. Excess fluid intake or fluid therapy is a simple cause of hypoproteinemia. This is due to the dilution.
- **Decreased A/G ratio**: It is generally due to decreased level of albumin and increased level of globulins.The conditions resulting in the reduced level of albumin and increased level of globulins have been discussed earlier.
- **Increased A/G ratio:** Generally albumin is not produced in excess. Any increase in the level of albumin is due to hemoconcentration as a result of dehydration.
- Decreased globulins: Newborn animals are physiologically hypoglobulinemic(failure of passive transfer of cholostral antibodies).
- When there is a failure in the formation of gamma globulins(Immunosuppression or immunodeficiency.

Lecture-22

Acute Phase Proteins (APPs)

Acute phase proteins (APPs) are defined as proteins that change their serum concentration by >25% in response to inflammatory cytokines (IL-1, IL-6, TNFα). The acute-phase response is considered part of the innate immune system, and APPs play a role in mediating such systemic effects as fever, leukocytosis, increased cortisol, decreased thyroxine, decreased serum iron, and many others. APPs can be categorized as positive (increasing serum concentration) or negative (decreasing serum concentration). Increased production of positive acute phase proteins is a sensitive indicator of inflammation which can occur prior to the development of an inflammatory leukogram.

Positive acute phase proteins

Positive acute-phase proteins increase in plasma concentration in response to inflammation (usually within 1-2 days). Positive APPs are further categorized as major, moderate or minor, depending on the degree of increase.

- **Major APP:** A protein with a low concentration in the serum of healthy animals (often <0.1 μg/dL), but upon stimulation will increase over 100 – 1000 fold, reaching a peak 24-48 hours after insult, then rapidly decreasing. An example of an major APP is Serum amyloid A.
- **Moderate APP:** Present in the blood of healthy animals, but increases 5 – 10 fold upon stimulation, peaking around 48 – 72 hours after insult, then decreases at a slower rate than major APPs.
- **Minor APP:** Increase only by 50 – 100% above resting levels and at a gradual rate.

The rapidity and magnitude of the increase in each acute phase protein varies depending on the species. The following table list the acute phase proteins that are major and moderate responders in various animal species.

Protein	Main function
Alpha-1-acid glycoprotein	Anti-inflammatory and immunomodulatory agent: has antineutrophil and anticomplement activity and increases macrophage secretion of IL-1 receptor antagonist. Binds to lipophilic and acidic drugs.
C-reactive protein	On bacteria, it promotes the binding of complement, facilitating phagocytosis. Induction of cytokines Inhibition of chemotaxis and modulation of neutrophil function

	Neutralizes deleterious effects of histones
Ceruloplasmin	Copper transport (for wound healing, collagen formation and maturation) Antioxidant Reduces the number of neutrophils attaching to endothelium
Haptoglobin	Binds free hemoglobin (limiting Hb iron availability for bacterial growth) Natural antagonist for receptor-ligand activation of the immune system Inhibition of granulocyte chemotaxis and phagocytosis
Serum amyloid A	Chemotactic recruitment of inflammatory cells to sites of inflammation Induction of inflammatory cytokines (via surface receptors, including Toll like receptor) Inhibition of myeloperoxidase release and lymphocyte proliferation Involved in lipid metabolism and transport immunomodulatory (via the inflammasome)

Negative acute phase proteins

Negative acute phase proteins decrease in plasma concentration by greater than 25% in response to inflammation. This reduction can occur rapidly (within 24 hours) or may decrease gradually over a period of days. The two main negative acute phase proteins are albumin and transferrin. The mechanism by which their concentrations decrease is likely multifactorial, including decreased production by the liver in response to inflammatory cytokines, and possibly increased loss or increased proteolysis.

- **Albumin**
  - Reduced production of albumin allows greater increase in the amount of amino acids available for positive APP production.
  - Albumin concentration falls gradually and reduction in concentration is more noticeable in chronic inflammatory disease.
- **Transferrin**
  - Usually measured to assess iron status.
  - Ovotransferrin is the avian analog, but it is a positive acute phase protein.

Lecture-23

Enzymes of diagnostic values

Enzyme diagnostics is one of the branches of enzymology. It has two main directions:

1) Use of enzymes as reagents for determination of normal and pathological components in serum, urine, gastric juice etc.

2) Determination of enzyme activity in biological material with a diagnostic purpose.

Serum enzymes are divided into 3 groups:

1. ***Cellular enzymes*** enter the blood from different organs. Their activity in serum depends on enzyme content in organs, molecular weight, intracellular localization, rate of elimination. Cellular enzymes are divided into non-specific and organ specific.

2. ***Secretory enzymes*** are synthesized by cells, enter the bloodstream and fulfil their specific functions in the circulatory system. These are enzymes of coagulation system and fibrinolysis, choline esterase etc.

3. ***Excretory enzymes*** are synthesized by glands of GIT and enter the blood (amylase, lipase).

Enzymes synthesis, functioning and breakdown take place continuously and simultaneously; providing their given concentration and activity. Enzymes are localized in different cellular compartments (cytoplasm, lysosomes, cellular membrane, mitochondrions). That is why increased activity of certain enzymes can indicate the degree of severity of cellular damage. Here, we have provided information about enzymes which are most frequently used in clinical practice for diagnosis, prognosis and therapy monitoring of different pathologies. Their determination in blood serum has high clinical significance.

1. **Transaminases**

- These are enzymes involved in the trasfer of amino from an amino acid to a keto acid.
- Two aminotransferases are in use in diagnostic enzymology. They are:

  Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT).

  ***Aspartate Transaminase*: (SGOT/GOT):**

  (Also known as **S**erum **G**lutamate **O**xaloacetate **T**ransaminase)
- Both the enzymes are widely distributed in the body tissues such as heart, liver, skeletal muscle, kidney and erythrocytes.
- Damage to any of these tissues may increase plasma AST level.
- Causes of rise in plasma AST
- In vitro hemolysis.

- Circulatory failure with shock and hypoxia·
- Myocardial infarction·
- Acute viral or toxic hepatitis·
- Cirrhosis·
- Cholestatic jaundice·
- Skeletal muscle disease·
- Severe hemolytic anemia·
- After surgery.
- ALT is increased in hepatocellular injury in dog and cat. It is not useful in evaluating chronic liver disease.
- ALT may also be elevated in corticosteriod treatment. This enzyme is not useful in evaluating hepatic disease in horse, cow, sheep, goat and pig.
- Elevation of AST is more specific than that of ALT in evaluating hepatic disorders in large animals.

  Alanine Transaminase Also know as Alanine transaminase **(ALT)** or **S**erum **G**lutamate **P**yruvate **T**ransaminase (SGPT/GPT)
- In most conditions where AST is elevated there is a concurrent smaller raise in ALT.
- In hepatitis plasma levels of ALT may exceed those of AST.

  Alkaline Phosphatase

  Alkaline Phosphatase are group of enzymes which hydrolyze phosphates at high pH.

  This enzyme is present in high concentrations in liver, bone (osteoblast) placenta and intestinal epithelium.

  Each of these tissues contain specific isoenzymes of ALP.

  In general serum alkaline phosphatase activity is increased in bone and liver diseases.

  - Bone Diseases
  - Osteomalacia and Rickets.
  - Paget's disease of bone.
  - Carcinoma in bone.
  - Liver Diseases
  - Intra and extra hepatic cholestasis.
  - In lesions and tumour.

Acid Phosphatase (ACP)

- This enzyme is present in high concentrations in the prostate gland, liver, rbc, platelets and bone.
- And it is elevated in the plasma of some patients with prostatic cancer.

Lactate Dehydrogenase (LDH)

- This enzyme catalises the reversible interconversion of lactate and pyruvate.
- This enzyme is present in all cells of the body.
- Serum LDH is increased in liver, heart, skeletal muscle and kidney diseases and also in hepatopoietic and neoplastic disease.
- A better evaluation of the cause of an elevation of LDH can be ascertained by evaluating the isoenzymes separately.
- All LDH isoenzymes are found in varying concentrations in all the tissues.
- There are five well defined LDH isoenzymes.
- This enzyme exists in the body as a Tetramer.
- Two monomers H and M can combine in various proportions.
- Predominant elevation of LD1 and LD2 occurs after myocardial infarction.
- Predominant elevation of LD2 and LD3 occurs in acute leukemia.
- Elevation of LD5 occurs after damage to the liver or skeletal muscle.
- The means of identification of these isoenzymes is usually by serum electrophoresis.

Creatine Kinase

- Creatine kinase consists of two protein sub units (dimer) M and B, which combine to form three isoenzymes(BB, MM and MB).
- BB is confined to the brain.
- Most of the CK normally present in the plasma is the MM, which comes from skeletal muscle.
- An increase is seen with the skeletal muscle damage. Cardiac muscle contains MB type isoenzyme.
- A raised CK-MB is a characteristic of myocardial infarction.

Gama Glutamyl Transferase (GGT)

- This enzyme is present in high concentration in the liver, kidney and pancreas.
- Very high plasma activity is due to:
- Alcoholic hepatitis.

- Induction by anticonvulsant drugs.
- Cholestative liver dissorders.
- GGT is sensitive than ALP for the deduction of cholestatic disease in horse, cattle, sheep and pig.
- GGT appears in serum as a result of increased synthesisi rather than as a result of leakage from the cells.
- In acute hepatic injury ALT and AST are generally elevated and ALP may be normal.
- In chronic disease with cholestasis, ALP and GGT are elevated and ALT AST may be normal or slightly increased.

Amylase

- Amylase breaks down starch and glycogen to maltose.
- It is present at high concentrations in pancreatic juice and in saliva.
- The plasma activity of this enzyme is very high in acute pancreatitis.

Lipase

- It is found in the pancreas.
- Increased amounts of this enzyme indicates disease and inflammation of the pancreas.
- Lipase is not present in saliva so this test is more useful in the identification of pancreatic disorders.

Lecture-24

Metabolism of Xenobiotics

Xenobiotics is a compound that is foreign to the body; is a chemical which is found in an organism but which is not normally produced or expected to be present in body. Endogenous: Pigments, hormonenonendogenous: Such as drugs, food additives, pollutants, toxin, etc. Most of these compounds are subject to metabolism (biotransformation).

Definition of the biotransformation

Conversion of lipophilic xenobiotics to water-soluble chemicals by a process catalyzed by enzymes in the liver and other tissues. In most cases, biotransformation lessens the toxicity of xenobiotics, but many must undergo the process to exert their toxic effects.

Purpose of biotransformation

1. facilitates excretion: To Converts lipophilic to hydrophilic compounds.
2. Detoxification/inactivation: To converts chemicals to less toxic forms
3. Metabolic activation: To converts chemicals to more toxic active forms

General Metabolic Pathways:

Liver is the main site for biotransformation. Approximately 30 different enzymes catalyze reactions involved in xenobiotic metabolism; however, this note will only cover a selected group of them.It is convenient to consider the metabolism of xenobiotics in two phases. phase Ⅰ and phase II.

Phase I: The reactions of Phase I are oxidation, reduction and hydrolysis.

Phase II: These are the conjugation reactions, involving compounds such as glucuronic acid, amino acids (glycine), glutathione, sulfate, acetate and methyl group.

Generally, detoxification of a compound involves phase I as well as phase II reactions. For instance, oxidation followed by conjugation is the most frequent process in the metabolism of xenobiotics.

Lecture-25

Cytochrome p450 system

Cytochromes p450 (CYPs) are a superfamily of enzymes containing heme as a cofactor that function as monooxygenases. In mammals, these proteins oxidize steroids, fatty acids and xenobiotics and are important for the clearance of various compounds, as well as for hormone synthesis and breakdown. Cytochrome P450 enzymes are primarily found in liver cells but are also located in cells throughout the body. Within cells, cytochrome P450 enzymes are located in a structure involved in protein processing and transport (endoplasmic reticulum) and the energy-producing centers of cells (mitochondria).

cytochrome P450 enzyme system: A group of enzymes involved in drug metabolism and found in high levels in the liver. These enzymes change many drugs, including anticancer drugs, into less toxic forms that are easier for the body to excrete.

Salient features of cytochrome P450 :

- Multiple forms of cytochrome P450 are believed to exist, ranging from 20 to 200. Atleast 6 species have been isolated and worked in detail.
- They are all hemoproteins, containing heme as the prosthetic group.
- Cytochrome P 450 species are found in the highest concentration in the microsomes of liver. In the adrenal gland, they occur in mitochondria.
- The mechanism of action of Cytochrome p450 is complex and is dependent on NADPH.
- The Cytochrome p450 is an inducible enzyme.
- Molecular mass is about 55 kDa.
- Exihibit broad substrate specificity.
- Cause introduction of one atom of oxygen into the substrate and one intowater.
- The hydroxylated products are more water soluble.

Annexure-1

REFERENCE VALUES FOR SERUM CHEMISTRY FOR ANIMALS OF DIFFERENT SPECIES

Component	Units	Canine	Feline	Equine	Bovine	Porcine	Ovine
Albumin	g/dl	2.5-3.6	2.3-3.4	2.7-4.2	2.7-4.3	1.9-3.3	2.4-3.9
Bilirubin (total)	mg/dl	0.1-0.3	0.1-0.2	0.5-2.1	0.1-0.3	0.1-0.2	0.1-0.4
Calcium	mg/dl	9.0-10.8	7.4-10.5	10.6-13.0	7.9-10.0	8.0-12.0	10.4-13.0
Cliloride	mEq/L	110-118	116-125	97-104	94-104	100-105	98-115
Cholesterol	mg/dl	108-266	38-186	50-143	87-254	36-54	50-140
Creatinine	mg/dl	0.5-1.4	0.7-1.8	1.0-1.9	0.7-1.1	1.0-2.7	1.2-1.9
Globulin	g/dl	2.4-4.0	2.6-4.5	2.1-3.8	2.5-4.1	5.3-6.4	3.5-5.7
Glucose	mg/dl	77-120	58-120	76-127	37-71	65-95	50-80
Hemoglobin	g/L	130-190	90-150	110-170	80-150	100-180	80-160
Magnesium	mg/dl	1.8-2.4	2.0-2.2	2.2-2.8	1.8-2.3	2.7-3.7	2.2-2.8
Phosphorous (inorganic)	mg/dl	2.4-6.1	2.6-7.9	2.0-4.3	4.6-9.0	5.3-9.6	5.0-7.3
Potassium	ppm	4.2-5.6	4.0-5.3	2.4-5.2	4.0-5.3	4.9-7.1	4.0-6.0
Protein (total)	g/dl	5.4-7.1	5.7-7.9	5.5-7.3	5.9-7.7	7.0-8.9	6.0-7.9
Sodium	ppm	145-153	151-158	136-142	136-144	139-152	136-154
Blood Urea Nitrogen	mg/dl	7-25	18-33	12-26	10-26	8-24	18-31