MODULE-1: BLOOD - FUNCTIONS AND PROPERTIES

Learning objectives

- This module deals with
  - composition and functions of blood
  - properties of blood

BLOOD

- Blood is a fluid connective tissue that flows all over the body in the vessels of the cardiovascular system.

FUNCTIONS OF BLOOD

- **Transportation**
  - Transport oxygen from lungs to tissues and carbon dioxide from tissues to lungs
  - Transport nutrients from GI tract to liver and other cells
  - Transport hormones from endocrine glands to target cells
  - Transport waste products from cells to excretory organs
- **Regulation**
  - Maintains homeostasis
  - Regulates pH / acid base balance
  - Regulates body temperature and water balance
  - Regulates osmotic pressure
- **Protection**
  - Provides immunity

COMPOSITION OF BLOOD

- Blood is composed of fluid in which the cells and cell fragments are suspended. The fluid portion is known as the *plasma*, and the suspended cellular elements are the
  - Erythrocytes (RBCs)
  - Leukocytes (WBCs) and
  - Thrombocytes (platelets)
- Plasma constitutes about 55 to 70% of the blood volume, while the cellular components account roughly about 30-45% of the total blood. It performs numerous functions that are essential for life and health.

SPECIFIC GRAVITY OF BLOOD

- Specific gravity is the ratio of weight of a given volume of a fluid to the weight of the same volume of distilled water measured at 25°C.
- Plasma protein concentration is largely responsible for the specific gravity of the plasma. The cellular elements called the corpuscles have higher specific gravity than the plasma.

Species	Specific gravity
Goat	1.042 (1.036 - 1.051)
Pig	1.045 (1.035 - 1.055)
Dog	1.048 (1.045 - 1.052)
Cat	1.050 (1.045 - 1.057)
Sheep	1.051 (1.041 - 1.061)
Cattle	1.052 (1.046 - 1.061)
Horse	1.053 (1.046 - 1.059)

- The copper sulphate drop falling method is commonly used for the assessment of specific gravity of blood and plasma. Various factors influence the specific gravity of the blood.

Specific gravity is increased in the following conditions

- Polycythemia: High altitude, polycythemia vera, newborns etc.
- Severe dehydration: Excessive fluid loss such as in vomiting, diarrhoea.
- Hemoconcentration: Loss of plasma as in burns.

Specific gravity is decreased in the following conditions

- Pregnancy: Increase in plasma volume causes hemodilution.
- Anaemia.
- Renal diseases: Loss of albumin, oedema etc.
- Hemodilution: Hypersecretion or prolonged treatment with glucocorticoids.
- Starvation and malnutrition: Decrease in plasma protein.
- Intravenous fluid transfusions.

VISCOSITY OF BLOOD

- Viscosity is influenced by concentration of RBCs and plasma proteins. Among plasma proteins, the viscosity is mainly contributed by the *gamma globulins* which provide the resistance to blood flow and helps the normal pumping activity of the heart.
- It is normally about five times greater than water. Viscosity of blood is determined using **viscosimeter**.
- An *increase* in the viscosity of blood is seen in conditions like polycythemia, congestive heart failure, jaundice, vomiting, diarrhoea etc, whereas a *decrease* in the viscosity is commonly noticed in anemia, oedema etc.

PLASMA

- It is the fluid portion of the blood. It is yellow to colourless depending on the quantity, species of the animal and animal's diet.
- The plasma colour is golden yellow in dog, colourless or slightly yellow in sheep and goat, while it is highly yellow coloured in horse and cow which is chiefly due to bilirubin, and to some extent by the carotene, xanthine and other pigments.

Icterus index

- It is a measure of the *yellow colour of the plasma*. It is measured by comparing the colour of the plasma with a standard solution of potassium dichromate.
- Plasma is obtained by adding anticoagulant to whole blood to prevent clotting and centrifuging it for settling down of the cells.

Composition of Plasma

- Plasma is made up of water to the extent of 91 - 92%; Solids : 8 - 9%; the solid portion of plasma includes proteins: 6 - 8 g %: Organic and inorganic compounds: 2 - 3 %

Organic	Carbohydrates : Glucose, Lactate, Pyruvate
Inorganic	Macro level **:** Fe, Cl, SO_4, PO_4, Micro level : Mn, Co, Cu, Zn, Iodide.
Plasma proteins	Albumin, Globulin, and Fibrinogen
Non Protein Nitrogenous (NPN) compounds	Urea, Uric acid, Creatine, Creatinine, Salts of Ammonia
Lipids	Neutral fat, Cholesterol, Phospholipids, Lecithin
Others	Enzymes, Hormones, and Vitamins

SERUM

- In the absence of the anticoagulants, the blood comes out of the blood vessels and gets coagulated to form a blood clot. On shrinking it discharges a clear watery liquid called the *serum***.**
- Serum is also obtained by collecting the blood without the addition of anticoagulant and allowing it to clot. Later, upon shrinkage of the blood clot the serum is extruded.
- It differs from the plasma in lacking fibrinogen, prothrombin and other coagulation factors which are involved in blood coagulation.

REACTION OF THE BLOOD

- The normal pH of the blood is 7.4.
- The venous blood is slightly towards acidic side because of the increased content of CO_2, whereas the arterial blood is slightly more towards alkaline side.
- The plasma is more alkaline than the corpuscles.
- The blood pH range:
    - Dog : 7.32- 7.68
    - Cattle : 7.35 - 7.50
    - Horse : 7.35 - 7.43
    - Fowl : 7.56.
- Excessive production of metabolic acid products causes abnormal reduction in the alkaline reserve leading to the condition referred to as ***acidosis*****,** whereas abnormal increase in the alkaline reserve is called as ***alkalosis***.

MODULE-2: PLASMA PROTEINS AND LIPIDS

Learning objectives

- This module deals with
  - types of plasma proteins
  - functions and normal concentration of plasma proteins

PLASMA PROTEINS

- Plasma proteins contribute about 7% of the total organic molecules of the plasma. The major proteins present in plasma are:
  - Albumin (55%),
  - Globulins (38 %) and
  - Fibrinogen (7 %).
- Liver functions as a chief site of synthesis of plasma proteins, albumin, fibrinogen, prothrombin and some of the globulins. While the gamma globulins are synthesised extra-hepatically in the lymph nodes and in the Mononuclear Phagocytic System (MPS) of spleen and bone marrow.
- The ratio of albumin to globulin is 1:0.7. There are species variations in the ratio of plasma proteins *i.e.* albumin: globulin ratio. In humans, sheep, goats and dogs, the albumin predominates over globulin.
- In horses, pigs, cow and cats albumin globulin ratio is equal. In new born animals (except rodents and primates plasma gamma globulin is absent or found in minute quantity.

Separation of plasma proteins

- *Salting out method:* Using different concentrations of ammonium sulphate solution.
- *Electrophoresis:* Paper, starch gel, polyacrylamide gel electrophoresis are used for the separation of plasma protein fractions.

PLASMA PROTEIN FRACTIONS

Prealbumin

- Transports thyroxine and Vitamin A.

Albumin

- Two fifth is intravascular and the rest occur extravascularly. It provides colloidal osmotic pressure because of its high concentration and low molecular weight and non-diffusible property through blood vessels, thus prevents excessive passage of fluid from the blood into the interstitial tissue and serves to control the fluid balance between blood and tissues.
- It contributes to the amino acid pool with tissue protein and helps in transport of some anions and cations.

Globulins

- Includes fractions α1 and α2, β1 and β2 and γ globulins which include IgA, IgD,IgE, IgG and IgM.
    - *α - globulins* act as carrier proteins of bilirubin, lipids, steroids and thyroxine.
    - *α_1 globulins* are protease inhibitors and functions to inhibit WBC elastase and other WBC proteases and activated factor XI. The *α_1* lipoproteins transport lipids, fat soluble vitamins and hormones.
    - *α_2 globulins* are the macroglobulins and inhibit plasmin, thrombin and kallikrein.
    - *β - globulins* are*:*
        - *Ceruloplasmin:* Transports copper.
        - *Ferroxidase:* Transferrin transports iron.
        - *Haptaglobulin*: Transports Hb to liver after haemolysis.
        - *Apolipoproteins:* Transports triglycerides, phospholipids and cholesterol.
        - *Hemopexin:* Aids the transport of heme from lysed RBCs.
        - *Transcortin:* Transports cortisol.
        - *Transcobalamins I & II:* Transports cobalt.
    - *γ - globulins*
        - They provide viscosity to the blood, thus maintain normal blood pressure. Synthesised by -lymphocytes and plasma cells, functions to provide immunity and also helps transport of vitamin D.
        - The γ globulins are the immunoglobulins (Ig) and are classified into the following types:
        - *IgG* is responsible for most of the *humoral immunity* of the organism. It can cross placenta.
        - *IgM* is the second major immunoglobulin of the serum and it is typically the first immunoglobulin increase in concentration in serum during *primary immune response*. These are naturally occurring antibodies against erythrocytes in certain incompatible blood types.
        - *IgA* is a glycoprotein found in external secretion such as saliva, tears, colostrum etc. It forms the primary immunoglobulin in the colostrum and is responsible for natural passive immunity in the neo-natal calf, foal, lamb, kid and piglets. Plays an important role in local defence by protecting various body surfaces e.g., the intestinal, respiratory and uro-genital tracts, mammary gland and the eyes from bacterial and viral invasions. IgA does not cross the placenta. It is most abundantly found in normal animals.
        - *IgD* is involved in B-cell differentiation to form 'clones'.
        - *IgE* is involved in hypersensitivity and allergic responses. It causes the release of histamine from basophils and mast cells.

Fibrinogen

- Fraction from the liver, functions as a precursor to form a mesh work of fibrin threads and play a key role in blood coagulation.
- It influences the suspension stability of the erythrocytes. Increased concentration of fibrinogen and globulins alter the colloidal state of the blood, hastens agglutination of RBCs and settling.

FUNCTIONS OF PLASMA PROTEINS

1. Function as source of amino acids for the synthesis of tissue proteins. The amino acids of plasma protein and tissue proteins are in a state of dynamic equilibrium.
2. Provide colloidal osmotic pressure and helps to regulate fluid balance. Around 80% of the colloidal osmotic pressure is contributed by albumin.
3. They act as blood buffer and regulate acid-base balance, thus maintains *normal* pH of *7.4*.
4. Fibrinogen and various clotting factors are essential for coagulation of blood.
5. Influence the suspension stability of RBCs in the blood.
6. Contributes to the *viscosity* of plasma (by *gamma globulins*), thereby providing for peripheral resistance, which is essential for efficient cardiac function.
7. As carrier proteins they are invloved in transport of copper, iron, heme, bilirubin, thyroxine, cortisol, sex hormones, vitamin A, vitamin D, fatty acids, triglycerides, phospholipids and cholesterol.
8. Immunoglobulins provide specific antibody against specific antigen.

PLASMA PROTEIN CONCENTRATION

Species	Total protein	Albumin	Globulin	Fibrinogen
Horse	6.0 - 8.0	2.8 - 3.8	2.8 - 3.8	0.2-0.4
Cow	7.0 - 8.5	3.0 - 3.8	3.6 - 4.4	0.2-0.5
Sheep	6.0 - 8.0	3.5 - 4.5	2.5 - 3.5	0.2-0.4
Goat	6.5 - 7.5	3.7 - 4.5	2.4 - 3.2	0.2-0.5
Pig	6.5 - 8.5	3.5 - 4.0	3.5 - 4. 0	0.2-0.4
Dog	6.0 - 7.8	3.4 - 4.4	2.7 - 3.2	0.1-0.4
Cat	6.0 - 7.4	3.0 - 3.8	2.5 – 3.5	0.1 – 0.4
Fowl	4.0 - 5.2	1.2 - 3.8	2.3 - 3.3	--

PLASMA LIPIDS

- **Lipids** are heterogenous molecules soluble in organic solvents but not in water. They are esters of fatty acids, formed by the reaction of fatty acids with glycerol.
- **Blood lipids** (or blood fats) are lipids in the blood and they are present either free or bound to other molecules. Blood lipids are mainly fatty acids, triglycerides, lipoproteins and cholesterol.
- Since lipids are insoluble in water they are mostly transported in a protein covering, and the density of the lipids and type of protein determines the fate of the lipid and its influence on metabolism.

- The concentration of blood lipids depends on dietary intake, absorption from the intestine and excretion and uptake and secretion from cells.

FATTY ACIDS

- Fatty acids are present in different forms {as in chylomicrons, Very low density lipoproteins (VLDL), Low density lipoproteins (LDL)} in blood. In addition, the fatty acids released from adipocytes exist in the blood as free fatty acids.
- Short- and medium chain fatty acids are absorbed directly into the blood via intestine capillaries and travel through the portal vein. Long-chain fatty acids are too large to be directly released into the intestine capillaries. Instead, they are coated with cholesterol and protein (protein coat of lipoproteins) into a compound called as *chylomicrons*. The chylomicrons enter into lymphatic capillary and then into the bloodstream (having bypassed the liver).
- The concentration of blood fatty acids increase temporarily after a meal which increases the uptake of fatty acids in different cells of the body like liver cells, adipocytes and muscle cells. This uptake is stimulated by insulin from the pancreas.
- Some of the fatty acids taken up by the liver is converted into VLDL and again secreted into the blood.
- When the concentration of fatty acids in the blood decreases, this triggers adipocytes to release stored fatty acids into the blood as free fatty acids, in order to supply the energy for the muscle cells and other cells.

LIPOPROTEINS

- Lipoproteins are complex aggregates of lipids and proteins that increases the solubility of lipids and enable their transport throughout the body.
- Lipoproteins are synthesised mainly in the liver and intestines.
- The most abundant lipid constituents of lipoproteins are triacylglycerols, free cholesterol, cholesterol esters and phospholipids (phosphatidylcholine and sphingomyelin); fat-soluble vitamins and anti-oxidants are also transported in this way.
- The lipoproteins contain different protein components called apoproteins (or apolipoproteins). Apoproteins are required to solubilise the non-polar lipids in the circulation. These proteins determine the overall structures and metabolism, and their uptake in liver and peripheral tissues.
- Lipoproteins are classified as chylomicrons (CM), very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), based on the relative densities on ultracentrifugation.
- Based on the relative mobilities on electrophoresis, lipoproteins can also be classified into α, pre-β and β lipoproteins that correspond to HDL, VLDL and LDL, respectively.

PHYSICAL PROPERTIES AND LIPID COMPOSITIONS OF LIPOPROTEIN CLASSES

	CM	VLDL	LDL	HDL
Density (g/ml)	0.94	0.94-1.006	1.006-1.063	1.063-1.210
Total lipids (wt%)	99	91	80	44
Triacylglycerol	85	55	10	6

Cholesterol esters	3	18	50	40
Cholesterol	2	7	11	7
Phospholipids	8	20	29	46

- Lipoproteins are spherical in shape with the core containing non-polar lipids, triacylglycerols and cholesterol esters, and a surface layer consisting of phospholipids and non-esterified cholesterol, which also serve to present a hydrophobic face to the aqueous phase.
- The principal role of the chylomicrons and VLDL is to transport triacylglycerols as a source of fatty acids from the intestines or liver to the peripheral tissues.
- *Chylomicrons:* They are largest of the lipoproteins; they are formed in the intestinal mucosa and transport triacylglycerols from the intestinal mucosa to the liver or to other tissues; they carry mostly fats in the form of triglycerides and cholesterol. In the liver, chylomicron particles release triglycerides and some cholesterol.
- *VLDL:* They are synthesised in liver. The liver converts the excess fatty acids (dietary or synthesised) into very low density lipoproteins (VLDL) and secretes them into plasma; in the plasma they are converted to intermediate density lipoproteins(IDL), and then to low-density lipoprotein (LDL) particles. VLDL transports triacylglycerols from liver to peripheral tissues.
- *LDL:* It is the main transporter of cholesterol to the peripheral tissues. When they are present in excess they are strongly associated with the formation of atheromatous (plaque formation within arterial wall) disease within the arteries. For this reason, LDL is referred to as "bad cholesterol".
- *HDL:* They are synthesised in liver. The HDL removes excess cholesterol from peripheral tissues and delivers them to the liver for excretion in bile in the form of bile acids, a process that has been termed 'reverse cholesterol transport', helps to lower blood cholesterol level and hence HDL is commonly called "good cholesterol".
- After being transported to the liver by HDL, cholesterol is delivered to the intestines via bile production. However, 92-97% is reabsorbed in the intestines and recycled via enterohepatic circulation

CHOLESTEROL

- It is lipid, waxy steroid found in the cell membranes and transported in the blood plasma of all animals.
- It is an essential component of mammalian cell membranes where it is required to establish proper membrane permeability and fluidity.
- In addition, cholesterol is an important precursor molecule for the biosynthesis of bile acids, and several fat soluble vitamins, Vitamin D_3 and the steroid hormones, including the adrenal gland hormones such as cortisol and aldosterone as well as the sex hormones like progesterone, estrogens, and testosterone and their derivatives.
- Within the cell membrane, cholesterol also functions in intracellular transport, cell signalling and nerve conduction.
- Since cholesterol is essential for life, it is primarily synthesized *de novo,* within the body, with smaller contributions from the diet.
- Excessive levels of cholesterol in blood circulation however are strongly associated with progression of atherosclerosis.

- The fate of cholesterol in the blood is highly determined by its constitution of lipoproteins - some types (LDL) favour transport towards body tissues and others (HDL) towards the liver for excretion into the intestines.
- The average amount of *blood cholesterol* varies with age, typically rising gradually as age advances.
- *Hyperlipidemia* refers to the elevated or abnormal levels of cholesterol and triacylglycerols in the blood. One of the most clinically relevant lipid substances is cholesterol, especially on atherosclerosis and cardiovascular disease.
- *Lipemia* is a term used to refer marked hyperlipidemic condition resulting in plasma presenting a milky white appearance.
- Postprandial hyperlipidemia is most common in animals consuming fatty diet; hence to evaluate lipid status, blood samples have to be taken from fasting animals.
- Hyperlipidemia is noticed in dogs and man with hypothyroidism – main lipid increased is cholesterol. Pancreatitis, uncontrolled diabetes, cholestasis, hyperadrenocorticism are some other conditions causing hyperlipidemia.
- *Hypercholesterolemia* is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease.

Sources

- Animal fats are complex mixtures of triglycerides with phospholipids and cholesterol; hence, all foods containing animal fat contain cholesterol to varying extents.
- Cholesterol is not present in plant based food sources. However, plant products such as flax seeds and peanuts contain cholesterol-like compounds called *phytosterols*, which are suggested to help lower serum cholesterol levels.
- About 20–25% of total daily cholesterol production occurs in the liver; other sites of high synthesis rates include the intestines, adrenal glands and reproductive organs.
- Synthesis within the body starts with one molecule of acetyl CoA and one molecule of acetoacetyl-CoA. HMG-CoA reductase is an important enzyme in the cholesterol synthesis. Total cholesterol is the sum of HDL cholesterol, LDL cholesterol and 20% of triglycerides.

Blood cholesterol levels in human beings

Type	Level	Remarks
Total cholesterol	< 200 mg/dl	Desirable level. Low risk for coronary disease
	240 & above	Hypercholesterolemia, high risk for coronary heart disease
HDL cholesterol	<40 mg/dl	Low HDL levels is the risk factor for cardiac disease
	60 & above	High HDL, protective to heart
LDL cholesterol	< 100 mg/dl	Desirable level
	> 160 mg/dl	High, risk of heart disease
Triglycerides	< 150 mg/dl	Normal
	>200 mg/dl	High

Total blood cholesterol levels (mg/100 ml) in animals

Species	Blood cholesterol levels (mg/100 ml)
Horse	75-150
Cow	60-190
Sheep	52-90
Goat	80-130
Pig	80-134
Dog	135-270

MODULE-3: ERYTHROCYTE (RBC)

Learning objectives

- This module deals with
  - Functions and composition of erythrocytes
  - Shape, structure and size of erythrocytes

FUNCTIONS OF ERYTHROCYTES

- Transport hemoglobin which in turn carries oxygen from the lungs to the tissues.
- RBCs contain large quantity of carbonic anhydrase which catalyzes the reversible reaction between carbon dioxide and water. Thereby makes it possible for the water of the blood to transport enormous quantities of carbon dioxide from the tissues to the lungs in the form of bicarbonate.
- Hemoglobin in the cells is an excellent acid base buffer. Therefore, the RBCs are responsible for most of the acid base buffering power of whole blood

SHAPE OF RBC

- The mammalian RBCs are usually non-nucleated and non-motile cells, biconcave circular disc with central pale spot. Its shape differs in various species of animals:
  - *Dog, Cow, Sheep:* Markedly biconcave.
  - *Horse and cat:* Shallow concaving,
  - *Goat:* Very shallow or flat surfaced erythrocytes.
  - *Camel and Deer:* Elliptical and sickle shaped.
  - *Cold blooded animals (amphibians and birds):* Elliptical and nucleated.

Significance of biconcavity of RBCs

- It increase the surface area thus facilitating the exchange of oxygen and carbon dioxide carried by the RBCs.

STRUCTURE OF RBC

- The cell membrane of RBC is made up of lipids (*lecithin, cephalin and cholesterol*) and glycoprotein encloses a spongy inner structure called the *stroma*.
- The RBC membrane protein, *Spectrin* forms the inner lining of the membrane, whereas the outer layer is formed by glycoproteins, have the blood group antigens. The cell membrane is highly permeable to lipid soluble substances, glucose, urea and water.
- Hemoglobin is deposited in the inter-spaces of the spongy stroma. The surface of mature erythrocyte is smooth, while the immature RBCs have relatively rough surface.

SIZE OF RBC

- Average diameter of RBC ranges from 4.1 to 7.5 µm .

Species	Size (µm)
Goat	4.1
Sheep	5.0
Cattle	5.6
Horse	5.6
Pig	6.2
Cat	6.5
Dog	7.3
Man	7.5

- Surface area, varies from 57-67m^2 / kg body weight in mammals. It is lowest in goat (lesser diameter) and highest in man (greater diameters).

COMPOSITION OF ERYTHROCYTES

- Erythrocyte contains 62 - 72% water and 35% solids. Of the solids, 95% is contributed by Hb and the remaining 5% by cell and stromal protein, lipids, phospholipids, cholesterol, cholesterol esters, neutral fat and vitamins.

RBC METABOLISM

Energy is required for RBCs to

- To maintain the shape and flexibility of the cell membrane.
- To preserve high K^+, low Na^+ and low Ca^{++} ions within the RBCs against the concentration gradient of these ions of plasma.
- To maintain iron in ferrous (Fe^{++}) state (to reduce ferric to ferrous state, NADH and NADPH are required).
- To generate reduced glutathione (anti-oxidant); this helps to maintain the ferrous state.

- To generate 2,3 Diphospho glycerate (DPG) for O_2 dissociation.
- *Mitochondria are absent in mature erythrocytes*. These cells derive their energy from glucose metabolism via anaerobic Embden- Meyerhoff (EM) pathway (90%) and oxidative pentose cycle (10%) which produce NADH and NADPH. Kreb's cycle is very much reduced in activity.

CONCENTRATION OF RBCs

- The concentration of RBC depends on various factors such as interspecies, intraspecies and diurnal variation, age, sex, environment, exercise, nutritional status, climate and altitude.

Concentration of RBC in domestic animals (millions/ mm^3 of blood)

Species	Concentration of RBC
Fowl	3.0 (2.8 – 3.2)
Pig	6.5 (5.8- 8.0)
Dog	6.8 (5.5-8.5)
Sheep	12.0 (8.0-16.0)
Cattle	7.0 (5.0-10.0)
Goat	13.0 (8.0-18.0)
Horse	6.5 (6.5-12.5)
Cat	7.5 (5.0-10.0)
Man	5.4 (5.0-6.0)
Women	4.8 (4.0-5.0)

MODULE-4: ABNORMALITIES OF RBC CONCENTRATION

Learning objectives

- This module deals with
  - Abnormalities of RBC concentration
  - Erythrocyte indices

ABNORMALITIES OF RBC CONCENTRATION

1. Polcythemia
2. Oligocythemia
3. Anaemia

POLYCYTHEMIA

- It is otherwise known as *erythrocytosis*. It is a condition of increased number of RBCs in the circulation. It is of two types.
  - Physiological (secondary) polycythemia

        - An increase in RBCs occurs as a compensatory measure (in high altitude of 14000 to 17000 feet to compensate low PO_2). Whenever tissues become hypoxic because of too little oxygen in the atmosphere, for example at high altitude or because of failure of delivery of oxygen in the tissues as in cardiac failure, then the blood forming organs automatically produce large quantities of extra RBCs i.e 30% above the normal.
        - Increased Hb requirement during heavy muscular exercise to meet increased oxygen demand. In sports animals (racehorse, hunting dogs) RBC elevation is a normal feature.
        - Increased environmental stress / temperature, the spleenic contraction, and increased RBC synthesis by the bone marrow cause increased number of RBCs into the circulation.
        - Hemoconcentration due to water loss that occurs in vomiting, diarrhoea, prolonged high fever and burns also causes polycythemia.
    - Pathological polycythemia
        - Due to decreased O_2 supply to the tissue, chronic carbon monoxide poisoning, myeloid (bone marrow) cancer, pulmonary emphysema, repeated hemorrhage.
        - Polycythemia vera is the condition due to bone marrow cancer (myeloid leukemia). It occurs as a result of genetic aberration in the hemocytoblastic cell line that produces the blood cells.

OLIGOCYTHEMIA

- Reduction in the number of erythrocytes in the circulation is called as oligocythemia
- *Physiological oligocythemia* occurs due to hemodilution; RBC number per unit volume is reduced. **Example:** *pregnancy*.
- *Pathological oligocythemia* is also known as *anaemia*.

ANEMIA

- Abnormal reduction in the number of the erythrocytes or the hemoglobin content in the blood or both.

Causes

- Excessive whole blood loss occurs in hemorrhage or by blood sucking parasites (Hookworms, ticks), increased destruction of RBCs by the reticuloendothelial cells.
- Impaired RBC production and Hb synthesis, due to deficiency of Fe, Cu, Vitamin B12 and folic acid.
- Hemolytic:
    - Disease caused by blood parasites, (babesiosis) or drugs like sulphanamides, antimalarial drugs and high doses of aspirin (analgesic)

Anemia due to defective blood formation:

- Aplastic anemia
    - It occurs due to lack of functional bone marrow caused by excessive x-ray treatment or bone marrow cancer, certain industrial chemicals, drugs etc.

- Anemic anemia
  - *Megaloblastic anemia:*
    - It is due to deficiency of iron, folic acid, Vitamin B_{12} (extrinsic factor) and intrinsic factor of the gastric mucosa.
  - *Microcytic and hypochromic anemia:*
    - It results due to deficiency of iron results in small sized, decreased number of RBCs and low Hb content.
  - *Macrocytic and hyperchromic anemia:*
    - Lack of extrinsic factor, the Vitamin B_{12} causes decreased number of RBCs, large sized RBCs and high Hb content because the erythroblasts cannot proliferate rapidly enough to form normal number of RBCs, the cells that are formed are mostly oversized, bizarre in shape and have a fragile membrane.
  - *Pernicious anemia:*
    - It occurs due to the deficiency of the intrinsic factor of the gastric mucosa that interferes with the Vitamin B_{12} absorption.

Anemia due to excessive blood loss or increased RBC destruction:

- Hemorrhagic anemia:
  - Excessive blood loss due to accident, peptic ulcers etc.
- Hemolytic anemia:
  - Following acute destruction of RBCs (haemolysis) the number of RBCs is below normal, but the RBC size and Hb content are normal, known as normocytic and normochromic anemia.

Causes

- Blood parasites: Eg. babesiosis, theileriosis and trypanosomiasis;
- Chemicals: Copper, lead, nitrate and nitrite.

Anemia due to abnormal structure of RBC :

- In some hereditary diseases the defects are with the RBC membrane - e.g., *sickle cell anemia*, defects in the globin chain structure (*thalassemia*) or its synthesis or the deficiency of the enzymes of the RBCs energy system, the pyruvate kinase and glucose 6 phosphate dehydrogenase (G.6-PD).
  - Sickle cell anemia:
    - In this type of anemia the cells contain an abnormal type of hemoglobin called as *Hb "S"*. It is caused by abnormal composition of β chains of the hemoglobin.
    - When this type of hemoglobin is exposed to low concentration of oxygen it precipitates into long crystals inside the erythrocytes. These crystals elongate the cell and it gives the appearance of sickle shape.
    - The precipitated hemoglobin also damages the cell membrane so that the cells become highly fragile leading to anemia.
  - Thalassemia:
    - It is otherwise known as *Cooley's anemia or Mediterranean anemia*. It occurs due to defect in the synthesis of α or β peptide chains to form

hemoglobin or due to deficiency of enzymes of the RBC energy system, pyruvate kinase and G-6-PD thereby depressing the hemoglobin synthesis.

ERYTHROCYTIC INDICES

- These indices help in the diagnosis various types of anemia (microcytic vs. macrocytic or normocytic).
- Mean Corpuscular Volume (MCV):
  - It expresses the average cell size of the erythrocyte.

- Mean Corpuscular Hemoglobin (MCH):
  - It gives the average weight of Hb present in the erythrocytes.

$$MCH\,(pg) = \frac{Hb\,(g\%) \times 10}{No.\,of\ RBCs\,/\,mm^3 \times 106}$$

- Mean Corpuscular Hemoglobin Concentration (MCHC):
  - It is the average percentage of the mean corpuscular volume that the Hb occupies.

$$MCHC\,(g\%) = \frac{Hb\,(g\%) \times 100}{PCV\,/\,dl}$$

NORMAL RANGE OF ERYTHROCYTE INDICES IN DOMESTIC ANIMALS

Species	MCV (fl)	MCHC (%)	MCH (pg)
Dog	60-77 (70)	32-36 (34)	20-24
Cat	39-55 (45)	30-36 (33)	13-17
Cow	40-60 (52)	30-36 (33)	19
Sheep	23-48 (33)	31-38 (33)	10-14
Goat	15-30 (23)	35-42 (38)	8
Horse	34-58 (46)	31-37 (35)	18
Pig	50-68 (63)	30-34 (32)	16-20

MODULE-5: HAEMOLYSIS

Learning objectives

- This module deals with lifespan and fate of erythrocytes.

LIFE SPAN OF ERYTHROCYTES

- Life span of erythrocytes (days)

Cattle	Sheep	Goat	Horse	Dog	Cat	Pig	Poultry
125-150	140-150	125-150	140-150	100-120	70-80	51-79	20-30

SITE OF DESTRUCTION OF ERYTHROCYTES

- In most of the domestic animals bone marrow functions as a chief site of destruction of erythrocytes, whereas in man it is the spleen.
- In the birds liver acts as an organ of destruction of erythrocytes.

FATE OF ERYTHROCYTES

- The erythrocytes have a remarkable capacity to change their shape when they pass through the capillaries but they become less deformable when they reach the end of their life span.
- Two types of destruction of erythrocytes takes place,
    1. Intravascular hemolysis
    2. Extravascular hemolysis

INTRAVASCULAR HEMOLYSIS

- About 10% of aged RBCs undergo intravascular hemolysis within the capillaries due to loss of compressibility of RBCs caused by increased membrane permeability and osmotic change.
- When this occurs the hemoglobin is released, which combine with haptoglobulin which is removed by the cells of the mononuclear phagocytic system (MPS).

EXTRAVASCULAR HEMOLYSIS

- About 90% of the aged RBCs are directly destroyed by the mononuclear phagocytic system (MPS).

- The Hb and proteins are catabolised by the MPS cells. The MPS (also known as reticulo-endothelial system) includes the histiocyte or macrophages, stellate or Kupffer cells of the sinusoids of the liver, spleen, mononuclear cells of bone marrow and lymph nodes.
- The globin of the Hb is degraded to amino acids and is reutilized. Iron removed from the heme is stored in the MPS cells in the form of ferritin or hemosiderin and utilised for the synthesis of hemoglobin or enters the plasma and combine with apotransferrin to form transferrin. The transferrin enters the bone marrow to produce more erythrocytes.
- The heme is converted to bile pigments, biliverdin (a green pigment) and then reduced to bilirubin (a yellow pigment). The free bilirubin enters the plasma, binds with albumin and transported to liver. In the liver bilirubin is conjugated with glucuronic acid, secreted in bile to enter intestine. Large intestinal bacteria reduce the bilirubin to urobilinogen, most of that are excreted in feces in the oxidised form of urobilin or stercobilin which impart colour to feces.
- Part of the urobilinogen is reabsorbed into the enterohepatic circulation and reexcreted in bile. Some of the urobilinogen in the plasma enters the kidneys to be excreted in urine as urobilin.
- Globin protein portion of hemoglobin is broken down to amino acid and used in the formation of new hemoglobin or other proteins.

Hemolysis caused by external agents like

- *Blood parasites:* Babesiosis, theileriosis, trypanosomiasis and sarcocystosis.
- *Chemicals:* Copper, lead, nitrate and nitrite poisoning.

MODULE-6: ERYTHROPOIESIS

Learning objectives

- This module deals with
  - Erythropoiesis
  - Regulation of erythropoiesis

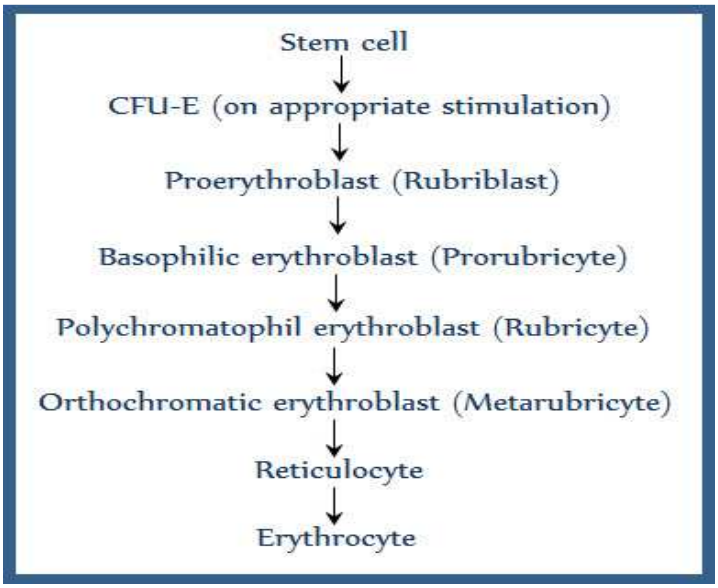
HEMATOPOIESIS

- It is the processes of formation of erythrocytes, leukocytes and platelets in the body. Formation of erythrocytes and leukocytes respectively are known as erythropoiesis and leukopoiesis.
- During embryonic state the blood islands of pander of the yolk sac functions as a site of hematopoiesis. The mesenchymal cells of the liver, spleen, bone marrow and lymph glands are the hemopoietic organs in early fetal life.
- The bone marrow *is* concerned with the production of erythrocytes, granulocytes and platelets during postnatal life, whereas the lymphocytes production occurs in the lymphoid tissues of lymph glands, Payer's patches of intestine, spleen and thymus.
- The lymphoid tissues of the bone marrow and also the spleen are the sites of production for monocytes. In ruminants, hemolymph nodes (hemal) functions as spleen. It takes part in the erythropoiesis during the foetal period, while granulopoiesis, is more prevalent in postnatal life.

- The mesenchymal cells of the yolk sac produce primitive stem cells, which give rise to the pleuripotent stem cells (colony forming units - CFU). These stem cells give rise to five different blast cells, *viz*.
    - Proerythroblast to form RBC
    - Myeloblast to form neutrophils, eosinophils and basophils
    - Monoblast to form monocyte
    - Lymphoblast to form lymphocyte
    - Megakaryoblast to form platelets. Depending on the microenvironment, *i.e*., the location of the stem cells and the growth factors, the stem cells differentiate into progenitor cells of different blood cells (Committed Stem Cells-CSC). A CSC that produces erythrocytes is called colony-forming unit-erythrocyte (CFU-E). Similarly, CFU that produce granulocytes and monocytes are designated as CFU-GM.
- The stem cells continue to divide throughout the life of the animal and a part of the cells remains as pleuripotent stem cells and retained in the bone marrow to maintain supply of stem cells.
- The pleuripotent stem cells differentiate to form the CSC. Several hemopoietic growth factors and differentiation factors stimulate the growth and differentiation of these stem cells into a particular progenitor cells.
- Cytokinins are the growth factors that regulate the formation of blood cells. Two cytokinins that stimulate red cell and WBC formation are the colony stimulating factors and interleukins. Erythropoietin increases erythrocyte precursor formation.

ERYTHROPOIESIS

- From stem cell, the formation of reticulocyte takes about 72 hours and conversion of reticulocyte to erythrocyte requires 48 hours. Thus RBC formation requires 5 days time.
- Under appropriate stimulation, CFU-E progenitor cells produce proerythroblast. Hb synthesis begins in polychromatophil erythroblast and maximum synthesis occurs in orthochromatic erythroblast.
- The metarubricyte ejects the nucleus to become the reticulocyte that contains some mitochondria, ribosomes and endoplasmic reticulum. In 1-2 days, they develop into erythrocytes and enter circulation.



REGULATION OF ERYTHROPOIESIS

- The level of oxygen in the tissue is the principle regulatory factor of erythropoietic activity of the bone marrow. The kidney cells, during hypoxia, releases erythrogenin (erythropoietin releasing factor) from the glomeruli, which in turn acts on erythropoietinogen, an $\square_2$ globulin of plasma and converts it into free erythropoietin (hemopoietin). Kidney produces 90% of erythropoeitin and liver produces about 10%.
- Erythropoietin as a hormone stimulates hemopoietic stem cells of bone marrow to produce the committed stem cells-proerythroblast, thus initiates erythropoiesis. It stimulates,
    - The proliferation of rubriblast by mitosis in the developing rubricytes.
    - Accelerates maturation of the rubricytic cells.
    - Induces the release of reticulocytes into the circulation.

THE ROLE OF VITAMINS AND MINERALS IN ERYTHROPOIESIS

1. Vitamin B_{12} and folic acid are essential for the maturation of erythrocytes. Vitamin B_{12} is required for DNA synthesis and folic acid for RNA synthesis. Macrocytic anemia is a very common in Vitamin B_{12} and folic acid deficiencies.
2. Thiamine (B1), Pantothenic acid, Nicotinic acid, Vitamin E and pyridoxine (B_6), riboflavin, biotin and ascorbic acid are essential for erythropoiesis. Deficiency of Vitamin B_6 causes *microcytic hypochromic anaemia* in pigs. Pantothenic acid deficiency results in deficiency of ALA synthatase in birds and animals. Normocytic anemia in swine and primates is due to Vitamin E deficiency.
3. Minerals such as iron, copper and cobalt are essential for erythropoiesis. Iron acts as an integral part of Hb which is absolutely essential for Hb synthesis. Copper acts as a co-

factor in ALA dehydrase in Hb synthesis. It is part of the enzyme ferroxidase which is necessary for oxidation of ferrous iron to ferric form and is necessary for the incorporation of iron into Hb. *Copper deficiency is common in pigs*, which may interfere with Fe absorption and utilization. In ruminants cobalt plays a key role for the synthesis of Vitamin B_{12} by the rumen bacteria which in turn is required for the normal production of erythrocytes.

RETICULOCYTE

- A low percentage (1 to 3%) of erythrocytes in circulation has a network of bluish threads within the cell and is called *reticulocytes.*
- These cells are immature RBCs, which have entered into the circulation at times of need from blood forming tissues.
- In some diseases or due to excessive loss of blood or destruction of RBCs, the reticulocytic number increases in circulation. These cells have less or no O_2 carrying capacity.

MODULE-7: HAEMOGLOBLIN

Learning objectives

- This module deals with
    - Hemoglobin and its properties
    - Types of hemoglobin
    - Regulation and concentration of hemoglobin

HEMOGLOBIN

- Hemoglobin is the iron-containing oxygen-transport metalloprotein present in the red blood cells of all vertebrates that carries oxygen from the lungs to the tissues and carbon dioxide from the tissues back to the lungs.
- The oxygen carrying capacity of hemoglobin is 60% more than that of plasma.
- It also functions as a buffer in the regulation of acid base balance.
- A hemoglobin molecule is a complex substance consisting of conjugate protein composed of a pigment heme and a protein, globin.
- The protein portion of each of these chains is called "globin". The a and b globin chains are very similar in structure. In this case, **α** and **ß** refer to the two types of globin.
- The globin is a conjugated protein and heme contains iron in ferrous state.
- Hemoglobin contains four polypeptide chains namely two alpha and two beta chains. Each of the four chains unites with a heme group resulting in a hemoglobin molecule.

HEMOGLOBIN BIOSYNTHESIS

- Hemoglobin (Hb) is synthesized in a complex series of steps.
- The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol.
- Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature.

Heme synthesis

- Heme is synthesized in a complex series of steps involving enzymes in the mitochondrion and in the cytosol of the cell. The first step in heme synthesis takes place in the mitochondrion, with the condensation of succinyl CoA and glycine to form delta-amino levulinate. This molecule is transported to the cytosol where a series of reactions produce a ring structure called protoporphyrin IX.
- Many enzymes concerned with heme synthesis are intra-mitochondrial, limited to erythroid precursors including reticulocytes.
- The ALA synthetase is the rate-limiting enzyme of the Hb synthesis, present within the mitochondria.

Globin synthesis

- After heme is synthesized within the mitochondria, 4 heme molecules combine with 4 globin polypeptides to form one molecule of haemoglobin.
- The globin molecule of haemoglobin differs among the species, whereas there is no difference in the heme portion.

FACTORS INFLUENCING HEMOGLOBIN SYNTHESIS

- The amount of Hb in the blood is influenced by age, sex, muscular activity, season, excitement etc. Erythropoietin stimulates RNA and DNA synthesis, the cell division, heme synthesis and hemoglobin production.
- At an oxygen pressure (PO_2) of 100 mm of Hg in the lung, the Hb forms loose and reversible combination with oxygen, the oxyhemoglobin, but at low oxygen pressure of 40 mm of Hg at tissue level, it readily releases oxygen to the tissues for complex metabolic process.

CONCENTRATION OF HEMOGLOBIN IN VARIOUS SPECIES

Species	Hb (g/dl)
Dog	12-18 (15)
Cat	8-15 (12)
Cow	8-15 (11)
Sheep	8-16 (12)
Goat	8-14 (11)
Horse	11-19 (15)
Pig	10-16 (13)

TYPES OF HEMOGLOBIN

- Based on physiological functions, the hemoglobins are typed as adult hemoglobin and fetal hemoglobins. Electrophoretically, the Hbs are classified as HbA, HbB, HbC and HbF. Human beings show three types of Hb, HbA (98%), HbA2 (2%) in the adult and HbF in fetus and new born. HbA has 2 μ -chains and 2 ß -chains; HbA2 is represented by 2 μ and 2 delta - chains. HbF has 2 μ -chains and 2 γ -chains.
- In adult sheep HbA (2 μ, 2 ß) is electrophoretically fast and has higher O2 affinity than HbB with 4γ chains. Sheep having HbA or HbB under anemia or hypoxic condition develop another type of Hb, the HbC, which partially or completely replaces the HbA. Such a change is also observed in goat. HbC is the naturally occurring Hb in sheep during growth period. HbF has higher affinity for O2. In many animal species fetal hemoglobin (HbF) is replaced by the adult types within 4 to 8 weeks after birth. In adult cat the HbA and HbB are found in the same erythrocyte.
- Some of the Hb variants, HbS, HbC, HbE are associated with specific hematologic disorders. HbS is responsible for sickle-cell anaemia in Negro race. HbC and HbE cause failure of synthesis of alpha or beta chains thus results in alpha or beta thalassemia.

DERIVATIVES OF HAEMOGLOBIN

Oxyhemoglobin

- Hemoglobin has an important physiological relationship with oxygen. Oxygen forms loose and reversible combination with hemoglobin called oxyhemoglobin when the erythrocytes passes through the pulmonary capillaries. Since there are four ferrous atoms in the hemoglobin molecule, four molecules of oxygen are transported by a molecule of hemoglobin.

$$Hb + 4O_2 \longleftrightarrow Hb4O_2$$

- As blood is transported through the systemic capillaries hemoglobin loses its oxygen to the tissues and becomes hemoglobin again. Hemoglobin shows progressive increase in the affinity for O_2 after the first two molecules of O_2 are taken up by the heme.
- The oxygen carrying capacity of the hemoglobin is dependent on the pigment it contains which in turn depends on the iron content for oxygen combining capacity.
- The amount of iron present in the blood is minute about 0.334% of the hemoglobin molecule or 0.04 to 0.05% of the blood itself. Each gram of Hb combines with a maximum of 1.34 ml of oxygen.
- In the lung (PO_2 100 mm Hg) the oxygen binds with Hb which shows 97% saturation. One hundred ml of blood containing approximately 15 grams of Hb, can carry approximately 19.4 ml of oxygen.
- In the tissue capillaries, (PO_2 40 mm Hg) Hb is 72% saturated and contains 14.4 ml of oxygen per 100 ml of blood, which indicates oxygen release from the Hb into the tissues. Thus under normal resting conditions about 5 ml of oxygen is transported by each 100 ml of blood during each cycle to the tissues. During heavy exercise this is increased to about 15 times normal. The oxygen hemoglobin dissociation curve is "S" shaped or sigmoid shaped.

Myoglobin (*Muscle hemoglobin*)

- It is a true hemoglobin and functions to store oxygen in the muscle. It contains only one heme and a polypeptide chain. It contains only one iron atom and can therefore store only one molecule of O_2.
- Its molecular weight is approximately 17,000, which is four times less than Hb. Hence it can pass through glomerulus.
- The oxygen dissociation curve with myoglobin is hyperbolic (very steep). The appearance of myoglobin in the urine is referred to as myoglobinuria or azoturia, which is a very characteristic symptom of monday morning sickness in horse.

Carboxyhemoglobin (*HbCO*)

- Hb has 200 times more affinity for carbon monoxide than oxygen.

Hb+ CO ☐ HbCO

- Carbon monoxide firmly attaches with the Fe^{++} molecules of heme, thus interferes with the transport of O_2 as oxy Hb. 0.1% of CO in inspired air will convert 20% of Hb into HbCO within 30 to 60 minutes.
- Oxygen under higher partial pressure is the only means to reverse the reaction.

$$HbCO + \uparrow PO_2 \rightarrow Hb\,O_2 + CO$$

Methemoglobin (*ferrihemoglobin*)

- It is formed by the oxidation of ferrous iron to ferric iron. During the circulation of blood about 1% of methemoglobin is formed by the oxidation of ferrous iron to ferric iron. Ferrihemoglobin cannot combine with oxygen, hence is useless as a respiratory pigment in the blood. It produces dark colored blood. Glutathione (GSH) in the erythrocytes prevents the excessive oxidation of ferrous iron into ferric iron.
- Chemicals like nitrates, sulphanamides, aminophenol and acetanilide cause increased concentration of methemoglobin in the blood. Horse blood at normal conditions shows significant amounts of methemoglobin. The normal blood of dog and cat has about 1% of methemoglobin.

ABSORPTION SPECTRA

- When white light is passed through a solution of hemoglobin or one of its derivatives, certain wavelengths are absorbed. The resulting spectrum is termed as absorption spectra; the region of absorption is known as absorption bands.
- They can be seen by examining the solution with a spectroscope.
- When white light is examined spectroscopically, a series of colours known as spectrum (VIBGYOR) is obtained.
- When sun light is examined, certain black vertical lines called as Fraunhofer's lines are found at definite places in the spectrum; these lines are designated as A, B, C, D, E, etc. In lamp light, these lines are not seen.
- When hemoglobin solution or its derivatives are examined in certain concentrations spectroscopically, absorption bands of definite size, appearance, and position are noticed.
- Hence, spectroscopic examination helps to identify these pigments in solution. E.g. Dilute oxy-Hb solution shows two absorption bands between line D and E; adding a reducing agent (produces reduced-Hb) gives one band at line D.
- Carboxy-Hb shows two bands but adding a reducing agent does not produce a single band.
- Met-Hb shows a band between line C and D.

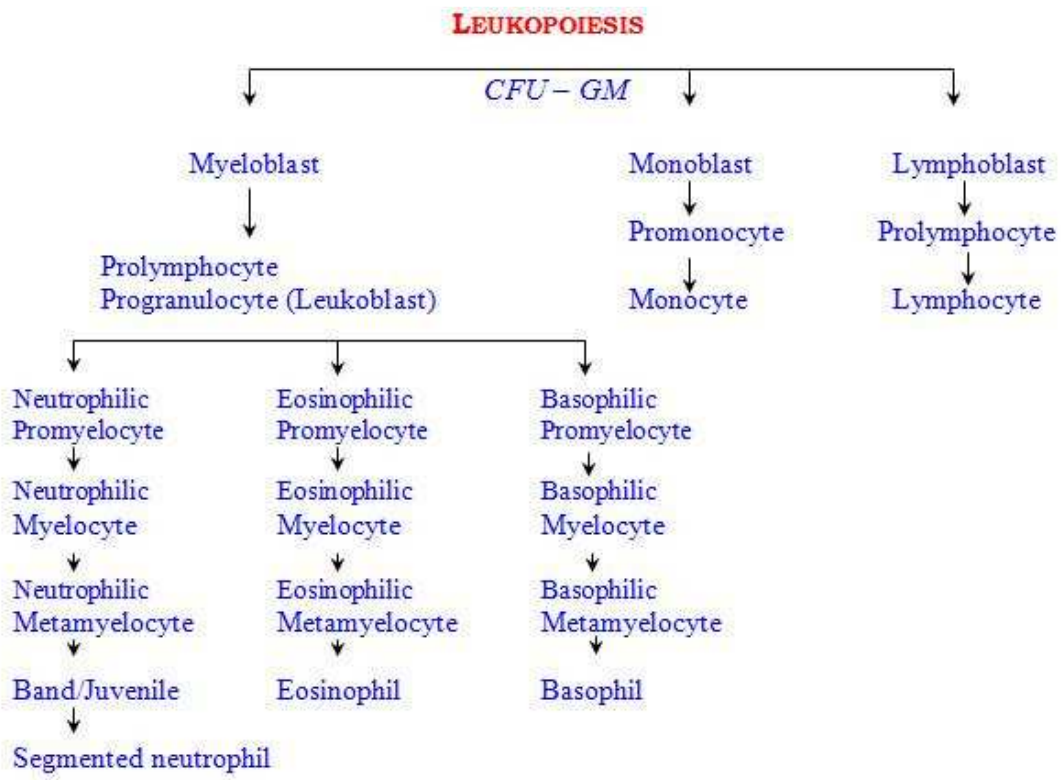
MODULE-8: LEUKOCYTES (WBC)

Learning objectives

- This module deals with
  - production of leukocytes
  - types of leukocytes
  - fate and concentration of leukocytes

LEUKOPOIESIS

- The process of formation of leukocytes is known as *Leukopoiesis*. Leukocytes are produced from the pleuripotent stem cells.



LEUKOCYTES

- Leukocytes are large sized, nucleated cells, lesser in number and do not have hemoglobin content. These cells show polymorphic forms, which differ morphologically and functionally.
- Majority of the WBCs is larger than RBCs; while RBCs are present in millions per ml of blood, the WBCs are present in thousands per ml of blood.
- The ratio of WBC to RBC varies from 1:100 in chicks to 1:1300 in goats, 1:600 in dog and cat, 1:800 in cattle 1:1000 in horse 1:1200 in sheep and 1:700 in man.

Types of leukocytes

- Based on the staining nature of the cytoplasmic granules when subjected to Leishman's stain, leukocytes are classified as granulocytes, which are further classified into three groups the neutrophils, eosinophils and basophils.
- Some of the leukocytes have cytoplasmic granules but they are non-visible even after staining with Leishman's stain, hence they are known as agranulocytes. The lymphocytes and monocytes form two groups of this type.

Site of production of WBCs

- The granulocytes and the megakaryocytes are produced from the myelocytes of the bone marrow hence often referred as myeloid series of blood cells.
- The lymphocytes and the plasma cells are formed from the lymphoid tissues such as lymph nodes, spleen and lymphoid tissues of the bone marrow, gut etc.
- The monocytes are produced by the mononuclear phagocytic system (MPS) cells of spleen and bone marrow (reticuloendothelial system cells).

NEUTROPHILS

- Neutrophils are produced in the bone marrow from the extravascular neutrophilic myelocytes. They are numerously found in the blood of most animals.
- The nucleus of mature cells is divided into lobes, usually five lobes and are stained blue or purple by Leishman's stain. They have abundant and fine granular cytoplasm which take up the neutral stain.
- These granules store lysosomes, which contfain hydrolytic enzymes, proteolytic enzymes and lipases to digest the invading organisms.
- The oxidative enzymes of the lysosome produce hydrogen peroxide, which attack the bacterial cell wall to cause bactericidal effect.
- Neutrophils are highly motile, responds to chemotaxis and actively phagocytic, thus serve as a first line of defence against invading organisms, (bacteria, viruses and cellular remnants).
- At the onset of infection neutrophils produce pyrogens which act on thermo-regulator centre of the brain results in fever. This rise in body temperature slows the reproduction process of bacteria and viruses
- Immature forms of neutrophils are characterized by unsegmented or less number of nuclear lobes and are referred to as *juvenile or band* cells. Pseudo neutrophils and *heterophils* are comparable to neutrophils, which are present in rabbit and poultry respectively.
- Heterophils contain large rod or spindle shaped granules, which are acid in reaction and stain red or pink with eosin.

Abnormalities

Neutrophilia

- It indicates more number of neutrophils in the circulation.
- Physiological neutrophilia occur in conditions like exercise, emotion, pregnancy, lactation and parturition.
- Abnormal or pathological neutrophilia may be due to acute inflammation following injury, surgery, burns, arthritis and acute infection by pyrogenic bacteria**.**
- Shift to left is a term used to describe an increase in the number of immature neutrophils (Band cells) in the circulation which is characteristic of bacterial infections. The shift to left is clinically helpful in diagnosis of traumatic reticulopericarditis (TRP).

Neutropenia

- It indicates reduction in neutrophils in the circulation, which is very common in viral infections and chronic infections like TB, brucellosis and protozoal and fungal infections.
- Injection of antiinflammatory drugs (cortisol) and antibacterial drugs (chloramphenicol and sulphanamides) may result in neutrophilia.

EOSINOPHILS

Eosinophils

- They are large cells which contain large cytoplasmic granules that stain red or purple eosin stain. They have bilobed nucleus, connected by a thin filament.
- The cytoplasmic granules contain enzymes, which are rich in oxidases, and peroxidases. Eosinophils are produced from the bone marrow and are highly motile, but less phagocytic.
- Eosinophils function to detoxify the proteins of the parasites, phagocytise antigen-antibody complexes, the inflammatory products of the mast cells and basophils.
- These cells have antiheparin, and anti- histaminic substances, thus act as an anti-inflammatory and anti- allergic agent. Eosinophils release profibrinolysin, which is then activated to fibrinolysin, and causes the dissolution of old blood clot.

bnormalities

- *Eosinophilia:* It is increased number of eosinophils in the circulation. This condition is common in G.I. parasitic infections, allergic disorders like bronchial asthma, allergic rhinitis, in skin diseases like eczema and dermatitis, drug reactions following penicillin and sulphanamides administration.
- *Eosinopenia:* It is decreased number of eosinophils in circulation. This condition occurs following stress , administration of ACTH or cortisol etc.

BASOPHILS

Basophils

- These cells are found in the blood in lower concentration. They have irregular shaped nucleus and the granules are stained blue by the basic dye of Leishman's stain. They originate in the bone marrow and have slight or no phagocytosis.
- Extravascularly, basophils enlarge and become tissue mast cells. Both basophils and mast cells release heparin (anticoagulant), and vasodilator substances histamine, serotonin and bradykinins. Thesevasodilators causes increased blood flow and reddish colour (hyperemia) at the site of infection followed by increased permeability of the plasma and proteins from the blood vessels, a process calledinflammation. Basophils and mast cells have receptors for immunoglobin E.

Abnormalities

- *Basophilia:* It is increase in basophils, and it is seen in allergic conditions and hematological malignancies.

LYMPHOCYTES

- These cells are characterised by well defined centrally placed nucleus, surrounded by cytoplasm. According to the size and the ratio of nucleus to cytoplasm, these cells are further divided into 3 types.
    - *Small lymphocytes:* These are small sized cells with a large notched nucleus.
    - *Medium lymphocytes:* These are medium sized, which have notched nucleus surrounded by thin rim of cytoplasm.
    - *Large lymphocytes:* These have comparatively small sized nucleus with broad rim of cytoplasm.
- Lymphocytes are actively motile cells with amoeboid movement and are mainly concerned with the development of immunity against specific disease organisms. They have no phagocytic properties.

Abnormalities

- *Lymphocytosis:* This condition indicates *increased* number of lymphocytes in the circulation, which is common in viral infection (eg) infectious hepatitis.
- *Lymphopenia:* This condition indicates *decreased* number of lymphocytes in circulation and is seen in cases of TB, acute stress and glucocorticoid injection.

MONOCYTES

- These are the largest of WBCs, characterised by kidney or bean shaped nucleus.
- Monocytes are actively motile, pinocytic and also highly phagocytic, which may migrate into the tissue spaces and become macrophages. These cells are much more powerful phagocytes than neutrophils by their ability to engulf much large sized particles, necrotic tissues and more number of microorganisms.
- Monocytes leave the blood and are attracted to the tissues by chemotaxis and lymphokines and they become macrophages, example reticular cells of lymph nodes, spleen, bone marrow and Kupffer cells of the liver sinuses.

Abnormalities

Monocytosis:

- This condition indicates increased number of monocytes in the circulation and is seen in hematological malignancies, endocarditis, typhoid, TB, brucellosis and prolonged blood parasitic infections. Eg: trypanosomiasis.

NORMAL VALUES OF LEUCOCYTES IN DOMESTIC ANIMALS

Animal	Total leukocytes ($x10^3$/ cu.mm)	Differential Leukocyte Count (%)					
		Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
		Band	Mature				
Dog	6.0-17.0 (11.5)	0-3(0.8)	60-77 (70)	12-30 (20)	3-10 (5)	2-10 (4)	Rare
Cat	5.5-19.5 (12.5)	0-3 (0.5)	35-75 (59)	20-55 (32)	1-4 (3)	2-12 (5)	Rare
Cow	4-12 (8)	0-2 (0.5)	15-45 (28)	45-75 (58)	2-7 (4)	2-20 (9)	0-2 (0.5)
Sheep	4-12 (8)	Rare	10-50 (30)	40-75 (62)	0-6 (2.5)	0-10 (5)	0-3 (0.5)
Goat	4-13 (9)	Rare	30-48 (36)	50-70 (56)	0-4 (2.5)	1-8 (5)	0-1 (0.5)
Horse	5.5-12.5 (9)	0-2 (0.5)	30-65 (49)	25-70 (44)	1-7 (4)	0-11 (4)	0-3 (0.5)
Pig	11-22 (16)	0-4 (1)	28-47 (37)	39-82 (53)	2-10 (5)	1-11 (3)	0-2 (0.5)
Chicken	20-30	-	25-30	55-60	10	3-8	1-4

FATE OF WBCs

1. It is very difficult to assess the life span of leukocytes because the WBCs move between blood and tissue.
2. The life span of granulocytes is normally 9 days but once they are released into circulation from bone marrow their life span is 4 to 8 hours.
3. During infection, the life span in shortened. “B” lymphocytes live for 3-4 days only but “T” lymphocytes may live for 1-3 years in tissues and return to circulation many times.
4. The monocytes also have short transit time in the blood (24 hours) before migrating to the tissues where they become tissue macrophages and can live for months or even years.

ABNORMALITIES OF LEUKOCYTES

- Leukocytosis:
  - It is an increase in the number of leukocytes which is an indication of presence of some infection.

- Leukocytosis may be physiological (related to time of day, meal, exercise, epinephrine, stress) or pathological. Bacterial infection shows leukocytosis with neutrophilia, whereas viral infections result in neutropenia.

- Leukemia:
  - Pathologically, cancer of leukocyte producing tissues results in abnormally high white cell count which is known as *leukemia*.

PLASMA CELLS

- Plasma cells are white blood cells which produce large volumes of antibodies.
- They are transported by the blood plasma and the lymphatic system and originate from the bone marrow.
- They are otherwise known as plasma B cells , plasmocytes , and effector B cells .
- They are present in lymph nodes ,spleen and diffuse lymphoid tissue of alimentary and respiratory tract.

STRUCTURE OF PLASMA CELLS

- Plasma cells are large lymphocytes round in shape containing a granular cytoplasm which stains with basic dyes.
- The nucleus is eccentric in position and typically represents clumps of chromatin in a radiating manner, resembling a "cart -wheel" or clock face in appearance.
- They have a considerable nucleus-to-cytoplasm ratio.
- The cytoplasm contains a conspicuous Golgi apparatus and abundant endoplasmic reticulum .
- Immunoglobulins are localised in the endoplasmic reticulum where it sometimes forms aggregates called as Russel bodies.

FORMATION OF PLASMA CELLS

- When specific B-lymphocytes are stimulated by specific antigen, they enlarge and become lymphoblast, some of which further differentiate to immature plasmablasts which finally forms, the plasma cells.

FUNCTIONS OF PLASMA CELLS

- These cells are rarely seen in the circulation, which are formed by the lymphoid tissues (spleen).
- They play a very important function in body defence mechanism.
- They are involved in the production of humoral antibodies.
- Plasma cells are concerned with the synthesis, storage and release of immunoglobulins at a very rapid rate.
- A plasma cell can only synthesize an antibody of a single specimen, either IgM or IgG or IgA except in primary immune response when a plasma cell producing IgM initially, may later switch over to the synthesis of IgG antibody.
- Mature plasma cells are end cells and survive only a few weeks and die after a few cell division.

MODULE-9: IMMUNITY

Learning objectives

- This module deals with
  - immunity and its types
  - production of antibody and its mechanism of action

TYPES OF IMMUNITY

- It is the ability of the body to resist the tissue damage by destroying the disease organisms or neutralising their toxins.

Types of immunity

- Innate immunity
- Acquired immunity

INNATE IMMUNITY

- This type of immunity is *present from birth*. This type of immunity is non specific and act on many organisms. It prevents first entry of microorganisms into the tissues or if the organisms has gained entry, eliminates them prior to the occurrence of the disease.
- This type of immunity is not effective on subsequent exposure to the same organisms. Non specific elimination of microorganisms is done by two methods:
  - Phagocytosis: It is ingestion and killing of microorganisms by specialised cells such as neutrophils, monocytes, macrophages etc.
  - Opsonisation: It is the process of coating the microorganisms with some of the proteins found in the plasma to make them more phagocytosable.

ACQUIRED IMMUNITY

- The development of immunity against specific organisms, the bacteria, virus and foreign tissues or toxins is referred to as *acquired immunity*.
- This type of immunity is also known as *adaptive or specific immunity*. It is caused by a special immune system that forms antibodies or activated lymphocytes that attack and destroy the microorganisms.

TYPES OF ACQUIRED IMMUNITY

  - Humoral immunity or B cell immunity
  - Cell mediated immunity or T cell immunity
- In mammals, shortly before or after birth the stem cells or the committed lymphoid progenitor cells migrate either to thymus, liver and bone marrow (in mammals) or to *bursa of Fabricius* (in birds) where they are processed and differentiated.
- The `T' lymphocytes are processed in the thymus and `B' lymphocytes are processed in bursa, liver and bone marrow. These lymphocytes then get into the circulation and localised in spleen and lymph nodes as clones of `T', and `B' lymphocytes.

- Thymus plays a key role in the development of cellular immunity shortly before birth or few months after birth. The T-lymphocytes provide cellular immunity, and the B-lymphocytes provide humoral immunity, by producing specific antibodies. There are different types of B and T cells, each of which responds specifically to one particular antigen.
- All the different B and T cells capable of forming a specific antibody or activated T cells against a specific antigen which are called “clone of lymphocytes”.
- The invading organisms provide proteins, large polysaccharides and large lipoprotein complexes, which function as an antigen. The antigen stimulates specific clone of lymphocytes.
- Macrophages in lymphoid tissues stimulated by antigen releases interleukin I, and it promotes growth of specific lymphocytes. The helper T cells release interleukin II, which stimulate B cells to produce specific antibodies. Those B-lymphocytes specific for an antigen enlarge and form lymphoblasts, which differentiates to plasmablast and finally to plasma cells. The plasma cells produce antibodies very rapidly.
- When these sensitised plasma cells are activated by an antigen, specific antibodies, the gamma globulins- IgG (75%), IgM, IgA, IgD and IgE (during allergy) are produced by them as primary response. However these antibodies have weak potency and short life.
- Some of the lymphoblasts remain dormant and function as memory T and B cells, which on subsequent stimulation by the same antigen (booster) produce more potent secondary response. On exposure to a specific antigen, the T cells of the specific clone proliferate and release large numbers of activated T cells, which neutralises the antigen. The memory T cells are retained in the lymphoid tissues.
- Humoral immunity is developed as specific antibody (gamma globulin) by the “B”-lymphocytes, rarely persists more than few months or utmost few years. On the other hand, the specific clones of “T” lymphocytes on stimulation by the very same antigens are converted into three major groups of “T” lymphocytes.
- Cytotoxic or killer “T” cells: These are the direct attack cells killing the micro-organisms. They have the lysosomal enzymes, the peroxidases, phosphatases and oxygenases and produce H_2O_2, which is lethal to the invading organisms.
- Helper `T' cells: These cells are very sensitive even to very small quantities of the specific antigen, release interleukin-2 (lymphokines), and activate the other two types of “T” cells, the cytotoxic and suppressor “T” cells and also the “B” lymphocytes. Helper “T” cells are not capable of secreting antibodies by themselves. It promotes the production of lymphokines.
- Lymphokines act as a macrophage migration inhibition factor, stop the chemotactically attracted macrophages and neutrophils in the infected area to effect more efficient phagocytosis, and thus limit the spread of the infection.
- Suppressor or regulatory “T” cells: Regulate the activities of both the helper and killer “T” cells. The activated “T” lymphocytes provide the cell-mediated immunity, which is far more persistent for many years than humoral immunity.

ACTIVE IMMUNITY

- Active immunity involves both the cell-mediated and humoral immunity as well as the innate immune system .
- Active immunity is induced in the host itself by the antigen, and lasts for a longerperiod of time.

TYPES OF ACTIVE IMMUNITY

Naturally acquired active immunity

- Naturally acquired active immunity occurs when an individual is exposed to live pathogens, and develops antibodies against specific pathogens which leads to immunological memory.
- In some cases, the immunity may be life-long as in the case of small pox, measles, chicken pox, yellow fever etc. while in some others the immunity may be lost after only a few years (e.g., diphtheria, tetanus) or even for lesser period (e.g., influenza, pneumonia).

Artificially acquired active immunity

- Artificially Acquired Active Immunity occurs when an antigen is intentionally introduced into a body to be immunized, the latter develops immunity .e.g., vaccine, toxoids.
- This immunity is artificial because the antigens are intentionally or purposely introduced, and it is active because the recipients immune system synthesizes antibodies in response.
- Vaccines provide usually long-term immunity.
- Killed and attenuated strains of bacteria and viruses are widely used forms of immunization against many diseases

PASSIVE IMMUNITY

- Passive immunity is the transfer of active immunity, in the form of readymade antibodies, from one individual to another.
- Passive immunity provides immediate protection to the individual, but the duration of passive immunization is relatively short, a few days to several weeks when compared to years for active immunity. This is due to the natural degradation of injected antibody from the circulation without internal replacement.
- Passive immunity can occur naturally, when maternal antibodies are transferred to the fetus through the placenta, and can also be induced artificially, when high levels of human (or horse) antibodies specific for a pathogen or toxin are transferred to non- immune individuals.

TYPES OF PASSIVE IMMUNITY

Naturally acquired passive immunity

- When antibodies produced in the body of an individual (called donor) are naturally transferred into the body of other individual (called recipient), the latter develops immunity, called naturally acquired passive immunity, in its immune system.
- This immunity is natural because the transfer of antibodies from donor to recipient occurs under natural conditions, and it is passive because the recipient does not synthesize antibodies but picks them up from the donor.
- This type of immunity is significant mainly in the survival of the neonates. The neonate passively acquires antibodies from its mother. The antibodies may pass from the immune mother to the fetus across the placental barrier.

- Certain antibodies are also transferred from mother to young ones through colostrum and milk during lactation. These antibodies, called **maternal antibodies,** remain with the young ones for certain period and after the specified time the immune state disappears. The maternal antibodies generally provide resistance against diseases.

Artificially acquired passive immunity

- Artificially acquired passive immunity is a short-term immunization induced by the transfer of antibodies to another individual i.e, produced in one individual or animal and then administered to the other individual, which can be administered in several forms; as human or animal blood plasma, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, and in the form of monoclonal antibodies (MAb).
- Currently passive immunization is mainly used for prophylaxis following exposure to such diseases as rubella and infectious hepatitis. This is usually accomplished by injecting the patient with gammaglobulin which has been extracted from the blood of immune persons.
- Artificially acquired passive immunity is immediate but short-lived (only for two to three weeks .
- Other examples of this type of immunization are injections of hyperimmune serum and antiserum.

ANTIBODY

- These are the gamma globulins or immunoglobulins made up of a combination of light and heavy polypeptide chains. Each heavy chain is paralleled by a light chain at one of its end, which form the variable portion.
- This variable region with both light and heavy chains provides very specific binding site to the specific antigen. Specific Antibodies differ from each other based on the different amino acid sequence in the light and heavy chains of the variable portion.
- On the other hand the free ends of the two heavy chains form the constant portion of the antibody which establishes the properties like diffusibility, adherence to tissue and attachment to complement complex.

MECHANISMS OF ACTION OF ANTIBODIES

- Agglutination in which antigens in the bacteria are bound by antibodies to form a clump.
- Precipitation in which the soluble antigen is rendered insoluble and precipitated.
- Neutralization, to cover the binding site of the toxin thus makes the virus non-virulent.
- Lysis, by the lysosome following the attachment with the antibody with the cell membrane of the invaders.
- Lymphocytes are motile, but not phagocytic in function. During viral infection, these cells come in contact with specific virus and release specific interferons, which inhibits the multiplication of that virus. Interferon also circulates throughout the body and protects other body cells from that virus.

NATURAL KILLER CELLS

- Natural killer cells (also known as NK cells, K cells, and killer cells) are a type of lymphocyte (a white blood cell) and a component of innate immune system.
- NK cells play a major role in the host-rejection of both tumours and virally infected cells.

- These cells are cytotoxic and contain small granules in their cytoplasm with special proteins such as perforin and proteases known as granzymes.
- When NK cells comes in close contact with target cells, perforin forms pores in the cell membrane of the target cell through which the granzymes and associated molecules enter, inducing apoptosis or death of the cell.
- NK cells are activated in response to interferons or macrophage-derived cytokines and control viral infections.

MODULE-10: BLOOD COAGULATION

Learning objectives

- This module deals with
  - mechanism of blood coagulation or blood clotting
  - mechanism of prevention of blood clotting in blood vessels

MEGAKARYOCYTE

- These are the myeloid cells of the bone marrow, rarely seen in the circulation as giant cells.
- Its fragments provide the platelets or thrombocytes.

PLATELETS OR THROMBOCYTES

- They are disk-shaped structures, derived from the megakaryocytes of the bone marrow.
- Mammalian platelets are non-nucleated, whereas they are nucleated in birds and reptiles.
- In mammals, platelets originate from the megakaryocytes of the bone marrow.
- In birds they arise from large mononucleated cells of bone marrow.
- Thrombocytes are about 3 μm in diameter.

STRUCTURE OF PLATELETS

- The thick glycocalyx of the platelet cell membrane contains clotting factors like fibrinogen, factor V, VII and IX. The phospholipid of the cell membrane is the precursors for prostaglandins and platelet factors.
- Platelet cytoplasm have two types of granules; the *alpha and dense granules*. They also have contractile fibrils containing actin and myosin, lysosomes, mitochondria and glycogen.

a - Granules	Functions
Albumin	Maintains colloidal osmotic pressure
β - Thromboglobulin	Inhibitor of Prostacycline formation
Factor V/Proaccelerin	Involved in blood coagulation

Fibronectin	Helps platelet adhesion with extracellular membrane
Platelet derived growth factor	Necessary for the growth of endothelial cells & Fibroblast
TGF – β	Involved in tissue repair
von Willebrand factor (vWF)	Helps platelet adhesion
Factor XIII	Fibrin stabilizing factor

Dense Granules	**Functions**
ADP	Involved in platelet activation
ATP	Provides energy
Calcium	Provides principle stimulus to blood coagulation
Serotonin	Vasoconstriction
Actin & Myosin	Contractile proteins, involved in clot retraction
Mitochondria	Powerhouse to synthesis ATP
Lysosomes	To digest the damaged tissues
Glycogen	Primary source of energy

- Platelets produce thromboxane A_2, which produces platelet aggregation and contraction of platelet (Clot retraction).
- Platelets survive for 8-11 days in circulating blood.

FUNCTIONS OF PLATELETS

- Their main function is in blood coagulation and clot retraction.
- They provide platelet phospholipid for coagulation and carry several clotting factors on their surface.
- They prevent hemorrhage when blood vessels are injured by transforming into platelet plugs, which is an important mechanism for closure of minute ruptures in blood vessels.

NORMAL PLATELET COUNT IN DOMESTIC ANIMALS

- **Mean platelet count /ml of blood**

Dog	Cat	Cow	Sheep	Goat	Horse	Pig
3,00,000	4,50,000	5,00,000	4,00,000	4,50,000	2,25,000	5,20,000

HEMOSTASIS

- It means prevention of blood loss from damaged vessels.

VASCULAR SPASM

- Injury or trauma to the blood vessels stimulates reflex constriction of the blood vessels through the sympathetic division or the local myogenic spasm by the action of ***serotonin (5-hydroxy tryptamine)*** to close even the large blood vessels to prevent excessive blood loss.
- When the injury is severe with extensive tissue damage (Crushing, laceration) this spasm is strong and bleeding is less.
- Smooth cut causes weak spasm and bleeding is severe.

FORMATION OF PLATELET PLUG

- Platelets have contractile proteins, the actin and myosin, factor XIII, the fibrin stabilising factor, enzyme systems for the synthesis of cAMP, ATP, ADP and prostaglandins (PGG_2, PGH_2, $PG1_2$ and PGF_2). Platelets are activated by their contact with collagen, which is present in the subendothelial membrane or by substances like ADP, serotonin and thromboxane A_2 released from damaged cells.
- Immediately after the vascular endothelial damage, the subendothelial collagen attracts the platelets to the site of injury. The platelets attach to the injured surface. They then undergo a series of complex physical and biochemical changes like swelling of the platelets, projection of radiating processes, *(pseudopods)* from the platelets and their adherence with the endothelial wall of the blood vessels. Factors vWF and fibronectin from subendothelium and platelets help in platelet adhesion.
- This reaction in turn stimulates release of Ca^{++} which stimulates the enzyme systems of the platelets and causes the release of ADP and thromboxane-A_2 which activate other platelets resulting in adherence of more number of platelets in the damaged endothelial wall forming the platelet plug.
- This plugs the injury on the blood vessel and prevents blood loss. Thromboxane-A_2 is the most potent platelet-aggregating agent that lowers platelet cAMP and also causes vasoconstriction. PGG_2 and PGH_2 also cause platelet aggregation, whereas PGI_2 produced by the normal endothelium is a vasodilator which acts as a powerful inhibitor of platelet adhesion / aggregation.
- Platelet aggregation is prevented by prostacyclins produced by endothelial cells of the artery and lungs. In normal blood flow, prostacyclin level is more than thromboxane and aggregation is prevented. When arterial walls are damaged, prostacyclin level is reduced and thromboxane level becomes high leading to platelet aggregation.

BLOOD COAGULATION OR BLOOD CLOTTING

- Many substances present in tissue and blood affect coagulation. Substances that promote coagulation are called as procoagulants, and those inhibiting coagulation are anticoagulants.
- Normally in the blood, anticoagulant activity predominates and blood does not coagulate. But, when a vessel is ruptured, the procoagulants activity in the damaged area becomes more and the blood clots.
- Clot is the meshwork of fibrin threads running in all directions to entrap the blood cells, platelets and plasma. This is achieved by the activation of series of clotting factors of the blood by two mechanisms:
    - The intrinsic and
    - Extrinsic systems

THREE STAGES OF CLOT FORMATION

- Formation of prothrombin activator in response to rupture of blood vessel or damage to the blood itself.
- Conversion of prothrombin into thrombin by the catalytic activity of prothrombin activator.
- Conversion of fibrinogen into fibrin thread by the enzymatic activity of thrombin.

INTRINSIC AND EXTRINSIC MECHANISM

Intrinsic or endogenous mechanism

- When the blood come in contact with foreign surface (other than intact vascular endothelium) like disrupted endothelium, negatively charged surface or glass, it causes a sequence of enzymatic reactions to initiate the coagulation mechanism.
- Blood drawn in a glass tube clots by this mechanism.

Extrinsic or exogenous mechanism

- These sequential enzymatic reactions occur when the blood from the damaged blood vessel contacts extra-vascular tissue factor, the tissue thromboplastin, released from the surrounding tissues.
- Tissue thromboplastin is not present normally in the blood but released when the surrounding tissue is damaged.

BLOOD CLOTTING CASCADE

- Most of the clotting factors are inactive proteolytic enzymes and when activated produce enzymatic activities leading to cascading reaction of clotting process.
- The extrinsic mechanism is *fast acting*. The cascade reactions of clotting process involving so many factors helps even a low amount of stimulus (damage) to produce large amounts of final end product (clot).

- In the intrinsic mechanism, injury to the blood alters the factor XII and the platelets of the blood. When the factor XII comes in contact with collagen or a wettable surface, it becomes a proteolytic enzyme called activated XII (XIIa).
- The blood trauma also damages the platelets, release platelet phospholipid, which is essential for subsequent reactions. In the extrinsic mechanism, the damaged tissue releases a complex of factor called *tissue thromboplastin* contains phospholipids and a glycoprotein which acts as proteolytic enzyme.

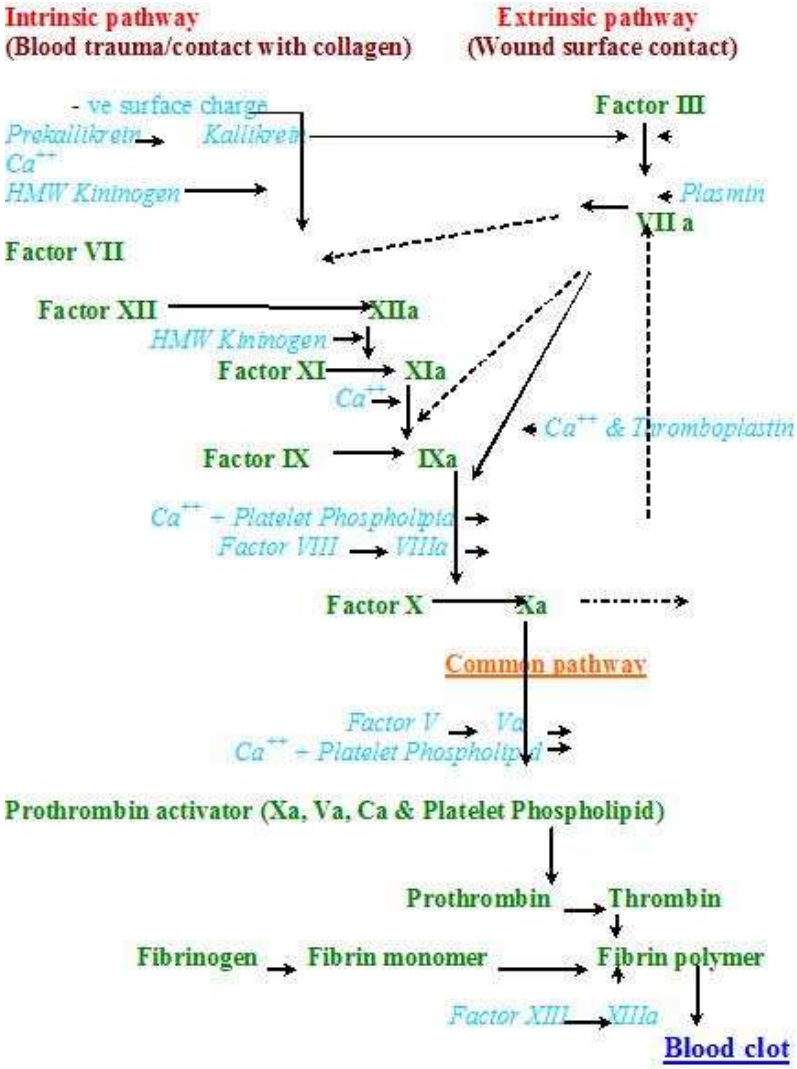
CONVERSION OF PROTHROMBIN TO THROMBIN

- Both intrinsic and extrinsic mechanisms results in the formation of a complex called prothrombin activator complex, which includes activated Factor X, (acts as protease on prothrombin); Factor V (accelerates protease activity) and phospholipid (further activates the whole process).
- The prothrombin activator acts on prothrombin and converts it to thrombin. The thrombin converts fibrinogen to fibrin monomers. It also activates more and more of Factor V, which further accelerates prothrombin activation.

Clotting factors	Proteins except factor IV
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Tissue thromboplastin
Factor IV	Ca^{++} ion
Factor V	Labile factor/Proacclerin
Factor VII	Serum prothrombin conversion accelerator/Proconvertin
Factor VIII	Antihemophlic factor C
Factor IX	Christmas factor/Antihemophlic factor B
Factor X	Stuart factor
Factor XI	Plasma thromboplastin antecedent
Factor XII	Hageman factor
Factor XIII	Fibrin stabilizing factor
VWF	Von Willebrand factor
Prekallikrein	Fletcher factor
HMWK	High molecular weight kininogen
Fibronectin	

- Prothrombin is a plasma protein, an $alpha_2$ globulin (molecular weight 68,700) produced from the liver. Vitamin K is required for the formation of prothrombin and clotting factors VII, IX and X. Thrombin is formed from prothrombin by the action of prothrombin activator and Ca^{++}.

BLOOD CLOT FORMATION



CONVERSION OF FIBRINOGEN TO FIBRIN

- Fibrinogen is a high molecular weight plasma protein produced from liver. The proteolytic enzyme thrombin acts on fibrinogen and remove 4 low molecular weight peptides from each molecule of fibrinogen resulting in formation of fibrin monomer which polymerises with other fibrin monomers and forms fibrin threads.
- The fibrin-stabilising factor, present in platelets and to small extent in plasma, causes covalent bonds between the fibrin monomers and cross linkage between adjacent fibrin threads thus adding strength to the fibrin meshwork, the clot.

- Clot, is composed of fibrin threads running in all directions and entrapping blood cells, platelets and plasma. The fibrin threads adhere to damaged surface of blood vessel and close the opening in the blood vessel thereby prevent blood loss.
- *Clot retraction:* Within a few minutes after a clot is formed, it begins to contract and expresses most of the fluid from the clot within 30 to 60 minutes. The fluid that is expressed out of the clot is called serum.
- Platelets are necessary for clot retraction. This is caused by the contraction of actin and myosin of the platelets by using ATP of platelets.

FATE OF CLOT

Fate of clot

- Once a blood clot is formed, it can follow either of the following two courses.
    - The clot may be invaded by fibroblasts (the formation and invasion is stimulated by platelet derived growth factor) within few hours of its formation. The fibroblasts promote connective tissue formation within the clot and within 7-10 days the clot is organised into fibrous tissue.
    - When the hemorrhage occur in large quantities and when the blood clot forms in the tissues, some special substances formed within the clot will dissolve the clot (lysis of clot).

Lysis of blood clot

- The plasma contain a glycoprotein called plasminogen or profibrinolysin. During the formation of clot, this is trapped within the clot.
- This can be activated to plasmin or fibrinolysin by tissue plasminogen activator (tPA) released from the damaged tissues and vascular endothelium. The plasmin acts as proteolytic enzyme and digests fibrin threads leading to dissolution of clot.

PREVENTION OF BLOOD CLOTTING IN BLOOD VESSELS

- The important factors that prevent the process of clotting within the vascular system are:
    - Smooth endothelium, which prevents contact activation of intrinsic system
    - Glycocalyx layer adsorbed to the inner surface of the endothelium that repels clotting factors and platelets
    - Protein bound with endothelium which binds thrombin and removes its effect; this protein also activates a plasma protein called protein C which inactivates the activated factor V and VIII. Damage to endothelium causes loss of smoothness and the glycocalyx layer and thus initiates coagulation.
- Anticoagulants in blood also prevent clotting within the vessels. Important anticoagulants are
    - Fibrin threads formed during clotting
    - A globulin known as antithrombin III, heparin and alpha$_2$ macroglobulin .
- The thrombin formed during clotting becomes adsorbed to fibrin and thus prevents further spreading of the blood clotting. Thrombin not adsorbed to fibrin combines with antithrombin and gets inactivated. Heparin increases the effectiveness of antithrombin in removing thrombin. The heparin and antithrombin also inactivates the activated factors XII, XI, and X. The alpha$_2$ macroglobulin acts similar to antithrombin.

- Clotting time, or coagulation time is the time required for the blood to clot after it is drawn out from the blood vessels. When blood is exposed to air, it clots within a short time. Clotting time is less than 5 min. in most of the domestic animals except cow and horse. Average clotting time (in minutes):
    - Horse : 11.5,
    - Sheep : 2.5,
    - Cow : 6.5,
    - Pig : 3.5,
    - Dog : 2.5,
    - Human : 5.0.
- The bleeding time is the time required for bleeding to stop after puncture of the skin. The blood is blotted away every 30 seconds or the wound is immersed in physiological salt solutions: (ranges from 3- 6 min)
- The prothrombin time is a measure of the clotting time of plasma to which an excess of thromboplastin has been added so that this coagulation factor will not be a limiting one. Since oxalated plasma is used, $CaCl_2$ is also added to the plasma.
- Longer the clotting time, the smaller the prothrombin concentration. Prothrombin time in dogs: 9-12 sec. in sheep 13-25 sec.

CAUSES OF EXCESSIVE BLEEDING

- Vitamin K deficiency: This vitamin is required for hepatic synthesis of prothrombin factors V, VII, IX and X and deficiency leads to reduced synthesis of these coagulation factors resulting in excessive bleeding.
- Hepatitis or cirrhosis of liver: Results in less synthesis of coagulation factors derived from liver.
- Thrombocytopenia: Reduction in platelet count, which may occur in irradiation of bone marrow, drug sensitivity and some infections.
- Hereditary hemorrhagic disorders
    - *Hemophilia A* due to deficiency of factor VIII. It is congenital, seen in dogs and also reported in cat and horse.
    - *Hemophilia B:* caused by reduction in factor IX. Seen in dogs and it is inherited.
    - *Von Willebrand's disease*: Inherited diseases resembles hemophilia A caused by reduction in platelet function and factor VIII. Deficiency of factor IX is reported in cattle.

MODULE-11: BLOOD GROUPS

Learning objectives

- This module deals with
    - blood group systems and blood typing
    - blood group and its significance in animals

BLOOD GROUPS

- Blood is divided into different groups based on the type of antigen present in the cells. The blood group antigen are glycolipids or glycoproteins having a molecular weight of 20,000 to 30,000 present on the surface of the erythrocytes.

- The antigens are highly specific and the specificity of the antigens appears to depend upon carbohydrate portion of the molecule. Based on the antigenic type present on the surface of the RBCs cell membrane blood can be grouped into different groups or types.
- Landsteiner (1900) was the first to identify four blood groups, A, B, AB and O in human beings.
- About 70 blood groups have been identified in man, but the ABO, Rh, MNS, P and Lewis groups are best known. Among these five, ABO and Rh systems are widely used for blood grouping in human beings.

O-A-B BLOOD GROUPS

- There are two related antigens namely type A and type B are present on the surfaces of the RBCs among most of the population. These antigens are inherited and an individual may have either one or both nor do neither of them are present in their cells.
- The genes on two adjacent chromosomes, one gene on each chromosome determine the O-A- B blood groups. There may be any one type of the three types O, A, B and six possible combinations of genes are known. Type O is always functionless as there are no antigens present.
- The antigens (type A and type B) present on the surface of the RBCs are known as Agglutinogens and the plasma antibodies that cause agglutination are known as Agglutinins.
- Based on the presence or absence of agglutinogens in the RBCs blood is grouped.
    - Group A - when only type A antigen is present
    - Group B - when only type B antigen is present
    - Group AB - when type A and B antigens are present
    - Group O – when neither A nor B are present.

Blood Group	Antigens on RBCs	Antibodies in Sera	Genotypes
A	A	Anti-B	*AA or AO*
B	B	Anti-A	*BB or BO*
AB	A and B	Neither	*AB*
O	Neither	Anti-A and anti-B	*OO*

- During blood transfusion, if the blood between recipient and donor are mismatched, there will be reaction of antigen (agglutinogen) with the antibody (agglutinin) causing clumping or agglutination of the erythrocytes. These agglutinated RBCs are carried by the leukocytes to the RE system where they are lysed releasing Hb. Sometimes, mismatching of recipient and donor's blood leads to immediate hemolysis of the RBC's in the circulating blood, caused by the complement system.
- AB group of blood is designated as universal recipient
- O group of blood is commonly referred as universal donor

ANTIGEN ANTIBODY REACTION

- Generally, antibodies present in the donor's plasma, which would be active against the recipient's red cells, do not produce such a reaction because of the rapid dilution in the recipient's circulation.

- Serious problem of antigen-antibody reaction result from antibodies present in the recipients plasma reacting with the donor's RBCs. This agglutination of the donor's erythrocytes may produce systemic thrombosis in the blood vessels.

Blood groups of recipient (Antibody)	Blood groups of the donor(Antigen)			
	A	B	AB	O
A (Anti B)	--	+	+	--
B (Anti A)	+	--	+	--
AB	--	--	--	--
O (Anti A, B)	+	+	+	--

(+ Agglutination or clumping of RBCs and - No agglutination)

BLOOD TYPING

- Determination of the blood groups of the recipient and donor prior to transfusion is done by matching. RBCs are diluted with saline and one portion is mixed with anti A antibody and the other with anti B antibody. The mixture is observed under the microscope.
- If the RBCs have become clumped antigen antibody reaction is said to have taken place which indicates mismatching.

RBCs	Sera	
	Anti A	Anti B
A	+	-
B	-	+
AB	+	+
O	-	-

Rh SYSTEM

- Rh antigens are transmembrane proteins with loops exposed at the surface of red blood cells. They are named after the rhesus monkey in which they were first discovered.
- They are used for the transport of carbon dioxide and/or ammonia across the plasma membrane. In Rh system, spontaneous agglutinins do not occur. There are six Rh antigens (Rh factors) C, D, E, c, d and e. Of these six types D is widely prevalent and is considered to be more antigenic than others. The presence of D antigen indicates Rh^{+}.
- In this system there is no immediate reaction when blood transfusion is effected between Rh^{+} and Rh^{-} individuals. When Rh^{-} individual receives Rh^{+} blood, the development of antibody or agglutinin occurs only after 2 to 4 weeks.
- Hence, the transfusion reaction is usually delayed and mild. If the same person has subsequent blood transfusion with the same antigen, there will be enhanced antigen - antibody reaction.

ERYTHROBLASTOSIS FETALIS

- It is a disease of the fetus and newborn infants characterized by progressive agglutination and phagocytosis of RBC's. The mother is Rh^-, father Rh^+, the baby inherits Rh^+ from the father. Usually the Rh^- mother develops anti-Rh agglutinins only when the Rh $^+$ child develops by inheriting the Rh^+ factor from its father.
- The child's Rh^+ antigen enters the maternal system and causes development of Rh^+ antibodies. Placental diffusion of this antibody causes hemolytic conditions in the subsequent Rh^+ new born infants. This disease condition is characterised by varying degrees of anaemia and jaundice in the newborn infants depending upon antibody reaction by the mother.
- This condition can be prevented by passively immunising the mother against Rh^+ factor.

BLOOD GROUPS IN ANIMALS

- In animals, the antigens representing the blood group are not strongly antigenic and occurrence of the natural antibody in their blood is rare. However, naturally occurring antibodies to some red cell antigens can be found in normal animals that lack the respective antigens.
- In domestic animals, the initial transfusion of whole blood will not result in serious problem. However, subsequent transfusions with the similar isoantigen can cause enhanced antigen-antibody response, which may produce the clinical symptoms like muscular trembling, salivation, dyspnea, and hemoglobinuria.
- In dogs, the blood groups are known as DEA system. They include 1.1, 1.2, and 3-8. DEAs 1.1 and 1.2 are present in 60% of the canine populations and these dogs are considered as A positive and the others in which DEA 1.1 and 1.2 blood groups are not present as A negative. The A negative dogs do have antibodies against A positive blood.
- In horses eight blood *groups* A, C, D, K, P, O, T and U has been identified. Hemolytic icterus occurs in newborn foals due to their erythrocytic destruction by the isoantibodies from the colostrum. This condition is very common in blood containing antibody for A or C.
- In cattle more than sixty erythrocyte antigenic factors, which are divided into 11 groups. They are designated as A, B, C, F- v, J, L, M, N, S, Z and R'-S. Anti- J is the naturally occurring antibody.
- In sheep, there are seven antigenic groups A, B, C, D, M, R–O and X-Z of which anti- R is the naturally occurring antibody of the R - O antigen.
- In the goats, five blood group antigens have been identified, A, B, C, M and J.
- In pigs, thirteen blood group systems, A, B, C, E, F, G, H, I, J, K, L, M and N have been identified . `A' antigen possesses naturally occurring antibody.
- In cats, three blood groups have been identified A, B and AB. Type A is most common and they have antibodies against A isoantigens. Type AB is very rare in occurrence.

SIGNIFICANCE OF BLOOD GROUPS IN ANIMALS

- To identify monozygotic twins from dizygotic twins.
- Some blood group systems correlate with economic traits, eg: milk fat, milk yield. Hence, useful for selection of animals for breeding.
- To solve parentage problem if there is any dispute.

Precautions for blood transfusion in animals

- The blood with anti-isoantibody A in pig, J in cattle, R in sheep and A or C in horse should be avoided for blood transfusion.

MODULE-12: BLOOD VOLUME, PCV & ESR

Learning objectives

- This module deals with
  - volume of blood in various species
  - packed cell volume
  - erythrocytes sedimentation rate

BLOOD VOLUME

- It makes up about 6 to 8% of the body weight. Blood volume measurement plays an important role when blood transfusion is attempted.
- It is also important to interpret PCV, Hb, RBC, and hematological parameters. These values are altered when the blood volume changes e.g. hemoconcentration and hemodilution.
- Blood volume is influenced by body type, body size, age, sex, breed, nutrition, pregnancy, and lactation, physical and metabolic activities. Males show higher blood volume than females.
- Blood volume increases with pregnancy, muscular activity, stress, and high temperature, whereas starvation, hemorrhage, burns, dehydration, anemia and cold decrease the blood volume.

AVERAGE BLOOD VOLUME IN VARIOUS SPECIES

Blood Volume (ml)	Cattle	Horse	Sheep	Goat	Pig	Dog	Cat	Poultry
Blood volume (ml)	40,000	40,000	3,200	2,400	8,000	1,600	240	160
% of body wt.	8	10	8	6	-	7	-	6.5

MEASUREMENT OF BLOOD VOLUME

Principle

- The volume of any fluid compartment of the body can be measured by placing a substance in the compartment and allowing it to disperse through the fluid. The extent to which the substance gets diluted in the fluid is measured.
- The substance used for determining blood volume must be capable of dispersing throughout the blood easily and must remain in the circulatory system for a longer period of time till the measurements are made.
- Blood volume may be measured indirectly by two methods
    - Plasma volume method
    - Erythrocyte volume method

Plasma volume method

- Plasma volume can be measured by dye dilution or by radioisotope. Blood sample from the experimental animal is collected and the plasma of the pre injected anticoagulant-added blood serves as a blank.
- Known quantity of Evan's blue dye/ (T-1824), or radioisotope ^{131}I, when injected, combines with the plasma proteins and disperses throughout the circulatory system in about 10 minutes.
- Blood samples are collected sequentially at 5 minutes interval for the next 15 to 30 minutes. These blood samples are centrifuged to get RBC free plasma. The concentrations of the dye or the radioactivity of isotope in the plasma samples can be measured *spectrophotometrically* at 620 nm or by the *scintillation counter* respectively.

Erythrocyte volume method

- This can be determined by radio active ^{32}P, ^{59}Fe and ^{51}Cr. Blood sample from the experimental animal is collected to get plasma free erythrocytes.
- Small quantity of ^{51}Cr is mixed with these RBCs which are then incubated at 36 °C for 30 minutes to activate the binding process of ^{51}Cr with the RBCs. Wash the erythrocytes with saline to remove the free ^{51}Cr. These radioactive erythrocytes are then injected into the circulation of the experimental animal. After proper mixing of these RBCs in the circulation, blood samples are collected after 10 minutes.
- RBCs from the blood samples are separated and the radioactivity of pre and post injected RBC samples are determined by scintillation counter.

$$\text{Erythrocyte volume (ml)} = \frac{\text{Quantity of radioactivity injected}}{\text{Radioactivity / ml of RBCs}}$$

$$\text{Blood volume (ml)} = \frac{\text{Plasma volume} \times 100}{100 - \text{PCV} \times 0.96} \quad \text{(or)} \quad \frac{\text{Erythrocyte volume} \times 100}{\text{PCV}}$$

- The trapped plasma value may interfere with the PCV. Hence the correction factor for trapped plasma is introduced as 0.96 for blood volume determination.
- Starvation, haemorrhage, hot environment and water deprivation reduce the blood volume.

PACKED CELL VOLUME

Packed cell volume (PCV) or Hematocrit (%)

- It is percent volume of packed blood cells present in whole blood after centrifugation at 3000 rpm for 30 minutes. Hematocrit is measured by two methods.

Wintrobe hematocrit (macro method)

- Wintrobe tubes have uniform 3 mm bore, calibrated by 10 cm scales with millimetre divisions. The wintrobe pipette with a long but narrow delivery tube is used to fill about 1 ml of blood into the wintrobe tube without air bubbles.
- Horse and dog blood require a relative centrifugal force of 2,260 G (generally 3000 rpm) for 30 minutes, while it is for 60 minutes in the case of cattle and pig blood. The blood of sheep and goat require higher relative centrifugal force than 2,260 G.

True PCV = Venous PCV **x** 0.96 (correction factor for trapped plasma)

- The RBCs settle to the bottom of the hematocrit tube leaving the plasma above the packed erythrocytes. A thin white or yellow layer called the buffy coat occurs in between these two and it indicates the WBC or the leukocytes.
- The amount of trapped plasma in the erythrocyte column varies with PCV, size of erythrocytes and the degree of rouleaux formation. Canine blood is characterised by large blood cells and greater tendency for rouleaux formation. Similarly equine blood is characterised by extreme rouleaux formation, hence less plasma is trapped.

Microhematocrit method

- PCV may be estimated by blood filled capillary tubes (32 mm x 0.8 mm) centrifuged at 14,000 G for 2 minutes. In general, the PCV is approximately three times the Hb concentration. PCV values ranges between 38 to 45% in most of the domestic animals.

Factors influencing PCV

- High temperature, muscular exercise, stress, pregnancy, hemorrhage, dehydration and burns, anemia, polycythemia and leukemia.

NORMAL PCV VALUES IN DOMESTIC ANIMALS

Species	PCV (%)
Dog	37-55 (45)

Cat	24-45 (37)
Cow	24-46 (35)
Sheep	24-50 (38)
Goat	19-38 (28)
Horse	32-52 (42)
Pig	32-50 (42)

ERYTHROCYTE SEDIMENTATION RATE (ESR)

- It is a measure of the settlement of the erythrocytes in millimetre for a given period usually 30 minutes or one hour when citrated blood filled standard hematocrit tube is placed in an absolutely vertical position.
- The length of storage of the blood sample and temperature of storage may influence ESR. The ESR test is generally done as part of complete hematological investigations.
- Suspension stability or sedimentation of RBCs in the blood is influenced by RBCs and plasma characteristics. Rouleaux formation or the agglutination of RBCs influences the ESR.
- Increasing concentration of fibrinogen or the globulin hastens agglutinations of RBCs. ESR is positively influenced by rouleaux formation and plasma contents of fibrinogen, α_2 globulins and gamma globulins, while it is negatively influenced by reticulocytes and plasma content of the albumin.
- Fibrinogen and a globulin are commonly elevated in variety of inflammatory diseases e.g., pleurisy, pericarditis, peritonitis, whereas hemoconcentration and glomerulonephritis may adversely affects the ESR by altering the influence of albumin fraction.
- The speed of settling of RBCs is inversely proportional to the number of erythrocytes in the given sample. Among the domestic animals, the erythrocytes of ruminants show little or low natural tendency to form rouleaux, whereas the equine blood shows high tendency to form rouleaux formation.
- Increased ESR is very common in increased cholesterol: lecithin ratio, pregnancy, acute general infections (septicaemia), and rheumatic fever, TB, arthritis, toxaemia, malignant tumours, hypothyroidism and glomerulonephritis.
- Decreased ESR is seen in afibrinogenemia, polycythemia, allergy etc.

ESR value (mm)

ESR (mm)	Cattle	Sheep	Goat	Dog	Cat	Horse	Chicken
30 minutes	--	--	--	1-5	--	15-38	0-1
60 minutes	--	--	--	6-10	7–27	--	1 – 3

- The ESR merely helps in evaluating the health status of animals, but it is not helpful in diagnosing any specific diseases

Factors influencing ESR

- Size and shape of erythrocytes, specific gravity of erythrocytes, specific gravity of plasma, viscosity of plasma, fibrinogen and globulin content, temperature.

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MODULE-13: HEART - STRUCTURE, CONDUCTION SYSTEM AND TRANSMISSION OF IMPULSE

Learning objectives

- This module deals with
  - structure of the heart
  - working mechanisms of the heart

ANATOMY OF THE HEART

- Heart is situated in the middle mediastinal space. It has two anterior chambers, the atria and two posterior chambers, the ventricles. The heart is separated into left and right sides by a septum. The atria and ventricles communicate with each other through the atrio-ventricular orifices, which are guarded by the AV valves, the bicuspid or mitral valve and tricuspid valve on the left and right sides, respectively.
- Chorda tendinae are the elastic structures attached to the valves with the internal muscular projections of the heart known as the papillae and prevent the evertion of the valves during ventricular contraction.
- AV valves prevent the back flow of blood into the atria during ventricular contraction (systole). Of the four chambers, the left ventricular wall is much thicker to provide effective force during its contraction to pump its contents throughout the body.
- The venous system provides blood to right atrium of the heart and the blood is pumped from the right ventricle to the lung; hence, it is referred to as venous or pulmonary side.
- The left atrium of the heart receives the oxygenated blood from the lungs and the left ventricle pumps it to the peripheral systemic organ which is known as systemic or arterial side. Blood vessels between aorta and vena cava supplying blood to all organs except to the lungs are called systemic circulation.
- The blood vessels of lungs constitute pulmonary circulation. Pulmonary circulation and heart are collectively termed as central circulation. In the orifices of the aorta and the pulmonary artery the Semilunar valves are present which prevent the back flow of blood from aorta and pulmonary artery into the ventricles during ventricular relaxation (diastole).
- The major function of the cardio vascular system is to transport oxygen, nutrients, water electrolytes and hormones to the tissues and remove the metabolic wastes such as CO_2, urea, creatinine etc from tissues to the lung and excretory organ like kidney

CARDIAC MUSCLE

- Heart is composed of three major types of cardiac muscle namely:
  - Atrial,
  - Ventricular and
  - Special excitatory and conductive muscle fibres.
- The atrial and ventricular muscle fibres contract similarly to skeletal muscle fibres except the duration of contraction is longer. The excitatory and conductive muscle fibres contract only feebly but exhibit rhythmicity and varying rates of conduction providing an excitatory system that controls rhythmical beating of the heart.
- Cardiac muscle fibres are arranged in a lattice network, fibres divide, recombine and spread again. The cardiac muscle fibres contain intercalated discs that contain many gap junctions through which action potential can spread from one to other cardiac muscle cell very easily.
- When one of the cardiac muscle cells is excited, and the action potential spreads to all of them, hence the cardiac muscle obey all or none law.
- The heart is composed of two separate functional syncytia, the atrial and the ventricular walls. They are separated from each other by fibrous tissue surrounding the valves between atria and ventricles, but are connected to each other by specialised conduction system fibres. This type of two functional syncytia allows the atria to contract a short time before ventricular contraction which is important for effectiveness for pumping of the heart.

CONDUCTION SYSTEM OF THE HEART

- The specialized excitatory and conduction fibres show feeble contractions only because of very few contractile fibres, but they conduct impulses very rapidly through out the heart.
- In mammals, S.A. node (Sino auricular node) is the specialized structure of the cardiac muscle located at the junction of the right atrium and cranial vena cava. It has an inherent property of generating its own action potential at periodical interval because of low resting membrane potential (- 55 mV).
- The SA node normally controls the rate of the heart; hence it is called as pace maker. The pacemaker dominates the normal rate and rhythm of the heart. In sub mammalian species, frog, this function is taken up by sinus venosus. This rhythm is called as ectopic rhythm.
- Most of the cardiac fibres including the conduction system have the ability of self-excitation and can produce automatic rhythmical contractions. In some pathological conditions of the mammalian heart, the excitatory impulses originate outside the S. A. node referred as ectopic foci in which the heart rate will be less than normal.
- S.A. node spreads its impulse through atrial muscular wall and interatrial bundles to A.V. node (atrio -ventricular node) which lies in the septal walls of the right atrium cranio dorsal to tricuspid valve.
- It conducts the action potentials to common bundle of His or AV bundle which then runs into the ventricular septum where it divides into a right and left bundle branches that run underneath the septal endocardium. At the ventricular apex, these branches finally terminate as purkinje fibres form a net work of conductive system in the ventricular muscle.
- The AV node and the AV bundle is the only route for the conduction of impulse from atria to ventricles. In the conduction system, the AV node shows a delay in the propagation of the action potential, the nodal delay for a period of 50 - 150 ms.

BASIC MECHANISMS OF MEMBRANE POTENTIAL

Three major factors cause the membrane potential.

- Differential permeability of the membrane to diffusion of ions
  - During resting state the cell membrane is 50 to100 times more permeable to K^+ ions out side the cell membrane through the *leak channels* than Na^+ ions due to their concentration gradient. This contributes accumulation of positive charges immediately outside the membrane.
  - The resting membrane is almost completely impermeable /very slightly permeable to Na^+ ions.
- The Na^+ - K^+ electrogenic pump
  - It generates positive membrane potential out side the cell by actively pumping 3 Na^+ ions out of the cell for every 2 K^+ ions pumped into the cell against their concentration gradient .
- Trapping of negatively charged anions inside the cell
  - Many intracellular anions are large molecules proteins, organic SO_4 and PO_4 are trapped within the cell and are attracted to the inner surface of the cell membrane generate negative electrical potential inside the cell.
  - These three factors are the primary cause of membrane potential. The magnitude of this potential produced by diffusion of ions can be predicted by the *Nernst equation*.

EVENTS OF ACTION POTENTIAL

- The action potential is caused by a sequence of changes or events occurring in the membrane permeability to Na^+ and K^+ ions. When an excitable cell is stimulated the cell membrane permits the inflow of Na^+ ions into the cell to generate the action potential.
- Action potential is the rapid changes in the membrane potential from its normal negativity to positive potential inside the cell membrane which last for few milliseconds and then returns back to its original resting negative potential level.
- *Polarised membrane* is the resting cell membrane with a normal negative resting membrane potential.
- *Depolarization stage*:
  - It is the *first event* of action potential and is characterized by rapid increase in the permeability to Na^+ ions (5000 folds) to interior of the cell generating more positive electrical potential inside of the cell.
  - This is followed by a gradual inactivation of Na^+ channels (closure) that occur within another few milliseconds and the membrane becomes impermeable to Na^+ ions. It is associated with gradual opening of***voltage gated K*$^+$ *channels*** to allow K ions outflow to the exterior of the cell membrane. The potential inside the cell is re-established to its normal resting level (- 90mV). This stage is called as the***repolarisation*** *stage*
  - Higher concentration of K^+ ions in the exterior of the cell towards the end of the action potential continues for a short period creates more negativity inside referred to as ***hyper-polarised*** *state*. At this state, re-excitation of the cell will not occur.
- The *final event* is characterized by ***electrogenic pump mechanism***, which aids in the transport of three Na^+ ions to the exterior for every two K^+ ions to interior of the cell and create the normal resting potential (- 90 mV) on the inside of the cell membrane.

CHANNEL SYSTEMS OF CELL MEMBRANE

- Na – K leakage channels
- Voltage gated Na channels and K channels

Na – K leakage channels

- These channels are 100 times more permeable to K^+ ions than Na^+ ions and are exceedingly important in determining the level of normal resting membrane potential.

Voltage - gated channels of the cell membrane

- The *voltage-gated Na^+ channel* causes both depolarisation and repolarisation of the nerve/muscle membrane during the action potential.
- The *voltage-gated K^+ channel* also plays an important role in establishing the repolarisation of the membrane. These two voltage-gated channels are present in the cell membrane.
    - Voltage - gated Na^+ channel
        - The voltage gated Na^+ channels have two gates, the ***external gate or activation gate which*** opens to outside of the cell. The other gate is at the interior end and opens to inside of the cell referred as***internal gate or inactivation gate.***
        - The activation gate is closed during resting stage (- 90 mV), while the inactivation gate is opened. This prevents free passage of Na^+ ions from outside to interior of the cell.
        - During depolarisation stage, the resting membrane potential drops from – 90 mV or to - 70 to - 50 mV causes opening of the activation gate due to conformational changes results in increased Na^+ ion permeability as much as 500 to 5000 folds into the cell through the channel system.
        - A gradual increase to positivity following the opening of the activation gate also closes the inactivation gate comparatively at a slow speed.
        - The time lapse between the activation and inactivation of the channels causes the passage of Na^+ ions to the interior of the cell for few milliseconds.
        - During repolarisation stage, the inactivation gate is completely closed and prevents Na^+ ion entry from outside to the interior of the cell membrane. The inactivation gate will not reopen until the disturbed membrane potential returns nearly to the original resting membrane potential level of -70 to- 90 mV.
    - Voltage - gated potassium channel
        - This channel has only one gate at the interior of the membrane. It may either close or open to the interior of the cell.
        - During resting stage this gate is *inactivated* and prevents the passage of K^+ ion to the exterior of the cell through this channel system. When the electrical potential drops towards zero, Na^+ channels get inactivated causes the activation of K^+ channels to allow increased K^+ ions diffusion to outside of the cells.
    -

ELECTROCHEMICAL CHANGES DURING ACTION POTENTIAL

Spike potential *(over shoot)*

- It is the steep change in the negativity of the cell from - 90 mV to more positivity of + 40 mV in the membrane potential due to rapid increase in the permeability of the Na^+ ions to the interior of the cell.

Positive after potential

- During repolarisation, the membrane potential drops little more negative than the normal resting value of – 90 mV due to excess of K^+ ion diffusion to out side the cell. This is caused by the prolonged opening of K^+ channels for several milliseconds even after repolarisation.

Resting membrane potential in cardiac muscle

- The resting membrane potential in cardiac muscle is – 90 mV, but has long action potential (250 msec).
  - S.A. node = - 50 to - 55 mV
  - Cardiac muscle = - 85 to - 95 mV
  - Conduction system = - 90 to -100 mV
  - Ventricle muscle = -100 to -105 mV

ACTION POTENTIAL IN CARDIAC MUSCLE

- Cardiac muscle has an inherent property of generating its own action potentials rhythmically, independent of nerve stimulation. This occurs in the peacemaker cells of the S.A. node, which depolarises faster than any other parts of the heart.
- The onset of action potential in cardiac muscle is slower but prolonged than skeletal muscle which lasts for 150 msec. in atria and 300 m.sec. in ventricle. In cardiac muscle, repolarisation does not occur immediately after depolarisation The positivity generated as the spike potential by the depolarisation remains as a plateau near the peak. This plateau lasts for a few-hundred m.sec prolongs the contraction of the cardiac muscle.
- *The reason for the prolonged action potential* in cardiac muscle cells is due to the presence of two separate channel systems
  - Voltage activated Na channel (fast channel)
  - Voltage activated Na - Ca channel (slow channel)
- Activation of the fast Na channels causes the spike potential of the action potential, whereas the slow channel prolongs the passage of Ca^{++} and Na^+ ions into the interior of the cell, thus establishes the plateau in the action potential.
- The inflow of Ca^{++} ions in to the cardiac muscle cells decreases K^+ permeability by about 5 fold. This delays the K^+ ion permeability to outside which in turn delays the re-polarization process of the action potential in cardiac muscle. This prolonged action potential (250 – 300 m.sec) provides longer contraction period in the cardiac muscle cells than the skeletal muscles. Hence, cardiac muscles do not functionally tetanized.

ACTION POTENTIAL IN PACEMAKER CELL

- In the cells of SA node, the resting potential is -55 mv only due to naturally leaky nature of these cells to Na^+ ions cause spontaneous depolarisation and the resting potential gradually rise up to - 40 mv. At this voltage, the fast Na^+ and Na^+- Ca^{++} channels are opened leading to development of self-excitation and heart beat. This is followed by opening of the K^+ channels causes diffusion of greater number K^+ ions to out side the cell causing the hyperpolarization (-55 to -60 mv).
- After this event, the K^+ channels close and Na^+ ion leaks to the interior of the cells to repeat the cycle.
- In cardiac muscles, the action potential is prolonged and it is referred as *plateau of action potential*.

VELOCITY OF CONDUCTION

Atrial muscles	0.05 - 0.1 m/sec
A.V. node	0.02 m/sec
Bundle of His and Purkinje fibres	1.5-4.0 m/sec
Cardiac muscles	0.3-0.5 m/sec

- The right atrium begins to depolarize about 0.01 sec before the left atrium. Conduction velocity is fastest in Purkinje fibres but slowest in A.V. nodal fibres.
- The A.V. node delays allow the atria to discharge their blood into the ventricles before ventricular systole. Of the two syncytia of the heart, the atrial syncytia cause the atrial contractions a short time ahead of the ventricular contraction.

NORMAL HEART RATE IN ANIMALS

-

Species	Cow	Horse	Sheep	Goat	Pig	Dog	Cat
Heart rate/min	48-84	28-40	70-80	70-80	70-120	70-120	120-140

PROPERTIES OF MYOCARDIUM

- Rhythmicity: The cardiac muscles have an inherent ability to generate their own impulses spontaneously at regular intervals known as rhythmicity without the influence of nerves and hormones,. This property can be demonstrated by coronary perfusion of the isolated mammalian heart using *Ringer's solution*.
- Contractility: Contraction of cardiac muscle differs from other contractile tissues in the following aspects:
- All or none principle: If at all the cardiac muscle responds to a stimulation, it contracts with maximum strength (i.e. all contractions of the cardiac muscle are maximum) when stimulated with either minimum or maximum strength of stimulus under the prevailing physical and chemical environment. This is due to the syncytial nature of the cardiac muscle.

- Staircase phenomenon or Treppe: When an effective stimulus is applied repeatedly with very short interval (1-2 seconds), the cardiac muscle responds with increasing degree of strength for the first 3 to 5 stimuli, after that the response becomes constant. When the interval between two stimuli of same strength is very short, the physico chemical changes known as beneficial effects, occurring during the first response persist and facilitate greater response for the second stimuli.
- Refractory period: This period of unresponsiveness of cardiac muscle when it is responding to a stimulus, it may not show another response for a subsequent stimulation for a shorter period. Following first stimulation, there is a total absence of response for a very brief period, called as *absolute refractory period*. This is followed by a short period of partial responsiveness referred as relative refractory period, during which the cardiac muscle can be stimulated by a stronger stimuli.
    - The cardiac muscle is in absolute refractory period (atria 0.15 sec., ventricle 0.25 sec.) during systole and in the relative refractory period (atria 0.03 sec, ventricles 0.05 sec) during early diastole.
    - The refractory period is *longer in cardiac muscle* than skeletal muscles, hence cardiac muscle can not be tetanized by a series of rapid stimuli.

MODULE-14: CARDIAC CYCLE AND HEART SOUNDS

Learning objectives

- This module deals with
    - normal and abnormal heart sounds in animals
    - pressure and volume changes in the atria and ventricles of the heart

CARDIAC CYCLE

- It is defined as sequence of events such as pressure and volume changes, cardiac sounds and changes in the electrical potential occurring during the period between two successive ventricular contractions.
- It is characterised by *one complete contraction (systole) and relaxation (diastole)* and indicates a complete heart beat.
- These periods are not equal in duration, when *heart rate is 75/minute, one cardiac cycle lasts for about 0.8 sec. in which* ventricular systole is 0.3 sec. and diastole is 0.5 sec.
- In atria, the systole lasts for 0.1 sec. and diastole 0.7 sec. During ventricular systole, the atria are not contracting and during atrial systole, ventricles are in diastole.

PRESSURE CHANGES IN THE ATRIA

- The intraatrial pressure records three major positive waves.
- 'a' wave: Results from atrial systole. Atrial systole causes an increase in the atrial pressure resulting in the opening of the AV valves and blood flows into the ventricles.
- 'c' wave: It results due to bulging of the AV valves into the atria during ventricular systole. As ventricular systole is initiated, the AV valves close and this increases the ventricular pressure causing the AV valves to bulge into the atria. This causes a second increase in

atrial pressure. As ventricular ejection takes place there will be a drop in the atrial pressure.

- 'v' wave: It results due to continuous inflow of blood into the atria during ventricular ejection. When blood flows into the atria from the vena cava of pulmonary vein, there is dilatation of the atrial walls and intraatrial pressure increases producing 'v' wave during atrial diastole.

PRESSURE AND VOLUME CHANGES IN THE VENTRICLES

- Isovolumetric contraction
  - Ventricular contraction starts at the peak of R wave in the ECG. This periods marks the onset of ventricular contraction. The ventricular pressure exceeds than that of the atrial pressure causing closure of AV valves. The volume of the ventricles do not change and the pressure rises rapidly. The ventricles remain as a closed chamber whereas AV and SL valves are closed.
- Maximum Ejection Phase
  - Pressure inside the ventricles exceeds the aortic pressure causing opening of the SL valves and ejection of ventricular blood rapidly into the pulmonary artery. It lasts until the peak of the arterial pressure curve.
- Reduced Ejection
  - During the onset of this phase pressure reaches a maximum. The pressure in the left ventricle falls below that of the aortic pressure and there is a decline in the outflow of blood from the ventricles. There is a progressive decrease in ventricular volume.
- Protodiastole
  - This marks the onset of ventricular relaxation and there is a rapid drop in ventricular pressure. The pressure falls below that of the aorta and pulmonary artery, while the atrial pressure gradually increases.
- Isovolumetric Relaxation
  - The AV valves and SL valves closes and these is no blood flow from the atria to the ventricles. Ventricles remain as closed chamber and there is myocardial relaxation with no increase in fibre length. When the intraventricular pressures fall below that of atrial pressure, AV valves open, at the end of this period. During this period ventricles remain partially filled with blood and they eject only 50-65% of the diastolic volume of blood.
- Rapid filling
  - It begins with the opening of AV valves as the atrial pressure rises causing blood to flow rapidly from the atria to the ventricles.
- Reduced filling (diastasis)
  - There is slow filling of blood from the atria to the ventricles. At the end of this period, there is onset of atrial contraction.
- Atrial systole
  - It is marked by the beginning of artial contraction (a) wave in which the two chambers are in communication. The atrial systole pressure wave peaks and begins to decline by the onset of ventricular isovolumetric contraction, thereby completing one cardiac cycle. During atrial systole 70-80% of the ventricles get filled but during rapid heart rate ventricular filling is reduced due to shorter time for diastolic filling. During exercise, atrial systole forces an increased 20-30% of the atrial blood into the ventricles thereby increases the ventricular pressure and volume.

- Pressure changes in the right ventricles are qualitatively similar but quantitatively lower than that of left ventricle. The right ventricle functions as volume pump and the left ventricle as pressure pump.

CARDIAC SOUNDS

- These are the distinct sounds produced during systole (S_1) and diastole (S_2) of the cardiac cycle, which can be recorded by the phonocardiogram.
- The first heart sound "lub", is systolic sound indicates the firm closure of AV valves, occur during ventricular systole. It is associated with the vibration of the valves (valvular factor) and also by the vibrations of the chordae tendinae and contracting ventricular muscle fibres (muscle factors). This sound is louder, low pitched but longer duration.
- The second heart sound "dub" is the diastolic sound developed during the closure of the aortic and pulmonary valves at protodiastole phase of the cardiac cycle.
- In addition to the above two normal sounds, occasional sounds like third heart sound (S_3) is produced early in diastole at the end of rapid ventricular filling due to sudden tension of chordae tendinae. The S_4 sound also referred as atrial sound, is due to the contraction of the atrial muscles which causes tension of the A.V. valves. These two sounds are very common in horses but pathological in dogs and cats. Usually in other animals the S_3 sound indicates congestive heart failure whereas S_4 indicates some degree of A.V. bundle block.

CARDIAC MURMURS

- Prolonged sounds of the heart occurring during normally silent intervals of cardiac cycle are referred as murmurs or adventitious heart sounds, which is due to valvular insufficiency or stenosis (narrowing) of the orifices. There are two types of cardiac murmurs.
- Systolic murmurs:
  - It occurs due to improper or defective closure of the mitral or tricuspid valves or due to stenosis of pulmonary or aortic valves.
- Diastolic murmurs:
  - It is caused by insufficiency of pulmonary or aortic valves or by stenosis of AV valves.
  - Endocarditis and erysepalus infection (in hogs) cause valvular insufficiency, which is very similar to that of rheumatic fever in man.

MODULE-15: CARDIAC OUTPUT AND ITS REGULATION

Learning objectives

- This module deals with
  - cardiac outputs - its determination and regulation
  - regulation of heart rate
  - metabolism and energetics of working myocardial cells

STROKE VOLUME

- The definite volume of blood pumped into the aorta / pulmonary trunk by each ventricular contraction (left / right) is referred to as stroke or pulse volume or the systolic discharge.

- If the stroke volume is expressed based on body weight it is known as stroke index.

$$\text{Stroke index} = \frac{\text{Stroke volume (ml)}}{\text{Body weight (kg)}}$$

- A portion of the blood is left in the ventricles even after the ventricular systole, which is known as end systolic volume. Similarly, the volume of blood present in the ventricles at the end of the diastole is referred to as end diastolic volume.

CARDIAC OUTPUT OR MINUTE VOLUME

- It is the volume of blood ejected by either the left or the right ventricles into the greater vessels, the aorta or pulmonary artery in a minute.

$$\text{Cardiac output (L / min)} = \text{Stroke volume x pulse rate}$$

- In most animals, the cardiac output is 10% of body weight in kgs. Cardiac out put of right ventricle will be normally equal to that of left ventricle.
- If this minute volume is expressed on the basis of body surface it is referred to as cardiac index (L / m^2 / min).

$$\text{Cardiac index} = \frac{\text{Minute volume}}{\text{Surface area of the body}}$$

- Venous return is the quantity of blood flowing from the great veins, vena cava in to the right atrium each minute.

DETERMINATION OF CARDIAC OUTPUT

Cardiac output can be measured by

- Direct Fick method
- Indicator dilution method
- Flowmeter method

Direct Fick method

Fick's principle

- The amount of substance taken up by the circulation per unit time equals the arterial level of the substance minus the venous level times the blood flow (Cardiac output). According to Fick's principle, cardiac output can be determined by measuring oxygen consumption/ minute and arterio-venous oxygen difference.

- Samples of blood are collected from artery and mixed venous blood sample drawn from right ventricle or pulmonary artery using catheter. Oxygen consumption/ minute is determined by spirometry.
- Amount of O_2 present in both arterial and venous samples are determined and the difference in the O_2 content, the atrio-venous O_2 difference is expressed as per cent volume.

$$\text{Cardiac output (L)} = \frac{\text{O2 consumption (ml) / minute}}{\text{Mean arterio-venous o2 difference} \;/\; \text{100 ml blood}}$$

Indicator dilution method

- This method is based on the principle that if a known quantity of a substance is mixed with an unknown volume of fluid the volume can be determined from the concentration of the substance in the fluid. The volume equals the amount of substance (in milligrams) divided by concentration of substance (in milligrams/litre).
- A known amount of indicator (Cardio green dye) is injected into a vein; arterial blood is sampled continually or at short intervals, frequently. The average concentration of dye in arterial blood is determined by the dye dilution curve, which is drawn using the sampling time interval and the log of dye concentration for each time sample. The duration of the curve for one complete circulation of the dye is determined from the curve.

$$\text{Cardiac output} = \frac{\text{Amount of dye injected x 60}}{\text{(Mean concentration of the dye / L) x Duration of curve}}$$

Flowmeter method

Magnetic flowmeter method

- The electromagnetic flowmeter used measures the electromotive force generated when the blood flowing within a vessel of known diameter passes through a magnetic field at right angles to the magnetic lines of force. Cardiac output is determined by placing the flowmeter probe around the root of aorta.

FACTORS INFLUENCING CARDIAC OUTPUT

- Muscular exercise (4-6 times increase), pregnancy, hypoxia, active digestion, stress and increased body temperature

Circulation time

- It is the time required for one complete circulation.

$$\text{Circulation time} = \frac{\text{Total blood volume (ml)}}{\text{Minute volume (ml)}}$$

- This can be measured either by injecting dyes like ferrocyanide or fluorescine or injecting radioactive chemicals.

Cardiac reserve

- It is the maximum percentage that the cardiac output can increase above normal. Cardiac reserve can be measured by exercise test.
- It is an adaptive mechanism of the heart by which cardiac output can be increased to maintain a balance between right atrial input and left ventricular output.
- This is achieved by
    - Increase in stroke volume
    - Increase in heart rate
    - Hypertrophy of the left ventricle
- During exercise the cardiac output can rise up to 400% above normal. If cardiac reserve is inadequate congestive heart failure or cardiac decompensation results which causes stasis of blood in the pulmonary system and right atrium.

REGULATION OF CARDIAC OUTPUT

- Heart functions (cardiac output, heart rate) can be regulated by
    - Intrinsic mechanisms, operating within the myocardium itself and
    - Extrinsic mechanisms (by factors outside the heart).

INTRINSIC REGULATION OF HEART

Frank's Starling Mechanism/Heterometric Regulation

- Regulation of the cardiac function is independent of the nervous or humoral control. This was proved by the Starling's experiment using Starling's heart lung preparation i.e., isolating the heart from the body so as to remove humoral and neural influences. The heart is perfused through the aorta with an oxygenated solution containing nutrients essential to maintain normal cardiac function. In this preparation, contractility of the heart is studied by allowing the isolated heart to do work under various degrees of pressure and load made in the pulmonary artery or aorta. Starling's law of heart was derived from these studies and was initially known as Heterometric autoregulation and it states that the force or contraction is a function of the initial length of the muscle fibres i.e upto a physiological limit, greater the initial length, greater is the force of contraction.
- An increase in the venous return to the heart increases the input of blood into the ventricles which stretches the ventricular wall and induces a stronger contraction during systole. According to this law the heart makes adjustments to load upon the ventricles during each beat without any neural or humoral influences.
- Preload is the amount of stretch on the heart prior to contracttion. The load is directly proportional to the volume of blood in the ventricles or end diastolic volume. Greater ventricular filling during diastole, stretches the muscle fibres, causes increased fibre

length and a greater amount of Ca^{++} release during systole resulting in increased force of contraction and stroke volume.

- After Load is the pressure that must be exceeded by the ventricles before blood can be ejected through the semilunar valves is called as after load. The resistance is determined by the diameter of the arterioles and pre-capillary sphincters which is controlled by the sympathetic system which in turn, controls the tone of the arteriole muscles as well as the diameter of the blood vessels. Any factor that increases after load, will increase end systolic volume and decrease stroke volume.

Homeometric autoregulation

- This type of regulation occurs when aortic blood pressure increases due to increase in cardiac output or increase in peripheral resistance to blood flow. When venous return is normal but the arterial pressure is increased by peripheral resistance, causes a reduction in the ventricular discharge for few beats and accumulation of ventricular blood volume. This causes subendocardial ischemia resulting in reduced contractile strength. Subsequently, the subendocardial ischemia is relieved by vascular autoregulation, the contractile strength increases without changing the muscle fibre length to pump normal blood volume against increased arterial resistance known as *homeometric autoregulation* and the ventricular volume returns to normal. The significance of homeometric regulation is to allow the ventricles to eject the same stroke volume against varying degrees of arterial resistance without an increase in end diastolic pressure or increase in myocardial fibre length during diastole.
- Cardiac output will change to match changing metabolic demands of the body. The outputs of both ventricles must be identical, and also equal to the venous return of blood from the body. During exercise of the muscles, the increased blood flow through the blood vessels is due to increased metabolism. This causes increased venous return and preload to right ventricular and pulmonary circulation. Consequently, more blood is delivered to the left ventricle and cardiac output increases. There will also be increased contractility and heart rate from the sympathetic activity associated with exercise to meet tissue requirements.

EXTRINSIC REGULATION OF HEART

- Heart regulates its cardiac output based on the functional blood requirement of different parts of the body by adjusting its rate. Heart rate is directly proportional to metabolic activity, but inversely proportional to the size of the animal. Large animals have a slower heart rate than smaller ones.
- Athletic animals (Hare) and animals trained for exercise (Greyhound) has slower heart rate than rabbit or untrained animals of the same species.
- Heart rate is also slow in male and adult animals than in female and young ones.
- Extrinsic regulation of heart may be achieved by two means:
    - Nervous regulation and
    - Chemical regulation

NERVOUS REGULATION OF HEART RATE

- Sympathetic and parasympathetic divisions of ANS play a key role in the regulation of heart rate.
- The nerves regulate to a larger extent the rate rather than the force of contraction either directly or indirectly.

- The two atria are especially well supplied with large number of both sympathetic and parasympathetic nerves, but the ventricles are supplied mainly by sympathetic nerves and fewer parasympathetic fibres.

DIRECT NERVOUS CONTROL

- Cardiac activity is regulated by cardio accelerator (augmentor) nerves and cardio inhibitory nerves.

Cardio accelerator nerve fibres

- Sympathetic nerves to the heart originate as post ganglionic fibres from the inferior, middle and superior cervical and first 4-5 thoracic ganglia, run along with vagus to form the cardiac nerves and plexus which pass to the heart along with great veins and arteries.
- Sympathetic division of the ANS provides two sets of accelerator fibres, the vago-sympathetic fibres**,** originating from the cervical ganglion and run along the vagal fibres and the cardio sympathetic fibres from the 2, 3, 4, 5^{th} thoracic nerves which function in emergency. Neurotransmitter of the sympathetic system is nor-epinephrine, which mainly act through b_1-receptors.
- Sympathetic fibres supply the atria, S.A. node, A.V. node and the ventricles stimulation of which causes increase heart rate and force of contraction referred to as positive chronotropic and positive inotrophic effects, respectively.

Cardio inhibitory nerve fibres

- Vagus, the X^{th} cranial nerve is the parasympathetic nerve originates from the dorsal nucleus of the medulla, form cardiac plexus with sympathetic nerves of the cervical ganglia. Vagus descends along with sympathetic fibres and terminates on the S.A. node in mammals and ventricles in amphibians.
- In mammals, vagus supplies fibres to the S.A. node, atrial muscles, A.V. node and A.V. bundle. The vagus nerve on stimulation produces inhibitory effect on heart resulting in negative chronotropic and negative inotropic effects. The inhibitory effect is more by right vagus than the left because SA node receives more fibres from the right vagus.
- Stimulation of vagus results in complete inhibition of heart beat (vagal inhibition) for a short duration. Following this the ventricles, start weak contractions called the vagal escape due to the shifting of the pacemaker from S.A. node to ventricular site, the ectopic foci**.**
- Principally, parasympathetic stimulation causes a marked decrease in heart rate and a slight decrease in contractility. Both the sympathetic and parasympathetic fibres of ANS constantly discharge impulses to the heart to keep the heart in tonic state.
- Under normal resting state, the parasympathetic nerves to heart are in tone. Vagal tone is particularly more in athletic animals. Hence, the heart rate is slower in athletic animals.
- Cutting the vagi accelerates heart rate. The sympathetic nerves are more functional only during emergency to meet excessive O_2 and nutrients demand of the metabolically active tissues. Stimulation of sympathetic fibres increases the heart rate, tachycardia and parasympathetic decreases heart rate, bradycardia.

INDIRECT NERVOUS CONTROL OF HEART

- Indirect nervous control of cardiac activity is principally mediated by reflex mechanisms, which are essential for the maintenance of blood pressure (BP). The reflexes are of two groups.
- Cardio accelerator reflex (*Bain bridge reflex*)
    - When the blood volume in the right side of the heart has increased with accumulation of blood, the stretch receptors of the right atrial wall get excited and the impulses are carried through vagus to the vasomotor centre of the medulla.
    - It causes inhibition of the vagal tone to the heart, but stimulates the vasoconstrictor centre. This result in increased force of contraction of the heart and constriction of the blood vessels, the BP returns to normal.
- Cardio inhibitory reflex
    - The two baroreceptors, the carotid sinus and the aortic body are very sensitive to increased BP and play a key role in the regulation of BP and heart rate.
    - Carotid sinus, pressure sensitive stretch receptors is located at the bifurcation of the internal and external carotid artery sense the BP changes and transmits the sensory impulses to cardio inhibitory area of the vasomotor centre of the medulla through afferent nerve, the sinus nerve, a branch of the glossopharyngeal nerve.
    - Aortic body pressure receptors is the thickened portion of the aortic arch get stimulated by increase in BP and the impulses are transmitted through aortic or cardio depressor nerve, a branch of vagus to the cardio inhibitory area of the medulla.
    - On stimulation of these two receptors by increased BP, inhibits vascular sympathetic nerves resulting in vasodilatation. The efferent impulses from cardio inhibitor centres pass through vagus causes reduction in heart rate and also vasodilatation. The sinus and aortic nerves are called as *buffer nerves*.
    - The signals regarding the drop in BP are transmitted to cardio accelerator area of the vasomotor centres of the medulla causes inhibition of the activities of cardio inhibitor centres, whereas the cardio accelerator centre and the sympathetic division are stimulated resulting in vasoconstriction and increased heart rate.

CHEMICAL REGULATION OF THE HEART

Cations

- Na^+ and K^+ ions favour diastole, whereas Ca^{++} ion favours systole. Sudden infusion of the Ca^{++} ions into the heart causes calcium rigor or systolic arrest.

Catecholamines

- Epinephrine and norepinephrine are the neurotransmitters of sympathetic division, acts on β_1 receptors and exerts positive chronotropic (atria and ventricles) and inotropic effects on heart. β_1 receptors are found in S.A. node, A. V. node and the ventricles.
- Stimulation of α_1 receptor (SA node, myocardium) causes positive inotropic effect in the atrial and ventricular muscles. Catecholamine increases the stroke volume and cardiac output during emergency / stress.

Acetyl choline

- The neurotransmitter of parasympathetic division exhibits negative chronotropic (decreased rate) and inotropic (decreased force of atrial contraction) effects, thus decreases cardiac output.

Metabolic acid end products

- CO_2 and other metabolic acid products, H^+ ions, lactic acid lower the p^H below normal, causes increased rate and force of contraction of the heart.

METABOLISM AND ENERGETICS OF WORKING MYOCARDIAL CELLS

- Cardiac muscle requires a continuous supply of oxygen and therefore myocardial cells under normal conditions, utilize aerobic metabolic system that provides a constant supply of high energy phosphate for mechanical and chemical working of the heart.
- The major fuel required for cardiac metabolism is free fatty acids followed by glucose and lactate with a minor contribution from amino acids, ketones and pyruvates.
- Myocardial metabolism has three phases:
    - Energy Liberation or Production: It is the stage during which energy is released from the carbon-hydrogen bonds of substrates including fatty acids, glucose, lactate and other compounds used by the heart.
    - Energy conservation or storage: It is the transfer of the energy into synthesis of high energy phosphate compounds such as ATP, creatine phosphate, AMP, ADP.
    - Energy Utilization: It is the release of this stored chemical energy into shortening or development of tension of cardiac contraction.
- Cardiac muscles cells contains larger number of mitochondria that are rich in enzymes of citric acid cycle and oxidative phosphorylation for muscle contraction. ATP is utilized by the myocardial cells for muscle contraction which is broken down to ADP and inorganic phosphate (Pi).
- The increased amount of ADP and inorganic phosphate (Pi) formed stimulates oxidative phosphorylation, the citric acid cycle and glycolysis thereby increasing the high energy phosphate bond production. Creatine phosphate also provides an immediate source of high energy phosphate bonds.

MODULE-16: CORONARY CIRCULATION

Learning objectives

- This module deals with
    - coronary circulation and its regulation
    - coronary reflexes
    - metabolic control
    - coronary vasodilators and
    - vasodilator chemicals

CORONARY CIRCULATION

- It starts as coronary orifices in the aorta and supplies the blood to the heart muscles through right and left coronary arteries. These orifices are in free communication with aorta both during systole and diastole.
- The main coronary artery branches into right and left coronary arteries. The left coronary artery is more important in dog, cat and ruminants, whereas in man, swine, horse and some primates the right coronary artery is more prominent.
- The left coronary artery gives of the left circumflex branch which runs along the auriculo-ventricular groove and distributes about 80% of the total blood flow to the heart.
- It supplies mainly the atria and left ventricle, A.V. node, inter ventricular septum. The right coronary artery nourishes the right ventricular muscles.
- The venous system of the heart comprises the great cardiac veins, which empties through the coronary sinus drains the blood supplied by the left coronary artery into the right atrium of the heart, whereas the anterior cardiac veins collect the blood supplied by the right coronary artery and discharge their blood into the right atrium. Small amount of blood is drained through the thebesian veins directly into the right ventricle and other chambers of the heart.
- About 4% of the cardiac output flows through the coronary arteries to meet the very high oxygen demand by the cardiac muscles under normal condition (10-15 ml of O_2 is taken by cardiac muscle from each 100 ml of blood as against 5 ml/100 ml in skeletal muscles). This blood supply may be increased to 40% during heavy muscular exercise.
- In the left coronary artery the blood flow is decreased during systole because of the high coronary resistance developed by the contraction of ventricular muscles. However, this gets reversed during ventricular diastole which shows increased coronary blood flow. On the other hand the right coronary artery shows increased blood flow during systole and lower during diastole.
- If a coronary artery is gradually blocked by stenosis, there may be a progressive development of new blood vessels from the pre-existing capillaries, known as the *collateral vessels.*

REGULATION OF CORONARY CIRCULATION

- Heart rate
  - Higher the heart rates, lesser the duration of diastole, and lowers the coronary flow.
- Aortic blood pressure
  - Increase in aortic pressure causes increased coronary flow.
- Peripheral resistance
  - More the peripheral resistance greater will be the aortic pressure and coronary flow increases.
- Autonomic control
  - Both the sympathetic (ß - adrenergic receptors (vasodilators), α - *receptors* (vasoconstrictors) and parasympathetic vasodilators are the primary autonomic control on the coronary arteries. Stimulation of $ß_1$ and $ß_2$ adrenergic receptors results in vasodilatation in major parts of the large coronary arteries, whereas the vasoconstrictor tone by α adrenergic receptors regulates the resting in coronary blood flow. The overall effect of stimulation of sympathetic nerves is coronary vasodilatation. Vasodilatation by vagus is less important.

- Norepinephrine and neuropeptide Y are the neurotransmitters of sympathetic nerves of which neuropeptide Y is strong vasoconstrictor particularly in coronary vasculature.
- Acetyl choline, the neurotransmitters of the parasympathetic system acts on the cholinergic receptors of the endothelium, causes the release of endothelium derived relaxing factor (EDRF). The EDRF acts on the smooth muscles of the blood vessel causes vasodilatation.

CORONARY REFLEXES

- Neurally mediated coronary vasomotor reflex effects can be elicited from the carotid sinus and aortic body.
- Increased venous inflow reflexly stimulates vagus and causes reflex vasoconstriction in abdominal and thoracic organs to divert increased coronary flow by vasodilatation.

METABOLIC CONTROL

- The most important controlling factor of coronary circulation is oxygen demand. Lack of oxygen in tissue release *adenosine*, which is a potent vasodilator and other metabolic products like CO_2, H^+ and lactic acid also produce vasodilatation.

VASODILATOR CHEMICALS

- Nitro-glycerine,
- Halothane,
- Nikethamide,
- Caffeine,
- Camphor and
- Nitrates.

HORMONES

Vasodilatation

- Glucagon,
- Insulin,
- Thyroxine,
- Serotonin,
- Histamine,
- PGE_2,
- Prostacyclin,
- VIP and
- Substance P

Vascoconstrictor

- Vasopressin,
- PGF_2□,

- Thromboxane A_2,
- Leukotriene D_4 and
- Angiotensin II are the vasoconstrictor hormones**.**
- *Endothelin* is the most potent of the all mammalian vasoconstrictor substances.

MODULE-17: ELECTROCARDIOGRAPHY (ECG)

Learning objectives

- This module deals with
  - recording of electrical activity of the heart
  - significance of ECG

ELECTROCARDIOGRAPHY

- Electrocardiography (ECG) is the recording of the electrical changes occurring during the process of depolarization and repolarization of the heart. The instrument, the electrocardiograph is used to take the recording known as elctrocardiogram.
- Einthoven (1908) first recorded ECG using bipolar leads. The depolarisation and repolarisation of cardiac muscle generate electrical current. It flows in a circuitous route from depolarized to polarized area, which can be measured by the electrodes (leads) which are placed on the surface of the body surrounding the heart.
- ECG is recorded as deflection or waves using lead systems. A lead is connection of two points /parts of the body through electrodes and wires with the electrocardiograph. If the electrical impulse travels towards the positive pole, a positive (upward) deflection occurs. If the impulse moves towards negative lead the deflection is negative (downward).

COMMON LEAD SYSTEMS

Bipolar limb leads (by Einthoven)

  - Lead I: Right arm, (- ve) & Left arm (+ ve)
  - Lead II: Right arm (- ve) & Left leg (+ ve)
  - Lead III: Left arm (- ve) & Left leg (+ ve)
  - The right hind leg is connected to the earth.
- The triangle formed by the three lead systems with the heart at the centre is referred to as *Einthoven's triangle*.

Augmented unipolar limb leads

- Basically, an augmented unipolar limb lead compares the electrical activity at the reference limb (right arm, or left arm or left leg) to the sum of the electrical activity at the other two limbs. Unipolar leads permit more precise location of direction of electrical potential within the myocardium.
  - Lead aVR: Right arm (+ ve) & Left arm and left leg (- ve)
  - Lead aVL: Left arm (+ ve) & Right arm and left leg (- ve)
  - Lead aVF: Left leg (+ve) & Right and left arm (- ve)

- Among the augmented unipolar limb leads only aVR shows inverted deflections when compared to others.

Unipolar chest leads (Exploring/ precordial/ chest leads)

- These leads record the potential from the anterior surface of the heart, from right side to left side. A central chest lead is placed at different locations on the chest and these leads are designated as V leads. The common positions of V leads are
    - V1 – 4th intercostal space at the right sternal border
    - V2 – 4th intercostal space at the left sternal border
    - V3 – equidistant between V2 and V4
    - V4 – at mid clavicular line in the 5th intercostal space
    - V5 – anterior axillary line at the same level as V4.
    - V6 – midaxillary line in the 5th intercostal space
    - V7 – posterior axillary line in the 5th intercostal space
    - V8 – posterior scapular line
- These electrodes are positive poles, which are compared with the average voltage across the three standard leads (Lead I to III). These leads record the potentials from the anterior surface of the heart and provide additional information about right and left heart enlargement.

ECG WAVES

- Deflection of normal ECG are referred as P, QRS and T waves

P wave

- It is a positive wave that indicates the sum of all the electrical potentials produced during the depolarisation of both atria and the spreading of the electrical activity from S.A node throughout the atrial musculature. It slightly precedes atrial systole and initiates the atrial contraction. There is a very brief interval between P wave and atrial systole, which is due to time lag between electrical and mechanical events.

QRS wave

- *QRS complex* shows the spreading depolarization wave through the ventricular muscles, thus initiates ventricular systole (depolarization). QRS wave precedes isovolumetric contraction.

T wave

- The positive (upward) deflection precedes ventricular diastole and indicates the initiation of ventricular repolarization (relaxation). ECG has no separate deflection or wave for atrial relaxation, which is due to the fusion of this deflection with QRS complex.

ECG INTERVALS

- P-Q or P-R interval is the duration of time between the beginning of the P wave and the beginning of the QRS wave. It indicates the time of excitation wave travel from SA node to purkinje system which includes short A.V nodal delay after the atrial contraction to permit complete ventricular filling. P-R interval indicates atrio ventricular conduction time. Normally P-R interval is 0.1 sec.
- Q-T interval is the duration from the beginning of Q wave to the end of T wave which indicates the duration from initiation of ventricular depolarization to completion of repolarization or ventricular contraction.
- S-T interval is the duration between the beginning of S wave and the beginning of T wave during which the ventricles remain depolarized.

AVERAGE HEART RATE PER MINUTE AND ECG INTERVALS

Species	Average heart rate	Intervals in seconds		
		PR	QRS	QT
Cattle	70 (48 – 84)	0.18	0.09	0.39
Horse	35 (28 - 40)	0.30	0.11	0.52
Swine	100 (70-120)	0.13	0.06	0.32
Sheep/goat	100 (72-120)	0.13	0.04	0.28
Dog	100 (70-120)	0.10	-	0.06
Cat	130(120-140)	0.07	0.04	0.17

- The time between two successive P waves is P-P interval, corresponds to time between atrial contractions which can be used to calculate the number of atrial contractions per minute.
- Similarly, R-R interval can be used to calculate the ventricular rate.

SIGNIFICANCE OF ECG

- It is a non-invasive method to evaluate cardiac function.
- To diagnose ventricular hypertrophy.
- To evaluate conduction system blocks, myocardial infarction and drug effects etc.

MODULE-18: ECHOCARDIOGRAPHY

Learning objectives

- This module deals with
  - principles of echocardiography
  - types of echocardiography

ECHOCARDIOGRAPHY

- Echocardiography is a non invasive tool for imaging the heart of the surrounding intrathoracic structures to diagnose various cardiac diseases to assess cardiac function.

Uses

- To evaluate size of cardiac chambers
- To study the thickness and movement of the wall
- To study the structure and movement of the valves
- To detect pericardial and pleural fluid
- To find out congenital cardiac anomalies
- To identify mass lesions within and adjacent to the heart
- To diagnose valvular and myocardial pathology

PRINCIPLE OF ECHOCARDIOGRAPHY

- Echocardiography employs high frequency sound waves greater than 20,000 Hz. These ultrasound waves when passed through the tissues are reflected back from the tissues based on the acoustic impedance of the tissue. The amount of reflection of ultrasound waves depends on the difference in the acoustic impendance between two adjacent tissues. i.e. greater the difference, greater in the reflection. Bone tissue and air/tissue interfaces are highly reflective. Bone has a very high acoustic impedance whereas air has a very low acoustic impedence, as well as the soft tissue.
- Echocardiography is usually performed in the inter spaces within the cardiac windows. Ultrasound waves are transmitted through the tissue at a known speed depending upon the type of tissue through which it travels. Ultrasound obeys the laws of geometric optics with regard to reflection, transmission and refraction.
- When an ultrasound wave meets an interface of differing acoustic impedance, the wave is reflected, refracted and absorbed. The intensity of the ultrasound beam decreases as it travels away from the transducer because of beam divergence, absorption, scatter, and reflection of wave energy at tissue interfaces. The largest ultrasound reflection occurs when the ultrasound beam is perpendicular to the imaged structure, creating a strong reflection or echo.
- These reflected ultrasound waves are then received by the transducer and processed by the ultrasound machine to create an image. The transducer acts as a receiver over 99% of the time. The echocardiographic images obtained are displayed on the monitor and can be recorded on videotape, thermal paper, radiographic film or computer disc.
- The frequency of the ultrasound waves emitted by the transducer markedly influence the quality of the image obtained and the depth of tissue that can be imaged successfully. Higher frequency ultrasound waves have a shorter wavelength and yield better resolution of small structures close to the skin surface.
- However, more energy is absorbed and scattered with high frequency ultrasound and thus, high frequency transducers have less penetrating ability. Conversely, a lower frequency transducer will have a greater depth of penetration but poor resolution. The transducer selected for echocardiography should be the highest frequency available that will penetrate to the depths needed to image the heart in its entirety. Frequencies generally used for veterinary echocardiography range from 2.25-3.5 MHz for adult horses and cattle and 3.5-10.0 MHz for small animals, small ruminants, foals, calves and exotics.

TYPES OF ECHOCARDIOGRAPHY

- There are three types of echocardiography, used clinically:
  - M-mode,
  - Two-dimensional (2-D, B-mode or real time), and
  - Doppler echocardiography.

M-mode Echocardiography

- It is a one-dimensional ("ice-pick") view of the cardiac structures moving over time. The echoes from various tissue interfaces along the axis of the beam are moving during the cardiac cycle and are swept across time, providing the dimension in time. The lines on the recordings correspond to the position of the imaged structures in relation to the transducer and other cardiac structures at any instance in time. More accurate placement of the M-mode cursor within the heart is performed by using the two-dimensional (2-D) real-time image as a guide. The M-mode echocardiogram uses a high sampling rate and can yield cleaner images of cardiac borders, allowing the echocardiographer to obtain more accurate measurements of cardiac dimensions and more critically evaluate cardiac motion.
- Standard M-mode views are obtained from the right parasternal position. The standard M-mode views utilized in the veterinary medicine include the left ventricle (at the level of the chordae tendineae), the mitral valve and the aortic root (aorta/left artrial appendage) view.

Two-Dimensional Echocardiography

- Two-dimensional echocardiography allows a plane of tissue (both depth and width) to be imaged in real time. Thus, the anatomic relationships between various structures are easier to appreciate than with M-mode echocardiographic images. An infinite number of imaging planes through the heart are possible, however, standard views are used to evaluate the intra and extracardiac structures. The standard views are obtained from either the right parasternal window in all species and from the left parasternal window in adult large animals or in other species when imaging the heart from the left side is desirable.

Doppler echocardiography

- Doppler imaging allows evaluation of blood flow patterns, direction, and velocity; thus, it permits documentation and quantification of valvular insufficiency or stenosis and cardiac shunts. Estimation of blood flow and cardiac output can also be made. Doppler echocardiography is based on detection of frequency changes (the Doppler shift) occurring as ultrasound waves reflect off individual blood cells moving either away from or toward the transducer. Calculation of blood flow velocity is possible when the flow is parallel to the angle of the ultrasound beam.
- Two types of Doppler echocardiography are used clinically:
  - Pulsed wave and
  - Continous wave.
  - Pulsed wave

        - Pulsed wave (PW) Doppler uses short bursts of ultrasound transmitted to a point (designated the "sample volume") distant from the transducer.
        - The advantage of this type of Doppler is that blood flow velocity, direction and spectral characteristics from a specified point in the heart or blood vessel can be calculated.
        - The main disadvantage is that the limited.
    - Continuous wave
        - Continuous wave (CW) Doppler uses dual crystals so that ultrasound waves can be simultaneously and continuously sent and received.
        - There is no maximum measurable velocity (Nyquist limit) with CW so high velocity flows can be measured.
        - The disadvantage with CW Doppler is that sampling of blood flow velocity and direction occurs all along the ultrasound beam, not in a specified area.
- Color flow Doppler echocardiography is a form of PW Doppler ultrasonography which combines the M-mode and 2-D modalities with blood flow imaging. With color flow Doppler, multiple sample volumes are analyzed along multiple scan lines. The mean frequency shift obtained from these many sample volumes is color-coded for direction and velocity. Several types of mapping are usually available. Most systems code blood flow toward the transducer as red and flow away as blue. Differences in relative velocity of flow can be accentuated, and the presence of multiple velocities and directions of flow (turbulence) can be indicated by different maps which utilize variations in brightness and color.

MODULE-19: PULSE, REGULATION OF HEART AND CIRCULATION – HEMODYNAMICS

Learning objectives

- This module deals with
    - pulse
    - velocity of pulse wave
    - venous pulse
    - physiology of circulation (Hemodynamics)
    - distribution of blood in the systemic circulation
    - compliance (capacitance)
    - blood velocity Resource

PULSE

- It is a wave of expansion and elongation followed by recoiling of the arterial walls and due to the forceful entry of the blood from the aorta during each heart beat. It originates from aorta spreads throughout arterial system and disappears at the arterioles.
- Systolic rush of blood into aorta causes steep increase in the distension of the arterial walls represents anacrotic limb of the pulse wave, whereas the elastic recoiling of the distended arteries causes a drop in the declining slope in the pulse wave, referred as catacrotic limb.
- During the end of systole and the beginning of isometric relaxation phase of cardiac cycle, there will be drop in the intra-ventricular pressure causes the back flow of blood in to the aorta. This results in closure of the aortic valve and the distension of the root of the aorta. This creates a drop in the intra-arterial pressure characterized by dicrotic notch or incisura of the pulse.

VELOCITY OF PULSE WAVE

- It is greater than blood velocity
- Rigid vessels have more velocity than elastic arteries
- Velocity is less in large central arteries than in small peripheral arteries
    - Aorta : 3-5 m/sec
    - Large artery : 7-9 m/sec
    - Small artery : 15-40 m/sec

VENOUS PULSE

- It is the pulsation seen in the large veins near the heart.
- It is of two types
    - If the wave travels towards heart it is referred as *positive venous pulse*. Downward movement of AV valves, lengthening of the atrium followed by a drop in the intra atrial pressure, gradual atrial filling (v wave of the atria), all result in positive venous pulse.
    - Bulging of AV valves during ventricular systole causes *negative venous pulse* (away from heart) in the jugular vein.

PHYSIOLOGY OF CIRCULATION (HEMODYNAMICS)

- Circulation is a continuous circuit through which the same amount of blood flow through each subdivision of circulatory system is maintained by the heart.
- In a resting animal about 25% of the blood volume is in the *central circulation* (pulmonary and coronary circulation) and the rest 75% in the *systemic circulation*.

DISTRIBUTION OF BLOOD IN THE SYSTEMIC CIRCULATION

    - Arteries and arterioles : 15%
    - Capillaries : 5%
    - Venules and veins : 80%
- Aorta is the biggest blood vessel, originates from the left ventricle of the heart and continues as large arteries. Both the aorta and the large arteries have more elastic tissues with less smooth muscle; hence known as elastic vessels. Arteries are the high-pressure passage way and deliver blood to capillaries. These vessels function in the maintenance of blood pressure during diastole by their elastic recoiling effect.
- The large arteries form the main arterial branches thar give rise to the terminal branches or smaller arteries, which have smooth muscles in their walls. They are continued as the small arterioles or the true arterioles, which are continued as meta-arterioles. Small arteries and arterioles have thick walls with less elastic tissue and more of smooth muscle, called as muscular vessels. These arterioles are gates of the systemic circulation, which may constrict or dilate to regulate blood flow to capillaries.
- Capillaries are tiny tubular network composed of only a thin layer of endothelium that favours selective permeability of water, oxygen, nutrients, CO_2 and other metabolic waste between blood and tissues and they are known as exchange vessels. In capillaries the blood cells flow in single layer with RBCs in the centre and WBCs in the periphery. Capillaries form the highest cross-sectional area in the circulatory system.
- The true capillaries are interconnected which forms the place of nutrient exchange. At the point the capillaries branch off from metarterioles, the capillaries are enriched by smooth

muscle called precapillary sphincter. The metarterioles and the precapillary sphincters are not innervated and are controlled by local conditions in the tissues.

- Blood flows from the arteriole directly into metarteriole then leads to capillaries and capillary like channels which connect directly with veins called as A - V shunts.
- When the tissues are at rest and large amount of blood is not required, the sphincters may be completely closed. When large amount of nutritive blood flow is required for active tissues, the metarterioles and the sphincters dilate to shunt the blood nearly the entire length into the capillary bed. The precapillary sphincter undergoes periodical contraction at intervals of 15 second to 3 minutes, called as vasomotion.
- Vasomotor mechanism causes either vasoconstriction (decreased diameter) or vasodilatation (increased diameter) of the arteries and arterioles. It involves an active contraction of the smooth muscles of arterioles and precapillary sphincters, whereas vasodilatation is a passive event involving only relaxation of smooth muscle.
- Arteriovenous anastomoses or shunt occurs between arteries and veins. It is not involved either in blood flow through true capillaries or in the exchange of materials between blood and extracellular fluid. These shunts are primarily by passing the resistance pathways of the precapillary area in tissues during a period of low requirement for nutritive blood supply. In dogs arteriovenous anastomose is primarily functional in thermoregulation allows increased blood flow to the tongue for evaporatory heat loss through panting. This structure is under separate neural and hormonal control.
- The terminal vascular beds consist of small arterioles, metarterioles, capillaries and venules forms microcirculation. Capillaries continue as venules, a thin walled network which unit to form terminal veins. Venules and veins are thin walled structures contain elastic tissues and smooth muscles. Veins function as a blood reservoir called as capacitance vessels. When dilated, they can store large amount of blood and by constriction shift blood volume and affect cardiac venous return.
- The veins continue as vena cava, which drains its content into the right atrium. Vena cava has the highest diameter.
- Veins in the extremities have valves. These valves prevent back flow of blood, thus permits unidirectional blood flow towards heart. There are no valves in the major abdominal and thoracic veins.
- Hemal nodes are the nodal structure found in cattle and sheep on the course of small blood vessels. They are similar in structure and function like spleen. Erythropoiesis occurs during fetal period and granulopoiesis occur in postnatal life.

COMPLIANCE (CAPACITANCE)

- It is the total storage capacity in a given portion of the circulatory system for every mm Hg of pressure rise.
- Veins have about 24 times more capacitance than arteries since about 80% of the total blood volume is located within the venous system.

TYPES OF ACTIVE IMMUNITY

- The blood flows at a steady rate through a long smooth vessel with each layer of blood remaining in the same distance from the wall i.e it flows in streamlines. The central portion of the blood stays in the centre of the vessel. This type of flow is called as laminar flow or streamline flow. If the blood flows in all direction in the vessel and continually mixing within the vessel it is called as turbulent flow. In turbulent flow, the blood flows cross wise in and along the vessel forming whirls in the blood called as eddy currents.

Turbulent flow occurs when the rate of blood becomes too great or when it passes an obstruction in a vessel or when it passes over a rough surface.

- Blood flow through the blood vessel is determined entirely by two factors
  - The pressure difference between the two ends of the vessel, which is the force that pushes the blood through the vessel
  - The impediment to blood flow the vessel known as the vascular resistance
- Blood flow through the vessel is calculated by Ohm's Law

$$\mathbf{Q = \Delta P/R}$$

  - Where,
  - Q = Blood flow
  - ΔP = Pressure difference between the two ends of the vessel (P_1-P_2)
  - P_1 = Pressure at the beginning of the vessel
  - P_2 = Pressure at the end of the vessel
  - R = Resistance
- According to this formula, the blood flow is directly proportional to the pressure difference but inversely proportional to the resistance

BLOOD VELOCITY

- It indicates the direction and the rate of blood flow in the blood vessels. It is directly proportional to the minute volume but inversely proportional to the cross sectional area of the blood vessel and circulation time. Velocity is high in the arteries and veins but slow in capillaries.
- The arterial portion of the circulatory system shows progressive increase in the cross sectional area due to extensive branching of the arterial tree, thus causes progressive fall in the velocity towards capillary bed. On the contrary, the venous system shows decreasing cross sectional area towards heart that results in progressive increase in blood velocity.
- In *dogs*, the cross-sectional area of aorta is 0.8 cm^2, large arteries is 3.0 cm^2 and in capillaries it is 600 cm^2.

Parts	Velocity of blood flow (cm/sec)
Aorta	13
Large arteries	6
Arterioles	0.3
Capillaries	0.05
Venules	0.1
Veins	1.0
Vena cava	9

- The distance travelled by a column of blood in a given time through a specific blood vessel is known as *linear blood velocity*.

$$\text{Mean velocity (mm/sec)} = \frac{\text{Blood volume / min}}{\text{Cross section}}$$

The peak velocity occurs during the period of maximal ventricular ejection followed by reduced velocity during the period of reduced ventricular ejection.

MODULE-20: BLOOD PRESSURE, VENOUS PRESSURE AND ARTERIA PRESSURE

Learning objectives

- This module deals with
  - blood pressure
  - mean circulatory filling pressure
  - determination of arterial, capillary and venous blood pressure
  - factors influencing production and maintenance of BP
  - normal BP in animals
  - regulation of arterial pressure
  - role of veins in BP regulation
  - hormonal regulation

BLOOD PRESSURE

- It is the pressure exerted by the circulating blood against any unit area of the blood vessel wall. Stephen Hales (1730) demonstrated the existence of pressure in the blood vessels. Average pressure in the aorta is 98 mm Hg and the mean pressure in veins is 3 mm Hg and this pressure difference (98-3 = 95) moves the through the systemic vessels. This pressure difference between the aorta and veins is the perfusion pressure.
- BP is highest in aorta (98 mm Hg), moderate in capillaries and lowest in the vena cava (3 mm Hg). This pressure gradient favours the blood flow through the blood vessels.
- During ventricular systole the arteries show the highest pressure, referred to as systolic pressure. It is due to increased blood volume and distension of the arterial walls. During ventricular diastole, blood continues to flow out of the aorta into small arteries, the volume of blood in the large arteries decreases, the arteries become less distended and blood exert less pressure in the arteries. The minimum pressure reached in the blood vessels before each new ventricular ejection is referred to as diastolic pressure.

Pulse pressure = the difference between systolic and diastolic pressure

- The pulse pressure increases as the blood flows from aorta to distal arteries and then becomes less and less when the blood moves towards periphery. It disappears in the arterioles and capillaries.

- *Systolic pressure:* Indicates the total kinetic energy imparted to the blood by the heart.
- *Diastolic pressure:* Reflects the state of peripheral vessels and load on vascular wall.
- *Pulse pressure:* Ventricular output and measure the variations of kinetic energy of heart.
- *Mean pressure* is the average pressure in a blood vessel over a given period of time. This represents potential energy that draws blood through the systemic circulation.

- This formula is useful to find out mean pressure in major arteries distal to aorta but not in aorta because the pattern of arterial pressure pulsation change as the pulse moves away from the heart. The pressure pulses are more triangular in large arteries not in aorta.
- Arterial BP is always expressed in mm Hg whereas capillary and venous pressure can be expressed as mm H_2O.

MEAN CIRCULATORY FILLING PRESSURE

- This eventual pressure in the static circulatory system when there is no pressure difference between the aorta and the vena cava and the pressure throughout the circulatory system which is called mean circulatory filling pressure, and it is is 7 mm Hg.
- This pressure is caused by the static blood distending the blood vessels; the vessels being elastic, they recoil and this recoiling accounts for the pressure in the static circulation.

DETERMINATION OF ARTERIAL BLOOD PRESSURE

- It helps in diagnosing the defects of heart and circulatory system.
- It can be measured by two means,
    - oThe direct and
    - oThe indirect (clinical) methods

Direct method

- Experimentally the carotid artery can be connected to any of the following devices such as, the mercury manometer, membrane manometer or the optical manometer to record BP. Mercury manometer is a `U' glass tube containing mercury in one limb and 10% sodium citrate in the opposite limb to balance the mercury.
- The limb with sodium citrate is connected to carotid artery through a tube with a cannula at its end. The float over the mercury column will record the BP over the kymograph.

Indirect method

- Clinically, BP is measured by sphygmomanometer (mercury manometer). It has an inflatable cuff connected to the manometer through a rubber tube. By another tube, the cuff is connected to an inflating bulb. A deflating valve is attached to the inflating bulb to deflate the air from the cuff.
- The cuff is placed in the arm (brachial artery) in human, thigh region in dog (femoral artery), tail region in cattle (middle coccygeal artery) which may be inflated by the inflating bulb. When the pressure in the cuff exceeds the pressure of the artery, the

blood vessel collapses and no blood flow through it. If the stethoscope is placed below the level of the cuff there won't be hearing of any sound of blood flow. The pulse also completely disappears.

- When the pressure in the cuff is released gradually by the deflating valve the pressure of the cuff slowly drops. When the cuff pressure is less than the pressure of the blood vessel, the blood begins to flow through the partially opened artery producing the sounds that are called Korotkoff sounds. The pulse and sound reappear.
- The pressure indicated by the manometer when the first sound is heard represents the systolic pressure. As the cuff is further deflated, more blood returns to the artery the sound gradually reduces (muffles) and disappears, the normal pulse is re-established. The pressure at muffling or at disappearance of sound indicates diastolic pressure.
- When the BP is measured using stethoscope, the method is called as auscultatory method. In palpatory method pulse is used to find systolic pressure only.
- Capillary pressure can be measured by introducing a micropipette into the capillary. In human, the capillary pressure at arteriolar and venous end is 45 and 22 mm H_2O, respectively.
- Venous pressure is very low and can be estimated by inserting a needle into the vein. Saline is allowed to flow into the vein from a long vertical graduated tube.
- The height of the vertical column is adjusted so that the flow ceases, the height of the top of the saline column above the heart is the venous pressure in mm H_2O. It is very low in great veins and negative at atria. BP determination helps in diagnosing disorders of heart and circulatory system.

Ultrasound Method

- In this method the Korotkoff sounds are amplified by using piezoelectric microphones mounted within or below the cuff. The electric signal obtained is amplified to increase the audibility.

Microphone Method

- In this method ultrasound is used to detect arterial wall movement as pressure is decreased in the blood pressure cuff.

DETERMINATION OF CAPILLARY PRESSURE

- Capillary pressure can be measured by introducing a micropipette into the capillary.
- In human, the capillary pressure at arteriolar and venous end is 45 and 22 mm H_2O, respectively.

DETERMINATION OF VENOUS PRESSURE

- Venous pressure is very low and can be estimated by inserting a needle into the vein.
- Saline is allowed to flow into the vein from a long vertical graduated tube.

- The height of the vertical column is adjusted so that the flow ceases, the height of the top of the saline column above the heart is the venous pressure in mm H_2O.
- It is very low in great veins and negative at atria.
- BP determination helps in diagnosing disorders of heart and circulatory system.

FACTORS INFLUENCING PRODUCTION AND MAINTENANCE OF BLOOD PRESSURE

- Heart beat
- Peripheral resistance
- Elasticity of arteries
- Volume flow
- Diameter of the blood vessels

HEART BEAT

- During systole, the arterial pressure increases.
- The rate at which blood enters the arterial system exceeds the rate at which it drains through arterioles and capillaries, increasing the pressure.
- During diastole, the BP decreases since blood passes out of arteries into capillaries.
- When other three factors remain constant, an increase in rate of the heart raises BP and decrease in heart rate will cause a fall in BP.

PERIPHERAL RESISTANCE

- It is caused by internal friction produced by the viscosity of blood and mostly present in arterioles and capillaries (peripheral circulation).
- BP is directly proportional to peripheral resistance. When most capillaries are opened peripheral resistance decrease causes reduction in BP.
- Resistance varies inversely with the fourth power of the radius of the vessels and directly proportional to the viscosity, and length of the vessel. Total peripheral resistance (TPR) is the resistance to flow of blood in the whole of systemic vessels.

$$\text{TPR} = \frac{\text{Aortic pressure (BP)}}{\text{Cardiac output}}$$

or

BP = TPR x Cardiac output

ELASTICITY OF ARTERIES

- The elastic recoil of large arteries is responsible for the continuous flow of blood in the arterioles and capillaries.
- During systole when more blood enters aorta and large arteries, they expand to store the blood i.e. potential energy is stored and during diastole, the stored blood is released due to elastic recoil of the walls of arteries causing blood to flow into arterioles during diastole.
- Arterial stiffening increases systolic pressure (due to reduced expansion of artery) and decreases diastolic pressure (since less blood is stored by reduced expansion).

VOLUME FLOW

- An increase and decrease in the blood volume through the circulatory system causes either increase or decrease in BP.
- When hemorrhage occurs in a large artery, the BP falls immediately due to drop in the blood volume.
- On moderate hemorrhage and when small arteries are cut, the fall in BP will be negligible because of the compensatory vasomotor mechanism and spleenic constriction.

NORMAL BP IN ANIMALS

BP	Cow	Horse	Sheep	Pig	Dog	Cat	Giraffe	Human
Systolic	140	130	140	140	120	140	260	120 mm.Hg
Diastolic	95	95	90	80	70	90	160	70 mm.Hg

REGULATION OF ARTERIAL PRESSURE

- Two mechanisms regulate the arterial pressure.
- Rapidly acting short term regulatory mechanism operates through nerves or hormones.
- Long term control of arterial pressure.
- When the BP undergoes change, after few hours to few days the nerves loose their power to control pressure because receptors adapt.
- Long-term mechanisms come into play, which involve renal fluid volume-pressure control mechanisms.

SHORT TERM REGULATION

- Nervous regulation
  - Baroreceptor reflex
  - Atrial volume receptor reflex
  - Bain bridge reflex
  - Psychogenic responses

        - Defense alarm reaction
        - Vaso vagal syncope
    - Chemoreceptor reflex
- Hormonal regulation
    - Renin- angiotensin system
    - Vasopressin

BARORECEPTOR REFLEX

- Arterial blood pressure is monitored by pressure sensitive nerve endings known as *baroreceptors; Carotid sinus and aortic body* located in the carotid bodies and aortic arch are sensitive to stretch of the arterial wall. The frequency of impulses generated from baroreceptors is proportional the BP.
- They send afferent impulses through sinus and aortic nerves (Buffer nerves) to vasomotor areas of the CNS which monitor beat to beat changes in BP.
- The CNS reflexly alters cardiac output via sympathetic and parasympathetic divisions of ANS altering the vascular resistance to keep blood pressure at a set point by either vasoconstriction or vasodilatation.
- The vasomotor area of the brain responds to an increase in BP by decreasing sympathetic activity and enhancing parasympathetic activity. Heart rate and force of contraction are decreased and stroke volume is decreased.
- Sympathetic inhibition causes vasodilatation of arterioles results in decreased peripheral resistance. All of these effects restore arterial BP to normal. A decrease in BP produces opposite effect to those considered above.

ATRIAL VOLUME RECEPTOR REFLEX

- This reflex is initiated by *stretch/ volume receptors* located in the walls of the left and right atria which are sensitive to change in atrial blood volume (increase or decrease).
- When blood volume is decreased due to hemorrhage and dehydration the CNS receives fewer impulses from the volume receptors and CNS reflexly increases the sympathetic activity to heart and blood vessels, but reflexly decreases parasympathetic activity.
- The volume receptor reflexly stimulates the thirst center of the hypothalamus to increase blood volume by drinking of water. It also activates renin-angiotensin-aldosterone system to conserve sodium along with water. ADH secretion is also enhanced to facilitate water reabsorption from the renal tubule.

BAIN BRIDGE REFLEX

- This reflex operates when the venous return in increased

PSYCHOGENIC RESPONSES

- Two important psychogenic responses are the

- Defense- alarm reaction
- Vaso-vagal syncope

- Defense alarm reaction
  - It is known as fight or flight and emotional response to a threatening situation such as physical injury or trauma.
  - It involves increased sympathetic and decreased parasympathetic activity which includes increased heart rate and stroke volume, vasoconstriction in kidneys, splanchnic organs and skin and vasodilatation in coronary vessel and skeletal muscles and increased BP.
  - There is enhanced secretion of ADH, angiotensin II and ACTH, retention of water and sodium ions to improve blood volume.
- Vaso vagal syncope
  - It occurs in response to certain emotional situation in which some animals faint due to decrease in BP.
  - It involves a decrease in sympathetic activity and increase in parasympathetic activity causing vasodilatation and decrease in TPR.
  - Heart rate and cardiac output decreases with a drop in BP resulting in inadequate blood flow to brain and fainting.

CHEMORECEPTOR REFLEX

- Carotid bodies and aortic bodies contain chemoreceptors.
- The excess CO_2 or H^+ from the body fluids or lack of oxygen or blood supply to these receptors stimulate the chemical sensory impulses through sinus and aortic nerves to vasomotor centre and BP is reflexly elevated to eliminate excess quantity of CO.

ROLE OF VEINS IN BP REGULATION

- Sympathetic stimulation of veins decreases the capacity of veins, venous return to heart increases and cardiac output is increased. Hence BP elevated.

HORMONAL REGULATION

- The sympathetic system influences the cardiovascular system through the release of neurotransmitter norepinephrine (NE) and also activates adrenal medulla to release epinephrine(EPN).
  The EPN and NE activate membrane receptors called adrenergic receptors on the cardiac muscle cells and on the endothelial or smooth muscle cells of blood vessels.
- The adrenergic receptors include α and β receptors which are subdivided into α1 & α2 andβ1&β2.
  The EPN and NE act through α receptors and causes arteriolar vasoconstriction.
- Resistance to blood flow is increased and total peripheral resistance (TPR) increases.
- These hormones also produce venoconstriction (α receptor activation), blood is displaced to central circulation and ventricular preload increases.

- The hormones also activate β receptors in heart and increases rate and force of cardiac contraction resulting in increased stroke volume, TPR, preload and resulting in elevation of BP.
  EPN has an affinity for both α and β receptors causes increased cardiac output, decreased peripheral resistance in skeletal muscle (vasodilatation) and elevated BP, whereas the NE has high affinity for α receptors but low affinity for β receptors causes vasoconstriction and rise in BP.

RENIN-ANGIOTENSIN SYSTEM

- Decrease in renal arterial pressure, ECF or blood volume, stimulation of sympathetic nerves or disturbance in ECF sodium level causes release of renin from the juxta glomerular apparatus of the kidneys.
- The renin, a proteolytic enzyme, splits angiotensinogen**,** a plasma α_2 globulin (synthesised from the liver) to form angiotensin-I by the enzymatic action in the kidney.
- Angiotensin–I is converted to angiotensin-II by the enzyme system present in the capillary endothelium of lungs. The angiotensin-II is a very powerful vasoconstrictor and destroyed by angiotensinases in peripheral capillaries.
- Angiotensin-II stimulates aldosterone release from adrenal cortex.
- Aldosterone acts on the thirst centre of the hypothalamus, and renal tubule causes increased water intake and retention of sodium and water from the renal filtrate.
- Aldosterone also causes vasoconstriction.
- All the three actions of aldosterone elevate the BP to normal.

VASOPRESSIN

- Its physiological role is related to long term regulation of BP brought about by water reabsorption from the kidneys.
- In hemorrhage, large amounts of ADH are released to bring about vasoconstriction.
- When the BP undergoes change, after few hours to few days the nerves loose their power to control pressure because receptors adapt.
- Long-term mechanisms come into play, which involve renal fluid volume-pressure control mechanisms.

LONG TERM REGULATION

- Long term control is brought about by renal regulation of blood volume.
- Blood volume has a direct effect on cardiac output and therefore affects blood pressure.

Direct renal mechanism

- An increase in BP or blood volume causes increased filtration rate in the kidney.
- As filtration rate exceeds the reabsorption rate in the kidney tubules, more urine is produced resulting in increased fliud loss and decreased blood volume.

Indirect renal mechanism

- If arterial BP declines, Renin angiotensin system comes into play. Angiotensin II also stimulates ADH release from the posterior pituitary gland.
- ADH increases water reabsorption from the kidney tubules.
- Increased sodium and water reabsorption from the kidneys leads to increased blood volume and BP.

MODULE-21: CONTROL OF BLOOD VESSELS AND VASOMOTOR REFLEXES

Learning objectives

- This module deals with
  - vasomotor mechanism
  - control of arterioles
  - control of capillaries
  - microcirculation
  - control of veins
  - vasomotor reflexes

VASOMOTOR MECHANISM

- It deals with the maintenance of the diameter of the blood vessels (arteries and arterioles) to maintain BP, thus regulates the blood flow to various organs or tissues according to their demand.

Functions

- Regulates blood flow to different organs according to their demand. *i.e.* shifting of blood from one organ to another on demand.
- Regulates peripheral resistance, thus the BP.

CONTROL OF ARTERIOLES

- It involves
  - Neural regulation
  - Hormonal regulation

NEURAL REGULATION BY VASOMOTOR CENTERS

- It is located bilaterally in the medullary reticular formation and lower pons and includes the
  - Vasoconstrictor and
  - Vasodilator centers.

- This centre receives sensory signals through vagus and glossopharyngeal nerves. The output signals from these centers regulate their vasoconstrictor or vasodilator activities on blood vessels through sympathetic and parasympathetic divisions of ANS.
- Vasoconstrictor centre is a bilaterally located centre, extends from the middle pons to upper spinal cord. It projects its vasoconstrictor fibers through sympathetic system. Under normal conditions, the vasoconstrictor area transmits continuous signals through the sympathetic vasoconstrictor nerve fibers which maintain a partial state of contraction of the blood vessels called as sympathetic vasoconstrictor tone.
- Vasodilator centre is located close to the vasoconstrictor area medially in the floor of the ventricles of the medulla oblongata that inhibits the vasoconstrictor area producing vasodilatation.
- Vasoconstrictor and vasodilator centers show reciprocal inhibition. These centers are controlled by higher neurons from hypothalamus and cerebral cortex and also from sensory impulses from the peripheral organs and chemical composition of blood.

VASOCONSTRICTOR NERVES

- The sympathetic nerves (thoraco-lumber outflow) innervate the small arteries, large arterioles and veins.
- They provide peripheral resistance and function to maintain the tonus of the arterioles, thus regulate BP.
- On the other hand, the capillaries and precapillary sphincters are free from sympathetic innervations.
- The venules have fewer adrenergic fibres than large veins which themselves are less richly innervated than the arterioles.
- Sympathetic innervation to veins causes either constriction or dilatation of the veins and regulates BP by altering the blood volume of the circulatory system.
- Norepinephrine is the sympathetic neurotransmitter.
- Sympathetic stimulation also causes the release of epinephrine from adrenal medulla.
- Both epinephrine and norepinephrine acts through α and β receptors on the blood vessels.
- NEP excites mainly α receptors and causes vasoconstriction.
- By its action on α1-adrenergic receptors, causes vasoconstriction in arterioles of all organs of the body.
- Epinephrine acts both on α and β adrenergic receptors.
- Its action on α adrenergic receptors in cutaneous and renal arterioles causes vasoconstriction.
- Epinephrine causes vasodilatation in the cardiac and skeletal muscles via its effect on β receptors.
- Neuropeptide-Y potentiates the vasoconstrictor effect of adrenergic receptors in primates.
- All vasoconstrictor fibers are sympathetic fibers and these fibers are in tone.
- Vasoconstrictor or vasodilator effect can be produced by altering vasoconstrictor tone without involving vasodilator fibers.
- Stimulation of sympathetic fibers to veins causes vasoconstriction.

VASODILATOR FIBRES

- These are of three types, parasympathetic, sympathetic cholinergic and antidromic fibers. These fibers do not exert tonic activity on blood vessels.
- Parasympathetic vasodilator fibers
  - These are the cranio-sacral outflow as chorda tympani (branch of facial nerve), glossopharyngeal, vagus and pelvic nerves.
  - The neurotransmitter of these fibers is acetyl choline, which act through the cholinergic muscarinic M_3 type receptors, located on the endothelial cells and the smooth muscle cells of most arterioles.
  - M_3 receptors are innervated by parasympathetic fibers in the coronary circulation and in the external genitalia and by sympathetic cholinergic fibers in skeletal muscles.
  - Activation of M_3 receptors on the endothelial cells causes vasodilatation and it also releases nitrous oxide from endothelial cells.
  - This nitrous oxide also produces vasodilatation by acting through smooth muscle cells of arterioles.
  - Stimulation of these fibers results in vasodilatation in coronary vessels, tongue, salivary gland, external genitalia, bladder and rectum.
- Sympathetic vasodilators
  - Two types of fibers are adrenergic and cholinergic vasodilator fibers.
    - Sympathetic adrenergic fibers
      - Stimulation of β_1 receptor causes relaxation of renal arterioles.
      - Sympathetic stimulation of β_2 adrenergic receptors causes vasodilatation.
      - Epinephrine acts on β_2 adrenergic receptors to effect vasodilatation of coronary vessels, skeletal muscle, pulmonary and splanchnic arterioles and systemic veins.
      - β_2 receptors are found on the coronary and skeletal muscle arterioles. These receptors are not innervated by sympathetic nerves but they respond to circulating epinephrine released from the adrenal medulla in response to sympathetic stimulation.
      - It causes anticipatory increase in blood flow to heart and skeletal muscles during fight or flight reaction.
    - Cholinergic sympathetic vasodilator fibers
      - They are limited to the arterioles of active skeletal muscles, causes an anticipatory increase in blood flow even before exercise to overcome fatigue (in dog and cat).
      - Sympathetic cholinergic fibers to cholinergic muscarinic M_3 receptors cause cutaneous vasodilatation.
- Antidromic fibers
  - These fibers originate from dorsal roots of spinal cord and show bidirectional conduction of impulses.
  - The antidromic fibers divide at their peripheral end, one branch supplying the receptors of the skin or muscle and the other to the nearby arterioles.

- The receptor are sensitive to trauma, heat, cold and frostbite.
- When the receptor are stimulated, the impulses reache the blood vessel concerned by travelling in opposite direction (antidromic) and result in vasodilatation. Since the reflex operates on the sensory nerve and its branch to the blood vessel without involving CNS, it is referred as the axon reflex.

HUMORAL REGULATION OF ARTERIOLES

- Effect of epinephrine, norepinephrine and renin - angiotensin on arterioles has already been dealt.
- ADH, angiotensin II, dopamine, prostaglandins and high cAMP act on epsilon receptors will result in vasoconstrictions.
- Atrial natriuretic factor (ANF) release is stimulated by stretching of atria due to increased blood volume. In the kidney tubules it causes increased GFR, decreased Na^+ reabsorption (natriuresis), diuresis by inhibition of renin, aldosterone and ADH activities. It also exhibits vasodilator effect through cGMP mechanism.
- Neuropeptide Y has direct vasoconstrictor properties, regulates the release of atrial natriuretic factor and angiotensin-II.
- The vasoconstrictor actions of serotonin and K^+ and norepinephrine are potentiated by neuropeptide Y, whereas it inhibits renin release. It is involved in moment by moment regulation of blood pressure and blood flow.
- Vasoactive inhibitory polypeptide (VIP) causes vasodilatation and renin release.
- Serotonin by its effect on S_1 and S_2 receptors causes vasodilatation and vasoconstriction, respectively. Serotonin and K^+ ions stimulate the release of atrial natriuretic factor; tend to reduce BP by vasodilatation.
- Prostaglandins are local hormones, synthesized by vascular endothelium. PGE_2 and prostacyclin (PGI_2) are vasodilators, whereas thromboxane A_2 and $PGF_{2\alpha}$ are vasoconstrictors.
- Acetylcholine, insulin, glucagon and prostaglandins are coronary vasodilators, whereas vasopressin (ADH) and oxytocin are coronary vasoconstrictors.
- Thyroxine causes increased blood flow through coronary vessels.
- Bradykinin and histamine cause very powerful vasodilatation and play a key role in the regulation of blood flow in the skin and gastrointestinal glands.
- Histamine acts on H_1 and H_2 histamine receptors cause the release of endothelium derived relaxing factor (EDRF) and low cAMP, results in vasodilatation.
- High CO_2, H^+ and lactic acid, the metabolic products cause local vasodilatation.
- Adenosine, ATP, K^+ ions are the vasodilator substances.
- An increase in Ca^{++} ions causes vasoconstriction, whereas increased concentrations of Na^+,K^+ and Mg^+ result in vasodilatation.
- GABA and glycine are the neurotransmitters of δ receptors cause vasodilatation**.**
- Adenosine released during tissue anoxia stimulates adenosine A_2 receptors activates cAMP mechanism results in profound vasodilatation. ADP and ATP causes release of nitrous oxide from endothelial cells, act on P_2 *receptors* cause vasodilatation**.**

CONTROL OF CAPILLARIES

Local control of blood flow

- Capillaries regulate the local blood supply to the tissues according to the need of the tissues.

MICROCIRCULATION

- Metabolic control of blood flow to an organ is the most important local control mechanism.
- Increased blood flow in tissues in response to increased metabolic rate is called as active hyperemia.
- When the metabolic rate of a tissue increases, its O_2 consumption also increases leading to increased rate of production of metabolic products like CO_2, adenosine and lactic acid. Simultaneously, O_2 concentration decreases in the interstitial fluid. This causes the dilatation of arterioles, relaxation of precapillary sphincters and opening of more capillaries to facilitate increased blood flow to the tissues to deliver more O_2 and removal of accumulated metabolic products.
- Autoregulation of blood flow is also a metabolic control phenomenon. If metabolic rate of an organ does not change but arterial-venous pressure is increased above normal, additional blood flow to that organ is achieved due to increased pressure. The additional blood flow accelerates removal of metabolic products and increases oxygen delivery to the tissues. Hence, the concentration of metabolic vasodilator products decreases. These changes cause the arterioles to constrict and blood flow returns to normal.

CONTROL OF VEINS

- On sympathetic stimulation adrenaline causes venous constriction but has no influence on peripheral resistance to increase venous return to the heart to maintain normal cardiac output and BP.
- In dog and cat, the sympathetic vasoconstrictor fibres (spinal nerves from lumbar 2-4) control the vasomotion in the veins of the hind legs.
- The splanchnic nerve supplies both vasoconstrictor and vasodilator fibres to the portal veins.

Factors causing venous blood flow

- Low pressure in the veins
- Unidirectional blood flow by valves
- Higher intra- abdominal pressure
- Tonicity of the skeletal muscles

Venous pump/muscle pump

- The veins contain valves to direct the blood flow only towards the heart.
- Movement of the legs or tension on the leg muscles causes the propulsion of the blood towards the heart and the venous pressure is lowered. This pumping mechanism is referred as venous pump.

VASOMOTOR REFLEXES

- There are two types of vasomotor reflexes.
- Pressor reflex

o When the BP falls below normal, the impulse frequency passing through the buffer nerves decreases causes the stimulation of cardio accelerator and vasoconstrictor centers of the medulla resulting in vasoconstriction to maintain BP to normal level.

- Depressor reflex
  - oA rise in BP above normal causes increased sensory impulse frequency through buffer nerves to the medullary vasomotor centers causes inhibition of vasoconstrictor and sympathetic centre, but stimulates vagal centre. It results in vasodilatation and decreased heart rate to establish normal BP.

Other reflexes

- Hypothalamic reflex
  - oThermal stimuli - causes vasoconstriction during cold and vasodilatation by heat.
- Cerebral cortex reflex
  - oStress, emotion, pain and exercise acts through dilator centre, vasodilatation in skeletal muscles.
- Chemoreceptor reflex
  - oHypoxia, high CO_2 and H^+ ion causes vasodilatation.
- Stretch reflex
  - o Mesenteric stretch causes distension of intestine which in turn causes vasodilatation.

MODULE-22: ADAPTATION OF CIRCULATION DURING EXERCISE AND SHOCK

Learning objectives

- This module deals with
  - o mechanisms of adaptation of circulation during exercises
  - o shock - definition, classification and stages

ADJUSTMENT OF CIRCULATION DURING EXERCISE

- The response of circulatory system to exercise is an integration of nervous system, endocrine, musculoskeletal and cardiovascular system. During exercise the demands on the circulatory system greatly varies. In the most of the human beings there is an increase in oxygen consumption to five fold within in a few minutes of intense exercise. This increase in oxygen consumption is due to increased demand of oxygen by the working muscle.
- During exercise the mechanoreceptors present in the muscles detect changes in the tension of the muscle and send afferent sensory information to the brain, thereby activating the cardiovascular control centre in the medulla.
- The cardiovascular control centre reduces the activity of the parasympathetic nervous system and increases the activity of sympathetic nervous system, changing the efferent signals going to the heart and the arteriolar smooth muscle.
- This change in parasympathetic and sympathetic activity has greater effect on cardiac output. Cardiac output can be increased 4-8 times the value at rest in humans where as in a trained thoroughbred horse a ten fold increase occurs during maximum exercise.
- At the onset, parasympathetic activity decreases causing an increase in heart rate. At the same time, increase in muscular activity and breathing improves the function of respiratory and skeletal muscle pumps causing an increase in venous return to the heart, resulting in increased stroke volume. Therefore, during initial stages of exercise, the cardiac output increase is due to increase in heart rate and stroke volume.
- Next, sympathetic stimulation of the heart increases, causing an increase in both heart rate and contractility. During the later stage of exercise, increase in heart rate, increases cardiac output rather than increase in stroke volume.
- In addition to changes in cardiac output, blood flow pattern also changes during exercise. During rest, skeletal muscle receive only about 20% of cardiac output, whereas during exercise they receive 88% of total cardiac output *i.e.*, blood flow to skeletal muscles is increased from 1.2 l/min at rest to 2.2 l/min during exercise. These changes in blood flow are as a result of vasodilation of the arterioles of skeletal muscle and heart. The increase in activity causes a generalised vasoconstriction of the arterioles of other organs.
- In skeletal muscle, local release of paracrine factors causes vascular smooth muscle to release by opposing vasoconstrictive effects of α-adrenergic receptor stimulation. These factors together cause an intense local vasodilatation thereby increasing the blood flow to the muscles.
- Vasodilatation of the arterioles of skeletal muscles and vasoconstriction of arterioles of other organs causes a decrease in the total peripheral resistance.
- As a result blood pressure increases slightly during exercise. This stimulates the baroreceptor reflex and brings back the BP to the normal by decreasing cardiac output or total peripheral resistance. The afferent signal from the muscle mechanoreceptors also changes the set point of the baroreceptor reflex allowing BP to increase slightly during exercise.

SHOCK

- Shock is an acute circulatory state in which the cardiac output is insufficient to maintain adequate blood flow to the tissues.
- Any condition causes a sudden decrease in circulating blood volume or increase in volume of the vascular system reduces the mean circulatory pressure results in shock.

CAUSES OF SHOCK

- Shock may be caused either by decreased cardiac output or without decreased cardiac output
- Shock caused by decreased cardiac output
  - Cardiac abnormalities include myocardial infarction, severe heart valve dysfunction, and cardiac arrhythmias etc decrease cardiac ability to pump blood, called as *cardiogenic shock***.**
  - Factors decreasing venous return due to reduced blood volume, decreased vascular tone and obstruction to blood flow.
- Shock caused by without diminished cardiac output
  - Cardiac output may be normal or may even be greater than normal. This can result from
    - Excessive metabolism of the body so that the normal cardiac output is not adequate.
    - Abnormal tissue perfusion.
  - These conditions are present in septic shock (blood poisoning) due to massive bacterial infection.
- Tissue deterioration is the end stage of circulatory shock

CLASSIFICATION OF SHOCK

- Shock can be classified into
  - *Hemorrhagic shock* due to blood loss (hypovolumic).
  - *Cardiogenic shock* due to myocardial damage.
  - *Septic or endotoxic shock* due to massive bacterial infection. The infection is carried by blood from tissue to tissue causing extensive damage. Septic shock may result in peritonitis, generalized infection by streptococci or staphylococci, generalized gangrenous infections etc.
  - *Anaphylactic shock* is due to immunological reaction. Antigen-antibody reaction provokes the release of harmful chemical substances like histamine, slow reacting substance (SRS), eosinphil chemotactic factor which causes cardiac failure and vascular fluid loss. Histamine and SRS increase capillary permeability and bronchospasm.

STAGES OF SHOCK

- Compensated or recovering shock: Blood volume and pressure are reduced. Compensatory mechanisms try to maintain adequate blood flow to vital organs and the patient can recover without transfusion.
- Progressive or degenerative shock: Blood pressure progressively falls despite compensatory mechanisms like reflex tachycardia, vasoconstriction of skin, muscle and splanchnic areas and fluid shift from interstitial compartment to plasma. When B.P falls to 60 mm Hg or less, functions of vital organs heart, lungs, CNS etc. are impaired. Transfusion and other treatments can arrest this stage to restore normality.
- Irreversible shock: Cardiac output and B.P. are very low; vital functions are depressed. Effects of transfusion are transient, B.P. falls and the patient dies of myocardial failure and cerebral depression.

Positive feedback in progressive shock

- Progressive shock if untreated, leads to irreversible shock because of a vicious cycle in which, with each degree of increase in shock, the cardiac output and B.P. further decreases and death occurs. Five feedback mechanisms account for this vicious cycle response.
- As shock is advancing, cerebral blood flow decreases progressively causing depression of the vasomotor and respiratory centers.
    - oVasomotor failure: Vascular tone is reduced because of reduced blood supply to vasomotor centre. The sympathetic discharge is reduced; this lead to decrease in means circulatory pressure; venous return leads to reduced cardiac output.
    - oIncreased capillary permeability: After many hours of capillary hypoxia and lack of nutrients leads to increased capillary permeability and fluid loss to ECF. Reduced blood volume further reduces cardiac output.
    - oBlockage of minute blood vessels: Addition of acids and deterioration products of tissues causes agglutination of local blood due to sluggish blood flow in capillaries; lead to clotting and further impeding blood flow.
    - oCardiac depression: When arterial pressure falls, coronary blood flow is reduced leads to myocardial hypoxia and reduction in the cardiac output.
    - oAt an arterial pressure of 50 mm Hg or less the positive feedback exceeds the negative feedback gains and the circulatory system collapses.

Negative feedback resistance to shock

- Regulatory mechanisms of the circulatory system tend to maintain cardiac output and B.P. at a normal level operate as a negative feedback control. These mechanisms have very short response time in restoring the cardiac output and BP.
- This include the baroreceprtor reflex, chemoreceptor reflex, central nervous ischemic responses. The renin-angiotensin-aldosterone and renal fluid balance mechanisms respond gradually over a period of several hours.

- Within minutes to few hours, the renin-angiotensin mechanism shows vasoconstriction and shifting of fluids into blood to maintain the normal blood volume and BP.

MODULE-23: REGIONAL AND FETAL CIRCULATION

Learning objectives

- This module deals with
  - cerebral, cutaneous and skeletal muscle circulation
  - splanchnic circulation
  - blood flow to rumen, spleen and liver
  - fetal circulation
  - changes in circulation after birth

CEREBRAL CIRCULATION

- Cerebral circulation constituents about 14% of the cardiac output. The arteries that supply the brain are the anterior, middle and posterior arteries of both sides through a common arterial pathway, the circle of Willis.
- In most of the species this circle is supplied by the internal carotid arteries and vertebral arteries, whereas in cat, dog, sheep and goat the circle is supplied by a vascular net, the rete mirable. This carotid rete aids in thermoregulatory mechanism of the brain.
- The circle of the Willis freely communicates with the contralateral side of the brain from both the carotid and vertebral arteries. There are no arteriovenous anastomoses in the brain.
- The walls of the capillaries are non porous and are separated by the nerve cells by neuroglia which prevents diffusion of high molecular weight substances thereby forming a blood brain barrier.
- The blood is drained through the internal jugular vein or through the vertebral venous plexus and external jugular vein.
- The cerebral blood vessels receive both sympathetic and parasympathetic nervous system.

Regulation of cerebral circulation

- Cerebral circulation is controlled primarily by auto regulatory mechanisms mediated by local pH changes.
- An increase is arterial PCO_2 or a decrease in PO_2 causes vasodilatation. Potassium ions produce vasodilatation whereas an increase in bicarbonate concentration causes vasoconstriction.
- The cerebral blood flow is decreased in chronic alkalosis and increased in chronic acidosis. Neural regulation of blood flow plays only a secondary role.

CUTANEOUS CIRCULATION

- The skin is supplied by a dense network of cutaneous arterioles under the dermis layer. These arterioles inturn divide into metarterioles which inturn give rise to capillary loops. These capillary loops provide a greater surface area for heat exchange mechanism.
- The venules form an extensive sub papillary venous plexus. Arteriovenous anastomoses communicate between the smaller arteries, arterioles and venous channels and are located in the distal parts of the extremities, nose, lips, ears etc. These vessels are wide and have low resistance connections that serve as shunts and allow blood to bypass superficial capillary tube.
- The cutaneous vessels are innervated by sympathetic adrenergic vasoconstrictor fibres. Alpha and beta adrenergic receptors are found in the cutaneous arterioles and only alpha receptors in the arteriovenous anastomoses. The venous plexus have separate neural connections and undergo a marked vasoconstriction which minimizes the amount of blood to the skin.
- The major function of the cutaneous vessels is the regulation of body temperature. It aids in the heat conservation and heat dissipation mechanisms. Heat conservation is brought about by vasoconstriction thereby reducing the blood flow to the skin.
- Heat dissipation occurs during increased environmental temperature wherein increased blood flow to the skin occurs in order to increase the heat loss from the body.
- It is brought about by vasodilatation in which increased blood temperature stimulates the hypothalamus which inturn inhibits vasoconstriction thereby increasing the blood flow and causes evaporative heat loss.
- Heat exchange mechanisms also occur via radiation, conduction and convection.

SKELETAL MUSCLE CIRCULATION

- Under normal resting condition, blood flow to the skeletal muscle is 15% and this varies according to the activity of the muscle. Blood flow to the skeletal muscle is controlled by the alpha adrenergic sympathetic system tone on the blood vessels.
- The neurotransmitter substance involved is the norephinephrine which stimulates alpha receptors in the vascular smooth muscle. These fibres also produce vasoconstriction and decreases the blood flow. This vasoconstriction is reflexely influenced by arterial baroreceptors, chemoreceptors and cardiac baroreceptors.
- On the other hand, epinephrine, has a slight to moderate vasodilator effect by stimulating the beta receptors. In certain species like cats and lower animals, sympathetic vasodilator fibres are present that have acetylcholine as the neurotransmitter.
- During rest and at exercise, blood flow to the skeletal muscle in autoregulated and is not affected by the level of metabolites. But local metabolites play role in vasodilatation.
- Vasodilator factors activated during muscle contraction are anoxia, increased Co_2 tension, lactic acid, hydrogen ions, bradykinin, histamine, acetyl choline, ATP, adenylic acid, potassium ions etc.

SPLANCHNIC CIRCULATION

- The splanchnic circulation consists of three parts:
  - oThe mesenteric bed, supplying the gastrointestinal tract;
  - oThe splenic bed and
  - oThe hepatic bed.
- A unique feature of this circulatory system is that the combined outflow from the mesenteric and splenic constitutes the major portion of the inflow of the hepatic through the portal vein.
- All of the splanchnic flow reaches the liver, 70 per cent arrives there via the portal vein from the stomach, intestine, spleen, and pancreas and the remaining 30 per cent comes via the hepatic artery. Together these fractions constitute the splanchnic flow, the hepatic blood flow, and the total through the hepatic veins.
- Three mechanisms operate to regulate blood flow at the local level.
  - oEnhanced metabolism of parenchymal cells of the villi which lowers tissue PO_2 and increases vasodilator metabolites, relaxing arteriolar smooth muscle and precapillary sphincters to increase mucosal blood flow,
  - oIncreased muscle activity metabolically produces active hyperemia of the muscularis mucosae and
  - oIntrinsic myogenic response to stretch autoregulates blood flow, when blood pressure fluctuates.

BLOOD FLOW TO RUMEN, SPLEEN AND LIVER

Rumen blood flow

- Feeding and the products of rumen digestion, carbon dioxide and volatile fatty acids, increases rumen blood flow.

Splenic Circulation

- In certain mammals (dog, cat, horse, guinea pig) the spleen serves as a reservoir of blood. Normally blood is stored in the venous sinuses and is released into the circulation when the need arises (as during exercise, anoxia and hemorrhage). This splenic emptying mechanism is under sympathetic regulation.
- Stimulation of splenic nerve fibers results in decrease in arterial inflow and increase in venous outflow. Norepinephrine mediates arteriolar inflow constriction and epinephrine-mediates emptying.

Hepatic Circulation

- The hepatic blood supply from the hepatic artery and portal vein is governed by hepatic arterial vascular resistance, the arterial tone in the vascular beds of the gastrointestinal tract, pancreas, and the spleen and the intrahepatic portal venous vascular resistance.

- The total hepatic blood flow in humans, cats and dogs contributes about 70 to 75% of the total hepatic blood flow.
- Sympathetic nerve stimulation increases hepatic arterial and portal venous tone. Since the liver, is a major blood reservoir, sympathetic stimulation rapidly mobilizes large amounts of blood for redistribution to vital organs under stress conditions.
- Epinephrine decreases hepatic artery flow and total hepatic blood volume. The hepatic vasculature contains both alpha and beta-adrenergic receptors.
- The hepatic arterial vascular bed exhibits relatively weak local control of its blood flow through both myogenic and metabolic types of auto regulation.
- The portal system lacks such intrinsic local control mechanisms. A partial reciprocity between the hepatic artery and the portal vein helps maintain total liver blood flow constant.
- Vasopressin has three effects on the liver circulation,
    - Hepatic arterial constriction,
    - Mesenteric vasoconstriction, and
    - Dilator action on intrahepatic portal resistance vessels. Angiotensin II causes marked vasoconstriction of both hepatic arterial and portal beds.

FETAL CIRCULATION

- The fetal lungs are nonfunctional before birth and therefore the heart pumps large quantities of blood through the placenta.
- Special anatomical arrangements operate in the fetal circulatory system which is different from the adult.
- Blood returns from the placenta through the umbilical vein passes through the ductus venosus, bypassing the liver.
- Most of the blood that enters the right atrium from the inferior vena cava passes across the posterior region of the right atrium and then through the foramen ovale directly into the left atrium.
- Therefore, well oxygenated blood from the placenta enters mainly the left ventricle of the heart rather than the right ventricle and is pumped by the left ventricle into the vessels of the head and forelimbs.
- The blood entering the right atrium from the superior vena cava passed downward through the tricuspid valve into the right ventricle. This blood is deoxygenated and is pumped into the pulmonary artery and through the ductus arteriosus into the descending aorta and through the two umbilical arteries into the placenta where deoxygenated blood becomes oxygenated.

CHANGES IN CIRCULATION AFTER BIRTH

- Primary changes occurs in the pulmonary and systemic vascular resistance at birth.
    - Loss of tremendous blood flow through the placenta and increases the systemic vascular resistance. This increases the pressure in the aorta, left atrium and left ventricle.

- Decrease in pulmonary vascular resistance due to expansion of lungs. This increases blood flow to lungs with a reduction in the pressures of pulmonary artery, right ventricles and right atrium.

- Closure of foramen ovale.
- Closure of ductus arteriosus.
- Closure of ductus venosus

MODULE-24: STRUCTURE OF KIDNEY

Learning objectives

- This module deals with structure and functions of kidney.

FUNCTIONS OF KIDNEY

- The important function of the kidneys is to rid the body of waste materials that are either ingested or produced by metabolism.
- To control the volume and composition of the body fluids. A balance between the intake and output is maintained in large part by the kidneys. This regulatory function of kidneys maintains the stable environment of cells necessary for them to perform various activities.
- Kidneys perform most of their important function by filtering the plasma and removing the substances from the filtrate at variable rates, depending on the needs of the body. Ultimately, they clear unwanted substances from the filtrate by excreting them in the urine while returning substances that are needed back to the blood.

Multiple Functions of Kidney

- Excretion of metabolic waste products & foreign chemicals, drugs & hormone metabolites -urea, creatinine, uric acid, end products of hemoglobin breakdown and metabolites of various hormones.
- Regulation of water and electrolyte balance.
- Regulation of arterial pressure.
- Regulation of acid-base balance.
- Regulation of RBC production.
- Regulation of 1,25 Dihydroxy Vit D_3 production.
- Gluconeogenesis: Kidneys synthesize glucose from amino acids and other precursors during prolonged fasting.
- Formation of urine.
- Concentration of urine and reabsorption of essential electrolytes.

ANATOMY OF KIDNEY

- The two kidneys are located on the posterior wall of the abdomen, outside the peritoneal cavity.
- The functional unit of the kidney is the nephron. Nephron numbers vary considerably among species and within species and their numbers are relatively constant.
- The kidney consists of two regions such as outer cortex and an inner region, the medulla.

Nephron Components

- The glomerular capsule (Bowman's capsule) is the dilated blind end of the nephron and consists of the invaginated capillary tuft called as the glomerulus.
- In the mammals the blood from the renal artery is delivered to the afferent arteriole which divides into numerous glomerular capillaries.
- The capillaries coalesce to form the efferent arteriole which conducts blood away from the glomerulus and is returned to the systemic circulation through the renal vein.
- The glomerular capsule is lined by a layer of epithelial cells.
- The area between the glomerular tuft and the Bowman's capsule is known as the Bowman's space and it is the site of collection of the glomerular filtrate which is directly funneled into the proximal tubule.
- The nephron is continued from the glomerular capsule by proximal tubule which is composed of proximal convoluted portion and the proximal straight portion.
- Convoluted portion is within the cortex and the straight portion extends about half way into the outer medulla.
- The loop of Henle consist of descending thin limb which is continuous from the proximal straight tubule, the ascending thin limb that terminates at the junction of the inner and outer medulla (cortical nephrons lack a thin ascending limb) and the ascending thick limb that returns to the glomerulus of origin in the cortex and passes between the afferent and efferent arterioles.
- The distal nephron begins at this point and consist of distal tubule, the connecting tubule, cortical collecting tubule and collecting duct (outer medullary and inner medullary).
- The distal tubule, connecting tubule and cortical collecting tubule are collectively referred to as distal convoluted tubule.

BLOOD SUPPLY

- Blood flow to the kidneys is normally 22% of the cardiac output.
- The renal artery enters the kidney and branches to form the interlobar arteries, arcuate arteries, interlobular arteries and afferent arterioles which lead to the glomerular capillaries, where large amount of fluid and solutes (except plasma proteins) are filtered to begin urine formation.
- The distal ends of the capillaries of each glomerular coalesce to form the efferent arteriole which leads to a second capillary network, the peritubular capillaries surrounding the renal tubules
- Renal circulation is unique, in that it has two capillary beds:
    - oGlomerular capillaries and

- Peri-tubular capillaries, separated by efferent arterioles which help to regulate the hydrostatic pressure.

- The glomerulus has a high pressure of 60 mm Hg and peritubular capillaries have a low pressure of 13 mm Hg which helps in rapid fluid filtration.
- The peritubular capillaries empty into vessels of the venous system which run parallel to the arteriolar vessels and progressively form the interlobular vein, arcuate vein, interlobar vein and renal vein.

Vasa recta

- The peritubular capillaries branches to form the vasa recta into the medulla and lie side by side with the loops of henle.
- Like the loops of henle, the vasa recta return toward the cortex and empty into the cortical veins.
- They are associated with long looped nephrons.
- They play an essential role in the formation of concentrated urine.

JUXTAGLOMERULAR (JG) APPARATUS

- When the thick segment of the ascending limb of the loop of Henle returns to its glomerulus of origin in the cortex, it passes in the angle between afferent and efferent arterioles and continues as the distal tubule. The side of the tubule that faces the glomerulus comes in contact with the arterioles, the contact epithelial cells are more dense than the other epithelial cells and are collectively called as macula densa. Macula densa marks beginning of the distal tubule.
- The smooth cells of the afferent and efferent arterioles that make contact with the macula densa are specialized smooth muscle cells and are called as Juxta glomerular cells or Granular cells. Juxta glomerular cells have secretory granules that contain renin, a proteolytic enzyme.
- The space between the macula densa and the afferent and efferent arterioles and the space between the glomerular capillaries is known as mesangial region/Extra glomerular mesangial cells or Lacis cells.
- JG cells are involved in feed back mechanism that assist regulation of renal blood flow and glomerular filtrate rate.

INNERVATION

- Innervation to the kidney is provided by fibres from the sympathetic division of the autonomic nervous system which assists in the regulation of renal blood flow (RBF), glomerular filtration rate (GFR), salt and water reabsorption by the nephron.
- RBF and GFR is brought about by vasoconstriction initiated by reflexes through the vasomotor centre in the medulla and pons.
- Increased sympathetic tone elicits renin secretion from granular cells and enhances sodium reabsorption from nephron segments.

TYPES OF NEPHRONS

- Mammalian kidney has two principal types of nephrons and are classified based on
  - Location of their glomeruli
  - Depth of penetration of the loops of Henle into the medulla.
- Those nephrons with glomeruli in the outer and middle cortices are called ***cortical nephrons.*** They are associated with the loop of Henle that extend to the junction of the cortex and medulla or into the outer zone of the medulla, *e.g.*, Marine aquatic mammals.
- Those nephrons with glomeruli in the cortex close to the medulla are known as ***juxtaxmedullary nephrons***. They are associated with loops of Henle that extend more deep into the medulla, e.g.,mammals in arid region desert animals such as Kangaroo and rat.

MODULE-25: GFR AND RBF

Learning objectives

This module deals with

- how blood is filtered in the kidney and what are the factors that regulate filtration?
- autoregulation of renal blood flow and filtration rate.

GLOMERULAR FILTRATION RATE (GFR)

- It is the quantity of GF formed each minute in all the nephrons of both the kidneys/kg body weight.
- In humans it is about 125 ml/min. Total quantity of GFR formed /day = 180 L. Over 99% of the filtrate is reabsorbed in the tubules, the remainder passing into the urine.

Filtration fraction

- It is the percentage of the renal plasma flow that becomes Glomerular Filtrate.
- The normal plasma flow through both the kidneys is 650 ml/min but the normal GFR in both the kidneys is 125 ml/min, hence the average filtration fraction is 19%

FACTORS AFFECTING GLOMERULAR FILTRATION RATE (GFR)

- Three factors that determine the filtration pressure are
  - Glomerular pressure
  - Plasma colloidal osmotic pressure (COP)
  - Bowman's capsular pressure

- Greater the glomerular pressure, greater is the filtration.
- Greater the plasma COP and Bowman's capsular pressure, lesser is the filtration.
- Effect of renal blood flow
    - oGFR is affected by the rate of blood flow through the nephrons. Since a very large portion of plasma is filtered through the glomerular membrane, the COP in the glomerulus is high which opposes further filtration. Therefore, a portion of plasma fluid is not filtered until new plasma flows into the glomerulus. Greater the plasma flow, greater the filtration rate.
- Effect of afferent arteriolar constriction
    - oAfferent arteriolar constriction decreases the rate of blood flow into the glomerulus and decreases GFR, causing decreased filtration rate whereas dilatation increases glomerular pressure as well as GFR.
- Effect of efferent arteriolar constriction
    - oConstriction of the efferent arteriole increases the resistance and outflow from the glomeruli, increases the glomerular pressure and also GFR initially. But when blood stagnates in the glomerulus for a prolonged period, increase in plasma COP occurs which causes a fall in GFR. Net effect is slight increase in GFR.
- Effect of sympathetic stimulation
    - oMild to moderate stimulation of sympathetic nerves causes afferent arteriolar constriction and decreases GFR. Strong sympathetic stimulation causes great reduction in the glomerular blood flow and glomerular pressure resulting in fall of GFR to zero level.
- Effect of arterial pressure
    - oWhen arterial pressure increases from 100 to 200 mm Hg afferent arteriolar constriction occurs automatically by autoregulation, thus prevents a major rise in glomerular pressure (GP) and GFR increases to only 15-20%.

AUTOREGULATION OF RENAL BLOOD FLOW (RBF) AND GFR

- RBF and GFR remains almost relatively constant when the systemic arterial pressure changes from 75 mm Hg to 160 mm Hg. This ability of the RBF and GFR to resist severe changes in the arterial pressure is called as 'autoregulation of RBF and GFR'. It is an intrinsic mechanism which is independent of the nerve supply.
- Two theories have been proposed to explain autoregulation
    - oMyogenic Theory
    - oJG Theory
- Myogenic theory
    - oAccording to this theory, the increase in BP would expand an artery and it would respond by contracting. In this way, RBF would be decreased and glomerular HP reduced. The reduced glomerular HP reduces GFR.
    - oA reduction in BP causes less tension and blood vessel would dilate to increase RBF and glomerular HP with subsequent increase in GFR. That is, when arterial pressure rises, arterioles are stretched, once stretched, arterioles contract forcefully. This decreases RBF to normal level. A decrease in arterial pressure dilates the blood vessels which increases RBF and GFR.

- JG theory
  - oJuxta glomerular apparatus (JGA) contains renin (a proteolytic enzyme). Renin is released when:-
    - Reduced GFR
    - Reduced glomerular pressure
    - Increased sympathetic stimulation of kidneys
  - oThe last two occur during low BP and always cause reduced GFR. Reduced GFR causes reduced sodium concentration in the tubular fluid as it flows past the macula densa and this low sodium causes release of renin from JGA. Once renin is released from JG cells it diffuses into the blood of afferent arteriole and circulates through out the body.
  - oIn the blood it splits a renin substrate, an alpha 2 globulin to angiotensin I, a decapeptide. Angiotensin I is rapidly converted to angiotensin II by converting enzyme which is present in high concentration in the lungs. Angiotensin II is a powerful vasoconstrictor and causes vasoconstriction through out the body thereby increasing the BP. Some amount of Angiotensin II is formed in the glomerulus, *i.e.* in the JG cells.
  - oAngiotensin II causes marked constriction of efferent arteriole which increase glomerular pressure and also GFR but decreases RBF. Increase in pressure increases GFR but decrease in blood flow decreases GFR and in effect there is a less change in GFR.
  - oDecreased blood flow to peritubular capillaries decreases peritubular pressure, which causes increased tubular reabsorption, so excretion is reduced. When efferent arterioles are constricted, GFR is normal, excretion of waste products such as urea, creatinine is normal. At the same time there is an increase in tubular reabsorption of salt and water. Therefore, effect of angiotensin is to conserve water and salt while allowing normal excretion of waste products.
  - oAngiotensin II constricts the efferent arterioles to a greater extent than that of afferent arteriole. Reabsorption of water and salt by renin-angiotensin system helps to control arterial BP.

Tubulo glomerular feedback

- The mechanism of tubulo glomerular feedback (TGF) is also associated with the JG theory of autoregulation. TGF refers to alteration in GFR with changes in the tubular flow rate.
- It is mediated by the macula densa cells of the JG apparatus. These cells sense changes in the sodium and chloride to their region. If GFR is increased because of increased glomerular HP there will be increase in macula densa flow and sodium and chloride delivery intiates a response that returns GFR and macula densa flow towards the normal by afferent arteriole constriction (which lowers glomerular HP).
- When renal blood flow falls too low it decreases the GFR thereby, decreases in sodium and chloride delivery to the distal tubule which initiate a signal from macula densa and dilates the afferent arterioles and it increases glomerular blood flow and glomerular pressure. This causes the GFR to increase to normal.

MODULE-26: FORMATION OF URINE

Learning objectives

This module deals with

- Formation of urine
- Absorptive capabilities of different tubular segments
- Reabsorption and secretion in different regions of tubules
- Functions of the urinary bladder
- Uremia or Azotemia

URINE FORMATION

- Three processes are involved in the urine formation in the nephrons :
  - Glomerular filtration
  - Tubular reabsorption
  - Tubular secretion

GLOMERULAR FILTRATION

- Glomerular filtrate is the fluid that is filtered through the glomerular membrane into the Bowman's capsule.

Glomerular capillary membrane

- Glomerular capillaries are relatively impermeable to proteins so that the filtered fluid, glomerular fluid (GF) is essentially protein free and devoid of cellular elements.
- The glomerular capillary membrane has three layers:
  - Endothelium of the capillary
  - Basement membrane
  - A layer of epithelial cells (podocytes) surrounding the outer surface of the capillary basement membrane.
- The endothelial cells lining the glomerulus are perforated by thousands of small holes called fenestrae.
- Surrounding the endothelium is the basement membrane composed of a meshwork of collagen and proteoglycan fibrillae which can filter large amount of water and small solutes.
- The final layer contains epithelial cells called podocytes lining the outer surface of the glomerulus. These cells are not continuous but consists of many finger like projections which form slit pores through which glomerular filtrate filters.
- Therefore, GF must pass through three different layers before it enters the Bowman's capsule.

- The permeability of the glomerular membrane is 100 to 1000 times as great as that of the usual capillary and is because of the pores of the endothelium which are approximately 100 nm diameter and also slit pores approximately 25 nm wide.
- Despite the tremendous permeability of glomerular membrane, it has a high degree of selectivity for the sizes of the molecules that it allows to pass.

Molecular weight	Permeability	Example
5000	1.0	Inulin
30,000	0.5	Very small protein
69,000	0.005	Albumin

- Therefore, plasma proteins are completely impermeable.

Reasons for high degree of selectivity

- Size of the molecule: Pores in the membrane allow molecules with a diameter upto 8 nm.
- Pores are lined with a strong negative charges and electrostatic repulsion of the protein molecules (proteins are electronegative) prevent their filtration.

Dynamics of Glomerular filtrate

- The energy for the filtration is provided by the heart in the form of hydrostatic pressure of the blood inside the glomerular capillaries and the colloidal osmotic pressure of the fluid within the Bowman's space through the capillary membrane into the Bowman's capsule. On the other hand, colloid osmotic pressure in the blood and the hydrostatic pressure in the Bowman's space oppose filtration.
- The colloidal osmotic pressure in the Bowman's capsule is negligible due to very low protein content. The colloidal osmotic pressure in the glomerular capillaries increases, since $1/5^{th}$ of the fluid portion of the plasma in the capillaries filters into the capsule increases the protein concentration about 20% as blood passes from arterial to venous end of the glomerular capillaries.
- The colloidal osmotic pressure of the blood entering the capillaries is 28 mm Hg which rises to 36 mm Hg by the time the blood reaches the venous side and so the average colloidal osmotic pressure is 32 mm Hg.

Filtration pressure

- It is the net pressure forcing the fluid through the glomerular membrane equals to the glomerular pressure minus sum of glomerular colloidal osmotic pressure and capsular pressure.
- For example, If Glomerular Hydrostatic Pressure = 60 mm Hg, Colloidal Osmotic Pressure in glomerulus = 32 mm Hg and capsular pressure (Bowmans capsule Hydrostatic Pressure) = 18 mm Hg
- Then, the Filtration pressure = 60-(18+32) = 10 mm Hg.

Tubular transport

- Transport of fluid from the Bowman's capsule to the renal pelvis is accomplished by a difference in the hydrostatic pressure.
- Tubular reabsorption involves transport of water and solutes from the tubular fluid to the peritubular capillaries.

Tubular secretion

- It is the transport of solute from the peritubular capillaries to the tubular fluid.

TUBULAR REABSORPTION

- Reabsorption plays a major role than the secretion in the formation of urine. More than 90% of the water in the GF is reabsorbed as it passes through the tubules. Some substances such as sodium, glucose and amino acids are almost completely reabsorbed so that their concentration decreases almost to zero before the fluid becomes urine so that they are conserved by the body and not excreted and lost by the urine.
- Basic mechanisms of absorption is by two process
  - Active transport
  - Passive transport

Active transport through the tubular wall

- Tubular epithelial cells have a brush border at the luminal surface. It is composed of microvilli which increases surface area of the lumen. The base of the cell rests on the basement membrane. Basal channels in the basement membrane increases the surface area. Epithelial cells are attached to each other near the brush border forming tight junction or zona occludens.
- Sodium, glucose, amino acid, calcium, potassium, phosphate and urate ions are actively transported. They are transported through carrier proteins.

- Uniport
  - Transport by a carrier for a single compound (e.g., sodium) and is unidirectional.
- Symport or co-transport
  - Transport of two compounds on the same carrier in the same direction (e.g., sodium plus glucose, or sodium plus amino acid).
- Antiport or counter transport
  - Movement of a compound in one direction driven by the movement of a second compound in opposite direction (e.g., Na^{2+} and H^{+} antiport).
- Sodium reabsorption
  - About 65% of Na^{2+} is reabsorbed in the proximal tubule. The energy required for this mechanism is derived from the Na^{2+}-K^{+} ATPase (sodium pump) located in the basolateral membrane of the proximal tubule epithelial cells.
- Electrochemical Gradient

- Sodium ions are actively transported from the interior of the tubular epithelial cells to the peritubular space across the basal membrane. Therefore, intra-cellular Na^{2+} is lowered, creating a chemical gradient for Na^{2+} (lumen concentration higher) between the tubular lumen and tubular epithelial cell. It also causes a low negative intracellular electrical potential (-70 mv) which in turn causes the Na^{2+} to diffuse from the tubular lumen into the cell through the brush border. This mechanism is uniport or unidirectional Na^{2+} transport. Chloride ion readily diffuses from the tubular lumen to the peritubular space through tight junction between tubular epithelial cells because of transepithelial electrical potential difference (lumen negative) created by Na^{2+} transport.

- Antiport or counter transport of sodium ion
  - Diffusion of Na^{2+} because of electrochemical gradient is coupled through a carrier protein with H^{+} diffusing in the opposite direction from the cell interior to the tubular lumen. HCO_3^- in the cell can diffuse through the basolateral membrane to the peritubular space or move into the tubular lumen in counter transport to Cl^- diffusion into the cell.
- Chloride driven sodium ion transport
  - As more of HCO_3^- is being reabsorbed in to the peritubular space Cl^- gradient favours diffusion of Cl^- through the leaky tight function from the tubular lumen into the peritubular space and is accompanied by diffusion of Na^{2+} in the same direction to maintain electrical neutrality.
- Glucose and amino acid reabsorption
  - These are reabsorbed by symport or co-transport. They are coupled with specific carriers that require Na^{2+} binding and diffuse into the cell. Inside the cell Na^{2+} and glucose or amino acid separates from the carrier. The Na^{2+} is actively transported by Na^{2+}- K^{+} ATPase to the peritubular space. Glucose and amino acids are then transported by facilitated diffusion.

Passive transport of water and other solutes

- After the diffusion of solute (Na^{2+}, Cl^- , HCO_3^-, glucose, amino acid) into the peritubular space, an osmotic gradient is established, where by a greater osmotic pressure is present in the peritubular space. Therefore, water diffuses from the peritubular lumen and tubular cells into the peritubular space.
- As 65% of Na^{2+} is reabsorbed, similarly 65% of water is reabsorbed from the proximal tubule (an additional amount for other osmotically active substances such as glucose, amino-acid).
- As water is reabsorbed, urea and other non-actively reabsorbed solutes get concentrated in the tubular lumen.
- A chemical concentration gradient is established for these substances and they are reabsorbed down their concentrated gradient. The extent of their reabsorption depends on the permeability of the tubular epithelium for the solute.
- Urea permeability for the proximal tubule is much less than that of water and more than 50% of the amount of urea in the GF continues beyond the proximal tubule. There is no

permeability of tubular membrane for reabsorption of creatinine, inulin, mannitol and sucrose and therefore once these are filtered, their total quantity appears in the urine.

- Reabsorption of proteins and peptides
  - Proteins with a molecular weight of less than 69,000 are reabsorbed in the proximal tubule and not lost in the urine. They are reabsorbed by endocytosis and subsequently degraded by cellular lysosomes to amino acids. The amino acids move from the cell to the peritubular space by facilitated diffusion. Small peptides are hydrolyzed at the luminal brush border of proximal tubule and the resultant amino acids is taken into the cell by co-transport mechanism.

TUBULAR SECRETION

- Several substances are transported from the peritubular capillaries into the interstitial fluid and then to the tubular lumen via the tubular epithelial cells.
  - Antiport of H^+ along with Na^{2+} reabsorption in the proximal tubule and distal tubule. H^+ is secreted throughout the nephron.
  - K^+ transport is unique, in that it is reabsorbed in some parts of the tubule and secreted in others. It is reabsorbed in the convoluted portion of proximal tubule and secreted in the straight portion of proximal tubule. It is secreted and reabsorbed in the distal nephron.
  - Ammonium ions are synthesised in the epithelial cells and diffuse into the tubular fluid.

ABSORPTIVE CAPABILITIES OF DIFFERENT TUBULAR SEGMENTS

- Proximal tubule:
- 65 % of the reabsorption and secretion take place. Only 35% of the GF leaves the proximal tubule.
- Thin segment of Loop of Henle:
  - Permeability of the epithetical cells of descending limb of Loop of Henle is great and occurs by simple diffusion. It is highly permeable to water and moderately permeable to urea and Na^{2+}.
- Ascending limb of Loop of Henle:
- It is less permeable to water and more permeable to urea.
- Thick segment of Loop of Henle and distal tubule
- They have rudimentary brush border and cells adapted for Na^{2+} transport against concentration gradient. They are impermeable to water and urea. Here, active absorption of Na^{2+} and active secretion of K^+ is controlled by aldosterone.
- Collecting tubule
  - Final concentration of urine takes place in the collecting tubule. It has two functional units, cortical and medullary portion. Cortical portion is impermeable to urea and medullary portion is moderately permeable to urea. Permeability of collecting tubule to water is determined by the concentration of Antidiuretic hormone (ADH) in the blood. Large amount of ADH causes

collecting tubule to be highly permeable to water. In the absence of ADH, very little water is reabsorbed. They can secrete H^+ into the tubule.

REABSORPTION AND SECRETION IN DIFFERENT REGIONS OF TUBULES

- Water transport is by osmotic diffusion.
- Proximal tubule : 65% reabsorption.
- Loop of Henle : 15%.
- Distal tubule : 10%.
- Collecting duct : 9.3%.
- Urine : 0.7%.
- Glucose, proteins, amino acids, vitamins and acetoacetate ions are completely reabsorbed by active process in proximal tubule.
- 50% of urea is reabsorbed.
- Creatinine is not reabsorbed but some quantities are secreted in proximal tubule.
- About 86% of urate ions are reabsorbed.
- Sulphate, phosphate and nitrates are transported similarly like urates.
- K^+ ion is secreted in distal tubule and collecting tubule.
- H^+ ions are actively secreted in proximal tubule, distal tubule and collecting tubule.

Transport maximum

- Substances such as glucose that are actively absorbed by a carrier transport, there is a maximum rate at which they can be reabsorbed. This is known as transport maximum (T_m).
- When T_m is exceeded in the nephron, it appears in the urine, e.g., in Diabetes mellitus, the movement of glucose from the plasma to the cells is impaired because of lack of insulin. Glucose concentration increases causing plasma and tubular loads to increase. When increased tubular load exceeds the availability of the carriers molecules for glucose reabsorption, excess glucose flows through the tubules into to the urine. As glucose is retained within the tubules it contributes to an increase in osmotic pressure and therefore water also remains in the tubular fluid. The point at which the glucose first begins to appear in the urine, 175 mg/dl is known as the renal threshold for glucose. The T_m for the kidney is reached when all the nephrons are reabsorbing to their maximum ability.
- T_m for glucose = 320 mg/min

FUNCTIONS OF THE URINARY BLADDER

- It provides an expandable reservoir for urine, which is continuously flowing from pelvis of the kidney through ureters.

Micturition

- It is the process in which the urinary bladder empties when it becomes filled with urine.

Passage of Urine from the kidney to the bladder

- Urine is secreted continuously though at a varying rate and it passes through the collecting ducts into the pelvis of the kidney and carried to the urinary bladder by the ureters. The ureters contain muscles capable of contraction, which helps in propulsion of urine along the tube into bladder. Each ureter is innervated by both sympathetic and parasympathetic nerves. As urine collects in the pelvis, pressure increases which initiates peristaltic contraction beginning from pelvis and spreading down along the ureters to force urine towards bladder.
- Parasympathetic stimulation increases frequency and sympathetic stimulation decreases frequency of peristalsis of ureters.

Filling and emptying of bladder (Micturition Reflex)

- At the junction of ureter with bladder, an ureterovesicular valve is present which prevents reflux of urine from bladder. The urinary bladder possess the power of accommodation to increase in its contents without increase in internal pressures up to about 150 mm H_2O. So, as urine enters the bladder, its walls becomes distended. Out flow of urine into the urethra is prevented by sphincters at the neck of the bladder (internal and external sphincters).
- When the pressure in the bladder reaches 150 mm water, contraction of bladder wall begins, relaxation of sphincter occurs and urination or micturition occurs. Contraction of abdominal muscles and contraction of diaphragm assist the emptying of the bladder. The urinary reflex may be assisted by voluntary effect; may also be opposed voluntarily. This is accompanied by inhibition of spinal centres of micturition and by contraction of external sphincter which surrounds the external part of the urethra. The desire to urinate arises from stimulation of receptors in the wall of the bladder by stretch and contraction of musculature.
- Nerves from sympathetic and parasympathetic divisions of ANS supply the bladder. The preganglionic sympathetic fibres leave from spinal cord in 2^{nd} to 4^{th} lumbar nerves to the posterior mesentric ganglion and postganglionic fibres reach the bladder through hypogastric nerves.
- In all mammals, parasympathetic nerves cause contraction of whole bladder and they are main motor nerves. They are inhibitory to internal sphincter.
- Complete emptying of the bladder depends upon maintenance of bladder muscle contraction and sphincter relaxation. This is achieved by two reflex systems.
- Receptors are present in the bladder walls that are stimulated during contraction of urinary bladder. Another reflex arises from receptors in the wall of urethra. Urine flow through urethra helps to maintain bladder contraction and relaxation of external sphincter.
- The tone of the bladder decreases as the bladder becomes emptied of its contents and this is accompanied by contraction of sphincter.
- The cessation of micturition involves voluntary control and voluntary regulation.

UREMIA OR AZOTEMIA

- Urea is the chief nitrogenous end product of protein metabolism and is excreted by the kidneys in the urine of mammals. It is also found in the blood and lymph. Uremia is a toxic condition that occurs due to retention of urea is the blood. It results due to
  - oRenal failure.
  - oIncreased production of urea in the liver due to high protein diet, drugs, increased breakdown of protein etc.
  - oDecreased elimination of urea due to reduced blood flow to kidney, obstruction of urinary tract etc.
  - oDehydration
  - oChronic infection of the kidney.
- The symptoms of uremia are lethargy, depression, nausea, vomiting, deep breathing, dizziness, coma and convulsions. In these cases, there is usually a smell of urine in all the animal's secretions.

TERMINOLOGIES

- Diuresis: Increased urine formation.
- Polyuria: Increased excretion of urine. It may be due to the deficiency of ADH.
- Oliguria: Reduced excretion of urine
- Anuria: Complete cessation of urine formation.
- Dysuria: Difficult or painful micturition.
- Stranguria: Slow dropwise painful discharge of urine caused by spasm of urethra and bladder.

MODULE-27: RENAL FUNCTION TESTS

Learning objectives

- This module deals with the tests required to detect functional disorders of the kidney.

KIDNEY FUNCTION TESTS

- Renal clearance is the measurement of the kidney's ability to remove substances from the plasma.
- Clearance measurements are used to determine Renal Blood Flow (RBF), Renal Plasma Flow (RPF), Glomerular Filtration Rate (GFR), Filtration Fraction (FF) and how different substances are handled by the kidney tubules (reabsorbed or secreted) and to compare the kidney function values for diagnostic purposes.

PLASMA CLEARANCE

- It is used to express the ability of the kidneys to clean or clear the plasma of various substances. e.g., If the plasma passing through the kidneys contains 1 mg of a substance in each ml and 1 mg of this substance is also excreted into the urine each minute, then 1 ml/min of the plasma is cleared of the substance.

- Plasma clearance is an excellent measure of kidney function and the clearance rate of different substances are determined by analyzing the concentration of substance simultaneously in plasma and urine and measuring the rate of urine formation.

CLEARANCE AS AN ESTIMATE OF GFR

- A substance to measure GFR must be freely filtered at the glomerulus and should not be reabsorbed or secreted by the tubular epithelium after it enters the nephron. Inulin, a fructose polysaccharide is the substance that is most commonly used.

$$GFR = \frac{U_x \times V}{P_x}$$

- Mannitol, is another polysaccharide used to estimate GFR.

CREATININE CLEARANCE

- In clinical conditions it can be used to measure GFR and kidney functions. Creatinine is freely filtered and not reabsorbed by the tubules.
- In some species (not in dogs) about 10% is secreted by the tubules. It can't be used in birds because creatinine is either secreted or reabsorbed from the tubules to a greater extent.

MEASUREMENT OF RPF

- The substance used must be freely filtered at the glomerulus and must not be reabsorbed from the tubular lumen and must be secreted by the tubular epithelium so that all of the substances in the blood perfusing the tubules is removed before the blood leaves the kidney. Therefore, if all the substance in the plasma that perfuses the kidneys is excreted in the urine the rate of its excretion is the same as its plasma load.
- Para Amino Hippuric acid (PAH) is

PLASMA LOAD AND TUBULAR LOAD

- Plasma load of a substance is the total amount of substance in the plasma that passes through the kidneys each minute.

- Eg: If the concentration of glucose in the plasma is 100 mg/100 ml and 600 ml of plasma passes through both the kidneys each minute, then the plasma load of glucose is 600 mg/min.
- A fraction of plasma load that is filtered as GF is referred to as tubular load.
- For example, if 125 ml of GF is formed each minute with a glucose concentration of 100 mg% then the tubular load of glucose is 125 mg (100 x 1.25) glucose/min.
- used to measure RPF.

MODULE-28: COUNTER CURRENT MECHANISM

Learning objectives

This module deals with

- renal mechanism of urine formation
- hyperosmolality of the medullary fluid
- permeability of the tubules
- counter current mechanism
- excretion of dilute urine
- excretion of a concentrated urine

RENAL MECHANISM OF URINE FORMATION

Renal mechanism of concentrated and dilute urine formation

- Concentrated urine is formed by passive water reabsorption from the tubules while many solutes in the tubular fluid are absorbed by active process.
- Dilute urine is formed by absorption of solutes alone and excretion of water in the urine.

Dilution or concentration of urine depends on

- Hypertonicity/osmolality of the interstitial fluid in the renal medulla.
- Dilution of the tubular fluid by the thick ascending limb and distal tubules by solute reabsorption which allows formation of dilute urine.
- Variation in the water permeability of collecting ducts due to ADH, which determines the final concentration of urine.
- For concentration of urine, there is generation of hypertonic medullary fluid and increase water reabsorption in the distal tubule.
- For generating hypertonic medullary fluid, *two factors* are essential:
    - Reabsorption of osmotically active substance by the tubules into the medulla.
    - Removal of water from the interstitium by the vasa recta.
- These two factors are produced by counter current mechanism.

HYPEROSMOLALITY OF THE MEDULLARY FLUID

- Normal osmolality of the GF as it enters the proximal tubule is 300 milli osm/L. But osmolality in the medullary interstitial fluid is higher reaching a maximum in the inner most regions of the medulla, it increases from 300 milli osm/L to 1200 milli osm/L. The cause of this increased osmolality is the active transport of both sodium and chloride out of the Loop of Henle's ascending limb and slight reabsorption of sodium actively from the collecting tubule into the interstitial fluid.

Also the cause for accumulation of sodium chloride in medulla is:

- Sluggish blood flow through vasa recta which helps to prevent removal of sodium chloride from the interstitial fluid.
- Presence of counter current mechanism in the Loop of Henle and vasa recta.

PERMEABILITY OF THE TUBULES

- Descending limb of Loop of Henle: High permeability for water and no permeability for sodium, chloride and urea
- Ascending thin limb of Loop of Henle: No permeability for water, highly permeable to sodium, chloride and moderately permeable to urea.
- Ascending thick limb of Loop of Henle: Permeable to sodium, chloride, low permeability to water and urea.
- Distal tubule: Permeable to sodium, chloride and low permeability for water and urea.
- Cortical collecting tubule, outer medullary collecting duct and inner medullary collecting duct: Sodium reabsorption is stimulated by aldosterone and water and urea reabsorption by ADH.

COUNTER CURRENT MECHANISM

- A counter current system of tubules or vessels exists where the inflow of fluid runs parallel to, counter to, and in close proximity to the outflow for some distance.
- These characteristics are common to the anatomical arrangements of the Loops of Henle and vasa recta. In the kidney, two counter current systems operate.
    - oCounter current multiplier - Loops of Henle
    - oCounter current exchanger – Vasa recta

Counter current multiplier

- The ascending thick limb of the Loop of Henle is permeable to solutes and so the solutes diffuse into the medullary interstitial fluid with retention of water in the tubule, thereby diluting the tubular fluid. This creates a small osmotic gradient between the tubular and peritubular fluids (interstitial fluid). This osmotic gradient is being multiplied vertically

by counter current flow in the descending thin limb (permeable for water and not for solutes).

- Water diffuses from the lumen of the descending thin limb into the interstitial fluid. Therefore, the tubular fluid of the descending thin limb increases in osmotic concentration as it descends to the inner most region of the medulla. When thin tubular fluid enters the ascending thin limb (permeable for solute and not for water), sodium chloride diffuses readily outward into the inner medullary interstitial fluid and urea diffuses inward into the tubular fluid.
- Continued active secretion by the ascending thick limb, concentration of tubular fluid in the descending thin limb and diffusion from the lumen of the ascending thin limb into the medullary interstitial fluid establishes a vertical osmotic gradient. Therefore, each time sodium chloride makes the circuit around the Loop of Henle, this multiples the concentration of sodium chloride in the medulla and hence, Loop of Henle is called as counter current multiplier.

Countercurrent exchanger - Vasa recta

- It is a counter current system in which transport between the outflow and inflow is entirely passive. Vasa recta is permeable to water and solutes through out their length.
- In the descending limb of the vasa recta, water is drawn by osmosis from the plasma of vasa recta to the hyperosmotic peritubular fluid (created by counter current multiplier) and the solutes diffuse from the peritubular fluid into the vasa recta.
- In the ascending limb, solutes diffuse back into the peritubular fluid and water is drawn by osmosis back into the vasa recta. Net result is that the solutes responsible for medullary gradient are mostly retained in the medulla and the vasa recta carry only slightly more solutes than are brought to them.
- Blood flow in the vasa recta is sluggish because an increased rate of medullary blood flow results in decreased time required for diffusion of solute from the ascending limb back to peritubular fluid resulting in gradual loss or wash out of medullary gradient. All the excess salt removed from the interstitial fluid has to be replaced to maintain an osmotic gradient.

Recirculation of urea

- It is a mechanism for concentration of urea in the medulla. Urea is concentrated in collecting tubule and diffuses through the walls of collecting tubule into the medullary interstitial fluid. From there, it is reabsorbed in the Loop of Henle and flows with tubular fluid in the ascending limb through distal tubule into collecting tubule and again out of the collecting tubule into the medullary interstitial fluid. Urea circulates several times before it flows into the urine and it causes urea to accumulate in high concentration in medullary interstitium. This counter current multiplier system helps in concentration of urine and also ensures constant excretion of urea when urine output is low.
-

EXCRETION OF DILUTE URINE

- When there is excess of water in the body and a reduction in plasma osmolarity, kidneys can excrete a dilute urine with a concentration as low as 50 mOsm/L. This can be achieved by reabsorbing only the solutes and not water in the distal parts of the nephron.
- The total amount of solute excreted remains constant but the urine formed is very dilute and urine osmolarity decreases from 600 to about 100 mOsm/L.
- The glomerular filtrate initially formed has the osmolarity similar to that of plasma (300 mOsm/L). Inorder to excrete excess water it is necessary to dilute the filtrate as it passes along the tubule which is achieved by reabsorption of solutes to a greater extent than water.
- As the fluid flows through the proximal tubule solutes and water are reabsorbed in equal amount and there is a little change in osmolarity but when it reaches down the descending limb of loop of Henle water is reabsorbed by osmosis and the tubular fluid reaches the osmolarity of renal medulla, *i.e*. it becomes hyperosmotic.
- In the ascending limb of loop of Henle sodium, potassium and chloride are actively reabsorbed. Therefore, the fluid becomes dilute as it flows to distal tubule with osmolarity decreasing to 100 mOsm/L (osmolarity is one third of that of plasma).
- When the fluid passes to late distal tubule and collecting tubule there is little reabsorption of sodium chloride. In the absence of ADH, collecting tubule is impermeable to water and additional reabsorption of solutes causes tubular fluid to become even more dilute, decreasing its osmolarity to as low as 50 mOsm/L.
- Therefore, failure of reabsorption of water and continued reabsorption of solutes leads to a large volume of dilute urine.

EXCRETION OF A CONCENTRATED URINE

- For formation of a concentrated urine two basic requirements are essential
    - oHigh level of ADH which increases the permeability of distal tubule and collecting tubule to water.
    - oHigh osmolarity of the renal medullary fluid which provides osmotic gradient for reabsorption of water in the presence of ADH.
- When the tubular fluid leaves the loop of Henle and flows into the distal convoluted tubule in the renal medulla, fluid is dilute with a osmolarity of 100 mOsm/L.
- As fluid flows into the cortical collecting tubule, the amount of water reabsorption is dependent on plasma concentration of ADH. In the presence of high concentration of ADH the cortical collecting tubule becomes highly permeable to water and large amounts of water are reabsorbed.
- As the fluid flows into the medullary collecting tubule there is further water reabsorption and when the fluid reaches the end of the collecting ducts the osmolarity reaches 1200 mOsm/L which is similar to renal medullary osmolarity.

- Therefore, by reabsorbing as much as water as possible, kidneys form a highly concentrated urine excreting normal amount of solutes in urine and increasing the ECF volume.

HORMONAL REGULATION OF RENAL FUNCTION

ADH and water conservation

- ADH is synthesised in the cell bodies of hypothalamic nuclei (supraoptic nuclei) and transported to nerve fibre endings in the posterior lobe of the pituitary where it is stored in the secretory granules.
- Its release in the blood is controlled by osmoreceptors in the hypothalamus that are close to the supraoptic nuclei. It regulates water conservation. Increase in plasma osmolality stimulates osmoreceptors in the hypothalamus causing release of ADH which decreases the ECF volume. Fear and pain also causes release of ADH.

Aldosterone

- Adrenal cortex regulates K^+ and Na^+ concentration. It acts on tubules causing Na^+ reabsorption and K^+ excretion. Aldosterone increases Na^+ reabsorption from distal tubules by increasing Na^+ transport protein; salt free diet causes increased aldosterone secretion resulting in increased Na reabsorption.

Renin - Angiotensin system

- Renin is activated by reduced circulating blood volume as in hemorrhage. Decreased sodium concentration in the distal convoluted tubule and sympathetic stimulation also causes release of renin.

Parathyriod hormone

- Ca^{2+} and PO_4 excretion in urine is regulated at the proximal tubule by the action of parathyroid and thyrocalcitonin from thyroid. Parathyroid hormone (PTH) causes decrease in PO_4 reabsorption and increase in PO_4 excretion in urine. PTH increases mobilisation of Ca^{2+} from bone and absorption from intestine resulting in increased plasma Ca^{2+} level and decreased Ca^{++} excretion.

Atrial natriuretic peptide

- Myocardial cells of the atria release the ANP when the atria are stretched during high volume of blood. It has the following functions.
  - Increases the GFR by causing vasodilatation of afferent arterioles and vasoconstriction of efferent arterioles.
  - Inhibits angiotensin II stimulated absorption of Na^+ and water in proximal tubules.
  - Reduces water reabsorption in collecting tubules.

- Inhibits aldosterone release.
- Decreases the response of the collecting tubules and collecting ducts to ADH.

Local hormones

- Erythropoietin produced from kidney regulates erythropoiesis.
- Renin is produced from kidney.
- Prostaglandin from kidney acts as blood pressure lowering agents. PGE is natriuretic.

MODULE-29: ACID BASE BALANCE

Learning objectives

This module deals with

- various buffer systems of the body
- how kidney plays a major role in maintaining pH of the blood?

INTRODUCTION

- The normal blood pH is 7.4. Maintenance of normal blood and extracellular pH within the narrow limits is essential for homeostasis. The pH usually refers to the hydrogen ion (H^+) concentration and has a widespread effect on the function of the body systems. Any disturbance in the H^+ ion concentration leads to imbalance of pH.
- When pH rises above the optimal value it is referred to as alkalosis and if pH drops below optimum level it is referred as acidosis.

Regulation of hydrogen ion concentration

- Three primary buffer systems are involved in regulation of H^+ ion concentration in the body fluids to prevent alkalosis or acidosis.
  - Chemical acid base buffer systems
  - Respiratory regulation of acid base balance
  - Renal control of

CHEMICAL ACID BASE BUFFER SYSTEMS

- When there is a change in the H^+ ion concentration, the body fluids react immediately to minimise the change. Chemical buffers act by converting either strong acids or bases into weaker acids or bases. The various chemical buffers are:
- Bicarbonate
  - This is the most important buffer system in the body. Bicarbonate combines with H^+ ions to form carbonic acid in the tubular fluid, which then dissociates to CO_2

and H_2o. The CO_2 formed is removed by the lungs and the HCO_3^- formed in the cells is reabsorbed from the filtrate to the blood.

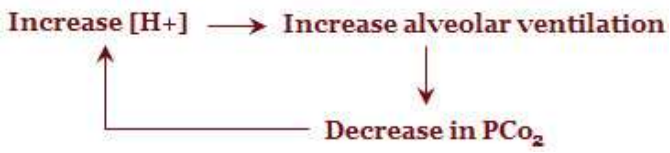
- Phosphate buffer
  - It plays a major role in buffering renal tubular fluid and ICF. The two main elements of the phosphate buffer are HPO_4^{2-} (base) and $H_2PO_4^-$ (weak acid). Hydrogen ions from strong acids are captured by converting a weak base to a weak acid and strong base captured by conversion of a weak acid to a weak base.
- Protein buffer
  - It is an intracellular buffer present in high concentrations in the blood. Hemoglobin molecule forms the second most important blood buffer and is present in the form of proteinate ions (Hb^-). These basic ions, with their weak acids (HHb) form a buffer pair. When an acid is added to the blood,

  - This reaction shifts to the right and the ratio of base to acid is decreased. Hemoglobin is a powerful buffer and it binds with protein, CO_2 and H^+ ions. In the tissues CO_2 passes into the RBC where it combines with water to form carbonic acid which is catalyzed by the enzyme carbonic anhydrase. Carbonic acid then dissociates into HCO_3^- and H^+ ions. The H^+ ions binds to hemoglobin to form HHb and the HCO_3^- ions passes back to the plasma in exchange for chloride ions. In the lungs this process is reversed where in H^+ ions bind to hemoglobin and recombine with bicarbonate to form CO_2 which passes into the alveoli.
- Ammonia buffer system
  - Ammonia is formed by the hydrolysis of glutamine in the presence of enzyme glutaminase in the tubular cells which freely diffuses into luminal fluid and continues with H^+ ions to form NH_4^+ ions. This NH_4^+ ions combines with chloride ions and is excreted as ammonium chloride in the urine.
  - acid base balance

RESPIRATORY REGULATION OF ACID BASE BALANCE

- The respiratory system acts as the second line of defense against acid base disturbances. An increase in PCO_2 of ECF, decreases the pH, while a decrease in PCO_2, increases the pH. Therefore, by adjusting the PCO_2, the lungs effectively regulate the H^+ ion concentration of the ECF.
- An increase in ventilation removes CO_2 from ECF thereby reducing the H^+ ion concentration. Similarly, a decrease in ventilation, increases CO_2 thus increasing H^+ ion concentration in ECF.
- Arterial PCO_2 is inversely proportional to alveolar ventilation, i.e., if alveolar ventilation falls, PCO_2 rises. Therefore, relatively small changes in ventilation has a profound effect on H^+ ion concentration and pH.
- Respiratory system acts as a typical negative feedback controller of H^+ ion concentration.

- An increase in the H^+ concentration above the normal, stimulates the respiratory system and alveolar ventilation increases. This decreases the PCO_2 in ECF and reduces H^+ ions concentration back to normal.



RENAL CONTROL OF ACID BASE BALANCE

- The kidneys regulate acid-base balance by excreting either an acidic or basic urine.
- Excretion of either an acidic or a basic urine removes acids or basic from the ECF. Large numbers of HCO_3^- ions are filtered in the urine and if they are excreted into the urine, it removes base from the blood. Similarly, large numbers of H^+ ions are secreted into the urine, and if they are excreted, it results in loss of acid from the blood. If more HCO_3^- ions are filtered than the H^+ secreted, there will be net loss of base from ECF.
- The kidneys regulate ECF H^+ ions through three basic mechanisms:
    - oTubular Secretion of H^+ ions
    - oReabsorption of filtered bicarbonate ions
    - oCombination of excess H^+ ions with phosphate and amino buffers

Tubular secretion of H^+ ions

- H^+ ions are secreted in the proximal tubule, thick ascending loop of Henle and distal tubule by sodium hydrogen counter transport. This occurs by means of active transport of sodium ions into the cell and H^+ ions from the tubular cell into the tubular lumen against the concentration gradient provided by sodium-potassium ATP pump. For each H^+ ion secreted, one HCO_3^- ion is reabsorbed. When CO_2 diffuses into the tubular cells, formed by metabolism, CO_2 combines with water, forms H_2CO_3 which dissociates into HCO_3^- and H^+ ions. H^+ ions are secreted from the cell into the lumen by sodium-hydrogen counter transport.
- The sodium moves into the cell by concentration gradient established by sodium-potassium ATP ase pump in the basolateral membrane. H^+ ions are also secreted in the distal tubule and collecting ducts and transported through H^+ pump by H^+ ATP ase mechanism.

Reabsorption of filtered bicarbonates

- The filtered bicarbonate ions are not easily reabsorbed across the tubular membrane. The bicarbonates combines with H^+ ions to form H_2CO_3 which then dissociates to form Co_2 and H_2O. This CO_2 moves across the tubular membrane and diffuses immediately into the tubular cell.

- Inside the cell, it combines with H_2O to from H_2CO_3 in the presence of carbonic anhydrase and H_2Co_3 dissociate to HCO_3^- and H^+ ion. Therefore for energy H^+ ion formed in the tubular epithelial cells, a HCO_3^- ion is formed and reabsorbed into the blood.

Combination of excess H^+ with phosphate buffer

- When excess of H^+ ions are secreted, only a small fraction of is excreted in the ionic form (H^+) in the urine and the remaining H^+ ions combines with buffers such as phosphate and ammonia buffer in the tubular fluid as urine can be acidified to a pH of about 4.5.
- The phosphate buffer system is composed of HPO_4^{2-} and $H_2PO_4^-$. Both are concentrated in the tubular fluid because of poor reabsorption. Excess H^+ ions combines with HPO_4^{2-} to form H_2PO_4 which in turn are excreted as sodium salt (NaH_2PO_4).

Combination of excess H^+ with ammonia buffer system

- This buffer system is composed of ammonia (NH_3) and ammonium ion (NH_4^+). Ammonium ion is synthesized from glutamine which is actively transported into the tubular epithelial cells. Inside the cell, glutamine is metabolised to form NH_4^+ and two HCO_3^- ions. The NH_4^+ is secreted into the tubular lumen by countercurrent mechanism in exchange for sodium which is reabsorbed. The HCO_3^- moves across the basolateral membrane along with reabsorbed Na^+ into the blood. Therefore for each molecule of glutamine metabolised in the proximal tubule, two NH_4^+ ions are secreted into the urine and two HCO_3^- ions are reabsorbed into the blood.
- In the collecting tubule, formation of NH_4^+ ions occurs by a combination of NH_3 (ammonia) with H^+ ions and then excreted as NH_4^+. The collecting ducts are permeable to NH_3 and form NH_4^+. Hydrogen ions react with NH_3 and form NH_4^+. For each NH_4^+ excreted, one HCO_3^- reabsorbed in to the blood.

ELECTROLYTES

- Sodium / Potassium: Sodium reabsorption and H^+ ion excretion are interlinked. Sodium reabsorption is controlled by the hormone, aldosterone and ion exchange proteins that exchange sodium for H^+ ions or K+ ions. Therefore, the changes in aldosterone secretion may influence acid-base balance.
- Chloride: The bicarbonate and chloride ions are the two abundant negative ions in the plasma. In order to maintain electroneutrality any change in chloride must be accompanied by opposite change in bicarbonate concentration. Therefore, chloride concentration may also influence acid base balance.

ACID BASE BALANCE DISTURBANCES

- The pH of the ECF is determined by the rate of conjugate base to their weak acids. The total amount of buffer base in whole blood including HCO_3, Hb and other bases of lesser importance is called buffer base (B.B). These bases are known as metabolic components determining blood pH. Acid base disturbance involves either the gain or loss of strong acid or the gain or loss of base (Cl^- or HCO_3^-) by the ECF.

Metabolic acidosis

- The gain of strong acid or loss of base from the ECF is known as metabolic acidosis. Acidemia will be present in metabolic acidosis. It occurs in:
  - Ketosis.
  - Diabetes mellitus in which ß hydroxy butyric acid, acetone, aecto acetic acid are produced.
  - Renal acidosis in which there is failure of HCO_3^- reabsorption and loss in the urine.
  - Diarrhoea where pancreatic juice containing HCO_3 is not reabsorbed and is lost.
- In all these cases, HCO_3^- falls either as a result of a reaction with acid or due to direct loss from ECF and pH falls. This results in fall of all blood buffer bases. Usually there is no change in plasma PCO_2 . However, a fall in pH results in increased alveolar ventilation and therefore a fall in PCO_2. Decreased PCO_2 will bring the ratio of conjugate base to weak acid back to normal. However, the total bases will be less than normal and this requires renal correction - the excretion of H^+ and restoration of plasma $HCO3^-$.
- The acidemia stimulates secretion of H^+ ion by the renal tubule. This ensures reabsorption of all HCO_3 ions from tubular fluid and the excess H^+ ions will begin to acidify the urine. For each H^+ ion secreted, one HCO_3 will be reabsorbed into the plasma. This holds good for short-term stress and in severe conditions, therapeutic action is required.

Metabolic alkalosis

- This process involves the gain of base (OH or HCO_3 ions) or loss of strong acid by ECF.
- Metabolic alkalosis is present in
  - Persistent vomiting, in which gastric acid is lost from the body.
  - K^+ deficiency in which renal tubules secretes large amount of H^+ ions into urine.
  - Injection of HCO_3 solutions.
- In all these cases, there is an increase in $HCO3^-$ in ECF, resulting in increased base content. The response of the body is opposite to the one observed in metabolic acidosis. Alkalemia results in rise in pH which will depress pulmonary ventilation and PCO_2 will rise. This respiratory compensation thus will bring pH back to normal. Renal correction consists of decreased secretion of pH ions and so increased excretion of HCO_3^- ions.

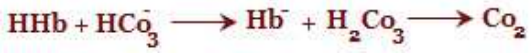
Respiratory acidosis

- If excretion of CO_2 by the lungs falls below the rate of CO_2 production in the body, respiratory acidosis develops.

- There will be an increase in blood PCO_2 (hypercapnia) and the primary defect will be in the inability of lungs to expire CO_2 at a normal rate. This may be due to
  - Depression of respiratory centres in CNS.
  - Abnormality of chest wall or respiratory muscles which prevents enlargement of thorax.
  - Obstruction to gas movement in lungs.
- A rise in PCO_2 causes increase in H_2CO_3 and buffer reaction prevents the fall of pH caused by rise in H_2CO_3.
- Renal compensation then follows. Low pH stimulates secretion of H^+ into urine with a rise in plasma $HCO3^-$.

Respiratory alkalosis

- When alveolar hyperventilation occurs, the expiration of CO_2 may exceed the rate of its production within the body and respiratory alkalosis develops.
- There will be low plasma PCO_2 (hypocapnia) and alkalemia.
- Hyperventilation is caused by abnormal stimulus to respiratory centres either directly as in NH_3 toxicity or through hypoxemia acting through peripheral chemoreceptors.
- Buffer reaction follows:



- Thus HCO_3 falls and Hb rises. The renal compensation begins, alkalemia depresses H^+ ion secretion by renal tubules and excretion of filtered HCO_3 rises. This results in further fall of plasma HCO_3 and the ratio of HCO_3 to H_2CO_3 moves back to normal.

MODULE-30: FLUID AND ELECTROLYTE BALANCE

Learning objectives

This module deals with

- maintainence of fluid and electrolyte balance by the kidneys during dehydration.

INTRODUCTION

- Regulation of fluid by the body is necessary to maintain homeostasis. If the water or electrolyte equilibrium is affected, many body functions fail to proceed at normal rates.

Water

- Water is a major constituent of all living things. Most of the ions and molecules that make up living matter have chemical and physical relationships with water.

- The total body water content varies among different species, age, sex, nutritional factors etc. Water content is highest in the new born animal and declines as age advances.
- An adult contains 60% of water by weight depending upon age and the amount of body fat. Body fat is inversely proportional to the body water content. For example, a very lean animal will contain 70% of water whereas very fat animal will have only 40% of total body water.

Body fluid Compartments

- Body fluid is present in three different compartments namely, intracellular fluid and extracellular fluid, which in turn is divided into interstitial fluid and plasma.
- In a lean animal, 50% of water is present intracellular, 15% in the interstitial spaces and 5% in the blood plasma. Apart from this, water is also present in the transcellular fluids such as in CSF, aqueous humor of the eye, synovial fluids, urine, bile etc.
- Water molecules can rapidly penetrate most of the cell membrane. If an osmotic or hydrostatic pressure gradient exists between body fluid compartments, a shift of water will occur. Addition of an isotonic NaCl solution to the ECF causes equal distribution of water extracellularly and intracellularly.
- If a hypertonic NaCl solution is added, water would begin to shift into the plasma, while, an addition of hypotonic NaCl solution shifts the water into the cell.

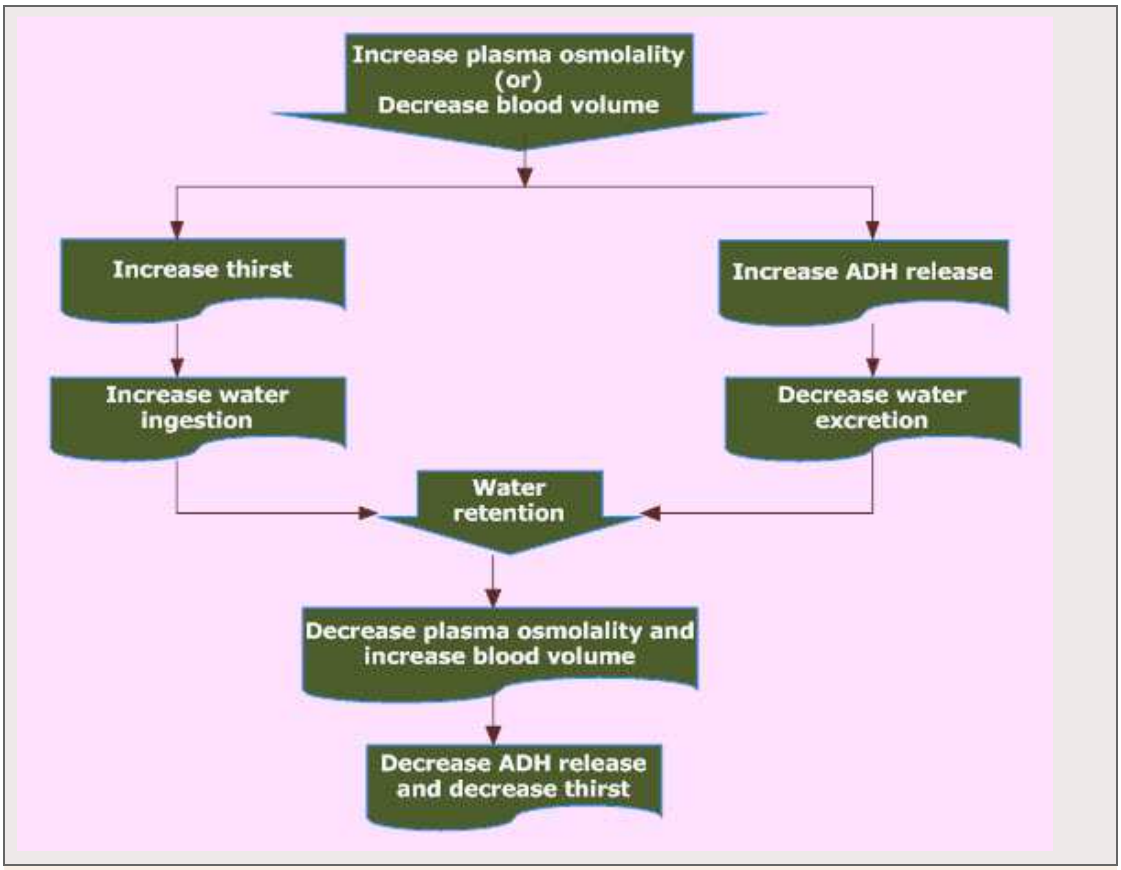
Water balance

- The total amount of water in the body almost remains relatively constant. The body gains water either by ingestion or as end product of cellular metabolism. Similarily loss of water occurs in urine, from the skin, expired gases, faeces etc.

THIRST

- Loss of water from the body is continuous and when there is deficiency of water, specific controls of water intake act to correct the deficit. Water deprivation causes the sensation of thirst and drive to drink water. Several mechanisms aid in the control of the amount of water to be ingested so that it equals the deficit of body water.
- A decrease in body water causes thirst which is characterized by dryness of the throat and mouth due to decreased salivary secretion. The centre for thirst and drinking behaviour is located in the hypothalamus of the brain.
- Water deficiency causes a rise in osmotic and sodium concentration in the ECF and a decrease in the circulating fluid volume together with a fall in BP.
- A fall in the BP is detected by the baroreceptors and the renin relating cells of the kidney which stimulates the thirst centre in the hypothalamus.
- As a result, thirst and drinking of water occurs. A fall in arterial BP alone also causes the release of renin from the juxta glomerular cells of the afferent arterioles of the kidney glomeruli. Renin acts on angiotensinogen to form angiotensin I, which in turn in is converted to angiotensin II by the converting enzyme. In most of the animals angiotensin II causes an increase in drinking water.

- During water deprivation, excretion of water by the kidney is controlled primarily by the Antidiuretic Hormone (ADH) secreted from the posterior pituitary gland. ADH acts on the nephrons of the kidney and causes increased reabsorption of water thereby decreasing the excretion of water with urine.
- The oxidation of food stuffs also yields some amount of water. The oxidation of each gram of carbohydrate, fat and protein yields about 0.6 ml, 1.1 ml and 0.4 ml of water. This metabolic water constitutes 5 to 10% of the total water intake in animals.



DEHYDRATION

- Dehydration in the animals results due to reduced or absence of intake of water and continuous loss of body water.
- Dehydration involves both loss of water and electrolytes. The process of dehydration may be slow or rapid depending on the relative rates of water and electrolyte loss. In a simple dehydration due to lack of water under moderate environmental conditions, causes the animals to seek and drink water. There will be a decrease in urine volume output. These changes can be seen when the dehydration is about 1-2% of the body weight.
- Severe dehydration occurs when the water loss is 10% of the body weight. The immediate source of water lost from the body is the ECF. If the rate of water loss is vey rapid, there

is drastic reduction in ECF volume. In slow dehydration, there will be a shift of water from the cells into the ECF.

- During water deprivation loss of water from the body causes an increase in ECF osmolality and a decrease in ECF volume. This stimulates the drinking behaviour and a reduced urine output. Electrolytes are also excreted from the body in proportion to water loss inorder to prevent a further rise in osmoconcentration.
- In early dehydration, sodium chloride is excreted in the urine. There is a shift of intracellular water to extracellular fluid. Cellular potassium is also excreted in the urine. Therefore, after a long period of dehydration depletion of both water and primary electrolytes occurs. In human, the limit to which the water can be lost is about 15-25% of the body weight before death occurs.

Adaptation to water lack

- Some of the species that are found in the desert regions such as camel, donkey, kangaroo rats etc., have acquired adaptation to resist water lack. During high summer temperature, these animals have to expend increased amount of water to control their body temperature in the desert regions as there is little amount of rainfall and natural vegetation which contains less water.
- Under these conditions, the animals have to obtain water either through vegetation or through metabolic water.
- The water obtained through these mechanisms are small as the actual amount of water formed from the oxidation of fat, carbohydrates and protein are 0.12 g/kcal, 0.14 kg/Kcal and 0.10 g/kcal.
- The camel has a remarkable ability to conserve water and can withstand a water loss of 25%. During the day when the heat stress is greatest, the camel can rise its body temperature thereby storing heat and saving water.
- During the cooler night this stored heat is dissipated and the body temperature is brought back to normal.
- The camel's summer fur also aid in reducing the solar heat gain. Camel conserves water by excreting dry faeces. The water lost through respiration also is very low in camels because of their ability to reduce the relative humidity of expired air to below 100%. This is due to the hygroscopic nature of the nasal secretions. The camel also has the ability to rehydrate rapidly as it can ingest $1/4^{th}$ of its body weight as water in a few minutes. The oval biconcave RBCs of the camel are highly resistant to osmotic hemolysis.
- The donkeys can also withstand dehydration upto 30% and can rehydrate rapidly by drinking 20.5 litres of water in 2.5 minutes.
- Sheep also has remarkable ability to withstand heat stress and water lack. It can resist 30% dehydration and minimize solar heat absorption by increasing the wool surface temperature upto 87°C. It dissipates heat by means of panting.

ELECTROLYTES

- An electrolyte in any chemical that dissociates to ions when dissolved in a solution. Ions can be positively charged (cations) or negatively charged (anions). The major electrolytes found in the body are sodium, potassium, calcium, magnesium, chloride, phosphate, sulphate and bicarbonate. The primary electrolytes of the ECF are sodium, chloride and bicarbonate and in the ICF are potassium and phosphates.

Composition of major electrolytes in the body fluids (mOsm/Kg H_2O)

Ions	Plasma	Interstitial Fluid	ICF
Sodium	146	142	14
Potassium	4.2	4.0	140
Chloride	105	108	4
Bicarbonate	27	28.3	10

Sodium

- It is the major cation of ECF. About 45% of sodium stored is found in ECF, 45% in the bone and the remaining intracelullarly. It plays an important role in the excitability of muscles and neurons and in regulating the fluid balance in the body.
- A constant sodium equilibrium in maintained in the ECF by two mechanisms-long term and short term control. The short term control is achieved by the ADH- thirst control system whereas long term control is by both ingestion and urinary excretion of sodium.

Short term Control (ADH-Thirst Control System)

- If ECF sodium rises, it stimulates the release of ECF and thirst. Water gained by this mechanism, dilutes the ECF thereby restoring normal sodium level. This increases the blood volume and a slight increase in BP, which in turn increases the GFR and excretion of excess sodium and water there by restoring the ECF volume to its normal level.

Longterm Control (Salt hunger/ Sodium ingestion)

- A deficiency of sodium in the ECF causes increased excretion of water due to inhibition of the release of ADH. A decline in osmolality decreases the GFR with a subsequent increase in reabsorption of sodium and water. Many sodium deficient animals have a strong behavioural drive to salt to replace the deficiency of sodium. This is called as salt hunger and is commonly seen in ruminants.
- An increase in angiotensin II stimulates the salt hunger. A decrease in the plasma sodium and BP stimulates the release of aldosterone from the adrenal cortex via the Renin-Angiotensin system. Aldosterone enhances the reabsorption of sodium from the renal tubule. Aldosterone also increases the secretion of potassium ions from the renal tubules into the lumen.

Urinary excretion of Sodium

- Sodium is filtered at the glomerulus and most of it is reabsorbed in the renal tubules. If the plasma sodium or the GFR increases, the amount of sodium filtered into the tubule will also increase but the reabsorption of sodium will not increase.
- An increase in the arterial BP results in the release of Atrial Natriuretic factor (ANF) from the atria of the heart. This hormone inhibits renin and aldosterone release which results in increased excretion of sodium and inhibition of reabsorption of sodium. Along with sodium, water will also be lost thereby bringing the BP back to normal level.

Potassium

- It is the major cation of intra cellular fluid and about 89% of the total body content of potassium is present in the ICF. Potassium is important for the functioning of excitable cells and in the regulation of fluid levels with in the cells.
- Potassium output in usually equal to the potassium input. Almost nearly all the potassium filtered by the kidney glomeruli is reabsorbed by proximal convoluted tubule and is secreted in the distal tubule and the collecting ducts. Potassium reabsorption by the tubular cells occurs by active transport of potassium into the cells in exchange for sodium through the Na/K ATPase system.
- Potassium concentration is regulated by the aldosterone. An increase in ECF potassium results in the aldosterone release, which increases the potassium excretion in the urine and returning of the plasma potassium to its normal level. Potassium is secreted in exchange for the sodium ion in the tubular cells. Hydrogen ion secretion competes with K^+ for Na^+ with which it exchanges. Therefore, an increased H^+ secretion depresses K^+ secretion. Likewise an increased reabsorption of Na^+ facilitates increased K^+ secretion. An excess of aldosterone results in hypokalemia whereas deficiency of aldosterone causes hyperkalemia.

Chloride and bicarbonate

- The sodium ions are balanced electrically with the chloride and bicarbonate ions. The chloride ions are regulated secondarily to sodium and bicarbonate ions.
- The excretion or reabsorption of sodium ions is accompanied by chloride ions. Similarily, chloride ions are excreted along with the bicarbonate ions to maintain electroneutrality in the ECF.
- The bicarbonate ion is unique in that it is formed or removed rapidly by the body.
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- Bicarbonates are formed and removed by carbon dioxide.

MODULE-31: FORMATION AND EXCRETION OF URINE IN BIRDS

Learning objectives

This module deals with

- structure of kidney in birds
- formation of urine in birds

EXCRETION IN BIRDS

- Urine formation in birds is almost similar to the mammals but still then there are some notable differences:
  - Presence of two major types of nephrons which are functionally different.
  - Presence of renal portal system.
  - Formation of uric acid instead of urea as the end product of nitrogen metabolism.
  - Post renal modification of the urine in the ureter.
- In the birds, the ureters transport the urine to the cloaca, which is the common collection site for digestive, reproductive and urinary organs.
- There is no urinary bladder in birds.

NEPHRON TYPES

- Avian kidneys are characterized by having two major types of nephrons:
  - Reptilian type and
  - Mammalian type
- The *reptilian type* nephrons are located in the cortex and it lacks the loop of Henle. It has no capacity to concentrate the urine, *i.e.*, there is no tubular transport system and whatever solute and water is present in the filtrate, directly passes to the cloaca.
- *Mammalian type* of nephrons have well defined loop of Henle. It has the capacity to concentrate the urine. In this tubular transport system is present.

RENAL PORTAL SYSTEM (RPS)

- An unique feature of avian kidney is its Renal Portal System (RPS) which provides an extra branch of blood flow to the renal tubules along with peritubular capillaries.
- Venous blood from portal vein gives one branch to the kidney and this branch provides the microcapillaries which perfuse the tubules along with peritubular capillaries.
- Both the branches of capillaries are interconnected and drained by the venous system to the posterior venacava. When it leaves these capillary network and enters the renal vein there is a valve which regulates the transition of the blood in these capillary network.

URIC ACID FORMATION AND EXCRETION

- The metabolic end product of protein and amino acids in reptiles and birds is the uric acid (instead of urea in mammals).
- Uric acid is formed in liver and also in kidneys from ammonia.
- Uric acid is freely filterable at the glomerulus, and it is also secreted by the tubules.
- Tubule secretion accounts for 90% of total uric acid eliminated.

- The renal portal system may provide a greater quantity of blood to the tubules for the secretion of uric acid by the tubules. Since greater quantity of uric acid is available in the tubules, which exceeds the solubility, the uric acid is precipitated. It passes through the tubules in the precipitated form and appears in the urine as a white coagulum. Since the uric acid is not in solution, it does not contribute to osmotic pressure, and thus avoids obligatory water loss.

MODIFICATION OF URETERAL URINE

- The post renal modification of urine is possible in the birds when the urine reaches the cloaca water is drawn back to colon and cecum due to antiperistaltic movement of colon.
- So when urine is exposed to colon and cecum for water absorption sodium is also reabsorbed.

CONCENTRATION OF AVIAN URINE

- Renal response to ADH in birds is similar to that of the mammals.
- In addition to the action of ADH on the tubular cells it also controls the functioning of reptilian and mammalian type of nephrons.

URINE CHARACTERISTICS AND FLOW

- Birds urine when mixed with faeces is cream coloured and contains thick mucous.
- The precipitated uric acid is mixed with mucus and mucus facilitates the carrying of uric acid in the urine.

MODULE-32: LYMPH AND CSF

Learning objectives

This module deals with

- formation of lymph and cerebrospinal fluid
- composition of lymph and cerebrospinal fluid

LYMPH

- Lymph is a clear colorless fluid somewhat similar to plasma. It is derived as a left out fluid at venous end of the capillary bed which is filtered out of the arterial end.
- It contains few or no RBCs, numerous leukocytes, inorganic salts, glucose, NPN substances and some proteins. Lymph derived from the intestine during absorption contains lipids which gives milky white appearance known as chyle.

- Lymphatic vessels originate as blind lymphatic capillaries in between the tissues to collect the tissue fluids, which are not absorbed by the venous system. Lymph capillaries are highly permeable and have less resistance to the entry of macromolecule proteins.
- The lymphatic capillaries form a complex network throughout the tissues and combine to form small lymphatic vessels. This in turn unites to form large lymphatic vessels.
- All the lymphatic vessels of the left side of head, arm and chest and the posterior portion of the body drain their lymph into the thoracic duct, whereas the right lymphatic duct receives the lymph from the right side of the head, neck, foreleg and thorax.
- These two lymphatic vessels empty their contents into the venous system at the juncture of the right subclavian vein and internal jugular vein.
- Lymphatic vessels resemble veins, but their walls are much thinner than veins. They have valves throughout their course to direct the lymph flow towards heart and prevent back flow of lymph.
- Lymph glands or nodes are discrete nodular structures scattered along the course of lymphatic vessels. These nodes function as filters to foreign agents (bacteria).
- Lymphatic glands have lymphocytes and plasma cells, produce antibodies and macrophages against foreign invaders, thus act as a first line of defense organ against infective organisms. Lymph nodes are absent in brain, bone marrow and also in birds.

FORMATION OF LYMPH

- At venous capillary end, only about 90% of the fluid portion of plasma that comes out from the capillary bed of the blood vessels is reabsorbed due to pressure gradient. The remaining 10% constitute the lymph which enters the lymph channels and return to the circulatory system.
- The movement of the lymph inside the vessel is caused by the pressure difference between two ends of the lymphatic system, contractions of the muscles during movement of the body, contractions of the smooth muscles of the lymphatic vessels and presence of valves which prevent back flow.

Forces causing filtration (at the atrial end)

- Forces tending to move fluid outward
  - oCapillary pressure : 30.0 mm Hg
  - oNegative pressure of interstitial fluid : 3.0 mm Hg
  - oI.F. colloidal osmotic pressure : 8.0 mm Hg
  - oTotal outward force : 41.0 mm Hg
- Forces tending to move fluid inward
  - oPlasma colloidal osmotic pressure : 28.0 mm Hg
  - oNet filtration pressure = Out pressure – In ward pressure
  - o41.0 - 28.0 = 13.0 mm Hg.

Force causing reabsorption (At the venous end)

- Forces tending to move fluid outward

- oCapillary pressure : 10.0 mm Hg
- oNegative pressure of interstitial fluid : 3.0 mm Hg
- oI.F colloidal osmotic pressure : 8.0 mm Hg
- oTotal outward force :21.0 mm Hg

- Forces tending to move fluid inward
  - oPlasma colloidal osmotic pressure : 28.0 mm Hg
  - oNet absorption pressure = Inward pressure - Outward pressure
  - o28.0 - 21.0 = 7.0 mm Hg.

Factors determining fluid movement across the capillary wall

- Capillary pressure or capillary hydrostatic pressure tends to move fluid outward.
- Interstitial fluid pressure tends to move fluid inward when it is positive and outward when it is negative.
- Plasma colloid osmotic pressure (Oncotic pressure) tends to cause osmosis of fluid inward.
- Interstitial fluid colloid osmotic pressure tend to cause osmosis of fluid outward.
-

FUNCTIONS OF LYMPH

- The lymph helps in the reabsorption of the fluid from the tissue spaces and adds it to the circulatory system, thus maintains a constant fluid equilibrium.
- It filters the harmful infectious agents (bacteria) from the body.
- It absorbs and transports the fatty substances from the intestine in the form of chyle.
- It helps in the absorption of macromolecular proteins from the interstitial spaces, which are then added to the blood.

CEREBROSPINAL FLUID

- It is formed by the choroid plexus of the lateral, third and fourth ventricles of the brain.

- CSF is periodically absorbed by the arachnoid villi of the subarachnoid space. This structure is quite large in man and horse, but generally small or microscopic in other domestic animals.
- The piamater covering the surface of the brain and spinal cord also contributes small quantities of CSF. This fluid circulates throughout the sub arachnoid space between the piamater and arachnoid membrane, ventricles of the brain and central canal of spinal cord.
- CSF contains a very small quantity of protein. The concentration of sodium and chloride are higher and potassium, urea and glucose are lower in CSF than plasma. The pH is same as blood. It does not contain cellular elements except very few lymphocytes.
- CSF secretion is an active process and is not affected by the blood pressure or CSF pressure. The choroid plexus provides some selectivity of permeability for certain substances of the blood and the CSF is not identical with the blood plasma. It contains

lower concentration of proteins, K^+ and glucose than plasma, but has higher concentration of Na^+ ions. Thus the choroid plexus functions as blood-cerebrospinal fluid barrier which protects the CNS from the influence of a variety of substances.

- CSF is absorbed by the arachnoid villi of the arachnoid membrane, which project through the venous sinuses of the duramater.
- The CSF serves partly as a nutritive medium for the brain and spinal cord as well as cushioning these structures against shock. It also aids in the transport of some peptide hormones and other substances of the brain into the circulation.

EDEMA

- It is the abnormal accumulation of the tissue fluids due to excessive filtration by increased capillary pressure or poor reabsorption at the venous end of the capillaries.
- Edema occurs in
    - oIncreased capillary pressure
    - oIncreased capillary permeability
    - oDecreased concentration of plasma proteins and
    - oObstruction of lymph vessels.
- The low plasma colloidal osmotic pressure due to hypoproteinemia, obstruction of the lymphatic vessels by tumours or by filarial worms, vasodilator substances of allergic reaction etc causes increased capillary permeability leading to edema.
- Chronic congestive heart failure due to improper drainage of fluids into the right heart results in fluid accumulation in the peritoneum and is referred to as *ascites***.**

MODULE-33: JOINTS AND BONE METABOLISM

Learning objectives

This module deals with

- the types of joints and composition of synovial fluid
- hormonal regulation of bone metabolism

JOINTS

- Joint is a junction between two bones. It is also called as articulation. Joints are classified into three types
- Fibrous Joints:
    - oThese are immovable and the bones are firmly united by fibrous tissue. **Eg.:** Joints found between the skull bones.
- Cartilaginous Joints:
    - oThese joints are slightly movable. **Eg.:** Intervertebral disks between the bodies of adjacent vertebrae in the spine.
- Synovial Joints:

- These are freely movable joints, *e.g.*, shoulders joint, stifle joint etc. The movement of a joint is normally controlled and united by the action of muscles and ligaments and tendons. All synovial joints have some common characteristics. They have a capsule with an outer fibrous layer consisting of collagen fibres running from the periosteum of one bone to the other.
- Ligaments are located in and around the capsule. The inner lining layer of the joint surface is the synovial membrane which produces synovial fluid that lubricates the joint surfaces. Synovial fluid is a transparent viscous liquid and it provides lubrication to the joint provides lubrication to the joint.
- Some bones contain special areas of dense fibro cartilage between the articulating cartilage surfaces and are known as menisci. **Eg.:** Bones that bear great weight. The function of the menisci is to cushion compressive forces acting on the ends of the bones.

SYNOVIAL FLUID

- Synovial fluid is a thick, viscous liquid found in joint cavities, tendon sheath and bursae.
- It contains small amounts of albumin, globulin, mucin and hyaluronic acid but no fibrinogen.
- Synovial fluid functions as a lubricant to reduce friction in the joints and help to nourish the articular cartilages.

BONE METABOLISM AND HOMEOSTASIS

- Bone consists of both organic and organic components. The major inorganic compound is calcium phosphate which accounts for 2/3rd of the bone weight. This calcium phosphate interacts with calcium hydroxide to from hydroxyapatite.
- Other inorganic compounds include calcium carbonate, sodium, magnesium and fluoride.
- The remaining organic portion of the bone is made up of cells such as osteoblasts, osteocytes, osteoclasts etc and osteoid such as collagen fibers, proteoglycans, glycoproteins etc. The osteoid is secreted by osteoblasts.
- Bone mineral metabolism is important for calcium homeostasis. During remodelling of the bone, minerals are released into the ECF through the action of osteoclasts and these minerals are removed from the blood by the osteoblasts during bone formation. Under normal conditions the rate of bone resorption is equal to the rate of bone formation.
- There are three major hormones that regulate calcium homeostasis,
  - Parathyroid hormone(PTH),
  - Calcitonin and
  - Vitamin D metabolite (1,25, Dihydroxy cholecalciferol).

EFFECT OF PTH IN THE BONE

- PTH is released from the parathyroid gland in response to low blood calcium levels. The action of PTH on the bone is calcium resorption from the skeletal reserves into the ECF. Release of calcium from the bone occurs in 2 phases. They are:
  - Rapid phase and
  - Slow phase.

- Rapid phase
  - This phase is termed as osteocytic osteolysis. It results from activation of the already existing osteocytes and osteoclasts present in the bone. During this phase there is an increased flow of calcium from the bone fluid to the ECF by osteocyte-osteoblast pump.
  - PTH causes removal of bone salts from two areas in the bone, one from the bone matrix in the vicinity of the osteocytes lying in the bone itself and two, in the vicinity of the osteoblasts along the bone surface. When this osteocyte - osteoblast pump is activated by PTH, bone fluid calcium concentration falls and calcium and phosphorous salts are absorbed from the bone.
  - When the pump is inactivated, bone fluid calcium concentration rises and calcium and phosphorous salts are redeposited in the matrix.
  - PTH has receptors on the cell membrane of both osteoblast and osteocytes When stimulated by PTH, calcium permeability of the osteocytic membrane increases thus allowing calcium to diffuse into the membrane cells from the bone fluid to the ECF.
- Slow phase
  - This phase results from proliferation of the osteoclasts followed by increased osteoclastic reabsorption of the bone itself. PTH do not have receptors on the osteoclasts. Activation of osteoclastic system occurs in two stages,
    - Immediate activation of the osteoblasts that are already formed.
    - Formation of new osteoblasts.
  - PTH stimulates the conversion of osteoprogenitor cells to osteoclasts. PTH binds to the receptors present on the surfaces of osteoblasts causing the cells to contract and thereby exposes the minerals in the matrix to osteoclasts. The minerals and organic components released from the bone are phagocytized by osteoclasts and transported across the cell to be released into the ECF. When the animal is in the positive calcium balance, PTH secretion subsides and osteoblasts finish the reversal and formation phases of bone remodelling.

CALCITONIN

- It is the *second major hormone* that regulates calcium. It is the secreted by the 'C' cells within the thyroid gland in response to high blood calcium levels.
- Calcitonin has receptors on the osteoclasts and directly act on these cells to inhibit bone resorption by blocking osteoclastic osteocytic activity.

1, 25 DIHYDROXY CHOLECALCIFEROL (1,25 DHCC)

- It is the third major hormone involved in calcium metabolism and is a vitamin D metabolite. The action of 1,25 DHCC in bone formation is primarily indirect. It acts on the intestine to increase the efficiency of absorption of calcium and phosphorous absorption via calcium binding protein .
- Osteoblasts have receptors for 1,25 DHCC. It causes osteoclastic resorption and calcium mobilization from the bone.
- Not only bone helps in the maintenance of blood calcium level it also acts as a buffer of the blood pH. It can release cations or anions in response to acidosis or alkalosis.

EFFECT OF ESTROGEN

- Estrogen receptors are present on osteoblasts and osteoclasts. In the osteoclast, estrogen increases cell proliferation and enhances expression of genes that encode for growth factors, enzymes, matrix proteins and cytokines that alter bone remodeling.
- Estrogen inhibit production of cytokines associated with bone resorption such as interlukins (IL–I, IL-6 and IL-11). Estrogen also stimulates synthesis of transforming growth factor-beta (TGF- ß), Insulin like growth factor (IGF)–I and IGF-binding proteins. Proteins involved in bone remodeling, such as bone morphogenic protein and osteoprotegenin are also regulated by estrogen. In estrogen deficiency these is an upward regulation in the maturation of osteoclasts and osteoblasts, with an over all increase in bone remodeling.
- The major action of estrogen on bone is inhibition of bone resorption. Estrogen induces apoptosis and reduces the lifespan of the osteoclast. The synergy of several cytokines enhance osteoclast recruitment, differentiation and activity. The blockage of the productive of cytokines such as IL and tumour necrosis factor μ (TNF μ) by osteoblasts prevents bone resorption in an estrogen deficient state.