COMPARATIVE ANIMAL PHYSIOLOGY

M.Sc., ZOOLOGY First Year

Semester – II, Paper-II

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M.Sc., ZOOLOGY – Comparative Animal Physiology

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A^{+}' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the doorstep of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao

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M.Sc. – Zoology
SEMESTER-II
202ZO24: COMPARATIVE ANIMAL PHYSIOLOGY

Syllabus

Course Objectives/Course outcomes:

CO1: Ability to compare and contrast the physiological adaptations of different animals in different environments

CO2: Understanding the challenges in animals face in maintaining homeo- stasis such as thermo regulatory and various physiological such as metabolism, respiration, circulation, Osmo regulation and excretory system

CO 3: To recognize and analyze the mechanisms in animals to regulate their internal; environment in response to external stimuli.

CO 4: To integrate knowledge of molecular, cellular and organosomal physiology to understand the animal function.

CO 5: Appreciation of diversity of life and the remarkable adaptations that allow animals to survive and thrive in different environments.

UNIT-I:
Transformation of energy in animals: Bio-energetics; diversity in operations, Factors regulating enzyme activity, energy producing reactions, proteolytic enzymes, pathways of cellular metabolism. Nutrition impairment and stress. Learning outcome In the topic of transformation of energy in the environment and diversity in operations can be regulation and the energy reactions with the enzymes in the cellular metabolic can be estimated in their unit.

UNIT-II:
Digestion: Process of digestion and absorption; energy balance; Basal Metabolic Rate. Respiration: Mechanism of gaseous exchange in animals; neural and chemical regulation.
Blood: Composition and function of blood; respiratory pigments and their functions. Circulation: Comparative account of circulatory system in animals. Learning outcome: In the process of digestion, we can learn regarding the food digested in body, respiration through different necessary organs the way of circulation in different living organisms can be identified.

UNIT-III:
Thermoregulation in poikilotherms and homeotherms. Muscles: Structure and function of muscles; Theories of muscle contraction. Nervous system: Neurons, action potential, neural control of muscle tone and posture; propagation of nerve impulse and synaptic transmission in animals. Learning outcome Thermoregulation is the process to different temperature adaptations in the environment that how living organisms can live in different environment conditions through movement and nervous system and propagation in different animals by the students can learn regarding the above said themes.

UNIT-IV:

Excretion and Osmoregulation: Comparative account of structure and function of kidneys in animals; regulation of water and electrolyte balance. Endocrinology and reproduction: Endocrine glands in animals, mechanism of hormonal action; Hormonal regulation in reproduction; growth and development -Regeneration, moulting and metamorphosis. Learning outcome: The detailed out comes from this chapters in regarding sensory organs, excretory and osmoregulation and endocrinology and reproductive process in different living organisms which are confined to aquatic and terrestrial organisms

UNIT-V:

Chromatophores and Significance of chromatophores and colour change in animals-Photo-receptors, Phono- receptors, Tango receptors, and Chemoreceptor's occurrence and Functional significance of Bioluminescence. Learning outcome: The detailed out comes from this chapters in regarding sensory organs, excretory and osmoregulation and endocrinology and reproductive process in different living organisms which are confined to aquatic and terrestrial organisms.

REFERENCE BOOKS:

1. Eckert H. Animal Physiology: Mechanisms and Adaptation. W.H. Freeman & Company.
2. Floray E. An Introduction to General and Comparative Animal Physiology. W.B. Saunders Co., Philadelphia.
3. Goel KA and Satish KV. 1989. A Text Book of Animal Physiology, Rastogi Publications, Meerut, U.P.
4. Hoar WS. General and Comparative Physiology. Prentice Hall of India, New Delhi.
5. Lehninger AL. Nelson and Cox. Principles of Biochemistry. Lange Medical Publications, New Delhi.
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7. Schmidt-Nielson K. Animal Physiology. Cambridge University Press, Cambridge.

CODE: 202ZO24

M.Sc DEGREE EXAMINATION
Second Semester
Zoology:: Paper II – Comparative Animal Physiology
MODEL QUESTION PAPER

Time : Three hours **Maximum : 70 marks**

Answer ONE question from each Unit. **(5 x 14 = 70)**

UNIT – I

1. a) Discuss about various factors regulating enzyme activity.

(or)

b) Write a note on

i Proteolytic enzymes

ii Stress

UNIT – II

2. a) Explain about neural and chemical regulation of respiration.

(or)

b) write about functions of blood with a note on respiratory pigments and their functions.

UNIT – III

3. a) Explain the structure and function of muscles.

(or)

b) Write a note on

i Action potential

ii Propagation of nerve impulse

UNIT – IV

4. a) Describe the structure and functions of kidneys in mammals.

(or)

b) Discuss about endocrine glands in animals.

UNIT – V

5. a) Define bioluminescence and explain the functional significance of bioluminescence.

(or)

b) Write a note on

i) Photoreceptors

ii) Chemoreceptors

CONTENTS

LESSON-1
BIOENERGETICS

OBJECTIVES:

1. To understand the fundamental principles of energy flow and transformation in living organisms, with special reference to the role of energy-rich compounds such as ATP, GTP, cAMP, and cGmp.
2. To explain the thermodynamic basis of biological reactions by analysing the relationship between free energy, enthalpy, and entropy, and distinguishing between exergonic and endergonic processes.
3. To study major energy-producing pathways such as glycolysis, the TCA cycle, oxidative phosphorylation, and photosynthesis, and to evaluate their significance in ATP generation, metabolic regulation, and cellular functions.

STRUCTURE:

1.1 INTRODUCTION TO BIOENERGETICS:

Bioenergetics is the study of the flow and transformation of energy in living organisms, focusing on how cells acquire, store, and utilize energy to perform vital biological functions. It explains how chemical energy derived from nutrients or sunlight is converted into usable forms, mainly adenosine triphosphate (ATP), which serves as the universal “energy currency”of the cell. By applying principles of thermodynamics, bioenergetics explores exergonic and endergonic reactions, the relationship between free energy, enthalpy, and entropy, and the role of high-energy compounds such as ATP, GTP, Camp, and Cgmp in metabolism, signaling, and cellular regulation.

1.1.1 Bioenergetics:

Concept of free energy, endergonic and exergonic reaction, Relationship between free energy, enthalpy and entropy; Redox potential: Energy rich compounds; classification; biological significances of ATP and cyclic AMP Bioenergetics.

Bioenergetics means study of the transformation of energy in living organisms. The goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics. In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work (such as mechanical work) or for other processes (such as chemical synthesis and anabolic processes in growth), when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy. Adenosine triphosphate (ATP) is the main "energy currency" for organisms; the goal of metabolic and catabolic processes is to synthesize ATP from available starting materials (from the environment), and to break- down ATP (into adenosine diphosphate (ADP) and inorganic phosphate) by utilizing it in biological processes. In a cell, the ratio of ATP to ADP concentrations is known as the "energy charge" of the cell. A cell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation. Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is hydrolyzed (broken down by water) to adenosine diphosphate and inorganic phosphate. Here it is the thermodynamically favorable free energy of hydrolysis that results in energy release; the phosphoanhydride bond between the terminal phosphate group and the rest of the ATP molecule does not itself contain this energy.

1.1.2 Energy and Metabolism:

All living organisms need energy to grow and reproduce, maintain their structures, and respond to their environments. Metabolism is the set of life-sustaining chemical processes that enables organisms transform the chemical energy stored in molecules into energy that can be used for cellular processes. Animals consume food to replenish energy; their metabolism breaks down the carbohydrates, lipids, proteins, and nucleic acids to provide chemical energy for these processes. Plants convert light energy from the sun into chemical energy stored in molecules during the process of photosynthesis.

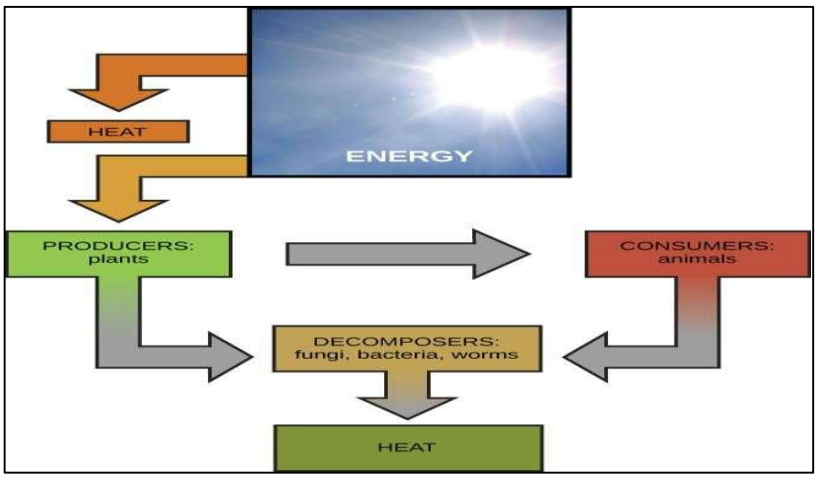


Figure-1: Most energy comes from the sun, either directly or indirectly: Most life forms on earth get their energy from the sun. Plants use photosynthesis to capture sunlight, and herbivores eat those plants to obtain energy. Carnivores eat the herbivores, and decomposers digest plant and animal matter energy and metabolism

1.1.3 Cellular metabolism:

Every task performed by living organisms requires energy. Energy is needed to perform heavy labor and exercise, but humans also use a great deal of energy while thinking and even while sleeping. For every action that requires energy, many chemical reactions take place to provide chemical energy to the systems of the body, including muscles, nerves, heart, lungs, and brain.

The living cells of every organism constantly use energy to survive and grow. Cells break down complex carbohydrates into simple sugars that the cell can use for energy. Muscle cells may consumer energy to build long muscle proteins from small amino acid molecules. Molecules can be modified and transported around the cell or may be distributed to the entire organism. Just as energy is required to both build and demolish a building, energy is required for both the synthesis and breakdown of molecules. Many cellular processes require a steady supply of energy provided by the cell's metabolism. Signaling molecules such as hormones and neurotransmitters must be synthesized and then transported between cells. Pathogenic bacteria and viruses are ingested and broken down by cells. Cells must also export waste and toxins to stay healthy, and many cells must swim or move surrounding materials via the beating motion of cellular appendages like cilia and flagella.

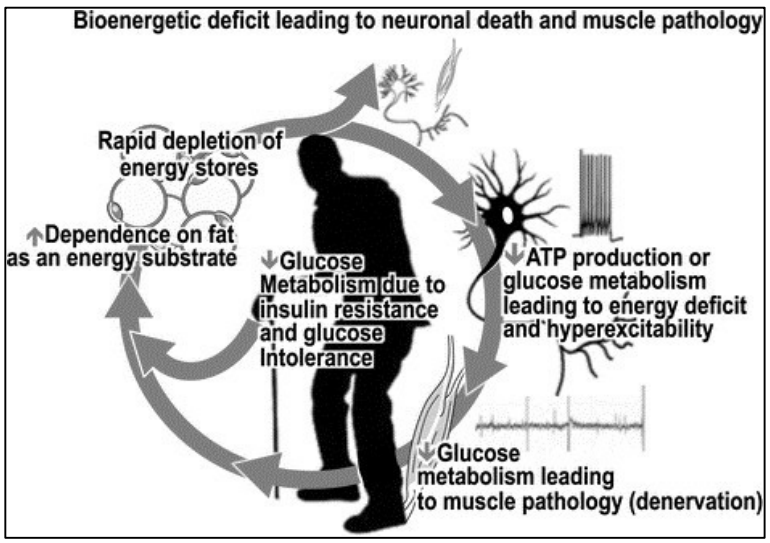


Figure 2: Bioenergetic deficit leading to neuronal death and muscle pathology

1.2 FORMS OF ENERGY IN BIOLOGICAL SYSTEMS:

1.2.1 Bioenergetics and chemical reactions:

Scientists use the term bioenergetics to discuss the concept of energy flow through living systems such as cells. Cellular processes such as the building and breaking down of complex molecules occur through step-by-step chemical reactions. Some of these chemical reactions are spontaneous and release energy, whereas others require energy to proceed. All of the chemical reactions that take place inside cells, including those that use energy and those that release energy, are the cell's metabolism. Energy is a property of objects which can be transferred to other objects or converted into different forms, but cannot be created or destroyed. Organisms use energy to survive, grow, respond to stimuli, reproduce, and for every type of biological process. The potential energy stored in molecules can be converted to chemical energy, which can ultimately be converted to kinetic energy, enabling an organism to move. Eventually, most of energy used by organisms is transformed into heat and dissipated.

1.2.2 Kinetic energy:

Energy associated with objects in motion is called kinetic energy. For example, when an airplane is in flight, the airplane is moving through air very quickly—doing work to enact change on its surroundings. The jet engines are converting potential energy in fuel, to the kinetic energy of movement. A wrecking ball can perform a large amount of damage, even when moving slowly. However, a still wrecking ball cannot perform any work and therefore has no kinetic energy. A speeding bullet, a walking person, the rapid movement of molecules in the air that produces heat, and electromagnetic radiation, such as sunlight, all have kinetic energy.

1.2.3 Potential energy:

What if that same motionless wrecking ball is lifted two stories above a car with a crane? If the suspended wrecking ball is not moving, is there energy associated with it? Yes, the wrecking ball has energy because the wrecking ball has the potential to do work. This form of energy is called potential energy because it is possible for that object to do work in a given state. Objects transfer their energy between potential and kinetic states. As the wrecking ball hangs motionlessly, it has 0% kinetic and 100%potential energy. Once the ball is released, its kinetic energy increases as the ball picks up speed. At the same time, the ball loses potential energy as it nears the ground. Other examples of potential energy include the energy of water held behind a dam or a person about to skydive out of an airplane.

1.2.4 Chemical energy:

Potential energy is not only associated with the location of matter, but also with the structure of matter. A spring on the ground has potential energy if it is compressed, as does a rubber band that is pulled taut. The same principle applies to molecules. On a chemical level, the bonds that hold the atoms of molecules together have potential energy. This type of potential energy is called chemical energy, and like all potential energy, it can be used to do work. For example, chemical energy is contained in the gasoline molecules that are used to power cars. When gas ignites in the engine, the bonds within its molecules are broken, and the energy released is used to drive the pistons. The potential energy stored within chemical bonds can be harnessed to perform work for biological processes. Different metabolic processes break down organic molecules to release the energy for an organism to grow and survive.

1.3 TYPES OF BIOENERGETICS REACTIONS:

1.3.1 Exergonic Reaction:

- Exergonic implies the release of energy from a spontaneous chemical reaction without any concomitant utilization of energy.
- The reactions are significant in terms of biology as these reactions have an ability to perform work and include most of the catabolic reactions in cellular respiration.
- Most of these reactions involve the breaking of bonds during the formation of reaction intermediates as is evidently observed during respiratory pathways. The bonds that are created during the formation of metabolites are stronger than the cleaved bonds of the substrate.
- The release of free energy, G, in an exergonic reaction (at constant pressure and temperature) is denoted as
  $\Delta G = G \text{ products} - G \text{ reactants} < 0$

1.3.2. Endergonic Reactions:

- Endergonic in turn is the opposite of exergonic in being non-spontaneous and requires an

input of free energy. Most of the anabolic reactions like photosynthesis and DNA and protein synthesis are endergonic in nature.

- The release of free energy, G, in an exergonic reaction (at constant pressure and temperature) is denoted as
  $\Delta G = G \text{ products} - G \text{ reactants} > 0$

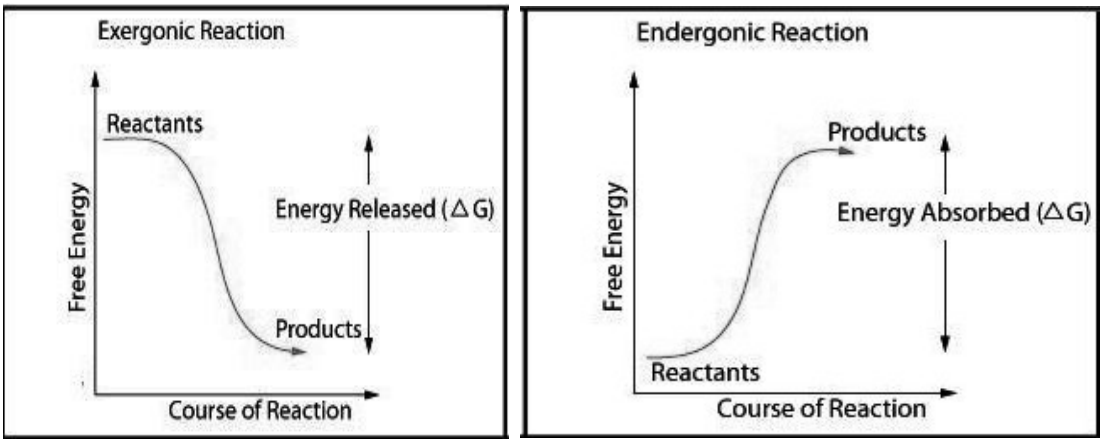
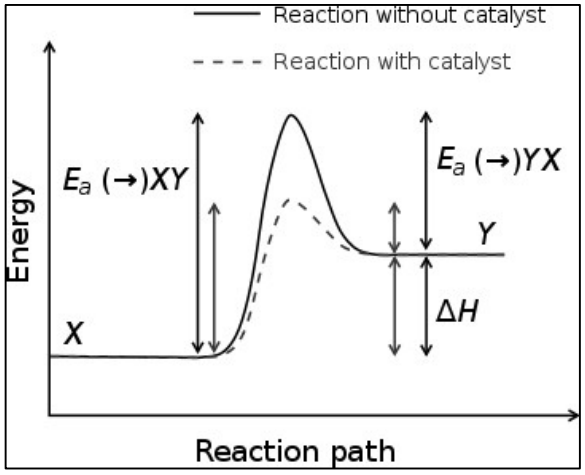


Figure 3: Exergonic reaction & Endergonic reaction

1.3.3. Activation Energy:

- Activation energy is the energy which must be available to a chemical system with potential reactants to result in a chemical reaction. Activation energy may also be defined as the minimum energy required starting a chemical reaction.

Figure 4: Activation energy



Photosynthesis, another major bioenergetics process, is the metabolic pathway used by plants in which solar energy is used to synthesize glucose from carbon dioxide and water. This reaction takes place in the chloroplast. After glucose is synthesized, the plant cell can undergo photophosphorylation to produce ATP.

1.4 MECHANISMS OF ENERGY TRANSFER:

1.4.1 Cotransport:

In August 1960, Robert K. Crane presented for the first time his discovery of the sodium-glucose co-transport as the mechanism for intestinal glucose absorption.[24] Crane's discovery of co-transport was the first ever proposal of flux coupling in biology and was the most important event concerning carbohydrate absorption in the 20th century.

1.4.2 Chemiosmotic theory:

One of the major triumphs of bioenergetics is Peter D. Mitchell's chemiosmotic theory of how protons in aqueous solution function in the production of ATP in cell organelles such as mitochondria. This work earned Mitchell the 1978 Nobel Prize for Chemistry. Other cellular sources of ATP such as glycolysis were understood first, but such processes for direct coupling of enzyme activity to ATP production are not the major source of useful chemical energy in most cells. Chemiosmotic coupling is the major energy producing process in most cells, being utilized in chloroplasts and several single celled organisms in addition to mitochondria.

1.5 MAJOR BIOENERGETIC PROCESSES:

1. Glycolysis:

Glycolysis is the process of breaking down glucose into pyruvate, producing net eight molecules of ATP (per 1 molecule of glucose) in the process. Pyruvate is one product of glycolysis, and can be shuttled into other metabolic pathways (gluconeogenesis, etc.) as needed by the cell. Additionally, glycolysis produces equivalents in the form of NADH (nicotinamide adenine dinucleotide), which will ultimately be used to donate electrons to the electron transport chain.

2. Citric acid cycle:

The citric acid cycle is a process of cellular respiration in which acetyl coenzyme A, synthesized from pyruvate dehydrogenase, is first reacted with oxaloacetate to yield citrate. The remaining eight reactions produce other carbon- containing metabolites. These metabolites are successively oxidized, and the free energy of oxidation is conserved in the form of the reduced coenzymes FADH2 and NADH. These reduced electron carriers can then be re-oxidized when they transfer electrons to the electron transport chain.

3. Oxidative phosphorylation

Oxidative phosphorylation and the electron transport chain is the process where reducing equivalents such as NADPH, FADH2 and NADH can be used to donate electrons to a series of redox reactions that take place in electron transport chain complexes. These redox reactions take place in enzyme complexes situated within the mitochondrial membrane. These redox reactions transfer electrons "down" the electron transport chain, which is coupled to the proton motive force. This difference in proton concentration between the mitochondrial matrix and inner membrane space is used to drive ATP synthesis via ATP synthase.

4. Gluconeogenesis:

Gluconeogenesis is the opposite of glycolysis; when the cell's energy charge is low (the concentration of ADP is higher than that of ATP), the cell must synthesize glucose from carbon- containing biomolecules such as proteins, amino acids, fats, pyruvate, etc. For example, proteins can be broken down into amino acids, and these simpler carbon skeletons are used to build/ synthesize glucose.

5. Ketosis:

Ketosis is a metabolic process whereby ketone bodies are used by the cell for energy (instead of using glucose). Cells often turn to ketosis as a source of energy when glucose levels are low; e.g. during starvation.

6. Photosynthesis:

Photosynthesis, another major bioenergetic process, is the metabolic pathway used by plants in which solar energy is used to synthesize glucose from carbon dioxide and water. This reaction takes place in the chloroplast. After glucose is synthesized, the plant cell can undergo photophosphorylation to produce ATP.

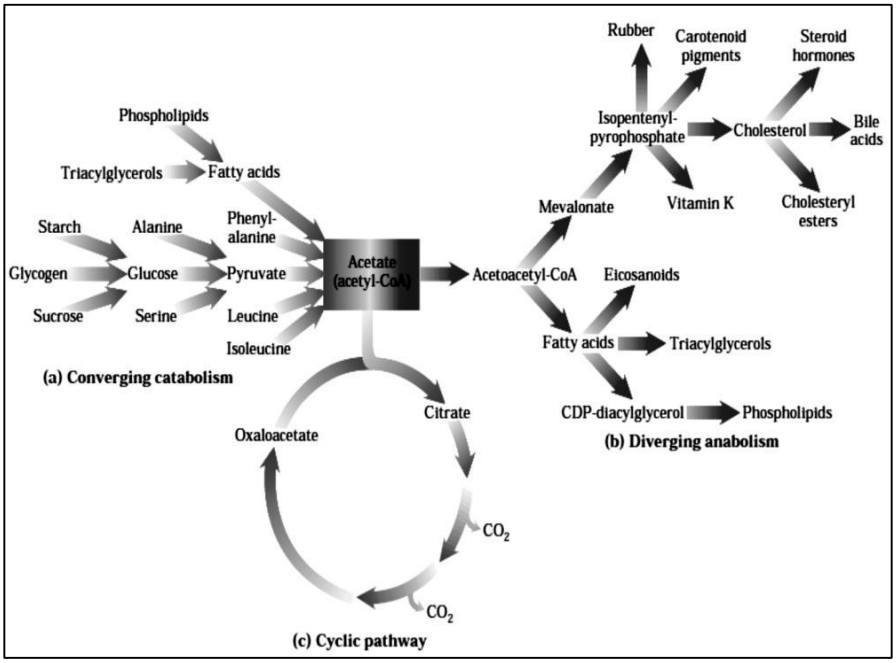


Figure 5: Amphibolic nature of metabolic pathways

1.6 THERMODYNAMICS IN BIOENERGETICS:

1.6.1 Bioenergetics Relationship Between Free Energy, Enthalpy & Entropy:

Every living cell and organism must perform work to stay alive, to grow and to reproduce. The ability to harvest energy from nutrients or photons of light and to channel it into biological work is the miracle of life.

- ***1st Law of Thermodynamics:*** The energy of the universe remains constant.
- ***2nd Law of Thermodynamics:*** All spontaneous processes increase the entropy of the universe.

The important state functions for the study of biological systems are:
The Gibbs free energy (G) which is equal to the total amount of energy capable of doing work during a process at constant temperature and pressure.

- If ΔG is negative, then the process is spontaneous and termed exergonic.
- If ΔG is positive, then the process is nonspontaneous and termed endergonic.
- If ΔG is equal to zero, then the process has reached equilibrium.

The Enthalpy (H) which is the heat content of the system. Enthalpy is the amount of heat energy transferred (heat absorbed or emitted) in a chemical process under constant pressure.

- When ΔH is negative the process produces heat and is termed exothermic.
- When ΔH is positive the process absorbs heat and is termed endothermic.

The Entropy (S) is a quantitative expression of the degree of randomness or disorder of the system. Entropy measures the amount of heat dispersed or transferred during a chemical process.

- When ΔS is positive then the disorder of the system has increased.
- When ΔS is negative then the disorder of the system has decreased.

The conditions of biological systems are constant temperature and pressure. Under such conditions the relationships between the change in free energy, enthalpy and entropy can be described by the expression where T is the temperature of the system in Kelvin. $\Delta G = \Delta H - T\Delta S$

[ΔG = Gibbs Free Energy; ΔH = Change in Enthalpy; T = Temperature in K; ΔS = Change in Entropy]

Three Thermodynamic Quantities

Quantity	Symbol	Measures	Units
Enthalpy	*H*	Heat	Energy
Entropy	*S*	Disorder	Energy/K
Free energy	*G*	Reactivity	Energy

Figure 6: Three thermodynamic quantities

1.7 ENERGY-RICH COMPOUNDS AND REACTIONS:

High energy phosphates act as energy currency of cell. – Three major sources of high energy phosphates taking part in energy conservation or energy capture.

1.7.1 Oxidative phosphorylation:

- In metabolic pathway, cells use enzymes to oxidize nutrients, thereby releasing energy which is used to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.
- The process that accounts for the high ATP yield is known as oxidative phosphorylation.
- In glycolysis and the citric-acid cycle generate other products besides ATP and GTP, namely NADH and FADH2. These products are molecules that are oxidized (i.e., give up electrons) spontaneously. The body uses these reducing agents (NADH and FADH2) in an oxidation-reduction reaction.

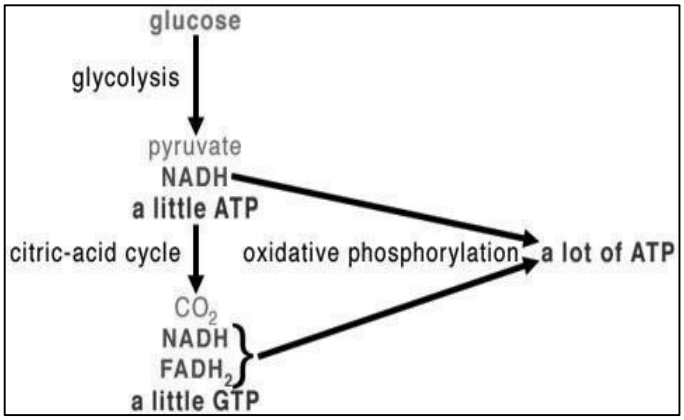


Figure 7: Oxidative phosphorylation

2. Glycolysis:

- Cells use the glycolysis pathway to extract energy from sugars, mainly glucose, and store it in molecules of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). The end product of glycolysis is pyruvate, which can be used in other metabolic pathways to yield additional energy.
- During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

Reactions catalyzed	ATP used	ATP formed
Stage 1:		
1.Glucokinase (for phosphorylation)	1	
2.phosphofructokinase I (for phosphorylation)	1	
Stage II:		
3. glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain)	6	
4. Phosphoglycerate kinase (substrate level phosphorylation)	2	
Stage IV:		
5. Pyruvate kinase (substrate level phosphorylation)	2	
Total net gain 2	**08**	**10**

Table 1: ATP utilization and production during glycolysis

In the anaerobic phase oxidation of one glucose molecule produces **4 – 2 = 2 ATP.**

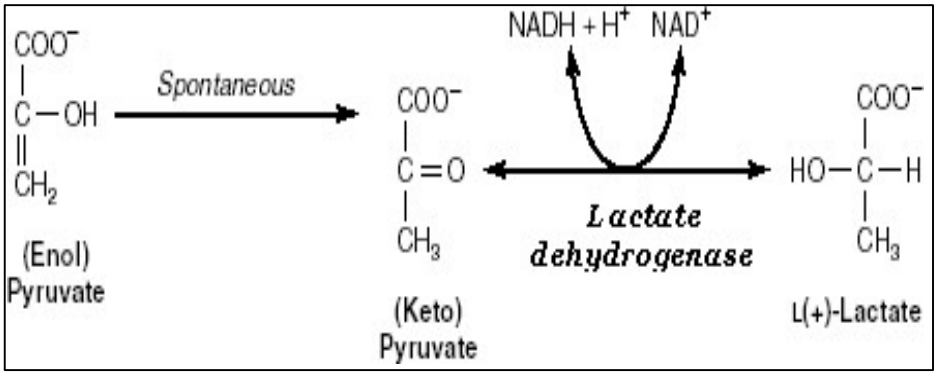


Figure 8: Conversion of Pyruvate to Lactate-by-Lactate Dehydrogenase

3. TCA Cycle:

- The citric acid cycle (CAC)-also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP).

- If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated. As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

Reactions	No.of ATP formed
1. 2 isocitrate → 2 α-ketoglutarate (2 NADH + 2H$^+$) (2 × 3)	6
2. 2 α-ketoglutarate → 2 succinyl CoA (2 NADH + 2H$^+$) (2 × 3)	6
3. 2 succinyl CoA → 2 succinate (2 GTP = 2ATP)	2
4. 2 succinate → 2 Fumarate ($2\ FADH_2$) (2 × 2)	4
5. 2 malate → 2 oxaloacetate (2 NADH + 2H$^+$) (2 × 3)	6
Total No.of ATP formed	24

Table 2: ATP Yield from Citric Acid Cycle (TCA Cycle)

Energy shuttles:

i. **NADH:** An energy shuttle which delivers high energy electrons to the electron transport chain where they will eventually power the production of 2 to 3 ATP molecules. When this electron shuttle is not carrying high energy electrons, meaning it has been oxidized (lost its electrons), it is left with a positive charge and is called NAD+.

ii. FADH2: Another energy shuttle that carries high energy electrons to the electron transport chain, where they will ultimately drive production of 1 to 2 ATP molecules. The oxidized form of FADH2 is FAD and happens just like in NADH.

High energy molecules:

i. ATP: The basic energy currency of the cell. It's a form of energy that cells can use right away.

ii. GTP: Similar to ATP, GTP can be easily converted to ATP in the

4. Energy Released by Hydrolysis of Some Phosphate Compounds

Type	Example	Energy Released (kcal/mol)
Acyl phosphate	1,3-bisphosphoglycerate (BPG)	−11.8
Acetyl phosphate	–	−11.3
Guanidine phosphates	Creatine phosphate	−10.3
	Arginine phosphate	−9.1
Pyrophosphates	Ppi → 2Pi	−7.8
	ATP → AMP + Ppi	−7.7
	ATP → ADP + Pi	−7.5
	ADP → AMP + Pi	−7.5
Sugar phosphates	Glucose-1-phosphate	−5.0
	Fructose-6-phosphate	−3.8
	AMP → Adenosine + Pi	−3.4
	Glucose-6-phosphate	−3.3
	Glycerol-3-phosphate	−2.2

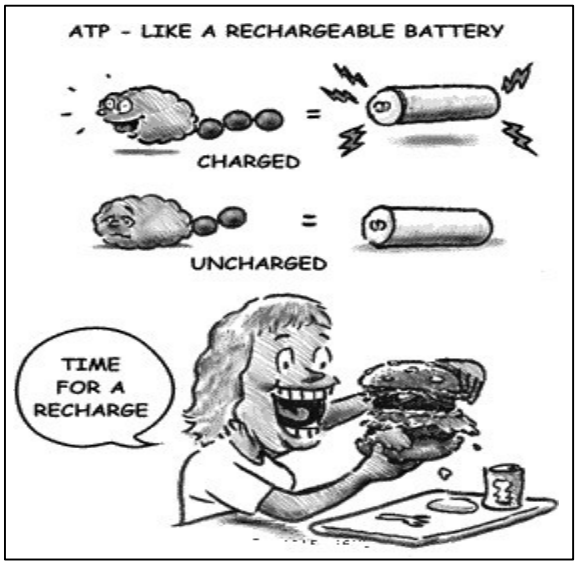


Figure 9: ATP- like a rechargeable battery

1.8 BIOLOGICAL ENERGY CARRIERS:

1.8.1 Adenosine triphosphate (ATP)

- Adenosine-5'-triphosphate (ATP) is a multifunctional nucleotide used in cells as a coenzyme.

- It is often called the "molecular unit of currency" of intracellular energy transfer. ATP transports chemical energy within cells for metabolism.
- It is produced by photophosphorylation and cellular respiration and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division.
- One molecule of ATP contains three phosphate groups and it is produced by *ATP synthase* from inorganic phosphate and adenosine diphosphate (ADP) or adenosine monophosphate (AMP).
- The structure of this molecule consists of a purine base (adenine) attached to the 1' carbon atom of a pentose sugar (ribose). Three phosphate groups are attached at the 5' carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter- convert ATP, ADP and AMP. When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.

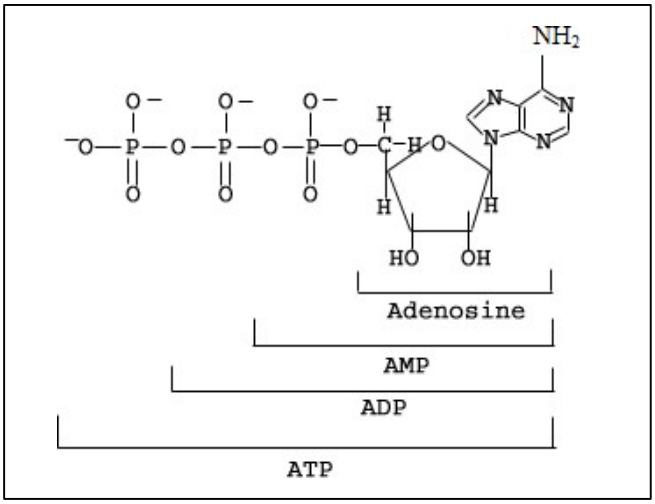


Figure 10: Structure of Adenosine Triphosphate (ATP) and Its Components

The three main functions of ATP in cellular function are:

1. Transporting organic substances such as sodium, calcium, potassium through the cell membrane.
2. Synthesizing chemical compounds, such as protein and cholesterol.
3. Supplying energy for mechanical work, such as muscle contraction.

- The standard amount of energy released from hydrolysis of ATP can be calculated from the changes in energy under non-natural (standard) conditions, then correcting to biological concentrations. The energy released by cleaving either a phosphate (Pi) or pyrophosphate (Ppi) unit from ATP at standard state of 1 M are:

$ATP + H2O \rightarrow ADP + Pi \ \Delta G^{\circ} = -30.5$ Kj/mol (-7.3 kcal/mol)

$ATP + H2O \rightarrow AMP + Ppi \ \Delta G^{\circ} = -45.6$ Kj/mol (-10.9 kcal/mol)

These values can be used to calculate the change in energy under physiological conditions and the cellular ATP/ADP ratio (also known as the Energy Charge). This reaction is dependent on a number of factors, including overall ionic strength and the presence of alkaline earth metal ions such as Mg2+ and Ca2+.Under typical cellular conditions, ΔG is approximately -57 Kj/mol (-14 kcal/mol).

1.8.2 Cyclic Adenosine Monophosphate:

(Camp, cyclicAMPor3'-5'-cyclicadenosine monophosphate)

- It is a second messenger important in many biological processes. Camp is derived from adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the Camp-dependent pathway.
- Camp is synthesized from ATP by adenylyl cyclase located on the inner side of the plasma membrane. *Adenylyl cyclase* is activated by a range of signaling molecules through the activation of *adenylyl cyclase* stimulatory G (Gs)-protein-coupled receptors and inhibited by agonists of *adenylyl cyclase* inhibitory G (Gi)-protein-coupled receptors. Liver *adenylyl cyclase* responds more strongly to glucagon, and muscle adenylyl cyclase responds more strongly to adrenaline.
- Camp decomposition into AMP is catalyzed by the enzyme *phosphodiesterase*.
- Function: Camp is a second messenger, used for intracellular signal transduction, such as transferring the effects of hormones like glucagon and adrenaline, which cannot pass through the cell membrane. It is involved in the activation of *protein kinases* and regulates the effects of adrenaline and glucagon. It also regulates the passage of Ca2+ through ion channels. cAMP and its associated kinases function in several biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism by activating protein kinase

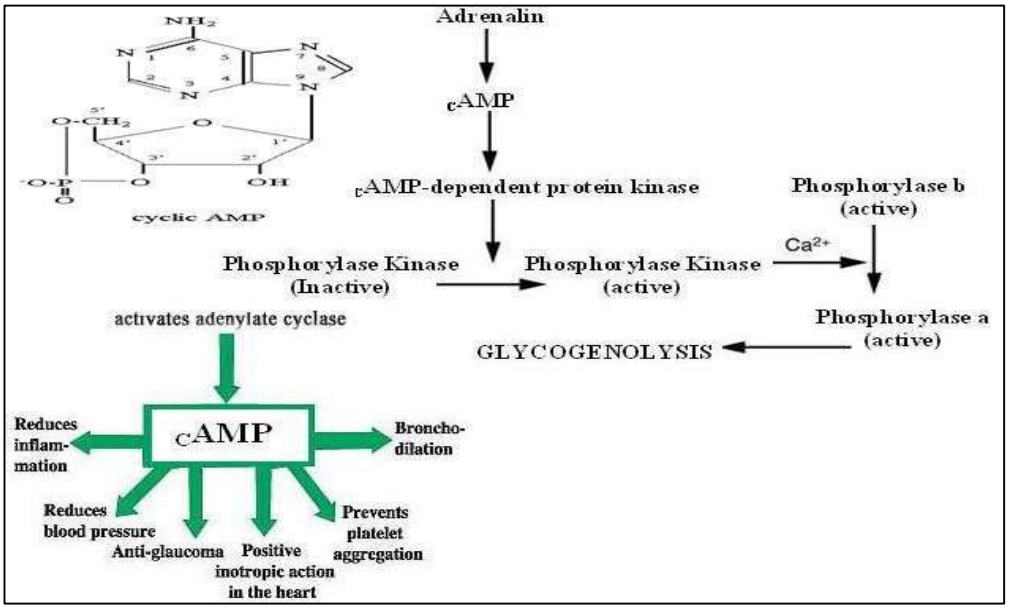


Figure 11: Role of Camp in Glycogenolysis and Physiological Functions

1.8.3 GUANOSINE TRIPHOSPHATE (GTP):

Guanosine-5'-triphosphate (GTP) is a purine nucleoside triphosphate. It can act as a substrate for both the synthesis of RNA during the transcription process and of DNA during DNA replication. It also has the role of a source of energy or an activator of substrates in metabolic reactions, like that of ATP, but more specific. It is used as a source of energy for protein synthesis and gluconeogenesis. GTP is essential to signal transduction, in particular with G-proteins, in second-messenger mechanisms where it is converted to Guanosine diphosphate (GDP) through the action of GTPases.

USES:

Energy transfer

GTP is involved in energy transfer within the cell. For instance, a GTP molecule is generated by one of the enzymes in the citric acid cycle. This is tantamount to the generation of one molecule of ATP, since GTP is readily converted to ATP with nucleoside-diphosphate kinase (NDK).

Genetic translation

During the elongation stage of translation, GTP is used as an energy source for the binding of a new amino-bound Trna to the A site of the ribosome.

Mitochondrial function

The translocation of proteins into the mitochondrial matrix involves the interactions of both GTP and ATP.

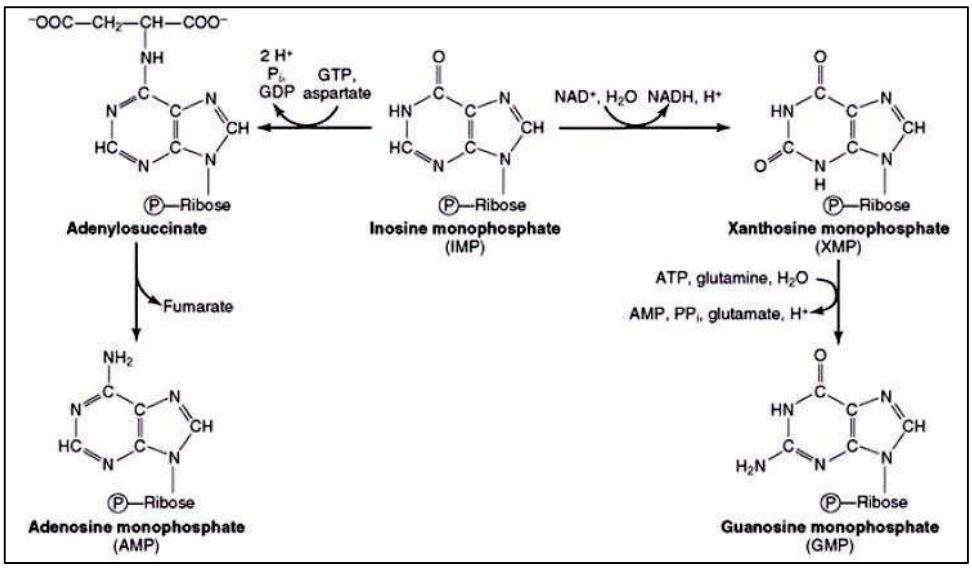


Figure 12: Synthesis of AMP and GMP from IMP.

1.8.4 Cyclic Guanosine Monophosphate:

- Cyclic guanosine monophosphate (cGmp) is a cyclic nucleotide derived from guanosine triphosphate (GTP).
- Cgmp acts as a second messenger much like cyclic AMP. Its most likely mechanism of action is activation of intracellular protein kinases in response to the binding of membrane-impermeable peptide hormones to the external cell surface.
- Synthesis: Guanylate cyclase (GC) catalyzes cGmp synthesis. This enzyme converts GTP to cGmp.

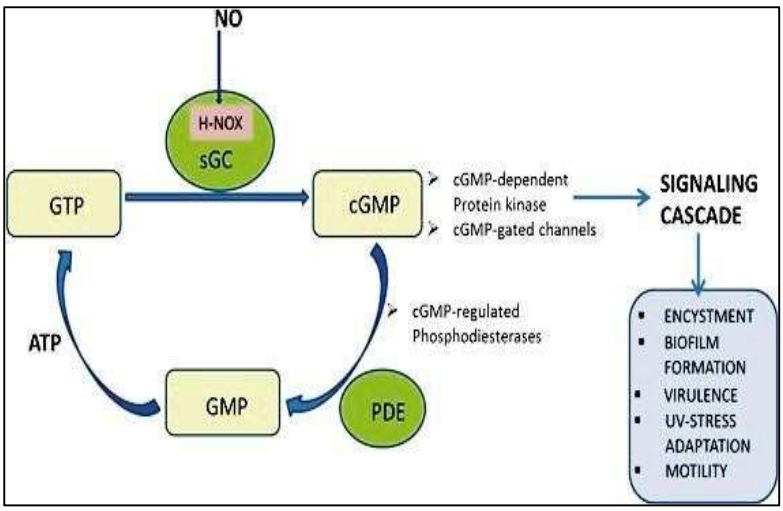


Figure 13: cGmp Signaling Pathway

Schematic representation of synthesis, degradation, and function of cGmp. The three targets of cGmp molecules are

(i) cGmp dependent protein kinases,
(ii) cGmp gated ion channels and
(iii) cGmp dependent phosphodiesterase's.

While phosphodiesterase's are involved in the degradation of cGmp to GMP, the protein kinases and activation of ion channels are subsequently involved in various bacterial signaling pathways. GTP: Guanosine 5′-triphosphate; Sgc: soluble guanylate cyclase; NO: nitric oxide; H-NOX: Heme-Nitric oxide/Oxygen domine; cGmp: cyclic guanosine 3′,5′-monophosphate; PDE: Phosphodiesterase; GMP: guanosine 3′,5′-monophosphate; ATP: adenosine 5′-triphosphate.

Effects

- cGmp is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. It also relaxes smooth muscle tissues. In blood vessels, relaxation of vascular smooth muscles leads to vasodilation and increased blood flow.
- cGmp is a secondary messenger in phototransduction in the eye.
- cGmp is involved in the regulation of some protein-dependent kinases.

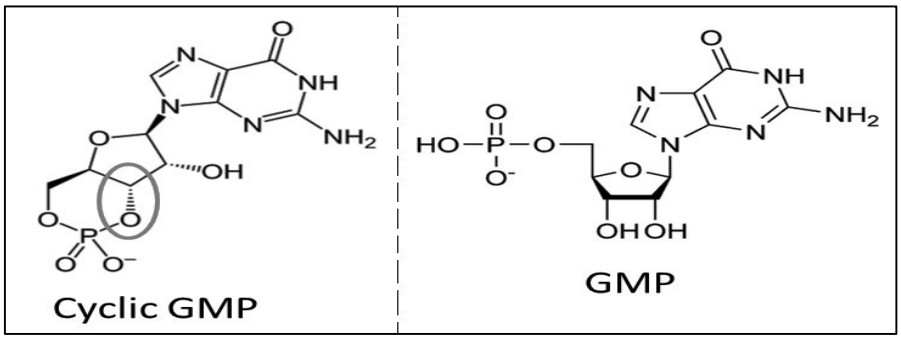


Figure 14: Cyclic GMP & GMP

1.9 SUMMARY:

All living organisms need energy to grow and reproduce, maintain their structures, and respond to their environments; metabolism is the set of the processes that makes energy available for cellular processes. Metabolism is a combination of chemical reactions that are spontaneous and release energy and chemical reactions that are non-spontaneous and require energy in order to proceed. Living organisms must take in energy via food, nutrients, or sunlight in order to carry out cellular processes. The transport, synthesis, and breakdown of nutrients and molecules in a cell require the use of Pathways.

1.10 TECHINICAL TERMS:

ATP, High-energy phosphate compounds, Substrate-level phosphorylation, Oxidative phosphorylation, Amphibolic pathway, Redox reactions, Chemiosmotic theory, Cytochromes, cAMP, cGmp

1.11 SELF ASSESSMENT QUESTIONS:

1. What are bioenergetics and why is it important in living systems?
2. Differentiate between kinetic energy, potential energy, and chemical energy with biological examples.
3. Explain the difference between exergonic and endergonic reactions.
4. What is activation energy? How do enzymes help reduce it?
5. Describe the chemiosmotic theory of ATP synthesis?
6. Name the major bioenergetic processes in the cell and briefly state their significance.

1.12 SUGGESTED READING:

1) Eckert H. Animal Physiology: Mechanisms and Adaptation. W.H. Freeman & Company.
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- **Prof. M. JAGADISH NAIK**

LESSON- 2

FACTORS REGULATING ENZYME ACTIVITY

OBJECTIVES:

- To study how environmental conditions such as temperature, Ph, enzyme concentration, and substrate concentration influence the rate of enzyme-catalyzed reactions.
- To understand the role of cofactors, coenzymes, and prosthetic groups in enhancing enzymatic activity and stability.
- To analyze the mechanisms of enzyme regulation through reversible and irreversible inhibitors and their biological significance.

STRUCTURE:

2.1 INTRODUCTION TO ENZYMES:

Enzymes are biological catalysts whose activity is strongly influenced by their surrounding environmental conditions. The efficiency of an enzyme-catalyzed reaction depends on several factors such as temperature, Ph, enzyme concentration, substrate concentration, and the presence of cofactors, activators, or inhibitors. Each enzyme has an optimum temperature and Ph at which it functions most effectively, while extreme changes can denature the enzyme and alter its active site. Similarly, the availability of substrate and enzyme molecules, as well as the regulation by cofactors or inhibitors, play a crucial role in determining the overall rate of biochemical reactions. Understanding these factors is essential for explaining enzyme regulation in metabolic pathways, industrial applications, and medical contexts.

2.2 FACTORS AFFECTING ENZYMES:

The activity of an enzyme is influenced by its environmental conditions. Changes in these conditions can alter the rate of the reaction catalyzed by the enzyme. In nature, organisms adjust the conditions of their enzymes to maintain an optimum rate of reaction when necessary, or they may possess enzymes that are adapted to function efficiently in the extreme environments in which they live. Several factors affect the rate of enzymatic reactions, including temperature, pH, enzyme concentration, substrate concentration, and the presence of inhibitors or activators.

2.2.1 Temperature:

Increasing temperature increases the kinetic energy that molecules possess, leading to more random collisions between molecules per unit time. Since enzymes catalyze reactions by randomly colliding with substrate molecules, increasing temperature increases the rate of reaction, forming more product. As temperature increases more bond, especially the weaker ionic bonds will break as a result of this strain. As temperature increases, initially the rate of reaction will increase. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease. The rate of enzyme activity increases with as temperature increases until the optimum temperature because of increased kinetic energy, then falls to zero as the enzyme is denatured. The temperature at which the maximum rate of reaction occurs is called the enzyme's optimum temperature. This is different for different enzymes. Most enzymes in the human body have an Optimum Temperature of around 37.0 °C temperature.

- Increasing temperature increases the Kinetic Energy that molecules possess. In a fluid, this means that there are more random collisions between molecules per unit time.
- Since enzymes catalyze reactions by randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product.
- However, increasing temperature also increases the Vibrational Energy that molecules have, specifically in this case enzyme molecules, which puts strain on the bonds that hold them together.
- As temperature increases, more bonds, especially the weaker Hydrogen and Ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the Active Site to change shape.
- This change in shape means that the Active Site is less Complementary to the shape of the Substrate, so that it is less likely to catalyze the reaction. Eventually, the enzyme will become Denatured and will no longer function.
- As temperature increases, more enzymes' molecules' Active Sites' shapes will be less Complementary to the shape of their Substrate, and more enzymes will be Denatured. This will decrease the rate of reaction.
- In summary, as temperature increases, initially the rate of reaction will increase, because of increased Kinetic Energy. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.

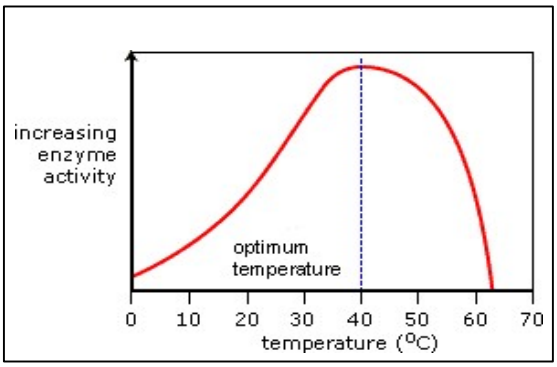

Figure 1: Factors Affecting Enzyme Activity

- The temperature at which the maximum rate of reaction occurs is called the enzyme's Optimum Temperature. This is different for different enzymes. Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.

2.2.2 Ph-Acidity and Basicity

Ph-Acidity and Basicity Ph measure the acidity and basicity of a solution. It is a measure of the hydrogen ion (H+) concentration, and therefore a good indicator of the Hydroxide Ion (OH-) concentration. It ranges from Ph1 to Ph14. Lower Ph values mean higher H+ concentrations and lower OH concentrations. Acid solutions have Ph values below 7, and Basic solutions (alkalis are bases) have Ph values above 7. Deionized water is Ph7, which is termed 'neutral'.

Each enzyme has its own range of Ph in which it will work. H+ and OH- Ions are charged and therefore interfere with hydrogen and ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, it's Active Site. Different enzymes have different optimum Ph values. This is the Ph value at which the bonds within them are influenced by H+ and OH- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. AT the optimum pH, the rate of reaction is at an optimum. If pH increases or decreases much beyond this optimum, the ionization of groups at the active site and on the substrate may change, effectively slowing or preventing the formation of the enzyme substrate complex. At extreme Ph, the bonds which maintain the tertiary structure hence the active site – are disrupted and the enzyme is irreversibly denatured. Any change in Ph above or below the optimum will quickly cause a decrease in the rate of reaction, since more of the enzyme molecules will have active sites, whose shapes are not (or at least are less) complementary to the shape of their substrate.

- pH measures the acidity and basicity of a solution. It indicates the concentration of hydrogen ions (H^+) and, therefore, serves as a good indicator of the hydroxide ion (OH^-) concentration. The pH scale ranges from 1 to 14. Lower pH values indicate higher H^+ concentrations and lower OH^- concentrations.
- Acidic solutions have pH values below 7, while basic (alkaline) solutions have pH values above 7. Deionized water has a pH of 7, which is considered neutral.
- H^+ and OH^- ions are charged particles that can interfere with the hydrogen and ionic bonds holding an enzyme together, as they are attracted to or repelled by the charges formed by these bonds. This interference changes the enzyme's shape and, importantly, the structure

of its active site.

- Different enzymes have different optimum pH values the pH at which their bonds are influenced by H^+ and OH^- ions in such a way that the shape of the active site is most complementary to the substrate. At this optimum pH, the rate of reaction is highest.
- Any change in pH above or below the optimum rapidly decreases the rate of reaction, as more enzyme molecules will have active sites whose shapes are no longer or are less complementary to their substrates.

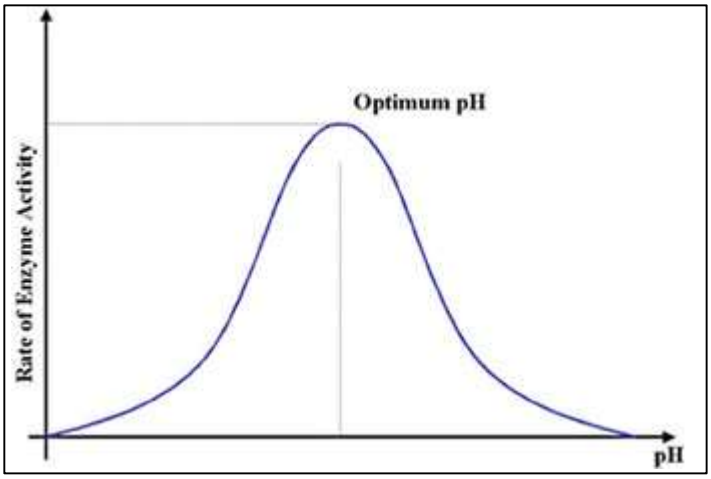

Figure 2: The Effect of Ph on the Rate of Enzyme Activity

- Small changes in Ph above or below the Optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in Ph can cause enzymes to Denature and permanently lose their function.
- Enzymes in different locations have different optimum pH values because their environmental conditions vary. For example, the enzyme pepsin functions best at around pH 2 and is found in the stomach, which contains hydrochloric acid (pH 2).

Small changes in Ph above or below the optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in Ph can cause enzymes to denature and permanently lose their function. Enzymes in different locations have different optimum Ph values since their environmental conditions may be different. For example, the enzyme Pepsin functions best at around Ph=2 and is found in the stomach, which contains Hydrochloric Acid (Ph=2). Most enzymes have an optimum Ph that falls within the physiological range of 7.0-7.5.

2.2.3 Concentration

- Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme-catalyzed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its Metabolism.
- Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from preceding at a higher rate.
- If it is the limiting factor, increasing concentration will increase the rate of reaction up to a point, after which any increase will not affect the rate of reaction. This is because it will no longer be the limiting factor and another factor will be limiting the maximum rate of reaction.

- As a reaction proceeds, the rate of reaction will decrease, since the Substrate will get used up. The highest rate of reaction, known as the Initial Reaction Rate is the maximum reaction rate for an enzyme in an experimental situation.

2.2.4 Substrate Concentration

Changing the enzyme and substrate concentrations affect the rate of reaction of an enzyme catalyzed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its metabolism. A. Substrate Concentration If we keep the concentration of the enzyme constant and increase the concentration of the substrate, it leads to increase in the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed. However, after a certain concentration, any increase will have no effect on the rate of reaction, since substrate concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

- Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.
- However, after a certain concentration, any increase will have no effection the rate of reaction, since Substrate Concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

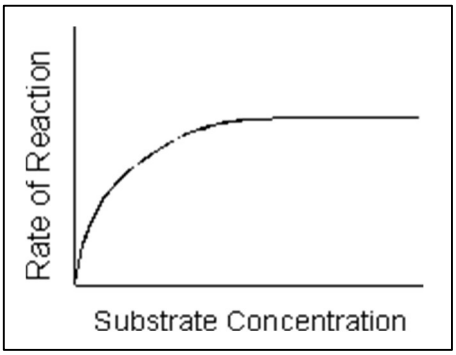

Figure 3: The Effect of Substrate Concentration on the Rate of Reaction

2.2.5 Enzyme Concentration

- Increasing Enzyme Concentration will increase the rate of reaction, asmore enzymes will be colliding with substrate molecules.
- However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.

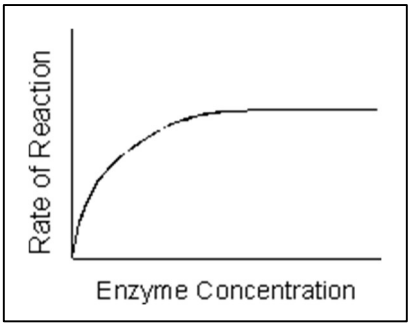

Figure 4: The Effect of Substrate Concentration on the Rate of Reaction

2.2.6 Cofactors:

Many enzymes require cofactors to function properly. There are three main types of cofactors; coenzymes, inorganic ions and prosthetic groups.

1. Coenzymes are organic molecules, which often contain a vitamin molecule as part of their structure. Coenzymes become loosely bound to the enzyme and move away from the enzyme once the reaction is completed. One coenzyme, e.g. NAD+ may react with many different enzymes in many different types of reaction. NAD+ transfers hydrogen in reactions involving dehydrogenase enzymes.
2. Inorganic metal ions are also known as enzyme activators. They change the charge in the active site, enabling the enzyme substrate complex to form. Some become intimately bound to the enzyme, e.g. Fe2+ in catalase. Most others accelerate the binding between the enzyme and the substrate, e.g. Mg2+ in phosphotransferases.
3. Prosthetic groups are coenzymes that bind permanently to the enzyme molecule and remain there even after the reactions are complete, e.g. FAD (flavin adenine dinucleotide). Like NAD+ it carries hydrogen atoms, this time with oxidase enzymes.

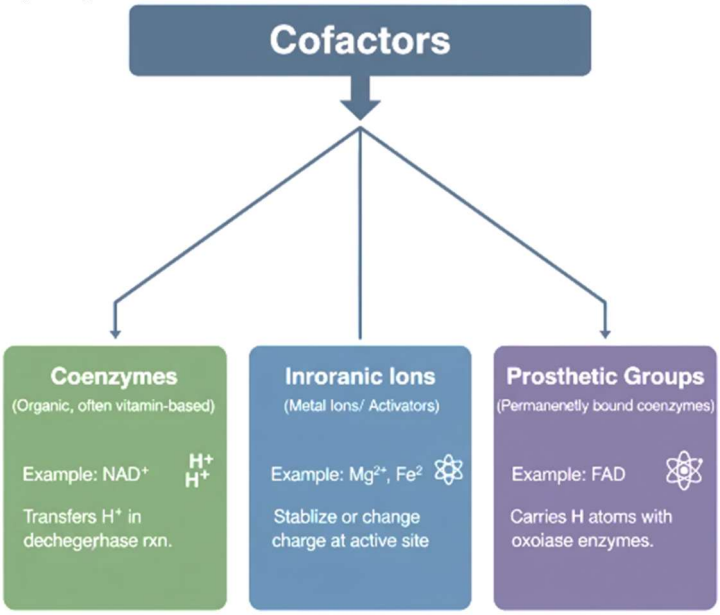

Figure 5: Classification of enzyme cofactors

2.2.7 Inhibitors:

Inhibitors slow down the rate of reaction. As such, they are an essential form of cellular control, allowing enzyme reaction rate to be slowed when necessary. Some enzymes are inhibited by the end product of the reaction they catalyze.

(a) Reversible inhibitors:

There are two types of reversible inhibitor:

- Competitive reversible inhibitor
- Non-competitive reversible inhibitor

Competitive reversible inhibitors are structurally similar to the normal substrate and compete with the normal substrate for the active sites However, if the concentration of the normal substrate is increased, reversible inhibitors are displaced from the active site and the normal enzyme substrate complex can form. Non-competitive reversible inhibitors react with the enzyme but not at the active site. They change the shape of the whole enzyme, including the shape of the active site, hence the reaction cannot proceed and no products are formed on those

enzymes.

(b) Irreversible inhibitors Irreversible:
inhibitors bind covalently and permanently to the enzyme, preventing normal enzyme function. For example, Aspirin is an irreversible inhibitor of cyclooxygenase, an enzyme involved in the synthesis of prostaglandins. Substances such as mercury, iron and arsenic bind irreversibly to the SH (sulfhydryl) group on enzymes.

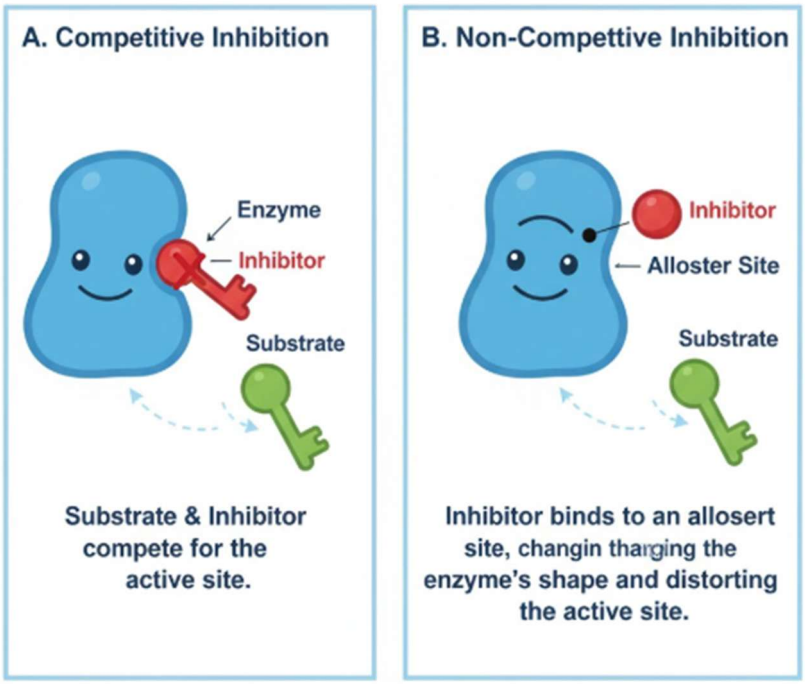

Figure 6: Mechanism of Enzyme Inhibition

2.2.8 Enzyme Specificity:
Enzymes are highly specific biological catalysts. This specificity arises from the precise interaction between the enzyme and its substrate, often compared to a "lock and key" mechanism. Only the correctly shaped substrate (the "key") fits into the enzyme's active site (the "lock").
Some enzymes exhibit "induced fit," where the active site slightly changes shape to accommodate the substrate more snugly. Enzyme specificity ensures that cellular reactions occur in an organized and regulated manner.
Types of specificity:

- **Absolute specificity**: The enzyme acts only on one substrate (e.g., urease acts only on urea).
- **Group specificity**: Acts on a group of related compounds (e.g., hexokinase acts on glucose, fructose, etc.).
- **Linkage specificity**: Acts on a particular type of bond (e.g., lipase acts on ester bonds).
- **Stereochemical specificity**: Acts on specific isomers (e.g., L-amino acid oxidase acts only on L-amino acids).

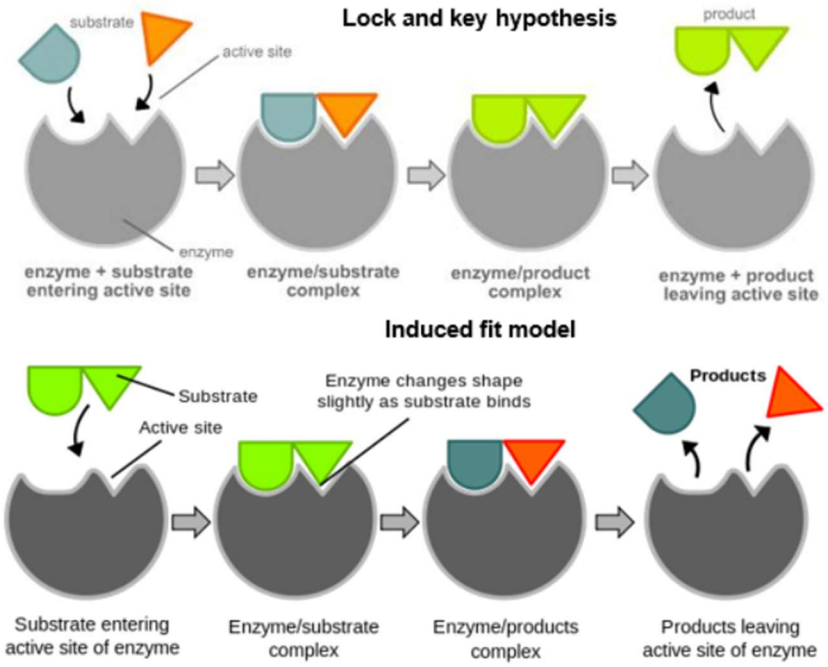

Figure 7: Lock-and-Key vs. Induced Fit model

2.2.9 Applications of Enzymes:

Enzymes play a vital role in various industrial, medical, and research applications. They are widely used because they are efficient, specific, and operate under mild conditions of temperature and ph.

1. **Industrial Applications:**

- Food industry: Amylases in bread-making, proteases in cheese production, and pectinases in fruit juice clarification.
- Detergent industry: Protease and lipase enzymes break down protein and fat stains at lower washing temperatures.
- Textile and leather industries: Enzymes are used for bio-polishing fabrics and softening leather.

2. **Medical and Pharmaceutical Applications:**

- Enzymes are used in diagnostic tests, e.g., glucose oxidase in blood sugar testing.
- Therapeutic enzymes like streptokinase dissolve blood clots.
- Enzymes help produce antibiotics, vitamins, and other biochemicals.

3. **Environmental Applications:**

- Enzymes are used in bioremediation to break down pollutants and waste.
- Enzymes in composting accelerate the decomposition of organic matter.

Application Area	Industry Example	Enzyme Class/Example	Function/Role
Industrial (Food)	Baking	Amylases, Proteases	Hydrolyze starch and proteins to improve dough handling and volume.
Industrial (Beverages)	Brewing/Winemaking	B-Glucanases, Pectinases	Clarify liquids, reduce viscosity, and aid in filtration.
Industrial (Detergents)	Laundry	Lipases, Proteases, Cellulases	Break down fats, proteins (stains), and improve fabric color/feel.
Industrial (Chemical)	Pharmaceutical Synthesis	Lipases, Oxidoreductases	Used in highly stereoselective reactions (biocatalysis) to produce single-enantiomer drugs.
Medical (Diagnostics)	Biosensors/Assays	Glucose Oxidase, Uricase	Measure metabolite levels (e.g., blood glucose) for disease monitoring.
Medical (Therapy)	Thrombolysis	Streptokinase, Urokinase	Systemically degrade fibrin clots to treat stroke or heart attack.
Medical (Therapy)	Cancer Treatment	L-Asparaginase	Depletes L-asparagine, a required nutrient for certain tumor cells (like acute lymphoblastic leukemia).

table 1: Industrial and Medical Uses of Enzymes

2.3 SUMMARY:

Enzymes are biological catalysts whose activity depends on environmental conditions such as temperature, Ph, enzyme and substrate concentration, and the presence of cofactors or inhibitors. Each enzyme functions best at an optimum temperature and Ph, while extremes can denature it by altering its active site. Increasing temperature or substrate concentration initially increases reaction rate until saturation or denaturation occurs, and enzyme concentration similarly boosts activity only until another factor becomes limiting. Cofactors such as coenzymes, metal ions, or prosthetic groups are often required to aid catalysis, whereas inhibitors competitive, non-competitive, or irreversible reduce or block activity, allowing fine control of metabolic pathways. Understanding these factors is crucial for explaining enzyme regulation in biological systems, medicine, and industry.

Factor	Effect on Enzyme Activity	Example/Optimum Condition
Temperature	Increases rate until denaturation	37°C for human enzymes
pH	Alters shape of active site	Pepsin: pH 2, Amylase: pH 7
Substrate concentration	Increases rate until saturation	Enzyme active sites fully occupied
Enzyme concentration	Increases rate until substrate limiting	More enzyme = faster reaction
Cofactors	Enhance activity	Mg^{2+} for kinases
Inhibitors	Decrease or stop reaction	Cyanide inhibits cytochrome oxidase

2.4 TECHINICAL TERMS:

Catalyst, Active Site, Denaturation, Optimum Temperature, Optimum Ph, Substrate Concentration, Cofactor, Prosthetic Group, Competitive Inhibition, Non-competitive Inhibition

2.5 SELF-ASSESSMENT QUESTIONS:

1. What is meant by the optimum temperature of an enzyme, and why is it important?
2. How does Ph affect enzyme activity and the shape of the active site?
3. Why does increasing substrate concentration beyond a certain point not increase the reaction rate?
4. Differentiate between competitive and non-competitive inhibitors with examples.
5. What role do cofactors and coenzymes play in enzyme activity?

2.6 SUGGESTED READINGS:

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LESSON- 3

PROTEOLYTIC ENZYMES

OBJECTIVES:

- To study the occurrence, types, and classification of proteolytic enzymes with respect to their site of action, catalytic mechanism, and Ph activity.
- To understand the functional significance of proteases in biological processes such as digestion, metabolism, cell regulation, and disease mechanisms.
- To explore the industrial and medical applications of proteases and their importance in food processing, pharmaceuticals, and biotechnology.

STRUCTURE:

3.1 INTRODUCTION:

Proteases, also known as proteolytic enzymes or peptidases, are a large and diverse group of enzymes that catalyze the hydrolysis of peptide bonds in proteins, thereby converting them into shorter peptides and amino acids. They are universally present in all living organisms including plants, animals, microorganisms, and even some viruses, where they play essential roles in digestion, metabolism, cell signaling, protein recycling, and regulation of numerous biological processes. Due to their high specificity and catalytic efficiency, proteases are not only crucial for physiological functions such as digestion, blood coagulation, immune responses, and apoptosis, but also have wide-ranging applications in the food, pharmaceutical, detergent, leather, and textile industries.

3.2 PROTEOLYTIC ENZYMES:

Definition: "Protease is an enzyme that catalysis the hydrolysis of peptide bonds present in proteins."

In most of the living organisms, protease enzymes are essential for digestion and absorption of proteins. Proteases or proteolytic enzymes are found in all living organisms, e.g. bacteria, algae, plants and animals and in some of the viruses too. They are involved in the catabolism and digestion of proteins and also in cell signaling.

Proteolytic enzymes catalyze the hydrolysis of the peptide bonds between amino acid residues of proteins. They are often referred to as proteases or peptidases. The International Union of Biochemistry and Molecular Biology, however, recommends the term peptidase. Proteases have been used for a long time for the benefit of humans. Hundreds of years ago humans learned to convert milk to curd by storing it in bags made of calf stomach. Rennet, whose main component is chymosin, a protease, was responsible for the conversion of milk to curd. Cheese making is still considered a craft in nonindustrial setups that relies on rennet for coagulating milk proteins. With a growing concern over the reduction of the impact of industries on the environment, there is a visible shift toward less chemical-intensive processes. Enzymes have provided an eco-friendly alternative to harsh or toxic chemicals. The leather industry, for instance, has introduced alkaline proteases into the leather treatment process, which has successfully helped in reducing the pollution and water consumption in addition to improving leather quality. Enzymes as biological catalysts offer the advantages of high specificity and high catalytic activity over chemical catalysts. They also provide flexibility in terms of temperature of the reactions to be catalyzed, as enzymes of several kinds are available that are active at various temperatures. The proteolytic enzymes are used in the food, dairy, detergent, leather, and pharmaceutical industries and many others.

Most proteases are produced as a zymogen, the inactive form. Zymogens are activated by environmental cues or upon encountering their specific substrate, which may bring about a conformational change. Another strategy employed by nature is the use of sequences of up to 100 amino acid residues known as activation segments. The activation segments when cleaved allow the zymogen to function. Proteolytic enzymes are involved in crucial biological reactions occurring at the cellular to the organism level. At the cellular level proteolytic enzymes are involved in several pivotal biological reactions related but not limited to cell cycle, degradation of misfolded proteins, apoptosis, and mediation of immune responses. At the sub organism level their role has Proteolytic enzymes are ubiquitous because they are indispensable for any kind of living organism. They are produced by all organisms whether prokaryote or eukaryote.

3.2.1 Classification of proteolytic enzymes:

Proteolytic enzymes are classified under the hydrolase category of enzymes. There are other criteria, also, by which the proteolytic enzymes are classified depending on the site of their action.

- **Exopeptidases**
- **Endopeptidases**

i. **Exopeptidases** cleave peptide bonds near the termini of the polypeptide chains. Exopeptidases that cleave the bond from the N-terminus and produce a single amino acid residue or a di- or tripeptide are referred to as **aminopeptidases.** The exopeptidases that act on the C-terminus and produce either a single amino acid residue or dipeptides are referred to as **carboxypeptidases**.

ii. **Endopeptidases** cleave the bonds away from the termini. The proteolytic enzymes are classified according to the mechanism of action based on the catalytic amino acid residue involved, in four categories:
   - Serine proteases
   - Aspartate proteases
   - Cysteine proteases
   - Metalloproteases.

a. **Serine proteases** may be an exo- or endopeptidase according to their mode of action and are active at neutral and alkaline Ph; e.g., subtilisin, which is an important component of detergents, is classified as a serine protease.
b. **Aspartic proteases** are exopeptidases active at acidic Ph; e.g., chymosin is used in making cheese.
c. **Cysteine proteases** are usually active at neutral Ph and often require the presence of a reducing agent. Papain is a well-known cysteine protease.
d. **Metalloproteases** have a characteristic requirement of a divalent cation for their activity; e.g., collagenase is a metalloprotease. They may be active in alkaline or acidic Ph.

3.2.2 Types of Protease Enzymes:

There are many different types of proteases, that take part in various biochemical processes. On the basis of the site of the peptide bond cleavage, proteolytic enzymes are divided into groups:

- **Exopeptidase:** They catalyse the cleavage on terminal peptide bond, e.g. aminopeptidases, carboxypeptidases, etc.
- **Endopeptidase:** They facilitate the cleavage of internal peptide bonds of proteins, e.g. pepsin, trypsin, chymotrypsin, elastase, etc.
- **Oligopeptidases** refers to enzymes, that act on a specific peptide bond.

Different types of protease enzymes remain active in the different Ph range, e.g. acid proteases, alkaline or basic proteases and neutral proteases.

3.2.3 Functions of protease enzymes:

Protease enzymes are essential for many biological processes. They are required for the regulation of various metabolic and cellular processes.

- They are proteolytic, they help in digestion and catabolism of proteins. They catalyze the hydrolysis of peptide bonds and convert them to amino acids, which is then absorbed and utilized by cells.
- They are required for the blood coagulation process.
- Protease enzymes are involved in the cell division, growth, apoptosis and migration.
- Protein recycling and transport across membranes.
- They are involved in the activation of precursor proteins and zymogens.
- Proteases provide immune support and regulate the process of tumor growth, metastasis, inflammation, etc.
- They may help in wound healing and muscle soreness.

Proteolytic enzymes catalyse the hydrolysis of peptide bonds. Catalysis facilitates the nucleophilic attack of an activated water molecule on the peptide bond. Serine, cysteine and threonine proteases function by forming an acyl-enzyme intermediate, which then gets hydrolysed by water to get the product and enzyme is set free. Proteolytic enzymes range from general to specific, e.g. digestive protease enzyme, trypsin can cleave many proteins into smaller fragments, whereas enzymes like thrombin, which takes part in blood clotting are highly specific. Many protease enzymes are present in an inactive form. Being proteins themselves, these precursors get converted to an active form by another protease enzymes. It helps in the regulation and control of the activity. E.g. **trypsinogen, chymotrypsinogen, procarboxypeptidase, proelastase,** etc.

Protease enzyme is a group of proteolytic enzymes, which hydrolyse the peptide bonds present in proteins to convert it to shorter polypeptides and amino acids. They play a major role in the digestion and absorption of dietary proteins. They also play a role in blood coagulation, support immunity, activation of precursor proteins, cell signalling, protein recycling, apoptosis, etc. Proteolytic enzymes are found in both animals and plants. In humans, proteases are mainly present in pancreatic juice and gastric secretions to break down dietary proteins. Trypsin and chymotrypsin are secreted by the pancreas and pepsin is secreted by the chief cells in the stomach. Proteolytic enzymes are naturally found in various food sources such as roots, leaves and fruits of papaya contain papain, a protease enzyme. Pineapple fruits contain a proteolytic enzyme known as bromelain. Fruits like papaya, fig, pineapple, kiwi, etc. contain proteases. Proteases help in the digestion of proteins. Proteases from fruits are used as dietary supplements, treating wounds and also in food processing industries. An example of a protease enzyme is pepsin. It is secreted by peptic of chief cells of gastric mucosa as a proenzyme called pepsinogen. Pepsinogen gets converted into pepsin by the action of HCl secreted by gastric glands. It converts proteins to short-chain polypeptides known as peptones and proteoses.

Protease Enzyme Name	Function
Trypsin	Found in pancreatic juice and breaks proteins and peptones and proteoses to Dipeptides
Chymotrypsin	Found in pancreatic juice and breaks proteins and peptones and proteoses to Dipeptides
Carboxypeptidase	Found in pancreatic juice and breaks proteins and peptones and proteoses to Dipeptides
Elastase	Present in pancreatic juice and digests elastin
Nuclease (ribonuclease and deoxyribonuclease)	Present in pancreatic juice. They split nucleic acid to nucleotides
Collagenase	It digests collagen
Dipeptidase	Found in intestinal secretion. Breaks dipeptides to amino acids
Pepsin	Present in stomach and converts proteins to smaller peptides – proteoses and Peptones
Rennin	Secreted by chief cells of the stomach and curdles milk protein
Thrombin	Involved in blood coagulation
Plasmin	Involved in blood coagulation
Renin	Secreted by juxtaglomerular cells of the kidney and converts angiotensinogen to angiotensin
Hyaluronidase	Present in the acrosome of sperms and helps in penetration of sperm into the ovum during fertilization
Insulinase	Present in the kidney and liver. It degrades insulin
Chymases, tryptases	They are present in mast cells and involved in allergic reactions and Inflammation
Cathepsin, Neurolysin	Present in immune cells

Table 1: Protease Enzyme and its functions

3.3 PROTEOLYTIC ENZYMES FROM ANIMALS:

Pancreatic trypsin, chymotrypsin, pepsin, and chymosin (rennet) are the most important industrial proteases of animal origin. Chymotrypsin from pancreatic juice is used for diagnostic purposes. These enzymes were being obtained from slaughtered cattle; however, to meet the demands from industry, recombinant versions are being produced and tested for efficacy.

Trypsin is a serine protease and is used in preparing medium for growing bacteria for research and industrial purpose. The performance of animal derived trypsin compared to recombinant trypsin for use in clinical applications was similar. Chymotrypsin is obtained from animal pancreatic extract and thus is an expensive enzyme. It has diagnostic and other analytical applications. Rennet is a pepsin-like protease, which is produced in the stomach of nursing mammals. Rennet from calf had a huge demand in the dairy industry for cheese production that is now being met by fungal enzymes. The quality of the cheese made with recombinant chymosin has been shown to be comparable with that of cheese made with calf rennet.

3.3.1 Production of Proteolytic Enzymes:

Enzyme-mediated processes are economically comparable to chemical process nowadays. Therefore, the reduction in the cost of enzyme production is a positive stimulus for the commercialization of enzyme-based processes. Proteolytic enzymes are one of the most important groups of industrial enzymes and account for nearly 60% of total enzyme sales.

According to a global food enzymes market report, it is expected that the global food enzyme market. Proteolytic enzymes find applications in industrial processes such as detergent, textile, leather tanning, dairy, pharmaceutical preparation, cosmetics, peptide synthesis, Proteolytic enzymes can be produced by plants, animals, and microorganisms. The inability of the plant and animal proteolytic enzymes to meet current world demands has led to an increased interest in microbial sources. The relative ease of genetic manipulation and biodiversity of microorganisms make them a highly used source of proteolytic enzymes. Microbial proteases account for 40% of the total worldwide enzyme sales. Microbial proteolytic enzyme production depends on the microorganism, medium composition, physicochemical properties, and the method of production. It is estimated that around 30e40% of the cost of production of industrial enzymes can be attributed to the cost of the growth medium. Selection of the microorganism is important to obtain the desired product. The microorganism should be able to secrete large amounts of proteolytic enzymes and give, adequate yields in a short time period. The production of proteolytic enzymes is also affected by the medium components such as carbon and nitrogen sources and supplementation of mineral salts. Easily metabolizable carbon sources such as maltose, starch, molasses, wheat bran, and coffee pulp and coffee husk enhance the production of proteolytic enzymes. Corn steep liquor, soybean meal, fish meal, and yeast extract enhance the production of proteolytic enzymes, whereas free amino acids decrease the production. Proteolytic enzyme production is also affected by the physicochemical properties such as Ph, temperature, moisture content, incubation period, inoculation size, and aeration.

Proteolytic enzyme production is enhanced by vitamins such as biotin and growth promoters such as 1-naphthylacetic acid. Microbial proteolytic enzymes are produced by fermentation methods. The success of fermentation depends upon the usage of low-cost raw materials, enzyme productivity, and ease of product recovery from the fermentation broth.

3.3.2 Protease Enzymes classification & Types

i.Protease: Branden & Tooze,

An enzyme that hydrolyzes the peptide bond– works without consuming energy because peptide bond hydrolysis is exothermic– releases ~ 2 kcal/mol when the bond breaks– resonance structure that gives it partial double bond characteristics makes the bond kinetically stable although thermodynamically unstable– study the mechanism of serine protease– determinants of specificity.

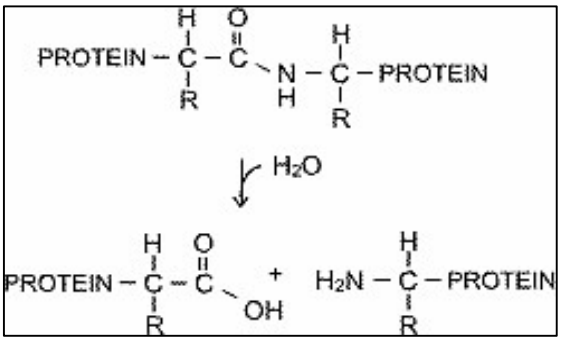

Figure 1: Hydrolysis

ii.Role of protease in disease development

implicated in numerous hereditary diseases normal developmental process and lifecycle of pathogens (virus and parasite) both depend on protease activity cancer needs proteases to break loose and metastasize structure-based design of protease inhibitor has a potential of regulating disease propagation. Peptide bond hydrolysis

- Proteases accelerate peptide bond hydrolysis
- Often form an acyl-enzyme intermediate which is subsequently resolved
- Tetrahedral transition state is formed at two different time points
- End result is an addition of water (i.e. hydrolysis) but water does not attack the main chain directly

Table 1

Peptidases used in structure-based drug design.

Peptidase	Biological function	Disease
Cysteine peptidases		
Cathepsin B	Antigen processing	Acute pancreatitis, cancer
Cathepsins L and S	Lysosomal proteolysis	Inflammation
FP-2	Haemoglobin degradation	Malaria
Caspase-1	Maturation of interleukin 1-β	Ameliorate inflammation, endotoxic shock
Caspase-3 and -7	Executioner caspases in apoptosis	Neuronal and cardiac ischemic injuries
Calpain-1 and -2	Degradation of cytoskeletal proteins	Stroke, neural injuries
Picornain cysteine peptidases	Processing of viral pro-protein	Virus infection
Serine peptidases		
Thrombin	Proteolysis of fibrinogen	Thrombosis
Factor Xa	Conversion of prothrombin to thrombin	Thrombosis
Factor VIIa	Activation of factors IX and X	Thrombosis
Urokinase	Activation of plasminogen	Cancer
Flavivirus peptidases	Processing of polyprotein	Viral infection
DPP-4	Processing of hormone precursors	Type 2 diabetes mellitus
20S proteasome	Ubiquitin-dependent protein degradation	Cancer
Aspartic peptidases		
HIV peptidase	Processing of viral pro-protein	HIV infection
Renin	Processing of angiotensinogen	Blood pressure
Memapsin-2	β-Secretase activity	Alzheimer's disease
Plasmepsin	Haemoglobin degradation	Malaria
Metallo peptidases		
Angiotensin-converting enzyme	Conversion of angiotensin	Hypertension
Botulinium neurotoxin	Cleavage of SNAP proteins	*Clostridium* and tetanus infection
Anthrax lethal factor	Cleavage of MAPKK	*B. anthracis* infection
Matrix metallopeptidase-1	Degradation of connective tissue	Tissue damage in tumour invasion
FtsH	Elimination of misfolded proteins	Neurological diseases
Carboxypeptidases B and U	Cleavage of tissue plasminogen activator	Blood coagulation
PSMA	Liberates glutamate from Ac-Asp-Glu in brain	Marker for prostate cancer

Table 2: Peptidases used in structure-based drug design

proteolysis, Process in which a protein is broken down partially, into peptides, or completely, into amino acids, by proteolytic enzymes, present in bacteria and in plants but most abundant in animals. Proteins in food are attacked in the stomach by pepsin and in the small intestine mainly by trypsin and chymotrypsin from the pancreas. Proteolytic enzymes are secreted as zymogens, which are themselves converted by proteolysis to their active forms. Many other zymogens or precursors undergo proteolysis to form active enzymes or proteins (e.g., fibrinogen to fibrin). In cells, proteolytic degradation of old proteins is part of cellular maintenance.

iii.Role of Proteolytic Enzymes in Digestion:

Dietary protein is digested by many proteases' enzymes present in the digestive tract. Pepsin present in the gastric juice as pepsinogen, which in presence of HCl, gets converted into pepsin. Pepsin partially hydrolyses proteins into proteoses and peptones. Gastric juice of infants contains renin, which hydrolyses milk protein. The partially digested proteins are acted on by pancreatic enzymes in the small intestine. Pancreatic secretion has many proteolytic enzymes present as an inactive precursor-chymotrypsinogen, trypsinogen, procarboxypeptidase.

Enterokinase secreted by intestinal mucosa converts trypsinogen to trypsin, which in turn activates other proteolytic enzymes. Proteins, peptones and proteoses are acted on by pancreatic proteolytic enzymes to form dipeptides. The enzyme dipeptidase present in the succus entericus converts dipeptides to amino acids.

The enzymatic splitting of large and complex molecules into smaller ones is effective only if the enzyme molecules come into direct contact with the molecules of the material they are to digest. In animals that ingest very large pieces of food, only the molecules at the surface are exposed to the digestive enzymes. Digestion can proceed more efficiently, therefore, if the bulk food is first mechanically broken down, exposing more molecules for digestion. Among the variety of devices that have evolved to perform such mechanical processing of food are the teeth of mammals and the muscular gizzards of birds. Human digestion begins in the mouth. Their food is chewed and mixed with saliva, which adds moisture and contains the enzyme amylase, which begins to break down starches.

The tongue kneads food into a smooth ball (bolus), which is then swallowed. The bolus passes through the pharynx and esophagus into the stomach, propelled by peristaltic muscular contractions. In the stomach the food is then mixed by peristaltic contractions (about three per minute) with highly acidic gastric juices secreted there. The hormone gastrin stimulates the secretion of these juices, which contain water, inorganic salts, hydrochloric acid, mucin, and several enzymes. The food, now in a semiliquid state called chyme, passes from the stomach into the duodenum, the first section of the small intestine, where the greatest part of digestion takes place. The chemical reactions involved in digestion can be clarified by an account of the digestion of maltose sugar. Maltose is, technically, a double sugar, since it is composed of two molecules of the simple sugar glucose bonded together. The digestive enzyme maltase catalyzes a reaction in which a molecule of water is inserted at the point at which the two glucose units are linked, thereby disconnecting them.

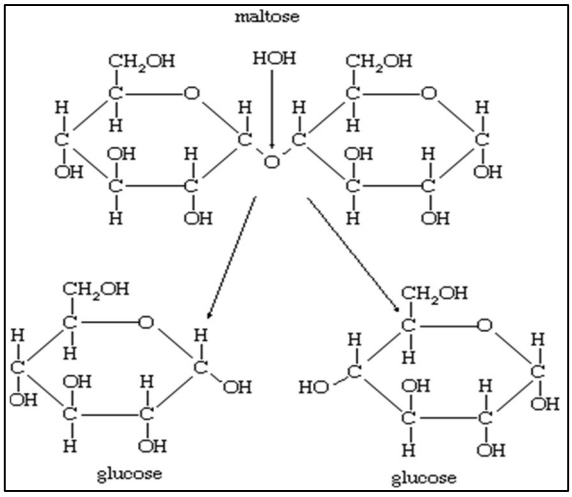

Figure 2: hydrolysis of maltose

And chemical terms, the maltose has been hydrolysed. All digestive enzymes act in a similar way and thus are hydrolysing enzymes. One of the most common uses for proteolytic enzymes is to improve the digestion and absorption of dietary protein. Pancreatic enzyme replacement therapy (PERT) is often used in the treatment of pancreatic insufficiency, cystic fibrosis, certain types of cancers such as pancreatic, colorectal and stomach cancer, or after gastric or pancreatic Taking proteolytic enzyme supplements helps those with a deficiency or lack of these enzymes properly break down and digest dietary protein. Both foods and supplements containing proteolytic enzymes can aid protein digestion. Several animal studies have shown that kiwifruit extract helps improve the breakdown and digestion of proteins, especially meats, milk, cheese, fish and eggs Another study found that when people with indigestion took a supplement containing proteolytic enzymes, they experienced a significant improvement in bloating, abdominal pain, belching, heartburn and loss of appetite.

Many other nutrient molecules are much more complex, being polymers, or long chains of simple component units. Starch, for example, is a carbohydrate, like maltose, but its molecules are composed of thousands of glucose units bonded together. Even so, the digestion of starch is essentially the same as the digestion of maltose: each linkage between adjacent glucose units is hydrolyzed, with the result that the starch molecule is split into thousands of glucose molecules.

Protein molecules also are polymers, but their constituent units are amino acids instead of simple sugars. Proteolytic enzymes (i.e., protein-digesting enzymes) split the protein chains by hydrolyzing the bonds between adjacent amino acids. Because as many as 20 different kinds of amino acids may act as building blocks for proteins, the complete digestion of a protein into its amino acids requires the action of several different proteolytic enzymes, each capable of hydrolyzing the bonds between particular pairs of amino acids. Fat molecules too are composed of smaller building-block units (the alcohol glycerol plus three fatty acid groups); they are hydrolyzed by the enzyme lipase.

Various other classes of compounds are digested by hydrolytic enzymes specific for them. Not all of these enzymes occur in every organism; for example, few animals possess cellulase (cellulose-digesting enzyme), despite the fact that cellulose constitutes much of the total bulk of the food ingested by plant-eating animals. Some nonetheless benefit from the cellulose in their diet because their digestive tracts contain microorganisms (known as symbionts) capable of digesting cellulose. The herbivores absorb some of the products of their symbionts' digestive activity.

So far, emphasis has been placed on the role of digestion in converting large complex molecules into smaller simpler ones that can move across membranes, which thus permits absorption of food into cells. The same processes occur when substances must be moved from cell to cell within a multicellular organism. Thus, green plants, which do not have to digest incoming nutrients, digest stored material, such as starch, before it can be transported from storage organs (tubers, bulbs, corms) to points of utilization, such as growing buds.

iv. Certain Proteolytic Enzymes May Have Cancer-Fighting Properties

Test-tube and animal studies have shown that some proteolytic enzymes may help fight cancer cells. One test-tube study demonstrated that bromelain inhibited the growth and induced the death of human stomach cancer and colon cancer cells. Similar study found that bromelain extracted from pineapple stems exerted cancer-fighting effects on colon cancer cells. It suggested that both bromelain and bromelain-containing foods like pineapple may help prevent colon cancer. Another recent test-tube study showed that both bromelain and papain stopped growth and caused cell death in human bile duct cancer cells. Though these results are promising, human studies are needed to examine the efficacy and safety of proteolytic enzymes in the treatment of certain cancers.

3.4 ADVANCED INDUSTRIAL APPLICATIONS OF PROTEOLYTIC ENZYMES:

3.4.1 Enzymes in Detergent Formulations (The Subtilisin Family)

Proteolytic enzymes, especially those from the **subtilisin family** (a class of serine proteases), are one of the largest enzyme applications globally.

- **Mechanism of Stain Removal:** Stains from food (like grass, blood, egg) and human secretions are primarily composed of **proteins**. Subtilisin and other alkaline proteases (active at high pH) act by **hydrolyzing** these large, insoluble protein molecules into smaller, water-soluble peptides and amino acids. This makes the stain detach easily from the fabric during the wash cycle.
- **Engineering for Industry:** Industrial proteases must be robust. They are often engineered for:

1. **Thermostability:** To survive high washing temperatures (though modern trends favor lower temperatures).
2. **Alkaline Stability:** To remain active in high-pH detergent solutions.
3. **Compatibility:** To resist denaturation by surfactants, bleaching agents, and other components in the detergent mixture. **Directed evolution** and **site-directed mutagenesis** are key biotechnological tools used to optimize these enzyme properties.

3.4.2 Proteases in Biocatalysis and Peptide Synthesis

In the pharmaceutical and fine chemical industries, proteases are increasingly used as **biocatalysts** for their remarkable specificity, offering an environmentally friendly alternative to traditional organic synthesis.

- **Stereoselectivity:** Chemical catalysts often produce a mixture of mirror-image molecules (**enantiomers**), but for pharmaceuticals, only one enantiomer is typically active. Enzymes like proteases can execute reactions with **high stereoselectivity**, producing the desired single enantiomer with high purity, which is critical for drug safety and efficacy.
- **Reverse Hydrolysis (Peptide Synthesis):** Proteases normally perform hydrolysis (breaking bonds with water). However, under conditions of low water activity (high substrate concentration or use of organic solvents), the reaction equilibrium can be shifted, allowing the enzyme to catalyze **peptide bond formation** (the reverse reaction). This process is known as **enzymatic peptide synthesis** and is an eco-friendly route to

manufacturing specific dipeptides or short peptides for use in medicine or as artificial sweeteners (like aspartame).

3.5 ADVANCED MEDICAL AND BIOTECHNOLOGICAL APPLICATIONS:

The text mentions roles in blood coagulation and cancer; here we explore specific therapeutic and diagnostic applications at the molecular level.

3.5.1 Therapeutic Thrombolytic Enzymes:

The use of enzymes to dissolve blood clots (thrombolysis) is a critical intervention for myocardial infarction (heart attack) and ischemic stroke.

- **Key Enzymes: Streptokinase** (derived from bacteria) and Urokinase (naturally occurring in urine and blood) are prominent examples.
- **Mechanism of Action:** These enzymes are not direct proteases of the clot protein (fibrin) itself. Instead, they act as activators. They specifically convert the circulating inactive precursor plasminogen into its active form, plasmin. Plasmin is the true enzyme that then cleaves the fibrin meshwork of the clot, leading to its dissolution. This targeted activation cascade is a hallmark of highly regulated proteolytic systems.
- **Engineered Thrombolytics:** Recombinant tissue plasminogen activator (rt-PA) is a highly engineered version that offers greater specificity by preferentially activating plasminogen that is already bound to the fibrin clot, thus reducing the risk of systemic bleeding.

3.5.2 Protease Inhibitors in Drug Design:

Proteases are essential for the life cycle of many pathogens and the propagation of diseases like cancer, making their inhibitors crucial therapeutic agents.

- **Targeting Viral Replication:** Many viruses, including HIV and the Hepatitis C Virus (HCV), produce their necessary structural and functional proteins as a single, long polypeptide chain (polyprotein). They rely on their own specific viral proteases to cleave this polyprotein into individual, active enzymes and proteins. HIV protease inhibitors (like Ritonavir and Lopinavir) and HCV protease inhibitors (like Teleprevir) function by binding tightly to the viral protease's active site, blocking this maturation step and effectively stopping viral replication. This is a classic example of structure-based drug design.
- **Targeting Cancer Metastasis:** Tumour cells often overexpress matrix metalloproteases (MMPs). These MMPs break down the extracellular matrix (ECM), the protein barrier surrounding cells. By degrading the ECM, the tumour cells gain the ability to metastasize (spread) to distant sites. Therefore, the development of MMP inhibitors is a continuous area of research in cancer therapy, aimed at blocking the invasion process.

3.5.3 Proteases in Diagnostic Assays

Beyond therapy, proteases are vital components in clinical diagnostics.

- **Immunoassay Signal Amplification:** In highly sensitive tests like ELISA (Enzyme-Linked Immunosorbent Assay), a detection antibody is often conjugated to a protease. After the substrate is added, the protease acts to amplify the signal by rapidly converting a non-coloured substrate into a detectable product, which is often a colour change or a fluorescent signal.
- **Biomarker Detection:** Certain elevated protease levels in bodily fluids can serve as a biomarker for disease. For instance, high levels of Prostate-Specific Antigen (PSA), which is a serine protease, are used in screening for prostate cancer. Monitoring the level of specific, circulating proteases can provide a simple, quantifiable method for early disease detection or tracking disease progression.

3.6 SUMMARY:

Proteolytic enzymes may aid the digestions of protein, reduce symptoms of irritable bowel syndrome, decrease inflammation, ease muscle soreness and speed recovery after surgery. Early-stage research suggests they may even help fight cancer cells.

3.7 TECHINICAL TERMS:

Zymogen, Hydrolysis, Endopeptidase, Exopeptidase, Catalytic Mechanism, Active Site, Cofactor, Proteolysis, Apoptosis, Metalloprotease

3.8 SELF-ASSESSMENT QUESTIONS:

1. What is the role of protease enzymes?
2. Where can proteolytic enzymes be found?
3. What fruit contains proteolytic enzymes?
4. What is an example of a protease?

3.9 SUGGESTED READINGS:

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LESSON- 4

ENERGY-PRODUCING PATHWAYS

OBJECTIVES:

- understand metabolism and distinguish between anabolic and catabolic pathways in living systems.
- To explain the role of ATP as the energy currency of the cell and how it is produced through carbohydrate metabolism.
- To describe the stepwise process of energy production-glycolysis, oxidation of pyruvate, citric acid cycle, electron transport chain-and alternative pathways such as fermentation.

STRUCTURE:

4.1 INTRODUCTION:

Energy is the capacity to do work. All living organisms require a continuous supply of energy to perform biological processes such as growth, movement, and reproduction. This energy is obtained mainly through the breakdown of nutrients, primarily glucose, via a series of biochemical pathways collectively known as metabolism.

4.2 METABOLISM:

The processes of making and breaking down carbohydrate molecules illustrate two types of metabolic pathways. A metabolic pathway is a step-by-step series of interconnected biochemical reactions that convert a substrate molecule or molecules through a series of metabolic intermediates, eventually yielding a final product or products. For example, one

metabolic pathway for carbohydrates breaks large molecules down into glucose. Another metabolic pathway might build glucose into large carbohydrate molecules for storage. The first of these processes requires energy and is referred to as anabolic. The second process produces energy and is referred to as catabolic. Consequently, metabolism is composed of these two opposite pathways:

1. Anabolism (building molecules)
2. Catabolism (breaking down molecules)

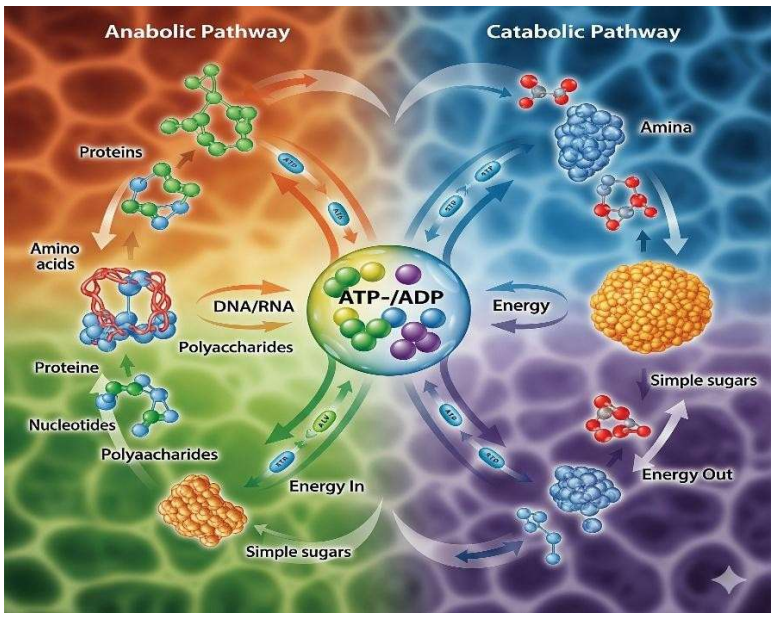

Figure 1: Anabolic and catabolic pathways: Anabolic pathways

are those that require energy to synthesize larger molecules. Catabolic pathways are those that generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.

4.3 METABOLIC PATHWAYS:

Chemical reactions in metabolic pathways rarely take place spontaneously. Each reaction step is facilitated, or catalyzed, by a protein called an enzyme. Enzymes are important for catalyzing all types of biological reactions.

A metabolic pathway is a series of chemical reactions in a cell that build and breakdown molecules for cellular processes. Anabolic pathways synthesize molecules and require energy. Catabolic pathways break down molecules and produce energy. Because almost all metabolic reactions take place non spontaneously, proteins called enzymes help facilitate those chemical reactions.

1. Anabolic Pathways:

Anabolic pathways require an input of energy to synthesize complex molecules from simpler ones. One example of an anabolic pathway is the synthesis of sugar from CO. Other examples include the synthesis of large proteins from amino acid building blocks and the synthesis of new DNA strands from nucleic acid building blocks. These processes are critical to the life of the cell, take place constantly, and demand energy provided by ATP and other high energy molecules like NADH (nicotinamide adenine dinucleotide) and NADPH.

2. Catabolic pathways:

Catabolic pathways involve the degradation of complex molecules into simpler ones, releasing the chemical energy stored in the bonds of those molecules. Some catabolic pathways can capture that energy to produce ATP, the molecule used to power all cellular processes. Other energy-storing molecules, such as lipids, are also broken down through similar catabolic reactions to release energy and make ATP.

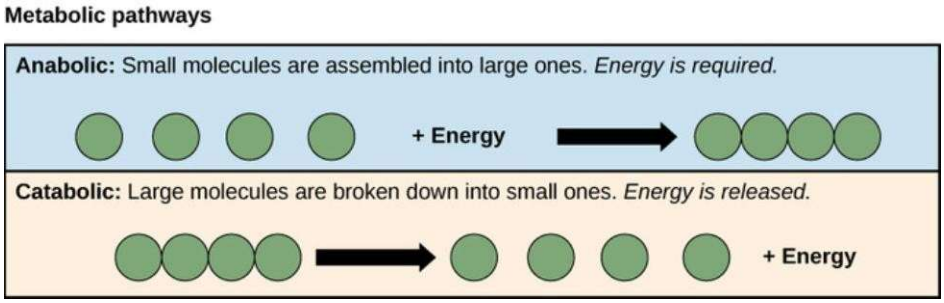

Figure 2: Metabolic Pathways

4.4 CARBOHYDRATE METABOLISM:

Carbohydrates are one of the major forms of energy for animals and plants. Plants build carbohydrates using light energy from the sun (during the process of photosynthesis), while animals eat plants or other animals to obtain carbohydrates. Plants store carbohydrates in long polysaccharides chains called starch, while animals store carbohydrates as the molecule glycogen. These large polysaccharides contain many chemical bonds and therefore store a lot of chemical energy. When these molecules are broken down during metabolism, the energy in the chemical bonds is released and can be harnessed for cellular processes.

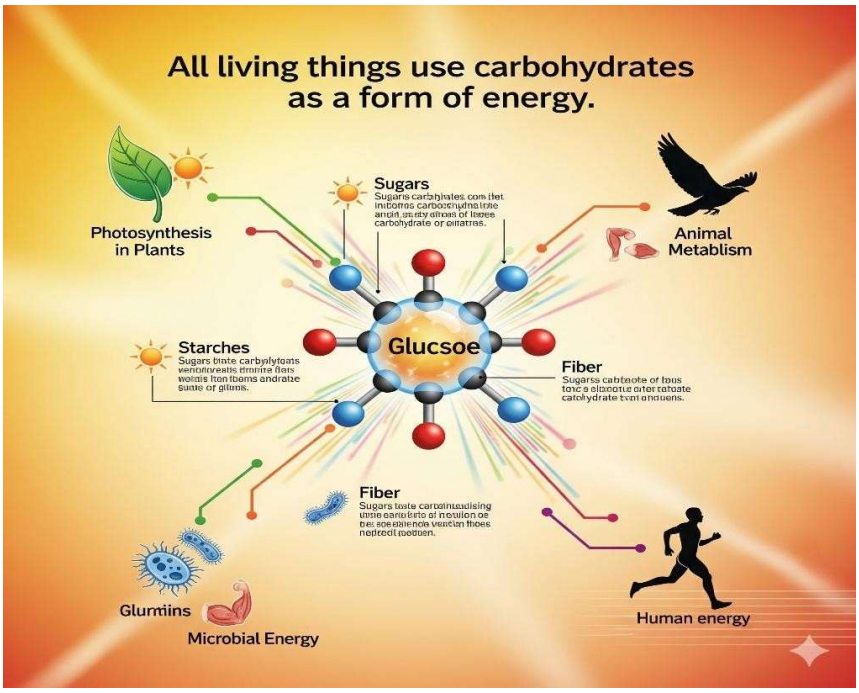

Figure 3: living things use carbohydrates as a form of energy

Plants, like this oak tree and acorn, use energy from sunlight to make sugar and other organic molecules. Both plants and animals (like this squirrel) use cellular respiration to derive energy from the organic molecules originally produced by plants

4.4.1 Cellular Respiration:

An electrical energy plant converts energy from one form to another form that can be more easily used. For example, geothermal energy plants start with underground thermal energy (heat) and transform it into electrical energy that will be transported to homes and factories.

This geothermal energy plant transforms thermal energy from deep in the ground into electrical energy, which can be easily used. Like a generating plant, living organisms must take in energy from their environment and convert it into to a form their cells can use. Organisms ingest large molecules, like carbohydrates, proteins, and fats, and convert them into smaller molecules like carbon dioxide and water. This process is called cellular respiration, a form of catabolism, and makes energy available for the cell to use. The energy released by cellular respiration is temporarily captured by the formation of adenosine triphosphate (ATP) within the cell. ATP is the principle form of stored energy used for cellular functions and is frequently referred to as the energy currency of the cell. The nutrients broken down through cellular respiration lose electrons throughout the process and are said to be oxidized. When oxygen is used to help drive the oxidation of nutrients the process is called aerobic respiration. Aerobic respiration is common among the eukaryotes, including humans, and takes place mostly within the mitochondria. Respiration occurs within the cytoplasm of prokaryotes. Several prokaryotes and a few eukaryotes use an inorganic molecule other than oxygen to drive the oxidation of their nutrients in a process called anaerobic respiration. Electron acceptors for anaerobic respiration include nitrate, sulfate, carbon dioxide, and several metal ions. The energy released during cellular respiration is then used in other biological processes. These processes build larger molecules that are essential to an organism's survival, such as amino acids, DNA, and proteins. Because they synthesize new molecules, these processes are examples of anabolism.

- In **aerobic respiration**, oxygen is the final electron acceptor, and the process mainly occurs in the mitochondria (eukaryotes) or cytoplasm (prokaryotes).
- In **anaerobic respiration**, other inorganic molecules such as nitrate, sulphate, or CO_2 act as electron acceptors.

4.4.2 Energy Production from Carbohydrates (Cellular Respiration)

The metabolism of any monosaccharide (simple sugar) can produce energy for the cell to use. Excess carbohydrates are stored as starch in plants and as glycogen in animals, ready for metabolism if the energy demands of the organism suddenly increase. When those energy demands increase, carbohydrates are broken down into constituent monosaccharides, which are then distributed to all the living cells of an organism. Glucose ($C_6H_{12}O_6$) is a common example of the monosaccharides used for energy production. Inside the cell, each sugar molecule is broken down through a complex series of chemical reactions. As chemical energy is released from the bonds in the monosaccharide, it is harnessed to synthesize high-energy adenosine triphosphate (ATP) molecules. ATP is the primary energy currency of all cells. Just as the dollar is used as currency to buy goods, cells use molecules of ATP to perform immediate work and power chemical reactions. The breakdown of glucose during metabolism is call cellular respiration can be described by the equation.

$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2 +6H_2O+\text{Energy (ATP)}$

4.4.3 Producing Carbohydrates (Photosynthesis)

Plants and some other types of organisms produce carbohydrates through the process called photosynthesis. During photosynthesis, plants convert light energy into chemical energy by building carbon dioxide gas molecules (CO_2) into sugar molecules like glucose. Because this process involves building bonds to synthesize a large molecule, it requires an input of energy (light) to proceed. The synthesis of glucose by photosynthesis is described by this equation

$$\mathbf{6CO_2+6H_2O+energy(light)\rightarrow C_6H_{12}O_6+6O_2}$$

As part of plants' chemical processes, glucose molecules can be combined with and converted into other types of sugars. In plants, glucose is stored in the form of starch, which can be broken down back into glucose via cellular respiration in order to supply ATP.

4.5 ATP IN LIVING SYSTEMS:

A living cell cannot store significant amounts of free energy. Excess free energy would result in an increase of heat in the cell, which would lead to excessive thermal motion that could damage and then destroy the cell. Rather, a cell must be able to handle that energy in a way that enables the cell to store energy safely and release it for use as needed. Living cells accomplish this by using the compound adenosine triphosphate (ATP). ATP is often called the "energy currency" of the cell and can be used to fill any energy need of the cell. Adenosine triphosphate ATP has three phosphate groups that can be removed by hydrolysis to form ADP (adenosine diphosphate) or AMP (adenosine monophosphate).The negative charges on the phosphate group naturally repel each other, requiring energy to bond them together and releasing energy when these bonds are broken.

ATP Structure and function:

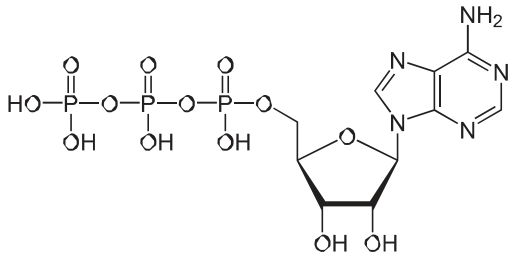

Figure 4: Adenosine triphosphate (ATP)

The core of ATP is a molecule of adenosine monophosphate (AMP), which is composed of an adenine molecule bonded to a ribose molecule and to a single phosphate group. Ribose is a five-carbon sugar found in RNA, and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in the formation of adenosine diphosphate (ADP); the addition of a third phosphate group forms adenosine triphosphate (ATP). The addition of a phosphate group to a molecule requires energy. Phosphate groups are negatively charged and, thus, repel one another when they are arranged in a series, as they are in ADP and ATP. This repulsion makes the ADP and ATP molecules inherently unstable. The release of one or two phosphate groups from ATP, a process called dephosphorylation, releases energy.

Energy from ATP:

Hydrolysis is the process of breaking complex macromolecules apart. During hydrolysis, water is split, or lysed, and the resulting hydrogen atom (H) and a hydroxyl group (OH) are added to

the larger molecule. The hydrolysis of ATP produces ADP, together with an inorganic phosphate ion (P), and the release of free energy. To carry out life processes, ATP is continuously broken down into ADP, and, like a rechargeable battery, ADP is continuously regenerated into ATP by the reattachment of a third phosphate group. Water, which was broken down into its hydrogen atom and hydroxyl group during ATP hydrolysis, is regenerated when a third phosphate is added to the ADP molecule, reforming ATP. Obviously, energy must be infused into the system to regenerate ATP. In nearly every living thing on earth, the energy comes from the metabolism of glucose. In this way, ATP is a direct link between the limited set of exergonic pathways of glucose catabolism and the multitude of endergonic pathways that power living cells.

4.6 GLYCOLYSIS:

Nearly all of the energy used by living cells comes to them from the energy in the bonds of the sugar glucose. Glucose enters heterotrophic cells in two ways.

- Through secondary active transport against the glucose concentration gradient.
- Through GLUT proteins (glucose transporters) that facilitate diffusion.

Glycolysis is the first pathway used in the breakdown of glucose to extract energy. It takes place in the cytoplasm of both prokaryotic and eukaryotic cells. Since it does not use oxygen, it is **anaerobic**.

- First phase of glycolysis requires energy.
- Second phase produces ATP and NADH, while glucose is converted into pyruvate.

Products of Glycolysis (per glucose):

- 2 Pyruvate
- 2 ATP (net gain)
- 2 NADH

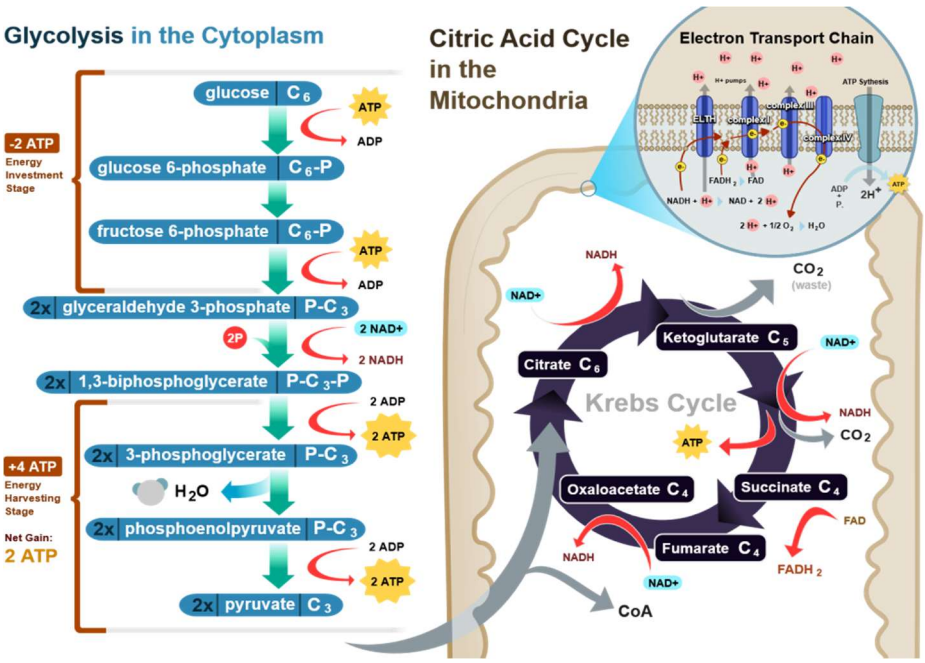

Figure 5: Cellular Respiration

Glycolysis is the first pathway of cellular respiration that oxidizes glucose molecules. It is followed by the Krebs cycle and oxidative phosphorylation to produce ATP.

4.6.1 The Second Half of Glycolysis

- 2 molecules of glyceraldehyde-3-phosphate enter the second half.
- Produces **2 NADH** and **4 ATP** molecules per glucose.
- Net gain: **2 ATP** (since 2 were consumed in the first half).

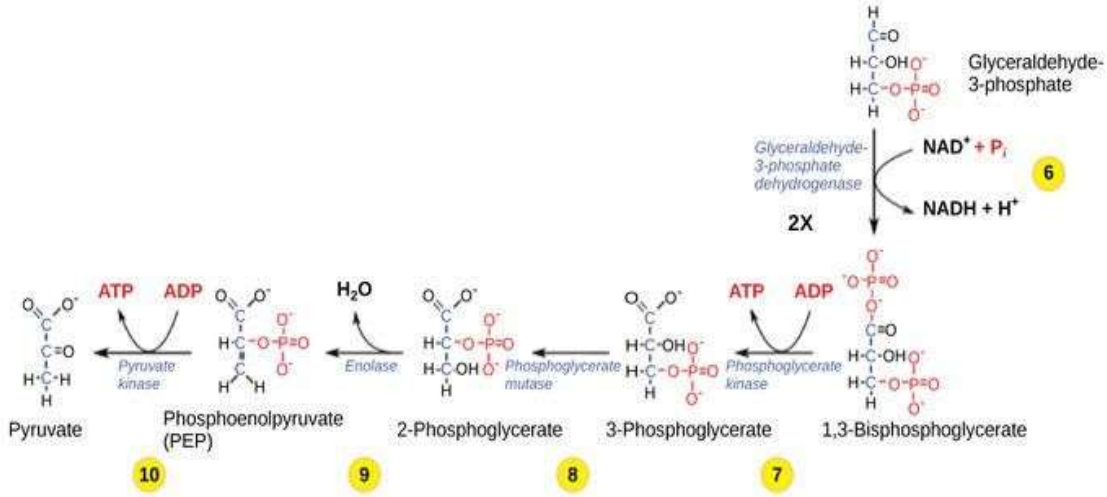

Figure 6: Second half of Glycolysis – Return on Investment

Enzymes that catalyze ATP-producing reactions are rate-limiting and must be sufficient for glycolysis to complete production of 4 ATP, 2 NADH, and 2 pyruvate per glucose. Red blood cells require glycolysis as their sole source of ATP because they lack mitochondria. Cancer cells and stem cells also rely on glycolysis as their main ATP source (aerobic glycolysis, or Warburg effect).

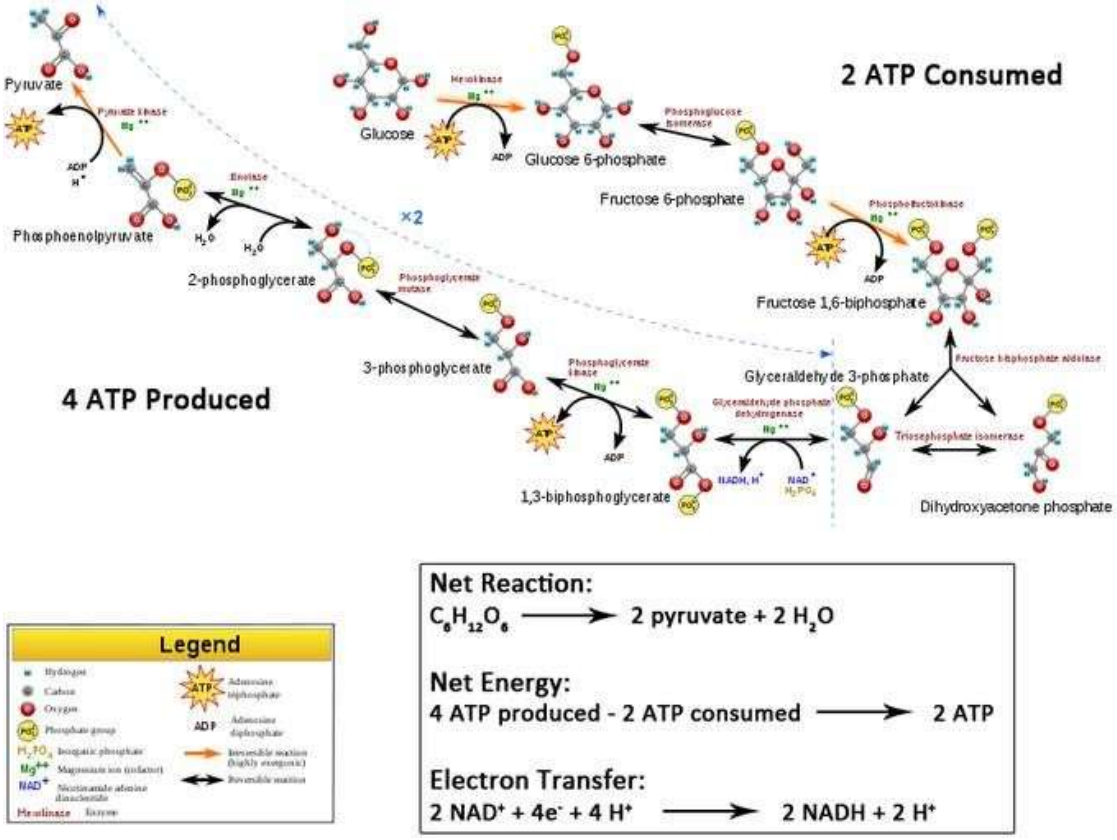

Figure 7: Glycolysis

4.7 OXIDATION OF PYRUVATE-BREAKDOWN OF PYRUVATE:

Before entering the citric acid cycle, pyruvate is converted into acetyl CoA. In order for pyruvate, the product of glycolysis, to enter the next pathway, it must undergo several changes to become acetyl Coenzyme A (acetyl CoA). Acetyl CoA is a molecule that is further converted to oxaloacetate, which enters the citric acid cycle (Krebs cycle). The conversion of pyruvate to acetyl CoA is a three-step process.

Steps:

Step 1: Carboxyl group removed → CO_2 released.

A carboxyl group is removed from pyruvate, releasing a molecule of carbon dioxide into the surrounding medium. (Note: carbon dioxide is one carbon attached to two oxygen atoms and is one of the major end products of cellular respiration.) The result of this step is a two-carbon hydroxyethyl group bound to the enzyme pyruvate dehydrogenase; the lost carbon dioxide is the first of the six carbons from the original glucose molecule to be removed. This step proceeds twice for every molecule of glucose metabolized (Remember: there are two pyruvate molecules produced at the end of glycolysis); thus, two of the six carbons will have been removed at the end of both of these steps.

Step 2: Hydroxyethyl group oxidized → acetyl group, transferred to NAD^+ → NADH.

The hydroxyethyl group is oxidized to an acetyl group, and the electrons are picked up by NAD, forming NADH (the reduced form of NAD+). The high- energy electrons from NADH will be used later by the cell to generate ATP for energy.

Step 3: Acetyl group transferred to CoA → Acetyl CoA.

The enzyme-bound acetyl group is transferred to CoA, producing a molecule of acetyl CoA. This molecule of acetyl CoA is then further converted to be used in the next pathway of metabolism, the citric acid cycle.

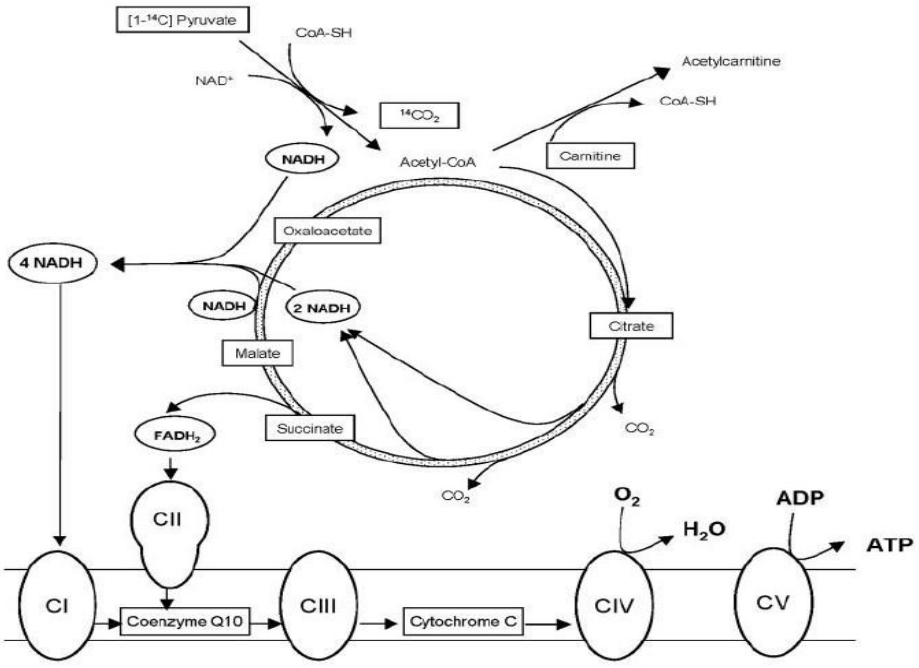

Figure 8: Breakdown of Pyruvate

4.8 CITRIC ACID CYCLE:

Citric Acid Cycle (Krebs Cycle / TCA Cycle)

Acetyl CoA enters the cycle by combining with oxaloacetate to form citrate. Acetyl CoA links glycolysis and pyruvate oxidation with the citric acid cycle. In the presence of oxygen, acetyl CoA delivers its acetyl group to a four-carbon molecule, oxaloacetate, to form citrate, a six-carbon molecule with three carboxyl groups. During this first step of the citric acid cycle, the CoA enzyme, which contains a sulfhydryl group (-SH), is recycled and becomes available to attach another acetyl group. The citrate will then harvest the remainder of the extractable energy from what began as a glucose molecule and continue through the citric acid cycle. In the citric acid cycle, the two carbons that were originally the acetyl group of acetyl CoA are released as carbon dioxide, one of the major products of cellular respiration, through a series of enzymatic reactions. For each acetyl CoA that enters the citric acid cycle, two carbon dioxide molecules are released in reactions that are coupled with the production of NADH molecules from the reduction of NAD molecules. For each molecule of acetyl CoA that enters the citric acid cycle, two carbon dioxide molecules are released, removing the carbons from the acetyl group. In addition to the citric acid cycle, named for the first intermediate formed, citric acid, or citrate, when acetate joins to the oxaloacetate, the cycle is also known by two other names. The TCA cycle is named for tricarboxylic acids (TCA) because citric acid (or citrate) and isocitrate, the first two intermediates that are formed, are tricarboxylic acids. Additionally, the cycle is known as the Krebs cycle, named after Hans Krebs, who first identified the steps in the pathway in the 1930s in pigeon flight muscle.

Main Events:

- 2 carbons from acetyl CoA released as CO_2.
- NAD^+ reduced to NADH.
- Produces ATP, NADH, $FADH_2$ for further energy production.

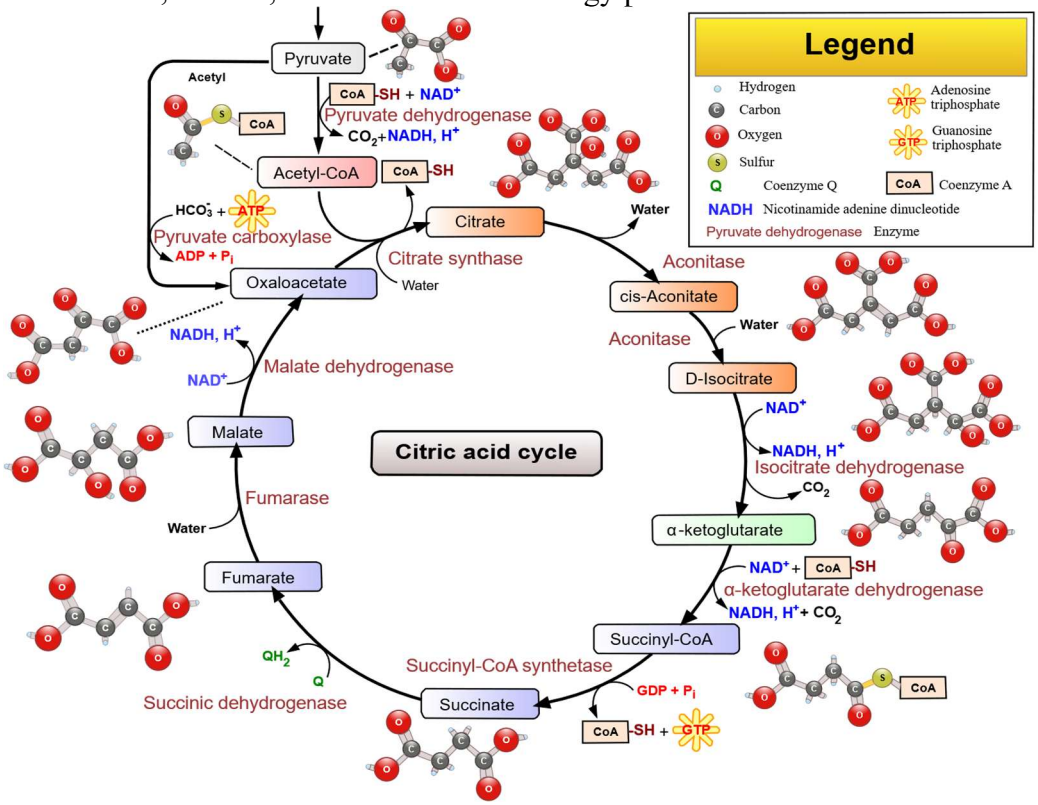

Figure 9: Acetyl CoA and the Citric Acid Cycle

4.9 ELECTRON TRANSPORT CHHAIN (ETC):

The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and FADH to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

Complexes:
Oxidative phosphorylation is the metabolic pathway in which electrons are transferred from electron donors to electron acceptors in redox reactions; this series of reactions releases energy which is used to form ATP. There are four protein complexes (labeled complex I-IV) in the electron transport chain, which are involved in moving electrons from NADH and FADH to molecular oxygen.

Complex I establish the hydrogen ion gradient by pumping four hydrogen ions across the membrane from the matrix into the intermembrane space.

Complex II receives FADH, which bypasses complex I, and delivers electrons directly to the electron transport chain. Ubiquinone (Q) accepts the electrons from both complex I and complex II and delivers them to complex III.

Complex III pumps' protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes.

Complex IV reduces oxygen; the reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water.

- Complex I: Pumps 4 H^+ across membrane.
- Complex II: Receives $FADH_2$, bypasses I.
- Ubiquinone (Q): Shuttles electrons to III.
- Complex III: Pumps protons, passes electrons to cytochrome c.
- Complex IV: Reduces O_2 to water.

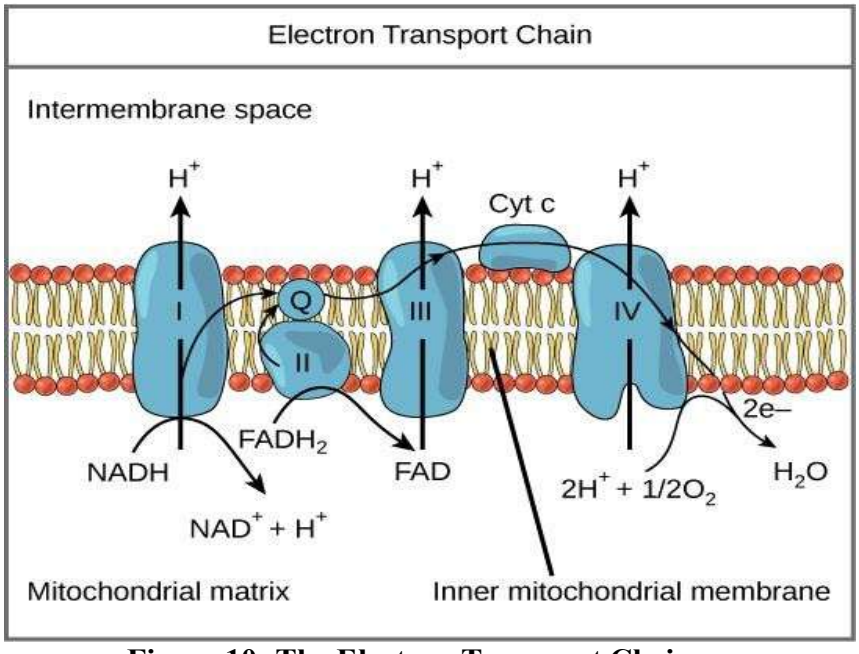

Figure 10: The Electron Transport Chain

4.10 FERMENTATION:

4.10.1 Lactic Acid Fermentation:

The fermentation method used by animals and certain bacteria (like those in yogurt) is called lactic acid fermentation. This type of fermentation is used routinely in mammalian red blood cells and in skeletal muscle that has an insufficient oxygen supply to allow aerobic respiration to continue (that is, in muscles used to the point of fatigue). The excess amount of lactate in those muscles is what causes the burning sensation in your legs while running. This pain is a signal to rest the overworked muscles so they can recover. In these muscles, lactic acid accumulation must be removed by the blood circulation and the lactate brought to the liver for further metabolism. The chemical reactions of lactic acid fermentation are.

Pyruvic acid + NADH ↔ lactic acid + NAD

Lactic acid fermentation is common in muscle cells that have run out of oxygen. The enzyme used in this reaction is lactate dehydrogenase (LDH). The reaction can proceed in either direction, but the reaction from left to right is inhibited by acidic conditions. Such lactic acid accumulation was once believed to cause muscle stiffness, fatigue, and soreness, although more recent research disputes this hypothesis. Once the lactic acid has been removed from the muscle and circulated to the liver, it can be reconverted into pyruvic acid and further catabolized for energy.

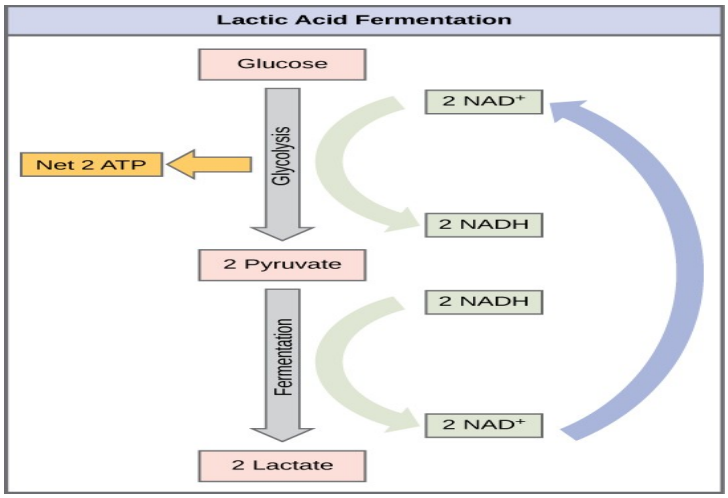

Figure11: Lactic Acid Fermentation

4.10.2 Alcohol Fermentation:

Another familiar fermentation process is alcohol fermentation, which produces ethanol, an alcohol. The use of alcohol fermentation can be traced back in history for thousands of years. The chemical reactions of alcoholic fermentation are the following (Note: CO does not participate in the second reaction):

Pyruvic acid → CO2 + acetaldehyde + NADH → ethanol + NAD+Produces ethanol + CO_2.

Steps:

1. Pyruvate → CO_2 + Acetaldehyde.
2. Acetaldehyde + NADH → Ethanol + NAD^+.

4.11 SUMMARY:

This lesson explains the process of metabolism, focusing on how organisms obtain and utilize energy. It covers the difference between anabolic and catabolic pathways, emphasizing carbohydrate metabolism as the primary source of energy. The central role of ATP as the energy currency of cells is highlighted, along with the sequential pathways of energy production: glycolysis, oxidation of pyruvate, citric acid cycle, and electron transport chain. The lesson also introduces fermentation as an alternative pathway when oxygen is limited. Together, these concepts provide a complete understanding of how living organisms manage and balance their energy needs.

4.12 TECHNICAL TERMS:

Metabolism, Anabolism, Catabolism, ATP (Adenosine Triphosphate), Glycolysis, Pyruvate, Acetyl CoA, Citric Acid Cycle (Krebs Cycle), Electron Transport Chain, Fermentation.

4.13 SELF-ASSESSMENT QUESTIONS:

1. Differentiate between anabolic and catabolic pathways with examples.
2. Why is ATP called the “energy currency” of the cell?
3. What are the end products of glycolysis?
4. Explain the role of acetyl CoA in the citric acid cycle.
5. How does fermentation differ from aerobic respiration?

4.14 SUGGESTED READINGS:

1. Eckert H. Animal Physiology: Mechanisms and Adaptation. W.H. Freeman & Company.
2. Floray E. An Introduction to General and Comparative Animal Physiology. W.B. Saunders Co., Philadelphia.
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- **Prof. M. JAGADISH NAIK**

LESSON- 5

NUTRITION & STRESS

OBJECTIVES:

- When we complete this unit, we will be able to define food, nutrition, and nutrients, and diet
- list the functions of food,
- understand the vital role of nutrition
- Identify the types of nutrients we get from food, and their functions.

STRUCTURE:

5.1 INTRODUCTION:

Every single cell in the human body is an outcome of the food we have ingested. Our mind and body are made of the food we eat and it continues until we live. The body parts, organs, muscles, blood and bones are all made from the food we have eaten. Food gives energy and stamina for work. It also gives emotional stability and security. Our appearance and feeling of good health and happiness also depends on the right kind of food and the quantity of food that we eat. Lot of research is being done and new findings published every day to increase our knowledge about food and nutrition, and to find ways to apply this knowledge in choosing the right foods to eat, so that our body is well nourished and healthy.

5.2 DEFINITION OF FOOD, NUTRITION, NUTRIENTS AND DIET:

Food is the very basis of our life. It contains different nutrients that are needed by the body for survival and sustenance. The food we eat is digested and converted into nutrients. These nutrients are absorbed and transported to different parts of the body, and utilized for the day-to-day functioning. At the end they are disposed of by further metabolism and transformed into the end products. We need to consume a variety of foods in order to remain healthy. Thus, we can define food as any solid or liquid which when eaten can supply any of the following:

- Material from which the body can produce movement, heat or other forms of energy
- Material for growth, repair and reproduction
- Substances necessary to regulate the production of energy or the processes of growth and repair.

The components of foods which have these functions are called nutrients. In other words, nutrients are vital in keeping a living thing alive and helping it to grow. The diet consists of those foods or mixtures of foods in the amounts which are eaten daily. A good diet provides adequate amounts of all the nutrients, without harmful excesses, from a wide range of foods.

Dietary habits are dependent on geographical and cultural traits and vary accordingly. Nutrition is the process by which living things receive the food necessary for them to be healthy. The science of nutrition is a study of all the processes of growth, maintenance and repair of the living body which depend upon the digestion and absorption of food and the study of that food. A nutritionist is an expert on the relationship between food and health and a dietitian is an expert on what one should eat under different conditions.

5.3 FUNCTIONS OF FOOD:

Let us now get acquainted with the major functions of food. As we know we eat when we are hungry, but food not only removes hunger, it also gives us a feeling of satisfaction and renewed strength. We also share food to express happiness, friendship, love and unity. Most families bond over mealtimes. Most meetings and important discussions in companies happen over lunches and dinners. Thus, food has many important functions in our lives. When we understand these functions, we appreciate how they affect our food intake and our physical and mental well-being.

- Physiological functions
- Socio-cultural Functions

Physiological functions the most important function of food is to build our body. We have seen that we probably weighed somewhere between 2.5 to 3.2 kg at birth and now weigh between 45 to 60 kg. This growth is the result of the food we ate from birth to adulthood. Now as an adult, our weight is constant which shows that we have achieved optimum growth. At this stage, the food we eat helps to maintain and renew worn out cells of our body and keep the body in good condition. Secondly, food provides the energy, our body needs for all its activities, voluntary and involuntary. We know that even when we sleep, many of our body's activities continue, like breathing, heartbeat, digestion, absorption of food, etc. These keep us alive without any effort on our part and are called involuntary activities. The work or activities we do when we are awake and our mind is in it like studying, walking, cooking or working at the desk, or playing a game of badminton, is called voluntary activity. The amount of energy we need for all these depends on the kind of activity and the time and energy we spend doing it. The third function of food is to regulate all the activities of the body and protect it from diseases and infections. Examples of some of the activities which regulate the body are given below:

- Beating of our heart for circulation of blood
- Maintenance of our body temperature
- Muscle contraction for voluntary and involuntary movements
- Removal of waste from the body in form of sweat, urine and feces, etc. Apart from these, vital nutrients in food also help to protect the body from various infections, diseases and from wearing out.

In addition, to meeting our physical needs, food also satisfies certain emotional needs. These include a feeling of security, love and attention. We feel secure when familiar foods are served which are also known as comfort foods. When a child comes home from school the mother knows that he/she is hungry and serves food. Thus, she expresses her love and attention. Many times, we miss our mother because of the attachment to her cooking.

When we share our lunch with a colleague, we express acceptance and friendship. If we are amongst friends, we try unfamiliar foods and enlarge our food experience. These are some of the positive aspects of food acceptance. There are occasions when we are hungry, but are unable to eat even a nutritious meal, because the foods are unfamiliar or we are unhappy or lonely. We need to understand these aspects about food acceptance, so that when we plan meals, we will not only think of nutrition, but also the persons for whom we plan.

Socio-cultural Functions We know how important food is in our social and cultural life. We serve refreshments at meetings and seminars or to visitors to create a relaxed atmosphere for an exchange of ideas. In most of our festivals and celebrations we have lunch and dinner with family and friends which binds us together. Food is also used as an expression of happiness.

For example, we distribute sweets when we pass examinations, buy a car or when there is a marriage feast. We also celebrate birthdays, anniversaries, promotions etc. by cutting cakes and enjoying food at parties. In religious functions like Easter, Ramzan, Rajo, Nuakhai, etc. we make special preparations to distribute food offerings. Thus, food helps to strengthen our social and cultural ties.

Food Habits Food intake is related to food habits, which is a powerful force in deciding what we eat. We tend to eat according to our set food habits and like all habits these may be good or bad. Formation of food habits have to be understood so that we can take appropriate measures towards building good food habits.

- Regional and Community Variations: Food habits are affected by food production and supply. For example, rice is the staple food in the East, West and South of India whereas wheat is popular in the North. This is because of agricultural practices of the region. Then the amounts and kinds of foods we eat depend on the money that one can spend. The geographic region, religion, community and family beliefs and practices that have developed over several generations, heavily influences a family's eating pattern. In spite of all these variations, all regional patterns can meet the nutritional needs of people if sufficient food from each group is included. A particular food chosen and the way it is prepared and seasoned is a matter of personal choice. Social customs like when and with whom and what to eat, will affect our exposure and hence our acceptance pattern.
- Personal Factors: The atmosphere in which we eat may modify our attitude towards food. A happy or an unhappy atmosphere affects our reactions to food and eating. Everyone has a personal response to the taste of foods. We may like pungent foods or acid foods while our friends may prefer mild or astringent foods. These basic influences affect our food habits, as we tend to adopt the food practices of our family. Our selection of foods should be based on the knowledge of food values. Convenience of food preparation also affects food habits of the present generation.
- Other Factors: Many other influences modify our food habits. When we move away from our region for education or work, we are exposed to new foods and our eating pattern is modified.

Food misinformation We spend a considerable part of our time and income to select and purchase foods. Besides food habits, our choices are also affected by prevalent misconceptions, we have about foods and food products. We are exposed to a variety of views about the foods and their nutritional contribution–through conversation, and through newspapers, magazines and books. People see and hear advertisements about foods and drinks. So their ideas about food are indirectly modified by what they hear and see. The internet, television and other multimedia sources also influence food habits to a great extent. Water: Some people think drinking water can help them lose weight but water cannot wash away the fat from the cells, or those extra calories we get from overeating. If we drink water instead of the calorie-rich soft drinks, we might cut down on our calorie intake. When a person or a child suffers from diarrhea and vomiting, some people restrict their water intake with the hope of stopping it, which is very wrong. We know it is very important to feed clean, boiled, cooled water, with added sugar, salt and lemon, to a person who has suffered loss of body fluids due to diarrhea or vomiting. Water intake in such a condition is crucial to prevent dehydration especially in children.

Cereal and cereal products: There is a wrong notion that starchy foods, such as rice and bread, are high in calories. Many dieters frequently reduce or cut out cereals from their diet. We need to remind them that basic cereals and plain breads (like chapatti) are not very high in calories. The calories come from the foods they add such as ghee, butter, cheese, jam, sugar, oil, chutney, etc. It is the extra calorie foods that they should omit, not the breads and cereals. Another misconception is that weight reduction is possible if we eat bread or chapati instead of rice.

Actually, both rice and wheat contain about the same number of calories. It is the total calorie intake that needs to be reduced not calories from a particular food. We have noticed that many people cut the sugar from tea or coffee to reduce their calorie intake but do not skip the biscuits, cake, or other snacks that are served with the tea or coffee. The teaspoon of sugar they skip is only 20calories, and the snacks they eat may add 50- 100 ‘calories. So they end up having 2 to 5 times the calories than sugar. Fats and oils: All vegetable oils (except coconut and olive oil) contain a high amount of PUFA (poly unsaturated fatty acids). It is important to understand that vegetable oils do not contain any cholesterol. To say that a particular brand of vegetable oil contains no cholesterol is intended to misguide us to think that other brands of vegetable oils contain cholesterol. When these are taken with fast food and junk food they create health hazards in the long run. Instead, if we make traditional cool drinks at home and other snacks and food items it will cost a fraction of the price, we pay for commercial weaning foods and children’s snacks and drinks. Another advantage would be that children would develop healthy food habits in this way, which will help them through the lifetime.

5.4 NUTRIENTS:

Understanding nutritional needs and translating it into practical diets requires a sound knowledge of nutrition. For that first, we need to review the nutritional components of the foods that we eat. The following paragraph will focus on this aspect.

5.4.1 Classification of nutrients:

All foods are classified into three broad categories: -

- Energy yielding foods (Carbohydrates and Fats)
- Body building foods (proteins)
- Protective and regulatory foods (vitamins, minerals and other foods)

5.4.2 Types of nutrients

The foods that we consume are composed of varying quantities of the following nutritionally important components: Carbohydrates Proteins Lipids Water Minerals Vitamins Fibre Phytochemicals and anti-oxidants Detoxifying agents If these nutritional components are consumed daily in the amounts and proportion required, then the chances are that we will maintain a good health.

5.4.3 Functions of nutrients

We use foods such as wheat, rice, dal, vegetables, fruits, milk, eggs, fish, meat, sugar, oils, on a daily basis in our diet. These foods are made up of the nutrients mentioned earlier. Let us get to know the functions of these nutrients in our body. Carbohydrates: We get about 70-80 per cent of our energy from carbohydrates. The energy content of foods is expressed in calories.

One gram of carbohydrate provides four calories. If we take these in excess of our body's need, the unused part is stored as glycogen in the muscle or converted to fat and stored for later use. The main carbohydrates in our diet are starches found in cereals, dals and tubers, sugar from sugarcane and fruits. Cereals and dals also provide a large part of the proteins, some minerals and vitamins. Proteins: As we know, proteins are present in all living tissues-both plant and animal. Next to water, protein is the most abundant component of our body. About one-sixth our body weight is protein. The main function of protein is building of new tissues, and maintenance and repair of those already built. Further, a number of regulatory and protective substances (enzymes, antibodies, hormones) in the body are made from proteins. We get about 8 to 15 per cent of our total energy from proteins. Energy supply is thus a secondary function of our dietary proteins. Each gram of protein gives four calories of energy to our body. Protein is present in vegetables and animal sources. Among the former, pulses, nuts and soybeans are good sources of protein. Among the latter, milk, fish, egg, meat, chicken and liver are rich sources. Paneer and khoa (condensed milk) are also good sources of protein. Fats: In India, about 10 to 30 per cent of the energy needs are met from oils and fats. One gram of oil or fat gives nine calories of energy to the body. Please remember that the fats and oils are concentrated sources of energy. We need fats as a medium for the absorption of fat- soluble vitamins. We need essential fatty acids, which vegetable oils provide. The oil we use in seasoning, the ghee or butter used as a spread, and the fat in eggs and meat, are the major sources of fats in our 5 diet. The oilseeds and nuts we use in food preparations also contribute some fat. Remember if we take more energy than our body needs, in any form, be it fats, carbohydrates 'or proteins, it is stored in our body as fat. Minerals: Our bones and teeth need minerals for their formation and maintenance. Iron is needed for formation of the red pigment in the blood. Minerals have an important role in the regulation of a number of body processes, e.g. muscle contraction, nerve stimulation, respiration, etc. we get the minerals that we need from a variety of foods. For example, we get calcium from milk and leafy vegetables, iron from leafy vegetables, dals and eggs, sodium from salt and other foods. Vitamins: We need different vitamins, for example, vitamin A, B-complex, C, D, etc. Our body needs these to grow and develop, to help our eyes, nerves and skin to remain healthy, and to protect us from infections.

We need very small amounts of these vitamins. We get these from a variety of foods like leafy vegetables, carrots, amla, guava, mosambi, oranges, pulses, whole grain cereals and eggs. Water: Our body contains approximately 60 to 65 per cent water. Hence, it is an essential part of our body structure. We know, water is a universal solvent. It carries food into the body, helps in the digestion and absorption of food and ensures elimination of waste from the body. Water helps to regulate body temperature. It acts as a lubricant in the mobile parts of our body, such as joints and prevents friction. We need about 5 to 6 glasses of water each day. We get it from the water we drink and beverages such as tea, coffee, etc.

5.5 DIGESTION OF FOOD:

Carbohydrates, fats and proteins that are in the food are much too large to be of use to the body. Before the body can use them, it has to break them down into units, which are small enough to be absorbed into the bloodstream. One of the commonest forms of carbohydrate in food is starch. Starch is made up of many small units called glucose. All carbohydrates must be broken down into small soluble units for utilization by the body. Digestion of carbohydrate starts when you begin chewing food. When we chew some bread or rice, we find it tastes sweeter as we keep chewing. This is because saliva contains a starch-splitting enzyme called ptyalin mixes with the starch in the food. In the stomach, little break down of carbohydrate occurs. In the intestines, however, the carbohydrate is completely hydrolyzed to glucose. Hydrolysis means break down of a large substance into smaller ones with the addition of water. Just like starch in the rice must be hydrolyzed, so also the protein in the pulses. Protein digestion begins in the stomach due to the action of the enzyme, pepsin. However, pepsin by itself does not complete the digestion. In the small intestine, protein digestion is completed by other enzymes (proteases) which are secreted by the pancreas. Fat digestion occurs mainly in the small intestine. Bile from the gall bladder breaks the fat into small particles. Thus, the enzymes have more access to the fat to hydrolyze them. Fats are also hydrolyzed during digestion. Just like carbohydrate and protein, digestion of fat takes place gradually, in stages. Digestion of any one carbohydrate, protein or fat is like starting with a large chain of beads and splitting this into pieces by removing one bead at a time. Not everything we eat needs to be digested, e.g., vitamins and minerals or simple sugars like glucose. However, many times, the minerals and vitamins are bound to the fat, protein or carbohydrate in your food. Thus, for these vitamins and minerals to be available for absorption, first they need to be separated from the complexes in which these are found in the food. Fat-soluble vitamins need to be dissolved in fat in order to be absorbed. Before absorption, the other nutrients are dissolved in water. Therefore, water is essential for absorption of most nutrients. Enzymes speed up hydrolysis just like a mixer hastens the grinding of food into smaller units. For hydrolysis to occur, water is necessary.

Any food that has not been digested and absorbed from the small intestine goes into the large intestine. Fibre is one substance in our diet that remains undigested. Fibre is present in fruits, vegetables, in the outer skin of cereal grains and pulses. Fibre has the property of absorbing water and swelling. It is not digested; it only increases the bulk (amount) of undigested material.

In addition, it soaks up water like a sponge. As a result, if we eat sufficient fiber every day, we can ensure that the muscles of our intestines are stimulated and defecation occurs regularly and constipation can be prevented. In the large intestine, no new enzymes are produced and therefore no further digestion takes place. The main task of the large intestine is to remove excess water from the undigested material. Remember that most of the break down stages in digestion involve hydrolysis and therefore need water. A lot of water passes into the large intestine. After this water is removed. The undigested material is removed from the body or excreted through the anus. Absorption of nutrients: Absorption is the process by which the products of digestion pass out of the digestive tract through the cells in the intestinal wall, into the bloodstream. Most nutrients are absorbed directly into our blood, which is then distributed to different parts of the body. Some nutrients are absorbed in the stomach, for example vitamin B 12. Minerals like calcium and iron are absorbed in the first portion of the small intestine. Most end products of digestion of carbohydrates, fats and proteins are absorbed in the small intestine.

5.6 ASSIMILATION OF FOOD IN THE BODY:

Assimilation of food in the body We learnt about all the nutrients that the body needs. What we eat becomes flesh and blood. The process by which the nature of food is changed in the body is called digestion. The digestive tract is nature's (or our body's) ingenious way of extracting nutrients from the food we eat. The digestive tract is selective. It breaks down the materials that are nutritious for the body into particles that we can assimilate. Those particles that are left undigested pass out from the other end of the digestive tract. Our body needs energy to perform its various activities, protein to replace the cells it has lost and to grow, various vitamins and minerals for bone and blood formation and for the body to work efficiently and smoothly. The digestive system: When the body requires food, it signals us to eat. This signal is hunger. The moment we eat something the digestive journey starts. As we swallow, the food slides across the gullet or esophagus passing over the entrance to our lungs. Whenever we swallow, the body automatically closes off all air passages, so that we do not choke. When we chew food, we break it up (grind it) into small particles with our teeth. From the mouth, the food goes into the stomach, where it stays for a few hours. The stomach breaks the food into still smaller particles, mixing it at the same time with acid and the enzyme pepsin, which chemically alters the protein in the food. From the stomach, the food enters the intestines.

We have a small intestine and a large intestine. The small intestine is actually not small at all. It is actually a 20 feet coiled tube within the abdomen. From here, food enters the large intestine. In the colon (latter portion of the large intestine), the body withdraws water, leaving behind semi-solid waste. The waste is held back here because of the strong muscles (called sphincter muscles) of the rectum. It would be inconvenient and embarrassing if one had to excrete continuously. When the body chooses to defecate, this muscle is relaxed and the waste material is voided. The path that food follows in the body is like this:

- Mouth (epiglottis)
- Gullet (esophagus)
- Stomach
- Small intestine
- Large intestine
- Rectum (anus).

For food to pass smoothly through the system, it must be mixed with water. If we drink too little water, the food becomes compact and moves very slowly.

For digestive enzymes to work on food, it should be finely divided and suspended in water so that every particle is accessible to the enzymes. Once digestion is complete and all the essential nutrients are absorbed, the residue which remains is excreted. It would be wasteful and inconvenient to excrete large amounts of water with these 6 residues. So, your body withdraws some water, leaving a semi-solid mass just smooth enough and easy to pass. This shows that food is altered physically in the digestive tract.

The enzymes of the digestive tract break down or digest carbohydrates, protein and fat into smaller units. This is chemical alteration of food. The body needs many nutrients, which it absorbs from food through the digestive tract. The digestive tract digests only carbohydrates, fats and proteins although the food we eat contains many other substances like vitamins, minerals, preservatives, colors, etc. Certain glands in the body contribute the digestive juices or enzymes. These are the salivary glands, gastric glands, the liver and the pancreas. The enzymes from these glands break down proteins, fats and carbohydrates.

Physical Alteration of Food

We chew or masticate food, and grind it. Chewing food helps to Increase the surface area of food, Break the cell walls of cells, releasing the nutrients. In the mouth, the tongue helps to mix the food with saliva. In the stomach, food is mixed and altered further by contraction and relaxation of stomach muscles (known as peristalsis). Fat, we know, does not mix with water but contraction and relaxation of the stomach muscles break up the fat into small particles and disperses it in the watery medium or liquid in the stomach. This dispersion of fat in the liquid is called emulsification

5.6.1 Nutritional status

Nutritional status is the level of nourishment of our body. Each of us would like to have a good nutritional status. So far, we have learnt that nutrition affects our body size, our brain development, our performance, our capacity to work and life span. Nutritional status shows the kind of nourishment our body gets from the foods we eat. If foods provide for our body's needs optimally, we look and feel healthy and enjoy a good nutritional status. Indicators of good nutritional status are:

1. **Measurement of body size:** We have learnt that body weight and height indicate our body size. In addition, the head, chest and mid arm circumferences give an idea of growth and development, especially of small children (0-5 years). Therefore, we record these body measurements at various stages of development to study the growth pattern and check the nutritional status.
2. **Weight:** Weight is a commonly used indicator of body size, as it reflects the level of food intake. The weight of children is recorded regularly to check if there is a normal gain. If we are a nutrition professional or a dietitian, then we also 'have the responsibility of planning diets for others both for health and in diseases and in addition, we will be counseling a large number of people on appropriate diets.

5.7 IMPAIREMENTS & STRESS:

Stress is a common problem in most societies. There are three main types of stress that may occur in our everyday lives: acute (a brief event such as a heated argument or getting stuck in a traffic jam), acute episodic (frequent acute events such as work deadlines), and chronic stress (persistent events like unemployment from a job loss, physical or mental abuse, substance abuse, or family conflict). Many of us may experience a combination of these three types. Our bodies react to all types of stress via the same mechanism, which occurs regardless if the stress arises from a real or perceived event. Both acute and chronic stressors cause the "fight-or-flight" response. Hormones are released that instigate several actions within seconds: pumping blood and oxygen quickly to our cells, quickening the heart rate, and increasing mental alertness. In prehistoric times, this rapid response was needed to quickly escape a dangerous situation or fight off a predator. However, all types of stress can trigger this response, as described in more detail below: A very small region at the base of the brain, called the hypothalamus, sets off the reaction and communicates with the body through the autonomic nervous system (ANS). This system regulates involuntary responses like blood pressure, heart rate, breathing, and digestion. The ANS signals nerves and the hormone corticotropin to alert the adrenal glands, located on the top of each kidney, to release a hormone called adrenaline into the blood. Adrenaline (also known as epinephrine) quickens the heart rate and increases blood pressure so more blood circulates to the muscles and heart to support a boost of energy. More oxygen in the blood is available to the heart, lungs, and brain to accommodate faster breathing and heightened alertness. Even one's vision and hearing may become sharpened.

If stress continues, the adrenal glands release another hormone called cortisol, which stimulates the release of glucose into the blood and increases the brain's use of glucose for energy. It also turns off certain systems in the body to allow the body to focus on the stress response. These systems include digestion, reproduction, and growth. These hormones do not return to normal levels until the stress passes. If the stress does not pass, the nervous system continues to trigger physical reactions that can eventually lead to inflammation and damage to cells. With acute stress, the event is brief and hormone levels will gradually return to normal. Acute episodic and chronic stress repeatedly trigger the fight-or-flight response causing a persistent elevation of hormones, leading to a risk of health problems: Nutrition Impact or recommended Diets or imbalance

- Digestive issues (heartburn, flatulence, diarrhea, constipation)
- Weight gain
- Elevated blood pressure
- Chest pain, heart disease
- Immune system problems
- Skin conditions
- Muscular pain (headaches, back pain, neck pain)
- Sleep disruption, insomnia
- Infertility
- Anxiety, depression

How Chronic Stress Affects Eating Patterns

Chronic stress can affect the body's use of calories and nutrients in various ways. It raises the body's metabolic needs and increases the use and excretion of many nutrients. If one does not eat a nutritious diet, a deficiency may occur.

Stress also creates a chain reaction of behaviors that can negatively affect eating habits, leading to other health problems down the road. Stress places a greater demand on the body for oxygen, energy, and nutrients. Yet people who experience chronic stress may crave comforting foods such as highly processed snacks or sweets, which are high in fat and calories but low in nutrients. People feeling stress may lack the time or motivation to prepare nutritious, balanced meals, or may skip or forget to eat meals. Stress can disrupt sleep by causing lighter sleep or more frequent awakenings, which leads to fatigue during the day.

In order to cope with daytime fatigue, people may use stimulants to increase energy such as with caffeine or high-calorie snack foods. The reverse may also be true that poor-quality sleep is itself a stressor. Studies have found that sleep restriction causes a significant increase in cortisol levels During acute stress, the hormone adrenaline suppresses the appetite. But with chronic stress, elevated levels of cortisol may cause cravings, particularly for foods high in sugar, fat, and calories, which may then lead to weight gain. Cortisol favors the accumulation of fat in the belly area, also called central adiposity, which is associated with insulin resistance and an increased risk of type 2 diabetes, cardiovascular disease, and certain breast cancers. It also lowers levels of the hormone leptin (that promotes satiety) while increasing the hormone ghrelin (that increases appetite).

Tips to Help Control Stress

Healthy diet. A balanced diet can support a healthy immune system and the repair of damaged cells. It provides the extra energy needed to cope with stressful events. Early research suggests that certain foods like polyunsaturated fats including omega-3 fats and vegetables may help to regulate cortisol levels. If you frequently rely on fast food because you are tired or too busy to prepare meals at home, consider meal planning, a practice that can help save time in the long run, ensure more balanced healthful meals, and prevent weight gain. Mindful eating. When we "stress-eat," we eat quickly without noticing what or how much we're eating, which can lead to weight gain. Mindful eating practices counteract stress by encouraging deep breaths, making thoughtful food choices, focusing attention on the meal, and chewing food slowly and thoroughly. This increases enjoyment of the meal and improves digestion. Mindful eating can also help us realize when we are eating not because of physiological hunger but because of psychological turbulence, which may lead us to eat more as a coping mechanism. Regular exercise. Physical activity will help to lower blood pressure and stress hormone levels. Aerobic exercise like walking and dancing increases breathing and heart rate so that more oxygen reaches cells throughout the body. This reduces tension in muscles, including the heart.

Meditation or deep breathing techniques. Fast, shallow breathing and erratic thoughts occur in response to stress. Therefore, take slow deep breaths to reduce muscular tension, lower the heart rate, and calm the mind. Whenever you feel stressed, breathe slowly, focusing on each in- and out-breath. Through this simple act, your parasympathetic nervous system kicks in and can help you calm down. If you'd like some guidance, try this short mindful breathing exercise.

Additionally, certain exercises like yoga and tai chi emphasize deep breathing and a focused mind. Research has also found that meditation training may lengthen or prevent the shortening of protein structures called telomeres. Telomeres generally shrink in length with age and in those experiencing chronic stress; this can lead to the death of cells and inflammation, which is associated with an increased risk of age-related dementia and cardiovascular disease.

Meditation practice has been associated in some studies with greater telomere activity and length in response to a reduction in anxiety, chronic stress, and cortisol levels. Mental health counseling or other social support. Feeling alone can add to stress. It can help to talk through feelings and concerns with a trusted individual. Often, just realizing that you are not alone and that your feelings are not unusual can help lower stress.

Practicing work-life balance. Use vacation and personal time, or just set aside an hour a day. A periodic escape from the pressures of work can do wonders to reduce stress, increase productivity, and decrease the risk of physical and mental illnesses that are associated with workplace burnout. Schedule fun activities or hobbies at least once a week. Gardening, reading, enjoying music, getting a massage, hiking in nature, and cooking a favorite recipe are examples of welcome stress relievers. Good sleep hygiene. Stress can cause a heightened sense of alertness, which delays the onset of sleep as well as cause interrupted sleep throughout the night. This can prevent one from entering the deeper sleep stages in which the body repairs and grows tissue and supports a healthy immune system. The REM (rapid eye movement) sleep stage in particular helps with mood regulation and memory. Aim for 7-9 hours of sleep a night by slowing down about 30 minutes before bedtime. Controlling stress through the other tips listed above can also improve sleep quality.

5.8 SUMMARY:

Words Meanings Absorption Calorie Diet Digestion Essential fatty acids Glycogen Health Nutrient Regulation of body temperature Respiration Solvent Vitamin The uptake of the products of digestion through the cell membrane of the digestive tract into the blood and lymph circulation. One calorie is the amount of heat required to raise the temperature of 1litre of water through 1°c. The unit used in Nutrition & Food Composition tables is kilocalories (kcal). It is 1000 times the unit of calorie used in Physics. (Noun)-All the foods eaten and drinks taken (Verb)-to eat only prescribed foods.

5.9 TECHINICAL TERMS:

Nutrients, Digestion, Assimilation, Metabolism, Enzymes, Amino acids, Emulsification, Nutritional status, Antioxidants, Homeostasis.

5.10 SELF-ASSESSMENT QUESTIONS:

1. What is the difference between food, nutrition, nutrients, and diet?
2. Explain the physiological and socio-cultural functions of food.
3. Classify nutrients and describe their functions.
4. Describe the process of digestion and assimilation of food in the human body.
5. How does stress affect nutrition and health?

5.11 SUGGESTED READINGS:

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LESSON- 6
DIGESTION

OBJECTIVES:

- Upon completion of this unit, you should be able to- State the pattern of digestion and absorption in different types of animals.
- List the digestive enzymes, their source and function
- Describe the mechanism of digestion, absorption and utilization of various food Components.

STRUCTURE:

6.1 INTRODUCTION:

All living organisms need organic raw materials to build up most of their own body molecules, and they require energy to operate the metabolic reactions that sustain life. The materials which provide the two primary requirements of life, namely, organic raw materials and energy, are called nutrients. A substance which taken to supply the necessary nutrients to the body is termed food, or diet. All organisms obtain their nutrients as food from their surrounding or habitat. The mode of obtaining of nutrients by the organisms is termed as nutrition. Nutrition is the sum of all those activities which are concerned with ingestion, digestion, absorption of digested food into blood, lymph, cytoplasm, oxidation of simple food to produce energy for growth, repair, synthesis of biomolecules and egestion. Depending on the quantity and functions nutrients are classified as macronutrients and micronutrients. Macronutrients e.g. carbohydrates, proteins and lipids are taken in large amount and required to produce energy and for growth and repair. Micronutrients e.g. vitamins and minerals do not provide energy and are required in very small amount but they are essential regulatory components of food, their deficiency can cause specific disease. The water we take in, plays an important role in metabolic processes and also prevents dehydration of the body. Biomacromolecules in food cannot be utilized by our body in their original form. They have to be broken down and converted into simple substances in the digestive system. This process of conversion of complex food substances to simple absorbable forms is called digestion and is carried out by our digestive system by mechanical and biochemical methods.

6.2 PATTERNS OF DIGESTION AND ABSORPTION IN ANIMALS:

Animals obtain their nutrients through a broad variety of feeding patterns. Sponges, for example, feed on small particles of food that enter their pores. Other aquatic organisms, such as sea cucumbers, wave their tentacles about and trap food on their sticky surfaces. Mollusks, such as clams and oysters, feed by filtering materials through a layer of mucus in their gills. Certain arthropods feed exclusively on fluids. Some animals feed on food masses, and they usually have organs for seizing, chewing, and consuming food. Herbivores are animals that eat only plants, while carnivores are animals that eat only other animals. Omnivores, which consume both plants and animals, are typified by humans.

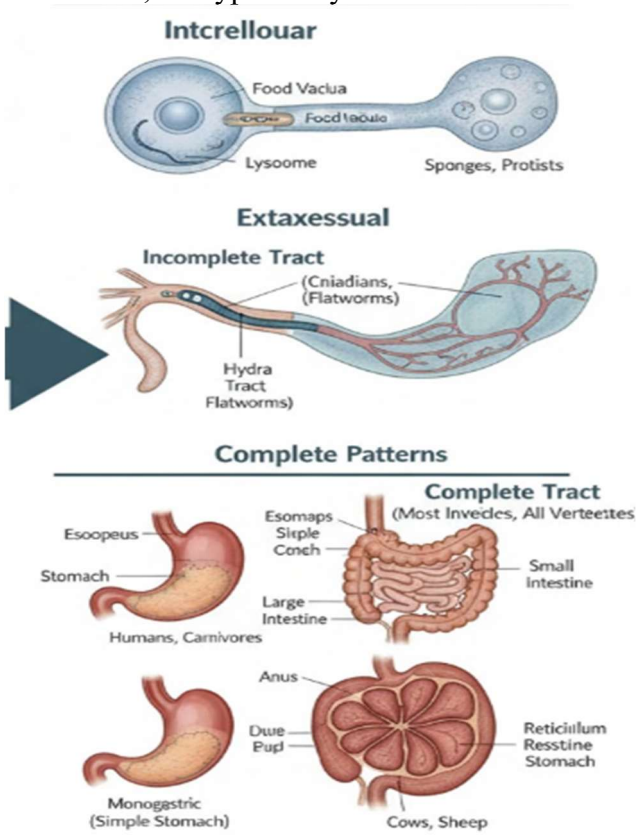

Figure 1: Digestion Patterns

6.2.1 Digestion in Herbivores:

Herbivores eat plant material. While no animal produces the digestive enzymes to break down the large cellulose molecules in the plant cell walls, micro-organisms like bacteria, on the other hand, can break them down. Therefore, herbivores employ micro-organisms to do the job for them. There are two types of herbivores: ruminants and non-ruminants. The **ruminants** like cattle, sheep and goats, house these bacteria in a special compartment in the enlarged stomach called the rumen. The second group has an enlarged large intestine and caecum, called a functional caecum, occupied by cellulose digesting micro-organisms. These **non-ruminant** herbivores include the horse, rabbit and rat. Plants are a primary pure and good source of nutrients; however, they aren't digested very easily and therefore herbivores have to eat large quantities of food to obtain all they require. Herbivores like cows, horses and rabbits typically spend much of their day feeding. To give the microorganisms access to the cellulose molecules, the plant cell walls need to be broken down. This is why herbivores have teeth that are adapted to crush and grind. Their guts also tend to be lengthy and the food takes a long time to pass

through it. Eating plants have other advantages. Plants are immobile so herbivores normally have to spend little energy collecting them. This contrasts with another main group of animals – the carnivores that often have to chase their prey.

6.2.2 Digestion in Carnivores:

Carnivorous animals like those in the cat and dog families, polar bears, seals, crocodiles and birds of prey catch and eat other animals. They often have to use large amounts of energy finding, stalking, catching and killing their prey. However, they are rewarded by the fact that meat provides a very concentrated source of nutrients. Carnivores in the wild therefore tend to eat distinct meals often with long and irregular intervals between them. Time after feeding is spent digesting and absorbing the food.

6.2.3 Digestion in Omnivores:

Many animals feed on both animal and vegetable material-they are **omnivorous**. There are currently two similar definitions of omnivores:

1. Having the ability to derive energy from plant and animal material.
2. Having characteristics which are optimized for acquiring and eating both plants and animals. Some animals fit both definitions of omnivores, including bears, raccoons, dogs, and hedgehogs. Their food is diverse, ranging from plant material to animals they have either killed themselves or scavenged from other carnivores. They are well equipped to hunt and tear flesh (claws, sharp teeth, and a strong, non-rotational jaw hinge), but they also have slightly longer intestines than carnivores, which has been found to facilitate plant digestion. The examples also retain an ability to taste amino acids, making unseasoned flesh palatable to most members of the species.

Classically, humans and chimpanzees are classified as omnivores. However, further research has shown chimpanzees typically consume 95% plant matter (the remaining mass is largely termites), and their teeth, jaw hinge, stomach Ph, and intestinal length closely matches herbivores, which many suggest classified them as herbivores. Humans, conversely, have chosen to eat meat for much of the archaeological record, although their teeth, jaw hinge, and stomach Ph, and intestinal lengths also closely match other herbivores. Per the classical definition, omnivores lack the specialized teeth and guts of carnivores and herbivores but are often highly intelligent and adaptable reflecting their varied diet.

6.3 ROLE OF DIGESTIVE ENZYMES:

Chemical processes are achieved by the different digestive enzymes.

6.3.1 Salivary Amylases and Lysozymes

These enzymes are produced by the salivary glands in oral cavity. The main function of these enzymes is to split the carbohydrates by the hydrolytic actions. The function of these enzymes is as follows:

Salivary amylases-30% of starch molecules are converted into maltose by salivary amylases at the Ph of 6.8.

Lysozymes-It acts against the bacterial infections. They are also called antibacterial agents.

6.3.2 Gastric Gland Enzymes:

Gastric glands are present in the mucosa of the stomach. The following types of cells are present in the gastric gland.

Mucous neck cells-These cells secrete mucus, which is used to protect the mucosal epithelium from concentrated HCl. This concentrated HCl is secreted by the oxyntic cells. Peptic or chief cells-These cells secrete the proenzyme pepsinogen. It is in an inactive form. This inactive proenzyme pepsinogen is activated by the chyme and converted into the active form of the enzyme which is called pepsin. Pepsin is used to convert the proteins into proteoses and peptones.

Parietal or oxyntic cells-These cells secrete concentrated HCl and intrinsic factor. Intrinsic factor plays an important role in the absorption of the vitamin B12. HCL is used to activate the proenzyme pepsinogen.

Finally, the following enzymes are secreted by gastric glands:
Pepsin-It is used to denature the proteins into peptones and proteases.
Rennin-It is a type of proteolytic enzyme which is present in the infant's gastric juice. **Gastric Lipase**-Small amount of lipase enzyme is secreted by the gastric gland. It is used to convert the di and monoglycerides into fatty acids and glycerol.

Enzyme Category	Enzyme Name	Source	Substrate	Product
Salivary Enzymes	Lingual lipase	Lingual glands	Triglycerides	Free fatty acids, and mono- and diglycerides
Salivary Enzymes	Salivary amylase	Salivary glands	Polysaccharides	Disaccharides and trisaccharides
Gastric enzymes	Gastric lipase	Chief cells	Triglycerides	Fatty acids and monoacylglycerides
Gastric enzymes	Pepsin*	Chief cells	Proteins	Peptides
Brush border enzymes	α-Dextrinase	Small intestine	α-Dextrins	Glucose
Brush border enzymes	Enteropeptidase	Small intestine	Trypsinogen	Trypsin
Brush border enzymes	Lactase	Small intestine	Lactose	Glucose and galactose
Brush border enzymes	Maltase	Small intestine	Maltose	Glucose
Brush border enzymes	Nucleosidases and phosphatases	Small intestine	Nucleotides	Phosphates, nitrogenous bases, and pentoses
Brush border enzymes	Peptidases	Small intestine	Aminopeptidase: amino acids at the amino end of peptides Dipeptidase: dipeptides	Aminopeptidase: amino acids and peptides Dipeptidase: amino acids
Brush border enzymes	Sucrase	Small intestine	Sucrose	Glucose and fructose
Pancreatic enzymes	Carboxy-peptidase*	Pancreatic acinar cells	Amino acids at the carboxyl end of peptides	Amino acids and peptides
Pancreatic enzymes	Chymotrypsin*	Pancreatic acinar cells	Proteins	Peptides
Pancreatic enzymes	Elastase*	Pancreatic acinar cells	Proteins	Peptides
Pancreatic enzymes	Nucleases	Pancreatic acinar cells	Ribonuclease: ribonucleic acids Deoxyribonuclease: deoxyribonucleic acids	Nucleotides
Pancreatic enzymes	Pancreatic amylase	Pancreatic acinar cells	Polysaccharides (starches)	α-Dextrins, disaccharides (maltose), trisaccharides (maltotriose)
Pancreatic enzymes	Pancreatic lipase	Pancreatic acinar cells	Triglycerides that have been emulsified by bile salts	Fatty acids and monoacylglycerides
Pancreatic enzymes	Trypsin*	Pancreatic acinar cells	Proteins	Peptides

Table 1: Small Intestine Enzymes

6.3.3 Small Intestine Enzymes:

In the small intestine, three major digestive juices are secreted. These are as follows: Bile juice, Pancreatic juice and Intestinal juice

Bile juice

Bile juice is secreted by the liver. It is a yellowish color fluid. The main function of bile juice is to digest the lipid molecules and to activate the lipase enzymes. Bile juice consists of the

following components- bile pigments (Bilirubin and biliverdin), Bile salts (Sodium carbonates, bicarbonates, sodium glycolate and taurocholate), Cholesterol, Phospholipids but no digestive enzymes. Bile juice helps to break down the lipid molecules into di and monoglycerides by the lipase enzymes.

Pancreatic juice enzymes

The pancreatic juices are secreted by the pancreas. Pancreatic juice consists of the following inactive enzymes. These enzymes are activated by the intestinal mucosal secretions.

- **Trypsinogen-**An inactive form of trypsinogen is converted into an active form, trypsin by enter peptidase (one of the intestinal mucosa secretions). Trypsin is used to convert the protein molecules into dipeptides.
- **Chymotrypsinogen** -An inactive form of chymotrypsinogen is converted into an active form, chymotrypsin. Proteins are denatured into dipeptides by chymotrypsin.
- **Procarboxypeptidase**-An Inactive form of procarboxypeptidase is converted into an active form, carboxypeptidase. Carboxypeptidase is also used to denature the protein.
- **Amylases**-Amylases are used to denature the polysaccharides into the disaccharides.
- **Lipases**-Lipases are used to convert the fats into diglycerides and monoglycerides.
- **Nucleases-**Nucleases are used to convert the nucleic acids into nucleotides and nucleosides.

Intestinal juice enzymes

The Intestinal mucous epithelium of the small intestine consists of brush border cells and goblet cells. The secretions of brush border cells and goblet cells from the intestinal juice in the small intestine. Intestinal juice consists of the following enzymes:

- **Disaccharidases-**It is also called maltase. Maltase's are used to convert the maltose into glucose.
- **Dipeptidases-**These enzymes are used to convert the dipeptides into simple amino acids.
- **Lipases-**Lipases are used to convert the diglycerides and monoglycerides into fatty acids and glycerol's.
- **Nucleosidases-**These enzymes are used to convert the nucleotides into nucleosides, sugars, and bases.
- **Lactases-**Lactases are used to convert the lactose into simple glucose.

6.4 DIGESTION, ABSORPTION AND ASSIMILATION OF VARIOUS FOOD STUFFS:

6.4.1 Mechanical Digestion of food:

This process comprises mastication, swallowing and churning of food in stomach.

Mastication

It is the chewing of food by various types of teeth. The teeth are admirably designed for chewing. The anterior teeth (incisors) provide a strong cutting action, and the posterior teeth (molars) provide a grinding action. Chewing aids the digestion of food for further action of digestive enzymes acts only on the surfaces of food particles; therefore, the rate of digestion is dependent on the total surface area exposed to the digestive secretions.

Swallowing (deglutition)

Swallowing is a complicated mechanism, principally because the pharynx serves respiration and swallowing both. Tongue helps in mixing of saliva with the food. Saliva moistens and lubricates the food, which changes into semisolid form called bolus. The bolus is then

swallowed through esophagus to the stomach. Peristalsis movement of alimentary canal also helps in swallowing.

Churning in stomach

The wall of stomach undergoes periodic movement as well as contraction producing churning movement called peristalsis, which results in breakdown of complex food into simpler form. The bolus after mixing with gastric juice, turn into fine soluble form known as chyme.

6.4.2 Chemical Digestion of Food:

It involves the breaking of covalent chemical bonds in organic molecules by
Digestive enzymes. Carbohydrates are broken down into monosaccharides, proteins are broken down into amino acids, and fats are broken down into fatty acids and glycerol.

Digestion of Carbohydrates

Ingested carbohydrates consist primarily of polysaccharides, such as starches (rice, bread), disaccharides, such as sucrose (table sugar) and lactose (milk sugar); and monosaccharides, such as glucose and fructose (found in many fruits). During the process of digestion, polysaccharides are broken down into smaller chains and finally into disaccharides and monosaccharides. Disaccharides are broken down into monosaccharides.

a) digestion of carbohydrates in mouth- Carbohydrate digestion begins in the oral cavity with the partial digestion of starches by salivary amylase. About 30 percent of starch is hydrolyzed here by this enzyme amylase (optimum Ph 6.8) into a disaccharide-maltose. Lysozyme present in saliva acts as an anti-bacterial agent that prevents infections.

b) digestion of carbohydrates in stomach and intestine- A minor amount of digestion occurs in the stomach through the action of gastric amylase and gelatinase. Carbohydrate digestion is continued in the intestine by pancreatic amylase. A series of disaccharidases enzymes that are released by intestinal epithelium digest disaccharides into monosaccharides.

Digestion of Proteins

Proteins are taken into the body from a number of dietary sources. Pepsin secreted by the stomach catalyzes the cleavage of covalent bonds in proteins to produce smaller polypeptide chains.

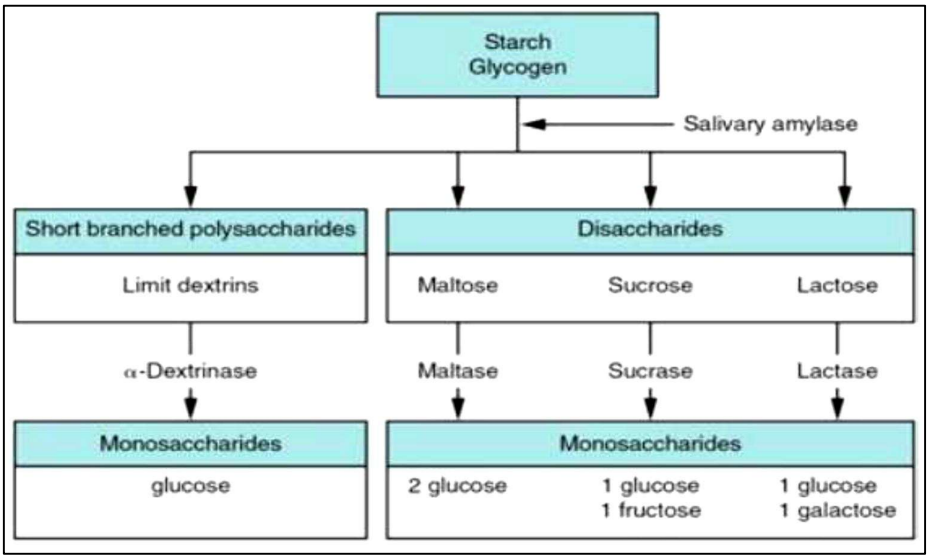

Figure 2: digestion of carbohydrates

a) digestion of protein in stomach and intestine- Gastric pepsin digests as much as 10%–20% of the total ingested protein. The mucosa of stomach has gastric glands. Gastric glands have three major types of cells namely:

- mucus cells: which secrete mucus
- peptic or chief cells; which secrete the proenzyme pepsinogen
- parietal or oxyntic cells; which secrete HCl and intrinsic factor (factor essential for absorption of vitamin B12).
- The stomach stores the food for 4-5 hours. The food mixes thoroughly with the acidic gastric juice of the stomach by the churning movements of its muscular wall and is called the chyme. The proenzyme pepsinogen, on exposure to hydrochloric acid gets converted into pepsin. Pepsin then converts proteins into proteoses and peptones (peptides). The mucus and bicarbonates present in the gastric juice play an important role in lubrication and protection of the mucosal epithelium from excoriation by the highly concentrated hydrochloric acid. HCl provides the acidic Ph (Ph 1.8) optimal for pepsin. Rennin is a proteolytic enzyme found in gastric juice of infants which helps in the digestion of milk proteins.

b) digestion of protein in intestine- The bile, pancreatic juice and the intestinal juice are the secretions released into the small intestine. Pancreatic juice and bile are released through the hepato-pancreatic duct. The pancreatic juice contains inactive enzymes trypsinogen, chymotrypsinogen, procarboxypeptidase. Trypsinogen is activated by an enzyme, enterokinase, secreted by the intestinal mucosa into active trypsin, which in turn activates the other enzymes in the pancreatic juice. Pancreatic proteinases (all secreted in their inactive forms) digest peptides into amino acids. Trypsinogen is activated by enterokinase (secreted by duodenum) into trypsin, which in turn activates the other 3 enzymes-chymotrypsinogen becomes chymotrypsin, pro-aminopeptidase becomes aminopeptidase, and pro-carboxypeptidase becomes carboxypeptidase.

Digestion of Lipids

Lipids are molecules that are insoluble or only slightly soluble in water. Lipids include triglycerides, phospholipids, cholesterol, steroids, and fat-soluble vitamins. The first step in lipid digestion is emulsification, which is the transformation of large lipid droplets into much smaller droplets. The emulsification process increases the surface area of the lipid exposed to the digestive enzymes by decreasing the droplet size. Emulsification is accomplished by bile salts secreted by the liver and stored in the gallbladder. Lipase digests lipid molecules. The vast majority of lipase is secreted by the pancreas. A minor amount of lingual lipase is secreted in the oral cavity, is swallowed with the food, and digests a small amount (<10%) of lipid in the stomach. The stomach also produces very small amounts of gastric lipase. The primary products of lipase digestion are free fatty acids and glycerol and few cholesterols and phospholipids.

6.4.3 Absorption:

Absorption is the process by which the end products of digestion pass through the intestinal mucosa into the blood or lymph. It is carried out by passive, active or facilitated transport mechanisms. Water moves by osmosis; small fat-soluble substances, e.g. fatty acids and 57 glycerol, are able to diffuse through cell membranes; while others are generally transported inside the villi by other mechanisms.

Source	Substance
Carbohydrates	Monosaccharides: glucose, galactose, and fructose
Proteins	Single amino acids, dipeptides, and tripeptides
Triglycerides	Monoacylglycerides, glycerol, and free fatty acids
Nucleic acids	Pentose sugars, phosphates, and nitrogenous bases

Table 2: Macronutrient Digestion

Passive transport:
Small amounts of monosaccharides like glucose, amino acids and some electrolytes like chloride ions are generally absorbed by simple diffusion. The passage of these substances into the blood depends upon the concentration gradients.

Active transport:
Active transport occurs against the concentration gradient and hence requires energy. Various nutrients like amino acids, monosaccharides like glucose, electrolytes like Na+ are absorbed into the blood by this mechanism. Some substances like glucose and amino acids are absorbed with the help of carrier proteins. This mechanism is called the facilitated transport. Fatty acids and glycerol being insoluble, cannot be absorbed into the blood. They are first incorporated into small droplets called micelles which move into the intestinal mucosa. They are re-formed into very small protein coated fat globules called the *chylomicrons* which are transported into the lymph vessels (lacteals) in the villi. These lymph vessels ultimately release the absorbed substances into the blood stream.

Part of the Alimentary Canal	**Substances Absorbed**
Mouth	Minimal absorption. – Certain medications (e.g., nitroglycerin).
Stomach	Water. – Alcohol. – Some drugs (e.g., aspirin). – Simple sugars.
Small Intestine	The primary site of absorption for most nutrients.- Carbohydrates: Glucose,fructose, and galactose. – Proteins: Amino acids, dipeptides, and tripeptides. – Fats: Fatty acids and monoacylglycerides (into the lymphatic system). - Vitamins: Both fat- soluble (A, D, E, K) and water-soluble vitamins. – Minerals: Electrolytes (e.g., sodium, calcium, iron). – Water: A large amount of water.
Large Intestine	Water: Reabsorption of a significant amount of water. – Electrolytes: Sodium and chloride. – Vitamins: Vitamins produced by gut bacteria, such as vitamin K and some B vitamins.

Table 3: Absorption of food substances in different parts of alimentary canal

6.4.4 Assimilation:

The absorbed food materials are transported by blood and lymph. Lymph is finally transferred to the blood circulation. The blood transports absorbed food materials to different body cells where food materials become integral component of the living protoplasm and are used for energy, growth and repair. This is called assimilation of food.

Assimilation of proteins
Amino acids are not stored but are taken up by the cells in connection with the synthesis of proteins. Proteins are used for growth, repair, etc. Excess amino acids can be converted into glucose and then to fat and are thus stored. This is an irreversible reaction. Amino acids can also be converted to glucose and used as fuel for the cell. During their conversion to glucose the amino acids are deaminated (removal of amino groups NH2). The liver is chief site for deamination, i.e., a process by which the amino group is removed from the amino acids resulting in the production of ammonia. The ammonia is soon converted into urea, which is filtered from the blood in the kidney.

Assimilation of carbohydrates
The excess of the monosaccharide's; the glucose, fructose and galactose are usually stored in the liver and muscle cells in the form of glycogen (glycogenesis). Whenever, there is a deficiency of glucose in the blood the glycogen is converted into glucose (glycogenolysis). Muscle glycogen is utilized during muscle contraction. Glucose is utilized in the production of energy for various body activities. A considerable amount of glucose is converted into fat and stored as such.

Assimilation of lipids
The fat is stored in the fat deposits of the body, such as subcutaneous layers, mesenteries, etc. The fat stored is a readily available source of fuel for the cells. Fat has important insulating properties in connection with the conservation of heat and maintenance of body temperature. Fat also plays a protective role as filling or around packing material and between organs. In the liver phospholipids are formed which are returned to the blood to be used by all the cells. In the liver cells the fats are converted into amino acids and carbohydrates. Vitamins, salts and water are also useful for various metabolic processes.

6.5 SUMMARY:

Digestion and absorption are vital processes that enable animals to convert complex food substances into simpler, usable forms of nutrients. Different animals exhibit diverse patterns of digestion depending on their mode of nutrition and structural adaptations of the digestive system. Digestive enzymes such as amylases, proteases, and lipases play a crucial role by catalyzing the hydrolysis of carbohydrates, proteins, and fats into absorbable units. The small intestine, with specialized structures like villi and microvilli, ensures efficient absorption of nutrients into the bloodstream. Assimilation then incorporates these absorbed nutrients into the body's metabolic pathways, providing energy and building materials essential for growth, repair, and maintenance of homeostasis.

6.6 TECHNICAL TERMS:

Digestion, Absorption, Assimilation, Enzymes, Hydrolysis, Proteases, Lipases, Amylases, Peristalsis, Villi

6.7 SELF-ASSESSMENT QUESTIONS:

1. Explain the different patterns of digestion and absorption found in animals.
2. What is the role of digestive enzymes in the breakdown of food?
3. How are carbohydrates, proteins, and fats digested and absorbed in the human body?
4. Define assimilation. How does it differ from absorption?
5. What structural adaptations in the small intestine aid in efficient absorption?

6.8 SUGGESTED READINGS:

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- **Prof. P.V. KRISHNA**

LESSON - 7
RESPIRATION

OBJECTIVES:

1. To study the types and mechanism of respiration in animals.
2. To understand the process of external and internal respiration and gas exchange.
3. To learn about respiratory structures, transport of gases, and control of respiration in humans.
4. To identify different respiratory mechanisms such as integumentary, gills, lungs, and tracheal respiration.
5. To understand the role of respiratory pigments and the oxygen–haemoglobin dissociation curve in respiration.

STRUCTURE:

7.1 INTRODUCTION:

Respiration is the important function of any animal for its survival. If an animal not respire it will expire. Respiration is defined as the process of receiving Oxygen and releasing of CO2 to the surrounding environment. All living organism organisms require a continuous and adequate supply of oxygen for cellular metabolic activity and during that process Carbon dioxide released as metabolic waste, which need to be removed. So, the respiration consists of all the physiological process that contribute to the uptake of Oxygen and the elimination of Carbon dioxide.

7.2 TYPES AND MECHANISM OF RESPIRATION:

In general human respiratory mechanism is divided to two types. They are external respiration and internal respiration.

- External Respiration
- Internal Respiration

. External Respiration:

External respiration is otherwise called as lung respiration or breathing. This type of respiration is carried out by lungs and its accessory structures. External respiration is also called breathing,

which includes two phases, namely inspiration and expiration. Inspiration is called intake of air or breathing in and expiration is called as the release of air or breathing out. During external respiration pulmonary gas exchange takes place between alveoli and the blood. It is the common available process in most of the higher animal groups. In some lower organisms, such as jelly fishes, earthworms, etc. skin plays an important role in respiration. In amphibians the purpose is served by the skin.

2. Internal Respiration:

The second type is called internal respiration or cellular respiration. During internal respiration, the gaseous exchange between the blood or other circulating fluid and the active cells of the organism takes place. Since it this happens at cellular level it is called cellular respiration. In this second phases, blood distributes O2 to the cells and receives CO2 from them through the tissue fluid. The internal respiration is chiefly concerned with the uptake of O2, release of energy and the production of CO2 by the cells.

Feature	External Respiration	Internal Respiration
Site	Lungs (alveoli)	Body cells
Type of process	Physical	Chemical
Function	Exchange of gases between lungs and blood	Energy production inside cells
Oxygen role	Taken from air into blood	Used to oxidize glucose
Carbon dioxide	Released from blood into air	Produced as waste in cells

Table 1: Comparison Between External and Internal Respiration

Energy Production in Respiration

During cellular respiration, glucose combines with oxygen to release energy stored in ATP.
Equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + Energy (≈38 ATP molecules).

- Aerobic Respiration – Occurs in the presence of oxygen; produces 38 ATP.
- Anaerobic Respiration – Occurs without oxygen (e.g., yeast or muscle cells); produces 2 ATP.

Types of Respiratory Mechanisms

The striking adaptability of animal's tor the procurement of oxygen is exemplified by the many modifications which have evolved to alleviate limitations imposed by simple diffusion. Organisms have met similar respiratory problems in different ways. 1 he elaboration of respiratory mechanisms has developed along with the evolution of efficient vascular (convection) systems. Krogh's admirable survey of respiratory adaptations should be consulted in this connection, as well as the extensive review of respiratory systems by Guieysse- Pellissier. Four main types of respiratory mechanisms are considered here: (1) integument, (2) gills, (3) lungs, and (4) tracheae.

1. Integument:

Cutaneous respiration plays a significant role in the respiratory economy of many invertebrates, and it is safe to say that it occurs to some extent in all animals. A circulating mechanism is required in aquatic animals, either cilia or movement of the organ as a whole, to move the water over the respiratory surfaces. In air-breathing forms a moist integument is essential for significant gas exchange through the skin, and mucous glands are generally found in cutaneous

air breathers, as the terrestrial isopods, molluscs, and amphibians. Cutaneous respiration is more common than generally recognized the diffusion constant (10’ times the diffusion coefficient) is defined as the number of cc. of oxygen (reduced to 0° C, 760 mm. Hg) passing through a distance of 1 n over an area of I sq. cm. with a pressure difference of 1 atmosphere because it will occur by necessity through all permeable membranes when the partial pressure of the gas establishes a sufficient gradient. Special respiratory mechanisms have evolved in some annelids (Nereis and Chaetopterns), but many members of this phylum respire through the general body surface. In the oligochaete, Drilocritis, a specialized "dorsal groove" in the epithelium permits the drawing of air bubbles below the water surface and the absorption of oxygen through this portion of the skin. The pulmonale gastropods (Limnaea and Helicosovia) can depend on their integuments when submerged for long periods of time, particularly at lower temperatures, although usually they take in aerial oxygen through the lungs. In blowfly larvae (Calliphora) that under experimental conditions 10 per cent of the total oxygen intake could be accounted for by cutaneous channels. Embryos, young larvae, and some “transparent” arthropods also respire through the body surface. The fish Acara (Chromidae) undergoes caudal differentiation with great increase in vascularity so that the tail becomes a specialized respiratory structure. From the time of Spallanzani, numerous investigators have studied the problem of gas exchange through the integument of vertebrates, many of which respire through the skin, particularly in eliminating carbon dioxide.

2. Gills:

Gills are respiratory appendages, generally well vascularized, and usually ciliated and motile, or located in the current of water flow. They are usually aquatic but may be aerial, and they are sometimes both. The respiratory functions in some cases have been combined or confused with other processes, such as salt absorption in the so-called “anal gills” of Citlex and Chirononnis Movement of water o\’er the surface of aquatic gills is mandatory to insure efficient respiratory exchange. The countercurrent principle of operation in fish, the water outside the gill surface and the blood of the adjacent capillaries inside Rowing in opposite directions, provides for rapid oxygen uptake and almost complete saturation as the blood leaves the gill filaments.

Dermal branchiae, the so-called papulae, are found in many of the echinoderms supplementing respiratory exchange through the tube feet. The papulae are evaginations of the body wall (extensions of the coelom), bringing the gently moving coelomic fluid in close association with the cilia-stirred external sea water. In Asterias a specialized region of the ambulacral system and a portion of the madreporite plate have been regarded as respiratory in nature. Polychaetes have evolved some elaborate gill structures, such as parapodia (Nereis), gills (*Arcnicola),* and branchial cornis has been shown by extirpation experiments to take up through the filaments of the branchial tufts approximately 37 per cent of the total oxygen consumed, the rest entering through the integument. Molluscs show a great range of respiratory mechanisms with a marked tendency toward the development of aerial gills. The primitive Chiton has six to eight aquatic gills in each pallial groove. Bivalves generally have two pairs of gills or ctenidia, each with two lamellae, situated in a water supply flowing through the mantle cavity. Gastropods are gill-bearing except for the 91eurocoele land snails, in which the “lung” is actually modified from the lining of the mantle cavity. Ancula, an opisthobranch snail, with both aquatic and aerial tendencies, possesses on its back a peculiar system of rigid gills which can extract oxygen from both air and water.

3. lungs:

In all cases the lung surfaces must be moist, and gas transport occurs across a thin water film. Lungs are of two general types, diffusion and ventilation, depending on the presence of renewal

mechanisms. All the lung-like organs considered here are parts of or outgrowths from the alimentary tract.

Water-Lungs:
Water-lungs are found in several invertebrate phyla and consist essentially of respiratory cavities filled with water, rhythmically drawn into and expelled from the body. Respiratory trees of holothurians are alternately filled and emptied by means of muscular movements of the body wall, and gas exchange occurs by simple diffusion between the lung water and body fluid. The entire hind-gut of the gephyrin worm, Jrechis caupo, constitutes a respiratory organ, muscular contractions of the cloacal region serving as the pumping mechanism. This thin-walled respiratory surface, adjacent to the coelomic fluid which is constantly agitated by ant peristaltic waves in the hind-gut, provides adequate gas exchange; water expelled from the hindgut contains less oxygen by about 40 per cent and more carbon dioxide than the surrounding medium. The 92eurocoele snails, Livinaea and Planorhis, are able to live under water for considerable periods of time, the lungs filling with water and aiding the skin in respiration. Water can be rapidly sucked into the "Endblase" or hindmost gut in the dragonfly larva (Aeschna) and brought in contact with the respiratory surfaces. Alimentary Mucosa.

Some vertebrates possess modified gastrointestinal epithelium which permits uptake of oxygen from swallowed air. Gastric respiration is known to occur in such tropical forms as Plecostomiis and Ancistrus, and intestinal respiration has been demonstrated in a great many other varieties of fish. In the loach, Cohitiis, gas exchange is indicated not only by the histologic nature of the mucosa but also by direct determination of the gas of the intestinal lumen, which shows less oxygen (15.7 per cent) and more carbon dioxide (3.0 per cent) than does air Gas Bladder. The gas bladder, considered by many to be the fore runner of the vertebrate lung, functions in respiratory exchange in many of the physostome (open-duct) teleosts, ganoids, and dipnoans. To be efficient as a respiratory organ, the gas bladder must have some renewal mechanism. Many gas bladders have a respiratory type of epithelium, partitions forming "alveoli," and their own blood supply derived from the pulmonary arch (Fig. 40). The gas bladder can serve merely as an accessory organ when the oxygen tension falls, as in the actinopterygian, Polypterus, or as the main respiratory mechanism in the true lungfishes, Protopterus, Lepidosiren, and Neoceratodus As a respiratory organ the gas bladder must give up oxygen and take on carbon dioxide so that analyses should indicate less oxygen and more carbon dioxide than in the inspired atmospheric air, a situation shown to exist in many physostome fish.

4. Tracheae:
Tracheae consist of a network of fine, chitin-lined tubes that branch throughout the body of insects and some arthropods. They open to the outside through small openings called spiracles, which are usually located along the sides of the body. These tubes repeatedly divide into finer branches called tracheoles, which penetrate tissues and reach individual cells. The ends of tracheoles contain tracheal fluid that facilitates the exchange of gases between the tracheal system and the cells. The chitin lining helps maintain the structural integrity of the tubes, preventing them from collapsing under pressure.

The primary function of the tracheal system is to deliver oxygen directly to body cells and remove carbon dioxide without relying on the circulatory system. Oxygen diffuses through the tracheae and tracheoles, reaching cells rapidly, while carbon dioxide follows the reverse path to exit the body. In larger insects, rhythmic body movements assist in ventilating the system, ensuring efficient gas exchange. This direct delivery system allows insects to maintain high metabolic rates, supporting energy-demanding activities like flight and rapid movement.

Despite its efficiency, the tracheal system imposes certain limitations. Since it relies mainly on diffusion, it is effective only in small to medium-sized organisms. Very large body sizes would prevent oxygen from diffusing quickly enough to reach all cells, which is why insects and arthropods are generally small. Nevertheless, the tracheal system is highly adapted to terrestrial life, allowing insects and other arthropods to remain active and survive in oxygen-variable environments.

7.2.1 Process of pulmonary respiration:

Respiration includes several processes which are listed below

i. **Ventilation** is the breathing of air with more oxygen into the lungs (inspiration), it is followed by expulsion of air with more carbon-di-oxide (expiration).
ii. **Diffusion** of oxygen from the alveoli into the blood inside surrounding capillaries.
iii. **Transport** of oxygen by the blood to the heart through the pulmonary vein.
iv. **Distribution** of oxygen by various arteries and their capillary network to all cells of the body. As the blood passes through tissue capillaries, it gives up oxygen (and nutrients such as glucose) to the body tissues and pick up the waste products of cellular respiration (Carbon dioxide and water).
v. **Exchange** of the oxygen and carbon dioxide between blood and body cells. Within body cells glucose and oxygen take part in a complex series of reactions which provide energy to power the cells. During this cellular respiration glucose is converted to carbon dioxide and water (Enzymatic oxidation).
vi. **Transporting** blood with carbon dioxide. Carbon dioxide is carried back in the blood to the heart then to the lungs where it diffuses into the alveoli and is breathed out of the body (External respiration)
vii. **Exchanging** of carbon dioxide with oxygen at the alveolar surface.
viii. **Expiration** of air with carbon dioxide from the lungs.

7.2.2. Mechanism of Breathing:

a. Inhalation:

Inhalation is initiated by the diaphragm and supported by the external intercostal muscles. Normal resting respirations are 10 to 18 breaths per minute, with a time period of 2 seconds. Under normal conditions, the diaphragm is the primary driver of inhalation. When the diaphragm contracts, the ribcage expands and the contents of the abdomen are moved downward. This results in a larger thoracic volume and negative (suction) pressure (with respect to atmospheric pressure) inside the thorax. As the pressure in the chest falls, air moves into the conducting zone. Here, the air is filtered, warmed, and humidified as it flows to the lungs. During forced inhalation, as when taking a deep breath, the external intercostal muscles and accessory muscles aid in further expanding the thoracic cavity.

b. Exhalation

Exhalation is generally a passive process; however, active or forced exhalation is achieved by the abdominal and the internal intercostal muscles. During this process air is forced or exhaled out. The lungs have a natural elasticity: as they recoil from the stretch of inhalation, air flows back out until the pressures in the chest and the atmosphere reach equilibrium. During exhalation ribcage muscles relax and the diaphragm moves up and relaxed (Fig. 2a & 2b). During forced exhalation, as when blowing out a candle, expiratory muscles including the abdominal muscles and internal intercostal muscles generate abdominal and thoracic pressure, which forces air out of the lungs.

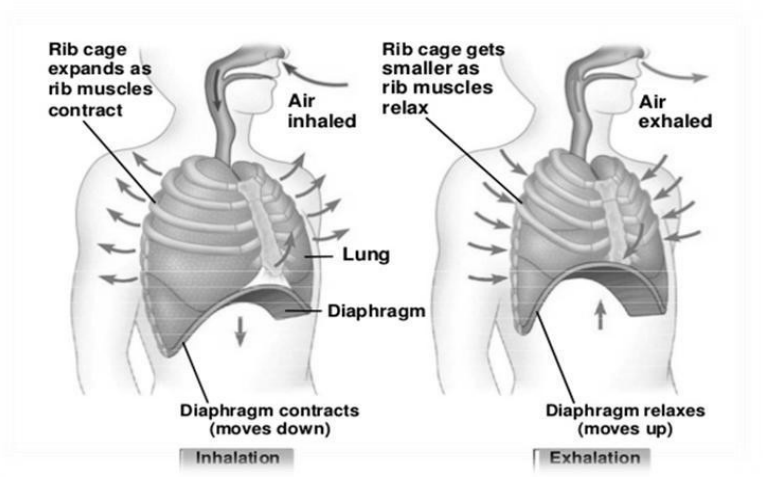

Figure 1: Mechanism of Breathing Inhalation and Exhalation

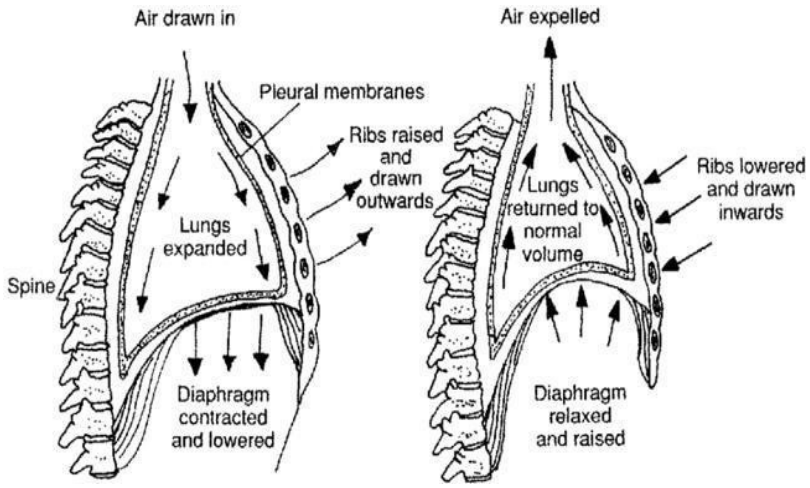

Figure 2: Contraction of diaphragm during inhalation and relaxation during exhalation

7.3. TRANSPORT OF GASES:

A. Gas exchange in the lungs:

The major function of the respiratory system is gas exchange between the external environment and an organism's circulatory system. In humans and mammals, this exchange facilitates oxygenation of the blood with a concomitant removal of carbon dioxide and other gaseous metabolic wastes from the circulation. As gas exchange occurs, the acid-base balance of the body is maintained as part of homeostasis. If proper ventilation is not maintained, two opposing conditions could occur: respiratory acidosis, a life-threatening condition, and respiratory alkalosis. Upon inhalation, gas exchange occurs at the alveoli, the tiny sacs which are the basic functional component of the lungs. The alveolar walls are extremely thin (95euroco. 0.2 micrometers). These walls are composed of a single layer of epithelial cells (type I and type II epithelial cells) in close proximity to the pulmonary capillaries which are composed of a single layer of endothelial cells. The close proximity of these two cell types allows permeability to gases and, hence, gas exchange. This whole mechanism of gas exchange is carried by the simple phenomenon of pressure difference. When the atmospheric pressure is low outside, the air from lungs flow out. When the air pressure is low inside, then the vice versa.

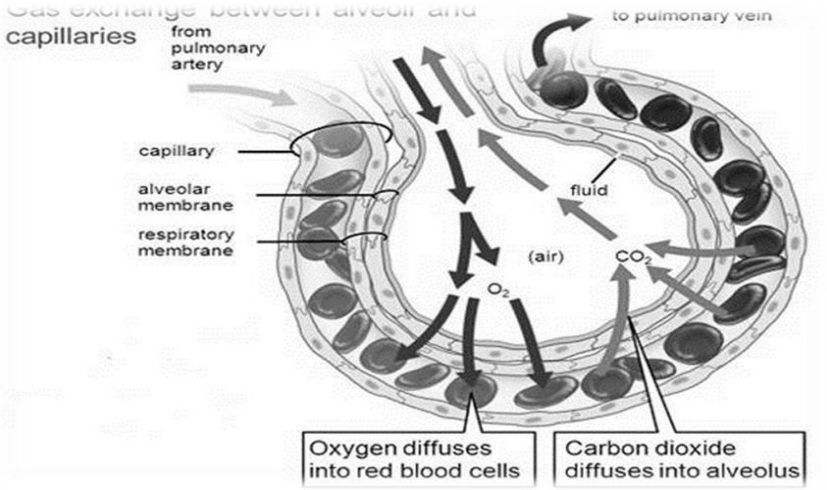

Figure 3: Exchange of gases (O2 and CO2) between alveoli and capillaries Gas exchange Mechanism of Gas Exchange in Alveoli

Gas exchange occurs in the alveoli by diffusion. Oxygen from alveolar air enters the capillary blood, while carbon dioxide moves from blood to alveoli and is exhaled.

B. Respiratory pigment:

A respiratory pigment is a molecule, such as hemoglobin in humans, that increases the oxygen-carrying capacity of the blood. The four most common invertebrate respiratory pigments are hemoglobin, haemocyanin, haemerythrin and chlorocruorin. Hemoglobin is bright red when oxygenated, and dark red when deoxygenated, oxygenated haemocyanin is blue in color, deoxygenated is almost colorless. Oxygenated chlorocruarin turns green where oxygenated haemeryhrtin is a violet to pink colour, and colorless when deoxygenated. All vertebrates use the hemoglobin respiratory pigment. Respiratory pigments such as hemoglobin and hemocyanin reversibly bind oxygen in the blood of many animals. This binding facilitates the transport of oxygen from the respiratory surfaces to the inner tissues. The oxygen binding properties of respiratory pigments are dependent upon both the partial pressure of oxygen and the pH of the blood. At equilibrium, the proportion of oxygenated to deoxygenated respiratory pigment has a sigmoid relation to the partial pressure of oxygen (PO2). When all of the respiratory pigment is combined with oxygen, the respiratory pigment is said to be saturated. Shifts in blood Ph can affect this equilibrium, causing more or less O2 to bind to the pigment at any given PO2. It is the changing nature of these binding properties that facilitates the uptake of environmental oxygen at the respiratory surfaces and its subsequent release in the inner tissues.

C. Maximum amount of O2 that can combine with the Hemoglobin of blood:

The blood of a normal person contains about 15 gm of Hb in each 100 ml of blood. One gm of Hb binds to 1.34 ml of O2 when fully saturated. Thus 100 ml of pre blood can combine with 20 ml of O2, when Hb is 100% saturated. On passing through tissue capillaries, this amount is reduced to 14.4 ml. Thus, 5ml of O2 is transported from the lungs to the tissue by each 100 ml of blood.

D. Oxygen-Hemoglobin dissociation curve:

The oxygen hemoglobin dissociation curve demonstrates a progressive increase in the percentage of hemoglobin bound with oxygen as the blood PO2 increases, which is called the percent saturation of the hemoglobin. As the blood leaving the lungs and entering the systemic

arteries usually have a PO2 of about 95mm Hg, it can be observed from the dissociation curve that the usual oxygen saturation of systemic arterial blood is about 97 percent. On the other hand, in normal venous blood returning from the peripheral tissues, the PO2 is about 40 mm Hg, and the saturation of the hemoglobin is about 75 percent. Hemoglobin in the blood automatically.

E. Factors that shift the Oxygen Hemoglobin dissociation curve: A number of factors can displace the dissociation curve in one direction or the other. When the blood becomes slightly acidic, with the Ph decreasing from the normal value of 7.4 to 7.2, the oxygen-hemoglobin dissociation curve shifts, on average about 15 percent to the right. Conversely, an increase in the Ph from the normal 7.4 to 7.6 shifts the curve similar amount to the left. In addition to Ph changes, several other factors are known to shift the curve.

Three of these, all of which shift the curve to the right, are

- Increased carbon dioxide concentration
- Increased blood pressure, and
- Increased/13-diphosphoglycerate (DPG),

A metabolically important phosphate compound present in the blood but in different concentration under different metabolic conditions. Increased delivery of oxygen to the tissues when carbon dioxide and hydrogen ions shift the oxygen-hemoglobin dissociation curve-

The Bohr Effect. A shift of the oxygen hemoglobin dissociation curve in response to changes in the blood carbon dioxide and hydrogen ions has a significant effect in enhancing oxygenation of the blood in the lungs and then again in enhancing release of oxygen from blood in the tissues.

This is called Bohr Effect. This can be explained as follows: As the blood passes through the lungs, carbon dioxide diffuses from the blood into the alveoli. This reduces the blood PCO2 and decreases the hydrogen ion concentration because of the resulting decrease in blood carbonic acid. Both these effects shift the oxygen-hemoglobin dissociation curve to the left and upward. Therefore, the quantity of oxygen that binds with the hemoglobin at any given alveolar PO2 now becomes considerably increased, thus allowing greater oxygen transport to the tissues.

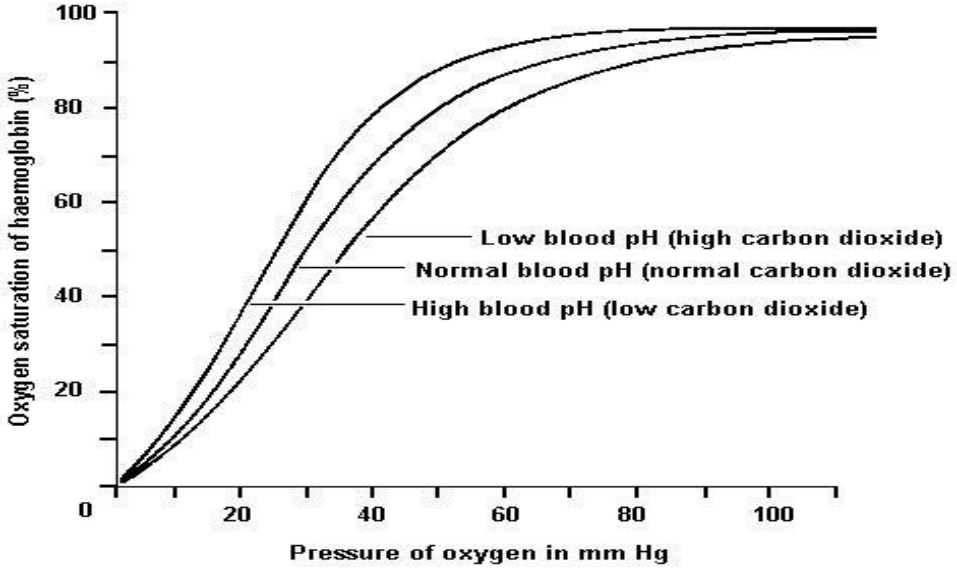

Figure 4: Shift of Oxygen-hemoglobin dissociation curve to the left by high Ph and low carbon dioxide.

Type	Description	Average Volume
Tidal Volume (TV)	Air inhaled/exhaled during normal breathing	500 mL
Inspiratory Reserve Volume (IRV)	Extra air inhaled after normal inspiration	3000 mL
Expiratory Reserve Volume (ERV)	Extra air exhaled after normal expiration	1000 mL
Residual Volume (RV)	Air remaining after forced exhalation	1200 mL
Vital Capacity (VC)	TV + IRV + ERV	≈4500 mL
Total Lung Capacity (TLC)	Sum of all volumes	≈6000 mL

Table 3: Lung Volume Graph – [Draw with labels TV, IRV, ERV, RV]

7.4. CONTROL OF RESPIRATION:

Oxygen must be supplied continuously and the amount of Oxygen present in the lungs, tissues and blood is only 1200ml which is enough for only 5 minutes. Oxygen deprivation causes loss of consciousness within 20 seconds and irreversible brain damage within about 4 minutes.

Respiration of lungs is under the control of the autonomic nervous system from parts of the brain stem, the medulla oblongata and the pons. This area of the brain forms the respiration regulatory center, a series of interconnected brain cells within the lower and middle brain stem which coordinate respiratory movements. The sections are the pneumotaxic center, the apneaustic center, and the dorsal and ventral respiratory groups. The activity of the respiration Centre is modified by the chemical composition of its fluid environment, as well as by the nervous influences. Control of respiration is due to rhythmical breathing generated by the phrenic nerve in order to stimulate contraction and relaxation of the diaphragm during inspiration and expiration. Ventilation is controlled by partial pressures of oxygen and carbon dioxide and the concentration of hydrogen ions. The control of respiration can vary in certain circumstances such as during exercise.

Disorders Related to Respiration

1. Asthma – Narrowing of airways causing difficulty in breathing.
2. Bronchitis – Inflammation of bronchi.
3. Emphysema – Damage to alveoli
4. Pneumonia – Infection causing fluid in alveoli
5. Tuberculosis – Bacterial infection damaging lung tissues.
6. COVID-19 – Viral infection affecting alveoli and gas exchange.

7.5 SUMMARY:

Respiration is the process of receiving oxygen and releasing carbon dioxide to the surrounding environment. This can be classified into external respiration and internal respiration. External respiration is carried out by the lungs and its accessory structures. The internal respiration is called as cellular respiration carried out by the blood and the cells of the tissues. The normal breathing consists of inhalation and exhalation. Intercostal muscles, diaphragm and ribcage assist the inhalation and exhalation. The gas exchange takes place in the moist surface of alveoli. The respiratory pigments in the RBC helps to carry oxygenated blood to the tissues. The respiration is controlled by the autonomic nervous system which is located in the brain stem, medulla oblongata and the pons.

7.6 TECHINICAL TERMS:

Aerobic, Anaerobic, External, Internal, Haemoglobin, Oxyhaemoglobin, Carbonic anhydrase, Medulla oblongata, Chemoreceptors, Tidal volume.

7.7 SELF-ASSESSMENT QUESTIONS:

1. What is respiration? Differentiate between external and internal respiration?
2. What are respiratory pigments? Discuss various respiratory pigments?
3. Discuss the process of gaseous exchange in respiratory organs and tissues?
4. Discuss the structure of Hemoglobin? Explain oxygen transport by hemoglobin?
5. Discuss the transport of CO2 by blood?

7.8 SUGGESTED READINGS:

1. Hall, J. E. 2015. Guyton and Hall Text book of Medical Physiology, 13th Edition, Relx India Pvt. Ltd.
2. Verma, P.S., B.S. Tyagi, V. K. Agarwal, 2000. Animal Physiology, S. Chand & Co. India.
3. Rastogi, S. L. 1997. Essential of Animal Physiology, New Age International Publishers, India.

- **Prof P.V. KRISHNA**

LESSON- 8
BLOOD

OBJECTIVES:

1. To understand the composition, functions, and regulation of blood and its components.
2. To study the processes of hemopoiesis, hemostasis, and the role of formed elements in maintaining homeostasis.
3. To explore the nature, types, and evolutionary significance of respiratory pigments in different organisms.

STRUCTURE:

8.1 INTRODUCTION:

Blood is a fluid connective tissue circulating in the body. It provides one of the methods of communication between the cells of different parts of the body. The blood carries oxygen and nutrients to the tissues and carbon dioxide and waste products from the tissues to the excretory organs. In addition, it conveys antibodies to the site of injury or disease and hormones or chemical messengers from the endocrine glands to various target organs. The blood is the medium of transportation to all these substances. The average human adult has more than 5 liters of blood in his or her body. Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug in a damaged blood vessel to prevent blood loss. Through the circulatory system, blood adapts to the body's needs. When you are exercising, your heart pumps harder and faster to provide more blood and hence oxygen to your muscles. During an infection, the blood delivers more immune cells to the site of infection, where they accumulate to ward off harmful invaders.

8.2 BLOOD COMPOSITION:

The blood is composed of faintly yellow transparent fluid known as plasma and numerous cells or corpuscles of different kinds floating in this liquid medium. The fluid plasma constitutes about 55% and the remaining 45% are occupied by corpuscles in the blood. The three main components of the blood are erythrocytes or red blood cells, leucocytes or white blood cells and blood platelets.

8.2.1 Erythrocytes or Red Blood Corpuscles:

In humans red blood cells lacks the nucleus. However, absence of nucleus increases the respiratory efficiency of the red blood corpuscles. All the vertebrates other than mammals have nucleated red cells. The red blood cells are biconcave non nucleated cells. The central part of the corpuscle is much thinner than the circumference, thus the term biconcave. In edge view the outline is like that of a dumb bell. This shape favors the flexibility and the absorbing and releasing of gases quickly. The red corpuscles are soft and flexible. They withstand much bending, squeezing and deformation as they are pushed through the narrow capillaries. The respiratory pigment of red corpuscle is hemoglobin. It is a complex protein having iron as one of the main constituents. In adults red blood cells are mostly produced in the red bone marrow and this process is called erythropoiesis. This also controlled by a feedback mechanism.

Deficiency of Oxygen following hemorrhage or if an individual lives at higher elevation where the Oxygen pressure in the atmosphere is low, more RBC are produced. Other than that, a hormone secreted by the kidney called erythropoietin also increase the production of erythrocytes. The normal erythrocyte count is usually higher in men compared to women and having 5 to 5.5 million per cubic millimeter of blood. The number varies with different pathological and pathological conditions. An abnormal raise in RBC count is called polycythemia. The life span of human erythrocytes is approximately 120 days. After that, these cells break down in the spleen. The protein part of the erythrocyte is converted into biliverdin, which is converted into a yellow pigment called bilirubin. This is carried by splenic and portal veins to the liver, where it is again changed into conjugated form and excreted in the bile as bile pigment.

8.2.2 Leucocytes or White Blood cells:

Unlike RBC the white blood cells or leucocytes contain a nucleus. The leucocytes resemble the amoeba cells. They vary in size from 8 to 15 μ. The average number of WBC is between 6,000 to 10,000 numbers per cubic millimeter of blood. In pathogenic condition there is the variation from the normal number. An increase in the number of white blood cells is called leucocytosis. A decrease below 6,000 is called leucopenia as in typhoid fever. The WBCs are divided as granular polymorphonuclear leucocytes and agranular or Mononuclear leucocytes.

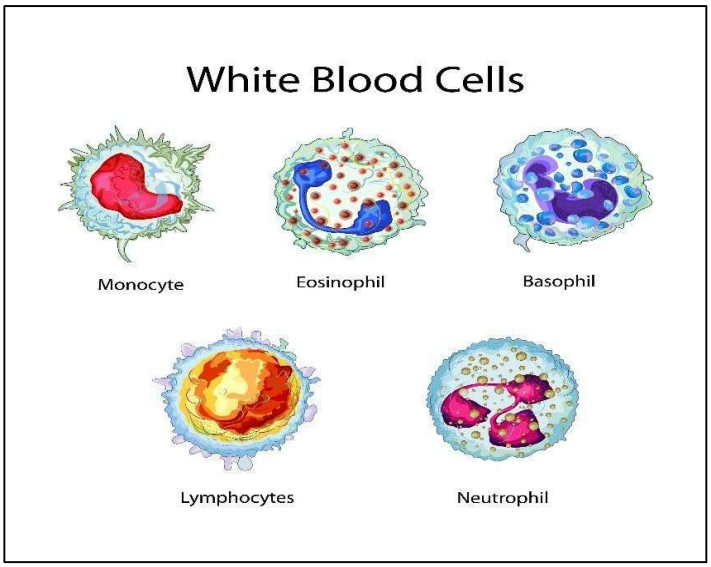

Figure1: **White blood corpuscles**

1. Granular Leucocytes

These cells are developed from the red bone marrow. The granulocytes constitute about 75% of the total white blood cells. Among them Neutrophils constitute approximately 70% followed by Eosinophils (4%) and Basophils (1%)

a. Neutrophils: The cytoplasm of Neutrophils are closely packed with fine, inconspicuous granules that absorb both acidic and alkaline dyes, thus producing neutral purple color. The nucleus has three to five lobes connected by thin chromatin strands. Fewer lobe indicates less mature cells. About three percent of neutrophils from a female show the sex chromosome attached to one end of the nuclear lobe by a thin stalk forming the so called - drumstick chromosome. Neutrophils protect the body against the invasions of bacteria. They are attached in large numbers to any area of the body which has been invaded micro- organism. Neutrophils leave the blood by squeezing themselves out through the walls of the capillaries in the infected area. This process is known as diapedesis. Thereafter they kill the organisms by digesting them by means of various enzymes, a process known as phagocytosis. The pus which may exude from an infected area consists of destroyed tissue, live microorganisms and dead neutrophils which have ingested more microorganisms than they could digest. The life span of neutrophils which remain in blood vessels is about 30 hours. After migrating from the blood capillaries into the inflamed tissue, they play a defensive role there and soon die.

b. Eosinophils: The diameter of the cell is nearly twice that of the red blood corpuscle. The cytoplasm is packed with coarse round granules which absorb acid dye such as eosin which is red. The nucleus is bilobed with a connecting isthmus. Eosinophils engulf the particles which are formed when antigens and antibodies react with each other. In allergic conditions such as asthma and parasitic infections of the digestive tract they are increased in numbers.

c. Basophils: The cytoplasmic granules absorb an alkaline or basic dye such as methylene blue and are stain dark blue or purple in color. The granules the granules are not as numerous as in eosinophils but they are large, spherical and almost cover the nucleus which is generally, S. shaped. Basophils are actively amoeboid and ingest small particles like carbon.

2. Agranular leucocytes:
They have few non-specific or no granules in the cytoplasm and the nucleus is spherical to kidney shaped. They comprise about 25% of the leucocytes.

a. lymphocytes: There are two types of lymphocytes and they are classified as small and large lymphocytes. Small lymphocytes are mostly found in the blood. It has a large nucleus surrounded by narrow rim of basophilic cytoplasm. The nucleus is dense and dark because of heavy chromatin masses. Large lymphocytes are primitive cells residing in the lymph nodes that give rise to the active small lymphocytes. The nucleus is largely kidney shaped.

b. Monocytes: Monocytes are largest of leucocytes and are relatively few in number. The nucleus varies from oval, round or indented to kidney shape and stains lightly than that of the lymphocytes. The abundant cytoplasm is slightly basophilic. Their function is closely resembled that of the neutrophils in that that they are actively mobile, phagocytic in action and will leave blood capillaries to ingest microorganisms

8.2.3 Blood platelets:

Blood Platelets arise from the megakaryocytes. They are 2-3 μ in diameter. There are approximately 300,000 platelets in the cubic millimeter of blood. The platelets are associated with blood clotting, both inside and outside of the blood vassals.

8.2.4 Blood plasma:

Blood plasma contains 90 to 92% water. Other constituents include blood proteins. They are serum albumin, which is derived from liver, serum globulin derived from lymphocytes, fibrinogen, which is derived from liver helpful for blood clotting and prothrombin, which served as factor II in the blood clotting which is also derived from liver. The blood plasma gives viscosity to the blood and also helpful to maintain the blood pressure. The plasma also contains minerals such as sodium chloride, sodium carbonate, potassium, magnesium etc which involve in the physiological process. Nutrient materials such as carbohydrate, protein and fats to maintain the body functions. Other than hormones, enzymes, antibodies and gasses are also present in the blood plasma.

8.3 HEMOPOIESIS:

Hemopoieses is derived from the Greek words for blood “and to make”. The bone marrow is the chief source of blood cells in children and adults. Cells are derived from the progressive differentiation of primitive haemopoietic stem cells, in the presence of soluble and cellular signals, and expression of key transcription factors. All skeletal bones are active sites of hemopoiesis in children, whereas in adults this is limited to a few sites such as the skull, vertebrae, ribs and scapulae. Haemopoietic tissue occupies most of the bone marrow in children, whereas this declines progressively in adults. Hemopoiesis takes place in the honeycomb spaces of trabecular bone, interspersed with fat cells that increase in number with age. The bone marrow microenvironment forms a stem cell niche around self-renewing haemopoietic progenitor cells and is important for controlling appropriate blood cell production. Hemopoiesis is considered to be clonal. A single multipotent stem cell is capable of repopulating the entire haemopoietic system, forming blood and immune cells. A multipotent haemopoietic stem cell can self-renew or differentiate into a multipotent progenitor (MPP). MPP differentiation produces common myeloid progenitors and common lymphoid progenitors.

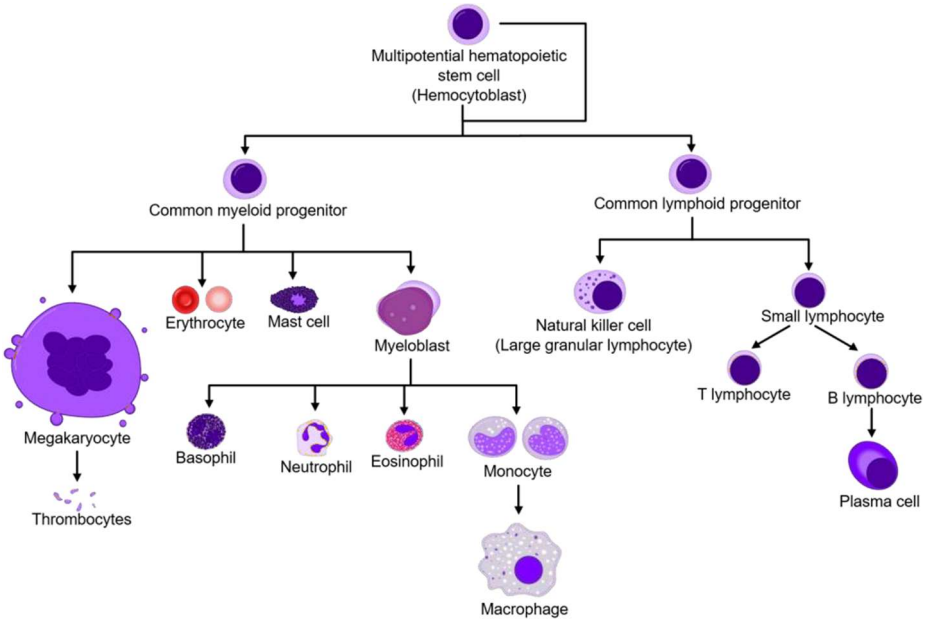

Figure 2: Hematopoiesis from pluripotent stem cell

Haemopoietic stem cells differentiate into MPPs, then Common myeloid progenitors CMPs, then megakaryocyte erythroid progenitors (MEPs), then megakaryocytes and finally platelets. Thrombopoietin (TPO), interleukin (IL)-6 and other cytokines and soluble growth factors stimulate maturation of megakaryocytes, where rounds of DNA replication occur without intervening cell divisions; this forms polypoid cells that have up to 64N. The resulting abundant cytoplasm facilitates the maturation of platelets crashing into sinusoids, so nuclei enter the circulation, being cleared by pulmonary macrophages. Erythropoietin (Epo) is chiefly produced by renal peritubular interstitial cells (and to some extent hepatocytes) in response to tissue hypoxia, through up-regulation of hypoxia-inducible factor 1 (HIF1). The von Hippel Lindau tumor suppressor gene (VHL) negatively regulates HIF1 via an E3 ubiquitin ligase. Erythropoietin is necessary for the differentiation of all stages of red cell development from the erythroid colony-forming unit phase onwards. This phase is preceded by the erythroid burst-forming unit stage. Red cell formation often occurs around a macrophage, which contains iron stores. In summary, form the hematopoietic stem cells, lymphoid progenitor and the myeloid progenitors are formed. From the lymphoid progenitors, the lymphocytes and natural killer cells are formed. The Myeloid progenitor is divided into Megakaryocyte progenitor which produce platelets, erythroid progenitor which produce the red blood cells and Myeloblasts, which produce macrophages.

8.4 FORMED ELEMENTS:

The formed elements are cells and cell fragments suspended in the plasma. The three classes of formed elements are the erythrocytes (red blood cells), leukocytes (white blood cells), and the thrombocytes (platelets). Erythrocytes, or red blood cells, are the most numerous of the formed elements. Erythrocytes are tiny biconcave disks, thin in the middle and thicker around the periphery. The shape provides a combination of flexibility for moving through tiny capillaries with a maximum surface area for the diffusion of gases. The primary function of erythrocytes is to transport oxygen and, to a lesser extent, carbon dioxide. Leukocytes, or white blood cells, are generally larger than erythrocytes, but they are fewer in number. Even though they are considered to be blood cells, leukocytes do most of their work in the tissues. They use

the blood as a transport medium. Some are phagocytic, others produce antibodies; some secrete histamine and heparin, and others neutralize histamine. Leukocytes are able to move through the capillary walls into the tissue spaces, a process called diapedesis. In the tissue spaces they provide a defense against organisms that cause disease and either promote or inhibit inflammatory responses. There are two main groups of leukocytes in the blood. The cells that develop granules in the cytoplasm are called granulocytes and those that do not have granules are called agranulocytes. Neutrophils, eosinophils, and basophils are granulocytes. Monocytes and lymphocytes are agranulocytes. Neutrophils, the most numerous leukocytes, are phagocytic and have light-colored granules. Eosinophils have granules and help counteract the effects of histamine. Basophils secrete histamine and heparin and have blue granules. In the tissues, they are called mast cells. Lymphocytes are agranulocytes that have a special role in immune processes. Some attack bacteria directly; others produce antibodies.

8.5 BLOOD VOLUME AND ITS REGULATION:

Blood volume is necessary to maintain adequate perfusion to all of the tissues in the body. Nearly all cells in the body require replenishment of nutrients and a removal system for waste, both of which the blood provides. When a tissue loses its blood supply, ischemia occurs which may lead to an infarct after some time. Depending on the location of this tissue, an infarct could have a fatal effect. An infarct of the heart is a myocardial infarction; an infarct of cerebral tissue is a stroke. Blood volume also functions in the maintenance of body osmolality. Osmolality refers to the balance of solutes and water within a solution, in this case, the blood. A properly functioning system maintains an osmolality of 275 to 295 mOsm/kg of water through water and sodium manipulation primarily at the kidney. When one of these two varies from the standard range, plasma osmolality changes and may increase or decrease plasma volume. Changing plasma osmolality results in an imbalance between intracellular and extracellular compartments. This imbalance can cause water entry or exit from cells. Overall, it may greatly increase or decrease blood volume. Increased blood volume is called hypervolemia and decreased blood volume is called hypovolemia.

8.6 HAEMOSTASIS:

The term hemostasis means the prevention of blood loss, whenever a blood vessel is severed or ruptured. It is the innate response for the body to stop bleeding and loss of blood. During hemostasis three steps occur in a rapid sequence. Vascular spasm is the first response as the blood vessels constrict to allow less blood to be lost. In the second step, platelet plug formation, platelets stick together to form a temporary seal to cover the break in the vessel wall. The third and last step is called coagulation or blood clotting. Coagulation reinforces the platelet plug with fibrin threads that act as a “molecular glue”.

Platelets are a large factor in the hemostatic process. They allow for the creation of the “platelet plug” that forms almost directly after a blood vessel has been ruptured. Within seconds of a blood vessel’s epithelial wall being disrupted platelets begin to adhere to the sub-endothelium surface. It takes approximately sixty seconds until the first fibrin strands begin to intersperse among the wound. After several minutes the platelet plug is completely formed by fibrin. Hemostasis is maintained in the body via three mechanisms:

1. Vasoconstriction: Vasoconstriction is produced by vascular smooth muscle cells, and is the blood vessel’s first response to injury. The smooth muscle cells are controlled by vascular endothelium, which releases intravascular signals to control the contracting properties. When

a blood vessel is damaged, there is an immediate reflex, initiated by local sympathetic pain receptors, which helps promote vasoconstriction. The damaged vessels will constrict (vasoconstrict) which reduces the amount of blood flow through the area and limits the amount of blood loss. Collagen is exposed at the site of injury; the collagen promotes platelets to adhere to the injury site. Platelets release cytoplasmic granules which contain serotonin, ADP and thromboxane A2, all of which increase the effect of vasoconstriction. The spasm response becomes more effective as the amount of damage is increased. Vascular spasm is much more effective in smaller blood vessels.

2. Platelet plug formation: Platelets adhere to damaged endothelium to form a platelet plug (*primary hemostasis*) and then degranulate. This process is regulated through thromboregulation. Plug formation is activated by a glycoprotein called Von Willebrand factor (Vwf), which is found in plasma. Platelets play one of major roles in the hemostatic process. When platelets come across the injured endothelium cells, they change shape, release granules and ultimately become sticky. Platelets express certain receptors, some of which are used for the adhesion of platelets to collagen. When platelets are activated, they express glycoprotein receptors that interact with other platelets, producing aggregation and adhesion.
Platelets release cytoplasmic granules such as adenosine diphosphate (ADP), serotonin and thromboxane A2. Adenosine diphosphate (ADP) attracts more platelets to the107eurocted area, serotonin is a vasoconstrictor and thromboxane A2 assists in platelet aggregation, vasoconstriction and degranulation. As more chemicals are released more platelets stick and release their chemicals; creating a platelet plug and continuing the process in a positive feedback loop. Platelets alone are responsible for stopping the bleeding of unnoticed wear and tear of our skin on a daily basis. This is referred to as primary hemostasis.

3. Clot formation Once the platelet plug has been formed by the platelets, the clotting factors (a dozen proteins that travel along the blood plasma in an inactive state) are activated in a sequence of events known as 'coagulation cascade' which leads to the formation of Fibrin from inactive fibrinogen plasma protein. Thus, a Fibrin mesh is produced all around the platelet plug to hold it in place; this step is called "Secondary Hemostasis". During this process some red and white blood cells are trapped in the mesh which causes the primary hemostasis plug to become harder: the resultant plug is called as 'thrombus' or 'Clot'. Therefore 'blood clot' contains secondary hemostasis plug with blood cells trapped in it. Though this is often a good step for wound healing, it has the ability to cause severe health problems if the thrombus becomes detached hemostasis method in medicine Some main types of hemostasis used in emergency medicine include:

- **Chemical/topical**- This is a topical agent often used in surgery settings to stop bleeding. Microfibrillar collagen is the most popular choice among surgeons because it attracts the patient's natural platelets and starts the blood clotting process when it comes in contact with the platelets. This topical agent requires the normal hemostatic pathway to be properly functional.
- **Direct pressure or pressure dressing**- This type of hemostasis approach is most commonly used in situations where proper medical attention is not available. Putting pressure and/or dressing to a bleeding wound slows the process of blood loss, allowing for more time to get to an emergency medical setting. Soldiers use this skill during combat when someone has been injured because this process allows for blood loss to be decreased, giving the system time to start coagulation.
- **Sutures and ties**- Sutures are often used to close an open wound, allowing for the injured area to stay free of pathogens and other unwanted debris to enter the site;

however, it is also essential to the process of hemostasis. Sutures and ties allow for skin to be joined back together allowing for platelets to start the process of hemostasis at a quicker pace. Using sutures results in a quicker recovery period because the surface area of the wound has been decreased.

- **Physical agents (gelatin sponge)**- Gelatin sponges have been indicated as great hemostatic devices. Once applied to a bleeding area, a gelatin sponge quickly stops or reduces the amount of bleeding present. These physical agents are mostly used in surgical settings as well as after surgery treatments. These sponges absorb blood,.

8.7 RESPIRATORY PIGMENTS:

A respiratory pigment is a molecule that increases the oxygen carrying capacity of the blood or in other words they are the substances which combine with oxygen reversibly and acts as the carrier or storage units for it. These pigments are generally carried by the blood. Their oxygen affinity determines the efficiency of these pigments and their use as the oxygen carrier. The oxygen affinity of these pigments is indicated by their *p*50 or partial pressure of oxygen at which they are half saturated with oxygen. The value of p50 is inversely proportional to the oxygen affinity of the pigment. Some of the common respiratory pigments are as follows:

i Hemoglobin:

Hemoglobin is the iron containing oxygen transport metalloprotein found in red blood cells (RBC) of all vertebrates as well as tissues of some invertebrates. With some very rare exception of leptocephalus (larvae of eel), all classes of vertebrates possess hemoglobin in their RBCs and their muscle contain myoglobin.

Structure: Hemoglobin is a conjugated protein made up of four subunits. Each subunit is made up of a protein part called globin and an iron containing protoporphyrin (heme) ring. Heme attaches to the polypeptide chain by a nitrogen atom to form one subunit of hemoglobin. Each of the four heme portions of the hemoglobin molecule contains an atom of Iron (Fe) which binds oxygen. The iron in the heme is in ferrous state (Fe2+). The protein moiety or globin varies considerably in size, amino acid composition, solubility and other physical properties from animal to animal. The iron content of mammalian hemoglobin is 0.336 % and the heme content is 4 %. In each unit of heme, the iron atom is joined by four of its co-ordination bonds to the four nitrogen atoms of protoporphyrin and one of the remaining six co-ordination bond is joined to the molecule of globin.

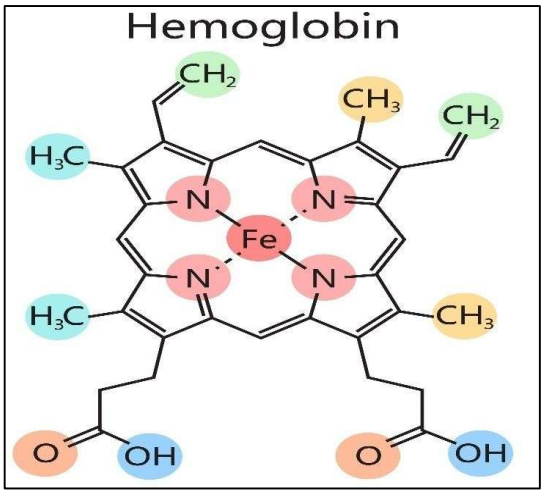

Figure 3: Structure of protoporphyrin (Heme) ring

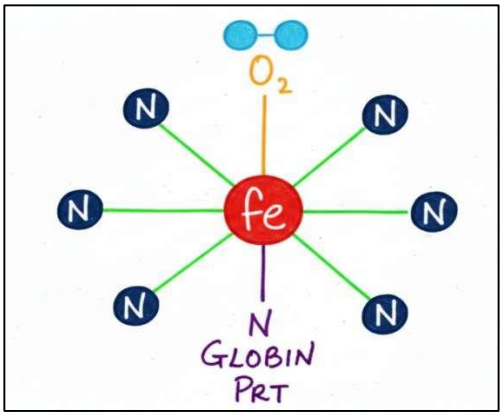

Figure 4: **schematic coordination of the ferrous ion.**

Hemoglobin devoid of oxygen is called deoxyhemoglobin. Adult blood contains two types of hemoglobin: 98 % hemoglobin A (HbA) and 20 % hemoglobin A2 (HbA2). HbA contains two α chains and two β chains (α2β2) and HbA2 contain a pair of α chain and a pair of δ chains (α2δ2). Each heme can combine with two atoms of oxygen to form oxyhemoglobin (HbO2). Oxygen carrying capacity of the hemoglobin is the function of the iron atom. This co-ordination of heme iron with oxygen is reversible and it depends on the partial pressure of oxygen (PO2). Oxyhemoglobin is formed when PO2 is high i.e. oxygen is present in excess and deoxyhemoglobin is formed when PO2 is low i.e. oxygen concentration is low. Since each hemoglobin molecule has four heme rings, it carries a total of 4 oxygen molecules. Hemoglobin is beautifully adapted to the task of oxygen carrier as it can pick up or release oxygen readily in response to the change in partial pressure of the oxygen in lungs, blood or tissues. Also, its affinity for oxygen changes with variations in Ph and CO2 concentrations. This is called Bohr Effect. Hemoglobin can also combine with CO2 or other gases. It can carry a small amount of the CO2 out of tissues. Hemoglobin can combine with 4 molecules of carbon monoxide (CO) and form carboxyhemoglobin. The affinity of hemoglobin is about 200 times high for CO than oxygen. It can therefore cause CO poisoning if inhaled in even in small quantities and result in death by anorexia (lack of oxygen).

ii Myoglobin:

Myoglobin (Mb) is found in vertebrate muscles and is an oxygen storage protein. It stores oxygen in resting skeletal muscles as oxymyoglobin (MbO2). Myoglobin protein sequence has low similarity with the hemoglobin monomeric chains but it shares a striking similarity with the quaternary structure of Hemoglobin with the same kind of helical and other secondary structures. In muscles, myoglobin binds O2 reversibly. It does not exhibit Bohr effect and its oxygen dissociation curve is hyperbolic. During muscle contraction when the demand for the oxygen is highest, oxygen dissociates from the Myoglobin and is available for oxidation. Humans have a large quantity of myoglobin in cardiac muscles only. In birds the flight muscles are rich in myoglobin. Even at low partial pressure of oxygen, when the Hb is only partially saturated, myoglobin can be fully saturated. Myoglobin can even accept O2 from hemoglobin and store it in muscle cells for later release.

iii Hemerythrin:

It is another respiratory pigment which is present in the blood of all sipunculid worms, a few polychaeta forms and the branchiopod *Lingula*. It is a rare, reddish violet, iron containing pigment. *Sipunculus* hemerythrin has a molecular weight of 105 kDa and contains 16 Fe2+ atoms. It binds one molecule of oxygen per iron atom.

iv Hemocyanin:

Hemocyanin or the blue pigment found mostly in mollusks and arthropods is a copper containing pigment. Squid hemocyanin is a decamer and each monomer contains two Cu+ atoms and they bind one O2 molecule per two Cu+ ions. Hemocyanin is a respiratory pigment present in various invertebrates, such as arthropods and mollusks. Unlike hemoglobin, hemocyanin circulates freely in the hemolymph rather than being confined within cells. This pigment contains copper atoms instead of iron, imparting a blue or green coloration. Hemocyanin binds oxygen directly to these copper atoms, which allows it to function effectively in low-oxygen conditions. Its efficiency in oxygen transport at low partial pressures makes hemocyanin particularly well-suited for organisms residing in cold environments, where oxygen availability may be limited.

v Erythrocruorin:

It is a large molecule consisting of multiple subunits and is found in many annelid worms and mollusks. *Limnodrillus* erythrocruorin consists of 108 subunits each having on heme group. Erythrocruorin is a respiratory pigment closely related to hemoglobin, found in some invertebrates. Like hemoglobin, it contains iron and can be responsible for a bright red coloration when oxygenated. It typically exists in a solution in the blood and exhibits a structure similar to that of hemoglobin, allowing it to perform similar functions in oxygen transport.

vi Chlorocruorin:

It is green in color and is found in certain annelids like *Spirographis*. It is present in coelomic cavity and serves to store rather than transport oxygen. Chlorocruorin is another respiratory pigment found in specific annelids, particularly within polychaetae worms. It contains iron atoms and is characterized by its green-colored protein, enabling effective oxygen binding and transport. Chlorocruorin demonstrates a high affinity for oxygen, making it advantageous for organisms living in low-oxygen environments, such as muddy sediments or deep-sea habitats. The color of chlorocruorin can vary depending on its concentration; it appears green when diluted and transitions to a reddish blue when concentrated.

vii Metalloprotein Composition:

Hemoglobin, erythrocruorin, and chlorocruorin contain iron, while hemocyanin utilizes copper for oxygen binding.

Location: Hemoglobin and erythrocruorin are intracellular, while hemocyanin and hemerythrin are extracellular.

Source Organism: Hemoglobin is present in almost all vertebrates, while hemocyanin is primarily found in arthropods and mollusks. Chlorocruorin is found in four families of marine polychaetas, and hemerythrin is present in sipunculids, priapulids, some brachiopods, and a single genus of annelids.

Coloration: Oxygenated hemoglobin appears bright red, while deoxygenated hemoglobin is crimson. Hemocyanin turns blue upon oxygenation and is colorless when deoxygenated. Erythrocruorin exhibits bright red oxygenated color and dark red when deoxygenated, while chlorocruorin is green when diluted and turns brown-red when concentrated.

8.8 EVOLUTIONARY SIGNIFICANCE OF RESPIRATORY PIGMENTS:

The evolution of respiratory pigments has played a crucial role in the adaptation of organisms to different environments and lifestyles. These pigments have undergone remarkable diversification and optimization to meet the oxygen demands of various organisms.

1. Adaptation to Low Oxygen Environments:
Organisms inhabiting low-oxygen environments, such as high-altitude regions or deep-sea ecosystems, have developed respiratory pigments with high oxygen affinity. This adaptation allows for efficient oxygen extraction from the environment and transport to tissues. Examples include the high-affinity haemoglobin variants found in high-altitude-dwelling mammals or the specialized haemoglobin of deep-sea fish.

2. Temperature Adaptation:
Respiratory pigments also exhibit adaptations to temperature variations. Some ectothermic organisms, such as reptiles and fish, have haemoglobins that display increased oxygen affinity at lower temperatures, ensuring efficient oxygen transport even in colder environments. This adaptation is essential for maintaining adequate oxygen supply during periods of reduced metabolic activity.

3. Evolutionary Transitions:
The evolution of respiratory pigments has witnessed transitions between different types of pigments across phylogenetic groups. For instance, some invertebrates, like certain crustaceans, have transitioned from using hemocyanin to haemoglobin-like molecules, possibly due to the advantages conferred by higher oxygen-carrying capacity.

4. Functional Convergence:
In certain cases, unrelated taxa have independently evolved similar respiratory pigments due to functional convergence. For example, the haemoglobins found in mammals, birds, and some reptiles are structurally similar and share oxygen-binding characteristics, despite arising through independent evolutionary lineages. This convergence highlights the importance of oxygen transport and the selective pressures that have shaped these adaptations.

8.9 SUMMARY:

Blood, a vital connective tissue, ensures transport of gases, nutrients, hormones, and defense components throughout the body. Its composition includes plasma, red blood cells, white blood cells, and platelets, each performing specialized roles such as oxygen transport, immunity, and clotting. The process of hemopoiesis in bone marrow maintains continuous production of blood cells, while hemostasis prevents excessive blood loss through vascular spasm, platelet plug formation, and coagulation. Respiratory pigments like hemoglobin, myoglobin, hemocyanin, erythrocruorin, and chlorocruorin enhance oxygen transport efficiency and have evolved to suit diverse environmental and physiological needs. Their variations across species highlight evolutionary adaptations to oxygen availability, temperature, and ecological niches.

8.10 TECHINICAL TERMS:

Erythropoiesis, Diapedesis, Polycythaemia, Bilirubin, Leucocytosis, Thrombopoietin, Oxyhaemoglobin, Myoglobin, Hemocyanin, Chlorocruorin

8.11 SELF-ASSESSMENT QUESTIONS:

1. What is the role of erythropoietin in red blood cell production?
2. Differentiate between granular and agranular leucocytes with examples.
3. Explain the steps involved in haemostasis.
4. How do respiratory pigments differ in vertebrates and invertebrates?

5. Why is the evolutionary adaptation of respiratory pigments significant in low-oxygen environments?

8.12 SUGGESTED READINGS:

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- **Prof. P.V. KRISHNA**

LESSON - 9

CIRCULATORY SYSTEM IN ANIMALS

OBJECTIVES:

1. To understand the structure, types, and evolution of circulatory systems in animals.
2. To study the anatomy and physiology of the human circulatory system and its components.
3. To analyse the mechanisms of blood flow, regulation, and adaptations across species.

STRUCTURE:

9.1 INTRODUCTION:

The efficient system of blood circulation is responsible for the maintenance of homeostatic mechanisms in the body. It is essential that the volume and the composition of the intracellular and extracellular fluids are maintained constant since the proper fluid balance would help the animal maintain its steady state. The volume of water and the concentration of the electrolytes are regulated through the circulatory.

9.2 CARDIOVASCULAR PHYSIOLOGY

All the animals, above the level of Helminthes have been gifted with a circulatory system which besides performing subsidiary function s like thermoregulation in higher forms .it is primarily concerned with the translocation of various substances like oxygen, carbohydrates, amino acids, fats, hormones, and vitamins to different tissues and even individual cells of the body and with the removal of metabolic waste from different parts of the body.

9.3 COMPONENTS OF CIRCULATORY SYSTEM:

The blood circulation has two purposes: it serves to supply nutrients and oxygen to the tissues, and removes wastes like carbon dioxide and others from the tissues. The essential components of the circulatory system are the heart, arteries, veins and the capillaries. Some information about their anatomy would be useful for the purpose of a better understanding of the circulatory process.

Arteries: The arteries are thick-walled and muscular vessels that carry the blood away from the heart. Structurally arteries are made up of three layers: intima, media and externa. They may be large, medium and small. The medium sized arteries contain well developed musculature and are further divisible into small arteries or arterioles. The walls of the arterioles also possess muscle layers which cause these vessels to constrict or dilate. This property of Vasoconstriction of arterioles is responsible for increasing the blood pressure to enable it to flow with a greater velocity. Arterioles are supplied with neurons which respond to sympathetic stimulation. In response to sympathetic stimulation chemical agents like norepinephrine are secreted with produce vasoconstriction. Other chemical agents like epinephrine, serotonin and angiotensin also produce the same effect. If the vasoconstrictor neuron is severed, the arteriole dilates. Vasodilation can also be caused by the action of chemicals like acetylcholine, bradykinin, histamine, etc.

Veins: Veins are thin walled with fewer elastic fibers. The three layers present in the arteries are also present in the veins, but they are much thinner. Small veins are called venules. T eh larger vins of the abdomen and lower limbs possess valves which open in the direction in which the blood is flowing, i.e. towards the heart.

Capillaries: Capillaries are very fine blood vessels which are composed of a thin wall made up of a single layer of flat endothelial cells. The cells have a basement membrane which is continuous. Capillary wall is so thin that it allows transfer of gases or substances through it. Two types of capillaries can be distinguished:

- True capillaries.
- Sinusoidal capillaries.

True capillaries are present in most tissues and have a lumen diameter ranging from 4 to 8 Ï. Sinusoidal capillaries are channels with irregular diameter ranging form 5 to 30 Ï. Such capillaries are generally found in blood forming tissues like thymus, lymph nodes, bone marrow, liver, spleen and adrenal cortex. The capillary walls are permeable, but the permeability is not the same throughout the body. The capillaries of the liver are most permeable. In conditions of trauma permeability increase to the extent that even cellular elements can also pass through it. Although the capillary walls are very thin, yet they are capable of withstanding pressures as high as 90 m Hg or even higher.

9.4 HEART:

All vertebrates are gifted with a pulsating heart which receives blood from different parts of the body by means of veins and pumps in into various parts of the body through arteries arising from the anterior end. The wall of the heart is composed of three layers namely, the endocardium, myocardium, and the epicardium. The endocardium consists of connective tissues lined with a thin layer of endothelium. The myocardium is the principal muscle layer

which is thin in the auricles and thick in the ventricle. The epicardium is made up of epithelial cells and connective tissue and remains lodged in a special coelomic chamber known as pericardial cavity. The heart is ventrally placed below the alimentary canal.The circulatory system is essential for transporting nutrients, oxygen, and waste products throughout the body. Different organisms have developed unique circulatory patterns to meet their metabolic needs. Vertebrates, for instance, have evolved hearts with varying chambers, from the two-chambered heart in fish to the four-chambered heart in birds and mammals. The human circulatory system, comprising the heart, blood vessels, and blood, ensures efficient circulation and overall bodily function.

9.5 HEART OF INVERTEBRATES:

Acoelomates do not possess blood vessels and hearts (exception Nemertines). Coelomic invertebrates have hearts which can be classified into three types:

1. Tubular hearts.
2. Pulsating hearts.
3. Ampullar hearts.

1. Tubular hearts

In arthropods, the systemic hearts consist of long, tubular contractile structures. The heart may be suspended in a large pericardial chamber by means of elastic ligaments or it may be free without any support. The heart is bathed in the surrounding hemolymph. In most arthropods (insects), the heart is held in position by special alary muscles and receives blood by means of lateral paired openings called ostia. The ostia are guarded by valves. These ostial openings close when the alary muscles contract, and the blood is pushed through the artery. Consequent upon the contractions of the heart, a negative pressure is created within the pericardial chamber thereby forcing the fresh supply of blood from the haemocoel into the heart thoughted ostia. The entire heart may show wave of contraction. In case of crustaceans, the blood passes from the heart into the arteries and arterioles and from there to the gills. The veins then bring back the blood to the pericardial chamber. The heart of tunicates is a convoluted tube situated in the pericardium. The heart pumps blood in one direction for some time and then the direction of the flow is reversed.

2. Pulsating hearts

Pulsating hearts are characteristic of annelids which have a closed circulatory system. These pulsatile hearts contract in peristaltic fashion. In the earthworm, rhythmic pulsatile movements are observed in the dorsal tubular vessel from the posterior end to the anterior end. The lateral vessels, commonly known as hearts, also beat rhythmically independent of each other. In Hirudin aria, there are two lateral channels which show alternate contractions.

3. Ampullar hearts

In certain animals ampullar hearts or accessory booster hearts are present which function as booster pumps to force the blood with increased pressure. Such accessory hearts are commonly found in cephalopods and insects. In cephalopods, these hearts help in forcing the blood into small peripheral vessels. In insects they are situated at the base of the antennae. Wings and legs. In aphids. Booster hearts force the extracellular fluids into the legs.

9.6 HEART OF VERTEBRATES:

The hearts of vertebrates are known as chambered hearts. Chambered hearts are also found in molluscs where one or two auricles and one ventricle are present. In the vertebrate series, fishes

have two chambers in the heart, the auricle and the ventricle. In addition, two antechambers, the sinus venosus and the conus arteriosus are also present. The sinus venosus opens into the auricle and the conus arteriosus springs from the ventride. The venous blood entering the sinus venosus is brought into the auricle from where the blood comes into the ventricle which distributes arterial blood through the conus arteriosus. The two chambered hart attained more specialization in its structure with the evolution of land vertebrates. In reptiles for the first time the ventricle became incompletely divided by an incomplete ventricular septum. This ventricular septum became complete in birds and mammals. Birds and mammals have four chambered hearts, having two auricles and two ventricles. These hearts are highly specialized in their structure and function.

9.7 STRUCTURE OF MAMMALIAN HEART:

The heart of mammals has attained a high functional efficiency. In order to understand the physiology, we can examine the human heart. In man the heart is situated in the thoracic cavity slightly displaced towards the left side. T eh wall of the heart is composed of three layers, namely, the endocardium, myocardium and the epicardium. The endocardium consists of connective tissues lined with a thin layer of endothelium. The myocardium is the principal muscle layer which is thin in the auricles and thick in the ventricle. The epicardium is make up of epithelial cells and connective tissue. The heart is enclosed in a pericardial membrane. The space between the pericardium and epi µ 119euroco is known as pericardial space which contains a fluid. The pericardial fluid lubricates the heart.

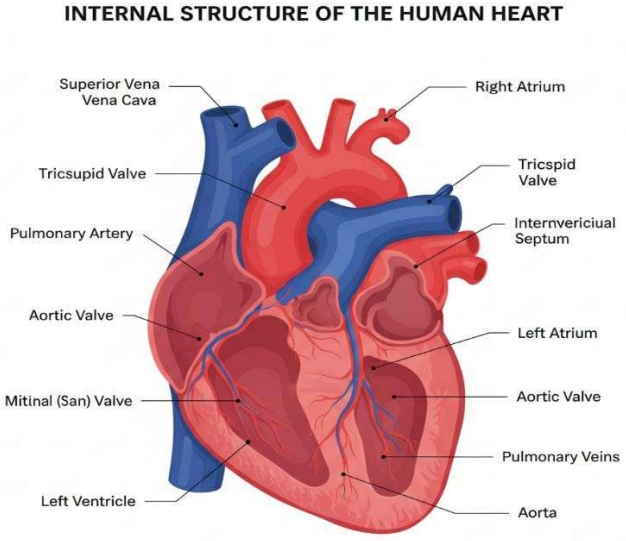

Figure 1: internal structure of heart

9.8 CIRCULATORY PARRERNS IN DIFFERENT GROUPS OF ORGANISMS:

Transport Mechanism in Organisms: Different groups of organisms have evolved diverse methods of transport. These methods ensure the delivery of nutrients, oxygen, and other essential substances to their cells. They also facilitate the removal of waste and harmful substances produced by various metabolic processes.

Types of Circulatory Patterns: Two types of circulatory patterns exist:

- Open Circulatory System: Found in arthropods and molluscs, where blood pumped by the

heart flows through large vessels into open spaces or body cavities known as sinuses.

- Closed Circulatory System: Present annelids and chordates, where the heart pumps blood through a closed network of blood vessels.

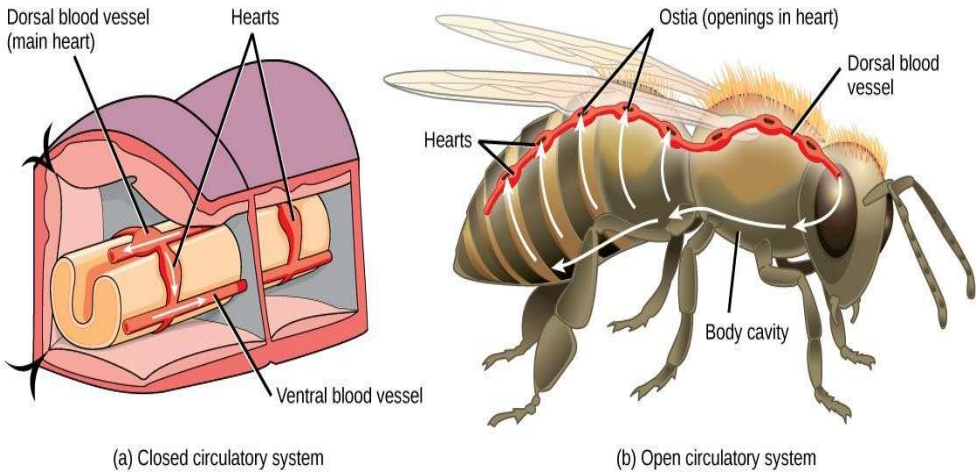

Figure 2: **a.Closed circulatory system b. open circulatory system**

9.8.1 Circulatory Systems in Vertebrates:

All vertebrates feature a muscular, chambered heart. A brief outline of circulatory systems in different groups of vertebrates:

- **Fishes** have a 2-chambered heart with an atrium and a ventricle. In fish, the heart pumps out deoxygenated blood, which is oxygenated by the gills and supplied to the body parts, from where deoxygenated blood is returned to the heart (single circulation).
- **Amphibians and reptiles** (except crocodiles) have a 3-chambered heart with two atria and a single ventricle. In amphibians and reptiles, the left atrium receives oxygenated blood from the gills/lungs/skin and the right atrium gets the deoxygenated blood from other body parts. However, they get mixed up in the single ventricle, which pumps out mixed blood (incomplete double circulation). Crocodiles,
- **birds and mammals** possess a 4-chambered heart with two atria and two ventricles. In birds and mammals, oxygenated and deoxygenated blood received by the left and right atria, respectively, passes on to the ventricles of the same sides. The ventricles pump it out without any mixing up, i.e., two separate circulatory pathways are present in these organisms; hence, these animals have double circulation.

9.9 COMPONENTS OF HUMAN CIRCULATORY SYSTEM:

It (also called the blood vascular system) consists of muscular chambered heart, a network of closed branching blood vessels and blood, the fluid which is circulated. Heart: It is situated in the thoracic cavity, in between the two lungs, slightly tilted to the left and is the size of a clenched fist.

Heart Chambers:

Our heart has four chambers, two relatively small upper chambers called atria and two larger lower chambers called ventricles (separated from each other by walls called septum).

1. **Septal Openings**:
However, each of these septa is provided with an opening (guarded by valves– tricuspid or bicuspid) through which the two chambers on the same side are connected. Valves: The valves in the heart allow only unidirectional flow of blood, preventing any backward flow.

2. **Cardiac Cycle**:
The sequential pumping of the heart, which is cyclically repeated, is called the cardiac cycle, and it consists of the systole (contraction) and diastole (expansion) of both the atria and ventricles. Heart Rate and Stroke Volume: The heart beats 72 times per minute (beats per minute/bpm), and during a cardiac cycle, each ventricle pumps out approximately 70 ml of blood, which is called the stroke volume. Cardiac Output: The stroke volume multiplied by the heart rate (bpm) gives the cardiac output. The body has the ability to alter the stroke volume as well as the heart rate and, thereby, the cardiac output. Example: the cardiac output of an athlete will be much higher than that of an ordinary man.

3. **Blood Vessels:**
The blood flows strictly by a fixed route through the Blood Vessels- the arteries, veins and capillaries. Anatomy of arteries and veins each artery and vein consists of three layers:

- **Tunica Intima:** an inner lining of squamous endothelium
- **Tunica Media**: a middle layer of smooth muscle and elastic fibers, the,
- **Tunica Externa**: external layer of fibrous connective tissue with collagen fibers, the tunica externa.

The tunica media is comparatively thin in the veins. Oxygenation Status of Arteries and Veins: All arteries except the pulmonary artery (which carries blood from the heart to lungs) carry oxygen-rich blood, and all veins except the pulmonary vein (which carries blood from the lungs to heart) carry deoxygenated blood. Capillaries: These are the smallest and most numerous of the blood vessels. These form the connection between arteries and veins. The primary function of capillaries is the exchange of materials between the blood and tissue cells.

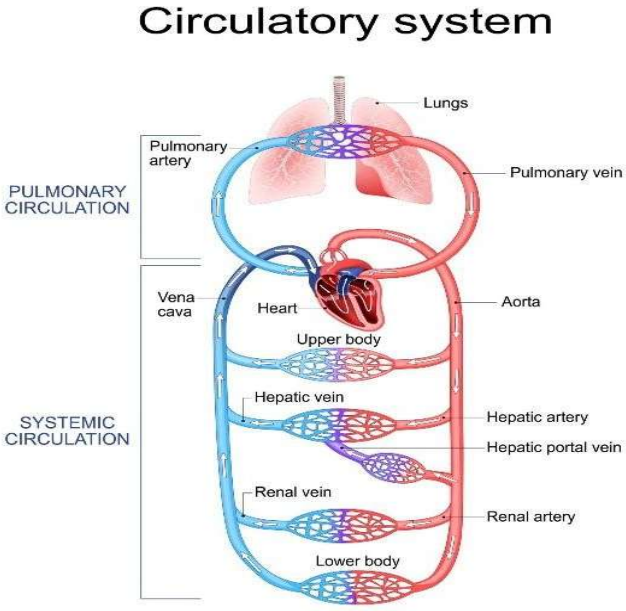

Figure 3: Course of circulation

The circulatory system is the primary method used to transport nutrients and gases through the body. Simple diffusion allows some water, nutrient, waste, and gas exchange in animals that are only a few cell layers thick; however, bulk flow is the only method by which the entire body of larger, more complex organisms is accessed the circulatory system is effectively a network of cylindrical vessels: the arteries, veins, and capillaries that emanate from a pump, the heart. In all vertebrate organisms, as well as some invertebrates, this is a closed-loop system, in which the blood is not free in a cavity. In a closed circulatory system, blood is contained inside blood vessels and circulates unidirectionally from the heart around the systemic circulatory route, then returns to the heart again.

As opposed to a closed system, arthropods-including insects, crustaceans, and most mollusks-have an 'open' circulatory system. In an open circulatory system, the blood is not enclosed in blood vessels but is pumped into an open cavity called a hemocoel and is called hemolymph because the blood mixes with the interstitial fluid.

As the heart beats and the animal moves, the hemolymph circulates around the organs within the body cavity and then reenters the hearts through openings called ostia. This movement allows for nutrient exchange, and in some organisms lacking direct gas exchange sites, a basic mechanism to transport gasses beyond the exchange site. Because the gas exchange in many open-circulatory systems tends to be relatively low for metabolically-active organs and tissues, a tradeoff exists between this system and the much more energy-consuming, harder-to-maintain closed system.

The circulatory system varies from simple systems in invertebrates to more complex systems in vertebrates. The simplest animals, such as the sponges (Porifera) and rotifers (Rotifera), do not need a circulatory system because diffusion allows adequate exchange of water, nutrients, and waste, as well as dissolved gases. Organisms that are more complex but still only have two layers of cells in their body plan, such as jellies (Cnidaria) and comb jellies (Ctenophora) also use diffusion through their epidermis and internally through the gastrovascular compartment. Both their internal and external tissues are bathed in an aqueous environment and exchange fluids by diffusion on both sides. Exchange of fluids is assisted by the pulsing of the jellyfish body.

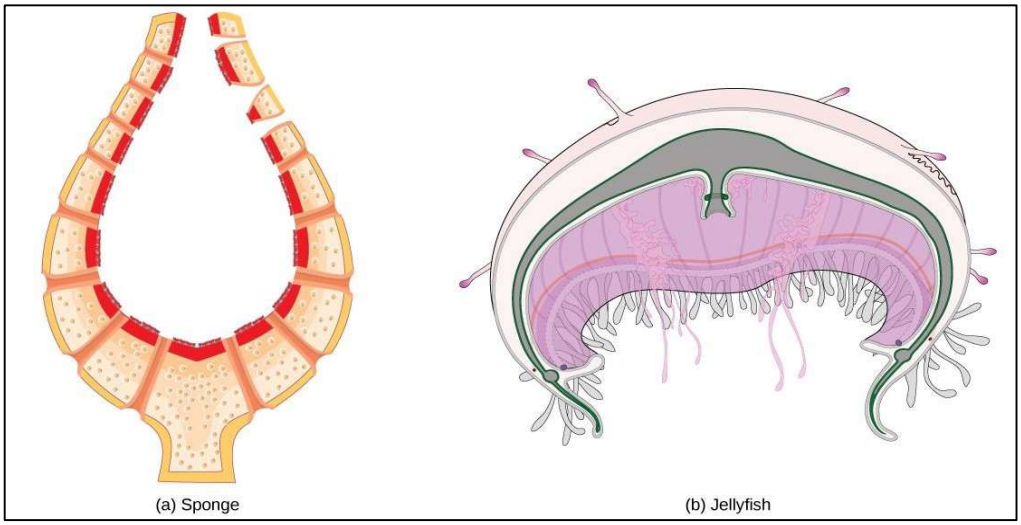

Figure 4: Simple animals consisting of a single cell layer such as the (a) sponge or only a few cell layers such as the (b) jellyfish do not have a circulatory system. Instead, gases, nutrients, and wastes are exchanged by diffusion.

For more complex organisms, diffusion is not efficient for cycling gases, nutrients, and waste effectively through the body; therefore, more complex circulatory systems evolved. In an open system, an elongated beating heart pushes the hemolymph through the body and muscle contractions help to move fluids. The larger more complex crustaceans, including lobsters, have developed arterial-like vessels to push blood through their bodies, and the most active mollusks, such as squids, have evolved a closed circulatory system and are able to move rapidly to catch prey. Closed circulatory systems are a characteristic of vertebrates; however, there are significant differences in the structure of the heart and the circulation of blood between the different vertebrate groups due to adaptation during evolution and associated differences in anatomy. The figure below illustrates the basic circulatory systems of some vertebrates: fish, amphibians, reptiles, and mammals.

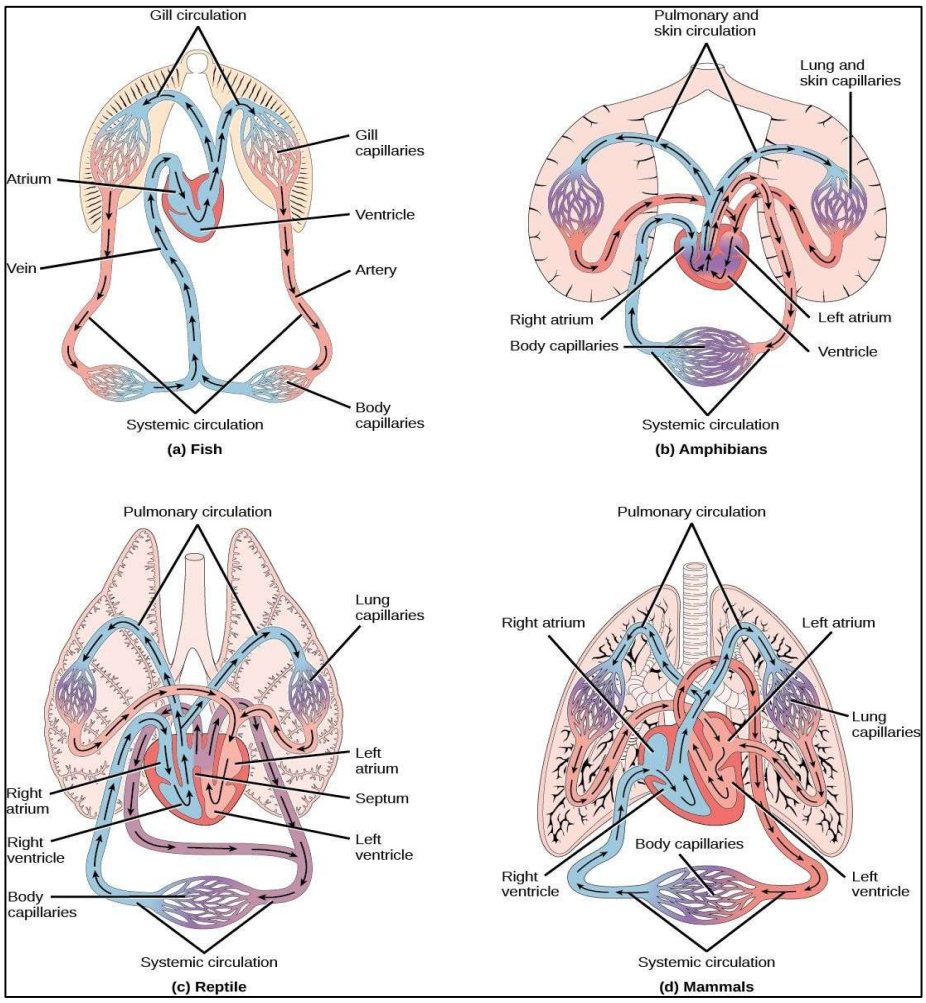

Figure 5: **(a) Fish have the simplest circulatory systems of the vertebrates. (b) Amphibians have two circulatory routes (c) Reptiles also have two circulatory routes. (d) Mammals and birds have the most efficient heart with four chambers that completely separate the oxygenated and deoxygenated blood.**

Fish have a single circuit for blood flow and a two-chambered heart that has only a single atrium and a single ventricle. The atrium collects blood that has returned from the body and the ventricle pumps the blood to the gills where gas exchange occurs and the blood is re-oxygenated; this is called gill circulation. The blood then continues through the rest of the body before arriving back at the atrium; this is called systemic circulation.

This unidirectional flow of blood produces a gradient of oxygenated to deoxygenated blood around the first systemic circuit. The result is a limit in the amount of oxygen that can reach some of the organs and tissues of the body, reducing the overall metabolic capacity of fish. In amphibians, reptiles, birds, and mammals, blood flow is directed in two circuits: one through the lungs and back to the heart, which is called pulmonary circulation, and the other throughout the rest of the body and its organs including the brain (systemic circulation). In amphibians, gas exchange also occurs through the skin during pulmonary circulation and is referred to as pulmocutaneous circulation.

Amphibians have a three-chambered heart that has two atria and one ventricle rather than the two-chambered heart of fish. The two atria (superior heart chambers) receive blood from the two different circuits (the lungs and the systems), and then there is some mixing of the blood in the heart's ventricle (inferior heart chamber), which reduces the efficiency of oxygenation. The advantage to this arrangement is that high pressure in the vessels pushes blood to the lungs and body. The mixing is mitigated by a ridge within the ventricle that diverts oxygen-rich blood through the systemic circulatory system and deoxygenated blood to the pulmocutaneous circuit. For this reason, amphibians are often described as having double circulation.

Most reptiles also have a three-chambered heart similar to the amphibian heart that directs blood to the pulmonary and systemic circuits. However, the ventricle is divided more effectively by a partial septum, which results in less mixing of oxygenated and deoxygenated blood. Some reptiles (alligators and crocodiles) are the most "primitive" animals to exhibit a four-chambered heart. Crocodilians have a unique circulatory mechanism where the heart shunts blood from the lungs toward the stomach and other organs during long periods of submergence, for instance, while the animal waits for prey or stays underwater waiting for prey to rot. One adaptation includes two main arteries that leave the same part of the heart: one takes blood to the lungs and the other provides an alternate route to the stomach and other parts of the body. Two other adaptations include a hole in the heart between the two ventricles, called the foramen of Panizza, which allows blood to move from one side of the heart to the other, and specialized connective tissue that slows the blood flow to the lungs. In mammals and birds, the heart is divided completely into four chambers: two atria and two ventricles. Oxygenated blood is fully separated from deoxygenated blood, which improves the efficiency of double circulation and is probably required for supporting the warm-blooded lifestyle of mammals and birds. The four-chambered heart of birds and mammals evolved independently from a three-chambered heart. The independent evolution of the same or a similar biological trait is referred to as convergent evolution.

9.10 SUMMARY:

Animals have a transport system to distribute substances uniformly in cytoplasm and throughout body called circulatory system. It is of two types- intracellular and extracellular circulation. Streaming movement of cytoplasm in unicellular animals (protozoans) is known as cyclosis. It is an example of intracellular circulation. Extracellular circulation includes canal system of sponges, gastro-vascular system in cnidarians, parenchymal circulation of Helminthes, open blood vascular systemin arthropods, molluscs except cephalopods and closed blood vascular system of annelids, cephalopods and vertebrates. Lymphatic system is considered as second circulatory system of vertebrates. Blood vascular system of higher animals includes heart, blood and blood vessels. Heart is a hollow, muscular, pulsatile organ that receives and pumps the blood through the circulatory system. Protochordates like *Herdmania* and *Amphioxus* have single chambered heart. Heart has two chambers in fishes,

three chambers in amphibians and four chambers in birds and mammals. Cockroach has 13 chambered tubular heart. On the basis of origin of cardiac impulses for heartbeat, hearts are of two types- neurogenic hearts and myogenic hearts. Lower animals like annelids, arthropods and molluscs have neurogenic hearts where cardiac impulses for heart beat are generated by neural tissues. Higher animals like vertebrates have myogenic hearts because cardiac impulses for heart beat are generated by the muscles themselves. Cyclic events during heart beat constitute a cardiac cycle. In human being the duration as a cardiac cycle is 0.8 seconds. It completes in three events- auricular systole (0.1 sec), ventricular systole (0.3 sec) and joint diastole (0.4 sec). Cardiac Output (CO) is the volume of blood pumped from heart (left ventricle) into the systemic aorta in one minute. It is calculated as the product of stroke volume (amount of blood pumped by left ventricle each time it contracts) and rate of heart beat. In human beings the value of CO is about 5 liters. Cardiac Index (CI) is the cardiac output per square meter of body surface area per minute. Itis about 3 liters/min/square meter for a normal healthy person. Cardiac Reserve (CR) means maximum amount of blood that can be pumped by left ventricle under the conditions of maximum needs. Cardiac reserve is 25-30 liters which is about 5-6 times of cardiac output.

9.11 TECHINICAL TERMS:

Haemolymph, Sinus venosus, Conus arteriosus, Double circulation, Pulmocutaneous, Cardiac cycle, Stroke volume, Vasoconstriction, Vasodilation, Pericardium

9.12 SELF-ASSESSMENT QUESTIONS:

1. Explain why circulation of fluids is required in organisms?
2. Describe the different types of circulation in animals.
3. Describe the types and number of chambers in hearts.
4. Describe the neurogenic and myogenic hearts.
5. Give a brief account of cardiac cycle, cardiac output, cardiac index.
6. Explain the ECG and its role in diagnosis of cardiac disorders.
7. Understand the hemostasis and mechanism of blood coagulation?
8. Examples of natural and synthetic anticoagulants

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- **Prof. P.V. KRISHNA**

LESSON- 10

THERMOREGULATION IN POIKILOTHERMS & HOMEOTHERMS

OBJECTIVES:

1. To understand the role of temperature in metabolism and survival of organisms.
2. To study mechanisms of thermoregulation in poikilotherms, homeotherms, heterotherms, and adaptations like hibernation, aestivation, and acclimatization.
3. To analyse the biological significance of temperature regulation in maintaining homeostasis and survival in extreme environments.

STRUCTURE:

10.1 INTRODUCTION:

Respiration is essential for the survival of the organisms. It is an involuntary process that is common to almost all the organisms. Although the mode of the respiration can be different for different groups of organisms depending on their habitat and other factors, it remains the central process for sustaining the life. All life forms have optimized their way of respiration and have evolved as such. They have the specialized organs to achieve that. In mammals the inhaled oxygen helps in the oxidation of the digested food products and thus in turn helps in production of energy for the other metabolic processes. It is a coordinated effort of many organ systems of the body which work tirelessly to maintain the required level of the oxygen in the body. Our bodies have been evolved in such a way that they need oxygen (O_2) for cellular oxidation and

in turn release carbon dioxide (CO_2). Any interference in this routine is not good for the body and may prove fatal. Active animal life is limited to a narrow range of temperatures, from a few degrees below the free going point of pure water (O°C) to approximately + 50O+C. We are now concerned with the temperature of the organism itself not the surroundings. In polar regions numerous fish and inverter Brates live in water at. 1.8^0C. At the other extreme, in hot springs a few animals can live at about 50^0C. Some blue green algae live above 70^0C and a few thermophilic bacteria survive above the boiling point of water. Most animals, among them all aquatic invertebrates, have nearly the same temperature as their surroundings. Birds and mammals, in contrast, usually maintain their body temperature nearly constant and independent of the environment. However, some other animals both vertebrates and vertebrates, can at times maintain a substantial difference between their own temperature and that of the surrounds. Each and every activity of living organism is the result of the biochemical processes that take place in their body. All such processes are so much temperature sensitive that even a slight change in temperature alters the rate of these reactions. Therefore, a situation arises a need for maintenance of a specific body temperature for the life of an animal to be carried on in a perfect order. The way in which the organisms maintain their body temperature within a certain limited range is termed as `129euroco regulation

(Poikkilothermic (Greek: Poikilos = changeable)

Homeothermic (Homoios = similar)

Heterotherms
Animals that at times have high and well-regulated body temperature, but at other times are more like cold. Blooded animals, are often called Heterothermic (Greek heteros = different).

Endothermic Animals
These animals are able to maintain a high body temperature by internal heat production.

Ectothermic animals
These animals depend on external heat sources, primarily solar radiation. The choice of terminology is primarily a matter of convenience. The question is not weather a certain terminology is right (or) wrong, but how useful it is for a given purpose.

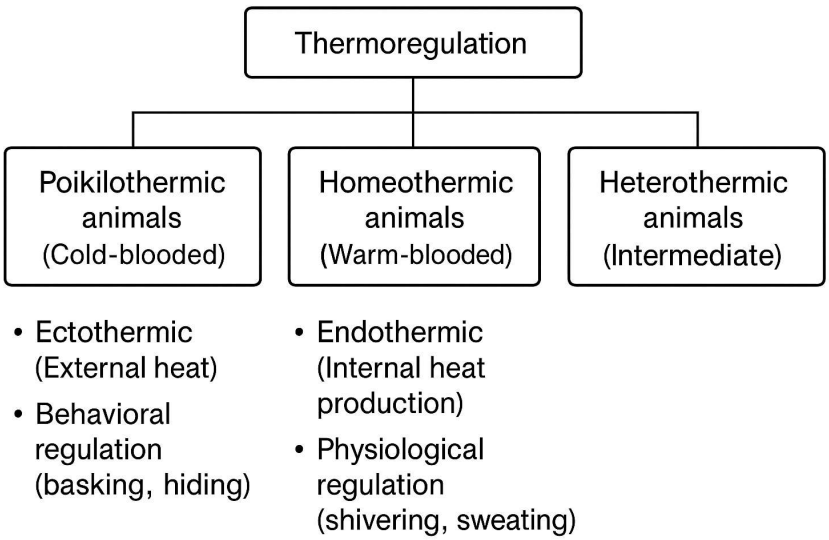

Figure 1: Types of Temperature Regulation in Animals

Thermoregulation is the ability of an organism to maintain its internal temperature within a tolerable range. Based on this, animals are classified into poikilothermic, homeothermic, and heterothermic types depending on how they control their body temperature.

10.2 EFFECTS OF TEMPERATURE ON METABOLISM:

As stated earlier, the physiological processes are highly temperature sensitive. Environmental temperature weathering. (or) falling manipulates, the rate of metabolic processes accordingly. The fact has been studied by various author's van Hoff describes this generalization in the following way. For every few degrees rise in temperature, the rate of bio-chemical reactions becomes almost double. This is known as Q10 law and is expressed as

$$Q10 = \frac{(Kt + 10)}{Kt}$$

Where Kv is the velocity constant at temperature.t. and Kt+10 is the velocity constant at temperature.t. and Kt+10 s the velocity constant at temperature (t+10). In case of poikilothermic animals, with the change in environmental temperature the rates of metabolic processes changes. But when find homeothermic animals the change in external temperature has no (or) very little effect on body temperature which remains almost constant. Also, we see that the poikilotherms having low body temperatures are lower grade of animals as compared to birds and mammals having higher body temperature. Thus, it can be said that higher body temperature favors more complex form of life.

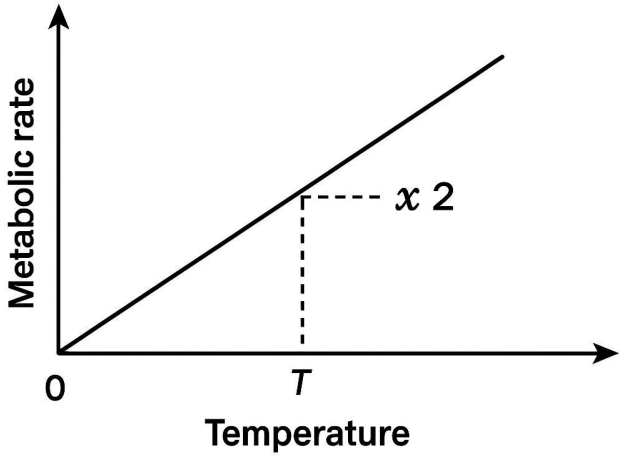

Figure 2: Effect of Temperature on Metabolism (Q_{10} Law)

The Q_{10} coefficient explains that the rate of biochemical reactions approximately doubles with every 10°C rise in temperature, up to a certain limit, beyond which enzymes denature and metabolic rate declines.

a. Effect of low temperature:

Varied kinds of responses are shown by animals to cold conditions. A few of them migrate to avoid the cold conditions, while the others who face such low temperatures either mould themselves accordingly (or) sure to be affected adversely. Lethal temperature for different animals varies according to the degree of exposure to temperature. There are number of theories explaining the cause of death because of cold but there is no general agreement and none of Thero are universally accepted.

b. Effect of high temperature:

The effect of life of animals due to high temperatures are much more severe than those of low temperature. Aquatic animals face less variations in temperature because of high specific heat of water (H2O) and thus their lethal temperatures are lower as compared to those of t and animals. It is evident that the higher temperatures are lowered as compared to those of land animals. After certain limit, if the temperature rises the body activities cease down and the animals finally die. Protoplasm constituting the animals contains proteins and enzymes will coagulate and get denatured at high temperatures. Thus effect the body activities in general and finally result in death. The excess of heat also leads to an increased viscosity of cellular fluid. With the result of which only vacuolation also takes place. This leads to the release of Ca^{++} ions within the cells and the Ca^{++} ions so released have a disruptive influence on the cell by affecting the permeability of plasma membrane. All these factors combinedly (or) alone affect the organisms adversely.

10.3 MECHANISM OF HEAT REGULATION IN POIKILOTHERMS:

The body temperature of poikilotherms changes according to that of the environment which they do not have any control on it. This is so because they lack the temperature regulating mechanisms. Still to a very small degree a regulation of temperature is run in these animals and this is by their behavioral and metabolic activities. Many reptiles (Snakes and Lizards) are often found to bath in sun light. This practice may increase their body temperature by even 20^0C. This is because of absorption of solar radiation and conduction by the substrate. By fluttering of wings also some insects manage to rise the temperature of their flight muscles to the extent to make the flight possible. This method of generating. Metabolic heat is also very efficient as in bees and can raise the temperature by 10-12^0C. Generally, in fish and other aquatic poikilotherms there is no appreciable difference between their temperature and that of the surrounding water. The heat produced as a result of muscular activity during moving is exchanged and equilibrium in temperature with that of surrounding water is maintained. Besides these, these are certain special methods also which are in practice to overcome the handicaps of being poikilotherms such as larvae of butterfly, Venessa group together in cold weather. This group as a whole is able to maintain a temperature of 1.5 to 2.0^0C higher than that of the surrounding air. Workers of Polites (A social wasp) fan with their wings to produce a cooling effect. In extreme hot weather they put water on their combs. Its evaporation produces cooling effects. In this way it is seen that in spite of lack of heat regulatory mechanisms, poikilotherms do have adopted different ways to adapt themselves to the hot (or) cold condition. Poikilotherms use behavioral adaptations like basking, burrowing, or wing vibration to regulate body temperature. These mechanisms are simpler than in homeotherms but vital for survival.

10.3.1 Concept of Poikilothermy and Homeothermy:

A poikilotherm is an animal whose internal temperature varies considerably. Poikilotherms have to survive and adapt to environmental stress. One of the most important stressors is temperature change, which can lead to alterations in membrane lipid order and can cause protein unfolding and denaturation at elevated temperatures. It is the opposite of a homeotherm, an animal which maintains thermal homeostasis. While the term in principle can apply to all organisms, it is generally only applied to animals, and mostly to vertebrates. Usually, the fluctuations are consequence of variation in the ambient environmental temperature. Many terrestrial ectotherms are poikilothermic. However, some ectotherms remain in temperature constant environments to the point that they are actually able to maintain a constant internal temperature (i.e. Are homeothermic). It is this distinction that often makes the term “poikilotherm” more useful than the vernacular “cold-blooded”, which is sometimes used to

refer to ectotherms more generally. Homeothermy is one of the three types of thermoregulations in warm-blooded animal species. Homeothermy's opposite is poikilothermy. A poikilotherm is an organism that does not maintain a fixed internal temperature but "ather fluctuates based on their environment and physical behavior. Homeothermy, homeothermy or homoiotherm is thermoregulation that maintains a stable internal body temperature regardless of external influence. This internal body temperature is often, though not necessarily, higher than the immediate environment (from Greek homoios "similar" and thermē "heat"). Homeothermy is one of the three types of thermoregulations in warm-blooded animal species. Homeothermy's opposite is poikilothermy. A poikilotherm is an organism that does not maintain a fixed internal temperature but rather fluctuates based on their environment and physical behavior. Homeotherms are not necessarily endothermic. Some homeotherms may maintain constant body temperatures through behavioral mechanisms alone, i.e., behavioral thermoregulation. Many reptiles use this strategy. For example, desert lizards are remarkable in that they maintain near constant activity temperatures that are often within a degree or two of their lethal critical temperatures.

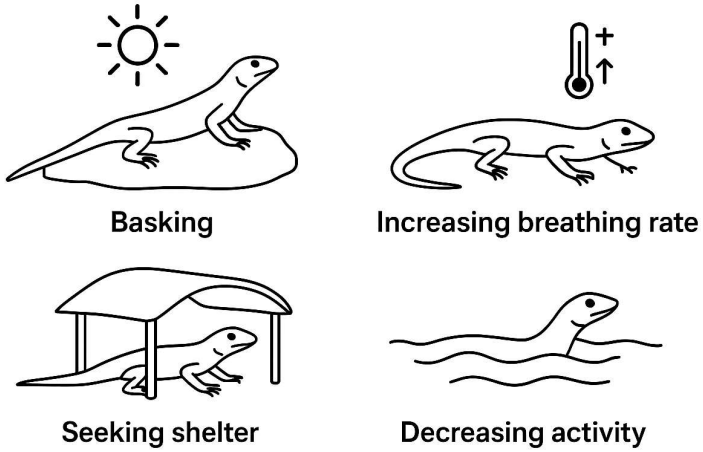

Figure 3: Behavioral and Physiological Adaptations in Poikilotherms

10.3.2 Survival mechanism in Poikilotherms and Homeotherms:

Poikilothermic animals must be able to function over a wider range of temperatures than homeotherms. The speed of most chemical reactions varies with temperature, and in order to function poikilotherms may have four to ten enzyme systems that operate at different temperatures for an important chemical reaction. As a result, poikilotherms often have larger, more complex genomes than homeotherms in the same ecological niche. Frogs are a notable example of this effect, though their complex development is also an important factor in their large genome. Because their metabolism is variable and generally below that of homoeothermic animals, sustained high- energy activities like powered flight in large animals or maintaining a large brain is generally beyond poikilothermic animals. The metabolism of poikilotherms favors strategies such as sit- and-wait hunting over chasing prey for larger animals with high movement cost. As they do not use their metabolisms to heat or cool themselves, total energy requirement over time is low. For the same body weight, poikilotherms need only 5 to 10% of the energy of homeotherms. **Adaptation in Poikilotherms**

- Some adaptations are behavioral. Lizards and snakes bask in the sun in the early morning and late evening, and seek shelter around noon.

The eggs of the yellow-faced bumblebee are unable to regulate heat. A behavioral adaptation to combat this is incubation, where to maintain the internal temperatures of eggs, the queen and

her workers will incubate the brood almost constantly, by warming their abdomens and touching them to the eggs. The bumblebee generates heat by shivering flight muscles even though they are not flying.

- Termite mounds are usually oriented in a north–south direction so that they absorb as much heat as possible around dawn and dusk and minimize heat absorption around noon.
- Tuna is able to warm their entire bodies through a heat exchange mechanism called the rete mirabile, which helps keep heat inside the body, and minimizes the loss of heat through the gills. They also have their swimming muscles near the center of their bodies instead of near the surface, which minimizes heat loss.
- Gigantothermy means growing to large size in order to reduce heat loss, such as in sea turtles and ice-age mega fauna. Body volume increases proportionally faster than does body surface, with increasing size; and less body surface area per unit body volume tends to 133eurocoe heat loss.
- Camels, although they are homeotherms, thermoregulate using a method termed "temperature cycling" to conserve energy. In hot deserts, they allow their body temperature

10.3.3 Cold Resistance and Cold Death, Heat Resistance and Heat Death:

Hypothermia is defined as a body core temperature below 35.0 °C (95.0 °F) in humans. Symptoms depend on the temperature. In mild hypothermia, there is shivering and mental confusion. In moderate hypothermia, shivering stops and confusion increases in severe hypothermia, there may be paradoxical undressing, in which a person removes their clothing, as well as an increased risk of the heart stopping. Hypothermia has two main types of causes. It classically occurs from exposure to extreme cold. It may also occur from any condition that decreases heat production or increases heat loss. Commonly this includes alcohol intoxication but may also include low blood sugar, anorexia, and advanced age. Body temperature is usually maintained near a constant level of 36.5–37.5 °C (97.7–99.5 °F) through thermoregulation. Efforts to increase body temperature involve shivering, increased voluntary activity, and putting on warmer clothing. Hypothermia may be diagnosed based on either a person's symptoms in the presence of risk factors or by measuring a person's core temperature.

The treatment of mild hypothermia involves warm drinks, warm clothing, and physical activity. In those with moderate hypothermia, heating blankets and warmed intravenous fluids are recommended. People with moderate or severe hypothermia should be moved gently. In severe hypothermia, extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass may be useful. In those without a pulse, cardiopulmonary resuscitation (CPR) is indicated along with the above measures.

Rewarming is typically continued until a person's temperature is greater than 32 °C (90 °F). Hypothermia is the cause of at least 1,500 deaths a year in the United States. It is more common in older people and males. One of the lowest documented body temperatures 5 from which someone with accidental hypothermia has survived is 13.0 °C (55.4 °F) in a near drowning of a 7-year-old girl in Sweden. Survival after more than six hours of CPR has been described. In individuals for whom ECMO or bypass is used, survival is around 50%. Deaths due to hypothermia have played an important role in many wars. The term is from Greekhupo, meaning "under", and thermal, meaning "heat".

The opposite of hypothermia is hyperthermia, an increased body temperature due to failed thermoregulation. Hypothermia usually occurs from exposure to low temperatures, and is frequently complicated by alcohol consumption. Any condition that decreases heat production, increases heat loss, or impairs thermoregulation, however, may contribute. Thus, hypothermia

risk factors include: substance use disorders (including alcohol use disorder), homelessness, any condition that affects judgment (such as hypoglycemia), the extremes of age, poor clothing, chronic medical conditions (such as hypothyroidism and sepsis), and living in a cold environment.

Hypothermia occurs frequently in major trauma, and is also observed in severe cases of anorexia nervosa. Hypothermia is also associated with worse outcomes in people with sepsis. While most people with sepsis develop fevers (elevated body temperature), some develop hypothermia. The heat death of the universe (also known as the Big Chill or Big Freeze) is a theory on the ultimate fate of the universe, which suggests the universe would evolve to a state of no thermodynamic free energy and would therefore be unable to sustain processes that increase entropy. Heat death does not imply any particular absolute temperature; it only requires that temperature differences or other processes may no longer be exploited to perform work.

In the language of physics, equilibrium (maximum entropy). This is when the universe reaches thermodynamic If the topology of the universe is open or flat, or if dark energy is a positive cosmological constant (both of which are consistent with current data), the universe will continue expanding forever, and a heat death is expected to occur, with the universe cooling to approach equilibrium at a very low temperature after a very long time period. The hypothesis of heat death stems from the ideas of Lord Kelvin, who in the 1850s took the theory of heat as mechanical energy loss in nature (as embodied in the first two laws of thermodynamics) and extrapolated it to larger processes on a universal scale. 6 The idea of heat death stems from the second law of thermodynamics, of which one version states that entropy tends to increase in an isolated system. From this, the hypothesis implies that if the universe lasts for a sufficient time, it will asymptotically approach a state where all energy is evenly distributed. In other words, according to this hypothesis, there is a tendency in nature to the dissipation (energy transformation) of mechanical energy (motion) into thermal energy; hence, by extrapolation, there exists the view that, in time, the mechanical movement of the universe will run down as work is converted to heat because of the second law.

10.4 THERMO REGULATION IN HOMEOTHERMS:

Homeotherms, in contrast to the poikilotherms definitely maintain a constant body temperature (which may, however, vary to a very small extent within a certain limited range).

The reptiles are the first animals to show some kind of 135euroco-regulatory device though they are at a very primary level. Birds and mammals are the groups that possess highly developed 135euroco-regulatory centers, situated in the hypothalamus and are quite efficient. Homeotherms regulates their body temperature in the following manner. Weather heat produced (or) lost it is adjusted against the environmental temperature by physical processes and the production is regulated by altering the metabolic rates (or) chemical regulation according to the body requirements.

In cold, the heat is lost from the body by radiation. This loss is reduced by lowering the temperature of body surface so as to lower down the heat gradient between the animal and its atmosphere. As a result, the heat given to the surroundings gets lowered and is conserved in the body of the animal itself. The loss of heat occurs by the process of conduction is quite much in homeotherms because of their higher metabolic rate. This may be reduced by the presence an insulating layer between the body surface and the tissue. Blood circulation in skin and the

presence of layer of fact below the skin which works as an insulating layer in combination with the four (or) feathers in homeotherms are the mechanism to combat the loss of heat by both of these probabilities.

The loss of water from the body in the form of sweeting is also a method of controlling temperature. More and more water is given out as the temperature rises. For this these are present the sweat glands in the skin of mammals. However, birds and marsupials etc. lack these glands and therefore have to use some other methods for evaporation of water. Animals inhabiting the polar regions (or) Arctic regions have to live in a permanently cold environment and accordingly possess a very thick coat of fur. Ex: polar bear just opposite to it is the case of desert animals they have to live almost permanently to hot climate. As a consequence, their dermal fat is aggregated to a limited peace in the body, so that the heat loss may take place through rest of the surface easily. Ex: Camel (Dermal fat concentrated in hump region).

- **Heat Gain:** Shivering, hormonal increase (thyroxine, adrenaline), vasoconstriction, insulation (fur, fat).
- **Heat Loss:** Sweating, panting, vasodilation, behavioural cooling (shade seeking)

Homeotherms regulate temperature through both physiological and behavioral mechanisms. The hypothalamus acts as the main control center coordinating heat production and loss.

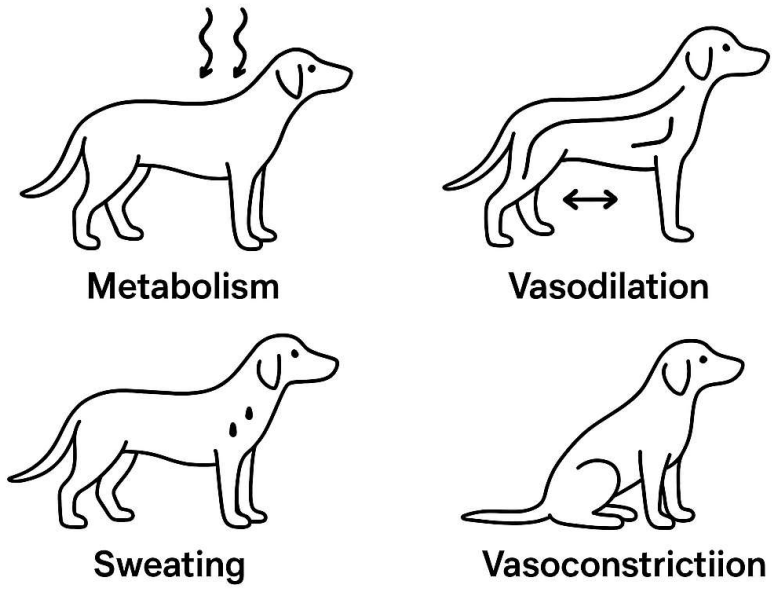

Figure 4: Heat Gain and Heat Loss Mechanisms in Homeotherms

10.5 HOMEOTHERMIC ANIMALS:

In aquatic homeotherms e.g. Seals, whales etc., body is so thickly insulated that the inside temperature is quite different from the body surface temperature, which is only slightly different from the body surface temperature, of surrounding water. The extremities of body are not found to be insulated in this way and to compensate this there is formed a thick network of arteries and veins so that the heat gets transferred to venous blood from arterial one and does not get lost. Homeothermic animals are further assisted by few chemical mechanisms in addition to these physical methods in regulating their body temperature increased activity of thyroid gland and of adrenal cortex increase metabolic activity in a few tissues as muscles, liver etc., leading to the increased heat production.

Control of homeothermy

Control of homeothermy by the central nervous system is mainly carried out by hypothalamus in the brain. There is one center for heat and three centers for cold stimulation. As soon as stimulus for heat is received the immediate response given to the skin is dilation of vessels and wetting. Similarly, the cold centers respond by constriction of blood, vessels, muscular contraction, shivering, retardation and finally cessation of sweating i.e. all the means of retaining head in the body. Pituitary gland controls the process by its hormonal secretary and nervous effects are brought about in addition by the motor neurons of spinal cord in case shivering and autonomic nervous system in sweating and blood vessel constriction

10.6 HETEROTHERMS:

Heterotherms Some low-grade mammals like Echidna, opossum, ornithorhynchus etc., are not very much efficient in maintaining a constant body temperature and large fluctuations of temperature is observed in them. They may have a temperature that is little bit lower than that of the environment. Similarly, a few birds and smaller mammals (endotherms) show diurnal variations in their body temperature and this is correlated with their changed metabolic activity during day and night.

Feature	Poikilotherms	Homeotherms	Heterotherms
Heat source	External	Internal	Mixed
Temperature stability	Variable	Constant	Intermediate
Example	Frog, Fish	Bird, mammal	Bat, Echidna

Table 1: Comparison of Poikilothermy, Homeothermy, and Heterothermy

10.7 HIBERNATION:

In cold climatic conditions the food is scarce and also the food requirement to conduct normal metabolism becomes very high. Both these lead to many deaths because of starvation. To prevent deaths from such situation many animals pass their winter in lethargic states. It is a pattern of adaptive hypothermia found in small animals like rodents, insectivores, bats, etc. Because of low body temperatures the heat losses get minimized and thus only a small amount of food is required by animal. Consequently, the metabolic rate reduces down. Most of the body activities come to a very minimum level however, vital activities such as heart beat, respiration etc., continue. During hibernation the animal lives entirely on reserve foods and because they perform very less activities, they are said to be in a sleeping state. Generally, the hibernating animals undergo this stage till winter lasts but there are a few in which the phenomenon is known to take place at other time also. For example, in bats diurnal changes and they sleep at day times, with a little search for food at night. Thus, they behave as hibernators every day and the long winter sleep found in them may be considered as an extension of this daily hibernation. The arousal from hibernation is a slow and complex process. Slowly and slowly the animal comes to its original metabolic rate and body temperature, the cause of this is thought to be shivering and non-shivering thermogenesis. Hibernation, in homeotherms, there is a mode of temperature regulation against the cold conditions and the animals behave like poikilotherms to some extent. If the environmental temperature goes below zero the animal regains its own body temperature rather than lowering so much and starts all its metabolic reactions. Thus, it is a well-regulated process by virtue of which hibernators tide over the most adverse climate and thus is biologically important as it preserves the species.

10.8 AESTIVATION:

Hibernation is a phenomenon adopted during hypothermic conditions and aestivation just opposite to it, used to tackle hyperthermic conditions. Many small animals as amoeba, mollusc, even fish are found to enter a state of protection. This follows a state of high ambient temperature. Along with this the decreased water quantity around them, induces conditions of hyperthermia. These protective coverings asset to avoid the conditions of tissue dehydration, enzyme loss, turn off metabolic mechanisms etc.

10.9 ACCLIMATIZATION:

It is a phenomenon correlating the climatic and metabolic changes. Earlier this term was used to include the metabolic changes associated with the change in temperature and humidity of climate but now it includes all the metabolic changes, because of changed O2 content of water, salinity food etc., with the climatic changes, the overall behavior of animals also changes. Salinity, humidity and other climatic factors change at every place and no animal has a constant environment. Thus, to define the process, the ecological factors of a particular place are to be taken as constant. Discontinuous distribution of animals all over the world and the migratory changes show that the animals oppose the changed climatic conditions and which do not move, either die (or) have to change themselves accordingly.

10.10 HOMEOSTASIS:

Animals have evolved in such a way that there is more control and less variability in internal environment with respect to changed temperature, concentration of different nutrients, Ph, O2 content, degree of hydration and other factors and this control is termed Homeostasis.

10.11 SUMMARY:

Temperature profoundly influences the physiology, metabolism, and survival of animals. Organisms display different strategies of thermoregulation: poikilotherms depend largely on environmental temperature, while homeotherms maintain nearly constant internal temperatures through efficient regulatory mechanisms involving hypothalamus, hormones, and insul
ation. Heterotherms show intermediate adaptations, sometimes maintaining high regulated temperatures and at other times behaving like poikilotherms. Environmental extremes cause challenges such as hypothermia and hyperthermia, while survival strategies like hibernation, aestivation, and acclimatization help animals cope with seasonal or climatic stresses. Ultimately, thermoregulation and homeostasis are crucial for sustaining life, energy balance, and evolutionary success.

10.12 TECHNICAL TERMS:

Thermoregulation, Poikilothermy, Homeothermy, Heterothermy, Endothermic, Ectothermic, Hypothermia, Hyperthermia, Acclimatization, Homeostasis

10.13 SELF-ASSESSMENT QUESTIONS:

1. Describe the mechanism of thermoregulation in a Homeotherm animal.
2. What is the significance of hibernation and aestivation in animals?
3. Describe adaptations of poikilothermic animals to extreme cold and hot conditions.

4. Write in detail the process of thermoregulation in Homeotherms and poikilotherms?

10.14 SUGGESTED READINGS:

1. Describe the mechanism of thermoregulation in a Homeotherm animal.Describe the tomer vertebrates undergo minter sleep mention the changes brought about by the during hibernation?
2. Write in detail the process of thermoregulation in Homeotherms and poikilotherms?

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Prof. K. SUMANTH KUMAR

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LESSON- 11
MUSCLES

OBJECTIVES:

- To study the structure and types of muscles (skeletal, cardiac, and smooth) and understand their functional adaptations in different animals.
- To understand the mechanism of muscle contraction, including the sliding filament theory and Szent-Györgyi's myosin fragment theory.
- To examine the functional properties of muscles, such as isotonic/isometric contraction, twitch, tetanus, summation, facilitation, and fatigue, and their physiological significance

STRUCTURE:

11.1 INTRODUCTION:

The way muscle is used by various animals differs a great deal (depending primarily on the function of the particular muscle). The demands on the flight muscles of an insect, which contract several hundred times per second, and on the muscle that closes the shells of a clam and remains contracted perhaps for several hours, are very different indeed. The best way to describe how muscle can serve different purposes is to examine some characteristic types of muscles and how they are modified to meet specific demands. Muscles may be differentiated into smooth and striated types though in strict sense these terms are very narrow to accommodate all the different varieties of muscles that exist. A purposeful locomotory movement certainly occurs in all animals. Among unicellular animals this is involved in the movement of the cell itself or in that of special structures of the cell such as the cilia, flagella, etc., but with the cellular differentiation in the multicellular animals this property of the movements gets restricted to the special muscle cells. These muscle cells are long and variously organized, the connective tissue binds them together. A general specialty of all muscle cells is their capacity to shorten in length, i.e., to contract. Sometimes the muscles do not shorten even on contraction such as when they are joined to the fixed structures or while maintaining a particular position due to the actin of the antagonistic muscles; but here to, the production of heat in these muscles indicates that they are expending energy in an attempt to contract.

11.2 TYPES OF MUSCLE:

Muscle is generally divided into three types. **Skeletal, Cardiac, and Smooth,** though smooth muscle is not a homogenous single category, skeletal muscle makes up the great mass of the somatic musculature. It has well ñ developed cross ñ striations, does not normally contract in the absence of nervous stimulation. Lacks anatomic and functional connections between individual muscle fibers, and is generally under voluntary control. Cardiac muscle also has cross ñ striations, but it is functionally syncytial and contracts rhythmically in the absence of external innervation owing to the presence in the myocardium of pacemaker cells that discharge spontaneously, smooth muscle lacks cross ñ striations. The type found is most hollow viscera is141eurocoenally syncytial and contains pacemakers that discharge irregularly. The type found in the eye and in some other locations is not spontaneously, active and resembles skeletal muscle.

1. Skeletal muscle (or) Striped (or) Striated Muscles
2. Smooth Muscle
3. Cardiac Muscle

1. Skeletal muscle (or) Striped (or) Striated Muscles:

The striated muscles exhibit an alternating arrangement of dark and light bands, crossed by thin dark lines under the polarizing microscope. According to Weismann (1913), the dark bands are doubly refracting, while the light bands are singly refracting. Under high power of a light microscope, the striated pattern appears as a regular alternation of isotropic “I” bands (light bands), through which light passes equally in all directions, and anisotropic “A” bands (dark bands), which refract light differently in different directions.

In vertebrate myofibrils, the length of an A band is usually about **1.5 µm**, while that of an I band is about **0.8 µm**. The “Z” line (from the German term *Zwischenscheibe*) bisects the I band.

In the middle of each A band lies a lighter region known as the “H” zone (or H band), named after the German scientist Hensen. The portion of the muscle fibre between two successive Z-lines is called a **sarcomere**.

Examination under the electron microscope reveals that the myofibril is composed of two types of filaments: thick and thin, with the thick filaments being about twice the diameter of the thin ones. The dense A band consists of overlapping thick and thin filaments; the lighter I band contains only thin filaments; while the H band contains only thick filaments.

The thin filaments, about **50 Å (5 nm) in diameter and 2 µm long**, are anchored at the Z line, which appears as a narrow dense region. The thick filaments, about **100 Å (10 nm) in diameter and 1.5 µm long**, lie in the centre of the sarcomere. Each thin filament is positioned between three thick filaments in a hexagonal arrangement. A single myofibril of about **1 µm diameter** contains roughly **5,000 filaments** in each cross-section of an A band

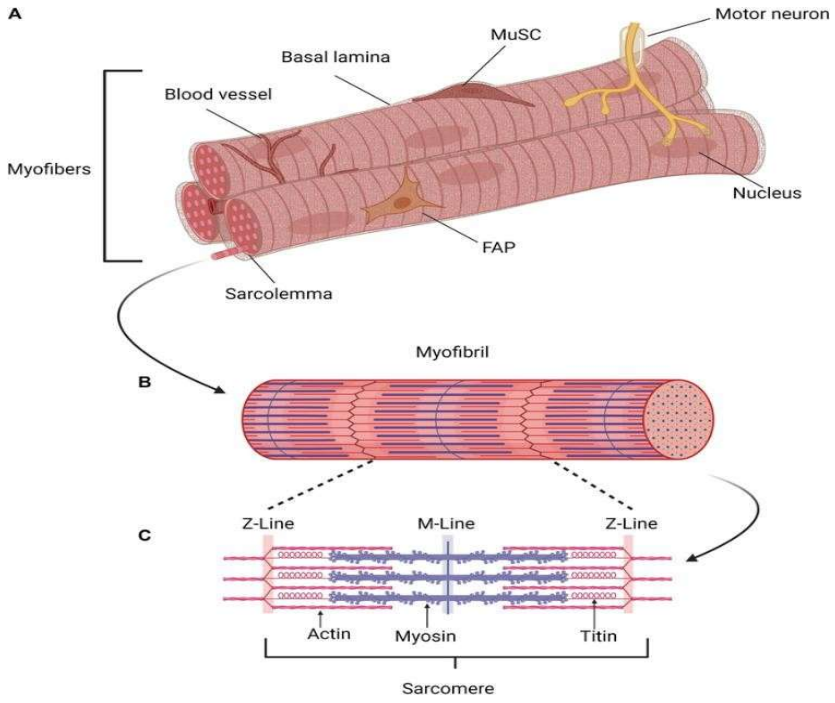

Figure 1: skeletal muscle structure.

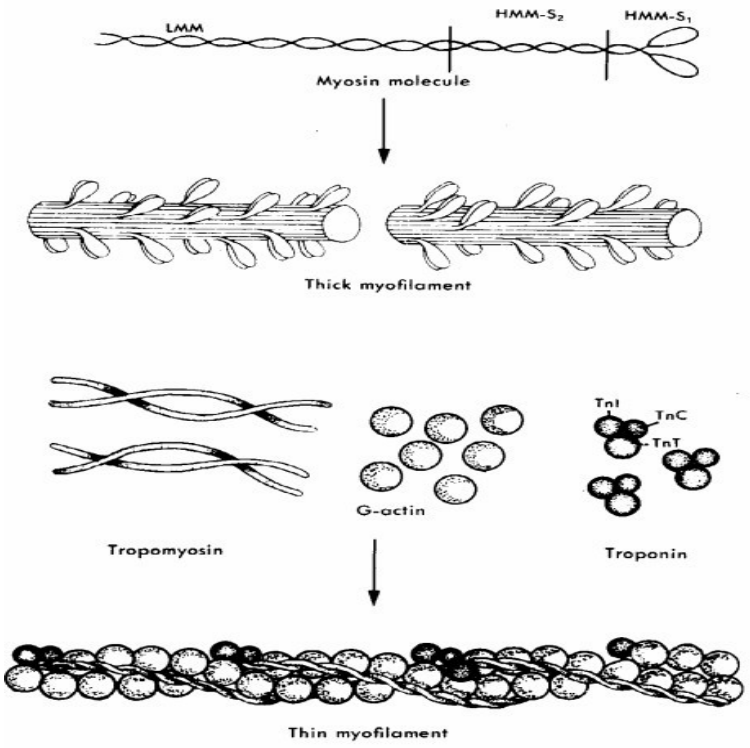

Figure 2: Myofilament

2. Smooth muscle:

In contrast to striated muscle in vertebrates, smooth muscle occurs in the stomach, intestines, bladder, ureters, uterus, bronchi, blood vessels, and other visceral organs.

Contraction of smooth muscle depends on actin and myosin filaments (the same contractile proteins found in striated muscle) and a supply of energy from ATP. However, smooth muscle lacks the regular striations characteristic of skeletal muscle because the arrangement of actin and myosin filaments is less organized.

Smooth muscle fibers are only a fraction of a millimeter long and are often oriented in many different directions. Unlike skeletal muscle, smooth muscle is not a discrete organ but usually forms an integral part of the wall of other organs.

In smooth muscle, the actin filaments are mainly oriented parallel to the long axis of the cell, which determines the direction of contraction. Contraction is triggered by a mechanism different from that in skeletal muscle. Although stimulation of smooth muscle also causes a rise in intracellular calcium ion concentration, the calcium mainly diffuses into the cell from the extracellular fluid rather than being released from intracellular stores. While this would be too slow for skeletal muscle contraction, diffusion is sufficiently fast for smooth muscle because these cells have a large surface area relative to their volume and they contract more slowly.

In blood vessels, smooth muscle contraction is regulated by chemical compounds released by endothelial cells lining the vessels. Contraction can also be initiated by electrical depolarization. When a small number of smooth muscle cells are electrically stimulated, the impulse spreads to neighbouring cells through gap junctions, which allow ions to flow directly between adjacent cells. This electrical coupling, similar to that in cardiac muscle, coordinates the activity of smooth muscle cells.

Contraction in smooth muscle differs significantly from that in skeletal muscle. The degree of shortening is variable, allowing smooth muscle to adjust its length over a wide range. Both the activation and velocity of contraction are slower. Moreover, smooth muscle can maintain contraction for prolonged periods with much lower energy expenditure than skeletal muscle.

3. Cardiac Muscle:

The muscle of the heart contains actin and myosin filaments arranged in cross striations identical to those in skeletal muscle. However, mitochondria are far more abundant in cardiac muscle, which is understandable given the constant demand for continuous work throughout the lifetime of the organism.

A key contrast with skeletal muscle is that once a contraction starts in the heart, it rapidly spreads to the entire muscle. Cardiac muscle consists of numerous branching cells that are connected end to end by specialized structures called intercalated discs. As fibers branch, they connect to adjacent fibers, forming a complex three-dimensional network. Intercalated discs maintain cell-to-cell cohesion and, at irregular intervals, contain gap junctions. These gap junctions provide areas of low electrical resistance, permitting the rapid spread of excitation from one fiber to the next throughout the heart.

Another important feature of cardiac muscle relates to the electrical phenomena during contraction. Like other muscle fibers, the cell membrane of cardiac muscle fibers undergoes action potentials during contraction. However, cardiac muscle fibers exhibit a long refractory period after each action potential. This allows the muscle to fully relax before the next

contraction, preventing sustained contraction (tetanus). This refractory period is essential for the rhythmic alternation between contraction and relaxation of the heart.

Contraction of the heart originates in pacemaker cells, which are specialized muscle cells. Because the repetitive contractions are generated within the muscle tissue itself, the vertebrate heart is described as myogenic. By contrast, the hearts of some invertebrates are neurogenic, meaning their contractions are initiated by nerve impulses.

Although the vertebrate heart is myogenic, it is still influenced by the autonomic nervous system:

- The vagus nerve carries parasympathetic fibers that release acetylcholine, causing the heart rate to slow.
- Sympathetic nerves release noradrenaline (norepinephrine), which increases the heart rate and strengthens the force of contraction.

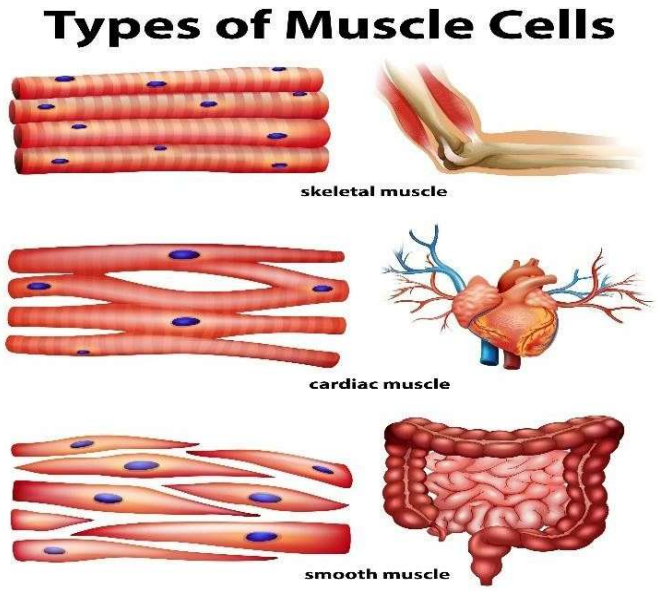

Figure 3: Skeletal muscle, Cardiac muscle, Smooth muscle

11.2.1 Properties of Muscle Tissue:

- Excitability (Irritability): Ability to respond to a stimulus.
- Contractility: Ability to shorten and thicken when stimulated.
- Extensibility: Ability to be stretched without damage.
- Elasticity: Ability to return to original shape after contraction or extension.
- Tonus: A state of partial contraction even at rest.

11.3 FUNCTIONS OF MUSCLE:

Muscles convert chemical energy into mechanical energy to perform work. A nerve impulse initiates this process by depolarizing the membrane of the muscle Fiber. Once activated, the structural components of the muscle fiber undergo changes, resulting in a shortening of muscle length. The energy for contraction is derived from exothermic, energy-yielding reactions.

Not all of the work done by the contractile elements is applied to lifting the load; part of it is spent on stretching the non-contractile elements of the muscle, such as the sarcoplasm,

sarcolemma, and connective tissue. The shortening of the contractile elements is termed contraction, while the return of the muscle to its resting length is called relaxation.

Relaxation, however, is not a passive process energy is consumed from the onset of contraction until nearly the end of relaxation. This entire energy-utilizing period is called the active state.

1. Isotonic and Isometric Contractions:

- **Isotonic contraction**: Occurs when a muscle contracts against a constant load that it can lift. The muscle changes in length while maintaining equal tension (tonus).
- **Isometric contraction**: Occurs when a muscle contracts against a load it cannot lift. The muscle develops tension but does not change in length.
- In isotonic contraction, there is a change in shape, whereas in isometric contraction, the shape remains unchanged.

2. Muscle Twitch (Simple Contraction):

A twitch is the response of a muscle to a single brief stimulus (e.g., an electric shock). It has three phases:

- **Latent period:** The interval between the stimulus and the beginning of contraction; muscle length remains constant.
- **Contraction period:** The phase during which the muscle shortens.
- **Relaxation period:** The phase during which the muscle returns to its normal length and tension.

3. Tetanus:

If a muscle fiber is stimulated repeatedly before it has completely relaxed, it can enter a state of continuous contraction called tetanus. This results from a high frequency of stimulation that prevents relaxation between successive contractions.

4. Summation

When a presynaptic nerve is stimulated more than once in rapid succession, the effects on the muscle can add up. In muscles, this results in:

- **Mechanical summation** – stronger contractions due to successive stimuli.
- **Electrical summation** – additive effects on the membrane potential.

If stimuli occur at short intervals, the resulting contraction is greater than that produced by a single stronger stimulus, because multiple fibres are excited. This additive effect of repeated stimuli is called summation.

5. Facilitation

Facilitation refers to the increased strength of contraction that results from repeated stimulations. Unlike summation, facilitation does not occur indefinitely and is limited to an optimal period. If stimulations are continued for too long, contractions gradually weaken and may stop altogether.

6. Fatigue

The failure of a muscle to contract after repeated stimulation is called fatigue. It can be demonstrated when individual twitches are induced continuously by electrical stimulation. During fatigue, contraction strength gradually decreases until Fibers fail to contract.
Although the exact cause is uncertain, fatigue has been attributed to:

- Depletion of metabolic energy sources.
- Accumulation of metabolic waste products.
- Loss of potassium ions from the postsynaptic cell and their accumulation in the extracellular space.
- Increased sodium ion concentration inside the postsynaptic cell.
- Depletion of stored neurotransmitter substances in the presynaptic terminals.

During intense exercise, oxygen supply may not meet energy demand. Muscles switch to anaerobic glycolysis, producing lactic acid. The extra oxygen required to convert lactic acid back to glycogen during recovery is called the **oxygen debt**.

Type	Colour	Contraction Speed	Fatigue Resistance	Function
Type I (Red)	Dark red	Slow	High	Endurance (e.g., posture)
TypeII (White)	Pale	Fast	Low	Quick bursts (e.g., sprinting)

Table 1: Types of muscle fibers

Motor unit:
A single motor neuron and all the muscle fibers it innervates together function as a motor unit. When the neuron fires, all the fibers in that unit contract simultaneously.
Smaller motor units-fine control (eye muscle)
Large motor units – powerful contraction (leg muscle)

Oxygen Debt and Recovery
During intense exercise, oxygen supply may not meet energy demand. Muscles switch to anaerobic glycolysis, producing lactic acid. The extra oxygen required to convert lactic acid back to glycogen during recovery is called the **oxygen debt**.

Experiments have shown that removing waste products formed during contraction, such as by washing the muscle in a balanced salt solution, can delay the onset of fatigue.

11.4 THEORIES OF MUSCLE CONTRACTION:

11.4.1 The sliding Filament Theory of Muscle Contraction:

The sliding filament mechanism of muscle contraction was proposed by Hugh Huxley (University College, London) and Jean Hanson, and independently by Andrew Huxley and Rolf Niedergerke in 1954 (later refined by F.A. Huxley, 1965, Cambridge).

According to this theory, the shortening of the sarcomere during contraction occurs due to the sliding of thin filaments (actin) inward between the thick filaments (myosin), thereby reducing the width of the I band and H zone, while the length of the A band remains constant.

The theory postulates that thin actin filaments are displaced with respect to thick myosin filaments during each cycle of contraction and relaxation.

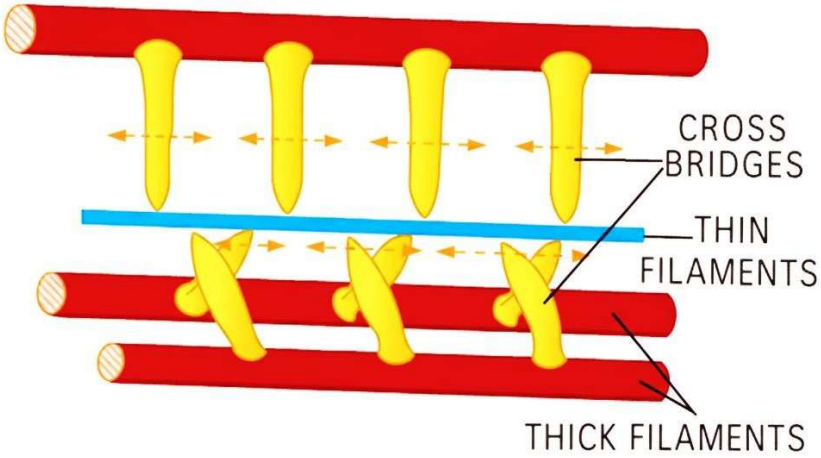

Figure 4: Position of cross bridge between myosin and actin filaments during sliding

1. The Contractile Process

When a muscle is in its resting state, the actin and myosin components of the actomyosin-ATP complex remain separated due to repulsive electric charges. Contraction involves a sequence of events:

Step 1: Excitation

- When a nerve impulse arrives at the neuromuscular junction, the sarcolemma of the muscle fibres is depolarized.
- The depolarization is transmitted inward via the T-tubule system to the sarcoplasmic reticulum (SR).
- Calcium ions are stored in the SR during rest, maintained by an ATP-dependent calcium pump.

Step 2: Release of Calcium and Activation of Troponin

- Excitation causes the release of Ca^{2+} ions from the SR.
- Calcium binds to the TnC (calcium-binding subunit) of the troponin complex.
- This induces a conformational change transmitted to tropomyosin, exposing the active sites on actin filaments.
- Actin can now interact with myosin, initiating contraction.

Step 3: Cross-Bridge Formation and Power Stroke

- Myosin heads contain ATPase activity, which hydrolyses ATP into ADP and Pi, releasing energy.
- Energized myosin heads (cross-bridges) attach to exposed active sites on actin.
- The myosin head bends, pulling the actin filament inward – this is called the power stroke.
- The cross-bridge then detaches, re-cocks, and attaches to the next site, producing a ratchet-like sliding motion.

Step 4: Sarcomere Shortening

- Repeated cross-bridge cycles cause actin filaments to slide inward past myosin, leading to sarcomere shortening and visible contraction of the muscle.
- The A band length remains constant, while the I band and H zone shorten.

2. Relaxation:

When nervous stimulation ceases, the sarcolemma repolarizes and muscle excitation stops. Calcium ions are actively pumped back into the sarcoplasmic reticulum by an ATP-dependent calcium pump. As Ca^{2+} concentration in the sarcoplasm falls, troponin-tropomyosin complex re-covers the actin binding sites. Myosin ATPase activity is inhibited, preventing further cross-bridge cycling. The muscle fiber returns to its resting state. ATP stores are replenished from ADP via phosphagens (e.g., creatine phosphate).

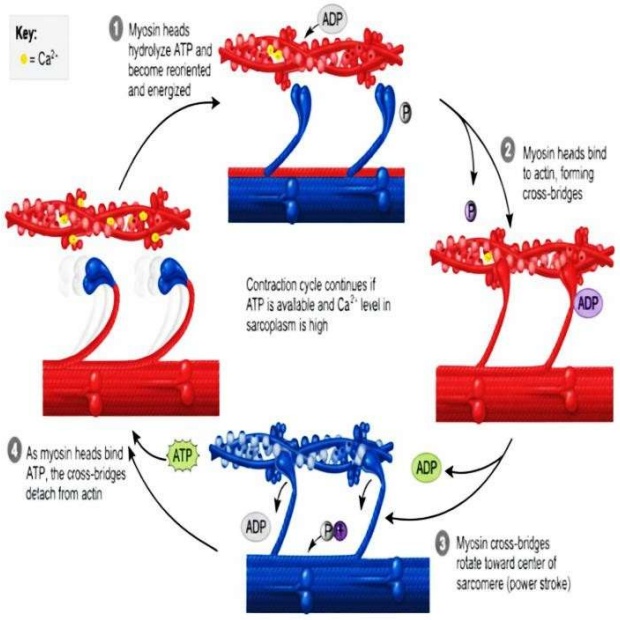

Figure 5: Mechanism of cross bridge formation and sliding of filaments

11.4.2 Andrew Szent-Györgyi's Theory of Muscle Contraction:

In the 1940s and 1950s, Andrew Szent-Györgyi demonstrated that myosin, the major contractile protein of muscle, could be split into different functional fragments using the enzyme trypsin. His studies provided important insights into how myosin interacts with actin and ATP during muscle contraction.

When myosin is treated with trypsin, it splits into two main fragments:

1. Light Meromyosin (LMM):

- Forms filaments but lacks ATPase activity.
- Does not interact with actin.
- Structurally, LMM is a two-stranded α-helical rod, about 850 Å long.
- It mainly provides the structural backbone of the thick filament.

2. Heavy Meromyosin (HMM):

- Does not form filaments, but has ATPase activity and binds to actin.

- Composed of a rod region attached to a double-headed globular region.

Can be further split into:

HMM S-1: Two globular sub fragments; each contains:
- An ATPase active site.
- An actin-binding site.
- Bound light chains, which are thought to modulate ATPase activity.

HMM S-2: A rod-shaped sub fragment that links the globular heads to the filament backbone.

Energy Metabolism in Muscle:
- ATP: Immediate source of energy.
- Creatine phosphate: Regenerates ATP rapidly during short intense activity.
- Glycogen: Stored carbohydrate, broken down by glycolysis.
- Aerobic & Anaerobic respiration: Oxygen availability determines endurance.
- Lactic acid formation: Responsible for muscle fatigue and oxygen debt.

11.5 SUMMARY:

Muscles are specialized tissues that convert chemical energy into mechanical work. They are classified as skeletal (striated), cardiac, and smooth, each with distinct structural and functional characteristics. Skeletal muscle has well-organized sarcomeres and contracts under voluntary control, cardiac muscle contracts rhythmically and is myogenic, while smooth muscle contracts slowly and efficiently in various visceral organs. Muscle contraction is driven by the interaction of actin and myosin filaments, regulated by calcium ions and ATP. The sliding filament theory explains contraction through the cyclical formation of cross-bridges between actin and myosin, whereas Szent-Györgyi's studies revealed the functional roles of myosin fragments (LMM and HMM) in filament structure, actin binding, and ATP hydrolysis. Muscle properties, including isotonic/isometric contraction, twitch, tetanus, summation, facilitation, and fatigue, demonstrate how muscles generate force and adapt to physiological demands.

Topic	Key Point
Types of Muscles	Skeletal, Smooth, Cardiac
Structural Unit	Sarcomere
Contractile Proteins	Actin and Myosin
Theory	Sliding Filament Theory
Energy Source	ATP
Types of Contraction	Isotonic, Isometric
Fatigue Cause	Energy depletion, metabolite buildup

11.6 TECHINICAL TERMS:

Sarcomere, Actin, Myosin, Sliding filament theory, Heavy meromyosin (HMM), Light meromyosin (LMM), Calcium ions (Ca^{2+}), ATPase, Isotonic and isometric contraction, Fatigue

11.7 SELF-ASSESSMENT QUESTIONS:

1. Describe the generation and conduction of nerve impulse in a typical neuron.
2. Give the detail structure of a synapse and explain the mechanism of impulse conduction through synapse.
3. Describe the structure and mechanism of vision in human eye.
4. Describe the comparison details of smooth muscles, cardiac muscles and skeletal muscles.
5. Describe the ultrastructure of skeletal muscle in detail.
6. With the suitable diagram, explain the sliding filament theory of muscle contraction.

11.8 SUGGESTED READINGS:

1. Dinesh Objective Biology. K.N. Bhatia and K. Bhatia, S. Dinesh & Company, Jalandhar, India.
2. Principles of Anatomy and Physiology. Gerard J. Tortora and Bryan Derrickson, John Wiley & Sons. USA.
3. Principle of human physiology. Cindy L. Stanfield. Pearson Publication, Boston Columbus Indianapolis New York.c) Circulatory system.

- **Prof. K. SUMANTH KUMAR**

LESSON- 12

NERVOUS SYSTEM

OBJECTIVES:

- To understand the structure and function of neurons, action potential, and propagation of nerve impulses.
- To explain the mechanisms of synaptic transmission, including electrical and chemical synapses with neurotransmitters.
- To analyse the neural control of muscle tone, reflexes, and posture through spinal cord, brainstem, cerebellum, and basal ganglia.

STRUCTURE:

12.1 INTRODUCTION:

The act of changing place or position by entire body or by one or more of its parts is called movement. Animals that can move are able to flee, hunt for prey, travel long distances or conquer new habitats. Movement may occur at cellular level, for instance cytoplasmic streaming or cyclosis in protozoans and swimming of gametes to achieve mother nature goal.

Motion at organ level, such as heart beat and rotation of eyeball or at the organism level, for example swimming by fish, flying by a bird and walking by a man show the dynamics of life.

With the evolution of neuromuscular systems, animals acquired the capacity for articulated movement, allowing them to develop complex patterns of behavior. Multicellular animals have structurally complex body organization. Due to this, there arises a need to have a system for coordinating various metabolic and homeostatic activities of different parts, organs, organ systems in the body. This is achieved by nervous and endocrine systems present in the body.

Higher animals involve muscles, skeleton and joints for locomotion. Movement results from cooperation between muscles and skeletal elements. The muscles provide force and the

skeleton offers hard surface for the force to work against. The muscular system contains muscle tissues and interconnects with both the nervous system and skeletal system. Nerves control the muscles and allow us to consciously direct movements. The nervous system allows for control and coordination of skeletal muscular movements that may be consciously predetermined, or may happen automatically, such as reflexes. Other parts of the nervous system control and coordinate subconscious body activities, including heart rate, gland secretions and smooth muscle movement in the digestive system. Some activities, such as breathing, can be controlled both subconsciously and consciously. The nervous system typically works quickly. It also allows us to integrate and store information, such as when you are learning. The nervous system transmits signals to different parts of the body to coordinate function. Electrochemical signals are processed in the brain and sent down the spinal cord, which runs the length of the back.

From the spinal cord, peripheral nerves send signals out to the extremities. Return signals come in through sensory nerves and either return to the spinal cord for processing or back to the brain. The spinal cord processes reflexes and repeated patterns.

12.2 NEURONS:

The nervous system serves as a centre for learning, memory, and coordination of body activities. In higher vertebrates, it accumulates memories from past experiences to influence behaviour. The medical science that studies the structure (anatomy), functions (physiology), and diseases (pathology) of the nervous system is called Neurology.

Division of the Nervous System

The nervous system is broadly divided into three parts:

1. **Central Nervous System (CNS)**
- Composed of the brain and spinal cord.
- Coordinates impulses received from receptors and transmits responses to effectors.

2. **Peripheral Nervous System (PNS)**
- Composed of cranial nerves (10–12 pairs) and spinal nerves.
- Acts as a communication link between receptors, CNS, and effectors.

3. **Autonomic Nervous System (ANS)**
- Innervates smooth muscles, cardiac muscles, and glands.
- Controls involuntary activities, e.g., peristalsis of the alimentary canal and heartbeat.
- Usually considered part of the PNS due to its connections with it.

Structure of neuron:

The basic functional units of the nervous system that transmit messages are cells called neurons. Neurons are specialized cells of the nervous system that transmit signals throughout the body. Signals travel through a neuron as electrical impulses. Neurons release chemical substances, known as neurotransmitters, to transmit information to other neurons, to muscles, or to glands. The chemical messages of the nervous system are transmitted over short distances, and their effects are short-lived. You may already know that neurons can do many different things from sensing external and internal stimuli, to processing information and also directing muscle actions.

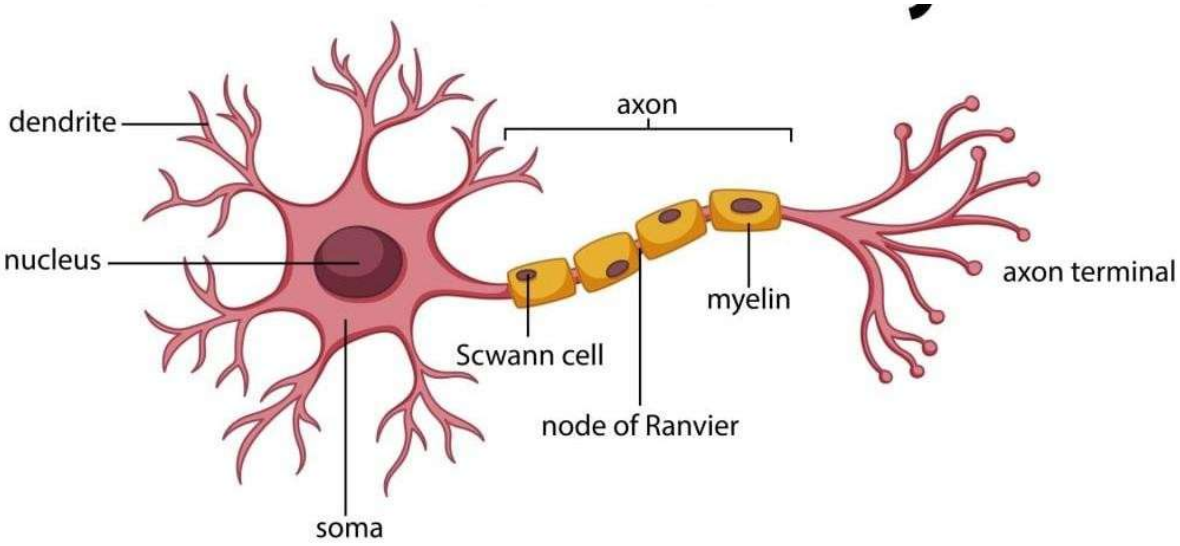

Figure 1: Neuron

1. Cell Body

The cell body of a neuron is also called the soma or perikaryon and may be round, stellate, pyramidal or fusiform in shape. Like any other cell it consists of a mass of cytoplasm with all its principal constituents surrounded by a cell membrane. The cell body contains a large nucleus with one or two nucleoli but there is no centrosome. In addition to the general features of a typical cell, the cytoplasm of a neuron has following distinctive characteristics.

2. Nissl granules/bodies:

These are basophilic granules, and composed of rough surfaced (with ribosomes) endoplasmic reticulum. These are concerned with protein synthesis in neurons. The proteins are needed for maintenance and repair, and for production of neurotrans enzymes. The Nissl bodies are present in the dendrites as well but are usually absent from the axon hillock and the axon.

3. Neurofibrils:

These consist of microfilaments and microtubules. These filaments provide a frame work of shape for cell body.

4. Dendrites:

The dendrites are multiple small branched processes which contain Nissl bodies and neurofibrils. Dendrites are the receptive processes of the neuron receiving signals from other neurons via their synapses with axon terminals.

5. Axon:

The axon is the single longer process of the nerve cell. It varies in length from a few microns to one meter. It arises from the conical extension of the cell body called axon hillock, which is devoid of the Nissl bodies. The part of the axon between the axon hillock and the beginning of myelin sheath is called the initial segment. In the axon, the cell membrane continues as axolemma and the cytoplasm as axoplasm. The axon terminates by dividing into a number of branches, each ending in a number of synaptic knobs also known as terminal buttons or axon telodendrion. Synaptic knobs contain macrovesicles in which chemical neurotransmitters are stored. Myelin sheath is present around the axon in the so-called myelinated nerve fibers. Myelin sheath which consists of protein–lipid complex is produced by glial cells called Schwann cells which encircle the axon forming around it a thin sleeve. Each Schwann cell

provides the myelin sheath for a short segment of the axon. At the junction of any two such segments, there is a short gap, i.e. periodic 1 μm constrictions at about 1 mm distance. These gaps are the nodes of Ranvier. There are some axons which are devoid of myelin sheath. Myelination of axons increases the speed of conduction, but greatly increases their diameter. Axons perform the specialized function of conducting impulses away from the cell body.

12.2.2 The basic functions of a Neuron:

If you think about the roles of the three classes of neurons, you can make the generalization that all neurons have three basic functions. These are to:

- Receive signals (or information).
- Integrate incoming signals (to determine whether or not the information should be passed along).
- Communicate signals to target cells (other neurons or muscles or glands).

12.2.3 Classes of neurons:

Based on their roles, the neurons found in the human nervous system can be divided into three classes: sensory neurons, motor neurons, and interneurons.

Sensory neurons
Sensory neurons get information about what's going on inside and outside of the body and bring that information into the CNS so it can be processed. For instance, if you picked up a hot coal, sensory neurons with endings in your fingertips would convey the information to your CNS that it was really hot.

Motor neurons
Motor neurons get information from other neurons and convey commands to your muscle's organs and glands. For instance, if you picked up a hot coal, it motors neurons innervating the muscles in your fingers would cause your hand to let go.

Interneurons
Interneurons, which are found only in the CNS, connect one neuron to another. They receive transmit information to other neurons (either motor neurons or interneurons). Depending upon the number of poles from which processes arise, neurons are divided into four categories.

- **Unipolar** neurons have a single pole, from which both the processes—axon and dendrite arise. True unipolar cells are present only in embryonic stage in human being.
- **Bipolar** neurons have two poles, one for axon and other for dendrite. Bipolar neurons are found in the vestibular and cochlear ganglia, in the nasal olfactory epithelium and as bipolar cells in the retina.
- **Multipolar Neurons:** Have many processes: one axon and multiple dendrites. Most common type of neuron in CNS, e.g., motor neurons.
- **Pseudo unipolar Neurons:** Start as bipolar neurons during development but fuse their processes into a single stalk that later splits. Found in dorsal root ganglia of spinal nerves; primarily sensory.

12.2.4 Properties of neuron:

1. Excitability: It is the ability of the nerve cells and fibers to enter into an active state called the state of excitation in response to a stimulus. Excitation arises at the receptors on account of various stimuli such as light, temperature on the organisms.
2. Conductivity: The excitation does not remain at the site of its origin. It is transmitted along nerve fibers. The transmission of excitation in a particular direction is called conductivity neuron-false, uni-one) is a kind of sensory neuron in the ripheral one nerve cell temperature, chemical, electrical or pressure which constantly act.

12.2.5 Neuroglia or Glia Cells:

Neuroglia, or glial cells, are part of the Nerve nutrients, oxygen and insulation and by eliminating harmful pathogens. They comprise approximately half of the total cellular composition of the central nervous system, and are found in all regions of the spinal cord and brain. Glia was first described in 1856 by the pathologist

Rudolf Virchow. There are six common types of glia cells in nervous system.

1. **Microglia:** clean up cellular debris via phagocytosis.
2. **Astrocytes:** support and repair Nervous system.
3. **Satellite:** form the brain-blood barrier within the central nervous system, function similarly to astrocytes.
4. **Ependymal:** form epithelial lining of the central nervous system fluid.
5. **Oligodendrocytes:** myelinates axons of the neurons in the central nervous system.
6. **Schwann Cells:** myelinates axons of the neurons in the peripheral nervous system. Nervous system that supports neurons by providing them initial. Neurons; form the brain-blood barrier within the central system

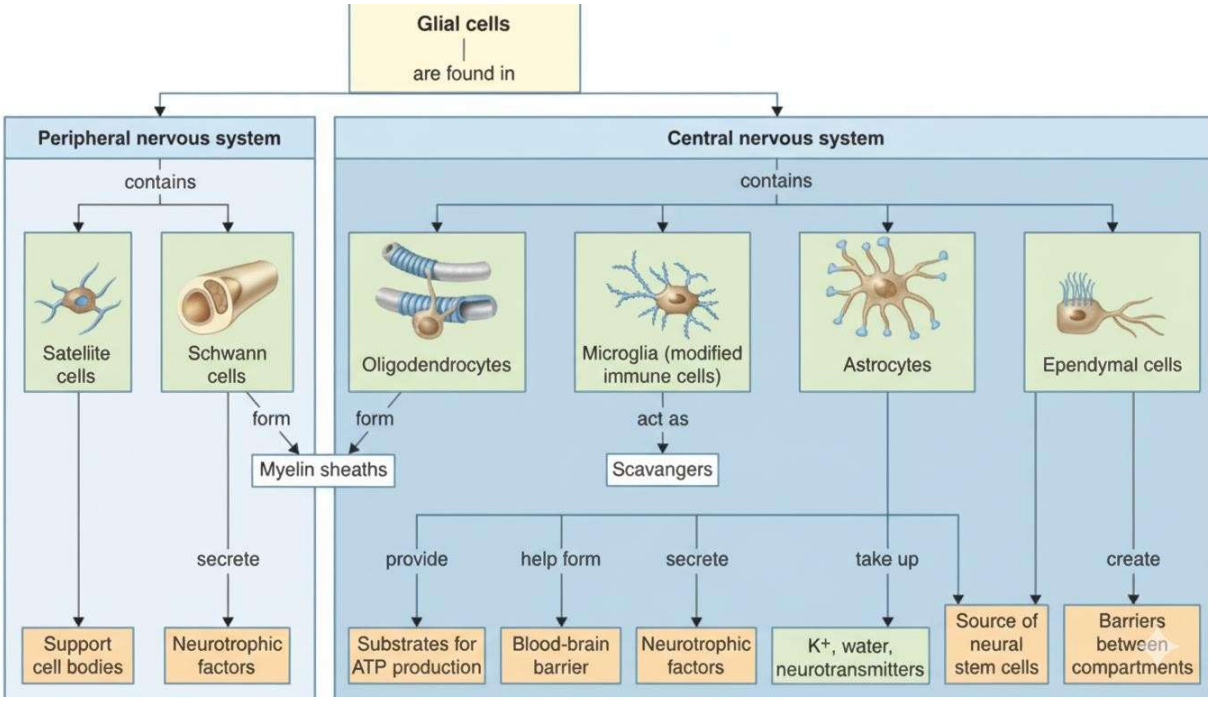

Figure 2: Types of glial cells and their function

12.3 ACTION POTENTIAL:

A wave of reversed polarity or depolarization (action potential) moving down an axon is called a nerve impulse. The most accepted theory of nerve impulse conduction is ionic theory proposed by Hodgkin and Huxley (1952). This theory states that nerve impulse is an electro-chemical event governed by differential permeability of neurilemma to Na^+ and K^+ which in turn is regulated by the electric field. Generation and propagation of nerve impulse through nerve cell completes in three sequential events i.e. polarization, depolarization and repolarization

Resting membrane potential:

In a resting nerve fiber (a nerve fiber that is not conducting an impulse), sodium ions (Na^+) predominate in the extracellular fluid, whereas potassium ions predominate in the intracellular fluid (within the fiber). Intracellular fluid also contains large number of negatively charged (anions) protein molecules. Na^+ are 10 times more outside the neuron and K^+ ions are 25 times more inside the cell. Thus, it makes a considerable difference between the ion concentration outside and inside the plasma membrane. It also causes a difference in electrical charges on either side of the membrane. The plasma membrane is electrically positive outside and negative inside. This potential difference across the plasma membrane is known as resting potential. These potential averages -70 mv (-60 to-90 mv) in inner side of membrane in respect to outer side. Due to different concentrations of ions on the two sides of the membrane, sodium ions tend to diffuse into the nerve fiber and potassium ions tend to diffuse out of the nerve fiber. The membrane of a resting nerve fibre is more permeable to potassium than to sodium. So, potassium leaves the nerve fiber faster than sodium enters it. This results in a higher concentration of cations outside the membrane compared to the concentration of cations inside it. This state of the resting membrane is called polarized state and makes its inner side electronegative to its outer side.

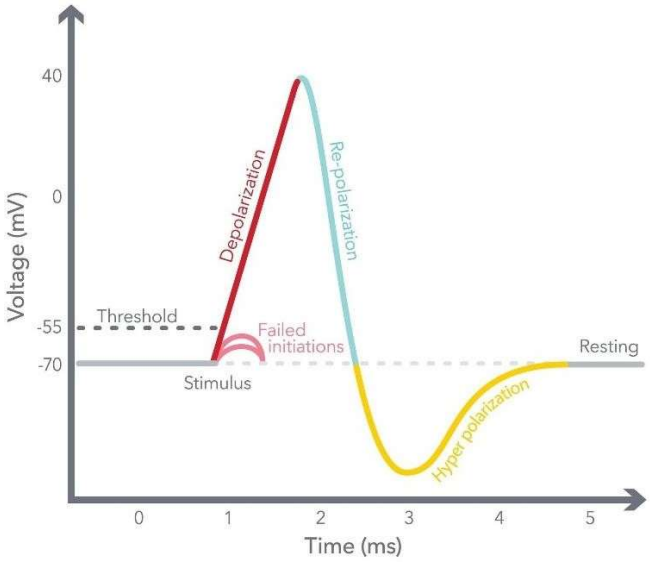

Figure 3: action potential

1. Depolarization:

When the nerve fiber is stimulated mechanically, electrically, thermally or chemically a disturbance is felt at the point of stimulation which gives rise to a local excitatory state. The membrane becomes permeable to sodium ions. Suddenly sodium ions rush and potassium ions diffuse out of the axon membrane. Due to the diffusion of ions, more sodium ions enter

the axon than potassium ions leave it, so that the positive and negative charges on the outside and inside of the axon membrane the outside and positively charged on the inside. The membrane with reversed polarity is said to inside the nerve fiber is reversed. The membrane is negatively charged once depolarized. Thus, the impulse is propagated as a wave of depolarization (reversed polarity). This wave of depolarization travelling down a nerve fiber is called action potential. In fact, the action potential "moves" in the manner of a spark moving along a fuse. This "moving" action potential constitutes the nerve impulse. The action potential (impulse) is the basic means of communication within the nervous system. The action potential of + 45 mv on inner side of axolemma in respect to its outer side is also called spike potential.

2. Repolarization:

With the increase of sodium ions inside the nerve cell, the membrane becomes less permeable to sodium ions whereas the permeability membrane to potassium ions increases. The sodium ions are pumped out of the cell and potassium ions are pumped into the cell until the original resting state of ionic concentration is achieved. Thus, this makes the membrane negative on inside and positive on outside, this process is called repolarization. Sodium Potassium Pump and Refractory Period the Na^+ and K^+ concentration gradients across the membrane of the cell are maintained by the activity of a protein called the Na^+ -K^+ ATPase, often referred to as the sodium-potassium pump. The sodium-potassium pump is a process of expelling out sodium ions and drawing in potassium ions against concentration and electrochemical gradient. Each Na^+ K^+ pump, for one ATP used, expels 3 Na^+ out for 2 K^+ taken in. The entire process of repolarization requires some time during which the nerve cannot be stimulated again. This period is called refractory period. The refractory period is very short, being only about one millisecond (1/1000 of a second). Thus, a nerve fiber can transmit about 1000 impulses per second. During repolarization, as the cell returns to its resting potential, the neuron is ready to receive another stimulus. In non-myelinated axons the impulse moves along the length of axon. Current generated in one channel acts as stimulus for next channel. This way every next channel is affected in succession

Moving the impulse ahead continuously towards the axon terminal. This is called stepwise transmission. It is comparatively a slower conduction and common in invertebrates. In myelinated neurons the axon is insulated by myelin sheath which is impermeable for ion exchange. The impulse generated at axon 161 euroco can affect the Na^+ channels only at the Nodes of Ranvier where axolemma is non myelinated. Thus, generated impulse moves forward node to node in jumping manner. It is known as saltatory conduction. It is comparatively faster and found in vertebrates.

3. Hyperpolarization:

The voltage-gated potassium channels are slow to close, causing an "overshoot" where too many K+ ions leave the cell. This makes the membrane potential temporarily more negative than the resting potential (e.g., -90 Mv). This is known as the **refractory period**, during which the neuron is less likely to fire another action potential. The sodium-potassium pump then works to restore the original ion balance, bringing the membrane back to its resting potential.

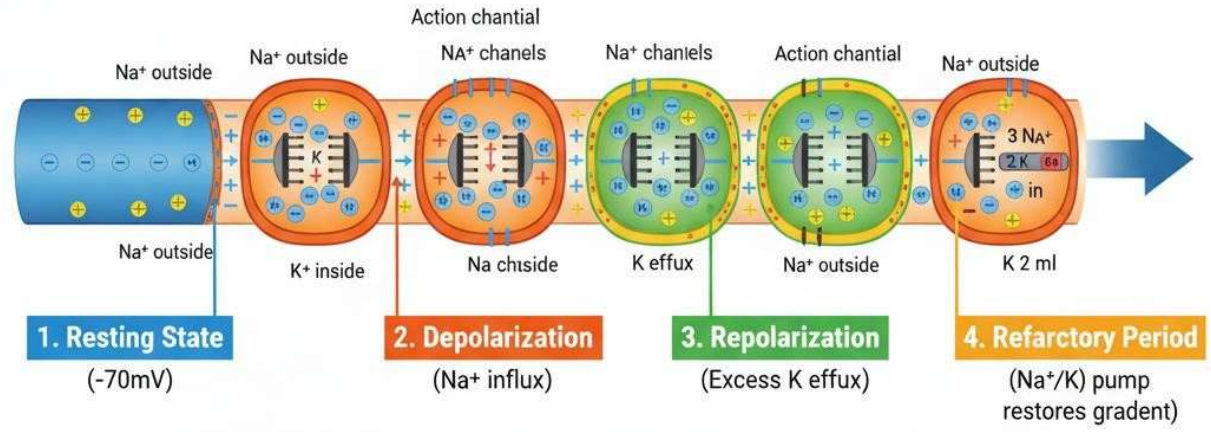

Figure 4: neuron action potential

12.4 PROPOGATION OF NERVE IMPULSE:

A nerve impulse is a wave of electrical activity that travels along the membrane of a neuron, allowing rapid communication between the nervous system and muscles or other organs. It is the result of depolarization and repolarization of the neuronal membrane, driven by the movement of ions across voltage-gated channels. The propagation of a nerve impulse is a fundamental property of excitable cells, including neurons and muscle Fibers, and is inseparably linked to irritability the ability of a cell to respond to a stimulus.

At rest, a neuron maintains a resting membrane potential of about -70 Mv, with the inside negatively charged relative to the outside. This is due to the high concentration of K^+ ions inside, Na^+ ions outside, and the presence of negatively charged organic molecules (e.g., acetate, pyruvate, lactate, amino acids) that cannot cross the membrane. The sodium-potassium pump (Na^+/K^+-ATPase) actively maintains these ionic gradients by pumping 3 Na^+ ions out and 2 K^+ ions in.

When a neuron is stimulated, a local depolarization occurs as Na^+ channels open, allowing Na^+ ions to enter the cell. This reversal of membrane potential triggers neighbouring regions of the membrane to depolarize in turn, creating a progressive wave of depolarization along the axon. The local flow of ions between depolarized and resting regions forms local circuit currents, which are the driving force for conduction. After depolarization, K^+ channels open, allowing K^+ ions to leave the cell, which restores the negative internal environment-his is called repolarization. Often, the membrane temporarily becomes more negative than the resting potential, a phase called hyperpolarization.

The all-or-none principle governs nerve impulses: if the depolarization reaches the threshold, a full action potential is generated; if not, the impulse does not occur. Following an action potential, the neuron enters a refractory period, divided into the absolute refractory period (no new impulse can be generated) and the relative refractory period (a stronger-than-normal stimulus can trigger a new impulse). The refractory period ensures that impulses propagate in one direction only and prevents overlap.

In **non-myelinated Fibers**, the action potential moves continuously along the membrane. In **myelinated Fibers**, the impulse jumps between nodes of Ranvier in a process called saltatory conduction, significantly increasing conduction speed. The speed of propagation depends on factors such as axon diameter, myelination, and temperature.

Propagation of a nerve impulse is therefore a highly coordinated sequence of **ionic movements and electrical changes**, allowing neurons to rapidly transmit information throughout the body, from sensory receptors to the central nervous system, and from the CNS to muscles and glands.

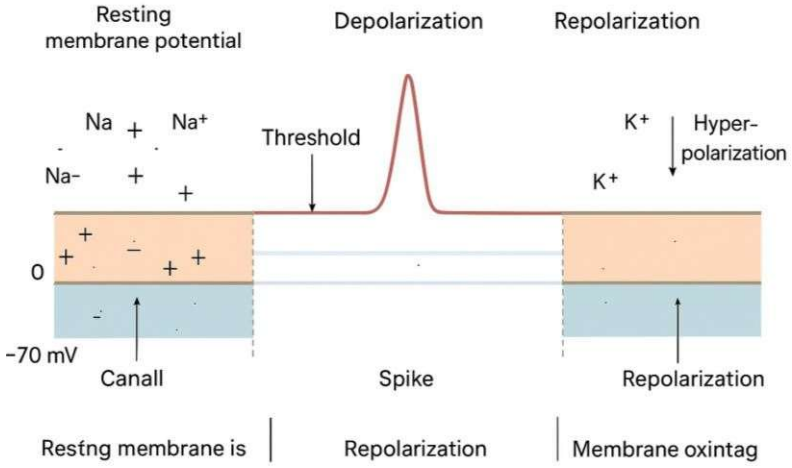

Figure 5: Propagation of nerve impulse

12.5 SYNAPTIC TRANSMISSION IN ANIMALS:

Intraneuronal transmission

Intraneuronal transmission is the process by which one neuron communicates with another. Sir Charles Sherrington (1861–1952) coined the term **"synapse"** for the specialized junction where one neuron influences another. A synapse can be defined as any specialized site where a neuron affects the activity of another neuron. Functionally, there are **two main types of synapses**: electrical and chemical.

1. Transmission of Nerve Impulse at a Chemical Synapse:

The process of chemical transmission across synapses was discovered by **Henry Dale** (1936). The chemical transmission of impulse occurs in following steps.

I. When an impulse arrives at a presynaptic knob, calcium ions from the synaptic cleft enter the cytoplasm of the presynaptic knob.
II. The calcium ions cause the movement of the synaptic vesicles to the surface of the knob. The synaptic vesicles are fused with the presynaptic membrane and get ruptured (exocytosis) to discharge their contents (neurotransmitter) into the synaptic cleft
III. The synaptic vesicles then return to the cytoplasm of the synaptic knob where they are refilled with neurotransmitter.
IV. The neurotransmitter of the synaptic cleft binds with protein receptor molecules on the postsynaptic membrane. This binding membrane, opening channels in the membrane and allowing sodium ions to enter the cell. His causes the depolarization and generation of action potential in the post the impulse is transferred to the next neuron
V. Having produced a change in the permeability of the postsynaptic membrane the neurotransmitter is immediately lost from the synaptic cleft. In the case of cholinergic synapses, acetylcholine (Ach) is hydrolyzed by an enzyme in high concentration at the synapse.
VI. The products of the hydrolysis are acetate and choline which are reabsorbed into the

synaptic knob where they are resynthesized into cleft. Binding action changes the membrane potential of the postsynaptic post-synaptic membrane. Thus acetylcholinesterase (AchE) which is present acetylcholine, using energy from ATP.

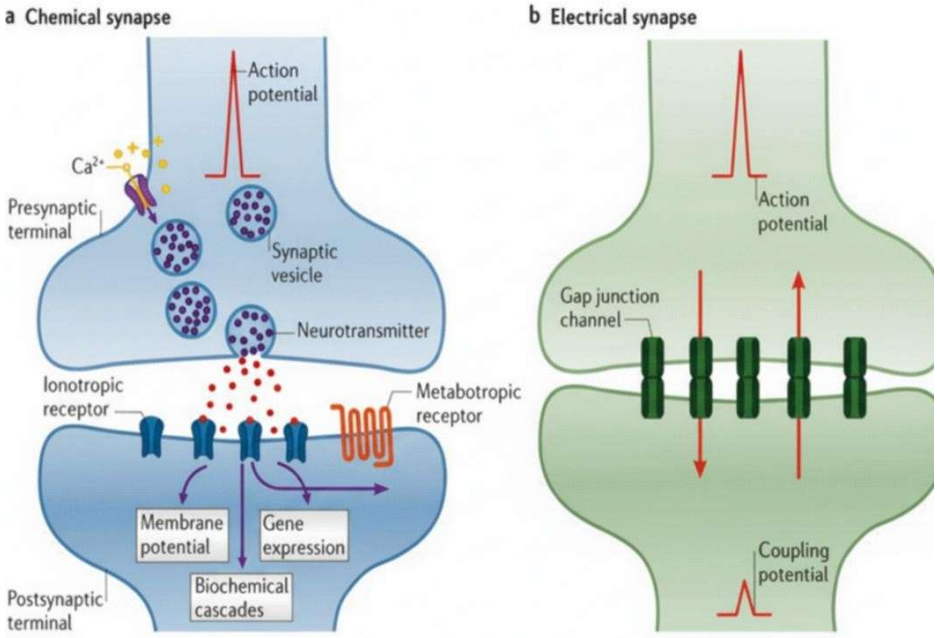

Figure 6: Transmission of impulse through (a) chemical synapse (b) electrical synapse

2. Transmission of Nerve Impulse at an Electrical Synapse:

At electrical synapse there is continuity between the pre continuity is provided by the gap junction between the two neurons. The gap junctions are small protein tubular structures that allow free movement of ions between the two neurons. Because of this, the action potential reaching the synaptic neuron. In electrical synapse there is minimal synaptic delay because of the direct flow of electrical current from one neuron into the other through gap junction. Thus, impulse transmission across an electrical synapse is always faster than the at across a chemical synapse. Most impulse transmission across the synapse between neurons takes place at the chemical synapses. Electrical synapses are relatively rare. It is found in the cardiac muscle smooth muscle fibers of intestine and the epithelial cells of lens. **Neuromuscular junction:** Impulses are conducted from a neuron to a muscle cell across an area of contact called neuromuscular junction. When a nerve fiber ends on a muscle fiber, forms motor end plate.

The motor end plates have vesicles and mitochondria. The vesicles secrete neurotransmitter (figure 4.8). When the motor impulse from the nerve is received on the motor end plates, a local depolarization occurs there resulting in fiber. Pre-synaptic and post-synaptic neurons. The pre-synaptic terminal produces potential change in the post the excitation of the muscle fiber.

- **Neuroglandular junction:** It is an area of contact between a neuron and glandular cells. There is also a gap which is bridged at the time of the transmission of the impulse by a neurotransmitter.
- **Synaptic delay:** Transmission of an impulse across a synapse is slower than its conduction along neuron. This is because of the time needed for the release of a neurotransmitter, its diffusion through the synaptic cleft, and its action on the post-synaptic membrane. The difference in the rate is called synaptic delay. It amounts to about half a millisecond at body temperature (37Oc).

- **Synaptic fatigue:** Repeated stimulation of the presynaptic knob may deplete the neurotransmitter, and this may fail to stimulate the post-synaptic membrane. This condition of the synapse is termed synaptic fatigue. It lasts for several seconds during which the neurotransmitter is re-synthesized. Synaptic fatigue is the only fatigue that affects the nervous tissue. Conduction of the nerve impulse along the neurons is not subject to fatigue.
- **Neurotransmitters:** Neurotransmitters are the chemical substances which are responsible for transmission of an impulse through a synapse.

Characteristics of neurotransmitters:

- A neurotransmitter should be synthesized by pre-synaptic neurons and stored in the vesicles, which are present in axon terminal. The synthesizing enzymes should be present in the nerve at storage site.
- A neurotransmitter should be released on stimulation of nerve.
- A neurotransmitter travels a very small distance between pre-synaptic membrane and postsynaptic membrane.
- A neurotransmitter is associated with an enzyme or enzyme system for its inactivation.
- A neurotransmitter when applied extrinsically should mimic the effects of the nerve stimulation.
- Drug which modifies the response to nerve stimulation should also modify the proposed transmitter action in a similar way.
- The most common neurotransmitter is **acetylcholine**, which is released at voluntary neural synapses, neuromuscular junctions, synapses of preganglionic nerve fibers, synapses of postganglionic parasympathetic nerve fibers. The nerve fibers which release acetylcholine neurotransmitter are called **cholinergic**. In the synaptic cleft it is broken down by enzyme **cholinesterase** into choline and acetic acid. The latter two are reabsorbed by axon terminal for synthesis of acetylcholine. Acetylcholine has excitatory effect except at the ends of parasympathetic nerves.
- **Noradrenaline** (nor-epinephrine, similar to adrenal hormone) is formed at synapses and neuromuscular junctions of postganglionic sympathetic nerve fibers. The nerve fibers are called **adrenergic**. The transmitter is broken by enzyme **monoaminoxidase**. It excites some regions and inhibits a few others. Peripheral nervous system generally uses three neurotransmitters- acetylcholine, noradrenaline and adrenaline. Central nervous system uses these and some additional neurotransmitters like gamma amino butyric acid (GABA), glycine, glutamic acid, serotonin, endorphins and nitric oxide. Glycine, dopamine and gamma amino butyric acid (GABA) are **inhibitory** transmitters. Glutamate is **excitatory**. Serotonin is inhibitor of pain pathways of spinal cord. It may also control mood and induce sleep. Nitric oxide is formed at the site of transmission. It does not alter membrane potential but brings about changes in metabolic functions that modify neuronal excitability.
- Dopamine can play a lot of different roles in the brain, depending on the location. It also plays a role in attention, problem-solving, and memory Serotonin is involved with mood, as well as your sleep cycle, pain control, and digestion. Serotonin can also help with forming blood clots and increasing sex drive.
- Acetylcholine (Ach) plays a major role in the formation of memories, verbal and logical reasoning, and concentration.
- GABA is also an inhibitory neurotransmitter that helps to balance any neurons that might be over-firing. GABA also plays a role in vision and motor control.
- Noradrenaline is an excitatory neurotransmitter that helps to activate the sympathetic

nervous system, which is your "fight or flight" response to a stress. Norepinephrine also plays a role in attention, emotion, sleeping and dreaming, and learning.

12.6 NEURAL CONTROL OF MUSCLE TONE AND POSTURE:

Neural control of muscle tone and posture is a complex, hierarchical process involving multiple levels of the central nervous system (CNS), from the spinal cord to the cerebral cortex. This system ensures the body maintains a stable position against gravity, both during static stances and dynamic movements.

12.6.1. Spinal Cord and Brainstem Reflexes:

The most basic level of control occurs at the **spinal cord**, where fundamental reflexes maintain muscle tone.

- **Stretch Reflex (Myotatic Reflex):** This is a monosynaptic reflex arc that is critical for maintaining muscle tone. When a muscle is stretched, **muscle spindles** (proprioceptors within the muscle) are activated. They send a signal via a Ia afferent fiber directly to the alpha motor neuron of the same muscle, causing it to contract. Simultaneously, the Ia afferent fiber activates an inhibitory interneuron that relaxes the antagonistic muscle. This feedback loop is the foundation of muscle tone and is what allows you to stand upright without constantly thinking about it.
- **Golgi Tendon Reflex:** This polysynaptic reflex is initiated by **Golgi tendon organs** (proprioceptors in tendons) which sense muscle tension. When tension becomes excessive, these organs send a signal that activates an inhibitory interneuron in the spinal cord, causing the muscle to relax. This acts as a protective mechanism to prevent muscle and tendon injury.

The **brainstem** contains nuclei and descending pathways that modulate these spinal reflexes. The **reticulospinal** and **vestibulospinal tracts** are the primary pathways.

- **Reticulospinal Tracts:** Originating from the reticular formation, these tracts control voluntary movement and a range of vital autonomic functions, including posture. They exert a powerful influence on spinal motor neurons, modulating the gain of spinal reflexes and adjusting muscle tone to support planned movements.
- **Vestibulospinal Tracts:** These tracts arise from the vestibular nuclei in the brainstem, which receive sensory information about head position and movement from the inner ear. They adjust muscle tone in the limbs and trunk to maintain balance and correct for shifts in posture.

12.6.2 Cerebellum:

The **cerebellum**, often called the "little brain," plays a crucial role in coordinating and fine-tuning motor movements, including posture. It does not initiate movement but acts as a comparator and a control centre for balance and coordination.

- **Feedback and Feedforward Control:** The cerebellum receives extensive sensory input from the spinal cord (proprioception) and motor commands from the cerebral cortex. It compares the intended movement with the actual movement and sends corrective signals back to the motor cortex and brainstem.

- **Postural Adjustments:** It works with the vestibular system to make continuous, subtle adjustments to muscle tone, ensuring the body remains stable in response to changing conditions, such as standing on an uneven surface.

12.6.3 Cerebrum and Basal Ganglia:

The **cerebral cortex** and **basal ganglia** are at the highest level of this hierarchy, involved in the planning and execution of voluntary movements.

- **Motor Cortex:** The primary motor cortex and premotor areas initiate and plan voluntary movements. They send signals down the **corticospinal tract** to the spinal cord, which directly influences alpha motor neurons.
- **Basal Ganglia:** This group of subcortical nuclei is involved in the selection and initiation of voluntary movements, as well as the suppression of unwanted movements. They modulate the activity of the descending pathways that control posture and muscle tone. Dysfunctions in the basal ganglia, such as in Parkinson's disease, can lead to severe issues with muscle tone (e.g., rigidity) and posture.

12.7 SUMMARY:

Interneuron transmission is the process by which neurons communicate through specialized junctions called synapses. Electrical synapses involve direct ionic flow through gap junctions, enabling rapid conduction, while chemical synapses depend on neurotransmitters that diffuse across the synaptic cleft to produce excitatory or inhibitory responses. The action potential, generated by ion exchange across the neuronal membrane, propagates along axons via continuous or saltatory conduction. Neuroglial cells support neurons, and neurotransmitters regulate signaling. Integration of neuronal activity allows precise control of muscle tone, posture, and reflexes through coordinated actions of the spinal cord, brainstem, cerebellum, and basal ganglia.

12.8 TECHINICAL TERMS:

Depolarization, Hyperpolarization, Saltatory conduction, Excitatory Postsynaptic Potential (EPSP), Inhibitory Postsynaptic Potential (IPSP), Neurotransmitter, Synaptic cleft, Myelination, Resting membrane potential, Reflex arc.

12.9 SELF-ASSESSMENT QUESTIONS:

1. What is the difference between electrical and chemical synapses?
2. How is resting membrane potential maintained in a neuron?
3. What is meant by excitatory and inhibitory postsynaptic potentials?
4. Why is conduction faster in myelinated neurons compared to non-myelinated neurons?
5. Explain the role of the cerebellum in maintaining posture and coordination.

12.10 SUGGESTED READINGS:

1 General and Comparative Physiology – William S. Hoar
2 Comparative Physiology ñ C.L.Processor

- **Prof. K. SUMANTH KUMAR**

LESSON- 13
EXCREATION

OBJECTIVES:

- To understand the structure, types, and functions of kidneys and nephrons in animals, with emphasis on their role in osmoregulation and homeostasis.
- To explain the mechanisms of excretion including urine formation, urea production, and modes of nitrogenous waste elimination (ammonotelic, ureotelism, uricotelism, genteelism).
- To analyse the comparative excretory adaptations in different organisms, highlighting how kidneys and associated organs maintain water balance, ionic regulation, and removal of metabolic wastes.

STRUCTURE:

13.1 INTRODUCTION:

A number of byproducts are produced in the animal body as a result of carbohydrates, protein and fat metabolism. Some of these may be fatal. Therefore, they are either to be eliminated or are to be converted into less toxic substances. Thus, the removal of such waste products becomes necessary. On the other handsome of the metabolic by product perform useful functions in the body for example water, is a metabolic byproduct and is excreted out in large amount as urine. In some other forms, metabolic water is the only available source of water, so it is rigorously conserved. Similarly, carbon dioxide serves as an important component in the synthetic and regulatory mechanism in animals and plants. The same is true with urea which is one of the main excretory end products in a large number of animals but discharges some useful physiological functions in many other forms. In a healthy man the normal blood urea level is 172euroco. 0.01to 0.04percent and if it rises to about 0.05percent, a serious pathological state

of uremia develops. Excretion has an important role in maintaining homeostasis in the body. If excretory system any how fails, waste products will accumulate in the body, causing disturbance in osmoregulation, ionic regulation, and acid- base balance and finally death may occur. All living organisms need organic raw materials to build up most of their own body molecules, and they require energy to operate the metabolic reactions that sustain life. All organisms obtain their nutrients as food from their surrounding or habitat. Depending on the quantity and functions nutrients are classified as macronutrients and micronutrients.

Macronutrients e.g. carbohydrates, proteins and lipids are taken in large amount and required to produce energy and for growth and repair. Micronutrients e.g. vitamins and minerals do not provide energy and are required in very small amount but they are essential regulatory components of food, their deficiency can cause specific disease. Animals obtain their nutrients through a broad variety of feeding patterns. Herbivores eat plant material. Carnivorous animals like those in the cat and dog families, polar bears, seals, crocodiles and birds of prey catch and eat other animals. Many animals feed on both animal and vegetable material they are omnivorous. There are currently two similar definitions of omnivores. The process of conversion of complex food substances to simple absorbable forms is called digestion.

Digestion in animals is carried out by means of mechanical and chemical processes. Mechanical digestion comprises mastication, swallowing and churning of food in stomach. Chemical digestion of food is achieved by different types of hydrolytic digestive enzymes. These enzymes are secreted by salivary glands, gastric glands, pancreas and intestinal glands in their secretions. End products of digestion are absorbed through the intestinal mucosa into the blood or depending upon the form in which nitrogenous waste is excreted from the body, The organisms are grouped as under into three categories: Ammonotelic, Uricotelic and Ureotelic.

All aquatic invertebrates, bony fishes and aquatic amphibians are ammonotelic organisms. All terrestrial animals like insects, reptiles, and birds excrete uric acid as nitrogenous wastes. Those animals that excrete their nitrogenous waste mainly in the form of urea are known as ureotelic and the phenomenon is known as ureotelism. Ureotelic animals include Ascaris, earthworm (both are ammonotelic and ureotelic), cartilaginous fishes like sharks and sting rays, semi-aquatic amphibians such as frogs and toads, aquatic or semi aquatic reptiles like turtles, terrapins and alligators, and man and all other mammals. Other excretory wastes include guanine, creatine, creatinine, TMO, Ornithuric acid, hippuric acid, bile pigments, hormones and drugs. Excretion in humans occurs through the kidney, sweat glands, lungs, liver, skin and alimentary canal. Kidneys are the chief excretory organs and are mainly concerned with the excretion of urea in the form of urine. Liver converts toxic ammonia (NH3) into much less toxic urea which is excreted in urine. Urea is the end product of protein metabolism (amino acid metabolism). Urea is synthesized in liver and transported to kidneys for excretion in urine.

Urea is produced through urea cycle or Krebs-Henseleit cycle or Ornithine cycle. In Human beings urine is formed by kidneys. There are three major processes of urine formation in human body.

13.2 FUNCTIONS OF KIDNEY:

We take in a lot of things in our body the whole day. They need to be simultaneously excreted out of the body to maintain the body's steady state. This process of removal of chemical wastes from our body is known as **excretion**. There are a number of substances that our body needs

to get rid of. Some of them are carbon dioxide, nitrogenous wastes, excessive salts and vitamins, water and bile pigments. Excretion in humans occurs through the **kidney**, sweat glands, and lungs etc.

Kidney

Kidneys are the chief excretory organs and are mainly concerned with the excretion of urea in the form of urine. The function of our kidney is monitored and regulated by the feedback Mechanisms which involve hypothalamus, juxtaglomerular apparatus, and the heat. Human Kidneys are located at about the T12 to L3 vertebrae, whereas the right is lower due to slight displacement by the liver. Each kidney weighs about 125-175 g in males and 115-155 g in Females. They are about 11-14 cm in length, 6 cm wide, and 4 cm thick, and are directly covered by a fibrous capsule. A frontal section through the kidney reveals an outer region called the renal cortex and an inner region called the medulla. The renal columns are connective tissue Extensions that radiate downward from the cortex through the medulla to separate the most characteristic features of the medulla, the renal pyramids and renal papillae. The papillae are bundles of collecting ducts that transport urine made by nephrons to the calyces of the kidney for excretion. The renal columns also serve to divide the kidney into 6-8 lobes and provide a supportive framework for vessels that enter and exit the cortex. The, pyramids and renal columns taken together constitute the kidney lobes.

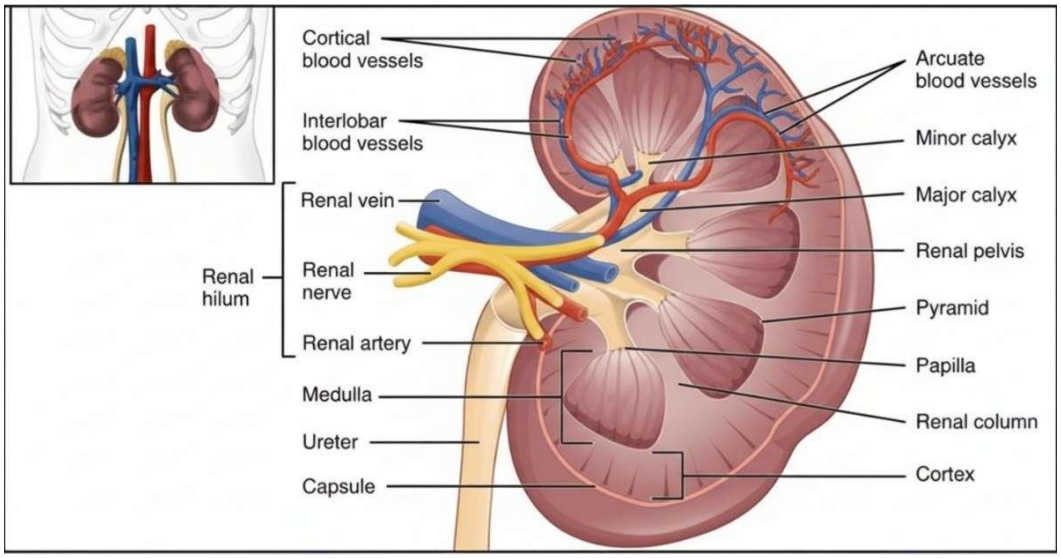

Figure 1: kidney

When there is excessive loss of fluid from the body, osmoreceptors are activated which stimulate the hypothalamus to release ADH helps in reabsorption of water from the latter parts of the tubule and prevents the loss of water from the body. When the fluid volume of body increases, osmoreceptors are switched off and the release of ADH is suppressed. ADH may also increase the glomerular blood flow. When the glomerular blood flow decreases, juxtaglomerular cells release renin which converts angiotensin in blood to angiotensin I which is further converted to angiotensin II. This causes an increase blood to angiotensin I. It is further converted to angiotensin II. This causes an increase in glomerular blood pressure.

Another function of angiotensin II is activation of the adrenal cortex to release aldosterone which causes reabsorption of sodium ion and water from the distal parts of the tubule. his also leads to the release of antidiuretic hormone (ADH) from the neurohypophysis. This hormone

increases water reabsorption in the kidneys, which raises blood volume and thereby increases blood pressure. Another function of angiotensin II is activation of the adrenal cortex to release aldosterone which causes reabsorption of sodium ion and water from the distal parts of the tubule. This also leads to antidiuretic hormone from neuro-hypophysis. This warm blood pressure thereby increasing the increase in glomerular blood pressure. It converts angiotensin in hormone an increase in blood pressure and glomerular filtration rate. This entire mechanism is known as the renin-angiotensin mechanism.

The Atrial Natriuretic Factor (ANF) is released when there is an increase in blood flow to the atria of the heart. It can cause a decrease in blood pressure by dilating blood vessels.

Bowman's capsule is invaginated by a tuft of some 40-50capillary loops, each covered by special epithelial cells known as Podocytes. Podocytes have slit pores through which ultra filtration occurs and forms the Glomerulus. The glomerular capillary tuft surrounded by its capsule is termed as Malpighian corpuscle. Blood enters the capsule through afferent renal arteriole and after passing through glomerular capillary network it is collected by the efferent renal arteriole.

Renal tubule: The long portion of the nephron following the Bowman's capsule is termed as renal tubule. It is divided into the following regions-

1. The proximal convoluted tubule,
2. The loop of Henle (Descending limb and ascending limb)
3. The distal convoluted tubule
4. Collecting duct

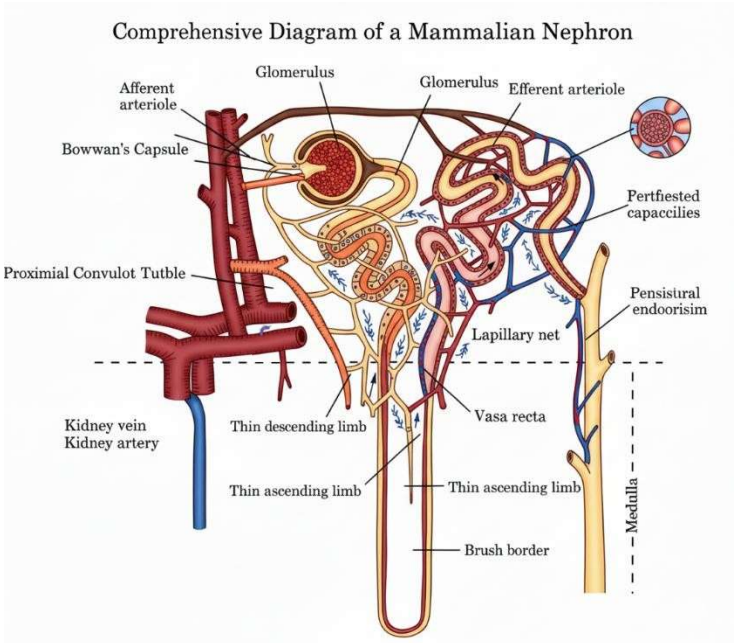

Figure 2: Structure of single kidney tubule and its blood vessels

13.3 TYPES OF NEPHRONS IN MAMMALIAN KIDNEY:

1. Cortical Nephrons:

The nephrons situated in cortical region are, called cortical nephrons. In cortical nephrons, loop of Henle is short. In a kidney 15 to 35% nephrons are cortical nephrons.

2. Juxtamedullary nephrons:

Nephrons situated near medulla part are, called juxtamedullary nephrons It is formed by the DCT and glomerular afferent arteriole. It is so named because it lies next (juxta)to the glomerular. In juxtamedullary nephrons, loop of Henle is long and parallel blood vessels, called vasa rectae are present. In a kidney 65 to 85% nephrons are juxtamedullary nephrons. It works only in condition of stress. It consists of three types of cells.

I. **The macula densa**, a part of the distal convoluted tubule of the same nephron. It acts as a chemoreceptor and are stimulated by decreased NaCl concentration and thereby cause release of rennin.
II. **Juxtaglomerular cells** or granular cells which secrete rennin. These cells are smooth muscle cells of afferent arteriole which supply blood to the glomerulus. They are baroreceptors and respond to changes in the pressure gradient. They are innervated by sympathetic nerves.
III. **Extraglomerular mesangial cells (Laci's cells)** These cells are located at the junction between afferent and efferent arterioles. They are contractile and play a role in regulation of GFR.

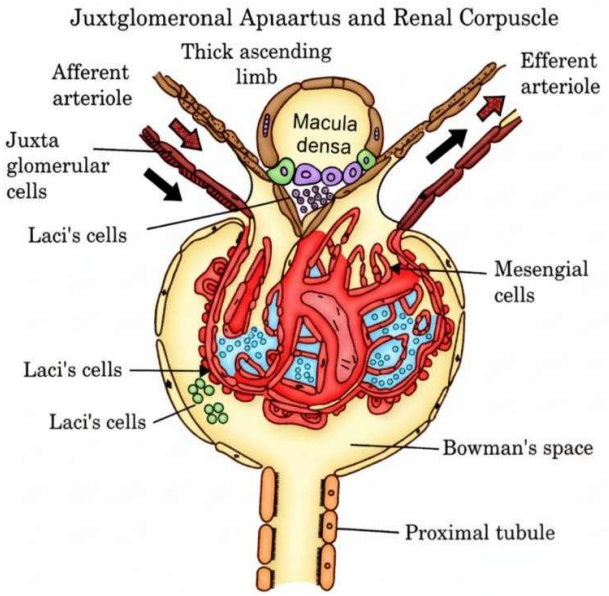

Figure 3: Juxtaglomerular apparatus and renal 176eurocoel

13.4 TYPES OF NITROGENOUS WASTE IN DIFFERENT ANIMALS:

Excretory products and their types

Metabolism of carbohydrates and fats produces CO2and H2O which are easy to remove. They are effectively removed through lungs (expired air), skin (sweat) or kidneys (urine). Other excretory products such as bile pigments (formed by the breakdown of RBCs), drugs etc. are removed in liver. Metabolism of proteins produces nitrogenous wastes such as ammonia, which is the basic nitrogenous catabolites of protein, formed by breakdown of amino acids is finally removed from Kidney. Depending upon the form in which nitrogenous waste is excreted from the body, the organisms are grouped as under into three categories: **Ammonotelic**, **Uricotelic** and **Ureotelic**

1. Ammonotelic organism:

Those animals which excrete their nitrogenous waste in the form of ammonia are known as **ammonotelic**. Ammonia is highly soluble in water with which it forms ammonium hydroxide

(NH4OH) which can damage cells directly by its alkaline caustic action. Excretion of ammonia requires large amounts of water, so that more water loss from the body. That is why such a mode is suitable for aquatic organisms which have a constant access to water. Ammonia is the first metabolic waste product of protein metabolism and no energy is required to produce ammonia. All aquatic invertebrates, bony fishes and aquatic amphibians are ammonotelic organisms.

2. Uricotelic organism:

Those animals which excrete their nitrogenous waste mainly in the form of uric acid and urates are known as **uricotelic**. The phenomenon is known as uricotelism. Elimination of uric acid requires lesser amount of water, comparatively less soluble in water and is less toxic as compared to ammonia. All terrestrial animals like insects, reptiles, and birds excrete uric acid as nitrogenous wastes.

3. Ureotelic organism:

Those animals that excrete their nitrogenous waste mainly in the form of urea are known as **ureotelic** and the phenomenon is known as ureotelism. Ureotelic animals include *Ascaris*, earthworm (both are ammonotelic and ureotelic), cartilaginous fishes like sharks and sting rays, semi-aquatic amphibians such as frogs and toads, aquatic or semi aquatic reptiles like turtles, terrapins and alligators, and man and all other mammals. Urea is less toxic and less soluble in water than ammonia. Hence, it can stay for some time in the body. Sharks retain large quantity of urea in their blood, therefore, blood osmotic pressure approaches that of sea water, which Minimizes water loss from their body.

Other excretory waste:

1. **Guanine:** Spiders excrete guanine and are said to be guanotelic and their mode of excretion is called guanotelism.
2. **Creatine and Creatinine:** Muscle cells contain molecules of creatine phosphate, which are highly energy molecules and serve for storage of bioenergy like ATP. Excess amount of this phosphate is however, excreted out as such, or after being changed into creatinine. The latter is passed out through urine.
3. **Tri-methylamine-oxide (TMO):** Marine teleost fishes excrete a large proportion of their nitrogen as trimethylamine oxide (TMO). Large amounts of this compound is also stored in their body for osmoregulation, (i.e., to minimize loss of water and entry of salts).
4. **Ornithuric acid:** It is excreted in small amount by birds and is formed by a combination of benzoic acid (formed during fat metabolism) with the amino acid ornithine. **Hippuric acid-** It is formed when benzoic acid is combined with glycine. It is less toxic.
5. **Bilirubin and Biliverdin:** These are the bile pigments which are formed in the liver due to breakdown of hemoglobin of worn-out RBCs. These are excreted through bile. In jaundice, level of bilirubin is high in the blood resulting yellow skin, white eyes, etc.
6. **Allantoin:** It is formed from uric acid as a result of an oxidation reaction catalyzed by the enzyme uricase. Higher primates including man do not have enzyme uricase. Allantoin is an excretory product of embryos of amniotes. In a very young embryo, the excretory matter is stored in allantois.
7. **Bile Salts:** Bile salts are the sodium and potassium salts of bile acids, which are conjugated with glycine or taurine. The bile acids (glycocholic acid and taurocholic acid) are derived from cholesterol.
8. **Heavy metals**: The liver also excretes heavy metals like lead, arsenic and bismuth. The other substances excreted in bile are heavy metals such as copper and iron, some toxins, some bacteria like typhoid bacteria, cholesterol, lecithin and alkaline phosphatase. Heavy metals and drugs are also excreted in the saliva.

9. **Carbon Dioxide and Water:** It is mainly expelled out by lungs. Some carbon dioxide is also excreted through sweat and defecation. Excess of water is a waste product and is eliminated in urine, feces, sweat and expired air.
10. **Drugs, Hormones and Other Substances:** The liver is well known for its ability to detoxify or excrete into bile many drugs, including sulfonamides, penicillin, ampicillin and erythromycin. Several hormones secreted by the endocrine glands are either chemically altered or excreted by the liver, including thyroxine and essentially all the steroid hormones such as estrogen, cortisol and aldosterone. The excess of water-soluble vitamins like vitamin B complex and vitamin C is removed from the body in urine. Sebaceous glands (oil glands) secrete an oily secretion called sebum that contains some lipids such as sterols, other hydrocarbons and fatty acids. Sudoriferous glands (sweat glands) in the skin and gastrointestinal tract also expel heat which is the result of various metabolic processes.

13.5 EXCRETORY ORGANS:

1. Invertebrate Excretory Organs:

Many invertebrates such as flatworms use a nephridium as their excretory organ. At the end of each blind tubule of the nephridium is a ciliated flame cell. As fluid passes down the tubule, solutes are reabsorbed and returned to the body fluids.

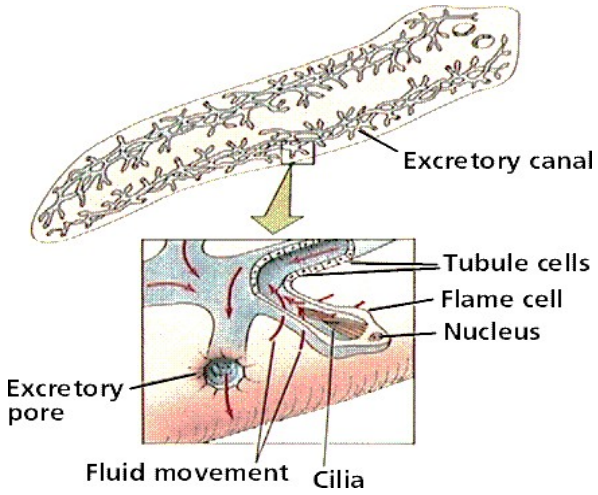

Figure 4: Excretory system of a flatworm

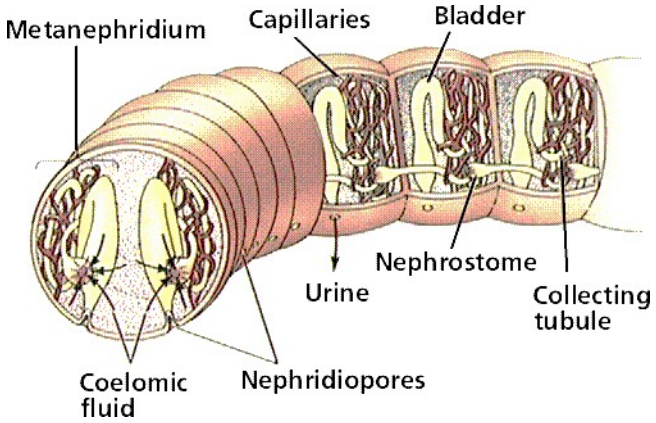

Figure 5: Excretory system of an earthworm

Body fluids are drawn into the Malpighian tubules by osmosis due to large concentrations of potassium inside the tubule. Body fluids pass back into the body; nitrogenous wastes empty into the insect's gut. Water is reabsorbed and waste is expelled from the insect.

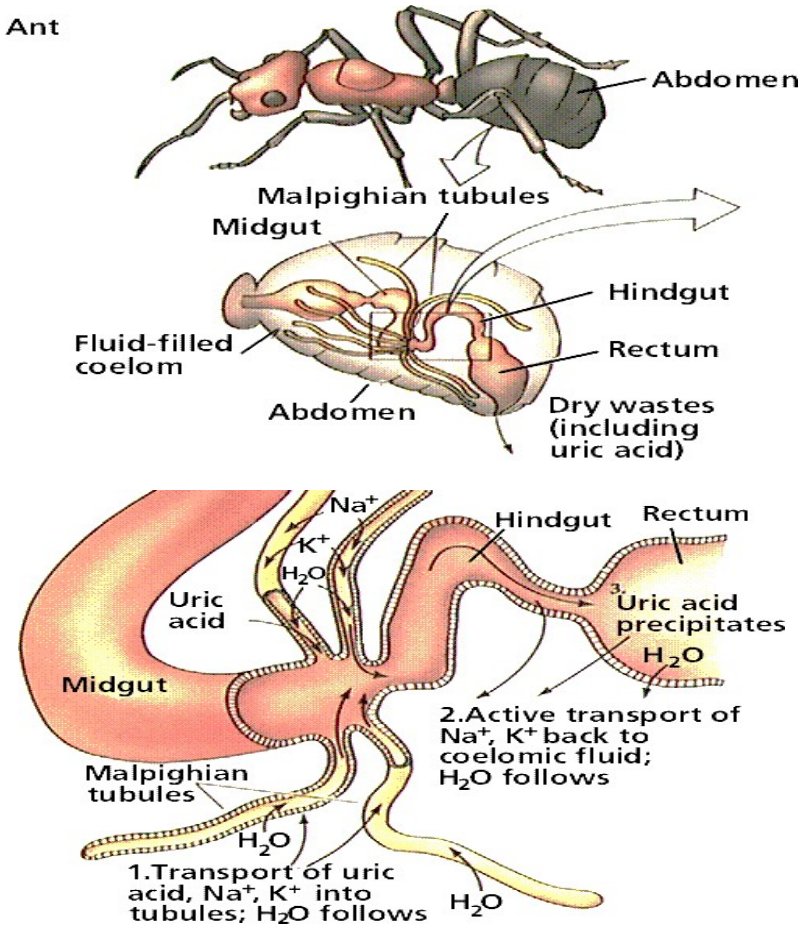

Figure 6: Excretory system of an Ant

2. Vertebrate Excretory Organs:

All vertebrates have paired kidneys. Excretion is not the primary function of kidneys. Kidneys regulate body fluid levels as a primary duty, and remove wastes as a secondary one.

The Human Excretory System

The urinary system is made-up of the kidneys, ureters, bladder, and urethra. The nephron, an evolutionary modification of the nephridium, is the kidney's functional unit. Waste is filtered from the blood and collected as urine in each kidney. Urine leaves the kidneys by ureters, and collects in the bladder. The bladder can distend to store urine that eventually leaves through the urethra.

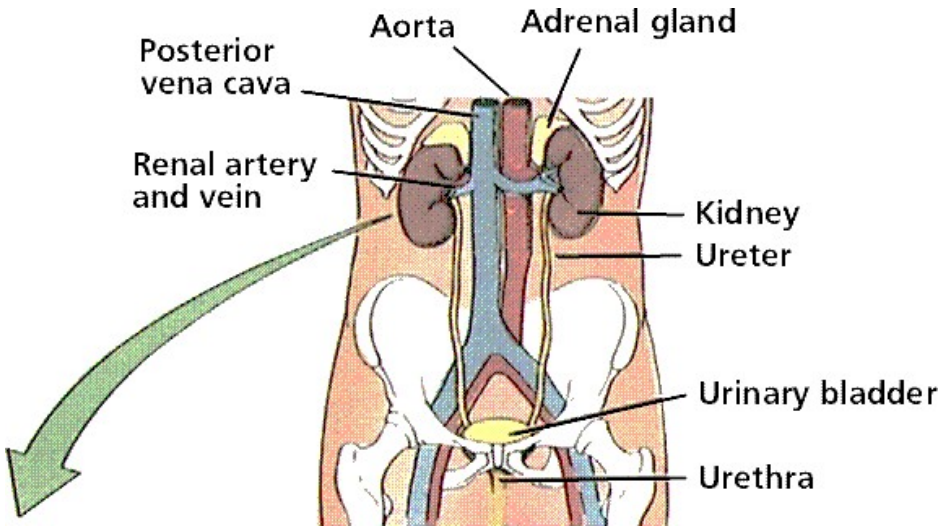

Figure 7: Excretory system of a Human

13.6 UREA PRODUCTION:

13.6.1 Krebs Hensleit cycle (ornithine cycle):

Liver converts toxic ammonia (NH3) into much less toxic urea which is excreted in urine. Urease the end product of protein metabolism (amino acid metabolism). Urea is synthesized in liver and transported to kidneys for excretion in urine. Urea is produced through urea cycle which was discovered by Hans Krebs and Kurt Henseleit (1932), hence it is known as **Krebs-Henseleit cycle or Ornithine cycle**. The individual reactions, however, were described in more detail later on by Ratner and Cohen. Urea has two amino (-NH2) groups, one derived from NH3 and the from aspartate. Carbon atom is supplied by CO2. Urea cycle includes five steps involving five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol (the cytoplasm minus the mitochondria and endoplasmic reticulum).

1. **Synthesis of Carbamoyl Phosphate:** Carbamoyl phosphate synthase 1 (CPS 1) of mitochondria catalyzes the condensation of NH4+ ions with CO2 to form carbamoyl phosphate. This step consumes two ATPs.
2. **Formation of Citrulline:** Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarboxylase. Ornithine is regenerated and used in urea cycle. Ornithine and citrulline are basic amino acids.
3. **Synthesis of arginosuccinate:** Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. This step requires ATP.
4. **Cleavage of arginosuccinate:** Arginosuccinate cleaves arginosuccinate to give arginine and fumarate. Fumarate liberated here provides a connecting link with Krebs cycle, gluconeogenesis
5. **Formation of Urea:** Arginase is the fifth and final enzyme that cleaves arginine to form urea and ornithine. This ornithine enters mitochondria for its reuse in the urea cycle. The urea cycle (also called ornithine cycle) is irreversible.

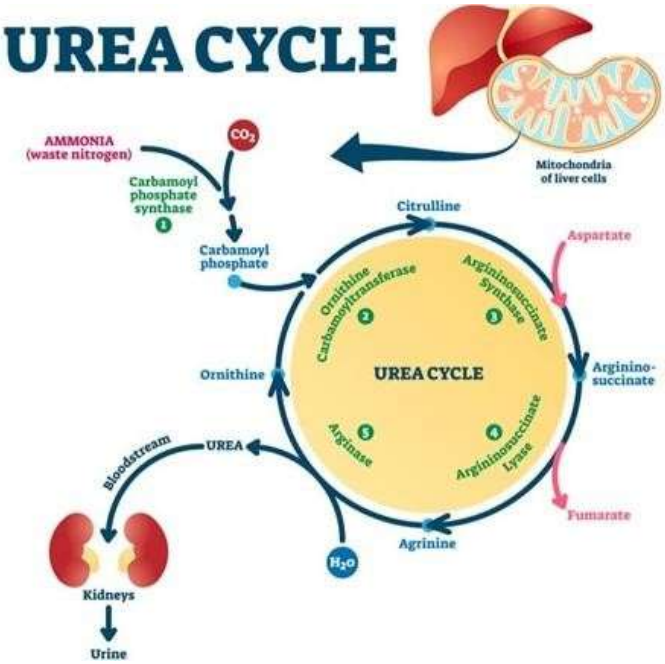

Figure 8: Mechanism of Urea formation (Krebs-Henseleit cycle)

13.7 URINE FORMATION:

In Human beings urine is formed by kidneys. Each human kidney consists of about one million of fine convoluted tubules called uriniferous tubules or nephrons. There are three major processes of urine formation in human body.

- Glomerular Filtration or Ultrafiltration
- Tubular Reabsorption or Selective reabsorption
- Tubular Secretion

1. Glomerular Filtration (=Ultrafiltration):

On an average 1100-1200 ml of blood is filtered by the kidneys per minute. The glomerular capillaries are narrower than the afferent renal arterioles. Therefore, the blood pressure in the glomerular capillaries becomes very high so that there is continuous process of ultrafiltration (filtration under pressure) through the semi-permeable glomerular capillaries. Thus, water and many dissolved substances from the blood are filtered into the lumen of the Bowman's capsule through its walls. The glomerular filtrate contains a large amount of water and other dissolved sub-stances such as urea, uric acid, creatinine, amino-acids, glucose, sodium, potassium, vitamins, etc. Nephrons filter 125 ml of body fluid per minute; filtering the entire body fluid component 16 times each day. In a 24-hour period nephrons produce 180 liters of filtrate, of which 178.5 liters are reabsorbed. The remaining 1.5 liters forms urine.

Regulation of glomerular filtration: An increase in blood pressure tends to stretch the afferent arteriole which increases the blood flow to the glomerulus. When the wall of the arteriole contracts, the diameter of the afferent arteriole is reduced that increases the flow of blood. Juxtaglomerular apparatus (JGA) cells secrete enzymes like renin that modulate blood pressure and thus renal blood flow. This regulates GFR. Blood vessels of the kidney are innervated by nerve fibers of the sympathetic neural system. When activated, the nerve fibers bring about constriction of renal arteries and cause decrease in renal flow and glomerular filtration rate.

2. Tubular Reabsorption:

From the Bowman's capsule, the glomerular filtrate enters the proximal convoluted tubule. Absorption of selected materials takes place from the filtrate into the blood of the peritubular capillaries or vasa recta. It is termed the tubular reabsorption. Reabsorption involves both passive and active transport across the tubular epithelium. As already stated, the glomerular filtrate in the Bowman s capsule resembles blood plasma in composition except for plasma proteins and fats. Therefore, it is almost isotonic to the plasma.

Selective reabsorption at Proximal Convoluted Tubule (PCT): About 65 per cent of the glomerular filtrate is normally reabsorbed in the proximal convoluted tubule before reaching the loop of Henle. Glucose, amino acids, vitamins, hormones, sodium, potassium, chlorides, phosphates, bicarbonates, much of water and some urea from the filtrate are absorbed. Sulphates and creatinine are not reabsorbed. Sodium and potassium are reabsorbed by primary active **Selective reabsorption at loop of Henle:** It consists of descending limb and ascending limb.

- **Descending limb of loop of Henle:** As the filtrate flows in it, its water is reabsorbed due to increasing osmolality of interstitial fluid. Sodium and other solutes are not reabsorbed here. The filtrate becomes hypertonic to blood plasma.
- **Ascending limb of loop of Henle:** It is impermeable to water but permeable to K^+, Cl^- and Na^+ and partially permeable to urea. Thus, in the thick ascending limb of the loop of Henle sodium, potassium, calcium, magnesium, and chloride are reabsorbed. The filtrate becomes hypotonic to blood plasma.

Selective reabsorption at Distal convoluted tubules (DCT): There is active reabsorption of sodium ions from the filtrate under the influence of aldosterone (hormone secreted by the cortex of adrenal glands). Chloride ions are also reabsorbed in the distal convoluted tubules. Water is reabsorbed here under the influence of antidiuretic hormone (ADH) secreted by posterior lobe of pituitary gland. This makes the filtrate isotonic to blood plasma.

Selective reabsorption at collecting duct: A considerable amount of water is reabsorbed in the collecting duct under the influence of ADH. Sodium is reabsorbed in the collecting duct under the influence of aldosterone. The filtrate is now called urine. Thus, urine is hypertonic to blood and isotonic to medullary fluid.

3. Tubular Secretion:

The cells of the renal tubule no. only remove substances from the filtrate by the process of re-absorption and send them to the blood capillaries (peritubular) but also excrete additional wastes from the blood stream into the filtrate by the process of secretion. Thus, tubular secretion is the opposite of tubular reabsorption. It occurs as follows: Creatinine, hippuric acid, pigments, drugs including penicillin are actively secreted into the filtrate in the proximal convoluted tubule from the interstitial fluid. Hydrogen ions and ammonia are also secreted into the proximal convoluted tubule. Urea enters the filtrate by diffusion in the thin segment of the ascending limb of loop of Henle. Potassium, hydrogen ions, ammonia, HCO3- ions are secreted by active transport into the filtrate in the distal convoluted tubule. Maximum hydrogen secretion occurs in the proximal convoluted tubule. Removal of hydrogen ions and ammonia from the blood in the proximal convoluted tubule and distal convoluted tubule helps to maintain the pH of the blood between 6 to 8 (pH of blood is usually 7.4). Tubular secretion probably plays a minor role in the function of human kidneys but in animals like marine fishes and desert amphibians, whose nephrons do not possess developed glomeruli, their urine is formed mainly by the tubular secretion of urea, creatinine and mineral ions. Kidneys excrete about 1.5 liters of urine in a day.

- Glomerular filtration
- Tubular reabsorption
- Tubular secretion-Kidneys produce about 1.5 liters of urine in a day. Human is urine is slightly acidic (Ph 6.8) and pale yellow in color due to presence of urochrome (urobilin's).

Cells produce water and carbon dioxide as by-products of metabolic breakdown of sugars, fats, and proteins. Chemical groups such as nitrogen, sulfur, and phosphorous must be stripped, from the large molecules to which they were formerly attached, as part of preparing them for energy conversion. The continuous production of metabolic wastes establishes a steep concentration gradient across the plasma membrane, causing wastes to diffuse out of cells and into the extracellular fluid.

Single-celled organisms have most of their wastes diffuse out into the outside environment. Multicellular organisms, and animals in particular, must have a specialized organ system to concentrate and remove wastes from the interstitial fluid into the blood capillaries and eventually deposit that material at a collection point for removal entirely from the body.

Regulation of Extracellular Fluids Excretory systems regulate the chemical composition of body fluids by removing metabolic wastes and retaining the proper amounts of water, salts, and nutrients. Components of this system in vertebrates include the kidneys, liver, lungs, and skin. Not all animals use the same routes or excrete their wastes the same way humans do. Excretion applies to metabolic waste products that cross a plasma membrane. Elimination is the removal of feces.

Nitrogen Wastes:
Nitrogen wastes are a byproduct of protein metabolism. Amino groups are removed from amino acids prior to energy conversion. The NH_2 (amino group) combines with a hydrogen ion (proton) to form ammonia (NH_3). Ammonia is very toxic and usually is excreted directly by marine animals. Terrestrial animals usually need to conserve water. Ammonia is converted to urea, a compound the body can tolerate at higher concentrations than ammonia. Birds and insects secrete uric acid that they make through large energy expenditure but little water loss. Amphibians and mammals secrete urea that they form in their liver. Amino groups are turned into ammonia, which in turn is converted to urea, dumped into the blood and concentrated by the kidneys.

Water and Salt Balance:
The excretory system is responsible for regulating water balance in various body fluids. Osmoregulation refers to the state aquatic animals are in: they are surrounded by freshwater and must constantly deal with the influx of water. Animals, such as crabs, have an internal salt concentration very similar to that of the surrounding ocean. Such animals are known as Osmo conformers, as there is little water transport between the inside of the animal and the isotonic outside environment. Marine vertebrates, however, have internal concentrations of salt that are about one-third of the surrounding seawater. They are said to be osmoregulatory. Osmo regulators face two problems:

prevention of water loss from the body prevention of salts diffusing into the body.
Fish deal with this by passing water out of their tissues through their gills by osmosis and salt through their gills by active transport. Cartilaginous fish have a greater salt concentration than seawater, causing water to move into the shark by osmosis; this water is used for excretion. Freshwater fish must prevent water gain and salt loss. They do not drink water, and have their

skin covered by a thin mucus. Water enters and leaves through the gills and the fish excretory system produces large amounts of dilute urine. Terrestrial animals use a variety of methods to reduce water loss: living in moist environments, developing impermeable body coverings, production of more concentrated urine. Water loss can be considerable: a person in a 100-degree F temperature loses 1 liter of water per hour.

Excretory System Functions: Collect water and filter body fluids.

1. Remove and concentrate waste products from body fluids and return other substances to body fluids as necessary for homeostasis.
2. Eliminate excretory products from the body.

13.8 SUMMARY:

The excretory system plays a vital role in maintaining homeostasis by removing harmful metabolic wastes, regulating water balance, and maintaining ionic equilibrium in the body. The kidney, as the chief excretory organ in vertebrates, filters blood through nephrons and forms urine via glomerular filtration, tubular reabsorption, and tubular secretion. Nitrogenous wastes are excreted in different forms ammonia, urea, uric acid, and guanine depending on the organism's habitat and adaptation, classifying them as ammonotelic, ureotelic, uricotelic, or guanotelic. Urea is synthesized through the ornithine (Krebs-Henseleit) cycle in the liver and excreted by kidneys. Excretion in invertebrates occurs through specialized structures such as flame cells, nephridia, and Malpighian tubules, while vertebrates rely primarily on kidneys. Hormonal control, particularly by ADH, aldosterone, renin, and atrial natriuretic factor, regulates water and salt reabsorption to balance blood pressure and glomerular filtration. Thus, the excretory system integrates with circulatory and endocrine systems to ensure survival across diverse environments.

13.9 TECHINICAL TERMS:

Anti-diuretic, Bowman's capsule, Collecting tubule, Creatinine, Deamination, Diuretic, Glomerulus, Loop of Henle, Guanotelism.

13.10 SELF-ASSESSMENT QUESTIONS:

1. Describe the mechanism of digestion and absorption of fat in mammals.
2. Explain how different components of food are absorbed in alimentary canal?
3. Describe the structure and function of human kidneys.
4. Give a detailed account of types of nitrogenous wastes and their elimination in different animals.
5. Describe the mechanism of urine formation in mammals.
6. Describe the role of different parts of nephron in urine formation.

13.11 SUGGESTED READINGS:

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- **Prof. K. SUMANTH KUMAR**

LESSON- 14

OSMOREGULATION

OBJECTIVES:

- To study the mechanisms of osmoregulation
- To compare Osmo conformers and osmoregulatory
- To relate osmoregulation with survival and homeostasis

STRUCTURE:

14.1 INTRODUCTION:

When two aqueous solutions of different concentrations are separated by a semipermeable membrane that allows water but not solute to pass, water moves from the solution with lower solute concentration (hypotonic) to the solution with higher solute concentration (hypertonic) until the concentrations on both sides become equal (isotonic). This movement of water across a semipermeable membrane is called osmosis**.**

All living organisms maintain proper water and salt balance in their bodies through specialized mechanisms, collectively termed osmoregulation. The term “osmoregulation” was first coined by Hober to describe the various processes that control water movement and volume in organisms. It is not enough to regulate water alone; the internal ionic or molecular concentration must also be controlled, as there are large differences in ionic concentrations in various parts of the body. The processes that maintain internal ionic balance are called ionic regulation**,** while the regulation of external ionic concentrations is inseparable from internal regulation. Therefore, osmoregulation includes both water balance and ionic balance.

Osmoregulation is closely linked to other homeostatic functions**,** such as Ph and temperature regulation, because these also depend on maintaining proper water and ionic concentrations in the body.

14.2 ISOTONIC, HYPOTONIC AND HYPERTONIC ANIMALS:

Depending on their osmoregulatory mechanisms, animals can be classified as:

1. **Isotonic(or)Isosmatic:**

The concentration of body fluids in these animals is the same as that of their surrounding medium. They do not face significant problems of osmoregulation as long as they remain in that medium. Most marine animals are isotonic because their body fluid concentration closely resembles that of seawater.

2. **Hypotonic(or)Hypoosmotic:**

Animals living in a medium with lower salt concentration face excess hydration, as water continuously enters their bodies and dilutes their body fluids. Such animals have evolved special mechanisms to remove the excess water. For example, freshwater protozoans and crustaceans eliminate extra water through contractile (pulsating) vacuoles and their excretory organs.

3. **Hypertonic(or)Hyperosmatic:**

Animals living in a medium with higher salt concentration face the risk of dehydration, as water tends to leave their body. These animals evolve regulatory mechanisms to conserve water. For instance, teleost fishes in seawater constantly lose water to their surroundings and must actively compensate for this water loss.

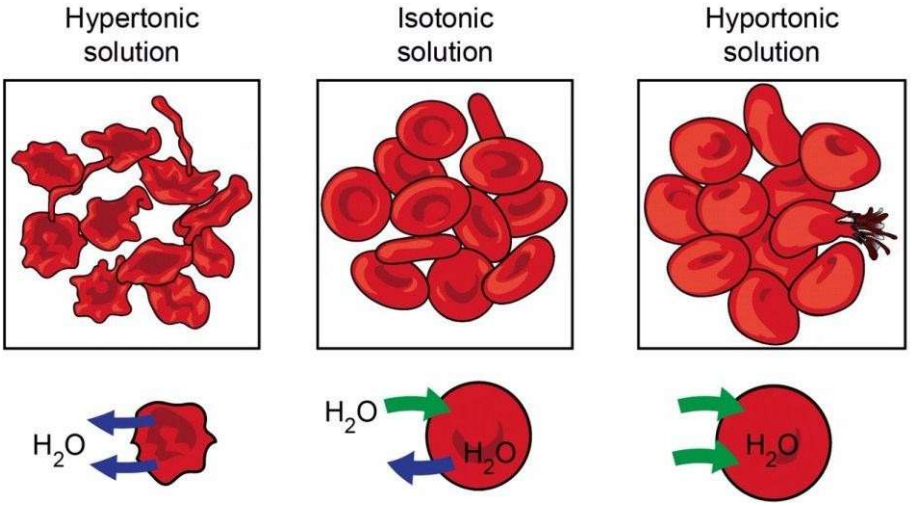

Figure 1: a) Cells placed in a hypertonic environment tend to shrink due to loss of water; b) the blood maintains an isotonic environment so that cells neither shrink nor swell in a hypotonic environment; c) cells tend to swell due to intake of water

14.3 ANIMALS RESPONSE TO OSMOTIC CONDITIONS OF THE MEDIUM:

Animals exhibit two patterns of response to osmotic conditions of environment for their survival.

- Animals may be osmotically dependent and their body fluid concentration change according to the medium. Such animals are called as osmocon formers or poikilosmotic. These animals can tolerate wider variation in their internal osmotic concentration.
- Animals which are osmotically stable or independent i.e. when concentration of the medium changes, the internal concentration of the body fluid remain unchanged. Such animals are called as osmoregulators or homeosmotic. These animals can withstand a wider environment range.

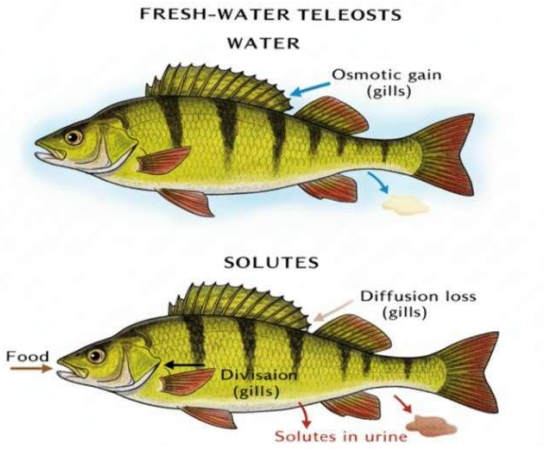

Figure 2: osmotic regulation

14.4 POIKILOSMOTIC AND HOMEOSMOTIC ANIMALS:

1. Poikilosmotic Animals:

These are aquatic invertebrates whose tissues can tolerate dilutions of body fluids and adjust their internal medium to match the external environment. They are Osmo conformers internal osmotic concentration changes with external salinity.

a. Stenohaline Animals

- Poikilosmotic animals that tolerate only slight changes in salinity.
- Example: The marine spider crab (*Maia*) gains weight when transferred to 80% seawater due to rapid water entry. Although its excretory organs eliminate excess water via dilute urine, salts are lost too, making body fluids even more dilute. Since the crab cannot replenish salts, it dies within ~18 hours.
- Another example: *Arenicola* (marine worm).

b. Euryhaline Animals

- Poikilosmotic animals that can withstand wide fluctuations in salinity.
- They commonly live in estuaries, river mouths, or seashores were salinity changes
- greatly.
- Example: The sea mussel (*Mytilus*) tolerates seawater dilution up to 4% and survives.
- Other examples: *Phascolosoma* (sipunculid worm), *Aurelia* (jellyfish), and *Aplysia* (sea hare).

2. Homeosmotic Animals (Osmostable):

- These are regulators that maintain a stable osmotic concentration of body fluids independent of external salinity.
- They are also called osmoregulators.
- Examples include most freshwater animals and many vertebrates.

Osmoregulatory adaptations in freshwater homeosmotic animals:

1. Presence of an impermeable cuticle prevents excessive water entry.
2. Restriction of semipermeable surfaces to small areas (respiratory and digestive surfaces).

3. Development of excretory organs to produce hypotonic urine (expel excess water).
4. Possession of special cells (e.g., chloride cells in gills) to actively absorb salts from the surrounding medium to compensate for salt loss through urine.
5. Tolerance of dilute body fluids minimizes metabolic energy expenditure.

14.5 MECHANISM OF OSMOREGULATION:

14.5.1 Osmoregulation in Freshwater Animals:

1. **General Mechanism:** Freshwater animals live in a hypotonic medium where their body fluids are more concentrated than the surrounding water. Consequently, water tends to enter their bodies by osmosis while salts are lost to the environment. To regulate this imbalance, freshwater animals eliminate the excess water through excretory structures and replenish the lost salts by absorbing them through gills, skin, and parts of the alimentary canal.

2. **Protozoa:** In freshwater protozoans such as *Amoeba, Euglena,* and *Paramecium*, osmoregulation is carried out by the contractile vacuole. This organelle rhythmically contracts to expel excess water that enters the cell, thereby preventing the dilution of body fluids. Parasitic protozoa that live in isotonic conditions, however, do not possess a contractile vacuole.

3. **Crustacea:** In freshwater crustaceans like *Palaemon*, the blood remains hypertonic to the external medium, causing water to diffuse inward through permeable gills. The excess water is eliminated by antennary or green glands. Since salts are lost along with urine, chloride cells located in the gills actively absorb sodium, potassium, and chloride ions from the surrounding medium, even when present in very low concentrations.

4. **Mollusca:** Freshwater mussels such as *Unio* and *Anodonta* maintain a higher osmotic pressure than the external environment, resulting in water entering their tissues by osmosis. This water is removed by the kidneys, which excrete it as dilute urine.

5. **Teleosts:** Freshwater teleosts or bony fishes also live in a hyperosmotic state relative to their environment. Water continuously enters their bodies through the skin, gills, and the lining of the buccal cavity. Their kidneys are highly developed and secrete large amounts of dilute urine, while simultaneously reabsorbing useful solutes. The chloride cells in the gills further aid in maintaining salt balance by absorbing ions from the external medium.

6. **Elasmobranchs:** Certain freshwater elasmobranchs such as *Pristis* and *Carcharhinus giganteus* retain a small quantity of urea in their blood and tissues, which raises their osmotic concentration. As a result, water enters their bodies, but it is efficiently removed by the large glomerular kidneys.

7. **Amphibia:** Freshwater amphibians, particularly frogs, face the problem of water entry through their smooth and permeable skin. This excess water is eliminated in the form of dilute urine by the kidneys. Any salts lost through urine are compensated for by reabsorption from the urine itself and through absorption of ions directly from the environment across the skin.

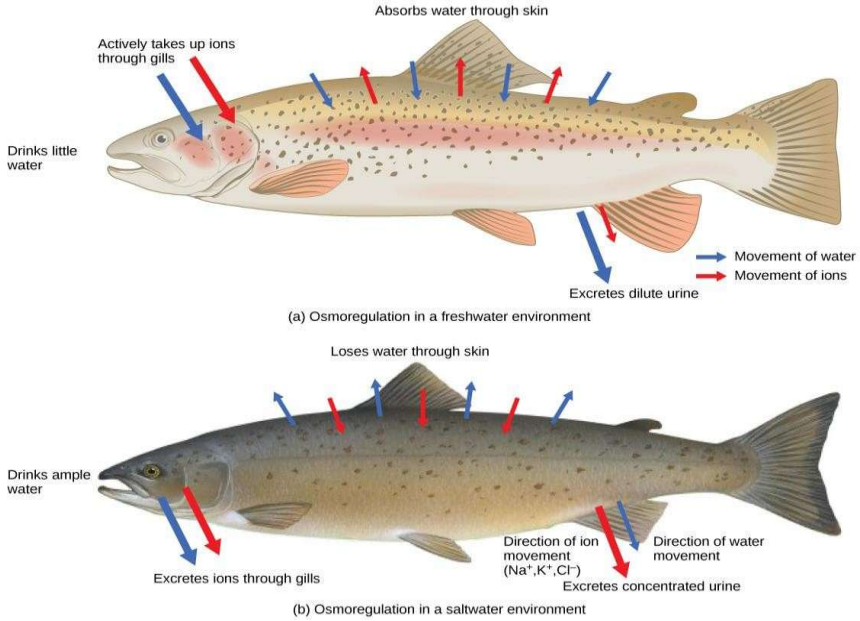

Figure 3: a. **Osmoregulation in fresh water animals b. Osmoregulation in salt water animals**

14.5.2 Osmoregulation in Marine Animals:

1. **Hagfish:** A few marine animals such as the hagfish (*Myxine*) maintain body fluids that are about as salty as the surrounding seawater, which prevents them from losing water by osmosis. However, their glomerular kidneys play an important role in ionic regulation. These kidneys remove calcium, magnesium, and sulfate ions while reabsorbing potassium and chloride, thereby maintaining ionic balance in the body.
2. **Marine Teleosts:** In marine bony fishes such as *Opsanus* and *Cophius*, the body fluids are hypotonic to the surrounding seawater, having only about one-third the solute concentration of the marine medium. This condition causes continuous water loss through gills and oral epithelia, exposing the fish to the risk of osmotic desiccation. To counter this, marine teleosts drink seawater, which restores their water balance but introduces excess salts into the body. The problem of salt overload is resolved by specialized chloride-secreting cells in the gills that actively excrete the excess salts. To further minimize water loss, these fishes possess aglomerular kidneys, and the distal convoluted tubule of the nephron is absent, leading to the production of very little urine compared to freshwater fishes.
3. **Marine Elasmobranchs:** Cartilaginous fishes such as *Scoliodon* and *Stegostoma* maintain body fluids that are isotonic to seawater, but they achieve this isotonicity in a unique way. Unlike teleosts, they retain high concentrations of urea in their blood, making their osmotic concentration equal to that of the surrounding seawater. However, their ionic composition remains different. Ionic regulation is maintained by a specialized rectal gland, which excretes excess salts, since their gills lack chloride-secreting cells.
4. **Migratory Fishes:** Certain migratory fishes shift between freshwater and marine environments during their life cycles. Catadromous fishes such as the eel (*Anguilla bengalensis*) migrate from freshwater to seawater, while anadromous fishes like salmon (*Salmo fario*) migrate from the sea to freshwater for spawning. These fishes adjust their osmoregulatory mechanisms according to the medium. In freshwater, the eel's blood is hypertonic to the environment, and it behaves like a freshwater teleost, producing dilute

urine and absorbing salts. In contrast, when in seawater, the eel's blood becomes hypotonic to the environment, and it compensates for osmotic desiccation by drinking seawater and excreting excess salts through chloride-secreting cells of the gills.

5. **Marine Reptiles:** Marine reptiles such as turtles, sea snakes, and iguanas maintain osmotic and ionic balance in a manner similar to marine teleosts. They consume seawater and salty prey, absorbing the required water but eliminating excess salts through specialized salt glands. In turtles, these glands are located in the head and excrete concentrated NaCl solutions, often seen as tears when they come ashore to lay eggs. This adaptation prevents salt accumulation in their bodies and helps them survive in marine habitats.
6. **Marine Birds:** Seabirds including petrels, cormorants, penguins, and herring gulls also regulate their salt balance with the help of specialized nasal salt glands. These glands excrete highly concentrated NaCl solutions, often through the nostrils, which can reach concentrations five times greater than that in the blood. This adaptation allows marine birds to drink seawater and consume salty food without suffering from dehydration.
7. **Marine Mammals:** Whales, dolphins, porpoises, and seals differ from other marine animals in that they do not drink seawater. Instead, they meet their water requirements from the metabolic water produced by the oxidation of food. These mammals conserve water efficiently, as they do not possess sweat glands and thereby avoid unnecessary water loss. This adaptation enables them to survive in a saline environment without the risk of dehydration.

14.5.3 Osmoregulation in terrestrial:

1. **Water Balance and Sources of Water:** Since terrestrial animals do not always have automatic access to either fresh or salt water, they must regulate their body water content through a balance of gains and losses. Water is gained by drinking fluids, consuming water- rich foods, and through metabolic water formed in mitochondria during the oxidative breakdown of nutrients. Oxidation of 1 gram of glucose yields about 0.6 grams of water, oxidation of 1 gram of protein yields only 0.3 grams, whereas fat oxidation produces about 1.1 grams of water due to its high hydrogen content.
2. **Prevention of Water Loss:** The main challenge for terrestrial animals is preventing water loss. This is achieved through various structural and physiological adaptations. Many animals possess a highly impermeable cuticle or skin covered with scales, feathers, or fur, often reinforced with keratin or wax, which reduces evaporative water loss. In reptiles and birds, water loss is minimized by excreting nitrogenous waste in the form of uric acid crystals, a semisolid excretion that conserves water. Additional reabsorption of water from the rectum or cloaca further enhances conservation. Mammals and some birds possess Henle's loop in their kidneys, a specialized structure that enables the production of concentrated urine with minimal water loss.
3. **Absorption of Water from Environment:** Certain desert reptiles, such as the lizard *Moloch horridus*, have the unique ability to absorb water directly through their skin. Acting like a blotting paper, their body surface absorbs water during rainfall or when humidity is high, enabling them to sustain themselves in arid environments.
4. **Dependence on Metabolic Water:** In extremely dry habitats, animals rely heavily on metabolic water for survival. A prime example is the Kangaroo rat (*Dipodomys*), which lives its entire life without drinking water. It prefers fatty seeds, which yield high amounts of water upon oxidation. Adaptations of the Kangaroo rat include the absence of sweat glands, nocturnal activity to avoid daytime heat, highly concentrated urine, and extremely dry feces. Water loss through respiration is minimized by its elongated nasal

passages, which allow condensation and recovery of water from exhaled air. However, a high-protein diet can be detrimental, as it increases nitrogenous waste while yielding less water, potentially leading to dehydration.

5. **Human Water Regulation:** In humans, water intake averages about/1300 millilitres daily from food and drink, with an additional 200 millilitres derived from oxidation of nutrients. Loss occurs via moist exhaled air, sweat, faces, and urine, with the kidneys being the primary regulators. A healthy adult excretes around 1,500 millilitres of urine daily, though this amount may vary between 500 and/1300 millilitres depending on hydration status and physiological needs.
6. **Water Management in Reptilian and Avian Eggs:** Reptiles and birds lay cleidoic eggs with hard shells and a limited water supply. The developing embryo depends mainly on metabolic water and converts nitrogenous wastes into uric acid crystals, which accumulate harmlessly in the allantoic sac until excretion after hatching.
7. **Tolerance of Dehydration and Temperature Fluctuations:** Some terrestrial animals, such as the camel, exhibit remarkable tolerance to dehydration and internal temperature fluctuations. Camels can survive water loss exceeding 25% of their body weight and may go without drinking for weeks. Their body temperature can fluctuate between 34°C and 45.5°C without activating sweat glands, saving up to 5 Liters of water per day. Additionally, they excrete highly concentrated urine, further minimizing water expenditure.
8. **Behavioural and Hormonal Adaptations:** Terrestrial animals also employ behavioural strategies such as restricting activity to cooler periods, burrowing to avoid heat, and inhabiting humid regions to reduce desiccation. Hormonal regulation further assists in osmoregulation. Antidiuretic hormone (ADH) enhances water reabsorption from kidney tubules, while aldosterone regulates ionic balance by increasing sodium reabsorption and promoting potassium excretion. These mechanisms ensure efficient water conservation and homeostasis in terrestrial environments.

14.6 SUMMARY:

All living organisms need organic raw materials to build up most of their own body molecules, and they require energy to operate the metabolic reactions that sustain life. All organisms obtain their nutrients as food from their surrounding or habitat. Depending on the quantity and functions nutrients are classified as macronutrients and micronutrients. Macronutrients e.g. carbohydrates, proteins and lipids are taken in large amount and required to produce energy and for growth and repair. Micronutrients e.g. vitamins and minerals do not provide energy and are required In very small amount but they are essential regulatory components of food, their deficiency can cause specific disease. Animals obtain their nutrients through a broad variety of feeding patterns. Herbivores eat plant material. Carnivorous animals like those in the cat and dog families, polar bears, seals, crocodiles and birds of prey catch and eat other animals. Many animals feed on both animal and vegetable material they are omnivorous. There are currently two similar definitions of omnivores The process of conversion of complex food substances to simple absorbable forms is called digestion. They need Osmo conformer organisms try to match the osmolality of their body with their surroundings, thus eliminating the need for spending energy on osmoregulation. Most invertebrate seawater organisms are Osmo conformers. Osmo regulator organisms maintain their internal osmolality, which can be extremely different from that of the surrounding environment, through physiological processes. Higher animals like some fishes, reptiles, birds and mammals are good examples of osmoregulatory.

14.7 TECHNICAL TERMS:

Osmoregulation, Osmo conformers, Osmo regulators, Hypoosmotic, Isotonic, Ionic regulation, Contractile vacuole, Chloride cells, Homeostasis

14.8 SELF-ASSESSMENT QUESTIONS:

1. Describe the role of different parts of nephron in urine formation.
2. Give a detailed account of osmoregulation in vertebrates.
3. Describe the role of different factors and components of kidney regulation.
4. Explain how marine birds regulate their water and electrolyte balance?
5. Write a brief note osmoregulation in marine mammals.
6. Explain why nitrogenous wastes are harmful to store in body?

14.9 SUGGESTED READINGS:

1. Essentials of Animal Physiology. Author, S. C. Rastogi. Publisher, New Age International,1971. ISBN, 8122412793, 9788122412796
2. Animal Physiology V Edn. Adaptation and environment Knut Schmidt. Nielsen Cambridge Low Price Editions.
3. A Test book of Animal Physiology, K.A. Goel and K.V. Sastri.
4. Dogel Eckert David Randall Animal Physiology Surjeet Publications, New Delhi.

- **Dr. N. GOPAL RAO**

LESSON- 15
ENDOCRINOLOGY

OBJECTIVES:

- To understand the structure, function, and regulation of the endocrine and neuroendocrine systems.
- To study the hormones produced by different glands and their physiological effects.
- To explore the mechanisms of hormone action and intercellular communication in maintaining homeostasis.

STRUCTURE:

15.1 INTRODUCTION:

In living organisms' various activities performed by cells, tissues and organ systems in the body are well coordinated by the interplay of different types of communication systems, which include a) Neural b) Endocrine and c) Neuroendocrine d) Paracrine and e) autocrine. In this chapter we discuss mainly the Endocrine and Neuroendocrine systems as many of the bodyís chemical messenger systems interact with one another to maintain homeostasis.

15.2 STRUCTURE AND FUNCTIONING OF DIFFERENT ENDOCRINE GLANDS AND TISSUES:

The endocrine system is responsible for the synthesis and secretion of hormones, which act as chemical messengers in the body. These hormones are transported by the circulatory system to different cells, where they bind to specific receptors and trigger physiological reactions. Some

hormones influence only particular tissues called **target tissues**, as only these tissues possess the corresponding receptors. For example, **Adrenocorticotropic Hormone (ACTH)** secreted by the anterior pituitary acts specifically on the adrenal cortex to stimulate secretion of adrenocortical hormones. On the other hand, some hormones are more general in action, affecting a wide range of cells. For instance, **growth hormone** from the anterior pituitary promotes overall growth of body tissues. Certain hormones may also act locally, reaching their site of action through specialized microcirculation.

In close association with the nervous system, the endocrine system coordinates and integrates functions across all physiological systems to maintain internal homeostasis. The endocrine glands exist either as **discrete organs** such as the pituitary, thyroid, parathyroid, and adrenal glands, or as **endocrine tissues within other organs**, like the pancreas, kidneys, testes, ovaries, placenta, brain, and gastrointestinal tract.

The structure of endocrine glands reflects their high secretory activity. Endocrine secretory cells are characterized by prominent nuclei and abundant cytoplasmic organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, secretory vesicles, and lysosomes. The glands are typically composed of islands of epithelial secretory cells, supported by connective tissue rich in blood and lymphatic capillaries. Since they lack ducts, they are also called **ductless glands**. The secretions are released into interstitial spaces and rapidly absorbed into the bloodstream.

Properties of Hormones:

As chemical messengers, hormones exhibit certain defining properties:

- They are composed of small, soluble organic molecules.
- They are effective even at very low concentrations.
- They are transported via blood circulation from their site of secretion.
- They act on specific **target tissues** that are different from the site of production.
- Their action is highly specific, as only target cells with the proper receptor molecules respond to them

Endocrine glands:

In vertebrate animals the endocrine glands are located in various locations in the body. The chief function of these glands is to secrete hormones which act upon the target cells producing desired effect. The location, structure and functioning of each endocrine gland is presented hereunder.

15.2.1 PITUITARY GLAND:

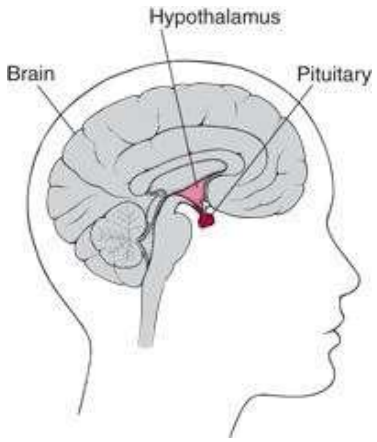

Figure 1: Pituitary gland

The pituitary gland, also called the hypophysis, is a small, pea-sized, rounded structure located at the base of the brain, lying just beneath the third ventricle in a bony cavity called the sella turcica. It is suspended from the hypothalamus and consists of two distinct parts the anterior and posterior pituitary which differ in embryological origin, structure, and function.

i Anterior Pituitary (Adenohypophysis):

The anterior pituitary is formed as the epithelial upgrowth of the roof of the primitive buccal cavity known as Rathke's pouch.

This is also called as the adenohypophysis.

It represents the specialized glandular epithelium covering the anterior portion of the posterior pituitary.

The adenohypophysis shows a cleft, the vestigial lumen of the Raschke's pouch. This vestigial cleft divides the major part of the anterior pituitary from a thin zone of tissue lying opposed to the posterior pituitary known as pars intermedia.

The secretary cells of the anterior pituitary have been recognized as chromophils and chromophobes based on their affinity for the histological stains. The chromophils are further identified into acidophils and basophils according to the staining properties.

Recent studies using Immunohistochemical techniques, based on the nature of the secretory product, the secretory cells have been recognized under five types;

a. somatotrophs, the cells responsible for the secretion of growth hormone
b. mammographs (lectotrophs): the prolactin secreting cells, c)
c. corticotrophs: the adrenocorticotrophic hormone (ACTH) secreting cells d)
d. thyrotrophs: cells secreting thyrotrophin (TSH) and e)
e. gonadotrophs: cells responsible for the secretion of FSH and LH.

The somatotrophs and monotrophs are acidophils while the thyrotrophs, gonadotrophs and corticotrophs are basophils.

ii Posterior Pituitary (Neurohypophysis):

The posterior pituitary also called the neurohypophysis or pars nervosa, is derived from the downward growth of the nervous tissue from the hypothalamus, to which it remains attached by the pituitary stalk This part originates as an extension of the brain and does not synthesize the hormones but only stores and releases them. Posterior pituitary contains the non-myelinated axons of the neurosecretory cells and the cell bodies of which are located in the hypothalamus. The neurosecretory axons are supported by cells called pituicytes, which are similar in structure and function to the neuroglial cells of the Central Nervous System. (CNS).

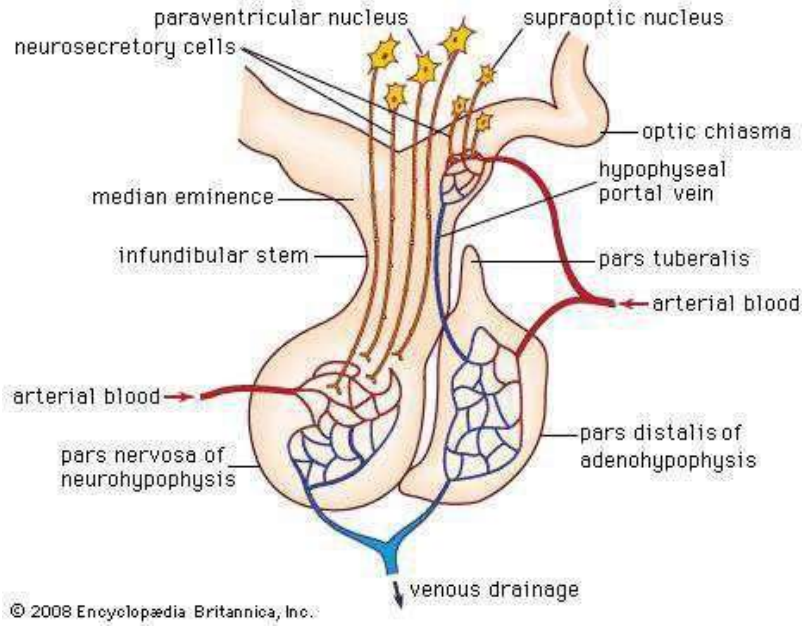

Figure 2: Vascular connection between the pituitary gland and hypothalamus

Hormones secreted by the pituitary:

The type of secretions and the mode of formation of the secretary products differ greatly between the anterior and posterior parts of the pituitary gland. The anterior pituitary secretes both the trophic and direct acting hormones A trophic hormone is one, which stimulates other endocrine glands to release their hormones. E.g. thyroid stimulating hormone (TSH); adrenocorticotrophic hormone (ACTH) and the gonadotrophic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The tropic hormones are produced and stored by the anterior pituitary. These six trophic hormones pass into the blood vessels that leave the pituitary gland and exert their effects on the specific target organs distributed throughout the body.

These include growth hormone, prolactin, FSH, LH. TSH and ACTH. The release of the GTH and prolactin is stimulated and also is inhibited by the hypothalamus, whereas the release of the other four is regulated by the negative feedback of hormones from the target glands acting as receptors in the hypothalamus and anterior pituitary. These six types of hormones stimulate the release of the target gland hormones and as the levels of these rises they inhibit the secretion of the hypothalamus. And the pituitary hormones. When the blood levels of these target hormones fall below a certain level inhibition of the Pituitary and Hypothalamus ceases allowing increased secretion from these glands. This is an example of negative feedback mechanism. The direct acting hormones are Growth hormone (GH) and Prolactin (Recently, it was established that prolactin exhibits a trophic action on the endocrine tissues of the ovary in some animals).

The posterior pituitary secretes two hormones, antidiuretic hormone (ADH) also called vasopressin and Oxytocin, both of which act directly on the non- endocrine tissues. ADH is synthesized in the neuron cell bodies of the supraoptic nucleus while the oxytocin is synthesized in the neuron cell bodies of the paraventricular nucleus of the hypothalamus. These hormones pass down the axons of the hypothalamus hypophysial tract, through the pituitary stalk, to the posterior pituitary where they are stored in the distended terminal parts of the axons. Since the process involves both the nervous and endocrine system, the response is called

neuroendocrine response. They result in neuroendocrine reflexes – a type of behavioral pattern. Release of these posterior pituitary hormones is controlled directly by the nerve impulse passing down the axons from the hypothalamus process that is referred to as neurosecretion Nerve terminals from the specialized neurosecretory cells release two distinct groups of chemical substances known as releasing factors and inhibiting factors into the blood capillaries at the hypothalamus end of the portal system. These pass to the pituitary-end where they cause the release of the six types of hormones known as trophic hormones. The trophic hormones are produced and stored by the anterior pituitary gland. These six hormones pass into blood vessels that leave the pituitary gland and exert their effects on the specific target organs distributed throughout the body. These include the growth hormone, prolactin, FSH, LH, TSH and ACTH. Hypothalamic control of the anterior pituitary secretion is mediated by specific hypothalamic releasing hormones e.g. thyroid stimulating hormone releasing hormone (TSHRH). Exceptions to this rule are prolactin secretion, which is under the control of dopamine and secretion of growth hormone which is controlled by releasing and inhibitory hormones.

These releasing and inhibitory hormones are conducted from the median hypothalamic eminence to the anterior pituitary by a unique system of portal veins (the pituitary portal system) The pars intermedia of the pituitary gland synthesizes and secretes melanocyte stimulating hormone (MSH), which functions in maintenance of the skin color in the animals.

15.2.2 HYPOTHALAMUS:

Hypothalamus is situated at the base of the fore brain, immediately beneath the thalamus and is present above the pituitary gland. It plays a dominant role in collecting information from the other regions of the brain. The information is passed to the pituitary gland, which directly or indirectly regulates the activity of the other endocrine glands.

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15.2.3 PARATHYROID GLAND:

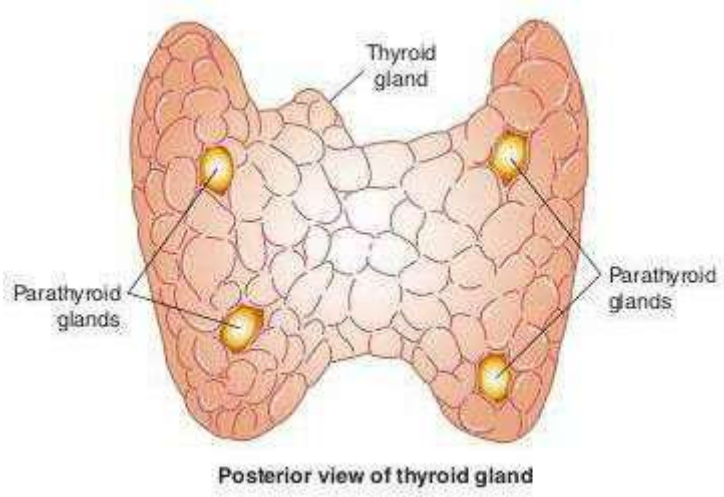

Figure 3: posterior view of thyroid gland

Parathyroid glands are small oval endocrine glands closely associated on each side with the thyroid gland. These glands are derived embryologically from the third and fourth branchial (pharyngeal) pouches. They are usually found embedded in the thyroids. The thin fibrous capsules of the parathyroid gland give rise to delicate septa, which divide the cells into nodules of secretory cells. The glandular cells are of two types: the principal or chief cells and Oxyphil cells. These cells are arranged in the form of clusters, ribbons or glands.

The parathyroid gland regulates the serum calcium and phosphate levels via parathyroid hormone, called the parathormone (PTH). The parathormone increases the serum calcium levels in three ways: a) by direct action on bone, increasing the rate of osteoclastic resorption and promoting breakdown of the bone matrix b) by direct action on the kidney, increasing the renal tubular reabsorption of calcium ions and inhibiting the resorption of phosphate ions from glomular filtrate and c) by promotion of the absorption of calcium from the small intestine; this effect involves Vitamin D. The activity of the parathyroid gland is controlled by the simple negative feedback mechanism.

Hyper activity of the gland is known to reduces the calcium levels in the plasma and tissues due to calcium excretion in the urine. It also reduces the rate of excretion of phosphate and as a result there is an increase of phosphate levels in the plasma membrane.

15.2.4 THYROID GLAND:

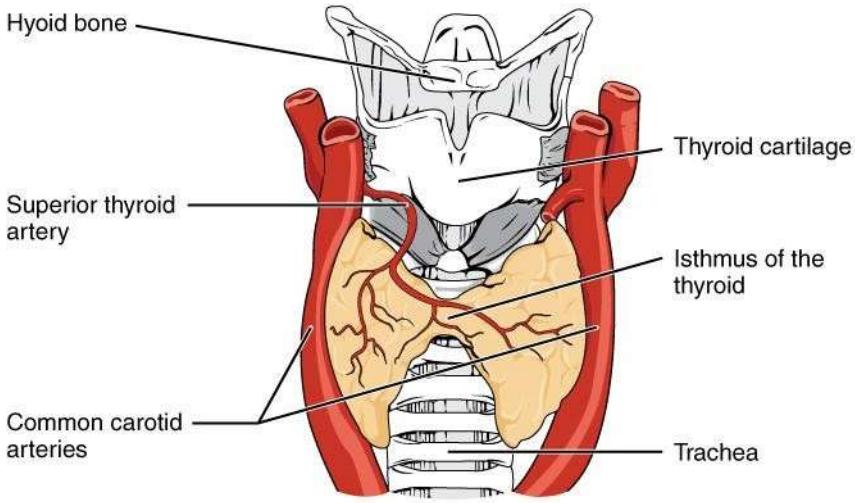

Figure 4: thyroid gland

The thyroid gland is unique in that it stores large amounts of hormone in an inactive form, in extracellular compartments in the center of the follicles, in contrast to other endocrine glands, which store only small quantities of hormone in intracellular sites. Thyroid gland is located in the neck region, in the lobes present on each side of the trachea and larynx connected by a band of tissue, and is enveloped by a fibrous capsule from which fine collagenous septa extend into the gland dividing into distinct lobules. The major portion of the gland develops from the epithelium, down growth of the epithelium in the mouth cavity during the fetal development, while the calcitonin- secreting cells are derived from the ultimobranchial element of the fourth branchial pouch in the fetus. It is made up of hundreds of thousands of tiny follicles- the functional units, which are about 0.1 mm in diameter. Each follicle is a hollow spheroidal structure composed of a single layer of cuboidal epithelial cells bounded by a basement

membrane. These cells secrete the hormones T3 and T4. They become columnar in shape and develop microvilli on inner surface when the gland is activated by thyroid stimulating hormone (TSH) from the anterior pituitary.

The thyroid gland produces three active hormones triiodothyronine (T3), thyroxin (T4) and calcitonin. T3 and T4 regulate metabolic rate, growth and development of the organism while calcitonin is involved in the regulation of calcium levels in the blood. The main effect of the thyroxin is to control the basal metabolic rate. Protein synthesis is stimulated by both the Thyroxin and Growth hormone, leading to an increase in growth, particularly of the selected systems.

The levels of the T4 circulating in the blood controls its release from the thyroid gland by negative feedback mechanisms involving the hypothalamus and the anterior pituitary. If excess of T4 is present in the blood it switches off its own production by switching off the production of the thyroxin releasing hormone (TRH) by the hypothalamus and TSH by the anterior pituitary. This is referred to as the feedback mechanism.

Over activity or under activity of the thyroid produces a swelling in the neck in human beings, referred to as Goiter. The symptoms include an increase in the heart and ventilation rate and body temperature. Extreme hyperthyroidism is termed the thyrotoxicosis and is associated with the increased excitability of the cardiac muscle. Hypothyroidism is the result of deficiency of enzyme system in hormone production, due to lack of TSH production in the anterior pituitary or iodine deficiency in the diet. Thyroxin deficiency at birth leads to poor growth, mental retardation, and a condition known as cretinism. The deficiency at a later period in life gives rise to a condition, myxoderma. The symptoms include reduction in metabolic rate, accompanied by decreased O2 consumption, ventilation, heart rate and body temperature. Mental activity becomes slower; weight increase due to formation and storage of semi fluid under the skin. Face of the individual becomes puffy, swelling of the tongue, rough skin and loss of hair from the scalp.

15.2.5 ADERNAL GLAND:

Adrenal glands (ad, to; renes, kidney) are a pair of glands located just above each kidney. Each gland is composed of two types of cells of different origin and these cells function independently. The outer region commonly called the cortex forms about 80% of the gland and the inner region the medulla, is closely linked to the nervous system.

1. Adrenal cortex:

The adrenal cortex is distinguishable into three histological zones named according to the arrangement of the secretory cells.: Zona glomerulosa in which the cells are arranged in rounded clusters, Zona fasciculata cells arranged in parallel cords at right angles to the capsules and zona reticularis, in which the small closely packed cells arranged in irregular cords. The Zona glomerulosa secretes the mineralocorticoid hormones principally aldosterone. It acts directly on the renal tubules to increase sodium and therefore water retention thus increasing extracellular fluid volume and the arterial blood pressure. Aldosterone secretion is independent of ACTH. The Zona fasciculata secretes glucocorticosteroid hormones, principally Cortisol, which has wide ranging metabolic effects. It raises the blood glucose levels and increases cellular synthesis of glycogen. Cortisol secretion is controlled by the hypothalamus via the anterior pituitary hormone ACTH. By this

means it promotes the secretion of glucocorticoids, which adjust body metabolism. The Zona reticulata secretes small quantities of androgens and glucocorticoids. All these steroid hormones are formed from a molecule called the cholesterol, which is synthesized by the cortex and also taken up from circulation following the absorption from the diet. Steroids diffuse through the cell membranes and attach to the cytoplasmic receptor proteins. These pass into the nucleus, attach to specific areas of the chromosome and switch on or of certain genes.

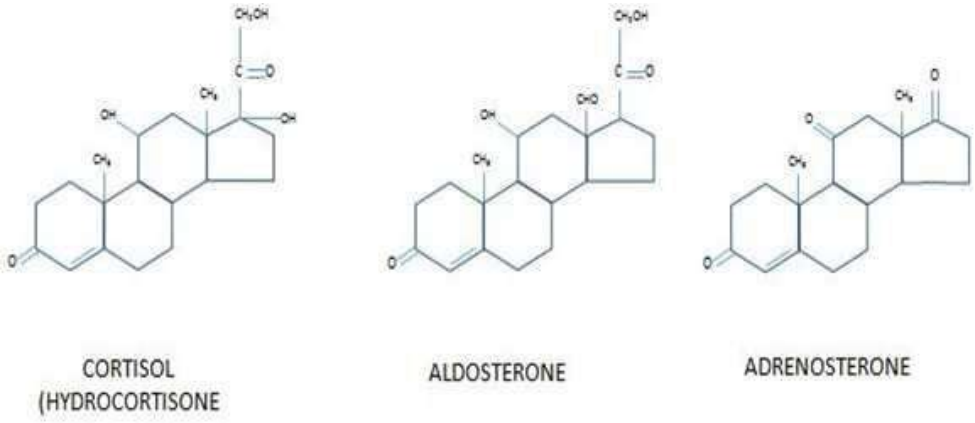

Figure 5: Some Adrenocortical Hormones

2. Adrenal medulla:

Adrenal medulla forms the center of the adrenal gland and is richly supplied with nerves and blood vessels. It is supplied by long cortical secretory arteries composed of closely packed clusters of secretory cells. The medullary capillaries drain in to the central vein of the medulla. The secretory cells are exposed to fresh supply of arterial blood rich in adrenocorticosteroids, which are known to influence the synthesis of adrenaline by the medulla.

It secretes catecholamine hormones noradrenaline and adrenaline, under the direct control of the sympathetic nervous system. The adrenal medullary hormones are stored in the membrane-bound dense core cytoplasmic granules and are released only in response to nervous stimulation. Secretion of catecholamines by the sympathetic nervous system. Acute physical and psychological stresses in human beings initiate release of adrenal medullary hormones. The released catecholamines act on adrenergic receptors present throughout the body particularly in the heart, blood vessels bronchioles, visceral muscle and skeletal muscle producing physiological effects. Adrenaline promotes glycogenolysis in the liver and skeletal muscles during stress situations.

15.2.6 PANCREAS:

Pancreas is located associated with the intestines in vertebrates. Pancreas has both exocrine and endocrine functions. It is a major exocrine gland. The bulk of the gland is made up of cells which surround the numerous branches of the pancreatic duct. A ring of cells called acinus surrounds each branch. These acinar cells are exocrine cells. They secrete the pancreatic juice. The secretary endocrine cells with rich supply of blood become clustered and are known as islets of Langerhans after its discoverer Paul Langerhans in 1868. These contain a small number of large cells known as alpha cells and many smaller cells known as beta cells scattered

throughout the exocrine glandular tissue and the blood capillaries. The islets vary in size. They contain a variety of cell types each is responsible for secretion of one type of hormone. The endocrine pancreas contains secretory cells of several types. Traditionally the glucagon's, insulin and somatostatin secreting cells have been designated as alpha, beta and delta cells respectively.

The important secretary products of the endocrine pancreas are insulin and glucagon's. They play an important role in carbohydrate metabolism. The hormone insulin was isolated by Bauting, Best and Maclod in 1921. Insulin is known to be secreted by the beta cells and the glucagon's by alpha-cells. These two hormones function antagonistically on the glucose level in the blood. Insulin is known to be released in response to a rise in blood glucose level (> 90 mg per 100 cc of blood) It is carried in the plasma, bound to beta globulin Insulin promotes the uptake of glucose by most cells particularly those of liver, skeletal muscle and adipose tissue, thus lowering the plasma glucose concentration. Production of insulin is regulated by a negative feedback mechanism. A rise in blood sugar is detected by beta cells in the pancreas, which in response produce more insulin. As the blood sugar gets lower, beta cells reduce the output of the insulin. A deficiency of insulin production leads to a disorder known as diabetes mellitus. Glucagon is released in response to fall in blood glucose level below normal. Its role is to increase blood glucose level and its main target is liver. Glucagon stimulates the conversion of glycogen to glucose and the process is called glycogenolysis. It also functions in the breakdown of proteins and fats to glucose and conversion of lactic acid to glucose-called glucone oogenesis. Glucagon has no effect on the muscle glycogen. Regulation of glucagon's secretion is similar to that of insulin, but here alpha cells are involved and they respond to falling blood glucose levels.

Four other types of endocrine cells are known to be present in the islets of Langerhans in the Pancreas, scattered, single or in smaller groups between the exocrine acini and also along the ducts. Their secretory products include somatostatin (inhibits insulin and glucagon's secretion) vasoactive intestinal peptide (VIP) and pancreatic polypeptide (PP). Another cell type, the enterochromaffin cell (EC), appears to secrete several different peptides including motilin, serotonin and substance

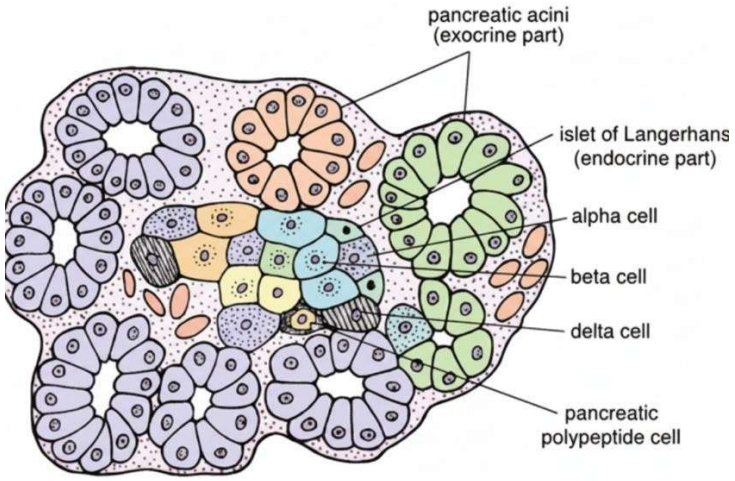

Figure 6: part of the T.S. of Pancreas showing alpha and beta cells of islet of Langerhans

15.2.7 PINEAL GLAND:

The Pineal gland is a small organ 6-8 mm long represents an evagination of the posterior part of the of the roof of the 3rd ventricle in the mid ventral line of the brain. The pineal is connected to the brain via a short stalk containing nerve fibers, many of which communicate with the hypothalamus. In reptiles and other lower vertebrates, the penial lies at or near the skin surface and functions as a photoreceptor organ secreting the hormone melatonin, which lightens the skin color by acting on melanophores. The pineal consists of two main cell types: Pinacocytes and neuroglial cells. Pinacocytes are modified neurons while the neuroglial cells are similar in structure to the astrocytes of the CNS

Melatonin synthesis from the amino acid Tryptophan is induced by darkness and inhibited by the light. Information received by the retina of the eye in the form of light is converted by Pineal into a chemical signal melatonin. It is now known that melatonin regulates the circadian rhythms of the body and also functions on seasonal reproduction in animals, effect the ageing and regulates the immune system.

Gastrointestinal Endocrine System:

Neuroendocrine cells are found scattered in the gastrointestinal tract and in the pancreatic and biliary ducts. These cells are known to secrete hormones, which include Gastrin, secretin, CCK. Serotonin, enter glucagon. Somatin, substance P, vasoactive intestinal peptide (VIP), bombesin, gastric inhibitory polypeptide (GIP), motilin and pancreatic polypeptide (PP). These hormones collectively regulate and coordinate most aspects of gastroinstinal activity in close association with the autonomic nervous system. While some of the hormones are true endocrine hormones that act on target organs at a distance, others are locally acting mediators known as paracrine hormones. Some act by neurotransmitter activity and are called the neuroendocrine hormones and indeed they act as neurotransmitters with in the Central Nervous System. F.

Respiratory Endocrine System:

Similar to the ones in the gastrointestinal tract, the lower respiratory tract contains scattered peptide and amine secreting endocrine cells, which are involved in local or autonomic ally mediated regulation of respiratory tract function particularly among children. These endocrine cells are scattered individually in epithelium and produce a variety of secretory products such as serotonin, calcitonin, bombesin and leu-enkephalin.

Hormones as major vehicles for intercellular communication

Intercellular communication is a result of the signals sent and signals received by the cells. Most cells produce signals in the form of either specific cell surface receptors or secreted molecules. Some signals are effective only if the signaling cell is in direct contact with the signaled cells. Other signals are effective only on cells that are near by.

Hormones produced by the specialized endocrines constitute the best-studied signals. The hormones are effective over a long range. Each cell is programmed to respond to a select group of hormonal signals in a specific way. The responding cell should have the receptor that interacts with the signaling molecule otherwise the signal is ignored. Further the type of response evoked by a signal-sensitive cell depends upon the way in which the signal binding receptor is hooked to the other signal-relay chains in the cells. The various aspects of hormone-

receptor interaction and the direct biochemical consequences of these interactions and the ways in which the hormonal circuits are regulated are of prime importance. In all vertebrate animals, hormones belong to the following chemical groups a. Polypeptides b. derivatives of Amines. C. Steroids d. Fatty acids. Hormones that are released by the presence of another circulating hormone are usually under the control of an endocrine gland; a small quantity of the initial hormones becomes amplified at each stage in the pathway and this phenomenon is referred to as cascade effect.

Polypeptide hormones:

All peptide hormones that contain signal peptides; direct them in to the lumen of the endoplasmic reticulum. Polypeptide hormones are generally stored in secretory granules after their passage through the endoplasmic reticulum and Golgi apparatus. Release of these hormones into the blood stream is accomplished by fusing of the secretary granule membranes with the plasma membrane. Polypeptide hormones are removed from the circulation by serum and cell surface proteases, by endocytosis, followed by lysosomal degradation and by glomerular filtration in the kidney.

Amino acids and their derivatives:

The hormones Thyronine (T4), and triiodothyronine (T3) and the epinephrine also called the adrenaline are amino acid derivatives. These are mostly associated with carrier proteins in the serum. These carrier proteins are called Tyrosine- binding globulin.

Steroid hormones:

Steroid hormones Cortisol, Corticosterone, aldosterone, and Testosterone are the derivates of the Cholesterol. These are taken up by the liver and metabolized to inactive forms, which are extracted in to the bile duct or back into the blood for removal by the kidneys. These are associated with the carrier proteins transporting for cortisol and other sex- steroid binding proteins

15.3 MECHANISM OF HORMONAL ACTION:

Hormones are chemical messengers synthesized by endocrine glands that regulate the activity of target cells and organs. The mechanism by which hormones exert their effects depends on their chemical nature and the location of their receptors. Hormonal actions can be broadly classified based on whether the hormone is water-soluble (peptide/protein and catecholamines) or lipid-soluble (steroid and thyroid hormones).

1. Hormone-Receptor Interaction:

Hormones act by binding to specific receptors located either on the cell membrane or inside the cell. These receptors are proteins that recognize and bind the hormone with high specificity. The receptor-hormone interaction is the first and critical step in initiating a hormonal response.

Target specificity: Only cells possessing the appropriate receptor respond to a given hormone.
Receptor types: Membrane-bound receptors: Found on the plasma membrane, typically for water-soluble hormones.
Intracellular receptors: Located in the cytoplasm or nucleus, generally for lipid-soluble hormones.

The binding of a hormone to its receptor triggers a series of intracellular events collectively termed signal transduction.

2. Mechanism of Action of Water-Soluble Hormones:

Water-soluble hormones include peptide hormones (like insulin, glucagon) and catecholamines (like epinephrine and norepinephrine). These hormones cannot cross the lipid bilayer of the plasma membrane and, therefore, act through cell-surface receptors.

- **Hormone Binding:** The hormone binds to a specific G-protein coupled receptor (GPCR) or enzyme-linked receptor on the cell membrane.
- **Activation of Second Messengers:** Hormone binding activates intracellular signalling pathways, often through second messengers such as cyclic AMP (Camp), cyclic GMP (Cgmp), calcium ions (Ca^{2+}), or inositol triphosphate (IP_3).
- **Signal Amplification:** Each activated receptor can activate multiple intracellular molecules, leading to **amplification** of the signal. For example, one molecule of epinephrine binding to its receptor can activate hundreds of molecules of adenylate cyclase, producing thousands of Camp molecules.
- **Physiological Response:** Second messengers modulate cellular processes by activating protein kinases, opening ion channels, or modifying enzyme activities. For instance, insulin binding leads to the activation of kinases that promote **glucose uptake** into muscle and adipose tissue.
- **Termination of the Signal:** Hormonal effects are terminated by:
  - Degradation of the hormone.
  - Inactivation of second messengers (e.g., Camp breakdown by phosphodiesterase).
  - Internalization or desensitization of receptors.

3. Mechanism of Action of Lipid-Soluble Hormones

Lipid-soluble hormones include **steroid hormones** (cortisol, aldosterone, estrogen, testosterone) and **thyroid hormones (T3, T4)**. These hormones **readily cross the plasma membrane** due to their lipophilic nature.

- **Hormone Entry into the Cell:** Lipid-soluble hormones diffuse through the phospholipid bilayer of the target cell membrane.
- **Formation of Hormone-Receptor Complex:** Once inside, the hormone binds to **cytoplasmic or nuclear receptors,** forming a **hormone-receptor complex**.
- **Translocation to the Nucleus:** The complex enters the nucleus (if not already there) and binds to **specific DNA sequences** called hormone response elements (HREs).
- **Regulation of Gene Expression:** Binding of the hormone-receptor complex to DNA modulates transcription of target genes, leading to **synthesis of new proteins or enzymes**. These proteins mediate the physiological effects of the hormone.
- **Physiological Response:** The new proteins may affect metabolism, growth, differentiation, or other cellular functions. For example: Cortisol induces the synthesis of gluconeogenic enzymes in the liver. Thyroid hormones stimulate the production of metabolic enzymes, increasing basal metabolic rate.
- **Termination of Hormonal Action:** Hormone levels decline due to metabolism in liver and kidney. Receptor-hormone complexes dissociate. Newly synthesized proteins degrade over time, ending the response.

4. Signal Amplification and Cascade Effect:

A key feature of hormonal action is **signal amplification**, where a small quantity of hormone produces a large cellular response. This occurs through:

- Activation of multiple second messengers by a single hormone-receptor interaction.
- Activation of enzyme cascades downstream of the second messenger.

Example: Epinephrine activates adenylate cyclase → Camp increases → protein kinase A activated → phosphorylase kinase activated → glycogen phosphorylase activated → rapid glycogen breakdown in liver cells.

5. Hormone Feedback Mechanisms:

Hormonal actions are tightly regulated by feedback mechanisms to maintain homeostasis:

- **Negative feedback:** Most common; rising hormone levels inhibit further hormone secretion (e.g., T4/T3 inhibit TRH and TSH release).
- **Positive feedback:** Less common; hormone levels stimulate additional secretion (e.g., oxytocin during childbirth).

6. Neuroendocrine Integration:

Some hormones, particularly those of the **posterior pituitary** (oxytocin and ADH), demonstrate **neuroendocrine action**, where nervous system signals directly trigger hormone release. This ensures rapid and coordinated responses to physiological stimuli.

15.4 SUMMARY:

The endocrine and neuroendocrine systems coordinate physiological activities in living organisms by secreting hormones, which act as chemical messengers. Endocrine glands, both discrete and scattered in organs, secrete hormones into the bloodstream to act on specific target tissues. Key glands include the pituitary, hypothalamus, thyroid, parathyroid, adrenal, pancreas, pineal, gastrointestinal, and respiratory endocrine cells. Hormones are classified as polypeptides, amino acid derivatives, steroids, or fatty acids and operate via specific receptors, often under feedback control. These systems work closely with the nervous system to maintain homeostasis, regulate metabolism, growth, development, and stress responses, and ensure proper intercellular communication.

15.5 TECHNICAL TERMS:

Adenohypophysis, Neurohypophysis, Chromophils, Trophic hormones, Thyroglobulin, Triiodothyronine (T3), Thyroxine (T4), Parathormone (PTH), Melatonin, Islets of Langerhans, Glucagon, Insulin, Cortisol, Aldosterone, Catecholamines

15.6 SELF-ASSESSMENT QUESTIONS:

1. Differentiate between the anterior and posterior pituitary in terms of structure and function.
2. Explain the role of tyrosine in thyroid hormone synthesis.
3. How do insulin and glucagon regulate blood glucose levels?

4. Describe the negative feedback mechanism controlling thyroid hormone secretion.
5. List the main hormones secreted by the adrenal cortex and their functions.
6. Mechanism of hormonal regulation?

15.7 SUGGESTED READINGS:

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2. Floray E. An Introduction to General and Comparative Animal Physiology. W.B. Saunders Co., Philadelphia.
3. Goel KA and Satish KV. 1989. A Text Book of Animal Physiology, Rastogi Publications, Meerut, U.P.
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- **Dr. N. GOPAL RAO**

LESSON- 16

HORMONAL REGULATION ON REPRODUCTION

OBJECTIVES:

- To understand the role of hormones in reproduction in vertebrate animals
- To understand the hormonal regulation of reproduction in males and females.
- To analyse the physiological and behavioural roles of pheromones in reproduction.
- To examine feedback mechanisms controlling hormone secretion and reproductive processes.

STRUCTURE:

16.1 INTRODUCTION:

The process of reproduction:
In animals the process of reproduction is controlled by different hormones. In animals the hormones start acting on the gonads when the animal becomes an adult and is capable of participating in the process of reproduction. The time of onset of maturity differs in different animals. The attainment of puberty is also under the hormonal control.

16.2 HORMONES ON REPRODUCTION:

1. Gonadotropin Releasing Hormone (GnRH):

GnRH is a neuropeptide (a decapeptide) that is produced in the hypothalamic surge and tonic centers. In the male and the female, the target tissue is the anterior pituitary gland, specifically Gonadotroph cells. In males and females, secretion of GnRH results in the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the anterior pituitary gland. GnRH-producing neurons are stimulated into production in response to spontaneous rhythms and by sensory impulses from sensory inputs derived from the external environment. Alterations in the internal conditions of the body can also result in altered GnRH production. For example, in some species such as the sheep, there is seasonal sexual activity and the cerebral cortex, hypothalamus, pituitary and testes interact to regulate functions further along the signaling chain.

In females when the estrogen concentration prior to ovulation reaches a certain threshold, large quantities of GnRH are released in the form of a surge. This results in a corresponding peak in LH that stimulates ovulation. In females this surge center is often called the **preovulatory center**. In males this surge center becomes inactivated during fetal life due to the brain maturation effects of estradiol (see section below) being able to pass through the blood brain barrier in males, please see the reproductive development of the brain for more details. In males there are between 4-12 GnRH peaks per day. Plasma concentrations of LH peak approximately 10minspostGnRHsurge.

Although the hypothalamus via GnRH stimulates the secretion of LH and FSH, it cannot regulate LH and FSH independently. Therefore, another hormone produced from the developing ovarian follicle in the female and 217euroco cells in the male acts as a negative feedback mechanism for FSH. Sex hormones also alter the level of production of GnRH from the hypothalamus via a negative feedback system. High concentrations of progesterone or testosterone will reduce the secretion of GnRH and also therefore the secretion of LH and FSH.

2. Luteinizing Hormone (LH):

LH is a type of glycoprotein that is produced in the anterior pituitary via gonadotroph cells and serves to regulate the function of the gonads. In males LH stimulates the production and secretion of testosterone from the testes via Leydig cells. In females LH stimulates the production of estrogens and progesterone from the ovary via theca interna cells and luteal cells. Concentrations of LH increase during ovulation and with the formation of the corpora lutea with progesterone secretion. The secretion of LH is regulated via the secretion of GnRH. As shown previously, in males there are between 4 to 12 GnRH pulses per day and this therefore means that LH also peaks throughout the day. During these peaks, the production and secretion of testosterone increase. Testosterone secretion also is pulsatile.

3. Follicle Stimulating Hormone (FSH):

FSH is a type of glycoprotein that is produced in the anterior pituitary via gonadotroph cells. FSH secretion is regulated by GnRH from the hypothalamus. The target tissue of FSH in males are the Sertoli cells within the testes and in the female the granulosa cells of the ovary. FSH stimulates the maturation of germ cells within the testes and ovaries. In the female it also stimulates follicular Development and estrogen synthesis. In the male FSH also stimulates the secretion of inhibin which has negative feedback directly to the anterior pituitary. Although GnRH is released in a pulsatile fashion and the other gonadotropic hormone LH is therefore also pulsatile, FSH concentrations do not fluctuate as much as that of LH. This is because of the added regulatory feedback mechanism of inhibin within the regulatory pathways for FSH secretion.

4. Prolactin (PRL):

Prolactin is a protein that is produced from by the anterior pituitary via lactotroph cells. This hormone exerts a stimulatory effect on milk synthesis within the mammary glands. It has also been shown to have some degree of gonadal function in some domestic species and rodents. In birds increased concentrations of prolactin have been linked with brooding behaviors and the associated metabolic changes that birds undergo during brooding. Prolactin secretion is regulated by the hypothalamus which produces several neurohormones that affect prolactin concentrations. The most important within this is **dopamine** (or prolactin inhibitory hormone, PRL-IH) which exerts a totally dominant inhibitory action on prolactin synthesis. The hypothalamic regulation of prolactin secretion is via signals from the central nervous system. Prolactin synthesis is increased when the mother is suckling via a reflex stimulation of the teats.

This stimulation reflex reduces the secretion of dopamine and increases the hormone prolactin releasing hormone (PRL-RH).

Once prolactin binds to its target receptors within the mammary gland cells, it activates an intracellular tyrosine kinase. When this occurs in the developing animal this binding can also cause the differentiation of mammary epithelial cells during pregnancy. The half-life of prolactin is approximately 20mins. Estradiol can also have an effect on the prolactin producing cells within the anterior pituitary and is responsible for increased concentrations of prolactin in females undergoing puberty and may also contribute to the increased concentrations during late pregnancy.

5. Oxytocin (OT):

OT is a neuropeptide (a octapeptide) which is synthesized in the **hypothalamus** and stored in the posterior pituitary. OT is primarily involved in upregulating the activity of smooth muscle cells in the uterus and the smooth muscles surrounding the alveoli ducts of the mammary glands. At parturition, OT causes strong contractions from the myometrium. OT is also essential for 'milk let-down in most domestic species. OT binds to receptors in the membrane of target cells which activates phospholipase C. OT facilitates the generation of the driving pressure behind pushing the milk towards the large excretory ducts and the teats.

6. Estradiol (E2):

Estradiol (E2) is a steroid hormone and is part of the estrogen group of hormones and is the principal estrogen in females. Estrone and estriol are chemically similar to estradiol but are found in lower concentrations and have a lower estrogenic activity. Production of estrogens occurs in the ovary via granulosa cells, the placenta and the Zona reticularis of the adrenal cortex. In males in it is produced in Sertoli cells found in the testes. Estradiol is synthesized from cholesterol.

Where estrogens stimulate growth of follicles in the ovaries, estrogens secreted from the ovary in the follicular phase (proestrus and estrous) lead to hypertrophy of the epithelium and the endometrium. Secretory glands within the uterus enlarge and secretion is initiated leading to thickening of tissues. The blood vessels supplying the uterus and external genitalia dilate and blood flow to these areas increases significantly. Oedema occurs within the uterus and surrounding connective tissues. Estrogen also causes increased uterine muscle tone. In the cervix estrogens stimulate increased mucus secretion and the vaginal epithelium becomes keratinized. In males the target tissue is the brain where it causes maturation of the brain during development. This maturation process ensures the appropriate development of male sexual behaviors. E_2 in the male also inhibits long bone growth.

7. Progesterone (P4):

Progesterone is a steroid hormone that along with estrogens is based on a cholesterol molecule produced by the corpus luteum and the placenta using cholesterol as the base molecule. Progesterone is produced by the corpus luteum as well as by the feto-placental unit and in the zona reticularis of the adrenal cortex (to a lesser extent). More detailed information regarding corpus luteum formation and regression please use the links. Progesterone prepares the uterus for reception of fertilized oocytes and is transported via the blood bound to plasma proteins. Progesterone also prepares the mammary tissues for milk production as well as inhibiting female reproductive behaviors associated with estrous.

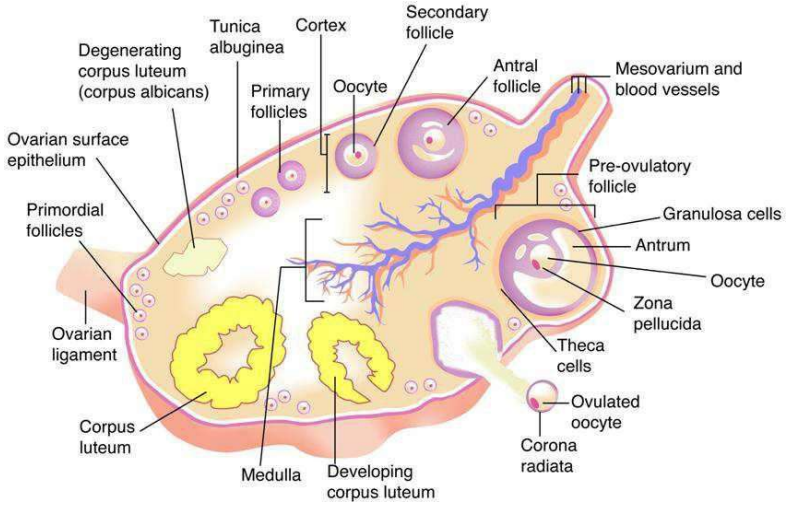

Figure 1: ovary

16.3 EFFECTS OF HORMONES IN REPRODUCTION:

The concentration of progesterone increases after ovulation increasing the growth of glands found in the endometrium resulting in increased secretion. These secretions include mucin, carbohydrates and specific proteins that are designed for nourishment of the embryo prior to implantation. Progesterone also stimulates the growth of the endometrium and 220eurocoele smooth muscle cells to ensure that they do not contract during fetal development. Once near term, the concentration of progesterone decreases, altering the ratio between progesterone and estrogen. This stimulates myometrial activity and prepares the uterus for parturition.

Progesterone During Pregnancy

During pregnancy the plasma concentration of progesterone is maintained at an elevated level. Progesterone also inhibits secretion of FSH and LH (negative feedback at hypothalamic level by inhibiting GnRH) and thus also prevents the ovulation of follicles during the luteal phase and during pregnancy. In most domestic species the corpus luteum persists for the entire length of gestation.

The exception to this rule is the mare in which the progesterone concentration falls during the later stages of pregnancy. This is due to the regression of the corpus luteum around day 180 of the330-340daygestationperiod. It is possible to use the relative concentration of progesterone as an aid to pregnancy diagnosis, for example in cattle. However, for a definitive diagnosis a high level of progesterone is required on two separate samples due to the overlap between the luteal phase and pregnancy.

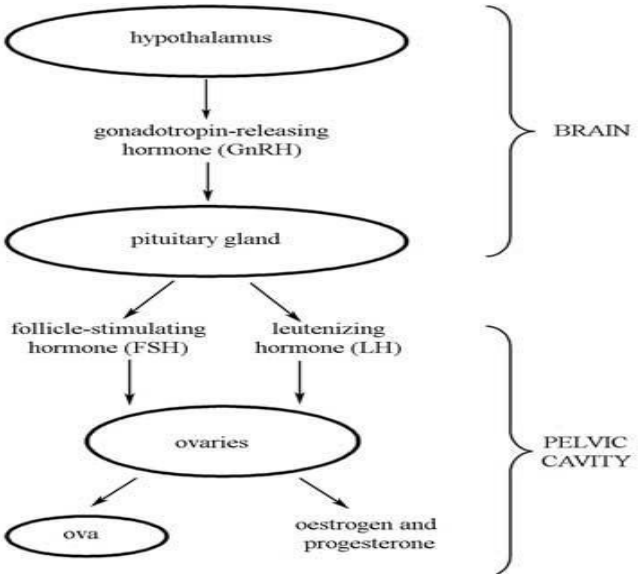

Figure 2: Flow chart showing hormonal control of oogenesis Testosterone (T)

The male sex hormone is called testosterone and this hormone is required for **spermatogenesis**. Testosterone is a steroid hormone that is produced in the 221euroc cells within the testes. A relatively high concentration of testosterone is maintained within the testicular tissue and testosterone is circulated around the body by diffusion of the hormone from the spermatic cord into the testicular veins and arteries. The primary action of testosterone is anabolic growth, spermatogenesis promotion and promotion of secretion from the accessory sex glands.

Male sex hormones are regulated by negative feedback systems that operate at various levels within the male sex hormone system. The starting point for the production of testosterone (and therefore the production of spermatozoa) is the hypothalamus. The hypothalamus contains neuroendocrine cells that are capable of secreting a substance called **Gonadotropin-releasing hormone** or GnRH. GnRH stimulates basophilic cells in the adenohypophysis, via the "portal system" to secrete two intermediate hormones within the male sex hormone cycle; **Luteinizing hormone** (LH)and **Follicle-Stimulating-Hormone** (FSH).

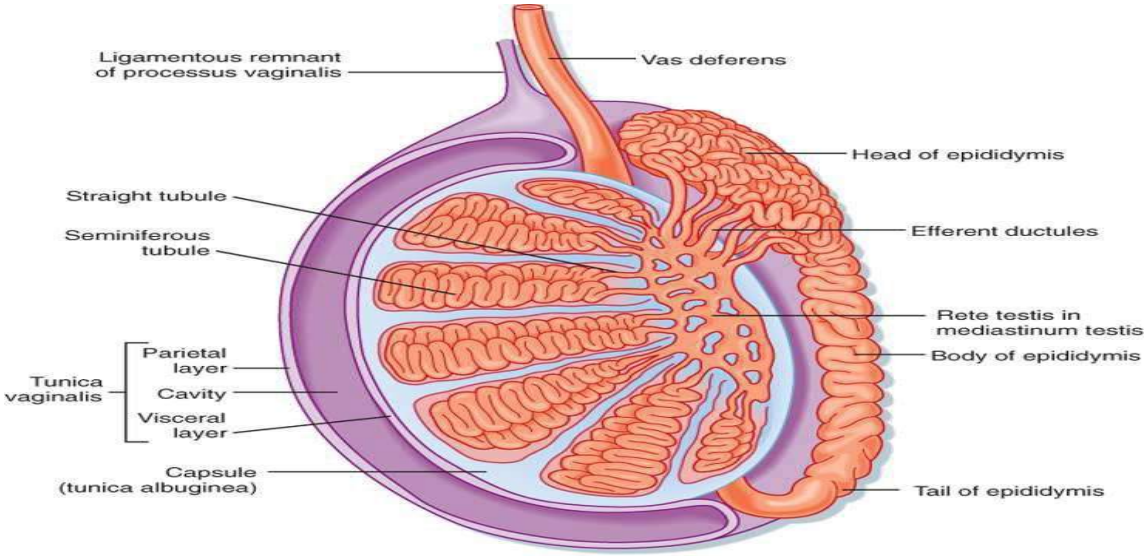

Figure 3: L.S. of testis

The secretion of GnRH is pulsatile and can vary greatly throughout the day and/or year, and therefore the secretion of LH and FSH are also pulsatile (although the plasma concentration of FSH does not fluctuate as much as LH due to the effect of Inhibin, see below). The activity of GnRH neuroendocrine cells is determined by spontaneous rhythms and by sensory impulses. Cycles such as seasonal sexual activity are controlled by this pulsatile system. In male animals there are generally 4 to 12 GnRH pulses per day.

Testosterone Regulation

When LH binds to the Leydig cells, it stimulates the cellular messenger **Camp** to **activate protein kinase A**. Protein kinase A undergoes a series of phosphorylation's that in turn activate a series of enzymes that synthesis testosterone from the cholesterol base molecule. A portion of the testosterone produced in the Leydig cells diffuses into the Sertoli cells that are positioned adjacent to the Leydig cells in the testes but separated by a basal lamina. This secreted testosterone is converted to the female sex hormone estradiol in the Sertoli cell and as with the testosterone, a proportion diffuses into the blood, becoming part of the negative feedback system for LH. Testosterone inhibits the secretion of GnRH from the hypothalamus and therefore secretion of LH from the pituitary gland. If the testes are removed via castration, blood concentrations of LH and FSH will increase as there is only limited negative feedback.

Effects of Male Sex Hormones

Testosterone plays a crucial role in the development of male sex organs during fetal growth where increased production of testosterone causes penis growth and development of accessory sex glands during puberty. Testosterone also affects a number of other characteristics of the male, often called the "secondary sex characteristics". Testosterone is able to bind to receptors in the cytosol of cells in the same manner as other steroid hormones and these hormone-receptor complexes are then able to bind to DNA in the nucleus resulting in alterations in the level of transcription of specific genes. Testosterone has a number of anabolic effects stimulating the development and growth of the skeleton and skeletal muscles. Muscle masses show a general increase and in certain body regions such as the neck of stallions or bulls there is obvious hypertrophy. Testosterone also alters behavior in terms of increasing the degree of sex drive and as a result of the action in several areas of the brain, behavior can become more aggressive. The **larynx** of males also enlarges during puberty and the vocal cords lengthen resulting in a deeper and stronger voice.

Testosterone also causes an increase in the level of pheromones to be secreted by glands in the skin which attract and evoke sexual behavior in females. Glands use in scent marking and territorial marking are also activated by testosterone. In certain species, tusks, antlers and horns are also stimulated to develop.

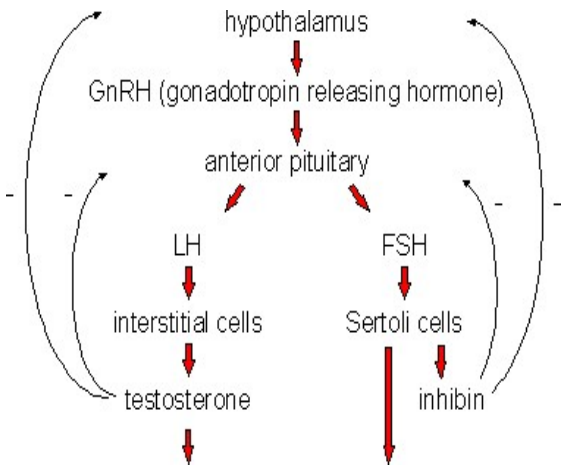

Figure 4: Flow chart showing hormonal control of spermatogenesis

Inhibin

Inhibin is a type of glycoprotein that is synthesized within the granulosa cells of ovarian follicles in females and in 224 Eurico cells located in the seminiferous tubules within the testes in the male. In both males and females, the target organ for inhibin is the adenohypophysis, specifically the gonadotrophic cells (basophilic cells).

In the male inhibin production is stimulated via androgens. Inhibin inhibits FSH secretion, which together with decreased concentrations of LH and testosterone results in decreased spermatogenesis and therefore decreased sperm output and quality.

In females some studies have suggested that inhibin may also be produced by the placenta. In females' inhibin inhibits FSH secretion. It does however not have any effect on the secretion of LH. When inhibin is secreted, a relatively higher concentration of LH is secreted from the anterior pituitary gland than FSH. Therefore, during follicle development, the increased LH concentration causes cessation of the recruitment of further follicles under the effect of FSH. The hormonal changes resulting from the production of inhibin cause some of the previously recruited follicles to undergo atresia. Inhibin in the female can also be diminished by GnRH and enhanced by insulin-like growth factor-1 (IGF-1).

Activin

Activin is a glycoprotein that is produced within granulosa cells in females and 224euroco cells in the male. Activin is thought to play an almost directly opposite role to that of inhibin and is involved in many physiological functions including stimulation of FSH synthesis and other roles including cell proliferation, cell differentiation, apoptosis and homeostasis. The target tissue for activin in the male is the epididymis where it enhances spermatogenesis via increased FSH secretion. Activin also enhances the effect of LH on the testes. In the female activin has an effect on the anterior pituitary gland, specifically on gonadotroph cells, resulting in increased FSH secretion. The increased concentrations of activin results in increased FSH binding on the female follicle and FSH-induced aromatization (increased synthesis of estrogens). Activin also enhances the action of LH in the ovary. A further non-reproductive role of activin is its role in skin lesions where it is thought to stimulate keratinocytes.

Prostaglandin F2α

Prostaglandin is a C2O fatty acid and is produced within the uterine endometrium and vesicular glands. Estradiol stimulates prostaglandin synthesis while progesterone inhibits it. The target tissue in the female is the corpus luteum, uterine myometrium and ovulatory follicles. In the

female PGF2α cause luteolytic and can also cause the induction of tone and contractions within the uterus. It plays an important role in parturition in ruminants. If a pregnancy is to remain viable then luteolytic needs to be avoided and this is achieved where concentrations of PGF2α remain below a threshold level allowing the corpus luteum continue to secrete progesterone and thus maintain pregnancy. There are two main factors involved in the regulation of uterine secretions of PGF2α; oxytocin secretions from the corpus luteum and molecules secreted by the developing embryo that facilitate the maternal recognition of pregnancy.

Oxytocin secretion via the corpus luteum stimulates endometrial production of PGF2α and by the end of the luteal phase the concentration of oxytocin and the number of oxytocin receptors within the endometrium allow the production of enough PGF2α to breach the threshold level and cause luteolysis. During pregnancy the embryonically produced pregnancy recognition molecules inhibit the secretion of PGF2α from the endometrium ensuring that luteolysis cannot occur.

Normally the concentration of PGF2α in arterial blood is relatively low due to extensive metabolism by PGF2α-dehydrogenase (in especially the lungs). These levels are below the threshold required to cause luteolysis as PGF2α production in early gestation is low. The ovarian artery is wrapped around the uterine vein. This creates a countercurrent mechanism by which the lipid soluble prostaglandins are able to diffuse from the uterine vein into the ovarian artery. During the latter stages of the luteal phase as PGF2α production increases luteolysis will occur as PGF2α Is able to reach its target in the ovary before being metabolized in systemic circulation.

Horses and pigs do not pose this countercurrent mechanism. In these spp. The [PGF2α-dehydrogenase] in systemic circulation is much lower in order to induce luteolysis when Prostaglandin concentration rises.

Prostaglandin (PGE2)

PGE2 is another form of prostaglandin that is produced by the ovary, uterus and embryonic membranes. This form of prostaglandin also has other important roles including vasodilation, smooth muscle relaxation, and inhibition of the release of noradrenaline from sympathetic nerve terminals. In females its target tissue is the cervix (it is a potent cervical dilator), corpus luteum and the oviduct where it helps induce ovulation and the secretion of progesterone from the corpus luteum. PGE2 also plays an important role during labor where it aids the softening of the cervix in animals with a soft-type cervix (equine and human) and aids stimulation of uterine contractions. It can thus be used to prepare the tract for parturition.

Human Chorionic Gonadotrophin (Hcg)

Hcg is a form of glycoprotein that is 226eurocoele226 within the trophoblast cells of a blastocyst. Hcg is particularly important in primate reproduction where it has a similar effect to LH in stimulating the continued production of progesterone and estrogens. This represents part of the system involved in fetal-maternal communication and pregnancy recognition. Primate blastocysts therefore produce Hcg in relatively high concentrations during the first 3 months of pregnancy. Hcg has also been suggested to play a role in defense of the embryo from the maternal immune system during the initial stages of pregnancy. In males Hcg increases the growth of the fetal testes. As Hcg is only produced by embryonic cells, the presence of this hormone within maternal blood can be used for pregnancy confirmation.

Placental Lactogen (PL)
Placental lactogen is a form of protein that is produced by the placenta and is chemically close in composition to growth hormone. The primary target tissue of PL are the mammary glands where they stimulate the growth of alveoli during pregnancy. PL is also referred to as Chorionic Somatomammotropin (CS).

Relaxin
Relaxin is produced mainly by the corpus luteum in most species and in the placenta (main contributor in the equine) and ovaries throughout pregnancy. During pregnancy relaxin prevents the initiation of uterine contractions, together with progesterone. Relaxin accumulates trough tout pregnancy and is released in large amounts a few days before parturition. Its target organs are the cervix, vagina, pubic symphysis and related structures. Relaxin is responsible for the softening and relaxation of connective tissues in the cervix, muscles and ligaments in the pelvis prior to parturition. Estradiol priming is required for this. This relaxation of tissues via relaxin is performed in conjunction with prostaglandin.

16.4 PHEROMONES:

The term *pheromone* is derived from the Greek word *Pherein*, meaning "to transfer excitement," and was first proposed by Karlson and Luscher in 1959. Pheromones are chemical substances released by one animal into the external environment, which then evoke a physiological or behavioral response in another individual of the same species. They are generally volatile and odorous fatty acids, detected by other animals through olfaction. Karlson (1960) defined pheromones as chemical signals exchanged between individuals of the same species that trigger specific behaviors or developmental processes. Because they act outside the body, 227eurocoeles are also known as **ectohormones** and play important roles in attracting or repelling individuals, identifying sex and status, and regulating physiological processes in other individuals.

16.4.1 Types and Functions of Pheromones:

- **Releaser or Sex Pheromones:** These pheromones cause immediate behavioural responses, especially related to mating. They are highly potent but degrade quickly. For instance, the female silkworm moth secretes *bombykol*, and the female gypsy moth secretes *gyplure* to attract males over long distances.
- **Trail Pheromones:** Used by social insects to guide others to food sources or new nest sites. Ants, for example, mark their paths with volatile hydrocarbons, and these trails attract other ants. The pheromone trails require continuous renewal because they evaporate quickly. Some insects, like aphids, release geraniol to indicate food sources.
- **Alarm Pheromones:** These pheromones warn members of a species about predators or threats. Aphids release alarm pheromones when attacked, while *Vespula squamosa* and *Polistes exclamans* use them to alert colonies of danger.
- **Primer Pheromones:** Unlike releaser pheromones, primer pheromones trigger long-term developmental changes in the recipient rather than immediate behavioural responses.
- **Territorial Pheromones:** These mark and defend an individual's territory. In cats and dogs, for example, territorial pheromones are present in urine and deposited on landmarks to indicate the boundaries of their territory.

16.4.2 Differences Between Hormones and Pheromones:

Pheromones differ from hormones in several ways. They are produced by exocrine glands rather than endocrine glands and are transmitted through the external environment instead of

circulating within the body. Pheromones are usually more species-specific than hormones and elicit specific behavioural, developmental, or reproductive responses in other individuals of the same species, whereas hormones primarily regulate the organism's own physiological processes.

16.4.3 Hormonal Control of Reproduction and Parental Care:

Endocrine glands play a vital role in reproduction by producing hormones that coordinate the function of reproductive organs. Hormones regulate the development and maturation of eggs and sperm, the reproductive behaviour of mates, and parental care to ensure offspring survival. Many animals reproduce seasonally, with reproduction timed to periods of optimal environmental conditions such as favourable temperatures and abundant food. Environmental cues, particularly photoperiod (day length), help synchronize reproductive cycles with these favourable conditions, ensuring successful mating, development, and care of young.

16.5 SUMMARY:

Reproduction in animals is intricately regulated by a network of hormones that control gamete development, mating behavior, pregnancy maintenance, and parturition. GnRH, LH, FSH, prolactin, oxytocin, estrogens, progesterone, testosterone, inhibin, activin, prostaglandins, Hcg, PL, and relaxin work together through feedback mechanisms to synchronize reproductive events. Pheromones complement hormonal control by influencing behavior, attraction, territoriality, and developmental changes, ensuring successful reproduction and parental care in different species.

16.6 TECHINICAL TERMS:

GnRH, LH, FSH, Prolactin, Oxytocin, Estradiol, Progesterone, Testosterone, Inhibin, Activin

16.7 SELF-ASSESSMENT QUESTIONS:

1. Explain the role of GnRH in regulating reproductive hormones.
2. How do LH and FSH differ in their target tissues and functions?
3. Describe the effects of progesterone during pregnancy.
4. What are the main types of pheromones and their functions?
5. How does testosterone influence male reproductive behavior and secondary sexual characteristics?

16.8 SUGGESTED READINGS:

1. Eckert H. Animal Physiology: Mechanisms and Adaptation. W.H. Freeman & Company.
2. Floray E. An Introduction to General and Comparative Animal Physiology. W.B. Saunders Co., Philadelphia.
3. Goel KA and Satish KV. 1989. A Text Book of Animal Physiology, Rastogi Publications, Meerut, U.P.
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- Dr. N. GOPALA RAO

LESSON-17
REGENERATION

OBJECTIVES:

1. To understand the mechanisms and types of regeneration across animal groups.
2. To study cellular and molecular processes, including stem cells, gradients, and genes involved in regeneration.
3. To analyse the evolutionary and functional significance of regeneration in survival and adaptation.

STRUCTURE:

17.1 INTRODUCTION:

Regeneration is the biological process by which animals restore lost or damaged tissues, organs, or body parts. This remarkable ability varies widely across the animal kingdom, from simple organisms like Hydra and Planaria to more complex animals such as amphibians, crabs, and mammals. Regeneration can involve simple tissue replacement, dedifferentiation of cells, or compensatory growth of functional tissues. The mechanisms of regeneration are broadly classified into morphallaxis, epimorphosis, and compensatory regeneration, each with distinct cellular and molecular strategies.

17.2 TYPES OF REGENERATION:

Throughout the animal kingdom, stem cells are involved to regrow tissues or organs that have been lost. We can designate such regrowth or repair as normal and routine regeneration. There are numerous examples of stem-cell mediated regeneration, such as, continuous production and replacement of blood cells form hematopoietic stem cells in bone marrow; regrowth of hair shafts from stem cells in hair follicles etc. but such routine regeneration is not the topic of discussion in this chapter. The regeneration mechanisms, which are not routine and peculiar in specific animals, are three types:

- When regeneration occurs mainly by repatterning of existing tissues and re-establishment of boundaries, it is called morphallaxis**.** In this process, there are little growth and seen in *Hydra.*
- The regeneration process that involves dedifferentiation of adult tissues to form undifferentiated mass of cells, which are again respecified into lost structures, is called epimorphosis**.** There is new growth of correctly patterned structures. Epimorphosis is seen in planarian flatworms and in limb regeneration of urodeles amphibians.
- A third type of regeneration is found in mammalian liver, called **compensatory** regeneration. In this process, each differentiated cell type divides and produces cells like itself and they maintain their differentiated functions. There is no dedifferentiation of adult cells; hence no mass of undifferentiated tissues is formed.

17.3 MORPHALLAXIS REGENERATION IN HYDRA:

Hydra is freshwater coelenterate (phylum Cnidaria). It has a hollow tubular body, about 0.5 cm long, with head at one end foot at another. Head or distal end consists of a conical **hypostome** with mouth at its tip and a set of tentacles surrounding the mouth. The tentacles are used to catch small prey animals upon which *Hydra* feed. The foot or proximal end is the **basal disc,** which enables *Hydra* to stick to an underwater substratum.

Hydra is diploblastic animal that is having only two germ layers. Body wall is composed of two cell layers –outer epithelium or ectoderm and inner epithelium or endoderm. Two layers are separated by a basement membrane and there is no true mesoderm layer. There are about 20 different cell types in the body of *Hydra,* which include nematocytes (stinging cells), secretory cells, nerve cells, interstitial cells etc. Interstitial cells act as stem cells, which can give rise to other cells. But interestingly, interstitial cells have little role in regeneration of this genus.

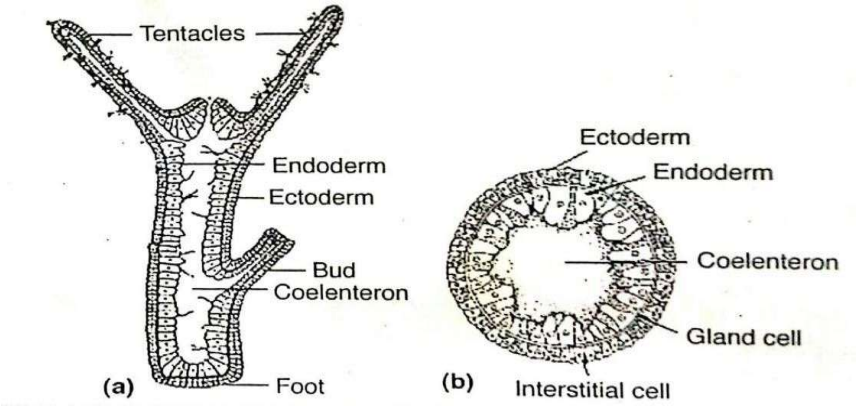

Figure 1: Structure of an adult Hydra (a) and a transverse section showing two germ layers (b

A. Body of *Hydra* is under dynamic growth:

Like most animals, body of *Hydra* is not stable. A well- nourished *Hydra* is always in a dynamic state of growth. Cells of both epithelial layers constantly undergo mitosis, and as the tissues grow, cells are displaced along the body column towards head or foot. In order to maintain size, an adult *Hydra* needs to shed the excess cells continuously. Cells are shed through two extremities-at the tips of tentacles and at the basal disc. In this process of

continuous growth, cells are continually changing their relative positions along the body column. Thus, each cell plays several roles, which depends upon the age of the cell.

Most excess cells are used when new Hydra is produced from the body column by asexual budding. (Hydra can reproduce sexually too, but only in unfavorable conditions like low temperature, crowding etc.). Budding generally occurs at body column at about two-third of the way down from head. During budding, an evagination occurs in the body wall that forms a new column. Ultimately, a head develops in the distal end of the column and it detaches from the parent body as a small new Hydra. During continuous growth, changing position and role, and during asexual budding, there must be some dynamic mechanisms for repatterning of cells. These mechanisms enable Hydra to have a remarkable capacity of regeneration.

When the body column of a *Hydra* is cut transversely into two halves, the lower portion regenerates a head and upper portion a foot. If the body column is cut transversely in several small pieces, each piece regenerates to become small Hydra. The structure cells will regenerate at cut surface depends on their relative position, indicating a well-defined polarity**.** The polarity is even maintained in small pieces; the distal end regenerates a head and proximal end become basal disc. When a small piece of body column regenerates, there is no initial increase in size. Thus, regenerated Hydra becomes a small one, which after feeding attains a normal size. Even, a piece of body column lacking interstitial cells (stem cells) regenerate normally.

Since each cell retains its plasticity, the regeneration of Hydra is called morphallaxis**.** Head of Hydra acts both as organizer and inhibitor Each part of body column of Hydra along apical – basal axis is potentially able to form both head and a foot. But there is series of morphogenetic gradients that allow the head to form at one part and basal disc at another.

Early grafting experiments showed that a small portion of hypostome region of *Hydra* into middle (gastric) region of another Hydra induced a new body axis with complete head with tentacles. Similarly, grafting of a portion of basal region in the same region of another Hydra induced a new body column with basal disc at its tip. Thus, it appears that Hydra has two organizing regions, one at each end-the hypostome and basal disc, which give Hydra its overall polarity.

Grafting experiments also indicate that the hypostome produces an inhibitor of head formation, effectiveness of which becomes lesser with distance from the head. (This inhibitory property normally prevents inappropriate head formation in intact animals). When a body fragment of just below the head (sub hypostomal region)

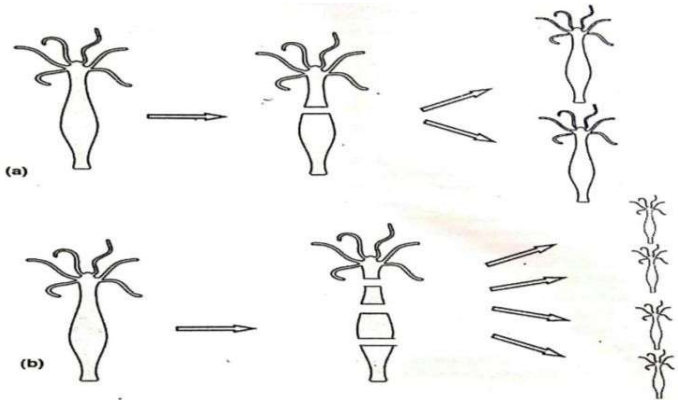

Figure 2: Regeneration in Hydra (a) when cut transversely into two halves and (b) when cut transversely into several pieces.

(i) Grafted into gastric region, no new head is induced and the grafted tissue simply absorbed into body.
(ii) Grafted into same region and the head of host Hydra is removed, the graft tissue induces a new axis with head.
(iii) Grafted near the foot, the graft tissue induces a new body axis with head, while the original head of the host is in position.

Results from these experiments suggest that:

(a) Formation of additional head is normally prevented by an inhibition mechanism.
(b) The inhibitory mechanism act through a gradient with its highest effectiveness at the head.

I When head is removed [experiment (ii)], effectiveness of head inhibitory Mechanism is lost.

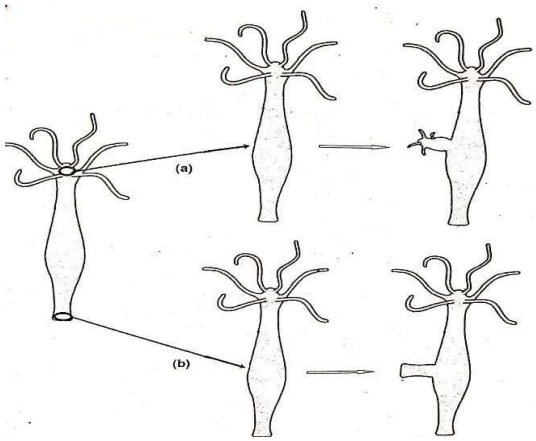

Figure 3: Grafting experiments in Hydra. (a) Hypostome region into middle position of another Hydra, (b) basal region at same position of another Hydra.

B. Basal disc activation and inhibition gradient:

Similar like head activation and inhibition gradients, there is a source of both foot activation and inhibition at the basal disc. The gradient of head inhibition is highest at head region, lower down the body column and lowest at basal disc. Similarly, gradient of foot inhibition is highest at basal disc, lower upward the body column and lowest at head. The inhibition gradients for the head and the foot are crucial in determining where a bud can form.

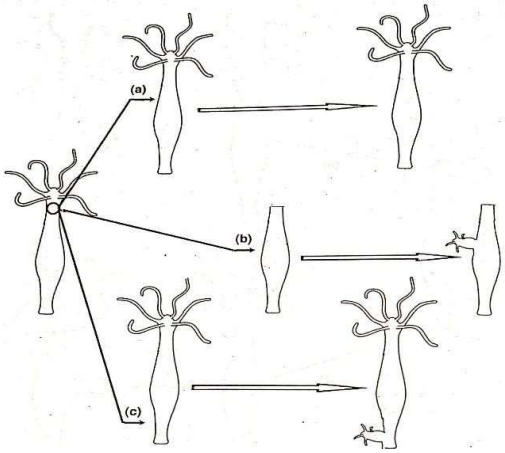

Figure 4: Results of grafting of sub hypostomal region into (a) gastric region. gastric region but head is removed and (c) foot region of another hydra

The region of body column of a growth *Hydra* that is about two –third down the trunk has the both inhibitor at minimal. This region is known as bud formation region.

C. Two gradients caused by head in *Hydra:*

Experiments with regeneration of *Hydra* indicate that two gradients are set up along the body column by the head, one is a gradient in positional value and other is a gradient of head inhibitor. The gradient in positional value seems to control two incidences –resistance to head inhibition and head –inducing ability.

The gradient in resistance to head inhibition can be determined by the ability of different regions of the body to suppress head formation. This gradient in resistance decreases with distance from the head. Thus, when region 1 (resistance to head inhibitor is high) is transplanted near foot (insufficient head inhibitor), a new head is regenerated. But when region 5 (resistance to head inhibitor is low) is transplanted near foot, inhibition is sufficient to prevent head regeneration.

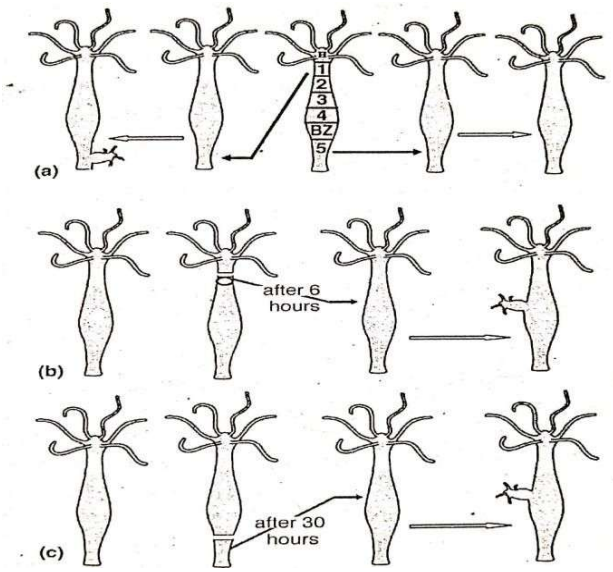

Figure 5: Head of Hydra set up a gradient in positional value along body column, which control resistance to head inhibition (a), and head inducing ability (b) & (c).BZ Budding zone; H = Hypostome.

The gradient in head inducing ability also decreases with distance from head i, e, high at head end low at foot end. This gradient in head inducing ability is determined by a gradient in positional value, which is evident from the differences in time required for different regions of body column to acquire head inducing property. It is already mentioned that region 1 from an intact *Hydra,* when transplanted to gastric region of another individual, no axis is induced. When the head of donor Hydra us cut, region 1can induce a new axis when transplanted 6 hours after head amputation. The region 5 forms a new axis when transplanted 30 hours after amputation. Thus, more down the axis of amputation, longer the remaining cells take to acquire head –inducing property.

A simple model assumes that head inhibitor is a diffusible factor that is secreted by head. The factor diffuses down the body column and is degraded at foot end. The gradient in positional value is supposed to be an intrinsic property of cell. Both gradients are linear, decreases constantly with distance form head.

According to this model

I. In an intact *Hydra,* level of inhibitor is greater than the threshold set by the positional value, hence head regeneration is inhibited.

II. After head removal, concentration of inhibitor started falling, lowest at the cut end. As the inhibitor concentration falls below threshold set by local positional value, the positional value increases at that enc. So, when the head region is removed, the first necessary step in regeneration of *Hydra* is to specify a new head region at the cut end.

III. Once positional value increased to normal alike head region, the cells start to secrete inhibitor, which prevent another head formation elsewhere. After new head specification, inhibitory gradient is re-established but that takes time. In the meantime, the regenerated small *Hydra* grows to adult size.

D. Chemicals and genes affecting regeneration of *Hydra*:

Chemicals of activators and inhibitors are found to be peptide in nature. Three peptides associated with head activation are Heady, Head Activator and Hym-301. First two are key molecules for head formation and inhibition of bud; the third peptide has a role in regulating the number of tentacles. Several other small peptides are known to affect foot formation, but details of their functions are yet to be sort out.

Several genes are found active in head (hypostome) organizer area, which suggests that a set of signals is conserved over millions of years of evolution and acts as organizer throughout animal kingdom. The Hox *genes,* which control body pattern in many animals, are involved in regional patterning of Hydra. Some *Hox* genes express in regional pattern and seem to be involved to specify head –foot positional values.

A Hydra version of goosecoid *gene,* an organizer specific gene in vertebrates, is expressed in the hypostome region, just above where tentacles will appear. When hypostome is brought in contact with trunk of an adult *Hydra,* it induces the expression of *brachyury* gene. The usual tissue in which *Brachyury* expresses in mesoderm that *Hydra* lacks; it suggests that the head of *Hydra* correspond to blastopore of other animals. Another organizer-specific gene is *Hywnt,* the *hydra wnt* homolog, has restricted expression to the apical tip of body axis, which is the *Hydra* head organizer. During head regeneration both *wnt* and *Hydra β-catenin* genes are expressed at the tip within one hour after head amputation. A significant upregulation of *Hydra β-catenin* expression is observed in the prospective budding zone, just before evagination begins.

Understanding regeneration in *Hydra* allows insight into organizer and developmental gradients, which evolved early in animal kingdom. So, it is logical that complex body patterns of higher animals evolved from simple body plan of animals like *Hydra.*

17.4 REGENERATION IN PLANARIA:

Planarians are free-living flatworms (phylum Platyhelminthes), primitive but organized. They are triploblastic, bilaterally symmetrical, acoelomate and unsegmented. Except digestive and simple nervous system, they lack circulatory, respiratory and skeletal structures. They reproduce through two methods-asexually by fission and sexually through producing sperms and eggs by males and females respectively.

Planarians have long been known for their high regenerative capacity. If a Planaria is cut into two halves, the anterior half will regenerate a new tail from the cut surface and the posterior half will regenerate a head from its cut surface. Even a small fragment cut from its body can give rise to an intact animal. Apparently, regenerative power of planaria seems like of *Hydra,* but there are basic differences in the mechanisms of regeneration. The process of regeneration in planarians involves two main events blastema formation and pattern formation.

A. Blastema formation:

When a planaria is cut, there is a muscular contraction limiting the area of the cut. The epithelium around the wound rapidly closes up and the cut surface is covered by a thin film of epidermal cells from the stretched old epidermis. Below the wound epithelium, mass of undifferentiated cell accumulates in a few layers to give rise to an outgrowth, called regeneration blastema. Blastema is generally used to designate regeneration bud containing undifferentiated proliferating cells. But in planaria, there is no cell division in regeneration blastema. The cells in blastema of planarians come dividing neoblast cells in the proximal region. The blastema grows by continuously incoming cells, which then differentiate over a few days to form the missing body part.

Planarians can grow or shrink on a daily basis through continuous cell turnover, and it depends on food supply. Neoblast cells with large nuclei consist about 20% of total cells in the body of planarians. Neoblasts are the only dividing cell population in planarians and perform as stem cells. When one neoblast cell divides, one daughter cell produces a differentiated cell in the body and other daughter cell remains as stem cell. Neoblasts have developmental totipotency and can produce 12-15 histologically distinguished and differentiated cell types found in the body of planarians. The presence of neoblast cells in body is evitable for regeneration in planaria, because irradiation of these cells results in failure of regeneration.

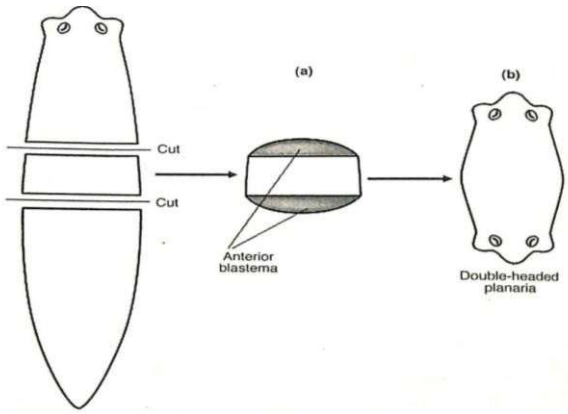

Figure 6: A short segment from middle part of body of Planaria forms anterior blastema at both ends (a) resulting a double-headed worm (b).

17.5 REGENERATION IN AMPHIBIANS:

A. Pattern formation:

The cells in the blastema have to decide first about that of polarity, whether to be a head dud or tail bud. Little is known about determination of polarity, but it is suggested that origin of would epithelium is important. When the wound epithelium originates from dorsal epidermis, it becomes anterior blastema producing head. Similarly, if the wound epithelium originates from ventral epidermis, it becomes posterior blastema producing tail.

Involvement of some mechanism to control polarity is suggested by errors in regeneration. In some species of planaria, short segment from middle part of body form anterior blastema at both ends resulting a double headed worm. In this case, blastema in posterior end of the segment lacks some signal that normally causes it to become tail blastema. Existence of some signaling process associated with planarian regeneration is also evident. When a second head is grafted near the original one and the original one is amputated, its regeneration is prevented by the grafted one.

The undifferentiated cells originating after division of neoblasts accumulate within blastema and then differentiate into new structures following a distal-proximal sequence. Ultimately, lost structural pattern is restored in proportion of normal body size. The epithelium enclosing blastema cells induce expression of several homebox genes. These genes, such as *otx, pax6,* probably maintain the cell proliferation below the wound surface and induce some early pattern formation.

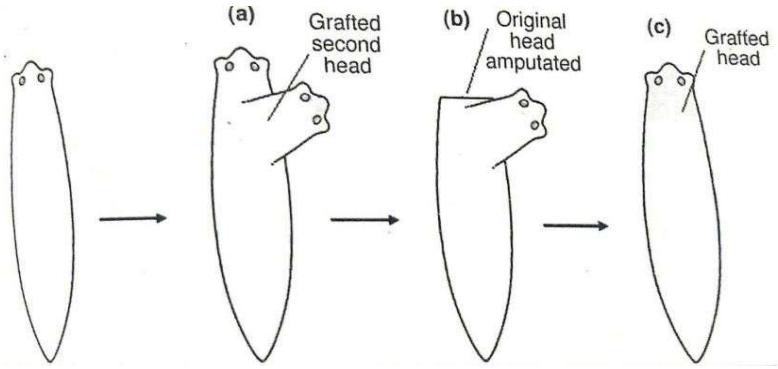

Figure 7: When a second head is grafted near original head (a) and the original one is amputated (b), its regeneration is prevented (c)

There are two theories existing on the origin of blastema cells. First is neoblast theory**,** which is current view and put forward that neoblast cells give rise to the cell population involved in regeneration. Recent electron microscopic studies put forward a dedifferentiation theory**.** According to this theory, differentiated cells below blastema undergo dedifferentiation during regeneration. Gland cells lose secretary properties, muscle cells lose their fiber system and they transform into neoblasts. Thus, planarian regeneration includes both normally existing neoblasts and transformed neoblasts from other cell types. The regeneration of planaria should have a special class between morphallaxis and epimorphosis.

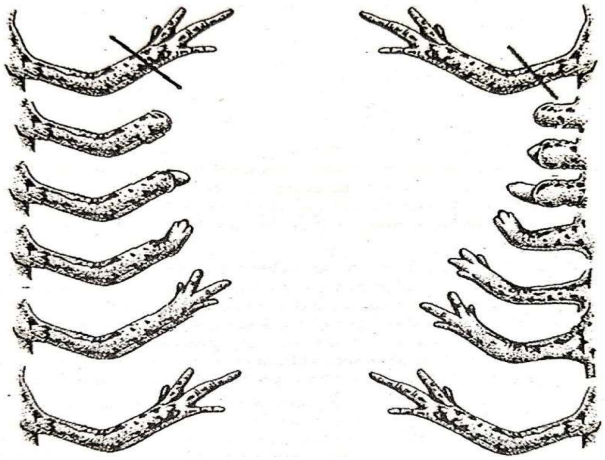

Figure 8: Urodel amphibians have remarkable capacity of regeneration of only the missing part. When a wrist is amputated, only the wrist is regenerated (left figures). When arm is amputated in middle region (right figures), entire portion is regenerated.

B. Regeneration in urodele amphibians:

A remarkable capacity of regenerating body structures like limb, tail, jaws etc. is found in urodele amphibians. An adult limb of urodele consists of many differentiated cell types which are organized and arranged to form the structure. So, the central question of regeneration in urodeles is the origin of cells, which give rise to regenerated structures. Are there any reserve cells or the existing differentiated cells change their character and dedifferentiate to give rise to all other cell types? Another point is that only the missing part is reconstructed; for example, when a wrist is amputated, only the wrist is regenerated. So, there is a positional knowledge about where the limb is severed on regeneration involves new growth, it is epimorphic in nature.

C. Formation of Regeneration Blastema:

Following amputation of a salamander limb, there is a rapid formation of plasma clot. Within 8-12 hours, epidermal cells from remaining structure migrate over the wound surface to form a wound epidermis. Initially single –layered, the wound epidermis proliferates and forms an apical ectodermal cap.

Within 3-4 days of amputation, the cells below ectodermal cap undergoes dedifferentiation. Fibroblasts, nerve cells, muscle cells, bone cells all lose their differentiated properties and detach from each other. Genes in these differentiated tissues are down-regulated. Thus, at the cut edge, mass of a dedifferentiated, indistinguishable proliferating cells aggregated below the ectodermal cap. The cell mass is called regeneration blastema and continue proliferation. In the next weeks, the limb regenerates and these cells differentiate into muscle, cartilage,242eurocoele tissue etc. Such process of conversion of one tissue into another is called trans differentiation or metaplasia**.**

Cells of blastema originate locally from the mesenchyme tissue of the stump, near the site of amputation. Majority of them come from dermis but also from cartilage and muscle. Multinucleated muscle cells, which have stopped mitosis, can be changed to uninucleate cells in culture medium, in presence of thrombin. Thrombin is familiar as one key protein in blood clotting, but also involved in dedifferentiation in some cases. Msx1 is a transcription factor that is known to prevent myogenic differentiation in mammals. Its characteristic expression is found in undifferentiated mesenchymal cells and dedifferentiated muscle cells, which undergo regeneration.

Whether cells differentiated into muscle or cartilage in the regenerating limb remain true to its type or whether dedifferentiated cells can differentiate into different cell types, is a question. In urodele amphibians, the second statement is correct. Cultured limb muscle myotubes, which are multinucleated and left cell cycle, are labeled and introduced into regenerating limbs. Labeled unnucleated cells are found in blastema after a week. These unnucleated cells, derived from myotubes, proliferate and give rise to new muscle cells as well cartilages. Thus, blastema creates an environment that induces cells to dedifferentiate. These cells then act as progenitor cells for regenerating limb. Thus, blastema provides necessary environment containing cues for both dedifferentiation and redifferentiation into other cell types.

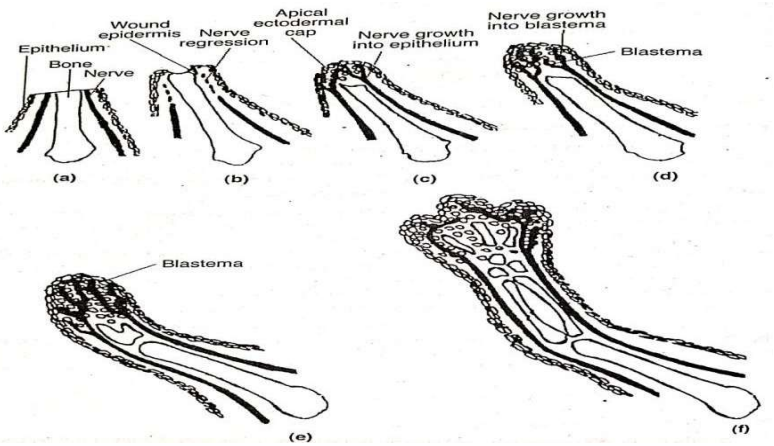

Figure 9: Urodel amphibians have remarkable capacity of regeneration of only the missing part. When a wrist is amputated, only the wrist is regenerated (left figures). When arm is amputated in middle region (right figures), entire portion is regenerated.

Normally mature skeletal muscle cells never divide, so entering cell cycle by the muscle cells is a key feature of salamander limb regeneration. During differentiation of vertebrate muscle, precursor cells withdraw from cell cycle after myoblasts fuse into myotubes. The regenerating cells of salamander contain Rb protein (a product of *Rb* gene) that is involved in cell cycle regulation, but it is inactivated by phosphorylation, so the cell re-enter cell cycle and divide. Local activation of thrombin is also associated with the ability of muscle cells to re-enter cell cycle.

D. Requirements for proliferation of Blastema Cells:

Proliferation of blastema cells is dependent on several factors:

1. Normal limb generation requires presence of minimum nerve supply. In limbs denervated before amputation, a blastema is formed but failed to grow. Nerve cells, therefore, seems to release some growth factor, that is essential for proliferation of blastema cells. Glial growth factor, a member of neuregulin family, is one likely candidate for maintaining high rate of cell division.

In an interesting experiment, it is found that when nerve is removed from a limb in early development, it can regenerate complete limb in absence of any nerve supply. It suggests that dependence of proliferation of blastema on nerve is imposed on the limb after the ingrowth of nerves.

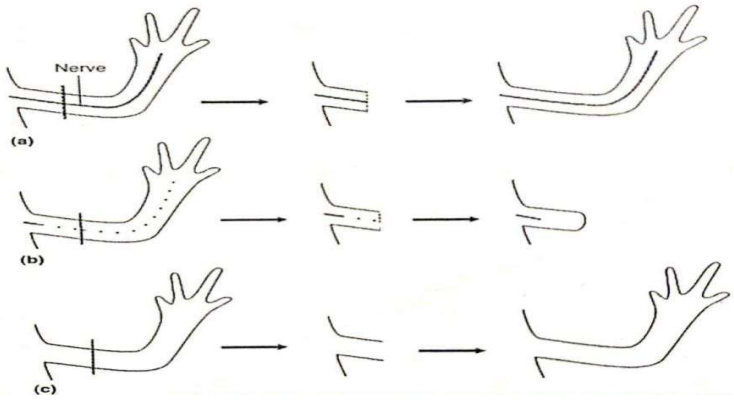

Figure 10: Proliferation of blastema cells in urodels is dependent on nerve supply except the limbs which are without nerve at early development. Regeneration of (a) Normal limb, (b) Denervated and (c) Aneurogenic limb.

2. The wound epidermis provides the signals to the underlying cells to dedifferentiate forming blastema and also its proliferation. The signals include Fibroblast growth factors (Fgf1 and Fgf2 is also important in regeneration by restoring the expression of a developmental gene *Distal –less* (Dx1) in the epidermis. Local application of Fgf2 can result in regeneration of denervated limb.

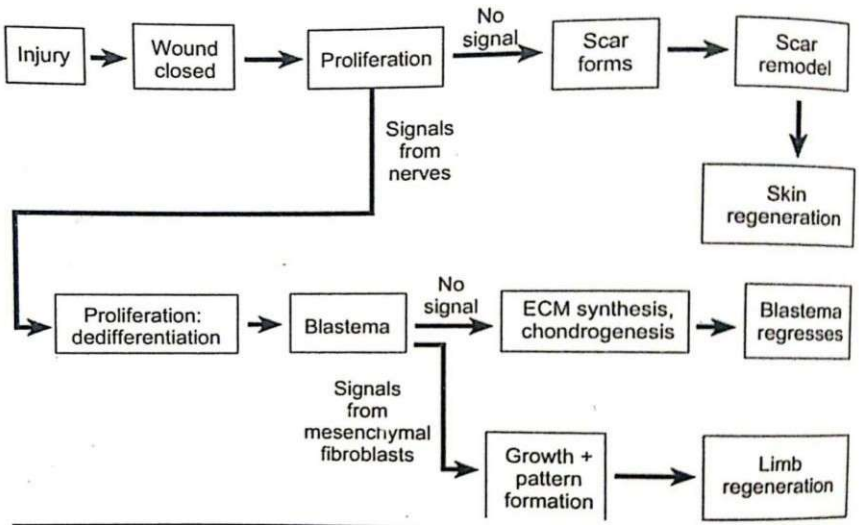

Figure 11: A model is forwarded that includes both nerve-dependent and mesenchyme- dependent steps of regeneration of urodel limb

Fgf2 servers as an angiogenesis factor because regenerating limb requires normal blood supply after amputation. Fgf2 also promotes mitosis and patterning of limb generation. Apical mesenchyme of blastema release some other Fgf2 (4, 8, 19), which are necessary as positional signals for successful regeneration.

A model of limb regeneration is forwarded that include both nerve-dependent and mesenchyme-dependent steps:

- After amputation, dermal cells proliferate and migrate over wound.
- Without signals from neurons, these cells form a scar and allow only healing (skin regeneration).
- In presence of neural signals these cells proliferate and differentiate to form blastema.
- Without signals from mesenchyme, the blastema regresses.
- In presence of mesenchymal signals, the blastema is patterned to regrow the limb.

E. Positional value and Pattern Formation in Regeneration of Urodele Limb:

Whether limb regeneration and embryonic limb development involve same mechanisms is not known but some relationship must exist between the two. Regeneration always follows the direction distal to the amputated surface and allows replacement of lost parts. If the hand is cut at wrist, only carpals and digits are regenerated; if cut at the middle of humerus, regeneration occurs distal to that point. Thus, positional value along the axis is of great importance. The blastema appears to have morphogenetic autonomy; when grafted to a neutral location permitting growth, it regenerates structures appropriate to the position from which it was taken.

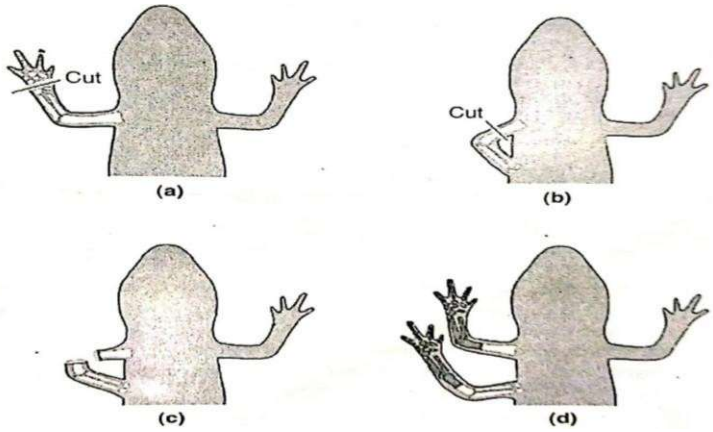

Figure 12: Distal end of limb of an urodel is amputated at wrist (a) and inserted into belly of same animal (b). When this limb is cut at mid-humerus level (c), radius and ulna are formed at proximal level (d).

A classic experiment showed that blastema is not simply replacing missing parts. Distal end of a limb is amputated at wrist and inserted into belly of same animal to establish a blood supply to that part. This limb was then amputated at mid-humerus level. Both cut surfaces regenerate distally, the attached part to the belly had a radius and ulna at proximal level.

Blastema is larger in size than embryonic limb bud. But it has a set of positional value along its proximal –distal axis, alike that set up during embryonic development. The retention of embryonic process, like the ability to specify new positional values is one of the main features of epimorphic regeneration in urodel amphibians.

Cells in amputated stumps can recognize the discontinuity in positional values. When a distal blastema is grafted to a proximal stump, the stump and blastema have different positional values. A normal limb is regenerated from the proximal stump by intercalary growth and cells from wrist blastema give rise to entire limb.

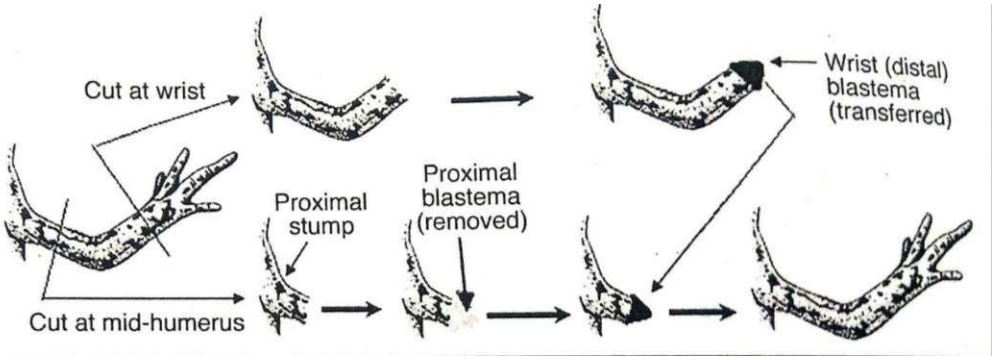

Figure 13: A wrist (distal) blastema can form entire limb from proximal stump.

Knowledge of pattern formation comes from the molecular basis of positional information. Recently, a cell surface protein is identified that is expressed in a graded manner along the proximal distal axis of salamander blastema. A cell surface protein, known as Prod1, is equivalent of mammalian cell surface protein CD59. Concentration of Prod1 has about twofold difference between proximal and distal blastema. Probably this protein guides the cells to determine positional identity.

Gradient in Prod1 evidently shows that cell-surface proteins are involved in regeneration. Transplantation experiments suggest that proximal distal positional values in urodel limb

regeneration are encoded as a graded property at the cell surface. Cell behavior relevant to axial specification is a function of the expression of this property. At present, it is not clear to what extent blastema cells inherit positional value and to what extent they are subject to signals inducing expression of positional values.

Relationship between *Hox* gene expression and positional value is becoming understood for both embryonic limb development and limb regeneration. Though initially *Hox* gene expression in regenerating limb is not same as embryonic limb development, but later becoming same as limb regenerate. During embryonic limb development, *Hoxa* genes are expressed with temporal and spatial co-linearity along proximal distal axis. During regeneration of salamander limb, two *Hox* genes –*Hoxa* and *Hoxd* are expressed together in the cells of stump within 48 hours of amputation. Experiments show that most distal region of blastema is divided into distinct zones within 4 days, which will become the proximal –distal regions of the limb.

F. Role of Retinoic Acid in Limb Regeneration:

Retinoic acid (RA) has crucial effect on regeneration of amphibian limb. RA is synthesized in wound epidermis of regenerating limb and form a gradient in the blastema along proximal-distal axis. This gradient probably informs the cells about their position in limb axis. RA can differentially activate the *Hoxa* gene across blastema and specify pattern in the regenerating limb. During normal regeneration, probably RA is secreted by wound epidermis and /or apical ectodermal cap. This RA induces gene activation for cell proliferation, down –regulates specific genes for differentiated cells and activates *Hox* genes, which inform the cells about their position in the limb and how much they have to grow.

When regenerating limb is exposed to RA, the blastema becomes proximalized, that means the regenerating limb has been cut at a more proximal place. When a limb is cut at middle of radius and ulna, and treated with RA, the regeneration occurs not only distal to the cut but also production of a new limb. Thus, RA can change the proximal distal positional value of blastema towards more proximal. RA probably affects proximalization by increasing the concentration of Prod1. RA change positional values in blastema by activating *meis* homebox gene, which are involved in specifying proximal identity.

17.6 REGENERATION IN CRABS:

Crabs have the incredible ability to regenerate lost limbs, such as claws or legs, which is vital for their survival. This process is intricately linked to their molting cycle, allowing the growth of new limbs under the protection of a newly formed exoskeleton. Regeneration helps crabs maintain their functionality and adapt to environmental challenges.

Process of Regeneration in Crabs

Autotomy: Crabs can voluntarily shed damaged or injured limbs at specific breakpoints to prevent further harm or escape predators.

Limb Bud Formation: Following autotomy, a limb bud forms at the site of the lost appendage. This bud is made up of undifferentiated cells that proliferate and differentiate to develop a new limb.

Molting Cycle: The regeneration process is closely tied to molting. Since the exoskeleton limits growth, the new limb develops underneath it. When the crab molts, the old exoskeleton is shed, revealing the partially regenerated limb.

Growth and Maturation: The newly regenerated limb is initially smaller and less functional. Over successive molting cycles, the limb grows and matures until it matches the original in size and capability.

Importance of Limb Regeneration

- **Survival:** Allows crabs to escape predators even after losing limbs.
- **Functionality:** Restores critical functions like locomotion and feeding.
- **Adaptation:** Demonstrates their resilience and ability to recover from injuries, maintaining ecological balance.

17.7 COMPENSATORY REGENERATION:

Compensatory regeneration is observed in higher organisms, where damaged tissues or organs recover their functional capacity without restoring the original structure. Unlike complete regeneration, which restores both form and function, compensatory regeneration emphasizes functional recovery.

Examples of Compensatory Regeneration

1. **Liver Regeneration in Mammals:**
   - The liver can regenerate its functional capacity after partial removal or damage.
   - Remaining liver tissue undergoes cellular proliferation to compensate for the loss.
   - The regenerated tissue does not replicate the exact structure of the original but restores vital functions like metabolism and detoxification.

2. **Kidney Regeneration:**
   - In certain species, kidneys exhibit limited compensatory regeneration.
   - Surviving nephrons increase their functionality to compensate for damaged ones.

3. **Heart Regeneration in Zebrafish:**
   - Zebrafish can regenerate damaged cardiac tissue, restoring heart function through the replacement of lost cells.

Mechanisms of Compensatory Regeneration

1. **Cellular Proliferation:** Surviving cells divide and expand to replace lost or damaged tissues.
2. **Tissue Remodeling:** Existing tissues reorganize to optimize functionality.
3. **Gene Activation:** Specific genes are activated to support repair and functional recovery.

Importance of Compensatory Regeneration:

- **Survival in Higher Organisms:** Enables recovery from injuries while maintaining essential bodily functions.
- **Functional Recovery:** Prioritizes restoring critical processes over structural replication.
- **Evolutionary Adaptation:** Demonstrates the ability of organisms to thrive despite injury or tissue loss.

17.8 SUMMARY:

Regeneration represents an evolutionary spectrum from simple tissue repatterning to complex dedifferentiation and compensatory growth. Hydra demonstrates morphallaxis, Planaria and urodeles show epimorphosis, crabs regenerate limbs via molting, and mammals rely on compensatory regeneration. Molecular signals, stem cells, gene regulation, and positional gradients are central to all regenerative processes. Studying these mechanisms provides insights into development, repair, and potential regenerative medicine applications.

17.9 TECHINICAL TERMS:

Morphallaxis, Epimorphosis, Compensatory regeneration, Blastema, Dedifferentiation, Apical ectodermal cap, Positional value, Neoblast, Autotomy, Homeobox genes

17.10 SELF-ASSESSMENT QUESTIONS:

1. What are the main types of regeneration and their differences?
2. How does morphallaxis in Hydra differ from epimorphosis in Planaria?
3. Explain the role of blastema in urodele limb regeneration.
4. What molecular signals regulate head and foot regeneration in Hydra?
5. How does compensatory regeneration differ from complete regeneration in mammals?

17.11 SUGGESTED READINGS:

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2. Gilbert SF. 2006. Developmental Biology, 8th Edition. Sinauer Associates Inc., Publishers, Sunderland, USA.
3. Schatten H and Schatten G. 1989. Molecular Biology of Fertilization. Academic Press, New York.

- **Dr. N. GOPALA RAO**

LESSION- 18

MOULTING AND METAMORPHOSIS

OBJECTIVES:

- To understand the biological processes of moulting and metamorphosis across different animal groups.
- To study the hormonal regulation, mechanisms, and adaptive significance of these processes.
- To compare different types of metamorphosis, including progressive and retrogressive changes, in evolutionary context

STRUCTURE:

18.1 INTRODUCTION:

Molting is the biological process by which animals shed old outer layers such as skin, exoskeleton, feathers, or fur to allow growth or replace worn-out structures. It is often regulated by hormonal changes and environmental cues and requires a significant amount of energy. During molting, animals may become more vulnerable to predators or environmental stresses, but the process is essential for survival, growth, and adaptation.

18.2 MOULTING IN ARTHROPODS (Ecdysis):

In arthropods like insects, spiders, and crustaceans, molting is essential for growth. Because their **exoskeleton** (hard outer shell) doesn't expand, they must shed it to get bigger. This process is called **ecdysis**.

Molting in arthropods is a complex process controlled by hormones, ensuring it happens at the right time. The key hormone is **ecdysone**, also known as the "molting hormone."

- **Brain's Role**: The process begins in the arthropod's brain, which secretes a hormone that stimulates glands in the thorax.
- **Ecdysone Release**: These glands then release ecdysone into the bloodstream. When a certain concentration of ecdysone is reached, it signals the cells in the epidermis (the layer of tissue that secretes the exoskeleton) to begin the molting process.

This hormonal cascade is a brilliant evolutionary adaptation that allows the animal to prepare its new skin before shedding the old one.

The Process

1. **Preparation (Apolysis):** This is the most critical preparatory phase, triggered by ecdysone. The epidermis separates from the old exoskeleton. Enzymes are secreted into the space between the old and new layers. These enzymes begin to digest the inner, softer layer of the old exoskeleton. The nutrients from this digested layer are reabsorbed and used to build the new exoskeleton.
2. **Shedding (Ecdysis):** After the new exoskeleton is fully formed underneath the old one, the animal is ready to shed. It often takes in large amounts of air or water, which causes its body to swell. This internal pressure is enough to rupture the old exoskeleton along pre-determined **suture lines** (areas of weakness). The animal then slowly pulls its limbs and head out of the old shell. This is a very delicate process, and an arthropod can get stuck and die if it can't complete the molt.
3. **Hardening (Sclerotization):** Right after shedding, the new exoskeleton is soft and vulnerable. The animal pumps fluid into its body to expand the new cuticle, giving it room to grow. Over a few hours or days, the new exoskeleton hardens and darkens in a process called **sclerotization**. This involves cross-linking protein chains, making the new cuticle rigid and protective. Until this process is complete, the animal is highly susceptible to predation.

Examples

- **Insects:** A caterpillar molts several times as it grows, shedding its skin to become a larger caterpillar before its final molt into a pupa.
- **Crabs:** A crab will hide after molting as its new shell hardens. During this time, it's called a "soft-shell" crab.

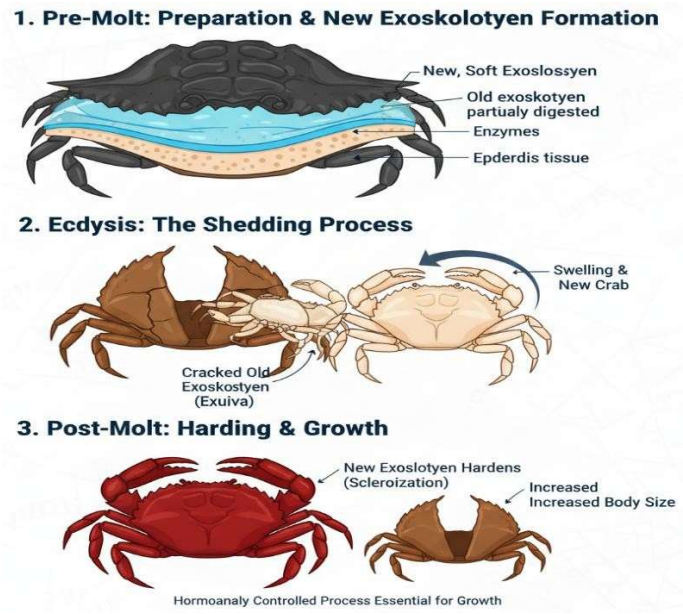

Figure 1: Crab exoskeleton shedding process

18.3 MOULTING IN VERTEBRATES:

Many vertebrates also molt, but it's often a less dramatic process focused on replacing worn-out feathers, fur, or skin.

Reptiles

Reptiles like snakes and lizards shed their skin to grow and remove parasites.

- **Preparation**: The process is triggered by hormonal changes. A new layer of skin begins to form beneath the old one. During this time, the reptile's skin may appear dull or cloudy, especially over the eyes in snakes, which affects their vision.
- **Apolysis**: The old skin separates from the new layer. In snakes, this entire outer layer, including the scales and the cap over the eye (the spectacle), loosens.
- **Shedding**: The reptile actively aids in the shedding process.

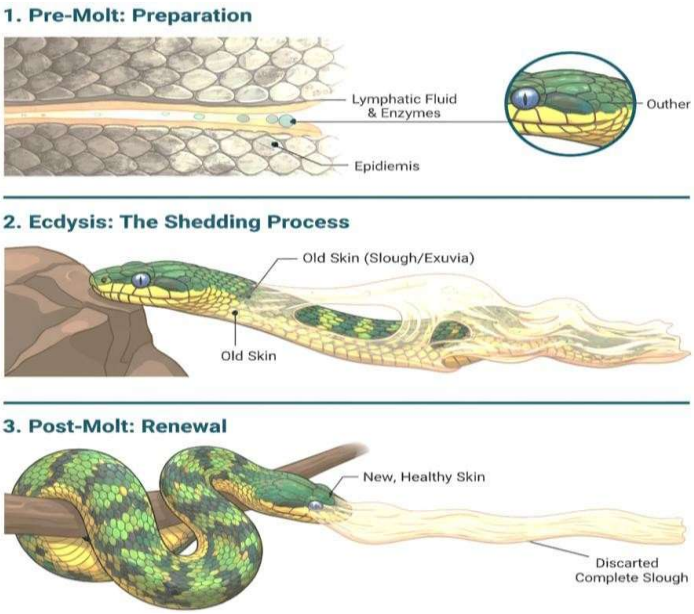

Figure 2: **snake showing the skin shedding**

- **Snakes:** A snake's outer skin doesn't grow with it. Before a molt, its skin becomes dull and its eyes appear cloudy as a new skin layer form beneath the old one. A snake will rub its head and body against a rough surface like a rock or branch to create a tear in the skin, often near the mouth. It then "peels" out of the old skin by crawling, leaving the shed skin (called a **slough**) behind in a single, continuous piece.
- **Lizards and Turtles:** These animals usually shed their skin in flakes and pieces rather than a single layer. A lizard might rub against objects or even eat the shed skin. Turtles shed the outer scutes (plates) on their shell and the skin on their legs and neck.

Post-Shedding: The new skin is fresh and vibrant, and the reptile's vision, which was cloudy during the process, returns to normal. The new skin is ready to protect the animal.

Birds

Birds molt to replace old, worn-out feathers. Feathers are dead structures made of keratin and cannot repair themselves. This process is essential for flight, insulation, and display.

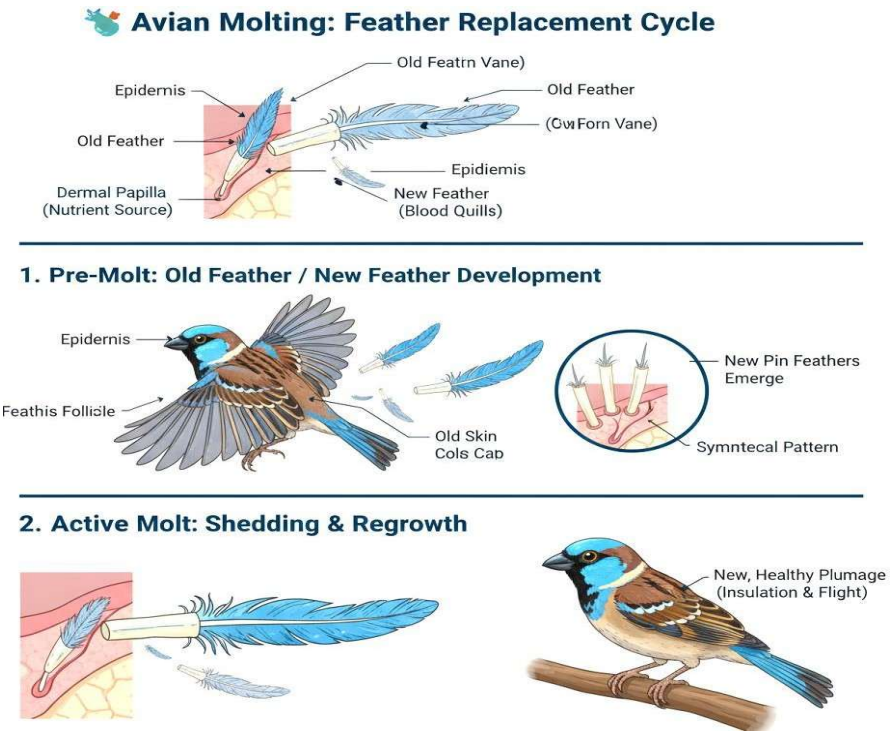

Figure 3: Birds showing feather replacement

Timing and Energy

Moulting is an **energetically costly** process. Growing new feathers requires a lot of protein and nutrients. Because of this, a bird's body prioritizes the moult by timing it to avoid other high-energy activities like:

- **Breeding and nesting:** Raising young is demanding, so most birds moult after the breeding season is over.
- **Migration:** Birds need to be in peak physical condition for long-distance flights, so they typically complete their moult before or after migration.

The Moulting Process

The process of shedding old feathers and growing new ones is controlled by **hormones**, primarily triggered by changes in day length and temperature.

- **Gradual Moult:** The majority of bird species use a gradual moulting strategy. They shed a few feathers at a time in a symmetrical pattern across their wings and tail to ensure they can still fly. This ensures the bird is never left vulnerable or flightless.
- **Simultaneous Moult:** Some birds, particularly large waterfowl like ducks, geese, and swans, shed all their flight feathers (the long feathers on their wings) at once. This makes them **temporarily flightless** for several weeks. During this period, they seek out safe, secluded areas with access to food and water to protect themselves from predators.

Types of Moults

Birds can have different types of moults throughout their lives:

- **Juvenile Moult:** The first moult a bird undergoes, where it replaces its soft downy feathers with its first adult plumage.
- **Annual Moult:** A complete moult that occurs once a year, replacing all feathers. This is common in many birds.
- **Pre-alternate Moult:** Some species undergo an extra, partial moult before the breeding season to acquire a more colourful, vibrant "nuptial" plumage for attracting a mate.

Mammals
Many mammals, such as dogs and cats, "moult" by shedding their fur. This is a continuous process for some species but can also be seasonal, driven by changes in temperature and daylight.

Unlike the ecdysis of arthropods, which is for growth, mammalian moulting is about maintaining a healthy and functional coat. The process is a hormonal response to environmental cues, mainly the amount of daylight. As days shorten in the fall, a hormone called **melatonin** increases, prompting the growth of a thicker winter coat. When days lengthen in the spring, melatonin levels decrease, signalling the shedding of the heavy coat.

- **Coat Structure**: A mammal's fur isn't a single layer. It's usually a combination of **guard hairs**, which are long, coarse, and protect against elements, and **underfur**, which is dense and soft, providing insulation. In winter, the underfur grows much thicker. Some animals, like the Arctic fox, even grow hollow guard hairs to trap more air for better insulation.
- **Energy Cost**: Moulting is an energy-intensive process, requiring a lot of protein and nutrients to grow new hair. Animals often schedule their moulting to avoid other high-energy activities like reproduction or migration.

1. Seasonal Moult: This is a crucial adaptation for animals in temperate and arctic climates. The change in coat colour and density provides both camouflage and thermal regulation.
- **Arctic Fox:** An excellent example of seasonal moulting. The fox's coat changes from a thin, Gray-brown summer coat to a dense, white winter coat, helping it blends into the snow. In spring, it sheds the white coat for a darker one to match the sawing landscape.
- **Deer:** Deer undergo a very noticeable seasonal moult. Their thick, greyish-brown winter coat is shed in the spring, often in patchy clumps, to reveal a shorter, reddish summer coat. This change helps them stay cool and provides better camouflage in the summer landscape.

2. Continuous Molt: Many domestic animals, like dogs and cats, shed year-round, but this process becomes more pronounced during certain seasons. For breeds with a dense undercoat (e.g., Huskies, German Shepherds), the spring "blowout" where they shed large amounts of fur is very noticeable. This is their body's way of getting rid of the heavy winter insulation.

3. Juvenile Moult: Many young mammals shed their baby coats to grow their first adult coats. This often involves a change in texture and colour. For instance, a young mouse's soft, juvenile coat is replaced by a course, adult coat.

18.4 METAMORPHOSIS:

In many animals, embryonic development ends with the formation of a stage that does not resemble a young adult-called larva. Larva is a free-living and normally sexually immature stage in life cycle. Most often larval stage has separate life style and separate name than the adult stage, and specific functions like growth and dispersal. The transition from the larval to adult stage entails an amazing sequence of events known as metamorphosis: we generally use to say that larva undergoes metamorphosis to become adult. Existence of larval forms is so widespread in animal world that it is expected to have some biological pay off. Adult sea urchin leads a sedentary life; the pluteus larva of sea urchin can travel on ocean currents, hence help in dispersal. Adults of many species of moths live for a brief period, they do not eat, even have no mouth parts; their post-embryonic larval stage or caterpillar feeds for several months and only growth period of the life cycle. In many land-dwelling amphibians, larval forms are transition from the embryo's strictly aquatic existence to adult's terrestrial existence.

If the living conditions experienced by larva are more hospitable than those experienced by adult, the time of life cycle spend as larva and as adult may vary adaptively. As for example, larval forms of some species of lamprey exist for several years. When larvae metamorphose into adults, they exist for a few weeks of breeding and die. Similarly, silk moths have a long period of larval existence in comparison to adult life. Metamorphosis is reactivation of developmental process, during which the entire organism is remodeled. The changes not only occur at morphological level, but also at physiological and behavioral level. In fact, metamorphosis prepares an organism for its new mode of life style during adult stage. Even though metamorphosis is widespread, we shall restrict our discussion in two groups of animals, one invertebrate the insects and one vertebrate – the amphibians.

18.4.1 Metamorphosis in Insects:

Post-embryonic stages of insects (and other crustaceans) are encased within rigid exoskeleton, called cuticle. Cuticle is a non-cellular layer containing protein, chitin, wax and calcium carbonate, and secreted by underlying epithelial layer. Because the cuticle has limited capacity for expansion, growth of body requires periodical shedding of it with the shedding of old cuticle, a new one is formed by underlying epithelial layer. The new cuticle is larger and softer, thus allow growth. The process of replacement of older cuticle by the new one is called molting or ecdysis, and the stage between successive molts is called an instar. Development of insects and other arthropods is punctuated by molting, number of which varies with species**.**

Life Cycle in Insects:

Insects have three major strategies of development. Springtails (order Collembola) and silver fish (order Thysanuran) have no larval stage, so they undergo direct development, and are called **ametabolous**. After hatching, these insects have a transitory stage called **pronymph.** After pronymph stage, they appear as a miniature adult (preadult) and grow larger through successive molts until reaching adult stage.

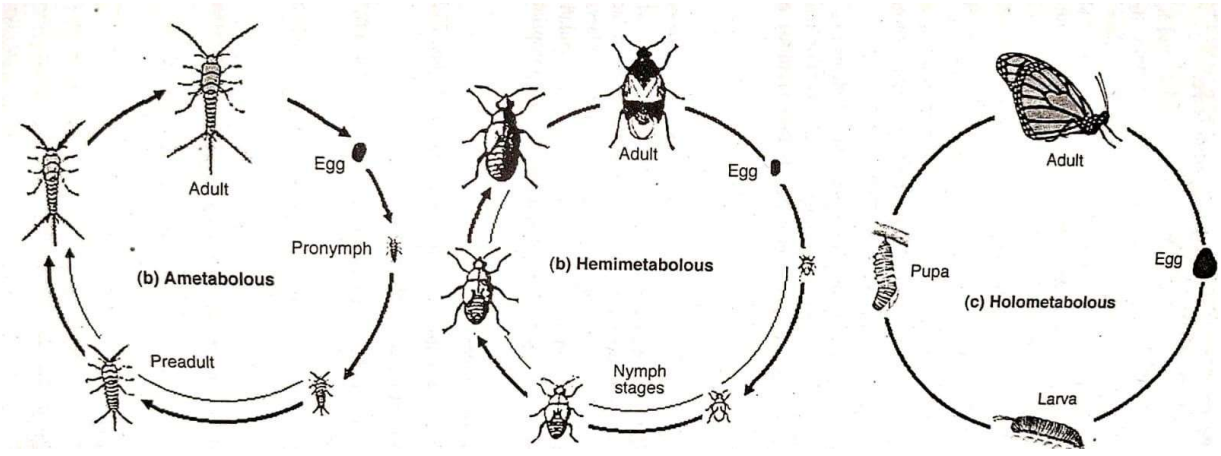

Figure 4: Types of life cycle in insects. (a) Ametabolous, (b) Hemimetabolous and (c) Holometabolous.

Grasshoppers (order Orthoptera), dragon files (order Odonata) and bugs (order Hemiptera) undergo indirect development through **hemimetabolous** (incomplete) metamorphosis. They hatch into a paranymph stage, which then molts into a **nymph –** a stage alike immature adult.

Through successive molting and nymphal instars, these insects grow in size; develop wing, genital organs and other adult structures. The sexually mature adult with wings is called **imago,** which emerges after final molt. In many other insects like files (order Diptera), moths and butterflies (order Lepidoptera), beetle (order Coleoptera), post-embryonic stages hatch from egg is called larva (a caterpillar or grub or maggot). Larva grows larger, though successive instars and undergoes series of molts. After final larval instar, the insect undergoes an encasement, the process is called **pupation** and the encased stage is called **pupa.** The molt of final larval instar to become pupa is called metamorphic molt.

During pupation, adult structures develop to replace larval structures and after definite time period (influenced by external environment), adult insect or imago emerges from pupal case. This type of development is called holometabolous or complete metamorphosis.

18.4.2 Metamorphosis in Amphibians:

The class of vertebrates designated as amphibians is due their dual life (amphi=double; bios = life). That means; to complete their life cycle, they undergo partly aquatic-partly-terrestrial existence. The morphological changes occurred during leaving aquatic existence and entering terrestrial existence is generally metamorphosis in amphibians. In anurans (frogs and toads), metamorphic changes are more dramatic; include almost all organs and regarded as complete metamorphosis. In urodeles (salamanders and newts), changes are less drastic than anurans and in some partial metamorphosis occurs. Metamorphic changes in amphibians, mainly in anurans are summarized in table 1.

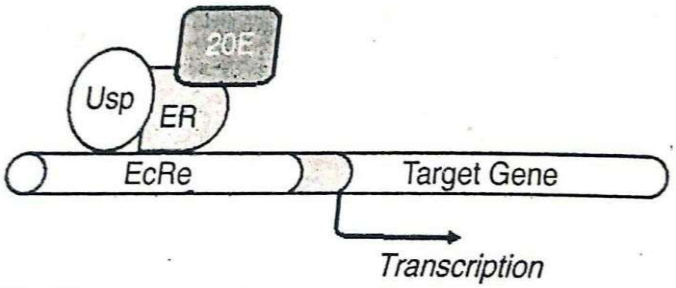

Figure 5: Usp and 20E receptor protein (ER) heterodimer with 20E regulates gene transcription.

Table 1: Important metamorphic changes in amphibians

Organ System	Larval	Adult
Locomotory	Tail with tail fin	Legs. Tail absent in anurans but retained in urodels. In some fins are also retained.
Respiratory	Gills, skin, lungs	Lungs and skin. In some urodels, external gills are retained.

Nutritional	Herbivore, long spiral gut, small mouth.	Carnivore, short gut, wide mouth with long tongue.
Excretory	Mostly ammoniotelic	Mostly ureotelic
Nervous and senses	Lateral line sense organ, no nictitating membrane and eyelids	Nictitating membrane and eyelids present, no lateral line sense organ.
Retinal pigment	Prophyropsin	Rhodopsin
Integument	Thin epidermis and dermis; no mucous or granular gland	Stratified epidermis with keratin; well developed dermis with mucous and granular glands.

Morphological and Biochemical changes during Amphibian metamorphosis:

Amphibian metamorphosis is initiated by hormones, mainly Thyroxine (T4) and Triiodothyronine (T3). These hormones are transported through blood circulation to the larval organs, which respond either of four ways.

- Development of new structures
- Cell death
- Remodeling of existing structures, and
- Biochemical specification

1. Development of New Structures:

- Limbs of the adult amphibians emerge on metamorphosing tadpole. Paired hind limbs appear first, followed by paired forelimbs. As they grow out from body axis, new neurons proliferate and differentiate in spinal cord, which send axons to the newly formed limbs.
- Both nictitating membrane and eyelids emerge in the eye. At the same time, position of eye becomes frontal from their original lateral position in larva. With this movement of eye position, adult frogs acquire a binocular field of vision. Paired lungs develop and circulatory system also changes with development of carotid and systemic arch.

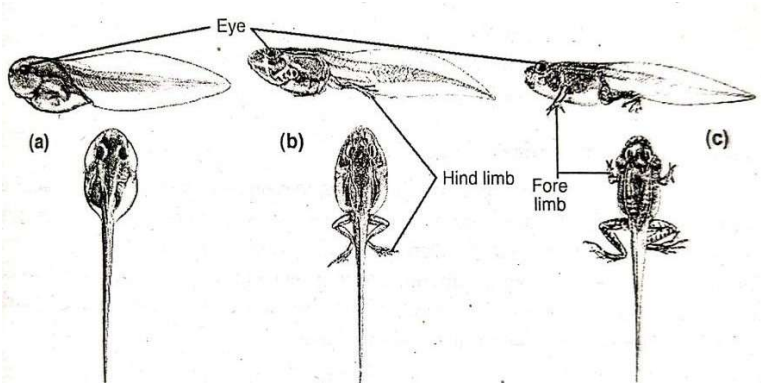

Figure 6: In Metamorphosis of amphibians, new structures develop in tadpole (a). Paired hind limbs appear first, followed by forelimbs (b). Position of eye becomes frontal from lateral in tadpoles developing a binocular vision in adult (c). Upper row – lateral view; lower row – dorsal view.

2. Cell Death:

- Degeneration of tail occurs. It is evident that first part of tail regresses by means of programmed cell death or apoptosis. In later metamorphosis, remnants of larval tail are destroyed by phagocytosis.
- Gills those are important for larval respiration degenerates.
- When adult red blood cells become functional, larval red blood cells are digested by macrophages in liver and spleen.
- Lateral line sense organ that helps in hearing and swimming also degenerates.

3. Remodeling of Structures:

Larval intestine is long and coiled, designed for digesting plant diets. Larval intestine is converted into shorter intestine of adult frog for carnivorous diet. The cells of adult intestine are derived from functional cells of larval intestine. Adult nervous system is remodeled as neurons grow and innervate new organs. However, during restructuring, some neurons die, some are born and some change their specificity.

Almost all structural component of head in adult is remodeled along with the change in the shape of skull and lower jaw. Some skeletal elements die, some proliferate and some are remodeled.

4. Biochemical Respecification:

In association with morphological changes, certain new proteins are induced in existing adult cells. Tadpoles are ammonotellic while adult frogs are mostly ureotelic an adaptation to terrestrial life. During metamorphosis, liver cells start to synthesize enzymes which are necessary to produce urea from ammonia and certain carbon-di-oxide. It is known that T3 induces a transcription factor that activates expression of urea-cycle genes and suppresses genes for ammonia synthesis.

18.5 RETROGRESSIVE METAMORPHOSIS:

In majority cases of metamorphosis, we witness that adult individuals develop more advanced features than their larval forms. It is not that larvae are less adapted, but adult structures are better than their larval counterparts. Naturally we denote this type of metamorphosis as progressive. But there are examples in animal kingdom, where key features of the group are present in larvae disappear in adults after metamorphosis. Such type of metamorphosis is called retrogressive.

Retrogressive metamorphosis is excellently evident in urochordates, particularly in ascidians. Ascidians or sea squirts are solitary or colonial marine chordates, adult are sessile, but larvae are free-swimming.

1. Larva:

Larvae are also called ascidian tadpoles, usually do not feed, swim actively for a few days and select a suitable substratum to settle permanently as adult. The free-swimming larva exhibit chordate characters: -

a. Small pharynx bearing slits,
b. Tubular nerve cord extending into tail, and
c. A flexible notochord.

At the beginning of pharynx, there is a distinct endostyle. Pharyngeal slits open into bilateral atrium that opens to exterior through atrial pore. In solitary species of ascidians, gut is not well-

developed in non-feeding larva and anus is absent. In majority colonial species gut is fully differentiated with mouth and anus. Their feeding begins shortly after settlement. The heart and pericardium arise from pharynx and lie behind endostyle

In most solitary and colonial species, notochord is tubular. Walls of notochord are made up of single layer of epithelial cells covered by a sheath of collagen fibers. The epithelial layer encloses a lumen that is fluid-filled. As a result, the notochord is tubular, closed at both ends and is turgid. Brain composed of a rudimentary cerebral ganglion and a large visceral ganglion that sends nerve to various parts of body. A hypophysis is present beneath cerebral ganglion. Dorsal nerve cord arise from visceral ganglion has a 263eurocoele surrounded by ependymal cells and nerve tracts. Dorsal nerve cord with notochord and tail muscles forms axial complex.

A sensory vesicle near the atrial pore includes a light-sensitive ocellus and a gravity-sensitive otolith. These sensitive structures help the larva as navigational equipment's, during swimming and searching a place for attachment. The active body of larva is covered by an acellular tunic secreted by underlying epidermis. The tunic is covered by an outer and inner cuticular layer. From outer cuticle tail fin of larva arise. At the anterior part of larva, adhesive papillae are formed from epidermis, which help the larva to attach to a suitable substratum.

2. Metamorphosis:

After a short free-swimming life, larva attaches with a suitable substrate in a shaded place. The attachment is made by the adhesive papillae and the larva immediately begins to metamorphose. Most of the chordate features in larva, namely notochord, nerve cord and tail disappear.

The epithelial cells in the notochord contract and separate from each other. The fluid from the lumen leaks and the notochord becomes limp. The axial complex is actively absorbed into body. Within body, the axial complex is broken down by phagocytosis and its components are used to rebuild the young adult. Outer tunic layer, sensory vesicles and visceral ganglion are lost. The pharynx persists and enlarges, slits in pharyngeal wall increase in number and each slit subdivides repeatedly to form small openings called stigmata. The pharynx becomes barrel-shaped and called branchial basket. After attachment, the metamorphosing tadpole starts feeding.

3. Adult:

In sedentary life, adult ascidians need only feeding system, circulatory system, nervous system and reproductive system. Whole animal is enclosed in a tunic composed of unique protein tunicin and a polysaccharide resembling cellulose. Tunic is almost transparent, below which a single-layer epidermis is present. Within tunic, branchial basket, atrial cavity and visceral system are enclosed, and the basal part of tunic helps the individual to attach with substratum.

Water circulates through the body of adult ascidians. Water enters into branchial basket through incurrent siphon. Small finger like sensory tentacles encircling incurrent siphon test the quality of entering water and prevent entrance of large particles. Water passes from branchial basket to atrium through numerous stigmata. From atrium, water exits through excurrent siphon.

Within branchial basket, endostyle produces mucous, which bind the food particles. Row of cilia, lining the internal chamber of branchial basket collect the sheet of mucous containing food and convey it to the gut.

Tubular heart contracts to push blood out to organs and tunic. After several minutes, blood

flows in reverse direction in same vessel to return the blood to heart. Blood contain amoebocytes, which are phagocytes.

Nervous system includes a cerebral ganglion, located between two siphons. From this ganglion, nerves arise to supply siphons, gills and other visceral organs. A sub neural gland below this ganglion persists from larval hypophysis, function of which in adult is not known.

Solitary ascidians reproduce sexually, but colonial ascidians reproduce both by sexually and asexually. All ascidians are hermaphrodites but self-fertilization, usually does not occur. Asexual reproduction in colonial ascidians involves budding.

18.6 HARMONAL REGULATION IN INSECT METAMORPHOSIS:

1. Hormonal Influence on Insect metamorphosis:

The general pattern of hormonal regulation on metamorphosis is very similar among insect species. Three hormones are recognized to play crucial role in molting and metamorphosis – prothoracicotropic hormone, ecdysone and juvenile hormone.

Prothoracicotropic hormone (PTTH) is, in fact, a neurohormone secreted by the neurosecretory cells so called brain. PTTH is a peptide hormone with 109 amino acids and molecular weight about 40,000; its release is triggered by neuronal, hormonal or environmental signals. PTTH stimulates the production and release of ecdysone form the prothoracic gland situated near the brain.

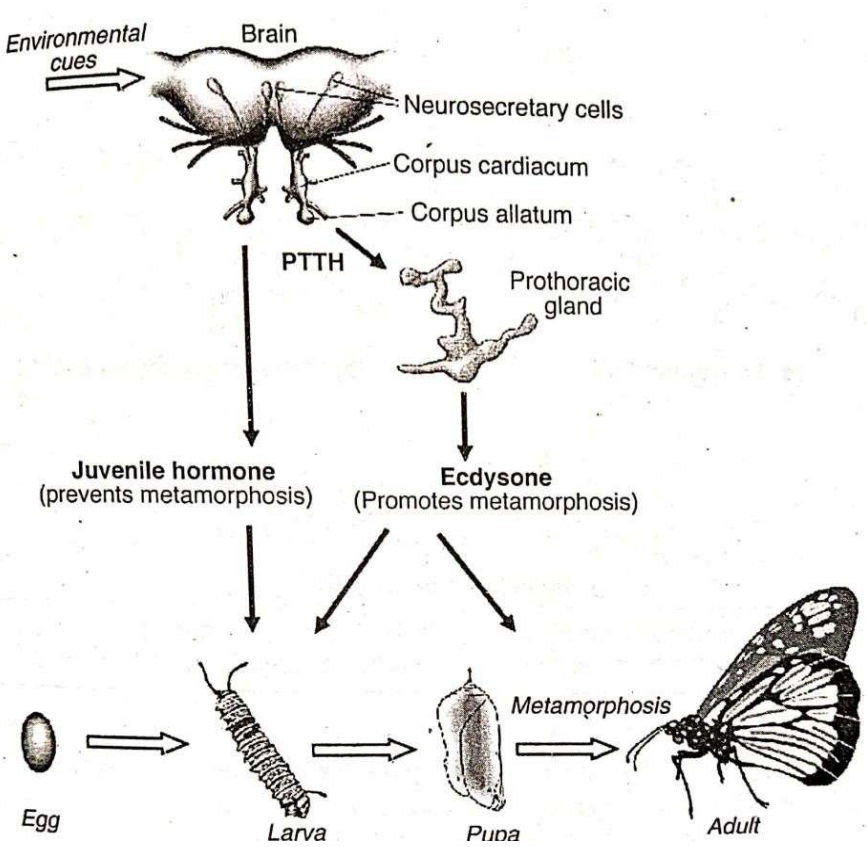

Figure 7: Neurosecretory cells of so-called brain of a holometabolous insect, which secretes a neurohormone PTTH to trigger metamorphosis.

Ecdysone (Ec) is a steroid ($C_{27}H_{44}O_6$) molecule and is a prohormone that is modified in

peripheral tissues and fat bodies in 20-hydroxyecdysone (20E). 20E ($C_{27}H_{44}O_7$) is the active form that initiates and coordinates each molt and regulates changes in gene expression occurring during metamorphosis.

Juvenile hormone (JH) is secreted by a pair of endocrine glands, the corpora allata, situated and attached to the posterior of the brain. JH is a hydrophobic molecule of acyclic sesquiterpenoid ($C_{16}H_{26}O_3$) class. JH does not cause molt, but play a vital role in its regulation. When JH level is relatively low, 20E stimulates pupal molt. In some insects, corpora allata and prothoracic glands are fused into one called ring gland, as observed in *Drosophila.*

Hormonal interactions during molt and metamorphosis:

PTTH secretion produces waves of ecdysone production, hence initiating pulses of 20E. During each larval molt, concentration of 20E in hemolymph rises to trigger a change in commitment of epidermal cells. A second pulse of 20E initiates differentiation processes during molting, stimulated by 20E, epidermal cells of body surface withdraw from cuticle and produce a molting fluid containing a proenzyme. This proenzyme, upon activation, digests the old cuticle. The epidermis then generates a new cuticle that is initially distensible and expands as the larva (now in next instar) grows in size. Then the new cuticle hardens and becomes nonelastic; the cycle is repeated.

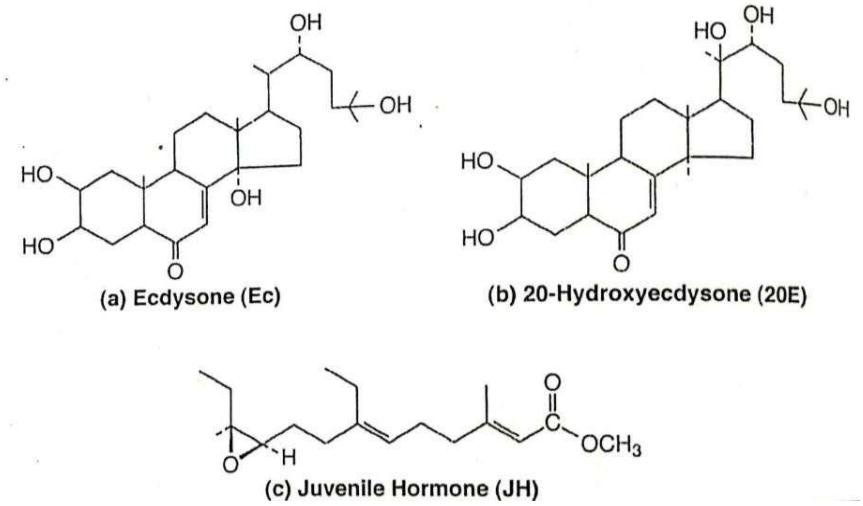

Figure 8: Hormones playing crucial role in molting and metamorphosis in insects. Ecdysone (Ec) (b) 20-Hydroxyecdysone (20-E) and (c) Juvenile Hormone (JH).

It is JH that determine whether the result of a molt will be an increase in larval size or pupation or metamorphosis. As long as JH is present in relatively high concentration, molts result in a new larval instar. In last larval instar, neuronal signal from brain to corpora all ate inhibits JH production. A drop in JH level causes PTTH secretion from brain, which in turn, stimulates secretion of ecdysone from prothoracic gland. This pulse of pulse of 20E in presence of low level of JH causes pupal development. In the final step, 20E acts in absence of JH, the imaginal disc differentiates and the molt results in adult.

During metamorphosis of *Drosophila,* two major pulses of 20E occur. At the end of third instar, first pulse of 20E initiates morphogenesis of leg and wing imaginal discs. At this time larval hind gut degenerates and larva stops eating. The second pulse of 20E occurs several hours after the first one; it activates pupa-specific characters and later molt in absence of JH. The result is

adult fly. Beside PTTH, 20E and JH, several other factors are involved in insect metamorphosis. A cascade of hormones controls the events of eclosing-muscular movements and rupture of the puparium that allow the emergence of adult fly. There are receptor molecules in the target tissues, which are3 synthesized at particular time to make the tissue responsive.

Like other steroid hormones, 20E diffuses across cell membranes and interacts with cytoplasmic receptor proteins. In order to the active, 20E –receptor complex requires (ER) another protein produced by a gene known as *ultraspiracle (usp)* in *Drosophila.* Usp protein dimerizes with 20E receptor protein (ER); the heterodimer with 20E becomes an active complex that regulates gene transcription.

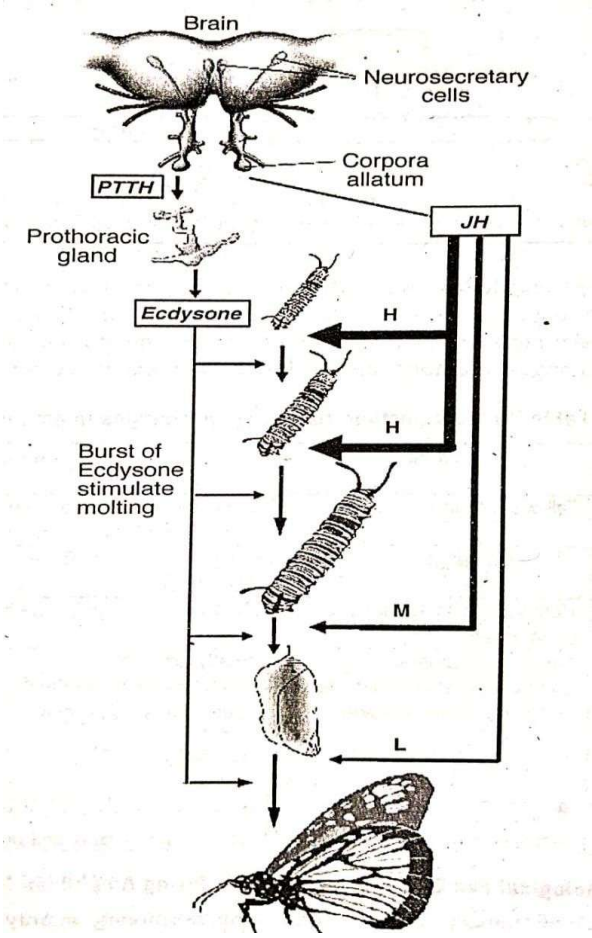

Figure 9: It is JH that determine whether molt will be an increase in larval size or pupation or metamorphosis. Burst of JH secretion – H = high, M = Moderate and L = low.

18.7 HARMONE REGULATION IN AMPHIBIAN METAMORPHOSIS:

Role of thyroid hormones in metamorphosis of amphibians was demonstrated in 1912. When tadpoles were fed extract of horse thyroid gland, they metamorphosed prematurely. Complementarily, surgical removal of developing thyroid gland of tadpoles was done in 1916. Such thyrodectomized tadpoles failed to metamorphose and grew into giant tadpoles. Later, it was found that hypophysectomy produced similar effect. Subsequent studies evidently showed that amphibian metamorphosis is regulated by thyroid hormones- tetraiodothyronine/thyroxine (T4) and triiodothyronine (T3). In absence of T4 and/or T3 metamorphosis will not occur, the tadpoles simply grow into large aquatic creatures.

It is now known that some environmental factors, especially temperature, and an endogenous developmental program in brain trigger the secretion of a hypothalamic hormone – the Thyrotropin-Releasing-Hormone (TRH). TRH stimulates.

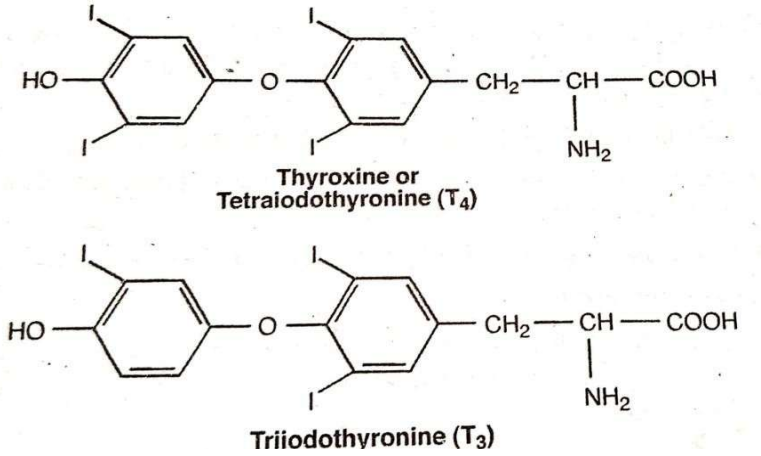

Figure 10: Thyroid hormones play a key role in amphibian metamorphosis. (a) Thyroxine or Tetraiodothyronine (T3), (b) Triiodothyronine (T4).

Anterior pituitary to synthesize and release Thyroid-Stimulating-Hormone (TSH) or thyrotropin. TSH controls the function of thyroid gland to synthesize and release T4 and T3. Interestingly, the effective hormone in tadpoles to drive TSH release pituitary is Corticosterone-Releasing-Hormone (CRH) that also triggers the release of Adrenocorticotropic Hormone (ACTH). ACTH stimulates adrenal cortex to secrete corticosteroids, which partly regulate the production of enzyme deiodinase (type II) to convert T4 to T3 in target tissues. Corticosteroids also modify the action of thyroid hormones in some target tissues. Recently known that thyroid hormones stimulate pituitary to secrete another hormone – Prolactin, which also play some role in regulating metamorphosis in amphibians.

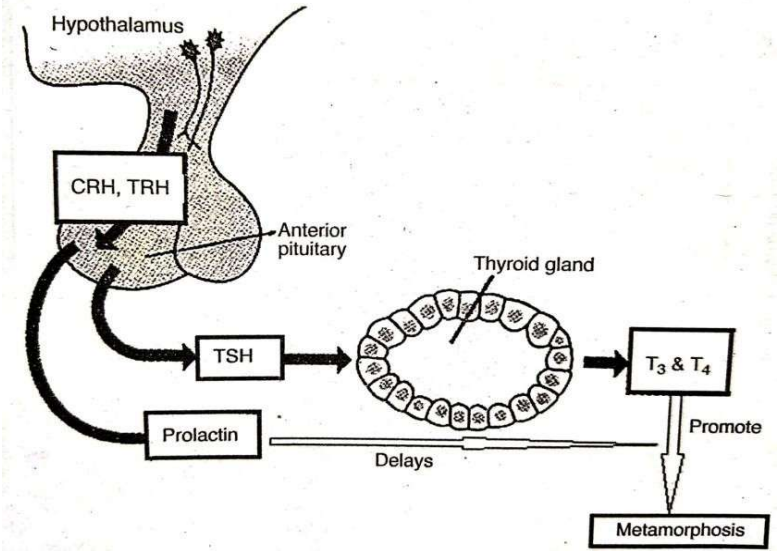

Figure 11: Hormonal interaction in regulating thyroid function during metamorphosis in frog

Role of Prolactin in amphibian metamorphosis:

It is now known that thyroid hormones stimulate pituitary gland to secrete prolactin, which has

some regulatory role in metamorphosis. When mammalian prolactin is injected into tadpoles, they show slower metamorphic changes. It is suggested that prolactin interferes with the T3 and T4 receptors. Recent findings reveal that prolactin level increases as metamorphic climax approaches. Experiments with transgenic tadpoles expressing high level of prolactin show that prolactin prevent tail resorption. Such tadpoles, however, metamorphose, but juvenile frogs appear with persistent tail. There are many questions about interaction between T3 and prolactin; there is no doubt that prolactin counteracts some of many effects of T3.

Metamorphic events are regulated by levels of thyroid hormones:

Sequence of metamorphic changes is controlled by concentration of thyroid hormones. In fact, thyroid hormone concentration increases during the progressive changes of metamorphosis. Some changes occur early, when thyroid hormone concentration is low; some changes occur later after the thyroid hormone concentration is higher. These kinds of observations support that each of the different local responses to T3/T4 has a threshold concentration. This threshold model is useful in understanding amphibian metamorphosis, but it cannot throw any light on the mechanism involved. Again, molecular studies reveal that timing of events in amphibian metamorphosis is even complex than just hormone concentration.

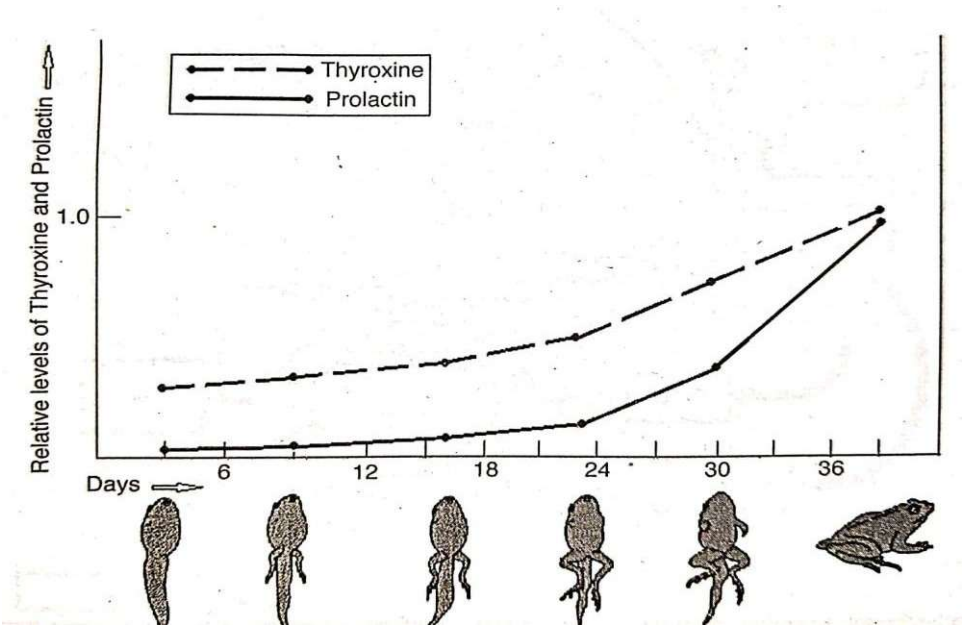

Figure 12: Relative levels of Thyroxine and Prolactin during metamorphosis in amphibians

Depending on thyroid hormone concentration, metamorphosis in amphibians can be divided into stages. In the initial stage premetamorphosis, thyroid glands of tadpoles start secreting low amount of T4 and very low amount of T3. This initial T4 secretion is brought about by CRH. In this stage, limb growth begins and continues. In pro metamorphosis stage, when mature thyroid glands produce more hormones, lungs begin to develop and changes in head region begin. In metamorphic climax stage, concentration of T3/T4 rises dramatically. In this stage, major changes like resorption of gill, remodelling of gut, tail resorption etc. occur. The high level of T3/T4 produce a negative feedback loop to lower TSH and probably CRH productions, so that thyroid hormone secretion is appropriate for juvenile frog/toad. In fact, metamorphic events are down-regulated once metamorphic climax is reached.

Response of thyroid hormones is tissue/organ specific:

T3 alone causes many changes in metamorphosis of amphibians. The same hormone causes some tissues to develop and differentiate while causing other tissues to degenerate. The type of response (differentiation or degeneration) is determined by other factors present in different tissues. Degeneration of tadpole's tail occurs relatively rapidly, because bony structures do not extend to tail. Tail regression takes place by apoptosis followed by enzymatic digestion by the macrophages. Tail epidermis lacks epithelial stem cells and fails to generate new skin like head or trunk epidermis.

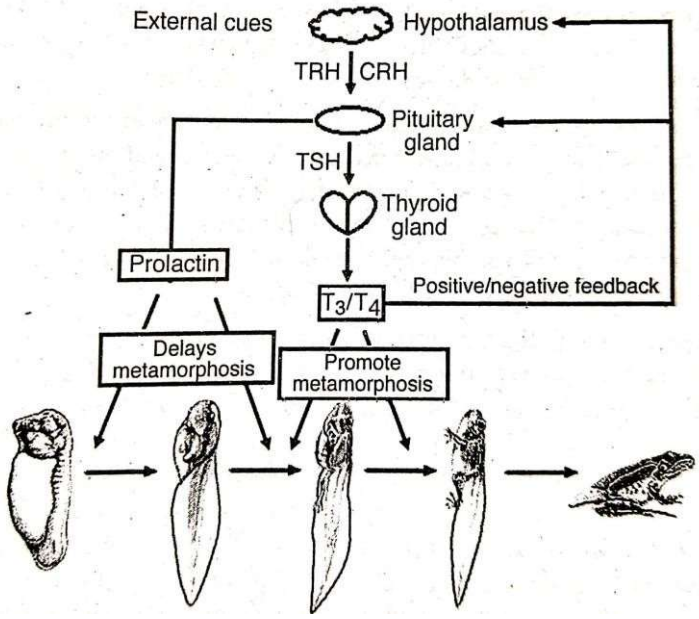

Figure 13: **Level of T3/T4 produces a positive/negative feedback loop to higher/lower secretion of TSH/CRH, which in turn results thyroid hormone secretions appropriate to juvenile frog/toad**

Metamorphosis not only involves dramatic morphological changes, some fundamental metabolic pathways also altered. Rod segments of tadpole's retina contain porphyropsin, which is vitamin-A_2 based visual pigment. Vertebrates inhabiting freshwater have vit-A2 based visual pigment in their eye. During metamorphosis, the metabolic pathways to produce vit-A2 shift to produce vit-A_1, a character of terrestrial vertebrates. As a result, the visual pigment of adult frog's eye is rhodopsin instead of porphyropsin

Tadpoles like other aquatic animals, are ammonotelic, i.e., their excretory end product is ammonia. Ammonia, though toxic, is easily dissolved, diluted and excreted by the aquatic animals. During metamorphosis, enzymatic machinery of tadpole liver changes to produce urea as major excretory end product. Synthesis of urea from ammonia requires four urea-cycle enzymes. Changes in visual pigments and urea cycle are triggered by an increase in T3 concentration.

Some experiments are dramatical to demonstrate the organ-specific responses to thyroid hormones. A tail tip is transplanted to trunk region and an eye cup is placed in the tail. The tail in the trunk undergoes degeneration but the eye cup retains its progressive development though placed in degenerating tail. Thus, programmed-cell-death is organ-specific and specific organ/tissue will die when it receives the signal. Such programmed-cell-death is crucial in morphogenesis.

The response of each tissue to thyroid hormone is specific to that particular tissue. Isolated tadpole tail survives in simple culture medium for long time. Addition of very low levels of T3 in the medium induces macroscopic changes, characteristic tail resorption during metamorphosis. The process includes failure of epithelial stem cells to replace epidermis, increased accumulation of lysosomal enzymes and-apoptosis of muscle cells. When prolactin is added to the medium with Ty, these changes do not occur; thus, both thyroid hormones and prolactin act locally and specifically. Though it is said that every tissue or organ is affected by thyroid, at least one tissue seems to be unresponsive. Ventral retina is activated by thyroid hormones, in which neurons proliferate to form ipsilateral axons. Dorsal retina is not responsive to thyroid hormone and does not generate new neurons. Dorsal retina expresses Hormone receptors are also essential in metamorphosis

18.8 HARMONE RECEPTORS ARE ALSO ESSENTIAL IN METAMORPHOSIS:

In metamorphosis of frog, thyroxine (T4) is released into blood by thyroid glands, which is then converted by target tissues into T3 by an enzyme deiodinase. T3 is a more active hormone that binds with Thyroid-Hormone-Receptors (TR) with much higher affinity than T4 resulting gene activation. Thus, threshold model is restated that levels of both thyroid hormones and their receptors (TRs) are essential for producing response during metamorphosis.

There are at least two types of TRs Tra and TRß. Tra is present widely in all tissues even the animal does not develop a thyroid gland. TRß is directly activated by thyroid hormones. Before metamorphosis, TRß level is very low; during metamorphosis, intracellular level of TRß increases as the levels of T3 and T_1 increase.

Each TR joins with a molecule called retinoic acid-receptor (RXR). This heterodimer (TR-RXR) binds with thyroid hormone and influence transcription. The TR-RXR complex is a transcriptional repressor but when T3 is added to this complex, T3-TR-RXR activates some genes and their transcription. **Thus,** it can be concluded that local tissue-specific responses and the regulation of hormone sensitivity are controlled by TR levels. Again, prolactin seems to decrease TR expression, which support that prolactin counteracts, at least, some actions of thyroid hormones.

18.9 SUMMARY:

Molting and metamorphosis are fundamental biological processes essential for growth, survival, and reproduction across animals. Molting involves shedding of old skin, feathers, fur, or exoskeleton, as seen in arthropods, reptiles, birds, and mammals. Metamorphosis, on the other hand, is a dramatic transformation from larva to adult, found in insects, amphibians, and some chordates. Retrogressive metamorphosis reduces larval complexity to simpler adult forms, as in tunicates. Both processes are tightly regulated by hormones such as ecdysone, juvenile hormone, and thyroid hormones, which ensure precise timing and coordination. Together, these processes highlight evolutionary adaptations that enable animals to thrive in diverse habitats.

18.10 TECHNICAL TERMS:

Moulting, Ecdysis, Exoskeleton, Metamorphosis, Juvenile Hormone, Ecdysone, Thyroxine, Morphallaxis, Epimorphosis, Retrogressive Metamorphosis

18.11 SELF-ASSESSMENT:

1. Define moulting and explain its significance in arthropods.
2. Differentiate between hemimetabolous and holometabolous metamorphosis in insects.
3. Describe the role of thyroid hormones in amphibian metamorphosis.
4. What is retrogressive metamorphosis? Give one example.
5. Explain how juvenile hormone regulates metamorphosis in insects

18.12 SUGGESTED READINGS:

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- **Prof. K. VEERIAH**

LESSON- 19
CHROMOTOPHORES AND RECEPTORS

OBJECTIVES:

- To understand the structure, origin, types, and classification of chromatophores in different animal groups.
- To study the mechanisms of physiological and morphological colour change and their adaptive functions such as camouflage, communication, thermoregulation, and UV protection.
- To analyse the biological, ecological, and biomedical significance of chromatophores, including their role in research, medicine, and applied sciences.
- To study the types, structures, and functions of receptors with emphasis on phono receptors and tango receptors.
- To understand the evolutionary development of sensory systems in invertebrates and vertebrates. C
- To analyse the adaptive significance of receptors in survival, communication, and environmental interaction.

STRUCTURE:

19.1 INTRODUCTION:

Chromatophores are specialized pigment-containing and light-reflecting cells that impart color to the skin, scales, and eyes of many animals, particularly ectothermic vertebrates like fish, amphibians, and reptiles, as well as invertebrates such as crustaceans and cephalopods. These cells originate from the neural crest during embryonic development and are responsible for a wide range of color patterns. Chromatophores play essential roles in **camouflage, communication, thermoregulation, and protection from UV radiation**.

In mammals and birds, melanocytes serve as the functional equivalent of chromatophores. The diversity of chromatophore subtypes melanophores, xanthophores, erythrophores, iridophores, leucophores, and cyanophores illustrates the complex mechanisms animals use to adapt to their environments.

19.2 CHROMOTOPHORES:

Chromatophores are referred to as pigment-containing cells or groups of cells that produce colour. They are present in various creatures, including fish, amphibians, reptiles, crabs, and cephalopods. Melanocytes are a kind of cells that are responsible for the colouration of mammals and birds. Other membrane expansions into the cytoplasm of some prokaryotes, such as cyanobacteria, are called chromatophores and contain pigments.

In ectothermic animals, chromophores are produced in the neural crest during embryonic development and play a significant role in imparting skin and eye colour. Erythrophores (red), xanthophores (yellow), iridophores (iridescent/reflective), leucophores (white), melanophores (black/brown), and cyanophores (blue) are the mature chromatophores that are divided into subclasses based on their colour (more accurately “hue”) under white light.

Several species can quickly alter their colour through systems that move pigment and reposition reflecting plates within chromatophores. Metachrosis, also known as physiological colour change, is the term used to describe this phenomenon, which is frequently used as a form of camouflage.

Classification:

Following Sangiovanni's chromoforo, the word chromatophore has been coined to describe pigment bearing cells originating from the neural crest of coldblooded cephalopods and vertebrates. The name derives from the Greek terms chroma, which indicates "color," and phoros, which means "bearing". The term chromatocyte (from the Greek kytos, referring to "cell") has been coined to describe the cells responsible for color in mammals and birds. In such animals, just a single cell type, the melanocyte, has been discovered. It wasn't until the 1960s that chromatophore cells were thoroughly understood enough to have been classed just on the basis of their looks. Even if the biochemistry of the pigments could be more relevant to scientific knowledge of how cells function, this classification system survives to this day**.**

There are the different categories of chromatophores:

- **Melanophores:** Contain dark brown or black **melanin** pigment. When the pigment disperses, the skin appears dark; when it aggregates at the centre, the skin becomes lighter, revealing other underlying colours.
- **Xanthophores:** Contain yellow **pteridine** pigments.
- **Erythrophores:** Contain red/orange **carotenoid** pigments.
- **Iridophores:** These are not pigment cells. They contain reflective platelets made of crystalline guanine. They produce **iridescent** colours by reflecting light at different wavelengths through constructive interference.
- **Leucophores:** Similar to iridophores, but with more disorganized crystals that scatter light randomly, producing a **white** or silvery appearance.
- **Cyanophores:** These contain blue pigments, although they are less common than other types.

Erythrophores and Xanthophores:

Chromatophores are classified as either erythrophores or xanthophores depending on whether they are predominantly composed of red/orange carotenoids or significant quantities of yellow

pteridine pigments. The overall colour, however, relies on the ratio of red and yellow pigments when vesicles carrying carotenoids and pteridine are present in the same cell. Consequently, the difference between these chromatophores is not always evident.

Xanthophores are chromophores that hold a number of yellow pteridine pigments, while erythrophores comprise mostly red/orange carotenoids. However, vesicles containing pteridine and carotenoids can occasionally be detected in the very same cell, so in that situation, the overall color is determined by the red-to-yellow pigment ratio. As a result, it's not always easy to tell the difference between these chromatophores. Pteridines could be made among most chromatophores from guanosine triphosphate, but xanthophores seem to have additional metabolic pathways that allow them to acquire yellow pigment. Carotenoids, on the other hand, are processed and transferred to erythrophores. This was initially established by feeding carotene-deficient crickets to typically green frogs. The red/orange carotenoid color 'filter' was not found in the erythrophores because carotene was not included in the frogs' diet. The frogs seemed blue rather than green as a result of this.

Leucophores and Iridophores:

Iridophores are chromatophores that reflect light utilising crystalline plates of guanine-based chemochromes also known as guanophores or guanochromes. They produce iridescent hues when lighted as a result of constructive light interference. Iridophores produce bright blue or green colours by utilising biochromes as coloured filters, which causes an optical phenomenon called Tyndall or Rayleigh scattering.

Leucophores, a related class of chromatophores, are primarily found in the tapetum lucidum of several fish. They use crystalline purines (usually guanine) to reflect light, like iridophores. Leucophores have more structured crystals than iridophores, which reduces diffraction. They emit a white shine when exposed to white light.

Melanophores:

Because of its ability to absorb light, eumelanin, a kind of melanin found in melanophores, gives them their dark brown or black appearance. It is dispersed throughout the cell in vesicles called melanosomes. which are small vesicles that are scattered all throughout the cell. In a sequence of catalyzed chemical processes, eumelanin is made from tyrosine. It's a complicated compound having pyrrole rings and dihydroxy indole and dihydroxyindole-2- carboxylic acid units. Tyrosinase is a crucial enzyme in the production of melanin. Whenever this protein is faulty, no melanin is produced, leading to albinism in some forms. Various pigments are packed alongside eumelanin in certain amphibian species. In the melanophores of phyllo medusa frogs, for instance, a novel deep (wine) red pigment has been discovered.

To produce skin, hair, and eye colour, humans have just one type of pigment cell, melanocytes the mammalian equivalent of melanophores. Melanophores are by far the most extensively researched chromatophore, partly because the cells are typically relatively simple to visualise due to their size and contrasting colour. However, the biology of melanophores and melanocytes differ from one another.

Chromatophores in Humans: To produce hair, skin, and eye color, humans only have a single type of pigment cell, the mammalian analogue of melanophores. Melanophores are the most commonly researched chromatophore for such a reason, as well as the fact that their large

quantity and contrasting color make them extremely simple to see.

Cyanophores

Some bacteria and algae contain light-emitting organelles called cyanophores. They are in charge of providing these organisms with their characteristic blue-green hue. Phycobilins and phycoerythrin are the two types of proteins that make up cyanophores. The blue hue comes from phycobilins, whereas the red tint comes from phycoerythrin. Instead of pigments, structural coloration includes approximately all of the bright blues seen in animals and plants.

In cells called cyanophora, certain varieties of Synchiropus splendidus do have vesicles of a cyan biochrome of unknown chemical structure. Despite their restricted taxonomic range, cyanophores (along with other unique chromatophore types) may be found in other amphibians and fish. Brightly colored chromatophores with unknown pigments have been identified in both poison dart and glass frogs, while erythro-iridophores, atypical dichromatic chromatophores, were identified in Pseudochromis Diadema.

19.3 FUNCTIONS AND APPLICATIONS:

Animals have colour-changing pigment cells or chromophores in their skin. They serve as a means of communication, temperature regulation, and camouflage. Melanin, a pigment found in chromophores, absorbs light. The melanin is concentrated, and the pigment cells appear dark when constricted. The melanin is scattered when the cells grow, giving the cells a light appearance.

Chromatophores are often used in practical research. Zebrafish larvae, for example, are used to examine how chromatophores coordinate and communicate to produce the continuous horizontal striped pattern seen in adult fish. This is regarded as a helpful model system in the field of evolutionary developmental biology for understanding patterning.

Additionally, human conditions or diseases like albinism and melanoma have been modelled using chromatophore biology.

Chromatophores are also studied as a biomarker of blindness in cold-blooded species because animals with specific visual abnormalities cannot effectively adapt to light settings.

Mechanisms of Colour Change:

- **Physiological Colour Change:** This is a **rapid** process (seconds to minutes) that involves the movement of pigment granules within the existing chromatophore cells. This is primarily controlled by the nervous system and hormones. For example, in a chameleon, nerve impulses cause pigment to either disperse or aggregate.
- **Morphological Colour Change:** This is a **slow** process (hours to days) that involves a change in the number or size of the chromatophores themselves or the amount of pigment they produce. This is typically a long-term adaptation to a persistent change in the environment.

19.4 SIGNIFANCE OF COLOUR CHANGE:

The ability to change colour is critical for survival and has a variety of functions:

1. Camouflage (Crypsis): This is the most common use. Animals blend in with their background to avoid being seen by predators or to sneak up on prey. A flounder can match the colour and texture of the ocean floor, and a cuttlefish can instantly mimic a checkerboard pattern.

2. Communication: Colour changes can serve as a form of social signalling. Animals may use colour to:

- **Attract mates:** Males often display bright, vibrant colours during courtship.
- **Intimidate rivals:** Some animals flash aggressive colours to deter competitors.
- **Warn predators (Aposematism):** Bright, conspicuous colours can signal that an animal is venomous or unpalatable, warning off potential threats.

3. Thermoregulation: Some cold-blooded animals use colour change to regulate their body temperature. A lizard can darken its skin to absorb more sunlight and heat up when cold, and then lighten its skin to reflect heat and cool down when it's hot.

4. Protection from UV Radiation: Darkening the skin with melanin can protect the animal from harmful ultraviolet rays, similar to how humans get a tan.

Control of Colour Change:

The regulation of colour change is a complex interplay between the nervous and endocrine systems.

1. **Nervous System Control:** In fast-changing animals like cephalopods (octopuses and squid), the nervous system directly controls the muscles around each chromatophore, allowing for nearly instantaneous colour and pattern changes.
2. **Hormonal Control:** In vertebrates like fish, amphibians, and reptiles, hormones play a significant role.

- **Melanocyte-stimulating hormone (α-MSH):** Promotes the dispersion of melanin, leading to skin darkening.
- **Melatonin and Melanin-concentrating hormone (MCH):** Induce the aggregation of melanin, causing the skin to lighten.

These hormonal and nervous signals are often triggered by external stimuli detected by the eyes, such as changes in light, temperature, or the presence of a predator.

RECEPTORS:

Receptors are specialized sensory structures present in animals that detect and receive stimuli from the external or internal environment. They act as biological transducers, converting physical, chemical, or mechanical energy into electrical impulses that are transmitted through sensory neurons to the central nervous system for interpretation. Receptors are highly essential for survival, enabling organisms to respond to changes, maintain balance, regulate behaviour, and interact with their surroundings. Based on the nature of the stimulus they detect, receptors are classified into mechanoreceptors, phono receptors, tango receptors, chemoreceptors, photoreceptors, thermoreceptors, and electroreceptors.

19.5 RECEPTORS IN INVERTEBRATES:

1. Mechanoreceptors:

Mechanoreceptors respond to touch, vibration, pressure, and movement. In cnidarians, **tactile receptors** called **cnidocils** trigger nematocyst discharge for defence. Earthworms have sensory

cells on the body surface, while molluscs use tentacles. In arthropods, tactile hairs and bristles function as mechanoreceptors. **Proprioceptors** are stress and posture receptors present in insects, especially in wings and legs, for flight coordination. The **campaniform sensilla**, for instance, are dome-shaped structures that detect stress and strain on the exoskeleton. **Equilibrium receptors** such as **statocysts** in molluscs and arthropods contain statoliths that stimulate sensory hairs to detect balance and orientation. The statoliths, often calcium carbonate granules, shift with gravity, bending the sensory hairs to signal the animal's position.

2. Phono receptors:

Phono receptors detect vibrations or sound. In invertebrates, true hearing is absent, but they perceive substrate or air vibrations. Insects like moths and crickets have **tympanal organs** sensitive to sound waves. These are thin membranes stretched over an air sac, with associated sensory neurons that vibrate in response to sound. In moths, these organs can detect the echolocation signals of bats, triggering evasive action. Spiders possess **lyriform organs** and sensilla hairs for vibration reception. The lyriform organs are tiny slits on the exoskeleton that detect minute vibrations, helping spiders find prey caught in their webs. These help in predator detection, communication, and mating.

3. Tango receptors:

Tango receptors are touch-sensitive receptors that detect mechanical contact or pressure. In annelids and molluscs, tango receptors occur as tactile cells on the skin and tentacles. In arthropods, sensory bristles and cuticular hairs serve as tango receptors. These are connected to sensory neurons at their base, which fire in response to bending or pressure. They help in locomotion, prey capture, and orientation in the environment.

4. Chemoreceptors:

Chemoreceptors perceive taste and smell. In insects, they occur on antennae, palpi, mouthparts, and tarsi for detecting food, mates, or danger. The antennae of male moths, for example, have highly sensitive chemoreceptors that can detect a single molecule of a female's pheromone. In molluscs, the **osphradium** tests water quality and food particles. These receptors play roles in feeding, reproduction, and social communication.

5. Photoreceptors:

Light-sensitive receptors vary from simple to complex. Protozoans like *Euglena* possess a **stigma** (eyespots) for light detection. This allows them to orient themselves towards light for photosynthesis. *Planaria* has pigmented **ocelli**, which can detect light intensity and direction but do not form images. *Nereis* has pigmented eyes, while cephalopods have camera-type eyes that are functionally analogous to, but structurally different from, vertebrate eyes. Arthropods possess **compound eyes** made of **ommatidia**, each with its own lens and photoreceptor cells. This structure provides a wide field of vision and excellent motion detection.

The **mosaic vision** produced by these eyes is a compilation of images from each ommatidium, creating a detailed view of the surrounding environment.

6. Thermoreceptors:

Invertebrates lack specialized thermoreceptors but possess temperature-sensitive cells scattered across the body. Insects such as mosquitoes detect body warmth of hosts through antennae. This is a crucial adaptation for blood-feeding insects to locate prey.

19.6 RECEPTORS IN VERTEBRATES:

1. Mechanoreceptors:

Mechanoreceptors in vertebrates are highly developed. **Tango receptors**: Free nerve endings, **Meissner's corpuscles**, **Merkel's discs**, and **Pacinian corpuscles** in skin detect touch, pressure, and vibration. The Pacinian corpuscle is a classic example: a sensory nerve ending encased in layers of connective tissue. It responds to deep pressure and vibration by deforming the layers, which in turn deforms the nerve ending and triggers an action potential.

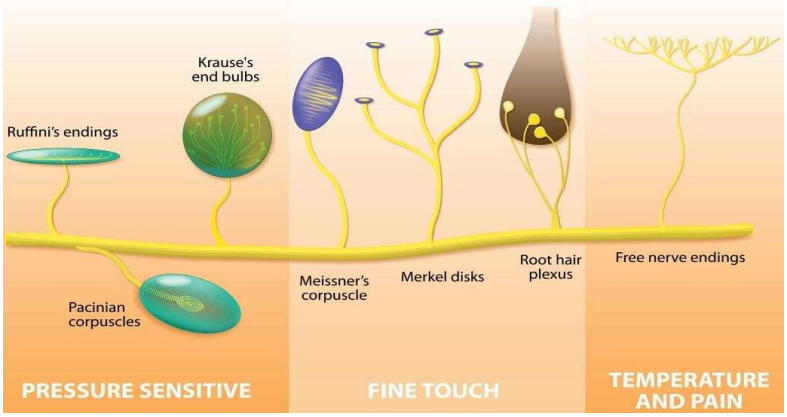

Figure 1: cutaneous receptors

Proprioreceptors: Present in muscles, tendons, and joints, these detect stretching and muscle movement. They are essential for **kinesthesia**, or the sense of body position and movement. **Equilibrium receptors**: Located in the inner ear. The semicircular canals, utricle, and saccule detect balance through the movement of fluid (endolymph) and small stones (**otoliths**), which stimulates hair cells.

2. phono receptors (Auditory receptors):

Phono receptors in vertebrates are represented by the ear. In fishes, the **lagena** detects sound, and the lateral line system detects pressure changes and low-frequency vibrations in water. In amphibians and reptiles, the auditory system develops further with the addition of the middle ear, containing a single bone, the **stapes** (or columella). In mammals, the ear is divided into three parts:

- **External ear**: collects sound.
- **Middle ear**: contains a chain of three ossicles (malleus, incus, stapes) that amplify and transmit vibrations.
- **Inner ear**: contains the snail-shaped **cochlea**, where the **organ of Corti** resides. Hair cells in the organ of Corti are the actual auditory receptors, converting vibrations into nerve impulses.

This enables perception of a wide range of frequencies, aiding in communication and survival.

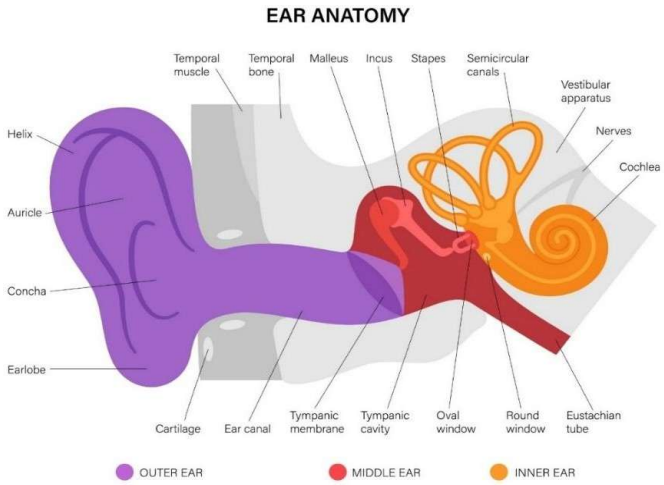

Figure 2: Ear Anatomy

3. Tango receptors:

Tango receptors in vertebrates are well-developed cutaneous mechanoreceptors. Examples include free nerve endings for pain, Meissner's corpuscles for light touch, and Pacinian corpuscles for deep pressure. They are concentrated in sensitive areas like fingertips, tongue, and lips, allowing fine discrimination and object recognition.

4. Chemoreceptors:

- **Olfactory receptors:** in the nasal cavity that detect volatile odors. These are G-protein coupled receptors that can bind to a wide variety of odorant molecules.
- **Gustatory receptors:** on the tongue that detect sweet, sour, salty, and bitter tastes. These are clustered in **taste buds** on the tongue's surface.
- **Jacobson's organ:** (vomeronasal organ) in reptiles and some mammals for pheromone detection.

5. Photoreceptors:

Photoreceptors are highly advanced in vertebrates. The mammalian eye is a camera-type structure with three layers:

- **Sclera & cornea:** (outer layer).
- **Choroid:** with iris & ciliary body (middle layer).
- **Retina:** (inner layer) containing **rods** (for dim light and motion detection) and **cones** (for bright light and colour vision). Rods contain the pigment **rhodopsin**, while cones contain **photopsins**.

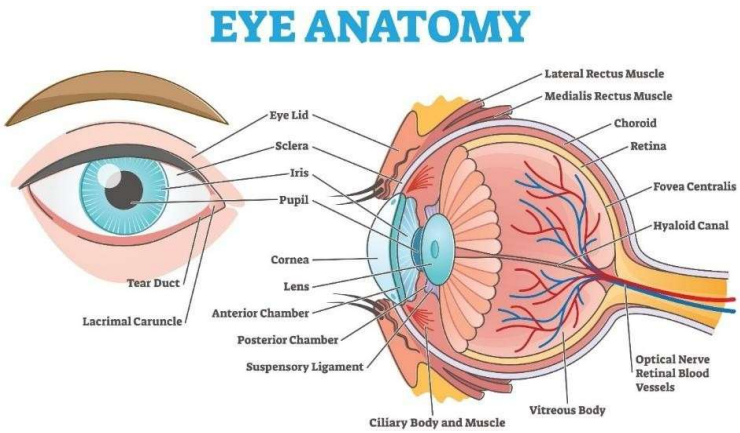

Figure 3: Eye Anatomy

Impulses generated are transmitted via the optic nerve to the brain.

6. Thermoreceptors:
General thermosensitive cells are scattered across the vertebrate skin. Fishes detect minor temperature changes for migration. Snakes possess specialized **pit organs** to detect infrared radiation, allowing them to locate warm-blooded prey even in darkness. These organs are incredibly sensitive, able to detect temperature differences as small as 0.003°C.

7. Electroreceptors:
Present in aquatic vertebrates like electric rays, sharks, and eels. They sense electric fields in water and may generate electric discharges for navigation, communication, or stunning prey. Sharks, for example, have **ampullae of Lorenzini**, which are electroreceptors that can detect fishes use electroreception for orientation in murky habitats.

19.7 SUMMARY:

Chromatophores are pigment cells that determine the coloration and patterns of many animals. They are classified into different types based on the pigment or reflective material they contain, such as melanophores (black/brown), xanthophores (yellow), erythrophores (red/orange), iridophores (iridescent), leucophores (white), and cyanophores (blue). These cells not only provide external color but also enable animals to undergo rapid or long-term color changes through physiological or morphological mechanisms. The ability to change color serves multiple adaptive purposes including camouflage, courtship signaling, thermoregulation, and UV protection. Beyond biology, chromatophores are studied in biomedical research, evolutionary developmental biology, and applied sciences such as biomimetic camouflage technologies.

Receptors are specialized sensory structures that detect and convert environmental stimuli into electrical signals for the nervous system. Invertebrates possess simple receptors like tactile hairs, ocelli, tympanal organs, and osphradia, whereas vertebrates have highly advanced systems such as camera-type eyes, complex ears, and specialized cutaneous receptors. phono receptors detect sound and vibration, tango receptors detect touch and pressure, while other receptors perceive light, chemicals, temperature, and electricity. These structures are vital for survival, communication, feeding, defence, and adaptation in diverse environments

19.8 TECHNICAL TERMS:

Chromatophore, Melanophore, Xanthophore, Erythrophore, Iridophore, Leucophore, Cyanophore, Metachrosis, Melanocyte, Tyrosinase, Phono receptor, Tango receptor, Tympanal organ, Cochlea Pacinian, corpuscle Ommatidia, Statocyst, Osphradium, Pit organ

19.9 SELF-ASSESSMENT:

1. Define chromatophores and describe their origin.
2. Differentiate between melanophores, iridophores, and leucophores with examples.
3. Explain the difference between physiological and morphological colour change.
4. How do chromatophores contribute to thermoregulation and UV protection?
5. Discuss the significance of chromatophores in research and medicine.
6. What are phono receptors? Describe their structure and function in insects and mammals.
7. Explain the role of tango receptors in invertebrates and vertebrates.

8. Differentiate between mechanoreceptors, chemoreceptors, and photoreceptors with examples.
9. Describe the structure of the mammalian ear and its role as a phono receptor.
10. What is the significance of electroreceptors in aquatic animals?

19.10 SUGGESTED READINGS:

1. Alberts, B. *Molecular Biology of the Cell* (latest edition).
2. Campbell, N.A. *Biology*. Pearson Education.
3. Wolpert, L. *Principles of Development*. Oxford University Press.
4. Bagnara, J.T. & Hadley, M.E. (1973). *Chromatophores and Colour Change: The Comparative Physiology of Animal Pigmentation*.
5. Articles from *Journal of Experimental Biology* on chromatophore function in fish and cephalopods.

- **Prof. K. VEERAIAH**

LESSON- 20

BIOLUMINESCENCE

OBJECTIVES:

1. To understand the evolutionary origin, biochemical mechanism, and ecological significance of bioluminescence across different organisms.
2. To analyse the distribution and adaptive roles of bioluminescent systems in terrestrial and marine ecosystems.
3. To explore the applications of bioluminescence in science, medicine, and biotechnology, along with future prospects.

STRUCTURE:

20.1. INTRODUCTION:

'Bioluminescence' refers to the phenomenon of chemically induced emission of light (or other electromagnetic radiations) by a living organism. It is a common occurrence frequently observed in various organisms, ranging from simple ones like bacteria to complex animals like deep-sea fish or fireflies, and even some fungi. The first accounts of bioluminescence are found in the works of Dioscorides and even Pliny the Elder, who believed that certain bioluminescent organisms had medicinal properties. There are accounts of coal-miners using dried fish skins, and even bottled fireflies as safe light sources. Charles Darwin also wrote about the glowing oceans in his travails. E. N. Harvey conducted extensive studies on this phenomenon, and wrote the first detailed account of all natural bioluminescent forms. In biochemical terms, the phenomenon of bioluminescence occurs due to an interaction of a substrate luciferin with an enzyme luciferase. Shimomura et al. were the first to obtain crystalline luciferin from the sea firefly. In this chapter, we explore the origins of bioluminescence in nature, its distribution,and the many ecological roles that it plays. Furthermore, the harnessing of this phenomenon for human use and the future prospects have also been discussed in brief.

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organisms had medicinal properties. There are accounts of coal-miners using dried fish skins, and even bottled fireflies as safe light sources. Charles Darwin also wrote about the glowing oceans in his travails. E. N. Harvey conducted extensive studies on this phenomenon, and wrote the first detailed account of all natural bioluminescent forms. In biochemical terms, the phenomenon of bioluminescence occurs due to an interaction of a substrate luciferin with an enzyme luciferase. Shimomura et al. were the first to obtain crystalline luciferin from the sea firefly. In this chapter, we explore the origins of bioluminescence in nature, its distribution, and the many ecological roles that it plays. Furthermore, the harnessing of this phenomenon for human use and the future prospects have also been discussed in brief.

20.2. THE EVOLITION OF BIOLUMINESCENCE:

Since bioluminescence has proven to be an energy-expensive process, the evolution of bioluminescence in nature must be of some ecological or biological significance, or must offer some evolutionary advantage to the organism. This is certainly true, because there are multiple incidences of the evolution of bioluminescence, all completely independent from each other, and showing a convergent evolution pattern. This trait is found in multiple species spanning different phyla. Some even show symbiotic association with microbes. All these species use this phenomenon for a diverse range of applications including evasion of predators, luring prey and even attracting mates. Since bioluminescence is so widespread in nature, scientists have been speculating the cause of its origin and selection in the first place.

The first speculation was made by E. N. Harvey himself, who believed that it had something to do with respiratory chain proteins, some of which may have had fluorescent groups or side chains. Owing to the extensive research that he conducted, his theory gained some attention and credibility. It was, however, soon disproved. Some even state that bioluminescence may have merely evolved as a by-product of other metabolic functions, having no importance of its own. However, the repetitive and independent origins of bioluminescence in nature must mean that this trait does confer a significant evolutionary advantage to the species that exhibits it. One theory, proposed by Seliger et al. in 1993, stated that luciferases were actually a group of mixed function oxygenase's. According to him, bioluminescence evolved primarily as a means of intra-specific or inter-specific interaction in the dark, deep-sea biome. Rees et al. conducted an independent study on coelenterazine, which is a marine luciferin. They came to the conclusion that bioluminescence may have evolved as a biochemical pathway, mainly for the disposal of peroxide, superoxide, and other harmful oxygen species produced in the course of metabolism. This may have additionally been favored by the acute absence of illumination in the dark depths of the ocean.

Bioluminescence may have undergone natural selection as these species may have progressed deeper in the dark depths of the ocean, where the selective pressure for anti-oxidant defense naturally subsided. As is clear from the above discussion, there was a unanimous agreement among many that bioluminescence may have evolved in the deep-sea ecosystem. Even today, the vast depth of the ocean abounds in various species that exhibit this trait. These may range from microbes like bacteria and dinoflagellates to complex organisms like crustaceans, molluscs, jellyfish, various bony fish, and even cartilaginous fish like sharks. As of today, bioluminescence has many more purposes apart from free radical disposal, like camouflage, counter-illumination, warning colorations, predation or courtship, which have been discussed in further subsections.

20.3. DISTRIBUTION:

As stated earlier, bioluminescence has emerged independently in nature on multiple occasions. Nearly 700 to 800 genera spanning 13 phyla, including both prokaryotic as well as eukaryotic species, have been reported to exhibit this trait. The evolutionary trends of bioluminescence show exemplary convergent evolution in many cases, because of the almost similar purposes this trait serves in various species, or because of the similarity in the biochemistry of the molecules involved

Bioluminescent organisms are found in both terrestrial as well as aquatic habitats. However, the aquatic species are exclusively limited to marine ecosystems, and a freshwater bioluminescent system is yet to be reported. For the sake of simplicity, the distribution of this trait has been discussed separately for bacteria, fungi and protists, and higher animals have been discussed separately.

1. Bacterial bioluminescence:

It is a common belief that bacterial bioluminescent systems were among the first to originate in nature. Bioluminescent bacteria are present in both terrestrial as well as aquatic habitats, and can be found all over the world. In fact, these bacteria can easily be sourced from any tissue or detritus lying on beaches, or even from uncooked seafood. The glowing oceans, which are a spectacular result of these microorganisms, have been described in detail in the travails of Darwin, and can be observed, or rather enjoyed at various locations all over the world. Bioluminescent bacteria mainly belong to the class Gammaproteobacteria, and are confined to three genera, namely Vibrio, Photobacterium and Xenorhabdus. Out of these, Vibrio and Photobacterium are mostly found in marine ecosystems, whereas Xenorhabdus inhabits terrestrial habitats. New strains of bioluminescent bacteria are still being discovered. A remarkable fact about bacterial bioluminescence is that all bacterial bioluminescent systems are exactly alike in terms of biochemistry, i.e., they all rely on flavin mononucleotide (FMN), myristic aldehyde and NADH, and also oxygen. Bioluminescent bacteria may exist as free-living, symbiotic or even pathogenic forms. However, a completely obligate bacterial symbiotic system is yet to be observed in nature.

For example, Vibrio fischeri has been known to colonize specialized “light organs” in the fish Monocentris japonicus, and also exhibits mutualistic relationship with Hawaiian squid Euprymnia scolopes, and various species from the genus Photobacterium have been known to exhibit symbiosis with various fish, molluscs, etc. and even cause diseases in some others. However, there has been no genetic alteration in the bacterial genome for the said symbiosis. Though the animals showing the said symbiosis have developed exclusive modifications like light organs, they do not show any endosymbiotic behavior. The development of the said specialized organs may even be influenced by the presence of the symbiotic bacterial population. One hypothesis account for the emergence of bioluminescence in bacteria because it promotes such symbiotic behavior, conferring a survival advantage to the microbes. The symbiotic behavior may further be promoted because of the fact that the luminescent machinery of the bacteria is instrumental in getting rid of the reactive oxygen species produced in the host tissue. The symbiotic microbes are obtained externally, and the hosts show some degree of selectivity towards the symbiont. It appears that the host organisms ‘choose’ the colonizing symbiont according to the availability as per the depth which they inhabit. Furthermore, the said hosts can even dump the symbiont cells in order to keep their population in check. Terrestrial bioluminescent bacteria are rare, and are known to infect nematodes that

parasitize glowworm larvae. Upon the death of the larva, predators and scavengers ingest the carcasses, hence dispersing the bacteria as well as the nematode. Other than that, bioluminescent bacteria have been observed to inhabit various depths of the ocean, and are found even in sediments, seawaters, saline lakes, etc.

2. Fungal bioluminescence:

Of all the bioluminescent systems that have been studied, fungal bioluminescence remains by far the most poorly investigated of them all, even though fungi are the only terrestrial eukaryotes that exhibit bioluminescence, besides animals. This might be owing to the fact that most initial attempts at determining the enzymatic nature of fungal bioluminescence were failures, and have only recently been confirmed successfully. The study of fungal bioluminescence has thus gained sudden prominence, and a genetically encodable bioluminescent system for eukaryotes has been developed. Kaskova et al. conducted an extensive study of the fungal bioluminescence and color modulation mechanisms. Out of all the fungal species that have been documented till date, only about 71 to 80 fungal species have been known to exhibit bioluminescence. All of the said species have been unequally classified into four distinct lineages that are not so closely related. "Honey Mushrooms" of the Armillaria lineage, the causative species for foxfire phenomenon, and the "Jack-o-Lantern Mushrooms" from the Omphalotus lineage are common examples of bioluminescent fungi.

The origin of fungal bioluminescence can be attributed to a single evolutionary ancestry, the proof of which has been given by cross-reactions between the luciferins and luciferases of distant lineages to yield light successfully. The purpose behind the emergence of fungal bioluminescence still remains elusive. Speculations have been made by Oliveira et al. that it may serve as a mode of attraction for insects, facilitating entomophilous spore dispersal, as seen in some species of Neonothopanus. Furthermore, the same study revealed that there is some semblance of circadian control to make this entire affair more energy efficient by increasing bioluminescence at night. However, this is not true for all fungal species, wherein this trait may simply be a luminous by-product of metabolism, without a definite purpose. The evolutionary feasibility of such cases is yet to be determined.

3. Bioluminescence in protists:

Among protists, the chief groups that exhibit bioluminescence are Radiolaria (or Radiozoa), and Dinoflagellates, which are both exclusively marine. Both of these are described as follows:

- **Bioluminescent radiolaria:** Among all the *radiozoa,* only two genera, namely *Collozoum* and *Thalassicola* are known to exhibit bioluminescence. Both of these belong to the order Collodaria, and use coelenterazine as substrate. Bioluminescence has also been reported in some other deep-sea species like **Aulosphaera spp.** and **Tuscaridium cygneum .**
- **Bioluminescence in dinoflagellates**: Dinoflagellates are a group of cosmopolitan protistan organisms having an ancient evolutionary history, which form one of the most important groups of phytoplankton in the aquatic ecosystems. They are the only photosynthetic organisms that are capable of bioluminescence, and are the most dominant contributors to the occurrence of this phenomenon in the upper ocean. Common phenomena like the "Red Tides" and the bioluminescent bays of Jamaica are because of the dramatic increase in the population of *Gonyaulax* and other dinoflagellate species. *Gonyaulax polyedra* is supposedly the most studied dinoflagellate species. Other common bioluminescent genera are *Ceratium, Protoperidinium, Pyrocystis, Noctiluca,* and *Alexandrianum.* There have been

inaccurate records of bioluminescent dinoflagellate species in the past, because of the presence of both bioluminescent as well as non-bioluminescent strains belonging to the same species. Difference in the ability has been observed even between cells of the same strain. The chemical structure of dinoflagellate luciferin (sourced from Pyrocystis lunula) is remarkably unique, similar only to that found in euphausiids (krill). This perhaps is an example of dietary linkage, as krill are known to source their luciferin from the food they consume. Dinoflagellate luciferin is believed to be a derivative of chlorophyll. Unlike most species that are autotrophic in nature, some heterotrophic species even supplement their luciferin synthesis with chlorophyll-rich diets. Dinoflagellates produce bioluminescence with the help of specialized cell organelles called "scintillons", which enable them to glow only in response to shear or physical disturbance/turbulence in the surrounding water. This glow is not persistent, but occurs in brief flashes. The intensity of these flashes may be affected by various factors like exposure to prior illumination, nutritional state of the cell, or even because of a diurnal rhythm. There are evidences of a circadian rhythm that is operational in dinoflagellates, and also photoinhibition of bioluminescence during daytime. The synthesis and destruction of luciferin is not the only method of regulation though; cellular redistribution of luciferin has been reported to be affected by the said circadian rhythm. The intensity of the flashes also differs from species to species. Dinoflagellates prioritize bioluminescence second only to reproduction, to an extent that there have been reports of cannibalism under nutritional stress in order to support bioluminescence. As far as the ecological purpose of bioluminescence in dinoflagellates is concerned, we are still unclear as to why these organisms take such measures to sustain it.

The exact ecological context of this trait still remains unclear, maybe because of a lack of in-situ studies. Some studies show that the flashes of light have a startling effect on copepods (the prime predators of dinoflagellates), which dart away from the prey. Another speculation, called the "Burglar Alarm" hypothesis, states that the brief flashes produced by the cells upon coming in contact with a grazer (for example, a copepod) in turn attracts a predator of higher trophic level, hence protecting the cell from its own predator. This hypothesis is widely accepted, although there are no sufficient evidences of the same.

Furthermore, this hypothesis does not point out any clear advantage to the dinoflagellate. To conclude, bioluminescence in dinoflagellates seems to be a useful but unnecessary evolutionary trait, as an accurate ecological context is yet to be determined. In order to gain more knowledge on the same, coastal blooms can be harnessed as natural laboratories to study dinoflagellate bioluminescence in further detail.

20.4. BIOLUMINESCENCE IN ANIMALS:

As it is expected, the complexity of bioluminescence certainly upgrades as we proceed upwards in the tree of life. There are no plants (terrestrial or aquatic) that exhibit bioluminescence. Fungal bioluminescence is rare, and has been discussed in the previous sections. Coming to bioluminescence in animals, there is a strong agreement that the evolution of bioluminescence first occurred in the ocean, as the oceanic ecosystem offers many favorable conditions like optical homogeneity, stability of environment, large areas that are almost or completely perpetually dark and a large diversity of organisms that can engage in a variety of ecological interactions. This, and the fact that both luminous as well as non-luminous prey in the ocean are rich in luciferins ensures that the emergence of bioluminescence in the ocean must have been a comparatively easy process. The phenomenon of bioluminescence is so significant in the oceanic ecosystem, that it serves as the predominant source of illumination in many parts

of the ocean. Furthermore, courtships involving bioluminescence have been reported to show higher species accumulation rates than those without bioluminescence. The presence of many independent coelenterazine-mediated bioluminescent systems, nine different phyla to be exact, indicates dietary linkage, as coelenterazine is procured by most species mainly through their diet. Bioluminescence is encountered most commonly in the topmost 1-kilometer layer of the ocean, and is doubtlessly the most efficient mode of communication in the oceanic ecosystem.

The ability to glow is strongly habitat dependent because of various selection forces described earlier, and it is observed that there is a marked difference in the occurrence of this trait as we go deeper in the ocean. Bioluminescence is also common in the terrestrial ecosystems, though it is nowhere as abundant as in the ocean. Various worms and arthropods are known to exhibit complex behaviors related to this phenomenon. It is clear that bioluminescence has a powerful impact on behavioral and ecosystem dynamics. In this section, bioluminescence has been followed as a trait through various animal phyla, both terrestrial and aquatic, and its ecological significance is simultaneously discussed.

1. Bioluminescence in ctenophores:

Comb jellies are the phylogenetically the most basic examples of bioluminescence in animals. Many species like *Mnemiopsis* use calcium activated coelenterazine as their bioluminescent substrate. Some species, for example *Beroe forskalii* are known to produce myriad, cascading wave-patterns of intrinsic glow on their bodies, and some even emit a haze of glowing particles to startle the predator as a defensive measure, coupled with an escape response. A majority of pelagic species are likely to exhibit bioluminescence. The photo-proteins involved in bioluminescence in various genera like *Mnemiopsis* and Beroe have been studied, and are known to depend on calcium ions for their activity. Many comb jellies like *Pleurobrachia* and some species of the genus Beroe also show a startling display of rather colorful lights, in various wavelengths found in the visible spectrum. This was mistakenly believed as bioluminescence in the past. However, the said lights were not actually “produced” in the organism itself, as was evident in some studies. This iridescence was rather found to be a result of refraction of ambient light through the moving combs as the organism swims around.

2. Bioluminescence in cnidarians:

Cnidarians in both pelagic as well as benthic zones, including corals, anemones, hydroids and medusae are known to exhibit bioluminescence. All of them use the luciferin coelenterazine as the substrate for their biochemical pathways (hence the name “coelenterazine”). Most of the pelagic siphonophores encountered show bioluminescence.

The most common examples of bioluminescent coelenterates are the shallow-living hydrozoan Crystal Jelly (*Aequorea victoria)*, the sea pansy Renilla and also the bamboo corals from the pelagic zone. Anatomically, light producing centers, or phorocytes, may be clustered or widely scattered all over the body, located around the endodermal layer. The bioluminescent system of *Renilla* has been studied extensively, and attempts have been made to triangulate and engineer the genes from the source into various eukaryotic (plant) systems. Cnidarians use bioluminescence for various defensive, aggressive as well as warning purposes. Some jellyfish show glowing wave patterns on their umbrellas, and even emit clouds of glowing particles as a part of their escape response. Siphonophores use bioluminescence to attract prey within reach of their cnidocytes. Some jellyfish are also known to show aposematic glow, which is indicative of distastefulness. Cnidarians can gain a lot from aposematic

bioluminescence, as it would not only warn the predators of the unpalatability of the individual, but also protect them from any physical injuries. However, many predator species like leatherback turtles use this to their advantage, and easily locate prey like jellyfish.

3. Bioluminescence in annelids:

Bioluminescence in annelids has independently emerged in several lineages, resulting in a rich taxonomic diversity spanning across 45 different genera in 13 lineages of clitellates and polychaetes. They are found in diverse terrestrial and aquatic habitats all across the globe.

Clitellates are the only terrestrial annelids known, including potworms and earthworms from families Lumbridae and Megascolidae. Most of them emit brief flashes, and secrete a slimy coelomic fluid packed with bioluminescent granules under mechanical, chemical or electrical stimulation. The same trend is seen in benthic species from the family Chaetopteridae. This is basically a form of aposematism or advertisement of distastefulness or toxicity, due to which predator species avoid such individuals from a distance. In the marine ecosystems, polychaetes are the predominant annelid species in both pelagic as well as benthic zones. Unlike their terrestrial counterparts, marine annelids show an interesting diversity of adaptations of bioluminescence, which they use for a variety of functions. The swarming behaviors of Chaetopterus and Odontosyllis spp. and their flashing patterns have been studied in detail. The bioluminescent "bombs" of the deep-sea genus Swima are detonated upon the slightest disturbance, facilitating an almost ninja-like distraction while the animal swims to safety. Several members of the family Tomopteridae are known to produce golden yellow light, which is quite rare in aquatic ecosystems. Scale worms (family Polynoidae) emanate flashes when disturbed, and even break off one or more bioluminescent scales or even whole parts of the body as decoys or sacrificial lures for the predator while they flee. Some species even shoot sticky glowing mucus at the predators to hamper their mobility, distracting them while making them even more conspicuous. Arrow worms (Chaetognatha) are also known to adapt similar defensive measures. Light production also wards of symbiotic bacteria that overcrowd the tubules of some annelids. Bioluminescence is also used as a mode of intraspecific communication in annelids. Some members of the families Syllidae and Cirratulidae exhibit bioluminescence as a part of their mating behaviors. Elaborate bioluminescent courtship displays of the genus Odontosyllis are even known to align with lunar cycles.

4. Bioluminescence in molluscs:

Bioluminescence in molluscs is represented by many unusual taxa, for example the bivalve Pholas, the biochemical machinery of which has been extensively studied. Also, the sea-firefly Cypridina is a specimen of significance, as its bioluminescent system was among the first to be studied and analysed in detail. The only bioluminescent organism from freshwater ecosystem, the snail Latia neritoides, is also a mollusc. Also, the terrestrial snail Dyakia striata is another bioluminescent organism that has been studied in great detail. Also, the snail Hinea brasiliana uses flashes of blue light as an aposematic signal to ward off predators.

Cephalopods are the prominent representatives of bioluminescent molluscs, and some of these may have been the source behind the fables of the mythical Kraken. Among squids alone, there are about 70 bioluminescent genera, both symbiotic and intrinsic. Most luminescent cephalopods use coelenterazine as substrate for bioluminescence. Squids are almost flamboyant in their exhibition of bioluminescence. Euprymna is known to be symbiotic with the bioluminescent bacteria Vibrio fischerii to form exclusive light organs [10] which it uses

for counter illumination. The vampire squid Vampyroteuthis has light organs all over its body, and it even shoots glowing particles from the tips of its tentacles. The squid Taningia danae has light organs on the tips of its arms, which it uses for intraspecific communication as well as to lure, stun and baffle prey. Even some octopods are known to use bioluminescence to lure prey into their glowing suckers. Cephalopods are also known to autotomize entire glowing arms as decoys if threatened. Some species of octopus also use bioluminescence in courtship displays. An interesting fact about sperm whales is that they hunt squid by triggering the burglar alarm mechanism around themselves to attract unsuspecting squids.

5. Bioluminescence in insects:

Insects are the most predominant terrestrial organisms that exhibit the phenomenon of bioluminescence. A majority of the bioluminescent insects are beetles (Coleoptera), click beetles (Elateridae), glowworms & railroad worms (Phengodidae), and fireflies (Lampyridae).

The biochemical mechanism of luminescence is similar in all of these, even though each of them emit a diverse palette of wavelengths. Other insects like lantern flies (Homoptera), springtails (Collembola), etc. also show bioluminescence. Among springtails, only two families exhibit bioluminescence upon mechanical stimulation. Bioluminescence occurs only during sexual phases, and is crucial for sperm transfer. Lantern flies, for example Fulgora lanternaria, emit bright white light when both the sexes fly together. Glowworms and Fungus gnats from the order Diptera show bioluminescence only in the larval stages, where they use their glow to attract prey and snare them in webs. The larvae of Arachnocampa luminosa are a prime example of such behavior. Female glowworm pupae also glow to attract males. Click beetles show bioluminescence in all stages of life. In the larval stage, bioluminescence serves as a tool to attract prey, as well as for defense. The pupae also glow when illuminated, and adults use bioluminescence for various functions like defense, mating communication and even general illumination. In glowworms, on the other hand, bioluminescence is only secondary to pheromone-mediated communication. Males are rarely bioluminescent, only in the sexual stages for seductive purposes, whereas larvae and females are very luminescent. The railroad worm Phrixothrix is highly aposematic, as its body is lined with bright green glowing patches, while it has red headlights, which is very rare among all animals. Fireflies are among the most studied bioluminescent systems, especially the north American Photinus pyralis. All life stages in fireflies are luminescent, and firefly larvae are known to use their glow for defensive purposes. Illumination patterns of fireflies may differ even for different individuals of the same species, and are highly encodable. Fireflies have specialized organs called lanterns in their abdominal segments, which can be controlled by the nervous system. Since bioluminescence in fireflies forms the basis of various complex interspecific as well as intraspecific interactions, visual sensitivity according to the environment, time of activity and other parameters has evolved in parallel.

The signaling systems in firefly species are highly encodable, species specific, and crucially timed for maximum efficiency. Synchronous flashes are seen in various species, sometimes in swarms spanning 30 meters, producing spectacular displays like the ones at Chaophraya river, Bangkok. The biological significance of such displays is still not understood. Due to the uniqueness of the signaling mechanism, some species have evolved to mimic other species-specific signals. For example, female fireflies of the genus Photuris mimic the female signal of Photinus macdermotti to attract and prey upon their males. Fireflies are also highly distasteful to predators, which is exhibited by their aposematic signals, a necessary counter measure to

compensate for their high conspicuousness. Today, fireflies are adversely affected by the growing numbers of artificial lighting systems, which hamper their signaling and even cause direct mortality in some cases.

6. Bioluminescence in crustaceans:

The evolutionary pathway of crustaceans reveals that bioluminescence has emerged multiple times. Many krill (euphausiids) are bioluminescent, showing biochemical pathways similar to diatoms. Sergestids use bioluminescence for counter-illumination purposes. Cypridinids are known to release puffs of bioluminescent particles, and also have elaborate mating behaviors involving bioluminescence.

7. Bioluminescence in other Arthropods:

Few luminous species of centipedes (Chilopoda) and millipedes (Diplopoda eg. Motyxia) have also been shown to exhibit bioluminescence. Millipedes are also known to show aposematic signaling as a warning for toxicity**.**

8. Bioluminescence in echinoderms:

Four out of the five classes of echinoderms, namely Ophiuroidea (brittle stars), Asteroidea (starfishes), Holothuroidea (sea cucumbers) and Crinoidea (sea lilies) are bioluminescent. Echinoderms mostly use coelenterazine dependent bioluminescent systems, although some of them also use a novel photoprotein. Bioluminescence is more commonly exhibited by echinoderms inhabiting deep seas. Many new are bioluminescent taxa still being discovered, and 70 ophiuroid species have been recognized to exhibit bioluminescence till date.

9. Bioluminescence in tunicates:

Many species of tunicates are known to exhibit bioluminescence, though planktonic tunicates are not as frequent exhibitors of the trait as planktonic larvacean Appendicularia. However, it cannot be ascertained accurately because some filter feeders (like Pyrosoma) may ingest and trap luminescent microbes and appear to be bioluminescent. Species like Balanoglossus (Acorn worms) and Ptychodera of the class Enteropneusta are also known to be bioluminescent. Also, the sessile adult Clavelina miniata glows green when stimulated.

10. Bioluminescence in fish:

Among vertebrates, fish are the only taxa that have the ability of bioluminescence. This trait is found in fish inhabiting all the depths of the ocean, but is most frequently encountered in specimens from the deepest recesses of the ocean. Bioluminescence is found in about 1500 species of marine bony fish spanning 43 families in 11 different orders, out of which some like the anglerfish, flashlight-fish (*Photoblepharon*) and pony-fish *(Leiognathus*) harbor symbiotic bacteria in discrete, specialized light organs, while others produce glow intrinsically. On the other hand, only a handful of shark species in three families of cartilaginous fish are known to exhibit bioluminescence. Unlike bony fish species, cartilaginous fishes do not rely on symbionts for bioluminescence, but use an altogether different, unknown bioluminescent system. Some other species like the midshipman fish *Porichthy*s and various lantern-fish obtain their respective luciferins from dietary sources.

Fish use the ability of bioluminescence for a variety of applications like communication, evading predators, luring prey. The latter is highly expressed in various taxa inhabiting the deep seas. Various anatomical modifications (like the light organs in various bony fish and the esca of anglerfish) harbor symbiotic bacteria, which enable the fish to use the bacterial emission with ample control on the intensity as well as distribution of the emission. Fish of the order *Stomiiformes* (like dragon-fish, etc.) have evolved most elaborately arranged photophores, including those emitting red light. Cookie-cutter sharks are interesting examples of both counterillumination and mimicry, as they bait their prey with non-luminescent patches on their bodies that look like small fish. Bioluminescence may also prove disadvantageous to some species in certain cases. For example, elephant seals follow bioluminescence to track down prey populations. Some studies have shown that seals prefer to hunt in locations where there are more bioluminescent individuals.

11. Future prospects:

Even though we still need to understand the dynamics and biochemistries of many bioluminescent systems in nature, humans have already begun to put bioluminescence to various applications. Bioluminescent mechanisms have been used in the diagnosis of various pathological conditions in the form of Green Fluorescent Proteins (GFP). Furthermore, attempts are being made to incorporate bioluminescent systems into plants to supplement illumination. However, these prospects are still in their developmental stages, and there are various challenges and issues that need to be tackled.

20.5. SUMMARY:

The emergence of bioluminescence in nature has occurred independently on multiple occasions, which certainly means that it confers some significant evolutionary advantage(s) which we are yet to understand fully. This is bolstered by the fact that there are so many species that exhibit this trait, and show a plethora of behavioral, anatomical and ecological trends so as to survive and thrive in various habitats. With a better understanding of these systems and their interactions, we will certainly be able to use this phenomenon to our advantage. However, there are some challenges that keep us from fully exploring certain bioluminescent systems. For example, the deep-sea bioluminescent systems are very hard to access, and thus in-situ observations are few and far between. With the advent of new tools and techniques, we shall be able to gain a better insight into the dynamics of these systems.

20.6. TECHINICAL TERMS:

Luciferin, Luciferase, cintillons, Aposematism, Counter-illumination, Symbiosis, Convergent evolution, Oxidative metabolism, Photophores, Circadian regulation

20.7. SELF ASSESSMENT QUESTIONS:

1. Explain the biochemical mechanism of bioluminescence with reference to luciferin and luciferase**.**
2. Discuss the evolutionary theories proposed for the origin of bioluminescence.
3. Compare bacterial, fungal, and protist bioluminescence in terms of occurrence and ecological roles.
4. How is bioluminescence used for camouflage and predator evasion in marine organisms?
5. Write short notes on the applications of bioluminescence in biotechnology and medicine**.**

20.8. SUGGESTED READINGS:

1. Alberts, B. *Molecular Biology of the Cell* (latest edition).
2. Campbell, N.A. *Biology*. Pearson Education.
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