PREFACE

In a bid to standardise higher education in the country, the University Grants Commission (UGC) has introduced Choice Based Credit System (CBCS) based on five types of courses viz. *core, discipline specific, generic elective, ability* and *skill enhancement* for graduate students of all programmes at Honours level. This brings in the semester pattern, which finds efficacy in sync with credit system, credit transfer, comprehensive continuous assessments and a graded pattern of evaluation. The objective is to offer learners ample flexibility to choose from a wide gamut of courses, as also to provide them lateral mobility between various educational institutions in the country where they can carry acquired credits. I am happy to note that the University has been accredited by NAAC with grade 'A'.

UGC (Open and Distance Learning Programmes and Online Learning Programmes) Regulations, 2020 have mandated compliance with CBCS for U.G. programmes for all the HEIs in this mode. Welcoming this paradigm shift in higher education, Netaji Subhas Open University (NSOU) has resolved to adopt CBCS from the academic session 2021-22 at the Under Graduate Degree Programme level. The present syllabus, framed in the spirit of syllabi recommended by UGC, lays due stress on all aspects envisaged in the curricular framework of the apex body on higher education. It will be imparted to learners over the *six* semesters of the Programme.

Self Learning Materials (SLMs) are the mainstay of Student Support Services (SSS) of an Open University. From a logistic point of view, NSOU has embarked upon CBCS presently with SLMs in English / Bengali. Eventually, the English version SLMs will be translated into Bengali too, for the benefit of learners. As always, all of our teaching faculties contributed in this process. In addition to this we have also requisitioned the services of best academics in each domain in preparation of the new SLMs. I am sure they will be of commendable academic support. We look forward to proactive feedback from all stakeholders who will participate in the teaching-learning based on these study materials. It has been a very challenging task well executed, and I congratulate all concerned in the preparation of these SLMs.

I wish the venture a grand success.

Professor (Dr.) Subha Sankar Sarkar

Vice-Chancellor

Netaji Subhas Open University

Undergraduate Degree Programme

Choice Based Credit System (CBCS)

Subject : Honours in Zoology (HZO)

Course : Animal Physiology, Molecular Biology & Evolution (Practical)

Course Code : CC - ZO - 02

First Print : December, 2021

Printed in accordance with the regulations of the
Distance Education Bureau of the University Grants Commission.

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UG : Zoology (HZO)

Course: Animal Physiology, Molecular Biology & Evolution (Practical)

Course Code : CC - ZO - 02

Unit-1 □ Preparation of haemin crystals

Structure

1.0 Objectives

Haemin can be produced from haemoglobin by the so-called Teichmann test, when haemoglobin is heated with Glacial Acetic Acid (saturated with saline). This can be used to detect blood traces and used in forensic purpose.

(i) It is important in medico – legal test for the detection of blood.

(ii) It helps to give an opinion as to proof whether a stain is blood or something else.

1.1 Introduction

Haemin is protoporphyrin IX containing a ferric iron (Fe^{3+}) ion with a coordinating chloride ligand that can be formed from a haem group, such as haem b found in the haemoglobin of human blood. Crystals are homogeneous solids, bounded by plane faces and having a geometric shape.

1.2 Procedure

(i) Chemical formula of Haemin - $C_{34}H_{32}ClFeN_4O_4$

(ii) IUPAC Name - Chloro [3,7,12,17-tetramethyl-8, 13-divinylporphyrin-2, 18-dipropanoato(2–) iron(III)

(iii) **Preparation**

A small amount of dry blood is taken on a glass slide and crushed to a fine powder with the help of the fused end of a glass rod or with a needle. Some crystal of common salt (NaCl) is added to it, which is also crushed to powder. The two are thoroughly mixed and two drops of Glacial Acetic acid added to it.

The mixture is covered with a cover slip and the slide heated over the flame of a spirit lamp. The reaction is complete with the beginning of boiling of the mixture and the slide is quickly removed from the flame.

The preparation is allowed to cool and examine under a microscope, initially under low magnification and then under high magnification.

Observed Structure

Man **Rat** **Guineapig**

Man: Rhomboidal plates and prisms, often arranged in star-shaped clusters with round edges

Rat: Narrow plates with varying width to needles, blunt at both the ends

Guineapig: Triangular plates often arranged in the form of squares

1.3 Summary

The formation and study of crystals will confirm the presence or absence of blood in a particular red coloured stain. Moreover, the shape and nature of crystals will help to identify the source of blood.

1.4 Questions

(i) What is the purpose of haemin crystal study?

(ii) Why dry blood sample is required for haemin crystal preparation?

Unit-2 □ Study of permanent histological sections of mammalian pituitary, thyroid, pancreas, adrenal gland, testis, ovary

Structure

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2.1 Mammalian pituitary gland

2.1.1 Objectives

To study the microanatomy of the pituitary gland in tissue level.

2.1.2 Introduction

The Pituitary gland is by far the most important endocrine organ of the body. It is the entire hormonal regulating system of our bodies.

2.1.3 Histological structure

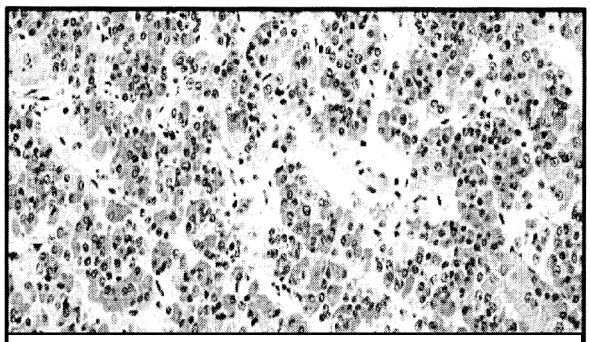
Its section reveals the following structures.

It is enveloped by a thin connective tissue capsule. It consists of the large glandular lobe, the Adenohypophysis, and the smaller cerebral lobe, the Neurohypophysis. The infundibulum connects the Neurohypophysis (*posterior lobe*,

pars nervosa) to the diencephalon. The intermediary lobe between anterior and posterior lobes is a part of the adenohypophysis.

The sagittal section distinctly shows the following parts of the pituitary gland;

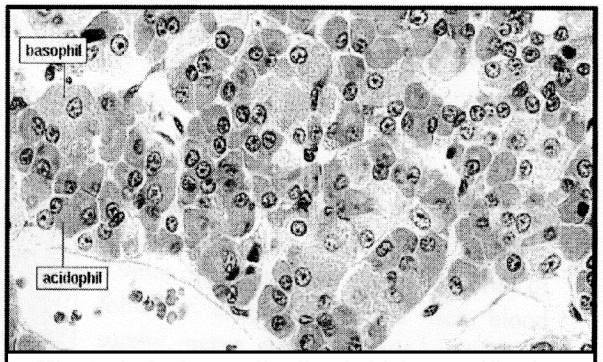
Pars distalis : The anterior lobe of the Adenohypophysis *(pars distalis)* consists of different types of epithelial cells. These are surrounded by reticular fibres and wide blood sinuses. The cells of the adenohypophysis are of three types based on their affinities to dyes, which are, acidophilic, basophilic or chromophobic cells. Acidophilic cells are round and contain a dense (acidophilic) population of granules.



Histological configuration of the pars distalis of mammalian pituitary in low magnification

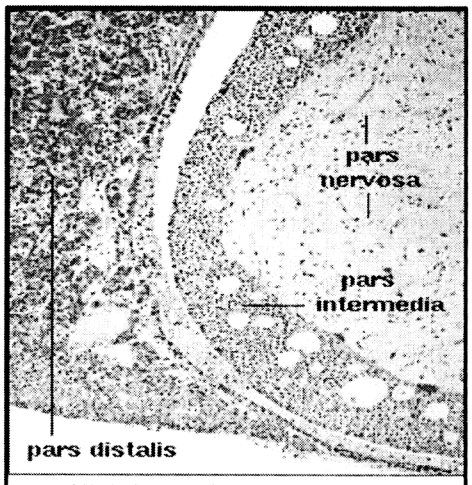
Basophilic cells are of varying sizes. There are quite a few types of basophilic cells viz. Gonadotropic basophilic cells, Thyrotropic basophilic cells, Adrenotropic basophilic cells, Lipotropic basophilic cells and Melanotropic basophilic cells. The chromophobic cells do not participate in the biosynthesis of hormones. They are considered as the precursors of hormone-producing cells.

Pars intermedia : The pars intermedia are considered to belong to the adenohypophysis, the reason being that the origin of both are same during development. Basophilic cells enter the dorsal hypophysis (basophil invasion). Colloid filled cysts are conspicuous elements in the intermediary lobe. They derive from the hypophyseal pouch (remnant of the Rathke pouch). The cysts are lined by either a single-layered epithelium or sometimes by a multilayered stratified epithelium at different levels of differentiation.



Histological of pars distalis of high magnification

Pars nervosa : The neurohypophysis consists of the posterior lobe (pars nervosa) and the infundibulum, including the eminentia mediana. The constituent cells of the neurohypophysis are neuroglia cells (pituicytes, protoplasmic glial cells), numerous unmyelinated nerve fibres, which stem from neurosecretory neurons of the hypothalamus, connective tissue and vessels. Routine staining procedures reveal a dense matted layer of fibres or a woven meshwork of fine, unmyelinated nerve fascicles (cross-sectioned or cut longitudinally). The meshwork of the nerve fascicles contains pituicytes and wide capillaries.



Histology of pars nervosa and pars intermadia

2.1.4 Questions

1. What are the histological differences between acidophilic and basophilic cells of pars distalis?
2. What are pituicytes?
3. What are neuroglia cells?

2.2 Mammalian thyroid gland

2.2.1 Objectives

To study the microanatomy of the thyroid gland in tissue level

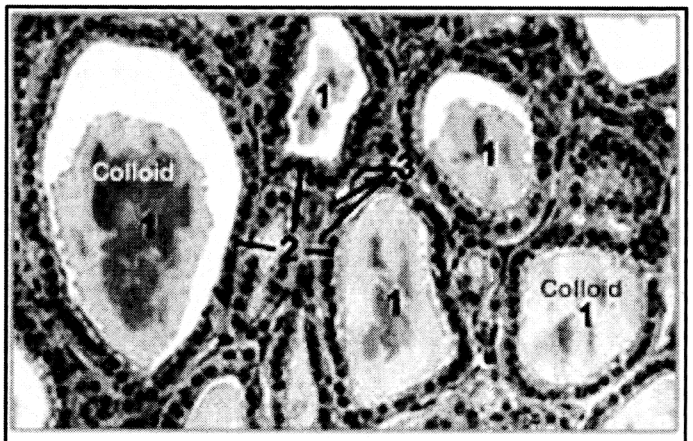
2.2.2 Introduction

At the microscopic level, there are three primary features of the thyroid — - follicles, follicular cells, and parafollicular cells, first discovered by Geoffery Websterson in 1664

2.2.3 Histological structure

There are three primary features of the thyroid gland — follicles, follicular cells, and para-follicular cells. Description of these features is given below:

Follicles: Thyroid follicles are small spherical sac like structures of 0.02 -0.9mm diameter. They consist of a rim of follicular cells that has a rich blood supply, nerve and lymphatic vessels are also present. The rim surrounds a core of colloid that consists mostly of thyroid hormone precursor proteins called thymoglobulin, an iodinated glycoprotein.



Histological feature of Thyroid Gland: 1. Follicles; 2. Follicular cells; 3. Endothelial cells

Follicular cells: The core of a follicle is surrounded by a single layer of follicular cells. When stimulated by thyroid stimulating hormone (TSH), these cells secrete the thyroid hormones T_3 and T_4. They do this by transporting and metabolising the thyroglobulin contained in the colloid. Follicular cells vary in shape from flat to cuboids to columnar, depending on the state of activity.

Para-follicular cells : Scattered among follicular cells and in spaces between the spherical follicles is another type of thyroid cell, para-follicular cells. These cells secrete calcitonin and so are also called as C cells.

2.2.4 Questions

1. What are the differences between follicular and para-follicular cells?
2. What is the nature of colloids in follicles?

2.3 Mammalian pancreas

2.3.1 Objectives

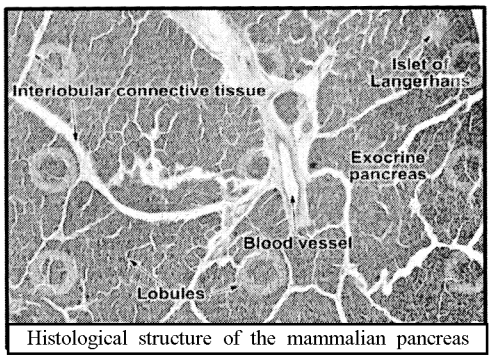
To study the microanatomy of the pancreas in tissue level.

2.3.2 Introduction

The pancreas contains tissue with an endocrine and exocrine role, and this division is also visible when the pancreas is viewed under a microscope.

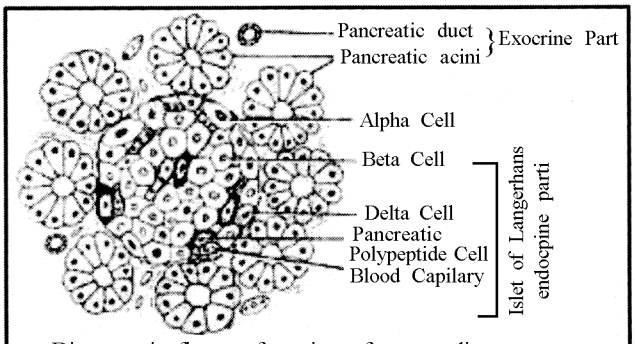
2.3.3 Histological structure

The pancreatic tissues with an endocrine role can be seen under staining as lightly-stained clusters of cells, called pancreatic islets (also called islets of Langerhans). Pancreatic islets contain **alpha cells, beta cells, delta cells,** and **F cells**, each of which releases a different hormone. These cells have characteristic positions, with alpha cells (secreting glucagon) usually situated around the periphery of the islet, and beta cells (secreting insulin) more numerous and found in the centre. Islets are composed of around 3,000 secretory cells, and contain several small arterioles and venules that allow the hormones secreted by the cells to enter the systemic circulation.



Histological structure of the mammalian pancreas

The majority of pancreatic tissue has a digestive role. These cells take darker-staining and clusters called acini, which are arranged in lobes that have thin fibrous wall. The cells of each acinus are granulated and secrete zymogens into small intercalated duct. The intercalated ducts drain into larger ducts within the lobule, and finally interlobular ducts. The ducts are lined by a single layer of column-shaped cells. There is more than one layer of cells as the diameter of the ducts increases.



Diagramatic figure of section of mammalian pancreas

2.3.4 Questions

1. What do you mean by exocrine and endocrine function of a gland?
2. What are the functions of alpha cells, beta cells and delta cells?
3. What will happen if alpha cells are missing from the pancreas of a mammal?

2.4 Mammalian adrenal gland

2.4.1 Objectives

To study the microanatomy of the adrenal gland in tissue level

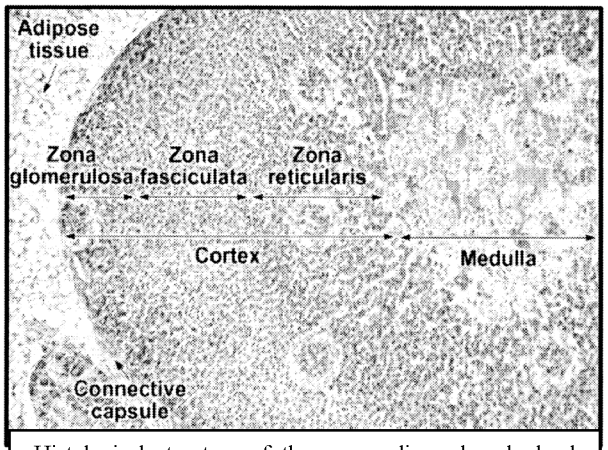
2.4.2 Introduction

The adrenal glands are situated over the kidneys, surrounded by a fatty capsule and lie within the renal fascia. A weak septum (wall) of connective tissue separates the glands from the kidneys. The adrenal glands are situated below the diaphragm, and are attached to the crura of the diaphragm by the renal fascia. This gland is also known as **supra-renal gland** and **emergency gland**. This gland secrets aldosterone, cortisol, androgens catecholamines, adrenaline and noradrenaline.

2.4.3 Histological structure

Each adrenal gland has two distinct parts, each with a unique function, the **Outer Adrenal Cortex** and the **Inner Adrenal Medulla**, both of which produce hormones. The histology of the parts is described below.

Adrenal Cortex : The adrenal cortex is the outermost layer of the adrenal gland. Within the cortex are three layers, called "zones". When viewed under a microscope each layer has a distinct appearance, and each has a different function. The three layers are zona glomerulosa, zona fasciculata and zona reticularis. The tissues of adrenal cortex secret different hormones like aldosterone, cortisol, and androgens.

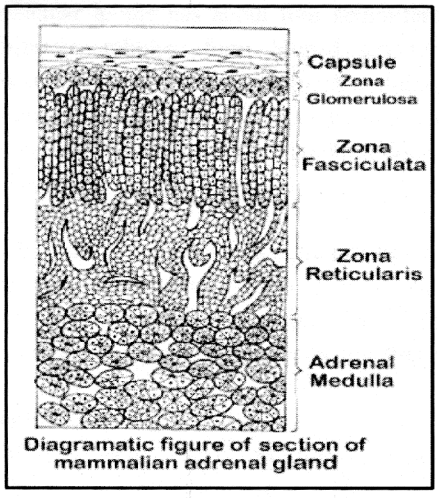


Histological structure of the mammalian adrenal gland

Zona glomerulosa : The outermost zone of the adrenal cortex is the zona glomerulosa. It lies immediately under the fibrous capsule of the gland. Cells in this layer form oval groups, separated by thin strands of connective tissue from the fibrous capsule of the gland. This layer is the main site for production of aldosterone. Aldosterone plays an important role in the long-term regulation of blood pressure.

Zona fasciculata : The zona fasciculata is situated between the zona glomerulosa and zona reticularis. It is the largest of the three layers, accounting for nearly 80% of the volume of the cortex. In this zone, cells are arranged in columns and radially oriented towards the medulla. The cells of this layer contain numerous lipid droplets in the cytoplasm, abundant mitochondria and a complex sER. Cells in this layer are responsible for producing glucocorticoids such as cortisol.

Zona reticularis : The innermost cortical layer, the zona reticularis, lies directly adjacent to the medulla. This layer contains small cells and form irregular cords and clusters, separated by capillaries and connective tissue. The cells contain relatively small quantities of cytoplasm and lipid droplets than the cells of other two zones, and sometimes display brown lipofuscin pigment. It produces androgens, mainly dehydro-epiandrosterone (DHEA), DHEA sulphate (DHEA-S), and androstenedione (the precursor to testosterone) in humans.



Diagramatic figure of section of mammalian adrenal gland

Adrenal Medulla : The adrenal medulla is at the centre of the adrenal gland, and is surrounded by the adrenal cortex. The chromaffin cells of the medulla are the body's main source of the catecholamines, adrenaline and noradrenaline.

The adrenal medulla is innervated by preganglionic nerve fibres, thus adrenal medulla is considered as a specialized sympathetic ganglion. Unlike other sympathetic ganglia, however, the adrenal medulla lacks distinct synapses and releases its secretions directly into the blood.

2.4.4 Questions

1. Why adrenal gland is called as emergency gland?
2. Name the hormones secreted from adrenal cortex.
3. Name the hormones secreted from adrenal medulla.

2.5 Mammalian testis

2.5.1 Objectives

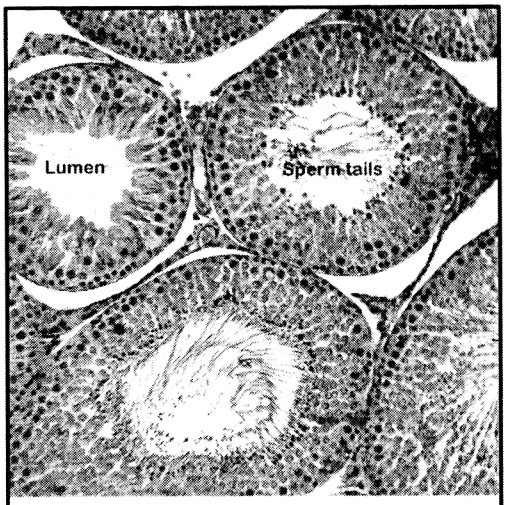
To study the microanatomy of the testis in tissue level

2.5.2 Introduction

Testes, the most important reproductive organ in males are of a very elaborate microscopic structure, which shall be understood in the following paragraphs. It also has certain very essential, endocrine properties.

2.5.3 Histological structure

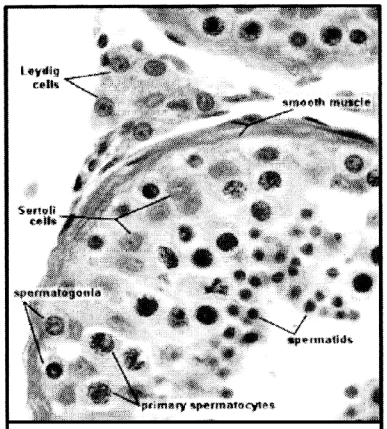
There is a connective tissue that surrounds the testes, called the Tunica Albuginea. The different components of the mammalian testes are seminiferous tubules, developing spermatozoa, myoid cells, seminiferous epithelium, interstitial tissues, Leydig cells, Sertoli cells. These are described below—



Histological morphology of section of mammalian testis

Seminiferous tubules : Each testicular lobule has about four seminiferous tubules. Seminiferous tubules, form looped structures, which lead into straight zones of the seminferous tubules. Seminiferous tubules contain cells of the gamete producing line and Sertoli cells, which support the gamete producing cells.

Myoid Cells : These cells share similar characteristic features with that of the smooth muscle cells. Myoid cells cause peristaltic waves of contraction that pass along the seminiferous tubules and propel spermatozoa towards the cpididymis.



Enlarged view of a seminiferous lobule

Seminiferous epithelium : The seminiferous epithelium is a continuous layer of Sertoli cells lined by prominent functional complexes. Compartments between the lateral boundaries of adjacent Sertoli cells contain spcrmatogonia resting on the basal lamina, primary spcrmatoeytes, secondary spcrmatocytes, spermatids and spermatozoa.

Interstitial tissue : There exists a space between seminiferous tubules, which is occupied by tissues which are the continuation of the tunica albuginea. These tissues are called the interstitial tissues. Interstitial tissue contains connective tissue ftbroblasts and collagenous fibrils that hold the spermatogenic tissue together.

Leydig cells : These cells exist in small clusters of cells. The extensive Golgi apparatus helps in the formation of smooth endoplasmic reticulum, which contains the enzymes for testosterone synthesis. There are present some interstitial fenestrated capillaries near Leydig cells. It is from here that the androgens enter the systemic circulation, and affect the target organs, in the body.

Sertoli cells : The seminiferous epithelium contains a continuous layer of tall columnar epithelial cells called, Sertoli cells. These cells support the spermatogenic cell lines. These cells rest on the seminiferous epithelial basal lamina and reach the lumen.

2.5.4 Questions

1. What are seminiferous tubules?
2. What are functions of Leydig cell?
3. What are the functions of Sertoli cells?
4. Where interstitial tissues are present?

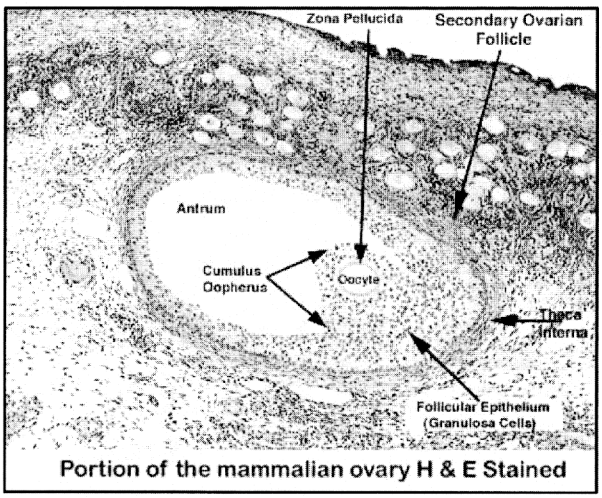
2.6 Mammalian ovary

2.6.1 Objectives

To study the microanatomy of the ovary in tissue level

2.6.2 Introduction

Ovary is the most important reproductive organ of the female reproductive system. This has very important endocrine roles as well, which control most of the female physiological processes.

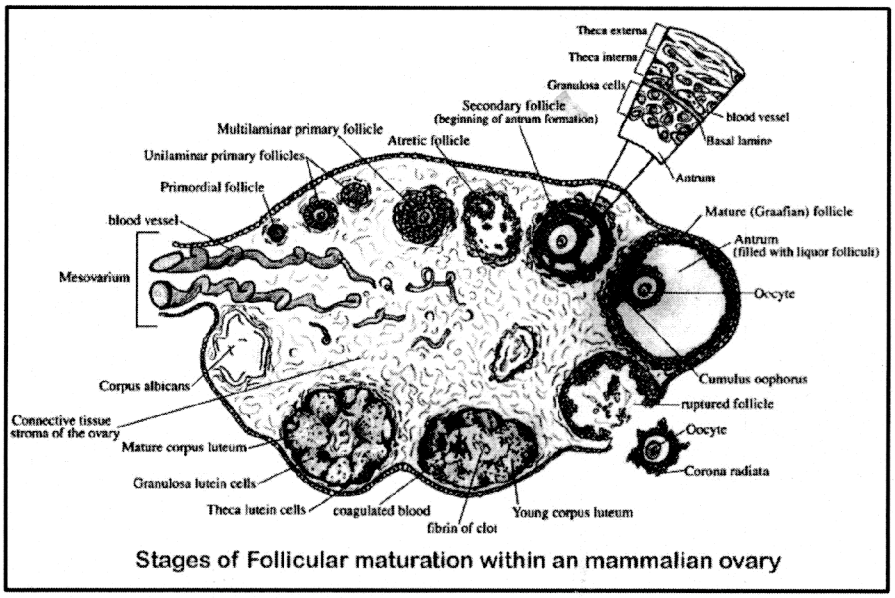


Portion of the mammalian ovary H & E Stained

2.6.3 Histological structure

The description of the histological section of ovary is explained below;

Germinal Epithelium : The surface of each ovary is covered by a simple cuboidal epithelium called, germinal epithelium. It is continuous with the simple squamous mesothelial covering of the mesovarium and uterus.



Stages of Follicular maturation within an mammalian ovary

Medulla : There are regional differentiation within the ovaries, the outer cortex and the inner medulla. The medulla is located at the centre of the ovary. It is composed of loosely packed connective tissue, coiled blood vessels, nerves and lymphatics. The medulla also contains the vestigial rete ovarii, an anastomosing network of closed ducts lined by low cuboidal epithelium.

Cortex : The ovarian cortex surrounds the centrally located medulla of the ovary. The cortex contains ovarian follicles in different stages of development. Ovarian follicles consist of an oocyte and a layer of follicular epithelial cells that varies in thickness. In certain, more mature follicles, an acellular, glycoconjugate-rich layer called the zona pellucida, surround the oocyte. Stromal cells and small blood vessels are present between the follicles. The stroma of the cortex forms a dense capsule called the tunica albuginea, which lies beneath the basement membrane of germinal epithelium.

Corpus luteum : The follicular structure that forms after the ovulation is converted into the corpus luteum, which is the major source of progesterone secretion.

2.6.4 Questions

1. What do you mean by germinal epithelium?
2. What is corpus luteum?
3. What is theca interna?

Unit-3 □ Study of permanent histological slides of duodenum, intestine, liver, lung, kidney, bone, cartilage

Structure

3.0 Objectives

After studying this unit, students would be able to understand about the microanatomy of the different organs & tissues of the animals.

3.1 Introduction

Histology or tissue structure study is the most important component for the understanding of the functions of the different organs, tissues or systems.

3.2 Histology of duodenum

Study of histological sections of mammalian of duodenum

The duodenum in cross section possess following three layers from the inner lumen of the intestine : mucosa, submucosa and muscularis.

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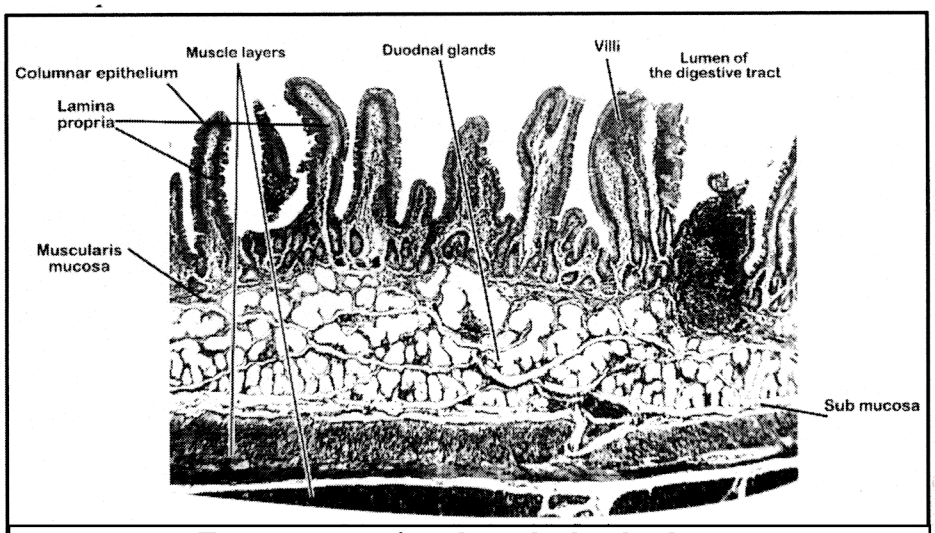
(1) The mucosa consists of simple columnar epithelium (lamina epithelialis), a connective tissue layer (lamina propria) and a smooth muscle layer (lamina muscularis). The intestinal epithelial cells (enterocytes) are overlaid by a layer of glycoproteins and mucin.

(2) The submucosa comprises loose connective tissue, numerous blood vessels and the Meissner's plexus.

(3) The muscularis consists of an inner circular and an outer longitudinal musculature between which the Auerbach's plexus lies.



Transverse section through the duodenum

(4) Typical for all sections of the small intestines are microvilli (hair like structures projecting from the surface), finger-shaped villi and circular folds of the mucosa and submucosa (valves of Kerckring). These structures increase the absorption area of the duodenum up to 1500 times.

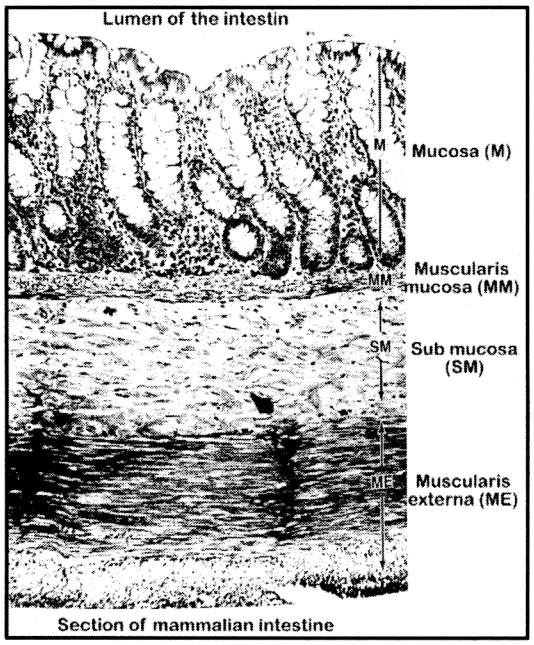
(5) The duodenum is rich of absorbing enterocytes, mucus producing goblet cells and peptide hormone producing endocrine cells.

(6) A characteristic feature of the duodenum is the Brunner's glands embedded in the submucosa. These produce - amongst others - mucous containing bicarbonate, which serves to neutralize the gastric acid. Furthermore, crypts of Lieberkuhn lie between the villi. Paneth cells are found in the lumen of these crypts. The Paneth cells play a role in the antimicrobial defence.

3.3 Histology of intestine

Study of histological sections of mammalian intestine

(1) Mucosa shows only crypts, there are no villi. Epithelium consists of simple columnar epithelium with numerous goblet cells. Lamina propria possess connective tissue with many crypts. Cells of the crypts are the same as in small intestine. Lymphatic nodules are frequent. Muscularis mucosae have two layers of smooth muscle.



(2) Submucosa has no glands. There are Meissners plexus.

(3) Muscularis externa is with inner circular and outer longitudinal smooth muscle layers.

(4) The longitudinal layer is not continuous but in the form of three ribbons. There present Aucrbach's nerve plexus.

(5) Serosa covered by mesothelium. Has fat-filled pouches called appendices epiploicae.

(6) Cells lining of the crypts are

(a) Surface columnar absorptive cells.

(b) Goblet cells.

(c) Enteroendocrine cells.

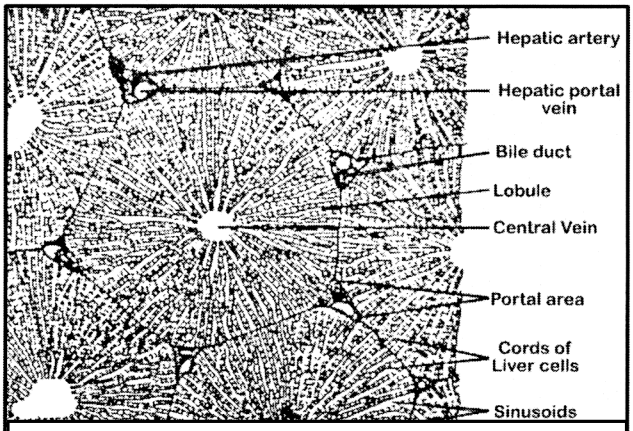
(d) Stem cells.

(e) M-cells.

3.4 Histology of liver

Study of histological sections of mammalian liver

(1) The liver histological section shows lobes with of a large number of lobules compactly held together.



Section through a part of liver lobe

(2) Polyhedral lobules are five to seven sided. Most of the cases these are hexagonal in shape. Each lobule is separated from one another by a connective tissue layer, called Glisson's capsule.

(3) An intra-lobular vein present at the centre of each lobule, known as central vein.

(4) Mostly triangular shaped portal canals are present at the junction of lobules. These portal canals consist of an inter-lobular hepatic portal vein, branches of hepatic artery, the interlobular bile ducts and lymphatic vessels.

(5) Cords of liver cells (hepatocytes) are radiating from the central vein. These cells are polyhedral with either coarsely granular cytoplasm or with vacuolated cytoplasm.

(6) Cords of liver cells are arranged in the form of branching and anastomosing plates, termed as muralium.

(7) The plates separate adjacent lacunae formed by blood containing sinusoids. Kupffer cells or stellate cells present on the walls of the sinusoids.

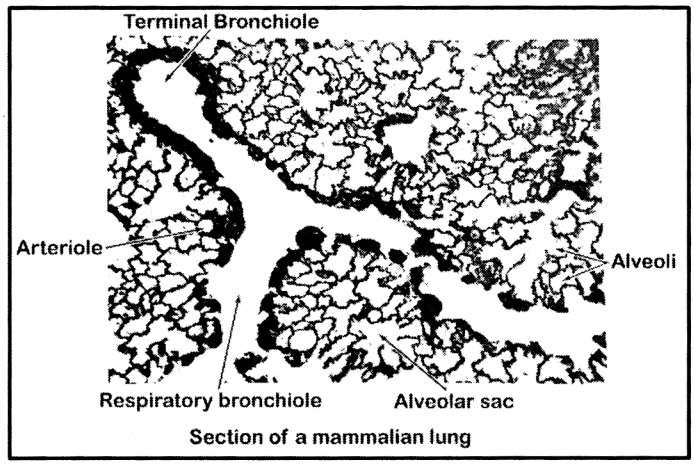
(8) Nucleus is large, round and vesicular. The number of nucleus may be one or two.

(9) The hepatic parenchyma consists of a continuous mass of cells traversed by a complex system of lacunae.

3.5 Histology of lung

Study of histological sections of mammalian lung

(1) The trachea divided into paired main bronchi. The bronchi begin a branching pattern, splitting next into lobar (secondary) bronchial branches and then again into segmental (tertiary) bronchi. The tertiary bronchi continue to divide into small bronchioles where the first change in histology takes place as cartilage is no longer present in the bronchioles. The end of the conduction portion of the lungs is at the final segment called the terminal bronchioles. The terminal bronchioles open into the respiratory bronchioles.



Section of a mammalian lung

(2) Specialized cells within the bronchioles collaborate to warm, moisturize, and remove particles that enter into the lung. These cells are the respiratory epithelium and comprise the entire respiratory tree. Most the respiratory epithelium is ciliated pseudostratified columnar epithelium. The following five types of cells are present in this region: Ciliated cells. Goblet cells, Basal cells, Brush cells and Neuroendocrine cells.

(3) There are full of empty spaces throughout the section. These spaces are called alveoli.

(4) The alveoli are enclosed by thin walls, portions of the bronchi walls, bronchioles and blood vessels.

(5) The thin wall of the alveoli consists of stroma containing anastomosing capillaries and a network of fine reticular and elastic fibres.

(6) Inter-alveolar septa bears pore for smooth diffusion.

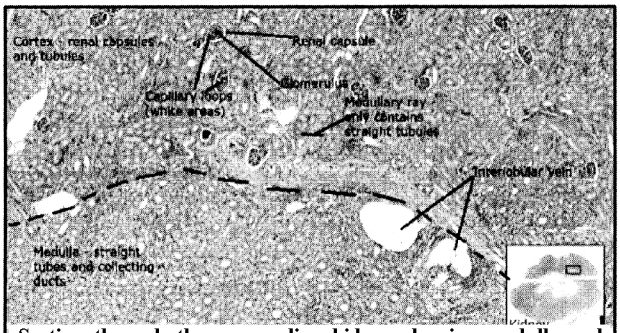
(7) The septa housed macrophages and fibrocytes.

(8) The alveoli are lined by squamosal epithelial cells.

3.6 Histology of kidney

Study of histological sections of mammalian kidney

(1) The kidneys form the first part of the urinary system and their principle function is to maintain electrolyte homeostasis and the acid-base balance.



Section through the mammalian kidney showing medulla and cortax inset transverse section of the kidney

(2) Kidney function is vital for regulating blood pressure and the kidneys are also a source of several important hormones such as erythropoietin, which regulates the production of red blood cells.

(3) Histologically, the renal parenchyma consists of four parts: glomeruli, tubules, interstitium and blood vessels.

(4) Glomeruli are complex vascular structures composed of a tuft of capillaries comprised of specialized

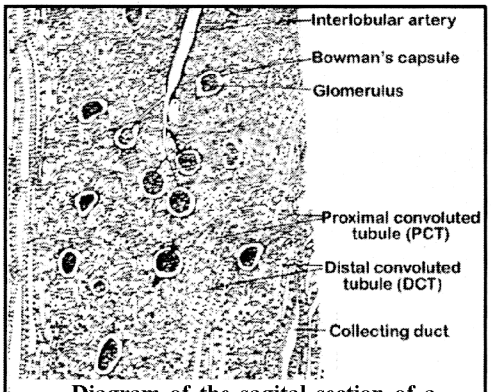


Diagram of the sagital section of a mammalian kidney

endothelial, epithelial and mesangial cells arranged around a relatively thick basement membrane. The afferent arteriole divides into 4-8 branches in the glomerulus. Each branch spread into networks of capillaries that form lobules and then rejoin the vascular pole to drain into the efferent arteriole.

(5) The tuft of capillaries lies within the lumen of the expanded proximal end of the nephron, or Bowman's space, which is lined with a layer of attenuated epithelial cells overlying a thick basement membrane. Together the epithelial cells and basement membrane comprise the Bowman's capsule.

(6) The function of the glomeruli is filtration of the blood that leads to the formation of urine.

(7) A complex tubular system begins at the urinary pole that extends to the renal papilla. The system comprises of the proximal convoluted tubule (PCT), the loop of Henle, distal convoluted tubule (DCT) and collecting duct.

(8) The PCT consists of convoluted and straight portions, lined with tall columnar cells with abundant, acidophilic cytoplasm rich in structures for active fluid transport.

(9) The loop of Henle has thin descending and thick ascending portions covered with cuboidal and columnar cells.

(10) The DCT is narrow and short and lined with low cuboidal cells.

(11) Cuboidal cells with pale acidophilic cytoplasm and central nuclei line the collecting ducts.

(12) The interstitium contains specialized interstitial cells and connective tissue elements. The larger renal blood vessels are structurally similar to those in other parts of the body.

3.7 Histology of bone

Study of histological sections of mammalian bone

(1) Bone is a strong, flexible and semi-rigid supporting tissue. It can withstand compression forces, and yet it can bend.

(2) Bone is made up of Cells and Extracellular matrix

(3) Cells of the bone are called **osteoblasts** and **osteocytes**. There are also two other cell types: **osteoprogenitor cells** and **osteoclasts**.

(4) Extracellular matrix is made up of an organic matrix (30%) containing proteoglycans, glycosaminoglycans, glycoproteins, osteonectin and osteocalcin.

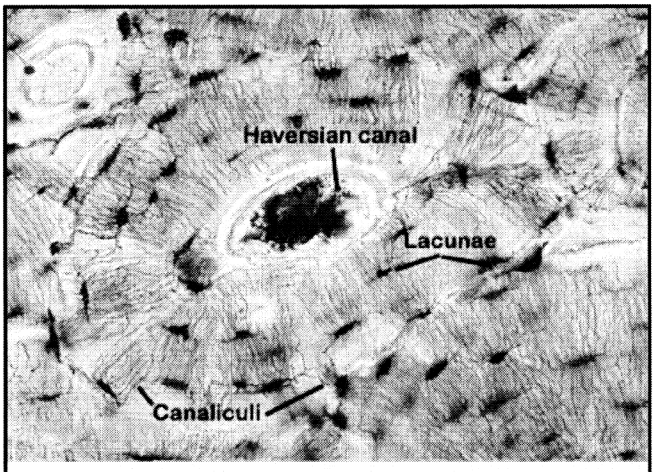
(5) There arc collagen fibres {mostly type 1 (90%), with some type V}.

(6) Only 25% of bone is water.

(7) Almost 70% of bone is made up of bone mineral called hydroxyapatite.

(8) Before the extracellular matrix is calcified, the tissue is called osteoid (bone-like) tissue.

(9) When the concentrations of calcium and phosphate ions rise high enough, they are deposited into the extracellular matrix, and the bone calcifies.



Photomicrograph thorugh the Haversian system of the mammalian bone

(10) Calcification occurs only in presence of collagen fibres - salts crystallize in the spaces between the fibres, and then accumulate around them.

(11) The bone is ensheathed by a vascular connective tissue, the periosteum.

(12) A thick wall surrounds a large central cavity containing bone marrow.

(13) The wall consists of circumferentially arranged bone lamellae and Haversian system, arranged in long axis of the bone

(14) In a Haversian system 20 to 40 lamellae lie concentrically around a central space, called Haversian canal.

(15) Bone is resistant to bending, twisting, compression and stretch. It is hard, because it is calcified, and the collagen fibres help the bone to resist tensile stresses.

3.8 Histology of cartilage

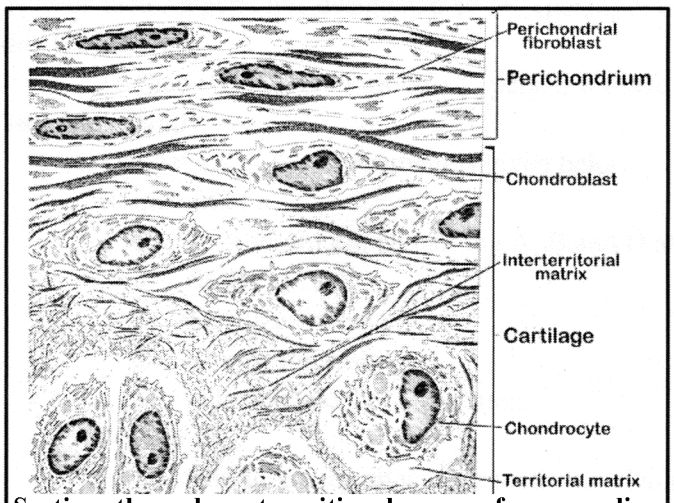
Study of histological sections of mammalian cartilage

(1) Cartilage is a connective tissue, therefore, it consists - matrix and cells

(2) In cartilage both matrix and cells undergoes a remarkable modification of structure and function. Sulphated compounds in the ground substance and a high proportion of water trapped in the ground substance allows cartilage to resist compression.

(3) The principal cell type in cartilage is the chondrocyte.

(4) Chondrocytes are surrounded completely by the matrix they produce.



Section through a transitional area of mammalian Perichondrium and Cartiage

(5) During preparation of sections the cells shrink, but the surrounding matrix remains firm. This creates the appearance of chondrocytes lying in cavities or "lacunae" in the matrix.

(6) It is to be remembered that in living cartilage there is no gap between the matrix and the cell.

(7) Three types of cartilage are found.

(a) **Hyaline cartilage:** The word hyaline means "glassy". Fresh hyaline cartilage has a translucent appearance, hence the term.

(b) **Elastic cartilage:** Due to a high proportion of elastic fibres and lamellae, this type of cartilage is yellowish in colour in the fresh state.

(c) **White fibro-cartilage:** As the name suggests, a high proportion of collagen fibres characterises this cartilage. It may be described as a transitional stage between dense connective tissue and cartilage, and indeed, blends imperceptibly with surrounding dense connective tissue.

(8) A special feature of cartilage is the absence of blood vessels in the main mass of the tissue, i.e., cartilage is avascular.

(9) Hyaline and elastic cartilage is covered by a fibrous layer called perichondrium.

(10) In hyaline and elastic cartilage the perichondrium carries the blood vessels of the cartilage.

(11) Exchange between blood and chondrocytes takes place through the matrix.

(12) The deeper layer of perichondrium contains "chondrogenic" cells which become chondrocytes as they produce matrix.

(13) White fibro-cartilage blends with the surrounding fibrous tissue, and therefore does not have perichondrium.

(14) Histological features of cartilage are best understood with the example of hyaline cartilage.

3.9 Questions

1. What is composition of renal corpuscle?
2. What are PCT and DCT? Mention their function.
3. In kidney, where renal corpuscles are mostly found?
4. What are bronchioles?
5. What are alveolar macrophages?
6. What do you mean by hepatic lobule?
7. What are the functions of hepatocytes?
8. What are villi and micro-villi?

Unit-4 □ Study of human blood group by agglutination reactions

Structure

4.0 Objectives

After studying this unit, students would be able to understand about the identification of blood group of a person for safe transfusion during emergency treatment.

4.1 Introduction

The serum of a person may cause agglutination of the RBC of another person. Depending upon the presence or absence of two agglutinogens A and B in RBC and two specific agglutinins anti-A (α) and anti-B (β) in the serum, human blood groups are designated as A, B, AB and O. An RBC may have either or both factors (A, B, AB) or none at all. A serum may have either or both factors (α, β, $\alpha\beta$) or none at all.

Similarly a person may possess Rhesus factor or not. Depending on the presence or absence of Rhesus factor a person is designated as "+" (positive) or "–" (negative) respectively.

4.2 Procedure

The details of the experiment is given below

Requirement

1. Anti serum kit, containing A, B and D antiserum
2. Slides

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3. Sterilized needle
4. Simple microscope
5. Absolute alcohol and cotton

Procedure

(a) Middle finger tip was cleaned with alcohol and pricked.

(b) Three drops of blood in three different points of a slide were taken.

(c) Three points of the drops were marked as A, B & D.

(d) Immediately poured one drop each of antiserum marked A, B & D and observed for few minutes and observed.

Observation & Conclusions

(1) 'A' and 'D' marked blood showed agglutination—the blood group is A+

(2) 'A' marked blood showed agglutination, but no agglutination in 'D'—the blood group is A–

(3) 'B' and 'D' marked blood showed agglutination—the blood group is B+

(4) 'B' marked blood showed agglutination, but no agglutination in 'D'—the blood group is B–

(5) Both 'A', 'B' and 'D' marked blood showed agglutination—the blood group is AB+

(6) Both 'A', 'B' marked blood showed agglutination, but no agglutination in 'D'—the blood group is AB–

(7) No agglutination in 'A', 'B' and 'D'—the blood group is O–

(8) No agglutination in 'A', 'B' and agglutination in 'D'—the blood group is O+

4.3 Questions

i. What is antiserum?

ii. What is the importance of blood grouping in man?

Unit-5 □ Isolation of DNA from fish blood (Demonstration)

Structure

5.0 Objectives

The aim of this study was to identify the most feasible method for the extraction of high quantity and quality DNA from fish blood. This process provides high yields of remarkably pure DNA from blood samples of fishes which is suitable for most downstream processes.

5.1 Introduction

DNA is reported to be the most appropriate molecule for the detection and identification of fish species in processed food products, offering numerous advantages over the analysis of proteins. DNA is present in all tissue types, has a greater stability at high temperatures, and the diversity afforded by the genetic code allows differentiation of closely related species. DNA extraction from blood samples is the primary requirements for the determination of genetic abnormalities, epigenetic studies and various diagnostic and preventive tests.

5.2 Procedure

Blood samples collection from the fishes

Blood samples will be freshly collected from the fish species namely *Tilapia, Channa, Labeo, Cyprinus.* etc. by puncturing the caudal vein with a micro-syringe

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or by decapitating the caudal portion and exposing the blood vessel. This fresh blood will be collected in anticoagulant EDTA tubes.

Reagents and Solutions

Blood DNA extraction kit (viz., **GenElute Blood Genomic DNA Kit, Sigma Aldrich**) may be used for isolation of genomic DNA from blood samples with some modifications.

(1) Resuspension solution

(2) Lysis solution C

(3) Column preparation solution

(4) Prewash solution

(5) Wash solution

(6) Elution solution (10 mM Tris-HCl, 0.5mM EDTA, pH 9.0)

(7) Proteinase K

(8) RNase A Solution

(9) Ethanol(95-100%)

DNA extraction procedure

The extraction procedures for fresh samples will be done in the following manner:

1. The blood of the number of fishes of same species will be collected by puncturing with a micro-syringe into the caudal vein and pooled and transferred to EDTA anticoagulant tubes.
2. The blood then equilibrated to room temperature before beginning of preparation.
3. 200µl of blood was transferred to 1.5ml micro-centrifuge tube and 20µl of Proteinase K solution was added to the tube.
4. For RNA-free genomic DNA, 20µl of RNase A solution was added.
5. 200µl of Lysis Solution C was added to the sample and vortex thoroughly for 15 seconds.

6. The sample was then incubated for at 55°C for 10 minutes in a water bath.
7. 500µl of column preparation solution was added to each pre-assembled GenElute Miniprep Binding Column and centrifuge at 12,000g for 1 minute. The flow was discarded through liquid.
8. 200µl of ethanol was added to lysatc (step 6). The solution was mixed thoroughly in vortex for 5-10 seconds.
9. Transfer entire content (of step 8) into the treated column (step 5).
10. The Column was centrifuged at 6500g for 1 minute and discarded the tube containing flow-through liquid and place the column in a new 2ml collection tube.
11. First wash of solution was done by adding 500µl of Prewash Solution to the column and centrifuge at 6500g for 1 minute.
12. For second wash, 500µl of Wash Solution was added to the column.
13. The column was centrifuged for 3 minutes at 12000g-16000g.
14. DNA was eluted by adding 200µl of the Elution Solution directly into the centre of the column.
15. The column was then centrifuged for 1 minute at 6500g. To increase elution efficiency, the column was incubated for 5 minutes at room temperature after adding the Elution Solution, then centrifuge.
16. Elute contains pure genomic DNA. The DNA can be stored for short term at 2-8°C and for long term storage, –20°C is recommended.

5.3 Summary

In this process the produced DNA is sufficiently suitable for studies of various genetic problems in fishes.

5.4 Questions

(i) Why DNA extraction is necessary from the tissues?

(ii) What will be the problem if samples from other tissues are collected for DNA extraction?

Unit-6 □ Quantitative estimation of DNA using spectrophotometer

Structure

6.0 Objectives

The aim of this study was to identify the most feasible method for the extraction of high quantity and quality DNA from fish blood. This process provides high yields of remarkably pure DNA from blood samples of fishes which is suitable for most downstream processes.

6.1 Introduction

DNA is reported to be the most appropriate molecule for the detection and identification of fish species in processed food products, offering numerous advantages over the analysis of proteins. DNA is present in all tissue types, has a greater stability at high temperatures, and the diversity afforded by the genetic code allows differentiation of closely related species. DNA extraction from blood samples is the primary requirements for the determination of genetic abnormalities, epigenetic studies and various diagnostic and preventive tests.

6.2 Procedure

Blood samples collection from the fishes

Blood samples will be freshly collected from the fish species namely *Tilapia*,

Channa, Labeo, Cyprinus, etc. by puncturing the caudal vein with a micro-syringe or by decapitating the caudal portion and exposing the blood vessel. This fresh blood will be collected in anticoagulant EDTA tubes.

Reagents and Solutions

Blood DNA extraction kit (viz., **GenElute Blood Genomic DNA Kit, Sigma Aldrich)** may be used for isolation of genomic DNA from blood samples with some modifications.

(1) Resuspension solution

(2) Lysis solution C

(3) Column preparation solution

(4) Prewash solution

(5) Wash solution

(6) Elution solution (l0mM Tris-HCl, 0.5mM EDTA, pH 9.0)

(7) Proteinase K

(8) RNase A Solution

(9) Ethanol(95-100%)

DNA extraction procedure

The extraction procedures for fresh samples will be done in the following manner:

1. The blood of the number of fishes of same species will be collected by puncturing with a micro-syringe into the caudal vein and pooled and transferred to EDTA anticoagulant tubes.
2. The blood then equilibrated to room temperature before beginning of preparation.
3. 200µl of blood was transferred to 1.5ml micro-centrifuge tube and 20µl of Proteinase K solution was added to the tube.
4. For RNA-free genomic DNA, 20µl of RNase A solution was added.
5. 200µl of Lysis Solution C was added to the sample and vortex thoroughly for 15 seconds.

6. The sample was then incubated for at 55°C for 10 minutes in a water bath.
7. 500µl of Column preparation solution was added to each pre-assembled GenElute Miniprep Binding Column and centrifuge at 12,000g for 1 minute. The flow was discarded through liquid,
8. 200µl of ethanol was added to lysate (step 6). The solution was mixed thoroughly in vortex for 5-10 seconds.
9. Transfer entire content (of step 8) into the treated column (step 5).
10. The column was centrifuged at 6500g for 1 minute and discarded the tube containing flow-through liquid and place the column in a new 2ml collection tube.
11. First wash of solution was done by adding 500µl of Prewash solution to the column and centrifuge at 6500g for 1 minute.
12. For second wash, 500µl of Wash Solution was added to the column.
13. The column was centrifuge for 3 minutes at 12000g-16000g.
14. DNA was eluted by adding 200µl of the Elution Solution directly into the centre of the column.
15. The Column was then centrifuged for 1 minute at 6500g. To increase elution efficiency, the column was incubated for 5 minutes at room temperature after adding the Elution Solution, then centrifuge.
16. Elute contains pure genomic DNA. The DNA can be stored for short term at 2–8°C and for long term storage, –20°C is recommended.

Quantification of extracted DNA by spectrophotometer

Known volumes of DNA extracts were diluted to 2 ml in double distilled water and aliquots of the diluted DNA were transferred to separate quartz cuvettes. The diluted DNA solutions were quantified and assessed for impurities by measuring the absorbance at 260 nm (A260) and 280 nm (A280) in a spectrophotometer. DNA concentrations were calculated by multiplying the A260 measurement by the dilution factor and then by 50, based on the relationship that an A260 of 1.0 equals 50 mg/ml pure DNA. DNA yields were calculated by multiplying the DNA concentration value by the final volume of DNA extracted with each method. DNA purities were determined by calculating the A260/A280 ratios. Samples calculated to have A260/A280 ratios of approximately 1.8-2.0 were assumed to be pure samples, free from protein and/or RNA contamination.

6.3 Summary

In this process the produced DNA is sufficiently suitable for studies of various genetic problems in fishes.

6.4 Questions

(i) Why DNA extraction is necessary from the tissues?

(ii) What will be the problem if samples from other tissues are collected for DNA extraction?

Unit-7 □ Quantitative estimation of RNA using orcinol reaction

Structure

7.0 Objectives

By studying this unit, students would be able to understand about estimation of the concentration of RNA by orcinol reaction.

7.1 Introduction

This is a general reaction for pentoses and depends on the formation of furfural when the pentose is heated with concentrated Hydrochloric Acid. Orcinol reacts with the furfural in the presence of Ferric Chloride as a catalyst to give a green colour, which can be measured at 665 nm.

7.2 Procedure

Requirements

1. Standard RNA solution - 200ug/ml in 1N Perchloric Acid/buffered saline.
2. Orcinol Reagent - Dissolve 0.lg of Ferric Chloride in 100 ml of concentrated HC1 and add 3.5 ml of 6% w/v orcinol in alcohol.
3. Buffered Saline - 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure

1. Pipette out 0.0, 0.2. 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.

2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 2 ml of orcinol reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortex/shaking the tubes and heat on a boiling water bath for 20 minutes.
6. Then cool the contents and record the absorbance in a spectrophotometer at 665 nm against blank.
7. Then plot the standard curve by taking concentration of RNA along X-axis and absorbance at 665 nm along Y-axis.
8. Then from this standard curve calculate the concentration of RNA in the given sample.

Observations and calculations

Volume of standard (200 Mg/ml) RNA	Volume of distilled water (ml)	Concentration of RNA (Mg)	Volume of Orcinol reagent (ml)	Incubate all test tubes in boiling water bath for 20 minutes and then cool to room temperature	Take reading in A665
0.0	1.0	00	2		
0.2	0.8	40	2		
0.4	0.6	80	2		
0.6	0.4	120	2		
0.8	0.2	160	2		
1.0	0.0	200	2		
1.0 Unknown	0.0	To be estimated	2		
Make a Standard Curve for RNA estimation by Orcinol reaction with the above data and put the data of the unknown sample, write in the result					

Result : The given unknown sample contains - ...µg RNA/ml.

7.3 Summary

RNA estimation requires expertise and sophisticated instrument handling. This method will give very precise data of RNA content of the sample.

7.4 Questions

(i) Why RNA estimation is required?

(ii) What are the advantages of Spectrophotometer over colorimeter?

Unit-8 □ Demonstration and preparation of permanent slide to demonstrate DNA, RNA and Proteins

Structure

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8.0 Objectives

By studying this unit, students would be able to understand about staining of the DNA molecule in histological slides

8.1 Introduction

Acid hydrolysis, designed to separate selectively 2 purine bases, namely adenine and guanin, from DNA molecule; staining of apurinic acid resulting from hydrolysis with Schiff reagent. This reagent can be used since free deoxyribose changes to aldehyde in acid environment. Feulgen reaction is highly selective for DNA. Moreover, this reaction allows a very precise localisation of DNA.

In this histochemical technique, the section is treated such that the substance under investigation produces a compound for which a specific test exists. Basic fuchsin is a magenta coloured dye which is rendered colourless when treated with hydrochloric acid and sodium bisulphate.

8.2 DNA by feulgen reaction procedure

Method of fixation

The tissue will be fixed using a saturated aqueous solution of Mercuric Chloride with 5% Glacial Acetic Acid. The mercurial deposits will then be removed from the tissue by treating with iodine (to convert into mercuric iodide which is soluble in alcohol) and washed in alcohol. Paraffin sections cut at 5-7 microns.

Reagents required for staining

1. N HC1 39.3ml of concentrated HC1 + distilled water up to 400ml
2. Preparation of Schiffs reagent: - 1g basic fuchsin + 1.9g Sodium metabisulphite (anhydrous) + 15ml of N HCl + distilled water. Shake periodically for first 2 hours. Add 0.5g of fresh activated charcoal, shake for 2 minutes. Filter, store in dark and refrigerate.
3. H_2SO_3, 36ml 10% Potassium metabisulphite + 30ml N HCl up to 600ml with distilled water.
4. Light Green stain - 0.25% light green in 70% alcohol.
5. Keep the stains in couplin jars filled to 40 ml and follow the staining procedure provided below.

Staining procedure

1. Bring paraffin section to water.
2. Wash in running water for 2 minutes.
3. Hydrolyse DNA by placing slides in N HCl at 60°C for 8 minutes
4. Rinse in distilled water for 1 minute.
5. Place in Schiffs reagent for $1^1/2$ hours.
6. Drain and rinse in 3 lots of sulphurous acid solution for 5 minutes each.
7. Wash in running water for 3 minutes.
8. Counter stain cytoplasm if desired in light green for 1 minute. Then wash in distilled water.
9. Dehydrate quickly through 70%, 90% and absolute alcohols.
10. Clear in Xylene, and mount in DPX.

8.2.1 Results and conclusion

Observe the slide under the microscope. DNA will take Red-purple in colour within Green background. Try a control run (i.e. section unhydrolysed).

8.2.2 Questions

(i) Why DNA staining is required in histology?

(ii) Is this procedure qualitative or quantitative estimation?

8.3 Demonstration and preparation of permanent slide to demonstrate: DNA or RNA by Methyl green-pyronine procedure (MGP)

8.3.1 Objectives

To identify DNA or RNA in a permanent slide.

8.3.2 Introduction

This is a simple method to demonstrate DNA and RNA in histological slides.

8.3.3 Procedure

The procedure is described below

Solutions required

A. 0.01 M phthalate buffer, pH 4.0

Potassium hydrogen phthalate ($KHC_8H_4O_4$) – 1.02g

Distilled Water–490ml

0.01 M HC1–about 1.0ml

Check the pH, and then add water to make volume up to 500 ml.

B. Dye mixture

Pyronine Y (Cl 45005; dye content > 90%): 0.03g

Methyl or ethyl green (Cl 42585 or 42590; must be close to 100% Dye content and free of violet impurities): 0.15g

C. Buffer pH 4.0: 100 ml

Procedure

(1) Paraffin sections of material fixed in Carnoy or aqueous formaldehyde are de-waxed and hydrated.

(2) Buffer will be used to prepare fixatives

(3) Stain in the dye mixture for 5 minutes

(4) Rinse in two changes of water, 5 seconds in each. Shake to drain off most of the water.

(5) Dehydrate by agitating vigorously in each of three changes of n-butanol. about 1 minute in each. The volume of butanol must be 50 ml for one or two slides in a coupling jar.

(6) Clear in xylene.

(7) Apply cover slips, using a resinous mounting medium.

8.3.4 Results

(a) The presence of DNA will be viewed as green or bluish-green.

(b) The presence of RNA will be viewed as bright pink red.

(c) Sulphated carbohydrates (mast cell granules, cartilage matrix, some types of mucus) are coloured metachromatically (orange) by the pyronine.

8.3.5 Questions

(i) What are the uses of staining DNA and RNA?

(ii) Why buffer is used for fixative preparation?

8.4 Demonstration and preparation of permanent slide to demonstrate: Proteins by mercurobromophenol blue

8.4.1 Objectives

Localization of total proteins in a permanent histological slide.

8.4.2 Introduction

This method is employed for the localization of total proteins for the following advantages,

(i) Even the minute quantity of proteins can be localized.

(ii) The dye binds itself to the basic proteins even when mercury is present and also with other proteins when mercury is absent.

(iii) Amount of dye bound is proportional to the quantity of proteins present.

8.4.3 Procedure

Preparation of reagent

1. **Fixative (FAA)**

   Ethyl alcohol (50% or 70%) 90ml+Acetic acid 5ml + Formalin (40%) 5ml.

2. **Stain**

   10 mg of Bromophenol Blue is dissolved in 100 ml of 10% Mercuric Chloride solution in 95% alcohol.

Staining procedure

(a) Deparaffinized sections were brought to absolute alcohol and incubated for 15 minutes in Mercuric Bromophenol Blue stain at room temperature.

(b) Sections were rinsed in 0.5% Acetic Acid for 5-10 minutes and differentiated in tap water until the sections were blue.

(c) Sections were air dried, cleared in Butanol and then Xylol and mounted with DPX.

Colour indication

Sites of proteins stain blue.

Control test for proteins: Trypsin method (Pearse, 1960)

Deparaffinized and hydrated sections were incuibated for 1 hour at 37°C in 0.05 M phosphate buffer at pH 8.9 containing 1 gm/ml of pure Trypsin. After washing the sections in water, sections were dehydrated up to 90% alcohol and stained with mercuric Bromophenol Blue. The sites of protein do not show blue colour.

8.4.4 Summary

Blue coloured portion of the tissue sections are confirmed sites of proteins.

8.4.5 Questions

(i) Why we will stain protein in a slide?

(ii) What is fixative?

Unit-9 □ Study of homology from suitable specimens/pictures

Structure

9.0 Objectives

By studying this unit, students would be able to understand about the origin of different organs having different functions

9.1 Introduction

Homologous organs are those organs which have the same basic structural design and origin but have different functions. For example: The forelimbs of human and the wings of birds and bats look different externally but their skeletal structure is similar.

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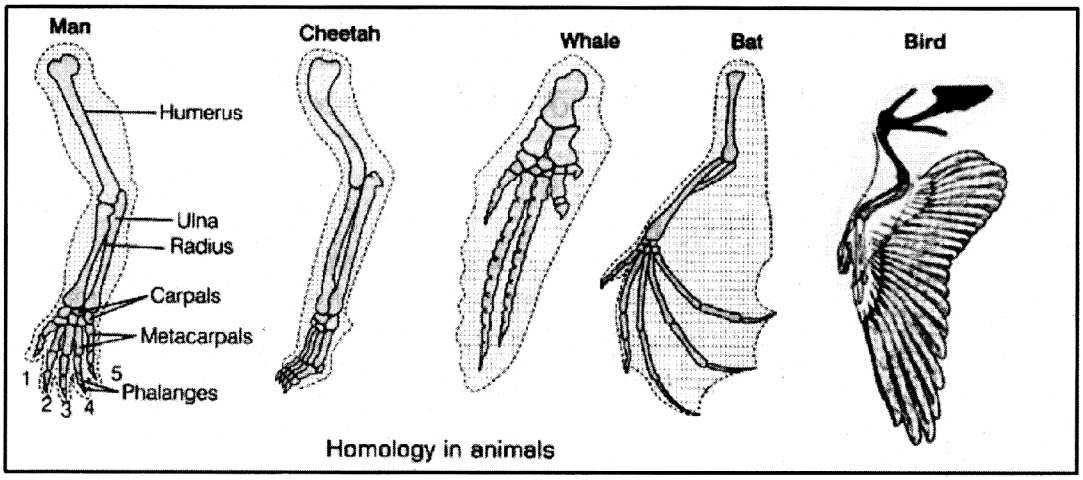
9.2 Procedure for homology study

External examination of the wings of the birds, flying mammal bat and the forelimb of a man, will not show any similarity. But on examination of the bones one by one, it can be seen that each of them has arm bone (humerus), hand bones (radius-ulna), wrist bones (carpals), palm bones (metacarpals), and fingers (phalanges). Of course, in terms of proportions of growth of each constituent bone, there are differences.



Homology in animals

For example, the fingers of bat are much longer. What this comparative study suggests is that basically the forelimbs of these two creatures are made up of the same parts, that is, they are anatomically similar.

9.2.1 Conclusions & Summary

Variations of fore limbs of animals are due to their adaptive modification suitable for living in a particular environment.

1. They differ morphologically.
2. They have similar internal structure.
3. They develop in related organisms.
4. Stages in the development are similar.
5. They perform different functions.
6. They have similar developmental pattern.
7. Homologous organs show adaptive radiation (divergent evolution).

9.2.2 Questions

(i) What is adaptive radiation?

(ii) What is divergent evolution?

9.3 Study of analogy from suitable specimens/pictures

9.3.1 Objectives

To study the origin of different organs in animals performing similar activity

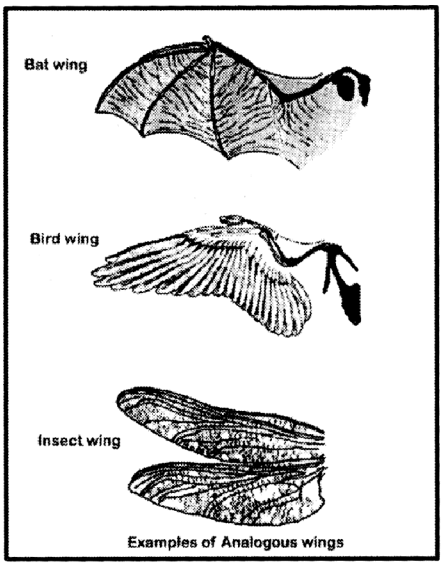
9.3.2 Introduction

Analogous structures are those structures in different species which perform the same function, have similar appearance and structure but are not evolved together; therefore, do not share a common ancestor.

9.3.3 Procedure for analogy study

On observation of the internal structure of the wings of butterfly, the shape and size it will be seen that it is membranous and is made up of thin cuticle. There are veins in the wing but there is no skeleton.

Now, observe the preserved specimen of a bat and a bird, and examine their wings. You will find skeletal support. What does this type of comparative study indicates?



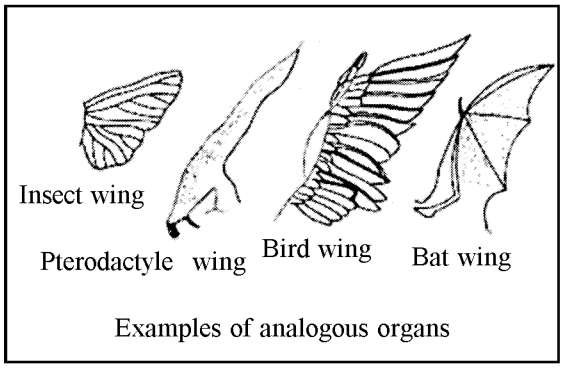
Examples of Analogous wings

It shows that the basic structures of wings of butterfly, bird and bat are different. In other words, they are anatomically different, although externally they look alike. Wings in these animals are used for flying. Such organs that differ anatomically and in embryonic mode of origin but perform similar function are said to be analogous organs.

9.3.4 Conclusions and Summary

It is evident that external morphological resemblance may not be originated from predecessors or genetic inheritance.

1. They show superficial resemblance.
2. Their internal structure is quite different.
3. They develop in unrelated organisms.
4. Stages in the development are different.



Examples of analogous organs

5. They have similar functions.
6. They have dissimilar developmental pattern.
7. Analogous organs show convergent evolution.

9.3.5 Questions

1. Why analogous organs evolve?
2. Give any other example than wing in animal world.

Unit-10 □ Phylogeny of horse with diagrams/ cut outs of limbs and teeth of horse ancestors

10.0 Objectives

By studying this unit, students would be able to understand about the pathway of evolution of *Equus* group from *Eohippus* to recent

10.1 Introduction

The phylogeny of horse is well studied with the osteological evidences. There are several items in the osteological survey available for the study. Here only limbs and teeth are considered for study of phytogeny of horse.

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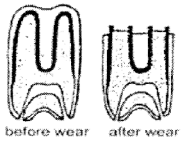
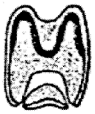
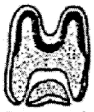
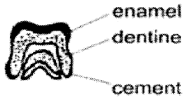
10.2 Limbs and teeth of horse ancestors

Changes of Teeth and limb structure during the evolutionary history of Modern Horse			
ERA	TEETH	FORE FOOT	HIND FOOT
Pleistocene	 ***Equus***	 One Toe, Splints of 2nd & 4th digits are visible	 One Toe, Splints of 2nd & 4th digits are visible
Pliocene	 ***Pliohippus***	 One Toe, Splints of 2nd & 4th digits are visible	 One Toe, Splints of 2nd & 4th digits are visible
Miocene	 note complete covering of cement ***Merichippus***	 Three Toes, Side toes are not touching the ground	 Three Toes, Side toes not touching the ground
Oligocene	 ***Mesohippus***	 Three Toes, Side toes touching the ground splint of 5th toe	 Three Toes, Side toes touching the ground
Eocene	 ***Eohippus***	 Four Toes	 Three Toes Splints of 1st & 5th digits
	Unknown five toed ancestor		

10.2.1 Conclusion

It is evident from the above figure that the molar teeth are becoming more complex with multiple crowns and the enamel of the teeth is organised. The size of the molars becomes much robust for perfect herbivorous habit.

The limbs are trimmed for smooth sailing through the land surface. The number of digits has been reduced from four toes to one toe in *Eohippus* to the *Equus*. This is the perfect adaptation for cursorial habit.

Therefore, it be concluded that horse is perfectly adapted for terrestrial, cursorial life both in food getting mechanism and speed adaptation.

10.2.2 Questions

(i) What are the main features of *Equus* evolution?

(ii) ''Reduction of digits helps in cursorial adaptation''—how?

10.3 Darwin's Finches with diagrams/cut outs of beaks of different species

Six finches are compared in the following table :

1.		**Warbler finch** Slender beak for catching small insects on the wing
2.		**Vegetarian tree finch** Curved parrot-like beak for crushing nuts
3.		**Insect-eating tree finch** Curved parrot-like beak for feeding on beetles and other insects
4.		**Woodpecker finch** Strong beak to pick up a stick which it pokes into trees to find insects
5.		**Cactus ground finch** Long straight beak for getting nectar out of cactus flowers
6.		**Large ground finch** Powerful blunt beak for crushing seeds

10.3.1 Objectives

Study of evolutionary changes according to the food habit for survival of group of birds in a confined area.

10.3.2 Introduction

Nature observation is a primary quality for a biologists or naturalist. One of the prominent naturalists, who recorded his observation critically, is Darwin. In this observation is the study of finches in Australia with comments of bird's evolution considered.

10.3.3 Procedure of study

Comparative chart of six specimens of finches as observed by Darwin

10.3.4 Conclusion & summary

It is evident from the above chart that the finches of an island survived with the change of their food habit. Otherwise, they may have to extinct from the earth. The reason of the extinction was limitation of food for the predecessor in a particular area.

(i) Limitation of food for the predecessor finches in Australian island.

(ii) Struggle for existence among finches.

(iii) Predecessor changed their food item.

10.3.5 Questions

(i) What are finches?

(ii) How they changed their food habits'?

(iii) What is the outcome of evolution in finches

Unit-11 □ Visit to any National Museum

Structure

11.0 Objectives

By studying this unit, students be would be able to understand about local and national heritage and scientific information.

11.1 Introduction

Visit any National Museum created for Zoological interest along with other preservations, before entering the museum, know detail of the museum by studying literatures and if possible take help of internet. A zoology student must visit zoology gallery and fossil gallery. If possible, explore other galleries of scientific and cultural interest.

11.2 Process of writing the field report

Write following information in field report book

(i) Name of the museum

(ii) Location of the museum

(iii) Date of establishment

(iv) History of inauguration

(v) Galleries visited

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(vi) Record of items observed in every gallery

(vii) Record of fossil items and their evolutionary importance

(viii) Record of stubbed zoological specimen, their occurrence and importance

(ix) Record of anthropological gallery

(x) Record of botanical gallery

(xi) Record of galleries of economical importance

(xii) Record of historical gallery

(xiii) Record of social gallery

11.3 Summary

A student after exit from the museum should be informative on how his/her past was? The demonstration of the museum personnel will be added advantage.

11.4 Questions

(i) What are the differences between stubbed and fossil specimen?

(ii) What is the National importance of the museum you visited?

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Glossary

Acini: An acinus is a round cluster of cells, usually epithelial cells, that looks somewhat like a knobby berry. The acini are lined with cuboidal/columnar epithelium that has a brush border in the lateral parts of the gland.

Adenohypophysis : A major organ of the endocrine system, the anterior pituitary (also called the adenohypophysis or pars anterior), is the glandular, anterior lobe that together with the posterior lobe (posterior pituitary, or the neurohypophysis) makes up the pituitary gland (hypophysis).

Adrenal cortex: Situated along the perimeter of the adrenal gland, the adrenal cortex mediates the stress response through the production of mineralocorticoids and glucocorticoids, such as aldosterone and cortisol, respectively. It is also a secondary site of androgen synthesis.

Adrenal medulla: The adrenal medulla, the inner part of an adrenal gland, controls hormones that initiate the flight or fight response. The main hormones secreted by the adrenal medulla include epinephrine (adrenaline) and norepinephrine (noradrenaline), which have similar functions.

Adrenaline: Adrenaline, also known as epinephrine, is a hormone, neurotransmitter, and medication. Adrenaline is normally produced by both the adrenal glands and certain neurons.

Agglutination: Agglutination is the clumping of particles. Agglutination is the process that occurs if an antigen is mixed with its corresponding antibody called isoagglutinin. This term is commonly used in blood grouping. Agglutinogens: An antigenic substance present in blood cells, bacteria, etc., which stimulates the formation of an agglutinin in blood serum.

Aldosterone: Aldosterone, the main mineralocorticoid hormone, is a steroid hormone produced by the zona glomerulosa of the adrenal cortex in the adrenal gland. It is essential for sodium conservation in the kidney, salivary glands, sweat glands and colon.

Alpha cells: The alpha cells of the islets of Langerhans produce an opposing hormone, glucagon, which releases glucose from the liver and fatty acids from fat tissue.

Androgens: Androgens are a group of hormones that play a role in male traits and reproductive activity. Present in both males and females, the principle androgens are testosterone and androstenedione.

Antiserum: Antiserum is human or nonhuman blood serum containing monoclonal or polyclonal antibodies that is used to spread passive immunity to many diseases.

Basic fuchsin: Basic Fuchsin is a dye composed of rosaniline, magenta II, pararosaniline, and new fuschsine. The fluorescent dye has been used for Ziehl-Neelson staining in order to detect acid-fast bacilli. It becomes magenta when dissolved in water; as a solid, it forms dark green crystals.

Beak: The beak, bill, and/or rostrum is an external anatomical structure of birds that is used for eating and for preening, manipulating objects, killing prey, fighting, probing for food, courtship and feeding young.

Beta cells: Beta cells (B cells) produce insulin and are the most abundant cells of the islet of Langerhans. Beta cells also secrete the hormone Amylin and called C-peptide, a by-product of insulin production.

Bromophenol Blue: Bromophenol blue is used as a pH indicator, a color marker, and a dye. It can be prepared by slowly adding excess bromine to a hot solution of phenolsulfonphthalein in glacial acetic acid.

Buffered Saline: Phosphate-buffered saline is a buffer solution commonly used in biological research. It is a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, in some formulations, potassium chloride and potassium dihydrogen phosphate. The buffer helps to maintain a constant pH.

Butanol: Butanol is a four-carbon alcohol with a formula of C_4H_9OH, which occurs in five isomeric structures, from a straight-chain primary alcohol to a branched-chain tertiary alcohol; all are a butyl or isobutyl group linked to a hydroxyl group.

Carpals: The carpal bones are the eight small bones that make up the wrist (or carpus) that connects the hand to the forearm.

Catecholamine: A catecholamine is a monoamine neurotransmitter, an organic compound that has a catechol and a side-chain amine. Catechol can be either a free molecule or a substituent of a larger molecule, where it represents a 1,2-dihydroxybenzene group. Catecholamines are hormones produced by the adrenal glands.

Corpus luteum: A corpus luteum is a mass of cells that forms in an ovary and is responsible for the production of the hormone progesterone during early

pregnancy. The role of the corpus luteum depends on whether or not fertilization occurs.

Cortex: The cortex is the outermost (or superficial) layer of an organ. Organs with well-defined cortical layers include kidneys, adrenal glands, ovaries, the thymus, and portions of the brain, including the cerebral cortex, the best-known of all cortices.

Cortisol: Cortisol is a steroid hormone, in the glucocorticoid class of hormones. When used as a medication, it is known as hydrocortisone. It is produced in many animals mainly by the zona fasciculata of the adrenal cortex within the adrenal gland. It is produced in other tissues in lower quantities.

Decapitating: Decapitation is the complete separation of the head from the body.

Delta cells: The delta cells produce somatostatin, a strong inhibitor of somatotropin, insulin, and glucagon; its role in metabolic regulation is not yet clear. Somatostatin is also produced by the hypothalamus and functions to inhibit secretion of growth hormone by the pituitary gland.

Dentine: Hard dense bony tissue forming the bulk of a tooth, beneath the enamel.

Digit: A digit is one of several most distal parts of a limb, such as fingers or toes, present in many vertebrates.

DNA: Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helix carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses.

EDTA: Ethylenediaminetetraacetic acid (EDTA) is a molecule called a chelating agent. A chelating agent is a claw-like substance that can grab and stick to other molecules.

Emergency gland: Adrenal called as the gland of emergency because it releases Adrenaline hormone, which is called a emergency hormone. When adrenaline is secreted into the blood it prepares our body for action.

Enamel: Enamel is the thin outer covering of the tooth. This tough shell is the hardest tissue in the human body. Enamel covers the crown which is the part of the tooth that's visible outside of the gums. Because enamel is translucent, light can pass through it.

Evolution: Evolution is change in the heritable characteristics of biological populations over successive generations. These characteristics are the expressions of genes that are passed on from parent to offspring during reproduction

Extinction: Extinction is the termination of an organism or of a group of organisms, usually a species. The moment of extinction is generally considered to be the death of the last individual of the species, although the capacity to breed and recover may have been lost before this point.

F cells: The F (or PP) cell is located at the periphery of the islets of Langerhans and secretes pancreatic polypeptide.

FAA: Formalin-acetic acid-alcohol (FAA) and formalin-propionic acid-alcohol (FPA) are good general-purpose fixatives. Increase concentration of acetic or propionic acid induces greater tissue swelling and to counteract alcohol shrinkage. Generally, tissues are killed and hardened within 18-24 hours when treated at room temperature.

Feulgen reaction: Feulgen stain is a staining technique discovered by Robert Feulgen and used in histology to identify chromosomal material or DNA in cell specimens. It is darkly stained. It depends on acid hydrolysis of DNA; therefore fixating agents using strong acids should be avoided.

Finches: The true finches are small to medium-sized passerine birds in the family Fringillidae. Finches have stout conical bills adapted for eating seeds and often have colourful plumage.

Follicles: A follicle is a small spherical or vase-like group of cells enclosing a cavity in which some other structure grows or other material is contained. Thyroid follicles make up the thyroid gland.

Follicular cells: Follicular cells (also called thyrocytes or thyroid epithelial cells) are the major cell type in the thyroid gland and are responsible for the production and secretion of the thyroid hormones thyroxine (T4) and triiodothyronine (T3).

Food habit: The term food habit refers to why and how people eat, which foods they eat, and with whom they eat, as well as the ways people obtain, store, use, and discard food. Individual, social, cultural, religious, economic, environmental, and political factors all influence people's eating habits.

Germinal Epithelium: The germinal epithelium is the innermost layer of the testicle. Germinal epithelium is also known as the wall of the seminiferous

tubule within the testes. The cells in the epithelium are connected via tight junctions. Germinal epithelium (female) is a layer of cells covering the ovary.

Glacial Acetic acid: Acetic acid, systematically named ethanoic acid, is a colourless liquid organic compound with the chemical formula CH_3COOH. When undiluted, it is sometimes called glacial acetic acid.

Glucagon: Glucagon is a peptide hormone, produced by alpha cells of the pancreas. It works to raise the concentration of glucose and fatty acids in the bloodstream, and is considered to be the main catabolic hormone of the body. It is also used as a medication to treat a number of health conditions.

Glucocorticoids: Glucocorticoids are a class of corticosteroids, which are a class of steroid hormones. Glucocorticoids bind to the glucocorticoid receptors that are present in almost every vertebrate animal cell.

Haemin: Haemin (haemin; ferric chloride heme) is an iron-containing porphyrin with chlorine that can be formed from a haem group, such as haem b found in the haemoglobin of human blood.

Haemoglobin: Hemoglobin or haemoglobin, abbreviated Hb or Hgb, is the iron-containing oxygen-transport metalloprotein in the red blood cells of almost all vertebrates as well as the tissues of some invertebrates. Haemoglobin in blood carries oxygen from the lungs or gills to the rest of the body.

Humerus: The humerus is the long bone in the upper arm. It is located between the elbow joint and the shoulder. At the elbow, it connects primarily to the ulna, as the forearm's radial bone connects to the wrist. At the shoulder, the humerus connects to the frame of the body via the Glenoid fossa of the scapula.

Insulin: Insulin is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body.

Interstitial tissues: The interstitial compartment is composed of connective and supporting tissues within the body - called the extracellular matrix - that are situated outside the blood and lymphatic vessels and the parenchyma of organs.

Islets of Langerhans: The pancreatic islets or islets of Langerhans are the regions of the pancreas that contain its endocrine (hormone-producing) cells, discovered in 1869 by German pathological anatomist Paul Langerhans. The pancreatic islets constitute 1 to 2% of the pancreas volume and receive 10-15% of its

blood flow. The islets of Langerhans contain alpha, beta, and delta cells that produce glucagon, insulin, and somatostatin, respectively.

Leydig cells: Leydig cells, also known as interstitial cells of Leydig, are found adjacent to the seminiferous tubules in the testicle. They produce testosterone in the presence of luteinizing hormone (LH).

Limb: A limb, or extremity, is a jointed, or prehensile, appendage of the human or other animal body. In the human body, the upper and lower limbs are commonly called the arms and the legs, respectively. Arms and legs are connected to torso or trunk.

Medulla: The inner region of an organ or tissue, especially when it is distinct from the outer region or cortex (as in a kidney, an adrenal gland, or hair).

Mercuric chloride: Mercuric chloride is the chemical compound of mercury and chlorine with the formula $HgCl_2$. It is white crystalline solid and is a laboratory reagent and a molecular compound that is very toxic to humans.

MGP: Matrix gla protein (MGP) is member of a family of vitamin-K2 dependent, Gla-containing proteins. MGP has a high affinity binding to calcium ions, similar to other Gla-containing proteins. The protein acts as an inhibitor of vascular mineralization and plays a role in bone organization.

Myoid cells: A peritubular myoid cell is one of the smooth muscle cells which surround the seminiferous tubules in the testis. These cells are present in all mammals but their organisation and abundance varies between species.

Neurohypophysis: The posterior lobe of the hypophysis (pituitary gland), which stores and releases oxytocin and vasopressin produced in the hypothalamus.

Noradrenaline: Norepinephrine. also called noradrenaline or noradrenalin, is an organic chemical in the catecholamine family that functions in the brain and body as a hormone and neurotransmitter.

Orcinol: Orcinol is an organic compound with the formula CtLjCgH^OtTh.

Para-follicular cells: Parafollicular cells (also called C cells) are neuroendocrine cells in the thyroid for which the primary function is to secrete calcitonin. They are located adjacent to the thyroid follicles and reside in the connective tissue. These cells are large and have a pale stain compared with the follicular cells.

Pars distalis: The bulk of the adenohypophysis is pars distalis. That tissue is composed of winding cords of epithelial cells flanked by vascular sinusoids. Three distinct cell types are seen among epithelial cells: acidophils, basophils, and chromophobes.

Pars intermedia: Pars intermedia is the boundary between the anterior and posterior lobes of the pituitary. It contains three types of cells - basophils, chromophobes, and colloid-filled cysts. The cysts are the remainder of Rathke's pouch.

Pars nervosa: The neural lobe or posterior lobe, this region constitutes the majority of the posterior pituitary and is the storage site of oxytocin and vasopressin. Sometimes (incorrectly) considered synonymous with the posterior pituitary, the pars nervosa includes Herring bodies and pituicytes.

Phalanges: The phalanges are digital bones in the hands and feet of most vertebrates. In primates, the thumbs and big toes have two phalanges while the other digits have three phalanges. The phalanges are classed as long bones.

Proteinase K: Proteinase K is a highly active serine protease with broad cleavage specificity on native and denatured proteins.

Protoporphyrin: Protoporphyrin IX is an organic compound, specifically a porphyrin, that plays an important role in living organisms as a precursor of other critical compounds like hemoglobin and chlorophyll. It is a deeply colored solid that is not soluble in basic water. The name is often abbreviated as PPIX.

Purine bases: Purines and Pyrimidines are nitrogenous bases that make up the two different kinds of nucleotide bases in DNA and RNA. The two-carbon nitrogen ring bases (adenine and guanine) are purines, while the one-carbon nitrogen ring bases (thymine and cytosine) are pyrimidines.

Radius-ulna: The radius or radial bone is one of the two large bones of the forearm, the other being the ulna. It extends from the lateral side of the elbow to the thumb side of the wrist and runs parallel to the ulna. The radius is shorter and smaller than the ulna.

Rhesus factor: Rhesus *(Rh)* factor is an inherited protein found on the surface of red blood cells. The Rh blood group system is one of forty-five known human blood group systems. It is the second most important blood group system, after the ABO blood group system.

RNA: Ribonucleic acid is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life.

Seminiferous tubules: The seminiferous tubules are the site of the germination, maturation, and transportation of the sperm cells within the male testes. Seminiferous tubules are made up of columnar Sertoli cells surrounded by spermatogenic cells on the epithelial interior and stem cells exteriorly.

Sertoli cells: Sertoli cells are the somatic cells of the testis that are essential for testis formation and spermatogenesis. Sertoli cells facilitate the progression of germ cells to spermatozoa via direct contact and by controlling the environment milieu within the seminiferous tubules.

Spectrophotometer: The spectrophotometer is an optical instrument for measuring the intensity of light relative to wavelength. Electromagnetic energy, collected from the sample, enters the device through the aperture (yellow line) and is separated into its component wavelengths by the holographic grating.

Spermatozoa: The mature motile male sex cell of an animal, by which the ovum is fertilized, typically having a compact head and one or more long flagella for swimming. A spermatozoon joins an ovum to form a zygote.

Supra-renal gland: The adrenal glands (also known as suprarenal glands) are endocrine glands that produce a variety of hormones including adrenaline and the steroids aldosterone and cortisol. They are found above the kidneys. Each gland has an outer cortex which produces steroid hormones and an inner medulla.

Teiehmann test: Ludwig Karl Teichmann was a Polish anatomist and physician who made an enduring contribution to forensic science with his discovery of the Teichmann test for haemoglobin. Also called the Teichmann crystal, this is a test that is used on dried stains to determine whether or not blood is present. Teichmann's test (haemin crystal test) is done by converting haemoglobin to haemin crystals which is converted to salt in the presence of halogen and forms rhombic crystals.

Trypsin: Trypsin is a serine protease found in the digestive system of many vertebrates, where it hydrolyzes proteins. Trypsin is formed in the small intestine when its proenzyme form, the trypsinogen produced by the pancreas, is activated.

Tunica albuginea: The tunica albuginea is the fibrous envelope of the corpora cavernosa penis. It consists of approximately 5% elastin, an extensible tissue that is primarily made up of the amino acids glycine, valine, alanine, and proline.

Xylol: Xylene, xylol or dimethylbenzene is any one of three isomers of dimethylbenzene, or a combination thereof. With the formula $(CH_3)2C_6H_4$, each of the three compounds has a central benzene ring with two methyl groups attached at substituents.

Zona fasciculata: The zona fasciculata constitutes the middle and also the widest zone of the adrenal cortex, sitting directly beneath the zona glomerulosa. Constituent cells are organized into bundles or "fascicles". The zona fasciculata chiefly produces glucocorticoids (mainly cortisol in the human), which regulates the metabolism of glucose, especially in times of stress, it is stimulated by the hormone Adrenocorticotropic hormone (ACTH) which is released from the anterior portion of the pituitary and axised upon this adrenal gland.

Zona glomerulosa: The zona glomerulosa is the outermost layer, in which cells arranged in arcuate formations produce mineralocorticoids, mainly aldosterone.

Zona pellucida: The thick transparent membrane surrounding a mammalian ovum before implantation. The zona pellucida is an extracellular matrix composed of three glycoproteins: ZP1, ZP2, and ZP3. Receptors on the sperm plasma membrane attach to ZP3.

Zona reticularis: The zona reticularis is the innermost layer of the adrenal cortex, lying deep to the zona fasciculata and superficial to the adrenal medulla.

Zymogens: Digestive enzymes are released in inactive forms called zymogens. This is necessary to prevent the digestive enzymes from digesting the cells that produce them. In a zymogen, also called a pro-enzyme, is an inactive precursor of an enzyme, part of the protein blocks the active site of the enzyme. Cleaving off this peptide activates the enzyme.