PREFACE

With its grounding in the "guiding pillars of Access, Equity, Equality, Affordability and Accountability," the New Education Policy (NEP 2020) envisions flexible curricular structures and creative combinations for studies across disciplines. Accordingly, the UGC has revised the CBCS with a new Curriculum and Credit Framework for Undergraduate Programmes (CCFUP) to further empower the flexible choice based credit system with a multidisciplinary approach and multiple/ lateral entry-exit options. It is held that this entire exercise shall leverage the potential of higher education in three-fold ways – learner's personal enlightenment; her/his constructive public engagement; productive social contribution. Cumulatively therefore, all academic endeavours taken up under the NEP 2020 framework are aimed at synergising individual attainments towards the enhancement of our national goals.

In this epochal moment of a paradigmatic transformation in the higher education scenario, the role of an Open University is crucial, not just in terms of improving the Gross Enrolment Ratio (GER) but also in upholding the qualitative parameters. It is time to acknowledge that the implementation of the National Higher Education Qualifications Framework (NHEQF), National Credit Framework (NCrF) and its syncing with the National Skills Qualification Framework (NSQF) are best optimised in the arena of Open and Distance Learning that is truly seamless in its horizons. As one of the largest Open Universities in Eastern India that has been accredited with 'A' grade by NAAC in 2021, has ranked second among Open Universities in the NIRF in 2024, and attained the much required UGC 12B status, Netaji Subhas Open University is committed to both quantity and quality in its mission to spread higher education. It was therefore imperative upon us to embrace NEP 2020, bring in dynamic revisions to our Undergraduate syllabi, and formulate these Self Learning Materials anew. Our new offering is synchronised with the CCFUP in integrating domain specific knowledge with multidisciplinary fields, honing of skills that are relevant to each domain, enhancement of abilities, and of course deep-diving into Indian Knowledge Systems.

Self Learning Materials (SLM's) are the mainstay of Student Support Services (SSS) of an Open University. It is with a futuristic thought that we now offer our learners the choice of print or e-slm's. From our mandate of offering quality higher education in the mother tongue, and from the logistic viewpoint of balancing scholastic needs, we strive to bring out learning materials in Bengali and English. All our faculty members are constantly engaged in this academic exercise that combines subject specific academic research with educational pedagogy.We are privileged in that the expertise of academics across institutions on a national level also comes together to augment our own faculty strength in developing these learning materials. We look forward to proactive feedback from all stakeholders whose participatory zeal in the teaching-learning process based on these study materials will enable us to only get better. On the whole it has been a very challenging task, and I congratulate everyone in the preparation of these SLM's.

I wish the venture all success.

Professor Indrajit Lahiri
Vice-Chancellor

Netaji Subhas Open University

Four Year Undergraduate Degree Programme

Under National Higher Education Qualifications Framework (NHEQF) & Curriculum and Credit Framework for Undergraduate Programmes

Subject : Honours in Zoology (NZO)

Course Title : Histology, Osteology and Developmental Biology (Practical)

Code : 6CC-ZO-04

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Histology

Osteology

Developmental Biology

Histology

Unit - 1 ❑ Demonstration of paraffin block preparation of tissue

Structure

1.1 Objectives

The primary objective of this unit is to provide instructions and knowledge for paraffin embedding of fixed, histological tissue or any other biological materials. Paraffin embedded samples can, among other applications, be stained for histological analysis. Fixation of biological materials are needed before starting this protocol.

1.2 Introduction

Before going in to the details of this unit , all the learners should remember that there are some pre requisite for completion of this laboratory process. Actually unit 1,2,3 and

4 are all meant for the preparation of biological materials for microscopic study of histological and histochemical structures of any biological materials. Following are the chronological process adopted for the microscopic study of biological materials.

a) Biological sample (tissue) collection and dissection

b) Tissue fixation with the help of requisite fixatives , the fixation times depends upon the type of fixative used.

c) Dehydration of the fixed tissue with the help of graded alcohol (ethyl alcohol)

d) Cleaning of the fehydrated tissue with the help of xylene

e) Preservation of the cleaned tissue for future use with the help of clove oil or ceder wood oil.

f) Paraffine embedding and paraffin infiltration with the help of hot air oven

g) Block making with the help of paraffin

h) Block trimming for fixing the block in the microtome holder

i) Section cutting with the help of microtome (rotatary microtome generally used)

j) Section stretching and fixing in the glass slide with help of myers albumin

k) Staining of tissue section with the help of biological dye

l) Permanent slide preparation with the help of canada balsum or DPX mountant.

1.3 Paraffin block preparation of tissue.

Following are the sequential laboratory processes to complete the paraffin block making;-

1.3.1 Tissue collection/Biological material collection

Requisite tissues/materials will be collected from animal by dissecting the animal and after that tissues will be washed in the Ringers solution to clean the bloods and other unwanted materials. Then the requisite tissue is to be pieces into smaller size for better experimental result.

1.3.2 Tissue fixation

The broad objective of tissue fixation is to preserve cells and tissue components in a

‘‘life-like state’’ and to do this in such a way as to allow for the preparation of thin, stained sections. Of course during fixation and the steps that follow there are substantial changes to the composition and appearance of cell and tissue components and these are quite far removed from the ideal "life-like state". However, with care, we can produce consistent chemical and physical characteristics in tissue sections which allow patterns to be observed, morphological and chemical changes to be noted and comparisons made. Normally bouins fluid is used for generalized purposes and the fixation time is about 24 hrs.

1.3.2.1 Types of fixatives

Fixation of tissues can be achieved by chemical or physical means. Physical methods include heating, micro-waving and cryo-preservation (freeze drying). Chemical fixation is usually achieved by immersing the specimen in the fixative (immersion fixation). Fixative solutions may contain a single fixative agent dissolved in a solvent such as water or alcohol or more commonly, a buffer solution to stabilize pH. Some popular fixative solutions contain several different fixing agents in combination and are known as complex fixatives e.g. Bouins fluid.

1.3.3 Tissue dehydration

Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax. This process is commonly carried out by immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. A series of increasing concentrations is used to avoid excessive distortion of the tissue.

After fixation, dehydrate the fixed tissues in the graded ethyl alcohol in the following chronological orders:

70% EtOH (ethyl alcogol), 80% EtOH, 90% EtOH, 95% EtOH, 3x 100% EtOH(20 min each) canstore tissue in fresh 100% EtOH at -20?C.

1.3.4 Tissue cleaning

After dehydration tthe tissue must be cleaned with the help of xylene (pure) for 1 to 2 minutes and the process should be repeated for 2-3 times.

1.3.5 Paraffin infiltration

The tissue can now be infiltrated with a suitable histological wax. Although many different reagents have been evaluated and used for this purpose over many years, the paraffin wax-based histological waxes are the most popular. A typical wax is liquid at 60°C and can be infiltrated into tissue at this temperature then allowed to cool to 20°C, where it solidifies to a consistency that allows sections to be consistently cut. These waxes are mixtures of purified paraffin wax and various additives that may include resins such as styrene or polyethylene.

1.3.6 Paraffin Embedding and block making

Before sectioning, tissue samples are embedded in a material with similar mechanical properties. This step allows the tissue to be cut easily.

Now that the specimen is thoroughly infiltrated with wax, it must be formed into a "block" which can be clamped into a microtome for section cutting. This step is carried out using an "embedding centre"where a mold or paper boat (hand made embedding center) is filled with molten wax and the specimen placed into it. The specimen is very carefully orientated in the mold because its placement will determine the "plane of section", an important consideration in both diagnostic and research histology. A cassette is placed on top of the mold, topped up with more wax, and the whole thing is placed on a cold plate to solidify. When this is completed, the block with its attached cassette can be removed from the mold and is ready for microtomy.Paraffin should solidify in 30 minutes. When the wax is completely cooled and hardened (30 minutes) the paraffin block can be easily popped out of the mold; the wax blocks should not stick. If the wax cracks or the tissues are not aligned well, simply melt them again and start over.

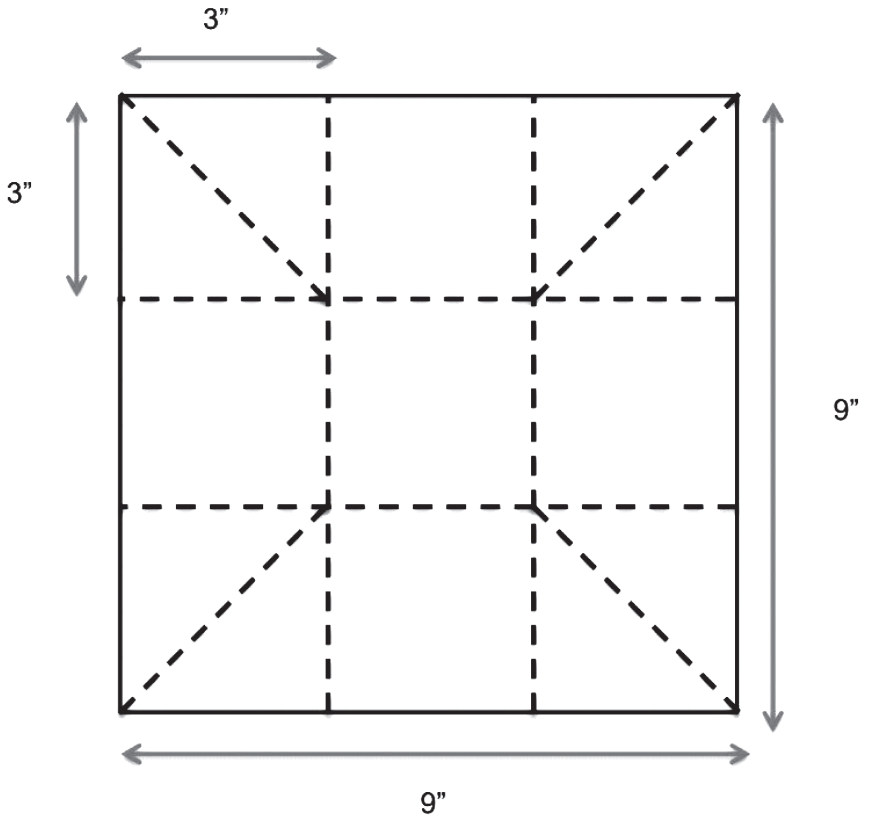


Figure: Schematic representation of paper boat making for paraffin embedding of tissue. Dotted line indicates the folding region of paper to make paper boat.

1.4 Conclusion

If a biological sample is to be sectioned with a microtome, it must be embedded in paraffin, or in plastic resin if the tissue is particularly hard.

Paraffin embedding is a standard technique used in clinical and research laboratories to create a formalin-fixed, paraffin-embedded (FFPE) block of tissue. Formalin-fixed tissue undergoes tissue processing and then is embedded in paraffin (wax) to create a FFPE block or paraffin block. The paraffin block can be cut using a microtome to generate thin sections of tissue contained in paraffin to be stained or paraffin tissue ribbons suitable for nucleic acid extraction. In addition, the FFPE blocks can be stored

at room temperature for years. After fixation in formalin or bouins fluid, tissues that are to be paraffin-embedded and are dehydrated by first using graded ethanol solutions, then graded xylene solutions, then finally liquid paraffin. The graded solutions gradually expose the sample to changes in hydrophobicity, minimizing damage to cells. After a short time in the liquid paraffin, the tissue is placed into a mold with more paraffin. The wax is allowed to solidify, forming a block that can be held in a microtome.

1.5 Summary

Embedding is the process in which the tissues or the biological samples are enclosed in a mass of the embedding medium using a mould. Since the tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatine, etc.

1.6 Glossary

Microtome: A kind of cuttimg tool used to produce a very thin sections of biological samples at the micron level.

Types of microtome: There are eight types of microtomes are now available in the market which are- Rotary microtome, Sledge microtome, Cryomicrotome, Cryostat, Ultramicrotome, Vibrating microtome, Saw microtome and Laser microtome. Rotary microtome are of two types , Spencer type and Erma type.

Embedding: Immersed in a medium coated by a medium like wax or resin or plastic.

Infiltration: Permeation of a liquid into something

1.7 Further reading

Bancroft JD, Stevens A, Turner DR (1990) Theory and practice of histological techniques. In: Gordon KC (ed) Tissue processing, 3rd edn. Churchill Livingstone, London.

Gray P (1958) Handbook of basic microtechnique, 2nd edn. McGraw-Hill, New York

Suvarna SK, Layton C, Bancroft JD (2013) Bancroft's theory and practice of histological techniques. In: Spencer LT, Bancroft JD (eds) Tissue processing, 7th edn. Churchill Livingstone-Elsevier, China

1.8 Model questions

1. What is the procedure for paraffin embedding?
2. How do you make paraffin wax blocks?
3. What are the uses of paraffin wax block?
4. What is the use of tissue block?
5. Why paraffin embedding?
6. What is the principle of embedding?

Unit - 2 ❑ Demonstration of microtome sectioning of tissue.

Structure

2.1 Objective

The main objective of this unit is to elaborate the procedure of sectioning the biological samples for cellular or histological studies through rotary microtome. In the previous unit you have learn the preparatory phase for microtome sectioning i.e the paraffin embedding and block making. In this unit you will learn how the block is prepared and trimmed for proper sectioning with the help of rotary microtome of any model.

2.2 Introduction

A microtome is a specialized precision cutting instrument, which accurately and repeatedly slices sections from a block of embedded tissue. Different kinds of microtomes are used to section paraffin and plastic embedded tissues as well as the specialized microtomes used to section frozen tissues. In any microtome a sharp knife and the tissue block are held in a fixed relation to each other. With each pass of the tissue past the knife it advances the tissue block a preset amount-the section thickness. For frozen

sections the section thickness typically ranges from 8 to 15µm, for wax sections 4-10µm, and for plastic histological sections 0.5-3µm(micro meter). In electron microscopy sections must be extremely thin, about 200× thinner than wax sections. Typically plastic sections used in transmission electron microscopy (TEM) are cut in the range 60-100 nm (nano meter).

2.3 Microtome sectioning of tissue

The quality of microtome section is strongly influenced by the steps described in the previous unit i.e fixation, dehydration,cleaning,infiltration and embedding . You should remember that of different steps fixation is utmost important because it helps to get good sections. Microtomy and Paraffin Section Preparation" is required a great training and knowledge. Following are the chronological steps for getting high quality microtome sections:

2.3.1 Microtome setup

The most important aspects of microtome set up are :-

1. High quality, sharp blades are always required for cutting good sections.
2. Blade tilt angle is always optimized for each microtome machine and blade type.
3. Blade tilt angle (which is 450) is never adjusted when conditions are changed (e.g. different microtome, new blade type, different wax etc.).
4. The leading edge of microtome knife or blade must be made thinner than the main body of the knife, in a wedge shape, to enable both cutting and physical inflexibility.
5. All microtome knives, whether resharpenable or disposable, have a wedge shaped part.
6. Thickness of section must be set up by adjustment screw before sectioning.

2.3.2 Carefully Trim Blocks

1. Blocks are carefully trimmed to expose tissue.
2. The last few sections are always cut at what will be the final thickness to polish the block face.
3. Blocks are never be roughly trimmed to save time.

4. Trimmed blocks are chilled on a cold wet surface and are always cold when cut (the surface of melting ice is excellent).

2.3.3 Block fixing with the microtome block holder

Final trimmed block is to be fixed with the block holder anf fixation must be with the axis of the block holder and it is necessary for cutting a good sections.

2.3.4 Cut Sections Slowly

1. The final sections from each block are cut gently with a uniform, slow rotation.
2. Never cut sections quickly with a rapid rotation of microtome because it will cause tissue damage and section damage.
3. Section ribbons must be 2-3 inches long which will help to stretch the sections and fixing them in the glass slides.

2.4 Conclusion

The biological sample sectioning through microtome requires skill and experience. Following are the most important factors for getting a best quality section :

1. Fixation is the most important step in performing histologic specimen preparation techniques .
2. Poorly fixed specimens are almost always more difficult to section .
3. A paraffin block will be difficult to section unless a well fixed specimen is properly processed using an appropriate schedule.
4. Where it is impossible to obtain sections from a block, it is probably due to the specimen has not been properly processed.
5. Embedding is an important step and careless embedding can make microtomy much more difficult to cut sections.
6. Locate microtome appropriately because position of the microtome on a stable bench, away from air drafts, doorways and passing staff.
7. Any air movement from air conditioners or other causes can make section handling very difficult.
8. Students must be familiar with the safety features of the microtome they are using and observe some basic rules when cutting sections.

9. The knife or blade should be removed from the microtome when the instrument is left unattended or when cleaning the instrument.
10. Generally a slow, uniform cutting stroke of microtome produces the best results.

2.5 Summary

Sectioning is accomplished by using a cutting apparatus called a rotary microtome, is a tool used to cut extremely thin slices of material, known as sections. The microtome will drive a knife (generally steel knife is used in rotay microtome) across the surface of the paraffin cube and produce a series of thin sections of very precise thickness. The objective is to produce a continuous ‘‘ribbon’’ of sections adhering to one another by their leading & trailing edges. The thickness of the sections can be preset, and a thickness between 5 - 10?m is optimal for viewing with a light microscope.

2.6 Glossary

Rotary microtome : This is the most commonly used microtome in routine laboratory. The cutting blade is kept in horizontal position, and the block containing tissue moves up and down with the help of rotatory handle attached with the microtome.

2.7 Further reading

Gray P (1958) Handbook of basic microtechnique, 2nd edn. McGraw-Hill, New York

Dey P. (2018) Basic and Advanced Laboratory Techniques in Histopathology and Cytology, Springer Nature Singapore Pte Ltd.

2.8 Model questions

1. What is tilt angle of knife or blade?
2. What do you mean by trimming?
3. Define embedding?
4. What is fixative?
5. What is difference between preservative and fixative?

Unit - 3 ❑ Stretching of tissue sections and fixing on glass slide.

Structure

3.1 Objective

In this unit students will learn the process by which microtome cut ribbons of sections of any biological materials will be stretched or flattened so that the sections will be affixed in the glass slides in chronolofical orders and will be stained by the required biological dyes.

3.2 Introduction

A microtome is a specialized precision cutting instrument, which accurately and repeatedly slices sections from a block of embedded tissue forming a ribbons of sections. The knife is usually tilted at 0 - 15"angulation on a microtome to allow a clearance angle between the cutting facet and the tissue block which will help to form a good ribbons of sections. Sections are removed in ribbons of ten, to allow easy location of serial sections.

3.3 Stretching or flattening of tissue sections and fixing on glass slide.

After completion of cutting all the ribbons of sections containing tissues are collected on a paper box which must be chilled so that no ribbons will be adhared with the paper

box. After that ribbons will be transfered either on flotation bath or on the glass slide laden by Mayers albumin containing some drops of water. The sections are then floated out on a water bath or glass slide containing drops of water set at 45 - 50°C, approximately 6 - 10°C lower than the melting point of the wax used for embedding the tissue. This is to flatten or stretching the sections and prepare them for mounting into the slider.

3.3.1 Materials required

Following materials are required to flatten and affix the sections on a glass slides :

a) Some good ribbons of sections which are generally cut between 6 - 10 micron thickness.

b) A paper box with smooth surface

c) Distilled water

d) one forcep

e) One needle

f) One blade

g) Some clean glass slides

h) One hot plate

i) One dropper

j) Myers albumin solution (Normally it is prepared in the laboratory by mixing 50 ml egg white or albumin, Glycerol 50 ml and Sodium salicylate 1gm)

k) One piece soft clean cloth for cleaning the slides

3.3.2 Process of stretching or flattening the sections

This is a process which needs experience, skill and patience because this process will enable us to get good sections for further microscopical studies. Following are process and precautionary measure to be taken.

1. First thing is to monitor the temperature carefully. The temperature will need to be 5 - 9 ?C below the melting point of the wax.

2. Then make sure the water which will be used is clean and free of bubbles and section waste (to avoid cross-contamination).

3. Take some grease free slides which will be covered with very thin layer of Mayers albumin which will act as adhesive for section adherence.
4. Then put a ribbon of sections containing 8-10 sections with the help of forcep on the Mayers albumin laden glass slide.
5. Pour some drops of distilled water with the help of dropper so that section can float easily but not move out of slides.
6. Put this section containing slides on the hot plate which is preset at 500 C teperature.
7. Leave the section on the water surface just long enough for it to flatten.
8. Always remember that over-expansion can spoil the morphology in susceptible sections.
9. When the floating sections are transformed from milky white to water transparent then you can sure the section flattening is complete.
10. Now took the slides and cut the sections containing ribbon with the help of blade and orient the sections on the sile in desirable manner.
11. Now take the slides with stretched or flattened sections and water and make efficient drainage of remnant water on the glass slide and carefully handle this process to prevent the section slipping down the slide,
12. Drain excess water from beneath the section before drying. This is vital if slides are dried flat on a hot plate.
13. Generally drying temperatures should not exceed 65 ?C.
14. Remember excessive heat can cause droplets of water underneath a section to boil and this will cause damage.
15. Dry sections for between 10 and 30 minutes.
16. When the water will completely dry the sections will automatically fixed or adhared with the Mayers albumin coated slides.

3.4 Conclusion

Trimming is an important phase which is required when the block has not been cut before to remove the top layer of wax and expose the actual tissue before cutting the

actual sections. Before stretching and afixing the sections on the slide mark the specimen side of the slide with pencil to make it clear which side to pick up the section on.

3.5 Summary

Creating great paraffin sections using a rotary microtome takes a great deal of skill and experience. ‘‘Microtomy and Paraffin Section Preparation’’ is a great training aid for new microtomists. The paraffin-embedded tissue block is sectioned to obtain the thin slices of the tissue specimen which is further stained (which will be discussed in the next unit) using suitable staining techniques and mounted to preserve the stained section for future reference.

3.6 Glossary

Block holder - where the tissue is held in position.

3.7 Further reading

Gray P (1958) Handbook of basic microtechnique, 2nd edn. McGraw-Hill, New York

Dey P. (2018) Basic and Advanced Laboratory Techniques in Histopathology and Cytology, Springer Nature Singapore Pte Ltd.

3.8 Model questions

1. What do you mean by flattening or stretching of sections?

Unit - 4 ❑ Haematoxylin and Eosin staining of mammalian tissue sections

Structure

4.1 Objective

In this unit learners will learn and able to develop their skill regarding the process of biological staining. Normally hematoxylin and eosin stains are used to study the tissue and cell structure. The Hematoxylin & Eosin procedure stains the nucleus and cytoplasm contrasting colors to readily differentiate cellular components. However, staining results are dependent on proper specimen processing, which involves tissue preservation, dehydration, clearing, and paraffin infiltration.

4.2 Introduction

The use of Hematoxylin and Eosin (H&E) is by far preferred for viewing cellular and tissue structure detail by pathologists. Because this stain demonstrates such a broad range of cytoplasmic, nuclear, and extracellular matrix features. We continue to use this simple and essential stain still today, which has remained unchanged for well over a century.

4.3 Haematoxylin and Eosin staining of mammalian tissue sections

Hematoxylin and Eosin stains are used in many areas of the histology laboratory, including frozen sections, fine needle aspirates, and paraffin fixed embedded tissues.

Hematoxylins are typically classified by the mordant used before staining. Mordants strengthen the positive ionic charge of the hematin. Mordants aids the bonding of the hematin to the anionic tissue component, which is most commonly chromatin. The type of mordant also influences the final color of the stained components. The most common mordant used in routine histology is aluminum ammonium sulfate (alum). This mordant causes the nuclei to be red in color, which is then changed to the more familiar blue color when the sample is later rinsed with a weakly basic solution.

There are several types of hematoxylin stains sre used like Mayer's hematoxylin (a water-based stain) , Harris hematoxylin (an alcohol-based stain) , Iron alum hematoxylin (a water based stain) etc. Similarly Eosin Y is the most commonly used form of eosin and may be used in both water and alcohol medium.In this regard one important point is to be remembered that the water which will be used for water wash of tissue sections after hematoxylin stain shoul be basic or alkaline in pH because it will help to change the hematoxylin from red to the traditional blue color.

4.3.1. Haematoxylin and Eosin staining protocol

Following is the baseline protocol and this should be follwed chronological order as written:

1. First sections affixed slides are to be selected for dewaxing and rehydrating
2. Slides are to be immersed in xylene for 5 min in a staining bottle
3. Then transfer the slides from xylene to 100% ethanol for 5 min
4. After that the slides are removed and then immersed in 90% alcohol for 5 min.
5. Now the slides are to be removed and then immersed in 70% alcohol for 5 min.
6. After that the slides are to be immersed in 50% alcohol for 5 min
7. Now the slides are to be immersed in 30% and then in water for 5 min.
8. It is now the time for hematoxylin staining because we have selected water medium hematoxylin or Iron alum hematoxylin. Staining time is 2-3 min.
9. Now remove the slides from hematoxylin stain and wash it in water for 2-3 times.

10. After that slides are to be again dehydrated through 30% to 90% alcohol in chronological order for eosin staining (because eosin is in 90% alcohol).
11. After remiving the slides from 90% alcohol are to be immersed in eosin solution for 2 min.
12. Now slides are to immersed in 100% ethanol for 5 min.
13. Now the slides are transferred into the xylene for 2 min.
14. After completion of this step the slides are to mounted by cover glass with the help of DPX or Canada Balsum mountant.
15. Now the mounted slides are to be transferred on the hot plate for drying.

(Important Point : The use of clean and fresh dewaxing reagents is essential for the removal of paraffin from the slide prior to the addition of the dyes.)

4.4 Conclusion

The Hematoxylin & Eosin stain provides a comprehensive picture of the microanatomy of organs and tissues. Hematoxylin precisely stains nuclear components, including heterochromatin and nucleoli, while eosin stains cytoplasmic components including collagen and elastic fibers, muscle fibers and red blood cells. The basic chronological steps in performing an Hematoxylin & Eosin stain are :

Remove the Wax, Hydrate the Sections, Apply the Hematoxylin stain, Differentiate the hematoxylin stain by rinsing in tap water (alkaline pH), Remove Excess background stain by rinsing 2-3 times in water, Apply the Eosin Counterstain, and at last Rinse, Dehydrate, Clear and Mount (Apply Cover Glass).

4.5 Summary

Hope all of you have understand the protocol and now you have to remember that hematoxylin is a reasonably simple dye to make. The dye itself is extracted from the tree Haematoxylum campechianum. Oxidation of the hematoxylin produces hematein, which is the actual dye used in an H&E stain. Addition of the mordant improves the ability of the hematein to attach to the anionic (negatively charged) components of the tissues. Always try to use freshly prepared solvents and staining reagents which gives the better result.

4.6 Glossary

Mordant: A mordant is a substance used to set or stabilize stains or dyes.

Routine Staining: that stains the different tissues with little differentiation except between nucleus and cytoplasm e.g. hematoxylin and eosin

Types of Hematoxylin: Hematoxylin solutions can be classified according to which mordant is used e.g. Alum hematoxylin , Iron hematoxylin, Tungsten hematoxylin, Molybdenum hematoxylin, Lead hematoxylin and hematoxylin without mordant.

4.7 Further reading

Gray P (1958) Handbook of basic microtechnique, 2nd edn. McGraw-Hill, New York

4.8 Model questions

1. What is the source of hematoxylin stain ?
2. Why alkaline water is needed to wash the hematoxylin stained tissue?
3. Describe the classification of hematoxylin stain.
4. What do you mean by progressive staining ?
5. What do you mean by dewaxing ?

Unit - 5 ❑ Study of permanent slides of mammalian endocrine glands - pituitary, thyroid, pancreas, adrenal, testes and ovary

Structure

5.1 Objective

In this unit students will learn how the permanent slides made from hematoxylin & eosin stain looks like and also the different characteristic features present in the different endocrine glands of mammalian system. Not only that other objective of this unit is to help the students to learn the process of identification of different mammalian endocrine glands by observing the histological structures.

5.2 Introduction

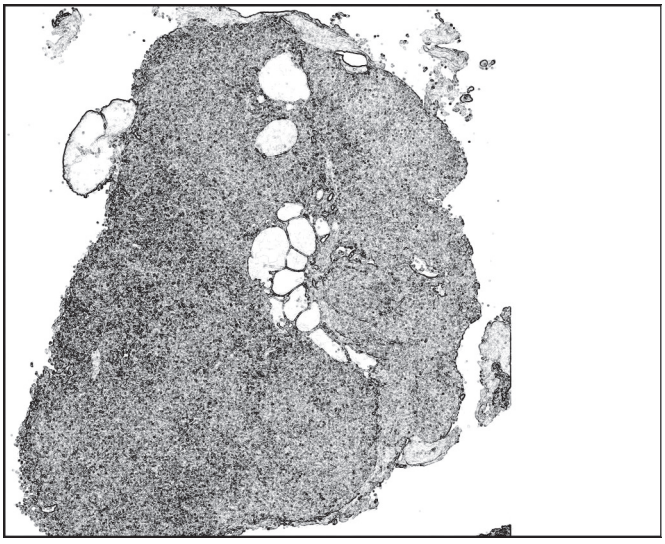
Endocrine glands are those glands which secretes the hormones and controls all most all the physiological and biochemical functions of the animal systems. In mammalian system there are nine endocrine glands present and of them some are single and others are more than one. The mammalian endocrine glands are Pituitary gland (Single), Pineal gland (Single), Thyroid gland (Single), Parathyroid gland (Four),

Thymus (Single), Adrenal gland (Two), Islets of Langerhans within the Pancreas (Multiple in number), Ovary (Two) and Testis (Two).

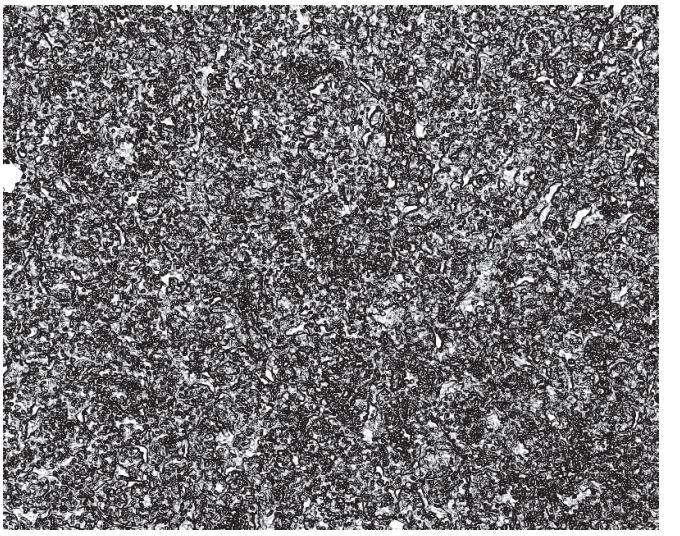
5.3 Study of permanent slides of mammalian endocrine glands

Study of sections of pituitary gland :

1. The section shows two parts one anterior and one posterior.
2. Anterior part is known as adenohypophysis and posterior part is know as neurohypophysis.
3. Along the posterior part of the the anterior lobe there is a narrow region called the pars intermedia.
4. Sections of anterior part shows two types of chromophils called acidophils and basophils.
5. There are two types of acidophil cells found which are Somatotrophs and Mammotrophs.
6. Similarly three types of basophil cells are found which are Corticotrophs, Thyrotrophs and Gonadotrophs.
7. The posterior part looks very different to the anterior part and contains non-myelinated axons which are the neurosecretory cells.



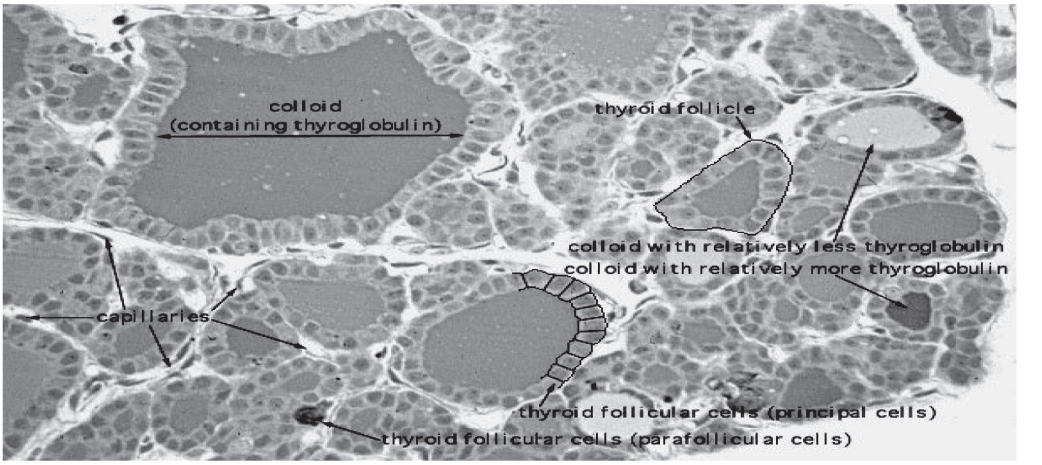
Sections of mammalian pituitary gland under low magnification showing two region



Sections of mammalian pituitary gland under high magnification showing anterior region

Study of sections of thyroid gland :

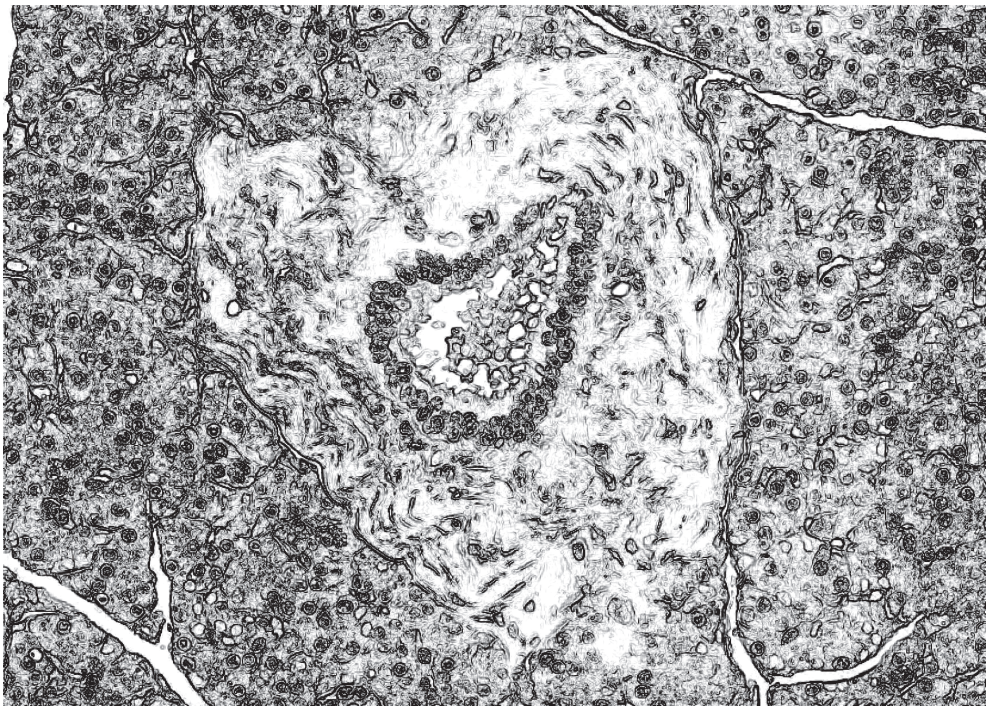
1. The section consists of innumerable number of follicles or acinus
2. Each follicle consists of an epithelium enclosing a cavity called follicular cavity
3. The cavity is filled up with viscous fluid called colloid.
4. Interfollicular space are filled up with connective tissue,adipose tissue and blood vessels.
5. The follicular cells shows basal round nucleus and apical tips of the cells extends microvilli in the cavity.
6. There are some parafollicular cells also present either singly or in group.
7. Central colloid is actually thyroid colloid which is composed of nucleoprotein, thyroglobulin and proteolytic enzymes.
8. The follicular cells are of different shapes.



Sections of mammalian thyroid gland under high magnification

Study of sections of pancreas gland : (Mixed gland)

1. Sections of the material reveal two different types of parenchymal tissue.
2. Light-stained clusters of cells are called islets of Langerhans.
3. The dark-stained cells form acini that are connected to ducts and known as acinar cells.
4. Acinar cells belong to the exocrine pancreas .
5. Light-stained clusters consists of four main cell types which are ? cellsm, ? cells, Delta cells and PP cells or gamma cells.
6. Islets of Langerhans cells arranged in clusters and cords and are crisscrossed by a dense network of capillaries.
7. Most islets of Langerhans cells are in direct contact with blood vessels, either by cytoplasmic processes or by direct apposition.
8. Surrounding the Islet is a thin layer of connective tissue capsule, which provides a slight barrier between the endocrine and exocrine cells.

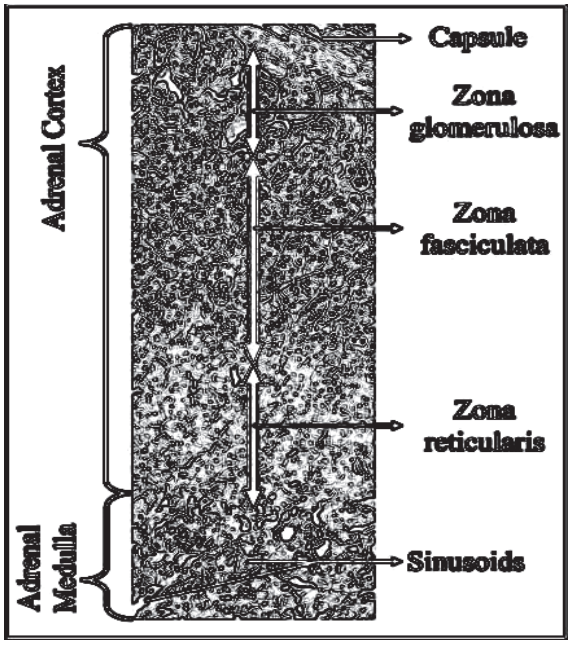


Sections of mammalian pancreas under high magnification

Study of sections of mammalian adrenal gland :

1. The section shows that the gland is encased in a connective tissue capsule that extends septae into the substance of the gland.
2. The most distinctive feature of the section is its partitioning into cortex and medulla.
3. Cells in the cortex region are arranged into three concentric zones which are thin, outermost zone- the zona glomerulosa , thick, middle zone -zona fasiculata, and thin, inner zone -zona reticularis .
4. Cells within zona glomerulosa tend to be columnar in shape and are arranged in irregular cords.
5. The zona fasiculata is the middle and largest of the three zones in the cortex.
6. Cells in the fasiculata zone are polyhedral and usually have a foamy appearance due to abundant lipid droplets.

7. In this zone the cells are arranged in distinctively straight cords that radiate toward the medulla.
8. The innermost zone of the cortex is the zona reticularis.
9. Cells within this zone are arranged in cords that project in many different directions and anastomose with one another.

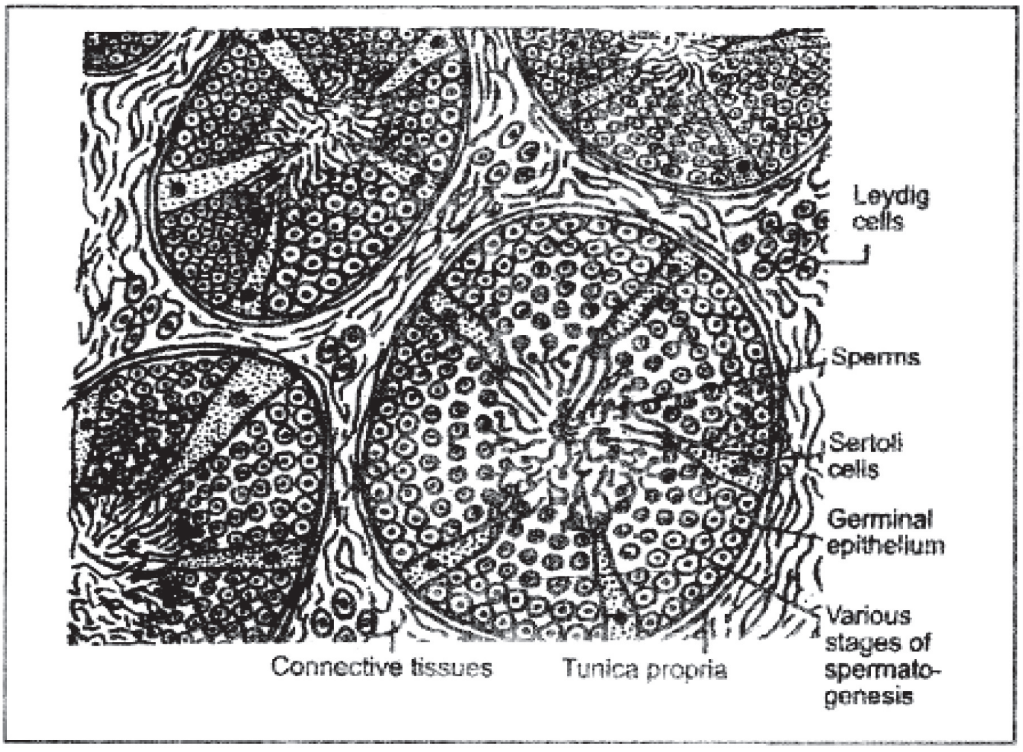


Sections of mammalian adrenal under high magnification

Study of sections of mammalian testis :

1. The section shows that it consists of 200-300 tubules which are known as seminiferous tubule.
2. The lining epithelium of seminiferous tubules undergoes mitotic proliferation forming spermatogonia.
3. The spermatogonia persists as such along the inner surface of the basement membrane.
4. Sertoli cells are slender, pillar like cells with large pale staining nucleus which are separated from one another by densely crowded spermatogenic cells.

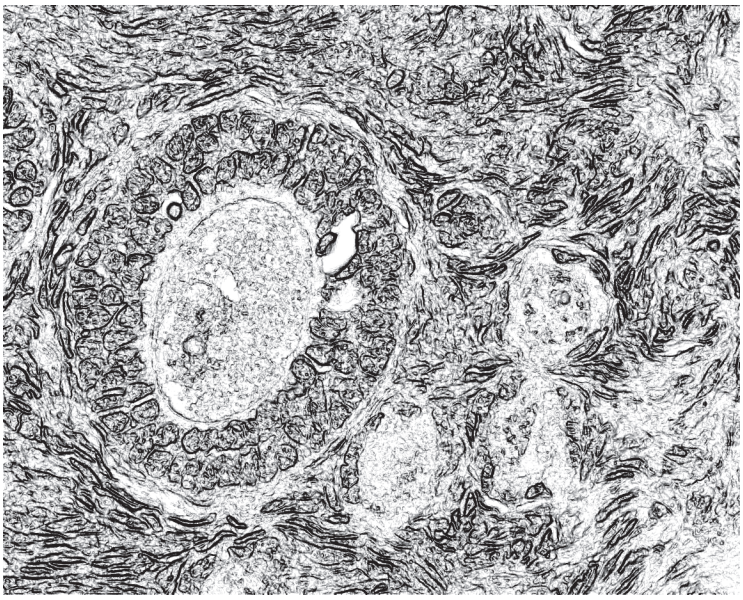
5. The nucleus of the primary spermatocytes stains deeply with distinctive chromatin pattern.
6. Inner to those primary spermatocytes are secondary spermatocyte with half the intensity of staining of the nuclear chromatin.
7. There are even smaller cells, the spermatids with spherical or elongated condensed nuclei which are nearer to the lumen.
8. There are number of spermatozoa which are seen attached to sertoli cells.
9. There are connective tissue containing blood vessels, nerves, lymph vessels and groups of interstial cells which are known as cells of Leydig are present in between seminiferous tubules.



Sections of mammalian testis under high magnification

Study of sections of mammalian ovary :

1. The surface of the section is covered by simple cuboidal epithelium.
2. Beneath the epidermal cover, occurs spindle shaped fibroblasts like stromal cells.
3. Between the stromal cells numerous fine collagenous fibers are arranged parallel to the surface to form the tunica albuginea.
4. The highly vascular medulla is relatively small with loosely arranged connective tissues, some elastic fibers and some smooth muscles.
5. Many primary follicles are present in the peripheral cortex forming a thick layer beneath the tunica albuginea.
6. Some of the follicles are primordial follicle, each of which has large central cell surrounded by a single layer of squamous follicular epithelium, i.e., granulosa cell.
7. One of a very few maturing follicle enlarge with large eccentric cell in the antrum and is supported by cumulus oophorus.
8. The mature follicle appears to bulge from the surface, this mature follicle is termed Graafian follicle.
9. Enlarged ruptured follicles contain lutein cells surrounding a blood clot and forms corpus luteum.
10. Degenerated or atretic follicle shows disorganization of the follicular cells with the sign of nuclear necrosis as well as shrinkage or distortion of the central cell, i.e., the oocyte and/or its nucleus.



Sections of mammalian ovary under high magnification

5.4 Conclusion

From the above microscopical studies of the sections of different endocrine glands in mammalian system it is clear that how hematoxylin and eosin stain can help histological studies in both normal and pathological states. It is also clear that there are some kinds of structural similarities lies in between mammalian and othe vertebrate animal system.

5.5 Summary

Endocrine tissue is made up of cells that produce secretions which are poured directly into blood. Endocrine cells lie in close apposition to blood capillaries or sinusoids. Secretions of endocrine cells are called hormones. Hormones travel through blood to target cells and influence their function.

5.5 Glossary

Suprarenal gland- It is the synonym of adrenal gland

Glands- Glands produce and secrete hormones that the body uses for a wide range of functions.

Hormones- Made by endocrine glands, hormones are chemical messengers.

Mixed gland- Functions as both exocrine and endocrine gland like pancreas.

5.7 Further reading

Smith, P.E. (1940) Bailey's Textbook of Histology, 10 th edit. Williams and Wilkins, Baltimore.

Cowdry, E. V. A (1938) Textbook of Histology. 2nd edit. Lea and Febiger, Philadelphia,.

Schafer, E. S. (1934) The Essentials of Histology. 13th edit. Lea and Febiger, Philadelphia.

Maximow, A., and Bloom, W. A (1938) Textbook of Histology. 3rd edit. Saunders, Philadelphia.

5.8 Model questions

1. How many endocrine glands are present in mammalian system?
2. How could you identify adenohypophysis of pituitary gland?
3. Name two hormones released from ovary.

Unit - 6 ❑ Study of permanent slides of mammalian organs - duodenum, intestine, liver, lung, kidney

Structure

6.1 Objective

In this unit students will learn how the permanent slides made from hematoxylin & eosin stain looks like and also the different characteristic features present in the different important organs.In this unit students will learn the important characters for the identification of histological structures present in different organs which are not glandular tissue.

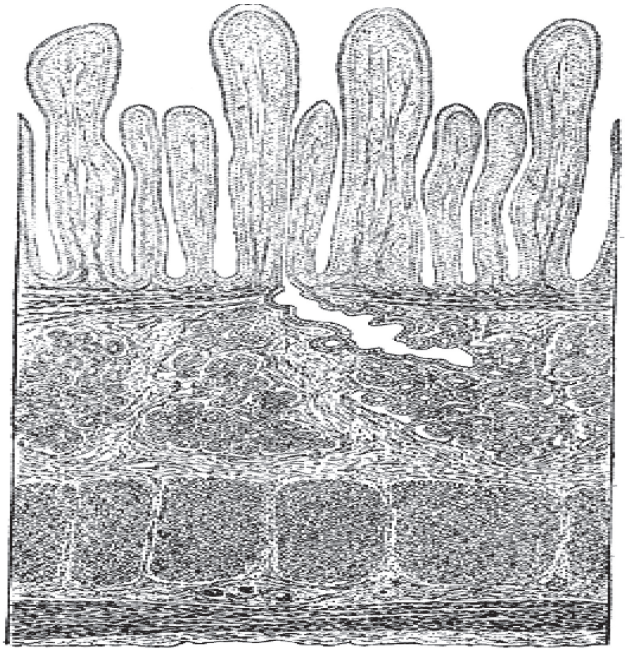
6.2 Introduction

Alimetary tract or gut,lung .liver and kidney are the most important organs inresponse to their functional attributes e.g. gut plays an important role in digestion and absorption of food materials ,lung plays the primary role respiration, liver plays a role in detoxification of body and kidney plays an important role in excretion. Therefore it is necessary to know their structural peculiarities.

6.3 Study of permanent slides of mammalian organs

Study of sections of mammalian duodenum :

1. Presence of Brunner's glands in the submucosa.
2. These glands are diagnostic of the duodenum.
3. The section shows the inner circular and outer longitudinal layers of smooth muscle in the muscularis externa.
4. Presence of the muscularis mucosae , which is often disrupted by Brunner's glands.
5. The section also shows the large broad villi of the mucosa.
6. The core of the villus is an extension of the lamina propria and also contains a blind ending lymphatic capillary, the lacteal.
7. The section shows the presence of goblet cells and the absorptive enterocytes with apical microvilli.
8. Paneth cells are observed at the base of the crypts but are not very well preserved.
9. Auerbach's (myenteric) plexi are also present between the layers of the muscularis externa.



Section of the mammalian duodenum in high magnification

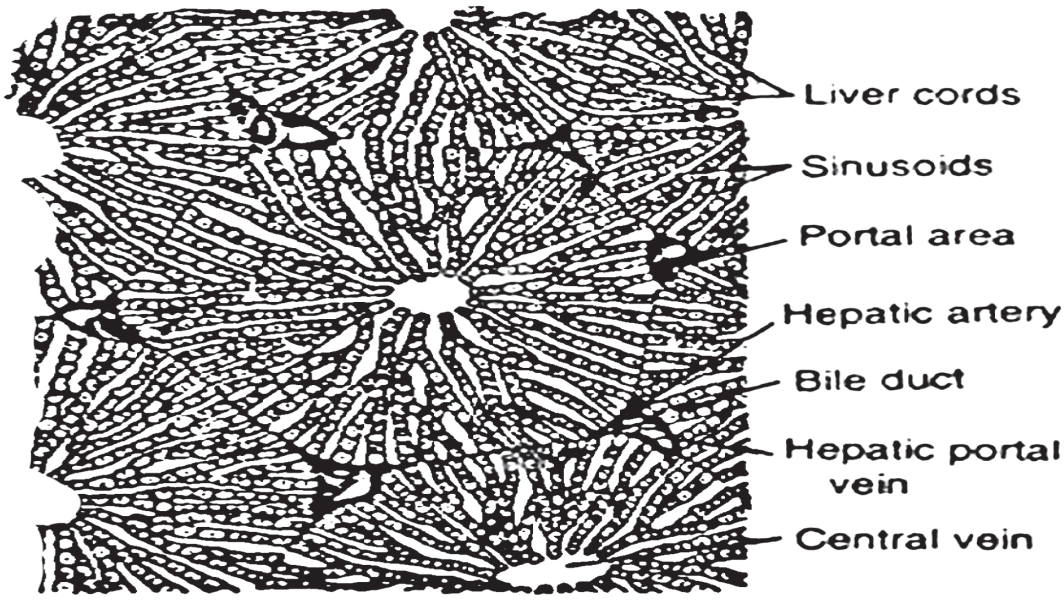
Study of sections of mammalian intestine :

1. The section shows four tissue layers:
2. Presence of serosa which is the outermost layer of the intestine and it is a smooth membrane consisting of a thin layer of cells .
3. The muscularis is a region of muscle adjacent to the submucosa membrane and it usually has two distinct layers of smooth muscle: circular and longitudinal.
4. The third layer is the submucosa is dense, consists of irregular connective tissue or loose connective tissue that supports the mucosa.
5. Presence of mucosa which is the innermost tissue layer and have a mucous membrane that secretes digestive enzymes and hormones.
6. The section also shows the presence of villi which are the part of the mucosa.
7. The section also shows the presence of Peyer's patches and these are actually the organized lymph nodes.

Section of the mammalian small intestine in high magnification

Study of sections of mammalian liver:

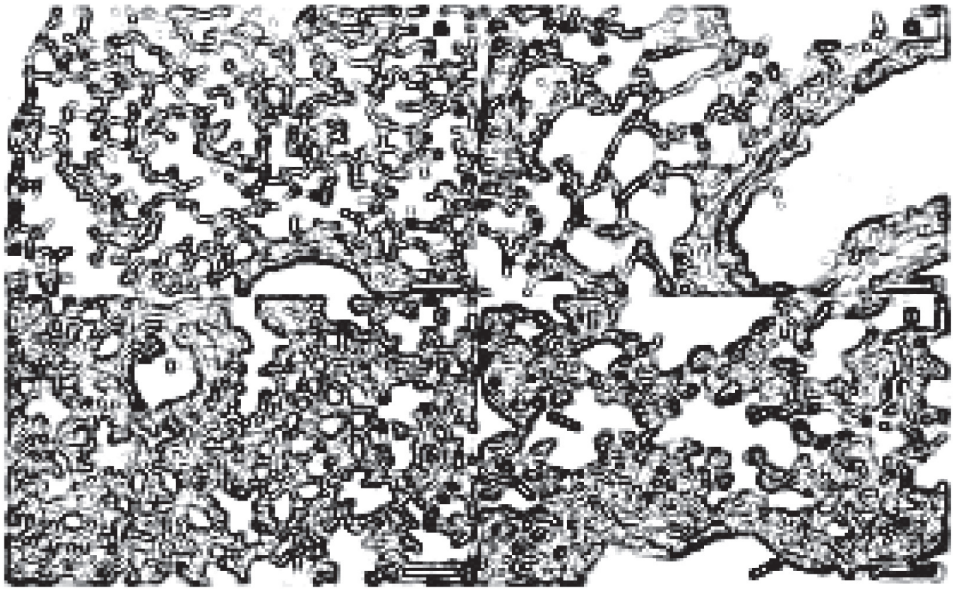
1. The outer surface of the section is composed of a fibrous tissue capsule.
2. The outermost surface is covered by a thin layer of mesothelial cells that arises from the peritoneum.
3. The section contain the acinus and the lobule.
4. The acinus is a unit that contains a small portal tract at the center and terminal hepatic venules at the periphery.
5. It is the smallest functional unit of the liver.
6. Secretory units called lobules are present, each lobules are hexagonal in shape.
7. Each lobule contains cord of polyhedral cells that converges on the central vein.
8. The hepatic cords are separated by slit like sinusoids, which have some phagocytic cells called the kupffer cells.
9. The polyhedral cells constitutes the trabeculae which have exocrine functions.
10. Peripheral to the lobules lies the arteries, veins, ductules and the bile duct.



Sections of the mammalian liver

Study of sections of mammalian lung:

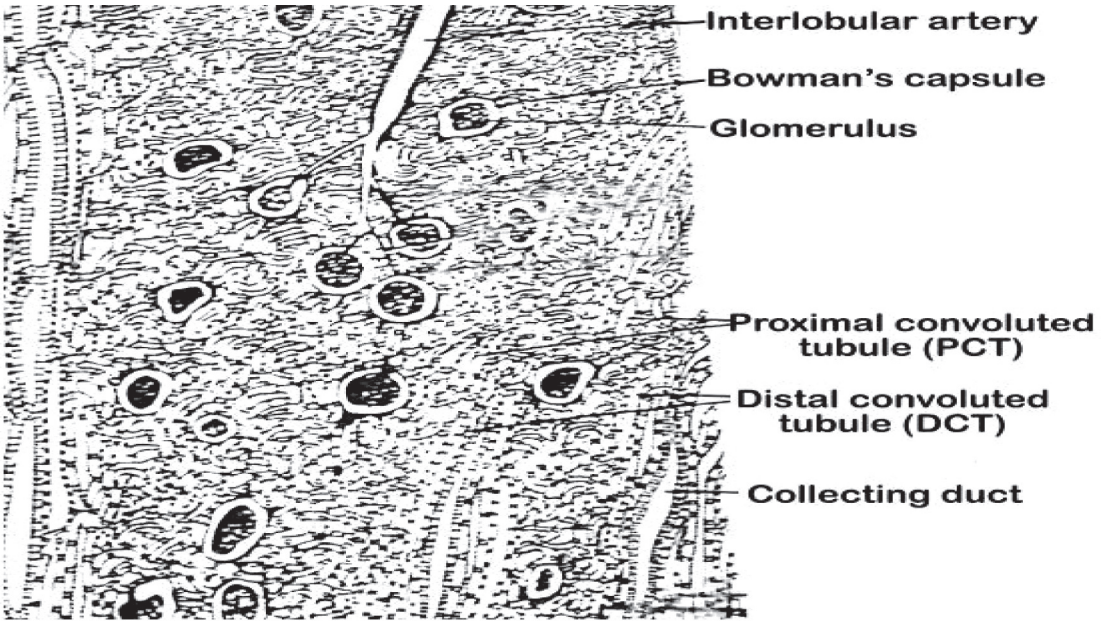
1. Section of the tissue reveals the presence of pseudostratified epithelium.
2. The section shows the presence of alveoli which are responsible for the spongy nature of the lung.
3. These alveoli are lined by flattened epithelial cells called pneumocytes with a single opening.
4. The alveolar wall or septum is made up of three tissue components: surface epithelium, supporting tissue, and an extensive network of continuous capillaries.
5. The trachea spits into paired main bronchi.
6. The bronchi begin a branching pattern, splitting next into lobar (secondary) bronchial branches and then again into segmental (tertiary) bronchi.
7. 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.
8. 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.
9. There are full of empty spaces throughout the section. These spaces are called alveoli.
10. The alveoli are enclosed by thin walls, portions of the bronchi walls, bronchioles and blood vessels.
11. The thin wall of the alveoli consists of stroma containing anastomosing capillaries and a network of fine reticular and elastic fibers.



Section of mammalian lung

Study of sections of mammalian kidney:

1. Section shows that it consists of four parts: glomeruli, tubules, interstitium and blood vessels.
2. Glomeruli are complex vascular structures composed of a tuft of capillaries comprised of specialized endothelial, epithelial and mesangial cells arranged around a relatively thick basement membrane.
3. 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.
4. Together the epithelial cells and basement membrane comprise the Bowman's capsule.
5. The function of the glomeruli is filtration of the blood that leads to the formation of urine.
6. The loop of Henle has thin descending and thick ascending portions covered with cuboidal and columnar cells.
7. Distal convoluted tubule is narrow and short and lined with low cuboidal cells.
8. Cuboidal cells with pale acidophilic cytoplasm and central nuclei line the collecting ducts.



Section of mammalian kidney

Osteology

Unit - 7 ❑ Method of preparation of animal skeleton

Structure

1.1 Objective

The primary objective of skeleton preparation is to study animal resource use at the time from animal remains for which only hard tissue such as bones and teeth remain.

Many skeletal diagrams and photographs that are helpful for identifying animal remains have been published. Strict identification however requires extant animal bone specimens rather than diagrams or photographs.We must therefore get an understanding of the relative positioning of all skeletal regions by preparing bone specimens. Skeleton specimen preparation provides a precious opportunity to gain anatomical knowledge of bones, muscles, tendons, nerves, etc.

3.7 Introduction

It is first necessary to obtain animals for specimens. When doing so, laws, regulations and ordinances related to capture of wild animals must be observed and ethical issues concerning collection of live specimens must be considered. The collection of animal specimen for skeleton preparation is the primary criteria. Methods of obtaining animal specimen include (1) collecting them yourself and (2) purchasing from dealers. (3) obtaining animals killed by hunting/extermination, (4) obtaining animals killed by accidents and (5) donation. (donations involve using carcasses of dead animals donated by zoos or aquariums as specimens.)

If an animal or animals obtained cannot be prepared as a specimen immediately, it should be frozen for temporary storage. Small animals can be preserved in ethanol (ethyl alcohol). In the case of prolonged storage, students should keep in mind the fact that ethanol evaporates. Formalin is also available as a preservative solution.

7.3 Method of preparation of animal skeleton

Preparation of animal specimens for skeleton preparation differs according to the size of the animal. Thus, here we shall concentrate on preparing medium size and large avian and mammal specimens. Following are the steps or phases for animal skeleton preparation :-

1. Obtaining scheduled animal specimen:

Normally animals killed by hunting/extermination involves using game animals or harmful animal that have been trapped for extermination or obtaining animals killed by accidents involves collecting road kill (because laws, regulations and ordinances related to capture of wild animals must be observed and ethical issues concerning collection of live specimens must be considered.)

2. Decortication / organ extraction of the collected animal specimen:

Removal of skin by cutting between the skin and muscle with a scalpel while pulling the skin. In the case of large mammals, skinning is facilitated by the force of gravity if the carcass is hung up for decortication. Next place the carcass on its back facing upward and extract the organs. When doing so, determine the sex by checking the internal reproductive organs (testicles, ovaries, uterus).

3. Disconnection of joints:

This should be done in the following chronological steps :-

a) Separate the cranium and atlas, scapula and ribs, and coxal bone and femur with a scalpel.

b) Roughly divide into cephalic region, trunk and front legs (left and right) and rear legs (left and right).

c) Because the scapula and ribs are not connected, the front legs can be easily separated from the trunk by inserting a scalpel from the rear.

d) The ligaments must be severed in order to separate the cephalic region and rear legs from the trunk.

e) Insert the scalpel ventrally between the cranium and atlas and separate by severing the large ligaments (alar ligaments and apical ligament of dens).

f) To separate the coxal bone and femur, sever the large ligament (femoral head ligament) that connects the acetabular notch and femur.

g) When separating joints, be careful not to damage the bones.

h) After severing the surrounding muscle as much as possible, the joint can be easily separated by pulling while twisting.

4. Excision of soft tissue:

Muscle tissue should be excised for each separated region. Work is facilitated by removing as much muscle tissue as possible. It is convenient to make a photographic record or drawings of the relative positioning of the carpals and tarsals when dissecting.

5. Decomposition of soft tissue:

Soft tissue such as remaining muscle tissue is generally decomposed flesh removal.

Methods of decomposing soft tissue include -

(1) boiling,

(2) using chemicals,

(3) using insects,

(4) decomposition by immersion in water, and

(5) decomposition by burying in the soil.

6. Precautionary measure -

When the soft tissue decomposes, throw away the water and wash the specimen. Use a colander with fine mesh when draining or washing, and be careful not to overlook fine bones or isolated teeth that may get mixed with the putrid matter.

Boiling is the most common method of removing flesh from bones. Bird bones are however thinner and more fragile than those of mammals and require more care when handling. Bones particularly adhere to wings and tail feathers, and may be lightly

scraped when decorticating.

7. Delipidation/bleaching:

Depending on the specimen, fat may fail to be removed and rise to the surface of the bone. Boiling is the easiest way to remove fat from bones. To bleach specimens, immerse in a diluted hydrogen peroxide solution.Bleaching can harm bones. You should therefore exercise caution when bleaching. Rinse well after immersing in hydrogen peroxide solution.

8. Drying:

It is important to dry specimens thoroughly before storing. If specimens are not sufficiently dried, fungus will grow after being put away.

9. Skeleton preparation requiring precaution:

The tongue of highier vertebrates contains bones called "hyoid bones." Be careful not to damage the hyoid bones when removing flesh from the skull. Male carnivores, chiroptera, and rodents are equipped with a baculum. Be careful not to damage the baculum. The baculum, for instance, is important for determining sex of buried dogs.

Similarly handling animal carcasses, etc., required proper sanitation. Students should use rubber gloves when preparing specimens in order to reduce the danger of infection. Apparatus used for preparing specimens must also be washed and disinfected.

7.4 Conclusion

In order to study animal remains, it is necessary to prepare bone specimens or skeleton of extant animals. The significance of this is the same as that of producing stone tools in order to get a good understanding of stone tools.

There is no way to learn how to prepare bone specimens other than experience gained by trial and error. Even if you fail at preparing a bone specimen, you will gain precious experience for thinking about use of past animal resources. Even if records related to specimens are incomplete and are of low biological value, as long as the species of the specimen is clear, it is a great specimen for identifying species of animal remains.

7.5 Summary

At the begining students should prepare a bone specimen or skeleton for identifying the species of a fish that has been eaten. Because preparation of fish specimens does not require special equipment or chemicals. Under ordinary circumstances all students have to do is boil in a pot. Natural history museums in Kolkata also have bone specimens, so when preparing specimens, students may consult with natural history museums in kolkata.

7.6 Glossary

Extant animal - Animals belongs to highier groups.

7.7 Further reading

William T. Hornaday, (1894) Taxidermy and Zoological Collecting: A Complete Handbook for the Amateur Taxidermist, Collector, Osteologist, Museum-Builder, Sportsman, and Traveller (fourth edition; New York: C. Scribner's Sons,).

Frederic A. Lucas (1925) The preparation of rough skeletons , New York,

7.8 Model questions

1. What type of bleaching agents are generally used to blesch the bones ?
2. What precautionary measures are to be taken during the skeleton preparation ?

Unit - 8 ❑ Study of Skulls of amphibia, reptile (Calotes), bird (fowl) and mammal (rat/guineapig and dog). [for amphibia and reptile photograph or virtual mode can be applied]

Structure

8.1 Objective

The primary objective of this unit is to elaborate the knowledge about skull structure in different vertebrate animals and their evolutionary trends. With this unit students also learn the identifying characters of each type of skull.

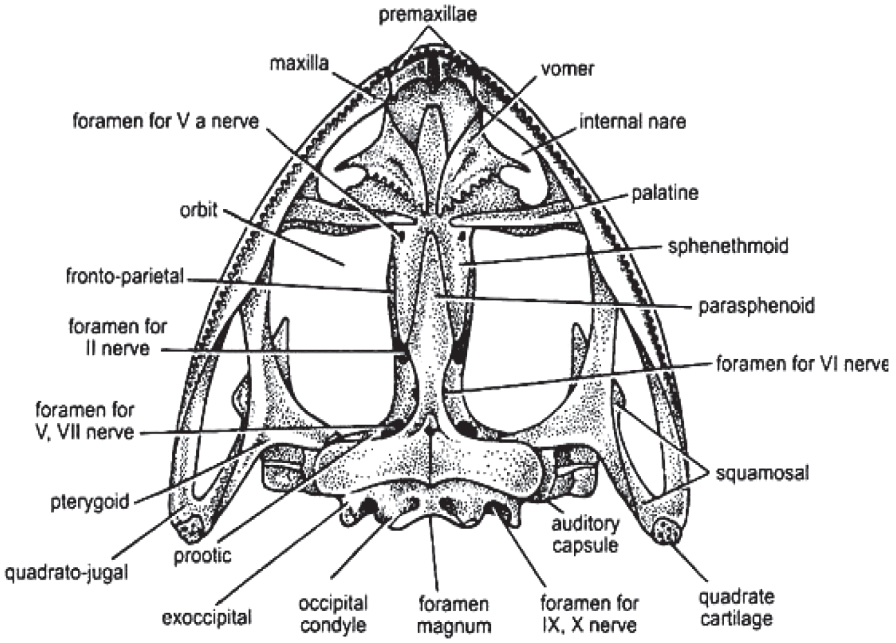
8.2 Introduction

Over time, skulls changed from a primitive collection of bony plates to the highly reinforced, structural marvels most vertebrates carry around today. In this unit students will learn the various changes occurred in different vertebrate groups.

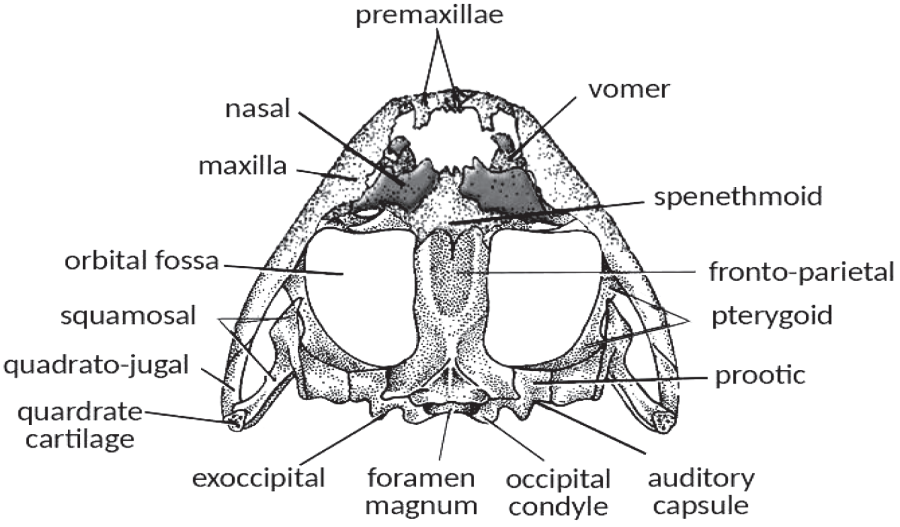
8.3 Study of Skulls of amphibia, reptile (Calotes), bird (fowl) and mammal (rat/guineapig and dog)

Skulls of amphibia (Frog)

1. The skull is dicondylic that means at the posterior end of the cranium is a foramen magnum surrounded by two exoccipitals. Each exoccipital bears at its posterior end a convexity, the occipital condyle which articulates with the concavity of the atlas vertebra.
2. The olfactory capsules have two nasals dorsally and two vomers ventrally, the vomers bear vomerine teeth.
3. A pair of special bones called septomaxillary (ethmoids) form the boundary of nostrils is present. They are associated with and surround the Jacobson's organ.
4. Optic Capsules are not fused with the skull.
5. The upper jaw has two halves, each half has an anterior premaxilla followed by a long maxilla, both bear teeth.
6. The mandible articulates with the quadrate cartilage.
7. Ventral anterior to the orbit is a slender, rod-like palatine.
8. At the posterior lateral end of cranium is present a large 3-rayed or Y-shaped pterygoid.
9. At the posterior dorsolateral end of cranium is the hammer-shaped bone, the squamosal.
10. The posterior part of the upper jaw has a small quadratojugal. Its broad posterior end unites with quadrate cartilage, which is a small thin rod forming the suspensorium.



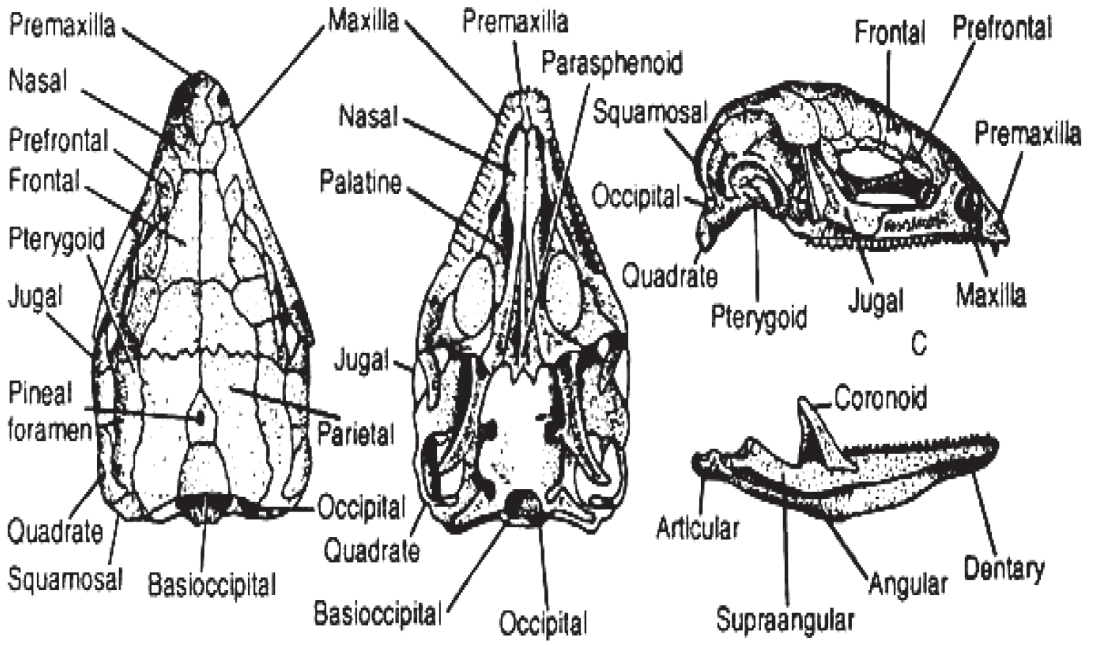
Frog skull ,Ventral view



Frog skull , Dorsal view

Skulls of reptile (Calotes):

1. The skull of Calotes is well-ossified but cartilaginous elements persist in the region of nares and inter-orbital septum.
2. The brain box or carnium is comparatively small and is covered by investing bones.
3. The roof of the skull is made up of a pair of parietals which are fused and bear a median parietal foramen, a pair of frontals and two nasal bones.
4. The premaxillae are situated anterior to and between the nasals while the sickle-shaped squamosals are attached to the posterolateral sides of the parietals by sutures.
5. The floor of the skull is composed of a flat and large basal bone formed by the fusion of occipital and sphenoidal elements.
6. On the roof of the orbit there are many small bones, of which the anterior and posterior ones are known as prefrontal and postfrontal, respectively.
7. The pterygoid forms a bridge between the quadrate (posteriorly) and palatine (anteriorly).
8. The vomer is small, anterior to palatine and articulates with premaxilla and maxilla.
9. The premaxillae and maxillae are furnished with small teeth.
10. The posterior part of the skull is made up of large exoccipitals which are continuous with the horizontal prootic processes.
11. The lateral wall of the cranium is mainly formed by a paired prootics.
12. The quadratojugal is not ossified.
13. The lower jaw is composed of two similar halves or rami. Each ramus, in addition to the slender persistent Meckels cartilage, consists of six pieces of investing bones which fuse with each other.
14. The lower jaw is articulated to the cranium through the quadrate which is movable and such attachment of lower jaw is streptostylic in nature.
15. The lower jaw bears teeth.
16. Hence it is the skull of Calotes (Reptilia) sp.

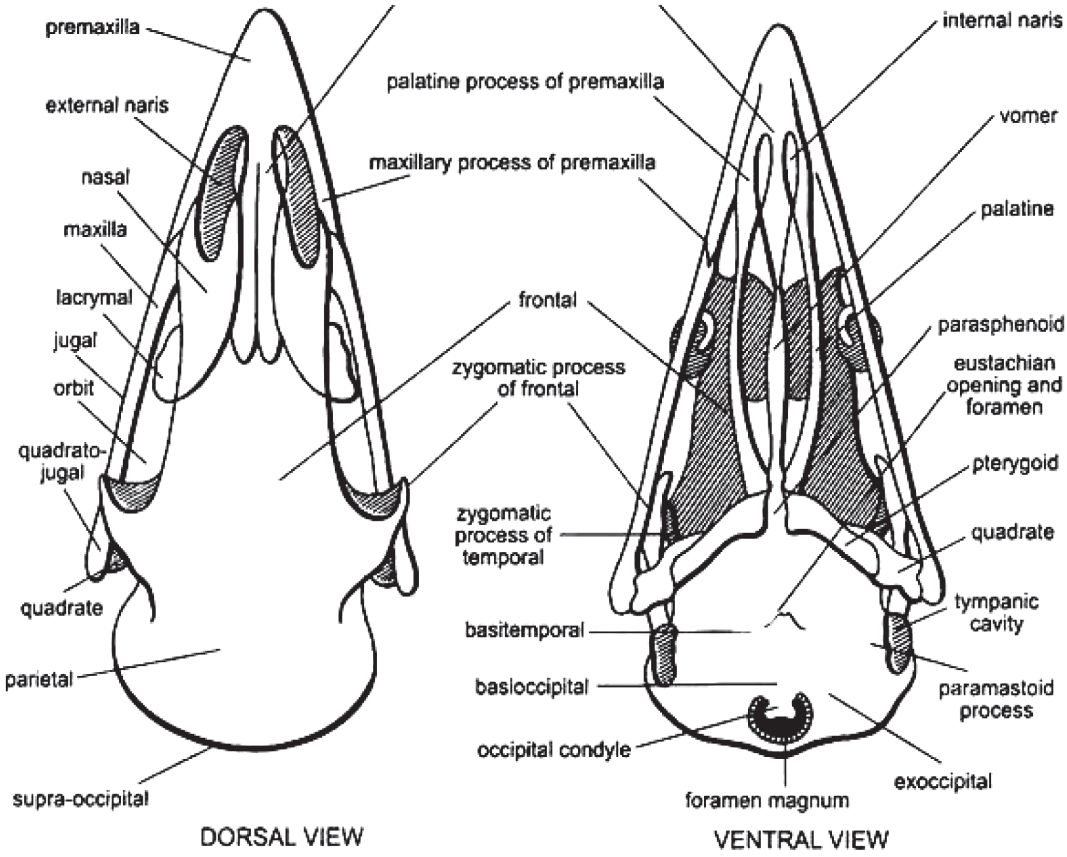


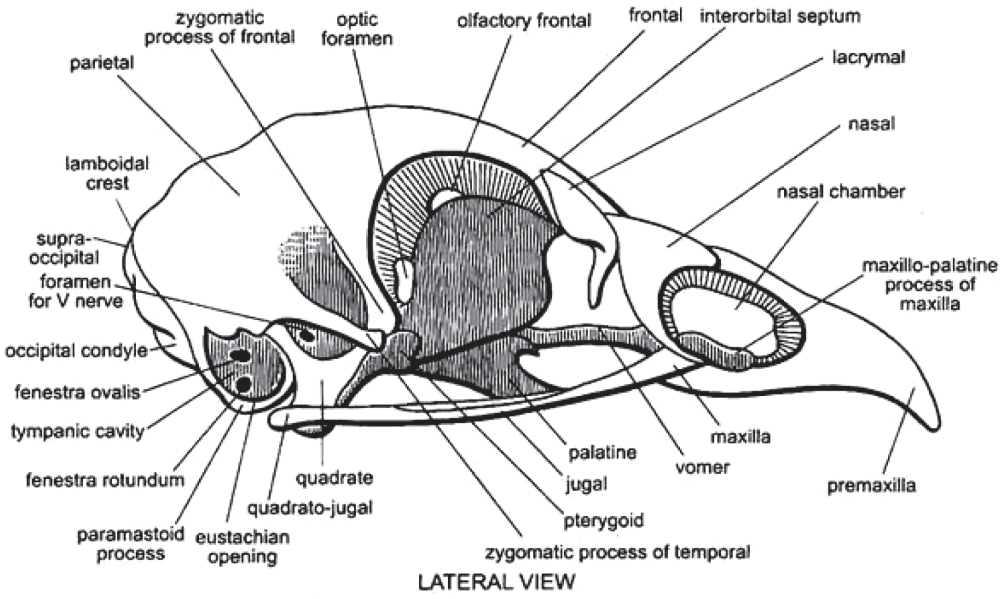
Skull of *Calotes sp.*

Skulls of bird (fowl) :

1. The skull is large but very light due to spongy bones.
2. There has been a complete fusion of bones so that it has no sutures, but it is not deviated from the reptilian type of skull.
3. Number of bones is reduced.
4. Jaw bones form a toothless beak.
5. Monocondylic, i.e., having a single occipital condyle.
6. Cranium is rounded and large for accommodating the well-developed brain.
7. Cranium does not extend forward into orbital region (tropibasic).
8. Large orbits separated by a thin membranous interorbital septum.
9. Autostylic jaw suspensorium.
10. In fowl, palate is schizognathous (short vomers and palatines meet together).

11. The lateral sides of parietal region are formed by the squamosals and alisphenoids and the floor by a large basisphenoid.
12. The roof of the frontal region consists of a pair of large frontal bones.
13. The two orbits are very large to accommodate the relatively massive eyes.
14. Each orbit is bounded anteriorly by the frontal and large lacrymal, dorsally by the frontal and posteriorly by the post-orbital process of frontal and alisphenoid.
15. The upper jaw on each side forms two bony arcades- the outer arcade or infra-orbital arcade or sub-orbital bar which is consisting of premaxilla, maxilla, jugal and quadrato- jugal and the inner arcade or palato-pterygo-quadrate bar is formed by pterygoid, palatine and quadrate.



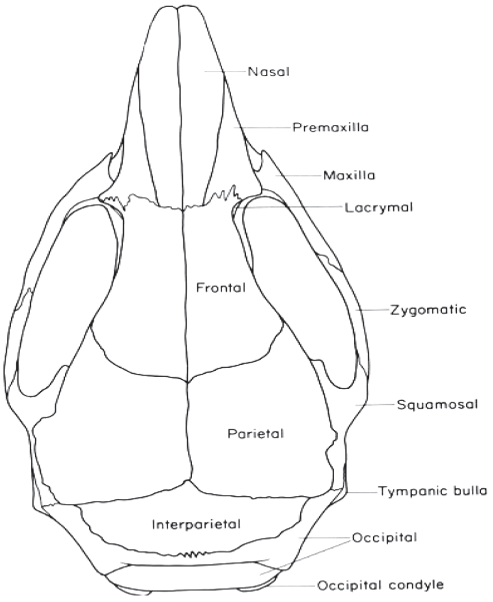


Skull of Fowl (Birds)

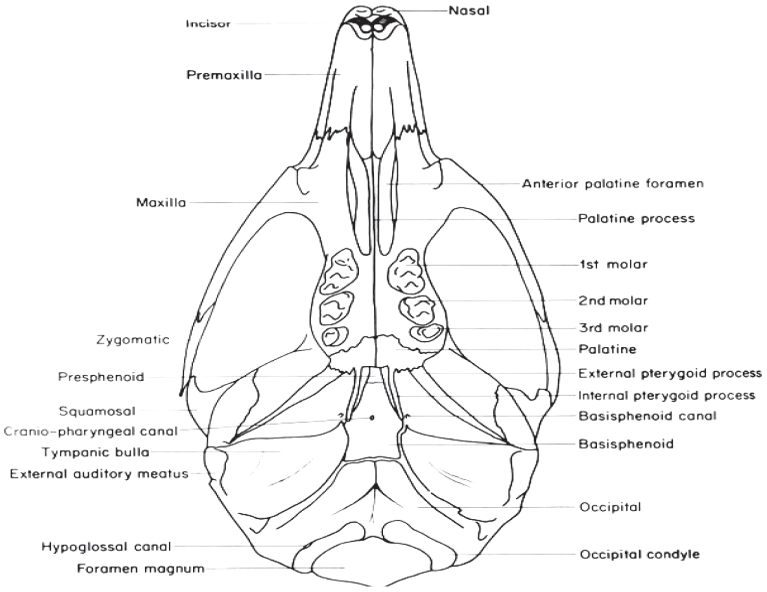
Skulls of Rat (Mammals) :

1. The Skull is longer and pointer .
2. Skull generally longer than 25 mm.
3. Front molars (and their tooth beds) strongly angled up towards front of skull.
4. Front molars large.
5. Tooth bed teardrop shaped, pointed towards rear.
6. Upper rim of eye sockets have a slight point into the sockets.
7. Strong ridge on top of skull from eye socket to back of skull, swoops back and down when viewed from the side.
8. Bottom-front of eye socket has no extension.
9. Distance of the two plates between cranial ridges is wider (side to side) than longer (front to back)
10. Palate extends beyond rear molars.
11. Skull is dicondylic t.e. two occipital condyles are present.
12. Teeth are heterodont, diphyodont and thecodont type.

13. Teeth are present in both the jaws.
14. The cranium is relatively large.
15. A secondary palaye is present.



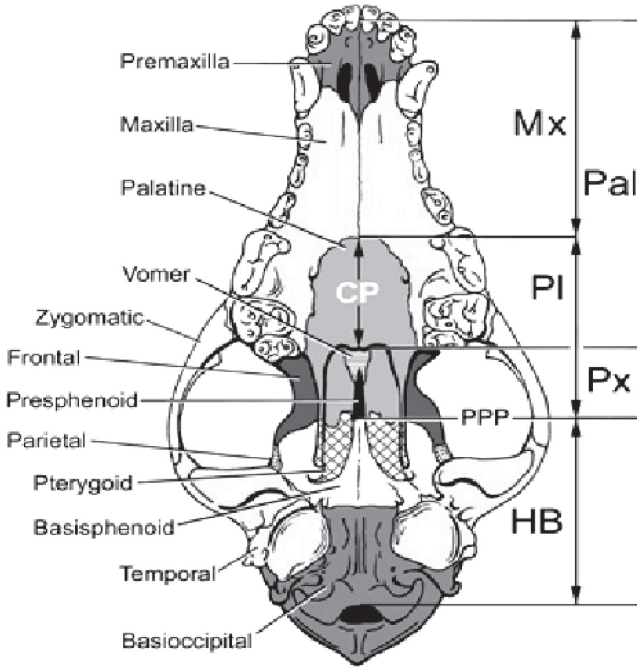
Dorsal view (Skull of Rat , Mammals)



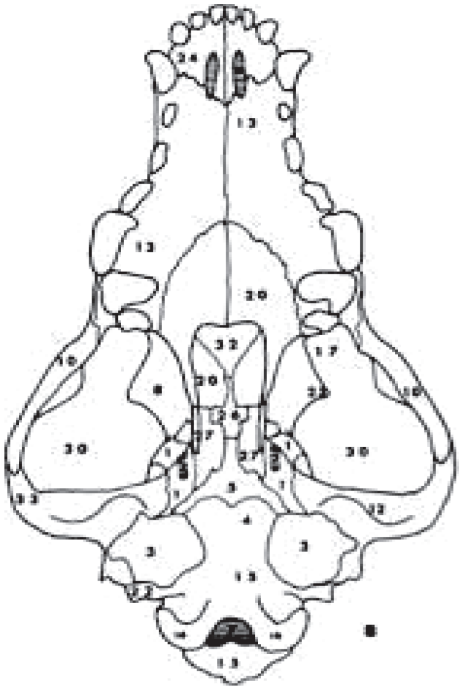
Ventral view (Skull of Rat , Mammals)

Skulls of Dog (Mammals) :

1. The skull of dog is short and high and strongly built.
2. The bones of skull,include: cranium, occipital bone, parietal bone, frontal bone, presphenoid bone, basisphenoid bone, temporal bone, ethmoid; ethmoidal bone, vomer, incisive bone, nasal bone, maxilla, zygomatic bone, palatine bone, lacrimal bone, pterygoid bone, mandible and hyoid apparatus.
3. The bones of the cranium include:
   i) **Parietal -** which forms most of the dorsal and lateral walls of the cranium
   ii) **Temporal -** which lies below the parietal bone on the caudolateral surface of the skull.
4. The most ventral part of the temporal bone forms a rounded prominence called the tympanic bulla which houses the structures of the middle ear.
5. The partition of the tympanic bulla is incomplete.
6. An opening into the tympanic bulla, is present which is called the external acoustic meatus.
7. At the side of the occipital condyles are the jugular processes, which are sites for muscle attachment.
8. Sphenoid lies on the ventral aspect of the skull forming the floor of the cranial cavity.
9. Sphenoid is penetrated by many small foramina through which nerves and blood vessels passes.
10. A well developed sagittal lambdoid crest which is a ridge-like bone is present on the dorsal midline surface of the skull and it is prominent .
11. Many of the bones of the skull are joined together by fibrous joints called sutures. Sutures are firm and immovable joints but allow for expansion of the skull in a growing animal.
12. Transverse glenoid fossa is present.
13. Dental formula is 3.4.1.3 / 2.4.1.3 that is 21.
14. The hyoid apparatus lies in the intermandibular space and consists of a number of fine bones and cartilages joined together in an arrangement that resembles a trapeze.



Ventral view (Dog skull)



Dorsal view (Dog skull)

8.4 Conclusion

The function of the skull is both structurally supportive and protective. The skull will harden and fuse through development to protect its inner contents: the cerebrum, cerebellum, brain stem, and orbits. The skull is a vital bone in the body as it houses the brain - one of the delicate organs in the body. It serves as the protection for the brain and the facial skeleton, which is more delicate as it consists mostly of thin-walled bones.

8.5 Summary

The skull is a vertebrate novelty. Morphological adaptations of the skull are associated with major evolutionary transitions, including the shift to a predatory lifestyle and the ability to masticate while breathing. These adaptations include the chondro cranium, dermato cranium, articulated jaws, primary and secondary palates, internal choanae, the middle ear, and temporomandibular joint. The incredible adaptive diversity of the vertebrate skull indicates an underlying bauplan that promotes evolvability.

8.6 Glossary

Cranium - Brain box

Chondro cranium- Cartilegenous cranium

Tympanic bulla - Tympanic cavity

8.7 Further reading

Hirasawa T, Kuratani S.(2013) Evolution of the vertebrate skeleton: morphology, embryology, and development. Zoological Lett. 1:2.

Bradley Adam & Pam Crabtree (2012) Comparative Osteology, Academic Press Inc., New York, London'

8.8 Model question

1. What is the difference between rat and dog skull ?
2. What is dental formulla ?
3. What is the characteristic feature of avian bone ?

Unit - 9 ❑ Axial and appendicular skeletal system of white rat and fowl.

Structure

9.1 Objective

The major objective of this unit is to inoculate the knowledge of axial and appendicular skeleton structure of two important animal rat and fowl. From this unit students will learn the major structural difference of axial and appendicular skeleton of mammalian and avian animal.

9.2 Introduction

The bvones of any vertebrate animal can be divided into two broad groups wcich are the axial skeleton and the appendicular skeleton. The axial skeleton comprises the bones found along the central axis traveling down the center of the body. The appendicular skeleton comprises the bones appended to the central axis.

9.3 Axial and appendicular skeletal system of white rat and fowl.

Axial skeletons

These are the main support of the body and consists of the skull, the bones of the inner ear (known as ossicles), the hyoid bone in the throat, and the bones of the vertebral column, including the sacrum and coccyx bones in the center of the pelvic girdle.

Appendicular skeletons

The appendicular skeleton are made up by the bones attached or appended to the axial skeleton. These are the bones of the limbs, hands, and feet, the bones of the pectoral (shoulder) girdles, and the coxal (hip) bones of the pelvic girdle.

Appendicular skeleton of rat

Pectoral girdle

It presented a rod like, curved, drumstick shaped clavicle.

Scapula is present as a triangular shaped structure with the cranial border being convex and circular in outline thereby making the cranial angle indistinct.

The caudal border was slightly concave with a thick pronounced caudal angle.

Humerus

The bone has two extremities (proximal and distal).

The proximal extremity present ed a prominent head, well defined neck and two tuberosities separted by an intertuberal groove.

The bone also presented a prominent deltoid tubercle at the middle of the s haft.

The distal extremity presented the supratrochlear fossa, olecranon fossa, trochlea, lateral, medial condyles and epicondyles with supracondylar fossa.

Radio-ulna bones

The ulna was the longer of the forearm bones, extending from the elbow to the carpus.

It got attached to the radius except two points proximally and distally hence presenting a body and 2 interosseus spaces respectively.

Each of the two bones ended up distally with a styloid process.

Carpus

It presented a series of 9 rather nodular bones with more or less irregular shapes and flattened articular surfaces.

These bones were arranged in two rows of 4 in the proximal and 5 in the distal rows.

Metacarpals and digits

It presented complete met acarpal and digital bones (5 each).

All the digits presented 3 phalanges except digit 1 which presented 2 phalanges only.

On the flexor surface of each met acarpophalangeal joint are 2 sessamoids.

Also on the flexor surface, at the distal end of the middle phalanges are single sessamoid bones.

The 1st digit which has only 2 phalanges its distal sessamoid lied at the base of the terminal phalanx.

Pelvic girdle

A distinct muscular tubercle was observed cranial to the acetabulum, having a less prominent gluteal line.

There was a prominent iliopectineal eminence, while the ischiadic spine was not well developed.

The tuber ischiadicum was prominent and had a single process.

There was a small notch on the caudal side of the tabula ossis ischii.

The symphysis pelvis was formed by symphysis pubis connecting the two caudal branches of the pubic bones (ramus caudalis & ossis pubis) and are fused .

The male pelvis also appeared larger.

Femur

The head of the femur was supported by a distinct neck.

Laterally, the greater trochanter arose dorsal to the level of the head.

The fovea capitis was absent but was replaced by an indistinct depression.

There were three trochanters on the femur: the greater trochanter (trochanter major), the lesser trochanter (trochanter minor) and a prominent trochanteric tertius, which formed a solid crest running from the greater trochanter to the mid-femoral shaft.

Tibia and Fibula

The tibia and fibula were separated by a wide cleft at their proximal two-thirds but were fused in the distal third.

The distal portion of the tibia bore a distinct depression, which in the fresh state was crossed by a thick tendon, turning it into a foramen.

Tarsus

There were 8 tarsal bones.

The proximal row consisted of the talus and calcaneus bones, and the tibial tarsal bone occurred medial to the talus while the central tarsal bone was in the distal part of the talus.

Metatarsus

There were five distinct metatarsal bones lying between the tarsal bones and phalanges.

The comparative lengths of the metatarsal bones were: III>IV>II>V>I.

Phalanges

There were two phalanges in the first digit and the other four digits had three phalanges.

The distal phalanges were arched and pointed to accommodate the curved nails.

Axial skeleton of Rat

Vertebral column

The vertebral column of rat consists of 7 cervical vertebrae, 13 thoracic vertebrae, 6 well-developed lumbar vertebrae, 4 sacral vertebrae , 31-36 caudal or coccygeal vertebrae.

1. Cervical Vertebrae

Of the seven cervical vertebrae the atlas and the axis are the largest while the rests were shorter and wider. The greatest diameter of the vertebral canal was observed at the atlas vertebra. The transverse processes were positioned caudolaterally in cervical vertebrae. There were ventral tubercles on the ventral surfaces of the bodies of the cervical vertebrae. Transverse processes is small in the 2nd-5th cervical vertebrae. Fovea costalis caudalis is present on the 7th cervical vertebra.

2. Thoracic Vertebrae

The caudal ends of the transverse processes were craniolaterally directed.

Fovea costalis caudalis was more distinct than fovea costalis cranialis.

Spinous processes of the thoracic vertebrae were caudodorsally inclined, and the dorsal processes of the last two thoracic vertebrae were wide and dorsally projected.

3. Lumbar Vertebrae

There were 6 well-developed lumbar vertebrae and each vertebra appeared uniform in length.

Their spinous process and transverse processes increased in size caudally as the spinous process became more erect.

Presence of the mammillary processes on all lumbar vertebrae .

The ventral crest was present on all of the lumbar vertebrae.

The transverse processes of the 3rd-5th lumbar vertebrae were larger than the other lumbar vertebrae.

The lumbar vertebrae were slightly larger than the thoracic vertebrae.

4. Sacral Vertebrae

4 sacral vertebrae are fused together to form Os sacrum.

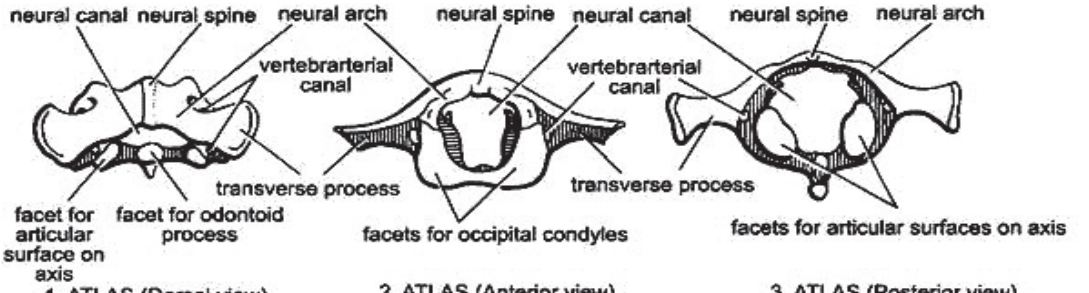
Although they appeared fused but the outlines of the transverse and articular processes were visible.

5. Coccygeal Vertebrae

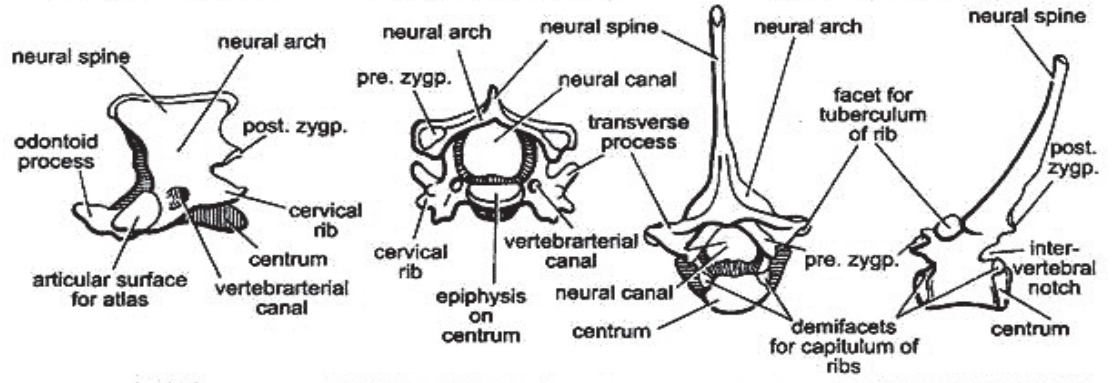
There were 31-36 caudal or coccygeal vertebrae forming the bone of the tail.

Cranial articular processes is present.

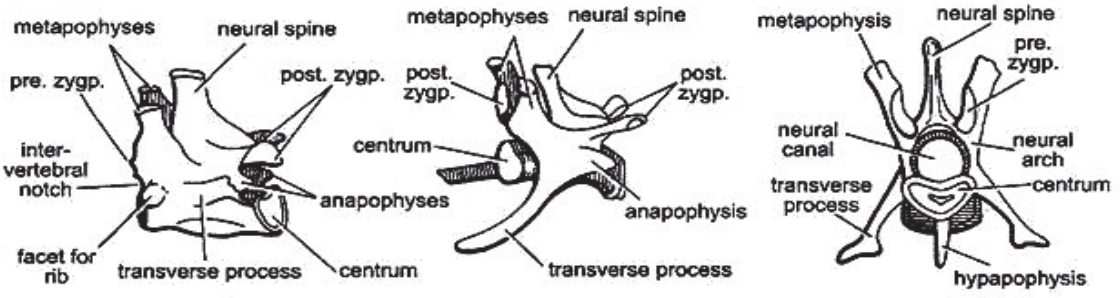
Hemal arch is present.



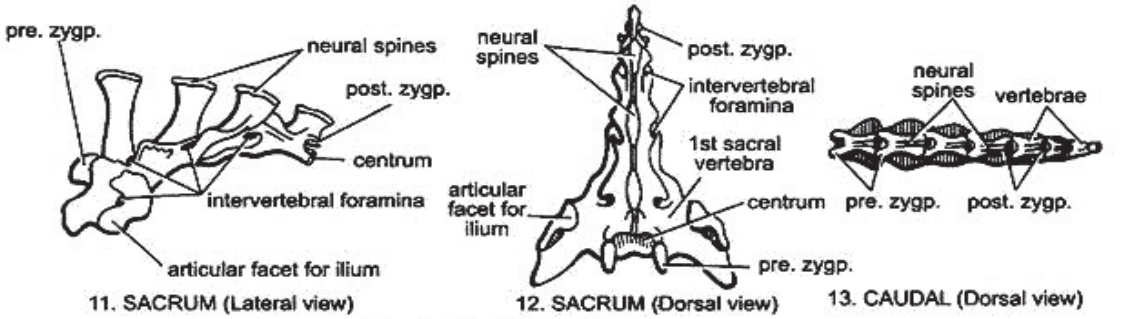
1. ATLAS (Dorsal view) 2. ATLAS (Anterior view) 3. ATLAS (Posterior view)



4. AXIS (Lateral view) 5. TYPICAL CERVICAL (Anterior view) 6. ANTERIOR THORACIC (Anterior view) 7. ANTERIOR THORACIC (Lateral view)



8. POSTERIOR THORACIC (Lateral view) 10. POSTERIOR LUMBAR (Lateral view) 9. ANTERIOR THORACIC (Anterior view)



11. SACRUM (Lateral view) 12. SACRUM (Dorsal view) 13. CAUDAL (Dorsal view)

Appendicular skeleton of Fowl

Pectoral girdle

The b.one is very stout and connected with sternum.

The both side of the bone consists of a scapula, coracoid and clavicle.

Scapula is long, flattened, slightly curved, sabre-shaped bone

The expanded anterior end of scapula is firmly united with the coracoid

Its anterior end bears a shallow depression forming a part of glenoid cavity.

The glenoid end of scapula is produced into an acromian process to provide articular surface to the clavicle and to form a part of the foramen triosseum.

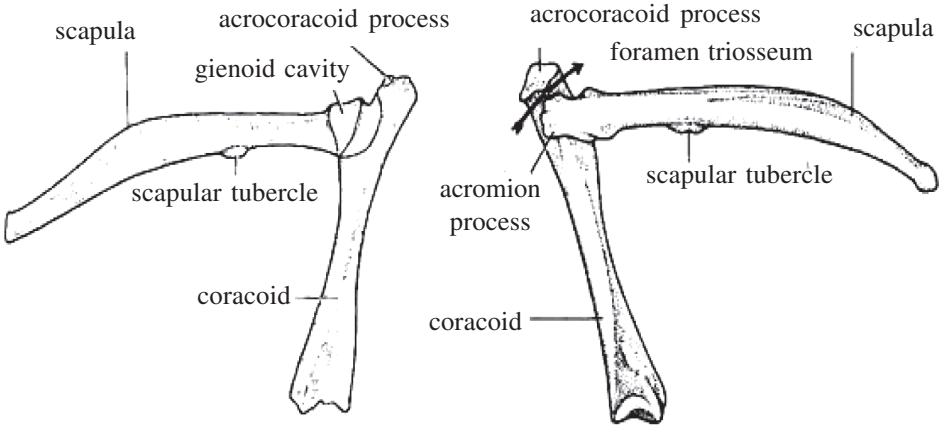
Coracoid is a stout, straight rod-like bone directed downwards and articulates with the coracoid groove on the antero-lateral edge of the sternum at the base of manubrium.

The upper end of coracoid on its inner side articulates with the scapula and on its outer side it bears a deep cup-shaped depression which forms the greater part of the glenoid cavity.

Clavicles are a pair of slender, curved delicate rod-like bones connected by their expanded upper ends with the scapula.

Ventrally the two clavicles are fused with a small interclavicle to form a laterally compressed disc or hypocleidium.

The V-shaped bone is known as fercula.



Pectoral girdle of Fowl

Humerus

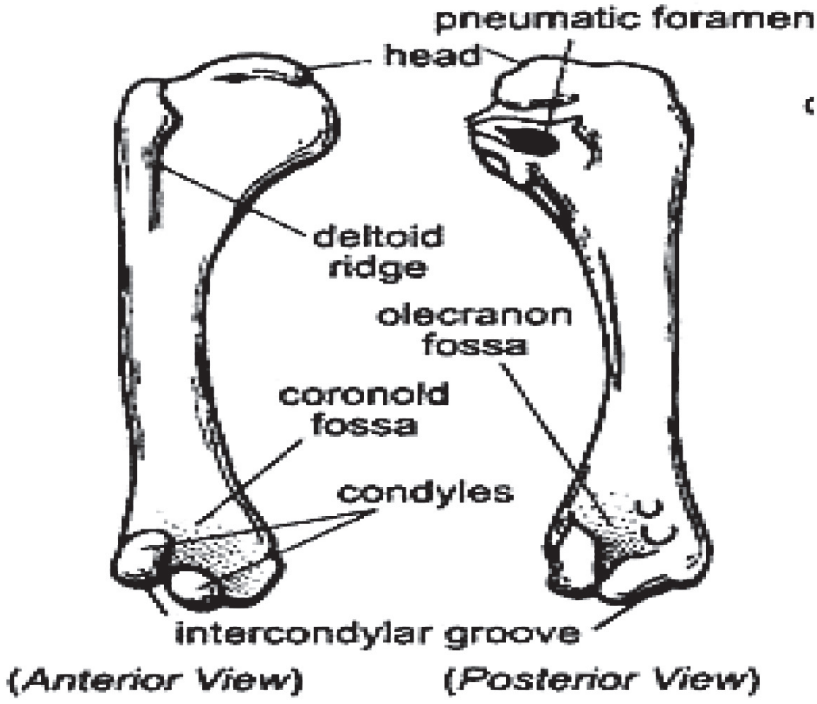
Humerus is the bone of upper arm of forelimb.

It is an elongated stout bone expanded at both the ends.

The proximal end is expanded into smooth convex surface the head which fits into the glenoid cavity of pectoral girdle.

The postaxial tuberosity is larger and has a pneumatic foramen on the dorsal side of the proximal end communicating with its air cavity.

The distal end possesses trochlea or condyles for the articulation with the radius and ulna.



HUMERUS

Radio-ulna bones

Radius and ulna are the bones of the forearm.

Radius is shorter, slender and straight bone.

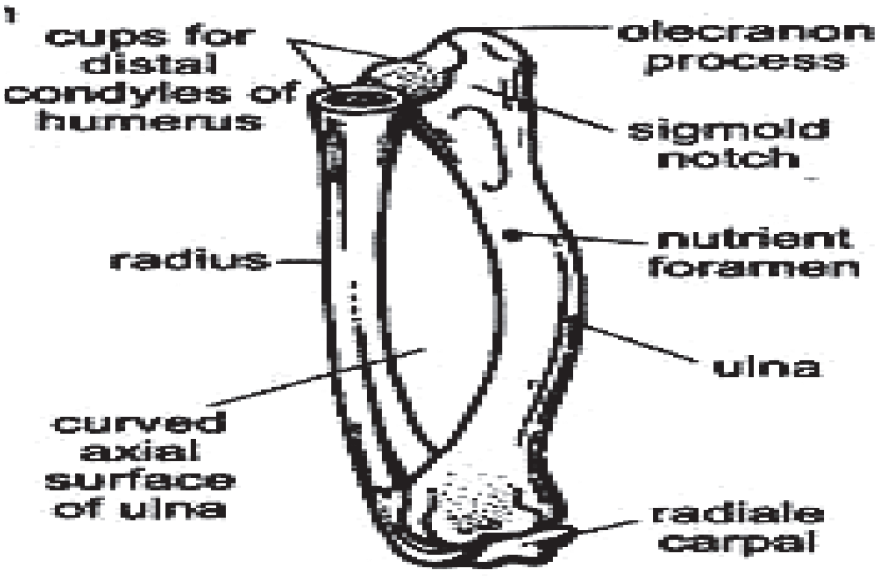
Proximally it has a cup-shaped articular surface for the outer trochlea of humerus.

Distally it bears a knob which articulates with the radiale carpel.

Ulna is stouter and larger than the radius and also slightly curved.

Proximally it bears a small projection, the olecranon process, and a large articular facet for the inner condyle of the humerus.

Distally it articulates with the ulnare carpal and radius.



RADIUS & ULNA

Pelvic girdle

The pelvic girdle consists of two separate halves lying on either side of the synsacrum.

Each half is known as os-innominatum.

Each os-innominatum is composed of ilium, ischium and pubis.

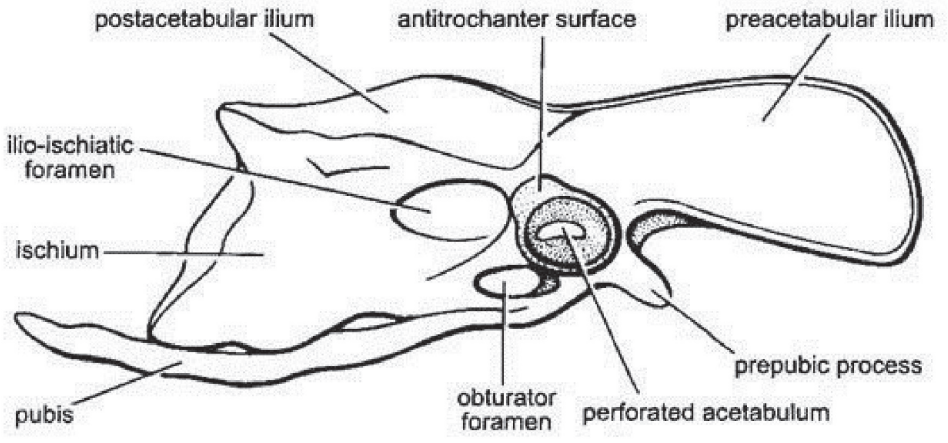
At the junction of three bones is present a concavity, the acetabulum, which provides surface for the articulation of the head of femur.

Ilium is an elongated and remarkably expanded bone extending both anterior and posterior to the acetabulum.

Ischium is also dorso-ventrally flattened bone projecting backwards behind the acetabulum and parallel to the posterior part of the ilium.

Pubis is a long, thin, curved, slender bone directed backward parallel to the ventral margin of ischium with which it is usually fused.

Ilium forms the dorsal part of acetabulum.



Pelvic girdle of Fowl

Femur

Femur is the bone of thigh of hindlimb.

It is short and powerful bone flattened at both the ends.

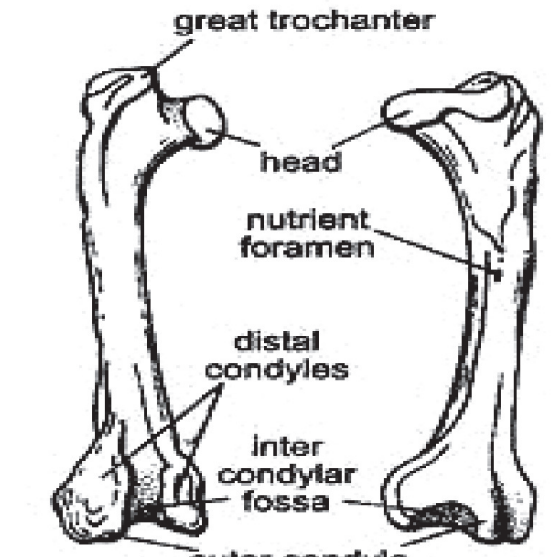
Proximally on the inner side it bears a rounded head which fits into acetabulum.

On the outer side of the head of the bone is an irregular prominent process the great trochanter.

Between the great trochanter and head is the articular surface for the antitrochanter of ilium.

Distally it has a deep groove or intercondylar fossa bounded laterally by two condyles for the articulation with the tibiotarsus.

Outer condyle receives the upper end of fibula.



FEMUR

Tibio-tarsus and Fibula

Tibio-tarsus and fibula are the bones of shank of hindlimb.

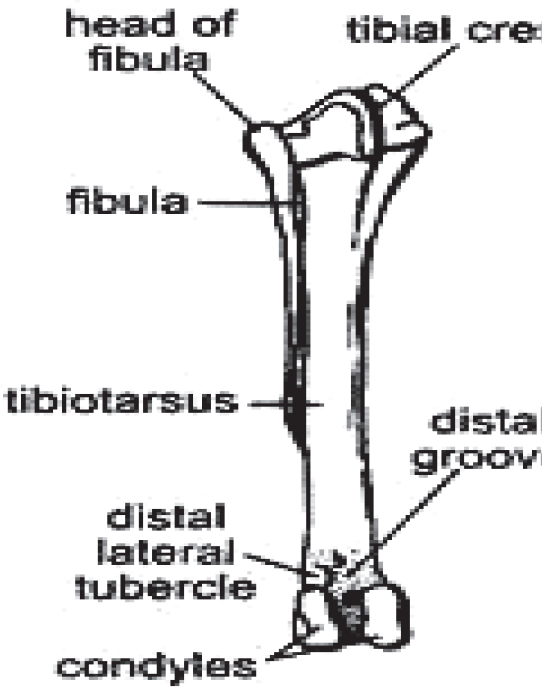
Tibiotarsus is a long and stout nearly straight bone found on the inner side of shank.

It is formed by the fusion with the proximal row of tarsals, i.e., astragalus and calcaneum.

The proximal end of tibiotarsus is expanded and has on its anterior face a median cnemial or tibial crest and two articular surfaces for the condyles of the femur.

Fibula is a smaller, slender bone closely applied to the outer surface of the tibiotarsus.

Proximally it articulates with the outer condyle of femur.



Axial skeleton of Fowl

Vertebral Column

The vertebral column of birds is peculiar in having a large number of cervical vertebrae (14 to 16 in number)due to long mobile neck; thoracic (seven in number), lumbar and sacral vertebrae are fused giving rigidity which is advantageous in flight. Caudal vertebrae are fused forming the pygostyle which supports the tail feathers. Epiphysis is lacking in long bones.

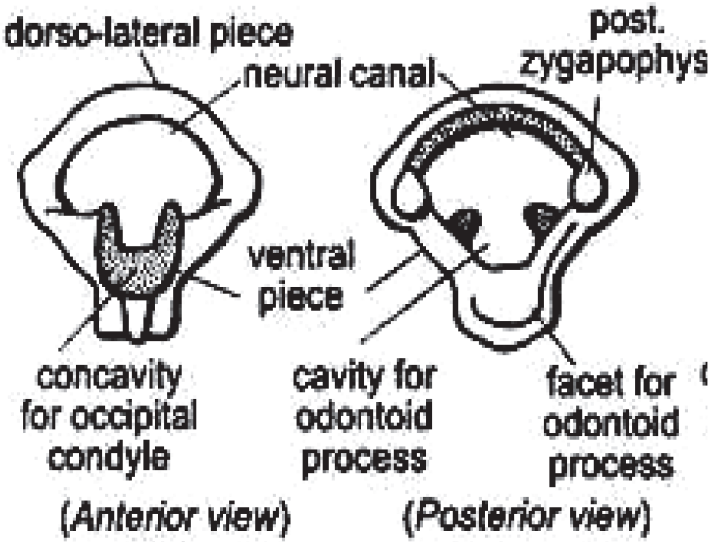
Atlas Vertebra or First vertebra

Atlas is the first vertebra of the cervical region.

It is small ring-like in appearance. Centrum, neural spine, transverse processes, ribs and prezygapophyses are absent.

It is formed of three pieces, a ventral and two dorso-lateral uniting mid-dorsally to form the neural arch.

Its anterior side bears a cup-like cavity to provide articulation to the single occipital condyle of the skull.



ATLAS

Axis Vertebra

Axis is the second vertebra of the cervical region.

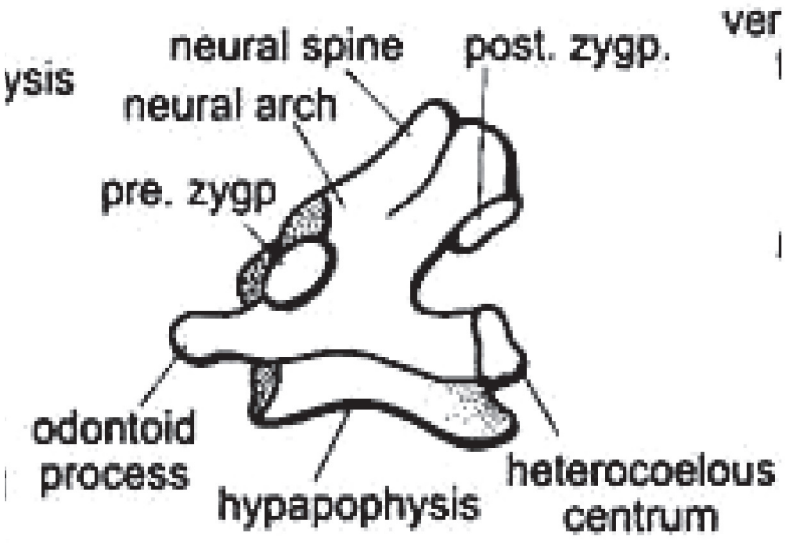
Transverse processes, ribs and vertebrarterial canal are absent.

Centrum is produced in front into a peg-like odontoid process by which it articulates within notch present on the dorsal side of the ventral piece of the atlas vertebra.

Neural spine is blunt.

Pre and post zygapophyses are present.

Head and atlas get mobility over the axis vertebra.



AXIS (*Lateral view*)

Typical Cervical Vertebra

The typical cervical vertebra has a long body or centrum.

The centrum is heterocoelous or saddle-shaped.

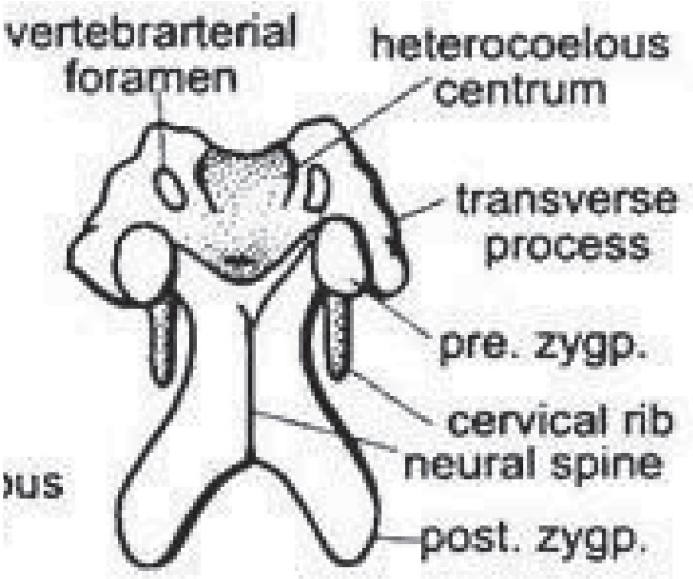
Anterior surface is concave from side to side and convex from above downwards, while the posterior surface is convex from side to side and concave from above downwards.

The neural arch is short and notched both anteriorly and posteriorly.

The neural spine is rudimentary.

Transverse processes are short and irregular, and arise laterally from the anterior end.

Prezygapophyses are flat .



(*Dorsal view*)

TYPICAL CERVICAL

Synsacrum

Synsacrum is a long compound bone formed by the fusion of 14 to 16 vertebrae, supporting the long ilium of the pelvic girdle on both sides.

The fused vertebrae of synsacrum are last thoracic having a pair of free thoracic ribs and have no hypapophysis, six lumbars, two sacrals and about seven caudal vertebrae.

All the fused vertebrae of lumbar region have free well-developed transverse processess which is applied against long ilia.

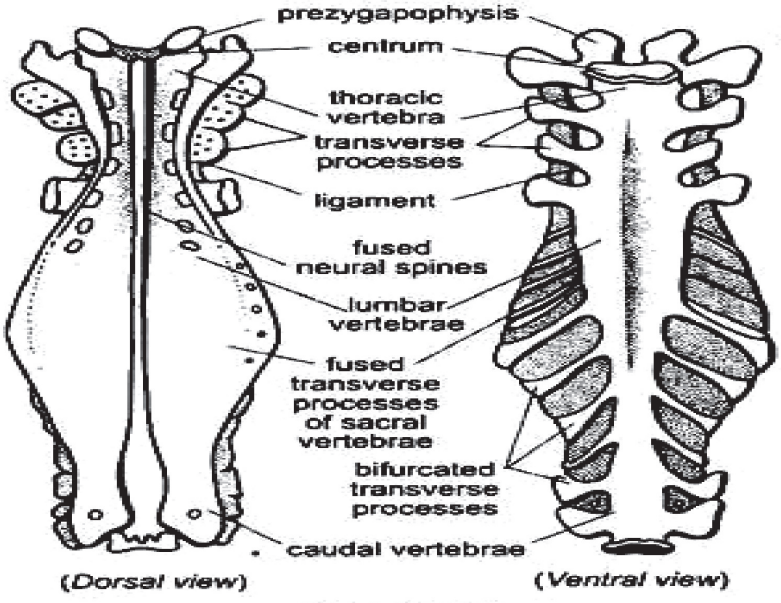
The neural spines of these vertebrae are also fused to form a vertical crest which is continuous in front with that of the last thoracic and attached with the dorsal margins of the ilia.

Two sacral vertebrae lie behind the lumbars and fused with them.

Their transverse processes are also united to form bony plates which support the ilia along their outer margins.

Sacral vertebrae are fused with their transverse processes.

The hindermost part of the synsacrum is formed by the remaining seven anterior caudal vertebrae.



SYNSACRUM

Caudal Vertebrae

Tail is short and has a few free vertebrae and pygostyle (fused caudal vertebrae).

Four or five free caudal vertebra are small.

Their centra are heterocoelous, and neural spines are small and bifid.

Transverse processes are somewhat cylindrical and directed downwards and outwards.

Pre and post zygapophyses are absent.

Pygostyle

Pygostyle is the last fused vertebra of the caudal region.

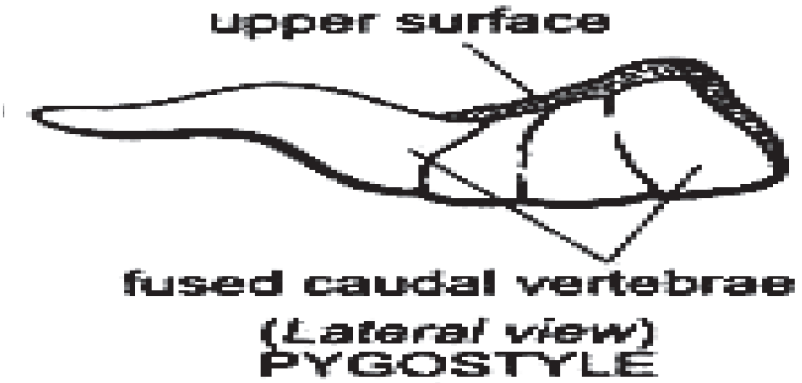
It is commonly known as ploughshare.

It is formed by the fusion of four or more of hindermost caudal vertebrae.

It looks like a vertically triangular and laterally compressed plate.

Centrum, neural spine, pre and post zygapophyses are absent.

It supports the rectrices (tail feathers) and provides attachment to caudal muscles.



9.4 Conclusion

Axial skeleton is made up of the bones in your head, neck, back and chest. Your appendicular skeleton is made up of everything else - the bones that attach (append) to your axial skeleton. Appendicular skeleton includes the bones in your shoulders, pelvis and limbs, including your arms, hands, legs and feet.

9.5 Summary

The appendicular skeleton is composed the bones that attach (append) to the axial skeleton. The appendicular skeleton includes the bones of the pectoral girdle (shoulders, arms, hands) and pelvic girdle (pelvis, legs and feet). In other words, the bones of the appendicular skeleton are attached, therefore hanging off the axial skeleton.

9.6 Glossary

Calvaria - the dome or roof of the skull, also known as the "skullcap"

Sutures - immoveable joints of the

Hyoid Bone - The only bone in body that does not directly articulate with any other bone.

9.7 Further reading

Hirasawa T, Kuratani S. (2013) Evolution of the vertebrate skeleton: morphology, embryology, and development. Zoological Lett. 1:2.

Bradley Adam & Pam Crabtree (2012) Comparative Osteology, Academic Press Inc., New York, London'

9.8 Model questions

1. What is pygostyle ?
2. What is synsaccrum?

Unit - 10 ❑ Carapace and plastron of turtle

Structure

10.1 Objective

The main objective of this unit is to help the students to learn about the skeletons of Testudines which are the most unique skeletons of any other type of reptile, because of the presence of a sell structure. The primary function of this skeletal structures is the protection of the animal.

10.2 Introduction

Turtles have a shell consisting of a dorsally arched carapace and a ventral flattened plastron,and the two are united by lateral bridges which are cut through to expose the internal surfaces of the armature. Carapace, plastron and lateral bridges consist of large dermal plates which are covered by broad quadrilateral sheets of horny epidermis, the corneoscutes.

10.3 Carapace and plastron of turtle

Carapace

The carapace contains axial endochondral skeletal elements and exoskeletal dermal bones.

The carapaceis formed by the fusion of about 50 bones.

The carapace of turtles consists of the ossified ribs of turtles which are fused with the dermal bone.

The spine and the expanded ribs are fused by ossification to the dermal plates which are present beneath the skin and so form a hard shell.

The shell is relatively uniform in structure.

The carapace usually has 38 scutes.

The scutes of the carapace are of five categories according to their placement :

i) The Nuchal - the scute directly above the head

ii) The Supracaudal - the scute directly above the tail

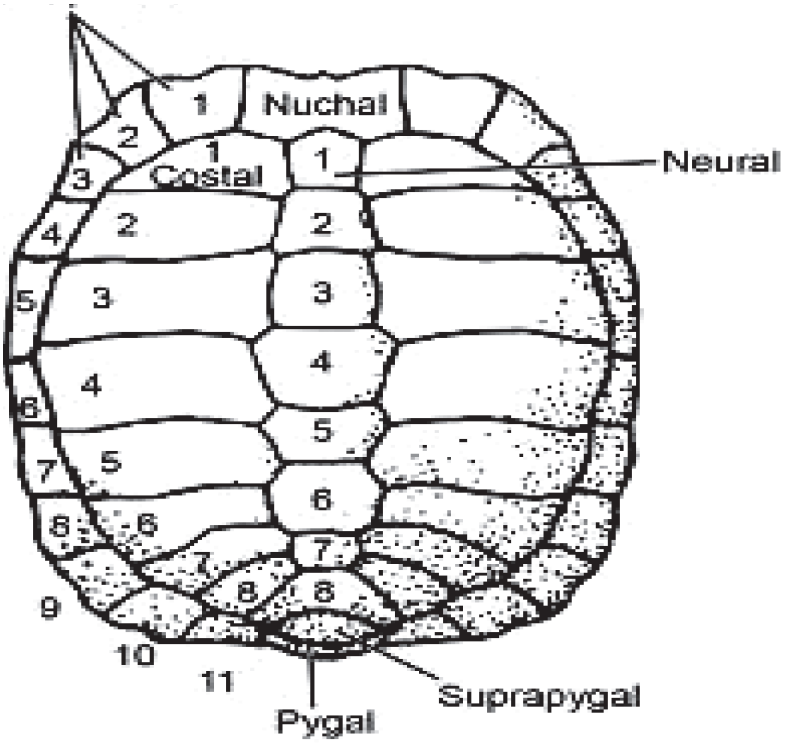
iii) The Vertebrals - a single line of scutes which run centrally from the head to the tail

iv) The Costals - run parallel to, and at either side of, the Vertebrals

V) The Marginals - flank the Costals and attach to the "bridge".

The Marginal scutes have a large influence on the overall shape of a tortoise's shell.

The inner plate of carapace is formed by the fusion of dermal bones with expanded ribs and vertebrae.



Carapace structure

Plastron

Plastron is also formed by the fusion of bones which include the clavicle (collar bones), bones between the clavicles, and portion of the ribs.

The clavicle (collar bone) becomes a part of the plastron known as the epiplastron.

The plastron is formed largely from dermal ossification.

The plastron consist of 9 bones which are:

i) The median endoplastron which lie in front and is derived from the interclavicle,

ii) The paired epiplastron derived from the clavicles.

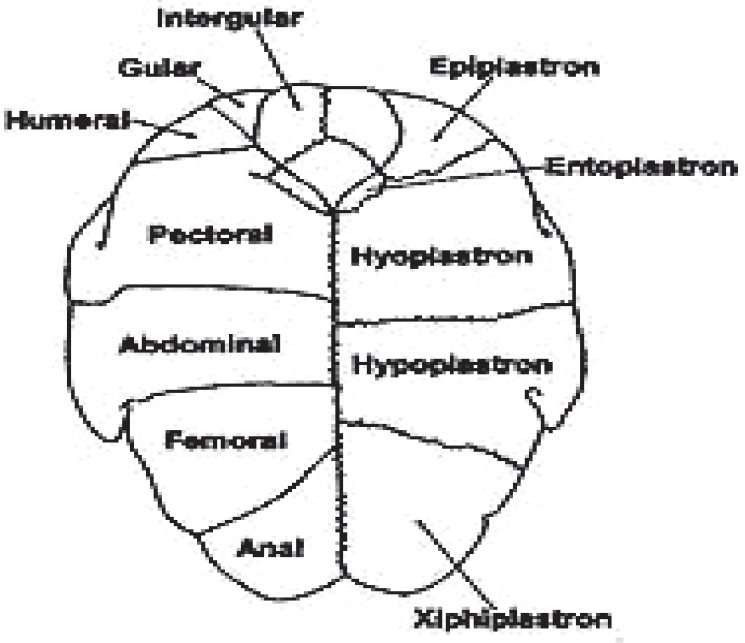
iii) Processess from the hyoplastron fuse with the first and fifth pleurals, forming a rigid connection between the plastron and carapace.

The scutes of the plastron are also separately categorized.

There are two scutes in each category.

Starting from the head moving down to the tail plastron have; Intergular, Gular, Humeral, Pectoral, Abdominal, Femoral and Anal.

Both carapace and plastron are heavily keratinized.



Plastron structure

10.4 Conclusion

Carapace is a protective exoskeleton on the dorsal side. Plastron is a protective shield that is typically on the ventral side. It is a convex structure. It can be a flat, convex or concave structure. Dermal plates of plastron on its internal face are paired epiplastrons, median entoplastron, paired hyoplastrons, paired hypoplastrons and paired xiphiplastrons.

10.5 Summary

The carapace and plastron each arose from two types of bone which are dermal bones that form in the skin and endochondral bone (bone arising from cartilage) derived from the skeleton. Evolution has intricately linked these two types of bone to produce the shell of modern turtles.

10.6 Glossary

Carapace - Protective external covering of some animal and also known as shell and present in Turtle, Crustacea and arachnids.

Scutes - a thickened horny or bony plate on a turtle's shell or on the back of a crocodile,

10.7 Further reading

Hirasawa T, Kuratani S.(2013) Evolution of the vertebrate skeleton: morphology, embryology, and development. Zoological Lett. 1:2.

Bradley Adam & Pam Crabtree (2012) Comparative Osteology, Academic Press Inc., New York, London'

10.8 Model questions

1. How many bones are fused to form carapace?
2. What is the difference between carapace and plastron ?

Developmental Biology

Unit - 11 ❑ Study of Life-history stages of Mosquito.

Structure

11.1 Objective

The primary objective of this unit is to learn about the life history stages of an important vector insect known as mosquito which is considered to be a most dangerous insect vector because of their disease spreading capacity like malaria,elephanticiaasis and dengue etc.

11.2 Introduction

Mosquitoes are one of the most intensely studied creatures on the planet. Their role in disease transmission and nuisance biting makes them worthy of such attention. There are over 3,500 species of mosquito on Earth and they are found on every continent except Antarctica. Yet, from this great diversity, only a small handful can carry the pathogens that cause disease and it is these species which have been studied most intensely. Mosquitoes are holometabolous insects. There are three primary types of mosquito found in India which are very important and they are Culex Mosquitoes , Anopheles Mosquitoes and Aedes Mosquitoes. Mosquito belongs to the Class Insecta or Hexapoda,Order Diptera and Family Culicidae.

11.3 Study of Life-history stages of Mosquito.

While all mosquitoes need standing water to reproduce, different mosquito species are found in different habitats. Some mosquitoes are considered "flood water" species that breed in temporary water habitats, while others are considered "permanent water"

mosquitoes and breed in water sources that remain for long periods of time. Other species have evolved so specifically that they will only lay their eggs in natural or artificial containers.

No matter what their preferred breeding habitat, all mosquitoes undergo the same four-stage life cycle: egg, larva, pupa, and adult, with the larval and pupal stages always being aquatic.

Mosquito Eggs

Depending on the particular species, the female mosquito lays her eggs either individually or in attached groups called rafts.

The eggs are placed either directly on the surface of still water, along its edges, in treeholes, or in other areas that are prone to flooding from rain, irrigation, or flooding.

In some species, the eggs may hatch within a few days of being laid, with the exact amount of time dependent on temperature.

Most eggs hatch into larvae within 48 hours during the peak of summer

The duration of egg stage is about 2-3 days.

The Larval Stage (Also called "wigglers")

Once the egg hatches, the larval stage begins.

The larvae of most mosquito species hang suspended from the water surface because they need air to breath.

An air tube, called a siphon, extends from the larva's posterior to the water surface and acts as a snorkel.

Larvae filter feed on aquatic microorganisms near the water's surface.

During growth, the larva molts (sheds its skin) four times.

The larval stage has four instars. (the stages between each molts are called instars.)

The length of the larval stage ranges from 4 to 14 days, varying with species, water temperature, and food availability.

Larvae are constantly feeding since maturation requires a huge amount of energy and food.

They hang with their heads down and the brushes by their mouths filtering anything small enough to be eaten toward their mouths to nourish the growing larvae.

They feed on algae, plankton, fungi and bacteria and other microorganisms.

The duration of larval stages is about 4-14 days.

The Pupal Stage or commonly called "tumblers"

The fourth instar larvae moults into the comma shaped stage called the pupa (or tumblers).

The pupa is lighter than water and therefore floats at the surface.

It takes oxygen through two breathing tubes called ''trumpets.''

In the pupal stage, no feeding occurs, however the pupa must still breathe air at the water's surface and is sensitive to light, shadows, and other disturbances.

Pupae are also physically active and employ a rolling or tumbling action to escape to deeper water, which is why they are commonly referred to as ''tumblers''.

The pupal stage lasts from 11/2 to 4 days, after which the pupa's skin splits along the back allowing the newly formed adult to slowly emerge and rest on the surface of the water.

The span of pupal stages is about 1-4 days.

Adult Mosquitoes

The male adult mosquito will usually emerge first and will linger near the breeding site, waiting for the females.

Mating occurs quickly after emergence due to high adult mortality rates.

As much as 30% of the adult population can die per day.

The females compensate for this high rate by laying large numbers of eggs to assure the continuation of the species.

Male mosquitoes will live only 6 or 7 days on average, feeding primarily on plant nectar, and do not take blood meals.

Females with an adequate food supply can live up to 5 months or longer, with the average female life span being about 6 weeks.

To nourish and develop her eggs, the female usually must take a blood meal in addition to plant nectar.

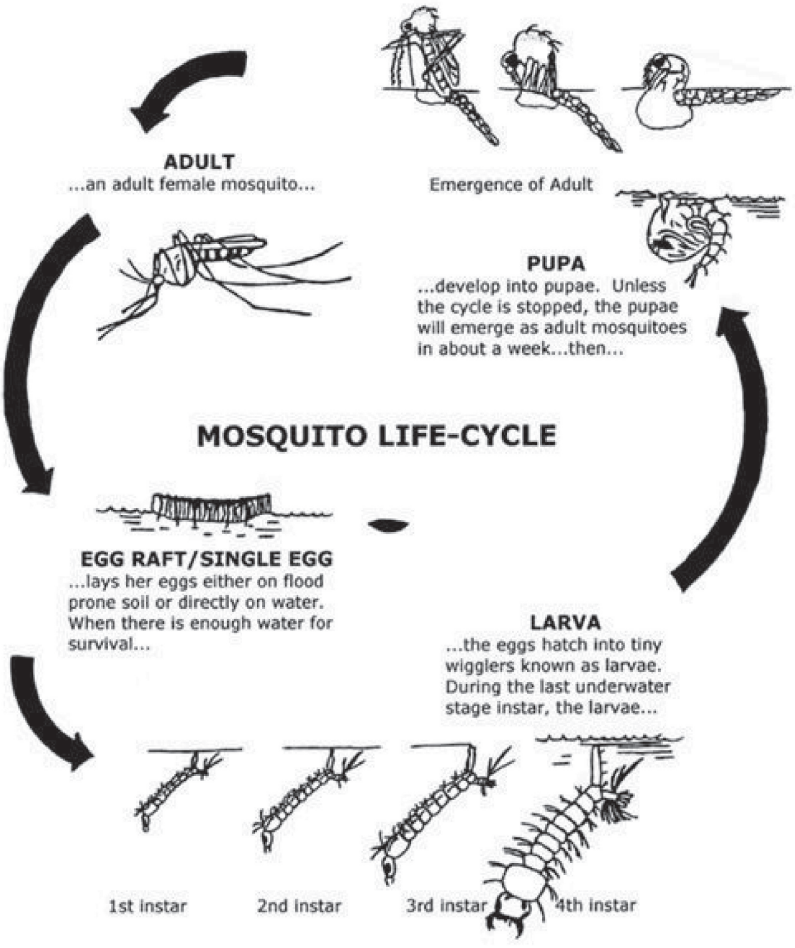
The life span of adult stages is about 2 weeks to several weeks and it depends upon the environmental condition.

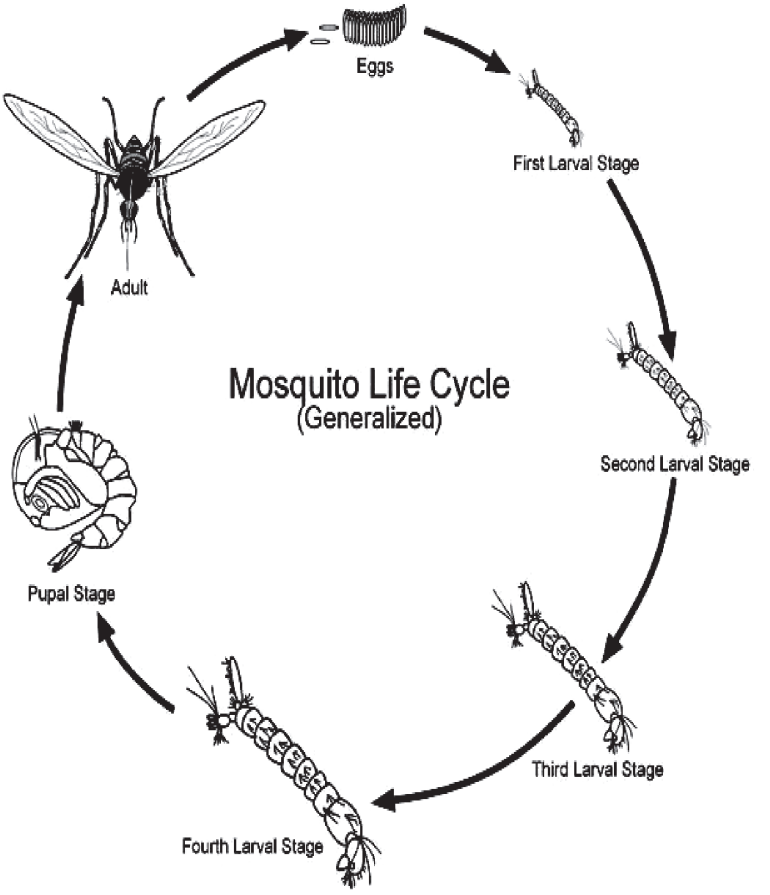
11.4 Conclusion

All mosquitoes go through four distinct stages (egg-larvae-pupa-adult) in their life cycle which can vary in length depending on temperature and food resources. In the summer time it takes mosquitoes 7-10 days to totally complete their life cycle from egg to adult.

11.5 Summary

The mosquito goes through four separate and distinct stages of its life cycle: Egg, Larva, Pupa, and Adult. During each stage of its life cycle, the mosquito looks distinctly different than any other life stage. This is known as a complete metamorphosis and each stage can be easily recognized by its unique appearance and morphology.





11.6 Glossary

Adult - The mature stage of a mosquito's life history

Instar - The form assumed by insects between molts. Mosquitoes develop through 4 larval instars.

Larva(e) - For mosquitoes: the feeding aquatic life stage of the mosquito

Pupa(e) - The intermediate stage between larva and adult, where the insect body structures change to those present on the adult

Vector - An organism which transports and transmits a parasite or pathogen from one host to another.

11.7 Further reading

Timothy C. Winegard (2019) The Mosquito: A Human History of Our Deadliest Predator, ? Dutton;

Busvine J.R., (1980), Insects and Hygiene: The biology and control of insect pests of medical and domestic importance, Third Edition, Chapman and

Hall, London.

Lehane M.J., (1991), Biology of Blood-sucking Insects, Harper Collins Academic, London

11.8 Model questions

1. How many types of mosquito are found in India ?
2. Which sex of the mosquito are haematophagous ?
3. How many larval instar are found in the life history of mosquito ?

Unit - 12 ❑ Study of life history stages of Toad/Frog

Structure

12.1 Objective

The primary objective of this unit is to diiscuss the process by whiich the toad is born and developed into the adult and how the aquatic larval stage is developed into the terrestrial adult stage.

12.2 Introduction

A life cycle refers to the stages or changes that an animal goes through while it's alive. A life cycle repeats itself (or goes in a circle) for each new generation of life.Toads breed during the rainy season. Males come by the side of a suitable pond or ditch and begin to croak loudly by inflating their vocal sac. Invited by the song, the females come to the water in a large number. This is followed by mating or sexual reproduction.

12.3 Study of life history stages of Toad/Frog

Eggs & Zygote stage

The eggs look like small black spots placed upon white strings of jelly, thus forming a convoluted ribbon which floats freely on the surface of the water.

This is the toad spawn.

The average number of eggs that an amphibian can lay ranges from 2,000 to 6,000.

The spermatozoa swim actively by lashing their tail and a swarm of them encircle every egg on the spawn.

Each egg is fertilized by a sperm, the process taking place externally, that is outside the body, in water.

The fertilized egg is known as the oosperm or zygote.

It is a single cell which is destined to develop into a toad.

Zygotes are left in water without and parental care.

Each zygote undergoes a period of rest and then begins to develop by repeated cell division known as cleavage .

The embryonic development goes on for about two weeks.

Eventually the embryo hatches out by rupturing the egg membrane which iis known as larva.

Larval stages

The freshly hatched young larva is known as tadpole bears no resemblance to its parent.

It has an ovoid head, a short trunk, and a slender compressed tail with a small vent near its root.

There is no limb, but there is an adhesive sucker on the ventral side, by which it attached to some submerged object.

There is no mouth and it cannot ingest any food and depends on the residue of yolk.

Three pairs of branched processes, called external gills, develop on the sides of the head which helps in respiration.

After a few days a mouth is formed near the sucker and a pair of horny jaws encircle the mouth.

The tail grows longer and develops a dorsal and a ventral fold.

It is thus converted into a fin.

Meanwhile, the limbs have been developing as buds.

The hind limbs appear, one on either side of the root of the tail.

The forelimbs remain under cover of the operculum for a time and then emerge by bursting through it.

When the limbs grow in size the animal enters into a period of fasting and the tail is gradually absorbed into its body.

Finally the mouth widens and the horny jaws are replaced by true bony jaws.

The animal changes its food and becomes a carnivore; consequently its gut becomes short and more or less straight.

The total larval development is completed in about twelve weeks.

Metamorphosis of the larva

The term metamorphosis is applied to the series of changes which a larva has to undergo for altering its structure and mode of life before it comes to resemble its parents.

During metamorphosis of the tadpole larva, fish-like characters are given up and toad-like characters are taken on.

The larva is thoroughly changed in its mode of respiration, locomotion, and nutrition.

The metamorphosis occurs rapidly towards the end of the tadpole's aquatic life.

The metamorphosis is under the control of thyroxine hormone which is secreted from thyroid gland.

Metamorphosis is hastened if tadpoles are fed artificially with the substance of the thyroid gland.

On the other hand, removal of thyroid from a tadpole completely stops metamorphosis.

Adult stage

Adulthood is the last stage of the toad life cycle.

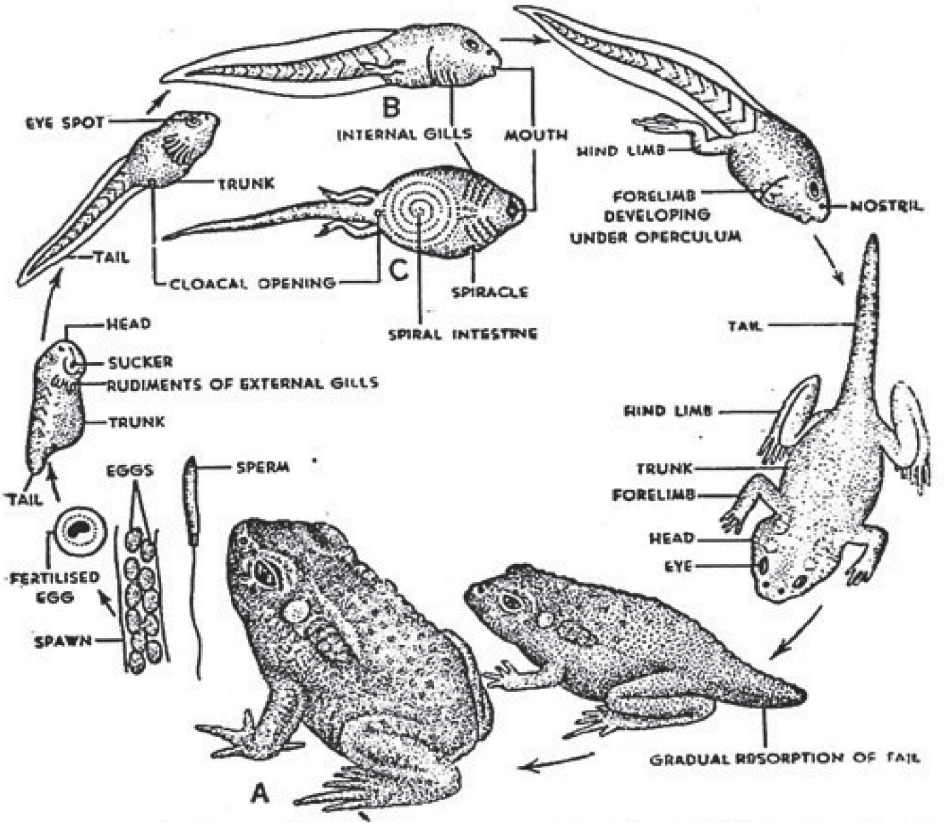
With the age, toads develop legs to move around on the ground and lungs to breathe oxygen.

They ultimately start to lose their tail.

It reaches maturity, the last step of the toad life cycle when its tail is absorbed.

Mature toads are more likely to spend the majority of their time on the ground and less time in the water. The tail gradually vanishes over time as it gets smaller and smaller. The tadpole develops appendages and sheds its gills at the same moment.

The adult toad is prepared to survive on land once this period of metamorphosis is over.



12.4 Conclusion

The life cycle of toad clearly iindicates that there is a transition from aquatic liife to terrestrial life with the help of changes in both morphological and physiological status. This change is commonly known as metamorphosis.Metamorphosis is totally regulated by pituitary and thyroid gland.Some environmental factors are also influencing the metamorphosis process.The amphibian metamorphosis is a type of complete metamorphosis.If there was no metamorphosis the tadpole remains in the larval state and if it is permanent then it is known as neotiny.If reproductive maturity occurs during neotiiniic period then iit iis known as paedomorphosiis.

12.5 Summary

In the life cycle of a toad, there are three stages. Eggs of a toad develop into a larva (tadpole) and later become an adult toad. These drastic changes in appearance and stages

are not as long as human growth. The changes in the stages from a larva to an adult are known as metamorphism. The life cycle of toad begin as an egg, hatch as tadpoles (newly hatched baby toads), grow into toadlets (teenage or not-fully grown toads), and finally become adult toads.

12.6 Glossary

Metamorphosiis - Profound changes in form from one liiife stage to next i.e. from tadpole to toad.

Spawn - Amphibian egg

Toadlet- young toad that has just completed its metamorphosis from a tadpole and left the pond.

Tadpole - Larval stages of toad

12.7 Further reading

Kentwood D. Wells (2007)- The Ecology and Behavior of Amphibians, University of Chicago Press, USA.

12.8 Model questions

1. What is metamorphosis ?
2. Which hormone played a major role in amphiibian metamorphosis ?
3. What do you mean by larva ?

Unit - 13 ❑ Study of Frog sperm and ova through permanent slides or photo-micrographs

Structure

13.1 Objective

The main aim of this unit is to discuss the structure of both male and female gamete of toad (Class-Amphibia) and how these will be identifiiied and their important characters.

13.2 Introduction

In amphibians, fertilisation is external, hence males and females produce a lot gametes and release in water to maximise the chances of fertilisation. Males of Class Arnphibia generally lack copulatory organs. The reproductive tracts open outside through cloacal aperture.Male frog gametes are sperm, and female frog gametes are eggs. Each gamete contains a single sex chromosome, chosen at random from one of the pair of adult sex chromosomes. Male frogs have an X-chromosome and a Y-chromosome, whilst female frogs have two X-chromosomes.Amphibian sperm has a flattened, acidic vesicle, called an acrosome, at the top of the sperm head. The acrosome contains several proteases which are discharged after acrosome reaction

13.3 Study of Frog sperm and ova through permanent slides

Structure of Sperm in Toad

The mature sperm measures on an average 0.03mm in length. It has an elongated solid

head with an anterior bead-like acrosome. The short middle piece is invisible but the tail appears as a gray filamentous extension about four or more times the length of the sperm head.The sperm head consists of mainly the nucleus and acrosome. Its shape, size and structure vary greatly in different groups of vertebrates.

The nucleus of the sperm occupies major part of the head and its shape, ultimately, determines the shape of the head of sperm. A large number of enzymes especially hydrolases are also present in the acrosome. It also contains two most important enzymes such as hyaluronidase and zona Iysin or acrosin which functions during sperm entry into the ovum

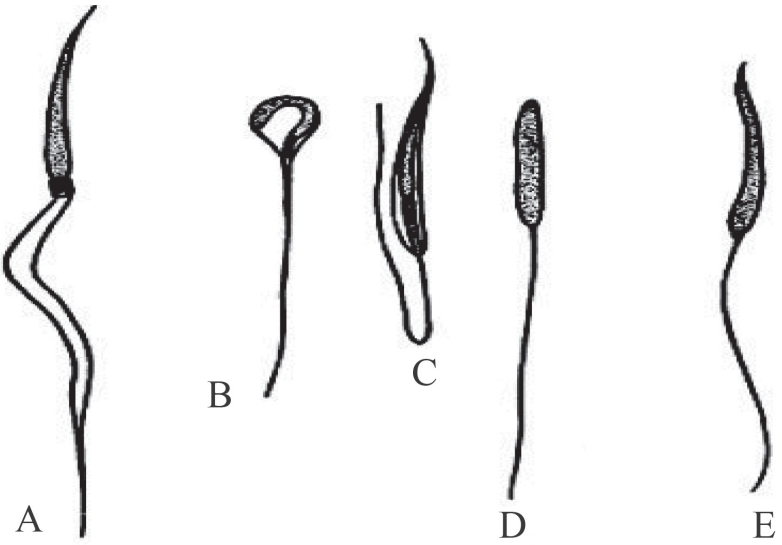
Structure of Egg in Toad

The egg for frog is about 2mm in diameter at the time of ovulation. It is surrounded by two accessory egg membranes in addition to the plasma membrane. Just outside the plasma membrane is a non living transparent membrane called vitelline membrane developed by the ovum itself. Outer to vitelline membrane is the jelly coat or albumen secreted by the walls of the oviduct. As soon as the egg reaches the water, the jelly coat swells up by the imbibitions of water and it protects the egg from injury and against infection by bacteria and other microorganisms. Frog's egg exhibits a well developed polarity and radial symmetry. The cytoplasm has two regions, the cortex and endoplasm.

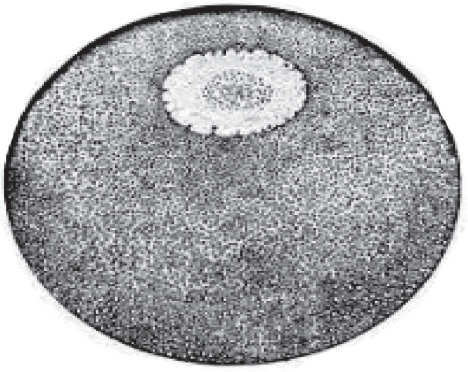
Mating posture of Toad

The female releases her eggs and the male releases his sperm at the same time. In order to make sure that the sperm reach the eggs, the male and female get into a mating posture called amplexus. The male climbs onto the female's back and clasps his forelegs around her middle. Frogs can stay in amplexus for hours or even days as the female releases as few as one or as many as several hundred eggs.

All Toads, eggs require moisture to develop, and most frogs abandon their eggs once they're fertilized. But not all eggs incubate underwater or without parental care. A few species carry their eggs in their vocal sacs or their abdomens. Others lay eggs in dry areas and keep the eggs moist with water or urine. Depending on the toad's species and the climate in which it typically lives, the eggs can hatch in a few days to a few weeks.



Amphibian spermatozoa



Amphibian egg

13.4 Conclusion

The spermatozoa of toad can not be seen with naked eyes. The spermatozoa is divided into three parts which are head part,middle part or neck part and tail part.

The head part iis oval iinshape and it contaiin nucleus. The ovum is often called an egg. The eggs appear as mustard-grain andcan be seen in naked eyes. The shape of the ovum of toad is rounded, one end is blackish and the other end is whitish in colour. At the

blackish end of ovum remains the cytoplasm and nucleus and in the whitish end portion remains the yolk. The amount of yolk in the ovum of the toad is plenty. Each ovum remains covered by a thin membrane. The egg having the yolk side is called a 'Telolecithal' egg.

13.5 Summary

In Toad the sexes are separate. Male and female toads are very much alike in external feature. During the breeding season, however, the female bulges out, being distended by her eggs, whereas the male remains slender. The male, on the other hand, produces cushion like thumb-pads at the bases of his first two fingers. This thickening is used for grasping the female. Lastly, the male can croak loudly because it has a vocal sac under its throat.

13.6 Glossary

Anura- Amphibians without tails

Exotic- a species not native to this country

Spawn- amphibian eggs.

13.7 Further reading

Blair, W. F., editor. [ed.] . (1972) Evolution of the genus Bufo. University of Texas Press, Austin.

Conant, R. (1958) A field guide to reptiles and amphibians. Houghton-Mifflin, Boston.

Goin, C. J., and O. B. Goin. (1971) Introduction to herpetology, 2nd ed. Freeman, San Francisco.

13.8 Model questions

1. What do you mean by sexual dimorphism ?
2. What is sex chromosome ?
3. How many regions are iin toad spermatozoa ?

Unit - 14 ❑ Study of developmental stages of chick embryo - whole mount permanent slides of 24hrs, 48hrs and 72 hrs embryo

Structure

14.1 Objective

The main objectiiive of this unit is that after completing this unit you will be able to identify the various stages of development in chick embryos from prepared permanent slides. In this unit you will observe under the microscope the developmental stages of chick embryo. In the present unit, you will study with the help of prepared slides, the salient developmental stages of chick observed by you under the compound microscope. You are expected to consult the present laboratory SLM (DSC-4) since the descriptions and labeled figures given in it will help you to study the developmental stages of chick properly.

14.2 Introduction

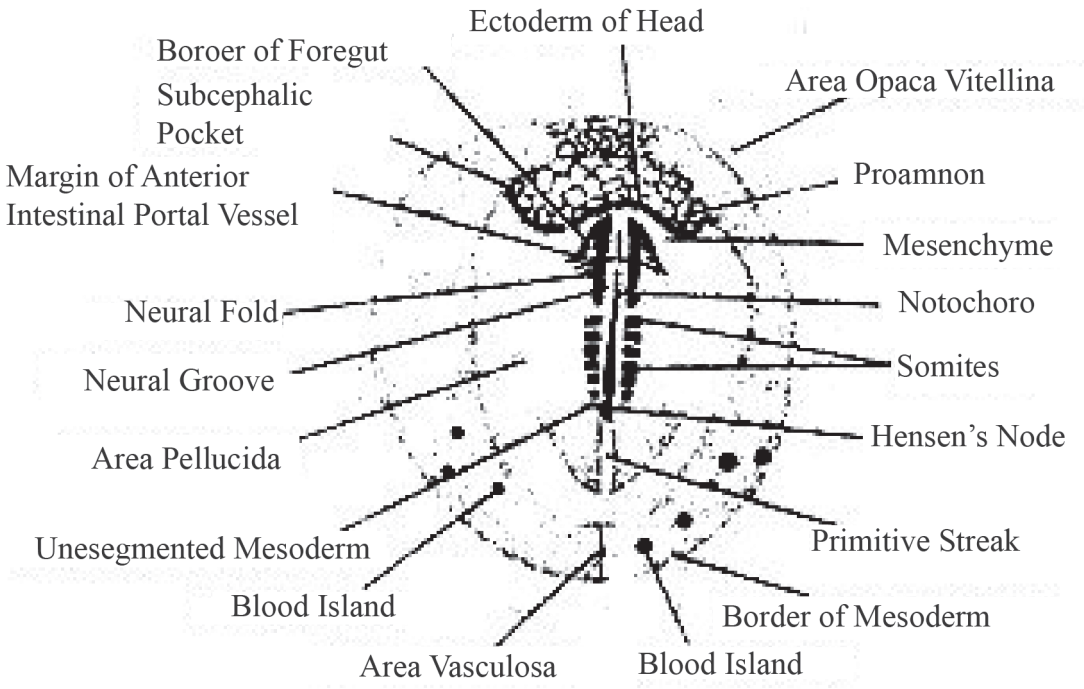
By tradition, the common domestic chick, Gallus gallus, is the popular choice in embryology laboratory because it is large, readily available, and useful in understanding the ontogenic phases of amniotes. Likewise, the phylogenetic relationship among most animals can be inferred from the development of the structures of a chick embryo.Since cleavage is over by the time the egg is laid, the chick embryo has already undergone the process of gastrulation.When the egg is laid some embryonic development has occurred and usulally stops untill iincubation to resume.Soon after incubation begins, a pointed thickened layer of cells becomes visible in the caudal or tail end of the embryo.

14.3 Study of developmental stages of chick embryo

The early stages of the chick embryos are commonly identified by the number of hours of incubation (at 38° - 39°C) needed to reach the point of development. Once the somites appeared, the number of somites may also be used.You should remember that in chick, the appearance of a primitive streak initiates the series of organogenesis. The embryo resembles a straight line surrounded by dense staining masses.

14.3.1 Whole Mount preparation of 24-Hour Chick Embryo

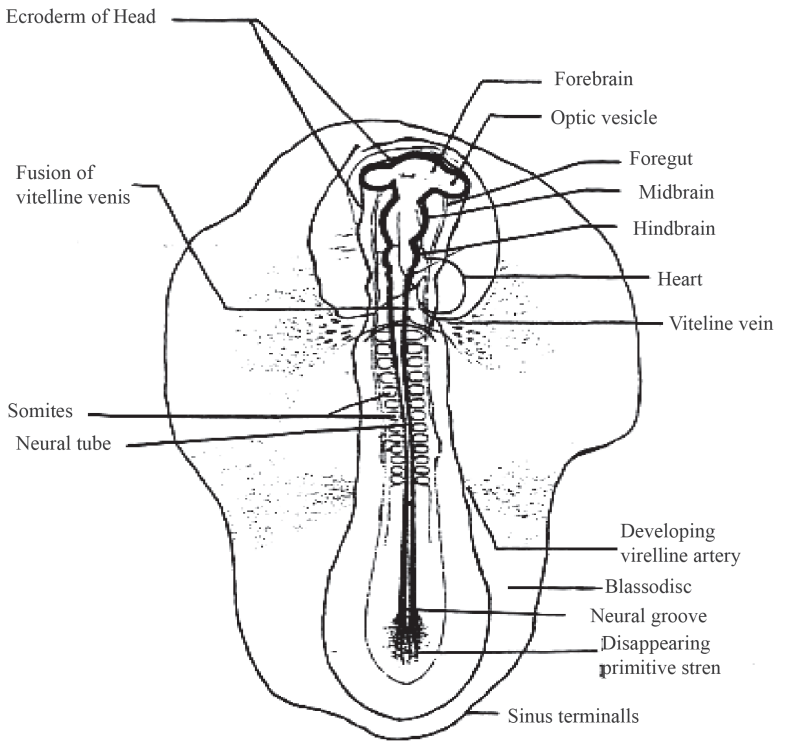
1. A one-day old chick embryo resembles a tall puppet with a veil, the head fold, and "swollen legs, the somites.
2. It is a four pairs of somites stage of chick.
3. These two parts are the most distinct features of the embryo in this stage due to its rapid growth and development.
4. The head elongated anteriorly via continuous cell proliferation and became elevated above the yolk region.
5. Generally four or sometimes five pairs of somites of the embryo serve as a benchmark of this stage.
6. At this stage the dark peripheral area opaca and central translucent and colourless area pellucida are distinctly visible.
7. In the anterior part is present the proamnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.
8. In the middle of area pellucida, in its posterior half runs a primitive streak with a primitive groove in its centre.
9. The primitive groove is bound by primitive folds.
10. The primitive streak and neural groove are separated by Hensen's node having a small depression in the centre-the Hensen's pit.
11. In the area pellucida embryonic and extra embryonic regions also become distinguished.
12. In front of Hensen's node the mesoderm of embryonic area differentiated into 3-4 pairs of mesodermal somites.
13. The foregut extends on either side into an amino-cardiac vesicle.



14.3.2 Whole Mount preparation of 48-Hour Chick Embryo

1. At this stage the area opaca and area pellucida are not visible.
2. The mesoderm, in front of Hensen's node, has given rise to 26-28 pairs of somites.
3. The extra embryonic area has grown in size.
4. Primitive streak has disappeared.
5. The brain has differentiated into telencephalon, prosencephalon, mesencephalon, metancephalon and mylencephalon.
6. The heart has been differentiated into ventricle and atrium.
7. The eye has been differentiated into optic cup and lens and optic vesicle has also developed sufficiently.
8. The head region has curved on right side due to cranial flexion.
9. Three pharyngeal gill-slits have also been differentiated.
10. Behind Hensen' node a tail bud has also developed.

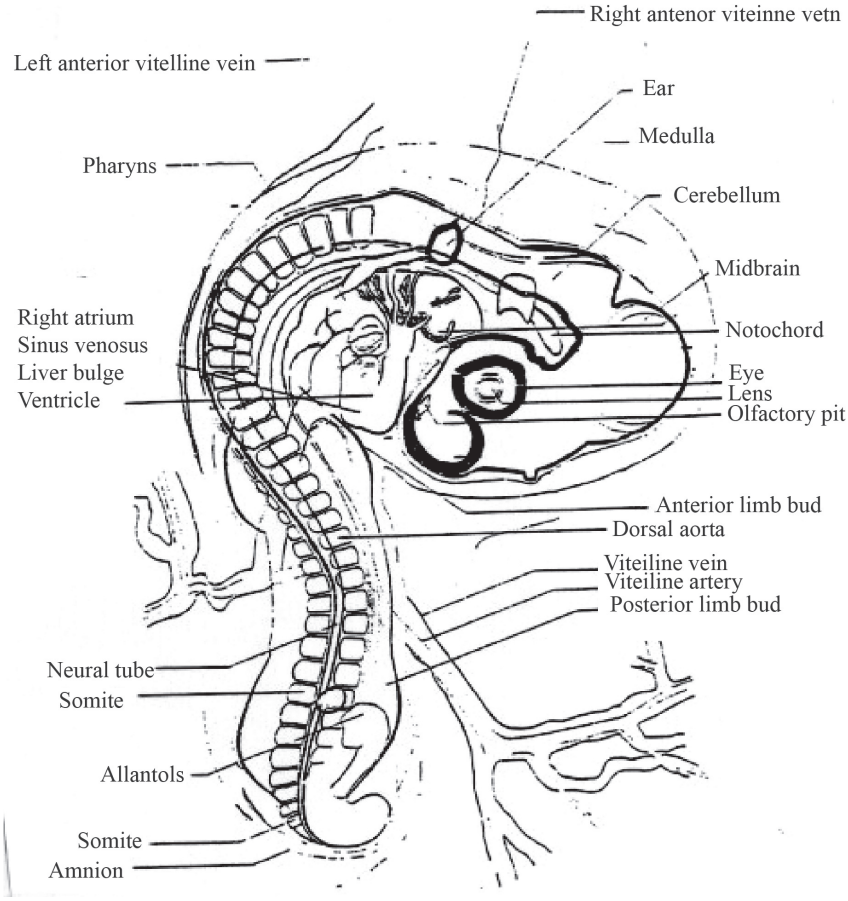
11. Lateral amniotic folds, anterior omphalomesentric vein and vitelline artery have appeared.
12. Two distinct circulatory systems are established, an embryonic system for the embryo and a vitelline system extending into the egg.



14.3.3 Whole Mount preparation of 72-Hour Chick Embryo

1. At this stage area opaca and area pellucida are not visible and distiguishable.
2. The extra embryonic area has grown in size.
3. Primitive streak has disappeared.
4. The mesoderm, in front of Hensen's node, has given rise to 36 pairs of somites.
5. The brain has differentiated into telencephalon, mesencephalon, metancephalon and mylencephalon.
6. The heart has been differentiated into ventricle and atrium.

7. The eye has differentiated into optic cup and lens and optic vesicle has also developed sufficiently.
8. The head region has bent on right side due to cranial flexion.
9. Four pairs of gill-slits have been differentiated.
10. Tail bud is greatly developed and has given rise to allentoic stalk and tail.
11. Lateral amniotic folds, vitelline artery and anterior omphalomesentric vein have developed.
12. In the middle region a pair of fore limb buds and in front of tail a pair of hind limb buds have developed, which will give rise to fore and hind limbs.
13. Olfactory pit, visceral arches, amnion, allantois and amniotic cavity have also developed.



14.3.4 How to prepare the whole mounts of Chick Embryo

As already described the egg of hen is telolecithal type and it develops only within a limited range of temparature which is only about 37 - 390C. It iis actually the body temperature of adult bird. Therefore the temperature of the incubator must be carefully controlled for proper development of the chick.

Materials Required

One fertilized hens egg , Incubator, forcep , Fine scissor, Scalpel , Petridish, Saline solutiion, Diistilled water, Different alcohol grade for dehydration purpose, Eosiin stain , One siimple miicroscope or binoccularmicroscope, Compound miicroscope for study.

Protocols

1. Clean the egg surface and then incubate it at 380C in incubator.
2. Remove the egg after desired time of iiincubation liike 24 hrs, 48 hrs and 72 hrs etc.
3. Now crack the egg at the broad end wiith the help of scalpel.
4. Remove the egg shell pieces gently till the opening of the egg shell is large.
5. Transfer the contents of the egg in a clean petridish containing enough saline.
6. Now observe the embryo under binocular or simple microscope.
7. Embryo will appear on the surface of the yolk approximately at the center.
8. Now hold the vitelline membrane with the help of forcep and cut iit close to the embryo.
9. Now carefully separate the embryo from the underlying yolk and transfer it to a watch glass.
10. Wash out the embryo so that all the attached yolk will be removed.
11. Now pass the embryo in graded alcohol for dehydration and after that stained the embryo iin Eosin stain which is dissolved in 90% alcohol.
12. After that pass the embryo in Absolute alcohol and then Xylene.
13. Now it is the time to mount the embryo in glass slide with the help of either Canada balsum or DPX mountant.
14. Place the slide on hot plate for drying up and further studies.

14.4 Conclusion

The chick has long been a selected model for studying developmental biology, owing to the ease with which the embryo can be accessed and manipulated inside the eggshell.

However, much of its first day of development following fertilization, which occurs inside the hen before the egg is laid (oviposition), remains shrouded in mystery.

14.5 Summary

As because the avian embryo has no anatomical connection to the hen, all of its nutritive requirements, except oxygen, must be contained within the egg. From very early on, the embryo develops special membranes external to its body to access the nutrients in the egg and to carry out essential bodily functions.There are four special membranes develops during the development of chick which are Yolk sac (used as a food source), Amnion (provides a shock-absorbing environment for proper development), Allantois (develops an extensive circulatory system connected to that of the embryo) and Chorion (fuses to the allantoin).

14.6 Glossary

Incubator- A machine repared for incubation of living thing at a particular temperature

Amnion- Extra embryonic membrane

Primitive streak- It is a region of the epiblast along which precursor cells of the mesoderm and the definitive endoderm ingress .

Animal pole - Where the nucleus of the egg is placed.

14.7 Further reading

Balinsky, B. I. (1975) Introduction to Embryology, 4th Ed. Saunders, Philadelphia.

Wolpert, L., R. Beddington, J. Brockes, T. Jessell, P. Lawrence and E. Meyerowitz. (1998) Principles of Development. Current Biology, Ltd., London.

S.F. Gilbert & A.M. Raunio (1997) Embryology. Constructing the Organism. Sinauer Associates, Sunderland, MA.

14.7 Model questions

1. How could you identify the chick embryo as 24 hrs of devbelopment ?
2. What is amnion ?
3. What is allantoin ?
4. What is the function of yolk in chick development
5. What do you mean by telolecithal egg ?