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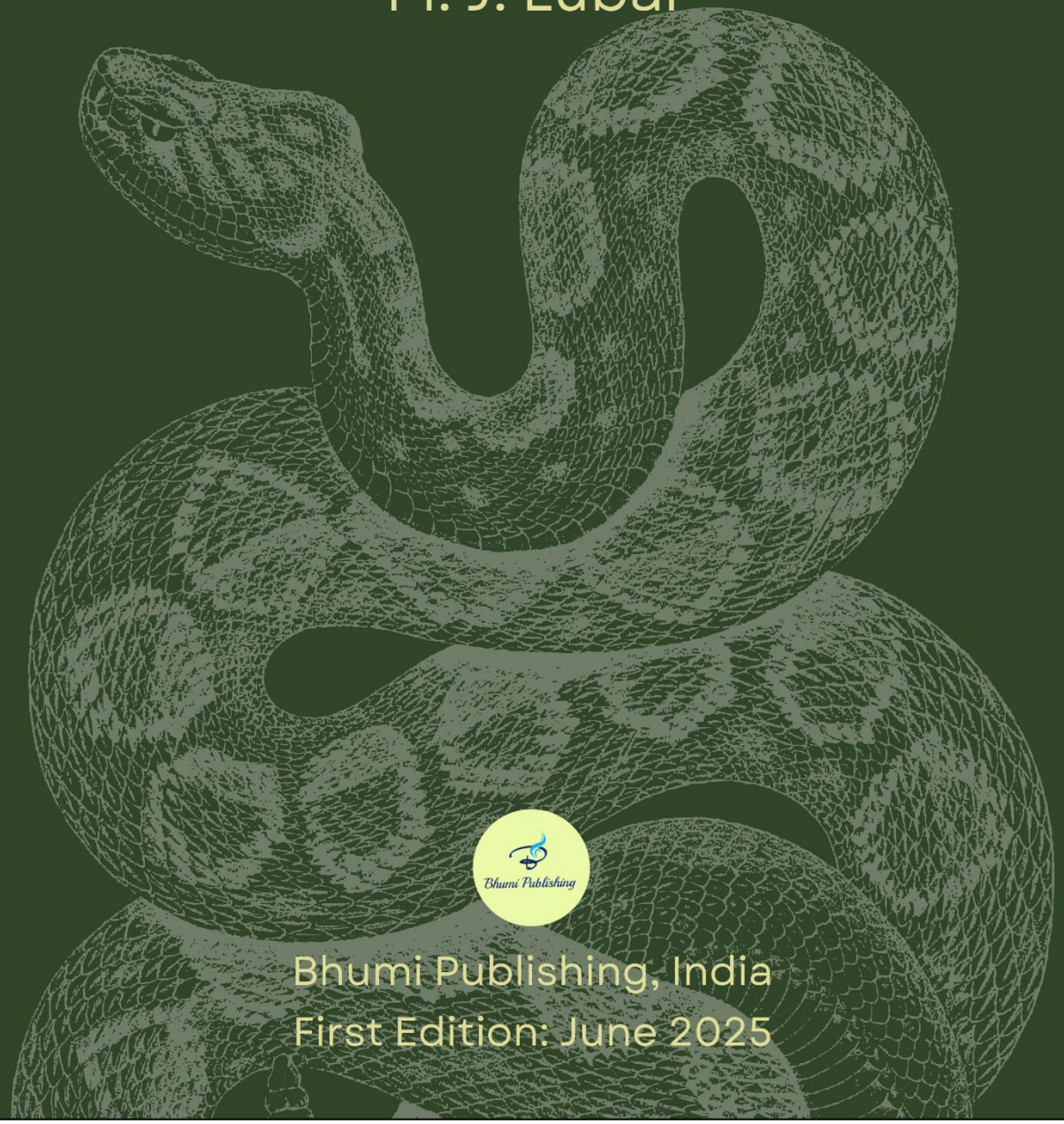
PRACTICAL HANDBOOK OF ZOOLOGY

(B. SC. II, SEM III)

AS PER NEP-2020 (2.0) SYLLABUS OF SHIVAJI UNIVERSITY, KOLHAPUR

K. J. Adate
V. V. Ajagekar
S. A. Vhanalakar
M. J. Lubal



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(IMPLEMENTED FROM JUNE 2025)

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PREFACE

This practical book for B.Sc. Part II, Semester III (NEP 2.0) Zoology (Major/Minor) has been meticulously designed to align with the curriculum of Shivaji University, Kolhapur. It aims to provide students with a comprehensive and hands-on learning experience in two fundamental areas of Zoology: Fundamentals of Chordates and Biochemistry.

We believe that practical learning is indispensable for a thorough understanding of biological concepts. This book is crafted to be user-friendly, providing clear instructions and theoretical background for each experiment. We encourage you to approach each practical session with curiosity and dedication, paying close attention to observations and developing critical analytical skills.

A compulsory study tour to a suitable location like a Sea Shore, Wildlife Sanctuary, National Park, or National Science Institute is an integral part of this curriculum. This immersive experience will provide you with invaluable exposure to animal diversity in its natural habitat, fostering a deeper appreciation for the subject. A detailed report of this tour will be a mandatory submission for your practical examination.

We are confident that this practical book will serve as an invaluable resource in your academic journey, fostering a deeper understanding and appreciation for the vast and intricate world of Zoology.

We wish you a successful and enriching learning experience.

- Authors

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B. Sc. PART – II SEMESTER – III (NEP 2.0)

MAJOR/MINOR ZOOLOGY PRACTICAL - III

(Based on Fundamentals of Chordates and Biochemistry)

UNIT I (FUNDAMENTALS OF CHORDATES)

1. Amphioxus

1. Systematic Position and Morphology
2. Transverse sections passing through
    a. Oral Hood b. Pharynx
    c. Intestine d. Tail

2. Rat: (Only Demonstration)

1. Systematic Position and Morphology
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3. Respiratory System
4. Excretory System
5. Male and Female Reproductive System
6. Heart
7. Brain

3. Study of Venomous and Non-Venomous Snakes

A. Identifying Characters of venomous and non-venomous snakes

B. Study of venomous snakes:
    a. Russell's viper b. Saw scaled viper c. Common krait
    d. Indian Cobra e. Sea Snake

C. Mild Venomous Snakes
    a. Cat snake b. Green vine snake

D. Study of Non- Venomous Snakes
    a. Python b. Rat snake
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E. First aid treatments (Simulation)

4. Dentition in Mammals: Rat, Sheep, Dog and Man

5. Aerial adaptations in birds- Morphological and Anatomical

UNIT – II (BIOCHEMISTRY)

1. **Biochemical tests**
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   B. Disaccharides - Sucrose, Lactose
   C. Polysaccharides – Starch
   D. Lipid
   E. Protein
2. Estimation of total protein by Lowry's method.
3. Estimation of casein from milk
4. Study of activity of salivary amylase under optimum conditions.
5. Effect of Temperature on activity of salivary amylase.
6. Effect of pH on activity of salivary amylase.
7. Detection of abnormal urine constituents.
8. Determination of Ascorbic acid/Vitamin C from a given sample.
9. Separation of Amino acids by paper chromatography.
10. Study Tour: Compulsory study tour to any suitable place to study animal diversity (sea Shore / Wildlife sanctuary / National Parks / National science Institutes) and submission of report at the time of practical examination.

1. Amphioxus

1. Systematic Position and Morphology

Systematic Position

Phylum: Chordata: Dorsal Tubular Nerve Cord, Gill-Slits and Notochord present

Group: Acraniata: No Head, Cranium or Brain.

Subphylum: Cephalochordata: Notochord persist along the entire body length

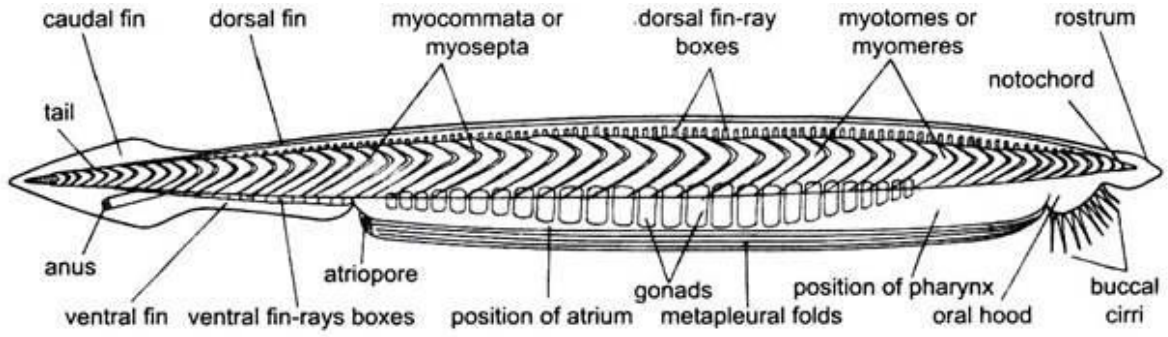
Class: Leptocardii: Numerous pharyngeal gill-slits.

Genus: *Branchiostoma*

Species: *lanceolatum*

Morphology

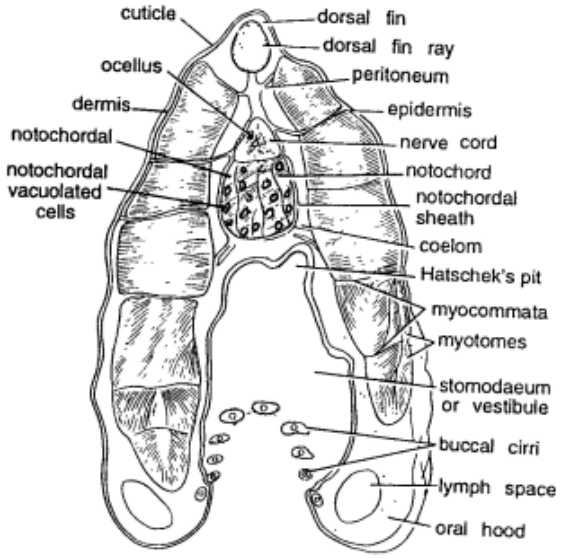
Amphioxus (commonly known as lancelet) inhabits clean, shifting and shallow coastal waters. It typically remains buried in the sand with only its anterior end exposed. Although it stays mostly buried during the day, it becomes active at night, swimming rapidly using swift, lashing movements of its tail. Amphioxus is a ciliary feeder, using cilia to direct food into its mouth.



- Commonly referred to as the *lancelet*.
- First discovered by Pallas in 1778.
- The adult measures less than 5 cm in length and has a superficial resemblance to a fish.
- Its body is elongated, laterally compressed, unpigmented and pointed at both ends—hence the name *Amphioxus* (meaning "both pointed").
- The anterior end extends forward into a structure called the *rostrum*.
- It possesses low, continuous dorsal, ventral, and caudal fins. Additionally, there are two lateral structures known as *metapleural folds*.
- The dorsal fin contains supportive structures called *fin rays*.

- The ventral mouth is surrounded by an *oral hood* bearing slender projections called *oral cirri*.
- The *atriopore* is a single, median, ventral opening, while the *anus* is located on the left side of the body.
- The body musculature is organized into metamerically arranged blocks called *myotomes*, which are separated by V-shaped partitions known as *myosepta* (or *myocommata*). These are composed of striated muscle fibers.
- A flexible *notochord* extends the entire length of the body, serving as an axial skeleton. A *nerve cord* runs just above the notochord.
- Amphioxus has 26 pairs of gonads aligned along the pharynx in a metameric pattern. The sexes are separate, no sexual dimorphism.

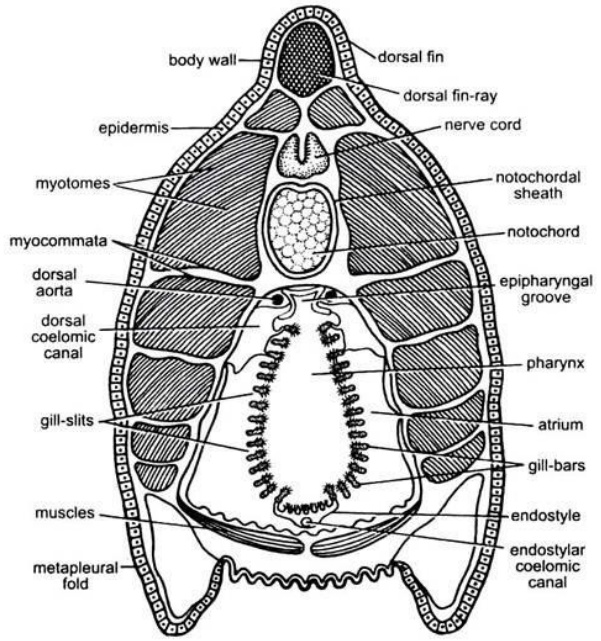
A) Transverse Section of Amphioxus Passing through Oral Hood



T.S. of amphioxus passing through oral hood

- Body Wall Composed of three main layers: Epidermis: Dermis and Muscle Layer: A thick layer of longitudinal muscles, arranged in segmented blocks (myotomes).
- Just beneath the dorsal epidermis lies a dorsal fin ray, providing internal support to the dorsal fin.
- Below the fin ray is the nerve cord, a tubular, glandular structure with a central cavity called the neurocoel.
- Beneath the nerve cord lies the notochord, which extends forward even into the rostrum in Amphioxus.
- The oral hood forms a prominent mid-ventral extension and encloses the vestibule, the entrance to the buccal cavity.
- The Dorsal wall of the buccal cavity has a sensory Hatscheck's groove.
- This region is involved in filter feeding and includes oral cirri (not always visible in all sections) that help in preventing large particles from entering.

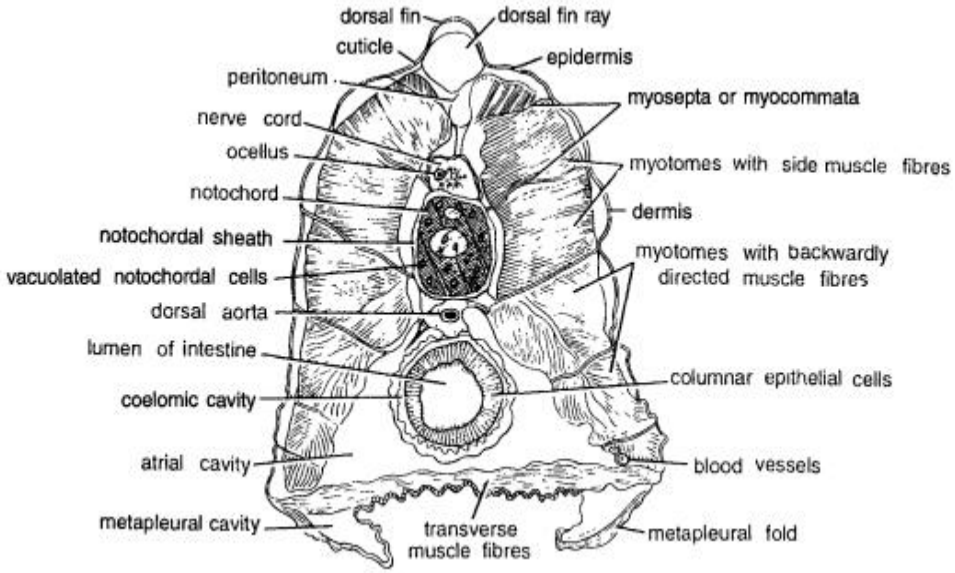
B) Transverse Section of Amphioxus Passing through Pharynx



T.S. of amphioxus passing through pharynx

- The body wall, composed of distinct layers: an outer cuticle, followed by the epidermis, a thin dermis and finally, a muscle layer.
- A significant portion of the dorsal side (more than three-fourths of the cross-section) is occupied by thick, segmented muscle bundles known as myotomes.
- Just beneath the epidermis, lies the dorsal fin ray.
- Ventral to the dorsal fin ray, we find the nerve cord, and situated beneath the nerve cord is the notochord. The notochord itself is encased in a notochordal sheath and filled with vacuolated notochordal cells.
- The ventral half of the cross-section is dominated by the large pharynx, which is surrounded by the atrial cavity and perforated by numerous gill slits.
- Internally, the pharynx features longitudinal bands of cilia. These cilia form an epipharyngeal groove along the mid-dorsal line and an endostyle, enclosing an endostylar canal, along the mid-ventral line.
- The lateral walls of the pharyngeal cavity are supported by several gill arches.
- In posterior sections of the pharynx, two metapleural folds, enclosing a metapleural cavity, can be observed.
- Depending on the specific section examined, a midgut diverticulum, also known as the liver, may be visible.

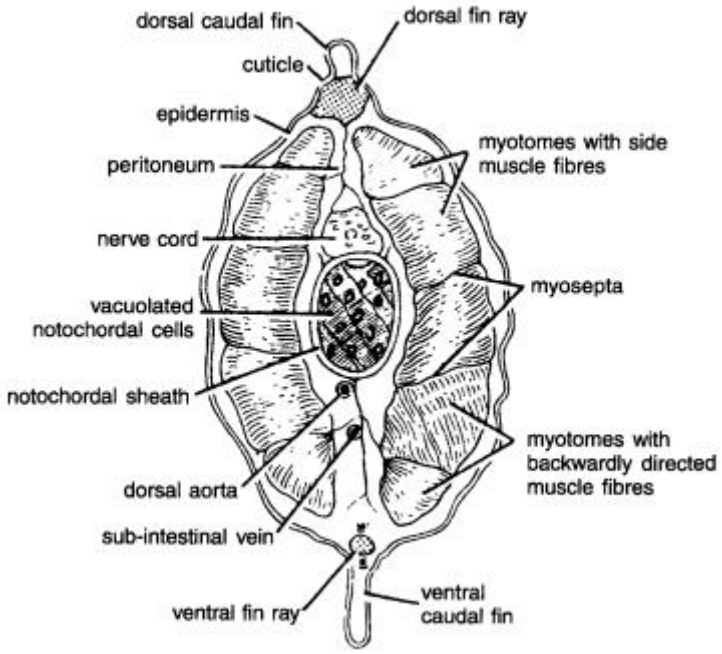
C) Transverse Section of Amphioxus Passing through Intestine



T.S. of amphioxus passing through intestine

- The body wall consists of a thin cuticle, a single-layered epidermis dermis and myotomes.
- The intestine is present centrally and appears as a circular or oval structure in cross-section.
    - It has a ciliated inner lining and a well-defined intestinal wall.
    - The intestinal lumen contains food particles and digestive enzymes.
- Surrounding the intestine is the coelomic cavity, which is reduced but present.
- The notochord is located dorsal to the intestine, and appears as a circular or oval rod composed of vacuolated chordal cells.
- Above the notochord lies the nerve cord, which contains a small central canal known as the neurocoel.
- The dorsal fin is present on the outer side, supported internally by the dorsal fin ray just beneath the epidermis.
- The atrium (or atrial cavity) surrounds the intestine and is lined by atriopore epithelium. This cavity collects water from the pharynx and channels it out through the atriopore.
- The metapleural folds (paired lateral extensions) are also visible in this section.
- Blood vessels, such as dorsal and ventral blood vessels, may also be observed near the notochord and gut region.

D) Transverse Section of Amphioxus Passing through Tail



T.S. of amphioxus passing through tail

- This section is relatively smaller in size and does not include any body openings.
- The body wall consists of a thin cuticle, a single-layered epidermis dermis and myotomes.
- Just beneath the epidermis of the dorsal side lies the dorsal fin ray, which supports the dorsal fin.
- Below the dorsal fin ray is the nerve cord, containing a central canal known as the neurocoel.
- Under the nerve cord lies the notochord, which consists of vacuolated chordal cells, serving as a flexible axial skeleton.
- Beneath the notochord are the caudal artery and caudal vein.
- This section lacks the alimentary canal, atrial cavity, coelom, and metapleural folds, as these structures are absent in the tail region.
- Posteriorly, the caudal fin, supported by fin rays, is present.

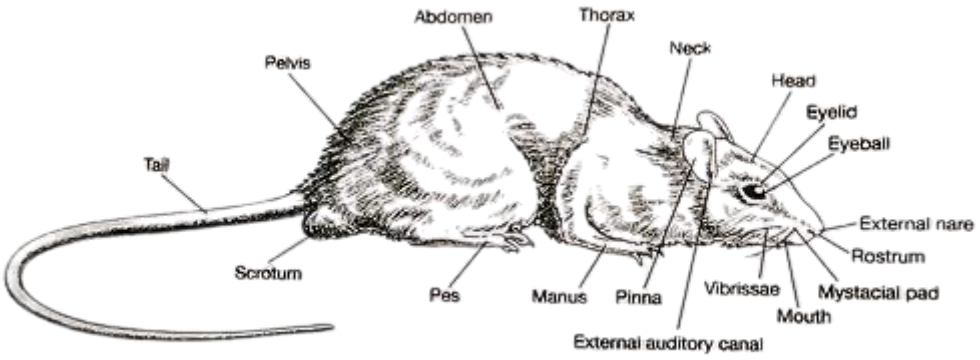
2. Rat: (only Demonstration)

1. Systematic Position and Morphology

1. Systemsatic Position

- **Kingdom:** Animalia
- **Phylum:** Chordata
- **Subphylum:** Vertebrata (Craniata)
- **Superclass:** Gnathostomata
- **Class:** Mammalia
- **Subclass:** Placentalia
- **Order:** Rodentia
- **Genus:** *Rattus*
- **Species:** There are numerous species of rats, the most common being the Brown Rat (*Rattus norvegicus*) and the Black Rat (*Rattus rattus*).

Rats are mammals belonging to the order Rodentia, the largest order of mammals, characterized by their continuously growing incisors used for gnawing. As chordates and vertebrates, they possess a notochord (present during embryonic development, later replaced by the vertebral column), a dorsal hollow nerve cord, pharyngeal slits (present during embryonic development), and a post-anal tail. Their placement within Mammalia signifies that they are warm-blooded, have hair or fur, possess mammary glands (in females), and typically give birth to live young.



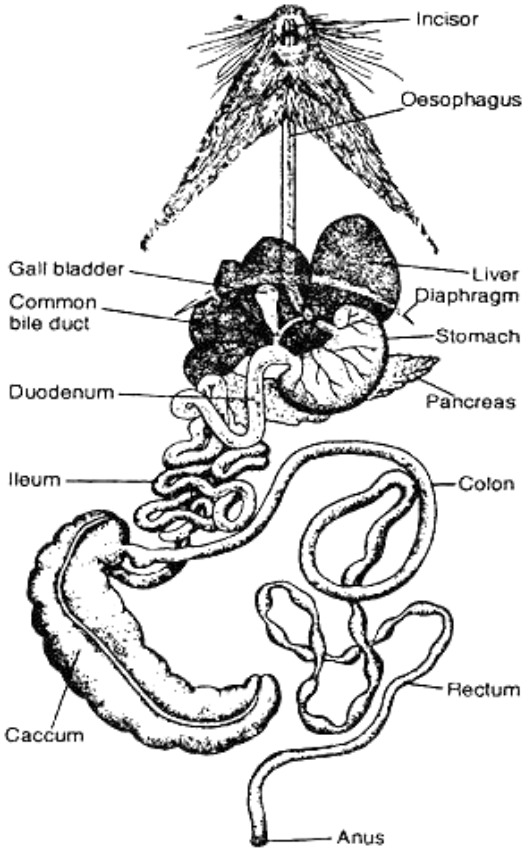
2. Morphology

Rats exhibit a morphology adapted for their diverse lifestyles, which can include burrowing, climbing and scavenging. Here are some key morphological features

- **Body Shape and Size:** Rats typically have an elongated, cylindrical body that tapers at both ends. Their size varies depending on the species, with body lengths ranging from about 16 to 40 cm (excluding the tail) and weights from 150 to 500 grams.

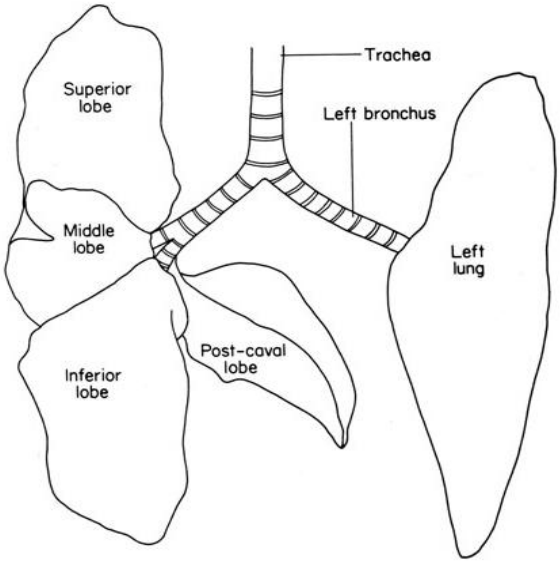
- **Head:** The head is generally conical, tapering to a pointed snout or muzzle.
    - **Snout:** Bears a pair of nostrils that can be closed underwater. Long, stiff, bristle-like sensory hairs called **vibrissae** (whiskers) are present around the mouth and nostrils, used for tactile sensing.
    - **Mouth:** Small with a cleft in the upper lip exposing large incisors.
    - **Eyes:** Located laterally on the head, with a large pupil and a **nictitating membrane** (a third eyelid) for protection.
    - **Ears:** A pair of rounded external ears (pinnae) are situated on the postero-lateral sides of the head, enclosing the external auditory canal.
- **Neck:** A short neck connects the head to the trunk, allowing for head movement.
- **Trunk:** The trunk is elongated and divisible into the thorax (chest) and abdomen (belly).
    - The female rat has **six pairs of nipples (teats)** on the ventral surface of the trunk (three thoracic, three abdominal).
    - Two pairs of limbs (forelimbs and hind limbs) are attached to the trunk.
    - The **anus** (outlet of the digestive tract) and external sex organs are located at the posterior end of the trunk.
- **Limbs**
    - **Forelimbs:** Smaller than the hind limbs, consisting of the upper arm (brachium), forearm (antebrachium) and hand (manus) with digits bearing claws.
    - **Hind limbs:** Larger and more powerful, adapted for locomotion, consisting of the thigh, lower leg, and foot (pes) with five toes, each with a pointed claw. The soles of the feet have pads.
- **Tail:** Typically, cylindrical, tapering, and as long as or longer than the body (depending on the species). The tail is covered by overlapping epidermal scales arranged in rings and is sparsely covered with short hairs or appears naked. It serves as a balancing organ, and in some species, it plays a role in thermoregulation.

2. Digestive System of Rat



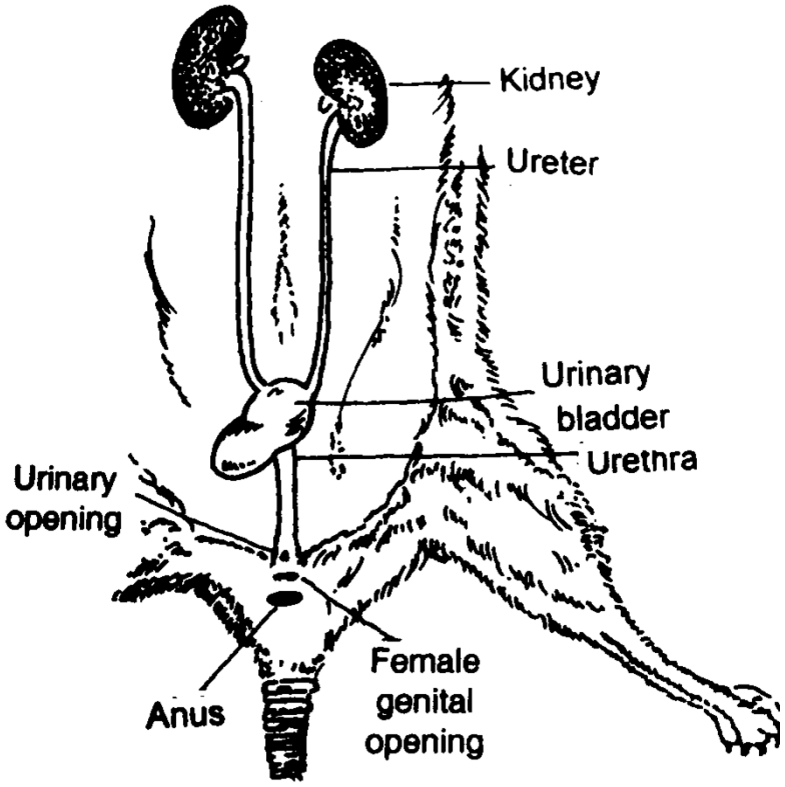
- It consists of an alimentary canal and digestive glands.
- Alimentary canal is made up of the mouth, oesophagus, stomach, intestine, caecum, colon, rectum and anus.
- Mouth- True mouth with cleft in lower lip. Ingestion of food
- Oesophagus- acts as a passage between mouth and stomach. It conducts the food from mouth to stomach by peristalsis.
- Stomach- acts as temporary storage for food & its partial digestion, secrets Hcl by oxyntic cells and peptic juice by gastric glands.
- Intestine is divided into– Duodenum, ileum, colon, caecum and rectum.
- Intestine – Anterior most part of the intestine called duodenum which forms a loop and entangles pancreas. The remaining part of the intestine is a long narrow ileum. It plays an important role in digestion of food and absorption of digested food.
- Caecum is present at the junction of ileum and colon. It contains cellulose digesting bacteria.
- Colon- it absorbs water from undigested food.
- Rectum- Stores undigested food and forms its pellets.
- Anus- Egestion of undigested food.

3. Respiratory System of Rat



- The rat respiratory system consists of two main sections: the respiratory tract and the respiratory organs.
- The respiratory tract includes the external nares, nasal cavities, internal nares, pharynx, larynx, trachea, and bronchi.
- The respiratory organs are a pair of lungs, located within the pleural cavity of the thorax.
- Unlike the left lung, which has a single lobe, the right lung of a rat is divided into four lobes.
- The primary function of the lungs is to oxygenate the blood.

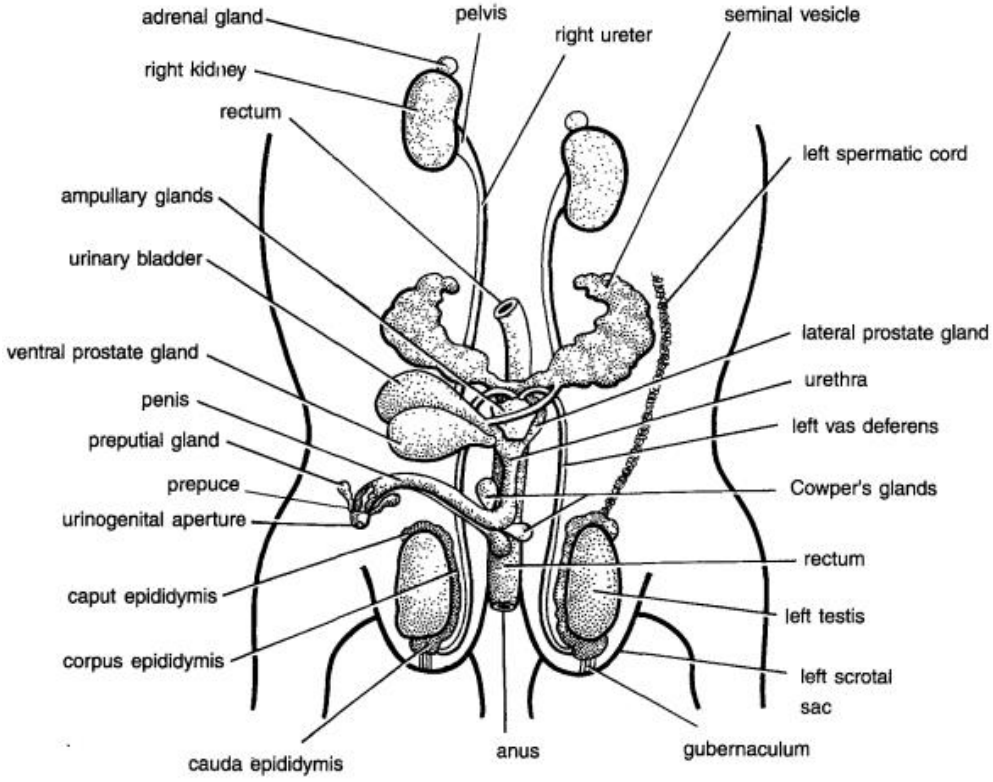
4. Excretory System of Rat



- Excretory system consists of a pair of kidneys, a pair of ureters, urinary bladder, urethra & urinary opening.
- Morphologically, kidneys appear bean shaped.
- It separates nitrogenous waste and excess water as osmoregulatory in function from blood.
- Ureters act as passage for urine towards the urinary bladder.
- Urinary bladder stores urine for some time.
- Urethra disposes of urine through the urinary bladder.

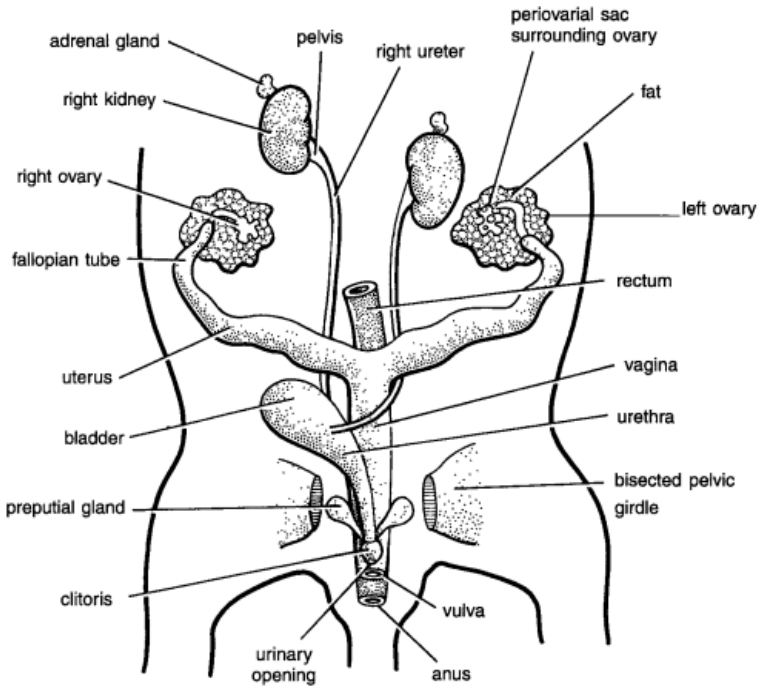
Reproductive System of Rat

Male Reproductive System of Rat



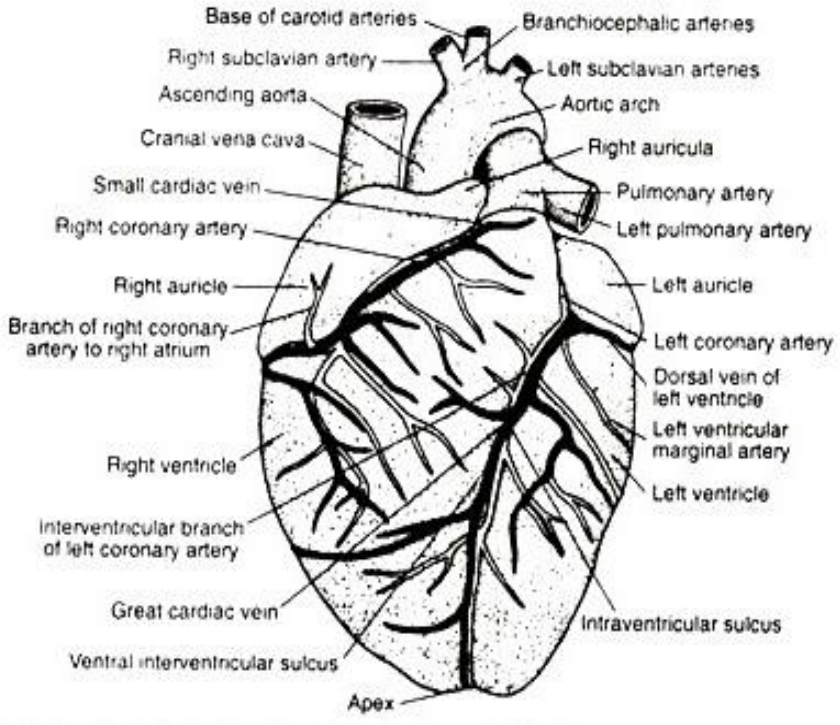
- Male reproductive system consists of a pair of testes pair of epididymis, a pair of vasa differentia, a pair of seminal vesicles, associated glands, urethra and penis.
- Testes produce sperms, vasa deferentia convey them from epididymis to seminal vesicles that secretes a major part of semen, and associated glands activate the sperms.
- The penis acts as an intermittent organ to transfer the sperm during coitus.
- In male the genital and excretory product use a common path for some distance & internally connected so it is called urinogenital system.

Female Reproductive System of Rat



- Female reproductive system consists of a pair of ovaries, a pair of oviducts, uterine horns & vagina.
- Ovaries produce mature ova, oviduct brings it in the uterus, by that time ova get fertilised & get implanted in the uterus where they get fully developed.
- Vagina receives penis during mating.
- It also acts as a parturition path during birth of young ones.
- In females, genital and excretory systems are not internally connected, so it is simply called the reproductive system.

Heart of Rat

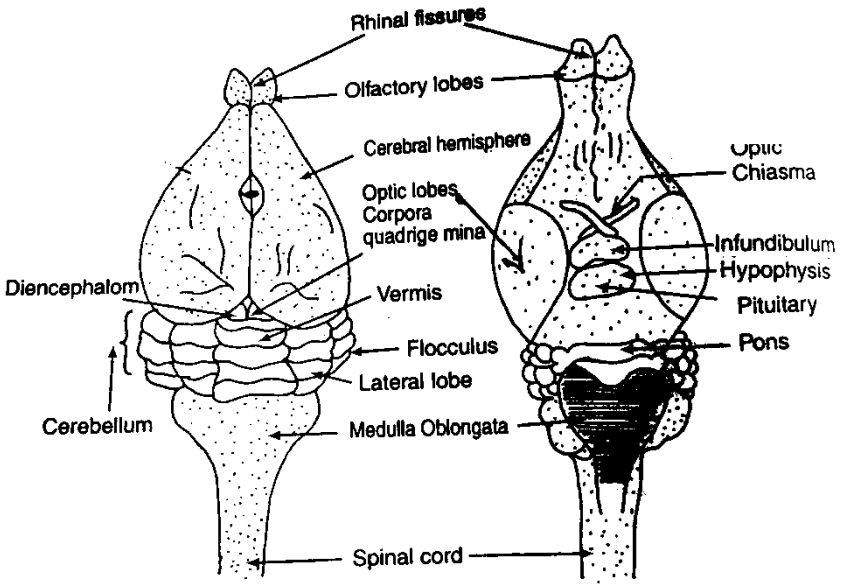


The rat heart is a four-chambered muscular organ that efficiently pumps blood throughout the body via a double circulatory system. Its structure, including the atria, ventricles, valves and septa, ensures the separation of oxygenated and deoxygenated blood and the unidirectional flow necessary for maintaining life. The rapid heart rate is a key physiological adaptation for a small mammal with a high metabolic rate.

The rat heart consists of four chambers

a) **Right Atrium:** Receives deoxygenated blood from the body through large veins called the **vena cava** (superior and inferior vena cava).
b) **Right Ventricle:** Receives deoxygenated blood from the right atrium and pumps it to the lungs through the **pulmonary artery** for oxygenation.
c) **Left Atrium:** Receives oxygenated blood from the lungs through the pulmonary veins.
d) **Left Ventricle:** Receives oxygenated blood from the left atrium and pumps it to the rest of the body through the aorta, the largest artery in the circulatory system. The left ventricle has thicker walls than the right ventricle because it needs to generate more force to pump blood to the entire body.
e) The heart rate of a rat is typically much faster than that of a human, ranging from **250 to 450 beats per minute** under normal conditions. This high heart rate is necessary to meet the relatively high metabolic demands of a small, active animal.

Brain of Rat

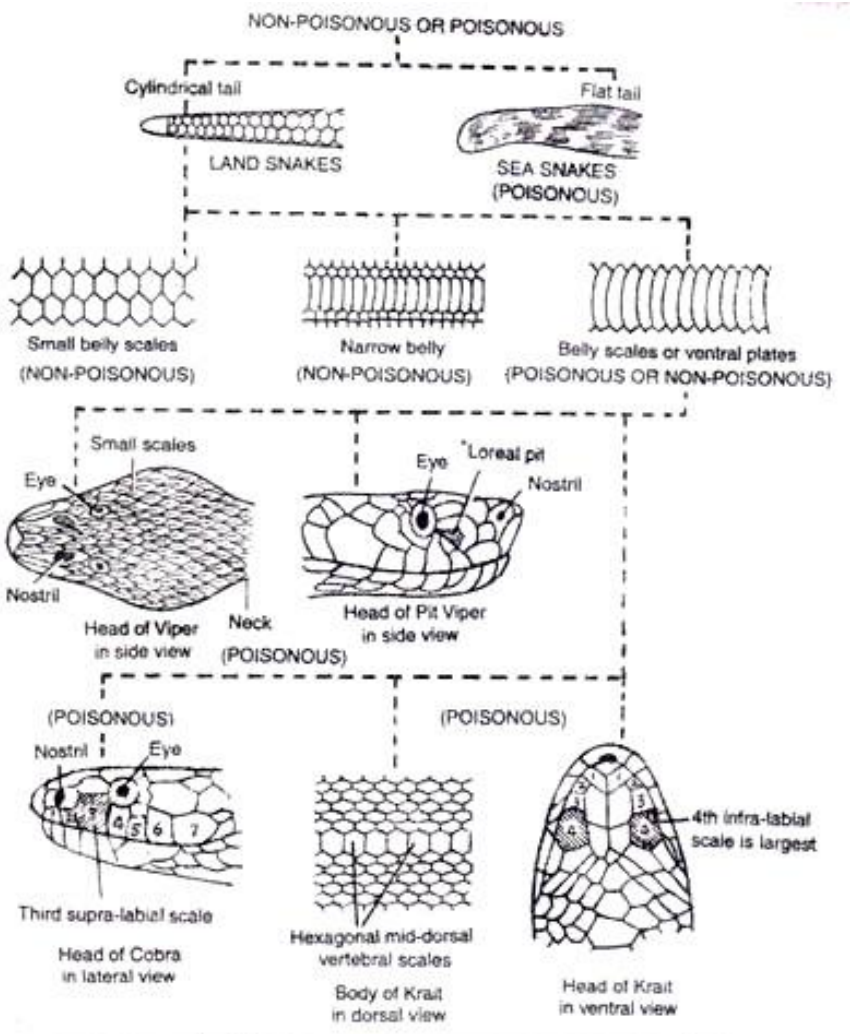


Dorsal View **Ventral View**

- Morphologically brain is divided into three parts
  - i) Fore brain or prosencephalon.
  - ii) Midbrain or mesencephalon.
  - iii) Hindbrain or rhombencephalon.
- **Forebrain consists of** i) Olfactory lobe (sense of smell)- highly developed
- ii) Cerebral hemisphere (Seat of intelligence, will power, memory and controls the voluntary actions)- highly developed iii) Diencephalon dorsally (provides nourishment to brain) and iv) iv) Infundibulum and hypophysis ventrally together forms pituitary body (hormonal and enzyme regulation).
- **Midbrain consists** of i) Optic lobes or corpora qudrigemina (sense of sight or vision)- Highly developed, ii) Cruracerebri - thick nerve bands ventrally (Transmission of information from forebrain to hindbrain and vice-versa.
- **Hindbrain consists of** i) Cerebellum made up of five lobes (function to control muscular and static (equilibrium)- Highly developed) ii) Medulla oblongata (Control involuntary activities like- respiration, digestion, heart beating, excretion etc.)

3. Study of Venomous and Non-Venomous Snakes

A. Identifying Characters of venomous and non-venomous snakes



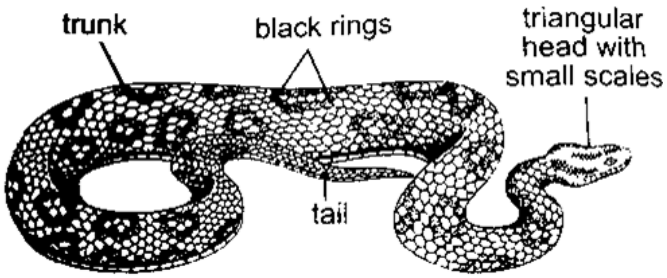
1. If the small scales are present on the belly and back, it is a non-poisonous snake.
2. If the belly scales are not broad enough to extend right across it, it is a non-poisonous snake.
3. If broad plates cover the entire width of the belly, it is poisonous or non-poisonous.
4. If small scales are present on the head, it is poisonous and a viper.
5. If small scales or shields are present on the head and a pit lies between the eye and the nostril, it is poisonous and a pit-viper.
6. If the dorsal side of the head has both small scales and large shields, the snake may or may not be poisonous.
7. If the third supra labial scale touches the eye and the nostril, the snake is a cobra or a coral snake. If the neck is with a hood and markings, it is a cobra. If the neck is without a hood and coral spots are present on the belly, it is a coral snake. Both cobra and coral snakes are poisonous.

8. If vertebral (scales on the middle of the back) are hexagonal and larger than other scales over the back and the fourth infra-labial scale is the largest, it is poisonous and a Krait snake.
9. If the snake has small scales and large shields on the head but does not have the characteristics of cobra, coral-snake or krait, then it is nonpoisonous.

B. Study of Venomous Snakes

A. Russell's Viper

The Russell's viper, a highly venomous snake, possesses distinct characteristics that make it both intriguing and dangerous.

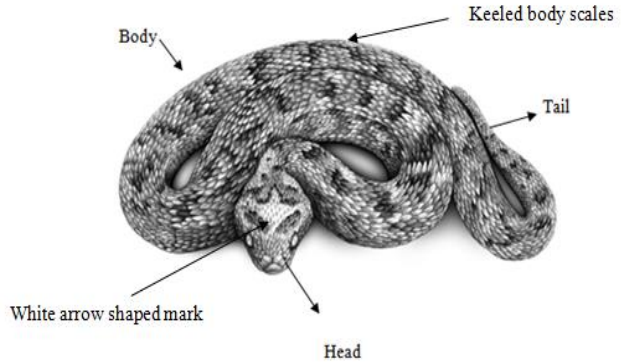


1. **Venom and Bite:** Russell's viper is notorious for its deadly haemotoxic venom. A bite from this snake can lead to severe consequences, causing tremendous pain, swelling and irritation at the site of the bite. In some cases, the venom can also lead to systemic effects, including potential heart failure, which can be fatal if not treated promptly.
2. **Dorsal Markings:** The snake's dorsal side features three distinctive longitudinal lines, forming a diamond-shaped pattern along its back.
3. **Coloration:** Russell's viper typically displays a pale brown color on its dorsal side, while the ventral parts appear yellowish white.

4. **Head Shape and Scales:** The viper's head is triangular in shape and covered with small scales. This characteristic head shape is a key identifier for distinguishing it from other snake species.
5. **Snout and Nasal Opening**: The snake's snout is angular and its nasal opening is quite prominent, contributing to its unique appearance.
6. **Eyes:** Russell's viper has striking golden eyes with an elliptical pupil, adding to its captivating yet intimidating appearance.
7. **Hissing Behavior:** When provoked or threatened, Russell's viper can produce a loud hissing noise, similar to the sound of a pressure cooker. This warning signal serves as a deterrent to potential threats.

B. Saw Scaled Viper

The Saw-scaled Viper is a venomous snake with distinct characteristics that make it both dangerous and fascinating.

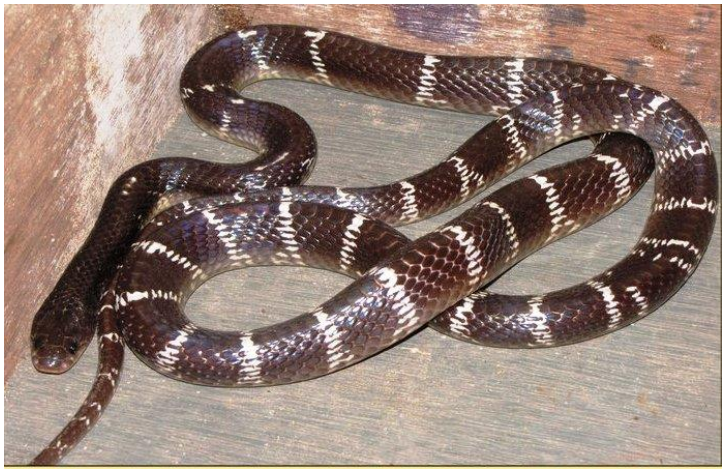


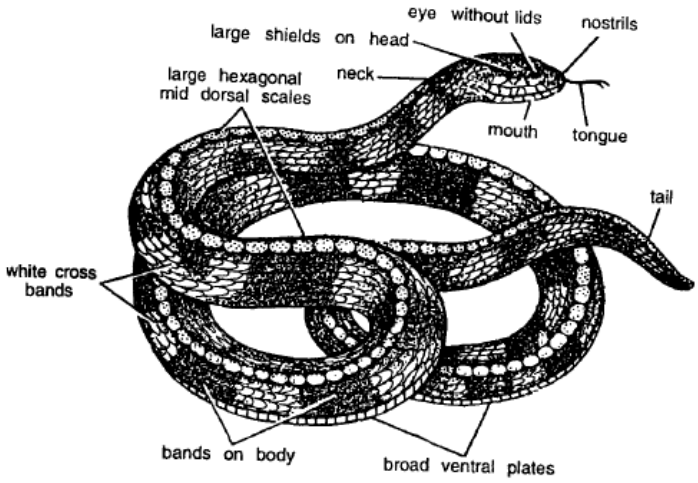
1. **Venom and Bite:** The Saw-scaled Viper's venom is potent and haemotoxic. A bite from this snake can result in severe consequences, including tremendous pain, swelling, and irritation at the bite site. In some cases, the venom can lead to systemic effects, potentially causing heart failure and even death if not promptly treated.
2. **Size:** This species is relatively small, with a maximum length of around 3 feet.

3. **Sound Production:** Unlike hissing, the Saw-scaled Viper produces a unique sound while moving. The friction between its rough scales and the ground creates a distinctive sizzling or saw-like sound, from which it derives its name.
4. **Head and Snout:** The snake's head is short, wide and pear-shaped, clearly distinct from its neck. The snout is short and rounded.
5. **Eyes:** Saw-scaled Vipers have large eyes that are positioned well forward on their head, aiding in their ability to detect movement and locate prey.
6. **Crown and Scales:** The crown of the head is covered with small, irregular imbricate scales, which may have a smooth or keeled texture. The dorsal scales on its body are keeled, providing a rough appearance.
7. **White Arrow Mark:** Behind the eyes, there is often a distinct white arrow-shaped mark on the head, which serves as a distinguishing feature.

C. Common Krait

The Common Krait, scientifically known as Bangarus, is a venomous snake with several distinctive features

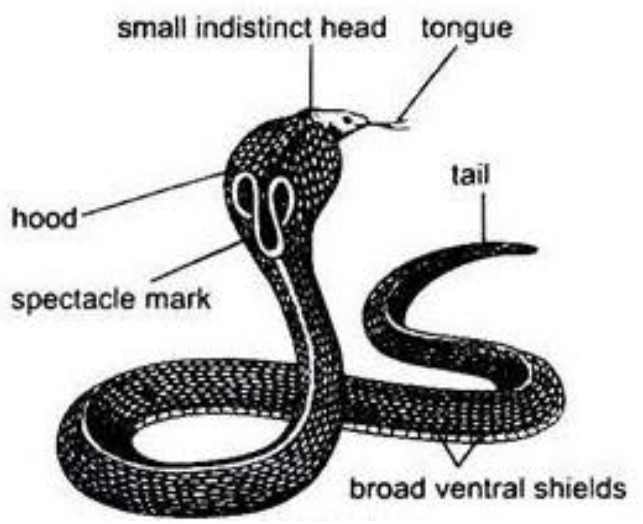




1. **Size and Name:** The Common Krait typically measures about one meter in length. It belongs to the Bangarus genus and is commonly referred to as the Krait.
2. **Coloration:** The snake's coloration is a striking combination of glittering blue or black, with two narrow white crossbars running both dorsally and ventrally. The ventral side is uniformly white.
3. **Infralabial Scales**: Among the scales surrounding the lips, the fourth infralabial scale is notably the largest.
4. **Eyes:** The Common Krait has eyes of moderate size and the pupil appears narrow in shape.
5. **Scales:** The vertebral scales, extending from the neck to the tail tip, take on a hexagonal shape. On the ventral side, the scales are smooth and entire.
6. **Subcaudal Scales:** The scales on the underside of the tail are arranged in a single row.
7. **Venom and Bite:** The Common Krait is highly venomous, and its venom is primarily neurotoxic. A bite from this snake can lead to severe symptoms, including tremendous pain, swelling, and continuous blood flow from the wound. Additionally, there can be internal bleeding in organs, which may result in death due to hemorrhages.

D. Indian Cobra

The Cobra, also known as the Indian cobra or Nag in Marathi, is a venomous snake with several distinguishing features and potent neurotoxic venom. Here are some key characteristics of the cobra

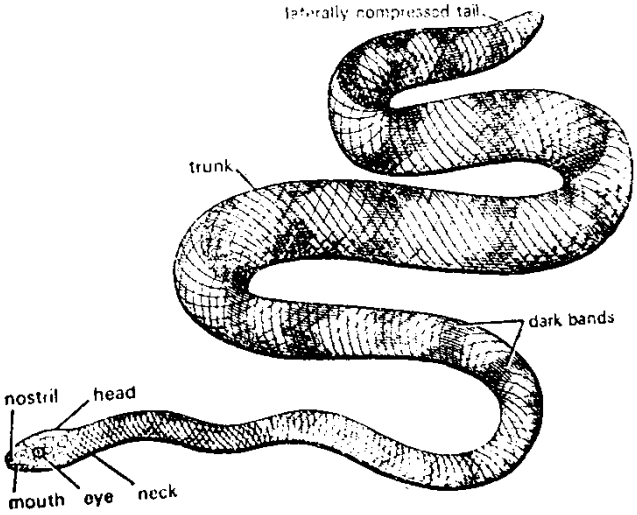


1. **Physical Appearance:** Cobras have a brown or black body covered with smooth, oblique scales arranged in 15 – 25 rows. Their subcaudals are divided into two rows.
2. **Hood and Hissing:** One of the most recognizable features of a cobra is the ability to expand its neck and cervical ribs to form a distinctive hood when threatened or excited. During such instances, it produces a loud hissing noise as a warning or prelude to an attack.
3. **Mark of Spectacle:** Dorsal to the hood, there is a prominent bicoelous mark resembling a figure of the number "ten" or a pair of spectacles, which adds to its unique appearance.
4. **Supra-labial and Eye Connection:** The third supra-labial scale of the cobra makes contact with both the eye and the nostril.
5. **Poison Fangs**: Cobras possess two long, hollow fangs in their upper jaw, through which they inject venom into their prey or when threatened.
6. **Diet:** Cobras are carnivorous and primarily feed on frogs, lizards, rats and young birds. They are known to be oviparous, meaning they lay eggs to reproduce.
7. **Venom:** The venom of the cobra is neurotoxic, targeting the nervous system of its prey. It is potent and can cause severe pain, swelling, and irritation in humans when bitten. In some cases, a cobra bite can lead to respiratory failure and even death if not promptly treated.

The cobra holds a significant place in cultural and natural history, being both feared and revered. Its striking appearance and venomous nature make it a captivating yet potentially dangerous species in the animal kingdom.

E. Sea Snake

The Sea Snake is a highly venomous snake, possessing unique characteristics that are well-adapted for life in the oceanic environment.

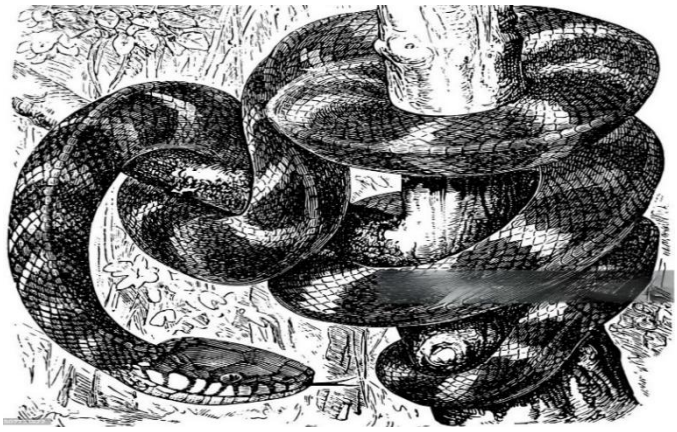


1. **Venom and Bite:** The Sea Snake is indeed a deadly and venomous species and its venom is primarily neurotoxic. A bite from this snake can have severe effects on the nervous system and can be potentially fatal.
2. **Body and Tail:** The Sea Snake has a long and slender body, which is well-suited for swimming in water. Its tail is laterally compressed, enabling efficient propulsion through the ocean currents.
3. **Coloration:** The snake's coloration typically comprises dark olive green on the upper part of its body, adorned with yellowish crossbars. On the ventral side, the color is predominantly white, which provides camouflage in the marine environment.
4. **Ventral Scales:** Unlike some terrestrial snakes, the Sea Snake has relatively small ventral scales, which minimize drag and facilitate smooth movement through water.
5. **Eyes:** Sea Snakes have small eyes with rounded pupils, adapted to function effectively in the underwater environment.
6. Carnivorous Nature: As carnivores, Sea Snakes primarily prey on fish and other marine organisms, making them well-suited for their oceanic habitat.

C. Mild Venomous Snakes

A. Cat Snake

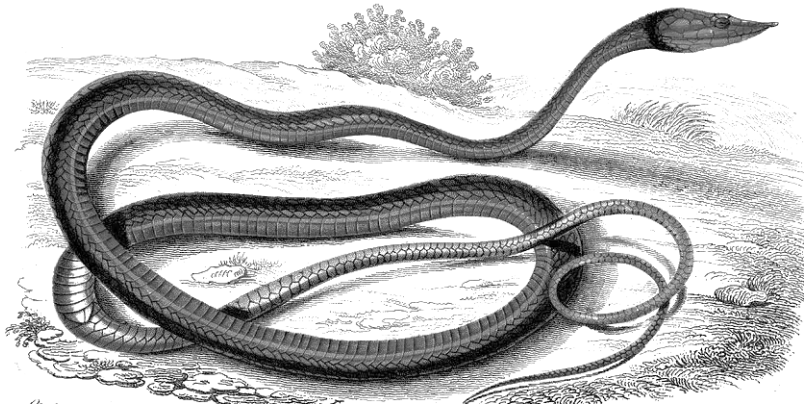
The Cat Snake, belonging to the genus *Boiga*, is a group of mildly venomous, predominantly nocturnal, arboreal snakes found across parts of Asia, Africa, and Australia. They are known for their distinctive cat-like eyes and often slender build.



- Appearance: Slender body with smooth, oblique scales; color varies (grey-brown, black/yellow); distinctive triangular head, broader than the neck.
- Eyes & Pupils: Large eyes with vertically elliptical (cat-like) pupils, specialized for nocturnal vision.
- Venom & Fangs: Rear-fanged with mild venom, effective for small prey; generally, not dangerous to humans.
- Behavior & Habitat: Largely arboreal (tree-dwelling) and nocturnal; active at twilight/night; may display warning behaviors (S-coil, hiss, tail vibrate).
- Diet: Carnivorous, primarily feeding on small vertebrates like lizards, frogs, birds/eggs, small mammals, and other snakes.
- Reproduction: Oviparous (egg-laying), with clutches of 3-15 eggs laid in protected spots**.**

B. Green Vine Snake

The Green Vine Snake, particularly species within the *Ahaetulla* genus (such as *Ahaetulla nasuta*, also known as the Long-nosed Whip Snake or Oriental Whip Snake), is a highly camouflaged and mildly venomous snake found across tropical Asia. Its remarkable resemblance to a vine makes it a master of stealth in its arboreal habitat.



- **Appearance:** Extremely long, slender, bright green to brownish with a pointed snout; often has a yellow side stripe.
- **Eyes & Vision:** Large, yellowish eyes with unique horizontal (keyhole-shaped) pupils provide excellent binocular vision for tree hunting.
- **Venom:** Rear-fanged with mild venom, effective for small prey; generally, not medically significant to humans, requires "chewing" for delivery.
- **Behavior & Camouflage:** Diurnal and highly arboreal; uses cryptic coloration and slender body to mimic branches; ambush predator; may display warning signs (inflating body, opening mouth) when threatened.
- **Diet:** Primarily carnivorous, feeding on lizards and frogs; occasionally eats small birds, eggs, or rodents.
- **Reproduction:** Oviparous (egg-laying), typically laying 6-10 eggs; young are precocial.

D. Study of Non- Venomous Snakes

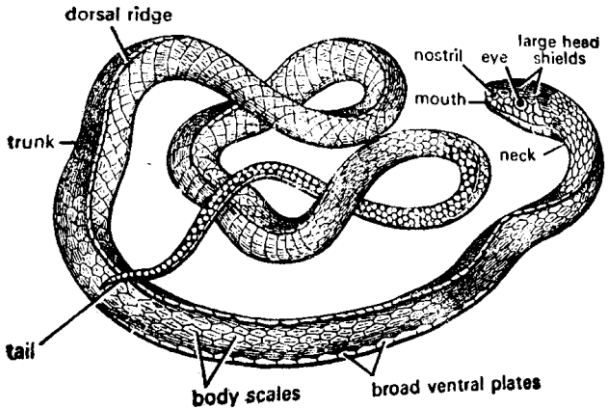
A. Python

Pythons are a family of non-venomous constrictor snakes found in the tropics and subtropics of the Eastern Hemisphere.

- **Distribution:** They are native to Africa, Asia and Australia.
- **Size:** They are among the largest snakes in the world. The reticulated python (*Malayopython reticulatus*) is the longest, reaching lengths of 7-8 meters (23-26.2 feet), with some individuals reaching up to 10 meters (32.8 feet).
- **Physical characteristics:** They have a triangular head, sharp, backward-curving teeth and powerful bodies.
- **Diet:** Pythons are carnivores and primarily kill their prey through constriction. They eat mammals, birds, amphibians and reptiles.
- **Human Interaction:** Python skin is used to make clothing and accessories. Some species, like the ball python (*Python regius*), are popular pets.
- **Reproduction:** Pythons are oviparous, meaning they lay eggs. Females typically incubate the eggs until they hatch.

B. Rat Snake

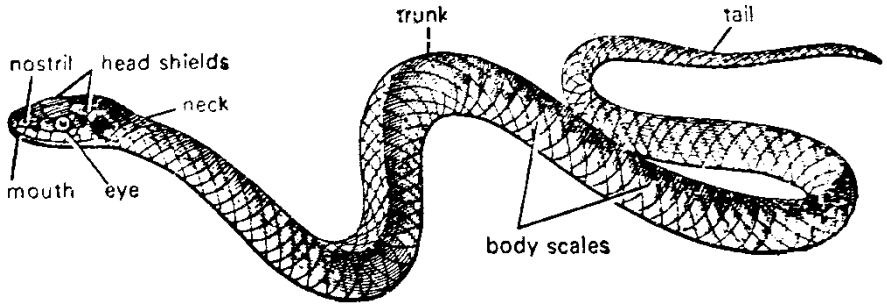
The Rat Snake, also known as Dhaman, is a non-poisonous snake with several unique characteristics



1. **Non-Poisonous:** The Rat Snake is not venomous and poses no immediate danger to humans. It is a harmless species.
2. **Size:** This snake can grow to a length of more than two meters, making it one of the larger snake species.
3. **Coloration:** Typically, the Rat Snake has a brown body with black cross bands on the posterior part of its body and tail. The under parts of the snake exhibit a faint yellow hue.
4. **Dorsal Ridge:** A notable feature of the Rat Snake is the presence of a dorsal ridge along the mid-dorsal line of its body.
5. **Head and Supralabial:** The head of the Rat Snake is distinct from its neck and the fourth and fifth supra labials (scales above the upper lip) come into contact with its eye.
6. **Reproductive Nature**: The Rat Snake is oviparous, meaning it lays eggs as part of its reproductive process.
7. **Bite Effects:** In the rare event of a bite, the Rat Snake may leave a mark from its teeth, but there is usually no continuous bleeding or significant swelling. It is essential to remember that Rat Snakes are not aggressive towards humans and usually bite only when provoked or threatened.

C. Checkered Keelback

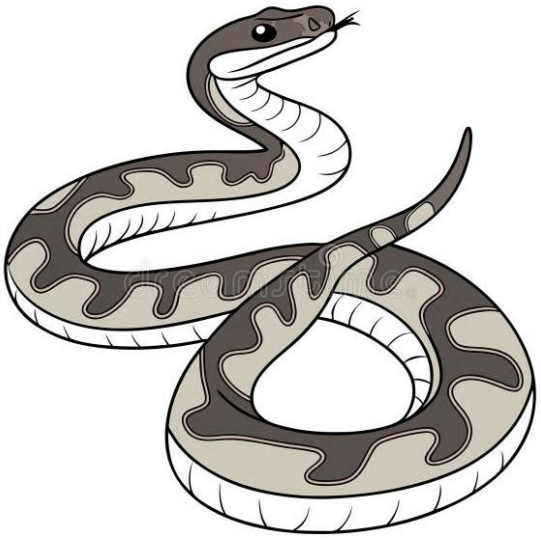
The Water Snake, also known as the Checkered Keel back, is a non-poisonous snake with distinctive features



1. **Body and Head:** The Water Snake has a stout body and a flat head, which is a common characteristic of snakes that inhabit aquatic environments. It is non-poisonous and poses no threat to humans.
2. **Coloration:** The snake's coloration is typically olive with black spots arranged in a chessboard-like pattern, creating an attractive and unique appearance.
3. **Scales:** The scales on the Water Snake's body are keeled and arranged in 19 rows, giving its skin a textured appearance.
4. **Ventral and Subcaudal Scales:** On the ventral side, the scales are rounded, while the subcaudal scales (found on the underside of the tail) are divided, aiding in movement through water.
5. **Supralabial Scales:** The snake has 9 supralabial scales, which are located above the upper lip and contribute to the unique patterning on its head.
6. **Diet and Reproduction:** As a carnivorous species, the Water Snake primarily preys on aquatic animals. It is oviparous, meaning it lays eggs as part of its reproductive cycle.

D. Sand Boa

The Sand Boa is a fascinating group of non-venomous snakes belonging to the family Boidae, specifically within the subfamily Erycinae. They are known for their adaptations to sandy, arid environments. While there are several species, the Kenyan Sand Boa (*Eryx colubrinus*) and the Indian Sand Boa (*Eryx johnii*) are among the most commonly discussed.



- Appearance: Heavy-bodied with a blunt, wedge-shaped head and a short, blunt tail; often camouflaged in sandy colors.
- Unique Features: Small eyes and nostrils are located high on the top of their head, ideal for remaining buried in sand.
- Habitat & Behavior: Primarily terrestrial and fossorial (burrowing); mostly nocturnal or crepuscular ambush predators.
- Diet: Feeds on small mammals (like rodents), lizards, and sometimes birds.
- Reproduction: Most are ovoviviparous, giving birth to live young.

E. First Aid Treatments (Simulation)

These are simulations for learning purposes. In a real snakebite situation, the most crucial step is to seek immediate medical attention at a hospital equipped to handle snake venom poisoning.

1. Immediately move the "patient" away from the snake to prevent further bites. Do not try to catch or kill the snake, but try to remember its appearance for identification by medical professionals if it can be done safely.
2. Calm and reassure the "patient," keeping them as still as possible to minimize venom spread.
3. Call for emergency medical help immediately (e.g., dial 108 in India). Clearly state the location and that it's a suspected venomous snakebite.
4. Carefully remove any jewelry (rings, bracelets, anklets) or tight clothing near the bite area, as swelling may occur.
5. Immobilize the bitten limb. Use a splint if available, or try to keep the limb still using whatever materials are at hand (e.g., tying it loosely to a stick or the uninjured leg). Keep the bitten limb at or slightly below the level of the heart, if possible, to slow venom spread. However, do not elevate the limb significantly.
6. Apply a pressure immobilization bandage *if you are trained in its proper use and the bite is from a neurotoxic snake (like a cobra or krait) and medical help is significantly delayed (over an hour)*. This involves wrapping firmly (but not so tightly as to cut off circulation) starting just above the bite and extending up the entire limb. The bandage should be about the tightness you would use for a sprained ankle. Do not use this technique for viper bites (like Russell's viper or saw-scaled viper) as it can worsen local tissue damage.
7. Monitor the "patient's" vital signs (breathing, pulse, level of consciousness) and be prepared to provide basic life support if needed.
8. Do not cut the wound, do not try to suck out the venom, do not use a tourniquet (unless specifically instructed by medical professionals in extreme circumstances), do not apply ice, and do not give the "patient" anything to eat or drink (especially alcohol or caffeine).
9. Continue to reassure the "patient" until medical help arrives.
10. When medical help arrives, provide them with any information you have about the snake's appearance and the timeline of events.

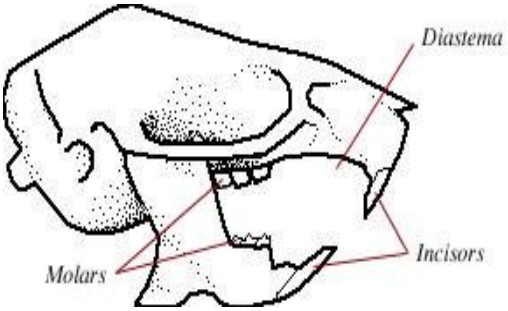
Key Takeaways for First Aid Simulation

- Prioritize getting the "patient" to a hospital as quickly as possible.
- Keep the "patient" calm and still.
- Immobilize the bitten limb (generally at or slightly below heart level).
- Only use pressure immobilization for suspected neurotoxic bites and when medical help is significantly delayed, and only if you are trained.
- Do not use harmful traditional remedies.
- Provide medical professionals with any information about the snake.

Remember, this is a simulation. Real snakebite situations can be frightening and complex. Knowing the correct first aid procedures and acting calmly can make a difference, but professional medical treatment with antivenom is essential for venomous snakebite

4. Dentition in Mammals: Rat, Sheep, Dog and Man

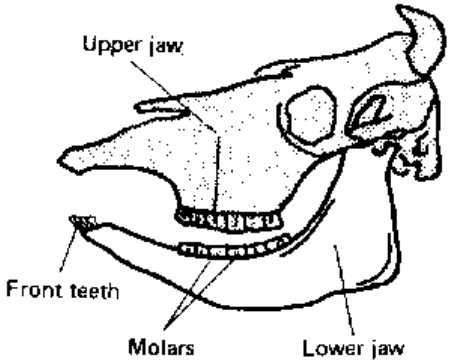
A. Dentition in Rat



Dentition in rat

- In Rat the total number of teeth in both jaws is 16. having dental formula 1.0.0.3 / 1.0.0.3
- Teeth are heterodont and thecodont.
- Incisors are grown continuously.
- Diastema (toothless gap) present.
- Canines and premolars are absent.
- Teeth are adapted for cutting.

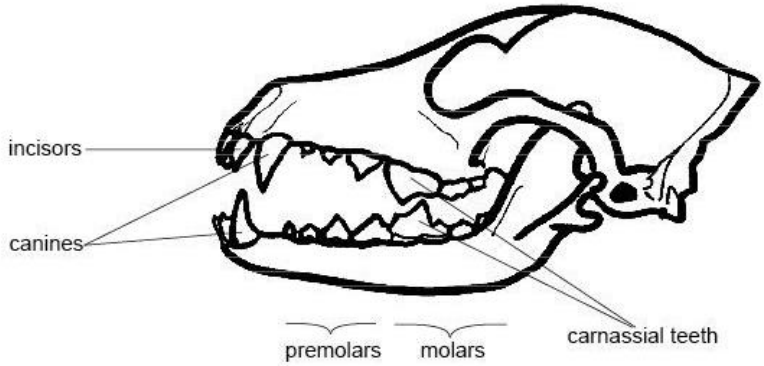
B. Dentition in Sheep



Dentition in sheep

- In Sheep/Cow/Goat the total number of teeth in both jaws is 32, having dental formula 0.0.3.3 / 3.1.3.3
- Teeth are heterodont, thecodont and selenodont (cusp is Crescentic).
- Diastema (toothless gap) present.
- Teeth are adapted for herbivorous habits i.e. for grazing.

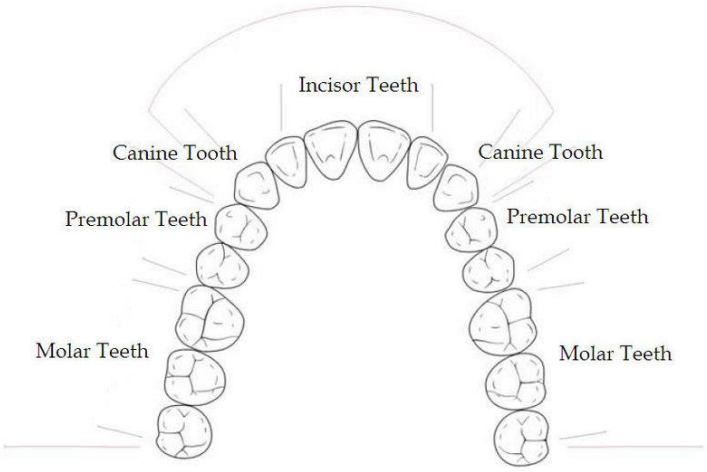
C. Dentition in Dog



Dentition in dog

- In Dogs the total number of teeth in both jaws is 42.
- Having dental formula 3.1.4.2 / 3.1.4.3
- Teeth are heterodont and thecodont.
- Canines are well developed and can be seen very easily.
- Teeth are adapted for cutting bones and tearing flesh.

D. Dentition in Man

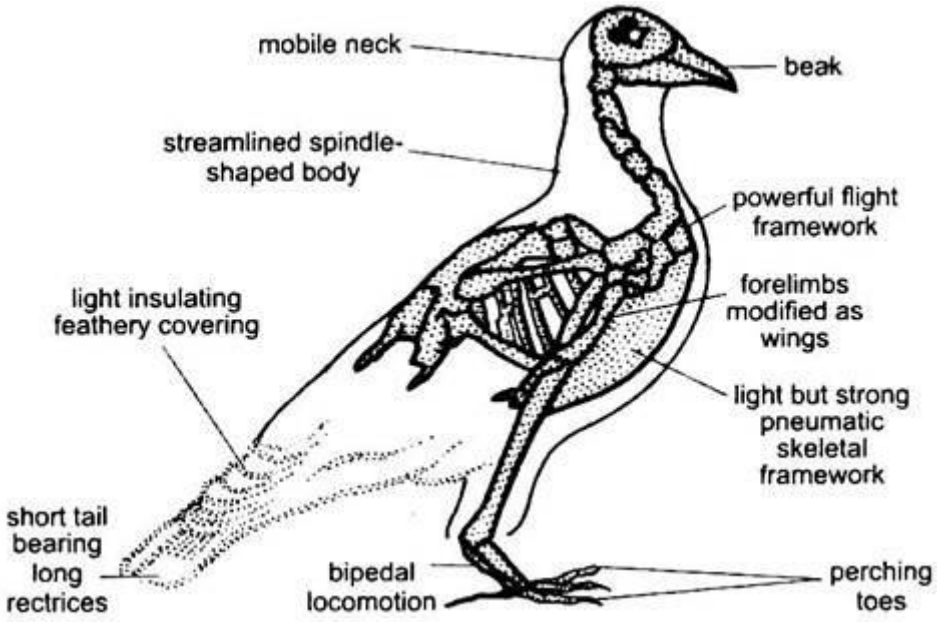


Dentition in man

- In Man the total number of teeth in both jaws are 32 with dental formula 2.1.2.3 / 2.1.2.3
- Teeth are heterodont, i.e. Incisors, Canines, Premolars and Molars.
- Diphyodont i.e. milk teeth are followed by permanent teeth.
- Thecodont i.e. rooted in the bony socket.
- Bunodont – blunt short cusps.
- Teeth are adapted to cutting, tearing and grinding the food.

5. Aerial Adaptations in Birds- Morphological and Anatomical

Birds exhibit a remarkable suite of morphological (External Structure) and anatomical (Internal Structure) adaptations that enable them to achieve and sustain flight. These adaptations work in concert to reduce weight, provide power, ensure balance and maneuverability and support the high metabolic demands of flying.



Morphological Adaptations

1. **Streamlined Body Shape:** Birds possess a fusiform or spindle-shaped body that tapers at both ends. This aerodynamic shape minimizes air resistance (drag) during flight, allowing for more efficient movement through the air.
2. **Feathers:** These are the defining characteristics of birds and are crucial for flight.
    - **Lightweight and Strong:** Feathers are made of keratin, making them light yet strong and flexible.
    - **Aerodynamic Surfaces:** Contour feathers create the smooth, aerodynamic shape of the wings and body. Flight feathers are specialized for generating lift and thrust.
    - **Insulation:** Feathers also provide insulation, helping birds maintain their high body temperature required for the energy demands of flight.
3. **Wings (Modified Forelimbs):** The forelimbs are highly modified into wings, the primary organs of flight.
    - **Large Surface Area:** Wings provide a large surface area to push against the air, generating lift.

- **Airfoil Shape:** The convex upper surface and concave lower surface of the wing create a difference in air pressure, resulting in an upward lift force.
- **Strong Leading Edge:** The thick, strong leading edge of the wing helps to maintain its shape during flight.

4. **Lightweight Beak (Absence of Teeth):** Birds have replaced heavy, bony jaws and teeth with a lightweight beak made of keratin. This significantly reduces the weight in the head, aiding balance in flight. The shape of the beak is adapted to their diet.
5. **Short Tail:** The tail is typically short and bears a fan of feathers. The tail acts as a rudder for steering, braking and balancing during flight and landing.
6. **Powerful Flight Muscles:** Large and well-developed pectoral (chest) muscles are anchored to a prominent **keel** on the sternum (breastbone). These muscles provide the powerful downstroke necessary for generating thrust. Smaller supracoracoideus muscles lift the wings during the upstroke via a tendon pulley system.
7. **Bipedal Locomotion:** Birds are bipedal, freeing their forelimbs entirely for flight. Their hind limbs are adapted for various functions like perching, walking, swimming, or grasping prey, depending on the species.
8. **Mobile Neck:** A long and flexible neck allows birds to maneuver their head for feeding, preening, and maintaining balance during flight.
9. **Center of Gravity:** The arrangement of organs and muscles is such that the center of gravity is located ventrally and slightly below the wings, providing stability during flight.
10. **Reduced Weight:** Besides the beak and feathers, other morphological features contribute to reduced weight, such as the absence of a urinary bladder (excreting uric acid as a semi-solid paste) and small reproductive organs (except during breeding season).

Anatomical Adaptations

1. **Pneumatic Bones:** Many of a bird's bones are hollow and filled with air sacs, which are extensions of the respiratory system. These "pneumatic bones" reduce overall body weight without compromising strength. Internal struts within the bones provide structural support.
2. **Fused and Reduced Bones:** The bird skeleton exhibits fusion of several bones, increasing rigidity and strength, which is essential for withstanding the stresses of

flight. For example, the thoracic vertebrae are fused, and the hand bones are reduced and fused to support the wing structure.

3. **Keeled Sternum:** As mentioned earlier, the sternum has a large, ventral keel that provides a broad surface area for the attachment of the powerful flight muscles.
4. **Efficient Respiratory System with Air Sacs:** Birds have a unique respiratory system that includes lungs and a network of air sacs that extend throughout the body cavity and into the bones. This system allows for a unidirectional flow of oxygenated air through the lungs, maximizing gas exchange and supporting the high metabolic rate required for flight.
5. **Efficient Circulatory System:** A large, four-chambered heart ensures complete separation of oxygenated and deoxygenated blood, delivering oxygen efficiently to the active flight muscles. Blood contains a high concentration of hemoglobin.
6. **High Metabolic Rate and Endothermic (Warm-bloodedness):** Birds are endothermic, maintaining a high and constant body temperature, which is necessary for the energy-intensive activity of flight. Their efficient respiratory and circulatory systems support this high metabolic rate.
7. **Specialized Sensory Organs:** Birds have highly developed vision, crucial for navigation, hunting, and avoiding obstacles during flight. Their eyes are often large relative to their head size. The cerebellum in the brain is also well-developed for coordination and balance.
8. **Absence of Urinary Bladder:** As mentioned morphologically, the absence of a urinary bladder is also an important anatomical adaptation for reducing weight.
9. **Rapid Digestion:** Birds have a digestive system adapted for processing food to meet their high energy demands. They have a gizzard for grinding food and a relatively short digestive tract to reduce weight.
10. **Single Ovary (in Females):** Female birds typically have only one functional ovary (usually the left one), which reduces body weight compared to having two.

These intricate morphological and anatomical adaptations demonstrate the powerful evolutionary pressures that have shaped birds into highly efficient masters of the air.

Unit – II (Biochemistry)

1. **Biochemical tests**

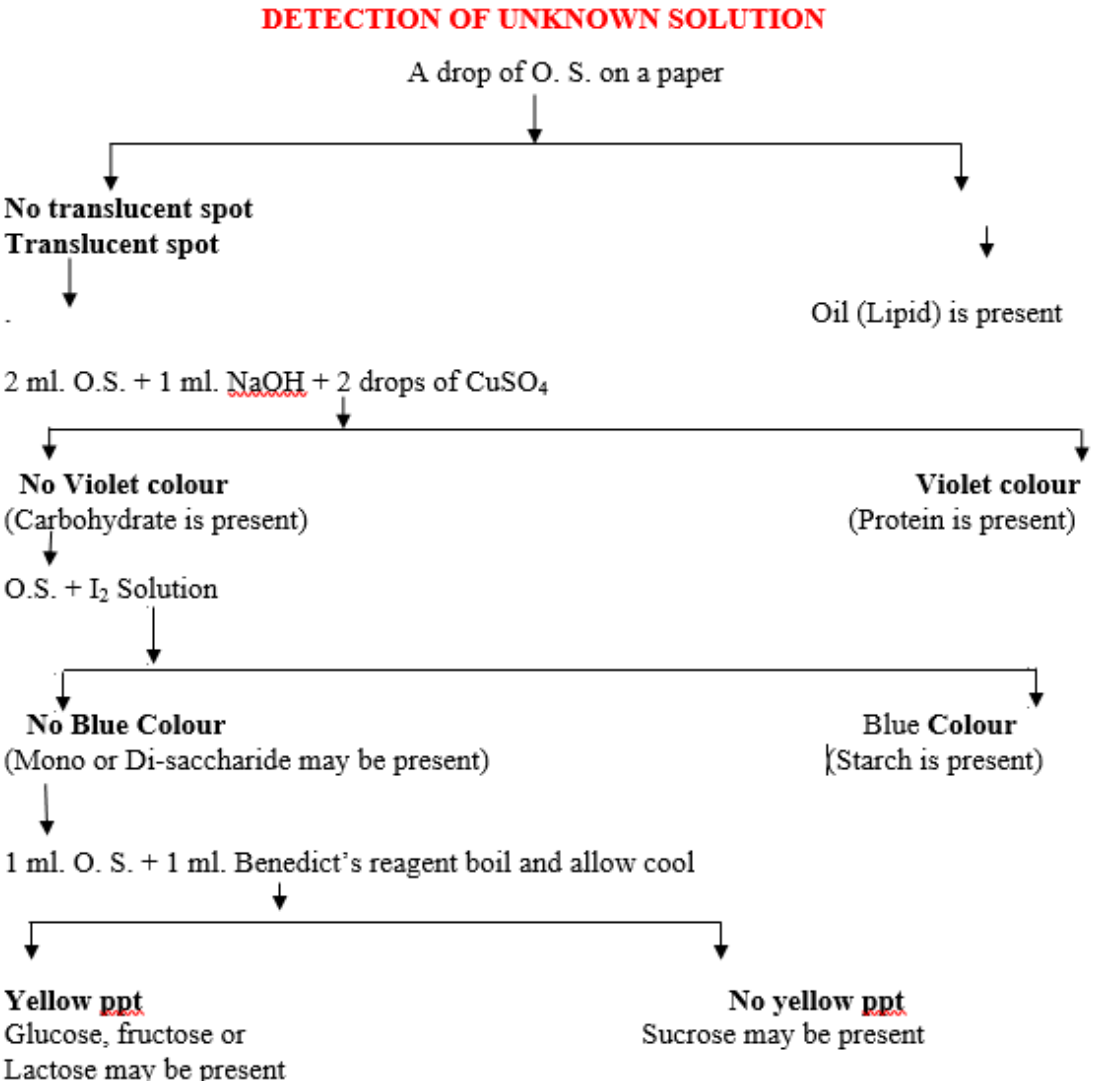
   A. Monosaccharides - Glucose, Fructose

   B. Disaccharides - Sucrose, Lactose

   C. Polysaccharides – Starch

   D. Lipid

   E. Protein



Tests For Oil (Lipid)

Test	Observation	Inference
Emulsification test 2ml. O.S.+ 2 – 3 ml. of water shake well	Droplets are observed	Oil confirmed
Sudan III test 3ml.O.S. + 2 drops of Sudan III stain. Take out one to two drops on a clean and dry slide. Observe under microscope	Brick red coloured droplets observed	Oil confirmed
Saponification test 1ml O.S. + 3 ml of alc. 10% NaOH boil, cool & add excess of Na_2SO_4	Soap is formed & it rises to the surface	Oil confirmed

Tests For Sucrose

Test	Observation	Inference
Fearson's test 4ml. O.S.+ 4 drops of 10% methylamine hydrochloride boil for 30 seconds and add 5 drops of 20% NaOH solution	Yellow colour appears which turns red	Sucrose present
Seliwanoff's test 1ml.O.S. + 3 ml Seliwanoff's reagent, boil for 30 seconds	Cherry red colour	Sucrose present
Inversion test O.S. + 2 drops of conc. H_2SO4 and boil for a minute, cool under tap water, neutralize it with 40 % NaOH then perform Benedict's test	Yellow or Brick red ppt	Sucrose present and confirmed

Tests For Glucose, Fructose and Lactose

Test	Observation	Inference
Benedict's test 1 ml. Benedict's reagent + 0.5 ml. O.S. boil & allow the test tube stand for 10 minutes.	Yellow ppt	Glucose, fructose, Lactose may be present
Tomer's test 2 ml. $CuSO_4$ + 2 ml. O.S. Mix well + 2 ml. NaOH boil	Yellow or Buff red ppt.	Fructose present
Fehling's test 1 ml. Fehling solution boils no change in colour + O.S. boil again	Brick red ppt.	Fructose present
Barford's test 1 ml. Barford's reagent + 5 ml. O.S. – boil.	Red ppt. settle at the bottom	Fructose is confirmed
C. T. For Glucose and Fructose		
Seliwanoff's test 1ml.O.S. + 3 ml Seliwanoff's reagent, boil for 30 seconds	Brick or Cherry red colour	Fructose present and confirmed
	If no brick or Cherry red colour	Glucose present and confirmed

Osazone test 5 ml. O.S. + 2 – 3 drops of Glacial acetic acid + a pinch of Phenyl hydrazine hydrochloride + 2 pinches of Sodium acetate, mix well, keep in boiling water bath for 5 - 20 minutes	Within 5 minutes **glucosazone** or **fructosazone** crystals forms	Glucose or fructose may be present
C. T. For Lactose		
Osazone test 5 ml. O.S. + 2 – 3 drops of Glacial acetic acid + a pinch of Phenyl hydrazine hydrochloride + 2 pinches of Sodium acetate, mix well, keep in boiling water bath for 5 - 20 minutes	**Lactososazone** crystal forms within 40-45 minutes	Lactose present and confirmed

Glucososazone: Needle-shaped crystals arranged like a broom

Lactososazone: Hedgehog or "pincushion with pins" or flower of "touch-me-not plant"

Estimation of Total Protein by Lowry's Method.

Aim: To determine the total concentration of proteins by Lowry's method.

Apparatus and Glassware required: Test tubes, Pipettes, Colorimeter, etc

Principle

The –CO-NH- bond (peptide) in the polypeptide chain reacts with copper sulphate in an alkaline medium to give a blue colored complex. In addition, tyrosine and tryptophan residues of protein cause reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of this method.

Reagents Required

1. Reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide.
2. Reagent B: 0.5% copper sulphate (CuSO4.5H2O) in 1% potassium sodium tartarate. Prepare fresh by mixing stock solutions.
3. Alkaline copper solution (Reagent C): Mix 50mL of reagent A and 1 mL of reagent B prior to use.
4. Diluted Folin's reagent (Reagent D): Dilute Folin-Ciocalteau reagent with an equal volume of 0.1 N NaOH
5. Standard: Dissolve 50mg BSA in 50 mL of distilled water in a volumetric flask. Take 10mL of this stock standard and dilute it to 50 ml in another flask for a working standard solution. One ml of this solution contains 200 μg proteins.

Procedure

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard into the series of labeled test tubes.
2. Pipette out 1 ml of the sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 5 ml of reagent C to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by overtaxing / shaking the tubes and allow standing for 10 min.
6. Then add 0.5 ml of reagent D rapidly with immediate mixing well and incubate at room temperature in the dark for 30 min.
7. Now record the absorbance at 660 nm against blank.

8. Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 660 nm along Y-axis.
9. Then from this standard curve calculate the concentration of protein in the given sample.

Observations and Calculations

Volume of standard BSA (ml)	**Volume of distilled water (ml)**	**Concof Protein (µg)**	**Volume of reagent C (ml)**		**Volume of reagent D (ml)**		AT 660 nm
0.0	1.0	00	5 ml	Incubate	0.5	Incubate	0.00
0.2	0.8	40	5 ml	At	0.5	At dark	
0.4	0.6	80	5 ml	Room	0.5	room	
0.6	0.4	120	5 ml	Temp	0.5	temp.	
0.8	0.2	160	5 ml	for	0.5	for	
1.0	0.0	200	5 ml	10	0.5	30	
1.0	0.0	?	5 ml	min	0.5	min	

Result: The given unknown sample contains …… µ g protein/ml.

Graph:

2. Estimation of Casein from Milk

Casein is the primary protein found in milk, comprising about 80% of the proteins in cow's milk and a significant portion in human milk as well. It's what gives milk its white color and is the essential ingredient in cheese. It can be estimated using several methods

Principle: Casein has an isoelectric point of around pH 4.6. At this pH, casein becomes least soluble and precipitates out of the milk. This precipitate can then be separated, dried and weighed to estimate the amount of casein present.

Procedure

1. **Defatting the Milk**

- Take a known volume of milk (e.g., 20 ml) in a beaker.
- Add an equal volume of saturated ammonium sulfate solution slowly while stirring. This step helps to precipitate out fats along with casein.
- Filter the mixture. The precipitate contains fat and casein.
- Transfer the precipitate to another beaker and add about 30 ml of water. Casein will dissolve, forming a milky solution, while fat remains undissolved.

2. **Casein Precipitation**

- Heat the milky solution to about 40°C.
- Add a 1% acetic acid solution drop by drop while stirring until casein starts to precipitate as white curds. The pH should reach around 4.6.

3. **Separation and Drying**

- Filter the precipitated casein using filter paper.
- Wash the precipitate with water to remove any remaining impurities.
- Allow the precipitate to dry completely, either by air drying or in a warm oven at a low temperature.

4. **Weighing and Calculation**

- Weigh a clean, dry watch glass.
- Transfer the dried casein precipitate onto the weighed watch glass and weigh again.
- Calculate the mass of casein by subtracting the weight of the watch glass from the final weight.
- Calculate the percentage of casein in the original milk sample using the formula:

$$\% \text{ Casein} = \frac{\text{Mass of dried casein}}{\text{Volume of milk taken (assuming density of milk is } \sim 1 \text{ g/ml)}} * 100$$

Study of Activity of Salivary Amylase under Optimum Conditions

Aim: Study of activity of salivary amylase under optimum conditions.

Principle: The salivary amylase is a starch digesting enzyme found in saliva. The polysaccharide starch is acted by salivary amylase and broken down into dextrin and finally into disaccharides. By using iodine solution as an indicator, the action of enzymes can be analyzed. As starch is broken up to dextrins, the iodine turns to a brown or red colour and it becomes pale brown or yellow when the reaction is completed.

Extraction of saliva: Clean the teeth, gargle with a mild antiseptic and rinse the mouth thoroughly with water. Hold a piece of sour foodstuff in front of the tongue. Collect the saliva from under the tongue with a pipette or medicine dropper. Filter it and use it as salivary amylase for its action.

Material: Starch solution (1%), Standard iodine solution, distilled water, test tubes, hot water bath, etc.

Procedure: As per shown in the table, label the 5 test tubes as A to E. Add original solution and other reagents as per the instructions in these test tubes. Observe the changes in colour during the experiment and note down the observations. Draw the conclusion based on the action of salivary amylase.

Sr. No.	Test Tube	Test	Observation	Inference
1	Test tube – A (Control)	5ml O. S. + I_2 solution	Blue colour suspension	Starch present
2	Test tube - B	5 ml O. S. + 5ml Saliva filtrate keep at room temperature and after some time add I_2 solution	Blue coloured suspension	Starch present (starch not digested)
3	Test tube - C	5ml O. S. + 5ml saliva incubate in water bath at 37 ^{0} C for 15 minutes		
4	Test tube - D	Half of the incubated solution from test tube – C + I_2 solution few drops.	No blue colour	Starch absent
5	Test tube - E	Half of the remaining incubated solution from test tube – C + 5ml Benedict's solution boil	Red ppt.	Reducing sugar present.

Result

1. In the test tube- B Salivary amylase does not act on starch at room temperature which is indicated by the blue colour of solution.
2. In the test tube- D starch is digested into reducing sugar at 37^0c by the action of salivary amylase hence the blue colour is disappeared.
3. In the test tube E – reduced sugars are present and confirmed by appearance of red ppt.

Effect of Temperature on Activity of Salivary Amylase

Aim: Study of effect of temperature on salivary amylase.

Principle:

The salivary amylase is a starch digesting enzyme found in saliva. The activity of salivary amylase is optimum at specific temperature. At very low and very high temperature the enzyme denatures and lost its activity. The effect of temperature can be studied by keeping temperature variation during the activity.

Material: Starch solution (1%), Standard iodine solution, distilled water, test tubes, hot water bath, etc.

Procedure: As per shown in the table, label the 4 test tubes as A to D. Add 5 ml original solution and 5 ml saliva solution in these test tubes. Keep the test tubes at 4, 20, 37 and 50 ^{0} C and observe the changes in colour and note down the observations. Draw the conclusion based on the temperature specific action of salivary amylase.

Sr. No.	Test Tube	Temp	Observation	Inference
1	Test tube – A	4 ^{0} C	Blue coloured suspension	No enzyme action
2	Test tube - B	20 ^{0}C	Blue coloured suspension	No enzyme action
4	Test tube - C	37 ^{0} C	Blur colour disappeared	Enzyme acted
5	Test tube - D	50 ^{0}C	Blue coloured suspension.	No enzyme action.

Result

1. In the test tube- A, B and D Salivary amylase does not act on starch at 4, 20 and 50 ^{0}C temperature which is indicated by the blue colour of solution.
2. In test tube- C starch is digested into reducing sugars at 37^{0}c by the action of salivary amylase hence the blue colour is disappeared.

Effect of pH on Activity of Salivary Amylase

Aim: Study of effect of pH on salivary amylase.

Principle

The salivary amylase is a starch digesting enzyme found in saliva. The activity of salivary amylase is optimum at specific pH. At very low and very high pH the enzyme denatures and lost its activity. The effect of pH can be studied by keeping pH variation during the activity.

Material

Starch solution (1%), Standard iodine solution, buffers of different pH, distilled water, test tubes, hot water bath, etc.

Procedure

As per shown in the table, label the 4 test tubes as A to D. Add 5 ml original solution and 5 ml saliva solution and add buffer solutions of different pH in these test tubes as shown in table. Keep the test tubes at 37 ^{0} C and observe the changes in colour and note down the observations. Draw the conclusion based on the pH specific action of salivary amylase.

Sr. No.	Test Tube	pH	Observation	Inference
1	Test tube – A	2.0	Blue coloured suspension	No enzyme action
2	Test tube - B	5.0	Blue coloured suspension	No enzyme action
4	Test tube - C	7 .0	Blur colour disappeared	Enzyme acted
5	Test tube - D	10.0	Blue coloured suspension.	No enzyme action.

Result

1. In the test tube- A, B and D Salivary amylase does not act on starch at 2, 5 and 10 pH which is indicated by the blue colour of solution.
2. In the test tube- C starch is digested into reducing sugars at 7.0 pH and 37^{0} C by the action of salivary amylase hence the blue colour disappears.

Detection of Abnormal Urine Constituents

Normal urine is primarily water with dissolved waste products like urea, creatinine, and uric acid. The presence of certain substances in urine that are not normally found there, or are present in abnormal concentrations, can indicate various underlying health issues. Detecting these "abnormal urine constituents" is a crucial part of urinalysis, a common diagnostic tool.

Test	Observation	Inference
Test For Sugar Benedict's test: Take 5ml Benedict's reagent in test tube then add 8drops of urine sample and Boil it for 1-2 min and observe.	Green or yellowish green or orange or red precipitate obtained	Reducing sugar confirmed Therefore urine contains reducing sugar **(Glycosuria)**
Test For Protein Heat coagulation test: Fill 3/4th of test tube with urine sample solution. Heat it at the top and observe. Then Add 1-2 drop of acetic acid to it and again observe.	White Coagulum or turbidity is obtained which after the addition of acetic acid gets intensified.	Albumin is confirmed. Therefore urine contains albumin **(Proteinuria)**
Test For Ketone Bodies Rothera's test: Saturate 5 ml of urine with Rothera's mixture and then add 1-2 ml of conc. Ammonia solution. Gently mix by rotation and allow to stand and observe	Permagnate colour or deep purple ring is observed at the junction of two layers	Acetone and acetoacetic acid present. Therefore, urine contains ketone bodies. **(Ketonuria)**
Test For Blood Benzidine Test: Take 2ml of urine in a test tube and boil for 5min. Cool it. Mix equal volume of benzidine solution (2-3ml) and H2O2 in a test tube and add the boiled cooled urine into the test tube	Blue colour solution later turns green	Blood is present in urine. Therefore urine contains blood **(Hematuria)**

Test	Observation	Inference
Hay's Sulphur Powder Test for Bile Salts Hay's Sulphur powder test: Take 3ml urine in a test tube and sprinkle a pinch of Sulphur Powder and observe. Control: Take 3ml water in a test tube and sprinkle a pinch of Sulphur powder and observe	Sulphur powder sinks down to the bottom of the test tube. Sulphur powder floats	Bile salts confirmed. Therefore urine contains bile salts.
Fouchet's Test for Bile Pigments Take 3ml urine solution in the test tube. add 2 ml 10% $BaCl_2$. until thick white precipitate is obtained. Filter it and to the precipitate on the filter paper add a few drops of Fouchet's reagent and observe.	Colour changes from yellow to pista green	Bile pigments confirmed. Therefore, urine contains bile pigments. **(Bilirubinuria)**

Result:

The given sample of urine contains …… so the sample is abnormal.

Determination of Ascorbic Acid / Vitamin 'C' from Given Sample

Ascorbic acid is a reducing agent and reacts with iodine (I_2) in a redox reaction. The ascorbic acid is oxidized to dehydroascorbic acid, while iodine is reduced to iodide ions (I). The endpoint of the titration is detected using starch as an indicator. In the presence of free iodine, starch forms a dark blue-black complex. As long as ascorbic acid is present, it will react with the iodine, preventing the formation of this blue-black color. Once all the ascorbic acid has been oxidized, the next drop of iodine solution will remain in the solution and react with the starch, indicating the endpoint.

Procedure

1. **Preparation of Iodine Solution (Titrant)**
    - Obtain or prepare a standardized iodine solution of known concentration. The concentration is usually expressed in Normality (N) or Molarity (M). *In a real lab, this solution is often standardized against a primary standard like sodium thiosulfate.*
2. **Preparation of the Sample Solution**
    - Obtain the sample containing Vitamin C (e.g., fruit juice, crushed tablet).
    - If the sample is solid (like a tablet), dissolve a known weight of it in a suitable solvent, usually a slightly acidic solution (e.g., with dilute sulfuric acid or acetic acid) to prevent oxidation of ascorbic acid. Make up to a known volume in a volumetric flask.
    - If the sample is a liquid (like juice), you might need to filter it to remove any particulate matter. For colored juices, dilution might be necessary to observe the endpoint clearly.
3. **Titration**
    - Pipette a known volume of the prepared sample solution into a conical flask.
    - Add a few drops of starch indicator solution to the conical flask. The solution should remain colorless or the original color of the sample at this stage.
    - Fill a burette with the standardized iodine solution. Record the initial volume of the iodine solution in the burette.
    - Slowly add the iodine solution from the burette to the sample solution in the conical flask while continuously swirling the flask.
    - Continue adding the iodine solution drop by drop until a faint but permanent blue-black color appears in the conical flask. This indicates that all the ascorbic

acid has reacted, and the excess iodine is reacting with the starch indicator to form the colored complex (the endpoint).

- Record the final volume of the iodine solution in the burette.

4. **Calculation**
    - Calculate the volume of iodine solution used in the titration by subtracting the initial burette reading from the final burette reading.
    - Use the stoichiometry of the reaction between ascorbic acid and iodine (1:1 molar ratio) and the concentration of the iodine solution to calculate the amount of ascorbic acid in the sample.

The reaction is

$C6H8O6$ (Ascorbic Acid) + I_2 → $C6H6O6$ (Dehydroascorbic Acid) + $2H^+$ + $2I^-$

The calculations involve

- Moles of (I_2) used = (Concentration of (I_2) solution) × (Volume of (I_2) solution used)
- Moles of Ascorbic Acid reacted = Moles of (I_2) used (due to 1:1 stoichiometry)
- Mass of Ascorbic Acid = (Moles of Ascorbic Acid) × (Molar mass of Ascorbic Acid, 176.12 g/mol)
- Finally, calculate the concentration of Ascorbic Acid in the original sample based on the weight or volume of the sample taken.

Example Calculation

Let's say:

- Concentration of iodine solution (I_2) = 0.01 M
- Volume of sample solution titrated = 25 mL (0.025 L)
- Volume of iodine solution used at the endpoint = 15 mL (0.015 L)

1. Moles of (I_2) used = 0.01 mol/L × 0.015 L = 0.00015 moles
2. Moles of Ascorbic Acid reacted = 0.00015 moles
3. Mass of Ascorbic Acid in the 25 mL sample = 0.00015 moles × 176.12 g/mol = 0.026418 g

To find the concentration in the original sample, you would need to know the initial amount of the sample you used (e.g., if 1 gram of tablet was dissolved in 100 mL, then this 0.026418 g was present in 25 mL of that 100 mL solution).

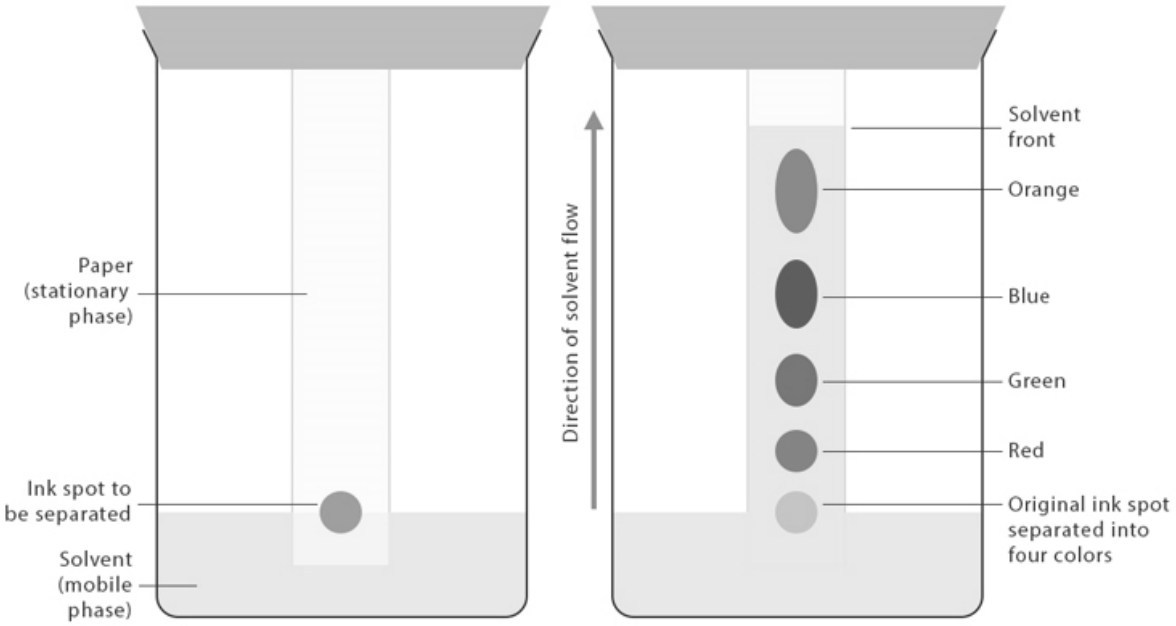
Important Considerations

- **Accuracy of Iodine Solution:** The accuracy of the iodine solution's concentration is crucial for accurate results.
- **Endpoint Detection:** A sharp endpoint is important. Sometimes, for dark-colored samples, it can be challenging to see the color change.
- **Interfering Substances:** Other reducing agents present in the sample might also react with iodine, leading to inaccurate results.
- **Freshness of Sample:** Ascorbic acid is easily oxidized, so it's best to analyze samples as fresh as possible or store them properly to prevent degradation.
- **Blank Titration:** Sometimes, a blank titration is performed using the solvent and indicator to account for any color changes not due to the reaction with ascorbic acid.

This iodometric titration method is a simple and widely used technique for determining Vitamin C content in various samples. However, for complex matrices or more accurate results, other methods like HPLC (High-Performance Liquid Chromatography) or spectrophotometric methods might be employed.

Separation of Amino Acids by Paper Chromatography

Paper chromatography is a simple, inexpensive and effective technique used to separate mixtures, including amino acids, based on their differing affinities for a stationary phase (paper) and a mobile phase (solvent).



Paper Chromatography

Principle

The separation relies on the differential partitioning of the amino acids between the stationary phase (the cellulose paper) and the mobile phase (the solvent).

- **Stationary Phase:** The paper, specifically the cellulose fibers, acts as the stationary phase. The hydroxyl groups of cellulose form hydrogen bonds with water molecules, creating a thin layer of adsorbed water that acts as the true stationary phase.
- **Mobile Phase:** A solvent mixture, often a combination of organic solvents and water, is used as the mobile phase. The choice of solvent is crucial for effective separation.

Amino acids will move at different rates depending on

1. **Solubility in the mobile phase:** Amino acids that are more soluble in the solvent will travel further up the paper.
2. **Adsorption to the stationary phase:** Amino acids that have a stronger affinity (e.g., through hydrogen bonding, hydrophobic interactions) for the cellulose paper and its adsorbed water will move slower.

Materials Required

- **Chromatography paper:** Whatman No. 1 filter paper is commonly used.
- **Chromatography tank/jar:** A tall glass jar or a specialized chromatography tank with a lid.

- **Solvent system:** A mixture of solvents (e.g., n-butanol: acetic acid: water in a specific ratio like 4:1:5, or phenol: water).
- **Amino acid mixture:** The sample to be separated.
- Standard amino acids: Pure samples of known amino acids for comparison.
- **Capillary tubes:** For spotting the samples.
- **Pencil and ruler:** For drawing the baseline.
- **Drying oven/air dryer:** To dry the chromatogram.
- **Locating agent (Ninhydrin solution):** To visualize the separated amino acids.
- **Sprayer:** To apply the locating agent.

Procedure

1. **Preparation of the Stationary Phase (Paper)**
    - Cut a strip or sheet of chromatography paper to fit the chromatography tank.
    - Draw a faint pencil line about 1-2 cm from one end of the paper. This is the **origin or baseline.**
    - Mark small, equally spaced points along this baseline, leaving enough space between them (e.g., 1-2 cm apart).
2. **Sample Spotting**
    - Using separate capillary tubes, carefully apply small, concentrated spots of the amino acid mixture and each individual standard amino acid onto the marked points on the baseline.
    - Allow each spot to dry completely before applying the next, or before proceeding, to ensure a concentrated spot. This can be done with a gentle air dryer.
3. **Preparation of the Mobile Phase (Solvent)**
    - Pour the chosen solvent system into the chromatography tank. The solvent level should be below the origin line on the paper, so the spots are not directly immersed.
    - Place the lid on the tank and allow the atmosphere inside to saturate with solvent vapor for some time (e.g., 30 minutes to 1 hour). This saturation helps ensure uniform solvent front movement.
4. **Chromatography Run**
    - Carefully suspend the spotted paper strip in the chromatography tank, ensuring that the bottom edge (below the origin line) is immersed in the solvent, but the spotted samples themselves are above the solvent level.
    - Secure the paper so it hangs straight and does not touch the sides of the tank.
    - Replace the lid.

- Allow the solvent to ascend the paper by capillary action. The solvent front will move up the paper, carrying the amino acids with it at different rates. This process can take several hours (e.g., 2-6 hours), depending on the solvent system and paper length.

5. **Drying the Chromatogram**
    - Once the solvent front has almost reached the top of the paper (or a predetermined distance), remove the paper from the tank.
    - Immediately mark the position of the solvent front with a pencil.
    - Hang the paper in a fume hood or use a drying oven/air dryer to completely evaporate all traces of the solvent.
6. **Visualization (Locating the Amino Acids)**
    - Amino acids are colorless, so they need to be visualized.
    - Evenly spray the dried chromatogram with Ninhydrin solution.
    - Heat the paper gently in a drying oven (e.g., at 60-80°C for 5-10 minutes) or allow it to air dry.
    - Ninhydrin reacts with the amino groups of amino acids (and primary amines) to produce a characteristic purple/pink color (Ruhemann's Purple). Proline, a secondary amine, produces a yellow color.

Data Analysis: Rf Value

After visualization, each amino acid will appear as a distinct colored spot. The identity of an unknown amino acid can be determined by comparing its **Rf (Retention Factor) value** with that of known standard amino acids run under identical conditions.

The Rf value is calculated as

Rf = Distance traveled by solvent front / Distance traveled by solute (amino acid)

- The Rf value is a constant for a given compound under specific chromatographic conditions (Stationary Phase, Mobile Phase, Temperature).
- By comparing the Rf values of the unknown spots with the Rf values of the known standards, the amino acids in the mixture can be identified.

Advantages of Paper Chromatography for Amino Acid Separation

- **Simplicity:** Relatively easy to set up and perform.
- **Cost-effective:** Requires minimal and inexpensive equipment.
- **Effective for simple mixtures:** Good for separating a few components.
- **Qualitative analysis:** Primarily used for identification.

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