B.Sc. ZOOLOGY LAB MANUAL

4th Semester

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Zoology

MIDNAPORE CITY COLLEGE

PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for UG Zoology fourth Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers and non teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

C8P: Comparative Anatomy of Vertebrates (Lab)

List of Practical

1. Study of placoid, cycloid and ctenoid scales through permanent slides/photographs.

2. Study of disarticulated skeleton of Toad, Pigeon and Guineapig.

3. Demonstration of Carapace and plastron of turtle.

4. Identification of mammalian skulls: One herbivorous (Guineapig) and one carnivorous (Dog) animal.

5. Dissection of Tilapia: Circulatory system, Brain, pituitary, urinogenital system.

C9P: Animal Physiology: Life Sustaining Systems Lab **Credits 02**

List of Practical

1. Determination of ABO Blood group
2. Enumeration of red blood cells and white blood cells using haemocytometer
3. Estimation of haemoglobin using Sahli's haemoglobinometer
4. Preparation of haemin and haemochromogen crystals
5. Recording of blood pressure using a sphygmomanometer

C10P: Immunology (Lab)

List of Practical

1. Demonstration of lymphoid organs.

2. Histological study of spleen, thymus and lymph nodes through slides/ photographs

3. Preparation of stained blood film to study various types of blood cells.

4. ABO blood group determination.

5. Demonstration of ELISA

STUDY OF PLACOID, CYCLOID AND CTENOID SCALES THROUGH PERMANENT SLIDES/PHOTOGRAPHS

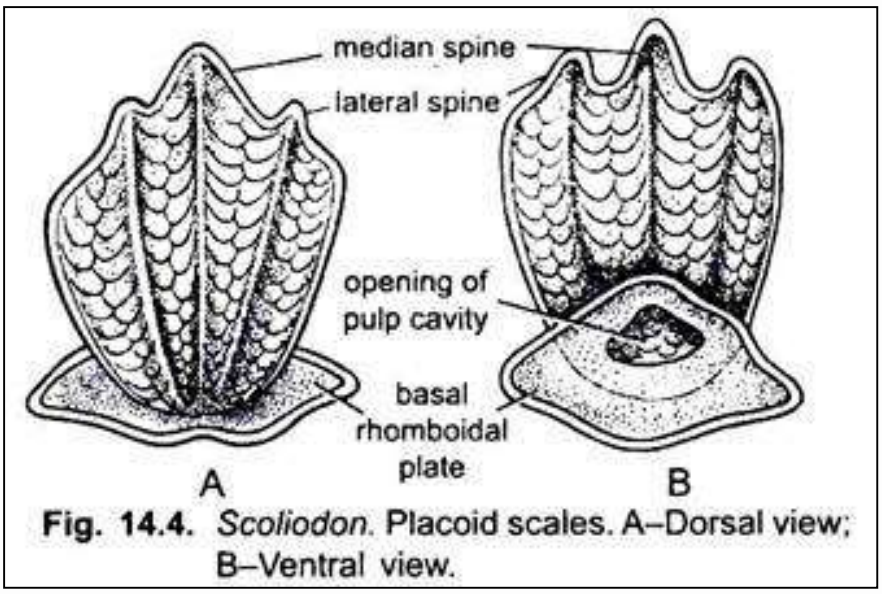
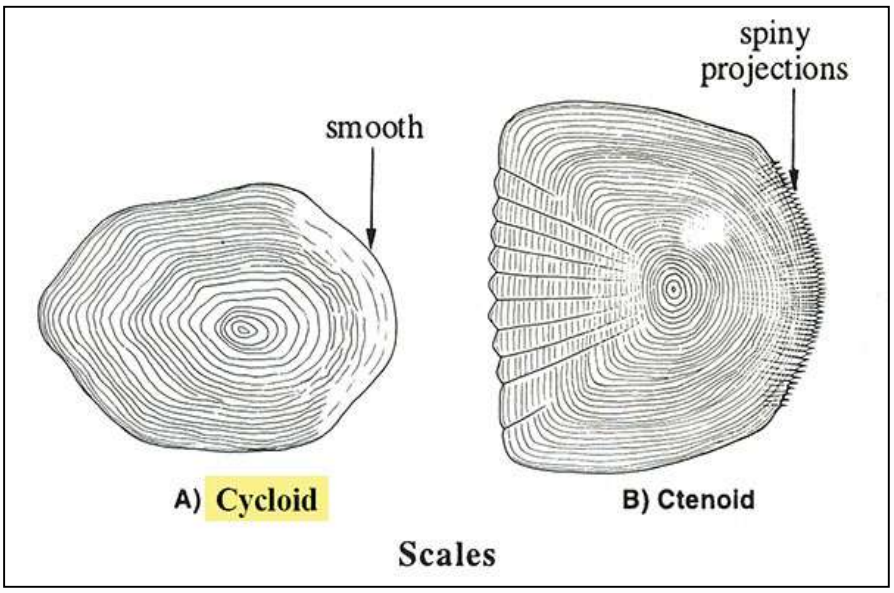


Fig. 14.4. *Scoliodon*. Placoid scales. A–Dorsal view; B–Ventral view.



DISARTICULATED SKELETON OF TOAD, PIGEON AND GUINEAPIG

Vertebrae

Precaudal or trunk vertebra of Teleost Fish

1. Ring like body.
2. Presence of **centrum**, neural arch, neural canal and neural spine.

Hence, the specimen seems to be a vertebra.

1. Completely **ossified** structure.
2. Centrum is prominently **amphicoelous** with a small central opening for the passage of **notochord.**
3. Presence of dorsal neural arch with a median, long, backwardly directed and pointed **neural spine.**
4. Neural arch bears, at its base, paired, anterior and posterior, small, thin processes called neural **zygapophyses.**

Hence, the specimen seems to be a vertebra of Teleost Fish.

1. Centrum bears a pair of ventrolateral, short, downwardly directed transverse processes or **basapophysis.**
2. **Haemal** arch and spine are absent.

Hence, the specimen seems to be a trunk vertebra of Teleost (Gk. *telos*, end; *osteon*, bone) **Fish.**

Caudal vertebra of Teleost Fish

Same up to **vertebra** as in **Teleost Fish.**

1. Absence of separate transverse process.
2. Presence of a ventrally disposed haemal arch with a central haemal canal.
3. Presence of a pointed and backwardly directed median haemal spine.
4. Presence of paired anterior and posterior haemal zygapophyses.

Hence, the specimen seems to be a caudal vertebra of Teleost Fish.

Atlas or first vertebra of toad

Same up to **vetebra** as in **Teleost Fish.**

1. Centrum is of **procoelous** type.
2. **Hypapophysis** and **chevron** bones are absent.
3. **Zygantrum** and **Zygosphene** are absent.
4. Articular surface for vertebra is absent.
5. Vertebra is of **notocentrous** type.

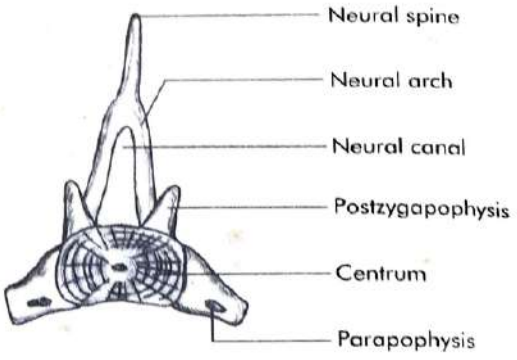


Fig. 6.21 Trunk vertebra of a teleost fish

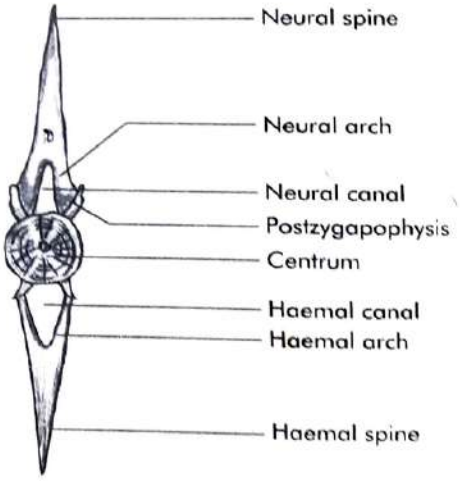


Fig. 6.22 Typical caudal vertebra of a teleost fish

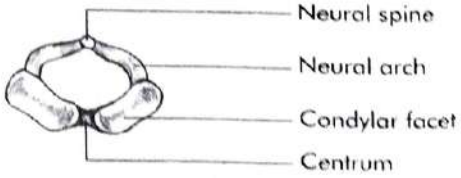


Fig. 6.23 Atlas vertebra of *Bufo* sp. (anterior view)

Hence, the specimen seems to be a vertebra of anuran Amphibia, Toad (e.g. *Bufo* sp.).

1. A ring-like shape with a large circular, wide neural canal.
2. Centrum is comparatively small.
3. It bears anteriorly two lateral and oval concavities called the condylar **facets.**
4. Neural spine is reduced.
5. Prezygapophysis and transverse processes are absent.

Hence, the specimen seems to be an Atlas vertebra of Toad (e.g. *Bufo* sp.).

Atlas or first vertebra of Pigeon

Same up to **vertebra** as in **Teleost Fish.**

[**Note:** Omit 'presence of neural spine' from point 2.]

1. Bones are lightweight, and **pneumatic.**

Hence, the specimen seems to be a vertebra of Aves (L. *avis*, bird), **Pigeon** (e.g. *Columba* sp.).

1. A ring-shaped body with a large, circular, wide neural canal.
2. Centrum is absent.
3. Ventral part of neural arch bears a median condylar facet on the anterior face.
4. Neural spine is absent.
5. Prezygapophysis and transverse process are absent.

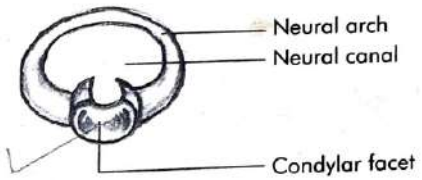


Fig. 6.24 Atlas vertebra of *Columba* sp. (anterior view)

Hence, the specimen seems to be an Atlas vertebra of Pigeon (e.g. *Columba* sp.).

Atlas or first vertebra of Guineapig

Same up to **vertebra** as in **Teleost Fish.**

1. A thick and heavy bony structure.
2. Presence of intervertebral foramen at the base of the transverse process (Vertebrarterial foramen or Transverse foramen).

Hence, the specimen seems to be a vertebra of mammal, Guineapig (e.g. *Cavia* sp.).

1. A ring-shaped body with large, circular, wide neural canal.
2. Centrum is absent.
3. A pair of ventro-lateral condylar facet is present anteriorly.
4. Neural spine is reduced.
5. Prezygapophysis is absent.
6. Well-developed, laterally directed, wing-like transverse process.
7. A small midventral hypapophysis is present.

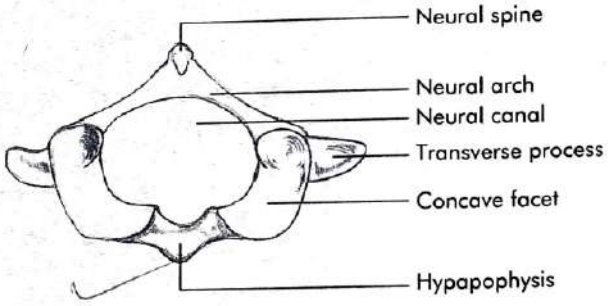


Fig. 6.25 Atlas vertebra of *Cavia* sp. (anterior end on view)

Hence, the specimen seems to be an Atlas vertebra of Guineapig (e.g. *Cavia* sp.).

Typical vertebra (second to eighth) of Toad (e.g. *Bufo* sp.)

Same up to **anuran amphibian vertebra** as in **Toad.**

1. Neural spine is a low median ridge, and is well built.

2. Presence of well-developed pre and postzygapophysis.
3. Long, narrow, flat, rod-like transverse process is laterally and slightly upwardly directed.

Hence, the specimen seems to be a typical vertebra of Toad (e.g. *Bufo* sp.).

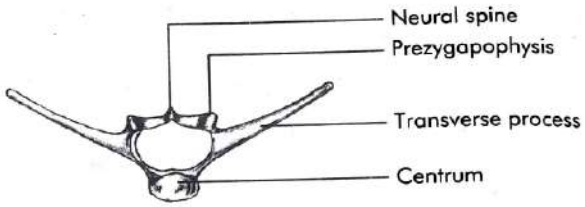


Fig. 6.26 Typical vertebra of *Bufo* sp. (anterior view)

Ninth (IXth) vertebra of Toad

Same up to **anuran amphibian vertebra** as in **Toad**.

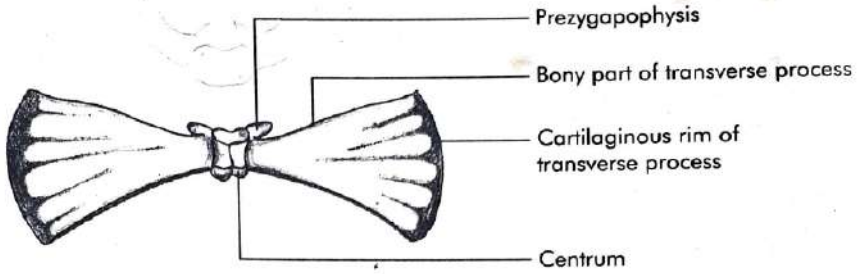


Fig. 6.27 9th vertebra of *Bufo* sp. (ventral view)

1. Neural spine is a low median ridge, and is well-built.
2. Neural canal is narrow and transversely elongated.
3. Prezygapophysis well-developed but postzygapophysis is absent.
4. Stout, fan-shaped and dorsoventrally flattened transverse process is laterally and slightly upwardly directed; lateral edge of the transverse process is bounded by cartilage.
5. Posterior convex end of centrum is bifid to form two condyles.

Hence, the specimen seems to be a ninth (IXth) vertebra of Toad (e.g. *Bufo* sp.).

Urostyle of Toad

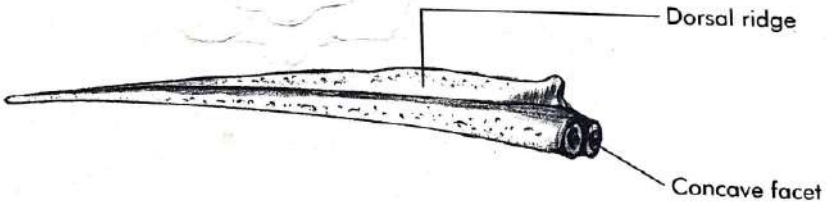


Fig. 6.28 Urostyle of *Bufo* sp. (lateral view)

Same up to **amphibian vertebra** as in **Toad.**

1. Unsegmented rod-like bone, the anterior end of which is broader than the posterior end.
2. Presence of continuous neural spine which gradually loses its height posteriorly.
3. Neural canal is narrow.
4. Presence of two concave facets at the expanded anterior end.

Hence, the specimen seems to be a Urostyle of Toad (e.g. *Bufo* sp.).

Axis or second vertebra of Pigeon

Same up to **vertebra** as in **Teleost Fish**.

1. Centrum is **heterocoelous.**
2. Bone is light-weight and pneumatic.

Hence, the specimen seems to be a vertebra of Aves, Pigeon (e.g. *Columba* sp.).

1. Centrum bears anteriorly a tooth-like **odontoid** process and ventrally a hypapophysis.
2. Neural spine short and stout.
3. Pre and postzygapophyses are present.
4. Transverse process is absent.

Hence, the specimen seems to be an Axis vertebra of Pigeon (e.g. *Columba* sp.).

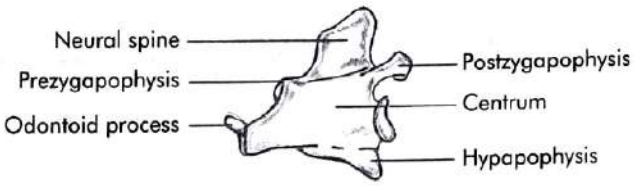


Fig. 6.29 Axis vertebra of *Columba* sp. (lateral view)

Axis or second vertebra of Guineapig

Same up to **vertebra** as in **Teleost Fish**.

1. Thick and heavy bony structure.
2. Centrum is **acoelous**.
3. Presence of intervertebral foramen at the base of the transverse process (Vertebrarterial foramen or transverse foramen).
4. **Epiphyses** are on the anterior and posterior surface of centrum.

Hence, the specimen seems to be a vertebra of mammal, Guineapig (e.g. *Cavia* sp.).

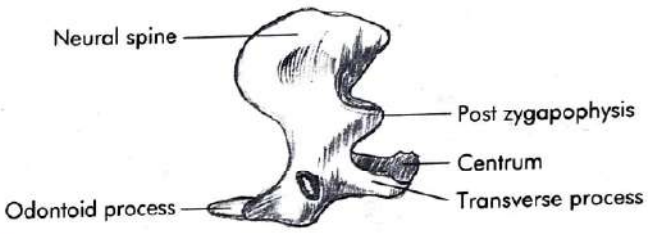


Fig. 6.30 Axis vertebra of *Cavia* sp. (lateral view)

1. Centrum bears anteriorly a tooth-like odontoid process, and ventrally a hypapophysis.
2. Thin, flat, plate-like and backwardly directed neural spine is present.
3. Pre and postzygapophyses well-developed.
4. Transverse process is short and rod-like, and is backwardly directed.

Hence, the specimen seems to be an Axis vertebra of Guineapig (e.g. *Cavia* sp.).

Cervical vertebra of Pigeon

Same up to **vertebra of Aves** as in **Pigeon**.

1. Saddle-shaped bone with elongated centrum.
2. Neural spine is poorly developed.
3. Neural arch is deeply notched anteriorly and posteriorly.
4. Pre and postzygapophyses are well-developed.
5. Presence of short and irregular shaped transverse process.

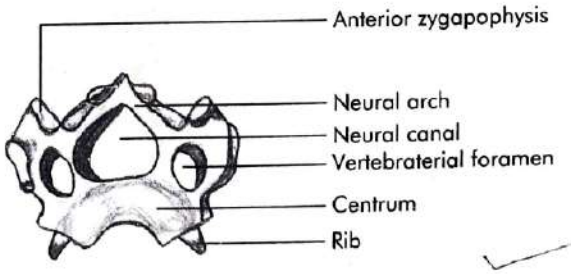


Fig. 6.31 Typical cervical vertebra of *Columba* sp.

6. Vertebrarterial foramen is present at the base of transverse process.
7. Short, slender, spine-like, ventral and backwardly directed vestiges of cervical rib is fused at the proximal end with the transverse process.

Hence, the specimen seems to be a cervical vertebra of Pigeon (e.g. *Columba* sp.).

Cervical vertebra of Guineapig

Same up to **vertebra of Mammals** as in **Guineapig**.

1. Short neural spine is directed vertically upwards.
2. Postzygapophysis is well developed.
3. Transverse process short and stout and is directed outward and downward.

Hence, the specimen seems to be a cervical vertebra of Guineapig (e.g. *Cavia* sp.).

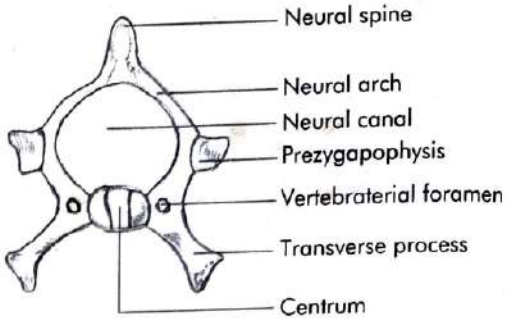


Fig. 6.32 Typical cervical vertebra of *Cavia* sp. (anterior view)

Thoracic vertebra of Pigeon

Same up to **vertebra of Aves** as in **Pigeon**.

1. Centrum stout and short bearing a ventral hypapophysis.
2. Prominent and vertically oriented neural spine is flat and plate-like in appearance.
3. Well-developed transverse process, directed laterally outwards, not perforated at the base.
4. **Capitular** and **tubercular facets** are present for the articulation of the two heads of the ribs on transverse process and centrum.
5. Pre and postzygapophysis well developed.

Hence, the specimen seems to be a thoracic vertebra of Pigeon (e.g. *Columba* sp.).

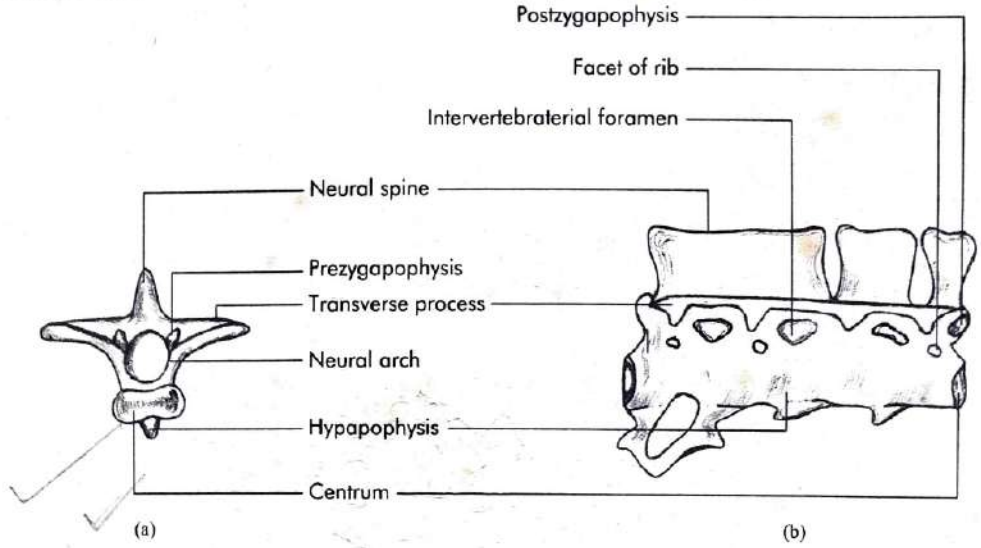


Fig. 6.33 (a) Free Thoracic vertebra (anterior view) and (b) Fused Thoracic vertebra (lateral view) of *Columba* sp

Thoracic Vertebra of Guineapig

Same up to **vertebra of mammal** as in **Guineapig**.

1. Centrum is short and thick.
2. Neural spine is long, slender, pointed and upwardly directed.
3. Transverse process is short and stumpy but not perforated at the base.
4. **Capitular** and **tubercular** facets are there for the articulation of the two heads of the ribs on transverse process and centrum.
5. Pre and postzygapophysis well-developed.

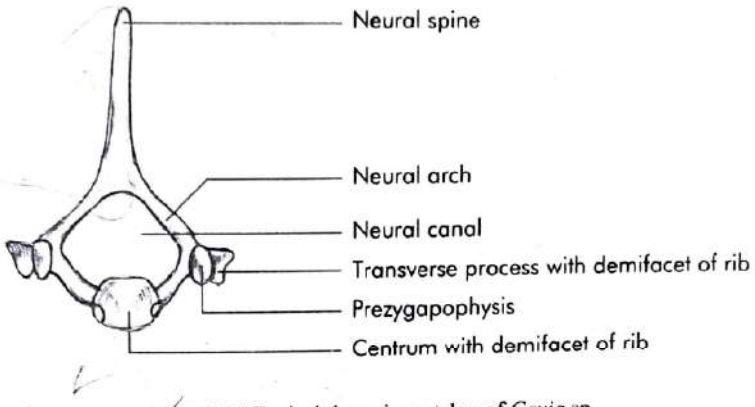


Fig. 6.34 Typical thoracic vertebra of *Cavia* sp

Hence, the specimen seems to be a thoracic vertebra of Guineapig (e.g. *Cavia* sp.).

Synsacrum of Pigeon

1. A large thin plate-like structure, narrow anteriorly but broad and rectangular posteriorly.
2. Elongated bony complex formed by the fusion of thirteen vertebrae [one thoracic, five **lumbar**, two **sacral** and five **caudal** vertebrae).
3. Each vertebra is composed of a centrum, neural arch, neural spine, transverse process, etc. Intervertebral foramens are also present.
4. Anteriormost vertebra with heterocoelous centrum, prezygapophysis, transverse process and facets for attachment of ribs.

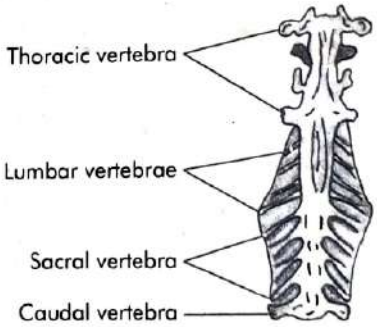


Fig. 6.35 Synsacrum of *Columba* sp. (ventral view)

5. Neural spines of all vertebrae fused to form a continuous neural crest which is very prominent anteriorly as compared to the posterior part.
6. Transverse process are thin, flat and well-developed, and completely fused together except for the first three vertebrae, to form a continuous lateral plate on each side.
7. Each of the seventh and eighth vertebra bears ventral, paired, lateral, rod-like process–the sacral ribs, originating from the centrum.

Hence, the specimen seems to be a synsacrum of Pigeon's (e.g. *Columba* sp.) **vertebral column.**

Pygostyle of Pigeon

1. Light-bony complex composed of laterally compressed segments.
2. Small plough-shaped, upturned structure made up of four posterior caudal vertebrae.

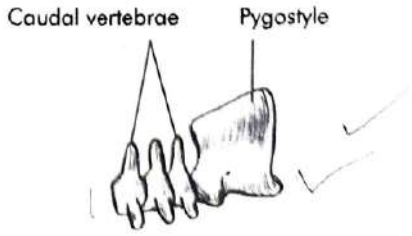


Fig. 6.36 Pygostyle with a few caudal vertebra of *Columba* sp. (lateral view)

Hence, the specimen seems to be a Pygostyle of Pigeon's (e.g. *Columba* sp.) **vertebral column.**

Lumbar vertebra of Guineapig

Same up to **vertebra of mammal** as in **Guineapig**.

1. Large and stout vertebra.
2. Neural spine short and thick, projecting obliquely forward and upward.
3. Transverse process thin, flat and wing-like, directed downward and outward.
4. Well-developed **metapophysis**, dorsolateral to the prezygapophysis and **anapophysis** below the postzygapophysis.
5. Hypapophysis in the midventral line.

Hence, the specimen seems to be a Lumbar vertebra of Guineapig (e.g. *Cavia* sp.).

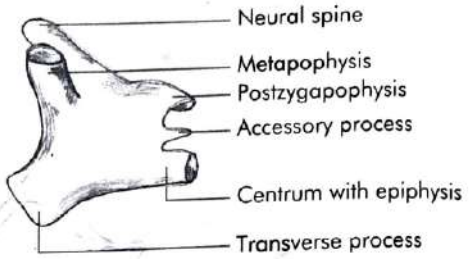


Fig. 6.37 Typical Lumber vertebra of *Cavia* sp. (lateral view)

Sacrum of Guineapig

1. Compound structure made up of several bones arranged one after another in an anteroposterior axis (two sacral and two caudal vertebrae).
2. Roughly triangular in shape, broad anteriorly and narrow posteriorly.
3. Each component bone is a vertebra being made up of centrum, neural canal, neural arch, transverse process, etc.
4. Centrum acoelous with epiphyses.
5. Neural canal is narrow.
6. Intervertebral foramina are present between adjacent vertebrae.
7. Transverse process on the anteriormost vertebra is stout, laterally expanded and has rough dorsal area.
8. Transverse process of posterior vertebrae are not laterally expanded and are steadily reduced in size.
9. Well-developed prezygapophysis on the anteriormost vertebra.
10. Neural spines are flattened and ridge-like.

Hence, the specimen seems to be a sacrum of Guineapig (e.g. *Cavia* sp.).

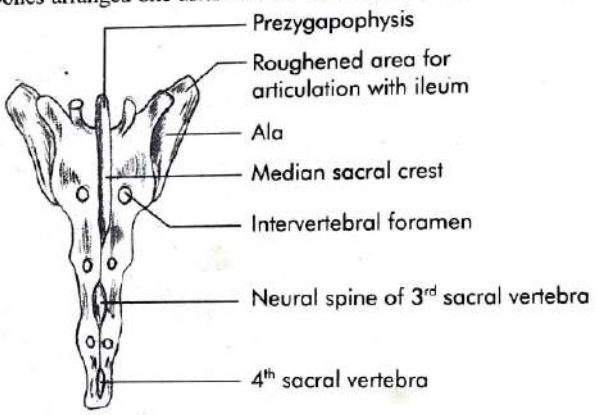


Fig. 6.38 Sacrum of *Cavia* sp. (dorsal view)

Caudal vertebra of Guineapig

Same up to **vertebra of mammal** as in **Guineapig**.

1. Centrum is short.
2. Neural spine is short and small.
3. Transverse process is **bifurcated**.

Hence, the specimen seems to be a caudal vertebra of Guineapig (e.g. *Cavia* sp.).

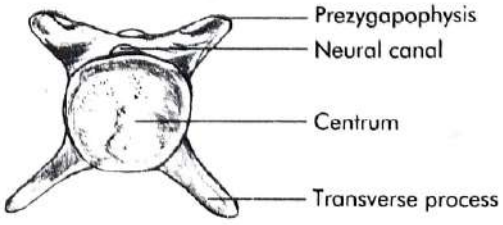


Fig. 6.39 Caudal vertebra of *Cavia* sp

Precaudal Vertebra of Snake

Same up to **vertebra** as in **Teleost Fish**.

1. Centrum is procoelous.
2. Hypapophysis is seen to have been arising from the centrum.
3. Presence of articular surfaces for the ribs.
4. Presence of pre and postzygapophysis.

Hence, the specimen seems to be a vertebra of reptile.

1. Neural spine is well-developed.

Hence, the specimen seems to be a vertebra of reptile belonging to the Order Squamata.

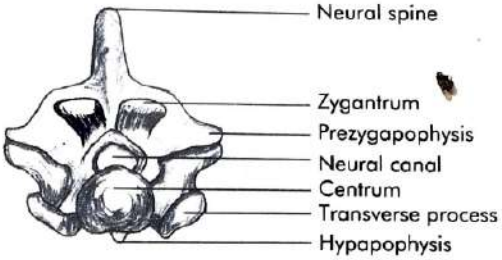


Fig. 6.40 Vertebra of snake (posterior view)

1. Presence of zygantrum and zygosphene.
2. Zygantrum is a hollow cavity on the posterior surface of the neural arch and zygosphene are wedge-shaped processes on the anterior face of the vertebrae.
3. Neural spine is large and flat.
4. Haemal arch is absent.
5. Transverse process is short.

Hence, the specimen seems to be a precaudal or typical trunk vertebra of an ophidian reptile (e.g. snake).

6.1.3 IDENTIFICATION OF VERTEBRATE APPENDICULAR SKELETON WITH REASONS

Girdles

Pectoral Girdle of Toad

1. A compound structure made up of bones and cartilages–seven paired and one unpaired. It consists of two similar halves united midventrally with **sternum** but separated dorsally.
2. Each half consists of a supra-scapula, **cleithrum, scapula, clavicle, precoracoid, coracoid** and **epicoracoid.**
3. Clavicle and cleithrum are the only dermal element of this bony complex.
4. Interclavicle is absent.

Hence, the specimen seems to be a pectoral girdle of an anuran amphibia.

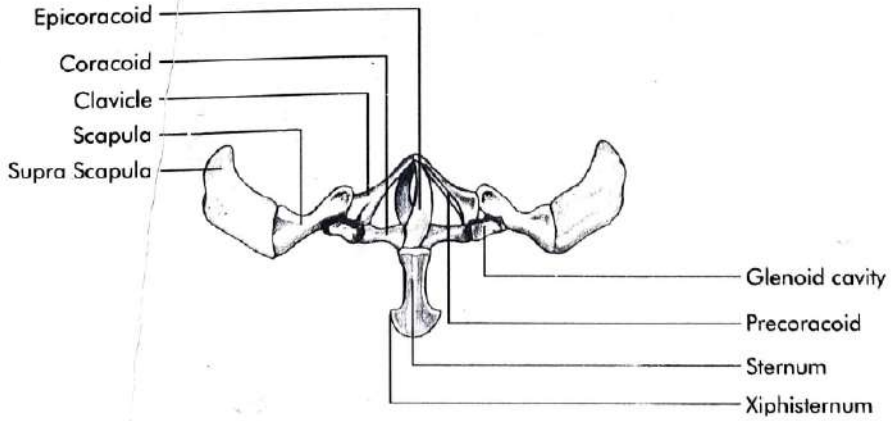


Fig. 6.41 Pectoral girdle of *Bufo* sp

1. Supra-scapula is a dorsally located, thin, flat and cartilaginous structure.
2. Cleithrum is a dorsally located thin and flat body.
3. Scapula is a thick and long dorsolaterally located structure which is expanded at both ends.
4. Coracoid is a stout, rod-like structure that is expanded in the middle and located posteroventrally.
5. At the junction of scapula and coracoid, ventrolaterally there is a deeply concave or cuplike depression, called the **glenoid** cavity.
6. Clavicle is a slender, bony, rod-like structure located anteroventrally.
7. Precoracoid is a narrow strip of cartilage, closely associated with clavicle.
8. Coracoid and clavicle is separated by a wide gap or coracoid foramen.
9. In between the triangular arch formed by clavicle and coracoid, a pair of cartilaginous, dagger-shaped epicoracoid is present. These two epicoracoids overlap on each other (the **arciferal** type of pectoral girdle).
10. Sternum is the unpaired bone, located mid-ventrally at the junction of two halves, the posterior terminal end of which is expanded to form cartilaginous **xiphisternum**. **Omosternum** and **episternum** are however, absent.

Hence, the specimen seems to be pectoral girdle of Toad (e.g. *Bufo* sp.).

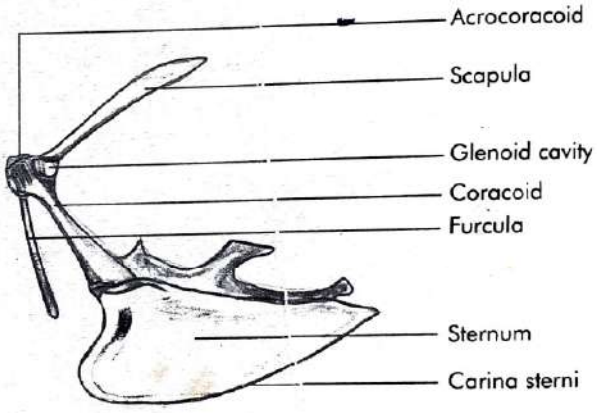


Fig. 6.42 Pectoral girdle of *Columba* sp. (one half with furcula and keel attached)

Coracoid and Scapula of Pigeon

1. Compound structure made up of two bones–coracoid and scapula.
2. Scapula is a sabre-shaped structure.
3. Coracoid is a strong, straight, pillar-like bone.
4. The bony structure is light and pneumatic.

Hence, the specimen seems to be coracoid and scapula of bird.

1. Coracoid and scapula are large and united with one another by ligaments, and meet nearly at an angle less than 90°.
2. **Acromion** and **acrocoracoid** process present.

Hence, the specimen seems to be coracoid and scapula of Carinatae (L. *carina*, keel).

1. Glenoid cavity is formed by the union of scapula and coracoid.
2. **Foramen triosseum** is present.

Hence, the specimen seems to be coracoid and scapula of Pigeon (e.g. *Columba* sp.).

Furcula of Pigeon

1. 'V' shaped structure, very light in weight.
2. Each limb is slender, slightly curved and flattened.
3. Dorsal end of each limb bears a concave articular facet.
4. Ventral end, where both the limbs meet, is thickened, but there is no disc-like structure [**Hypocleidium** (Gk. *hypo*, under; *kleidion*, little key): interclavicle].
5. **Symphysis** and suture absent.

Hence, the specimen seems to be a furcula of Pigeon (e.g. *Columba* sp.).

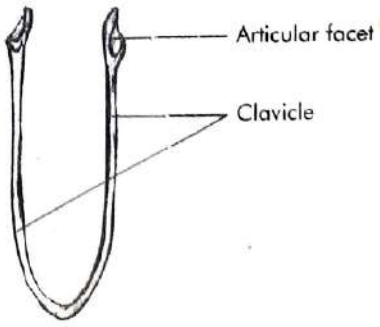


Fig. 6.43 Furcula of *Columba* sp.

Pectoral Girdle of Guineapig

1. Presence of glenoid cavity for the articulation with the head of humerus.
2. The structure is made up of two identical halves.
3. Each half consists of scapula, coracoid, supra-scapula and clavicle.

Hence, the specimen seems to be a pectoral girdle of mammal.

1. Thin, triangular, plate-like scapula, wide posteriorly and narrow anteriorly.
2. Presence of a dorsal, median and anteriorly directed ridge–the scapular spine.
3. Scapular spine is separated from the scapula anteriorly by a scapular notch.
4. Scapular spine bears anteriorly placed acromion and metacromion process.
5. Coracoid is rudimentary and present as a curved hook or a knob-like bone.
6. On the wide posterior border of scapula, suprascapula is present as a thin rim of cartilage.
7. Glenoid cavity is a shallow concavity at the narrow anterior end formed by the scapula.
8. Clavicle is a slender rod, greatly reduced in length.

Hence, the specimen seems to be a pectoral girdle of Guineapig (e.g. *Cavia* sp.).

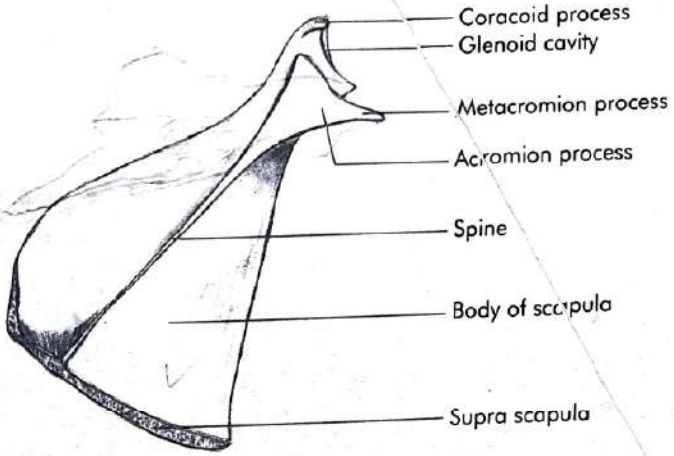


Fig. 6.44 Pectoral girdle of *Cavia* sp. (one half only)

Pelvic Girdle of Toad

1. Compound, strong, thick, long, bony complex made up of two identical halves. Each half consists of **ilium, ischium** and **pubis.**
2. Presence of a deep concavity—the acetabulum for the articulation of the head of the femur.

Hence, the specimen seems to be a Pelvic Girdle.

1. Ilium is a long curved bone directed anteriorly; iliac crest is absent.
2. Ischium is a large and flat bony plate.
3. Ischium of both halves are united in a median symphysis.
4. Pubis is cartilaginous (calcified) and triangular in shape. It is united with its counterpart of the other half in a median symphysis.
5. **Acetabulum** is located posterolaterally and is formed by ilium, ischium and pubis.
6. **Ypsiloid** cartilage is absent.

Hence, the specimen seems to be a pelvic girdle of an anuran Amphibia, Toad (e.g. *Bufo* sp.).

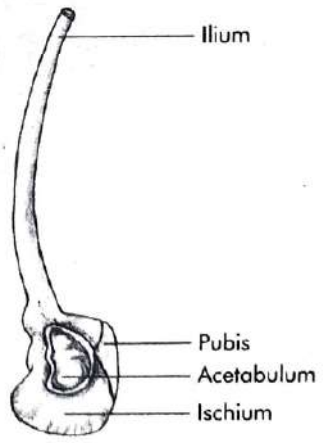


Fig. 6.45 Pelvic girdle of *Bufo* sp.

Pelvic Girdle of Pigeon

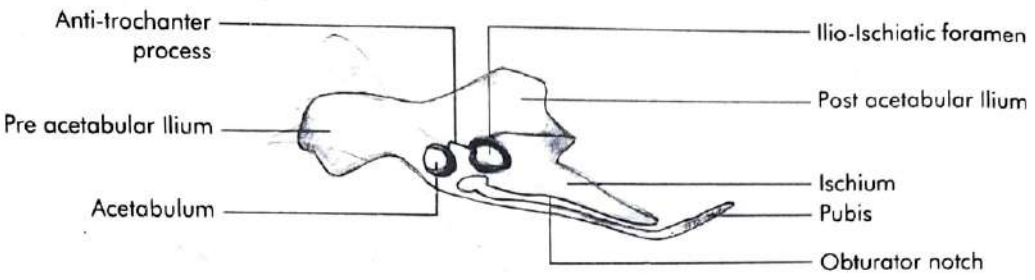


Fig. 6.46 Pelvic girdle of *Columba* sp

Up to **pelvic girdle**, same as in **Toad**.

1. Ilium is the broad and largest thin plate, flat and elongated, divided into concave anterior pre-acetabular part and a convex posterior post-acetabular part, bearing an anti-**trochanter** process.
2. Ischium is a broad, thin, flat and backwardly directed bone which is fused with the post-acetabular ilium.
3. Ilium and ischium is separated by ilio-ischiatic foramen.
4. Pubis is a slender, curved, elongated, backwardly directed bone which is placed parallel to the ischium by a narrow elongated space; presence of **obturator** notch and obturator foramen.
5. Acetabulum is a deep, perforated concavity which is completely ossified and formed by all the three bones.

Hence, the specimen seems to be Pelvic Girdle of Pigeon (e.g. *Columba* sp.).

Pelvic Girdle of Guineapig

Same up to **pelvic girdle** as in **Toad**.

1. Each half of the pelvic girdle (or os-innominatum) is the product of fusion of ilium, ischium and pubis.
2. Each half joins with its fellow bones ventroposteriorly by pubic symphysis.
3. Presence of a large obturator foramen.
4. Ilium is flat, elongated and stout with its anterior end expanded; tip of this end is thickened to form an iliac crest.
5. Ischium is short, stout, backwardly directed and L-shaped with an ischiatic spine, a **sciatic** notch and an ischial **tuberosity.**

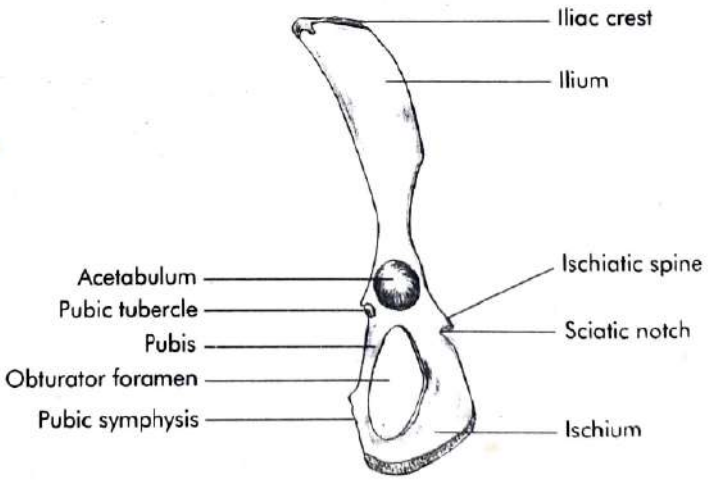


Fig. 6.47 Pelvic girdle of *Cavia* sp. (one half only)

6. Pubis is thin, flat and curved and joins posteriorly with the fellow bones through pubic symphysis.
7. Acetabulum is situated at the junction of ilium, ischium and pubis.

Hence, the specimen seems to be a pelvic girdle of mammal, Guineapig (e.g. *Cavia* sp.).

Limb Bones

Humerus of Toad

1. Single long bone made up of a narrow shaft, while the proximal and distal ends are broad and made up of calcified ridges.
2. Presence of knob-like head on the proximal end.
3. Presence of **deltoid** ridge.

Hence, the specimen seems to be a Humerus.

1. **Shaft** is markedly curved, stout and cylindrical.
2. Prominent deltoid ridge runs down the shaft longitudinally, just below the head.
3. Distal end bears spherical **trochlea** with **condyloid** ridge on either side.

Hence, the specimen seems to be a humerus of an anuran Amphibia, Toad (e.g. *Bufo* sp.).

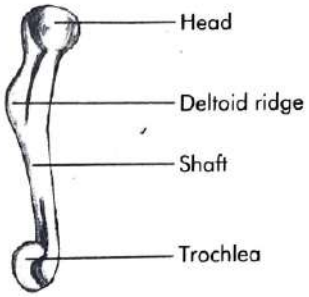


Fig. 6.48 Humerus of *Bufo* sp.

Humerus of Calotes

Up to **Humerus**, same as in **Toad.**

1. Shaft of the humerus is straight, narrow, slender and cylindrical.
2. Proximally it bears transversely elongated convexity, the head, a deltoid ridge, and a tuberosity.
3. Distal trochlear end is pully-like, and bears two articular condyles– the radial and ulnar condyles. These two condyles are again bordered by **ent-epicondyle** and **ect-epicondyle** respectively.
4. An ect-epicondylar foramen is present.

Hence, the specimen seems to be a Humerus of *Calotes* sp.

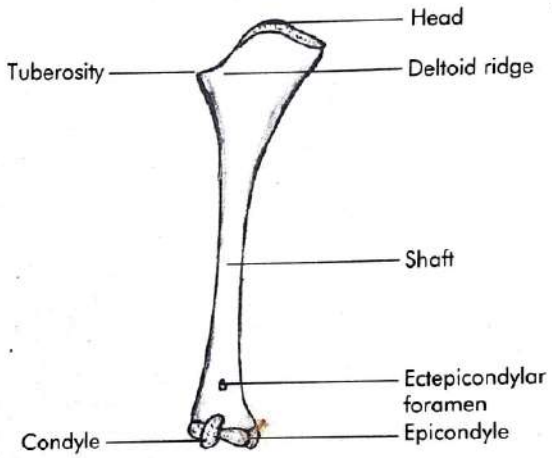


Fig. 6.49 Humerus of *Calotes* sp.

Humerus of Pigeon

Up to **Humerus**, same as in **Toad**.

1. Light and pneumatic bone.
2. Shaft region is large, stout, straight and somewhat flattened.
3. Proximal end greatly expanded and bears a median convex head, an outer large greater tuberosity, an inner small lesser tuberosity, and a prominent deltoid ridge which is continued downwards for a short distance from the lesser tuberosity.

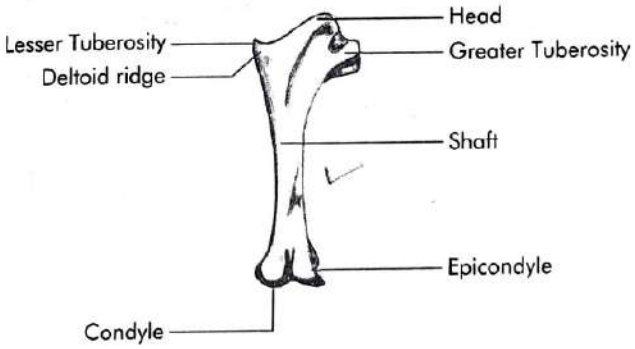


Fig. 6.50 Humerus of *Columba* sp.

4. Distal end bears radial and ulnar condyles and two lateral epicondyles.
5. Greater tuberosity bears a pneumatic foramen.
6. Epicondylar and supra-trochlear foramen absent.

Hence, the specimen seems to be a humerus of Pigeon (e.g. *Columba* sp.).

Humerus of Guineapig

Up to **characters of Humerus**, same as in **Toad**.

1. Stout, heavy bone.
2. Shaft region is large, stout, straight and flattened.
3. Proximal end bears a spherical head, an outer large greater tuberosity, an inner small lesser tuberosity, a shallow **bicipital** groove separating the tuberosities, and a moderately developed deltoid ridge extending down from greater tuberosity.
4. Distal end with a pulley-like trochlea and two lateral epicondyles.
5. Epicondylar foramen is absent.
6. Supratrochlear foramen is present.

Hence, the specimen seems to be a humerus of mammal, Guineapig (e.g. *Cavia* sp.).

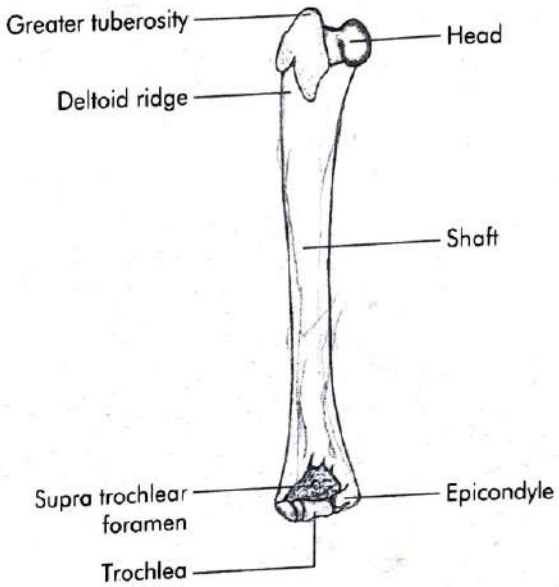


Fig. 6.51 Humerus of *Cavia* sp

Femur of Toad

1. Long, stout, rounded shaft of bone.
2. Proximal end bears rounded head, and distal end bears articulating surface.
3. Deltoid ridge is absent.

Hence, the specimen seems to be a Femur.

1. Shaft is cylindrical, thin and slightly curved.
2. Roughened, rounded head is made of calcified cartilage at the proximal end.
3. A small lateral crest runs down the shaft for a short distance, starting from a little distance below the head.
4. Distal end bears a laterally expanded, roughened, larger mass, with a concave articular facet.

Hence, the specimen seems to be a femur of anuran Amphibia, Toad (e.g. *Bufo* sp.).

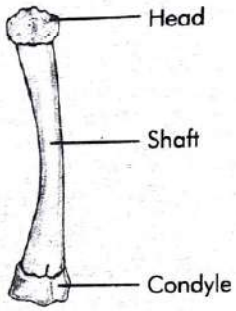


Fig. 6.52 Femur of *Bufo* sp.

Femur of Calotes

Same up to **Femur** as in **Toad**.

1. A laterally expanded head is present at the proximal end. A

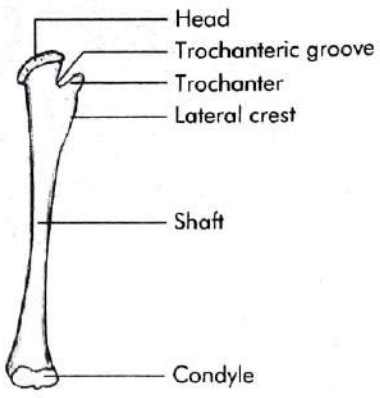


Fig. 6.53 Femur of *Calotes* sp.

well-developed lesser trochanter groove separating the head and trochanter is also present.
2. Small lateral crest is present.
3. Large, spindle-shaped tibial condyle and small fibular condyle is present distally. A patellar (intercondylar) groove is present ventral to the distal end.

Hence, the specimen seems to be a Femur of *Calotes* sp.

Femur of Pigeon

Up to **Femur**, same as in **Toad** plus one additional point given below.

1. Presence of trochanter at the proximal end, and at an angle to the shaft.

Hence, the specimen seems to be a Femur.

1. Light and pneumatic bone.
2. Shaft straight and cylindrical.
3. The proximal end bears a distinct rounded and knob-like head extending at right angle away from the shaft.
4. A prominent greater trochanter and a flat articular surface between the head and trochanter at proximal end of the shaft.
5. The distal end of shaft is provided with a pulley-like condyle separated by intercondylar groove.

Hence, the specimen seems to be a Femur of a bird, Pigeon (e.g. *Columba* sp.).

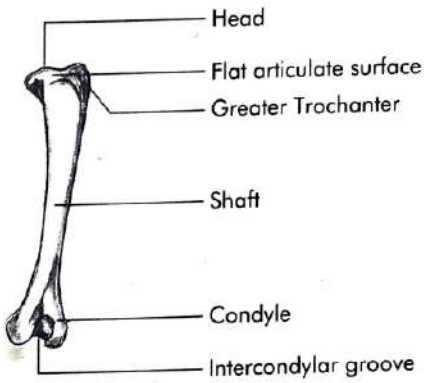


Fig. 6.54 Femur of *Columba* sp.

Femur of Guineapig

Up to **Femur**, same as in **Toad**.

1. Heavy bony structure.
2. Shaft straight and somewhat flattened.
3. The proximal end bears a distinct spherical head extending away from the shaft in greater angle and borne on a distinct neck.
4. A large greater trochanter, a small lesser trochanter and a deep trochanteric fossa at the proximal end of the shaft. Absence of any third trochanter.
5. A prominent lateral crest is present.
6. Distal end bears two large lateral condyles separated by a patellar or intercondylar groove.

Hence, the specimen seems to be a Femur of mammal, Guineapig (e.g. *Cavia* sp.).

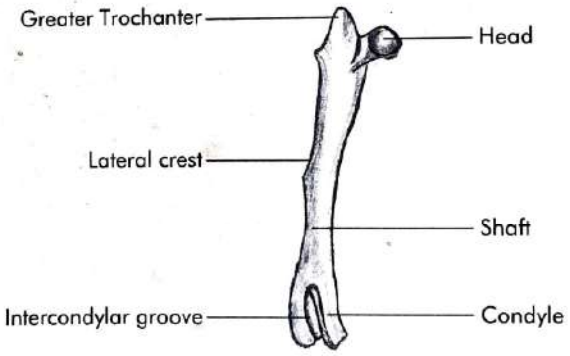


Fig. 6.55 Femur of *Cavia* sp.

Radio-Ulna of Toad

1. Compound structure made up of two elongated, unequal bones, radius and ulna, lying close together.
2. Proximal and distal ends are made up of calcified ridges.

Hence, the specimen seems to be a radio-ulna.

1. Radius and ulna are fused together.

2. Proximal end bears a concavity or semilunar notch and an upwardly directed projection or **olecranon** process.
3. Distal end of this compound bone is somewhat flattened and a groove indicates the fusion of two bones.
4. Distal end bears articular facets.
5. Proximal and distal ends are made up of calcified cartilage.

Hence, the specimen seems to be a Radio-ulna of an anuran Amphibia, Toad (e.g. *Bufo* sp.).

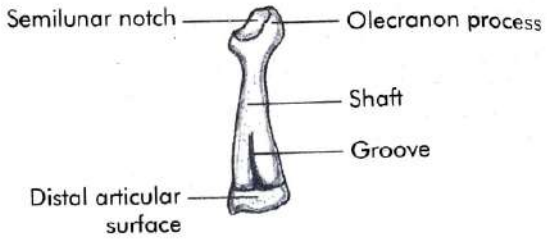


Fig. 6.56 Radio-Ulna of *Bufo* sp.

Radio-Ulna of Calotes

Up to **Radio-Ulna**, same as in **Toad**.

1. Bones are slender and almost equal in diameter, but unequal in length.
2. The longer bone is ulna; proximally it bears an olecranon process and a cup-shaped (semilunar) notch. Distally this bone is convex.
3. The shorter bone is radius. Its proximal end bears a shallow concave articular facet while distal end bears a **styloid** process.

Hence, the specimen seems to be a Radio-ulna of *Calotes* sp.

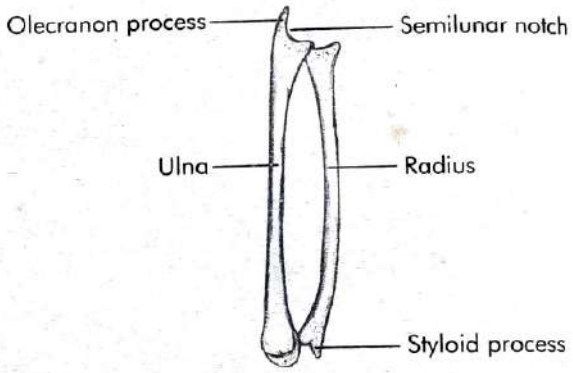


Fig. 6.57 Radio-Ulna of *Calotes* sp.

Radio-Ulna of Pigeon

Same up to **Radio-Ulna** as in **Toad**.

1. Light, pneumatic bone.
2. Bones are unequal in thickness.
3. Presence of slender and straight bone–the radius, and the stout and curved bone, ulna.
4. Radius and ulna lying close together at their proximal and distal ends, but remain widely separated in the middle.
5. The proximal end of ulna bears a cup like semilunar notch.
6. Proximal end of ulna forms an upwardly directed projection or olecranon process.
7. Proximal end of radius is slightly concave while distal end is convex.
8. Distal end bears articulating surface.

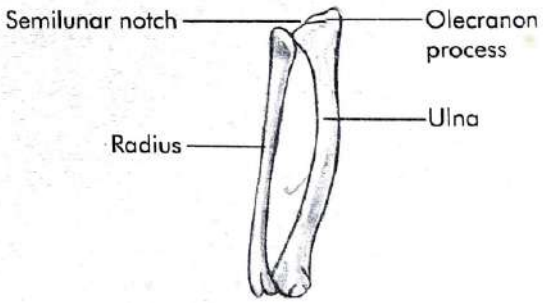


Fig. 6.58 Radio-Ulna of *Columba* sp.

Hence, the specimen seems to be a Radio-ulna of a bird, Pigeon (e.g. *Columba* sp.).

Radio-Ulna of Guineapig

Same up to **Radio-Ulna** as in **Toad**.

1. Stout and heavy bone.

2. Bones are of equal thickness.
3. Radius is small and slightly bent having a concave facet at the proximal end.
4. Ulna is long bone with a proximal olecranon process.
5. Two bones are united at the proximal and distal ends having a gap in the middle.
6. The proximal end of the ulna bears a deeply concave semilunar or sigmoid notch.
7. Distal ends of both radius and ulna bears styloid process.

Hence, the specimen seems to be Radio-Ulna of mammal, Guineapig (e.g. *Cavia* sp.).

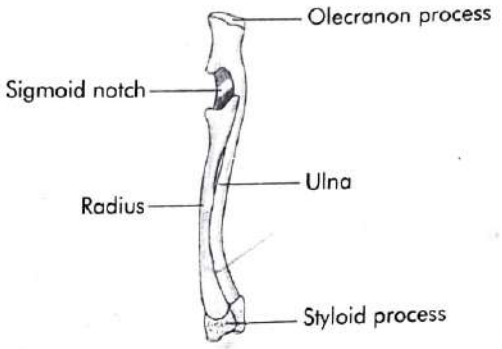


Fig. 6.59 Radio-Ulna of *Cavia* sp.

Tibio-Fibula of Toad

1. Compound bony structure made up of complete lengthwise fusion of two elongated bones (even longer than femur).
2. Proximal and distal ends of this bone are somewhat flattened; presence of proximal and distal groove mark the line of fusion of two bones.
3. Proximal and distal end is rough, laterally expanded and made up of calcified cartilages.
4. A **nutrient** foramen is present in the middle of the fused shaft region.
5. Proximal end of tibia bears an ill developed groove called **cnemial** or **tibial** crest.

Hence, the specimen seems to be Tibio-Fibula of an anuran Amphibia, Toad (e.g. *Bufo* sp.).

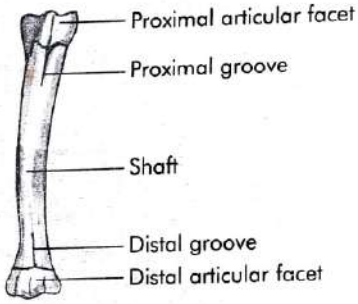


Fig. 6.60 Tibio-Fibula of *Bufo* sp.

Tibio-Fibula of Calotes

1. Compound bony structure made up of two elongated bones lying close together both at proximal and distal ends, but are widely separated in the middle. Bones are of unequal thickness.
2. The thicker bone is tibia, and it bears two articular surfaces and cnemial crest.
3. Fibula is the thinner bone; proximally it bears one articular surface.
4. There is one condyle at the distal end.

Hence, the specimen seems to be a Tibio-Fibula of *Calotes* sp.

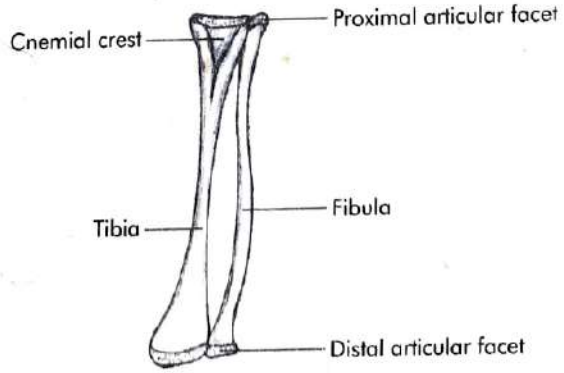


Fig. 6.61 Tibio-Fibula of *Calotes* sp.

Tibio-Tarsus and Fibula of Pigeon

1. Light and pneumatic bone.
2. Compound structure made up of two elongated bones lying close together.

3. Bones are grossly unequal both in length and in thickness.
4. The stouter and longer bone is the tibio-tarsus.
5. The short, slender and gradually tapering bone is the fibula.
6. At its proximal end, tibio-tarsus bears two articular concavities and two prominent crests—median cnemial (or tibial) and lateral cnemial (or tibial).
7. Distal end is pulley like and shows a smooth groove bounded by two articular condyles (epicondyles or **malleoli**).

Hence, the specimen seems to be a Tibio-Tarsus and Fibula of bird, Pigeon (e.g. *Columba* sp.).

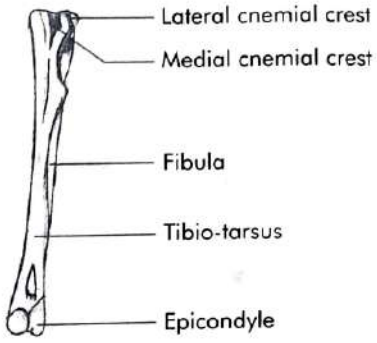


Fig. 6.62 Tibio-Tarsus and Fibula of *Columba* sp.

Tibio-Fibula of Guineapig

1. Heavy bony structure.
2. Compound structure made up of two elongated bones lying close together, narrowly separated proximally.
3. Bones are slightly unequal in length but grossly unequal in thickness.
4. The stout bone is tibia.
5. The thin and narrow (slender) bone is fibula.
6. At its proximal end, tibia bears two concave articular surfaces and a well developed cnemial (or tibial) crest.
7. Distal end of tibia bears two irregular articular surfaces.
8. Both ends of fibula is slightly expanded and fused with tibia.
9. Ends of this compound bone are covered by epiphyseal cartilagenous cap.

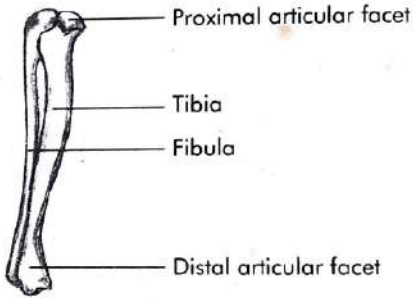


Fig. 6.63 Tibio-Fibula of *Cavia* sp.

Hence, the specimen seems to be a Tibio-fibula of mammal, Guineapig (e.g.*Cavia* sp.).

Carpometacarpus of Pigeon

1. Light and pneumatic bone.
2. Compound structure made up of two elongated bones fused together completely at the proximal and distal ends, but widely separated in the middle.
3. Bones are of unequal thickness.
4. One bone is stout and straight, while the other is slender and curved.
5. Proximal end bears large concavity and a stout, short rod like process.
6. Distal end bears three articular surfaces.

Hence, the specimen seems to be a Carpometacarpus of bird, Pigeon (e.g. *Columba* sp.).

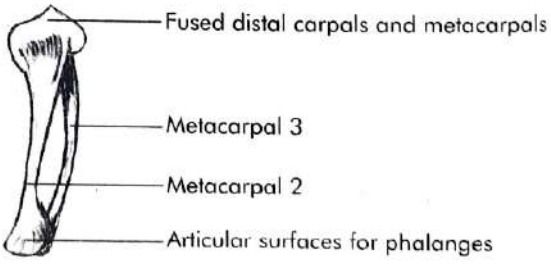


Fig. 6.64 Carpometacarpus of *Columba* sp.

Astragalus-Calcaneum of Toad

1. Compound structure made up of two elongated bones fused together at their proximal and distal ends, but with a wide gap in the middle.
2. Outer thicker and straight bone is **calcaneum** or **fibulare.**
3. Inner, thinner and slightly curved bone is **astragalus** or **tibiale.**
4. Proximal and distal ends are rough, covered by epiphyses, and bear articular facets.

Hence, the specimen seems to be an Astragalus-Calcaneum of anuran Amphibia, Toad (e.g. *Bufo* sp.).

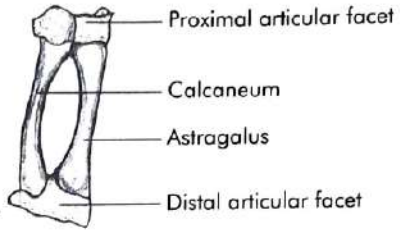


Fig. 6.65 Astragalus-calcaneum of *Bufo* sp.

Tarso-Metatarsus of Pigeon

1. Light and pneumatic bone.
2. Compound structure made up of three elongated bones completely fused together except at their distal ends.
3. Presence of two articular concavities, two foramina and a prominent ventral crest at the proximal end.
4. Presence of three pulley like tubercles and a single foramen at the distal end.

Hence, the specimen seems to be a Tarsometatarsus of bird, Pigeon (e.g. *Columba* sp.).

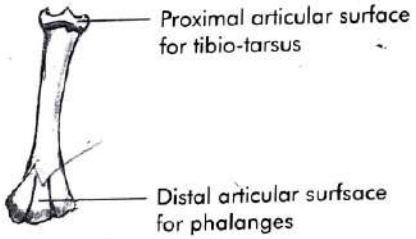


Fig. 6.66 Tarsometatarsus of *Columba* sp.

GLOSSARY OF TERMS USED IN SECTION 6

acetabulum (L. *acetabulum*, vinegar cup). Socket in pelvic girdle for head of femur.

acoelous (Gk. *a*, without; *koilos*, hollow). *appl.* vertebrae with flattened centra.

acrocoracoid (Gk. *akros*, tip; *korax*, crow; *eidos*, form). A process at dorsal end of coracoid in birds.

acrodont (Gk. *akros*, tip; *odous*, tooth). *appl.* teeth attached to the summit of a parapet of bone, as in lizards.

acromion (Gk. *akros*, summit; *omos*, shoulder). Ventral prolongation of scapular spine.

aegithognathous (Gk. *aigithos*, hedge sparrow; *gnathos*, jaw). Skull with maxillopalatines separate, vomers forming a wedge in front and diverging behind. *appl.* a type of palate found in Passeres.

amphicoelous (Gk. *amphi*, both; *koilos*, hollow). *pert.* vertebrae that are concave on both surfaces (*appl.* biconcave vertebral centra).

anapophysis (Gk. *ana*, up; *apo*, from; *phyein*, to grow). A small dorsal projection rising near transverse process in lumbar vertebrae.

arciferal (L. *arcus*, bow; *ferre*, to carry). *appl.* pectoral arch of toads where precoracoid and coracoid are separated and connected by arched epicoracoid.

astragalus (Gk. *astragalos*, ankle bone). Astragalus or talus refers to a tarsal bone in vertebrates.

atlas (Gk. *atlas*, a titan). The first cervical (L. *cervix*, neck) vertebra.

axis (L. *axis*, axle). The main stem or central cylinder; *appl.* second cervical vertebra.

basapophysis (Gk. *basis*, base; *apo*, away; *phyein*, to grow). A transverse process arising from ventrolateral side of a vertebra.

beak (O.F. *bee*, beak). Jaw or bill of birds.

bicipital (L. *bis*, twice; *caput*, head). *pert.* biceps; *appl.* fascia, an aponeurosis of distil tendon of the biceps brachii, *alt.* lacertus fibrosus; a groove, the intertubercular sulcus, on upper part of humerus.

IDENTIFICATION OF MAMMALIAN SKULLS: ONE HERBIVOROUS (GUINEAPIG) AND ONE CARNIVOROUS (DOG) ANIMAL.

1. Bony complex structure enclosing a central cavity.
2. Presence of **occipital condyle.**
3. Presence of **foramen magnum.**
4. Presence of **parietal** and **frontal** segment.
5. Presence of sensory capsules— **olfactory, optic** and **otic.**

Hence, the specimen seems to be a skull.

Skull of Guineapig

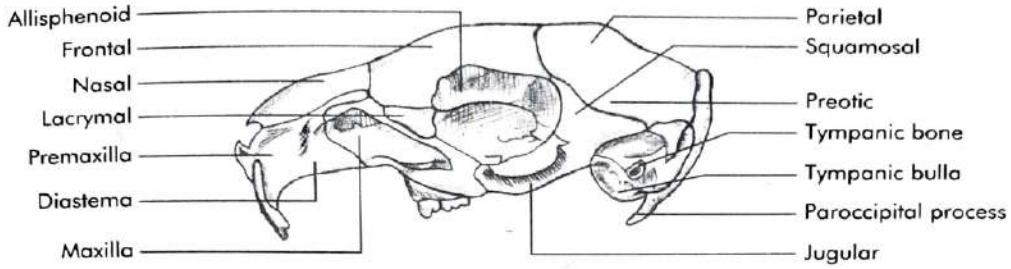


Fig. 6.13 Skull of *Cavia* sp. (lateral view).

Up to **skull** same as in **Toad**.

1. Sutures are prominent.
2. Two occipital condyles, borne on the exoccipital.
3. Presence of well-developed **zygomatic** arch.
4. Dentition is **thecodont** and **heterodont**.
5. Presence of secondary palate.
6. Foramen magnum is wide.

Hence, the specimen seems to be the skull of a Mammalia (L. *mamma,* breast).

1. Secondary palate is without posterior vacuities.
2. Bases of the zygomatic arch are not perforated.
3. Tympanic bone is fused with the skull as **tympanic bulla.**

Hence, the specimen seems to be the skull of an eutherian (Gk. *eu*, well; *therion*, small animal) **mammal.**

1. One pair of **incisor** is long, strong, chisel-shaped, and with enamel on the anterior face.
2. **Canines** are absent; a wide **diastema** is there between incisor and molar.
3. Temporal and orbital fossae are confluent.
4. Posterior border of the orbit is incomplete.
5. Presence of slender and outwardly curved zygomatic arch.
6. Jugal lies in the middle of zygomatic arch.

Hence, the specimen seems to be the skull of a mammal belonging to Order Rodentia (L. *rodere*, to gnaw: the largest order of placentals).

1. **Paroccipital** process is long and slender.
2. Infraorbital foramen is large.
3. Dental formula: $I\frac{1}{1}, C\frac{0}{0}, Pm\frac{1}{1}, M\frac{3}{3}$.

Hence, the specimen seems to be a skull of Guineapig (e.g. *Cavia* sp.).

Skull of Dog

Same up to **skull of eutherian mammal** as in **Guineapig**.

1. Presence of well-developed **sagittal** and **lambdoidal** crest.
2. Temporal and orbital fossae are confluent.
3. Tympanic bulla is well-developed.

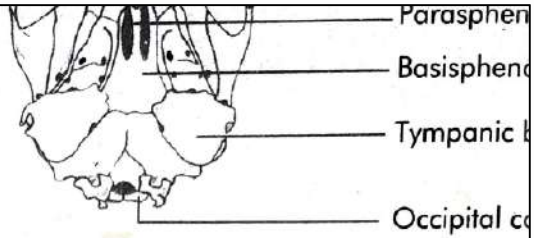


Fig. 6.14 Skull of Rat (ventral view)

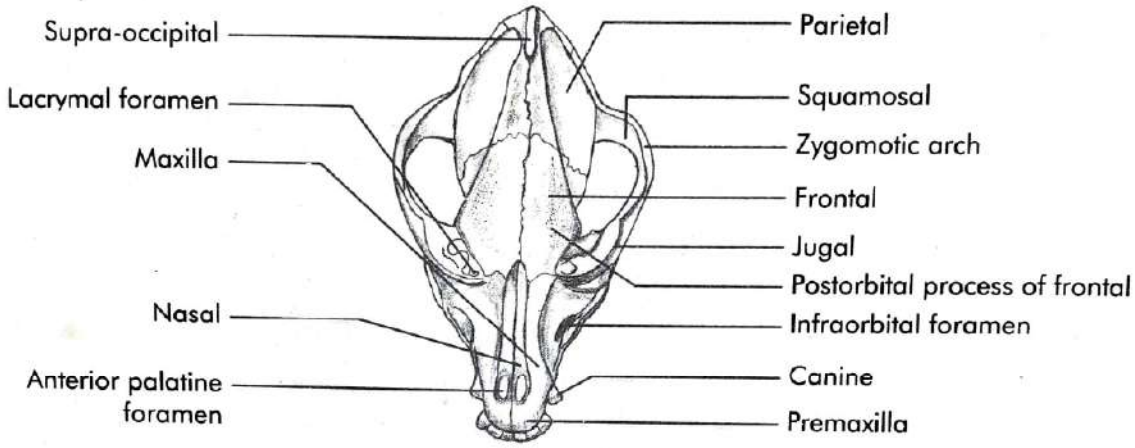


Fig. 6.15 Skull of *Canis* sp. (dorsal view)

4. Posterior border of orbit is incomplete.
5. Zygomatic arch is strong and outwardly curved.
6. Incisors are three in number, and are comparatively small.
7. Canine is large, pointed and slightly curved.
8. Presence of **carnassial** teeth (modified last upper premolar).

Hence, the specimen seems to be a skull of eutherian mammal belonging to Order Carnivora (L. *carn* flesh; *vorare*, to devour); **SubOrder Fissipedia** (L. *fissus*, cleft; *pes*, foot).

1. Facial part is elongated.
2. Well-developed paroccipital process not closely applied to the tympanic bulla.
3. Palate is fully ossified.
4. Upper molar is bluntly triangular.
5. Dental formula: $I\frac{3}{3}, C\frac{1}{1}, Pm\frac{4}{4}\ M\frac{3\text{ to }2}{4\text{ to }3}$.

Hence, the specimen seems to be a skull of Dog (e.g. *Canis* sp.).

DISSECTION OF TILAPIA: CIRCULATORY SYSTEM, BRAIN, PITUITARY, URINOGENITAL SYSTEM

Lata fish
(Ophiocephalus punctatus)

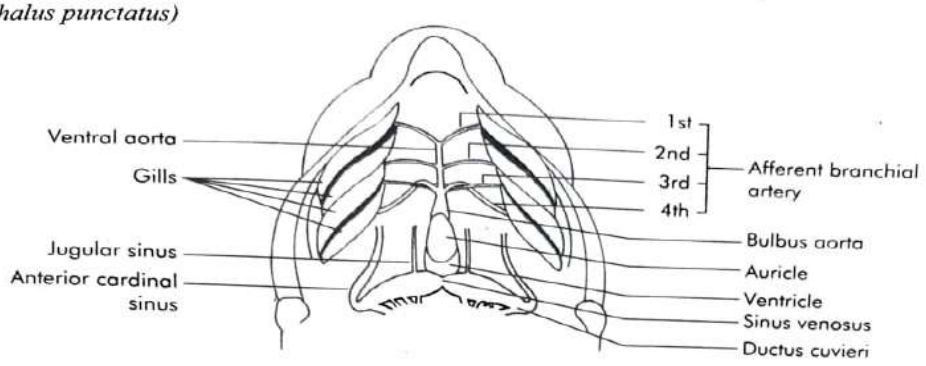


Fig. 11.23 Afferent branchial arterial system of Lata fish

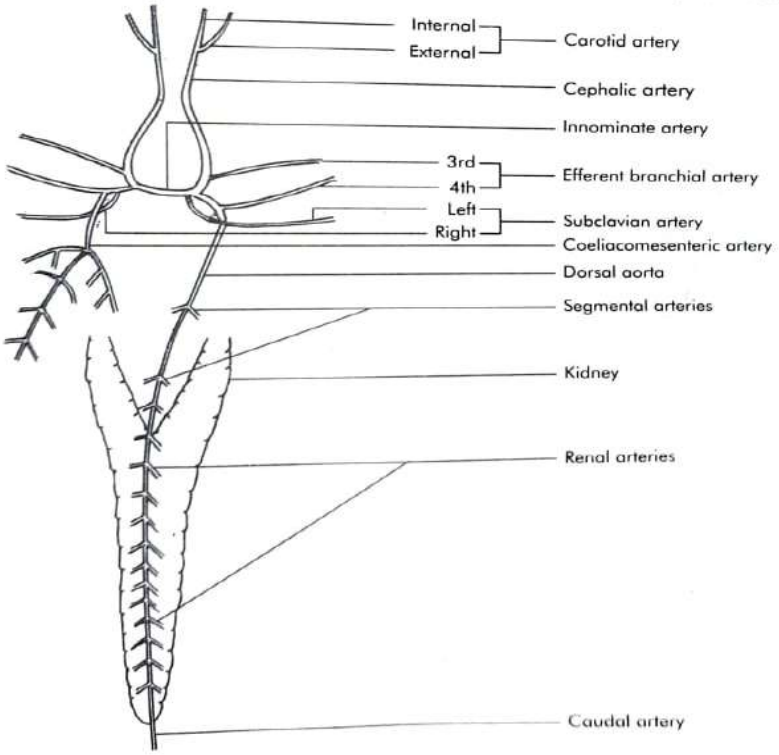


Fig. 11.24 Efferent branchial arterial system of Lata fish

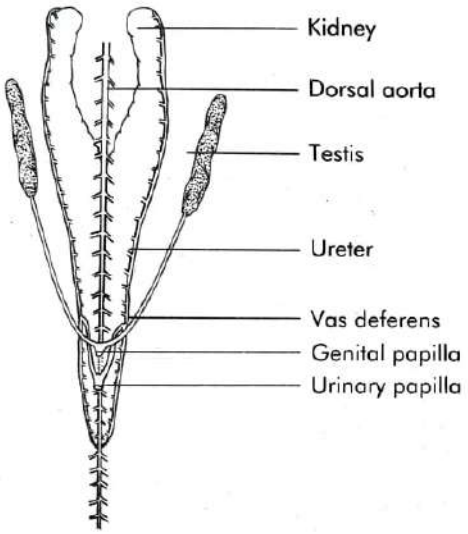


Fig. 11.26 Male urinogenital system of Lata fish

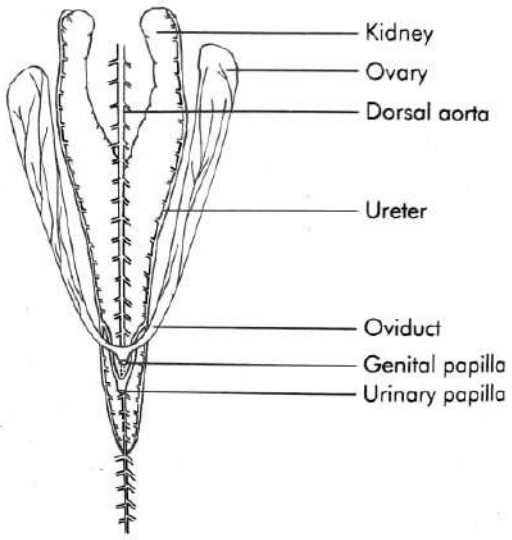


Fig. 11.27 Female urinogenital system of Lata fish

Tilapia fish
(Oreochromes sp.*)*

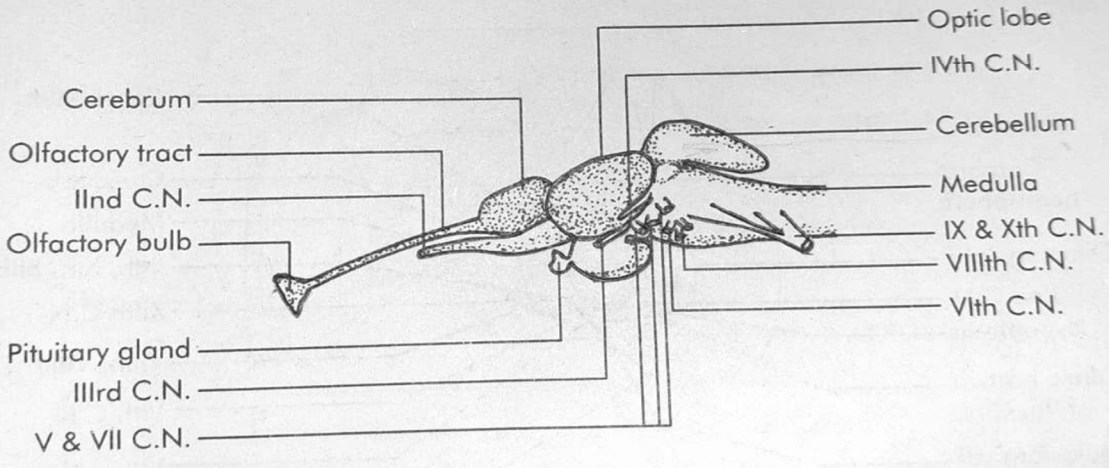
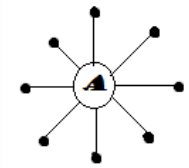
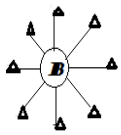
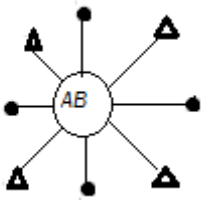
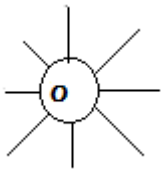
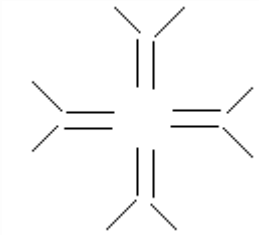
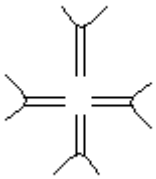
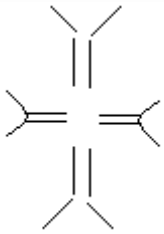


Fig. 11.28 Brain and pituitary gland of Tilapia fish [C.N. = Cranial nerve]

DETERMINATION OF HUMAN BLOOD GROUP

Principle:

In 1900 Karl Land Steiner grouped human blood into 4 group based on presence of two antigens on the surface of RBCs. This groups are designated as A,B, AB & O. This are commonly called ABO Blood group. This blood group system is determined on the basis of presence or is absence of antigen on RBC on antibody in blood group is designated as a blood group. B antigen is present outer membrane of RBC and anti-a antibody present blood plasma. This blood group is designated by B+ blood group. A,B both antigen is present outer membrane of RBC, this blood group is called AB+ blood group.
A, B both antigen is absent outer membrane of RBC, this blood is called O+.
In addition to antigen on ABO system the red cells of 80-85% also have an addition antigen called RH+ antigen or Rh factor.

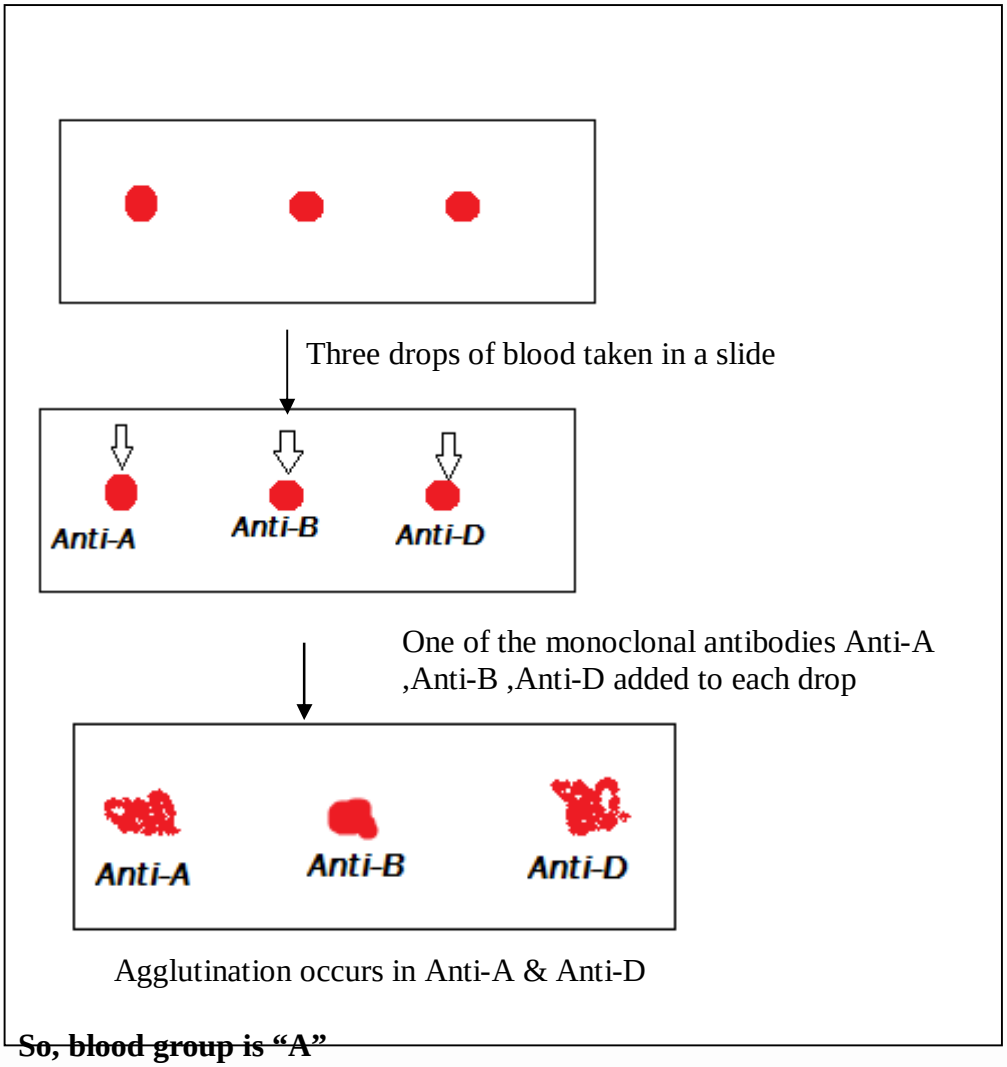
RBC TYPE	GROUP-A	GROUP-B	GROUP-AB	GROUP-O
				
ANTIBODY IN PLASMA	Anti-B 	Anti-A 	None	Anti-A Anti-B 
ANTIBODY IN RBC	A	B	AB	None

In 1940 Karl Land Steiner and Weiner reported that Rabbit sera contains antibodies against of Rhesus monkey that agglutinates RBC of some human being. This antigen is latter name as Rhesus factor (Rh Factor).
During RH determination blood Anti-D serum is mixed with blood and if there is agglutination then the blood is (Rh)(D) is agglutination then the blood is does not agglutinate the blood not have (Rh).

Material required:

1. Rectified spirit

2. Cotton
3. Sterile needle
4. Human blood
5. Glass slide
6. Anti-A(monoclonal)
7. Anti0B(monoclonal)
8. Anti-C(monoclonal)
9. Glass rod



So, blood group is "A"

Procedure:

1. Three area on a slide are marked and labelled them A,B and.
2. The finger tip is punctured by a sterile needle and three drops of blood is taken in a clean glass slide.
3. One drop of Anti-a is added to area 'A' are drop of anti 'Rh' is added to area 'B' and one drop of Anti Rh is added to area D.
4. With separate application glass rod blood is mixed reagent well.
5. After a few seconds the drop are examined.

Observation:

1. Agglutination occurs when reaction is made with Anti-A serum.
2. Agglutination does not occurs when reaction is made with Anti-B serum.
3. Agglutination occurs when tested with Anti-D serum.

Conclusion

On the basis of the above observation it can conclude that my blood group is A positive.

Precaution:

1. The blood should be mixed with antigen quickly, otherwise it will gel coagulated quickly.
2. The slide used should be dust free.
3. The drop of the blood and the drop of anti sera should be equal or else we can not conform if agglutination has occurred or not.

ENUMERATION OF RED BLOOD CELLS AND WHITE BLOOD CELLS USING HAEMOCYTOMETER

Introduction

Red blood cells (RBCs) are the round shape, biconcave discs, present in the blood that helps in the transport of gases throughout the body. The biconcave shape helps the RBCs in rendering the red cells quite flexible so that they can easily pass through the capillaries. On an average, the size of the Red Blood Cells (RBCs) is 7.2 – 7.4 mm (microns). The mature RBCs are non-nucleated cells with an Iron-containing pigment known as Hemoglobin which helps in the transport of oxygen from the lungs to tissues and carbon dioxide from tissues back to the lungs for excretion. The Average lifespan of Red Blood Cells (RBCs) is 100 – 120 days.

Aim

The purpose of performing Total Red Blood cell count is to know whether or not you are suffering from Erythrocytosis or Polycythemia (i.e. the increase in the no. of Red Blood Cells to more than 6.5 million/mm^3) or Erythrocytopenia or Erythropenia (i.e. the Decrease in the no. of Red Blood Cells to less than 3.5 million/mm^3).

Principle

Very large numbers of Red Blood Cells are present in the Blood Specimen. Practically, counting this amount of Red cells directly under the microscope is highly impossible. So, the Red Blood cells are counted by using a special type of chamber, designed for the counting of blood cells in the specimen, known as Hemocytometer or Neubauer's chamber.

For this, the blood specimen is diluted (usually in 1:200 ratio) with the help of RBC diluting fluid (commonly the Hayem's Fluid) which preserve and fix the Red blood cells. The Hayem's fluid is isotonic to the Red blood cells and does not cause any damage to it. The Normal Saline solutions can also be used for this but it causes the slight creation of red blood cells and allows rouleaux formation which may cause the errors in results.

After diluting the specimen, the content is charged on Hemocytometer / Neubauer's chamber and the cells are counted in the areas specific for RBC count.

Nowadays, two types of RBC Diluting fluid are commonly used in Laboratories –

- Hayem's RBC Diluting fluid
- Formalin Citrate diluting fluid

The composition of Hayem's diluting Fluid

COMPONENTS	QUANTITY
Mercuric Chloride	0.25 grams
Sodium sulfate	2.5 grams
Sodium chloride	0.5 grams

The Final pH of the solution (at 25°C) varies from 5.8 – 6.0 which depends on the composition and companies who manufacture it.

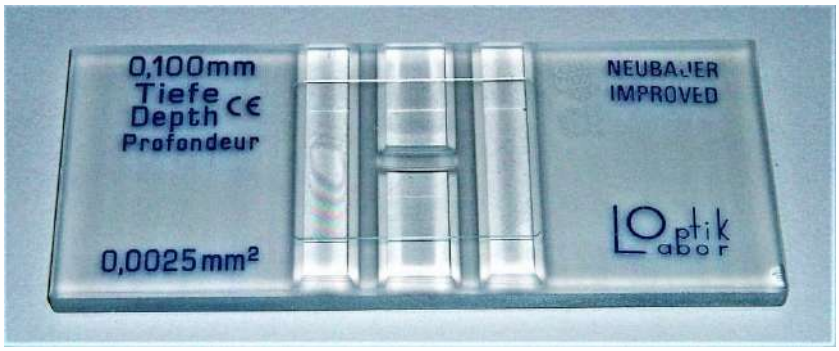
The composition of Formalin Citrate diluting fluid

COMPONENTS	QUANTITY
Trisodium citrate	3 grams
Formalin	1 ml
Distilled water	99 ml

This diluting fluid is commonly used because it is cheaper than the Hayem's fluid. However, Hayem's diluting fluid gives the better results.

Hemocytometer

This is a special type of glass chamber that is used for the cell counting, especially for Blood cells. Nowadays, most commonly Improved Neubauer's Chamber is used and in some laboratories, other types of chambers are also employed like Burkers chamber, Levy's chamber and Fusch – Rosenthal chamber etc.

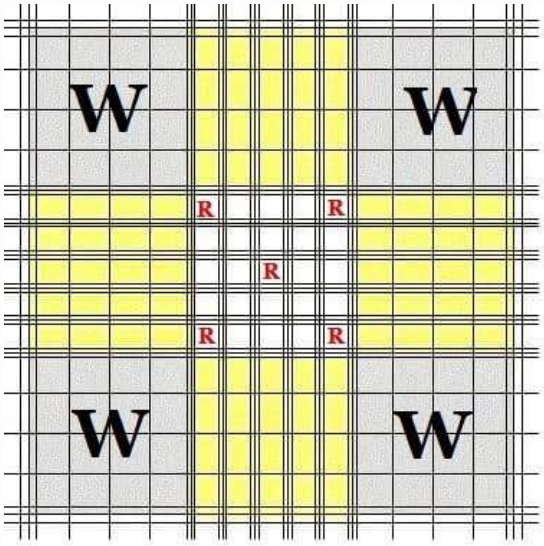


HEMOCYTOMETER

The Neubauer's Chamber has ruled the area of total 9 square mm and the depth is 0.1 mm as when the coverslip is placed on the surface of the counting chamber, the space between the bottom of the cover glass and the base of grooved area measures 0.1 mm in depth.

The central 1 square is highly ruled which is divided into 25 squares. Each square of the Central square is further subdivided into 16 small squares.

For RBC count the cells are counted in the 5 squares of the Central square as 4 Corner squares of the Central square (divided into 25 squares) and 1 central square of the Larger Central Square (divided into 25 squares).



R – RBC AREAS W – WBC AREAS

Each square of the Central Square (divided into 25 squares) contains 16 small squares so the total no. of the area to be counted for RBC Count –

16 × 5 = 80 small squares

Two Method has been developed for the Manual Estimation of Total Red Blood Cell Count Using Hemocytometer / Neubauer's chamber –

- *Microdilution Method*
- *Macrodilution Method*

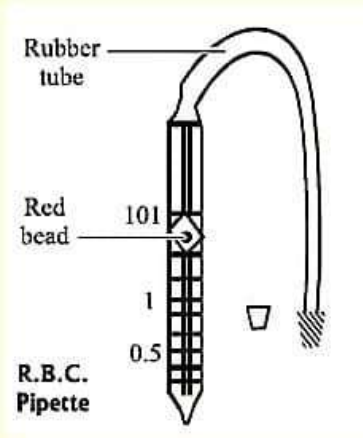
MICRODILUTION METHOD FOR THE ESTIMATION OF TOTAL RBCs USING HEMOCYTOMETER

Materials Required for the Total Red Blood Cell (RBC) Count by Microdilution Method –

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- RBC diluting fluid (preferably Hayem's fluid)
- Gauze piece or Cotton
- RBC pipette
- Hemocytometer a.k.a. Neubauer's Chamber
- Coverslip
- Microscope

A Brief Introduction to RBC Pipette

RBC pipette is a graduated pipette that gives the dilution of 1:100 and 1:200. It has two markings at the bottom as 0.5 and 1 and the top of the pipette is marked 101. It has a round shape bulb which contains the Red bead to mix the blood specimen and the diluting fluid. On the top, a rubber tube is attached to the pipette for sucking the blood specimen and diluting fluid.



RBC PIPETTE

When blood is sucked up to 0.5 mark and the diluting fluid up to 101 marks, gives the 1:200 dilution of Blood: Diluting fluid and When the Blood is sucked up to 1 mark and the diluting fluid up to 101, gives the 1:100 dilution of Blood: Diluting fluid which is commonly used in anemic patients. After sucking the Specimen & Diluting fluid, the content is gently mixed by rotating the pipette on its long axis to ensure thorough mixing of blood and diluting fluid.

Procedure of the Total Red Blood Cell (RBC) Count by Microdilution Method

⇒ Fill the RBC pipette up to the 0.5 mark with the blood specimen and wipe out the pipette externally to avoid false high results.

⇒ Fill the same pipette with the RBC diluting fluid (preferably Hayem's Fluid) up to the mark 101.

⇒ Be cautious that there should be no air bubble in the pipette bulb.

⇒ Mix the Blood and Diluting fluid in the pipette by rotating the pipette (horizontally) between your palms.

⇒ Take out the Neubauer's chamber / Hemocytometer from its case and clean it using a swab or gauze piece. Similarly, clean out the cover glass and place it over the grooved area of Hemocytometer.

⇒ Now, put the RBC pipette, mix the solution present in it again and then discard 1-2 drops from the pipette before charging the chamber.

⇒ Gently press the rubber tube of the RBC pipette, so that the next drop of fluid is in hanging position.

⇒ Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

⇒ Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.

⇒ After charging, wait for 3-5 min so that the cells settle down in the chamber & then focus the chamber under the microscope to calculate Red Cells.

MACRODILUTION METHOD FOR THE ESTIMATION OF TOTAL RBCs USING HEMOCYTOMETER

Materials Required

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- RBC diluting fluid (preferably Hayem's fluid)
- Hb pipette or Micropipette (0.02 ml or 20 μl)
- Hemocytometer / Neubauer's Chamber
- Gauze piece or Cotton swab
- Graduated Pipette (5 ml)
- Test tubes
- Cover Slip

Procedure

⇒ Take 3.98 ml of RBC diluting fluid in a Clean, Dry and Grease free Test tube.

⇒ Now add 0.02 ml or 20 μl of Blood Specimen to the tube containing diluting fluid with the help of micropipette or RBC pipette.

⇒ Mix well for few minutes and ready your Hemocytometer / Neubauer's Chamber.

⇒ Take out the Neubauer's chamber / Hemocytometer from its case and clean it using a swab or gauze piece. Similarly, clean out the cover glass and place it over the grooved area of Hemocytometer.

⇒ Now, take out the RBC pipette and fill it with the Diluted Specimen, mix the solution well and then discard 1-2 drops from the pipette before charging the chamber.

⇒ Gently press the rubber tube of the RBC pipette, so that the next drop of fluid is in hanging position.

⇒ Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

⇒ Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.

Using Micropipette instead of RBC pipette for charging the Hemocytometer

⇒ You can also use a micropipette instead of RBC pipette for charging the Hemocytometer. So, with a micropipette, carefully draw up around 20 μl of the diluted specimen. Press the knob of the pipette to make a hanging drop at the tip of the micropipette.

⇒ Now gently place the pipette tip against the edge of the cover glass and if required slowly expel the more liquid until the counting chamber is full. This process occurs by Capillary action, but care should be taken not to overfill the chamber. A volume of 10 μl is sufficient to fill out the one counting chamber.

⇒ After charging, wait for 3-5 min so that the cells settle down in the chamber.

Counting the red blood cells under microscope

⇒ Focus the ruling using the 10x Objective lens and then Count the RBCs in 5 small squares of the central square as described above, using the 40x Objective lens.

⇒ Count the cells which are lying on the right and lower lines of the 5 small squares but not the opposite line. In case of marginal cells, count the cells on 'L' line that is either on Right and Lower lines or Left and Upper lines.

Calculations

⇒ After counting the cells under the microscope, we know the No. of RBC in 5 squares of the central square. Let's consider it as 'N' no. of cells.
⇒ Now, the volume of the fluid inside the chamber is the product of Area and depth of the Hemocytometer / Neubauer's chamber.
⇒ The central area is the 1 sq. mm which is divided into 25 parts so the area is
25 squares = 1 sq. mm
⇒ Out of these 25 squares, the RBCs are counted in 5 squares. So the Area of 5 small squares is 5/25 i.e. 1/5
⇒ The depth of the Hemocytometer is 0.1 mm as described above in a short description of Hemocytometer.
⇒ Now Apply the Following formula to get the Total Red Blood Cell Count –
Total RBC Count = N × Dilution / Area × Depth
N × 200 (or 100 as the dilution is made) / (1/5 × 0.1)
Total RBC count = N × 10,000 / mm^3

Using the Above formula we can calculate the Total No. of Red Blood Cells present in the Blood Specimen.

NORMAL VALUES OF RED BLOOD CELLS
In Males – 4.8-5.5 million/mm^3
In Females – 4.5-5 million/mm^3

PRECAUTIONS
⇒ Use of Mouth pipettes (RBC pipette) is banned in many countries. However, in case you have to use it, be cautious that you should not intake the diluting fluid or Specimen.
⇒ Accurately measure the amount of specimen and Diluting Fluid to avoid any error in the results.
⇒ In case you are performing this test by Microdilution method, mix the specimen and diluting fluid appropriately by gently rotating in between your palms.
⇒ Carefully charge the Hemocytometer or Neubauer's chamber that it should not be overcharged and do not contain any air bubble in it.

Estimation of haemoglobin using Sahli's haemoglobinometer

INTRODUCTION

Hemoglobin (Hb or Hgb) is a red color pigment present in red blood cells (RBCs) comprises Fe2+ and Globin protein. It is Hemoglobin in RBCs that carries the oxygen from the lungs to the tissues and CO2 from body tissues to the lungs for excretion.

Hemoglobin (Hb or Hgb) is responsible for the appearance of Red color RBCs and blood. Hemoglobin is a chromoprotein consisting of Globin molecule attached to 4 red colored Heme molecules. Hemoglobin synthesis requires the coordinated production of Heme and Globin. Heme is a prosthetic group that medicates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the Heme molecule.

The Estimation of hemoglobin in the blood is commonly prescribed in various physiological and pathological conditions and as both diagnostic and prognostic test especially in case of suspected Anemia which can be caused by various factors.

Nowadays in many laboratories, the Hemoglobin estimation is done by using Automatic Hematology Analyzers but still in many other labs the following method is Commonly used to determine the Hemoglobin concentration in patient's blood.

- Sahli's Method a.k.a. Acid Hematin Method
- Cyanmethemoglobin Method (CMG) a.k.a Drabkin's Method

Principle

The principle of Sahli's Method or Acid hematin method is quite easy that when the blood is added to N/10 Hydrochloric acid (HCl), the hemoglobin present in RBCs is converted to acid hematin which is a dark brown colored compound. The color of the formed acid hematin complex corresponds to the Hemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with N/10 hydrochloric acid or distilled water until the color of acid hematin complex match with the color of the standard.

Reagents required

- N/10 hydrochloric acid (It is prepared by diluting concentrated hydrochloric acid 0.98 ml in distilled water and volume is made up 100 ml).
- Distilled water

Apparatus & equipments required

- Sahli's Apparatus
  - Hemoglobin pipette (0.02 ml or 20 µl capacity)
  - Sahli's graduated Hemoglobin tube
  - Thin glass rod Stirrer for Hemoglobin Tube
  - Sahli's Comparator box with brown glass standard

- Spirit swab
- Blood Lancet
- Dry cotton swab
- Pasteur pipette

Procedure

⇒ N/10 Hydrochloric acid is taken in Hemoglobin tube (has two graduations – one side gm/dl, and other side shows the Hb %age), up to the mark 20 – the lowest marking (yellow marking).

⇒ For capillary blood draw, boldly prick the tip of the middle or ring finger with the help of Blood lancet or pricking needle. Wipe out the first drop of blood and suck the blood from the second drop in Hb pipette up to the mark of 20 µl. Fill the Hb pipette by capillary action.

⇒ Wipe out the surface of the pipette with the help of tissue paper/ cotton so that excess blood may not be added to the Hb tube.

⇒ Dispense the blood into N/10 hydrochloric acid taken in the hemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer.

⇒ Place the tube at room temperature for 10 minutes for complete conversion of hemoglobin into acid hematin.

⇒ After the reaction completes, place the Hb tube in the column in Sahli's Comparator box and start diluting the dark brown coloured compound (Acid Hematin) formed in the Hb tube using the N/10 HCl or distilled water by adding drop by drop of it into the solution and mix with the help of stirrer after each addition.

⇒ This process is done until the endpoint comes matching the color of standard with the color of the test.

⇒ Once the color is matched with the standard brown glass, lift the stirrer up and note down the reading in Sahli's Hb tube by taking the lower meniscus in consideration.
⇒ Now add one more drop of distilled water and mix it properly with the help of stirrer. If color is still matching with the standard add another drop till it matches with the standard and note down the reading and, if it gets lighter after adding the first extra drop, it shows reading taken before dilution was correct. Note down that reading as the final result.
⇒ Reading of this method is expressed in Hemoglobin gm/dl (gram/100 ml) of blood.

Precautions
⇒ Sahli's apparatus especially the Hemoglobin pipette and Sahli's Hemoglobin tube should be clean and dry before use.
⇒ Suck the blood exactly up to the mark of 20 µl (0.02 ml) and air bubbles should not be present in the pipette with blood.
⇒ Mix well the acid and blood and wait for at least 10 minutes after adding the blood in acid.
⇒ Add distilled water drop by drop and mix well after each dilution. Avoid over dilution of the content.
⇒ The matching of color should be done against the natural source of light or electrical tube light (white light) to avoid any visual errors.
⇒ Blood sample and N/10 HCl acid should be taken in an accurate and precise amount in the Hb tube.
⇒ The Hb pipette should be wiped off properly in order to avoid the excess addition of

Advantages
⇒ It is the simple and easy method and may be done at any place because apparatus can be picked up anywhere.

Disadvantages
⇒ Visual intensity may be different for different individuals by this method, we are not able to measure the inactive hemoglobin.
⇒ This method estimates only oxy Hemoglobin. Carboxyhemoglobin and methemoglobin cannot be estimated.
⇒ The endpoint disappears soon so it is difficult to know the actual endpoint and also the Proper stable standard is not available
⇒ The resulting solution is not a clear solution but a suspension due to the action of hydrochloric acid on the proteins and lipids.

Normal values of hemoglobin

- Adult Male: 14-16 gm/dl
- Adult Female: 13-15 gm/dl
- Newborn: 16-18 gm/dl

Clinical significance of hemoglobin estimation

Hemoglobin estimation gives a brief idea of the pathological conditions to the physician so that your physician can easily understand the cause of pathology and prescribe an effective treatment for it.

Raised Hemoglobin Content

- Polycythemia Vera
- Associated with Hypoxia
- Cyanotic Congenital Heart disease
- High Altitudes
- Heavy smoking
- Methemoglobinemia
- Elevated erythropoietin levels
    - Tumors of Kidney, Liver, CNS, Ovary etc.
    - Renal Diseases (Hydronephrosis & Vascular impairment)
- Adrenal hypercorticism
- Therapeutic androgens
- Relative causes of high hemoglobin content
    - Dehydration – Water deprivation, Vomiting, Diarrhea
    - Plasma loss – Burns, Enteropathy

Reduced Hemoglobin Content

Low Hemoglobin value means anemia caused by the following conditions

- Leukemia
- Tuberculosis
- Iron deficiency anemia
- Parasitic infections severely in hookworm infection
- Sickle cell anemia
- Thalassemia
- Aplastic anemia
- Hemolytic anemia
- Loss of blood

Preparation of haemin and haemochromogen crystals

Introduction

Red blood cells of human beings contain a pigment called haemoglobin, which provides red colour to the blood. Haemoglobin is a conjugated chromoprotein and is composed of two parts; non-protein haem and the protein globin. Haem, also known as ferroprotoporphyrin, is made up of four pyrrole rings (tetrapyrrole) which hold iron in the ferrous state. The purpose of this exercise is to enable you to understand how haem component of the haemoglobin present in blood can be converted into specific crystals for the identification.

Materials required

Glass slides, Cover slips, Cotton, Pricking needles, Dropper, Spirit lamp, Compound microscope, Match box, 90% alcohol/Spirit, Takayama's reagent.

Preparation of Takayama's reagent: Add 3 mL of 10% NaOH, 3 mL of Pyridine, and 3 mL of saturated glucose solution in 7 mL of distilled water.

Principle

Hemochromogen, also spelled as Haemochromogen, is a compound of heme with globin modified by the action of alkali. The crystals of haemochromogen are prepared by the heating of blood with Takayama's reagent (the reagent has obtained its name from Masao Takayama who introduced the reagent in Japan in 1912). Heating ruptures the red blood cells and releases the haemoglobin. The protein also gets denatured but remains attached to the haem which is called ferrohaemochrome. During the process, the ferrous form of iron is converted to ferric form due to the presence of NaOH in Takayama's reagent. This oxidized haem is known as alkaline haematin. Haematin combines with pyridine, a nitrogenous compound present in Takayama's reagent, to form insoluble coloured crystals. These are called pyridine haemochromogen which appear as pink-coloured crystals. Saturated glucose solution in the reagent acts as a reducing agent in the reaction, which reduces the solubility of haemochromogen and forms numerous crystals. This property of haem present in human blood is used for identification of blood stains.

Haemoglobin + Heat + NaoH → Haem + Globin

Haem +Glucose+ Pyridine+ Takayama' Reagents → Pyridine Haemochromogen

Procedure

1. Take a cotton swab, soak in the 90% alcohol/spirit and sterilize the tip of your finger.
2. Prick the fingertip with the help of sterilized pricking needle.
3. Place a small drop of blood in the center of a clean slide.
4. Spread the blood drop a little with the help of a needle.
5. Add 2-4 drops of Takayama's reagent on the blood and place a cover slip taking care that bubbles do not appear.
6. Heat the material gently over low flame for 10-20 seconds.
7. Remove the slide from the flame, and then add 1-2 drops of Takayama's reagent from the side of the cover slip.
8. Keep the slide aside at room temperature for 4-5 minutes and examine under the microscope at low (x10) and then at high (x40) magnification power.

Observations

Pink needle-shaped crystals of pyridine haemochromogen appear which confirms the presence of hemoglobin.

Haemochromogen Crystals of Human Blood

Discussion

Haemochromogen crystals are used in forensic cases and medico-legal practices to distinguish blood stains from other red-coloured marks. The crystals can be prepared from fresh as well as dried blood, and thus, it also helps to identify old blood stains. In addition, minute traces of blood are enough to form the crystals.

Precautions

• Prepare Takayama's reagent afresh.
• Use Takayama's reagent carefully as pyridine is a noxious compound and can cause harmful effects if inhaled, swallowed or absorbed through the skin.
• Sterilize the finger with alcohol before pricking.
• Discard the first drop of blood.
• Avoid overheating of the slide. During summers, heating can be skipped.
• Keep the slide undisturbed at the time of cooling.

RECORDING OF BLOOD PRESSURE USING A SPHYGMOMANOMETER

Blood pressure is the force of blood against the walls of the arteries. Blood pressure is recorded as two numbers, the **systolic** pressure (the pressure when the heart beats) over the diastolic pressure (the pressure when the heart relaxes between beats).

Normal systolic pressure is 120 mmHg (millimetres of mercury) and the diastolic pressure is 80 mmHg, that would describe the blood pressure as '120 over 80', written 120/80.

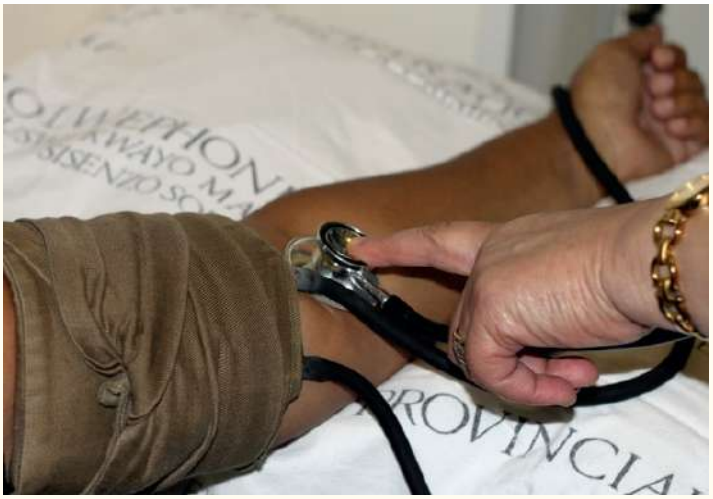


Figure 1. Sphygmomanometer (wall-mounted)

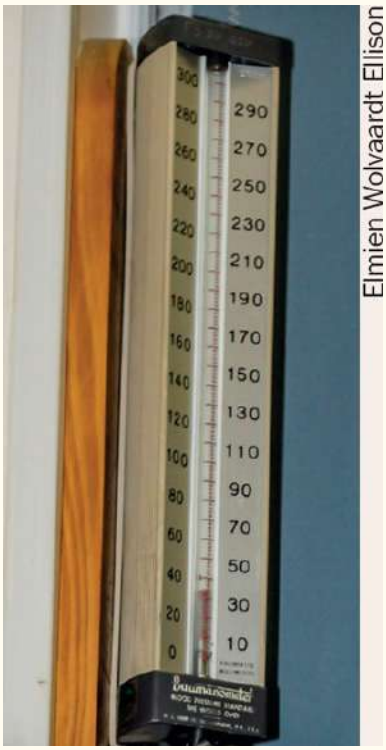


Figure 2. The arm is supported on a level surface. The cuff is around the upper arm and the stethoscope is over the brachial artery, in the bend of the elbow

Blood pressure may vary according to whether the patient is lying down, sitting or standing. It is normally recorded with the patient sitting.

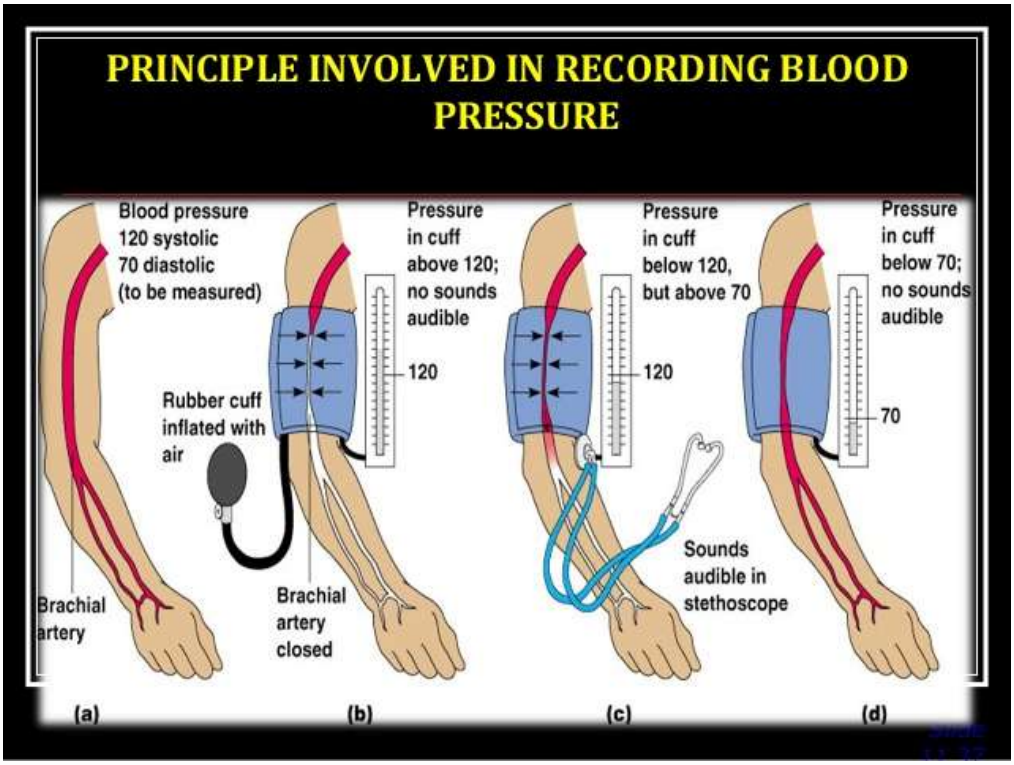
Required apparatus

- sphygmomanometer
- blood pressure cuffs: small, medium, large
- stethoscope
- chair

- patient's care notes or observation chart
- alcohol wipe

Procedure

- Ask the patient to loosen any tight clothing or remove long-sleeved garments so that it is possible to access the upper arm. Do not use an arm that may have a medical problem.
- Place the cuff around the upper arm and secure.
- Connect the cuff tubing to the sphygmo-manometer tubing and secure.
- Rest the patient's arm on a surface that is level with their arm.
- Place the stethoscope over the brachial artery (in the bend of the elbow) and listen to the pulse
- Pump up the cuff slowly and listen for when the pulse disappears. This is an indication to stop inflating the cuff.
- Start to deflate the cuff very slowly whilst watching the mercury level in the sphygmomanometer.
- Note the sphygmomanometer reading (the number the mercury has reached) when the pulse reappears: record this as the systolic pressure.
- Deflate the cuff further until the pulse disappears: record this reading as the diastolic pressure.
- Record these two measurements, first the systolic and then the diastolic (e.g., 120/80), in the patient's notes or chart.
- Tell the patient the blood pressure reading.
- Disinfect the stethoscope drum and ear pieces with the alcohol wipe.
- Wash and dry your hands.
- Report an extremely low or high reading to the clinically qualified person in charge of the patient's care.


PRINCIPLE INVOLVED IN RECORDING BLOOD PRESSURE
Blood pressure
120 systolic
70 diastolic
(to be measured)
Brachial artery
Rubber cuff inflated with air
Brachial artery closed
Pressure in cuff above 120; no sounds audible
120
Pressure in cuff below 120, but above 70
120
Sounds audible in stethoscope
Pressure in cuff below 70; no sounds audible
70
(a)
(b)
(c)
(d)

C10P: Immunology Lab

PREPARATION OF HUMAN BLOOD FILM

Introduction:

A well prepared blood smear is necessary for microscopic examination of blood. Blood smears are used to determine leukocyte differentials, to evaluate erythrocyte, platelet and leukocyte morphology, and, if necessary, to estimate platelet and leukocyte counts.

Principle:

The Clinical Pathology Laboratory uses the wedge technique for preparation of blood smears. This method produces a gradual decrease in thickness of the blood from thick to thin ends with the smear terminating in a feathered edge approximately 2 mm long.The smear is greater than 25 mm long and the feathered edge stops approximately 10 mm from the end of the slide. The blood film occupies the central portion of the slide and has definite margins on all sides that are accessible to examination by oil immersion.

The thin end of the film becomes thinner gradually and does not have grainy streaks, troughs, ridges, waves or holes – features that can result in an uneven distribution of leukocytes. In preparations from normal patients, the thin section of the smear occupies approximately 1/3 of the total area and, within that area; erythrocytes are distributed in a monolayer.

The thickness of the spread is influenced by the angle of spreader slide (the greater the angle, the thicker and shorter the blood smear), the size of the drop of blood and the speed of spreading. Glass cover slips are mounted on all blood smears to prevent damage to smear during examination, cleaning, handling and storage.

Preparation of Blood Film:

The slide should be clean. Place a small drop of blood, or one side about 1-2 cm from one end. Without delay place a spreader at an angle of 45° from the slide and move it back to make contact with the drop. The drop should spread out quickly along the line of contact of spreader with the slide.The moment this occurs, spread the film by rapid smooth forward movement of the spreader. The film should be 3-4 cm in length. The ideal thickness is such that there is some overlap of R.B.C. throughout most of its length with proper separation and lack of distortion of RBC's. the end from where the spread had ended is called tail end.The ideal zone to examine the blood film is the areas between tail and body. If the film is made

too thin or if a rough edged spread is used many leucocytes accumulate in edges and at tail. DLC should not be attempt on such a slide.

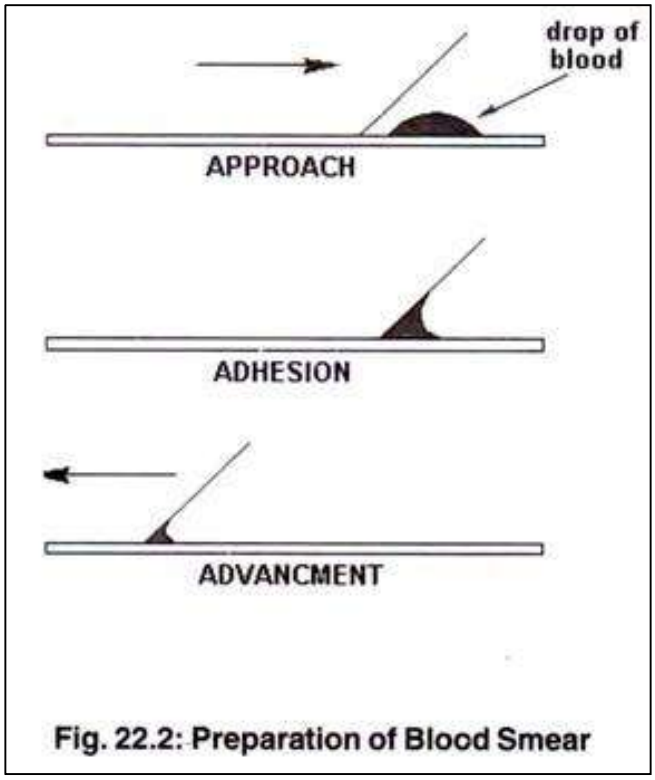


Fig. 22.2: Preparation of Blood Smear

Characteristics of an Ideal Blood Smear:

1. It should be in the central 2/3 of slide.

2. It should have straight lateral border and short tongue shaped tail.

Precautions to be taken during preparation:

1. Angle should be maintained at 45°.

2. Blood drop should be of proper size.

3. Spreader's edges should be smooth and it should be smaller than the slide on which smear is being made.

4. Pressure applied should be proper.

5. Drop should be pulled with spreader not pushed with it.

6. Preparation should be in one single stroke.

Staining of Blood Film:

Process of Staining:

The slide is covered with leishman stain for 2 mins. This much time is required for fixation. After 2 mins it is diluted with double the volume of buffer water. On adding buffer water a metallic shin will be formed, if the stain is dry. Allow this to stand for 15 min. after min, flood the slide with water to remove stain. Then wash under tap water wiping the back of slide with finger or cotton. Dry in air.

Precautions Suring Staining:

1. Time: Initial time 2 minutes, is important. After dilution increase of 1-2 minutes, does not alter staining.

2. Never let the stain dry on the slide otherwise stain deposits will make it impossible to count leucocytes (DLC).

3. Staining should be deposit free.

4. For washing the smear – let the water stream replace the stain. Don not throws the stain first.

HISTOLOGICAL STUDY OF SPLEEN, THYMUS AND LYMPH NODES

SPLEEN:

Identifying Characters:

1. The **stroma** is composed mainly of a network of reticular connective tissue.
2. The **parenchyma** is divided into two functionally and morphologically distinct compartments (**red pulp** and **white pulp**) divided by a tissue layer called the **marginal zone**.
3. Outside the marginal zone is the **perifollicular zone** which contains sheathed capillaries and blood-filled spaces without endothelial lining.
4. **Sinusoids** are found between the cords of Billroth.
5. The **inner layer** is mainly composed of T lymphocytes which is why it is also called the T-zone.
6. The **outer layer** has a more diverse cellular morphology, containing T and B lymphocytes.

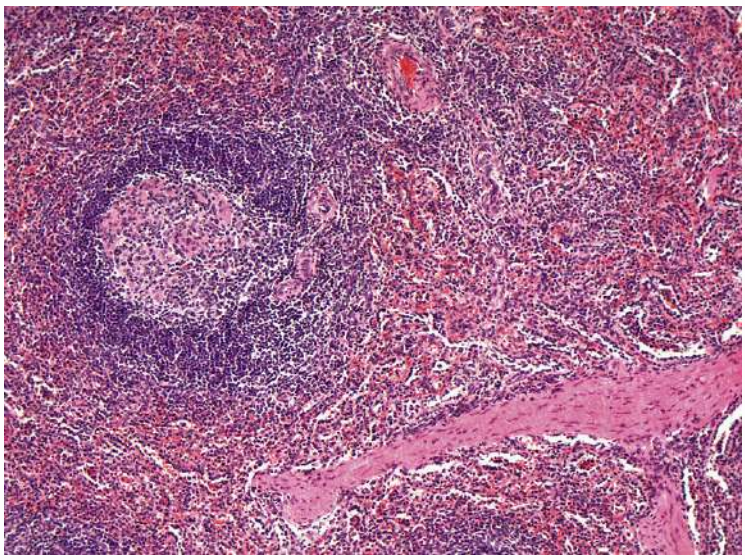


Fig 1: TS of mammalian spleen

THYMUS:

Identifying Characters:

1. The cortex stains more darkly than the medulla, because it contains more lymphocytes than the medulla.
2. The epithelial network in the cortex is more finely branched than in the medulla - and this gives this network the name 'reticular'.
3. The epithelial cells are connected to each other by desmosomes, and the intermediate filament protein keratin is present in their cytoplasm.
4. Presence ofHassal's corpuscles in the medulla.
5. The T-cell progenitors proliferate in the outer cortex.

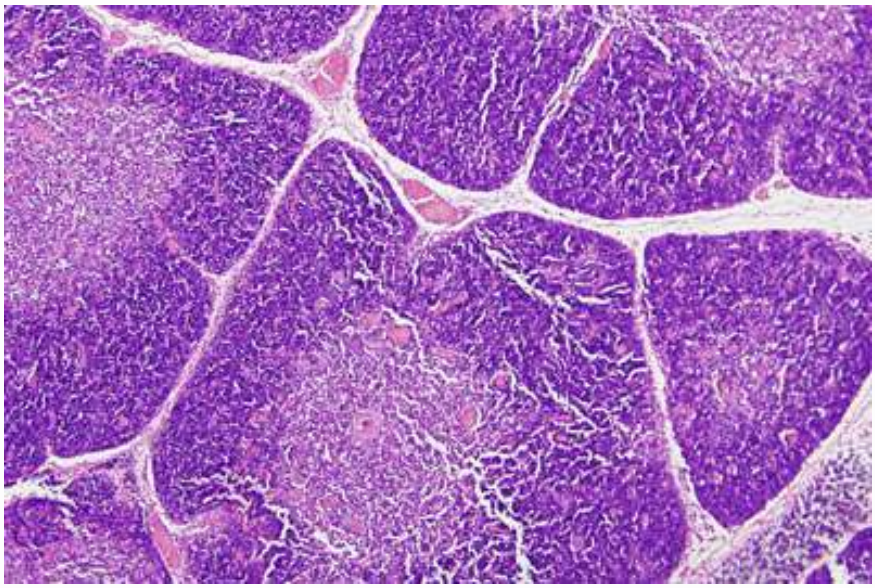


Fig 2: TS of mammalian thymus

LYMPH NODES:

Identifying Characters:

1. The nodes are covered by a capsule of dense connective tissue, and have capsular extensions, of connective tissue, called the trabeculae.
2. The lighter staining areas are germinal centres, where the B-cells proliferate into antibody secreting plasma cells.
3. The hilum is an indent on the concave surface of the lymph node where lymphatic vessels leave and blood vessels enter and leave.
4. The substance of a lymph node is divided into the outer cortex and the inner medulla.
5. Thin reticular fibers of reticular connective tissue form a supporting meshwork called reticulin inside the node.

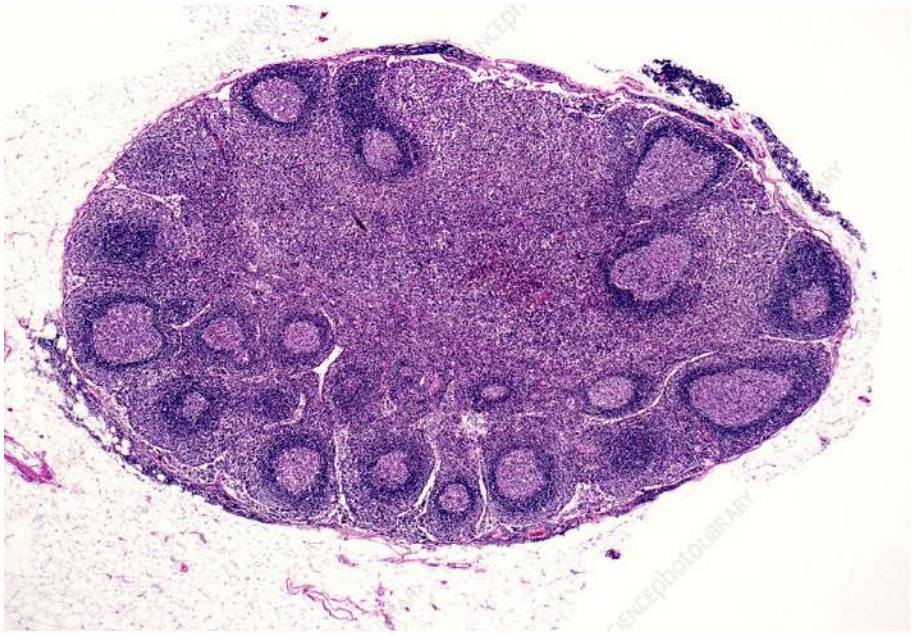


Fig 3: TS of mammalian lymph node

E L I S A

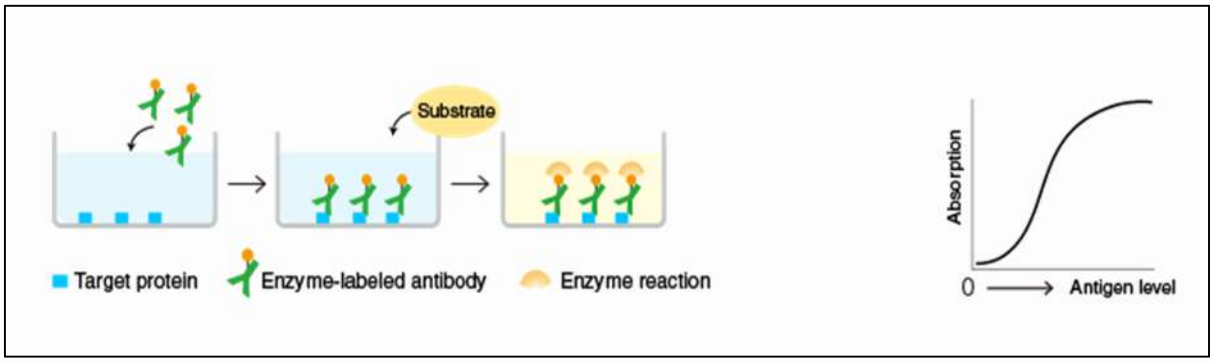
Introduction:

Enzyme-linked immunosorbent assay (ELISA) is a method of target antigen (or antibody) capture in samples using a specific antibody (or antigen), and of target molecule detection/quantitation using an enzyme reaction with its substrate.

Principle:

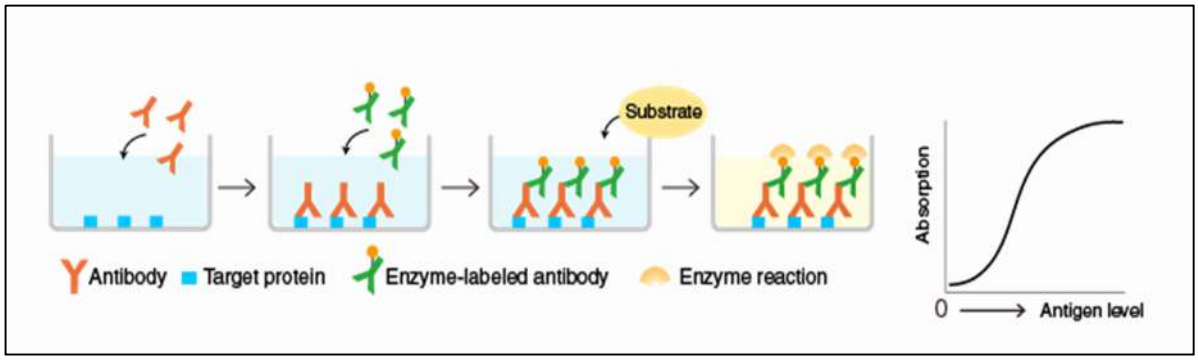
In ELISA, various antigen-antibody combinations are used, always including an enzyme-labeled antigen or antibody, and enzyme activity is measured colorimetrically.The enzyme activity is measured using a substrate that changes color when modified by the enzyme. Light absorption of the product formed after substrate addition is measuredand converted to numeric values.Depending on the antigen-antibody combination, the assay is called a direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA etc.

Direct ELISA:



A target protein (or a target antibody) is immobilized on the surface of microplate wells and incubated with an enzyme-labeled antibody to the target protein (or a specific antigen to the target antibody). After washing, the activity of the microplate well-bound enzyme is measured.

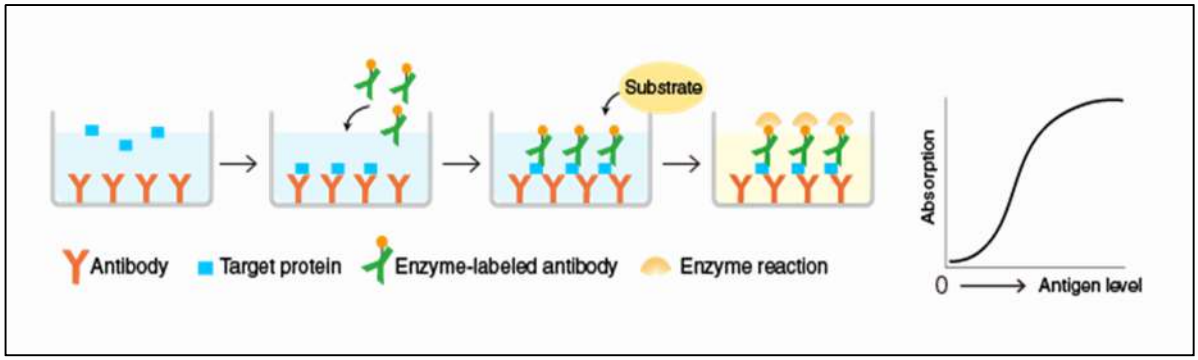
Indirect ELISA:



A target protein is immobilized on the surface of microplate wells and incubated with an antibody to the target protein (the primary antibody), followed by a secondary antibody against the primary antibody. After washing, the activity of the microplate well-bound enzyme is measured.Although indirect ELISA requires more steps than direct ELISA,

labeled secondary antibodies are commercially available, eliminating the need to label the primary antibody.

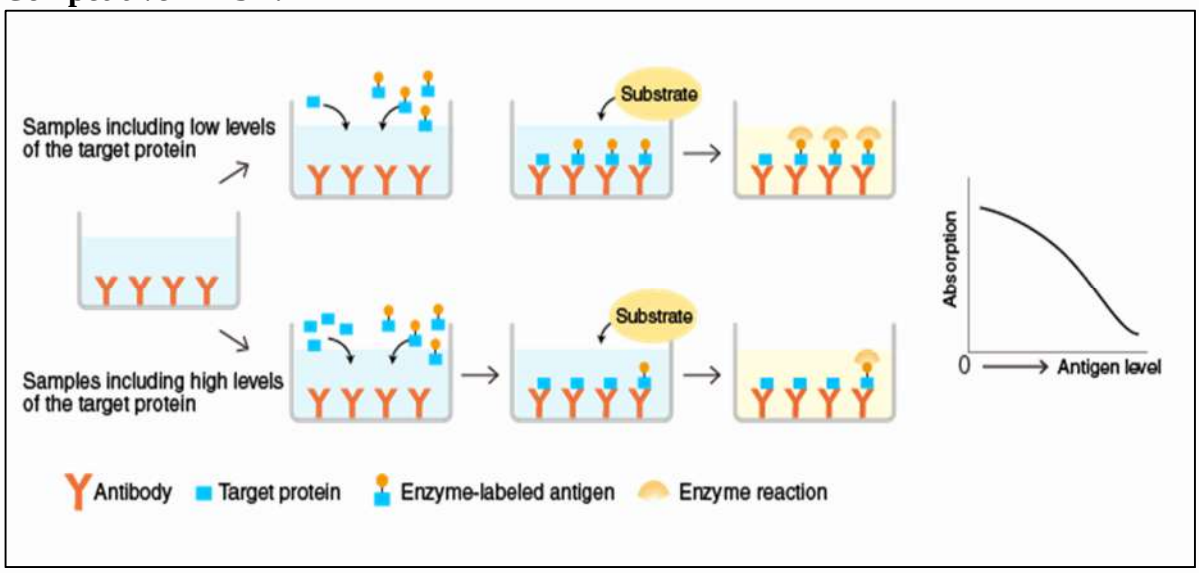
Sandwich ELISA:



An antibody to a target protein is immobilized on the surface of microplate wells and incubated first with the target protein and then with another target protein-specific antibody, which is labeled with an enzyme. After washing, the activity of the microplate well-bound enzyme is measured. The immobilized antibody (orange) and the enzyme-labeled antibody (green) must recognize different epitopes of the target protein.

Compared to direct ELISA, the sandwich ELISA (combining antibodies to two different epitopes on the target protein) has a higher specificity. Sandwich ELISA is useful for applications that require a high accuracy.

Competitive ELISA:



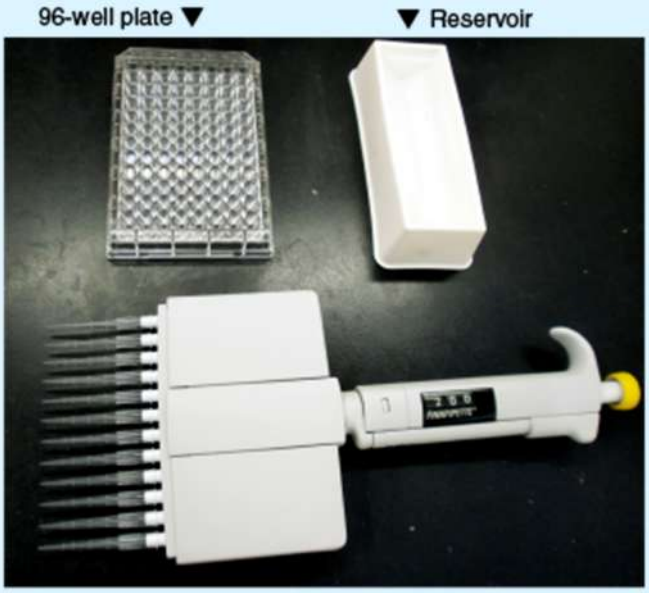
An antibody specific for a target protein is immobilized on the surface of microplate wells and incubated with samples containing the target protein and a known amount of enzyme-labeled target protein.After the reaction, the activity of the microplate well-bound enzyme is measured.

When the antigen level in the sample is high, the level of antibody-bound enzyme-labeled antigen is lower and the color is lighter. Conversely, when it is low, the level of antibody-

bound enzyme-labeled antigen is higher and the color, darker. The graph above and to the right illustrates the correlation between absorption and antigen levels in samples.

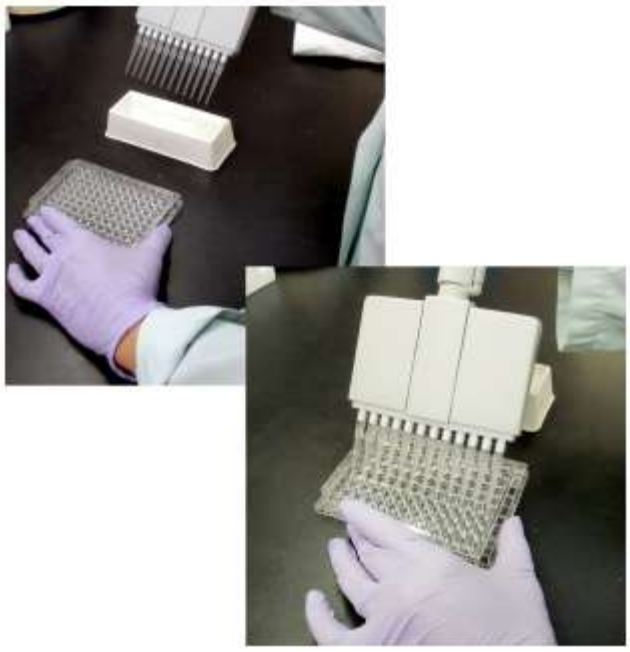
When a target antigen is a small molecule, such as histamine, pesticide, and dioxin, two antibodies cannot simultaneously bind to the antigen in sandwich ELISA. Competitive ELISA is useful for the measurement of low molecular weight targets.

ELISA Procedure:

Step-by-step procedure	
Preparation of reagents and equipment	 ▲ 12-Channel pipette

Immobilization of antibody
Add diluted antibody to each well of a 96-well ELISA plate. Seal the plate to prevent evaporation, and allow it to incubate at 4°C for 15-18 hours to immobilize the antibody.

Washing
Remove the diluted antibody, and wash 3 times with washing solution.

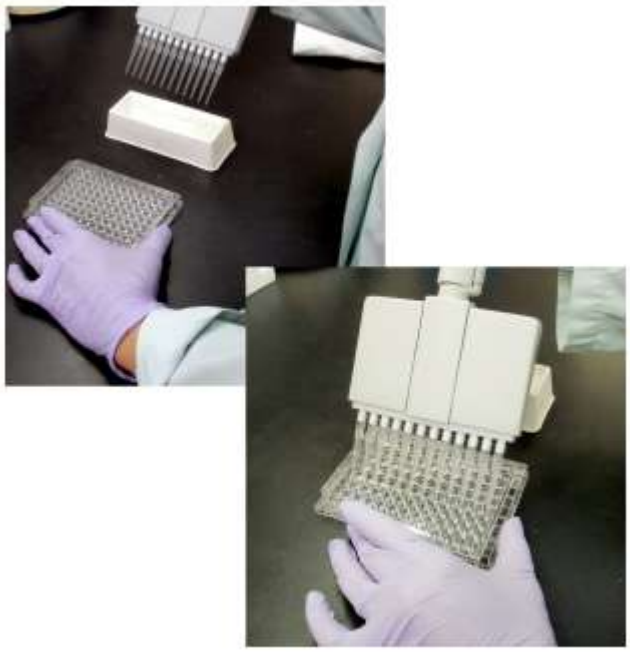


Blocking
Add blocking buffer to each well, and allow it to incubate at 37°C for 1 hour to reduce non-specific binding of the target protein to the well.

Washing
Remove the blocking buffer, and wash 3 times with washing solution.

Addition of samples
Dilute the samples with sample dilution buffer, and add 100 µL of each sample to each well. For the calibration curve, prepare a dilution series of the standard on the same plate. Allow it to incubate at 37°C for 1 hour.

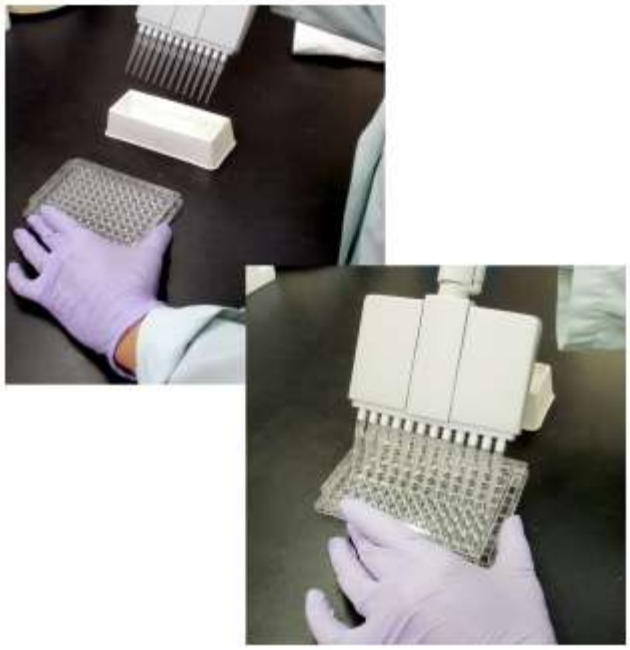
Washing
Remove the samples, and wash 5 times with washing solution.



Addition of the detection antibody
Dilute the detection antibody in sample dilution buffer, and add 100 µL to each well.
Allow it to incubate at 37°C for 1 hour.

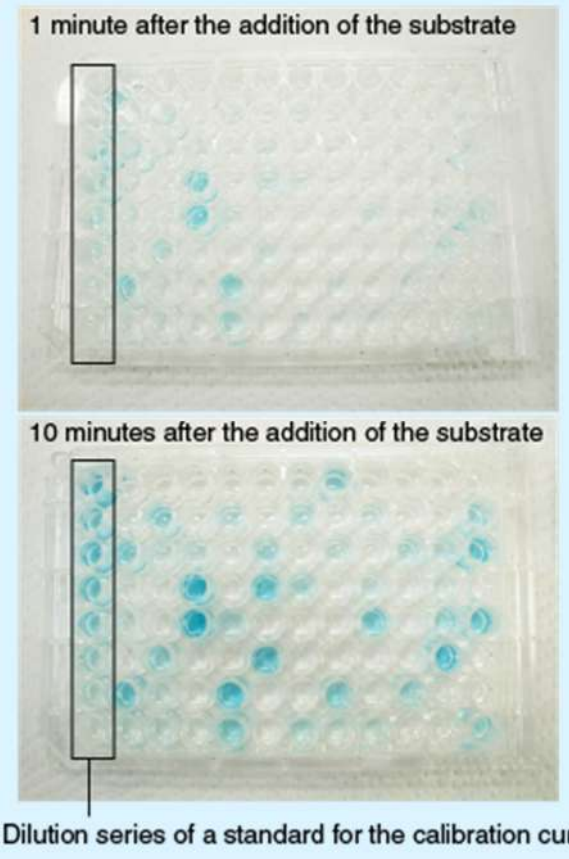
Washing

After reaction, remove the detection antibody, and wash 5 times with washing solution.



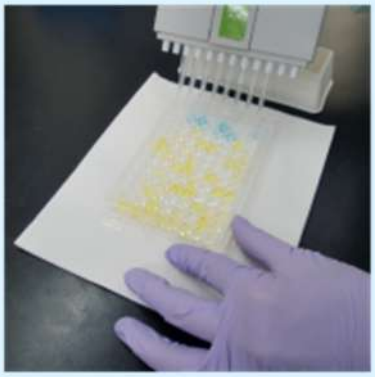
Addition of enzyme-labeled secondary antibody
Dilute an enzyme-labeled secondary antibody with sample dilution buffer, and add 100 µL to each well.
Allow it to incubate at 37°C for 1 hour.

Washing
After reaction, remove the secondary antibody, and wash 5 times with washing solution.



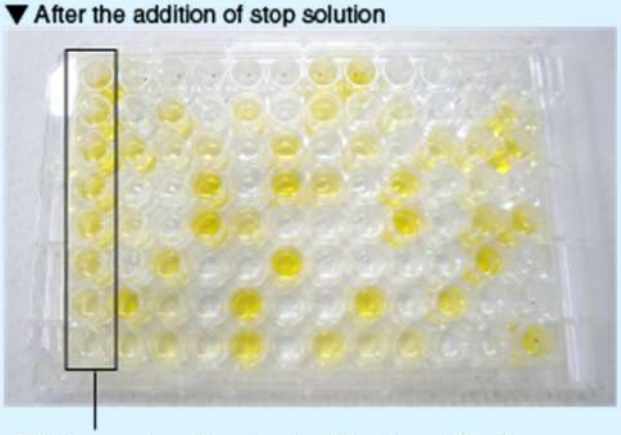
Add a substrate solution.
Allow it to incubate as the color develops.

Adding stop solution ▶



Add a stop solution to stop the reaction when the color is sufficiently developed.

▼ After the addition of stop solution



Dilution series of a standard for the calibration curve

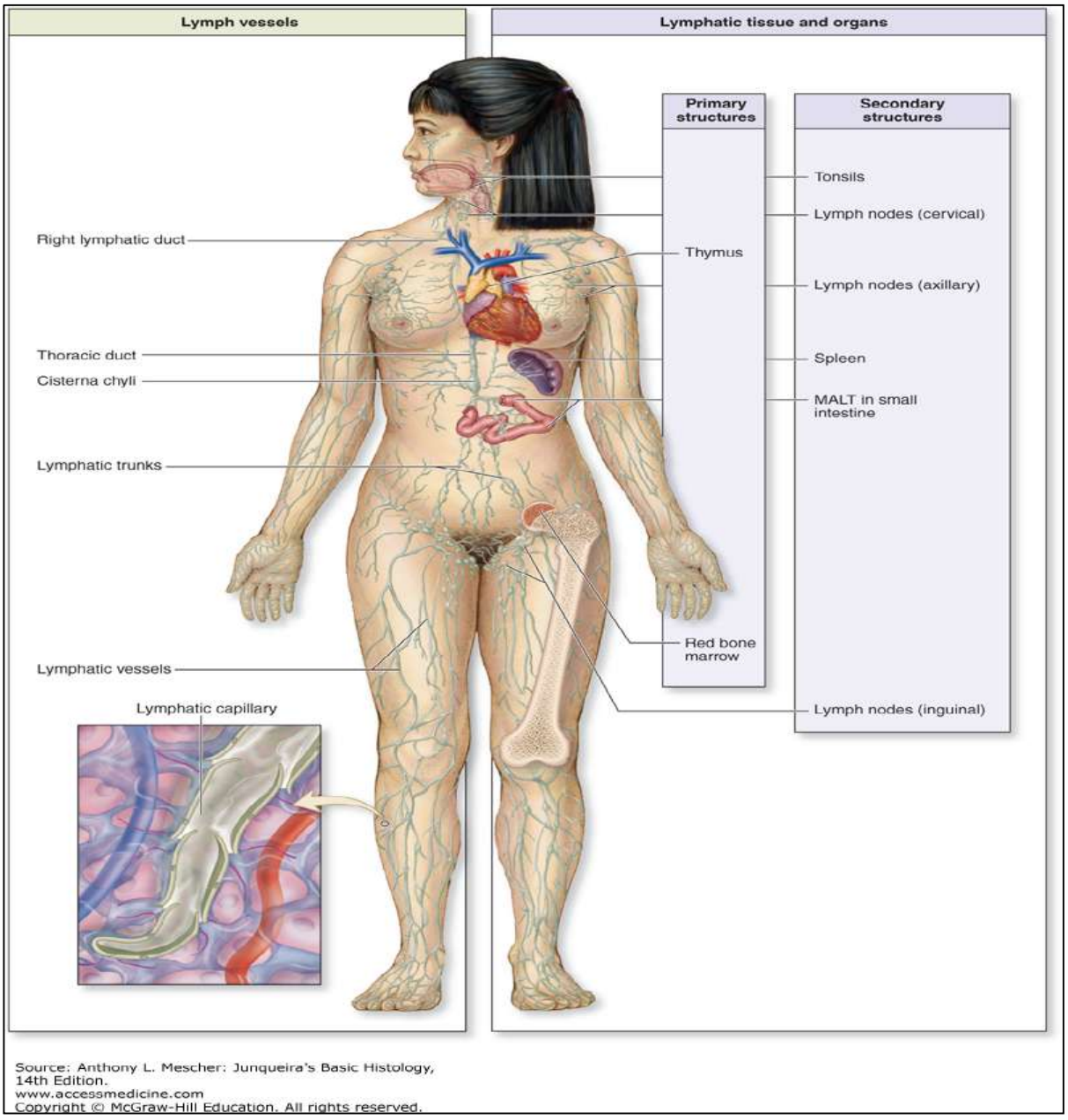
Plate reader ▼

Measure the absorption at 450 nm by a plate reader.

DEMONSTRATION OF LYMPHOID ORGAN

Introduction:

The lymphocytes and APCs for adaptive immunity are distributed throughout the body in the blood, lymph, and epithelial and connective tissues. Lymphocytes are formed initially in primary lymphoid organs (the thymus and bone marrow), but most lymphocyte activation and proliferation occur in secondary lymphoid organs (the lymph nodes, the spleen, and diffuse lymphoid tissue found in the mucosa of the digestive system, including the tonsils, Peyer patches, and appendix).

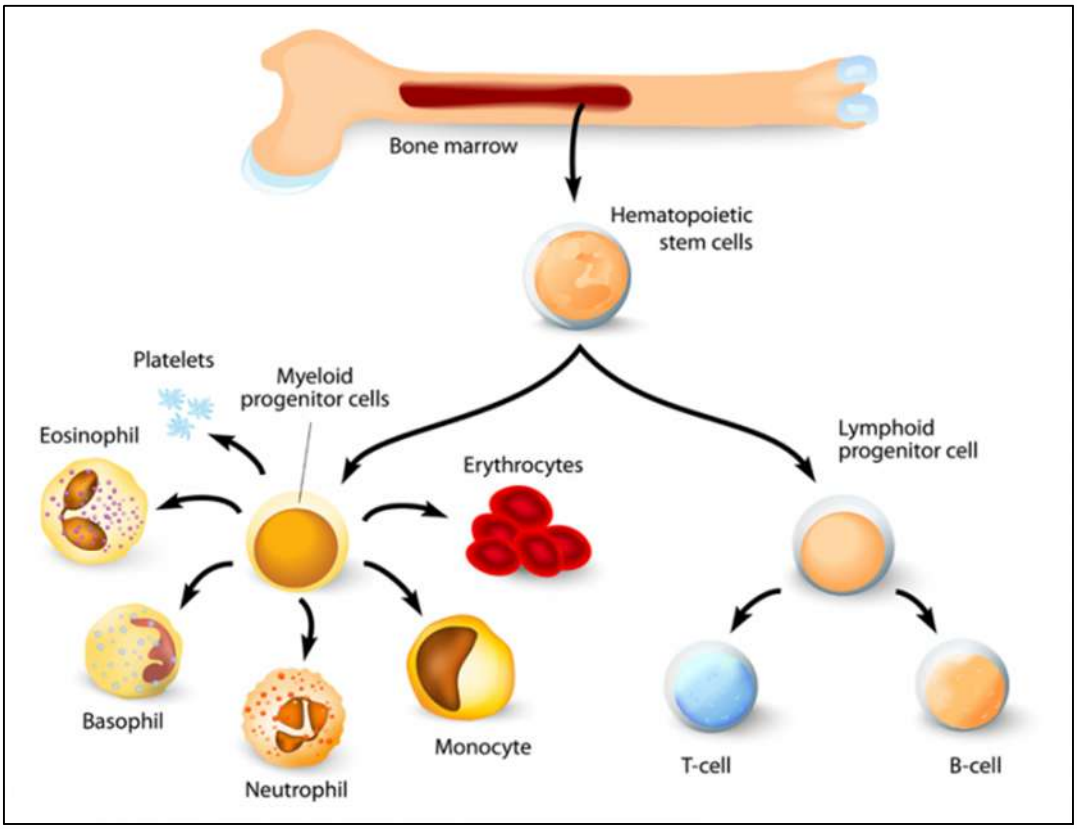


Primary lymphoid organs:

The primary (or central) lymphoid organs generate lymphocytes from immature progenitor cells. The thymus and the bone marrow constitute the primary lymphoid organs involved in the production and early clonal selection of lymphocyte tissues.

Bone marrow:

Bone marrow is responsible for both the creation of T cells and the production and maturation of B cells, which are important cell types of the immune system. From the bone marrow, B cells immediately join the circulatory system and travel to secondary lymphoid organs in search of pathogens. T cells, on the other hand, travel from the bone marrow to the thymus, where they develop further and mature. Mature T cells then join B cells in search of pathogens. The other 95% of T cells begin a process of apoptosis, a form of programmed cell death.

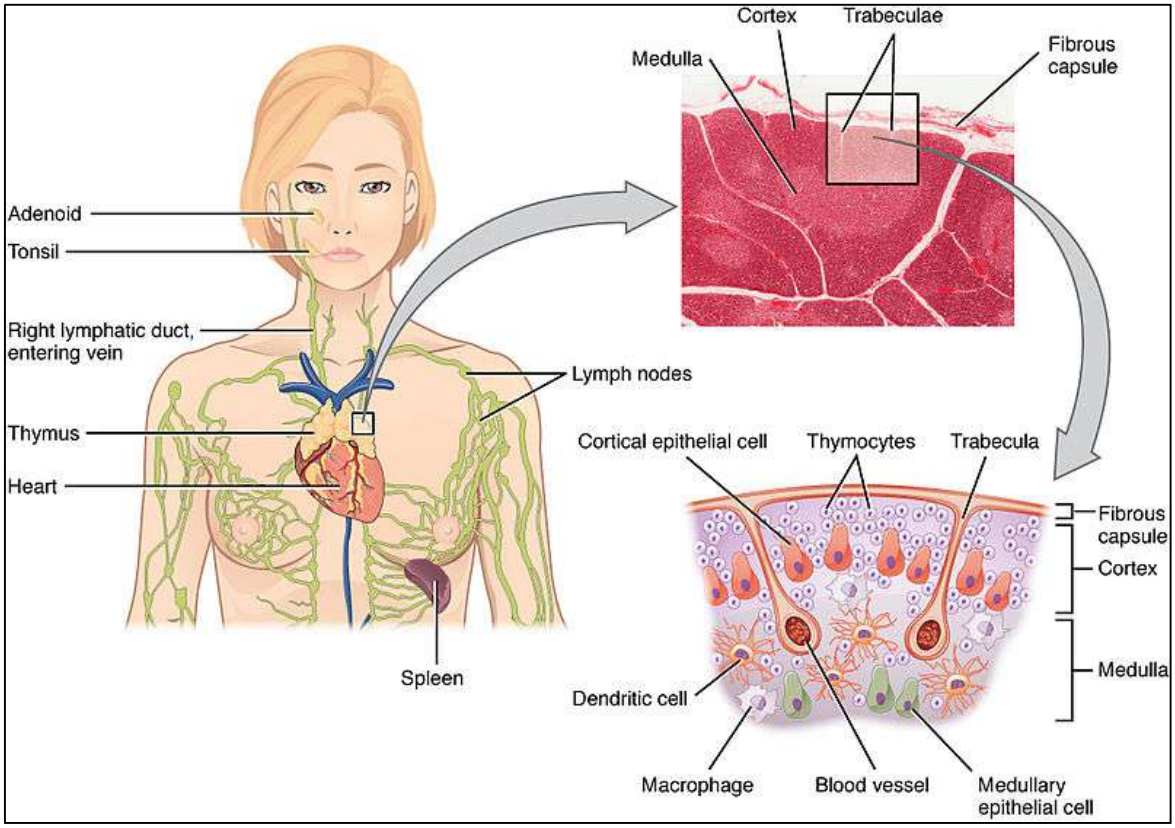


Thymus:

The thymus increases in size from birth in response to postnatal antigen stimulation. It is most active during the neonatal and pre-adolescent periods. At puberty, by the early teens, the thymus begins to atrophy and regress, with adipose tissue mostly replacing the thymicstroma. However, residual T lymphopoiesis continues throughout adult life. The loss or lack of the thymus results in severe immunodeficiency and subsequent high susceptibility to infection. In most species, the thymus consists of lobules divided by septa which are made

up of epithelium; it is therefore often considered an epithelial organ. T cells mature from thymocytes, proliferate, and undergo a selection process in the thymic cortex before entering the medulla to interact with epithelial cells.

The thymus provides an inductive environment for the development of T cells from hematopoietic progenitor cells. In addition, thymicstromal cells allow for the selection of a functional and self-tolerant T cell repertoire. Therefore, one of the most important roles of the thymus is the induction of central tolerance.



Secondary lymphoid organs:

The secondary (or peripheral) lymphoid organs (SLO), which include lymph nodes and the spleen, maintain mature naive lymphocytes and initiate an adaptive immune response. The peripheral lymphoid organs are the sites of lymphocyte activation by antigens. Activation leads to clonal expansion and affinity maturation. Mature lymphocytes recirculate between the blood and the peripheral lymphoid organs until they encounter their specific antigen.

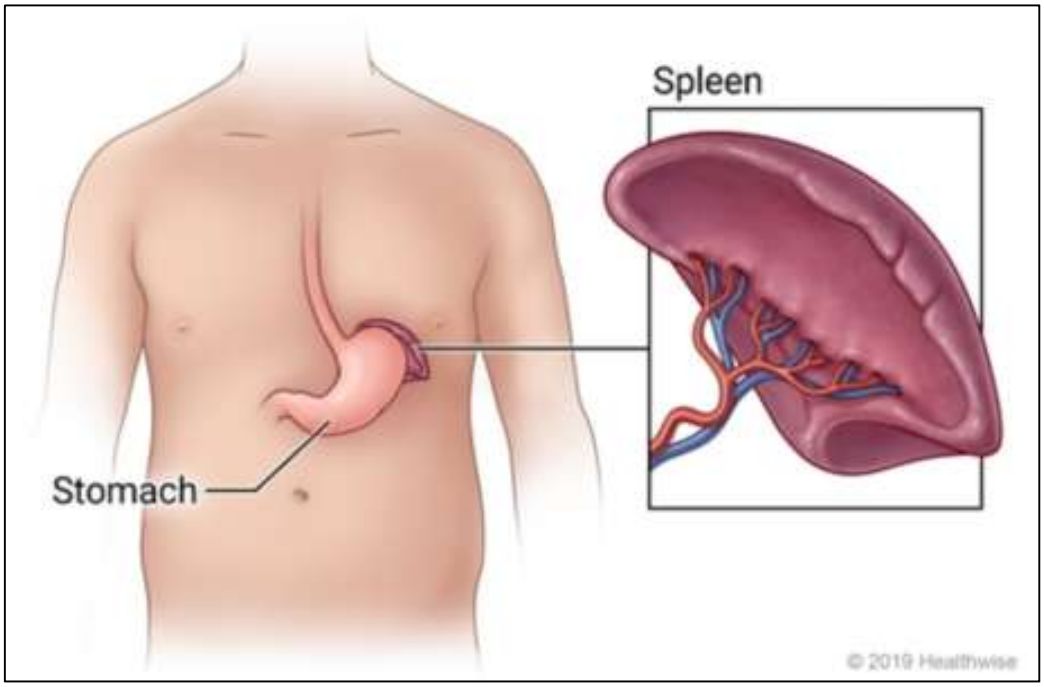
Spleen:

The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation. A study published in 2009 using mice found that the spleen contains, in its reserve, half of the body's monocytes within the red pulp. These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing. The

spleen is a center of activity of the mononuclear phagocyte system and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections.

Like the thymus, the spleen has only efferent lymphatic vessels. Both the short gastric arteries and the splenic artery supply it with blood. The germinal centers are supplied by arterioles called penicilliaryradicles.

Until the fifth month of prenatal development, the spleen creates red blood cells; after birth, the bone marrow is solely responsible for hematopoiesis. As a major lymphoid organ and a central player in the reticuloendothelial system, the spleen retains the ability to produce lymphocytes. The spleen stores red blood cells and lymphocytes. It can store enough blood cells to help in an emergency. Up to 25% of lymphocytes can be stored at any one time.



Lymph nodes:

A lymph node is an organized collection of lymphoid tissue, through which the lymph passes on its way back to the blood. Lymph nodes are located at intervals along the lymphatic system. Several afferent lymph vessels bring in lymph, which percolates through the substance of the lymph node, and is then drained out by an efferent lymph vessel. Of the nearly 800 lymph nodes in the human body, about 300 are located in the head and neck. Many are grouped in clusters in different regions, as in the underarm and abdominal areas. Lymph node clusters are commonly found at the proximal ends of limbs (groin, armpits) and in the neck, where lymph is collected from regions of the body likely to sustain pathogen contamination from injuries. Lymph nodes are particularly numerous in the mediastinum in the chest, neck, pelvis, axilla, inguinal region, and in association with the blood vessels of the intestines.

The substance of a lymph node consists of lymphoid follicles in an outer portion called the cortex. The inner portion of the node is called the medulla, which is surrounded by the cortex on all sides except for a portion known as the hilum. The hilum presents as a depression on the surface of the lymph node, causing the otherwise spherical lymph node to be bean-shaped or ovoid. The efferent lymph vessel directly emerges from the lymph node at

the hilum. The arteries and veins supplying the lymph node with blood enter and exit through the hilum. The region of the lymph node called the paracortex immediately surrounds the medulla. Unlike the cortex, which has mostly immature T cells, or thymocytes, the paracortex has a mixture of immature and mature T cells. Lymphocytes enter the lymph nodes through specialised high endothelial venules found in the paracortex.