T.Y. B.Sc ZOOLOGY

PRACTICAL V JOURNAL

GOGATE JOGALEKAR COLLEGE (AUTONOMOUS)
RATNAGIRI
DEPARTMENT OF ZOOLOGY

Corse code:- 25_USZOM 504
Topic:- Zoology Practical V
Credit :- 02

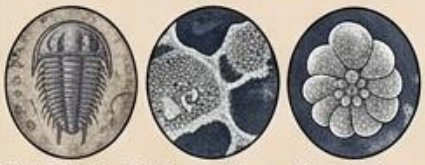
Invertebrate Phyla Diversity

Orders:
Cephalopoda, Anthozoa, Araneae, Polychaeta

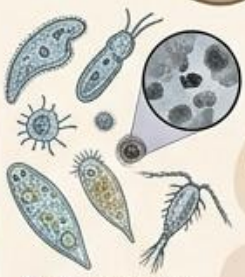
Palaeontology & Ancient Life

Trilobite Fossil Ammonite Shell Frachiopoda

Palaeontology & Ancient Life

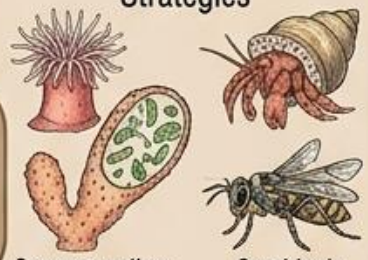


Trilobite Fossil Microontology Foraminifera



Protozoan and Plankton Studies

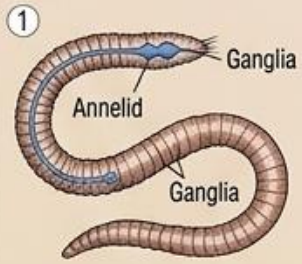
Coevolutionary Invertebrate Strategies



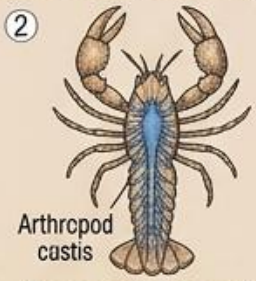
Commensalism Symbiosis

Invertebrate Body Plans & Germ Layers

Comparative Invertebrate Neurobiology

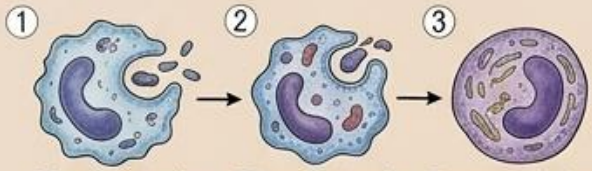


Ladder-like system

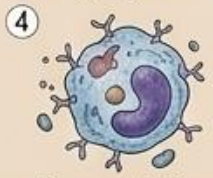


Segmental ganglia

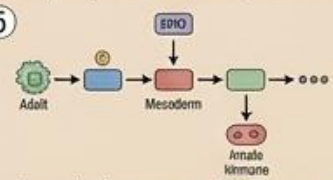
Invertebrate Immunology



Phagocytosis Phagocytosis Encapsulation



Encapsulation



Innate Immune Pathway

AUTHOR: DR. MADHURA MUKADAM

R. E. Society's

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AND

R. V. JOGALEKAR COLLEGE OF COMMERCE (AUTONOMOUS), RATNAGIRI

NAAC Accredited 'A' Grade (4th Cycle)

Ratnagiri – 415612, Maharashtra, India

CERTIFICATE

This is to certify that Mr./Ms. _____ of T. Y. B. Sc. (Bachelor of Science) has satisfactorily completed and submitted the Practical Journal of Zoology (Major I/II) prescribed under the autonomous syllabus for Semester V/VI during the academic year 20 – 20 . The journal contains a record of practical work performed by the student according to the syllabus of the Bachelor of Science (B.Sc.) Programme of this college.

Course Teacher

Head, Department of Zoology

Place: Ratnagiri

Date: / / 20

COURSE OUTCOMES

After successful completion of the **T.Y. B.Sc. Zoology – Major I Practical V Course**, students will be able to:

CO1: Apply knowledge of animal diversity, taxonomy and evolutionary concepts in the practical study of selected invertebrates.

CO2: Understand the relationship between structure and function through practical studies in animal physiology and biochemistry.

CO3: Analyse the effect of different physical and chemical factors on enzyme activity and interpret the resulting experimental data.

CO4: Understand fundamental concepts of haematology and immunology through laboratory-based observations and experiments.

CO5: Develop scientific skills for observation, data recording, analysis, interpretation and presentation of experimental results.

CO6: Demonstrate scientific attitude, laboratory ethics, safety awareness and responsible practical conduct.

PRACTICAL SKILLS DEVELOPED

After completing the practical course, students will be able to:

1. Identify selected invertebrate specimens based on diagnostic morphological characters.
2. Determine symmetry and coelomic conditions in representative invertebrates.
3. Apply the principles of binomial nomenclature and use basic taxonomic keys for identification.
4. Classify representative molluscan shells and recognise characteristic features of echinoderms.
5. Measure and record selected physiological parameters such as blood pressure and heart rate.
6. Perform experiments to study the effects of substrate concentration, temperature, pH and enzyme concentration on acid phosphatase activity.
7. Record experimental data in appropriate tables and perform relevant calculations.
8. Plot graphs and interpret experimental results.
9. Prepare and examine a blood smear and identify different types of white blood cells.
10. Identify major lymphoid organs and demonstrate selected immunological reactions.
11. Prepare neat, labelled scientific diagrams and maintain a systematic practical record.
12. Follow standard laboratory procedures, safety precautions and responsible scientific practices.

GENERAL LABORATORY RULES

1. Students must enter the laboratory only with the permission of the teacher concerned.
2. Students must wear a clean laboratory coat/apron during practical sessions wherever required.
3. The practical journal, record book and necessary stationery should be brought to every practical session.
4. Students should listen carefully to the instructions given by the teacher before starting an experiment.
5. The laboratory bench should be kept clean throughout the practical session.
6. All apparatus, specimens and materials should be handled carefully.
7. Reagents and chemicals should be used only in the quantities instructed by the teacher.
8. Labels on reagent bottles and chemical containers must be read carefully before use.
9. Reagents should not be returned to their original containers after use.
10. Mouth pipetting is strictly prohibited.
11. Students should not eat or drink inside the laboratory.
12. Mobile phones should be kept away unless their use is specifically permitted for academic purposes.
13. Microscope lenses should be cleaned only with appropriate lens-cleaning material.
14. Glassware and instruments should be cleaned after use and returned to their designated places.
15. Preserved specimens should be handled carefully and returned properly after observation.
16. Any breakage, spill, injury or malfunction of equipment should be reported immediately to the teacher or laboratory assistant.
17. Students should not perform any experiment that has not been authorised or explained by the teacher.
18. Unused chemicals and biological materials should be disposed of only as instructed.
19. Students should wash their hands thoroughly after completing practical work.
20. Discipline, cleanliness and responsible behaviour must be maintained throughout the practical session.

SAFETY PRECAUTIONS

1. Always follow the instructions provided by the teacher or laboratory staff.
2. Wear appropriate personal protective equipment, such as a laboratory coat, gloves or protective eyewear, whenever required.
3. Avoid direct contact with chemicals, stains, biological materials and preserved specimens.
4. Do not taste or intentionally smell any chemical or biological material.
5. Never pipette liquids by mouth; use appropriate pipetting devices.
6. Handle glassware carefully to prevent breakage and injury.
7. Use electrical instruments and equipment only after receiving proper instructions.
8. Keep water away from electrical connections and instruments.
9. Use heating equipment and water baths carefully to avoid burns.
10. Allow hot apparatus to cool before handling.
11. Chemicals should be used in the prescribed concentration and quantity only.
12. Any chemical spill should be reported immediately and cleaned according to laboratory instructions.

13. Broken glass should not be picked up with bare hands.
14. Biological materials should be handled hygienically and disposed of according to institutional laboratory procedures.
15. Human physiological observations, where included in the practical curriculum, should be conducted only under the supervision of the teacher and with appropriate consent and institutional guidelines.
16. Students should not interpret practical observations as a medical diagnosis.
17. In case of an accident or injury, inform the teacher immediately.
18. Know the location of the first-aid box, fire extinguisher and emergency exits.
19. Wash hands thoroughly before leaving the laboratory.
20. Follow all institutional and departmental safety guidelines applicable to the laboratory.

INSTRUCTIONS FOR MAINTAINING THE PRACTICAL JOURNAL

1. The practical journal should be maintained neatly and regularly throughout the academic year.
2. The student's name, class, division, roll number and academic year should be clearly written on the cover page.
3. An index of experiments should be maintained at the beginning of the journal.
4. Each experiment should be written according to the prescribed sequence.
5. Every experiment should include, wherever applicable:
    - Experiment Number and Title
    - Date
    - Aim
    - Principle/Theory
    - Requirements
    - Procedure
    - Observations
    - Calculations
    - Graphs
    - Diagrams
    - Result
    - Precautions
6. Observations should be recorded clearly and accurately during or immediately after the experiment.
7. Tables should be properly ruled, titled and labelled.
8. All calculations should show the relevant formula and appropriate units.
9. Graphs should be plotted neatly using appropriate scales, with clearly labelled X-axis and Y-axis.
10. Diagrams should be neat, properly labelled and drawn with a sharp pencil.
11. Scientific names should be written according to accepted nomenclatural conventions.
12. Corrections should be made neatly. Excessive overwriting and cutting should be avoided.
13. Blank pages should not be unnecessarily left between experiments.
14. Each completed practical should be submitted for checking and signature within the prescribed time.
15. The journal should be updated regularly and should not be completed hurriedly at the end of the term.
16. Students should maintain the journal as an authentic record of their practical work.

17. The completed practical journal should be submitted for examination as per the instructions of the Department and University.

EVALUATION SCHEME

The practical performance of students may be assessed through **continuous internal assessment and end-semester practical examination**, as per the applicable University regulations.

The evaluation may include the following components:

Component	Parameters for Assessment
Practical Performance	Understanding of the experiment, correct procedure, handling of apparatus and materials
Observation and Recording	Accuracy, completeness and systematic presentation of observations
Calculations and Graphs	Correct formulae, calculations, units, graphical representation and interpretation
Identification/Spotting	Identification of specimens, slides, models, instruments or other practical materials using diagnostic characters
Practical Journal	Completeness, neatness, regularity, labelled diagrams and authenticated record
Viva-Voce	Understanding of principles, procedures, observations and applications
Laboratory Discipline and Safety	Regularity, responsible conduct, cleanliness and adherence to laboratory safety rules

General Guidelines for Assessment

1. Students should be assessed on both **practical skills and conceptual understanding**.
2. Greater importance should be given to the student's ability to perform and interpret an experiment rather than merely reproducing theoretical information.
3. The practical journal should be checked periodically during the academic term.
4. Students should demonstrate proper handling of specimens, instruments and laboratory materials.
5. Viva-voce questions should assess understanding of the principle, procedure, observations and applications of the experiment.
6. Diagrams, observations, calculations and graphs should be assessed for accuracy and scientific presentation.
7. The final distribution of marks should strictly follow the **applicable syllabus, examination pattern and regulations**.

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Experiment No. 1

STUDY OF SYMMETRY IN INVERTEBRATES

Aim

To study and identify different types of symmetry in selected invertebrate specimens and understand their taxonomic significance.

Principle

Symmetry refers to the balanced arrangement of body parts around a central axis or plane. It is one of the fundamental characteristics used in animal classification and reflects the level of organisation and evolutionary advancement of an organism.
Based on body organisation, invertebrates exhibit three major types of symmetry:

1. **Asymmetry** – Body cannot be divided into equal halves by any plane.
2. **Radial Symmetry** – Body parts are arranged around a central axis, and multiple planes passing through the central axis divide the body into similar halves.
3. **Bilateral Symmetry** – Only one median sagittal plane divides the body into two equal mirror-image halves.

The evolution of symmetry is associated with increasing complexity of body organisation and adaptation to different modes of life.

Requirements

Specimens/Models

- Sponge (Sycon or Spongilla)
- Hydra
- Sea Anemone (Metridium)
- Adult Starfish (Asterias)
- Sea Urchin (Echinus)
- Liver Fluke (Fasciola)
- Earthworm
- Crab
- Pila
- Sepia

Apparatus

- Dissecting tray
- Hand lens
- Zoological charts/models

Procedure

1. Arrange all preserved specimens on a dissecting tray.
2. Observe the external morphology of each specimen.
3. Imagine passing different planes through the body.
4. Determine whether the body can be divided into equal halves.
5. Record the number of planes that produce identical halves.
6. Identify the type of symmetry exhibited by each specimen.

7. Compare symmetry patterns among different phyla.
8. Record observations in tabular form.

Special Note

Larval Echinoderms exhibit bilateral symmetry, whereas **adult echinoderms** exhibit pentaradial symmetry. This is considered important evidence of their evolutionary relationship with bilaterally symmetrical animals.

Result

The studied invertebrates exhibited three major types of symmetry:

- **Asymmetry** in Porifera (Spongilla, Sycon)
- **Radial Symmetry** in Cnidaria (Hydra, Aurelia, Sea Anemone) and adult Echinodermata (Asterias, Echinus)
- **Bilateral Symmetry** in Platyhelminthes, Nematoda, Annelida, Arthropoda, and Mollusca

Thus, symmetry serves as an important criterion in the classification and evolution of invertebrates.

Precautions

1. Handle preserved specimens carefully.
2. Observe specimens from different orientations before concluding symmetry.
3. Use a hand lens for observing small specimens.
4. Compare specimens with standard zoological charts.
5. Do not confuse the pentaradial symmetry of echinoderms with the true radial symmetry of cnidarians.

Observations

Specimen	Phylum	Type of Symmetry
Spongilla / Sycon	Porifera	Asymmetrical
Hydra	Cnidaria	Radial
Sea Anemone	Cnidaria	Radial
Liver Fluke (Fasciola)	Platyhelminthes	Bilateral
Ascaris	Nematoda	Bilateral
Earthworm	Annelida	Bilateral
Crab	Arthropoda	Bilateral
Pila	Mollusca	Bilateral
Sepia	Mollusca	Bilateral
Adult Starfish (Asterias)	Echinodermata	Radial (Pentaradial)
Sea Urchin (Echinus)	Echinodermata	Radial (Pentaradial)

Experiment No. 2

STUDY OF DIFFERENT TYPES OF COELOMS IN INVERTEBRATES

Aim
To study and identify different types of body cavities (coelom) in selected invertebrate specimens and understand their evolutionary significance.

Principle
The coelom is a fluid-filled body cavity located between the body wall and the alimentary canal. It is lined by mesodermal epithelium called the peritoneum and serves various functions such as providing space for internal organs, facilitating the circulation of nutrients, acting as a hydrostatic skeleton, and allowing independent movement of body organs.

Based on the presence and development of the body cavity, animals are classified into three major groups:

1. Acoelomates
Animals in which the space between the body wall and digestive tract is filled with mesodermal tissue, and no body cavity is present.
Examples: Planaria, Fasciola, Taenia

2. Pseudocoelomates
Animals possessing a body cavity that is not completely lined by mesoderm. The cavity persists from the embryonic blastocoel and is therefore called a pseudocoelom.
Examples: Ascaris, Wuchereria

3. Eucoelomates (True Coelomates)
Animals possessing a true coelom are completely lined by mesodermal peritoneum.
Examples: Earthworm, Leech, Prawn, Cockroach, Pila, Starfish
The development of a true coelom represents an important evolutionary advancement in animal organisation.

Requirements
Specimens/Models

- Planaria
- Ascaris
- Leech
- Prawn
- Cockroach
- Pila
- Starfish

Teaching Aids

- Charts showing transverse sections of body cavities
- Models illustrating coelom development
- Zoological atlas

Apparatus

- Hand lens
- Dissecting tray

Procedure

1. Arrange the preserved specimens on a dissecting tray.
2. Observe the external morphology of each specimen.
3. Examine charts and diagrams showing transverse sections.
4. Identify the region between the body wall and digestive tract.
5. Determine whether:
    - The cavity is absent.
    - The cavity is partially lined by mesoderm.
    - The cavity is completely lined by mesoderm.
6. Classify the specimen as acoelomate, pseudocoelomate, or eucoelomate.
7. Compare the organisation of body cavities among different phyla.
8. Record observations in tabular form.

Evolutionary Significance of Coelom

1. Provides space for the development of complex organs.
2. Facilitates the independent movement of internal organs.
3. Acts as a hydrostatic skeleton.
4. Enhances circulation of nutrients and wastes.
5. Represents a major evolutionary advancement in higher animals.

Result

The studied invertebrates exhibited different types of body cavities:

- **Planaria and Liver Fluke** were identified as **acoelomates** due to the absence of a body cavity.
- **Ascaris and Wuchereria** were identified as **pseudocoelomates** because the body cavity was not completely lined by mesoderm.
- **Earthworm, Leech, Prawn, Cockroach, Pila, and Starfish** were identified as **eucoelomates**, possessing a true coelom completely lined by mesoderm.

Thus, the coelom serves as an important taxonomic and evolutionary character in animal classification.

Precautions

1. Refer to standard transverse section diagrams during identification.
2. Observe the mesodermal lining before classifying specimens.
3. Distinguish between pseudocoelom and true coelom accurately.
4. Use well-preserved specimens and models.
5. Compare specimens belonging to different phyla for a better understanding.

Observations

Specimen	Phylum	Type of Coelom
Planaria	Platyhelminthes	Acoelomate
Ascaris	Nematoda	Pseudocoelomate
Leech	Annelida	Eucoelomate (Reduced Coelom)
Prawn	Arthropoda	Eucoelomate
Cockroach	Arthropoda	Eucoelomate
Pila	Mollusca	Eucoelomate
Starfish	Echinodermata	Eucoelomate

Diagrammatic Interpretation

Acoelomate Condition
Body Wall → Mesoderm → Gut
(No body cavity present)

Pseudocoelomate Condition
Body Wall → Mesoderm → Pseudocoel → Gut
(Cavity not completely lined by mesoderm)

Eucoelomate Condition
Body Wall → Mesoderm → True Coelom → Mesoderm → Gut
(Cavity completely lined by mesoderm)

Comparative Table

Character	Acoelomate	Pseudocoelomate	Eucoelomate
Body Cavity	Absent	Present	Present
Mesoderm Lining	Absent	Partial	Complete
Organ Development	Simple	Intermediate	Advanced
Examples	Planaria, Fasciola	Ascaris	Earthworm, Prawn

Experiment No. 3

BINOMIAL NOMENCLATURE AND IDENTIFICATION OF INVERTEBRATES USING TAXONOMIC KEYS

Aim

To identify selected invertebrate specimens using taxonomic keys and to understand the principles and rules of binomial nomenclature.

Principle

The immense diversity of living organisms necessitates a universal system of naming and classification. Binomial nomenclature is the scientific system of naming organisms developed by Carl Linnaeus and is accepted internationally.

According to this system, each organism is assigned a two-word scientific name:

1. **Genus** – The first word, written with an initial capital letter.
2. **Species** – The second word, written in lowercase letters.

For example:

- *Hydra vulgaris*
- *Ascaris lumbricoides*
- *Pila globosa*

Scientific names are written in italics when printed and underlined separately when handwritten.

Taxonomic identification is carried out using identification keys based on observable morphological characters. These keys help in placing organisms into appropriate taxonomic categories such as phylum, class, order, family, genus, and species.

Objectives

1. To understand the concept of binomial nomenclature.
2. To learn the rules governing scientific naming.
3. To identify invertebrate specimens using taxonomic keys.
4. To classify specimens into their respective phyla.

Requirements

Specimens / Models

- Sponge (*Spongilla*)
- Hydra
- Taenia
- Ascaris
- Leech
- Prawn
- Unio
- Starfish

Apparatus and Materials

- Taxonomic identification keys
- Classification charts
- Hand lens
- Preserved specimens/models
- Practical record book

Procedure

1. Arrange the preserved specimens on a laboratory table.
2. Observe the external morphology of each specimen.
3. Note important diagnostic characters such as:
    - Body symmetry
    - Level of organisation
    - Presence or absence of segmentation
    - Body cavity
    - Appendages
    - Shell or exoskeleton
    - Special organs
4. Refer to the dichotomous taxonomic key.
5. Follow the key steps until the specimen is identified.
6. Determine the phylum and scientific name of the organism.
7. Record the observations in tabular form.
8. Verify the scientific name according to binomial nomenclature rules.

Rules of Binomial Nomenclature

1. Scientific names consist of two words.
2. The genus name begins with a capital letter.
3. The species name begins with a small letter.
4. Names are written in italics when printed.
5. When handwritten, both words are underlined separately.
6. Scientific names are universally accepted and avoid confusion caused by local names.

Significance of Binomial Nomenclature

1. Provides a universal naming system.
2. Eliminates confusion caused by common names.
3. Indicates relationships among organisms.
4. Facilitates scientific communication worldwide.
5. Helps in accurate identification and classification.

Result

The given invertebrate specimens were successfully identified using taxonomic keys and classified into their respective phyla. The scientific names were recorded according to the rules of binomial nomenclature.

Precautions

1. Observe all morphological characters before using the taxonomic key.
2. Follow the identification key sequentially without skipping steps.
3. Use accepted scientific names only.
4. Ensure correct spelling of genus and species names.
5. Write scientific names according to international nomenclature rules.
6. Compare specimens with standard classification charts whenever necessary.

Simplified Identification Key

Step 1
Body with pores and a canal system → **Porifera**
Body without pores → **Go to Step 2**

Step 2
Tentacles surrounding the mouth present → **Cnidaria**
Tentacles absent → **Go to Step 3**

Step 3
Body dorsoventrally flattened → **Platyhelminthes**
Body cylindrical → **Go to Step 4**

Step 4
Body unsegmented and cylindrical → **Nematoda**
Body segmented → **Go to Step 5**

Step 5
Jointed appendages present → **Arthropoda**
Jointed appendages absent → **Go to Step 6**

Step 6
Soft body with shell → Mollusca
Pentaradial symmetry present → **Echinodermata**

Observations

Specimen	**Phylum**	**Scientific Name**
Sponge	Porifera	*Spongilla* sp.
Hydra	Cnidaria	*Hydra vulgaris*
Tapeworm	Platyhelminthes	*Taenia solium*
Roundworm	Nematoda	*Ascaris lumbricoides*
Leech	Annelida	*Hirudinaria granulosa*
Prawn	Arthropoda	*Palaemon* sp.
Freshwater Mussel	Mollusca	*Unio* sp.
Starfish	Echinodermata	*Asterias rubens*

Experiment No. 4

CLASSIFICATION OF MOLLUSCAN SHELLS

Aim

To study and classify different molluscan shells based on their external and internal morphological characteristics and to understand their taxonomic significance.

Principle

Molluscs possess a characteristic calcareous shell secreted by the mantle. The shell serves as a protective exoskeleton and exhibits considerable variation in shape, size, ornamentation, and structure among different classes of Mollusca.

Shell morphology is an important taxonomic character used in the identification and classification of molluscs. Features such as shell coiling, number of valves, aperture, hinge, growth lines, operculum, and internal structure help distinguish various molluscan groups.
Based on shell characteristics, molluscs are classified into different classes such as:

- Gastropoda
- Bivalvia
- Cephalopoda
- Polyplacophora
- Scaphopoda

Objectives

1. To study the morphology of molluscan shells.
2. To identify important shell characters used in classification.
3. To classify representative molluscan shells into their respective classes.
4. To understand adaptive modifications of shells in different molluscan groups.

Requirements

Specimens

- *Pila globosa* (Apple snail)
- *Unio* sp. (Freshwater mussel)
- *Sepia officinalis* (Cuttlefish)

Additional specimens (if available)

- *Turbo* sp.
- *Murex* sp.
- *Pinctada* sp.
- *Dentalium* sp.
- *Chiton* sp.
- *Nautilus* shell

Apparatus

- Hand lens
- Measuring scale
- Identification charts
- Dissecting tray

Theory

The molluscan shell is composed mainly of calcium carbonate and consists of three layers:

1. Periostracum

- Outermost organic layer.
- Composed of conchiolin.
- Protects the shell from dissolution.

2. Prismatic Layer

- Middle layer.
- Composed of calcium carbonate crystals.

3. Nacreous Layer

- Inner layer.
- Forms mother-of-pearl.
- Smooth and lustrous.

Different molluscan classes exhibit characteristic shell modifications:

Class	**Shell Type**
Gastropoda	Single spirally coiled shell
Bivalvia	Two lateral valves
Cephalopoda	Internal, external, or absent shell
Scaphopoda	Tusk-shaped shell
Polyplacophora	Eight dorsal shell plates

Procedure

1. Arrange the molluscan shells on a dissecting tray.
2. Observe each shell with the naked eye and hand lens.
3. Record the following features:
    - Shape
    - Size
    - Colour
    - Surface markings
    - Aperture
    - Number of valves
    - Hinge structure
    - Presence of operculum
    - Growth lines
4. Compare the observed features with standard identification charts.
5. Identify the specimen.
6. Classify the shell into its appropriate molluscan class.
7. Record observations in tabular form.

Identification Key

Step 1
Shell consisting of two valves → **Bivalvia**
Shell consisting of one valve → **Go to Step 2**

Step 2
Shell spirally coiled → **Gastropoda**
Shell not spirally coiled → **Go to Step 3**

Step 3
Shell internal and reduced → **Cephalopoda**
Shell tubular → Scaphopoda
Shell composed of eight plates → **Polyplacophora**

Adaptive Significance of Shells

1. Protection against predators.
2. Prevention of desiccation.
3. Mechanical support.
4. Maintenance of body shape.
5. Adaptation to aquatic and terrestrial habitats.

Result

The given molluscan shells were successfully identified and classified based on their morphological characteristics.

- *Pila* was classified under **Class Gastropoda**.
- *Unio* was classified under **Class Bivalvia**.
- *Sepia* was classified under **Class Cephalopoda**.

The shell characters proved useful for the taxonomic classification of molluscs.

Precautions

1. Handle shells carefully to avoid breakage.
2. Observe shell characters with a hand lens whenever necessary.
3. Examine hinge structures carefully in bivalves.
4. Distinguish external shells from internal shells.
5. Use standard identification charts for accurate classification.
6. Record observations systematically.

Observations

Specimen	Class	Diagnostic Features
Pila globosa	Gastropoda	Single spirally coiled shell with large aperture and operculum
Unio sp.	Bivalvia	Two equal valves joined by hinge ligament
Sepia officinalis	Cephalopoda	Internal calcareous shell (cuttlebone)
Nautilus sp.	Cephalopoda	External chambered shell
Dentalium sp.	Scaphopoda	Tusk-shaped tubular shell
Chiton sp.	Polyplacophora	Eight overlapping dorsal plates

Comparative Study of Representative Shells

Character	Pila	Unio	Sepia
Class	Gastropoda	Bivalvia	Cephalopoda
Shell Type	External	External	Internal
Number of Valves	One	Two	Absent externally
Coiling	Present	Absent	Absent
Hinge	Absent	Present	Absent
Operculum	Present	Absent	Absent

Experiment No. 5

STUDY OF CHARACTERISTIC FEATURES OF ECHINODERMS

Aim

To study the characteristic features of Echinoderms using preserved specimens and to identify diagnostic characters of the Phylum Echinodermata.

Principle

Echinoderms are exclusively marine, triploblastic, coelomate animals characterised by a unique **water vascular system**, **tube feet**, and **pentaradial symmetry** in adults. They occupy an important position among invertebrates because of their evolutionary relationship with chordates.

The phylum derives its name from the Greek words:

- **Echinos** = Spiny
- **Derma** = Skin

Echinoderms possess a calcareous endoskeleton composed of ossicles and spines. Their most distinctive feature is the **water vascular system**, which functions in locomotion, feeding, respiration, excretion, and sensory perception.

Adult echinoderms exhibit **pentaradial symmetry**, whereas larvae show **bilateral symmetry**, indicating their evolutionary affinity with higher animals.

Objectives

1. To study the external morphology of echinoderms.
2. To identify the components of the water vascular system.
3. To observe pentaradial symmetry.
4. To understand the adaptive significance of echinoderm characters.
5. To classify common echinoderms based on diagnostic features.

Requirements

Specimens

Primary Specimen:

- *Asterias rubens* (Starfish)

Additional Specimens (if available):

- *Echinus* (Sea Urchin)
- *Ophiura* (Brittle Star)
- *Holothuria* (Sea Cucumber)
- *Antedon* (Feather Star)

Apparatus

- Dissecting tray
- Hand lens
- Identification charts
- Zoological atlas

General Characteristics of Echinoderms

1. Exclusively marine animals.
2. Triploblastic and coelomate.
3. Organ-system level of organisation.

4. Adults exhibit pentaradial symmetry.
5. Larvae exhibit bilateral symmetry.
6. Endoskeleton composed of calcareous ossicles.
7. Presence of spines on the body surface.
8. Water vascular system present.
9. Tube feet are used for locomotion and feeding.
10. Regeneration capacity is well developed.

Classification of Common Echinoderms

Class	Example	Common Name
Asteroidea	*Asterias*	Starfish
Ophiuroidea	*Ophiura*	Brittle Star
Echinoidea	*Echinus*	Sea Urchin
Holothuroidea	*Holothuria*	Sea Cucumber
Crinoidea	*Antedon*	Feather Star

Procedure

1. Place the preserved specimen of *Asterias* in a dissecting tray.
2. Observe the general body shape and arrangement of arms.
3. Count the number of arms radiating from the central disc.
4. Observe the oral surface and identify the mouth.
5. Examine the ambulacral grooves extending along each arm.
6. Observe the tube feet emerging through ambulacral grooves.
7. Turn the specimen and observe the aboral surface.
8. Locate the madreporite near the central disc.
9. Identify spines and dermal ossicles.
10. Observe the arrangement of body parts to confirm pentaradial symmetry.
11. Record all observations.

Evolutionary Significance

1. Presence of true coelom.
2. Endoskeleton of mesodermal origin.
3. Bilateral larval stage suggests affinity with chordates.
4. Advanced organ systems.
5. Efficient locomotion through hydraulic mechanisms.

Result

The preserved specimen of *Asterias* exhibited the characteristic features of Phylum Echinodermata including:

- Pentaradial symmetry
- Calcareous endoskeleton
- Water vascular system
- Tube feet
- Madreporite
- Marine mode of life

Hence, the specimen was identified as an echinoderm belonging to Class Asteroidea.

Observations

External Features of Asterias

Character	Observation
Habitat	Marine
Body Shape	Star-shaped
Symmetry	Pentaradial
Number of Arms	Five
Central Disc	Present
Mouth	Present on oral surface
Tube Feet	Present
Ambulacral Grooves	Present
Madreporite	Present on aboral surface
Spines	Present
Endoskeleton	Calcareous ossicles present
Water Vascular System	Present

Identification of Important Structures

Oral Surface

- Mouth
- Ambulacral grooves
- Tube feet

Aboral Surface

- Madreporite
- Spines
- Anus
- Dermal branchiae (papulae)

Water Vascular System

The water vascular system is a hydraulic system unique to echinoderms.

Components

1. Madreporite
2. Stone canal
3. Ring canal
4. Radial canals
5. Tube feet

Functions

- Locomotion
- Feeding
- Respiration
- Excretion
- Sensory perception
-

Diagrammatic Features to Observe

1. Central disc
2. Madreporite
3. Ambulacral groove
4. Tube feet
5. Mouth
6. Anus
7. Spines

Comparative Study of Common Echinoderms

Character	Asterias	Ophiura	Echinus	Holothuria
Body Shape	Star-shaped	Disc with slender arms	Globular	Elongated
Arms	Present	Present	Absent	Absent
Tube Feet	With suckers	Without suckers	Present	Present
Spines	Moderate	Reduced	Long spines	Absent
Locomotion	Tube feet	Arm movement	Tube feet	Muscular body wall

Experiment No. 6

ESTIMATION OF BLOOD PRESSURE

Aim

To measure the systolic and diastolic blood pressure of an individual using a sphygmomanometer and to compare blood pressure values at rest and after exercise.

Background Information

Blood pressure (BP) is the lateral force exerted by circulating blood on the walls of the arteries. It is one of the vital physiological parameters used to assess the functional status of the cardiovascular system.

Blood pressure depends on several factors, including:

- Cardiac output
- Peripheral resistance
- Blood volume
- Elasticity of blood vessels
- Age, physical activity, and emotional state
-

Blood pressure is expressed as two values:

1. **Systolic Blood Pressure (SBP):** The maximum pressure exerted during ventricular contraction (systole).
2. **Diastolic Blood Pressure (DBP):** The minimum pressure exerted during ventricular relaxation (diastole).

Blood pressure is expressed in millimetres of mercury (mmHg) and is generally written as:
Systolic Pressure / Diastolic Pressure
For example: **120/80 mmHg**

Principle

Blood pressure is measured indirectly by the auscultatory method using a sphygmomanometer and a stethoscope.

When the pressure in the cuff exceeds the arterial pressure, the brachial artery is completely compressed and blood flow stops. As the cuff pressure is slowly released, blood begins to flow through the partially compressed artery, producing characteristic sounds known as **Korotkoff sounds**.

- The appearance of the first Korotkoff sound indicates the **systolic blood pressure**.
- The disappearance of the Korotkoff sounds indicates the **diastolic blood pressure**.

Requirements

Instruments

- Sphygmomanometer (Mercury or Digital)
- Stethoscope
- Stopwatch

Materials

- Volunteer/Subject
- Chair and table

Procedure

Measurement of Resting Blood Pressure

1. Seat the subject comfortably in a chair and allow him/her to rest for 5 minutes.

2. Ensure that the subject has not consumed tea, coffee, or performed vigorous exercise within the previous 30 minutes.
3. Place the subject's arm on a table at the level of the heart.
4. Wrap the cuff of the sphygmomanometer around the upper arm approximately 2–3 cm above the elbow.
5. Palpate the brachial artery in the cubital fossa.
6. Place the diaphragm of the stethoscope over the brachial artery.
7. Inflate the cuff to about 20–30 mmHg above the expected systolic pressure (approximately 160–180 mmHg).
8. Slowly release the cuff pressure at a rate of 2–3 mmHg per second.
9. Note the pressure at which the first tapping sound is heard. This is the **systolic blood pressure**.
10. Continue releasing the pressure and note the point at which the Korotkoff sounds disappear. This is the **diastolic blood pressure**.
11. Record the readings.

Measurement of Blood Pressure After Exercise

1. Ask the subject to perform mild exercise such as running in place or climbing stairs for 3–5 minutes.
2. Immediately repeat the blood pressure measurement using the above procedure.
3. Record the readings.

Observations

Table 1. Blood Pressure Measurements

Condition	Systolic Pressure (mmHg)	Diastolic Pressure (mmHg)
Resting		
Post Exercise		

Table 2. Pulse Pressure and Mean Arterial Pressure

Condition	Pulse Pressure (mmHg)	Mean Arterial Pressure (mmHg)
Resting		
Post Exercise		

Calculations

Pulse Pressure (PP)

[PP = Systolic Pressure – Diastolic Pressure]

Mean Arterial Pressure (MAP)

[MAP = Diastolic Pressure + (Pulse Pressure /3)]

Expected Outcome

- Blood pressure values generally increase immediately after exercise due to an increase in cardiac output and sympathetic stimulation.
- Systolic pressure rises significantly because the heart pumps blood more forcefully.
- Diastolic pressure may remain unchanged or show only a slight increase.
- After a period of rest, blood pressure gradually returns to normal.

Interpretation

- **Low Blood Pressure (Hypotension):** Less than 90/60 mmHg.
- **Normal Blood Pressure:** Approximately 120/80 mmHg.

- **Elevated Blood Pressure:** 120–129 mmHg systolic and less than 80 mmHg diastolic.
- **Hypertension:** Equal to or greater than 140/90 mmHg.

Regular monitoring of blood pressure is important in the diagnosis and management of cardiovascular disorders.

Result

The blood pressure of the subject was found to be:
Resting Blood Pressure =
Post-exercise Blood Pressure =
The systolic blood pressure increased following exercise due to increased cardiac output and enhanced sympathetic activity.

Normal Values

Parameter	Normal Value
Systolic Pressure	120 mmHg
Diastolic Pressure	80 mmHg
Pulse Pressure	40 mmHg
Mean Arterial Pressure	93 mmHg

Precautions

1. The subject should be relaxed and seated comfortably.
2. The cuff size should be appropriate for the arm circumference.
3. The cuff should be wrapped snugly around the upper arm.
4. The arm should be maintained at heart level.
5. The cuff pressure should be released slowly.
6. Avoid talking or moving during measurement.
7. Take at least two readings and calculate the average value.
8. Ensure proper placement of the stethoscope over the brachial artery.

Experiment No. 7

EFFECT OF EXERCISE ON HEART RATE

Aim

To study the effect of exercise on heart rate and to observe the recovery of heart rate after exercise.

Background Information

The heart rate is the number of times the heart beats in one minute and is usually expressed as beats per minute (bpm). In a healthy adult, the normal resting heart rate ranges from **60 to 100 beats per minute**, with an average of about **72 beats per minute**.

During physical exercise, the body's demand for oxygen and nutrients increases significantly. To meet this increased metabolic demand, the cardiovascular system responds by increasing the rate and force of cardiac contractions. Consequently, the heart pumps more blood to the active muscles.

After exercise, the oxygen demand decreases, and the heart rate gradually returns to its resting level. The speed of recovery of heart rate is considered an important indicator of cardiovascular fitness.

Principle

Exercise increases the metabolic activity of skeletal muscles, resulting in an increased demand for oxygen and nutrients. This stimulates the sympathetic nervous system and increases the secretion of adrenaline, leading to an increase in heart rate and cardiac output. The pulse rate, which reflects the heart rate, is measured before exercise, immediately after exercise, and during the recovery period.

Requirements

Materials

- Healthy volunteer
- Stopwatch or timer

Apparatus

- Chair
- Recording sheet and pen

Procedure

Measurement of Resting Pulse Rate

1. Ask the volunteer to sit comfortably and relax for 5 minutes.
2. Locate the radial artery on the wrist using the index and middle fingers.
3. Count the pulse beats for one minute using a stopwatch.
4. Record the resting pulse rate.

Measurement of Pulse Rate After Exercise

1. Ask the volunteer to perform mild exercise such as:
    - Running in place,
    - Climbing stairs, or
    - Jumping for 3 minutes.
2. Immediately after exercise, count the pulse rate for one minute and record it.

Measurement of Recovery Pulse Rate

1. Allow the volunteer to rest quietly for 5 minutes.
2. Again, count the pulse rate for one minute.

3. Record the recovery pulse rate

Observations

Table 1. Effect of Exercise on Heart Rate

Condition	Pulse Rate (beats/min)
Resting	
Immediately After Exercise	
Recovery (After 5 min)	

Table 2. Increase in Heart Rate

Parameter	Value
Resting Pulse Rate	
Maximum Pulse Rate After Exercise	
Increase in Pulse Rate	

Increase in Pulse Rate = Pulse Rate after Exercise – Resting Pulse Rate

Expected Outcome

- The resting pulse rate is usually between **60 and 100 beats per minute**.
- Immediately after exercise, the pulse rate increases due to increased oxygen demand and sympathetic stimulation.
- During the recovery period, the pulse rate gradually decreases and approaches the resting value.
- Individuals with good cardiovascular fitness generally show a faster recovery of heart rate.

Interpretation

- **Resting Phase:** The oxygen requirement of tissues is minimal, and therefore the heart beats at a normal resting rate.
- **Exercise Phase:** Increased muscular activity requires greater oxygen delivery, leading to an increase in heart rate and cardiac output.
- **Recovery Phase:** As the metabolic demand decreases, parasympathetic activity predominates, and the heart rate gradually returns to normal.

A rapid recovery of heart rate after exercise is considered an indicator of efficient cardiovascular function and good physical fitness.

Result

The pulse rate of the volunteer increased immediately after exercise and gradually returned toward the resting value during the recovery period.
Therefore, exercise has a significant stimulatory effect on heart rate due to increased metabolic demand and sympathetic activation.

Normal Values

Parameter	Normal Value
Resting Heart Rate	60–100 beats/min
Average Adult Heart Rate	72 beats/min
Pulse Rate After Mild Exercise	90–130 beats/min (approximately)
Recovery Time	3–10 minutes

Experiment No. 8

EFFECT OF SUBSTRATE CONCENTRATION ON ACID PHOSPHATASE ACTIVITY

Aim

To study the effect of varying substrate concentration on the activity of Acid Phosphatase and to understand Michaelis-Menten enzyme kinetics.

Background Information

Enzymes catalyse biochemical reactions by binding with substrate molecules to form an enzyme-substrate (ES) complex. The rate of reaction depends on the substrate concentration available to the enzyme.

At low substrate concentrations, many enzyme active sites remain unoccupied, and the reaction velocity increases rapidly with increasing substrate concentration. At higher substrate concentrations, all active sites become occupied, and the enzyme becomes saturated, resulting in a maximum velocity (Vmax).

Principle

Acid phosphatase (EC 3.1.3.2) hydrolyses p-nitrophenyl phosphate (pNPP) under acidic conditions.

Reaction:

p-Nitrophenyl Phosphate + H_2O

↓ Acid Phosphatase

p-Nitrophenol + Inorganic Phosphate

The p-nitrophenol produced develops a yellow colour in alkaline medium and is measured spectrophotometrically at 405 nm.

Requirements

Chemicals

- Acid phosphatase enzyme solution
- p-Nitrophenyl phosphate (PNPP 0.1 M) (1–8 mM)
- Citrate buffer (pH 4.8) 0.05 M
- 0.4 N NaOH
- Potato extract

Apparatus

- Test tubes
- Water bath (37°C)
- Micropipettes
- Colorimeter
- Cuvettes

Procedure

1. Prepare a set of six test tubes as shown in the observation table and label these test tubes as 1to 6.
2. Mix well and incubate in a water bath at 37 ^{0}C for exactly 15 minutes.
3. Stop the reaction by adding 1 mL 0.4 N NaOH.
4. Measure absorbance at 420 nm against a blank.

5. Record the readings.
6. Plot a graph of substrate values on the X-axis against O.D. on the Y-axis.

Expected outcome – As the substrate concentration increases, the reaction velocity of Acid Phosphatase also increases because more enzyme-substrate complexes are formed. However, once a certain substrate concentration is reached, all the enzyme's active sites become occupied, and the enzyme becomes saturated. At this point, the reaction attains its maximum velocity (Vmax), and any further increase in substrate concentration does not result in an increase in the reaction rate.

This phenomenon of enzyme saturation is characteristic of biological catalysts (enzymes) and distinguishes them from ordinary chemical catalysts, which do not exhibit substrate saturation. The graph obtained is a **hyperbolic Michaelis-Menten curve**, showing an initial rapid increase in reaction velocity followed by a plateau corresponding to Vmax.

Interpretation

- Low substrate concentration → low activity.
- Increasing substrate concentration → increased ES complex formation.
- High substrate concentration → enzyme saturation.
- Reaction reaches Vmax.
-

Result

The activity of Acid Phosphatase increased with increasing substrate concentration and ultimately reached saturation, consistent with Michaelis-Menten kinetics.

Precautions

1. Maintain constant temperature.
2. Prepare fresh substrate solution.
3. Use equal incubation time.
4. Measure absorbance immediately.

Observation Table

T. T. No.	**PNPP (Substrate)**	**PNPP (Con. mM)**	**Citrate Buffer pH 4.8 (ml)**	**Potato Extract (ml)**		**0.4 N NaOH (ml)**	**O.D. 420 nm**
1	0.1	1.0	1.9	0.5	Incubate in a water bath at 37 ^{0}C for 15 minutes.	1.0	
2	0.2	2.0	1.8	0.5		1.0	
3	0.3	3.0	1.7	0.5		1.0	
4	0.4	4.0	1.6	0.5		1.0	
5	0.5	5.0	1.5	0.5		1.0	
6 Blank	0.5	5.0	1.5	0.5 (DW)		1.0	

Total reaction mixture before addition of NaOH = 2.5 ml

For the Lineweaver-Burk Plot calculate 1/[S] and 1/[V] and then plot a graph

[S] = Substrate concentration (mM)

[V] = Reaction velocity (here, O.D. or absorbance at 420 nm is taken as a measure of enzyme activity)

Lineweaver-Burk Plot

Substrate	1/substrate	O. D	1/O.D.
0.1			
0.2			
0.3			
0.4			
0.5			

Experiment No. 9

EFFECT OF TEMPERATURE ON ACID PHOSPHATASE ACTIVITY

Aim
To study the effect of temperature on the activity of Acid Phosphatase and to determine its optimum temperature.

Background Information
Temperature is one of the most important factors affecting enzyme activity. Enzymatic reactions depend on the frequency of collisions between enzyme and substrate molecules. As temperature increases, the kinetic energy of molecules increases, resulting in more frequent and effective collisions and, consequently, an increase in reaction rate.

However, enzymes are proteins and are sensitive to high temperatures. Beyond a certain temperature, known as the **optimum temperature**, the three-dimensional structure of the enzyme begins to unfold or denature. This alters the shape of the active site, reducing its ability to bind the substrate and causing a decline in enzyme activity.

At low temperatures, the enzyme remains active but the reaction rate is slow due to reduced molecular movement. At the optimum temperature, the enzyme exhibits maximum activity. Further increase in temperature results in enzyme denaturation and loss of catalytic activity.

Principle
Acid phosphatase (EC 3.1.3.2) hydrolyses p-nitrophenyl phosphate (pNPP) under acidic conditions.
Reaction
p-Nitrophenyl Phosphate + H_2O
↓ Acid Phosphatase
p-Nitrophenol + Inorganic Phosphate

The p-nitrophenol produced develops a yellow colour in alkaline medium and is measured spectrophotometrically at 420 nm. The amount of p-nitrophenol formed is directly proportional to the activity of Acid Phosphatase.

Requirements
Chemicals
• Acid phosphatase enzyme solution (Potato extract)
• p-Nitrophenyl phosphate (PNPP 0.1 M)
• Citrate buffer (pH 4.8), 0.05 M
• 0.4 N NaOH

Apparatus
• Test tubes
• Water baths maintained at different temperatures (10°C, 20°C, 30°C, 40°C, 50°C and 60°C)
• Micropipettes
• Colorimeter/Spectrophotometer
• Cuvettes

Procedure

1. Label six test tubes as 1 to 6.
2. Prepare the reaction mixture as shown in the observation table.
3. Place each test tube in water baths maintained at different temperatures (10°C, 20°C, 30°C, 40°C, 50°C and 60°C).
4. Incubate the reaction mixtures for exactly 15 minutes.
5. Stop the reaction by adding 1 mL of 0.4 N NaOH.
6. Measure the absorbance at 420 nm against a blank.
7. Record the readings.
8. Plot a graph of temperature on the X-axis against O.D. on the Y-axis.

Expected Outcome

As the temperature increases, the activity of Acid Phosphatase also increases because the kinetic energy of enzyme and substrate molecules increases, resulting in more frequent molecular collisions and greater enzyme-substrate complex formation.

At a particular temperature, known as the **optimum temperature**, the enzyme exhibits maximum activity, and the rate of product formation is highest.

Beyond the optimum temperature, enzyme activity decreases sharply because the enzyme undergoes denaturation. Denaturation disrupts the enzyme's three-dimensional structure and alters the active site's conformation, reducing its ability to bind the substrate.

The graph obtained is a **bell-shaped curve**, showing an initial increase in enzyme activity with increasing temperature, followed by a decline beyond the optimum temperature due to enzyme denaturation.

Interpretation

• Low temperatures → reduced molecular movement and fewer enzyme-substrate collisions.

• Increasing temperature → increased kinetic energy and reaction rate.

• Optimum temperature → maximum enzyme activity.

• Higher temperatures → denaturation of the enzyme protein.

• Denaturation changes the active site and decreases catalytic efficiency.

Result

The activity of Acid Phosphatase increased with increasing temperature up to _____ °C, at which maximum activity was observed. Beyond this temperature, enzyme activity decreased due to thermal denaturation. Therefore, _____ °C represents the optimum temperature for Acid Phosphatase activity.

Observation Table

T.T. No.	Temperature (°C)	PNPP (ml)	Citrate Buffer pH 4.8 (ml)	Potato Extract (ml)	Incubation Time	0.4 N NaOH (ml)	O.D. at 420 nm
1	10	0.5	1.5	0.5	15 min	1.0	
2	20	0.5	1.5	0.5	15 min	1.0	
3	30	0.5	1.5	0.5	15 min	1.0	
4	40	0.5	1.5	0.5	15 min	1.0	
5	50	0.5	1.5	0.5	15 min	1.0	
6	60	0.5	1.5	0.5	15 min	1.0	
Blank	—	0.5	1.5	0.5 (DW)	—	1.0	

Total reaction mixture before addition of NaOH = 2.5 ml

Graph

X-axis: Temperature (°C)

Y-axis: Enzyme Activity (O.D. at 420 nm)

Experiment No. 10

EFFECT OF pH ON ACID PHOSPHATASE ACTIVITY

Aim
To study the effect of pH on the activity of Acid Phosphatase and to determine its optimum pH.

Background Information

The activity of an enzyme is greatly influenced by the pH of its surrounding medium. The ionization state of amino acid residues present at the active site of an enzyme depends upon the hydrogen ion concentration (pH). Any change in pH can alter the charge, shape, and conformation of the enzyme molecule, thereby affecting substrate binding and catalytic activity.

Each enzyme exhibits maximum activity only within a narrow pH range called the **optimum pH**. At this pH, the ionization state of the active site and substrate is ideal for the formation of the enzyme-substrate complex.

Acid phosphatase is an intracellular enzyme that functions optimally under acidic conditions. At pH values lower or higher than the optimum, the enzyme structure is altered, resulting in reduced catalytic efficiency and decreased reaction rate.

Principle
Acid phosphatase (EC 3.1.3.2) hydrolyses p-nitrophenyl phosphate (pNPP) under acidic conditions.

Reaction
p-Nitrophenyl Phosphate + H_2O
↓ Acid Phosphatase
p-Nitrophenol + Inorganic Phosphate

The p-nitrophenol produced develops a yellow colour in alkaline medium and is measured spectrophotometrically at 420 nm. The amount of p-nitrophenol formed is directly proportional to the activity of Acid Phosphatase.

Requirements
Chemicals
• Acid phosphatase enzyme solution (Potato extract)
• p-Nitrophenyl phosphate (PNPP 0.1 M)
• Buffer solutions of pH 3, 4, 5, 6, 7 and 8
• 0.4 N NaOH

Apparatus
• Test tubes
• Water bath maintained at 37°C
• Micropipettes
• Colorimeter/Spectrophotometer
• Cuvettes

Procedure

1. Label six test tubes as 1 to 6.
2. Prepare the reaction mixtures using buffers of pH 3, 4, 4.8, 6 and 7 as shown in the observation table.
3. Add equal volumes of pNPP substrate to all test tubes.
4. Add equal volumes of enzyme solution (potato extract).
5. Mix thoroughly and incubate all the tubes at 37°C for exactly 15 minutes.
6. Stop the reaction by adding 1 mL of 0.4 N NaOH.
7. Measure the absorbance at 420 nm against a blank.
8. Record the readings.
9. Plot a graph of pH values on the X-axis against O.D. on the Y-axis.

Expected Outcome

As the pH of the reaction medium changes, the activity of Acid Phosphatase also changes because the ionization state of amino acid residues at the active site is altered. At low and high pH values, the enzyme activity is reduced because the enzyme structure and substrate binding are adversely affected.

As the pH approaches the optimum value, the enzyme exhibits maximum catalytic activity due to favourable ionic conditions for enzyme-substrate complex formation. Beyond the optimum pH, enzyme activity decreases because alterations in the charge distribution and conformation of the enzyme reduce its catalytic efficiency.

The graph obtained is a **bell-shaped curve**, showing an increase in enzyme activity up to the optimum pH followed by a decline on either side of the optimum.

Interpretation

• Extremely acidic or alkaline pH alters the enzyme structure.
• Optimum pH provides ideal ionic conditions for substrate binding.
• Maximum product formation occurs near the optimum pH.
• Activity decreases on either side of the optimum pH.
• Changes in pH affect the ionisation of amino acid residues at the active site.

Result

The activity of Acid Phosphatase increased with increasing pH up to _____ and then decreased at higher pH values. Therefore, pH _____ represents the optimum pH for Acid Phosphatase activity.

Observation Table

Observation Table

Effect of pH on Enzyme Activity (Acid Phosphatase using PNPP as Substrate)

T.T. No.	PNPP (1 mM) (ml)	Citrate Buffer pH	Citrate Buffer (ml)	Potato Extract (ml)	Incubation Conditions	0.4 N NaOH (ml)	O.D. at 420 nm
1	0.5	3.0	1.5	0.5	37°C for 15 min	1.0	_____
2	0.5	4.0	1.5	0.5	37°C for 15 min	1.0	_____
3	0.5	4.8	1.5	0.5	37°C for 15 min	1.0	_____
4	0.5	6.0	1.5	0.5	37°C for 15 min	1.0	_____
5	0.5	7.0	1.5	0.5	37°C for 15 min	1.0	_____
6 (Blank)	0.5	4.8	1.5	0.5 ml Distilled Water	37°C for 15 min	1.0	_____

Total reaction mixture before addition of NaOH = 2.5 ml

Graph
X-axis: pH
Y-axis: Enzyme Activity (O.D. at 420 nm)

Experiment No. 11

EFFECT OF ENZYME CONCENTRATION ON THE ACTIVITY OF ACID PHOSPHATASE

Aim

To study the effect of enzyme concentration on the rate of reaction catalysed by Acid Phosphatase.

Background Information

Enzymes are biological catalysts that increase the rate of biochemical reactions by lowering the activation energy. The rate of an enzyme-catalysed reaction depends on several factors, including substrate concentration, temperature, pH, and enzyme concentration.

When the substrate concentration is present in excess and remains constant, the reaction rate is directly proportional to the enzyme concentration. Increasing the amount of enzyme increases the number of available active sites, allowing more substrate molecules to bind simultaneously and form enzyme-substrate complexes.

However, this direct relationship persists only as long as sufficient substrate molecules are available. Once the substrate becomes limiting, further increases in enzyme concentration do not significantly increase the reaction rate.

Principle

Acid phosphatase (EC 3.1.3.2) hydrolyses p-nitrophenyl phosphate (pNPP) under acidic conditions.

Reaction

p-Nitrophenyl Phosphate + H_2O
↓ Acid Phosphatase
p-Nitrophenol + Inorganic Phosphate

The p-nitrophenol produced develops a yellow colour in alkaline medium and is measured spectrophotometrically at 420 nm. The amount of p-nitrophenol formed is directly proportional to the activity of Acid Phosphatase.

Requirements

Chemicals

- Acid phosphatase enzyme solution (Potato extract)
- p-Nitrophenyl phosphate (PNPP 0.1 M)
- Citrate buffer (pH 4.8), 0.05 M
- 0.4 N NaOH

Apparatus

- Test tubes
- Micropipettes
- Water bath maintained at 37°C
- Colorimeter/Spectrophotometer
- Cuvettes

Procedure

1. Prepare different concentrations of enzyme by varying the volume of potato extract while keeping the substrate concentration constant.
2. Label four test tubes as 1 to 4 and one tube as blank.
3. Prepare the reaction mixtures as shown in the observation table.
4. Mix thoroughly and incubate all the tubes in a water bath at 37°C for exactly 15 minutes.
5. Stop the reaction by adding 1 mL of 0.4 N NaOH.
6. Measure the absorbance at 420 nm against the blank.
7. Record the readings.
8. Plot a graph of enzyme concentration on the X-axis against O.D. on the Y-axis.

Expected Outcome

As the enzyme concentration increases, the reaction velocity of Acid Phosphatase also increases because more active sites become available for substrate binding. Consequently, more enzyme-substrate complexes form, leading to increased product formation.

Since the substrate concentration is maintained in excess and remains constant throughout the experiment, the increase in enzyme activity is directly proportional to the increase in enzyme concentration.

The graph obtained is a **straight-line graph**, indicating a linear relationship between enzyme concentration and reaction velocity.

However, this linear relationship is observed only when the substrate is present in excess. If the substrate becomes limiting, further increases in enzyme concentration do not result in a proportional increase in the reaction rate.

Interpretation

• Low enzyme concentration → fewer active sites and lower product formation.
• Increasing enzyme concentration → increased enzyme-substrate complex formation.
• Since substrate is present in excess, reaction rate increases proportionally with enzyme concentration.
• A straight-line relationship indicates that enzyme concentration is the limiting factor.
• At very high enzyme concentrations, the relationship becomes non-linear if the substrate becomes limiting.

Result

The activity of Acid Phosphatase increased with increasing enzyme concentration, demonstrating that the reaction rate is directly proportional to enzyme concentration when the substrate is present in excess.

Observation Table

T.T. No.	PNPP (ml)	Citrate Buffer pH 4.8 (ml)	Potato Extract (ml)	D/W (ml)		0.4 N NaOH (ml)	O.D. at 420 nm
1	0.5	1.5	0.1	0.4		1.0	
2	0.5	1.5	0.2	0.3	**Incubation at 37°C for 15 min**	1.0	
3	0.5	1.5	0.3	0.2		1.0	
4	0.5	1.5	0.4	0.1		1.0	
5	0.5	1.5	0.5	0.0			
Blank	0.5	1.5	0.00 (DW)	0.5		1.0	

Total reaction volume in each tube = 2.5 ml

Graph
X-axis: Enzyme Concentration (ml of Potato Extract)
Y-axis: Enzyme Activity (O.D. at 420 nm)

Experiment No. 12

STUDY OF ENZYME INHIBITION IN ACID PHOSPHATASE

Aim

To study the effect of competitive and non-competitive inhibitors on the activity of Acid Phosphatase.

Background Information

Enzyme activity can be regulated by substances known as **enzyme inhibitors**, which decrease or completely prevent the catalytic activity of enzymes. Inhibition plays an important role in the regulation of metabolic pathways, drug action, and enzyme kinetics.

There are two major types of reversible enzyme inhibition:

Competitive Inhibition

In competitive inhibition, the inhibitor resembles the substrate and competes with it for binding at the enzyme's active site. Since both substrate and inhibitor cannot bind simultaneously, the inhibitor reduces the formation of enzyme-substrate complexes and decreases the reaction rate. The inhibition can be overcome by increasing the substrate concentration because a higher substrate concentration increases the probability of substrate binding to the active site.

Non-Competitive Inhibition

In non-competitive inhibition, the inhibitor binds to a site other than the active site, called the **allosteric site**. Binding of the inhibitor changes the three-dimensional structure of the enzyme and alters the configuration of the active site, thereby reducing catalytic activity.

Unlike competitive inhibition, this type of inhibition cannot be overcome by increasing substrate concentration because the enzyme itself becomes less effective.

Principle

Acid phosphatase (EC 3.1.3.2) hydrolyses p-nitrophenyl phosphate (pNPP) under acidic conditions.

Reaction

p-Nitrophenyl Phosphate + H_2O

↓ Acid Phosphatase

p-Nitrophenol + Inorganic Phosphate

The p-nitrophenol produced develops a yellow colour in alkaline medium and is measured spectrophotometrically at 420 nm. The amount of p-nitrophenol formed is directly proportional to enzyme activity.

In the presence of inhibitors, less p-nitrophenol is produced, resulting in lower absorbance values.

Requirements

Chemicals

- Acid phosphatase enzyme solution (Potato extract)
- p-Nitrophenyl phosphate (PNPP 0.1 M)
- Citrate buffer (pH 4.8), 0.05 M
- Competitive inhibitor (Phosphate solution)
- Non-competitive inhibitor (Sodium fluoride solution)
- 0.4 N NaOH

Apparatus

- Test tubes
- Micropipettes
- Water bath maintained at 37°C
- Colorimeter/Spectrophotometer
- Cuvettes

Procedure

Tube A (Control)

1. Add 0.5 mL PNPP substrate.
2. Add 1.5 mL citrate buffer (pH 4.8).
3. Add 0.5 mL potato extract.
4. Incubate at 37°C for exactly 15 minutes.
5. Stop the reaction by adding 1 mL of 0.4 N NaOH.
6. Measure the absorbance at 420 nm.

Tube B (Competitive Inhibitor)

1. Add 0.5 mL PNPP substrate.
2. Add 1.0 mL citrate buffer.
3. Add 0.5 mL phosphate solution (competitive inhibitor).
4. Add 0.5 mL potato extract.
5. Incubate at 37°C for exactly 15 minutes.
6. Stop the reaction by adding 1 mL of 0.4 N NaOH.
7. Measure the absorbance at 420 nm.

Tube C (Non-Competitive Inhibitor)

1. Add 0.5 mL PNPP substrate.
2. Add 1.0 mL citrate buffer.
3. Add 0.5 mL sodium fluoride solution (non-competitive inhibitor).
4. Add 0.5 mL potato extract.
5. Incubate at 37°C for exactly 15 minutes.
6. Stop the reaction by adding 1 mL of 0.4 N NaOH.
7. Measure the absorbance at 420 nm.

Blank

1. Add all reagents except enzyme solution.
2. Add distilled water instead of potato extract.
3. Measure absorbance against the blank.

Expected Outcome

The control tube exhibits maximum enzyme activity because there is no inhibitor present.
The competitive inhibitor decreases enzyme activity by competing with the substrate for the active site. However, the inhibition is only partial and can be overcome by increasing substrate concentration.

The non-competitive inhibitor causes a greater reduction in enzyme activity because it binds at an allosteric site and changes the conformation of the enzyme. Since the active site itself is altered, increasing substrate concentration cannot restore the original activity.
The graph obtained generally follows the order:
Control > Competitive Inhibitor > Non-Competitive Inhibitor
indicating that non-competitive inhibition produces the greatest decrease in enzyme activity.

Interpretation

- The control shows maximum enzyme activity.
- Competitive inhibition reduces activity by preventing substrate molecules from binding to the active site.
- Non-competitive inhibition reduces activity by altering enzyme conformation.
- Lower absorbance values indicate reduced product formation and therefore reduced enzyme activity.
- Non-competitive inhibition generally produces a greater decrease in activity than competitive inhibition.

Result

Both competitive and non-competitive inhibitors reduced the activity of Acid Phosphatase. The competitive inhibitor caused a moderate decrease in enzyme activity, whereas the non-competitive inhibitor produced a greater reduction in activity by altering the structure of the enzyme.

Observation Table

Without Inhibitor

T.T. No.	PNPP (ml)	Citrate Buffer pH 4.8 (ml)	Potato Extract (ml)		0.4 N NaOH (ml)	O.D. at 420 nm
1	0.1	1.9	0.5		1.0	
2	0.2	1.8	0.5	**Incubation at 37°C for 15 min**	1.0	
3	0.3	1.7	0.5		1.0	
4	0.4	1.6	0.5		1.0	
5	0.5	1.5	0.5			
Blank	0.5	1.5	0.5 (DW)		1.0	

With Inhibitor

T.T. No.	PNPP (ml)	Citrate Buffer pH 4.8 (ml)	Potato Extract (ml)		0.4 N NaOH (ml)	O.D. at 420 nm
1	0.1	1.9	0.5		1.0	
2	0.2	1.8	0.5	**Incubation at 37°C for 15 min**	1.0	
3	0.3	1.7	0.5		1.0	
4	0.4	1.6	0.5		1.0	
5	0.5	1.5	0.5			
Blank	0.5	1.5	0.5 (DW)		1.0	

Calculation
Relative Activity (%)
Absorbance of Test /Absorbance of Control X 100

Graph
Plot a **bar graph**:
X-axis: Treatment
Y-axis: Enzyme Activity (O.D. or Relative Activity %)
Bars:

- Control
- Competitive Inhibitor

Experiment No. 13

PREPARATION OF BLOOD SMEAR AND IDENTIFICATION OF WHITE BLOOD CELLS (WBCs)

Aim
To prepare a peripheral blood smear, stain it using Leishman stain, and identify different types of white blood cells (leukocytes) under a microscope.

Principle
Blood consists of plasma and formed elements, namely red blood cells (RBCs), white blood cells (WBCs), and platelets. White blood cells play an important role in the body's defence and immunity. They can be differentiated based on their size, shape, nucleus, cytoplasmic granules, and staining characteristics.

A thin blood smear is prepared on a clean glass slide and stained with Leishman stain, a Romanowsky stain containing methylene blue and eosin. The stain differentially colours the cellular components, allowing the identification of various leukocytes.
The five major types of leukocytes found in peripheral blood are:

1. Neutrophils
2. Eosinophils
3. Basophils
4. Lymphocytes
5. Monocytes

Each type possesses characteristic morphological features that can be observed under the oil immersion objective of a microscope.

Requirements
Materials
- Clean grease-free glass slides
- Sterile lancet or blood sample
- Cotton and spirit
- Dropper
- Tissue paper
- Immersion oil

Reagents
- Leishman stain
- Distilled water or phosphate buffer (pH 6.8)

Instruments
- Compound microscope with oil immersion objective (100×)

Procedure
A. Preparation of Blood Smear
1. Clean a glass slide thoroughly to remove grease and dust.
2. Place a small drop of blood near one end of the slide.
3. Hold another clean slide (spreader slide) at an angle of about 45° in front of the blood drop.
4. Draw the spreader backwards until it touches the blood drop, allowing the blood to spread along its edge.
5. Push the spreader smoothly and quickly forward to prepare a thin, uniform smear.

6. Allow the smear to air dry completely.

B. Staining of Blood Smear

1. Place the dried smear on a staining rack.
2. Flood the smear with Leishman stain and allow it to stand for 2 minutes.
3. Add an equal volume of distilled water or phosphate buffer and mix gently by blowing.
4. Allow staining for 8–10 minutes.
5. Wash the slide gently with distilled water.
6. Drain excess water and air-dry the slide.

C. Microscopic Examination

1. Place the stained slide on the microscope stage.
2. Observe first under low power to locate the evenly spread area.
3. Add a drop of immersion oil and observe under the oil immersion objective (100×).
4. Identify the different types of leukocytes based on their morphology and staining characteristics.

Result

A thin peripheral blood smear was successfully prepared and stained with Leishman stain. Different types of white blood cells—namely neutrophils, eosinophils, basophils, lymphocytes, and monocytes—were observed and identified based on their characteristic morphological features.

Observations

Cell Type	Morphological Features	Percentage in Normal Blood
Neutrophil	Multi-lobed nucleus (3–5 lobes), pale cytoplasm with fine granules	50–70%
Eosinophil	Bilobed nucleus, large orange-red granules	1–4%
Basophil	S-shaped nucleus obscured by large deep blue granules	0.5–1%
Lymphocyte	Large dark nucleus occupying most of the cell, scanty cytoplasm	20–40%
Monocyte	Largest WBC, kidney-shaped nucleus, abundant cytoplasm	2–8%

Identification of Leukocytes

1. Neutrophils

- Most abundant WBCs.
- Nucleus is divided into several lobes.
- Cytoplasm contains fine granules.
- Function: Phagocytosis of bacteria and foreign particles.

2. Eosinophils

- Possess a bilobed nucleus.
- Cytoplasm contains large red or orange granules.
- Function: Defence against parasitic infections and involvement in allergic reactions.

3. Basophils

- Least abundant WBCs.
- Large blue-purple granules often hide the nucleus.
- Function: Release of histamine and heparin during inflammation and allergic responses.

4. Lymphocytes

- Large spherical nucleus occupying most of the cell.
- Thin rim of cytoplasm.
- Function: Immunity and antibody production.

5. Monocytes

- Largest leukocytes.
- Kidney-shaped or horseshoe-shaped nucleus.
- Function: Phagocytosis and formation of macrophages.

Experiment No. 14

STUDY OF LYMPHOID ORGANS

Aim

To study the structure and functions of various lymphoid organs and understand their role in the immune system.

Principle

The immune system protects the body against pathogens and foreign substances. The organs involved in the production, maturation, storage, and activation of immune cells are collectively known as **lymphoid organs**.

Lymphoid organs are classified into:

1. Primary (Central) Lymphoid Organs

These are the sites where lymphocytes are produced and mature.

- Bone marrow
- Thymus

2. Secondary (Peripheral) Lymphoid Organs

These are the sites where mature lymphocytes encounter antigens and initiate immune responses.

- Spleen
- Lymph nodes
- Tonsils
- Peyer's patches and other mucosa-associated lymphoid tissues (MALT)

The study of histological slides and models of these organs helps in understanding their structural organisation and immunological functions.

Requirements

Materials

- Permanent histological slides of lymphoid organs
- Charts and models of lymphoid organs
- Compound microscope

Reagents

- Immersion oil (if required)

Instruments

- Compound microscope

Procedure

1. Observe the permanent slides or models of various lymphoid organs.
2. Identify the characteristic histological features of each organ.
3. Note the arrangement of lymphoid tissue and associated structures.
4. Correlate the structural features with the immunological functions of the organs.
5. Draw neat labelled diagrams of the observed lymphoid organs.

Result

The primary and secondary lymphoid organs were observed and identified based on their histological features. Their role in the development, maturation, activation, and functioning of immune cells was studied successfully.

Observations

A. Primary Lymphoid Organs

1. Bone Marrow

Histological Features

- Soft, vascular connective tissue present within bones.
- Contains hematopoietic stem cells and developing blood cells.
- Rich in sinusoids and reticular fibres.

Functions

- Formation of all blood cells (haematopoiesis).
- Production and maturation of B-lymphocytes.
- Source of precursor cells for T-lymphocytes.

2. Thymus

Histological Features

- Bilobed organ divided into lobules.
- Each lobule has an outer cortex and inner medulla.
- Presence of characteristic Hassall's corpuscles in the medulla.

Functions

- Maturation and differentiation of T-lymphocytes.
- Development of immunological self-tolerance.
- Secretion of thymic hormones such as thymosin.

B. Secondary Lymphoid Organs

1. Spleen

Histological Features

- Largest lymphoid organ.
- Surrounded by a connective tissue capsule.
- Consists of white pulp and red pulp.

Functions

- Filters blood and removes old RBCs.
- Stores blood and platelets.
- Initiates immune responses against blood-borne antigens.

2. Lymph Node

Histological Features

- Bean-shaped organ surrounded by a capsule.
- Divided into cortex, paracortex and medulla.
- Contains lymphatic sinuses and lymphoid follicles.

Functions

- Filters lymph.
- Site of antigen presentation and activation of lymphocytes.
- Produces antibodies and initiates immune responses.

3. Tonsils

Histological Features

- Aggregates of lymphoid tissue beneath the epithelium.
- Contain crypts and lymphoid follicles.

Functions

- Protect the entrance of the respiratory and digestive tracts.
- Initiate immune responses against inhaled and ingested pathogens.

4. Peyer's Patches

Histological Features

- Aggregated lymphoid nodules are present in the ileum.
- Located beneath the intestinal mucosa.

Functions

- Monitor intestinal microorganisms.
- Generate immune responses against pathogens entering through the digestive tract.

Summary of Lymphoid Organs

Organ	Type	Major Function
Bone Marrow	Primary	Formation of blood cells and maturation of B-cells
Thymus	Primary	Maturation of T-cells
Spleen	Secondary	Blood filtration and immune response
Lymph Node	Secondary	Filtration of lymph and immune surveillance
Tonsils	Secondary	Defence against inhaled and ingested pathogens
Peyer's Patches	Secondary	Intestinal immunity

Experiment No. 15

OBSERVATION OF PROTOZOAN MOVEMENT (PHAGOCYTOSIS SIMULATION)

Aim

To observe the movement of a suitable protozoan and demonstrate phagocytic uptake of particles using a model organism.

Principle

Phagocytosis is the process by which certain cells engulf and digest solid particles such as microorganisms, cell debris, or foreign materials. It is an important mechanism of innate immunity carried out by cells such as macrophages and neutrophils.

Certain protozoans, particularly amoebae and ciliates, exhibit a similar feeding mechanism in which they engulf food particles from their surroundings. Therefore, protozoa can serve as model organisms to demonstrate phagocytosis.

When dilute ink particles or finely suspended carbon particles are added to the culture medium, the protozoan engulfs these particles by extending pseudopodia or through its feeding structures, forming food vacuoles that can be observed under a microscope.

Requirements

Materials

- Protozoan culture (Amoeba, Paramecium, or another suitable protozoan)
- Dilute Indian ink or carbon particle suspension
- Clean glass slides
- Coverslips
- Dropper
- Tissue paper

Instruments

- Compound microscope

Reagents

- Pond water or culture medium

Procedure

A. Preparation of Temporary Mount

1. Place a drop of protozoan culture on a clean glass slide.
2. Observe the organism under low power to locate active individuals.
3. Add one drop of dilute ink suspension near the edge of the coverslip.
4. Gently place the coverslip over the preparation, avoiding air bubbles.

B. Observation of Phagocytosis

1. Observe the preparation under low power and then under high power.
2. Identify the movement of the protozoan.
3. Observe the uptake of ink particles by the organism.
4. Note the formation of food vacuoles containing black particles.
5. Record the changes in particle uptake at regular intervals.

Interpretation

The engulfment of ink particles by the protozoan resembles the process of phagocytosis performed by immune cells in higher animals. The formation of food vacuoles demonstrates the internalisation of foreign particles and their subsequent digestion.

Observations

Table 1: Observation of Phagocytic Uptake

Time	Observation of Particle Uptake
0 min	No particles or very few particles present inside the organism
5 min	Several particles seen entering the cell; formation of food vacuoles begins
10 min	Numerous particles enclosed within food vacuoles; active ingestion observed

Table 2: Movement of Protozoan

Type of Movement	Observation
Amoeboid movement	Slow movement by formation of pseudopodia
Ciliary movement (Paramecium)	Rapid movement due to coordinated beating of cilia

Diagram

Students should draw neat labelled diagrams showing:

1. Protozoan before ingestion of particles.
2. Protozoan showing food vacuoles containing ink particles.

Result

The protozoan exhibited active movement and engulfed the suspended ink particles, demonstrating a process analogous to phagocytosis. The uptake of particles and formation of food vacuoles were observed successfully.

Experiment No. 16

ANTIGEN–ANTIBODY INTERACTION BY PRECIPITATION REACTION

Aim

To demonstrate the antigen-antibody precipitation reaction and study the specificity of antigen-antibody interactions.

Principle

An antigen is a substance capable of inducing an immune response, while an antibody is a specific protein produced by plasma cells in response to an antigen.

When a **soluble antigen** is mixed with its **specific antibody (antiserum)** in suitable proportions, antigen-antibody complexes are formed. At an optimal antigen-antibody ratio, known as the **zone of equivalence**, these complexes become large and insoluble, resulting in the formation of a visible precipitate. This reaction is known as the **precipitation reaction**.

The reaction can be represented as:

Soluble Antigen + Specific Antibody → Insoluble Antigen-Antibody Complex (Precipitate)

The precipitation reaction forms the basis of several immunological tests used in disease diagnosis and detection of antibodies and antigens.

Requirements

Materials

- Clean test tubes
- Test tube stand
- Pipettes or droppers
- Marker pen

Reagents

- Soluble antigen solution
- Specific antiserum (antibody solution)
- Physiological saline (for control)

Instruments

- Incubator or water bath (optional)

Procedure

Preparation of Control Tube

1. Label one test tube as **Control**.
2. Add 1 mL of antigen solution.
3. Add 1 mL of physiological saline instead of antibody solution.
4. Mix gently.

Preparation of Test Tube

1. Label another test tube as **Test**.
2. Add 1 mL of antigen solution.
3. Add 1 mL of the corresponding antiserum.
4. Mix gently without shaking vigorously.

Incubation

1. Allow both tubes to stand at room temperature or incubate at 37°C for 15–30 minutes.
2. Observe the tubes against a dark background for the formation of a precipitate.

Observations
Observation Table

Tube	Contents	Observation
Control	Antigen + Saline	No precipitate observed
Test	Antigen + Specific Antiserum	White precipitate observed

Interpretation
The formation of a white precipitate in the test tube indicates that the antigen and antibody have specifically reacted with each other to form insoluble antigen-antibody complexes.
The absence of a precipitate in the control tube confirms that precipitation occurs only in the presence of a specific antibody.

Diagram
Students should draw a neat labelled diagram showing:

1. Test tube showing a clear solution (Control).
2. Test tube showing white precipitate at the bottom or throughout the solution (Test).

Applications of Precipitation Reactions

1. Detection of specific antigens and antibodies.
2. Diagnosis of infectious diseases.
3. Determination of antigenic relationships.
4. Estimation of immunoglobulin concentration.
5. Basis of immunological techniques such as immunodiffusion and immunoelectrophoresis.

Result
A visible white precipitate was formed in the test tube containing the antigen and its corresponding antibody, confirming the occurrence of an antigen-antibody precipitation reaction.

Conclusion
The experiment demonstrated the specificity of immune reactions and the formation of antigen-antibody complexes leading to precipitation. The reaction occurs only when a soluble antigen reacts with its corresponding antibody in an optimal proportion.