



NETAJI SUBHAS OPEN UNIVERSITY

STUDY MATERIAL

**POST GRADUATE
ZOOLOGY**

**Paper : 10
Group : A**

**Laboratory Course
(Endocrinology, Cell &
Tissue Structure)**



PREFACE

In the curricular structure introduced by this University for students of Post-Graduate degree programme, the opportunity to pursue Pos-Graduate course in a subject as introduced by this University is equally available to all learners. Instead of being guided by any presumption about ability level, it would perhaps stand to reason if receptivity of a learner is judged in the course of the learning process. That would be entirely in keeping with the objectives of open education which does not believe in artificial differentiation.

Keeping this in view, study materials of the Post-Graduate level in different subject are being prepared on the basis of a well laid-out syllabus. The course structure combines the best elements in the approved syllabi of Central and State Universities in respective subjects. It has been so designed as to be upgradable with the addition of new information as well as results of fresh thinking and analysis.

The accepted methodology of distance education has been followed in the preparation of these study materials. Cooperation in every form of experienced scholars is indispensable for a work of this kind. We, therefore, owe an enormous debt of gratitude to everyone whose tireless efforts went into the writing, editing and devising of proper lay-out of the materials. Practically speaking, their role amounts to an involvement in 'invisible teaching'. For, whoever makes use of these study materials would virtually derive the benefit of learning under their collective care without each being seen by the other.

The more a learner would seriously pursue these study materials the easier it will be for him or her to reach out to larger horizons of a subject. Care has also been taken to make the language lucid and presentation attractive so that they may be rated as quality self-learning materials. If anything remains still obscure or difficult to follow, arrangements are there to come to terms with them through the counselling sessions regularly available at the network of study centres set up by the University.

Needless to add, a great deal of these efforts is still experimental—in fact, pioneering in certain areas. Naturally, there is every possibility of some lapse or deficiency here and there. However, these do admit of rectification and further improvement in due course. On the whole, therefore, these study materials are expected to evoke wider appreciation the more they receive serious attention of all concerned.

Professor (Dr.) Subha Sankar Sarkar
Vice-Chancellor

Third Reprint : February, 2020

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

POST GRADUATE : ZOOLOGY

[M.Sc.]

Paper : Group
PGZO 10 : A

Writer

Units : 1 - 4 : Dr. Kamales Kr. Misra

Editor

Prof. Buddhadeb Manna

Notification

All rights reserved. No part of this book may be reproduced in any form without permission in writing from Netaji Subhas Open University.

Mohan Kumar Chattopadhyay
Registrar

PROCEEDINGS OF THE

ANNUAL MEETING

OF THE

AMERICAN

ASSOCIATION OF PHYSIOLOGISTS

HELD AT

THE UNIVERSITY OF CALIFORNIA

AT SAN FRANCISCO, CALIFORNIA

DECEMBER 1-5, 1907



Group - A

Endocrinology, Cell & Tissue Structure

| | | |
|----------------|---|--------------|
| Unit-1 | ☐ Staining and Identification of different Endocrine Glands | 7-14 |
| Unit- 2 | ☐ Identification of stages of oestrous-cycle in rat | 15 |
| Unit -3 | ☐ Identification of neurosecretory cells in cerebral ganglia (cockroach), demonstration of neurosecretory centre | 16-18 |
| Unit- 4 | ☐ Identification of blood cell types | 19-22 |
| Unit- 5 | ☐ Suggestive questions | 23 |



Chapter 1

Introduction to the Study of Psychology

Psychology is the scientific study of behavior and the mind.

Behavior is any response that can be observed and measured.

The mind is the internal state that cannot be observed directly.

Psychologists use the scientific method to study behavior.

The scientific method is a systematic way of gathering information.

It involves making observations, forming hypotheses, and testing them.

Psychologists study behavior at different levels of analysis.

These levels include biological, psychological, and environmental.

Unit 1 □ Staining and identification of different endocrine glands

Structure

1.1 Staining procedure of histological sections

1.2 Identification of different endocrine organs

1.2.1 Section of Thyroid Gland of Mammal

1.2.2 Section of Adrenal Gland of Mammal

1.2.3 Section of Pancreas of Mammal

1.2.4 Section of Testis of Mammal

1.2.5 Section of Ovary of Mammal

1.2.6 Section of Anterior Pituitary (adenohypophysis) of Mammal

1.1 Staining Procedure of Histological Sections

Requirements

a) Staining set containing

- i) Xylene
- ii) Absolute ethyl alcohol
- iii) 90% ethyl alcohol
- iv) 70% ethyl alcohol
- v) 50% ethyl alcohol
- vi) 30% ethyl alcohol
- vii) Distilled water
- viii) Delafield haematoxylin solution (1%)
- ix) Running tap water facility (otherwise ammonium water is needed)
- x) Distilled water
- xi) 30% ethyl alcohol

- xii) 50% ethyl alcohol
- xiii) 70% ethyl alcohol
- xiv) 90% ethyl alcohol
- xv) 1% eosin dissolved in 90% ethyl alcohol
- xvi) 90% ethyl alcohol (for quick washing of excess eosin)
- xvii) Absolute ethyl alcohol (preferably two vials)
- xix) Xylene
- xx) Mounting medium (Canada balsam or DPx)
- xxi) Cover slip

Staining

1. Immerse glass slides with paraffin sections of 5-6 μm thickness in xylene (not more than a minute, confirm that the paraffin is completely washed).
2. Immediately place the de-paraffinized slide in absolute alcohol for 1-2 minutes.
3. Immerse the slide in 90% alcohol for a maximum of 5 minutes.
4. Keep the slide in 70% alcohol for 5 -10 minutes (confirm that no yellowish colour in the sections remains, i.e. removal of remaining picric acid is important, otherwise eosin stain will be hampered).
5. Bring the slide in 50% alcohol for 5 minutes.
6. Place the slide in 30% alcohol for 2-5 minutes.
7. Immerse the slide in distilled water for 2-3 minutes.
8. Dip the slide in Delafield haematoxylin solution for maximum of 1 minute (the time depends on the quality/strength of the stain).
9. Place the stained slide in running tap water for at least 5 minutes (till the bluish colour appear in the tissue; check the slide under microscope to satisfy yourself that the tissue is stained properly, otherwise, bring the slide to distilled water and again stain in haematoxylin. Remember that every time wash the slide in distilled water before you stain in haematoxylin.).
10. Bring the stained slide to distilled water for 2 minutes.

11. Place the slide in 30% alcohol for 5 minutes.
12. Put the slide in 50% alcohol for 5 minutes.
13. Keep the slide in 70% alcohol for 5-10 minutes.
14. Immerse the slide in 90% alcohol for at least 5 minutes. (If possible with two changes).
15. Dip the slide twice or thrice in eosin solution.
16. Quickly wash in 90% alcohol and dip in absolute alcohol for 3-5 minutes with preferably two changes).
17. In the mean time keep ready your cover slip clean and put a drop of mounting medium on it.
18. Dip the slide in xylene for one minute (see that when you remove the slide, xylene should decant uniformly along the slide).
19. Place the slide over the cover slip facing the section downward just over a drop of mounting medium.
20. Clean the slide around the cover slip and look under the microscope.

1.2 Identification of Section of Different Endocrine Organs

1.2.1 Section of Thyroid gland of mammal (Fig. 1)

1. The whole tissue is enclosed in a thick connective tissue capsule.
2. Presence of thyroid follicles of irregular diameter.
3. Each follicle is surrounded by a thin basal lamina, a network of reticular fibres, and a rich plexus of capillaries.
4. Each follicle is lined by cuboidal or low columnar epithelium.
5. The epithelial cells are polarized towards the lumen.
6. Nucleus of the cells are round and basal in position.
7. Each follicle is filled with gelatinous material.
8. Pale-stained parafollicular cells are found in small groups at the base of the follicular epithelium.

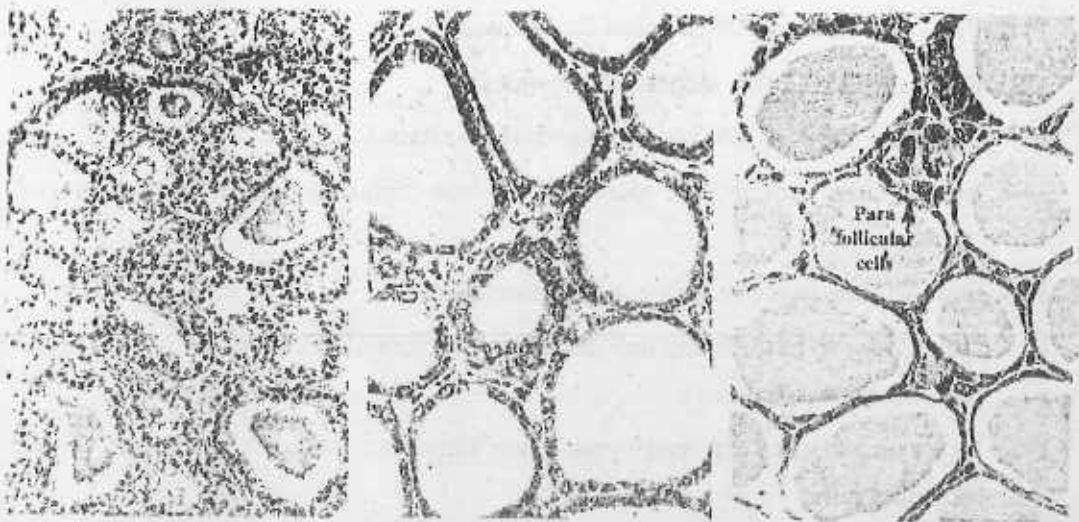


Fig. 1. Photomicrographs of sections of thyroid gland in three stages of activity. The height of the follicular cells is directly proportional to the glandular activity. Calcitonin-producing parafollicular cells are clearly shown at the right

1.2.2 Section of Adrenal gland of mammal (Fig. 2)

1. A thick cortex and a central medulla are distinguishable.
2. The section of the gland is enclosed by a connective tissue capsule.
3. Three concentric zones, zona glomerulosa, zona fasciculata, and zona reticularis are seen in the cortex.
4. Zona glomerulosa contains columnar epithelial cells, with heterochromatin nuclei and acidophilic cytoplasm, separated by thin connective-tissue septa.
5. Zona fasciculata contains pale-stained polyhedral cells arranged in long columns, and arranged radially to the medulla.
6. Zona reticularis is composed of networks of anastomosing cords, with smaller deep stained cells.
7. Medulla is composed of large epithelial cells in clusters with closely packed capillaries.
8. In medulla, two types of cells can be distinguished on the basis of staining properties.

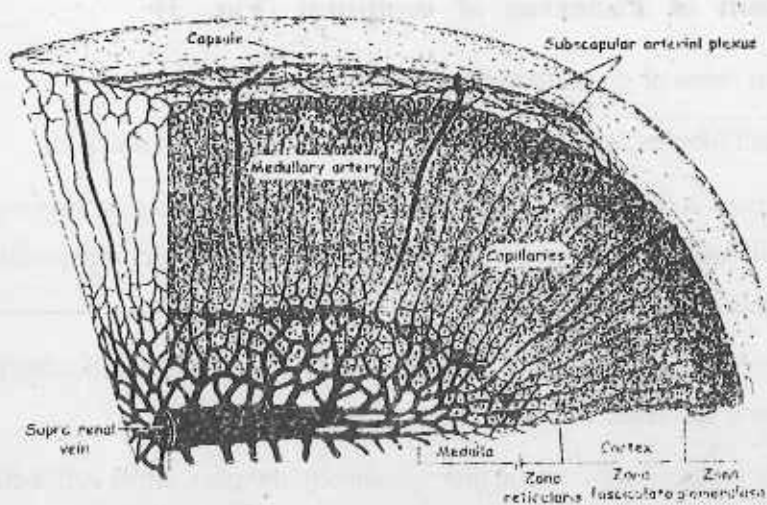


Fig. 2A. General architecture and blood circulation of the adrenal gland

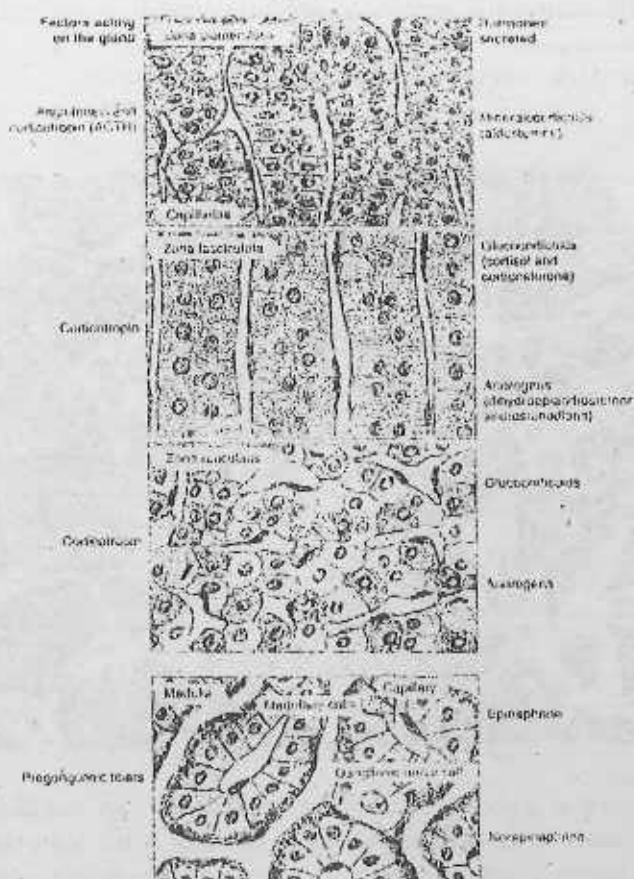


Fig. 2B. Section of adrenal gland, magnified

1.2.3 Section of Pancreas of mammal (Fig. 3)

1. Two types of glandular components are present.
2. Small lobules bound by loose connective tissue form the acini.
3. Acinous is composed of pyramidal epithelial cells surrounds lumen. Cells are with small nuclei and basophilic cytoplasm. Intercalated duct is associated with acini, thus it is an exocrine component of the gland.
4. There are aggregations of cells enclosed in a thin layer of reticular fibres scattered among the acini. These are islets of Langerhans.
5. Four types of cells can be distinguished in the islet, alpha cell, beta cell, D-cell, and F-cell.
6. These cells stains pink and possesses large vesicular nucleus.
7. Within the islet, rich network of capillaries are visible.

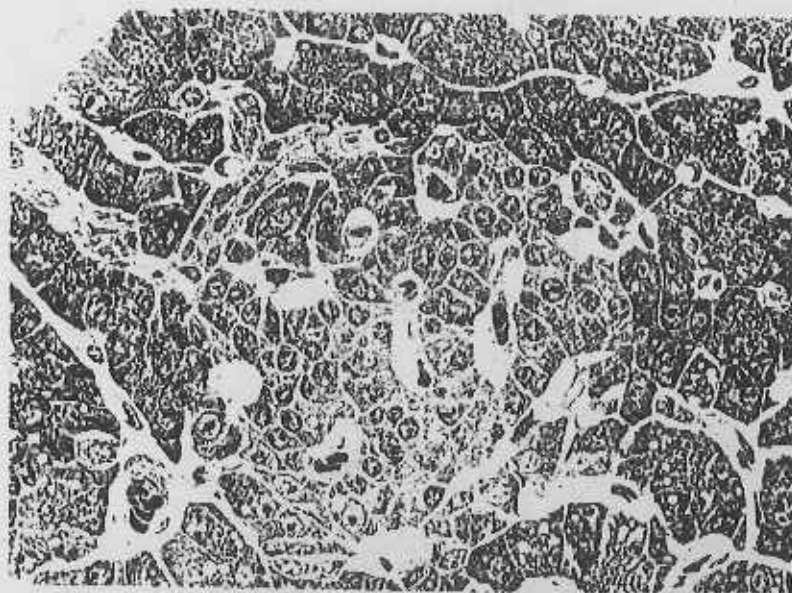


Fig. 3. Photomicrograph of a section of the pancreas. Note the islet of Langerhans, where the A cells appear mainly in the periphery as large cells with a dark cytoplasm. The other cells are mostly B cells. The islet is formed of cell cords and capillaries and is surrounded by pancreatic acinar cells

1.2.4 Section of Testis of mammal (Fig. 4)

1. Tissue section is surrounded by a thick collagenous connective tissue – tunica albuginea.
2. A number of round to ovoid seminiferous tubules present in each testicular lobule.
3. Each seminiferous tubule is composed of well-defined basal lamina, and germinal epithelium.
4. Flattened myoid cells adhere to the basal lamina.
5. Germinal epithelium consists of Sertoli cells and spermatogenic cell lineages.
6. Spermatogenic cell lineage consists of spermatogonium, primary and secondary spermatocytes, spermatids and bunches of sperms attached to Sertoli cells.
7. Interstitial cells are present in the space between seminiferous tubules.
8. Blood capillaries are present in inter-seminiferous tubular space.

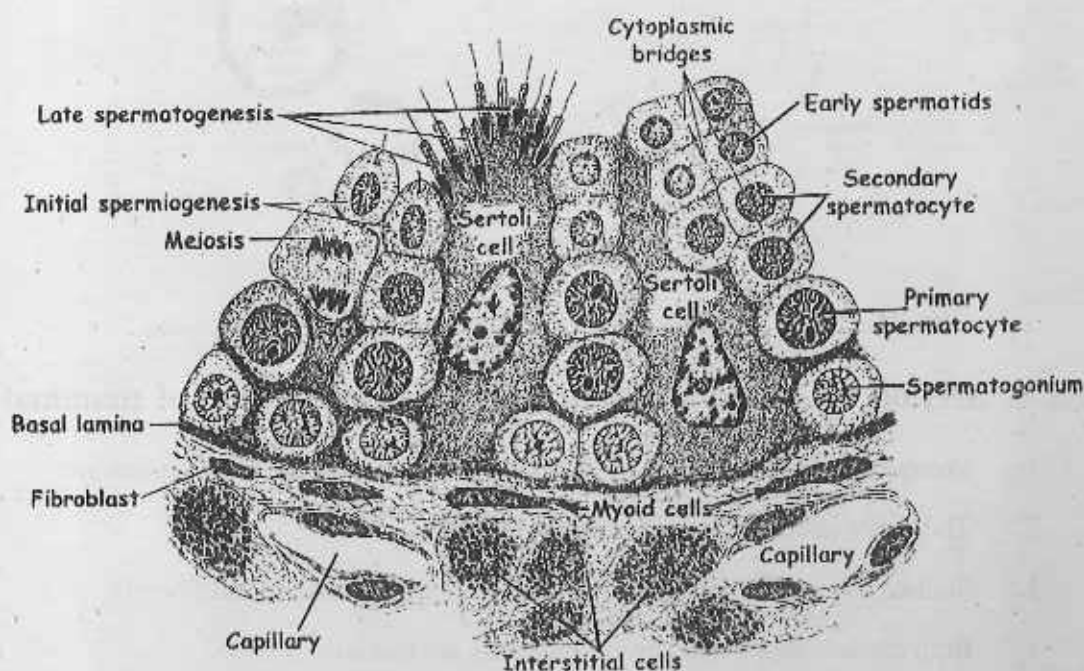


Fig. 4. Section of seminiferous tubule of mammal

1.2.5 Section of Ovary of mammal (Fig. 5)

1. The whole tissue is divided into stroma and medullary region, and surface of the tissue is covered by a simple squamous epithelium – the germinal epithelium.
2. Tunica albuginea is present under the germinal epithelium.
3. Ovarian follicles are embedded in the stroma.
4. A follicle consists of an oocyte surrounded by several layer of follicular cells – the granulosa cells.
5. Oocyte is covered by theca externa and interna, and filled with liquor folliculi.
6. Primary, secondary and mature (Graafian) follicles are present.
7. Corpora lutea and atretic follicle are present.

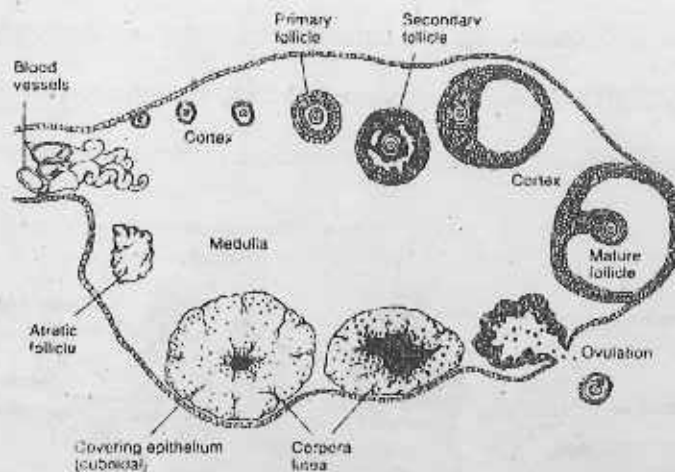


Fig. 5. Ovarian structures and their changes during the menstrual cycle

1.2.6 Section of Anterior Pituitary (adenohypophysis) of mammal

1. Presence of irregular cords of glandular cells interspersed with capillaries.
2. The sinusoids have a fenestrated endothelium.
3. Stellate, fibroblast-like cells with long branching processes are found.
4. Both chromophobe and chromophil cells are found.
5. Chromophil cells contain mainly 2 types of acidophil and 3 types of basophil cells.

Unit 2 □ Identification of stages of oestrous-cycle in rat

A. Proestrus

1. The smear is predominated by round and nucleated epithelial cells. (Fig. 6)

B. Estrus

1. The smear primarily consists of anucleated cornified cells. (Fig. 6)

C. Metaestrus

1. The smear consists of the same proportion among leukocytes, cornified, and nucleated epithelial cells. (Fig. 6)

D. Dioestrus

1. The smear primarily consists of a predominance of polymorphonuclear leukocytes. (Fig. 6)

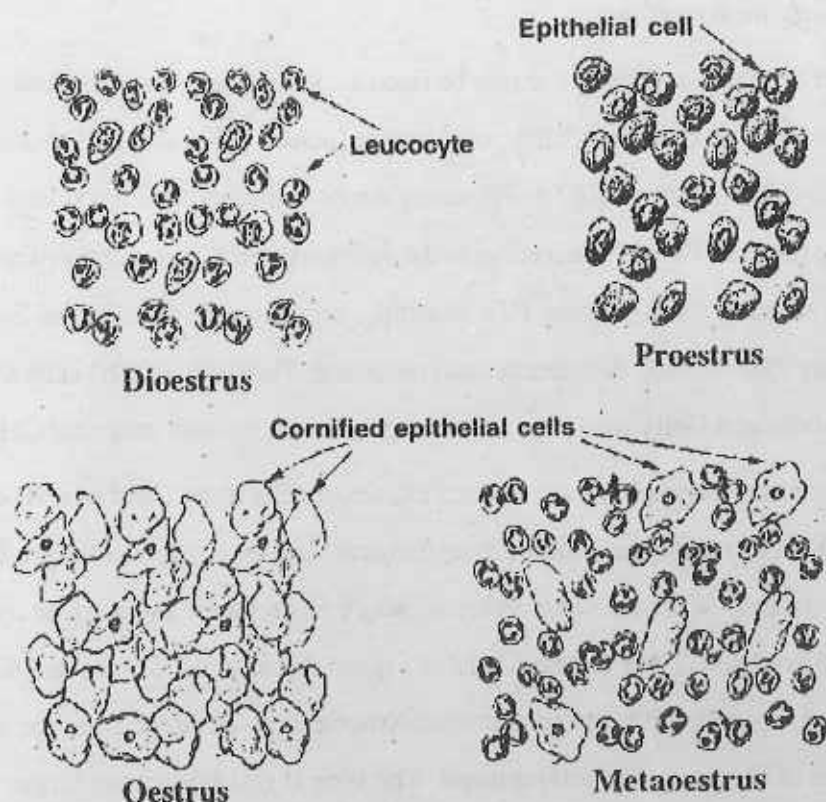


Fig. 6. Smears of different stages of Oestrus cycle in rat

Unit 3 □ Identification of neurosecretory cells in cerebral ganglia (cockroach), demonstration of neurosecretory centre

The neurosecretory system in cockroach consists of several sets of neurosecretory cells located in the brain and ventral nerve cord (Fig. 7). The majority of NSCs are found in the dorso-medial protocerebrum, the so-called pars intercerebralis (PI) and pars lateralis (PL). These NSCs project their axons toward a set of endocrine glands, the corpora cardiaca (CC) and corpora allata (CA), containing release sites for neurosecretory products. The CC and CA act as neurohemal organs. At the same time, neuropeptides that reach the CC and CA from the brain may act locally on the glandular cells of these organs and control the release of their hormones (Fig. 8).

Materials and methods.

In order to study the cells, tissue is to be fixed in Bouin fixative and the following stains: chrome hematoxylin-phloxin (CHP), paraldehyde fuchsin (PF), alcian blue-alcian yellow, paraldehyde thionin-phloxin (PTh- Ph) using Panov's method and paraldehyde thionin-paraldehyde fuchsin (PTh-PF) according to the following method: oxidation under standard conditions, staining for 10 min by PTh, washing, dehydration, staining for 2 min by PF, washing with 95% alcohol, dehydration and mounting. The study of C(r) cells to be carried out using Bouin and Helly fixatives, each being followed by both azan and CHP staining.

The two electrophysiologically distinct classes of cells (type I and type II) correspond to two distinct morphological and ultrastructural classes. Type I cells are the medial neurosecretory cells of the pars intercerebralis, which project their axons to the retrocerebral neuro-hemal complex. Their cell bodies have a mean diameter of 17 μm , and they contain neurosecretory granules 200 μm in diameter. Arborizations emanate from the axon in the anterior part of the protocerebral neuropil. The type II cell bodies are larger (38 μm in diameter). Their axons project into the contralateral circumesophageal connective. These

cells were usually multipolar, having somatic arborizations in the anterior protocerebral neuropile. The cell bodies contain vesicles 40 μm in diameter, numerous trophospongia, and a multi-layered glial envelope.

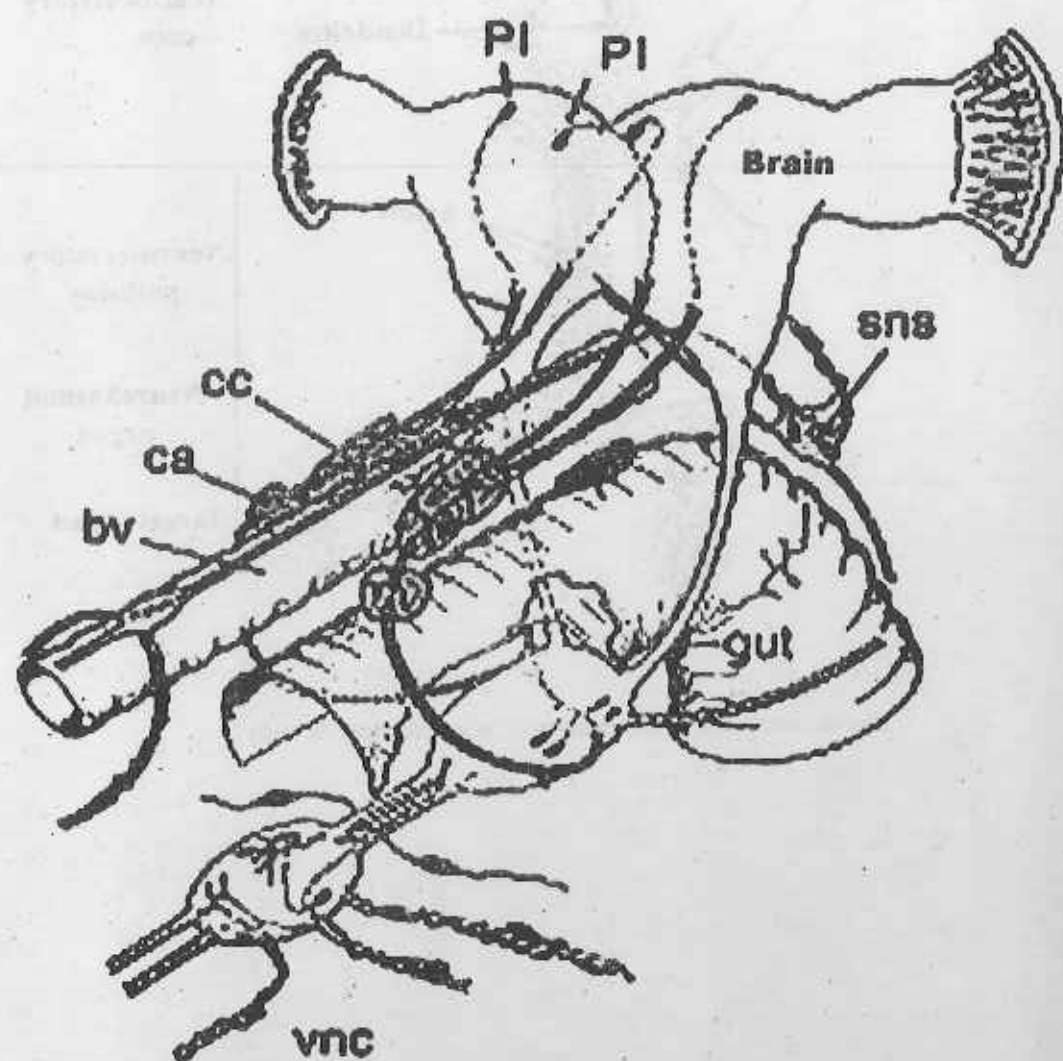


Fig. 7. Posterior-dorsal view of insect neuroendocrine system. Pars intercerebralis (PI), pars lateralis (PL) of the protocerebrum, the ventral nerve cord (vnc). Corpora cardiaca (cc) and corpora allata (ca), both of which are located close to the dorsal blood vessel (bv), prothoracic gland (ptg), stomatogastric nervous system (sns).

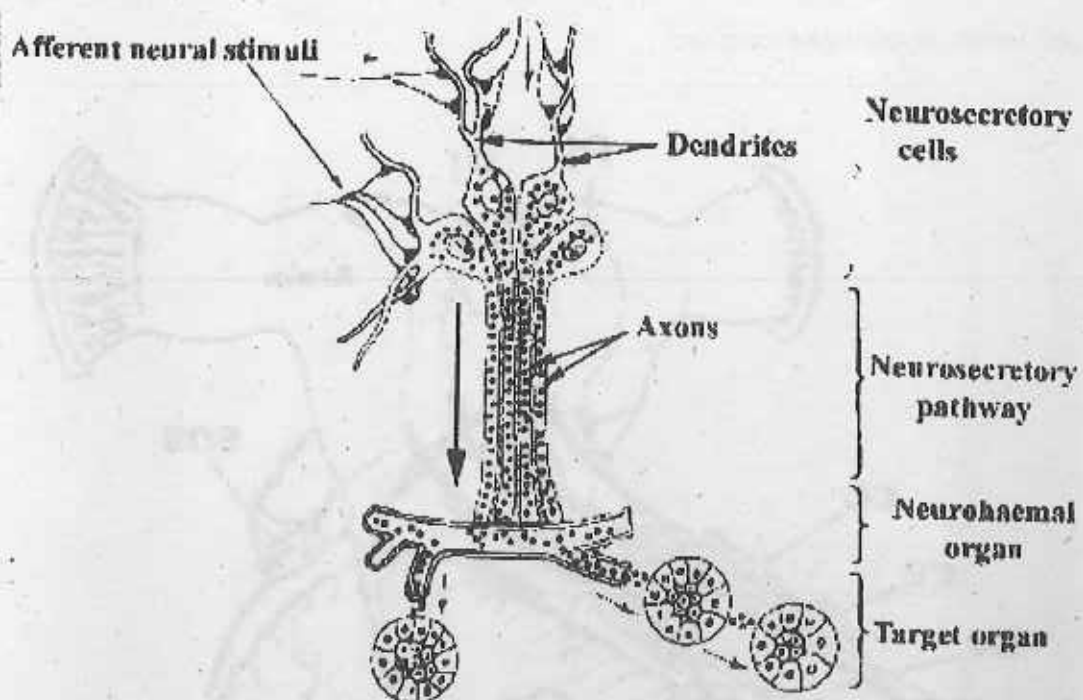


Fig. 8. Schematic representation of neurosecretory activity

Unit 4 □ Identification of blood cell types

Different types of WBC found in man

The classification is primarily made on the basis of presence and absence of granules in the cytoplasm. The 'granulocytes' contain granules in their cytoplasm but 'agranulocytes' are devoid of any granules. Granulocytes are further subdivided on the basis of their staining property in Leishman stain and number of lobes in the nucleus.

A. Granulocytes

1. Neutrophil

About 10 – 12 μm in diameter. Number of cells/ml in man 3000-6000

Structure : The nucleus is many lobed (2-7 lobes) and the granules in the cytoplasm take neutral stain. Sometime sex chromatin - like a drumstick is present in the nuclei. The cytoplasm contains fine neutrophilic granules, which appear pale-violate with Leishman's stain.

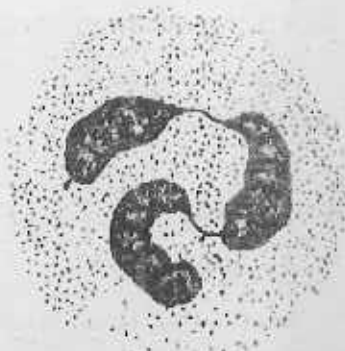
Function : Amoeboid and phagocytic in nature.

Fate : Live for 2 – 4 days. Undergo fragmentation in the blood stream and are also broken down in the reticulo-endothelial cells.

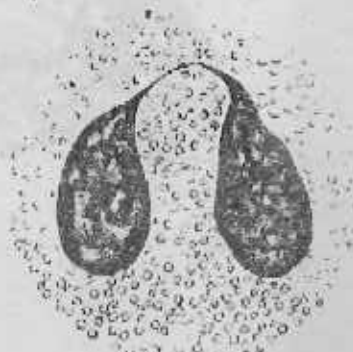
2. Eosinophil

About 10 – 12 μm in diameter. Number of cells/ml in man 150-400

Structure : The nucleus is commonly 2-3 lobed. The cytoplasm contains coarse granules, which are stained red (eosinophilic) in Leishman's stain.



Neutrophil



Eosinophil

Function : They are amoeboid but not phagocytic. They are very rich in histamine and defend against allergic conditions.

Fate : Live for 8 – 12 days. Die disintegrates and disappears.

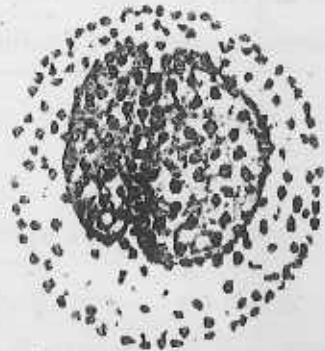
3. Basophil

About 8 – 10 μm in diameter. Number of cells/ml in man 0-100

Structure : The nucleus is lobed and slightly kidney-shaped. The cytoplasm contains granules of various sizes, which take deep blue (basophilic) colour in Leishman's stain.

Function : They are actively amoeboid. They secrete 5-hydroxy-tryptamine, heparin and histamine. They have role in anticoagulation.

Fate : Live for 12 – 15 days. Die, disintegrates and disappears.



Basophil

B. Agranulocytes

1. Monocyte

About 16 – 18 μm in diameter. Number of cells/ml in man 1500-2700



Lymphocyte



Monocyte

Structure : The nucleus is eccentric and kidney or horse shoe-shaped. A large amount of non-granular cytoplasm with vacuoles is present.

Function : They are motile. Engulf foreign bodies and bacteria, and generally digest them.

Fate : Undergo fragmentation in the blood stream and are also broken down in the reticulo-endothelial cells.

2. Lymphocyte

Number of cells/ml in man 350-800

a. Small lymphocyte

About 7.5 μm in diameter.

Structure : They are slightly larger than the red cells. The round nucleus occupies major part of the cell and relatively larger in size. The cytoplasm is basophilic and makes a thin rim around the nucleus.

Function : Manufacture β and γ fraction of serum globulin. They may be converted into fibroblast in the area of inflammation.

Fate : The average life span is 2 to 3 days. They leave the body and are destroyed by passing out through intestinal and other mucosa. In early childhood they make up about 50% of the total WBC count and diminish with age.

b. Large lymphocyte

About 10 - 12 μm in diameter.

Structure : The nucleus may be round, oval or kidney-shaped and situated in the centre of the cell. Cytoplasm is basophilic and forms comparatively wider zone around the nucleus.



Fate : The average life span is 2 to 3 days. They are considered to be the younger forms of small lymphocytes. In adults they are very few in numbers (4-8%) but are more frequent in children.

Unit 5 □ Suggestive questions

1. Stain the histological section provided. Draw, label and identify the tissue with specific characters.
2. Prepare a vaginal smear of the rat. Stain the smear. Draw, label and identify the phase of the oestrous cycle with distinctive character.
3. Identify with reasons :
 - i) Histological slide
 - ii) Stage of a oestrous cycle
 - iii) Blood film to identify one granulocyte
4. Laboratory note book
5. Viva voce

Notes



মানুষের জ্ঞান ও ভাবকে বইয়ের মাধ্যমে সঞ্চিত করিবার যে একটি প্রচুর সুবিধা আছে, সে কথা কেহই অস্বীকার করিতে পারে না। কিন্তু সেই সুবিধার দ্বারা মনের স্বাভাবিক শক্তিকে একেবারে অস্বল্প করিয়া ফেলিলে বুঝিকে বাবু করিয়া তোলা হয়।

—রবীন্দ্রনাথ ঠাকুর

ভারতের একটি mission আছে, একটি গৌরবময় ভবিষ্যৎ আছে, সেই ভবিষ্যৎ ভারতের উত্তরাধিকারী আমরাই। নূতন ভারতের মুক্তির ইতিহাস আমরাই রচনা করছি এবং করব। এই বিশ্বাস আছে বলেই আমরা সব দুঃখ কষ্ট সহ্য করতে পারি, অস্বাভাবিক বর্তমানকে অগ্রাহ্য করতে পারি, বাস্তবের নিষ্ঠুর সভাগুলি আদর্শের কঠিন আঘাতে ধুলিসাৎ করতে পারি।

—সুভাষচন্দ্র বসু

Any system of education which ignores Indian conditions, requirements, history and sociology is too unscientific to commend itself to any rational support.

—Subhas Chandra Bose

Price : Rs. 225.00

(Not for Sale to the Student of NSOU)