T.S. of all Endocrine gland.

Experiment-1:

Aim :- To study the permanent slides of all the endocrine glands in mammals.

A. T.S of Pineal Gland in Mammal :-

→ It is a "pinecore" shaped small gland located in the middle of the human brain in between the two hemispheres in an area called "epithalamus".

→ It is a major site for "Melatonin" secretion, which regulates the body's biological internal clock (Circadian Rhythm)- Sleep-wake cycle.

→ This gland is composed of Pinealocytes and supporting cells that resemble the astrocytes present in brain.

→ Pineal gland synthesizes "melatonin" and "serotonin" and they also produce "Neurosteroids".

→ Serotonin is the precursor of Melatonin. Serotonin is acetylated and methylated to yield melatonin within the pineal gland.

→ Melatonin Hormone regulates various activities :-

a) Metabolism
b) Pigmentation
c) Body Temperature
d) Defence capability
e) Sleep-wake cycle
f) Menstrual cycle.

→ Melatonin production is governed by light-Dark cycle. It contributes in the setting of biological clock.

→ Melatonin is antagonistic to the melanocyte. Make the skin pale colour.

B. T.S. of Mammalian Hypothalamus :-

→ Hypothalamus is a minute region, almost the size of an almond, present at the centre of the human brain, near the pituitary gland.

→ The structure of hypothalamus is made up of three main regions :- a) Anterior Region
b) Middle Region
c) Posterior Region

i)→ Anterior Region is also called as Supraoptic region :-
It regulates body temperature and maintains the circadian rhythm.

→ The major hypothalamic nuclei include supra optic and paraventricular nuclei.

→ The Hormones secreted from anterior region of Hypothalamus are :-

* Corticotropin releasing Hormone
* Thyrotropin releasing Hormone
* Gionadotropin releasing Hormone.
* Oxytocin
* Vasopressin
* Somatostatin.

ii)→ Middle region is also called as Tuberal region.
It contains Ventromedial nuclei - controls the apetite, and arcuate nuclei - that secretes Growth Hormone..

iii)→ Posterior Region - is also called as Mammillary region.
It contains Hypothalamic nuclei, which helps in body temp. regulation and mamillary nuclei which is involved in memory function.

→ Hypothalamus stimulates or inhibits many of the body's

C. T.S. of Mammalian Pituitary Gland :-

→ The Pituitary gland is also known as the "Hypophysis". It is a Pea-sized endocrine gland.
→ The pituitary gland is located in "Sella tursica", a depression in the sphenoid bone and attached to Infundibulum.
→ It is often referred to as the Master gland because it produces some of the important hormones in the body.
→ The pituitary gland is divided into three regions/parts :-
a) Anterior pituitary (Adenohypophysis).
b) Intermediate pituitary (absent in adult human being)
c) Posterior Pituitary (Neurohypophysis).

→ The Hormones secreted by the Anterior pituitary are :-
* Human Growth Hormone (GH) or Somatotropin Hormone (STH)
* Thyroid stimulating Hormone (TSH)
* Adrenocorticotropic Hormone (ACTH)
* Follicle Stimulating Hormone (FSH).
* Melanocyte stimulating Hormone (MSH)
* Prolactin (PRL)
* Leutinizing Hormone (LH).

→ The posterior pituitary is responsible for the storage and secretion of two Hormones i.e. Oxytocin (OT) and Antidiuretic Hormone (vasopressin)

→ There are some pituitary gland associated disorders :- Pituitary Dwarfism, Agromegaly (Gigantism). Diabetes Insipidus (↓ADH).

D. T.S. of Thyroid Gland :-

→ The thyroid gland is a ductless endocrine gland situated in the anterior/ front portion of the neck.

→ It is roughly resembles the shape of a butterfly. It is also one of the largest endocrine gland.

→ The primary function of the thyroid gland is to secrete two hormones, namely Triiodothyronine (T_3) hormone and the thyroxine hormone (T_4). (Tetraiodothyronine).

→ The thyroid gland is located in the anterior neck bet^n C_5 and T_1 vertebrae. It consists of two lobes and parathyroid glands are present on their posterior surface.

→ T_4 :-

Thyroxine is a hormone secreted by the thyroid gland in the bloodstream. It then travels to the organs such as Kidneys and liver where it gets converted into and gets converted into its active form Triiodo thyronine.

→ T_3 :-

It is a thyroid hormone that affects physiological processes such as growth, development, metabolism etc.

* Thyroid Gland Disorders :-

i) Goitre → Excessive enlargement of Thyroid Gland.

ii) Thyroid Cancer → Papillary thyroid cancer
→ Follicular thyrord cancer
→ Medullary cancer
→ Anaplastic thyroid cancer.

iii) Hyperthyroidism → Excessively produce a hormone "thyroxine"

iv) Hypothyroidism → Undersecretion of thyroid hormone.

v) Thyroid symptoms → Nerousness, weight gain, change in menstrual cycle, High Cholesterol level, muscle aches, Increased Heartrate.

e. T.S. of Parathyroid Gland :-

→ The parathyroid glands are small endocrine glands situated just below the thyroid glands in the neck.
→ They are usually four in number, two behind each thyroid gland.
→ They are very small, pea-sized and weigh about 50 mg. The gland function is to maintain the calcium and phosphorous levels in our bodies.
→ The two glands can be differentiated easily as the thyroid glands have a follicular structure, and the cells of a parathyroid gland are densely packed.
→ The parathyroid gland releases a parathyroid hormone (PTH); also known as parathormone or parathyrine.
→ PTH is a small peptide that maintains the homeostasis of calcium and phosphate and also acids in bone physiology.
→ Calcium :- The PTH functions to release calcium from the bone by stimulating the osteoblasts. It also promotes Ca^{2+} reabsorption in Kidneys.
Phosphorous :- Inhibit the absorption of phosphorous in kidney, but increase the absorption in the gastro-intestinal tract by activating vit-D.
Activation of Vit-D :- The PTH also helps in activation of vitamin-D by upregulating the enzyme. 1-α-hydroxylase.
→ Disorder :-
i) Hyperparathyroidism :- Overproduction of PTH ; releases Ca^{2+} more causes Hypercalcemia
ii) Hypoparathyroidism :- Low Level of PTH ; Hypocalcemia causes muscle cramps and Spasms.

F. T.S. of Adrenal Gland :-

Comments :-

→ Adrenal glands are paired organs present on top of each kidney at the anterior end.

→ Adrenal gland is composed of two distinct regions i.e.
outer vertically striated yellow coloured - Cortex.
Inner soft, highly vascular dark brown - Medulla.

→ Entire gland is covered by outermost layer → Fibrous capsule.

→ Adrenal cortex is further divided into three zones :-

a) Zona Glomerulosa :-

- It is found below capsule, comprising of columnar cells.
- It controls mineral and water balance as well as Fat and carbohydrate metabolism.
- It secretes "deoxycorticosterone" (not under Pituitary control).

b) Zona Fasciculata :-

- This is comprised of compressed cells.
- It secretes "corticosterone", which helps in carbohydrate metabolism (also causes lymphocyte disintegration and antibody release).

c) Zona Reticularis :-

- It is consist of pigmented reticular cells, secrete sex hormone.

→ The adrenal Medulla is innermost part. It has irregularly disposed cells and chromaffin cells (secrete catecholamines)

→ It also contains, elastic fibres, sinusoids (large blood spaces), blood vessels, (arteries, veins), granular cytoplasm.

→ Adrenal cortex has ectodermal origin, whereas medulla has neuroectodermal origin.

→ Adrenal cortex Hormones :-

Zona Glomerulosa → Mineralocorticoids (aldosterone)
Zona Fasiculata → Glucocorticoids (cortisol)
Zona Reticularis → Androgens (dehydroepiandrosterone) (DHEA)
(precursor of sex hormone)

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G1. T.S. of Testis :-

Comments :-

→ The testes are paired, oval-shaped glands located in the scrotum, external to the body in males.

→ Each testis is covered by a tough, fibrous capsule called "Tunica albuginea".

→ The testis is internally divided into numerous lobules, each containing seminiferous tubule, which are separated by connective tissue.

→ Seminiferous tubules are convoluted tubules with varifying diameter, coiled structures where sperm production (spermatogenesis) occurs.

→ T.S. of section shows tunica albuginea, cells, sperms, seminiferous tubule, and lumen of seminiferous tubule.

→ Interstitial cells (Leydig cells) are found in the spaces between seminiferous tubules and produce testosterone. This hormone is responsible for the development of male secondary sexual character.

→ Blood vessels, lymphatics and nerves supply the testes.

→ Primary function of testes is sperm production through spermatogenesis, which involves the differentiation of spermatogonia into mature spermatozoa.

→ Seminiferous tubules are lined with sertoli cells and contain different stages of developing sperm.

→ Seminiferous tubule is lined by a basement membrane of germinal epithelium. There are several kind of cells :-

Spermatogonia - lie along periphery of tubule

Primary spermatocyte - Largest cell and large nuclei

Secondary spermatocyte - smaller cells with deeply stained nuclei

Spermatid - small cluster of cells

→ Spermatozoa lie in the cavity of tubule.

H. T.S. of Ovary :-

Comments :-

→ The ovaries are paired, almond-shaped organs located in the pelvic cavity one on each side of the uterus in female.

→ The outermost layer is of Peritoneum which has cubical cells.

→ Just beneath peritoneum is germinal epithelium bounded by connective tissue called "tunica albuginea".

→ Germinal epithelium gives rise to oogonia, developing follicles and Graafian follicle.

→ Section shows young follicles and mature Graafian follicles and corpus luteum.

→ The cortex of the ovary contains the ovarian follicles, while the medulla contains blood vessels; nerve and lymphatics.

→ Follicles develop into mature Graafian follicle; which is surrounded by connective tissue or stroma.

→ Graafian follicle contains mature oocyte, which is surrounded by a thick transparent layer called zona pellucida surrounded by another layer corona radiate.

→ Corona radiata is surrounded by mass of cells called "discus proligerous" or "cumulus".

→ Corona radiate is covered by liquor foliculi and then by membrane granulosa.

→ Thick membrane granulosa is covered by thick layer called as theca folliculi.

→ Ovaries produce and secrete female sex hormones (estrogen, progesterone). These hormones regulate the female reproductive system and secondary sexual characteristics.

developmental stages and Metamorphosis

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Experiment no. - 2

* **Aim :-** Study of developmental stages and Metamorphosis in frog using the flow chart.

* **Theory**

Definition of Metamorphosis :-

Metamorphosis is a biological process by which an animal physically develops including birth transformation or hatching involving a conspicuous and relatively abrupt change in the animal's body structure through cell growth and differentiation.

Generally organisms with a larval stage undergo metamorphosis, and during metamorphosis the organism loses larval characteristics.

- Metamorphosis is the series of gradual change that takes place in the larva of frog to transform it into adult frog for terrestrial adaptation. Metamorphosis in frog brought about by the changes in the levels of thyroxine hormone.

Morphological Changes :-

Aquatic existence ⟶ Terrestrial existence.

Urodeles :- First living order of the class - Amphibia.

i. Resorption of the tail fin.
ii. destruction of the external gills.
iii. Change in the skin structure (e.g.- Salamander)

Anurans :- Second living order of the class - Amphibia.

i. More complicated
ii. Every organ is subjected to modification. (e.g.- Frog & Toad)

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- **Larval Structure respond to T_4 and T_3 in four ways :-**

i. Growth of New Structures
ii. Cell death during metamorphosis
iii. Remodelling.
iv. Respecification.

1) Growth of New structure :-

→ The limbs, nictitating membranes and eyelids emerge.
→ 'T_3' induces the proliferation and differentiation of new neurons to serve these organs.
→ Blocking T_3 activity prevents these neurons from forming and causes paralysis of the limbs.
→ Eyes move to the front of the head from their originally lateral position.
→ Ipsilateral pathway (belonging to or occuring on the same side of the body) emerge, enabling input from both eyes to reach the same area of the brain.

2) Cell death during Metamorphosis :-

→ T_3 causes the degeneration of the paddle like tail and the oxygen-procuring gills.
→ First part of tail resorption is caused by suicide, but that the last remnants of the tadpole tail must be killed off.
→ T_3 tells the muscle cells to kill themselves by apoptosis.
→ Later in Metamorphosis, the tail muscles are destroyed by phagocytosis.
→ Larval Red Blood Cells are specifically digested by macrophages in the liver and spleen.

3) Remodelling during Metamorphosis :-

→ Larval intestine is converted into a shorter intestine for a carnivorous diet.
→ Much of the nervous system is remodeled as neurons grow and innervate new targets.
→ The lateral line system of the tadpole degenerates and the ears undergo further differentiation.
→ The middle ear develops, as does the tympanic membrane characteristic of frog and toad outer ears.
→ Tadpoles experience a brief period of deafness as the neurons change targets.
→ The shape of the anuran skull also change significantly.

4) Biochemical Respecification :-

→ T_3 induces a new set of protein in existing cells. Tadpole are ammonotelic (excrete ammonia). However, many adult frog are ureotelic (excrete urea).
→ During Metamorphosis, the liver begins to synthesize the enzymes necessary to create ~~are~~ urea from carbon dioxide and ammonia.
→ T_3 may regulate this change by inducing a set of transcription factors that specifically activates expression of the urea cycles genes while suppressing the genes responsible for ammonia synthesis.
→ Tadpole Haemoglobin is changed into an adult hemoglobin that binds oxygen more slowly and releases it more rapidly than does tadpole haemoglobin.

Hormonal Control of Amphibian Metamorphosis :-

Metamorphic changes are due to :-

i) The secretion of the hormone thyroxine (T_4)

ii) The conversion of T_4 into the more active hormone, Tri-iodothyronine (T_3).

iii) The degradation of T_3 in the target tissues.

* T_3 binds to the nuclear thyroid hormone receptors (TRs). Thus T_3 and TRs are essential in each tissue.
* Concentration of T_3 depends on T_4 and 2 important enzymes. They are :-

a) Type II deiodinase :-

Removes an iodine atom from the outer ring of the precursor hormone (T_4) to convert it into the more active hormone (T_3).

b) Type III deiodinase :-

Removes an iodine atom from the inner ring of T_3 to convert it into an inactive compound that will eventually be metabolized to tyrosine.

Receptor Types :-

They are of 2 types :-

a) TRα

b) TRβ

→ TRα is widely distributed and is present before the organism has a thyroid gland.

→ TRβ is gene product, which is activated by hormone.

- TR binds and forms dimer with RXR. These dimers bind T_3 and effect transcription.
- TR-RXR is associated with gene promoters and enhancers and

repress transcription.

- When T_3 added to this complex, gene activation takes place.

Premetamorphosis :-

During this stage, the thyroid gland has begun to mature and is secreting low levels of T_4. The initiation of T_4 secretion may be brought about by corticotropin releasing hormone (CRH).

CRH may act directly on the frog pituitary, instructing it to release thyroid stimulating hormone (TSH), or it may act generally to make the body cells responsive to low amounts of T_3.

The tissues that respond earliest to the thyroid hormone, are those that express high levels of deiodinase II, and can thereby convert T_4 directly into T_3.

As the thyroid matures to the stage of prometamorphosis, it secretes more thyroid hormones. Many major changes (such as tail resorption), gill resorption and intestinal remodelling must wait until the metamorphic climax stage. The concentration of T_4 rises dramatically and TRβ levels peak inside the cells.

Prometamorphosis :- During prometamorphasis, the rising levels of thyroid hormones induces higher level of TRβ. At metamorphic climax, deiodinase II is expressed and the tail begins to be resorbed. Thus, the tail undergoes absorption only after the legs are functional. The frog brain also undergoes changes during metamorphosis, and one of the brain's functions is to down regulate metamorphosis, once metamorphic climax has been reached.

General Survey of All Endocrine glands in Dissected Rat

Experiment no.-3

* **Aim :-** General Survey / In-situ localization of endocrine glands in model Rat.
* **Principle :-**

Endocrine glands are distributed in different positions inside the organism which can be observed with the help of chart.

Endocrine Glands :-

Endocrine glands are ductless glands, which secretes chemical messenger known as "Hormones", the Hormones act via their specific receptor on the corresponding target tissue and give their physiological response.

- The study of science of structure and function of the endocrine gland and the diagnosis and treatment of disorders of the endocrine system is called endocrinology.
- Secretin was the first hormone discovered by Bayliss and Starling.
- Hormones are non-nutrient chemicals which act as intercellular messengers and produced in trace amounts.
- Neural system and endocrine gland, together called as Neuro-endocrine gland.
- The affective regulation of body function, control and co-ordinated not only by the neural system but also by the chemicals of endocrine system.
- Nerve fibres do not innervel into the cell but, cellular activities must be controlled for this purpose a special kind of system has developed that is endocrine system.

These are following endocrine glands :-

1) Pituitary Gland :-

Pituitary gland also called Hypophysis, it is situated beneath the cranium. It constitutes 2 major parts :-

Pituitary Gland

(Adenohypophysis) Anterior Pituitary	(Neurohypophysis) Posterior Pituitary
Pars distalis	Pars Nervosa
Pars tuberalis	Median Eminence
Pars intermedia	

* Seven Tropic Hormones from Anterior pituitary :-

i) Human Growth Hormone (GH) / Somatotropin (STH)
ii) Thyroid Stimulating Hormone (TSH)
iii) Adrenocorticotropic Hormone (ACTH)
iv) Follicle Stimulating Hormone (FSH)
v) Prolactin (PRL)
vi) Melanocyte Stimulating Hormone (MSH).
vii) Leuiteinizing Hormone (LH)

* Two hormones which are only stored in posterioor pituitary are :-

i) Oxytocin (OT) → Ejection of Milk.
ii) Vasopressin (ADH) → (Vasoconstrictor) → H_2O absorption.

2) Thyroid Gland :-

Thyroid gland is situated in the neck region, anterior to the larynx, two lobes, situated either side to the trachea. Two lobes are connected by "Isthmus". It synthesises and stores two hormones

T_3 and T_4 that help regulate the heart rate, blood pressure, body temperature, and at the rate at which food is converted into energy.

2 types of Cells are present :-

a) C-cells / Parafollicular Cells → Thyrocalcitonin Hormone.
b) Cuboidal Epithelium Cells → T_3 / T_4 Hormone.

3) Parathyroid Gland :-

Two pairs of parathyroid gland situated posterior to the thyroid lobes.
Each thyroid lobe consists of 2 parathyroid gland, produces hormone called, parathyroid hormone (PTH) / Collip's Hormone.
Significant role in the calcium balance in the body.

4) Thymus Gland :-

It is a lobular structure, located below the sternum and above the heart and aorta. It produces a group of peptide hormones called Thymosin, which strengthening the immune system. It regress after attainment of adult stage. Thymosin is necessary for T-cell development and production.

5) Adrenal Gland :-

One pair of adrenal gland are located at the top of superior kidney. So it is also called supra-opt renal gland.

Adrenal Cortex

Cortex (Outer)
- Zona Glomerulosa
- Zona Fasciculata
- Zona reticularis

Medulla (Inner)
- Epinephrine
- Norepinephrine

} (QF) Hormone.

- Mineralocorticoids → Aldosterone
- Glucocorticoids → Cortisol
- Sex Corticoids → Male → Androgen
  Female → Estrogen.

6) Pancreas :-

It is compound gland / mixed gland because it contains both parts :→

a) Exocrine → 98% → Pancreatic acini → Pancreatic Juice.
b) Endocrine → 2% → Islets of Langerhans → Hormone.

α cells → Glucagon → 17%.
β cells → Insulin → 71%.
δ cells → Somatostatin
F-cells → Pancreatic polypeptide } 12%.

7) Gonads :-

a) Testis (Male Reproductive Organ) :-

Male Gametes → Sperms
Sex hormone → Androgens } Cytogenic gland → Living cells as their secretion.

→ Situated in the scrotal sac (outside abdomen) to maintain temperature less than 2-2.5°C from normal body temperature.
→ Development of secondary sexual characters, stimulates spermatogenesis., stimulate CNS to maintain Male behaviour called [Libido / Sex drive / sexual urge]

b) Ovary (Female Reproductive Organ) :-

Female Gametes → Ova
Female Hormone → Estrogen and Progesterone } cytogenic gland.
→ ovary produces one ovum in each menstrual cycle from

the Giraafian Follicle.
→ Remnants of Giraafian follicles convert into Corpus luteum, which produces progesterone.
→ Estrogen is released by developing ovarian follicles.
→ In the absence of Fertilisation, corpus luteum degenerates into corpus albicans (white body).

8) Pineal Gland :-
It is extended from dorsal side of epithalamus.
It produces a hormone called Melatonin.
- Melatonin controls certain activity :-

a) Metabolism
b) Pigmentation
c) Body Temperature
d) Menstrual cycle
e) defense capability.
f) Sleep-wake cycle.

Primer Designing

Experiment no.-4

* Aim :- To study the process of designing of primers of any Hormone.

PCR (Polymerase Chain Reaction) :-

Aim :- Amplification of gene of interest.

Principle :- The double stranded DNA of interest is denatured to separate into 2 individual strands. Each strand is allowed to hybridise with a primer. The primer template duplex is used for DNA synthesis. Denaturation, Annealing and Extension are repeated again and again to generate multiple forms of target DNA.

Apparatus :- Thermocycler (High temperature is maintained), Deoxyribonucleotides, two sets of DNA primers, Taq-DNA polymerase (Taq - Thermus aequaticus), (RNA Primer - Oligo-nucleotide).

Process :- It includes the following three steps :-

i) Denaturation :-

At 94°C, the 2 template strands of gene of interest are separated at high temperature.

ii) Annealing :-

DNA primers are attached at 3'-end of the template. The Annealing step allows the hybridisation of the two oligonucleotide primers, which are present in excess to bind to their comple-mentary sites that flank the target DNA (Temperature - 50-60°C)

iii) Extension / Elongation :-

At 72°C, polymerisation of Deoxyribonucleotide is brought about by the Taq polymerase.

Requirement for PCR in Brief :-

i) DNA Template → DNA segment to be amplified.
ii) Taq. Polymerase → An enzyme to synthesize DNA copies obtained from Thermus acquaticus.
iii) Deoxynucleotide tri Phosphate (dNTPs) → Building Blocks of new DNA template.
iv) Primers → A short segment of DNA (forward and Reverse).
v) Buffer Solution → A suitable Chemical environment.
vi) Divalent cation → Mg^{2+} ions (act as cofactor to enhance the DNA polymerase activity.
vii) Monovalent ion → K^+ ions (Neutralize the charge present in DNA Backbone).
viii) PCR Machine → Thermal Cycler.

Note :-

→ PCR was invented by Kary Mullis (American biochemist) in 1983 at Cetus corporation.
→ PCR is one of the widely used amplification techniques due to its high sensitivity and good reproductivity / reproducibility.
→ Each cycle doubles the copy number of the amplified gene: ten cycles ideally produces 2 → 4 → 8 → 16 → 32 → 64 → 128 → 256 → 512 → 1026 → .... (2^{10}) copies. Thus, 30 cycle yields a ($2^{10\times3}$) = @ 10^9 fold amplification.

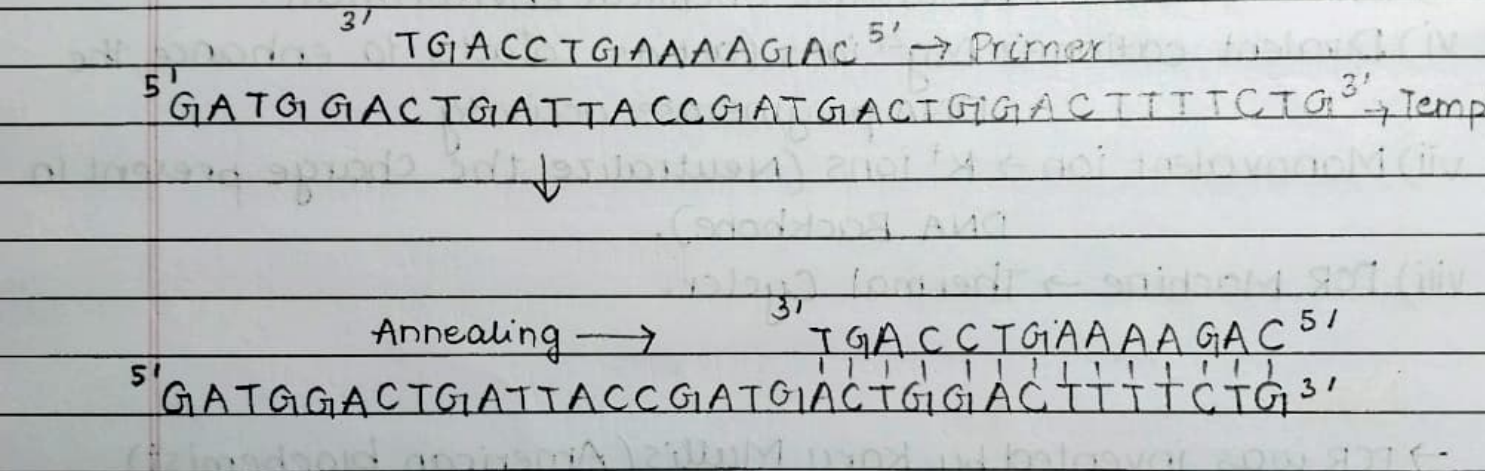
* Types of PCR :-

i) Multiplier PCR
ii) Long range PCR
iii) Single Cell PCR
iv) Fast Cycling PCR
v) Methylation Specific PCR (MSP)
vi) Digital PCR
vii) Hot start PCR
viii) High Fidelity PCR
ix) "RAPD" - Rapid Amplified Polymorphic DNA analysis
x) Insitu PCR.

Primer Designing :-

Definition :- A primer is a short synthetic oligonucleotide which is used in many molecular techniques from PCR to DNA sequencing.

These primers are designed to have a sequence which is the reverse compliment of a template or target DNA to which we wish the primer to anneal.



General Rules of Primer Designing :-

i) **Primer length :-**

Primers should be 18-24 bases in length.

- If too short then → less specificity.
- If too long then → low temperature template binding affinity and higher probability of formation of secondary structure.

ii) **Base composition :-**

G-C content should be 50-60% (G+C), because, there are 3 hydrogen bond present between G and C, provide good strength, then A+T, because between A and T, it has only 2 Hydrogen Bonds.

iii) Maximum 3'-end stability:-
Primers should end (3') in a G or C, or CG or GC, Prevent breathing of ends increases efficiency of priming.

iv) Melting Temperature (Tm):-
Temperature of which 50% of DNA duplex dissociates to become single stranded.

- Determination of optimal PCR annealing Temperature (Ta).

* Ta should be 5°C below than Tm.
* Tm between 52-60°C are preferred. So Ta will be between 47-55°C (5°C less than Tm)

Right Melting and annealing temperature, Appropriate hybridization stability.

v) Walace Rule:-
A rudimentary method to calculate the melting temperature of DNA is an equation known as the Walace Rule.
The eqn is:-

$$Tm = 4(G+C) + 2(A+T)$$

What to Avoid during Primer Designing:-

i) 3'ends of primers should not be complimentary (ie base pair) as otherwise primer dimer will be synthesized preferentially to any other product.

ii) Primer self complementarity (ability to form 2° structures such as hair pins) should be avoided.

iii) Runs of 3 or more Cs or Gs at the 3'ends of primers may promote mispriming at G or C with sequences (because of stability of annealing) and should be avoided.

Steps of Primer designing through Online Website :-

Google/Chrome (Search any box)

↓

Search - NCBI (National Centre for Biotechnology Information)

↓

Click on http://www.ncbi.nlm.nih.gov.

↓

Click on → All Database → Select → Nucleotide

Search any option → Any Hormone, Suppose we select Oxytocin Hormone → then search.

↓

Apply Filter → Homo sapiens

↓

Select on First option → Human oxytocin mRNA, complete cds (439 bp linear mRNA).

↓

We get complete information about that particular hormone, like Source organism, Author name, Title, Journal etc.

↓

Analyze the sequence → Click on → Pick Primers.

↓

Primer for target on one template

i) PCR Template → FASTA Sequence → M25650.1

ii) Primer Parameter :-

PCR Product Size → min^m - 70

max^m - 10,000 - change it to 200

Tm → min^m - 59, optimum - 60, max^m - 65

Max^m Tm difference → 3

iii) Exon / Intron Selection →

Keep all data as it is

Intron length range :- Min^m - 200, Max^m - 10,000.

iv) Primer Pair specificity checking parameters :-
Keep All data as it is.

↓

Select on ☐ show result in a new window.

↓

Get Primer

↓

This process takes time from minutes to several hours.

↓

Select on → 1st option : Homo sapiens Oxytocin/neurophysin 1 prepropeptide (OXT), mRNA → Submit.

↓

Wait for few minutes.

↓

We get → Graphical view of primer pairs.
And then, 10 primer pairs (detailed primer repeat).

↓

Choose according to your preference.

ELISA

Experiment - 5

Aim :- To study Elisa based Immuno hormone assay.
Estimation of plasma level of any hormone using ELISA.

Introduction :-

ELISA (Enzyme linked Immuno Sorbant Assay) is a plate based assay technique, which is used for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. An enzyme conjugated with an antibody reacts with colourless substrate to generate a coloured product. Such substrate is called chromogenic substrate A number of enzymes have been used for ELISA such as alkaline phosphatase, horse radish peroxidase and β-galactosidase.

Principle :-

- "ELISA" are typically performed in 96-well polystyrene plates.
- The serum is incubated in a well, each well contains a different serum.
- A positive control Serum and a negative control serum would be included among the 96 samples being tested antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface.
- After sometime, the plate is washed to remove serum and unbound antibodies or antigen with a series of wash buffer.
- To detect the bound antibodies or antigens, a secondary antibody that attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well.

- After an incubation period, the unbound secondary antibodies are washed off. When a suitable substrate is added, the enzyme reacts with it to produce a colour.
- This colour produced is measurable as a function or quantity of antigens or antibodies present in the given sample. The intensity of colour/optical density is measured at 450 nm.
- The intensity of the colour gives an indication of the amount of antigen or antibody.

* ELISA can be divided into two types :-

A. Quantitative ELISA :- It reflects the concentration of the target muscle in a sample.

B. Qualitative ELISA :- It provides a simple positive or negative result for a sample.

Four Main Types of "ELISA" :-

i) Direct ELISA :-

→ Simplest ELISA technique
→ The antigen in the sample is first immobilized to the wall of the wells of a microtitre plate.
→ The wells are then washed off thoroughly, leaving only the absorbed antigen.
→ An enzyme linked antibody, complementary to the antigen of interest, is then added to the wells, where it binds to the antigen.
→ The well is again washed. This leaves a bound antigen-antibody complex on the surface of well.
→ A substrate is then added, which will be converted by the enzyme-linked with antibodies into a detectable product.
→ This method is quicker and simpler than the other ELISA methods.

ii) Indirect ELISA :-

- Antibody can be detected or quantitatively determined by Indirect ELISA.
- A complementary antibody (primary antibody) is ~~washed~~ away the presence of antibody bound to the antigen de then added, which binds to the antigen forming a complex.
- After any free primary antibody is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme linked secondary antibody, which binds to the primary antibody.
- And free secondary antibody then is washed away and a substrate for the enzyme is added.
- The amount of coloured reaction products that form is measured by specialized spectrophotometric plate readers which can measure the absorbance of all of the wall.

The indirect ELISA is used to detect the presence of antibody against HIV.

iii) Sandwich ELISA :-

- An antigen can be detected or measured by a sandwich ELISA.
- In this, the antibody (rather than the antigen) is immobilized on a microtiter well.
- The sample containing antigen is added to the well, which binds to the antibody.
- Finally, a second different antibody to the antigen is added.
- This antibody is enzyme linked.
- After any free second antibody is removed by washing substrate (s) for enzyme linked with antibody is added and the coloured reaction is product (p) is measured.

The extent of reaction is directly proportional to the amount of antigen present.

iv) Competitive ELISA :-

→ This is perhaps the most complex of all ELISA types.
→ It involves the use of inhibitor antigen, so competitive ELISA is also known as inhibitor ELISA.
→ In competitive ELISA, the inhibitor antigen and the antigen of interest compete for binding to the primary antibody i.e. two antigens to compete with each other for binding to antibodies.
→ The unlabeled primary antibody is first incubated with the sample containing antigen of interest, leading to the formation of antigen antibody complex.
→ Since the antibody is excessive compared with the antigen, So there are free antibodies left.
→ The antigen antibody mixture is added to the plate coated with inhibitor antigen that can also bind to the primary antibody.
→ The free antibody in the mixture binds to the inhibitor antigen on the plate, while the antigen-antibody complexes in the mixture do not react and are therefore washed away.
→ The enzyme labeled secondary antibody is added to the plate and binds to the primary antibody bound to the inhibitor antigen on the plate.
→ Finally, a substrate is added to react with the enzyme and emit a visible signal for detection.

Preparation of Vaginal smear

Experiment No.- 06

* **Aim :-** Preparation of vaginal smears in Rat/Mouse.

* **Introduction :-**

The Gamete production in female vertebrates is cyclic and in most animals take place during seasons which are most favourable for the survival of their offspring. In mammals, other than primates, the reproductive cycle is known as estrous cycle.

Rats and mice have an estrous cycle of 4 to 5 days and the cycle is divided roughly into four stages, Estrous, Metaestrous, Diestrous and Proestrous.

* **Requirement :-**

Female Rat / Mice, Physiological Saline, Cotton swab, Giemsa stain, Microslides, Coverslips, DPX mount.

* **Procedure :-**

(i) The Rat / mice was anesthetised.
(ii) As soon as they were immobilised, they were loged on the dissection tray with the ventral side up.
(iii) A cotton swab was used (preferably on ear cleaning cotton bud was used) moistened with physiological saline.
(iv) It was inserted into the vagina of animal and rolled gently. The cells adhering to the lining of the vaginal wall will stick to the moist cotton swab.
(v) Cotton swab was removed and smear it on a clean slide.
(vi) The slide was drived by waxing in air for a few minutes.
(vii) Left the preparation in a petridish and a small quantity/amount of Giemsa stain was added to smear.
(viii) It was covered and left for 10 minutes.

ix) The slide was washed in distilled water and left for air dried.
x) Mounting of the slide was done with a cover slip using DPX mountant.

* Comments :-
In this experiment one will able to :-
i) Preparation of vaginal smear from rat or mice.
ii) Identifying the stages of the estrous cycle in the animal provided.

A. Proestrous :-
→ This is associated with commencement of enhanced ovarian activity under the influence of follicle stimulating hormone (FSH) from adenohypophysis.
→ This phase is analogous to proliferation phase of the menstrual cycle in primates.
→ The vaginal smear is determined by nucleated epithelial cell, which may occur single or in sheath. During this phase the ovaries become active and shows mature follicle cells and the uterus collects the fluids and it becomes contractile. It lasts for about 12 hours. It preceeds the next estrous.
→ Degeneration of old corpora lutea continues but new follicle mature rapidly.

B. Estrous :-
→ This is the period of heat during which ovulation occurs.
→ This period lasts for 9-15 hours under the influence of FSH and estrogen.
→ The female is receptive to the male only during this period, therefore ovulation and fertilization are well coordinated.
→ The uterus becomes enlarged and the vaginal mucosa proliferates

and the vaginal epithelium becomes squamous cornified.
→ A vaginal smear taken during this period shows squamous cells, indicates estrous phase.

C. Metestrous :-
→ In absence of copulation, this stage occurs shortly after ovulation and lasts for 10-14 hours.
→ A small corpus luteum is formed and some progesterone is secreted. A vaginal smear taken at this stage shows leukocytes with some cornified cells.
→ During this stage, the formation of corpus luteum may be observed in the ovary and the uterus diminishes in vascularity and contractivity.

D. Diestrous :-
→ Degeneration of old corpora lutea continues and new follicles formation started from this phase.
→ This stage lasts for 60-70 hours.
→ The corpora lutea regresses during this period and vaginal smear contains only leukocytes.

Observation :-
Observe under the microscope and Identify stages of estrous cycle on the slide.

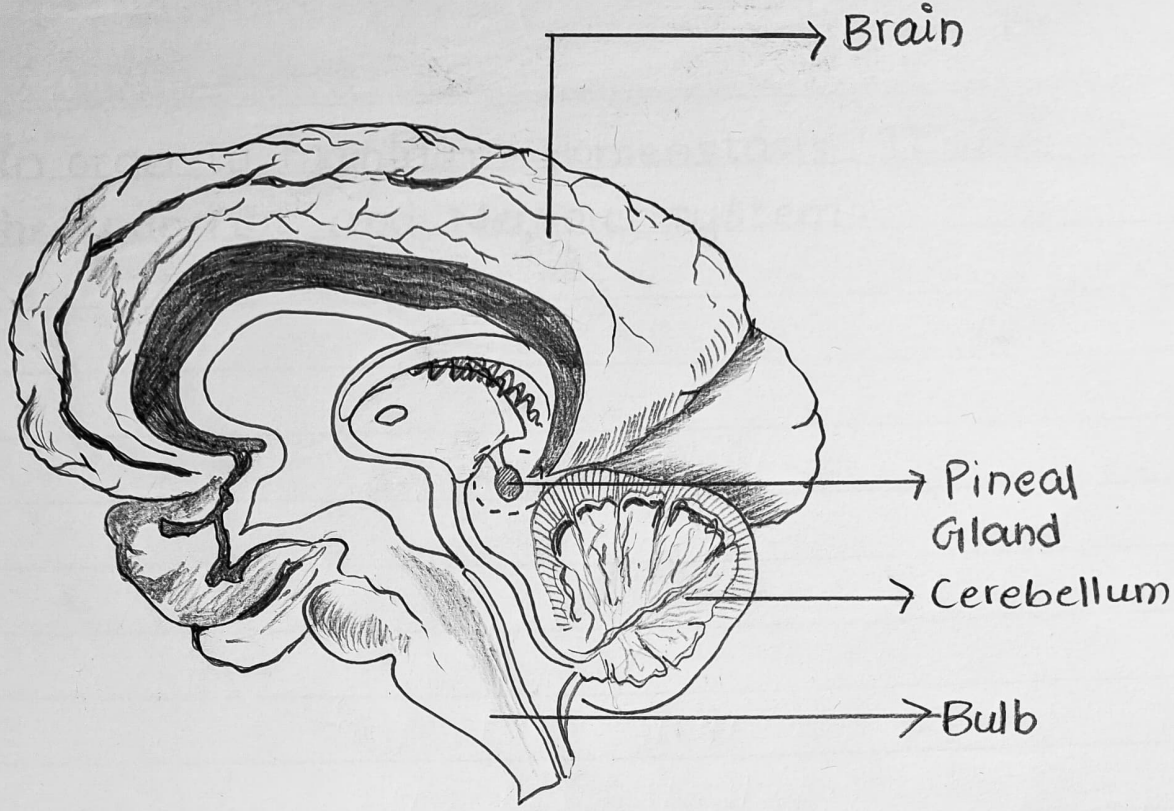
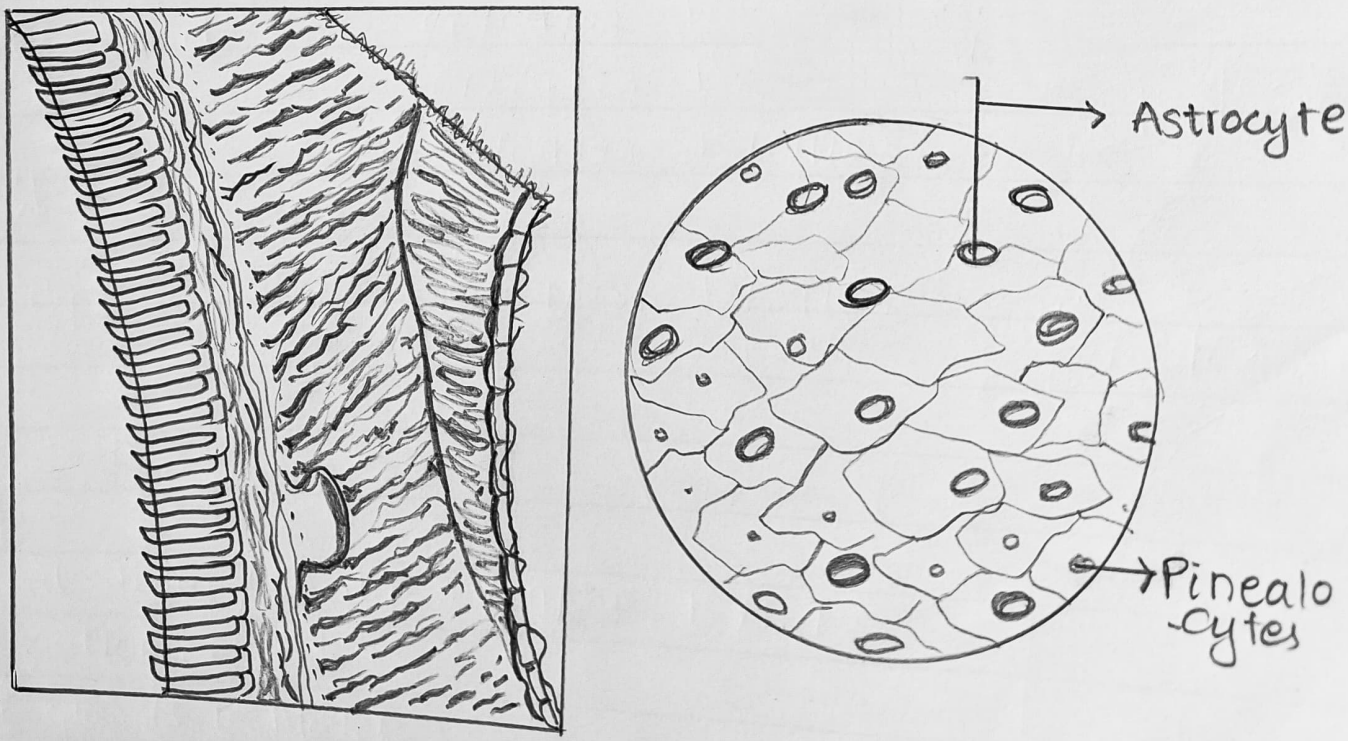
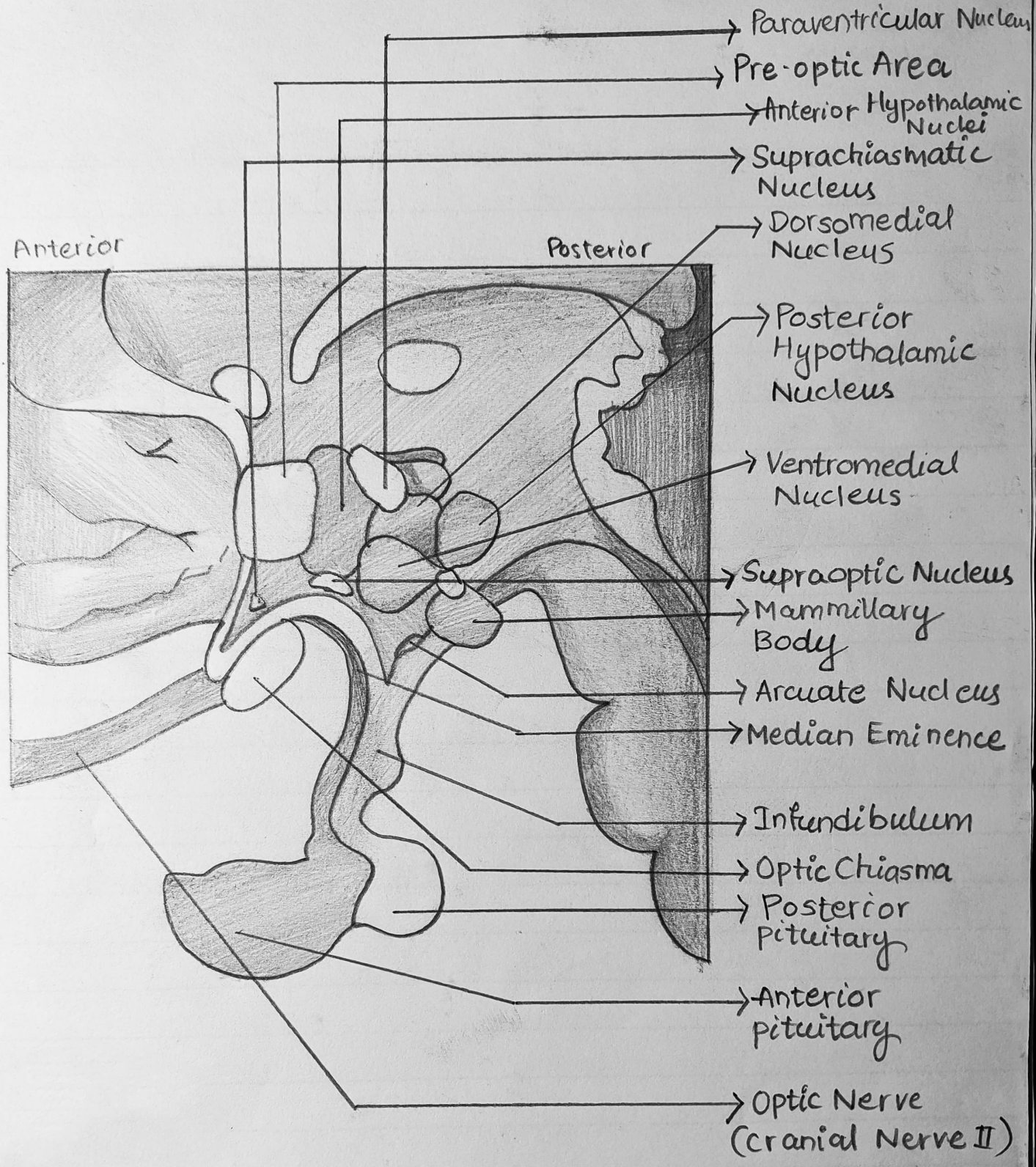


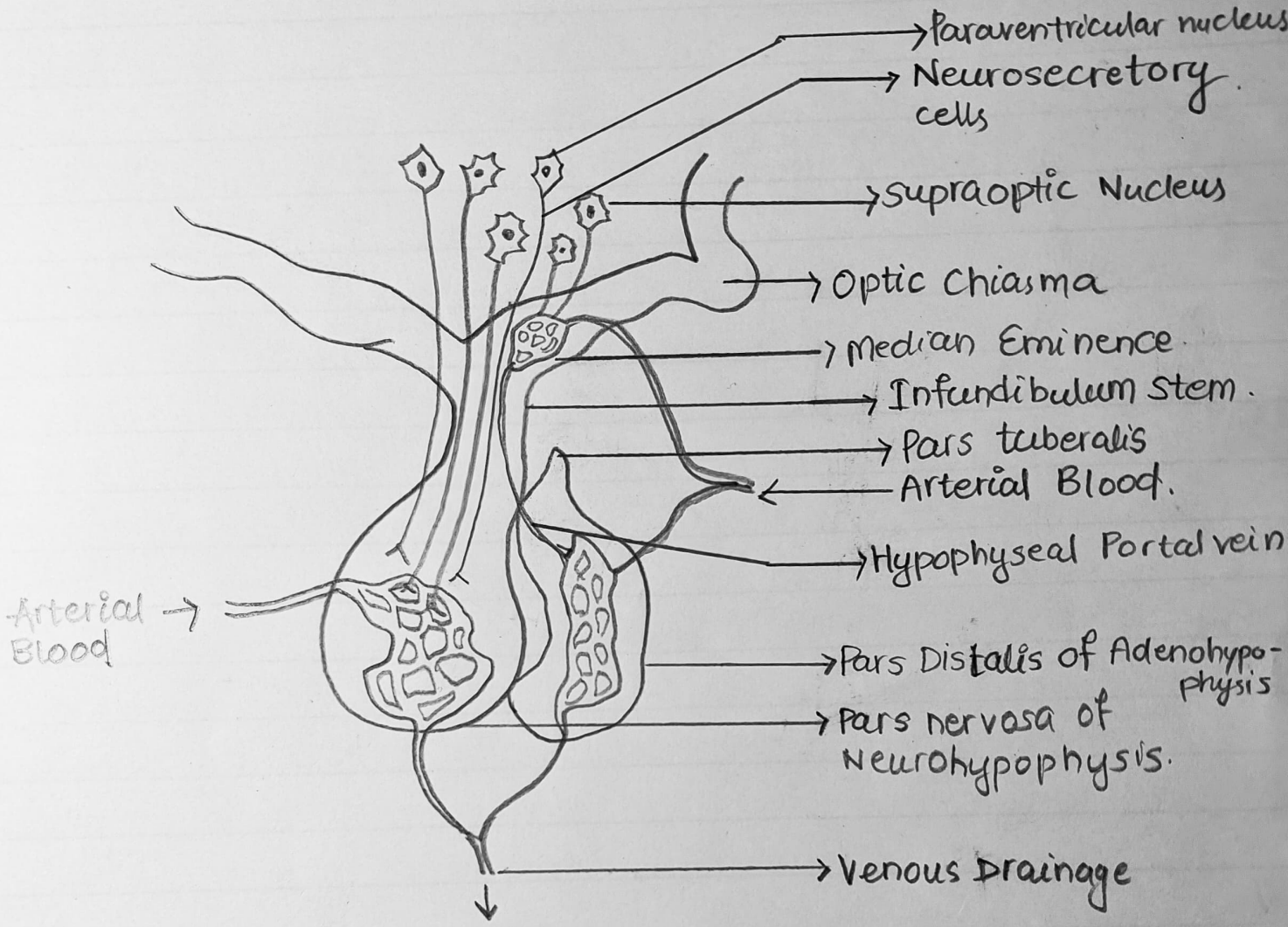
Fig:- Mammalian Pineal Gland



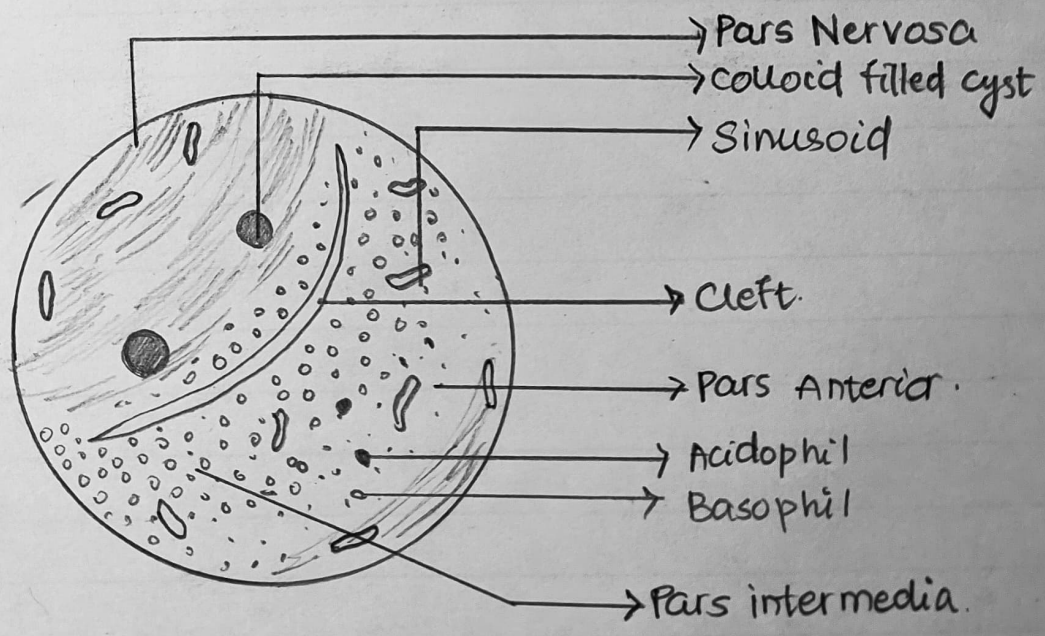
[Fig:- T.S of Pineal Gland]



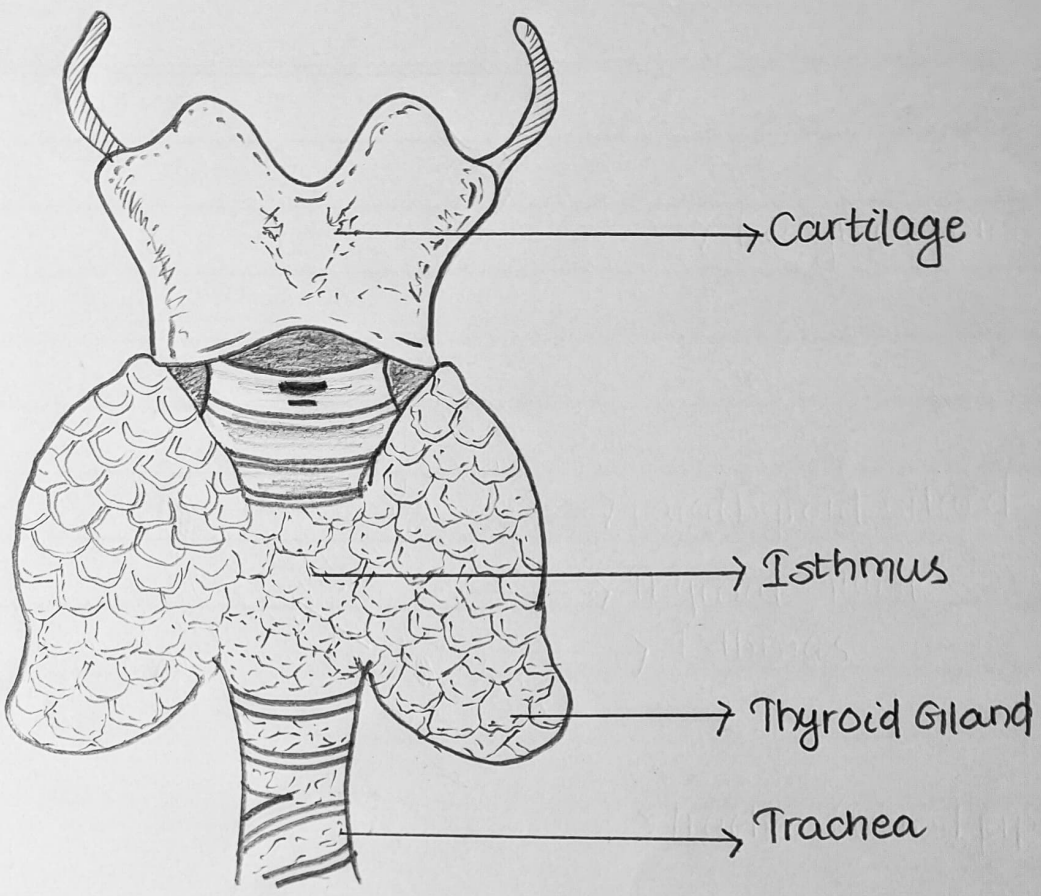
[Fig:- Mammalian Hypothalamus]



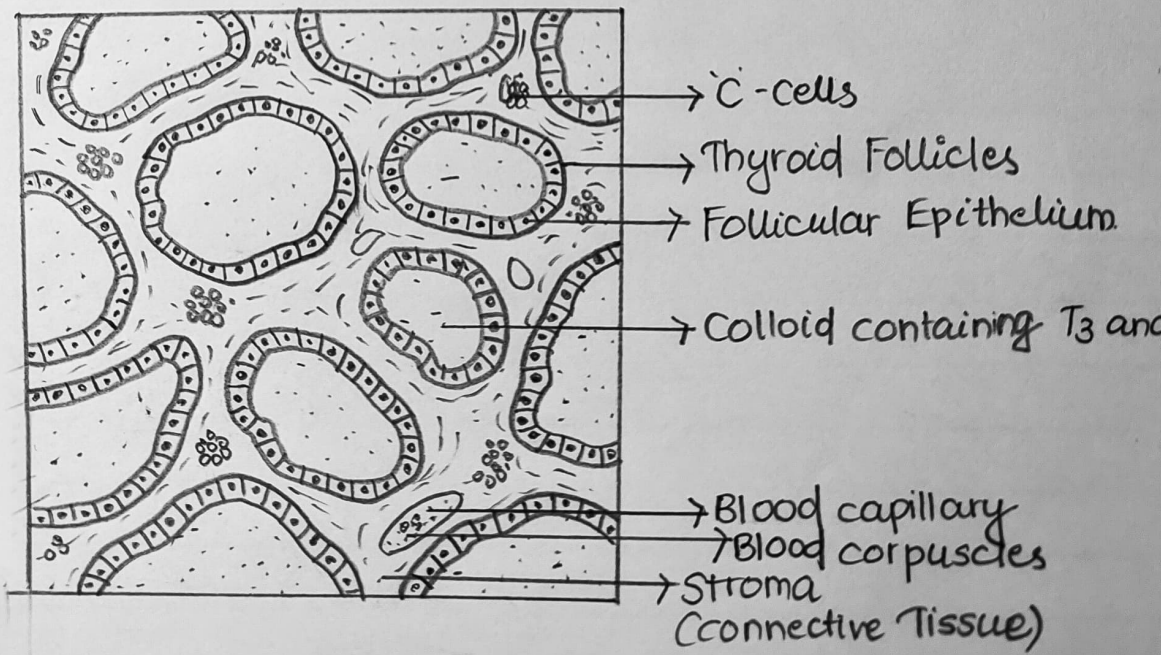
[Fig :- Mammalian Pituitary Gland]



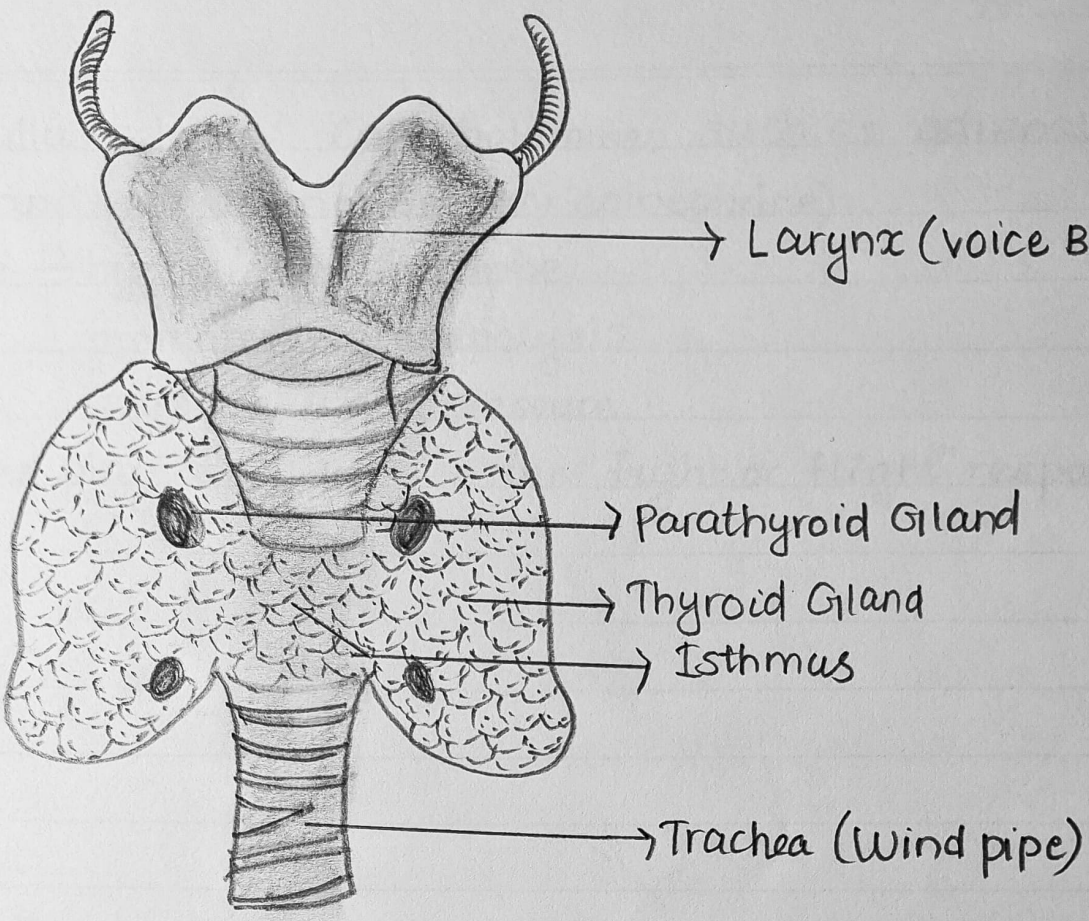
[Fig :- T.S of Pituitary Gland]



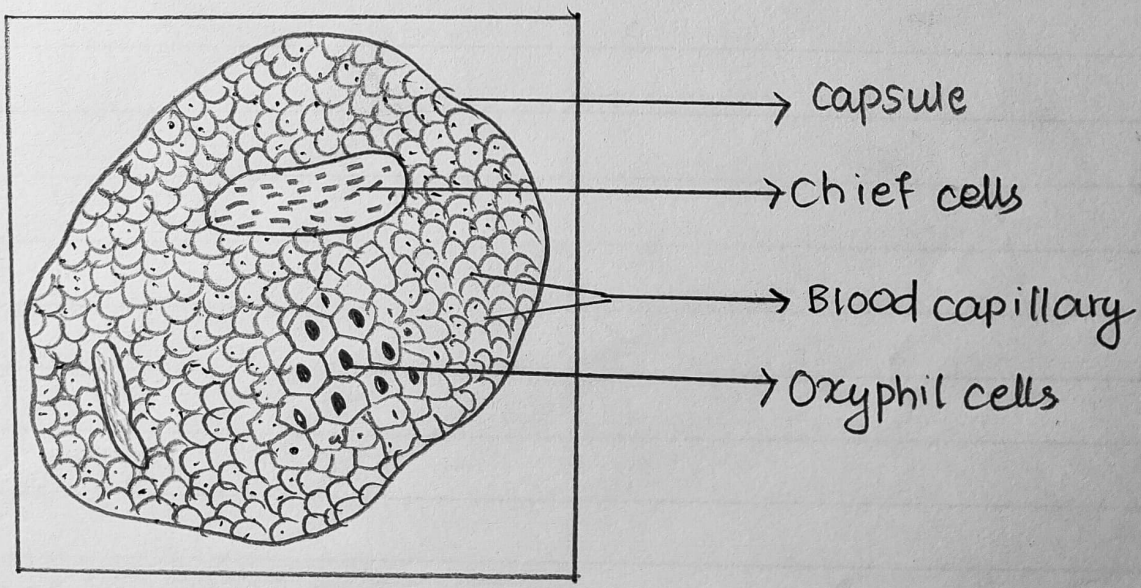
[Fig:- Human Thyroid Gland]



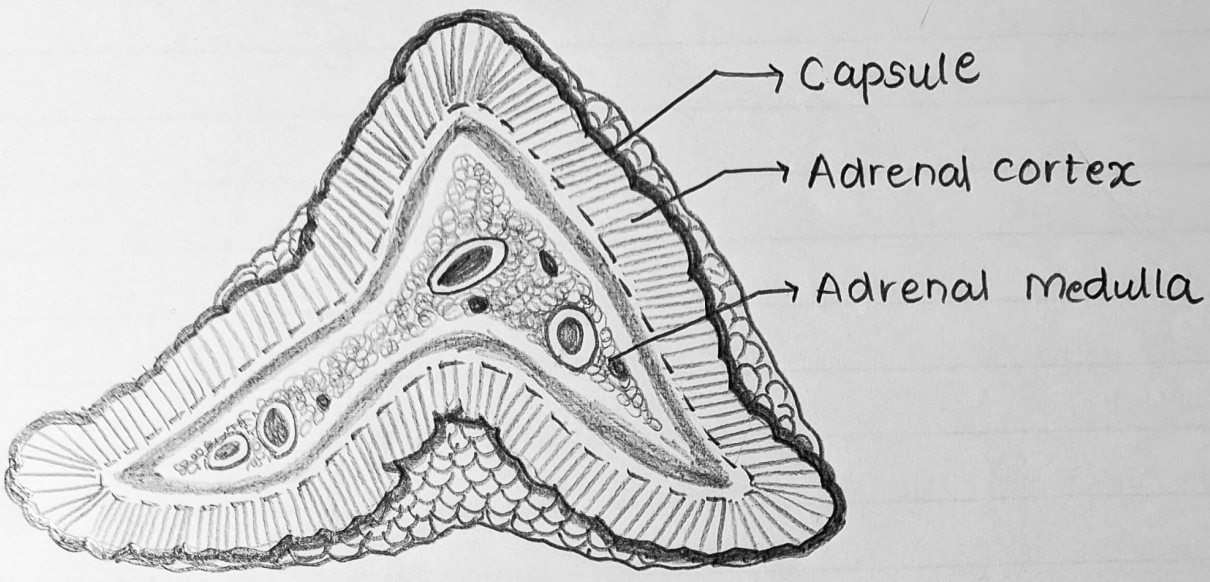
[Fig:- T.S. of Thyroid Gland]

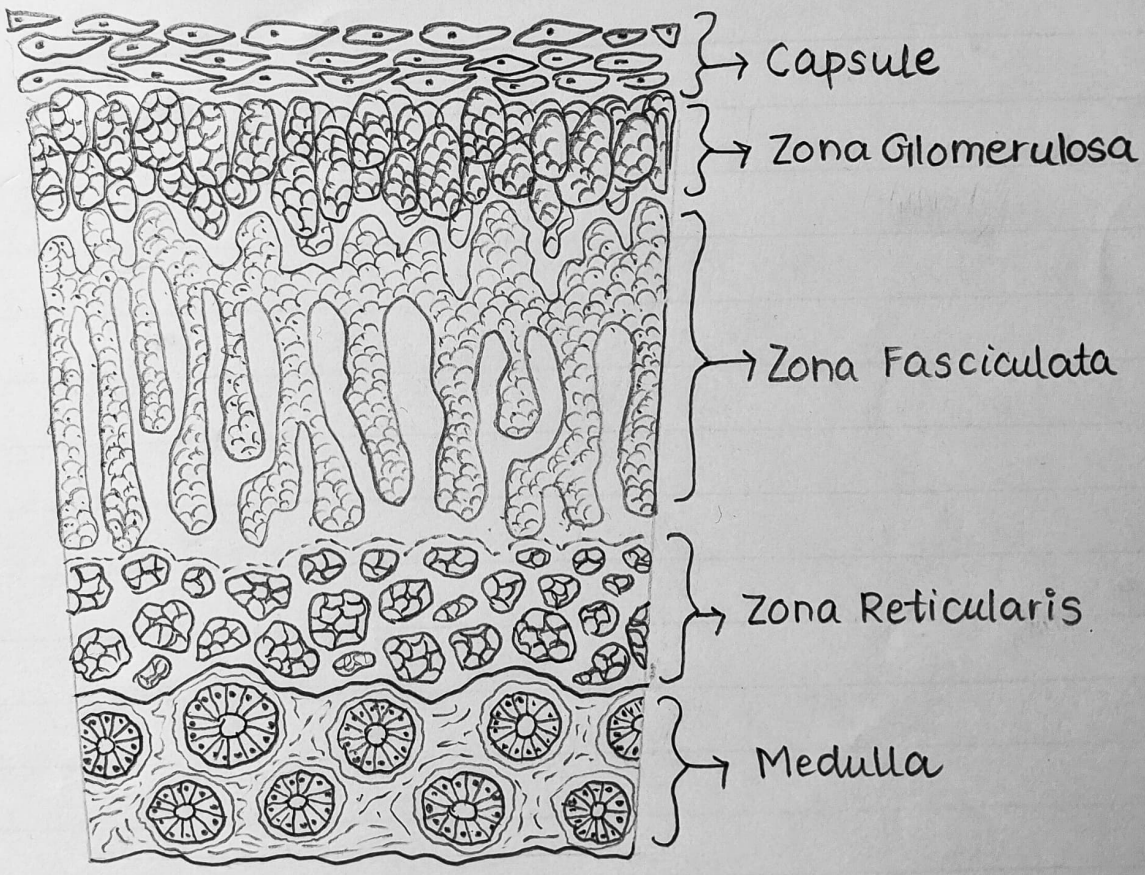


[Mammals Parathyroid Glands]

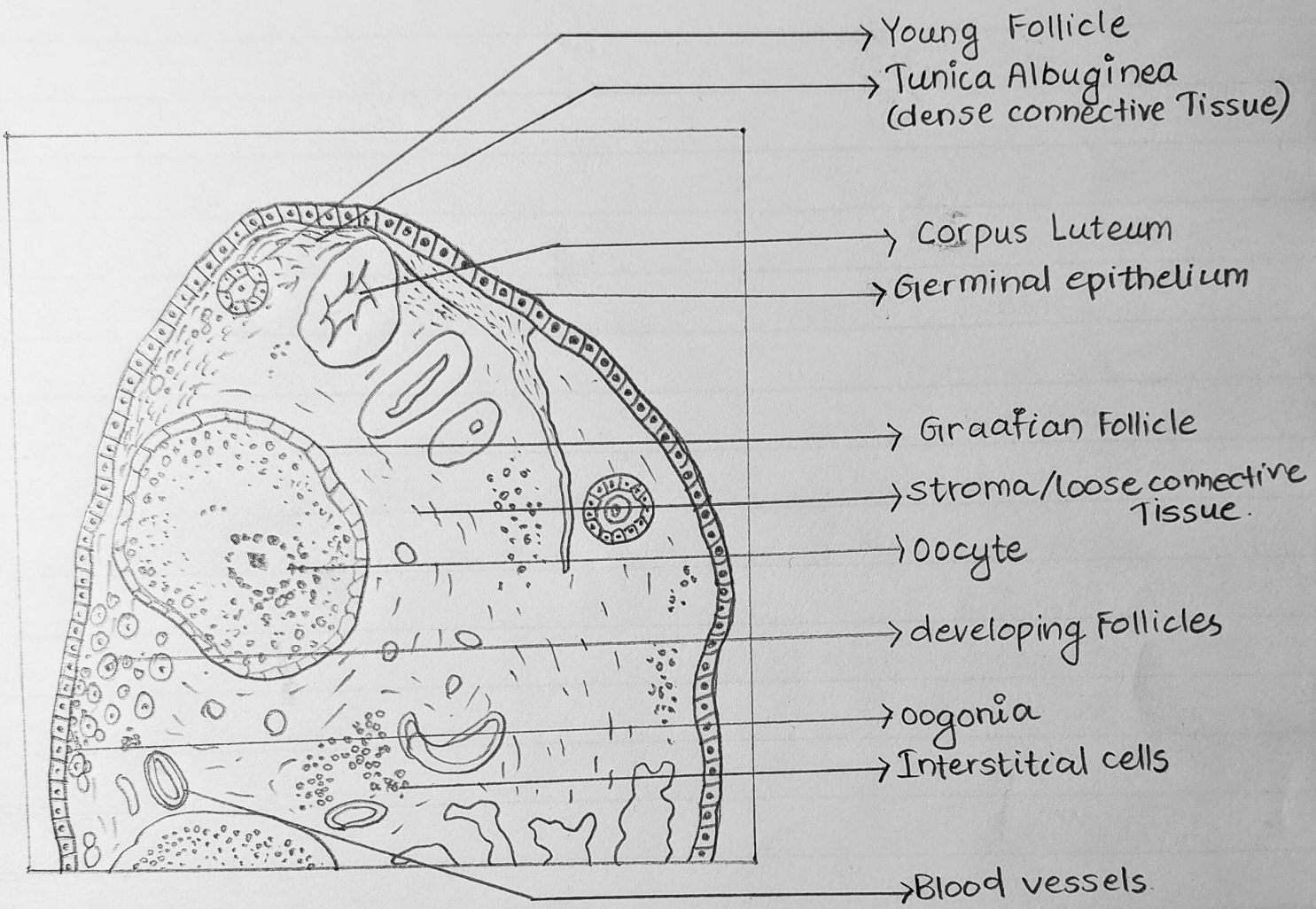


[T.S. of Parathyroid Gland]

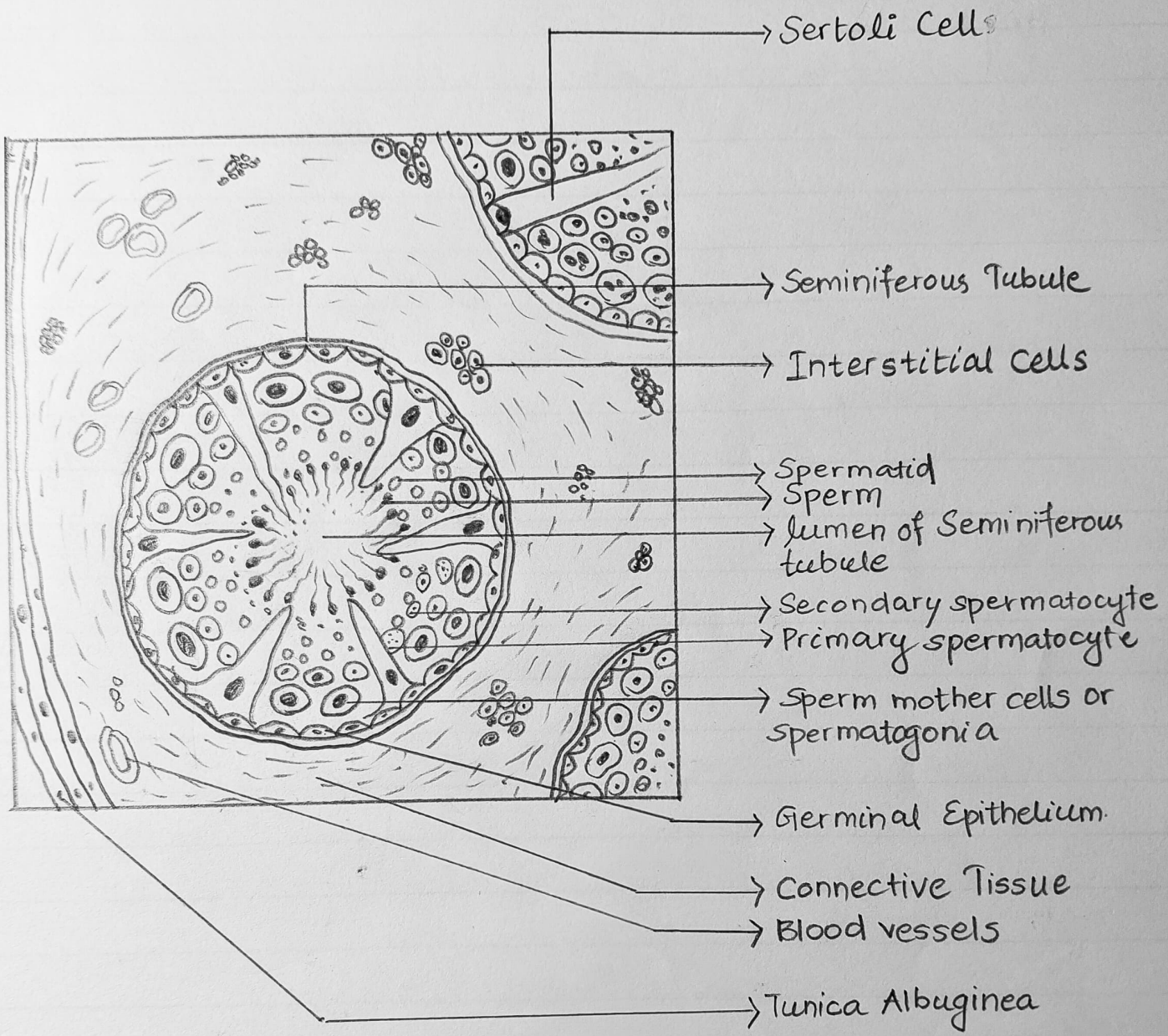




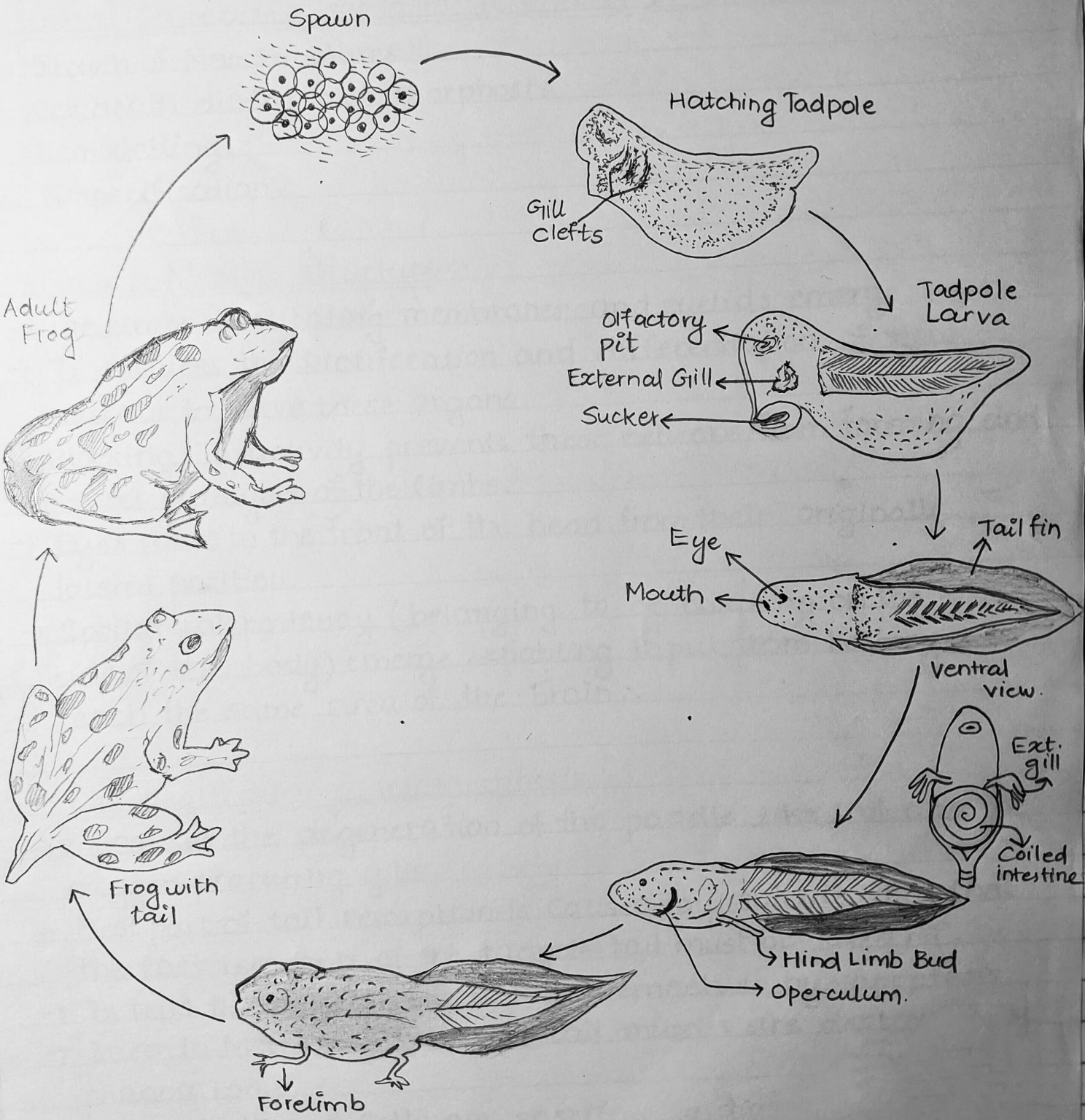
[Fig:- T.S. Section of Adrenal Gland]



[Fig:- T.S. of Ovary]



[Fig:- T.S. of Testis in Mammal]



[Fig:- Life cycle and Metamorphosis of Frog]

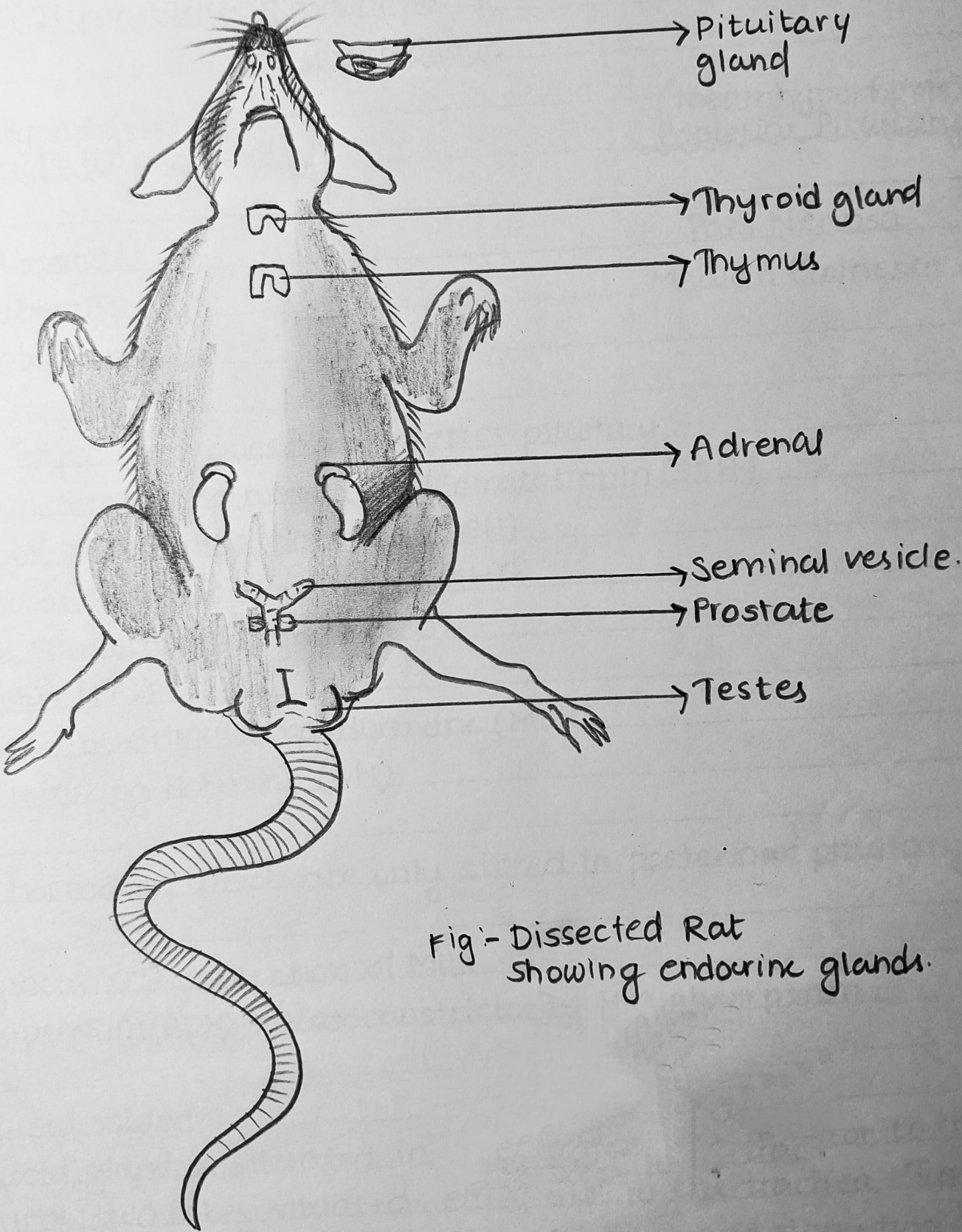
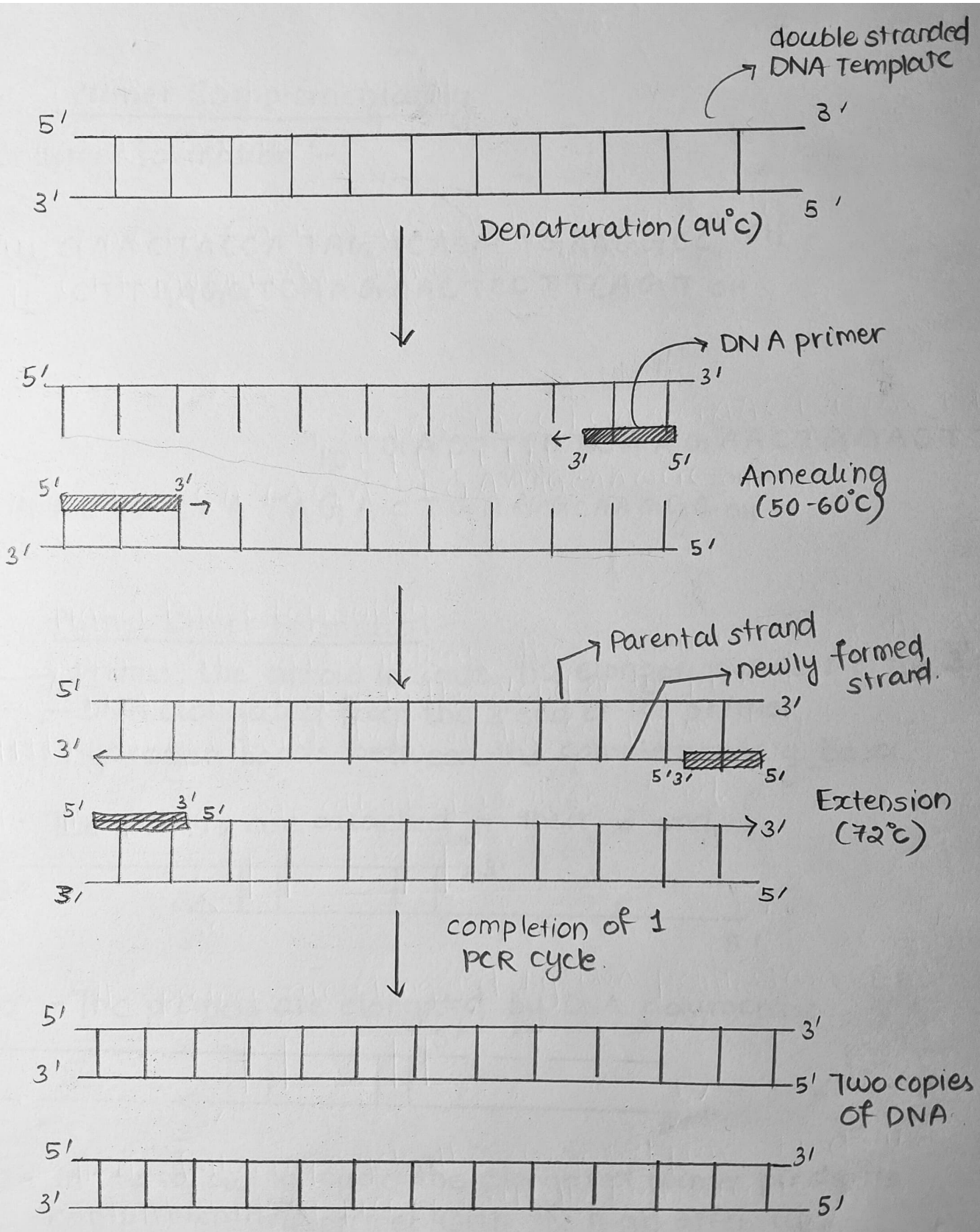


Fig:- Dissected Rat showing endocrine glands.



[Fig:- Steps involved in PCR]

Primer Complementarity

- Primer dimer Formation :-

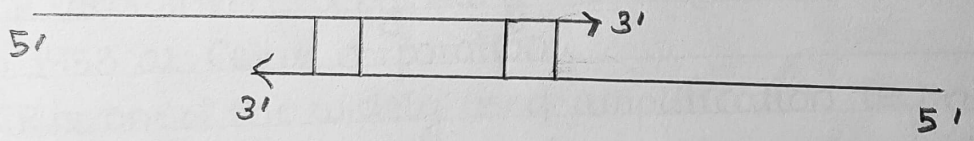
U. GAACTACCA TAGACACACTGAAGGGCC 3' OH
L. CTTGAGGTCAAGAACTCCTTCAGT OH

HO TGACTTCCTCAAGAACTGGACTTC
GAACTACCA TAGACTGAAGGCAAGGC OH

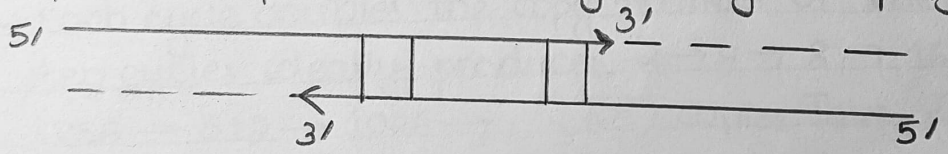
Primer Dimer Formation :-

→ Primer, the arrow indicates the elongation side i.e. the 3'end.
— — DNA elongated from the 3'end or the primer.
IIIII Hydrogen bonds between the complementary bases.

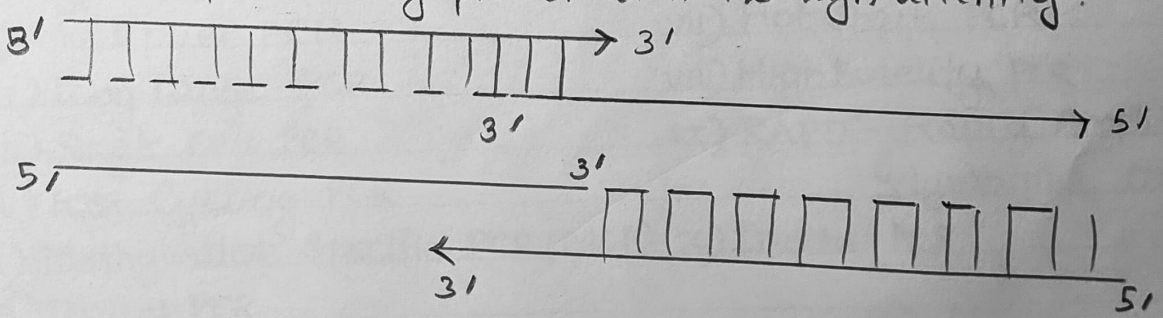
Step1 :- The primers are attached in their 3'-end.



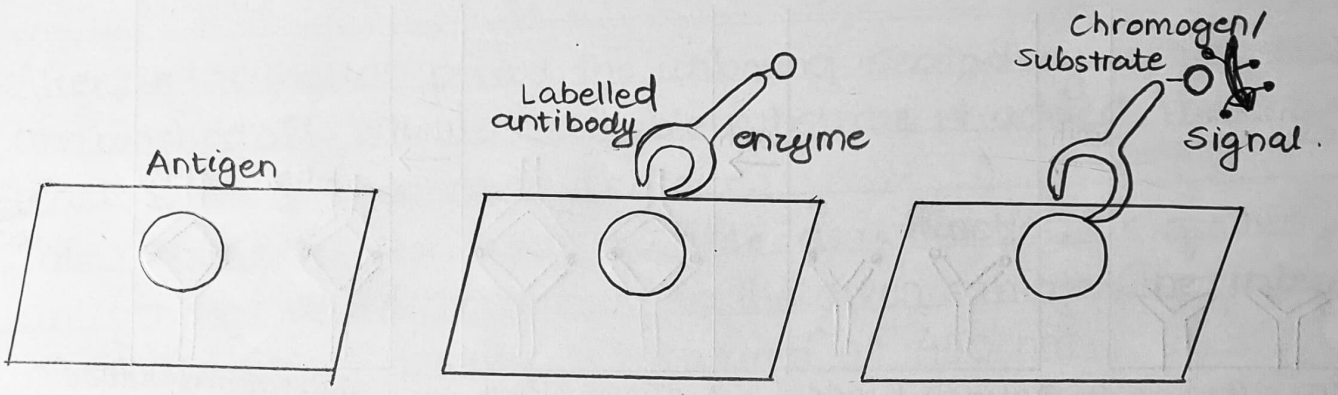
step 2 :- The primers are elongated by DNA polymerase.



step 3- In the following cycle the elongated primer binds its complementary primer with the high affinity.



i) Direct ELISA :-

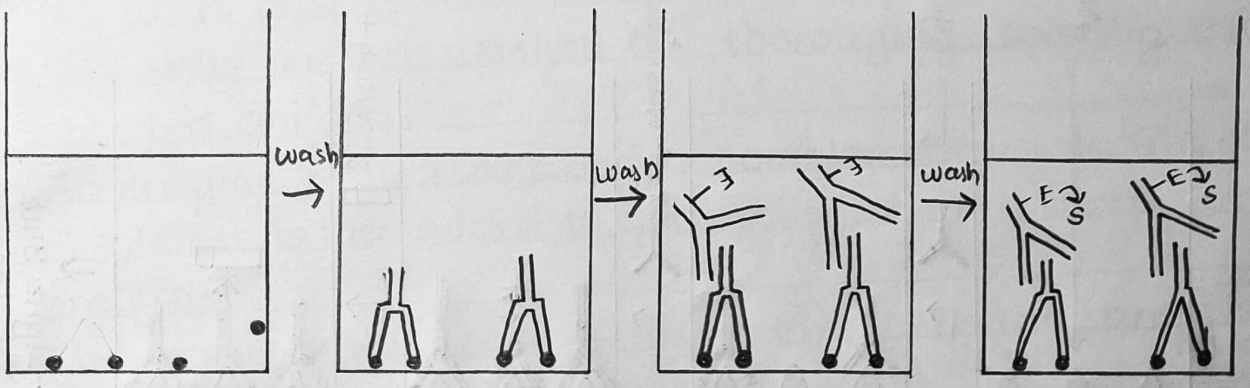


1. Antigen is coated by passive adsorption.

2. Antibody conjugated with enzyme is added and incubated with antigen and incubation.

3. Substrate / Chromophore is added and colour develops.

ii) Indirect ELISA :-



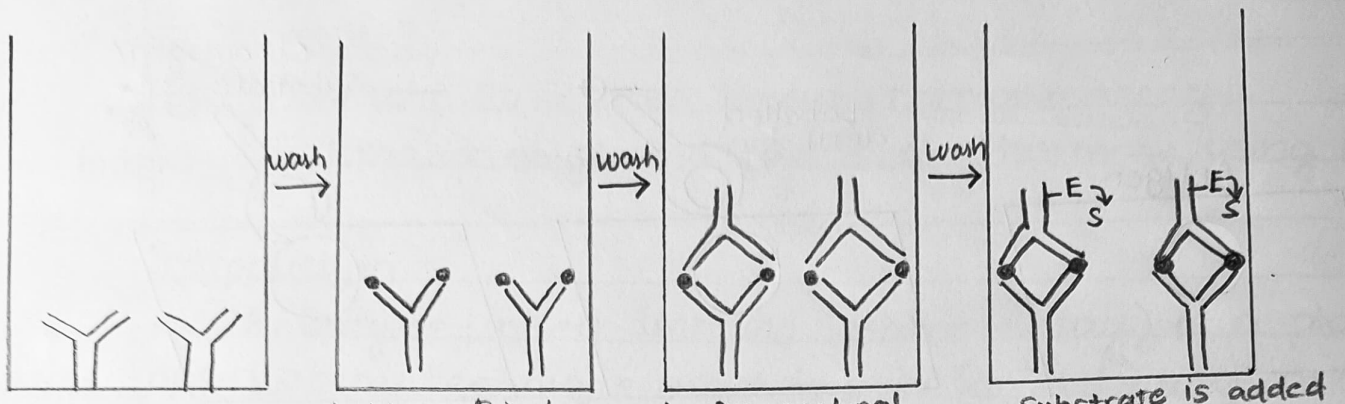
Antigen coated well

Specific antibody binds to antigen.

Enzyme linked antibody binds to specific antibody

Substrate is added and converted by enzyme into coloured product, the rate of colour formation is proportional to the amount of specific antibody

iii) Sandwich ELISA :-

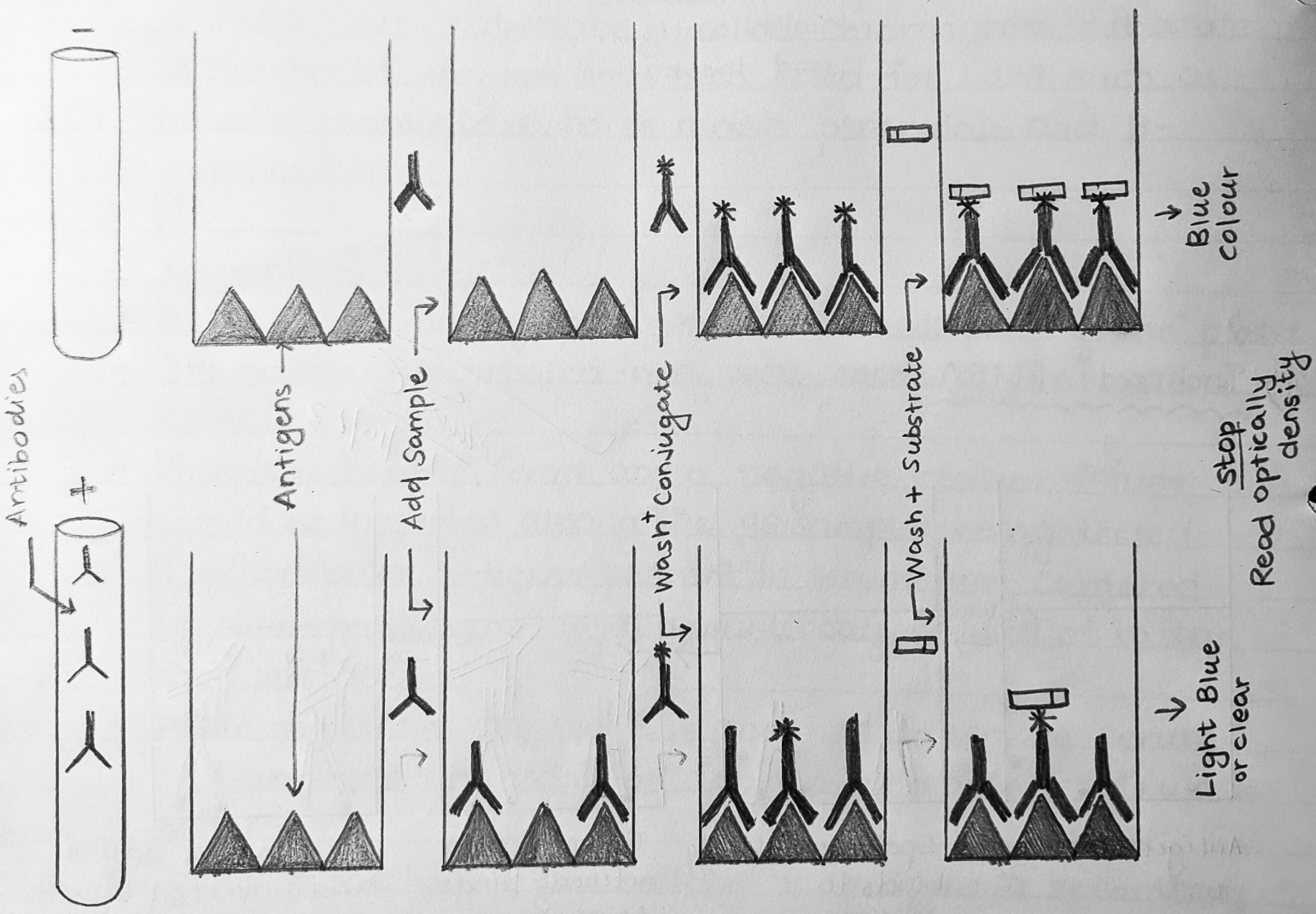


Monoclonal antibody coated well

Antigen Binds to Antibody

A 2° monoclonal antibody, linked to enzyme binds to immobilized antigen.

Substrate is added and converted by enzyme into colored product.



iv) Competitive ELISA

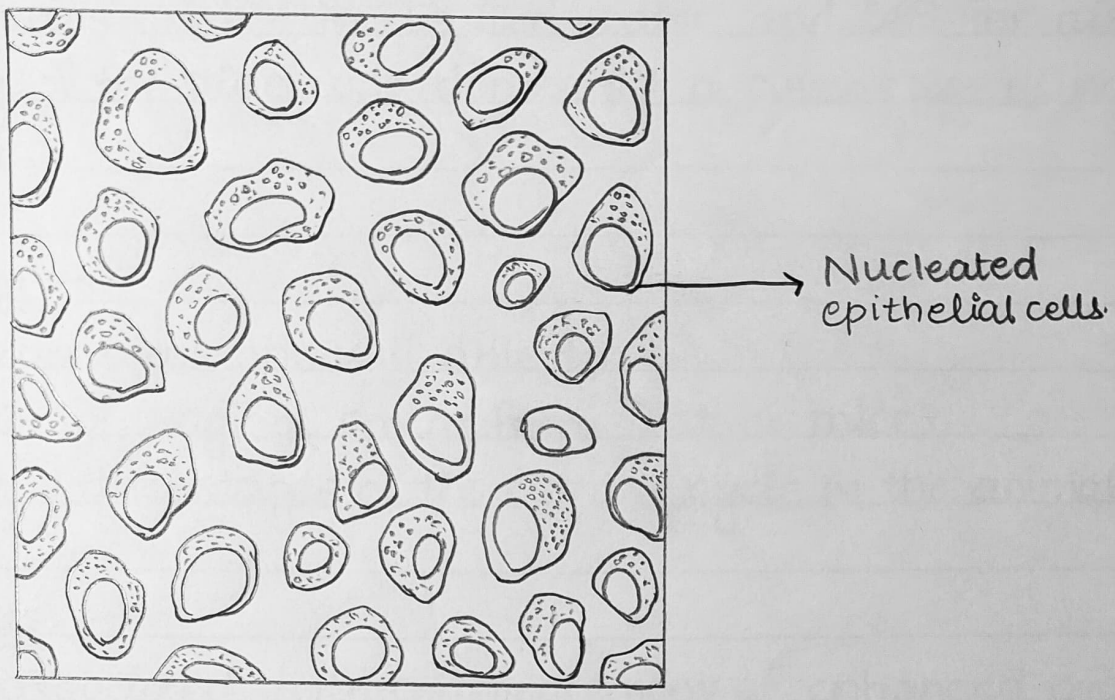


Fig:- Proestrous Phase.

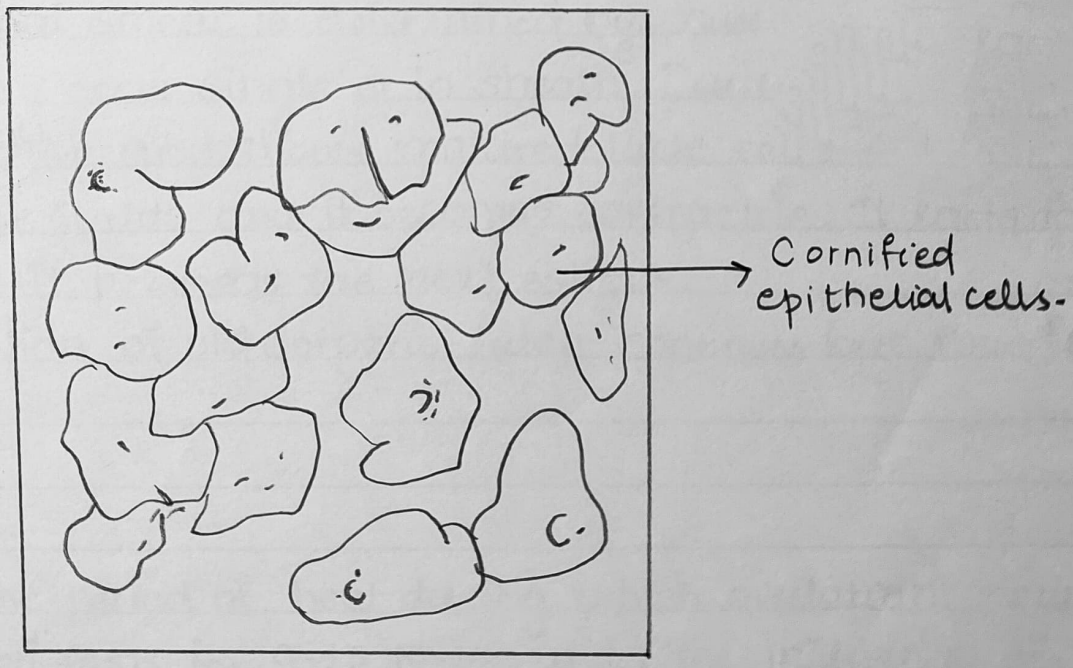


Fig:- Estrous phase

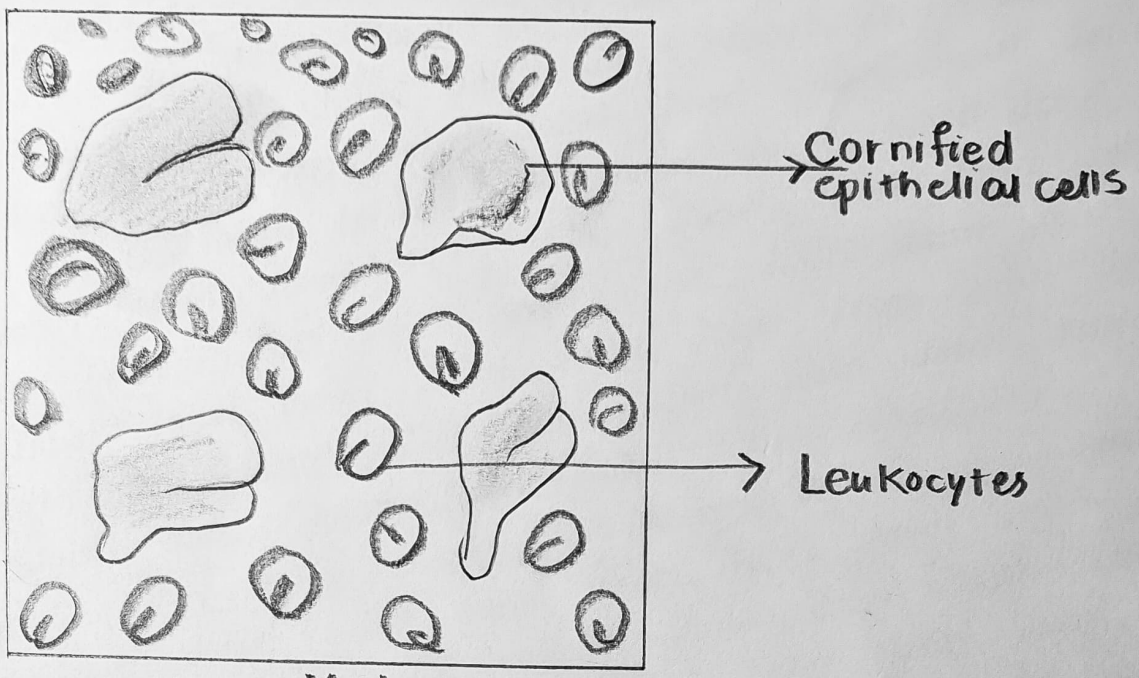


Fig :- Metestrus

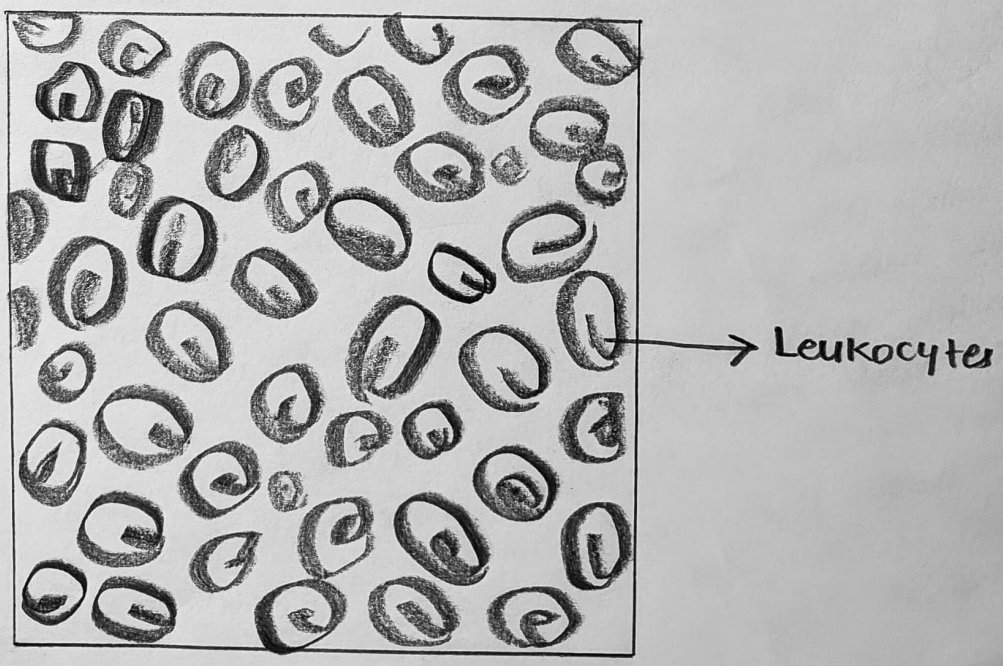


Fig :- Diestrus.

PRACTICAL PROTOCOLS

Experiment 1: Dissection and demonstration of endocrine glands

ABSTRACT:

This experiment outlines the procedures for dissecting and demonstrating the endocrine glands in lab-bred rats. The study aims to provide a hands-on understanding of the endocrine system in a common animal model. Endocrine glands play a vital role in regulating various physiological processes, and this practical exercise will enhance students' knowledge of their anatomy and location.

INTRODUCTION:

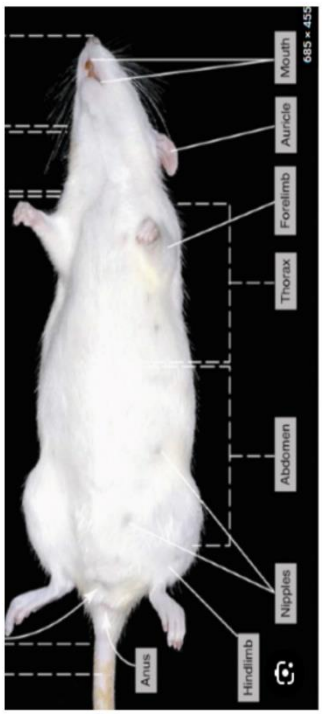
The endocrine system consists of several glands that secrete hormones, regulating numerous bodily functions. Understanding the anatomy and location of these glands is crucial for comprehending their role in maintaining homeostasis. In this practical demonstration, we will dissect lab-bred rats to identify and study the endocrine glands, including the pituitary gland, thyroid gland, parathyroid glands, adrenal glands, and pancreas.

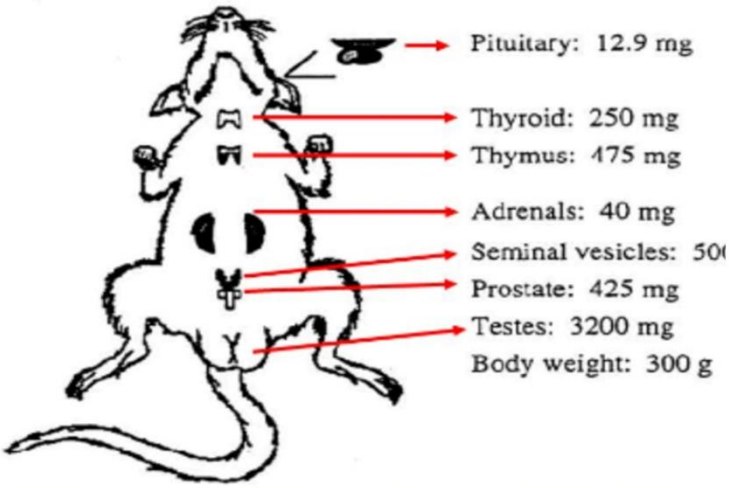
REQUIREMENTS:

1) Lab-bred rats (appropriate number)
2) Dissection tools (scalpel, scissors, forceps, and dissecting pins)
3) Dissection trays
4) Gloves and lab coats
5) Microscope (optional)
6) Charts or diagrams of rat anatomy
7) Formalin solution (for preserving specimens)

Method of Dissection:

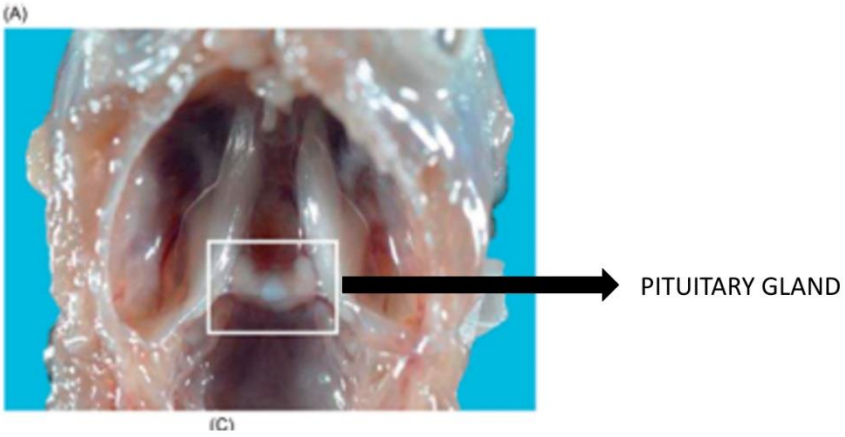
1. Begin by obtaining lab-bred rats, ensuring they have been appropriately anesthetized or euthanized, following ethical guidelines.
2. Place the rat in a supine position on the dissection tray.
3. Pin the limbs and secure the rat to the tray to expose the ventral side.
4. Using a scalpel, make a midline incision from the lower jaw to the pubic area, taking care not to damage the underlying structures.
5. Carefully lift and pin back the abdominal muscles to reveal the abdominal cavity.
6. Locate and identify the following endocrine glands:
    a. Pituitary gland (near the base of the brain)
    b. Thyroid gland (in the neck region)
    c. Parathyroid glands (located near or on the thyroid gland)
    d. Adrenal glands (on top of each kidney)
    e. Pancreas (located near the stomach and small intestine)
7. Examine the glands closely, noting their size, colour, and shape.
8. You can optionally use a microscope to observe tissue samples from these glands for a more detailed study.
9. Record your observations and findings in your lab notebook.





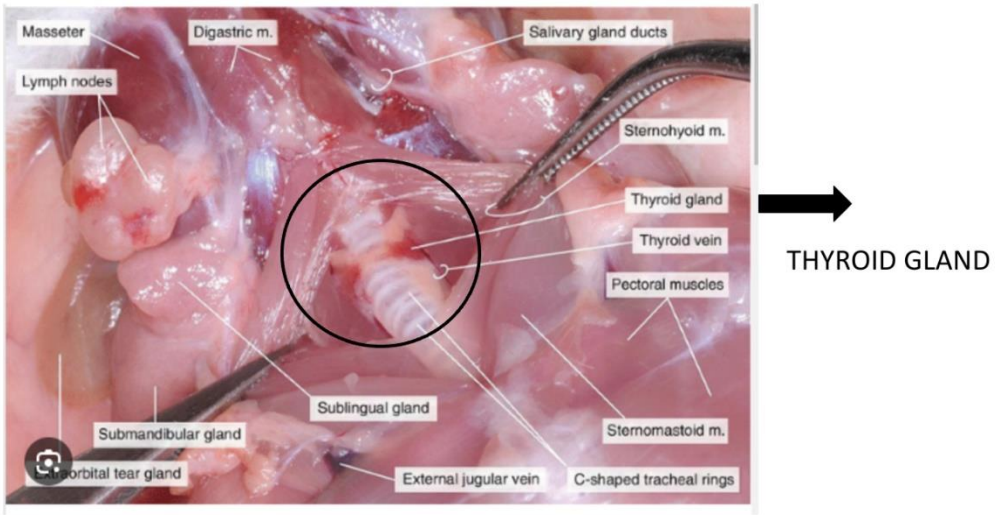
Pituitary Gland:

- The pituitary gland is situated near the base of the brain, just below the hypothalamus.
- After making the midline incision, gently lift the brain to expose the pituitary gland, which appears as a small, oval structure attached to the base of the brain.



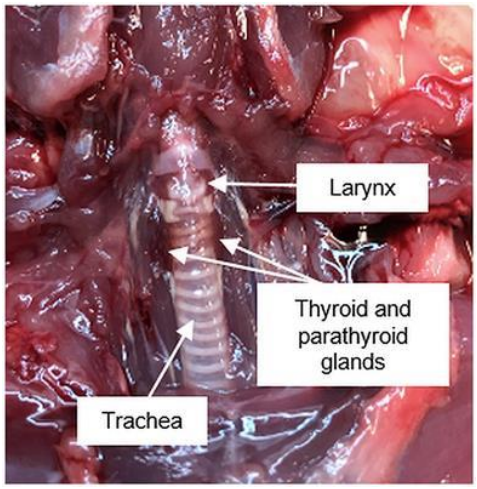
Thyroid Gland:

- The thyroid gland is in the neck region of the rat, near the trachea.
- Carefully remove the muscles and tissues in the neck area to reveal the thyroid gland, which typically has two lobes on either side of the trachea.



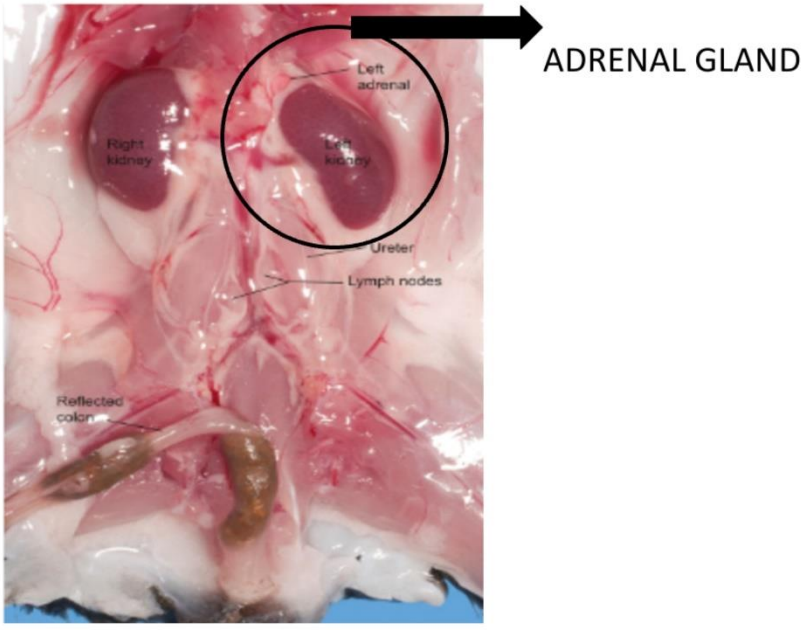
Parathyroid Glands:

- The parathyroid glands are usually located on or near the thyroid gland.
- These glands are very small and can be challenging to identify. They are typically embedded within the thyroid tissue. Examine the thyroid area closely for tiny, yellowish structures.



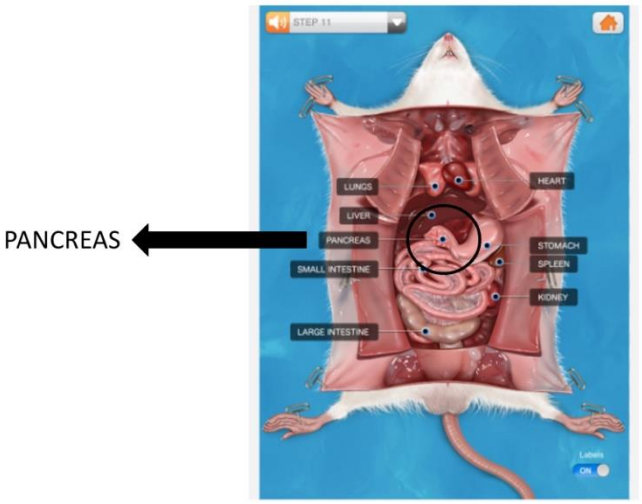
Adrenal Glands:

- The adrenal glands are found on top of each kidney.
- After exposing the abdominal cavity, locate the kidneys. The adrenal glands sit atop the kidneys and are often triangular or crescent shaped.



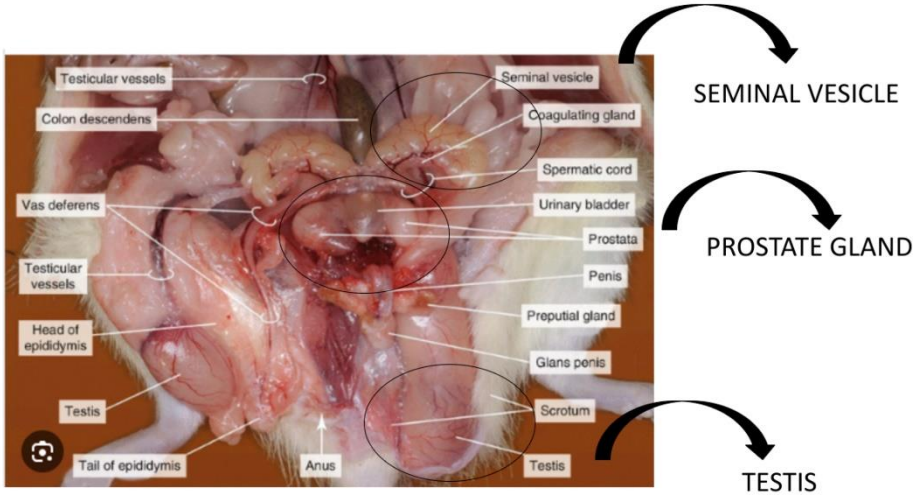
Pancreas:

- The pancreas is located near the stomach and small intestine, within the abdominal cavity.
- It appears as a pale, elongated structure near the stomach and the beginning of the small intestine.



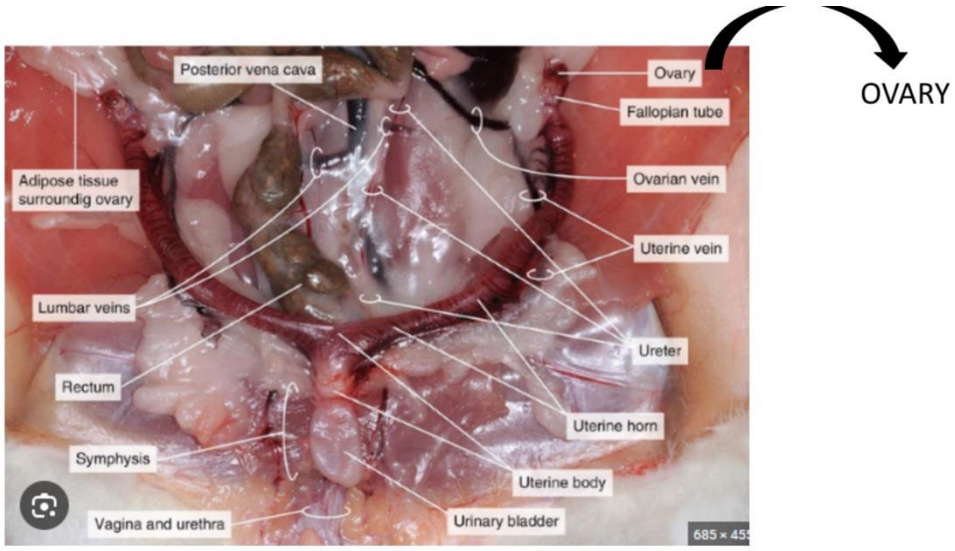
Testes:

- locate the testes in male rats. They are found in the scrotal sac, which is a pair of external pouches located between the hind limbs.
- Gently remove the testes from the scrotal sac by cutting the surrounding connective tissues. Be cautious not to damage the delicate structures.
- Examine the testes closely, noting their size, shape, and any distinguishing features.



Ovaries:

- In female rats, the ovaries are in the abdominal cavity near the lumbar region.
- Carefully lift and pin back the abdominal muscles to expose the abdominal cavity and locate the ovaries.
- The ovaries are small, bean-shaped structures that are usually located on each side of the uterus and attached to the body wall by ovarian ligaments.



Results:

The dissection and demonstration of the endocrine glands in lab-bred rats revealed the presence and location of the following glands:

I. Pituitary gland
II. Thyroid gland
III. Parathyroid glands
IV. Adrenal glands
V. Pancreas
VI. Testes
VII. Ovary

These glands are to be successfully identified and examined in the dissected rat specimen. Each gland's location and physical characteristics are to be noted .