PREFACE

In the curricular structure introduced by this University for students of Post Graduate degree programme, the opportunity to pursue Post Graduate course in Subjects 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 subjects 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 analyses.

The accepted methodology of distance education has been followed in the preparation of these study materials. Co-operation 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 a 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 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 part 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.

Prof. (Dr.) Manimala Das
Vice-Chancellor

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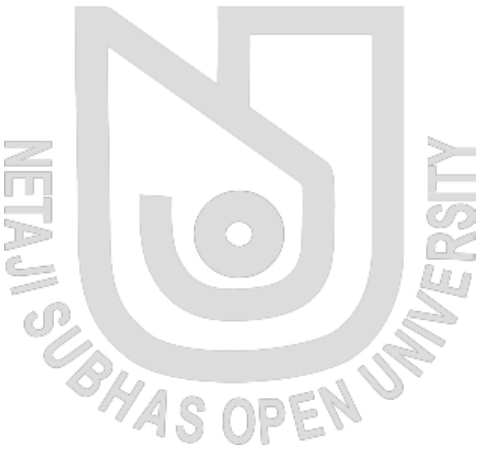
POST GRADUATE ZOOLOGY

[M.Sc]

Paper : PGZO 9

Group : B

Units	Writer	Editor
1-5	Dr. Kamales Kr. Misra	Dr. Bibhas Guha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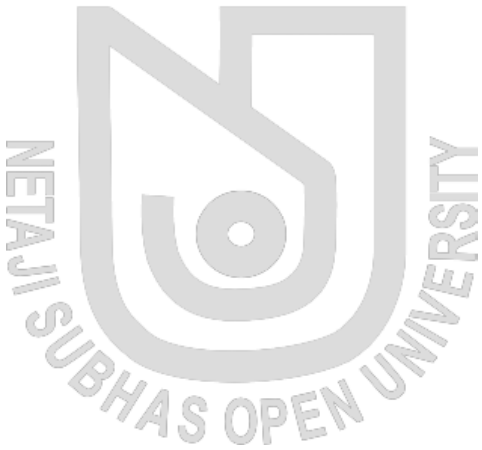
PGZO - 9
(Laboratory Course)

Group - B

Developmental Biology & Immunology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Unit 1 Studies of activated chick egg of different hours

Structure

1.1 Materials

(i) Fertilized egg of 24, 48, 72 and 76 hours
(ii) Forceps and scissors
(iii) Chick saline:
7.20 g NaCl
0.23 g $CaCl_2$ H_2O
0.37 g KCl
1 liter of distilled water
} Ingrediants are mixed and the pH is adjusted to 7.2 - 7.3
(iv) Petri dish/tumble
(v) Brush, needle
(vi) 2% Borax carmine solution
(vii) Binocular microscope/simple microscope

1.2 Preparation of chick embryo

The fertilized eggs are collected from recognized poultry farm having arrangements for incubation of eggs. A good poultry can supply eggs of definite hour of incubation. Eggs incubated for 24, 48, 72 and 96 hours are used for the study of development of chick. The eggs are cleaned with 50% alcohol to make germ free. A small pore at the broad end of the egg is made gently with the help of a scalpel. Then the shell is cut around the broad end with the help of scissors till the opening is large enough. The content of the incubated egg is poured in a large Petri dish / tumble filled with

chick saline, without damaging the vitelline membrane around the yolk. In the animal pole the embryo appears as a small white body on the surface of the yolk at the centre. The embryo is dissected out and kept it in a wash glass. The embryo is cleaned with a brush in the chick saline. Then the embryo is stained with 2% borax carmine solution. The embryo is thoroughly washed, dehydrated and mounted in DPX. Slide with stained embryo is examined under microscope.

1.3 Characters of 24 hour chick embryo (Fig. 1.1)

(i) Area vasculosa and area pelucida are distinct.
(ii) Primitive streak is much reduced at the posterior end.
(iii) Usually 4 pairs of somites are present in the middle of the body.
(iv) Neural folds are well developed at the anterior end.
(v) Primary optic vesicles in an early stage of development can be recognized.
(vi) Fore gut development initiates.

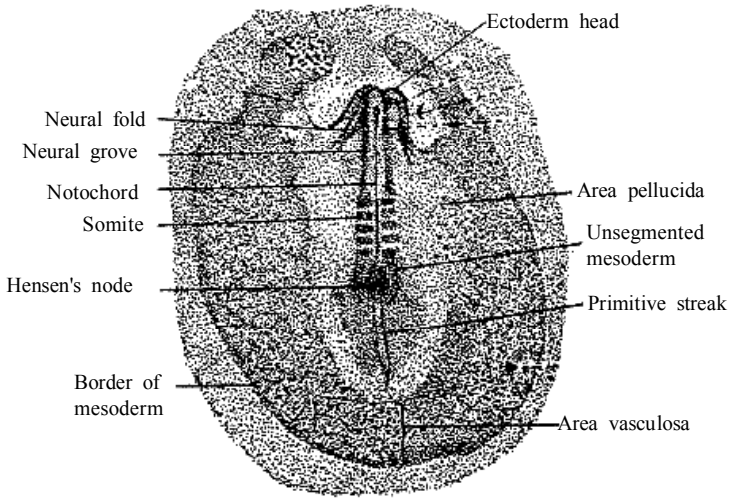

Fig. 1.1 : Diagrammatic view of a 24 hour chick embryo

1.4 Characters of 48 hour chick embryo (Fig. 1.2)

(i) Primitive streak is almost absent. Both cervical and cranial flexures are well developed.

(ii) Neural tube differentiated into brain and spinal cord.

(iii) Usually 26 pairs of somites are visible.

(iv) Anterior part of the embryo is twisted to the right.

(v) Pharyngeal pouches are visible.

(vi) Amnion development starts.

(vii) Optic cups are conspicuous.

(viii) Distinct auditory vesicles are present.

(ix) Three pairs of arterial arche arises from the ventral aorta.

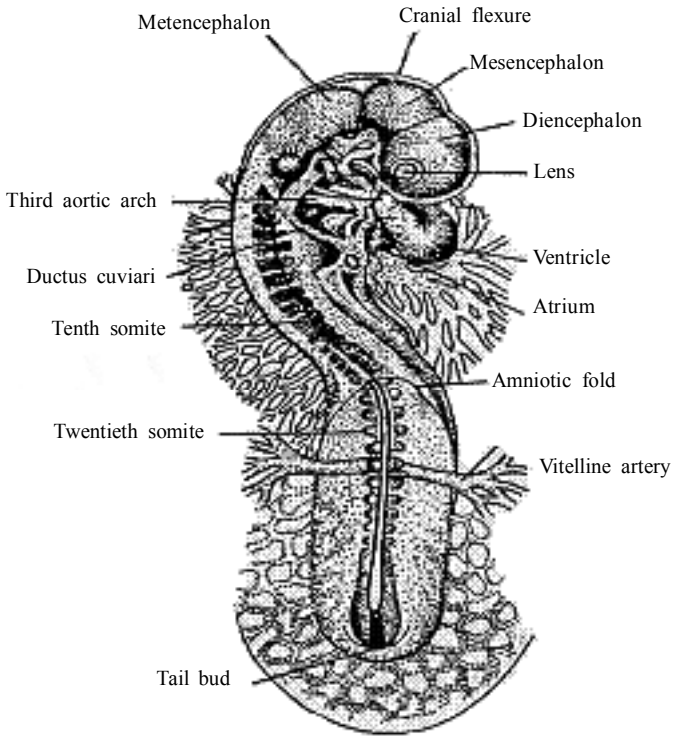

Fig. 1.2 : Diagrammatic view of a 48 hour chick embryo

1.5 Characters of 72 hour chick embryo (Fig. 1.3)

(i) Maximum cranial flexure is present.

(ii) Appendage's rudiments are visible.

(iii) Head fold grows back and lies between 10 to 18 somites.

(iv) Pharyngeal pouches are formed.

(v) Presence of 34 pairs of somites.

(vi) Posterior part of the body ends in a tail, which is curved inwardly.

(vii) Closed retina and lenses formed within optic cup.

(viii) Auditory vesicles are connected to the ectodermal aperture through ductus endolymphaticus.

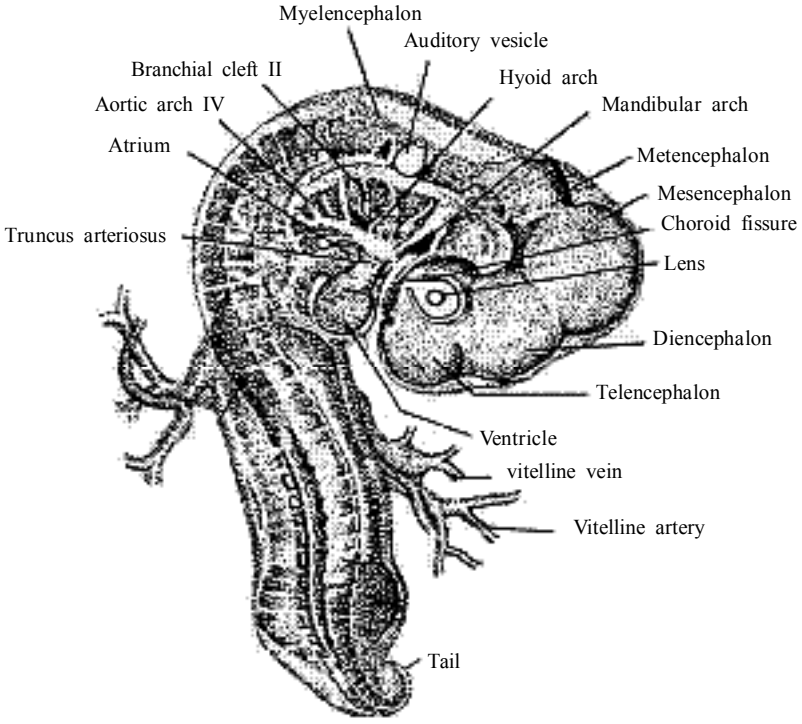

Fig. 1.3 : Diagrammatic view of a 72 hour chick embryo

1.6 Characters of 96 hour chick embryo (Fig. 1.4)

(i) The entire body turned through 90° and lies with its left side on the yolk.

(ii) The head and tail of the embryo are close together.

(iii) Optic cup shows more developed lens.

(iv) 41 pairs of somites.

(v) Fully formed heart is present with aorta and other blood vessels.

(v) Optic cup is nearly developed.

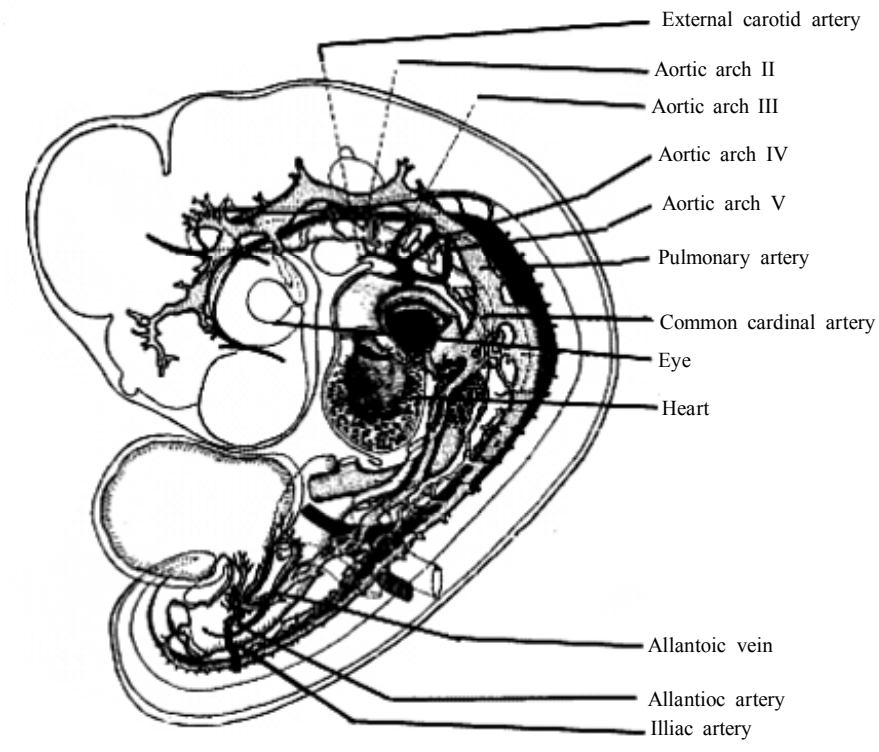

Fig. 1.4 : Diagrammatic view of a 96 hour chick embryo

Unit 2 Studies of egg and sperm of grasshopper (*Gesonula punctifrons*)

Structure

2.1 Squash preparation of grasshopper testis/ovary

Grasshopper provides the best tool for the study of sperms or eggs among the eukaryotes for laboratory students. The squash preparation of grasshopper testis is a very quick method.

Out of four common species of grasshopper, *Gesonula punctifrons* (short-horned grasshopper) of the order Orthoptera is easily available and best for chromosome study.

Grasshopper males are distinguishable from females by the presence of a red spot at the tarso-metatarsal joint and two pairs of abdominal appendages (a pair of anal cerci and a pair of anal style) instead of one pair in female (only anal cerci).

2.2 Location of testis

A pair of testes lobes is located on the dorsolateral side of alimentary canal at 3rd - 5th abdominal segment.

2.3 Chemicals and reagents

1. **Insect Ringer** (0.67%, isotonic solution; pH 6.2-7.0) : used to maintain the physiological integrity of cells.

   Composition: Sodium chloride (NaCl) 3.25 g, Potassium chloride (KCl) 0.17 g, Sodium carbonate ($NaHCO_3$) 0.10 g, Calcium chloride ($CaCl_2$) 0.06 g and Di-sodium hydrogen phosphate (Na_2HPO_4) 0.005 g, dissolved in 500 ml of distilled water.

2. **Aceto - alcohol (1:3) :** It acts as fixative. 1 part of glacial acetic acid mixed with 3 parts of ethyl alcohol.
3. **Aceto-orcein (1-2%):** It acts as chromosome dye. 1-2 gm of orcein powder was gently mixed in 100 ml of heated (60°C) 50% acetic acid, then cooled at room temperature and filtered. Final product was stored in brown bottle at 4°C.
4. **50% Acetic acid :** It is used for washing the excess stain. Equal volumes of glacial acetic acid and distilled water were mixed thoroughly.
5. **Lacto-aceto-orcein :** (1-2%): It acts as mounting medium. 1 or 2 gm of orcein powder was dissolved in heated solution containing 50 ml of 50% acetic acid and 50 ml of lactic acid.

2.4 Procedure

The grasshopper was pinned on a dissecting tray.

A mid - dorsal incision was given along the abdomen.

Targum was stretched and pinned laterally.

A few drops of ringer solution were added on the abdomen.

Reproductive organs were removed with the help of fine forceps and were kept in ringer solution in watch glass.

The adhering fat bodies were thoroughly removed with the help of needle and fine forceps to get rid of unwanted tissue.

The organs were kept in aceto-alcohol solution for 3-5 minutes covered by watch glass. The organs became white after putting on this solution.

Fixed follicles were kept in aceto-orcein solution for 40 - 45 minutes and covered by watch glass.

Stained follicles were washed in 50% acetic acid to remove excess stain.

A drop of lacto-aceto-orcein was added on a clean glass slide and the stained lobes were put on it and were covered with a cover slip.

Air-bubbles, if any were removed pressing with a needle.

The slide was then covered with blotting paper and the follicles were squashed with the help of thumb pressure.

The edge of the cover slip was sealed with nail - polish for observation under microscope.

Fig. 2.1 : Squash preparation of grasshopper sperm bundle

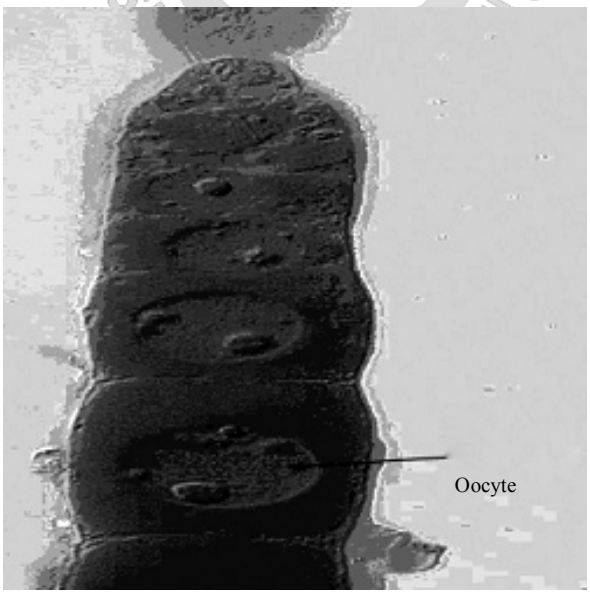

Fig 2.2 : Whole mount of grasshopper ovariole

Unit 3 Macrophage isolation from peritoneal fluid of mice

Structure

3.1 Introduction

Macrophage (MΦ) cells are key cells responsible for body's first line of defense, triggering initial response and resistance against various infections before the action of B and T cells. A macrophage is a White Blood Cell from the myeloid cell line that develops from monocytes (White Blood Cells with a single nucleus that can take in foreign substances) and migrated in to the bloodstream. Macrophages destroy certain types of invading cells, such as tumor cells and bacteria. Macrophages can be found in almost all the organs in different names. A large population of distinct macrophage cells can easily be isolated from peritoneal fluid.

3.2 Materials

(i) Inbred mice (healthy adult male, 20-25gm)
(ii) Disposable syringe with needle
(iii) Phosphate buffer saline (PBS), (0.85%NaCl + phosphate buffer 0.1M, pH 7.2)
(iv) Chloroform
(v) Petri dish
(vi) Slide and cover slips
(vii) Rectified spirit
(viii) Cotton
(ix) Glass distilled water
(x) Leishman/Giemsa stain
(xi) DPX
(xii) Microscope

3.3 Methods

One inbred mice was isolated from the cage and 2-3 ml PBS (preferably chilled) is injected in the peritoneal cavity and kept for 2 hours. After 2 hours the animal is anesthetized with chloroform. The peritoneal fluid is taken out with the help of the syringe and one drop of it is placed on a clean slide. (Presence of living macrophage cells is confirmed under light microscope.) A thin smear of the fluid is then made and subsequently semidried. The smear is covered with few drops of Leishman's stain and kept for 3-4 minutes. Then, few more drops of stain and same amount of glass distilled water are placed over the smear and kept for 15 minutes. Excess stain is decanted and the slide is washed in glass distilled water. The slide is dehydrated and mounted in DPX with a cover slip. The slide is observed under microscope with 10X x 40X magnification.

3.4 Observations

Fresh peritoneal fluid shows numerous macrophage cells. Stained predation shows macrophage cells with irregular outline having pseudopodia. Nucleus is vesicular and in some cells lobed nucleus is seen. Nucleus stains deep and located at the middle of the cell. The cytoplasm and the pseudopodia take light stain. Some cells are found to adhere with some small particle supposed to be foreign in nature, because the property of MÖ is to adhered foreign substances.

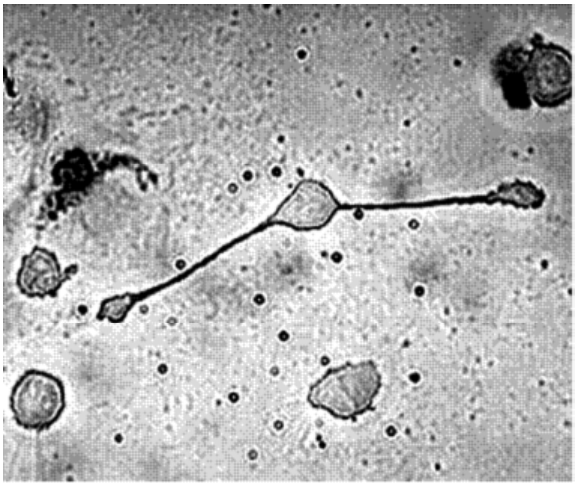

Fig. 3.1 : Photograph of a macrophage cell with enlarged pseudopodia

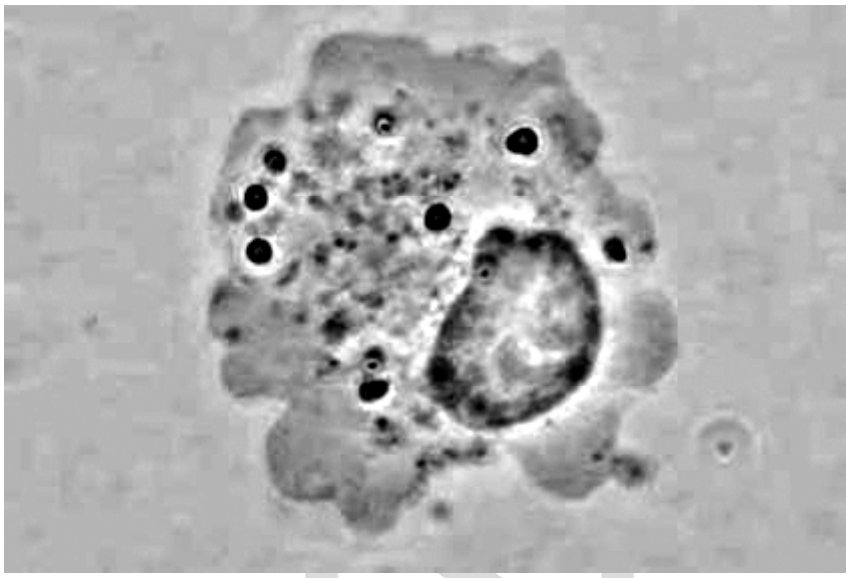

Fig. 3.2 Phagocytosis by macrophage

Unit 4 Identification of thymus, bursa and spleen

Structure

4.1 Thymus

1. The thymus lobe is subdivided into three gross areas: a connective tissue capsule, a dark peripheral margin called cortex and a lighter central area called the medulla.
2. The capsule contains small blood vessels that supply the cortex.
3. Immediately beneath the capsule there is a nearly continuous layer of epithelial reticular cells.
4. Deep to this bounding layer, lymphocytes completely fill the interstices of a three-dimensional network of stellate reticular cells with pale nucleus and acidophilic cytoplasm.
5. The lobe is subdivided into several lobules.
6. The cortex area of the lobe is made up of three types of cells: stromal cells, lymphocytes and macrophages.
7. Immature T-lymphocytes are packed tightly in the cortex.

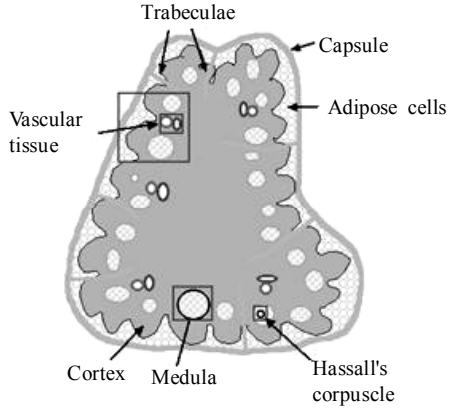

Fig. 4.1 : Diagrammatic features of a thymus lobule

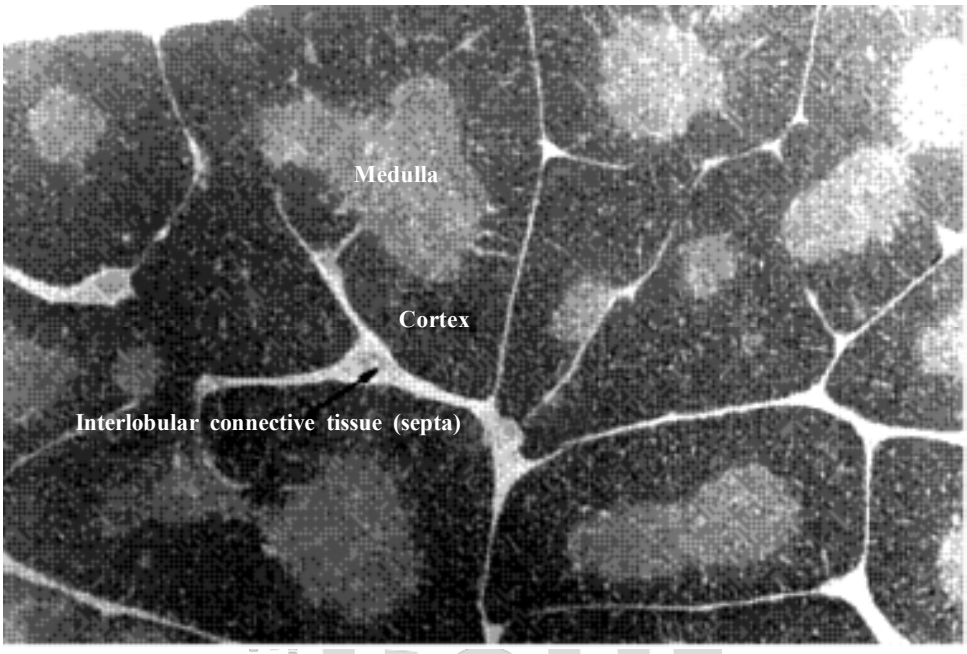

Fig. 4.2 : Histological features of a section of thymus gland

8. Macrophage are scattered throughout the cortex.
9. The medulla consists of the same cell composition as cortex.
10. The medulla is a lighter staining area because of decreased proportion of lymphocytes.
11. Cells of one type aggregate and become flattened and concentrically arranged to form bodies called Hassall's corpuscles.

4.2 Bursa

1. In birds, the **bursa of Fabricius** (Latin: *Bursa cloacalis* or *Bursa fabricii*) is the site of hematopoiesis, a specialized organ that is necessary for B cell (part of the immune system) development in birds. It is named after Hieronymus Fabricius who described it in 1621.

2 Mammals generally do not have an equivalent organ; the bone marrow is often both the site of hematopoiesis and B cell development

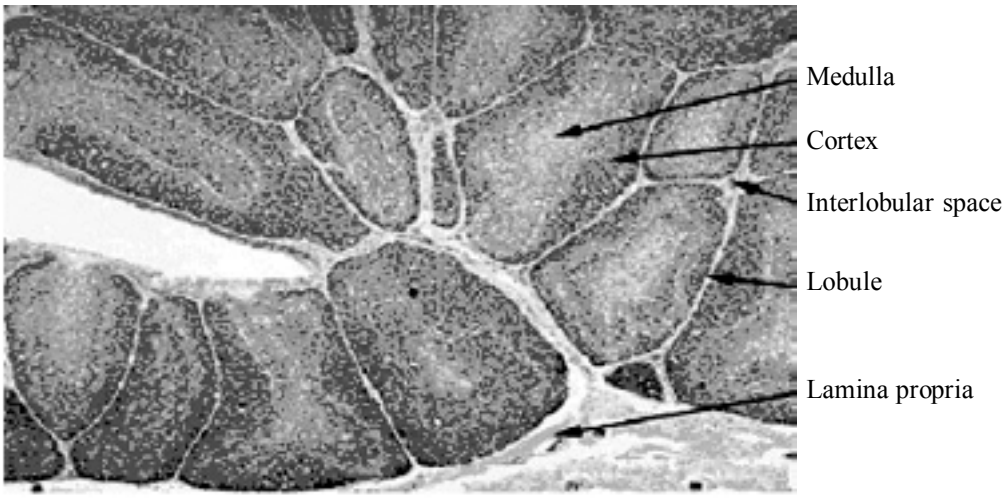

Fig. 4.3 : Section of bursa showing different parts

3. The 'B' in 'B cell' refers to bursa-derived. The fact that 'bone marrow', like bursa, starts with a 'B' is sheer coincidence.
4. The bursa is an epithelial and lymphoid organ that is found only in birds.
5. It develops as a dorsal diverticulum of the proctadael region of the cloaca.
6. The luminal (interior) surface of the bursa is plicated with as many as 15 primary and 7 secondary plicae or folds.
7. These plicae have hundreds of bursal follicles containing follicle-associated epithelial cells, lymphocytes, macrophages, and plasma cells.
8. In the bursa, these stem cells acquire the characteristics of mature, immune competent B cells.

Lymphoid stem cells migrate from the fetal liver to the bursa during ontogeny.

4.3 Spleen

1. It is a lymphoid organ that functions as a filter for the blood system.
2. It is a highly vascular organ, consisting of white pulp areas scattered throughout the red pulp tissues.
3. White pulp areas appear as dark grayish blue areas scattered throughout the red staining red pulp areas in haematoxylin-eosin stain.

4. White pulp is a lymphatic tissue consists of lymphocytes, plasma cells, macrophages and reticular cells all surrounding a major blood vessel.

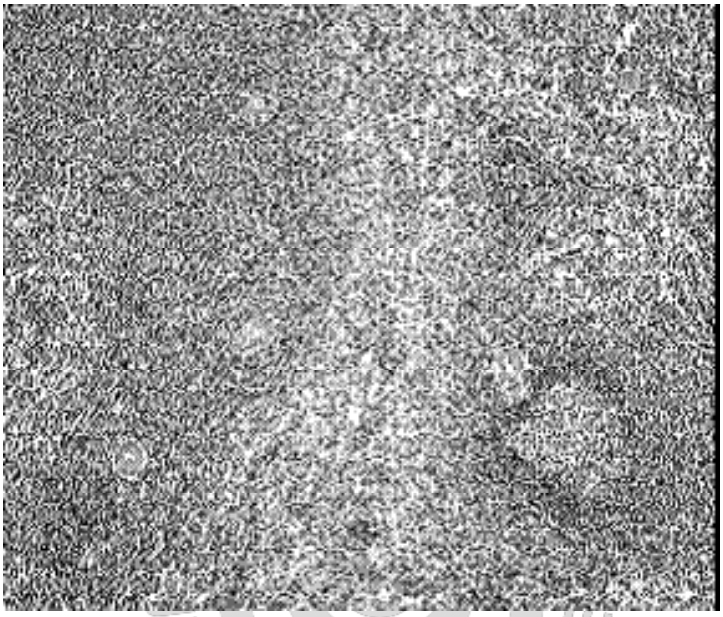

Fig. 4.4 : Section of spleen showing red and white oulps.

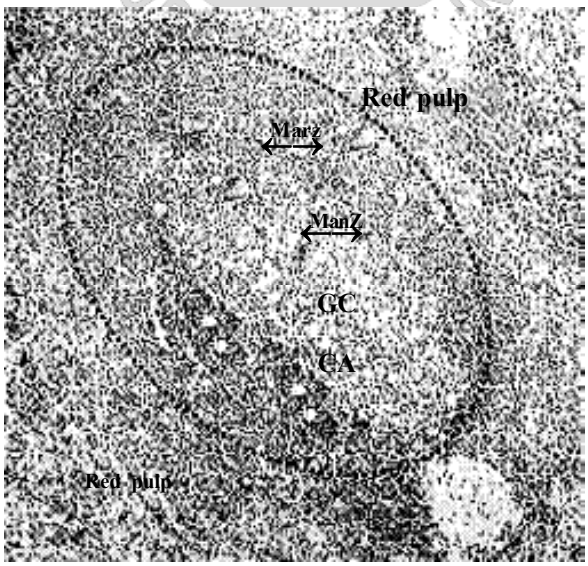

Fig. 4.5 : Enlarged view of white pulp

5. Red pulp consists of branching, anastomosing networks of sinuses separated from each other by a reticular meshwork. The reticular meshwork is often called splenic cord or Billroth's cords.
6. Surrounding the parenchymal tissue of the spleen is a highly collagenous capsule (GC), which is thickened at the hilus.
7. The capsule produces inward extensions called trabeculae that penetrate deep into the organ.
8. The periarteriolar lymphocyte sheath (PALS), the characteristic association of lymphocytes and blood vessels in the spleen. The lymphocytes are arranged along the arteries forming a sleeve or sheath. Collectively, the PALS are the "white pulp." The rest of the interior volume of the spleen is the splenic cords or Billroth's cords, collectively constituting the "red pulp," and the blood sinuses between them.

Unit 5 Antigen-antibody reaction by blood group test

Structure

5.1 Principle

The serum of a person may cause agglutination of the RBC of another person. Depending upon the presence or absence of two agglutinogens A and B in RBC and two specific agglutinins anti-A (α) and anti-B (β) in the serum, human blood groups are designated as A, B, AB and O. RBC may have either or both factors (A, B, AB) or none at all. Serum may have either or both factors (α, α, $\alpha\beta$) or none at all.

Similarly a person may possess Rhesus factor or not. Depending on the presence or absence of Rhesus factor a person is designated as '+' (positive) or '–'(negative) respectively.

5.2 Requirement

(i) Anti serum kit
(ii) Slides
(iii) Sterilized needle
(iv) Simple microscope
(iv) Absolute alcohol and cotton

5.3 Procedure

a) Middle finger tip was cleaned with alcohol and pricked.
b) Three drops of blood in three different points of a slide were taken.
c) Three points of the drops were marked as A, B & D.
d) Immediately poured one drop each of antiserum marked A, B & D and observed for few minutes.

5.4 Observation and inference

'X' marked blood showed agglutination. Therefore, the blood group of mine is 'X'

Blood group	Agglutinogen in RBC	Agglutinins in plasma	reaction with	
			anti-A serum	anti-B serum
O universal donor	none	a and b		
A	A	b	 agglutination	
B	B	a		 agglutination
AB universal recipient	A and B	none	 agglutination	 agglutination

Fig. 5.1 : The ABO blood groups. The apperance of the blood of each group when exposed to anti-A and anti-B is shown in the two right han coloums. Clumping of cells indicate reaction