ELECTRON MICROSCOPIC AND IMMUNOHISTOCHEMICAL STUDIES OF SPLEEN, THYMUS AND CAECAL TONSIL IN CHICKEN (*Gallus domesticus*)

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*Thesis submitted in partial fulfillment of the requirements for the degree of*

DOCTOR OF PHILOSOPHY

in

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*to the*

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2008
Dedicated to my Beloved Family Members
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T.A. KANNAN
ABSTRACT

Title : Electron microscopic and immunohistochemical studies of spleen, thymus and caecal tonsil in chicken (Gallus domesticus)

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Year : 2008

Light and electron microscopic and immunohistochemical studies on spleen, thymus and caecal tonsil were done in layer chicken of various age groups ranging from day-old to forty weeks.

The spleen was encapsulated by a connective tissue capsule and the trabeculae were poorly developed in all the age groups studied. The major cellular population of the white pulp included lymphoblasts, lymphocytes of various sizes, follicular dendritic cells and reticulum cells. PALS were found adjacent to the central artery.

The splenic red pulp was composed of pulp cords consisted of erythrocytes, reticular cells and lymphocytes of various sizes, macrophages, granulocytes, plasma cells and mast cells. The arterioles that continued into the red pulp formed sheathed capillaries or ellipsoids.

The thymic gland in chicken showed a thin connective tissue capsule. The connective tissue septa divided the gland into lobules with a dark outer cortex and a pale inner medulla. In thymic parenchyma, lymphocytes or thymocytes,
reticuloepithelial cells, myoid cells and macrophages were the predominant component and the other cell types occasionally observed were erythrocytes, granulocytes, mast cells and plasma cells.

Three types of reticuloepithelial cells were observed. The first and second types were distributed both in cortex and medulla. The third type was noticed in medulla and at the cortico-medullary junction. The Hassall’s corpuscles were composed of concentrically arranged reticuloepithelial cells. The centre of the corpuscles appeared either solid or cystic.

The myoid cells of the chicken thymus were found mainly in the medulla. In the present study, intracellular and intercellular cysts were observed in association with the Hassall’s corpuscles in all the age groups. The onset of involution was observed in twenty week-old birds and marked involutary changes were noticed in forty weeks.

The caecal tonsil revealed two types of lymphoid aggregations (germinal centre). The first type had an incomplete capsule and the second type was found encapsulated with connective tissue. The capsule of the germinal centre consisted of many layers of flattened reticular cells separated with an intercellular substance. The germinal centre consisted of lymphoblasts, lymphocytes of various sizes, reticular cells, plasma cells, mast cells and macrophages. In forty week-old birds, the lymphocytic population was observed to be comparatively reduced and more number of fibroblasts and collagen fibres were noticed. The caecal tonsil also had M cells with short and irregular microvillus.

A distinct difference was observed in the ratio of CD4 and CD8 cells in the spleen and thymus of different age groups. Nevertheless, the ratio narrowed down at forty weeks of age.

Flow cytometric analysis of CD4 and CD8 in the spleen and thymus from control and *Eimeria tenella* infected birds reflected the participation of these organs in cell mediated immunity.
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6 SUMMARY

REFERENCES
This is to certify that the thesis entitled “ELECTRON MICROSCOPIC AND IMMUNOHISTOCHEMICAL STUDIES OF SPLEEN, THYMUS AND CAECAL TONSIL IN CHICKEN (Gallus domesticus)” submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANATOMY to the Tamilnadu Veterinary and Animal Sciences University, Chennai-600 051 is a record of bonafide research work carried out by T.A. KANNAN, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Date: 
Place: Chennai-7

(GEETHA RAMESH) 
CHAIRPERSON

APPROVED
Chairperson : Dr. GEETHA RAMESH

Members : 1. Dr. S. USHAKUMARY
           2. Dr. G. DHINAKAR RAJ
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External examiner :
Date: 
Place: Chennai-7
**CURRICULUM VITAE**

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<tr>
<th>Name of the Candidate</th>
<th>T.A. KANNAN</th>
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<tr>
<td>Place of Birth</td>
<td>Salem</td>
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<tr>
<td>Major field of specialization</td>
<td>Anatomy</td>
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**Educational Status**

- Completed B.V.Sc. at Veterinary College and Research Institute, Namakkal in 1994.
- Completed M.V.Sc., Programme at Madras Veterinary College in 1994-96.

**Professional experience**

- Assistant Professor (from 27.10.1999 to till date) in Tamilnadu Veterinary and Animal Sciences University, Chennai.

**Marital Status**

- Married

**Permanent Address**


**Membership of Professional Society**

1. Life member, Tamil Nadu Veterinary Council
2. Life member, Indian Association of Veterinary Anatomists
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INTRODUCTION

Poultry industry is one of the fastest growing segments of the agricultural sector in India today. While the production of agricultural crops has been rising at a rate of 1.5 to 2 per cent per annum, that of eggs and broilers has been rising at a rate of 8-10 per cent per annum. As per the Economic Survey of 2007 – 08 in India, the total production of egg was 51 billion with the per capita availability of 42 eggs per annum during the year 2006 - 07. In Tamilnadu, egg production was 8.04 billion with the per capita availability of 123 eggs per annum in 2006-07. The state stands second in egg production of the country (Integrated Sample Survey, Tamilnadu, 2007) and India is now the world's fifth largest egg producer and the eighteenth largest producer of broilers (Mehta and Nambiar, 2002).

Chicken play an important role in the rural economy both on commercial raising and backward rearing. They are reared due to their wide range of production capabilities including meat, egg and its products (Leslie, 1975). Chicken have been used as experimental animals for studies on the immune system because T and B cells mature in the thymus and bursa of Fabricius respectively, as well as these organs can easily be manipulated (Khan et al., 1998).

Commercial production of broiler and layer flock is dependent on the immunological status of the birds. Genetically determined immunocompetence and environmental factors are responsible for varying susceptibility or resistance to infectious diseases of chicken (Bridle et al., 2006).

The primary and secondary lymphoid organs are the main components of immune system in vertebrates. Further, the lymphoid tissue in the digestive tract (GALT) is an important element in the immunological defense of the host particularly against intestinal pathogens (Erf, 2004).

The thymus gland is a central lymphoid organ in which bone marrow-derived T-cell precursors undergo differentiation, maturation eventually leading to migration of positively selected thymocytes to the peripheral lymphoid organs such as the spleen (Savino and Dardenne, 2000) and GALT including the caecal tonsil and the lymph nodes (Ciriaco et al., 2003).
Spleen is a principal organ of systemic immunity and its importance in disease resistance is accentuated by the scarcity of avian lymph nodes (John, 1994).

The avian spleen functions as a major blood filtering organ and is the major source of antibody production. It does not function as a reservoir of blood as in mammals and its function is not oriented towards oxygen provision (Jeurissen, 1991). The spleen also plays an important role in erythrocyte destruction, phagocytosis and antigen-antibody interactions (Burke and Simon, 1970).

The alimentary tract is incessantly invaded by foreign antigenic or harmful substances and their lumina are also sites for the proliferation of both beneficial and disadvantageous bacterial flora (Mead, 1989 and Kitagawa et al., 2000). Caecal tonsil, the immunodefence mechanism of the caecal environment regulates the proliferation of microflora in the caecum continuously and also prevents the invasion of extra caecal microorganisms. This is brought about by organization of enormous lymphoid nodules throughout the caecal mucous membrane forming tonsils (Kitagawa et al., 1998).

The immunocompetence of an individual is evaluated based on several parameters including circulating T lymphocyte populations such as CD3+, CD4+, CD8+, TCR+, TCR2+ and TCR3+ lymphocytes. The amount and proportion of T cell subsets in circulation and in organs have been correlated with disease susceptibility (Kitagawa et al., 1998).

It is well established that infection with *Eimeria* coccidian in poultry induces both cellular and humoral immune responses. In chicken, the immunity produced by coccidian infection has a variable effect upon the parasite depending on several factors, such as host age at immunization, strain of chicken, magnitude of the immunizing inoculum and its mode of administration (Lillehoj, 1994).

The invasion of the *Eimeria* sporozoites into the intestinal epithelium results in massive infiltration of macrophages, granulocytes and lymphocytes into the lamina propria. The macrophages modulate the severity of the infection and the lymphocytes, in particular CD4+ T cells, act as inducer of an effective immune response (Jeurissen and Veldman, 2002).
Extensive work on the immune developmental and functional aspects in poultry has been conducted in chicken which have been genetically selected for egg production. Due to genetic and environmental differences, avian immune developmental and functional aspects established in broilers may not be directly applicable to layers. Hence, considering the economic importance, an in depth study on the immune system in layer chicken is essential.

Little is known about age-related immunocompetence in commercially raised layer chicken with their individual management programmes and specific immunization protocols. Therefore, understanding age-related immunocompetence by evaluating circulating T lymphocyte population in apparently healthy commercially raised chicken is of direct relevance to developing breeding strategies as well as promoting health measures of the flock (Zekarias et al., 2002).

Hence, to bridge the gap in avian immune research with regard to understanding the ultrastructural details of the lymphoid organs and age related changes in T lymphocyte subset population, the present study was aimed at to explain the factors influencing the immunocompetence of the birds with the following objectives.

To study the electron microscopic structural details of spleen, thymus and caecal tonsils in the layer chicken of different age groups.

To study the occurrence of T and B lymphocytes in the spleen and thymus by immunohistochemical methods in the layer chicken of different age groups.

Flow cytometric evaluation of CD4 and CD8 count in spleen and thymus of layer chicken in different age groups.

To compare the changes in CD4 and CD8 count in spleen and thymus in control and experimentally infected chicken with Eimeria tenella.
CHAPTER - 2

REVIEW OF LITERATURE

A. Histomorphology

2.1. Spleen

3.2.4 Capsule and Trabeculae

Light microscopy

Hodges (1974) observed that the spleen of fowl was enclosed by a thin fibrous capsule limited externally by a flattened layer of peritoneal mesothelium. The capsule was made up of an outer layer of collagen fibres with few elastic fibres comprising one third of the total thickness and an inner layer composed mainly of a network of elastic fibres and fibroblasts comprising of two third of the thickness of the capsule.

Brown et al. (1987) stated that the spleen of mammals was surrounded by a thick connective tissue capsule, which consisted of layers of connective tissue and smooth muscle fibres. They stated that, spleen of horse had the thickest capsule, pig and ruminants had moderately thick capsule whereas cat had the thinnest capsule. The capsule and trabeculae consisted of collagen and elastic fibres with few smooth muscle cells. Arteries, veins, lymph vessels and nerves were found in the trabeculae.

Suchumzar and Welsch (1987) reported that the spleen of the Antarctic seal consisted of smooth muscle fibres in the capsule and trabeculae. The trabecular system was well developed in long diving weddell seals.

Nasu et al. (1992) reported that in dove, the spleen was found to be covered by a thin layer of connective tissue which extended into the parenchyma of the gland as trabeculae. Normally, the trabeculae were poorly developed and observed only at the hilus of the organ. Hence, the parenchyma of the spleen was not subdivided by the trabeculae as in mammals.
Venkatesan and Vijayaragavan (1997) in Japanese quail observed that there were no true trabeculae in the spleen.

**Electron microscopy**

Fukuta *et al.* (1969) described that in chicken, the trabeculae of the spleen were too less developed for the trabecular artery and vein to exist together than in mammal. Branches about 15 microns in caliber of the trabecular artery were the central arteries. They entered the white pulp, passing its centre axially and were deprived of the tunica adventitia. The central artery radiated as penicillar artery towards the peripheral part of the white pulp. The penicillar artery was seen divided three to five times in the white pulp.

Burke and Simon (1970) stated that the splenic capsule in rabbit was composed primarily of cells which had features of both smooth muscles cells and fibroblasts. These cells were stellate with dense, fibrillar cytoplasm, small mitochondria and glycogen-like granules. They were associated with large bundles of collagen and occasionally with some elastic tissue. The external aspect of the capsule was lined by a single layer of mesothelial cells, characterized by small irregular microvillous projections at their free border. The inner surface of the capsule was lined by a typical large sinus with endothelial cells of the usual type and a fenestrated basement membrane. A few reticulum cells and macrophages were found in the capsule adjacent to the sinus, and frequently a macrophage or reticulum cell could be found in the gaps of the capsular basement membrane. Elsewhere, capsular fibroblasts were found against the basement membrane (Moore *et al.*, 1964).

Burke and Simon (1970) further stated that the trabeculae of the spleen of rabbit varied in thickness and size and composed of fibroblasts, collagen, elastic and basement membrane-like material. The portions of the trabeculae adjacent to the red pulp also contained reticulum cells and macrophages and were bordered by a sinus lined by endothelial cells and a fenestrated basement membrane. In the white pulp, the trabeculae laid in immediate relation to the fibroblasts, lymphocytes and reticulum cells of this zone.
Kimura et al. (2001) observed the fine structure of contractile trabecules in the splenic red pulp of the rat by electron microscopy to elucidate their participation in the active contraction of the spleen. Numerous fine thready trabecules were developed in the red pulp. They were enveloped with a cytoplasmic layer of reticular cells and consisted of elongated smooth muscle cells, fascicles of collagenous fibrils and elastic fibres. Their fibrous components in the capsular ends extended in a triangular form of fan ribs into the fibrous tunica of the capsule. Smooth muscle cell-like interstitial cells (SIC) were situated in the interfibrous spaces.

Further, flattened SIC were affixed with cytoplasmic processes to the elastic lamina. The trabeculo-capsular junctions were represented on the elastic lamina by grouped or isolated circular patches with concentrically arranged triangular processes and were also observed on the capsular serosa by plaques with scarce microvilli of serosal cells. Smooth muscle cells of the fine trabecules were equipped on the cell surface with anchoring structures to extracellular fibrous elements. Close associations were also seen between the smooth muscle cells and elastic fibres which were terminated to the fascicles of collagenous fibers. Cell-to-cell connections were expressed by fibrous connections between spiny processes and a small number of punctate intermediate junctions and nexuses. Unmyelinated nerve fibres with adrenergic terminals were seen in the intercellular spaces.

3.2.5 Parenchyma

Lucas et al. (1954) observed that in the chicken, over 80 per cent of the substance of the spleen was divided equally into red and white pulp and the remainder of the total volume was taken up by blood vessels, connective tissue and other miscellaneous tissue. The dominant cell type of the whole spleen was small to medium sized lymphocyte.

In birds, the areas of red pulp and white pulp were not distinguished properly in contrast to that of mammals (Thorbecke et al., 1957; Fukuta et al., 1969; Hodges, 1974; King and McLelland, 1981 and Miyamoto et al., 1980).
The parenchyma of the spleen in the chicken consisted of a reticular network with numerous circumscribed areas, the white pulp surrounded by areas of red pulp. Several central arteries were seen within the white pulp areas and composed chiefly of lymphocytes. The red pulp areas were composed of numerous eosinophilic cells, macrophages and intermingled lymphocytes. Sinuses containing red blood cells were common in red pulp (Malewitz and Calhoun, 1958).

Bradley and Grahame (1960) reported that the underlying framework of splenic tissue in fowl consisted of a network of reticular cells and reticular fibres.

Hoffmann et al. (1977) identified the distribution of T and B lymphocytes in chicken spleen by immunohistochemical methods. He described that T cells were predominant in periarteriolar lymphatic sheath and in the red pulp cords. A few of these cells were also present in periellipsoid lymphatic tissue and in germinal centres. Majority of the cells in the germinal centre were B lymphocytes.

### 3.2.5.1 White pulp

**Light microscopy**

According to Lucas et al. (1954), the avian spleen differed from the mammalian spleen by not having germinal centres in the white pulp and by the absence of lymph vessels. On the contrary, Cooper et al. (1965 and 1967) described that in birds, the germinal centres of the white pulp and the corona of the lymphocytes, which surrounded them, were B-dependant. Two distinctly different types of lymphoid tissue were present in the spleen of chicken. One was seen along the arteries and arterioles as sheaths of small lymphocytes or as clusters of large and small lymphocytes, lymphoblasts and primitive reticular cells, which were thymus dependent. Second type was circumscribed round or oval lymphoid follicles, enclosed by a thin fibrous membrane, which always laid juxtaposition to a small artery. They resembled morphologically the follicle of the bursa of Fabricius and they were bursa dependant.
Payne (1971) stated that the white pulp in the chicken consisted of two types of tissue, a diffuse lymphoid mass enveloped the central arteries and their branches and germinal centres near the arteries.

According to Hodges (1974), the white pulp in the chicken was comparatively a diffuse network of reticular cells within which were scattered numbers of small, medium and large lymphocytes. At the junction of the red and white pulp was the marginal zone where both T and B lymphocytes were present in birds (Ford, 1975).

Ogata et al. (1977) reported that white pulp in the chicken was divided into four elements, such as periarterial lymphatic tissue, perivenous lymphatic tissue, periellipsoidal lymphatic tissue and germinal centres. The first elements appeared two days after hatching. The second, third and fourth elements appeared on the sixth day, third week and fourth week respectively. Plasma cells appeared on the second day of hatching and were more frequent at the periphery of the white pulp.

King and Mc Lelland (1981) reported that the white pulp in birds consisted of collection of small lymphocytes around the central arteries. Radiating out from the central arteries were penicillar arterioles, which, at the periphery of the white pulp, gave rise to sheathed capillaries. The sheathed capillaries were enclosed by reticular cells forming ellipsoids.

Olah and Glick (1982) stated that in chicken, the periarterial lymphatic sheath consisted of densely packed mass of medium and large sized lymphocytes and macrophages.

Jeurissen et al. (1992) stated that unlike the spleen of rodents, the spleen of chicken did not have a marginal zone between white and red pulp.

**Electron microscopy**

The fibroblasts of the white pulp in mammals often appeared flat and were stellate with extremely long cytoplasmic prolongations which were sometimes U-shaped. They had a strikingly dilated rough endoplasmic reticulum filled with a
very fine flocculent material. The smooth endoplasmic reticulum contained similar material, some of which appeared fibrillar. These fibroblasts together with the stellate projections of reticulum cells made the loose meshwork which compartmentalized the white pulp. This meshwork was not dense enough to form solid barriers or prevent the free passage of cells within the pulp (Galindo and Freeman, 1963).

Ogata et al. (1977) observed that the plasma cells in the white pulp of spleen of chicken were identified by their well developed system of rough endoplasmic reticulum which extended throughout the cytoplasm. The rough endoplasmic reticulum generally contained a fine flocculent material and occasionally Russell bodies. Their mitochondria were round, large and denser than in lymphocytes or reticulum cells, and had prominent cristae. There were numerous ribosomes both free and attached to the dilated endoplasmic reticulum.

In chicken, the follicular dendritic cells of the spleen were stellate in shape, sending out long, thin sheets of cytoplasm that folded and coiled into complex arrays. The cytoplasm of follicular dendritic cells lacked organelles of active secretory and endocytic vesicles such as rough endoplasmic reticulum and lysosomes. These anatomical features distinguished the follicular dendritic cells from other cell types, even those that were extended in shape (Chen et al., 1978).

Djaldetti et al. (1980) described in mouse spleen that the smooth surfaced lymphocytes formed the majority in the white pulp. They had high nucleo-cytoplasmic ratio. The nucleus was round or slightly indented with heterochromatin in the vicinity of the nuclear membrane. Cytoplasm contained fewer mitochondria and Golgi apparatus.

Olah and Glick (1982) stated that in birds the lymphocytes of the white pulp varied in size and were often difficult to differentiate from the reticular cells. The lymphocytes were round to oval and characterized by a high nucleo–cytoplasmic ratio. The cytoplasm was very sparse and inactive in appearance. There was occasionally a little perinuclear rough endoplasmic reticulum, a few free ribosomes and small mitochondria with few cristae. The smooth endoplasmic reticulum was in
the form of small vesicles dispersed randomly in the cytoplasm and the Golgi zone was not prominent. The reticulum cells were ovoid and stellate in shape. The cytoplasm contained phagocytic debris. These phagocytes were the macrophages, and were identical to the macrophages in the red pulp.

Gallego et al. (1995 and 1997) described that two types of follicular dendritic cells existed in the chicken spleen. First type showed filliform cell processes, the other type was provided with beaded dendrites. The first type was observed when the follicular dendritic cells bound the antigen on their surfaces.

3.2.5.2 Periarterial lymphatic sheath (PALS)

Light microscopy

Olah and Glick (1982) observed that the PALS of chicken spleen consisted of densely packed small lymphocytes, several medium sized and large lymphocytes as well as few macrophages.

Dellmann (1998) reported that T cells were concentrated adjacent to the tunica media of artery of white pulp in PALS of spleen of domestic animals whereas the peripheral region of the sheath contained a more diverse mixture of T cells, B cells, macrophages and dendritic cells.

Geetha Ramesh et al. (2001) reported that the periarterial lymphatic sheath was infiltrated with lymphocytes and reticular cells which were also commonly observed in larger nodules of white pulp in the post-pubertal age groups of rat, mice and guinea pig.

Electron microscopy

In chicken spleen, the germinal centres were located at the beginning of the central artery which was surrounded by PALS. The central artery had no branch crossing the PALS, and there was no histologically identifiable marginal zone in the chicken spleen. The central artery continued as penicilliform capillaries. The mid
portion of the penicilliform capillary was surrounded by the ellipsoid or Schweigger-seidel sheath (Olah and Glick, 1982).

2.1.2.3 Red pulp
Light microscopy

Burke and Simon (1970) described that the splenic red pulp in rabbit was composed of a complex series of anastomosing, tortuous sinuses which varied in size from small channels to large vascular pathways. These sinuses were separated from each other by solid partitions of red pulp called the Billroth or pulp cords. The Billroth cords varied in thickness from one cell to many. Within the cords were mainly reticulum cells, macrophages and fibroblasts, but plasma cells, leukocytes, platelets and red blood cells were also present. The Billroth cords were often irregularly partitioned by extra cellular reticulum which had the appearance of amorphous basement membrane-like material. This extra cellular reticulum sometimes was fibrillar, with a periodicity suggesting collagen.

Hodges (1974), King and Mc Lelland (1981) and Bacha and Bacha (2000) reported that the splenic red pulp in chicken was made up of a loose spongy tissue composed of ramifying cellular cords surrounded by venous sinusoids. The cellular cords were composed of basically the reticular cells, lymphocytes, macrophages and granulocytes.

Rose (1981) reported that in chicken, the red pulp was composed of lymphoid tissue, which was honeycombed by venous sinusoids to give an appearance of cellular cords. These cords consisted of reticular cells, lymphocytes, macrophages and granulocytes. Red pulp of chicken spleen was also said to be the site of plasma cell proliferation in birds.

Connolly et al. (1999) described that the red pulp contained haemopoietic tissue in the spleen of platypus. Geetha Ramesh et al. (2001) reported that in rat, mice and guinea pig, the red pulp consisted of splenic cords. The cords consisted of
erythrocytes, lymphocytes, macrophages, mast cells, plasma cells and giant cells. Isolated smooth muscle fibres were observed in the red pulp of spleen in rats and guinea pigs whereas they were totally absent in mice.

**Electron microscopy**

Roberts and Latta (1970) described that the sinuses of the red pulp in rabbit spleen were lined by endothelial cells which varied in size based on the size of the sinus. The larger endothelial cells were in the larger sinuses, did not have a prominent vesicular system or a well developed endoplasmic reticulum, and were less stellate than most of the endothelial cells which lined the medium and smaller sinuses. As the endothelial cells in medium and small sinuses were more stellate, their nuclei were not always present in every section. The nuclear portion of the cell bulged into the lumen of the sinus while the remainder of the cells were roughly cuboidal. The cytoplasm was characterized by a very large number of vesicles and vacuoles many of which were at the luminal surface. Occasionally, one of the vacuoles contained some dark, dense, coarse material which might perhaps represented a product of endothelial phagocytosis. There was also a well developed rough and smooth endoplasmic reticulum and the mitochondria were round with a light matrix and well developed cristae.

In the scanning electron microscopic studies on the mink spleen, Abe et al. (1989) observed that the red pulp consisted of lattice work formed by elongated endothelial cells with side processes and the spongy reticular tissue. The sinusoids were covered by reticular cells. Numerous sheathed arteries were found in the splenic cords.

**2.1.2.4 Ellipsoids**

**Light microscopy**

Hoshi and Mori (1975), Rose (1981) and Kenji et al. (1995) described that in birds, white pulp of spleen consisted of a collection of small lymphocytes surrounding
the central arteries. Penicillar arteries radiated out from the central arteries and at the periphery of the white pulp gave rise to sheathed capillaries. The sheathed capillaries were found enclosed by reticular cells forming the ellipsoid.

**Electron microscopy**

An ellipsoid of chicken spleen consisted of reticular cells and macrophages. The macrophages were fixed to the vascular wall by the connective tissue (Fukuta *et al.*, 1976).

Electron microscopic study of ellipsoid in the spleen of dog and cat revealed that the periarterial macrophage sheath were composed of a fine meshwork of reticular cells, reticular fibres which held tightly packed macrophages and interspersed blood cells. The cytoplasm of reticular cells was filled with thin filaments and possessed plasmalemmal dense bodies as found in smooth muscle cells (Blue and Weiss, 1981a and b).

Olah and Glick (1982) studied the chicken spleen and found that the ellipsoid was made up predominantly of reticular cells. The reticular cells were extremely polymorphic. Reticular fibres were seen between the reticular cells.

Unmyelinated adrenergic nerve fibres and nerve endings were observed in electron microscopic studies of ellipsoids in the spleen of dog (Blue and Weiss, 1981b) and chicken (Olah and Glick, 1982). The nerves were always in close association with the arteries of the pulp. They had a variable number of axons and one could usually discern the cytoplasm of the Schwann cell which surrounded them.

**2.2 Thymus**

The thymus, a primary or central lymphoid organ and was essential for the development of the peripheral lymphoid tissues and their associated adoptive immune function (Clawson *et al.*, 1967; Dellmann, 1998 and Dellman and Brown, 1998).
The major function of the thymus in vertebrates such as birds and mammals, was to provide appropriate micro environment within which cells of T-lineage could develop, proliferate, mature, generate their antigen receptor repertoire and leave the thymus to enter the pool of recirculating lymphocytes which helped to protect the animal against pathogens (Ritter and Crispe, 1992 and Romano et al., 1999).

2.2.1 Capsule
Light microscopy

Thymus gland of the chicken was covered by a connective tissue capsule and septa radiated from the capsule divide the parenchyma into lobules. These septa carried the blood vessels (Bradley, 1950).

Leeson and Leeson (1976) and Dellmann (1998) mentioned that the mammalian thymus was surrounded by a thin connective tissue capsule which consisted of collagen fibres together with a few elastic fibres. Interlobular trabeculae arose from the capsule and passed into the cortex of the lobule which never penetrated into the lobules beyond the limit between the cortex and medulla.

Hodges (1974) and Firth (1977) described that the thymus in birds was enclosed by a thin connective tissue capsule, composed of mainly coarse collagen fibres together with a few fine elastic fibres. External to the capsule were considerable amount of loose connective tissue and adipose tissue. Fine septa passed inwards from the capsule and divided the mass of the gland into lobules, which were again subdivided into segments by numerous small, short septules branching out from the main septa.

Kendall (1980) reported that the plasma cells and macrophages were seen scattered occasionally beneath the capsule in the thymus of aves.

The subcapsular cells of thymus in rabbits lied along the cortical surface. Some of these cells over the stroma were flat; resembling the fibroblast, while other cells bulged deeply into the cortex resembled the cortical reticular cells (Sainte et al., 1986).
Ritter and Crispe (1992) stated that in mammals, the septa carried both the vascular and neuronal supply to and from the thymus, while branches from the septa gave rise to the perivascular spaces within the thymus.

2.2.2 Parenchyma

2.2.2.1 Cortex

Light microscopy

Thymic lymphocytes or thymocytes in fowl and mammals were indistinguishable from the lymphocytes of the circulation formed the major component of the cortex. These cortical lymphocytes masked the reticular cells which formed a supporting framework. Occasionally, capillaries were seen in the cortex with erythrocytes and eosinophilic granulocytes (Bradley, 1950 and Trautmann and Fiebiger, 1957).

Thymus dependent line of lymphocyte was represented morphologically by small lymphocytes of circulation and the white pulp type of tissue in birds (Cooper et al., 1966).

Biester and Schwarte (1969) stated that the thymic cortex in birds contained abundant small and medium lymphocytes in the meshes of reticular cells with few plasma cells.

In Japanese quail, each lobule of the thymus had a cortex in which the stroma was infiltrated with thymocytes that were not distinguishable from the small sized lymphocytes. No germinal, or reaction centres of lymph sinuses were present (Fitzgerald, 1969).

In birds, bursa dependent lymphocytes were morphologically represented by large lymphocytes of the germinal centres and by the plasma cells (Kincade et al., 1971).
King and Mc Lelland (1981) stated that each lobule of the avian thymus consisted of an outer cortex and an inner pale medulla, both formed from a framework of scattered reticular cells and fibres which contained masses of small lymphocytes.

Firth (1977), Sabiha et al. (1998) and Bacha and Bacha (2000) stated that the division between cortex and medulla of the thymus in birds was not well demarcated as in bursa of Fabricius.

Lymphoblasts and medium sized lymphocytes predominated in the meshes of peripheral epithelial reticulum, where they showed mitotic divisions and produced small lymphocytes that differentiated in the deep cortex in mammals (Dellmann and Brown, 1998).

In mammals, the thymic cortex consisted mainly of an epithelial reticulum, lymphocytes and scattered macrophages (Connolley et al., 1999 and Bacha and Bacha, 2000).

Bearman et al. (1978) and Young and Heath (2000) reported that in infants, thymic cortex was packed with immature and maturing thymocytes. The lymphoblasts in the outer cortical zone divided to produce smaller, mature T cells.

**Medulla**

**Light microscopy**

Biester and Schwarte (1969) stated that the thymic medulla in birds had relatively more reticular cells and fewer lymphocytes and Hassall’s corpuscles and the same was reported by Ramakrishna et al. (1978) in Murrah buffalo calves.

In Japanese quail (Fitzgerald, 1969), medulla of each lobule was made up chiefly of reticular epithelial cells which were not phagocytic. Numerous thymic bodies or Hassall’s corpuscles were present.

Hodges (1974) reported that in fowl, the medulla contained fewer lymphocytes than the cortex. The reticular cell nuclei were seen in between the lymphocytes.
The medulla of the chicken thymus was comprised of numerous reticular cells which grouped to form a syncytium or supporting framework with less number of lymphocytes (Sabiha et al., 1998).

Connolley et al. (1999) reported that in platypus the thymic medulla consisted of numerous Hassall’s corpuscles, lesser lymphocytes and more reticular cells that in cortex.

2.2.2.3. Cellular components
2.2.2.3.1. Thymic lymphocyte/thymocyte

Light microscopy

The thymus of sixteen week-old chicken contained three types of lymphoid subpopulation which constituted 95 per cent. The first type, the medullary thymocytes were responsible for the graft versus host reaction. Other type, formed the bursal dependent lymphocytes differed from the circulating B cells and the third variety corresponded to the T cell population (Zucker et al., 1973).

King and Mc Lelland (1981) stated that in birds, the thymocytes in the medulla were less numerous and larger than those in the cortex. Hence, the medulla in histological sections was more lightly stained than the cortex.

The most numerous cells in the thymus of mammal were lymphocytes. Approximately five per cent of the lymphoid cells resided in the subcapsular area, 80-85 per cent was in cortex and remaining ten per cent of the lymphocytes were in the medulla (Ritter and Crispe, 1992).

Electron microscopy

Clawson et al. (1967) described the electron microscopic structure of thymocytes in normal two month-old chickens. Thymocytes had a thin rim of cytoplasm surrounding a nucleus with heavily clumped chromatin and mitochondria present occasionally in the cytoplasm. Larger lymphocytes had occasional well defined Golgi zone and paired centrioles and these organelles were found rarely in
small lymphocytes. The ribosomes were scattered mainly as a single unit with occasional clusters of two or three. They were not uniformly distributed throughout the cytoplasm but appeared to congregate only in specific cytoplasmic areas.

Maxwell and Trejo (1970) and Maxwell (1974) described in domestic fowl that the small and medium lymphocytes were rounded cells with peripheral pseudopodia and a large nucleo-cytoplasmic ratio. Relatively few cytoplasmic organelles were present; only some mitochondria, short strands of rough endoplasmic reticulum, many ribosome and dense membrane bound granules (Enbergs and Kriesten, 1968; Maxwell, 1974 and Hodges, 1977).

The electronmicrograph of the thymocytes in chicken showed multi-vesiculated bodies, pinocytotic vesicles and empty vacuoles and at times fat droplets (Maxwell and Trejo, 1970).

Discrimination between the small and medium lymphocyte in birds was made on the basis of amount of cell cytoplasm; in small lymphocyte it consisted of a narrow rim of cytoplasm surrounded the nucleus, but in the medium sized lymphocyte, it formed a moderately wide band (King and Mc Lelland, 1981).

A Golgi complex was present in both the cell types, being better developed in medium lymphocytes, and was frequently located opposite to nuclear indentation. The nuclear heterochromatin was more strongly condensed and peripherally arranged in the small than the medium lymphocytes (Simpson, 1968).

Ultrastructurally, small lymphocytes were tiny, round cells with a narrow rim of cytoplasm that contained largely free ribosomes. Their Golgi zones were small, lysosomes were rarely seen. Nuclear chromatin was densely packed particularly at the periphery of the nucleus (Ferrarini, 1980).

Lymphocyte populations acquired their special characteristics by residence in the thymus (to become T cells) or GALT (to become B cells) in mammals (Cheville, 1994).
2.2.2.3.2. Reticuloepithelial cells

**Light microscopy**

Reticuloepithelial cells which formed the supporting meshwork in the thymus of mammals were large cells with long processes, large nucleus with prominent nucleoli and eosinophilic cytoplasm (Weiss, 1966).

Firth (1977) stated that the stroma of both cortex and medulla of thymus in birds consisted of a network of reticular cells and their fibres.

Ritter and Crispe (1992) and Dellmann and Brown (1998) expressed that the epithelial cells in the cortex were smaller, stellate in shape, had very long cytoplasmic processes with pale nucleus formed a network throughout this region of the thymus. But the epithelial cells in the medulla were larger, oval shaped cells with shorter, spatula-like processes.

Boyd *et al.* (1983) reported that the reticuloepithelial cells released humoral factors essential for the production, development and maturation of the lymphocytes in bursa and thymus of chicken.

Ushiki (1986) reported that, the epithelial cells that formed a meshwork in the thymic parenchyma of rat were stellate in shape.

**Electron microscopy**

Three types of reticuloepithelial cells were observed in the thymus of birds. The first type was the large, pale cell found in the cortico-medullary junction. The second type was small and dark, found both in cortex and medulla along with the third type of cells. The third type of cell was of intermediate morphology of the first and second. These large pale whorls of epithelial cells were associated with Hassall’s corpuscles and cyst in the thymic medulla (Frazier, 1973).

Robert *et al.* (1978) demonstrated a supporting framework of epithelial reticular cells with long branched cytoplasmic processes joined by desmosomes in normal human thymus.
Van de Wijngaert et al. (1985) and von Gaudecker (1986) described that the human thymus consisted of six types of thymic epithelial cells under electron microscope. Type-1 cells were the sub-capsular epithelial cells, which lied along the surface of the basement membrane, these cells lined the septa and perivascular spaces. In the cortex, these Type-1 cells formed the blood-thymus barrier. Secretory granules were noticed in the cytoplasm.

Type-2, 3 and 4 cells represented different stages of a single class of epithelial cells. Type-2 cells were characterized by their long cytoplasmic processes that extended far from the cell body; intermeshed with Type-2 cells and surrounded small islands of cortical thymocytes. Type-3 was termed intermediate and Type-4 being electron dense, distorted dying cells in the deep cortex.

Type-5 and 6 cells were medullary epithelial cells. Type-5 cells had relatively short processes, occurred in small groups, the cytoplasm had no secretory granules. Type-6 cells formed a loose network in the medulla and cytoplasm, they contained secretory granules. Type-6 cells formed the Hassall’s corpuscles.

Ritter and Crispe (1992) described that the epithelial cells in mammals were characterized by the presence of tonofilaments and desmosomes. Mohammed et al. (2007) described six types of epithelial cells in the thymus of lung fish according to its location and ultra structure.

2.2.2.3.3. Myoid cell

Light microscopy

Myoid cells contained myofibrils and were commonly present in the thymus of birds and reptiles (Raviola and Raviola, 1967; Toro et al., 1969 and Curtis et al., 1972).

Gilmore and Bridges (1974) described that the myoid cells in thymus were occasionally seen in cortex but most numerous in the medulla. Myoid cells were few at hatch but increased in number to become a marked feature of the thymic medulla in twenty six week-old fowl.
Myoid cells formed the peculiar feature of the chicken thymus present occasionally in the cortex but numerous in the medulla from one day to eighty four weeks of age (Gilmore and Bridges, 1974 and Kendall, 1980). In duck, myoid cells were numerous in the medulla not in the cortex (Sugimura, 1972).

Robert et al. (1978) described that myoid cells were found rarely in the normal human thymus.

Geetha Ramesh and Vijayaragavan (1997) reported a few number of myoid cells in the thymus of neonatal buffalo calves in the medulla near the Hassall’s corpuscles. The functional significance of the myoid cell was still uncertain even in higher vertebrates (Zapata, 1996).

**Electron microscopy**

Gilmore and Bridges (1974) observed myoid cells of thymus in chicken and they described that the cytoplasm contained many elements of skeletal muscle. The nucleus contained dispersed chromatin, with condensed chromatin abutting on the nuclear membrane. Cytoplasm contained few mitochondria and smooth endoplasmic reticulum. But, these organelles with the nucleus were displaced peripherally by the mass of myofibrils which occupied the greater part of the cell. They further reported that the myofibrils in the myoid cells were present without the overall organization seen in a typical striated muscle fibre. Both thick and thin myofilaments were seen in most cells.

Chan (1992) described the presence of unmyelinated nerve fibres in close proximity to the myoid cells in the medulla of the thymus in chick. The unmyelinated axons contained predominantly clear vesicles and some dense core vesicles in the recesses of plasmalemma of Schwann cell. Small bundles of unmyelinated nerves were observed in the medulla of chick thymus, predominantly near the blood vessels or in the vicinity of myoid cells. The axoplasm contained mitochondria, a mixture of small, clear vesicles and occasionally large, dense-core vesicles. Neurofilaments and neurotubules were also found in some axons.
Chan (1995) described the myoid cells of chicken were round or elongated, distributed singly or in clusters in the medulla or at the cortico-medullary junction. The cytoplasm was packed with myofibrils arranged in circular bundles around the nucleus. The interfibrillar cytoplasm contained smooth endoplasmic reticulum, mitochondria and free ribosomes.

2.2.2.3.4. Macrophages

Light microscopy

Frazier (1973) opined that in the chick thymus, macrophages were present in moderate numbers, both in the cortex and medulla.

Robert et al. (1978) described that in normal human thymus macrophages were most numerous in the cortex where they often contained phagocytosed nuclear debris.

Macrophages were one of the principal cell types in the thymic parenchyma at the cortico-medullary zone in rat which acted in the disposal of degenerating lymphocytes and in the maintenance of blood thymus barrier by clearing up leakages of macromolecules (Salman and Cardingley, 1979).

Oliver and Dourain (1984) recorded two basic types of accessory cells, namely dendritic cells and macrophages in quail and chick thymus. They considered that the cells of this lineage entered the thymus during initial colonization of the epithelial thymic rudiment of haemopoietic cells.

Milicevic et al. (1987) identified three types of macrophages in rodents; cortical, with enclosed lymphocyte debris; cortico-medullary, with cytoplasmic vacuolar inclusions and medullary type.

Electron microscopy

Frazier (1973) described that the cytoplasm of macrophages of thymus in chicken contained phagocytosed material, vacuoles, granules, mitochondria and
endoplasmic reticulum. The nuclear membrane was often indented by the inclusions. Desmosomes were not present.

Ultrastructurally, resting macrophages had large elongate nuclei and a large volume of cytoplasm. Their ultrastructural appearance was a reflection of variable differentiation pathways, stages of maturation and states of activation in mammals (Unanue et al., 1976).

Macrophages, in the unstimulated state, were large, pale cells with highly developed Golgi complexes and many small primary lysosomes. Lysosomes contained potent acid hydrolases, lysozyme and other enzymes in mammals (Spicer et al., 1977 and Warnock et al., 1987).

Rappolee and Werb (1992) and Cross and Mercer (1993) described in mammals that the lysosomes were particularly developed in the macrophages. They contained accumulations of heterogeneous bodies in secondary lysosomes which represented phagocytosed material in the process of digestion.

2.2.2.3.5. Plasma cell

Light microscopy

Mature plasma cells in small groups of three or four were present throughout the parenchyma of the thymus in chicken (Thorbecke et al., 1957).

In mammalian thymus, Ham (1965) described that the plasma cells were rounded cell with an eccentrically placed spherical nucleus. The chromatin was arranged like the spokes of a wheel and gives the cart-wheel appearance.

Kendall and Frazier (1979) in their studies of avian thymus found plasma cells in almost all the thymic lobes examined. They were always present beneath the capsule in the perivascular space or within the connective tissue septa.
**Electron microscopy**

The Golgi region of the plasma cell was customarily very large and the cell membrane of the plasma cell often extended from the cell in finger-like processes (Movat and Fernando, 1962).

Berek (1992) described that the plasma cell was characterised by a well developed rough endoplasmic reticulum. Antibodies synthesised within the rough endoplasmic reticulum were processed and packaged within the Golgi prior to secretion. In plasma cells, heterochromatin did not reflect inactivity since the small part of the genome that is euchromatic was exceedingly active in maintaining the synthesis of many copies of a single antibody.

**2.2.2.3.6. Mast cell**

**Light microscopy**

Vijayaragavan (1988) reported that mast cells were commonly found in the cortex and medulla of avian thymus and were closely associated with the Hassall’s corpuscle.

The number of mast cells increased with age in the thymus, bursa of Fabricius and spleen in avian species (Karaca et al., 2006).

**Electron microscopy**

Wight and Mackenzie (1970) opined that the mast cells of avian and mammalian thymus were similar in their cytological descriptions both under light and electron microscopioic studies.

Valsala *et al.* (1986) studied the distribution and ultra structure of mast cells in the thymus of duck and found that these cells contained different types of membrane bound granules, some of which were electron dense and mottled. Their plasma membrane showed numerous large thin villi like profile parallel to the cell surface. The prominent structure in the cytoplasm was the presence of membrane bound round or oval granules.
Crivellato et al. (2005) described that the thymic mast cells in chicken were relatively small cells with a few secretory granules in electron microscopic study. Exocytosis was not seen but granules emptied in a piece meal degranulation fashion.

2.2.2.3.7 Granulocytes

Light microscopy

Granulocytes such as basophils, eosinophils and heterophils were commonly present in the medulla of chicken thymus (Lucas and Jamroz, 1961), in normal human thymus (Robert et al., 1978).

A review on the avian thymus gland by Kendall (1980) summarised that the cells characteristic of bone marrow such as small and large eosinophils, basophils and heterophils were found in the thymus of chicken, as in mallards, starlings, house sparrow and red billed queleas, in addition to the normal cellular constituents of the gland.

Electron microscopy

In pigeon, the eosinophils showed marked cytoplasmic lobulation and the characteristic bilobed nucleus. A few small, round mitochondria were present, and in some cells fine fibrils were seen adjacent to the cytoplasmic membrane (Maxwell and Siller, 1972).

Maxwell (1973) studied the heterophil granules of ducks and geese under electron microscope. The nucleus usually had up to three lobes, rich in heterochromatin, and a single nucleolus. The mitochondria were small and sparse. The Golgi apparatus and centrioles, if evident, were usually located centrally. Glycogen was normally present in the form of single granules and in some cases also as glycogen rosettes. Heterophils of both ducks and geese contained many empty smooth-walled vesicles and granules in their cytoplasm.

The avian basophil had a non-lobulated nucleus, normally situated towards one pole of the cell, with small aggregates of heterochromatin attached to its nuclear
membrane. Short strands of rough endoplasmic reticulum and lipid droplets, infrequently seen in heterophils, were not uncommon in basophils. The Golgi apparatus and centrioles were usually on one side of the cell. Mitochondria were few in number and single glycogen particles were much in evidence (Maxwell, 1973).

2.2.2.3.8 Erythrocytes

Light microscopy

Vijayaragavan (1988) described the presence of erythrocytes in various locations of the parenchyma of thymus in chicken. The erythrocytes were more in the medulla than in the cortex and were also present in the interlobular septa of the organ. The erythrocytes revealed various shapes of nuclei and contour of the cells, indicated various stages of development of the cell.

Kendall and Ward (1974) and Kendall (1979) in birds and Geetha Ramesh and Vijayaragavan (1998) in laboratory animals reported that the thymus of birds was capable of producing large numbers of erythrocytes which entered the circulation after physiological stress. In rodents, the small foci of erythropoiesis were widely separated, often scattered throughout the cortex. Erythropoiesis was also noticeable in the thymus of man too (Kendall and Singh, 1980).

2.2.2.3.9 Hassall’s Corpuscles

Light microscopy

Hassall’s corpuscles were found in the medullary tissue of the fowl thymus in an oval or round homogenous eosinophilic mass lined by faint concentric layers of flat reticular cells (Trautmann and Fiebiger, 1957 and Hodges, 1974).

According to Vijayaragavan (1988) the Hassall’s corpuscles in the thymus of white leghorn birds were found commonly in the medulla. The cortical ones were smaller in size with few cells, while the medullary ones constituted of more number of epithelial cells. Their association with macrophages, lymphocytes, mast cells and myoid cells were common. They were also associated with blood sinusoids.
Electron microscopy

Kohnen and Weiss (1964) and Mandel (1968a and b) identified groups of squamous epithelial cells under electron microscopic study of thymus in guinea-pig. These cells were concerned with the formation of Hassall's corpuscles. The prominent features of this cell type were abundance of cytoplasmic fibrils and desmosomes and they resembled keratinising squamous epithelial cells. The nucleus was oval or round and slightly indented. A nucleolus was usually seen and moderate numbers of mitochondria and ribosomes were present in the cytoplasm.

Frazier (1973) described that large Hassall's corpuscles were not often seen in the chick thymus, when present they were composed of concentric rings of squamous epithelial cells interconnected by many desmosomes.

Robert et al. (1978) described that the centre of the Hassall’s corpuscle was either solid or cystic under electron microscope. In the cystic type, the lining epithelial cells had microvilli. The central cells of the corpuscles showed degenerative nuclear and cytoplasmic changes.

2.2.3. Cyst

Light microscopy

Bridges et al. (1970) numerous intercellular cysts in the thymic medulla of cockerel’s up to the age of 41 weeks. The cysts were lined by epithelial cells with micro villi with a mucoprotein coat at the luminal surface.

According to Hashimoto and Sugimura (1976) in white pekin ducks rapid regression of the thymus occurred at 11 weeks of age by the formation of great number of cysts, in addition to the depletion of the cortical lymphocytes. Normally, the medulla of thymus consisted of small and large types of cystic structures, along with myoid cells and Hassall’s corpuscles.
Electron microscopy

Isler (1976) identified three types of epithelial cysts in the thymus of fowl. Largest being made-up of stratified columnar epithelium contained lymphoid cells. The cells bordered the lumen were columnar and their micro villi formed a brush border. The wall of the cyst contained lymphocytes and granular cells. Often the lumen of the cyst was filled with flocculent material and cell debris. Smaller vesicles without lymphoid cells were made up of a simple or stratified epithelium which contained typical mucous cell or columnar cell similar to the bordering cells of the larger cysts. The unicellular cysts of the reticuloepithelial cells were small and without microvilli. The unicellular cysts were presumed to be capable of producing factors to regulate the cell mediated immune response.

Chan (1986) observed variable number of intercellular and intracellular cysts in the medulla of two week-old chicks. The lining epithelium of the intercellular cysts varied from squamous to columnar or stratified and contained cystic cells, endocrine-like cells and lymphoid cells. Their luminal surface contained microvilli and occasional cilia. Endocrine-like cells were separated from the lumen by the cystic cells and their cytoplasm was characterized by the presence of membrane bound secretory granules. Cystic cells with single or multiple cysts, lined with microvilli, were also observed.

2.2.4. Involution

par Rodica Giurea (1977) observed that no thymic involution started until at least four months of age in chicken.

Involuntary changes of the thymus gland in birds were characterized by the thickening of the capsule, loss of interlobular septa, pyknosis of the thymocytes, extension of the area of the medulla, increased in size and number of Hassall’s corpuscles (Bhattacharya and Binaykumar, 1983).

Steinmann (1986) and Marinova (2005) described that in human, age related involution was characterized by a progressive reduction in size and weight due to the
loss of both thymic lymphocytes and stromal cells such as epithelial cell, dendritic cell and macrophages.

In the thymus of *Gallus domesticus*, the medullary thymic epithelial cells and cortico-medullary macrophagic cells were reduced during involution (Ciriaco *et al.*, 2003).

2.3. Caecal tonsil

Light microscopy

Caecal tonsils were the lympho-myeloid aggregations located at the proximal end of the each of the caecal pouches in chicken (Muthmann, 1913; Looper and Looper, 1929; Calhoun, 1932 and Payne, 1971).

The germinal centres of the caecal tonsils closely resembled the germinal centre of the spleen and were composed of lymphoblast, lymphocytes and primitive reticular cells. Plasma cells, mast cells and macrophages were also present. (Jankovic and Mitrovic, 1967; Payne, 1971 and Hoshi and Mori, 1973).

In *Gallus*, depending upon the age of the bird, the lymphoid aggregations occupied the initial 4-10 mm of the caecum (Glick *et al.*, 1978).

Olah and Glick (1979) observed two kinds of germinal centres in the chicken caecal tonsil under light microscope. The first type was located deep in the lymphatic tissue close to the muscle layer of the caeca. These germinal centres frequently had an incomplete capsule, and were not, therefore, separated from the diffuse lymphatic tissue. The second type of germinal centre was located closer to the epithelium and had a complete capsule separating it from the diffuse lymphatic tissue. Both the types of germinal centres possessed an outer dark cortical zone which contains smaller lymphoblasts and a central medullary area which contained larger lymphoblasts.

Rose (1981) and Bar-Shira and Friedman (2005) stated that in chicken large aggregates of lymphoid tissue occurred near the junction of each caecum with colo-rectum called as caecal tonsils.
Glick et al. (1981) considered that the structural design of the nodular units in chicken caecal tonsil corresponded to that of the mammalian palatine and lingual tonsils.

The lymph node tissues of pigs were also made-up of nodular units in a similar structural design (Hoshi et al., 1986). del Cacho et al. (1993a) described the presence of the follicular dendritic cells and interdigitating dendritic cells in the germinal centre of caecal tonsil in chicken. They also described that in the chicken caecum, lymphoid nodules were found in the distal region approximately three centimetres from the ilo-caecal junction.

Bar-Shira and Friedman (2005) opined that the chicken caecal tonsil contained predominantly T lymphocytes principally CD4+ cells from four days to two weeks of age. At six weeks of age, B-lymphocytes predominated, which occurred in both germinal centre and in sub epithelial zone. At eight weeks of age, CD8+ lymphocytes gradually became more numerous than CD4+.

Kitagawa et al. (1998) described that the caecal tonsil in chicken was made up of a fundamental structure, the nodular units. Each nodular unit was demarcated by narrow loose connective tissue. A fossula was observed in each nodular unit; the fossula was located centrally at varying depths and was surrounded by subepithelial lymphoid tissue with peripherally situated germinal centres. Several intestinal crypts originated from each fossula towards the peripheral margin of the nodular units. Epithelial mitoses were not observed in the fossulae but were frequent in the intestinal crypts.

Upon maturity of the immune system, most of the immunological activity with in the chick GALT was concentrated in the hindgut, specifically in the caecal tonsil and bursa of Fabricius (Bar-Shira and Friedman, 2005).

Electron microscopy

Olah and Glick (1979) described the electron microscopic structure of the germinal centre of chicken caecal tonsil. The capsule of the germinal centre consisted
of several layers of flattened and elongated reticular cells which were separated with an intercellular substance. The number of reticular cells in the cortical zone was higher than in the lighter medullary area. Ultra structurally, the reticular cells of the germinal centre were similar to that of the cells of the capsule. Apart from the reticular cells, the germinal centre consisted of large and small lymphoblasts and macrophages. A small lymphocyte-like cell was also observed in the germinal centre of chicken caecal tonsil. A cell containing granules of possible secretory nature had also been identified in the central zone of germinal centre of the caecal tonsil as well as the germinal centre of spleen and intramural nodes.

Microvilli of M cells were generally sparse and low compared with those of adjacent microvillous epithelial cells in man (Owen and Jones, 1974), in mice (Owen, 1977), in guinea-pig (Rosner and Keren, 1984) and in cattle (Parsons et al., 1991).

In rabbits, the microvilli were sparse and low in peyer’s patches (Neutra et al., 1987) but were tall and thick in caecal lymphoid nodules (Gebert and Hach, 1993 and Gebert and Bartels, 1995). These morphological characteristics of mammalian M cells, except for caecal lymphoid nodules of rabbit, were consistent with those of chicken M cells.

Jeurissen et al. (1999) observed M cells in the Meckel’s diverticulum and caecal tonsils of chicken under electron microscope. M cells were characterized by their short and irregular microvilli that discriminated the M cells from its neighbouring epithelial cells. The M cells were connected to neighbouring epithelial cells by desmosomes. Owen and Jones (1974) observed similar electron microscopic findings in M cells of human peyer’s patches.

The level of the nuclei of chicken caecal M cells differed from that of mammalian M cells, whose nuclei were situated from the middle to basal cytoplasm (Bye et al., 1984 and Uchida, 1987).
B. Immunohistochemistry

Most CD4^+CD8^- cells were helper/inflammatory T cells responding to exogenous antigen in association with major histocompatibility complex (MHC) class-II molecules; whereas, CD4^-CD8^+ cells are helper/inflammatory T cells responded to exogenous antigen in association with MHC class-I molecules and generally functioned as cytotoxic T cells (Littman, 1987 and Norment et al., 1988).

The tissue distribution of avian CD4 and CD8 molecule was very similar to that of mammalian CD4 and CD8 (Cooper et al., 1991 and Luhtala, 1997).

CD8 was a cell surface glycoprotein expressed primarily on thymocytes and cytotoxic T-lymphocytes (Parnes, 1986 and Fung-Leung et al., 1991). During T cell differentiation, CD8 was involved in positive and negative selection of thymocytes (Blackman et al., 1990 and Von Boehmer, 1994).

Similar to mammalian species, chicken T cell populations could be delineated into subsets based on their expression of cell-surface proteins. T cell subsets were broadly defined by their expression of molecules such as CD4, CD8 and T cell receptor (TCR). Each individual T cell expressed only one type of TCR on its surface (Cooper et al., 1991 and Gobel, 1996).

Major differentiation events regarding T cell development in the thymus appeared to be conserved between chickens and mammalian species. In chickens, CD4^-CD8^- thymocytes gave rise to CD4^-CD8^+ thymocytes, which gave rise to CD4^-CD8^- or CD4^-CD8^+ T cells. Within all CD4^+ and/or CD8^- thymocytes subsets, all types of TCR could be detected (Davidson and Boyd, 1992).

In Aves, mature CD4 or CD8 single-positive T cells from the thymus, populated in the secondary immune organs and travelled in the circulatory and lymphatic system. In the blood, CD4^-CD8^-TCR1^+ T cells as well as CD4^-CD8^- and CD4^-CD8^- T cells expressing either TCR2 or TCR3 had been identified. The same T cell population could be found in the spleen with addition of CD4^-CD8^-TCR1^+ T cells (Sowder et al., 1988 and Gobel, 1996).
In addition to marking the stage of T cell differentiation, the expression of certain cell-surface proteins could be related to the functional role of a T cell. T cell expressing both CD4 and CD8 molecules were considered immature T cells and they constituted the majority of cells in the thymus. Single-positive T cells, expressing either CD4 or CD8, were mature T cells in chicken (Gobel, 1996).

Kon-Ogura et al. (1993) depicted 17.32 per cent of CD4$^+$ and 29.62 per cent of CD8$^+$ in the spleen of seven weeks age white leghorn chicken by using flow cytometry analysis with monoclonal antibodies.

In healthy egg-type (Davidson and Boyd, 1992) and broiler (Erf, 1997) chickens, double-positive (CD4$^+$CD8$^+$) lymphocytes in the thymus (thymocytes) constituted the majority of thymocytes (>60 %).

Erf (1997) recorded that the proportions among CD4 and/or CD8 defined T cell populations in the thymus were not different at one or two week-old single comb white leghorn (SCWL) and broilers. By four weeks of age, the broilers had a lower percentage of double positive (CD4$^+$CD8$^+$) thymocytes and a higher percentage of CD4$^-$CD8$^+$ thymocytes than the SCWL chicks. Spleen from the broilers consistently contained a lower percentage of CD4$^+$CD8$^-$ T cells than those from SCWL chickens, whereas no strain differences were observed in the percentages of CD4$^-$CD8$^+$ and CD4$^+$CD8$^+$ T cells.

Erf et al. (1998) found that the ratio between CD4 and CD8 cells in thymus increased from two to seven weeks aged broiler chicken. In seven week old chicken, the per cent of CD4$^+$CD8$^-$ thymocytes was higher and the per cent of CD4$^+$CD8$^+$ thymocytes was lower as compared to two week old chicken. There was no difference in the per cent of CD4$^+$CD8$^+$ thymocytes between the two age groups. The ratio of CD4 and CD8 in spleen decreased with age from 0.44 in two week-old chicks to 0.12 in seven week-old chicken. In spleen of seven week-old chickens, the per cent of CD4$^+$CD8$^-$ lymphocytes was lower and the percent of CD4$^+$CD8$^+$ and CD4$^+$CD8$^+$ lymphocytes were higher than in two week old chicks.
Khan et al. (1998) studied the postnatal development of T cell subpopulation in the lymphoid organs of white leghorn chickens, using an immunohistochemical method. The chicken lymphoid organs were very small at hatch. Their further growth was correlated with the advancement of age of the chicken. CD3, CD4 and CD8 cells were the highest in number at 15 weeks. A significant decline was observed in T cell subsets at 78 weeks of age. T cell subpopulations in the lymphoid organs of postnatal white leghorn chickens peaked at about 15 weeks of age, indicating age-related development of T cells in these organs.

C. Experiment

Rose (1978) and Rose et al. (1979) carried out an experiment and evaluated the roles of T and B lymphocytes in immunity to infections with *Eimeria* species in thymectomized or bursectomized chickens.

Fitzgerald (1980) stated that the Apicomplexan protozoa of the genus *Eimeria* were the common cause of coccidiosis in domestic animals following ingestion of infective oocysts. Coccidial parasites underwent a complex life cycle ultimately impairing the gastrointestinal tract and resulted in nutrient malabsorption, body weight loss and in severe cases, death.

Cell mediated immunity (CMI) played a major role in host protection against coccidiosis in chickens (Bhogal et al., 1989; Martin et al., 1995 and Lillehoj and Trout, 1996) and mice (Rose et al., 1990 and Yun et al., 1998 and 2000).

Findly et al. (1993), Lillehoj (1994) and Bessay et al. (1996) studied the alterations in lymphocyte subpopulations and cytokine production during *Eimeria* infection in chicken and mice and investigated to clarify the nature of protective immunity.

Breed at al. (1997), Lillehoj and Choi (1998) and Choi et al. (1999) confirmed that gamma interferon was an important component in host protective cell mediated immunity in *Eimeria* infection in chicken.
Both macrophages and lymphocytes were the source of cytokine production in the intestine during *Eimeria* infection and thereby modulate the immune response (Lillehoj, 1994 and Beattie et al., 2001).

The invasion of the *Eimeria* sporozoites into the intestinal epithelium resulted in massive infiltration of macrophages, granulocytes and lymphocytes into the lamina propria. The macrophages modulated the severity of the infection, and the lymphocytes, in particular CD4 T cells, acted as inducer of an effective immune response (Jeurissen and Veldman, 2002).

Cuppen et al. (2006) conducted an experiment in chickens infected with a mixture of *Eimeria* by a single dose administered through gavage into the crop.

Infection by *Eimeria* promotes antibody and cell mediated immune responses. However, cellular immunity mediated by various cell populations, including T lymphocytes, NK cells and macrophages, played a major role in disease resistance (Lillehoj and Trout, 1996). There was increasing evidence of CD4 and intraepithelial lymphocyte (IEL) involvement during a primary infection, while T cell receptor alpha and beta chain positive CD8 IEL played a key role in secondary infection (Lillehoj, 1998).
CHAPTER-3

MATERIALS AND METHODS

Materials

The present study on electron microscopic and immunohistochemical studies of spleen, thymus and caecal tonsils in layer chicken was conducted at the Department of Veterinary Anatomy and Histology, Madras Veterinary College, Chennai 600 007. The birds for this study were procured from Poultry Research Station, Nandhanam, Chennai – 600 035.

Materials for electron microscopic and immunohistochemical studies were collected from six different age groups such as day-old, four, eight, twelve, twenty and forty weeks. Six birds were used in each age group.

Methods

Light microscopy

Tissue pieces were collected from spleen, thymus and caecal tonsils and were rinsed in normal saline and fixed in 10 per cent neutral buffered formalin and Bouin’s fluid. The fixed tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (Bancroft and Stevens, 2007). Tissue sections were cut at 3-5 micron thickness and used for the routine Haematoxylin-eosin staining method (Singh and Sulochana, 1978) and Masson’s trichrome method for collagen and muscle fibres (Luna, 1968).

Electron microscopy

Tissue samples from all the six age groups were collected for transmission electron microscopic studies. Small pieces of spleen, thymus, caecal tonsils (1-2 mm thickness) were collected and prefixed at 3 per cent glutaraldehyde and stored at 4\degree C. Subsequently, the tissues were washed, three changes (each 30 minutes) in cold sodium cacodylate buffer solution (pH 7.4) and post fixed in 1 per cent osmium tetroxide for two hours at 4\degree C. The tissues were then dehydrated in ascending grades
of alcohol (50, 70, 80, 90, 95 per cent and absolute ethyl alcohol), propylene oxide: epoxy resin mixture and embedded in Epon-araldite mixture. Semi thin (1 micron) sections were stained by toluidine blue. Ultra thin sections (600 Å to 900Å°) were prepared on Leica ultracut microtome, mounted on uncoated copper grids and stained with saturated solution of uranyl acetate and lead citrate. The ultra thin sections were examined under Phillips (Teknai-10) computer augmented transmission electron microscope operated at 60-kilowatt ampere (KVA).

Immunohistochemical study

CD4, CD8 count in spleen and thymus from the birds of different age groups under study was estimated by flow cytometry.

3.2.3.1 Preparation of lymphocytes from spleen and thymus

Lymphocyte suspensions were prepared from spleen and thymus as per the method of Wu et al. (2000) and the cell concentration was adjusted to 1.5 x 10^6 cells/ml in RPMI-1640 medium.

Protocol

4 The spleen and thymus were aseptically collected in RPMI-1640 medium containing antibiotics.
5 The spleen and thymus thus obtained were washed three times in medium containing antibiotic.
6 The capsule of these organs was then carefully removed using sterile forceps and scissors.
7 The spleen tissue was then teased well by using the scalpel blade and was filtered through cheese cloth.
8 The filtrate was then centrifuged at 1500 rpm for five minutes.
9 The supernatant was collected and centrifuged at 550 g for 15 minutes in the present work against 550 g for ten minutes as per the method of Wu et al. (2000).
10 The cell pellet was then washed two times with FACS buffer (PBS+3% horse serum + 0.1% sodium azide).
11 Ten microlitre of cell suspension was mixed with 10 microlitre of one per cent trypan blue to check the live and dead cells.
12 The cell concentration was adjusted to $1 \times 10^6$ cells/ml in FACS buffer.

3.2.3.2 Identification of lymphocyte subsets by immunofluorescence using flow cytometry

A single colour immunoflourcence staining procedure was followed as per Chan et al. (1988) with the following procedure.

1. 100 micro litre of cell suspension was taken in three microcentrifuge tubes (control without monoclonal antibodies, CD4 and CD8)
2. The cells were pelleted by centrifugation at 800 rpm for five minutes at 4°C.
3. Supernatant was discarded and ten micro litre of monoclonal antibodies (1 in 100 dilution in PBS) was added in CD4 and CD8 tubes except in the control tube. The control tube was added with 10 micro litre of FACS buffer. Mouse monoclonal antibodies such as mouse anti-chicken CD4: FITC (Cat. No. Serotec MICA 2164F) and mouse anti-chicken CD8a: FITC (Cat. No. Serotec MCA-2166F) that recognize the avian CD4 and CD8 respectively were used.
4. Tubes were mixed gently and incubated at 4°C for 45 minutes in dark.
5. After incubation the cells were washed with the 100 micro litre FACS buffer and pelleted by centrifugation at 800 rpm for 5 minutes.
6. The pelleted cells were then resuspended in 500 micro litre of FACS buffer. The cells were kept in ice until it was read in the flow cytometer.
7. The cells were then read in the FACS caliber Flow cymeter (Becton and Dickinson, USA).
8. The lymphocytes were gated based on the FSC and SSC characteristics and using the PBS control cells the background florescence was set as M1 and the fluorescence was set as M2.
9. Percentage of M2 values for different monoclonal antibodies was recorded from the single parameter histogram.

The ratio of CD4 and CD8 was calculated by dividing percentage of CD4$^+$CD8$^-$ cells by percentage of CD4$^-$CD8$^+$ cells.
Statistical analysis was carried out using the SYSTAT statistical analysis software (Systat Inc., Evanston, IL). Test results were considered significant of P<0.01. All data were expressed as the mean + SEM.

3.2.4 Experiment

Birds

Fifty white leghorn chicks were obtained from a commercial hatchery and reared in coccidian free environment until they were 15 days of age. Chicks were fed with coccidiostat free diet.

Parasites

Sporulated oocysts of *Eimeria tenella* was obtained from the Department of Veterinary Parasitology, Madras Veterinary College, Chennai 600 007.

Experimental design

On 15th day, fifty birds were divided into three groups consisted two infected and one control group.

Group-I

Ten birds served as non-infected control. Spleen and thymus from the control birds were removed at five, six and nine days post inoculation and flow cytometric analysis of CD4, CD8 cells was done.

Group-II

A single dose of $2 \times 10^4$ oocysts was given to twenty birds. Spleen and thymus were removed at five, six and nine days post inoculation and flow cytometric analysis of CD4, CD8 cells was done.
Group-III

Twenty birds were trickle infected with the split dose of 6750, 6750 and 6500 oocysts on three continuous days. Spleen and thymus were removed at five, six and nine days post inoculation and flow cytometric analysis of CD4, CD8 cells was done.

CD4, CD8 count in spleen and thymus from different groups was estimated by using a FACSCAN flow cytometer (Becton and Dickinson). Statistical analysis was carried out using the SYSTAT statistical analysis software (Systat Inc., Evanston, IL).
CHAPTER-4

OBSERVATIONS

A. Histomorphology

4.1 Spleen

4.1.1 Capsule and Trabeculae

Light microscopy

In day-old chicken, the spleen was found enclosed by a fibrous capsule which had a flattened layer of mesothelium externally (Plate-1). The capsule was noticed mainly with collagen fibres and few elastic and smooth muscle fibres in all the age groups studied. The cellular components of the capsule included the smooth muscle cells and fibroblasts (Plate-2). The thickness of the capsule was increased with more collagen fibres as age advanced (Plate-3). In the present study, trabeculae were poorly developed in all the age groups studied.

Electron microscopy

In all the age groups, the splenic capsule was composed of collagen bundles and a few elastic fibres. The capsule was also observed with smooth muscle cells and fibroblasts. These cells were stellate shaped (Plate-4) with fibrillar cytoplasm and mitochondria. External surface of the capsule was observed to be lined by a single layer of mesothelial cells with small microvillous projections at their free border. The inner surface of the capsule was found to have subcapsular sinus lined by endothelial cells, filled with erythrocytes (Plate-5). The presence of collagen fibres increased as age advanced in the present study.

4.1.2 Parenchyma

The parenchyma of the spleen was observed to have stroma consisted of red pulp, white pulp and blood vessels.
4.1.2.1 White pulp

**Light microscopy**

The white pulp appeared as islands enclosed by red pulp and the distinction between the two pulp was not marked in the present study (Plate-6).

Generally, the major cellular population of the white pulp included small, medium and large lymphocytes and stellate shaped reticular cells with cytoplasmic processes in the present study. Macrophages, plasma cells and fibroblasts were also noticed (Plate-7 and Plate-8).

The white pulp was composed of lymphocytes of various sizes and reticular cells. These cells were found to be diffusely distributed as clusters or follicles of formative stage in day-old and four week-old chicken (Plate-1). At the periphery of these follicles, arteries were observed (Plate-9).

From the age of eight weeks onwards more number of lymphoid follicles were seen and the number was observed to increase as age advanced (Plate-6).

In twenty week-old birds, round lymphoid follicles surrounded by a thin fibrous covering were noticed with smaller arteries at their periphery (Plate-9).

The white pulp was observed with numerous small lymphocytes around the central artery (Plate-10) as age advanced. The central artery of the lymphoid follicles of the white pulp continued as penicillar artery and observed at the periphery of the white pulp as arterioles.

**Electron microscopy**

In all the age groups studied, white pulp of the spleen was observed with predominant lymphocytes of various sizes and reticulum cells. These cells were arranged in the form of clumps (Plate-11). These clumps were separated by a meshwork composed of collagen, fibroblasts and reticulum cells (Plate-12). Germinal centres were not observed in all the age groups of the present study.
In day-old and four week-old birds, the lymphoblasts and small lymphocytes were observed more. In eight week-old birds, all the types of lymphocytes such as small, medium and large lymphocytes were noticed (Plate-13). Whereas in twenty week-old birds, the existence of large lymphocytes predominated and in forty week-old birds, the depletion of lymphocytes and the amount of collagen was maximum in the stroma was observed (Plate-14).

The lymphoblasts were characterised by large euchromatic nuclei and predominance of free poly-ribosomes. The small and medium sized lymphocytes were found to be round to oval shaped and were seen with high nuclear-cytoplasmic ratio. The cytoplasm was sparsely seen with occasional little perinuclear rough endoplasmic reticulum, a few ribosomes and mitochondria. The smooth endoplasmic reticulum was seen in the form of small vesicles dispersed in the cytoplasm and the Golgi zone was not prominent. Nucleus was observed mostly round or slightly indented with heterochromatin (Plate-15).

The reticulum cells were stellate shaped, they had smaller nuclear-cytoplasmic ratio and more organelles in the cytoplasm (Plate-16). Rough endoplasmic reticulum was found to be more (Plate-17). Well developed mitochondria, ribosomes and a prominent Golgi zone were also observed. The presence of reticulum cells were commonly observed in all the age groups studied.

The fibroblasts appeared flat and stellate with long cytoplasmic prolongations. They had rough endoplasmic reticulum seen filled with a fine flocculent material (Plate-18). The smooth endoplasmic reticulum also had similar material and some of them appeared fibrillar. The presence of fibroblasts were noticed in all the age groups studied.

The follicular dendritic cells were seen in all the age groups and confirmed by their large and irregular shaped nucleus. It had a little heterochromatin. The cytoplasm was found to be ramified as very thin processes in many directions (Plate-19). Some of the processes were observed long and straight. Mitochondria, rough and smooth endoplasmic reticulum, vesicles were rarely seen within the cytoplasm.
The plasma cells of the spleen were observed with well developed rough endoplasmic reticulum. There were larger and denser mitochondria and numerous ribosomes. The heterochromatic nucleus was eccentrically placed (Plate-20).

The macrophages were ovoid or stellate shaped and contained vacuoles and phagocytosed material. The nucleus was heterochromatic (Plate-21).

4.1.2.2 Periarterial lymphatic sheath

Light microscopy

Periarterial lymphatic sheath (PALS), a diffuse lymphatic sheath was observed adjacent to the central artery in all the age groups studied. It consisted of closely packed small lymphocytes and several medium to large sized lymphocytes. Few macrophages and plasma cells were occasionally found towards the periphery of the periarterial lymphatic sheath (Plate-22).

Electron microscopy

The white pulp contained the central artery which was observed to have smooth muscle layer. The lumen of the artery was lined by endothelial cells and muscle cell (Plate-23). Adjacent to the central artery, a diffuse lymphatic tissue, the periarterial lymphatic sheath was noticed with densely packed lymphocytes of various sizes and was also infiltrated with reticular cells (Plate-24). There were no structural changes noticed in periarterial lymphatic sheath of spleen in different age groups. A few macrophages were also observed in the PALS. Very few reticular fibres formed a meshwork around the periarterial lymphatic sheath separated it from the adjacent red pulp.

Nerve fibres were seen in close association with the arteries of the white pulp.
4.1.2.3 Red pulp

Light microscopy

In the present study, the red pulp was found to have splenic cords and venous sinusoids (Plate-2). In all the age groups, the splenic cords consisted of erythrocytes, reticular cells, lymphocytes, macrophages, plasma cells (Plate-25) and mast cells (Plate-26).

In the present study, the arterioles from the periphery of the white pulp observed to enter the red pulp as sheathed capillaries. These capillaries were found to be surrounded by reticular cells and macrophages formed the ellipsoids.

Electron microscopy

The splenic red pulp was composed of anastomosing sinuses lined by endothelial cells were noticed (Plate-27). These sinuses were found to be separated with each other by the pulp cords. These pulp cords consisted of erythrocytes, reticular cells, lymphocytes of various sizes, macrophages, granulocytes, plasma cells and mast cells (Plate-28 and Plate-29).

The erythrocytes were present both in the sinuses and in the pulp cords which had different shapes. Macrophages were seen with phagocytic vacuoles which contained the degraded erythrocytes or leukocytes (Plate-29). The structure of reticulum cell and lymphocytes were similar to that present in the white pulp.

The arterioles from the periphery of the white pulp were found to enter into the red pulp. In these arterioles, the lumen was surrounded by muscle cell and these arterioles continued into the red pulp and formed sheathed capillaries or ellipsoids. These ellipsoids were found to have a meshwork of polymorphic reticular cells, reticular fibres and a few macrophages.
4.2 Thymus

4.2.1 Capsule

Light microscopy

In chicken, the thymic gland was surrounded by a thin connective tissue capsule composed mainly of collagen fibres and a few elastic fibres. The connective tissue septa, from the capsule entered the gland and divided it into the lobules (Plate-30).

4.2.2 Parenchyma

The parenchyma was observed to have a dark outer cortex and a pale inner medulla within the lobule in day-old, four weeks and eight weeks of age groups (Plate-31).

4.2.2.1 Cortex

The outer cortex was packed with numerous lymphocytes of various sizes in all the age groups. Reticuloepithelial cells were also present in the cortex which formed the Hassall’s corpuscles.

4.2.2.2 Medulla

The inner medulla was noticed with various sizes of lymphocytes with predominant lymphoblasts in younger age groups. Numerous reticuloepithelial cells which formed the Hassall’s corpuscles were also noticed (Plate-31).

4.2.2.3 Cellular components

Light microscopy

Small and medium sized lymphocytes were found to be numerous and tightly packed in the cortex than the medulla in all the age groups studied. Lymphoblasts were noticed in the younger age groups.
The reticuloepithelial cells were stellate shaped with large nucleus and eosinophilic cytoplasm and found more in the medulla (Plate-32).

In all the age groups studied, myoid cells were noticed in the medulla. The myoid cells were seen as elongated, spindle shaped cell with striations (Plate-33). The presence of myoid cells was also confirmed by the special staining technique *viz.*, Masson’s trichrome (Plate-34). Number of myoid cells were found to be more in forty weeks of age.

Macrophages were also seen as one of the cellular components both in the cortex and medulla of all the age groups of chicken (Plate-35).

Mast cells and plasma cells were observed throughout the thymic parenchyma. Increased number of mast cells was observed in four week-old birds. Number of plasma cells were more in twenty week-old birds.

Various forms of granulocytes such as basophils, eosinophils and heterophils were commonly seen in the medulla of chicken. The presence of erythrocytes was observed in a small number in various locations of parenchyma in all the age groups studied. The erythrocytes were found to be more in the medulla than in the cortex (Plate-36).

The Hassall’s corpuscles were found to be a round, homogenous eosinophilic mass lined by flat reticular cells (Plate-37). Hassall’s corpuscles were commonly observed in the medulla of chicken thymus. However, the presence of Hassall’s corpuscles was also noticed in the cortical areas of day-old, four weeks and ten weeks age groups (Plate-31). The corpuscles present in the cortex were smaller in size with few cells whereas the medullary ones were larger with more number of reticuloepithelial cells.

The number of corpuscles were found to be more as age advanced, where hyalinised mass in the centre with concentric layers of reticuloepithelial cells were noticed (Plate-38). The corpuscles were normally associated with blood sinusoids.
The presence of unicellular cysts were common in all the age groups studied which occurred either free in the parenchyma or associated with the Hassall’s corpuscles (Plate-36). Multicellular cysts were noticed in the advanced age groups of chicken in the present study.

**Electron microscopy**

In the present study, the lymphocytes or thymocytes, reticuloepithelial cells, myoid cells and macrophages were observed as the predominant component of the chicken thymus in all the age groups. The other cell types observed were granulocytes, mast cells and plasma cells which were occasionally seen.

Lymphocytes were more numerous in the cortex than in the medulla. These cells had a thin rim of cytoplasm around a nucleus with clumped chromatin.

In the present study, small and medium lymphocytes were round cells with a narrow rim of cytoplasm which contained a few mitochondria and rough endoplasmic reticulum (Plate-39). Ribosomes were observed more and lysosomes were occasionally seen. However, the medium lymphocytes had moderately wide band of cytoplasm with better developed Golgi complex which was observed smaller in the small lymphocytes. Nuclear chromatin was found densely packed at the periphery of the nucleus which was more condensed in the small lymphocyte than the medium lymphocyte (Plate-40).

A greater proportion of large lymphocytes were seen in the medulla of the thymus in the present study. The nuclei of these cells contained one or more nucleoli and the chromatin was found to be less condensed (Plate-41). The cytoplasm was observed to be pale and a few strands of rough endoplasmic reticulum were present. The cytoplasmic-nuclear ratio was larger than that of small and medium sized lymphocytes.

Three types of reticuloepithelial cells were observed in the present study. The first type of epithelial cells seen in the cortex had long cytoplasmic processes. They had a pale nucleus which contained one or two nucleoli. The nuclear membrane
was observed to be intended (Plate-42). The cytoplasm also appeared pale with a few mitochondria, Golgi apparatus, ribosomes and vacuoles.

The second type of epithelial cell observed in the cortex had a much darker nucleus with an irregular outline and the nucleus was observed to be elongated (Plate-42). The cytoplasm was darker. However, vacuoles similar to the one present in the pale reticuloepithelial cells were observed in this type.

The first type of reticuloepithelial cells were more commonly observed in the cortex whereas the second type was also observed in the medulla.

A third type of reticuloepithelial cell was also observed in the cortico-medullary junction and in the medulla. These cells had a pale, oval nucleus which contained one or two nucleoli. Indentation of the nuclear membrane was not observed (Plate-43). The cytoplasm had mitochondria, ribosomes, a few rough endoplasmic reticulum and small, dark granules.

The myoid cells of the chicken thymus were found mainly in the medulla in all the age groups studied. The cytoplasm of these myoid cells contained skeletal muscle (Plate-44). The other organelles observed in the cytoplasm were few mitochondria and smooth endoplasmic reticulum. The myofibrils occupied greater part of the cell. The nucleus was found to contain dispersed chromatin with condensed chromatin.

Small bundles of unmyelinated nerves were observed in the parenchyma of the thymus in the present study (Plate-45).

Macrophages were observed both in the cortex and medulla of all the age groups. The cytoplasm had vacuoles, phagocytosed materials, granules, mitochondria and endoplasmic reticulum. The nucleus appeared round to oval in shape with little chromatin.

The plasma cells were observed within the connective tissue septa of all the age groups. The cells showed a very large Golgi region and well developed rough
endoplasmic reticulum within the cytoplasm. Conspicuous euchroamtin of the nucleus was a common feature of the plasma cell (Plate-46).

Mast cells in the chicken were small cells with a few secretory granules in the cytoplasm (Plate-47).

Granulocytes such as heterophils, eosinophils and basophils were commonly observed in the medulla of the chicken thymus. The cytoplasm of the heterophil had small and sparse mitochondria. The Golgi apparatus were located centrally. The nucleus was observed to be rich in heterochromatin and had two to three lobes and a single nucleolus.

Eosinophils present had a few small round mitochondria in the cytoplasm and characteristic bilobed nucleus.

The cytoplasm of the basophil had rough endoplasmic reticulum and lipid droplets. The Golgi apparatus was usually observed on one side of the cell. Mitochondria were few in number. The nucleus was non-lobulated, observed towards one pole of the cell with small aggregation of heterochromatin.

The erythrocytes were commonly seen in the medulla which were found to have irregular shapes (Plate-48).

The Hassall’s corpuscles were composed of reticuloepithelial cells interconnected by many desmosomes. These epithelial cells had abundance of cytoplasmic fibrils and desmosomes with few mitochondria and ribosomes. The nucleus was oval or round which was slightly indented (Plate-49). A nucleolus was also observed. The centre of the Hassall’s corpuscles was appeared either solid or cystic. The cystic corpuscles observed had cell debris within the cyst lumen (Plate-49 and Plate-50). However, some of them were seen empty. The lining epithelial cells had microvilli.

In the present study, two types of vesicles or cysts were observed in association with the Hassall’s corpuscles in all the age groups. Many epithelial cells in
the medulla formed the unicellular or intracellular (Plate-51) and multicellular or intercellular cysts. The cytoplasm of the intracellular cysts contained mitochondria, endoplasmic reticulum, Golgi apparatus, ribosomes, some small dense granules. The nucleus was pale, oval and had one or two nucleoli. These cystic cells were found attached to the neighbouring epithelial cells by desmosomes.

The intercellular cysts were found to be formed by two or three cells which varied from very pale to very dark in their appearance. The cytoplasm contained a few mitochondria and ribosomes and very little rough endoplasmic reticulum. The nucleus was found to be oval or spherical (Plate-52). The microvilli was seen projected into the lumen of the cyst. Some of the cysts appeared to be empty while some of them contained mucous droplets in this type of cyst also.

4.2.4 Involution

Light microscopy

The involution was characterized by thickening of the capsule of the thymus with more collagen fibres. Depopulation of cortical lymphocytes, invasion of connective tissue into the parenchyma (Plate-53), increased number of Hassall’s corpuscles and cysts were the features of involuting thymus.

The onset of involution was observed in twenty week-old birds and marked involutary changes were noticed in forty weeks.

Electron microscopy

The involutary changes of the thymus included the regression of lymphocytes the pyknotic nucleus in the present electron microscopic study. Numerous cysts were found in forty week-old birds (Plate-54).
4.3. Caecal tonsil

**Light microscopy**

These were the lymphoid aggregations (germinal centres) located at the junction of caecum and colo-rectum.

Two types of germinal centres were observed in all the age groups studied. The first type had an incomplete capsule and the lymphocytes were diffusely distributed. This type of germinal centre was noticed near the muscular layer of the caeca (Plate-55).

The second type of germinal centre was seen located in the lamina propria and closer to the surface epithelium of the villi of the caecum (Plate-56). This germinal centre was found encapsulated with connective tissue composed mainly of collagen fibres.

The cellular components of the germinal centres included lymphoblasts, lymphocytes of various sizes, reticular cells, plasma cells, mast cells and macrophages (Plate-57).

The number of both the types of germinal centres were found to be increased as age advanced (Plate-58).

**Electron microscopy**

The capsule of the germinal centre of the caecal tonsil of chicken consisted of many layers of flattened reticular cells separated with an intercellular substance. The cytoplasm of the reticular cells showed a number of smooth surfaced vesicles (Plate-59). The cell processes were seen with filamentous substance with small groups of scattered ribosomes.

The germinal centre consisted of reticular cells, large and small lymphocytes and macrophages.
The structure of the reticular cells were similar to that of the reticular cells of the capsule. The number of reticular cells in the periphery of the germinal centre was higher than the central area.

The large lymphocyte had a small amount of cytoplasm with ribosomes and few mitochondria. The nucleus was found to be heterochromatic and a well developed nucleolus. The small lymphocytes were round cells which had numerous ribosomes in their cytoplasm. A patchy chromatin pattern was observed in the nucleus (Plate-60). The other cellular components of the germinal centre included fibroblasts, plasma cells, macrophages and mast cells in all the age groups.

In forty week old-birds, the lymphocytic population was observed to be comparatively reduced and more number of fibroblasts and collagen fibres were noticed (Plate-61).

The caecal tonsil of the chicken also had M cells which were found associated with the surface epithelium of the villi of the caecum. These M cells were found to have short and irregular microvilli and were observed to be connected with the neighbouring epithelial cells by desmosomes. Some of the M cells were columnar shaped and some of them were found to be dome-shaped. The M cells possessed darker cytoplasm but the density was found to be less. The cytoplasmic processes were found to project at the apical surface (Plate-62). Numerous small vesicles with a few ribosomes, rough endoplasmic reticulum and mitochondria were present within the cytoplasm. The nucleus was larger and possessed less heterochromatin with a nucleolus.

The distal part of the villi of the caecum showed many small irregular cavities which corresponded to the lymphatic space of the villi.

**B. Immunohistochemistry**

In the flow cytometric analysis, the CD4 and CD8 count was recorded in the normal spleen and thymus of different age groups of chicken (Table-1).
In day-old birds, the mean of CD4 and CD8 ratio was 0.61 in spleen (Figure-1) and 0.92 in thymus (Figure-2). There was an increase in the ratio of CD4 and CD8 to 1.01 in the spleen (Figure-3) and there was a reduction to 0.67 in the thymus (Figure-4) of four week-old birds when compared to day-old birds.

In eight weeks, the mean of CD4 and CD8 ratio in spleen was 0.95 (Figure-5) and the ratio was found to be 0.72 in thymus (Figure-6) of the same age group.

The mean of the CD4 and CD8 ratio was found to be 0.62 in spleen (Figure-7) and 0.77 in thymus (Figure-8) of twelve week-old birds.

The ratio of CD4 and CD8 was observed to be 0.43 in spleen (Figure-9) and 0.78 in thymus (Figure-10) of twenty week-old birds.

In forty weeks of age, the ratio of CD4 and CD8 was recorded to 0.75 in spleen (Figure-11) and 0.67 in thymus (Figure-12).

The relationship between the mean CD4 and CD8 values in spleen and thymus of different age groups were recorded (Figure-13, 14 and 15).

C. Experiment

Spleen and thymus were collected from control and *Eimeria tenella* (sporulated oocysts) infected (Plate-63) birds and flow cytometric analysis of CD4 and CD8 was done (Table-2).

On five days post infection, the ratio of CD4 and CD8 count in spleen was 0.53 in group-II and 0.62 in group-III birds. However, the control birds (Group-I) showed a value of 0.99 (Figure-16). The ratio of CD4 and CD8 count in thymus was 0.76 in group-I, 0.80 in group-II and 0.77 in group-III (Figure-17).

On six days post infection, the ratio of CD4 and CD8 count in spleen was 0.58 in group-I, 0.38 in group-II and 0.40 in group-III birds (Figure-18). The ratio in thymus was recorded as 0.86 in group-I, 0.71 in group-II and 0.83 in group-III (Figure-19).
On nine days post infection, the ratio of CD4 and CD8 count in spleen was 0.88 in group-I, 0.37 in group-II and 0.44 in group-III birds (Figure-20). In thymus, the mean values were 0.74 in group-I, 0.78 in group-II and 0.81 in group-III (Figure-21).

The CD4 and CD8 per cent in spleen and thymus between groups were analysed statistically by using SYSTAT and the results were tabulated (Table 3).

The mean CD4 percentage of spleen on five days post infection showed a highly increase (P<0.01) trend in group-I (12.233± 1.0644) followed by group-III (9.790± 0.1351) when compared with control group (1.425 ± 0.003).

Statistical analysis revealed a highly significant difference (P<0.01) in the CD8 percentage on five days post infection in group-II (26.990 ± 0.2190) followed by group-III (15.896±0.0210) when compared with control (1.435±0.0034).

Statistical analysis of CD4 percentage in thymus on five days post infection revealed significant increase in both group-II and group-III (71.043 ±7.5031 and 60.763 ± 1.0607) when compared with group-I (0.251 ±0.0016).

Statistical analysis of CD8 percentage in thymus on five days post infection revealed significant increase in both group-II and group-III (78.266 ± 3.1968 and 78.940 ± 1.0808) when compared with group-I (0.333 ± 0.0021).

The mean CD4 percentage in spleen on six days post infection showed a higher trend in group-I (20.956 ± 0.0098). Among groups II and III, group-III revealed significant increase (11.673 ± 0.8781).

Statistical analysis of CD8 percentage in spleen (six days post infection) revealed no significant (P>0.05) variation between group-II and III (24.970 ± 0.4199 and 19.843 ± 5.0952). Whereas, the CD8 percentage was significantly higher in group-I (36.090 ± 0.1680).

Statistical analysis of CD4 percentage in thymus (six days post infection) revealed no significant variation between groups. However a non-significant (P>0.01)
increase in CD4 percentage was noted in group-I (60.540 ± 0.0068) when compared to group-II and III (55.003 ± 5.395 and 58.663 ± 1.7837).

Statistical analysis of CD8 percentage in thymus (six days post infection) revealed no significant variation between groups. However a non-significant (P>0.01) increase in CD8 percentage was noted in group-II (75.893 ± 3.1128) when compared to group-I and III (70.290 ± 0.0051 and 70.960 ± 1.3693).

Analysis of mean percentage of CD4 in spleen on nine days post infection revealed significant increase in group-III (13.773 ± 0.0566), followed by group-II (9.563 0. ± 2394) and group I (2.045 ± 0.0099).

Analysis of mean percentage of CD8 in spleen on nine days post infection revealed a significant increase in group-III (30.190 ± 2.1069) followed by group-II (25.600 ± 0.5075) and group-I (2.330 ± 0.0051).

Though statistical analysis revealed no significant variation between groups in CD4 percentage in thymus on nine days post infection, the mean percentage of group-II and III remain higher.

Statistical analysis revealed no significant difference between groups in CD8 percentage of thymus on nine days post infection.
Figure 1. A representative single parameter histogram for CD4 and CD8 count in spleen of day-old chick assessed by flow cytometry
(M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)

Figure 2. A representative single parameter histogram for CD4 and CD8 count in thymus of day-old chick assessed by flow cytometry
(M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)
Figure 3. A representative single parameter histogram for CD4 and CD8 count in spleen of four week-old chicken assessed by flow cytometry
(M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)

Figure 4. A representative single parameter histogram for CD4 and CD8 count in thymus of four week-old chicken assessed by flow cytometry
(M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)
Figure 5. A representative single parameter histogram for CD4 and CD8 count in spleen of eight week-old chicken assessed by flow cytometry (M1- Population of cells without CD4/CD8 marker, M2- Population of cells with CD4/CD8 marker)

Figure 6. A representative single parameter histogram for CD4 and CD8 count in thymus of eight week-old chicken assessed by flow cytometry (M1- Population of cells without CD4/CD8 marker, M2- Population of cells with CD4/CD8 marker)
Figure 7. A representative single parameter histogram for CD4 and CD8 count in spleen of twelve week-old chicken assessed by flow cytometry (M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)

Figure 8. A representative single parameter histogram for CD4 and CD8 count in thymus of twelve week-old chicken assessed by flow cytometry (M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)
Figure 9. A representative single parameter histogram for CD4 and CD8 count in spleen of twenty week-old chicken assessed by flow cytometry (M1 - Population of cells without CD4/CD8 marker, M2 - Population of cells with CD4/CD8 marker).

Figure 10. A representative single parameter histogram for CD4 and CD8 count in thymus of twenty week-old chicken assessed by flow cytometry (M1 - Population of cells without CD4/CD8 marker, M2 - Population of cells with CD4/CD8 marker).
Figure 11. A representative single parameter histogram for CD4 and CD8 count in spleen of forty week-old chicken assessed by flow cytometry. (M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)

Figure 12. A representative single parameter histogram for CD4 and CD8 count in thymus of forty week-old chicken assessed by flow cytometry. (M1: Population of cells without CD4/CD8 marker, M2: Population of cells with CD4/CD8 marker)
Figure-13 Mean CD4 and CD8 T cells in Spleen of different age groups

Age in weeks

Day-old 4 8 12 20 40

Mean Percentage CD4 CD8

0 5 10 15 20 25 30 35 40 45

Figure-14 Mean CD4 and CD8 T cells in thymus of different age groups

Age in weeks

Day-old 4 8 12 20 40

Mean Percentage CD4 CD8
Figure 15: CD4 and CD8 T cell ratios in spleen and thymus of different age groups.
Figure 16. A representative single parameter histogram for CD4 count in spleen of control and *Eimeria tenella* infected chicken (5 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker).

Figure 17. A representative single parameter histogram for CD8 count in spleen of control and *Eimeria tenella* infected chicken (5 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker).
Figure 18. A representative single parameter histogram for CD4 count in thymus of control and Eimeria tenella infected chicken (5 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker).

Figure 19. A representative single parameter histogram for CD8 count in thymus of control and Eimeria tenella infected chicken (5 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker).
Figure- 20. A representative single parameter histogram for CD4 count in spleen of control and *Eimeria tenella* infected chicken (6 days post infection) assessed by flow cytometry (M1- Population of cells without marker, M2- Population of cells with marker).

Figure- 21. A representative single parameter histogram for CD8 count in spleen of control and *Eimeria tenella* infected chicken (6 days post infection) assessed by flow cytometry (M1- Population of cells without marker, M2- Population of cells with marker).
Figure- 22. A representative single parameter histogram for CD4 count in thymus of control and *Eimeria tenella* infected chicken (6 days post infection) assessed by flow cytometry (M1- Population of cells without marker, M2- Population of cells with marker)

Figure- 23. A representative single parameter histogram for CD8 count in thymus of control and *Eimeria tenella* infected chicken (6 days post infection) assessed by flow cytometry (M1- Population of cells without marker, M2- Population of cells with marker)
Figure 24. A representative single parameter histogram for CD4 count in spleen of control and Elmeria tenella infected chicken (9 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker)

Figure 25. A representative single parameter histogram for CD8 count in spleen of control and Elmeria tenella infected chicken (9 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker)
Figure 26. A representative single parameter histogram for CD4 count in thymus of control and Eimeria tenella infected chicken (3 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker)

Figure 27. A representative single parameter histogram for CD8 count in thymus of control and Eimeria tenella infected chicken (3 days post infection) assessed by flow cytometry (M1: Population of cells without marker, M2: Population of cells with marker)
DISCUSSION AND CONCLUSION

A. Histomorphology

5.1 Spleen

5.1.1 Capsule and Trabeculae

Light microscopy

In fowl, the spleen was invested by a fibrous capsule which consisted of mainly collagen fibres and few elastic fibres in all the age groups studied which was externally limited by a flattened layer of mesothelium. This is in total agreement with the findings of Hodges (1974) in fowl and Brown et al. (1987) in mammals. Suchumzar and Welsch (1987) reported smooth muscle fibres in both capsule and trabeculae of spleen in Antarctic seal.

The cellular component of the capsule consisted of smooth muscle cells and fibroblasts which is in agreement with the findings of Bradley and Grahame (1960) in chicken.

Venkatesan and Vijayaragavan (1997) in Japanese quail opined that well defined trabeculae were absent but thin strands of connective tissue fibres invaded the parenchyma, surrounded the blood vessels which represented the trabecular component. A similar observation was made in the present study where the trabeculae were poorly developed in all the age groups of chicken as also reported by Nasu et al. (1992) in dove. However, Malewitz and Calhoun (1958) contradicts the above statement that the trabeculae were completely absent in chicken. Whereas, Fitzgerald (1969) stated that in Japanese quail, the spleen revealed a tortuous trabeculae and presence of well developed trabecular pattern as in mammals which was responsible for the division of parenchyma into smaller compartments (Brown et al., 1987).
**Electron microscopy**

In all the age groups, the splenic capsule was composed of collagen bundles and a few elastic fibres. The capsule was also observed with smooth muscle cells and stellate shaped fibroblasts with fibrillar cytoplasm which is in agreement with the findings of Burke and Simon (1970) in rabbits.

The external surface of the capsule was observed to be lined by a single layer of mesothelial cells with small microvillous projections at their free border. The inner surface of the capsule was found to have subcapsular sinus lined by endothelial cells, filled with erythrocytes. These findings were in accordance with the findings of Moore *et al.* (1964).

Kimura *et al.* (2001) observed the presence of contractile trabecules in the splenic red pulp of the rat by electron microscopy to elucidate their participation in the active contraction of the spleen. However, in the present study, the trabeculae were not well developed.

5.1.2 Parenchyma

The parenchyma was observed with a stroma consisted of red pulp, white pulp and blood vessels in chicken as reported by Lucas *et al.* (1954), Malewitz and Calhoun (1958) and Bradley and Grahame (1960) in fowl.

5.1.2.1 White pulp

**Light microscopy**

The white pulp appeared as islands enclosed by red pulp and the distinction between the two pulp was not marked in the present study which is similar to the findings of Thorbecke *et al.* (1957), Fukuta *et al.* (1969), Hodges (1974) Miyamoto *et al.*, (1980) and King and Mc Lelland (1981) in birds. This could probably be the reason for clear zonal demarcation of white and red pulp and absence of marginal
zone in the present study as in the spleen of rodents (Jeurissen et al., 1992 and 1994). However, Ford (1975) opined that a marginal zone was present at the junction of white and red pulp in birds.

The major cellular population of the white pulp included small, medium and large lymphocytes and stellate shaped reticular cells in all the age groups. This is in accordance with Olah and Glick (1982) in chicken. Whereas, Hodges (1974) in chicken stated that the white pulp was comparatively a diffuse network of reticular cells within which were scattered numbers of small, medium and large lymphocytes.

In day-old and four week-old chicken, the parenchyma consisted of diffusely distributed clusters or follicles of lymphocytes. At the periphery of these follicles, arteries were observed. These findings were in accordance with Cooper et al. (1965) in birds, Payne (1971) in chicken and Hashimoto and Sugimura (1980) in ducklings.

The cellular population in day-old and four week-old birds was consisted of lymphoblasts, small lymphocytes, reticular cells, heterophils and macrophages. The presence of more number of lymphoblasts, small lymphocytes and few heterophils at this stage reflected the transformation function of the organ from haemopoietic to lymphopoietic side which is in agreement with the report of Lucas and Jamraz (1961) in chicks.

Form the age of ten week onwards more number of lymphoid follicles were seen and the follicles were surrounded by a thin fibrous covering with smaller arteries at their periphery as reported by Cooper et al. (1967), Payne (1971) and Hodges (1974) in chicken.

The white pulp was observed with numerous small lymphocytes around the central artery as age advanced as in birds (King and Mc Lelland, 1981). The central artery of the lymphoid follicle of the white pulp continued as penicillar artery and observed periphery of the white pulp as sheathed capillaries which is in total agreement with the findings of Olah and Glick (1982) in chicken and Raviola (1994) in mammals.
The sheath like arrangement of lymphocytes consisted of small, medium and large sized lymphocytes with few plasma cells and macrophages adjacent to the central artery constituted periarterial lymphatic sheath which is in concurrence with the findings of Olah and Glick (1982) in chicken, Raviola (1994) in mammals and Geetha Ramesh et al. (2001) in laboratory animals.

**Electron microscopy**

In all the age groups studied, white pulp of the spleen was observed with predominant lymphocytes of various sizes and reticulum cells arranged in the form of clumps as reported by Olah and Glick (1982) in chicken and Burke and Simon (1970) in rabbits. However, Djaldetti et al. (1980) described in mouse spleen that the smooth surfaced lymphocytes alone formed the majority in the white pulp.

The lymphoblasts were characterised by large euchromatic nuclei and predominance of free polyribosomes which is similar to the findings of Cross and Mercer (1993) in mammals. This indicated the active stage of the lymphoblast and much of the gene activity during this stage is directed towards the synthesis of protein used in mitosis during the clonal expansion of the activated lymphocyte. These lymphoblasts are thought to be B lymphocytes which can form antibody and or develop into small, memory cells for subsequent antibody responses.

The small and medium sized lymphocytes were found to be round to oval shaped and were seen with high nuclear-cytoplasmic ratio. The cytoplasm was sparsely seen with occasional little perinuclear rough endoplasmic reticulum, a few ribosomes and mitochondria. The smooth endoplasmic reticulum was seen in the form of small vesicles dispersed in the cytoplasm and the Golgi zone was not prominent. Nucleus was observed mostly round or slightly indented with heterochromatin. This is in accordance with the findings of Olah and Glick (1982) in chicken and Djaldetti et al. (1980) in mouse.

The reticulum cells were stellate shaped, they had smaller nuclear-cytoplasmic ratio and more organelles in the cytoplasm. Rough endoplasmic reticulum was found to be more. Well developed mitochondria, ribosomes and a prominent Golgi zone
were observed. The presence of reticulum cells were commonly observed in all the age groups studied which is in agreement with the findings of Burke and Simon (1970) in rabbits.

The fibroblasts of the white pulp often appeared flat and were stellate with long cytoplasmic prolongations. They had rough endoplasmic reticulum seen filled with a fine flocculent material. The smooth endoplasmic reticulum also had similar material and some of them appeared fibrillar. This is in accordance with the findings of Galindo and Freeman (1963). They opined that these fibroblasts together with the stellate projections of reticulum cells made the loose meshwork which compartmentalized the white pulp. This meshwork was not dense enough to form solid barriers or prevent the free passage of cells within the pulp.

A single type of follicular dendritic cells (FDC) were seen in all the age groups and confirmed by their large and irregular shaped nucleus as reported in chicken (Chen et al., 1978 and Banchereau and Steinman, 1998). It had a little heterochromatin. The cytoplasm was found to be ramified as very thin processes in many directions. Some of the processes were observed long and straight. Mitochondria, rough and smooth endoplasmic reticulum and vesicles were rarely seen within the cytoplasm. This observation is contrary to the findings of Gallego et al. (1995 and 1997) who described two types of follicular dendritic cells in the spleen of chicken. The first type showed filiform cell processes and the other type was provided with beaded dendrites.

The plasma cells of the spleen were observed with well developed rough endoplasmic reticulum. There were larger and denser mitochondria and numerous ribosomes. The heterochromatic nucleus was eccentrically placed which correlates with the findings of Ogata et al. (1977) in chicken. Kopp (1990) reported that the heterochromatic nuclei did not reflect the inactivity as the small part of the genome that was euchromatic, was exceedingly active in maintaining the synthesis of many copies of a single antibody. Further, each plasma cell synthesizes and secretes antibodies that bind specifically to the antigen that initially activated the precursor B lymphocyte. Antigen-antibody binding is a major means of immune defense.
Antibodies synthesized within the rough endoplasmic reticulum are processed and packaged within the Golgi prior to secretion.

The macrophages were ovoid or stellate shaped and contained vacuoles and phagocytosed material. The nucleus was heterochromatic as observed by Burke and Simon (1970) in rabbits. These macrophages in the spleen were the important site of erythrocyte destruction which was evident by the presence of several partially digested fragments of old erythrocytes. According to Weiss (1964 and 1990), it also played a role in antigen presentation and secretion of mediators of the immune response in laboratory animals.

Nerve fibres were seen in close association with the arteries and arterioles of the white pulp which is in agreement with Galindo and Imaeda (1962) in mouse. Ballantyne (1968) in rat described that the splenic nerve fibres in these periarterial plexuses had butrylcholinesterase activity, and no acetylcholinesterase. He suggested that the splenic nerves were therefore of the postganglionic-sympathetic type and probably regulate the flow of blood in the spleen.

A diffuse lymphatic tissue, the periarterial lymphatic sheath (PALS) was noticed with densely packed lymphocytes of various sizes and was also infiltrated with reticular cells as reported by Olah and Glick (1982) in birds. There were no structural changes noticed in periarterial lymphatic sheath of spleen in different age groups. A few macrophages were also observed in the PALS. Very few reticular fibres formed a meshwork around the periarterial lymphatic sheath separated it from the adjacent red pulp. The lymphocytes concentrated close to the central artery were might be thymus dependent cells while those in the peripheral portion along with the plasma cells and macrophages are Bursa dependent lymphocytes as reported by Raviola (1994) and Dellmann (1998) in animals.

5.1.2.2 Red pulp

Burke and Simon (1970) in rabbits, King and Mc Lelland (1981) and Bacha and Bacha (2000) in chicken reported that the splenic red pulp in chicken was made up of a loose spongy tissue composed of ramifying cellular cords surrounded by
venous sinusoids which was also observed in the present study. Connolly et al. (1999) reported the presence of haemopoietic tissue in the red pulp of the spleen in platypus.

The cellular population of the red pulp of the spleen consisted of erythrocytes, reticular cells, lymphocytes, macrophages, plasma cells and mast cells which is in accordance with the findings of Rose (1981) in chicken and Geetha Ramesh et al. (2001) in laboratory animals.

**Electron microscopy**

In the present study, the splenic red pulp was composed of anastomosing sinuses lined by endothelial cells were noticed. The cellular composition of the pulp cords consisted of erythrocytes, reticular cells, lymphocytes of various sizes, macrophages, granulocytes, plasma cells and mast cells which is in accordance with the findings of Roberts and Latta (1970) in rabbit and Abe et al. (1989) in mink spleen.

The ellipsoids or sheathed capillaries were found to have a meshwork of polymorphic reticular cells, reticular fibres and a few macrophages as reported by Olah and Glick (1982) in chicken. These ellipsoids may phagocytose the erythrocytes and foreign macromolecules transported by the blood stream as reported by White et al. (1970) in chicken.

**5.2 Thymus**

**5.2.1 Capsule**

**Light microscopy**

In the present study, the thymic gland was surrounded by a thin connective tissue capsule composed mainly of collagen fibres and a few elastic fibres. Fine connective tissue septa from the capsule entered into the parenchyma and divided it into the lobules. These are in agreement with the findings of Bradley (1950), Hodges (1974), Firth (1977) in birds and Bhattacharya and Binaykumar (1983) in
chicken. According to Ritter and Crispe (1992) the septa of the thymus carried vascular and neuronal supply to and from the thymus in mammals.

5.2.2 Parenchyma

The parenchyma was observed to have a dark outer cortex and a pale inner medulla within the lobule in day-old, four weeks and ten weeks of age groups. The ratio of cortex to medulla of the thymus in day-old chick reversed in adult age group which is in agreement with the findings of Hodges (1974) in fowl and King and Mc Lelland (1981) in birds.

5.2.2.1 Cortex

The outer cortex contained abundance of small, medium and large lymphocytes that were tightly packed in all the age groups. Numerous reticuloepithelial cells were also present in the cortex which formed the Hassall’s corpuscles. This observation is in total agreement with Bradley (1950) and Kendal (1975) in birds. Cooper et al. (1966) opined that those small lymphocytes in the cortex represent thymus dependent lymphocytes in birds. Similarly, bursa dependent lymphocytes were morphologically represented by large lymphocytes and by plasma cells (Kincade et al. 1971).

5.2.2.2 Medulla

The medulla of the chicken thymus was comprised of numerous reticular cells which grouped to form a syncytium or supporting framework with less number of lymphocytes which is in agreement with the findings of Sabiha et al. (1998) in chicken and Connolly et al. (1999) in platypus.

5.2.2.3 Cellular components
Light microscopy

Small and medium sized lymphocytes were found to be numerous and tightly packed in the cortex which imported a darker appearance than the medulla in all the age groups studied. This is in agreement with the findings of
King and Mc Lelland (1981) in birds and Ritter and Crispe (1992) in human. Lymphoblasts were noticed in younger age groups in the present study which confirmed the findings of Dellmann and Brown (1998) in mammals. Thymus dependent line of lymphocyte was represented morphologically by small lymphocytes of circulation and the white pulp type of tissue in birds (Cooper et al., 1966).

The reticuloepithelial cells were stellate shaped with large nucleus and eosinophilic cytoplasm and found more in the medulla as reported by Weiss (1964) in mammals and Firth (1977) in birds. Boyd et al. (1983) reported that these reticuloepithelial cells released humoral factors essential for the production, development and maturation of the lymphocytes in bursa and thymus of chicken.

The myoid cells were observed in the medulla of all the age groups studied. A similar finding was observed by Gilmore and Bridges (1974) and Kendall (1980) in chicken and Geetha Ramesh and Vijayaragavan (1997) in buffalo calves. But Robert et al. (1978) described that myoid cells were rare in human thymus. The number of myoid cells increased as age advanced in the present study. This leads to a postulation that these myoid cells directly or indirectly, may be related with the involution of the organ, which is functional synchronization with the amount of sex hormone present in the blood (Vijayaragavan, 1988). Whereas Bockman (1968) assumed that the myoid cells were involved in the mechanism of tolerance to muscle self-antigen in human, but Mandel (1968a and b) opined that the thymic striated muscles may act as a local source of antigen for the self recognition of skeletal muscle in guinea pig.

Frazier (1973) opined that in the chick thymus, macrophages were present both in the cortex and medulla which is similar in the present study also. The presence of macrophages can be considered as one of the common defensive mechanism existing in birds, parallel to the findings of Kendall (1984) in man. Kolsch (1968) stated that macrophages mediate in the immune responses to certain antigens, in addition to their role as scavengers in AKR mice, as efficient presentation of antigen to thymic lymphocytes by other cells is necessary in any normal immune response. Contrary to this, Robert et al. (1978) in human and Salman and Cordingley (1979) in
rat opined that these macrophages merely as one of the phagocytic cells in the thymus.

Plasma cells with round and eccentrically placed nucleus was observed in all the age groups and found to be more in number in twenty week-old birds which is in agreement with the findings of Thorbecke et al. (1957) in birds. Kendal and Frazier (1979) stated that they were always present beneath the capsule in the perivascular space or within the connective tissue septa in avian thymus. However, Biester and Schwarte (1969) have reported the presence of plasma cells in the cortex of the thymus in birds and Gorgollon and Anaya (1977) have reported their presence in the medulla of thymus in dog. The findings of the present study is in contrary to the view of Ham and Cormack (1979) who have expressed that there is no possibility for the occurrence of plasma cells in the thymus, in view of its protective barrier and absence of any antigen necessary for their formation. But there is every possibility for the formation of plasma cells within the thymus itself, by the transformation of the lymphocytes, as reported in guinea pig by Murray and Woods (1964). Further, Murray and Woods (1964) have stated that B lymphocytes may form plasma cells in the thymus, by entering through the blood vessels of the medulla, which are permeable even to macro-molecules.

Vijayaragavan (1988) reported that mast cells were commonly found in the cortex and medulla of avian thymus and were closely associated with the Hassall’s corpuscle which is similar in the present study also. However, Karaca et al. (2006) reported that the number of mast cells increased with age in the thymus, bursa of Fabricius and spleen in avian species.

Various forms of granulocytes such as basophils, eosinophils and heterophils were commonly seen in the medulla of chicken is in agreement with the findings of Lucas and Jamroz (1961) in chicken and Robert et al. (1978) in human thymus.

The presence of extravascular erythrocytes was observed in a small number in various locations of parenchyma and particularly found to be more in the medulla.
than in cortex. A similar observation was made by Vijayaragavan (1988) and Kendall (1980) in chicken.

The Hassall’s corpuscles were found to be a round, homogenous eosinophilic mass lined by flat reticular cells. Hassall’s corpuscles were commonly observed in the medulla of chicken thymus in the present study is in accordance with the findings of Trautmann and Fiebiger (1957) in birds. The numbers of corpuscles were found to be more as age advanced, where hyalinised mass in the centre with concentric layers of reticuloepithelial cells were noticed which is in agreement with Vijayaragavan (1988) in chicken.

The presence of unicellular cysts were common in all the age groups studied which occurred either free in the parenchyma or associated with the Hassall’s corpuscles in the present study is similar to the findings of Bridges et al. (1970) in cockrel’s and Hashimoto and Sugimura (1976) in white pekin ducks.

**Electron microscopy**

In the present study, the lymphoid cells or thymocytes, reticuloepithelial cells, myoid cells and macrophages were observed as the predominant component of the chicken thymus in all the age groups. The other cell types observed were granulocytes, mast cells and plasma cells which were occasionally seen which is in total agreement with Frazier (1973) in chick.

Electron microscopic details of the lymphocytes in the present study are similar to the findings of Clawson et al. (1967), Enbergs and Kriesten (1968), Maxwell and Trejo (1970), Maxwell (1974), Hodges (1977) in chicken and Murray et al. (1965) in rats.

Three types of reticuloepithelial cells were observed in the present study. The pale reticuloepithelial cells in the cortex were similar in morphology to those present in the thymus of rat (van Haelst, 1967), the mouse (Hoshino, 1963), the guinea pig (Izard, 1966a and b) and the monkey (Chapman and Allen, 1971). The dark reticuloepithelial cells in the cortex and medulla of the chick thymus are
somewhat unusual and have rarely been observed in the mammalian thymus. However, Izard and Harven (1968) described a "dense reticular cell" present in low numbers in the thymus and lymph nodes of mice; the number of these cells was greatly increased in leukemic mice. Ito and Hoshino (1966) described a dark reticuloepithelial cell in the medulla of the hamster thymus. However, this cell appears to have a relatively pale nucleus. A third type of epithelial cell was observed in the cortico-medullary junction and in the medulla. These cells had a pale, oval nucleus which contained one or two nucleoli. Indentation of the nuclear membrane was not observed. The cytoplasm had mitochondria, ribosomes, a few rough endoplasmic reticulum and small, dark granules. Mandel (1968a and b) observed a similar type of cell in guinea pig. These undifferentiated epithelial cells possibly represent a reserve of epithelial cells which are able to differentiate and replace some of the other, more differentiated forms.

The myoid cells of the chicken thymus were found mainly in the medulla in all the age groups studied. Similar type of striated muscle cells or myoid cells have been found in thymic tissue from humans, amphibians, reptiles, birds and various mammals (van de Velde and Friedman, 1966, 1967; Strauss et al., 1966; Henry, 1968; Toro et al., 1969 and Bridges et al., 1970). However, Morris (1971) and Sugimura (1972) have reported their presence in the medulla of the ten to twelve month old ox, and they are present in the normal adult human thymus (Henry, 1968). The myoid cells seen in the present study are structurally similar to those described in the frog thymus (Toro et al., 1969).

Two main theories have been put forward to explain the presence of myoid cells within the thymus: (1) that aberrant mesodermal elements from the branchial arches become incorporated accidentally into the thymus during its embryological development (van de Velde and Friedman, 1966; Mandel, 1968; Frazier, 1973) and (2) they develop within the thymus as an intrinsic part of the organ (Kapa et al. 1968; Bockman and Winborn, 1969 and Toro et al., 1969). These latter authors suggested that the myoid cells develop from the epithelial cells of the thymic cytoreticulum. Toro and Olah (1967) and Toro et al. (1968) have also observed this process in the development of myoid cells during the in vitro culture of embryonic rat thymus tissue.
The number of myoid cells increased as age advances in the present study does not support the first theory that they represent the outcome of an embryological accident. Hence, the present study supports the second theory that the cells develop as an integral part of the normal thymus and as such it is reasonable to suggest that they play a physiological role therein.

The function of the myoid cells is still unknown. However, Raviola and Raviola (1967) and Rimer (1980) found that myoid cells play no physiological role because myofilaments are organized in random directions and myoid cells have no anchoring apparatus. But in the present study, myofilaments were shown to arrange regularly, and cross striations were clear. Hence, we are inclined to think that myoid cells push lymphatic cells to circulation around hatching as Toro et al. (1969) suggested, though anchoring apparatus was not found in this study.

Macrophages in the present study are similar in structure to those described by Frazier (1973) in chick thymus, and may be differentiated from the reticuloepithelial cells by the absence of desmosomes and presence of phagocytosed material in the cytoplasm.

The structure of plasma cells in the present study is in agreement with the findings of Frazier (1973) in chicken who found that plasma cells were often found near the blood vessels and increase in number with increasing age of the chicken.

Mast cells in the chicken were small cells with a few secretory granules in the cytoplasm is similar to the findings of Burnet (1965) in mice and Crivellato et al. (2005) in chicken. He proposed that the thymus is a site of mast cell development in chicken embryos. Castells (1999) opined that mast cells played an active role in antigen presentation to T cells and direct interaction between mast cell and B cells provided signals for specific IgE production.

Kendall (1980) described that the cells characteristic of bone marrow such as small and large eosinophils, basophils and heterophils were found in the thymus of chicken, as in mallards, starlings, house sparrow and red billed queleas, in addition to
the normal cellular constituents of the gland. Similar findings were observed in the present study too.

The structure of heterophil and basophil in the present study is in accordance with the findings of Maxwell (1973) in ducks and geese. Eosinophils in the chicken had a few small round mitochondria in the cytoplasm and characteristic bilobed nucleus which is similar to the findings of Maxwell and Siller (1972) in pigeon.

The erythrocytes were commonly seen in the medulla which was found to have irregular shapes in the present study. This is in confirmation with the concept forwarded by Ward (1972) and Kendall (1975), who were of the opinion that haemopoiesis takes place in the thymus on increased demand for blood during breeding season.

The Hassall’s corpuscles in the present study are similar to the findings of Robert et al. (1978) who described that the centre of the Hassall’s corpuscle was either solid or cystic under electron microscope. In contrast Frazier (1973) described that large Hassall's corpuscles were not often seen in the chick thymus, when present they were composed of concentric rings of squamous epithelial cells interconnected by many desmosomes. The association of dieing cells and macrophages with Hassall’s corpuscles in birds proved beyond doubt that Hassall’s corpuscles were the repository for a great number of old cells as opined by Blau (1973) and Olsson and Classon (1975). This finding is contrary to the findings of Senelar et al. (1976) in guinea pig who were of the opinion that Hassall’s corpuscles are the privileged areas for maturation of the medullary lymphocytes.

The presence of unicellular/intracellular and multicellular/intercellular cysts in association with the Hassall’s corpuscles in all the age groups is in agreement with the findings of Chan (1986) in chicken. In the present study, the cells of the intercellular cysts contained dense secretory granules which are similar to the findings of Frazier (1973) in chicken. This is good evidence to support the idea that the thymus is an endocrine gland, and secretes a hormone that induces lymphoid cells to acquire immunological competence (Goldstein et al., 1970). Whereas, Hoshino (1963),
van Haelst (1967) and Mandel (1968a and b) are of the opinion that cystic epithelial cells show cytoplasmic features suggestive of a secretory function.

Further, Clark (1963) provided some evidence to suggest that cystic epithelial cells in the mouse thymus secrete a sulphated mucoid lymphopoietic hormone. Henry (1966) reported that a mucoid substance is regularly secreted by the epithelial cells forming the Hassall's corpuscles in the human thymus. Pfoch (1971) suggests that "clear vesicles" present in reticulum cells of the rat thymus are the morphological equivalent of the production of a humoral thymus factor. However, Cesarini et al. (1968) and Chapman and Allen (1971) considered that the granular inclusions in cystic epithelial cells may be related to a lymphocyte stimulating hormone. However Izard (1966a and b) suggested that dense granules present in epithelial cells in the guinea pig thymus were kerato-hyaline granules linked to keratinisation but he did not exclude the possibility of the presence of another form of endocrine secretion, especially a colloid or steroid secretion.

5.2.3 Involution

Light microscopy

In the present study, marked involutary changes were noticed in forty weeks of age and was characterized by thickening of the capsule of the thymus with more collagen fibres, depopulation of cortical lymphocytes, invasion of connective tissue into the parenchyma, increased number of Hassall’s corpuscles and cysts which are in agreement with Bhattacharya and Binaykumar (1983) in chicken and Steinmann (1986) and Marinova (2005) in human.

Electron microscopy

The involutary changes of the thymus included the regression of lymphocytes, pyknotic nucleus and numerous cyst in the present study which is similar to the findings of Clarke and Mac Lennan (1986) in mammals.
5.3. Caecal tonsil

**Light microscopy**

Two types of germinal centres were observed in all the age groups studied which are in accordance with the findings of Olah and Glick (1975 and 1979) in chicken. The first type had an incomplete capsule and was noticed near the muscular layer of the caeca. This may represent a specific site of uncommitted cell proliferation and differentiation. Olah et al. (1975) opined that in chicken, plasma cells of the caecal tonsil, in general, are found deep in the tunica propria. The location of plasma cells and plasmablasts in the caecal tonsil suggest that their lymphoid precursors could originate from the first type of germinal centre.

The second type of germinal centre located closer to the surface epithelium of the villi of the caecum and was encapsulated with connective tissue. This is in agreement with Olah and Glick (1979) in chicken. Hence, it is presumed that the development of second type of germinal centre may depend upon antigenic influence which pass from the lumen to the germinal centre through the surface epithelium or via the vascular system.

The cellular components of the germinal centre of the caecal tonsils were similar to the findings of Jankovic and Mitrovic (1967), Payne (1971), Hoshi and Mori (1973), Glick et al. (1981), Gomez et al. (1998) and Kitagawa et al. (1998) in chicken. Upon maturity of the immune system, most of the immunological activity with in the chick gut-associated lymphoid tissue (GALT) was concentrated in the hind gut, specifically in the caecal tonsil and bursa of Fabricius (Bar-Shira and Friedman, 2005).

**Electron microscopy**

In the present study, the capsule of the germinal centre of the caecal tonsil of chicken consisted of many layers of flattened reticular cells separated with an intercellular substance. The cytoplasm of the reticular cells showed a number of smooth surfaced vesicles which is similar to the findings of Olah and Glick (1979) in chicken.
The germinal centre consisted of reticular cells, large and small lymphocytes and macrophages which is in agreement with Olah and Glick (1975) in chicken.

In the present study, M cells were found in the germinal centre associated with the surface epithelium of the villi of caecum. The structure of M cells was similar to the findings of Kitagawa et al. (2000).

Particulate antigens are transported within minutes by M cells to intraepithelial lymphocytes and into the follicular dome (Opstelen, 1984 and Ermak et al., 1994). Mammalian M cells have been shown to take up various substances actively (Gebert et al., 1996). During active pinocytosis and phagocytosis of luminal contents, M cell surface membranes are considered to be depleted by vesicle formation, resulting in attenuation of surface projections (Owen and Ermak, 1990).

In the chicken, lack of inactive uptake of foreign materials has been reported in caecal tonsil M cells (Kato et al., 1992). In the present study, however, small pits in the apical and lateral membranes and numerous small vesicles in the apical cytoplasm were detected in M cells of chicken caecal tonsils. In addition, M cells possessing few microvilli were found. These phenomena suggest that active uptake and transportation of small particulates by chicken M cells occurs under intact conditions. It has been suggested that M cells cannot modify exogenous substances they endocytose, since mammalian M cells are known to contain few lysosomes in the cytoplasm (Owen et al., 1981, 1986; Brandzaeg and Bjerke, 1990).

**B. Immunohistochemistry**

In spleen, the mean CD4 and CD8 population was found to be the highest at day one and lowest at twenty weeks of age. Nevertheless, an increase in both the T cell population was detected at forty weeks of age. Bridle et al. (2006) have detected an increase in the circulating CD4 and CD8 T cell populations by eighty weeks. Although we have not assessed the circulating T cell populations in our study, the increase in the population of these cells in spleen at two hundred days concurs with the findings of Bridle et al. (2006). Among the two cell types studied, CD8 cells
were always higher than the CD4 cells, with significant difference (P<0.05) at day one.

The distribution pattern of CD4 and CD8 cells in thymus was slightly different from that of spleen. Thymus had more CD4 cells on day one followed with twelve weeks of age. In contrast, CD8 cells were more on twelve weeks followed with day one. CD4 and CD8 cell populations were lowest on four weeks. Although we did not observe any set pattern of distribution of these cell types in the spleen and thymus of normal chicken, the results we have recorded concurs with some of the earlier reports (Davidson and Boyd, 1992) in that substantial populations of these cells could be detected at two and seven weeks of age.

In the present study the CD4 and CD8 ratio at four weeks of age was 1.01 in spleen and 0.67 in thymus whereas the reverse condition was recorded by Erf (1997) in spleen and thymus of commercial broilers and specific pathogen single comb white leghorn birds.

In the present study the CD4 and CD8 ratio in spleen was decreased from four weeks of age (1.01) to eight weeks of age (0.95). Erf et al. (1998) recorded decreased in CD4 and CD8 ratio between two and seven weeks of age in commercial broiler chicken. Further Erf et al. (1998) also found that there was an increase CD4 and CD8 ration in thymus between two weeks (1.20) and seven weeks (2.30). A similar increase CD4 and CD8 ratio in thymus was observed in the present study between four weeks (0.67) and eight weeks (0.72).

A distinct difference was observed in the ratios of CD4 and CD8 cells in the spleen and thymus at different age. At day one the ratio was less in spleen and high in the thymus. However, at eight weeks, the CD4 and CD8 cell ratio was high in spleen and low in thymus. Nevertheless, the ratios narrowed down at forty weeks of age.
C. Experiment

In the present study, an increase in CD4 and CD8 count was observed in spleen on five days post infection in group-II and group-III when compared to control. However, on six days post infection, there was a depletion of CD4 and CD8 count in spleen in group-II and group-III when compared to control. This is in agreement with the findings of Allen and Fetterer (2002) who observed that depletion of CD4 cells in spleen in primary infection with *Eimeria tenella*. This indicated that CD4 cells are important effectors of resistance to primary *Eimeria tenella* infection. Similarly Breed *et al.* (1997) observed that there was a depletion of CD8 count in caecal tonsils during primary infection with *Eimeria acervulina* and *Eimeria tenella*.

Further, it was theorized that the effect of reduction in the number of CD8 cells served as a transporters for sporozoites. The importance of CD8 cells in protective immunity to *Eimeria tenella* infection was also confirmed by the sharp increase in the CD8 count in peripheral blood during primary infection (Breed *et al.*, 1997). Whereas Bessay *et al.* (1996) found an increase in the proportion of CD4 and CD8 cells among the intraepithelial lymphocytes (IEL) of caecal tonsils on six and eight days post infection. They concluded that *Eimeria* infections seem to rapidly induce the T cell count locally at the site of parasite development that resulted in dramatic modification of the T cell subsets in IEL.

The strain of the birds used as well as those of the parasites was believed to play an important role in the dynamics of immunity. Lillehoj (1994) found that SC strain of chicken had a better immunity and a significant increase in CD8 IEL, when compared to TK chickens after infection with *Eimeria acervulina*. It is possible that the results obtained in this trial may be influenced by the type of birds used for experimentation.

In the present study, there was an increase in the CD4 and CD8 count in spleen on nine days post infection in group-II and-III when compared to control birds. This is in partial agreement with the findings of Swinkels *et al.* (2006). They observed that there was no increase in CD4 count in the duodenum of broilers infected with high dose of *Eimeria acervulina* on seven and nine days post infection. However, they
documented an increase in CD8 cells in the infected birds on seven and nine days post infection.

In the present study, there was a significant increase in the CD4 and CD8 count in thymus on five days post infection in group-II and-III when compared to control birds. This is in agreement with the findings of Sasai et al. (1997) and Asheg (2003) in *Eimeria* infection in chicken. This finding documented the primary role of thymus in the protective immune response against infection.

However, in the present study there was no significant difference in CD4 and CD8 count in thymus on six and nine days post infection between and control and infected birds.