HISTOLOGY AND AGE RELATED INVOLUTARY CHANGES OF THE THYMUS OF GIRIRAJA BIRDS (*Gallus domesticus*) *

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Abstract

Erythrocytes were maximum post hatch. Thymic nurse cells, myoid cells (round forms) were seen more during involution. Regression started at 8 weeks, but recrudescence was seen at 14 weeks. hjkLamellated Hassel's corpuscles and metaplasia of reticuloepithelial cells were common features of involuting thymus. Intracellular cysts and apoptotic changes were recorded.

Key words*: involution, giriraja, thymus, histology.*

The thymus gland in the fowl consists of three to eight irregular shaped lobes, pale pink in colour, situated on either side of the neck, close to the jugular vein (King and McLelland,1983). It is a primary lymphoid organ where T cells differentiate and participate in cell mediated immune response. Literature available on age related changes of the thymus of Giriraja birds are sparse. Hence the present study was envisaged.

Materials and Methods

A total of 72 birds were reared separately at the U.A.S. poultry farm, Bangalore from day old to 24 weeks and the thymus was collected from six birds each every alternate week. Tissue pieces were fixed in 10% neutral buffered formalin, Bouin's fluid, lymphatic tissue fixative and Zenker's acetic fluid. The tissues were processed by paraffin embedding and cut at 4 mm thickness. They were stained by Hematoxylin and Eosin, Van Geison's stain for collagen fibres, Masson's Trichrome method, method for reticulin (Kiernan, 1981), Weigert's elastic stain and Methyl green Pyronin method for the demonstration of DNA and RNA (Kiernan, 1981).

Results and Discussion

The thymus of the perinate birds contained many immature erythrocytes. The macrophage activity at day old stage was maximum (Fig. 1) which may be due to the selection process (Ross *et al.,* 1998).

The thymus of day old birds comprised of pyramidal or polygonal lobules. Its cortex consisted of densely packed small and medium sized lymphocytes making it to appear deeply basophilic. Division of the cortex and medulla started at first week and was distinct by the third week of age. During the first week the cortex contained huge pale eosinophilic structures containing more than one lymphocyte frequently undergoing mitotic divisions. By fifth week a basophilic cortex and an eosinophilic medulla were clear.

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Heterophils were numerous in the peripheral cortical area. Similar observations were made by King and McLelland (1983) in fowl.

The mitotic index of the cortex was high in the first four weeks and then decreased, however at 16 weeks the index increased again which may be due to the effect of prolactin having a mitogenic effect associated with breeding cycles (Ibars *et al.,* 1997).

By third week many lobules possessed a common medulla and by the end of the sixth week, the medulla was found to open into the interlobular connective tissue. The blood vessels appeared from the fourth week onwards. By the end of the sixth week, pale eosinophilic medulla showing reticuloepithelial cells with fewer lymphocytes compared to darkly stained basophilic cortex was distinctly noticed. As differentiation of cortex and medulla progressed, the reticuloepithelial cells were more apparent in the lightly stained medulla.

The reticular cells were observed between the lymphocytes and also occurring frequently in small groups devoid of other cell types. Mild alkaline phosphatase activity as well as acid phosphatase activity was recorded by the thymic reticuloepithelial cells and stroma which were in accordance with the findings of Fennel and Pearse (1961).

Numerous thymic bodies or Hassal's corpuscles occurred in the medulla. They were composed of degenerative reticular cells which have formed a concentric mass. The centre of the mass was degenerated so that it had a cystic or calcified appearance. Avian type Hassal's corpuscles as described by Kendall (1980) characterised by vesiculated reticuloepithelial cell clusters surrounding a homogenous mass were recorded in the present study. They represented an abortive attempt at keratinisation by the epithelial cells (Tizzard,1987).

The myoid cells were seen in the medulla as round or oval or elongated, strongly eosinophilic structures (Fig. 2) which appeared to be syncitial in nature. Their cytoplasm was strongly eosinophilic and fibrous (Itoh,1983). During the involutary phase the round forms were more in number and were supported by the observations of Van develde *et al*. (1967).

In transmission electron microscopic studies the myofibrils were found in the cytoplasm of some cells similar to the description of myoid cells given by Chan (1995).

A continuous layer of reticuloepithelial cells was noted around the blood vessels. This component of blood thymic barrier had three components namely the capillary with its basement membrane, the perivascular space and reticuloepithelial cell with its basement membrane. Toivanen and Toivanen (1987) stated that the blood thymic barrier was present in the cortex and not in the medulla. However, in this study the presence of germinal centres were very few and were present mostly in the involuting thymus in the medulla. At this stage

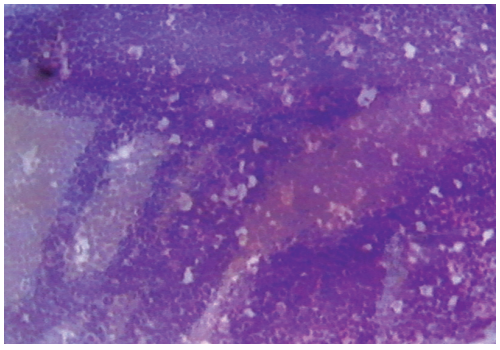

Fig. 1. Phagocytic activity in day old thymus. H & E X 450

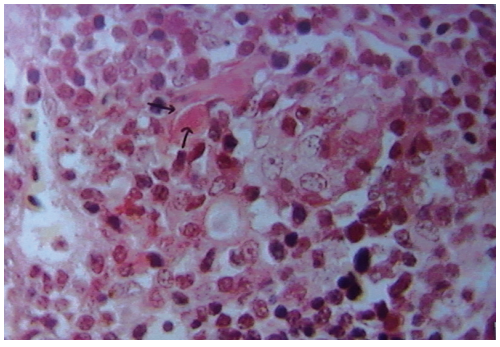

Fig. 2. Cross and longitudinal sections of Myoid cells. Masson's Trichrome X 1000

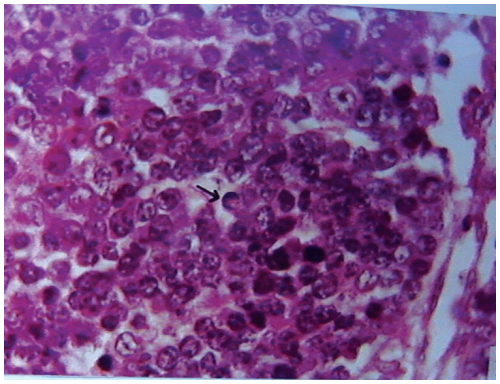

Fig. 3. Nuclear toroids of apoptotic lymphocytes. H & E X 1000

degeneration of the blood thymic barrier cells also occurred.

Ham and Cormack (1979) reported that protective blood thymic barrier prevented the occurrence of plasma cells in the thymus. In the present study however plasma cells and germinal centres were found within the thymus which suggested that thymus in addition to its role as a primary lymphoid tissue may function as a secondary lymphoid organ. In support occasional secondary lymphoid follicles were also observed.

The involutary changes were seen from eighth week onwards which progressed till 14th week of age, after which a re-enlargement and restoration of thymic architecture was noticed indicating recrudescence. The re-enlarged thymus regressed very slowly thereafter. But complete involution was not seen even at 24 weeks of age. Involutary changes in the thymus gland were characterised by thickening of the capsule, loss of interlobular septa, pyknosis of thymocytes, extension of area of medulla, increase in size and number of Hassal's corpuscles and myoid cells and accumulation of lipid granules in the medulla. These observations were also noted by Bhattacharya and Binay Kumar (1983) in fowl.

Metaplasia of epithelial reticular cells into epithelial myoid cells were seen. Round and elongated forms of myoid cells were more and found associated with blood vessels. Towards the later stages, myoid cells showed granular and strand like fibrous nature in their cytoplasm. Empty spaces were evident around these myoid cells with advancement of age.

Frequent capped appearance of lymphocyte nucleus referred to as toroids (Fig.3) and horse shoe forms of the nuclei were noticed which increased in number during later stages of involution. These were also noted by Wyllie and Duvall (1998) and were a feature of apoptotic cells. Sparsely disributed round or oval masses of intensely eosinophilic materials were seen in the involuting thymus. These were similar to the description of apoptotic bodies given by Vegad (1995). Rapid ingestion of these bodies by phagocytes accounts for their rare occurrence.

Some cells of the medulla, probably the reticuloepithelial cells, appeared cystic and were alcian blue positive, which were probably intracellular cysts. Others were squamous and formed Hassal's corpuscles. The cysts comprised of smaller vesicles lined by simple or stratified columnar cells and containing mucus. Some were large intracytoplasmic vesicles in the reticuloepithelial cells.

The weight of the thymus reduced at 14 weeks with a subsequent increase at 16 weeks followed by further decrease which is an evidence for recrudesence. The corticomedullary ratio increased till three weeks and then decreased till 14 weeks after which it increased drastically indicating reappearance of the cortex, an observation further supporting recrudesence. Similar observations were recorded in some species of birds by Kendall (1980). A decrease in cortical lymphocytes and increase in fat and connective tissue were also seen. The cortex decreased with age and by six months it was noted to be absent. Lipid substances were noted in the medulla of thymus at involution.

The size of the thymus vary considerably, its relative size being greatest in newborn and its absolute size being greatest at puberty. An inverse weight relationship existed between the growth of the thymus and sexual maturity. The absolute weight of the thymus was maximum at 16 weeks, the time of sexual maturity while the relative weight was highest at day old being 1.31 which is in total agreement with Tizzard (1987).

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