



# AGE RELATED HISTOCHEMICAL CHANGES OF THE BURSA AND THYMUS OF DOMESTIC FOWL\*

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## Abstract

*Histochemical changes of bursa and thymus of Giriraja birds from day old to 24 weeks of age was studied. Intense acid phosphatase and mild alkaline phosphatase activity was seen in lining epithelium of bursa. The follicle associated epithelium showed positive PAS and alcian blue reaction. Medullary cysts of bursa and intracellular cysts of thymus also stained positive. Lipids were seen from 14<sup>th</sup> week onwards in thymus.*

**Key words:** *Histochemistry, thymus, bursa, involutary*

Birds possess a unique lympho-epithelial gland, the bursa of Fabricius, located dorsal to the cloaca (Hodges, 1974), while the thymus gland consists of three to eight irregular shaped lobes, and is situated on either side of the neck close to the jugular vein (King and McLelland, 1981). Bursa and thymus are primary lymphoid organs where B and T cells differentiate and participate in humoral and cell mediated immune response respectively. While elaborate studies have been undertaken on immune system of chicken, not much work has been done on age-related histochemical changes of the lymphoid organs of Giriraja birds.

Giriraja is a disease resistant bird with body weight and egg production three times than the local bird. In the present work an attempt has been made to study the

histochemistry of the bursa and thymus in various age groups of Giriraja birds.

## Materials and Methods

A total of 72 birds were reared separately at the UAS poultry farm, Bangalore from day old to 24 weeks. Bursa and thymus were collected from six birds each every alternate week

Cryostat sections of 12 $\mu$ m thickness were obtained from fresh tissues and were stained by Gomori's alkaline phosphatase cobalt method, Gomori's acid phosphatase method and Oil red 'O' in propylene glycol method for lipids (Singh and Sulochana, 1978). The sections were also subjected to Periodic acid Schiff's reaction (Singh and Sulochana, 1978), Alcian blue method for mucosubstances at pH 2.5, Toluidine blue method for metachromasia (Luna, 1968).

## Results and Discussion

### Bursa

1. Cortex and Medulla- Medulla at the involuting stage presented acid phosphatase reaction (Fig. 1) which may be indicative of degenerative changes. The cortex and corticomedullary junction showed very mild reaction throughout the period of the study. Alkaline phosphatase activity was seen in a few isolated areas of follicular cortex which was suggestive of intracellular metabolism and differentiation of bursal cells.

\*Part of M.V.Sc. thesis submitted by the first author to U.A.S., Bangalore

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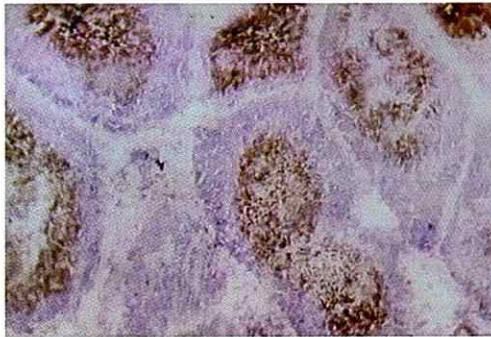
3. Professor(Retd.)

4. Associate Professor

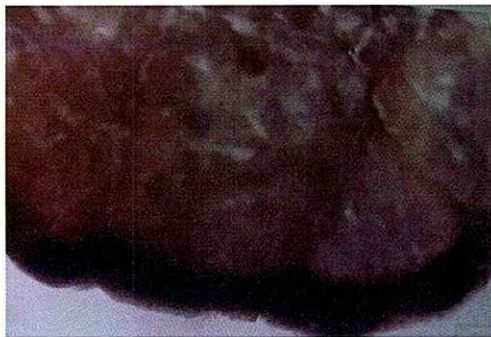
2. The Corticomedullary Border- It was PAS positive while the reticuloepithelial network became slightly alcian blue positive at the terminal stages of involution.

3. The Lining Epithelium- of the bursa showed intense acid phosphatase till about 18 weeks after which it became non specific. Interfollicular epithelium (IFE) and the epithelium lining the follicles (FAE) during involution showed intense reaction. Mild alkaline phosphatase activity was observed in the bursal epithelium of all age groups (Fig. 2), being strong initially during the first week and decreasing with the advancement of age. The reaction was variable from 18<sup>th</sup> week onwards.

The lining epithelium of bursal mucosa



**Fig. 1.** Acid Phosphatase positive reaction in medulla of bursa Acid Phosphatase X 100



**Fig. 2.** Alkaline Phosphatase positive reaction in lining epithelial cells of bursa Alkaline Phosphatase X 100

showed PAS positive reaction and was Alcian blue positive in all the age groups studied (Fig. 3). The reaction was distinctly absent in the FAE (Follicle associated epithelium) and was intense in the IFE and associated crypts of IFE. The interfollicular epithelium released mucin into the bursal lumen, which was indicated by the alcian blue positive reaction in this region in agreement with the observations of Farner *et al.* (1983)

4. Follicles- The infolded epithelium and those lining follicles during involution stages were also positive which indicated the presence of acid mucopolysaccharides. This along with intense acid phosphatase reaction was an indication of the presence of glycoprotein which were the basis for antibodies biochemically (Sabiha, 1993). The bursal epithelium, mast cells and cystic fluid stained metachromatically with toluidine blue.



**Fig. 3.** Alcian blue positive reaction in lining epithelial cells of bursa Alcian Blue X 100

5. Cysts- Large cysts lined by simple squamous epithelium enclosing a central core of mucoid substances were found in the medulla at 8 weeks and were formed by the epithelialisation of follicles and secretion of mucoid matter into the lumen formed. Their number increased with age. The cystic fluid stained metachromatically with toluidine blue. Simple tubular glands lined by columnar cells were noticed from 10 weeks of age closely associated with the cysts. These findings concurred with those observed by Scala *et al.* (1989) in the involuted bursa of duck. These glands stained positive for alcian blue and were suggestive of mucous secreting glands.

6. Lipids- The gradual replacement of the follicles by lipids was seen from 8<sup>th</sup> week onwards (Fig. 4). The lipid substances were



**Fig. 4.** Diffuse lipid droplets in involuting bursa Oil Red O X 100

localized in the lamina propria between the 10<sup>th</sup> and 18<sup>th</sup> week while masses of adipose tissue in follicles and huge random deposits were seen by 22<sup>nd</sup> to 24<sup>th</sup> week of age.

#### Thymus

Mild alkaline phosphatase activity was noticed in the thymus of all age groups studied. Cortex showed slight reaction from 8<sup>th</sup> to 16<sup>th</sup> week. Pale reaction was seen in the Hassal's corpuscle associated areas. Capsule showed strong reaction initially. Endothelium wherever present showed positive reaction. Circumscribed areas with central reactive masses probably germinal centres were seen in the 16<sup>th</sup>, 22<sup>nd</sup> and 24<sup>th</sup> week.

1. Stroma- 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). Acid phosphatase activity was noticed in the thymic medulla at 10 weeks and in stroma between 14 to 16 weeks of age. Perivascular spaces wherever present gave positive reaction.

Macrophages showed Alcian blue positive material in their cytoplasm. Macrophages were also present in the cortex and medulla, supported by the observations of Riddell (1987). They were found to have slightly alcian blue positive material. Kendall (1980) found the macrophage contents to be PAS positive confirming their secretory nature.

Toluidine blue staining showed mast cells with characteristic metachromatic granules in the parenchyma of most age groups studied.

2. Lipids- Lipid substances were seen as faint diffuse, droplets by 8<sup>th</sup> week, widespread and homogenous by 12<sup>th</sup> week and localizing in connective tissue septa by 14<sup>th</sup> week onwards. Focal depositions and wide spread lipid accumulations were seen in the 22<sup>nd</sup> and 24<sup>th</sup> week respectively.

3. Cysts- Some cells of the medulla, probably the reticuloepithelial cells, appeared cystic and were alcian blue positive, which in turn might be the intracellular cysts noted by Riddell (1987).

#### Acknowledgements

The authors are thankful to the Indian Council of Agricultural Research, New Delhi, for providing financial support for the work.

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