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Histochemistry of Bursa of Fabricius and Thymus in Guinea Fowl *(Numida meleagris)**

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Abstract

The study was carried out on bursa of Fabricius and thymus of guinea fowl to elucidate its histochemical features. The tissue samples were collected from day old to six months at monthly interval and the results are discussed.

Key words: Histochemistry, Bursa of Fabricius, Thymus, Guinea fowl

The bursa of Fabricius and thymus are the central lymphoid organs of the guinea fowl. The bursa of Fabricius can act as a primary lymphoid organ for B - cell lineage and controlling the production of antibodies by B-lymphocytes (Glick, 1991). The thymus produces precursors of cells involved in specific cell mediated immune responses (T-cells) and these cells co-operate with B - cells in the production of antibodies.

It has a significant role in immune system of birds. Hence, the present study was undertaken due to its importance in immunological competence and to provide histochemical details of bursa of Fabricius and thymus in guinea fowl.

Materials and Methods

Totally 42 samples of bursa of Fabricius and thymus were collected from apparently healthy guinea fowls (pearl variety) from Poultry Research Station, Madhavaram Milk Colony, Chennai-51 from day old to six months of age. The tissues samples were fixed in 10 per cent neutral buffered formalin, Bouin's fluid and formal calcium. Fixed tissues were processed for paraffin block preparation and sections of 3-5 µm thickness were made and utilized for this study. Freshly collected unfixed frozen tissues of 20 µm thickness were utilized for demonstration of lipids and enzymes. Periodic acid-Schiff (PAS) technique for Glycogen, Alcian blue method at pH 2.5 for acid mucopolysaccharides, Millon reaction for tyrosine, Oil red O method for lipids, Gomori lead method for acid phosphatase, Naphthol AS-BI method for alkaline phosphatase and α Naphthyl acetate method for non-specific esterase (Bancroft and Stevens, 1996).

Results and Discussion

In the bursa of fabricius follicle associated epithelium and inter follicular epithelium showed strong PAS reaction (Fig.1) as observed by Gulmez and Aslan (1999) in native geese, Indu *et al*. (2005) in White Pekin ducks, Jayachitra *et al*. (2009) in turkeys. The undifferentiated epithelial cells at the corticomedullary junction and eosinophilic homogenous mass noticed in the medulla also showed mild PAS reaction as mentioned by Gulmez and Aslan (*loc. cit*) in native geese and Leena *et al*. (2009) in Giriraja birds. Apical portion of surface epithelium was alcian blue positive (Fig. 2), similar observation was made by Aita *et al*. (1992) in chicken. The Positive reaction of PAS and Alcian blue indicated that the presence of glycogen and acid mucopolysaccharides respectively in bursa of Fabricius. This along with intense acid phosphatase reaction was an indication of the presence of glycoprotein which were the basis for antibodies biochemically (Sabiha, 1993).

Tyrosine containing proteins were noticed within the lymphoid follicle and epithelium of the bursa of Fabricius (Fig. 3) as observed by Jayachitra *et al*. (*loc.cit*) in turkeys. These disulphide proteins could be attributed to their role in the active transport of sugars and amino

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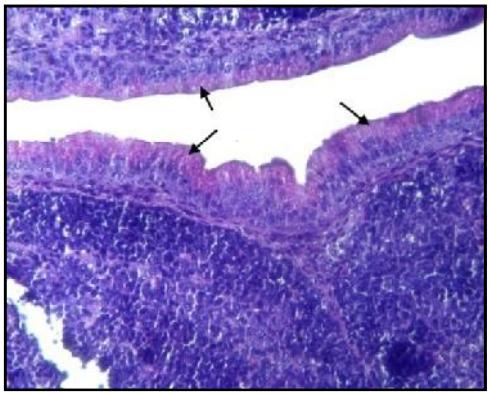

Fig 1. Showing strong PAS reaction in both Follicle Associated Epithelium (FAE) and Interfollicular Epithelium (IFE) **Periodic Acid Schiff x400**.

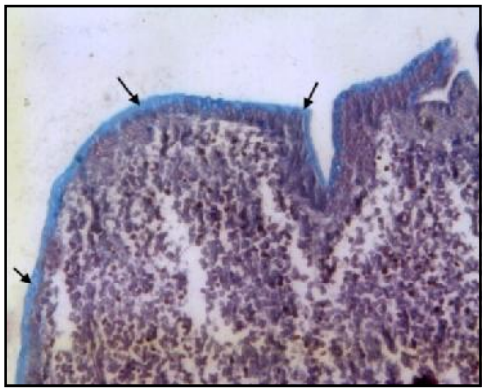

Fig 2. Showing alcian blue reaction in the apical portion of surface epithelium. **Alcian blue x400.**

Fig 3. Showing tyrosine positive proteins in the lymphoid follicle. **Millon reaction x400.**

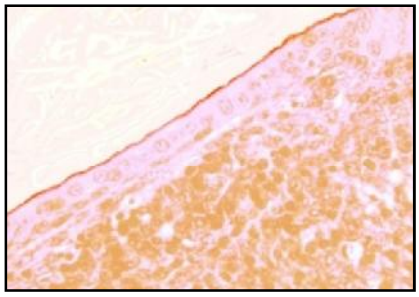

Fig 4. Showing acid phosphatase activity in the surface epithelium. **Gomori lead method x400.**

acids across the cell membrane as pointed out by Lehninger (1975). Lipid droplets were noticed in the subepithelial and interfollicular connective tissue as well as in the lymphoid follicles of the bursa of Fabricius. This is in accordance with the findings of Indu *et al.* (*loc.cit*) in ducks, Leena *et al.* (*loc.cit*) in Giriraja birds and Jayachitra *et al.* (*loc.cit*) in turkeys. The increased amount of lipids found in the aged birds is probably because of their lowered metabolic activities as in toad (Hara and Yamada, 1963).

As mentioned by Indu *et al.* (*loc.cit*) in ducks and Leena *et al.* (*loc.cit*) in Giriraja birds, acid phosphatase activity was noticed in the surface epithelium (Fig. 4) and lymphoid follicles. Acid phosphatase is considered to be related to B lymphocyte maturation and is regarded as a B cell marker in the chicken (Graczyk, 1987; Sur, 2001). Surface epithelium (Fig. 5) and lymphoid follicle showed alkaline phosphatase activity in the early age groups and it gradually disappeared as age advances. Which is in agreement with Indu *et al.* (*loc. cit*) in White Pekin duck, Leena *et al.* (*loc.cit*) in Giriraja birds. The alkaline phosphatase activity in thymus is associated with lymphocyte maturation and lymphocyte- stimulating factor production (Rakhawy *et al.*, 1976). Non-specific esterase activity was observed in the undifferentiated epithelial cells, macrophages and lymphocytes of lymphoid follicle. These are in accordance with Lupetti *et al.* (1983) in chicken and Saifuddin *et al.* (1988) in Shaver cockerels.

The Hassall's corpuscles of thymus were showed strongly PAS positive reaction (Fig. 6) and mild alcian blue positive (Fig.7). Some of the reticular cells were faintly PAS positive, Bhattacharya (1983) in chicken and Gulmez and Aslan (*loc.cit*) in native geese. These indicated that the presence of glycogen and acid mucopolysacharides respectively. This along with intense acid phosphatase reaction was an indication of the presence of glycoprotein which were the basis for antibodies biochemically (Sabiha, *loc. cit*).

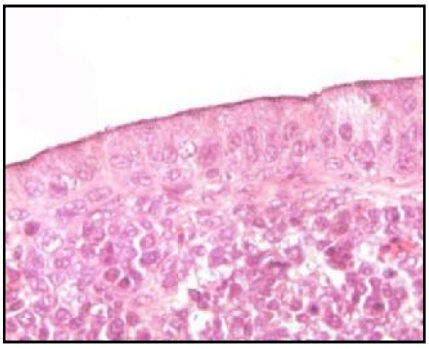

Fig 5. Showing alkaline phosphatase activity in the surface epithelium. **Naphthol AS-BI method x400.**

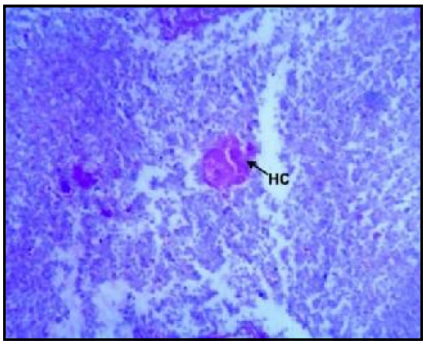

Fig 6. Showing strong PAS positive in the Hassall's corpuscles and reticuloepithelial cells. **Periodic Acid Schiff x400.**

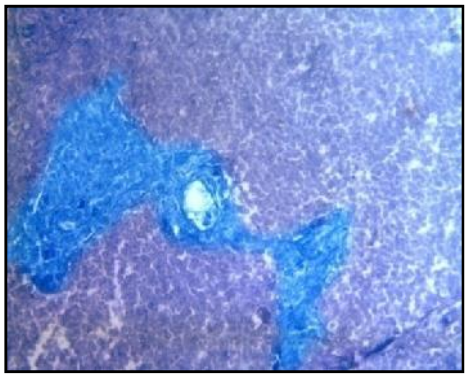

Fig 7. Showing alcian blue positive Hassall's corpuscles and adjacent cells. **Alcian blue x400.**

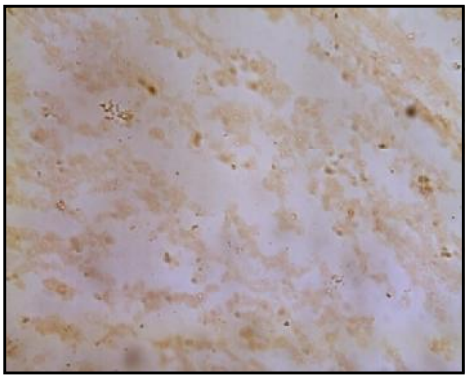

Fig 8. Showing tyrosine positive proteins in lymphoid follicle. **Millon reaction x400.**

Tyrosine containing proteins were seen in the epithelial cells, thymocytes and macrophages (Fig. 8) as observed by Muthukumaran *et al*. (2011). These disulphide proteins could be attributed to their role in the active transport of sugars and amino acids across the cell membrane as pointed out by Lehninger (*loc.cit*). Lipid granules were uniformly distributed throughout the thymus gland of guinea fowl (Fig. 9). Similar distribution was observed by Fennell and Pearse (1961) in chicken. The increased amount of lipids found in the aged birds is probably because of their lowered metabolic activities as in toad (Hara and Yamada, *loc.cit*).

The acid phosphatase activity was present in the Hassall's corpuscles, reticuloepithelial cells and the intense reaction was observed in the medullary reticular cells, corticomedullary border and interlobular septa. This is in agreement with Bhattacharya (1982) and Fennell and Pearse (*loc.cit*) in chicken. Leena *et al*. (*loc.cit*) reported that, the acid phosphatase activity was noticed in the thymic medulla at ten weeks and in stroma between 14-16 weeks of age in Giriraja birds. This enzyme is involved in the T lymphocyte maturation and phagocytic activity (Basso *et al*., 1980).

Alkaline phasphatase activity was observed in the Hassall's corpuscle, subcapsular region, perivascular space and corticomedullary border of the thymus in guinea fowl as reported by Leena *et al*. (*loc.cit*) in Giriraja birds. The alkaline phosphatase activity in thymus is associated with lymphocyte maturation and lymphocyte stimulating factor production (Rakhawy *et al*. *loc.cit*).

Summary

The study revealed that the strong PAS positive material was located in both interfollicular epithelium and follicle-associated epithelium. The tyrosine containing proteins and lipid droplets were also noticed in the lymphoid follicles. The enzyme activities like acid phospha-

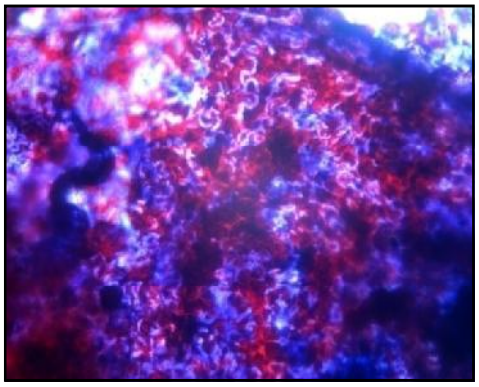

Fig 9. Showing lipid droplets in the cortical region of thymus. **Oil red O x 400.**

tases, alkaline phosphatases and non-specific esterases were noticed in the epithelium and lymphoid follicle of the bursa of Fabricius. The central amorphous core of the Hassall's corpuscles showed strong PAS reaction and alcian blue reaction. Tyrosine containing proteins were noticed in the epithelial cells, thymocytes and macrophages. The enzyme activities like acid phosphatases and alkaline phosphatases were noticed in the perivascular and corticomedullary border of the thymus in guinea fowl.

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