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Research article

THE STRUCTURE OF BURSA OF FABRICIUS IN THE LONG-LEGGED BUZZARD (*BUTEO RUFINUS*): HISTOLOGICAL AND HISTOCHEMICAL STUDY

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The bursa of Fabricius (BF) is a lymphoepithelial organ found only in birds. Differences in morphology of BF could play an important role in immune response. The objective of this study was to investigate the histological and histochemical characteristics of the bursa of Fabricius in the long-legged buzzard (*Buteo rufinus*). The material for the study comprised bursa samples obtained from three long-legged buzzards with permission of the General Directorate of Nature Protection and National Parks (Ankara, Turkey). Briefly, interfollicular epithelium (IFE) was shown to be columnar in shape and not to contain goblet cells. Reticular fibers were located in interfollicular septae. Each lymphoid follicle in the bursa of Fabricius in the long-legged buzzard was remarkably linked to the follicle associated epithelium (FAE). Namely, FAE has been reported to stimulate antibody production by transferring antigens to the medulla and have a leading role in developing of local immune response. Among the others, the species-specific differences in bursa of Fabricius morphology of long-legged buzzard (*Buteo rufinus*) also might support the continuity of this species in nature.

Key words: bursa of Fabricius, histochemistry, long-legged buzzard

INTRODUCTION

The bursa of Fabricius (bursa cloacalis or bursa Fabricii) is a lymphoepithelial organ which was first described by Hieronymus Fabricius in the 17th century [1] and found exclusively in birds. It opens into the proctodeal region of the cloacae with a short duct. The bursa develops as a blind, sac-like and dorsal evagination of the cloaca wall [2]. Embryological its short duct originated from the ectoderm while the epithelium of the bursa of Fabricius (BF) is derived from the endoderm of the hind gut, follicle associated epithelium (FAE) and partly from the mesoderm [3,4]. However at different ages of maturation in avian species the bursa of Fabricius begins involution [5,6].

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Briefly, histologically the BF wall is composed of the *Tunica mucosa, Tunica muscularis* and *Tunica serosa*. The *T. mucosa* consists of the *Lamina epithelialis, Lamina propria* and *Lamina submucosa* while the muscular layer is absent [7]. The mucosa contains several plications and a large number of polyhedral lymphoid follicles [8], composed of cortex, medulla and corticomedullary border [9,10]. The surface comprised two kinds of epithelium: follicle associated epithelium (FAE) covering the apex of each lymphoid follicle and the interfollicular epithelium (IFE) covering the space between the lymphoid follicles [3,11].

The role of BF in immunity was first described by Glick *et al.* [12] when the bursa was surgically removed in young chicken. Now it is known that the bursa of Fabricius is a gut-associted lymphiod organ responsible for the maturation and differentiation of B lymphocytes, and immunoglobulin isotype switch [1,13]. It is not only a primary lymphoid organ but also a secondary lymphoid organ due to the presence of a T cell-dependent area i.e. the diffusely infiltrated area (DIA) which is located on the dorsal bursal duct [14,15]. At the same time it was indicated that the bursa of Fabricius has an influence on B cell development based on the production of the differentiation factor of B cells in its epithelium [16,17].

The BF histological structure was investigated broadly in various bird species [7,18-20]. There are no related data on BF in the long-legged buzzard (*Buteo rufinus*). The goal of this study was to investigate the structure of the bursa of Fabricius in the long-legged buzzard using histological and histochemical methods.

MATERIAL AND METHODS

Material

The investigated, bursa of Fabricus were taken from three healthy long-legged buzzards provided by permission of the General Directorate of Nature Protection and National Parks (Ankara, Turkey).

Methods

Tissue samples were fixed in 10% formalin solution for 24 hours and alcohol-formol solution for 48 hours. Following routine histological processing the fixed samples were embedded in paraplast and serial sections at 5-7 μm were prepared. The modified Mallory's triple staining was used to show the general structure of the bursa of Fabricius. Methyl green pyronin (MGP) staining was applied to the sections to observe the plasma cells and the silver staining technique of Gordon and Sweets was performed for the reticular cells and reticulum fibers. For the disclosure of glycogen Periodic Acid Schiff (PAS) staining was applied to the sections.

To identify the T lymphocytes, tissue specimens were first fixed in cold formol-sucrose solution (pH 6.8) at +4 ^{0}C for 22 h, then in Holt's solution at +4 ^{0}C for 22 h. Finally,

specimens were cut into 8 μm thick sections on a cryostat. Alpha naphtyl acetate esterase (ANAE) enzyme staining was applied to these sections at pH 6.4 by Mueller *et al*. [21]. The sections were examined under light microscope (Olympus BX51, Japan).

RESULTS

The dissected bursa of Fabricius of the long-legged buzzard were oval in shape. The inner surface of the bursa was composed of several mucosal folds (plicae). The wall structure of the bursa consisted of T. mucosa, T. muscularis and T. serosa layers (Figure 1). Two different types of epithelial cells were observed on the plications: follicle associated epithelium (FAE) simple squamous and cuboidal in shape, associated with the lymphoid follicle as well as interfollicular epithelium (IFE) which is columnar in shape. No goblet cells were seen in the epithelium (Figures 1 and 2).

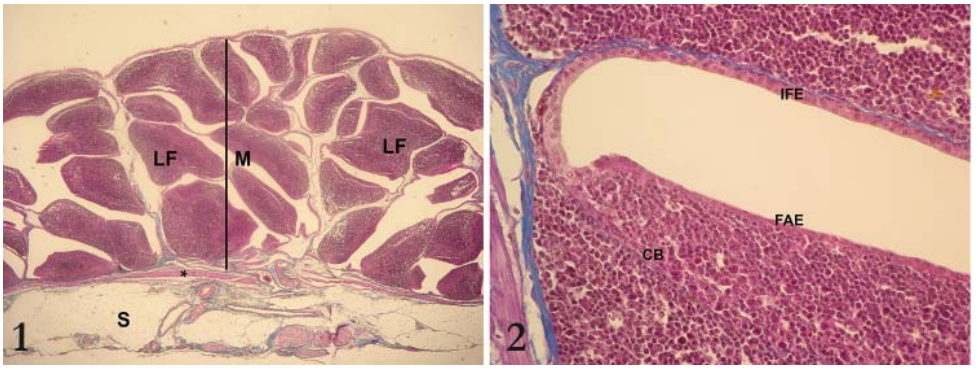
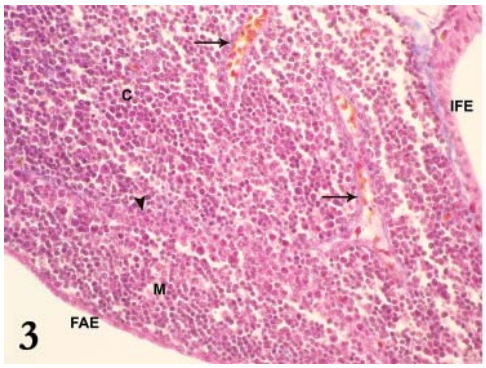


Figure 1: Long-legged buzzard bursa of Fabricius. M: Muocosa layer, *: Muscularis layer, S: Serosa layer, LF: lymphoid follicle. Triple.

Figure 2. Long-legged buzzard bursa of Fabricius. FAE: Follicle associated epithelium, IFE: Interfollicular epithelium, CB: Corticomedullary border. Triple.

Numerous lymphoid follicles are present in the mucosal layer. The surface of each follicle was associated with FAE. The follicles were divided into the cortex and medulla by the corticomedullary border. The corticomedullary border is a continuation of IFE and is cuboid in shape (Figures 1 and 2). The cortex of the lymphoid follicles adjacent to the connective tissue and medulla was close to FAE as well. The capillary vessels with connective tissue were located in the cortex but not in the medulla (Figure 3). The smooth muscular layer lies beneath the mucosa encircled by the T. serosa. (Figure 1)

Follicle associated epithelium as well as the smooth muscle cells were weakly PAS-positive (Figure 4). The reticular fibers and reticulum cells were located in the cortex of BF (Figures 5 and 6). Pyroninophilic cells were observed in lymphoid follicles and plasma cells mostly were beneath the IFE (Figure 7). Numerous ANAE-negative, and rare ANAE-positive lymphocytes bearing one or two granules inside the cytoplasm were determined in the lymphoid follicles of bursa of Fabricius. (Figure 8)



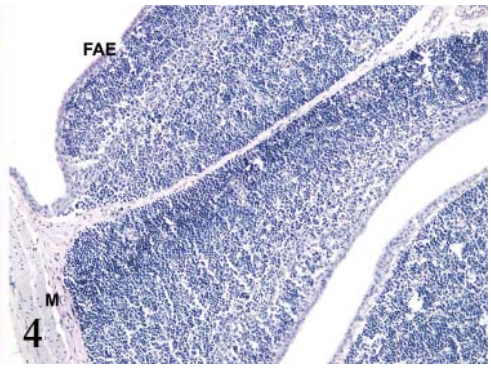
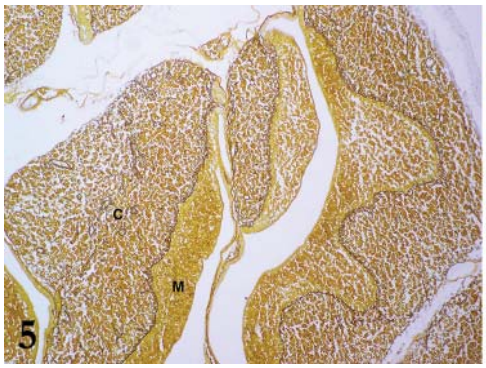


Figure 3. Long-legged buzzard bursa of Fabricius. C: Cortex, M: Medulla, arrows: Capillary vessels, arrowhead: Corticomedullary border, FAE: Follicle associated epithelium, IFE: Interfollicular epithelium. Triple.
Figure 4. Long-legged buzzard bursa of Fabricius. M: Smooth muscle fibers, FAE: Follicle associated epithelium. PAS.



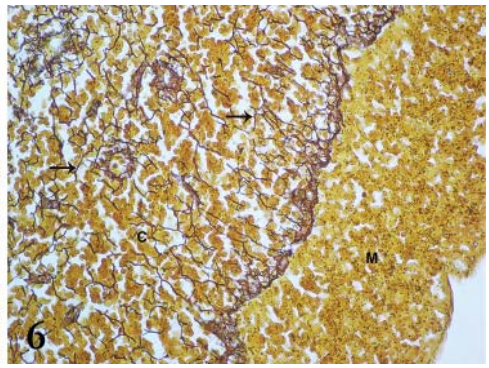
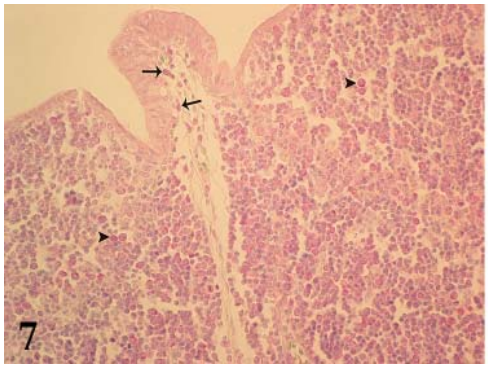


Figure 5. Long-legged buzzard bursa of Fabricius. C: Cortex, M: Medulla. Silver staining.
Figure 6. Long-legged buzzard bursa of Fabricius. C: Cortex, M: Medulla, arrows: Reticulum fibers. Silver staining.



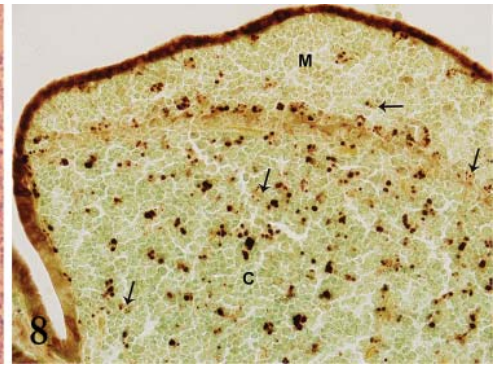


Figure 7. Long-legged buzzard bursa of Fabricius. Arrows: Plasma cells, arrowheads: Pyroninophilic cells. MGP.
Figure 8. Long-legged buzzard bursa of Fabricius. C: Cortex, M: Medulla, arrows: ANAE positive lymphocytes. ANAE staining.

DISCUSSION

In avian species, pre-B cells originating from the bone marrow, proliferate, differentiate and develop into mature B-lymphocytes able to respond to the antigen in the bursa of Fabricius [22]. Although Nickel *et al.* [23] have suggested that the bursa of Fabricius is round or pear-shaped in all avian species the morphology may vary among avian species. Bursa of Fabricius of the long-legged buzzard is oval in shape. It was determined that the wall structure of bursa of Fabricius, is similar to BF structure in turkeys [7] and geese [24], consists of mucosa, muscular and serosa layers and the mucosa layer extends into the lumen of the organ forming plicae.

Both IFE and FAE of the BF differ structurally. It was reported that IFE has the features of a single layer of prismatic epithelium [23], while other authors [25,26] reported that it has features of pseudostratified columnar epithelium. IFE has been proposed to have goblet cells in pigeons [18] and geese [24] and in the current study IFA is shown to be columnar in shape without goblet cells in the long-legged buzzard. Furthermore, FAE in the BF was considered to be a part of the surface epithelium [27,28]. Also, Bocman and Cooper [3] have identified its pinocytotic activity. FAE cells have been reported to be prismatic and similar to absorptive M cells in mammals [3]. In addition, FAE has been reported to stimulate antibody production by transferring antigens to the medulla following pinocytosis and have a leading role in developing of local immune response [29]. FAE was stated to be patchy-settled in IFE [13]. However, in our study, FAE was observed to continue uninterrupted along the surface of the follicle rather than being patchy. Besides, each lymphoid follicle in the bursa of Fabricius in the long-legged buzzard is remarkably associated with FAE. Compared with other avian species all histological characteristics of FAE in long-legged buzzard suggest that the immune system response in this avian species could be more effective also by increasing the pinocytotic activity.

Each lymphoid follicle consists of a cortex and a medulla. The cortex and medulla are separated from each other with a layer composed of undifferentiated epithelium [9, 10] which is in continuation with IFE or epithelium called corticomedullar border epithelium [14] and capillary vessels [30]. This layer has been reported to be absent in quail [31] and it was found to be similar to those of turkey [7] and chicken [32].

As a result of PAS staining for glycogen, FAE cells and smooth muscle cells in the BF of the long-legged buzzard were seen to have weak PAS positivity. Reticular epithelial cells (REC) [11,33], which aid in the differentiation and maturation of B lymphocytes, were found to be rare in the cortex of lymph follicles and numerous in the medulla. These cells were reported to form a supporting network for lymphocytes and other cells in the medulla [32]. RECs [11] that are proposed to be star-shaped due to cytoplasmic extensions and reticular fibers were found to be present in the cortex and absent in the medulla of the BF in the long-legged buzzard.

Furthermore, T lymphocytes stimulation is needed for the activation of B lymphocytes within lymphoid follicles of the bursa of Fabricius. Antibody production starts with

the conversion of activated B-lymphocytes into plasma cells [33]. The majority of lymphoid cells in the bursa of Fabricius produce IgM (+) and a small portion IgG (+) and IgA (+) [34]. Gulmez and Aslan [24] have suggested that plasma cells are located in lymphoid follicles, whereas Karadag Sari and Kurtdede [7], Hodges [2] and Frazier [32] have suggested that plasma cells are located between lymphoid follicles and in the connective tissue under the IFE. Pyroninophilic cells were seen in lymphoid follicles in the bursa of Fabricius of the long-legged buzzard, whereas plasma cells were found in the connective tissue, especially under the IFE.

More than 90% of cells found in the bursa of Fabricius are known to consist of B-lymphocytes [35]. Alpha naphthyl acetate esterase is a lysosomal enzyme. This enzyme, which was reported to be formed during the first stages of T lymphocyte development, is present in T lymphocytes but not in B-lymphocytes. The ANAE positive reaction was expressed by one or two localized granules within T lymphocytes [21,36]. Numerous ANAE-negative, but only rare ANAE-positive lymphocytes were located in the lymphoid follicles of bursa of Fabricius in the long-legged buzzard.

The species-specific differences in bursa of Fabricius morphology of long-legged buzzard (Buteo rufinus) might support the continuity of this species in nature among other avian species.

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STRUKTURA BURZE FABRICIJUS RIĐEG MIŠARA (*BUTEO RUFINUS*): HISTOLOŠKA I HISTOHEMIJSKA STUDIJA

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BAKIR Buket

Bursa Fabricius (BF) je limfoepitelni organ, prisutan samo kod ptica. Razlike u građi BF mogu značajno uticati na imunski odgovor. Cilj ove studije je bio da se ispitaju histološke i histohemijske specifičnosti u građi i strukturi burze kod riđeg mišara (*Buteo rufinus*). Ispitivanjem su obuhvaćeni uzorci BF uzeti od tri primerka riđeg mišara, uz dozvolu Uprave za zaštitu prirode i nacionalnih parkova (Ankara, Turska). Ukratko, interfolikularni epitel (IFE) kod ove vrste je imao cilindričan izgled i u njemu nisu bile prisutne peharaste ćelije. Retikularna vlakna su bila prisutna u septama interfolikularno. Svaki limfoidni folikul burze Fabricijus riđeg mišara je bio u prisnoj vezi sa folikularno asociranim epitelom (FAE). Naime, postoje podaci o ulozi FAE u transferu antigena ka meduli i stimulaciji proizvodnje antitela i značajnoj ulozi u razvoju lokalnog imunskog odgovora. Pored ostalog, species-specifične razlike u građi BF kod riđeg mišara (*Buteo rufinus*) važne su takođe, kako bi se obezbedio kontinuitet generacija ovih ptica u prirodi.