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Histological and Histometrical Investigations on Bursa of Fabricius and Thymus of Native Geese

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Abstract:The present work was undertaken to show the histological structure and histometrically features of thymus and bursa of Fabricius of native geese.

The general histologic structure of bursa of Fabricius was similar to the structure of many other birds stated in literature.

By histometrical measurements, it was determined that the mean thickness of cortex and medulla of the lymphoid follicles in plicae were $89.06 \pm 3.23 \mu$ and $196.8 \pm 3.08 \mu$ respectively. The mean diameters of lymphocytes in cortex were $4.54 \pm 0.08 \mu$ while those of plasma cells were $6.36 \pm 0.33 \mu$. The mean diameters of lymphocytes in medulla were $4.49 \pm 0.08 \mu$, and that of plasma cells were $6.68 \pm 0.34 \mu$.

In comparison of the diameters of lymphocytes in cortex with those of in medulla, it was seen that the statistical difference in both diameters was insignificant ($p > 0.01$) while the diameters of the plasma cells were statistically significant ($P < 0.01$).

The general histologic structure of the thymus as the bursa of Fabricius was similar to other birds however there were also some differences. The number of lobes was found about 6-9 in right about 5-9 in left in geese. There was not any lobulation in the first cranial lobes. Plasma cells and myoid cells were located in cortex and medulla and myoid cells have diameters 13-33 μ .

Hassal's corpuscles in the medulla had both vacuoles and laminated structures approximately at the same range.

By histometrical measurements of thymus, the mean diameters of lymphocytes in cortex were $3.80 \pm 0.08 \mu$ while those of plasma cells were $6.03 \pm 0.31 \mu$.

The mean diameters of lymphocytes in medulla were $4.10 \pm 0.08 \mu$, and those of plasma cells were $5.83 \pm 0.31 \mu$.

In comparison of the diameters of lymphocytes of lymphocytes in cortex with those of in medulla the statistical difference in both diameters was significant ($P < 0.01$), but the difference in the diameters of plasma cells was statistically insignificant ($P > 0.01$).

In comparison of these two organs, the difference in both diameters of lymphocytes and plasma cells was statistically significant ($P < 0.01$).

Key Words: Bursa of Fabricius, Thymus, Histology, Geese.

Yerli Kazların Timus ve Bursa Fabricius'unda Histolojik ve Histometrik İncelemeler

Özet: Bu çalışmada yerli kazların timus ve bursa Fabricius'larının histolojik yapısı ve histometrik özelliklerinin ortaya çıkarılması amaçlandı.

Bursa Fabricius'un genel histolojik yapısı literatürlerde bildirilen diğer bir çok kanatlıninkine benzerdi.

Histometrik ölçümlerde bursa Fabricius plikaları içerisindeki lenf folliküllerinin korteks kalınlıkları ortalaması $89 \pm 0.63.23 \mu$, medulla kalınlıkları ortalaması ise $196.8 \pm 3.08 \mu$ olarak saptandı. Lenfositlerin ortalama çapları kortekste $4.54 \pm 0.08 \mu$, medullada $4.49 \pm 0.08 \mu$ olarak ölçülürken plazma hücrelerinin ortalama çapları kortekste $6.36 \pm 0.34 \mu$ olarak ölçüldü. Korteks ve medulladaki lenfositlerin çapları karşılaştırıldığında aradaki farklılığın istatistiksel açıdan önemsiz ($P > 0.01$), plazma hücrelerinin çapları arasındaki farklılığın ise önemli olduğu ($P < 0.01$) belirlendi.

Timusun genel histolojik yapısında bursa Fabricius gibi diğer kanatlılarla benzerlik göstermekle birlikte bazı farklılıklarında olduğu belirlendi. Lob sayısı sağda 6-9, solda 5-9 olarak belirlendi. En kranialdeki loblarda lobulasyon yoktu. Plazma hücreleri ve 13-33 μ arasında değişen çapa sahip olan miyoid hücreler hem korteks hemde medullada gözlemlendi. Medulladaki Hassal cisimciklerinde vakuolleşme ile birlikte yaklaşık olarak aynı oranda laminasyonda görüldü.

Timus korteksindeki lenfositler ortalama $3.80 \pm 0.08 \mu$, plazma hücreleri $6.03 \pm 0.31 \mu$ çapa sahipken medullada lenfositlerin ortalama çapı $4.10 \pm 0.08 \mu$, plazma hücrelerinininki ise $5.83 \pm 0.31 \mu$ olarak saptandı. Korteks ve medulladaki lenfositlerin çapları karşılaştırıldığında farklılığın istatistiksel açıdan önemli ($P < 0.01$) fakat plazma hücrelerinin çapları arasındaki farklılığı önemsiz ($P > 0.01$) olduğu belirlendi.

Her iki organ arasındaki karşılaştırmalarda hem lenfosit hemde plazma hücre çapları arasındaki farklılıkların istatistiksel açıdan önemli ($P < 0.01$) olduğu saptandı.

Anahtar Sözcükler: Bursa Fabricius, Timus, Histoloji, Kaz.

Introduction

Bursa of Fabricius and thymus are primary lymphoid organs of the fowls (1). These two organs resemble each other respect to their epithelial derivation, lymphoid nature, growth during embryonic and early postnatal life due to the fact that these organs begin to involute before the development of sexual maturity (2, 3).

The Bursa of Fabricius:

The bursa of Fabricius, which is peculiar to birds, is the dorsal diverticulum of the proctodeal wall of the cloaca (1, 2, 3, 4). The wall of the bursa of Fabricius is composed of tunica mucosa, tunica muscularis and tunica serosa (1, 2, 5).

Tunica mucosa forms plicae towards the bursal lumen while the numbers of these plicae have been demonstrated about 12-14 in the Helmeted Guinea Fowl (6). While some investigators (1, 2, 5, 6) has demonstrated that the surface of plicae is covered with pseudostratified epithelium, the other (3, 4) have noticed that it is covered with simple columnar epithelium.

Connective tissue under the epithelium is full of the follicles (1, 2, 3, 5, 6, 7). There are different ideas about structure of the lymph follicles. Freedman has mentioned about the presence of two types folicles with button like or projection like formation.

The Thymus:

The thymus is a lymphoepithelial organ and therefore plays an important role in the defence mechanism against infections. It is also believed to exert an influence on the development of other lymphoreticular organs (4). The lobes of thymus of fowl string along each side of the neck are close to the jugular vein and their number is different in birds from one another. The structure of thymus of some birds is different from each other by the number of lobes it contains. Goose, native duck and pekin duck have 6-9, 5-6, 4-7 lobes on the right side and 5-9, 5-7, 4-6 lobes on the left side respectively (8). Guinea fowl has 7 lobes on the right side and 6 lobes on the left side (9). Water fowl has 5-6 lobes (4, 10).

Histological structure of the thymus of the fowl resembles that of the mammal. Each lobe consist of lobules which are partly separated by connective tissue. The lobule consist of an outer dark cortex and inner pale medulla. Islands of reticular cells occur in the medulla; the cells are usually vacuolated rather than laminated unlike those of mammalian corpuscles (3).

Structures possessing rounded, laminated and cornified structure of typical mammalian corpuscles are only infrequently seen in the fowl thymus (2). Myoid cells have been found both in embryonic and adult thymuses in several vertebrate species, including man (11). Thymic myoid cells produce macrophage lineage cell stimulatory factors (12).

The present study was undertaken to show the histologic structure of thymus and bursa of Fabricius of native geese species in Kars city, Turkey and to find out statistical variance in these two tissues by measuring the diameters of myoid cells in thymus and the wideness of cortex and medulla in bursa of Fabricius histometrically.

Material and Methods

In this study, 10 native geese at 1 year old were used as subjects.

Thymus and bursa of Fabricius were fixed in bouin's and formol-alcohol solution and paraffin-embedded. These were sectioned at 6 μ .

The paraffin section were stained with Crossmon's triple stain (13) and haematoxylin and eosin for routine examinations. For the basement membran and glycoproteins, periodic acid Schiff (P.A.S.) were used. A methyl green-pyronin method was applied to identify the plasma cells. The major cells of the thymus were stained with Weigert's haematoxylin and van Gieson's stains (14). The diameter of lymphocytes and plasma cells and also the thickness of cortex and medulla in bursa of Fabricius were measured by using ocular micrometer. By finding the minimum and maximum values and the means of all measurements, t-test was used as a statistical method (15).

By comparing the diameters of lymphocytes and plasma cells in both cortex and medulla of thymus and bursa of Fabricius, it was tried to determine whether these differences are statistically significant or not.

Results

The Bursa of Fabricius

It has been reported that the wall of the organ consist of tunica mucosa, tunica muscularis and tunica serosa. It was determined that tunica mucosa forms the plicae at different length and thickness which prolongate towards lumen. The numbers of plicae were about 11 to 13. The surface of plicae was surrounded by pseudostratified

epithelium. Epithelium cells had usually an ovoid nucleus located below the midline of the cell and more than one nucleolus. Among these cells, more granulated P.A.S. positive cells and goblet cells were observed. The epithelium cells was surrounded by a P.A.S. positive basement membran (Figure 1).

Each plica was completely filled with follicles separated by connective tissue. The forms and sizes of follicles were different from one to another. The follicles had an inner pale medulla and outer dark cortex. The minimum, mean and maximum thickness of medulla were measured 103.6 μ , 196 \pm 3.08 μ and 316.2 μ ,

respectively. The minimum, mean and maximum thickness of cortex were measured 20.72 μ , 89.06 \pm 3.23 μ and 202.05 μ , respectively. Some follicles were in a direct contact with epithelium while others were completely surrounded by a connective tissue. Epithelium cells located at the basis of surface epithelium enter into follicles together with the basement membrane which surrounds them and separates medulla from cortex. The follicles connected with epithelium did not have cortex at connection areas. Also, an increase of epithelium cells was observed between pseudostratified epithelium and medulla. This situation was not observed at the other parts of surface epithelium (Figure 2).

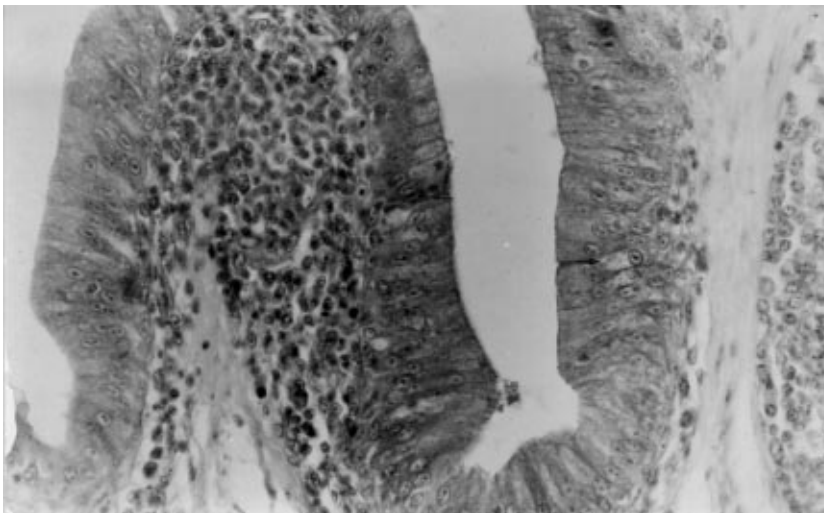


Figure 1. Surface epithelium of bursal plicae. (→) Goblet cells. (←) Bold P.A.S. (+) cells P.A.S. x 40

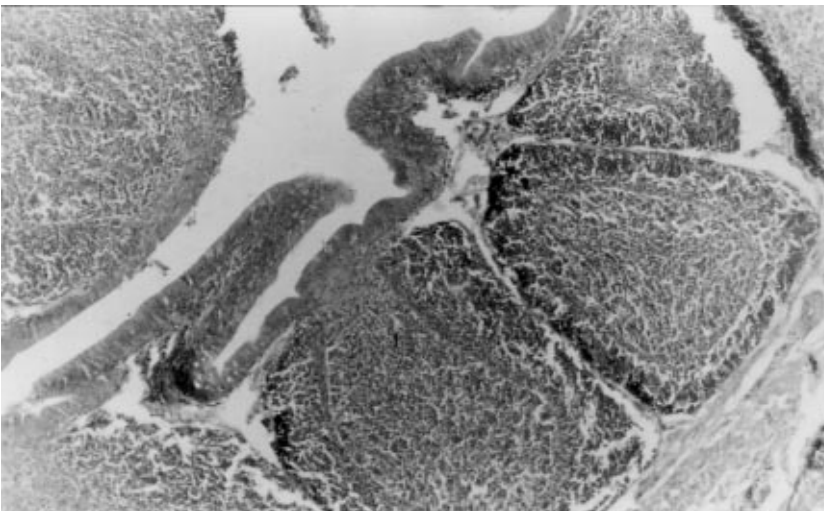


Figure 2. The lymph follicles (f) related to the surface epithelium. P.A.S. x 10.

	Bursa of Fabricius				Thymus			
	Cortex		Medulla		Cortex		Medulla	
	Lymphocyt	Plasma Cells	Lymphocyt	Plasma Cells	Lymphocyt	Plasma Cells	Lymphocyt	Plasma Cells
Min.-Max.	2.51-7.52	5.02-8.53	2.01-8.02	5.02-8.53	2.01-6.02	4.51-8.02	2.01-6.02	4.51-8.02
$\bar{X}\pm S_{\bar{X}}$	4.54 \pm 0.08	6.36 \pm 0.33	4.49 \pm 0.08	6.68 \pm 0.34	3.80 \pm 0.08	6.03 \pm 0.31	4.10 \pm 0.08	5.83 \pm 0.31

Table 1. Minimum, Maximum and Mean Diameters of Lymphocytes and Plasma Cells in Bursa of Fabricius and Thymus.

The minimum, mean and maximum diameters of lymphocytes in cortex were 2.51 μ , 4.45 \pm 0.08 μ and 7.52 μ , respectively. Also the minimum, mean and maximum diameters of lymphocytes in medulla were 2.01 μ , 4.49 \pm 0.08 μ and 8.02 μ , respectively (Table 1). Statistical difference in both diameters was insignificant ($p>0.01$) (Table 2).

Both in cortex and medulla, there were reticulum cells, the cytoplasm of which were P.A.S. positive, among the lymphocytes. The connective tissue under surface epithelium rarely had eosinophilic granulocytes. The plasma cells which cytoplasm stained in red with methyle green pylonin were generally located in periphers much less than in internal area. Plasma cells in cortex were dispersed about in cortex (Figure 3). The minimum, mean and maximum diameters of plasma cells in cortex were 5.02 μ , 6.36 \pm 0.33 μ and 8.53 μ , respectively. The minimum, mean and maximum diameters of plasma cells in medulla were 5.02 μ , 6.68 \pm 0.34 μ and 8.53 μ ,

respectively (Table 1). In comparison the diameters of plasma cells in cortex with those in medulla, the statistical difference in both diameters was found significant ($p<0.01$), (Table 2).

At the center of some follicles there were a group of P.A.S. positive stained cells the cytoplasm of which is totally or locally vacuolated and degenerated nucleus formed a group. But there were also some healthy reticulum cells in these groups. There were macrophages among the lymphocytes. Both in medulla and cortex, some cells which were stained darker than reticulum cells with P.A.S. stain were determined, but they could not be identified.

Tunica muscularis surrounding the mucosa, had been formed by an outer layer of longitudinal fibers and an inner layer of circular fibers. Tunica muscularis was observed outside of tunica muscularis. Blood vessels of subserosa under the tunica serosa were also seen in connective tissue of tunica mucosa and tunica muscularis.

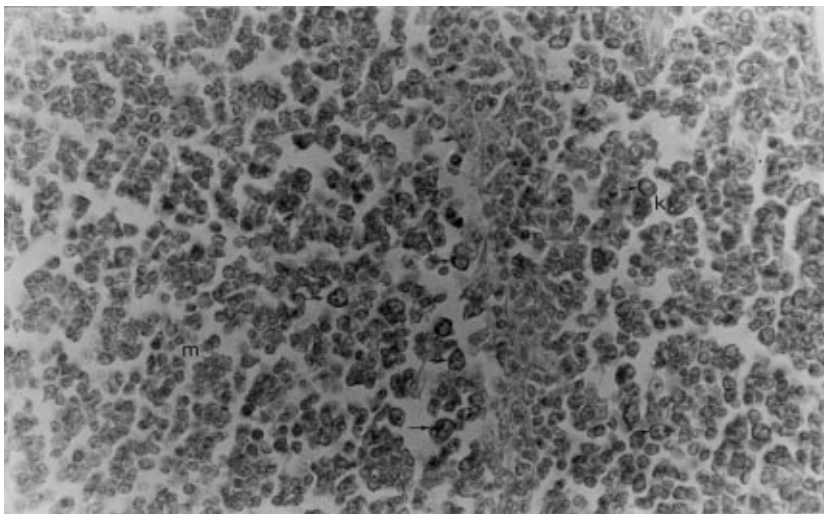


Figure 3. Plasma cells in the cortex and medulla of the bursa of Fabricius. (m) Medulla, (k) Cortex, (arrows), Plasma cells. Methyl green-pyronin x 40

		Bursa of Fabricius		Thymus	
		Medulla		Medulla	Cortex
		Lymphocyt	Pl. Cell.	Lymphocyt	Pl. Cell.
Bursa of	Cortex	Lymphocyt	0.444 ^{n.s}	6.683*	3.932*
		Pl. Cell.		2.959*	4.673*
Fabricius	Medulla	Lymphocyt		6.262*	3.498*
		Pl. Cell.		5.630*	7.244*
Thymus	Medulla	Lymphocyt			2.804*
		Pl. Cell.			1.907 ^{n.s}

* $p<0.01$ n.s: non significant

Table 2. T-values Obtained by the Comparison of the Diameters of Lymphocyt and Plasma Cells (Pl. Cell.) in Cortex with Those in Medulla of Bursa of Fabricius and Thymus and of the Cells Between two Organs and Their Statistical Results.

While we observe numerous blood vessels at the cortex of follicles, we could not observe any in the medulla.

The Thymus

The lobes of the thymus glands of the geese were situated along the jugular vein on both sides of the neck. The number of lobes was about 6 to 9 on the right side and 5 to 9 on the left side. The gland was enclosed by a connective tissue capsule. A connective tissue originated from capsule enters into lobes dividing them into lobules. There was not any lobulation in the first cranial lobes. It was pointed out that the lobules had an outer cortex and an inner pale medulla. The cortex was seen to be separated by connective tissue while the medulla was seen to be completed.

There were more lymphocytes than those of medulla. The minimum, mean and maximum diameters of lymphocytes in cortex were 2.01 μ , 3.80 \pm 0.08 μ and 6.02 μ , respectively. The minimum, mean and maximum diameters of lymphocytes in medulla were 2.01 μ , 4.10 \pm 0.08 μ and 6.02 μ , respectively (Table 1.) When the diameters of lymphocytes in cortex were compared with those in medulla, it was determined that the difference was statistically significant (Table 2). There were epithelial reticulum cells among lymphocytes. These cells were separated or in groups especially in medulla.

While the pale stained nucleus were round in single cells, cells in group had round or oval nucleus according to the shape of cells. Round nuclei had generally one nucleolus, but oval nucleuses had many nucleoli. The cytoplasm of epithelial reticulum cells was P.A.S. positive and had many cytoplasmic protrusions.

There were many Hassal's corpuscles fairly different in shape and size in medulla. Hassal's corpuscles had both

acidophilic and basophilic masses. Strong P.A.S. positive Hassal's corpuscles had both vacuoles at different size and laminated structures approximately at the same range (Figure 4).

Myoid cells stained in yellow with van Gieson were observed both in medulla and cortex. It could not be determined, whether these cells have striations or not. In the longitudinal sections of myoid cells there were fibrillar structures. Pale stained nucleus was centrally placed in some cells but eccentrically placed in many of cells. The nucleoli were single and dark stained. The diameters of myoid cells were about 5-8 μ (Figure 5).

Plasma cells stained red with methyl green-pyronin were located in cortex and medulla, especially around of blood vessels in connective tissue dividing the organ to lobules (Figure 6, 7). The minimum, mean and maximum diameters of plasma cells in cortex were 4.51 μ , 6.03 \pm 0.31 μ and 8.02 μ , respectively. Also, the minimum, mean and maximum diameters of plasma cells in cortex with those in medulla it was seen that the differences were not statistically significant ($P < 0.01$) (Table 2).

When the diameters of lymphocytes and plasma cells in bursa of Fabricius were compared with the diameters of lymphocytes and plasma cells in thymus, it was determined that the differences among those measurements were statistically significant ($P < 0.01$) (Table 2).

Eosinophil granulocytes were found in cortex more than those in medulla. Macrophages located both in cortex and in medulla. Blood vessels originated from capsule of organ with connective tissue scattered through cortex and medulla.

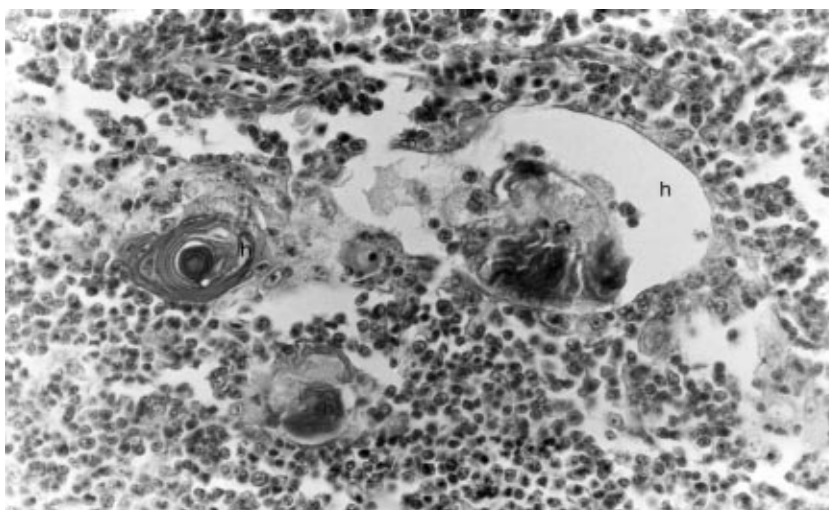


Figure 4. Thymic Hassal's corpuscle (h). Triple stain x 40.

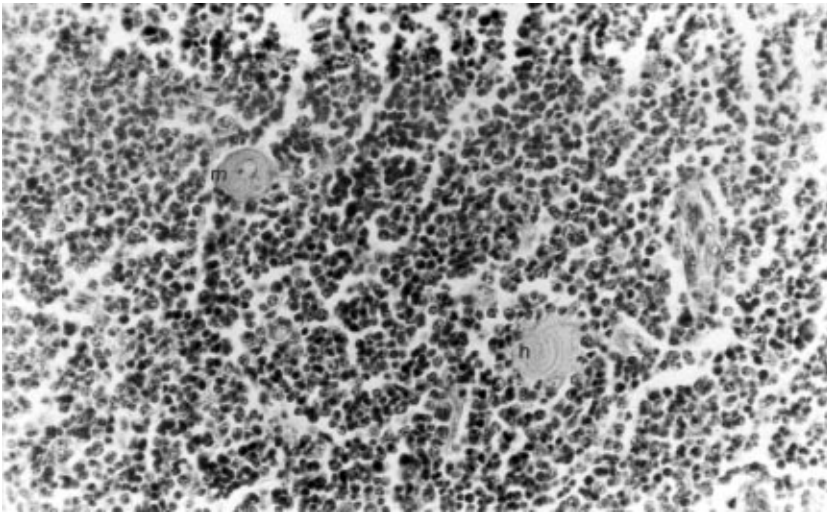


Figure 5. The myoid cell (m) and Hassal's corpuscle (h) in the thymus. Weigert's haematoxylin and von Gieson x 40.

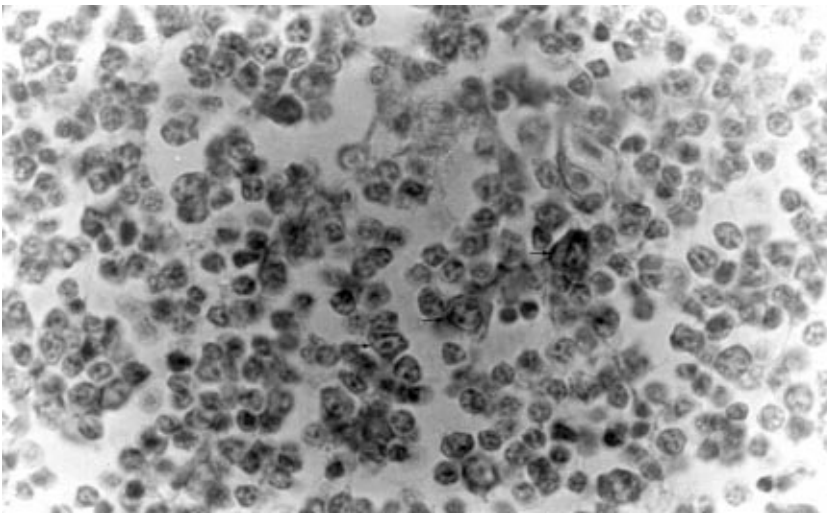


Figure 6. Plasma cells (arrows) in the cortex of the thymus. Methyl green-pyronin x 100.

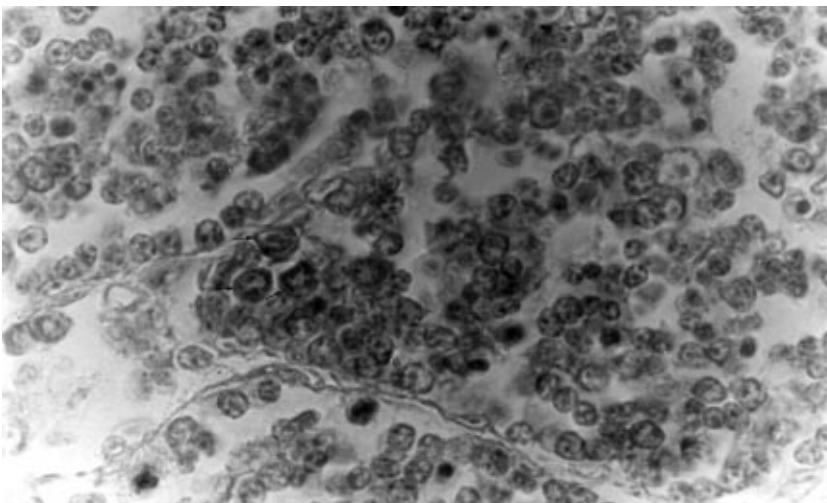


Figure 7. Plasma cells (arrows) in the cortex of the thymus. Methyl green-pyronin x 100.

Discussion

Bursa of Fabricius

Our observations on the general histologic structure of bursa of bursa of Fabricius was similar to many other birds stated in literature, but there were also some differences.

While the number of plicae on mucosa has been stated about 12 in domestic fowl (2, 3) and about 12-14 in the Helmeted Guine fowl (6), it has been stated about 11-13 in the chicken (16). In this study, we found plicae about 11-13 in the geese.

Many investigators have different ideas about the surface epithelium covering the inner surface of bursa of Fabricius. While some investigators (1, 2, 5, 6) have reported that the surfaces of plicae were covered with a pseudostratified epithelium, the others have stated that they were covered with a simple columnar epithelium. Hodges, (2) has stated that there were three definitive types of surface epithelium cells in the chick. Type I is an oval cell, with round or oval nucleus and their cytoplasm contain P.A.S.-positive granules. Type III is a narrow goblet cell containing a hyper chromatic nucleus and P.A.S.-positive secretory matter (2). Hasa (5) has stated that it could not determine the goblet cells, but Onyeausi et al. (6) have determined the goblet cells among the surface epithelium cells. The surface epithelium in the geese are pseudostratified. Most of epithelium cells, an oval nucleus located below the midline of the cell and more than one nucleolus were observed. There were a few cells including P.A.S.-positive stained granules and goblet cells. These findings confirmed that of Hodges (2).

Findings about the general construction of lymph follicles filled the connective tissue under the epithelium were similar to those of some other investigators. The lymph follicles related to the surface epithelium demonstrated by Betti et al (16) were found in the geese, too.

While Yılmaz et al. (17) pointed out that the plasma cells were seen nearly throughout the bursa of Fabricius, Özcan (18) has stated that these cells were located in the medulla rather than cortex and especially at the outer sides of medulla under the cortex. In this study it was seen that, in the bursa of Fabricius of geese, plasma cells were generally located peripherally in the medulla and homogenically in the cortex and these findings confirmed that of Özcan (18).

Glick and Olah (19) have found that the bursa of Fabricius have secretory cells consisting a central, located

or elongated nucleus and sometimes granules in them. Also, we observed dark red stained P.A.S.-positive cells both in the medulla and cortex but it could not be found out whether these cells were secretory cells.

Contrary to Onyeausi et al., (6), Hodges (2) has demonstrated that there were blood vessels in medulla. In this study, it was not seen blood vessels in medulla.

The Thymus

The number of lobes of thymus was variable according to bird species. This number has been stated about 7 on each sides of the neck in the domestic fowl by Hodges (2); about 3-8 by King (3) about 7 in right and 6 in left in Guinea fowl by Onyeausi (9); about 7-9 on each side of neck of the domestic fowl by Gilmore and Bridges (20). We found about 6-9 in lobes right and 5-9 in left in geese as did Bahadır et al. (8). Hodges (2) and King (3) has mentioned that the structure of thymus were alike that of mammalian and contained lobules made by a separation of lobes with a connective tissue, however, the lobulation was much less, also some lobes has none in Guinea fowl (9) lobulation was observed in all lobes but no lobes was seen in the most upper cranial small one.

Hodges (2) has stated that the lamination and cornification peculiarity of Hassal's corpuscles were rare in birds. King (3) has stated that the reticulum cells which produce Hassal's corpuscles did not form laminae contrary to those of in mammals; these cells were vacuolated. In this study vacuolation and lamination were seen in some range in Hassal's corpuscles.

Our findings about mean diameters of lymphocytes in thymus were similar to that of Hodges (2). Histochemical findings were also similar to that of Bodey et al. (21).

Van de velde and Freedman (11) and Chan (22) have observed cross striated myoid cells in thymus. Immunohistochemical observations have confirmed that myoid cells exist mainly in the medulla and that some myoid cells exist in the interlobular connective tissue region in the chicken. After the immunohistochemical procedures cross striations were clear, though it was difficult to identify cross striations in specimens which were stained only with hemotoxylin and eosin (23). In the thymus of the turtle, myoid cells are scattered about in the lobule, in the thymus of the snake, they were mainly concentrated in the central region of the lobule and decreased in number towards the periphery; in the thymus of the pigeon they were located both in the medulla and at the boundary region between cortex and medulla (24). Myoid cells were occasionally seen in

cortical areas but were most numerous in the medulla. The cells were about 30 μ in diameter and of various shapes. In older birds ovoid or round cells predominated while elongated forms were frequently seen in younger birds. The plane of section has often failed to pass through the nuclear region but examination of serial sections always has revealed an eccentrically placed nucleus. The nucleus was about 10 μ long diameter and oval in shape. The majority of nuclei have contained a single, discrete nucleolus. The cytoplasm was coloured bright yellow with Weigert's haematoxylin and van Gieson's stain and was red after Masson's trichrome stain (20). In this study it was seen that the myoid cells coloured yellow with van Gieson were seen both in cortex and in medulla. The nucleus was usually located eccentrically but sometimes centrally. The striation was not seen in the cells. The myoid cells were about 13 to 33 μ in diameter and nucleus were about 5 to 8 μ in diameter. These findings confirmed that of Gilmore and Bridges (20).

Many different opinions have been put forward about the existence and location of plasma cells. Some

investigators (25); Yılmaz et al., (17) have stated that these cells present mostly in cortex, Özcan (18) has claimed that they present in medulla, while Thorbecke et al. (26) have reported that they present only in outer areas of medulla. We observed plasma cells both in medulla and in cortex in geese.

As a result, the thymus and bursa of Fabricius in geese is similar to the birds, however some structural differences in thymus have been identified in this study. When the diameters of cells in cortex and medulla of bursa of Fabricius follicles are compared, (Table 2) it was found that the diameters of plasma cells were different; and when the diameters of cells in cortex and medulla of thymus are compared, the diameters of lymphocytes were different, which is statistically significant. Furthermore, it was found that the diameters of lymphocytes and plasma cells in these two organs were different, which is statistically significant. All these data are thought to be the morphological indicators that different types of lymphocytes originate from thymus and bursa of Fabricius.

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