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Light and Electron Microscopic Studies of the Bursa of Fabricius in Turkeys

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Summary

The present study was carried out using both light and electron microscopies to investigate the micro structure of bursa of Fabricius in turkeys. Tissue samples were obtained from 20 turkeys at 1 month old were provided from Bolca Turkey Company, (Bolu, Turkey). The microscopic structure of bursa of Fabricius was composed of tunica mucosa, tunica muscularis, and tunica serosa. The tunica mucosa was made up by the plicae which projected through the lumen of bursa of Fabricius. The lamina epithelialis was comprised two different epithelium which were interfollicular epithelium (IFE) and follicle associated epithelium (FAE). Microvilli were seen on the apical surfaces of interfollicular epithelium cells which contained mucous granulles in their cytoplasm. Microfolds were detected on the apical surfaces and many vacuoles and vesicles in the cytoplasm of the follicle associated epithelium cells. The follicle associated epithelium was supported by the supporting cells which infolded into the medulla and formed lamellated epithelial bodies. The plicae of bursa of Fabricius contained lymphoid follicles which were divided into cortex and medulla. Lymphocytes, lymphoblasts, reticular epithelial cells (REC) and macrophages were observed in the cortex and medulla. Although many secretory dendritic cells (SDC) were seen in the medulla, they were not seen in the cortex. Tonofibrils and lipid droplets in the cytoplasm of the reticular epithelial cells which were abundant in the medulla and rare in the cortex were observed. Many alpha naphtyl acetate esterase (ANAE)-negative and a few ANAE-positive cells were detected in the cortex and medulla of the lymphoid follicles. Plasma cells were also detected beneath the interfollicular epithelium and at the interfollicular region, and pyroninophilic cells were seen in the lymphoid follicles as well.

Keywords: *Bursa of Fabricius, Light microscopy, Electron microscopy, Turkey*

Hindilerin Bursa Fabricius'u Üzerinde Işık ve Elektron Mikroskobik Çalışmalar

Özet

Bu çalışmanın amacı, hindilerde bursa Fabricius'un yapısını ışık ve elektron mikroskobik olarak incelemektir. Çalışmada 20 adet hindi bursa Fabricius'undan alınan doku örnekleri materyal olarak kullanıldı. Bursa Fabricius'un duvar yapısının tunika mukoza, tunika muskularis ve tunika seroza katmanlarından oluştuğu görüldü. Tunika mukozanın lumene doğru uzanan plikalar yaptığı gözlendi. Lamina epitelyalisin interfoliküler epitel (IFE) ve foliküle ilişkili epitel (FAE) olmak üzere iki farklı epitelden oluştuğu tespit edildi. IFE hücrelerinin apikal yüzlerinde mikrovilluslar ve sitoplazmalarında mukus granülleri görüldü. FAE hücrelerinin apikal yüzlerinde mikrofoldlar ve sitoplazmalarında ise çok sayıda vezikül ve vakuol göze çarptı. FAE'in alt kısmında medulaya doğru devam ederek timusdaki Hassal cisimciklerine benzer lamelli epitelyal cisimcikleri şekillendiren destekleyici hücreler katmanının yer aldığı belirlendi. Plikalarda korteks ve medula bölümlerinden oluşan lenf folikülleri tespit edildi. Medulada ve kortekste lenfosit, lenfoblast, retikuler epitelyal hücreler (REC) ve makrofajlar gözlendi. Ayrıca medulada çok sayıda gözlenen sekretorik dendritik hücrelere (SDC) kortekste rastlanmadı. Kortekste az medulada ise bol bulunan REC'in sitoplazmalarında tonofibril ve yağ damlacıkları görüldü. Lenf foliküllerinin korteks ve medulasında az sayıda alfa naftil asetat esteraz ANAE-pozitif ve çok sayıda ANAE-negatif hücreler belirlendi. İnterfoliküler epitelin hemen altında ve interfoliküler bölgede plazma hücreleri ile lenf foliküllerinde pironinofilik hücreler görüldü.

Anahtar sözcükler: *Bursa Fabricius, Işık mikroskop, Elektron mikroskop, Hindi*

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INTRODUCTION

The bursa of Fabricius of avian species is a lymphoepithelial organ which possesses several mucousal folds and opens into the cloaca by a short duct [1,2]. Embriyologically, its short duct is originated from ectoderm while epithelium of the bursa of Fabricius is from endoderm of the last intestine and the other tissue components from mesenchyme [2,3]. Involuted through sexuel maturation, this organ is a primary lymphoid organ playing essential role on the maturation of B lymphocyte, and is responsible for immunoglobulin isotype switch [4].

The wall of the bursa of Fabricius is composed primarily of the tunica mucosa, tunica muscularis, and tunica serosa. The tunica mucosa is the thickest component projecting several plicae throughout the lumen [5]. The plicae comprise two kinds of epithelium; firstly the follicle associated epithelium covering the lymph follicle and secondly, the interfollicular epithelium veiling the space between the follicles [6,7].

Each of the plicae contains several lymphoid follicles. They possess two components, cortex and medulla. The cortex and medulla are unrelated from one another [5]. Both a capillary layer and an epithelium called either corticomedullary border epithelium [8,9] or undifferentiated epithelium [10], detach the cortex from the medulla. Reticular epithelial cells, lymphocytes, lymphoblasts, and macrophages are found in the cortex. Thus, it contains thin arterioles and venules, and capillaries [2]. Additionally, medulla includes RECs, SDCs, B lymphocytes, T lymphocytes in small numbers and macrophages [10,11]. The lymphoid cell content of the medulla is composed by lymphocytes and several lymphoblasts usually located peripherically. Blood are deprived of medulla layer [2]. More than 90% of the lymphocytes of the bursa of Fabricius are B lymphocytes [12,13]. The T lymphocytes are mostly found in the cortex, localized particularly along the corticomedullar region [11].

The tunica muscularis contains externally, longitudinal, and internally circular smooth muscle layers in some species whereas either a single longitudinal or two longitudinal muscle layers containing a circular layer are seen in other species. It is covered externally by a thin serosa layer [2].

The aim of the current study was to determine the ultrastructure of the bursa of Fabricius in turkey.

MATERIAL and METHODS

Animals

One-month-old turkeys (n=20) provided from Bolca Turkey Company (Bolu, Turkey) were used in this study. Tissue samples were removed from turkeys under sodium pentobarbital anesthesia.

Light microscopic examinations

The tissue samples of the bursa of Fabricius were fixed in 10% formaldehyde for 24 h, then they were put in ethyl alcohol, methylbenzoate, and benzol series and embedded in paraplast. Modified triple staining of Mallory [14] was performed on 6 μm thick sections. To identify the T lymphocytes, tissue specimens were first fixed in cold formol-sucrose solution (pH 6.8) at +4°C for 22 h, and then in Holt's solution at +4°C for 22 h. Finally, specimens were cut into 8 μm thick sections on a cryostat. Alpha naphtyl acetate esterase (ANAE) was applied to these sections at pH 6.4 by Mueller et al[15]. To observe the plasma cells and pyroniniphilic cells, tissue samples were also fixed in alcohol-formol solution for 48 h, followed by ethyl alcohol, methylbenzoate, and benzol series, and embedded paraplast. Methyl green pyronin staining was performed on the 7 μm thick sections of those tissue blocks [16].

Electron Microscopic Examinations

The tissue samples were first fixed by glutaraldehyde-paraformaldehyde, then postfixed in osmium tetroxide and finally they were embedded in araldit M after being put in alcohol series and propylen oxcyde. Toluidin blue pyronin staining was applied to 1 μm semi-thin sections of those tissue blocks. Those semi-thin sections were observed and thin sub-sections at 300-400 Å were chosen from indicated areas, stained whith uranyle acetate and lead citrate, in accordance with the method of Veneable and Coggeshall[17] and examined in the Carl Zeiss EM

9S-2 model transmission electron microscope.

RESULTS

Light Microscopy

Several plicae were observed inside the bursa of Fabricius, projecting throughout the lumen. The wall structure of the organ was composed of the tunica mucosa, tunica muscularis, and tunica serosa. The sublayers of the tunica mucosa were constituted by the lamina epithelialis, lamina propria, and lamina submucosa, while the muscular layer was absent. Two different epitheliums were observed in the lamina epithelialis; the follicle associated epithelium (FAE) covering the lymphoid follicles, and the interfollicular epithelium (IFE) *(Figure 1)*. IFE was observed to display the characteristics of pseudostratified columnar epithelium whereas FAE possessed columnar epithelial cells. Supporting flat cells, containing flat nucleus were also present just beneath the FAE *(Figure 2)*. Some of them were also observed throughtout the medulla, forming lamellated epithelial bodies, which were similar to the thymic Hassal bodies *(Figure 2)*. Each follicle was shown to be divided into two parts; externally located cortex and internally located medulla *(Figure 1)*. An eminent corticomedullary border was formed with the capillaries from cortical side and the cuboidal epithelial cells from medullary side *(Figure 1)*. The capillaries were not depicted to cross the medulla and consequenthly, there was no capillary vessel in the medulla. Beneath the tunica mucosa, externally located longitudinal and internally lied circular smooth muscle layers were observed in the tunica muscularis. This tunica layer was hence encircled by the tunica serosa which had a mesothelial in nature.

Most of the lymphocytes in the bursa of Fabricius were ANAE-negative, while some ANAE-positive lymphocytes containing 1-2 reddish-brown cytoplasmic granules were eridenced both in the cortex and medulla, particularly along the cortico-medullary border *(Figure 3)*. Diffuse black-brown stained reticular cells were also detected along with the ANAE-positive and ANAE-negative lymphocytes in the lymphoid follicles *(Figure 3)*. The ANAE-positive and ANAE-negative lymphocytes were seen in the FAE and also among the supporting cells. Moreover, plasma cells were present densely in the connective tissue beneath the epithelium, and more scarcely in the interfollicular connective tissue *(Figure 4)*. The number of the pyronino-philic cells observed in the cortex was lower than in the medulla.

Electron Microscopy

Microvilli were seen on the apical surfaces of the IFE cells *(Figure 5)*. Numerous mucous granules were also observed in the apical cytoplasma of the cells *(Figure 5)*. Plasma cells were found to be located in the connective tissue beneath the epithelium *(Figure 5)*. Short, thick and irregular microfolds were determined on the apical surfaces of the FAE cells *(Figure 6)*. Intercellular areas of the FAE were widened and the cells were related apically by the intercellular connections. Several vacuoles and vesicles were observed in the apical cytoplasma of the FAE cells that contained an eminent nucleus *(Figure 6)*. A prominent Golgi zone, several mitochondria, endoplasmic reticulum, lysosomes, and free ribosomes were also detected, FAE cells were determined to be lack of basal membrane *(Figure 6)*.

Beneath the FAE supporting flat cells were attached by desmosomes and their cytoplasma contained a lot of tonofibrils. SDCs and lympho-cytes were seen around those cells *(Figure 7)*. Supporting cells, SDCs, and macrophages were found in the lamellated epithelial bodies formed by the supporting cells in the medulla. However, neither lymhocyte nor lymphoblast was found in those areas.

Cuboidal epithelial cells of the cortico-medullary border were bound by desmosomes. Several lymphocytes, lymphoblasts, RECs, and macrophages were observed in the medulla and in the cortex. Hence, the SDCs determined several in the medulla, were absent in the cortex. RECs were more frequently observed in the medulla than that of in the cortex. A lot of tonofibrils and lipid drops were shown in the RECs which contained several cytoplasmic extensions *(Figure 8)*. Those extensions were attached by desmosomes, particularly in the medulla *(Figure 8)*. These cells encircled by

lymphocytes and lymphoblasts.

DISCUSSION

Several contradictory suggestions have been raised regardling the IFE of the bursa of Fabricius. Some [18,19] reports indicated the IFE to be pseudostratified epithelium type while others [8,20] documented that to be a single layer columnar epithelium. In the present study conducted on turkey, the IFE was a pseudostratified columnar epithelium, and electron microscopic observations confirmed the presence of long microvilli on the edge of the IFE cells that contained severel mucous granules in their cytoplasma, as shown by Ciriaco et al.[21]. Moreover, intercellular areas among the FAE cells were wide and there were several macrophages along the FAE, as reported by Lupetti et al.[9]. The FAE was also shown to contain lymphocytes. Olah and Glick [11] have reported that tonofibrils are present in the supporting cells that attach to the FAE cells through desmosomes. Furtheremore, secretory dendritic cells have been reported among the supporting cells [11,22]. Lamellated epithelial bodies constituted by supporting cells and some macrophages, SDCs, lymphocytes and lymphoblasts were found in the medulla of several lymphoid follicles as it was previously described [11]. Cytoplasma of the SDC in the medulla contained electron dense and round, oval, and irregular granules [10,23]. SDCs similar to Hassal corpuscules, have been documented to be present either in the FAE [24], or among the supporting cells beneath the FAE [11] .

The cortex of the lymphoid follicles has been documented to contain RECs, lymphocytes, lymphoblasts, mitosis figures and macrophages, as well as thin arterioles and venules, and capillaries. The capillaries were not depicted to cross the medulla and consequenthly, there was no capillary vessel in the medulla [2], whereas lymphocytes, lymphoblasts, SDCs, RECs, and macrophages were present [10,11]. Consequently, the corticomedullary border was formed by cortical capillaries and by a cuboidal epithelium, called either undifferentiated epithelium [8,9] or corticomedullary border epithelium [10]. The cellular distribution between cortex and medulla of lymphoid follicles observed in the current study was quite comperable to data obtained in other bird species.

The RECs essentially found in the medulla, exhibited tonofilaments and lipid drops in the cytoplasma. They also presented long cytoplasmic extensions, which participated with desmosomes to intercellular connections [25].

Olah and Glick [11] reported that the frequency of T lymphocytes in both cortex and medulla differed greatly among the lymphoid follicles. Nevertheless, T lymphocytes were essentially found in the cortex, mostly located along the corticomedullary border. They also reported that some T cells were observed in the IFE but not in the FAE. B and T lymphocytes can be identified on the basis of their enzyme content in the cytoplasma. T lymphocytes express a non specific esterase, ANAE, in several secretory granules, whereas B lymphocytes do not synthetize it. Consequently, ANAE positive lymphocytes (usually showing 1-2 reddish brown granules) were considered T cells, and ANAE negative lymphocytes as B cells [15,26].

In the lymphoid follicles of the turkey, ANAE positive lymphocytes (T cells) were found not only in the cortex, scarcely in the medulla but also in the FAE. In addition, B lymphocytes (ANAE negative lymphocytes) were also detected in the FAE and among supporting cells. These findings are in agreement with the study of the Lupetti et al.[27] which demonstrated that lymphocytes were needed for the FAE development.

While Gulmez and Aslan[28] observed a homogeneous distribution of plasma cells between the cortex and the periphery of the medulla, Hodges[2] claimed that these cells were absent in the bursal follicle, and in fact, they were disseminated in the connective tissue among the follicles and beneath the epithelium. In the present study, the plasmocyte localisation was similarly in the connective tissue, as reported by Hodges [2], but several pyroninophilic cells at different development periods and mitosis figures were also scarcely observed in the cortex and medulla.

A thick muscular tunica has been reported to encircle the tunica mucosa of the bursa of Fabricius. The tunica mucosa comprises two layers in same species; externally longitudinal

layers, internally located circular layer while it, sometimes, contains either a longitudinal layer or two longitudinal layers surrounding a circular layer [2]. It has thus, been reported to be covered by a thin tunica serosa which is characteristic with mesothel [2,5] . The tunica muscularis observed in our study, possessed externally longitudinal and internally circular muscle layers. The tunica serosa located as a loose connective tissue was also observed on the outermost surface of the organ.

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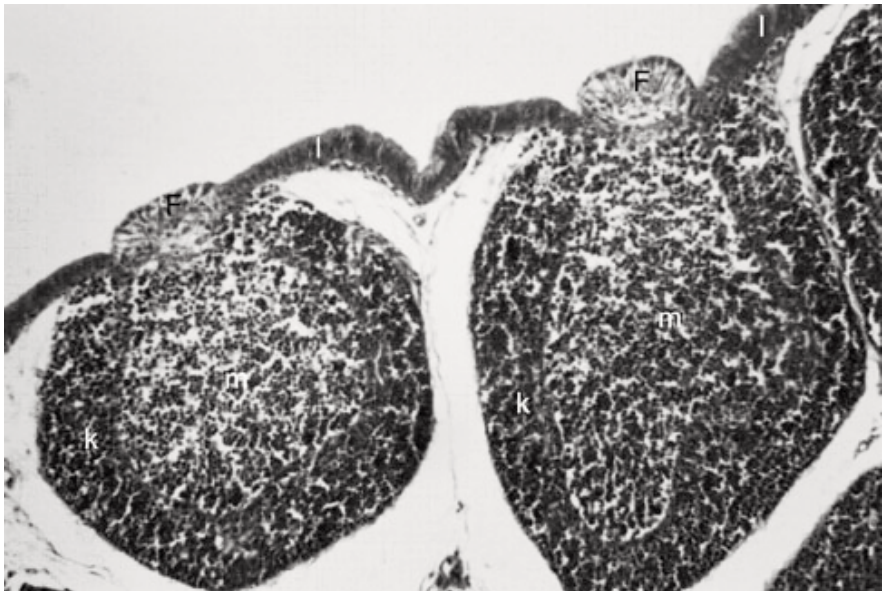

Fig 1. Lamina epithelialis. I: interfollicular epithelium, F: follicle associated epithelium, m: medulla, k: kortex, thin arrow: capillary vessel, thick arrow: epithelial cells. Triple. x 145 magnification.

Şekil 1. Lamina epitelyalis: I: interfoliküler epitel, F: folikülle ilişkili epitel, m: medulla, k. korteks, ince ok. kapillar damar, kalın ok: epitel hücreleri. Triple. x 145.

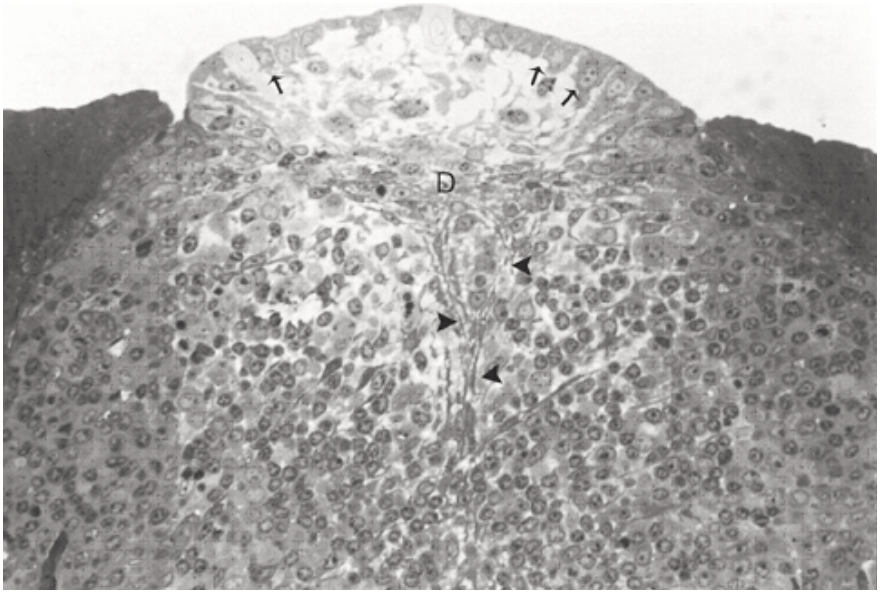

Fig 2. FAE cells (arrows) and supporting cells (D). arrowhead: supporting cells which infolded into the medulla. Toluidin blue pyronin. x 560 magnification.

Şekil 2. FAE hücreleri (oklar) ve destekleyici hücreler (D). ok başı: destekleyici hücrelerin medulanın içine doğru devam etmesi. Toluidin blue pironin. x 560.

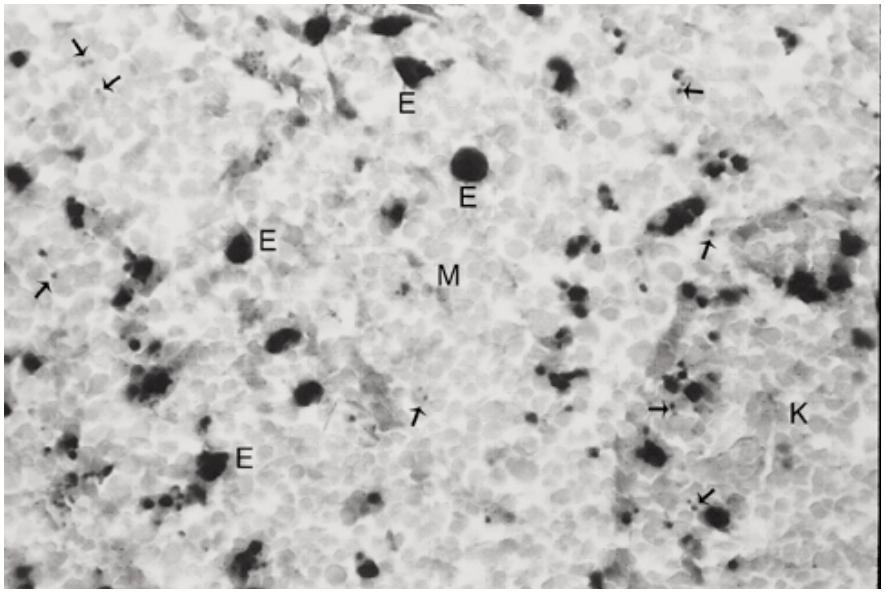

Fig 3. ANAE-positive and ANAE-negative lymphocytes in lymph follicles. arrows: ANAE-positive lymphocytes, K: kortex, M: medulla, E: reticular epitelyal cell. ANAE. x 470 magnification.

Şekil 3. Lenf foliküllerinde ANAE-pozitif ve ANAE-negatif lenfositler. oklar: ANAE-pozitif lenfositler, K: korteks, M: medula, E: retikulum hücresi. ANAE. x 470.

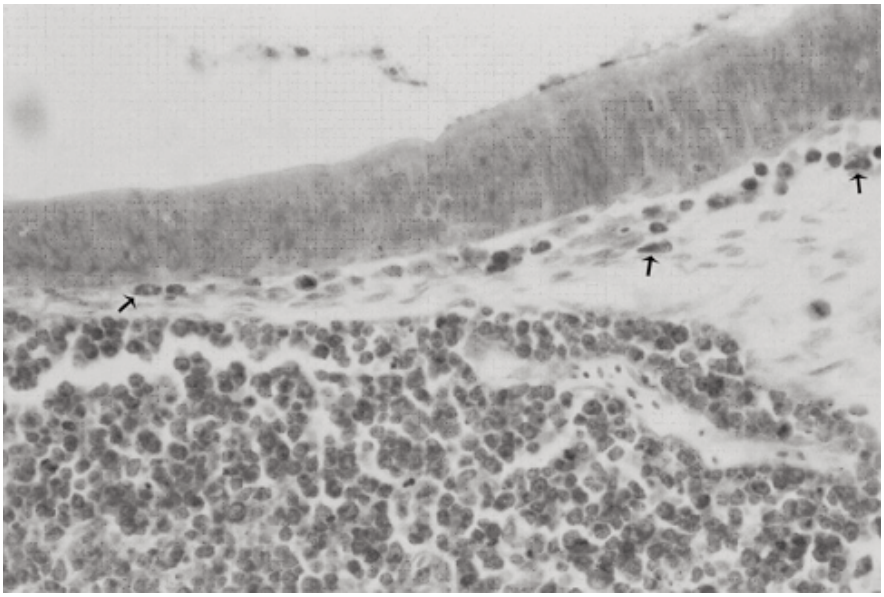

Fig 4. Plasma cells in the connective tissue beneath the interfollicular epithelium (arrows). Methyl green pyronin. x 560 magnification.

Şekil 4. İnterfoliküler epitelin altındaki bağ dokuda plazma hücreleri (oklar). Metil green-pironin. x 560.

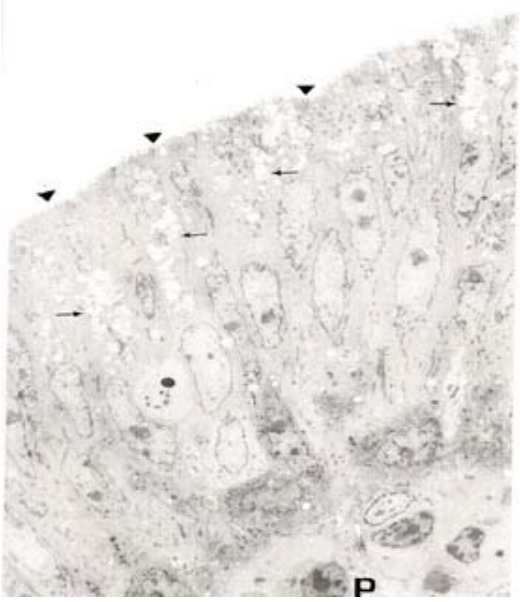

Fig 5. Interfollicular epithelium. arrow: mucous granules, arrowhead: microvilli,
p: plasma cell. x 2 860 magnification.

Şekil 5. İnterfoliküler epitel. ok: müköz granüller, ok başı: mikrovilluslar,
p: plazma hücresi. x 2 860.

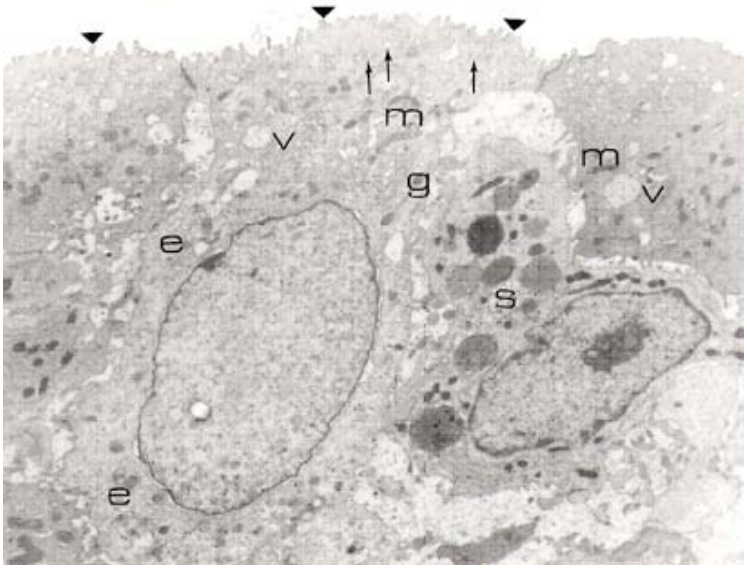

Fig 6. Follicle associated epithelium. arrowhead: microfold, arrow: vesicle, v: vacuol, m: mitochondria,
g: golgi zone, e: endoplasmic reticulum, S: secretory dendritic cell. x 6 600 magnification.

Şekil 6. Foliküle ilişkili epitel. ok başı: mikrofold, ok: vezikül, v: vakuol, m: mitokodriyon, g: Golgi aygıtı,
e: granüllü endoplazma retikulumu, S: sekretorik dendritik hücre. x 6 600.

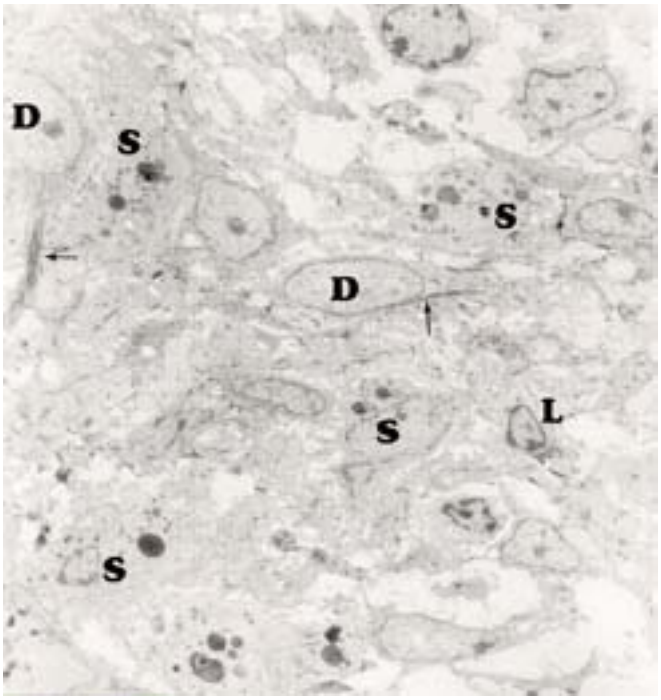

Fig 7. Supporting cells (D). S: secretory dendritic cell, L: lymphocyte, arrow: tonofibril.
x 3 200 magnification.

Şekil 7. Destekleyici hücreler (D). S: sekretorik dendritik hücre, L: lenfosit, ok: tonofibril .
x 3 200.

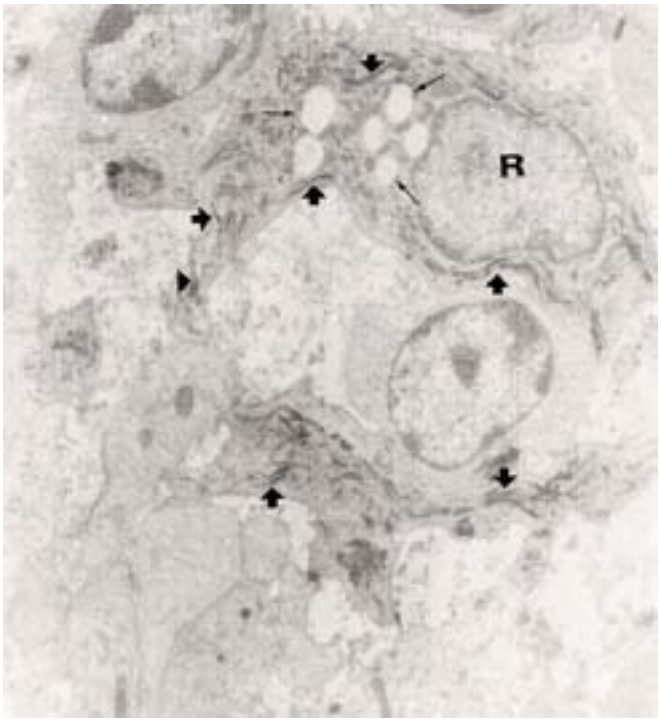

Fig 8. Reticular epithelial cell (R) in medulla. thin arrow: lipid drop, thick arrow: tonofibril,
arrowhead: desmosome. x 8 500. magnification.

Şekil 8. Medulada retiküler epitelyal hücre (R). İnce ok: yağ damlacığı, kalın ok: tonofibril,
ok başı: dezmozom. x 8 500