# Histology and ultrastructure of the lymph nodes of the Egyptian water buffalo (*Bus bubalus*)

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## Abstract

Prescapular, femoral, mesenteric, mediastinal lymph nodes from 5 buffalo calves and 5 buffalo bulls were studied using both light and transmission electron microscopes. The lymph nodes were surrounded with a thin capsule of dense connective tissue and smooth muscles. Trabeculae of similar structure extended from the capsule dividing the parenchyma into incomplete lobules. Subcapsular and trabecular lymphatic sinuses were lined with endothelial cells resting on a basement membrane and supported with a reticular fiber network. Fine reticular fiber network was extended through the lumen of these sinuses. The parenchyma was divided into cortex and medulla separated with ill defined paracortical area. The cortex was formed from lymphoid follicles and interfollicular diffuse lymphocytes. Primary and secondary follicles were observed. The medulla was formed from medullary cords of lymphocytes separated by lymphatic sinuses. These sinuses were lined with a discontinuous epithelium and crossed with reticular fibers. Numerous lymphocytes and some macrophages were observed in the sinuses. High endothelial venules were found in the paracortical area. Several lymphocytes were observed infiltrating the wall of these venules. The lymph node of the Egyptian water buffalo showed a typical structure compared with the majority of mammals with no age related structural variation. These lymph nodes play an essential role in regional immunity throughout the life of this animal and considered a mirror reflecting the health status in meat inspection.

## Introduction

Lymph nodes are independent secondary lymphatic organs lying in the course of lymphatic vessels. They play an essential role in regional immune response (Dellmann and Brown, 1981 and Picker and Siegelman, 1999) and have a veterinary importance in meat inspection, as they reflect the health of the region they drain (Wilson, 1991). The histology of the lymph nodes were studied in different animal species including human (Forkert et al., 1977), Goat ( Faroon et al., 1989), rat (Ushiki et al., 1995), mouse (Crivellato and Mallardi, 1998), Seal (Welsch, 1997), the dromedary camel (Taher, 1962, Mosallam, 1978, Taher et al., 1979 and 1989a,b, Abdel-Magied et al., 2001 and Zidan, 2004). The lymph nodes have a general structure in all mammals studied before, with some species variations (Dellmann and Brown, 1981). The same authors added that, the lymph nodes are surrounded by a capsule and trabeculae of connective tissue, a reticular meshwork permeates the parenchyma; afferent lymph vessels enter the lymph node at its periphery. Efferent lymphatics leave at the hilus. The parenchyma of the lymph nodes is organized into outer cortex of lymphoid follicles and inner medulla of medullary cords separated by lymphatic sinuses.

Paracortical area of small lymphoctes extended between the cortex and medulla. They added that, the cortical and medullary tissues are reversed in the pig. In the same time the lymph nodes of the dromedary camel showed some peculiarities, Taher (1962), Mosallam (1978), Abdel-Magied et al. (2001) and Zidan (2004) explained that the parenchyma of the dromedary camel was not defined into a cortex, paracortex and medulla, but intermingled in a some what spleen like fashion. In spite of the great importance of the Egyptian water buffalo *(Bus bubalus)* as a source of milk, meat and hide production in Egypt, and to the best of my knowledge there is no available published data on the histology of the lymph nodes of this important animal. Therefore the present work aimed to describe the histology and ultrastructure of the lymph nodes of the Egyptian water buffalo *(Bus bubalus)*. This framework should also throw a light on the function of the lymph nodes in the buffalo and may be useful for physiological, immunological and pathological studies.

## Material and Methods

*Lymph nodes*

The Specimens were obtained in the slaughter house from 10 clinically healthy animals: 5 male buffalo calves (2-3 months) and 5 buffalo bulls (2-3 years) directly after slaughtering for human consumption in the slaughter house of Faculty of Agriculture, Alexandria University. Specimens were obtained from each animal from prescapular, femoral, mesenteric, mediastinal lymph nodes. Each lymph node was sampled as follow:

1. Small samples were fixed in 10% phosphate buffered formaldehyde and used for routine histology
2. Fresh samples were fixed immediately in 6% solution of phosphate buffered gluteraldehyed (pH 7.4) at 4°C (McDowell and Trump, 1976) and processed for transmission electron microscopical examination.

*Routine histology*

The fixed specimens were processed for paraffin sections. Four □m thick Sections were prepared and stained using Mayer's hematoxylin and eosin (H&E) stain., Gomori's silver impregnation stain (Bancroft and Stevens, 1982) and Crossmon trichorome stain (Boeck, 1989)

*Transmission electron microscopy*

Fresh specimens, about 1 $mm^3$ in size were obtained from the each lymph node and fixed in 4% phosphate buffered glutaraldehyde pH 7.4 for 2 hours. at 4 °C (Mc Dowell and Trume, 1976). After postfixation in 1% solution of phosphate buffered osmium tetroxide at 4°C, specimens were dehydrated in ascending grades of ethanol and embedded in Epon (Serva, Heidlberg, Germany) according to standard protocols (Hayat, 1986). Semithin sections (1 □m) were prepared, stained with toluidine blue and examined by the light microscope. The suitable areas for electron microscopic examination were selected. Then, ultrathin sections (50-70 nm) were cut by a diamond knife and stained with uranyl acetate followed by lead citrate. The sections were observed with a Zeiss EM10 electron microscope (Zeiss, Oberkochen, Germany) at 80 KVs.

## Results

The lymph nodes of the Egyptian water buffalo were composed of stroma of capsule and trabeculae which enclosed parenchyma formed from outer cortex and inner medulla. The paracortical area was ill defined (Fig.1). The capsule was fairly thin formed from dense connective tissue and smooth muscles (Figs. 1& 2). The trabeculae extended from the capsule into the parenchyma. These trabeculae have the same structure of the capsule with fewer smooth muscles. Both of the capsule and trabeculae were continuous with a reticular network of reticular fibers which support the parenchyma (Figs. 3&4).

Subcapsular lymphatic sinuses were extended under the capsule. The subcapsular sinuses are connected to trabecular lymphatic sinuses which surround the trabeclae. Both sinuses were supported with a reticular fiber network. Fine reticular fiber trabeculae were observed extended through the lumen of these sinuses (Figs. 3, 4&5). Each sinus was lined with endothelial cells resting on a basement membrane. Lymphocytes were the main cells in the sinus lumen in addition to macrophages (Fig. 6).

The cortex was formed from lymphoid follicles and interfollicular tissue (Fig. 7). Primary and secondary lymphoid follicles were observed. The lymphoid follicles were supported with a reticular fiber network. These reticular fibers were rarely observed in the germinal centers (Fig. 8). The interfollicular tissue was formed mainly from diffuse lymphocytes.

High endothelial venules were extended in the in the ill defined paracortical area. These venules were lined with high endothelial cells resting on a basement membrane. Several lymphocytes were observed infiltrating the wall of these venules (Figs. 9, 10&11). Dendretic cells were distributed among the lymphocytes in the paracortical area (Fig. 12).

The medulla was formed from medullary cords separated by lymphatic sinuses. The medullary cords were formed from diffuse lymphocytes supported with reticular fiber network (Figs. 13&14). Other cells were observed in the medullary cords including macrophages, plasma cells and granulocytes (Figs. 15&16). The lymphatic sinuses which were distributed among the lymphatic cords were variable in sizes and crossed with reticular fibers (Fig. 14). These lymphatic sinuses were lined with discontinuous endothelial cells resting on a basement membrane. Lymphocytes were the main cellular component observed in the sinus lumen. Some macrophages were also associated to the lymphocytes (Figs. 17&18).

## Discussion

The present study described the histology and ultrasructure of the lymph nodes of the Egyptian water buffalo. The present finding shows that the buffalo lymph node is surrounded with a fairly thin capsule formed from connective tissue and smooth muscles. Trabeculae extended from the capsule into the parenchyma. These trabeculae have the same structure of the capsule. The smooth muscles were described in the capsule of the lymph node of the camel (Taher 1962, Abdel-Magied et al., 2001 and Zidan, 2004), Goat (Faroon et al., 1989) and Seal

(Welsch et al., 1997). The contraction of these muscles may accelerate lymphatic flow from the lymphatic sinuses through efferent lymphatics.

Subcapsular lymphatic sinuses were extended under the capsule. These sinuses are connected to trabecular lymphatic sinuses. Both sinuses were lined with continuous endothelial cells resting on a basement membrane. Fine reticular fiber network was extended through the lumen of these sinuses providing an attachment for various cells mostly macrophages and lymphocytes. Williams et al. (1995) explained that, the lymph flowing through these sinuses is exposed to the action of macrophages and lymphocytes adhering to the reticular network.

The current work found that the parenchyma of the buffalo lymph node is similar to most animal species in arrangement, where the lymph node is divided into cortex harboring the lymph follicles and medulla containing medullary cords and free from any follicles (Dellmann and Brown, 1981). The presence of secondary follicles is indicative for the role of lymph node in antibody production.

The fine reticular fibers network around the lymphoid follicles described in the current work were described in human spleen as a myofibroblastic cells (Steiniger et al., 1997).

High endothelial venules were extended in the paracortical areas. Lymphocytes, which form part of recirculating pool, migrate across the wall of the high endothelial venules from the blood to the lymph stream (Gowans and Knight, 1964 and Anderson and Anderson, 1976). In the present study, several lymphocytes were observed in the wall of the high endothelial venules. That may suggest the role of the high endothelial venules in the buffalo lymph node in lymphocytes recirculation. Lymphocytes of the blood recirculate through the lymph nodes by migration through the cuboidal endothelium lining the high endothelial venules and filtered again into the lymphatic sinuses to the efferent lymphatics (Dellmann and Brown, 1981). The high endothelial venules form an important site of lymphocytes migration. They allow extensive movement of lymphocytes from blood stream into the paracortical area and probably vice verse. (Williams et al., 1995 and Butcher et al., 1999).

In agreement with Crivellato and Mallardi (1998) and Abdel-Magied, et al. (2001), the current work showed that, the medullary lymphatic sinuses were lined with discontinuous endothelial cells, this discontinuity of the endothelium allow the free passage lymphocytes and macrophages. This finding differs from that of Taher et al. (1989a) who mentioned that the endothelial lining of these sinuses in the camel was continuous.

There are numerous fine reticular trabeculae crossing the medullary sinuses make their lumina labyrinthine and provides large area for attachment of various cell types in the lumen. This may help the medullay sinuse in doing a perfect filtration of the lymph.

The lymph node of the Egyptian water buffalo showed a typical structure compared with most mammals with no age related structural variation. These lymph nodes play an essential role in regional immunity throughout the life this animal and considered a mirror reflecting the health status in meat inspection.

## References

Abdel-Magied, E.M., Taha, A.A.M., Al-Qarawi, A.A. and Elfaki, M.G. 2001: The parotid, mandibular and lateral retropharyngeal lymph nodes of the camel (*Camelus dromedarius*). Anat. Histol. Embryol. 30 (4): 199-203.

Anderson, A. O. and Anderson, N. D. 1976: Lymphocytes emigration from high endothelial venules in rat lymph nodes. Immunology. 731-748.

Bancroft, J. and Stevens, A. 1982: Theory and practice of histological techniques. 2nd Ed. Churchill Livingstone. Edinbergh, London, Melbourne and New York. PP. 110-111.

Boeck, p. 1989: Romeis Mikroskopische Technik, 17 Auflage. Urban & Schwarzenberg, München, Wien and Baltimore. PP. 502-503.

Butcher, E., Williams, M., Youngman, K., Rott, L. and Briskin, M. 1999: Lymphocyte trafficking and regional immunity. Adv. Immunol. 72:209-253

Crivellato, E. and Mallardi, F. 1998: the sinus endothelial cell architecture in the mouse lymph node. Structural peculiarities and close correlation with the fibroblastic reticular cells. J. Submicrosc. Cytol. Pathol. 30 (4): 495-502.

Dellman H. D. and E. M. Brown, 1981: Textbook of Veterinary Histology. Lea & Febiger, Philadelphia pp. 170-176.

Faroon O.M., Henry, R.W. and Al-Bagdadi, F.K. 1989: SEM and TEM study Caprine superficial lymph nodes. Histol. Histopathol. 4 (2): 173-181.

Forket, P.G., ThliverisJ. A. and Bertalanffy. F.D. 1977: Structure of sinuses in human lymph node. Cell Tissue Res. 14; 183(1): 115-130.

Gowans J. L. and E. J. Knight, 1964: The rout of re-circulation of lymphocytes in the rat. Proceeding of the Royal Society of London (biology) 159: 257-282.

Hayat, M. 1986: Basic techniques for transmission electron microscope.
Academic Press, Baltimor, PP 42-43.

Mc-Dowell, E.M. and F. Trump, 1976: Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100: 405-415

Mosallam, El-S. M. M. (1978): Histological and histochemical studies of the lymphoreticular and glandular tissue of the pharyngeal region of the one humped camel *(Camelus dromedarious).* M.V.sc. Thesis. Fac. Vet. Med. Cairo Univ.

Picker, L. J. and Siegelman, M. H. 1999: Lymphoid tissues and organs. In Paul, W. E., ed., Fundamental Immunology, Lippincott-Raven, Philadelphia, PA. pp. 479–532.

Steiniger, B., P. Barth, B. Herbst, A. Hartnell, and P.R. Crocker, 1997: The species-specific structure of microanatomical compartments in the human spleen: strongly sialoadhesin-positive macrophages occur in the perifollicular zone, but not in the marginal zone. Immunol. 92, 307-316.

Taher, El-S. M. 1963: Study of the lymph nodes of the camel. Doctorate thesis. Fac. Vet. Med. Cairo Univ. Cited after Mosallam, El-S. M. M. (1978): Histological and histochemical studies of the lymphoreticular and glandular tissue of the pharyngeal region of the one humped camel *(Camelus dromedarious)*. M.V.sc. thesis. Fac. Vet. Med. Cairo Univ.

Taher, El-S. M., El-Sakhawy, M., Moussallam El-S., and El-Bargeesy, G. 1989a: Studies of the lymphatics and lymph node sinuses of one–humped camel fetuses *(Camelus Dromedarius)*. Egypt. J. Histol. 12 (1) 35- 42.

Taher, El-S. M., El-Sakhawy, M. A., Moussallam El-S., Moussa, M.H.G. and El-Bargeesy, G. 1989b: Studies of the lymphoid tissue of lymph nodes of one–humped camel fetuses *(Camelus Dromedarius)*. Egypt. J. Histol. 12 (1) 23-33.

Ushiki, T., Ohtani, O. and Abe, K. 1995: Scanning electron microscopic studies of reticular framework in the rat mesenteric lymph node. Anat. Rec. 241(1): 113-122.

Welsch, U., Schwertfirm, S., Skirnisson, K. and Schumacher, U. 1997: Histological, histochemical, and fine structural observation on the lymph node of common seal (*Phoca vitulina*) and the grey seal (*halichoerus grypus*).Anat. Rec. 247 (2): 225-242.

Wilson, A., 1991: practical meat inspection , Oxoford: Blackwell Scientific Publication. pp 37-46.

Williams, P., Bannister, L., Berry M., Collius, P., Dyson, M., Dussek, J. and Ferguson, M. 1995: Gray's anatomy 39 Ed. Churchill Livingstone, New York, Edinburgh and London. pp. 1431-1437.

Zidan, M. 2004: Histological, histochemical and immunohistochemical study of the lymph nodes of the Dromedary camel. Alex. J. Vet. Sci. 20, 137-148.

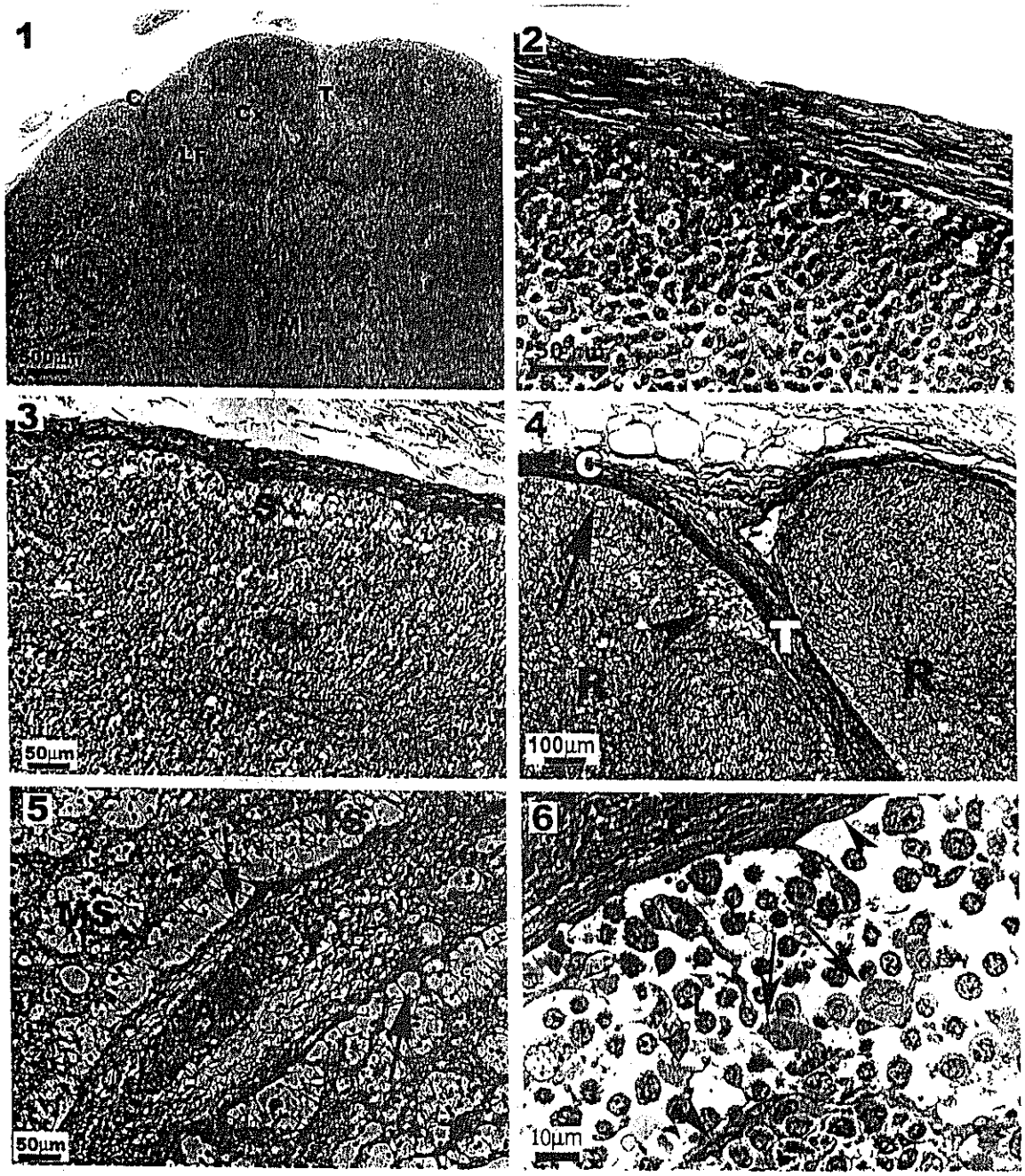


**Fig. 1.** The buffalo lymph node has a thin capsule (C) and trabeculae (T) enclosing the cortex (Cx) and medulla (M). LF = lymphoid follicles. Trichrome stain.

**Fig. 2.** The capsule (C) is fairly thin and consists of connective tissue rich in collagen fibers and moderate smooth muscles. Trichrome stain.

**Fig. 3.** The capsule (C) is rich in reticular fibers which extended through the subcapsular sinus (S). The cortex (Cx) is supported with a reticular fiber network. Gomori's stain.

**Fig. 4.** The trbecula (T) originates from the capsule (C). Both are continuous with a reticular network (R) supporting the parenchyma. Arrow = subcapsular sinus, arrowheads = trabecular sinus. Gomori's stain.

**Fig. 5.** The trabecula (T) is rich in reticular fibers which are extended through the trabecular sinuses (TS). Medullary sinuses (MS) are drained with trabecular sinus (arrows), A= trabecular artery. Gomori's stain.

**Fig. 6.** A semithin section showing a subcapsular sinus lined with endothelial cells (arrow heads) and containing lymphocytes (L) and macrophages (arrows).C = capsule. Toluidine blue.

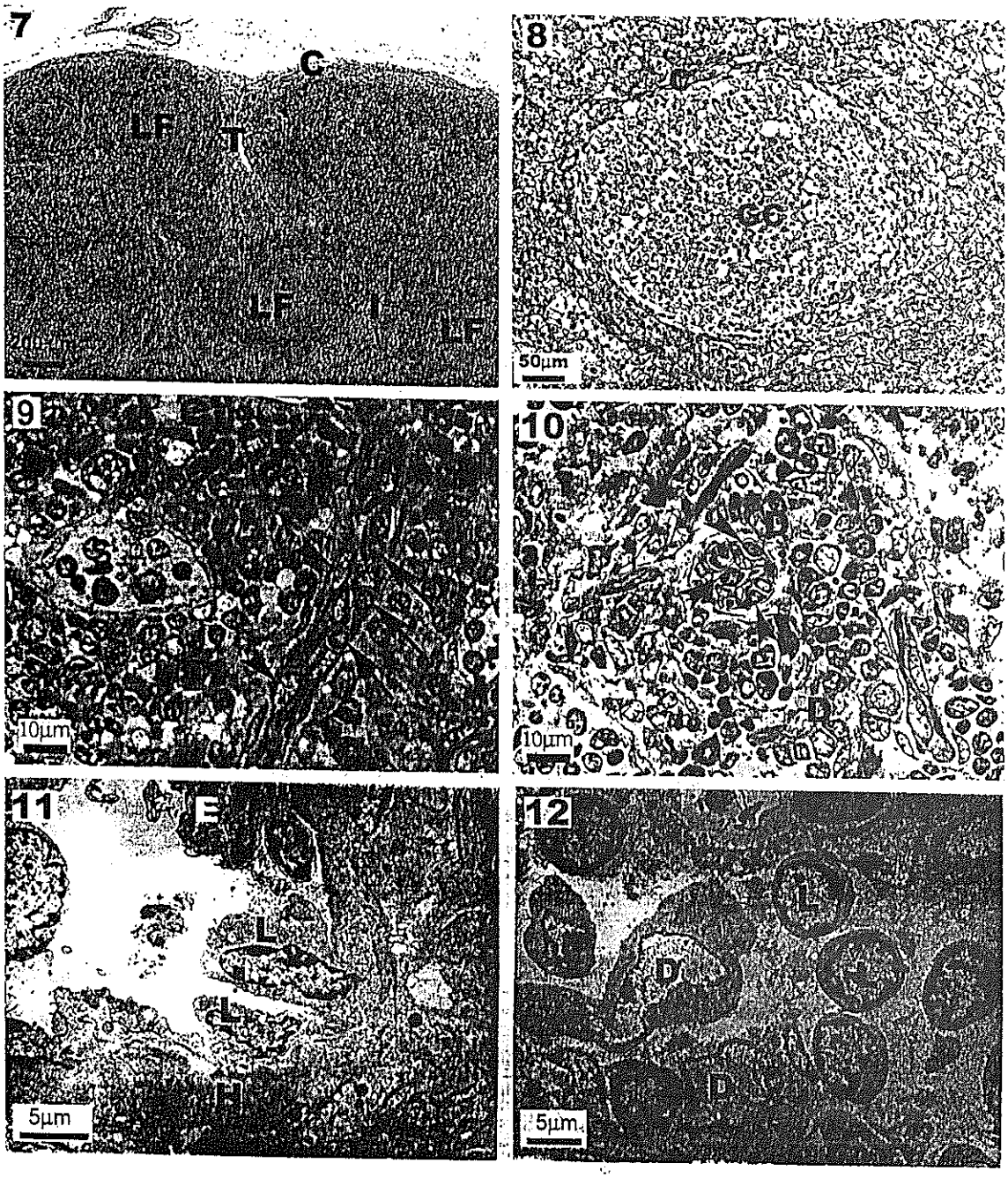


Fig. 7. The cortex is formed of lymphoid follicles with clear germinal centers (LF). I = interfollicular diffuse lymphocytes. C = capsule T = trabecula. Trichrome stain.

Fig. 8. The lymphoid follicle is enclosed within a reticular fiber network. The fibers are condensed in the corona (C) and very rare in the germinal center (GC). Gomori's stain.

Fig. 9. Longitudinal section of a high endothelial venule in the paracortical area. Several lymphocytes migrate through the wall (arrows). Arrow heads = High endothelial cells. S = medullary lymphatic sinus. Toluidine blue.

Fig. 10. Cross section of a high endothelial venule revealing different stages of lymphocyte migration (arrow heads). D = dendretic cells. Toluidine blue.

Fig. 11. Electron micrograph of a high endothelial venule lined with high endothelial cells (E). L = migrating lymphocytes.

Fig. 12. Electron micrograph showing dendretic cells (D) extended among several lymphocytes (L) in the paracortical area.

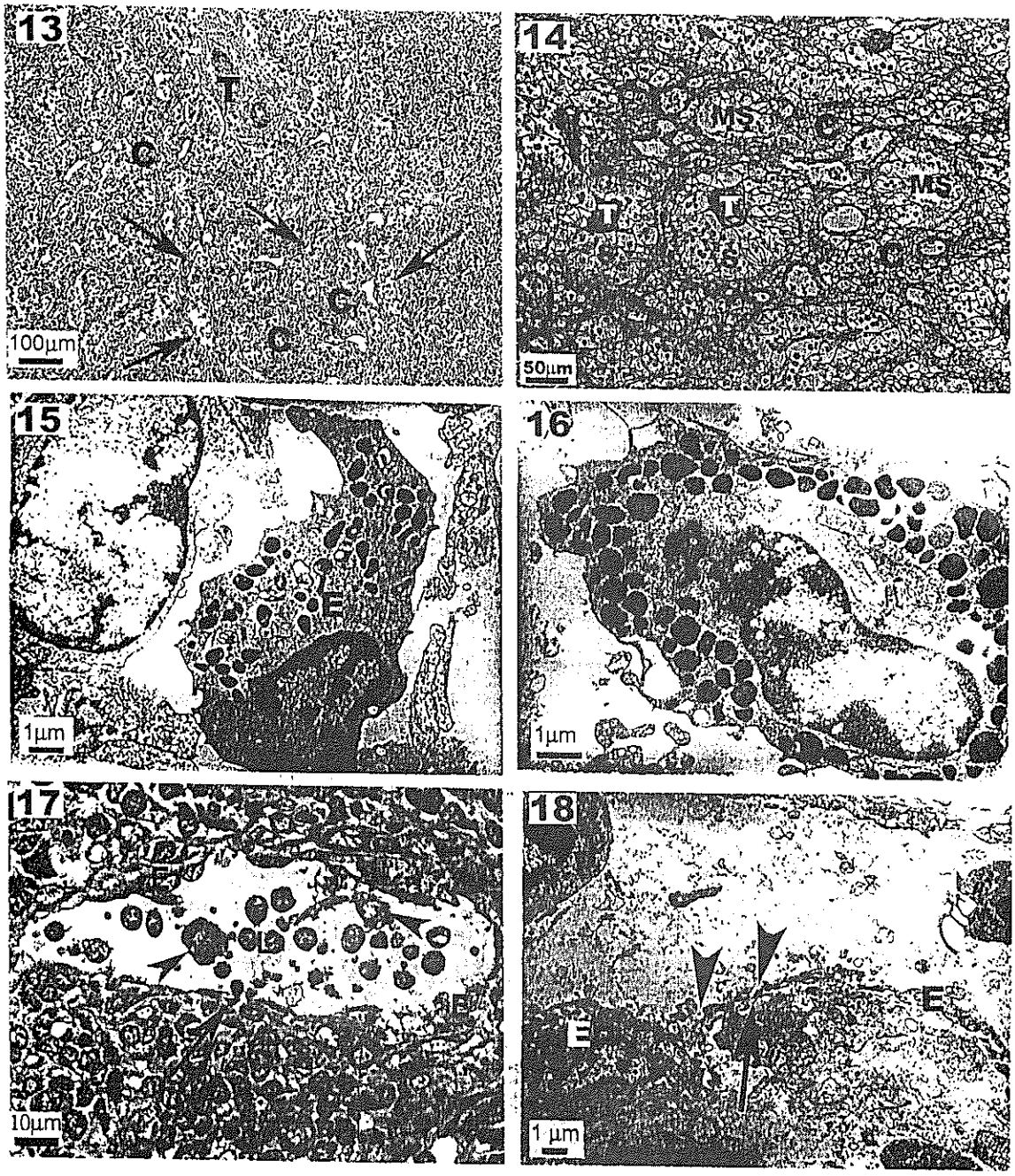


Fig. 13. The medulla is consisted of medullary cords (C) separated by medullary sinuses (arrows). T = trabecula. Trichrome stain.
Fig. 14. The medullary cords (C) are supported with reticular fibers. The medullary sinuses (MS) and the trabecular sinuses (S) are crossed with reticular fibers. T = Trabeculae. Gomori's stain.
Fig. 15. Electron micrograph of an eosinophil located in the medullary cord, showing the charactrestic cytoplasmic granules.
Fig. 16 . Electron micrograph of a basophil located in the medullary cord.
Fig. 17. The medullary sinus is lined with endothelial cells (E) and containing lymphocytes (L) and macrophages (arrow heads). Arrow = migrating lymphocyte. Toluidine blue.
Fig. 18. Electron micrograph of lymphatic sinus lined with discontinuous endothelial (E) resting on a basement membrane (arrow). Arrowheads = gaps.

الملخص العربى

## التركيب النسيجى و الدقيق للعقد الليمفية فى الجاموس المصرى

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تم إجراء هذه الدراسة على العقد الليمفية العنقيه السطحية والتحت حرقفية و المساريقية و الحاجزية فى الجاموس المصرى. أخذت العينات من خمس عجول جاموس وخمس ثيران جاموس. تم تجهيز و فحص العينات باستخدام المجهر الضوئى والإلكترونى الناقل.

ولقد أظهرت الدراسة أن العقد الليمفية محاطة بحافظة رقيقة من النسيج الضام والعضلات الملساء. يمتد من الحافظة حويجزات لها نفس تركيب الحافظة. ويمتد تحت الحافظة جيب ليمفى يتصل بجيوب ليمفية أخرى موجودة حول الحويجزات وتبطن الجيوب الليمفية بخلايا طلائية مفلطحة ويمتد خلال هذه الجيوب ألياف شبكية رقيقة وتحتوى على خلايا ليمفاوية ولاقمات كبيرة.

يتكون الجزء الوظيفى فى العقد الليمفية من قشرة ولب بالإضافة إلى منطقة جار القشرة الغير واضحة.ولقد كانت القشرة مكونه من حويصلات ليمفاوية بينها خلايا ليمفاوية وتكون اللب من أحبال ليمفاوية من الخلايا الليمفاوية واللاقمات الكبيرة والخلايا المحببة ويمتد بين الأحبال الليمفاوية جيوب ليمفية مبطنة بخلايا طلائية مفلطحة غير متصلة لتسمح بمرور الخلايا الليمفاوية وكذلك اللاقمات الكبيرة من خلالها. ويوجد فى منطقة الجار قشرة أوردة ذات خلايا طلائية مرتفعة وقد وجد عدد من الخلايا الليمفاوية المهاجرة بين هذه الخلايا الطلائية.

ولقد أوضحت هذه الدراسة أن العقد الليمفية فى الجاموس المصرى تشابه مثيلاتها فى معظم الثدييات فى التركيب وأنها تلعب دورا هاما فى المناعة للمنطقة التى ترشح سائلها الليمفاوى وبذلك يكون لفحصها فى عملية الرقابة الصحية على اللحوم أهميه كبيره لأنها تعكس الحاله الصحيه للمنطقه الخاصه بها.