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Cellular Components of the Hemal Node of Egyptian Cattle

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***Abstract*—**10 clinically healthy hemal nodes were collected from male bulls aged 2-3 years. Light microscopy revealed a capsule of connective tissue consisted mainly of collagen fiber surrounding hemal node, numerous erythrocytes were found in wide subcapsular sinus under the capsule. The parenchyma of the hemal node was divided into cortex and medulla. Diffused lymphocytes, and lymphoid follicles, having germinal centers were the main components of the cortex, while in the medulla there was wide medullary sinus, diffused lymphocytes and few lymphoid nodules. The area occupied with lymph nodules was larger than that occupied with non-nodular structure of lymphoid cords and blood sinusoids. Electron microscopy revealed the cellular components of hemal node including elements of circulating erythrocytes intermingled with lymphocytes, plasma cells, mast cells, reticular cells, macrophages, megakaryocytes and endothelial cells lining the blood sinuses. The lymphocytes were somewhat triangular in shape with cytoplasmic processes extending between adjacent erythrocytes. Nuclei were triangular to oval in shape, lightly stained with clear nuclear membrane indentation and clear nucleoli. The reticular cells were elongated in shape with cytoplasmic processes extending between adjacent lymphocytes, rough endoplasmic reticulum, ribosomes and few lysosomes were seen in their cytoplasm. Nucleus was elongated in shape with less condensed chromatin. Plasma cells were oval to irregular in shape with numerous dilated rough endoplasmic reticulum containing electron lucent material occupying the whole cytoplasm and few mitochondria were found. Nuclei were centrally located and oval in shape with heterochromatin emarginated and often clumped near the nuclear membrane. Occasionally megakaryocytes and mast cells were seen among lymphocytes. Megakaryocytes had multilobulated nucleus and free ribosomes often appearing as small aggregates in their cytoplasm, while mast cell had their characteristic electron dense granule in the cytoplasm, few electron lucent granules were found also, we conclude that, the main function of the hemal node of cattle is proliferation of lymphocytes. No role for plasma cell in erythrophagocytosis could be suggested.

***Keywords*—**Cattle, Electron microscopy, Hemal node, Histology, Immune system.

I. Introduction

CATTLE are among the most important domesticated animals. They could serve different functions: working animal, meat provider, milk provider [1]. The population of cows in Egypt is continuously increasing and estimated to be about 5.02 million heads. They produce about 3.21 million metric tons of milk and about 0.32 million metric tons of meat, representing about 53.88 % of the total milk production and 46.69 % of the total meat production, respectively [2].

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The hemal node (HN) is a hematopoietic and lymphoid organ that is found in some mammals such as humans, rats and ruminants. In ruminants, HNs are located in the subcutaneous region of the head, the mesenteric region and along the large blood vessels such as the aorta in the thorax and abdomen [3]-[5].

The histological structure of hemal node resembles that of lymph nodes and consists of a lymphoid region, in which the cortex and paracortex are recognized, and a sinusoid region, in which the lymphatic cord and sinus are formed [4], [6], [7].

The hemal nodes were involved in blood storage and filtration, platelet formation and immunological defense [8], [9] suggest that the hemal node has an important role in both cellular and humoral immunity as well as the lymph node and the spleen in cattle.

In all mammals, the hemal nodes were covered by a thin capsule of connective tissue; subcapsular blood sinus and lymphatic nodule were present under capsule [10], [11]. A reticular meshwork extended through the interior of hemal node of sheep containing a large number of free blood cells, many macrophages, lymphocytes, and plasma cells [12]. The lymphoid tissue of hemal nodes in roe deer contained lymphocytes, plasma cells, granulocytes and reticular cells, only mast cells could not be observed [13]. Reference [14] also found that the parenchyma of hemal node of buffalo was composed of irregular lymphoid cords rich in erythrocytes, macrophages, and plasma cell. The density of cells in the lymphoid cords of hemal node of Egyptian water buffalo were reduced in number in old ages and hyaline masses appeared at 7 years [11].

The plasma cell of hemal node of sheep and buffalo showed numerous cytoplasmic prolongations suggestive of motility and phagocytic activity, their irregular perinuclear zones contained granular material of similar density to that of rough endoplasmic reticulum and some showed morphological evidence of various phases of erythrophagocytosis [15], [16].

The sinusoidal endothelial cells of hemal node were spread out into thin sheets of cytoplasm which exhibited one or more zonule occludentes where they were related to an adjacent endothelial cell. Occasionally wide gaps were present between adjacent endothelial cells through which lymphocytes appeared to be migrating. The nuclei of the sinusoidal endothelial cells were irregular in outline with less condensed chromatin [7]. In Korean native goat the sinuses of hemal nodes were lined by endothelial –like reticular cells which had euchromatic rich nuclei and many cytoplasmic processes, surrounding collagen fibrils [17].

Megakaryocytes, with multilobed nuclei and granulated basophilic cytoplasm were seen in the Canadian Friesian cattle hemal nodes by [18].

II. Materials and Methods

Fresh specimens of hemal nodes were collected from 10 clinically healthy male cows aged (2-3) years. The animals were slaughtered for human consumption in the abattoir of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Two hemal nodes were obtained from each animal, from the mesenteric and perirectal regions. Each hemal node was examined macroscopically and dissected.

For light microscopy, specimens were fixed in 10% phosphate-buffered formaldehyde and processed for paraffin sectioning. Sections (4µm) were prepared and stained by Haematoxylin and Eosin stain and Trichrome stain [19].

For transmission electron microscopy, pieces of 1mm were cut from the hemal nodes and quickly fixed in 6% solution of phosphate buffered gluteraldehyde pH 7.4 for 6 hours. at 4ºC [20]. After initial fixation, the tissues were washed in several changes of cold (4ºC) 0.1 M phosphate buffer every 15 minutes for 2 hrs. The tissues were postfixed in 1% solution of osmium tetroxide in cold (4ºC) 0.1 M buffer pH 7.2 for 2 hrs. Then, they were rapidly dehydrated through ascending grades of ethyl alcohol then transferred to propylene oxide and placed in a 1:1 mixture of propylene oxide and epoxy araldite [21]. Semi- thin sections (1µm) were cut firstly, stained with toludine blue, and viewed with light microscope to select the suitable areas for the electron microscope examination. The ultrathin sections (60-100 nm) were cut by a glass knife with LKB microtome, and then they were stained with uranyle acetate followed by lead citrate [21]. These sections were examined with Joel 100 cx electron microscope operating at 80 K.

For scanning electron microscopy specimens were immediately immersed in a fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2) at 4°C. Once fixed, the samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid, and finally dehydrated in increasing concentrations of ethanol series. The samples were then critical-point dried in carbon di-oxide, fixed on stubs with colloidal carbon and coated with gold palladium in a sputtering device. Finally, specimens were examined and photographed with a Jeol scanning electron microscope operating at 15 Kvs, at EM unit, Faculty of science, Alexandria University.

III. Results

The hemal node of cow was encapsulated by connective tissue capsule consisted mainly of collagen fibers, several trabeaculae originated from the capsule and extended into the parenchyma of the hemal node (Figs. 1, 3). A wide subcapsular sinus was present under the capsule and it was filled with numerous erythrocytes (Fig. 2). Elongated smooth muscle cell was found near the subcapsular sinus with contracted nucleus (Fig. 4). Some adipose tissue could be seen attached to the external surface of the capsule.

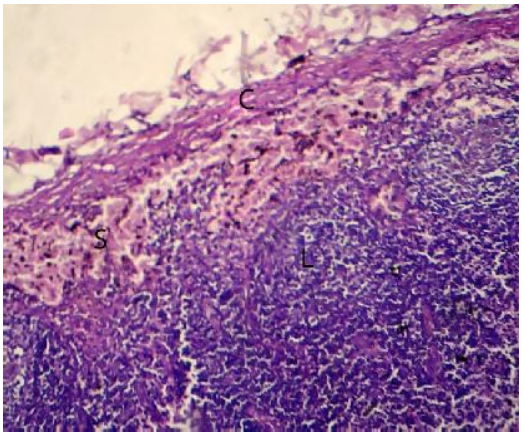

Fig. 1 Light photomicrograph of cow hemal node showing capsule (C), subcapsular sinus (S) and lymphoid nodules (L). Mic. Mag.x100. Stain H&E

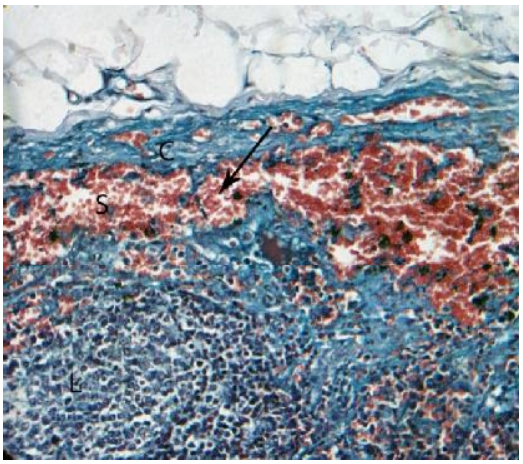

Fig. 2 Light photomicrograph of cow hemal node showing capsule (C), subcapsular sinus (S), erythrocytes (arrow) and lymphoid nodules (L). Mic. Mag.x400. Stain Trichrome

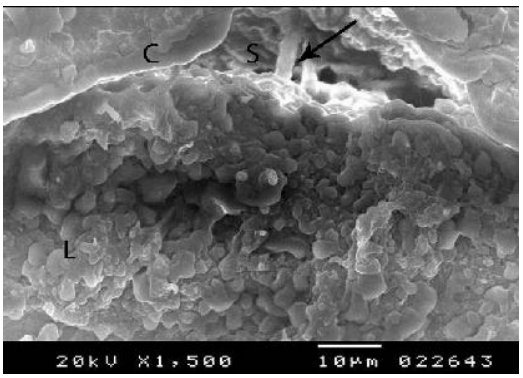

Fig. 3 Scanning electron micrograph of cow hemal node showing capsule (C), subcapsular sinus (S), trabeaculae dividing subcapsular sinus (arrow) and lymphoid nodules (L). Mic. Mag.x1500

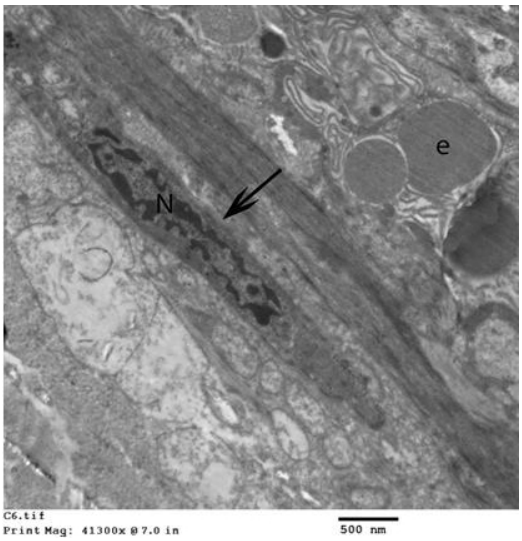

Fig. 4 Transmission electron micrograph of smooth muscle cell (arrow) near subcapsular sinus of cow hemal node (S). Note contracted nucleus (N). Erythrocytes (e) (x3000)

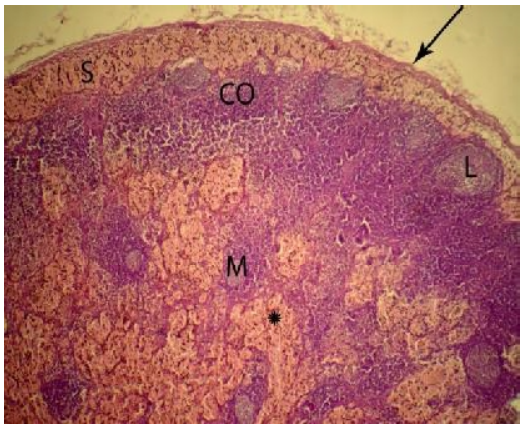

Fig. 5 Photomicrograph of cow hemal node showing capsule (arrow), subcapsular sinus (S), cortex (CO), medulla (M), lymphoid nodules (L) and medullary sinus(astrik). Mic. Mag.x100. Stain H&E

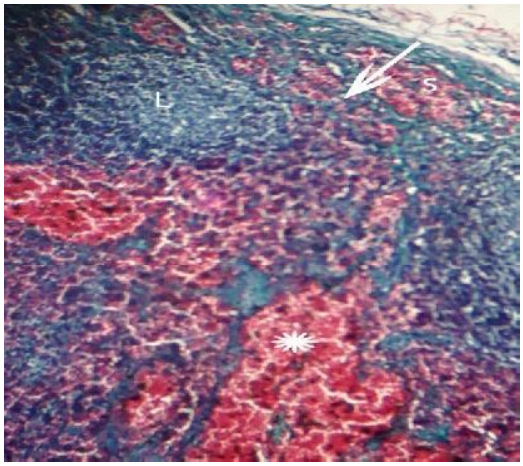

Fig. 6 Light photomicrograph of cow hemal node showing subcapsular sinus (S) interrupted with some trabeculae of collagen fibers (arrow) lymphoid nodules (L) and the medullary sinus (asterisk). Mic. Mag.x400. Stain Trichrome

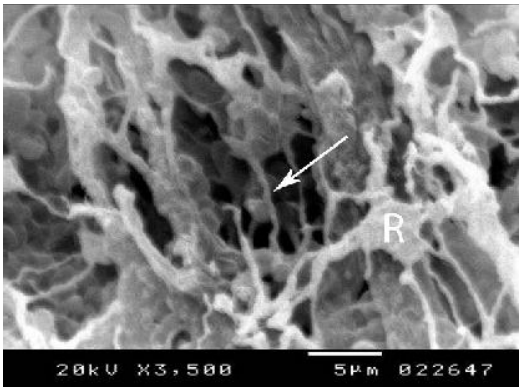

Fig. 7 Scanning electron micrograph of cortex of cow hemal node showing reticular cell (R) and reticular fibers (arrow). Mic. Mag.x3500

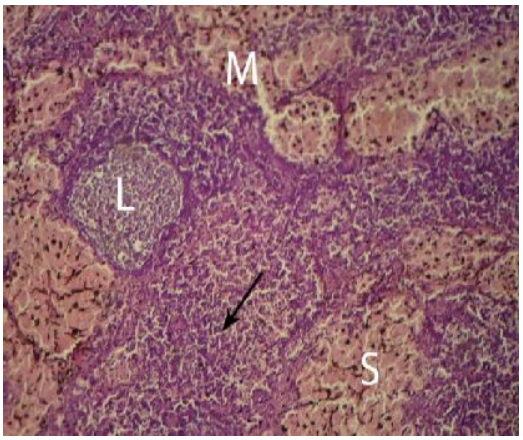

Fig. 8 Photomicrograph of the medulla (M) showing diffuse lymphocytes (arrow), medullary sinus (S) and lymphoid nodules (L). Note the medullary sinus (S). Mic. Mag.x400. Stain H&E

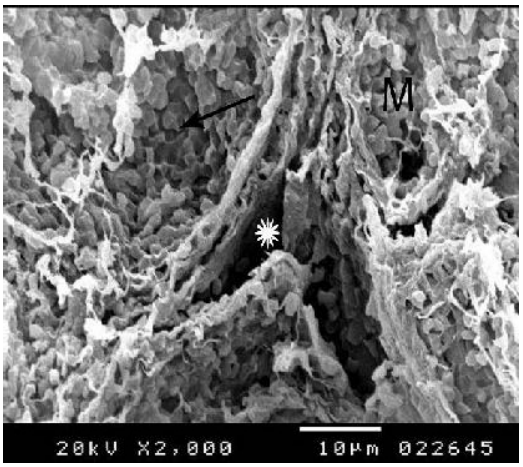

Fig. 9 Scanning electron micrograph of the medulla (M) showing, medullary sinus (asterisk) and diffuse lymphocytes (arrow). Mic. Mag.x2000

The parenchyma of cow hemal node was consisted of outer cortex and inner medulla. The cortex consisted of diffused lymphocytes, and lymphoid follicles, some of them had germinal center (Fig. 5). The subcapsular sinus was

continuous with the trabecular sinus and the medullary sinus (Fig. 6). The lymphoid follicles and diffused lymphocytes was supported by reticular cells and reticular fibers (Fig. 7). The Medulla was consisted of, diffused lymphocytes, a large medullary sinus and few lymphoid nodules (Figs. 8, 9).

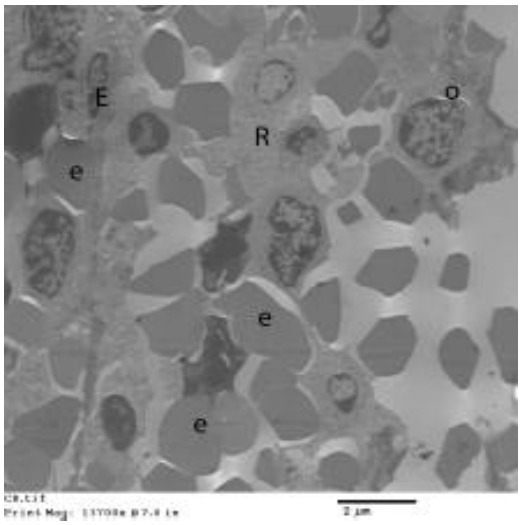

Fig. 10 Transmission electron micrograph of cow hemal node showing reticular cell (R), erythrocytes (e), lymphocytes (o) and sinusoidal endothelial cell (E). Mic. Mag (x1000)

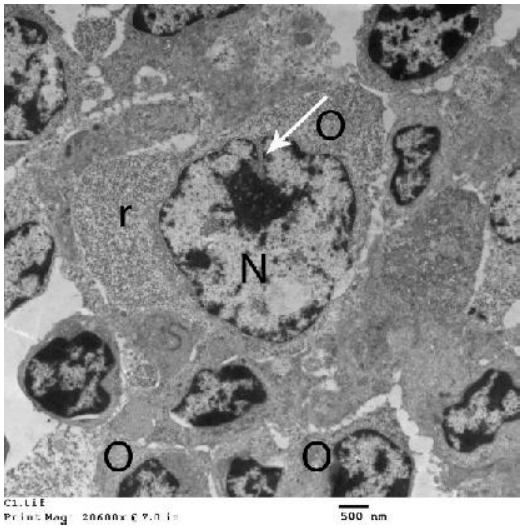

Fig. 11 Electron micrograph of lymphocytes (O) depicting numerous ribosomes (r), nucleus (N) with clear nuclear membrane indentations (arrow) Mic. Mag (x1500)

By TEM the cells of hemal node of cow included elements of circulating erythrocytes intermingled with lymphocytes, plasma cells, mast cells, reticular cells, macrophages, megakaryocytes and endothelial cells lining the blood sinuses (Fig. 10). Those endothelial cells were squamous with long cytoplasmic extensions and large fenestrations allowing lymphocytes to pass through.

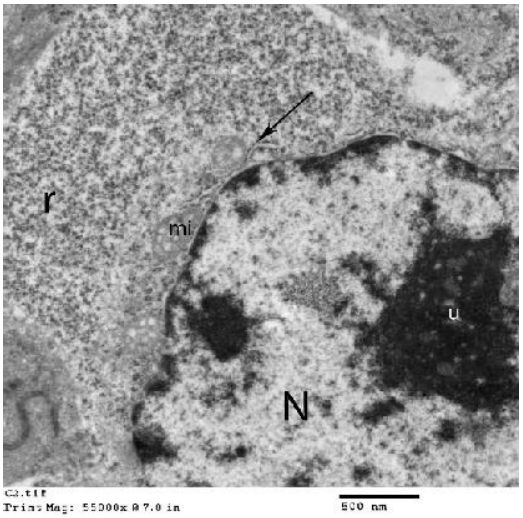

Fig. 12 Higher magnification of the previous photo showing numerous ribosomes (r), mitochondria (mi), rough endoplasmic reticulum attached to nuclear membrane (arrow). Nucleus (N) with clear nucleolus (u). Mic. Mag (x4000)

The lymphocytes had cytoplasmic processes extending between adjacent erythrocytes and they were somewhat triangular in shape, (Figs. 10, 11). Their cytoplasm had ribosomes and few mitochondria. Nuclei were, lightly stained with clear nuclear membrane indentation and clear nucleoli. They were triangular to oval in shape. Occasionally rough endoplasmic reticulum was attached to nuclear membrane (Fig. 12).

The reticular cell had cytoplasmic processes extending between adjacent lymphocytes, they were elongated in shape. Rough endoplasmic reticulum, ribosomes and few lysosomes were seen in their cytoplasm. Nucleus was elongated in shape with less condensed chromatin (Fig. 13).

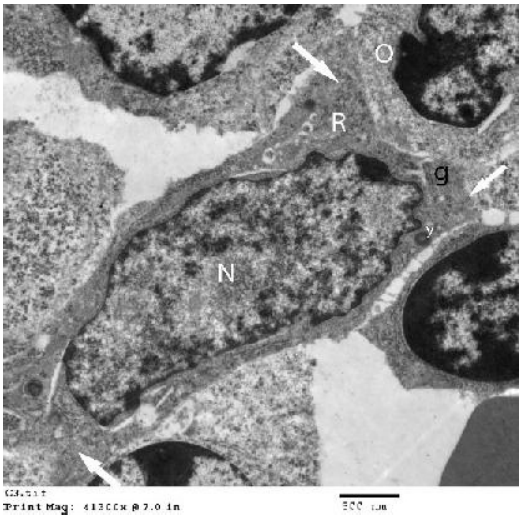

Fig. 13 Transmission electron micrograph of a reticular cell (R) with oval nucleus (N). Note the cytoplasmic processes (arrows) extended between the lymphocytes (O), rough endoplasmic reticulum (g) and lysosomes (y). Mic. Mag (x3000)

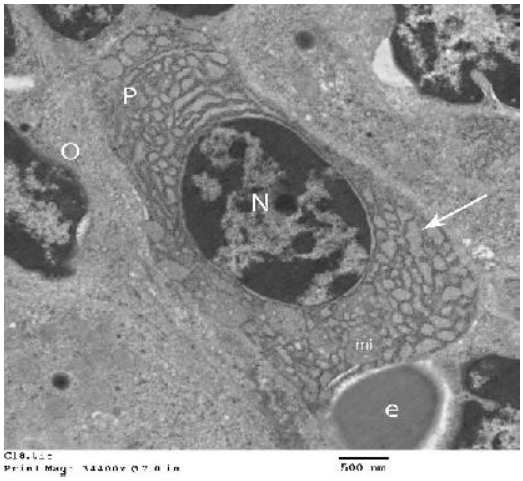

Fig. 14 Transmission electron micrograph of a plasma cell (p) of cow hemal node showing dilated rough endoplasmic reticulum containing electron lucent material (arrow), mitochondria (mi), nucleus (N). A lymphocyte (O) and Erythrocytes (e). Mic. Mag (x2500)

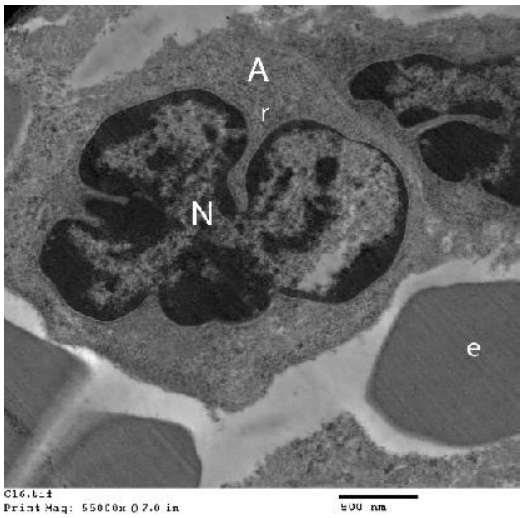

Fig. 15 Transmission electron micrograph of a megakaryocyte (A) showing extensive granular cytoplasm rich in ribosomes (r). Note multilobulated nucleus (N). Erythrocytes (e). Mic. Mag (x4000)

Numerous dilated rough endoplasmic reticulum containing electron lucent material occupying the whole cytoplasm of Plasma cell, which was oval to irregular in shape, few mitochondria were found. Nuclei were centrally located and oval in shape with heterochromatin emarginated and often clumped near the nuclear membrane (Fig. 14).

Megakaryocytes and mast cells were seen among lymphocytes, megakaryocytes had multilobulated nucleus and free ribosomes often appearing as small aggregates in their cytoplasm (Fig. 15), while in the cytoplasm of mast cell contained characteristic electron dense granule, few electron lucent granules were found also (Fig. 16).

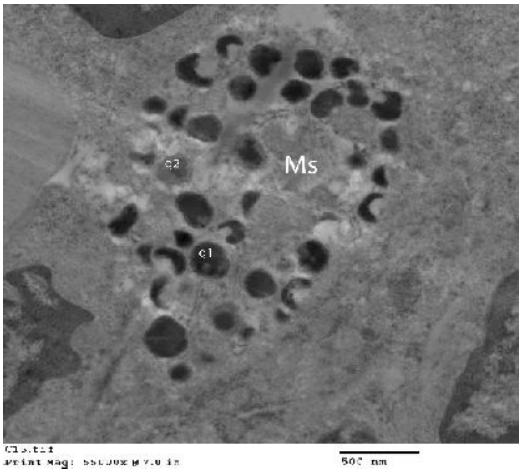

Fig. 16 Transmission electron micrograph of a mast cell (Ms) of cow hemal node depicting electron dense granule (g1) and electron lucent granule (g2). (x4000)

IV. Discussion

This study revealed that the histological structure of the hemal node may resembles that of lymph nodes especially in the cortex in which the lymphoid nodules appears and a medulla in which the lymphatic cords and sinus were formed [4], [6], [7] but the difference was the appearance of lymphoid nodules in the medulla of hemal node of cattle**.** The lymph nodules occupied larger area than that occupied with non-nodular structure of lymphoid cords and blood sinusoids and this result was in contrast to [11] in the hemal node of buffalo. So this means that the area of lymph nodules (site of lymphocyte proliferation, [14] was large, so the filtration of the blood may not be the main function of the hemal node in cattle in contrast to buffalo where filtration of blood may be the main function, [11]. Therefore, this study we may deduce that the principle function of the hemal node of cattle is proliferation of lymphocytes.

In the present study, the transmission electron microscope revealed, smooth muscle cell near the subcapsular sinus with contracted nucleus, but with light microscope we could not notice any smooth muscle cells even with the trichrome stain, and this confirms that smooth muscles may be found very rarely and this disagree with the results of [11] in the buffalo hemal node which had many layers of smooth muscle in the lower layers of the capsule near the subcapsular sinus, and this result confirms the previous conclusion that the main function of hemal node in cattle is not the blood filtration.

Plasma cell had numerous dilated rough endoplasmic reticulum containing electron lucent material occupying the whole cytoplasm, with few mitochondria but no cytoplasmic processes extending between the surrounding erythrocytes as in the hemal node of sheep and buffalo [15], [16], so we suggested that the plasma cell has no role in erythrophagocytosis and this is confirmed by the absence of any sort of union between plasma cells and macrophages and a very few number of macrophages in cattle hemal node.

Mast cells could not be observed in the hemal node of deer [13], but in this study we could observe many mast cells with their characteristic granules. Reference [22] highlighted the important role of mast cells in protection against infection with a variety of organisms, although these cells were best known for their role in mediating allergic diseases.

Megakaryocytes were observed among lymphocytes, having multilobulated nucleus and free ribosomes often appearing as small aggregates in their cytoplasm and this suggested a role for the hemal node in platelet formation although no platelet could be observed in the sections prepared and examined. Reference [23] observed megakaryocytes, while [24] observed erythropoiesis in only 1 of 86 haemolymph nodes of goats in which erythropoiesis had been activates experimentally, also [18] proved the occurrence of both erythropoiesis and erythrophagocytosis in bovine hemal nodes which are not clear in this study in the cattle hemal nodes.

V. Conclusion

Cow hemal node was different from that of buffalo in being mainly for proliferation of lymphocytes and no clear role could be concluded in blood filtration or erythrophagocytosis or platelet formation.

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