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CASTING METHODS OF SCANNING ELECTRON MICROSCOPY APPLIED TO HEMAL LYMPH NODES IN RATS

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ABSTRACT

Hemal lymph nodes of the para-aortic group in rats were examined by scanning electron microscopy (SEM) using the corrosion cast technique. The vasoarchitecture of hemal lymph nodes was described in cast specimens filled from the abdominal aorta. A dense system of capillaries arranged in the mode of a meshwork could be represented in the nodal cortex. The postcapillary venule (PCV) with wide vascular lumina provided the prevailing vessel type of the deep cortex. Sphincter-like narrowings of the capillary lumen appeared at the sites where capillaries merged with the PCV. These vascular segments were interpreted as structures controlling the hemodynamics of the joining PCV thereby providing appropriate conditions for homing lymphocytes. No evidence was obtained from the casts for an open circulation in hemal lymph nodes. The intranodal lymphatic pathways were studied in specimens interstitially injected with resin. The fine lymphoid tissue spaces were characterized by special cast patterns reflecting the structural differentiation of certain nodal compartments. Afferent lymphatics could be clearly identified in casts with a retrograde filling from the subcapsular sinus. Very spacious intermediary sinuses and wide-meshed medullary sinuses could be represented in those casts as well, thus allowing ample space for the encounter of large macrophages with red blood cells.

The gross anatomy of lymph nodes from different regions of the body is characterized by a framework of connective tissue consisting of the capsule and trabeculae, which embrace the specific reticular tissue filled with abundant cells, mainly lymphocytes, plasma cells, and macrophages. Hemal lymph nodes are distinguished from ordinary lymph nodes by a high content of red blood cells, most of them in a state of phagocytosis (erythrophagia). This salient characteristic accounts for the brownish or reddish color, which hemal lymph nodes show in a mature state.

Hemal lymph nodes have been the object of several studies since their first description in human by Gibbes (1884) (1). Most investigations on these peculiar structures have been carried out in rats (2-8), where they are located close to the kidney and pancreas. Hemal lymph nodes also have been studied in carnivora and rodents (9), goat (10), and pig (11) (for reviews see 2,12,13).

The results of all these studies formerly obtained on the basis of light microscopy and recently also by electron microscopy have left several open questions regarding the fine structural features as well as the general biological significance of hemal lymph nodes. Thus, even those questions have not been answered conclusively up to now, as to whether hemal lymph nodes are provided with afferent lymphatics. Moreover, no certainty exists in respect to the questions, from which source the red cells derive and how these cells enter the intrinsic lymph spaces.

In contrast to common lymph nodes (2,14-17), thus far, hemal lymph nodes have not been examined with the help of corrosion casting of scanning electron microscopy (SEM). Thus, the aim of the present cast study was to obtain new information on the blood vasculature and the lymphatic space system of hemal lymph nodes using the cast technique. For that purpose hemal lymph nodes of the para-aortic group in rats were cast by intra-arterial as well as interstitial injection of Mercox®. In parallel experiments hemal nodes of this region were examined using additional techniques such as transmission electron microscopy (TEM) and confocal laser scanning microscopy of nodes marked by different fluorescent indicators. The results of these approaches will be reported subsequently (12,18).

MATERIAL AND METHODS

Twelve adult rats (Wistar strain) of both sexes were used in this study. Under anesthesia with ether followed by Trapanal® (100 mg/kg body weight) intraperitoneally the rats were killed by thoracotomy and a cut through the heart.

For corrosion casting the blood vascular system was rinsed with 38°C warm heparinized Ringer solution via the left cardiac ventricle. Then, the abdominal cavity was opened and a cannula was introduced into the abdominal aorta just before the branching site of the renal arteries. Ten ml Mercox® (Vilena Comp., Tokyo, Japan) mixed with 0.2 g catalyst were injected into 4 rats using hand pressure. After having left the rats at room temperature for one hour, the pararenal group of hemal lymph nodes was prepared from the retroperitoneal tissue space and, after removal, macerated in potash lye at 45°C for two days. The specimens were washed under running water and posttreated with 10% hydrochloric acid. Thereafter, the specimens were cleansed in bidistilled water, air dried, mounted on stubs with carbon and examined in the AMR 1200 scanning electron microscope (E. Leitz, Germany) at accelerating voltages of 5-15 kv.

In 8 rats, Mercox® was applied into the hemal lymph nodes of the para-aortic group by interstitial injection. In this series of experiments, the lymph nodes were carefully prepared at their locations behind and above the renal vein on both sides. Then a small amount of Mercox® (0.1-0.2 ml) was placed with a fine cannula just beneath the capsule. After hardening of the resin, these hemal lymph nodes were processed in a similar way as described above for the specimens injected via the aorta.

RESULTS

Corrosion Casts of the Blood Vascular System

Casts injected from the abdominal aorta clearly showed the vascular supply of hemal lymph nodes. Specimens with complete filling of both the arterial and venous system allowed total view of the capillary network of the cortical zone along with larger arteries and veins of the hilum *(Fig. 1)*. The basic architecture of the blood vascular system was consistent in all hemal lymph nodes examined. A dense capillary network was found in the cortical zone. It contained capillaries of constant calibers with diameters between 6 and 8 µm. These vessels formed strains of partly parallel running, partly interweaving vessels, thus embracing meshes of cast-free spaces *(Fig. 2)*. The meshes were roundish to polygonal in shape and reached dimensions up to 100 µm.

At higher magnification shallow ovoid imprints created by endothelial nuclei occurred on the cast surface of the cortical capillaries. At some sites also slight ring-like furrows could be detected, which probably contributed to subendothelial cellular elements like pericytes. Casts of small arterioles feeding the capillary meshwork were provided by a regular pattern of ring-like depressions. Some of them exhibited circular structures in the mode of "plastic

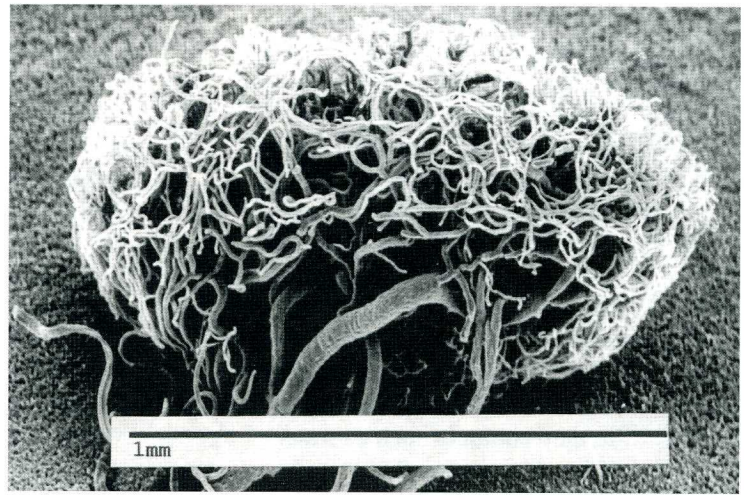


Fig. 1. Low power micrograph of a cast of a rat hemal lymph node of the pararenal group. The capillary network of the cortex and the nodal arteries and veins at the hilum are well seen.

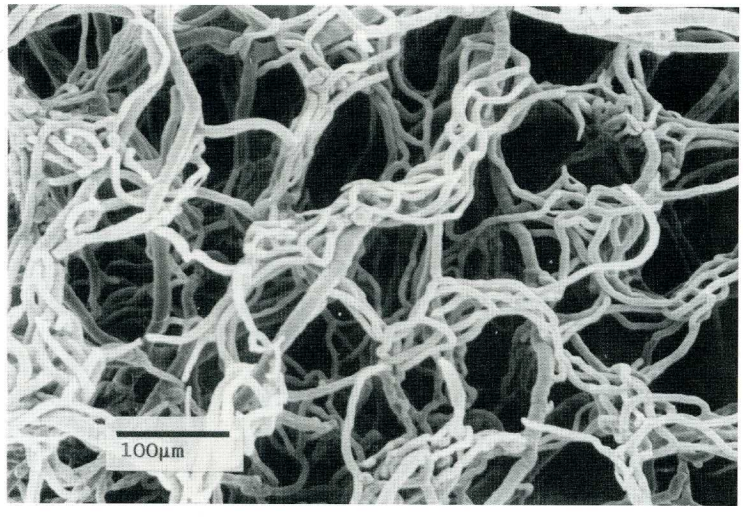


Fig. 2. Sectional area of the cortex of a hemal lymph node showing a meshwork of microvessels consisting of bundles of capillaries, which surrounds cast-free spaces.

strips". Specimens with an incomplete filling state allowed a view on the vascular organization of the paracortical and the outer medullary zones, in which postcapillary venules (PCV) with diameters of 30-50 µm dominated *(Fig. 3)*. This type of vessel was characterized by a regular outline produced by high protruding endothelial cells causing a corresponding irregular profile on the cast surface *(Fig. 4)*. Casts of PCVs therefore were clearly distinguished from those of the thin-walled veins and venules. In the transitorial zone, between cortex and paracortex, capillaries joined abruptly the initial segment of the PCVs. Next to the site of confluence the capillary casts contained segments of sphincter-like narrowings reducing the capillary lumen to 40% *(Fig. 5)*. At the merging point of both vessels the smooth cast profile of the capillary was replaced by the irregular profile of the PCV and a sudden step of the vascular lumen occurred with an increased diameter up to a level of 30-50 µm *(Fig. 5)*. At sites where venules with diameters of 15-20 µm merged into the PCVs, sphincter-like segments were missing, although great differences of the calibers between both types of vessels existed *(Fig. 6)*.

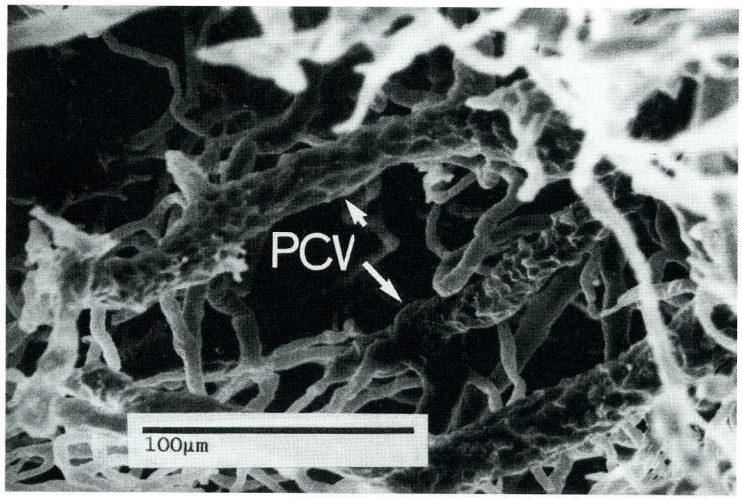


Fig. 3. Vascular cast with an incomplete filling of Mercox® allowing a view into the innermost cortical area with many postcapillary venules (PCV).

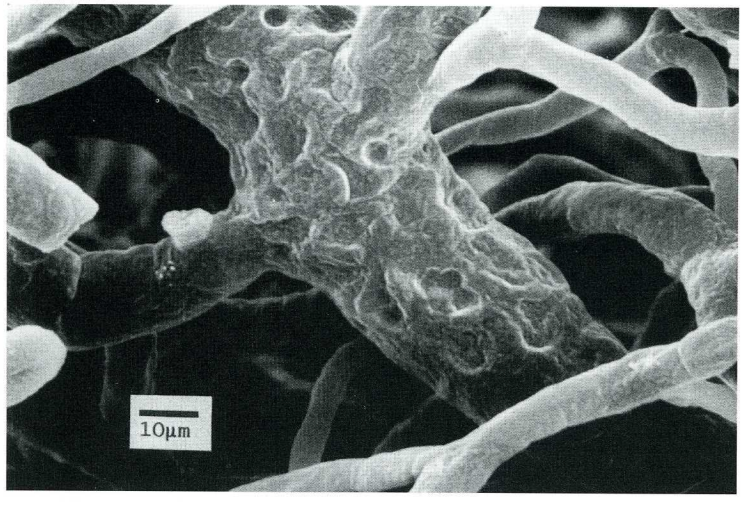


Fig. 4. High power micrograph of a postcapillary venule. The cast of this type of vessel is characterized by an irregular surface profile created by the bulging endothelial cells.

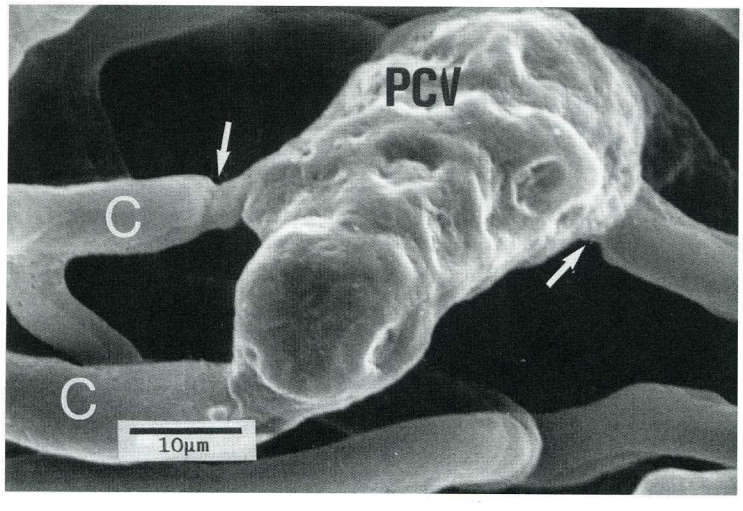


Fig. 5. Initial segment of a post-capillary venule (PCV) with merging capillaries (C)> Note the sphincter-like narrowing of the capillary lumen (arrows) just before the junction with the PCV where a sudden change of the vascular caliber occurs.

Fig. 6. Initial segment of a post-capillary venule (PCV) receiving influx from several venules (VU). Sphincter-like structures are missing at the merging site of the venules in contrast to the capillary (see Fig. 5).

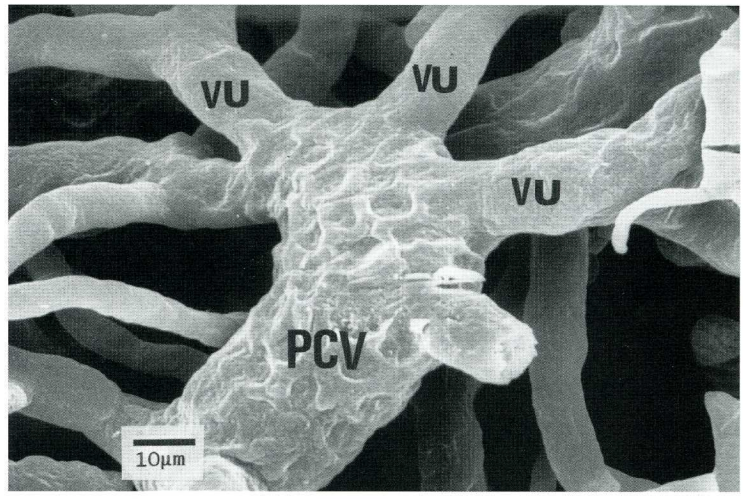


Fig. 7. High power micrograph showing the characteristic endothelial imprints appearing at the cast surfaces of an intranodal vein (V), and two intranodal arteries (A.).

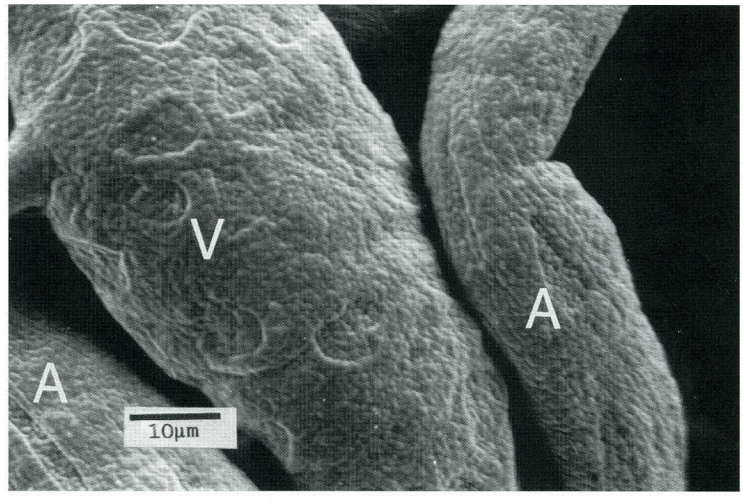
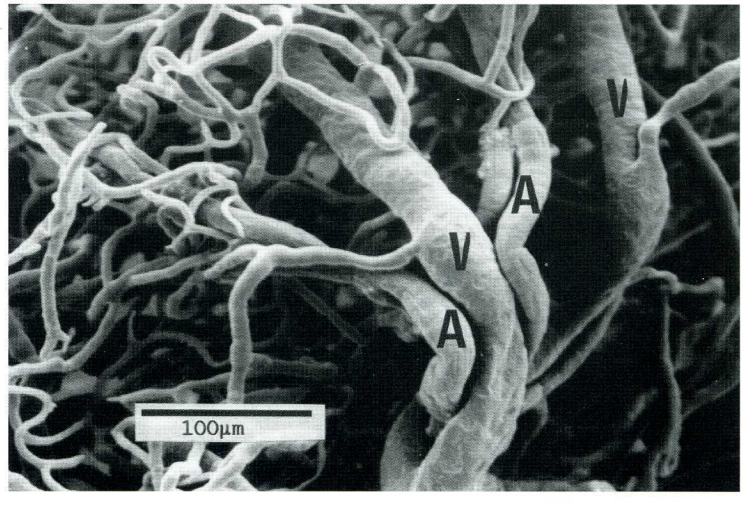


Fig. 8. The intranodal arteries and veins of the medullary area are arranged to form a bundle of hiliar vessels. From the intranodal artery (A) several small arterioles branch off. Single venules unite with the intranodal vein (V).



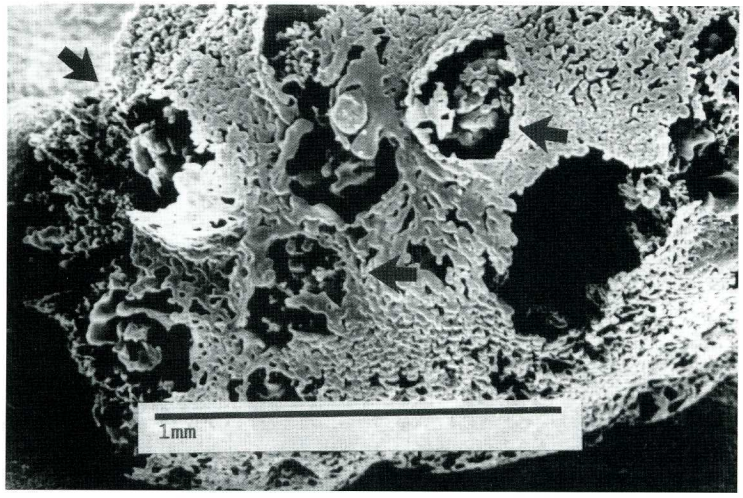


Fig. 9. Low power micrograph of a hemal lymph node injected with the resin from the subcapsular space. The subcapsular sinus forms a sheet covering the system of fine lymphoid spaces of the cortex. The latter become recognizable at sites, where the filling of the sinus is incomplete. Note the dome-like protrusions of the subcapsular sinus at some places (arrows).

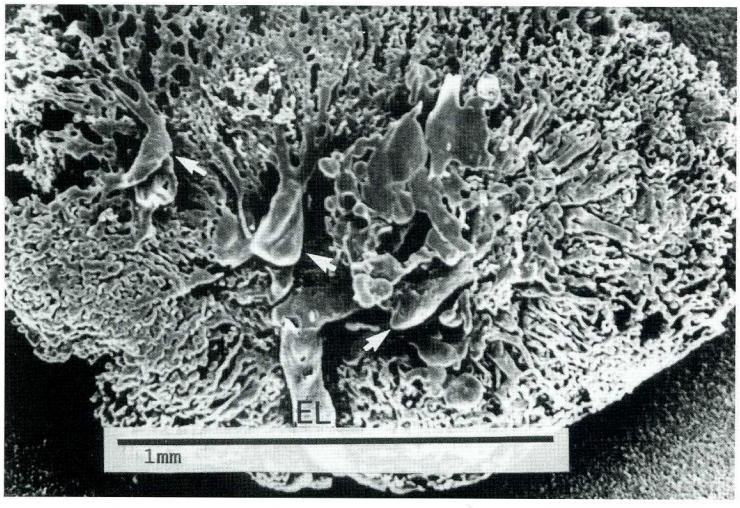


Fig. 10. View of the hilum of a specimen with interstitial filling. In this nodal area spaces of the subcapsular sinus fuse to form bigger units (arrows). At the hilum proper the cast of the terminal medullary sinus and that of the efferent lymphatic (EL) becomes well visible.

On the course into the medullary area the PCVs fused into larger veins. There, the irregular cast surface of these vessels was replaced by a smoother surface profile with roundish or ovoid imprints of the endothelial nuclei which characterized the bigger nodal venous vessels *(Fig. 7)*.

Near the hilum the nodal arteries and veins were arranged in close formation *(Fig. 8)*. Casts of arteries had smaller diameters (20-30 µm) than those of veins (40-60 µm) and exhibited a pattern of circular furrows on the surface created by muscle cells. The arteries gave off small branches while running towards the cortex, where final twigs as small arterioles joined the cortical capillary system. Small venules united with the medullary veins within the medullary zone. At the hilum one to three nodal arteries and one to two nodal veins appeared in the casts. The number of these vessels depended upon the size of the hemal node studied.

Corrosion Casts with Interstitial Filling

Interstitially injected casts of hemal lymph nodes showed the subcapsular sinus as a convex-shaped layer composed of small

Fig. 11. This micrograph shows an afferent lymphatic of the hemal lymph node cast by retrograde filling from the subcapsular space. When penetrating the nodal capsule the main stem of the lymphatic divides into smaller branches, which join the subcapsular sinus (appearing in the upper half of the image).

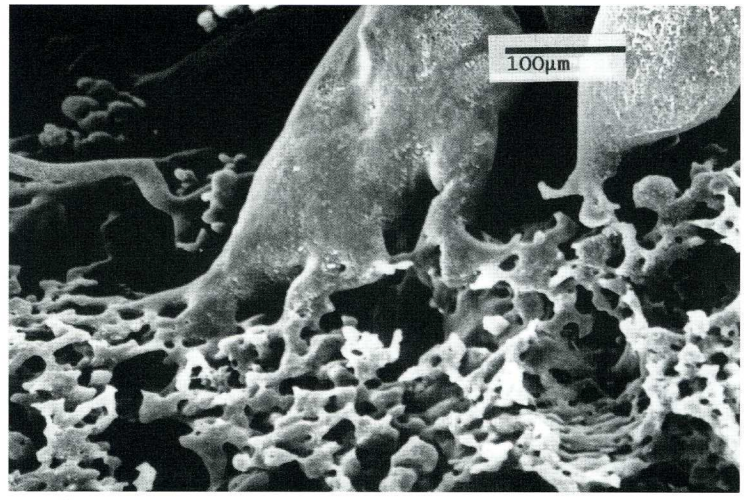


Fig. 12. Cast of an efferent lymphatic of a hemal lymph node with sharp indentations indicating the site of bicuspid valves.

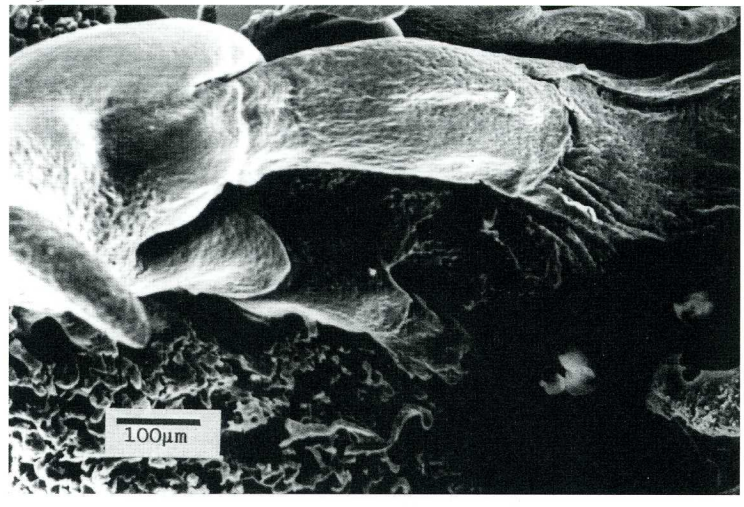
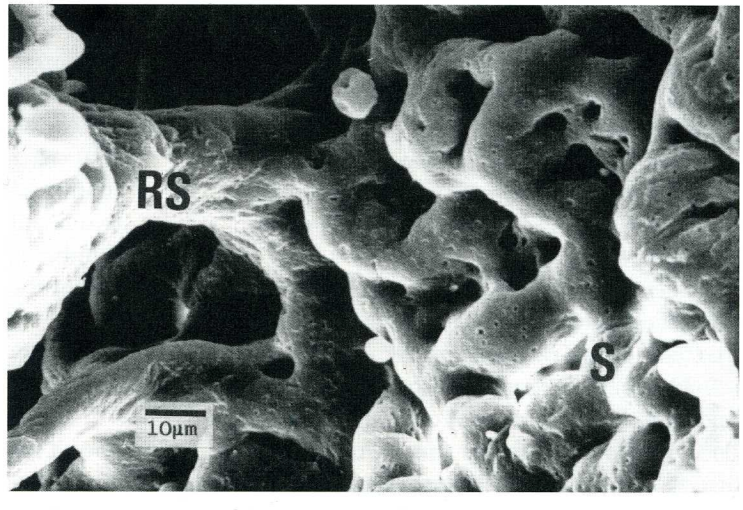


Fig. 13. High power micrograph of an interstitially filled cast with view on the inner surface of the subcapsular sinus (S)> A tube-like radial sinus (RS) connect the subcapsular sinus with the medullary sinus.



confluent ducts, which in complete casts, outlined the nodal shape and dimensions *(Figs. 9,10)*. In the abhilar area some dome-like protruding structures could be recognized in the cast sinus layer, which in most specimens exhibited an incomplete filling state. At those and other sites, the casts of the cortical tissue spaces became visible *(Fig. 9)*. At the hilum, the ducts of the subcapsular sinus merged into bigger units.

At some sites the casts of afferent lymphatics appeared, which merged into the cast of the subcapsular sinus. Valve structures were replicated as sharp indentations in the casts of these vessels. While passing the capsule, the afferent lymphatics branched and gave off several twigs, which united with the cast of the subcapsular sinus *(Fig. 11)*. At the hilum one or two well-developed efferent lymphatics formed prominent cast structures *(Fig. 10)*. Deep indentations indicated sites and an arrangement of well developed valve lips in this kind of vessel *(Fig. 12)*. Three to five afferent and 1-2 efferent lymphatics were found in each interstitially injected cast of a hemal lymph node studied. The calibers of the afferent lymphatics varied between 80-120 µm while those of the efferent vessel were 100-300 µm.

The outer cast surface of the subcapsular sinus was smooth, while that facing the tissue area of the cortex was irregularly shaped with bulging parts. Between the subcapsular sinus and the casts of the lymphoid tissue spaces of the nodal cortex a small cast-free space existed. Exceptions were those parts of the subcapsular sinus, from which intermediary sinuses began to join the medullary sinus. Some intermediary (radial) sinuses displayed tube-like shapes with smooth surfaces *(Fig. 13)*. Other sinuses of that type achieved larger extensions and were characterized by coarse, club-shape configurations and regular surface profiles *(Fig. 14)*.

Casts of the cortical and medullary tissue area showed a system of irregular structures as an equivalent pattern of the fine lymphoid tissue spaces. In the medullary area this intricate spatial system was characterized by a loose arrangement of small confluent cast structures, while a denser pattern of small ducts was reflected by casts of the paracortex and cortical zone. In the nodal cortex the system of small spaces in most parts was arranged around cast-free areas with extensions of about 100 µm *(Fig. 15)*. A pattern of glomerular structures was recognizable just beneath the dome-like structures of the subcapsular sinus mentioned above *(Fig. 9)*.

DISCUSSION

The present study deals with a unique lymphatic organ designated "hemal lymph node", which in contrast to ordinary lymph nodes exhibits a number of features not previously well understood. These include uncertainties about functional and immunological significance of hemal lymph nodes in general and their most prominent characteristic, the phenomenon of erythrophagia, in particular. Thus, questions concerning how red cells seep into the node are still debated as are the existence of afferent lymphatics. Some investigators maintain that red blood cells drain either directly into the intranodal sinus via open capillaries (19), whereas others favor migration of red cells across the vascular endothelium into the lymphoid tissue (6). The postcapillary venules have been regarded as vessels that facilitate transendothelial diapedesis of red blood cells similar to homing lymphocytes (5). Even a reflux mechanism via the efferent lymphatic has been suggested, which enables red blood cells to enter hemal lymph nodes across lymphatic-venous anastomoses between the efferent lymphatic and the renal vein (2).

The present SEM study on casts of the nodal blood vasculature as well as the intranodal lymphatic pathway system of hemal lymph nodes reveals that the basic morphology concerning both systems appears consistent with that described in common lymph nodes (20). In hemal lymph nodes the capillary network of the cortex is highly

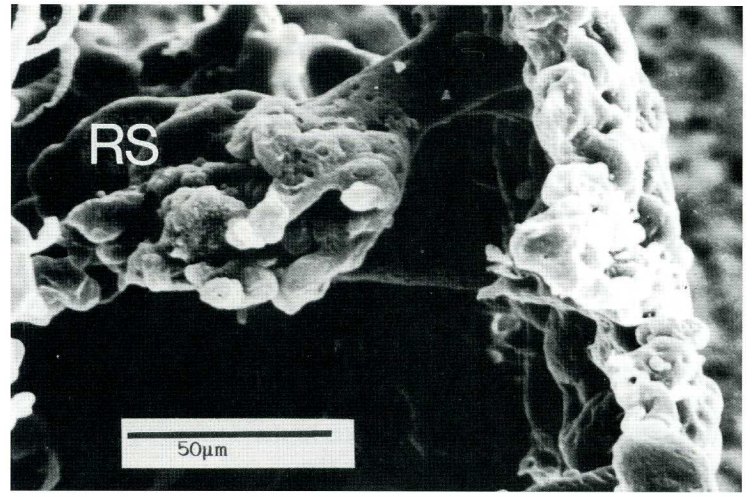


Fig. 14. Photo micrograph shows the cast of a spatial club-like intermediary sinus (RS). Its surface is characterized by an irregular profile.

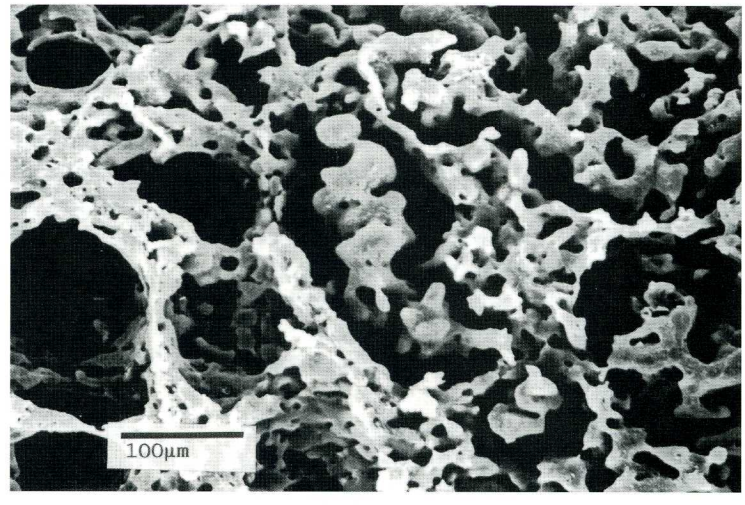


Fig. 15. Cast with the arrangement of lymphoid tissue spaces from the nodal area, where the deep cortex (left) unites with the medulla (right). A different pattern of fine spaces and ducts exists between both areas reflecting the special structural differentiation of these two nodal compartments.

vascular and seems to reflect the structure of the reticular tissue with its compartments. The occurrence of dome-like structures, especially evident in interstitially injected casts, suggests that the cortex exhibits a tissue differentiation similar to lymphoid follicles, except that germinal centers are commonly absent in hemal lymph nodes (21).

Casts of the intranodal blood vascular system also demonstrate that hemal lymph nodes are provided with an extraordinarily high number of postcapillary venules (PCV). In cast preparations the initial segment of that type of venule is clearly separated from the confluent capillaries and venules of the cortex. Sphincter-like structures characterize the last capillary segment at the merging point with the PCV. This phenomenon, described here for the first time, along with the sudden increase of the vascular caliber seems of high functional significance from a rheological point of view. Blood cells are forced to reduce their speed drastically, while passing from the capillary into the PCV. Accordingly, for homing lymphocytes, favorable conditions are achieved for rolling and fixing on the endothelial lining as preceding steps for transendothelial diapedesis.

Inasmuch as such a mechanism is relevant to the migration of red blood cells into the lymphoid tissue, as assumed by some investigators (5), the present study does not definitively clarify.

Moreover, no evidence was obtained from the cast specimens for the assumption (6,18) that red blood cells directly enter the intranodal sinuses by open communications between both systems or can pass the capillary wall. If this was so, equivalent structures such as extravasation of cast material indicating leaking capillary vessels and venules should be expected. Such artifacts, however, were not found in the arterially injected specimens.

The existence of afferent lymphatics, which has been questioned in the past (19), is clearly shown by casts with interstitial filling in the present study. In these casts, it is clear that hemal lymph nodes are provided with an extraordinarily spacious lymphatic sinus system, with a preference of the intermediary and medullary sinuses. These lymphatic sinuses offer ample space even to a great number of large macrophages for an encounter with red blood cells. Here the hemal lymph node can fulfill important immunological functions such as antigen presentation of autologous red blood cells on the base of erythrophagia. On the other hand, the system of fine interstitial lymphoid spaces, which could be represented in interstitially filled cast specimens appears less suitable as a site for large cellular complexes consisting of system may instead serve as a traffic site for small lymphoid cells and macrophages having ingested red blood cells and lost their rosette feature.

In summary, the following points of this study are emphasized: 1) Hemal lymph nodes in rats exhibit a basic architecture of the blood vascular system and of lymphatic pathways similar to other lymph nodes. 2) The cortical capillary system is highly vascular and postcapillary venules are prominent in hemal lymph nodes. 3) The sudden size increment of vascular caliber from the capillaries and venules to the postcapillary venule along with sphincter-like narrowings of the joining capillaries provide favorable rheological conditions for the homing of lymphocytes. 6) The intermediary and medullary sinus of hemal lymph nodes are extensive and thereby provide ample space for the interaction between erythrocytes and large macrophages.

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