THE MICROCIRCULATION OF THE HUMAN LYMPH NODE

by

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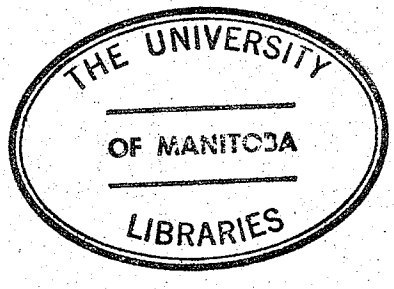
A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE



ACKNOWLEDGEMENTS

I would like to thank Doctor F.D. Bertalanffy, my advisor, for his support and invaluable assistance in the preparation of this thesis. I would also like to thank Doctor John Clancy Jr. for his encouragement, direction and interest in this investigation. To Rodica Schurath, for her technical assistance, to Brenda Bell and Charles Storton for their excellent photography, my sincere thanks.

ABSTRACT

The blood supply, the lymphatic spaces and their interrelationship within the lymph nodes of human cadavers are displayed in three dimensions by the use of a casting technique. Histologic sections are used for correlation. The morphology and topography of blood vessels, including the postcapillary venule, are visualised. These blood vessels show a characteristic arrangement of microvascular units. Microcirculatory units, in which lymphatic sinusoids are found in close relationship with the microvascular units, are seen in the cortex of the lymph node.

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INTRODUCTION

The blood and lymphatic systems within the lymph node constitute a significant component of the immunological apparatus. Some of the earliest descriptions of the blood supply of the lymph node were by His in 1859 (20), and Frey in 1861 (12). After reviewing their publications, Calvert (3) examined a similar problem in the mesenteric lymph node of the dog, and concentrated his study particularly on the blood supply to lymphatic follicles. He noted that an artery entered at the proximal pole, and branched in a fan-like pattern to supply the follicle. The capillaries emptied into small veins, situated at the periphery. More recently, the morphology of the blood microcirculation in dog and rat lymph nodes was demonstrated by Herman et al (18), employing stereomicroangiography. Their observations disclosed that the blood supply assumed a definite uniform pattern. Medullary cords exhibited the most extensive blood supply with numerous capillaries woven throughout the lymphoid tissue. Within the primary nodule, an arteriole gave rise to a capillary network, organised into a vascular unit. The secondary nodule contained a scarcity of vessels within the "germinal center", but curved venules surrounded the nodule. Long, slender blood vessels proceeded directly to the lymph node cortex, supplying there the undifferentiated lymphoid tissue. These vessels formed a plexus of capillaries. Apart from this dense capillary supply to the cortex, numerous vessels of larger calibre were likewise present. They were venules, many of which were lined by cuboidal endothelium, and hence could be identified as postcapillary venules.

The postcapillary venule is one aspect of the blood microcirculation that has received much attention ever since it was first described by Schulze in 1925 (30), as a peculiar venule with a high endothelial lining. Gowans and Knight (15) identified it as that vessel from which recirculating lymphocytes migrated from the blood to the lymphatic system within the lymph node. Benninghoff et al (2) studied the role of the postcapillary venule in the microcirculation of the lymph node in the dog. They disclosed that the cortical lymphoid tissue was endowed with a dense capillary supply that drained into postcapillary venules in the subcortex or at the cortico-medullary junction. Postcapillary venules received blood exclusively from the cortical tissue, without any contribution by medullary capillaries. The converse was claimed by Fukuda (13), who demonstrated in the rabbit that both cortical and medullary capillaries collected toward postcapillary venules.

Observations on lymphatic microcirculation were based on the injection of inert particles, such as india ink and acacia-graphite mixtures (10), or colloidal carbon (25), into afferent lymphatic vessels. Such studies demonstrated primarily a barrier function of the lymph node. Since the particles were lymph-borne, examination of tissue sections exhibited areas of deposits corresponding to the system of lymphatic microcirculation. Drinker et al (10) demonstrated by injection of india ink, that the carbon particles were first observed in the afferent lymphatic vessels. They were thereafter encountered in the subcapsular sinus, and later in the intermediary sinuses, before they finally departed the lymph node by the efferent vessel at the hilus. In the cortex of the node,

clear rounded spaces, corresponding to follicles, were present, devoid of carbon particles, as were also the medullary cords. The areas that were devoid of the ink particles were those portions of the node where lymphocytes with other cells were densely packed and the barrier was not caused by an intact sinus wall (10).

Other studies were directed to elucidate both the blood and lymphatic microcirculation concurrently to ascertain their interrelationships (6,7,13). The term "microcirculatory unit" was defined by Davidson et al (6) to be a lymphatic "sinusoidal sphere into which entered a polar artery leading to capillary loops, larger postcapillary venules and draining veins". Fukuda (13) employed that same term to include arterioles, capillaries and venules of the lymph node; hence he referred only to the blood system, though he stressed at the same time the importance of "the interrelation between the lymphatics and the blood vessels".

The stereoscopic structure of the microcirculation of the blood and lymphatic system in the human lymph node, including their interrelationship, remained unreported so far. Therefore, the present study employed a casting technique to visualise in three dimensions the lymphatic and blood microcirculation, including the postcapillary venules, in the human lymph node. Double casts, with two different colours of the injection medium, were prepared to display the interrelationship between the two systems. Histologic sections were used to correlate with the anatomical observations.

MATERIALS AND METHODS

Lymph nodes from five fresh and seven embalmed human cadavers were examined. The subjects ranged in age between 15 and 80 years. The injection medium was Microfil*, a brightly coloured radio-opaque silicone rubber compound, which on injection into vessels, produces a three-dimensional cast.

The general plan of study was to prepare three groups of lymph nodes. In the first group, lymph nodes were prepared so that they displayed the course of blood vessels, while the second group of nodes revealed their lymphatic microcirculation. The third category was a double cast combination to demonstrate simultaneously the interrelationship between the blood and lymphatic microcirculation within the same lymph node from the same specimen. The observations achieved by the casting technique were correlated morphologically by histologic sections of the lymph nodes from the same subject when feasible.

The inguinal and axillary lymph nodes of one side of the body were injected to visualise the blood supply, while the inguinal nodes of the other side served to outline the lymphatic spaces. In the embalmed cadavers, the femoral artery on one side was cannulated for introduction of the embalming fluid. Therefore, the inguinal nodes on that particular side were reserved for intra-lymphatic injections of Microfil, inasmuch

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*Canton Bio-medical Products Limited, Boulder, Colorado, U.S.A.

as the initial incision produced excessive leakage to facilitate a proper filling of blood vessels.

1. Blood Vascular System

To visualise the topography of blood vessels within lymph nodes, Microfil was injected into the main arteries supplying the particular region. Inguinal and axillary nodes were prepared for examination in the following manner:

Inguinal Nodes - An incision was made approximately four inches below the inguinal ligament, coinciding with the apex of the femoral triangle. The femoral and profunda femoris arteries were clamped. A second incision was placed in the abdominal wall proximal to the inguinal ligament, approximately above the mid-inguinal point. The Microfil was mixed with diluent and catalyzed with the curing agent. Fifty cc of this casting compound was then injected by manual pressure into the external iliac artery. The cannula, syringe and clamps were left in place until the compound had set. The gel time of the medium was approximately 100 minutes. Minimum tissue damage was produced during the injection procedure as the casting compound flowed smoothly and efficiently into the vessels. However, any leakage, if it occurred, reduced the pressure in the vessels, which were then improperly filled.

Following the superficial clearing of the subcutaneous fat, the filled nodes were revealed as masses of colour. Hence, the lymph nodes were located without difficulty. The node with the largest lymphatic vessels was left in situ for a subsequent intra-lymphatic injection to

produce a double blood and lymphatic vessel cast. A different node on the same side was removed for histologic sectioning. The remaining nodes were carefully dissected out, removed, cleared of fat, placed into saline and further processed to produce blood vessel casts.

Axillary Nodes - Through an incision on the medial aspect of the arm, the brachial and profunda brachii arteries were ligated just as they emerged from the axilla. The subclavian artery was exposed proximal to the clavicle. Thirty cc of the casting compound was injected into the latter vessel under manual pressure. After the compound had gelled, the nodes were carefully dissected, removed, cleared of fat, and placed into saline.

II. Lymphatic System

Inguinal and aortic lymph nodes were prepared for examination of the lymphatic system.

The technique in this study of injecting lymph vessels was a modification of that generally employed in lymphography (8).

In view of the considerable technical difficulties often associated with intra-lymphatic injections of other lymph nodes, the inguinal nodes were selected to demontrate lymph microcirculation, because they were more readily accessible than most other lymph nodes.

Lymph nodes were carefully exposed and the vessels partially cleared by dissection. They were not entirely freed from the surrounding fat as they were of small calibre, extremely fragile, and seemed to have lost their distensibility because of post-mortem changes. The general condition of vessels was not appreciably better in the fresh

body than that of the embalmed cadaver. The largest lymphatic vessel of the lymph node was selected for injection. Before cannulation, a loose tie suture was applied to the vessel with black linen thread. To immobilise the vessel, a scalpel handle was passed transversely behind it. It was held perfectly still with the left hand while with the right a 30 gauge lymphography needle was inserted through the wall into the lumen. The suture was tightened to hold the needle in place. Care was taken not to perforate the vessel wall at other points in this process. A syringe was then connected to the tubing, and Microfil was slowly injected into the vessel by manual pressure. That pressure had to be carefully regulated and applied gently as the vessels ruptured most readily. If such occurred, the particular nodes had to be abandoned entirely, because the extensive leakage of the medium obscured the other vessels to such a degree that it was infeasible to determine whether the compound was flowing within or merely along or around the vessels. Following successful cannulation, the Microfil was mixed with diluent, catalyzed with the curing agent and slowly injected by manual pressure into the lymphatic vessel. The mixing of the casting compound was performed after cannulation, because the operation required considerable time and the medium might gel before the lymphatic vessel was ready for injection. The instruments were left in place until the compound had set. To achieve a satisfactory degree of filling, it was often necessary to inject more than one lymphatic vessel of the same node. The node with the vessels were dissected out and removed for further processing.

Aortic nodes were obtained by the following approach. The ribs were sawed bilaterally at the level of the mid-clavicular line and were lifted out together with the sternum. The diaphragm was freed from its costal attachment. Incisions were made in the anterior abdominal wall to free and mobilise the abdominal contents. Sample aortic nodes were removed for histologic sections. Five cc Microfil was injected into the thoracic duct just below the diaphragm with the compound infused in a caudal direction. In some subjects, the thoracic duct was obstructed by a whitish deposit and the nodes failed to become filled. In such instances, all the aortic nodes were removed for histologic processing.

III. Combined Blood and Lymphatic Systems

To produce a double cast revealing the relationship between blood and lymphatic microcirculation, two different colours of Microfil were employed. The inguinal nodes were selected because their vessels filled readily and the nodes were more accessible. The previously described procedure was employed for the injection of lymph vessels (II), except that a different colour medium was used for the injection of the arterial system of that node. After the compound had set, the nodes were removed for processing.

Preparation of Lymph Node Casts

a) The nodes remained in normal saline for approximately 24 hours.

b) They were dehydrated by passing them for 24 hours through graded dilutions of ethyl alcohol (25%, 50%, 75%, 95%, and absolute alcohol).

c) The tissue was cleared by immersion in toluene, and

d) stored in synthetic oil*.

Examination of Casts

Each cleared lymph node was bissected with a razor blade. The two halves were placed into a petri dish and covered with the storage oil. A slide was placed over the sections to flatten them. The external and cut surfaces were examined with incident light, using an Ortholux microscope with Ultrapak objectives. Morphology of the surfaces were recorded by colour photography with an Orthomat+ camera.

Histologic Preparations

Lymph nodes for histologic sections were fixed in Davidson's solution. They were then bissected with a razor blade, and embedded in paraffin. The tissue was sectioned at 8 μ with a Sorvall Microtome JB-4, and stained with hematoxylin and eosin. The sections were derived from the cut surface to facilitate a correlation of observations from the cut surface of the casts.

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+Ernst Leitz Gmbh, 6330 Wetzlar, Postfach 210-211.

RESULTS

Blood System

Blood vessels in fresh bodies filled more completely with Microfil than cadavers that had previously been embalmed. Despite varying degrees of filling, all nodes exhibited an extensive blood supply not readily evident from histologic sections (Fig. 12). The venous system comprised a more extensive proportion than the arterial supply, which by comparison was rather scanty. Veins were characteristically larger and more tortuous than arteries, which as a rule were slender and straight (Fig. 4). Veins and arteries did not accompany one another in the lymph node in a parallel fashion, but rather took independent courses.

A main artery generally entered the node at the hilus and was of smaller caliber than the draining vein (Fig. 1). That artery branched as it coursed through the node. From these primary tributaries smaller arteries were given off to supply the medulla. They formed intricate plexuses which were extremely intertwined. Fine arterial vessels ramified in the region of the medullary cords, leaving the sinuses as clear spaces (Fig. 2). Examination of casts in 50 μ sections, which reduced superimposition, did not add significantly to any further interpretation. Consequently, vessels could not be traced adequately for a more detailed description.

One or more arteries commonly entered the hilus of the lymph node. Arterial branches from the main artery, or arteries, travserved the substance of the node to attain the opposite cortex (Fig. 3). In the cortex,

Fig. 1 Blood supply of the axillary lymph node (x10). Note the small artery (A) and the large draining vein (V), both at the hilus. Observe also the blood vessels surrounding the node.

Fig. 2 Blood supply of the medulla of the axillary lymph node, observed from the sectioned surface (x40). Note the fine mesh of intertwining blood vessels, leaving the sinuses as clear areas.

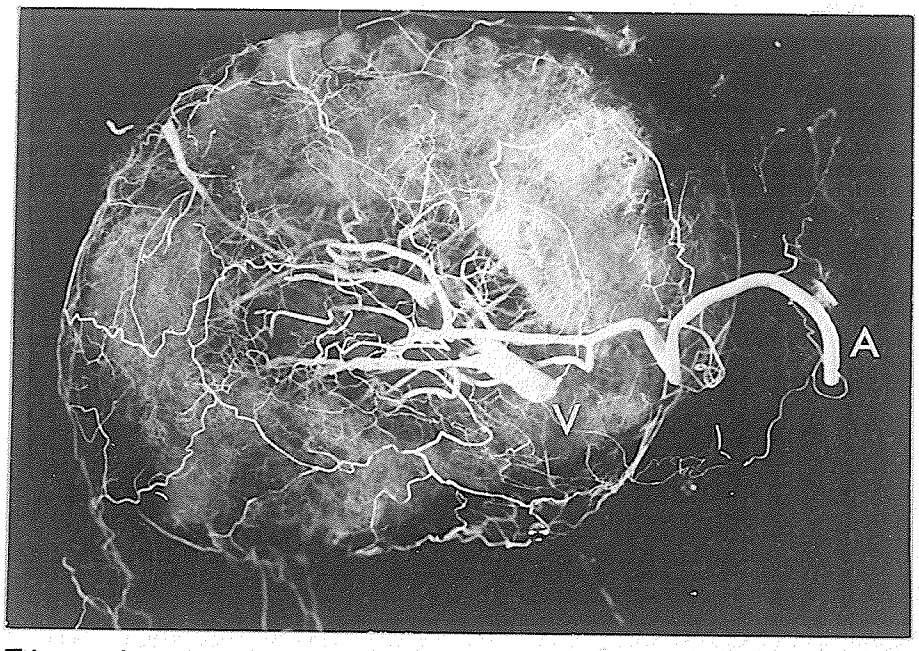

Fig. 1

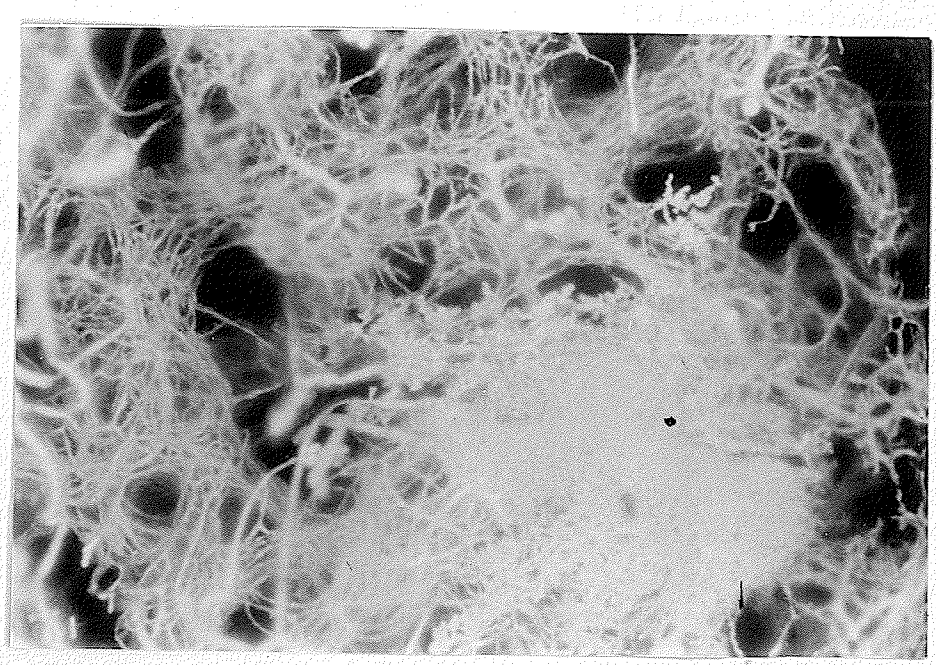

Fig. 2

Fig. 3 Blood supply of the axillary lymph node, seen from the sectioned surface (x10). Note the main artery (A) giving off branches as it proceeds towards the cortex (C) of the node. The arrow points to an arterial branch leading to the cortical capillary networks.

Fig. 4 Blood supply of the inguinal lymph node, seen from the sectioned surface (x15). Note the linear artery (A) and the tortuous and large vein (V). Observe the more extensive venous supply in contrast to the arterial supply. A lobular vascular unit with two subunits is indicated by the bracket.

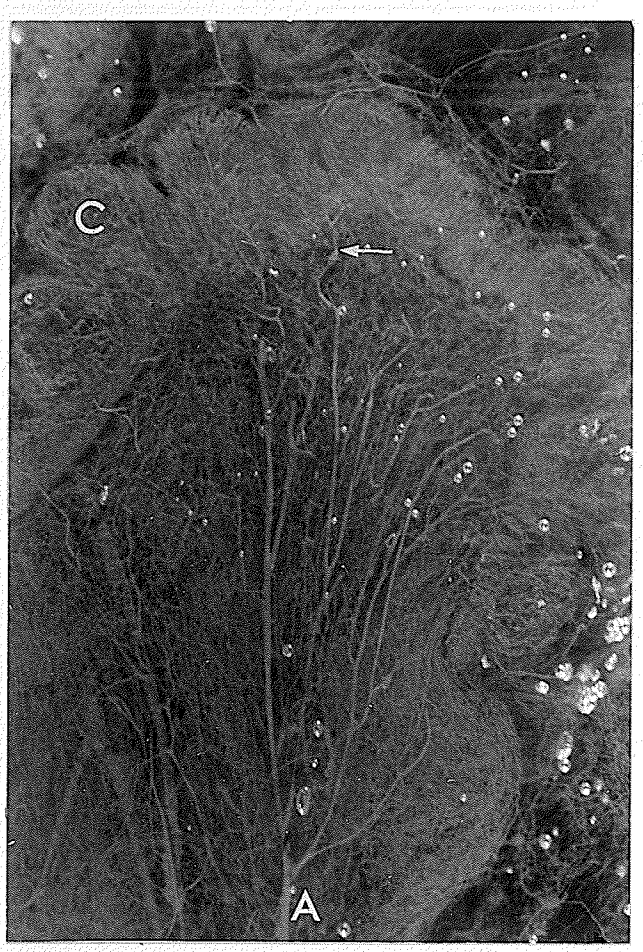

Fig. 3

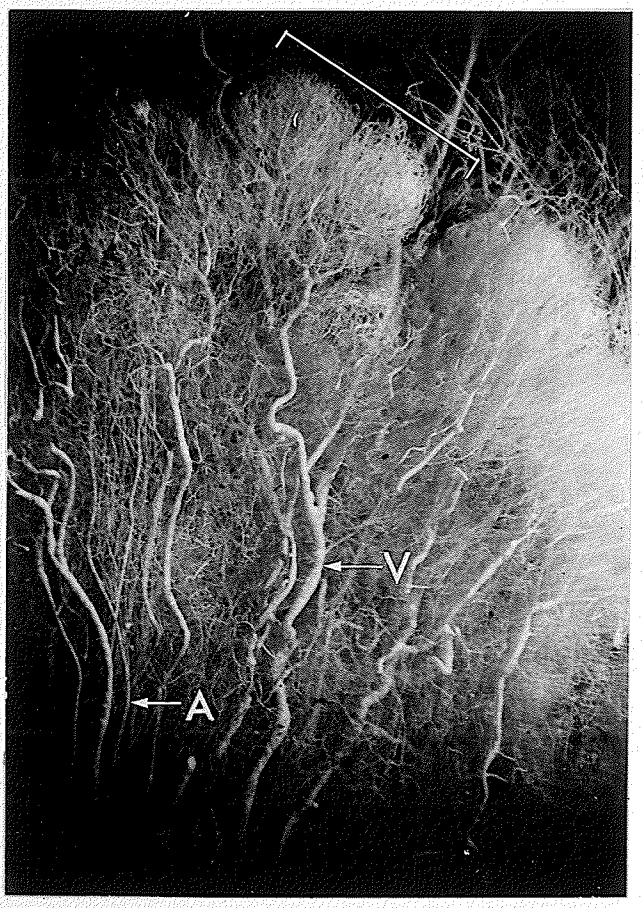

Fig. 4

networks of capillaries formed a subcapsular capillary arcade where arterioles gave rise to capillary vessels, which looped back to drain into venules. Lobular vascular units could be demarcated, and each of them was usually subdivided into smaller units (Fig. 4). In nodes with more complete filling, the plexus of blood vessels was frequently arranged in a specific manner. It formed small microvascular units which were scattered throughout the lymphoid tissue (Fig. 5A). At higher magnification, they were observed to consist of a precapillary arteriole at one pole, and a glomerular-shaped capillary cluster with a postcapillary venule emerging from the opposite pole (Fig. 5B). The comparable venule exhibited a significantly larger size than the arteriole, the latter being also more linear and slender. These microvascular units varied in size in different nodes, and corresponded to the "follicles" in the histologic sections (13).

Lymphatic System

The lymph nodes underwent segmental filling with Microfil, and more than one lymphatic vessel had to be injected to obtain an adequate representation.

An afferent lymphatic vessel to a node branched into a number of rootlets before penetrating the capsule from the periphery to drain into a subcapsular sinus. Examination of the external surface of the inguinal node revealed discrete dome-shaped clustered units (Fig. 6A). The cast of the cut surface displayed the subcapsular sinus and cortex as a superficial marginal rim. The sinus exhibited a crenated margin, following the outline of the dome-shaped units visible on the external surface. In some specimens, the cortex was thicker and more prominent, and the flow

Fig. 5 Blood supply of the inguinal lymph node.

A External surface (x10). Note the numerous microvascular units scattered throughout the node.

B Sectioned surface of the same node (x40). Note the components of a microvascular unit - an arteriole (Pa) branching to form a capillary cluster (c), collecting towards the larger postcapillary venule (Pv). A second microvascular unit is situated above the labelled unit. Note the proximity of the microvascular units to the margin of the cortex, outlined by the fine blood vessels in the lower portion of the figure.

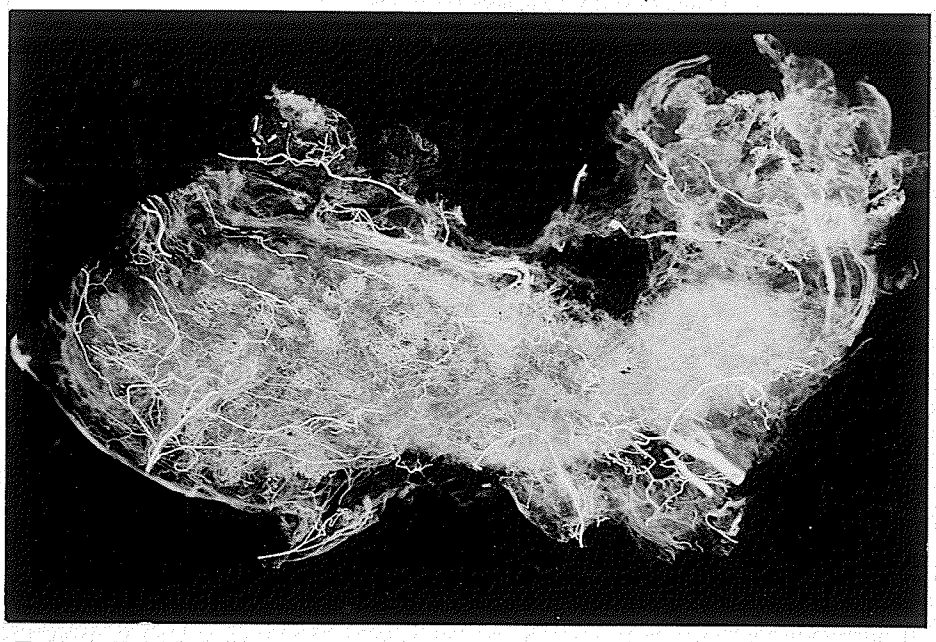

Fig. 5A

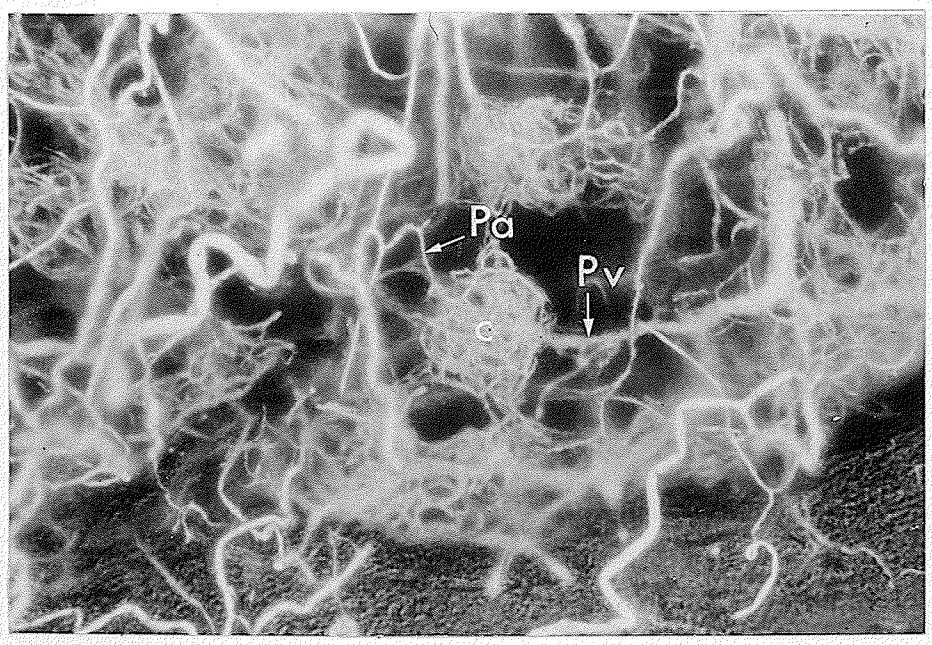

Fig. 5B

Fig. 6 Inguinal lymph node following intra-lymphatic injections. Female, aged 15 years; cause of death - carcinoma of the tongue.

A External surface (x10). Note branching afferent lymphatic vessels (L), leading to dome-shaped lymphatic spaces located external to follicles.

B Sectioned surface (x10). Note the prominent cortex (C) with extensive permeation of the injection medium. Observe the lack of clear spaces indicating follicles. The intermediary sinuses are more prominent as well. Compare with Fig. 7. Note also the undulating outline of the subcapsular sinus (S).

Fig. 6A

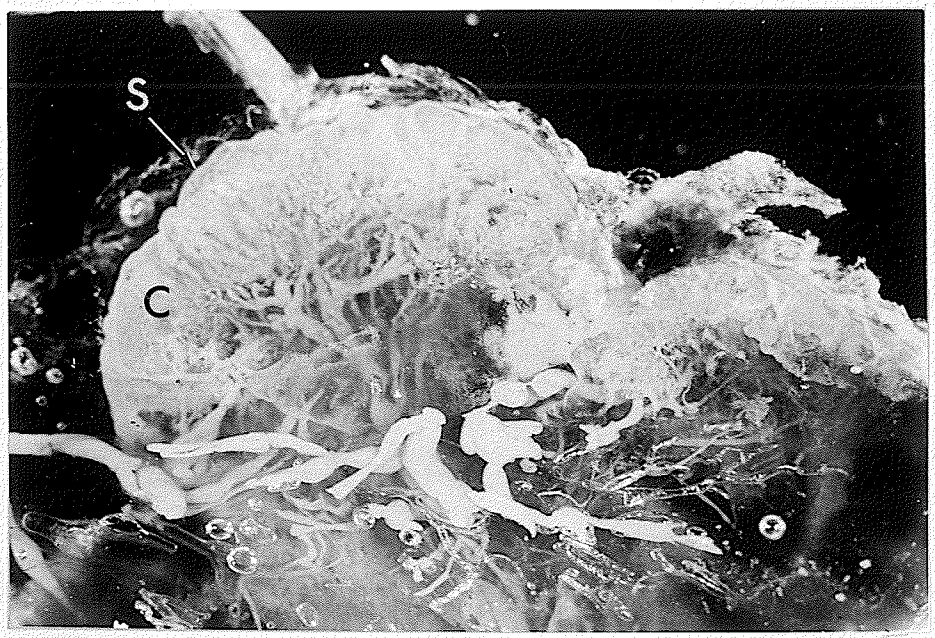

Fig. 6B

of the casting compound was correspondingly greater (Fig. 6B). In other nodes, the cortex was thinner and more porous (Fig. 7). Rounded clear spaces were present in the cortex corresponding to "follicles" (10). From the cortex, numerous lymphatic canaliculi converged onto larger ones, draining eventually into the efferent lymphatic trunks leaving the node at the hilus (Figs. 6B, 7). These canaliculi represented the medullary sinuses. Some of them had a beaded appearance indicating the position of valves. In an ultra-structural study of the lymph node, Nopajaroonsri et al (25) reported valves to be absent in the sinuses, except in the large medullary sinuses close to the efferent lymphatic vessels. That information aided in the identification of the approximate boundaries between medullary and efferent lymphatic trunks leaving the node.

In the present study, intra-lymphatic injections were performed on lymph nodes from the inguinal and aortic regions. The cast of aortic nodes exhibited an appearance quite different from the cast of inguinal nodes, described above. The external surface of aortic nodes did not possess any dome-shaped aggregates. Lobular units were present instead (Fig. 8A). From the cut surface, these lobules were discerned to intercommunicate, and the draining canaliculi observed in the inguinal nodes were absent (Fig. 8B). Examination of the histologic sections revealed "follicles" scattered throughout the node (Fig. 9). This was in contrast to the inguinal node where lymphocytes were concentrated at the periphery in a well-demarcated cortex (Fig. 10).

Numerous nodes in this study revealed the presence of hard whitish deposits resembling calcium within their substance, rendering histologic

Fig. 7 Inguinal lymph node following intra-lymphatic injections. Male, aged 80; cause of death - pneumonia and carcinoma of left lung. Sectioned surfaces (x10). Note the thin, porous marginal rim of cortical sinusoids (C), whence lymphatic canaliculi lead to large efferent trunks at the hilus.

S = subcapsular sinus. Compare with Fig. 6B.

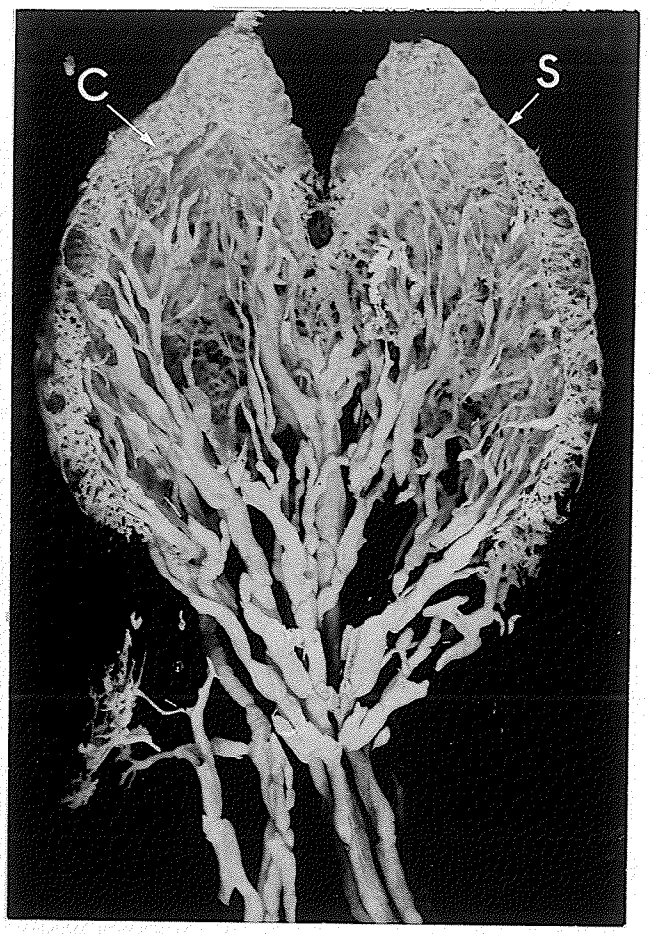

Fig. 7

Fig. 8 Aortic node following injection into the thoracic duct.

A External surface (x10). Note the branching afferent lymphatic vessel (L) leading to the lymph node. Observe the lobulated external surface of the node. Compare with Fig. 6A, where the external surface of the inguinal lymph node presents discrete dome-shaped units.

B Sectioned surface (x10). Note intercommunicating lobular-shaped aggregates. Observe the absence of well-defined canaliculi evident in the inguinal nodes in Figs. 6B and 7.

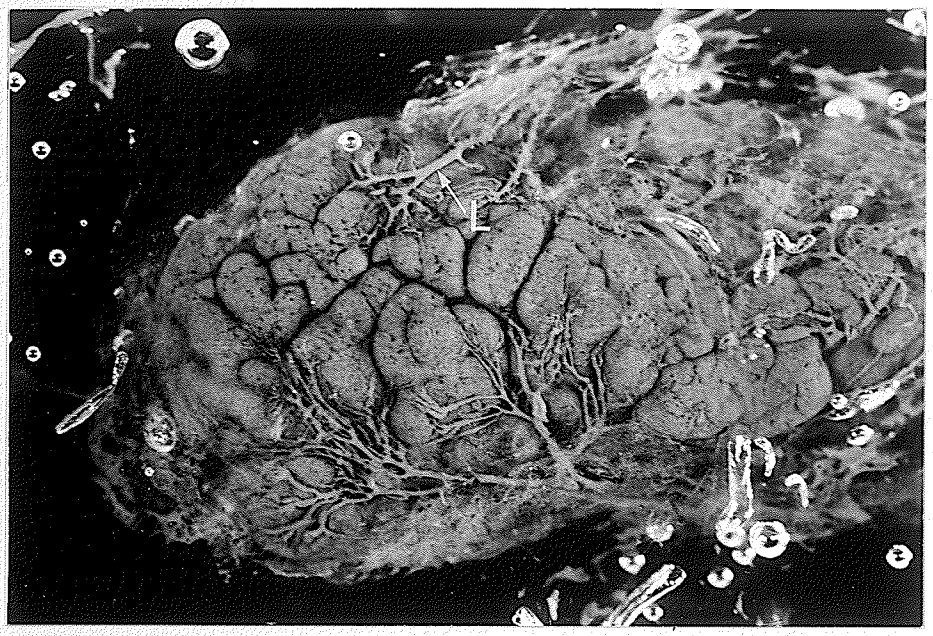

Fig. 8A

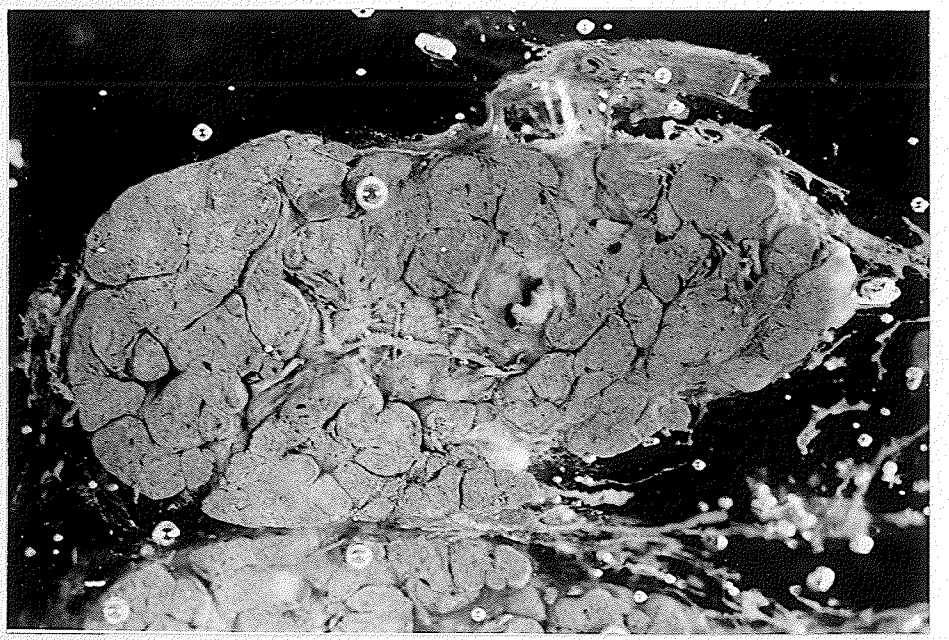

Fig. 8B

Fig. 9 Histologic section of aortic node (H and E x25, 8 μ section). Note the numerous follicles scattered throughout the node.

Fig. 10 Histologic section of inguinal node (H and E x2.5, 8 μ section). Note the well-defined cortex and hilus in contrast to Fig. 9.

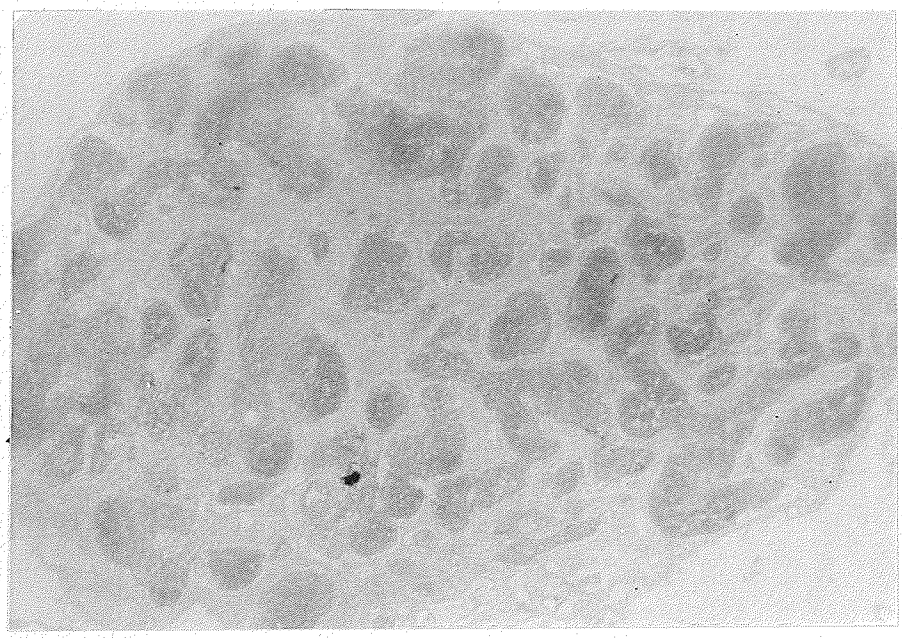

Fig. 9

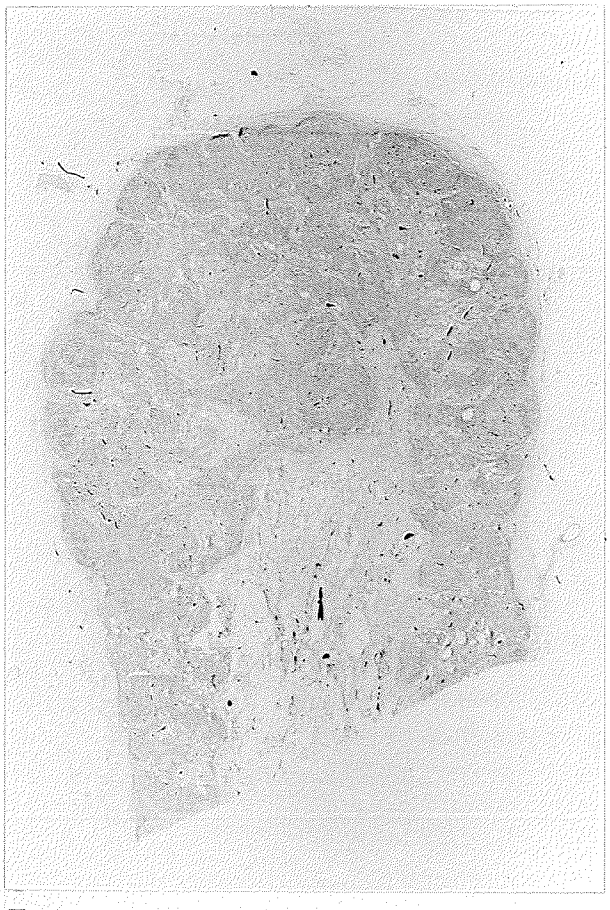

Fig. 10

sectioning infeasible. Consequently, it was infeasible to obtain histologic preparations from some of the examined lymph nodes. In other nodes, the rubbery casting medium pulled out of the blood vessels during sectioning, rendering these sections unsatisfactory for study. However, in many specimens such problems fortunately did not arise, and adequate histologic sections were obtained.

Combined Blood and Lymphatic Systems

Injection with two different colours of the casting compound was performed to demonstrate the interrelationship between the blood and lymphatic system. Yellow Microfil was injected into the afferent lymphatic vessel, and red medium into the regional artery. Microcirculatory units were observed in the cortical lymphoid tissue beneath the subcapsular sinus. These units consisted of spherical spaces surrounded by dome-shaped lymphatic sinusoids through which lymph percolated. Within the hollow cores, where histological "follicles" were situated, the blood vessels of the microvascular units were situated (Fig. 11A,B).

Fig. 11 Inguinal lymph node following intra-arterial and intra-lymphatic injections. Yellow = lymphatic spaces, red = arterial vessels.

A Sectioned surface (x10). Note the clear spaces with fine blood vessels beneath the subcapsular sinus (S). The arrow points to a microcirculatory unit.

B Sectioned surface (x60). Note the clear area surrounded by lymphatic sinusoids and fine blood vessels within the core.

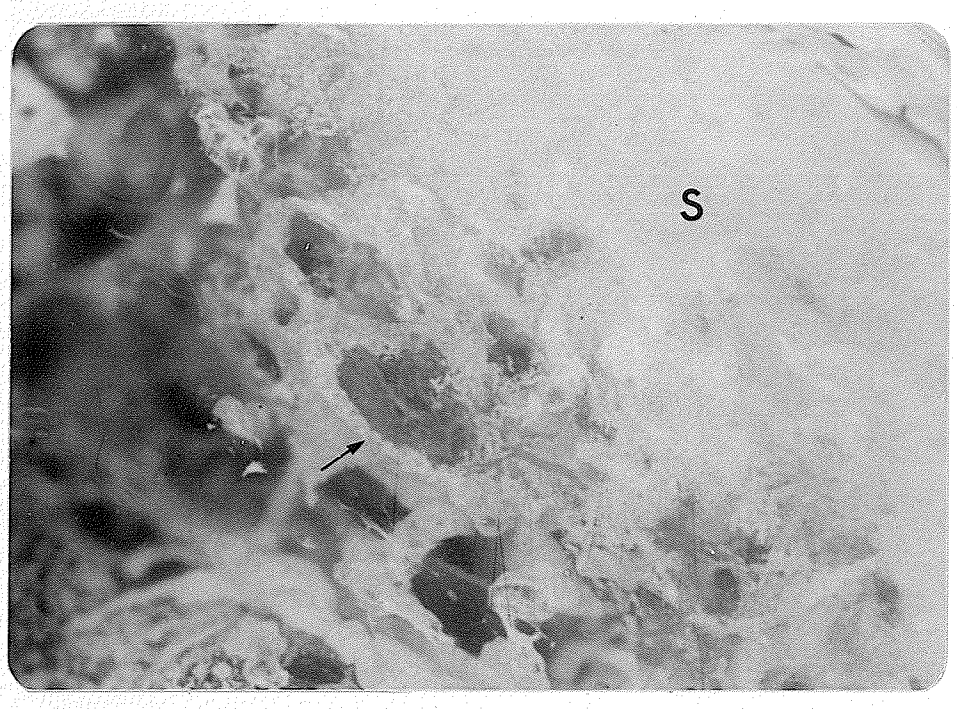

Fig. IIA

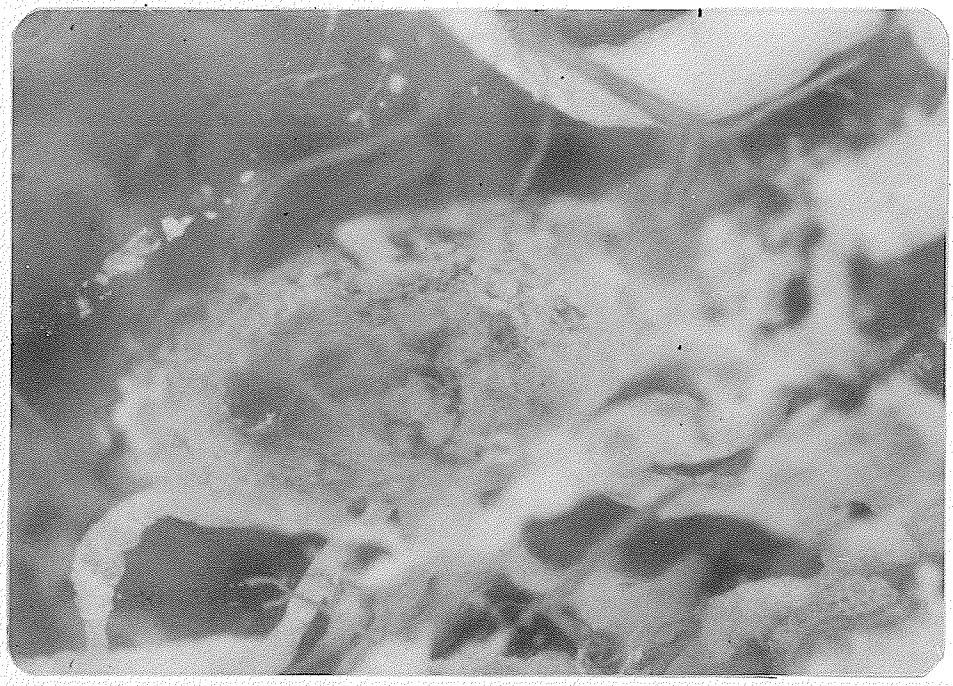

Fig. IIB

Fig. 12 Histologic section of inguinal lymph node (H and E x50, 8 μ section). Note the injection medium in the blood vessels in the cortex (Co) deep to the capsule (Cp). Observe the paucity of blood vessels.

Fig. 13 External surface of the inguinal node following intra-arterial injection (x10). Observe how the leakage of the injection medium accentuated the microvascular units.

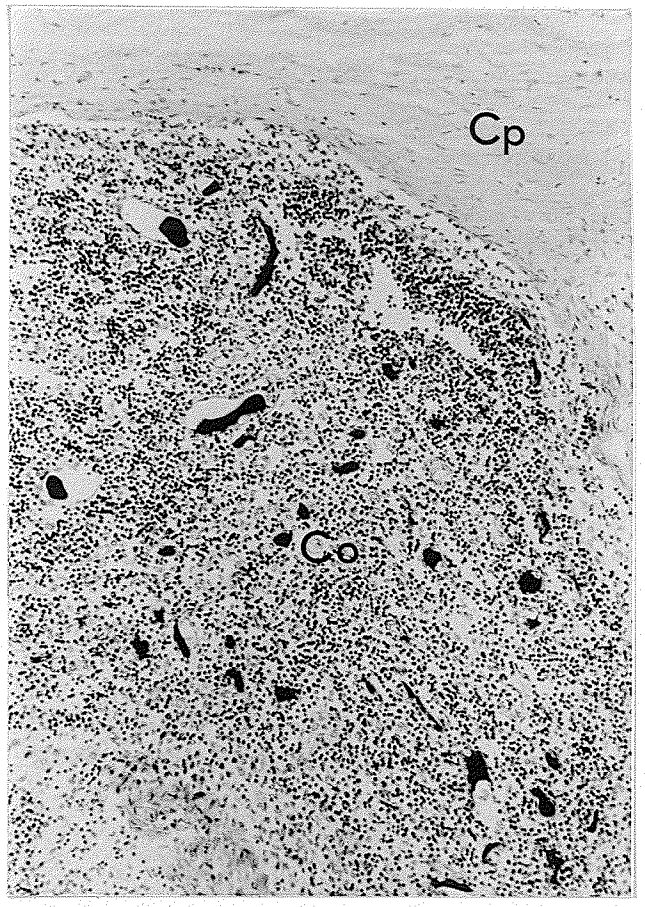

Fig. 12

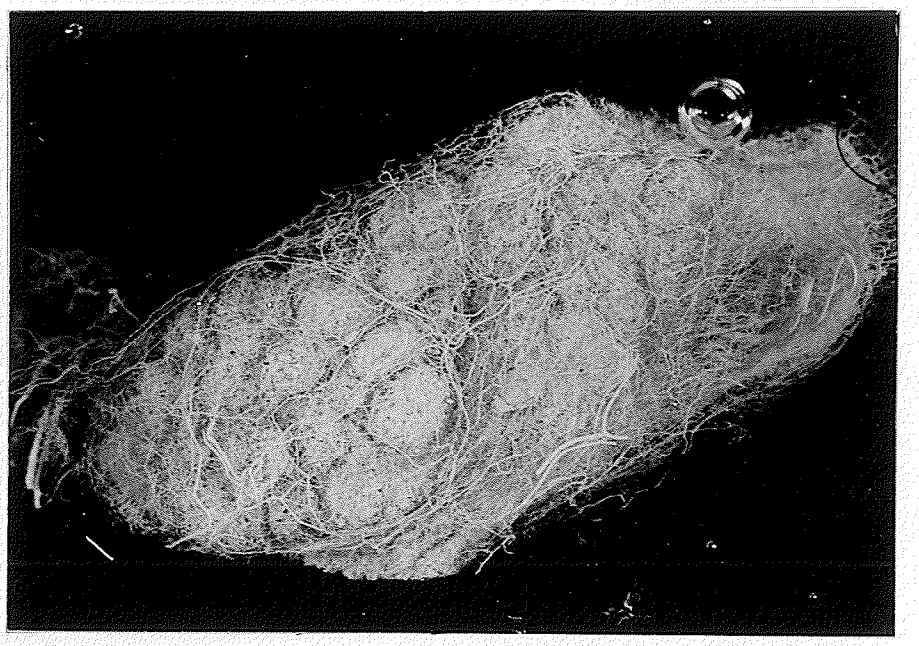

Fig. 13

DISCUSSION

The morphological architecture of the vascular system in human lymph nodes can be visualised by the use of a casting technique. As the continuity of structures is preserved, the topography of blood vessels and lymphatic sinusoids, including their interrelationship, can be accurately ascertained by that approach.

During injections of the arterial system, Microfil seeped from the cortical vessels in the majority of nodes. Yet, a leakage from vessels of the medulla was rare. Tiny globules of the rubber compound accentuated the blood microvascular units (Fig. 13). Fukuda (13) likewise encountered extravasation when injecting colloidal iron (Fesin) in saline solution into the femoral arteries of adult rabbits. Fesin particles were encountered around capillary networks in the cortical areas of popliteal lymph nodes. He concluded that the site of exudation of plasma from blood vessels to the lymphatic spaces were these capillaries. Herman et al (19) likewise noted extravasation of the injected medium in nodes during the primary immune response, whereas control nodes had minimal, if any, leakage. Vascular changes during the immune response might allow for this phenomenon. However, Dabelow (5), in his extensive study on the blood supply of the lymphatic system, stressed that he could not demonstrate any kind of extravasation. These divergent observations regarding extravasation may account for the globules of Microfil observed in the cortex of lymph nodes prepared by arterial injection. The leakage may hence not be merely ascribable to a breakdown of vessels in the course of post-mortem changes.

Active germinal centres were not encountered in the histologic sections of the nodes, at least not in the inguinal group of lymph nodes, which were mainly examined in this study. These superficially situated lymph nodes rarely contained well-developed centres (11); inguinal nodes exhibited few active centres, if any, after the age of twenty (9). Consequently, it was infeasible to study the blood vessels supplying germinal centres. However, follicles were present and they corresponded histologically to microvascular units. Their appearance confirmed a similar finding in the work of Fukuda (13). During the primary immune response, Herman et al (19) have reported increased vascularity within the lymph node, with an even redistribution of capillary and postcapillary venules. However, it is unknown whether any reorganization of the blood microcirculation occurs with retrogression of germinal centres commencing at puberty in superficial lymph nodes (9).

Perfusion of the lymphatic system with india ink or other inert particles, or dyes, does not reveal aspects of lymph node architecture of the type as they can be elucidated in a stereologic study. In their investigation on the structure of lymphatic sinuses, Drinker et al (10) injected india ink and acacia-graphite mixtures into adult dogs. Particles were encountered deposited in the marginal and intermediary sinuses. However, in the medulla, the particles remained confined within the sinus walls, clearly delineating their boundaries. Particles penetrated the cortical sinuses and lymphoid tissue from the marginal sinus. The boundaries of the cortical sinuses were less clearly defined than those of the medulla. Areas of densely packed lymphocytic aggregations remained

comparatively clear of ink, apparently providing an effective barrier for the carbonaceous matter (10).

Observations derived from the casts in the present study confirmed the aforementioned findings. Microfil filled through the afferent lymphatic vessels, the subcapsular sinus and thence permeated the cortical lymphoid tissue, as this was also achieved by india ink injections (10). The areas of aggregated lymphocytes in the follicles remained clear also of the casting compound, and appeared as rounded, empty spaces (Fig. 11A,B). They corresponded to the "light areas" of Drinker et al (10). The medullary sinuses were displayed as well-defined canaliculi, with the casting compound remaining confined within the limits of the sinus walls. Leakage was occasionally observed, but this was probably brought about by a breakdown of the formerly intact sinus walls in the course of post-mortem changes.

Electron microscope studies in mice of the sinuses and reticulum of the lymph node revealed the presence of pores between the lining-cells of both the subcapsular and medullary sinuses (23,24,25). Moe (24) described discrete cell junctions with gaps estimated to be about 140A in diameter, although the presence of larger interstices of 0.1 μm and up to 1.0 μm had been noted. However, such larger gaps were infrequent. It is unknown whether these larger crevices occurred selectively in the cortex to allow a more efficient lymphatic perfusion. The reticuloendothelial cells were separated from the lymphoid parenchyma by a perisinusoidal space, containing a reticulum of ground substance and collagenous fibrils. A definite basement membrane was absent (4). Nossal et al (26) noted in

rats that the barrier between the lymphoid parenchyma and medullary sinuses was not complete, and cells were observed in transit between the two regions. However, another study on murine lymph node reticulum reported that the inner wall of the subcapsular sinus was the only sinus lining with an incomplete structure (4). Many free cells were present, occluding the gaps in that inner subcapsular lining. In a separate labelling study on antigen capture in lymphoid follicles, it was pointed out that the sinus-lining phagocytic cells of the inner wall of the marginal sinus contained the label for just a very short time (27). The authors gave support to the proposal by Clark (4) that these sinus-lining cells had migrated elsewhere, and were replaced by new cells. This concept of a dynamic sinus-lining on the inner wall of the subcapsular sinus, as well as the larger incidence of gaps ascribable to the extensive cell movement in the cortex, might be some of the factors allowing for the free flow of Microfil into the cortical lymphoid tissue. Cell migration in the medulla is considerably less and hence gaps between the lining cells are also less frequent. This might have contributed to the diminished perfusion of medullary lymphoid parenchyma. It is noteworthy that there is a difference in antigen distribution between the medullary and cortical follicles (26,27). The bulk of the antigen in the medulla was phagocytosed by macrophages within the medullary sinuses. Most antigen encountered in the medullary cords were within macrophages. Free antigen occurred in the sinuses but not so within the cords (26). Antigen in the cortex was retained in follicles, but was extracellular, and associated with plasma membranes of cell processes. Antigen percolated between the

cells through the inner wall of the marginal sinus to terminate in these follicles (27).

The size of the particles in the casting compound employed in this study ranged from 1.0 μm to 2.5 μm. Data on the dimensions of the gaps in the sinus-lining, if they exist, are unavailable in the human lymph node. Therefore, quantitative comparison cannot be made.

Postcapillary structures were not observed in the medullary region of the node. That feature was in agreement with the report by Benninghoff et al (2), who observed in dog lymph nodes that the postcapillary venules did not receive blood directly from the medulla containing blood vessels in close proximity to lymphatic sinuses. However, Fukuda (13) noted that in rabbits the postcapillary venules did receive blood from the cortex as well as some from the medullary cords.

In rabbits, two to five capillaries collected to one postcapillary venule (19). In the present study of human nodes, a greater number of capillaries formed one postcapillary venule (Fig. 5B). In dogs, these venules traversed the cortex "in a direction from the surface into the depths" to join draining veins (2). In contrast, in the human lymph nodes, the position of the venules exhibited a random distribution.

Postcapillary venules, through which recirculating lymphocytes migrated to the lymphatic system, were described characteristically as being present in the deep cortex* of the lymph node (1,15,16,17). In the human lymph nodes examined in this study, postcapillary venules were

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*deep cortex = paracortex = diffuse cortex

encountered in both the deep cortex and in that region of the cortex close to the marginal sinus (Fig. 5B). This area could be designated as superficial cortex. However, divisions of the cortex into deep, mid- and superficial zones were difficult to delineate. Postcapillary venules, associated with microvascular units, were scattered throughout the cortex, without any definite concentration in any particular zone. The porportion of high endothelial postcapillary venules could not be determined.

Recirculating thoracic duct lymphocytes were identified to be both T and B cells, with the majority being T lymphocytes (29). The "homing" patterns of these two subpopulations of lymphocytes were the topic of several reports (1,16,17,21,28). General agreement prevails that T lymphocytes "homed" to the deep cortex, or the thymus-dependent area, whereas B cells settled in follicular regions. Howard et al (21) demonstrated labelled B cells around germinal centers in the deep and superficial cortex. Moreover, these cells were noted in one of their illustrations [Fig. 9, see Howard et al (21)] to lodge in a "band beneath the sinus", and not assuming a circular configuration demarcating follicles. The authors concluded that the deep cortex was the zone of recirculation for T lymphocytes, and the follicular areas in the outer cortex those "regions through which B lymphocytes normally recirculate". It was further demonstrated that the recirculation of B lymphocytes occurred on a "large scale" (22). Gutman and Weissman (17) noted that the postcapillary venules served as a common site of transfer for both T and B cells, and thereafter, T cells remained in the diffuse cortex whereas B cells migrated to follicles. It was interesting to note in human lymph

node sections that postcapillary venules became displayed in the outer as well as in the deep cortex, concurring with the descriptions in the preceding paragraph. These venules were an integral component of the microvascular units correlating histologically with follicles. The possibility that B cells might migrate through postcapillary venules situated within or close to follicles cannot be discounted. Another investigation would have to study that problem.

This investigation was undertaken to examine the microcirculation of the blood and lymphatic system within the human lymph node. It was revealed by the casts produced by intra-lymphatic injections, that there was a difference in structure and appearance between inguinal and aortic nodes. This might conceivably signify a specialisation of function, with the aortic nodes experiencing a more intense follicular activity than the inguinal group of nodes. The difference in the perfusion pattern between medulla and cortex might be related in some manner to a corresponding pattern of antigen distribution in these two regions. It was observed further than an intimate interrelationship existed between the microvascular unit and the surrounding lymphatic sinusoids. That arrangement has been comprised with the term microcirculatory unit, providing a functional anatomical configuration with important elements of the immune apparatus in close proximity. The morphology and distribution of the microvascular units in man had not previously been displayed stereologically. The revelation of the close association between the postcapillary venules and the microvascular units could well provide an anatomical basis for the traffic of cells within the immune system of the human lymph node.

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