THE MICROCIRCULATION OF THE HUMAN LYMPH NODE

by

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MASTER OF SCIENCE

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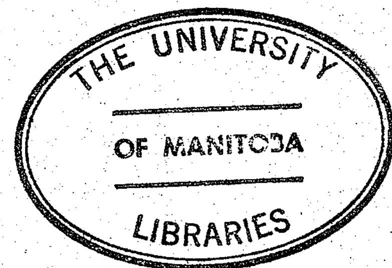

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ABSTRACT

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.

I. 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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*Dow Corning 710

+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.