Studies on the Structure and Permeability of the Microvasculature in Normal Rat Lymph Nodes

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The structure and permeability of the microvasculature in normal rat lymph nodes was studied by regional perfusion techniques. The results indicated that characteristic vascular units supplied each cortical lobule of lymphatic tissue. Numerous arteriovenous communications and venous sphincters innervated by unmyelinated nerve fibers were found in this vascular bed. These specialized vascular structures permitted regional control of blood flow through high endothelial venules. Lymphocytes migrated across these venular walls by moving through intercellular spaces in the endothelium and between gaps in the laminated, reticular sheath. No direct anastomoses between blood vessels and lymphatics were seen, but tracer studies with horseradish peroxidase suggested that functional lymph node--venous communications were present in the walls of high endothelial venules. (Am J Pathol 80:387-418, 1975)

The microvasculature has been generally accepted as an important functional component of lymphatic tissues. Early anatomists described a rich network of arborizing arteries, capillaries, and veins which supported the intense metabolic activities of lymph nodes. Other investigators emphasized the associations between lymphocytic proliferation and nodal vascularity by demonstrating that vascular patterns varied as germinal centers waxed and waned in the cortex. In addition to its nutritive functions, the nodal microcirculation has been reported to regulate fluid and cellular exchange between blood and lymph. Studies by Gowans and his colleagues clearly demonstrated that recirculating lymphocytes left the blood stream and entered lymph nodes by emigrating across the walls of venules lined with high endothelium. Other reports indicated that the volume and content of efferent lymph might be determined by redistribution of fluid between lymph channels and blood vessels within the node. These specialized functions cannot be adequately explained by previous descriptions of the nodal vascular

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anatomy, and the scant information available on mechanisms which regulate blood flow in lymphatic tissues.\textsuperscript{12}

The present study describes a series of light and electron microscopic observations on the microvasculature of normal and perfused rat axillary lymph nodes. The results indicate that distinct vascular units supply each lobule of lymphatic tissue. Numerous arteriovenous communications and venous sphincters innervated by unmyelinated nerve fibers are present in this vascular bed. These specialized vascular structures provide a unique system for regulating blood flow within high endothelial venules (HEVs) and probably influence fluid and cellular exchange within the node.

\section*{Materials and Methods}

\subsection*{Animals}

Adult Wistar rats (Microbiological Associates, Walkersville, Md.) of both sexes, weighing between 180 to 220 g were used in these studies.

\subsection*{Anesthesia}

Rats were anesthetized for surgical procedures by intraperitoneal injections with aqueous solutions of chloral hydrate at dosages of 360 mg/kg body weight.

\subsection*{Regional Perfusion With Alcian Blue Dye}

A 2\% solution of alcian blue dye (8 GS, Chroma Gesellschaft, Schmid & Co., Stuttgart, Germany) was prepared in physiologic saline at pH 6.8. This was passed through a filter unit (0.45-\(\mu \) grid membrane, Nalge Co., Rochester, N.Y.) immediately prior to injection. Axillary nodes were perfused by retrograde injection of dye into brachial arteries. A skin incision was made from the mid axilla along the ventral aspect of the upper forelimb. The brachial artery was exposed using sharp dissection to reflect overlying muscles. A 30-gauge needle was inserted into the distal segment of this vessel and advanced through its lumen until the needle tip was situated about 5 mm from the origin of the lateral thoracic artery. Then 0.2 to 0.4 ml of alcian blue dye was slowly injected from a 1 ml syringe. The injection pressure slightly exceeded rat systolic blood pressure and resulted in the flow of dye admixed with blood into the lateral thoracic and regional arteries supplying axillary nodes. The same injection techniques were employed in other animals where blood had been flushed from their vascular systems by intracardiac infusions of heparinized 10\% dextran in saline. The microvasculatures of submandibular and mesenteric nodes were stained in \textit{vivo} by direct injection of alcian blue dye into carotid and superior mesenteric arteries.

\subsection*{Preparation of Nodes Perfused With Alcian Blue}

Rats were killed by cervical dislocation immediately after perfusion. The regional lymph nodes were excised and fixed in 3\% glutaraldehyde, 0.1 M cacodylate at pH 7.3 for 18 hours. These nodes were sectioned at 150 \(\mu\) on a Smith-Farquhar tissue chopper, and the sections were cleared overnight in 100\% dimethyl sulfoxide. Cleared slices were mounted in glycerine and examined by light microscopy. Detailed microvascular tracings were made using a Leitz Prado microslide projector, and vascular structures were photographed with a Zeiss photomicroscope. After selecting specific blood vessels for ultrastructural studies, these tissue slices were removed from their temporary mounts. Sites containing
selected vascular structures were excised, washed in 0.1 M cacodylate, and prepared for electron microscopy.

Techniques for Studying Vascular Permeability

Several different tracer materials were used to evaluate vascular permeability. The colloidal carbon was a shellac-free, nontoxic suspension (Gunther Wagner, Hanover, Germany) which had been heated to remove phenol, filtered, and diluted in Hanks' solution to a final concentration containing 25 mg of carbon/ml. The other tracers employed were 0.05% horseradish peroxidase (Type II, Sigma Chemical Co., Saint Louis, Mo.) in Hanks' solution, and stabilized colloidal thorium dioxide (Thorotrast, Fellows Testagar, Detroit, Mich.). These agents were administered by regional arterial perfusion or direct injection into afferent lymphatics of the mesenteric node. The distribution and possible exchange of these tracers between blood and lymph were evaluated by standard ultrastructural techniques. In studies using horseradish peroxidase, reaction product was developed using the dianinobenizidine tetrahydrochloride–hydrogen peroxide methods described by Graham and Karnovsky. 13

Preparation of Normal Lymph Nodes

Several methods were used to correlate vascular anatomy with histologic organization of the nodal parenchyma. Some nodes were fixed in 10% formalin and studied in routine histologic sections stained with hematoxylin and eosin. Other nodes were snap-frozen in liquid nitrogen, and 4 µ cryostat sections were stained with aldehyde fuchsin to localize elastic tissue. Glutaraldehyde-fixed lymph nodes were examined by electron microscopy and by light microscopic study of 1 µ sections stained with toluidine blue.

Electron Microscopy

Lymph nodes were minced in a drop of cold 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 and then placed in fresh fixative for 2 to 4 hours at 4 C. After washing with 3% sucrose in 0.1 M cacodylate, these tissues were postfixed in 1% osmium tetroxide in Millonig's buffer at pH 7.2. These tissue fragments were washed in 70% alcohol, dehydrated through graded alcohols to toluene, and embedded in araldite. One-micron sections were cut with glass knives on a Sorvall MT-1 and stained with toluidine blue. Thin sections were cut at 600 A with a diamond knife on a Sorvall MT-2 ultramicrotome and mounted on 200-mesh copper grids. Thin sections were stained with uranyl acetate and or lead citrate and examined at magnifications ranging from 1 6 X 10^9 to 63 X 10^9 on an AEI 801 electron microscope.

Results

Selection of Optimal Perfusion Technique

Microscopic examination of cleared slices demonstrated that regional perfusion with alcian blue dye stained the endothelial lining of lymph node blood vessels (Figures 1–3). The distribution and characteristics of this staining varied with different perfusion techniques. Rapid injection of this cationic dye into the arterial circulation caused erythrocytic sludging and the formation of granular precipitates in the vascular lumen. These occluded many nodal vessels and prevented staining of capillary beds. However, these undesirable effects were minimized by slow injection rates (0.05 ml/min) where alcian blue produced uniform staining of the entire
nodal vasculature (Figure 1). Arteries were readily identified in these preparations since postexcisional contraction made these vessels appear more darkly stained than adjacent veins. High endothelial venules (HEVs) exhibited a characteristic “cobblestone” appearance in these cleared preparations, since alcian blue stained luminal, lateral, and basal surfaces of this endothelium (Figure 2). The nodal vasculature showed similar staining patterns after flushing the vascular system with dextran-saline solutions. While this technique prevented erythrocytic aggregation and precipitation of plasma proteins, it resulted in passive dilatation of the microvascular bed. Because of this loss of vasomotor tone and possible ultrastructural changes related to ischemic injury, dextran perfusion techniques were not used extensively in these experiments.

Alcian blue perfusion facilitated the demonstration of endothelial glyocalyx in routine electron micrographs (Figure 3). This could not be attributed to surface precipitation of denatured plasma proteins, since this amorphous layer was seen in nodal vessels after flushing plasma from the vascular lumens. Direct measurements showed that the thickness of this electron-dense endothelial coat varied from 490 ± 12 Å in capillaries to 1280 ± 108 Å in HEVs.

**Microvascular Patterns in Normal Lymph Nodes**

The vascular patterns observed in this study correlated closely with the histologic organization of the lymph node (Figure 1–3, Text-figures 1 and 2). Lobules of cortical lymphatic tissue were demarcated by fibrous trabeculae extending from the capsule towards the hilus. The blood vessels supplying each of these regions were designated as vascular units. In axillary lymph nodes from normal rats, lobules of cortical lymphatic tissue were aligned longitudinally and shared a common sinusoidal and medullary cord system. These nodules were further divided into primary, secondary, and tertiary nodules, and undifferentiated lymphoid parenchyma (Text-figure 2). Since this relatively simple organizational pattern was seen in all axillary nodes, these organs were selected for detailed study of the microcirculation.

One or two arteries (average diameter 50 to 70 μ) entered axillary nodes at the hilus. These arteries immediately divided into longitudinal branches which passed through the medulla. These vessels gave off branches which passed vertically through medullary parenchyma and supplied medullary capillary arcades through small side branches. Two or three of these vertically oriented arteries entered the base of each cortical lobule and continued arborizing as they linked with cortical capillary arcades (Figure 1). Some of these branches terminated in a small cluster of
Text-figure 1—Structure of the vascular units supplying lobules of lymph node cortex. This diagram was made by tracing the projected image of lymph node vasculature stained by alcian blue perfusion. Arteries are shown in black, arteriovenous communications are numbered (1–7), and high endothelial venules are designated by stippling.

arterioles and capillaries which formed basket-like plexuses around ger-
minal centers (Text-figure 1). While germinal centers appeared relatively
avascular, occasional capillaries and one to two thin arterioles were seen
within each follicle. These arterioles passed through the center of these
colloides and anastomosed with the sides of small arterial vessels which
arched around germinal centers. Many arteriovenous communications
(AVCs) were seen beneath the subcapsular sinus, where they formed loops
extending through the cortical capillary arcades and joined directly with
high endothelial venules (Figure 4). Some AVCs passed through fibrous
trabeculae and linked the vascular units of adjoining lymphatic lobules.
AVCs frequently appeared narrowed and stretched as they passed over
enlarging cortical nodules. Capillaries were randomly dispersed through
the deep cortex and formed anastomosing arcades beneath the subcap-
sular sinus. These drained into small venules lined by low endothelium.
Two to five of these postcapillary venules emptied into larger venules lined by high endothelial cells. These HEVs appeared randomly distributed in lymphatic lobules, and occasionally extended from beneath the subcapsular sinus to the medulla. Each main trunk received three to five branches lined with high endothelial cells and two to three branches lined with flat endothelium (Text-figures 1 and 2). These side branches freely anastomosed with each other and formed intricate venous plexuses in the deep cortex. The luminal diameters of these vessels progressively increased as HEVs neared the medulla, where these vessels merged into segmental veins lined by flat endothelium (Figure 2). Segmental veins coursed through the medulla and joined larger efferent vessels near the hilus. Focal constrictions were seen in venular walls at the junctions between segmental veins and at sites where these vessels joined larger veins deep in the medulla (Figure 5). Histologic findings described in a subsequent section of this report demonstrated that these constrictions...
were caused by contracted venous sphincters (VSs) (Figure 5). The rich capillary arcades surrounding medullary cords had a dual drainage system. Capillaries situated near the corticomedullary junction drained into small venules which extended into the cortex and joined with HEVs. The capillaries located deeper in the medulla emptied into venules which drained directly into segmental veins (Text-figure 3).

The pericapsular blood vessels formed a separate and distinct vascular bed which was not directly linked to the nodal microcirculation (Figure 6). When the capsule was viewed en face, parallel arteries and veins formed serpentine patterns as they coursed through this fibrous structure and followed capsular indentations overlying interlobular trabeculae. The

TEXT-Figure 3—This schematic diagram illustrates the vascular anatomy of the rat lymph node. The location of high endothelial venules (HEV) between arteriovenous communications (AVC) and venous sphincters (VS) provides a unique system for regulating blood flow in these venules. Cross-hatching indicates the distribution of lymphatic sinuses. Afferent lymphatics (AL), efferent lymphatics (EL), and germinal centers (GC) are shown in this drawing.
arteries branched into small arterolar twigs, which terminated in a rich capillary network spread out over the capsular surface. These capillaries drained into postcapillary venules which emptied into arborizing veins lined by low endothelium. AVCs were rarely seen in the capsule and surrounding adipose tissue. This relatively simple vascular pattern was quite similar to that seen in serosal surfaces and the mesentery.

Microstructure of Lymph Node Blood Vessels

Histologic and ultrastructural techniques were used to describe vascular morphology in normal and perfused nodes. The study of isolated vascular structures was facilitated by localizing blood vessels in cleared tissue slices and then examining these sites by electron microscopy. Conventional clearing agents such as xylene could not be used in these preparations since this resulted in the loss of membrane lipids, but clearing in dimethyl sulfoxide minimized these alterations.

Specialized Arterial Vessels

The arborizing arteries in rat lymph nodes showed the same structural features seen in other vascular beds. In addition, two types of specialized arterial vessels were found in nodes, and these deserve further comment. Germinal centers were surrounded by a cluster of small arterioles, capillaries, and veins. While some of these vessels passed radially for short distances into the substance of these follicles, germinal centers typically presented a relatively avascular appearance. One or two narrow arterial vessels entered the inferior pole and passed through the center of these follicles to anastomose in an end-to-side fashion with AVCs passing over the upper surface. Electron micrographs demonstrated that these vessels had structural characteristics of metarterioles (Figure 7). In cross sections, these vessels had luminal diameters ranging from 3 to 5 μ and were lined by one to two endothelial cells resting upon a thin basal lamina. This endothelium was surrounded by a discontinuous perivascular coat composed of smooth muscle cells or pericytes containing cytoplasmic microfibrils. These vessels were ensheathed by reticular fibers and cytoplasmic processes from reticular cells.

AVCs were found throughout the lymph node cortex and formed prominent arcades just beneath the subcapsular sinus. Aldehyde fuchsin stains demonstrated longitudinally oriented, elastic tissue fibers in these vessels. Ultrastructural studies showed that these AVCs resembled terminal arterioles (Figure 8 and 9). These vessels had luminal diameters ranging from 6 to 15 μ and were lined by three to five elongated endothelial cells. This endothelium was surrounded by a distinct basement
membrane and a simple muscular wall composed by a monolayer of smooth cells. Numerous vesicles were seen along the outer surface of these muscle cells. Focal discontinuities in the Schwann cell sheath were observed at sites where unmyelinated nerve fibers passed through the adventia and approximated the outer borders of smooth muscle cells (Figure 9). Varicosities were frequently found in these exposed nerve segments (Figure 10).

**Lymph Node Capillaries**

Rich networks of fenestrated and nonfenestrated capillaries were present in the cortex and medulla of normal rat lymph nodes. Fenestrated capillaries were lined by one to two endothelial cells. Fenestrae were present along segments of attenuated endothelial cell cytoplasm where they were covered only by basement membrane (Figure 11). Multiple processes extended from pericytes and caused focal indentations along the abluminal surfaces of endothelial cells. Basement membrane extended around the circumference of these vessels, but it did not separate pericytes from endothelial cells. These capillaries were surrounded by a thick sheath derived from reticular fibers. Nonfenestrated capillaries were lined by one to two endothelial cells with abundant cytoplasm (Figure 12). Numerous pinocytotic vesicles were dispersed in their cytoplasm, and complex interdigitations were observed at junctions between adjacent cells. This endothelium was surrounded by basement membrane and pericyte processes extended about some of these capillaries.

**Postcapillary Venules**

Capillaries drained into postcapillary venules through end-to-end and end-to-side anastomoses. These venules had luminal diameters ranging from 8 to 12 μ and were lined by two to four low endothelial cells. When compared with capillary endothelium, these cells showed a relative increase in their content of cytoplasmic organelles. Postcapillary venules were surrounded by a delicate basement membrane which extended onto outer surfaces of reticular cell plates at sites where these formed junctions with the abluminal surfaces of endothelial cells. A single layer of reticular cell plates and adventitial cells separated these venules from the adjacent interstitium (Figure 13).

**High Endothelial Venules**

Postcapillary venules emptied into specialized venules formed by polygonal endothelial cells and a complex reticular sheath. Mitotic figures were occasionally seen at these junctions when there was an abrupt tran-
tion from flat endothelium to cuboidal endothelial cells. The perivascular sheath showed progressive lamination as two successive layers of reticular cell plates formed about HEVs near these sites (Figure 13). These HEVs increased in size as they received additional vascular branches and passed towards the medulla. In proximal segments, these venules had luminal diameters ranging from potential spaces to 8 μ and were lined by four to six endothelial cells. Lumenal diameters measured 30 to 40 μ in dilated segments near the corticomedullary junction where these vessels were lined by fourteen to eighteen endothelial cells. Electron micrographs showed that most of these endothelial cells possessed abundant cytoplasm with faint electron density. Numerous free ribosomes, occasional polysomes, and sparse endoplasmic reticulum were seen in these cells. Their cytoplasm was dominated by a prominent Golgi apparatus. Each cell contained six to eight elongated mitochondria and two to three residual bodies with crystalline and globular osmiophilic inclusions. Typical Wiebel-Palade bodies were not seen in these endothelial cells, but one or two multivesicular bodies were usually found in their cytoplasm. These cells had large nuclei with condensed peripheral chromatin which contained ten to twelve nuclear pores and one to two nucleoli.

These endothelial cells formed a continuous monolayer lining HEVs. Adjacent cells were linked together by macular tight junctions located near their luminal and basal surfaces. Foot processes extended from basilar portions of these cells and formed an interlocking network along the abluminal surfaces of this endothelium. Some of these processes entered the perivascular sheath where they formed junctions with reticular cells. This endothelium rested upon a thin basal lamina which divided to cover external surfaces of reticular cell processes in the perivascular sheath.

HEVs were surrounded by a complex, reticular sheath which appeared to spiral about these vessels as they coursed across the cortex. This sheath was formed by two to three layers of overlapping cytoplasmic plates derived from reticular cells. Amorphous ground substance and a few collagen bundles separated layers of this sheath. Collagen bundles extended into the basement membrane covering the outer surfaces of these plates and formed focal attachments which individually linked each layer to the surrounding nodal parenchyma.

Numerous lymphocytes were present within the lumens and walls of HEVs. Some of these cells were attached to endothelial surfaces. Other lymphocytes appeared to be migrating through potential spaces between endothelial cells by insinuating themselves into gaps between macular junctions and following a tortuous path around interdigitating foot
processes. Occasional lymphocytes appeared to be contained within the cytoplasm of a single endothelial cell, but the majority of these lymphocytes were clearly migrating intercellularly. Flocculant deposits of basement membrane material covered the surfaces of lymphocytes crossing the basal lamina. Lymphocytes were seen between laminations of the reticular sheath and moving through gaps between overlapping reticular cell plates. Despite this evidence for intense cellular traffic from HEVs, there was no sign of extravasation of red cells or platelets from these venules.

While HEVs retained these basic structural characteristics as they progressively enlarged and passed across the cortex, definite changes appeared as these venules approached the medulla when they merged into segmental veins. There were no apical junctional complexes between adjacent high endothelial cells in these sites. This resulted in the formation of wide apical gaps which gave the endothelium a peg-like appearance. There was gradual transition from high to low endothelium at the corticomedullary junctions where high endothelial cells assumed a fusiform configuration. The laminated sheath surrounding HEVs terminated near these junctions.

**Segmental Veins**

Segmental veins drained centripitally through the medulla towards the hilum (Figures 5 and 14). These veins lacked muscular walls, but they did contain elastic fibers which stained with aldehyde fuchsin. Segmental veins appeared readily distensible, and their luminal diameters varied between 50 and 150 μ. Electron micrographs showed that these veins were lined by endothelial cells with ruffled borders, numerous vesiculatory processes, and pinocytotic vesicles. These cells contained numerous mitochondria and ribosomes. Adjacent cells were joined together by interlocking junctional complexes and rested upon a thin basement membrane. These veins were surrounded by sheaths containing cytoplasmic processes from reticulum cells and fibroblasts loosely organized within a collagenous matrix.

**Venous Sphincters**

Alcian blue perfusion studies showed segmental narrowings at junctions between converging segmental veins and at sites where these veins joined larger efferent vessels (Figure 5). Serial 1-μ sections through these sites demonstrated subintimal smooth muscle bundles surrounding each narrowed segment. Noncontracted venous sphincters were occasionally seen at these junctions in random sections of normal nodes (Figure 14). Electron
microscopy showed that variable numbers of smooth muscle cells 3-17 formed each sphincter (Figures 14-16). Unmyelinated nerves accompanied by Schwann cell processes passed through the adventia and approximated sphincteric smooth muscle bundles (Figure 15). Varicosities were observed in these nerve fibers near segments of smooth muscle membrane which contained pinocytotic vesicles.

**Topographic Relations Between Lymph Node Vasculature and Lymphatic Sinuses**

In some rats, the mesenteric node microvasculature was stained by regional perfusion with alcian blue dye, and dilute suspensions of colloidal carbon were injected into the afferent lymphatics. Cleared slices from these nodes were examined to define the relations between nodal blood vessels and lymphatic sinuses. Carbon particles clearly delineated the subcapsular, intermediate, and medullary sinuses in these preparations (Figure 17). The rich vascular arcades in the outer cortex closely approximated the subcapsular sinus and surrounded perforating branches extending from this sinus. No lymphatic vessels were seen within the relatively avascular germinal centers. In the deep cortex, the intermediate sinuses formed a complex, freely anastamosing network of lymph capillaries and larger channels which surrounded HEVs. Blood capillaries were randomly dispersed in the parenchyma about this lymphatic network. Large lymphatic sinuses passed centripetally through the medulla and joined with the subcapsular sinus at the hilus. The rich capillary networks surrounding medullary cords were in close proximity to these sinuses.

Examination of random thick sections and electron micrographs further emphasized the spatial associations between lymph and blood vessels within these nodes. Fenestrated and nonfenestrated capillaries in the superficial cortex were frequently found within .5 to 5 μ of the subcapsular sinus and its branches. Occasionally, proximal segments of HEVs extended into the cortex and lay just beneath the subcapsular sinus. At these sites, the walls of HEVs which juxtaposed the sinus were lined by low endothelial cells. In the deep cortex, HEVs were separated from lymph sinuses by reticular sheaths filled with small lymphocytes. Arterioles and venules usually coursed through the center of medullary cords, while fenestrated and nonfenestrated capillaries passed more superficially. At some sites, these capillaries were separated from adjacent sinus lining cells only by thin layers of collagen bundles and amorphous ground substance. However, direct anastomoses between blood capillaries and lymphatic vessels were not seen in these studies.

Although these lymphatic channels varied in size, they displayed a similar structure throughout the node. They were lined by a single layer of
endothelial cells containing elongate nuclei, numerous ribosomes and strands of RER, and cytoplasmic granules of varying size. No basement membranes were seen around these sinuses. The sinus endothelial cells formed a porous covering. Adjacent cells were joined together by focal, electron-dense junctional complexes which left patent junctions along the loosely overlapping cell borders. Lymphocytes and macrophages were frequently seen migrating between these intercellular gaps.

Neuropharmacologic Regulation of Lymph Node Microvasculature

Since these small axillary nodes were not suitable for conventional hemodynamic studies, regional perfusions with alcian blue dye were used to evaluate neuropharmacologic controls of this vascular bed. The nerves supplying axillary nodes were surgically interrupted in 10 rats. Alcian blue infusion demonstrated dilated cortical and medullary capillaries, widened AVCs, and a complete absence of contracted VSs in these nodes. Identical findings were observed in 6 rats where phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline, and French, Philadelphia, Pa.) was given intraperitoneally at dosages of 4 mg/kg body weight 30 minutes prior to dye infusion. The injection of 0.2 mg of levarterenol bitartrate (Levophed, Winthrop Laboratories, New York, N.Y.) into axillary arteries immediately prior to dye infusion resulted in minimal capillary staining, dilatation of AVCs, and constriction of numerous VSs. Similar changes were seen after regional perfusion of axillary nodes with 0.2 mg of epinephrine. None of these vasomotor effects could be reproduced in the nodal microvasculature of rats whose vascular system had been flushed with dextran–saline solutions.

Permeability of Lymph Node Vessels

Regional perfusion techniques were employed to study vascular permeability and possible exchange of tracer materials between blood and lymph vessels within the node. The distribution of these tracers was determined by macroscopic, light, and electron microscopic examination of nodes at sequential time intervals after perfusion.

Distribution of Tracers Following Intraarterial Injections

When lymph nodes were excised within 1 minute after intraarterial injections of horseradish peroxidase (molecular weight, 40,000), reaction product was found at intravascular and extravascular sites. With light microscopy, the lumens of all nodal vessels appeared uniformly stained by reaction product (Figure 18). Transudation of peroxidase occurred within focal areas of the capsule, superficial cortex, and medullary cords. Reac-
tion product appeared concentrated within reticular fibers and a con-
tinuous gradient of decreasing staining intensity was observed in fibers
coursing across the cortex. Reticular fibers extending into the laminated
sheaths of HEVs were darkly stained by reaction product, but there was
no apparent leakage of peroxidase from the lumens of these venules.
When these nodes were examined by electron microscopy, numerous
fenestrated capillaries were found at sites of transudation in the cortex and
medulla. Equally dense deposits of reaction product were present on both
sides of the fenestral membranes in these vessels. The adjacent reticular
fibers were darkly stained. Peroxidase activity was confined within the
lumens of nonfenestrated capillaries in the same areas. In HEVs, this
tracer extended from the lumen into intercellular spaces between adjacent
endothelial cells, but did not penetrate beyond basal junctional com-
pleses. Lymphocytes migrating through these intercellular spaces were
surrounded by reaction product.

In lymph nodes examined 5 to 10 minutes after regional arterial infu-
sions of peroxidase, reaction product was uniformly distributed along
reticular fibers of the cortex. Peroxidase activity was present within the
lumens and in tissue spaces about fenestrated capillaries. Nonfenestrated
capillaries showed staining of their lumens, intercellular clefts and base-
ment membranes. When reaction product was found in the adjacent inter-
stitium, numerous darkly staining pinocytotic vesicles were seen on the
abluminal endothelial surfaces of these nonfenestrated capillaries. The
reticular fibers and sheaths surrounding HEVs were stained by peroxidase.
Extravasation of tracer from these venules was seen only at sites where
lymphocytes had perforated the basement membrane and were in-
sinuated between endothelial and reticular cell processes. Horseradish
peroxidase appeared to bind on the surfaces of vesiculatory processes
which extended into lumen from endothelial cells in segmental veins.
Peroxidase was actively pinocytosed by this endothelium, but free reac-
tion product was not present in the surrounding interstitium. Similar sur-
face binding of peroxidase was not seen in other blood vessels in these
preparations.

Lymph nodes were examined by electron microscopy at 1 to 5 minutes
following injections of colloidal thorium dioxide (70 Å) into their regional
arteries. These particles were contained within lumens of arterioles, capil-
laries, and veins. In HEVs, this tracer penetrated between adjacent en-
dothelial cells to the level of the basal foot processes. Occasionally, these
particles extravasated from HEVs at focal sites where lymphocytes were
crossing the basal lamina and entering the reticular sheath.

Intraarterial perfusion with colloidal carbon (350 to 450 Å) produced
similar results. The carbon tracer was retained within the lumens of blood vessels in these nodes. Carbon particles were seen in gaps between endothelial cells in HEVs, but this tracer did not pass beyond basal, interendothelial junctions near the basement membrane. High endothelial cells endocytosed carbon particles at the luminal interface, but there were no signs of transendothelial transport of these carbon particles to the abluminal space during the 30-minute observation period used in these studies.

Distribution of Tracers After Intralymphatic Injections

In these studies, 0.02 to 0.05 ml of a solution containing 0.05% horseradish peroxidase and 0.05% trypan blue was injected into the subserosal lymphatics of the small bowel. Flow of these tracers into the mesenteric lymphatics was monitored by following the distribution of trypan blue staining with a dissecting microscope. Regional nodes were excised 1 to 5 minutes after entry of dye into their marginal sinuses. Peroxidase reaction product was found evenly distributed in the subcapsular and intermediate lymphatic sinuses in nodes excised within 1 minute of injection (Figure 19). Densely stained monocytic cells were seen in the buffer zone between the subcapsular sinus and the superficial cortex. Reaction product appeared to concentrate within reticular fibers in the outer cortex, and numerous, darkly stained fibers passed from these areas and extended into the reticular sheaths of HEVs. Reaction product was seen in intercellular spaces between endothelial cells and within the lumens of these HEVs. No peroxidase activity was found in the lumens of other blood vessels examined at this time interval. Nodes excised at 5 minutes postinjection showed a similar distribution of reaction product in lymph sinuses and reticular fibers. The lumens of arterial and capillary vessels contained peroxidase activity, but the intensity of staining was less intense than that observed in the wall and lumens of HEVs. A similar distribution of peroxidase reaction product was seen in nodes when the thoracic duct was ligated immediately prior to injection to prevent peroxidase recirculation via the efferent lymphatics.

Intralymphatic injections with dilute colloidal carbon stained subcapsular, intermediate, and medullary sinuses of the regional nodes. While this tracer appeared concentrated within these sinuses by light microscopy, ultrastructural examination demonstrated that carbon particles readily crossed the patent junctions of this sinus endothelium. Carbon particles were scattered in the nodal interstitium but did not penetrate the walls of arteries or capillaries. These tracer particles frequently appeared concentrated in the ground substance of the reticular sheaths surrounding
HEVs. Isolated carbon grains were occasionally seen in interendothelial spaces where lymphocytes were migrating across these venular walls.

**Discussion**

In the present study regional arterial infusions with alcian blue dye were used to stain the lymph node microvasculature *in vivo*. This technique avoided the irregular filling and overdistension of blood vessels produced by conventional perfusion methods. While other reports indicated that this cationic dye combined with negatively charged proteins and carbohydrates at physiologic pHs, the endothelial cell components stained by this dye remained uncertain. This study demonstrated that alcian blue (8 GS) precipitated plasma proteins within vascular lumens. While this precipitate may have facilitated the demonstration of vascular surfaces in cleared tissues, this mechanism could not explain the endothelial staining seen after flushing plasma from the vascular bed with dextran-saline solution. Since endothelial surface coat appeared as an electron-dense layer in ultrastructural studies of these nodes, it seemed likely that alcian blue dye bound directly to negatively charged radicals in the glycocalyx of these cells. This binding probably preserved endothelial surface coat and enhanced its staining by osmium and the other stains for electron microscopy.  

Previous studies established that the vascular pattern in lymph nodes closely corresponded to the histologic organization of the nodal parenchyma. Early anatomists described a system of progressively arborizing arteries and veins which radiated from the hilus and supplied capillary beds in the medullary cords, cortex, and pericapsular adipose tissue. While this general distribution of nodal vessels has been widely accepted, there has been prolonged controversy over the anatomy of the cortical microcirculation. Calvert considered individual lymph follicles as the basic structural units of the cortex, and described a separate, ramifying blood supply within each nodule. This concept became untenable when subsequent studies showed that the cortex formed a continuous layer diffusely populated with lymphocytes, which was divided into irregular lobules by fibrous trabeculae extending in from the capsule. Several reports clearly demonstrated that the number and structure of follicles interspersed within these lobules varied with antigenic stimulation. Dabelow provided the first accurate description of the cortical vasculature and the variations induced by antigenic challenge. His perfusion studies indicated that the lymph node cortex appeared to be formed by wedge-shaped lobules which corresponded to the vascular bed supplied by a single artery entering at the corticomedullary junction. The ap-
pearance of this vascular bed altered following regional stimulation with bacterial antigens. Dabelow observed clusters of capillaries forming glomerulus-like structures in developing primary nodules and suggested that these matured into secondary follicles with relatively avascular centers. Recently, Herman et al. defined similar vascular patterns within cortical lobules demarcated by septal trabeculae and described sequential changes in these vascular units as germinal centers dissolved and reformed in nodes stimulated with Salmonella antigen.

Several reports emphasized that the venous system in lymph nodes possessed distinctive characteristics. Heudorfer demonstrated that arteries and veins followed separate paths as they coursed across the nodal parenchyma. Thomé first described the high endothelium within lymph node venules. Perfusion studies by Schnlze indicated that this high endothelial lining was found within postcapillary venules of the nodal cortex, and this anatomic location was generally accepted in subsequent studies of these vessels. Although some reports indicated that many postcapillary venules in cortical beds were lined by flat endothelium, this observation was attributed to flattening of high endothelial cells in venules distended by perfusion.

The general vascular pattern seen in these normal rat lymph nodes was similar to that described by other investigators. Lymphatic lobules were partially demarcated by fibrous trabeculae and these formed the major subdivisions of the cortex. Each lobule was supplied by one to two small arteries entering at the corticomedullary junction. These arteries branched as they crossed the cortex and terminated in cortical capillary arcades. This study clearly demonstrated that capillaries drained into small venules lined with low endothelium, and these subsequently joined with HEVs. Each lobule was drained by two to three HEVs, and side branches from these venules formed a freely anastomosing plexus in the deep cortex. Since capillaries were never seen connecting directly with venules lined by high endothelial cells, the term high endothelial venule (HEV) was used to identify these specialized vessels in this study. Medullary cords were supplied by small arterial branches which terminated in rich capillary arcades. Some of these capillaries drained directly into medullary veins, and others emptied into small venules which passed into the deep cortex and linked with HEVs.

The presence of collateral circulation between lymph nodes and the surrounding adipose tissue has been discussed repeatedly. Frey reported that lymph nodes received a dual blood supply formed by vessels entering at the hilus and others which penetrated through the capsule. Subsequent vascular perfusion studies reportedly demonstrated that
some branches from the hilar arteries passed through the cortex and terminated in capillary beds within the perinodal fat. Dabelow's suggestion that these vessels provided one means for diverting blood away from the cortical capillary beds has never been substantiated. Recent descriptions of the nodal microvasculature made no mention of possible vascular connections between the node and surrounding tissue. We were unable to demonstrate communicating branches in this study. Although occasional vessels appeared to extend between the nodal parenchyma and pericapsular fat in the microscopic examination of cleared tissue slices, this was an artifact caused by the depth of focus. When the continuity of these vessels was traced by careful focusing, it was apparent that these arteries and veins were merely following the curvature of the lymph node surface. Experiments indicating that lymph nodes survived after occlusion of the main nodal artery can probably be explained by rapid development of collateral circulation at the capillary level.

Previous studies have provided little information on structures which regulate regional blood flow in lymphatic tissues. Most investigators concluded that capillary blood flow in lymph nodes was determined by contraction of pericapillary arterioles and precapillary sphincters described in other vascular beds. Dabelow suggested that AVCs might redistribute blood flow within the node, but he was unable to provide an unequivocal demonstration of arteriovenous anastamoses in his preparations. Although some reports described AVCs within mesenteric lymph nodes, these findings were not substantiated by recent studies of the nodal vasculature. This difficulty in demonstrating these shunts within lymph nodes by routine perfusion techniques can probably be attributed to postmortem constriction of AVCs which expells the injected tracer materials. In the present study, in vivo injections of alcian blue dye demonstrated numerous AVCs in the lymph node cortex. These vessels directly linked the arterial circulation with low endothelial venules interspersed between capillary beds and HEVs. These AVCs contained longitudinal bundles of elastic tissue which probably permitted these vessels to maintain their integrity as they were stretched and displaced by expanding germinal centers. Electron micrographs showed that these vessels possessed the typical structural characteristics seen in AVCs at other sites. No myoneural junctions were seen in these vessels, but segments of unmyelinated nerve fibers containing varicosities closely approximated smooth muscle cells in the walls of AVCs. The Schwann cell sheaths were interrupted at these sites, providing an innervation pattern similar to that described in other blood vessels. The structure and distribution of these
vessels indicated that AVCs contributed in regional hemodynamic controls by shunting blood around the cortical capillary network.

Little is known about the regulation of venous blood flow in lymph nodes. Other studies have shown that venous tone and segmental contraction or dilation of veins influenced efferent blood flow in many vascular beds. If similar mechanisms are operative in rat lymph nodes, they must be restricted to large veins near the hilus, since the walls of postcapillary venules, HEVs, and segmental veins lacked smooth muscle. The existence of specialized sphincters for regulating venous blood flow has been debated repeatedly. Recent studies demonstrated venous sphincters about terminal branches of portal veins in the monitor lizard, but there have been no convincing descriptions of similar sphincters in other vascular beds. In the present study, venous sphincters composed of subintimal bundles of smooth muscle were found at sites where segmental veins joined with other medullary veins. These sphincters were seen in varying states of contraction within normal nodes, and ultrastructural studies indicated that these smooth muscle bundles were innervated by denuded segments of unmyelinated nerve fibers. The presence of these sphincters in the terminal ends of segmental veins suggested that these structures provided a regional mechanism for regulating pressure and flow of efferent blood from cortical lobules and the adjacent medullary cords. The walls of segmental veins contained elastic tissue, and their luminal diameters varied from 50 to 70 \( \mu \) when venous sphincters were open to more than 150 \( \mu \) in vessels where sphincters were contracted. These findings indicated that segmental veins might serve as capacitance vessels to accommodate the volume and pressure changes produced by contraction of venous sphincters.

Victor reported that the oxygen consumption of axillary lymph nodes in normal mice was fifteen to twenty times greater than that observed in resting skeletal muscle. The nodal vascular supply appeared well adapted to meet this nutritional demand since blood flow measurements demonstrated relatively high flow rates in these tissues, which could be greatly augmented by the local accumulation of metabolites. However, there is little information on the neuropharmacologic controls of this vascular bed. Several investigators have shown that both myelinated and unmyelinated nerves follow the distribution of blood vessels within lymph nodes. Lundgren and Wallenten found that sympathectomy and acetyl choline infusions increased blood flow in mesenteric nodes. Stimulation of sympathetic nerve fibers caused an initial phase of vasoconstriction followed by falling resistance in this vascular bed sug-
gestive of blood-shunting through arteriovenous pathways. The present studies revealed microvascular changes which were entirely consistent with these quantitative measurements of blood flow in lymph nodes. Regional denervation and α-adrenergic blockade with phenoxybenzamine resulted in dilatation of AVCs, VSs, and all capillary beds. Infusions with pharmacologic dosages of epinephrine and norepinephrine into the regional circulation caused shunting of blood away from capillary beds through widened AVCs and constriction of numerous VSs. While these results suggested that the muscular tone of precapillary sphincters, AVCs, and VSs were influenced by sympathetic nerves and catecholamines, further studies are needed to define the neuropharmacologic mediators which regulate these structures under physiologic conditions.

HEVs have been generally accepted as specialized blood vessels which serve as the major site for entry of recirculating lymphocytes into lymph nodes. While most investigators assumed that blood flow through HEVs was determined by capillary flow, the present study demonstrated that HEVs were situated between AVCs and VSs. These findings suggested that blood flow within HEVs could vary from rapid flow to complete stasis. The regional hemodynamic controls provided by these specialized microvascular structures could certainly influence fluid and cellular exchange from HEVs. While these venules lacked elastic tissue in their walls, they did exhibit structural characteristics which might minimize fluid leakage due to elevated venous pressure or lymphocyte emigration.

The light and electron microscopic appearance of high endothelial cells noted in the present study was quite similar to that described by other investigators. These polygonal cells possessed abundant cytoplasm and measured 10 to 15 μ in height. Adjacent cells were linked together by discontinuous junctions located near the luminal and basal surfaces, and by a complex network of interlocking basal foot processes. Flattening of high endothelial cells has been observed within congested HEVs in normal lymph nodes. Recent studies in this laboratory showed that distension of these venules during perfusion fixation caused overlapping margins of these endothelial cells to flatten and seal intercellular spaces. These findings suggested that high endothelium was specially constructed to maintain vascular integrity during venous distension. Ultrastructural observations by Marchesi and Gowans were interpreted as showing that lymphocytes emigrated from HEVs by passing directly through the cytoplasm of endothelial cells. However, other investigators reported that lymphocytes moved through intercellular spaces between adjacent endothelial cells. Examination of random electron micrographs in the present study supported this intercellular route of migration.
Dabelow and Schoefl suggested that the soft, easily deformable cytoplasm of high endothelial cells closed about the surfaces of these migrating cells and minimized vascular leakage at sites where lymphocytes emigrated from HEVs. The intimate associations between endothelial cells and migrating lymphocytes observed in the present study were entirely consistent with this thesis.

Several studies have shown that HEVs were surrounded by a complex perivascular sheath infiltrated with lymphocytes. However, there have been no reports on the detailed structure of this sheath. In the present study, serial electron micrographs showed that this sheath was composed of two to three layers of overlapping reticular cell plates. These were linked to the reticular meshwork of the node by collagen bundles which attached to the external surface of each plate. This laminated sheath surrounded HEVs throughout their entire length and terminated abruptly at proximal and distal ends of these vessels. Numerous lymphocytes were seen migrating through potential spaces between layers of this sheath. These cells moved radially across successive layers of the laminated sheath by insinuating themselves into gaps between overlapping reticular cell plates. This unique structure appeared to provide vascular support without impeding the movement of lymphocytes into the adjacent cortex. The possible roles of this sheath in regulating vascular permeability and providing conduits for the movement of lymphocytes across the cortex are currently being investigated in this laboratory.

Direct exchange of fluid, proteins, and cells between blood and lymphatic vessels within the node has been debated repeatedly. Previous studies demonstrated altered vascular permeability in antigen-stimulated nodes which resulted in the extravasation of blood cells and dye particles into lymph sinuses, and increased lymph flow through the efferent lymphatics. The evidence for similar exchange in normal nodes is more controversial. Several investigators suggested that blood capillaries drained directly into lymph sinuses of hemal lymph nodes. However, recent studies indicated that these were specialized nodes found in retroperitoneal areas, which lacked afferent lymphatics. Shulze concluded that fluid and cells could be exchanged between blood and lymph through "stomata" in the walls of HEVs within normal lymph nodes, but subsequent experiments showed that his observations were based upon perfusion artifacts. Other reports indicated that tracers infused under pressure into the afferent lymphatics could be recovered in venous blood draining from the node. These results were attributed to valve-guarded connections between lymph sinuses and blood vessels, but these investigators were unable to demonstrate flow of tracer particles through
these sites in histologic sections. While no convincing demonstrations of anastomoses between lymphatics and blood vessels within the nodes have been reported, there is evidence indicating that functional lymph node–venous communications exist.47,48

The present study demonstrated that lymph sinuses closely approximated blood vessels within the cortex and medulla. Other reports concluded that fenestrated capillaries were not present in lymphatic tissues, but typical examples of both fenestrated and nonfenestrated capillaries were observed in these rat lymph nodes.

While there was no apparent selective distribution of these vessels at different nodal sites, fenestrated capillaries were frequently found in the superficial cortex near the marginal sinus. The close proximity of these capillaries to lymph sinuses lined by porous endothelium could facilitate fluid exchange between blood and lymph. However, no evidence for direct anastamoses between blood vessels and lymph sinuses was seen during the microscopic examination of cleared slices and tissue sections prepared from 80 nodes perfused with tracer materials.

When the permeability of nodal vessels was studied by extraarterial injections of colloidal carbon or Thorotrast, most of these tracer particles were retained within vascular lumens. A few particles penetrated intercellular spaces between high endothelial cells, but extravasation of these tracers from HEVs was seen only at focal sites where lymphocytes were insinuated across the basement membrane. These findings supported the concept that high endothelial cells minimized vascular leakage during lymphocytic emigration.55,60 Intrarterial injections with horseradish peroxidase resulted in prompt transudation of this tracer in the cortex and medullary cords. Since Wistar rats were employed in these studies, the leakage of this tracer could not be attributed to altered vascular permeability induced by horseradish peroxidase.60 This tracer appeared to pass through fenestrated capillary membranes and enter the nodal interstitium where it concentrated in reticular fibers. Within 1 minute after injection, these fibers showed a continuous gradient of decreasing staining intensity as they extended from these sites across the cortex. By 5 minutes, all reticular fibers were uniformly stained, and peroxidase activity was concentrated in the reticular sheaths surrounding HEVs. These findings suggested that reticular fibers might serve as conduits for conducting this tracer across the cortex. The possible transport of macromolecules in this manner is not without precedent. Fraley and Weiss described similar movement of Thorotrast particles along collagen bundles in the rat diaphragm and postulated that these fibers functioned as "wicks" for transporting solutes and colloidal particles. Since peroxidase did not leak from
the lumens of HEVs, concentration of peroxidase within the sheaths surrounding these venules indicated that this tracer might pass through these vascular walls to reenter the bloodstream. This concept was supported by demonstrating that peroxidase given by intralymphatic injections flowed through these venular walls and entered the lumens of HEVs. A similar pattern of transudation from cortical capillaries and reabsorption in HEVs was suggested in tracer studies reported by Fukuda. In addition, peroxidase-positive vesicles were seen concentrated on the abluminal surfaces of nonfenestrated capillaries in the present study. This finding suggested that peroxidase might be transported back into the blood by active pinocytosis in capillary endothelial cells.

Intralymphatic injections showed that both carbon and Thorotrast particles readily crossed the porous endothelial lining of lymph sinuses. Endocytosis and transport of these extravasated particles by vascular endothelium was not seen in these acute experiments, but this sequence of events has been observed at later time intervals by other investigators. Injections with peroxidase resulted in passage of this tracer from lymph sinuses into reticular fibers and through intercellular spaces in the wall of HEVs to enter these venular lumens. There was no evidence for similar flow of this tracer into other nodal vessels. The luminal staining of HEVs was unaltered by thoracic duct ligation and could not be attributed to recirculation of this tracer via the efferent lymphatics. These observations supported Fukuda's suggestion that the walls of HEVs may serve as functional lymph node-venous communications in normal animals. The transport of macromolecules at this site could certainly be influenced by unique system of regional hemodynamic controls seen in this vascular bed. Fukuda postulated that antibodies produced in the node might pass directly into the blood stream at this site. This may be correct, but it is also possible that exchanges between lymph and blood in the wall of HEVs could contribute in regulating lymphocyte migration.

Recent studies have shown that lymphatic nodules were constituted by clones of marrow-derived lymphocytes producing antibody to single antigens. The vascular supply within these nodules varied during different developmental stages. Primary nodules composed by tightly packed small and medium lymphocytes and macrophages were supplied by clusters of capillaries interspersed within each nodule. There has been considerable controversy over the origin and distribution of blood vessels in mature germinal centers. In the present study, these follicles were surrounded by a basket-like plexus of capillaries, venules, and arterioles passing through the marginal zone. This appearance could readily be explained by the displacement and condensation of existing cortical vessels.
as these nodules expanded by cellular proliferation. The mid-portions of these follicles appeared relatively avascular. Typical, metarterioles passed through the center of these follicles, but relatively few capillaries were seen at these sites. Several investigators have suggested that extensive fluid transudation occurred from blood vessels located near the center of these follicles. There was no evidence of peroxidase leakage at these sites in the present study. This tracer transuded from capillaries in the cortex and mantle zones and gradually flowed towards the centers of these follicles along reticular fibers. Kojima and his colleagues reported a similar centripetal flow of tracer materials into germinal centers. Further studies will be required to determine whether the scanty vascular supply in the central regions of mature follicles correlates with diminished lymphocytic proliferation or prolonged sequestration of antigen of these sites.

References
LYMPH NODE MICROVASCULATURE

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Figure 1—Cleared section from an axillary lymph node, where the microvasculature was stained in vivo by regional perfusion with alcian blue dye. Arteries (A) exhibit dense staining produced by postexcisional contraction. Arteriovenous communications (AVC), metarterioles (MA), cortical and medullary capillary arcades (arrows), and high endothelial venules (HEVs) are shown in this preparation. (× 47) Figure 2—Multibranched high endothelial venules (HEVs) display "cobblestone" luminal contours in these cleared sections. The gradual transition to smooth lining is at the junction with a segmental vein (S). (× 250) Figure 3—An amorphous, electron-dense glycocalyx (GL) is seen on the surface of this high endothelial cell (EN) from a node where the vasculature was stained by regional perfusion with alcian blue. (Lead citrate, × 56,000)
Figure 4—This cleared section from an alcian blue perfused node shows an arteriovenous communication (AVC) between and artery (A) and a high endothelial venule (HEV) (× 220).

Figure 5—A focal constriction is shown near the terminal end of a segmental vein in this cleared section (× 200). Serial 1-μ sections through this site demonstrated a sphincter (VS) composed of circumferential smooth muscle bundles (see inset) (Inset, toluidine blue, × 625).
**Figure 6**—This *en face* view shows the appearance of the pericapsular vascular bed in cleared sections (x 25).

**Figure 7**—This metarteriole in a germinal center is surrounded by a pericyte process containing microfibrils (MF) (Lead citrate, x 10,000).

**Figure 8**—This arteriovenous communication in the lymph node cortex is surrounded by smooth muscle cells (Sm) and a monolayer of adventitial cells. Nerve fibers (N) are seen near the adventitia of this vessel. (Lead citrate, x 2200)

**Figure 9**—Unmyelinated nerve fibers (U) cross the adventitia and approximate smooth muscle cells in the wall of this arteriovenous communication. In this electron micrograph, the unmyelinated fiber is not surrounded by Schwann cell processes. (Lead citrate, x 15,000)

**Figure 10**—A varicosity (V) is shown in this unmyelinated nerve fiber beneath a focal discontinuity in the Schwann cell sheath (Sc). This bare nerve segment faces smooth muscle cells in the wall of an arteriovenous communication. (Lead citrate, x 45,000)
**Figure 11**—This electron micrograph shows a fenestrated capillary (F) in the lymph node cortex. Pericytes indent the abluminal surface of endothelial cells. The capillary is ensheathed by a thick mat of collagen (C) and ground substance. (Lead citrate, × 10,000)

**Figure 12**—The appearance of nonfenestrated capillaries in the lymph node cortex 10 minutes after intraarterial infusion with horseradish peroxidase (HRP). Reaction product (arrows) surrounds an erythrocyte (RBC) in the capillary lumen and is contained within pinocytotic vesicles in endothelial cells. (Unstained section, × 10,000)

**Figure 13**—This electron micrograph shows the abrupt transition from low to high endothelium at the junction between a postcapillary venule (PCV) and a high endothelial venule (HEV) (Lead citrate, × 6500).
Figure 14—This longitudinal section through a segmental vein shows circumferential bundles of subintimal smooth muscle in an open venous sphincter (VS) (Toluidine blue, × 440).

Figure 15—This electron micrograph shows a Schwann cell (SC) associated with four unmyelinated nerve fibers (U) lying within the adventitia adjacent to a smooth muscle cell (SM) of a venous sphincter (Lead citrate, × 20,000).

Figure 16—This electron micrograph demonstrates the detailed structure of the sphincteric smooth muscle bundle shown in Figure 17 (Lead citrate, × 5700).
Figure 17—This cleared section of a mesenteric node shows colloidal carbon within the subcapsular (Sub), intermediate sinuses (IS), and medullary sinuses (ms) following intralymphatic injection. The intermediate sinuses form a lymphatic plexus surrounding cortical nodules and high endothelial venules. (× 60)  

Figure 18—This unstained section shows the distribution of horseradish peroxidase activity in a mesenteric node 10 minutes after intraarterial injection. Note the intense staining within blood vessel lumina (BV) and relatively faint staining of intermediate sinuses and reticular fibers. (× 240)  

Figure 19—Following intralymphatic injections of horseradish peroxidase, intermediate sinuses (IS) and reticular fibers stain darkly with diaminobenzidine reaction product. Peroxidase activity is seen within the perivascular sheath, between endothelial cells, and in the lumina of high endothelial venules. (Unstained section, × 240)