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LIGHT AND SCANNING ELECTRON MICROSCOPY OF THE PORCINE MESOMETRIAL AND PARAOVARIAN LYMPHATIC NETWORKS

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ABSTRACT

Uterine lymphatics were examined in 38 pigs using light microscopy, stereomicroscopy, and scanning electron microscopy. Uterine lymphatics were initially filled with plastic materials: Microfil for stereomicroscopy, Mercox®-corrosion cast for scanning electron microscopy (SEM), and Micropaque for x-ray photographs. Lymph precollectors of the uterine horns formed two superficial layers, ventral and dorsal. At the level of the ovary, precollectors joined to become collector lymph vessels, which entered nearby lymph nodes. Among the lymph vessels emanating from the uterus, there was a characteristic band of lymphatics that bordered on the isthmus of the oviduct. These passed toward the ovary to form the paraovarian lymphatic plexus. Segments of collector lymphatics were longer than precollectors, had thicker walls consisting of endothelial cells, smooth muscle (uniformly forming a continuous band around lymphatics) and fibroblasts. Both precollector and collector lymphatics were covered and surrounded by a fine network of blood microvessels (vasa-vasorum) especially well seen in corrosion casts (SEM).

The lymphatic system apart from its role in transport of tissue fluid, proteins and immune cells has also been implicated as a potential site for transmitting hormonal information among neighboring tissues and organs (1). Daniel et al (2), for example, pointed out the role of efferent lymph vessels of endocrine organs (testis, ovary, adrenal gland, thyroid) in the transport of hormones. They stressed (2) that hormonal concentrations in regional lymph vessels were often higher than those in peripheral blood but lower compared with levels in venous blood directly draining the respective organ. Similar data were reported by Magness and Ford (3,4) for estradiol and estrone in lymph vessels of the porcine uterus.

Morphologic studies of the lymphatics of female reproductive organs have previously concentrated on the lymphatics of the ovary, uterus and oviduct. Few investigators have pursued lymphatics within the uterine broad ligament probably because these collector lymph vessels have thus far been poorly defined with respect both to structure and function. In this study, we examined the lymph vessels of the mesometrium in the pig using a combination of light microscopy, stereomicroscopy, and scanning electron microscopy (vascular corrosion casts) as a continuation of our earlier studies to better delineate the morphologic basis for the participation of lymphatics in the counter-current transfer of hormonal secretions between uterine-ovarian blood and lymph.

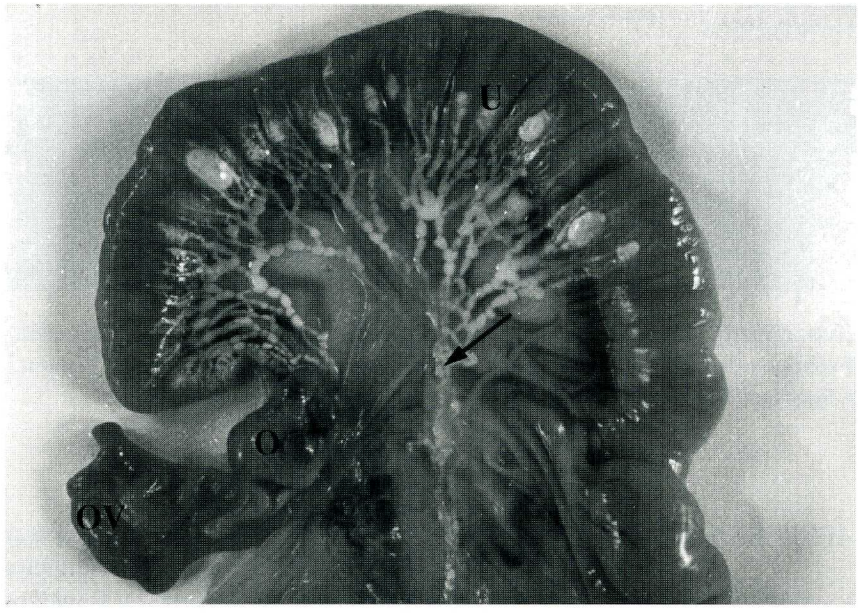

Fig. 1. Lymphatics draining the uterus filled with Microfil injected subserosally into the dorsal uterine wall. Arrows indicate sites where precollectors become collector vessels. U=uterus, O=ovary, OV=oviduct. x0.25

MATERIALS AND METHODS

Thirty-eight mature pigs (approximately 1 year old 90-120 kg BW) were studied. The reproductive organs including the broad ligament of the uterus, the adjacent segments of the ventral aorta and posterior vena cava were removed promptly after slaughter. The blood vessels of the reproductive organs were perfused via cannulae inserted into the ovarian and uterine arteries until the perfusate (physiologic saline with added heparin—30,000 IU/l at 37-39°C) washed out from the utero-ovarian veins was clear.

Filling of Lymph and Blood Vessels With Plastic (Microfil)

To highlight the lymphatic network, Microfil (Compound for Microvascular Injection—Flow Tek, Inc., Boulder, Colorado) was slowly interstitially injected to fill the lymph vessels emanating from the uterus. The injections were made along the uterine horn into the connective tissue of the subserosa at 1-2 cm intervals. Concomitantly, Microfil of a different color was instilled into the blood vessels of the broad ligament via the uterine and ovarian arteries. Filling of the blood vasculature was deemed sufficient when Microfil drained freely from the utero-ovarian vein. The tissues filled with Microfil were left for 2 hours at room temperature for solidification of the plastic followed by fixing and clearing in increasing glycerin concen-trations (50, 75, 85, and 100%; 24 h in each solution, respectively). Stereomicroscopy was used for subsequent observations and documentation.

Filling of Lymph Vessels With Barium (Micropaque)

Lymph vessels were filled with Micropaque (Nicholas Lab, Ltd., Slough SL1 4AU, England), similar to that described for Microfil (see above) and morphology assessed by plain x-rays.

Preparation of Corrosion Casts (Lymph and Blood Vessels) for SEM

The lymph and blood vessels were filled with Mercox resin (Vilne Comp., Ltd., Tokyo, Japan). To prolong polymerization, each

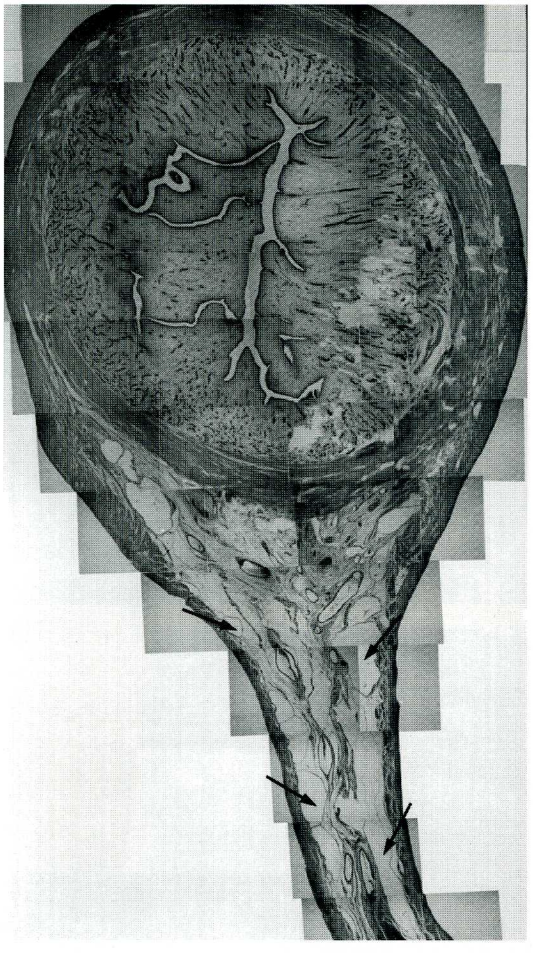

Fig. 2. Photographic montage of cross-section through the middle part of the uterine horn and mesometrium. Arrows show the dorsal and ventral bands of precollector lymph vessels (H&E x250).

resin was mixed with methyl methacrylate monomer and catalyst (4:1:0.1) (5,6). The blood vessels of the broad ligament of the uterus were also filled via the ovarian and uterine arteries. Injections were carried out manually at a flow rate of 5-7 ml/min. When resin flowed from the utero-ovarian vein, the arterial cannulae were sealed. In several specimens, blood vessels were also filled via the utero-ovarian vein. Lymphatics were filled after casts of the blood vasculature in the broad ligament were obtained (7). In this way, the network of smaller blood vessels and their connections with larger blood vascular trunks acted as a scaffold which protected lymph vessel segments from distortion and damage during corrosion cast processing. Lymphatics were filled with resin by slowly manually injecting the connective tissue of the uterine subserosa at 1-2 cm intervals, and after resin polymerization (~ 30 min), preparation was performed as previously described (7).

RESULTS

The most developed network of lymph vessels emanating from the uterus was seen after bilateral (dorsal and ventral) instillation of microfil into the mesometrium *(Fig. 1)*. The cross-sectional image of the middle portion of the uterine horn and its mesometrium *(Fig. 2)* confirm the presence of ventral and dorsal superficial layers of precollector lymph vessels. At the level of the ovary, precollector lymph vessels joined and became collector ducts which course towards nearby lymph node(s) *(Fig. 1)*. Lymph vessels exiting the uterine wall at the site bordering the isthmus of the uterine tube *(Figs. 3,5)* were particularly noteworthy. A single injection of resin was sufficient to fill 2-3 precollector lymph vessels. From there, lymphatics passed in parallel and formed a characteristic band *(Figs. 3-5)* near the oviduct. From there the band passed towards the ovary through an area of microvascular network of the ovarian ligament (*ligamentum ovarii proprium*) *(Fig. 3)*. A paraovarian lymphatic plexus (about 2 cm long), consisting of tightly packed bundles made delineation of individual lymph vessels extremely difficult *(Figs.4,5)*. The collector lymph vessels leaving this paraovarian plexus drained to nearby lymph nodes in close proximity to paraovarian blood vessels *(Fig. 5)*. Separate segments (lymphangions) (8) were partitioned by bicuspid valves *(Fig. 6)*. Segments of precollector lymph vessels close to the uterus and along the paraovarian lymphatic plexus were short (the width often

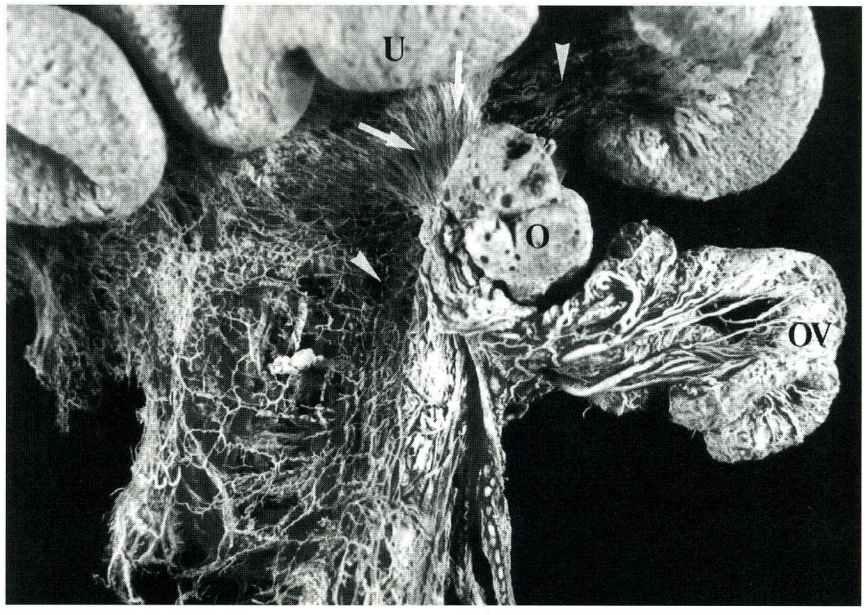

Fig. 3. Photograph of lymphatics (arrowheads) and blood vessels of ovarian ligament (ligamentum ovarii proprium) (arrows) in the reproductive organs of the pig. U=uterus, O=ovary, OV=oviduct. Corrosion cast, stereomicroscopy, x0.25.

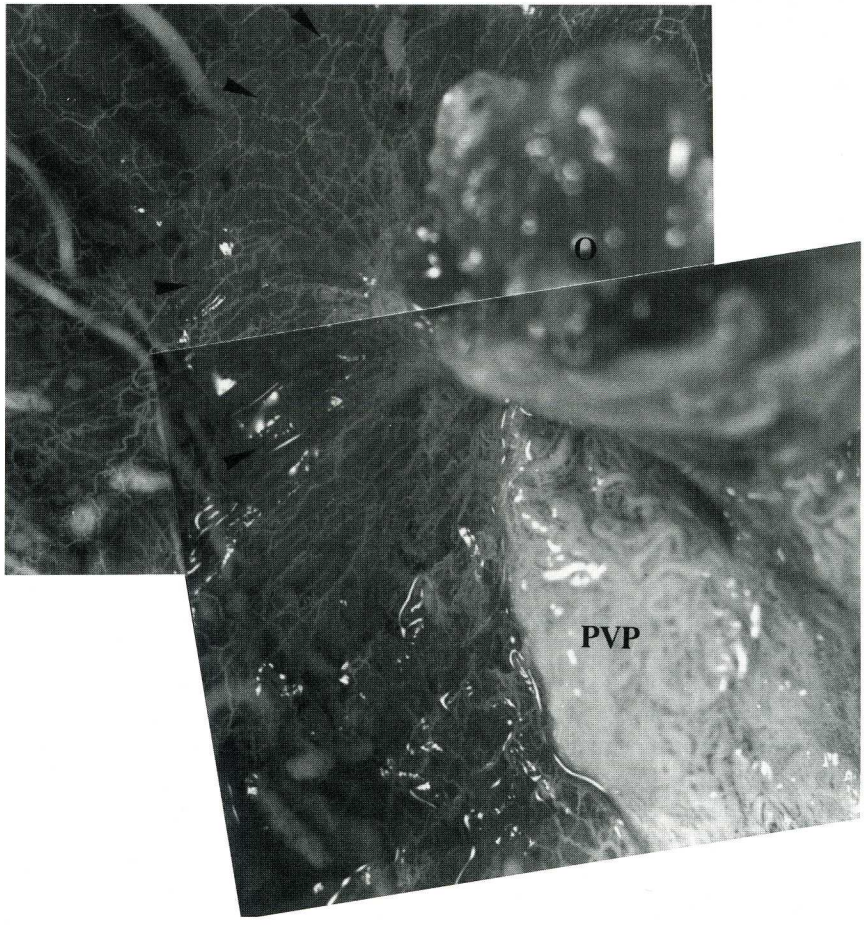

Fig. 4. Paraovarian lymphatic plexus showing lymph and blood vessels filled with Microfil of different colors. O=ovary, PVP=paraovarian blood vascular plexus; arrowheads point to the paraovarian lymphatic plexus. Stereomicroscopy, x1.25.

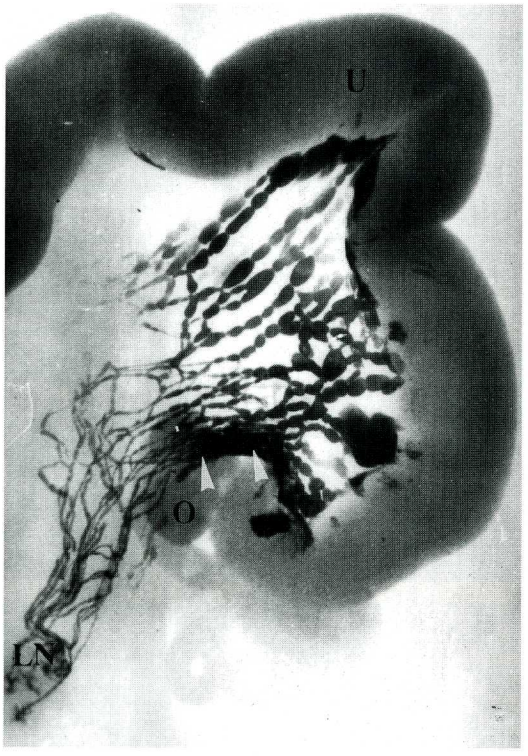

Fig. 5. Plain roentgenogram of the porcine uterus demonstrating the paraovarian lymphatic plexus after filling with Micropaque. U=uterus, O=ovary, LN=lymph node. Arrowheads point to the paraovarian lymphatic plexus. Micropaque, x0.25.

exceeded the length) or spheroid, whereas collector vessels were typically narrow and elongated *(Fig. 4)*.

Corrosion casts of the blood vessels and lymphatics of the broad ligament further clarified the topographic relationship of the two vasculatures. Lymphatic segments, both precollectors and collectors, were densely intertwined with small blood vessels *(Figs. 7-9)*.

DISCUSSION

These morphologic features extend earlier work (7,9-11) regarding the topography of porcine uterine lymphatic vessels. The broad ligament has been proposed as a site for hormonal regulation of reproductive processes (13), and it has even been suggested that the lymphatic system is pivotal to countercurrent transfer of these reproductive hormones (12-16).

Our studies on the structure of the uterine lymphatic network support the findings of others (1). Hunter et al (17) established two lymphatic plexi within the porcine broad ligament: the lymph plexus associated with the utero-tubal junction and the lymph plexus in the broad ligaments supplying the upper uterine horn. These workers, however, did not provide illustrative confirmation. Our studies document that lymphatics leaving the uterus near the oviduct form a prominent network (termed paraovarian lymphatic plexus) in the area of the ovarian ligament. This paraovarian lymphatic plexus is characterized by the presence of short, bulb-like, closely arranged segments. According to previously accepted nomenclature, these vessels have been classified as precollector lymph vessels, whereas lymphatics leaving the lymphatic plexus and draining towards pelvic lymph nodes are collector lymphatics. Both precollector and collector lymphatics are composed of segments separated by valves (i.e., lymphangions) similar to other uterine lymphatics. The paraovarian lymphatic plexus is enveloped by a dense network of small blood vessels (vasa lymphaticorum).

Physiological studies in sheep (16,18), cattle (19), and pig (12-15,20,21) have examined transfer of biologically active substances between the uterus and ovary. For example, in sheep, radiolabeled prostaglandin ($^3HF_{2\alpha}$) administered into uterine lymphatics was later located in ovarian corpus luteum (16). Similarly in cows, a high gradient of $PGF_{2\alpha}$ concentration between uterine lymph and ovarian arterial plasma suggested a local transfer of prostaglandin from uterus to ovary (19). Kotwica et al (14) further demonstrated that steroids infused into the musculature of the porcine uterine broad ligament (probably in the vicinity of the paraovarian lymphatic plexus) reached the

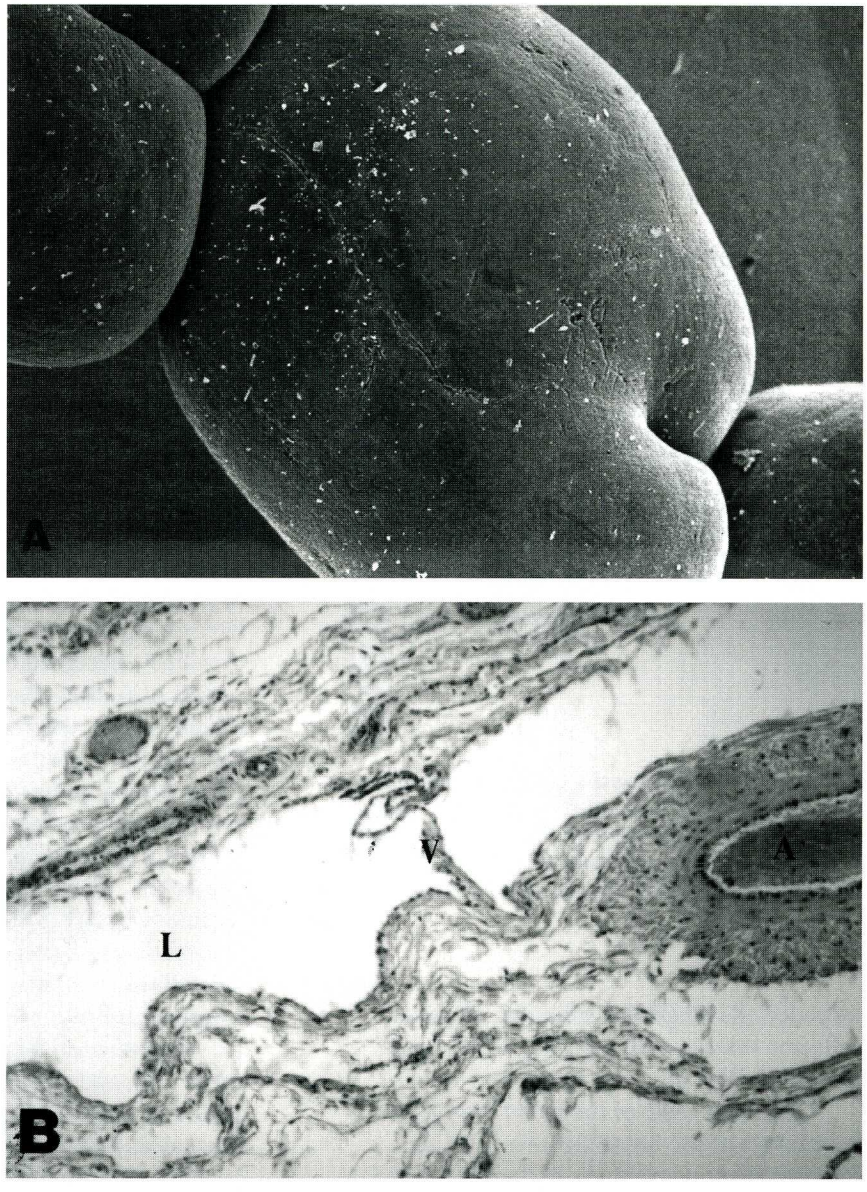

Fig. 6. A. Scanning photograph of precollector lymphatic segments. Deep notches produced by valves mark the ball-like shapes of the lymphangion (SEM x50). B. Thin-walled collector lymphatic vessel. Valve visible between two segments. L=lumen of the lymphatic, V=valve, A=artery, H&E x125.

bloodstream via the ovarian artery. Krzymowski et al (12) formulated a theory that steroids and prostaglandins diffused from lymph into arterial blood via capillaries of the broad ligament according to a "favorable concentration gradient" (first step). Subsequently, blood that flowed from these capillaries locally transported high concentration of these agents (hormones, prostaglandins) into a venous plexus encompassing the wall of the ovarian artery. Differences in hormone concentrations within these blood vessels and the ability of the vascular wall to bind these substances facilitated transfer into arterial blood (second step). Our morphologic studies demonstrating the presence of lymphatics leaving the uterus, joining with an intricate paraovarian

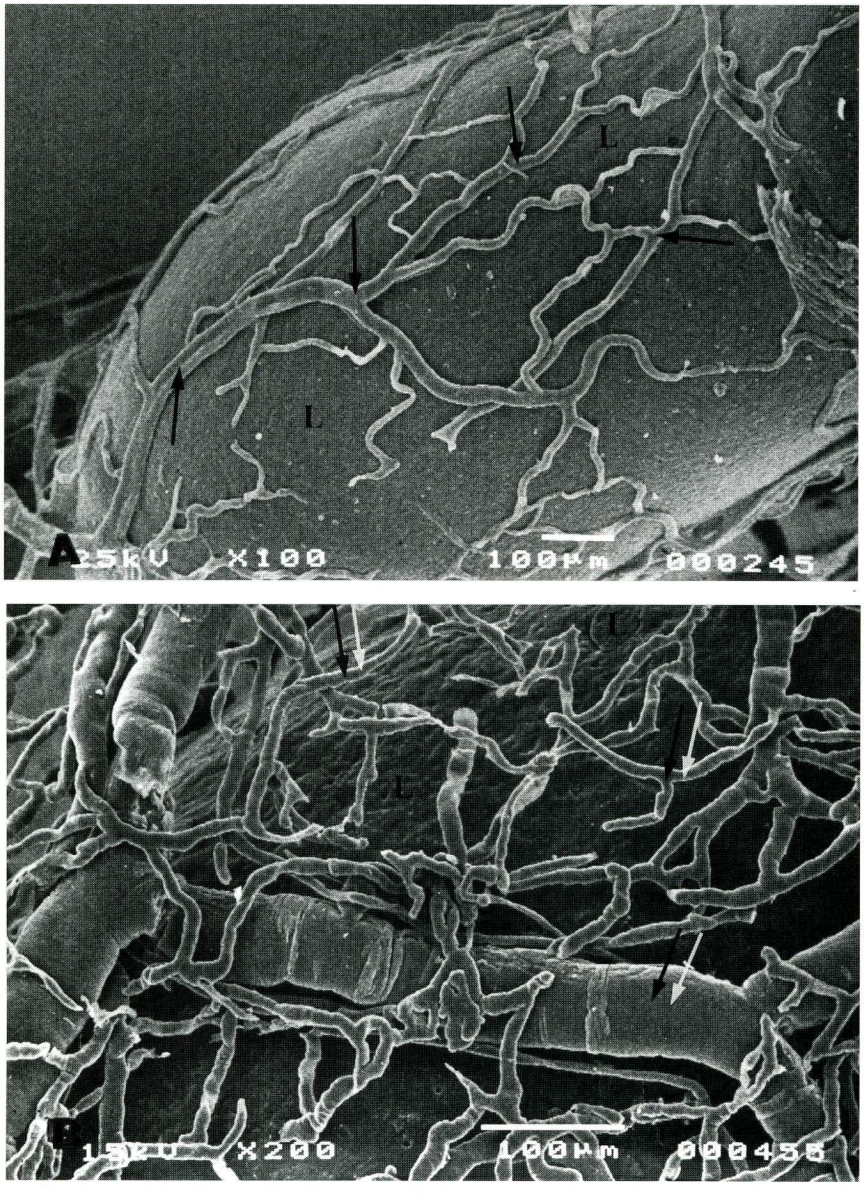

Fig. 7. Corrosion casts of lymphatics covered by microvascular blood network (vasa-lymphaticorum). L=lymph vessels, arrows indicate blood vessels. A=precollector lymph vessels, SEM, x100, B=collector lymph vessels, SEM, x200.

lymphatic plexus, prominence of a vasa-lymphaticorum, and structural characteristics of lymphatics themselves conform to this "countercurrent" secretory theory.

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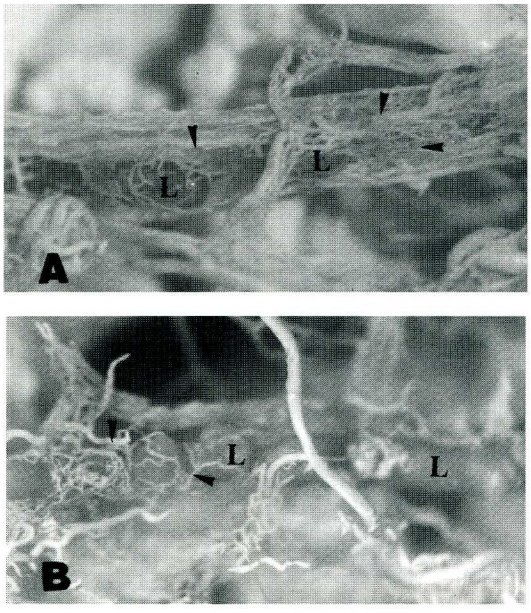

Fig. 8. Collector lymph vessels (L) wound around by small blood vessels (arrowheads). Corrosion cast, stereomicroscopy, x2.5.

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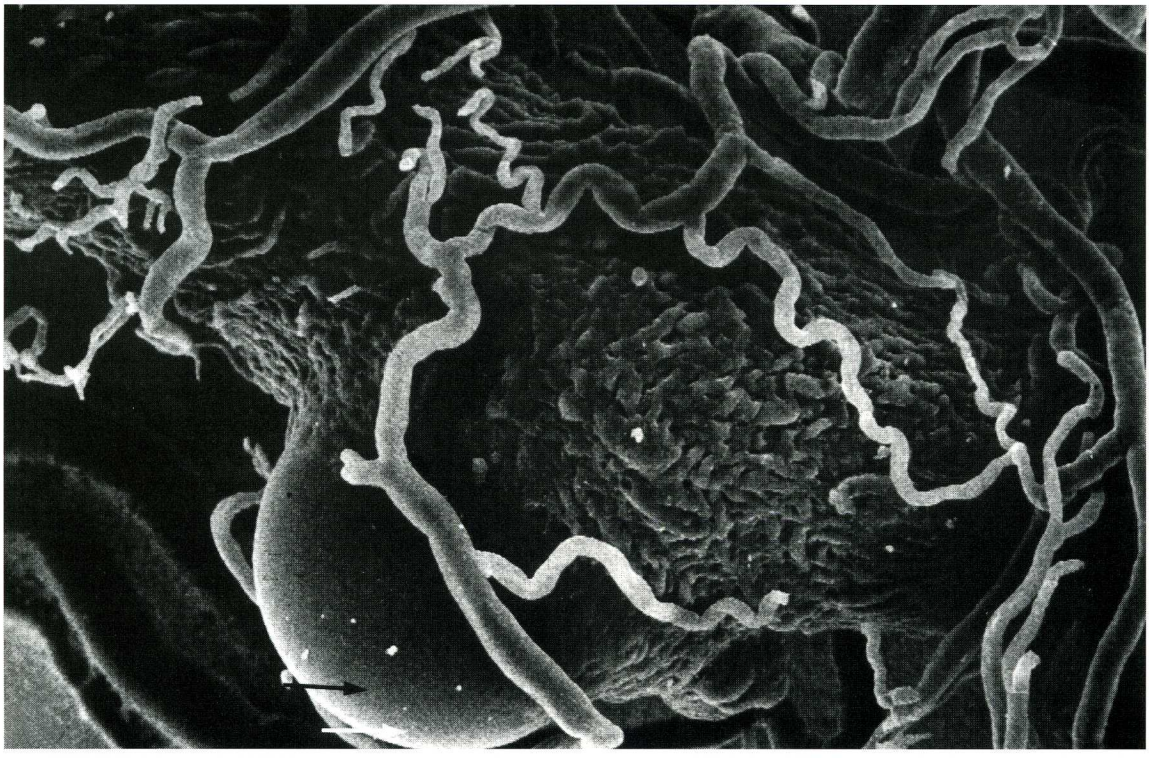

Fig. 9. Segment of a collector vessel close to a lymph node. Note diversified plicas on the cast surface and the site where small lymphatics join them (arrows). Note also the delicate networks of blood vessels (vasa-vasorum) surrounding the lymphatic segment, SEM 150.

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