MORPHOLOGY OF LYMPHATICS IN HUMAN VENOUS CRURAL ULCERS WITH LIPODERMATOSCLEROSIS

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ABSTRACT

A morphological evaluation of lymphatic vessels of skin leg ulcers was performed in 39 human subjects with longstanding venous insufficiency and lipodermatosclerosis. Light and electron microscopy demonstrated that the superficial fibrin and inflammatory cell layers and intermediate blood capillary layer of the ulcer bed, which were primarily granulation tissue, did not contain lymphatics. Moreover, lymphatic capillaries were present only sporadically in the transition zone from granulation tissue to the deeper collagenous scar layer of the ulcer. In some instances, in the deepest part of the ulcer bed near the crural fascia, there were one or two thicker lymphatic collectors with valves, which were continuations of collectors from the plantar foot region. Lymphatics were present at the border of the ulcer and in lipodermatosclerotic skin, but the endothelium and muscle lining layer were partially destroyed. Lymphatic capillaries were characterized by open interendothelial junctions in conjunction with subendothelial edema. In lipodermatosclerotic skin, the morphologic changes suggest that absorption of interstitial fluid and lymph is markedly disturbed adjacent to the ulcer bed, which likely contributes to both slow healing and high recurrence of skin ulcers associated with longstanding venous insufficiency.

Venous crural ulcer is a skin defect presenting as an excavation into the deeper layers of the skin and subcutaneous tissue, typically with secondary infection (1). Disintegration of the epidermis and dermis, and other severe morphological changes reaching to the crural fascia characterize skin ulcer pathology. The borders of the ulcer are thickened and the surrounding skin is transformed by chronic inflammation (lipodermatosclerosis).

Both blood and lymph vessels are affected in these lesions. Many factors have been proposed in the causation of ulceration and lipodermatosclerosis such as venostasis and tissue anoxia (2), reduced tissue fibrinolytic activity (3,4), white cell trapping (5-7), impaired endothelial nitric oxide synthesis (8), and presence of local arteriovenous anastomoses (9-11). These pathomechanisms cause structural changes in blood vessels, that alter their physiologic parameters to allow increased transendothelial and interendothelial permeability to proteins and erythrocytes (12,13), the existence of fibrin cuffs and microthrombi [which are also seen in several other types of skin ulcers (14-21)], with a reduction in the number of microvessels but increased blood flow in the adjacent periulcer soft tissue (21).

During these pathobiological processes, there is release of platelet activating factor and oxygen radicals that lead to disruption of the muscular pump and valves of the lymphatics (22,23). In chronic venous insufficiency, dilatation of the lymphatics and increased intralymphatic pressure accompany

increased venous pressure (24-26). Fluorescent microlymphography has also shown that in chronic venous insufficiency there is obliteration of part of the superficial skin lymphatic capillary network, dilatation of the remaining lymphatics and an increase in their permeability (27-29). One group of investigators using isotope lymphography described increased flow in lymphatics and increased accumulation of radiotracers in the inguinal lymph nodes in patients with lower extremity edema of venous origin (30). In contrast, others using similar technique described decreased function of lymphatics in venous edema, both in superficial varices and in chronic venous insufficiency with venous ulcers (31-33). Indirect contrast X-ray lymphography has shown irregular filling of lymphatics (34), their dilatation with extra-vasation of contrast material, and dermal backflow in chronic venous insufficiency (35).

When contrast material is injected into the scar of a crural ulcer, the initial lymphatics do not visualize but contrast is seen to enter lymphatic collectors (36). Indirect methods of examination (indirect lymphography and isotope lymphography) have depicted lymphovenous connections around the ulcer (36). In the post-thrombotic syndrome, isotope lymphography has revealed decreased and occasionally absent lymphatic transport (37). Incomplete and non-homogenous filling of dermal lymphatics has been seen adjacent to venous ulcers by direct contrast lymphography (38). Morphological studies have shown dilatation of the Golgi apparatus and mitochondrial destruction of the endothelium of dermal lymphatics adjacent to venous ulcers in the post-phlebitic syndrome (39). Electron microscopy in chronic venous insufficiency has shown collapse of paraulcer skin lymphatic capillaries with closed interendo-thelial junctions (40).

Our anatomic study focused on the dermal lymphatic drainage changes that develop with a venous crural ulcer and lipodermatosclerosis, and to determine the morphological basis for physiologic derange-ments in a peripheral leg venous ulcer.

MATERIALS AND METHODS

The study was performed on 39 human subjects with leg venous crural ulcers from 6 months to 6 years of age. In 34 subjects (20 females, 14 males) aged 44-70 years, the ulcer was located near the medial malleolus and was sampled 24 hours post-mortem. In 5 patients (3 females, 2 males), aged 42-60 years, biopsies and/or excisions were taken for electron microscopy of the ulcer site during an operative procedure.

The post-mortem specimens were processed as follows:

1)   In 8 cases, to visualize lymphatics, Gerota mass [Paris blue (Ferrum Feroxyanide in oil)] was slowly injected interstitially into the base of the ulcer and into the adjacent lipodermatosclerotic tissue. The latter region was excised, fixed in 10% formol, dehydrated in alcohol, cleared in methylsalicylate and observed under a dissecting microscope.

2)   In 20 cases, the crater of the ulcer and its borders were cut into 4 quadrants and blocks were taken from each quadrant for light microscopy. Histological sections were stained with hematoxylin-eosin, Masson blue trichrome and resorcin-fuchsin (a modifi-cation of the Weigert stain for elastin).

3)   In 6 cases, subepidermal veins in the lipodermatosclerotic skin were dissected, cannulated and injected with colored pigments of 5% gelatin. Samples were processed histologically.

4)   Electron microscopy. In 5 patients, the crater of the ulcer and its borders were excised. Samples were taken from the superficial, intermediate and deep layers of the ulcer bed. Samples of the ulcer and its surrounding tissue, including lipodermato-sclerotic tissue up to 20 cm from the ulcer, were initially fixed in Karnovsky solution then further fixed in 2% osmium tetroxide and embedded in araldite. Semi-thin sections were stained with toluidine blue and fuchsin-
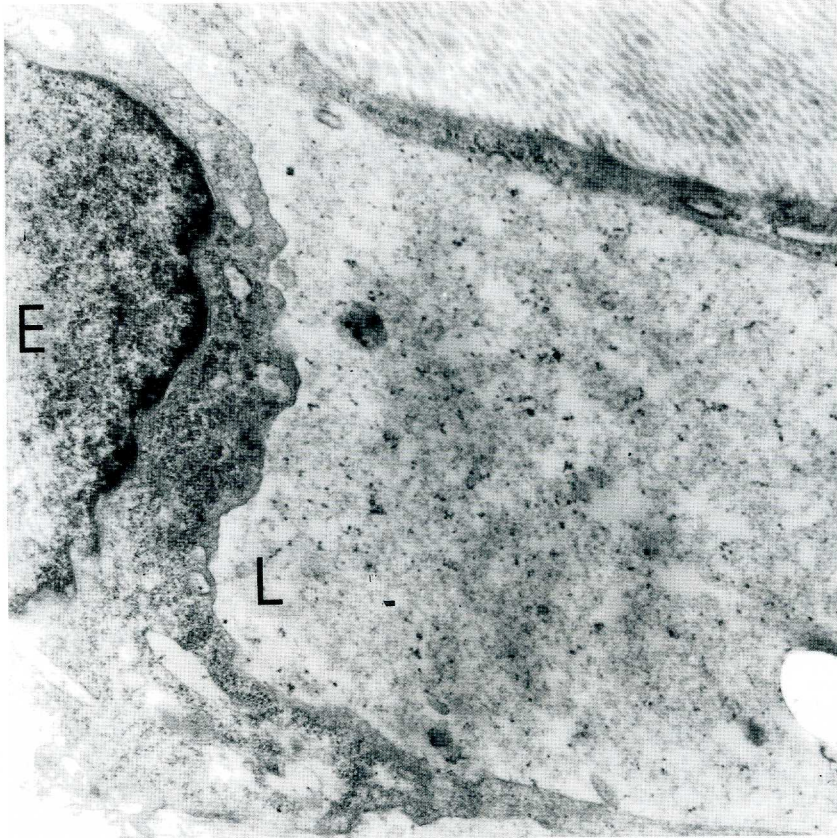
Fig. 1. Male 53 years. Lymphatic capillary photomicrograph (L) at the transition of the intermediate and deep layer of a crural ulcer. E–endothelial cells. Electron photomicrograph, orig. magnification x5600.

toluidine. Thin sections were contrasted with uranyl acetate and lead citrate; then both were observed under a JEM and Philips electron microscope.

In each patient, the cause of the crural ulcer was post-thrombotic syndrome and chronic venous insufficiency. The causes of death in the subjects where the ulcer was processed post-mortem varied: most often from trauma to the thorax, head, and abdomen, or myocardial infarction and stroke. Subjects with a history of, or clinical and laboratory evidence of ischemic disease of the lower extremities or diabetes mellitus were excluded from the study. The size of the ulcers when measured at their greatest diameter ranged from 3 to 12 cm.

Pathologic Findings

With respect to the localization of blood vessels and pathological structures, the ulcer specimens were divided into three zones: superficial, intermediate and deep. The superficial layer extended from the fibrin of the collagen and inflammatory cell layer to the apex of the blood capillaries. The intermediate layer was rich in vessels and contained blood capillaries, arterioles, post-capillary venules, and granulation tissue. The deep layer of the ulcer was located at the fascia and contained thick collagen fibers with large, partially or completely thrombosed veins. In the superficial layer, after injection as well as with light and electron microscopy,
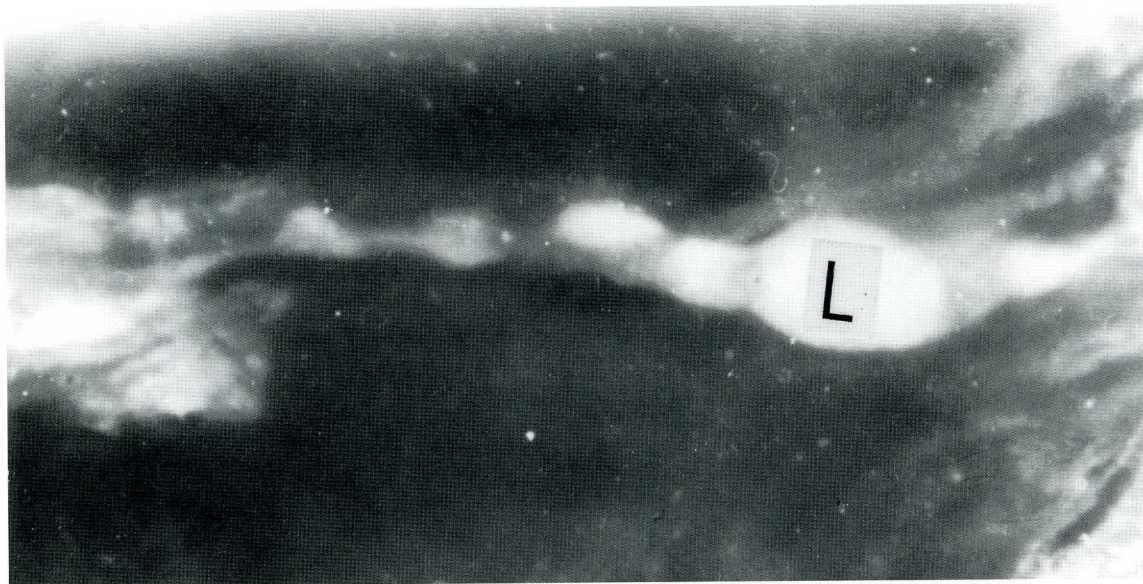
Fig. 2. Female 62 years. Lymphatic collector (L) injected with Gerota's mass, traversing the fascia at the base of a chronic (4 years) venous crural ulcer. The bead-like appearance and narrowing correspond to the location of intraluminal valves. Cleared specimen, orig. magnification x5.
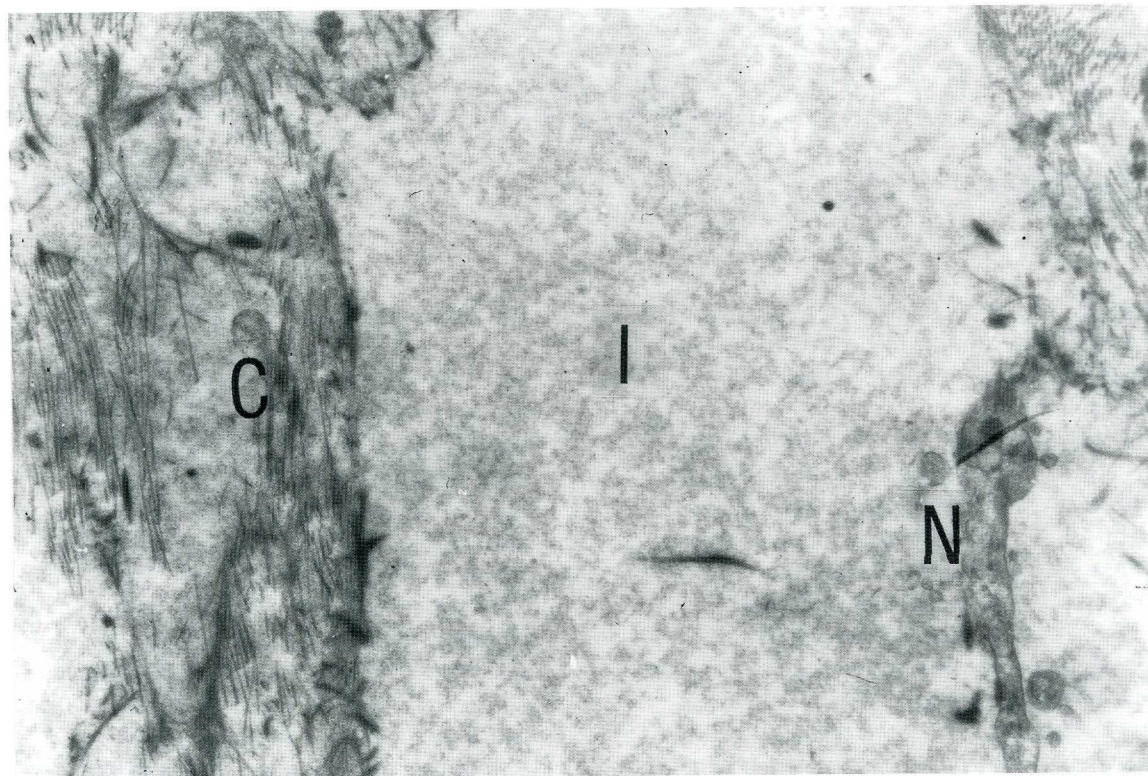

Fig. 3. Female 53 years. Dilated interstitial space (I) in a crural ulcer between fine collagen fibers (C) and processes of the cells (N) filled with amorphous edema fluid. Electron photomicrograph, orig. magnification x3900.
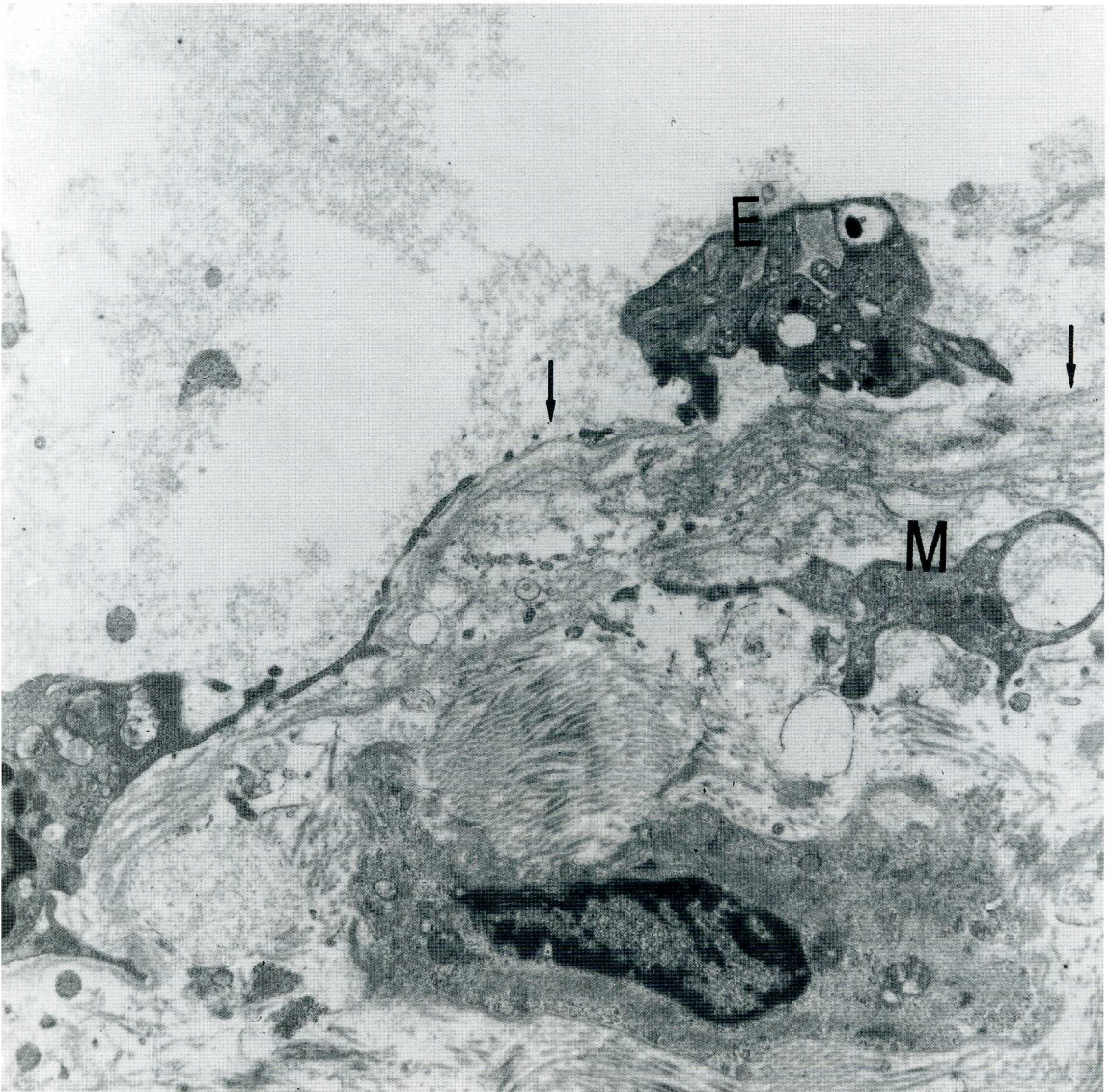
Fig. 4. Male 46-year old. Chronic venous insufficiency of legs more than 10 years with an open skin ulcer for 3 years. Lymphatic vessel at the periphery of the ulcer in lipodermatosclerotic skin. The endothelium of the lymphatic is atrophic and in some parts absent (arrows). The endothelial cells (E) are vacuolized and apoptotic. Smooth muscle cell with a vacuole (M) is seen. Electron photomicrograph orig. magnification x4500.

no lymphatic capillaries were identified in any specimen. In the intermediate layer with the Gerota mass, lymphatics also were not depicted, whereas the injected material rapidly appeared in the veins. With electron microscopy, in 3 out of 5 cases, we observed only 1 or 2 lymphatic capillaries (Fig. 1) and they were identified in only 2 of the 40 samples in several regions of the ulcer. With light microscopy at the transition of the intermediate and deep layers, in 3 ulcers we identified only a single lymphatic. In the deep layer close to the crural fascia, in 3 of the 8 ulcers injected with Gerota mass, only 1 or 2 larger dilated lymphatic collectors with valves were observed (Fig. 2). These lymphatics were

Fig. 5. Male 46 years. Lipodermatosclerosis. Smooth muscle cells in the wall of a lymphatic collector showing different stages of damage. Part of one muscle cell is transformed into a myofibrocyte (R), while a part of a second smooth muscle cell is disintegrating with the development of vacuoles (V) and destruction of myofibrils (D). In another smooth muscle cells vacuoles (V) are seen. Electron photomicrograph, orig. magnification x4000.

part of the lymphatic collectors, which we observed caudally from the ulcer–from the plantar and ankle region. In 5 cases, the interstitially injected Gerota mass did not fill lymphatics, but passed into the veins. In the intermediate layer, electron microscopy showed edematous dilated spaces between variously preserved collagen fibers, inflammatory cell infiltration and endothelial sprouting. The spaces were filled by interstitial fluid containing amorphous debris *(Fig. 3)*. Lymphatic vessels were dilated at the edges of the ulcer and up to 20 cms from the ulcer base in both the lipodermatosclerotic and normal looking skin which, however, contained venous varices. Electron microscopy showed various degrees of lymphatic changes from unremarkable to severely damaged *(Figs. 4-13)*. With less severe changes, there was abundant pinocytosis and vacuolization in the endothelial cells. In some lymphatics the endothelium was atrophic, in others hypertrophic with increased numbers of microfilaments. With more severely damaged lymphatics, the wall was edematous with defects of the endothelial lining and apoptosis of the endothelial cells. Collagen fibers protruded into the lumen of the lymphatics through the endothelial defects. Smooth muscle cells in the wall of the lymphatic collectors were vacuolized, in some parts transformed into myofibrocytes, in other parts disintegrated and their cell cytoplasm filled with cellular debris. Interendothelial junctions were both closed and widely open.
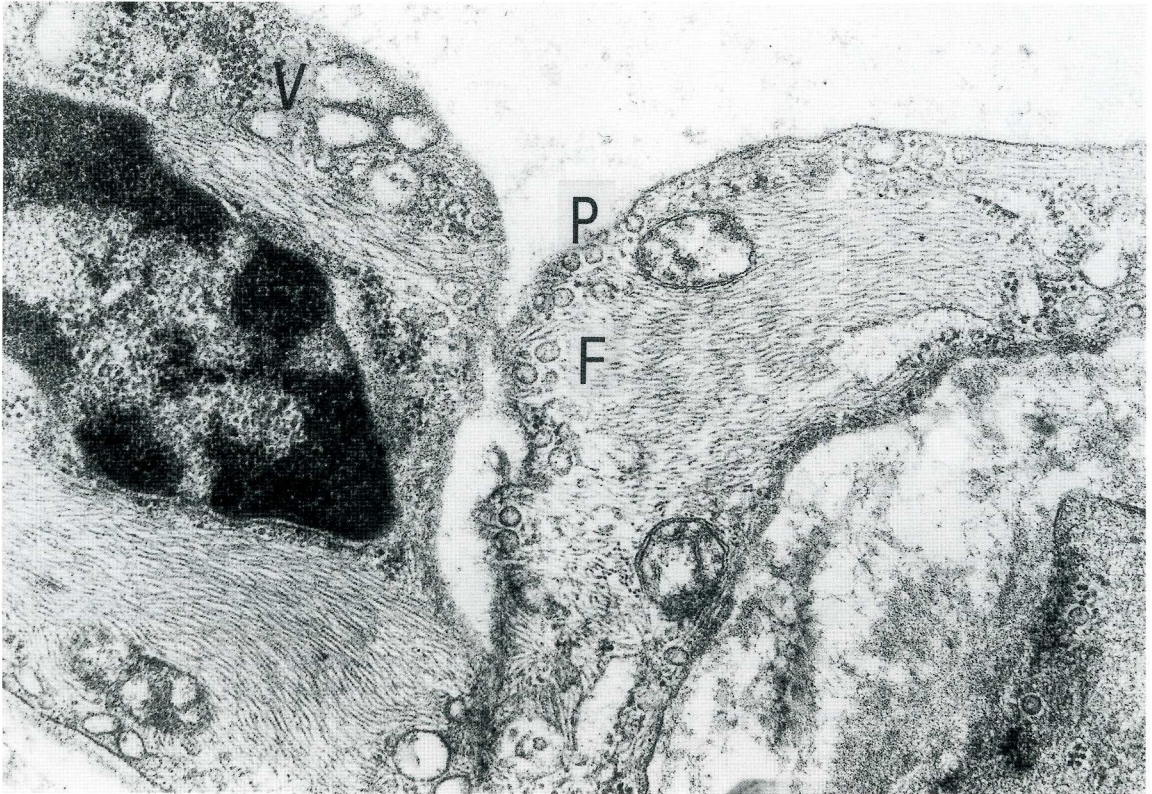
Fig. 6. Lipodermatosclerosis. Proliferation of microfilaments (F), vacuoles (V), pinocytosis (P), and dilation of mitochondria in a lymphatic endothelial cell are seen. Electron photomicrograph, orig. magnification x20000.

No lymphatics were completely collapsed. A schematic diagram of the lymphatic drainage of a venous crural ulcer is shown in *Fig. 14.*

DISCUSSION

Lymphatic vessels were best identified using electron microscopy and dye injections. In histological sections of the ulcers, it was often difficult to delineate between lymphatics and blood vessels. There was better differentiation in lipodermatosclerosis where dilated lymphatic vessels were filled with lymph and veins were injected with colored pigments.

In clinical practice, healing of a venous crural ulcer is typically slow and difficult. The morphological basis for this sluggish healing of such an ulcer and persistent lipodermatosclerosis are the chronic changes in the microcirculation as described in the Introduction (1-22). In addition, there are also alterations in the lymphatic drainage surrounding the skin ulcer (27-39).

Our morphological findings demonstrated that lymphatic vessels were absent in superficial and almost entirely absent in the intermediate layer of the ulcer, whereas the transition zone between the intermediate and deep layers, occasionally displayed lymphatic capillaries. In the deep layer in the connective tissue adjacent to the fascia where larger veins were partially or completely obliterated, there were seen sporadic lymphatic collectors with valves. These deep parafascial lymphatic collectors were likely continuations of lymphatic collectors from the plantar and dorsal part of the foot. Their diameters
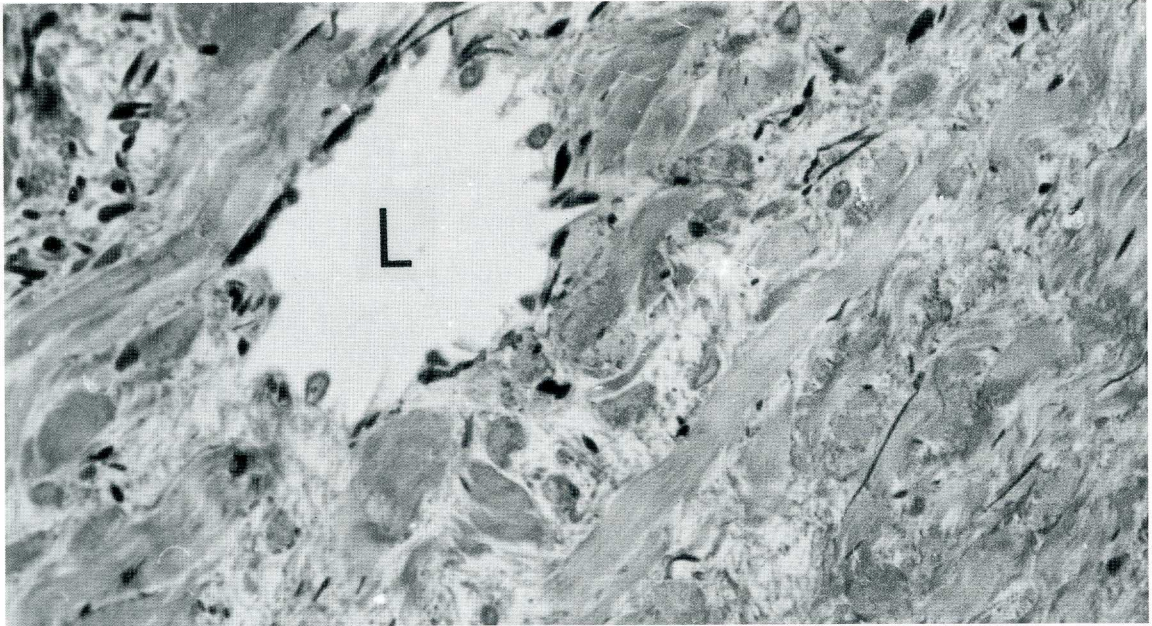
Fig. 7. Female 55 years. Chronic venous insufficiency of legs for 7 years with crural ulcer for 3.5 years. Dilated lymphatic vessel of the dermis (L) taken from an area where the varicose veins were present 16 cm above the skin ulcer. Semi-thin section, toluidine blue, orig. magnification x80.
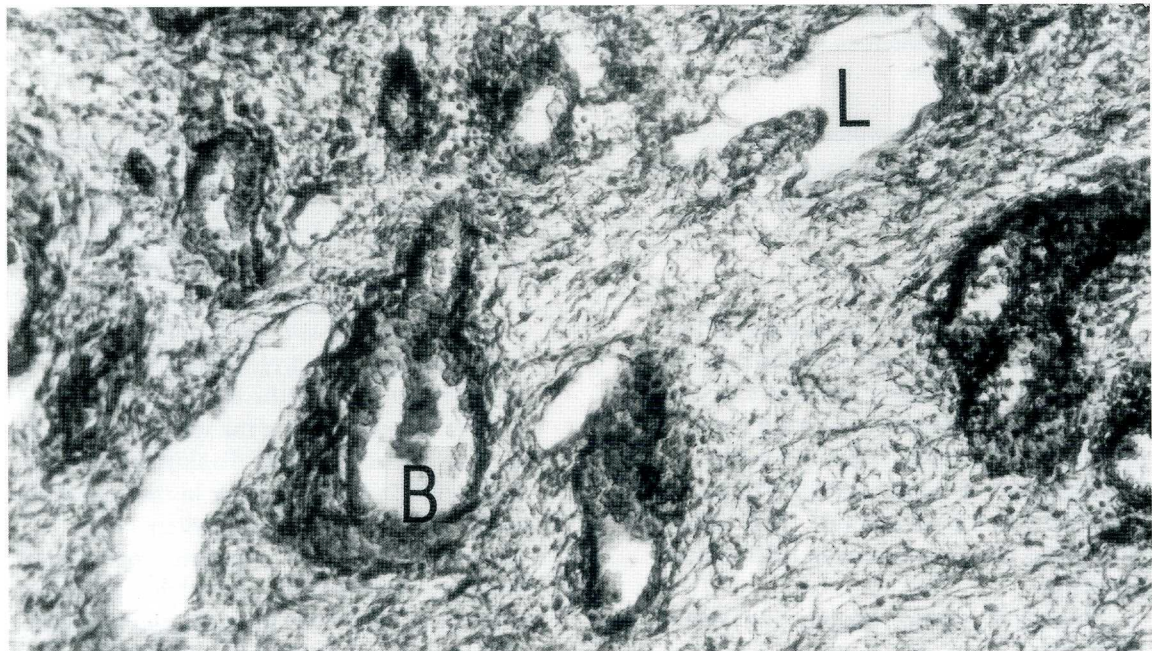

Fig. 8. Female 60 years. Chronic venous insufficiency with peripheral edema. Blood vessels (B) injected with red pigments in 4% gelatin. Dilated lymphatic vessel (L). Hematoxylin-eosin, orig. magnification x80.
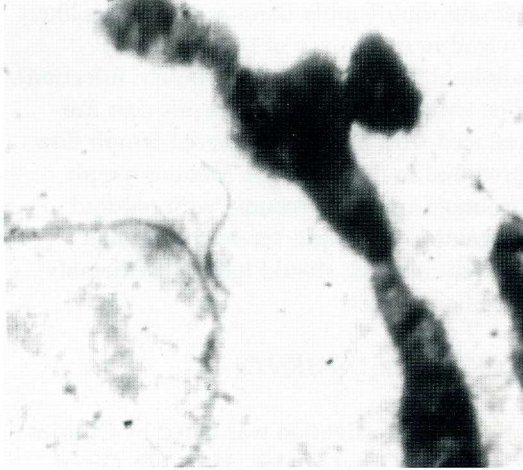
Fig. 9. Male 40 years. Lipodermatosclerotic skin-one and half cm from the border of a crural ulcer. Injected and dilated lymphatic capillary with an irregular contour. Cleared specimen, orig. magnification x20.

ranged from 0.3 to 1.3 mm. The edema that was present in the histological samples of the ulcer suggested stagnation of interstitial fluid. Whether such edema is "toxic" to the ulcer

healing (41-42) and retards healing (43) remains an open question. Findings of inadequately developed lymphatic drainage of the ulcer were covered from the view of general pathological classification. The upper two thirds of the ulcer was mostly granulation tissue; the lower third was dense collagenous scar tissue. A common characteristic of both tissues was insufficiency of lymphatic vessels. The findings in the lymphatics in the periphery of the ulcer and its surroundings showed several alterations, some of which (for example the increase in microfilaments in the endothelium) was speculatively interpreted as "compensatory" with lymphatics diverting edema fluid away from the ulcer region. Partial or complete destruction of lymphatic endothelium and lining muscle cells with transformation into myofibrocytes likely disturbs the contractility of the lymphangions and resorption of tissue fluid. Open junctions, the presence of wide interendothelial channels, and subendothelial edema support stagnation of interstitial fluid and lymph. Dilation of lymphatics, damaged
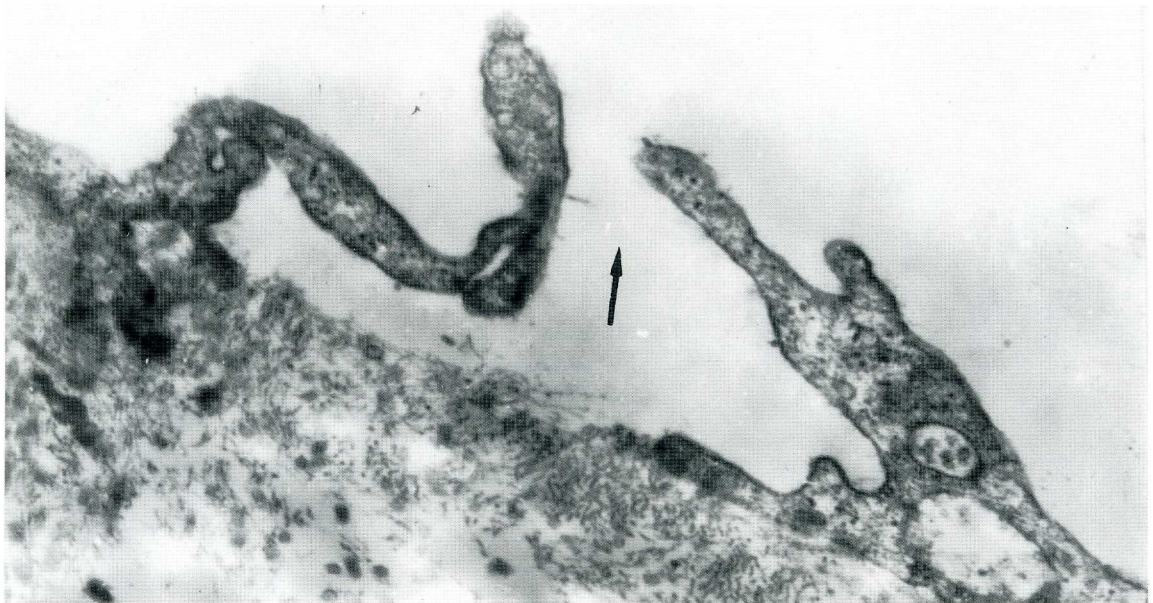

Fig. 10. Female 57 years. Chronic venous insufficiency with a crural ulcer and lipodermatosclerosis. Lymphatic capillary with wide open interendothelial junction (arrow) in lipodermatosclerotically altered skin. Electron photomicrograph, orig. magnification x33000.

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lymphatic endothelium and smooth muscle cells, wide open junctions and subendothelial edema in the lipodermatosclerotic skin around the ulcer are key morphological features of longstanding venous insufficiency. In contrast to others (40), we observed open interendothelial junctions and not collapsed lymphatics. Inadequate lymphatic drainage of the ulcer along with variably damaged lymphatics of lipodermatosclerotic skin are responsible for accumulation of edema fluid aggravated by "toxic" catabolic byproducts. Together with a disrupted network of prefascial lymphatic capillaries, i.e., lymphatic microangiopathy (27-29), and insufficient lymphatic drainage by the deep subfascial collectors (37), there is a distinctly unfavorable milieu that restricts healing of a crural ulcer. One can anticipate even if healing of the ulcer occurs predominantly by scarring that the adjacent lymphatic vessels remain significantly damaged and thus likely to lead to recurrence of the ulcer with minimal further insult (e.g., injury, infection). The lymphatics that traverse the scar are reduced with markedly lowered lymph flow proximal to the scar (44). Manual lymph drainage and compression, with reduced stagnation of lymph, venous blood, and interstitial fluid, should have a predictably beneficial effect.
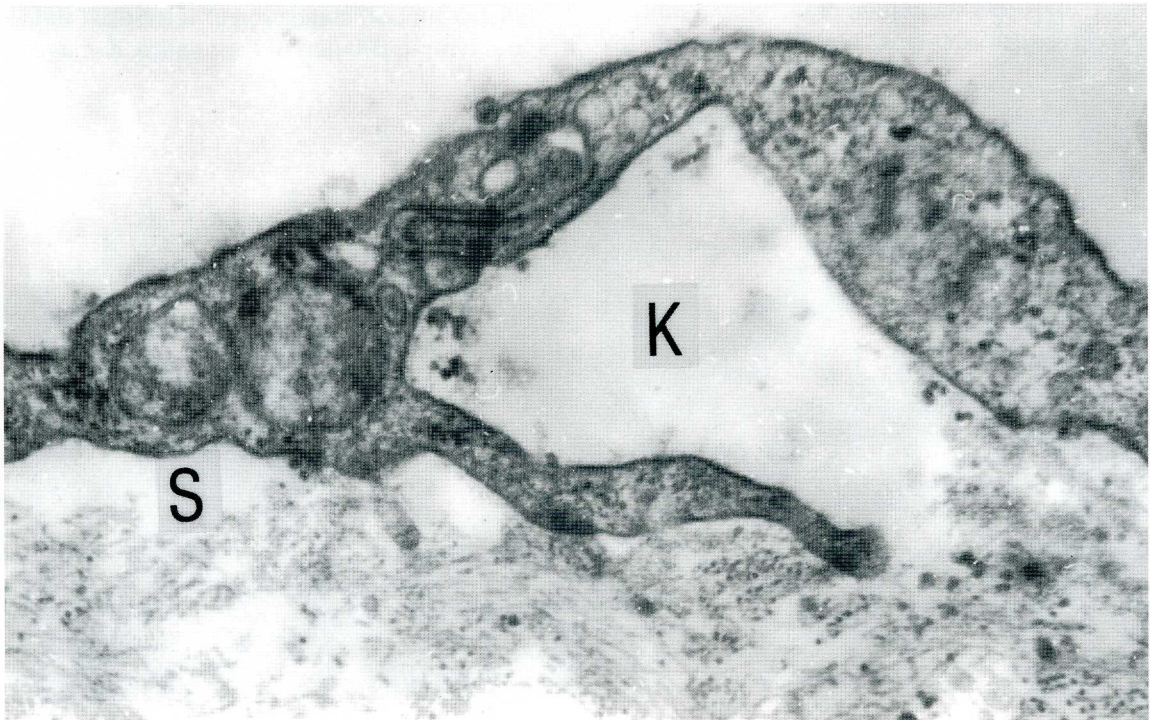
Fig. 11. Lymphatic taken from the same region as that in Fig. 7. Dilated interendothelial gaps in the form of channels (K). S–subendothelial interstitial edema. Electron photomicrograph, orig. magnification x20000.
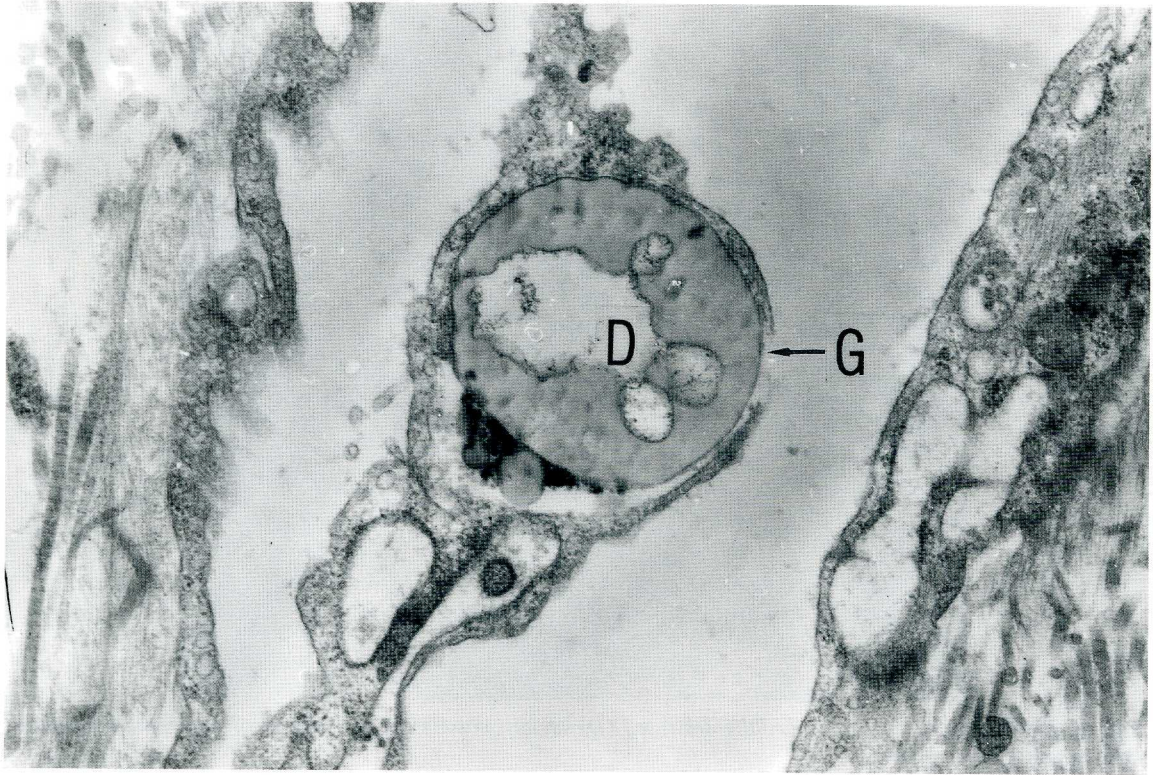
Fig. 12. Lipodermatosclerosis. A partially destroyed cell passing through an endothelial gap (G) of a lymphatic capillary. Electron photomicrograph, orig. magnification x12500.

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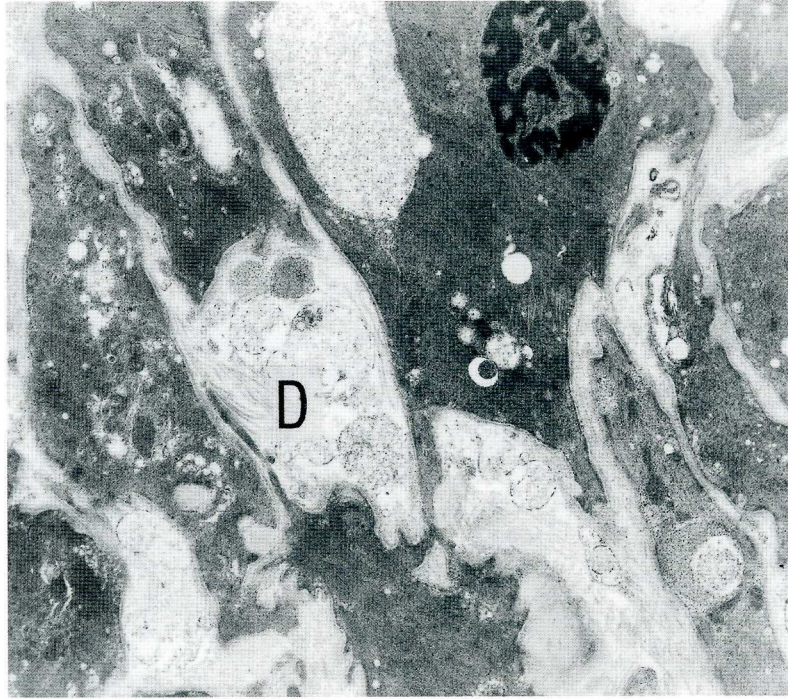16. Leu, AJ, HJ Leu, UK Franzeck, et al:

Fig. 13. Lipodermatosclerosis. A partially destroyed (D) smooth muscle cell in the wall of a lymphatic collector. Orig. magnification x4500.
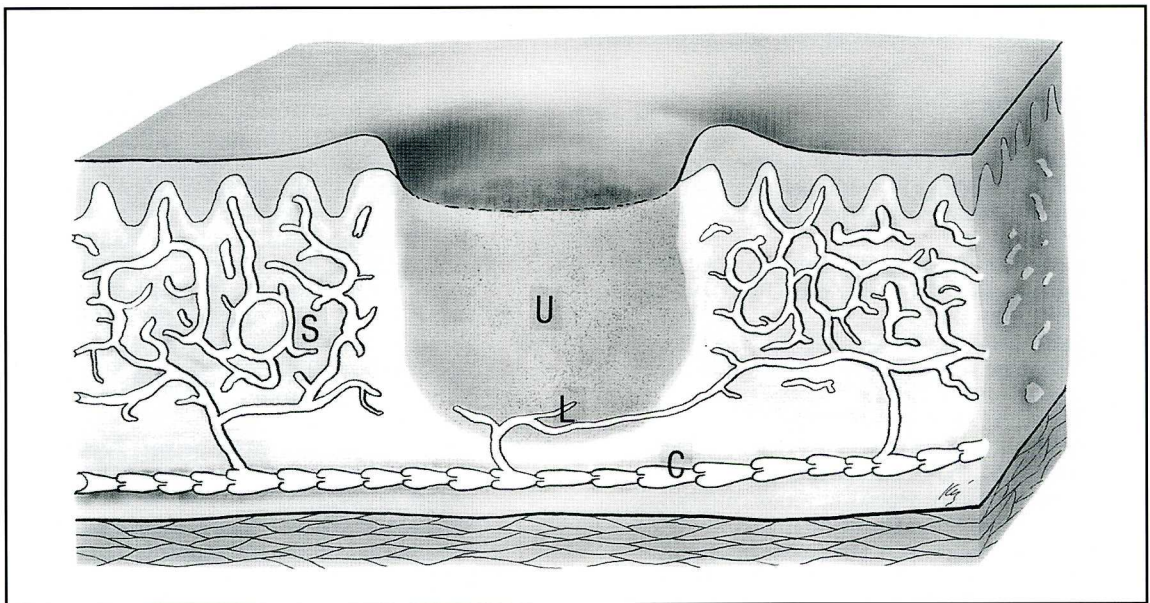

Fig. 14. Schematic diagram of the lymphatic drainage of a venous crural ulcer. Lymphatics with various degrees of morphological damage exist in the lipodermatosclerotic periphery of the ulcer bed (S). In the upper two thirds of the ulcer (U), lymphatics are absent. Lymphatics (L) are present sporadically in the transition zone between the intermediate and lower third of the ulcer bed, where they connect to lymphatics of lipodermatosclerotic skin and ultimately to the deep lymphatic collectors (C) on the underlying fascia.

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