

LYMPHATICS IN THE AORTA OF RATS TREATED WITH A SOY-BEAN OIL EXTRACT (LIPOFUNDIN)

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ABSTRACT

Lipofundin-S 20% (a soy-bean oil extract) when administered intravenously to rats resulted in dilation of aortic lymphatics at the media-adventitia junction. Increased endothelial permeability as demonstrated by intraaortic colloidal iron uptake with dynamic insufficiency of lymphatic drainage is suggested as the basis for dilatation of intraaortic adventitial lymphatics.

In earlier studies (1,2) cholesterol feeding produced dilation of adventitial lymphatics in the rat aorta. In these dilated lymphatics electron microscopy revealed lipid droplets in the endothelial cells. It was surmised that excess cholesterol feeding led to dynamic insufficiency of lymph transport (lymph flow exceeded drainage capacity) and lymphatic dilation ensued.

In other studies (3-8) dilatation of adventitial lymphatics occurred in the aorta and other blood vessels after lymphatic occlusion, with changes in the aortic wall that resembled media necrosis (5-7,9,10). Other types of physiologic damage (e.g. hypoxia, hypertension) (11-13) to the blood vessel wall, enhanced lymph drainage by increasing endothelial permeability and also induced lymphatic dilatation. Similar findings have been described by other workers (4,14-16).

The present study examined aortic

adventitial changes in rats induced by Lipofundin, a soy-bean oil extract, used clinically for parenteral nutrition.

MATERIALS AND METHODS

A total of 62 Wistar rats were used. Forty-two rats received 1 ml/100g B.W. Lipofundin-S 20% (Braun, Melsungen) three times daily into the tail vein and 20 other rats received two injections a day. 20 non-treated rats served as controls. Rats were killed after seven days of treatment (day eight) after half-day starvation. Just prior to sacrifice, colloidal iron (Ferrelcit Natterman Koln) was injected intravenously. Other details of the aortic preparations have been reported earlier (17,18).

Specimens were fixed in part by perfusion and in part by immersion using 4.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Specimens were washed and postfixed in 5% OsO₄ for two hours. After repeated washing they were dehydrated in an ascending series of alcohols and embedded in Durcupan ACM. Semi-thin sections were stained with methylene blue-azure II and basic fuchsin. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a JEM 100 B electron microscope. Sections were cut using an LKB III ultramicrotome and Reichert-ultrakat I microtome.

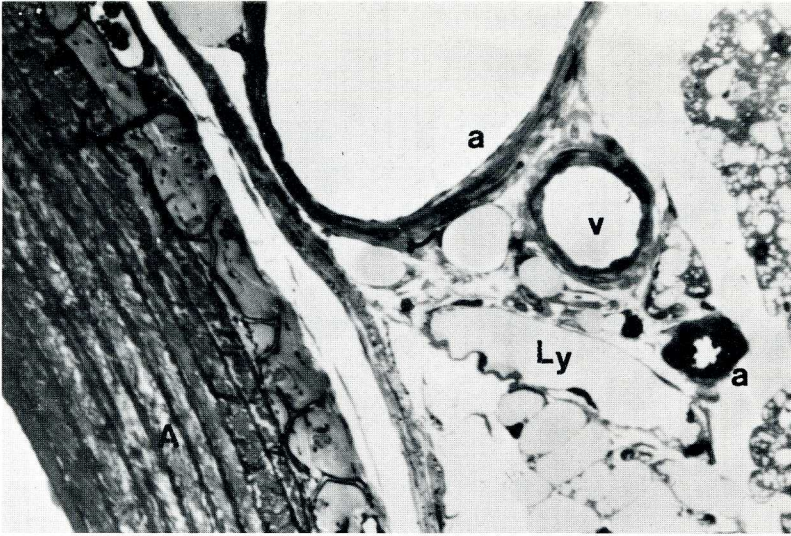


Fig. 1: Light micrograph of a semi-thin section of the rat aorta after administration of Lipofundin. Beside the artery (a) and vein (v) in the aortic (A) adventitia, a lymphatic (Ly) with thin wall is also seen.

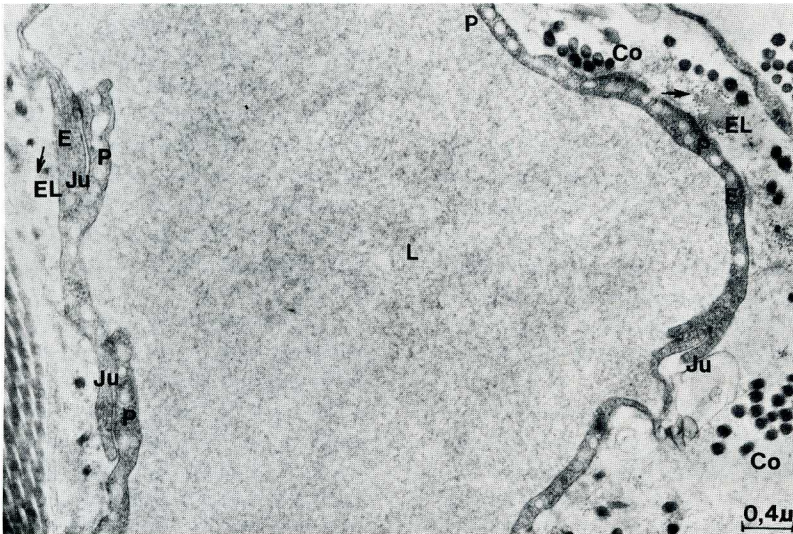


Fig. 2: A markedly dilated lymphatic. The endothelial cells of the lymphatic are thin, form several junctions and display pinocytotic (P) activity. In the lumen (L) finely dispersed granular substance appears. Around the lymphatic endothelium collagen (Co) and elastic fibers (EL) are seen with microfilaments (Ju) readily.

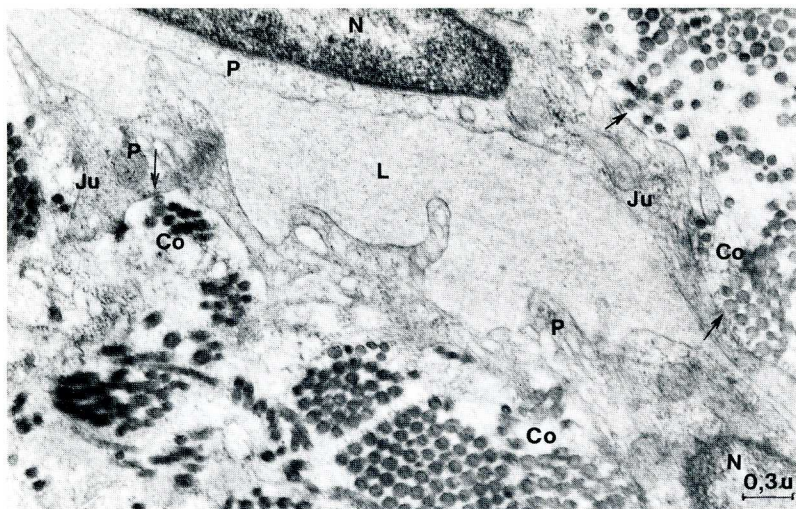


Fig. 3: Lymphatic-endothelial cells (E) forming junctions with each other and containing pinocytotic vesicles (P). In the lumen (L) is granular substance. In the outer zone collagen fibers (Co) are attached to the endothelial cells occasionally along a path as far as the membrane (\rightarrow). The nucleus (N) of the endothelial cell is also seen.

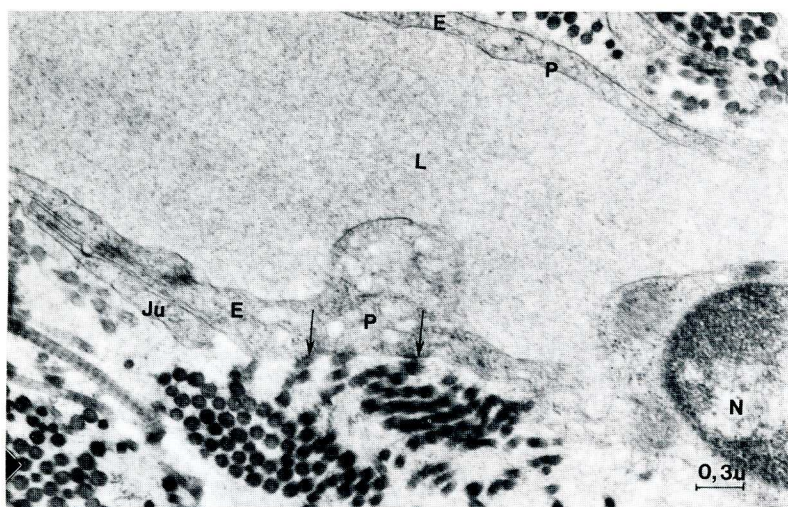


Fig. 4: Endothelial cells (E) of the lymphatic form junctions (Ju) with each other and contain pinocytotic vesicles (P). In the lumen (L) is finely dispersed granular substance. A detail of nucleus (N) of the endothelial cell is also seen. The collagen fibers (Co) are attached directly to the lymphatic (\rightarrow).

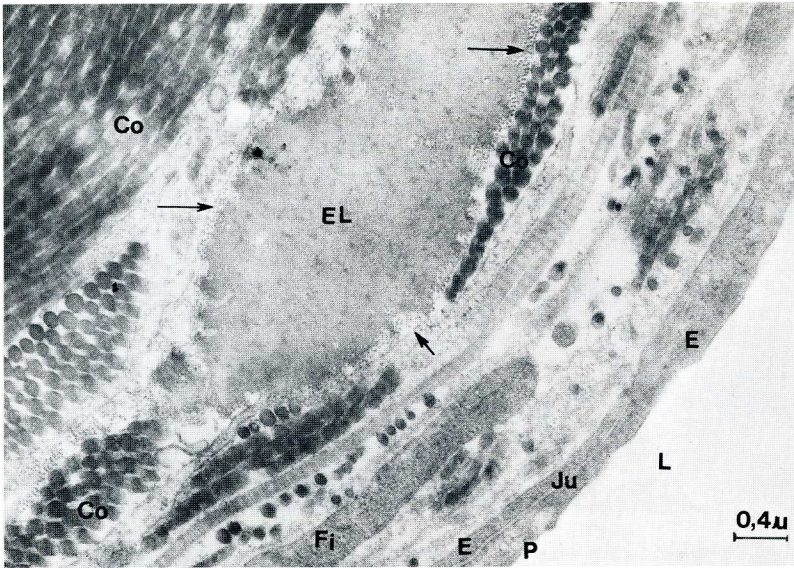


Fig. 5: In the aortic adventitia among the numerous collagen fibers (Co), an elastic fiber (EL) is seen (at the edge) with fine fibrillar structure (\rightarrow). Among the collagen fibers under the process of a fibrocyte (Fi) two endothelial cells (E) form a junction (Ju). L=lumen.

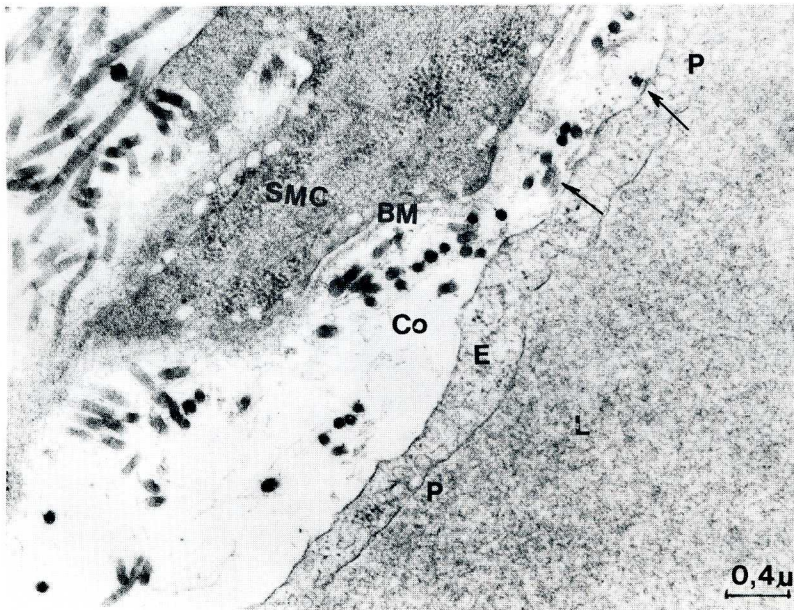


Fig. 6: Endothelial cell (E) of a lymphatic loaded with pinocytotic vesicles (P). In the lumen (L) is a granular substance. Between a smooth muscle cell (SMC) in the innermost layer of the media and the lymphatic, collagen fibers (Co) attach directly to the membrane of the lymphatic endothelial cell (\rightarrow). BM=basement membrane of the smooth muscle cell.

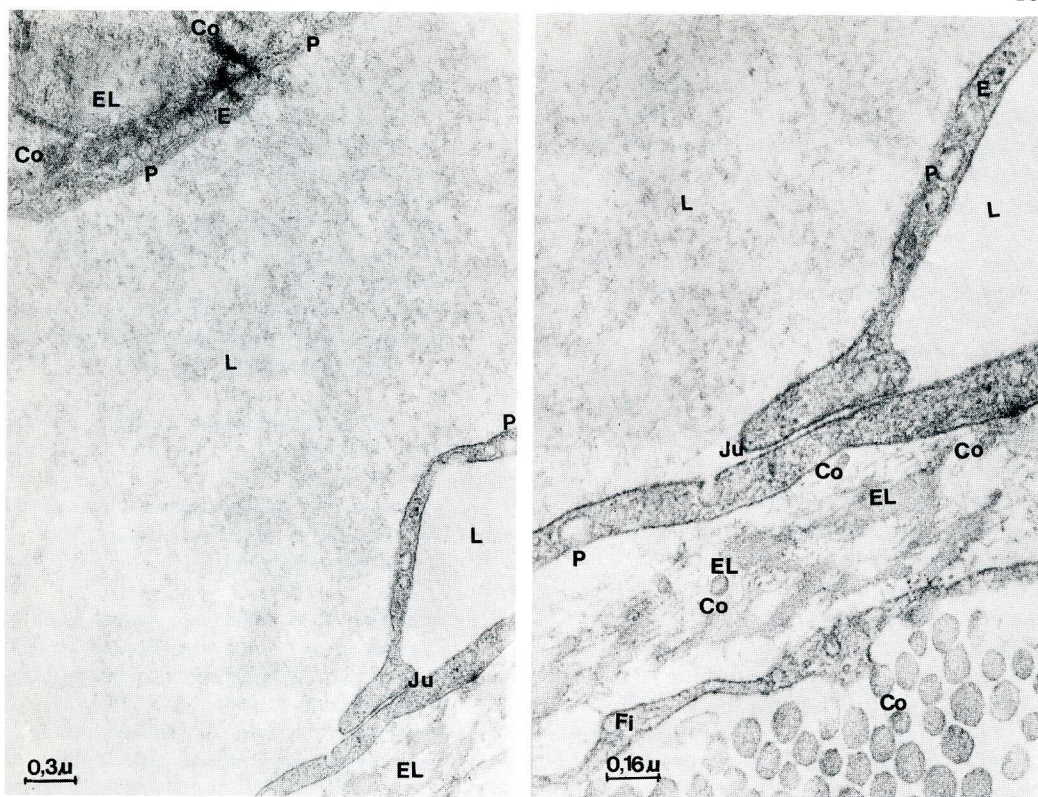


Fig. 7: a) Detail of the opposite walls of an adventitial lymphatic. Endothelial cells (E) contain pinocytotic vesicles (P). Directly adjacent to the endothelial cells are elastic (EL) and collagen fibers (Co). Two endothelial cells forming a foot-like junction (Ju) subdivide the lumen (L). b) Magnification of Fig. 7a. Fi=process of a fibroblast.

RESULTS

Semi-thin sections showed both dilated arterioles and even more dilated veins in the aortic adventitia. Moreover, in some specimens only lumina were seen which sometimes contained a faintly stained substance with low protein content and which were lined with a single endothelial cell layer (Fig. 1). Lymph vessels were not visible in control rats.

Under electron microscopy these lumina were lined with endothelial cells and appeared in the aortic adventitia. The endothelial cells contained pinocytotic vesicles and some of the cells formed junctions (Fig. 2). The endothelial cells of the lymphatics were thin, elongated and formed junctions along a long path (Fig. 3). Collagen fibers joined the

lymphatics directly and they appeared attached to the membrane of the lymphatic endothelium (Fig. 4). In some areas the lymphatics and collagen fibers were located in the immediate vicinity of the smooth muscle cells in the outer layer of the adventitia, whereas in other zones elastic fibers appeared between collagen bundles and microfibrils at the junction of the adventitia and media (Fig. 5, 6). Occasionally, the endothelial cells, like tiny feet, were attached to the surface of the endothelial cells lining the lumen thereby forming lymphatic branches (Fig. 7). In the endothelial cells of certain lymphatics, lysosome-like iron particles (ferrosome) were observed which corresponded to the colloidal iron injected prior to sacrifice.

DISCUSSION

Plasma flow and substances filtered into vessel walls are carried away via adventitial lymphatics (1,2,9,12,13). The anatomical existence of an intraaortic lymphatic drainage system has been demonstrated after cholesterol feeding or with lymphatic occlusion (1,8,19), while the function of these lymphatics has been depicted by severe alterations in the aorta (5,7) that develop with lymph congestion after lymphatic interruption.

Development of intraaortic lymphatic dilatation and iron particles in endothelial cells after Lipofundin, a soy-bean oil extract, suggests that in the initial phase at least there is accelerated fluid transport across the aortic wall with administration of this agent (20). With exaggerated flow and transport lymphatics distend within the adventitia and become visible. The lymphatic cells that project into the lumen, function as valves and help to determine the direction of flow (21). Although Lipofundin-S has been used for parenteral nutrition clinically it should be emphasized that in the experimental model the dosage and infusion rate differ considerably from its administration clinically. For parenteral nutrition a dosage of 1-2g fat/kg B.W./day and an infusion rate of not more than 0.2g fat/kg B.W. are recommended whereas in our study as much as 6g fat/kg B.W./day were given with rapid injections within two minutes. Nonetheless with this exaggerated dosage the importance in capacity of the intraaortic lymphatic system has been revealed.

Overall, these results suggest that where fluid flow through the aortic wall increases, lymph flow within the adventitia plays a major role in drainage transport. Blockage or dynamic insufficiency of this intraaortic lymphatic network may lead to aortic intramural injury such as atherosclerosis.

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