

Regeneration of the Deep Cervical Lymphatics

— Light and Electron Microscopic Observations —

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Summary

Regeneration of the lymphatic vessels following ligation of the deep cervical lymphatics was studied in 35 rabbits using both light and electron microscopes. The newly formed lymphatics were evident under the electron microscope in the first post-operative week, in which the restoration of the affected endothelial cells of the lymphatics and reaction of the free cells such as fibroblasts and macrophages were observed.

The objective of the study was to determine the changes which would occur with lymphatic obstruction in the lymphatic wall and, whether or not lymphatic vessels would form locally as a collateral route. Regeneration of lymphatic vessel has been studied using light microscopy (1, 2) and microlymphangiography (3, 4, 5). While the relatively low magnifications of those technics allow for the survey of the newly formed vessels and collateral routes, the difficulty in a correct identification of lymphatics is considerable. Electron microscopy studies revealed that lymphatics possess specific characteristics which differentiate them from blood vessels (6, 7, 8, 9, 10). We used the light and electron microscopes to observe the regeneration of lymphatics and our findings are reported herein.

Materials and Methods

In thirty-five male rabbits weighing from 2,500 to 3,000 g, the deep cervical lymphatics were ligated at a distance of 3 cm proximal from the deep cervical lymph nodes. The distal pieces of the lymphatics were excised at 8 intervals from 3 hours to 27 weeks after ligation. The tissues were fixed in a paraformaldehyde-glutaraldehyde mixture buffered at pH 7.4 with sodium cacodylate for four hours

(Karnovsky) (11) and post-fixed in 2% OsO₄ buffered with 0.1M sodium cacodylate. The specimens were then dehydrated and embedded in epoxy resin. Sections were stained with toluidine blue for light microscopy and with uranyl acetate and lead citrate for electron microscopy.

Results

Light microscopic observations: From three hours to one day after ligation, numerous lymphocytes appeared in the subendothelial area and most were destroyed on the third day. One week after ligation, numerous fibroblasts, macrophages and many small vessels had invaded in the lymphatic wall. Consequently, the thickness of the lymphatic wall appeared to be from four to seven times thicker than that of the normal wall (Figs. 1, 2). One of the micrographs clearly demonstrated a connection between the newly formed vessels and the ligated lymphatics. When India ink was injected into the ligated vessels at 30 minutes before excision, we found that only some of the newly formed vessels contained India ink. Erythrocytes were observed in most of the vessels not stained with India ink. Two weeks after ligation, connective tissue fibers were markedly increased in number while the cellular elements decreased in number. The thickness of the wall was decreased by two-thirds of the thickness formed in one week. From 4 to 27 weeks, the fibers were gradually arranged along the endothelium, so that the thickness of the wall further decreased, although such was still thicker than the normal one (Fig. 3). Vasodilatation of small blood



Fig. 1

Fig. 1. Light micrograph of a deep cervical lymphatic wall in a normal rabbit. X 360

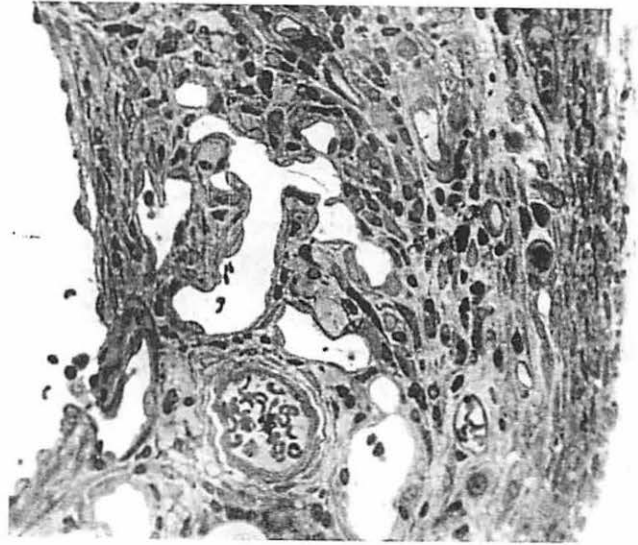


Fig. 2

Fig. 2 A portion of the wall of an occluded lymphatic vessel one week after ligation. Numerous cells and many small vessels are visible in the wall. X 360

vessels surrounding the lymphatic wall was still evident.

Electron microscopic observations: The endothelial cells of the occluded lymphatics were markedly flattened within a period of 3 hours to one day after ligation. The cells were simply opposed to each other. Neither extensive overlap, nor patent junction was noted. The pinocytotic vesicles were increased in one day even though there was a temporary disappearance within 3 hours. The lymphatic lumen and the surrounding interstitial area contained a flocculent precipitate and numerous lymphocytes, most of which were damaged within one to three days. The alterations of the cellular elements forming the lymphatic wall reached a peak 3 days after ligation. Most of the endothelial cells showed an electron dense appearance, in which swollen mitochondria, enlarged rough surfaced endoplasmic reticulum and affected nucleus were seen. In addition, finger or fungi-form projections of the endothelial cells extending into the subendothelial area were frequently

observed (Fig. 4). The smooth muscle cells and fibroblasts were also more or less affected. One week after ligation, the cell organelles of the endothelial cells showed a tendency toward recovery. The fibroblasts displayed an active fiber formation. The number of damaged lymphocytes decreased, whereas that of macrophages and plasma cells increased. Many minute vessels, which were not detectable by light microscopy were observed among those cells in the subendothelial area, as showed in figure 5. Such were surrounded by a layer of endothelial cells, in which numerous free ribosomes were visible. The basal lamina was faint and discontinuous. It was difficult to determine whether such were primitive blood vessels (12) or lymphatics (13), although erythrocytes are not seen in the lumen. On the other hand, in the larger vessels, it was possible to differentiate the lymphatics from the blood vessels. The differentiating was demonstrated more clearly in the fourth week (Fig. 6). The newly formed blood vessels were more evident in the first week, whereas the lymphatic vessels were prominent in the



Fig. 3

Fig. 3 A portion of an occluded lymphatic vessel four weeks after ligation. X 360

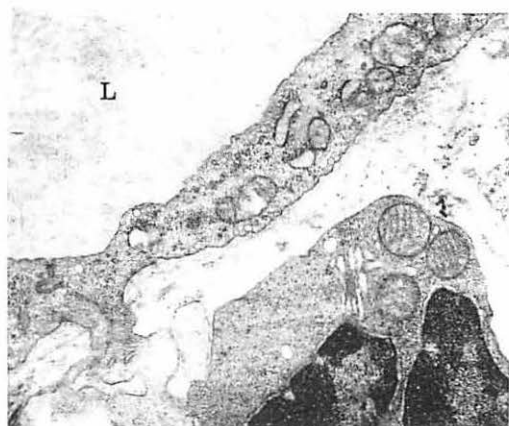


Fig. 4

Fig. 4 Electron micrograph of a portion of an occluded lymphatic vessel (L) three days after ligation showing a protrusion of the endothelium. X 12,000

fourth week. The proliferation of smooth muscle cells and elastic fibers was observed in the wall of the ligated lymphatics for periods from 7 to 27 weeks. The intracytoplasmic filaments of the endothelial cells also increased in number during this time.

Discussion

Using an electron microscope, the regeneration of the lymphatic vessels following ligation of the deep cervical lymphatics was confirmed in the first postoperative week. The first sign after the ligation was extravasation of lymph from the occluded lymphatics. Alterations of the cellular elements forming the wall then followed. Restoration of the affected endothelial cells of the lymphatics and reaction of the free cells such as fibroblasts and macrophages began one week after ligation, as reported previously (14). At this time, regeneration of the lymphatic vessels occurred in the subendothelial area and was accompanied by invasion of the blood capillaries sprouting from the pre-existing blood vessels

along the ligated lymphatics. Growth of the blood capillaries preceded that of the lymphatics as reported by *Clark and Clark* (15), and *Yoffey* (16). The finger or fungi-form projections extending from the abluminal surface of the endothelial cells of the occluded lymphatics as shown in figure 4 probably represent a sprouting of the regeneration of the lymphatics in the recovery and following active reaction stages. The considerable proliferation of smooth muscle cells, elastic fibers and intracytoplasmic filaments of the endothelial cells in the later stage suggests that an autocontraction plays a much greater role in ligated lymphatics.

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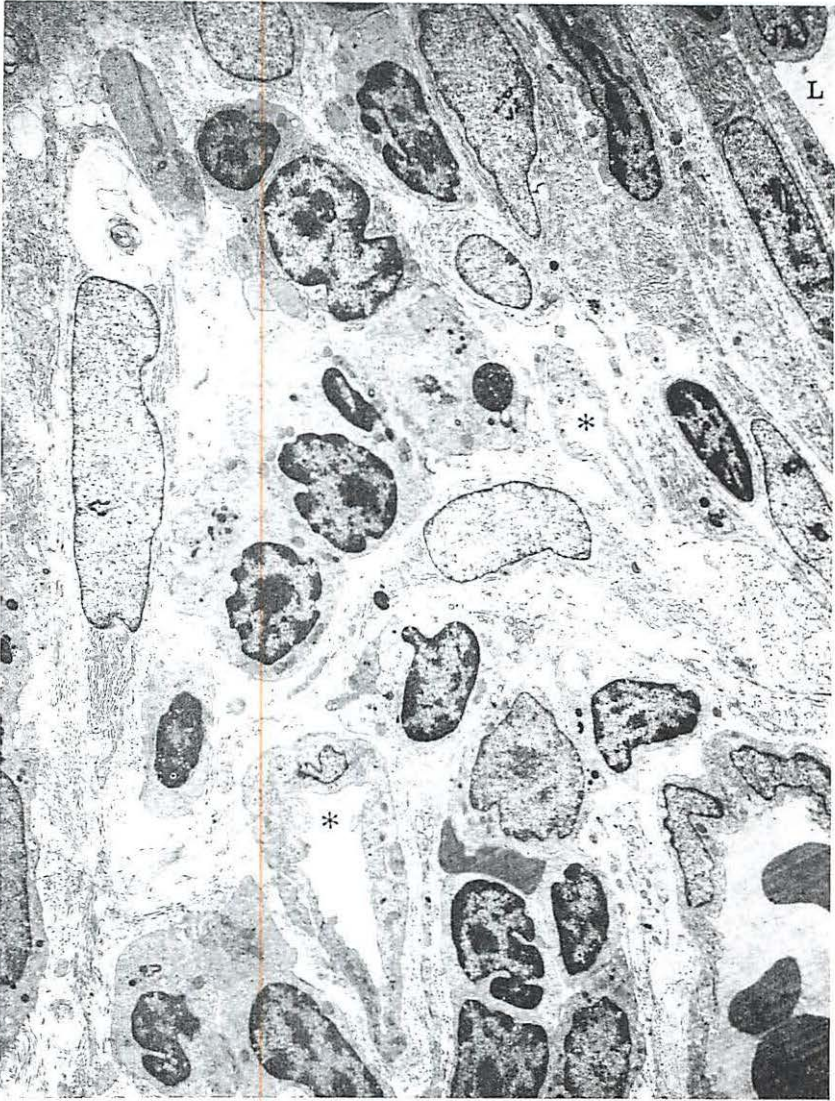


Fig. 5 Electron micrograph of a portion of the wall of an occluded lymphatic vessel one week after ligation. Note the numerous fibroblasts, macrophages, lymphocytes and two newly formed vessels (*) in the subendothelial area. At the upper right, the lumen of the ligated lymphatic (L) is visible. X 3,750

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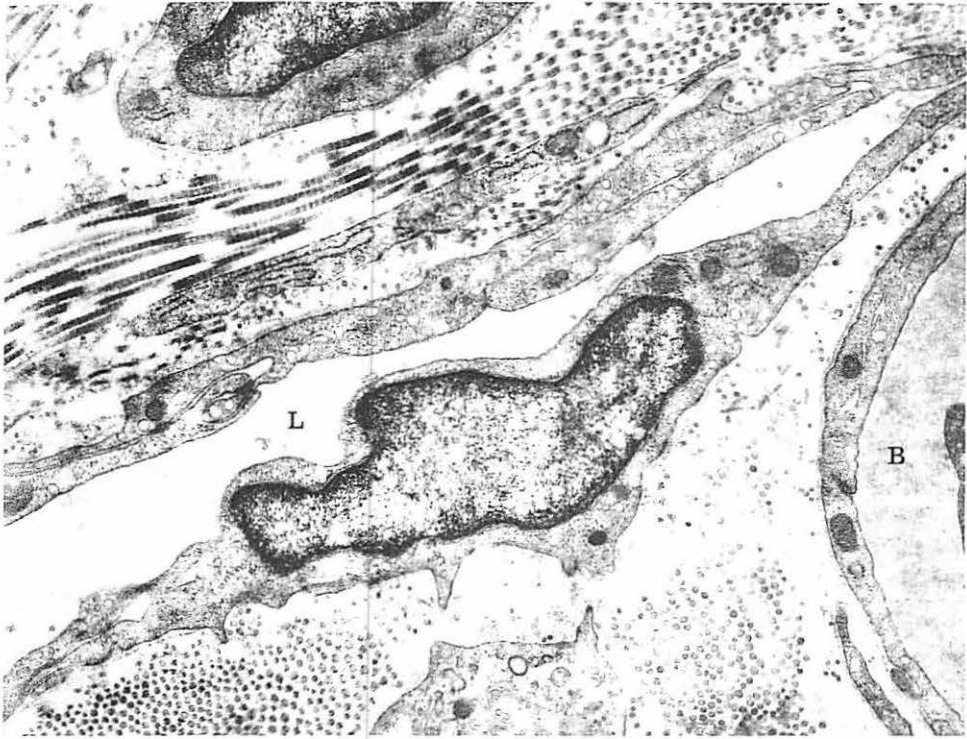


Fig. 6 A newly formed lymphatic vessel (L) and a blood capillary (B) in the ligated lymphatic wall four weeks after ligation. Structural features of the lymphatic vessel are quite different from that of the blood capillary. X 11,000

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