No pathology was found by histologic examination of the right iliac lymph nodes. The small lymph node metastasis on the left side could, of course, not cause any changes in the contralateral lymph flow.

The possible lymphographic demonstration of the inferior epigastric lymph nodes is of importance in patients scheduled for pelvic lymphadenectomy under X-ray control. The radiograms available in the operating theatre are in antero-posterior projection only. The inferior epigastric nodes are projected over the small pelvis and may, unless recognized in advance, easily be mistaken for nodes left behind in the internal iliac region (Fig. 2), where complete dissection is particularly difficult (4, 5).

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Lymphology 7 (1974) 160-168

Distribution and Ultrastructure of the Initial Lymphatics of Some Skeletal Muscles in the Rat

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Summary

The distribution and ultrastructure of initial lymphatics capillaries were studied in m. spinotrapezius, m. gastrocnemius, and m. soleus of the rat. It was found out that the initial lymphatic network starts in the form of blind capillary processes in the area of stems of terminal venules. The lymphatic capillaries converge from these areas into larger perimysial spaces, where they fuse into a larger lymphatic capillary having 30-60 μm in diameter. This capillary runs along together with the blood vessels into the muscular hilus, where it divides into 2-3 branches, also of capillary character.

The ultrastructure of all followed parts of the lymphatic network was quite congruent with the general description of the structure of the lymphatic capillaries. Multiple mast cells were detected in the close neighbourhood of the terminal venules and lymphatic capillaries, and so their possible influence on the dynamics of the contacts of endothelial cells of the lymphatic capillaries is discussed in connection with the regulation of the lymph production in the skeletal muscle.

Introduction

The structure and function of the terminal vascular network of the skeletal muscles has been recently studied in detail. In a number of studies it has been pointed out that even the intramuscular lymphatic vessels may, beside the venous part of the vascular network, significantly participate in the drainage of many products of the muscular metabolism (10, 18, 22, 27, 28). Experimental studies of this kind, however, lack an exact morphological correlation, as the recent data on the distribution and structure of intramuscular lymphatic vessels are mostly very poor, considerably not uniform, and mostly of relatively old date.

Some authors like *Bartels* (2), *Eisler* (6), *Yoffey and Courtice* (33) and many others were of the opinion that muscles, especially the small ones, have no lymphatic vessels of their own. According to their views the tissue fluid is, during muscular contractions, expelled from the muscular fibres towards the surface of the muscle, where it is resorbed in the fascial lymphatic network. On the other hand *Aagaard* (1), *Jossifow* (11), *Nadezhdin* (21) detected, with the help of the injection method, that there are lymphatic vessels situated parallelly with blood vessels in most of the intramuscular septa. They also found out that a delicate tridimensional network of lymphatic capillaries, starting in internal perimysia, opens into these lymphatic vessels. This scheme was rapidly accepted and has been still handed down, even if *Shdanow* (25) warned against the generalization of the presented theory knowing that the method of injecting intramuscular lymphatic vessels is rather rough and brings with artefacts in most cases.

Later on, *Kozma and Gellért* (13, 14), who proved the presence of initial lymphatic capillaries in perimysium internum with histological methods, made these findings substantially more precise. In a number of other studies dealing with the morphology of various parasites and their localization in the skeletal muscles (29, 30, 31) the problem was being dealt with more in detail but, nevertheless, no exhausting survey of the distribution and ultrastructure of the intramuscular lymphatic network has been presented.

We met this problem in studying the architecture and ultrastructure of the terminal blood vessels in m. spinotrapezius of the rat (26). In evaluating the preparations from this vaslular region we found, in a lot of places, also various parts of the lymphatic vessels (unpublished results) in the close neighbourhood of blood vessels. Due to the above mentioned lack of literary data we were not able to identify precisely these mostly haphazard parts of the lymphatic network. Hence we decided to complete our previous reports by a systematic study of the distribution and ultrastructure of the intramuscular lymphatic network. The obtained results are presented in this study.

Material and Methods

On the basis of the above mentioned data we supposed that the lymphatic vessels would be localized mostly in the neighbourhood of the blood vessels. Hence we took the muscle with a very simple architecture of blood vessels, enabling an easy detection, identification, and differentiation from the lymphatic vessels, as the main object of our study. This was a narrow muscular stripe on the ventral border of m. spinotrapezius of the rat, whose terminal network had been previously described (26) both from the point of view of its architecture and ultrastructure. Our findings in this respect can be shortly summarized as follows: in the middle of the stripe we see both the arteriole and venule, from which terminal arterioles alternating with terminal venules branch off at regular intervals

(Fig. 1). In addition to this area the lymphatic vessels were looked for, for orientation reasons, in larger intramuscular septa of the trapezius proper and in the spaces of hili of m. soleus and m. gastrocnemius.

Eight rats (Wistar) of both sexes, weight 120-370 g, were used for the investigation. The correspondent muscular areas were prepared and fixed "in situ" with the help of 1% solution of OsO_4, buffered after *Millonig* (20) for about 5 minutes. Afterwards these parts of the muscle were removed (the ventral stripe of the spinotrapezius as a whole, from the remaining muscles only blocks of the volume of cca 1 mm^3) and put into the same fixation for 2 hours at 4°C. Dehydratation in alcohol and embedding in Epon 812 followed. The blocks from spinotrapezius proper and from the hili of the soleus and gastrocnemius were cut "in toto", from the ventral stripe of the spinotrapezius we cut smaller blocks containing terminal vessels, central vessels, and the area of the hilus, as shown in Fig. 1. Semi-thick sections were prepared with the help of the ultramicrotome TESLA BS 490 A, and stained with toluidine blue and pyronine. Ultra-thin sections of grey up to golden colour were contrasted with uranylacetate and lead citrate after *Reynolds* (23) and observed in the table electron microscope TESLA BS 242 E.

Results

1. Light microscopic findings

In studying the semi-thick sections we did not find any lymphatic vessels in the area of the stems and branches of the terminal arterioles, nor in the branches of capillaries, in the close proximity of individual muscular fibres and superficial fascia of the muscles. The only regions where there was regular occurrence of initial lymphatic capillaries, were the areas of the stems of the terminal venules, especially in places where postcapillary venules opened into them (Fig. 2). The lymphatic capillaries started here, blind, as simple endothelial tubes of 9-12 μm in diameter, having a curved wall with minute finger-like processes. From the blood capillaries they evidently differentiated in the diameter of their lumen and by the curved wall. The lymphatic capillaries in all cases very closely approached the walls of the postcapillary and terminal venules, so that they were closely related even with the mast cells, richly scattered in the adventitia of this part of the venous network. The described initial lymphatic capillaries followed the course of the terminal venules and, together with them, got into the centrally localized intramuscular septum, in which the central arteriole and venule are situated (Fig. 3).

In this region the lymphatic capillaries connected into a larger vessel which was oriented the same as the blood vessels and ran mostly between them (Fig. 4). The diameter of this lymphatic vessel varied mostly between 14-40 micrometers, but in places where it was running more closely to the adjacent arteriole and venule its diameter decreased to only 2-3 μm. Even this vessel had an irregularly curved wall, formed only by the endothelium, had no valves, and hence it was classified as a lymphatic capillary. The close relation of the mast cells to the wall of the venules and the lymphatic capillaries was evident even at this level.

While this collecting capillary did not branch in the ventral stripe of the spinotrapezius, there were a lot of intramuscular septa in the spinotrapezius proper, being irregularly branched into 2-3 branches of 1-10 μm in diameter (Fig. 5). From the septal spaces the collecting lymphatic capillary followed the course of the blood vessels up to the space of the hilus. Here it was already constantly divided into 2-3 branches of 30-60 μm in diameter, localized again in the close proximity of the adventitia of the venule (Fig. 6).

Quite the same relations in the localization of the lymphatic vessels were found even in the hili of m. gastrocnemius and m. soleus, where the lymphatic branches were situated

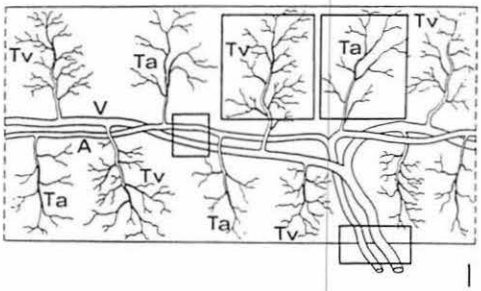

Fig. 1. Scheme of arrangement of the vascular network in the ventral stripe of m. spinotrapezius. Rectangles contain spheres cut for individual procedures after embedding. A – arteriole, Ta – terminal arteriole, Tv – terminal venule, V – venule.

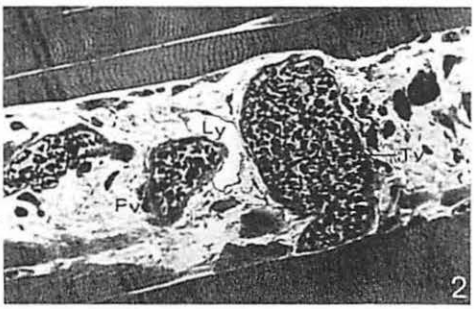

Fig. 2. Ventral stripe of m. spinotrapezius. Initial lymphatic capillary (Ly') lies between the terminal venule (Tv) and the postcapillary venule (Pv). x 430.

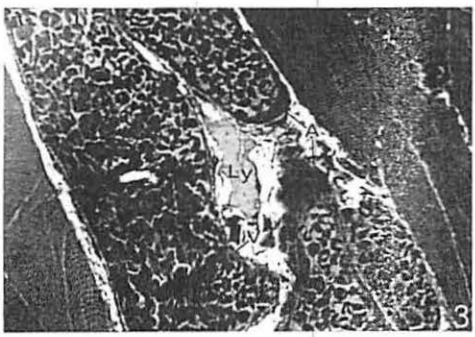

Fig. 3. Ventral stripe of m. spinotrapezius. Collecting lymphatic capillary (Ly) runs between the arteriole (A) and venule (V) in the intramuscular septum. x 600.

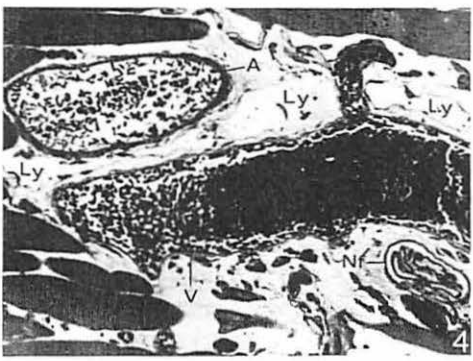

Fig. 4. Longitudinal section through the intramuscular septum of the ventral stripe of m. spinotrapezius, containing arteriole (A), venule (V), collecting lymphatic capillary (Ly) and myelinized nerve fibres (Nf). x 380.

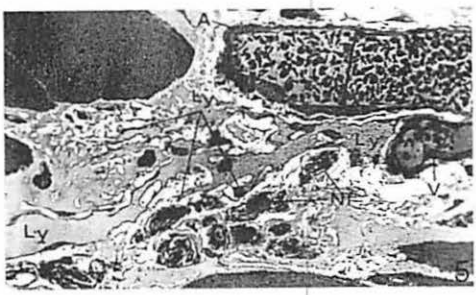

Fig. 5. Longitudinal section through a larger intramuscular septum of m. spinotrapezius proper. Collecting lymphatic capillary (Ly) runs in the proximity of arteriole (A), venule (V) and nerve fibres (Nf). In its middle part it is divided into 2-3 thinner branches. Initial lymphatic capillaries (Ly') open into it from the periphery. x 430.

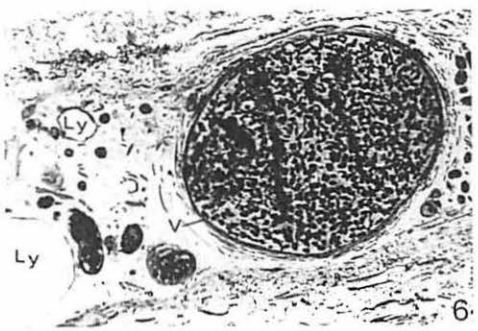

Fig. 6. Transversal section through the hilus of the ventral stripe of m. spinotrapezius. Lymphatic capillaries lie in a close proximity of the adventitia of the hilar venule (V). x 430.

in the close proximity of the hilus veins (Fig. 7 and 8). At this level as well the wall of the lymphatic vessels was made up of the endothelium only, being, however, far less curved if compared with the initial areas. The same as in all more peripheral areas, not a least hint of any presence of valves was detected here.

The distribution of the intramuscular lymphatic network, as detected in the light microscope for the ventral stripe of the spinotrapezius, is schematized in Fig. 9.

2. Electron microscopic findings

The ultrastructure of the wall of the lymphatic capillaries was studied in all above mentioned areas. It was found out that in all followed levels the intramuscular lymphatic vessels had a congruent submicroscopic structure, corresponding with the structure of the lymphatic capillaries in all directions.

The nuclei of endothelial cells (Fig. 10) had a nearly oval form and their longer axis was roughly parallel with the longitudinal axis of the capillary. The surface of nuclei was mostly curved, sometimes even lobular. In the area of the nucleus the endothelial cells were about 2-3 μm thick. In the immediate proximity of the nuclei most of the cellular organelles were localized. Most of them were oval protracted mitochondria, rough endoplasmic reticulum, and multiple ribosomes, localized free in the cytoplasma. In a lot of cases we also observed the presence of centrioles. The peripheral areas of the endothelial cells were far thinner if compared with the nuclear area, i.e. about 0.25-0.40 μm or, in some extreme cases, only 0.05 μm (Fig. 11). The luminal as well as abluminal surfaces of the endothelium had multiple vesicles of the same character as the pinocytotic vesicles of blood capillaries.

The external surface of the endothelium was covered by an incontinuous but well apparent basal membrane. In a close proximity of the endothelial cells we found multiple, minute bundles of collagen fibres with various space orientation, as well as solitary fibrocytes. In no cases there were any pores or fenestrations in the endothelium, that are typical for the blood capillaries in a number of body areas.

Areas of contact of neighbouring endothelial cells were also studied in great detail. In our material there were two different types of contact of these cells:

1. Relatively most frequently the endothelial cells were simply shifted in the intervals of about 1-2 μm (Fig. 12 and 13). In most of the areas the cellular membranes were isolated by a narrow intracellular space and did not merge into close contacts (tight junctions). Rather very frequently we observed local dilatations of the central part of the intercellular spaces forming clefts (Fig. 12).

2. In a minority of cases we observed that the neighbouring endothelial cells were mutually wedged in the form of simple or multiple digitations (Fig. 14). Even in this type of contact there was a narrow intercellular space, but in a lot of places the cellular membranes approached more closely so that these places reminded of areas of maculae adherentes. Completely open spaces between endothelial cells (patent junctions) were not detected in any of the observed areas of the lymphatic network.

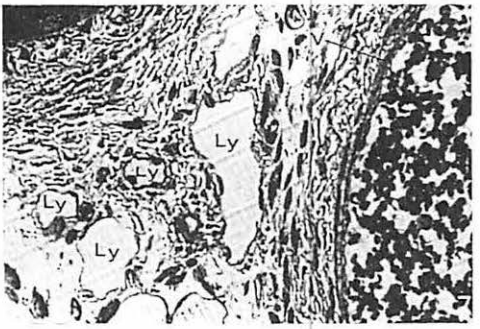

Fig. 7. Transversal section through the hilus of m. gastrocnemius. Externally from the adventitia of the hilar vein (V) run the lymphatic capillaries (Ly). x 580.

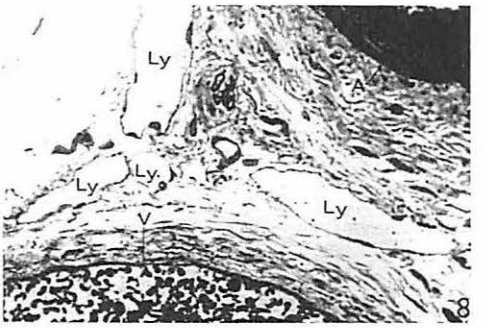

Fig. 8. Transversal section through the hilus of m. soleus. Large lymphatic capillaries (Ly) are localized between the hilar artery (A) and the vein (V). x 530.

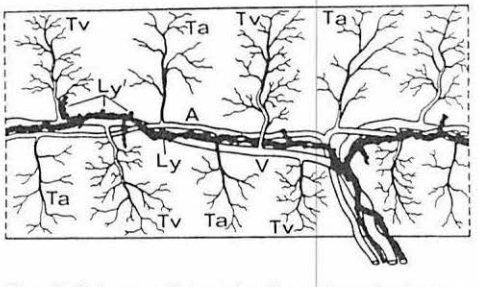

Fig. 9. Scheme of the blood and lymphatic network of the ventral stripe of m. spinotrapezius. A – arteriole, Ly – initial lymphatic capillaries, Ly – collecting lymphatic capillary, Ta – terminal arteriole, Tv – terminal venule, V – venule.

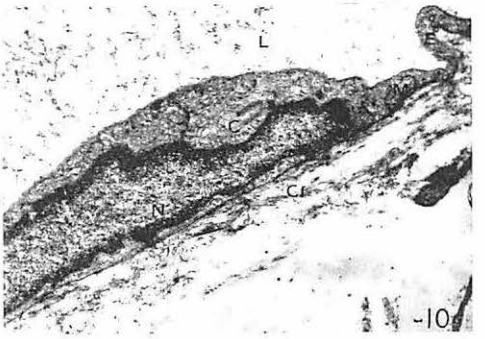

Fig. 10. Nuclear area of the endothelial cell (E) from the wall of the collecting lymphatic capillary. C – centriole, Cf – collagen fibres, L – lumen, M – mitochondrion, N – nucleus. x 15,360.

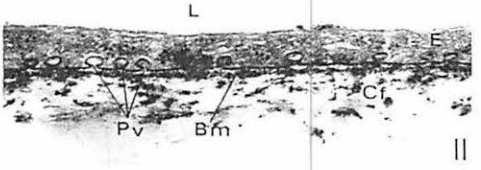

Fig. 11. Peripheral area of the endothelial cell (E) from the wall of the collecting lymphatic capillary. Bm – basal membrane, Cf – collagen fibres, L – lumen, Pv – pinocytotic vesicles. x 41,100.

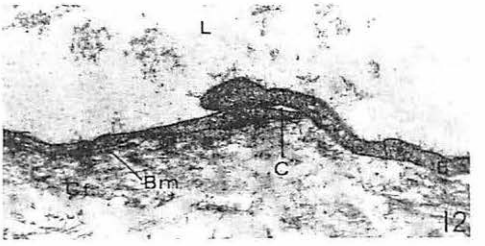

Fig. 12. Dilated middle part (cleft) in a simple type of contact of endothelial cells. Bm – basal membrane, C – cleft, E – endothelial cells, Cf – collagen fibres, L – lumen. x 23,100.

Fig. 13. Simple type of contact of neighbouring endothelial cells (E) from the wall of the lymphatic capillary. Cf – collagen fibres, L – lumen, M – mitochondrion, x – place of contact. x 15,360.

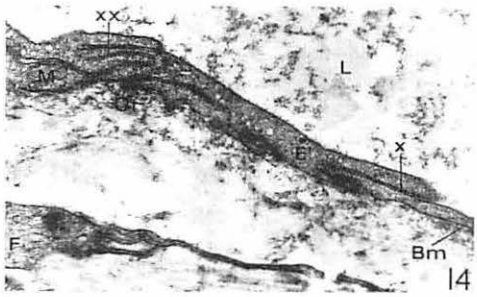

Fig. 14. A more complicated type of the contact of neighbouring endothelial cells (E) in the wall of the collecting lymphatic capillary. On the right – simple digitation (x), on the left – multiple digitation (xx). Bm – basal membrane, F – fibrocyte, Cf – collagen fibres, L – lumen, M – mitochondrion. x 23,100.

Discussion

The presented findings can be compared with the above mentioned observations of elderly authors who followed the distribution of the intramuscular lymphatic vessels with the help of the injection method, as well as with the results concerning the ultrastructure of initial lymphatic vessels in other organs.

In a complete disagreement with the findings of *Bartels* (2), *Eisler* (6), *Yoffey and Courtice* (33) and in agreement with the recent findings (32) it was found out that the lymphatic vessels may be detected both in relatively small and thin muscles as well as in more massive muscles. Our light microscopic observations confirm the findings of *Kozma and Gellért* (13, 14), *Šlais* (30, 31) and of other authors, i.e. that the initial areas of the intramuscular network may be detected in perimysial spaces and in greater intramuscular septa. Their conclusions may be made more precise in several aspects. First of all it was found out with certainty that the initial lymphatic capillaries are localized exclusively in the close proximity of the stems of the terminal venules and do not project into the areas of terminal arterioles or individual muscular fibres, as known for example for the myocardium.

The lymphatic vessels were not observed in the fascia of the studied muscles. Besides, in contradiction to the findings of *Aagaard* (1), *Gatzalov* (7), *Nadezhdin* (21) and of other authors we did not find the presence of the tridimensional network of initial lymphatic capillaries. This contradiction may be explained by the fact that the muscles of rat, used for the experiments, especially the ventral stripe of the spinotrapezius, are flat, and relatively thin, so that a relatively simple architecture of the lymphatic network may suffice for their drainage. The authors mentioned under studied mostly human muscles, which are far more massive, have stronger muscular fibres and a more complicated internal architecture. Hence we may suppose that in agreement with the more complicated internal architecture of the human muscles even their lymphatic network has a more complicated architecture. We come to this conclusion on the basis of our finding that in larger intramuscular septa of m. spinotrapezius proper the greater collecting lymphatic capillaries made more delicate branches in many places, resembling simple mesh of the tridimensional network. As shown in the studies of *Nadezhdin* (21) and *Šlais* (30) the initial lymphatic network has the form of simple capillaries in these massive muscles.

The ultrastructure of the lymphatic capillaries has recently been described in detail in the studies of *Casley-Smith* (4, 5), *Leake* (15, 16), *Schakhlamow* (24). We have found out that our findings are, in general, congruent with their description as well as with the observations of *Bullon and Huth* (3), *Horstmann and Breucker* (9), *Klika* et al. (21), i.e. especially as far as the description of the structure of the endothelial cells, their discontinuous basal membrane, pinocytotic activity etc. are concerned. Our observation differs only in that we have not found open intercellular contacts in the endothelium which, according to a number of data, belong to the normal picture of lymphatic capillaries. In spite of this we are of the opinion that the intramuscular lymphatic vessels function in the same way as in other areas, and thus enable the transport of a part of the interstitial fluid except pinocytosis, as well as the dilatation of their contacts. Our detection of minute clefts in the area of contacts confirms our opinion that the close contacts are only one of the functional forms of the lymphatic capillaries, not a constant fixed condition. An actual answer to this problem may be obtained by a special study only, if performed by the same way as e.g. in the study of *Casley-Smith* (5) and *Leak* (15, 16, 17).

In this connection we consider for significant to have found mast cells in a relative close proximity of terminal venules and lymphatic capillaries, this localization being very significant from the point of view of both structure and function. It has been known for a long time, namely, that these cells contain a large amount of vasoactive substances of the type of histamine, serotonin and heparine. These substances participate considerably in the transport of fluids through the wall of the terminal venules in the course of an inflammatory process, due, above all, to the fact that they dilate the contacts of the neighbouring endothelial cells (8, 19). It is very likely that the mast cells may influence even the dynamics of the contact places of the endothelial cells of lymphatic capillaries in a similar way. It has been a general knowledge, namely, that both the production and quality of the lymph in the muscle considerably vary due to various functional conditions. The localization of mast cells in the proximity of lymphatic capillaries supports the hypothesis that, in addition to other factors, these cells may influence the endothelium of the lymphatic capillaries due to their activity, and so participate in the regulation of the transport of the interstitial fluid into the initial processes of the lymphatic network. This hypothesis, however, requires an exact verification by experiments.

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