Lymphology 13 (1980) 202–206

Diabetic Lymphangiopathy. An Optical and Electron Microscopic Study

A. Kaufmann, B. Molnár, C. Craciun, A. Itcus

Institute of Medicine and Pharmacy, Surgery Clinic II and "Babes-Bolyai" University, Department of Biology, Cluj-Napoca, Romania

Summary

The lymphatic capillaries in the tegument of the dorsal side of the leg in patients with diabetes mellitus, and especially in those with clinically manifested macroangiopathy, present morphopathological modifications at the level of the endothelium and the basal membrane. Ultrastructurally, the modifications appear as dilatations of the intercellular space, dislocation or desquamation of the endothelium, an exaggerated growth of the micropinocytic vesicles, the appearance of the intracellular vacuoles and of the lysosomes. These modifications point to an increase in the endothelium permeability, which is an important phenomenon in the diabetic microangiopathy.

Introduction

At present, it is possible to prognosticate diabetes mellitus based on the vasculopathies observed in diabetic patients (1). Numerous studies, associating optical and electron microscopy, have been dedicated lately to the diabetic microangiopathy (2, 3, 4, 6). The lymphatic system, an important return circulatory pathway, has been less studied. The works of *Romani* (7, 8), based on optical microscopy, demonstrated that in the course of diabetes mellitus an important implication of the lymphatic capillaries is present, which seems to be more pronounced and to appear earlier than that of the blood capillaries. Situated at the limit between the blood circulation and the surrounding mesenchyme, the lymphatic vessels form an important link of the circulatory system. At the same time, the lymphatic vessels constitute an important pathway for dissemination of the infections, to which the diabetics are particularly succeptible.

In this paper we are presenting the results of our research on the lymphatic capillaries in the skin of the dorsal side of the legs of patients with diabetes mellitus, as compared to the results obtained in healthy subjects.

Materials and Methods

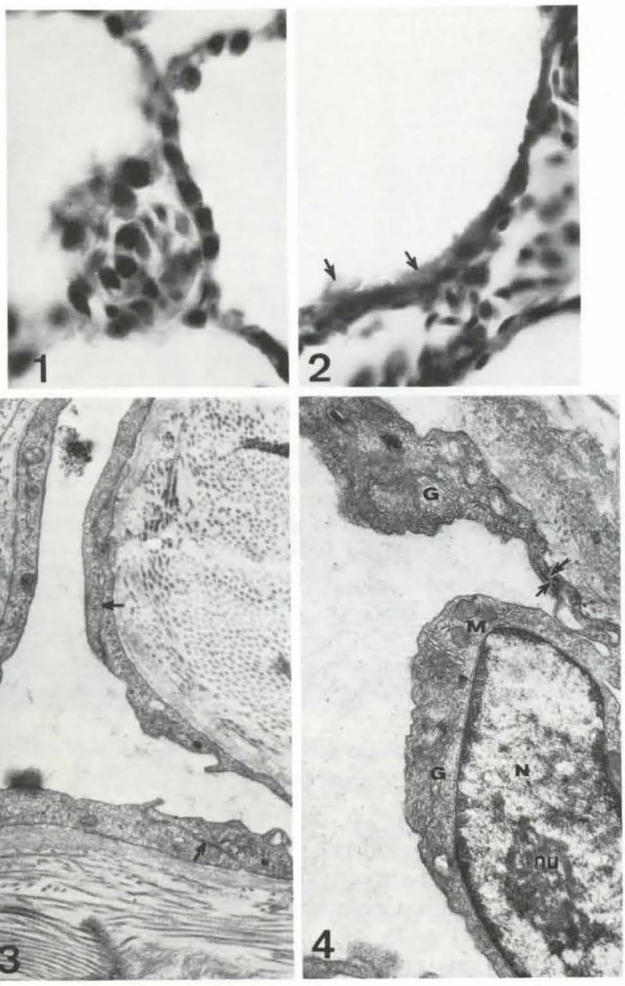
Cutaneous biopsies were performed on three groups of people: 1) Healthy subjects (control group of 20), with a normal glycemia, normal glucose tolerance tests and no hereditary diabetic antecedents. 2) Subjects with a diabetic risk (5 women having children heavier than 4 kg at birth) 3) Diabetic subjects (40 persons with declared diabetes and clinically manifested macroangiopathy of the type of obliterating arteriopathies).

The excised pieces were fixed in the Bouin-Holland and Serra mixtures. From the material embedded in paraffin, serial transverse and longitudinal sections, 7 μm in thickness, were than obtained and coloured with haemalaun-eosin and PAS.

A bilateral lymphography was performed on a group of the diabetic patients which presented obliterating arteriopathy, predominantly manifested in one leg, with chracteristic oscillometric and arteriographic values.

For electron microscopy the pieces were fixed in a 2.5% glutaraldehyde solution in 0.15 M phosphate buffer, washed in 3 consecutive phosphate buffer baths and postfixed in 1% osmic acid. The dehydration was done in successive acetone baths and embedding in vestopal W. The sections were cut with an ultrami-

0024–7766/80 1600–0202 $ 02.00



Figs. 1 and 2 Light microscopy.
Fig. 1 Healthy subject (control). Basal membrane having a weak positive PAS reaction. 70x.
Fig. 2 Diabetic subject. The basal membrane has an intense positive PAS character 70 x
Figs. 3 and 4 Ultrastructure of the lymphatic capillary in healthy subjects (control).
Fig. 3 Intercellular space between endothelial cells not dilated (arrows). 11.000 x.
Fig. 4 Endothelial cells with well contoured Golgi apparatus (G), mitochondria (M), nucleus (N) and nucleolus (nu). 12.200 x

crotome LKB III, contrasted with uranyl acetate and lead citrate and examined in a Tesla BS-613 electron microscope.

Results

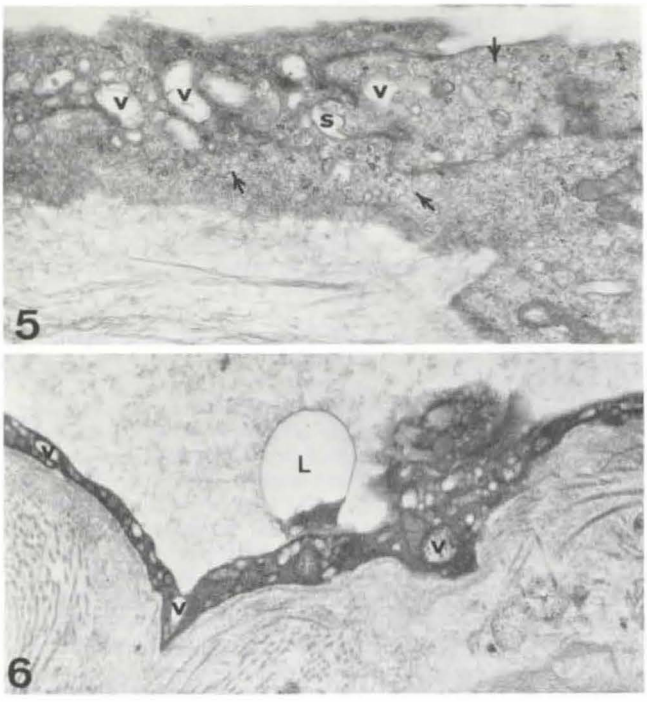
Light microscopy

The lymphatic capillaries on the dorsal side of the legs can be easily observed at the level of the dermal papilla. In longitudinal sections they are seen as tubular formations, with a sinuous pathway consisting in a succession of moderate dilatations. The basal membrane, with a thickness just within optical limits, presents a barely evident positive PAS reaction (Fig. 1). In diabetic patients, the basal zone of certain capillaries is slightly thickened and has a strong positive PAS reaction. The reaction intensity is not uniform in all the patients, and even in the same patient, one can also note the presence of normal lymphatic vessels. It can also be observed that the distribution of the positive PAS substance in the basal zone of the capillary wall is not uniform, but exaggerately concentrated at different levels, covering the endothelium in some places (Fig. 2, arrows).

Electron microscopy

1) Healthy subjects (control group)

The lymphatic capillaries have an endothelium of the continuous type, composed of flattened



Figs. 5 and 6 Ultrastructure of the lymphatic capillary in subjects with diabetic risk.
Fig. 5 Endothelial cells containing a large number of micropinocytic vesicles in the cytoplasm (arrows), dilated intercellular spaces (S) and intracellular vacuoles (V). 15.500 x.
Fig. 6 Intracellular vacuoles (V) and a large lysosome (L). 15.500 X.

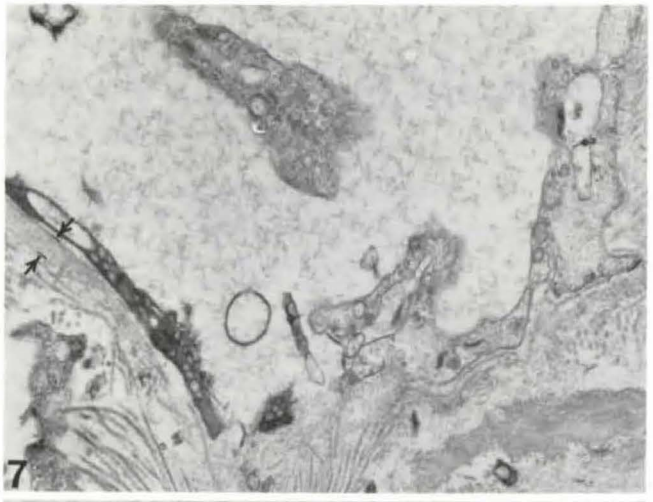
elongated cells. The lateral limits of the cells are perpendicular to the capillary lumen, or they intertwine, forming overlapping layers. The cellular junctions toward the lumen (zonula occludens) as well as those toward the basal portions (zonula adherens, rarely desmosomes) connect the cells uninterruptedly (Figs. 3 and 4, arrows). The cellular cytoplasm shows a slight pinocytotic activity. Intracellular vesicles are rarely observed. The basal zone is thin, in some places irregular in thickness. The nucleus (N) and the organelles have the usual aspect of the endothelial cells (Fig. 4).

2) Subjects with a diabetic risk.
The lymphatic capillaries have an endothelium with a high electron density (Fig. 5). Dilatations of the intercellular space are visible (Fig. 5, S). The cellular cytoplasm is characterized by an intense pinocytotic activity, manifested through the presence of numerous micropinocytic vesicles and the formation of vesicles of 150–250 nm in diameter (Figs. 5 and 6, V). In some capillaries, large vacuoles of a lysosomic nature appear (Fig. 6, L).

3) Diabetic subjects with clinically manifested macroangiopathy.

In this case, the morphopathological modifications of the lymphatic capillaries endothelium are very visible. The endothelium presents a pronounced desquamation process (Fig. 7). As a result of this process in some places, the endothelium is completely missing (Fig. 8). The basal zone, sometimes lacking in clarity, is thickened, especially at the level of the desquamating endothelium (Fig. 7), arrows). The nucleus is intensely electron dense, with a weakly contoured membrane. The Golgi complex and the endoplasmic reticulum are difficult to see or missing in our micrographs. Free ribosomes, often associated in a rosette like shape, are disposed around the intracellular vesicles and in the space between these vesicles.

Our lymphographic results did not reveal modifications of the lymphatic collectors, at the level studied.



Figs. 7 and 8 Ultrastructural aspects of the lymphatic capillaries in diabetic subjects with clinically manifested macroangiopathy.
Fig. 7 Dislocated endothelial cells presenting an intense process of desquamation. 16.200 x.
Fig. 8 Parts of a lymphatic capillary devoid of the endothelium (arrows). 7.500 X.

Discussion

From the analysis of our results, it is clear that in the diabetic patients the small lymphatic vessels undergo important morphopathological modifications in both the endothelium as well as the basal zone. These modifications are more pronounced in the patients with clinically manifested macroangiopathy. Ultrastructurally, the modifications appear as dilatations of the intercellular space, dislocations and desquamations of the endothelium and excessive increase of the pinocytotic activity. This is reflected by the presence of numerous microvesicles at the endothelium level as well as the appearance of intracellular vacuoles and lysosomes.

All these morphopathological data seem to imply a change in membrane permeability of the lymphatic endothelium, as envisaged by the permeability increase and the induction of other structural alterations, resulting in a deficient activity of the endothelium. This suggestion is also based on the experimental findings of *Karaganov and Senatova* (9), and their statement that the incipient stages of the vascular permeability increase are connected to the deregulation of the pinocytotic activity, manifested by the enhancement of microvesicles transport in the endothelial cells. In this sense, the presence of the intracellular vacuoles, of the dilated intercellular spaces, of the lysosomes and the dislocation or desquamation of the endothelium, observed in our diabetic patients, may be considered as indicators of the degree of vascular permeability in the microcirculatory pathway. Similarly, taking into consideration the ultrastructural modifications mentioned above, we could explain our optical microscopic observations regarding the thickening of the basal membrane and the increase of its positive PAS character.

The differences in the parietal structures between arteries and lymphatic vessels, as well as the composition differences between circulating fluids of the two types of vessels can not be neglected when interpreting our results. The normal lymphographic aspect does not

exclude participation of the lymphatic system to the general suffering of the diabetic vasculopathy (5).

References

1 *Mincu, I., S. Cimpeanu:* Angiopatia diabetică, Ed. Medicală, Bucureşti, 1970
2 *Azerad, E.:* La microangiopathie diabétique. Fictions et réalités. La Nouv. Presse Méd., 45 (1973) 3027–3036
3 *Băcanu, S.G.:* Congrena diabetică, Ed. Facla, Timişoara, 1973
4 *Giacomoni, Ph., Ph. Babin:* Aspects ultra-structuraux de la microangiopathie diabétique. Angeiologie, 26, 6 (1974) 243–250
5 *Kaufmann, A.:* Limfografia, Ed. Medicală, Bucureşti, 1970
6 *Kaufmann, A., B. Molnár, A. Iţcus, B. Palmariu:* Modifications au niveau du système lymphatique dans le diabète sucré. 10th Int. Congr. Angiology, Tokyo, 1976
7 *Romani, J.D.:* Les lésions pariétales des capillaires lymphatiques du derme chez les diabétiques. La Presse Méd., 79 (1971) 228–234
8 *Romani, J.D.:* Histologie semi-quantitative de la micro-angiopathie diabétique. Angeiologie, 26, 6 (1974) 251–256
9 *Karaganov, J.L., I.D. Senatova:* Role of vesicular transport in mechanism of leakage's appearance in microvessel's walls. Proc. 10th Int. Congr. Anatomists and 8th Ann. Meet. Jap. Assoc. Anatomists, Tokyo (1975) 349

Dr. Aurel Kaufmann, Surgery Clinic II, Str. Clinicilor, 3400 – Cluj-Napoca, Romania

International Society of Lymphology

2nd German-Japanese Angiological Congress

The second German-Japanese Angiological Congress with lymphological topics will be held in Tokyo, March 15–20, 1981.
For further information please contact:
Yoshio Mishima, M.D., c/o Dept. of Surgery, Univ. of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

*

5th International Congress of Radiology

The fifth International Congress of Radiology will take place in Brussels, Belgium, June 24–July 7, 1981. There are separate sessions for radiotherapy and nuclear medicine and radiodiagnosis including ultrasonography and computerized tomography. One session will be devoted to "limits of effectiveness of lymphology".
For further information please contact:
Secretary General ICR, c/o Foire Internationale de Bruxelles, Parc des Expositions, B 1020 Bruxelles, Belgium

*

FORTHCOMING CONVENTIONS:

8th International Congress of Lymphology

The 8th International Congress of Lymphology will be held at the Meridien Hotel, Montreal, Canada, from September 20th to 25th, 1981. Informations about room reservations, social program including banquet, tours and tennis tournament will be sent to the members of ISL in October 1980.

The main topics of the scientific program are the following:

I – Basic sciences:
anatomy, physiology, pharmacology, immunology, tumor spread, pathology, hematology, microcirculation

II – Clinical investigations of lymph system:
Radiology: lymphography, angiography, computed tomography, ultrasonography, nuclear medicine

III – Oncology:
radiation therapy, chemotherapy, surgery, immunotherapy, endolymphatic therapy

IV – Pathology:
lymphoma, tumors other than lymphoma

V – Angiopathies

VI – Lymphedema

VII – Intestinal lymphatics

VIII – Others – Free communications

IX – Poster – Session

Attention! Deadline for submission of abstracts has been changed to February, 25, 1981

Fort further information please contact:
Dr. *Maurice Falardeau*, general secretary, 8th International Congress of Lymphology, Hopital Notre Dame, 1560 Sherbrooke St. East, Montreal, P.Q., Canada H2L 4K8