

## Histochemical Change of the Endothelial Basal Lamina of the Diabetic Lymphatic Vessel\*

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### Summary

Electron microscopic periodic acid methenamine silver staining of lymphatic endothelial basal lamina taken from diabetic patient has shown a positively changed basal lamina, which is an early sign of the changes and plays an important role in pathological tissue changes in the disease.

It has been suspected that not only the blood vessel but also the lymphatics may play a role in pathological tissue changes in diabetes. Periodic acid methenamine silver staining (PAM), which is considered to stain at least a part of polysaccharide, has been performed using the subcutaneous lymphatic vessel of healthy human (1). And it has been disclosed that the endothelial basal lamina is negatively stained, whereas the membrane system of the endothelial cell is positive. This experiment was performed to see the changes of the lymphatics in diabetes.

### Methods

The subcutaneous lymphatic vessel was taken under local anesthesia from a 39 year-old diabetic male with abnormal glucose tolerance test, fixed in glutaraldehyde, dehydrated, embedded in Epon and the ultrathin sections were stained on the gold grid by periodic acid methenamine silver after *Yajima* (2). Some tissues are post-fixed in osmium tetroxide dehydrated, embedded in Epon as usual and sections were stained with uranyl acetate and lead. The light microscopic observations were done after HE and PAS staining fixing the tissue in formalin.

### Results

The light microscopic observation after staining the sections with HE and PAS has revealed no remarkable changes. The PAM staining of the lymphatic vessel shows a positively changed basal lamina of the endothelial cell as well as positive membrane system. The collagen fibers are also stained (Fig. 1). The controls stained

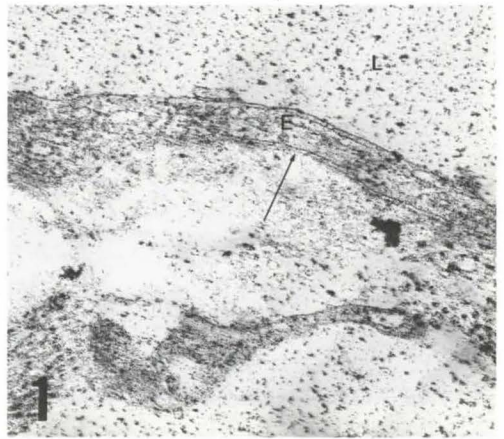


Fig. 1 PAM staining of the subcutaneous lymphatic vessel taken from the dorsal foot of a 39 year old diabetic male. The membrane system as well as the basal lamina (L) shows positive silver particle. Fixation; glutaraldehyde and osmium tetroxide. Embedding; Epon 812. x 38 000

in the same way without periodic acid shows also positive collagen fibers and chromatin of the nucleus which are considered to be unspecific for the staining, although the basal lamina has turned out to be negative and

\* A part of the work was presented in the 5th International Congress of Lymphology, 1975.

\* This work is supported by the Grants from the Japanese Ministry of Education

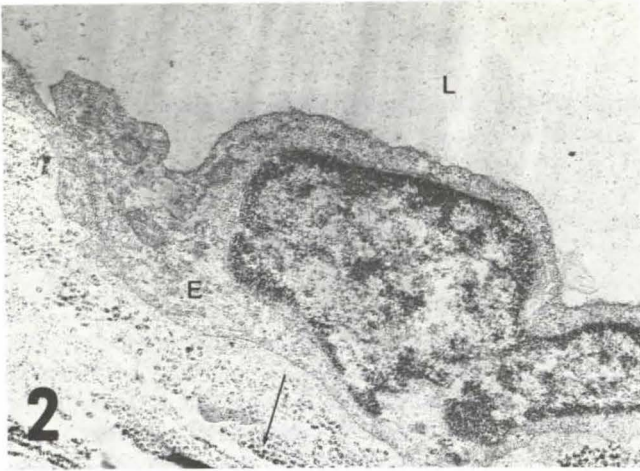


Fig. 2 Control specimen stained in the same way without periodic acid in the solution. The nuclear chromatin and collagen fibers (E) reveal positive staining, which is considered to be unspecific for the staining. The membranes are also partly weakly positive. x 10 000

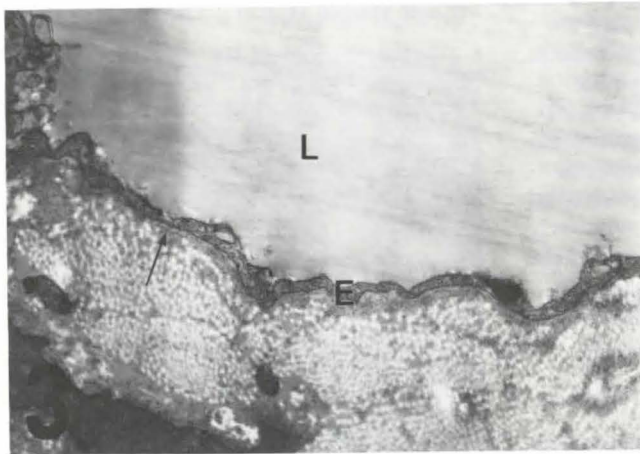


Fig. 3 The lymphatic fixed in glutaraldehyde and osmium, embedded in Epon and stained with uranyl acetate and lead as usual. The continuous basal lamina (L) is observed. x 18 000

some of the membranes are only weakly stained (Fig. 2). There is no difference in findings in specimens stained for 60 minutes and those for 120 minutes. Another control stained without silver has proved no positive staining at all. The usual electron microscopic examinations staining the sections by uranyl acetate and lead citrate after postosmification of the tissue have revealed a never interrupted basal lamina of the lymphatic endothelial cell (Fig. 3).

#### Discussion

It is to be further investigated whether this PAM staining is specific for polysaccharide or not. However the staining is for at least a

part of the substance, since *Yajima* has performed the staining on the tissue before and after digestion by hyaluronidase and neuraminidase and confirmed the nature of the PAM staining (2). The duration of staining is enough for 60 minutes, because the further incubation does not make any difference but extent of contamination. The positive stained collagen fibers and the nuclear chromatin are thought to be unspecific for the PAM, since the control staining without periodic acid shows also positive findings.

The light microscopic examinations shows no remarkable changes and this patient is considered to be in the early stage of diabetic change. It is not to be concluded whether this change in the lymphatic is due to arterio-

sclerosis which is, sooner or later, occurred in case of diabetes. But diabetic change of the tissue is not advanced in this patient and also the ocular fundus and X-ray chest findings have revealed no evidence of arteriosclerosis. Therefore the basal lamina change in the patient is looked upon as diabetic and not as arteriosclerotic one.

It is highly suspected that this change of the basal lamina of the diabetic lymphatic vessel may produce a changed permeability and a disturbed metabolism of its wall inducing a disordered function of the vessel

with deposit of some substances in the tissue which is to be carried away under normal condition by the normal functioning lymphatic vessel. This accumulation of polysaccharide in the basal lamina of the lymphatic may play an important role in pathological tissue change in case of diabetes.

#### References

- 1 *Ohkuma, M.*: Periodic acid methenamine silver staining of the human subcutaneous lymphatic vessel. *Acta Dermatovenerol.* 55 (1975) 51
- 2 *Yajima, G., K. Aihara*: Periodic acid methenamine silver staining. *Igaku no Ayumi* 76 (1971) 328

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