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PERIODIC ACID METHENAMINE SILVER (PAM) STAINING OF THE HUMAN SUBCUTANEOUS LYMPHATIC VESSEL

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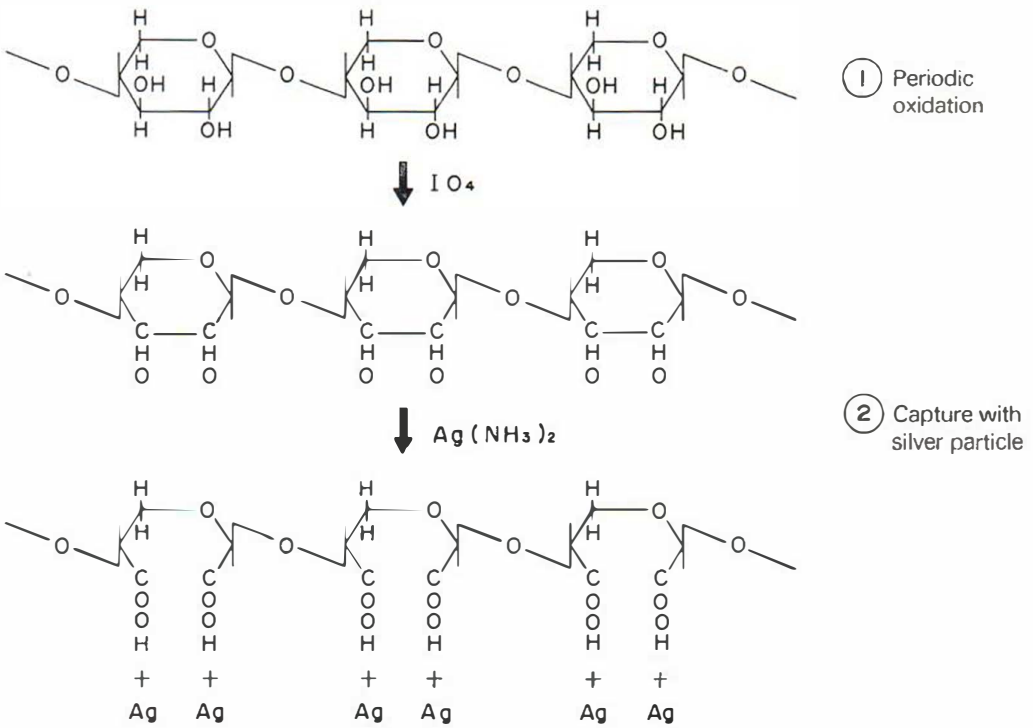
Abstract. The fine structure of the subcutaneous lymphatic vessel taken from the dorsum pedis of the human adult was investigated without and after impregnation by periodic acid methenamine silver (PAM). To check the stainability of another tissue, the renal glomerulus of the rat was also examined in the same way as outlined above. The membrane system of various cells stains positive. No special surface coat of the endothelial cell of the lymphatic vessel was detected. The fine intracellular filament as well as a continuous basal lamina of the endothelial cell were almost PAM-negative, although a part of the basal lamina was occasionally weakly positive. The chemical composition of the basal lamina of the lymphatic vessel is considered to differ from that of the glomerular blood capillary of the kidney, which is PAM-positive. This difference is thought to be due to the smaller polysaccharide content in the former than in the latter.

Key words: Lymphatic vessel; Basal lamina; PAM staining

Periodic acid methenamine silver staining for electron microscopy has been tried by many investigators (1, 2, 3, 5, 6, 7, 8). It is not certain which substance this PAM method stains selectively. How-

Table I. *Possible mechanism of periodic acid methenamine silver staining suggested by Yajima* (7)



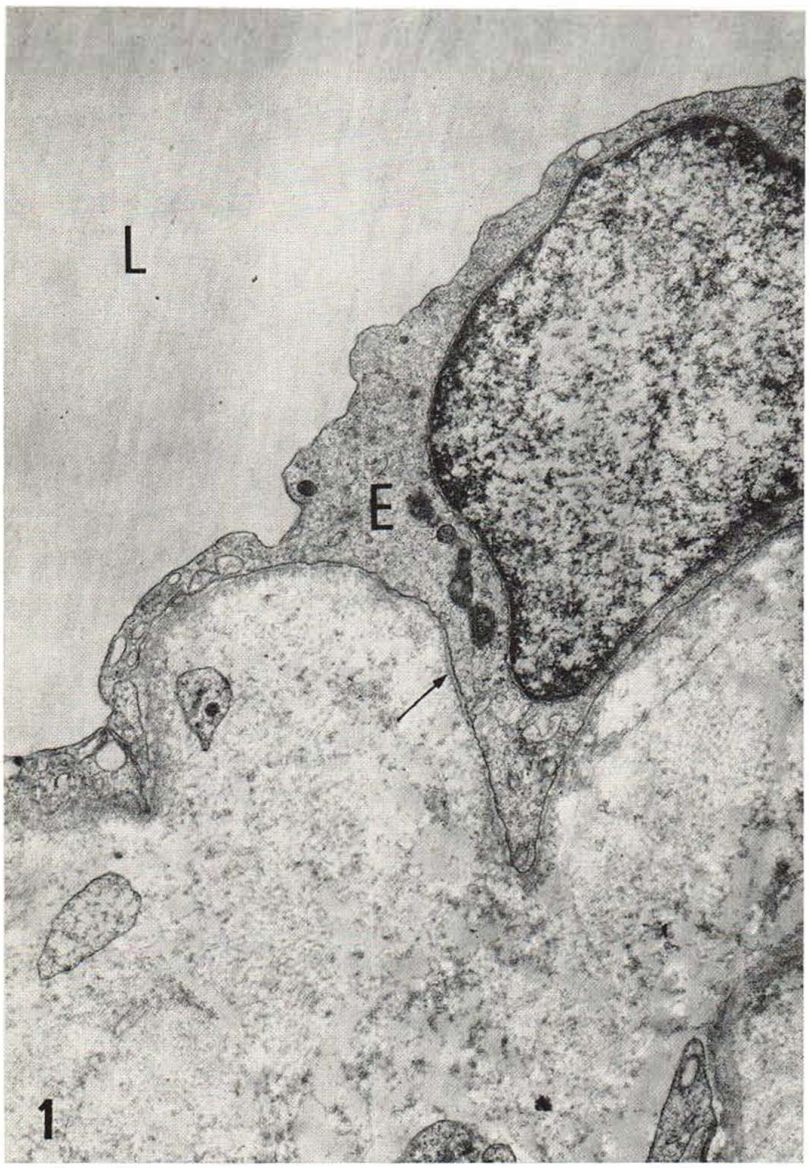


Fig. 1. Endothelial cell (*E*) of the subcutaneous lymphatic vessel is surrounded by continuous basal lamina (↗). In the connective tissue area there are numerous collagen fibers. *L*, lumen. Stained with uranyl acetate and lead citrate. × 13 000 (Figs. 1–3: human subcutaneous lymphatic vessel).

ever, Yajima & Aihara (7) have used this method to stain a variety of tissues, including renal glomerulus, pulmonary alveolar lining layer, axon of the neuromuscular junction, collagen fiber, and virus particle, before and after digestion by bovine testicular hyaluronidase as well as by neuraminidase. The PAM-positive substance disappeared or diminished in amount after digestion by the above enzymes. Yajima & Aihara also made comparisons with the results in the fine structure of different tissues stained by ferric oxide (Gasic et al.), colloidal iron (Rinehart & Abul-Haj), colloidal iron (Mowry), lanthanum nitrate (Revel & Karnasky), ruthenium red (Luft), alcian blue (Ohkura), silver proteinate (Thiery) and phosphotungustic acid (Marinozzi). It is concluded that this PAM method stains at least a part of polysaccharides by the staining mechanism shown in the Table I.

This is the first report on PAM staining of the subcutaneous lymphatic vessel of the human dorsum pedis, which is normally used to inject contrast medium for lymphography.

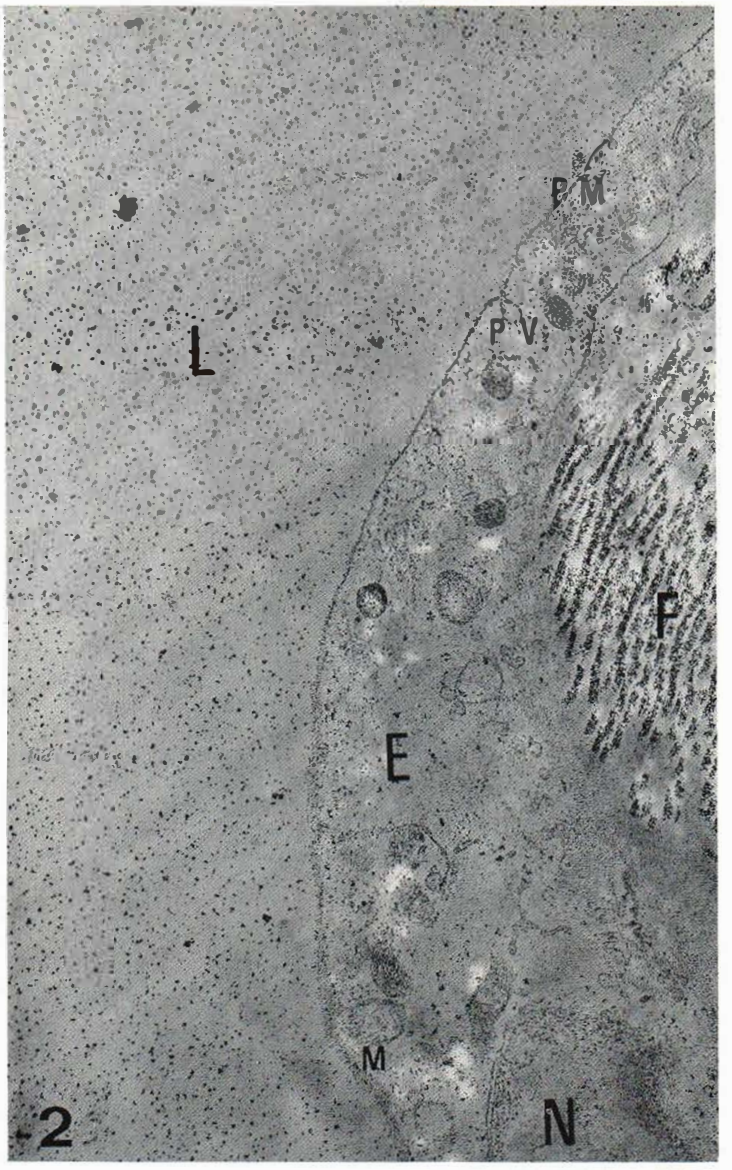


Fig. 2. The plasma membrane (*PM*), membrane of pinocytotic vesicle (*PV*), of mitochondrion (*M*) and of nucleus (*N*) are all positively stained, whereas the basal lamina of the lymphatic endothelium (*E*) is negative. Collagen fibers (*F*) in the connective tissue area are also stained by this method. *L*, lumen. PAM staining, × 20 000.

MATERIAL AND METHOD

The mixture of 1 ml of 1% hydrochloric procaine and 1 ml of patent blue was injected into the dermis between the 1st and 2nd toe of two subjects, 43 and 74 years old. Three hours later, the blue-stained subcutaneous lymphatic vessel of the dorsum pedis was exposed, excised, and fixed with 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2–7.4) for 2 hours at 4°C. Postfixation was performed by 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hours, dehydrated in ethanol and embedded in Epon 812. PAM impregnation after Yajima (7) was applied for ultrathin sections on a gold grid for 90 and 120 minutes at 60°C. For the control, several sections were incubated in the same solution but without silver nitrate and some others were stained in the same way as above though without the oxidating procedure with periodic acid. To understand the normal fine structure of the lymphatic vessel, some sections were stained with uranyl acetate and lead citrate as usual.

In order to make a comparison with the basal lamina of another tissue, the kidney of Sprague-Dawley rat was excised under ether anesthesia, prepared and stained by PAM in the same way, and the glomerular capillary examined.

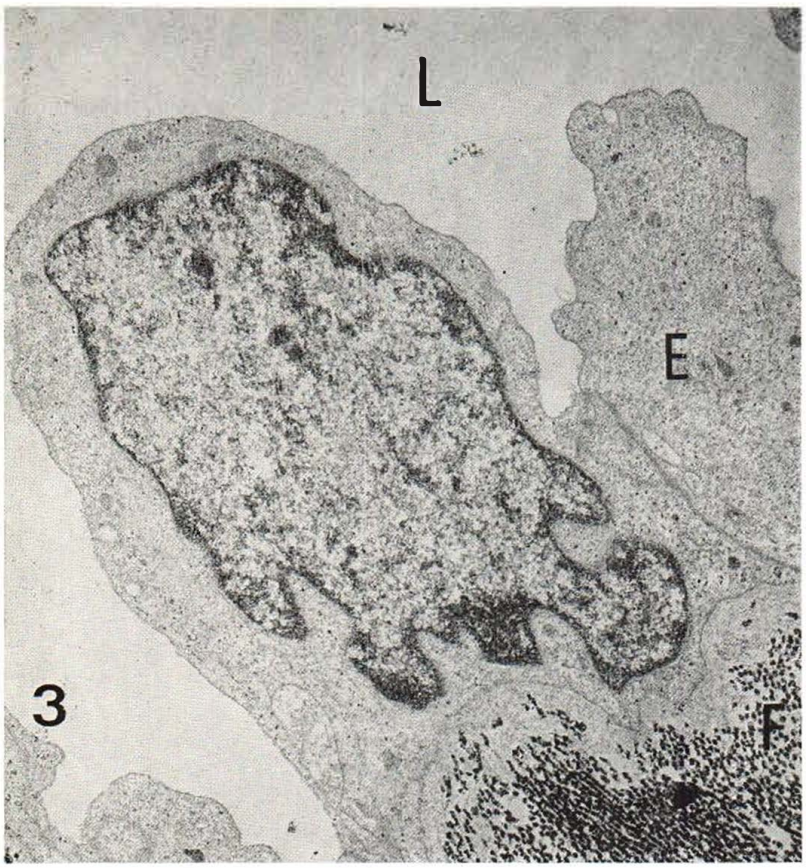


Fig. 3. Silver deposit of the membranes of the endothelial cell (*E*) is smaller in amount and the basal lamina of the endothelium is still negative, yet collagen fibers show the same strong reaction. *L*, lumen. PAM staining without oxidation by periodic acid, ×11 500.

RESULTS

Fine structure of the lymphatic vessel

In the lumen there are fine, fuzzy materials, fibrin, and sometimes red blood corpuscles. The contour of the lumen is irregular with rich luminal and abluminal endothelial projections.

The endothelial cell is rarely defective. It is always surrounded by the continuous basal lamina (Fig. 1), with which some fibrils are connected. The endothelial cell contains less pinocytotic vesicles but otherwise cellorganelles similar to those of the lymphatic capillary endothelium (3).

The smooth muscle cell in the wall shows myofilaments in the cytoplasm as well as a number of pinocytotic vesicles, especially near the plasma membrane and has an uninterrupted basal lamina. Some muscle cells have a vacuolated cytoplasm with myelin-like figures. Elastic fibers in the wall are less numerous and less regularly arranged than those of the blood vessel wall. Connective tissue cells are sporadically encountered in the lymphatic wall.

PAM staining of the lymphatic vessel

The plasma membrane, nuclear envelope, endoplasmic reticulum, mitochondrial membrane, and membrane of the endothelial cell vesicle all stain positive. The lysosome and a part of the Golgi complex also stain positive. However, the cytoplasmic filament and the basal lamina do not stain at all (Fig. 2), though some parts of the latter show a slight deposit of silver.

The findings in the smooth muscle cell are similar to those in the case of the lymphatic endothelial cell. Collagen fibers in the wall are strongly positive and some are especially strongly stained on the fiber surface. By contrast, extracellular fine fibrils and elastic fibers are not stained. There is almost no difference in intensity of silver deposit between the sections incubated for 90 and 120 minutes. The ultrathin specimens stained without an oxidating procedure with periodic acid, but otherwise stained in the same way, show smaller silver deposits in the membranes of the lymphatic endothelium and the

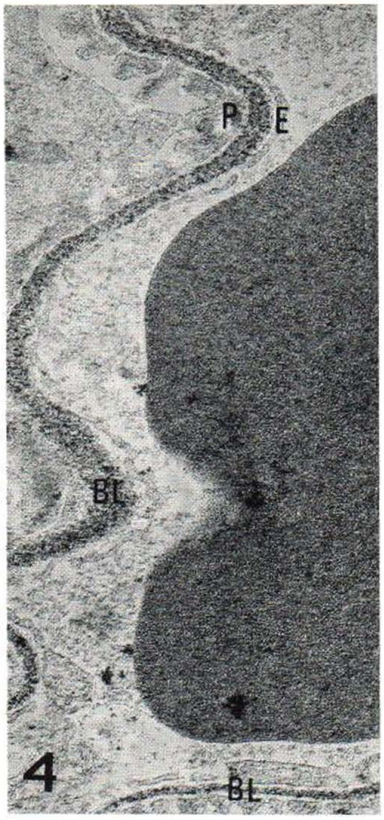


Fig. 4. Three layers of the basal lamina (*BL*) of the glomerular capillary endothelium (*E*) show a strong deposit of silver and the plasma membranes of the endothelial cell (*E*) and of the podocyte (*P*) are also positive. PAM staining of glomerulus of rat kidney, ×25 000.

same strong reaction of the collagen fibers (Fig. 3). Another control, stained in the silver nitrate deficient solution but otherwise prepared as above, revealed no silver particles in the tissue.

PAM staining of glomerular capillary of rat kidney

All three laminae of the basal lamina of the capillary —lamina densa, rara externa et interna—show clear and massive deposits of silver, which are continuous with the mesangial matrix. The positive cellorganelles of the endothelial cell and of the podocyte are almost the same as those of the lymphatics (Fig. 4).

DISCUSSION

A luminal surface coat of the lymphatic endothelium cannot be observed, even with PAM staining. It is not clear which leaflet of the cell membranes it is that stains positive, since the size of a deposited silver particle is greater than the width of the leaflet. Kajikawa (1) has described how a mature collagen fiber shows silver deposition in accordance with its periodicity, whereas an immature one shows impregnation of the surface. In this experiment both types of collagen fiber are observed. PAM impregnation has been used to selectively stain the basal laminae of the renal glomerular capillary, of the lung, of the intestinal epithelium, of the pancreas acinus, and of the thyroid gland (7). However, the basal lamina of the iris capillary has been reported to be negative with this stain (8). After staining, the basal laminae of the lymphatic endothelium and of the smooth muscle cell are usually negative though occasionally very weak positive, possibly because of a reduced or totally lacking polysaccharide content. The author's observations have shown that the basal lamina of the subcutaneous lymphatic vessel taken from the dorsum of the foot of a diabetic patient shows a strong silver impregnation with PAM staining. This fact also supports the above explanation.

The author is inclined to consider that the basal lamina differs in its chemical composition depending on the adjacent cell and tissue, even though its fine structure appears to be similar in all cases after the usual staining with uranyl acetate and lead.

Rambourg (4, 5) has suggested that PAM-positive chromatin, nucleolus, and ribosome are not specific for the staining, as they are positively stained even after omission of oxidation with periodic acid. The positively stained collagen fibers in this investigation are also impregnated even without the oxidating procedure, which suggests that these positive substances are not necessarily specific for the staining. The duration of incubation in the staining procedure is considered to be sufficiently long, since the stainability of the specimens incubated for 90 and 120 minutes was the same.

The side effect of injected patent blue and hydrochloric procaine is not to be excluded because of the technical difficulty of performing the control experiments.

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