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ENZYME-HISTOCHEMICAL IDENTIFICATION OF THE HUMAN LYMPHATIC CAPILLARY BY ADENYLATE CYCLASE

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ABSTRACT

This investigation was performed in order to establish a new histochemical method for the identification of lymphatic capillaries. The microvasculature in specimens from human foreskin was examined for adenylate cyclase and alkaline phosphatase activity by light and electron microscopy. Lymphatic capillaries showed positive adenylate cyclase reactivity and negative alkaline phosphatase reactivity whereas the blood capillaries showed a positive reaction for alkaline phosphatase and a negative one for adenylate cyclase. The presence of adenylate cyclase activity in the endothelium of the lymphatic capillary may relate to microvascular function including the transcapillary exchange of water and solutes.

Understanding the function of dermal lymphatic capillaries in normal and pathologic conditions may elucidate the etiology, pathophysiology, diagnosis, and treatment of certain skin diseases. It is difficult to distinguish lymphatic from blood capillaries by light microscopy. A variety of techniques have been employed including identification of Factor VIII-associated antigen and the presence of Weibel Palade bodies to aid in this distinction. However Factor VIII and Weibel Palade granules are present in both types of capillaries (1,2). Although the existence of Weibel Palade granules in the endothelial cells of dermal lymphatics in the healthy state has not been demonstrated, the authors have noted their presence in the endothelium of cutaneous lymphangiomas. Therefore, neither Factor VIII-associated antigen nor Weibel Palade granules are of diagnostic value in differentiating lymphatic from blood capillaries. A specific immunohistochemical stain for the identification of dermal lymphatics has not been established (3), although the presence of 5'-nucleotidase activity in the wall of non-dermal lymphatic capillaries has been described (4-6). We have also demonstrated the presence of alkaline phosphatase, aminopeptidase, peroxidase and acid para-nitrophenyl phosphatase in blood but not lymphatic capillary endothelium (7). The elucidation of enzyme activity in endothelial cells with dermal lymphatic and blood capillaries may shed light on the different functions of the two types of vessels. The present study was designed to investigate adenylate cyclase activity in the wall of human lymphatic capillary.

MATERIALS AND METHODS

Human foreskin from 16-, 18-, 35-, and 42-year old patients with phimosis were excised under 1% lidocaine hydrochloride local anesthesia. Each specimen was divided in two and each half frozen in liquid nitrogen and stored at -80°C in Tissue Tek's OCT compound (embedding medium for frozen tissue specimens) until examined for adenylate cyclase or alkaline phosphatase activity; the other half was prepared for

electron microscopy by conventional methods and examined in an Hitachi 12A electron microscope at 100 kV.

In order to detect adenylate cyclase activity, 8μ frozen sections were cut and processed along with the appropriate controls according to Howell and Whitfield (8). To determine alkaline phosphatase activity, 8μ frozen sections were processed according to Burstone (9). For electron microscopy, sections were washed in HEPES (N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid)-buffer with 0.25M sucrose, fixed in 1% glutaraldehyde for two hours at 4°C, dehydrated in graded ethanol, and re-embedded by placing an inverted Beem capsule filled with the embedding medium over the slide, incubating and "popping" off the section according to the method of Nakane (10). Reaction-positive areas were trimmed, sectioned, and stained in uranyl acetate for electron microscopy.

Specimens which showed either macro- or microscopic pathological changes were excluded.

RESULTS

Ultrastructurally, dermal lymphatic capillaries were characterized by a wide, irregular lumen and a thin wall with a discontinuous basal lamina, open endothelial junctions, and absence of pericytes (11) *(Fig. 1)*. Blood capillaries, in contrast, showed a high endothelium, a continuous basal lamina, and a regular rounded contour *(Fig. 2)*.

A summary of the findings for adenlate cyclase activity is shown in *Table 1*. The lymphatic capillary showed a positive, but discontinuous reaction which was more pronounced after 90 minutes incubation, although there was more contamination after this prolonged period of time. *Fig. 3A*

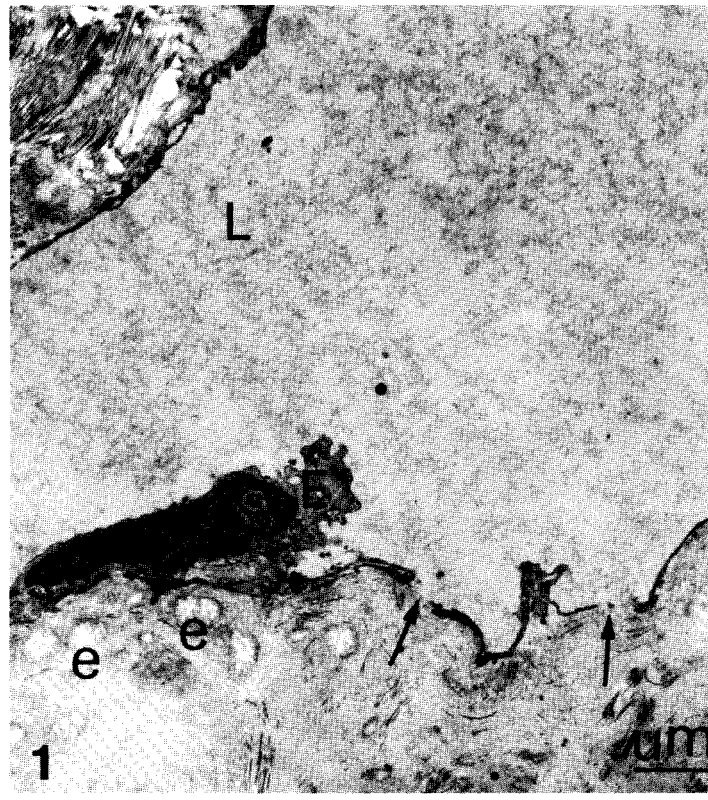


Fig. 1. An electron micrograph of a lymphatic capillary in the foreskin of an 18-year old man. Note the wide, irregular lumen (L); protruding nucleus (E); open endothelial junctions (arrows); discontinuous basal lamina and elastic fibers (e). x2,000.

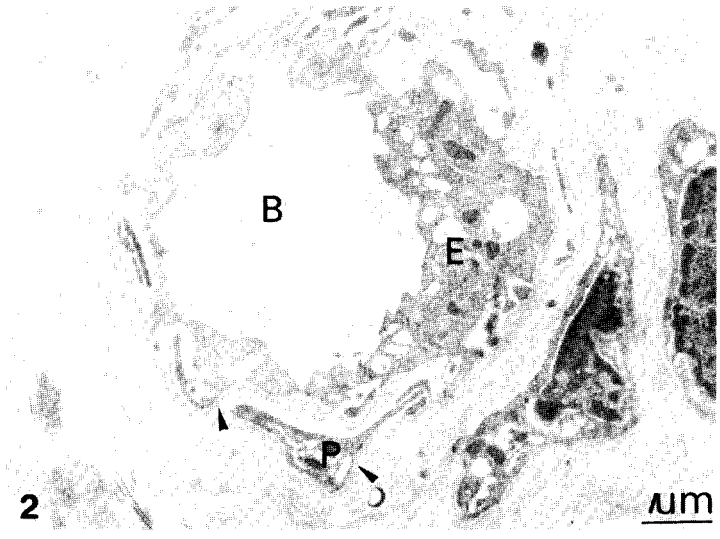
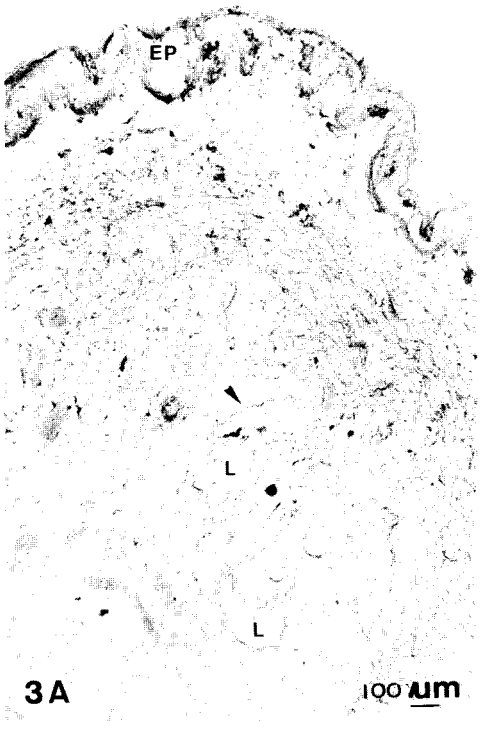


Fig. 2. An electron micrograph showing a blood capillary of the same specimen taken in Fig. 1. *Note the rounded lumen (B); regular contour; high endothelial cell (E); continuous basal lamina (arrows); pericyte (P). x3,000.*

TABLE 1
Light Microscopic Staining Reactions
(- none; ± weak; + moderate; ++ strong)

Agent	Incubation Time (min)	Temp (°C)	Capillary	
			Lymphatic	Blood
Adenylate cyclase	60	37	+	-
	90	37	++	-
Substrate-free medium	60	37	-	-
5mM alloxan in medium	60	37	-	-
Medium + L-p-bromotetramisole*	60	37	+	-
	90	37	++	-
Preincubated in 1% glutaraldehyde	10	4	±	-
	60	4	-	-
Preincubated in buffer	60	60	-	-
Alkaline phosphatase	30	37	-	+
	60	37	-	++
Substrate-free medium	60	37	-	-
Medium + L-p-bromotetramisole*	60	37	-	-
Preincubation in buffer	60	60	-	-

*0.1mM



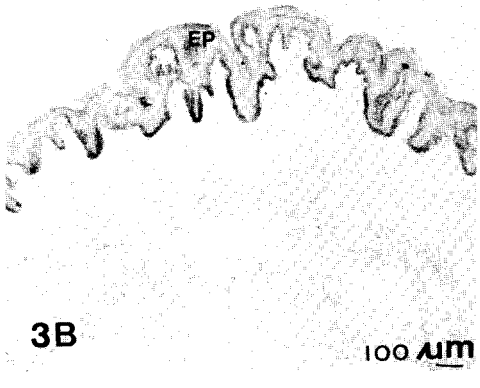


Fig. 3. A. Light micrograph of collapsed (arrowhead) and open (L) lymphatic capillaries in the foreskin of a 16-year old man. Adenylate cyclase activity is seen as a discontinuous black line in the vessel wall. x13. B. Light micrograph of a control for Fig. 3, *incubated in a substrate-free medium. x13.*

is a light micrograph of skin with lymphatic vessels showing a positive reaction for adenylate cyclase activity, whereas *Fig. 3B* is a control. *Figs. 4 and 5* are enlargements of the two lymphatic capillaries shown in *Fig. 3A*. *Fig. 6* is an electron micrograph of a lymphatic capillary showing positive adenylate cyclase activity; the blood capillaries were negative for this enzyme on light microscopy.

DISCUSSION

We previously showed that blood capillary endothelium is enzymatically more active than lymphatic endothelium (7). However, lymphatic endothelium has shown 5'-nucleotidase activity (4-6) and in the present study adenylate cyclase activity. The enzyme 5'-nucleotidase catalyzes the conversion of 5'-adenosine monophosphate to adenosine, and adenylate cyclase catalyzes the reaction by which adenosine triphosphate is converted to cyclic adenosine monophosphate (8,12). The latter reaction is necessary for a number of cell activities including differentiation, division, hormonal reactions, and calcium metabolism (8,12,13). We propose that adenylate cyclase is associated with the active transport of water and electrolytes and thus plays a role in lymph formation.

It can be seen in *Figs. 3A, 4 and 5* that the adenylate cyclase activity in the lymphatic endothelium is not uniform. Possible explanations for this finding are: 1) the endothelium was damaged during processing; 2) two distinct types of endothelial cells exist; 3) the section was tangentially cut; or 4) parts of the endothelial lining are too thin to be detected by light microscopy.

There is disagreement with respect to the enzyme inhibiting effect of lead compounds in the incubation medium (14). The present study, however demonstrates that lead nitrate does not interfere with enzyme activity as the lymphatic vessels show a positive staining reaction.

Contrary to the work of Szumanska et al (15) and Vorbrodt et al (16), we have

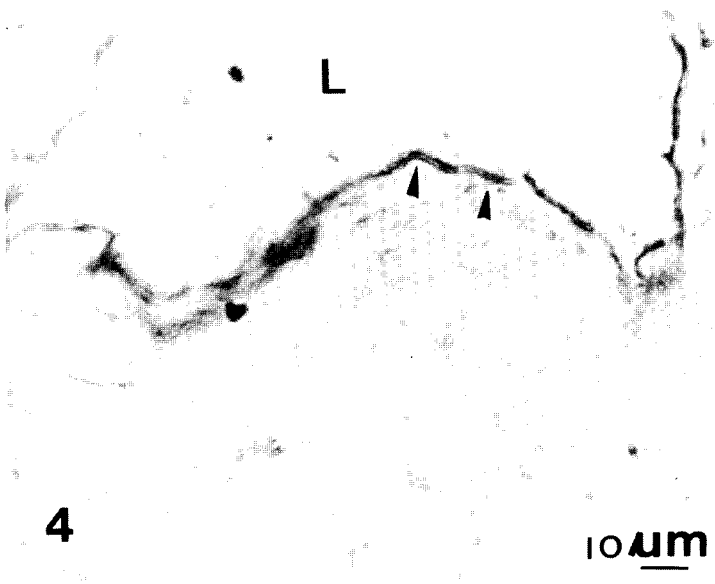


Fig. 4. A higher magnification of one of the lymphatics shown in Fig. 3A. *Note a discontinuous dark line denoting adenylate cyclase activity; in places the line is doubled (arrowheads). L=lumen. x150.*

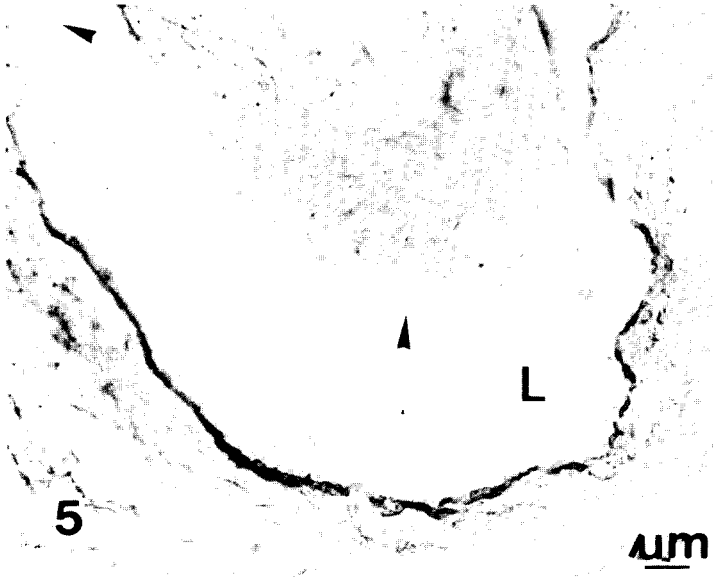


Fig. 5. A higher magnification of another lymphatic shown in Fig. 3A. *Note discontinuous or inconsistent staining for adenylate cyclase activity; unstained areas (arrowheads). L=lumen. x150.*

demonstrated a negative reaction for adenylate cyclase in the endothelium of blood vessels. This discrepancy may relate to differences in animal species, in organs, size of the vessel, sensitivity of the reaction, and light vs. electron microscopic findings. Whereas most cells are involved with cyclic adenosine monophosphate and adenylate cyclase activity, enzyme activity may vary in different cell types. This investigation has demonstrated a difference in adenylate cyclase activity between blood and lymphatic capillaries; however, the findings do not necessarily signify that blood capillaries are totally devoid of adenylate cyclase.

In conclusion, the histochemical

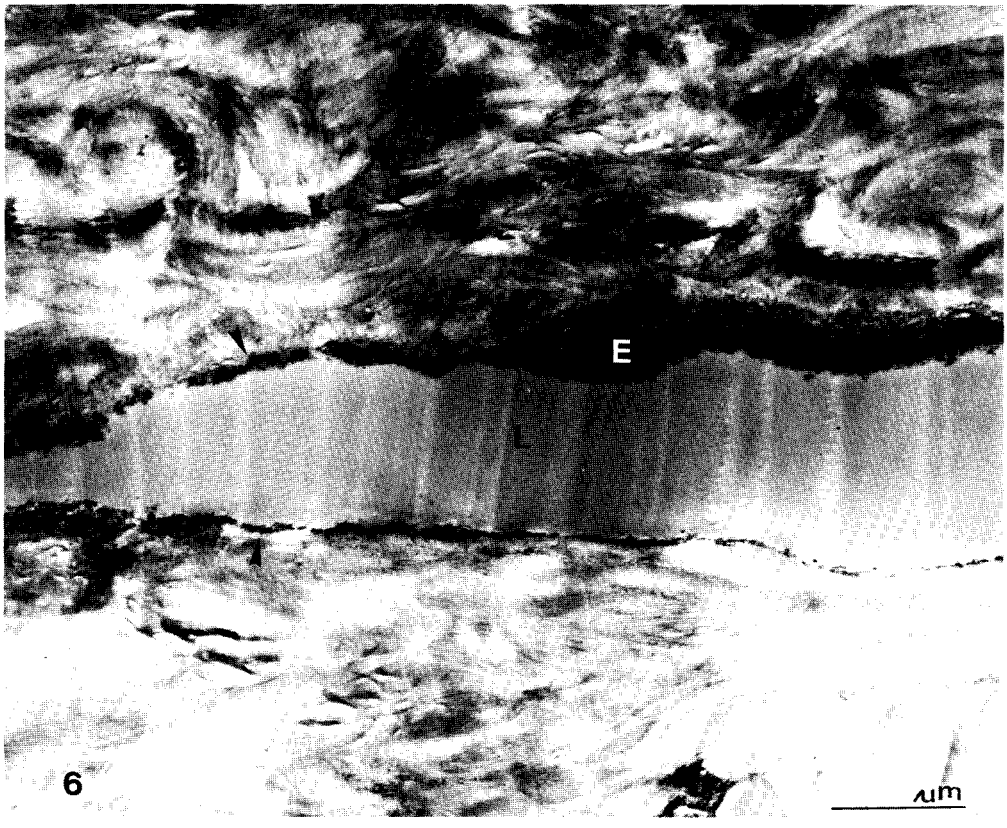


Fig. 6. An electron micrograph of a lymphatic capillary in the foreskin of a 42-year old man. Adenylate cyclase activity (arrowheads) is seen in the endothelial layer (E). L=lumen. x2,000.

method described for the identification of adenylate cyclase activity may histologically differentiate between blood and lymphatic capillaries of the dermis.

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