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BRIEF COMMUNICATION

ENZYME-HISTOCHEMICAL STAINING OF DERMAL LYMPHATIC CAPILLARIES BY GUANYLATE CYCLASE

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ABSTRACT

Human foreskins were examined for enzyme-histochemical staining of microvessels using guanylate cyclase, an enzyme similar to adenylate cyclase. Like 5'-nucleotidase (which hydrolyzes 5'-adenosine monophosphate to adenosine), and adenylate cyclase (which converts adenosine triphosphate to cyclic AMP), guanylate cyclase selectively stains positive for lymphatic capillaries and therefore may be another useful histochemical marker to differentiate dermal lymph from blood capillaries.

Vetter (1) and Nishida and Ohkuma (2) have demonstrated that lymph capillaries but not blood capillaries stain positive for 5'nucleotidase (1) and adenylate cyclase (2). Guanylate cyclase is an enzyme closely linked to adenylate cyclase, and, accordingly, we examined the histochemical staining characteristics of this enzyme in microvessels of human foreskin for its utility in distinguishing lymph from blood capillaries.

MATERIALS AND METHODS

Human foreskins were removed under local anesthesia using 1-2% lidocaine hydrochloride from 4 adult men for treatment of phimosis. The specimens were cut into small pieces, promptly immersed in liquid nitrogen and stored at -80°C. Cryosections were incubated in medium for 60 minutes at 37°C using the method of Saito et al (3) for guanylate cyclase. Control pieces were incubated without the substrate (guanylyl imidodiphosphate), and preincubated in 1% glutaraldehyde/HEPES buffer for 60 minutes.

To identify lymphatic capillaries, a positive microvessel on cryosection was shown to lack pericytes on electron microscopy after Epon-embedded block was trimmed for ultrathin section. Other cryosections were stained for alkaline phosphatase for 60 minutes (4) for blood capillaries and compared with stained microvessels.

RESULTS

Microvessels which lacked pericytes (i.e., lymph capillaries) as demonstrated by electron microscopy *(Figs. 1,2)* stained positive for guanylate cyclase *(Figs. 3-5)* in contrast to blood capillaries which stained positive for alkaline phosphatase *(Fig. 6)*. The blood capillary was negative for guanylate cyclase. Only a small portion of the lymphatic capillary membrane was negative for guanylate cyclase. Controls were negative.

DISCUSSION

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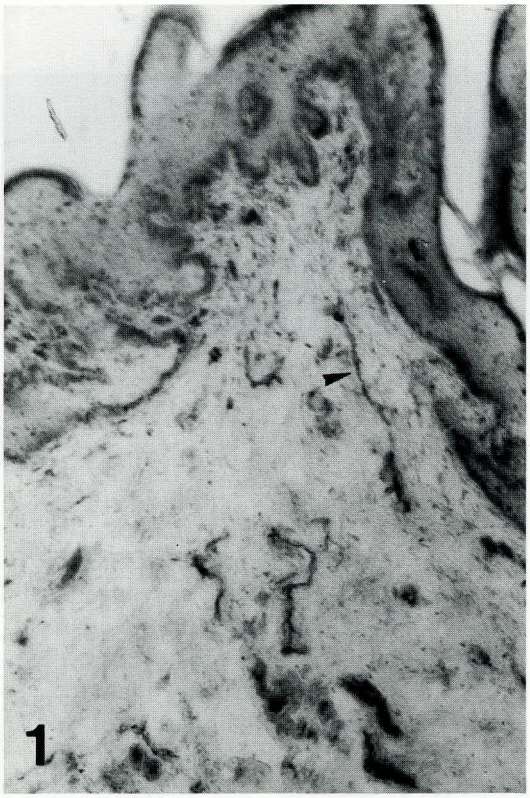

Fig. 1. A guanylate cyclase positive capillary (arrowhead) was chosen for electron microscopy. x75 (see Fig. 2)

Guanine triphosphate is converted to cyclic 3,5'-guanine monophosphate by guanylate cyclase, cyclic GMP to 5'-guanosine monophosphate by phosphodiesterase and 5'-guanosine monophosphate to guanosine by 5'-nucleotidase. Goldberg et al (6) suggest that adenylate and guanylate cyclases, despite seemingly antagonistic function, maintain balanced activity. Inhibition of these enzyme reaction by lead nitrate is controversial. LeMay et al showed that boiled tissue maintains cyclic AMP activity if adenylate cyclase is added to the reaction (7). Cutler, on the hand (8), showed that lead nitrate inhibited adenylate cyclase reaction. Fujimoto et al (9) maintained that this inhibition by lead nitrate is of minimal consequence. Our control specimens with tissue omitting the guanylate immunodiphosphate substrate and "overfixing" the tissue in 1% glutaraldehyde are sufficient to block lead nitrate inhibition. Immunohistochemical staining with anti-cyclic GMP antibody also shows positivity for lymph but not blood capillaries (10).

Whenever a linear structure bulges at one end under microscopy, it is probably a lymphatic rather than blood capillary or nerve ending. If the capillary lacked a pericyte by electron microscopy and did not stain for alkaline phosphatase, the microvessel was considered to be a lymphatic capillary.

Concentration of cyclic GMP in a cell is controlled by 1) activity of guanylate cyclase, 2) phosphodiesterase, 3) extracellular escape of cyclic GMP, 4) binding with protein which is resistant to dissolution. Which factor is the most important to account for high levels of cyclic GMP in a lymph capillary is unknown. Guanylate cyclase which is activated by acetyl choline is located in the membrane and is regulated by calcium transport. Perhaps guanylate cyclase contributes to imbibition of fluid and electrolytes into initial lymphatics and their transport in contractile lymphatics. Simultaneous histochemical staining of microvessels using alkaline phosphatase (for blood capillaries) and guanylate cyclase *(Fig. 7)* (for lymphatic capillaries) make differentiation of these microvessels easier (11). A small part of the lymphatic capillary often stains negative for guanylate cyclase but the reason is unclear. It may relate to different kinds of endothelial cells, the capillary membrane being too thin to be recognized as positive using light microscopy, an artifact related to tangential sectioning, or the membrane lining has been damaged or partially desquamated in preparation for histology (i.e., during cryosectioning).

CONCLUSIONS

The microvessels of human foreskin were histochemically stained for guanylate cyclase. Lymph capillaries were positive whereas blood capillaries were negative. Along with adenyl cyclase and 5'nucleotidase which selectively stain positive for lymph capillaries and

Fig. 2. The microvessel shown in Fig. 1 demonstrates positive staining endothelial cells for guanylate cyclase (arrowheads) and lacks pericytes consistent with a lymphatic capillary. Electron micrograph x6,700

alkaline phosphatase which stains positive for blood capillaries, histochemical enzyme staining of the microvessels with guanylate cyclase may also help distinguish lymph from blood capillaries.

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Fig. 3. Other serial sections were stained for guanylate cyclase. Collapsed capillaries (arrowheads) stain positive in the superficial dermis. x200

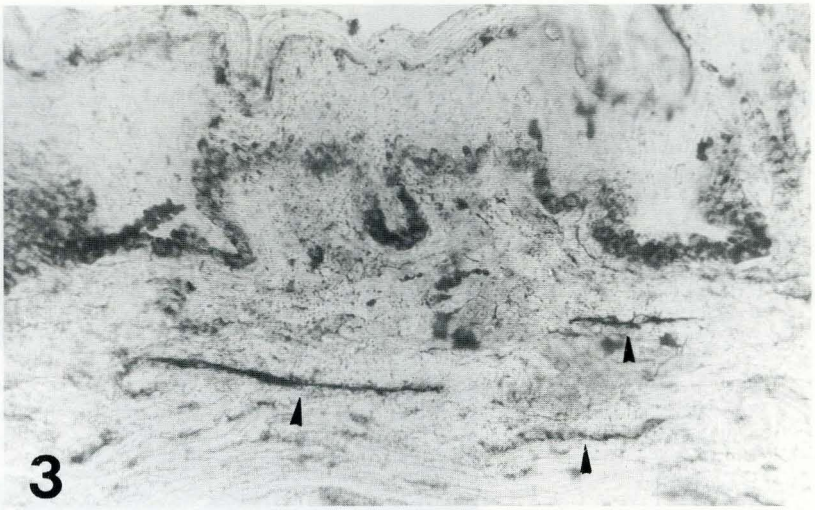

Fig. 4. The lymphatic capillaries (arrowheads) and the intima of a larger lymphatic vessel (L) stain positive for guanylate cyclase. x200

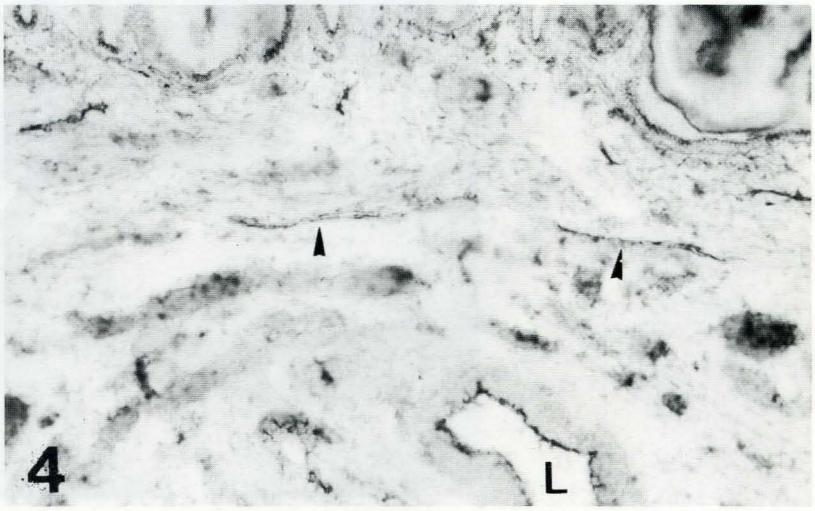

Fig. 5. A collapsed lymphatic capillary possesses slightly dilated portion (arrowhead). x150

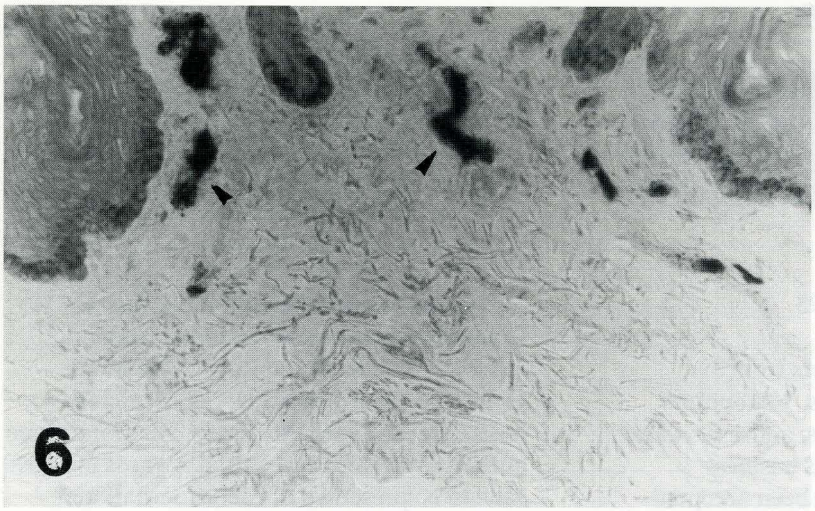

Fig. 6. Other serial sections stained for alkaline phosphatase demonstrate positive microvessels with a different structure consistent with blood capillary (arrowheads). x200

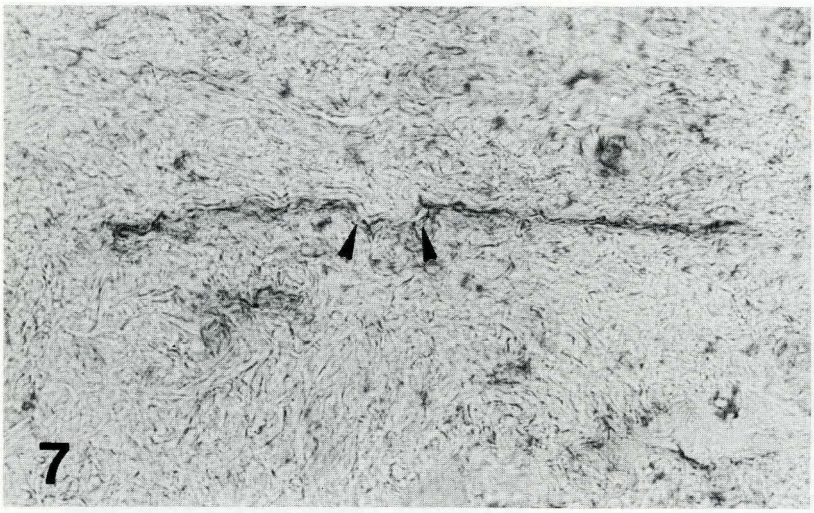

Fig. 7. For purposes of comparison the same foreskin has been stained for adenylate cyclase. A slightly dilated lymphatic (arrowheads) is stained positively. x300

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