Lymphology 25 (1992) 134-138

BRIEF COMMUNICATION

ENZYME-HISTOCHEMISTRY OF THE FORESKIN MICRO-VASCULATURE USING FRUCTOSE-1,6-DIPHOSPHATASE

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ABSTRACT

Microvascular capillaries (blood and lymph) in human foreskin were examined for staining characteristics for fructose-1,6-diphosphatase (FDPase). Based on enzyme-histochemistry and electron microscopy, both blood and lymph vessels and microvessels as well as dermal smooth muscle all stain positively but with varying intensity for FDPase.

A number of studies aim to distinguish blood and lymph microvasculature using enzyme-histochemistry. For example, blood capillaries stain more intensely than lymph capillaries for alkaline phosphatase, aminopeptidase, endogenous peroxidase, and acid p-nitrophenyl phosphatase (1). Fructose-1,6-diphosphatase (FDPase) is an enzyme which hydrolyzes one phosphate group from fructose-1,6-diphosphate and is considered an important component for glycogenesis as exemplified in the sperm tail as a source of energy. Because lymphatics also require energy for intrinsic propulsion in the transport of lymph, we examined the presence of this enzyme in blood and lymph vessels and capillaries.

MATERIALS AND METHODS

Human foreskins were removed during circumcision under local anesthesia in four adults (age 16-33 years). The specimens were cut into pieces, promptly frozen in liquid nitrogen and stored at -80°C in OCT compound and later examined according to the method of Saito and Ogawa (2). The cryosections were incubated for 60 min at 37°C in 5.6ml of 0.2M Tris-HCl buffer, pH 8.5, fructose-diphosphate (sodium salt) (8.4mg), 15mM magnesium sulfate (10.4ml), 0.5% lead citrate (16.0ml) and Aq. bidest (8.0ml) with the pH adjusted to 9.5. In controls either the substrate (fructose diphosphate) was omitted or inhibited by adding 5mM-p-chlorbenzoic acid. Subsequently, after appropriate washing, staining with 1% ammonium sulfide, fixation in paraformaldehyde and embedding in glycerin-jelly were carried out for final sections.

For identification of lymphatic capillaries, every other cryosection after serial sectioning was stained for alkaline phosphatase using Burstone's incubation method (3) and for 5' nucleotidase after Wachstein and Meisel's incubation method with the addition of L-bromoteramisole (4). The remaining excised foreskin was embedded for electron microscopy.

RESULTS

Capillaries with a large lumen, thin wall irregular shape, luminal and abluminal wall projections, that stained negative for

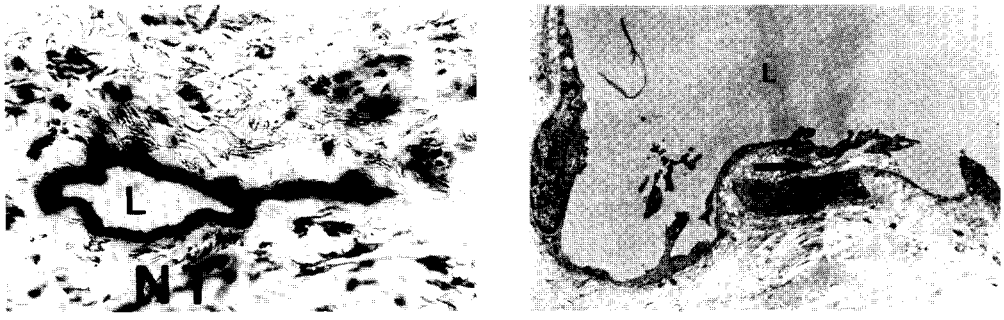

Fig. 1. Left—foreskin section showing lymphatic capillary (L) staining positively for 5'nucleotidase. Right—ultrastructure showing characteristics of lymphatic (L) capillary with thin irregular endothelial cell with luminal and abluminal projection (P), open interendothelial junction (arrow) and interrupted basal lamina (x4000).

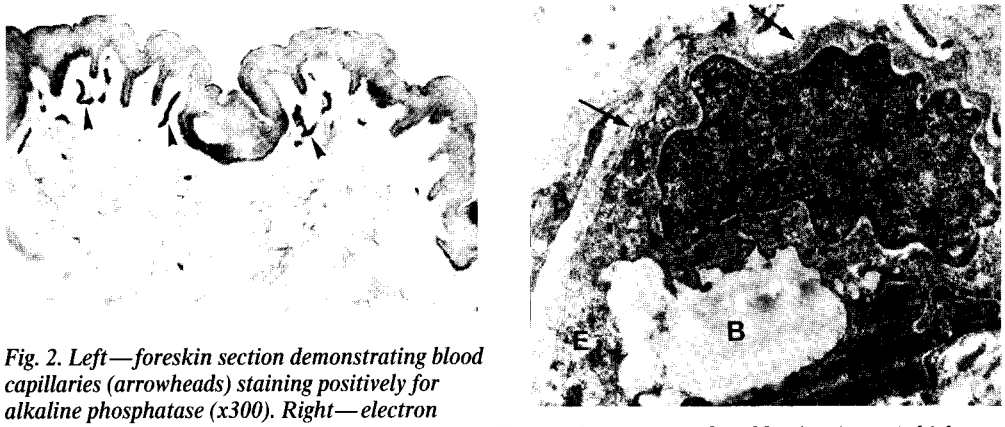

Fig. 2. Left—foreskin section demonstrating blood capillaries (arrowheads) staining positively for alkaline phosphatase (x300). Right—electron micrograph showing characteristics of blood (B) capillary with continuous basal lamina (arrow), high endothelium (E), and comparatively narrow lumen (x20,000).

alkaline phosphatase and positive for 5'nucleotidase (5-8) *(Fig. 1,2)* and on ultrastructure showed absent, scanty basal lamina and open endothelial junctions (9-11) were considered lymphatic capillaries *(Fig. 1)*. In contrast, microvessels with a small, regular round lumen with thick wall that stained positive for alkaline phosphatase and negative for 5'nucleotidase, and on electron microscopy showed an intact, uninterrupted (basal lamina) with closed interendothelial junction *(Fig. 2)* were deemed blood capillaries.

Fructose-1,6-Diphosphatase (FDPase)

Both blood and lymph vessels and microvessels in the subcutis stained positively for FDPase *(Figs. 3,4)*. Intradermal muscle also stained positively for FDPase *(Fig. 5)*. In contrast, control sections (without substrate or enzyme inhibition with p-chlorbenzoic acid) were without dermal reaction *(Fig. 5)*. Light microscopy of the excised foreskin did not show noteworthy pathologic change.

COMMENT

Both lymph and blood vasculature stain positively for FDPase which may reflect the vasomotion capabilities of both circulatory systems. In unpublished observations, we have noted that "portwine" stains which are

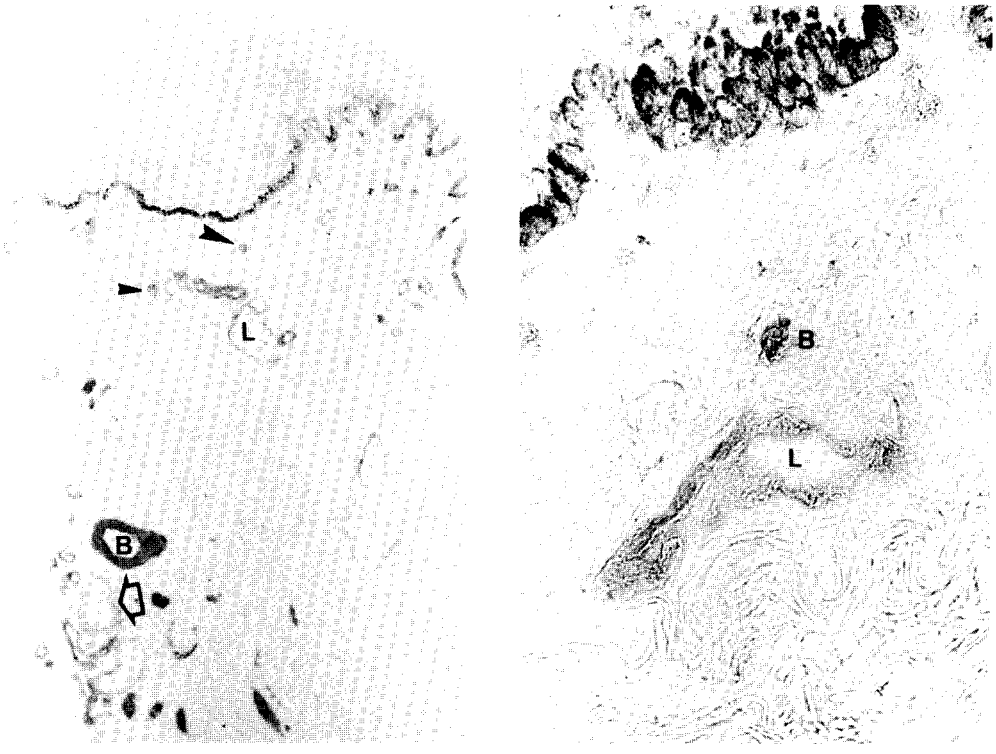

Fig. 3. Left—light micrograph (human foreskin) shows positive staining for fructose-1,6-diphosphatase (FDPase) in both blood vessel (B) and capillaries (arrowhead) and lymph vessel (white arrow) and lymph capillary (L) (x30). Right—another section showing positive staining for FDPase in the wall of both lymphatic (L) and blood (B) capillary (x400).

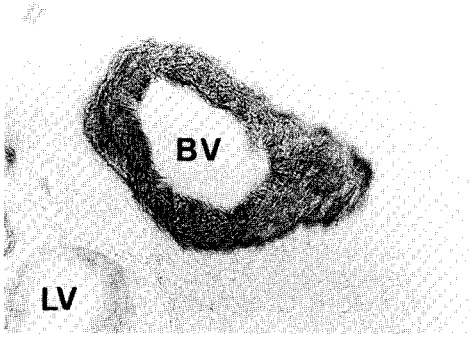

Fig. 4. High power light micrograph showing both a blood vessel (BV) and lymphatic vessel (LV) staining positive for FDPase.

largely a stagnant blood pool react weakly or not at all with FDPase. Because FDPase is also seen in smooth muscle cells, one may speculate that this enzyme is localized in the myosin filament of the endothelial cells (both blood and lymphatic). Whereas FDPase stain may not be specific for the circulatory system (it also stains muscle) it nonetheless may be a useful enzyohistochemical staining technique for histologic differentiation of various skin diseases. Although it may be difficult at times to distinguish blood capillaries from sweat glands in skin cryosections, the absence of sweat glands in foreskin precluded possible confusion in this study.

More recently adenylate cyclase has also been found in human cutaneous lymphatic capillary (12). Of interest, this enzyme plays both an inhibitory and regulatory role in fructose kinetics as well as anaerobic and aerobic glycolysis.

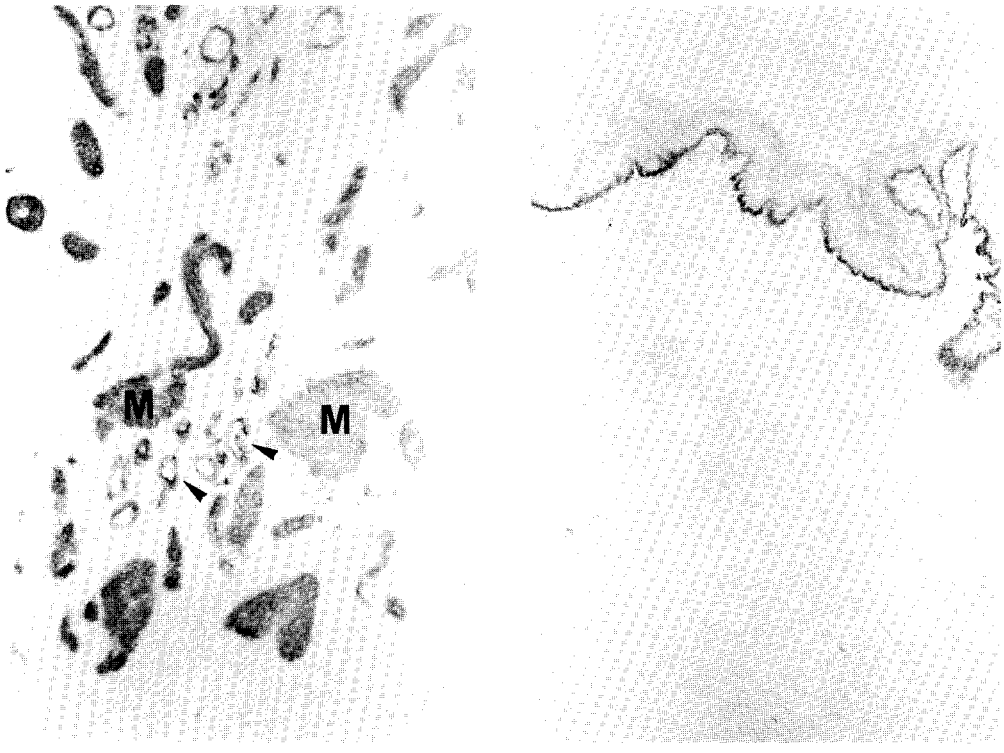

Fig. 5. Left—dermal muscle (M) and possibly small lymphatics (arrowheads) staining positively for FDPase (x150). Right—substrate free control section shows dermis entirely negative for FDPase. The epidermal basal layer is positive and probably represents melanin (x40).

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